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Ionizing and ultraviolet radiation-induced reversion of sequenced frameshift mutations in *Escherichia coli*: a new role for *umuDC* suggested by delayed photoreactivation

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Summary

The ultraviolet (UV) and γ radiation-induced reversion of the *trpA21*, *trpA9813*, and *trpE9777* sequenced-frameshift mutations were studied in *Escherichia coli* K-12 with or without the plasmid pKM101. Radiation induced the reversion of all 3 frameshifts, and pKM101 enhanced this reversion 10–50-fold. Factors influencing the differential radiation revertability of frameshifts are discussed. The two most revertable frameshifts, *trpE9777* and *trpA9813*, were used as probes to understand the role of the *umuDC* genes in radiation-induced frameshift reversion. Unlike the UV radiation-induced reversion of base-substitution mutations, the reversion of these frameshifts was *not* enhanced in a *uvrA umuC* strain by photoreactivation after a post-UV-irradiation incubation. The UmuDC proteins are suggested to have functions in the radiation induction of frameshifts that are more complex than are their functions in the induction of base substitutions.

Small insertions or deletions of DNA that do not involve multiples of 3 nucleotides cause a shift in the reading frame of mRNA, and therefore are called frameshift mutations (reviewed in Roth, 1974). The fraction of ionizing radiation-induced mutations that are composed of frameshifts and other insertions and deletions is, e.g., 0.47 in *Salmonella typhimurium* (Hartman et al., 1971), 0.43 in *Neurospora crassa* (Malling and de Serres, 1973), and 0.61 in bacteriophage T4 (Conkling et al., 1976). UV radiation also induces frameshifts in bacteria, but their fraction seems smaller, e.g., 0.27 in *S. typhimurium* (Hartman et al., 1971) and 0.35–0.40 in *Escherichia coli* (Miller, 1985).

Since a frameshift can often be phenotypically reverted by a second, nearby frameshift that restores the proper translational reading frame to the affected gene (e.g., Roth, 1974), and since radiation induces frameshifts (see above), it seems surprising that UV radiation would revert only one (i.e., *trpE9777*) of thirteen *trp* frameshift mutations in *E. coli* (Kato and Nakano, 1981), and that even that particular frameshift mutation could not be reverted by ionizing radiation (Glickman et al., 1980; incorrectly listed as *trpE997*). In contrast, Imray and MacPhee (1981) detected

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TABLE 1

BACTERIAL STRAINS USED

Stanford Radiology number	Genotype [a]	Source, reference or derivation [b]
SR114	*uvrA6*, otherwise as SR749	AB1886, S. Linn
SR248	*leuB19 metE70 thyA36 deo(C2?) bioA2 lacZ53 malB45 rha-5 rpsL151*	KH21, R.B. Helling
SR250	*leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 rpsL151*	Sargentini and Smith, 1984
SR350	*uvrA6 uvrB230 phr-2*, otherwise as SR250	Youngs and Smith, 1978
SR353	*sulA1 uvrA155 trpE65*	$WP2_s$, E.M. Witkin
SR716	*trpE9777*	W3110 *trpE9777*, C. Yanofsky
SR749	*argE3 hisG4 leuB6 Δ(gpt – proA)62 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rfbD1 mgl-51 kdgK51 rpsL31 supE44 rac*	AB1157, B.J. Bachmann
SR960	*ilvA700*::Tn*5 thyA deo* λ^r	CBK007, K.J. Shaw
SR1018	*umuC122*::Tn*5*, otherwise as SR749	GW2100, G.C. Walker
SR1023	pKM101, otherwise as SR250	SR250 × TA100, Ap^r Sm^r
SR1119	*deoC araD139 Δ(lac)U169 malE7*::Tn*5* f16B *relA rpsL*	T5M7, T. Silhavy
SR1120	*malE7*::Tn*5*, otherwise as SR749	SR749 × Plvir · SR1119, Kn^r
SR1165	*umuC122*::Tn*5*, otherwise as SR749	SR749 × Pl::Tn*9*cts · SR1018, Kn^r
SR1181	*pyrF1189*::Tn*1 rpsL*	TH1161, S. Harayama
SR1265	*pyrF1189*::Tn*1*, otherwise as SR749	SR749 × Plvira · SR1181, Ap^r
SR1268	*uvrA6*, otherwise as SR749	SR1120 × Pl Tn*9*cts · SR114, Mal^+
SR1273	*trpA21 mel-1 supE57 supF58*	Ymel *trpA21*, C. Yanofsky
SR1275	*trpA9813*	W3110 *trpA9813*, C. Yanofsky
SR1276	*trpE9777*	W3110 *trpE9777*, C. Yanofsky
SR1282	*trpA21*, otherwise as SR749	SR1265 × Plvira · SR1273, Pyr^+
SR1284	*trpA9813*, otherwise as SR749	SR1265 × Plvira · SR1275, Pyr^+
SR1285	*trpE9777*, otherwise as SR749	SR1265 × Plvira · SR1276, Pyr^+
SR1286	*trpE65*, otherwise as SR749	SR1265 × P1::Tn*9*cts · SR353, Pyr^+
SR1327	SR1282 (*trpA21*) carrying pKM101	SR1282 × SR1023, Ap^r Met^+ Thy^+
SR1329	SR1284 (*trpA9813*) carrying pKM101	SR1284 × SR1023, Ap^r Met^+ Thy^+
SR1330	SR1285 (*trpE9777*) carrying pKM101	SR1285 × SR1023, Ap^r Met^+ Thy^+
SR1340	*Δ(trpEA)2 tna*	*tna Δ(trpEA)2*, C. Yanofsky
SR1548	*avrA6 pyrF1189*::Tn*1*, otherwise as SR749	SR1268 × Plvira · SR1181, Ap^r
SR1562	*uvrA6 trpE65*, otherwise as SR749	SR1548 × Plvira · SR1286, Pyr^+
SR1699	*ilvA700*::Tn*5 Δ(trpEA)2 tna*	SR1340 × Plvira · SR960, Kn^r
SR1747	*metE70 Δ(trpEA)2 tna*	SR1699 × Plvira · SR248, Ilv^+
SR1817	*uvrA6 trpE9777*, otherwise as SR749	SR1548 × Plvira · SR716, Pyr^+
SR1821	*uvrA6 umuC122*::Tn*5 trpE9777*, otherwise as SR749	SR1817 × Plvira · SR1018, Kn^r
SR1849	*uvrA6 umuC122*::Tn*5*, otherwise as SR749	SR1562 × Plvira · SR1018, Kn^r
SR1864	*uvrA6 trpA9813*, otherwise as SR749	SR1548 × Plvira · SR1275, Pyr^+
SR1872	*uvrA6 phr-2 trpE65* Gal^+, otherwise as SR749	SR1562 × Plvira · SR350, Gal^+
SR1873	*uvrA6 umuC122*::Tn*5 trpA9813*, otherwise as SR749	SR1864 × Plvira · SR1018, Kn^r
SR1878	*uvrA6 phr-2 umuC122*::Tn*5 trpE65* Gal^+, otherwise as SR749	SR1872 × Plvira · SR1165, Kn^r
TA100	*hisD3052 ΔuvrB rfa* /pKM101	B.A.D. Stocker

[a] Genotype nomenclature is that used by Bachmann (1983). All strains are *E. coli* K-12 $F^-\lambda^-$ except SR1273 (F^+, λ^+), SR353 (*E. coli* B/r), and TA100 (*S. typhimurium*).

[b] Ap^r, Sm^r, and Kn^r indicate resistance to ampicillin, streptomycin, and kanamycin, respectively.

ionizing radiation-induced reversion for two frameshift mutations in *S. typhimurium*.

In an attempt to understand these conflicting reports, we have assayed in *E. coli* the UV and γ radiation-induced reversion of the *trpE9777*, *trpA21*, and *trpA9813* frameshift mutations in the presence or absence of the plasmid pKM101. Since the nucleotide sequences of these 3 frameshifts are known, factors influencing their differential revertability are discussed. We have shown that the radiation induction of frameshift reversion in *E. coli* occurs much more often than suggested by others, and have discussed essentially why the reversion of the hyper-revertable *trpE9777* frameshift is just as good a model for frameshift reversion as is the reversion of less-revertable frameshift mutations. We then used *trpE9777* (and *trpA9813*) reversion to test one model for the function of the *umuDC* genes in the radiation induction of frameshifts.

Materials and methods

Bacteria. Strains used are listed in Table 1. Bacteriophage P1 transductions were performed generally as described by Miller (1972). Strains were tested for P1 lysogeny.

Media. YENB was yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. Trp-0 was a 0.4% glucose-salts medium (Ganesan and Smith, 1968), supplemented with arginine, histidine, leucine, proline and threonine all at 1 mM, thiamine · HCl at 0.5 μg/ml, and Noble agar (Difco) at 1.6%, and was dispensed at 27 ml per petri dish. Arg-0 was Trp-0 with tryptophan in place of arginine. Trp-1 and Arg-1.5 were Trp-0 and Arg-0 containing YENB at 1 or 1.5% (v/v), respectively. PB was Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0. Strains carrying the plasmid pKM101 were cultured in YENB containing ampicillin at 50 μg/ml and dimethyl sulfoxide at 0.74% (v/v), and were assayed for mutagenesis on plates supplemented with adenine at 10 μg/ml (Waleh and Stocker, 1981).

Preparation and irradiation of cells. Logarithmic-phase cells were prepared by diluting an overnight culture, 1 : 500, into YENB or YENB plus ampicillin and shaking at 37 °C until an optical density (OD) at 650 nm of 0.4 was attained. Cultures were pelleted by centrifugation (6 min, 6000 × *g*), washed twice, and resuspended in PB at OD = 0.05 or 0.2 for UV irradiation or at OD = 2, 5 or 20 for γ-irradiation. Washed cells at OD = 5 corresponded to 1.3×10^9 colony-forming units (CFU) per ml. UV (254 nm) and γ (^{137}Cs, oxic) irradiation procedures have been described (Sargentini and Smith, 1983). After irradiation, UV-irradiated cells were concentrated by centrifugation if required. For mutation assays, 0.2-ml cell samples were spread onto quadruplicate mutant-selection plates (duplicate for photoreactivation experiments), e.g., Trp-1 and Arg-1.5. Plates were incubated 3–4 days at 37 °C.

Mutant frequency. The calculation of radiation-induced mutant frequency has been described (Sargentini and Smith, 1980). In general, the listed mutant frequency (e.g., Trp^+ per 10^8 cells) is the frequency of radiation-induced mutants corrected for spontaneous "plate" mutants and the killing of "preexisting" spontaneous mutants. The latter correction was not done for the mutant assay described in Table 2. The frequency of preexisting spontaneous mutants was determined by plating nonirradiated cells on Trp-0 or Arg-0 plates, as

TABLE 2

γ-RADIATION-INDUCED REVERSION OF *E. coli trpE9777* STRAINS

γ-Radiation dose (krad)	SR1276 (W3110 *trpE9777*) $Trp^+/10^8$ cells	SR1285 (AB1157 *trpE9777*) $Trp^+/10^8$ cells
0	1	0
10	22	12
20	65	34
30	107	65
40	144	80
50	208	129

Logarithmic-phase, YENB-grown cells were γ-irradiated in PB, diluted to about 2×10^6 colony-forming units (CFU) per ml with YENB, shaken 16 h at 37 °C, washed twice with PB, diluted 10-fold and spread at 0.2 ml (1×10^8 CFU) per Trp-0 plate for Trp^+ mutant selection. This procedure was designed to mimic that of Glickman et al. (1980).

appropriate. Radiation survival and plate and induced mutants were determined on Trp-1 or Arg-1.5 plates.

Photoreactivation. Plated cells were photoreactivated for 40 min at room temperature through plastic petri dish lids with a bank of 4 parallel 48-inch Sylvania 34-W Lite White Super Saver II lamps. The lamp centers were 9 cm apart, and were arranged 4 cm above the agar surface. Plates were arranged parallel to the lamps with one row of plates centered below each lamp.

Several control experiments were performed to test our procedure for the delayed photoreactivation effect on UV-radiation mutagenesis in a *umuC* strain (see Results). The 40-min photoreactivation time used gave the maximum mutagenesis for both frameshift and base-substitution reversion; times of 10–60 min were tested (data not shown). The 90-min postirradiation incubation time before photoreactivation gave the most base-substitution reversion; times of 30–240 min were tested. None of these times of postirradiation incubation before photoreactivation had any effect on frameshift reversion. Other light sources such as blacklight and purple light also produced the delayed photoreactivation effect (data not shown), and a *phr* mutation blocked the effect (Fig. 1).

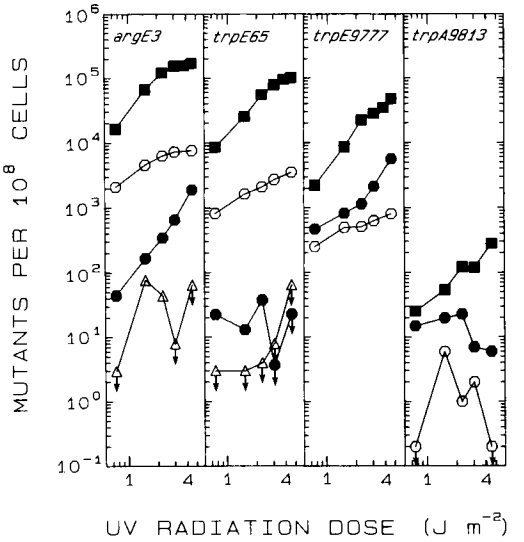

Fig. 1. Effect of delayed photoreactivation on UV radiation-induced reversion of ochre base-substitution (oc) and frameshift (fs) mutations in *Escherichia coli uvrA umuC* strains. The *uvrA* (■), *uvrA umuC* (●,○), and *uvrA umuC phr* (△) strains were UV irradiated, plated, incubated for 90 min at 37 °C, photoreactivated (open symbols) or not (closed symbols), and further incubated for 3–4 days at 37 °C before scoring for UV-radiation mutagenesis. Data are the means from two or more experiments per strain. When the calculated value for UV-radiation-induced mutants per plate was negative, it was replaced by 0.1 to determine a theoretical upper limit for the mutant frequency. These theoretical data are shown with arrows. For simplicity, some data for *argE3* and *trpE65* are combined from 2 or 3 isogenic strains. Strains used for *argE3* (oc) are: SR1562 and SR1817, (■); SR1821, SR1849, and SR1878, (●); SR1821 and SR1849, (○); and SR1878, (△). For *trpE65* (oc), strains are: SR1562 (■); SR1849 and SR1878, (●); SR1849, (○); and SR1878, (△). For *trpE9777* (fs), strains are: SR1817, (■); and SR1821, (●, ○). For *trpA9813* (fs), strains are: SR1864, (■); and SR1873, (●, ○).

Results

The γ-radiation-induced reversion of the *trpE9777* mutation was studied in *E. coli* K-12 strains W3110 *trpE9777* (the original mutant strain that we received from Dr. C. Yanofsky and call SR1276 in our lab) and SR1285 (an AB1157 transductant strain carrying *trpE9777*). The mutation assay procedure was similar to that used by Glickman et al. (1980). In contrast to their results, however, we found the *trpE9777* mutation to be readily reverted by γ-radiation (Table 2).

To gain a further understanding of radiation-induced frameshift reversion, isogenic *E. coli trp* mutants, with and without the mutagenesis-enhancing plasmid pKM101 (Mortelmans and Stocker, 1976), were tested for spontaneous mutability and UV and γ radiation mutability using a plate-reversion assay (Tables 3 and 4, respectively). The *trpA21* and *trpA9813* mutations showed significant UV and γ radiation reversion. The *trpE9777* mutation was by far the most radiation revertible mutation studied. Except for the spontaneous reversion of *trpA21*, where it had no effect, the plasmid always increased the spontaneous reversion and the UV and γ radiation-induced reversion of the frameshifts studied. Whereas pKM101 has been shown to afford some protection to UV and γ radiation (Walker, 1977; Francia et al., 1984), under our experimental conditions, which were somewhat different than those used by others, pKM101 enhanced radiation lethality to a small degree (Tables 3 and 4).

TABLE 3

EFFECT OF PLASMID pKM101 ON UV-RADIATION-INDUCED REVERSION OF *trp* FRAMESHIFT MUTATIONS IN *Escherichia coli* [a]

Mutation studied [b]	pKM101	Spontaneous mutants per plate	UV-radiation induction of Trp^+ mutants per 10^8 cells and lethality [c]						
			10	20	30	40	50	60	70 J m^{-2}
trpA21	–	69	–	–	7.1	13.8	8.4	12.9	15.2
	+	62	17	52	138	267	–	–	–
trpA9813	–	2	–	–	10	17	25	29	52
	+	22	54	135	556	1078	–	–	–
trpE9777	–	46	–	–	940	1460	2580	3130	6170
	+	254	7100	12600	35000	91300	–	–	–
Surviving fraction	–	–	–	–	0.66	0.50	0.41	0.34	0.19
	+	–	0.88	0.68	0.38	0.18	–	–	–

[a] Logarithmic-phase cells were irradiated in PB (see Materials and methods). Data are the means of 2 Expts. per strain. Values for spontaneous mutants have been corrected for the preexisting mutants (always less than 10% of the spontaneous mutants), and represent the spontaneous mutations occurring during growth on the plates. The approximate number of radiation-induced mutants per plate equals $(Trp^+/10^8)$ (10^{-8}) (surviving fraction) (cells plated), where 1.3×10^7 cells were spread per plate for strains SR1285 and SR1330, and 8.4×10^8 cells were spread per plate for the other strains.

[b] Strains without and with pKM101, respectively, were SR1282, SR1327 (*trpA21*); SR1284, SR1329 (*trpA9813*); and SR1285, SR1330 (*trpE9777*).

[c] Surviving fractions, combined from isogenic strains differing only in their *trp* genotype, are given in the lower part of the table for the relevant radiation doses.

The revertants of the *trpE9777* mutant tended to produce colonies that were either 0.2–0.6 mm or 1–2 mm in diameter on the mutant-selection plates. To quantitate the possible role of suppressor mutations in the reversion of *trpE9777*, we selected 10 revertants of each size-class from both

TABLE 4

EFFECT OF PLASMID pKM101 ON γ-RADIATION-INDUCED REVERSION OF *trp* FRAMESHIFT MUTATIONS IN *Escherichia coli* [a]

Mutation studied [b]	pKM101	Spontaneous mutants per plate	γ-Radiation induction of Trp^+ mutants per 10^8 cells and lethality [c]				
			5	10	15	20	25 krad
trpA21	–	82	–	6.2	5.2	6.4	11
	+	105	3.3	7.9	21	23	–
trpA9813	–	2	–	1.4	1.8	2.7	4.8
	+	20	12	26	37	61	–
trpE9777	–	56	–	35	56	73	117
	+	207	158	370	549	1473	–
Surviving fraction	–	–	–	0.65	0.52	0.43	0.26
	+	–	0.80	0.58	0.31	0.20	–

[a] Same as for Table 3, but cells spread per plate were 3.5×10^8 for SR1285, 1.4×10^8 for SR1330, and 1.5×10^9 for other strains.

[b] See Table 3.

[c] See Table 3.

the γ and UV radiation experiments (40 revertants, total), and tested, after cloning, whether they could donate, by bacteriophage P1 transduction, the Trp^+ character to a strain, SR1747, deleted for the *trp* operon. All of the radiation-induced Trp^+ revertants were able to donate the Trp^+ phenotype to the deletion recipient, which strongly suggests that none of the donors were frameshift suppressor mutants (data not shown).

The *umuC* gene is required for UV and most of γ-radiation frameshift reversion (Kato and Nakano, 1981; Sargentini and Smith, 1984). Here we show that the presence of the *mucAB* genes (i.e., carried on pKM101) enhanced both UV and γ radiation frameshift reversion (Tables 3 and 4). These genes produce proteins that are very analogous in structure and function to the UmuDC proteins (Perry et al., 1985).

Bridges and Woodgate (1984, 1985) have shown that *uvrA umuC* cells, which are deficient in UV-radiation mutagenesis when assayed for base-substitutions, do show mutagenesis if photoreactivated after a post-UV-irradiation incubation (see Discussion). To determine whether this "delayed photoreactivation" mechanism also functions for frameshift reversion, UV-irradiated *uvrA umuC* cells carrying *trpE9777* or *trpA9813* were photoreactivated after various times of post-UV-irradiation incubation. Although delayed photoreactivation enhanced the UV-radiation-induced reversion of two ochre nonsense (i.e., base-substitution) mutations, *argE3* and *trpE65*, it reduced the UV-radiation-induced reversion of *trpE9777* and *trpA9813* in the same or related strains (Fig. 1).

Discussion

Kato and Nakano (1981) reported that the *trpA21, trpA540,* and *trpA9813* mutations are not reverted by UV radiation. We studied a higher dose range than that used by Kato and Nakano ($1-4\ Jm^{-2}$) and, while we did not detect reversion of *trpA540* (data not shown), we did detect significant UV radiation-induced reversion of the *trpA21* and *trpA9813* mutations, and the reversion frequencies were enhanced 10–50-fold by the presence of plasmid pKM101, depending on whether the strains are compared at equal doses or at equal-killing doses (Table 3). These results are in agreement with those for *S. typhimurium*, which indicate that UV radiation is generally capable of reverting frameshifts, especially in the presence of plasmid pKM101. Such reversions are presumably due to the intragenic induction of second frameshifts, which restore the proper translational reading frame, or they may be due to intergenic frameshift suppressor mutations (reviewed in Roth, 1974).

Glickman et al. (1980) reported that the *trpE9777* mutation was not revertible by ionizing radiation in *E. coli*. In contrast, we detected substantial γ radiation-induced reversion of *trpE9777* in the original strain (using 2 different samples received 5 years apart from Dr. C. Yanofsky; data not shown) and in another strain background (Table 2), and this reversion was enhanced 15–20-fold in the presence of plasmid pKM101 (Table 4). Since the data of Glickman et al. were for a strain that they constructed, we suggest either that *trpE9777* is not reverted by radiation in their strain background (KMBL 3835), or that their *trp* mutation is not *trpE9777*. As for UV radiation, we also detected γ radiation-induced reversion of the *trpA21* and *trpA9813* mutations, and this was enhanced by pKM101 (Table 4). These results, especially for the strains carrying plasmid pKM101, are in agreement with those for *S. typhimurium* (MacPhee and Schoeffel, 1981; Imray and MacPhee, 1981) and indicate that frameshift mutations in *E. coli* can be reverted by ionizing radiation.

How does one account for the fact that the *trpE9777* mutation is so much more revertible than the other 3 frameshift mutations that we have studied? Several possibilities can be discussed. First, +1 frameshift mutations (e.g., *trpE9777*, Fig. 2) often revert phenotypically through the production of frameshift suppressing mutations in tRNA genes (reviewed in Roth, 1974). Thus, the *trpE9777* strain could have additional sites for mutagenesis that might help explain its high sensitivity to reversion. However, external suppressors of *trpE9777* did not appear to play a role in our results (data not shown). Second, frameshift mutagenesis generally depends on sequences of repeated nucleotides that allow misalignment (Streisinger et al., 1966; Ripley, 1982; de Boer and Ripley, 1984). Streisinger and Owen

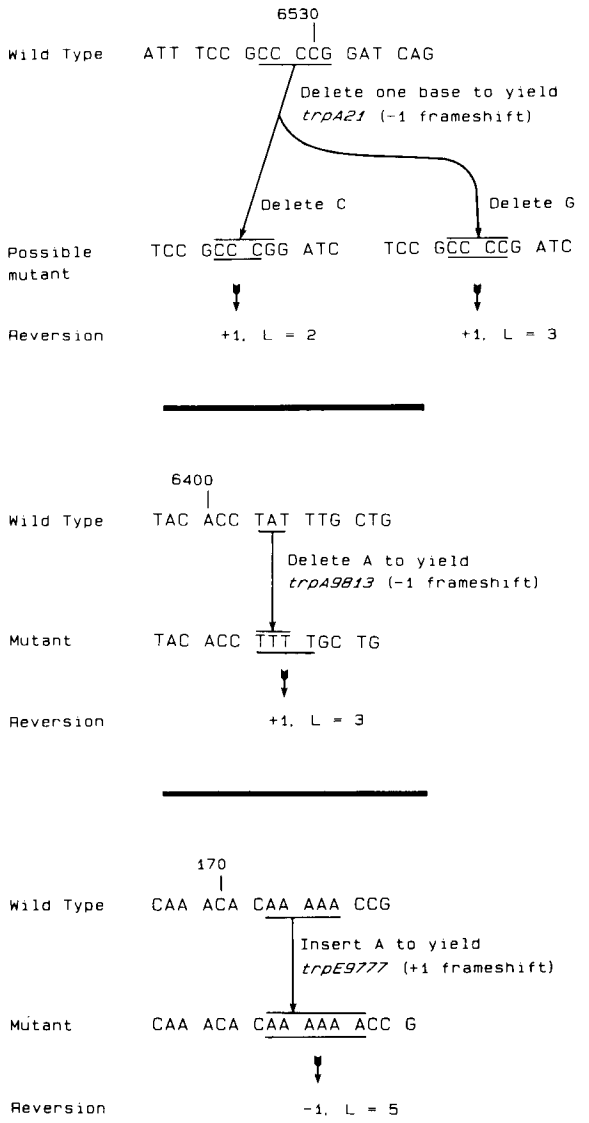

Fig. 2. Nucleotide sequences for *trpA21*, *trpA9813*, and *trpE9777* frameshift mutations. Wild-type *trpA* and *trpE* sequences are from Yanofsky et al. (1981). The *trpE9777* mutation is described by Bronson and Yanofsky (1974). The possible sequences for the *trpA21* and *trpA9813* mutations were determined by comparing the wild-type *trpA* sequence with data summarized by Siegel and Vaccaro (1978). Underlined sequences are potential sites for misalignment according to the work of Streisinger et al. (1966). Numbers associated with the underlined sequences indicate the more likely types of frameshift mutations that might occur at those sites to revert the strain to tryptophan prototrophy.

(1985) have shown that the frequency of spontaneous and proflavine-induced small additions and deletions, at a given site, are related to L, the maximum number of pairing bases in the misaligned stretch. Thus, for an increase in L from 3 to 4 or 4 to 5, a 20–150-fold increase in mutant frequency occurs at different sites in bacteriophage T4 (Streisinger and Owen, 1985). The possible L values are 2 or 3 for *trpA21*, 3 for *trpA9813*, and 5 for *trpE9777* (Fig. 2). These increasing L values correlate with the respective increasing radiation revertability of these mutations.

While the L value correlates with frameshift revertability, additional factors are relevant in frameshift mutagenesis. Miller (1985) showed a large variability in the susceptibility to UV-radiation-induced frameshifts in several runs of 4 or 5 AT pairs in the *lacI* gene, and concluded that the surrounding DNA sequence must play an important role in frameshift inducibility. Also in the *lacI* gene, UV radiation showed a 20-fold preference for the induction of -1 frameshifts over $+1$ frameshifts (Miller, 1985). Note that this last observation is also consistent with the hyper-revertability of *trpE9777* over the other frameshifts in our studies (Fig. 2). Therefore, one may conclude that both UV and γ radiation can revert frameshifts in *E. coli*, and that the DNA sequence of the *trpE9777* mutation provides several explanations for its high sensitivity to radiation-induced reversion.

The ability of pKM101 to enhance the radiation-induced reversion of frameshifts (Tables 3 and 4) underscores the requirement for the UmuDC proteins (Kato and Nakano, 1981; Sargentini and Smith, 1984) in this type of mutagenesis in *E. coli*. Bridges and Woodgate (1984, 1985) have shown that the need for the UmuDC proteins in UV radiation-induced base-substitution can be circumvented by photoreactivating UV-irradiated *E. coli umuC* cells after a post-irradiation-incubation period. They have suggested that RecA protein facilitates the misincorporation of nucleotides opposite noncoding DNA lesions, and that the UmuDC proteins somehow stabilize this misincorporation, and thus allow DNA synthesis to proceed beyond the misincorporated nucleotides. This model can also explain *umuDC*-dependent γ radiation base-substitution mutagenesis (Sargentini and Smith, 1984).

To explain the radiation induction of frameshifts, Streisinger and Owen (1985) have suggested that when DNA synthesis is blocked by DNA lesions, the gaps in the nascent DNA allow local denaturation and renaturation with strand misalignment. The larger the *L* value, the more stable the misalignment. Since in this model the replication complex still must get by the lesion that induced the gap, one can imagine that it is this step that accounts for the similar involvement of the *umuDC* and *mucAB* genes in the radiation induction of both base substitutions and frameshifts (Mortelmans and Stocker, 1976; Kato and Shinoura, 1977; Kato and Nakano, 1981). Consistent with this notion is the lack of involvement of these genes in frameshift mutagenesis caused by 9-aminoacridine or ICR191 (Thomas and MacPhee, 1985), which because of their property of DNA intercalation, are thought to increase frameshift fixation either by stabilizing DNA misalignments at spontaneous regions of single-stranded DNA (Roth, 1974), or by producing regions of lesion-free single-stranded DNA through *uvrB*-dependent excision repair (Rene et al., 1986).

If the role of the *umuDC* and *mucAB* genes in the radiation induction of frameshifts is to allow the bypass of a lesion [known to be a cyclobutane dipyrimidine in the case of *trpE9777* (Yamamoto, 1985)] that induced the gap that induced the frameshift, one could expect that delayed photoreactivation would facilitate UV-radiation frameshift induction in a *uvrA umuC* strain just as it facilitates base substitution. However, our results do not support this notion, and seem to indicate that UmuDC proteins may play a direct role in strand misalignment. Another possibility is that the UmuDC proteins function in the reinitiation of replication on a misaligned primer. This would be an essential part of frameshift mutation fixation, and would be independent of the problem of bypassing the gap-inducing lesion. We suggest that in the absence of the UmuDC proteins, that the replication complex more efficiently recognizes the distorted duplex formed by strand misalignment, and either doesn't reinitiate DNA synthesis or it may even correct it with the DNA polymerase-associated 3′–5′-exonuclease. This model does not exclude a second role for the UmuDC proteins in allowing lesion bypass at the gap-inducing lesion, as proposed by Bridges and Woodgate (1984, 1985).

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