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Genetics

UV-induced mutagenesis of phage S13 can occur in the absence of the RecA and UmuC proteins of *Escherichia coli*

(mutagenic repair/Weigle repair/photorepair/SOS system)

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ABSTRACT **The UV-induced mutagenesis of phage S13 that accompanies Weigle repair is known to require the products of the *recA* and *umuDC* genes, as does the UV-induced mutagenesis of the *Escherichia coli* chromosome. I found that UV-induced mutagenesis of phage S13 occurred in the absence of both the RecA and UmuC functions when the irradiated phage was photoreactivated. Furthermore, UV-induced phage mutations were produced in a *recA*$^-$ *umuC*$^-$ cell even without photoreactivation and in the absence of any other known UV repair mechanism, at a frequency 29% of that found after photoreactivation and 7% of that found after Weigle repair, implying that DNA synthesis can proceed past a dimer at an unexpectedly high frequency even when unaided by the UmuC–RecA SOS repair functions. The unaided DNA synthesis appears capable of producing mutations in the vicinity of a pyrimidine dimer; by aiding synthesis past a dimer, a repair mechanism may disclose a mutation without having any active role in producing it.**

UV-induced mutagenesis is generally considered to result from mutagenic repair of UV-damaged DNA. It has been assumed that some aspect of the repair process is directly responsible for producing mutations, an assumption that is reflected in the often-used term "error-prone repair."

The mutagenic repair process in *Escherichia coli* that functions in the dark requires the expression of the *recA* and *umuDC* genes; these are part of the SOS system, a set of genes repressed by the LexA protein and induced by damage to DNA (1). In response to a signal caused by DNA damage, the RecA protein is activated to a protease state, in which condition it plays two roles in mutagenic repair: the first is an indirect role, namely the induction of all the SOS genes, including the *umuDC* genes; the second is a direct involvement, observed when the SOS system is derepressed, of some aspect of RecA protease function in the repair process (1, 2). The recombinase function of RecA is not needed for mutagenic repair of *E. coli* (3) and phage (unpublished data) DNA.

In the case of UV-damaged phage, for which mutagenic repair was first discovered (4), the phenomenon is called Weigle reactivation (or W-repair) and Weigle mutagenesis. W-repair, accompanied by mutations, occurs in phage S13 (5) and the mutagenic specificity has been partly determined (6). The dependence on the *recA* and *umuDC* genes has been demonstrated for W-repair of phage S13 (unpublished data) as well as for repair of apurinic sites (7). The experiments described here deal entirely with this phage. Because phage S13 is single-stranded, neither excision repair nor recombinational repair can occur in single infection with UV-irradiated phage. In the dark only W-repair is known to repair the UV damages to the phage; in the light there is in addition photoreactivation.

The present work is a study of UV-induced mutagenesis in the absence of the RecA and UmuC functions. While this work was in progress, Bridges and Woodgate (8) showed that UmuC was not always required for UV-induced mutations of *E. coli*. The present work goes beyond this in two respects. It will be shown that mutants are produced not only in the absence of UmuC function but also in the absence of RecA function. It will be shown further that UV-induced mutants can be produced without the assistance of any known repair function, a result that implies that the DNA replication complex can replicate past a UV lesion unassisted.

MATERIALS AND METHODS

Bacteria and Phage. The bacterial strains described in the tables were all derived from the temperature-resistant *E. coli* K-12 strain IT1819 [*recA441 sulA dinD*::Mud(*lac*) S13^s], a derivative of GW1000 (9). Mutant combinations were constructed by phage P1 transduction. Cotransduction with *srl*::Tn*10* was used to introduce *recA*$^+$ (from strain DB4243) and Δ*recA* (10). Transduction of Tn*5* was used to introduce *umuC122*::Tn*5* (11) and *lexA*(Def)*71*::Tn*5* (ref. 12; Def = defective). The presence of the *umuC* mutation was confirmed by the increased sensitivity to UV light and the absence of W-repair (13). The presence of the *lexA*(Def) mutation was confirmed by the derepression of the *lac* gene that was fused to *dinD* (9). The formation of IT1874 (Δ*recA umuC*) by the introduction of Δ*recA* into IT1872 (*recA*$^+$ *umuC*) was confirmed by the substantial increase in UV sensitivity.

The same lysate of phage S13 was used for all of the experiments; the lysate was made by inoculation of *E. coli* C (AP1) with the contents of a single plaque. Phage were assayed on plates containing ≈30 ml of salt-free L agar (5) and a 2.5-ml top layer of 0.9% nutrient agar. In the photoreactivation experiments (see Table 1), the phage were plated for plaques at 33°C; in the dark experiment (see Table 3), the phage were plated at 30°C. Temperature sensitivity was determined at 43°C.

UV Inactivation and Avoidance of a Watch Glass Artifact. A suspension of phage S13 in 0.05 M ammonium acetate (pH 7.0) was exposed to UV light from a 15-W germicidal lamp while being shaken in a watch glass at room temperature. Inactivation of phage S13 is exponential for at least 10 orders of magnitude provided special precautions are taken to overcome an apparent shielding effect that has been inferred to arise from microscopic imperfections on the surface of the watch glass, which allows amounts of the small virus to be shielded from the UV light by the glass (unpublished data). If the virus suspension remains in the same watch glass throughout the irradiation, the survival levels off between

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Abbreviations: W-repair, Weigle reactivation or repair; ts, temperature-sensitive.

10^{-4} and 10^{-5}. This artifact was avoided by the following procedure: after each exposure of the virus to a dose that reduced the survival by roughly 2.5 orders of magnitude, the viral suspension was poured into a fresh watch glass, thereby leaving practically all of the shielded viruses behind. A dose of 22 J/m^2 reduced survival by 1 order of magnitude.

Photoreactivation. The irradiated phage S13 were plated with each of the several bacterial strains as indicator, and the agar plates were incubated at 33°C overnight 50 cm beneath a bank of six 15-W white fluorescent bulbs (F15T8/W) screened by 0.25 inch (0.63 cm) plate glass. The photoreactivable sector P_s is defined as

$$P_s = 1 - \log S_l / \log S_d$$

where S_l and S_d are the fractions of irradiated viruses surviving when plated in the light and in the dark, respectively (14). P_s is the fraction of lethal UV hits that are photorepaired. A repair sector W_s can be defined for W-repair that is strictly analogous to P_s.

Scoring for Temperature-Sensitive Mutants. Phage were plated with 4×10^8 logarithmic-phase cells at permissive temperatures to give ≈40 plaques per plate. Therefore, the multiplicity of infection was 5 for $S = 2 \times 10^{-8}$ and 0.010 for $S = 1 \times 10^{-5}$. Individual plaques that formed at permissive temperatures were picked and distributed in a dilute broth solution into the 96 wells of a microtiter plate. Agar plates overlaid with *E. coli* C indicator were each spotted with samples from 48 wells that were all transferred at one time by a 48-pin stamp. One set of plates was incubated at 43°C to test for temperature sensitivity, and a duplicate control set was incubated at 30°C. Because phage S13 can form plaques in less than 3 hr at 37°C, it was necessary to heat the high-temperature plates to 43°C rapidly; otherwise, the temperature-sensitive mutants would have had time to grow at the intermediate temperatures. Rapid heating was accomplished by placing the plates in a 55°C incubator for 30 min before transfer to 43°C.

The phage were scored as temperature sensitive (ts) if the spots at 43°C were absent or were very faint. To avoid any bias, the plates were scored "blind." Each experiment involved a comparison of "light" versus "dark" (see Table 1) or "irradiated" versus "nonirradiated" (see Table 3). The two comparison sets of spot tests at 43°C were made on plates coded by random numbers. After the plates were spotted, they were arranged in numerical order and were scored after incubation at high temperature by another person with no access to the plate code.

The effective temperature at which the spots developed could be compared in a rough way from one set of experiments to another by the size of the plaques within the spots. Although the spots contained confluent plaques, individual plaques could be seen around the periphery of a spot. The smaller the size of the average plaque, the higher the effective temperature to which the plates were exposed.

RESULTS AND DISCUSSION

The following experiments grew out of an effort to test the photoreactivability of UV lesions in phage S13 that are involved in mutagenic W-repair. In these experiments it was observed that photoreactivation itself, under conditions where W-repair could not occur, resulted in a higher mutation frequency than when the UV-irradiated phage were plated in the dark. Mutants were scored by their ts phenotype at 43°C. When irradiated phage were plated on the unirradiated host strain IT1870 (*lexA*$^+$ *recA*$^+$ *umuD*$^+$ *umuC*$^+$), the plates that were incubated in the light yielded a higher frequency of ts mutations than those incubated in the dark (Table 1). Because of the presence in the host strain of the LexA repressor of the SOS system and the absence of activated RecA protein that could cleave the repressor, there should have been no induction of the SOS system and consequently no W-repair. It is known, however, that mere infection with a single-stranded virus can activate RecA protein slightly (15); a small amount of W-repair might occur as a result. This possibility was eliminated in strain EST1779; despite deletion of the *recA* gene, the mutagenic effect remained the same, proving that the mutagenic process does not depend in any way on the RecA protein. In this strain the RecA protein was unavailable for cleavage of the LexA repressor and the consequent induction of *umuD umuC*; it was also unavailable for its other (direct) role in W-repair.

Table 1. Mutation frequency of UV-irradiated phage S13 plated in the light and in the dark

Cell strain	Strain number	Mutation frequency*	
		Light	Dark
lexA$^+$ *recA*$^+$ *umuC*$^+$	IT1870	0.063 ± 0.011 (33/524)	0.024 ± 0.007 (14/575)
lexA$^+$ Δ*recA* *umuC*$^+$	EST1779	0.060 ± 0.013 (23/382)	0.005 ± 0.004 (2/384)
lexA$^+$ *recA*$^+$ *umuC*	IT1872	0.044 ± 0.009 (23/528)	0.021 ± 0.006 (12/575)
lexA$^+$ Δ*recA* *umuC*	IT1874	0.058 ± 0.013 (21/357)	0.010 ± 0.004 (6/575)
lexA(Def) Δ*recA* *umuC*$^+$	IT1865	0.056 ± 0.010 (35/621)	0.017 ± 0.006 (8/474)
Average		0.056 ± 0.005 (135/2412)	0.016 ± 0.003 (42/2583)

The virus was irradiated as described until the surviving fraction, S, was reduced in the dark to 3×10^{-8}. The photoreactivable sector, P_s, was 0.20 ± 0.02, implying that one-fifth of the lethal damages were repaired in the light. The permissive temperature for plaque development was 33°C; ts mutants were scored by inability to grow at 43°C. Light treatment of unirradiated phage infecting strain IT1874 was not mutagenic (0 of 354 mutants).

*Each parenthesis contains the actual number of mutants counted divided by the total number of viral plaques screened.

That UV mutagenesis of phage S13 did not require a functional *umuC* gene was shown by the result found for IT1872, a result also obtained for mutagenesis of *E. coli* by Bridges and Woodgate (8). I also ruled out the possibility that the *umuC* and *recA* genes could substitute for each other in the mutagenic process by showing that there was an undiminished mutagenic effect on irradiated phage S13 in the *recA umuC* double mutant (IT1874).

In the last case (IT1865) in Table 1, although the *lexA*(Def) mutation derepressed the SOS genes, W-repair of phage S13 still could not occur because it is absolutely dependent on the second role of the RecA protease (unpublished data).

The results in the light for the several strains were consistent and averaged overall to a mutation frequency of 0.056 ± 0.005, which was 3.5 ± 0.7 times the mutation frequency in the dark (and the latter frequency will be shown below to be still much higher than the spontaneous mutation frequency of unirradiated phage). The probability that the difference in overall mutation frequency between light- and dark-treated viruses could have occurred purely by chance is less than 10^{-10}. Table 2 provides a comparison of the mutation frequency accompanying photorepair with that accompanying W-repair; in terms of mutations per repaired lesion, which is a specific frequency, photorepair is roughly one-fourth as effective as W-repair. Photorepair by itself should not be mutagenic because it does not direct replication of the complementary strand but simply monomerizes a cyclobutane dimer. Light treatment alone is not mutagenic (see the legend to Table 1). The elevated mutation frequency

Table 2. Comparison of photorepair and W-repair of UV-irradiated phage S13

	UV survival	Repair sector	Lesions repaired per virus	ts mutations per virus*	Mutations per lesion repaired
Photorepair	3×10^{-8}	0.20 ± 0.02	3.5 ± 0.4	0.058 ± 0.005	0.017 ± 0.002
W-repair	8×10^{-6}	0.18 ± 0.02	2.1 ± 0.2	0.150 ± 0.025	0.071 ± 0.014

Photorepair data from Table 1. W-repair (unpublished data) was obtained by plating on unirradiated strain EST1640, which contains *lexA*(Def)*71*::Tn*5 recA441*.
*Corrected for multiple mutations per virus.

that we observe after photoreactivation of irradiated phage S13 must be due to rescue of already mutated DNA. Therefore, the repair is postmutagenic.

What is the origin of the mutations for which neither the *umuC* nor the *recA* gene is needed? It is possible that before replication is completely halted by the UV lesion, a noncomplementary base is inserted opposite the distorted DNA template. If the subsequent replication block persists, then the mutant will not survive. When the dimer is repaired by photoreactivation, the replication block is removed and the mutation, already in existence, is disclosed.

It will now be seen that the replication block can often be overcome even in the absence of known repair mechanisms. That was examined by measuring the ts mutation frequency of irradiated phage S13 that had been plated on strain IT1874 in the dark. For comparison, plaques from unirradiated phage S13 were picked at the same time and scored for ts mutants at the elevated temperature together with survivors of irradiated viruses. The irradiated phage S13 showed highly significant increases in the frequency of ts mutations relative to the unirradiated control (Table 3); the probability that the differences between the irradiated and unirradiated viruses could have occurred purely by chance is $\approx 2 \times 10^{-4}$ and $\approx 1 \times 10^{-2}$ for the results, respectively. Therefore, mutations were produced and disclosed without the benefit of any known repair mechanism.

If we return to Table 1, we can now appreciate the implication of the fact that the mutation frequency in the dark was 0.29 ± 0.06 times that in the light. Since Table 3 shows that the dark value was well above the spontaneous control, it follows that replication proceeded successfully past a dimer without the need of repair in roughly 30% of the cases in which mutations accompanied photorepair and in roughly 7% of the cases (compare Table 2) in which mutations accompanied W-repair.

Technique can affect the measured mutation frequencies. The mutation frequency of irradiated phage S13 incubated in the dark was 0.016 ± 0.003 in Table 1 and 0.0086 ± 0.0021 for the comparable UV fluence in Table 3. But comparisons are only meaningful when all plates are incubated together, as they were for each experiment represented by a separate line of Tables 1 and 3; that is essential to ensure nearly identical test conditions, particularly in regard to temperature. The mutation frequency is very sensitive to the temperature of the agar plates because the phenotype of phage mutants can change from ts to ts$^+$ by a drop of only 0.5°C in the incubator. The effective temperature of the plates depends on how rapidly they are heated because phage S13 plaques develop in <2.5 hr at temperatures >40°C. The water content of the plates (i.e., their freshness) and the crowdedness of the incubators change the rate of heating of the plates and, therefore, their sensitivity for detecting ts mutants. Under the crowded conditions of the huge experiments described in Table 3, individual plaques, which were often visible within the spots made by ts$^+$ phage at the high temperature, were larger than usual, indicating that the scoring for ts mutants occurred at a lower-than-usual temperature. That would account for the lower frequency in the dark of UV-induced mutants in Table 3 compared with those in Table 1.

Table 3. Mutation frequency of UV-irradiated and nonirradiated phage S13 in the *lexA*$^+$ Δ*recA umuC* mutant host IT1874 in the dark

	Mutation frequency	
S	UV-irradiated	Nonirradiated
2×10^{-8}	0.0086 ± 0.0021 (17/1965)	0.00056 ± 0.00056 (1/1775)
1×10^{-5}	0.0040 ± 0.0012 (12/2973)	0.00067 ± 0.00048 (2/2973)

To eliminate the possibility that the mutants found after UV irradiation might have been selected by resistance to UV, 10 of those mutants, 5 from each group, were chosen randomly for study of their UV sensitivity. Mutants and wild type were irradiated under identical conditions ($S \cong 1 \times 10^{-5}$). The value of *S* for the mutants was 0.99 that of the wild type with a standard deviation of 0.09 for the distribution of the 10 mutants. The mutants were all found to be phenotypically distinct from each other as indicated by plaque morphologies on various indicators at different temperatures, implying that they are all different genotypically.

EXTENDED DISCUSSION

By having mutations in both the *umuC* and *recA* genes, it was possible to induce mutations in phage S13 under conditions where no mutagenic repair process is known to function. A potential contribution of genetic recombination to the repair process had to be ruled out because multiple infection of cells would provide a small chance for recombinational repair of the irradiated single-stranded phage DNA. The *recA* deletion mutation reduced even that chance by elimination of the primary recombination mechanism; but there is still a secondary mechanism of recombination that can function in *E. coli* in the absence of RecA activity (16). For UV-irradiated phage, however, the secondary mechanism does not work (17). That mechanism produces recombination only if gene *A* of phage S13 can function, which in turn requires supercoiled replicative-form DNA (18). Without some type of prior repair, the UV-inactivated phage cannot provide the replicative-form template needed for gene *A* to function; thus, recombinational repair is also eliminated as a factor in these experiments. For the lower dose shown in Table 3, the low multiplicity of infection also ruled out recombinational repair. Since no known repair function contributes to the mutations, what is usually termed mutagenic repair does not seem to be involved; even in the case of photorepair, the repair process appears to be postmutagenic rather than mutagenic.

The results here suggest that the DNA replication machinery of *E. coli*, unaided by repair mechanisms, can produce mutations when replicating dimer-containing DNA. The mutation frequency is roughly 7% of that found when there is a functioning W-repair mechanism, which requires the RecA, UmuC, and UmuD proteins. I suggest that the unaided replication complex proceeds with difficulty into the region of the dimer, occasionally producing mutations by inserting noncomplementary bases; usually, however, replication fails to continue completely past the dimer. One role of W-repair may be to disclose the presence of mutations by assisting the blocked replication complex to proceed past the dimer to

complete the complementary DNA strand, which then can produce viable progeny.

It might be argued that the mutations (Table 3) could conceivably have arisen in cells mixedly infected with UV-damaged and undamaged phage, where the UV-damaged phage might possibly stimulate untargeted mutagenesis (reviewed in ref. 1) of the undamaged phage. This mechanism appears unlikely for two reasons. First, in the case $S = 1 \times 10^{-5}$, the amount of mixed infection was too low to account for the mutation frequency. Second, the untargeted mutagenesis of phage S13 is *recA*-dependent (19) and so could not occur in strain IT1874.

It is unclear whether W-repair itself actively produces mutations. W-repair of phage S13 produced 4.2 ± 1.0 times more mutations per repaired lesion than did photorepair (Table 2). Although the two sets of experiments were not done together, W-repair appears to be more mutagenic than photorepair. Nevertheless, it cannot be concluded that W-repair contributes actively to the mutation process, in contrast to its merely disclosing the mutation, because there are at least three other explanations that might account for the higher mutation frequency observed for W-repair than for photorepair. They depend on apparent differences in the mechanism of photorepair and W-repair and on the assumption that the UV-induced mutations arise where replication is blocked.

The first explanation is that, when there are several UV dimers in the same DNA molecule, as would occur for the UV doses used in the experiments described here, the number of effective replication blocks should be greater with W-repair than with photorepair (Fig. 1). This is because all dimers are probably equally susceptible to photorepair, not just the dimer that is blocking replication at the moment. Accordingly, photorepair is shown in Fig. 1 to be capable of eliminating dimers in random order. There is no information on how W-repair works, but I assume it functions only at the lesion currently blocking replication (Fig. 1); the consequence of that assumption is that, for the same number of repairable

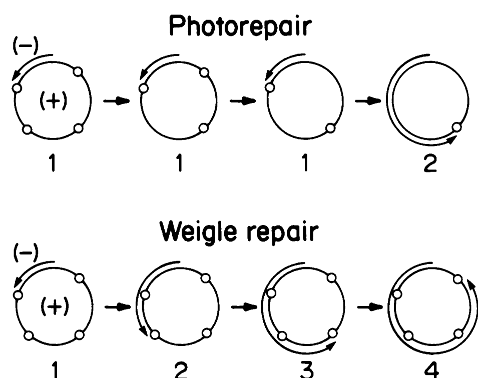

FIG. 1. Comparison of the number of replication blocks encountered in photorepair with the number in W-repair. In this example the phage S13 viral (+)-strand is shown with four repairable dimers (h = 4), each of which in turn will become a block to replication of the complementary (−)-strand, except for those dimers that get repaired while replication is stalled at an upstream dimer. The number under each circle is the total number of replication blocks encountered through that stage of replication. In the case of photorepair, the figure illustrates a fairly typical situation in which, by chance, replication is actually blocked at only two of the four dimers because of the random order of their repair. In contrast, in the case of W-repair, it is assumed that repair can only occur at the site that is currently blocking replication. Therefore, for W-repair each of the four dimers will block replication in succession. Whether W-repair actually eliminates the dimer is not critical to the analysis. (If, as shown in the figure, the dimer should remain after the (−)-strand replicates past the block, it could eventually be removed by excision repair.)

lesions per phage genome, W-repair must overcome more blocks to replication than does photorepair, with each replication block being a potential source of mutations.

Quantitatively, if B_h is the average number of blocks to replication when there are h repairable lethal hits (i.e., dimers) per phage genome, then for W-repair we have $B_h = h$, but for photorepair we have $B_h = 1 + 1/2 + 1/3 + \cdots + 1/h$. (The harmonic series for B_h was derived from a recursive formula described in the *Appendix*.) For the example $h = 4$, depicted in Fig. 1, the average number of replication blocks would be given by $B_4 = 2.1$ for photorepair, compared with $B_4 = 4$ for W-repair. In this case W-repair would give twice the mutation frequency of photorepair.

The second explanation follows from the picture of a replication complex struggling to proceed past a dimer in the absence of repair mechanisms. The further replication proceeds in the immediate vicinity of the dimer before being aided by a repair mechanism, the greater should be the chance of a mutation occurring. It is possible that W-repair may not occur until replication advances further, on the average, into the blocked site than is the case for photorepair, inasmuch as photorepair can occur independently of replication. That would provide an additional reason for W-repair to be more mutagenic than photorepair.

The third explanation is that a mutation will not necessarily be fixed if it is followed by photorepair because a mismatch would be created that could be eliminated by mismatch repair (20–22). The phage S13 sequence of Lau and Spencer (personal communication) contains one G-A-T-C *dam* methylation site; this implies that after photorepair the mutant (−)-strand would be corrected in more than half of the cases (23).

The results in Tables 1 and 3 show that there are at least three different conditions under which mutations may appear in UV-irradiated phage S13; the location of the mutations may be specific for each condition. (*i*) In the absence of repair, it is possible that an incorrect base can be inserted at any position opposite a distorted template during DNA synthesis. (*ii*) Mutations disclosed by photorepair would be expected to occur predominately on the 3′ side of a lesion if the eventual need for photorepair means that the unaided DNA synthesis could not have occurred already opposite the 5′ side. (*iii*) W-repair like photorepair, should passively disclose mutations on the 3′ side of a lesion; however, it is difficult to predict whether mutations should also be found on the 5′ side because so little is known about how W-repair works, particularly regarding its ability to contribute actively to the production of mutations.

It is not known at what exact place DNA synthesis is blocked relative to a DNA lesion or, indeed, if it is always blocked at the same place. Synthesis might even proceed past the lesion and still become blocked at the distal side because of the distorted template. A comparative analysis of the location relative to pyrimidine sequences of UV-induced mutations isolated by W-repair, photorepair, and without either type of repair would help to locate the block.

In the first analysis of the specificity of mutants induced by W-repair, Howard and Tessman (6) examined 16 induced ts mutants of phage S13. Eleven were C → T changes in the irradiated strand. The remaining 5 either were also C → T or were not single-base transitions. To explain this preference for C → T mutations, I have proposed a noninstructive mechanism of W-repair in which the purine adenine is often indiscriminately inserted at the growing end of the newly synthesized (−)-strand when replication is blocked by a dimer (24). The indiscriminate use of adenine could also explain how DNA synthesis bypasses apurinic sites (25). Since it now appears that repair acts at least in part by disclosing the mutagenic effects of unaided replication, the possibility that the unaided replication complex can add

adenine to the growing chain when blocked by a lesion should be examined. Preferential incorporation of adenine has been observed to occur during *in vitro* synthesis opposite apurinic/apyrimidinic sites (26).

I found here that UV-irradiated DNA could be mutated and, therefore, replicated without the assistance of known UV repair mechanisms. Mismatch repair, so far as it is understood, could play a role in fixing the mutations but not in their formation. While it is not possible to completely rule out undiscovered repair mechanisms, it would seem that the terms mutagenic repair and error-prone repair, though convenient labels, may be misnomers; it is even conceivable that W-repair would be found to be antimutagenic if the mutation frequency were considered only for those lesions for which replication is successful, which cannot yet be done for unaided replication. Since the detailed composition of the replication complex, including the proofreading as well as the polymerization components, must be critical in determining the kinds of mutations that are likely to occur, it is apparent that there are many opportunities for mutagenic specificities to vary according to the organism and type of UV lesion.

It is possible that some UV-induced mutations are actively produced by a repair mechanism, while others are only disclosed by a repair mechanism. It is widely thought that all UV-induced mutations are of the first type, when actually there is no evidence as yet that mutations of that type even exist.

APPENDIX

For photorepair of circular DNA containing h repairable lesions we can evaluate B_h, the average number of replication blocks, by introducing a related quantity b_h, defined as the average number of additional replication blocks that subsequently will be encountered after repair of the current replication block. Clearly,

$$B_h = 1 + b_h. \quad [1]$$

If the lesion that is currently blocking replication is the next one to be repaired then $b_h = B_{h-1}$; the probability of this occurring is $1/h$. If that lesion is not the next one to be repaired, then $b_h = b_{h-1}$; the probability of that occurring is $1 - 1/h$. Therefore,

$$b_h = (1/h)B_{h-1} + (1 - 1/h)b_{h-1}. \quad [2]$$

By substituting Eq. **1** into Eq. **2** and knowing that $b_1 = 0$, all values of b_h can be found, after which Eq. **1** yields the harmonic series for B_h.

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