Aspects of the Ultraviolet Photobiology of Some T-Even Bacteriophages

Leslie A. Smith and John W. Drake

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709-2233

ABSTRACT

Bacteriophage T4 DNA metabolism is largely insulated from that of its host, although some host functions assist in the repair of T4 DNA damage. Environmental factors sometimes affect survival and mutagenesis after ultraviolet (UV) irradiation of T4, and can affect mutagenesis in many organisms. We therefore tested the effect of certain environmental factors and host genetic defects upon spontaneous and UV-induced mutagenesis and survival in T4 and some related T-even phages. Plating at pH 9 enhances UV resistance in T4 by about 14% compared to pH 7. The host cAMP regulatory system affects host survival after UV irradiation but does not affect T4 survival. Thermal rescue, the increasing survival of irradiated T4 with increasing plating temperature, occurs also in phage T6, but only weakly in phages T2 and RB69; this temperature effect is not altered by supplementing infected cells with additional Holliday resolvase (gp49) early in infection. Phage RB69 turns out to have almost 50% greater UV resistance than T4, but has a genome of about the same size; RB69 is UV-mutable but does not produce *r* mutants, which are easily seen in T2, T4, and T6. Spontaneous mutagenesis in T4 shows no dependence on medium and little dependence on temperature overall, but mutation rates can increase and probably decrease with temperature at specific sites. UV mutagenesis is not affected by incubating irradiated particles under various conditions before plating, in contrast to phage S13.

MUCH of our understanding of the processing of DNA damage, and particularly of damage induced by ultraviolet (UV) irradiation, derives from studies employing the classical bacteriophage T4 system (reviewed in Kreuzer and Drake 1994). Partly because T4 DNA contains glucosylated 5-hydroxymethylcytosine (5HMC) instead of cytosine, T4 DNA metabolism is largely independent of that of its host *Escherichia coli* (Drake and Kreuzer 1994), thus facilitating genetic analysis. In addition, host transcription and translation are both inhibited shortly after infection (Kutter *et al.* 1994), so that functions that would normally be induced in the host by DNA damage cannot be induced following T4 infection. In addition, constitutive host DNA repair functions are usually unable to operate on T4 DNA, perhaps because of its 5HMC content.

The survival of UV-irradiated T-even phages depends primarily on the activity of three phage-encoded systems (reviewed in Kreuzer and Drake 1994). The excision repair of cyclobutane dimers is carried out by the T4 *denV* system; the host *uvrABC* system does not operate on T4. Recombination repair is directed by a set of several T4 genes, including *uvsW*, *uvsX*, and *uvsY*; the host *recA* system does not operate on T4. The third T4 system is replication repair, a poorly understood process thus far observed only in T4; it depends upon at least the phage genes *32* (encoding a protein-binding single-stranded DNA) and gene *41* (encoding a replicative DNA helicase). However, there are distinct if minor host contributions to the repair of damage in T4 DNA. The efficiency of the *denV* system is enhanced by host *polA*-encoded DNA polymerase I. The repair of alkylation damage depends at least partly on host functions. Photoreactivation operates exclusively via the host's *phr*-encoded enzyme. Therefore, when either environmental conditions or genetic perturbations are observed to affect the survival of UV-irradiated *E. coli*, we attempt to test the same factors for their effect on the survival of UV-irradiated T4. Several such tests are described here.

UV mutagenesis in T4 also requires the functions of the *uvsW*, *uvsX*, and *uvsY* genes (reviewed in Drake and Ripley 1994a). UV mutagenesis in T4 is indifferent to the functional states of the *E. coli recA*, *umuC*, and *umuD* genes that are required for most UV mutagenesis of the host. We have long been concerned about possible environmental effects on spontaneous mutagenesis, and we report here tests for the effects of both growth temperature and medium. In addition, we have inquired whether the handling of phages immediately after irradiation affects the subsequent mutant frequency, as is the case in at least one other phage system.

Both survival and mutagenesis after UV irradiation depend on temperature in T4 (Conkling and Drake 1984b). We also report here a survey of the distribution of such "thermal rescue" among some T-even phages, and a test of the possibility that it reflects the expression of T4 gene *49*, which encodes the phage Holliday resolvase.

MATERIALS AND METHODS

Strains: Except where noted, all bacterial and phage strains derive from our long-established collection. T4B was the T4

Corresponding author: John W. Drake, Laboratory of Molecular Genetics E3-01, NIEHS, Research Triangle Park, NC 27709-2233.
E-mail: drake@niehs.nih.gov

strain in these experiments and most of its *rII* mutants are described in Drake (1963). T2L was obtained from Gisela Mosig (Vanderbilt University) and RB69 from Jim Karam (Tulane University).

The *E. coli* K-12 *crp* and *cya* mutants were obtained from George Weinstock and are described in Salles and Weinstock (1989); crp^+ cya^+ is strain MC4100 [*araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 ptsF25 deoC1*]; Δ*crp* is strain GE1050 = MC4100 Δ*crp*::*cam*^{r}; and Δ*cya* is strain GE1068 = MC4100 Δ*cya854*.

E. coli K-12 strain BL21DE3 carrying the plasmid pet11.49 (pet 11 bearing and expressing T4 gene *49*) was obtained from Gisela Mosig and is described as pET49 in Mosig *et al.* (1991). The control plasmid was constructed by inserting pet11 [obtained from Susanna Clark, National Institute of Environmental Health Sciences (NIEHS)] into BL21DE3 (obtained from W. C. Copeland, NIEHS). Background expression of the plasmid was sufficient to permit plaque formation by the gene-*49* mutant *amE727*. gp49 expression was further induced by adding IPTG to 12 μM to cells at 37° at 1×10^8/ml for 25 min before T4 infection.

Media: Our standard LB medium contains, per liter: Bacto tryptone, 10 g; Bacto yeast extract, 5 g; NaCl, 5 g; glucose, 1 g. Our standard M9 salts solution (M9S) contains, per liter: KH_2PO_4, 3 g; Na_2HPO_4, 6 g; NaCl, 3.5 g; NH_4Cl, 1 g; $FeCl_3$ at 0.16 mg/ml stored frozen, 1 ml. 50×Mg-G contains, per liter: $MgSO_4$, 6.65 g; glucose, 200 g. 25×CA contains, per liter: Bacto casamino acids, 200 g. M9 medium is prepared by adding 2% of 50×Mg-G to M9 salts solution. M9CA medium is prepared by adding 4% of 25×CA to M9 medium. The compositions of diluting (D) broth and top and bottom agars are given in Conkling and Drake (1984a).

UV irradiation: Irradiation procedures and subsequent plating methods are described in Conkling and Drake (1984a). In general, phages were diluted to negligible UV absorbancy in M9S at pH 7 and irradiated in a hand-agitated watch glass using a Champion 15-watt low-pressure mercury germicidal lamp at a dose rate of about 0.25 J m^{-2} sec^{-1}. T4 UV-survival curves display variable shoulders depending on the history of the stock, followed by a terminal slope, followed by irregular upward deviations due to multiplicity reactivation on the plate (Kreuzer and Drake 1994; Drake and Ripley 1994a; Drake and Ripley 1994b). When measurements are conducted with care (Drake and Ripley 1994b), terminal slopes are reproducible to within 5% (Wachsman and Drake 1987).

Thermal rescue experiments were performed approximately according to Conkling and Drake (1984b). B cells were grown to about 2×10^8/ml at 37° in LB medium, 0.4-ml samples were placed at 20° or 43°, 0.1 ml of phage was added, incubation was continued for 10 min, 2.5 ml of supersoft agar was added, and the tubes were immediately poured onto plates previously equilibrated at 20° or at 37°. After 1.5 min, the 37° plates were moved to the 43° incubator. After 2 hr, all plates were shifted to 37° for overnight incubation. The 20° manipulations were conducted in a cold room equipped with a 20° incubator. Because the ratio of plaque-forming units to cells in the plating mixtures was roughly 10^{-3}, little or no multiplicity reactivation occurs in these experiments, and none was detected in control experiments designed to probe this possibility in T4.

Plating-pH experiments: LB, bottom agar and top agar were prepared at the usual pH 7 or at pH 9 (adjusting with NaOH). *E. coli* B plating cells were grown in LB of the indicated pH. The intrinsic buffering capacities of these media are low, so that the LB media had measured pH values of 6.9–7.3 and 9.0–9.1 when inoculated (the latter falling to to about 8.1 by the time the cells were used), the top agars had pH values of 6.1–7.1 and 8.2–9.1, and the bottom agars had pH values of 6.9–7.0 and 8.6–9.0. After overnight incubation, all plates had pH values of about 8.5.

Spontaneous mutagenesis: General methods and commentary are given in Drake and Ripley (1994a) and Dressman *et al.* (1997). Several stocks are grown in parallel from small, mutant-free inocula using BB cells, and their mutant frequencies are then determined. In forward-mutation experiments, stocks are plated on B cells and *r* mutants are counted; these are rapid-lysis mutants that produce large, sharp-edged plaques that contain fewer phage particles than do the small, fuzzy-edged wild-type plaques. In reversion experiments, the stocks are plated on BB cells to measure total particles and on KB cells to measure rII^+ revertants; KB cells are a strain of K-12(λ) and *rII* mutants cannot multiply on λ lysogens. We report median mutant frequencies; the median is a more reproducible measure of mutation than is the mean because of the clonal nature of the distribution of mutant frequencies (Luria and Delbrück 1943). In all of the present experiments, stock titers were sufficiently similar so that relative mutant frequencies were the same as relative mutation rates (Drake 1991); therefore, we did not convert frequencies into rates.

Induced mutagenesis: UV mutagenesis was performed according to Conkling and Drake (1984a); for the holding experiments, irradiated samples were held in LB medium. Hydroxylamine mutagenesis was performed according to Freese *et al.* (1961).

TAFE gel analysis: Transverse alternating field electrophoresis was performed according to Wang and Lai (1995) by the incomparable Joan Graves (National Institute of Environmental Health Sciences).

RESULTS

Survival after ultraviolet irradiation

Effect of plating pH: When *E. coli* K-12 is grown at pH 7 and pH 9, it displays increased resistance to killing by UV irradiation at the higher pH, and this difference persists in both $polA^-$ and $recA^-$ backgrounds (Schuldiner *et al.* 1986; Goodson and Rowbury 1990). When T4 is irradiated at pH 7 and then plated at roughly pH 7 and pH 9 on pH-adapted cells, it, too, displays a higher survival at the higher pH (Figure 1). In four such measurements, the mean terminal slope ± SEM at pH 9 relative to that at pH 7 was 0.86 ± 0.02.

Because the T4 particles were all irradiated at pH 7, the difference in UV sensitivity must reside not in lesion density but in lesion processing after infection. The component of lesion processing that is affected by the pH difference remains unknown in both *E. coli* and T4. Because of the small (14%) difference in T4 survival between the two pH values, and the difficulties of controlling pH values without developing new media, we chose not to pursue this difference further.

Effect of host *cya* and *crp* genotypes: In *E. coli*, cyclic AMP is a global regulator of cellular functions. This regulation is disabled in *cya* and *crp* mutants. Such mutants display markedly increased resistance to killing by UV irradiation; the difference persists in $uvrA^-$, $lexA^-$, and $recA^-$ backgrounds and includes the survival of irradiated phage λ (Puyo *et al.* 1992). There is no discern-

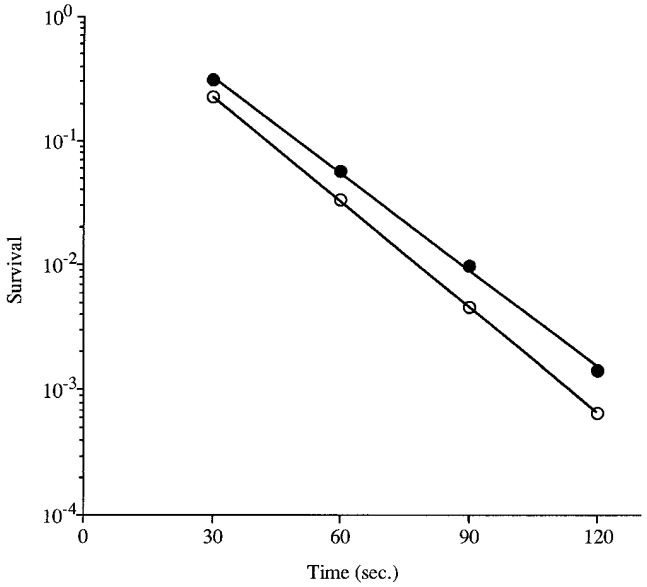

Figure 1.—Effect of pH on survival of UV-irradiated T4. Phage particles were irradiated at pH 7 in M9S and then plated using cells grown in LB media and both top and bottom agars adjusted to approximately pH 7 (top curve) or pH 9 (bottom curve) (see materials and methods).

ible effect of host *cya* or *crp* mutations on the UV sensitivity of phage T4 (Table 1).

Thermal rescue: When phage T4 is UV-irradiated, plated at various temperatures, held for 2 hr, and then shifted to 37° for overnight incubation, it displays steadily increasing survivals with increasing temperature (Conkling and Drake 1984b). This "thermal rescue" (higher survivals at higher temperatures) is due to the cold-sensitivity of recombination repair and is not observed in T4 strains defective in *uvsW*, *uvsX*, or *uvsY*.

Thermal rescue among T-even phages: We surveyed thermal rescue after UV irradiation among several T-even phages, including the classical T2-T4-T6 series plus phage RB69, which appears to be highly diverged from T4 (Russell and Huskey 1974; Wang *et al.* 1995). Table 2 compares survival at 20° with that at 43° using UV doses yielding 43°-survivals in the neighborhood of 10^{-3}; different UV doses were used for different phages because T2 and T6 are roughly twice as UV-sensitive as T4

TABLE 1

Survival of UV-irradiated T4 in *cya*$^-$ or *crp*$^-$ host cells

Host genotype	Relative survival[a]
B *cya*$^+$*crp*$^+$	1.00
K-12 *cya*$^+$ *crp*$^+$	1.02
K-12 Δ*cya*	0.97
K-12 Δ*crp*	1.02

[a] Slope of the linear portion of each survival curve normalized to the mean slope of the four determinations. None of the differences are significant.

TABLE 2

Thermal rescue in T-even phages

Phage	Sec UV	$S_{20°}$	$S_{43°}$	$S_{20°}/S_{43°}$
T2	45	8.2×10^{-4}	1.7×10^{-3}	0.48
T4	120	4.4×10^{-4}	2.7×10^{-3}	0.17
T6	45	2.4×10^{-4}	9.9×10^{-4}	0.24
RB69	180	2.3×10^{-3}	4.2×10^{-3}	0.56

S is the surviving fraction at the indicated temperature after the indicated UV dose.

because of their lack of a functional *denV* excision-repair system, and RB69 is less UV-sensitive than T4 (see below).

We observed slightly less thermal rescue with phage T4 than did Conkling and Drake (1984b), whose $S_{20°}/S_{43°}$ was about 0.13. T4 shows stronger thermal rescue than the other tested T-even phages, but the difference between T4 and T6 is small and probably not significant. T2 and RB69 show only weak thermal rescue; the RB69 effect is seen even at small doses and appears to reside primarily in the shoulder rather than the terminal slope, but the data on T2 are less clear on this issue (data not shown). We conclude that the strength of thermal rescue varies considerably among T-even phages, the data suggesting two groups (T4 and T6 versus T2 and RB69).

Role of gene 49 in thermal rescue: Because thermal rescue is a property of recombination repair and because the early production of gp49 (the T4 "Holliday resolvase" that cleaves recombinational intermediates) is likely to be temperature-dependent (Barth *et al.* 1988; Mosig *et al.* 1991), Gisela Mosig suggested to us that thermal rescue might reflect a temperature-dependent paucity of gp49 early in infection. To test this notion, we plated UV-irradiated T4 at 20° and 43° on cells carrying a plasmid expressing (or not) an IPTG-inducible gp49. The gene-*49* mutant *amE727*, which is unable to grow on *su*$^-$ cells, forms plaques on the plasmid-bearing strain only when the plasmid carries gene *49* (data not shown). When we conducted thermal-rescue experiments using wild-type T4 particles, we could discern no effect of gp49 supplementation upon the $S_{20°}/S_{43°}$ ratio (Table 3), and therefore conclude that the expression of gene *49* is not the cold-sensitive component of thermal rescue.

TABLE 3

Effect of gp49 supplementation on thermal rescue

Host strain	IPTG	$S_{20°}/S_{43°}$[a]
K-12 BL21DE3 pet11	−	0.26
	+	0.21
K-12 BL21DE3 pet11.49	−	0.20
	+	0.23

[a] Survival of wild-type T4 when plated at 20° relative to 43°; average of two experiments.

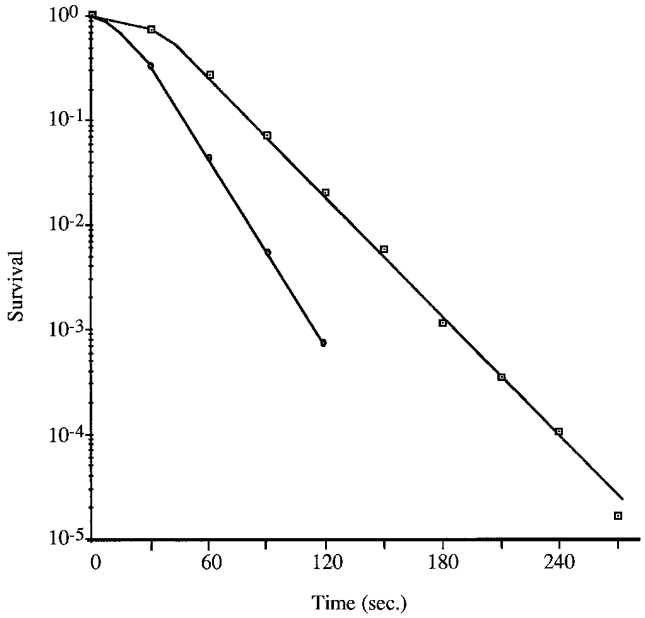

Figure 2.—The UV sensitivity of phage RB69 (top curve) compared to that of T4 (bottom curve).

The UV sensitivity of RB69: RB69 is considerably less sensitive to UV irradiation than is T4 (Figure 2). It also differs strikingly from T4 in not exhibiting upward deviations at survivals below about 10^{-3} caused by multiplicity reactivation on the plate. Under our conditions and using terminal slopes, T4 is reduced about tenfold in titer by 35.25 sec of irradiation and RB69 by 51.9 sec; the RB69 / T4 ratio is about 1.47. Because the genetics of RB69 are poorly explored, it is impossible to distinguish between an RB69 DNA repair system not operating in T4, or the more effective operation of one or more systems already active in T4; indeed, the absence of multiplicity reactivation on the plate suggests that RB69 may be *less* efficient than T4 in interchromosomal recombination repair (Kreuzer and Drake 1994).

It is, however, possible to test the hypothesis that RB69 has greater UV resistance because it has less DNA. T4 has about 172 kbp (kilobase pairs) of DNA per normal particle (including its genomic terminal redundancy). If RB69 had the same overall efficiency of repair as T4, its UV sensitivity would be proportional to the size of its chromosome, so that RB69 would contain about 172 / 1.47 = 117 kbp of DNA. We used TAFE gel analysis to determine the size of RB69 DNA (Figure 3), which we estimate by linear extrapolation to be 173.6 kbp based on 172 kbp for T4. (RB69 also contains a minority of 0.3-sized genomes akin to T4's incomplete "petit" genomes.) Thus, although the length of terminal redundancy in RB69 is unmeasured, its genome size is likely to be very close to that of T4 and the other T-even phages. Our results are consistent with the dimensions of RB69, which are indistinguishable from those of T4 (H.-W. Ackermann, personal communication).

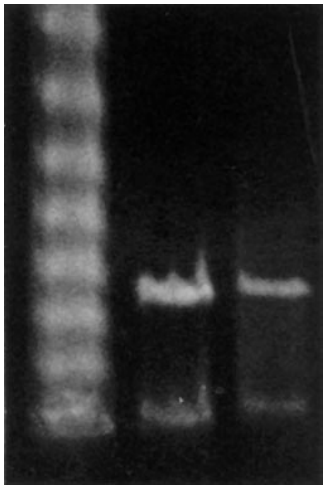

Figure 3.—TAFE analysis of the RB69 chromosome. Lane 1 = phage λ DNA ladder in 50-kbp increments, lane 2 = T4 DNA, lane 3 = RB69 DNA.

Spontaneous and UV-induced mutagenesis

Spontaneous mutagenesis: Both endogenous and unrecognized exogenous mutagens are likely to act upon intracellular DNA, and free phage particles spontaneously accumulate mutations by at least two mechanisms (reviewed in Drake and Ripley 1994a). Therefore, as a prelude to studies on UV mutagenesis, we explored the effects of some key environmental variables on spontaneous mutagenesis in T4.

Growth temperature: Growth temperature has been known for decades to affect mutation rates in a variety of organisms, including phage T4 where Bessman and Reha-Krantz (1977) reported that revertant frequencies of *rII* mutants grown at 30° were generally higher than at 43°; relative revertant frequencies at 30° *vs.* 43° were: *rUV199* (A:T) ≈ 20, *rUV373* (A:T) ≈ 0.7, *rUV248* (A:T) ≈ 10, *rUV183* (A:T) ≈ 11, *rUV4* (A:T) ≈ 13, *rSM51* (G:C?) ≈ 7, *rUV13* (G:C) ≈ 0.3, *rUV6* (fs) ≈ 1.8, and *rUV58* (fs) ≈ 1.9. Here, ''A:T'' means a mutant that is reverted by transition-inducing base analogues but not by hydroxylamine (which induces G:C → A:T); "G:C" means a mutant that is reverted by hydroxylamine and "?" indicates that it reverted to a nonwild phenotype; and "fs" means a mutant that is reverted by proflavin and thus by a base addition or deletion mechanism; for more details, see Drake and Ripley (1994a). Thus, the reversion of most A:T mutants showed about an order-of-magnitude dependency on growth temperature, two G:C mutants showed opposite dependencies, and two frameshift mutants showed small and probably insignificant dependencies on growth temperature.

We sought both to confirm these often strong effects and to inquire whether the sum of all detectable mutations arising in a large mutational target also depends on growth temperature. The results appear in Table 4. First, we confirm the dependence of reversion on

TABLE 4

Effect of growth temperature on mutant frequencies

Mutational pathway	Growth T	No. of stocks	Median mutant frequency	Relative mutant frequency (30°/43°)
rUV4 → r^+	30°	5	8.2×10^{-8}	3.1
	43°	5	2.7×10^{-8}	
rUV183 → r^+	30°	5	3.2×10^{-6}	6.0
	43°	5	0.5×10^{-6}	
rUV199 → r^+	30°	5	1.5×10^{-6}	6.2
	43°	5	0.2×10^{-6}	
r^+ → *r*	30°	7	8.4×10^{-4}	1.4
	37°	7	5.2×10^{-4}	
	43°	7	6.2×10^{-4}	

growth temperature in three of the mutants tested previously. However, our 30°/43° ratios (3, 6, and 6) are smaller than those of the 1977 experiments (13, 11, and 20, respectively), perhaps because of unrecognized procedural differences. Second, we screened mutagenesis in roughly 4 kb of T4 mutational target by scoring *r* mutants. Any kind of mutation can probably be detected in the *r* genes, but base pair substitutions are poorly detected in *rIIA* and *rIIB*, which tolerate most missense mutations (Koch and Drake 1970) and make up most of the mutational target. We observed little or no temperature dependency of *r* mutant frequencies, the 1.4-fold difference being neither statistically significant on the basis of the number of mutants scored (data not shown) nor likely to be reproducible based on historical experience; note also that the 37° value was lower, not higher, than the 43° value. However, there is a fundamental difference between our reversion and forward-mutation experiments: all stocks were grown in BB cells, which do not select for or against *rII* mutants in reversion and forward-mutation tests (because *rII* mutants express the r^+ phenotype on BB cells) but which do select to some extent against non-*rII r* mutants in forward-mutation tests because r^+ phages out-compete *r* phages in mixed infections due to the advantage of lysis inhibition (Hershey 1946). Although *rII* mutants comprise roughly 70% of spontaneous *r* mutants, growth temperature might affect the strength of selection against the non-*rII r* mutants and thus either increase or decrease mutant frequencies independently of the underlying mutation rate.

Growth media: We routinely use two different growth media for survival and mutagenesis experiments (see materials and methods), with variations depending on momentary strategies. LB (Luria-Bertani) medium is a rich broth medium whose components are mixed before sterilization by autoclaving. LB medium darkens upon autoclaving, when diverse chemical interactions undoubtedly occur among its many components. Some of these interactions may generate mutagens. We therefore compared mutant frequencies in both reversion and forward-mutation tests in stocks grown using LB sterilized by filtration through a 0.45-μm Millipore filter (color of an American lite beer; code "LB-F"), by autoclaving for 10 min at 20 psi, about 252°F (color of a good bitter; code "LB-A"), and by overautoclaving for 40 min (approaching the color of a stout; code "LB-O"). The stocks were then plated using standard top and bottom agars.

M9 medium is prepared by autoclaving a solution of most of its salts and, upon cooling, adding separately autoclaved solutions of $MgSO_4$-plus-glucose and of casamino acids. The last darkens considerably upon autoclaving. We compared *r* mutant frequencies in forward-mutation tests in stocks grown using M9 medium supplemented with casamino acids sterilized by the same three methods described above for LB medium (coded M9CA-F, M9CA-A, M9CA-O, respectively).

The results of these tests appear in Table 5. In the forward-mutation tests, there was no difference between LB and M9 media, nor among the various methods of medium preparation. The potentially more sensitive reversion tests also revealed no significant differences; historically, only differences of twofold or more are likely to be reproducible in such tests (Santos and Drake 1994). (*rUV7* and *rUV48* are G:C mutants; *rUV357* and *rUV375* are A:T ochre mutants; and *rUV74* appears to be a frameshift mutant that can also revert by a G:C → T:A transversion that provides a translational reinitiation site.)

The mutant frequency in UV-irradiated T4 during postirradiation holding: Irradiated T4 samples can be stored for weeks without change in their *r* mutant frequencies (Drake 1966). However, the interval soon after irradiation was not explored in those experiments, and early changes might have gone undetected. More recently, mutants have been shown to accumulate when UV-irradiated phage S13, which contains single-stranded DNA, is held for brief intervals (such as 30 min at 37°) before plating (Tessman and Kennedy 1991; Tessman *et al.* 1992; Tessman *et al.* 1994). This accumulation displays step-like kinetics and has been interpreted as reflecting some unusual kinetics of deamination of cyto-

TABLE 5

Effect of growth medium on mutant frequencies

Mutational pathway[a]	Medium	No. of stocks	Median mutant frequency
$r^+ \rightarrow r$	LB-F	7	1.0×10^{-3}
	LB-A	7	1.0×10^{-3}
	LB-O	7	1.1×10^{-3}
	M9CA-F	7	1.0×10^{-3}
	M9CA-A	7	1.0×10^{-3}
	M9CA-O	7	1.0×10^{-3}
rUV7 $\rightarrow r^+$	LB-F	5	2.6×10^{-7}
	LB-A	5	2.3×10^{-7}
	LB-O	5	2.0×10^{-7}
rUV48 $\rightarrow r^+$	LB-F	5	2.2×10^{-7}
	LB-A	5	3.5×10^{-7}
	LB-O	5	4.0×10^{-7}
rUV74 $\rightarrow r^+$	LB-F	5	3.6×10^{-7}
	LB-A	5	6.2×10^{-7}
	LB-O	5	7.0×10^{-7}
rUV357 $\rightarrow r^+$	LB-F	5	5.7×10^{-8}
	LB-A	5	7.8×10^{-8}
	LB-O	5	5.8×10^{-8}
rUV375 $\rightarrow r^+$	LB-F	5	0.6×10^{-7}
	LB-A	5	0.7×10^{-7}
	LB-O	5	1.1×10^{-7}

sine residues trapped within cyclobutane pyrimidine dimers. We therefore conducted experiments in which irradiated T4 particles were plated as soon as possible after irradiation or were held under various conditions that might promote "cytosine" deamination and then plated. Note that T4 DNA contains exclusively 5-HMC, whose deamination produces a thymine analogue that appears to be insusceptible to repair (Ripley and Drake 1984).

The results of some of these tests appear in Table 6, where holding for "0" min means that the particles were plated within 5 min of irradiation. We observed no significant effect of the holding regimen upon the frequency of mutants. The difference between T4 and S13 may reflect the single-stranded *vs.* double-stranded state of their DNAs, or differences in the way lesions are processed after infection.

TABLE 6

Effect of holding regimen upon UV mutagenesis on phage T4

Sec UV	Holding conditions: Min.	Temp.	pH	Survival $\times 10^3$	*r*/total	*r* mutants per 10^3 survivors
0	0				10/15,300	0.7
107	0			1.5	45/8,800	1.3
	60	21°	7		47/7,600	1.5
120	0			0.6	19/34,000	2.2
	60	21°	7		15/31,200	2.1
0	0				14/11,900	1.2
110	30	37°	7.3	1.3	36/14,100	2.6
			8.9		31/13,900	2.2

The mutability of phage RB69: Jim Karam (personal communication) told us that RB69 does not sport *r* mutants. This is surprising because T2, T4, and T6 all do, and the RB69 gp43 replicase/exonuclease has the same average fidelity as the corresponding T4 enzyme (Dressman *et al.* 1997). We therefore explored this difference ourselves. RB69 produces small, slightly sharp-edged plaques on B, B/4 and BB cells, and produces plaques on K-12 strains KB (a λ lysogen) and CR63 (not a λ lysogen), which are only slightly smaller and sharper than those of T4, suggesting some lysis inhibition. RB69 stocks grown in KB cells lyse somewhat like stocks of T4 r^+ mutants but produce lower titers. (Stocks of RB69 grown in B cells lyse slowly if at all, and produce low titers.) Paddison *et al.* (1998) constructed PCR primers that amplified a putative T4 *rI* homolog from several T-even phage DNAs, and also produced a weak signal from RB69 DNA; however, the sequence and functional significance of this signal remains uncharacterized.

We explored this situation further by screening 27,500 plaques on B cells and 10,000 plaques on KB cells; no typically large *r* mutants were observed. We then performed hydroxylamine mutagenesis using a dose that would produce ≥1% *r* mutants in T4; at a survival of 0.003, no typical *r* mutants were seen among 24,500 RB69 plaques. Thus, Jim Karam was quite correct to say that RB69 does not sport typical *r* mutants. However, RB69 does produce a few small, sharp-edged mutant plaques on KB cells. While rare ($\leq 5 \times 10^{-4}$) in untreated phages, they rise to a frequency of about 10^{-3} in the survivors ($S = 3.4 \times 10^{-4}$) of 210 sec of UV irradiation. These mutants may be similar to the classical *minute* mutants once studied in T2 and T4, which probably result from sublethal mutations in any of many genes required for phage replication. On the other hand, they may be *r* mutants with markedly reduced burst sizes.

DISCUSSION

The general rule that most T4 DNA metabolism is indifferent to the *E. coli* genotype has been extended to the host cAMP global regulatory system which, when inactivated by mutations in *cya* or *crp*, results in increased survival of *E. coli* after UV irradiation (Puyo *et al.* 1992) but does not affect the survival of T4 (Table 1). The basis of the effect in *E. coli* is difficult to assign because it persists in both *uvrA*$^-$ and *recA*$^-$ backgrounds; because the *uvrA*$^-$ *recA*$^-$ double mutant was not tested, it is possible that the mutations enhance the efficiency of both excision repair and recombination repair in *E. coli*.

Higher pH values in the plating medium also increase UV survival in both *E. coli* (Schuldiner *et al.* 1986; Goodson and Rowbury 1990) and T4 (Table 1), but

by only 14% in T4 compared to roughly threefold in *E. coli*. In *E. coli* the effect persists in both *polA*$^-$ and *recA*$^-$ backgrounds, presenting the same barrier to interpretation noted just above. The small magnitude of the effect in T4 renders further analysis difficult, but it would be interesting to know if the effect is mediated via a T4 or a host mechanism.

Thermal rescue, the increased survival of phage T4 at higher temperatures, displays two patterns among four T-even phages, being much stronger in T4 and T6 than in T2 and RB69 (Table 2). The idea that the early expression of gene *49* may be sufficiently temperature sensitive to be rate limiting to recombination repair was disproved in tests in which gp49 was provided early in infection from a plasmid-borne gene (Table 3). Thus, other than being a known property of recombination repair, thermal rescue remains a mystery.

By some criteria, RB69 is strongly diverged from T4 (Russell and Huskey 1974; Wang *et al.* 1995). RB69 displays the highest UV resistance reported thus far for a T-even phage (Figure 2). The genetics of this phage have been little studied, so that the basis for this difference remains unknown except that it is not the result of a smaller genome (Figure 3). The structure of the RB69 DNA polymerase and proofreading exonuclease (gp43) is now described (Wang *et al.* 1997), and RB69 gp43 can substitute for that of T4 yet support high replication fidelity (Dressman *et al.* 1997). Therefore, RB69 genetics is likely to be more closely studied in the future.

Because of anecdotal unpublished reports that particular batches of medium sometimes produce stocks with higher or lower mutant frequencies, it was something of a relief to find that the rate of spontaneous mutation in T4 is robustly independent of the composition and history of the medium (Table 5). While we confirmed an old observation that the revertant frequency in T4 *rII* stocks depends on growth temperature (Bessman and Reha-Krantz 1977), it was also pleasing to find that the overall mutation rate ($r^+ \rightarrow r$) depends hardly at all on temperature, removing one more potential variable from T4 forward-mutation experiments. It is perhaps also pleasing that the frequency of UV-induced *r* mutants does not detectably depend upon handling conditions between the times of irradiating and plating, as does UV mutagenesis in phage S13 (Tessman and Kennedy 1991; Tessman *et al.* 1992; Tessman *et al.* 1994).

We thank Joan Graves for performing the TAFE analysis of RB69 DNA, and Holly Dressman and Roel Schaaper for critical reading of the manuscript.

LITERATURE CITED

Barth, K. A., D. Powell, M. Trupin and G. Mosig, 1988 Regulation of two nested proteins from gene *49* (recombination endonuclease VII) and of a λ RexA-like protein of bacteriophage T4. Genetics **120:** 329–343.

Bessman, M. J., and L. J. Reha-Krantz, 1977 Studies on the biochemical basis of spontaneous mutation. V. Effect of temperature on mutation frequency. J. Mol. Biol. **116:** 115–123.

Conkling, M. A., and J. W. Drake, 1984a Isolation and characterization of conditional alleles of bacteriophage T4 genes *uvsX* and *uvsY*. Genetics **107:** 505–523.

Conkling, M. A., and J. W. Drake, 1984b Thermal rescue of UV-irradiated bacteriophage T4 and biphasic mode of action of the *WXY* system. Genetics **107:** 525–536.

Drake, J. W., 1963 Properties of ultraviolet-induced *r* II mutants of bacteriophage T4. J. Mol. Biol. **6:** 268–283.

Drake, J. W., 1966 Ultraviolet mutagenesis in bacteriophage T4. I. Irradiation of extracellular phage particles. J. Bacteriol. **91:** 1775–1780.

Drake, J. W., 1991 A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA **88:** 7160–7164.

Drake, J. W., and K. N. Kreuzer, 1994 DNA transactions in T4-infected *Escherichia coli*, pp. 11–13 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, D.C.

Drake, J. W., and L. S. Ripley, 1994a Mutagenesis, pp. 98–124 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, D.C.

Drake, J. W., and L. S. Ripley, 1994b Induced mutagenesis and isolation of T4 mutants, pp. 447–451 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, D.C.

Dressman, H., C.-C. Wang, J. D. Karam and J. W. Drake, 1997 Retention of replication fidelity by a DNA polymerase in a homeologous environment. Proc. Natl. Acad. Sci. USA **94:** 8042–8046.

Freese, E., E. Bautz and E. B. Freese, 1961 The chemical and mutagenic specificity of hydroxylamine. Proc. Natl. Acad. Sci. USA **47:** 845–855.

Goodson, M., and R. J. Rowbury, 1990 Habituation to alkali and increased u.v.-resistance in DNA repair-proficient and -deficient strains of *Escherichia coli* grown at pH 9.0. Lett. Appl. Microbiol. **11:** 123–125.

Hershey, A. D., 1946 Mutation of bacteriophage with respect to type of plaque. Genetics **31:** 620–640.

Koch, R. E., and J. W. Drake, 1970 Cryptic mutants of bacteriophage T4. Genetics **65:** 379–390.

Kreuzer, K. N., and J. W. Drake, 1994 Repair of lethal DNA damage, pp. 89–97 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, D.C.

Kutter, E., T. White, M. Kashlev, M. Uzan, J. McKinney *et al.*, 1994 Effects on host genome structure and expression, pp. 357-368 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, D.C.

Mosig, G., A. Luder, A. Ernst and N. Canan, 1991 Bypass of a primase requirement for bacteriophage T4 DNA replication in vivo by a recombination enzyme, endonuclease VII. New Biologist **13:** 1195–1205.

Paddison, P., S. T. Abedon, H. K. Dressman, K. Gailbreath, J. Tracy *et al.*, 1998 The roles of the bacteriophage T4 *r* genes in lysis inhibition and fine-structure genetics: a new perspective. Genetics **148:** 1539–1550.

Puyo, M.-F., P. Calsou and B. Salles, 1992 UV resistance of *E. coli* K-12 deficient in cAMP/CRP regulation. Mutat. Res. **282:** 247–252.

Ripley, L. S., and J. W. Drake, 1984 Bacteriophage T4 particles are refractory to bisulfite mutagenesis. Mutat. Res. **129:** 149–152.

Russell, R. L., and R. J. Huskey, 1974 Partial exclusion between T-even bacteriophages: an incipient genetic isolation mechanism. Genetics **78:** 989–1014.

Salles, B., and G. M. Weinstock, 1989 Interaction of the CRP-cAMP complex with the *cea* regulatory region. Mol. Gen. Genet. **215:** 537–542.

Santos, M. E., and J. W. Drake, 1994 Rates of spontaneous mutation in bacteriophage T4 are independent of host fidelity determinants. Genetics **138:** 553–564.

Schuldiner, S., V. Agmon, J. Brandsma, A. Cohen, E. Friedman *et al.*, 1986 Induction of SOS functions by alkaline intracellular pH in *Escherichia coli*. J. Bacteriol. **168:** 936–939.

Tessman, I., and M. A. Kennedy, 1991 The two-step model of UV mutagenesis reassessed: deamination of cytosine in cyclobutane dimers as the likely source of the mutations associated with photoreactivation. Mol. Gen. Genet. **227:** 144–148.

Tessman, I., M. A. Kennedy and S.-K. Liu, 1994 Unusual kinetics of uracil formation in single and double-stranded DNA by deamination of cytosine in cyclobutane pyrimidine dimers. J. Mol. Biol. **235:** 807–812.

Tessman, I., S.-K. Liu and M. A. Kennedy, 1992 Mechanism of SOS mutagenesis of UV-irradiated DNA: mostly error-free processing of deaminated cytosine. Proc. Natl. Acad. Sci. USA **89:** 1159–1163.

Wachsman, J. T., and J. W. Drake, 1987 A new epistasis group for the repair of DNA damage in bacteriophage T4: replication repair. Genetics **115:** 405–417.

Wang, C.-C, L.-S. Yeh and J. D. Karam, 1995 Modular organization of T4 DNA polymerase. Evidence from phylogenetics. J. Biol. Chem. **270:** 26558–26564.

Wang, J., A. K. M. A. Sattar, C. C. Wang, J. D. Karam, W. H. Konigsberg *et al.*, 1997 Crystal structure of a pol α family replication DNA polymerase from bacteriophage RB69. Cell **89:** 1087–1099.

Wang, M., and E. Lai, 1995 Pulsed field separation of large supercoiled and open-circular DNAs and its application to bacterial artificial chromosome cloning. Electrophoresis **16:** 1–7.