JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2440–2448
0021-9193/92/082440-09$02.00/0

Vol. 174, No. 8

Cell Density-Dependent Modulation of the *Vibrio fischeri* Luminescence System in the Absence of Autoinducer and LuxR Protein†

PAUL V. DUNLAP* AND ALAN KUO

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Received 18 November 1991/Accepted 31 January 1992

Expression of the *Vibrio fischeri* luminescence genes (*luxR* and *luxICDABEG*) in *Escherichia coli* requires autoinducer (*N*-3-oxohexanoyl homoserine lactone) and LuxR protein, which activate transcription of *luxICDABEG* (genes for autoinducer synthase and the luminescence enzymes), and cyclic AMP (cAMP) and cAMP receptor protein (CRP), which activate transcription of the divergently expressed *luxR* gene. In *E. coli* and in *V. fischeri*, the autoinducer-LuxR protein-dependent induction of *luxICDABEG* transcription (called autoinduction) is delayed by glucose, whereas it is promoted by iron restriction, but the mechanisms for these effects are not clear. To examine in *V. fischeri* control of *lux* gene expression by autoinducer, cAMP, glucose, and iron, *lux*::Mu dI(*lacZ*) and *lux* deletion mutants of *V. fischeri* were constructed by conjugation and gene replacement procedures. β-Galactosidase synthesis in a *luxC*::*lacZ* mutant exhibited autoinduction. In a *luxR*::*lacZ* mutant, complementation by the *luxR* gene was necessary for luminescence, and addition of cAMP increased β-galactosidase activity four- to sixfold. Furthermore, a *luxI*::*lacZ* mutant produced no detectable autoinducer but responded to its addition with induced synthesis of β-galactosidase. These results confirm in *V. fischeri* key features of *lux* gene regulation derived from studies with *E. coli*. However, β-galactosidase specific activity in the *luxI*::*lacZ* mutant, without added autoinducer, exhibited an eight- to tenfold decrease and rise back during growth, as did β-galactosidase and luciferase specific activities in the *luxR*::*lacZ* mutant and luciferase specific activity in a Δ(*luxR luxICD*) mutant. The presence of glucose delayed the rise back in β-galactosidase and luciferase specific activities in these strains, whereas iron restriction promoted it. Thus, in addition to transcriptional control by autoinducer and LuxR protein, the *V. fischeri lux* system exhibits a cell density-dependent modulation of expression that does not require autoinducer, LuxR protein, or known *lux* regulatory sites. The response of autoinducer-LuxR protein-independent modulation to glucose and iron may account for how these environmental factors control *lux* gene expression.

Induction of luminescence in *Vibrio fischeri* is controlled by the cell density-dependent accumulation of a species-specific, diffusible autoinducer (*N*-3-oxohexanoyl homoserine lactone) produced by *V. fischeri*. During growth of *V. fischeri* cells in batch culture, autoinducer gradually accumulates in the medium and in cells. When it reaches a threshold concentration, autoinducer triggers the transcription of genes encoding the luminescence enzymes (11, 18–20, 30, 35, 37). Studies of the cloned *V. fischeri* luminescence genes (the *lux* genes, *luxR* and *luxICDABEG*) in *Escherichia coli* have shown that this induction (termed autoinduction) also requires the LuxR protein, which, together with autoinducer, activates transcription of *luxICDABEG* (genes for autoinducer synthase and the luminescence enzymes) and represses the divergently transcribed *luxR* gene. Conversely, cyclic AMP (cAMP) and cAMP receptor protein (CRP), which are required for luminescence in *V. fischeri*, activate transcription of *luxR*, thereby apparently stimulating synthesis of the LuxR protein to a level sufficient for interaction with autoinducer, and repress expression of *luxICDABEG* in *E. coli* (4, 6, 8, 10, 12, 14–17, 21–23, 35, 43, 50).

Besides autoinducer and cAMP, some environmental factors control autoinduction of luminescence in *V. fischeri*. These include glucose, the presence of which delays induction of luminescence (43), and iron and oxygen, low levels of which lead to induction of luminescence at a lower cell density (27, 40). These factors could play a role in the bioluminescent (light organ) symbiosis of *V. fischeri* with monocentrid fish; limitation of glucose, iron, or oxygen in the symbiosis might promote bacterial light production while limiting the growth of *V. fischeri* cells (16, 27, 39). Control of *lux* gene expression by iron, analogous to that in *V. fischeri*, was recently shown for *E. coli* containing the cloned *lux* genes, and results of that study indicated that iron operates indirectly in the *lux* system through a mechanism distinct from direct transcriptional control of *luxICDABEG* by autoinducer and LuxR protein (12). Consequently, the autoinduction phenomenon appears to be more complex than previously thought.

In the present study, gene replacement procedures were used to construct transcriptional *lux*::*lacZ* fusion mutants of *V. fischeri* with which to examine *lux* gene regulation. The effects of glucose and iron on expression from the *luxR* and *luxICDABEG* promoters were assessed. The results confirm information on control by autoinducer, LuxR protein, and cAMP derived from studies of the cloned *lux* genes in *E. coli*, and they indicate the presence of a cell density-dependent form of regulation that underlies transcriptional control of *luxICDABEG* but that is independent of autoinducer and LuxR protein. Glucose and iron apparently operate through this autoinducer-LuxR protein-independent modulation.

* Corresponding author.

† Contribution 7964 from the Woods Hole Oceanographic Institution.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics[a]	Source or reference
E. coli K12		
PD1734	POI1734 [Mu *cts*, Mu dI1734 (Km^r *lacZYA*)] *recA56*	15
PD100	Δ(*argF-lac*)*U169*	14
S17-1	RP4 *tra*$^+$	48
V. fischeri		
MJ-1	*lux*$^+$ (parent strain)	43
MJ-100	MJ-1, spontaneous Nx^r strain	This study
MJ-112	MJ-100 *luxR*::*lacZ* Mu dI1734 Nm^r	This study
MJ-141	MJ-100 *luxI*::*lacZ* Δ(*luxICD*) Nm^s	This study
MJ-181	MJ-100 *luxC*::*lacZ* Mu dI1734 Nm^r	This study
MJ-184	MJ-100 *luxD*::*lacZ* Mu dI1734 Nm^r	This study
MJ-201	MJ-100 Δ(*luxR luxICD*) Nm^s	33
Plasmids		
pJE202	pBR322 with 9-kb *Sal*I fragment of *V. fischeri* DNA (*luxR luxICDABEG*) Ap^r	21
pSUP102	pACYC184, RP4 *mob*$^+$, Cm^r Tc^r	48
pNL121	pSUP102 with 9-kb *Sal*I *lux* fragment of pJE202	This study
pWH112	pNL121 with *luxR*::Mu dI(*lacZYA*) Nm^r	This study
pWH181	pNL121 with *luxC*::Mu dI(*lacZYA*) Nm^r	This study
pWH184	pNL121 with *luxD*::Mu dI(*lacZYA*) Nm^r	This study
pJE411	pJE202 with *luxI*::Mu dI1681 (*lacZYA* Nm^r)	22
pWH141	pNL121 with 9-kb *Bgl*II-*Pst*I *luxI*::*lacZYA* fragment of pJE411 replacing 4-kb *Bgl*II-*Pst*I *lux* fragment (Δ*luxICD*), Nm^s Cm^r	This study
pWH201	pWH112 with Δ(Mu dI) Δ(*luxR luxICD*) Cm^r Nm^s	33
pPD749	p*tac-luxR* Ap^r	15
pSUP202	pBR325, RP4 *mob*$^+$, Ap^r Cm^r Tc^r	48
pLR001	pSUP202 with 1.5 kb-*Sal*I p*tac-luxR* fragment of pPD749, Ap^r Cm^r	This study
pWH202	pNL121 with Δ(*luxDA*) Cm^r *luxR*$^+$	This study
pHK555	pACYC184 with *luxICDABEG luxR*::Mu Δ(*c nerAB*) dI 1681 (Km^s *lacZYA*) Cm^r	31

[a] Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant; Nx^r, nalidixic acid resistant; Nm^r, neomycin resistant; Nm^s, neomycin sensitive; Tc^r, tetracycline resistant.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The strains used in this study are derivatives of *E. coli* K-12 and *V. fischeri* MJ-1 and are listed in Table 1. The *E. coli* strains were grown on LB agar (47) with the appropriate antibiotics to assure plasmid maintenance, except that for conjugative matings both *E. coli* and *V. fischeri* strains were grown without antibiotics on LBS (10), which contained 10 g of tryptone, 5 g of yeast extract, 3 ml of glycerol, 340 mM NaCl, and 50 mM Tris-HCl, pH 7.5. For growth studies of recombinant *V. fischeri* strains, cells were grown without antibiotics in 3- or 30-ml volumes with aeration at 28°C (10, 14) in a filter-sterilized artificial seawater-based (38) medium (artificial seawater HEPES [ASH]), which contained 300 mM NaCl, 10 mM KCl, 50 mM $MgSO_4$, 10 mM $CaCl_2$, 5 g of tryptone, 3 g of yeast extract, 3 ml of glycerol, and 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 7.5. ASH broth was supplemented with glucose, *N*-3-oxohexanoyl homoserine lactone (*V. fischeri* autoinducer), cAMP, and ethylenediamine dihydroxy acetic acid (EDDHA) as indicated.

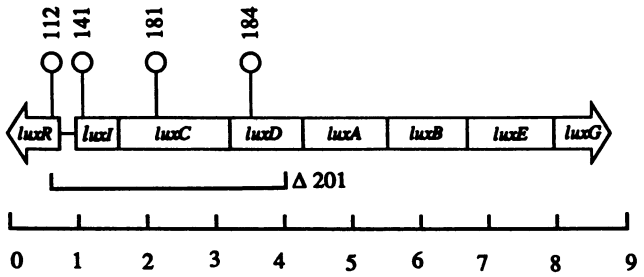

FIG. 1. Organization of the *V. fischeri lux* genes and map locations of the Mu dI(*lacZ*) insertions and the *lux* gene deletion used to construct *lux*::*lacZ* fusion mutants and a *lux* deletion mutant of *V. fischeri*. A map of *lux* DNA in pNL121 showing the two divergent transcriptional units, *luxR* and *luxICDABEG*, is shown. Markers and numbers indicate the positions of Mu dI(*lacZ*) insertions in *luxR* (pWH112), *luxI* (pWH141), *luxC* (pWH181), and *luxD* (pWH184), with *lacZ* transcribed in the direction of the arrows. In the construction of pWH141 (see Materials and Methods), DNA from the 3′ end of *luxI* to the 5′ end of *luxD* was removed. The bracket, labelled Δ201, denotes the extent of the *lux* gene deletion in pWH201.

Construction of *lux*::*lacZ* fusion plasmids. To construct *lux*::*lacZ* fusions for mobilization into *V. fischeri*, the *lux* gene-containing 9-kb *Sal*I fragment of pJE202 (21) was transferred to the *Sal*I site of pSUP102 (*mob*$^+$) (48). The resulting vector, pNL121, conferred on *E. coli* strains the ability to make light. Both pSUP102 and pNL121 could be mobilized by conjugation from *E. coli* S17-1 (*tra*$^+$) (48) to *V. fischeri* MJ-100 (a spontaneous nalidixic acid-resistant strain, isolated by the procedure of Miller [36]) at frequencies of 10^{-2} to 10^{-4} per donor cell, with selection for resistance to chloramphenicol (30 μg · ml^{-1}) and nalidixic acid (20 μg · ml^{-1}). However, pNL121 (but not pSUP102) was unstable in *V. fischeri* MJ-100; serial transfer of the transconjugant strains in the absence of antibiotic selection resulted in loss of the super-bright phenotype (conferred presumably by the supernumerary *lux* genes), resistance to chloramphenicol, and the plasmid, as determined with plasmid "miniprep" and gel electrophoresis procedures (this and other standard procedures, except as indicated, were those of Sambrook et al. [44]). This suggested that double homologous recombination occurred readily between the vector-borne *lux* genes and the chromosomal *lux* genes.

Mu dI1734 (*lacZ*) insertions in the *lux* genes of pNL121 were constructed according to the procedure of Castilho et al. (5), using *E. coli* PD1734, as described previously (15). The positions and orientations of Mu dI insertions in specific *lux* genes were identified by phenotypic screening and restriction endonuclease mapping procedures, as described previously (15). Positions of insertions are accurate to approximately ±100 bp. Fusions representative of those in *luxR* (pWH112), *luxC* (pWH181), and *luxD* (pWH184) were chosen for further study (Fig. 1). Of the 120 clones examined, however, apparently none contained a Mu dI insertion in *luxI*. Therefore, a different procedure was used to construct a mobilizable *luxI*::*lacZ* fusion vector. A plasmid containing a Mu dI1681 insertion in *luxI*, pJE411 (22), was digested with *Bgl*II (one site in *luxD*, two sites in Mu dI) and *Pst*I (one site in *luxR*, one site in the vector [pBR322], and four sites in Mu dI), and the resulting 9-kb *luxI*::*lacZ* fusion

fragment was isolated away from other *Bgl*II- and *Pst*I-generated fragments on low-melting-point agarose. Similarly, pNL121 was digested with *Bgl*II and *Pst*I, and the 11-kb fragment containing the vector with the 3′ end of *luxR* and the 3′ end of *luxD* (including *luxABE*) was isolated on low-melting-point agarose. The fragments were ligated together in the gel overnight at 15°C, and the ligation mixture was used to transform (25) *E. coli* PD100, with selection for resistance to chloramphenicol. A *luxI*::*lacZ* fusion vector, pWH141, was recovered by this procedure; its construction was confirmed by screening and mapping procedures, as indicated above. The cloning procedure eliminated most of *luxI*, all of *luxC*, and part of *luxD* plus essentially all of the Mu dI DNA (including the neomycin resistance determinant [Nm^r]) except for *lacZYA*.

A *lux* deletion vector, pWH201, was constructed by digestion of pWH112 with *Bgl*II (two sites in Mu dI1734 and one site in *luxD*) followed by ligation of the vector (33). This procedure removed most of the *luxR*-inserted Mu dI1734 (including the Nm^r determinant) and a 3-kb portion of the *lux* genes from the 5′ end of *luxR* through the *lux* regulatory region, *luxI*, *luxC*, and the 5′ end of *luxD* (33) (Fig. 1), as confirmed by restriction mapping.

Construction of *V. fischeri lux*::*lacZ* fusion mutants and a *lux* deletion mutant. *E. coli* S17-1 was transformed with the *lux*::*lacZ* fusion vectors (pWH112, pWH141, pWH181, and pWH184) and the *lux* deletion vector (pWH201) and was then mated with *V. fischeri* MJ-100. For matings, mid-exponential-phase cultures of S17-1 containing each vector and MJ-100 were spotted together (15 μl each) on LBS plates, which were incubated overnight at 28°C. Growth from each mating spot was then resuspended in 1 to 2 ml of LBS broth, and 100-μl portions were spread on plates of LBS containing nalidixic acid, chloramphenicol, and neomycin (200 $\mu g \cdot ml^{-1}$) (except for cultures containing pWH141 and pWH201, which are sensitive to neomycin [Nm^s]). *V. fischeri* transconjugants arose in 2 to 4 days. Purified transconjugants were luminous and, except for pWH201, produced β-galactosidase. To recover strains in which the fusion or deletion replaced the chromosomal *lux* genes, purified transconjugants were transferred one to several times on LBS plates containing nalidixic acid and neomycin (except that neomycin was not used for strains receiving pWH141 or pWH201) but lacking chloramphenicol. Strains arose readily that produced no visibly detectable luminescence (after 15 min of dark adaptation), that were sensitive to chloramphenicol, and that lacked the vector (as determined with ethidium bromide-stained agarose gels of plasmid minipreps [44]). Replacement of the native *lux* genes with the *lux*::*lacZ* fusions or with the *lux* deletion in the *V. fischeri* chromosome (MJ-112 [*luxR*::*lacZ*], MJ-141 [*luxI*::*lacZ*], MJ-181 [*luxC*::*lacZ*], MJ-184 [*luxD*::*lacZ*], and MJ-201 [Δ*luxR luxICD*] [33]) was confirmed by Southern hybridization analysis using the Genius nonradioactive labelling kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) according to the manufacturer's recommendations. For the *lux*::*lacZ* strains, the 8.1-kb *Bgl*II-*Hin*dIII *lacZYA* fragment of MudI 1681 (5) was used as the probe (Fig. 2). For MJ-141, consistent with the *lacZ* insertion in *luxI*, no autoinducer was detected in ASH medium conditioned (18) by MJ-141 grown to an optical density at 660 nm (OD_{660}) of 1.0, with MJ-1 as the assay organism.

Construction of *luxR*-complementation plasmids. To construct pLR001, a derivative of pSUP202 that contains a functional *luxR* gene, the 1.5-kb *Sal*I *ptac-luxR* fragment of pPD749 (15) was subcloned into the *Sal*I site of pSUP202

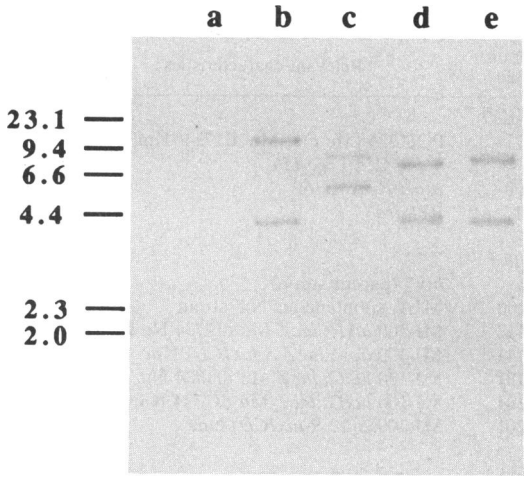

FIG. 2. Southern blot analysis of chromosomal DNA of *V. fischeri lux*::*lacZ* fusion strains. The DNA was digested with *Eco*RI (which cuts once in *lacZ*) and *Sal*I and electrophoresed on a 0.8% agarose gel. The blot was hybridized with the 8.1-kb *Bgl*II-*Hin*dIII *lacZYA* fragment of Mu dI1681, as described in Materials and Methods. Lane a, MJ-100 (*lux*$^+$); lane b, MJ-112 (*luxR*::*lacZ*); lane c, MJ-141 (*luxI*::*lacZ*); lane d, MJ-181 (*luxC*::*lacZ*); lane e, MJ-184 (*luxD*::*lacZ*). Fragment length standards (*Hin*dIII-digested phage lambda DNA) in kilobases are indicated at the left.

(48). To generate pWH202, a *luxR*-containing plasmid based on pSUP102, pNL121 was digested with *Bgl*II, which cuts in *luxD*, and *Xho*I, which cuts in *luxA* (21), the overhanging ends were blunt ended with Klenow fragment, and the plasmid was religated. Plasmid constructions were confirmed by restriction mapping. Complementation of the defect in *luxR* in *E. coli* S17-1 containing pWH112 (*luxR*::*lacZ luxICDABE*) by transformation with pLR001 restored the ability of this strain to produce luminescence.

Determination of cellular luminescence, luciferase activity, and β-galactosidase activity. The light-measuring equipment and standard to calibrate the equipment have been described previously (14, 24, 26), as have the procedures for measuring luminescence of broth cultures (14). The procedure for measuring luciferase activity in cell extracts involved a reaction with excess reduced flavin mononucleotide, decanal, and oxygen (42). Cell extracts for luciferase assays were prepared as described previously (10). Luciferase activity is based on cell extracts from 1 ml of culture (14), and luciferase specific activity is activity per milliliter per unit of cell density (OD_{660}). β-Galactosidase activity was measured by the $CHCl_3$-sodium dodecyl sulfate method of Miller (36), as described previously (14), and β-galactosidase specific activity is activity per milliliter per unit of cell density (OD_{660}). Assay mixtures were centrifuged (11,000 × *g*, 1 min, room temperature) before the A_{420} was measured. Phase-contrast microscopy of *V. fischeri* cultures (MJ-1, MJ-112, and MJ-201) revealed no significant changes in cell size or shape over the range of cell densities considered in this study.

Chemicals. Antibiotics, cAMP, *n*-decylaldehyde, EDDHA, flavin mononucleotide, HEPES, Tris, and 5-bromo-4-chloro-3-indolyl-β-galactoside (X-Gal) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Deferrated EDDHA was prepared by the method of Rogers (41). Synthetic, pure *V. fischeri* autoinducer was generously provided by A. Eberhard.

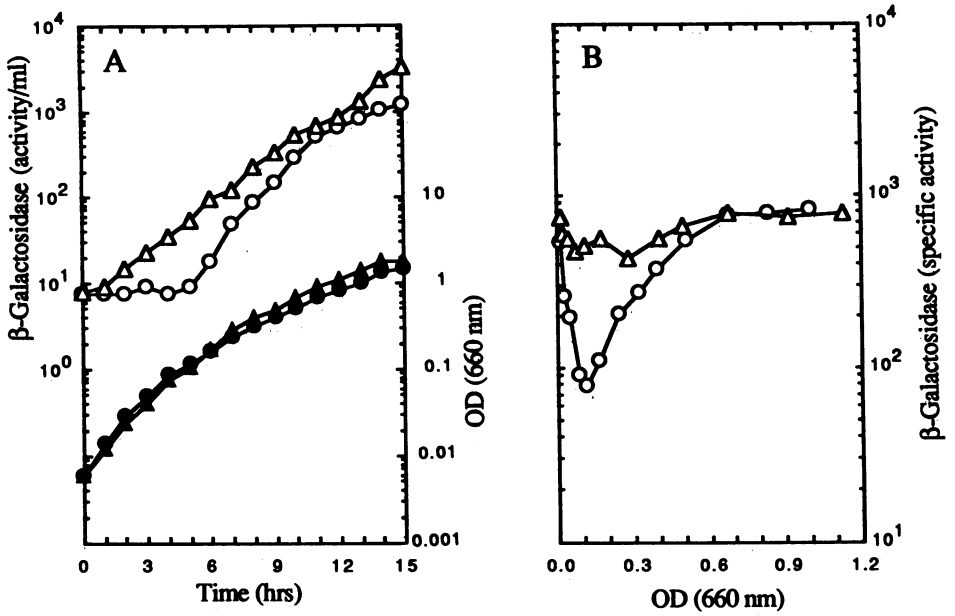

FIG. 3. Cell density-dependent autoinduction of β-galactosidase synthesis in *V. fischeri* MJ-181 (*luxC*::*lacZ*). (A) β-Galactosidase activity per milliliter (open symbols) during growth (OD_{660}, closed symbols); (B) β-galactosidase specific activity (defined in Materials and Methods). Symbols: ○ and ●, no addition; △ and ▲, autoinducer (0.2 μM) added.

RESULTS

Construction and characterization of *lux*::*lacZ* transcriptional fusion mutants of *V. fischeri* MJ-100. Gene replacement mutants of *V. fischeri* MJ-100 (Table 1) with Mu dI(*lacZ*) fusions (Fig. 1) in *luxR* (MJ-112), *luxI* (MJ-141), *luxC* (MJ-181), and *luxD* (MJ-184) were constructed as described in Materials and Methods. These strains produced no visibly detectable luminescence but produced β-galactosidase; they were resistant to neomycin (except MJ-141, see Materials and Methods) and were sensitive to chloramphenicol, and they exhibited no plasmid bands on agarose gels. These traits are consistent with replacement of the parental *lux* genes with the *lux*::*lacZ* fusions and with subsequent loss of the vectors used to deliver the fusions. When transferred repeatedly on nonselective medium (LBS agar without antibiotics), these strains exhibited no changes in these traits, which indicated that the fusions were stable. The presence of the *lacZ* gene at a single location in the chromosome of each fusion strain and its hybridization to chromosomal fragments of the appropriate sizes were confirmed by Southern blot analysis (Fig. 2). No hybridization of the *lacZ* gene occurred with DNA from MJ-100 (Fig. 2).

Cell density-dependent autoinduction of *luxICDABEG* transcription. With the construction and characterization of *V. fischeri lux*::*lacZ* fusion mutants, we were able to examine in *V. fischeri* the control of *lux* gene expression by autoinducer, LuxR protein, and cAMP, using β-galactosidase and luciferase as transcriptional reporters. First, to confirm autoinduction of transcription from the *luxICDABEG* promoter, β-galactosidase synthesis in *V. fischeri* MJ-181 (*luxC*::*lacZ*) was examined. In this strain, the regulatory genes *luxI* (putative autoinducer synthase gene) and *luxR* (putative autoinducer receptor protein gene) are intact. Consequently, MJ-181 cells were expected to induce β-galactosidase synthesis in an autoinducer-dependent fashion similar to the induction of luciferase synthesis in *V. fischeri* MJ-1. This was found to be the case. When cells of MJ-181 were inoculated into fresh medium, levels of β-galactosidase remained constant for 4 to 5 h during growth and were then induced to a level approximately 100- to 300-fold higher than preinduction levels (Fig. 3A). Addition of a high concentration of *V. fischeri* autoinducer (0.2 μM) eliminated the lag in β-galactosidase synthesis (Fig. 3A). On a specific activity basis, β-galactosidase decreased 8- to 10-fold and then rose back during culture growth, and addition of autoinducer eliminated the decrease (Fig. 3B). These results are consistent with a dependence on autoinducer for *luxICDABEG* transcription, and the pattern and magnitude of expression are very similar to results obtained for luciferase synthesis in *V. fischeri* and in *E. coli* containing plasmids with the intact *lux* genes, as well as to those for β-galactosidase synthesis in *E. coli* containing a plasmid with a Mu dI(*lacZ*) insertion in *luxC* (14, 18, 37). Thus, levels of β-galactosidase produced by *V. fischeri* reliably reported *luxICDABEG* transcription. Similar results for autoinduction of β-galactosidase synthesis were obtained with MJ-184 (*luxD*::*lacZ*).

Complementation of luminescence in a *luxR*::*lacZ* fusion mutant with the *luxR* gene. Studies with *E. coli* containing the cloned *V. fischeri lux* genes have demonstrated that the LuxR protein, along with autoinducer, through its activation of *luxICDABEG* transcription, is required for the expression of luminescence (6, 12, 15, 17, 21, 22, 31, 45, 46, 49). To examine this requirement in *V. fischeri*, we constructed a *luxR*-complementing plasmid, pLR001 (a derivative of pSUP202), that contains the *luxR* gene under control of the *tac* promoter (see Materials and Methods and Table 1). Conjugative delivery of pLR001 into *V. fischeri* MJ-112 from *E. coli* S17-1 restored the ability of MJ-112 to produce luminescence; the transconjugant *V. fischeri* colonies arising on selection plates (LBS agar containing chloramphenicol and nalidixic acid) produced a high level of luminescence. However, a more detailed analysis of the function of LuxR protein was precluded by poor viability of MJ-112 containing pLR001, due apparently to instability of the *luxR* vector in

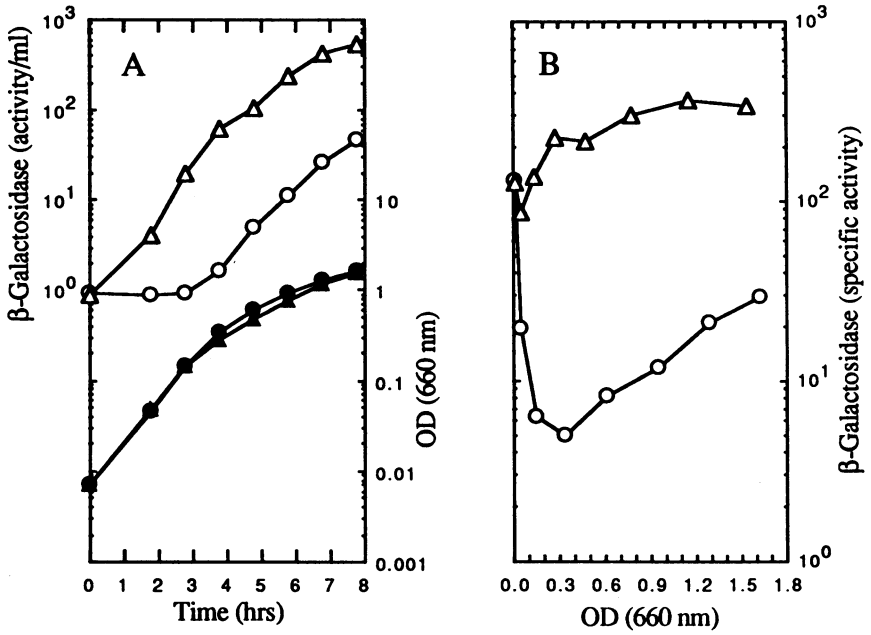

FIG. 4. Cell density-dependent modulation of β-galactosidase synthesis in *V. fischeri* MJ-141 (*luxI*::*lacZ*). (A) β-Galactosidase activity per milliliter (open symbols) during growth (OD_{660}, closed symbols); (B) β-galactosidase specific activity. Symbols: ○ and ●, no addition; △ and ▲, autoinducer (0.2 μM) added.

MJ-112. Similar complementation of luminescence in MJ-112 by the *luxR* gene and similarly poor viability of MJ-112 containing a *luxR*-complementing vector were observed when the *luxR* gene was present on another vector, pWH202 (a derivative of pSUP102; see Materials and Methods and Table 1). Nonetheless, these results confirm in *V. fischeri* the requirement for LuxR protein in luminescence.

cAMP control of transcription from the *luxR* and *luxICDABEG* promoters. Studies with *V. fischeri* and with *E. coli* containing the cloned *V. fischeri lux* genes have shown that cAMP and CRP are required for autoinduction of luminescence (10, 14, 15, 17). cAMP and CRP activate transcription from the *luxR* promoter and repress transcription from the *luxICDABEG* promoter. These responses occur both in the absence and in the presence of the LuxR protein (14, 15, 17). To determine if cAMP exerts a similar control in *V. fischeri*, we examined the effect of added cAMP on *V. fischeri* MJ-112 (*luxR*::*lacZ*). In this strain, transcription from the *luxR* promoter can be monitored by assaying for β-galactosidase while transcription from the *luxICDABEG* promoter can be monitored simultaneously by assaying for luciferase (14, 15, 17). In MJ-112, addition of cAMP (5 mM) stimulated β-galactosidase synthesis four- to sixfold (e.g., β-galactosidase specific activity at an OD_{660} of 0.5: no addition, 25; with cAMP, 140) and decreased luciferase synthesis by three- to fourfold (e.g., luciferase specific activity [10^5] at an OD_{660} of 0.5: no addition, 8.1; with cAMP, 1.9). The addition of cAMP had no appreciable effect on growth rate. These results are very similar to the effects of cAMP on transcription from the *luxR* and *luxICDABEG* promoters in *E. coli* containing recombinant *lux* plasmids (14, 15, 17). They confirm in *V. fischeri* cAMP control of *lux* gene expression.

Cell density-dependent modulation of *luxICDABEG* expression in the absence of autoinducer. Results consistent with a dependence on autoinducer and LuxR protein for *luxICDABEG* transcription are described above. However, normal cellular levels of autoinducer and LuxR protein can mask regulation underlying and contributing to the autoinduction pattern of *lux* gene expression (12, 14, 15, 17). We therefore examined in greater detail *V. fischeri* strains with *lacZ* transcriptional fusions in *luxI* and *luxR*. Initially, MJ-141 (*luxI*::*lacZ*) was used to critically assess the requirement for autoinducer in the autoinduction pattern of *luxICDABEG* expression. This strain lacks most of *luxI*, and consistent with this defect, it produced no detectable autoinducer (see Materials and Methods). However, the *luxR* gene of MJ-141 is intact; this permitted MJ-141 to respond to added autoinducer and permitted its response to be monitored by assay of β-galactosidase. It was anticipated that addition of autoinducer would activate (via LuxR protein) transcription from the *luxICDABEG* promoter and thereby lead to an increase in the levels of β-galactosidase. In the absence of added autoinducer, it was anticipated that β-galactosidase specific activity in this strain would remain at a low constant level.

β-Galactosidase activity in MJ-141 was substantially lower than in MJ-181 (in which the *luxI* and *luxR* genes are intact), and the addition of a high concentration of autoinducer (0.2 μM) stimulated β-galactosidase activity in this strain to levels approaching those in MJ-181 (Fig. 4A). These results, along with those shown above for MJ-181 (Fig. 3A), confirm the requirement for autoinducer in the LuxR protein-dependent activation of *luxICDABEG* transcription.

However, MJ-141 exhibited an unexpected modulation of β-galactosidase synthesis. In the absence of added autoinducer, the level of β-galactosidase remained constant for a period of approximately 3 h, as the cell density increased over 10-fold, and it then increased rapidly at a rate faster than the increase in cell density (Fig. 4A). On a specific activity basis, the level of β-galactosidase in MJ-141 initially decreased 8- to 10-fold and then rose back (Fig. 4B), in a fashion similar to that shown for MJ-181 (Fig. 3B); in the presence of high levels of autoinducer the decrease did not occur. This pattern of enzyme expression, presented as activity per milliliter or as OD_{660}-specific activity, is strik-

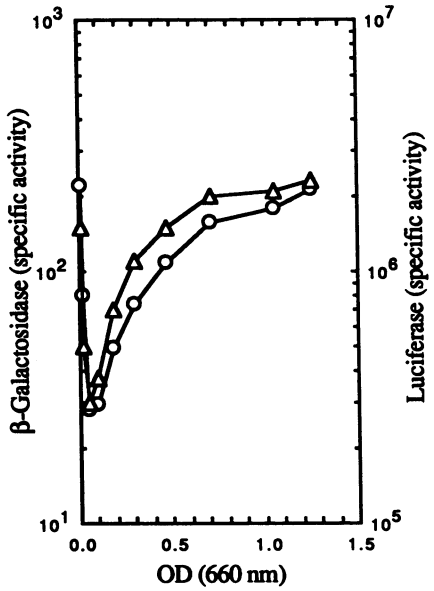

FIG. 5. Cell density-dependent modulation of β-galactosidase and luciferase synthesis in *V. fischeri* MJ-112 (*luxR*::*lacZ*) during growth in batch culture. Symbols: ○, β-galactosidase specific activity; △, luciferase specific activity.

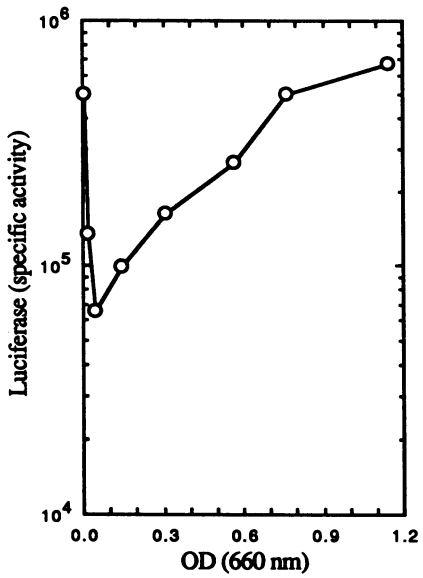

FIG. 6. Cell density-dependent modulation of luciferase synthesis in *V. fischeri* MJ-201 [Δ(*luxR luxICD*)] during growth in batch culture. Symbol: ○, luciferase specific activity.

ingly similar to that described for autoinducer-dependent induction of luciferase synthesis in *V. fischeri* MJ-1 (18, 37) and β-galactosidase synthesis in MJ-181 (Fig. 3). These results indicate that *luxICDABEG* exhibits a cell density-dependent modulation of expression even in the absence of autoinducer.

Cell density-dependent modulation of *luxR* and *luxICDABEG* expression in the absence of LuxR protein. A possible explanation for the autoinducer-independent modulation of *luxICDABEG* expression is that the LuxR protein activated transcription from the *luxICDABEG* promoter even in the absence of autoinducer (45). To examine this possibility, expression from the *luxICDABEG* promoter in *V. fischeri* MJ-112 (*luxR*::*lacZ*) was examined in detail by monitoring luciferase synthesis. The *luxR* gene is inactivated in this strain, so the autoinducer-independent modulation was expected not to occur if it was attributable to the LuxR protein.

However, instead of remaining constant as anticipated for an absence of modulation, luciferase specific activity decreased 8- to 10-fold and then rose back to its initial level during culture growth (Fig. 5). Therefore the autoinducer-independent modulation observed with MJ-141 (Fig. 4) is not attributable to the LuxR protein. Furthermore, β-galactosidase specific activity (expression from the *luxR* promoter) for cells from the same experiment also exhibited an 8- to 10-fold decrease and rise back (Fig. 5). These results indicate that both *luxICDABEG* and *luxR* are subject to a cell density-dependent modulation that does not require autoinducer or the LuxR protein.

Cell density-dependent modulation of the *lux* region in a Δ(*luxR luxICD*) mutant of *V. fischeri*. The results described above did not exclude the possibility that the *lux* regulatory region is involved in the autoinducer-LuxR protein-independent modulation. Proteins other than LuxR, such as LexA and σ^{32} (2, 51, 52), could interact with *lux* regulatory sequences and account for the observed modulation of expression. To consider this possibility, we next examined *V. fischeri* MJ-201. This strain lacks the entire *lux* regulatory region as well as the genes encoding the putative autoinducer synthase and LuxR protein [Δ(*luxR luxICD*)] (33). The deletion eliminates all sites known or proposed to be involved in *lux* gene regulation. The *luxAB* genes in this strain are intact, however, so luciferase activity levels provided a way of monitoring expression of the *lux* region.

Luciferase activity was very low in MJ-201, which is consistent with the lack of the *luxICDABEG* promoter and the absence of the genes encoding the putative autoinducer synthase and LuxR protein. Nonetheless, luciferase activity could be quantified. Remarkably, luciferase activity exhibited modulation; specific activity decreased 8- to 10-fold during culture growth and then gradually rose back to its initial level (Fig. 6). These results indicate that the autoinducer-LuxR protein-independent modulation of the *lux* system is unrelated to the presence of the *lux* regulatory region or the *lux* regulatory genes. Therefore, it is not likely that interaction with *lux* regulatory sites by proteins such as LexA or σ^{32} accounts for this modulation.

Control of autoinducer-LuxR protein-independent modulation of the *lux* region by glucose and iron. Some environmental factors can influence autoinduction of luminescence in *V. fischeri*. For example, addition of glucose to complete medium leads to faster growth and a delay in autoinduction of luminescence until a higher cell density is attained (43), and restriction of iron leads to slower growth and earlier autoinduction (i.e., at a lower cell density) (27, 28). The effects of glucose and iron have been documented for *E. coli* containing the cloned *V. fischeri lux* genes, but no direct connection between *lux* gene transcriptional control and glucose or iron has been established (12, 14). Consequently, as an alternative to the possibility that these factors operate through direct transcriptional control of *luxICDABEG* by autoinducer and LuxR protein, we tested whether glucose or iron could influence the autoinducer-LuxR protein-independent modulation of the *lux* system.

The effects of added glucose on β-galactosidase and luciferase syntheses were examined with *V. fischeri* MJ-112

(*luxR*::*lacZ*). With or without added glucose, cell density-dependent modulation was observed; both β-galactosidase specific activity (*luxR* expression) and luciferase specific activity (*luxICDABEG* expression) decreased 8- to 10-fold and then rose back gradually during culture growth. However, in the presence of added glucose (10 mM), cells of MJ-112 grew more rapidly, and the rise back in β-galactosidase activity was delayed compared with that in the absence of added glucose (e.g., β-galactosidase specific activity at an OD_{660} of 0.7: no addition, 155; with glucose, 45), as was the rise back in luciferase activity (e.g., luciferase specific activity [10^5] at an OD_{660} of 0.7: no addition, 20.0; with glucose, 6.0).

To examine the response of MJ-112 to iron, the iron chelator EDDHA was used. Addition of EDDHA to complete medium provides an effective way of restricting the availability of iron (12, 27, 41). Regardless of the presence or absence of EDDHA, cells of MJ-112 exhibited cell density-dependent modulation of the *lux* system. However, in the presence of EDDHA (10 μM), cells of MJ-112 grew more slowly and the rise back of β-galactosidase was stimulated (i.e., occurred earlier in culture growth) (e.g., β-galactosidase specific activity at an OD_{660} of 0.3: no addition, 110; with EDDHA, 290), as was the rise back in luciferase synthesis (e.g., luciferase specific activity [10^5] at an OD_{660} of 0.3: no addition, 3.2; with EDDHA, 5.8).

To determine if the effects of glucose and iron on the *lux* system observed with MJ-112 were related to the presence of the *lux* regulatory genes (*luxR* and *luxI*) or the *lux* regulatory region, *V. fischeri* MJ-141 (*luxI*::*lacZ*) and MJ-201 [Δ(*luxR luxICD*)] were also examined. For MJ-141, results similar to those described above for MJ-112 for the effects of EDDHA on growth and β-galactosidase synthesis were obtained (e.g., β-galactosidase specific activity at an OD_{660} of 0.3: no addition, 4.0; with EDDHA, 23.0). Furthermore, for MJ-201, results comparable to those for MJ-112 for the effects of glucose and EDDHA on luciferase synthesis were obtained (e.g., luciferase specific activity [10^5] at an OD_{660} of 0.3: no addition, 0.5; with EDDHA, 1.7; with glucose, 0.3). These results indicate that the effects of glucose and iron are not attributable to the presence of the *lux* regulatory genes or the *lux* regulatory region. Glucose and iron apparently exert their control over expression of the *lux* system by influencing the autoinducer-LuxR protein-independent modulation.

DISCUSSION

Chromosomal *lux*::*lacZ* transcriptional fusion mutants of the bioluminescent symbiotic bacterium *V. fischeri* MJ-1 were constructed in this study by conjugation and gene replacement procedures. Construction of these mutants permitted analysis of *lux* gene control by regulatory factors (autoinducer, LuxR protein, and cAMP) involved in autoinduction and by environmental factors (glucose and iron) for which the mechanisms of action on the *lux* system are not known. Key features of models for *lux* gene regulation derived from studies with *E. coli* containing the *lux* genes were confirmed, and a previously hidden aspect of *lux* gene regulation, a cell density-dependent modulation in the absence of autoinducer and LuxR protein, was revealed. The autoinducer-LuxR protein-independent modulation apparently underlies and contributes to the autoinduction pattern, and its response to glucose and iron helps explain how these environmental factors control *lux* gene expression.

Previously, there were no reported studies involving the genetic manipulation of *V. fischeri*. In this study, conjugative transfer of mobilizable plasmids (48) from *E. coli* to *V. fischeri* was found to be effective for the delivery of cloned and mutated *lux* genes back into *V. fischeri*. Rapid loss from *V. fischeri* cells of vectors carrying *lux* genes facilitated construction of chromosomal *lux*::*lacZ* fusion mutants and a *lux* deletion mutant of *V. fischeri*; homologous recombination apparently operates effectively in this species. However, detailed complementation studies probably will require construction of a *recA*-like derivative of *V. fischeri*, since *lux* sequence-containing vectors are unstable in *V. fischeri*. Expression of the *E. coli lacZ* gene served as an effective reporter of *lux* gene expression in *V. fischeri*. Active β-galactosidase was synthesized by *V. fischeri* containing the *lacZ* gene, and its synthesis was regulated in a fashion consistent with known *lux* gene transcriptional control (see below). We noticed, however, that *V. fischeri* expressing *lacZ* at a high level, but not *V. fischeri* lacking *lacZ*, grew poorly on plates containing X-Gal; apparently X-Gal cleavage products are toxic to this species. This sensitivity could serve as the basis of a selection procedure for loss of *lacZ*-containing transposons and plasmids and for down mutations in *V. fischeri* promoters fused to *lacZ*. The gene transfer and gene replacement methods used here should be applicable to the analysis of other genes in *V. fischeri*.

The construction of *V. fischeri lux*::*lacZ* transcriptional fusion mutants permitted key components of current models for *lux* gene regulation, derived from studies of *E. coli* containing the cloned *lux* genes, to be analyzed and confirmed for *V. fischeri*. In *V. fischeri*, β-galactosidase synthesis in a *luxC*::*lacZ* mutant exhibited autoinducer-mediated cell density-dependent induction (Fig. 3) similar to that described for luciferase synthesis in *V. fischeri* and in *E. coli* containing the intact *lux* genes and to that for β-galactosidase synthesis in *E. coli* containing a *lux* plasmid with a *lacZ* insertion in *luxC* (14, 18, 37). As shown previously with *E. coli* (6, 12, 15, 17, 21, 22, 31, 45, 46, 49), the LuxR protein was required for luminescence in a *luxR*::*lacZ* mutant of *V. fischeri*. Furthermore, cAMP controls *lux* gene expression similarly in *E. coli* (14, 15, 17) and *V. fischeri*, activating transcription from the *luxR* promoter while repressing transcription from the divergent *luxICDABEG* promoter. Finally, the requirement for autoinducer in LuxR protein-dependent activation of *luxICDABEG* transcription was confirmed with a mutant unable to synthesize autoinducer (MJ-141, *luxI*::*lacZ*) (Fig. 4).

Experiments with the *luxI*::*lacZ* mutant, moreover, revealed a previously hidden aspect of *lux* gene regulation, autoinducer-LuxR protein-independent modulation. β-Galactosidase activity levels in the *luxI*::*lacZ* strain exhibited an 8- to 10-fold decrease and rise back during growth of the culture (Fig. 4B), a pattern that is strikingly similar to that of autoinduction of luciferase synthesis in *V. fischeri* (18, 37). In contrast to autoinduction, however, this cell density-dependent modulation does not require autoinducer, LuxR protein, or regulatory sites involved in controlling *lux* gene expression; both β-galactosidase and luciferase levels in a *luxR*::*lacZ* fusion strain and luciferase levels in a Δ(*luxR luxICD*) mutant also exhibited the 8- to 10-fold decrease and rise back (Fig. 5 and 6). Therefore, the pattern of *V. fischeri lux* gene expression observed in batch culture and called autoinduction apparently is composed of two cell density-dependent phenomena, transcriptional control of *luxICD ABEG* by autoinducer and LuxR protein and autoinducer-LuxR protein-independent modulation. This implies that regulation at the gene transcription level per se cannot be inferred simply from the autoinduction pattern of the bacte-

rial luminescence system. The contribution of autoinducer-LuxR protein-independent modulation to autoinduction appears to be substantial, providing a baseline pattern of *luxICDABEG* expression that is enhanced transcriptionally 10- to 100-fold by autoinducer and LuxR protein (Fig. 3 and 4). Consequently, the autoinducer-LuxR protein-independent modulation may have complicated the screening for *luxI*::*lacZ* (Mu dI) insertions in this study (see Materials and Methods), and it might account for the cell density-dependent response to autoinducer of *E. coli* containing the cloned *V. fischeri lux* genes with a *luxI* mutation (1).

Although autoinducer-LuxR protein-independent modulation does not require autoinducer, LuxR protein, or *lux* regulatory sequences, it is influenced by glucose and iron, environmental factors known to control luminescence (12, 14, 27, 28, 43) but for which the mechanisms of control are unclear. Glucose has been thought to retard synthesis of LuxR protein by decreasing cellular levels of cAMP (14, 43). Iron has been proposed to block *luxICDABEG* transcription by associating with an iron-binding repressor protein, thereby delaying the accumulation of autoinducer (28). However, the relationships between glucose, cellular cAMP levels, and *luxR* transcription have not been defined, and evidence inconsistent with an iron-binding repressor protein functioning in the *V. fischeri lux* system has been obtained (12). Consequently, no direct link between luminescence and glucose or iron has been established. Instead, the influence of glucose and iron on the *V. fischeri* luminescence system appears to be indirect (12), operating through autoinducer-LuxR protein-independent modulation rather than through direct transcriptional control by autoinducer and LuxR protein. This implies that the glucose effect (43) is not related to an interaction of cAMP and CRP with *lux* regulatory sequences. The effect of oxygen on the *V. fischeri* luminescence system (40), which was not addressed in this study, will provide an interesting comparison with the effects seen here with glucose and iron.

The lack of a requirement for autoinducer, LuxR protein, or the *lux* regulatory region suggests that autoinducer-LuxR protein-independent modulation results from effects outside of the *lux* region. It is known in this regard that cell density-dependent changes in DNA replication initiation occur during batch culture growth (7) and that products of genes proximal to the origin of replication can exhibit as much as eightfold modulation simply due to changes in the dosage of those genes (3, 34). The location of the *lux* genes on the *V. fischeri* chromosome has not been reported, but studies with *V. fischeri* (13) show a decrease and rise back in specific DNA content that reflect the pattern of autoinducer-LuxR protein-independent modulation of the *lux* system described here. Possibly, then, the link between this modulation and control by autoinducer and LuxR protein relates to the dosage of the *lux* regulatory genes, modulation of which has been proposed to influence the cell density at which induction of the luminescence system occurs (12). However, the relationships between cell density-dependent changes in DNA replication initiation and factors that alter the growth rate of *V. fischeri* cells (e.g., presence of glucose and restriction of iron) remain to be determined, as does whether such control might be mediated by an inducer or by an inhibitor (18). As an alternative mechanism, DNA supercoiling in *V. fischeri* might exhibit cell density-dependent effects or growth rate effects and thereby modulate expression of the *lux* system, as shown for the expression of a virulence gene in *Shigella flexneri* (9). The mechanism for autoinducer-LuxR protein-independent modulation of the *lux* system and the basis for its response to environmental factors may have relevance for other bacterial systems controlled by autoregulatory factors, such as A-factor control of antibiotic synthesis in *Streptomyces griseus* (29, 32).

ACKNOWLEDGMENTS

We thank J. Ray for technical assistance, A. Eberhard for pure autoinducer, A. Pühler for pSUP vectors, and L. Gibson for comments on the manuscript.

This work was supported by a Woods Hole Oceanographic Institution Independent Study Award (to P.V.D.) and an Ocean Ventures Fund Award (to A.K.).

REFERENCES

1. **Adar, Y. Y., J. Kuhn, and S. Ulitzur.** 1991. The effect of external inducer on *E. coli* containing the *lux* operon of *V. fischeri* with a *luxI* deletion during different stages of growth, p. 63–66. *In* P. E. Stanley and L. J. Kricka (ed.), Bioluminescence and chemiluminescence, current status. John Wiley & Sons, Chichester, England.
2. **Baldwin, T. O., J. H. Devine, R. C. Heckel, J.-W. Lin, and G. S. Shadel.** 1989. The complete nucleotide sequence of the *lux* regulon of *Vibrio fischeri* and the *luxABN* region of *Photobacterium leiognathi* and the mechanism of control of bacterial bioluminescence. J. Biolumin. Chemilumin. **4:**326–341.
3. **Bird, R. E., J. Louarn, J. Martuscelli, and L. Caro.** 1972. Origin and sequence of chromosome replication in *Escherichia coli*. J. Mol. Biol. **70:**547–566.
4. **Boylan, M., A. F. Graham, and E. A. Meighen.** 1985. Functional identification of the fatty acid reductase components encoded in the luminescence operon of *Vibrio fischeri*. J. Bacteriol. **163:** 1186–1190.
5. **Castilho, B. A., P. Olfson, and M. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158:**488–495.
6. **Choi, S. H., and E. P. Greenberg.** 1991. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent lux gene activating domain. Proc. Natl. Acad. Sci. USA **88:**11115–11119.
7. **Churchward, G., E. Estiva, and H. Bremer.** 1981. Growth rate-dependent control of chromosome replication initiation in *Escherichia coli*. J. Bacteriol. **145:**1232–1238.
8. **Devine, J. H., C. Countryman, and T. O. Baldwin.** 1988. Nucleotide sequence of the *luxR* and *luxI* genes and the structure of the primary regulatory region of the *lux* regulon of *Vibrio fischeri* ATCC 7744. Biochemistry **27:**837–842.
9. **Dorman, C. J., N. N. Bhriain, and C. F. Higgins.** 1990. DNA supercoiling and environmental regulation of virulence gene expression in *Shigella flexneri*. Nature (London) **344:**789–792.
10. **Dunlap, P. V.** 1989. Regulation of luminescence by cyclic AMP in *cya*-like and *crp*-like mutants of *Vibrio fischeri*. J. Bacteriol. **171:**1199–1202.
11. **Dunlap, P. V.** 1991. Organization and regulation of bacterial luminescence genes. Photochem. Photobiol. **54:**1157–1170.
12. **Dunlap, P. V.** Iron control of the *Vibrio fischeri* luminescence system in *Escherichia coli*. Arch. Microbiol., in press.
13. **Dunlap, P. V.** Mechanism for iron control of the *Vibrio fischeri* luminescence system: involvement of cyclic AMP and cyclic AMP receptor protein and modulation of DNA level. J. Biolumin. Chemilumin., in press.
14. **Dunlap, P. V., and E. P. Greenberg.** 1985. Control of *Vibrio fischeri* luminescence gene expression in *Escherichia coli* by cyclic AMP and cyclic AMP receptor protein. J. Bacteriol. **164:**45–50.
15. **Dunlap, P. V., and E. P. Greenberg.** 1988. Control of *Vibrio fischeri lux* gene transcription by a cAMP receptor protein-LuxR protein regulatory circuit. J. Bacteriol. **170:**4040–4046.
16. **Dunlap, P. V., and E. P. Greenberg.** 1991. Role of intercellular chemical communication in the *Vibrio fischeri*-monocentrid fish symbiosis, p. 219–253. *In* M. Dworkin (ed.), Microbial cell-cell interactions. American Society for Microbiology, Washington, D.C.

17. **Dunlap, P. V., and J. M. Ray.** 1989. Requirement for autoinducer in transcriptional negative autoregulation of the *Vibrio fischeri luxR* gene in *Escherichia coli*. J. Bacteriol. **171:**3549–3552.
18. **Eberhard, A.** 1972. Inhibition and activation of bacterial luciferase synthesis. J. Bacteriol. **109:**1105–1109.
19. **Eberhard, A., A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, and N. J. Oppenheimer.** 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry **20:**2444–2449.
20. **Eberhard, A., T. Longin, C. A. Widrig, and S. J. Stranick.** 1991. Synthesis of the *lux* gene autoinducer in *Vibrio fischeri* is positively autoregulated. Arch. Microbiol. **155:**294–297.
21. **Engebrecht, J., K. Nealson, and M. Silverman.** 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell **32:**773–781.
22. **Engebrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA **81:**4154–4158.
23. **Engebrecht, J., and M. Silverman.** 1987. Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. Nucleic Acids Res. **15:**10455–10467.
24. **Friedrich, W. F., and E. P. Greenberg.** 1983. Glucose repression of luminescence and luciferase in *Vibrio fischeri*. Arch. Microbiol. **134:**87–91.
25. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
26. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotropic sources. J. Opt. Soc. Am. **53:**1410–1415.
27. **Haygood, M. G., and K. H. Nealson.** 1985. The effect of iron on the growth and luminescence of the symbiotic bacterium *Vibrio fischeri*. Symbiosis **1:**39–51.
28. **Haygood, M. G., and K. H. Nealson.** 1985. Mechanisms of iron regulation of luminescence in *Vibrio fischeri*. J. Bacteriol. **162:**209–216.
29. **Horinouchi, S., Y. Kumada, and T. Beppu.** 1984. Unstable genetic determinant of A-factor biosynthesis in streptomycin-producing organisms: cloning and characterization. J. Bacteriol. **158:**481–487.
30. **Kaplan, H. B., and E. P. Greenberg.** 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J. Bacteriol. **163:**1210–1214.
31. **Kaplan, H. B., and E. P. Greenberg.** 1987. Overproduction and purification of the *luxR* gene product: the transcriptional activator of the *Vibrio fischeri* luminescence system. Proc. Natl. Acad. Sci. USA **84:**6639–6643.
32. **Khokhlov, A. S., L. N. Anisova, I. I. Tovarova, E. M. Kleiner, I. V. Kovalenko, O. I. Krasilnikova, E. Y. Kornitskaya, and S. A. Pliner.** 1973. Effect of A-factor on the growth of asporogeneous mutants of *Streptomyces griseus*, not producing this factor. Z. Allg. Mikrobiol. **13:**647–655.
33. **Kuo, A., and P. V. Dunlap.** 1991. Evidence of a physiological role for the luminescence system in *Vibrio fischeri*, abstr. I-24, p. 194. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991.
34. **Masters, M., and P. Broda.** 1971. Evidence for the bidirectional replication of the *Escherichia coli* chromosome. Nature (London) New Biol. **232:**137–140.
35. **Meighen, E. A., and P. V. Dunlap.** Physiological, biochemical and genetic control of bacterial bioluminescence. Adv. Microb. Physiol., in press.
36. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase. Occurrence, mechanism and significance. Arch. Microbiol. **112:**73–79.
38. **Nealson, K. H.** 1978. Isolation, identification, and manipulation of luminous bacteria. Methods Enzymol. **57:**153–166.
39. **Nealson, K. H.** 1979. Alternative strategies of symbiosis of marine luminous fishes harboring light-emitting bacteria. Trends Biochem. Sci. **4:**105–110.
40. **Nealson, K. H., and J. W. Hastings.** 1977. Low oxygen is optimal for luciferase synthesis in some bacteria. Arch. Microbiol. **112:**9–16.
41. **Rogers, H. J.** 1973. Iron-binding catechols and virulence in *Escherichia coli*. Infect. Immun. **7:**446–456.
42. **Rosson, R. A., and K. H. Nealson.** 1981. Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch. Microbiol. **129:**299–304.
43. **Ruby, E. G., and K. H. Nealson.** 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*: a model of symbiosis based on bacterial studies. Biol. Bull. **151:**574–586.
44. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Shadel, G. S., and T. O. Baldwin.** 1991. The *Vibrio fischeri* LuxR protein is capable of bidirectional stimulation of transcription and both positive and negative regulation of the *luxR* gene. J. Bacteriol. **173:**568–574.
46. **Shadel, G. S., R. Young, and T. O. Baldwin.** 1990. Use of regulated cell lysis in a lethal genetic selection in *Escherichia coli*: identification of the autoinducer binding region of the LuxR protein from *Vibrio fischeri* ATCC 7744. J. Bacteriol. **172:**2946–2954.
47. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments in gene fusions, p. 217. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Simon, R., M. O'Connell, M. Labes, and A. Pühler.** 1986. Plasmid vectors for the genetic analysis and manipulation of rhizobia and other Gram-negative bacteria. Methods Enzymol. **118:**640–659.
49. **Slock, J., D. VanRiet, D. Kolibachuk, and E. P. Greenberg.** 1990. Critical regions of the *Vibrio fischeri* LuxR protein defined by mutational analysis. J. Bacteriol. **172:**3974–3979.
50. **Swartzman, E. S. Kapoor, A. F. Graham, and E. A. Meighen.** 1990. A new *Vibrio fischeri lux* gene precedes a bidirectional termination site for the *lux* operon. J. Bacteriol. **172:**6797–6802.
51. **Ulitzur, S.** 1989. The regulatory control of the bacterial luminescence system—a new view. J. Biolumin. Chemilumin. **4:**317–325.
52. **Ulitzur, S., and J. Kuhn.** 1988. The transcription of bacterial luminescence is regulated by sigma 32. J. Biolumin. Chemilumin. **2:**81–93.