The 109-amino acid Nun protein of prophage HK022 excludes superinfecting bacteriophage λ by blocking transcription elongation on the λ chromosome. Multiple interactions between Nun and the transcription elongation complex are involved in this reaction. The Nun NH2-terminal arginine-rich motif binds BOXB sequence in nascent λ transcripts, whereas the COOH terminus binds RNA polymerase and contacts DNA template. Nun Trp108 is required for interaction with DNA and transcription arrest. We analyzed the role of the adjacent Lys106 and Lys107 residues in the Nun reaction. Substitution of the lysine residues with arginine (K106R/K107R) had no effect on transcription arrest in vitro or in vivo. Nun K106A/K107A was partially active, whereas Nun K106D/K107D was defective in vitro and failed to exclude λ. All mutants bound RNA polymerase and BOXB. In contrast to Nun K106R/K107R and K106A/K107A, Nun K106D/K107D did not cross-link DNA template. These results suggest that transcription arrest is facilitated by electrostatic interactions between positively charged Nun residues Lys106 and Lys107 and negatively charged DNA phosphate groups. These may assist intercalation of Trp108 into template.

Coliphage HK022 Nun protein excludes superinfecting bacteriophage λ by blocking elongation of early λ transcripts (Fig. 2A; Refs. 1 and 2). Nun belongs to the arginine-rich motif (ARM) family of RNA binding proteins (Fig. 1), which includes human immunodeficiency virus proteins Tat and Rev, as well as λ N protein (3–5). N promotes transcription antitermination on λ DNA templates and is required for the growth of λ phage (6). During transcription elongation, the Nun NH2-terminal ARM motif recognizes the NUT BOXB stem-loop sequence on nascent λ pL and pR operon transcripts, recruiting Nun to the transcription elongation complex (TEC), and blocking attachment of N (Fig. 2B; Refs. 7 and 8). The Nun COOH terminus binds to RNA polymerase (RNAP) (9) and contacts DNA template 7–8 bp promoter-distal to the 3′-OH end of the transcript (10). In vitro, Nun alone blocks elongation of the TEC (11), whereas in vivo, Nun acts in association with four Escherichia coli host factors, NusA, NusB, NusE, and NusG (11–13). Host transcription repair-coupling factor releases Nun-association TEC (14). The penultimate Nun tryptophan residue (Trp108) mediates the interaction of Nun with the template in the TEC, possibly by intercalating into double-stranded DNA template (15).

In this article, we investigate the role of Nun residues Lys106 and Lys107 in transcription arrest and λ exclusion. We showed previously that substitution of these basic residues with aspartate (Nun K106D/K107D) strongly inhibited transcription termination in vitro (16). Nun K106D/K107D could, however, prevent translation of the λ N gene in an rnc host (16). This suggested that Nun K106D/K107D bound BOXB, and, along with E. coli Nus factors, blocked ribosomal access to the adjacent N ribosome-binding site (16). In this paper, we show that Nun Lys106 and Lys107 are not required for binding to RNAP or BOXB but are instead involved in binding to DNA template. We propose that favorable electrostatic interactions between Nun Lys106 and Lys107 and template phosphate groups assist intercalation of Nun Trp108 into the λ DNA template.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Bacteriophage—Standard bacterial techniques used in strain construction, e.g. transformation, transduction, and media preparation are as described previously (17). The bacterial host is W3110. Strains carrying either pl-L-nutL-lacZ or chromosomal transcriptional fusion (18), or r-protein-galETK chromosomal transcriptional fusion (2), are described in detail below. All plasmids were constructed from pET-21d vector (Novagen). Plasmids expressing wild-type Nun, K106D/K107D, and T-Nun (Nun V96) are as described previously (16). Plasmids expressing K106R/K107R, K106A/K107A, K106D/K107D were made as described previously (9). For T-Nun purification, additional steps were applied. After anion exchange chromatography with Mono S HR 5/5 (Amerham Biosciences), fractions containing T-Nun protein were combined and concentrated to 500 μl with Centricon YM-10 (10,000 molecular weight cutoff) (Amicon). Subsequently, T-Nun was size-separated from other proteins with Superose 6 column (Amersham Biosciences). E. coli holo RNAP was purchased from Epicenter (Madison, WI). For cross-linking experiment, Nun mutants, 110C, K106A/K107A110C, K106D/K107D110C, and K106D/K107D110C were purified as described previously (9).

Mutagenesis—K106D/K107D and T-Nun protein (Nun V96) protein mutants were constructed from the pIT7 NunII plasmid as described previously (9). The K106A/K107A and K106R/K107R mutations were made using QuickChange site-directed mutagenesis technique (Stratagene) from the pIT7 NunII plasmid. The primers used to make these mutations were as follows: K106A/K107A, GCT CAC CAG CGA CCA ACC AAC AGA GCG TGG TCA TAA AAG CTT GC and GCA AGC TTT TAT GAC CAC GCT; K106R/K107R, GCT CAC CAG CGA CCA ACC AAC AGA GCG TGG TCA TAA AAG CTT GC and GCA AGC TTT TAT GAC CAC GCT; K106A/K107A110C, K106D/K107D110C, were purified as described previously (9).

Proteins—For in vitro transcription, BOXB binding, and RNA polymerase binding experiments, wild-type and Nun mutants, K106A/K107A, K106R/K107R, and K106D/K107D were purified as described previously (9). For T-Nun purification, additional steps were applied. After anion exchange chromatography with Mono S HR 5/5 (Amerham Biosciences), fractions containing T-Nun protein were combined and concentrated to 500 μl with Centricon YM-10 (10,000 molecular weight cutoff) (Amicon). Subsequently, T-Nun was size-separated from other proteins with Superose 6 column (Amersham Biosciences). E. coli holo RNAP was purchased from Epicenter (Madison, WI). For cross-linking experiment, Nun mutants, 110C, K106A/K107A110C, K106D/K107D110C, and K106D/K107D110C, were purified as described previously (9).

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**Nun Residues Lys<sup>106/107</sup> Promote Binding to λ DNA**

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VKTI YVNPQ SGQRN KVSDR GLTSR<sub>25</sub>

DRRRI ARWEK RIAYA LKNGV TPGFN<sub>93</sub>

AIDDG PEYKI NEDPM DKVDK ALATP<sub>5</sub>

FPDRV EKIED EKYED VMHRV VNHAH<sub>108</sub>

QRNPN KK<sup>77</sup>K<sub>78</sub> S<sub>10</sub>

**Fig. 1. Amino acid sequence of HK022 Nun protein.** The Arg region is italicized, His<sup>95</sup>, His<sup>96</sup>, and His<sup>108</sup> are underlined, Arg<sup>102</sup> is shadowed, Lys<sup>106</sup> and Lys<sup>107</sup> are in bold, and Trp<sup>108</sup> is outlined. Amino acid sequence of HK022 Nun protein. **A** Amino acid sequence of HK022 Nun protein. **B** HK022 Nun. **C** HK022 Nun.

K107R, and K106D/K107D Nun was used, respectively, as a template. The primers used to make these mutations were as follows: 110C, CCA GCG AAA CCC AAA CAA AAA GTG GTC ATG CTA AAA AAT GCT TGC and GCA AGC TTT TAG CAT GAC CAC TTT TTT GGG TTT CGC TGG: K106A/K107A110C, GCT CAC CGA CAG CAC ACC GAG GCG TGG TCA TGA AAG GTT GC and GCA AGC TTT TAG CAT GAC CAC TTT TTT GGG TTT CGC TGG: K106R/K107R110C, GCT CAC CGA CAG CAC ACC GAG GCG TGG TCA TGA AAG GTT GC and GCA AGC TTT TAG CAT GAC CAC TTT TTT GGG TTT CGC TGG.

**β-Galactosidase and Galactokinase Assays—** Cultures were grown in LB + 50 µg/ml of ampicillin at 32 °C and shifted to 42 °C for 1 h with shaking to OD<sub>600</sub> = 0.6 for β-galactosidase assays (19) and for 7 h to OD<sub>600</sub> = 0.5–0.7 for galactokinase measurements (20). The shift to 42 °C initiates transcription from the λ pL promoter (β-galactosidase assays) or pR promoter (galactokinase assays).

**Efficiency of λ Platein—** Laws of strains were plated on top agar on LB or LB + 50 µg/ml ampicillin. Efficiency of λ platein was determined by spotting dilutions of λ phage and incubating overnight at 37 °C.

**In Vitro Transcription Assay—** This assay was based on two-step, multiround transcription reaction. In the first step, open complex formation was allowed by preincubating E. coli RNAP (20 nm), DNA template (10 nt) in 50 µl of transcription buffer containing 20 mM Tris acetate (pH 7.9), 60 mM potassium acetate, 2 mM magnesium acetate, 0.2 mg/ml bovine serum albumin, 1 mM DTT, and 5% glycerol at 32 °C for 10 min. In the second step, transcription was initiated by addition of 5 µM ATP, CTP, GTP, UTP and 1–2 µCi of [α-<sup>32</sup>P]ATP. Transcription products were then phenol/chloroform-extracted, ethanol-precipitated under 2.5 volumes of 100% ethanol (Sigma), and resuspended in 20 µl of gel loading buffer II (Amicon). Extracted transcripts were electrophoresed in 10% polyacrylamide, 8 M urea gel, and analyzed with a Fujilm ST-503 densitometer.

**Templates for in Vitro Transcription, BOXB RNA Synthesis, and Cross-linking—** For in vitro transcription assay, a λ DNA fragment (35,253–35,718) carrying pL-nutL was amplified from λ DNA/HindIII markers (Fermentas, Hanover, MD) by PCR using GeneAmp (Roche Diagnostics) and the following two primers: TCA GAT CTC TCA CCT ACC AAA C and AGG GCG GTT AAC TGG TTT TG. The product was then purified using the QIAquick PCR purification kit (Qiagen). For the nutR-boxB template, two complimentary DNA oligonucleotides with 5' EcoRI and 3' HindIII restriction sites (MWG Biotech) annealed. The oligonucleotides used were GCG GAA TTA ACA TTC CAG ACC TGA AAA AGG GCA TCA AAT AAG CGG CCC and GCG GAG GCT TTT TCA GGG GCG GAC TTA GTT TCC. The PCR product was then digested with EcoRI and HindIII restriction enzymes at 37 °C for 30 min. Linearized pGEM-3Z was purified with the PCR purification kit (Qiagen), and the boxB template was purified by nucleotide removal kit (Qiagen).

**Cross-linking—** Cross-linking was determined by addition of a 50-µl stop solution containing 375 mM sodium acetate (pH 5.2), 62.5 mM EDTA, and 1 µg of yeast tRNA. Transcription products were then phenol/chloroform-extracted, ethanol-precipitated under 2.5 volumes of 100% ethanol (Sigma), and resuspended in 20 µl of gel loading buffer II (Amicon). Extracted transcripts were electrophoresed in 10% polyacrylamide, 8 M urea gel, and analyzed with a Fujilm ST-503 densitometer.

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Nun Residues Lys<sup>106</sup> and Lys<sup>107</sup> Promote Binding to λ DNA

### RESULTS

**Effect of Mutations in Nun Residues Lys<sup>106</sup> and Lys<sup>107</sup> on Termination Activity in Vivo**—Nun arrests transcription on the λ chromosome at many different sites distal to the λ nut sequences (21, 22). Interaction between Nun and λ DNA template does not, therefore, involve specific DNA base contacts. Instead, it depends on interactions between the planar aromatic Nun Trp<sup>106</sup> residue and double-strand DNA. Trp<sup>106</sup> may intercalate into DNA (15). Nun residues Lys<sup>106</sup> and Lys<sup>107</sup> lie adjacent to Trp<sup>106</sup> (Fig. 1). We asked whether these basic amino acids have a role in Nun-dependent transcription termination in vivo, perhaps by neutralizing negative DNA charges and allowing W108 to interact with template.

Plasmids expressing Nun mutants were transformed into E. coli strains carrying Nun-sensitive transcriptional chromosomal fusions. N8499 carries λ pL-nutL-lacZ, and N7499 carries λ pR-cro-nutR-Tr1-galETK. The λ c1857 repressor controls both promoters. Transcription from the λ pL or λ pR promoter was initiated by shifting cultures from 32 to 42 °C, which inactivates the repressor. Nun termination efficiency was determined by β-galactosidase assay (Table I) or galactokinase assay (Table II).

As shown in Table I, replacement of either or both lysines with arginine, another basic amino acid (K106R, K107R, and K106D/K107D) had no effect on Nun activity in the λ pL-nutL operon. Alanine substitution of either Lys<sup>106</sup> or Lys<sup>107</sup> slightly decreased termination activity. Substitution of both lysines (Nun K106A/K107A) reduced transcription termination to 79% wild-type levels. Replacement of a single lysine with a negatively charged aspartate residue also reduced Nun activity; K106D and K107D were 89 and 91% as active as wild-type Nun, respectively, and K106A/K107A had no terminal activity. Substitution of either Lys<sup>106</sup> or Lys<sup>107</sup> with aspartate reduced Nun termination efficiency 3–4-fold. As reported previously (16), substitution of both lysines with aspartate increased galactokinase levels more than 2-fold relative to wild type.

### Table I

| Mutation       | β-Galactosidase | Termination |
|----------------|-----------------|-------------|
| β-Galactosidase | Units | % |
| pET-21d        | 832  | 0 |
| Wild-type Nun  | 8    | 100 |
| K106R          | 4    | 100 |
| K107R          | 12   | 100 |
| K106R/K107R    | 15   | 99  |
| K106A          | 44   | 96  |
| K107A          | 36   | 97  |
| K106A/K107A    | 180  | 79  |
| K106D          | 95   | 89  |
| K107D          | 83   | 91  |
| K106D/K107D    | 429  | 49  |

### Table II

| Mutation  | Galactokinase | Termination |
|-----------|---------------|-------------|
|            | Units | % |
| PET21d     | 2.3   | 0  |
| Wild-type Nun | 0.4  | 100 |
| K106R      | 0.3   | 100 |
| K107R      | 0.5   | 90  |
| K106R/K107R| 0.5   | 90  |
| K106A      | 0.9   | 70  |
| K107A      | 0.6   | 85  |
| K106A/K107A| 2.4   | 0   |
| K106D      | 1.8   | 25  |
| K107D      | 1.7   | 30  |
| K106D/K107D| 5.9   | 9%  |

<sup>a</sup> The high galactokinase unit represents suppression of termination at Tr1.
Substitutions at Nun Positions 106 and 107 Affect λ Exclusion—Nun residues Lys<sup>106</sup> and Lys<sup>107</sup> also play a key role in λ exclusion (Table III). Phage λ failed to form plaques on strains expressing wild-type Nun or Nun variants K106R, K107R, and K106R/K107R. Similarly, Nun K106A and K107A completely excluded λ. However, single aspartate substitutions (K106D, K107D) partially inhibited exclusion (efficiency of plating = 67.5%). Furthermore, Nun K106A/K107A and K106D/K107D failed entirely to block λ plating (efficiency of plating = 1%). These results indicate that position 106 or 107 must carry a positively charged amino acid for Nun to block transcription on infecting λ chromosomes.

Effect of Mutations on Lys<sup>106</sup> and Lys<sup>107</sup> in Transcription Arrest in Vitro—We next tested the Nun mutants in a minimal in vitro transcription system. In this assay, Nun arrests transcription without releasing stalled TEC. As template, we used a 466-bp (35,253–35,718) DNA fragment containing pL-nutL, which was amplified from λ genomic DNA/HindIII (Fig. 3). Nun arrest activity is indicated by reduction in run-off transcripts (RO) and the appearance of specific short RNAs (NA; Fig. 3, lanes 3, 4, and 6). As reported previously (9), T-Nun (T), a truncated form of Nun that lacks the COOH-terminal 13 amino acid residues, was entirely inactive (Fig. 3, lane 2). Nun K106A/K107A (AA) and K106R/K107R (RR) both arrested transcription, although the former was less active than K106R/K107R (Fig. 3, lanes 4 and 6). Nun K106D/K107D failed completely to arrest transcription (Fig. 3, lane 5). This in vitro data further supports the hypothesis that the electrostatic interaction between Nun residues Lys<sup>106</sup> and Lys<sup>107</sup> and the DNA template facilitates Nun-dependent transcription arrest.

BOXB and RNAP Binding by Wild-type and Nun Lys<sup>106</sup>/Lys<sup>107</sup> Mutants—In addition to contacting DNA template, Nun transcription termination entails two additional binding reactions. First, Nun is recruited to the TEC by binding of the NH<sub>2</sub>-terminal ARM domain to the BOXB elements of the λ nascent transcript NUT sequences (Fig. 2B; Refs. 7 and 8). Second, Nun binds to RNA polymerase, either in the TEC or in solution (9, 10). Binding is Zn<sup>2+</sup>-dependent and is promoted by Nun residues His<sup>83</sup>, His<sup>98</sup>, and His<sup>100</sup> (Fig. 2B).

We asked whether Lys<sup>106</sup> and Lys<sup>107</sup> played a role in these interactions. The ability of Nun mutants, K106A/K107A, K106R/K107R, and K106D/K107D to bind BOXB was tested by a native gel mobility shift assay (Fig. 4A). All mutants bound BOXB with approximately wild-type efficiency. Averaging three independent experiments, under these conditions wild-type Nun bound 47% (±6.5%) of input BOXB, whereas Nun K106D/K107D bound 48% (±15.7%).

Binding of wild-type and mutant Nun to RNAP was compared using size exclusion chromatography on a Sephadex G-100 superfine column (Fig. 4B). Under our assay conditions, wild-type Nun binds to RNAP (Fig. 4B, lane 2), whereas T-Nun is retained in the column (Fig. 4B, lane 6). Wild-type Nun, the arrest-proficient Nun mutants, K106A/K107A and K106R/K107R, and the arrest-defective Nun mutant K106D/K107D, all bound RNAP (Fig. 4B, lanes 2–5). These results indicate that

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Table III

| Mutation     | Efficiency of plating |
|--------------|-----------------------|
| PET-21d      | 1.0                   |
| Wild-type Nun| 1.0                   |
| K106R        | <10<sup>-6</sup>      |
| K107R        | <10<sup>-6</sup>      |
| K106R/K107R  | <10<sup>-6</sup>      |
| K106A        | <10<sup>-6</sup>      |
| K107A        | <10<sup>-6</sup>      |
| K106A/K107A  | 1.0                   |
| K106D        | <10<sup>-4</sup>      |
| K107D        | <10<sup>-4</sup>      |
| K106D/K107D  | 1.0                   |

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*H. C. Kim and M. E. Gottesman, unpublished results.
substitutions at Lys<sup>106</sup> and Lys<sup>107</sup> that abrogate transcription arrest do not affect the ability of Nun to bind to BOXB or RNAP.

Lys<sup>106</sup> and Lys<sup>107</sup> Promote Nun-DNA Contacts—Nun can be photochemically cross-linked to λ DNA template in a Nun-arrested TEC (10). Cross-linking is dependent on Nun residue Trp<sup>108</sup>. The results reported above suggest that Lys<sup>106</sup> and Lys<sup>107</sup> help position Trp<sup>108</sup> at the template by neutralizing DNA negative charges.

To demonstrate cross-linking, we used Nun derivatives carrying an additional C110 residue linked by a disulfide bond to the photoreactive cross-linker, AET (Fig. 5; Ref. 10). The 251 bp pl-nut<sub>L</sub> DNA fragment described under “Materials and Methods” was used as a transcription template after dephosphorylation and 5′-end labeling with [γ-<sup>32</sup>P]ATP. Transcription was performed as described in the legend to Fig. 5. As reported previously (10), AET-Nun did not cross-link [<sup>32</sup>P]DNA template in the absence of a TEC (Fig. 5, lane 2). Incubation of the TEC with Nun110C yielded an Nun-AET- [<sup>32</sup>P]DNA complex that migrated slower in the gel than the labeled template marker (Fig. 5, lane 3). As expected, the complex was sensitive to DTT, which reduces the disulfide bond in Nun-S-S-AET- [<sup>32</sup>P]DNA complex (Fig. 5, lane 4). Nun K106W/K107R110C showed the same cross-linking pattern as wild-type Nun (Fig. 5, lanes 3 and 7). K106A/K107A110C formed less complex with template DNA than wild-type Nun (Fig. 5, lanes 3 and 5). Finally, K106D/K107D110C failed to cross-link template (Fig. 5, lane 6). We conclude that the transcription termination/arrest defect of K106D/K107D is explained by its inability to contact template DNA.

**DISCUSSION**

Prophage HK022 Nun protein and phage λ N protein bind the same NUT sequences in a nascent transcript (7, 21). However, Nun induces transcription arrest, whereas N both accelerates the rate of transcription elongation and suppresses transcription termination (6). The functional differences between Nun and N are specified by their unique carboxyl-terminal regions (23). The COOH terminus of Nun is required for Nun transcription arrest both in vivo and in vitro. A truncated variant of Nun, T-Nun, which lacks Nun residues 97–109, is completely inactive in blocking transcription elongation (9). T-Nun binds BOXB sequences within NU1 and NU5 but, unlike wild-type Nun, fails to interact with RNAP (9, 10) or DNA template.3 The contribution of Nun carboxyl-terminal residues to transcription arrest has been explored by genetic and biochemical analyses. Histidine residues His<sup>93</sup>, His<sup>98</sup>, and His<sup>100</sup> coordinate Zn<sup>2+</sup> (15) and are required for binding to RNAP.2 The penultimate Nun residue, Trp<sup>105</sup>, plays a critical role in Nun arrest. There are suggestions that Trp<sup>105</sup> may intercalate in the λ template, allowing Nun to act as a brake to TEC translocation. Thus Nun does not arrest transcription on single strand DNA (15). Furthermore, although Nun W108A or Nun W108L fails to terminate λ transcription, substitution of Trp<sup>105</sup> with another aromatic planar amino acid, tyrosine, is innocuous (15). Finally, Nun arrests transcription at many sites promoter-distal to NUT (21, 22). This suggests that specific amino acid-nucleotide interactions are not involved in Nun transcription arrest.

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arrest. Nun W108A, although it interacts with a TEC, does not form stable contacts with DNA (10). Interaction of Nun with template occurs only in the context of a TEC (Ref. 10 and this work) and entails contacts between Nun and RNAP. Nun H93A, like W108A, does not cross-link template (10).

Two basic residues, Lys\textsuperscript{106} and Lys\textsuperscript{107} lie adjacent to Trp\textsuperscript{108} (Fig. 1). We present evidence above that Lys\textsuperscript{106} and Lys\textsuperscript{107} facilitate the proper positioning of the Nun carboxyl terminus near the template by electrostatic interactions with DNA. Thus, K106D/K107D is defective for exclusion and for transcription termination in vivo and fails to arrest transcription in vitro. Importantly, K106D/K107D does not cross-link with DNA template. In contrast, K106R/K107R, which preserves the basic charges at positions 106 and 107, is fully active in transcription arrest and in cross-linking DNA. K106A/K107A cross-links template DNA with an efficiency intermediate between wild-type or K106R/K107R and K106D/K107D.

The defect in the Nun pathway of K106D/K107D is accounted for entirely by inability to interact with template DNA. The mutant protein binds BOXB and RNAP in vitro with wild-type efficiency. Evidence for binding of K106D/K107D to BOXB in vivo has been reported previously (16). We conclude that Lys\textsuperscript{106} and Lys\textsuperscript{107} act to neutralize negative charges on DNA template, allowing Nun to interact with template and to block translocation of the TEC.

In general, Nun proteins mutated at residues 106 and 107 are less active at nutR than at nutL. Thus, K106D/K107D and K106A/K107A induce 49% and 79% termination at nutL, respectively, and are entirely inactive at nutR. In this respect, Nun mutants act like host nus and rpoC mutants, which suppress Nun at nutR but have little effect at nutL (2, 24). Two factors contribute to the difference between nutL and nutR. First, nutL lies only 34 bp from pL, whereas nutR is separated by 227 bp from pR (14). The proximity of nutL to its cognate promoter may block access of host transcription repair-coupling factor to the Nun-arrested TEC, thus prolonging the half-life of the complex (14). Second, the λ pL operon includes the RNase III-sensitive site, rIII, which lies just distal to nutL (Fig. 2A; Refs. 25–27). rIII enhances Nun activity, perhaps by making contacts with Nun residues.\textsuperscript{4}

\textsuperscript{4}R. S. Washburn, H. C. Kim, and M. E. Gottesman, manuscript in preparation.

Alanine scanning of the Nun 13 carboxyl-terminal amino acids (Asn\textsuperscript{102}–Ser\textsuperscript{109}; Fig. 1) revealed that an R102A substitution reduced termination efficiency to 67% at pL-nutL and completely abolished activity at pR-nutR (data not shown). In contrast to Lys\textsuperscript{106} and Lys\textsuperscript{107}, however, substitution with another basic amino acid (R102K) was equally disabling. The role of Arg\textsuperscript{102} in transcription termination, therefore, appears to be unrelated to its charge.

Acknowledgment—We thank Robert Washburn for helpful discussions.

REFERENCES

1. Robert, J., Sloan, S. B., Weisberg, R. A., Gottesman, M. E., Robledo, R., and Harberrecht, D. (1987) Cell 51, 483–492
2. Robledo, R., Gottesman, M. E., and Weisberg, R. A. (1990) J. Mol. Biol. 212, 635–643
3. Burd, C. G., and Dreyfuss, G. (1994) Science 265, 615–621
4. Lazinski, D., Gradzielska, E., and Das, A. (1989) Cell 59, 207–218
5. Weiss, M. A., and Narayanan, N. (1996) Biopolymers 48, 167–180
6. Das, A. (1992) J. Bacteriol. 174, 6711–6716
7. Chattopadhyay, S., Hung, S. C., Stuart, A. C., Palmer, A. G., III, Garcia-Mena, J., Das, A., and Gottesman, M. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12131–12135
8. Oberto, J., Weisberg, R. A., and Gottesman, M. E. (1989) J. Mol. Biol. 207, 675–693
9. Watnick, R. S., and Gottesman, M. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1546–1551
10. Watnick, R. S., and Gottesman, M. E. (1999) Science 286, 2337–2339
11. Hung, S. C., and Gottesman, M. E. (1995) J. Mol. Biol. 247, 428–442
12. Modgrijde, J., Mah, T. F., and Greenblatt, J. (1995) Genes Dev. 9, 2831–2845
13. Robledo, R., Atkinson, B. L., and Gottesman, M. E. (1991) J. Mol. Biol. 220, 613–619
14. Washburn, R. S., Wang, Y., and Gottesman, M. E. (2003) J. Mol. Biol. 329, 655–662
15. Watnick, R. S., Herring, S. C., Palmer, A. G., III, and Gottesman, M. E. (2000) Genes Dev. 14, 731–739
16. Kim, H. C., Zhou, J. G., Wilson, H. R., Mogilnitskiy, G., Court, D. L., and Gottesman, M. E. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 5308–5312
17. Silhavy, T., Berman, M., and Enquist, L. (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Wilson, H. R., Kameyama, L., Zhou, J. G., Guarneros, G., and Court, D. L. (1997) Genes Dev. 11, 2204–2213
19. Miller, J. H. (1992) A Short Course in Bacterial Genetics: A Laboratory Manual for Escherichia coli and Related Bacteria, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
20. Adhya, S., and Miller, W. (1979) Nature 279, 492–494
21. Baron, J., and Weisberg, R. A. (1992) J. Bacteriol. 174, 1983–1989
22. Hung, S. C., and Gottesman, M. E. (1997) J. Mol. Biol. 11, 2670–2678
23. Henthorn, K. S., and Friedman, D. I. (1996) J. Mol. Biol. 257, 9–20
24. Burova, E., Hung, S. C., Chen, J., Court, D. L., Zhou, J. G., Mogilnitskiy, G., and Gottesman, M. E. (1999) Mol. Microbiol. 31, 1783–1793
25. Kameyama, L., Fernandez, L., Court, D. L., and Guarneros, G. (1991) Mol. Microbiol. 5, 2853–2865
26. Lozener, H. A., Dahlberg, J. E., and Szybalski, W. (1976) Virology 71, 262–277
27. Steege, D. A., Cone, K. C., Queen, C., and Rosenberg, M. (1987) J. Biol. Chem. 262, 17651–17658
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J. Biol. Chem. 2004, 279:13412-13417.
doi: 10.1074/jbc.M313206200 originally published online January 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M313206200

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