Rates of Spontaneous Mutation in Bacteriophage T4 Are Independent of Host Fidelity Determinants

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ABSTRACT

Bacteriophage T4 encodes most of the genes whose products are required for its DNA metabolism, and host (*Escherichia coli*) genes can only infrequently complement mutationally inactivated T4 genes. We screened the following host mutator mutations for effects on spontaneous mutation rates in T4: *mutT* (destruction of aberrant dGTPs), *polA*, *polB* and *polC* (DNA polymerases), *dnaQ* (exonucleolytic proofreading), *mutH*, *mutS*, *mutL* and *uvrD* (methyl-directed DNA mismatch repair), *mutM* and *mutY* (excision repair of oxygen-damaged DNA), *mutA* (function unknown), and *topB* and *osmZ* (affecting DNA topology). None increased T4 spontaneous mutation rates within a resolving power of about twofold (nor did *optA*, which is not a mutator but overexpresses a host dGTPase). Previous screens in T4 have revealed strong mutator mutations only in the gene encoding the viral DNA polymerase and proofreading 3′-exonuclease, plus weak mutators in several polymerase accessory proteins or determinants of dNTP pool sizes. T4 maintains a spontaneous mutation rate per base pair about 30-fold greater than that of its host. Thus, the joint high fidelity of insertion by T4 DNA polymerase and proofreading by its associated 3′-exonuclease appear to determine the T4 spontaneous mutation rate, whereas the host requires numerous additional systems to achieve high replication fidelity.

BACTERIOPHAGE T4 has evolved a replication strategy that insulates most aspects of its DNA metabolism from that of its host *Escherichia coli*. Instead, T4 encodes the corresponding functions in its own genome. T4 distinguishes its DNA by inserting 5-hydroxymethylcytosine (5-hmC) instead of cytosine and, after DNA replication, by glucosylating its 5-hmC. [For recent reviews of DNA replication, recombination, repair and mutation in T4, see DRAKE and RIPLEY (1994), GREENBERG *et al.* (1994), KREUZER and DRAKE (1994), KREUZER and MORRICAL (1994), MOSIG (1994) and NOSSAL 1994).] As a result, few defects in host DNA metabolism affect T4 DNA replication, and host genes can rarely complement mutational defects in their T4 counterparts. For instance, DNA repair in T4 is indifferent to the *uvrABC* and *recA* systems, although T4 is subject to photoreactivation by the host *phr* system.

DNA-based microbes display a common spontaneous mutation rate of about 0.0033 per genome per DNA replication (DRAKE 1991). Thus, their average rates of mutation per base pair vary inversely with genome size. The T4 genome is about 30-fold smaller than the *E. coli* genome, and T4 has an average spontaneous mutation rate per base pair about 30-fold larger than that of *E. coli*. While a general picture is emerging of how *E. coli* determines its spontaneous mutation rate (SCHAAPER 1993), it has been less clear how T4 does so. Do host genes involved in maintaining accurate replication of DNA also affect the fidelity of T4 DNA replication?

Few host genes have been tested for their ability to influence spontaneous mutation in T4. Despite the privatization of T4 DNA metabolism, it seemed likely to us that some host genes might assist T4 in maintaining the fidelity of its DNA replication. Were these genes also to assist the maintenance of replication fidelity in the host, their mutant alleles would exhibit mutator activity. We therefore systematically examined nearly all known host mutator mutants for effects on spontaneous mutation in T4, using sensitive tests for the T4 mutational pathways most likely to be affected. The results were uniformly negative. In view of our current understanding of the evolution and mechanics of spontaneous mutation in T4, this set of negative results suggests that the virus has evolved a powerful but remarkably simple strategy to achieve its mutation rate.

MATERIALS AND METHODS

Media: L broth and Drake top and bottom agars were used throughout [CONKLING and DRAKE (1984a) and references therein].

Strains and growth conditions: T4 strains (Table 1) consisted of wild-type T4B and various *rII* mutants chosen because their reversion pathways are fairly well understood. Unless otherwise indicated, stocks were grown by plating on the host of choice, picking a plaque with the corner of a sterile paper strip, briefly introducing this into 2 ml of the same host strain grown

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TABLE 1

T4 mutants

Mutation[a]	Map segment[b]	Type[c]	Other relevant characteristics	References[d]
r131	A6c	FS	At $(A{\cdot}T)_6$ run	1
r360	B1a	A·T	Ochre mutation in B1	1, 2
r2074	B1a	BPS	Amber mutation in B1	1, 2
rEM84	B1b1	BPS	Amber mutation in B1	1, 2
rFC11	B1a	FS/fs	Large target size	1, 2, 3
rFC47	B1b2	FS/fs	Large target size	1, 2, 3
rHB74	B1b1	BPS	Amber mutation in B1	1, 2
rHB118	A2h1	BPS	Amber mutation in B1	4
rNT332	B1b1	BPS	Amber mutation in B1	1, 2
rUV7	B7a	G·C		5
rUV13	A1b2	G·C		5
rUV48	B9a	G·C		5
rUV256	A3d	G·C		5
rUV357	B1a	A·T	Ochre mutation in B1	1, 5
rUV363	B7b	G·C		5
rUV375	B1a	A·T	Ochre mutation in B1	1, 5
rX27	B1b1	A·T	Ochre mutation in B1	1, 2
tsCB120			DNA polymerase mutation	6, 7

[a] All *r* mutations reside in an *rII* cistron in a T4B background. The *ts* mutation resides in gene *43* and was backcrossed five times against T4B.

[b] *rII* gene segments defined by deletion mapping (BENZER 1962).

[c] BPS = capable in principle of reversion by any base-pair substitution except G·C → A·T because the corresponding UAG codon resides in a permissive region (see text). FS = capable of reversion to a wild or nearly wild phenotype by a frameshift mutation (addition or deletion of one or a few base pairs); reverted by proflavin but not by base analogs; *FC11* produces a (−1) reading frame shift, while *FC47* produces a (+1) shift. fs = capable of reversion to a tiny-plaque morphology by a frameshift mutation (see text). A·T = susceptible to reversion by 2-aminopurine but not by hydroxylamine, and therefore capable of reversion by A·T → G·C transitions but not by G·C → A·T transitions, and potentially capable of reversion by any transversions. G·C = susceptible to reversion by hydroxylamine and therefore capable of reversion by G·C → A·T transitions, and potentially capable of reversion by G·C → A·T transitions and by any transversions.

[d] 1 = PRIBNOW *et al.* (1981), 2 = BARNETT *et al.* (1967), 3 = RIPLEY *et al.* (1986), 4 = BENZER and CHAMPE (1961), 5 = DRAKE (1963a), 6 = DRAKE *et al.* (1969), 7 = RIPLEY (1975).

to ~10^8/ml in L broth, and incubating on a rotary shaker at 37° lysis; stock titers were typically (4–8) × 10^{10}/ml.

E. coli strains are described in Table 2. In most instances, the mutator strains are otherwise isogenic with the control strain.

Reversion rates: For stocks grown to 10^{10}–10^{11} particles, relative mutation rates are proportional to relative mutant frequencies and are insensitive to variations in stock titer (DRAKE 1991). Thus, we compared revertant frequencies and did not convert to mutation rates. For reversion tests, we grew five or more stocks in parallel on the host strains to be compared. We present median rather than mean revertant frequencies because means (but not medians) are strongly affected by "jackpot" stocks containing unusually high frequencies of revertants. Medians are the most reproducible value under conditions where random variation is clonal rather than Poisson in nature. Historically, such medians are reproducible in this laboratory to within a factor of about two when presented as relative medians (experimental median ÷ control median) for stocks grown in parallel. In the few instances where an increase of more than twofold was detected when the particles were propagated on a mutator *vs.* an isogenic non-mutator host strain, the measurement was repeated.

Forward mutation rates: The considerations that apply to revertant frequencies also apply to frequencies of *r* mutants, which arise via mutations in about five cistrons encompassing roughly 5 kbp. To estimate total mutant frequencies, five T4B stocks were grown on each host strain and were screened visually for R mutants (large, sharp-edged plaques) on plates displaying about 800 plaques. Screening continued until about 25 (or more) mutant plaques were detected, providing a sampling variance that was usually ≤25%; substantially more plaques (53–1516) were counted to estimate the total population size.

To estimate frequencies of deletion mutations, a few hundred wild-type T4B plaques were picked from the host strains of interest with the corner of a sterile paper strip (a "pickate," containing 10^{6-7} particles) and replated until one R plaque (the phenotype of an *r* mutant) was obtained from most of the pickates; thus, all R plaques were of independent origin. The pickates were first classified by spotting roughly 0.01 ml with a sterile paper strip onto lawns of KB cells, on which the RI phenotype (produced by *rI* and *rV* mutations) consists of R plaques, the RII phenotype (produced by *rIIA* and *rIIB* mutations) consists of no plaques (except for rare revertants), and the RIII phenotype (produced by *rIII* mutations plus leaky or rapidly reverting *rII* mutations) consists of wild-type plaques. Because *rII* mutants whose stocks contain fewer than ~10^{-9} revertants when plated on KB cells almost always carried deletions (CONKLING *et al.* 1976; J. W. DRAKE, unpublished results), we screened progressively more particles from each *rII* isolate by (i) plating 0.1-ml samples from the *rII* pickates, (ii-iii) growing low-titer stocks (~10^{-10}/ml), spot-testing from them, and then plating ~10^{-8} particles on KB cells, and (iv) growing high-titer stocks (~10^{-11}/ml) and plating 8 × 10^8 particles on KB cells. The non-reverting *rII* mutants were classified as deletions.

RESULTS

Host mutators: The relevant characteristics of the *E. coli* mutator mutants (plus *optA*) are listed in Table 3. The mutators include alleles of genes acting before, during, after, and extrinsic to DNA replication, and will be discussed later.

Selection of *rII* tester strains: *rII* mutants were chosen for their ability to revert by the pathways expected

TABLE 2

***E. coli* strains**

Strain[a]	Mutator genotype[b]	Full genotype[b] and other characteristics	Source[c]	References[d]
P90C	*mut*$^+$	*araΔ(lac-proB)*$_{XIII}$ *thi-1*		1
KA796	*mut*$^+$	= P90C	RMS	2
NR8039	*mutH*	KA796 + *mutH101*	RMS	3
NR8041	*mutS*	KA796 + *mutS101*	RMS	3
NR8040	*mutL*	KA796 + *mutL101*	RMS	3
NR9082	*mutT*	KA796 + *azi mutT1*	RMS	4
NR9373	*mutY*	KA796 + *mutY*::Tn*5* from PR68	RMS	5
NR11040	*mutM*	KA796 + *mutM*::mini-tet from TT101	RMS	6
NR11070	*mutYM*	KA796 + *mutY*::Tn*5 mutM*::mini-tet	RMS	5, 6
NR9458	*dnaQ*	KA796 + *zaf-13*::Tn*10 mutD5*	RMS	7
NR9601	*mut*$^+$	*ara-9 fhuA1 lacZ118 tsx-3 supE44 galK2 hisG4 rfbD1? trp-3 rpsL8* or *rpsL99 malA1 metE46 mtl-1 thi-1*	RMS	8
NR9699	*polC*	NR9601 + *zae-502*::Tn*10 dnaE919*	RMS	8
MAF100	*mut*$^+$	*lexA3 malE*::Tn*10*	RMS	9
MAF102	*uvrD*	MAF100 + *Δ(uvrD)288::kan* from SK6776	RMS	9
FC36	*mut*$^+$	P90C + Rif^r	PLF	10
PFB40	*polA*	FC36 + *fadAB101*::miniTn*10 polA1*	PLF	11
PA610	*mut*$^+$	*thr leu purE his lys argH thi ara lacY gal malA mtl xyl str*^{r} *tonA supE*	PG	12
HR40	*optA*	PA610 + *optA1*	PG	13
CC105	*mut*$^+$	P90C + F′ *laclZ proB*$^+$		14
S90C/CC105	*mut*$^+$	= CC205 = CC105 + Str^r	JHM	15
(MutA)	*mutA*	S90C/CC105 + *mutA*	JHM	15
1*P90C	*mut*$^+$	= DB-1 = P90C + F′ *lacIZ*	JHM	16
(TopB)	*topB*	= DB100 = 1*P90C + *topB*::miniTn*10*	JHM	17
S90C	*mut*$^+$	P90C + Str^R	MFG	18
SH2101	*polB*	S90C + *polB* Δ1::ΩSm-Sp	MFG	18
TP2101	*mut*$^+$	*xyl-7 argH1 ΔlacX74* (= *lacZ*)	PL	19
TP2600	*osmZ*	TP2101 + *bglY2600*	PL	19
B	*mut*$^+$	Wild type, displays all *r* mutants	SB	20
BB	*mut*$^+$	Wild type, host for *rII* mutants	SB	20
KB	*mut*$^+$	Wild type, restricts *rII* mutants	SB	20

[a] Entries in parentheses were named by the donator by appending the relevant added mutation to the parental strain name.

[b] Canonical gene names are as in BACHMANN (1990) when provided; see Table 3 for synonyms.

[c] RMS = ROEL M. SCHAAPER, PLF = PATRICIA L. FOSTER, PG = PETER GAUSS, JHM = JEFFREY H. MILLER, MFG = MYRON F. GOODMAN, PL = PHILIPPE LEJEUNE, SB = SEYMOUR BENZER.

[d] 1 = MILLER *et al.* (1977), 2 = SCHAAPER *et al.* (1985), 3 = GLICKMAN and RADMAN (1980), 4 = SCHAAPER and DUNN (1987), 5 = RADICELLA *et al.* (1988), 6 = MICHAELS *et al.* (1991), 7 = SCHAAPER and CORNACCHIO (1992), 8 = OLLER *et al.* (1993), 9 = WASHBURN and KUSHNER (1991), 10 = CAIRNS and FOSTER (1991), 11 = DELUCIA and CAIRNS (1969), 12 = CHASE and RICHARDSON (1977), 13 = SAITO and RICHARDSON (1981), 14 = CUPPLES and MILLER (1989), 15 = MICHAELS *et al.* (1990), 16 = WHORISKEY *et al.* (1991), 17 = SCHOFIELD *et al.* (1992), 18 = ESCARCELLER *et al.* (1994), 19 = LEJEUNE and DANCHIN (1990), 20 = BENZER (1955).

to be promoted if a host mutator acted on T4 with the same specificity it displays on its own genome.

The base-pair substitution (BPS) tester mutants carry amber mutations (specifying UAG codons) within the first 174 bp of the *rIIB* cistron. This region is dispensable for many *rIIB* functions and is insensitive to almost all missense mutations but is fully sensitive to chain-terminating mutations (DRAKE 1963b; NELSON *et al.* 1981; PRIBNOW *et al.* 1981). Thus, these mutants should be able to revert by any base-pair substitution except G·C → A·T (which produces the cognate UAA ochre codon). This expectation was confirmed by testing their ability to form plaques on amber-suppressor host strains inserting tyrosine (generated from an amber codon by G·C → C·G and G·C → T·A), glutamine (A·T → G·C), serine (A·T → C·G), and lysine (A·T → T·A) (data not shown). The sequences surrounding these mutations are known (PRIBNOW *et al.* 1981), and each has different nearby base pairs. Nonsense suppressors in the T4 genome do not suppress *rII* nonsense codons (DRAKE and RIPLEY 1983) because T4 tRNAs are expressed too late to rescue an *rII* defect.

The A·T tester mutants carry ochre mutations (specifying UAA codons), again within the first 174 bp of the *rIIB* cistron, and can revert by A·T → any base pair.

The G·C tester mutants carry mutations scattered throughout the *rII* locus. They are reverted by hydroxylamine (DRAKE 1963a) and thus can revert via G·C → A·T transitions, but other pathways are not excluded.

The frameshift tester mutants are of two types. One, *r131*, resulted from the reduction of a run of six consecutive A·T base pairs to five and usually reverts back to six (PRIBNOW *et al.* 1981; STREISINGER and OWEN 1985). The other two, *rFC11* and *rFC47*, reside in the early portion of the *rIIB* cistron. *rFC11* produces a (−1) and *rFC47* a (+1) reading frame shift. Both mutations can revert by intragenic suppressors over about 120 bp bounded by out-of-frame stop codons (RIPLEY *et al.* 1986). The revertants may display a wild (FS) phenotype or a partially mutant (fs) phenotype consisting of small

TABLE 3

Properties of host mutator mutants

Mutator gene[a]	Other names	Strongest pathways[b]	Mutator factor[c]	Enzymology[d]	References[e]
optA			1	(dGTP → dG + PPP) dGTPase increased 50×	1–3
mutT		A·T → C·G	10^3	dG*TPase where G* = 8-Oxo, etc.	4–8
polA	*resA*	BPS, Fs	10	Pol I: DNA repair polymerase; induces SOS?	9–12
polB		BPS?	30	Pol II: DNA repair polymerase	13
polC	*dnaE*	BPS, Fs	40[f]	Pol III: DNA replication polymerase	14–16
dnaQ	*mutD*	Ts > Tv	10^2	Pol III: DNA proofreading 3′-exonuclease[g]	17, 18
mutH	*mutR*	Ts, Fs	10^3	Methyl-directed DNA mismatch repair: MutH recognizes GATC, MutS recognizes mispairs, MutL promotes excision, UvrD unwinds dsDNA	16, 19–21
mutL		Ts, Fs	10^3		
mutS		Ts, Fs	10^3		
uvrD	*mutU*	BPS?	10^3	DNA helicase II	22–24
mutY	*micA*	G·C → T·A	30	Adenine DNA glycosylase on G·A, 8-oxoG·A	25, 26
mutM	*fpg*	G·C → T·A	15	8-oxoG and purine ring-opened DNA glycosylase	25, 26
mutYM			10^3		25
mutA		A·T → T·A	10	Unknown	27
topB	*mutR*	Δ	6	DNA topoisomerase III (relaxes supercoils)	28, 29
osmZ	*bglY*	Δ	10^2	Histone-like protein affecting DNA supercoiling	30, 31

[a] Canonical name and synonyms are as in BACHMANN (1990) if listed therein; see also RILEY (1993).

[b] BPS = ill defined base-pair substitutions. Fs = frameshifts (base additions and/or deletions). Ts = transitions. Tv = transversions. Δ = deletions. ? = at least that pathway is promoted.

[c] Typical factor of increase in *E. coli*.

[d] A number of gene products are or may be involved in aspects of host DNA metabolism in addition to the listed function. For instance, PolA may function not only in diverse excision repair systems but also in DNA replication, and DNA helicase II is involved in fidelity processes other than DNA mismatch repair, including recombination and the SOS response.

[e] Frequently, only key or recent references are provided as useful points of entry. 1 = MYERS *et al.* (1987), 2 = BEAUCHAMP and RICHARDSON (1988), 3 = SETO *et al.* (1988), 4 = COX (1976), 5 = AKIYAMA *et al.* (1988), 6 = BHATNAGAR and BESSMAN (1988), 7 = SCHAAPER *et al.* (1989), 8 = MAKI and SEKIGUCHI (1992), 9 = VACCARO and SIEGEL (1978), 10 = ENGLER and BESSMAN (1979), 11 = FIX *et al.* (1987), 12 = BATES *et al.* (1989), 13 = ESCARCELLER *et al.* (1994), 14 = MO *et al.* (1991), 15 = OLLER *et al.* (1993), 16 = SCHAAPER (1993), 17 = SCHAAPER (1988), 18 = SCHAAPER (1989), 19 = LEONG *et al.* (1986), 20 = SCHAAPER and DUNN (1987), 21 = MODRICH (1991), 22 = SIEGEL (1973), 23 = WASHBURN and KUSHNER (1991), 24 = R. M. SCHAAPER (personal communication), 25 = MICHAELS *et al.* (1992), 26 = MICHAELS and MILLER (1992), 27 = MICHAELS *et al.* (1990), 28 = WHORISKEY *et al.* (1991), 29 = SCHOFIELD *et al.* (1992), 30 = HIGGINS *et al.* (1988), 31 = LEJEUNE and DANCHIN (1990).

[f] BPS values for the *dnaE919* allele (OLLER *et al.* 1993) used in our T4 experiments; with *dnaE173*, values were ~10^4 and ~10^5 (MO *et al.* 1991).

[g] A defect in proofreading sometimes saturates the *mutHSL* system, in which case both *dnaQ* and *mutHSL* mutator specificities and factors apply; the listed value probably represents mainly the proofreading contribution.

to tiny plaques on KB cells; the efficiency of scoring of fs revertants is variable from day to day but is moderately consistent between different plates on a particular day. These two mutants revert by a variety of frameshifting mechanisms (RIPLEY and SHOEMAKER 1983).

Effects of host mutators in reversion tests: In each case, *rII* tester mutants were used which could respond to the mutational specificity of the host mutator if it acted on T4 sufficiently strongly to produce a result at least twofold above the background. (Note, however, that the background revertant frequency of an *rII* tester mutant is often the sum of several pathways, not all of which might be affected by the mutator.) The results are displayed in Table 4 in roughly the order in which the mutators might affect T4 mutation rates: before, during, after, or extrinsic to DNA replication. For the large-reversion-target frameshift mutants *rFC11* and *rFC47*, both FS and fs revertants are recorded. In the first round of tests, *rII* mutants grown on some of the mutators displayed median revertant frequencies that were more than twofold over the background (in eight of 68 tests: *rHB74* on *mutT*, *mutH*, *mutS*, *mutYM*, and *mutA*; *rNT332* on *uvrD*; *rHB118* on *uvrD* and *mutA*). None of these differences persisted in a second round of tests, and the first results with *rHB74* were probably the consequence of an anomalously low control value. Overall, there was no discernible host mutator effect on spontaneous mutation rates in T4.

Because we focused on mutator effects, the few relative medians of <0.5 (in 5 of 68 tests: *rFC47* on *polA*; *rHB74* on *polC* and *uvrD*; *rNT332* on *mutS* and *mutY*) were not remeasured, but are more likely to represent sampling deviations than true antimutator effects because of their low frequency.

The *mutT* mutator specifically enhances A·T → C·G transversions. It did not detectably affect T4 (Table 4). However, if an amber *rII* tester mutant reverts strongly by transitions but weakly by transversions, an increase in transversions might go undetected. Some of the tests were therefore repeated in a T4 gene *43* (DNA polymerase) *tsCB120* background, because *tsCB120* strongly reduces the frequency of A·T → G·C transitions (DRAKE *et al.* 1969) but only modestly increases transversions at A·T sites (RIPLEY 1975). The results appear in Table 5. *tsCB120* reduced the reversion of *rHB74* by about threefold, suggesting that *rHB74* reverts primarily by transitions in a wild-type background. *tsCB120* increased the reversion of *rEM84* by about threefold (in

TABLE 4

Effects of *E. coli* mutator mutations on bacteriophage T4

Host mutator gene[a]	T4 *rII* tester mutant[b]	Mutations detected[b]	Control revertants per 10^8	No. of control stocks	Relative revertant frequency[c]
optA	*UV7*	From G·C	26	5	0.7
	UV13	From G·C	64	5	0.6
	UV48	From G·C	24	5	0.6
	UV256	From G·C	91	5	0.8
	UV363	From G·C	75	5	0.6
	360	From A·T	16	5	1.1
	UV357	From A·T	93	5	2.1
	UV375	From A·T	25	5	0.6
	X27	From A·T	31	5	1.1
mutT	*EM84*	BPS	12	10	0.9
	NT332	BPS	7.7	10	0.7
	HB74	BPS	4.6	10	3.1
			9.8	10	1.8
	2074	BPS	53	10	0.7
polA	*EM84*	BPS	35	5	0.6
	2074	BPS	133	5	0.6
	131	FS	45	5	0.4
	FC11	FS	40	5	0.6
		fs	21	5	1.7
	FC47	FS	15	5	1.2
		fs	89	5	0.3
polB	*EM84*	BPS	16	5	1.5
	2074	BPS	190	5	1.1
	HB74	BPS	31	5	0.9
	131	FS	45	5	1.8
	FC11	FS	87	5	1.0
		fs	257	5	1.0
	FC47	FS	71	5	1.2
		fs	207	5	1.3
polC	*EM84*	BPS	8.2	5	2.1
	NT332	BPS	13	5	0.8
	HB74	BPS	63	5	0.4
	2074	BPS	156	5	0.8
	HB118	BPS	9.2	5	0.5
dnaQ	EM84	BPS	34	5	0.9
	NT332	BPS	5.3	5	1.3
	HB74	BPS	7.8	5	1.9
	2074	BPS	58	5	1.2
	HB118	BPS	14	5	0.5
mutH	*EM84*	BPS	12	10	1.0
	NT332	BPS	7.7	10	1.0
	HB74	BPS	4.6	10	2.8
			9.8	10	1.1
	2074	BPS	53	10	1.4
mutL	*EM84*	BPS	12	10	1.7
	NT332	BPS	7.7	10	1.1
	HB74	BPS	4.6	10	1.7
	2074	BPS	53	10	1.3
mutS	*EM84*	BPS	12	10	1.2
	NT332	BPS	7.7	10	0.4
	HB74	BPS	4.6	10	3.8
			9.8	10	2.1
	2074	BPS	53	10	0.6
uvrD	*EM84*	BPS	18	5	0.6
	NT332	BPS	6.1	5	4.1
			16	5	1.7
	HB74	BPS	46	5	0.4
	2074	BPS	91	5	0.7
	HB118	BPS	30	5	3.9
			41	5	1.4
mutY	*EM84*	BPS	12	10	1.5
	NT332	BPS	7.7	10	0.4
	HB74	BPS	4.6	10	1.8
	2074	BPS	53	10	0.9

TABLE 4

Continued

Host mutator gene[a]	T4 *rII* tester mutant[b]	Mutations detected[b]	Control revertants per 10^8	No. of control stocks	Relative revertant frequency[b]
mutM	*EM84*	BPS	12	10	0.7
	NT332	BPS	7.7	10	0.7
	HB74	BPS	4.6	10	1.8
	2074	BPS	53	10	1.0
mutYM	*EM84*	BPS	12	10	1.4
	NT332	BPS	7.7	10	0.9
	HB74	BPS	4.6	10	2.7
			9.8	10	1.4
	2074	BPS	53	10	0.7
mutA	*EM84*	BPS	11	5	0.9
	NT332	BPS	6.5	5	0.7
	HB74	BPS	6.2	5	3.8
			25	5	1.0
	2074	BPS	62	5	1.7
	HB118	BPS	2.6	5	4.2
			34	5	2.9

[a] See Tables 2 and 3 and the Discussion for properties of the *E. coli* mutator strains; *mutYM* = *mutY mutM*.
[b] See Table 1 and the Results section for properties of the *rII* tester strains.
[c] Median revertant frequency of five *rII* stocks grown on the mutator host divided by median revertant frequency of stocks grown on the otherwise isogenic non-mutator host.

a test that was duplicated to confirm the result), suggesting that *rEM84* frequently reverts by transversions in a wild-type background. When the *rII-ts* double mutants were grown in otherwise isogenic *mutT*$^+$ and *mutT* cells, there was again no *mutT* effect on the reversion of the *rII* mutation.

Effects of host deletion mutators: Reversion tests for large deletions were not available, and the more tedious method described in MATERIALS AND METHODS was used instead. First, median R mutant frequencies were measured for wild-type T4 grown on isogenic Mut and Mut$^+$ strains. Next, a set of independent *rII* mutants was collected and screened for members displaying $\leq 10^{-9}$ revertants. Such *rII* mutants were classified as harboring deletions based on previous experience. The results appear in Table 6. Neither host mutator affected either median R mutant frequencies or the fraction of nonreverting *rII* mutants.

DISCUSSION

Expectations and reliabilities: Mutator mutations mark genes whose wild-type gene products are likely to assist in maintaining the fidelity of genomic replication. A host mutator mutation might affect rates of spontaneous mutation in phage T4 either if the wild-type allele reduced T4 mutation rates, or if the mutator allele increased T4 mutation rates. Either possibility seemed unlikely where T4 encodes its own version of the afflicted host function, but an effect seemed possible for some others.

Historically, both the reversion and the forward-mutation tests are usually reproducible to within twofold, and we therefore regarded a twofold increase as interesting if reproducible. Except for one cluster of results based on a low control value, there were very few factors of increase greater than twofold, and none of these were reproduced in subsequent tests. While effects larger than twofold could have escaped detection if the tester system recorded the sum of several pathways of which only one was increased by the host mutator, the use of several tester mutants with each mutator host reduces this possibility. There was little overall tendency for the relative reversion frequencies to fall above *vs.* below unity (40 *vs.* 34), and none at all (35 *vs.* 34) when the results based on the lower-than-average *rHB74* control value are ignored.

Host mutations tested previously for effects on mutation in T4: Various *rpoB* mutations (rif^r, stl^r, and the double mutant) stimulate the reversion of diverse T4 base pair substitutions by up to fivefold (ALIKHANIAN *et al.* 1976). The mechanism is unknown.

An *ung* mutation inactivates the deoxyuracil glycosylase which removes the mutagenic products of deaminated cytosines, and is a weak *E. coli* mutator (DUNCAN and WEISS 1982). The glycosylase does not excise thymine and thus should not excise 5-hmC in T4. An *ung* mutation does not affect T4 mutation rates (SIMMONS and FRIEDBERG 1979; RIPLEY and DRAKE 1984).

Host cells induced for the SOS response (*e.g.*, for *recA* and *umuDC*) are mutators (MILLER and LOW 1984), but there were no differences in the frequencies of mutant plaques in T4 stocks grown on uninduced cells and plated on induced cells, nor were survival or mutagenesis after ultraviolet irradiation affected (CONKLING and DRAKE 1984b). The assay would probably have detected an increase over the background had the mutation rate increased severalfold during plaque growth on the plates.

TABLE 5

Tests for host *mutT* action on T4 in a *43ts* background

T4 *rII* tester mutant	T4 gene *43* background	Host mutator genotype	No. of control stocks	Median revertant frequency	Relative revertant frequency
HB74	ts^+	mut^+	3	26.3	
	tsCB120	mut^+	3	7.8	0.30
EM84	ts^+	mut^+	3	6.3	
	tsCB120	mut^+	3	17.1	2.7
	ts^+	mut^+	5	5.9	
	tsCB120	mut^+	5	18.5	3.1
HB74	*tsCB120*	mut^+	5	6.8	
		mutT	5	9.2	1.3
EM84	*tsCB120*	mut^+	5	11.0	
		mutT	5	10.2	0.9

See Table 4 footnotes for descriptions of terms. In the top six entries, the $mutT^+$ host was BB cells.

TABLE 6

Effects of *E. coli* deletion-mutators on bacteriophage T4

Relevant host genotype	Median R mutant frequency ($\times 10^3$)	Nonreverters among *rII*	
		No./No.	Frequency
$topB^+$	0.98	5/113	0.044
topB	1.03	4/132	0.030
$osmZ^+$	1.57	2/81	0.025
osmZ	1.69	6/169	0.036
$topB^+$ + $osmZ^+$		7/194	0.036

Revertant frequencies of several T4 amber mutants were reported to be sharply increased in a *recBC sbcA* si^+ host (KANNAN and DHARMALINGAM 1987) for reasons unknown.

***optA*:** The *optA1* mutant 50-fold overproduces a dGTPase (MYERS *et al.* 1987; BEAUCHAMP and RICHARDSON 1988; SETO *et al.* 1988) and restricts the growth of T4 gene *43* mutants that turn over dNTPs rapidly (GAUSS *et al.* 1983). *optA1* produces little or no mutator effect in *E. coli* when resistance to rifampicin or nalidixic acid is scored (WURGLER-MURPHY 1993), perhaps because it reduces the dGTP pool size only about fivefold (MYERS *et al.* 1987). In T4, *optA1* might enhance mutation at G·C sites and/or reduce mutation at A·T sites. Neither outcome was detected. This result may reflect the observations that "thymineless" mutagenesis (SMITH *et al.* 1973) and "cytosineless" mutagenesis (WILLIAMS and DRAKE 1977) in T4 only occur in sharply reduced burst sizes.

***mutT*:** This was the first *E. coli* mutator to be studied intensively. An early paper (PIERCE 1966) reported that *mutT* produced a 3.3-fold increase in the median frequency of T4 *q* mutants, no effect on the reversion of a frameshift mutant, and a 24-fold increase in the median frequency of revertants of *rAP129*, which can revert by G·C → A·T transitions and perhaps by other pathways as well. A later report (COX and YANOFSKY 1969) described tests with 17 mostly *rII* mutants whose reversion was usually indifferent to the allelic state of the host at the *mutT* locus; however, two 10-fold antimutator effects and one eightfold mutator effect were tabulated. *mutT* specifically promotes A·T → C·G transversions (by factors of 10^3–10^4) arising via $A_{template}$·G_{primer} mispairs, and its wild-type allele encodes a dGTPase which degrades abnormal, mutagenic forms of dGTP, including the 8-oxo derivative (YANOFSKY *et al.* 1966; COX 1976; AKIYAMA *et al.* 1988; BHATNAGAR and BESSMAN 1988; SCHAAPER *et al.* 1989; MAKI and SEKIGUCHI 1992). However, we detected no *mutT* effect, even when the possibly obscuring role of transitions in the spontaneous background was reduced by means of an "antimutator" mutation in the viral DNA polymerase.

How, then, does T4 protect itself against the mutagenic ravages of aberrant dGTPs? At least three possibilities can be imagined: channeling, a T4 homolog of MutT, and strong discrimination against the mutagenic version(s) of dGTP during DNA synthesis.

Channeling, in which a dNTP is synthesized within the replication complex and then is rapidly incorporated into DNA (GREENBERG *et al.* 1994), might reduce the time during which dGTP could be oxidatively converted into the 8-oxo derivative or might reject abnormal precursors of dGTP, although either process would have to be much more efficient for T4 than for *E. coli* to explain the lack of a *mutT* effect on T4. Alternatively, MutT might act within the host replication complex but be unable to enter the T4 replication complex. In that case, T4 might contain its own version of *mutT*. We therefore searched the T4 genome for a homolog of the *E. coli* or *Proteus vulgaris mutT* sequences using the algorithms FASTA (PEARSON and LIPMAN 1988), BLAST (ALTSCHUL *et al.* 1990) and PIMA (SMITH and SMITH 1992). No similarities were detected, suggesting that T4 encodes no MutT homolog.

Instead, T4 may protect itself against mutagenic forms of dGTP by its intrinsically high fidelity of DNA replication. T4 DNA polymerase discriminates more strongly against the incorporation of 8-oxo-dGTP *in vitro*, and its

3′-exonuclease proofreads the insertion more strongly, than does the Klenow fragment of *E. coli* polymerase I. As a result, the analog is much less mutagenic *in vitro* with the T4 enzyme than with *E. coli* polymerase I, or with the *Tth* or mammalian γ polymerases (PAVLOV *et al.* 1994). If fidelity is further improved by additional replication proteins [for reviews see YOUNG *et al.* (1992) and DRAKE and RIPLEY (1994)], then T4 may require no additional protection against this source of mutations.

***polA*:** Mutator mutations in *polA* produce base-pair substitutions and frameshift mutations in *E. coli* (VACCARO and SIEGEL 1978; ENGLER and BESSMAN 1979; FIX *et al.* 1987; BATES *et al.*, 1989). Pol I (= PolA) also contains both 3′- and 5′-exonuclease activities, and *polA* mutations reduce excision repair in T4 exposed to ultraviolet irradiation (MAYNARD SMITH *et al.* 1970), methyl methanesulfonate (EBISUZAKI *et al.* 1975), ethyl methanesulfonate (RAY *et al.* 1972) or hydroxylamine (JANION 1982), especially in the presence of host *xth* (3′-exonuclease III) or *nfo* (endonuclease IV) mutations (SAPORITA *et al.* 1989). However, we detected changes in neither base-pair substitution nor frameshift mutation frequencies.

***polB*:** Mutator mutations in *polB* produce at least base-pair substitutions in *E. coli* (ESCARCELLER *et al.* 1994) and Pol II (= PolB) can synthesize past abasic template sites and thus generate mutations (TESSMAN and KENNEDY 1994). A *polB* mutation may slightly reduce the survival of T4 particles treated with methyl methanesulfonate (NISHIDA *et al.* 1976). However, a *polB* mutator detectably enhanced neither base-pair substitution nor frameshift mutagenesis in T4.

***polC* and *dnaQ*:** *polC* (= *dnaE*) encodes Pol III (= PolC), *dnaQ* (= *mutD*) encodes proofreading 3′-exonuclease ϵ, and both are components of the *E. coli* replicative complex. A *polC* mutator produces both base-pair substitutions and frameshift mutations (MO *et al.* 1991; OLLER *et al.* 1993; SCHAAPER 1993). A *dnaQ* mutator produces at least base-pair substitutions, with transitions in substantial excess (SCHAAPER 1988, 1989). T4 gene *43* encodes the viral homolog of these two host proteins. Because gene *43* amber mutations are lethal if unsuppressed, the host proteins are unable to substitute for the viral protein. We therefore expected to observe no host mutator effects, and detected none.

***mutH*, *mutL*, *mutS* and *uvrD*:** These genes encode the *E. coli* methyl-instructed DNA mismatch repair system (MODRICH 1991). Their mutator alleles promote frameshift mutations (especially in repeating sequences), and transitions more than transversions (LEONG *et al.* 1986; SCHAAPER and DUNN 1987; SCHAAPER 1993).

Although a *uvrD* mutation was reported to reduce slightly the survival of T4 particles treated with methyl methanesulfonate (NISHIDA *et al.* 1976), it had no effect on several T2 or T4 base-pair substitution pathways (SIEGEL 1973). The UvrD helicase operates in other pathways of host DNA metabolism, including recombination and the SOS response, but is unlikely to affect T4 DNA replication because mutationally blocking the action of both T4 DNA helicases abolishes DNA synthesis (GAUSS *et al.* 1994).

While T4 also encodes a DNA-adenine methylase (HATTMAN 1983), its function remains obscure and there is no evidence that T4 encodes an antimutagenic mismatch-repair system of its own. For instance, no mutator mutation marking such a system has been recovered; an obvious candidate marker for parental strand *vs.* progeny strand (achieved in the host by hemimethylation of GATC sequences) is 5-hmC glucosylation, but mutational inactivation of glucosylation does not produce a mutator phenotype (DRAKE 1964); and T4 displays a high rate of mutation in repeated sequences (PRIBNOW *et al.* 1981; STREISINGER and OWEN 1985), as do *E. coli* mutants deficient in *mutHLS* mismatch repair. The T4 cyclobutane pyrimidine dimer glycosylase/endonuclease DenV cuts looped-out sequences and reduces recombinationally constructed heteroduplexes to homoduplexes by loop removal (without strand orientation) (BENZ and BERGER 1973; BERGER and BENZ 1973), but a *denV* mutant is not a mutator (DRAKE 1966). Other reports of mismatch reduction *in vitro* (BERGER and PARDOLL 1976; SOLARO *et al.* 1993) describe systems without strand orientation and thus unable to reduce mutation rates.

It was therefore not surprising that none of these four host mutators detectably affected spontaneous mutation rates in T4.

***mutY* and *mutM*:** Like *mutT*, these mutators inactivate functions that protect *E. coli* against DNA damage by oxygen radicals, $mutY^+$ encoding an adenine DNA glycosylase acting on G·A and 8-oxoG·A mispairs and $mutM^+$ encoding a DNA glycosylase acting on 8-oxoG and several other purine derivatives (MICHAELS *et al.* 1992; MICHAELS and MILLER 1992; BOITEUX *et al.* 1992). The result is a mutator specificity (G·C → T·A) which is the reverse of the *mutT* specificity. The *mutYM* double mutant is a much stronger mutator than either component singly (MICHAELS *et al.* 1992). We expected these mutators to affect T4, but we detected no changes. Perhaps the combined fidelities of insertion and proofreading of mispairs opposite a template 8-oxoG by the T4 replication apparatus suffice.

***mutA*:** This is a weak mutator favoring A·T → T·A mutations by an unknown mechanism (MICHAELS *et al.* 1990). It was without obvious effect on T4.

***topB* and *osmZ*:** The *topB* (= *mutR*) gene encodes DNA topoisomerase III (which affects DNA supercoiling), and a *topB* mutation is a deletion mutator (WHORISKEY *et al.* 1991; SCHOFIELD *et al.* 1992). The *osmZ* gene (= *bglY*) encodes a histone-like protein that also affects DNA supercoiling, and an *osmZ* mutation is also

a deletion mutator (HIGGINS *et al.* 1988, LEJEUNE and DANCHIN 1990). Although it is unknown whether either of these gene products can function in T4 DNA metabolism, the host gyrase genes (*gyrAB*) partly complement defects in the T4 topoisomerase genes *39*, *52* and *60* (MUFTI and BERNSTEIN 1974; MCCARTHY 1979). However, neither *topB* nor *osmZ* detectably affects either overall mutation rates or the proportion of deletion mutations in T4.

Untested host mutators: A *dam* (DNA adenine methylase) mutant is deficient in methyl-directed DNA mismatch repair and has a mutator phenotype (GLICKMAN 1979), but was not tested here because mutations in four other steps in the same pathway were each without effect. A mutation in *miaA*, or limitation of *miaA*$^+$ bacteria for iron, leads to tRNA undermodification and increases G·C → T·A rates about sixfold (CONNOLLY and WINKLER 1989, 1991); we excluded this mutator from our tests because of its small mutator effect and obscurity of mechanism. The *mutC* mutator (MICHAELS *et al.* 1990) has an unknown mechanism and was not available. Mutations in *E. coli* genes involved in the repair of methylated bases produce no or a tiny mutator effect (REBECK and SAMSON 1991), and mutations in *ada*, *tag*, and *ogt* were therefore not tested for effects on T4, although such mutations may affect the survival of T4 particles treated with methyl methanesulfonate (JANION 1982; RADANY *et al.* 1987). A number of other mutator mutations were not tested because they inhabit one or another of the genes whose mutator alleles we did test, or were equivocal or contradictory in their reported mutator activities.

T4 mutators: All strong T4 mutator mutations reside either in gene *43* (T4 DNA polymerase and proofreading activities) or in genes affecting dNTP pool sizes (DRAKE and RIPLEY 1994). Weaker mutators reside in genes encoding other proteins involved in DNA replication [*30* (DNA ligase), *32* (single-stranded-DNA binding protein), *39*, *52* and *60* (DNA topoisomerase), *41* (DNA helicase), *58/61* (DNA primase), *46* and *47* (DNase), and *44*, *45* and *62* (polymerase accessory proteins)]. Most of these mutators were discovered among conditional mutations in known genes, and only a few selections have been conducted for mutator mutations as such. These mutator selections (REHA-KRANTZ *et al.* 1986; REHA-KRANTZ 1988) would probably have detected 20-fold increased mutation rates efficiently (L. J. REHA-KRANTZ, personal communication). Nearly all of the many mutators thus recovered, including all strong mutators, reside in gene *43*. Thus, experience to date indicates that large increases in rates of spontaneous mutation in T4 are produced by mutations in only two classes of T4 genes, those severely affecting dNTP pool sizes and those directly involved in DNA replication.

The mutational economies of *E. coli* and T4: In *E. coli*, the incorporation error rate is $\sim 10^{-5}$ per base pair replicated and the proofreading error rate is $\sim 10^{-2}$, providing a replication error rate of $\sim 10^{-7}$ (SCHAAPER 1993). This value is further reduced to 6×10^{-10}, the observed mutation rate (DRAKE 1991), by a set of mismatch repair systems.

In T4, the incorporation error rate *in vitro* is $\sim 6 \times 10^{-5}$ for a mutant polymerase deficient in proofreading (T. A. KUNKEL, personal communication), while the proofreading defect increases the mutation rate ~760-fold (FREY *et al.* 1993) (or 650-fold in a different proofreading-deficient mutant, REHA-KRANTZ *et al.* 1991), providing a total replication error rate of $\sim 8 \times 10^{-8}$. However, the correct T4 value must in fact be 2×10^{-8}, the mutation rate observed *in vivo* (DRAKE 1991). The difference between "2" and "8" may reflect the increased replication fidelity provided by other proteins of the replication complex; for reviews see YOUNG *et al.* (1992) and DRAKE and RIPLEY (1994).

Thus, the available evidence suggests that bacteriophage T4 achieves its spontaneous mutation rate by highly accurate replicative DNA insertion and proofreading mechanisms, and does not require additional fidelity-enhancing systems.

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