ISOLATION AND CHARACTERIZATION OF CONDITIONAL ALLELES OF BACTERIOPHAGE T4 GENES *uvsX* AND *uvsY*

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ABSTRACT

The bacteriophage T4 *uvsW*, *uvsX* and *uvsY* gene functions are required for wild-type levels of recombination and for normal survival and mutagenesis after treatments with ultraviolet (UV) and ionizing radiations. The ability of *uvsX* and *uvsY* mutations to suppress the lethality of gene *49* mutations was used to select temperature-sensitive and amber alleles of these two genes. (*uvsW* mutations do not suppress gene *49* mutations.) A simple and powerful complementation test was developed to assist in assigning *uvs* mutations to genes. The amber alleles of *uvsX* and *uvsY* behave as simple null alleles, fully suppressing a gene *49* defect, enhancing UV killing and abolishing UV mutagenesis. However, the properties of the *ts* alleles of *uvsX* and *uvsY* demonstrated that suppression of a gene *49* defect, sensitivity to UV-induced inactivation and UV mutability can be partially uncoupled. These results prompt the hypothesis that radiation mutagenesis occurs during DNA chain elongation past template damage within a recombinational intermediate rather than within a conventional replication fork.

ERROR-prone repair denotes an enzymatic process by which the lethality of induced or spontaneous lesions in DNA is circumvented in a manner that fails to achieve the original nucleotide sequence, thus producing mutations. A wide variety of organisms from bacteriophages to mammals exhibit error-prone repair (DRAKE and BALTZ 1976). Here we describe the isolation and properties of conditional mutations of the error-prone repair system of bacteriophage T4.

Error-prone repair in T4 is mediated by at least three genes: *uvsW*, *uvsX* and *uvsY* (CONKLING, GRUNAU and DRAKE 1976; DRAKE 1982). *uvsX* maps between genes *41* and *42* (CHILDS 1980), whereas *uvsY* and *uvsW* map between genes *24* and *25* (MAYNARD SMITH and SYMONDS 1973; HAMLETT and BERGER 1975). UV or γ irradiation of *uvsW*, *uvsX* or *uvsY* mutants typically reveals decreased mutagenesis and increased inactivation (GREEN and DRAKE 1974; CONKLING, GRUNAU and DRAKE 1976; this report), and the mutants also exhibit decreased recombination frequencies and burst (and plaque) sizes (*e.g.*, HAMLETT and BERGER 1975). Mutations in *uvsX* and *uvsY* lead to an arrest of DNA synthesis about halfway through the latent period, whereas mutations in

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uvsW permit rates of DNA synthesis equal to or greater than those of the wild type (HAMLETT and BERGER 1975; CUNNINGHAM and BERGER 1977; MELAMEDE and WALLACE 1980).

The assignment of *uvsW*, *uvsX* and *uvsY* to a common repair pathway is based on experiments showing that their mutant alleles are mutually epistatic for sensitivity to UV-induced inactivation (BOYLE 1969; HAMLETT and BERGER 1975; WAKEM and EBISUZAKI 1981). However, the same mutations often exhibit incompletely additive effects upon recombination (HAMLETT and BERGER 1975; DEWEY and FRANKEL 1975; CUNNINGHAM and BERGER 1977), suggesting that the system is functionally complex.

DEWEY and FRANKEL (1975) isolated extragenic suppressors of gene *49* at two loci, *fdsA* and *fdsB*. Gene *49* mutants accumulate highly branched structures, presumably intermediates in recombination, that cannot be packaged (KEMPER and BROWN 1976), and gene *49* specifies endonuclease VII, which resolves such recombinational intermediates (MIZUUCHI *et al.* 1982). *fdsA* maps between genes *41* and *42* and fails to complement the arrest of DNA synthesis caused by a *uvsX* mutation, whereas *fdsB* maps between genes *24* and *25* and fails to complement a *uvsY* mutation. This led CUNNINGHAM and BERGER (1977) to conclude that *fdsA* and *fdsB* were synonymous with *uvsX* and *uvsY*, respectively; thus, the suppression of gene *49* mutations would result from reduced formation of recombinational intermediates and a correspondingly greater efficiency of packaging. We have confirmed these assignments using an alternative complementation test and have also shown that *uvsW* mutations do not suppress gene *49* mutations. The suppression of gene *49* defects was used as a selective device, and conditional (amber and temperature-sensitive) alleles of genes *uvsX* and *uvsY* were isolated and examined for sensitivity to UV-induced inactivation and mutagenesis, as well as ability to suppress the gene *49* mutation. Our analysis of the *ts* mutants demonstrates that the previously fully coupled phenotypes of suppression of gene *49* mutations, increased sensitivity to UV inactivation and decreased sensitivity to UV mutagenesis can be partially dissociated.

MATERIALS AND METHODS

Bacterial and phage strains: These are described in Tables 1 and 2, respectively.

Media and general methods: Bacteria were grown in L broth (10 g Bacto tryptone, 5 g Bacto yeast extract, 5 g NaCl and 1.1 g glucose per liter) or in M9CA medium (3 g KH_2PO_4, 6 g Na_2HPO_4, 1 g NH_4Cl, 3.5 g NaCl, 0.16 mg $FeCl_3$, 133 mg $MgSO_4$ and 4 g glucose per liter, pH 7.0, supplemented with Difco casamino acids to 0.8%). Phage stocks were grown by stabbing with paper strips from a 4- to 6-hr plaque into 5 ml of cells at 10^8/ml, incubating 6 hr in a rotary water bath and completing lysis with chloroform. Phage for plating were diluted in D broth (2 g Bacto tryptone and 5 g NaCl per liter). All plating was done on Drake agar (10 parts Bacto tryptone, 1 part Bacto yeast extract, 5 parts NaCl, 0.2 parts glucose and 10 parts Bacto agar). Bottom agar contained 26.2 g Drake agar per liter, 35 ml per plate. The soft agar overlay contained 17 g Drake agar per liter, 2.5 ml per plate. The super-soft agar overlay contained 10.7 g Drake agar per liter, 2.5 ml per plate. Phage were assayed on BB cells at 37° unless otherwise specified. For UV irradiation, phage were suspended in M9S buffer (M9 without $MgSO_4$, glucose or casamino acids).

Irradiation of extracellular phage particles: For UV irradiation, phage were diluted 100-fold into M9S and irradiated in a 100-mm watch glass with constant agitation by using a Champion 15 watt

TABLE 1

E. coli *strains*

Strain	Relevant characteristics	Reference
B	Reveals most *r* mutants	BENZER (1955)
BB	Reveals *rI* but not *rII* mutants	
KB	K12(λ), restricts growth of *rII* mutants	
CR63	K12 amber suppressor (*suI*$^+$); reveals *rI* but not *rII* mutants	BRENNER and BECKWITH (1965)
XA101c(λ)	Amber suppressor (*suI*$^+$)	MILLER *et al.* (1977)
XA90Nc(λ)	Nonsuppressor isogenic with XA101c(λ)	

TABLE 2

T4 strains

Strain or genotype	T4 background	Relevant characteristics	Reference
T4B	B	Wild type	BENZER (1955)
T4D	D	Wild type	DOERMAN and HILL (1953)
x	D	Canonical *uvsX* mutant	HARM (1963)
px	B	"Purified" (backcrossed) *x*	DRAKE (1973)
y	B	Canonical *uvsY* mutant backcrossed into B	BOYLE and SYMONDS (1969)
v	B	Canonical *uvsV1* mutant backcrossed into B; lacks exonuclease V	HARM (1963); YASUDA and SEKIGUCHI (1970)
m22, m33	D	Canonical *uvsW* mutants	HAMLETT and BERGER (1975)
amE727x1	D	Gene *49* amber mutant	DEWEY and FRANKEL (1975)
tsC9	D	Gene *49 ts* mutant	
fdsA2, fdsB7	D	Extracistronic suppressors of gene *49* mutants	
rUV7, rUV13	B	*rII* mutants reverting by G:C → A:T transitions	DRAKE (1963)
rUV183, rUV199	B	*rII* mutants reverting by A:T → G:C transitions	
rUV58, rUV113	B	*rII* mutants reverting by frameshift mutation	

low-pressure mercury germicidal lamp at a dose rate of about 0.25 J m^{-2} sec^{-1} and were plated under dim yellow light to avoid photoreactivation. For γ irradiation, phage were suspended in 2% Bacto nutrient broth and exposed to a ^{137}Cs source at a dose rate of about 3560 rad/min as determined by ferrous sulfate dosimetry (H. DUCOFF, personal communication). Irradiated stocks were stored at 4° and assayed within 1 week, during which time no change in viability or mutant frequency could be detected.

Measuring survival and mutagenesis in irradiated phage: Phage were preadsorbed to, and then plated on, either B or BB cells and incubated at 37° unless otherwise indicated. For preadsorption, phage were mixed with log-phase cells at 3–5 × 10^8/ml at ratios of fewer than 0.1 total particles

per cell, incubated at 37° (unless otherwise specified) for 10 min and then plated. In control experiments, B and BB cells gave equal efficiencies of plating for irradiated stocks. Lethal hits were determined from the linear portions of survival curves (log S *vs.* dose), one hit being the dose required to reduce survival by the factor e^{-1}.

To score $r^+ \rightarrow r$ mutations, control and irradiated samples were preadsorbed to and plated on B cells using super-soft agar overlays and incubated at 37° for 12–16 hr. Under our plating conditions, preadsorption and the use of super-soft agar overlays optimize plaque morphologies and the detection of *r* mutants in irradiated samples. Plates screened for mutants had fewer than 500 plaques, since the efficiency of detection of *r* plaques (especially mixed plaques) decreased above 600 plaques per plate. Since a large proportion of induced *r* mutants appear in aberrant, mottled plaques, all plaques exhibiting a possible *r* component were picked and replated on B cells; generally about 70% of the isolates contained *r* mutants. At least 20,000 plaques were screened to obtain each *r* frequency. Since the efficiency of *r* detection may vary from day to day, only samples screened on the same day were directly compared. Mutants were induced with single-hit kinetics.

The methods described above were used for all measurements except those involving temperature-sensitive (*ts*) and amber (*am*) mutations of *uvsX* and *uvsY*, which often resided in gene *49*$^-$ backgrounds. These mutants required different host cells and different incubation conditions. Survival frequencies of control and irradiated samples of the *ts* mutants were determined by preadsorbing to CR63 cells at either 32° or 42° for 10 min and plating the complexes on CR63 cells. (CR63 cells suppressed the gene *49* amber allele present in the *uvsts* mutants, and 32° was permissive for the gene *49 ts* allele present in the *uvsam* mutants.) Plates were incubated at either 32° or 42° for 2 hr, shifted to 37° and incubated an additional 10–14 hr. This temperature shift optimized and standardized plaque morphologies; control experiments revealed no change in survival frequencies when the temperature was shifted later than 2 hr. Unirradiated control samples plated with the same efficiencies on CR63 cells at both 32° and 42°; but see CONKLING and DRAKE (1984) concerning plating efficiencies of irradiated T4 at different temperatures.

Mutation frequencies of the *ts* mutants of *uvsX* and *uvsY* were determined for the $r^+ \rightarrow rI$ pathway; *rI* mutants exhibit an R plaque phenotype on CR63 cells, whereas *rII* mutants exhibit an R^+ phenotype. Samples were plated under conditions similar to those described above, but on CR63 cells, and were incubated at either 32° or 42° for 2 hr prior to shifting to 37°.

Survival frequencies for the *uvsam* mutants were determined by preadsorbing to the isogenic suppressing and nonsuppressing hosts XA101c(λ) and XA90Nc(λ), respectively, and then plating at 32° (a temperature permissive for the gene *49 ts* allele present in the background). Unirradiated control samples plated with the same efficiencies on both hosts at 32°.

Mutation frequencies of the amber mutants of *uvsX* and *uvsY* were determined for the $r^+ \rightarrow rI$ pathway; *rI* mutants produce an R plaque phenotype on the XA hosts, whereas *rII* mutants are excluded by the λ prophage. Samples were screened as described above but were plated on XA101c(λ) or XA90Nc(λ) at 32°.

Constructing rII uvs *double mutants*: Log-phase BB cells were mixed with parental *rII* and *uvs* mutants at phage to cell ratios of 1 and 5, respectively. The complexes were diluted 50-fold into L broth at 10 min and incubated at 37° for an additional 50 min before completing lysis with chloroform. The progeny were plated on B cells and small *r* plaques were picked. These isolates were screened for UV sensitivity and for their ability to recombine with the parental *rII* mutant: isolates with increased UV sensitivity failing to recombine with the parental *rII* mutant were *rII uvs* double mutants.

Plate-screening technique for UV sensitivity: Certain experiments required us to screen many plaques for relative UV sensitivity. Therefore, a plate-screening technique was developed that could distinguish sensitive and resistant phages. Log-phase BB cells concentrated to 10^9/ml in M9S were mixed with phage at a phage to cell ratio of about 10^{-6}. After about 10 min, 0.3 ml of the mixture was plated in the standard manner. These plates were incubated for 4 hr at 37°, irradiated with about 72 J/m^2 and reincubated at 37° for 18 hr. Plaques from phage with increased UV sensitivity were small relative to plaques from phage with wild-type sensitivity. Reconstruction experiments demonstrated that UV-sensitive plaques could be distinguished among wild-type plaques at frequencies of about 1%, whereas wild-type plaques could be distinguished among UV-sensitive plaques at frequencies of about 0.1%.

Recombination complementation tests: The ability of new *uvs* alleles to complement the recombi-

nation-defective phenotype of reference alleles of *uvsX* or *uvsY* was measured in recombination tests between two *rII* mutants, *rUV7* and *rUV183*. Log-phase BB cells at 5×10^8/ml were infected with an average of five phages of each genotype per cell. After 10 min at 37° the complexes were diluted 50-fold into L broth and incubated an additional 50 min in a rotary shaker water bath before completing lysis with chloroform. Progeny were assayed on BB cells for total phages and on KB cells for rII^+ recombinants.

UV survival complementation tests: The ability of new *uvs* alleles to complement the reference *uvsX* or *uvsY* alleles was also measured as restoration of wild-type UV sensitivity. Log-phase BB cells, concentrated in L broth to 10^9/ml, were mixed with an equal volume of parental phage; in uniparental controls the average phage to cell ratio was 10, whereas in biparental tests each phage was applied at a ratio of 5. After 10 min at 37° the mixture was diluted 100-fold into iced M9S and was immediately irradiated at a dose rate of about 2.9 J m^{-2} sec^{-1} in a 100-mm watch glass with constant agitation. Unirradiated and irradiated samples were immediately assayed for survival using BB-plating cells and super-soft agar overlays. All manipulations were performed within 18 min of the initial infection; no bursts were detected within this time.

Linkage analysis of new uvsX *and* uvsY *alleles*: The linkage of new *uvs* mutations to loci defined by canonical mutations was made by crossing each new *uvs 49*$^-$ double mutant with double mutants containing reference *uvsX* or *uvsY* mutations plus the same gene *49* mutation, using phage to cell ratios of 5 for each parent. The crosses were conducted, and their progeny plated, under conditions that were permissive for the gene *49* allele (CR63 cells for *amE727x1* and 32° for *tsC9*), and thus expected to be permissive for all progeny genotypes. To distinguish *uvs*$^+$ segregants among the several *uvs*$^-$ progeny classes, isolated plaques were picked and scored for suppression of the *49*$^-$ mutation by spot testing under conditions that were nonpermissive for the single *49*$^-$ mutant: *uvs*$^+$ segregants fail to grow under such conditions.

Hydroxylamine mutagenesis: Phage were suspended in freshly prepared solutions having final concentrations of 1.39 M $NH_2OH \cdot HCl$, 1.4 M NaCl, 13 mM Na_2HPO_4 and 4 mM $MgCl_2$, pH 6.0. Treatment was at 47° and was terminated by diluting at least 50-fold into iced D broth. Surviving and mutated phages were assayed immediately following the treatment.

Effect of UV on reversion: Most studies of radiation-induced mutagenesis in phage T4 have monitored $r^+ \rightarrow r$ forward mutation rates. This requires the visual screening of many plaques and, for optimal accuracy, the isolation and retesting of all apparent mutants. A system assaying revertants by differential plating might, therefore, offer some advantages. Encouraged by reports of such a system (YAROSH 1978; YAROSH *et al.* 1980; MUFTI 1980), we obtained the phage and host strains used by these workers. As we explored the system, however, we encountered difficulties.

All differential plating systems harbor the danger that plating efficiencies may differ at different plating densities or with different hosts or media. Therefore, we first tested whether UV-irradiated phages, plated at varying concentrations on the selective host, exhibited a constant revertant frequency. For the initial tests we used the gene *51* mutant *amS1* because its spontaneous and induced revertant frequencies were the highest among the three tester strains, thus reducing the numbers of plates required for scoring. (We first detemined by a reconstruction experiment that this high frequency was not due to selection for *am*$^+$ revertants during the growth of stocks. Care was also taken to use stocks with low spontaneous revertant frequencies.) Some typical results are shown in Table 3. In this table, the "host cell index" is both the cell concentration in the preadsorption tube and the number of cells per plate. Plating mode A refers to the direct-plating conditions generally used in this laboratory, mode B to conditions similar but not necessarily identical with those described by the developers of the system, mode C to experiments in which the phages were passaged through a permissive host before assaying revertants and mode A′ to experiments in which cells were added to phage, rather than phage to cells, in the adsorption vessel. The accuracies of the measurements were limited by the numbers of revertants counted, which are indicated in the final column in parentheses.

Several points became clear from experiments such as 1, 4, 6 and 9. First, although *am*$^+$ frequencies increased following UV irradiation, most of this increase disappeared at the lower phage to cell ratios. Second, *am*$^+$ frequencies in irradiated populations decreased with the decreasing phage to cell ratios in both plating modes (A and B). Third, this decrease in *am*$^+$ frequency largely disappeared if the cells were added to the phage in the adsorption vessel (mode A′), rather than the phage to the cells (mode A).

Experiments such as 11 and 12 tested whether passaging irradiated phages through permissive

TABLE 3

Revertant frequencies after UV irradiation

Tester strain[a]	Experiment	Host cell index[b]	Plating mode[c]	Surviving fraction[d]	Phage to cell ratio[e]	Apparent revertant frequency (total am^+ or r^+ plaques)[f]
amS1	1	6.1	A	1.0	0.044	24 (252)
				0.0020	0.041	140 (29)
					0.0041	36 (3)
			A′		0.041	120 (25)
					0.0041	95 (8)
	4	6.8	A	1.0	0.038	32 (450)
					0.0038	18 (273)
				0.0017	0.038	230 (49)
					0.0038	56 (2)
			A′	1.0	0.038	27 (380)
					0.0038	16 (236)
				0.0017	0.038	160 (35)
					0.0038	170 (6)
	6	20	B	1.0	0.011	9 (78)
					0.0011	10 (86)
				0.0052	0.011	23 (39)
					0.0011	14 (6)
	9	12	B	1.0	0.049	18 (662)
				0.0046	0.049	92 (205)
					0.0061	34 (35)

[a] Mutation *amS1* resides in gene *51* and *rUV375* in *rIIB*. Deletion *r1605* has its left end in *rIIA* and its right end in *rIIB*; "*r1605*/r^+" indicates an artificial mixture with the wild type.

[b] The host cell index indicates the cell concentration, in units of 10^8/ml, in the adsorption tube. For plating modes A, A′, B and D it also indicates the total number of nonpermissive cells, in units of 10^8, per plate.

[c] In general, one part of phage and nine parts of cell suspension were mixed with vigorous hand swirling and warmed toward the temperature of the soft-agar water bath, usually about 46°–48°; 20 parts of melted soft agar at about 52° were added within a minute or less; and 3-ml aliquots were plated through a large-bore pipette using an automatic pipetting device, all within about 18 min after the first mixing. The plates were incubated at 30°–31° overnight and were examined with care when plaque densities were very low in order to avoid being misled by bubbles, small plaque sizes and odd bits of debris. Plating mode A: BB nonpermissive cells, CR63 permissive cells, L broth and Drake agars, phage added to cells in adsorption vessel. Plating mode A′: same as A except cells added to phage in adsorption vessel. Plating mode B: S/6/5 nonpermissive cells, CR63 permissive cells, H broth and Hershey agars. Plating mode C: passaging protocol of YAROSH (1978); adsorption exceeded 99%, and burst sizes ranged from 83 to 790. Plating mode D: same as B, but K594(λ) nonpermissive cells.

[d] A surviving fraction of 1.0 indicates no UV irradiation. The irradiation buffer of YAROSH (1978) was used.

[e] Total phage particles (whether alive or UV inactivated) per cell as determined from plaque assays of unirradiated phage and colony assays of cells but uncorrected for plating efficiency difference of 0.9–1.1 between permissive and nonpermissive hosts as determined for each experiment using wild-type T4B.

[f] Apparent revertant frequencies per 10^7 survivors or progeny, uncorrected for plating efficiency differences. Total numbers of am^+ or r^+ plaques counted are given in parentheses; total numbers of *am* or *r* plaques counted were usually much greater.

TABLE 3—Continued

Tester strain[a]	Experiment	Host cell index[b]	Plating mode[c]	Surviving fraction[d]	Phage to cell ratio[e]	Apparent revertant frequency (total am^+ or r^+ plaques)[f]
	11	2.1	C	1.0	0.025	22 (134)
				0.0054	0.025	25 (3)
	12	2.7	C	1.0	0.027	20 (94)
				0.0044	0.027	7 (6)
rUV375	15	6.4	D	1.0	0.10	0.21 (17)
				0.013	0.051	0.27 (2)
r1605/r$^+$	17	6.2	D	1.0	0.054	0.77 (31)
				0.012	0.11	2.0 (6)
					0.022	0.42 (1)

cells before assaying am^+ frequencies would reveal stronger increases than upon direct plating. No increase was detected.

The second mutant (*am52* in gene *37*) produced two types of revertant plaques, large and small, on selective lawns. We did not further explore this strain because of uncertain scoring of the small-plaque revertants among the survivors of UV irradiation, which itself irregularly reduces plaque sizes.

We turned finally to the reversion of *rUV375* and to the behavior of a mixture (*r1605* and r^+) of a nonreverting deletion seeded with wild-type particles to a frequency similar to that usually displayed as a result of the spontaneous reversion of *rUV375*. As illustrated in experiments 15 and 17, we could obtain no evidence for UV-induced increases in r^+ frequencies with either stock.

The accuracies of many of the revertant frequencies in Table 3 are limited by the small numbers of am^+ or r^+ plaques counted. Our general conclusions are unaffected by this constraint, however, since each rests upon a strong consensus of repeated measurements. Most frequencies calculated from small samples derive from experiments in which the plaque density was 0.1–0.001 per plate, frequencies that frustrated our desire to improve accuracy.

Our experience with these systems suggests that the results reported previously may have been influenced to an unknown extent by reactivation events occurring on the plates. This problem had been addressed (YAROSH *et al.* 1980) by calculating the degree to which multiplicity reactivation contributed to the observed values. This approach has two important limitations. First, their experimental conditions may have varied in some details between reversion and multiplicity-reactivation experiments, rendering extrapolation somewhat uncertain. Second, YAROSH *et al.* (1980) assumed in their calculations that the phage particles were randomly distributed among the cells. However, in experiments of this type, cell densities during adsorption are often high, which may favor nonrandom adsorption and thereby increase reactivation effects. Such a bias may have been responsible for the curious differences in the results from experiments in which the order of addition of phage and cells was reversed (A and A′).

RESULTS

Phenotypic and allelic relationships among fdsA, fdsB, uvsW, uvsX *and* uvsY *mutations*

UV and γ inactivation and mutagenesis: Table 4 describes the UV-induced inactivation and induction of *r* mutants in wild-type T4, the gene *49* mutants *amE727x1* and *tsC9* and the repair-defective mutants *fdsA2, fdsB7, px, y, m22*

TABLE 4

UV-induced inactivation and mutagenesis

Strain or genotype	Mutated gene(s)	Lethal hits/sec[a]	*r* mutants/10^6 sec	*r* mutants/10^4 lethal hits
T4B		0.061	20	3.3
T4D		0.061	ND[b]	ND
amE727x1	*49*	0.063[c]	ND	ND
tsC9	*49*	0.063[d]	ND	ND
px	*uvsX*	0.10	~3	~0.3
fdsA2	*uvsX*	0.10	~3	~0.3
fdsA2 amE727x1	*uvsX 49*	0.10[e]	~2	~0.2
fdsA2 tsC9	*uvsX 49*	0.10[f]	~3	~0.3
y	*uvsY*	0.10	~2	~0.2
fdsB7	*uvsY*	0.10	<1	<0.1
fdsB7 amE727x1	*uvsY 49*	0.10	ND	ND
fdsB7 tsC9	*uvsY 49*	0.10	ND	ND
m22	*uvsW*	0.080	<0.1	<0.1
m33	*uvsW*	0.080	<0.1	<0.1

[a] Means from ten or more determination per genotype, among which the largest variation in individual determinations was 10% or less. The UV dose rate was about 0.25 J m^{-2} sec^{-1}.
[b] Not determined.
[c] Assayed on CR63 cells.
[d] Assayed at 32°.
[e] Assayed on both BB and CR63 cells.
[f] Assayed at both 32° and 42°.

TABLE 5

γ-induced inactivation and mutagenesis

Strain or genotype	Mutated gene	Lethal hits/krad	*r* mutants/10^9 rad	*r* mutants/10^4 lethal hits
T4B		0.018	18	9.9
px	*uvsX*	0.029	~3.5	~1.2
fdsA2	*uvsX*	0.028	~2.5	~0.9
y	*uvsY*	0.031	~1.5	~0.5
fdsB7	*yvsY*	0.027	~2.0	~0.7
m33	*uvsW*	0.024	<0.1	<0.1

and *m33*. Table 5 describes the γ-induced inactivation and induction of *r* mutants in wild-type T4, *fdsA2*, *fdsB7*, *px*, *y* and *m33*. As expected, both *fdsA2* and *fdsB7* resemble the reference *uvsX* and *uvsY* mutants, showing increased sensitivity to inactivation and decreased sensitivity to mutagenesis by either UV or γ irradiation. Both *uvsW* mutants decreased mutagenesis, thus placing the *uvsW* gene in the error-prone repair pathway. The UV sensitivities of double mutants of *fdsA2* or *fdsB7* with gene *49* amber or *ts* alleles were the same under conditions permissive or nonpermissive for the gene *49* alleles (Table 4). Thus, although a *uvsX* and a *uvsY* mutation suppress the lethality of gene *49* mutations, neither amber nor *ts* mutations of gene *49* suppress the UV sensitivities of either the *uvsX* or the *uvsY* mutation.

TABLE 6

Recombination complementation tests

Infecting genotypes[a]	Recombinant frequencies (% rII^+) in individual experiments[b] 1	2	3	Interpretation
T4B + T4B	3.42	2.92	3.44	Self-cross
fdsA2 + *fdsA2*	1.67	1.44	1.40	Self-cross
fdsB7 + *fdsB7*	0.94	0.48	0.88	Self-cross
px + *px*	1.16	1.16	1.13	Self-cross
y + *y*	1.45	1.24	1.27	Self-cross
fdsA2 + T4B	2.52	2.10	2.75	Codominance
fdsB7 + T4B	2.55	2.30	2.65	Codominance
px + T4B	2.32	2.04	3.19	Codominance
y + T4B	2.59	2.27	2.88	Codominance
fdsA2 + *fdsB7*	2.16	2.61	2.64	Complementation
fdsA2 + *y*	2.70	2.31	2.86	Complementation
fdsB7 + *px*	2.38	2.04	2.55	Complementation
px + *y*	2.74	2.27	2.72	Complementation
fdsA2 + *px*	1.36	0.96	1.57	Noncomplementation
fdsB7 + *y*	1.24	0.83	1.03	Noncomplementation

[a] The *fds* or *uvs* genotypes present in each *rUV7* × *rUV183* cross; all were 49^+.

[b] In the top five tests, each rII^+ value is a single determination. In the bottom ten tests, each rII^+ value is the mean of reciprocal tests (for instance, *fdsA2 rUV7* × *fdsB7 rUV183* and *fdsA2 rUV183* × *fdsB7 rUV7*). The range of values from reciprocal crosses within any experiment was less than the range among the three experiments in a single line.

Suppression of gene 49 *mutations by* uvsW, uvsX *and* uvsY *mutations*: In the early stages of this study, the mutations *m33* (*uvsW*), *px* (*uvsX*) and *y* (*uvsY*) were combined with the gene *49* mutation *amE727x1* and subsequently crossed out again to confirm the genotypes of the double mutants. Suppression of the gene *49* defect was achieved by inserting the *uvsX* or *uvsY* mutation but not the *uvsW* mutation. These results confirm numerous published and unpublished observations concerning suppression by *uvsX* and *uvsY* mutations, plus the mention by WAKEM and EBISUZAKI (1981) of the inability of a *uvsW* mutation to suppress a gene *49* defect.

Recombination complementation tests: Table 6 lists the rII^+ recombination frequencies from *rUV7* × *rUV183* crosses and the influences of *fdsA2, fdsB7, px* and *y* mutations upon these frequencies. The top five entries show the reductions in recombination frequencies caused by the four tested alleles. The next four entries show that the effects of these alleles upon recombination are approximately codominant with the wild-type allele. The following four entries show complementing combinations, that is, combinations that produce recom-

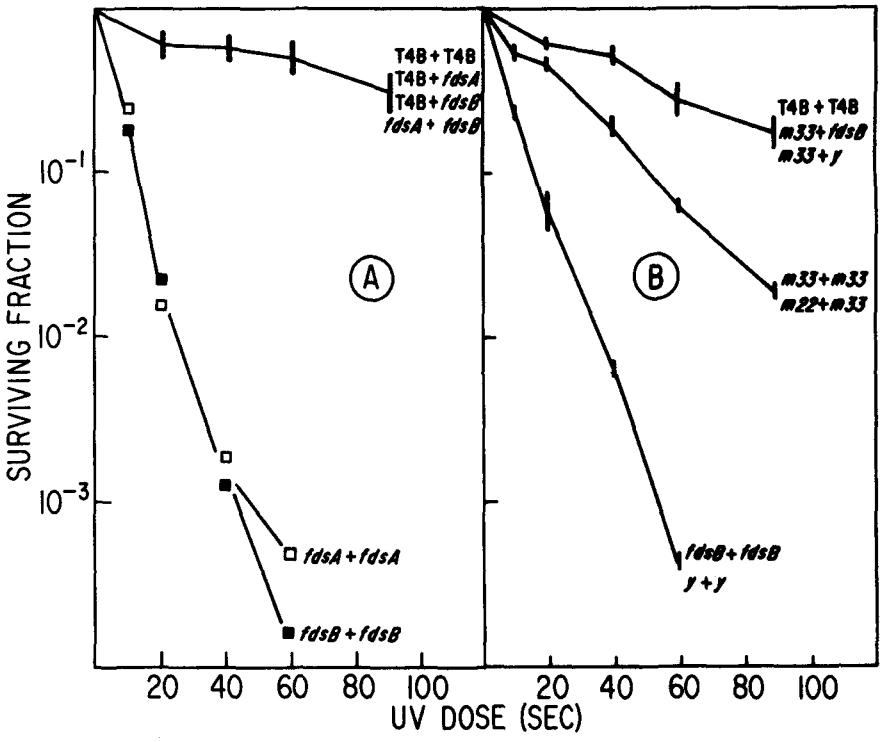

FIGURE 1.—UV survival complementation tests. Cells were infected with an average of five particles of each genotype, incubated for 10 min, UV irradiated and immediately plated. A: Complementation between *fdsA2* (*uvsX*) and *fdsB7* (*uvsY*). B: Complementation patterns of the *uvsW* mutants *m22* and *m33* and the *uvsY* mutants *fdsB7* and *y*.

bination frequencies similar to those produced by combinations of one of the mutant alleles with the wild type. The bottom two entries show noncomplementing combinations. While these results support the conclusions that *fdsA2* is an allele of *uvsX* and that *fdsB7* is an allele of *uvsY*, they also illustrate the complexities and low sensitivities of recombination complementation tests of alleles of the *WXY* system.

UV survival complementation tests: The ability of various *fds* and *uvs* alleles to complement each other was measured as the restoration of wild-type resistance to UV inactivation. When free phages are irradiated, mutations of the *WXY* system reduce the slopes of their survival curves by less than twofold. However, when 10-min phage-cell complexes were irradiated, the differences became much greater. Furthermore, mixed infections with a mutant and a wild-type allele always produced the wild-type level of UV sensitivity. Thus, whereas these mutant alleles are codominant in recombination complementation tests, they are recessive in UV sensivitity complementation tests.

Some typical results are shown in Figure 1, where *fdsA2* (*uvsX*) and *fdsB7* (*uvsY*) are seen to complement each other fully, as do *m33* (*uvsW*) and either *fdsB7* or *y* (both in *uvsY*). (Note that the *uvsW* shows an intermediate UV sensitivity, as it also does in the case of free phages.) Allelic combinations,

however, did not complement (*e.g.*, *fdsA2* and *x*, or *fdsB7* and *y*, or *m22* and *m33*). Taken together, our results completely confirm the previous picture concerning allelism in the *WXY* system. However, the *px* mutant failed to complement the *uvsY* mutant *fdsB7* but was fully recessive to the wild type. Note that *px* did display complementation in the recombination test (Table 6). Thus, *px* is not a simple *uvsX* mutant. It presumably accumulated one or more modifiers during its derivation (see DISCUSSION).

Isolation and properties of conditional alleles of uvsX *and* uvsY

Isolation of uvsX *and* uvsY ts *alleles*: The gene *49* amber mutant *amE727x1* was used to select *ts* alleles of *uvsX* and *uvsY*. We chose 32° as the temperature at which activity of the *uvs ts* gene products would be required and 42° as the temperature at which the *uvs ts* gene product would be inactive. Because a *uvsX* or *uvsY* defect suppresses a gene *49* mutation, *ts* isolates were identified by their ability to grow on BB cells at 42° but not at 32° (and to grow on CR63 cells at both temperatures). Of ten mutants temperature sensitive for suppression, six later proved to contain *ts* alleles of *uvsX* and four to contain *ts* alleles of *uvsY*.

Isolation of uvsX *and* uvsY am *alleles*: The gene *49* temperature-sensitive mutant *tsC9* was used to select *am* alleles of *uvsX* and *uvsY*. This allele of gene *49* is active at 32°, and the CR63 amber suppressor defines *uvs* amber alleles. Isolates unable to grow on CR63 cells at 42°, although growing on CR63 cells at 32° and on BB cells at both temperatures, were putative *uvs* amber alleles. *tsC9* was first mutagenized with hydroxylamine. After 30 min of treatment the frequency of *fds* suppressors increased from 0.38 to 3.3%. The mutagenized sample was plated on BB cells and incubated at 42° to select suppressors of the gene *49 ts* allele, and 1008 isolates were spotted on CR63 and BB cells at both 32° and 42°. Two isolates had an amber phenotype for suppression of the gene *49* mutation. One was later shown to be an allele of *uvsX* and the other of *uvsY*.

Assigning the mutations: Linkage relationships were determined by crossing the new 49^- *uvs?* double mutants against 49^- recombinants (see MATERIALS AND METHODS). Complementation relationships were tested using the UV survival test. Each mutant fell unequivocally into *uvsX* or *uvsY* (data not shown).

Properties of the conditional alleles: Each mutation (in its original 49^- background) was tested for its ability to suppress a gene *49* mutation and for its sensitivity to UV inactivation and mutagenesis under both permissive and nonpermissive conditions.

The kinetics of UV-induced inactivation and mutagenesis for the *uvsX* amber mutant are shown in Figure 2, and the properties of both amber mutants are listed in Table 7. As expected for amber mutants, they appear to alternate between fully active and fully inactive states according to the suppressor state of the host.

The corresponding responses of one of the more strongly temperature-sensitive *uvsX* mutants is shown in Figure 3, and the properties of all the *ts* mutants are listed in Table 8. These properties are complex and will be considered in more detail in the DISCUSSION.

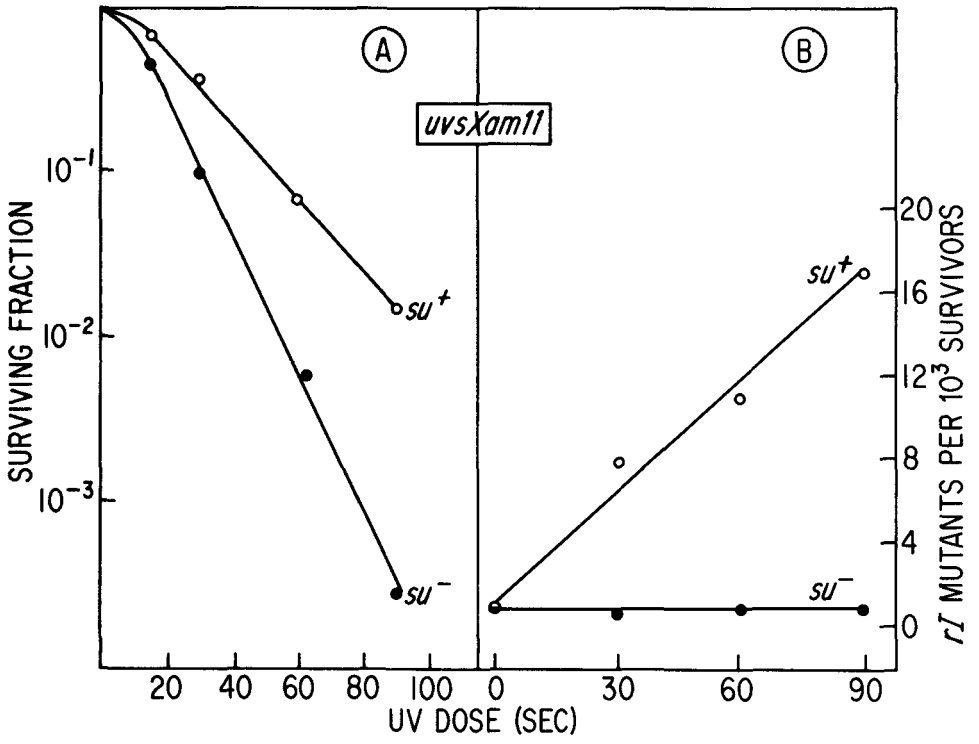

FIGURE 2.—Suppression-dependent responses of the *uvsXam11* mutant (in a *tsC9* background). A: UV inactivation. B: UV mutagenesis.

TABLE 7

Properties of amber alleles of uvsX *and* uvsY

	Suppression of *49ts* allele (efficiency of plating, 42°/32°)[b]		UV sensitivity (lethal hits/sec)[c]		UV mutability ($r^+ \rightarrow rI$)			
					$rI/10^6$ sec		$rI/10^4$ lethal hits	
Allele[a]	su^+	su^-	su^+	su^-	su^+	su^-	su^+	su^-
uvs$^+$	0.003	0.003	0.063	0.063	16	14	16	23
fdsA2	0.9	0.9	0.10	0.10	~3	~3	<1	<1
uvsXam11	0.003	0.8	0.067	0.10	16	<1	24	<1
uvsYam11	0.004	0.9	0.067	0.091	11	<1	17	<1

[a] All also contain *tsC9*.

[b] Plating efficiency at high *vs.* low temperature on indicated cells: su^+ = XA101c(λ), su^- = XA90Nc(λ).

[c] Dose rate about 0.25 J m^{-2} sec^{-1}.

When the *49*$^-$ *uvs* double mutants were backcrossed to the wild type, both *uvs* and *49*$^-$ segregants were recovered; due to the incomplete temperature sensitivity of some of these mutants and to the confounding effects of thermal rescue (see CONKLING and DRAKE 1984), it was necessary to resort to irradiating 10-min complexes (as in the UV survival complementation tests) to achieve ready identification of these mutants. Within the range of variation character-

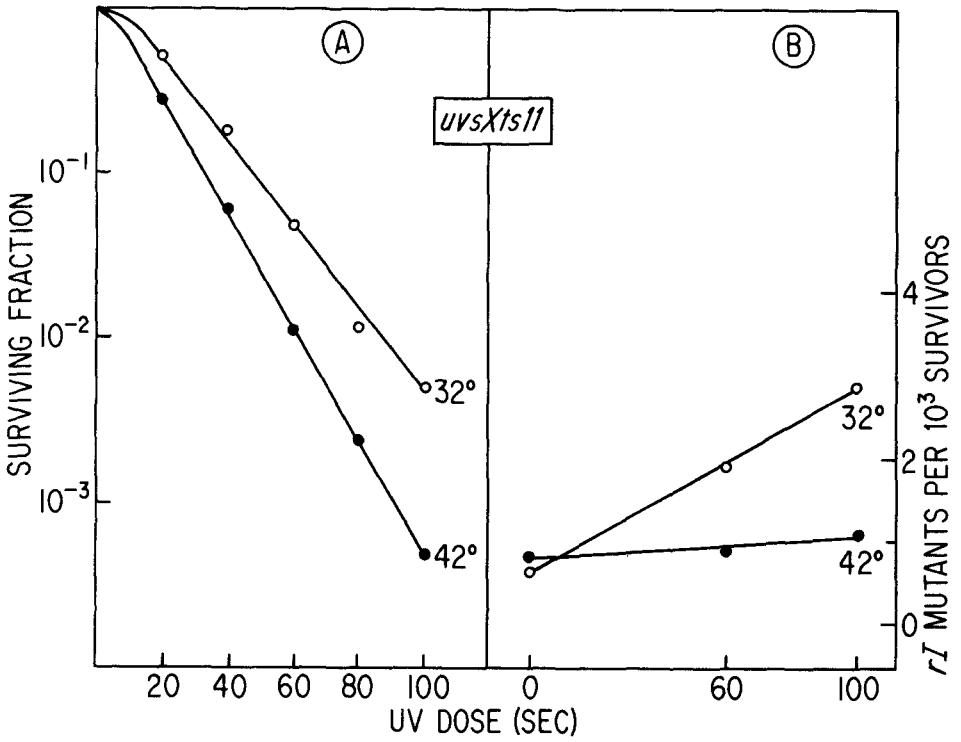

FIGURE 3.—Temperature-dependent responses of the *uvsXts11* mutant (in an *amE727x1* background). A: UV inactivation. B: UV mutagenesis.

TABLE 8

Properties of ts *alleles of* uvsX *and* uvsY

	Suppression of *49am* allele (efficiency of plating, su^-/su^+)[b]		UV sensitivity (lethal hits/sec)[c]		UV mutability ($r^+ \rightarrow rI$)			
					$rI/10^6$ sec		$rI/10^4$ lethal hits	
Allele[a]	32°	42°	32°	42°	32°	42°	32°	42°
uvs$^+$	0.001	0.001	0.068	0.066	21	20	3.4	3.2
fdsA2	0.9	0.9	0.091	0.091	~2	~2	~0.2	~0.2
uvsXts11	0.001	0.71	0.064	0.083	16	~3	2.6	~0.4
uvsXts12	0.001	0.83	0.063	0.074	22	~3	3.5	~0.4
uvsXts13	0.001	0.63	0.058	0.071	28	~4	4.8	~0.6
uvsXts14	0.0006	0.72	0.063	0.091	13	~3	2.1	~0.3
uvsXts15	0.0005	0.70	0.057	0.070	33	~3	5.6	~0.4
uvsXts16	0.0007	0.79	0.061	0.075	21	14	2.6	1.7
uvsYts11	0.0008	0.52	0.061	0.075	11	~5	1.8	~0.7
uvsYts12	0.0006	0.57	0.054	0.063	46	12	8.3	1.9
uvsYts13	0.0003	0.83	0.058	0.071	38	7	6.5	1.0
uvsYts14	0.08	0.63	0.059	0.079	~2	~5	~0.3	~0.7

[a] All also contain *amE727x1*.

[b] Plating efficiency on Xa90Nc(λ)su^- *vs.* XA101c(λ)su^+ at indicated temperature.

[c] Values determined from the linear portions of survival curves; averages of three measurements with ranges less than 20% (usually less than 10%) of the tabulated values; dose rate about 0.25 J m^{-2} sec^{-1}.

TABLE 9

Effects of px *and* y *upon* rII *revertant frequencies*

rII allele	Experiment	*rII*$^+$ × 10^8 in various *uvs* backgrounds[a]		
		uvs$^+$	*px*	*y*
rUV7	1	44	67	59
	2	63	64	59
	3	37	29	23
	Rel[b]	1	1.1	1.0
rUV13	1	6	10	8
	2	19	18	15
	3	30	23	25
	Rel[b]	1	0.9	0.9
rUV183	1	59	61	61
	2	78	52	77
	3	42	38	57
	Rel[b]	1	0.8	1.1
rUV199	1	58	60	49
	2	45	66	80
	3	22	10	25
	Rel[b]	1	1.1	1.2
rUV58	1	8	15	27
	2	17	25	69
	3	17	29	48
	Rel[b]	1	1.7	3.5
rUV113	1	108	61	77
	2	52	64	70
	3	67	75	73
	Rel[b]	1	0.9	1.0

[a] All stocks were initiated from 4-hr plaques. Each revertant frequency is the median value from four stocks grown in parallel for each experiment.

[b] Sums of the three *uvs* values normalized to sum of the *uvs*$^+$ values. (The same ratios were obtained if the three relative values were determined first and then averaged.)

istic of measurements of this type, the segregants exhibited the same inactivation and mutation patterns as did their double-mutant parents. Their complementation patterns were unchanged.

Tests for uvsX *and* uvsY *mutator activities*

Previous studies had suggested that some alleles of *uvsX* and *uvsY* might enhance the reversion of certain *rII* mutations (*e.g.*, WILLIAMS and DRAKE 1977). However, this possibility had never been vigorously explored. Therefore, *px* and *y* were paired with various well-characterized *rII* mutants whose revertant frequencies were then determined. The *rII* mutants included two that revert by G:C → A:T transitions (*rUV7* and *rUV13*), two that revert by

A:T → G:C transitions (*rUV183* and *rUV199*) and two that revert by frameshift mutations (*rUV58* and *rUV113*). The experiments were performed two or three times in M9CA medium and once in L broth. Table 9 summarizes the results of three independent sets of measurements. With the exception of modest but consistent increases in the reversion of *rUV58* in *uvs* backgrounds, there was no significant effect upon spontaneous mutation rates.

DISCUSSION

Complementation in the WXY *system*: The *uvsW*, *uvsX* and *uvsY* genes, together with a number of other genes, act in a repair pathway that processes many kinds of DNA damage (see HAMLETT and BERGER 1975; CUNNINGHAM and BERGER 1977; WAKEM and EBISUZAKI 1981; and references therein). Although mutants of the *WXY* system are viable, their burst and plaque sizes are reduced, and most aspects of their phenotypes are tedious to measure: the magnitude of the change is small compared with experimental variability, the measurement itself is cumbersome (*e.g.*, $r \rightarrow r^+$ or profiles of DNA synthesis) or the mutations exhibit codominance or interact additively. Two alterations, however, are both large and easy to measure and are, therefore, particularly useful for selecting new mutations and for analyzing their complementation patterns: the suppression of gene *49* mutations and the UV survival of phage-cell complexes after 10 min of development.

The UV survival test expands the difference between uvs^+ and uvs^- genotypes from less than twofold (for free phages) to more than 100-fold (for 10-min complexes). The molecular basis of this expansion is obscure, but it is likely to involve the several interacting components of the Luria-Latarjet effect: the size of the pool of replicating DNA, the phenomenon of multiplicity reactivation and postreplication recombinational repair. LURIA and LATARJET (1957) demonstrated that the shoulders of T4 inactivation curves become much greater, and their terminal slopes decrease, at increasing times after infection. After the onset of DNA synthesis, the production of multiple copies of the genome should increase the shoulder and the terminal slope of the inactivation curve by providing the opportunity for multiplicity reactivation, and SYMONDS, HEINDL and WHITE (1973) showed that Luria-Latarjet stabilization is indeed reduced in mutants of the *WXY* system. CUNNINGHAM and BERGER (1977) demonstrated that DNA synthesis is partially defective in cells infected by *uvsX* or *uvsY* mutants but not by *uvsW* mutants; correspondingly, our UV survival test reveals greater sensitivities of *uvsX* or *uvsY* than of *uvsW* mutants. Finally, postreplication recombinational repair would also be expected to contribute to the survival of 10-min complexes, and it too should be reduced in mutants of the *WXY* system.

Our results demonstrate that *uvsW* mutations resemble *uvsX* and *uvsY* mutations in their sensitivity to γ inactivation and their resistance to both UV and γ mutagenesis. On the other hand, *uvsW* mutations differ from *uvsX* and *uvsY* mutations by their inability to suppress gene *49* mutations and, as reported in the following paper (CONKLING and DRAKE 1984), in quantitative and temporal aspects of their expression. These differences are likely to reflect

the different patterns of DNA synthesis mentioned above. Taken as a whole, our results also confirm and extend those of CUNNINGHAM and BERGER (1977), who showed that *fdsA* is identical with *uvsX* and *fdsB* is identical with *uvsY*, and those of HAMLETT and BERGER (1975), who showed that *uvsW* differs from *uvsY*.

In the UV survival complementation test, *px* failed to complement either *y* or *fdsB7* but was not dominant to the wild type; similar results were reported by MELAMEDE and WALLACE (1977, 1980) using complementation tests based on rates of DNA synthesis. The *px* strain was obtained by backcrossing Harm's T4D*x* with T4B and selecting for both wild-type plaque size and UV sensitivity (DRAKE 1973). However, because all other mutant alleles of the *WXY* system exhibit reduced plaque sizes, and because the parental *x* mutation does complement both *y* and *fdsB7*, it seems likely that *px* bears one or more modifier mutations that interfere with complementation; indeed, HAMLETT and BERGER (1975) demonstrated the ease with which modifiers arise in the *WXY* system, thus signaling the need to beware of derivatives producing better plaques or plating efficiencies. It seems unlikely that complementation by *px* is inhibited by a modifier residing in *uvsY*, because *px* complements both *y* and *fdsB7* in our recombination complementation tests. However, the formal possibility exists that certain domains of *uvsY* are involved in the determination of UV sensitivity but not of recombination. The *px* anomaly indicates the desirability of examining *uvs* alleles with diverse complementation tests.

Conditional uvs *alleles*: The ability of *uvsX* and *uvsY* mutations to suppress gene *49* mutations was exploited to select conditional mutations. Both temperature-sensitive and amber mutations were isolated. Although suppressors of gene *49* mutations have also been identified in genes *46*, *47* and *59* (WAKEN and EBISUZAKI 1981) and gene *32* (MINAGAWA, YONESAKI and FUJISAWA 1983), all 12 of our suppressors were alleles of *uvsX* or *uvsY*.

The amber mutations behaved as expected of suppressible mutations with respect to UV inactivation and mutagenesis. In contrast, the *ts* mutants exhibited some surprising properties. All were TS for UV inactivation, but the magnitudes of the differences, when normalized to the mild cold sensitivity of the wild type (see CONKLING and DRAKE 1984), ranged from 21 to 51% excess sensitivity at 42° compared with 32°. The TS mutants were immutable even at 32°, or about equally mutable at 32° and 42°, or hypermutable at 32°, or immutable at either temperature. Most importantly, sensitivity to inactivation and to mutability could vary independently, and neither was fully correlated with suppression of a gene *49* defect (which was always strong because of the manner of selection of the *ts* mutants). For instance, *uvsXts11* and *uvsXts14* were strongly TS for both inactivation and mutagenesis; *uvsXts12* and *uvsXts13* were only moderately TS for inactivation but still strongly TS for mutagenesis; *uvsXts15*, *uvsYts12* and *uvsYts13* were moderately TS for inactivation, strongly TS for mutagenesis, but hypermutable at 32°; *uvsXts16* and *uvsYts11* were moderately TS for inactivation but hardly TS for mutagenesis; and *uvsYts14* was strongly TS for inactivation but immutable at either temperature. Thus, the mutants as a set demonstrate that the three traits of suppressor activity, UV inactivation and UV mutagenesis can be at least partially uncoupled.

Speculations and implications: Although current information is not sufficient to attempt comprehensive models of *WXY* function, there are certain points worth raising.

It is tempting to speculate that the *WXY* system specifies as few as two fundamental processes: recombination and damage bypass. Defects in the former would be expected to cause most of the traits of *uvs* mutants: recombination deficiency (by definition); suppression of gene *49* mutations (see, for example, WAKEM and EBISUZAKI 1981 and MIZUUCHI *et al.* 1982); defects in DNA synthesis (see MOSIG 1983 for review); increased sensitivity to inactivation by many DNA-damaging agents because of poor recombinational repair, multiplicity reactivation and/or Luria-Latarjet stabilization; and reduced burst and plaque sizes because of a combination of the above. On the hypothesis that inadequately templated bypass of DNA damage generates mutations, defects in bypass would be expected to abolish mutability and might also contribute to the damage-induced reduction of DNA synthesis and viability. However, it may be the case that *only* the recombinational defect is fundamental: the ability of the T4 DNA polymerase to synthesize past a lesion might reflect, not some alteration in its intrinsic fidelity, but rather an inherent ability but one expressed only in the vicinity of recombinational intermediates, which are themselves likely to be generated at increased frequencies by DNA damage.

Even if it directs but a single fundamental process, the *WXY* system is complex. For instance, its putative null mutations are fully epistatic with respect to UV survival but are codominant and/or additive with respect to recombination and burst size. Its *ts* mutations dissociate suppression of gene *49* mutations, UV survival and UV mutagenesis. Even their expression patterns are complex (CONKLING and DRAKE 1984). These observations strongly suggest that the *WXY* proteins interact with each other and with other gene products and/or that they possess specialized functional domains. Although such complexity is hardly without precedent in T4 (see, for instance, BRESCHKIN and MOSIG 1977), it certainly requires that the analysis of any particular aspect of the system (for instance, mutagenesis) take carefully into account the logical and functional web in which the phenomenon is embedded.

An interesting evolutionary argument follows from the differential effects of certain *uvs ts* mutations upon UV survival and UV mutagenesis—for instance, the high 30° UV survival but UV immutability of *uvsYts14* (and, to a lesser extent, of *uvsXts12, 13* and *15*) and the normal 30° UV survivals but hypermutabilities of *uvsYts12* and *13*. A number of bacteria altogether lack UV mutability but exhibit excellent resistance to inactivation, probably because of efficient excision and/or recombination repair (see DRAKE and BALTZ 1976). Even in *E. coli*, which is highly UV mutable, mutations exist that abolish the mutagenic response but hardly decrease survival (KATO and SHINOURA 1977). Thus, only a small increment of survival need be associated with the phenomenon so often called "error-prone repair." This, in turn, suggests that survival *per se* may not have been the fundamental selective advantage of the mutagenic process but instead that the very mutations it generates provided its evolutionary success. Alternatively, the *E. coli* SOS and T4 *WXY* systems may indeed have evolved more to prevent lethality than to generate mutations but may be

directed primarily toward lesions other than those produced by UV and ionizing radiations; for instance, the mutagenic component of the SOS system seems to provide substantial protection against photodynamic lethality mediated by isopsoralen (MILLER and EISENSTADT 1983).

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