JOURNAL OF BACTERIOLOGY, May 2009, p. 3172–3176 Vol. 191, No. 9
0021-9193/09/$08.00+0 doi:10.1128/JB.00040-09

Activation and Repression at the *Escherichia coli ynfEFGHI* Operon Promoter[▽]

Meng Xu, Stephen J. W. Busby,* and Douglas F. Browning

School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

Received 14 January 2009/Accepted 15 February 2009

Induction of the *Escherichia coli* K-12 *ynfEFGHI* operon in response to anaerobiosis is repressed by nitrate ions. In this study, we show that the global transcription factor FNR is a class II activator at the *ynfEFGHI* promoter and that NarL represses activation by binding to a single target that overlaps the promoter −10 element. Electromobility shift assays show that NarL does not prevent RNA polymerase binding and suggest that repression may involve a quaternary NarL-FNR-RNA polymerase-promoter complex.

Expression of the *Escherichia coli dmsABC* operon, which encodes a dimethyl sulfoxide reductase, is induced in response to anaerobiosis but repressed by nitrate ions. Thus, the *dmsA* promoter is activated by FNR binding to a single site centered at position −41.5 (i.e., between bp −41 and −42) upstream of the transcript start and is repressed by NarL binding to multiple sites (1, 5, 9). The *ynfEFGHI* operon encodes proteins that are paralogues of the *dmsABC* dimethyl sulfoxide reductase (12). Recent genomic studies of the FNR and NarL regulons revealed that *ynfEFGHI* expression is also activated by FNR and repressed by NarL (4, 8), but the different promoter elements were not defined experimentally. Thus, here, we have used a combination of biochemistry and genetics to characterize the *ynfE* promoter.

Identification of *ynfE* promoter elements. The starting point of this work was the construction of the *ynfE100* fragment, illustrated in Fig. 1A, that carries 200 bp of the *Escherichia coli* K-12 chromosome sequence upstream from the *ynfE* translation start codon. To investigate the *ynfE* promoter, we exploited potassium permanganate footprinting to monitor open complex formation on an end-labeled fragment, using purified RNA polymerase (RNAP) holoenzyme, purified FNR, and purified NarL. Recall that permanganate cleaves T's within single-stranded DNA, enabling the detection of DNA unwinding at promoters (15). Results illustrated in Fig. 1B show that, with RNAP alone, no unwinding is detected, and with FNR present, opening is clearly observed, and this opening is suppressed by NarL. The upstream end of the region of unwinding corresponds to the hexamer 5′ TAGACT 3′, which is the likely −10 hexamer element. Hence, in Fig. 1, the *ynfE* promoter sequence is numbered with the upstream T of this hexamer as position −12 with respect to the putative transcription start point at +1, in accord with the start point previously assigned by Kang et al. (8), after analysis of FNR-dependent transcription in vitro.

DNA sites for FNR and NarL in the *ynfE* regulatory region. Using DNase I footprinting, we studied the binding of purified FNR and NarL to the *ynfE100* sequence. Results in Fig. 2 show that FNR and NarL each protect ~30 bp, consistent with binding of a dimer of each protein to a single target. Inspection of the region protected by FNR reveals a sequence that corresponds to the consensus for FNR (16), centered at position −40.5. Similarly, the region protected by NarL reveals a sequence that corresponds to the NarL consensus (6), centered at position −18.5 (Fig. 1A).

In vivo assays of *ynfE* promoter activity. The *ynfE100* fragment and derivatives carrying a substitution in the proposed −10 element (p11G) or in the putative target for FNR (p45C) were cloned into the *lac* expression plasmid vector, pRW50 (11), to generate *ynfE*::*lac* fusions. Figure 3 illustrates measurements of *lac* expression. As expected, the activity of the starting *ynfE* promoter is low under aerobic conditions and induced during anaerobiosis but repressed by nitrate (Fig. 3A). Consistent with our assignments for the −10 element and DNA site for FNR, induction is suppressed by either the p11G or p45C substitution (Fig. 3A). The experiment illustrated in Fig. 3B shows that induction of the *ynfE* promoter is lost in an *fnr* mutant host and that repression by nitrate is substantially relieved in a *narL* host. These results confirm that FNR and NarL, respectively, are responsible for activation and repression. Interestingly, the residual nitrate-dependent repression in the *narL* mutant host is lost in an *narL narP* double mutant, in which NarP, a NarL homologue, is inactivated (Fig. 3B).

FNR, NarL, and RNAP binding. Figure 4 shows autoradiographs from electromobility shift assays designed to investigate how NarL represses FNR-dependent activation of the *ynfE* promoter. Results in panels A and B show that both FNR and NarL give a single clear bandshift with some smearing at higher concentrations, which is consistent with the existence of a single strong binding site for each protein with some weaker nonspecific binding. Together, NarL and FNR produce a supershifted band, which shows that NarL and FNR can bind together. The experiment illustrated in Fig. 4C shows that, as expected, together, FNR and RNAP result in a supershifted complex. Figure 4C also shows that NarL fails to prevent

* Corresponding author. Mailing address: School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom. Phone: 44-121-414-5439. Fax: 44-121-414-5925. E-mail: s.j.w.busby@bham.ac.uk.

▽ Published ahead of print on 27 February 2009.

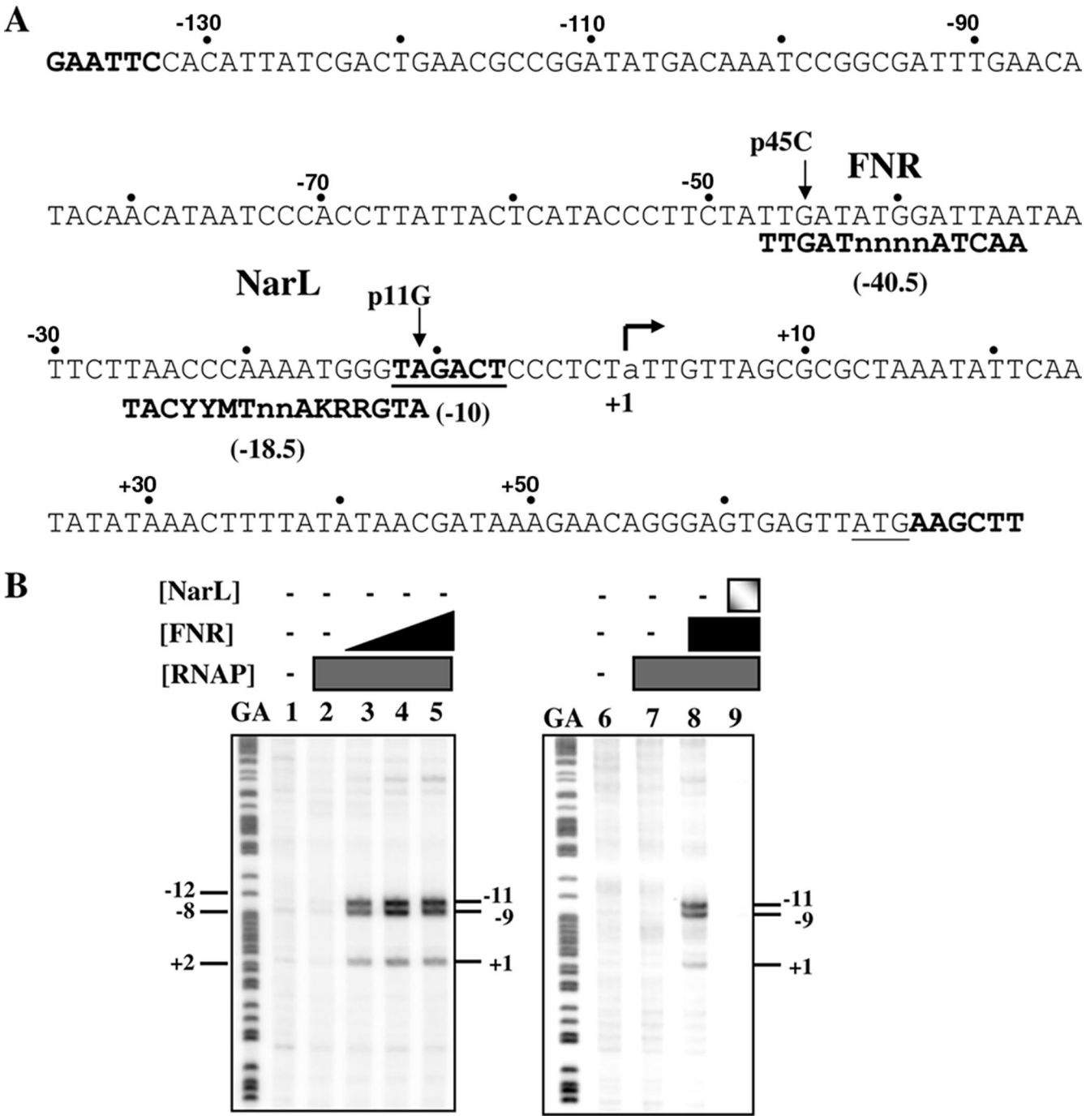

FIG. 1. Organization of the *E. coli ynfE* promoter region. (A) Base sequence of the *ynfE100* fragment that carries sequence from position −132 to +69 with respect to the proposed *ynfE* promoter transcript start (+1; designated by a lowercase letter and bent arrow), with flanking EcoRI and HindIII sites shown in boldface. The fragment was amplified by PCR from *E. coli* K-12 strain MG1655 genomic DNA (4). The proposed −10 hexamer element is in boldface and underlined, and the *ynfE* translation start ATG is underlined. Proposed DNA sites for FNR and NarL are aligned with the cognate consensus binding sequences (where Y = C or T, M = A or C, K = G or T, and R = A or G), and the center of each site is indicated (6, 16). Positions of the p11G and p45C substitutions in the −10 hexamer and FNR binding site, respectively, are indicated. (B) The end-labeled *ynfE* promoter fragment was incubated with purified RNAP, FNR, and NarL as indicated and subjected to potassium permanganate footprinting. The incubation buffer contained 20 mM HEPES (pH 8.0), 50 mM potassium glutamate, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 500 μg ml^{-1} bovine serum albumin. The experiment was performed as described by Browning et al. (3), using FNR with the DA154 substitution that renders FNR active under aerobic conditions, which had been overexpressed and purified as described by Wing et al. (20). NarL was generated after overexpression and purification of an NarL-maltose binding protein fusion and was used after activation with acetyl phosphate (7, 10). RNAP holoenzyme was purchased from Epicentre Technologies (Madison, WI). The concentrations of FNR were as follows: lane 3, 0.13 μM; lanes 4, 8, and 9, 0.25 μM; and lane 5, 0.5 μM. NarL and RNAP were used at concentrations of 0.2 μM and 50 nM, respectively. Gels were calibrated with Maxam-Gilbert sequencing reactions, and the locations of permanganate-induced cleavage are shown.

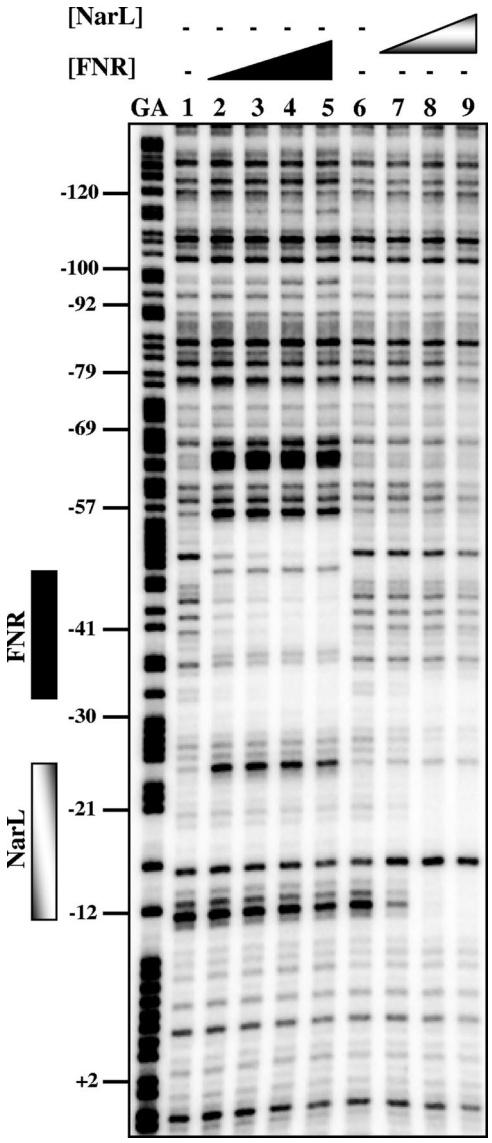

FIG. 2. DNase I footprinting of FNR and NarL binding to the *ynfE* promoter. End-labeled *ynfE* promoter fragment was incubated with either FNR DA154 or NarL and subjected to DNase I footprint analysis. The incubation buffer contained 20 mM HEPES (pH 8.0), 50 mM potassium glutamate, 5 mM $MgCl_2$, 1 mM dithiothreitol, 500 μg ml^{-1} bovine serum albumin, and 25 μg ml^{-1} herring sperm DNA. The concentrations of FNR DA154 were as follows: lanes 1 and 6 to 9, no protein; lane 2, 0.5 μM; lane 3, 1 μM; lane 4, 2 μM; and lane 5, 3 μM. The concentrations of NarL were as follows: lanes 1 to 6, no protein; lane 7, 0.4 μM; lane 8, 0.8 μM; and lane 9, 1.6 μM. The experiment was performed as described by Browning et al. (3), and gels were calibrated using Maxam-Gilbert sequencing reactions. Relevant positions are indicated, and the locations of FNR and NarL binding sites are shown by boxes.

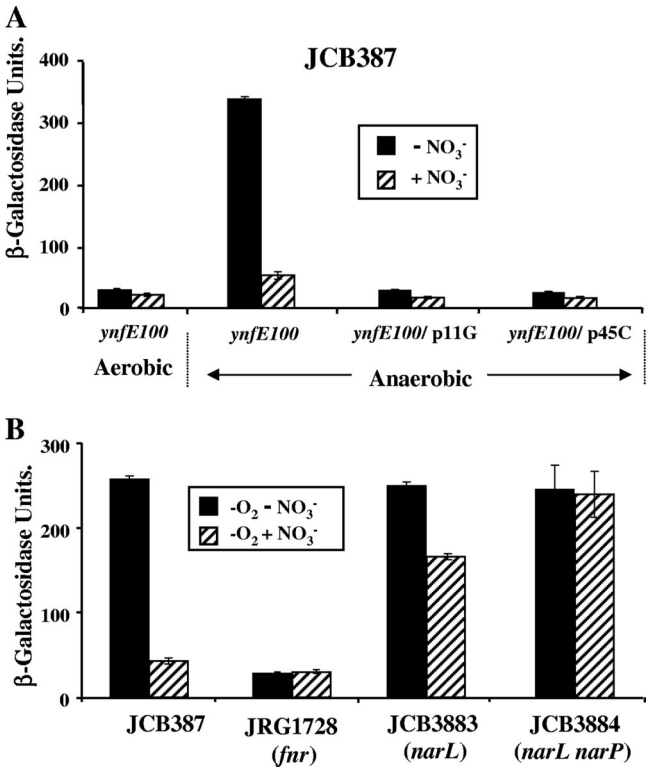

FIG. 3. Measurements of *ynfE* promoter activity. The figure illustrates measurements of β-galactosidase activity in different hosts carrying *ynfE* promoter::*lac* operon fusions in plasmid pRW50. Assays were performed using the Miller protocol (13) as in our previous work (2, 3). Cells were grown aerobically or anaerobically at 37°C in Lennox broth supplemented with 0.4% glucose with or without 20 mM sodium nitrate as indicated. Panel A shows activities measured in the *E. coli* K-12 Δ*lac* host strain, JCB387 (18), containing pRW50 derivatives with the starting *ynfE* promoter or the p11G or p45G substitutions. Panel B shows measured β-galactosidase activities in JCB387 cells and *fnr*, *narL*, and *narL narP* derivatives, described in reference 18, carrying pRW50 containing the *ynfE100* promoter fragment.

RNAP binding and that, in the presence of NarL, a new highly retarded complex appears that, most likely, contains NarL, FNR, and RNAP bound together to the *ynfE* promoter. Figure 4D shows an expanded segment of the autoradiograph to highlight the appearance of this new band.

Conclusions. The global transcription factor FNR controls the adaptation of *Escherichia coli* to growth under anaerobic conditions (16). At most target promoters, FNR binds to a site centered near position −41.5 and activates transcription by interacting directly with the RNAP σ and α subunits. Such promoters, known as class II promoters, are distinct from class I promoters, where FNR binds further upstream and interacts only with the RNAP α subunits (19). Here we present experimental evidence to show that the functional DNA site for FNR at the *ynfE* promoter is likely located at position −40.5 and, thus, as at the *dmsA* promoter (9), FNR functions as a class II activator. Many FNR-activated promoters are also regulated by NarL and its homologue, NarP, which share the same consensus target sequence and orchestrate adaptation to the presence of nitrate ions (6, 17). Thus, the *dmsA* promoter is repressed by NarL binding to multiple sites spread across 80 bp covering the entire promoter (1, 5). In contrast, the *ynfE* promoter has a single strong DNA site for NarL (and likely for NarP). Very little is known about how NarL and NarP repress transcription, and it is often assumed that they simply block access of RNAP to target promoters (1). Our results suggest that repression by NarL at the *ynfE* promoter may be unusual and might be due to locking of the FNR-RNAP-promoter

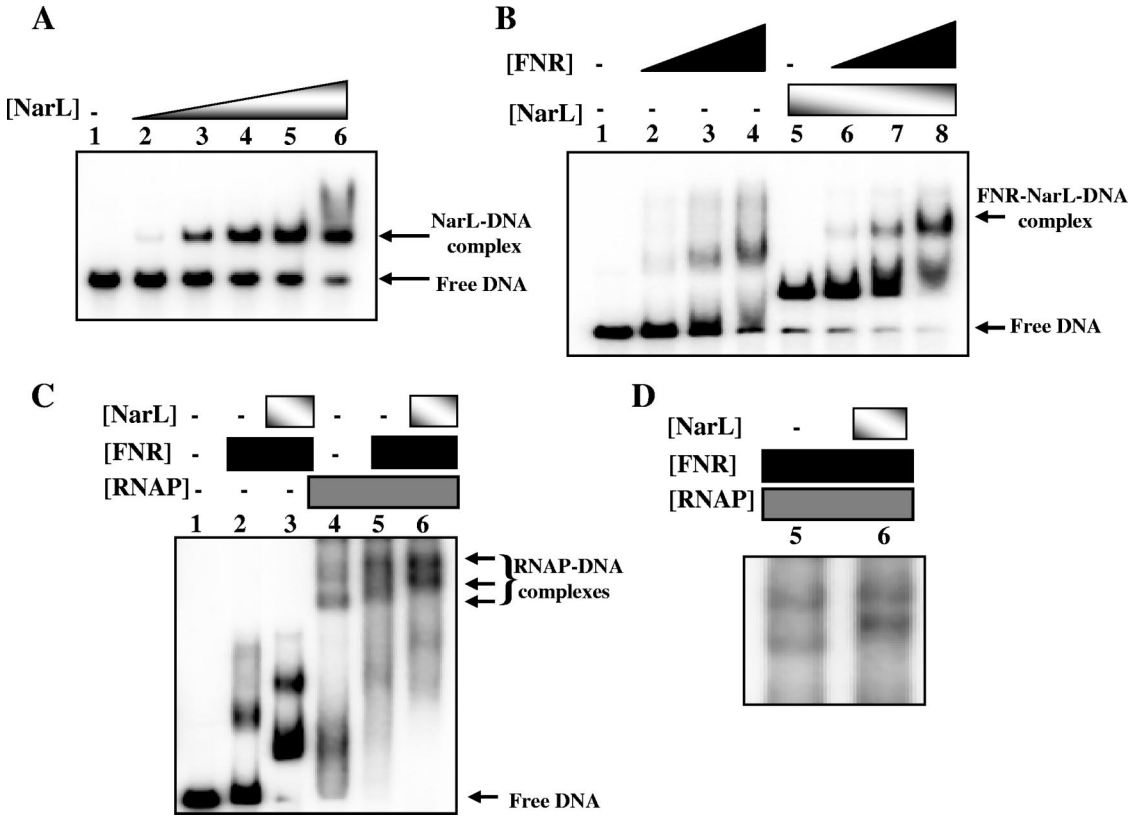

FIG. 4. Electromobility shift assays with the *ynfE* promoter fragment. Experiments were performed with labeled *ynfE100* promoter fragment using the protocols described by Browning et al. (2). The incubation buffer contained 10 mM potassium phosphate (pH 7.5), 100 mM potassium glutamate, 1 mM EDTA, 50 μM dithiothreitol, 5% glycerol, 25 μg ml^{-1} herring sperm DNA, and 50 mM acetyl phosphate. (A) The fragment was incubated with NarL. Lane 1, no protein; lane 2, 0.2 μM; lane 3, 0.4 μM; lane 4, 0.8 μM; lane 5, 1.6 μM; and lane 6, 3.2 μM. (B) The fragment was incubated with both FNR DA154 and NarL as indicated. The concentrations of FNR DA154 were as follows: lanes 1 and 5, no protein; lanes 2 and 6, 1 μM; lanes 3 and 7, 2 μM; and lanes 4 and 8, 3 μM. The concentrations of NarL were as follows: lanes 1 to 4, no protein; and lanes 5 to 8, 1.6 μM. (C) The fragment was incubated with 1.5 μM FNR DA154, 1.6 μM NarL, and 174 nM RNAP as indicated. FNR and NarL were preincubated with the fragment before RNAP was added. (D) Expansion of the top right segment of panel C, covering lanes 5 and 6, to highlight the NarL-induced shift of *ynfE* promoter complexes with RNAP.

ternary complex, as has been shown for some other repressors (14). Since the biological function of the *ynfEFGHI* operon remains uncertain (12), the significance of this awaits discovery.

This work was generously supported by a Wellcome Trust Programme Grant.

REFERENCES

1. **Bearson, S. M., J. A. Albrecht, and R. P. Gunsalus.** 2002. Oxygen and nitrate-dependent regulation of *dmsABC* operon expression in *Escherichia coli*: sites for Fnr and NarL protein interactions. BMC Microbiol. **2:**13.
2. **Browning, D. F., C. M. Beatty, A. J. Wolfe, J. A. Cole, and S. J. W. Busby.** 2002. Independent regulation of the divergent *Escherichia coli nrfA* and *acs*P1 promoters by a nucleoprotein assembly at a shared regulatory region. Mol. Microbiol. **43:**687–701.
3. **Browning, D. F., D. C. Grainger, C. M. Beatty, A. J. Wolfe, J. A. Cole, and S. J. W. Busby.** 2005. Integration of three signals at the *Escherichia coli nrf* promoter: a role for Fis protein in catabolite repression. Mol. Microbiol. **57:**496–510.
4. **Constantinidou, C. C., J. L. Hobman, M. D. Patel, C. W. Penn, J. A. Cole, and T. W. Overton.** 2006. A reassessment of the fumarate and nitrate reduction regulon and transcriptomic analysis of the effects of nitrate, nitrite, NarXL and NarQP as *Escherichia coli* adapts from aerobic to anaerobic growth. J. Biol. Chem. **281:**4802–4808.
5. **Cotter, P. A., and R. P. Gunsalus.** 1989. Oxygen, nitrate, and molybdenum regulation of *dmsABC* gene expression in *Escherichia coli*. J. Bacteriol. **171:** 3817–3823.
6. **Darwin, A. J., and V. Stewart.** 1996. The NAR modulon systems: nitrate and nitrite regulation of anaerobic gene expression, p. 343–359. *In* E. Lin and A. Lynch (ed.) Regulation of gene expression in *Escherichia coli*.fs R. G. Landes Company, Austin, TX.
7. **Darwin, A. J., K. L. Tyson, S. Busby, and V. Stewart.** 1997. Differential regulation by the homologous response regulators NarL and NarP of *Escherichia coli* K-12 depends on DNA binding site arrangement. Mol. Microbiol. **25:**583–595.
8. **Kang, Y., K. D. Weber, Y. Qiu, P. J. Kiley, and F. R. Blattner.** 2005. Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. J. Bacteriol. **187:**1135–1160.
9. **Lamberg, K. E., and P. J. Kiley.** 2000. FNR-dependent activation of the class II *dmsA* and *narG* promoters of *Escherichia coli* requires FNR-activating regions 1 and 3. Mol. Microbiol. **38:**817–827.
10. **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* K-12. J. Mol. Biol. **241:**150–165.
11. **Lodge, J., J. Fear, S. Busby, P. Gunasekaran, and N.-R. Kamini.** 1992. Broad host range plasmids carrying the *Escherichia coli* lactose and galactose operons. FEMS Microbiol. Lett. **95:**271–276.
12. **Lubitz, S. P., and J. H. Weiner.** 2003. The *Escherichia coli ynfEFGHI* operon encodes polypeptides which are paralogues of dimethyl sulfoxide reductase (DmsABC). Arch. Biochem. Biophys. **418:**205–216.
13. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
14. **Rojo, F.** 1999. Repression of transcription initiation in bacteria. J. Bacteriol. **181:**2987–2991.
15. **Savery, N., T. Belyaeva, and S. Busby.** 1996. Protein-DNA interactions, p.

1–5 and 21–33. *In* K. Docherty (ed.), Gene transcription: DNA binding proteins. BIOS Scientific Publishers, Oxford, United Kingdom.
16. **Spiro, S., and J. R. Guest.** 1990. FNR and its role in oxygen-regulated gene expression in *Escherichia coli*. FEMS Microbiol. Rev. **6:**399–428.
17. **Stewart, V.** 2003. Nitrate- and nitrite-responsive sensors NarX and NarQ of proteobacteria. Biochem. Soc. Trans. **31:**1–10.
18. **Tyson, K. L., J. A. Cole, and S. J. W. Busby.** 1994. Nitrite and nitrate regulation at the promoters of two *Escherichia coli* operons encoding nitrite reductase: identification of common target heptamers for NarP- and NarL-dependent regulation. Mol. Microbiol. **13:**1045–1055.
19. **Wing, H. J., S. M. Williams, and S. J. W. Busby.** 1995. Spacing requirements for transcription activation by *Escherichia coli* FNR protein. J. Bacteriol. **177:**6704–6710.
20. **Wing, H. J., J. Green, J. R. Guest, and S. J. W. Busby.** 2000. Role of activating region 1 of *Escherichia coli* FNR protein in transcription activation at class II promoters. J. Biol. Chem. **275:**29061–29065.