MUTATIONAL SPECIFICITY OF ULTRAVIOLET LIGHT IN
ESCHERICHIA COLI WITH AND WITHOUT THE R PLASMID pKM101

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ABSTRACT

Plasmid pKM101 provides UV protection and increases the frequency of spontaneous and UV-induced mutations in Escherichia coli. By analyzing reversion patterns of defined trpA alleles, we showed that pKM101 altered the mutational specificity of UV-induced mutations. Certain UV-induced base-pair substitutions were strongly enhanced, while others were decreased in frequency in the presence of pKM101. This result suggests an interaction between cellular misrepair and an error-prone repair function(s) provided by pKM101. We have also examined UV mutational specificity in the absence of pKM101 and found the following: (1) UV preferentially enhances missense, as well as nonsense, intergenic suppressor mutations; (2) UV causes all possible base-pair substitutions as well as frameshift mutations; (3) G·C base pairs are more susceptible to UV mutagenesis than A·T base pairs at the same nucleotide positions; and (4) UV-induced mutations can occur at nucleotide positions that are not part of pyrimidine-pyrimidine sequences.

CERTAIN R plasmids, such as pKM101 and its parent R46, have been shown to confer the following characteristics upon their host cells: (1) increased spontaneous mutagenesis (mutator effect) (MORTELDMANS and STOCKER 1976; WALKER 1977; FOWLER, McGINTY and MORTELDMANS 1979), (2) increased susceptibility to UV-induced mutations, and (3) reduced susceptibility to killing by UV irradiation (UV protection) (MACPHEE 1972, 1973; MORTELDMANS and STOCKER 1976; WALKER 1977). In Escherichia coli, each of the above plasmid properties of pKM101 or R46 depends on the recA+ lexA+ genotype (WALKER 1977), but not upon the functions of the uvrA, polA, lig, recB, recC or recF loci (TWEATS et al. 1976); in Salmonella typhimurium LT2, the same properties also depend upon the recA+ genotype (MORTELDMANS and STOCKER 1976). Because the ability of pKM101 or R46 to provide host UV protection and to induce UV mutagenesis depends on a functional recA gene, it has been suggested that these and similar plasmids may participate in a hypothesized error-prone repair system in the host (HOWARTH 1965, 1966; MONTI-Bragadin, BABUDRI and SAMER 1976; MORTELDMANS and STOCKER 1976; WALKER 1977).
A synergistic interaction has been observed in mutation production between a tif sfiA mutant and pKM101 (Doubleday, Green and Bridges 1977; Walker 1977), suggesting that pKM101 and related plasmids may interact with a cellular error-prone repair system. However, recent results of Goze and Devort (1979) suggest that inducible cellular error-prone repair is not elevated by the presence of pKM101.

In this paper, we use reversion analysis of defined trpA alleles (Yanofsky, Ito and Horn 1966) to determine the specificity of UV-induced mutations in E. coli strains with and without pKM101. If pKM101 and related plasmids increase UV mutagenesis by enhancing a cellular error-prone repair system, the mutational specificity should remain the same after the addition of pKM101 since mutational events will be generated by the same mechanism in both cases. A significant difference in UV mutational specificity between strains with and without pKM101 would indicate that the plasmid-enhanced mutagenesis occurs through a mechanism other than enhancement of a host error-prone repair system.

In addition, we are able to confirm and extend earlier observations on the specificity of UV mutagenesis in E. coli. Previous studies had shown that nonsense suppressor sites were particularly susceptible to UV mutagenesis (Bridges, Dennis and Munson 1967; Osborn et al. 1967). The trpA reversion system provides an opportunity to examine the susceptibility of several missense sites to UV mutagenesis. Coulondre and Miller (1977) have shown that UV induced all possible types of base-pair substitutions in the lacI gene. We are able to compare their results with the spectrum of reversion events induced by UV in the trpA gene where the nucleotide sequence has been recently determined (Nichols and Yanofsky 1979).

An abstract of a portion of this material has been published (Fowler et al. 1980).

MATERIALS AND METHODS

Strains: Nomenclature of the bacterial strains is that of Demerec et al. (1966) and Novick et al. (1976). The tryptophan synthetase A gene mutants used in this study were obtained from Charles Yanofsky (Yanofsky, Ito and Horn 1966; Brammar, Berger and Yanofsky 1967; Berger, Brammar and Yanofsky 1968; Drapeau, Brammar and Yanofsky 1968), and the trpE9777 frameshift mutant was obtained from Eli Siegel (Siegel and Vaccaro 1978). All trpA alleles and trpE9777 have been transduced into E. coli strain KD1088 thr leu Δ (tonB-trpAB) his arg, as previously described (Degnen and Cox 1974; Fowler, Degnen and Cox 1974). S. typhimurium LT2 strains SL 3810 pyrE125 rfa-F38 and TA100 hisG46 rfa Δ (gal-chl-bio-uvrB) (pKM101) were supplied by Bruce Stocker (Kuo and Stocker 1972) and Bruce Ames (McCann et al. 1975) respectively. The origin of pKM101 has been previously described (Mortelmans and Stocker 1979).

Media: VB medium is the minimal salts medium of Vogel and Bonner (1956). Minimal plates contained minimal medium solidified with 1.5% agar and supplemented with 0.2% glucose and 1 μg of thiamine-hydrochloride per ml. Required amino acids were added at 50 μg/ml to liquid and solid media and uracil, indole and chloramphenicol were added to solid media at 15 μg/ml, 10 μg/ml and 12.5 μg/ml, respectively. Trp+ revertants were selected on minimal plates supplemented with casamino acids (Difco Laboratories) at 2.5 mg/ml and
UV mutagenic specificity

limiting tryptophan at 0.4 μg/ml. L-broth is 1% tryptone (Difco), 0.5% yeast extract (Difco) and 0.5% NaCl. Nutrient broth is 0.8% nutrient broth (Difco). Tryptone plates consisted of 1% tryptone (Difco) and 0.5% NaCl solidified with 1% agar. Plasmid pKM101, which confers resistance to ampicillin (Mortelmans and Stocker 1979), was monitored on minimal plates supplemented with 25 μg of ampicillin per ml. The saline for dilutions and washing was 0.85% NaCl.

Conjugations and detecting the presence of plasmid pKM101: Transfer of pKM101 has been previously described (Mortelmans and Stocker 1976; Fowler, McGinty and Mortelmans 1979). Continued presence of pKM101 in randomly selected UV-induced Trp+ revertants of pKM101-containing trpA strains was checked by the presence or absence of revertant growth on ampicillin plates. No spontaneous losses of pKM101 were detected.

UV mutagenesis: To measure frequencies of UV-induced reversion to Trp+ for each of the trpA and trpE strains, with and without pKM101, 0.1 ml washed samples (10^8 cells) were taken from overnight L-broth cultures and spread on minimal plates supplemented with casamino acids and limiting tryptophan (0.4 μg/ml). Five to 7 plates were exposed to 39.0 J/m^2 UV from a Sylvania germicidal lamp, and the same number were left unirradiated. The UV dose was measured with a UV meter. Irradiated (and unirradiated) plates were wrapped in aluminum foil to prevent possible photorepair and Trp+ colonies were counted after 5 days of incubation at 37°. The number of UV-induced revertants per 10^8 survivors was calculated by subtracting the mean number of colonies per unirradiated plate from the mean number per irradiated plate and then correcting for the fraction of irradiated bacteria surviving determined by viable counts as described below.

To determine UV survival, dilutions were made from an overnight 37° L-broth culture. Dilutions were spread on the surface of tryptone plates and exposed to varying UV doses under a bactericidal lamp. Irradiated (and unirradiated) plates were wrapped in foil to prevent possible photorepair, incubated for 48 hr at 37° and colonies counted. (Control experiments showed that survival counts were similar when minimal plates supplemented with casamino acids and tryptophan were used instead of tryptone plates.)

Identification of Trp+ revertants: UV-induced Trp+ revertants for each trpA strain, with and without pKM101, were isolated by purifying 34 to 193 Trp+ colonies from irradiated and unirradiated selective plates. Every Trp+ colony was utilized per plate or a sector of a plate to insure an unbiased random selection of Trp+ revertants. Because of the high UV-induced reversion frequencies of some trpA alleles, fewer than 10^8 cells/ml of these strains were plated in order to allow purification of revertant colonies.

The classification of Trp+ revertants of trpA strains was based upon one genetic and two physiological tests. First, purified revertants were tested for indole glycerol phosphate (IGP) accumulation. Wild-type revertants do not accumulate IGP; partial revertants do (Allen and Yanofsky 1963). All Trp+ revertants were tested for IGP accumulation. Second, various partial revertants can often be distinguished quantitatively by their sensitivity to 5-methyl tryptophan (5-MT) (Lester and Yanofsky 1961). Partial or suppressed revertants growing on agar plates show much larger zones of inhibition than do full revertants around disks impregnated with 5-MT (Cox, Degnene and Scheppe 1972; Fowler, McGinty and Mortelmans 1979). This allows the placement of revertants into discrete classes that correspond to known base-pair substitution events. The 5-MT inhibition test was used to classify all revertants of trpA alleles 23, 46 and 58. Since the IGP accumulation test is accurate for full revertants, only occasional 5-MT inhibition tests were performed on revertants of trpA alleles 3, 11, 78, 88 and 223, since further separation of partial revertants provides no additional useful data. Known full and partial revertants containing pKM101 were utilized as controls to demonstrate that the presence of the plasmid had no effect on the 5-MT resistance or IGP accumulation phenotypes of the revertants. Third, suppressed revertants of trpA alleles 3, 11, 23, 46, 58, 78 and 88 were distinguished from intragenic revertants by P1 transduction, as previously described by Fowler, Degnene and Cox (1974).

Transductions: P1 transductions were carried out as described by Miller (1972) using the mutant P1cm1 clr100, which includes a transposon, Tn9, conferring resistance to chlorampheni-
icol. This mutant makes clear plaques at high temperatures (42°), but turbid plaques at low temperatures (32°). Lysogens were prepared by infecting donor cells with P1em1 clr100 and then selecting for chloramphenicol-resistant cells. High-titer lysates were made from heat induction, 40° for 35 min and a shift to 37° for an additional 60 min.

RESULTS

Enhancement of UV-induced reversion of trpA mutations by pKM101: The UV-induced Trp+ reversion frequency for each trpA strain, with and without pKM101, is shown in Table 1. The UV-induced reversion frequency was significantly enhanced by pKM101 for base-pair substitution trpA alleles 3, 23, 46, 88, and 223, as well as for the trpE9777 frameshift allele. Only a small proportion of the total Trp+ revertants for each of the three trpA frameshift alleles was UV-induced; the remaining revertants resulted from frequent background spontaneous events (Table 1). Since the small number of UV-induced revertants for each of these alleles is statistically unreliable, their P+/P- ratios were not

<table>
<thead>
<tr>
<th>trpA allele</th>
<th>Survival (%)</th>
<th>Revertant colonies/plate</th>
<th>Induced revertants per 10^8 survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-</td>
<td>p+</td>
<td>p-</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>63</td>
<td>516</td>
</tr>
<tr>
<td>11</td>
<td>49</td>
<td>62</td>
<td>141</td>
</tr>
<tr>
<td>23</td>
<td>36</td>
<td>54</td>
<td>214</td>
</tr>
<tr>
<td>46</td>
<td>34</td>
<td>62</td>
<td>229</td>
</tr>
<tr>
<td>58</td>
<td>43</td>
<td>60</td>
<td>1388</td>
</tr>
<tr>
<td>78</td>
<td>40</td>
<td>63</td>
<td>662</td>
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<tr>
<td>88</td>
<td>40</td>
<td>61</td>
<td>8</td>
</tr>
<tr>
<td>223</td>
<td>51</td>
<td>65</td>
<td>139</td>
</tr>
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<td>9813 (fs)</td>
<td>35</td>
<td>63</td>
<td>191</td>
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<td>21 (fs)</td>
<td>41</td>
<td>59</td>
<td>296</td>
</tr>
<tr>
<td>540 (fs)</td>
<td>51</td>
<td>67</td>
<td>317</td>
</tr>
<tr>
<td>E9777 (fs)</td>
<td>47</td>
<td>69</td>
<td>309</td>
</tr>
</tbody>
</table>

† Ratios of colony counts on tryptone plates, irradiated/nonirradiated, for trpA strains (P-)
and for pKM101-bearing derivatives (P+). Each value is the average of three experiments where the range did not exceed ±10% from the given value.
‡ Mean number of Trp+ revertant colonies/plate for 5–7 minimal plates supplemented with limiting tryptophan (0.4 μg/ml), each inoculated with ca. 10^8 washed cells of the indicated trp strain or its pKM101-bearing derivative and irradiated (39.0 J/m²) before incubation.
§ The mean number of revertant colonies per irradiated plate was corrected for spontaneous revertants by subtracting the mean number of revertant colonies per nonirradiated plate; the mean number of UV-induced revertants per plate inoculated with ca. 10^8 bacteria, thus calculated, was divided by the proportion of bacteria surviving irradiation to calculate the number of UV-induced revertants per 10^8 survivors.
$$ The figures in parentheses are the numbers of spontaneous revertants per 10^8 cells.
* Significantly different from a ratio of 1.0 at 0.05 level (t-test).
** Significantly different from a ratio of 1.0 at 0.01 level (t-test).
¶ Frameshift mutations.
$$ Ratio not calculated.
|| trpE frameshift mutation.
calculated. Nonetheless, it is apparent that the addition of pKM101 to these strains greatly increased the number of UV-induced revertants. Only the trpA58 allele produced fewer UV-induced revertants with pKM101, and this decrease was statistically insignificant (Table 1).

**Specificity and frequency of UV-induced reversion of trpA alleles in the absence of pKM101:** The base-pair substitutions caused by UV were elucidated by determining the reversion spectrum of trpA base-pair substitution mutants (DRAPEAU, BRAMMAR and YANOFSKY 1968; YANOFSKY, ITO and HORN 1966). YANOFSKY and co-workers have determined amino acid changes in the tryptophan synthetase A protein resulting from several of the trpA mutants and subsequent revertant classes. The data, together with the recently elucidated DNA sequence for the trpA gene (NICHOLS and YANOFSKY 1979), allow the deduction of genetic code changes at many mutant and revertant sites.

Figure 1 shows the amino acid substitutions and the corresponding base-pair substitutions at positions 49, 183, 211 and 234 in the tryptophan synthetase A protein and trpA mutants used in this study. Since the reversion pattern of a given trpA allele can often be deduced from IGP accumulation and 5-MT inhibition tests, independently of data on amino acid sequences, base-pair changes can often be ascertained directly from IGP and 5-MT phenotypes of Trp+ revertants.

YANOFSKY, ITO and HORN (1966) considered the possible amino acid replacements that could arise from single base-pair substitutions in some of the mutant codons of the trpA alleles used in this study. Most of the possible amino acid replacements that have not been recovered in Trp+ revertants can be assumed to have chemical properties that would make them incompatible with a functional tryptophan synthetase A protein (YANOFSKY, ITO and HORN 1966).

It is still possible that Trp+ revertants might have been induced by pKM101 or UV that have not been previously characterized and, thus, have unidentified base-pair changes. If such revertants have phenotypes that are indistinguishable from known revertant classes, they would not have been detected as unique and could bias our results. However, a variety of potent mutagens and mutator genes failed to produce new phenotypic classes of Trp+ revertants (COX, DEGNEN and SCHEPPE 1972; FOWLER, DEGNEN and COX 1974; PERSING, McGINTY and FOWLER 1980) that differ from those originally described by YANOFSKY and co-workers (YANOFSKY, ITO and HORN 1966; DRAPEAU, BRAMMAR and YANOFSKY 1968). Therefore, the existence of cryptic revertant classes seems a remote possibility.

The characterization and distribution of UV-induced revertants of the trpA mutants, based upon the IGP accumulation and 5-MT inhibition tests, is shown in Table 2. The trpA mutants, revertants and inferred base-pair substitutions are taken from the data of YANOFSKY and co-workers (DRAPEAU, BRAMMAR and YANOFSKY 1968; YANOFSKY et al. 1964; YANOFSKY, ITO and HORN 1966; NICHOLS and YANOFSKY 1979). Several components of UV mutational specificity can be inferred from these data. As shown by the presence of UV-induced revertants (column 7), all possible transitions and transversions were induced
Figure 1.—Amino acid substitutions observed at positions 49, 183, 211 and 234 in the tryptophan synthetase A protein and their corresponding mRNA codons (YanoFSky, Ito and Horn 1966; YanoFSky et al. 1967; Drapeau, Brammar and YanoFSky 1968). FR, Full revertant; PR, partial revertant. In older literature, the amino acid positions were numbered one less than that given here because the original amino acid sequencing of the tryptophan synthetase A protein failed to detect one amino acid [YanoFSky et al. 1967].
<table>
<thead>
<tr>
<th>trpA allele (1)</th>
<th>Revertant class (2)</th>
<th>5-MT sensitivity (3)</th>
<th>IGP accumulation (4)</th>
<th>Distribution of UV-induced revertants</th>
<th>UV-induced revertant colonies/10⁸ survivors</th>
<th>Inferred base pair substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>I FR</td>
<td>R</td>
<td>—</td>
<td>0.12 (97) 0.36 (193)</td>
<td>146 712</td>
<td>4.9**</td>
</tr>
<tr>
<td></td>
<td>PR§</td>
<td>S</td>
<td>+</td>
<td>0.88 (97) 0.64 (193)</td>
<td>1070 5/5§§ 1266 8/8</td>
<td>1.2</td>
</tr>
<tr>
<td>11</td>
<td>I FR</td>
<td>R</td>
<td>—</td>
<td>0.60 (107) 0.18 (89)</td>
<td>172 67</td>
<td>0.4**</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>S</td>
<td>+</td>
<td>0.40 (107) 0.82 (89)</td>
<td>114 5/5 307 7/7</td>
<td>2.7**</td>
</tr>
<tr>
<td>23</td>
<td>I FR</td>
<td>R</td>
<td>—</td>
<td>0.21 (101) 0.64 (157)</td>
<td>122 601</td>
<td>4.9**</td>
</tr>
<tr>
<td></td>
<td>II PR</td>
<td>S</td>
<td>+</td>
<td>0.22 (101) 0.05 (157)</td>
<td>127 47</td>
<td>0.4**</td>
</tr>
<tr>
<td></td>
<td>III PR</td>
<td>S</td>
<td>+</td>
<td>0.45 (101) 0.26 (157)</td>
<td>261 244</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>IV PR</td>
<td>S</td>
<td>+</td>
<td>0.11 (101) 0.04 (157)</td>
<td>64 2/2 38 3/4</td>
<td>0.6*</td>
</tr>
<tr>
<td>46</td>
<td>I FR</td>
<td>R</td>
<td>—</td>
<td>0.34 (93) 0.46 (192)</td>
<td>221 377</td>
<td>1.7*</td>
</tr>
<tr>
<td></td>
<td>II PR</td>
<td>S</td>
<td>+</td>
<td>0.16 (93) 0.38 (192)</td>
<td>104 311</td>
<td>3.0*</td>
</tr>
<tr>
<td></td>
<td>III PR</td>
<td>S</td>
<td>+</td>
<td>0.49 (93) 0.15 (192)</td>
<td>318 6/6 123 5/5</td>
<td>0.4**</td>
</tr>
</tbody>
</table>
### TABLE 2—Continued

| trpA allele | Revertant class | 5-MT sensitivity† | IGP accumulation | Distribution of UV-induced revertants || UV-induced revertant colonies/10⁶ survivors || Inferred base-pair substitutions |
|-------------|----------------|-------------------|------------------|-----------------------------------------|---------------------------------------------|-----------------------------------|
|             |                |                   |                  | **P**⁻ | **P**⁺ | **P**⁻ | **P**⁺ | **P**⁻/**P**⁺ |                                      |                                |
| 58          | I FR           | R                 | —                | 0.02 (111) | 0.06 (108) | 64     | 167     | 2.6**        | A·T → G·C                               |                                |
|             | II PR          | S                 | +                | 0.02 (111) | 0.11 (108) | 64     | 306     | 4.8**        | A·T → C·G                               |                                |
|             | III PR         | S                 | +                | 0.96 (111) | 0.82 (108) | 3079 7/7 | 2278 14/14 | 0.7*        | G·C → A·T                               |                                |
| 78          | I FR           | R                 | —                | 0.04 ( 83) | 0.35 (131) | 65     | 611     | 9.4**        | A·T → C·G                               |                                |
|             | PR             | S                 | +                | 0.96 ( 83) | 0.65 (131) | 1560 4/12 | 1135 3/7 | 0.7*        | —                                        |                                |
| 88          | I FR           | R                 | —                | 0.79 ( 34) | 0.43 ( 95) | 15     | 34      | 2.3**        | A·T → C·G                               |                                |
|             | PR             | S                 | +                | 0.21 ( 34) | 0.57 ( 95) | 4 5/5  | 45.6/6  | 11.3**       | —                                        |                                |
| 223         | I FR           | R                 | —                | 0.55 ( 59) | 0.53 (107) | 78     | 343     | 4.4**        | A·T → C·G                               |                                |
|             | PR             | S                 | +                | 0.45 ( 59) | 0.47 (107) | 63     | 305     | 4.8**        | —                                        |                                |

† The UV dose used was 39.0 J/m². Additional experiments with UV fluences of 19.5 and 78.0 J/m² resulted in data (not shown) similar to those presented here.

‡ Trp⁺ revertants that demonstrate an inhibitory response to 5-MT similar to that of the wild type are designated resistant (R); whereas, revertants less tolerant than wild type are termed sensitive (S).

§ “Distribution” is the decimal fraction of the total Trp⁺ revertants analyzed from each trpA allele that, based upon the 5-MT sensitivity and IGP accumulation tests, can be placed into discrete revertant classes. Numbers in parenthesis indicate total number of revertants analyzed for that trpA strain without pKM101 (P⁻) and with pKM101 (P⁺). “Distribution” is based solely on UV-induced revertants; the estimated proportions of spontaneous revertants for each class have been omitted from the distribution calculations.

∥∥ The number of UV-induced revertants per 10⁶ survivors for each revertant class is calculated by multiplying the number of UV-induced revertants per 10⁶ survivors (Table 1) times the distribution fraction for that revertant class.

* Significantly different from a ratio of 1.0 at 0.05 level (t-test).

** Significantly different from a ratio of 1.0 at 0.01 level (t-test).

§ The partial revertants for trpA alleles 3, 78, 88 and 223 were not placed into discrete classes by the 5-MT inhibition test. Therefore, these partial revertants for each allele may result from more than one mutational event.

 §§ The fractions are the ratios of the numbers of revertants found to be intergenic suppressors to the numbers of revertants tested for suppression.

¶ Base-pair or change not known.
by UV. These numbers of UV-induced revertants were calculated after subtracting the estimated numbers of spontaneous revertants for each class and therefore represent only UV-induced revertants.

There is only one site available for directly observing whether transitions or transversions are favored by UV mutagenesis. At the trpA58 site (Figure 1 and Table 2, column 10) an A·T→G·C transition results in full revertants, while an A·T→C·G transversion at the same A·T base pair results in a class of partial revertants. As can be seen from Table 2, columns 7 and 10, UV induced the A·T transition and transversion to the same extent.

There is an indication that UV preferentially causes substitutions of G·C base pairs in the trpA system. Both the trpA11 and trpA88 mutants resulted from base-pair substitutions of the same nucleotide pair at position 49 (Figure 1). However, as can be seen in Table 2, columns 7 and 10, the number of UV-induced full revertants of trpA11 resulting from G·C→C·G transversions was more than 10 times the number of full revertants of trpA88 resulting from A·T→C·G transversions. The same nucleotide position is involved in both of these transversion events. A second example of preferential UV mutagenesis of G·C base pairs involved revertants of trpA23 and trpA46. Both mutants result from base-pair substitutions in the same codon at position 211 (Figure 1). Among the unambiguous reversion events, III PR trpA23 revertants and II PR trpA46 revertants involve the same nucleotide position (Figure 1). UV induced the III PR trpA23 revertant class (C·G→A·T) 2 to 3 times more frequently than the II PR trpA46 revertant class (A·T→T·A).

The nucleotide sequence of the trpA gene (Nichols and Yanofsky 1979) reveals whether or not a base pair has to be part of a pyrimidine dimer in order to be mutated by UV. Known reversion events at the trpA23, A46, A58, A78 and A223 sites involve base pairs that are part of a pyrimidine-pyrimidine sequence (Nichols and Yanofsky 1979). Defined base-pair substitutions at position 49 giving rise to wild-type revertants of trpA alleles 3, 11 and 88 involve base pairs that are not part of a pyrimidine-pyrimidine sequence (Nichols and Yanofsky 1979) and therefore lack the potential to form pyrimidine dimers. Whereas full revertants of trpA88 (A·T→C·G) were not strongly induced by UV, full revertants of trpA3 (A·T→T·A) and trpA11 (G·C→C·G) were induced as strongly, or more strongly, by UV as were identical transversion events at other trpA sites (Table 2, columns 7 and 10) that have the potential to produce pyrimidine dimers. The lack of potential for producing a pyrimidine dimer does not prevent a base pair from being mutated by UV.

The obvious preference for UV-induced changes at sites that generate intergenic trpA suppressors is also shown in Table 2. All of the trpA base-pair substitution mutants, with the possible exception of trpA223 (which was not tested), had at least some intergenic suppressors among the partial revertants induced by UV (Table 2, column 7). All the UV-induced IV PR class members of trpA23, III PR class members of trpA46, III PR class members of trpA58
and PR class members of \textit{trpA} alleles 3, 11 and 88 tested were intergenic suppressors.

Intergenic suppression of \textit{trpA}58 is thought to result from a G·C→A·T transition in a glycine transfer RNA gene (Squires and Carbon 1971). This observation is supported by the finding that this class (III PR) of \textit{trpA}58 revertants was preferentially enhanced by 2-aminopurine (Persing, McGinty and Fowler 1980) and by sodium bisulfite (Spivak and Fowler, unpublished data), a mutagen that specifically induces G·C→A·T transitions (Mukai, Hawryluk and Shapiro 1970). Although the specific base-pair substitutions for other intergenic mutants of the \textit{trpA} system are unknown, none occurred as frequently as the G·C→A·T event that produces \textit{trpA}58 suppressors (Table 2, column 7).

\textit{Specificity of pKM101-induced mutagenesis:} The characterization and distribution of UV-induced Trp$^+$ revertants enhanced by pKM101 is also shown in Table 2. A summary of the enhancement of \textit{trpA} base-pair substitutions is shown in Table 3.

Among the revertant classes with known base-pair substitutions, pKM101 enhanced UV-induced A·T substitutions, with transversions occurring more readily than transitions, but pKM101 did not enhance UV-induced G·C base-pair substitutions of any type. In fact, at our limited number of G·C sites, pKM101 actually decreased the number of UV-induced changes compared to the number that occurred in its absence (Table 2, columns 7, 8, 9 and 10; Table 3). All of the G·C base-pair substitutions were readily induced by UV in the absence of pKM101 (Table 2, column 7). Whereas the intergenic suppressor sites of \textit{trpA} alleles A23, A46 and A58 were strongly mutated by UV without pKM101 (Table 2, column 7), the plasmid decreased the number of these UV-induced suppressors. On the other hand, pKM101 appeared to increase strongly the number of UV-induced intergenic suppressors of \textit{trpA}11 and \textit{trpA}88.

Clearly, the presence of pKM101 altered the UV mutational specificity for base-pair substitutions. It also appears that the mutational specificity of pKM101 is similar for spontaneous and UV-induced base-pair substitutions (Table 3; Fowler, McGinty and Mortelmans 1979); A·T transversions were most strongly enhanced.

\begin{center}
\textbf{Table 3}
\end{center}
\textit{A summary of the enhancement of UV-induced base-pair substitutions at \textit{trpA} sites by pKM101}

\begin{center}
\begin{tabular}{|c|c|}
\hline
\textbf{Base-pair substitution} & \textbf{UV-induced revertant colonies/10$^9$ survivors} \\
\hline
A·T→G·C & 2.6 (A58) \\
A·T→C·G & 4.8 (A58), 9.4 (A78), 2.3 (A88) \\
A·T→T·A & 4.9 (A3), 3.0 (A46) \\
G·C→A·T & 0.7 (A58) \\
G·C→C·G & 0.4 (A11), 0.4 (A23) \\
G·C→T·A & 0.9 (A23) \\
\hline
\end{tabular}
\end{center}
DISCUSSION

Specificity of UV mutagenesis in the absence of pKM101: The trpA reversion system of E. coli is advantageous for studying mutational specificity since amino acid substitutions are known at several sites and the entire trpA gene has been sequenced (Nichols and Yanofsky 1979). However, only a limited number of sites within the trpA gene are available to observe base-pair substitutions and frameshift mutations. Particularly unfortunate is the absence of reversion sites within trpA where G·C→A·T transitions can be detected. This precludes the determination of UV mutational hotspots within the trpA region. Also, all of the trp mutants used except trpA223 and trp A540 were UV induced (YanoFSKY, Ito and Horn 1966; Drapeau, Brammar and Yanofsky 1968; Yanofsky et al. 1971) and therefore may be located at sites particularly prone to UV mutagenesis.

Several aspects of UV mutational specificity have been elucidated or confirmed in this study. Mutations that lead to intergenic trpA suppressors are strongly induced by UV (Table 2). For example, UV readily induced mutations that produced intergenic suppressors of trpA46 and trpA88, although spontaneous intergenic suppressors of these alleles were not recovered. This sensitivity to UV induction applies to sites that generate missense suppressors (all of the trpA base-pair substitution mutants except trpA88 being missense mutants [YanoFSKY, Ito and Horn 1966; Drapeau, Brammar and Yanofsky 1968]), as well as sites that lead to nonsense suppressors (trpA88 being an amber mutant [Drapeau, Brammar and Yanofsky 1968]). The sensitivity of sites for nonsense suppression to UV mutability when cells are plated on broth or casamino acids has long been known (Bridges, Dennis and Munson 1967), and it appears that missense suppression sites are also susceptible.

Our results on the specificity of UV mutagenesis indicate that all possible transitions and transversions, as well as frameshift mutations, are induced (Tables 1 and 2), results consistent with those of CoulonDRe and Miller (1977), who analyzed forward mutations in the E. coli lacI gene, and with a preliminary study of UV mutagenesis by Yanofsky with the trpA system (cited by Witkin 1969).

G·C→C·G transversions are strongly enhanced by UV at the two available trpA sites, thereby certifying the UV-inducibility of this event, which occurred infrequently at a small number of sites in the lacI gene (CoulonDre and Miller 1977). Although G·C and A·T base pairs are both susceptible to UV mutagenesis, G·C base pairs seem to be more mutable than A·T base pairs when compared at the same site. Six of the seven original isolations of trpA base-pair substitution mutants by UV mutagenesis involved changes of G·C base pairs, several of these with many subsequent independent isolates (YanoFSky, Ito and Horn 1966; Drapeau, Brammar and Yanofsky 1968).

The importance of pyrimidine dimers as a type of UV-induced lesion that leads to mutations has long been recognized (Deering and Setlow 1963; Setlow, Boling and Bollum 1965; Witkin 1969), although other UV-induced
photoproducts also probably produce mutations (Meistrich and Drake 1972). The \( \text{trpA} \) alleles 3, 11 and 88 all are UV-reverted to wild type. However, the base pair involved in each case is not part of a pyrimidine-pyrimidine sequence (Nichols and Yanofsky 1979), and UV mutagenesis cannot be dependent on the altered base pair being part of a pyrimidine dimer or other photoproduct involving adjacent pyrimidines.

According to a current hypothesis, UV mutagenesis in \( \text{E. coli} \) occurs through a \( \text{recA} \)-dependent, inducible, error-prone repair system called SOS repair (reviewed by Witkin 1976). The system is induced by UV radiation or other agents that damage DNA or terminate its replication (Defais et al. 1971; George, Devoret and Radman 1974). This postulated inducible repair system is thought to act on UV damage that is not repaired by other repair systems, damage such as gaps created by the prevention of DNA replication past pyrimidine dimers. In order to utilize altered DNA templates, such as dimers, it has been suggested that this repair system involves polymerase activity that lacks 3'→5' exonuclease proofreading activity (Villani, Boiteux and Radman 1978). Mutations then would be expected to occur with this SOS repair system even with a normal template strand because of the lack of 3'→5' proofreading activity (untargeted mutagenesis). All types of base-pair substitutions should occur, and a mutated base pair would not have to be part of a dimer.

Earlier studies on UV mutational specificity in \( \text{E. coli} \) indicated that UV preferentially causes G·C→A·T transitions (Osborn et al. 1967; Cheung and Bockrath 1970; Person et al. 1974). Each of these studies involved mutations occurring at sites that generated nonsense suppressors. More recently, Coulondre and Miller (1977) showed that UV induces all base-pair substitutions within the \( \text{lacI} \) gene, although G·C→A·T “hot-spots” dominated the spectrum. We found that mutations, probably G·C→A·T transitions, which lead to suppression of \( \text{trpA58} \), were induced by UV more frequently than were either mutations at other suppressor-producing sites or direct reversion events within the \( \text{trpA} \) gene. Unfortunately, we have no sites available within the \( \text{trpA} \) gene to observe the UV induction of G·C→A·T transitions; and therefore we cannot compare their frequency with that of other base-pair substitutions. However G·C→A·T transitions are readily induced in the \( \text{trpA} \) gene since the majority of UV-induced isolates of \( \text{trpA} \) base-pair substitution mutants resulted from these events (Yanofsky, Ito and Horn 1966; Drapeau, Brammar and Yanofsky 1968). Within the limitations of the \( \text{trpA} \) reversion system, our results on UV mutational specificity agree with the studies of Coulondre and Miller (1977) utilizing the \( \text{lacI} \) gene of \( \text{E. coli} \), Drake (1963) with the \( \text{rII} \) gene of phage T4 and Prakash and Sherman (1973) with the \( \text{cycl} \) gene of yeast. These authors concluded that UV induces a variety of mutational alterations, with “hot-spots” occurring for particular events.

Specificity of UV-induced mutations enhanced by \( \text{pKM101} \): Our data clearly indicate (Table 3) that \( \text{pKM101} \) enhanced UV-induced mutations only at A·T base pairs, particularly transversions. The presence of \( \text{pKM101} \) actually de-
increased UV-induced base-pair substitutions at G•C base pairs; pKM101, then, does not function merely by enhancing the ability of the host cell to perform error-prone repair.

We conclude that cellular and plasmid-mediated error-prone repair systems are not identical, a conclusion also reached by Goze and Devoret (1979) who used a different experimental approach. Todd, Monti-Bradagin and Glickman (1979) showed that plasmid R46, the parent of pKM101 (Mortelmans and Stocker 1979), alters the mutational specificity of methyl methanesulfonate (MMS) when introduced into host strains. Since MMS is thought to mutate by misrepair (Drake and Baltz 1976), the authors concluded that cellular and plasmid-mediated misrepair of MMS-induced DNA damage occurred through different systems. However, it has not been resolved whether MMS and UV mutagenesis involve the same cellular error-prone repair system (Drake and Baltz 1976; Moreau and Devoret 1977).

Our data further indicate that, although cellular and pKM101-associated error-prone repair are not part of an identical system, they do interact, implying they share one or more steps in the same pathway. This is shown by the decrease in UV-induced substitutions at G•C sites and the increase at A•T sites caused by pKM101.

Mutants of pKM101 have been isolated that have simultaneously lost the UV-protecting effect, ability to enhance UV mutagenesis and spontaneous mutator activity (Walker 1978a; Shannabuch and Walker 1980). This would suggest that all of the above plasmid activities are coded by the same gene(s) (Walker 1978a). We previously showed that pKM101 preferentially enhances spontaneous mutations involving transversions at A•T base pairs (Fowler, McGinty and Mortelmans 1979). This is the same mutational preference pKM101 possesses in the enhancement of UV-induced revertants and provides further support for a limited number of plasmid genes being involved in repair and mutagenic activities.

A precise mechanism for the involvement of pKM101 and related plasmids in UV protection and in spontaneous and UV mutagenesis has not been elucidated. We suggest that pKM101 codes for a constitutive component of an error-prone repair system that results in spontaneous mutagenesis in the host (Goze and Devoret 1979). This repair activity can be enhanced through induction by treatments such as UV exposure that damage DNA or terminate its replication (Walker 1978b). The plasmid error-prone repair component may then compete with a similar cellular product to carry out one or more steps in error-prone repair. One possibility for this cellular component might be the umuC gene product (Kato and Shinoura 1977) as suggested by Walker and Dobson (1979) since pKM101 suppresses the effects of umuC mutants (Walker and Dobson 1979).

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LITERATURE CITED


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