Photolyase Confers Resistance to UV Light but Does Not Contribute to the Symbiotic Benefit of Bioluminescence in Vibrio fischeri ES114

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Recent reports suggest that the selective advantage of bioluminescence for bacteria is mediated by light-dependent stimulation of photolyase to repair DNA lesions. Despite evidence for this model, photolyase mutants have not been characterized in a naturally bioluminescent bacterium, nor has this hypothesis been tested in bioluminescent bacteria under natural conditions. We have now characterized the photolyase encoded by phr in the bioluminescent bacterium Vibrio fischeri ES114. Consistent with Phr possessing photolyase activity, phr conferred light-dependent resistance to UV light. However, upon comparing ES114 to a phr mutant and a dark ΔluxCDABEG mutant, we found that bioluminescence did not detectably affect photolyase-mediated resistance to UV light. Addition of the light-stimulating autoinducer N-3-oxo-hexanoyl homoserine lactone appeared to increase UV resistance, but this was independent of photolyase or bioluminescence. Moreover, although bioluminescence confers an advantage for V. fischeri during colonization of its natural host, Euprymna scolopes, the phr mutant colonized this host to the same level as the wild type. Taken together, our results indicate that at least in V. fischeri strain ES114, the benefits of bioluminescence during symbiotic colonization are not mediated by photolyase, and although some UV resistance mechanism may be coregulated with bioluminescence, we found no evidence that light production benefits cells by stimulating photolyase in this strain.

Bacterial bioluminescence is well understood biochemically and genetically (15, 27, 39); however, the functional significance of bioluminescence for bacteria remains uncertain (35). Generating luminescence presumably confers a selective advantage(s) under some circumstances, but this must be weighed against apparently significant energy costs (15, 21). Several hypotheses have been offered to explain how bioluminescence might be advantageous to the growth or survival of light-producing bacteria, and they propose that the value of luminescence might be due to the consumption of reducing equivalents, the reduction of oxygen, or an effect of light itself (29, 35). Recently, it was suggested that bioluminescence confers upon bacteria by stimulating the light-dependent photolyase-mediated repair of DNA lesions (7–9, 23, 43). Photolyase is an enzyme that uses light to drive the “photoreactivation” repair of pyrimidine dimers, such as those caused by UV light, in adjacent base pairs on the same strand of DNA (42), and theoretically photolyase could be activated by bioluminescence. This led to the suggestion that bioluminescence may have evolved to stimulate photolyase in an otherwise dark environment (8).

Proponents of the hypothesis that luminescence benefits cells by stimulating photolyase have reported several experiments that support this model. Consistent with a role in repairing pyrimidine dimers, bioluminescence in Vibrio harveyi is stimulated by UV irradiation (7, 9). Furthermore, dark lux mutants of V. harveyi (9) and of other bacteria (23) were more sensitive to UV light than their respective wild-type parents, while Escherichia coli clones carrying V. harveyi bioluminescence-generating lux genes were more UV resistant. Also, in mixed cultures, a dark luxA mutant of V. harveyi was outcompeted by its bioluminescent parent when the cultures were exposed to UV light (8). Importantly, the relative sensitivity of dark strains in these experiments could be compensated for, at least in part, by exposure to external light, consistent with a benefit mediated by light itself (8, 9, 23). Finally, perhaps the most compelling evidence of a connection between bioluminescence and photolyase is the fact that the UV resistance conferred upon E. coli by the lux genes is dependent upon phr, the gene encoding photolyase (23).

Despite this evidence, it is not yet certain whether bioluminescence plays a significant role in photoreactivation. Some of the experiments described above are open to alternative interpretations. For example, bioluminescence could influence stress resistance and DNA repair through pathways that do not involve photolyase, and experiments such as those involving lux expression in E. coli may not be relevant to bioluminescent bacteria in the environment. Moreover, photolyase mutants have not been characterized in a naturally bioluminescent bacterium, nor has a connection between photolyase and bioluminescence been tested in bioluminescent bacteria under natural conditions where the bacteria produce light.

We tested the relationship between bioluminescence and photolyase in Vibrio fischeri ES114. The genome of this bioluminescent bacterium has been sequenced (34), allowing us to identify phr and generate a photolyase mutant, and we were also able to observe the bacterium under conditions where it is naturally luminescent, in the light organ of its host, Euprymna scolopes. We found that although bioluminescence enables V. fischeri to fully colonize its host (41), this effect is not mediated

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by photolyase. Taken together with analyses of cultured cells, our results cast doubt on the importance of bioluminescence in photoreactivation in this bacterium.

**MATERIALS AND METHODS**

**Bacteria and culturing techniques.** The bacterial strains, plasmids, and oligonucleotides used in this study are described in Table 1. Plasmid constructs were transformed and maintained in E. coli strain DH5α or DH5αpir, with the latter used specifically for plasmids containing the R6Ky replication origin. The conjugal helper plasmid pEV5104 was maintained in E. coli strain CC118pir. E. coli was grown in LB medium (28) or brain heart infusion medium, and V. fischeri was grown in either SWT medium, which contained 5 g of tryptone, 3 g of yeast extract, 3 ml of glycerol, and 700 ml of Instant Ocean (Aquarium Systems, Mentor, Ohio) per liter, or in LBS medium, which contained 10 g of tryptone, 5 g of yeast extract, 3 ml of glycerol, and 700 ml of Instant Ocean (Aquarium Systems, Mentor, Ohio) per liter, or in LB medium (28) or brain heart infusion medium, and V. fischeri was grown in either SWT medium, which containe d5go f tryptone,3go f yeast

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Chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.). When added to LB medium for selection of E. coli, ampicillin, chloramphenicol, and kanamycin were used at concentrations of 100, 20, and 40 µg ml⁻¹, respectively. When added to brain heart infusion medium for selection of E. coli, erythromycin was used at a concentration of 1 µg ml⁻¹. When added to LB medium for selection of V. fischeri, chloramphenicol, erythromycin, and kanamycin were used at concentrations of 2, 5, and 100 µg ml⁻¹, respectively. To add N-3-oxo-hexanoyl homoserine lactone (3-oxo-C₆-HSL), a 200 µg ml⁻¹ stock solution was prepared in ethyl acetate, an appropriate volume was dispensed into flasks or tubes, the ethyl acetate was allowed to evaporate, and then the medium was added so that the final concentration of 3-oxo-C₆-HSL was 200 ng ml⁻¹.

**Genetic techniques and analyses.** We generated plasmids using standard cloning and DNA manipulation techniques. DNA ligase and restriction enzymes were

### TABLE 1. Bacterial strains, plasmids, and oligonucleotides

<table>
<thead>
<tr>
<th>Strain, plasmid, or oligonucleotide</th>
<th>Relevant characteristics</th>
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<tr>
<td><strong>V. fischeri</strong></td>
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<td>ESI14</td>
<td>Wild-type isolate from <em>Euprymna scolopes</em></td>
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<td>EW1</td>
<td>ESI14 <em>aph</em> (allele exchanged from pELW1)</td>
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<td>ESI14 <em>aph</em> ΔluxCABEG (EW1 with lux allele exchanged from pEV5103)</td>
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<td>ESI14 ΔluxCABEG</td>
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<td>JRM100</td>
<td>ESI14 mini-Tn7-ermR (inserted at intergenic att site)</td>
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<td>CC118pir</td>
<td>Δ(aar-lux) araD ΔlacX74 galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1; lysogenized with <em>pir</em></td>
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<td>DHSα</td>
<td>F'-898ΔlacZ∆M15 Δ(lacZYA-argF)U169 deoR supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1</td>
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<tr>
<td>DHSαpir</td>
<td>DHSα lysogenized with <em>pir</em></td>
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<td><strong>Plasmids</strong></td>
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<td>pCR-Blunt II-TOPO</td>
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<td>pEV5118</td>
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<td>JBPHOTO6</td>
<td>5′ CAC CTC AGA AGC AGG CTT TAG CTG AGC TGC 3′</td>
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<td>5′ GGG GTA CCC GAT GCC TTG CGA TGG GTT TAT ATC 3′</td>
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*ampR, ampicillin resistance (bla); chmR, chloramphenicol resistance (cat); ermR, erythromycin resistance; and kanR, kanamycin resistance (aph).
obtained from New England Biolabs (Beverly, Mass.), and oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, Iowa). Plasmids were purified using Qiagen Mini-prep kits (Qiagen Inc., Valencia, Calif.), and DNA was recovered from restriction and ligation reactions with the DNA Clean and Concentrator-5 Kit (Zymo Research, Orange, Calif.). We used the ZeroBlunt-TOPO PCR Cloning Kit (Invitrogen, Carlsbad, Calif.) to clone PCR products into ZeroBlunt II-TOPO. PCR was performed with an iCycler (Bio-Rad Laboratories, Hercules, CA) using KOD HiFi DNA Polymerase (Novagen, Madison, Wis.). In addition to using a high-fidelity polymerase, we sequenced the cloned PCR products to ensure that unintended base pair alterations were not incorporated into our constructs. DNA sequencing was conducted at the University of Michigan DNA Sequencing Core Facility, and sequences were analyzed using Sequencher 4.1.2 (Gene Codes Corp., Ann Arbor, Mich.). Sequencing was completed using the primer oligonucleotides JBP-TOPO5, JBP-TOPO6, JBP-TOPO7, and JBP-TOPO8 (Table 1). Protein sequence comparisons were generated with BLAST (1), and similarities were calculated using the BLOSUM62 scoring matrix (19).

Details of plasmid construction are included in Table 1. To generate the Δphr mutant allele, we PCR amplified phr and flanking sequences from V. fischeri ES114 using primers JBP-TOPO1 and JBP-TOPO2, cloned this PCR product into pCR-Blunt II-TOPO, and then subcloned the phr-containing fragment into the small, mobilizable vector pEV8118 (10). We then used primers JBP-TOPO3 and JBP-TOPO4, which are directed outward from the start and stop codons of phr and contain Smal recognition sites near their 5′ ends, to PCR amplify the vector and sequences flanking phr. The template was then destroyed by DpnI treatment, and the PCR product was digested with Smal and self-ligated to generate pALZ6s, which contains the genomic region surrounding phr but with the sequences between the phr start and stop codons replaced by 5′-CCGCGG-3′. pALZ6s was ligated to pBluescript KS(−) to generate pEW1, a mobilizable ColE1-containing vector for allelic exchange of the in-frame phr deletion. To generate the pEW phr complementation vector pEW5, we specifically PCR amplified phr using primers JB-TOPO9 and JB-TOPO10, cloned this PCR product into pCR-Blunt II-TOPO, and then subcloned a phr-containing fragment into shuttle vector pVS105 (11).

Mutant alleles and the stable vectors for complementation were transferred to V. fischeri from E. coli by triparental mating using the conjugative helper plasmid pEV8104 (37). For mutant construction, recombinational marker exchange was scored by screening for the appropriate antibiotic resistance phenotype, and putative double recombinants were screened by PCR to determine which had reverted to wild type and which had incorporated the Δphr or ΔΔphr phrDABEG mutant allele. The nonbioluminescent phenotype of the ΔΔphr phrDABEG mutant also confirmed the replacement of the wild-type lux allele.

**Measurement of UV resistance.** V. fischeri cells were grown in 3 ml of LBS in 18-mm tubes shaken at 28°C until the A595 was between 0.2 and 0.5. The cells were then pelleted by centrifugation, resuspended in filter-sterilized Instant Ocean, and diluted 10-fold in Instant Ocean, and 500-μl aliquots were plated into wells in two 24-well microtiter plates. This pelleting and resuspension did not appear to affect the bioluminescence (data not shown). One microtiter plate was exposed, with the lid removed, to a preset intensity (in μW cm⁻²) of UV light in a UV-Stratalinker 1800 (Stratagen, La Jolla, Calif.), while the other plate was left unexposed. The cells were allowed to recover for 2 h before they were dilution plated onto LBS agar to determine the UV survival frequency, which was defined as the percentage of CFU ml⁻¹ in the UV-treated sample relative to the number of CFU ml⁻¹ in the untreated sample. To determine the influence of light on UV survival, cell suspensions were exposed in four parallel microtiter plates, and two of these microtiter plates were exposed to UV light in a UV-Stratalinker that had been placed in a darkroom. Following exposure, one UV-treated plate and one untreated plate were allowed to recover for 2 h under white fluorescent laboratory lights, while the remaining plates for the “dark” treatment were kept in the darkroom during recovery and were dilution plated under a dim red light.

**Euprymna scolopes colonization assays.** E. scolopes hatchlings were infected with V. fischeri using previously described inoculation procedures (32, 36) and overnight exposures of the squid to Instant Ocean containing V. fischeri. Within each experiment, similar numbers of mutant or wild-type cells were present in the respective inocula. Inoculant strains were pregrown unshaken in 5 ml of SWT in 50-ml conical tubes at 28°C so that the A595 was between 0.3 and 0.8, the cultures were diluted in Instant Ocean to between 1,000 and 3,000 total CFU ml⁻¹, and E. scolopes juveniles were exposed to the inocula for between 12 and 14 h before being rinsed in V. fischeri-free Instant Ocean. The squid were homogenized at 48 h postinoculation, and the homogenates were serially diluted and plated onto LBS. Following overnight incubation, the colonies were counted to determine the number of CFU per animal. To generate mixed infections with both strains EW1 and JRM100, E. scolopes hatchlings were exposed to mixed inocula with roughly equal numbers of EW1 and JRM100 cells for 18 h before being rinsed in inoculum-free Instant Ocean. Ratios of EW1 to JRM100 were determined after dilution plating the cells on LBS by patching 50 colonies on LBS containing erythromycin. Relative competitive indices (RCIs) were calculated by dividing the ratio of EW1 to JRM100 in the squid after 48 h by the ratio of these strains in the inoculum.

**RESULTS**

The goal of this study was to characterize photolyase in *Vibrio fischeri* strain ES114, which is naturally bioluminescent and was originally isolated from the light organ of the Hawaiian bobtail squid, *E. scolopes*. Specifically, we sought to test whether there is a relationship between photolyase and bioluminescence both in culture and during symbiotic colonization.

**Bioinformatic characterization of phr in V. fischeri ES114.** Analysis of the ES114 genome (34) revealed one putative photolyase (phr) gene. This open reading frame (ORF), which was designated VFA0753 by the genome project, is located on chromosome II and encodes a protein that is 44% identical and 60% similar to the photolyase of *E. coli* K12 (Fig. 1). The ORF was annotated by the genome project as photolyase, and we refer to it here as *phr*. The genetic context of VFA0753 further supports its *phr* designation, as the gene immediately upstream of it, VFA0752, encodes a protein that is homologous to YbgA, which is encoded by the ORF immediately upstream of *phr* in *E. coli* (Fig. 1). Moreover, ORFs encoding proteins similar to YbgA are also upstream of putative photolyase genes in *Vibrio cholerae* (17), *Vibrio vulnificus* (6), and *Vibrio parahaemolyticus* (25), although in each of these vibrios a putative transcriptional regulator is encoded in between ybgA and *phr*. In each of these four vibrios, the ORFs encoding proteins similar to Phr and YbgA are also found on chromosome II, although homologs of VFA0751 (Tyrr), VFA0754, and VFA0756 (Fig. 1) are on chromosome I in these vibrios.

The genetic organization near *V. fischeri* *phr* indicates that it
may be expressed on a polycistronic transcript (Fig. 1). *phr* overlaps VFA0752 by 7 bp, and it is separated by only 59 bp from VFA0754, strongly suggesting that it is cotranscribed with the former and possibly with the latter. VFA0754 was annotated by the genome project as encoding methionine sulfoxide reductase, an enzyme that is similar to photolyase in that it repairs damaged macromolecules. However, we found no significant similarity between the protein encoded by VFA0754 and characterized methionine sulfoxide reductases, and we have annotated this ORF as encoding a hypothetical protein (Fig. 1).

The Δ*phr* mutant lacks light-dependent UV resistance “photoreactivation” activity. To test whether *phr* encodes a functional photolyase, we generated and characterized mutant EW1, which contains an in-frame deletion of the gene. Both EW1 and its wild-type parent, ES114, displayed a dose-dependent sensitivity to UV light; however, ES114 possessed a light-dependent mechanism of UV resistance that was absent in the mutant (Fig. 2). ES114 also appeared slightly more resistant to UV light than the mutant, even when recovery was in the dark, possibly due to minor photoreactivation stimulated by the darkroom red light or to Phr-assisted excision repair, which has been observed in *E. coli* (31). Restoring the *phr* gene in trans to the Δ*phr* mutant complemented its UV sensitivity (Fig. 3), indicating that this phenotype was not due to effects on VFA0752, VFA0754, or other genes. Taken together, our data indicate that *V. fischeri* *phr* encodes the bacterium’s photoreactivating photolyase.

Bioluminescence does not appear to stimulate photolyase in *V. fischeri* ES114. Over a range of UV doses, we saw no difference in the UV sensitivity, or the photoreactivation capacity, of ES114 or the dark ΔluxCDABEG mutant EVS102 (Fig. 2), suggesting that bioluminescence does not play a role in photoreactivation. On the other hand, the cells used in these experiments had been grown to low cell density and were relatively dim. It has been reported that exposure to UV light increases expression of the *lux* genes in some bacteria even at low cell density (7, 9); however, we considered the possibility that this was not the case in ES114 and that the similarity between ES114 and EVS102 might reflect the relatively dim luminescence ES114 generates in culture (3). We therefore amended the cultures with 200 ng ml⁻¹ of the autoinducer 3-oxo-C₆-HSL, an amount similar to that found in the light organ (4), which induced luminescence ~500-fold to levels comparable to that seen in the symbiosis. Although addition of 3-oxo-C₆-HSL stimulated luminescence in ES114, there was still no detectable difference between ES114 and the ΔluxCDABEG mutant EVS102 with regard to UV sensitivity or photoreactivation (Fig. 4).
Interestingly, the addition of 3-oxo-C6-HSL appeared to increase resistance to UV light when the cells recovered from UV exposure in the dark; however, this trend was seen in the wild type, the dark mutant EVS102, and the Δphr mutant EW1 (Fig. 4), indicating that this effect is not directly related to photolyase. For each of the three experiments, and when data for the three strains were pooled, the effect of 3-oxo-C6-HSL was significant (P < 0.05) enhanced the survival of cells incubated in the dark following UV exposure in two out of three independent experiments. When for the three strains were pooled, the effect of 3-oxo-C6-HSL was significant (P < 0.05) in all three experiments.

We also examined ES114, the dark ΔluxCDABEG mutant EVS102, and the Δphr mutant EW1 under conditions where bioluminescence is naturally induced and is beneficial to the bacteria, during symbiotic colonization of the E. scolopes light organ. Consistent with other studies (41; J. L. Bose, C. S. Rosenberg, and E. V. Stabb, unpublished data), we found that populations of the lux mutant in squid light organs 48 h after inoculation were three- to fourfold lower than the symbiotic populations in ES114-infected animals (Fig. 5). However, colonization by the phr mutant EW1 was indistinguishable from that of ES114 (Fig. 5). In some instances, symbiotic defects can be detected only if strains are forced to compete for colonization; however, as shown in Fig. 6, EW1 was not outcompeted by JRM100, a marked derivative of ES114 with competitive advantage (26). Juvenile squid were exposed to an ~1:1 mixture of the strains at a total concentration of 2,500 CFU ml⁻¹ for 14 h, and the RCI of EW1, defined as the ratio of EW1 to JRM100 in the squid divided by the ratio of these strains in the inoculum, was determined for each coinoculated animal 48 h after inoculation. The RCI is plotted as a circle. A dotted line marks the RCI of 1, where the strain ratio in the squid matches that in the inoculum and there is no difference in strain competitiveness.

![FIG. 5. Symbiotic colonization of E. scolopes hatchlings by the wild type, ΔluxCDABEG mutant EVS102, or Δphr mutant EW1. Average colonization levels 48 h after inoculation with the indicated strain are shown, with standard errors (n = 13 or 14).](image1)

FIG. 5. Symbiotic colonization of E. scolopes hatchlings by the wild type, ΔluxCDABEG mutant EVS102, or Δphr mutant EW1. Average colonization levels 48 h after inoculation with the indicated strain are shown, with standard errors (n = 13 or 14).

We cannot rule out the possibility that bioluminescence does benefit ES114 or other bacteria by stimulating photolyase-mediated DNA repair in V. fischeri ES114. Nonetheless, we believe that several theoretical considerations make photoreactivation an unlikely natural role for bioluminescence. For example, photoreactivation in bacteria is optimally stimulated by 380-nm- to 440-nm-wavelength light (12, 22), but bioluminescent bacteria usually emit blue (~500-nm-wavelength) light, with different strains emitting light with wavelengths ranging from 476 nm to 540 nm (33, 39). To our knowledge, photolyase has not been closely studied in a bioluminescent strain, but it has been examined in V. parahaemolyticus, a species that includes bioluminescent strains and is a close relative of V. harveyi and V. fischeri. In nonluminescent V. parahaemolyticus WP28, photolyase is maximally stimulated by light in the 375-nm to 425-nm range, 475-nm light is less than 10% as effective for photoreactivation, and light at >500 nm is complemented with the phr gene in trans (Fig. 3). However, although bioluminescence enabled the bacteria to fully colonize the E. scolopes light organ, as reported elsewhere (41), the phr mutant was unaffected in host colonization (Fig. 5 and 6).

Thus, in the light organ environment where the bacteria are naturally bioluminescent and where bioluminescence is advantageous for the bacteria, the benefits of bioluminescence are not mediated by photolyase. Moreover, bioluminescence did not detectably stimulate photoreactivation in culture, even when bioluminescence was stimulated by the addition of the autoinducer 3-oxo-C6-HSL (Fig. 4). Taken together, our data do not support the model that bioluminescence functions to stimulate photolyase-mediated DNA repair in V. fischeri ES114.

**DISCUSSION**

We have shown that the V. fischeri ES114 phr gene (ORF VFA0753) encodes the photolyase enzyme of ES114 and that the benefit of bioluminescence for this bacterium during host colonization is not mediated by stimulation of Phr. ORF VFA0753 encoded the only photolyase (Phr) homolog in the ES114 genome, an in-frame deletion mutant of phr lacked photoreactivation (Fig. 2 and 4), and this phenotype could be
ineffective (30). Thus, unless the photolyases of bioluminescent \textit{V. parahaemolyticus} strains are highly diverged from that of WP28, there will be relatively little overlap in the bioluminescence and photoreactivation spectra in these strains.

The possibility that bioluminescence is not well attuned to the absorption requirements of photolyase raises the issues of efficiency and energetics. Hastings and Nealson calculated that the energy of each photon emitted by bioluminescence is equivalent to the hydrolysis of six ATP molecules (15). Even more energy would be required if the bioluminescence efficiency is <100\%, as is likely the case, and this does not even take into account the considerable energy devoted to Lux protein synthesis (16, 21). Moreover, it seems likely that most of the photons generated will not be absorbed and utilized by photolyase, and this is supported by our observation that there was no detectable difference in the intensities of bioluminescence emitted from cultures of the wild type and the photolyase mutant (data not shown). Thus, energy devoted to bioluminescence-mediated photoreactivation could conceivably be equivalent to the hydrolysis of tens, hundreds, or possibly more ATP molecules per lesion repaired. This seems a remarkably inefficient process, although the energy cost might be outweighed by the importance of maintaining genetic integrity.

Perhaps more problematic is the question of whether there is a dark environment where bioluminescent bacteria would benefit from light-mediated activation of photolyase. \textit{Vibrio} spp. are common near the ocean surface (13), where they are exposed to UV light. However, in this setting, the bacteria would also be exposed to light that could support photoreactivation, and at increased depths, UV light is filtered out to a greater degree than the visible light that would support photoreactivation (2, 18). Although photolyase also binds to DNA lesions other than pyrimidine dimers, and in \textit{E. coli} it stimulates excision repair of such lesions (31), these examples do not constitute photoreactivation and do not require light. Until a natural condition is found under which photoreactivation is beneficial in an environment lacking light, the evidence suggests that bioluminescence functions to activate photolyase arguably reflects artificial conditions.

One unexpected finding of our study was that \textit{phr} is located on chromosome II in \textit{V. fischeri}, \textit{V. cholerae}, \textit{V. vulnificus}, and \textit{V. parahaemolyticus} (6, 17, 25, 34). In members of the family \textit{Vibrionaceae}, chromosome II contains a higher relative representation of unusual and presumably niche-specific genes than does the larger chromosome I, which has a higher representation of housekeeping genes. Although photolyase might be considered one of the housekeeping genes found on chromosome II, it could also reflect the fact that the benefit of having \textit{phr} is specific to certain environments, such as those exposed to UV light. In this regard, it is interesting to note that \textit{Photobacterium profundum} strain SS9, a member of the \textit{Vibrionaceae} that is adapted to deep, dark marine environments, lacks a photolyase gene (40).

Another interesting and unexpected finding was that the addition of 3-oxo-C6-HSL to stimulate bioluminescence also appeared to increase the survival of \textit{V. fischeri} exposed to UV light and then kept in the dark but that this UV resistance was mediated by a mechanism independent of bioluminescence or photolyase (Fig. 4). This phenomenon was statistically significant ($P < 0.05$) for each strain in two out of three experiments, and it could provide an alternative explanation for the UV sensitivity of undefined (e.g., chemically induced) dark mutants. If bioluminescence and some mechanism(s) of UV resistance are coregulated, then some regulatory mutants might be both dark and UV sensitive without a direct causal relationship between bioluminescence and UV resistance.

The functional significance of bioluminescence for light-producing bacteria remains uncertain, and the observation that bioluminescence allows \textit{V. fischeri} ES114 to fully colonize its host is equally mysterious. Several plausible explanations have been offered (35), suggesting that bioluminescence acts as a sink for excess reductant (5), as an antioxidant (24, 38, 41), or as a symbiotic signal to the host that is required for light organ tissue to develop into an environment receptive to colonization (M. McFall-Ngai and C. Whistler, personal communication). Given our results and the arguments above, we are skeptical of the idea that the driving force behind the evolution of bioluminescence was that it played a significant role in stimulating photolyase. There may not be a single, simple role for bioluminescence, and it seems likely that bioluminescence may serve different purposes depending on the bacterium and the environment. Our laboratory and others are actively investigating these multiple possible functions of bioluminescence.

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