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Detection of DNA Damage by Use of *Escherichia coli* Carrying recA'::lux, uvrA'::lux, or alkA'::lux Reporter Plasmids

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Plasmids were constructed in which DNA damage-inducible promoters recA, uvrA, and alkA from *Escherichia coli* were fused to the *Vibrio fischeri* luxCDABE operon. Introduction of these plasmids into *E. coli* allowed the detection of a dose-dependent response to DNA-damaging agents, such as mitomycin and UV irradiation. Bioluminescence was measured in real time over extended periods. The fusion of the recA promoter to luxCDABE showed the most dramatic and sensitive responses. *lexA* dependence of the bioluminescent SOS response was demonstrated, confirming that this biosensor’s reports were transmitted by the expected regulatory circuitry. Comparisons were made between luxCDABE and luxZ fusions to each promoter. It is suggested that the lux biosensors may have use in monitoring chemical, physical, and genotoxic agents as well as in further characterizing the mechanisms of DNA repair.

Bacterial repair of DNA damage is mediated by at least two inducible systems, the recA-independent, *ada*-controlled adaptive response and the recA-dependent, *lexA*-controlled SOS response. The former responds specifically to the presence of methylated phosphotriesters generated by DNA alkylation (39). This signal activates the *ada* gene product, which in turn triggers the transcription of genes such as *ada, alkA, alkB,* and *aid* (7, 20, 28, 39). In contrast, the nature of the specific inducing signal of the SOS response is not yet fully defined (21, 23, 53). Upon SOS induction, the recA gene product is converted into an active and specific protease (21). Activated RecA protein cleaves the *LexA* repressor and other repressors such as the phage λ CI product (22, 35), resulting in the transcriptional derepression of several genes, among them *uvrA, recA,* those needed in the lytic pathway of phage λ (17), and others, such as *sulA,* that couple DNA damage to cell division (14, 15). Recent reviews have focused upon one or both of these repair systems (39, 53).

Activation of such repair systems is a measure of the mutagenic and genotoxic effects of various chemical and physical treatments. Many of the gene products, however, are difficult to assay because of the nature of their enzymatic activities and the particular substrates upon which they act. Thus, investigators have used relatively inexpensive and rapid alternative approaches. Measuring the reversion of specific auxotrophic bacterial mutations is the strategy used in the Ames tests (1, 24), and detecting restoration of bioluminescence is used in the Mutatox assay (47). The use of various transcriptional fusions also allows detection of agents that interact with DNA. The *umu* test (31), the *rec-lac* test (30), the SOS chromotest (34), and the Pro-Tox assay (32) exploit the ease with which β-galactosidase specific activities can be determined (26), while the biochemical prophage induction assay (11, 37) measures additional effects of the SOS response. The use of these assays by several pharmaceutical companies in Europe, Japan, and the United States has allowed generation of large data sets cataloging the genotoxic and mutagenic effects of many substances. The Ames reversion tests, however, remain the recommended battery for bacterial mutagenesis studies (13).

This report presents the construction and initial characterization of an alternative panel of easily assayed transcriptional fusions useful for genotoxicity studies. Promoters for three *Escherichia coli* genes, recA, uvrA, and alkA, have each been fused to the promoterless *Vibrio fischeri* luxCDABE operon present within the broad-host-range, multicopy plasmid pUCD615 (36). These fusions in *E. coli* allow visualization of the transcriptional responses induced by DNA damage, without the need to perform enzyme assays or to add luciferase substrates exogenously, since the full lux operon encodes not only the catalytic luciferase (LuxAB) but also the enzymes required to shunt fatty acyl metabolites from the central metabolism and to convert them to the aldehyde substrate for luciferase. Comprehensive reviews of the physiological, genetic, and biochemical regulation as well as the applications of bacterial luminescence have been published (25, 46).

These biosensors thus report the presence of genotoxic doses of stressors by an increase in the production of light. At the same time, the presence of nonspecific toxicants may also be monitored by their inhibitory effect on luminescence. In contrast to other bacterial strains that produce light in response to specific toxicants, such as naphthalene (6), these strains are part of a panel of biosensors that utilize less-specific stress responses to report heat shock and protein damage (48–51) and oxidative stress (2–4).

**MATERIALS AND METHODS**

**Enzymes and chemicals.** All chemicals used were analytical grade. Mitomycin and 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) were purchased from Sigma. Hydrogen peroxide was obtained from J. T. Baker, and ethanol was obtained from Quantum Chemical Corp. Restriction endonucleases, T4 DNA ligase, the Silver Sequence kit, and Wizard DNA Clean Up columns and reagents were supplied by Promega. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and medium components were purchased from Gibco BRL.

**Plasmids and *Escherichia coli* K-12 strains.** Transcriptional fusions were constructed by directional cloning of PCR-amplified (40) promoter region DNA (GeneAmp PCR reagent; Perkin-Elmer Cetus, Norwalk, Conn.) from *E. coli* W3110 (12) into the multiple cloning site of the promoterless luxCDABE plasmid, pUCD615 (36). DNA sequences were obtained from the database compiled...
TABLE 1. E. coli strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>W3110</td>
<td>F-</td>
<td>12</td>
</tr>
<tr>
<td>RFM443</td>
<td>F' gokR2 lac74 rpsL200</td>
<td>9</td>
</tr>
<tr>
<td>DM800</td>
<td>F' metA20 lacY1 or Z4 λ' thi-1</td>
<td>27</td>
</tr>
<tr>
<td>DM803</td>
<td>As DM800, also lecA&lt;sup&gt;400&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>DPD2794</td>
<td>pRecALux3/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>DPD2818</td>
<td>pUvrALux1/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>DPD2844</td>
<td>pAlkALux1/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>DPD2850</td>
<td>pRecALux3/DM800</td>
<td>This study</td>
</tr>
<tr>
<td>DPD2851</td>
<td>pRecALux3/DM803</td>
<td>This study</td>
</tr>
<tr>
<td>ACV1002</td>
<td>pRS550/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>ACV1003</td>
<td>pRecALac1/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>ACV1004</td>
<td>pUvrALac2/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>ACV1005</td>
<td>pAlkALac3/RFM443</td>
<td>This study</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUCD615</td>
<td>Amp&lt;sup&gt;+&lt;/sup&gt; Kan&lt;sup&gt;+&lt;/sup&gt; multiple cloning site upstream of luxCDABE</td>
<td>36</td>
</tr>
<tr>
<td>pRecALux3</td>
<td>As pUCD615, but recA&lt;sup&gt;+&lt;/sup&gt;:luxABE</td>
<td>This study</td>
</tr>
<tr>
<td>pUvrALux1</td>
<td>As pUCD615, but uvrA&lt;sup&gt;-&lt;/sup&gt;:luxABE</td>
<td>This study</td>
</tr>
<tr>
<td>pAlkALux1</td>
<td>As pUCD615, but alka&lt;sup&gt;-&lt;/sup&gt;:luxABE</td>
<td>This study</td>
</tr>
<tr>
<td>pRS550</td>
<td>Amp&lt;sup&gt;+&lt;/sup&gt; Kan&lt;sup&gt;+&lt;/sup&gt; multiple cloning site upstream of lacZ</td>
<td>45</td>
</tr>
<tr>
<td>pRecALac1</td>
<td>As pRS550, but recA&lt;sup&gt;+&lt;/sup&gt;:lacZ</td>
<td>This study</td>
</tr>
<tr>
<td>pUvrALac2</td>
<td>As pRS550, but uvrA&lt;sup&gt;-&lt;/sup&gt;:lacZ</td>
<td>This study</td>
</tr>
<tr>
<td>pAlkALac3</td>
<td>As pRS550, but alka&lt;sup&gt;-&lt;/sup&gt;:lacZ</td>
<td>This study</td>
</tr>
</tbody>
</table>

Induction of plate cultures was performed either by exposing the plated cells to UV (254 nm) at doses from 0.5 to 2.000 J m<sup>-2</sup> on an uncovered agar surface with a Stratalinker 2300 (Stratagene) irradiation unit or by supplementing LB agar with mitomycin (final concentration = 0.1 μg ml<sup>-1</sup>) and incubation at 26°C. All E. coli liquid cultures were grown in LB medium (26) at 26°C with shaking. Overnight culture media contained 25 μg of kanamycin sulfate ml<sup>-1</sup>, while fresh dilution cultures were inoculated from overnight cultures in LB medium without out kanamycin (4). These cultures were allowed to grow at 26°C for 2 to 3 h (to 10 to 20 Klett units [1 × 10<sup>8</sup> to 2 × 10<sup>8</sup> cells ml<sup>-1</sup>) prior to induction. Induction of liquid cultures took place in opaque white 96-well microtiter plates (Dynex) containing 50 μl of the bacterial culture added to 50 μl of an appropriate dilution series of test compounds, which were dissolved in LB medium. UV induction was performed by transferring cells into a sterile petri dish, exposing the uncovered cells to UV as described above, and then transferring 100-μl aliquots to the 96-well plates. Luminescence was most conveniently measured in a microplate lumimeter as described previously with each of two duplicate samples being read 20 times per time point (48). In some cases, luminescence was measured at 26°C with a 1219 RackBeta liquid scintillation counter (LKB/Wallac) as per the manufacturer’s instructions for detecting chemiluminescence. For these measurements, 50-μl aliquots of samples and inducers were placed in colorless sterile 1.5-ml microcentrifuge tubes (without caps), which were then placed in standard capped scintillation vials. Quadruplicate samples were read every 20 min.

The lumimeter chamber was modified to allow for accurate temperature control at 26°C. The scintillation counter was placed in a 26°C temperature-controlled room. The sample holding compartment of the scintillation counter was maintained at 26°C.

Luminescence values are presented as relative light units (RLU [as per the particular instrument’s output]). Response ratios are the RLU of the induced samples divided by the RLU of a matched, untreated control (49).

RESULTS

The response of E. coli DPD2794 (containing the recA<sup>-</sup>:lux fusion) to mitomycin is shown in Fig. 1. The kinetic profile of the recA response had a 50- to 60-min lag followed by an increase in bioluminescence. The response was dose dependent in the range of 0 to 2 μg of mitomycin ml<sup>-1</sup> (Fig. 2); mitomycin concentrations higher than 2 μg ml<sup>-1</sup> led to a decrease in luminescence below the control levels (not shown). This was accompanied by a loss of viability, as judged by colony formation capacity (from 3 × 10<sup>10</sup> CFU ml<sup>-1</sup> without mitomycin to 4 × 10<sup>7</sup> CFU ml<sup>-1</sup> at 4 μg of mitomycin ml<sup>-1</sup>). Loss of viability was noted only at the high concentration of
mitomycin. Exposure of DPD2794 to UV irradiation in a range from 3.1 to 2,000 J m$^{-2}$ resulted in increased bioluminescence, which was maximal at 10 J m$^{-2}$. The presence of H$_2$O$_2$ and MNNG also induced bioluminescence of DPD2794 (Fig. 2). In addition, DPD2794 responded to 0.03 to 2.0 mg of ethidium bromide ml$^{-1}$ (data not shown). In a tolC (10, 44) background, recA::lux was more sensitive to mitomycin (data not shown). This is somewhat surprising, since the compound is not hydrophobic and the tolC efflux system is thought to expel hydrophobic xenobiotics from the cell.

Transformation of plasmid pRecALux3 into isogenic strains DM800 and DM803 yielded strains DPD2850 and DPD2851, respectively. DM803 carries a lexA$^\text{ind}$ repressor that is not a substrate for the SOS-activated recA-dependent cleavage, while the isogenic DM800 contains the cleavable wild-type LexA repressor (27). Results from challenging these strains with 2 mg of mitomycin ml$^{-1}$ (Fig. 3) indicate that the recA response that is reported by this transcriptional fusion is lexA dependent.

In contrast to the induction of DPD2794, DPD2818 (containing the uvrA::lux fusion) had a more delayed and less dramatic response to mitomycin (Fig. 4A) and UV exposure (Fig. 4B). There was consistently a longer lag phase between the beginning of the exposure to the inducer (time 0) and the initial evidence of response. The magnitude of the response (in RLU), although much smaller, was nevertheless dose dependent. Viability of cells was diminished at high levels of UV exposure (data not shown).

The response of strain DPD2844 (containing the alkA::lux fusion) to MNNG yielded a dose-responsive kinetic profile (Fig. 5). A 40- to 50-min lag was followed by a large rise in luminescence in samples treated with MNNG.

To test that the responses measured were not specific to the lux operon reporter, promoters were recloned so that they...
TABLE 2. Comparison of responses of lux and lac promoter fusions

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Inducer</th>
<th>Conc or dose of inducer</th>
<th>Response ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lux</td>
<td>lactZ</td>
<td>lux lac</td>
</tr>
<tr>
<td></td>
<td>lux</td>
<td>lactZ</td>
<td>lux lac</td>
</tr>
<tr>
<td>recA</td>
<td>Mitomycin</td>
<td>1 μg ml⁻¹</td>
<td>16.7 8.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 μg ml⁻¹</td>
<td>4.8 2.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 μg ml⁻¹</td>
<td>1.8 1.1 ± 0.2</td>
</tr>
<tr>
<td>UV</td>
<td></td>
<td>1,000 J m⁻²</td>
<td>7.7 6.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 J m⁻²</td>
<td>5.2 5.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 J m⁻²</td>
<td>4.6 3.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 J m⁻²</td>
<td>2.4 1.6 ± 0.09</td>
</tr>
<tr>
<td>uvrA</td>
<td>Mitomycin</td>
<td>1 μg ml⁻¹</td>
<td>41.0 23.5 ± 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 μg ml⁻¹</td>
<td>12.0 5.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 μg ml⁻¹</td>
<td>5.0 1.9 ± 0.2</td>
</tr>
<tr>
<td>UV</td>
<td></td>
<td>1,000 J m⁻²</td>
<td>52.5 21.5 ± 4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 J m⁻²</td>
<td>25.0 8.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 J m⁻²</td>
<td>2.5 1.1 ± 0.3</td>
</tr>
<tr>
<td>alkA</td>
<td>MNNG</td>
<td>1 mg ml⁻¹</td>
<td>4,000 3.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 mg ml⁻¹</td>
<td>500 2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 mg ml⁻¹</td>
<td>10 1.6 ± 0.7</td>
</tr>
</tbody>
</table>

Response ratios were calculated by dividing the bioluminescence displayed by the treated sample by the bioluminescence of the untreated sample. A response ratio of 1.0 represents no induction. The time of measurement for the recA fusion (strain DPD2794) was 90 min after treatment, that for the uvrA fusion (DPD2818) was 30 min after treatment, and that for the alkA fusion (DPD2844) was 90 min after treatment. Each value is the average of duplicate samples that have been read 20 times each by the luminometer.

Response ratios were calculated by dividing the bioluminescence displayed by the treated sample by the specific activity at 90 min of the untreated sample. A response ratio of 1.0 represents no induction.

Large response ratios are due to a very small amount (2 × 10⁻⁴ RLU) of bioluminescence in the untreated sample.

DISCUSSION

This study describes promoter fusions that allow the detection of sublethal levels of DNA-damaging agents. While in this report three promoters have been fused to luxCDABE, they represent a wide variety of genes involved in DNA repair. The noninvasive protocol using lux fusions allows real-time reporting of the transcriptional activation of SOS and adaptive response-regulated operators.

Furthermore, the presence of transcriptional fusions on a multicopy plasmid does not appear to affect their ability to be regulated by the SOS response, as shown by the lacZ dependency for recA induction. These results confirm earlier reports showing that multicopy plasmids bearing an SOS-responsive promoter do not titrate all available LexA repressor molecules in the cell (19, 29). Preliminary comparisons of DPD2794 and a single-copy (chromosomal) analog indicate qualitative differences in sensitivity and duration of the response to stressors and a lower background luminescence of the single-copy integrant (11a). Our results are in agreement with those of others who found that the transcription of recA (as measured by recA-lacZ expression on a prophage) is induced about 10-fold upon induction with UV at 5 J/m² (43). In similar, earlier studies with sulA-lacZ, via Mu d(Ap lac), transcriptional fusions indicate a 20- to 40-fold induction within a period of 90 min following UV irradiation at 10 J/m⁻² (14). The induction of multicopy uvrA::lux occurs later than that of recA::lacZ. This is consistent with findings that RecA-mediated cleavage of the LexA repressor results in a programmed cascade of gene expression (27). Some genes, such as uvrA, not expressed at all in the absence of the DNA damage signal, are expressed only after the SOS system has been activated for a prolonged period (18, 23). These two distinct SOS response reporters can thus be used differentially, depending upon the amount of basal level luminescence that is preferred.

Comparisons of the multicopy luxCDABE and lacZ reporter plasmids indicate a greater sensitivity when the former is used. Additionally, the comparative ease of measuring large numbers of samples noninvasively and the ability to collect data points continuously (i.e., in real time) are significant practical advantages of the luxCDABE format not reflected in Table 2.

It is important to note that the kinetic measurements made by different instruments (luminometers versus scintillation counters) cannot be compared directly. Rather, proportionate induction can be compared. However, in this regard, calculation of response ratios may be skewed toward large numbers...
if the value of the baseline expression values incorporated into the ratio’s denominator are extremely low. Also, when measurements approach the limit of detection of the instrument (see baseline, Fig. 5), then the machine noise can be problematic when trying to calculate response ratios. In some cases, difference curves of kinetic data, subtracting the response of untreated controls from treated controls, might be more informative. It is recommended that comparisons be made with data obtained with the same instrumentation and that kinetic data be analyzed prior to the calculation of response ratios. Any potential toxicant could be compared with defined concentrations of a known stressor, providing internal standards that would diminish day-to-day variation. The production of light is dependent on numerous metabolic factors in the growing culture. The sensitivity of this sensor system to changes in the physiological state (temperature, aeration, etc.) is a reason for variation in baseline bioluminescence.

This set of biosensors allows for the rapid measurement of environmental stressors at sublethal, although inducing, concentrations when compared to the process of scoring for revertants on selective media, the basis of the Ames test. The added advantage of using the recA reporter is that the induced level of recA transcription provides a significant baseline level of light emission. Decreasing luminescence from this baseline can be interpreted as toxicity associated with particular stressors, as was the case with mitomycin at concentrations exceeding 2 μg/ml (data not shown). Even though the background luminescence of recA is relatively high, the sensor is still quite sensitive, being in the middle of the 7 orders of magnitude range of the commercial luminometer used in most of these studies. Having both a “lights on” as well as a “lights off” reporter gives this system an advantage over assays such as Microtox, in which only the measurement of the loss of bioluminescence is quantitated. Moreover, a real-time reporter allows facility in the collection of detailed kinetic data without the manual intervention to make cell extracts or to add exogenous substrates. Coupled with the automation inherent in the microtiter plate luminometer format, large numbers of compounds can be analyzed easily. Even the use of a scintillation counter allows the assay of numerous samples. With the exception of the industrial wastewater samples, each of the agents tested here has previously been shown to cause damage to DNA by other assays; therefore, induction of each of the agents tested here has previously been shown to cause damage to DNA by other assays; therefore, induction of recA from the plant did not elicit the same response from that strain. Studies of recA and other promoters: lux fusion strains and their use in determining toxicant levels in water have been detailed elsewhere (4). Presently, studies are under way in which unknown or untested treatments are being assayed for their ability to induce these biosensors. Preliminary results indicate that insonification (high-frequency ultrasound) and the accompanying acoustic cavitation will induce DPD2794 in a dose-dependent manner that is possibly due to damage caused by reactive oxygen species (52). Additional studies utilize some of these biosensors to measure the change in sensitivity toward DNA-damaging agents as cells depart from the logarithmic phase of growth (51a). Future studies include investigating whether synergistic effects such as those affecting heat shock promoter:luxCDABE fusions (50) might also be operable with these DNA damage sensors. This system also offers a feasible way to further characterize other factors that influence the regulation of the SOS response, such as the effects of nucleotide pools (33) and pH (8) on LexA conformational equilibria. Finally, as was demonstrated with the influent and effluent water samples from an industrial facility, this set of biosensors may be used to detect the presence of genotoxic agents, as purified compounds or mixtures of unknown composition, in pharmaceutical and environmental applications.

ACKNOWLEDGMENTS

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