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Diffusion of Autoinducer Is Involved in Regulation of the *Vibrio fischeri* Luminescence System

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The enzymes for luminescence in *Vibrio fischeri* are induced by the accumulation of a species-specific metabolite (autoinducer) in the culture medium. Tritium-labeled autoinducer was used to study the mechanism of autoinduction. When ^{3}H-autoinducer was added to suspensions of *V. fischeri* or *Escherichia coli*, cellular concentrations equaled external concentrations. For *V. fischeri*, equilibration of ^{3}H-autoinducer was rapid (within 20 s), and >90% of the cellular tritium remained in unmodified autoinducer. When *V. fischeri* or *E. coli* cells containing ^{3}H-autoinducer were transferred to autoinducer-free buffer, 85 to 99.5% of the radiotracer escaped from the cells, depending on the strain. Concentrations of autoinducer as low as 10 nM, which is equivalent to 1 or 2 molecules per cell, were sufficient for induction, and the maximal response to autoinducer occurred at about 200 nM. If external autoinducer concentrations were decreased to below 10 nM after induction had commenced, the induction response did not continue. Based on this study, a model for autoinduction is described wherein autoinducer association with cells is by simple diffusion and binding of autoinducer to its active site is reversible.

The marine bacterium *Vibrio fischeri* possesses an inducible system for light production. The inducer, *N*-(3-oxohexanoyl) homoserine lactone, termed autoinducer, is produced by *V. fischeri*, is species specific, and accumulates in the culture medium. When autoinducer reaches a critical extracellular concentration, induction of the light-emitting enzyme, luciferase, and other enzymes involved in luminescence commences (2, 3, 8, 10). Autoinduction is an environmental sensing mechanism; under conditions wherein autoinducer accumulates, the bacteria respond by synthesizing the luminescence enzymes. Thus, when cells are grown in batch cultures, there is a lag in luminescence during early exponential growth phase followed by a dramatic increase in light emission later in exponential growth phase (10, 12).

V. fischeri has been isolated from seawater where it occurs at low population densities ($<10^2$/ml), and it also has been isolated as the bacterial symbiont within specialized light-emitting organs of certain marine fishes such as the Japanese pinecone fish, *Monocentris japonicus* (16–18). In the symbiosis with *M. japonicus*, *V. fischeri* occurs at densities of approximately 10^{10} cells per ml of light organ fluid (17). Autoinducer serves as a pheromone which signals cells in a light organ symbiosis to luminesce (2, 6). In seawater, autoinducer does not accumulate, and the symbiosis-related function, luminescence, is not induced (11, 17).

Although several species of luminous marine bacteria have been reported to display autoinduction (2, 6, 10, 14), certain recent advances involving *V. fischeri* make it an ideal model for investigating the molecular details of this cell density-dependent control system (3–5, 8). A fragment of *V. fischeri* DNA that encodes all the functions necessary for light production in *Escherichia coli* and which also specifies regulatory elements for autoinduction has been cloned and genetically characterized. This 9-kilobase DNA fragment contains the two *lux* operons. One, operon R, encodes five polypeptides required for luminescence and one polypeptide required for autoinducer synthesis. The other, operon L, encodes a protein, termed the receptor because it is required together with a sufficient concentration of autoinducer to activate transcription of operon R and thus induce luminescence (4, 5). Of particular importance for this study, carrier-free, tritium-labeled, biologically active autoinducer has been synthesized for use as a radiotracer (8).

Many important questions concerning autoinduction remain. For example, nothing is yet known about how or even whether the receptor interacts with *lux* DNA, how autoinducer interacts with the receptor, or how autoinducer associates with and dissassociates from cells. As a first step in understanding the details of autoinduction, we describe herein how autoinducer enters and exits cells.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Two strains of *V. fischeri* were used: *V. fischeri* MJ1, a brightly luminescent strain (17), and *V. fischeri* B61, a strain that produces dim light because it makes very little autoinducer (1, 10). The *E. coli* strain used was a derivative of HB101 described elsewhere (4); this strain contained one of three plasmids (pJE202, pJE411, or pBR322) as indicated. Both pJE202 and pJE411 are recombinant plasmids derived from pBR322 and *V. fischeri* DNA. pJE202 encodes the enzymes for luminescence and specifies regulatory functions necessary for luminescence in *E. coli* (*lux* operons L and R). pJE411 encodes one regulatory function: the autoinducer receptor function (4). *E. coli* containing pJE202 and pJE411 and *E. coli* containing neither of these plasmids were obtained from J. Engebrecht. *E. coli* was transformed with pBR322 purchased from International Biotechnologies, Inc., New Haven, Conn., by procedures described elsewhere (9).

The culture medium for *V. fischeri* B61 and MJ1 contained the following ingredients per liter: 5 g of tryptone (Difco Laboratories, Detroit, Mich.), 3 g of yeast extract (Difco), 3 ml of glycerol, 50 mmol of Tris base, 19 mmol of NH_4Cl, 0.33 mmol of $K_2HPO_4 \cdot 3H_2O$, 1.8 nmol of $FeSO_4 \cdot 7H_2O$, and

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500 ml of seawater salts solution (seawater salts solution consisted of 0.6 M NaCl, 0.1 M $MgSO_4 \cdot 7H_2O$, 0.02 M $CaCl_2 \cdot 2H_2O$, 0.02 M KCl). The medium was brought to volume with deionized water. The pH was adjusted to 7.0 with concentrated HCl, and the medium was sterilized by autoclaving. *E. coli* was cultured in L broth (19) adjusted to pH 7.5 with 10 N NaOH before autoclaving, after which ampicillin (80 μg/ml) was added to assure plasmid maintenance.

In all cases, cells were grown in 5-ml batches of culture medium contained in tubes (16 by 150 mm). Inocula for *V. fischeri* B61 cultures were 0.2 ml of mid-logarithmic phase cultures that had been stored in liquid nitrogen. For other bacteria, cultures were inoculated to a density of 0.03 to 0.05 (optical density at 660 nm; Spectronic 21; Bausch & Lomb, Inc., Rochester, N.Y.) with overnight cultures. Cultures were incubated with shaking at 22 and 30°C for *V. fischeri* and *E. coli*, respectively. For all strains, cultures were grown to an optical density at 660 nm of 1.0. As determined by direct counting procedures (7), this corresponded to 2.5×10^9 cells per ml for each strain of *E. coli* and 5×10^9 cells per ml for both strains of *V. fischeri*.

Preparation of cell suspensions. Cultures of *V. fischeri* were harvested by centrifugation (8,000 × *g* at 4°C for 5 min) and then suspended in nutrient buffer containing 0.01 M potassium phosphate (pH 7.0), 0.3 M NaCl, 0.05 M $MgSO_4 \cdot 7H_2O$, 0.05% tryptone (Difco), and 0.03% glycerol. For later use in determinations of cellular autoinducer concentrations, cells were suspended to a density of 5×10^{10}/ml, and for bioassays, *V. fischeri* B61 was suspended at a density of 5×10^8 cells per ml. *E. coli* cells were harvested by centrifugation as described above and suspended to a density of 2.5×10^{10}/ml in a buffer containing 0.01 M potassium phosphate (pH 7.0), 0.05% tryptone, and 0.03% glycerol.

Measurement of cellular autoinducer concentration. Per milliliter, each assay mixture contained 0.4 ml of cell suspension (2×10^{10} *V. fischeri* cells or 1×10^{10} *E. coli* cells) and 0.6 ml of suspending buffer (0.01 M potassium phosphate [pH 7.0], 0.3 M NaCl, 0.05 M $MgSO_4 \cdot 7H_2O$ for *V. fischeri* or 0.01 M potassium phosphate [pH 7.0] for *E. coli*) containing the desired concentration of ^{3}H-autoinducer. Unless otherwise specified, after a 5-min incubation period (22 to 25°C for *V. fischeri*, 30°C for *E. coli*), duplicate 0.1-ml samples were centrifuged through 0.1 ml of Versilube F(50) silicone fluid (General Electric Co., Waterford, N.Y.) into 0.05 ml of an aqueous solution of 2% trichloroacetic acid and 10% glycerol. Centrifugation was in 0.5-ml-capacity microcentrifuge tubes for 30 s in a Coleman microcentrifuge. The amounts of radiolabel in the suspending buffer above and the cell pellet below the silicone fluid were determined by standard scintillation counting procedures.

For determinations of cell volumes, either the freely permeable [^{14}C]ethylene glycol (5 μCi/ml of assay mixture; specific activity, 3 mCi/mmol) or the impermeable [^{14}C]dextran (1 μCi/ml of assay mixture; specific activity, 1.4 mCi/g) was used in place of ^{3}H-autoinducer. By measuring the amount of radiolabel associated with cells pelleted through silicone fluid, cell volume was calculated as described elsewhere (15).

This technique for measuring cell volume and cellular concentrations of radiolabeled compounds was based on previously described methods (13).

Measurement of autoinducer outflow from cells. Exit of autoinducer from cells was measured by comparing cellular autoinducer concentrations determined as described above with concentrations remaining after cells had been washed twice (by microcentrifugation at 13,000 × *g* at 22 to 25°C for 1 min) and resuspended in nutrient buffer. The incubation period between each wash was 5 min.

Autoinducer bioassays. Biological activity of autoinducer preparations was determined by a previously described technique (8). Autoinducer preparations (0.05 ml or less of an aqueous solution) were added to 1 ml of a *V. fischeri* B61 cell suspension contained in 20-ml glass scintillation vials, and luminescence was measured over time with a Turner model 20 photometer.

Analysis of cellular autoinducer by HPLC. Cells were incubated in buffer with ^{3}H-autoinducer as described above and then pelleted by centrifugation (13,000 × *g* at 4°C for 1 min in a microcentrifuge). The cell pellet was extracted twice with 0.2 ml of ethyl acetate. The extracts were then pooled, 10 nmol of unlabeled autoinducer was added as a carrier, and part of this material was used to determine total radioactivity extracted. A 0.1-ml sample of the material was dried, suspended in 0.1 ml of water-methanol (75:25), and subjected to analytical high-pressure liquid chromatography (HPLC; C_{18} reverse phase column; solvent system, 75% water–25% methanol; flow rate, 2 ml/min) as described previously (8).

Chemicals. *N*-(3-Oxohexanoyl) homoserine lactone (autoinducer) and *N*-[3-oxo-(4,5-$^3H_2$)-hexanoyl] homoserine lactone (^{3}H-autoinducer) were synthesized as described in a previous publication (8). HPLC-grade solvents were obtained from Fisher Scientific Co., Pittsburgh, Pa. [^{14}C]ethylene glycol was purchased from ICN Pharmaceuticals, Inc., Irvine, Calif., and [^{14}C]dextran, with an average molecular weight of 70,000, was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Comparison of cellular and external concentrations of autoinducer. With the rapid centrifugation procedure, it was demonstrated that when ^{3}H-autoinducer was added to *V. fischeri* B61, tritium associated with cells. Assuming that the cell-associated tritium was part of an unmodified compound and by using the measured value for cell volume, the internal autoinducer concentration could be calculated and compared with external concentrations. The assumption that cellular radiolabel was in the form of unmodified autoinducer is supported by the demonstration that approximately 90% of the tritium associated with cell pellets after centrifugation was extracted with ethyl acetate (a solvent used to extract autoinducer from aqueous solutions [10]) and that the HPLC elution profile of this extracted, tritium-labeled material was indistinguishable from that of ^{3}H-autoinducer (Fig. 1).

For *V. fischeri* B61 incubated with various concentrations of autoinducer, the ratio of cellular to external molarity was close to 1 (Table 1). Time course experiments demonstrated that the equimolar internal and external concentrations were established by the first sampling point (20 s; data not shown). The concentrations of autoinducer tested included those which resulted in the induction of luminescence. Under the conditions of our bioassays, a clear response (increase in luminescence) was observed when autoinducer was added to 10 nM, and the rate of induction was proportional to the concentration of autoinducer added up to 200 nM (Fig. 2). The rate of induction was not further increased by the addition of higher concentrations of autoinducer (data not shown). Because the volume of *V. fischeri* B61 was 3.5 μl/10^{10} cells, it appears that in the presence of 1 or 2 molecules of added autoinducer per cell, some induction occurred and approximately 40 molecules per cell resulted in the maximal rate of induction.

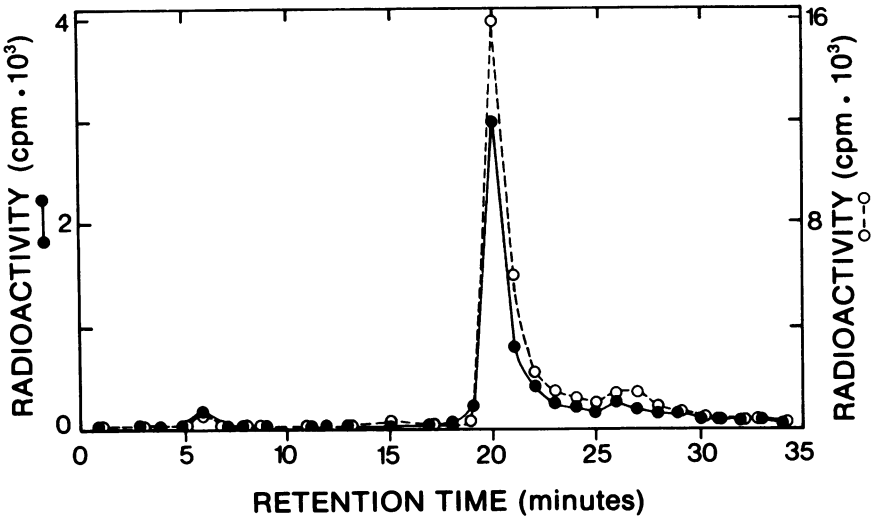

FIG. 1. HPLC comparison of ^{3}H-autoinducer with tritium-labeled material extracted from *V. fischeri* B61 that had been loaded with ^{3}H-autoinducer. Symbols: ○, ^{3}H-autoinducer (specific activity, 10 mCi/mmol); ●, material extracted from cells.

It seemed possible that the inability of *V. fischeri* B61 to concentrate autoinducer was related to the low, uninduced levels of luminescence enzymes in this strain. Perhaps an autoinducer transport system is coinduced with other luminescence-related proteins. To test this hypothesis, we measured the amount of radiolabel associated with cells of *V. fischeri* MJ1 that had induced levels of luminescence enzymes. As with *V. fischeri* B61, *V. fischeri* MJ1 did not concentrate tritium; the cellular autoinducer concentration was equivalent to the external concentration (Table 1). This was also true of *E. coli* containing a recombinant plasmid (pJE202) with all of the *V. fischeri* genes necessary for luminescence and autoinduction, of *E. coli* containing a recombinant plasmid (pJE411) that encodes receptor but that has a polar mutation in the first gene of operon R (4), and even of *E. coli* containing only the plasmid vector (pBR322) with no *V. fischeri* DNA (Table 1).

TABLE 1. Cellular and external concentrations of ^{3}H-autoinducer

Organism	External autoinducer concn[a]	Cellular autoinducer concn[b]	Ratio of cellular to external autoinducer concns
V. fischeri B61	19 nM[c]	20 nM	1.1
V. fischeri B61	200 nM[c]	170 nM	0.9
V. fischeri B61	2.2 μM[d]	2.3 μM	1.0
V. fischeri B61	24.8 μM[e]	21.2 μM	0.9
V. fischeri MJ1	280 nM[c]	280 nM	1.0
E. coli (pJE411)	190 nM[c]	200 nM	1.1
E. coli (pJE202)	190 nM[c]	140 nM	0.7
E. coli (pBR322)	190 nM[c]	190 nM	1.0

[a] Calculated from the amount of radiolabel above the silicone fluid after centrifugation.

[b] Calculated from the amount of radiolabel centrifuged through silicone fluid and the cell volume. Corrected for the small amount of extracellular material carried through the silicone fluid (as determined with [^{14}C]dextran). The volume for both strains of *V. fischeri* was 3.5 μl/10^{10} cells, and for *E. coli* it was 5 μl/10^{10} cells regardless of which plasmid was present.

[c] Specific activity of the ^{3}H-autoinducer added, 50 Ci/mmol.

[d] Specific activity of the ^{3}H-autoinducer added, 5 Ci/mmol.

[e] Specific activity of the ^{3}H-autoinducer added, 0.5 Ci/mmol.

Exit of autoinducer from cells. The observation that autoinducer entered cells and that cellular concentrations rapidly equilibrated to external concentrations (Table 1) suggests that cells are freely permeable to autoinducer, as

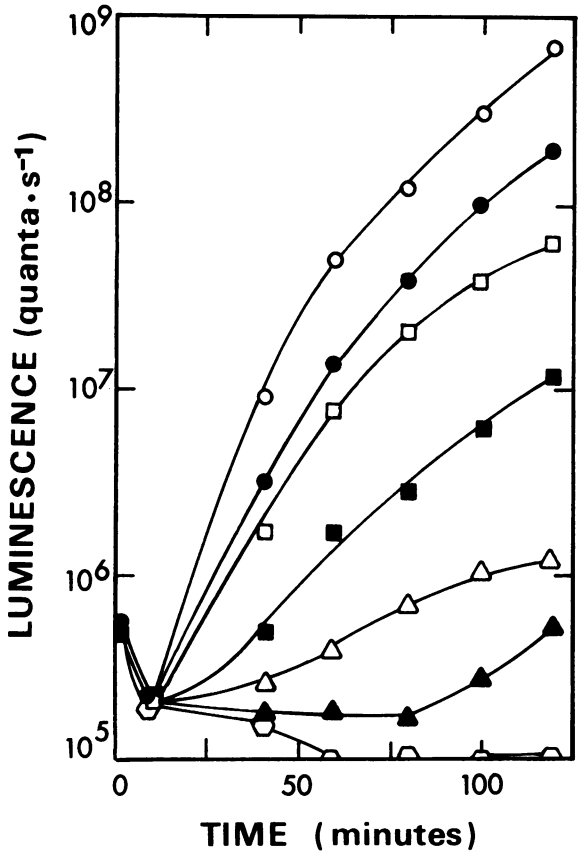

FIG. 2. Bioassay for autoinducer. Luminescence as a function of time with different concentrations of autoinducer: 200 nM (○), 100 nM (●), 50 nM (□), 25 nM (■), 10 nM (△), 5 nM (▲), none added (⬡).

TABLE 2. Exit of ^{3}H-autoinducer from cells

Organism	Radioactivity in loaded cells (cpm/10^{10} cells)[a]	Radioactivity retained in cells suspended in autoinducer-free buffer (cpm/10^{10} cells)	% Released[b]
V. fischeri B61	8.5×10^4	3.6×10^3	96
V. fischeri MJ1	8×10^4	4×10^2	99.5
E. coli (pJE411)	1.2×10^5	1.7×10^4	85
E. coli (pJE202)	1.3×10^5	2.0×10^4	96
E. coli (pBR322)	9.3×10^4	4.2×10^3	86

[a] Specific activity of ^{3}H-autoinducer added was 50 Ci/mmol.
[b] The percentage of counts in loaded cells that was not retained after suspension in autoinducer-free buffer.

they are to water or ethylene glycol. If this hypothesis is correct, then a decrease in external autoinducer concentration should lead to a decrease in cellular autoinducer concentration (back diffusion). When cells of either *V. fischeri* strain or any recombinant *E. coli* described above were loaded with ^{3}H-autoinducer (140 to 280 nM), washed, and then suspended in autoinducer-free buffer, cellular levels of autoinducer decreased 85 to 99.5%, the exact value depending on the strain used (Table 2). When similar experiments were performed with [^{14}C]ethylene glycol-loaded cells, no cellular radioactivity was detected after removal of the external [^{14}C]ethylene glycol. Furthermore, in a time course experiment in which *V. fischeri* B61 was preloaded with ^{3}H-autoinducer (200 nM) and then suspended in autoinducer-free buffer, the radiolabel remained with the cells for at least 50 min (the duration of this experiment). Thus, the small amount of radiolabel remaining after the removal of external ^{3}H-autoinducer was tightly associated with the cells.

Back diffusion of cellular autoinducer appears to stop transcriptional activation of *lux* genes. To test the possibility that transcriptional activation of operon R is reversed by back diffusion of autoinducer, *V. fischeri* B61 was incubated in the presence of autoinducer, and after the onset of transcriptional activation, the cells were centrifuged and suspended in autoinducer-free medium, and luminescence was monitored (Fig. 3). When the initial autoinducer concentration was 200 nM, the final extracellular autoinducer concentration was below the level required for a response in autoinducer bioassays (Fig. 2), and in this case luminescence did not continue to increase (Fig. 3). However, when the initial concentration was 2,000 nM, the final extracellular concentration remained high enough for some transcriptional activation, and in this case luminescence continued to increase. This together with the other controls described (Fig. 3) demonstrated that the manipulations involved in these experiments (centrifugation, resuspension, etc.) did not stop transcriptional activation of the luminescence genes. This experiment supports the contention that transcriptional activation of the luminescence genes involves a reversible association of autoinducer with cells.

DISCUSSION

This report demonstrates that *V. fischeri* and *E. coli* are freely permeable to autoinducer. When autoinducer was added to cell suspensions, internal concentrations approximated external concentrations (Table 1), and equilibration was rapid (within 20 s). For *V. fischeri* B61, over 90% of the cellular autoinducer appeared unmodified (Fig. 1). When cells were transferred from a buffer containing autoinducer to autoinducer-free buffer, autoinducer escaped from the cells (Table 2). Apparently, autoinducer was capable of diffusion into or out of cells, and the fact that similar results were obtained with a brightly luminous and a dim strain of *V. fischeri* and with *E. coli* containing no *V. fischeri* luminescence genes argues that no specific uptake or export systems are involved with autoinduction.

The increase in luminescence of *V. fischeri* cells ceased after back diffusion of autoinducer (Fig. 3); thus, the small amount of radiolabel that remained tightly associated with cells (Table 2) does not appear to be related to regulation of *lux* gene transcription. Apparently, autoinducer must associate in a reversible manner with some cellular component, presumably the 27,000-dalton receptor protein (5). This protein is required for cells to respond to autoinducer (4); however, binding of autoinducer to this protein has not yet been investigated.

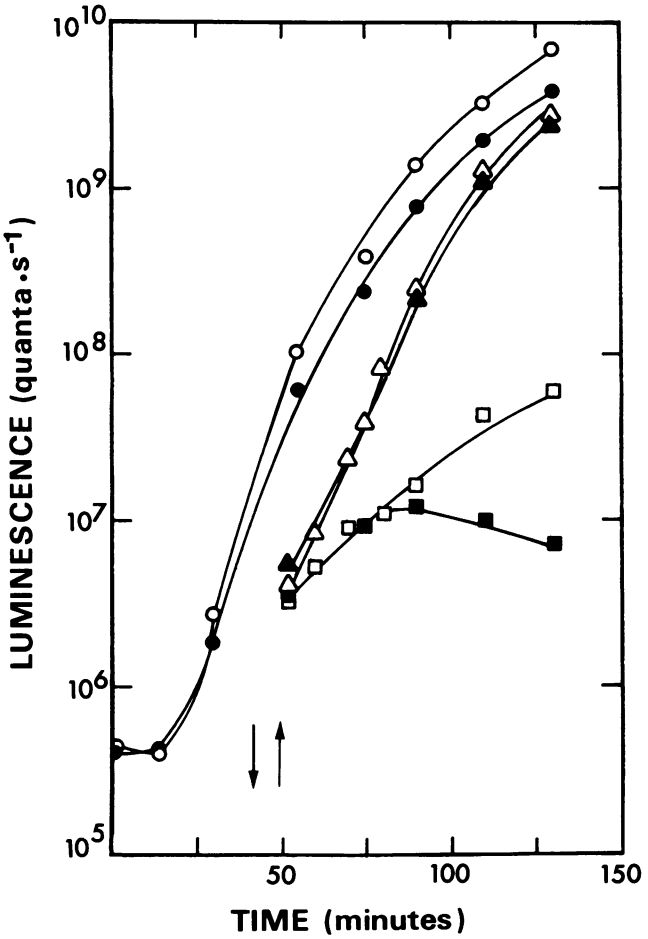

FIG. 3. Luminescence of *V. fischeri* B61 after dilution of external autoinducer. Induction in the presence of 200 nM (●) or 2,000 nM (○) ^{3}H-autoinducer; autoinduction of cells centrifuged from and resuspended in 200 nM (▲) or 2,000 nM (△) ^{3}H-autoinducer; luminescence of cells centrifuged from 200 nM (■) or 2,000 nM (□) ^{3}H-autoinducer and suspended in autoinducer-free buffer. Centrifugations and suspensions were at 40 to 48 min as indicated by the arrows. For assays wherein cells were suspended in autoinducer-free medium, the final external ^{3}H-autoinducer concentrations were determined to be 0.3 nM (■) and 7.7 nM (□). The specific activity of the 200 nM ^{3}H-autoinducer was 50 Ci/mmol, and that of the 2,000 nM ^{3}H-autoinducer was 5 Ci/mmol.

This study is consistent with and adds detail to the model for autoinduction suggested by Engebrecht et al. (4). As that model indicates, when external autoinducer concentrations reach a critical level, transcription of operon R is activated. Furthermore, autoinducer must interact with an operon L gene product. We now add to this model the suggestion that autoinducer is synthesized within cells and diffuses out. As environmental concentrations of this sensory molecule increase, so will cellular concentrations, again as a result of simple diffusion, and at concentrations in the range of 10 nM or approximately 2 molecules per cell, there is some activation of operon R. This activation appears to increase with increasing autoinducer concentrations, up to about 40 molecules per cell (Fig. 2). Another feature of our more detailed model is that the interaction of autoinducer with its receptor is reversible in nature.

The results of this study indicate that autoinduction is an extremely sensitive sensory system and one that, as might be expected, seems an ideally suited regulatory system with respect to the ecology of *V. fischeri*. In light organs of *M. japonicus* for example, *V. fischeri* cells are very densely packed (17), and the synthesis of small amounts of autoinducer should raise cellular concentrations of this diffusible molecule to the low level required for synthesis of the luminescence enzymes. If a cell was excreted into the surrounding seawater, its autoinducer concentration would drop rapidly, again as a result of simple diffusion. From our experiments (Fig. 3), it is expected that this would effect a rapid decrease in transcription of operon R. This system can therefore provide for a sensitive and rapid sensory response to changes in environmental conditions. Furthermore, no energy need be spent on transport (uptake or export) of autoinducer, and a minimal number of gene products are required for autoinduction. In fact, genetic analyses indicate that only two specific genes are required for autoinduction, one encoding the receptor and the other apparently encoding an enzyme that can synthesize autoinducer from substrates that must generally occur in *E. coli* (4). With recent advances in the molecular genetics of the *V. fischeri* luminescence system (4, 5) and with the availability of radioactive autoinducer (8), it should now be possible to study the interactions of autoinducer and its receptor and to study the mechanism by which they serve to activate transcription.

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LITERATURE CITED

1. **Baumann, P., L. Baumann, and M. Mandel.** 1971. Taxonomy of marine bacteria: the genus *Beneckea*. J. Bacteriol. **107**:268–294.
2. **Eberhard, A.** 1972. Inhibition and activation of bacterial luciferase synthesis. J. Bacteriol. **109**:1101–1105.
3. **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.
4. **Engebrecht, J., K. Nealson, and M. Silverman.** 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell **32**:773–781.
5. **Engebrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. U.S.A. **81**:4154–4158.
6. **Greenberg, E. P., J. W. Hastings, and S. Ulitzur.** 1979. Induction of luciferase synthesis in *Beneckea harveyi* by other marine bacteria. Arch. Microbiol. **120**:87–91.
7. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
8. **Kaplan, H. B., A. Eberhard, C. Widrig, and E. P. Greenberg.** 1985. Synthesis of *N*-[3-oxo-(4,5-$^3H_2$)-hexanoyl] homoserine lactone: biologically active tritium-labelled *Vibrio fischeri* autoinducer. J. Labelled Compd. Radiopharm. **22**(4):387–395.
9. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 250–251. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase: occurrence, mechanism and significance. Arch. Microbiol. **112**:73–79.
11. **Nealson, K. H., and J. W. Hastings.** 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. **43**:496–518.
12. **Nealson, K. H., T. Platt, and J. W. Hastings.** 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**:313–322.
13. **Pick, U., and R. E. McCarty.** 1980. Measurement of membrane ΔpH. Methods Enzymol. **69**:538–546.
14. **Rosson, R. A., and K. H. Nealson.** 1981. Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch. Microbiol. **129**:299–304.
15. **Rottenberg, H.** 1979. The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. Methods Enzymol. **55**:547–569.
16. **Ruby, E. G., E. P. Greenberg, and J. W. Hastings.** 1980. Planktonic marine luminous bacteria: species distribution in the water column. Appl. Environ. Microbiol. **39**:302–306.
17. **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.
18. **Ruby, E. G., and K. H. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore seawater. Limnol. Oceanogr. **23**:530–533.
19. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 217. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.