JOURNAL OF BACTERIOLOGY, Mar. 1995, p. 1388–1392 Vol. 177, No. 5
0021-9193/95/$04.00+0

Escherichia coli NusG Protein Stimulates Transcription Elongation Rates In Vivo and In Vitro

E. BUROVA,[1] S. C. HUNG,[1] V. SAGITOV,[2]† B. L. STITT,[3] AND M. E. GOTTESMAN[1,2]*

Institute of Cancer Research[1] and Department of Microbiology,[2] College of Physicians and Surgeons of Columbia University, New York, New York 10032, and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140[3]

Received 17 October 1994/Accepted 17 December 1994

The rate of transcription elongation in *Escherichia coli* was reduced when cells were depleted of NusG. In a purified in vitro system, NusG accelerated the transcription elongation rate. The stimulation of the rate of transcription elongation by NusG appears to result from the suppression of specific transcription pause sites.

The *Escherichia coli nusG* gene encodes an essential transcription factor that has been implicated in a variety of cellular and viral termination and antitermination processes (2, 9, 10, 21, 22). NusG is required, along with other Nus factors, for effective antitermination by N protein of λ phage in vitro (2, 8, 10, 11, 15). The *nusG4* mutation restores N activity in a *nusA1* host, suggesting that NusG is also involved in antitermination in vivo (22). NusG has been shown to stimulate Rho-dependent termination in vivo (21).

In vitro experiments indicate that NusG binds directly and selectively to Rho (9) and more weakly to core RNA polymerase (8). NusG both stimulates and changes the pattern of Rho-dependent termination at the λ tR1 and *trp t′* terminators (9, 14). A model has been proposed in which NusG serves as a bridge between RNA polymerase and Rho, thus helping to recruit Rho into the termination complex (9). Indeed, recent evidence indicates that NusG stably associates with stalled elongation complexes only if Rho is bound to the nascent RNA and that the presence of NusG in the complex leads to a slower off-rate of Rho from the transcript (13).

The participation of NusG in factor-dependent termination and antitermination processes, both of which involve modulation of transcription elongation, suggests the possibility that NusG may directly affect the rate at which RNA polymerase synthesizes RNA molecules. In this paper, we demonstrate that NusG increases the rate of transcription elongation both in vivo and in vitro. The ability of NusG to suppress transcriptional pausing probably accounts for its acceleration of the overall rate of transcription elongation.

NusG depletion slows the rate of transcription elongation. We first asked if depletion of NusG affected the rate of transcription elongation in *E. coli* cells. Strains SS287 and SS294 are derivatives of N99 that carry a *nusG*::*Kn* chromosomal insertion and a *nusG*$^+$ plasmid. SS287 carries the *rep ts* plasmid pSS119; SS294 carries the *rep*$^+$ plasmid pSS120. Depletion of NusG in SS287 occurs when the cells are shifted from 32 to 42°C, inducing the loss of the pSS119 plasmid (1, 21).

The rate of RNA chain growth can be estimated from the time of appearance of β-galactosidase after IPTG (isopropyl β-D-thiogalactosidase) induction of *lacZ* (5). This technique has been used recently to demonstrate altered rates of elongation by certain RNA polymerase mutants (3). To measure the effect of NusG depletion on the initial kinetics of β-galactosidase induction, SS287 and SS294 were grown in Luria-Bertani medium at 32°C until early log phase and shifted to 42°C for 2.5 h. Cultures were maintained in exponential growth by dilution into fresh media. Optical density at 650 nm did not exceed 0.3. Thirty minutes prior to induction, 5 mM cyclic AMP was added to both cultures to decrease the catabolite repression of the *plac* promoter. After the cultures were cooled to 25°C for 5 min, β-galactosidase was induced with 1 mM IPTG. Duplicate 0.5-ml samples were withdrawn every 30 s into tubes containing 0.5 ml of ice-cold chloramphenicol (0.1 mg/ml) to stop protein elongation and were immediately frozen in an ethanol-dry ice bath. After sampling was performed, the tubes were incubated for 15 min at 37°C to allow β-galactosidase monomers time to assemble into active tetramers. β-Galactosidase was measured as described previously (12).

The kinetics of β-galactosidase induction in normal and NusG-depleted cells is shown in Fig. 1. After an initial lag, the slopes of the curves of β-galactosidase activity in cells depleted of NusG and in control cells were identical (Fig. 1). Thus, NusG depletion does not reduce the rate of promoter clearance, affect *lacZ* mRNA stability, or affect the translation of the *lacZ* transcript. Under these conditions, the difference in lag between the cultures reflects the time required for the synthesis of the first full-size transcript; i.e., the lag is a function of the elongation rate (3, 5).

Figure 1 shows a lag of 2.9 min before the appearance of β-galactosidase activity after induction of SS287, compared with a lag of 2.3 min for SS294. This difference of 0.6 min represents an apparent decrease of ~20% in the rate of transcription elongation after depletion of NusG. The residual concentration of NusG protein, estimated by Western blot (immunoblot) analysis, is less than 10% of the initial level after 2.5 h of depletion at 42°C, although 4 h of heating is required for complete depletion of the protein (21). At 2.5 h, cell growth has not yet slowed in strain SS287 (21). We were thus able to avoid the reduction in transcription elongation that is associated with a reduced growth rate (23). It is possible that full depletion of NusG might further decrease the rate of transcription elongation.

NusG accelerates transcription elongation in vitro. Figure 2A shows the effects of NusG addition on the kinetics of transcription of a λ p_L operon template in vitro. Reactions were done in two steps. In the first step, transcription was synchronized at position +15 of the nascent transcript by omitting UTP. The reaction mixture (10 μl, including *E. coli* RNA

* Corresponding author. Mailing address: Institute of Cancer Research, College of Physicians and Surgeons of Columbia University, 701 West 168 St., New York, NY 10032. Phone: (212) 305-6900. Fax: (212) 305-1741.

† Present address: Ranet Center, Inc., Riverdale, NY 10471.

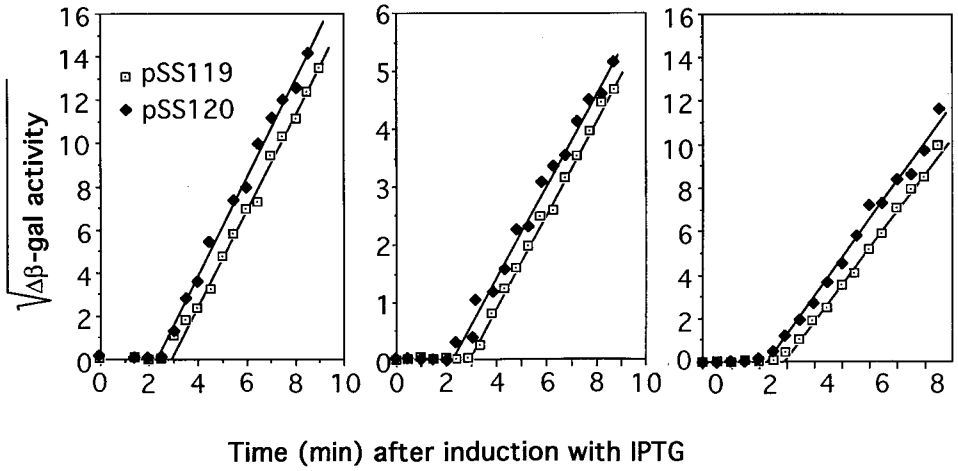

FIG. 1. Effect of NusG depletion on the initial kinetics of β-galactosidase induction. β-Galactosidase activity is plotted as $(\Delta\beta)^{1/2}$ versus time of sampling, where Δβ is β-galactosidase at $(T = t) - (T = 0)$. The lines were obtained from a least-squares fit of the datum points. The lag time for appearance of β-galactosidase is determined by the *x*-intercept of the line for β-galactosidase activity, as discussed elsewhere (17). SS287 and SS294 are *nusG*::*Kn* derivatives of the N99 strain that carry either pSS119 or pSS120. Results of three independent repetitions of this experiment are presented to show that the relative difference in the lag time for appearance of β-galactosidase between the two strains is reproducible. The variation in level of β-galactosidase observed among these three experiments reflects day-to-day variations in enzyme activity.

polymerase [20 nM; Boehringer Mannheim Biochemicals], DNA template [a 452-bp *Bgl*II-*Hpa*I restriction fragment of λ phage DNA containing the p_L promoter and 321 bp of downstream sequence; 20 nM], ApU [0.5 mM], ATP [2 μM], CTP [2 μM], GTP [2 μM], and [α-^{32}P]ATP [1 μCi, >1,500 Ci/mmol] in a transcription buffer containing 20 mM Tris-acetate (pH 7.9), 60 mM potassium acetate, 2 mM magnesium acetate, 0.2 mg of bovine serum albumin per ml, 1 mM dithiothreitol, and 5% glycerol) was incubated at 32°C for 15 min to allow the formation of 15-meric ternary complexes. In the second step, an equal volume of prewarmed mixture containing the same buffer, 20 μg of rifampin per ml, all four nucleoside triphosphates (NTPs) (each adjusted to a final concentration of 2 μM), and, where indicated, 100 nM purified NusG protein were added, and the samples were incubated at 32°C for the indicated times. Note that elongation was slowed by limiting the NTP concentrations. The reaction was stopped by the addition of EDTA to 50 mM, and transcription products were analyzed by electrophoresis on a 6% polyacrylamide–8.8 M urea gel followed by autoradiography. A series of transcription pause sites was evident, as well as the runoff transcript, the amount of which increased with time. The data of this experiment are presented graphically in Fig. 2B through E.

The time of appearance of runoff transcript was clearly accelerated by NusG. At 6 min, about 50% of the RNA polymerases reached the end of template in the presence of NusG, compared with less than 5% in its absence. Thus, at least half of the RNA polymerases respond to NusG. The presence of NusG did not prevent pausing or change the location of the pause sites. However, the occupancy of the three pause sites was reduced by NusG (Fig. 2C through E). This suggests that NusG accelerates the rate of transcription elongation by partially suppressing pausing.

In addition, the data shown in Fig. 2A reveal that NusG increased the efficiency of resuming elongation from a stalled transcription complex. The stalled complexes formed in the absence of UTP (Fig. 2A, time zero lane) were mobilized more efficiently in the presence of NusG when UTP was added to resume elongation.

We have also shown that NusG accelerated the rate of transcription elongation and suppressed pausing on a λ pR-tR1 template. Furthermore, the effect of NusG could be seen at NTP concentrations of 50 μM each (data not shown).

The effects of NusG on elongation during transcription of a template carrying the *trp t′* site were previously reported (14). Although Nehrke and Platt do not draw this conclusion, their experiments reveal that NusG also accelerates the rate of elongation and suppresses pause sites on this template (Fig. 4A in reference 14).

NusG suppresses pausing at the *trpL* pause site. To describe more quantitatively the effect of NusG on pausing, we used the artificial template developed by Landick and coworkers (7; described in detail in reference 16). This template contains the strong *trpL* pause site (Fig. 3A).

Transcription was initiated with ApU dinucleotide. Elongating RNA polymerase molecules were synchronized at position +20 by omitting UTP from the transcription mixture. The complexes were purified on a Sephadex G-50 column, and aliquots containing approximately 0.1 pmol of purified stalled complex were incubated for 5 min with or without 2 pmol of NusG. Transcription elongation was then resumed by addition of 50 μM each of the four NTPs. Aliquots of the reaction mixture were removed at various times, and the transcripts were resolved by 10% polyacrylamide–8 M urea gel electrophoresis (Fig. 3B). Figure 3C shows that at 30 s more than 25% of all transcripts had not read through the *trpL* pause site in the absence of NusG, compared with only 15% in the presence of NusG. These results indicate that NusG partially suppressed the *trpL* pause. The addition of NusG reduced the pause half-life twofold, from 30 to 15 s, but did not significantly affect the probability with which RNA polymerase paused at the site (Fig. 3C). Note that NusG had little or no effect on the efficiency of the Rho-independent *trpL* attenuator (Fig. 3B).

Pausing at *trpL* is induced by an RNA hairpin that forms in the paused transcription complex (6). Sequence modelling suggests that the pause sites shown in Fig. 2 are likewise associated with RNA hairpins. Whether NusG would suppress pause sites that lack a plausible hairpin structure remains to be determined.

The ability of NusG to suppress pausing at certain sites probably explains its stimulatory effect on the in vitro overall elongation rate, although NusG might also affect other fea-

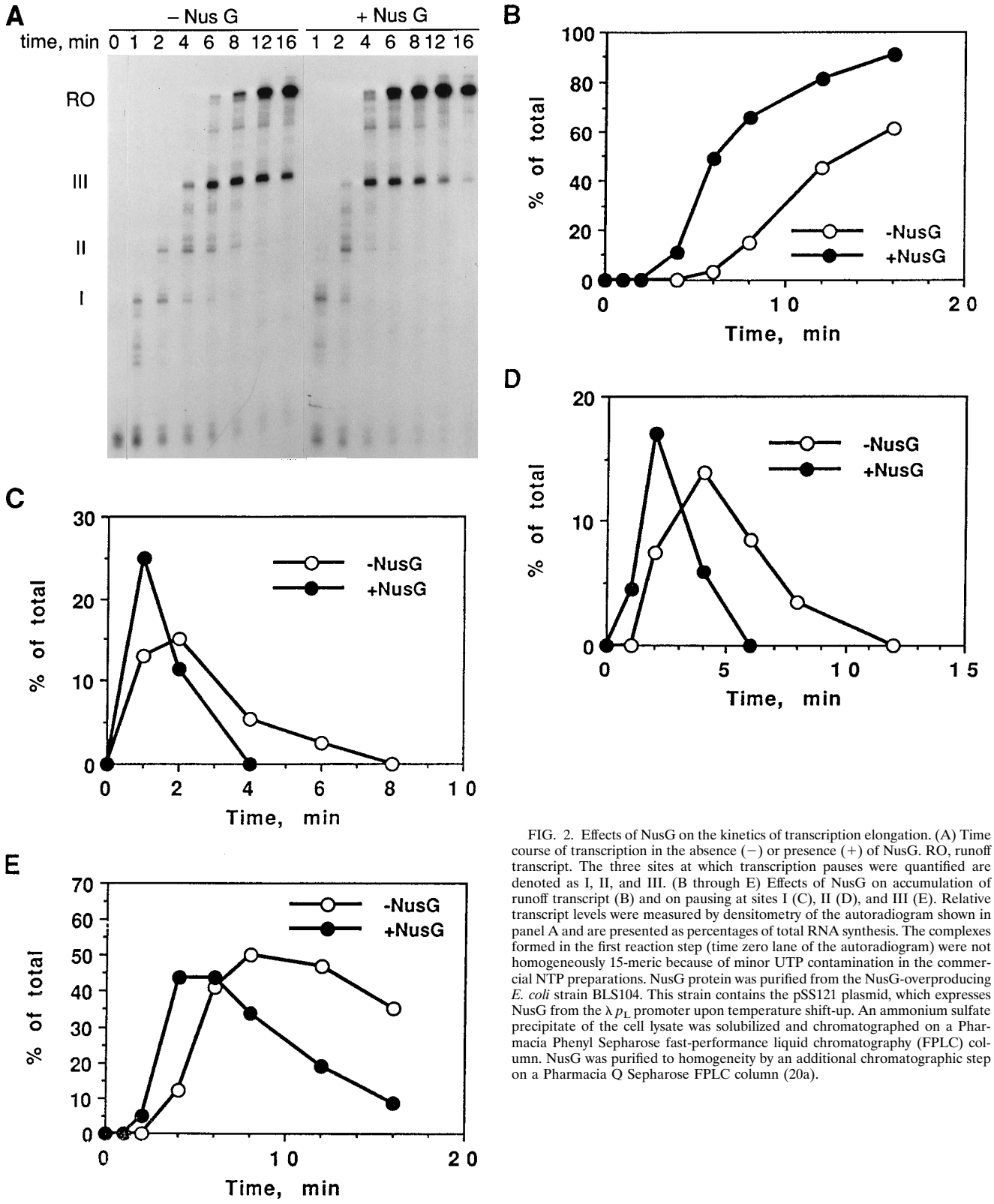

FIG. 2. Effects of NusG on the kinetics of transcription elongation. (A) Time course of transcription in the absence (−) or presence (+) of NusG. RO, runoff transcript. The three sites at which transcription pauses were quantified are denoted as I, II, and III. (B through E) Effects of NusG on accumulation of runoff transcript (B) and on pausing at sites I (C), II (D), and III (E). Relative transcript levels were measured by densitometry of the autoradiogram shown in panel A and are presented as percentages of total RNA synthesis. The complexes formed in the first reaction step (time zero lane of the autoradiogram) were not homogeneously 15-meric because of minor UTP contamination in the commercial NTP preparations. NusG protein was purified from the NusG-overproducing *E. coli* strain BLS104. This strain contains the pSS121 plasmid, which expresses NusG from the λp_L promoter upon temperature shift-up. An ammonium sulfate precipitate of the cell lysate was solubilized and chromatographed on a Pharmacia Phenyl Sepharose fast-performance liquid chromatography (FPLC) column. NusG was purified to homogeneity by an additional chromatographic step on a Pharmacia Q Sepharose FPLC column (20a).

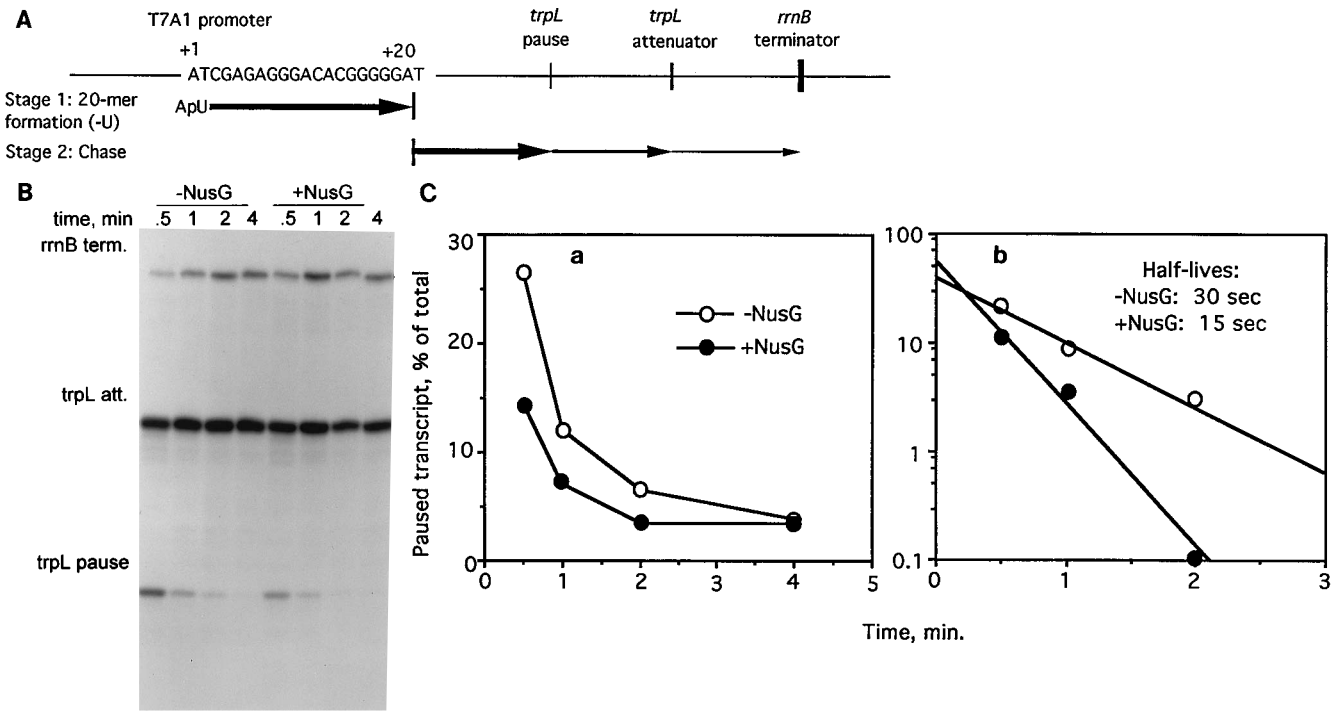

FIG. 3. Effect of NusG protein on RNA polymerase pausing at the *trpL* pause site. (A) Transcription template used in these experiments. (B) Autoradiogram of a gel analysis of the transcription products. Positions of the bands corresponding to transcripts ending at the *trpL* pause, the *trpL* attenuator (att.), and the *trpL* terminator (term.) are shown. (C) Percentage of transcripts paused at the *trpL* pause site at a given time. (a) Pause RNA from a reaction with a T7 A1 promoter-*trpL* fusion DNA template. Under the conditions of this experiment, all the transcripts reached the pause site or proceeded beyond it by the 0.5-min time point. The effect of NusG on pausing and termination at the *trpL* attenuator and the *rrnB* terminator was not quantified in this experiment because of the more complicated kinetics of formation of the corresponding bands. (b) Determination of paused transcription complex half-lives. The data for the paused transcript concentrations are shown in the semilogarithmic plots used to determine the exponential rate of disappearance of the complexes from the pause. Linear regression analysis was used to obtain the lines and the pause half-lives shown.

tures of transcription elongation as well. Though the slowing of elongation may be a secondary effect of NusG depletion, the correlation between the in vivo and in vitro results suggests that NusG directly accelerates transcriptional elongation in vivo. NusG binding to core RNA polymerase might induce a conformational change in the ternary elongation complex, resulting in the facilitated incorporation of nucleotide substrates. Alternatively, NusG might weaken the affinity between the nascent transcript and the RNA binding site of the polymerase.

The absence of NusG in standard transcription mixtures may help to explain why the elongation rate is slower in vitro than in vivo (4). We also note that the effects of NusG are the opposite of those of NusA, another *E. coli* transcription elongation factor. NusA binds to core RNA polymerase to slow the overall transcription elongation rate and to enhance pausing at certain sites (4, 18–20, 24). Whether NusG and NusA affect a common step in RNA chain elongation is not known.

This work was supported by NIH grant GM37219.

We thank Susan Sullivan and Alexander Gragerov for useful discussions. Paul Rubock provided excellent technical help.

REFERENCES

1. **Hashimoto-Gotoh, T., and M. Sekiguchi.** 1977. Mutations to temperature sensitivity in R plasmid pSC101. J. Bacteriol. **131:**405–412.
2. **Horwitz, R. J., J. Li, and J. Greenblatt.** 1987. An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda. Cell **51:**631–641.
3. **Jin, D. J., R. R. Burgess, J. P. Richardson, and C. A. Gross.** 1992. Termination efficiency at Rho-dependent terminators depends on kinetic coupling between RNA polymerase and Rho. Proc. Natl. Acad. Sci. USA **89:**1453–1457.
4. **Kassavetis, G. A., and M. J. Chamberlin.** 1981. Pausing and termination of transcription within the early region of bacteriophage T7 DNA *in vitro*. J. Biol. Chem. **256:**2777–2786.
5. **Kepes, A.** 1969. Transcription and translation in the lactose operon of *Escherichia coli* studied by in vivo kinetics. Prog. Biophys. Mol. Biol. **19:**199–236.
6. **Landick, R., and C. Yanofsky.** 1984. Stability of an RNA secondary structure affects *in vitro* transcription pausing in the *trp* operon leader region. J. Biol. Chem. **259:**11550–11555.
7. **Landick, R. L., J. Stewart, and D. N. Lee.** 1990. Amino acid changes in conserved regions of the β-subunit of *Escherichia coli* RNA polymerase alter transcription pausing and termination. Genes Dev. **4:**1623–1636.
8. **Li, J., R. J. Horwitz, S. McCracken, and J. Greenblatt.** 1992. NusG, a new *Escherichia coli* elongation factor involved in transcription antitermination by the N protein of phage λ. J. Biol. Chem. **267:**6012–6019.
9. **Li, J., S. W. Mason, and J. Greenblatt.** 1993. Elongation factor NusG interacts with termination factor ρ to regulate termination and antitermination of transcription. Genes Dev. **7:**161–172.
10. **Mason, S. W., and J. Greenblatt.** 1991. Assembly of transcription elongation complexes containing the N protein of phage λ and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10. Genes Dev. **5:**1504–1512.
11. **Mason, S. W., J. Li, and J. Greenblatt.** 1992. Host factor requirements for processive antitermination of transcription and suppression of pausing by the N protein of bacteriophage λ. J. Biol. Chem. **267:**19418–19426.
12. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
13. **Nehrke, K. W., and T. Platt.** 1994. A quaternary transcription termination complex: reciprocal stabilization by Rho factor and NusG protein. J. Mol. Biol. **243:**830–839.
14. **Nehrke, K. W., F. Zalatan, and T. Platt.** 1993. NusG alters Rho-dependent termination of transcription *in vitro* independent of kinetic coupling. Gene Expr. **3:**119–133.
15. **Nodwell, J. R., and J. Greenblatt.** 1991. The *nut* site of bacteriophage λ is made of RNA and is bound by transcription antitermination factors on the surface of RNA polymerase. Genes Dev. **5:**2141–2151.
16. **Sagitov, V., V. Nikiforov, and A. Goldfarb.** 1993. Dominant lethal mutations near the 5′ substrate binding site affect RNA polymerase propagation. J. Biol. Chem. **268:**2195–2202.

17. **Schleif, R., W. Hess, S. Finkelstein, and D. Ellis.** 1973. Induction kinetics of the L-arabinose operon of *Escherichia coli*. J. Bacteriol. **115:**9–14.
18. **Schmidt, M. C., and M. J. Chamberlin.** 1984. Amplification and isolation of *Escherichia coli* nusA protein and studies of its effects on *in vitro* RNA chain elongation. Biochemistry **23:**197–203.
19. **Schmidt, M. C., and M. J. Chamberlin.** 1987. NusA protein of *Escherichia coli* is an efficient transcription termination factor for certain terminator sites. J. Mol. Biol. **195:**809–818.
20. **Sigmund, C. D., and E. A. Morgan.** 1988. NusA protein affects transcriptional pausing and termination *in vitro* by binding to different sites on the transcription complex. Biochemistry **27:**5622–5627.
20a. **Stitt, B. L.** Unpublished data.
21. **Sullivan, S. L., and M. E. Gottesman.** 1992. Requirement for *E. coli* NusG protein in factor-dependent transcription termination. Cell **68:**989–994.
22. **Sullivan, S. L., D. F. Ward, and M. E. Gottesman.** 1992. Effect of *Escherichia coli nusG* function on λ N-mediated transcription antitermination. J. Bacteriol. **174:**1339–1344.
23. **Vogel, U., and K. F. Jensen.** 1994. The RNA chain elongation rate in *Escherichia coli* depends on the growth rate. J. Bacteriol. **176:**2807–2813.
24. **Yager, T. D., and P. H. von Hippel.** 1987. Transcript elongation and termination in *Escherichia coli*, p. 1241–1275. *In*: F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.