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Genetics

Role of RecA protein in untargeted UV mutagenesis of bacteriophage λ: Evidence for the requirement for the *dinB* gene

(SOS mutagenesis/UV damage/error-prone replication)

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ABSTRACT **Untargeted UV mutagenesis of bacteriophage λ—i.e., the increased recovery of λ mutants when unirradiated λ infects UV-irradiated *Escherichia coli*—is thought to be mediated by a transient decrease in DNA replication fidelity, generating mutations in the newly synthesized strands. Using the bacteriophage λ *cI857* → λ *c* mutation system, we provide evidence that the RecA protein, shown previously to be required for this mutagenic pathway, is no longer needed when the LexA protein is inactivated by mutation. We suggest that the error-prone DNA replication responsible for UV-induced untargeted mutagenesis is turned on by the presence of replication-blocking lesions in the host cell DNA and that the RecA protein is required only to derepress the relevant *din* gene(s). This is in contrast to mutagenesis of irradiated bacteria or irradiated phage λ, in which activated RecA* protein has a second role in mutagenesis in addition to the cleavage of the LexA protein. Among the tested *din* genes, the *dinB* gene product (in addition to the *uvrA* and *uvrB* gene products) was found to be required for untargeted mutagenesis of bacteriophage λ. To our knowledge, a phenotype associated with the *dinB* gene has not been reported previously.**

UV radiation generates mutations in *Escherichia coli* and in some of its phages by means of an inducible error-prone pathway (SOS repair) controlled by the *recA* and *lexA* genes (1–3). It has been proposed that mutagenesis in SOS-induced cells is mediated by a transient inducible decrease in fidelity of the replication complex that facilitates error-prone *trans*-lesion synthesis and generates mutations that are both targeted (at the site of UV photoproducts) and untargeted (at undamaged DNA sites) (4, 5). Genetic and DNA sequencing studies support the distinction between these two types of SOS-induced mutations in phages (6–11) and in bacteria, in which targeted mutations seem to predominate (refs. 5, 12, 13, and references therein). Bacteriophage λ has been used as a probe for cellular mutagenic processes induced by DNA-damaging agents (14) and has permitted the detection of the inducible cellular mutator effect (SOS mutator effect), which generates mutations in undamaged DNA (untargeted mutagenesis; refs. 15, 6, 16).

It has been shown that mutations within the *umuC* and *umuD* genes result in a considerable reduction in the level of radiation- (UV and ionizing) induced mutations in bacteria (17). In phage λ, mutagenesis of unirradiated phage induced by UV irradiation of the host cell prior to infection (i.e., untargeted mutagenesis) has been shown to be independent of the *umuC* function (9, 18), whereas mutations within the *umuC* gene affect Weigle reactivation (19, 20) and mutagenesis of irradiated phage (i.e., targeted mutagenesis) (18, 20, 21). Although the exact role of UmuD and UmuC proteins in SOS mutagenesis is not yet known, it has been speculated that the *umuD/C* function is required for the elongation of DNA synthesis past noncoding lesions in damaged DNA (18, 20, 22).

Certain mutations within the *recA* and *lexA* genes can suppress targeted and untargeted mutagenesis in bacterial and phage DNA (6, 23, 24). DNA damage induces a modification of the RecA protein into an activated form (RecA*) that promotes proteolytic cleavage of the LexA protein, the repressor of several DNA damage-inducible genes (*din* genes), including *umuC* and *umuD*. Activation of RecA and cleavage of LexA are manifested in the induction of the SOS response. Two *recA* alleles, *recA441* (at 42°C) (25, 26) and *recA730* (27), express SOS functions in the absence of any DNA-damaging treatment. Strains carrying *recA441* (at 42°C) or *recA730* have an elevated spontaneous mutation rate that is *umuD/C*-dependent (28, 29).

Some mutations in the *lexA* gene inactivate the LexA repressor (30), leading to constitutive expression of the *din* genes (31). In *recA*$^+$ strains, the presence of such a defective *lexA* [*lexA*(Def)] allele does not result in high spontaneous mutability of phage or bacteria, indicating that the derepression of *umuD/C* and the other *din* genes is itself not sufficient for the expression of SOS mutagenesis; activation of RecA protein is still required (21, 32). This observation and other genetic data suggest that, in addition to its regulatory role, the RecA* protein has another role in SOS mutagenesis (21, 29, 30, 32). It has been suggested that this second role of RecA* is to reduce the fidelity of DNA replication, resulting in the SOS mutator effect (29, 32, 33).

Using unirradiated phage λ as a probe for SOS-induced cellular mutator activity, we show here that untargeted mutagenesis of phage λ can occur in the absence of RecA protein and that its expression requires damage to the host cell DNA and derepression of *din* gene(s) other than *umuD/C*. Consequently, we investigated several *din* genes, whose functions are as yet unidentified, and found that a mutation in the *dinB* gene (34) suppresses untargeted mutagenesis of phage λ.

MATERIALS AND METHODS

Strains. *E. coli* K-12 derivatives are listed in Table 1. The presence of the *lexA51* mutation, formerly called *spr-51* (30), was verified by determining the level of RecA protein by $NaDodSO_4$/polyacrylamide gel electrophoresis (10%) (40). Note that all strains with *lexA51* also carry *lexA3* (30). The absence of RecA protein in strains carrying the del(*recA-srl*)306 mutation was checked by P. L. Moreau (Laboratoire d'Enzymologie, Gif sur Yvette, France) with rabbit anti-RecA serum using the nitrocellulose electrophoretic blotting method described by Moreau and Roberts (41). Stocks of λ *cI857* (42) with low background of spontaneous *c* mutants were made by heat induction of C600 lysogens.

UV Irradiation, Phage Assay, and Measurement of λ Clear-Plaque Mutation Frequency. These procedures were performed as described (36), with the most important points

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Table 1. Strains used

Strain	Relevant genotype	Source or ref.
AB1157	*recA*$^+$, *lexA*$^+$	35
PC10	As AB1157 but *leu*$^+$	36
AB2494	*recA*$^+$, *lexA1*	35
DM1420	*sfiA*, *recA*$^+$, *lexA3*, *lexA51*	30
PCR10	As PC10; also *recA1*, *srl*::Tn*10*	36
ABL306	As PC10; also del(*srlR-recA*)306, *srlR301*::Tn*10*	P1 transduction of del(*srl-recA*)306 from JC10289 (37) in PC10
ABL5101	As DM1420; also *recA1*, *srl*::Tn*10*	P1 transduction of *recA1* from PCR10 in DM1420
ABL5143	As DM1420; also *recA430*, *srl300*::Tn*10*	P1 transduction of *recA430* from IC373 (38) in DM1420
ABL5136	As DM1420; also del(*srlR-recA*)306, *srlR301*::Tn*10*	P1 transduction of del(*srl-recA*)306 from JC10289 (37) in DM1420
JC3890	del(*uvrB*, *chl*$^-$, *bio*$^-$, *phr*$^-$, *pgi*$^-$)301	39
GW1010	*dinA1*::Mud(Ap^r, *lac*)	34
GW1030	*dinB1*::Mud(Ap^r, *lac*)	34
GW1040	*dinD1*::Mud(Ap^r, *lac*)	34
GW1070	*dinF1*::Mud(Ap^r, *lac*)	34

being that (*i*) host bacteria were grown in a rich liquid medium, "869," containing bactotryptone (10 g/liter), yeast extract (5 g/liter) and NaCl (5 g/liter) and (*ii*) the plates were incubated 48 hr at 32°C. Infective centers were detected on a lawn of JC3890 (39). Mixed plaques containing *c* and *c*$^+$ phages can be detected easily under the following conditions: 0.2 ml of a saturated culture of the indicator strain is added to infective centers (maximum, 20,000) and plated with 2.5 ml of top agar on wet and well-filled plates. Plates and top agar are standard tryptone broth media described by Kaiser (43).

The results are an average of at least three experiments. Single burst experiments were done as described (44).

RESULTS

To investigate the role of RecA protein in untargeted mutagenesis of phage λ, we used the forward mutation system in the immunity control region of phage λ, resulting in a clear-plaque phenotype. The advantages of the forward mutation system $c^+ \rightarrow c$ (in particular, the use of the thermosensitive *cI857* mutation) to measure targeted and untargeted induced mutagenesis have been discussed (16). The clear-plaque mutation assay in phage λ has revealed a qualitative difference in phage mutant bursts produced by UV-irradiated host bacteria depending on whether the infecting phage has been irradiated or not (6, 9, 18). For damaged phage, the clear plaques result from bursts of phage containing predominantly or exclusively mutant phage (mixed and pure bursts). In contrast, when the phage are intact, the clear plaques detected result only from bursts containing predominantly wild-type phage, with a small number of mutant phage (mixed burst).

RecA Control of Untargeted Mutagenesis of Phage λ. Untargeted mutagenesis induced in unirradiated phage λ by preirradiation of the host cell appears to depend on *recA* and *lexA* genes (6, 16). Indeed, UV-irradiated *lexA*(Ind$^-$), *recA1*, or del(*srl-recA*)306 mutant strains show very little untargeted mutagenesis (Fig. 1). The plating efficiency and the phage yield of unirradiated phage λ are not affected differently in irradiated *lexA*(Ind$^-$), *recA*, and *recA*$^+$ strains, although the former are more UV-sensitive (ref. 44; data not shown).

Untargeted Mutagenesis of Phage λ in *lexA*(Def) Mutants Does Not Require RecA Protein. As is the case for spontaneous mutagenesis in bacteria, the spontaneous clear-plaque mutation frequency of phage λ was identical in a wild-type strain and in a *lexA51*(Def) mutant strain that lacks functional LexA protein (30), indicating that derepression of *din* genes in unirradiated host cells is not sufficient by itself for the generation of untargeted mutations in phage λ. However, in UV-irradiated hosts, the level of untargeted mutagenesis is enhanced in a *recA*$^+$, *lexA*(Def) strain relative to the *recA*$^+$, *lexA*$^+$ parental strain (Fig. 1).

Surprisingly, we found that UV-induced untargeted mutagenesis of phage λ is expressed in a del(*srl-recA*) strain at the same level as in a *recA*$^+$, *lexA*$^+$ strain when the *recA* deletion is associated with the *lexA51*(Def) mutations (Fig. 1).

It is unlikely that the difference observed in the mutation frequencies of unirradiated phage λ replicating in irradiated del(*srl-recA*) and *lexA*(Def) del(*srl-recA*) strains arises from a difference in phage production. The phage yield and mean

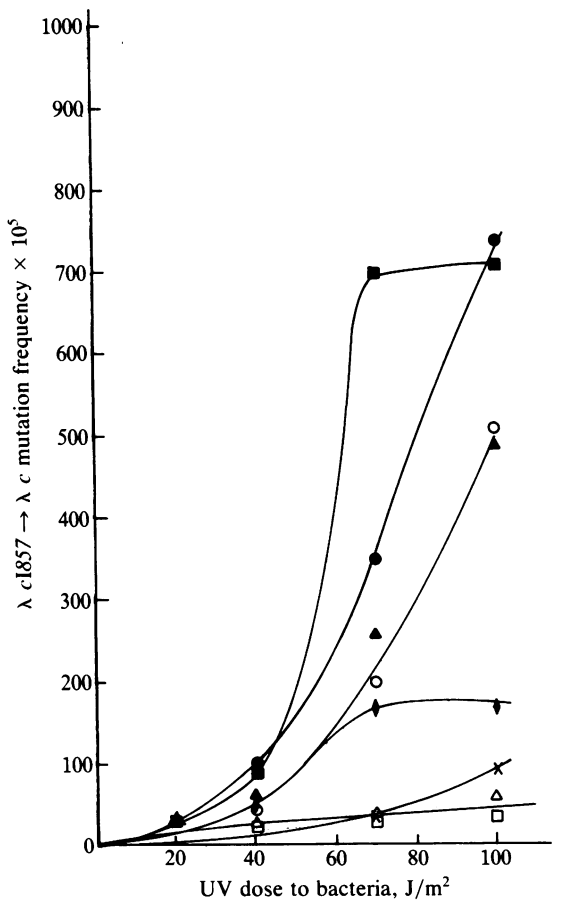

FIG. 1. Influence of different *recA* alleles on mutagenesis of unirradiated phage λ induced in UV-irradiated *lexA*$^+$ (open symbols) and *lexA*(Def) (closed symbols) host bacteria. The abscissa shows UV doses given to bacteria before infection. The ordinate shows the frequency of infective centers containing clear-plaque mutants. Relevant genotypes of host bacteria are for PC10, *recA*$^+$, *lexA*$^+$ (○); for ABL306, del(*srl-recA*)306 (△); for PCR10, *recA1* (□); for DM1420, *lexA51*, *recA*$^+$ (●); for ABL5136, *lexA51* del(*srl-recA*)306 (▲); for ABL5101, *lexA51*, *recA1* (■); for ABL5143, *lexA51*, *recA430* (♦); for AB2494, *lexA1*, *recA*$^+$ (×).

burst size were similar in the two irradiated strains (data not shown). Moreover, it has been shown that there is little dependence on the burst size of UV-induced untargeted mutation frequency in phage λ (6). These results suggest that untargeted mutagenesis of phage λ no longer requires RecA protein when the LexA protein is inactivated by mutation. This is in contrast to targeted mutagenesis of phage λ [ref. 21 and our results (not shown)] and to bacterial mutagenesis (29, 32), where activated RecA* protein has a second function in addition to LexA cleavage.

We observed no increase of pure bursts of clear-plaque mutants when UV-irradiated phage λ infected a UV-irradiated *lexA*(Def) del(*srl-recA*) strain. However, an increase in mixed bursts was observed that reaches the level observed for untargeted mutagenesis (data not shown). Therefore, as with *umuC* mutant hosts (18), the nature of mutagenesis of irradiated phage λ in irradiated *lexA*(Def) del(*srl-recA*)306 is not the same as that found in the isogenic *recA*$^+$, *lexA*$^+$ strain.

In the same *lexA51*(Def) background, the *recA1* and *recA430* mutations affect the extent of untargeted mutagenesis of λ differently relative to *recA*$^+$ and *recA* deletion strains (Fig. 1). The *recA430* mutation (formerly, *lexB30*), which renders the RecA protein partially deficient in LexA cleavage without affecting its activity in DNA recombination (45, 46), has an inhibitory effect on the expression of untargeted mutagenesis of λ in a *lexA*(Def) strain. In contrast, the *recA1* mutation, which suppresses recombination and protease activity of RecA protein (47, 48), appears to enhance untargeted mutagenesis (Fig. 1). These results suggest that the only essential role of RecA protein in untargeted mutagenesis of λ is to derepress *din* genes that are required, together with damage in the host DNA, to induce untargeted mutagenesis in phage λ DNA. The RecA protein, although dispensable, can influence the error-prone synthesis leading to untargeted mutagenesis.

Dependence of Untargeted Mutagenesis of Phage λ on the *dinB* Gene. We have investigated the effect of several *din* mutations on untargeted mutagenesis of λ and found that an insertion mutation within the *dinB* gene inhibits the expression of untargeted mutations of phage λ (Fig. 2). This inhibition was observed by measuring either forward mutagenesis (Fig. 2) or the reversion of an amber mutation λ *Ram221* → λ R^+ (data not shown). The failure to increase the mutant yield in unirradiated phage λ after UV irradiation of the *dinB1* strain does not reflect a defect in λ growth under these conditions: the plating efficiency and burst size (measured in single burst experiments) were the same in the *dinB1*, *dinA1*, and wild-type strains (data not shown). However, *dinB1* deficiency does not prevent UV-induced mutagenesis in bacteria or mutagenesis in irradiated phage λ (data not shown).

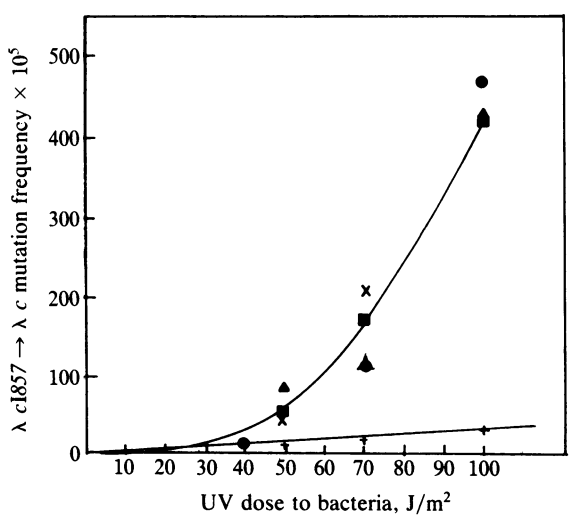

FIG. 2. Influence of insertion mutations in *din* genes on UV-induced untargeted mutagenesis of phage λ. ●, AB1157; ■, *dinA1*::Mud; +, *dinB1*::Mud; ▲, *dinD1*::Mud; ×, *dinF1*::Mud. The plating efficiency in *din* mutants is the same as in the wild-type strain.

DISCUSSION

We have shown that induction of untargeted mutagenesis in unirradiated λ phage by UV irradiation of the host bacteria prior to infection no longer requires the RecA protein when the LexA repressor is inactivated by mutation. Moreover, our results indicate that among the functions controlled by the LexA repressor, the *dinB* gene product is needed for untargeted mutagenesis in phage λ DNA. To our knowledge, a phenotype associated with the *dinB* gene has not been reported previously. The fact that some UV-induced SOS mutations can occur in the absence of RecA protein is in contrast to other data on SOS-induced mutagenesis in bacteria (29, 32) and in irradiated phage λ [ref. 21 and our results (not shown)] showing that activated RecA* has a second function in addition to the cleavage of LexA product.

What is the biochemical nature of untargeted mutagenesis of phage λ? Is it the manifestation of the SOS error-prone replication that has been postulated to be induced in damaged cells or is it a secondary effect of DNA damage? Sequencing of forward mutations has shown that a large proportion of the untargeted mutagenic events in phage λ and M13 are frameshifts (9, 11). It was suggested (9) that these untargeted frameshift mutations do not depend on the SOS system but on some other secondary effect of irradiation. However, our results support the hypothesis that untargeted mutagenesis of phage λ is part of SOS mutagenesis since it requires function(s) under the control of the LexA repressor.

The genetic requirements for the SOS mutator effect when generated by the presence of proteolytically active *recA* alleles (*recA730* or *recA441* at 42°C in the presence of adenine) are more similar to requirements for targeted UV mutagenesis than to those for untargeted UV mutagenesis of phage λ. Spontaneous bacterial mutations in *recA730* and *recA441* (at 42°C) are *umuC*-dependent (28, 29) and require a second role of RecA* protein. These conditions of activation of the SOS functions increase, hardly, if at all, spontaneous mutagenesis in phage λ (refs. 9, 21; P. Caillet-Fauquet and G.M.-M., unpublished data). Moreover, the *recA441*-mediated spontaneous mutagenesis has a strong specificity (49) that is very different from UV-induced untargeted mutations in phage λ or M13 (9, 11). Therefore, the UV-induced untargeted mutagenesis of phage λ may be different from the genetically (*recA441* or *recA730*) induced mutator effect. One can suggest (2, 49) that the mutator effect in *recA441* or *recA730* is actually targeted and reflects mutagenic repair of cryptic lesions that have arisen in bacterial DNA through cellular metabolism. These cryptic lesions would not induce error-prone replication but would be converted into mutations by the SOS system.

The fact that mutagenesis of unirradiated phage λ is still increased by UV irradiation of *recA730* or *recA441* hosts indicates that in the absence of DNA damage, the SOS mutagenic functions are not fully expressed in these two *recA* mutants (refs. 9, 21; P. Caillet-Fauquet and G.M.-M., unpublished data). This observation and results presented in this paper suggest that DNA damage, in addition to promoting the proteolytic activity of RecA protein, has a second function in the activation of the error-prone replication responsible for untargeted mutagenesis of phage λ. Other observations also support this notion—e.g., excision repair is necessary for untargeted mutagenesis in phage λ (50) but not in single-stranded DNA phage φX174 or M13 (G.M.-M. and

P. Caillet-Fauquet, unpublished data). This suggests that the processing of UV photoproducts by excision repair in host cell DNA produces an effector for the generation of untargeted mutagenesis in phage λ DNA. The *dinB1* mutation, like the *uvrA* and *uvrB* mutations, suppresses untargeted mutagenesis but not targeted mutagenesis of phage λ. Therefore, the *dinB* gene product might be involved in the activation of untargeted mutagenesis, similarly to excision repair.

Is the same mechanism involved in the production of targeted and untargeted mutations in phage λ? Untargeted mutagenesis of phage λ differs from targeted mutagenesis in several ways. (*i*) It has different genetic requirements; untargeted mutagenesis is suppressed specifically by several mutations [e.g., *polA* (6), *uvrA/B/C* (ref. 50; G.M.-M. and P. Caillet-Fauquet, unpublished results), *dinB* (this paper)] and it is expressed normally in *umuC* mutants (9, 18). (*ii*) RecA protein is dispensable for its expression if the LexA repressor is eliminated by mutation. (*iii*) It is susceptible to mismatch repair (16). (*iv*) The size of mutant bursts is much smaller (6, 9, 18). (*v*) It has a different mutation spectrum (9, 11). However, the difference in the genetic requirements depending on the presence or absence of damage in the target DNA could reflect different pathways of activation of the same SOS mutagenic function. It is difficult at present to assess whether untargeted and targeted mutagenesis are produced by different SOS mutagenic activities or whether there is a common mechanism for the fixation of both types of mutations but with different limiting steps.

The fact that untargeted mutations are susceptible to mismatch repair has suggested that they arise in undamaged λ DNA as replication errors introduced in the newly synthesized strand (16). A transiently induced low-fidelity replication of DNA has been postulated to be induced by the presence of lesions in the DNA and to be required for the bypass of blocking lesions (4, 5). Moreover, DNA polymerase III has been implicated in targeted mutagenesis in bacterial DNA (51) and untargeted mutagenesis in phage λ DNA (36). Three steps might be limiting for mutagenic bypass of blocking lesions: (*i*) misincorporation opposite damaged bases; (*ii*) inhibition of the exonucleolytic removal of the newly incorporated mismatched base; and (*iii*) elongation of the newly synthesized DNA chain past the damage in the template strand. A two-step model for UV mutagenesis in *E. coli* DNA was proposed recently (33): (*i*) misincorporation opposite a photoproduct, which can be mediated directly by RecA* protein, and (*ii*) bypass of blocking lesions, which requires $umuD^+$ and $umuC^+$ alleles. In phage λ DNA, it has been demonstrated that untargeted mutations can occur in the absence of RecA protein (this paper) and of the *umuC/D* functions (18), whereas mutagenesis on damaged DNA requires a second role of RecA* protein and the *umuC/D* functions (21). These results suggest that these gene products are no longer needed for the first misincorporation step but are only needed for the second and the third steps mentioned above. However, to draw this conclusion, we have to assume (*i*) that the same misincorporation function generates targeted and untargeted mutations—i.e., misincorporation opposite damaged and undamaged bases—and (*ii*) that the unirradiated phage λ mutagenesis observed in irradiated *recA*, *lexA*(Def) and *umuC* mutants has the same biochemical basis as that observed in the $recA^+$, $lexA^+$ host.

In summary, our results indicate that SOS-induced error-prone replication can operate in UV-irradiated cells in the absence of RecA protein if the LexA repressor is inactivated by mutation. RecA protein, although dispensable, can influence the error-prone DNA synthesis leading to untargeted mutagenesis. To be detected in unirradiated phage λ DNA, untargeted mutagenesis requires a damaged host replicon and some of the *din* gene products (*uvrA,B* and *dinB*). Our results also suggest that untargeted mutagenesis of phage λ is different from the mutagenic function mediated by the *recA441* and *recA730* alleles.

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