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Control of *Herbaspirillum seropedicae* NifA Activity by Ammonium Ions and Oxygen

E. M. SOUZA,[1]* F. O. PEDROSA,[1] M. DRUMMOND,[2] L. U. RIGO,[1] AND M. G. YATES[1]

Department of Biochemistry and Department of Pharmacology, Universidade Federal do Paranà, Curitiba, PR, Brazil,[1] and John Innes Centre, Norwich, United Kingdom[2]

Received 10 March 1998/Accepted 29 October 1998

The activity of a truncated form of *Herbaspirillum seropedicae* NifA in different genetic backgrounds showed that its regulatory domain is involved in nitrogen control but not in O_2 sensitivity or Fe dependence. The model for nitrogen control involving PII could thus apply to the proteobacteria at large. NifA may have a role in controlling ADP-ribosylation of nitrogenase in *Azospirillum brasilense*.

In gram-negative diazotrophs, *nif* (nitrogen fixation) gene expression is controlled by the σ^{54}-dependent activator NifA. Ammonium and molecular oxygen regulate NifA activity by mechanisms that differ significantly between taxa, and in some organisms ammonium also inhibits nitrogenase activity by ADP-ribosylation. Like most σ^{54}-dependent activators, NifA proteins comprise three domains. The well-conserved central and C-terminal domains have activator and DNA-binding functions, respectively (9, 22), while the N-terminal domains have an ill-defined regulatory function. Sequence identity between the regulatory domains is too low to confidently infer a common fold on this criterion alone, but their position within otherwise homologous structures makes structural similarity probable.

Whether they share a function between taxonomic groups is less sure. Arsène et al. (1) have shown that in *Azospirillum brasilense*, a member of the alpha subdivision of the proteobacteria, the N-terminal domain of NifA mediates nitrogen control. This may not be true of the symbiotic rhizobia in the group, which export ammonium to the host plant, and largely lack nitrogen regulation at the level of *nif* expression. Rhizobial NifA proteins are intrinsically redox sensitive, probably owing to a characteristic C-terminal extension of the central domain (12), a feature also present in the *A. brasilense* and *Herbaspirillum seropedicae* proteins (1, 27).

In *Klebsiella pneumoniae*, *Enterobacter agglomerans*, and *Azotobacter vinelandii*, members of the gamma subdivision of the proteobacteria, NifA is not oxygen sensitive but its activity is controlled by the NifL protein, which in the presence of high ammonia or oxygen concentrations binds to NifA, inhibiting activation. NifL has not been found outside this group of bacteria. Because nitrogen control is impaired in *nifL* mutants, it is widely assumed that NifL channels the signal for nitrogen control, a view we question here.

Within the beta subdivision of the proteobacteria, *nif* regulation is best characterized in *H. seropedicae* (2, 27). Here we show that *H. seropedicae* NifA is active in *A. brasilense* and that this activity is repressed by ammonium. It is inactive, however, in *Escherichia coli* under all conditions tested, suggesting the need for a factor absent from the enteric background. A truncated NifA protein lacking the N-terminal domain drives *nifH* transcription in both *E. coli* and *A. brasilense* with or without ammonium. This shows that the regulatory domain negatively regulates NifA activity and indicates that it is involved in the response to the nitrogen status of the cell. We further show that the activities of both the full-length and truncated NifA proteins of *H. seropedicae* are O_2 sensitive and Fe dependent.

To examine the activity of *H. seropedicae* NifA proteins in *E. coli*, plasmids expressing the truncated and full-length genes from the *lac* promoter were constructed (Table 1). A *Mae*I fragment containing *nifA* with its ribosome-binding site was cloned into pTZ18 (19) digested with *Hin*cII, giving pEMS130. To remove the regulatory domain, the region between positions 1370 (corresponding to Val 203) and 2530 of *nifA* (27) was amplified by PCR with primer 1 (5′-ACTCGGTACCGTA ATCGGCATTT-3′), containing a *Kpn*I site, and primer 2 (5′-CGGGGGATCCATCAATACAAC-3′). A product of about 1.1 kb was isolated, digested with *Kpn*I and *Bgl*II (located at position 1693 in the *nifA* coding region), and cloned into pEMS130 digested with the same restriction enzymes to yield pEMS131, and its sequence was verified.

The *nifA* plasmids were transformed into *E. coli* ET8894 carrying pRT22, a *K. pneumoniae nifH-lacZ* fusion plasmid. Fresh cultures grown in Luria-Bertani (LB) medium supplemented with antibiotics were used to inoculate 2 ml of NFDM medium containing glutamine (10 μg/ml), antibiotics, 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), and, when necessary, 20 mM NH_4Cl, in 7-ml bijou bottles. The β-galactosidase activities (20) were determined after shaking at 30°C and 120 rpm for 20 to 24 h either under air ($+O_2$) or under a nitrogen atmosphere ($-O_2$).

The native NifA protein of *H. seropedicae* encoded by pEMS130 failed to activate expression of *nifH-lacZ* to above background levels in all conditions tested, suggesting that expression of the regulatory gene was grossly impaired in this background. The gene was therefore cloned into the overexpression vector pDK7 (17), and NifA synthesis was induced to the point at which the protein was clearly visible on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but even so no expression of the *K. pneumoniae nifH-lacZ* fusion was observed (data not shown), indicating that the native *H. seropedicae* NifA is transcriptionally inert in the enteric background (Table 2). By contrast, activation by a comparable construct carrying *K. pneumoniae* NifA was 3 orders of magnitude above the background level (Table 2). However, the N-terminally truncated *H. seropedicae* NifA activated *nifH* expression to 100 times higher than the background activity and to 15% of the level obtained with *K. pneumoniae* NifA (pNH11) (Table 2). Expression mediated by truncated *H. sero-*

* Corresponding author. Mailing address: Department of Biochemistry and Department of Pharmacology, Universidade Federal do Paranà, C. Postal 19046, CEP 81531-990, Curitiba–PR, Brazil. Phone: 5541 266 4398. Fax: 5541 266 2042. E-mail: souzaem@bio.ufpr.br.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or phenotype	Reference
Strains		
H. seropedicae SMR54	*nifA*	27
A. brasilense		
FP2	Nif^+	23
FP9	*ntrC*	23
FP10	*nifA*	23
E. coli ET8894	*lacZ ntrC*	10
Plasmids (vector)		
pDK7(pBR322)	Cm^r *lacI*^{q}, overexpression from p*tac*	17
pRT22(pACYC177)	Cm^r *K. pneumoniae nifH-lacZ*	27
pEMS130(pTZ18)	Cb^r *H. seropedicae nifA*	This work
pEMS131(pTZ18)	Cb^r *H. seropedicae* Δ^R*nifA*	This work
pEMS135(pLAFR3.18)	Tc^r *H. seropedicae nifA*	This work
pEMS136(pLAFR3.18)	Tc^r *H. seropedicae* Δ^R*nifA*	This work
pNH11(pBR322)	Cb^r p*tac-nifA* (*K. pneumoniae*)	13
pTZ18	Cb^r	19
pLAFR3.18	Tc^r IncP cosmid with pTZ18 cloning nest	28

pedicae NifA was unaffected by 20 mM ammonium but reduced to background levels by oxygen (air).

The effect of Fe on the activity of truncated *H. seropedicae* NifA was examined by omitting Fe from an NFDM culture of *E. coli* ET8894(pRT22/pEMS131). This reduced β-galactosidase activity by 50%. The specific Fe chelator Desferal (250 μg/ml) reduced it by about 85% (Table 3), and EDTA had a similar effect (data not shown). The effect of Desferal was partially reversed by $FeSO_4 \cdot 7H_2O$ (20 μg/ml), with the β-galactosidase activity restored to the level observed in the absence of added Fe or Desferal. Neither $CoCl_2$, $MnCl_2$, nor $Ni(NO_3)_2$ reversed the effect of Desferal (not shown), demonstrating a specific requirement of truncated NifA activity for iron. In contrast, *K. pneumoniae* NifA expressed in *E. coli* ET8894 from pNH11 was unaffected by Desferal or EDTA (Table 3), as previously observed (12). Clearly, truncated *H. seropedicae* NifA activity was Fe dependent whereas *K. pneumoniae* NifA was not.

To examine the activity of *H. seropedicae* NifA proteins in *A. brasilense*, the *nifA* inserts were excised from pEMS130 and pEMS131 and inserted into the *incP lacZ* α-complementing vector pLAFR3.18 (28), yielding pEMS135 and pEMS136, respectively, in which *nifA* was transcribed from the *lac* promoter as before. Fresh cultures of the *nifA* mutant strain *A. brasilense* FP10 (*nifH*::*lacZ*) carrying these plasmids were harvested and resuspended in NFbHP medium (23) containing 100 μg of glutamate per ml and 20 mM NH_4Cl when indicated. When required, oxygen was removed by flushing the flask with nitrogen and the final oxygen concentration was adjusted to 0.5 to 1%. Where indicated, 0.2 mM EDTA and 20 μg of $FeSO_4 \cdot 7H_2O$ per ml were added. The cultures were shaken for 5 h at 30°C and 120 rpm, and the β-galactosidase activity was determined. When expressed in *A. brasilense* FP10 (*nifA*), the full-length NifA of *H. seropedicae* activated a chromosomal *nifH*::*lacZ* fusion only under low oxygen tensions (0.5%) and in the absence of ammonium (Table 4). However, the N-terminally truncated NifA protein was active with or without ammonium, provided the oxygen tension was around 0.5%. Fe was also required for maximum activation of the *nifH* promoter by both the truncated and native NifA proteins (Table 4).

The effect of *H. seropedicae* NifA on nitrogenase activity in *A. brasilense* Nif mutants was determined by using acetylene reduction in semisolid NFbHP medium (23). Both intact and truncated NifA proteins of *H. seropedicae* complemented the *nifA A. brasilense* strain FP10 (Table 5). The nitrogenase activity of the FP10(pEMS135) transconjugant, expressing intact *H. seropedicae* NifA, was repressed completely by 10 mM

TABLE 2. Activation of the *K. pneumoniae nifH* promoter by the *H. seropedicae* N-terminally truncated NifA protein in *E. coli*[a]

Plasmids in ET8894	Activator	β-Galactosidase activity (Miller units)		
		−N/−O	−N/+O	+N/−O
pRT22/pNH11	NifA (*K. pneumoniae*)	19,582	20,904	20,800
pRT22/pEMS130	NifA (*H. seropedicae*)	21	15	19
pRT22/pEMS131	Δ^R NifA (*H. seropedicae*)	2,884	31	3,016

[a] *E. coli* ET8894 containing the indicated plasmids was grown in LB medium as described in Materials and Methods. Cells were inoculated in NFDM medium plus glutamine (10 μg/ml) and IPTG with the following additions: N-free minimal medium under nitrogen (−N/−O), N-free minimal medium under air (−N/+O), and minimal medium plus NH_4Cl (20 mM) under nitrogen (+N/−O). β-Galactosidase activities were measured after 20 to 24 h of incubation at 30°C.

TABLE 3. Effect of iron and Desferal on the activity of the *H. seropedicae* N-terminally truncated and *K. pneumoniae* NifA proteins in *E. coli*[a]

Addition	β-Galactosidase activity (Miller units)	
	ET8894(pRT22/pNH11)	ET8894(pRT22/pEMS131)
1. −Fe	20,060	1,671
2. −Fe/+Desf	20,904	430
3. +Fe/+Desf	ND	1,612
4. +Fe	ND	2,750

[a] *E. coli* ET8894 containing the indicated plasmids was grown in LB medium as described in Materials and Methods. Cells were inoculated in NFDM medium plus glutamine (10 μg/ml) with the following additions: 1, N-free minimal medium under nitrogen and no Fe added; 2, treatment 1 plus Desferal (250 μg/ml); 3, treatment 2 plus $FeSO_4 \cdot 7H_2O$ (20 μg/ml); 4, treatment 1 plus $FeSO_4 \cdot 7H_2O$ (20 μg/ml). β-Galactosidase activities were measured after 20 to 24 h of incubation at 30°C. ND, not determined.

TABLE 4. Effect of ammonium ions, oxygen, and Fe on the activity of the *H. seropedicae* NifA proteins expressed in *A. brasilense*[a]

Treatment	β-Galactosidase activity (Miller units)	
	FP10(pEMS135) (*nifH*::*lacZ*)	FP10(pEMS136) (*nifH*::*lacZ*)
1. −N/−O	152	148
2. −N/+O	7	15
3. +N/−O	12	207
4. +N/+O	2	6
5. −Fe	8	2
6. −Fe/+EDTA	6	2
7. +EDTA/+Fe	124	132

[a] *Azospirillum brasilense* FP10 (*nifA*) carrying either pEMS135 (native NifA) or pEMS136 (N-terminally truncated NifA) was grown in NFbHP plus ammonium ions (20 mM) in air at 30°C overnight. The cells were then centrifuged and resuspended in NFbHP containing glutamate (0.1 mM) and derepressed for NifA activity under the following treatments: 1, absence of NH_4Cl and 0.5% oxygen; 2, absence of NH_4Cl and air; 3, NH_4Cl (20 mM) and 0.5% oxygen; 4, NH_4Cl (20 mM) and air; 5, treatment 1 but without added Fe; 6, treatment 5 plus EDTA (0.2 mM); and 7, treatment 6 plus $FeSO_4 \cdot 7H_2O$ (20 μg/ml). β-Galactosidase activities were measured after 4 h at 30°C.

TABLE 5. Effect of ammonium on the nitrogenase activity of *A. brasilense* strains carrying *H. seropedicae* NifA proteins[a]

Strain (mutation)	Plasmid (activator)	Nitrogenase activity (nmol of $C_2H_4 \cdot min^{-1} \cdot mg$ of $protein^{-1}$)	
		−N	+N
FP2 (wt)	None	19.5	0
FP2 (wt)	pEMS135 (NifA)	20	0
FP2 (wt)	pEMS136 (Δ^R NifA)	18.7	8.6
FP9 (*ntrC*)	None	0	0
FP9 (*ntrC*)	pEMS135 (NifA)	0	0
FP9 (*ntrC*)	pEMS136 (Δ^R NifA)	40.7	25.7
FP10 (*nifA*)	None	0	0
FP10 (*nifA*)	pEMS135 (NifA)	7.1	0
FP10 (*nifA*)	pEMS136 (Δ^R NifA)	21.2	10.1

[a] *A. brasilense* strains were grown in NFbHP medium plus NH_4Cl (20 mM) and inoculated (20 μl) into semisolid NFbHP medium with or without NH_4Cl (10 mM). Nitrogenase activity was measured after 18 to 24 h of incubation at 30°C as described in Materials and Methods. wt, wild type.

ammonium, whereas the nitrogenase activity of the FP10 (pEMS136) transconjugant, expressing truncated NifA, fell by only 50%. In the presence of 10 mM NH_4Cl, the wild-type strain of *A. brasilense*, FP2, carrying pEMS136 had only 50% of the maximum nitrogenase activity observed without ammonium. In contrast, *A. brasilense* FP9 (*ntrC*) was fully complemented by truncated NifA with or without ammonium, but not by native NifA under either condition (Table 5).

Surprisingly, truncated NifA failed to complement the *nifA* mutant of *H. seropedicae* Smr54, although the full-length form expressed from the same promoter gave growth rates on atmospheric nitrogen comparable to that of the wild type, suggesting that this truncated NifA is inactive in its own background.

These data have a number of implications. The inactivity of intact NifA in *E. coli* suggests that the enteric background either lacks a factor necessary for function of the full-length activator or contains one that inhibits its function; the former is more likely, as gross overexpression of NifA, which might be expected to titrate out any inhibitor, failed to activate *nifH* transcription. The deficient factor is not NtrC, since similar low activities were observed in Ntr^+ *E. coli* strains. A strong candidate is the PII protein, which is reversibly uridylated according to the nitrogen status of the cell and controls both nitrogen assimilation and Ntr transcription. Several bacterial species, including *H. seropedicae*, are now known to contain two or more PII-like proteins with obvious sequence similarity but distinct functions (3, 8, 30), so interspecific differences may well explain the failure of the *E. coli* PII proteins to relieve inhibition of full-length *H. seropedicae* NifA. Although the full-length *H. seropedicae* NifA is inactive in *E. coli*, the truncated form lacking the regulatory domain does drive *nifH* transcription. This implies that the N-terminal domain of *H. seropedicae* NifA negatively regulates activator function and is acted upon by PII (or another factor). The observation that addition of ammonium to the medium has no significant effect on the transcriptional activity of the truncated NifA is consistent with a role for the regulatory domain in nitrogen control.

Such a role is confirmed by results obtained in the *Azospirillum* background. Expression of the chromosomal *nifH-lacZ* fusion in *A. brasilense* FP10 was activated by intact *H. seropedicae* NifA, but only under ammonium deprivation, whereas the truncated NifA activated expression irrespective of nitrogen status. The complementation pattern of the Nif^- *A. brasilense* mutants by these two NifA proteins also suggests that ammonia inhibition is mediated by the N-terminal domain of NifA. *A. brasilense* FP9 (*ntrC*) is Nif^- and was complemented by the *ntrC* gene of either *K. pneumoniae* (23) or *A. brasilense* (18). Our observation that the N-terminally truncated *H. seropedicae* NifA complements the FP9 Nif^- phenotype, whereas the constitutively expressed full-length NifA fails to do so, suggests that FP9 either lacks an activator or contains an inhibitor which acts on the N-terminal domain of the full-length NifA. FP9 has a very low level of *glnB* expression (7), which suggests that the *glnB* product, PII, participates in the modulation of the NifA protein activity of *H. seropedicae*.

Recently, Arsène et al. showed that PII of *A. brasilense* may be required to activate the *A. brasilense* NifA protein by a mechanism involving the N-terminal domain (1). Since an involvement of the regulatory domain in nitrogen control has now been found in two of the major divisions of the proteobacteria, we suggest that it may in fact be a general property of NifA proteins. In the gamma proteobacteria, *nifL* mutants lack nitrogen regulation, which has widely been interpreted to mean that NifL senses and transmits a nitrogen deficit signal to NifA. However, we earlier noted the possibility that NifA itself may receive the nitrogen deficit signal, and that both it and NifL must be in the "derepressed" conformation to relieve mutual binding, or whatever other mechanism might be responsible for activator inhibition (10). This model predicts that in diazotrophs of the gamma proteobacteria, PII or its paralogue GlnK interacts not primarily with NifL but with the activator. In the rhizobia, which have evolved to fix more nitrogen than necessary to sustain the bacterial population, the NifA regulatory domain may either be vestigial or have a second function, e.g., sensitivity to metal supply. Interestingly, the only known instance of natural loss of the regulatory domain of NifA occurs in the symbiont *Rhizobium trifolii* (16).

Our finding that the *H. seropedicae* NifA resembles the *Bradyrhizobium japonicum* and *A. brasilense* NifAs in retaining oxygen sensitivity and iron dependence when the regulatory domain is removed supports the suggestion that the C-terminal extension to the central domain with conserved cysteine residues, a feature common to all three proteins, is involved in the response to oxygen.

A. brasilense differs from *H. seropedicae* in directly regulating the activity of nitrogenase through ADP-ribosylation of the Fe protein, which in ammonium normally reduces enzymatic activity to 1 to 2% of uninhibited levels. In the presence of truncated *H. seropedicae* NifA this inhibition is largely lacking, which cannot easily be ascribed to unregulated *nifA*-dependent transcription, since switch-off is normally more rapid than would be expected of a mechanism involving the decay of *nif* products of any kind. This finding therefore suggests either that the truncated form of NifA constitutively binds and titrates out a factor, possibly PII, necessary for communicating nitrogen excess to the ADP-ribosylation system or that NifA itself is involved in posttranslational control, an entirely novel function for this transcriptional regulator.

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REFERENCES

1. **Arsène, F., P. A. Kaminski, and C. Elmerich.** 1996. Modulation of NifA activity by P_{II} in *Azospirillium brasilense*: evidence for a regulatory role of the NifA N-terminal domain. J. Bacteriol. **178:**4830–4838.
2. **Baldani, J. I., V. L. D. Baldani, L. Seldin, and J. Döbereiner.** 1986. Characterization of *Herbaspirillum seropedicae* gen. nov. sp. nov., a root-associated

nitrogen-fixing bacterium. Int. J. Syst. Bacteriol. **36:**86–93.
3. **Benelli, E. M., E. M. Souza, S. Funayama, L. U. Rigo, and F. O. Pedrosa.** 1997. Evidence for two possible *glnB*-type genes in *Herbaspirillum seropedicae*. J. Bacteriol. **179:**4623–4626.
4. **Berger, D. K., F. Narberhaus, and S. Kustu.** 1994. The isolated catalytic domain of NIFA, a bacterial enhancer-binding protein, activates transcription in vitro: activation is inhibited by NIFL. Proc. Natl. Acad. Sci. USA **91:** 103–107.
5. **Beynon, J. L., M. K. Williams, and F. A. Cannon.** 1988. Expression and functional analysis of the *Rhizobium meliloti nifA* gene. EMBO J. **7:**7–14.
6. **Cannon, W., and M. Buck.** 1992. Central domain of the positive control protein NifA and its role in transcriptional activation. J. Mol. Biol. **225:**271–286.
7. **de Zamaroczy, M., A. Paquelin, and C. Elmerich.** 1993. Functional organization of the *glnB-glnA* cluster of *Azospirillum brasilense*. J. Bacteriol. **175:** 2507–2515.
8. **de Zamaroczy, M., A. Paquelin, A. Peltre, K. Forchhammer, and C. Elmerich.** 1996. Coexistence of two structurally similar but functionally different P_{II} proteins in *Azospirillum brasilense*. J. Bacteriol. **178:**4143–4149.
9. **Drummond, M., P. Whitty, and J. Wootton.** 1986. Sequence and domain relationships of *ntrC* and *nifA* from *Klebsiella pneumoniae*: homologies to other regulatory proteins. EMBO J. **5:**441–447.
10. **Drummond, M. H., A. Contreras, and L. A. Mitchenall.** 1990. The function of isolated domains and chimaeric proteins constructed from the transcriptional activators NifA and NtrC of *Klebsiella pneumoniae*. Mol. Microbiol. **4:** 29–37.
11. **Fischer, H. M.** 1994. Genetic regulation of nitrogen fixation in rhizobia. Microbiol. Rev. **58:**352–386.
12. **Fischer, H.-M., T. Bruderer, and H. Hennecke.** 1988. Essential and nonessential domains in the *Bradyrhizobium japonicum* NifA protein: identification of indispensable cysteine residues potentially involved in redox reactivity and/or metal binding. Nucleic Acids Res. **16:**5.
13. **Henderson, N., S. Austin, and R. A. Dixon.** 1989. Role of metal ions in negative regulation of nitrogen fixation by the *nifL* gene product from *Klebsiella pneumoniae*. Mol. Gen. Genet. **216:**484–491.
14. **Hill, S., S. Austin, T. Eydmann, T. Jones, and R. Dixon.** 1996. *Azotobacter vinelandii* NIFL is a flavoprotein that modulates transcriptional activation of nitrogen-fixation genes via a redox-sensitive switch. Biochemistry **93:**2143–2148.
15. **Huala, E., and F. M. Ausubel.** 1989. The central domain of *Rhizobium meliloti* NifA is sufficient to activate transcription from the *R. meliloti nifH* promoter. J. Bacteriol. **171:**3354–3365.
16. **Iismaa, S. E., and J. M. Watson.** 1989. The *nifA* gene product from *Rhizobium leguminosarum* biovar *trifolii* lacks the *N*-terminal domain found in other NifA proteins. Mol. Microbiol. **3:**943–955.
17. **Kleiner, D., W. Paul, and M. J. Merrick.** 1988. Construction of multicopy expression vectors for regulated over-production of proteins in *Klebsiella pneumoniae* and other enteric bacteria. J. Gen. Microbiol. **134:**1779–1784.
18. **Machado, H. B., M. G. Yates, S. Funayama, L. U. Rigo, M. B. R. Steffens, E. M. Souza, and F. O. Pedrosa.** 1995. The ntrBC genes of *Azospirillum brasilense* are part of a nifR3-like-ntrB-ntrC operon and are negatively regulated. Can. J. Microbiol. **41:**674–684.
19. **Mead, D. A., E. Szczesna-Skorupa, and B. Kemper.** 1986. Single-stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Protein Eng. **1:**67–74.
20. **Miller, J.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Morett, E., W. Cannon, and M. Buck.** 1988. The DNA-binding domain of the transcriptional activator protein NifA resides in its carboxy terminus, recognises the upstream activator sequences of *nif* promoters and can be separated from the positive control function of NifA. Nucleic Acids Res. **16:** 11469–11488.
22. **Morett, E., and L. Segovia.** 1993. The sigma 54 bacterial enhancer-binding protein family: mechanism of action and phylogenetic relationship of their functional domains. J. Bacteriol. **175:**6067–6074.
23. **Pedrosa, F. O., and M. G. Yates.** 1984. Regulation of nitrogen fixation (*nif*) genes of *Azospirillum brasilense* by *nifA* and *ntr* (*gln*) type gene products. FEMS Microbiol. Lett. **23:**95–101.
24. **Popham, D. L., D. Szeto, J. Keener, and S. Kustu.** 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. Science **243:**629–635.
25. **Schmitz, R. A., L. He, and S. Kustu.** 1996. Iron is required to relieve inhibitory effects on NifL on transcriptional activation by NifA in *Klebsiella pneumoniae*. J. Bacteriol. **178:**4679–4687.
26. **Souza, E. M., F. O. Pedrosa, H. B. Machado, M. Drummond, and M. G. Yates.** 1995. The N-terminus of the NifA protein of *Herbaspirillum seropedicae* is probably involved in sensing of ammonia, p. 260. *In* I. A. Tikhonovich, N. A. Provorov, V. I. Romanov, and W. E. Newton (ed.), Nitrogen fixation: fundamentals and applications. Kluwer Academic Publishers, Dordrecht, The Netherlands.
27. **Souza, E. M., S. Funayama, L. U. Rigo, M. G. Yates, and F. O. Pedrosa.** 1991. Sequence and structural organization of a *nifA*-like gene and part of a *nifB*-like gene of *Herbaspirillum seropedicae* strain Z78. J. Gen. Microbiol. **137:**1511–1522.
28. **Staskawicz, B., D. Dahlbeck, N. Keen, and C. Napoli.** 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. glycinea. J. Bacteriol. **169:**5789–5794.
29. **Tuli, R., and M. J. Merrick.** 1988. Over-production and characterisation of the *nifA* gene product of *Klebsiella pneumoniae*—the transcriptional activator of *nif* gene expression. J. Gen. Microbiol. **134:**425–432.
30. **van Heeswijk, W., D. Hoving, D. Molenaar, B. Stegeman, D. Kahn, and H. V. Westerhoff.** 1996. An alternative PII protein in the regulation of glutamine synthetase in *Escherichia coli*. Mol. Microbiol. **21:**133–146.