RESEARCH LETTER

FNR-mediated regulation of bioluminescence and anaerobic respiration in the light-organ symbiont *Vibrio fischeri*

Alecia N. Septer¹, Jeffrey L. Bose³, Anne K. Dunn¹,² & Eric V. Stabb¹

¹Department of Microbiology, University of Georgia, Athens, GA, USA; and ²Department of Botany and Microbiology, University of Oklahoma, Norman, OK, USA

**Abstract**

*Vibrio fischeri* induces both anaerobic respiration and bioluminescence during symbiotic infection. In many bacteria, the oxygen-sensitive regulator FNR activates anaerobic respiration, and a preliminary study using the light-generating *lux* genes from *V. fischeri* MJ1 cloned in *Escherichia coli* suggested that FNR stimulates bioluminescence. To test for FNR-mediated regulation of bioluminescence and anaerobic respiration in *V. fischeri*, we generated *fnr* mutants of *V. fischeri* strains MJ1 and ES114. In both strains, FNR was required for normal fumarate- and nitrate-dependent respiration. However, contrary to the report in transgenic *E. coli*, FNR mediated the repression of *lux*. *ArcA* represses bioluminescence, and *P_{arcA-lacZ}* reporters showed reduced expression in *fnr* mutants, suggesting a possible indirect effect of FNR on bioluminescence via *arcA*. Finally, the *fnr* mutant of ES114 was not impaired in colonization of its host squid, *Euprymna scolopes*. This study extends the characterization of FNR to the *Vibrionaceae* and underscores the importance of studying *lux* regulation in its native background.

**Introduction**

*Vibrio fischeri* is a model for investigations of bioluminescence and mutualistic symbioses, two fields connected by the importance of oxygen. O₂ is a substrate for the luminescence-producing enzyme luciferase, and luciferase may benefit *V. fischeri* by generating a more reduced environment in or near cells (Visick *et al.*, 2000; Timmins *et al.*, 2001). Reduction of O₂ could be especially advantageous for this facultative anaerobe when it is colonizing animal tissue and may minimize the host’s ability to generate reactive oxygen species (Visick *et al.*, 2000). Luminescence emanating from bacteria colonizing the symbiotic light organ of the host indicates that O₂ is present; however, evidence suggests that luciferase is O₂ limited in this environment (Boettcher *et al.*, 1996) despite its high affinity ($K_m$~35 nM) for O₂ (Bourgois *et al.*, 2001). Moreover, anaerobic respiration is apparently induced in symbiotic *V. fischeri* (Proctor & Gunsalus, 2000), consistent with the idea that [O₂] is low in the light organ.

One regulator that might control anaerobic respiration and luminescence in response to [O₂] is FNR (so named for its role in fumarate and nitrate reduction). FNR regulates genes during the switch between aerobic and anaerobic growth in *Escherichia coli* and other bacteria, and it often activates genes responsible for anaerobic respiration (Browning *et al.*, 2002; Reents *et al.*, 2006; Fink *et al.*, 2007). Although FNR is expressed during both aerobic and anaerobic growth, it is only functional under microaerobic or anaerobic conditions due to its dependence on an oxygen-labile 4Fe–4S center (Khoroshilova *et al.*, 1995, 1997; Lazazzera *et al.*, 1996; Kiley & Beinert, 1998). Under anaerobic conditions, [4Fe–4S]-FNR forms a functional dimer that binds DNA at a 5’-TTGAT(N₄)ATCAA-3’ FNR-box sequence (Eiglemeier *et al.*, 1989), and it activates or represses transcription depending on the location of binding relative to the promoter (Wing *et al.*, 1995; Meng *et al.*, 1997; Marshall *et al.*, 2001).

FNR was reported to activate bioluminescence in transgenic *E. coli* carrying the *V. fischeri* MJ1 *luxR-luxICDABEG* region, which encodes the autoinducer-dependent *lux* activator LuxR, the autoinducer synthase LuxI, and the Lux proteins that produce bioluminescence (Muller-Breikreutz & Winkler, 1993). Although FNR-mediated regulation of luminescence is cited frequently (Meighen, 1994; Spiro, 1994; Sitnikov *et al.*, 1995; Ulitzur & Dunlap, 1995; Stevens & Greenberg, 1999), these data were only presented in
whether FNR contributes to symbiotic competence.

We have examined fnr in two V. fischeri strains: ES114 and MJ1. ES114’s genome is sequenced, and its symbiosis with the squid Euprymna scolopes can be reconstituted in the laboratory (Ruby et al., 2005; Stabb, 2006); however, like most isolates from these animals, ES114 is not visibly luminescent in culture (Boettcher & Ruby, 1990). In contrast, MJ1 has bright luminescence typical of isolates from the pinecone fish Monocentris japonica, but this symbiosis is not yet experimentally tractable. The genes required for luminescence and autoinduction are similar in the two strains, with the luxICDABEG operon adjacent to and divergently transcribed from luxR (Gray & Greenberg, 1992). However, there are differences in the luxR-luxI intergenic region, and notably there is a putative FNR box in MJ1 that is absent in ES114. Our goals were to examine V. fischeri to assess FNR’s regulation of luminescence and anaerobic respiration, and to determine whether FNR contributes to symbiotic competence.

Materials and methods

Bacteria and media

The bacterial strains used in this study are described in Table 1. Escherichia coli was grown in Luria–Bertani (Miller, 1992) or in M9 (Sambrook et al., 1989) supplemented with 1 mg mL\(^{-1}\) casamino acids, 40 mM glycerol, and 40 mM of either sodium nitrate or sodium fumarate. Vibrio fischeri was grown in Luria broth plus salt (LBS) (Stabb et al., 2001), sea water tryptone (SWT) (Boettcher & Ruby, 1990), wherein seawater was replaced with Instant Ocean (Aquarium Systems, Mentor, OH), sea water tryptone at high osmolarity (SWTO) (Bose et al., 2007), or in a defined salts medium (Adin et al., 2009) with 40 mM glycerol as a carbon source, 1 mg mL\(^{-1}\) casamino acids, and 40 mM of sodium nitrate or sodium fumarate. Agar (15 mg mL\(^{-1}\)) was added to solidify media for plating. Anaerobic growth on plates was assessed using the GasPak EZ Anaerobic Container System from Becton, Dickinson and Company (Sparks, MD). Antibiotics were added as described previously for selection (Stabb & Ruby, 2002), and N-3-oxo-hexanoyl homoserine lactone (3-oxo-C6-HSL) autoinducer was added to the media at 140 nM.

Genetic manipulations

Cloning was performed using standard procedures, with plasmids transformed in E. coli strain DH5\(\alpha\) or DH5\(\alpha\)2pir, as described previously (Bose et al., 2008). Cloned PCR products were sequenced to ensure that unintended alterations were not incorporated. Sequencing was conducted at the University of Michigan DNA Sequencing Core Facility or at the University of Georgia Molecular Genetics Instrumentation Facility. Plasmids were mobilized into V. fischeri from E. coli by triparental mating using strain CC118\(\alpha\)pir with pEVS104 as a helper (Stabb & Ruby, 2002), and mutations were placed on the chromosome by allelic exchange. Parent strains and plasmids used for allelic exchange are listed in Table 1.

Key plasmids and oligonucleotides are described in Table 1, and an overview of allele construction follows. To mutate fnr, an ~3.3-kb region of the V. fischeri genome centered on fnr was PCR amplified with primers EVS97 and EVS98 using ES114 or MJ1 genomic DNA as a template, and the fragments were ultimately subcloned into pEVS136 and pJLB69, respectively (Table 1). We generated Δfnr::tmpR alleles by replacing the ClaI to AvrII fragment of fnr with the trimethoprim-resistance gene (tmpR) from pJLB1 (Dunn et al., 2005) on a BstB1 to AvrII fragment, resulting in tmpR replacing an internal 255-bp fragment beginning in the middle of fnr, with tmpR in the same orientation as fnr. The ES114-derived Δfnr::tmpR allele was placed in pJLB5 and pJLB70, and the MJ1-derived Δfnr::tmpR allele was used in pCDW5. For complementation of E. coli with ES114 fnr, we ligated the fnr-containing BsrBI–PstI fragment from pEVS136 into Smal– and PstI-digested pDAM5, generating pJLB6. To place lacZ under control of the arcA promoter, we PCR amplified an ~3.1-kb fragment containing an engineered lacZ (Tomich et al., 1988) using pVSV3 (Dunn et al., 2006) as a template and primers JBLACZ1 and JBLACZ2 (Table 1). We cloned this product into Smal-digested pA14 and pJLB55 (Bose et al., 2007), which carry regions flanking arcA from ES114 and MJ1, respectively, with the sequence between the start and the stop codons of arcA replaced by a 6-bp Smal recognition site. The P_\text{arcA-lacZ} alleles contain the arcA start codon, followed by a 5′-CCC-3′ proline codon, and then the lacZ reporter (Tomich et al., 1988) from its second codon onward. These ES114- and MJ1-derived alleles were subcloned into pAS31 and pJLB139, respectively.

Growth and luminescence

Overnight cultures in LBS were diluted 1 : 1000 into SWTO and incubated at 24 °C with shaking (200 r.p.m.). Aerobic cultures contained 50 mL of SWTO in 250-mL flasks. For anaerobic cultures, aerobically grown overnight cultures were diluted 1 : 10 in LBS before inoculation of 0.2 mL into 20 mL SWTO in 165-mL sealed bottles with a headspace containing 5% CO\(_2\), 10% H\(_2\), and 85% N\(_2\). Samples (500 µL each) were removed periodically and culture optical density \(\text{OD}_{595\text{nm}}\) was determined using a BioPhotometer (Brinkman Instruments, Westbury, NY) or a SmartSpec 3000 (BioRad Laboratories, Hercules, CA). After measuring OD\(_{595\text{nm}}\), cuvettes were covered with parafilm and shaken vigorously for ~10 s to aerate the sample, followed by
determination of luminescence using a GLOMAX 20/20 luminometer (Promega, Madison, WI).

**Quantitative reverse transcriptase (RT)-PCR**

Triplicate aerobic cultures of ES114 and JB1 were grown in LBS to an OD$_{595\text{ nm}}$~2.1. Samples (1 µL each) were removed, added to microcentrifuge tubes containing 1/5 volume 5% (v/v) phenol, pH 4.3, with 95% (v/v) ethanol, and placed on ice for 30 min. Samples were centrifuged and the pellets were stored at $-80 \degree C$ overnight. Pellets were thawed, and RNA was isolated using Absolutely RNA Mini-preps (Stratagene, La Jolla, CA). RNA was treated using the Turbo DNA-free kit (Applied Biosystems, Foster City, CA),
and RNA quantity and purity were assessed using a Biotek Synergy 2 plate reader with Take3 Multi-Volume Plate and software (Winooski, VT). RNA was then stored at −80 °C. cDNA was synthesized using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), and reactions were cleaned using a DNA Clean & Concentrator-5 kit (Zymo Research, Orange, CA). cDNA was quantified using the Synergy 2 plate reader. Real-time PCR was performed using the MyIQR Single-Color Real-Time PCR Detection System (BioRad Laboratories), and reactions were set up using the BioRad IQ SYBR Green Supermix. Primers AS1310RTF2 and AS1310RTR2 were used to determine the level of VF1310 cDNA. ES114 genomic DNA was used to generate a standard curve. Real-time PCR data were analyzed using BioRad IQ™5 software.

**lacZ reporter expression**

To determine P<sub>arcA-lacZ</sub> reporter expression, strains were grown overnight in LBS and diluted 1:1000 in 20 mL SWTO in 250-mL baffled flasks and grown at 24 °C with shaking to an OD of ~0.1. Four hundred microliters were inoculated to inoculate 20 mL SWTO in anaerobic bottles. These were incubated at 24 °C with shaking until peak luminescence was reached. Strains were also grown aerobically in 20-mL SWTO in 50-mL conical tubes at 28 °C with shaking to 1 °C. The next day, the pellet was thawed and resuspended in Z-buffer for determination of β-galactosidase activity expressed as Miller units as described previously (Miller, 1992).

**Symbiotic colonization assays**

Inoculant strains were grown unshaken in 5 mL of SWT in 50-mL conical tubes at 28 °C to an OD<sub>595</sub> of approximately 0.3–1.0, and cultures were diluted in Instant Ocean to a density no higher than 1700 CFU mL<sup>−1</sup>. In each experiment, the inoculant density of wild-type and mutants strains was equivalent, and this was checked by plating the inocula on LBS. Hatchling squid were placed in these inocula for up to 14 h before being rinsed in V. fischeri-free Instant Ocean. To study infection kinetics, the squid were placed in 5 mL of inoculant in scintillation vials, and the onset of luminescence was monitored using an LS6500 scintillation counter (Beckman Coulter, Fullerton, CA). For mixed-strain competitions, hatchlings were exposed to an inoculum containing an ~1:1 ratio of wild type and mutant. At 48-h postinoculation, individual squid were homogenized and dilution plated on LBS. The resulting colonies were patched onto LBS with added trimethoprim to determine the ratio of strains in each animal. Inocula were similarly plated and patched to determine the starting ratio. The relative competitiveness index (RCI) was determined by dividing the mutant to wild-type ratio in each animal by the ratio of these strains in the inoculum. The mean RCI was calculated from log-transformed data.

**Results**

**Identification of V. fischeri fnr**

BLAST searches (Altschul et al., 1990) of the V. fischeri ES114 genome revealed the similarity of ORFs VF1308 and VF1309 to the N and C termini of E. coli FNR, respectively (Fig. 1a). We suspected that a sequencing error had led to the misannotation of fnr as two genes, and we therefore cloned and sequenced the region spanning VF1308 and VF1309. We found five errors in the genome database, leading to an erroneously predicted truncation of VF1308, which we corrected in GenBank (Mandel et al., 2008). In the revised sequence, VF1308 encodes a protein that is the same length as, and shares 84% identity with, E. coli FNR. This ES114 FNR is identical to the previously deposited V. fischeri MJ1 FNR (accession no. CAE47558). Importantly, the residues necessary for interactions with RNA polymerase (Williams et al., 1997; Lonetto et al., 1998; Blake et al., 2002; Lambregt et al., 2002), 4Fe–4S center assembly (Spiro & Guest, 1988; Kiley & Beinert, 1998), and DNA recognition (Spiro et al., 1990) in E. coli are conserved in V. fischeri FNR. Using TransTermHP (Kingsford et al., 2007), we also found a likely Rho-independent transcriptional terminator downstream of fnr (Fig. 1a and b). Given the 142-bp spacing and strong putative terminator between fnr and VF1310 (Fig. 1b), it seems likely that these are expressed on separate transcripts. Using quantitative RT-PCR, we found that the fnr<sup>+</sup>tmpR allele in mutants described below did not affect the transcript levels for VF1310.

We next generated mutants disrupted in the putative fnr in V. fischeri ES114 and MJ1. We did not observe any attenuation of these strains under aerobic growth conditions, consistent with the role of FNR in other bacteria. Escherichia coli fnr mutants do not grow anaerobically with nitrate or fumarate as an electron acceptor (Lambden & Guest, 1976), and we found that V. fischeri fnr mutants were similarly attenuated. Specifically, when grown with minimal medium under anaerobic conditions, ES114 and MJ1 displayed nitrate- or fumarate-dependent growth on a non-fermentable carbon source (glycerol) that was lacking in the fnr mutants (e.g. Fig. 1c). Restoring fnr by replacing the fnr<sup>+</sup>tmpR allele with the wild-type allele by a crossover exchange back into these mutants recovered the ability to respire anaerobically. We restored the wild-type fnr allele on the chromosome in this way (replacing fnr<sup>+</sup>tmpR) rather than providing it in trans due to concerns that fnr provided in multiplicity can show uncharacteristic effects such as gene...
activation under aerobic conditions (Reyes-Ramirez & Sawers, 2006) and a narrowing of the difference between better and poorer FNR activation sites (Scott et al., 2003). However, because our V. fischeri-derived allele-replacement constructs were not appropriate (homologous) for exchange into E. coli, we provided the putative fnr of V. fischeri ES114 to E. coli in trans on plasmid pJLB6, which restored anaerobic respiration of E. coli fnr mutant PC2 on nitrate (Fig. 1d). Taken together, our results indicate that the putative V. fischeri FNR is similar in both sequence and function to E. coli FNR.

Repression of luminescence by FNR

We tested whether FNR regulates lux expression by monitoring the luminescence of strains grown aerobically or anaerobically (Fig. 2a and b). The luminescence of the fnr mutants was similar to that of their parent strains under aerobic conditions (Fig. 2a). FNR is inactivated by oxygen, and we therefore also assessed lux expression anaerobically. Luciferase uses oxygen as a substrate, and so anaerobic cultures do not luminesce; however, as with all luminescence measurements, samples removed from anaerobic bottles were shaken for ~10 s to saturate luciferase with oxygen before measuring luminescence. When grown anaerobically, luminescence was higher in fnr mutant EVS601 than in MJ1 (Fig. 2b). The magnitude of this difference varied between 1.5- and 20-fold, and averaged eightfold, in five experiments. The luminescence of ES114 and fnr mutant JB1 was below the background, appearing the same as a dark ΔluxCDABEG strain (data not shown), which raised the possibility that FNR regulates lux in ES114, but that the overall luminescence is below detection. To test this possibility, we added the luminescence-stimulating autoinducer 3-oxo-C6-HSL to anaerobic cultures of ES114 and its corresponding lux promoter.

We considered the possibility that increased luminescence in V. fischeri fnr mutants could result from increased availability of luciferase's substrates due to the physiological effects of this global regulator. To test this possibility, we disrupted fnr in a background where the luxCDABEG genes are under the control of LacIq and a non-native promoter. In this background, FNR had no significant effect (P > 0.05) on luminescence (Fig. 2c). Thus, the repressive effect of FNR on luminescence is dependent on the native lux promoter.

The luxCDABEG operon can be subject to positive feedback regulation, because the autoinducer synthase LuxI generates 3-oxo-C6-HSL, which, in combination with LuxR, stimulates luxCDABEG transcription. Given the amount of 3-oxo-C6-HSL added exogenously to the cultures (Fig. 2c), we predicted that endogenously produced autoinducer

Fig. 1. Genomic context and function of fnr in Vibrio fischeri. (a) Gene arrangement around fnr in V. fischeri ES114. Numbers represent the corresponding VF### ORF designation. Stem-loop icons indicate the positions of Rho-independent transcriptional terminators predicted using TransTermHP (Kingsford et al., 2007), with a confidence score of 100 in each case. ‘#aa’ indicates the number of amino acids encoded by each ORF. VF1308 and VF1309 (black arrows) indicate ORFs with similarity to the N and C termini of Escherichia coli FNR, respectively. The striped arrow shows the complete fnr based on our sequence revision. (b) The predicted Rho-independent transcriptional terminator between fnr and VF1310. (c) Growth of V. fischeri MJ1, fnr mutant EVS601, and restored fnr+ strain JB27 along with ES114, fnr mutant JB1, and restored fnr+ strain JB28 on defined medium with glycerol and fumarate, incubated in anaerobic jars at 28 °C. (d) Escherichia coli MC4100 and fnr mutant PC2 with vector pDMAS or pJLB6, which contains the V. fischeri ES114 fnr, grown on M9 medium with glycerol and nitrate in anaerobic jars at 37 °C.
would have no further stimulatory effect, and therefore the
effect of FNR on luminescence in this experiment would not
have a significant LuxI-mediated positive-feedback compo-
nent. We examined luxI point mutant VCW2G7 and found
that, as predicted, it achieved the same luminescence as the
wild type under anaerobic conditions with added 3-oxo-C6-
HSL (data not shown).

**Analysis of FNR boxes**

It was suggested that a putative FNR box upstream of luxR
might underpin the FNR-mediated regulation of lumines-

cence in MJ1 ( Muller-Brekreutz & Winkler, 1993); however,

ttempts to define a footprint using FNR*, an *E. coli* FNR
derivative that is active aerobically (Kiley & Reznikoff,
1991), failed to show binding to this site (A.M. Stevens,
pers. commun.). To further explore how FNR might affect
luminescence, we conducted a ‘Virtual Footprint’ analysis
with the PRODORIC database (Munch et al., 2005), search-
ing the *V. fischeri* genome for FNR boxes using a weighted
consensus matrix based on data from *E. coli*. As expected,
high Position Weight Matrix (PWM) scores (> 7.0) were
skewed toward intergenic regions. Such putative FNR boxes
numbered in the hundreds, consistent with FNR’s global
role in *E. coli*, and these included intergenic regions up-
stream of genes involved in anaerobic metabolism (e.g.
upstream of nitrate and nitrite reductase genes). However,
the best FNR box matches in the lux intergenic region of
MJ1 and ES114 returned scores of 6.73 and only 5.88,
respectively. To put this in perspective, > 25 000 sites with
no skew toward intergenic regions returned scores ≥ 5.9.
Although we cannot rule out the possibility that FNR
directly binds to the lux intergenic region, we believe this
model is unlikely, especially in strain ES114.

**FNR-mediated repression of arcA**

Virtual Footprinting did suggest a possible indirect effect of
FNR on luminescence. The highest PWM score returned in
this analysis (7.67) was found in six intergenic regions, one
of which was upstream of arcA. In *E. coli*, FNR activates arcA
(Compan & Touati, 1994), and in ES114, ArcA strongly
represses the lux operon (Bose et al., 2007). If FNR activates
arcA in *V. fischeri*, this might explain FNR’s repressive effect

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**Fig. 2.** Luminescence per OD595 nm of *fnr* mutants. (a, b) Specific
luminescence is shown at different culture densities for *Vibrio fischeri*
ES114 (solid diamonds), ES114 *fnr* mutant JB1 (empty diamonds), MJ1
(solid squares), MJ1 *fnr* mutant EV5601 (empty squares), and dark
ΔluxCDABEG mutant EV5102 (solid triangles) grown in batch cultures
that were (a) aerobic (50 mL medium in 250-mL flask) or (b) anaerobic
(20 mL medium in 165-mL bottles with anaerobic headspace) at 24 °C
with shaking (200 r.p.m.). ES114, JB1, and EV5102 were excluded
from(b), because luminescence was not detected above the background
for these strains under these conditions. Bars in (b) indicate the SD
(n = 5). Error bars were excluded in (a), because they were generally
smaller than (and never extended above) the data symbols. (c) ES114
(wild type), JB22 (lacP 3-OH-C6-HSL), and their respective *fnr*
mutants (represented by hatched bars) JB1 and ANS25, respectively (Table 1),
were grown under anaerobic conditions. ‘AI’ indicates supplementation
with 140 nM 3-oxo-C6-HSL autoinducer, and ‘IPTG’ indicates that
isopropyl-B-D-thiogalactoside was added to 2 mM to induce luxCDABEG
expression in strains containing lacP 3-OH-C6-HSL. Data are the average
peak luminescence per OD595 nm with SD (n = 2). Asterisks indicate that
the *fnr* mutant was significantly (*P < 0.01) brighter than the correspond-
ing isogenic *fnr*-positive strain. Other comparisons were not significant
(*P > 0.05).
on luminescence. Using $P_{arcA}\cdot lacZ$ transcriptional reporters, we found that fnr was responsible for an ~2–4-fold activation of the arcA promoter(s) anaerobically in ES114 and MJ1 backgrounds (Fig. 3).

**FNR is not necessary for host colonization**

We tested whether FNR was important for symbiotic colonization by ES114 using established measures of symbiotic competence (Adin et al., 2009). The onset of symbiotic luminescence (Fig. 4a), colonization levels (Fig. 4b), and colonization competitiveness (Fig. 4c) were similar for ES114 and fnr mutant JB1 during the first 2 days of infection. The fnr mutant was also equally competitive up to 90 h after inoculation (data not shown). Furthermore, the fnr mutation did not appear to affect the symbiosis in a $\Delta arcA$ mutant background (data not shown). We conclude that FNR is not necessary for colonization during the first days of a symbiotic infection.

**Discussion**

In this study, we investigated the oxygen-sensitive regulator FNR in *V. fischeri*. *Vibrio fischeri* fnr complemented an *E. coli* fnr mutant, and like fnr in *E. coli*, it is required for fumarate- and nitrate-dependent anaerobic respiration. Moreover, our data and another recent bioinformatic analysis (Ravechev et al., 2007) suggest that the FNR-box recognition site is conserved in *V. fischeri*. For example, we observed fnr-mediated regulation of reporters for arcA (Fig. 3), dmsA (Dunn & Stabb, 2008), torE (Dunn & Stabb, 2008), and yfiD (data not show), which have predicted FNR boxes upstream. Taken together, FNR’s function in *V. fischeri* appears to be similar to that in its fellow gammaproteobacterium *E. coli*. As the first experimental examination of FNR in the *Vibroniaceae*, this study should underpin future efforts to understand FNR-mediated regulation in this important bacterial family.

We initiated this study largely because FNR is cited as an activator of luminescence in *V. fischeri* (e.g. see Meighen, 1994; Spiro, 1994; Sitnikov et al., 1995; Ulitzur & Dunlap, 1995; Stevens & Greenberg, 1999). However, that paradigm was based on a preliminary study that used the MJ1 lux genes cloned in *E. coli* (Muller-Breikreutz & Winkler, 1993). Our results appear to contradict that report, showing instead that FNR mediates repression of the luminescence-generating lux system in *V. fischeri* under anaerobic conditions (Fig. 2). It is perhaps not surprising that lux regulation should be different in transgenic *E. coli* than in *V. fischeri*. For example, LitR, which activates luxR transcription, is absent in *E. coli* (Fidopiastis et al., 2002). It is also possible that FNR does activate luminescence in *V. fischeri* under conditions different from those tested here, and that the discrepancy between our study and previous work simply reflects methodological differences.

Repression of the lux genes anaerobically may minimize the production of luciferase when its O$_2$ substrate is unavailable. This is consistent with the finding that luminescence is repressed by the ArcAB two-component regulatory system, which is more active under relatively reduced conditions (Bose et al., 2007). The observation that arcA::lacZ reporters showed a lower expression in the absence of fnr (Fig. 3) suggests that the effect of FNR on bioluminescence may at least in part be indirect and mediated by FNR’s stimulation of arcA. Consistent with this idea, fnr did not exert much influence on luminescence in *arcA* mutant backgrounds, although *arcA fnr* double mutants were noticeably attenuated in anaerobic growth (data not shown). We speculate that FNR may amplify the
repressive effect of ArcA on luminescence under reduced conditions. Although we cannot rule out the possibility that FNR exerts a direct effect by binding the lux region, as described above, we believe this model is unlikely. In either case, FNR apparently contributes to regulation that effectively turns off expression of the lux genes under ES114 under anaerobic conditions, which is easily rationalized, given that luciferase requires O₂ to generate light.

Given the suggestion that anaerobic respiration is important for symbiotic *V. fischeri* (Proctor & Gunsalus, 2000), and the fact that FNR can contribute to virulence factor production and/or colonization by pathogens (Baltes et al., 2005; Bartolini et al., 2006; Fink et al., 2007; Zigha et al., 2007), we hypothesized that fnr would play a role in the symbiotic light organ. However, the fnr mutant had no discernable attenuation in colonizing *E. scolopes* during the first 90 h of infection. *Vibrio fischeri*, like other members of the Vibrionaceae family, is a cosmopolitan member of marine communities that is found in fish gut tracts and sediments where [O₂] is low. Future studies may show the ecological relevance of FNR for *V. fischeri* in such environments outside *E. scolopes*.

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**Authors’contribution**

J.L.B. and A.N.S. contributed equally to this work.

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