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Role of Alternative Promoter Elements in Transcription from the *nar* Promoter of *Escherichia coli*

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The effects of mutations in the −10, −35, and Fnr box regions of the *narGHJI* promoter of *Escherichia coli* were determined by assaying the expression of β-galactosidase from *narG::lacZ* fusion plasmids under aerobic and anaerobic conditions. A 1-base change in the −10 hexamer completely abolished expression, whereas a 3-base change to create the consensus TATAAT resulted in significant aerobic as well as anaerobic expression. A mutation in the putative −35 hexamer did not affect anaerobic expression but reduced aerobic expression from the construction with the −10 consensus sequence. A mutation in the Fnr box severely reduced anaerobic expression but did not affect aerobic expression. When the complete 5′ region of the *nar* operon including the NarL box was present, nitrate stimulated both aerobic and anaerobic expression. Stimulation of expression by nitrate occurred in an *fnr* mutant but not in a *narL* mutant. We conclude that the rate of transcription of the *nar* operon is dependent on two distinct modes of transcription. One mode, which occurs at low levels, depends on the −10 and −35 hexamer sequences and is dramatically enhanced by changing the −10 sequence to the consensus TATAAT. The second depends on the −10 and Fnr box sequences but is independent of the −35 sequence. This second mode occurs at a very high level under anaerobic conditions when Fnr is activated and is also enhanced by changing the −10 sequence to the consensus TATAAT. NarL, activated by nitrate, stimulated both modes of transcription, indicating that it does not act through Fnr but that it directly affects the interaction of RNA polymerase with the promoter.

The expression of the *narGHJI* operon, which encodes the respiratory nitrate reductase of *Escherichia coli*, is controlled by two transacting factors, Fnr and NarL (5, 22). Fnr, activated by an unknown mechanism under conditions of oxygen depletion, induces the transcription of the *narGHJI* operon as well as a number of genes encoding enzymes of anaerobic metabolism (6, 11, 16, 22, 25). Fnr is thought to bind to a highly conserved palindromic DNA sequence (Fnr box) found in all Fnr-activated promoters centered at a position approximately 40 bp from the transcription start site (7, 10, 21). In the presence of nitrate, NarL further stimulates anaerobic transcription from the *narGHJI* promoter through interaction with a *cis*-acting sequence (NarL box) located approximately 200 bp upstream from the transcription start site (12).

In the *narGHJI* promoter (Fig. 1), the Fnr box is centered −41.5 bp from the transcription start site (27) and, as generally found for positively regulated promoters (16) and other Fnr-dependent promoters (7), the −10 and −35 sequences are poorly conserved analogs of the −10 and −35 consensus sequences recognized by the σ^{70}-RNA polymerase of *E. coli*. On the basis of promoter reconstruction studies (27) we concluded that anaerobic transcription is dependent on the sequence of the −10 region and the critical positioning of the Fnr box and proposed that interaction of Fnr with the Fnr box replaces the function of a −35 region in anaerobic transcription from the *narGHJI* promoter. We present here studies on the effects of specific base substitutions in the −10, −35, and Fnr box regions which establish the contribution of each of these regions to regulation of transcription under aerobic and anaerobic conditions.

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MATERIALS AND METHODS

Strains, growth conditions, and enzyme assay. *E. coli* strains used were RK65P (MC4100 *gyrA non nar-202*::Tn*10 pcnB*) (27), ES2001 (MC4100 *fnr-250 zcj-637*::Tn*10*) (19), and RK5278 (MC4100 *gyrA non nar-215*::Tn*10*) (23). For assay of β-galactosidase expression strains were grown at 37°C in M9 medium (15) supplemented with 0.5% glucose, 0.5% acid-hydrolyzed casein, 0.1 mM Na_2MoO_4, 0.1 mM Na_2SeO_3, and the appropriate antibiotics. Nitrate was added to 1% where indicated, and chloramphenicol was added to 30 μg/ml at the end of the growth period to prevent any anaerobic induction of enzyme expression during the preparation of cell suspensions. Anaerobic cultures were grown on a roller drum to mid-log phase (optical density at 600 nm of from 0.4 to 0.6) to avoid the limitations of oxygen supply which occur at higher cell densities in shaking cultures. Anaerobic cultures were grown overnight in stationary, filled Eppendorf tubes to an optical density at 600 nm of approximately 1.0. Harvesting cultures at earlier times did not significantly alter the level of β-galactosidase expressed by anaerobically grown cells. β-Galactosidase activity was determined according to the method of Miller (15), and each determination is the result of duplicate assays from three individual colonies.

Plasmid construction and site-directed mutagenesis. Site-directed mutagenesis was performed with the Muta-Gene Phagemid in vitro mutagenesis kit (Bio-Rad Laboratories) according to the instructions of the manufacturer, and oligonucleotides were purchased from Genetic Designs, Inc. To construct pMW61, a *Kpn*I site was introduced into pSL8RB2, a derivative of pSL800 (12), by using the oligonucleotide GCAAGGTACCGCTCT. The resulting plasmid was cut with *Nco*I, filled in, and cut with *Kpn*I, and the small fragment was ligated with the large fragment obtained by cutting pMW652 (27) with *Eco*RI, filling in, and cutting with

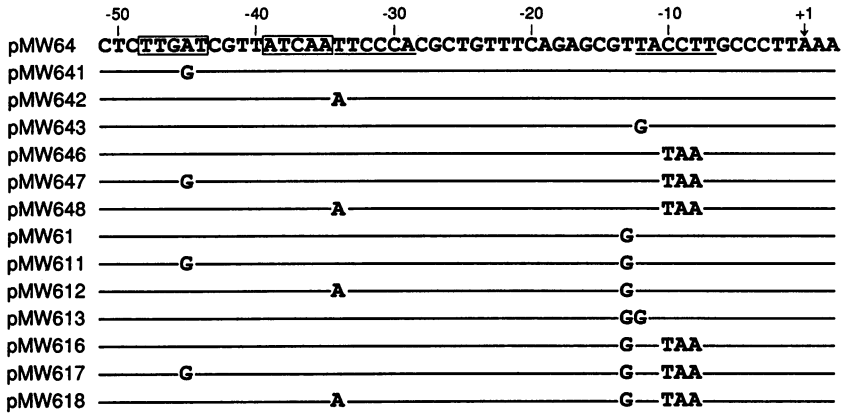

FIG. 1. Nucleotide sequence of the region of the *narGHJI* promoter controlled by anaerobiosis and changes introduced by site-directed mutagenesis. The highly conserved 5-base inverted repeat of the Fnr consensus sequence is boxed, and the putative −10 and −35 hexamers are underlined. The arrow above the sequence indicates the transcription start site (+1). Only bases differing from the wild-type sequence are shown for the mutant plasmids. pMW641, pMW642, pMW643, pMW646, pMW647, and pMW648 are derivatives of pMW64; pMW611, pMW612, pMW613, pMW616, pMW617, and pMW618 are derivatives of pMW61.

*Kpn*I. The oligonucleotides used to produce the T→G changes at −12 and to create the consensus −10 sequences in pMW64 (27) and pMW61 were, respectively, AGAGCG GVACCTTGCC and AGAGCGGTATAATGCCCTTAAAC. The oligonucleotides used to make the T→A changes at −34 and A→G changes at −45 in pMW64, pMW61, and both pMW64 and pMW61 with the consensus −10 sequences were, respectively, GTTATCAAATCCCACGC and CACT CTTGGTCGTTATC. All constructions were confirmed by DNA sequencing (14) with the Sequenase reagent kit purchased from U.S. Biochemical Corp.

RESULTS

To determine the relative roles of the −10, −35, and Fnr box sequences in the transcription of the *narGHJI* operon, we generated a set of specific base substitutions in two plasmids, pMW64 and pMW61. Both plasmids contain the *narGHJI* promoter region and 68 bp of the *narG* gene fused in frame with the *lacZ* gene to permit measurement of expression as β-galactosidase activity. The promoter region in plasmid pMW64 extends upstream 57 bp from the transcription start site and includes a functional Fnr box but not the NarL box region (27). Plasmid pMW61 was constructed to contain the complete *nar* promoter extending 200 bp upstream from the transcription start site and including the NarL box region. It also differed from plasmid pMW64 by a single base change (T to G at position −13) which had no effect on the regulation of expression from the truncated promoter (27) or from the complete promoter (data not shown). The sequence of the region mutagenized and the base substitutions for the pMW64 and pMW61 derivatives are given in Fig. 1. For all derivatives, expression of β-galactosidase was determined in strain RK65P (Δ*lac narG*::Tn*10 pcnB*). The *pcnB* mutation permitted us to measure β-galactosidase expression at low plasmid copy number (13), and the *narG*::Tn*10* insertion prevented any autogenous regulation of *nar* operon expression by chromosomally encoded nitrate reductase (3).

To confirm and extend our previous finding that multiple-base changes in the −10 region of the *narGHJI* promoter abolished anaerobic induction of expression (27), we determined the effect of specific sequence alterations in this region of plasmid pMW64 (Table 1). The wild-type promoter sequence has 3 bases homologous to the most highly conserved bases in the consensus −10 hexamer of *E. coli* (9). The alteration of one of those bases, T to G at position −12, completely abolished anaerobic induction (pMW643). However, when 3 bases were altered to create the consensus −10 sequence, TATAAT, there was an approximate 10-fold increase in both aerobic and anaerobic expression (pMW646). The same alterations were incorporated into plasmid pMW61, which contains the complete 5′ region of the *nar* operon including the NarL box. As shown in Table 1, anaerobic expression with pMW61 was approximately two-fold higher than that with pMW64 and, as expected, nitrate significantly stimulated anaerobic expression. Although not clearly documented by the data presented in Table 1, the low level of expression of β-galactosidase by aerobic cultures was measured at a level of significance (data not shown) which demonstrated that expression was increased four- to fivefold by nitrate. Changing the −10 sequence of pMW61 away from consensus (pMW613) abolished anaerobic induction, and the conversion of the −10 region to the consensus TATAAT resulted in a significantly increased expression under aerobic conditions which was stimulated an additional 20-fold by nitrate (pMW616). Anaerobic expression was also increased to an apparent maximum level of expression for this promoter, since it was not further increased by nitrate, and since it corresponded to the maximum level reached by any of the constructions studied. These results suggest that the −10 region sequence defines the effectiveness of the *nar* promoter in the absence of anaerobic activation by Fnr. Furthermore, they demonstrate that the stimulation of transcription by nitrate-activated NarL occurs under aerobic as well as anaerobic conditions.

To examine further the roles of the −35 and Fnr box regions in aerobic and/or anaerobic transcription, we determined the effects of specific base substitutions in these sequences (Table 1). Sixteen bases upstream from the −10 region in the *narGHJI* operon is a sequence (TTCCCA) homologous with 4 bases of the consensus −35 hexamer (TTGACA). This sequence is adjacent to the Fnr box (Fig. 1) and partially overlaps the right side of an extended consensus sequence (NNATCAANTTT) which has been proposed from a comparison of Fnr-dependent promoters (7). In order

TABLE 1. Expression of β-galactosidase in RK65P bearing plasmids with mutations in the *narGHJI* promoter

Plasmid	Mutation[a]	β-Galactosidase activity[b] during:			
		Aerobic growth	Aerobic growth with nitrate	Anaerobic growth	Anaerobic growth with nitrate
pMW64		9	6	230	240
pMW641	M FB	6	8	11	8
pMW642	M −35	8	9	280	170
pMW643	M −10	5	7	8	7
pMW646	C −10	110	120	2,260	1,990
pMW647	C −10/M FB	100	90	200	170
pMW648	C −10/M −35	44	53	3,700	3,400
pMW61		2	9	490	3,300
pMW611	M FB	3	7	4	50
pMW612	M −35	1	7	480	3,200
pMW613	M −10	2	4	4	31
pMW616	C −10	59	1,070	3,300	3,400
pMW617	C −10/M FB	32	1,150	140	1,400
pMW618	C −10/M −35	10	200	3,200	3,200

[a] Base changes away from consensus are designated M; changes to consensus are designated C. FB refers to the Fnr box, and −35 and −10 refer to the −35 and −10 hexamers, respectively.
[b] β-Galactosidase activity is expressed in Miller units (15).

to determine whether this region is involved in the activation of transcription, the T at −34, which is not part of the palindromic Fnr box, was changed to A, the base which occurs at the lowest frequency at that position in the −35 hexamer (9). This change had no apparent effect on expression under all conditions (pMW642 and pMW612). However, when the −35 mutation was introduced into the plasmids with the consensus −10 sequence, aerobic expression was significantly reduced while anaerobic expression was unaffected (pMW618) or significantly increased (pMW648). Transcription from the promoter with the −10 consensus sequence depends on the −35 sequence under aerobic conditions, but the increased level of transcription under anaerobic conditions apparently is not dependent on this sequence.

The hypothesis that aerobic transcription utilizes the −35 sequence while anaerobic transcription utilizes the Fnr box was confirmed by the effects of mutations in the Fnr box. When an Fnr box mutation (A to G at position −45) was introduced into pMW64 and pMW61 (Fig. 1) (pMW641 and pMW611, respectively), all anaerobic expression was abolished (Table 1). However, when the same mutation was introduced into the corresponding plasmids with the −10 consensus sequences, the level of aerobic expression of each was unaffected and anaerobic expression was reduced to essentially the same level as aerobic expression. Furthermore, with the pMW61 derivative (pMW617) aerobic expression was stimulated 20-fold by nitrate, confirming that the stimulation of transcription by NarL occurs in the absence of a functional Fnr box under both aerobic and anaerobic conditions.

The various plasmids were also expressed in the *fnr* mutant ES2001 to establish that the effects observed with the Fnr box mutations would also result from the absence of a functional Fnr protein (Table 2). Expression levels were higher in strain ES2001 because this strain does not carry a *pcn* mutation and because plasmids were present at high copy number. With both pMW64 and pMW61 the levels of expression were low and essentially the same under all conditions, as expected when there is no functional Fnr to interact with the Fnr box and stimulate anaerobic transcription. Expression with the consensus −10 derivative of pMW64 (pMW646) was significantly higher than with pMW64 but was virtually the same under all conditions. The same general results were observed with plasmid pMW61 and its consensus −10 derivative (pMW616), except that nitrate stimulated transcription with the consensus −10 derivative to the same degree under both aerobic and anaerobic conditions. These results confirmed that a functional Fnr protein as well as a functional Fnr box is required for anaerobic activation of transcription and that neither is required for the stimulation of transcription by nitrate.

TABLE 2. Effect of mutations in the −10 and −35 hexamers on expression from plasmids in an *fnr* mutant (strain ES2001)

Plasmid	Mutation[a]	β-Galactosidase activity[b] during:			
		Aerobic growth	Aerobic growth with nitrate	Anaerobic growth	Anaerobic growth with nitrate
pMW64		130	150	240	220
pMW646	C −10	1,330	1,340	1,870	1,600
pMW61		30	60	40	110
pMW612	M −35	17	31	36	73
pMW616	C −10	550	12,500	1,430	13,600
pMW618	C −10/M −35	110	2,000	330	4,130

[a] Mutations are designated as in Table 1.
[b] β-Galactosidase activity is expressed in Miller units (15).

TABLE 3. Expression from plasmids with a consensus −10 hexamer in a *narL* mutant (strain RK5278)

Plasmid	Mutation[a]	β-Galactosidase activity[b] during:			
		Aerobic growth	Aerobic growth with nitrate	Anaerobic growth	Anaerobic growth with nitrate
pMW61		12	16	800	380
pMW616	C −10	410	490	14,800	10,400
pMW617	C −10/M FB	370	430	880	600
pMW618	C −10/M −35	120	140	13,300	9,700

[a] Mutations are designated as in Table 1.
[b] β-Galactosidase activity is expressed in Miller units (15).

Furthermore, when the −35 mutation was included in the pMW61 derivatives, the level of expression promoted by nitrate was severely reduced (Table 2), demonstrating that transcription in the absence of Fnr depends on the −35 sequence under both aerobic and anaerobic conditions.

Expression of the pMW61 derivatives in the *narL* mutant RK5278 (Table 3) showed that nitrate stimulation of transcription involved a NarL-dependent mechanism regardless of the mode of transcription. In the absence of a functional NarL protein, nitrate did not stimulate expression either with pMW61 under anaerobic conditions or with the consensus −10 derivative (pMW616) under aerobic conditions. Similarly, it did not stimulate transcription which was exclusively Fnr dependent (pMW618) or that which was exclusively −35 sequence dependent (pMW617). Nitrate led to a slight decrease in transcription under anaerobic conditions, presumably due to the autoregulation of *nar* operon expression by the activity of nitrate reductase produced from the chromosome (3).

DISCUSSION

Promoter sequences of positively controlled genes in general do not conform well with the consensus elements present in promoters of constitutively expressed genes and are thought, therefore, to require the binding of transacting factors to initiate transcription (17). The −10 region of the *narGHJI* operon matches 3 of the 6 bases of the consensus −10 hexamer for the σ^{70}-RNA polymerase of *E. coli*. When 1 of the 3 homologous bases in this region was mutated, expression was abolished under all conditions, indicating that transcription is dependent on some homology in the −10 region. However, when the 3 nonhomologous bases were changed to create a consensus −10 sequence, there was significant expression aerobically which was raised to a high level by the addition of nitrate. It appears that partial homology in this region is essential for the physiological responsiveness of this operon in that some divergence from the consensus −10 sequence region is necessary to limit expression to anaerobic conditions.

The −35 regions of positively controlled genes often exhibit even less homology to the consensus hexamer for σ^{70}-RNA polymerase (17). However, the −35 region of the *narGHJI* operon is homologous in four positions to the consensus −35 sequence. Changing one of the most highly conserved bases (9) in this sequence away from consensus had little effect on anaerobic expression, and when the same mutation was made in promoters with consensus −10 regions, there was decreased aerobic expression while maximal anaerobic expression was maintained. These results demonstrated that there are two distinct modes of transcription for the *nar* operon. One mode depends on the −10 and −35 hexamer sequences and occurs in the absence of Fnr protein. The rate of this mode of transcription is very low, but it was dramatically enhanced by changing the −10 sequence to the consensus TATAAT. The second mode depends on the −10 and Fnr box sequences but is independent of the −35 sequence. This mode occurs at a high level only when Fnr is activated under anaerobic conditions, and it was also enhanced by changing the −10 region to the consensus sequence. While it is possible that two distinct forms of RNA polymerase are responsible for the two modes of transcription, the enhancement of each by the change to the consensus −10 sequence for the σ^{70}-RNA polymerase suggests that this form of the enzyme catalyzes both modes.

The pathway of transcription initiation consists of a number of steps, starting with the binding of RNA polymerase at the appropriate site and ending with elongation of the RNA molecule, and positive controlling factors have been found to act at various steps in the process (1). It is likely that Fnr affects an early step in transcription initiation such as site recognition or binding, since it appears to be compensating for the divergent −10 sequence. It may also affect later steps, since under anaerobic conditions in the presence of the consensus −10 sequence, expression increases to an apparent maximum level of expression. Sequence homology between Fnr and Crp and the interconversion of the DNA binding specificities of Fnr and Crp suggested that the mechanisms of transcription activation by both are similar (18, 20). Crp-binding sites are located at several discrete positions relative to the transcription start site (8). For certain Crp-activated promoters, the location is centered approximately 40 bp upstream, and it has been determined that 29 bp is the optimal spacing between the −10 hexamer and the center of the Crp-binding site (26). The center of the Fnr-binding site of the *nar* operon is also separated from the −10 hexamer by this number of bases. Unlike Crp, which can also activate transcription when bound 61.5 bp or farther upstream, Fnr does not induce expression when its binding site is positioned more than 41.5 bp upstream (27). However, it appears that Crp induces transcription by a different mechanism when bound at that position (8).

Our results demonstrate that Fnr-dependent transcription of the *nar* operon is not dependent on the −35 sequence which is required for the Fnr-independent mode of transcription. Chan et al. (4) have suggested that when RNA polymerase activity is independent of a −35 sequence, the overall architecture of RNA polymerase-promoter complexes varies so that the DNA is distorted and upstream regions become important. This may be true for the *nar* operon, since preliminary experiments in our laboratory indicate that DNA sequences upstream from the Fnr-binding site affect the rate of anaerobic transcription. Unfortunately,

it has not yet been possible to establish which regions of the promoter are protected by binding of Fnr (24).

Our observation that NarL-dependent nitrate stimulation of expression occurs with aerobic, Fnr-independent transcription as well as with anaerobic, Fnr-dependent transcription suggests that it involves an Fnr-independent mechanism. Our observations do not rule out the possibility that the single-base change from the wild-type promoter sequence at position −13, present in the complete promoter constructions, altered the mechanism involved from Fnr dependence to Fnr independence. However, this possibility seems unlikely on the basis of several published and unpublished observations. We have previously shown that except for a slight increase in expression under all conditions, the creation of two restriction enzyme sites between the −10 and −35 regions, which included the −13 base change, did not affect the relative increase resulting from Fnr activation of a truncated promoter under anaerobic conditions (27). More directly, with the −13 change in the complete promoter (plasmid pMV61, Table 1), nitrate induced the same relative increases in aerobic and anaerobic expression as it did with the wild-type promoter (data not shown), arguing against any change in the mechanism of regulation by NarL. In addition, Bonnefoy et al. (2) have also suggested that nitrate stimulation of *nar* operon expression occurs by an Fnr-independent mechanism, on the basis of their observations that a strain with a mutation in the *nar* promoter region, which led to derepressed aerobic, Fnr-independent expression, was nevertheless induced by nitrate under aerobic as well as anaerobic conditions. From these observations it seems likely that NarL stimulates transcription through an interaction with RNA polymerase which has already bound to the promoter by recognition of the sequence motifs which promote either aerobic or anaerobic transcription.

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REFERENCES

1. **Adhya, S., and S. Garges.** 1990. Positive control. J. Biol. Chem. **265**:10797–10800.
2. **Bonnefoy, V., M.-C. Pascal, J. Ratouchniak, and M. Chippaux.** 1986. Alteration by mutation of the control by oxygen of the *nar* operon in *Escherichia coli*. Mol. Gen. Genet. **205**:349–352.
3. **Bonnefoy, V., M.-C. Pascal, J. Ratouchniak, and M. Chippaux.** 1986. Autoregulation of the *nar* operon encoding nitrate reductase in *Escherichia coli*. Mol. Gen. Genet. **204**:180–184.
4. **Chan, B., A. Spassky, and S. Busby.** 1990. The organization of open complexes between *Escherichia coli* RNA polymerase and DNA fragments carrying promoters either with or without consensus −35 region sequences. Biochem. J. **270**:141–148.
5. **Chippaux, M., V. Bonnefoy-Orth, J. Ratouchniak, and M.-C. Pascal.** 1981. Operon fusions in the nitrate reductase operon and study of the control gene *nirR* in *Escherichia coli*. Mol. Gen. Genet. **182**:477–479.
6. **Chippaux, M., D. Guidici, A. Abou-Jaoude, F. Casse, and M.-C. Pascal.** 1978. A mutation leading to the total lack of nitrate reductase activity in *Escherichia coli* K-12. Mol. Gen. Genet. **160**:225–229.
7. **Eiglmeier, K., N. Honoré, S. Iuchi, E. C. C. Lin, and S. T. Cole.** 1989. Molecular genetic analysis of FNR-dependent promoters. Mol. Microbiol. **3**:869–878.
8. **Gaston, K., A. Bell, A. Kolb, H. Buc, and S. Busby.** 1990. Stringent spacing requirements for transcription activation by CRP. Cell **62**:733–743.
9. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15**:2343–2361.
10. **Jayaraman, P.-S., K. L. Gaston, J. A. Cole, and S. J. W. Busby.** 1988. The *nirB* promoter of *Escherichia coli*: location of sequences essential for regulation by oxygen, the FNR protein and nitrite. Mol. Microbiol. **2**:527–530.
11. **Lambden, P. R., and J. R. Guest.** 1976. Mutants of *Escherichia coli* K12 unable to use fumarate as an anaerobic electron acceptor. J. Gen. Microbiol. **97**:145–160.
12. **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. **263**:13700–13705.
13. **Liu, J., and J. S. Parkinson.** 1989. Genetics and sequence analysis of the *pcnB* locus, an *Escherichia coli* gene involved in plasmid copy number control. J. Bacteriol. **171**:1254–1261.
14. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
15. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355, 431. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Newman, B. M., and J. A. Cole.** 1978. The chromosomal location and pleiotropic effects of mutations of the *nirA*$^+$ gene of *Escherichia coli*: the essential role of *nirA*$^+$ in nitrite reduction and in other anaerobic redox reductions. J. Gen. Microbiol. **106**:1–12.
17. **Raibaud, O., and M. Schwartz.** 1984. Positive control of transcription in bacteria. Annu. Rev. Genet. **18**:173–206.
18. **Shaw, D. J., D. W. Rice, and J. R. Guest.** 1983. Homology between CAP and FNR, a regulator of anaerobic respiration in *Escherichia coli*. J. Mol. Biol. **166**:241–247.
19. **Sodergren, E. J., and J. A. DeMoss.** 1988. *narI* region of the *Escherichia coli* nitrate reductase (*nar*) operon contains two genes. J. Bacteriol. **170**:1721–1729.
20. **Spiro, S., K. L. Gaston, A. I. Bell, R. E. Roberts, S. J. W. Busby, and J. R. Guest.** 1990. Interconversion of the DNA-binding specificities of two related transcription regulators, CRP and FNR. Mol. Microbiol. **4**:1831–1838.
21. **Spiro, S., and J. R. Guest.** 1987. Regulation and overexpression of the *fnr* gene of *Escherichia coli*. J. Gen. Microbiol. **133**:3279–3288.
22. **Stewart, V.** 1982. Requirement of Fnr and NarL functions for nitrate reductase expression in *Escherichia coli* K-12. J. Bacteriol. **151**:1320–1325.
23. **Stewart, V., and C. H. MacGregor.** 1982. Nitrate reductase in *Escherichia coli* K-12: Involvement of *chlC*, *chlE*, and *chlG* loci. J. Bacteriol. **151**:788–799.
24. **Trageser, M., S. Spiro, A. Duchêne, E. Kojro, F. Fahrenholz, J. R. Guest, and G. Unden.** 1990. Isolation of intact FNR protein (M_r30000) of *Escherichia coli*. Mol. Microbiol. **4**:21–27.
25. **Unden, G., and J. R. Guest.** 1985. Isolation and characterization of the FNR protein, the transcriptional regulator of anaerobic electron transport in *Escherichia coli*. Eur. J. Biochem. **146**: 193–199.
26. **Valentin-Hansen, P., B. Holst, L. Søgaard-Andersen, J. Martinussen, J. Nesvera, and S. R. Douthwaite.** 1991. Design of cAMP-CRP-activated promoters in *Escherichia coli*. Mol. Microbiol. **5**:433–437.
27. **Walker, M. S., and J. A. DeMoss.** 1991. Promoter sequence requirements for Fnr-dependent activation of transcription of the *narGHJI* operon. Mol. Microbiol. **5**:353–360.