Vibrio fischeri lux Genes Play an Important Role in Colonization and Development of the Host Light Organ

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The bioluminescent bacterium Vibrio fischeri and juveniles of the squid Euprymna scolopes specifically recognize and respond to one another during the formation of a persistent colonization within the host’s nascent light-emitting organ. The resulting fully developed light organ contains brightly luminescing bacteria and has undergone a bacterium-induced program of tissue differentiation, one component of which is a swelling of the epithelial cells that line the symbiont-containing crypts. While the luminescence (lux) genes of symbiotic V. fischeri have been shown to be highly induced within the crypts, the role of these genes in the initiation and persistence of the symbiosis has not been rigorously examined. We have constructed and examined three mutants (luxA, luxC, and luxR), defective in either luciferase enzymatic or regulatory proteins. All three are unable to induce normal luminescence levels in the host and, 2 days after initiating the association, had a three- to fourfold defect in the extent of colonization. Surprisingly, these lux mutants also were unable to induce swelling in the crypt epithelial cells. Complementing, in trans, the defect in light emission restored both normal colonization capability and induction of swelling. We hypothesize that a diminished level of oxygen consumption by a luciferase-deficient symbiotic population is responsible for the reduced fitness of lux mutants in the light organ crypts. This study is the first to show that the capacity for bioluminescence is critical for normal cell-cell interactions between a bacterium and its animal host and presents the first examples of V. fischeri genes that affect normal host tissue development.

Bioluminescent bacteria are commonly found associated with marine animal tissues, as members of the enteric consortia, as opportunistic pathogens, or most notably as essentially pure cultures colonizing the light-emitting organs of certain squids and fishes (43). In at least some of these light organ associations, normal development of host tissues requires the presence of their species-specific luminous bacterial symbionts (28), which are obtained from the surrounding seawater by the newly hatched host (41, 52). The importance of these light organs to antipredatory defense and other behaviors of the hosts has been well documented (27). In contrast, two questions that focus on the initiation and development of light organ symbioses have remained unanswered: (i) why is it that only certain strains of bacteria are able to colonize and persist in these associations, and (ii) what are the bacterial signals that induce host tissue differentiation? We report here that the capacity to bioluminescence plays a critical part in the answers to both of these questions.

While relatively little is known about the symbiotic significance of light emission, much has been published about the biochemistry and regulation of bioluminescence in luminous bacteria. Bacterial luminescence is a product of the enzyme luciferase, which uses molecular oxygen from the surrounding environment to oxidize both an aliphatic aldehyde and a reduced flavin mononucleotide (reviewed in reference 30). The final products of this reaction are the corresponding aliphatic acid, oxidized flavin, and water, and an unstable intermediate in the reaction emits a photon of blue-green light.

There are at least eight lux genes comprising a regulon that encodes the proteins essential for luminescence in the best-studied luminous bacterium, Vibrio fischeri (30). The lux operon (Fig. 1), encoding luciferase (luxAB) and proteins required to synthesize the aldehyde substrate (luxCDE), is controlled by a number of factors, most notably the quorum-sensing regulator LuxR (13) and the autoinducer molecule 3-oxohexanoyl l-homoserine lactone (3-oxo-C6-HSL), synthesized by LuxI (11, 20). V. fischeri cells also synthesize a second autoinducer molecule, octanoyl l-homoserine lactone (C8-HSL) that, under some conditions, may stimulate transcription of the lux genes (10, 14, 21).

The process of quorum sensing, although first discovered in V. fischeri, is a widespread regulatory mechanism in gram-negative bacteria, particularly among a number of pathogens, which use various autoinducer molecules to modulate genes encoding virulence factors (5, 13, 37). To date, several organisms defective in their lux homolog have been constructed, and they display a reduction in virulence (39, 47); the defect can be at least partially restored by exogenous addition of synthetic autoinducer. Such studies provide strong evidence that quorum sensing plays an important role in pathogenic bacterium-host interactions. While not a pathogen, V. fischeri produces a persistent, benign infection of specific light-emitting tissues of a number of species of squids and fishes (43). The best studied of these cooperative bacterium-host associations is that between V. fischeri and the Hawaiian sepiolid squid, Euprymna scolopes (reviewed in references 29 and 41). The bacteria reside as a monospecific culture in epithelial cell-lined crypts of the light organ, which is used in the host’s nocturnal behavior (26).

Newly hatched juvenile squid are symbiont free and must acquire an inoculum of V. fischeri from the surrounding seawater (42, 52). As a result of colonization by V. fischeri, a
number of morphological and biochemical changes are triggered in the nascent light organ, leading to the development of the functional adult structure (32, 33). Among these changes is a dramatic swelling of the epithelial cells that line the bacterium-containing crypts (8, 32). The swelling, as well as several other developmental events, does not occur in the absence of colonization by V. fischeri cells (32).

This study presents evidence that the bioluminescence of V. fischeri cells plays an important role during the development of a successful light organ association. Specifically, we have examined luxA, luxI, and luxR mutants of a symbiosis-competent strain of V. fischeri for their relative abilities to (i) colonize and persist in the squid light organ and (ii) induce normal host developmental morphogenesis. Our results indicate a direct role of bacterial luciferase and/or its bioluminescence activity in both the normal induction of host development and symbiont persistence in this cooperative bacterial association.

MATERIALS AND METHODS

Bacterial strains and media. V. fischeri strain ESR1 (13), a rifampin-resistant derivative of wild-type strain ES114 (1), was used as the parent strain for all mutant constructions. Escherichia coli strain DH5α was used as the recipient for cloning experiments, and plasmids were passaged through a dam mutant E. coli strain (49) prior to introduction into V. fischeri cells (32).

This study presents evidence that the bioluminescence of V. fischeri cells plays an important role during the development of a successful light organ association. Specifically, we have examined luxA, luxI, and luxR mutants of a symbiosis-competent strain of V. fischeri for their relative abilities to (i) colonize and persist in the squid light organ and (ii) induce normal host developmental morphogenesis. Our results indicate a direct role of bacterial luciferase and/or its bioluminescence activity in both the normal induction of host development and symbiont persistence in this cooperative bacterial association.

FIG. 1. Plasmids used for construction of lux mutants. The region of the chromosome containing the lux regulon is shown, with arrows demonstrating the direction of transcription of the two transcriptional units. Relevant restriction enzyme sites are indicated. Plasmid constructs that were used to make mutations in the chromosomal copy of the lux genes are also shown. Black boxes indicate the location of the mutation resulting from either a deletion and gene replacement with the erm gene or a frameshift mutation (see Materials and Methods); the box with diagonal stripes depicts the location of a lacP/Plac cassette.
TABLE 1. Effects of autoinducer additions on the bioluminescence of *V. fischeri* ESR1 and its lux mutant derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>Luminescence (quanta/cell at OD&lt;sub&gt;600&lt;/sub&gt; of (0.1–1.5)) Alteration of luminescence by the addition of:&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3-oxo-C6-HSL</th>
<th>C8-HSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>24 4,400 ± 1,400 2.4 ± 0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KV150</td>
<td>(&lt;0.01) 870 ± 1,000 2.0 ± 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KV240</td>
<td>(&lt;0.01) 870 ± 1,000 2.0 ± 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KV267</td>
<td>(&lt;0.01) 1.0 ± 0.11 0.74 ± 0.06</td>
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* Ratio of the level of luminescence per cell for each strain grown in the presence and in the absence of the specified autoinducer; mean of three measurements, ± 1 standard deviation.

were obtained from either Promega (Madison, Wis.) or New England Biolabs (Beverly, Mass.). Plasmid pKv290 (Fig. 1) was constructed by insertion of the 8.8-kb *Sal* fragment from pHV2000 (38) containing the lux genes from *V. fischeri* strain ESI14 (17) into the *SalI* site of pBluescript (Stratagene, La Jolla, Calif.). The lux gene in that construct carries a 2-bp frameshift mutation near the end of the gene, rendering the protein inactive (38). Because this strain regains luminescence activity with the addition of 3-oxo-C6-HSL, the product of LuxL (Table 1), the mutation does not have any significant polar effects on *luxR* or its parent strain ESR1 was isolated and digested with *SalI*, treated with T4 DNA polymerase to fill in the overhanging ends of the DNA ligase. In the resulting plasmid, pKV44, the lux genes are in a large deletion, the lux gene is disrupted by a 2-bp frameshift mutation at the *SalI* site, and transcription of luxCDEAB is under the control of P<sub>lac</sub>. Construction of pKV19, which carries constitutively expressed *lacI* and replaced with the gene for erythromycin resistance (erm) contained on a 1.2-kb *Smal* fragment from pKV150 (30), resulting in *P<sub>lac</sub>*. To facilitate the subsequent recombination of the luxCDEABlux genes in the chromosome of *V. fischeri*, the region of the chromosome downstream of the *lux* gene was cloned (K. Visick and E. G. Ruby, unpublished data). The parental strain was used for the normal pattern of apoptotic cell death. Briefly, the squid were first anesthetized in a 1:1 mixture of filter-sterilized seawater and 7.5% MgCl<sub>2</sub> and then stained for 1 min in a solution containing 5 mg of acidophilic dye (0.01%) in seawater. Following a ventral dissection, the exposed light organs were visualized by epiphelomicroscopy to determine whether the typical pattern of apoptotic cell death was evident.

For the determination of cell swelling, the squid light organ tissues were prepared and analyzed with transmission electron microscopy (TEM) (31). Juvenile squid were placed in a 0.1 M sodium cacodylate–0.45 M NaCl buffer (pH 7.4) containing 2.5% glutaraldehyde and 2.5% paraformaldehyde and incubated for 2–3 h at 4°C. This fixation was followed with three 15-min washes with buffer alone, and the samples were then dehydrated in a graded ethanol series. Uranyl acetate (2%) was added to the 70% ethanol wash to increase contrast of the ultrathin sections. Next, the animals were embedded with Spurr, prepared according to the manufacturer’s protocol (Hymann Spurr, 24 h). Ultrathin sections were stained first with a solution of 5% uranyl acetate for 10 min and then with a 0.3% solution of lead citrate for 5 min (31).

Volumetric measurements of crypt epithelial cells. Stained ultrathin sections were visualized on a LEICA 912 transmission electron microscope, and TEM images were captured with a Proscan-CCD frame transfer camera. The epithelial cells were selected for measurement were taken from at least five different sections for each animal, all located deep in the interior of the crypt spaces. Previous studies have reported that the shape of crypt epithelial cells in juvenile squid is essentially columnar (8, 32); thus, we calculated whole-cell volumes from two-dimensional areas (height times width) by assuming that the width of the cell along the axis parallel to basal lamina is equal to its depth. The volume of the nucleus of each cell was similarly measured: unlike the case for total-cell volume (see below), no differences were observed in these values under any of the conditions reported in this study (data not shown). We therefore subtracted the nuclear volume from the whole-cell volume to obtain a calculated cytoplasmic volume.

RESULTS

Construction and luminescence phenotypes of *V. fischeri* lux mutants. To investigate the role of luminescence in the light organ association of *V. fischeri* with *E. scolopes*, mutants defective either for *luxA*, encoding one of the subunits of luciferase, or for *luxI* or *luxR*, two regulatory genes controlling *lux* gene expression, were constructed in the symbiosis-competent strain ESR1. Some characteristics of the *luxA* mutant have been described previously (49). The other two mutations were made in plasmid-borne copies of the *luxI* and *luxR* genes (Fig. 1) and recombined into strain ESR1. The genetic and physiological nature of the resultant mutants was confirmed both by Southern analysis (see Materials and Methods) and by their luminescence phenotype. In laboratory culture (SWT or CM), the growth rates of the lux mutants were indistinguishable from that of their parent, strain ESR1. In contrast, when cultures
were grown with an addition of 3-oxo-C6-HSL to achieve the full level of induction that occurs in the light organ (3), those strains that produced a very high level of luminescence (ESR1 and the lux mutant) exhibited a slightly lower rate of growth than those whose light emission remained low or absent (the luxA or luxR mutants) (data not shown). These results are consistent with previous reports that the synthesis of luciferase by highly induced cells requires a significant energy commitment (30).

Luminescence levels of the parent and the three lux mutants were assayed by growing the cells in media that either did or did not contain additions of an autoinducer (either 3-oxo-C6-HSL or C8-HSL). V. fischeri strain ESR1, like its wild-type parent (1), produces low concentrations of 3-oxo-C6-HSL in culture and thus emits a relatively low level of luminescence at cell densities below about 10^8 per ml (i.e., OD_{600} < 1). Thus, as expected, the luminescence of ESR1 was stimulated several thousand-fold by the addition of 3-oxo-C6-HSL (Table 1); in contrast, cells of ESR1 that were supplemented with C8-HSL increased their luminescence level only about twofold. Because the luxA mutant produces no active luciferase, it remains non-luminescent in the presence or absence of either autoinducer (Table 1). In the absence of a functional lux gene, V. fischeri cells produced a reduced, basal level of luminescence; however, upon addition of 3-oxo-C6-HSL, light emission of this strain was induced to essentially the same extent as it is in its parent strain (Table 1). Also like the parent strain, the luxR mutant showed only a slight induction of bioluminescence in response to the addition of C8-HSL. Interestingly, the luxR mutant produced a level of luminescence in culture that was very close to the uninduced level of the parent (Table 1). The addition of 3-oxo-C6-HSL to this mutant strain did not significantly alter bioluminescence levels, suggesting that the absence of LuxR prevented the cells from responding to the added autoinducer. Curiously, addition of C8-HSL to the culture caused a small decrease in the level of luminescence (Table 1); whether this effect is a significant one awaits further investigation.

Levels of colonization by the lux mutant strains. The lux mutants were assayed for the ability to colonize newly hatched juvenile E. scolopes. Measurements of bioluminescence, a central product of the symbiotic association, typically provide a noninvasive but indirect measure of colonization (42). Juvenile squid exposed to strain ESR1 showed the typical induction of luminescence, which becomes apparent between 12 and 24 h postinfection. However, animals colonized by either the luxA (luciferase) or the luxR or luxI (regulatory) mutant failed to produce detectable levels of luminescence (Fig. 2). Although in the light organ the luxI and luxR mutants are likely to produce some small level of luminescence that is masked by the surrounding animal tissue, additional measurements with a sensitive photometer (data not shown) showed that this activity was undetectable, thus representing less than 0.1% of that produced by the parent strain. These data indicate that the luxI and luxR mutants are uninduced in the light organ and that it is the bacterium, not the host tissue, that is the primary source of any quorum-sensing inducers.

A direct measure of the extent of colonization that was achieved by each of the strains was obtained by determining the number of CFU present in juvenile light organs at various times after the association had been initiated. Approximately 24 h after initiation, all three of the mutants (luxA, luxI, and luxR) achieved colonization levels that were indistinguishable from that of the parent strain (Fig. 3). However, by about 48 h postinfection, the three lux mutant strains exhibited a three- to fourfold reduction in the level of colonization compared to that of the parent strain. This reduced level of colonization remained unchanged for at least another 24 h (data not shown). There is no evidence that the reduction in CFU results from the death of a significant number of the lux mutant bacteria in the light organ: an examination of both luxA mutant and parent cells released from the light organ crypts after 48 h

![Graph showing luminescence levels over time](image)

**FIG. 2.** Relative luminescence over time of newly hatched E. scolopes juveniles exposed to either the parent strain ESR1 (▲), the luxA mutant strain KV150 (●), the luxI mutant strain KV240 (○), the luxR mutant strain KV267 (□), or the luxR luxI P_ux lux strain KV345 (△). A subset of the squid exposed to KV345 (■) were treated with IPTG (see Materials and Methods) to induce luminescence genes. ESR1-exposed animals treated with IPTG (●) served as a control for IPTG effects on the association.

![Graph showing colonization levels](image)

**FIG. 3.** Symbiotic colonization levels achieved by lux mutant strains of V. fischeri and their parent, strain ESR1. The number of CFU present in the light organs of juvenile E. scolopes exposed to either lux mutant V. fischeri strains or the parent strain was determined at two times after inoculation, 24 h (black bars) or 48 h (striped bars). Each bar represents an average value obtained with at least four animals (standard error of the mean ranges are indicated). Similar results were obtained in three other independent trials.
showed that at least 90% of the cells of both groups were viable in the symbiosis (data not shown). Because the common characteristic of these mutants is that, unlike the parent strain, they do not produce induced levels of luminescence in the squid light organ (Fig. 2), we hypothesized that (i) luminescence is a requirement for the normal persistence of V. fischeri in the symbiosis and (ii) because of the role of the luxI and luxR genes in regulating luminescence induction, they are essential to the normal symbiotic competence of V. fischeri.

**Competitive colonization defects of the V. fischeri lux mutants.** The ability of the luxA mutant strain to colonize the light organs of juvenile squid in coinfections with the parent (lux+) strain was also investigated. When presented to the host animal in a 1:1 ratio with parent strain ESR1, the luxA mutant showed signs of being outcompeted by the parental strain as early as 24 h postinoculation (Fig. 4). This competitive advantage of ESR1 over the luxA mutant occurred in spite of the relatively slower growth of induced ESR1 cells discussed above. Not surprisingly, at 48 h the mutants continued to exhibit a significant competitive defect. A similar defect was observed in the luxR mutant when it was competed against the parent strain (data not shown). Because both of these mutants were constructed by the insertion of an erm cassette (Fig. 1), we were concerned that the carriage of the cassette was itself the basis for the defect; however, insertions of this cassette into other chromosomal loci had essentially no effect in competitive experiments (data not shown). Taken together, these data provided support for the idea that luminescence plays a significant role in the ability of V. fischeri to colonize the light organ of E. scolopes juveniles and that this effect manifests itself as early as 24 h after colonization has been initiated.

**Defects in the induction of host development by V. fischeri lux mutants.** We also tested whether carriage of the luxA, luxI, or luxR mutation resulted in a defect in the ability of the bacteria to trigger normal symbiotic-induced changes in the host's program of light organ development. No differences were observed in the mutants' ability to induce the normal temporal and spatial pattern of apoptotic cell death in the ciliated surface of the light organ (data not shown). In contrast, defects were observed in the ability to trigger another morphological event that typically occurs in response to V. fischeri colonization, i.e., an increase in cytoplasmic volume, or cell swelling, within the epithelia that line the light organ crypt spaces. This swelling, or edema, results in the transformation of the initially columnar epithelial cells into more cuboidal ones. Cells of V. fischeri strain ESR1 (lux+) that infect juvenile squid induce the swelling within 48 h of inoculation, while the crypt epithelia of uninfected (aposymbiotic) juveniles remain unswollen (Fig. 5A and reference 32). In juvenile animals infected with either the luxA, luxI, or luxR mutant, the mean volume of the crypt cells in these animals was indistinguishable from that of uninfected animals (Fig. 5), demonstrating that all of the lux mutants are defective in triggering this specific host developmental event.

One possible reason that the lux mutants were unable to induce host cell swelling was their reduced level of colonization. Thus, we determined the cell volumes of crypt epithelia from squid colonized by an amino acid auxotroph of V. fischeri. As described previously (16), we found that squid infected by this auxotrophic mutant contained 5 to 10% of the bacteria present in organs colonized by the parent strain, but unlike the lux mutants, the auxotroph produced easily detected levels of luminescence in the light organ. Colonization by the auxotroph induced a degree of epithelial cell swelling that was as great as that observed with the parent (Fig. 5A); thus, the defect in cell swelling exhibited by the lux mutants is more likely to be related to their failure to produce a normal level of luminescence than to their inability to achieve a normal extent of colonization.

**Complementation of the luxA mutant colonization phenotype.** Because of the nature of the lux regulon and the mutations we had created in it, the possibility existed that the symbiotic defects described above were due to effects of the luxI or luxR mutations on some gene(s) other than those responsible for luminescence. To eliminate that possibility, we constructed a luxR luxA double mutant, strain KV345, in which transcription of the structural genes of the lux operon (luxCDABE) was placed under the control of Pران in the chromosome (Fig. 1). In culture, the level of luminescence of KV345 was induced >100-fold by the addition of IPTG (51); when this strain was used to infect juvenile E. scolopes, IPTG addition to the surrounding seawater produced a >50-fold increase in bioluminescence emission from the squid (Fig. 2). The level of colonization established by KV345, in the presence and absence of IPTG, was assayed 48 h after the initiation of the association. While IPTG addition restored luminescence capability and wild-type levels of colonization to this strain, it did not affect the colonization levels of either the parent strain or the luxR mutant (Fig. 6A). These results suggest that the luxR and/or luxI gene products may not play a significant role in early symbiotic colonization, apart from their requirement for the normal induction of the luminescence genes (Fig. 2).

We were concerned that a gene downstream of the luxCDABE genes might be playing a role in the observed colonization phenotypes as a result of a polar effect caused by the luxA mutation. To look for evidence of polarity, we complemented this mutation in trans with a wild-type copy of the luxA.
gene carried on pKV19 (49). The presence of pKV19, but not the parent vector alone, restored both the luminescence phenotype of the complemented luxA mutant strain (data not shown) and normal levels of symbiotic colonization at 48 h (Fig. 6B). These data do not support the hypothesis of polar effects but instead provide evidence for the importance of luminescence itself in the ability of V. fischeri to successfully persist in the juvenile squid light organ.

Complementation of the epithelial cell swelling defect. The formal possibility existed that the factors responsible for normal colonization and induction of host cell swelling are distinct. For example, the inability to induce host cell swelling
addition to this taxon level of specificity, different V. fischeri isolates have various colonization efficiencies (24, 35), suggesting that selectivity is active at even more subtle levels. Our studies of the symbionts of E. scolopes have resulted in the intriguing observation that none of the thousands of strains of V. fischeri that have been isolated from light organs has been found to be a nonluminous variant (41). Thus, this specificity apparently extends to the selection of particular genotypes of V. fischeri, i.e., those with intact lux genes. A competitive disadvantage for V. fischeri strains that are lux mutants is unexpected because luminescence is not an essential trait of cells growing in laboratory culture, and in fact, light emission requires a considerable energy commitment by these bacteria (26). Thus, the absence of dark variants in symbiotic populations is surprising because the light organ is an environment in which different strains compete for dominance (24), and one would predict that by gaining a mutation in luminescence activity, a dark strain of V. fischeri might achieve a slight growth advantage.

For this reason the apparent selectivity for lux$^+$ strains in nature suggested to us that maintaining the ability to luminesce might be of value not only to the host but to the bacterial symbionts as well. Indeed, the results presented here show that the symbiotic relationship in E. scolopes is profoundly affected by the inability of a colonizing symbiont to luminesce at normal levels. V. fischeri mutants defective for either structural (lux$^+$) or regulatory (luxI or luxR) luminescence genes exhibited a three- to fourfold decrease in the extent of colonization within 48 h of initiating the symbiotic association. In fact, the defect could be detected as soon as 24 h under the competitive conditions of a mixed inoculum with the lux$^+$ parent. While the basis for this more rapid appearance of the colonization defect is unknown, one possibility is that there may be a host challenge that is produced only as a response to the presence of luminescing cells. Colonization by the lux mutants alone would not elicit this additional challenge but a mixed infection would, exacerbating their defect.

These competition results further indicate that the presence in the symbiotic organ of wild-type, light-emitting cells does not complement the colonization defect of nonluminescing cells. Therefore, it is the luminescence activity of individual bacterial cells that is important, not any net effect of the symbiotic population as a whole. A similar competitive defect has been reported in experiments with a mutant defective for a periplasm-localized catalase enzyme (KatA), which is not rescued by the presence of wild-type V. fischeri cells (50). How this apparent cell-level selectivity is achieved remains to be determined.

A recent report has suggested that the products of luxI and luxR control the expression of several non-lux loci, including one required for V. fischeri to remain competitive in mixed symbiotic infections (4). Whether the luxR-dependent induction of these loci observed in culture occurs in the symbiosis, and/or is itself required for symbiotic competency, remains to be directly demonstrated. Our results indicate that the function of luxI and luxR during the establishment of a normal light organ colonization lies primarily with their role in the induction of bioluminescence. Examination of colonization by a luxI luxR double mutant that carried a tac promoter to control lux structural gene expression revealed that at least in juvenile squid, essentially wild-type levels of colonization could be achieved by this mutant as long as luminescence was artificially induced with IPTG. The direct role of the luxA gene was further substantiated by complementation studies that did not indicate a polar effect on any downstream gene critical for colonization. Taken together, these data suggest that in nature,
V. fischeri cells that have lost their luminescence capability will be unlikely to sustain a symbiotic relationship, particularly in the face of coinfesting wild-type V. fischeri. Population biology theory would predict that the host would gain a benefit from imposing such a restriction (40); however, no evidence directly linking bioluminescence capacity to symbiotic success has been previously reported.

Perhaps the most novel and intriguing result presented in this report is the observation that the lux mutants exhibited defects in the ability to trigger a normal host tissue response. Upon colonization by wild-type V. fischeri, light organ epithelial cells in direct contact with bacterial symbionts initiate a program of differentiation, including a significant increase in cytoplasmic volume (23, 32). One consequence of crypt epithelial cell swelling is to effectively reduce the volume of the crypt spaces, thus forcing a greater percentage of the bacterial population to be in direct contact with the host epithelial surface. This change is bacterium induced; in the absence of V. fischeri, the epithelial cells do not swell, and if the colonization is cured, the swelling subsides (8). All three of the lux mutants that were examined were deficient in inducing epithelial edema. Complementation studies with the luxA mutant demonstrated that the presence of an intact copy of this gene coordinately restored both luminescence and normal epithelial development. V. fischeri cells that carry amino acid auxotrophy mutations making them unable to fully colonize the light organ (16), but that are lux+, were capable of inducing cell swelling (Fig. 5B). Thus, we believe that the lux mutations’ effect on host development is due to a loss of luminescence function rather than to a reduced colonization level. We anticipate that there may be a number of bacterial activities that contribute to the induction of swelling and other host developmental events, and that the presence of luciferase and/or its luminescence activity is just one of them.

How does a lux mutation result in defects in both the effectiveness of colonization and the ability to induce host edema? Are these two symbiotic phenotypes linked and, if so, in what way? We hypothesize that the lux mutants express these symbiotic defects because they are less able to severely reduce the concentration of oxygen around them (44). Bacterial luciferase has an unusually high affinity for molecular oxygen, which is converted to water in the luminescence reaction. Specifically, its affinity constant (K_m) for oxygen is in the nanomolar range. Hypoxia has been demonstrated to have an edemic effect on epithelial cells in other systems (18, 25) and to result in exocytosis of cytoplasmic material (36). Such exocytosed material may be a source of nutrients to support the growing population of luminous symbiotic bacteria colonizing the crypt surfaces. If such a mechanism does, in fact, supply host-derived nutrients to light organ symbionts, then V. fischeri lux mutants may face a growth disadvantage because they are unable to create the severe hypoxic conditions that lead to an attendant swelling of the crypt epithelium.

These predictions are being currently tested using a V. fischeri strain carrying an altered luciferase enzyme that consumes normal levels of oxygen but produces no luminescence. Such studies should further our understanding of the patterns of cell-cell signaling that occurs between V. fischeri and E. scolopes. In addition, they will encourage an examination of whether symbiont-induced hypoxia is a general mechanism used by other luminous, or highly respiratory, bacteria as they establish cooperative or pathogenic associations with their hosts.

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