ULTRAVIOLET MUTAGENESIS AND ITS REPAIR IN AN *ESCHERICHIA COLI* STRAIN CONTAINING A NONSENSE CODON

STANLEY PERSON, JOHN ALLEN McCLOSKEY, WALLACE SNIPES AND RICHARD C. BOCKRATH

Biophysics Department, The Pennsylvania State University, University Park, Pennsylvania 16802

and

Department of Microbiology, University of Indiana Medical School, Indianapolis, Indiana 46204

Manuscript received July 9, 1973
Revised copy received July 29, 1974

ABSTRACT

Ultraviolet mutagenesis and its repair were studied mainly in WU36-10-89, a *uvr*⁻ strain of *Escherichia coli* containing a UAG mutation in a gene for leucine biosynthesis. Following ultraviolet (UV) irradiation revertants appearing with or without direct photoreactivation (PR) were classified according to the presence and type of suppressor they contained. We find UV mutation production to be quite specific. An analysis of revertants produced by UV indicates they are formed mainly from GC → AT and that the miscoding is due to a cytosine residue at the site of mutation in a cytosine-thymine (CT) dimer. We propose that the dimer serves as template during some aspects of repair replication and at the time of replication the C in the dimer directs the insertion of A in the complementary strand. We also note that C → A and T → G changes caused by a CT dimer occur much less frequently.

A hallmark of the effect of ultraviolet (UV) light on cells is the production and repair of pyrimidine dimers. Research in this area has been reviewed by SETLOW (1968a) and HOWARD-FLANDERS (1968). Elegant and incisive studies in these authors' laboratories have led to the characterization of two pathways for dark repair: excision and recombinational repair. The first pathway leads to dimer removal, whereas the second bypasses dimers. Recently it was shown that the first pathway consists of two types of excision repair, small and large patch, and that large patch repair is mediated by the *recA* and *recB* genes of *E. coli* (COOPER and HANAWALT 1972a). Although the average number of nucleotides in a repaired region is between 15 and 40 (LEY and SETLOW 1972) the large patches correspond to at least 1500 nucleotides (COOPER and HANAWALT 1972b). For the second pathway it appears from the data of HOWARD-FLANDERS' laboratory (HOWARD-FLANDERS *et al.* 1968; RUPP and HOWARD-FLANDERS 1968; HOWARD-FLANDERS, RUPP and WILKINS 1968) that a dimer cannot serve as a template during semi-conservative replication, so a gap occurs in the DNA strand opposite a dimer. Recombination in this region could leave the dimer in the parental DNA strand, but could bypass it by not using the dimer as template during recombi-

nation-associated DNA replication. From studies of the two pathways for dark repair it would appear that a cell either removes a dimer or bypasses it in attempting to survive. However, as a result of the experiments reported here we propose that a pyrimidine dimer can also serve as template during repair replication.

Pyrimidine dimers are important photoproducts as regards cell survival and mutation (for review see BRIDGES 1969; WITKIN 1969a). For example, UV mutagenesis is very efficient in excision-deficient strains of *E. coli*. Data exist which indicate that some types of mutations produced by UV, for example those giving rise to streptomycin resistance, may occur by different mechanisms in excision-deficient and wild-type bacteria, but both appear to be related to dimer formation and excision (NISHIOKA and DOUDNEY 1969). However, the finding that strains defective in a particular recombination function, e.g. *recA* in K12, give almost no mutations in response to UV (WITKIN 1969a, b; MIURA and TOMIZAWA 1968; KONDO *et al.* 1970) is not readily related to dimer formation.

The current studies were conceived in order to determine more about the nature of UV mutagenic lesions and their repair in bacteria. Bacterial reversion was used as an indicator of mutation production in an excision-deficient strain of B/r, WU36–10–89. In this strain, as in its parent, WU36–10 (BRIDGES, DENNIS and MUNSON 1967a; OSBORN and PERSON 1967), a gene in the leucine biosynthetic pathway contains a UAG nonsense mutation and a gene in the tryosine pathway contains a UAA nonsense mutation. We have analyzed numerous leucine revertants produced by UV in this strain and have determined the mode of reversion in each. Some revertants were also analyzed in the *uvr*$^+$ parent strain.

It is well established that exposure to UV, for strains auxotrophic because of UAA and UAG nonsense codons, results in the formation of some revertants containing nonsense suppressors (WITKIN 1966; BRIDGES, DENNIS and MUNSON 1967a; OSBORN and PERSON 1967; OSBORN *et al.* 1967). A particular revertant containing a suppressor may be analyzed as to suppressor class, class being synonymous with a particular amino acid insertion at the site of the nonsense codon (see DISCUSSION). This analysis was performed on revertants appearing after exposure to UV light with or without exposure to direct photoreactivating light. The changes in frequency of specific revertant classes, for these experimental conditions, are useful in determining the nature of the lesions responsible for UV mutation production. Some information exists concerning primary base sequence in suppressor tRNA molecules and from this the corresponding DNA primary base sequence may be inferred. This allows statements to be made concerning the specificity of the base changes produced by UV.

MATERIALS AND METHODS

Bacteria and bacteriophage: The bacterium WU36-10-89, a *uvr*$^-$ derivative of *E. coli* B/r, and WU36-10, its *uvr*$^+$ parent strain, were used in these studies. They were obtained from DR. EVELYN WITKIN (Douglass College, Rutgers University). The T4 bacteriophages used are those indicated in Table 1 and have been described previously (OSBORN *et al.* 1967; PERSON and OSBORN 1968).

Media: Cultures were grown in a minimal medium A-1 (PERSON and BOCKRATH 1964) supplemented with leucine and tyrosine (20 mg/liter). Two kinds of leucine revertant plates

TABLE 1

Response of UAG mutants of bacteriophage T4 to leu+ *revertants of strain WU36-10-89*

Response of T4 mutants*		Inferred cause of *leu*+ reversion
NG 75	NG 273	
—	—	Structural gene mutation
+	+	Class 1 UAG suppressor
+	—	Class 2 UAG suppressor

* +, lysis of revertant bacteria streaked on phage.
—, no detectable lysis of revertant bacteria streaked on phage.

were used. One contained A-1 supplemented with tyrosine (20 mg/liter) and leucine (2 mg/liter), and was hardened with Difco agar (15 g/liter). In the others leucine was replaced with nutrient broth (0.2 g/liter). Leucine revertant colonies were streaked onto a minimal agar plate containing tyrosine (20 mg/liter). After incubation, streaks were stored at 4° until tested for suppression by using T4 UAG mutants. Surviving bacteria were measured on what we call phage plates which contain tryptone broth (10 g/liter), yeast extract (5 g/liter) and sodium chloride (5.8 g/liter) hardened with Difco agar (12 g/liter). In preliminary experiments survival was also measured on leucine revertant plates supplemented with tyrosine (20 mg/liter) and limiting leucine (2 mg/liter). For the UV dose used there was little difference in survival.

Irradiations: A General Electric 15-watt germicidal lamp, #G8T5, emitting radiation mainly at 253.7 nm, was used. A 10-ml suspension was exposed to 40 ergs/mm^2 in a 100-mm glass petri dish. For photoreactivation (PR) a 406 nm band from a monochromator utilizing a high pressure mercury lamp was used. The incident dose rate was adjusted to approximately 800 ergs/mm^2/second. A 2.5-ml stirred sample of bacteria was irradiated in a quartz cuvette (1×1×3 cm) maintained at approximately 22°. Bacteria were exposed to an incident dose of 7.0×10^5 ergs/mm^2.

Isolation of revertants: Cultures were grown from 10^7 to $1–2 \times 10^8$ cells/ml with aeration at 37°. Cells were collected on a 0.22 μ Millipore filter, washed with minimal medium, and resuspended in the original volume of medium. One portion of the culture was used as a control and the remainder was irradiated with UV light. After UV irradiation, 0.1-ml samples were plated for reversion and 0.1 ml of a 10^5 dilution was plated for survival. Aliquots of control and UV-irradiated samples were then exposed to photoreactivating light or stored in the absence of photoreactivating light. After this treatment, samples were also plated for revision and appropriate dilutions were plated for survival. All platings were done immediately after the exposure to the inactivating and/or reactivating light on room temperature plates. After plating, the plates were immediately put at 37° and incubated for 2 days.

Phage streak tests: One-tenth ml of phage at 10^7 phage/ml was spread onto one-half of a phage plate and the liquid allowed to dry. Then bacteria from revertant stock plates were streaked with toothpicks across the phage plate. If phage had been spread on the right side of phage plate the bacteria were streaked from left to right .This gave a region of bacterial growth on the left side of the plate that was always used as a reference in deciding the extent of lysis. Ten revertant streaks were tested on each phage plate. The response of UAG phage mutants is indicated in Table 1. Since these were the first data obtained with a streak test, the response using this test was repeatedly checked with patterns of spot prints using a large number of mutant phages as described previously (Osborn *et al.* 1967). The phage streak test was found to be as dependable as the spot-print test.

RESULTS

Bacterial suspensions were exposed to UV and/or PR, plated and incubated on assay plates to allow revertant colonies to develop. Stock streaks from individual

TABLE 2

Analysis of spontaneous and UV-produced leucine revertants for uvr⁻ *and* uvr+ *strains*

Nature of revertant	Revertant class			Totals
	Structural gene	Class 1	Class 2	
a. *uvr*⁻ strain (WU36–10–89)				
1. Spontaneous revertants				
number	218	58	194	470
percent	46.4	12.3	41.3	100
2. UV-produced revertants				
net number	60.8*	29.9	1062	1152.7†
percent	5.3	2.6	92.1	100
b. *uvr*+ strain (WU36–10)				
1. Spontaneous revertants				
number	87	23	62	172
percent	50.6	13.4	36.0	100
2. UV-produced revertants (leucine-supplemented plates)				
net number	2.2	6.8	—0.8	8.3‡
percent	26.8	82.9	—9.8	99.9
3. UV-produced revertants (nutrient broth-supplemented plates)				
net number	17.2	2.6	64.2	84§
percent	20.5	3.1	76.4	100
c. Reversion frequencies and excision repair				
1. From line a2;UV, *uvr*⁻ strain				
revertants/10^8 survivors	213	105	3702	4020¶
2. From line b2;UV, *uvr*+ strain (leucine-supplemented plates)				
revertants/10^8 survivors	1.7	5.2	—0.6	6.3
3. From line b3;UV, *uvr*+ strain (nutrient broth-supplemented plates)				
revertants/10^8 survivors	4.3	0.7	16.1	21.1
4. Excision repair (line 1–2)				
revertants/10^8 survivors	211.3	99.8	3702.6	4013.7
percent	5.3	2.5	92.2	100
5. Excision repair (line 1–3)				
revertants/10^8 survivors	208.7	104.3	3685.9	3998.9
percent	5.2	2.6	92.2	100
6. Excision repair within each class (line 4/line 1)				
percent	99.2	95.0	100	—
7. Excision repair within each class (line 5/line 1)				
percent	98.0	99.3	99.6	—

* The net number of revertants in each class produced by UV has been adjusted to account for the number of revertants in a particular class that are of spontaneous origin. This correction, about 6 revertants/plate, is the source of non-integral and negative numbers.

† Obtained from a total of 12 plates, two in each of six experiments. The average cell survival was 16%.

‡ Obtained from a total of 10 plates in four experiments.

§ Obtained from a total of 31 plates in four experiments. The average cell survival, for plates supplemented with leucine or nutrient broth, was 93%.

¶ The number of spontaneous revertants/10^8 survivors was approximately 0.1 for both strains and supplements. About 10^7 cells were plated which subsequently divided to give about 6×10^9 cells/plate before the supplement became limiting.

revertant colonies were used as a source of bacteria and were streaked onto phage plates previously spread with particular nonsense mutants of T4 bacteriophage. The extent of lysis, or no lysis, for each revertant sample determined the revertant host as being permissive or non-permissive. The phage mutants and the classes of revertants indicated by characteristic patterns of lysis are summarized in Table 1.

An analysis of spontaneous and UV-produced leucine revertants of the *uvr*$^-$ (WU36–10–89) and *uvr*$^+$ (WU36–10) strains is shown in Table 2. In order that the only variable in these experiments be the extent of excision repair, both strains were exposed to the same UV dose, 40 ergs/mm^2. The number and percent distributions of revertants into classes for the *uvr*$^-$ and *uvr*$^+$ strains are listed in Table 2a and 2b, respectively.

We used equal numbers of revertant plates supplemented with either a limiting concentration of leucine or nutrient broth in all experiments because of the mutation frequency decline phenomenon (see DISCUSSION). As expected, we found no difference in the number or distribution of revertants into classes for spontaneous revertants for either strain, or for mutations produced by UV by the *uvr*$^-$ strains. Therefore, these data in Table 2 include similar numbers of revertants obtained from both types of plates. However, both the number and distribution into classes of UV-produced revertants were different for the *uvr*$^+$ strain. Therefore, these data are listed separately in Table 2.

The number of revertants/10^8 survivors produced by UV are listed in Table 2c. The average reversion frequency for this single UV dose was multiplied by the distribution of revertants into classes given in Table 2a and 2b to obtain the reversion frequency for each class. The differences in the number of revertants/10^8 survivors for the *uvr*$^+$ and *uvr*$^-$ strains represents the amounts of excision repair, and data for both types of supplements are shown in Table 2c (lines 4, 5). Finally, the extent of excision repair within each class was calculated and is shown in the bottom two lines of the table.

In a second set of experiments cultures of the *uvr*$^-$ strain were exposed to the same dose UV dose, and a portion of each culture was also exposed to PR light of 406 nm prior to plating for leucine revertants. The only variable in these experiments was the presence or absence of PR so that one could determine the extent of PR repair. The analysis of revertants exactly parallels the procedures just described for data presented in Table 2. The number and distribution of revertants into classes for cultures of the *uvr*$^-$ strain exposed only to UV are given in Table 3a, while revertants originating from cultures exposed additionally to PR are given in Table 3b. Reversion frequencies, the extent and distribution by class, and the percent of each revertant class repaired by PR are given in Table 3c.

DISCUSSION

In the present study we isolated leucine revertants occurring spontaneously and produced by UV in the presence and absence of excision and PR repair. We divided the revertants into classes to determine the effect of UV and repair on particular classes as opposed to the total revertant population. We conclude that spontaneous revertants occur in significant numbers for all revertant classes, and

TABLE 3

Analysis of UV-produced leucine revertants of a uvr⁻ *strain (WU36-10-89) repaired by PR*

Nature of revertant	Revertant class			Totals
	Structural gene	Class 1	Class 2	
a. UV-produced revertants				
net number	36.4*	15.2	560.6	612.2†
percent	5.9	2.5	91.6	100
b. UV+PR produced revertants				
net number	97.8	67.6	332.8	498.2‡
percent	19.6	13.6	66.8	100
c. Reversion frequencies and PR repair				
1. From a (UV)				
revertants/10^8 survivors	286	121	4440	4847
2. From b (UV+PR)				
revertants/10^8 survivors	46.8	32.5	159.6	238.9
3. PR repair (line 2—line 1)				
revertants/10^8 survivors)	239.2	88.5	4280.4	4608.1
percent	5.2	1.9	92.9	100
4. PR repair within each class (line 3/line 1)				
percent	83.6	73.1	96.4	—

* The net number of revertants in each class produced by UV has been adjusted to account for the number of revertants in a particular class that are of spontaneous origin. This correction, about 6 revertants/plate, is the source of non-integral and negative numbers.

† Obtained from 6 plates in 3 experiments. This is about one-half of the data given in Table 2 for the same conditions. The average cell survival was 15%.

‡ Obtained from 18 plates in 3 experiments. The average cell survival was 91%. The nine plates supplemented with nutrient broth had a considerably larger number of revertants/plate but the distribution into classes was so similar to that for plates supplemented with leucine that we decided to average the data from all 18 plates.

that the reversion frequencies and the distribution of revertants into classes are similar for both the *uvr*⁻ and *uvr*⁺ strains for plates supplemented with either leucine or nutrient broth (see Table 2a and 2b).

Nearly all of the revertants produced by UV for the *uvr*⁻ strain (1062 of 1152.7 or 92.1%) contained a class 2 UAG suppressor. Structural gene revertants and those containing a class 1 UAG suppressor were produced at 5.3% and 2.6%, respectively (see Table 2a). This striking specificity of UV mutagenesis in the *uvr*⁻ strains is our most interesting finding. The use of a *uvr*⁻ strain gives validity to the same result obtained earlier by CHEUNG and BROCKRATH (1970) using a *uvr*⁺ strain and plating conditions that give less than maximal excision repair.

The reversion frequencies (Table 2c) for the *uvr*⁺ strains exposed to UV are very low, being 640- and 190-fold less than that obtained for the *uvr*⁻ strain. The larger number was obtained for plates supplemented with leucine and the smaller number for plates supplemented with nutrient broth. In these experiments a single small UV dose (40 ergs/mm^2) was used so that both strains were exposed to a similar number and distribution of UV lesions. It is a well established fact, which we confirm here, that greater than 99% of the revertants observed using a *uvr*⁻ strain are repaired by a *uvr*⁺ strain.

The extent of excision repair of UV-produced mutations for *uvr*$^+$ strains is known to be affected by post-irradiation incubation conditions. This includes the nature of the supplement used in a revertant plate, a limiting concentration of the amino acid required by the auxotroph giving maximal repair and a limiting concentration of nutrient broth giving less repair. The general phenomenon, coined mutation frequency decline, was discovered by WITKIN (1956), who later showed the effect to be absent in *uvr*$^-$ strains (WITKIN 1966). We confirm these findings (see RESULTS). It has also been reported that mutation frequency decline occurs for suppressor mutations (WITKIN 1966) and does not occur for mutations to streptomycin resistance (*str*^{r}) (WITKIN and THIEL 1960) or for structural gene revertants (BRIDGES, DENNIS and MUNSON 1967b), leading these authors to the conclusion that this type of repair is specific for one or more nonsense suppressor mutations. Since revertants containing a class 1 UAG suppressor are 2.6%, and those containing a class 2 UAG suppressor are 92.1% of the total revertant population, the extensive repair due to mutation frequency decline is probably specific for potential revertants containing a class 2 UAG suppressor. We are not certain from the limited data reported here for the *uvr*$^+$ strain that the class 2 UAG suppressor is preferentially repaired. However, in other experiments using another strain of bacteria containing a UAG mutation and a higher dose of UV, we found that 41% of the revertants contained a class 2 UAG suppressor after maximal repair (OSBORN *et al.* 1967). Thus, in the presence of a nutrient broth supplement, potential revertants containing a class 2 UAG suppressor are especially refractory to repair, while in the presence of the required amino acid they are preferentially repaired.

We note that the UV-produced revertants of the *uvr*$^-$ strain reflect the number of revertants/10^8 survivors in the absence of excision repair while the data for the *uvr*$^+$ strain reflect the number of revertants/10^8 survivors that have escaped excision repair. The difference between the two represents the number of revertants/10^8 survivors that are subject to excision repair. Our data are very limited for the *uvr*$^+$ strains at the dose of UV used, representing only 8.2 and 84 net revertants tested for plates supplemented with leucine or nutrient broth, respectively. Therefore, the distributions of revertants among the classes, and the percent of revertants within any one class (see Table 2c) are not reliable, although it appears that all revertant classes are repaired. It would be desirable to use UV doses that give more comparable reversion frequencies for the two strains so that the subtraction process for any one class could give percentages between zero and 100. Nonetheless, the procedure used for bacteria differing only in the locus of a UAG mutation represents an interesting manner to investigate the extent of excision repair throughout the *E. coli* genome.

The only variable for the experiments reported in Table 2 is the extent of excision repair and the only variable for those reported in Table 3 is the extent of PR repair. The reversion frequency after UV + PR is sufficient to determine if there is any significant repair in all three revertant classes. We note for the data given in Table 3 that the distributions of revertants prior to PR and those repaired by PR (compare lines a and c3) are indistinguishable. Furthermore, 83.6% of

the structural gene revertants, 73.1% of the revertants containing a class 1 UAG suppressor and 96.4% of those containing a class 2 UAG suppressor are repaired by PR (Table 3c, line 4). These percentages imply that all of the revertants containing a class 2 UAG suppressor, and a large part, but not all, of those in the other two classes are caused by dimers. The base sequence analysis below shows that dimers do occupy the sites of mutation in all cases. We next asked ourselves whether we might ascertain the nature of the UV mutagenic lesion giving rise to these revertant classes from a consideration of our data and existing knowledge about the mechanism of UAG suppressor formation. This query is considered in the following sections.

Evidence that class 1 and 2 UAG suppressors arise by single base substitutions in DNA triplets specifying tRNA anticodons: We have previously inferred (PERSON and OSBORN 1968) that the class 1 and 2 suppressors for WU36–10 are are altered tRNA molecules inserting serine and glutamine, respectively, and that the alteration leading to such suppressors is a change in the DNA coding for the tRNA so that the anticodon becomes CUA (which is complementary to UAG). Primary base sequence analysis exists for another tRNA (*su3*) in three states: su^- (GOODMAN *et al.* 1970), as a UAG suppressor (GOODMAN *et al.* 1970), and after its conversion to a UAA suppressor (ALTMAN, BRENNER and SMITH 1971). It was found that the UAG suppressor was caused by a single base change in the anticodon giving rise to a CUA anticodon, and that the converted suppressor was due to a second change in the anticodon giving rise to a UUA anticodon. FOLK and YANIV (1972) have recently sequenced the two su^- glutaminyl-tRNA's of K12 and the presumptive anticodons were CUG and NUG where N is an unidentified base, perhaps 2-thiouridine. These two anticodons can become complementary to UAG and UAA, respectively, by single base substitutions. In consequence, class 1 and 2 UAG suppressors found in this study are probably formed by single base substitutions in DNA specifying seryl- and glutaminyl-tRNA's whose anticodons are one base different from UAG.

Inferred primary base sequences for before and after mutation: We next consider the specific base changes that would produce the class 2 UAG suppressor, class 1 UAG suppressor and structural gene revertants to see if the changes might arise from a pyrimidine dimer.

a) *The formation of the class 2 UAG suppressor*: Reference can be made to primary base sequence analysis of glutaminyl-tRNA's. For these two tRNA's the base adjacent to the 5′ side of the anticodon is U and that adjacent to the 3′ side is 2- methyladenosine (m^2A) (FOLK and YANIV 1972).

For the class 2 UAG suppressor the glutaminyl-tRNA would be derived from a tRNA whose anticodon is complementary to CAG, $tRNA_2^{Gln}$ of FOLK and YANIV. Therefore, the base sequence of the tRNA before (su^-) and after (su^+) suppressor formation would be:

(32)	(33)	(34)	(35)	(36)		(32)	(33)	(34)	(35)	(36)
U	C	U	G	m^2A	→	U	C	U	A	m^2A.

Suppressor formation results from a G → A change in position 35. The anticodon

is located in positions 33–35 from the 5′ end of the tRNA. The numbers are indicated as an aid in identifying possible sites for pyrimidine dimer formation.

The DNA coding for the *su*⁻ tRNA of bases 32–36 would be:

$$\begin{array}{ccccc} (32) & (33) & (34) & (35) & (36) \\ T & C & T & G & A \\ {}_{3'}A & G & A & C & T_{5'}. \end{array}$$

The lower strand would be that transcribed during tRNA synthesis. Pyrimidine dimers could be formed in positions 32–33 (TC), 33–34 (CT), and 35–36 (TC). Only the last one includes the site of mutation (position 35). UV leads to the formation of this suppressor at a large frequency and we conclude that it causes the C position 35 to code as T. This change, which could plausibly result from a TC dimer, would give rise to a G → A change in the tRNA.

b) *The formation of the class 1 UAG suppressor*: We assume that the seryl-tRNA that can be altered by mutation to form a class 1 UAG suppressor has an anticodon complementary to UCG, or is CGA. As in almost all tRNA's the base to the 5′ side of the anticodon is U and the base to the 3′ side is an altered A residue, designated A* below. For convenience we will use the same numbering of bases as was used for glutaminyl-tRNA. Therefore, the base sequence of the tRNA before (*su*⁻) and after (*su*⁺) suppressor formation would be:

$$\begin{array}{ccccc} (32) & (33) & (34) & (35) & (36) \\ U & C & G & A & A^* \end{array} \rightarrow \begin{array}{ccccc} (32) & (33) & (34) & (35) & (36) \\ U & C & U & A & A^*. \end{array}$$

Suppressor formation results from a G → U change in base 34.

The DNA coding for the *su*⁻ tRNA of bases 32–36 would be:

$$\begin{array}{ccccc} (32) & (33) & (34) & (35) & (36) \\ T & C & G & A & A \\ {}_{3'}A & G & C & T & T_{5'}. \end{array}$$

Pyrimidine dimers could be formed in positions 32–33 (TC), 34–35 (TC) and 35–36 (TT), but only the second one includes the site of mutation (34). If the C in this dimer coded as T the resulting anticodon would be CAA, which is not complementary to UAG and would, therefore, not give rise to a leucine revertant. If the C in the dimer coded as A (producing a G → U change in position 34 of tRNA) leucine revertants would be produced. Since 2.6% of the revertants for the *uvr*⁻ strain contain a class 1 UAG suppressor, we conclude that C in a dimer rarely codes as A.

c) *The formation of structural gene revertants*: Since the leucine gene specifies a UAG code word at some site in its mRNA we know that the DNA specifying UAG is:

$$\begin{array}{ccccc} (1) & (2) & (3) & (4) & (5) \\ x & T & A & G & y \\ {}_{3'}x' & A & T & C & y'_{5'} \end{array}$$

where x, y and their complements (x′, y′) are unknown. For convenience the bases are numbered 1–5. Pyrimidine dimers may be formed at positions 3–4 (CT), and at 1–2(xT) and 4–5(y′C) if x and y′ are pyrimidines. If the C in the 3–4 CT dimer coded as T with a high efficiency the triplet would become ATT

specifying the code word UAA. Since UAA is a second chain-terminating codon, leucine revertants would not be produced by this base change.

We observe that structural gene revertants are not produced efficiently by UV. We can make no other statements about coding changes of the C in the dimer since C → A and C → G would both result in the formation of tyrosine code words (UAU, UAC) at the site of the UAG mutation and it is not known if tyrosine insertion at this site results in the production of an active protein. However, if the T in the 3–4 CT dimer coded as G a serine code word (UCG) would result and we know that serine insertion does produce an active protein. Since 5.3% of the revertants for the *uvr*⁻ strain are structural gene revertants we conclude that a T in a CT dimer rarely codes as G.

d) *The formation of other suppressors by UV*: We have studied the formation of other suppressors that can be expected to be produced by C → T coding changes and in each case the suppressor is formed efficiently by UV. Base sequence examination showed, as above, that for each case a C occupied the site of mutation and was adjacent to a T, making dimer formation by UV possible. For example, class 1 and 2 UAG suppressors can be converted to the corresponding UAA suppressor in this manner (PERSON and OSBORN 1968). (Note that the anticodon complementary to UAG and CUA is specified by a DNA sequence CTA in one strand of DNA. If a dimer were formed in this CT sequence and if the C coded as T during repair replication the anticodon UUA, complementary to UAA, would be generated.) In addition to the earlier work of PERSON and OSBORN, we have shown that the same suppressor conversion is produced efficiently by UV in WU36–10–89 (*uvr*⁻) (unpublished results, this laboratory). It can also be shown that the class 2 UAA suppressor can be formed from an *su*⁻ strain by the same mechanism, and we have also found that this suppressor is produced efficiently in WU36–10–89 (unpublished results, this laboratory).

The efficiency of C in a CT dimer coding as T: The data from the experiments as well as base changes considered above for the three revertant classes are consistent with mutations being caused by a C in a dimer coding as T. We next calculate the expected reversion frequency if C codes as T in a CT dimer with a frequency of 1. We can then compare the observed value with the calculated value to determine the approximate miscoding frequency.

If a UV dose produced one CT dimer in the *E. coli* genome and reversion could occur only at one dimer site in the genome, the expected reversion frequency would be 1/total number of CT plus TC sequences. *E. coli* contains about 5×10^6 dinucleotide sequences and nearest-neighbor analysis shows that about 0.11 of dinucleotide sequences consist of CT and TC sequences (JOSSE, KORNBERG and KAISER 1961). It is also known that 7.4 dimers are produced/erg-mm^2 (HOWARD-FLANDERS and BOYCE 1966) in about the same number of dinucleotide sequences and that some of these dimers, approximately 0.4, are CT dimers (SETLOW, 1968b). For the dose used in our experiments (40 ergs/mm^2) the total number of dimers produced/genome is (7.4) (0.4) (40). Therefore the expected reversion frequency is about (7.4) (0.4) (40)/5×10^6 (0.11) = 2.2×10^{-4}. This calculation assumes that dimers are produced at random throughout the genome and that

reversion originating, for example, by the production of a class 2 UAG suppressor occurs at only one CT sequence in the entire genome. Unpublished evidence from our laboratory indicates the probable existence of three copies of the *su*⁻ gene that can be mutated to a class 2 UAG suppressor. Therefore the expected reversion frequency becomes 6.6×10^{-4}. We observe a frequency of 4.0×10^{-5} revertants/survivor at a dose of 40 ergs/mm^2 giving a C → T miscoding frequency of 4.0/66 or about 6%. For the specific mispairing scheme proposed it appears that in about 1 dimer of 16 the C in a CT dimer can code as T. If not all dimers are used as templates for repair replication the C → T coding efficiency would be correspondingly larger than 6%.

Direct UV mutagenesis by pyrimidine dimers: Mutations could occur at the site of a CT dimer if unexcised dimers in parental strands serve as templates for some type of DNA repair replication. If this occurred it would seem from our data that the C in a CT dimer might often code as T, resulting in the insertion of A as the complement to C. We have shown that this change leads to the specific formation of the class 2 UAG suppressor. Although the class 1 UAG suppressor and the structural gene (UAG) sites contain CT base sequences in the DNA information specifying the anticodon and codon, respectively, neither would cause reversion by a C → T coding change in a CT dimer formed at these sites. However, if CT dimers serving as templates caused random base insertions at high frequencies, then class 1 UAG suppressors would have been noted among the leucine revertants tested. Since few were detected we conclude that random miscoding does not occur often at the site of a CT dimer.

There are two possibilities whereby dimers might serve as templates during repair. Firstly, gaps are left across from dimers by semi-conservative replication of DNA and some of these might be filled by a polymerase that can use dimers as templates. In this proposal for direct UV mutagenesis, bacterial reversion will occur if recombination accompanies repair synthesis, but recombination would not be required for mutagenesis. However, single -strand termini due to gaps left by semi-conservative replication would be expected to increase recombination, and RUPP *et al.* (1971) have shown that sister-strand exchange is very frequent following UV in excision-deficient strains. Of course recombination through dimer bypass would cause recombination without mutation production. Secondly, the large-patch excision repair reported by COOPER and HANAWALT (1972b) extends over distances corresponding to 1500 nucleotides. Some of these large patches of excision will occur across from a dimer in the complementary strand. During the ensuing excision repair replication, the unexcised dimer in the complementary strand may serve as template. The large-patch repair is under the control of recombination genes, and independent of *pol*I activity (COOPER and HANAWALT 1972a). It has been shown previously that UV mutagenesis is also dependent upon the functioning of the same recombinational genes (MIURA and TOMIZAWA 1968; WITKIN 1969b; KONDO *et al.* 1970), and appears to be independent of *pol*I activity (WITKIN 1971). One might expect some of the dimers involved in mutation production to remain in DNA for subsequent generations, thereby giving rise to further mutations during succeeding template repair repli-

cation. BRIDGES and MUNSON (1968) have reported a continuous probability of mutation production for about four generations after UV exposure to *uvr*$^-$ cells growing in continuous culture.

There is no doubt that UV can produce mutations by more than one mechanism, especially at reduced frequencies, and the data of HILL, SQUIRES and CARBON (1970) and MEISTRICH and DRAKE (1972) may attest to this. However, the latter authors employed T4 bacteriophage in which C-containing dimers are not readily produced (see MEISTRICH and SCHULMAN 1969, p. 164) probably because there are no cytosine residues in T4.

Although UV may produce mutations at sites some distance from the original lesion, the simplest interpretation of our data is that suppressors arise by dimer formation at the mutagenic site.

SMITH and HANAWALT (1968) have considered a possible mode of mutation by cytosine in dimers which takes into account enhanced cytosine deamination when the 5–6 bond is saturated. Their suggestion, however, includes deamination of C to yield a uracil residue and dimer splitting by PR to reestablish a monomer U in place of the initial C. We found PR removes the mutagenic lesion and, consequently, we do not believe the cytosine in the dimer has deaminated. Furthermore, SETLOW and CARRIER (1966) found UV to produce cytosine-containing dimers and very little uracil-containing dimers in DNA. Possibly the imino tautomeric form of cytosine, a prerequisite for hydrolytic deamination and formation of the carbonyl group of uracil, prevails after dimer formation and offers hydrogen bonding analogous to that of uracil or thymine (the 3-nitrogen in the ring is protonated). It has been shown by PHILLIPS, BROWN and GROSSMAN (1966) that the imino tautomers of cytosine in template DNA select A during replication with an efficiency of one.

Significance of studies employing suppressor mutations: Several other workers have studied UV mutation production in *uvr*$^-$ strains of bacteria containing either a UAG or UAA chain termination mutation and concluded that suppressor mutations are caused by dimer formation (HILL 1965; KONDO and KATO 1966; WITKIN 1966; BRIDGES, DENNIS and MUNSON 1967b; NISHIOKA and DOUDNEY 1969). In the present study we have divided the revertant population into classes and by so doing have shown that it is predominantly a particular suppressor class (class 2) that is produced in response to UV. Furthermore, a CT dimer sequence is present at the site of mutation, and mutation would result if a dimer is formed and utilized as template in some aspect of repair replication. One must inquire as to the possible generality of such a result. A number of investigators have concluded that different mechanisms may produce suppressor mutations on the one hand and mutations to *str*^{r} on the other hand. The class 2 UAG and UAA suppressors arise by a forward mutation in the DNA specifying the anticodon of a tRNA and can only be formed by a particular C in a genome coding as T. *str*^{r} also arises by a forward mutation in DNA specifying a ribosomal protein. It should be noted that amino acid replacement analysis exists for three mutations from *str*^{s} to *str*^{r} (FUNATSA and WITTMAN 1972). In all cases a single amino acid replacement was indicated, occurring at the site of a possible TT dimer. In no case was mutation consistent with a GC → AT base change. Thus, it is possible that

str^r does not result from GC → AT base change while the class 2 UAG suppressor results exclusively from these base changes.

It has recently been reported that a third type of UV repair exists, an error-prone, UV-inducible repair system which is under the control of the *recA* and *lex* genes (WITKIN 1974; GEORGE, DEVORET and RADMAN 1974). This system may include an error-prone DNA polymerase with mutations produced during gap filling. If the polymerase could use dimers as templates then UV mutations could be solely explained by the presence of this inducible factor. In *uvr*$^-$ strains the gaps would be those left across from a dimer since dimers cannot serve as templates during semi-conservative replication. Our data require only that the C in a CT dimer miscodes as A during the error-prone gap filling and with a frequency that is large relative to random base changes at the dimer or other sites. In *uvr*$^+$ strains there are additionally the gaps left by the efficient excision of dimers, and data from the above authors indicate that mutations, although rare, may occur by an error-prone filling of these gaps. If such a polymerase is able to use an abnormal substrate, such as a dimer, as template, then it may indeed lack rigorous base pairing specificity thereby causing mutations at small frequencies during the filling of excision repair-initiated gaps.

It is a pleasure to acknowledge discussion of data and manuscript with DRS. MARY OSBORN and FRED D. FUNK as well as colleagues in our laboratories. The idea for using this type of bacterial system to study mutagenesis produced and repaired by UV is due to MARY OSBORN. Some data of this type using another bacterium may be found in her Ph.D. dissertation (The Pennsylvania State University, 1967). We thank DR. R. A. DEERING for a sustaining interest in our work as well as for help with the photoreactivation experiments, including the use of his monochromator. A special acknowledgment is made to DR. EVELYN WITKIN for the timely provision of strain WU36-10-89 and for her help with the manuscript. Most streak assays were done by LETHA MULLINS, JANE SCHEUCHENZUBER and PAULETTE TORCHIA. Supplies and some salaries were provided by grant AT(11-1)-3419 from the U. S. Atomic Energy Commission.

LITERATURE CITED

ALTMAN, S., S. BRENNER and J. D. SMITH, 1971 Identification of an ochre-suppressor anticodon. J. Mol. Biol. **56**: 195–197.

BRIDGES, B. A., 1969 Mechanisms of mutagenesis in cellular and sub-cellular systems. Ann. Rev. Nucl. Sci. **19**: 139–172.

BRIDGES, B. A. and R. J. MUNSON, 1968 The persistence through several replication cycles of mutation-producing pyrimidine dimers in a strain of *Escherichia coli*. Biochem. Biophys. Res. Comm. **30**: 620–624.

BRIDGES, B. A., R. E. DENNIS and R. J. MUNSON, 1967a Mutation in *Escherichia coli* B/r WP 2 *try*$^-$ by reversion or suppression of a chain-terminating codon. Mutat. Res. **4**: 502–504. ——, 1967b Differential induction and repair of ultraviolet damage leading to true reversions and external suppressor mutations of an ochre codon in *Escherichia coli* B/r WP2. Genetics **57**: 897–908.

CHEUNG, M. K. and R. C. BOCKRATH, 1970 On the specificity of UV mutagenesis in *E. coli*. Mutat. Res. **10**: 521–523.

COOPER, P. K. and P. C. HANAWALT, 1972a Role of DNA polymerase I and the *rec* system in excision-repair in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **69**: 1156–1160. ——, 1972b, Heterogeneity of patch size in repair replicated DNA in *Escherichia coli*. J. Mol. Biol. **67**: 1–10.

FOLK, W. R. and M. YANIV, 1972 Coding properties and nucleotide sequences of *E. coli* glutamine tRNAs. Nature New Biol. **237:** 165–166.

FUNATSA, G. and H. G. WITTMAN, 1972 Ribosomal proteins. XXXIII. Location of amino acid replacements in protein S12 isolated from *Escherichia coli* mutants resistant to streptomycin. J. Mol. Biol. **68:** 547–550.

GEORGE, J., R. DEVORET and M. RADMAN, 1974 Indirect ultraviolet reactivation of phage λ. Proc. Natl. Acad. Sci. U.S. **71:** 144–147.

GOODMAN, H. M., J. ABELSON, A. LANDY, S. ZADRAZIL and J. D. SMITH, 1970 The nucleotide sequence of tyrosine transfer RNAs of *Escherichia coli*. Europ. J. Biochem. **13:** 461–483.

HILL, C. W., C. SQUIRES and J. CARBON, 1970 Glycine transfer RNA of *Escherichia coli*. I. Structural genes for two glycine tRNA species. J. Mol. Biol. **52:** 557–559.

HILL, R. F., 1965 Ultraviolet-induced lethality and reversion to prototrophy in *Escherichia coli* strains with normal and reduced dark repair ability. Photochem. Photobiol. **4:** 563–568.

HOWARD-FLANDERS, P., 1968 DNA repair. Ann. Rev. Biochem. **37:** 175–200.

HOWARD-FLANDERS, P. and R. P. BOYCE, 1966 DNA repair and genetic recombination: Studies on mutants of *Escherichia coli* defective in these processes. Radiation Res. (Suppl.) **6:** 156–184.

HOWARD-FLANDERS, P., W. D. RUPP and B. M. WILKINS, 1968 In: *Replication and Recombination of Genetic Material.* Edited by W. F. PEACOCK. Australian Acad. Sci., Canberra.

HOWARD-FLANDERS, P., W. D. RUPP, B. M. WILKINS and R. S. COLE, 1968 DNA replication and recombination after UV irradiation. Cold Spring Harbor Symp. Quant. Biol. **33:** 195–205.

JOSSE, J., A. KORNBERG and A. D. KAISER, 1961 Enzymatic synthesis of deoxyribonucleic acid. VIII. Frequencies of nearest neighbor base sequences in deoxyribonucleic acid. Frequencies of nearest neighbor base sequences in deoxyribonucleic acid. J. Biol. Chem. **236:** 864–875.

KONDO, S. and T. KATO, 1966 Action spectra for photoreactivation of killing and mutation to prototrophy in UV sensitive strains of *E. coli* possessing and lacking photoreactivating enzyme. Photochem. Photobiol. **5:** 827–837.

KONDO, S., H. ISHIKAWA, K. IWO and T. KATO, 1970 Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics **66:** 187–217.

LEY, R. D. and R. B. SETLOW, 1972 Repair replication in *Escherichia coli* as measured by the photolysis of bromodeoxyuridine. Biophys. J. **12:** 420–431.

MEISTRICH, M. L. and J. W. DRAKE, 1972 Mutagenic effects of thymine dimers in bacteriophage T4. J. Mol. Biol. **66:** 107–114.

MEISTRICH, M. L. and R. G. SHULMAN, 1969 Mutagenic effects of sensitized irradiation of bacteriophage T4. J. Mol. Biol. **46:** 157–167.

MIURA, A. and J. I. TOMIZAWA, 1968 Studies on radiation-sensitive mutants of *Escherichia coli*. III. Participation of the *rec* system in induction of mutation by ultraviolet irradiation. Mol. Gen. Genet. **103:** 1–10.

NISHIOKA, H. and C. O. DOUDNEY, 1969 Different modes of loss of photoreversibility of mutation and lethal damage in ultraviolet light resistant and sensitive bacteria. Mutation Res. **8:** 215–228.

OSBORN, M. and S. PERSON, 1967 Characterization of revertants of *E. coli* WU36-10 and WP2 using amber mutants and an ochre mutant of bacteriophage T4. Mutation Res. **4:** 504–507.

OSBORN, M., S. PERSON, S. L. PHILLIPS and F. FUNK, 1967 A determination of mutagen specificity in bacteria using nonsense mutants of bacteriophage T4. J. Mol. Biol. **26:** 437–447.

PERSON, S. and R. C. BOCKRATH, 1964 Differential mutation production by the decay of incorporated tritium compounds in *E. coli*. Biophys. J. **4**: 355–365.

PERSON, S. and M. OSBORN, 1968 The conversion of amber to ochre suppressors. Proc. Natl. Acad. Sci. U.S. **60**: 1030–1037.

PHILLIPS, J. H., D. M. BROWN and L. GROSSMAN, 1966 The efficiency of induction of mutations by hydroxylamine. J. Mol. Biol. **21**: 405–419.

RUPP, W. D. and P. HOWARD-FLANDERS, 1968 Discontinuities in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**: 291–304.

RUPP, W. D., C. E. WILDE III, D. L. RENO and P. HOWARD-FLANDERS, 1971 Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J. Mol. Biol. **61**: 25–44.

SETLOW, R. B., 1968a The photochemistry, photobiology and repair of polynucleotides. Prog. Nuc. Acid. Res. **8**: 257–295. ——, 1968b Photoproducts in DNA irradiated *in vivo*. Photochem. Photobiol. **7**: 643–649.

SETLOW, R. B. and W. L. CARRIER, 1966 Pyrimidine dimers in ultraviolet-irradiated DNAs. J. Mol. Biol. **17**: 237–254.

SMITH, K. C. and P. C. HANAWALT, 1968 pp. 55–65. In: *Molecular Photobiology*. Academic Press, N.Y.

WITKIN, E. M., 1956 Time, temperature and protein synthesis. A study of ultraviolet-induced mutations in bacteria. Cold Spring Harbor Symp. Quant. Biol. **21**: 123–140. ——, 1966 Radiation-induced mutations and their repair. Science **152**: 1345–1353. ——, 1969a Ultraviolet-induced mutation and DNA repair. Ann. Rev. Microbiol. **23**: 487–514. ——, 1969b The mutability toward ultraviolet light of recombination-deficient strains of *Escherichia coli*. Mutation Res. **8**: 9–14. ——, 1971 Ultraviolet mutagenesis in strains of *E. coli* deficient in DNA polymerase. Nature New Biol. **229**: 81–82. ——, 1974 Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: Evidence that ultraviolet mutagenesis depends upon an inducible factor. Proc. Natl. Acad. Sci. U.S. **71**: 1930–1934.

WITKIN, E. M. and E. C. THEIL, 1960. The effect of post-treatment with chloramphenicol on various ultraviolet-induced mutations in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **46**: 226–231.

Corresponding editor: F. STAHL