Expression of Cloned Bacteriophage T4 *uvsW* and *uvsY* Genes in Rec^+ and Rec^- *Escherichia coli*

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Chimeric plasmids containing the *uvsY uvsW* region of the T4 genome were examined for the expression of these genes. Certain of these plasmids were shown to express the *uvsY* or the *uvsW* gene products by their ability to complement the UV sensitivity of infecting *uvsW* or *uvsY* mutant phage. Also, a chimeric plasmid containing both the *uvsW* and *uvsY* genes increases the survival of UV-irradiated, methyl methane sulfonate- or ethyl methane sulfonate-treated *recA* hosts.

T4 *uvsW* and *uvsY* are two mutants defective in a single recombination repair pathway for DNA damage (3, 8). These early genes map together between genes 24 and 25 in a region of the T4 genome otherwise occupied by late genes (28). Another gene, *uvsX* (9), which is also known to function in the same pathway (3), maps in the early region (28). T4 *uvsW* and *uvsY* are UV sensitive, recombination defective (3, 8), and sensitive to X and gamma rays (3, 26) and to a variety of chemical mutagens (1, 8, 16, 21, 30). The products of *uvsY* and *uvsW* (1206) have also been shown to be involved in an error-prone repair pathway leading to mutation induction (4, 7). Thus, the repair system that is defective in *uvsW* or *uvsY* mutants resembles in many ways the postreplication repair associated with the Rec system in *Escherichia coli*.

Cloning of restriction fragments of bacteriophage T4 has allowed the physical mapping of T4 genes (19) and facilitated the study of gene expression (14, 15, 23, 25, 27, 29). Because we have been interested in studying the functions of the *uvsY* and *uvsW* genes and ultimately hope to purify their products, we decided to determine whether these genes were present and active on chimeric plasmids carrying the *uvsW uvsY* region of the T4 genome.

In this study we show that *uvsW*⁺ and *uvsY*⁺ are present on certain of the chimeric plasmids examined and that they increase survival of UV-irradiated *uvsW* and *uvsY* mutant phage. These cloned genes also suppress certain RecA phenotypes when present in a *recA* host strain.

MATERIALS AND METHODS

Strains and plasmids. Bacteriophage T4⁺ (T4D) was originally supplied by A. H. Doermann. T4y⁺₁₀, derived from T4D, was obtained from J. Boyle. T4 *uvsW*

(m22), isolated by Hamlett and Berger (8), was supplied by J. Drake. The procedures and media used in the preparation of bacteriophage stocks and bacterial cultures were as described by Melamed and Wallace (16), except that M9 medium was routinely supplemented with thiamine.

The bacterial strains used are listed in Table 1.

Plasmid p656 was obtained from E. T. Young (University of Washington, Seattle) and carries an *EcoRI* restriction fragment of T4 cloned into the single *EcoRI* restriction site of pBR322. The plasmids p3.4, p1.3, p0.56, and p0.62 are subclones of p656 obtained after restriction with *HindIII*. These were also provided by E. T. Young. pKLM1 and pKLM4, provided by H. Krisch (Universite de Geneve, Switzerland), were constructed by inserting *BglII* restriction fragments of T4 into the unique *BamHI* site of pBR322. The T4 regions cloned are diagrammed in Fig. 1. Restriction sites are as reported by O'Farrell et al. (19).

Plasmid DNA was isolated for transformation by the rapid procedure of Kado and Liu (11). Transformation of plasmids into *E. coli* strains was carried out as described by Davis et al. (5).

Marker rescue. T4 genes carried on the hybrid plasmids were determined by marker rescue with the spot test procedure of Mattson et al. (15), and the T4am tester strains listed in Table 2 were obtained from J. Wood's laboratory.

Complementation of UV sensitivity of T4 *uvsW* and *uvsY* phages. In experiments with *uvsW* bacteriophages, plasmid-bearing bacterial strains were grown to log phase in 5 ml of M9 medium containing ampicillin (40 µg/ml). Cells were collected by centrifugation and then suspended in 10 ml of M9 medium containing ampicillin, but lacking Casamino Acids, and incubated overnight at 37°C with aeration to amplify the plasmids (2). Cells were again collected by centrifugation, suspended in H broth containing ampicillin (60 µg/ml), and incubated for 1.5 to 2 h at 37°C with aeration.

Bacteriophage were suspended in gel buffer (2.1 mM Na₂HPO₄, 1.1 mM KH₂PO₄, 6.8 mM NaCl, 2.9 mM K₂SO₄, 1.0 mM MgSO₄ · 7H₂O, 0.12 mM CaCl₂, 0.001% gelatin) at a titer of about 2 × 10⁹/ml and UV irradiated with a General Electric 15-W germicidal lamp at various doses as reported below. Dilutions

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TABLE 1. Bacterial strains

Strain	Relevant markers	Source
<i>E. coli</i> B	Sup ⁰	Laboratory collection
<i>E. coli</i> K-12 AB1157	<i>thr leu pro his arg thi rpsL sup-37</i> <i>supE44</i>	<i>E. coli</i> Genetic Stock Center
AB2463	<i>recA13</i> derivative of AB1157	<i>E. coli</i> Genetic Stock Center
CSR603	<i>thr leu pro phr-1 recA1 arg thi</i> <i>uvrA-6 rpsL supE44 gyrA</i>	<i>E. coli</i> Genetic Stock Center
M152	<i>recA3 rpsL</i> Sup ⁰	<i>E. coli</i> Genetic Stock Center
ED8689	<i>hsdR trpR</i> Sup ⁰	Patrick O'Farrell
K802	<i>hsdR met supII</i>	Geoffrey Wilson
RR1	<i>pro leu thi hsdR hsdM rpsL supE44</i>	Geoffrey Wilson

were then plated on the various plasmid-bearing strains in 0.4% Ionagar top agar on tryptone bottom agar plates.

For T4 *uvsY* experiments the protocol was essentially the same, except that the plasmids were not amplified by amino acid starvation. Freshly grown midlog-phase cultures of the plasmid-bearing strains were used as plating bacteria. Since the T4 *uvsY* mutant is an amber mutant, suppressor-negative bacterial strains were used.

UV sensitivity of plasmid-bearing strains of *E. coli*. Strains were grown to log phase in M9 medium plus ampicillin (40 µg/ml), and then plasmids were amplified by amino acid starvation as described above or by adding chloramphenicol to a concentration of 100 µg/ml and growing overnight at 37°C. Cells were then collected by centrifugation, washed with buffer to

remove chloramphenicol, suspended in H broth containing ampicillin (60 µg/ml), and incubated for 1.5 h at 37°C. The cells were again collected by centrifugation and suspended in gel buffer to a cell density (optical density at 600 nm) of approximately 0.1. These were incubated for 20 to 30 min at 37°C and then UV irradiated at various doses as reported below. Samples were diluted in buffer and plated in top agar on tryptone agar. Cells were irradiated in the dark and plated with a low level of illumination.

MMS sensitivity of plasmid-bearing strains of *E. coli*. The *E. coli* strains were grown and plasmids were amplified by amino acid starvation as described above. After overnight starvation, the cells were grown in 5 ml of H broth with ampicillin (60 µg/ml) for 1 h, collected by centrifugation, and suspended in 3 ml of M9 medium or M9 buffer (M9 medium lacking glucose

FIG. 1. (A) T4 restriction fragments from the late region of the T4 map from 100 to 120 kilobases cloned into pBR322. (B) Subclones of p656. Restriction map data are from O'Farrell et al. (19).

TABLE 2. Marker rescue of T4 genes from hybrid plasmids

T4 gene	Mutant <i>am</i>	Marker rescue of T4 genes from the following plasmids:						
		p656	p3.4	p1.3	p0.56	p0.62	pKLM1	pKLM4
29	B7	—	—	—	—	—	—	+
	B6	+	+	—	—	NT ^a	NT	NT
28	A452	+	+	—	—	—	—	+
27	N120	+	+	—	—	—	—	+
51	S29	+	+	—	—	—	—	+
26	S105	+	NT	—	—	—	NT	NT
	NG114	+	NT	—	—	NT	NT	NT
	N131	+	+	—	—	—	—	+
25	S52	+	—	+	—	NT	NT	NT
	NG216	+	—	+	—	NT	NT	NT
	NG473	+	—	+	—	NT	NT	NT
	NG67	+	—	+	—	—	—	+
	NG91	+	NT	—	—	NT	NT	NT
	NG542	+	NT	—	—	NT	NT	NT
24	N65	—	—	—	—	—	+	—
23	H11	—	NT	NT	NT	NT	+	—
22	B270	—	NT	NT	NT	NT	+	—

^a NT, Not tested.

and Casamino Acids). Then 0.5-ml samples of the cell suspensions were mixed with 0.5 ml of M9 buffer containing various concentrations of methyl methane sulfonate (MMS) as reported below. These were allowed to stand at room temperature for 1 h, diluted in buffer, and plated on tryptone agar.

EMS sensitivity of plasmid-bearing strains of *E. coli*. The *E. coli* strains were grown and plasmids were amplified as in MMS experiments, but the cells were suspended in minimal salts buffer (0.2 M Tris [pH 7.5] 10.5 g of K₂HPO₄, 4.5 g of KH₂PO₄, 1 g of (NH₄)₂SO₄, and 0.5 g of sodium citrate · 2H₂O per liter) (17). Various concentrations of ethyl methane sulfonate (EMS) were added to samples of the cell suspensions. These were incubated at 37°C for 2 h, diluted in buffer, and plated on tryptone agar.

RESULTS

Identification of T4 baseplate and head genes that flank the *uvsW uvsY* region of the T4 genome. Since there is no simple method for identifying *uvsW*⁺ or *uvsY*⁺ activity on cloned fragments, we determined that the hybrid plasmids carried neighboring essential genes by doing marker rescue tests with amber mutants in a number of baseplate and head genes. This also ensured that the T4 fragments were not deleted from the pBR322 vector when plasmids were transferred to various host strains. The results of the marker rescue tests are listed in Table 2, and the genes carried on the various plasmids are shown in Fig. 1. p656 and pKLM4 contain gene 25, which

flanks *uvsY*, but not gene 24, whereas pKLM1 contains gene 24, which flanks *uvsW*, but not gene 25. Subclone p1.3 also contains gene 25. From these data, as well as from the restriction mapping data, p656, p0.56, and pKLM1 are presumptive carriers of *uvsW*, whereas p656 and p1.3 are presumptive carriers of *uvsY*.

Survival of UV-irradiated *uvsW* and *uvsY* mutant phage on plasmid-bearing bacterial strains. Bacteriophage T4 *uvsW* and *uvsY* mutants are more sensitive to UV irradiation than are wild-type phage (3, 8). We wanted to know whether the wild-type alleles of these genes present on plasmids in the *E. coli* host strains could affect survival of UV-irradiated mutant phage.

In early experiments with T4 *uvsW*, UV-irradiated phage were simply plated on midlog phase cultures of strains carrying plasmids thought to carry the *uvsW*⁺ allele (p656, p0.56, and pKLM1) and on strains carrying control plasmids (p3.4, p1.3, p0.62, and pKLM4)—that is, plasmids carrying T4 DNA outside of the region where *uvsW* was genetically mapped (8). Bacteriophage survival after irradiation was enhanced in strains carrying p656 or pKLM1 and somewhat less in those carrying p0.56. The effects were small and variable, however, so we attempted to increase the level of plasmid-encoded gene products by amplifying the level of plasmids in the strains.

FIG. 2. UV inactivation of T4 *uvrW* in *E. coli* hosts bearing various hybrid plasmids. (A) Symbols: ■, pKLM4/RR1; ○, p656/RR1; ▽, pKLM1/RR1; X, T4⁺ averaged data. (B) Symbols: □, p1.3/RR1; △, p0.56/RR1; ○, p656/K802.

We first tried amplification by chloramphenicol treatment, but this did not give satisfactory results because of the loss of viability that accompanies such treatment. We then tried amplification by amino acid starvation, which has been reported to differentially increase the rate

of synthesis of the products of cloned genes after the end of starvation (2).

Typical survival curves of UV-irradiated T4 *uvrW* and wild-type phage when plated on various plasmid-bearing strains are shown in Fig. 2A and B. Strains with plasmids carrying the *uvrW*⁺ allele do enhance the survival of *uvrW* mutant phage, but not wild-type phage.

When experiments with UV-irradiated *uvrY* phage were done, it was necessary to use amber suppressor-free hosts (ED8689 and M152) since the *uvrY* mutant is an amber mutant. The strains have no amino acid requirements, so *uvrY* phage were plated on nonamplified cultures. Plasmids presumed to carry the *uvrY*⁺ allele (p656 and p1.3) clearly enhanced the survival of UV-irradiated *uvrY* phage (Fig. 3).

Survival of UV-irradiated plasmid-bearing Rec⁻ and Rec⁺ *E. coli* strains. It has been shown previously that *uvrW* and *uvrY* function in a single DNA repair pathway (8). Symonds et al. (24), have noted that the repair system which is impaired in *uvrY* mutants resembles the post-replication repair now known to be associated with the RecA system in *E. coli*. Therefore, we were interested in finding whether cloned *uvrW*⁺ and *uvrY*⁺ genes have any effect on survival of *recA* bacterial strains.

FIG. 3. UV inactivation of T4 *uvrY* in an *E. coli* host strain (ED8689) bearing various hybrid plasmids. Symbols: ○, p656; □, p1.3; △, p0.56; ●, p3.4.

FIG. 4. UV inactivation of *E. coli recA3* (M152) bearing various hybrid plasmids. Symbols: ○, p656; □, p1.3; △, p0.56; ●, p3.4; ▲, p0.62.

Experiments were carried out with three different *Rec*⁻ strains (M152, AB2463, and CSR603) carrying different *recA* alleles. One strain, CSR603, also carries a mutation in *uvrA* and is extremely UV sensitive. All of the *recA* strains tested showed increased survival after UV irradiation if they were carrying p656, which carries both *uvsW*⁺ and *uvsY*⁺. None of the other plasmids tested (pKLM1 was not tested) had this effect on survival. Survival curves for two of the *recA* strains are shown in Fig. 4 and 5. When the same experiment was done in a *Rec*⁺ background (RR1 or AB1157) there was no protective effect of p656 (Fig. 6); in fact, *Rec*⁺ strains carrying p656 consistently seemed more UV sensitive than did the other plasmid-bearing strains.

Effect of plasmids on survival of *Rec*⁻ bacterial strains after treatment with MMS or EMS. MMS and EMS are both DNA-damaging agents, and MMS is known to act as a mutagen through the SOS system in *E. coli*. (12). When the *RecA* strain AB2463 bearing various plasmids was treated with MMS (Fig. 7A) or EMS (Fig. 7B), we found that the presence of p656, which carries both *uvsW*⁺ and *uvsY*⁺, was associated with increased survival.

DISCUSSION

We have shown that the *uvsW*⁺ and *uvsY*⁺ genes are present on certain *EcoRI*, *HindIII*, and *BglIII* restriction fragments of T4 cloned into pBR322. The genes are active on these plasmids in that they enhance survival of UV-irradiated mutant phage.

Complementation was more efficient with *uvsY*⁺ than with *uvsW*⁺, which could be due to a number of reasons, including differential expression or stability of *uvsY* and *uvsW* proteins in the plasmid-bearing strains or perhaps to differences in plasmid copy number in the various hosts used. Previously the cloned *denV*⁺ gene of T4 was shown to increase resistance to UV irradiation of T4 *denV* mutants (14), and recently Takahashi and Saito (25) found that a cloned T4 fragment carrying *uvsW* and *uvsY* increased survival of MMS-treated mutant phage.

It is interesting that the cloned *uvsW*⁺ and *uvsY*⁺ genes are also able to increase the surviv-

FIG. 5. UV inactivation of *E. coli recA1 uvrA phr-1* (CSR603) bearing various hybrid plasmids. Symbols: ○, p656; □, p1.3; △, p0.56; ■, pKLM4.

FIG. 6. UV inactivation of *E. coli* *recA*⁺ (RR1) bearing various hybrid plasmids. Symbols: ○, p656; □, p1.3; △, p0.56; ●, p3.4.

al of UV-irradiated, MMS- and EMS-treated *recA* bacterial host strains. Although the *denV* gene product was shown to increase the survival of UV-irradiated, T4-infected *uvrA*⁻ hosts (6, 10) and cloned *denV*⁺ was shown to enhance the survival of *uvrA*⁻ strains bearing this plasmid (14), the T4 *uvrX* gene product did not affect the survival of UV-irradiated or MMS-treated, T4-infected *recA*⁻ hosts (18). Since the T4 *uvrX* gene product acts in the same pathway as those of *uvrY* and *uvrW*, it might be expected the *uvrX*⁺ might complement *recA* in the same manner as *uvrY*⁺ and *uvrW*⁺, especially in light of the fact that *E. coli* *recA* protein has been shown to function for the T4 *uvrX* product during multiplicity reactivation (20). It is possible that less *uvrX* gene product was produced in phage-infected cells than in *uvrY* and *uvrW* plasmid-bearing hosts or that these two observations reflect actual differences in protein function.

Both *uvrY*⁺ and *uvrW*⁺ genes appear to be required for the observed increase in survival of *recA* *E. coli* treated with UV, MMS, or EMS since only *recA* strains bearing p656 exhibit this phenotype, whereas strains bearing p1.3 (*uvrY*) or p0.56 (*uvrW*) do not. Although *uvrW* and *uvrY* are known to function in genetic recombination and repair, their gene functions have not yet been determined. The fact that p656 suppresses both MMS- and EMS-induced killing implies that the phage gene products are complementing a recombinational aspect of RecA protein function rather than an SOS function, since

FIG. 7. EMS (A) and MMS (B) inactivation of *E. coli* *recA* (AB2463) bearing various hybrid plasmids. Symbols: ○, p656; △, p0.56; ●, p3.4; ■, pKLM4.

MMS-induced mutagenesis is almost entirely dependent on RecA induction, whereas only a minor component of EMS mutagenesis requires RecA function (13, 22). Whether these genes are protective in *recA* *E. coli* by providing another repair capacity, by inhibiting DNA breakdown, or by some other mechanism awaits further study.

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