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Compromised Factor-Dependent Transcription Termination in a *nusA* Mutant of *Escherichia coli*: Spectrum of Termination Efficiencies Generated by Perturbations of Rho, NusG, NusA, and H-NS Family Proteins[▽]

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The proteins NusA and NusG, which are essential for the viability of wild-type *Escherichia coli*, participate in various postinitiation steps of transcription including elongation, antitermination, and termination. NusG is required, along with the essential Rho protein, for factor-dependent transcription termination (also referred to as polarity), but the role of NusA is less clear, with conflicting reports that it both promotes and inhibits the process. In this study, we found that a recessive missense *nusA* mutant [*nusA*(*R258C*)] exhibits a transcription termination-defective (that is, polarity-relieved) phenotype, much like missense mutants in *rho* or *nusG*, but is unaffected for either the rate of transcription elongation or antitermination in λ phage. Various combinations of the *rho*, *nusG*, and *nusA* mutations were synthetically lethal, and the lethality was suppressed by expression of the N-terminal half of nucleoid protein H-NS. Our results suggest that NusA function is indeed needed for factor-dependent transcription termination and that an entire spectrum of termination efficiencies can be generated by perturbations of the Rho, NusG, NusA, and H-NS family of proteins, with the corresponding phenotypes extending from polarity through polarity relief to lethality.

The Nus proteins of *Escherichia coli* were initially identified as host factors needed for N-mediated transcription antitermination during lytic growth of λ phage, and they were subsequently also shown to participate in antitermination of transcription in the rRNA operons. Antitermination in both these instances is dependent on presence of specific "box" sequences that mediate assembly of a transcription elongation complex which is resistant to normal transcription termination signals (reviewed in references 17, 25, 40, 44, 48, and 55).

Of the various Nus proteins, NusA and NusG also function as general transcription accessory factors, serving to modulate both elongation and termination of transcription elsewhere in the genome (6, 40, 44, 48). The two proteins (both essential for viability in wild-type *E. coli*) have been reported to exert antagonistic effects on the rate of transcription elongation (increased by NusG and decreased by NusA), and they have also been implicated in the two different processes of transcription termination, namely, intrinsic or factor independent (NusA) and factor dependent (NusA and NusG). The latter is further described below. Recently, NusA has also been shown to be involved in mutagenesis and transcription-coupled repair of DNA damage, the detailed mechanisms for which remain to be elucidated (15, 16).

Factor-dependent (also known as Rho-dependent) transcription termination underlies the phenomenon of transcriptional polarity and refers to the process by which synthesis of untranslated nascent transcripts (such as, for example, downstream of either a premature stop codon within, or a normal stop codon at the end of, the coding sequence of a gene) is terminated. The mechanism is absolutely dependent on the essential Rho protein that binds the nascent transcript and is then believed to signal RNA polymerase to terminate transcription, but there is no consensus as yet on its details (reviewed in references 1, 3, 14, 40, 41, 43, and 48; see also references 21 and 31). As with their effects on transcription elongation, NusA and NusG have been reported to function antagonistically for Rho-dependent transcription termination as well, with NusG increasing its efficiency (7, 8, 11, 29, 35, 38, 52) and NusA decreasing it (8, 27, 32–34); indeed, it has been reported that mutations in *rho* can suppress the inviability associated with the loss of NusA (58). On the other hand, Gottesman and coworkers (9, 52–54) have suggested that both NusA and NusG act cooperatively to promote Rho-dependent termination.

NusA is a 55-kDa conserved bacterial protein comprised of an N-terminal domain; the three domains S1, KH1, and KH2 with RNA-binding motifs; and two C-terminal acidic repeat domains AR1 and AR2 (5, 6, 24, 44, 48, 49, 57). In the present study, we identified a recessive substitution mutation in *nusA* that confers relief of transcriptional polarity (much like missense mutations in *rho* or *nusG*) but has no apparent effect on transcription elongation or λ antitermination *in vivo*. The *nusA*(*R258C*) mutation (in the KH1 domain) was synthetically lethal with *rho* or *nusG* mutations, and the lethality could be suppressed by certain perturbations of the H-NS family of nucleoid proteins that have earlier been shown (29, 47) to restore the efficiency of tran-

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

Strain[a]	Genotype[b]
MC4100	Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*
RS445	MC4100 *galEp3*, λRS88 lysogen carrying P_{lac}-*lacZYA*
GJ3119	MC4100 *nusA1*
GJ5147	MC4100 *galEp3*, λRS45 lysogen carrying P_{lac}-H-19Bt_{R1}-*lacZYA*
GJ6504	MG1655 *lacI* $lacZ_{U118}$ *trpR55 trpE9777*
GJ6509	GJ6504 *rho*(*A243E*)
GJ6511	GJ6504 *nusG*(*G146D*)
GJ6520	GJ6509 Δ(*yefM-yoeB*)::Cm
GJ6524	GJ6504 *nusG*(*G146D*) Δ(*yefM-yoeB*)::Cm
GJ7360	GJ6520 *argE55*::Tn*10*
GJ7361	GJ6520 *ilv-500*::Tn*10*
GJ7362	GJ6524 *ilv-500*::Tn*10*
GJ7363	GJ6524 *argE55*::Tn*10*
GJ7368[c]	GJ6504 Δ(*yefM-yoeB*)::Cm *rho*(*R102S*, *A243E*) *zif-909*::Tn*10*dTet/pLG-H-NSΔ64
GJ7376*	GJ6524 *rho*(*Q32R*)/pLG-H-NSΔ64
GJ7387*	GJ6524 *nusA*(*R258C*)/pLG-H-NSΔ64
GJ7395*†	GJ7387 Cm^s
GJ7417‡	GJ6509 *nusA*(*R258C*) *truB*::Tn*10*dCm
GJ7419‡	GJ6504 *nusG*(*G146D*) *nusA*(*R258C*) *truB*::Tn*10*dCm
GJ10524	GJ6504 *nusA*(*R258C*)
GJ10557	GJ6524 *rho*(*Q32R*)
GJ10572	GJ5147 *nusA*(*R258C*)
GJ10573	RS445 *nusA*(*R258C*)
GJ10581‡	GJ6504 *rho*(*Q32R*) *nusA*(*R258C*) Δ(*yefM-yoeB*)::Cm
GJ10591‡	GJ6524 *rho*(*A243E*)
GJ10668[d]	GJ7419 *rho*(*A243E*)
GJ10669‡	GJ7419 *rho*(*Q32R*)
GJ10697	GJ10527 *rpoB8 btuB*::Tn*10*
GJ10699	*rpoB8 btuB*::Tn*10*
GJ10700	*btuB*::Tn*10*
GJ10702	GJ10527 *btuB*::Tn*10*
GJ10753†	GJ6504 *rho*(*Q32R*) Δ(*yefM-yoeB*)
GJ10793§	GJ10572 *rho*(*R102S*, *A243E*) *zif-909*::Tn*10*dTet
GJ10794§	GJ10573 *rho*(*R102S*, *A243E*) *zif-909*::Tn*10*dTet
GJ10795	GJ5147 *rho*(*Q32R*)
GJ10796	GJ5147 *nusG*(*G146D*) *rho*(*Q32R*)
GJ10797	GJ10572 *rho*(*Q32R*)
GJ10798	RS445 *rho*(*Q32R*)
GJ10799	RS445 *nusG*(*G146D*) *rho*(*Q32R*)
GJ10800	GJ10573 *rho*(*Q32R*)
GJ10801	GJ5147 *rho*(*R102S*, *A243E*) *zif-909*::Tn*10*dTet
GJ10802	RS445 *rho*(*R102S*, *A243E*) *zif-909*::Tn*10*dTet
GJ10811§	GJ10572 *nusG*(*G146D*) *argE55*::Tn*10*
GJ10812§	GJ10573 *nusG*(*G146D*) *argE55*::Tn*10*
GJ10813§	GJ10572 *rho*(*A243E*) *ilv-500*::Tn*10*
GJ10814§	GJ10573 *rho*(*A243E*) *ilv-500*::Tn*10*
GJ10817§	GJ10799 *nusA*(*R258C*) *truB*::*Tn10*dCm
GJ10818§	GJ10796 *nusA*(*R258C*) *truB*::*Tn10*dCm

[a] Strains MC4100, RS445, GJ5147, GJ6504, GJ6509, and GJ6511 have been described in the accompanying study (47), and strain GJ3119 was described by Harinarayanan and Gowrishankar (29). Strains GJ6520 and GJ6524 were constructed by S. Aisha (unpublished); all other strains were constructed in this study. *, the indicated strain also carries a Tn*10*dTet (37) insertion at undetermined chromosomal locations; †, the indicated strain was constructed from its ancestor by excision of the Cm^r marker in Δ(*yefM-yoeB*)::Cm by site-specific recombination with the aid of plasmid pCP20, as described previously (18); ‡, the indicated strain was maintained as a derivative with plasmids pHYD751 ($nusG^+$) or pHYD1201 (rho^+), as appropriate; §, the indicated strain was maintained as a derivative with plasmid pLG-H-NSΔ64.

[b] Genotype designations are as described previously (4). All strains are F^-. The *rho*(*A243E*) mutation is also interchangeably referred to as *rho-4*. References or sources for the mutations that were introduced by transduction into the strains are as follows: Δ(*yefM-yoeB*)::Cm (13); *argE55*::Tn*10*, *ilv-500*::Tn*10*, and *btuB*::Tn*10* (50); and *rpoB8* (51).

[c] The *zif-909*::Tn*10*dTet insertion was obtained in this study and shown to be 76% cotransducible with *rho*.

[d] Strain GJ10668 was maintained as a derivative with plasmids pHYD751 and pLG-H-NSΔ64.

scription termination in *rho* and *nusG* mutants (for reviews of the H-NS family proteins, see references 19, 20, 22, and 36). Our data lend support to the findings of Cardinale et al. (9) that NusA promotes factor-dependent transcription termination. Furthermore, our findings suggest that a spectrum of efficiencies of termination exists that extends from polarity at one extreme, through polarity relief, to lethality at the other extreme and that the position in this spectrum of any strain is determined by its complement of Rho, NusG, NusA, and H-NS family proteins.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The strains of *E. coli* used in the present study are listed in Table 1. Phage λNK1324, used for Tn*10*dCm

transposition, has been described earlier (37), and phage λ*c*I857 was from our lab stock. Media and growth conditions were largely as described in the accompanying study (47). Where necessary, the medium was supplemented with ampicillin (Amp), tetracycline (Tet), chloramphenicol (Cm), kanamycin (Kan), and IPTG (isopropyl-β-D-thiogalactopyranoside) at the described concentrations (47). Spectinomycin (Sp) supplementation was at 50 μg/ml.

Several plasmids were included (with salient features in parentheses): pACYC184 (p15A vector, Cm^r Tet^r [12]), pLG339 and pLG-H-NSΔ64 (pSC101 replicon vector and its derivative encoding H-NSΔ64, respectively, Kan^r [56]), pCP20 (pSC101-based Ts replicon encoding Flp recombinase, Amp^r [18]), pHYD2509 (pSC101 replicon encoding H-NSΔ64, Amp^r [47]), pHYD2546 (p15A replicon carrying *ydgT*, Cm^r [47]), and two plasmids that are IPTG dependent for replication (29), pHYD751 (*nusG*$^+$, Amp^r) and pHYD1201 (*rho*$^+$, Amp^r). The following three plasmids with *nusA*$^+$ were used: pHYD2554 (Amp^r, carrying the 10-kb EcoRI-HindIII fragment between kilobase coordinates 3310.06 and 3320.08 of the *E. coli* genome [45] cloned in a ColE1-based replicon, and obtained from Manjula Reddy); pHYD2557 (Cm^r, carrying a 2.3-kb PCR-amplified region between genomic nucleotide coordinates 3314061 and 3316393 [45] cloned in a pSC101-based Ts replicon and obtained from Ranjan Sen); and pHYD2556 (Sp^r, carrying the minimal *nusA*$^+$ open reading frame with its native ribosome-binding site between genomic nucleotide coordinates 3314061and 3315548 [45] cloned downstream of the *ara* regulatory region in a pSC101-based replicon and obtained from Ranjan Sen).

Isolation and genetic mapping of suppressors of *rho* and *nusG* derivatives expressing H-NSΔ64. For the selection of suppressors in which transcriptional polarity relief was restored, the parental strains were pLG-H-NSΔ64 derivatives of GJ6520 and GJ6524 (*rho* and *nusG*, respectively, both carrying the $lacZ_{U118}$ [Am] and *trpE9777* [Fr] mutations). (Although a procedure for Tn*10*dTet transposon mutagenesis [37] was attempted in obtaining the suppressors, subsequent experiments demonstrated that suppression was not caused by the transposon insertion [data not shown].) Selections were for $Anth^+$ (utilization of anthranilate [Anth] at 20 μg/ml to satisfy the auxotrophic requirement for tryptophan [Trp]), Mel^+ (utilization of melibiose as sole C source at 39°C), or both (47), that is, for restoration of transcriptional polarity relief of the *rho* and *nusG* derivatives even in the presence of H-NSΔ64.

The suppressors obtained were then analyzed as follows. Plasmid preparations from them were transformed into GJ6520 or GJ6524, as appropriate, to determine whether any of the mutations was on the pLG-H-NSΔ64 plasmid itself. For the non-plasmid-borne suppressors, phage P1 lysates prepared on the mutants were used to transduce pLG-H-NSΔ64-bearing derivatives of GJ7361 (*rho*) and GJ7362 (*nusG*) to Ilv^+, and GJ7360 (*rho*) and GJ7363 (*nusG*) to Arg^+ followed by scoring for the polarity-relief phenotypes. The first pair of strains carries an *ilv*::Tn*10* mutation and the second pair an *argE*::Tn*10* mutation that are linked to the *rho* and *nusG* loci, respectively; in this manner, suppressors mapping to either *rho* or *nusG* were sought to be identified.

The suppressor mutation in *nusA* was mapped with the aid of a transposon-tagging approach, as follows. Random transpositions of Tn*10*dCm were generated in GJ7395 following infection with λNK1324, as described previously (37). (GJ7395 is the derivative of the original *nusA* suppressor GJ7387 in which the FRT-flanked Cm^r insertion in Δ(*yefM-yoeB*) [13] was excised by site-specific recombination with the aid of plasmid pCP20, as described previously [18].) A P1 lysate prepared on a population of Cm^r cells was used for transduction into GJ6511/pLG-H-NSΔ64, with double selection for Cm^r $Anth^+$ colonies. One such clone was shown in subsequent transduction experiments to have the Tn*10*dCm insertion ca. 86% linked to the suppressor mutation. Molecular mapping of the transposon insertion was undertaken with the aid of an inverse-PCR approach as described previously (30), and the insertion was shown to be located after bp coordinate 3310756 on the *E. coli* genome (45), downstream of codon 14 in the *truB* open reading frame at the 71-min region of the chromosome. In additional crosses, the suppressor mutation was also shown to be 92% cotransducible with an auxotrophic Δ*argG*::Kan mutation (2) in this region (data not shown).

Scoring for synthetic lethal phenotypes in *rho* and *nusG* derivatives. Tests for synthetic lethality conferred by additional mutations in *rho* and *nusG* strains were performed with derivatives carrying the cognate *rho*$^+$ or *nusG*$^+$ genes on the IPTG-dependent replicons pHYD1201 or pHYD751, respectively, and then testing for growth on medium not supplemented with IPTG.

Other techniques. Procedures for P1 transduction (37), *in vitro* DNA manipulations and transformation (46), and β-galactosidase assays (37) were as described. The scoring of phenotypes associated with transcriptional polarity and its relief (including quantitation of the extent of polarity relief with the aid of P_{lac}-*lacZ* and P_{lac}-t_{R1}-*lacZ* fusion construct pairs wherein t_{R1} is a Rho-dependent terminator from lambdoid phage H19B), as well as determination of *in vivo* transcription elongation rates were undertaken as described in the accompanying study (47). The chromosomal *rho* and *nusA* genes were PCR amplified with the primer pairs 5′-TCCTCGACGCTAACCTGGC-3′/5′-ACATCGCCAGCGCGG CAT-3′ and 5′-TTACGCTTCGTCACC-3′/5′-GAAGGCGAAGCTTGTTCCC CACTT-3′, respectively, and sequenced on both strands with these primers, along with additional primers internal to the genes. The plasmid-borne *hns* gene was PCR amplified with a pair of flanking vector-based primers 5′-AAGTGCG GCGACGATAGT-3′ and 5′-CCGTCTTTCATTGCCATAC-3′ and sequenced on both strands with these primers.

RESULTS

Selection for suppressors restoring polarity relief in *rho* and *nusG* derivatives with H-NSΔ64. As described earlier (29, 47), the dominant-negative H-NSΔ64 variant (that comprises the N-terminal segment of 63 amino acids, from the 137-amino-acid native protein) restores transcriptional polarity in the *rho*(*A243E*) or *nusG*(*G146D*) mutants. In the present study, we obtained suppressors of the *hns* effect on *rho* and *nusG*, by selection for mutants in these backgrounds in which polarity relief had been reestablished at *trp* and *lac* loci bearing polar mutations in the first genes of each of the two operons (*trpE9777* [frameshift] and $lacZ_{U118}$ [amber], respectively). Derivatives exhibiting relief of transcriptional polarity at these loci were selected as $Anth^+$ and Mel^+ (at 39°C), respectively, as described above. A phenotype correlated with transcriptional polarity relief in the *rho* and *nusG* mutants is that of R-loop-dependent lethality with ColE1 family plasmids such as pACYC184, which is also reversed by H-NSΔ64 (26, 29, 47), and all of the Mel^+ $Anth^+$ suppressors obtained in the present study had also reverted to the pACYC184 lethality phenotype.

The *rho* and *nusG* strains used in the $Anth^+$ selections above were *hns*$^+$ on the chromosome and carried the gene encoding H-NSΔ64 on a pSC101-based plasmid (56). In several of the spontaneous Mel^+ $Anth^+$ derivatives that were obtained, the suppressor mutations were shown to be plasmid-borne, and sequencing of the *hns* gene on the plasmid revealed that the suppressor mutation in each of them had either (i) reverted the frameshift allele encoding H-NSΔ64 to *hns*$^+$ or (ii) led to a total loss of *hns* function, including the dominant-negative effect of H-NSΔ64 (data not shown). That the two sets of mutant plasmids had now become wild type and null, respectively, for H-NS function and had lost their dominant-negative character was confirmed by measurements of expression from a *proU-lac* transcriptional fusion (which is known to be H-NS regulated [56]), in derivatives that were chromosomally *hns*$^+$ or null *hns* and carrying the mutant plasmids (data not shown). These results served to reaffirm the strong effect of H-NSΔ64 in restoration of transcriptional polarity in the *rho* and *nusG* mutants.

Three chromosomal mutants obtained as $Anth^+$ Mel^+ in the selections above (Fig. 1) were further characterized. One of these, obtained in the *nusG* derivative with H-NSΔ64, was mapped to the *rho* locus and then identified as a mutation leading to a Q32R substitution in Rho; when the single mutant *rho*(*Q32R*) was constructed and tested, it exhibited moderate polarity relief ($Anth^+$ with *trpE9777*) and was killed with pACYC184, with both phenotypes being suppressed by H-NSΔ64 (Fig. 2). Another suppressor mutation, obtained in the parental *rho-4*, that is, *rho*(*A243E*), strain with H-NSΔ64, was also in *rho*, resulting in an additional R102S substitution in the protein (Fig. 1). These two results suggested that one

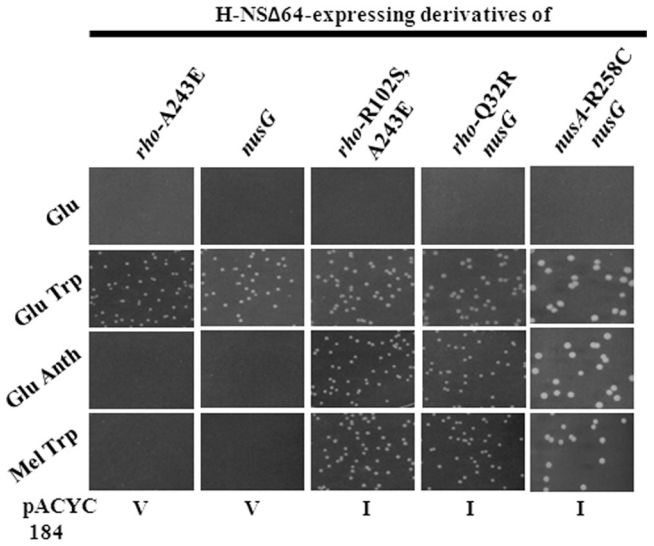

FIG. 1. Restoration of transcriptional polarity relief in suppressor derivatives of *rho*(*A243E*) and *nusG*(*G146D*) mutants expressing H-NSΔ64. The results shown are for the original suppressor isolates GJ7368 [*rho*(*R102S*, *A243E*)], GJ7376 [*rho*(*Q32R*) *nusG*], and GJ7387 [*nusA*(*R258C*) *nusG*], all carrying plasmid pLG-H-NSΔ64, as well as for pLG-H-NSΔ64-bearing derivatives of GJ6520 [*rho*(*A243E*)] and GJ6524 (*nusG*) as controls. Cultures were plated at a suitable dilution on a set of four minimal A medium-based plates, as shown. Glu, glucose (C source); Mel, melibiose (C source). The Mel Trp plates were incubated at 39°C. Indicated on the last row is the phenotype of pACYC184 transformants of the strains, after 40 h: V, viable; and I, inviable.

method of achieving polarity relief in H-NSΔ64-bearing derivatives is to increase the severity of deficiency in transcription termination beyond that conferred by the *rho-4* or *nusG*(*G146D*) mutations themselves. The *rho*(*R102S*, *A243E*) double mutant exhibited pronounced rich medium (LB) sensitivity that was not affected by the presence or absence of H-NSΔ64 (data not shown), and all experiments with this mutant were performed in minimal medium.

Identification of a transcription termination-defective *nusA* mutation. The third chromosomal suppressor mutation (that was obtained in the *nusG* parent with H-NSΔ64, Fig. 1) was shown by phage P1 crosses to be in neither *rho* nor *nusG* (data not shown). The mutation was then mapped, with the aid of a transposon-tagging approach as described in Materials and Methods, to the 71-min region of the *E. coli* chromosome, ca. 90% linked to *argG*. By transduction experiments, it was shown that the tagged mutation could also suppress the effects of H-NSΔ64 in a *rho-4* background (GJ7417) for both polarity relief and plasmid pACYC184 lethality (data not shown). Since *nusA* is in this location, we tested whether the suppressor mutation was in *nusA*. Introduction of any of three plasmids carrying *nusA*$^+$ (pHYD2554, -2557, or -2556, of which the last is a minimal *nusA*$^+$ construct) reversed the phenotypes of polarity relief and lethality with plasmids of the ColE1 family in the suppressor-bearing strain GJ7395 (data not shown). The *nusA* locus of the suppressor was then PCR amplified and sequenced, which showed that the gene had a mutation resulting in an Arg-to-Cys substitution at amino acid residue 258 in the KH1 domain of the protein (R258C).

Single mutant derivatives of *nusA* (without concomitant expression of H-NSΔ64) were then constructed and they exhibited several phenotypes. At the t_{R1} terminator (of lambdoid phage H19B) positioned upstream of a *lacZ* reporter gene on the chromosome, the *nusA*(*R258C*) mutation conferred moderate transcriptional polarity relief (Table 2) compared to that by the *rho*(*A243E*) or *nusG*(*G146D*) mutations (47). The *nusA* strain was also polarity relieved at *trpE9777* (Anth$^+$) and killed following pACYC184 transformation (Fig. 2). All of these phenotypes were suppressed by expression of H-NSΔ64 (see Table 2 and Fig. 2), as had been shown earlier also for the *rho* and *nusG* mutants, and the strain was also complemented by plasmid pHYD2556 (carrying minimal *nusA*$^+$ under P_{ara}) in an L-arabinose-dependent manner (Fig. 3).

As described in the accompanying study, overexpression of another protein of the H-NS family YdgT is associated with suppression of polarity relief and pACYC184 lethality phenotypes in *rho* and *nusG* strains (47) and, likewise, the *nusA* mutant phenotypes were also suppressed upon YdgT overexpression (Table 2, Fig. 2). These results established that the

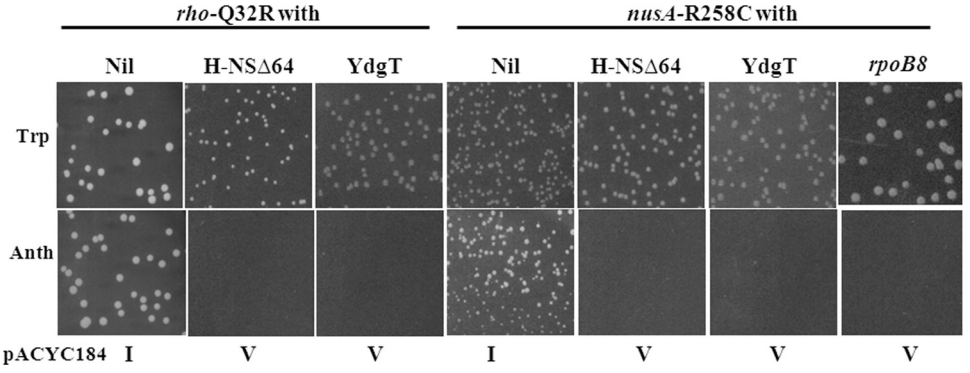

FIG. 2. Relief of transcriptional polarity in *nusA*(*R258C*) and *rho*(*Q32R*) single mutants GJ10524 and GJ10753, respectively, and their suppression by H-NSΔ64 and YdgT. H-NSΔ64 was provided from plasmids pHYD2509 and pLG-H-NSΔ64, respectively, in the *nusA* and *rho*(*Q32R*) mutants, while YdgT was expressed from plasmid pHYD2546 in both. The derivatives were plated at a suitable dilution on a pair of glucose-minimal A medium plates supplemented with Trp or Anth, as indicated. Given on the last row is the phenotype of pACYC184 transformants of the derivatives after 40 h: V, viable; and I, inviable. Also shown for the *nusA* mutant is suppression by *rpoB8*, for which strain GJ10598 was used.

TABLE 2. Polarity relief at phage t_{R1} terminator in the *nusA*(*R258C*) mutant and its suppression by H-NSΔ64 or YdgT expression

Strain[a]	Mean ± SE		
	β-Galactosidase sp act		% B/A
	$-t_{R1}$ (A)	$+t_{R1}$ (B)	
Wild type	9,400 ± 2,151	425 ± 79	5 ± 1
nusA	7,045 ± 1,451	1,327 ± 207	19 ± 4
nusA/vector	7,853 ± 1,335	1,157 ± 22	15 ± 3
nusA/H-NSΔ64	9,995 ± 1,985	532 ± 21	5 ± 1
nusA/YdgT	4,000 ± 7	246 ± 1	6 ± 0

[a] The strains (grown in LB medium) included those without ($-t_{R1}$) or with ($+t_{R1}$) the phage t_{R1} terminator upstream of the *lacZ* reporter gene and were, respectively: wild type, RS445 and GJ5147, and *nusA*, GJ10573 and GJ10572. Derivatives with vector, H-NSΔ64, and YdgT carried plasmids pLG339, pLG-H-NSΔ64, and pHYD2546, respectively.

nusA(*R258C*) mutant is defective for Rho-dependent transcription termination.

We also tested the effect of the *rpoB8* mutation in the *nusA* strain. RNA polymerase bearing the RpoB8 subunit exhibits a reduced rate of transcription elongation which is associated with suppression of transcription termination deficiency of *rho* mutants (31, 51). We found that, even in the *nusA*(*R258C*) strain, *rpoB8* suppressed both the Anth$^+$ phenotype associated with *trpE9777* and plasmid pACYC184 lethality (Fig. 2).

The *nusA*(*R258C*) mutant exhibited a normal growth phenotype and also was unaffected in its ability to support the growth of phage λ at all temperatures tested (see Fig. 4 for 42°C, data not shown for 30 and 37°C), whereas the *nusA1* mutant was λ resistant as previously reported (51).

Synthetic lethal phenotypes between *rho*, *nusG*, and *nusA* mutations. Our findings that the suppressor selection for polarity relief in the parental *rho-4* or *nusG* derivatives with H-NSΔ64 yielded mutations that were themselves associated with defective transcription termination led us to examine the synthetic phenotypes, if any, of the various mutant combinations in absence or presence of H-NSΔ64. The mutant combinations were assembled by P1 transductions in strains carrying the cognate *rho*$^+$ or *nusG*$^+$ gene on an IPTG-dependent replicon, so that the synthetic phenotypes (of both viability and transcriptional polarity relief) could then be determined on growth media not supplemented with IPTG.

By this assay, the mutant combinations *rho*(*A243E*) *nusA*(*R258C*) and *nusG*(*G146D*) *nusA*(*R258C*) exhibited synthetic lethality, and furthermore, transformation of these strains with a plasmid encoding H-NSΔ64 restored viability,

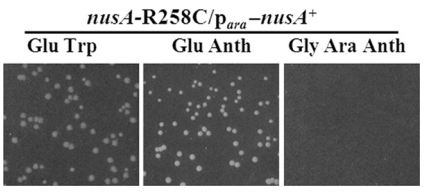

FIG. 3. Complementation of *nusA*(*R258C*) mutant by minimal *nusA*$^+$ plasmid. The *nusA*(*R258C*) mutant GJ10524 carrying plasmid pHYD2556 (P$_{ara}$-*nusA*$^+$) was plated at a suitable dilution on Trp- or Anth-supplemented plates with either glucose (Glu) or glycerol at 0.4% and L-arabinose at 0.2% (Gly Ara), as indicated.

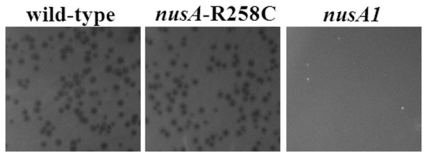

FIG. 4. Phage λ growth on *nusA*(*R258C*) mutant. A single suitable dilution of a λ*c*I857 phage lysate was plated on a lawn of strains GJ6504 (wild type), GJ10524 [*nusA*(*R258C*)], or GJ3119 (*nusA1*) and incubated at 42°C for 5 h.

but the resultant derivatives exhibited relief of transcriptional polarity at *trpE* and lethality with plasmid pACYC184 (Fig. 5A). Likewise, the *rho*(*A243E*) *nusG*(*G146D*) combination has previously been reported to be inviable (29), which was reproduced in the present study, and its H-NSΔ64 derivative was viable but polarity relieved (Fig. 5A). The combinations *rho*(*R102S*, *A243E*) *nusG*(*G146D*) and *rho*(*R102S*, *A243E*) *nusA(R258C)* were also inviable, and the latter was rescued by expression of H-NSΔ64 (data not shown). The *rho(Q32R)* mutation conferred a slow-growth phenotype with *nusA*(*R258C*) but not *nusG*(*G146D*), and the former was suppressed by H-NSΔ64 (Fig. 5A).

Two triple mutant combinations were tested (Fig. 5B): *rho*(*Q32R*) *nusG*(*G146D*) *nusA*(*R258C*) was viable only in the presence of H-NSΔ64, whereas *rho*(*A243E*) *nusG*(*G146D*) *nusA*(*R258C*) was inviable even with H-NSΔ64, that is, it could be maintained only in the simultaneous presence of both the IPTG-dependent replicon with *rho*$^+$ or *nusG*$^+$ and the plasmid expressing H-NSΔ64.

To quantitate the extent of polarity relief in many of the viable double and triple mutant combinations (with or without expression of H-NSΔ64), β-galactosidase activities were determined from pairs of P$_{lac}$-*lacZ* fusion derivatives with or without the intervening Rho-dependent t_{R1} terminator of lambdoid phage H19B, as described previously (47). The data from these experiments, presented in Table 3, were consistent with the notions that combining the individual mutations leads to additive effects on polarity relief and that H-NSΔ64 expression is ameliorative in this regard. Thus, for example, the *rho*(*Q32R*) mutation conferred polarity relief to the extents of, respectively, 30, 34, and 53% by itself and in combination with the *nusG* and *nusA* mutations in the absence of H-NSΔ64, which were then reduced to 15, 18, and 32%, respectively, in the presence of H-NSΔ64. The *rho*(*Q32R*) *nusA nusG* triple mutant (which is lethal without H-NSΔ64) exhibited 34% polarity relief with H-NSΔ64. Likewise, the *nusA rho*(*A243E*) and *nusA nusG* mutants with H-NSΔ64 were more polarity relieved (40 and 30%, respectively) than any of the corresponding single mutants with H-NSΔ64 [*nusA*, 5%; *rho*(*A243E*), 18%; and *nusG*, 17%] (see Table 2 and reference 47). In strains bearing the *rho*(*R102S*, *A243E*) allele alone or in combination with *nusA*, the same trend was observed. However, since the corresponding derivatives without the t_{R1} terminator exhibited 2- to 3-fold lower levels of *lac* expression than those not carrying this *rho* allele, the calculated extent of transcriptional readthrough was apparently elevated in these strains. [As mentioned above, strains with *rho*(*R102S*, *A243E*) are LB sensitive, and hence the derivatives had been cultured in a different minimal A medium for the enzyme assays.]

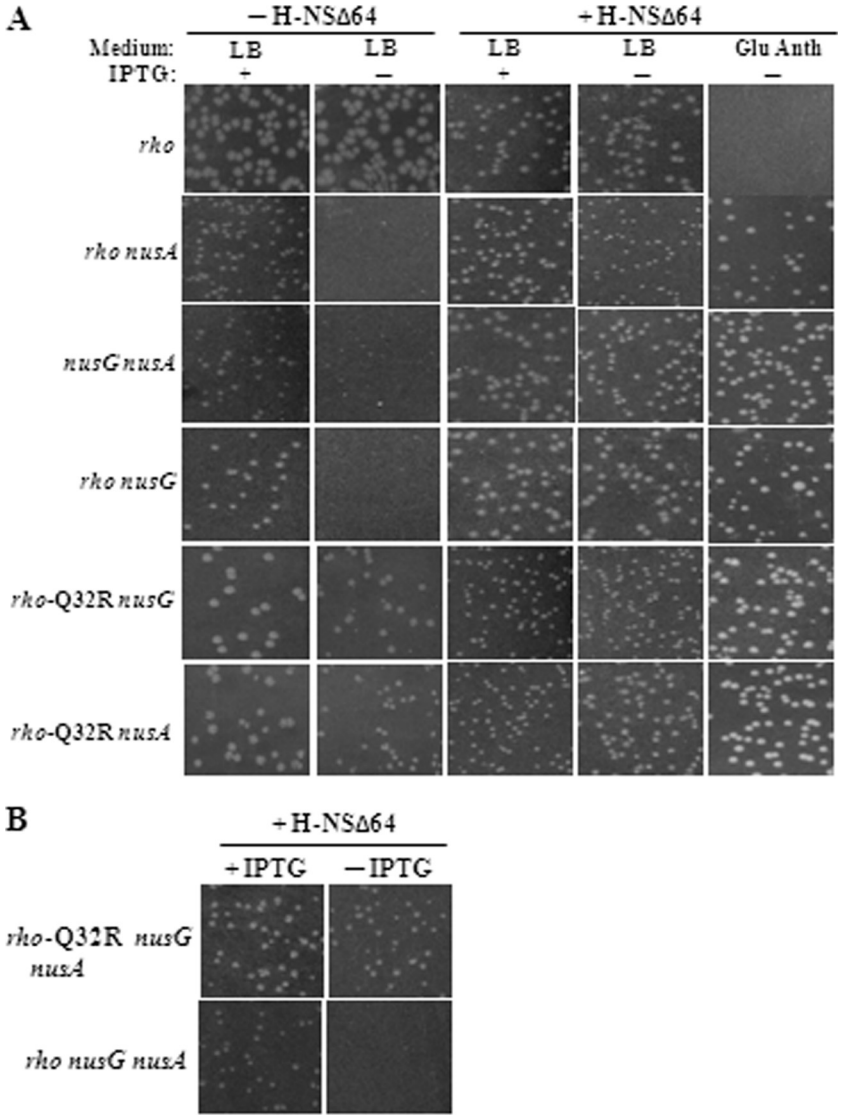

FIG. 5. Synthetic lethal phenotypes of *rho* and *nusG* mutants. Non-Q32R mutants designated *rho* carry the *rho-4* [that is, *rho*(*A243E*)] mutation. All strains also carry, as appropriate, the *nusG*$^+$- or *rho*$^+$-bearing plasmids (pHYD751 and pHYD1201, respectively) that are IPTG dependent for replication, so that the plasmids are retained only in growth medium supplemented with IPTG. Derivatives additionally expressing H-NSΔ64 from plasmid pLG-H-NSΔ64 are marked. (A) Cultures of the pHYD1201-bearing derivatives of strains GJ6509 (*rho*), GJ7417 (*rho nusA*), and GJ10581 [*rho*(*Q32R*) *nusA*] and of the pHYD751-bearing derivatives of strains GJ7419 (*nusG nusA*), GJ10591 (*rho nusG*) and GJ10557 [*rho*(*Q32R*) *nusG*] (including pLG-H-NSΔ64-carrying derivatives as indicated) were each plated at a suitable dilution on LB medium without or with IPTG supplementation and incubated for 20 h and on glucose-minimal A medium with Anth supplementation and incubated for 40 h. For the *nusG nusA* derivative without H-NSΔ64, small colonies were obtained on LB without IPTG, but they failed to grow upon subculturing, indicative of synthetic lethality. (B) Cultures of pHYD751- and pLG-H-NSΔ64-carrying derivatives of strains GJ10668 (*rho nusG nusA*) and GJ10669 [*rho*(*Q32R*) *nusG nusA*] were each plated as described above at a suitable dilution on LB without and with IPTG.

H-NSΔ64 was not able to suppress either the sickness of Δ*nusG* or Δ*nusA* mutants in the reduced-genome *E. coli* strain MDS42 (9, 42) or the inviability associated with these mutations or with Δ*rho* in wild-type *E. coli* (data not shown).

Transcription elongation rate is unaffected in *nusA* mutant. The NusA protein has been implicated in modulating the rate of transcription elongation (40, 44, 48) and thus in ensuring the coupling of transcription and translation (58); furthermore, the kinetic coupling model (31) posits that the efficiency of Rho-dependent termination is inversely related to the elongation rate of transcription. Hence, we considered the possibility that the *nusA*(*R258C*) mutant is termination-defective because of an inappropriately increased transcription elongation rate in the mutant leading to a mismatch in coupling of RNA polymerase with the pioneer ribosome and with Rho, which would then also explain its suppression by the *rpoB8* mutation (Fig. 2).

We therefore determined the rate of transcription elongation *in vivo* at the native *lacZ* locus following induction of the operon with IPTG, as previously described (31, 47), but no difference was observed between the pair of isogenic *nusA*$^+$ and *nusA*(*R258C*) strains (Fig. 6). Transcription elongation

TABLE 3. Polarity relief at phage t_{R1} terminator in viable double and triple mutants[a]

Genotype	Mean ± SE					
	Without H-NSΔ64			With H-NSΔ64		
	$-t_{R1}$ (A)	$+t_{R1}$ (B)	% B/A	$-t_{R1}$ (A)	$+t_{R1}$ (B)	% B/A
rho(*Q32R*)	5,728 ± 559	1,732 ± 339	30 ± 3	5,325 ± 314	832 ± 207	15 ± 3
rho(*R102S*, *A243E*)	2,275 ± 360	1,648 ± 161	74 ± 5	1,994 ± 462	738 ± 171	37 ± 0
rho(*Q32R*) *nusA*(*R258C*)	5,301 ± 451	2,818 ± 388	53 ± 5	4,682 ± 424	1,468 ± 232	32 ± 6
rho(*Q32R*) *nusG*(*G146D*)	4,156 ± 137	1,439 ± 214	34 ± 5	4,541 ± 664	832 ± 155	18 ± 1
rho(*A243E*) *nusA*(*R258C*)	ND[b]	ND	ND	4,465 ± 286	1,836 ± 425	40 ± 7
rho(*R102S*, *A243E*) *nusA*(*R258C*)	ND	ND	ND	2,144 ± 605	1,033 ± 169	52 ± 7
nusG(*G146D*) *nusA*(*R258C*)	ND	ND	ND	4,677 ± 546	1,370 ± 95	30 ± 4
rho(*Q32R*) *nusG*(*G146D*) *nusA*(*R258C*)	ND	ND	ND	3,779 ± 47	1,295 ± 78	34 ± 2

[a] The specific activities of β-galactosidase were determined for various pairs of strains without ($-t_{R1}$ [A]) or with ($+t_{R1}$ [A]) the phage t_{R1} terminator upstream of the *lacZ* reporter gene. The B/A ratios (%) given in the table are a measure of transcriptional readthrough or the polarity relief at the t_{R1} terminator. Where indicated, H-NSΔ64 expression was obtained by transformation of the strains with plasmid pLG-H-NSΔ64. The strain pairs ($-t_{R1}$ and $+t_{R1}$) used were as follows: *rho*(*Q32R*), GJ10798 and GJ10795; *rho*(*R102S*, *A243E*), GJ10802 and GJ10801; *rho*(*Q32R*) *nusA*(*R258C*), GJ10800 and GJ10797; *rho*(*Q32R*) *nusG*(*G146D*), GJ10799 and GJ10796; *rho*(*A243E*) *nusA*(*R258C*), GJ10814 and GJ10813; *rho*(*R102S*, *A243E*) *nusA*(*R258C*), GJ10794 and GJ10793; *nusG*(*G146D*) *nusA*(*R258C*), GJ10812 and GJ10813; and *rho*(*Q32R*) *nusG*(*G146D*) *nusA*(*R258C*), GJ10817 and GJ10818. Strains with the *rho*(*R102S*, *A243E*) mutation were grown in glucose-minimal A medium supplemented with Casamino Acids at 0.5%. All other strains were grown in LB medium.

[b] ND, not determined because these mutant combinations exhibited synthetic lethality in the absence of H-NSΔ64.

rates in the *rpoB8 nusA*$^+$ and *rpoB8 nusA*(*R258C*) pair of strains were also determined; although *rpoB8* itself was associated with a decreased elongation rate as has been reported earlier (31), once again the *nusA* mutation had no additional effect (Fig. 6). We conclude that NusA(R258C) does not grossly alter the transcription elongation properties of RNA polymerase *in vivo*.

DISCUSSION

A *nusA* missense mutant defective in Rho-dependent transcription termination. By using a suppressor selection approach in *rho* or *nusG* strains expressing H-NSΔ64, we have identified new mutations that affect the efficiency of Rho-dependent transcription termination, including a recessive missense mutation (R258C) in the KH1 domain-encoding region of the *nusA* gene. The *nusA* mutant is specifically affected for Rho-dependent termination, without any other apparent phenotype, such as growth sensitivity at low or high temperatures, λ phage resistance, or alteration of transcription elongation rates *in vivo*.

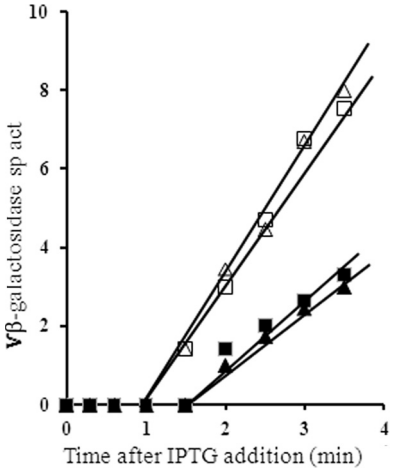

FIG. 6. Transcription elongation rate measurements *in vivo* for *nusA* and *rpoB8* mutants. For each culture grown in 0.4% glycerol-minimal A medium supplemented with Casamino Acids at 0.5%, the β-galactosidase specific activities were determined at various times after the addition of IPTG and the inflection point was identified on a curve plotting square root of enzyme specific activity against time, as described previously (31). The strains used were GJ10700 (wild type, □), GJ10702 [*nusA*(*R258C*), △], GJ10697 (*rpoB8*, ■), and GJ10699 [*nusA*(*R258C*) *rpoB8*, ▲].

An earlier study had shown that a G253D substitution in the same KH1 RNA-binding domain is associated with phenotypes of cold sensitivity and λ resistance (59). The *nusA1* and *nusA11* substitution mutations, both of which affect the S1 domain of the protein, confer transcriptional polarity relief, but they are associated with additional phenotypes such as λ resistance (*nusA1*) and temperature sensitivity (*nusA11*) (23, 39, 53). Our findings also support those of Cardinale et al. (9), who showed that a *nusA* insertion-deletion mutation, in the reduced-genome *E. coli* strain MDS42, renders transcription termination defective in the strain.

NusA in *E. coli* has been best studied as a transcription elongation factor and as a protein required for transcription antitermination in *rrn* operons and lambdoid phages (6, 17, 40, 44, 48). Previous reports have also suggested that NusA can indirectly affect Rho-dependent termination by its influence on the rate of transcription elongation and thereby on transcription-translation coupling (31, 58). However, we found that the *nusA*(*R258C*) mutant is unaltered for the rate of transcription elongation at the *lacZ* locus *in vivo*, which is consistent with recent findings that NusA's roles in transcriptional pausing and intrinsic termination are mediated solely by its N-terminal domain and are unaffected by substitutions in the KH1 or KH2 domains (28). Therefore, the simplest model to explain our results and those of Cardinale et al. (9) is that NusA participates directly in the process of Rho-dependent transcription termination, rather than indirectly through its effects on transcription elongation.

Spectrum of efficiencies of Rho-dependent termination and their associated phenotypes. Our studies demonstrate that, by combining different mutations in *rho*, *nusG*, and *nusA*, along with expression of YdgT or of variants of H-NS such as

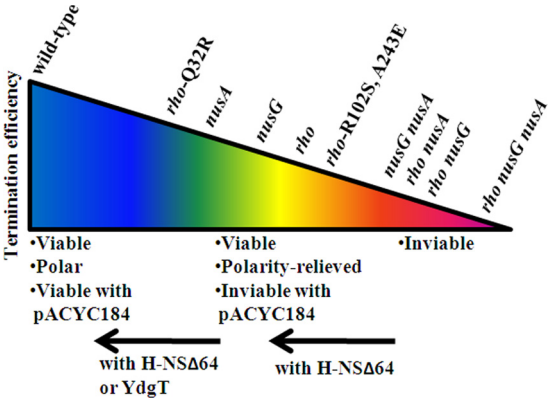

FIG. 7. Spectrum of Rho-dependent transcription termination efficiencies in the different mutants described in text, and the effects of H-NSΔ64 or YdgT thereon. The designations *rho*, *nusG*, and *nusA* without suffixes in the figure refer, respectively, to the *rho-4* [that is, *rho*(*A243E*)], *nusG*(*G146D*), and *nusA*(*R258C*) mutations.

H-NSΔ64, one can generate a wide spectrum of efficiencies of Rho-dependent termination *in vivo*. As depicted schematically in Fig. 7, the corresponding phenotypes would extend from viable cells exhibiting full transcriptional polarity (highest efficiency) through viable but polarity-relieved cells (intermediate efficiency) to inviable cells (lowest efficiency). Perturbations involving the H-NS family of proteins, as with expression of H-NSΔ64 or of YdgT, are associated with a shift in the spectrum from lower to higher efficiency, such as to convert synthetic lethal combinations to viable but polarity-relieved states, and polarity-relieved derivatives to those exhibiting full polarity. In the accompanying study (47), we suggested a model in which the polymeric H-NS scaffold is able to modulate Rho-dependent termination.

Some workers have speculated that Rho's essential function in *E. coli* may be different from its role in transcription termination (10, 54), and the fact that Cardinale et al. (9) could obtain viable insertion-deletion mutants of *nusA* or *nusG*, but not of *rho*, in the background of strain MDS42 may be seen as lending support to this notion. On the other hand, the existence of a spectral continuum as depicted in Fig. 7 suggests that inviability is merely an extreme consequence of decreasing efficiency of Rho-dependent termination. This is supported also by the observation that Δ*nusA* and Δ*nusG* derivatives of MDS42 exhibit hypersensitivity to the Rho inhibitor bicyclomycin (9, 54).

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