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Glucose Repression of Luminescence and Luciferase in *Vibrio fischeri*

W. F. Friedrich* and E. P. Greenberg

Department of Microbiology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA

Abstract. The autoinduction and glucose repression of luciferase synthesis in batch cultures and continuous cultures of *Vibrio fischeri* were investigated. As previously reported, a lag in luciferase synthesis occurred in glycerol-grown batch cultures and addition of D-glucose to the medium extended the lag period. A phosphate-limited chemostat culture with D-glucose as energy source (specific growth rate, $\mu = 0.45\,h^{-1}$) contained uninduced levels of luciferase. Luciferase activity increased to an induced level upon addition of c-AMP or autoinducer to such a chemostat culture while cell mass remained constant. Furthermore, when μ of a phosphate-limited chemostat culture containing D-glucose as energy source was decreased from 0.45 to $0.30\,h^{-1}$, luciferase activity increased from an uninduced to induced level. After exogenously added c-AMP or autoinducer was diluted out of a phosphate-limited continuous culture or after μ was increased to $0.45\,h^{-1}$, luciferase activity remained at an induced level. Apparently, luciferase in *V. fischeri* was subject to a catabolite repression by D-glucose that could be overridden by autoinduction or by an autogenous control element.

Key words: Luminescence – Luciferase – Induction – Repression – Chemostat – *Vibrio fischeri* – Marine bacteria

Luminous marine bacteria are common throughout the world's oceans. Within this extensive range they may exist as members of the bacterioplankton (Hastings and Mitchell, 1971; Ruby et al. 1980; Ruby and Nealson 1978; Yetinson and Shilo 1979) as mutualistic symbionts in the light-emitting organs of fishes (Herring and Morin 1978) as enteric bacteria of a variety of marine organisms (Hastings and Nealson 1977; Ruby and Morin 1979), as parasites of crustaceans (Baross et al. 1978; Harvey 1952), or as saprophytes on decomposing animal matter (Harvey 1952; Hastings and Nealson 1977). The luminescence reaction is controlled by a complex set of regulatory mechanisms which are thought to be important to the ability of these bacteria to exist in a wide variety of habitats; some where light-production is clearly useful and others where it is not (Hastings and Nealson 1977).

Bacterial luciferase, which catalyzes light emission, is in many cases an inducible enzyme. The inducers, referred to as autoinducers, are small molecules produced by the bacteria themselves, and excreted into the growth medium (Eberhard 1972; Eberhard et al. 1981; Nealson 1977; Rossen and Nealson 1981). Autoinduction has been most carefully studied in *Vibrio fischeri* and *Vibrio harveyi* where the autoinducers are species specific in that they do cross-react (Eberhard 1972; Greenberg et al. 1979). Autoinduction can actually be considered as an environmental sensing mechanism, a pheromone: under conditions where a species specific autoinducer can accumulate the bacteria are signaled to synthesize luciferase. Thus when cells are grown in batch cultures there is a lag in the synthesis of luciferase during early exponential growth phase followed by a dramatic increase in its synthesis later in exponential growth (Nealson 1977; Nealson et al. 1970).

Among the mechanisms other than autoinduction which regulate luciferase synthesis or activity, it has been demonstrated that D-glucose can repress luciferase synthesis in both *V. fischeri* and *V. harveyi* (Nealson et al. 1972; Ruby and Nealson 1976). Experiments with batch cultures of *V. harveyi* indicate the D-glucose effect on luciferase synthesis is catabolite repression as observed in other enteric bacteria (Nealson et al. 1972). However, in batch cultures of *V. fischeri* the D-glucose effect is temporary; once cells are adapted to a medium containing D-glucose, synthesis of luciferase is insensitive to D-glucose and the temporary D-glucose repression is not relieved by c-AMP (Ruby and Nealson 1976).

In other bacterial species an interplay between catabolite repression and inducer concentration has been observed. For example, catabolite repression of amidase in *Pseudomonas aeruginosa* can be overcome by addition of sufficient quantities of the inducer, acetamide, to the growth medium (Brammar and Clarke 1964; Clarke and Brammar 1964). Furthermore, specific growth rate affects the balance between catabolite repression and induction of amidase synthesis in *P. aeruginosa* (Clarke et al. 1968).

Because of the autoinduction phenomenon, interplay between inducer concentration and repression could confound results from batch culture experiments on the regulation of luciferase synthesis (and thus luminescence) in *V. fischeri*. To understand better the regulation of luciferase synthesis in *V. fischeri* we have employed chemostat cultures in order to establish conditions where autoinducer and D-glucose could be maintained at steady-state concentrations.

Materials and Methods

Bacterial Strain and Growth Media. The organism used was *Vibrio fischeri* MJ-1, originally classified as *Photobacterium*

* *Present address:* Ciba-Geigy Corp., Summit, NJ 07901
Offprint requests to: E. P. Greenberg

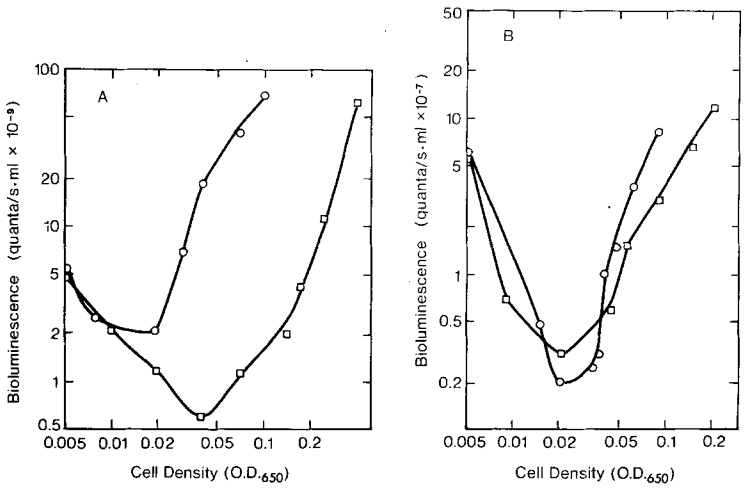

Fig. 1 A and B
In vivo luminescence in batch cultures of *Vibrio fischeri*. **A** Inoculum was from a glycerol-grown culture. **B** Inoculum was from a D-glucose-grown culture. Cells were inoculated into fresh medium containing either 12 mM glycerol (○) or 6 mM D-glucose (□) as energy source

fischeri MJ-1, Baumann et al. 1980; Ruby and Nealson 1976). The minimal media employed were similar to the medium described elsewhere (Nealson 1978; Rosson and Nealson 1981) except that DL-α-glycerophosphate (0.2 mM) was used in place of potassium phosphate, and ferric ammonium citrate (0.3 % w/v) was used in place of ferric sulfate in order to avoid formation of a precipitate. Either D-glucose or glycerol were used as energy source at concentrations as indicated.

Procedures for Batch Culture Experiments. Fifty-milliliter cultures contained in 250-ml Erlenmeyer flasks at 25° C in a reciprocating water bath shaker (150 strokes/min) were employed for experiments with batch cultures. Cultures were inoculated to an initial cell density (OD_{650}) of approximately 0.005. Inocula were from late-logarithmic phase cultures which had been incubated with shaking at 25° C. At various times after inoculation, 1-ml samples were removed from batch cultures for determinations of culture density, bioluminescence and luciferase content.

Procedures for Chemostat Experiments. The chemostat vessels employed were similar to those described by Jannasch (1967). Culture volume was 150 ml. Aeration and mixing were facilitated by means of a magnetic spin disc and passage of sterile air through the culture. Oxygen concentration in culture vessels was maintained at over 90 % saturation as measured with a presterilized oxygen electrode. Temperature in the chemostat vessels was maintained at 25° C. The inocula (10 ml) for chemostat cultures were from early-logarithmic phase batch cultures (cell density of 0.3 at OD_{650}) grown in the presence of the energy source supplied during the chemostat experiment (D-glucose or glycerol as indicated). Samples (5 ml) for analysis of cell mass, bioluminescence and luciferase content were removed from the chemostat vessels through sampling ports at the indicated times.

Measurements of Cell Mass. Immediately after sampling, the density of a culture was determined (OD_{650}, Bausch and Lomb Spectronic 21). For chemostat experiments cell mass is expressed as μg cell dry weight per ml of culture fluid as determined by techniques described elsewhere (Herbert et al. 1971). A culture density of 0.1 at 650 nm corresponds to 79 μg cell dry weight per ml.

Determination of Cellular Luminescence and Luciferase Activity. The light measuring equipment and standard to calibrate this equipment have been described previously (Hastings and Weber 1963). The techniques for measuring luminescence in a culture sample were described previously by Rosson and Nealson (1981). Assays for luciferase content were similar to those described by Rosson and Nealson (1981). Samples (4 ml) were passed through 0.22 μm membrane filters (Millipore Corp. Bedford, MA) and the filters were stored at −20° C. Cells on the membrane filters were lysed by treatment with lysis buffer (Nealson 1977, 1978). Luciferase activity was then determined by its reaction with reduced flavin mononucleotide and decanal.

Determination of D-glucose Concentration. The residual concentration of D-glucose in samples from chemostat culture vessels was determined by the glucose oxidase method. Reagents for these determinations were purchased from the Sigma Chemical Co. (St. Louis, MO).

Autoinducer. Purified *V. fischeri* autoinducer was provided by A. Eberhard (Eberhard et al. 1981), and was added to chemostat culture vessels as indicated by the procedures described by Rosson and Nealson (1981).

Results

Regulation of Luminescence in Batch Cultures of V. fischeri. When cultures of *Vibrio fischeri* were grown in minimal medium plus glycerol, induction of luminescence commenced at a cell density of 0.02. When cells from cultures grown in minimal medium plus glycerol were inoculated into minimal medium plus D-glucose, induction of luminescence did not occur until the cell density was nearly 0.05 (Fig. 1 A). However, if cells from cultures grown in minimal medium plus D-glucose were employed as the inoculum then induction of luminescence commenced at a cell density of 0.02 in minimal medium plus glycerol or plus D-glucose (Fig. 1 B). Induction of luminescence was temporarily suppressed by addition of D-glucose to cultures growing in minimal medium plus glycerol and c-AMP did not affect this supression (Fig. 2). These experiments with batch cultures are in agreement with those reported previously by Ruby and Nealson

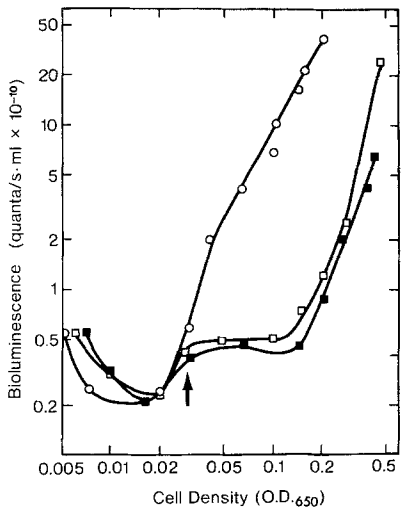

Fig. 2. Effect of c-AMP on luminescence of batch cultures in media containing D-glucose as carbon and energy source. A medium containing glycerol (12 mM) was inoculated with cells from a glycerol-grown culture: D-glucose (6 mM) added at arrow (■); D-glucose (6 mM) plus c-AMP (0.2 mM) added at arrow (□); and control to which no additions were made (○)

(1976) who concluded that the effect of D-glucose on luminescence in *V. fischeri* did not appear to be classical catabolite repression (as in *V. harveyi*), but is a temporary effect that can be eliminated by pre-exposure to D-glucose.

Regulation of Luciferase Activity in Chemostat Cultures of V. fischeri. At $\mu = 0.45\,h^{-1}$, a phosphate-limited chemostat culture supplied with D-glucose as the carbon and energy source exhibited basal or uninduced levels of luminescence and in vitro luciferase activity (Fig. 3). Apparently, the uninduced levels of luciferase were maintained by the excess of D-glucose and were not related to the autoinduction phenomenon directly. That is when D-glucose concentration in the reservoir was decreased so that it became limiting for growth, luminescence and luciferase content increased to fully induced levels even though cell mass decreased from 225 to 165 µg cell dry weight per ml.

The effects of c-AMP and autoinducer on phosphate-limited chemostat cultures were investigated to further understand the permanent repression of luciferase that was described above. Addition of 0.2 mM c-AMP to the culture vessel directly had little effect on cell mass but luminescence and luciferase content rose from uninduced to induced levels (Table 1). Thus in contrast to the temporary effect of D-glucose on luciferase in batch cultures, the effect in chemostat cultures was permanent and reversible by c-AMP. Furthermore, addition of autoinducer to the culture resulted in an increase in luciferase activity from uninduced to induced levels (Table 1). It is interesting that although additions to the cultures were made directly (and thus concentrations of autoinducer or c-AMP added were decreasing over time), the increase in luciferase activity was permanent. Apparently, once the D-glucose repression of luciferase activity had been overcome, the culture was no longer sensitive to the repression.

In phosphate-limited chemostat cultures where glycerol was employed as the energy source in place of D-glucose, luciferase activity was at an induced level although cell mass

Table 1. Effects of c-AMP and autoinducer on permanent repression of luciferase in phosphate-limited chemostats[a]

Energy source (µmol/ml)	Addition to culture vessel[b]	Cell mass[c] (µg dry wt/ml)	Glucose in culture vessel[c] (µmol/ml)	Specific luciferase activity[c] (quanta/s · mg dry wt)
Experiment I				
D-Glucose (2.9)	–	245	0.014	7×10^7
D-Glucose (2.9)	c-AMP	238	0.013	1×10^{10}
Experiment II				
D-Glucose (5.8)	–	257	2.9	5×10^8
D-Glucose (5.8)	Autoinducer	262	2.8	1×10^{10}
Experiment III				
Glycerol (12)	–	153	–	2×10^{10}

[a] In each experiment $\mu = 0.45\,h^{-1}$. In experiments I and II, parallel chemostat cultures utilizing medium from a single reservoir were maintained

[b] After steady-state had been established in each experiment additions were made as indicated. c-AMP was added to a final concentration of 0.2 µmol/ml and purified autoinducer was added to a final concentration of 0.13 ng/ml. Since these additions were made to the chemostat vessels directly, the concentrations of these compounds continually decreased. Concentrations of exogenously added c-AMP and autoinducer were calculated to be $< 10^{-10}$ µM and $< 10^{-16}$ µM at the termination point of these experiments

[c] Steady-state values

was less than in the presence of D-glucose (Table 1). Apparently, the observed repression is an effect of D-glucose and not an effect of phosphate limitation.

The effect of μ on luciferase activity was investigated by inoculating two different chemostats set at two different dilution rates with *V. fischeri*. At $\mu = 0.25\,h^{-1}$ with D-glucose as energy source and phosphate-limitation, luminescence and luciferase activity were at induced levels. When μ of such a culture was increased to $0.45\,h^{-1}$, luminescence and luciferase activity remained induced even though a culture inoculated at a dilution rate of $0.45\,h^{-1}$ exhibited uninduced levels of luciferase activity. Under these experimental conditions, two chemostat cultures were maintained at identical μ values and had identical values of cell mass but one culture possessed fully induced cells and the other possessed cells with uninduced levels of luciferase (Fig. 4). This is in fact consistent with experiments described above which indicated that cultures which contained induced levels of luciferase were not subject to glucose repression of luciferase synthesis.

Discussion

These studies on the effects of D-glucose on luciferase synthesis in chemostat cultures of *Vibrio fischeri* have provided information important to an understanding of the control mechanisms that operate to regulate luminescence in this light-organ symbiont. Furthermore, these studies provide a hypothesis that helps explain some of the effects of D-glucose on luciferase synthesis in batch cultures of *V. fischeri*.

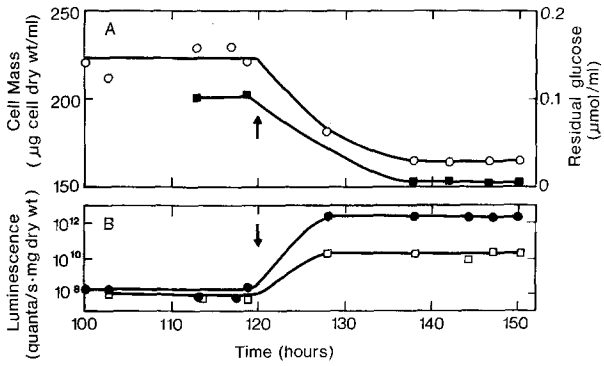

Fig. 3A and B. Effects of a switch from phosphate limitation to glucose limitation on a continuous culture of *V. fischeri*. The dilution rate was 0.45 h^{-1} at the time of inoculation and throughout the experiment. At arrow the concentration of D-glucose in the reservoir was decreased from 2.45 to 1.35 mM. **A** Cell mass (○) and D-glucose (■) in culture vessel. **B** Specific activity of luciferase in vitro (□) and in vivo luminescence of culture (●)

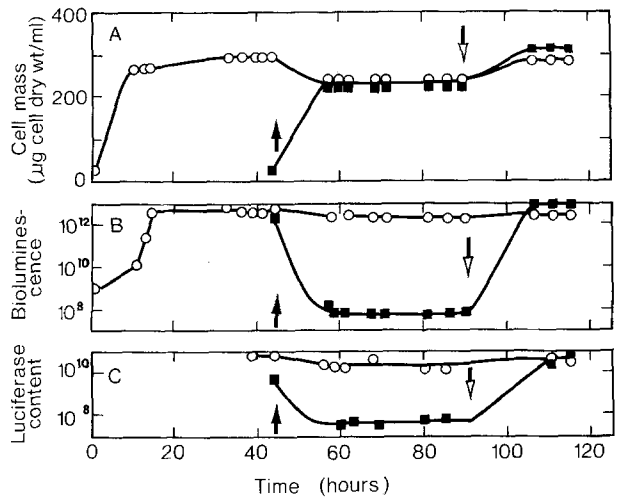

Fig. 4A—C. Effect of dilution rate on luciferase synthesis in phosphate-limited chemostat cultures (D-glucose as energy source). Culture 1 (○), culture 2 (■). At zero time culture vessel 1 was inoculated and dilution rate was set at 0.25 h^{-1}. At black arrow culture 2 was inoculated with cells from culture 1 and for both cultures, dilution rate was set at 0.45 h^{-1}. At white arrow dilution rates were shifted to 0.3 h^{-1}. **A** Cell mass, **B** in vivo bioluminescence (quanta/s·mg cell dry wt), **C** specific activity of luciferase (quanta/s · mg cell dry wt)

Under appropriate conditions D-glucose permanently repressed luciferase synthesis in phosphate-limited chemostat cultures (Figs. 3 and 4). Luciferase synthesis in such cultures was derepressed by exogenously added c-AMP or autoinducer (Table 1). The derepression was permanent in the sense that even after the exogenously added c-AMP or autoinducer was diluted out of the culture, luciferase remained at an induced level. Furthermore, at low specific growth rates (e.g. 0.25 h^{-1}), D-glucose did not repress luciferase synthesis in phosphate limited chemostat cultures and increasing the specific growth rate did not result in repression of luciferase synthesis (Fig. 4). This constitutes another demonstration that once a culture had attained an induced level of luciferase it was no longer subject to D-glucose repression of luciferase.

There are a number of possible explanations for these observations. It is possible that as is the case for the *lac* operon in *Escherichia coli*, the permease for the inducer is inducible itself (Tyler and Magasanik 1970). If this were the case, once induction had occurred, inducer would be transported more efficiently and could then overcome glucose repression and sustain full induction when it is at relatively low concentrations in the growth medium. Another possibility is that synthesis of autoinducer could be under the same transcriptional control as luciferase. In this case, once induction had commenced the rate of autoinducer synthesis would increase, thus effecting an increase in autoinducer concentration. This could serve to maintain the culture in a fully induced state. It should be pointed out, however, that there is a report indicating that autoinducer synthesis is constitutive in *V. fischeri* MJ-1 (Nealson 1977). Further studies are required to test these and other possible explanations for the autogenous control of luciferase synthesis in *V. fischeri*.

Experiments concerning effects of D-glucose on luciferase synthesis in batch cultures have been difficult to interpret (Ruby and Nealson 1976 and Figs. 1 and 2). This may be due to the variety of factors which have been shown to effect the catabolite repression exhibited by chemostat cultures. For example, the fact that D-glucose repression is transient rather than permanent in batch cultures may relate to the ability of high concentrations of autoinducer to overcome the repression phenomenon (Table 1). In batch cultures autoinducer concentration increases as growth proceeds (Nealson 1977), so eventually catabolite repression should be relieved. Furthermore, while the autogenous control element is not well understood it may relate to the adaptation of batch cultures to D-glucose (Fig. 1).

From batch culture experiments, Ruby and Nealson (1976) concluded that *V. fischeri* MJ-1 does not exhibit catabolite repression as defined by others (Tyler and Magasanik 1970; Ullmann and Monod 1968). It was speculated that this was related to the fact that *V. fischeri* MJ-1 occcurs as the specific light-organ symbiont of the Japanese pinecone fish, *Monocentris japonica*. In the light-organ, *Monocentris* could supply the bacterial culture with glucose and still achieve a high efficiency of light production. In such a situation *V. harveyi* would be expected to produce only low levels of luminescence due to catabolite repression (Ruby and Nealson 1976). Our studies with chemostat cultures indicate that *V. fischeri* does exhibit catabolite repression of luminescence. However, this repression is subject to additional layers of control (Figs. 3 and 4, Table 1) which would allow for high efficiency of luminescence in the light-organ where presumably the growth rate of *V. fischeri* is slow and autoinducer concentrations are high. The catabolite repression may serve in other host-associated habitats where *V. fischeri* has been found. Both *V. fischeri* and *V. harveyi* have been isolated from the intestines of various marine animals (Hastings and Nealson 1981). Maybe in the intestinal habitat, catabolite repression of luminescence serves an important function. Since *V. harveyi* has not been identified as a specific light-organ symbiont it may not require the additional levels of control exhibited by *V. fischeri* MJ-1.

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References

Baross JA, Tester PA, Morita R (1978) Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chinocetes tanneri.* J Fish Res Board Can 35:1141–1149

Baumann P, Baumann, L, Bang SS, Woolkalis MJ (1980) Reevaluation of the taxonomy of *Vibrio, Beneckea,* and *Photobacterium:* abolition of the genus *Beneckea.* Curr Microbiol 4:127–133

Brammar WJ, Clarke PH (1964) Induction and repression of *Pseudomonas aeruginosa* amidase. J Gen Microbiol 37:307–319

Clarke PH, Brammar WJ (1964) Regulation of bacterial enzyme synthesis by induction and repression. Nature 203:1153–1155

Clarke PH, Houldsworth MA, Lilly MD (1968) Catabolite repression and the induction of amidase synthesis by *Pseudomonas aeruginosa* 8602 in continuous culture. J Gen Microbiol 51:225–234

Eberhard A (1972) Inhibition and activation of bacterial luciferase synthesis. J Bacteriol 109:1101–1105

Eberhard A, Eberhard C, Burlingame AL, Kenyon GL, Oppenheimer NJ, Nealson KH (1981) Purification, identification and synthesis of *Photobacterium fischeri* autoinducer. In: Deluca M, McElroy W (eds) A symposium on bioluminescence and chemiluminescence; basic chemistry and analytical applications. Academic Press, New York, pp 113–120

Greenberg EP, Hastings JW, Ulitzur S (1979) Induction of luciferase synthesis in *Beneckea harveyi* by other marine bacteria. Arch Microbiol 120:87–91

Harvey EN (1952) Bioluminescence. Academic Press Inc. New York

Hastings JW, Mitchell G (1971) Endosymbiotic bioluminescent bacteria from the light organ of pony fish. Biol Bull 141:261–268

Hastings JW, Nealson KH (1977) Bacterial bioluminescence. Annu Rev Microbiol 31:549–595

Hastings JW, Nealson KH (1981) The symbiotic luminous bacteria. In: Starr MP, Stolp H, Truper HG,, Ballows A, Schlegel HG (eds) The prokaryotes, a handbook on habitats, isolation and identification of bacteria. Springer, New York, pp 1332–1345

Hastings JW, Weber G (1963) Total quantum flux of isotopic sources. J Optical Soc Amer 53:1410–1415

Herbert D, Phipps PJ, Strange RE (1971) Chemical analysis of microbial cells. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 5B, pp 209–344

Herring PJ, Morin JG (1978) Bioluminescence in fishes, pp 273–329. In: Herring PJ (ed) Bioluminescence in action. Academic Press Inc, New York

Jannasch HW (1967) Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol Oceanogr 12:264–271

Nealson KH (1977) Autoinduction of bacterial luciferase: Occurrence, mechanism and significance. Arch Microbiol 112:73–79

Nealson KH (1978) Isolation, identification and manipulation of luminous bacterial. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol 57, pp 153–165, Academic Press, New York

Nealson KH, Eberhard A, Hastings JW (1972) Catabolite repression of bacterial bioluminescence: functional implications. Proc Nat Acad Sci USA 69:1073–1076

Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 104:313–322

Rosson RA, Nealson KH (1981) Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch Microbiol 129:299–304

Ruby EG, Greenberg EP, Hastings JW (1980) Planktonic marine luminous bacteria: Species distribution in the water column. Appl Environ Microbiol 39:302–306

Ruby EG, Morin JG (1979) Luminous enteric bacteria of marine fishes: a study of their distribution, densities and dispersion. Appl Environ Microbiol 37:1230–1238

Ruby EG, Nealson KH (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

Ruby EG, Nealson KH (1978) Seasonal changes in the species composition of luminous bacteria in nearshore seawater. Limnol Oceanogr 23:530–533

Tyler B, Magasanik B (1970) Physiological basis of transient repression of catabolite enzymes in *Escherichia coli.* J Bacteriol 120:411–422

Ullmann A, Monod J (1968) Cyclic AMP as an antagonist of catabolite repression in *Escherichia coli.* FEBS Lett 2:57–60

Yetinson T, Shilo M (1979) Seasonal and geographical distribution of luminous bacteria in the eastern Mediterranean Sea and the Gulf of Elat. Appl Environ Microbiol 37:1230–1238

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Note Added in Proof

The notion that autoinducer synthesis is constitutive (Nealson 1977) is now under question. A recent genetic analysis indicates that as we have suggested, autoinducer synthesis is under the same transcriptional control as luciferase (J. Engebrecht, K. H. Nealson, and M. Silverman, 1983. Cell. In Press).