Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli* (umuC gene/recA/lexA regulation/operon fusion/SOS functions)

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ABSTRACT The product of the umuC gene is required for UV and chemical mutagenesis in *Escherichia coli*. By the use of the Mud(Ap, lac) bacteriophage, we have obtained an operon fusion of the lac structural genes to the promoter/regulatory region of the umuC gene. The strain containing the umuC::Mud(Ap, lac) fusion was identified on the basis of its UV nonmutability. Strains containing this putative null allele of umuC were (i) nonmutable by UV and other agents, (ii) slightly UV sensitive, and (iii) deficient in their ability to carry out Weigle reactivation of UV-irradiated bacteriophage A. The UV nonmutability of the strain could be suppressed by a derivative of the mutagenesis-enhancing plasmid pKM101. *β*-Galactosidase synthesis in umuC::Mud(Ap, lac) fusion strains was inducible by UV and other DNA-damaging agents. Genetic analysis of the regulation of *β*-galactosidase in umuC::Mud(Ap, lac) strains suggests that the lexA protein is the direct repressor of the umuC gene and that a function of the recA product, probably its protease activity, is required for the removal of the lexA repressor at the time of umuC induction.

In *Escherichia coli*, mutagenesis by agents such as UV light, methyl methanesulfonate (MeMes), and 4-nitroquinoline-1-oxide (4NQO) is not a passive process. Rather, there exists a cellular system that processes the DNA damage in such a way that mutations result. Mutagenesis is not a necessary consequence of DNA damage; if this system is inactivated, no mutations result (1–4).

This “mutagenesis system” is inducible. Its activity is observed in wild-type cells only after treatments that either damage DNA or block replication (1). This feature is perhaps best illustrated by the fact that UV-irradiated bacteriophage are only slightly mutagenic unless the cells that they infect have been exposed to such an inducing treatment (5, 6). In addition to increasing the mutation frequency of UV-irradiated bacteriophage, treatment of host cells with low levels of DNA-damaging agents also increases the fraction of surviving phage (5, 6). These inducible mutagenesis and reactivation activities have been called Weigle or W mutagenesis and W reactivation, respectively (2).

The ability of *E. coli* or its bacteriophage to be mutated by UV and chemical agents can be blocked by mutations at three bacterial loci, recA, lexA, and umuC (1, 3, 4). These mutations simultaneously reduce or eliminate W reactivation. Because of the association of an inducible mutagenesis activity with what appears to be an inducible repair activity, it has been proposed that mutations result from the operation of an “error-prone repair” system (1, 2). To date, the biochemical mechanism of these processes has not been established (7, 8), nor have the effects on mutagenesis and survival been rigorously shown to result from the same process.

The recA and lexA proteins coordinate the diverse set of SOS phenomena that occur when cells are treated with various DNA-damaging agents. In addition to the induction of error-prone repair, these include the induction of lambda prophage, the induction of the recA protein, and filamentous growth (1). The recA and lexA proteins regulate not only their own synthesis but also the expression of a set of cellular din (damage-inducible) genes (9) [including uvrA (9, 10), uvrB (10, 11), and sfa (12)] whose products are apparently required for at least some of these inducible responses. Although it is clear that the recA and lexA proteins are involved in the regulation of the SOS responses, it has not been established whether these proteins play additional mechanistic roles in some of the SOS responses. In particular, it is not yet clear whether the recA or lexA gene products participate in the actual processing of DNA damage that gives rise to mutations.

In contrast to the pleiotropic effects of the lexA and recA mutations, umuC mutations (3) specifically eliminate UV and chemical mutagenesis and reduce the efficiency of W reactivation. Thus, it is possible that the umuC gene product may play a key mechanistic role in the process of error-prone repair.

To better understand the molecular basis for the inducibility of the umuC-dependent mutagenesis and repair activities, we have isolated an operon fusion of the umuC promoter to the β-galactosidase structural gene. In doing so, we have obtained a putative null allele of the umuC gene. In this report, we describe the phenotype of this umuC mutant and analyze the regulation of the umuC gene.

METHODS AND MATERIALS

Strains. Bacterial strains used were all derivatives of *E. coli* K-12. Bacteriophage P1 transductions were essentially as described by Miller (13). The plasmids pGW200 (pKM101umuC::Tn5) and pGW249 (pKM101bla455::Tn5) were introduced into fusion strains by mating and selection on appropriately supplemented minimal plates containing kanamycin (25 μg/ml) (14).

Media. Bacteria were routinely grown in Luria broth and LB agar (13). Supplemented M9/glucose plates and liquid medium (13) were used for mutagenesis and UV-survival measurements (14) and for β-galactosidase assays (13).

Isolation of Nonmutable Fusion Strains. The screening procedure was adapted from that used by Kato and Shinoura (3). Ampicillin-resistant colonies of strain GW1101 containing random Mud(Ap, lac) insertions (9) were replicated onto two plates containing low levels (1 μg/ml) of histidine. These plates were then exposed to three 2-J/m² doses of UV light at 10-hr intervals and those that displayed a nonmutable phenotype (0–1 His+ papillae) on both replica plates were further characterized.

Abbreviations: MeMes, methyl methanesulfonate; 4-NQO, 4-nitroquinoline-1-oxide; MeNNG, N-methyl-N'-nitro-N-nitosoguanidine.
RESULTS

Isolation and Mapping of a umuC-lac Fusion. A fusion of the lac structural genes to the promoter/regulatory region of the umuC gene was obtained by using the Mud(Ap, lac) operon fusion vector (15). Mud(Ap, lac) is a derivative of the temperate bacteriophage Mu, which integrates into the bacterial chromosome with appreciable site specificity (16, 17). The phage carries the lactose structural genes but no promoter capable of initiating their transcription. However, when this phage integrates in a bacterial transcriptional unit, the lac genes can be expressed as a result of continued transcription into the phage genome. Such an insertion creates an operon fusion in which the synthesis of β-galactosidase has been placed under the control of the cellular regulatory locus. Moreover, the insertion of Mud(Ap, lac) in a gene, or proximal to it in its transcriptional unit, generates a mutation that generally results in the loss of function of that gene.

Our first step in obtaining a umuC::Mud(Ap, lac) fusion was to screen random insertions of Mud(Ap, lac) in the E. coli chromosome for those that made the cell nonmutable by UV. The bacterial strain we used for this screen had its own lac genes deleted and carried an ochre his<sup>−</sup> (18) and an ochre arg<sup>−</sup> mutation, each of which was revertable by UV. In addition, the strain carried a ura<sup>−</sup> mutation that inactivated the accurate ura<sup>−</sup>-dependent excision repair pathway and increased the sensitivity of the cells to UV mutagenesis (1). Colonies containing random insertions of Mud(Ap, lac) were replica plated to supplemented minimal plates containing a low concentration of histidine, and the plates were UV irradiated. After screening 17,000 independent Mud(Ap, lac) insertions, we were able to identify 11 mutants that had a complete or partial inability to carry out UV-induced his<sup>−</sup> to His<sup>+</sup> reversion. Of these, six probably contained Mud(Ap, lac) insertions in histidine biosynthetic genes as they still gave rise to UV-induced Arg<sup>−</sup> revertants at a normal frequency.

The approximate position of the Mud(Ap, lac) insertions in the remaining five "UV-nonmutable" strains was determined by Hfr "quick-mapping" experiments (19). One of these insertions was subsequently mapped to the umuC locus (3, 20) by P1 transduction. Previously, we had isolated an insertion of the transposable element Tn5 (kanamycin resistance) that is ~65% cotransducible with the known umuC<sup>36</sup> mutation. Transduction of this Tn5 from a umuC<sup>−</sup> strain into the nonmutable strain containing Mud(Ap, lac) resulted in loss of the Mud(Ap, lac) phage 50% of the time (12/24). All of those kanamycin-resistant transductants that had lost the Mud(Ap, lac) phage regained their ability to be mutated by UV. When this same Tn5 was transduced from a umuC<sup>−</sup> strain into the nonmutable Mud(Ap, lac) strain, none of the transductants that had lost Mud(Ap, lac) (0/25) regained the ability to be mutated by UV.

On the basis of this mapping data and the phenotypic characterization described below, we conclude that the UV nonmutability of this strain is due to an insertion of Mud(Ap, lac) in the umuC gene itself or proximal to it in the same transcriptional unit.

Properties of the umuC::Mud(Ap, lac) Mutant. The strains used are summarized in Table 1. As shown in Fig. 1, the strain carrying the umuC::Mud(Ap, lac) mutation was nonmutable with UV as are the known umuC mutants. An additional property of umuC mutants is that their nonmutability and their deficiency in W reactivation can be suppressed by the introduction of the drug-resistance plasmid pKM101 (21). This plasmid, in a recA<sup>−</sup>lexA<sup>−</sup>-dependent fashion, increases both the susceptibility of cells to mutagenesis and their resistance to killing by UV (22). We have previously suggested that a pKM101-encoded gene termed muc (mutagenesis::UV and chemical) may be an analog of the chromosomally encoded umuC gene (14, 21). Introduction of pGW249, a kanamycin-resistant muc<sup>−</sup> derivative of pKM101 (14), into the umuC::Mud(Ap, lac) strain suppressed the UV nonmutability of this strain (Fig. 1). Moreover, a muc<sup>−</sup> Tn5 insertion mutant of pKM101 that fails to suppress the UV nonmutability of known umuC mutants (14) similarly failed to suppress the UV nonmutability of the umuC::Mud(Ap, lac) strain (Fig. 1). In addition, the umuC::Mud(Ap, lac) mutation is recessive; the introduction of an F<sup>−</sup>umuC<sup>−</sup> episome made the strain mutable by UV again (data not shown).

umuC mutations generated by ethyl methanesulfonate mutagenesis cause a modest increase in the sensitivity of cells to

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**Table 1. Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant markers</th>
<th>Source</th>
</tr>
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<tr>
<td>GW1000</td>
<td>lacZ1619, tir-1, strA11, his-4</td>
<td>This laboratory</td>
</tr>
<tr>
<td>RB800</td>
<td>malE::Tn5, lexA3</td>
<td>R. Brent</td>
</tr>
<tr>
<td>GW1102</td>
<td>As GW1000, but malE::Tn5</td>
<td>P1-RB800 × GW1000</td>
</tr>
<tr>
<td>AB1886</td>
<td>uvrA6</td>
<td>This paper</td>
</tr>
<tr>
<td>GW1103</td>
<td>As GW1101, but umuC::Mud(Ap, lac), malE::Tn5</td>
<td>P1-AB1886 × GW1102</td>
</tr>
<tr>
<td>GW1104</td>
<td>As GW1103, but uvrA&lt;sup&gt;−&lt;/sup&gt;, malE::Tn5</td>
<td>P1-RB800 × GW1103</td>
</tr>
<tr>
<td>GW1105</td>
<td>As GW1103, but uvrA&lt;sup&gt;−&lt;/sup&gt;, malE::Tn5, lexA3</td>
<td>P1-RB800 × GW1103</td>
</tr>
<tr>
<td>DB6659</td>
<td>scr::Tn10, recA56</td>
<td>D. Botstein</td>
</tr>
<tr>
<td>GW1106</td>
<td>As GW1103, but uvrA&lt;sup&gt;−&lt;/sup&gt;, malE::Tn5, recA56, scr::Tn10</td>
<td>P1-DB6659 × GW1104</td>
</tr>
<tr>
<td>DM1187</td>
<td>spr-51</td>
<td>D. Mount (21)</td>
</tr>
<tr>
<td>GW1107</td>
<td>As GW1103, but uvrA&lt;sup&gt;−&lt;/sup&gt;, spr-51</td>
<td>P1-DM1187 × GW1105</td>
</tr>
<tr>
<td>GW1108</td>
<td>As GW1103, but uvrA&lt;sup&gt;−&lt;/sup&gt;, spr-51, recA56, scr::Tn10</td>
<td>P1-DB6659 × GW1107</td>
</tr>
<tr>
<td>GW1060</td>
<td>As GW1000, but uvrA215::Mud(Ap, lac)</td>
<td>Ref. 10</td>
</tr>
</tbody>
</table>

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**Fig. 1.** UV nonmutability of a umuC::Mud(Ap, lac) strain and suppression of the nonmutability by a pKM101 derivative. Exponentially growing cultures of cells in supplemented M9/glucose medium were UV irradiated and plated on supplemented M9/glucose plates containing histidine (1 μg/ml) (22). His<sup>−</sup> revertants were counted after 2 days of incubation at 30°C. ○, GW1101 [umuC<sup>−</sup>]; ●, GW1103 [umuC::Mud(Ap, lac)]; Δ, GW1103 (pGW249) [umuC::Mud(Ap, lac)/pKM101 blu485::Tn5]; △, GW1103 (pGW200) [umuC::Mud(Ap, lac)/pKM101 muc12::Tn5].
direct evidence that the synthesis of the umuC gene product is induced by DNA damage. As expected, the induction of the umuC gene product did not require the uvrA+/B+/C+-dependent endonucleolytic activity; the expression of the umuC::Mud(Ap, lac) fusion was also highly UV inducible in the original uvrA- background.

Control of umuC Expression. The mutability of umuC+ cells is dependent on the function of the recA and lexA proteins (1, 9). These proteins are known to regulate the expression of a variety of damage-inducible genes. Current genetic and biochemical evidence indicates that the lexA protein represses multiple cellular genes (10, 12, 23, 24) and that induction occurs when the recA protein proteolytically cleaves the lexA protein in response to DNA damage (25). To determine whether the umuC gene is likewise controlled by the recA and lexA proteins, we introduced recA+ and lexA+ (undiectable repressor) mutations into the umuC-lac fusion strain. As shown in Fig. 4, induction of β-galactosidase was abolished.

In an effort to further analyze this recA+ lexA+ dependence, we examined the effect of a putative null mutation of lexA, termed spr (23), on umuC expression. Introduction of the spr mutation into the umuC::Mud(Ap, lac) strain resulted in high-level constitutive synthesis of β-galactosidase; no further increase in β-galactosidase synthesis was seen after UV-irradiation (Fig. 4). Thus, the lexA protein appears to play a negative regulatory role in the control of the umuC gene. When a recA+ mutation was subsequently introduced into the spr umuC::Mud(Ap, lac) strain, high-level constitutive synthesis of β-galactosidase was still observed; once again, no increase in β-galactosidase expression was seen on UV irradiation (Fig. 4). Thus, once lexA activity is eliminated from a cell, recA function is no longer needed for umuC expression. These observations suggest that the lexA protein is the direct repressor of the umuC gene and that induction occurs when, in response to DNA damage, the lexA protein is cleaved by the recA protease (25).

In this analysis, we have assumed that the umuC::Mud(Ap, lac) mutant we isolated resulted from a simple insertion of Mud(Ap, lac) into the umuC translational unit. The formal possibility exists that the insertion of Mud(Ap, lac) into umuC was accompanied by a Mu-mediated deletion or rearrangement that resulted in the promoter of some other gene being fused to the lac genes of Mud(Ap, lac). We consider this unlikely as...
the \textit{umuC::Mud(Ap, lac)} fusion was identified not on the basis of its regulatory characteristics but rather by its nonmutable phenotype and the observed regulation of \(\beta\)-galactosidase is consistent with physiological and genetic studies of \textit{umuC}\(^+\)-dependent phenomena.

**Induction of \textit{umuC} Expression by Other Agents.** We have also examined the ability of other DNA-damaging agents besides UV to induce \(\beta\)-galactosidase synthesis in the \textit{umuC::Mud(Ap, lac)} fusion strain. MeMes, 4NQO, N-methyl-N'-nitro-N-nitrosoguanidine (MeNNG), and mitomycin C all proved to be effective inducers (Table 2). Reversion of the ochre \textit{arg}~ mutation by these first three agents was largely \textit{umuC}\(^+\)-dependent (Table 3). Mitomycin C was not a particularly effective mutagen, at least for this particular reversion, yet it was a highly effective inducer of \textit{umuC}. It is interesting to note that MeNNG, at 0.25 \(\mu\text{g}/\text{ml}\), was able to cause induction of \textit{umuC}. This is the same range of concentration of MeNNG that induces the "adaptive response" in related strains. The adaptive response, once induced, specifically prevents mutagenesis and killing by methylating and ethylating agents (26, 27).

The induction of the SOS phenomena and the expression of damage-inducible genes can be triggered by a variety of agents that damage DNA. Although the mechanism by which this induction occurs is not fully understood, it is likely that these agents lead to an increase in the concentration of an effector of the \textit{recA} protein (such as single-stranded DNA). The agents tested here are inducers of the general set of SOS responses, and it is likely that their effect on \textit{umuC} induction reflects their ability to generate an intracellular effector of the \textit{recA} protein.

**DISCUSSION**

Treatment of cells with DNA-damaging agents induces a \textit{umuC}\(^+\)-dependent system that can process DNA damage in such a way that mutations result. By isolating a fusion of the \textit{umuC} promoter to the \(\beta\)-galactosidase structural gene, we have shown that the inducibility of this system is due at least in part to the induction of the \textit{umuC} gene product.

The genetic analysis presented here indicates that the \textit{lexA} protein directly represses the \textit{umuC} gene. By similar genetic analyses and, in some cases, by biochemical experiments, this protein has now been shown to repress a number of cellular genes, including the \textit{lexA} gene itself (24), \textit{recA} (23), \textit{uvrA} (10), \textit{sfA} (12), and the \textit{dinA}, \textit{dinB}, and \textit{dinD} genes (ref. 9; unpublished results). Induction of these gene products occurs when the \textit{recA} protein is proteolytically activated in response to DNA damage and cleaves the \textit{lexA} repressor (25). Apparently, the use of a single regulatory protein such as the \textit{lexA} or \textit{crp} (28) proteins to control multiple cellular genes provides bacterial cells with an efficient mechanism for coordinate expression of genes that have diverse functions.

The demonstration that the \textit{umuC} gene product is inducible may help to clarify the role of the \textit{lexA} and \textit{recA} proteins in mutagenesis. The nonmutability (29) of \textit{lexA}\(^+\) [undiureducible repressor (25)] mutants is probably due at least in part to their inability to induce the \textit{umuC} gene product. It is unlikely that the \textit{lexA} protein functions mechanistically in mutagenesis as cells carrying putative null alleles of \textit{lexA} (\textit{spr} mutations) are fully mutable. In contrast, the \textit{recA} protein appears to have a second function in addition to inactivating the \textit{lexA} repressor protein. As shown above, cells carrying \textit{spr} mutations synthesize high levels of \textit{umuC} gene product in the absence of the \textit{recA} protein; however, they are nonmutable. This requirement for \textit{recA} function could be for its protease activity (30)—either to inactivate a second repressor or to proteolytically modify some protein. Alternatively, the requirement could be for those biochemical activities that have been associated with the \textit{recA} role in homologous recombination (31, 32).

The inducibility of the bacterial mutagenesis system implies that, for agents such as UV, MeMes, and 4NQO to be effective mutagens, they must have at least two properties: (i) they must induce the \textit{umuC} protein and (ii) they must introduce premutagenic lesions into DNA. The requirement for \textit{umuC} (and possibly \textit{recA}) induction is indicated by the nonmutability of cells carrying \textit{lexA}~ mutations. The need for premutagenic lesions can be inferred from a number of experiments—for example, the demonstration that the mutation frequency of bacteriophage infecting induced host cells is much higher when the phage have been irradiated (33). Thus, the efficiency of various mutagens is a reflection not only of their ability to introduce premutagenic lesions but also of their ability to induce the bacterial mutagenesis system that processes them. In addition, this suggests that certain combinations of mutagens may act synergistically.

The \textit{umuC} mutation isolated in this study is likely to be a null allele. The mutation is recessive and was detected after screening a relatively modest number (17,000) of random \textit{Mud(Ap, lac)} insertion mutants. The simplest interpretation of such a null allele is that the \textit{umuC} gene product is an active participant in the processing of DNA damage that results in mutations. Moreover, the inference that a nonmutable phenotype can result from a loss of cellular function suggests that the DNA damage resulting from UV and many chemical mutagens is not intrinsically mutagenic in bacteria. As the cells of many higher organisms, including humans, can be mutated by UV and many of these same chemicals, it seems reasonable to argue that these organisms possess analogous processing systems.

The phenotype of the \textit{umuC} insertion mutant is similar to that of cells carrying \textit{umuC} point mutations. In addition to becoming nonmutable, the cells are somewhat more sensitive to DNA damage and lose a component of the inducible W-rec-

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**Table 2. Induction of \(\beta\)-galactosidase in a \textit{umuC::Mud(Ap, lac)} fusion strain by various agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>(\beta)-Galactosidase units/OD\text{(\text{abs})} unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>MeMes</td>
<td>0.02 (\mu\text{g}/\text{ml})</td>
<td>5.6</td>
</tr>
<tr>
<td>4NQO</td>
<td>5 (\mu\text{g}/\text{ml})</td>
<td>64.7</td>
</tr>
<tr>
<td>MeNNG</td>
<td>0.25 (\mu\text{g}/\text{ml})</td>
<td>31.3</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>0.25 (\mu\text{g}/\text{ml})</td>
<td>67.3</td>
</tr>
</tbody>
</table>

An exponentially growing culture of GW1104 was split into several aliquots. One aliquot was untreated, and chemicals were added to the others to the concentrations shown. The cells were then incubated at 30\(^\circ\)C for 2 hr, and the \(\beta\)-galactosidase activity was determined.

**DISCUSSION**

**Table 3. Nonmutability of a \textit{umuC::Mud(Ap, lac)} strain with various agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>\textit{His}(^+) revertants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GW1000 (\textit{umuC}(^+))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>MeMes</td>
<td>1 (\mu\text{l})</td>
<td>55</td>
</tr>
<tr>
<td>4NQO</td>
<td>60 (\mu\text{g})</td>
<td>73</td>
</tr>
<tr>
<td>MeNNG</td>
<td>10 (\mu\text{g})</td>
<td>228</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>20 (\mu\text{g})</td>
<td>7</td>
</tr>
</tbody>
</table>

An 0.1-\text{ml} aliquot of an exponential culture was added to 2 ml of top agar containing the chemical and poured on a supplemented M9/glucose plate containing histidine at 1 \(\mu\text{g}/\text{ml}\). \textit{His}\(^+\) revertants were counted after 3 days of incubation at 30\(^\circ\)C.
tivation system. The association of the repair and mutagenesis deficiencies of the umuC mutant suggests that both the effects on mutagenesis and on recovery from UV damage may result from a single activity of the umuC protein.

Finally, it is interesting to note that the remaining component of W reactivation in a umuC mutant is abolished by the introduction of a recA mutation (21). The recA protein functions in the major excision repair pathway and, like the umuC gene product, is induced by DNA damage in a recA^lexA^-dependent fashion (9, 10).

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