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A NusG-Like Protein from *Thermotoga maritima* Binds to DNA and RNA

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The NusG-like protein from *Thermotoga maritima* was expressed in *Escherichia coli* and purified to homogeneity. Purified *T. maritima* NusG exhibited a generalized, non-sequence-specific and highly cooperative DNA and RNA binding activity. The complexes formed between nucleic acid and *T. maritima* NusG were unable to penetrate a polyacrylamide or agarose gel. The affinity of the protein for DNA was highest in buffers containing about 50 mM salt. The DNA-protein complexes could not be stained with ethidium bromide, were resistant to digestion by *Taq*I endonuclease, were able to be transcribed in vitro by *T. maritima* RNA polymerase, and contained a minimum of about 30 to 40 monomers of NusG per kb of duplex DNA. The protein had comparable affinities for duplex DNA and RNA but a lower affinity for single-stranded DNA. Electron microscopy showed that the DNA in the complex is condensed within a large structure that resembles the complex between DNA and histone-like protein Hc1 from *Chlamydia trachomatis*. Neither the wild-type *T. maritima nusG* gene nor a deletion derivative more similar to the *E. coli* gene was able to substitute for the essential *E. coli nusG*. Two variants of the NusG protein were constructed, expressed, and purified: one contains only the entire 171-amino-acid insertion that is unique to *T. maritima* NusG, and the other has only the sequences present in NusG homologs from *E. coli* and other eubacteria. Both variants exhibited similar DNA and RNA binding behavior, although their apparent affinities were 5- to 10-fold lower than that of the wild-type *T. maritima* NusG.

Nus factors endow the DNA-dependent RNA polymerase of *Escherichia coli* with the ability to read through both factor-dependent and factor-independent transcription termination signals on the template DNA. These Nus factors function in a multimeric complex with the RNA polymerase that is assembled shortly after transcription initiation (for reviews, see references 7 and 12). Two of the factors, NusB and NusE (ribosomal protein S10), bind to RNA polymerase and nascent RNA, tethering the transcript to the elongating RNA polymerase (25). NusA binds directly to the RNA polymerase and slows the transcription elongation, causing RNA polymerase to pause at specific sites in many transcriptional units (11, 40). This factor is also critical for interaction of RNA polymerase with the λ antitermination protein N during bacteriophage propagation (7, 29, 30, 39).

NusG protein also binds to the elongating RNA polymerase and is believed to be essential for rho-dependent transcription termination events (17, 18, 37). The termination factor rho is an RNA-dependent ATPase with RNA helicase activity (4); if unimpeded, it causes destabilization of the RNA-DNA duplex within the transcription bubble at the site of elongation and triggers the release of the nascent transcript when polymerase is paused at a rho-dependent termination site (23, 24). The activity of rho is mediated through an interaction with NusG in the elongation complex. The in vivo depletion of NusG has been shown to result in the suppression of termination at a number of different rho-dependent termination signals (37, 38). NusG may also play a role in the regulation of termination at an attenuator site located upstream of genes encoding the β and β′ subunits of RNA polymerase in *E. coli* (20).

The *E. coli* gene encoding the 181-amino-acid NusG protein is essential for cell viability and is located in the *secE-nusG* bicistronic operon (8, 33). This operon is part of a larger cluster of essential genes that encode the translation factor EF-TuB, the ribosomal proteins L11, L1, L10, and L12, and the β and β′ subunits of RNA polymerase (2, 28).

We recently cloned and characterized a complex transcription unit containing a *nusG*-like gene from the hyperthermophilic eubacterium *Thermotoga maritima* (19). The operon contains five tRNA genes in the 5′ mRNA leader, the *rpmG* gene encoding ribosomal protein L33, a diminutive version of *secE*, an enlarged version of *nusG*, and in the distal position, the *rplKAJL* genes encoding ribosomal proteins L11, L1, L10, and L12. The NusG protein of *T. maritima* has 353 amino acid residues and a mass of 40 kDa; within this protein, there is a 171-amino-acid sequence after residue 45 that is absent in the corresponding NusG homologs from *E. coli* and other eubacteria. *T. maritima* NusG contains a relatively high content of charged amino acids (121 of 353 residues) and is moderately basic (57 acidic and 64 basic residues; pI = 9.0) (19). The charged residues are evenly distributed along the length of the molecule. Because the protein is basic, because the *E. coli* homolog is involved in transcription, and because the *rpmG-secE-nusG-rplJAKL* operon of *T. maritima* exhibits a number of unusual transcriptional features, we initially examined the ability of the purified protein to interact with *nusG* operon DNA. We have found that the *T. maritima* NusG protein has a generalized DNA and RNA binding activity. We describe the characteristics of that binding in this paper.

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TABLE 1. Duplex DNA fragments used for DNA band shift assays and electron microscopy

Fragment	Size (kb)	Source	Description	Reference
*Xba*I-*Hin*dIII	5.2	pUC-TB4	Sequence that encodes part of the β subunit of the *T. maritima* RNA polymerase	27
*Msp*I digestion products	1.053–0.009	pPD990	A *Sac*I-*Eco*RI 3.8-kb *T. maritima* genomic fragment containing the *nusG* gene and surrounding region in plasmid pGEM-7Zf(+)	18a
*Hin*dIII-*Hin*dIII	3.5	pGEM-L12	Linearized plasmid pGEM-7Zf(−) that contains the *T. maritima* L12-encoding gene	18a
*Hin*dIII-*Eco*RI	3.0	pGEM-L1	The larger *Hin*dIII-*Eco*RI fragment of the plasmid pGEM-7Zf(−) containing the *T. maritima* L1-encoding gene	18a
	0.7	pGEM-L1	The smaller *Hin*dIII-*Eco*RI fragment of the plasmid described immediately above (the *T. maritima* L1-encoding sequence)	18a
*Sma*I-*Sma*I	2.7	pUC18	Plasmid pUC18 linearized by *Sma*I digestion	41

MATERIALS AND METHODS

Expression of the *T. maritima nusG* gene in *E. coli*. The *E. coli* strains JM101, JM109, and BL21(DE3)pLysS were used for the cloning and expression of the *nusG* encoding gene of *T. maritima* (36). The gene was amplified by the PCR. The sequence of the 5′ primer (oD7; 5′-GAGAATTCATGAAGAAAAAGTG GTAC-3′) contains the first six codons of *nusG* and an 8-nucleotide extension at its 5′ end containing the *Eco*RI recognition sequence (underlined). The sequence of the 3′ end primer (oD8; 5′-ACAAGCTTTCACTCGATTTTCTCCA C-3′) is complementary to the last six codons of *nusG*, and the 8-nucleotide extension at the 5′ end carries a *Hin*dIII recognition site (underlined). Amplification was carried out in a 100-μl mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.0 mM $MgCl_2$, 50 μM deoxynucleoside triphosphate, 100 μg of gelatin per ml, 40 pmol of both primers, 2.5 U of *Taq* DNA polymerase (Pharmacia), and 10 pg of plasmid pPD990, which contains the *T. maritima nusG* gene (19). The reaction cycles were as follows: cycle 1, 90 s at 94°C, 30 s at 55°C, and 90 s at 72°C; cycles 2 to 31, 15 s at 94°C, 30 s at 55°C, and 90 s at 72°C; cycle 32, 5 min at 72°C. The amplified DNA fragment was gel purified, digested by *Eco*RI and *Hin*dIII, and cloned into the *Eco*RI and *Hin*dIII sites of pGEM-7Zf(+) (Promega). Several clones were sequenced. The *Eco*RI-*Hin*dIII fragment with the correct NusG-coding sequence was recloned into the expression vector pKK223-3 (Pharmacia) to give plasmid pPD1077. The T7 expression vector pET-3a (36) was cut with *Nde*I, and pPD1077 was cut by *Eco*RI; the linear plasmids were blunt ended by treatment with nuclease S1 and Klenow fragment of *E. coli* DNA polymerase I. The plasmids were then recut with *Bam*HI. The linearized vector pET-3a and *nusG*-containing fragment were recovered from an agarose gel and ligated together to yield plasmid pPD1078. The *E. coli* strains JM101 and JM109 were transformed with pPD1077, and BL21(DE3)pLysS was transformed with pPD1078. Under inducing conditions, these transformants exhibited a high-level expression of the NusG protein of *T. maritima.*

Purification of NusG protein. JM109 harboring pPD1077 or BL21(DE3)pLysS harboring pPD1078 was grown at 37°C in 2 liters of YT medium containing appropriate antibiotics [ampicillin (100 μg/ml) for JM109/pPD1077 and ampicillin and chloramphenicol (25 μg/ml) for BL21(DE3)pLysS/pPD1078]. When the A_{600} of the culture reached a value between 0.6 and 1.0, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to each culture to a final concentration of 0.4 mM. Each culture was grown for an additional 3 h, and the cells were harvested at 4°C by centrifugation. The cells were washed with buffer A (50 mM Tris-HCl [pH 8.0], 0.35 M NaCl, 10 mM $MgCl_2$, 1 mM EDTA) and resuspended in 50 ml of buffer A. The cell suspensions were sonicated at 1-min intervals in an ice-water mixture for 8 min [the cells of BL21(DE3)pLysS/pPD1078 can also be lysed by a freeze-thaw cycle]. The cell lysates were centrifuged at 27,000 × *g* for 25 min to remove cell debris. An equal volume of 1 M NaCl was added to the cleared cell lysates, and streptomycin sulfate (20%, wt/vol) was added slowly to the cell lysates with stirring on ice to a final concentration of 4%. Stirring was continued for an additional 15 min. The solutions were then centrifuged at 18,800 × *g* for 10 min. The supernatants were heated at 75°C for 30 min and centrifuged at 10,800 × *g* for 15 min. Solid ammonium sulfate was added to the supernatants to a final concentration of 24% (wt/wt), and the supernatants were stirred on ice for 20 min and centrifuged at 15,900 × *g* for 15 min. The resulting pellet was dissolved in 15 ml of buffer B (25 mM sodium phosphate [pH 7.0], 50 mM NaCl), dialyzed overnight at 4°C against the same buffer with several changes, and then clarified by centrifugation. The cleared protein solution was applied to a column (2.0 by 15 cm) of CM Sepharose CL-6B (Pharmacia) which had been equilibrated with buffer B. The column was washed with 10 column volumes of buffer B and eluted with a linear NaCl gradient of 50 to 300 mM in 25 mM sodium phosphate buffer (pH 7.0). The column profile was obtained by plotting the A_{280} of each fraction. The fractions across the peak were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The fractions containing NusG were pooled and concentrated 10- to 15-fold with a Centriprep-10 (Amicon, Beverly, Mass.). The concentrated NusG solution was dialyzed overnight at 4°C against buffer B. All of the purification steps were carried out at room temperature as described above unless specified otherwise. The protein concentration was determined with a bicinchoninic acid protein assay reagent and by the procedure recommended by the manufacturer (Pierce, Rockford, Ill.).

Expression of NusG variant proteins. Site-directed mutagenesis was used to delete the 171-amino-acid insertion within *T. maritima* NusG essentially as described previously (32). The oligonucleotide oD17 (5′-GTAGATTCTGA TCTCTTTTCTTGTTGAAACTACCTCT-TCAGGAATAACAAT-3′) used for the deletion is complementary to nucleotides 1178 to 1155 (underlined) and 1715 to 1689 (not underlined), which encode eight amino acid residues (I-38 to V-45) in the N-terminal part and nine amino acid residues (S-217 to Y-225) in the central region of NusG, respectively (19). The deletion mutant was amplified by PCR and cloned into pET-3a to produce plasmid pPD1082 as described above. The deletion mutant was reamplified from pPD1082 with T7 promoter primer and oD23 (5′-AAGGATCCTCAGTGGTGGTGGTGGTGGTGCTCGATTT TCTCCACTTCAGAAC-3′). Similarly, the region encoding the 171-amino-acid insertion was amplified from plasmid pPD1077 with primers oD24 (5′-TTGGA TCCTCAGTGGTGGTGGTGGTGATAATCCACCACTTCGACTCTTC C-3′) and oD25 (5′-AGCATATGTTGGACGCCACCAGCCCTTCC-3′). Primers oD23 and oD24 contain a complementary sequence encoding six consecutive histidine residues and a *Bam*HI recognition sequence (underlined) near the 5′ end, and primer oD25 carries an *Nde*I recognition sequence (underlined). The PCR products were cloned into pGEM-T vector (Promega), and several clones were sequenced for verification. The deletion mutant was recloned between *Xba*I and *Bam*HI sites in pET-3a, and the central domain mutant was cloned into *Nde*I and *Bam*HI sites of pET-3a; both were transformed into *E. coli* BL21(DE3) pLysS. The strains were grown in YT medium containing 100 μg of ampicillin per ml and 25 μg of chloramphenicol per ml and induced by IPTG as described above. To purify the mutant proteins, wet cell pellets (0.5 g) were suspended in 1.5 ml of 50 mM Tris-HCl (pH 8.5), 10 mM 2-mercaptoethanol (β-ME), and 1 mM phenylmethylsulfonyl fluoride and lysed by freeze-thawing. The cell extracts were recovered after centrifugation; for each, 500 μl of extract was mixed with 200 μl of Ni-nitrilotriacetic acid resin from Qiagen (50% slurry equilibrated in buffer C [20 mM Tris-HCl {pH 8.5} 100 mM KCl, 20 mM imidazole, 10 mM β-ME, 10% glycerol]) for 30 min on ice. The mixtures were centrifuged, the resin was washed three times with 1 ml of buffer C, and the variant NusG proteins were eluted three times with 200 μl of buffer D (20 mM Tris-HCl [pH 8.5], 100 mM KCl, 100 mM imidazole, 10 mM β-ME, 10% glycerol). The eluted proteins were more than 80% pure as judged by SDS-PAGE.

DNA and RNA band shift assays. Double-stranded plasmid DNA and single-stranded M13 derivatives were purified by ethidium bromide-CsCl ultracentrifugation (32). Plasmids were digested with restriction enzymes, and linear fragments were isolated from agarose gels. Table 1 lists the sizes and the origins of the duplex fragments used in the experiments described in this paper. RNA was produced by in vitro transcription from the SP6 promoter in a 20-μl reaction mixture containing 1 mM (each) ATP, GTP, and CTP, 0.4 mM UTP, 80 nM [α-^{32}P]UTP (about 10 μCi), 6 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.5 at 25°C), 2 mM spermidine, 10 mM NaCl, 20 U of RNase inhibitor, 15 U of SP6 RNA polymerase, and 15 μg of DNA template per ml. After incubation at 39°C for 1 h, an additional 15 U (1 μl) of SP6 RNA polymerase was added and incubation was continued for an additional 2 h. The reaction mixture was electrophoresed through a 5% polyacrylamide gel, and the ^{32}P-labeled RNA was located by autoradiography and soaked out of the gel slice overnight at room temperature in diethylpyrocarbonate-treated water. The amount of purified RNA was estimated by liquid scintillation counting.

For band shift assays, purified NusG protein and its mutant variants were mixed with DNA or RNA and incubated at the stated temperatures for the indicated period of time. The standard assay was 65°C for 2 h. Unless otherwise indicated, a typical buffer was 33 mM NaCl and 17 mM sodium phosphate (pH 7.0). When necessary, binding reactions were terminated by freezing in a dry-ice–ethanol bath, thawed at 0°C, mixed with 5 μl of electrophoresis loading solution (50% glycerol [vol/vol], 0.2% bromophenol blue, 0.2% xylene cyanol), and electrophoresed in either agarose or polyacrylamide gels in 0.5× Tris-

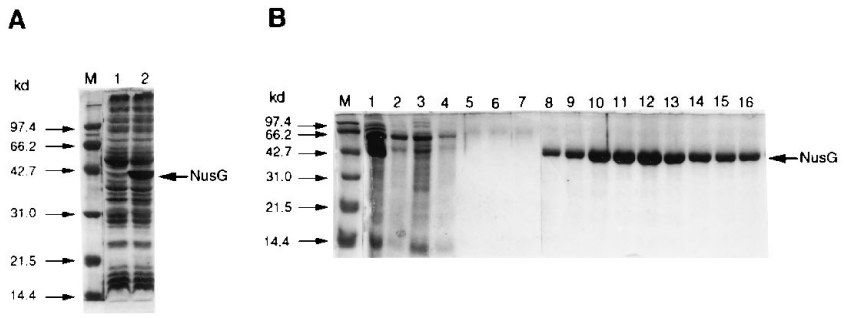

FIG. 1. Overexpression and purification of the *T. maritima* NusG protein. (A) Lysates of JM109 (lane 1) and JM109/pPD1077 grown in the presence of IPTG (lane 2) were electrophoresed on a 12% polyacrylamide–SDS gel. (B) Samples of material at various stages of NusG purification are visualized on a 16% polyacrylamide–SDS gel. Lanes: 1, supernatant after heating cell extract to 75°C for 30 min and centrifugation; 2, CM Sepharose CL-6B column flowthrough; 3 and 4, column washes with buffer B; 5 to 16, column fractions eluted with a linear gradient (50 to 300 mM) of NaCl; not all the column fractions are shown. For each gel, a molecular mass standard (M) was included. The sizes of the protein standards are in kilodaltons.

borate-EDTA. The gels were stained with ethidium bromide. When DNA 3′ or 5′ end labeled with ^{32}P or RNA labeled with ^{32}P was employed, complexes were visualized by autoradiography.

Electron microscopy. Linearized plasmid pUC18 (0.056 pmol) was incubated with NusG (5 pmol) at 37°C for 2 h in 50 μl of binding buffer (20 mM triethanolamine, 1 mM dithiothreitol [pH 7.5], various concentrations of KCl) (the normal NaCl-Na phosphate buffer interferes with electron microscopy sample preparation). The formed complexes were fixed for 15 min at 37°C with 0.2% glutaraldehyde. One-half of the assay mixture was used for agarose gel electrophoresis to confirm complex formation; the other 25 μl was used for adsorption to mica after the addition of 75 μl of binding buffer; Mg^{2+} was not used for adsorption. At higher protein concentrations, the DNA was more completely sequestered in complex, and often larger aggregates containing more than one DNA molecule was observed. Single DNA molecules with two separate sites of NusG nucleation were very seldom or never observed. Other details of the mica technique were described elsewhere (35).

Quantitation of NusG protein in *T. maritima* cell by Western blotting (immunoblotting). A culture of *T. maritima* was grown to the mid-log phase as described previously and harvested by centrifugation (15). Cell extracts were prepared in SDS lysis buffer. Serial dilutions of purified NusG were separated alongside a specified amount of *T. maritima* total cellular protein by SDS-PAGE and electrotransferred to an Immobilon membrane (Millipore). The membrane was blocked with 3% gelatin in Tris-buffered saline (20 mM Tris-HCl, 0.5 M NaCl [pH 7.5]) for 1 h and washed twice with washing buffer (0.05% Tween 20 in Tris-buffered saline). The membrane was then incubated with the antiserum against *T. maritima* NusG (diluted 1:30,000 with washing buffer containing 0.1% gelatin) for 4 h. After the membrane was washed twice with washing buffer, it was incubated for 1 h with secondary antibodies (peroxidase-conjugated goat anti-rabbit immunoglobulin G [Jackson ImmunoResearch Laboratories]), which had been diluted 1,000-fold in the washing buffer containing 1% gelatin. The membrane was washed subsequently with the washing buffer and Tris-buffered saline. The NusG protein was detected by the chemiluminescence method. The membrane was incubated in a solution containing 1.25 mM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma), 2.7 mM H_2O_2, 68 μM 4-hydroxycinnamic acid (Sigma), and 92 mM Tris-HCl (pH 8.6) and then exposed to either an X-ray film or a Bio-Rad chemiluminescence imaging screen. The amount of NusG protein in total cell extracts was determined by comparison with the calibrated standard generated with purified NusG.

P1 transduction. Genetic transduction with bacteriophage P1 was carried out essentially as described previously (34). Briefly, P1 lysate was prepared from donor *E. coli* SS294, which carries a chromosomal *nusG* that is disrupted with a kanamycin insertion and a wild-type *nusG* on plasmid SS120 (37). The wild-type *T. maritima nusG* and the deletion variant, missing the region encoding the extra 171 amino acids, were cloned into plasmid pKK223-3 (Pharmacia Biotech, Piscataway, N.J.). These plasmids along with the control plasmid pBRU, containing the wild-type *E. coli nusG*, were used to transform strain JM101. The transformants were transduced with P1. Overnight cultures from single colonies were centrifuged, resuspended in 2.5 ml of solution containing 10 mM $MgSO_4$ and 5 mM $CaCl_2$, and mixed with various amounts of the P1 lysate (2×10^9 PFU/ml). The mixtures were incubated at 30°C for 30 min without shaking. Sodium citrate (0.1 ml, 1 M) and 1 ml of growth medium containing 0.4 mM IPTG were added to the mixtures, and the mixtures were incubated without shaking at 37°C for 1 h to allow phenotypic expression. The mixtures were then centrifuged and resuspended in 0.1 ml of medium containing 0.4 mM IPTG and 20 mM sodium citrate, mixed with 2.5 ml of soft agar (45°C), and plated in medium containing 50 μg of kanamycin per ml. Transductants were grown in medium containing 50 μg of kanamycin per ml and 100 μg of ampicillin per ml.

In vitro transcription. To construct the DNA templates for the in vitro transcription assay, a 170-bp *Eco*RI-*Ava*I fragment containing a natural *T. maritima* promoter was isolated and cloned into the multiple cloning site of plasmid pGEM3-Zf(+). A 230-bp *Apa*I-*Xba*I fragment containing a *T. maritima* transcription attenuator was first cloned into pGEM11-Zf(+), and then a *Bam*HI-*Xba*I fragment with the attenuator was excised and fused into the *Bam*HI-*Xba*I sites downstream from the *Eco*RI-*Ava*I fragment in the multiple cloning site of pGEM3-Zf(+). A 93-bp fragment containing a *T. maritima* transcription terminator was obtained by PCR amplification with one primer carrying an *Xba*I site and *Hin*dIII site; the PCR product was digested with *Xba*I and *Hin*dIII and cloned downstream from the attenuator. The DNA fragments containing the promoter, attenuator, and terminator were all isolated from plasmid pPD990 containing the *secE-nusG-rplKAJL* operon of *T. maritima* (19). Template 1, containing promoter, attenuator, and terminator elements, was a 500-bp *Eco*RI-*Hin*dIII fragment, and templates 2 and 3, with only promoter and attenuator elements, were either a 430-bp *Eco*RI-*Hin*dIII fragment or a 760-bp *Pvu*II fragment.

These DNA templates (about 0.1 pmol) were incubated with 0.2 pmol (about 0.1 μg) of purified *T. maritima* RNA polymerase (a generous gift of Thomas Hermann and Hermann Heumann, Max Planck Institute for Biochemistry, Berlin, Germany) in 5 μl of buffer containing 50 mM Tris-HCl (pH 9.0 at 25°C) and 50 mM NaCl at 75°C for 10 min to form the binary complex. The binary complex was then added to a reaction mixture (preheated at 75°C for 1 min) containing 1 mM (each) ATP, GTP, and CTP, 0.4 mM UTP, 80 nM [α-^{32}P]UTP (about 10 μCi), 6 mM $MgCl_2$, 50 mM Tris-HCl (pH 9.0 at 25°C), 0.05 μg of bovine serum albumin per μl, 12.5 mM sodium phosphate (pH 7.0 at 25°C), and 37 mM NaCl (all final concentrations) in 20 μl of total reaction volume, and the mixture was incubated at 75°C for 20 min. Between 0 and 250 pmol of purified *T. maritima* NusG protein was added to transcription assays. The transcription was stopped by adding 20 μl of stop solution (0.6 M sodium acetate, 0.1 M EDTA, 0.2 mg of yeast tRNA per ml) and 60 μl of distilled water. The mixture was then extracted once with phenol-chloroform (1:1, vol/vol), and the RNA was precipitated by ethanol. The RNA was dried, dissolved in 10 μl of RNA loading buffer (90% deionized formamide, 50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue), and analyzed by electrophoresis through an 8% polyacrylamide–8.0 M urea gel.

RESULTS

The *nusG* coding region was amplified by PCR from the *T. maritima* genomic clone pPD990, cloned, sequenced to verify amplification fidelity, and finally recloned into the expression vectors pKK223-3 and pET-3a to give pPD1077 and pPD1078, respectively. In plasmid pPD1077, the *T. maritima nusG* gene is under the control of the *tac* promoter, and in plasmid pPD1078, the gene is under the control of a T7 RNA polymerase promoter. In *E. coli* strains harboring these expression plasmids, synthesis of the *T. maritima* NusG protein was tightly regulated and high-level expression was dependent on IPTG induction.

The expression of the *T. maritima* NusG protein from plasmid pPD1077 induced by the addition of IPTG to mid-log phase cells is illustrated in Fig. 1A. To purify the protein, the cell lysate was heated to 75°C for 30 min and heat-labile *E. coli* proteins were removed by centrifugation. *T. maritima* NusG was further purified by adsorption to a CM Sepharose column

and eluted with a linear gradient of NaCl. The eluted protein was essentially homogeneous as judged by SDS-PAGE (Fig. 1B). With polyclonal antibodies produced against the purified recombinant protein, we have shown by Western blotting that the single cross-reacting protein in *T. maritima* cell extracts has the same size as judged by electrophoretic mobility as the recombinant protein produced in *E. coli*. This indicates that the length of the designated *T. maritima nusG* open reading frame is correct and that the encoded protein is nearly twice the size of the NusG protein of *E. coli* (20 kDa). Antibodies raised against *E. coli* NusG cross-react with both natural and recombinant *T. maritima* NusG proteins (data not shown).

Binding activity of *T. maritima* NusG to duplex DNA. The highly purified *T. maritima* NusG bound cooperatively to double-stranded DNA nonspecifically. At salt concentrations between 5 and 300 mM NaCl, at incubation temperatures between 0 and 80°C, and at times greater than 30 s, *T. maritima* NusG formed a complex with DNA that was unable to penetrate the agarose gel matrix. Binding was not dependent on size (for molecules greater than 500 bp), base composition, or sequence. Binding to any target DNA tested was inhibited by an excess of poly(dI-dC).

To illustrate the binding cooperativity of *T. maritima* NusG to DNA, we used as targets a collection of 3′-end-labeled fragments generated by *Msp*I digestion of plasmid pPD990 (Fig. 2). The >30 fragments tested range from 9 to 1,053 bp in length. At low NusG concentrations, no DNA fragments, large or small, were shifted from their normal migration position. At intermediate NusG concentrations, larger fragments were preferentially shifted. The electrophoretic mobility shift of the three largest fragments (all greater than 500 bp) occurred simultaneously, whereas somewhat higher protein concentrations were required to shift smaller fragments. These results imply that assembly of complexes occurs most efficiently on DNA fragments greater than about 500 bp in length.

The amount of *T. maritima* NusG protein required to form a complex with DNA fragments between 0.7 and 5.2 kb in length was determined under standard assay conditions (33 mM NaCl, 17 mM sodium phosphate [pH 7.0], 65°C for 2 h) in titration experiments. Either the amount of DNA was held constant and the amount of NusG was varied or the amount of NusG was held constant and the amount of DNA was varied. To summarize, the results indicated that the transition between free DNA and complex occurs over a narrow range of protein concentrations. The critical concentration necessary for complex formation was about 30 to 40 molecules of *T. maritima* NusG per kb of DNA. At lower protein concentrations, no interaction as judged by electrophoretic mobility was detected, whereas at higher protein concentrations, all DNA was sequestered in complexes and retained at the electrophoretic origin. These complexes were compact and refractive to ethidium bromide staining. At NaCl concentrations above about 100 mM, complexes were less compact and were stained by ethidium bromide.

Accessibility of the *T. maritima* NusG-DNA complex to restriction by *Taq*I. The state of the complex between *T. maritima* NusG protein and duplex DNA was further investigated by accessibility to restriction endonuclease digestion. The restriction enzyme *Taq*I has a temperature optimum of 65°C and can efficiently cleave DNA over a wide range of salt concentrations. In this assay, parallel samples of linear DNA at 65°C in buffers containing between 10 and 150 mM NaCl were incubated in the absence or presence of *T. maritima* NusG protein (80 monomers per kb of DNA). After 2 h, *Taq*I endonuclease was added and digestion was continued for 1.5 h. One aliquot from each sample was electrophoresed directly on a standard agarose gel, and another was mixed with a small amount of SDS (0.2% final concentration) and run on a gel containing 0.2% SDS (Fig. 3). This concentration of SDS is sufficient to disrupt the *T. maritima* NusG-DNA complex so that the intact fragment or *Taq*I digestion products can be electrophoresed into the gel and visualized.

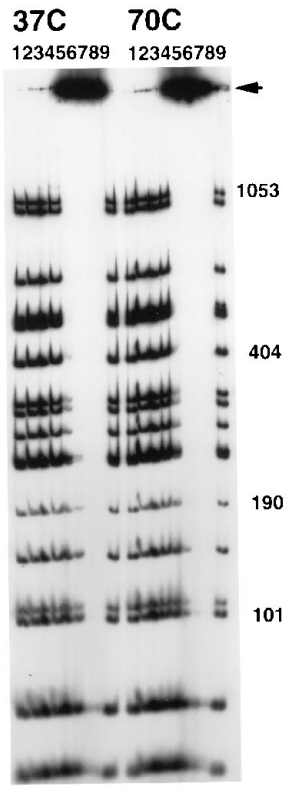

FIG. 2. Binding of *T. maritima* NusG to a ladder of 3′-end-labeled fragments. Plasmid pPD990 was digested with *Msp*I and end labeled by Klenow filling with [α-^{32}P]dCTP. Approximately 0.1 μg of this 3′-end-labeled DNA ladder was incubated with increasing amounts of purified *T. maritima* NusG protein. The buffer was 33 mM NaCl–17 mM sodium phosphate (pH 7.0), and the incubation was at either 37 or 70°C (as indicated on the top of the gel) for 20 min in a 20-μl reaction volume. The binding mixture was electrophoresed through a 6% polyacrylamide (29:1, acrylamide-bisacrylamide) gel with 0.5× Tris-borate-EDTA at 120 V for 20 h. The gel was dried and then exposed to an X-ray film. The amounts of NusG (in picomoles) are 0, 0.04, 0.08, 0.2, 0.4, 0.8, 2, 4, and 0 (lanes 1 to 9, respectively). The sizes of the fragments in the ladder are 1,053, 765, 577, 489, 457, 404, 368, 359, 328, 281, 255, 240, 190, 147, 110, 101, 65, 49, 26, 25, 22, 14, 13, 11, and 9 bp, from top to bottom, respectively (some fragments have the same length; fragments shorter than 49 bp were run off the gel). The electrophoretic origin and the length of several of the fragments are indicated.

In the native gel, it is apparent that the DNA fragment in samples not containing *T. maritima* NusG protein was efficiently restricted by *Taq*I at salt concentrations below 100 mM NaCl. At 150 mM NaCl, *Taq*I cleaved less efficiently and restriction of the DNA was incomplete. In samples containing *T. maritima* NusG protein and run on the native gel, the DNA was retained as a complex in the well because the number of monomers of protein per kilobase of DNA exceeded the critical value of about 30 to 40. When the *T. maritima* NusG-DNA complexes that had been digested with *Taq*I were dissociated with 0.2% SDS and run on an SDS gel, the DNA was protected from *Taq*I restriction by *T. maritima* NusG binding at salt concentrations below 50 mM NaCl. At salt concentrations above 50 mM NaCl, *Taq*I can digest the DNA within the NusG-DNA complex nearly to completion. At 150 mM NaCl,

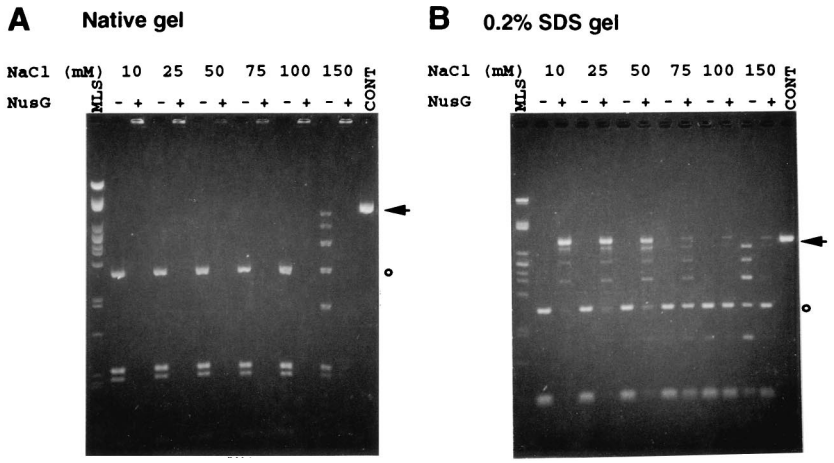

FIG. 3. Susceptibility of *T. maritima* NusG-duplex DNA complexes to restriction by *Taq*I. In parallel samples, a 3.5-kb linear DNA fragment (0.09 pmol) in 5 mM sodium phosphate buffer (pH 7.0) containing between 10 and 150 mM NaCl was prepared without or with NusG protein (1 μg; 25 pmol). The volume of each sample was 19.5 μl, and the first incubation was for 2 h at 65°C. At the end of the first incubation, a 0.5-μl mixture containing 1 U of *Taq*I endonuclease and sufficient Mg^{2+} to bring the final concentration to 3.0 mM was added to each sample. The second incubation was at 65°C for 1.5 h. (A) One aliquot from each sample was mixed with a standard loading solution and run on a standard agarose gel (1.0%). (B) A second aliquot was mixed with loading solution containing SDS (0.2% final concentration) and run on an SDS-containing 1.0% agarose gel (0.2% final concentration). The arrow to the right of each gel indicates the position of the control 3.5-kb DNA linear fragment. The 3.5-kb DNA fragment is cleaved by *Taq*I to produce a 2.0-kb fragment (indicated by a small circle to the right of each gel) and a number of other smaller subfragments.

*Taq*I was able to digest DNA in the complex much more efficiently than it digests free DNA. This result suggests that at salt concentrations below 50 mM, the DNA-protein complex is either more compact or more static and therefore resistant or inaccessible to digestion by *Taq*I endonuclease. We cannot explain the activation of *Taq*I nuclease by *T. maritima* NusG in high-salt buffer. Essentially identical results were obtained with the enzyme *Pvu*II at 37°C; at NaCl concentrations below 50 mM, NusG effectively protected DNA from *Pvu*II digestion, whereas at NaCl concentrations above 50 mM, the DNA was digested to completion (data not shown).

The effect of different salts on the *T. maritima* NusG-DNA interaction was investigated by replacing NaCl with KCl in a *Taq*I probing experiment. In KCl, the *T. maritima* NusG-DNA complex appears to be somewhat less compact than it does in NaCl; DNA molecules were completely digested by *Taq*I at KCl concentrations of 50 mM and above, whereas DNA molecules were still partially protected at NaCl concentrations as high as 75 mM (Fig. 4). At KCl concentrations below 50 mM, the DNA molecules were effectively protected from *Taq*I restriction (data not shown).

Binding activity of *T. maritima* NusG to single-stranded DNA. We then tested whether *T. maritima* NusG can bind to single-stranded as well as duplex DNA. We found that when we mixed NusG (2 μg; 50 pmol) with single-stranded M13 DNA (200 ng; 0.085 pmol) to give a ratio of about 80 monomers of protein per kb of single-stranded DNA, a complex that was retained in the well of the electrophoresis gel formed within 30 s at 65°C. When incubated for less than 30 min, the complexes were visible with ethidium bromide staining; with incubation times of greater than 30 min, the complexes could no longer be stained. The complexes were at least partially dissociated by incubation with SDS (>0.1%; data not shown).

The stoichiometry of the interaction between *T. maritima* NusG and single-stranded DNA was examined by titrating a constant amount of single-stranded M13 DNA (0.84 pmol) with various amounts of protein (1.25 to 160 pmol per assay). At a protein concentration above 20 pmol, all of the DNA was sequestered in complexes and retained at the electrophoretic origin. The protein concentration required to achieve complete complex formation was 40 pmol; this corresponds to about 66 monomers of *T. maritima* NusG per 1,000 bases of single-stranded DNA. In the reciprocal experiment, a constant amount of *T. maritima* NusG (25 pmol) was titrated with increasing amounts of single-stranded DNA. At DNA concentrations of 0.63 pmol per assay and below, virtually all of the DNA was sequestered; this corresponds to greater than 54 *T. maritima* NusG monomers per 1,000 bases of DNA (Fig. 4).

Competition between single-stranded and duplex DNA for *T. maritima* NusG binding. We investigated the preference of

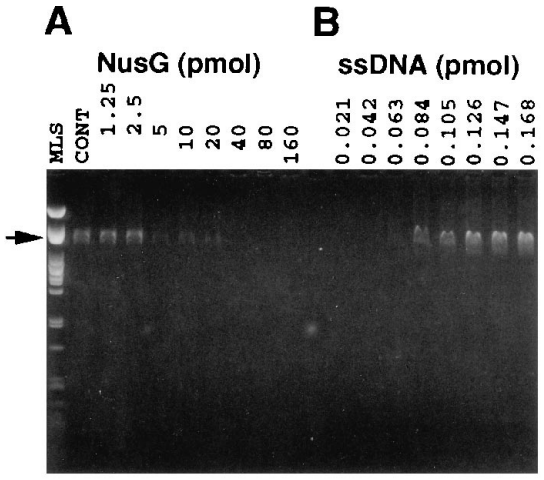

FIG. 4. Stoichiometry of *T. maritima* NusG–single-stranded DNA complexes. Assays were carried out in 15-μl volumes containing 33 mM NaCl and 17 mM sodium phosphate (pH 7.0), and incubations were for 2 h at 65°C. (A) The amount of DNA was constant (0.084 pmol), and the amount of NusG was increased from 1.25 to 160 pmol. (B) The amount of NusG protein was constant (25 pmol), and the amount of DNA was increased from 0.021 to 0.168 pmol. The position of free DNA is indicated by an arrow. The lane designated MLS is a molecular length standard (λ *Pst*I).

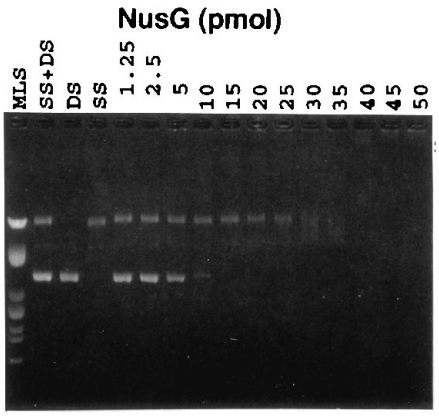

FIG. 5. Competition between single-stranded and duplex DNA for *T. maritima* NusG binding. Assays were carried out in 15-μl volumes containing 33 mM NaCl and 17 mM sodium phosphate (pH 7.0), and incubations were for 2 h at 65°C. Each assay contained 0.085 pmol of a 3.5-kb duplex DNA fragment and 0.085 pmol of single-stranded M13 DNA. The amount of NusG protein was increased from 1.25 to 50 pmol per assay (indicated at tops of lanes). After incubation, the samples were electrophoresed through a 1.0% agarose gel and stained with ethidium bromide. Lanes: MLS, molecular length standard (λ *Pst*I); SS+DS, single-stranded plus duplex DNA without protein; DS, duplex DNA without protein; SS, single-stranded DNA without protein.

NusG for single-stranded or duplex DNA in a competition experiment, where equal molar amounts of a 3.5-kb duplex DNA fragment and a 7.2-kb single-stranded M13 DNA (0.085 pmol of each) were mixed together and titrated against increasing amounts of *T. maritima* NusG protein (1.25 to 50 pmol). At a protein concentration above 10 pmol per assay, all of the 3.5-kb duplex DNA was sequestered in complexes and retained at the electrophoretic origin (Fig. 5). At the same time, at least some single-stranded DNA remained visible in the uncomplexed or partially complexed state at a protein concentration of 40 pmol per assay (Fig. 5). These results indicate that *T. maritima* NusG binds preferentially and more efficiently to duplex DNA.

Electron microscopic visualization of NusG-DNA complexes. Complexes formed at different salt concentrations between *T. maritima* NusG protein and linear DNA were examined by electron microscopy. Plasmid pUC18 (0.056 pmol) was linearized by digestion with *Sma*I and incubated with *T. maritima* NusG protein (5 pmol; corresponding to about 33 monomers per kb of DNA) for 2 h at 37°C. Linear DNA in the absence of NusG protein is shown in Fig. 6A. At 10 mM KCl, regular structures of *T. maritima* NusG with one or a few DNA molecules can be seen (Fig. 6B). In some cases, only part of the DNA was covered with protein. At 33 mM KCl, large complexes were formed (Fig. 6C). In these complexes, the *T. maritima* NusG protein was concentrated in the dark center, with thick and thin fibers of DNA extending from the core. These are presumably the tight structures containing DNA that cannot be stained with ethidium bromide and are resistant to cleavage by *Taq*I endonuclease. These large complexes closely resemble the structures formed between DNA and the histone-like protein Hc1 from *Chlamydia trachomatis* (6). At 80 mM KCl, the large compact structures were no longer apparent (Fig. 6D). Only a few sites of protein nucleation on individual DNA molecules were apparent; these structures are clearly susceptible to *Taq*I cleavage (see above). The gel patterns of *T. maritima* NusG-DNA complexes in buffer containing triethanolamine and KCl were similar to those of complexes in buffer containing sodium phosphate and NaCl (data not shown).

The electron microscopy visualizations clearly suggest that *T. maritima* NusG binding to DNA is cooperative (Fig. 6). Within any single DNA molecule, only one particular *T. maritima* NusG-protein complex (big or small) was observed; complexes appeared to be expanded from these sites of nucleation. In electron microscopy experiments carried out at 33 mM KCl, *T. maritima* NusG bound preferentially to duplex DNA (linear or circular) rather than single-stranded DNA (data not shown).

Binding activity of *T. maritima* NusG to RNA. Since *E. coli* NusG is a part of transcription elongation and termination machinery, we asked whether *T. maritima* NusG has any affinity for RNA. ^{32}P-labeled RNA was incubated with various amount of the purified *T. maritima* NusG. Similar to the DNA binding activity, *T. maritima* NusG bound to RNA cooperatively: at low protein concentrations, few RNA-*T. maritima* NusG complexes were formed, whereas at high protein concentrations, the complexes were retained at the electrophoretic origin (Fig. 7). In competition experiments, the affinities of *T. maritima* NusG RNA and DNA were comparable (data not shown).

Abundance of NusG in *T. maritima*. The abundance of the NusG-like protein in *T. maritima* was estimated by quantitative Western blotting. Binding of antibody to *T. maritima* NusG was detected by chemiluminescence and quantitated by densitometry with a phosphorimager. The amount of *T. maritima* NusG (e.g., Fig. 8, lane 8) was estimated to be 35 ng/5 μg of total protein or about 0.7% of total protein in *T. maritima*.

Binding activities of two NusG variants to RNA and DNA. Since *T. maritima* NusG has a 171-amino-acid insertion after codon 45, which is absent in other eubacterial counterparts, we wondered which domain is responsible for the observed binding activities to RNA and DNA. Two mutant variants were constructed, one which contains only the 171-amino-acid insertion, and the other which carries all residues except the insertion (we refer to these as the central domain variant and the deletion variant, respectively). Both variants carry six consecutive histidine residues (6×his tag) at their C-terminal ends; this allows rapid and efficient purification of the proteins from expressed *E. coli* cells.

Surprisingly, both purified derivatives exhibited nucleic acid-binding activities (data not shown). The affinity of each for DNA appeared to be 5 to 10 times lower than that of wild-type *T. maritima* NusG in a gel shift assay. Although a high temperature (65°C versus 37°C) appeared to enhance the binding of the deletion derivative to DNA, the binding behaviors of the two variant proteins to both DNA and RNA were remarkably similar. This suggests that both variant proteins contain a nucleic acid binding motif.

As a control, the cell pellet from the *E. coli* strain BL21(DE3)pLysS harboring only the expression vector pET-3a was used as the source of proteins; DNA binding activity was not observed in the final elution fraction from this strain. In addition, gel slices containing the DNA-protein complexes were isolated from a low-melting-point agarose gel and treated with β-agarase, and the released complexes were precipitated by acetone and analyzed by SDS-PAGE; we confirmed that the derivative proteins were present in the complexes.

Functional analysis of the *T. maritima* NusG protein. *T. maritima* is a descendant of the deepest branch within the eubacterial array; it is more divergent from *E. coli* than is virtually any other eubacterial species (1, 15). Nonetheless, it is clearly apparent that the *nusG* genes from these two organisms are evolutionarily related (19). Alignments of the two NusG proteins show 43% amino acid identity, and both are posi-

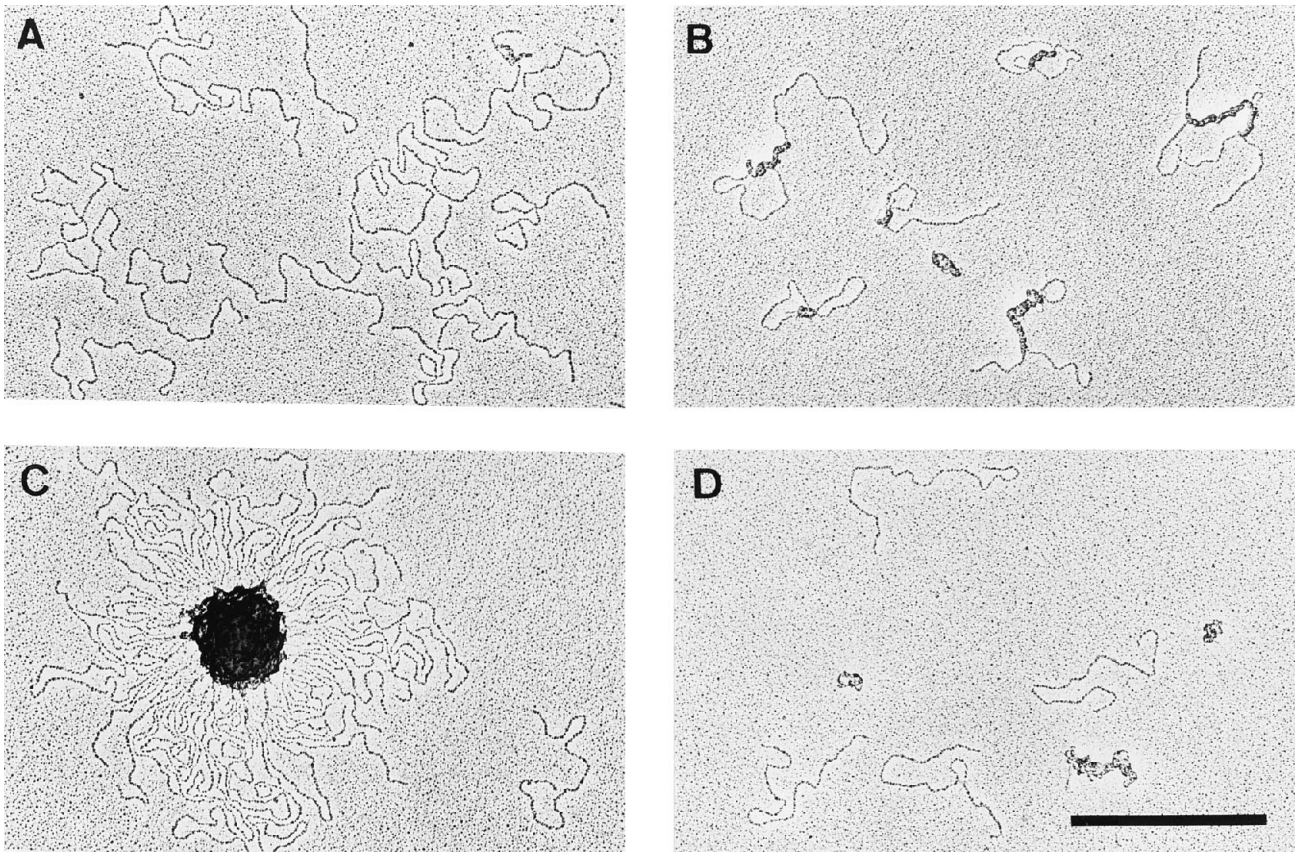

FIG. 6. Electron microscopic visualization of *T. maritima* NusG-DNA complexes at different salt concentrations. Plasmid pUC18 (0.056 pmol) was linearized by digestion with *Sma*I and visualized directly (A). The linear DNA was also mixed with purified NusG protein (5 pmol; corresponding to 33 NusG monomers per kb of duplex DNA) for 2 h at 37°C in buffer containing 20 mM triethanolamine (pH 7.5), 1 mM dithiothreitol, and either 10 mM (B), 33 mM (C), or 80 mM (D) KCl. Samples were processed as described in Materials and Methods. Magnification, ×50,000; bar, 0.5 μm.

tioned between *secE* and *rplK* within a cluster containing other essential tRNA, ribosomal protein, and RNA polymerase genes. The *T. maritima* protein has a 171-amino-acid insertion relative to other NusG proteins; however, it is uncertain whether the two types of NusG protein play functionally equivalent roles in the cell.

Neither the wild-type *T. maritima nusG* gene nor its deletion derivative was able to complement an *E. coli* strain carrying a functionally inactive chromosomal *nusG* gene. The failure to complement was not due to a failure in expression of the *T. maritima* proteins since both proteins accumulated in IPTG-induced *E. coli* cells. *T. maritima* NusG may not complement the *E. coli* protein (i) because it is inactive at the *E. coli* growth temperature of 37 to 42°C, (ii) because it is too divergent to maintain necessary molecular interaction in the heterologous system, or (iii) because it has no function in transcription elongation and termination (presumably the essential function

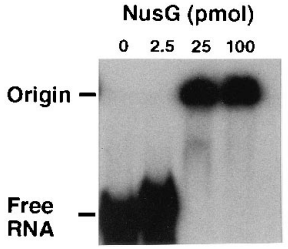

FIG. 7. RNA binding activity of *T. maritima* NusG. The ^{32}P-labeled RNA (5 pmol of a 189-nucleotide transcript that has sequence complementary to that of human U2 small nuclear RNA) was incubated with 0, 2.5, 25, or 100 pmol of NusG protein (indicated at tops of lanes) in 15-μl volumes containing 33 mM NaCl and 17 mM sodium phosphate (pH 7.0) for 30 min at 65°C. After incubation, the reaction mixtures were resolved on a 5% polyacrylamide gel and the gel was dried and exposed to an X-ray film.

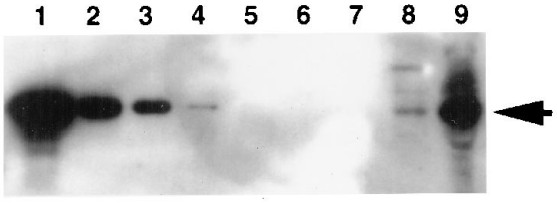

FIG. 8. Quantitation of *T. maritima* NusG protein in *T. maritima* total cell extracts. Various amounts of purified *T. maritima* NusG were separated by SDS-PAGE along with total *T. maritima* cell extract. The proteins were transferred to an Immobilon membrane, which was incubated in a solution containing polyclonal antibodies raised against purified *T. maritima* NusG. The NusG was detected with a chemiluminescence method and quantified as described in Materials and Methods. The amounts of purified *T. maritima* NusG were 1.0 μg, 0.1 μg, 50 ng, 10 ng, 1 ng, and 0.1 ng (lanes 1 to 6, respectively). The amounts of total *T. maritima* proteins loaded were 0.5 (lane 7), 5.0 (lane 8), and 50 (lane 9) μg. The signal intensity in lane 8 was between the intensities in lanes 3 and 4; the NusG amount in lane 8 was estimated to be 35 ng. Thus, the abundance for NusG was 0.7% of the total protein in *T. maritima.*

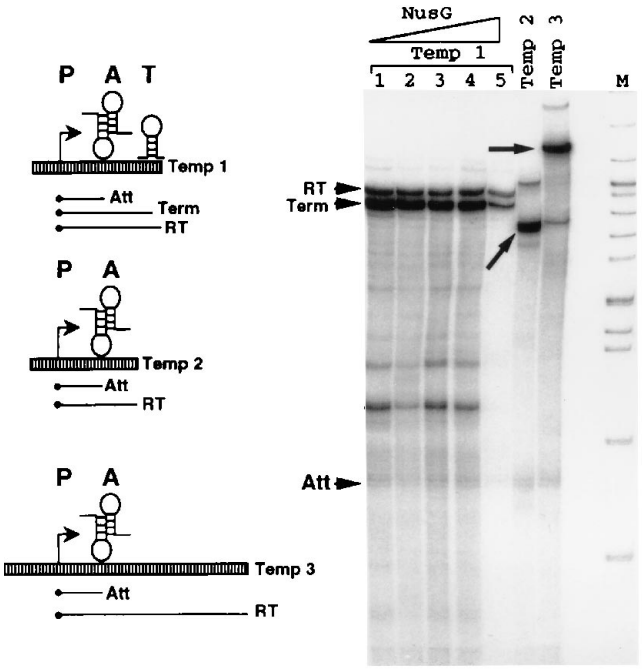

FIG. 9. Effect of purified *T. maritima* NusG protein on in vitro transcription with purified *T. maritima* RNA polymerase. The construction of recombinant DNA fragments containing authentic *T. maritima* promoter (P), attenuator (A), and terminator (T) elements and the in vitro transcription assay conditions are described in Materials and Methods. The cartoon structures of the linear templates (Temp) 1, 2, and 3 (0.50, 0.43, and 0.76 kbp, respectively) are depicted on the left. Lanes 1 to 5, transcripts produced with 0.1 pmol of template 1 DNA, 0.2 pmol of purified *T. maritima* RNA polymerase, and either 0 (lane 1), 0.25 (lane 2), 2.5 (lane 3), 25 (lane 4), or 250 (lane 5) pmol of purified NusG protein (major products are identified as the 410-nucleotide readthrough transcript initiated at the promoter and extending to the end of the fragment [RT], the 370-nucleotide transcript initiated at the promoter and terminated at the transcriptional terminator [Term], and the 160-nucleotide transcripts initiated at the promoter and terminated at the transcriptional attenuator [Att]); lanes Temp 2 and Temp 3, transcription assays without NusG by using templates 2 and 3, respectively (the arrows indicate the positions of the 340- and 560-nucleotide readthrough transcripts; transcripts terminating at the attenuator element are at the same position as they are with template 1); lane M, DNA standards of molecular length (from top to bottom) of 1,053, 765, 577, 489, 457, 404, 360, 328, 281, 255, 240, 190, and 147 nucleotides.

of the *E. coli* protein). NusG from another thermophile, *Thermus thermophilus*, was also unable to complement *E. coli* NusG despite the fact that its size (184 amino acids) and sequence are more similar to those of *E. coli* NusG than are the size and sequence of *T. maritima* NusG (13).

We investigated the effect of purified *T. maritima* NusG on in vitro transcription by purified *T. maritima* RNA polymerase (Fig. 9). The template was constructed by fusing DNA fragments containing natural promoter, attenuator, and terminator elements from the *secE-nusG-rplKAJL* operon of *T. maritima*. In vivo, the attenuator functions with an efficiency of about 50% and the terminator functions with an efficiency of nearly 100% (19). With this linear 0.5-kbp template DNA fragment, transcripts initiated efficiently at the natural promoter sequence and terminated at low efficiency at the transcription attenuator (<5%), at high efficiency at the terminator (about 65%), or at the end of the linear DNA template (about 30%) (Fig. 9). In addition, there appeared to be a relatively high background of nonspecific transcription termination along the entire template and some initiation from the end of the fragment. The addition of low concentrations (0.0125 to 1.25 μM) of *T. maritima* NusG protein had little or no effect on in vitro transcription. In contrast, the addition of 12.5 μM *T. maritima* NusG appeared to preferentially reduce the level of nonspecific or abortive termination but did not appreciably affect the ability of RNA polymerase to utilize or ignore natural attenuator and terminator sequences. As a control, two other linear DNAs with the natural terminator removed were used as a template for in vitro transcription. Both exhibit weak termination at the attenuator; because of the absence of the terminator, the predominant product is the runoff transcript.

DISCUSSION

There is strong biochemical and genetic evidence to suggest that, in *E. coli*, Nus factors form a multimeric complex with core RNA polymerase during transcriptional elongation (for reviews, see references 7 and 29). The complex is endowed with the ability to read through both factor-dependent and factor-independent transcription termination signals. An important component of this complex is NusG; it binds to and interacts with both core RNA polymerase and rho factor. The interaction with rho appears to stimulate rho-mediated transcriptional termination (17, 18, 21, 37).

In *E. coli*, the *nusG* gene is essential for cell viability; although the in vivo depletion of NusG appears to affect only rho-mediated termination, it remains to be established whether this is the essential function of NusG (9, 37). The gene encoding NusG is located in the *secE-nusG* bicistronic operon, adjacent to the *rplKAJLrpoBC* operon. Nuclease S1 protection assays indicate that the *secE-nusG* transcripts are about 5- to 10-fold less abundant than the ribosomal protein encoding transcripts (8, 9). With a quantitative Western blotting assay, Li et al. estimated that there are about 10,000 copies of NusG per cell (18). This value is about equal to the number of core RNA polymerase molecules and about fivefold below the number of ribosomes per cell (3).

Functional analysis of the *T. maritima* NusG protein. In *T. maritima*, the *secE* and *nusG* genes are cotranscribed with the downstream *rplKAJL* ribosomal protein genes (19). If the *nusG* cistron is efficiently translated, the stoichiometry of NusG and ribosomes should be about 1:1, and the protein is likely to be in substantial excess over the amount of core RNA polymerase (3). With a quantitative Western blotting assay (Fig. 8), we estimated the abundance of *T. maritima* NusG to be 0.7% of the total protein, a value 5- to 10-fold higher than that in *E. coli*. If the genome size and composition of a *T. maritima* cell are similar to those of a typical *E. coli*, there may be as many as 50,000 copies of NusG per cell; this would correspond to about five monomers per 1,000 bp of DNA.

Our results indicate that *T. maritima* NusG protein binds cooperatively to DNA and RNA in vitro. There appears to be no nucleic acid sequence specificity for the binding. Under the same ionic conditions, *E. coli* NusG fails to bind DNA as measured by our standard gel mobility shift assay. The *T. maritima* NusG protein binds to all DNA molecules tested, and the target DNA is released from the protein-DNA complex by an excess amount of the homopolymer poly(dI-dC). Binding occurs most efficiently on fragments greater than 500 bp in length.

Under the standard conditions of complex formation (33 mM sodium chloride, 17 mM sodium phosphate, 65°C for 2 h, 80 monomers of NusG protein per kb of DNA), DNA in the complex is resistant to cleavage by *Taq*I restriction endonuclease (Fig. 4). This means that either the DNA is completely sequestered by the *T. maritima* NusG protein or that the topology is altered in such a way that the restriction enzyme

cannot efficiently bind to, scan, or cleave the DNA at target sites.

Attempts were made, with DNase I, to footprint *T. maritima* NusG complexes on DNA; no specific protection was observed. Electron microscopic analysis indicates that within a single DNA molecule, only a single randomly positioned *T. maritima* NusG nucleation site occurs. These observations suggest that DNA sequences not in direct contact with *T. maritima* NusG protein are susceptible to DNase I cleavage. Since the position of nucleation is different from one molecule to the next, no distinct NusG footprint can be detected.

A number of observations suggest that the binding of *T. maritima* NusG protein to DNA is a cooperative process. First, there is a critical *T. maritima* NusG protein concentration (about 30 to 40 monomers per kb of target DNA) that must be exceeded to form complexes with DNA. Second, within any DNA molecule, only a single *T. maritima* NusG DNA complex was observed by electron microscopy. This means that additional binding of *T. maritima* NusG on the DNA occurs preferentially or exclusively at the site already bound. We do not know whether the form of *T. maritima* NusG protein that binds to DNA is monomeric or multimeric.

Proteins with generalized DNA binding activity. Two different low-molecular-weight histone-like proteins associated with the nucleoid of *E. coli* have been purified and characterized (reviewed in reference 10). The first, protein HU, is highly basic, resembling eukaryotic histones, and plays an important role in maintaining chromosome structure and superhelicity (5, 31). The HU protein present in 20,000 to 100,000 copies per cell binds DNA more tightly than RNA and single-stranded DNA more strongly than double-stranded DNA.

The second, protein H1, is neutral rather than basic and binds as a dimer once every 400 bp to duplex DNA. The abundance of H1 in the bacterial nucleoid is second only to that of HU (estimated to be about 20,000 copies per cell) (for a review, see reference 15). Mutations in the *osmZ* gene encoding the H1 protein are pleiotropic and influence the expression of many genes in a nonuniform fashion, presumably by affecting superhelicity and overall topology of the nucleoid (16). Both HU and H1 generally inhibit transcription by *E. coli* RNA polymerase; however, they can also enhance transcription of certain templates (10, 14).

At the present time, it is uncertain whether the NusG protein of *T. maritima* plays a role similar to that of HU and H1 in maintaining the structure of the nucleoid in this hyperthermophilic eubacterium. However, there is a close resemblance between *T. maritima* NusG-DNA complexes and the complexes between the histone-like protein Hc1 from *C. trachomatis* and DNA (6). Preliminary electron microscopic results indicate that *T. maritima* NusG protein induces topological ordering and condensation within the DNA (20a). This topological reordering may contribute in part to the resistance of the DNA to *Taq*I digestion.

Structural architecture of eubacterial NusG proteins. The majority of eubacterial NusG proteins are structurally conserved 20-kDa proteins that presumably play an essential and specialized role in rho-mediated transcription termination. The two structural exceptions to this pattern are the NusG proteins from *T. maritima* and *Streptomyces virginiae* (19, 22, 26). Unlike other NusG homologs, the *S. virginiae* protein contains an extra 125 amino acids at its amino terminus. The unique amino-terminal domain has a high proportion of acidic residues (35%) and contains four copies of the tetrapeptide Glu-Glu-Ala-Ala. It was suggested but not demonstrated that this acidic domain, absent from other NusG proteins, is the receptor for butyrolactone, an autoregulator for antibiotic production.

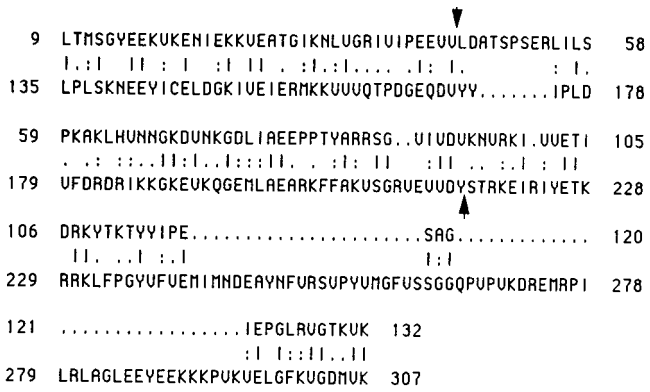

FIG. 10. Putative duplication within *T. maritima* NusG. The sequences between amino acid residues L-9 and K-132 and between L-135 and K-307 of *T. maritima* NusG, believed to be the result of an ancient duplication, are aligned with each other. The boundaries of the 171-amino-acid deletion present in the *T. maritima* deletion variant (and other eubacterial NusG proteins) are indicated by the arrows after V-45 and before S-217.

The *T. maritima* NusG protein contains a unique insertion of 171 amino acids after residue 45 that is absent from all other characterized eubacterial NusG homologous proteins (19). Examination of the sequence of the *T. maritima* NusG indicates that the segments between amino acid residues L-9 and K-132 and between L-135 and K-307 are the result of a tandem duplication event (Fig. 10). The two segments exhibit 33.3% identity and 53.8% similarity. The large number of gaps in the alignment imply that the duplication was an ancient event. In the *E. coli* and other eubacterial NusG proteins (and in the deletion variant of the *T. maritima* NusG that we constructed), only the N-terminal portion of the first repeat (up to and including V-45) and the C-terminal portion of the second repeat (beyond S-217) are retained. Surprisingly, both the deleted form of the *T. maritima* NusG lacking this insert and the insert itself were able to bind to DNA. It appears, therefore, that the nucleic acid binding activity of NusG has multiple components dispersed within the protein sequence.

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