Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9401–9405, December 1988
Biochemistry

NifA-dependent *in vivo* protection demonstrates that the upstream activator sequence of *nif* promoters is a protein binding site

(nitrogen fixation/DNA-binding proteins/positive control)

ENRIQUE MORETT AND MARTIN BUCK

Agricultural & Food Research Council, Institute of Plant Science Research, Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ, United Kingdom

Communicated by Boris Magasanik, August 29, 1988

ABSTRACT **Primer-extension analysis of the *Klebsiella pneumoniae nifH* promoter was used to determine changes in the accessibility of the promoter DNA to methylation after exposure of growing cells to dimethyl sulfate. Four guanine residues present in the *nifH* upstream activator sequence (UAS), the proposed NifA binding site, were protected from methylation and two guanine residues were hypermethylated when the transcriptional activator protein NifA was present in the cells. The interaction detected at the *nifH* UAS was independent of the alternative σ factor NtrA required for transcription of the *nifH* and other *nif* promoters. Mutations within the *nifH* UAS that diminish NifA-dependent transcriptional activation reduced the interaction at the UAS. It seems likely that the pattern of methylation protection observed in the *nifH* UAS is the result of NifA binding.**

The nitrogen fixation (*nif*) promoters of *Klebsiella pneumoniae* are among a class of promoters that require the σ factor encoded by the *ntrA* gene for expression (1, 2). The product of *ntrA* (NtrA, σ^{54}, also RpoN) is required for recognition of the −12,−24 consensus sequence characteristic of *ntrA*-dependent promoters (refs. 3–5, see Fig. 1). To date, all such promoters are positively activated. The positive activator protein specifically required for activation of *nif* genes is encoded by *nifA*. NifA has been proposed to bind the upstream activator sequence (UAS), identified in *nif* promoters from a variety of diazotrophic bacteria that are subject to activation by NifA (ref. 6, see Fig. 1). The UAS is located upstream (typically >100 base pairs) from the start site of transcription and can function when placed as far as 2 kilobases upstream (6). Transcriptional activation appears to involve the formation of a DNA loop between the UAS and downstream sequences, possibly to bring NifA bound upstream into the vicinity of the downstream RNA polymerase–NtrA complex (7).

Expression of *nifA* is positively controlled by the general nitrogen control protein NtrC, activating transcription of the *nifLA* promoter under conditions of nitrogen limitation (1, 2, 5). NifA and NtrC are functionally and structurally very similar. Each is predicted to have a helix-turn-helix super secondary structure within its C terminus, the proposed DNA-binding domain (8). Mutational analysis of the *K. pneumoniae* NtrC protein strongly supports this proposal (9). *In vitro* studies with purified NtrC have shown that NtrC binds to specific sites on two of the promoters that it activates. These are the *glnAp2* (10–12) and the *nifLA* (13, 14) promoters. Binding sites for NtrC are located upstream of the start of transcription in the *glnAp2* and *nifLA* promoters and it seems probable that activation of transcription at these promoters may require the formation of a DNA loop. NtrC in its phosphorylated and active form was shown to catalyze open complex formation at the *Escherichia coli glnAp2* promoter (10), indicating that this step limits expression from NtrA-dependent promoters when the activator is absent.

Mutational analysis of the *nifH* UAS has supported the suggestion that the $TGTN_{10}ACA$ motif (where N is any nucleotide), which characterizes the UAS of *nif* promoters, is a NifA binding site (15). We have now examined the occupancy of this site and mutant variants of it during transcriptional activation of the *nifH* promoter and conclude that the UAS is the site at which a protein, probably NifA, binds.

Table 1. Bacterial strains and plasmids

	Relevant genotype or characteristic(s)	Ref.
E. coli		
71–18	*ntr*$^+$ *lacI*^{q}	16
ET8000	*ntr*$^+$	17
ET8045	*ntrA*::Tn*10*	17
Plasmids		
pMB1	*nifH–lacZ* translational fusion, Cb^R	18
pRT22	pACYC177 based *nifH–lacZ* translational fusion, CA^R	19
pMB2	pMB1 with G → A at position −13	18
pMB52	pMB1 with C → T at position −12	18
pMB340	pMB1 with ΔT at position −18	20
pMB752	pMB1 with G → T at position −136	18
pMB86022	pMB1 with C → A at position −123	15
pMB131	pMB1 with ΔG at position −131	15
pSMM1	UAS alone	6
pMJ160	*nifA* expressed from *lac* promoter in pEMBL8, Cb^R	This paper
pMJ220	*nifA* expressed from *lac* promoter in pACYC184-based pMD220 vector, CA^R	This paper
pMJ221	pJM220 but Tyr-512 replaced by Phe in C terminus of NifA	This paper

Cb^R, carbenicillin resistance; CA^R, chloramphenicol resistance.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. These are listed in Table 1. An *Nru* I–*Acc* I restriction fragment spanning *nifA* and encoding 23 amino acids of *nifL* upstream of *nifA* and terminating 84 base pairs downstream of the *nifA* stop codon was used in the construction of plasmids pMJ160 and pMJ220.

Growth Medium. Bacteria were cultured in 2× YT to which the appropriate antibiotics [chloramphenicol (15 μg/ml) and carbenicillin (200 μg/ml)] were added to maintain the plasmids. Isopropyl β-D-thiogalactopyranoside was added (2 mM) to induce expression of *nifA* from the *lac* promoter. In all cases, except when pRT22 provided the *nifH* promoter,

Abbreviation: UAS, upstream activator sequence.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

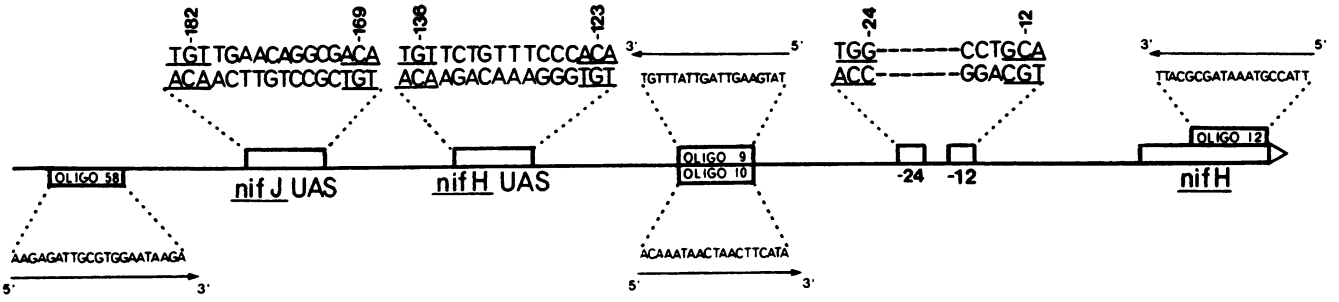

FIG. 1. Structure of the *nifH* promoter and oligonucleotide priming sites. The positions and sequences of the *nifJ* UAS, *nifH* UAS, and −12,−24 promoter element are shown. Oligonucleotides 9 and 12 were used as primers for the analysis of piperidine cleavage products corresponding to the top strand of the *nifJ* and *nifH* UASs and the *nifH* −12,−24 promoter element, respectively; oligonucleotides 58 and 10 were used to analyze the piperidine cleavage products corresponding to the bottom strand of the *nifJ* and *nifH* UASs and the −12,−24 promoter element, respectively.

nifA was expressed from pMJ220. For methylation experiments, 0.5 ml of a fully grown culture was added to 50 ml of medium in a 250-ml flask and bacteria were grown with shaking at 29°C (as transcriptional activation of *nif* promoters is temperature sensitive) until an A_{600} of 0.6–0.7 was reached.

DNA Methylation. Cultures (A_{600} of 0.6–0.7) were made 0.1% in dimethyl sulfate by the addition of 5 ml of fresh 1% dimethyl sulfate in saline phosphate (150 mM NaCl/40 mM K_2HPO_4/22 mM KH_2PO_4, pH 7.2. Cells were incubated for 3 min, rapidly collected by filtration on glass-fiber prefilters (AP15, Millipore), and washed twice with 150 ml of saline phosphate. Cells were recovered (typically 70%) by gently shaking the filters in 15 ml of saline phosphate followed by centrifugation. For the preparation of *in vitro* methylated DNA, 25 ng of pMB1 was incubated with 0.5% dimethyl sulfate at 20°C for 2 min and recovered by ethanol precipitation.

Primer-Extension Analysis. Plasmid DNA was isolated by alkaline lysis, briefly treated with RNase A, and repeatedly phenol-extracted. DNA isolated from a 50-ml culture was dissolved in 10 μl of 1 M piperidine, cleaved at methylated guanine residues by heating at 90°C for 30 min, lyophilized three times from 20 μl of H_2O, and dissolved in 36 μl of 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. To 6 μl of cleaved DNA ≈0.1 pmol of oligonucleotide primer (Fig. 1) 5′ labeled with [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) was added to a final volume of 10 μl in 10 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$. This mixture was boiled for 2 min then plunged into ice. The primer-cleaved DNA was used in a 12-μl extension reaction mixture containing 0.5 mM of each dNTP and 0.5 unit of the Klenow fragment of DNA polymerase I in 10 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$. The reaction was initiated at 37°C and continued for 20 min before termination by the addition of 4 μl of a formamide dye mixture. Samples were analyzed on sequencing gels, autoradiograms were scanned with an LKB Ultrascan model XL densitometer, and the peak area for each individual band was determined. The ratio of band peak area without NifA to that with NifA was calculated and plots were made of the natural logarithm of this quotient (21).

RESULTS

NifA-Dependent Methylation Protection of the *nifH* UAS. Fig. 2 shows the pattern of cleavage products obtained from *nifH* promoter DNA after exposure of cells harboring a multicopy *nifH* promoter plasmid to dimethyl sulfate. The primer-extension strategy used to visualize the cleavage products is illustrated in Fig. 1 (22). In the presence of NifA, cleavage products corresponding to guanine residues at position −136 of the top strand and at positions −123, −126, and −127 of the bottom strand (see Fig. 1) were clearly less abundant than when NifA was absent from the cells (Fig. 2 *A* and *B* and summarized in Fig. 4*A*). We interpret this to mean that when NifA is present in the cells a protein binds to the UAS and protects these guanine residues from methylation at the N-7 position. Hypermethylation of guanines at positions −125 and −133 of the UAS bottom strand was observed in the presence of NifA. Protection of the guanine at position −131 of the top strand was not seen in any experiment, consistent with the observation that mutations at this position of the UAS are silent (15). We observed (Fig. 2 *C* and *D*) no NifA-dependent change in the accessibility of bases in the −12,−24 region of the *nifH* promoter to dimethyl sulfate, the sequence that is recognized by RNA polymerase–NtrA (3–5, 23), or other significant changes in the pattern of methylation of the promoter DNA (see Fig. 4*B*). Measurements of β-galactosidase confirmed that the *nifH* promoter was being actively transcribed in these experiments (data not shown).

NifA-Dependent Protein Binding to the *nifJ* UAS. The UAS believed to be that of the *nifJ* promoter that is divergently transcribed with respect to *nifH* (24) is located on the *nifH* promoter plasmids pMB1 and pRT22 at position −169 with

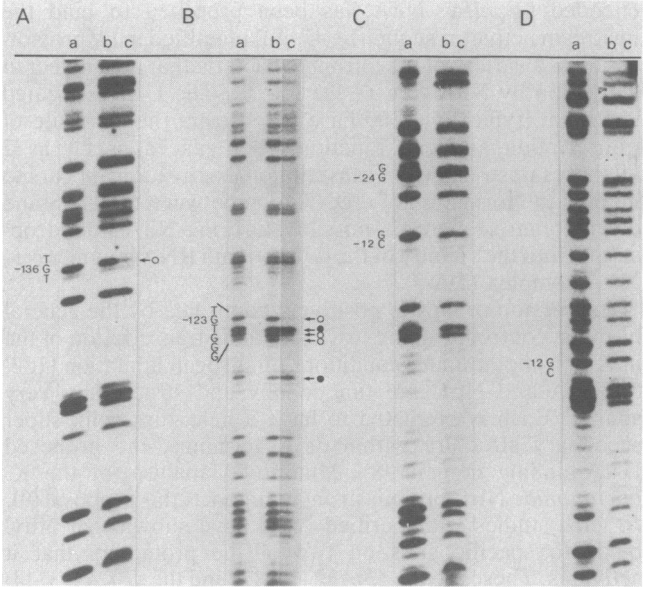

FIG. 2. NifA-dependent methylation protection and enhancement of the *nifH* UAS and −12,−24 *nifH* promoter element. Methylation was performed *in vitro* in the absence of proteins (lanes a) or *in vivo* when NifA was absent (lanes b) or present (lanes c). The arrows indicate guanine residues protected (○) or hypermethylated (●) when NifA was present in the cells. (*A* and *B*) Primer-extension products corresponding to the *nifH* UAS, top and bottom strands respectively. (*C* and *D*) Primer-extension products of the *nifH* −12,−24 promoter element, top and bottom strand, respectively.

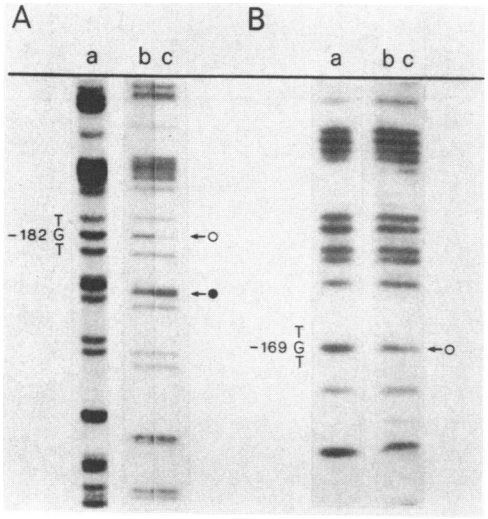

FIG. 3. NifA-dependent protein interaction at the *nifJ* UAS. Plasmid DNA with the *nifJ* UAS sequence was exposed to dimethyl sulfate *in vitro* (lanes a) or *in vivo* when NifA was absent (lanes b) or present (lanes c) in the cells. After methylation DNA was cleaved and the primer-extension products corresponding to the *nifJ* UAS top (*A*) or bottom (*B*) strand were analyzed. Guanine residues at which the most consistent NifA-dependent changes in methylation were observed are indicated by arrows. Both protection (○) and enhanced methylation (●) were detected. Consistently protected guanine residues were located in each TGT motif of the *nifJ* UAS half-site.

respect to the start site of *nifH* transcription (Fig. 1). The downstream *nifJ* promoter sequences are absent from both plasmids. We examined the *in vivo* methylation of guanine residues in the *nifJ* UAS in the presence and absence of NifA. Results (Fig. 3) demonstrate NifA-dependent protection of the guanines at positions −182 of the top strand and −169 of the bottom strand, corresponding to the guanines of the TGT motif of the *nifJ* UAS half-sites (Fig. 1). Hypermethylation of the guanine at position −174 on the top strand was observed. Densitometric analysis of the autoradiograms shows that the protection was not as marked as with the *nifH* UAS (Fig. 4*C*).

Modification of the Proposed DNA-Binding Domain of NifA. Protection of the *nifH* and *nifJ* UASs from methylation was not observed when a mutant form of NifA was present in the cells (Fig. 5). Substituting phenylalanine for tyrosine in the second helix of the helix-turn-helix motif of NifA, the predicted recognition helix (8), clearly diminishes NifA-dependent protein binding to the UAS. No protection of the *nifH* UAS was observed when cells were grown at 37°C, consistent with the observation that activation of the *nifH* promoter is greatly diminished at 37°C (data not shown).

Requirements for NifA-Dependent Protein Binding to UASs. Transversions in the guanine at position −136 or cytosine at position −123 or deletion of the guanine at position −131 of the UAS reduce activation of the *nifH* promoter by NifA (15). The former two mutations are in the half-site TGT motif, but in different half-sites. The latter mutation alters the spacing between the two half-sites from 10 to 9 base pairs. In all three cases NifA-dependent protein binding at the mutant UASs was found to be greatly diminished, as judged by their reduced protection from methylation (Fig. 6). Therefore, the reduced ability of NifA to activate *nifH* promoters with transversions at positions −136 or −123 or lacking the guanine at position −131 correlates with a diminished occupancy of the UAS.

Previous work had suggested that NifA binds to the UAS and that binding not only was dependent upon the integrity of the UAS but also required that the downstream sequences were present in cis (6, 18, 25). These conclusions are based on the observation that the *nifH* promoter, when present on

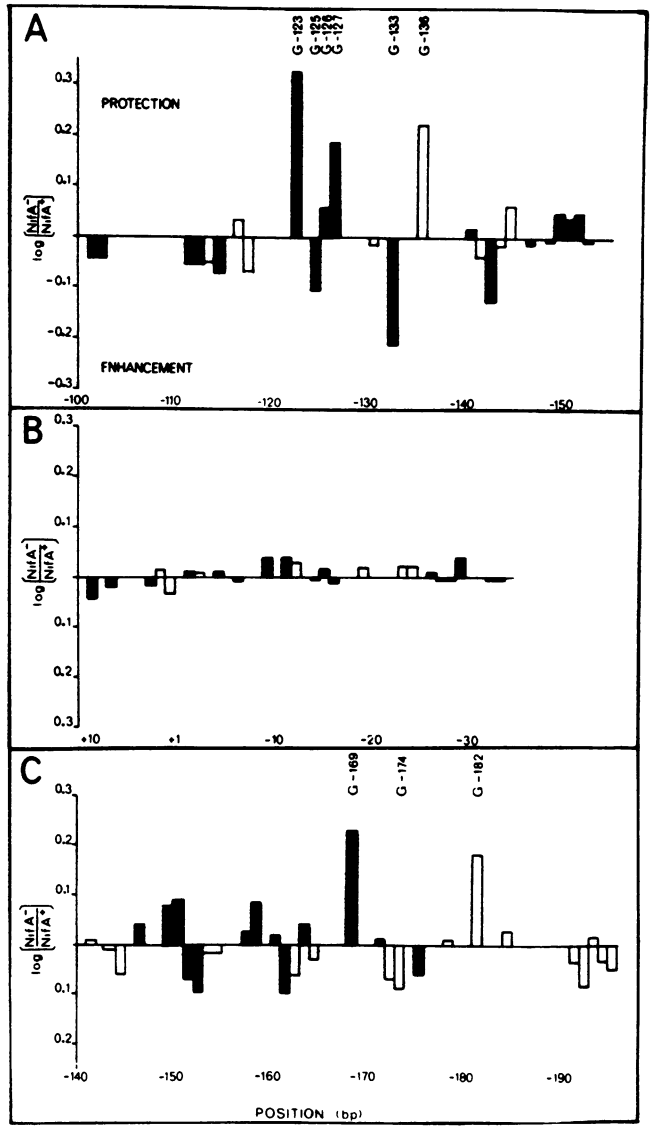

FIG. 4. Quantitative analysis of the reactivity of UASs to dimethyl sulfate in the presence and absence of NifA. The autoradiograms shown in Figs. 2 and 3 were densitometrically scanned. Each bar corresponds to a guanine residue and is plotted against its position in the primary promoter sequence. Positive values indicate methylation protection; negative values indicate enhancement of reactivity. Open bars indicate top-strand guanines; solid bars indicate bottom-strand guanines as defined in Fig. 1. The most consistent changes in the methylation pattern are indicated and were observed in at least three independent experiments. (*A*) *nifH* UAS. (*B*) *nifH* downstream promoter element. (*C*) *nifJ* UAS. The most protected guanine residues in the UASs were in the highly conserved TGT half-site motif. No consistent *nifA*-dependent change in the reactivity of the *nif* downstream promoter sequences to dimethyl sulfate was detected (*B*).

a high copy number plasmid, prevents expression of chromosomal *nif* genes. This phenomenon, called multicopy inhibition, is believed to result from the titration of NifA by the plasmid-borne promoter sequences making NifA unavailable to activate chromosomal *nif* genes. Multicopy inhibition is not displayed by *nifH* plasmids in which either the UAS is mutated or the conserved bases of the −12,−24 sequence are changed or if NifA is overexpressed (15, 18, 25). Plasmids bearing the UAS alone do not cause multicopy inhibition (6).

To examine the cis requirement for multicopy inhibition further, we examined *in vivo* NifA-dependent protein interactions at the *nifH* UAS when the conserved guanine at position −13 was mutated to adenine, the conserved cytosine at

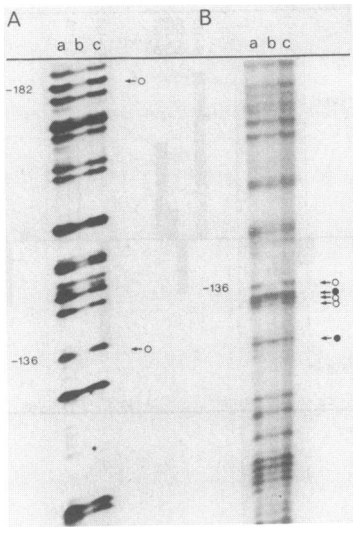

FIG. 5. Influence of the NifA Phe-512 mutant protein upon methylation protection of the *nifH* and *nifJ* UASs. Primer-extension products after the hybridization of the oligonucleotide 9 (*A*) (*nifH* and *nifJ* UASs top strand) or 58 (*B*) (*nifH* UAS bottom strand) to *in vivo* methylated pRT22 DNA are shown. Protection (○) and hypermethylation (●) of the UASs dependent upon wild-type (wt) NifA is not seen with a mutant (Phe-512) in which tyrosine has been replaced by phenylalanine in the NifA DNA-binding domain.

position −12 was mutated to thymine and the base at position −18 was deleted, all downstream mutations that relieve multicopy inhibition (18, 24). The NifA-dependent protection of the four UAS guanine residues observed with the wild-type promoter was seen with each of the three downstream promoter mutants analyzed. Results obtained with the transition at position −13 are shown in Fig. 7*b*. NifA-dependent methylation protection of the UAS was also observed when the −12,−24 region was absent (Fig. 7*c*) and in a *ntrA*⁻ mutant, a background in which the downstream sequences would not be participating in transcription (Fig. 7*d*).

Downstream Sequence Interactions in the *nifH* Promoter. RNA polymerase–NtrA is known to bind to the −12,−24 region of the NtrA-dependent *glnAp2* promoter to form a closed promoter complex (10, 23, 26). Therefore, an interaction with the *nifH* −12,−24 sequences is also anticipated. However, no differences in the methylation susceptibility of the guanine residues of the *nifH* downstream promoter element were observed when NtrA was either absent from cells, expressed from its chromosomal gene, or overexpressed (data not shown). This could reflect relatively weak binding of RNA polymerase–NtrA to the *nifH* promoter.

DISCUSSION

Results obtained with the *in vivo* methylation protection experiments have clearly demonstrated that the *nifH* UAS is a protein-binding site, occupied only when NifA is present. Occupancy of the UAS correlates with activation of the *nifH* promoter by NifA. Although unequivocal demonstration that NifA binds the UAS requires analysis of the interaction of purified NifA with the UAS *in vitro*, the following evidence supports this suggestion. (*i*) Multiple copies of the *nifH* promoter titrate NifA and mutations in the UAS relieve this effect (15, 25). (*ii*) NifA shows extensive structural homology to the characterized DNA binding and positive activator protein NtrC (8) and is, therefore, likely to function in a similar mode [when NtrC binding sites in the *nifLA* promoter are replaced with the *nifH* UAS, this hybrid promoter titrates NifA and is activated by NifA (6)]. (*iii*) If NifA were to activate transcription of the gene for the UAS-binding protein, and no evidence for such a gene exists, its expression is anticipated to be NtrA-dependent. However, protein-binding to the UAS was shown to be independent of NtrA. (*iv*) The UAS is protected from methylation when the C terminus of NifA is present in cells, although this truncated form of NifA is not adequate to activate transcription (unpublished data).

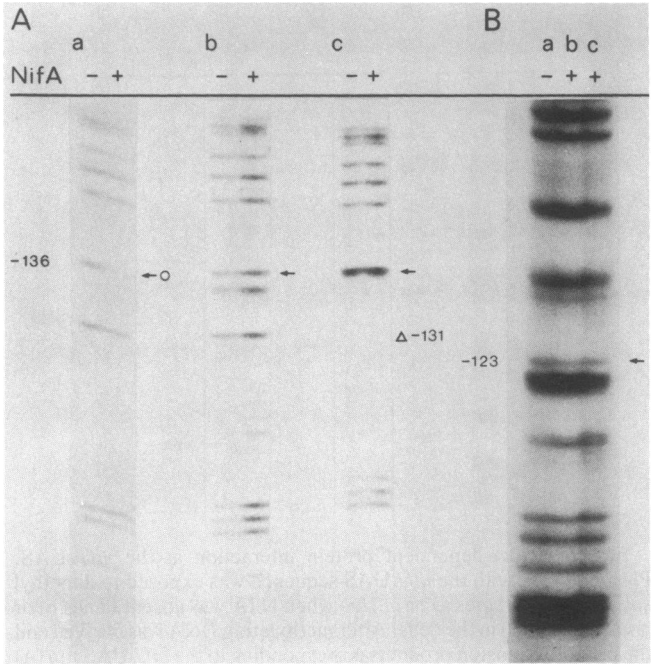

FIG. 6. Sequence requirements for NifA-dependent protein binding to the *nifH* UAS. Methylation protection experiments were carried out *in vivo* in the absence (−) or in the presence (+) of NifA. (*A*) Primer-extension products corresponding to the top strand of the *nifH* UAS. Lanes: a, wild-type promoter (pMB1); b, mutant UAS bearing a transversion at position −123 (pMB86022) (the termination product below the guanine at position −136 is not guanine-specific and sometimes appears as an extension product); c, mutant UAS deleted for the guanine at position −131 (pMB131). (*B*) Primer-extension products corresponding to the bottom strand of the *nifH* UAS. Lanes: a and b, wild-type promoter; c, mutant UAS with transversion at position −136 (pMB752). The arrows indicate guanines of the intact UAS TGT motif.

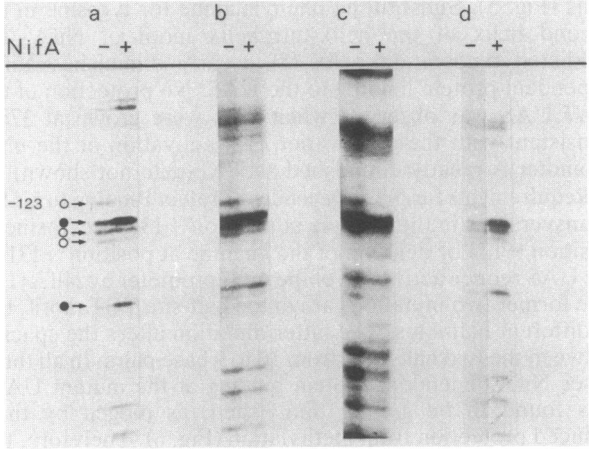

FIG. 7. Protein interaction at the *nifH* UAS is independent of the downstream promoter element. Growing cells with (+) or without (−) NifA were exposed to dimethyl sulfate and, after purification and cleavage, the plasmid DNA was hybridized with oligonucleotide 58 to analyze the bottom strand of the *nifH* UAS. (*a*) Wild-type promoter (pMB1). (*b*) Mutant promoter with a transition at position −13 (pMB2). (*c*) Mutant promoter deleted of sequences downstream of the UAS (pSMM1). (*d*) Wild-type promoter (pMB1) but assayed in a *ntrA*⁻ background.

Consistent with the postulate that the twofold rotational symmetry of the UAS is important for NifA binding (15), guanine residues in both half-site TGT motifs of the *nifH* and *nifJ* UASs were protected. Guanine-5 and -6 of one half-site of the *nifH* UAS were also protected. It is possible, therefore, that the sequence 5'-TGTNGG from position 1 to position 6 constitutes a UAS half-site to which NifA might bind well, given that the consensus $TGTN_{10}ACA$ is maintained. All *K. pneumoniae* UASs identified do in fact possess either one or both guanines at positions 5 and 6 in one or both half-sites (6). NifA sequences from *Rhizobium meliloti* (27), *Rhizobium leguminosarum* (28), *Bradyrhizobium japonicum* (29), and *Azotobacter vinelandii* (30) are available, and all show the C-terminal DNA-binding motif (8). The surface-exposed amino acids of the α-helix, which is predicted to be the recognition helix (31), are well conserved among these NifAs indicating that similar interactions to those identified in this paper and believed to be due to *K. pneumoniae* NifA could be established between other NifA proteins and their respective UASs. In support of this conclusion is the observation that the UASs from other diazotrophs also conform to the sequence $TGTN_{10}ACA$ often having guanines at positions 5 and/or 6.

Analysis of a number of prokaryotic DNA-binding proteins and their binding sites has led to a model in which bases 2, 4, and 5 of the recognition sequence half-site make specific contacts with the recognition helix (31). Guanine-4 in the *nifH* UAS half-site became hypermethylated rather than protected from methylation in the presence of NifA. This indicates that the N-7 of guanine-4 in the half-site does not make a protein contact. Hypermethylation might be due to a conformational change in the UAS when protein is bound. Alternatively, the bases that are hypermethylated could be placed in a more hydrophobic environment when the UAS is protein-bound, thus promoting their reactivity with dimethyl sulfate (32).

Although both upstream and downstream promoter sequences must be present in cis for NifA titration *in vivo*, we could not demonstrate a requirement for downstream sequences to protect the UAS from methylation. This may reflect the sensitivity of the *in vivo* assay, but it is possible that the unavailable form of NifA associated with multicopy inhibition is not the form that is simply bound to the UAS but one that is also engaged in transcriptional activation, perhaps involving an interaction with NtrA–RNA polymerase bound to the $-12,-24$ promoter element. This would explain why mutations in these downstream sequences relieve multicopy inhibition when they diminish occupancy of the downstream sequences by the polymerase complex or prevent a step in activation.

In conclusion, results obtained with the *in vivo* studies support a model in which a protein bound upstream at the UAS, probably NifA, participates in the activation of transcription by contacting the downstream promoter complex through a loop forming in the DNA between the UAS and the $-12,-24$ promoter element (7). Such a model seems applicable to systems subject to activation by NtrC and may also be applicable to other NtrA-dependent promoters—i.e., the dicarboxylic acid transport genes in *R. leguminosarum* (33) and the formate hydrogen lyase gene of *E. coli* (34).

We thank Ray Dixon, Martin Drummond, Mike Merrick, and Barry Smith for useful comments and discussion, and Beryl Scutt for typing the manuscript. E.M. was a recipient of a postgraduate fellowship from the Universidad Nacional Autónoma de México.

1. Gussin, G. N., Ronson, C. W. & Ausubel, F. M. (1986) *Annu. Rev. Genet.* **20,** 567–591.
2. Dixon, R. (1987) in *The Nitrogen and Sulphur Cycles,* eds. Cole, J. A. & Ferguson, S. (Cambridge Univ. Press, Cambridge, U.K.), pp. 417–438.
3. Hirschman, J., Wong, P.-K., Sei, K., Keener, J. & Kustu, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7525–7529.
4. Hunt, T. P. & Magasanik, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8453–8457.
5. Austin, S., Henderson, N. & Dixon, R. (1987) *Mol. Microbiol.* **1,** 92–100.
6. Buck, M., Miller, S., Drummond, M. & Dixon, R. (1986) *Nature (London)* **320,** 374–378.
7. Buck, M., Cannon, W. & Woodcock, J. (1987) *Mol. Microbiol.* **1,** 243–249.
8. Drummond, M., Whitty, P. & Wootton, J. (1986) *EMBO J.* **5,** 441–447.
9. Contreras, A. & Drummond, M. (1988) *Nucleic Acids Res.* **16,** 4025–4039.
10. Ninfa, A. J., Reitzer, L. J. & Magasanik, B. (1987) *Cell* **50,** 1039–1046.
11. Ames, G. F.-L. & Nikaido, K. (1985) *EMBO J.* **4,** 539–547.
12. Hawkes, T., Merrick, M. J. & Dixon, R. (1985) *Mol. Gen. Genet.* **201,** 492–498.
13. Wong, P.-K., Popham, D., Keener, J. & Kustu, S. (1987) *J. Bacteriol.* **169,** 2876–2880.
14. Minchin, S., Austin, S. & Dixon, R. (1988) *Mol. Microbiol.* **2,** 433–442.
15. Buck, M., Cannon, W. & Woodcock, J. (1987) *Nucleic Acids Res.* **15,** 9945–9956.
16. Gronenberg, B. (1976) *Mol. Gen. Genet.* **148,** 243–250.
17. MacNeil, T., Roberts, G. P., MacNeil, D. & Tyler, B. (1982) *Mol. Gen. Genet.* **188,** 325–333.
18. Buck, M., Khan, H. & Dixon, R. (1985) *Nucleic Acids Res.* **13,** 7621–7638.
19. Tuli, R. & Merrick, M. J. (1988) *J. Gen. Microbiol.* **134,** 425–432.
20. Buck, M. (1986) *J. Bacteriol.* **166,** 545–551.
21. Ogata, R. & Gilbert, W. (1979) *J. Mol. Biol.* **132,** 709–728.
22. Borowiec, J. A. & Gralla, J. D. (1986) *Biochemistry* **25,** 5051–5057.
23. Reitzer, L. J., Bueno, R., Cheng, W. D., Abrams, S. A., Rothstein, D. M., Hunt, T. P., Tyler, B. & Magasanik, B. (1987) *J. Bacteriol.* **169,** 4279–4284.
24. Shen, S.-c., Xue, Z.-t., Kong, Q.-t. & Wu, Q.-l. (1983) *Nucleic Acids Res.* **11,** 4241–4250.
25. Brown, S. E. & Ausubel, F. M. (1984) *J. Bacteriol.* **157,** 143–147.
26. Keener, J. D., Wong, P., Popham, D., Wallis, J. & Kustu, S. (1987) in *RNA Polymerase and the Regulation of Transcription,* eds. Reznikoff, W. S., Burgess, R. R., Dahlberg, J. E., Gross, C. A., Record, M. T. & Wichens, M. P. (Elsevier, New York), pp. 159–175.
27. Buikema, W. J., Szeto, W. W., Lemley, P. V., Orme-Johnson, W. H. & Ausubel, F. M. (1985) *Nucleic Acids Res.* **13,** 4539–4555.
28. Granger, P., Manian, S. S., Reilander, H., O'Connell, M., Preifer, U. B. & Puhler, A. (1987) *Nucleic Acids Res.* **15,** 31–49.
29. Fischer, H.-M., Bruderer, T. & Hennecke, H. (1988) *Nucleic Acids Res.* **16,** 2207–2224.
30. Bennett, L. T., Cannon, F. & Dean, D. R. (1988) *Mol. Microbiol.* **2,** 315–321.
31. Ebright, R. H. (1986) in *Protein Structure Folding and Design,* ed. Oxender, D. (Liss, New York), Vol. 1, pp. 207–219.
32. Johnsrud, L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5314–5318.
33. Ronson, C. W., Astwood, P. M., Nixon, B. T. & Ausubel, F. M. (1987) *Nucleic Acids Res.* **15,** 7921–7934.
34. Birkmann, A., Sawers, R. G. & Böck, A. (1987) *Mol. Gen. Genet.* **210,** 535–542.