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Structure Modification Induced in the *narG* Promoter by Binding of Integration Host Factor and NARL-P

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Interaction of integration host factor (IHF) with linear DNA fragments containing the *narG* promoter region induced an apparent sharp bend in the DNA centered at the IHF-binding site. Binding of NARL-P to two sites adjacent to the IHF site did not induce bending or modify the apparent bending induced by IHF.

Nitrate reductase is formed by *Escherichia coli* only under anaerobic conditions and is maximally induced when nitrate is present in the growth medium (15). This pattern of expression results from positive regulation of transcription from the *narGHJI* operon by three transacting factors, FNR, NARL, and integration host factor (IHF) (16). Transcription from the *narGHJI* promoter (*narG*p) under anaerobic conditions depends on the activation of FNR (14), which binds to sequences just upstream (−42 bp) from the transcription start site (18). The further increase in the level of expression which occurs in the presence of nitrate results from the phosphorylation of NARL (19) and the binding of NARL-phosphate (NARL-P) to two distinct regions of DNA which are located approximately −100 and −200 bp from the transcription start site (1, 6, 20). The stimulation of transcription promoted by NARL-P is dependent on the DNA-binding protein IHF (11), which binds to and protects a sequence located between (and partially overlapping) the two NARL-P-binding regions (6, 20). On the basis of the observation that specific binding of IHF induces bending of DNA (3, 4, 8), we proposed that a specific folded DNA-protein complex is generated when NARL-P and IHF bind which interacts with the RNA polymerase-*narG*p promoter complex and stimulates transcription (20). As one approach to determine the possible alterations in DNA structure which result from the concerted binding of these *trans*-acting factors to the *narG*p upstream region, we have explored the extent of apparent bending induced by independent or simultaneous binding of IHF and NARL-P to linear fragments of DNA containing the promoter and upstream *cis*-acting regions of *narG*p.

IHF bends *narG*p fragments. A modified version of the circular permutation assay (21) was used to analyze the interaction of IHF with the *narG*p region. For this purpose, specific DNA fragments of equal lengths (389 bp) were generated by PCR from the *narG*p region with the binding regions for the transacting factors positioned at different positions as diagrammed in Fig. 1. The ^{32}P-labeled fragments were prepared according to the method described by Krummel (5), with plasmid pSL800 (7) as the template, the products were purified with a Wizard PCR preparation kit (Promega), and the final DNA concentrations were determined by measuring the optical density at 260 nm. IHF was purified to apparent homogeneity from extracts of strain EM424 carrying the IHF-overproducing plasmid pNHab (obtained from Valley Stewart, Cornell University) by a method which combined the procedure described by Nash and Robertson (9) with purification on a Heparin Hitrap column (2).

Binding and retardation of the DNA fragments with purified IHF were assessed by electrophoresis on polyacrylamide gels (Fig. 2A). The binding reaction was carried out with a 20-ml reaction mixture containing 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.25 mg of bovine serum albumin per ml, 5% glycerol, and 1 mM dithiothreitol for 30 min. Loading buffer (4 ml of 0.25% bromphenol blue, 0.25% xylene cyanole, and 30% glycerol) was added, and the entire sample was run on a 5% polyacrylamide gel prerun for 2 h and developed in 40 mM Tris-acetate (pH 8.0) plus 1 mM EDTA for IHF alone and in 40 mM Tris-acetate (pH 8.0) when NARL-P was in the reaction mixture. After electrophoresis for approximately 1.5 h, the gel was dried and autoradiographed. The resulting films were scanned into the computer and digitized for reproduction. The unbound fragments all migrated to the same position (Fig. 2A, lanes 1 to 5), while single complexes formed with IHF which were retarded to dramatically different degrees for each fragment (lanes 7 to 11), suggesting that, as in the case of other DNA-bending systems (21), the end-to-end distances of the fragments are differentially altered depending on the position of an induced bend within each fragment. On the basis of the relative mobilities of the complexes, the position of the bend, which was approximated by the graphic method described by Wu and Crothers (21), is at −125 (Fig. 2B), which is within the region protected by IHF binding (Fig. 1, fragment 3). The angle of bending, which was estimated by the empirical formula developed by Thompson and Landy (17), is approximately 145°.

NARL-P and IHF bind independently. Possible interactions in the binding of IHF and NARL-P were explored by varying the amounts of each factor independently in gel retardation experiments with fragment no. 2 (Fig. 3). NARL-P, which was prepared from purified NARL (20), formed a complex which migrated as a band (lanes 11 to 15) more diffuse than that formed with IHF alone (lanes 1 to 5). With a saturating level of NARL-P and increasing amounts of IHF in the binding reaction mixture (lanes 6 to 10), a complex was formed in amounts proportional to that of the IHF added which migrated more slowly than the complex with IHF alone. Similarly, with a subsaturating level of IHF and increasing amounts of NARL-P (lanes 16 to 20), a proportional amount of the more slowly moving complex formed. In neither case was there any

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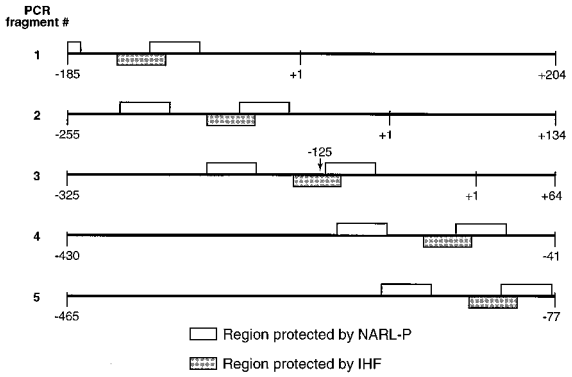

FIG. 1. Fragments of the *narG*p region generated by PCR. The numbers represent positions in the sequence of the *narGHJI* operon region (GenBank accession numbers X15996 and X16181) relative to the transcription start site (18). The boxes represent the sequences protected by IHF and NARL-P in DNase protection studies by Walker and DeMoss (20).

indication that either factor facilitated or interfered with the binding of the other.

NARL-P does not alter IHF-induced bending. The effects of NARL-P binding on the structure of the *narG*p region, either in the absence or presence of IHF, were examined by gel retardation with the *narG*p fragments described above (Fig. 4A). The complexes formed with NARL-P alone (lanes 2, 6, 10, 14, and 18) migrated to the same position for all five fragments, while those formed with NARL-P and IHF (lanes 4, 8, 12, 16, and 20) were retarded to different degrees in a pattern similar to that with IHF alone. However, in each case the combined factors formed a complex which was more retarded than that with IHF alone. As analyzed graphically in Fig. 4B, these results indicate that binding of NARL-P does not bend the DNA or alter the bending of DNA induced by IHF; neither the apparent center of bending nor the degree of

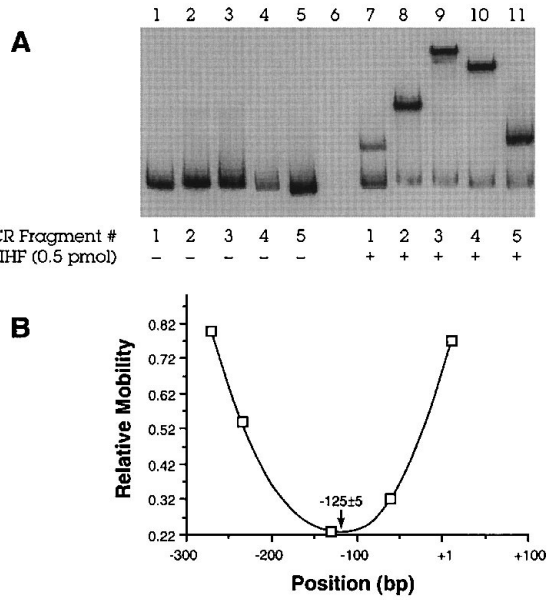

FIG. 2. Gel retardation of *narG* promoter PCR fragments with purified IHF. (A) Binding and electrophoresis as described in the text; (B) relative mobilities of the retarded bands in A plotted as a function of the position (in base pairs) of the center of each PCR fragment in the *narG* promoter region relative to the transcription start site.

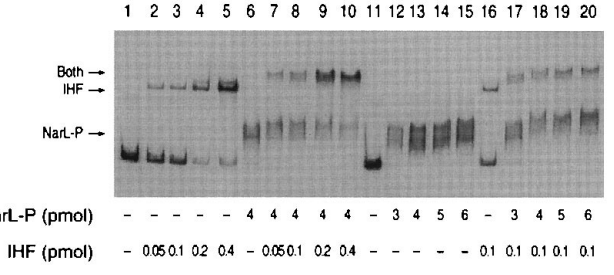

FIG. 3. Gel retardation of PCR fragment no. 2 with purified NARL-P and IHF. Binding and electrophoresis were as described in the legend to Fig. 2; NARL-P was purified and prepared as previously described (20).

bending, which was calculated according to the method described by Thompson and Landy (17), was significantly modified.

Structural implications. Interpreted as a simple bending model, these results suggest that IHF induces a sharp bend centered at the IHF-binding site which may promote an interaction between NARL-Ps bound at two specific sites located on either side of the putative bending center. Given the asymmetric distribution of the two NARL-P sites relative to the putative center of bending (Fig. 1, fragment 3), such a bend could bring the NARL-P bound to the upstream site to a position which could conceivably interact with both NARL-P bound to the downstream site and RNA polymerase bound to the promoter. Such an interaction of the two bound NARL-Ps is compatible with the fact that their binding sites are located on the same face of the DNA, i.e., the centers of the protected regions are separated by 104 bp, and the previous demonstration (7) that stimulation of transcription by NARL-P requires the appropriate phasing of the upstream site relative to the downstream sequences.

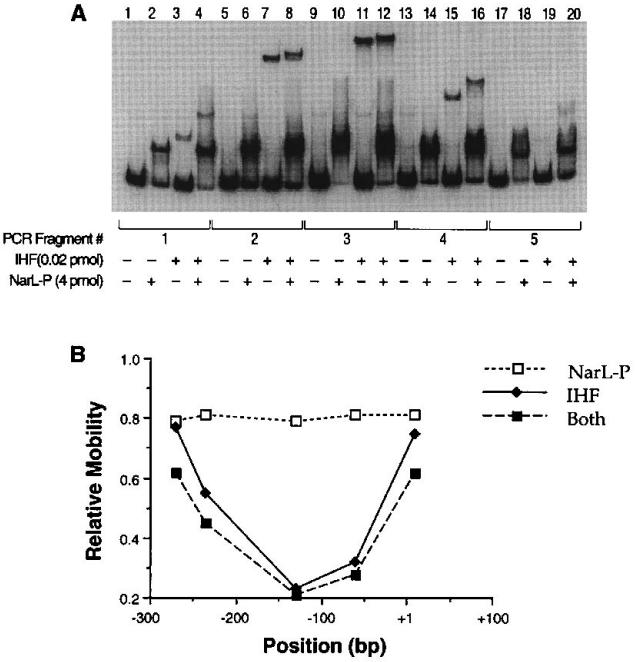

FIG. 4. Gel retardation of *narG* promoter PCR fragments with purified NARL-P and IHF. (A) Binding and electrophoresis were as described in the text; (B) relative mobilities of retarded fragments plotted as described in the legend to Fig. 2B.

This model for activation by the concerted binding of IHF and NARL-P to the *narG* promoter is similar to that proposed for the catabolite activator protein (CAP)-dependent activation of the maltose operons by MALT (12) in which specific CAP-induced bending is thought to promote a critical interaction between MALT proteins bound to sequences on either side of the bending site. The induction of protein-protein interactions between bound activation factors represents one of the general mechanisms by which global DNA-bending proteins may participate in the regulation of transcription from a number of inducible promoters (10, 13).

REFERENCES

1. **Dong, X.-R., S.-F. Li, and J. A. DeMoss.** 1992. Upstream sequence elements required for NarL-mediated activation of transcription from the *narGHJI* promoter of *Escherichia coli*. J. Biol. Chem. **267:**14122–14128.
2. **Flutowitz, M., and K. Appelt.** 1988. The integration host factor of *Escherichia coli* binds to multiple sites at plasmid R6K origin and is essential for replication. Nucleic Acids Res. **16:**3829–3834.
3. **Goodman, S. D., and H. A. Nash.** 1989. Functional replacement of a protein-induced bend in a DNA recombination site. Nature (London) **341:**251–254.
4. **Kim, S., and A. Landy.** 1992. Lambda INT protein bridges between higher order complexes at two distant chromosomal loci attL and attR. Science **256:**189–203.
5. **Krummel, B.** 1990. DNase I footprinting, p. 184–188. *In* M. A. Innis, D. H. Gelfand, J. J. Svensky, and T. J. White (ed.), PCR protocols. Academic Press, Inc., San Diego, Calif.
6. **Li, J., S. Kustu, and V. Stewart.** 1994. *In vitro* interaction of nitrate-responsive regulatory protein NARL with DNA target sequences in the *fdnG*, *narG*, *narK*, and *frdA* operon control regions of *Escherichia coli* K12. J. Mol. Biol. **241:**150–156.
7. **Li, S. F., and J. A. DeMoss.** 1988. Location of sequences in the *nar* promoter of *Escherichia coli* required for regulation by Fnr and NarL. J. Biol. Chem. **236:**13700–13705.
8. **Moitoso de Vargas, L. M., S. Kim, and A. Landy.** 1989. DNA looping generated by DNA bending protein IHF and two domains of lambda integrase. Science **244:**1457–1461.
9. **Nash, H. A., and C. Robertson.** 1981. Purification and properties of the *Escherichia coli* protein factor required for lambda integrative recombination. J. Biol. Chem. **256:**9246–9253.
10. **Perez-Martin, J., F. Rojo, and V. DeLorenzo.** 1994. Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. Microbiol. Rev. **58:**268–290.
11. **Rabin, R. S., L. A. Collins, and V. Stewart.** 1992. *In vivo* requirement of integration host factor for *nar* (nitrate reductase) operon expression in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **89:**8701–8705.
12. **Richet, E., D. Vidal-Ingigliardi, and O. Raibaud.** 1991. A new mechanism for coactivation of transcriptional activation: repositioning of an activator triggered by the binding of a second activator. Cell **66:**1185–1195.
13. **Schleif, R. F.** 1992. DNA looping. Annu. Rev. Biochem. **61:**199–223.
14. **Spiro, S., and J. R. Guest.** 1987. Regulation and overexpression of the *fnr* gene of *Escherichia coli*. J. Gen. Microbiol. **133:**3279–3288.
15. **Stewart, V.** 1988. Nitrate respiration in relation to faculative metabolism in enterobacteria. Microbiol. Rev. **52:**190–232.
16. **Stewart, V.** 1993. Nitrate regulation of anaerobic respiratory gene expression in *Escherichia coli*. Mol. Microbiol. **9:**425–434.
17. **Thompson, J. F., and A. Landy.** 1988. Empirical estimation of protein-induced bending angles: applications to 1 site-specific recombination complexes. Nucleic Acids Res. **16:**9687–9705.
18. **Walker, M. S., and J. A. DeMoss.** 1991. Promoter sequence requirements for Fnr-activation of transcription of the *narGHJI* operon. Mol. Microbiol. **5:**353–360.
19. **Walker, M. S., and J. A. DeMoss.** 1993. Phosphorylation and dephosphorylation catalyzed *in vitro* by purified components of the nitrate sensing system, NarX and NarL. J. Biol. Chem. **268:**8391–8393.
20. **Walker, M. S., and J. A. DeMoss.** 1994. NarL-phosphate must bind to multiple upstream sites to activate transcription from the *narG* promoter of *Escherichia coli*. Mol. Microbiol. **14:**633–641.
21. **Wu, H.-M., and D. M. Crothers.** 1984. The locus of sequence-directed and protein-induced DNA bending. Nature (London) **308:**509–513.