GAMMA-RAY MUTAGENESIS IN BACTERIOPHAGE T4

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ABSTRACT

^{137}Cs-gamma irradiation of bacteriophage T4 induces large deletions plus a variety of types of point mutations. All mutations arise with single-hit kinetics, and all by a misrepair process. The estimated point mutation rate is 1.5×10^{-9} per locus per rad.

IONIZING radiation mutagenesis has been studied in a large number of organisms for nearly 50 years, but no systematic investigation has been reported revealing the full spectrum of mutation pathways promoted, nor the underlying mechanisms by which the mutations arise. Although ionizing radiation mutagenesis has been observed in other phage systems (KAPLAN, WINKLER and WOLF-ELLMAUER 1960; FRADKIN 1964; KRIVISKY 1965; VAN DER ENT, BLOK and LINCKENS 1965; RÜGER and KAPLAN 1966; KRIVISKY and KZOJAN 1970; BLEICHRODT and VERHEIJ 1973, 1974; BRESLER *et al.* 1975), bacteriophage T4 is ideally suited for a more detailed analysis because of the high resolving power of the *rII* system and the availability of mutants which perturb the mutation process itself. The induction of mutations by ionizing radiation has previously been demonstrated both in phage T4 (BROWN 1966a,b; BRIDGES, DENNIS and MUNSON 1970; MUNSON and BRIDGES 1973) and in the closely related phage T2 (ARDASHNIKOV, SOYFER and GOLDFARB 1964).

We show here that ionizing radiation mutagenesis occurs by misrepair in phage T4, as it is already known to do in *Escherichia coli* (BRIDGES, LAW and MUNSON 1968; KONDO *et al.* 1970), *Saccharomyces cerevisiae* (LAWRENCE *et al.* 1974) and phage lambda (KRIVISKY and KZOJAN 1970; BRESLER *et al.* 1975). [BLEICHRODT and VERHEIJ (1973) mentioned that the induced reversion of a bacteriophage ϕX174 mutant did not require the host *recA*$^+$ misrepair system, but did not present specific data.] Misrepair mutagenesis, discovered by WITKIN (1969), is now known to occur in many (but perhaps not all) organisms (see reviews by DRAKE 1973, GREEN and DRAKE 1974 and DRAKE and BALTZ 1976). It is recognized by the properties of certain repair-defective mutants. These mutants typically exhibit increased sensitivities to the lethal effects of numerous agents, and concomitantly decreased sensitivities to their mutagenic effects. In addition, they frequently reduce recombination rates, and sometimes growth rates as well. Misrepair usually occurs during post-replication repair, probably not by strand transfer but instead by untemplated DNA synthesis past a genetic lesion which is incapable of pairing with any base (DRAKE and BALTZ 1976). The phage T4

mutants *x*, *y* and *1206* all produce the relevant repair-defective state (DRAKE 1973; GREEN and DRAKE 1974).

MATERIALS AND METHODS

Strains: Bacteriophage T4B was used throughout, the mutants *px* and *y* having previously been crossed out of T4D into a T4B background (GREEN and DRAKE 1974). Stocks were grown and titered on *E. coli* BB, except that *r* mutants were screened on B cells. The nonpermissive host for *rII* mutants, used to detect *r*+ revertants, was the lambda lysogen KB.

Media: Stocks were grown in L broth and phages were assayed on standard Drake agar (RIPLEY 1975) except where noted. Supersoft Drake agar, used for *r* mutant screening, contained only 10.7 gm/l of Drake agar mixture.

Ionizing radiation mutagenesis: Gamma irradiation was applied to phages suspended in 2% Difco Nutrient Broth using a ^{137}Cs source at a dose rate of 3560 rad/min as determined by ferrous sulfate dosimetry (H. DUCOFF, personal communication). Irradiated and control suspensions were stored at 4° without loss of viability or change in mutant frequency over many months. Survivor and mutant frequencies were determined by preadsorbing to and then plating on B cells using the supersoft agar overlay method (DRAKE 1966a). Since most *r* mutants appeared in aberrant plaques, all plaques exhibiting even a hint of an *r* component were picked and replated (by streaking with sterile paper strips) to confirm (about 70% of the time) the presence of *r* mutants.

Characterizing mutants: All confirmed *r* mutants were purified by replating and were repicked into a small volume. They were then spotted onto KB cells using sterile paper strips, and classified on the basis of their growth patterns into *rII* mutants (no growth, except for occasional revertants) and non-*rII* mutants (good growth). A few mutants produce weak growth on KB cells and are classified as leaky *rII* mutants, although these have occasionally proven on more detailed analysis to contain additional mutations, exterior to the *rII* cistrons, which result in poor growth on all K12 strains but good growth on B strains. Other *rII* mutants produce many revertants, but a decisive distinction between leaky and rapidly reverting *rII* mutants requires further tests (see below).

These same spot tests can also be used to separate the non-*rII* mutants into two categories, *rI* and *rIII*-like (BENZER 1957). The *rI* mutants produce large halos around the lysed spots, exhibit the *r* phenotype on BB cells, and map at the *rI* locus. The *rIII*-like mutants produce no halos around the lysed spots, exhibit the *r*+ phenotype on BB cells but rarely map at the *rIII* locus. Instead, most map within the *rII* cistrons, and are therefore merely very leaky *rII* mutants. (Little is known about the *rIII* locus, but the rarity of true *rIII* mutants suggest that only occasional *rIII* missense mutations can produce the *r* phenotype.)

The *rII* mutants were next mapped, first into cistron segments and then into sites, by recombination spot tests (BENZER 1957; DRAKE 1966a). They were then subjected to reversion analysis by means of base analogue spot tests and quantitative tests using hydroxylamine or proflavin (DRAKE 1966a).

Detecting deletions: A collection of *rII* mutants to be screened for deletions was first grown into low-titer stocks (about 10^{10}/ml) by stabbing mature plaques into about 2 ml of L broth containing BB cells at about 5×10^7/ml (or occasionally B cells, which strongly select for *r*+ revertants), incubating without agitation for about 4 hours, and adding a drop of chloroform to complete lysis. These stocks were then spotted onto KB cells, and any exhibiting reversion were discarded. The remainder were plated on KB cells in the ordinary manner (about 10^8 phages per plate) and reverting mutants were again discarded. The remaining mutants were then mapped by recombination spot tests against a set of *rII* mutants spanning the two *rII* cistrons, and multisite mutants were classified as deletions; total mutation rates were never high enough to produce significant numbers of multiple-point mutants.

Searching for duplications: Tandem duplications were sought on the assumption that they would revert rapidly (WEIL, TERZAGHI and CRASEMANN 1965; PARMA, INGRAHAM and SNYDER

1972). Mutants which exhibited numerous revertants in KB spot tests were repurified to exclude contamination and grown into high-titer stocks. These stocks contained up to 10^{-2} revertants, and therefore could be subjected neither to mapping nor to chemical reversion tests.

Two criteria were used to distinguish between rapidly reverting and leaky mutants. First, leaky *rII* mutants tend to produce a gradation of plaque sizes from normal to just visible on lambda lysogens, presumably because of revertants arising on the plate; whereas rapidly reverting mutants produce mainly normally-sized plaques. Second, mutants were preadsorbed to KB lambda lysogens, unadsorbed phages were inactivated with antiserum, and the complexes were plated on B cells: rapidly reverting mutants produce mainly $r+$ plaques (plus a minority of slightly mottled plaques), whereas leaky mutants produce mainly r plaques.

The rapidly reverting mutants were then subjected to "FUdR crosses" (SÉCHAUD *et al.* 1965; SHALITIN and STAHL 1965; DRAKE 1966b), which distinguish between mutants of large and small extent on the basis of their abilities to be incorporated into recombinational heteroduplex heterozygotes; the protocol was essentially that of SÉCHAUD *et al.* (1965), with chloramphencol present from 9 to 120 minutes and absent from 120 to 180 minutes. Each mutant, plus known deletion and point mutant controls, was crossed against the wild type in the presence of FUdR, which inhibits DNA synthesis in T4-infected cells. The progeny from these crosses were then plated on B cells and screened for mottled plaques, which arise from $r/r+$ heterozygotes. Mutants of large extent (deletions, and presumably also duplications) fail to form recombinational heteroduplex heterozygotes, and produce only a low background level of mottled plaques due to terminal redundancy heterozygosity. Point mutants, however, produce much larger frequencies of mottled plaques.

RESULTS

Figure 1 describes the gamma-ray inactivation of phage T4 and the induction of r mutants. Table 1 lists inactivation and mutation rates for T4 and its repair-defective mutants *px* and *y*. Both mutants sharply decreased induced mutation rates.

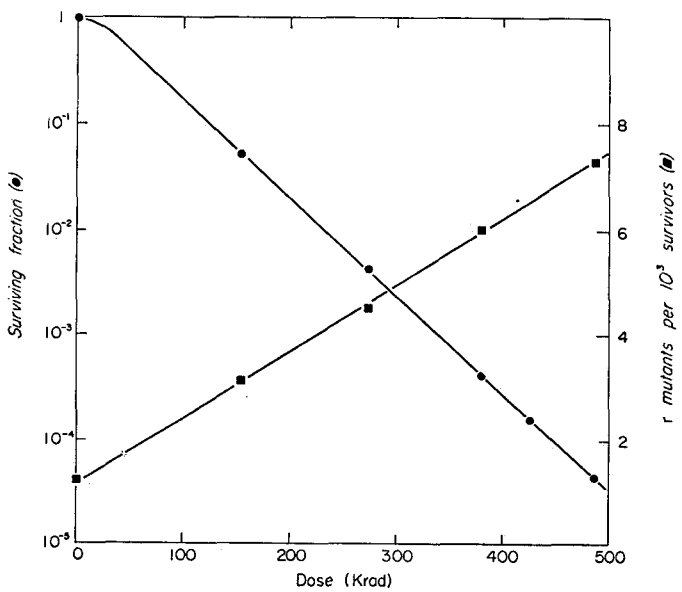

FIGURE 1.—Inactivation and mutagenesis of phage T4 by ^{137}Cs-gamma irradiation.

TABLE 1

Rates of inactivation and mutation

Strain	Krad per lethal hit	*r* mutants per 10^9 rad	*r* mutants per 10^4 lethal hits
T4	47.0	12.6	5.9
px	42.2 (90%)	3.8 (30%)	1.6 (27%)
y	39.3 (84%)	1.3 (10%)	0.52 (9%)

The mutation rates for *px* and *y* were determined at a single dose, 332 Krad. Figures in parentheses indicate percentages compared to the wild type. Lethal hits were determined from terminal slopes in all cases.

TABLE 2

rII *mutation rates*

Strain	Krad	Total *r* mutants	*r* per 10^8 survivors	*rII*/*r* tested	*rII* per 10^8 survivors	*rII* per 10^9 rad
T4	0	63	1.21	22/63	0.42	
	487	303	7.34	83/303	2.01	3.26
px	0	69	0.74	27/45	0.44	
	332	293	1.98	73/206	0.70	0.78
y	0	88	1.34	49/64	1.02	
	332	149	1.77	40/76	0.93	~0

Table 2 describes the *r* mutants obtained from control and irradiated populations, and shows that *px* and *y* sharply decreased rates of induction of *rII* as well as of total *r* mutants.

TABLE 3

rII *reversion patterns*

Krad	Number tested	Deletions	P(+)	BA(+) HA(—)	BA(+) HA(+)	BA(—) P(—)
0	21	2	15	3	1	0
(%)		(9.5)	(71.4)	(14.3)	(4.8)	(0)
487	76	21	28	10	13	4
(net %)		(32.4)	(27.7)	(12.9)	(20.4)	(6.7)

Abbreviations: P = proflavin, BA = base analogs (2-aminopurine and/or 5-bromouracil), HA = hydroxylamine, (+) = reversion induced, (—) = reversion not induced, (net %) = per cent of induced mutants after subtracting contributions from the spontaneous background using the 4.8-fold factor of increase of *rII* mutants in the irradiated sample compared to the control sample.

Table 3 describes the susceptibilities of *rII* mutants from control and irradiated populations to chemically induced reversion. When spontaneous backgrounds are subtracted, it becomes clear that deletions comprised almost a third of the induced mutants. Frameshift mutants (revertible by proflavin) and base pair substitution mutants (revertible by base analogues) were induced at about the

same rate, and a majority of the mutants revertible by base analogues were also capable of reverting by G:C → A:T transitions (revertible by hydroxylamine).

Sixty *rII* point mutants from the sample irradiated with 487 Krad were mapped into a total of 36 sites, but five of these mutants could not be subjected to reversion analysis because of leakiness or rapid reversion. Of the 36 sites, 29 contained one mutant apiece, two contained two mutants each, two contained three mutants each, one contained four mutants, one five mutants and one 12 mutants. The sites containing four and 12 mutants apiece corresponded to the two large *rII* mutational hot spots (BENZER 1957). The expected total number of mutants at these two sites contributed solely from the spontaneous background was about 13, indicating no special reactivity of these sites to misrepair mutagenesis. A similar lack of special reactivity was also observed following ultraviolet irradiation (DRAKE 1963, 1966a).

Table 4 describes *rII* deletion mutant frequencies among control and irradiated populations. As was the case with point mutants, the induction of deletions was markedly decreased by *px* and *y*. Table 4 also demonstrates that deletions were induced in the wild type with linear kinetics. When the 0- and 487-Krad data were fitted to equations on the assumption that all point mutants arise with linear kinetics, but that deletions might arise either with linear or with dose-squared kinetics, the predicted numbers of deletions in the 154-Krad sample were 44.2 and 19.9, respectively, compared to the observed number of 49. The induced deletions were also observed to differ qualitatively from deletions of spontaneous origin. The two spontaneous deletions mapped entirely within the *rII* cistrons, as do most spontaneous *rII* deletions (TESSMAN 1962). Most (18/21) of the mapped deletions from the 487-Krad-irradiated population exhibited left ends within the *rII* cistrons but extended at least to the right end of the *rIIB* cistron, and therefore probably extended into the adjacent region, which is devoid of esssential genes. About two of these 21 deletions were likely to have been of spontaneous origin, however, and may correspond to the three deletions located entirely within the *rII* cistrons.

Since gamma irradiation induced deletions, it might also have induced tandem duplications. On the assumption that tandem duplications would behave like

TABLE 4

rII *deletion mutant frequencies*

Strain	Krad	Deletions per *rII* tested	Deletions per 10^8 survivors	Deletions per 10^9 rad
T4B	0	2/21	0.04	
	154	49/276	0.23	1.21
	487	21/76	0.56	1.06
px	0	1/27	0.03	
	332	3/73	0.08	0.16
y	0	6/49	0.16	
	332	8/40	0.35	0.58

deletions and fail to form recombinational heteroduplex heterozygotes, therefore, a collection of rapidly reverting mutants was subjected to "FUdR crosses" (Table 5). The deletion mutant control produced the expected low frequency of mottled plaques and the three point-mutant controls produced the expected high frequencies. None of the rapidly reverting mutants produced the low frequencies of mottled plaques expected of tandem duplications. Control experiments in which some of the mutants were crossed against themselves instead of against the wild type failed to produce significant frequencies of mottled plaques, indicating that the high frequencies produced by the rapidly reverting mutants were not the result of reversion, but must instead have been recombinational heteroduplex heterozygotes.

DISCUSSION

Gamma irradiation of bacteriophage T4 induces *r* mutations at a linear rate of 12.6×10^{-9} per rad. The induction of mutations by ionizing radiations is also usually linear with dose in *E. coli*, most bacteriophages (see references in the INTRODUCTION) and eucaryotes (ABRAHAMSON *et al.* 1973). However, deletions are induced with two-hit kinetics in at least some eucaryotes (e.g., *Neurospora crassa*, DE SERRES, MALLING and WEBBER 1967); *e* (but not *b* or *c*) mutations are induced with three-hit kinetics in *Serratia marcescens* phage kappa (RÜGER and KAPLAN 1966); and lambda *vir* mutants are induced with approximately two-hit kinetics (KRIVISKY 1965; KRIVISKY and KZOJAN 1970; BRESLER *et al.* 1975). Although BROWN (1966a) reported that the reversion of a T4*rII* mutant

TABLE 5

Frequencies of heterozygotes from "FUdR crosses"

Mutant*	Type†	Revertant frequency	Per cent heterozygotes
rXS15	deletion	$<10^{-8}$	$\leqslant 0.44$
rUV20	frameshift	1×10^{-7}	11.0
	(control)		($\leqslant 0.53$)
rUV124	frameshift	2×10^{-8}	8.6
rUV200	transition	2×10^{-8}	11.7
rHS7-187	rapid reverter	2.4×10^{-3}	7.0
	(control)		($\leqslant 0.96$)
rXRR5	rapid reverter	1.1×10^{-2}	9.3
rXRR14	rapid reverter	1.2×10^{-2}	9.3
	(control)		($\leqslant 1.6$)
rXRR16	rapid reverter	1.3×10^{-2}	9.8
rXRR17	rapid reverter	1.2×10^{-2}	11.3

* *rXS15* is a spontaneous *rII* deletion mutant; the *rUV* point mutants were described by DRAKE (1963); the *rHS* mutant originated from a collection of heat-induced mutants but may be of spontaneous origin, since heat does not induce frameshift mutants (BALTZ, BINGHAM and DRAKE 1976); and the rapidly reverting *rXRR* mutants originated from the present collection of gamma-ray-induced mutants.

† The controls consist of the mutant crossed against itself instead of the wild type.

was nonlinear, his pooled data indicate a linear response except at the highest dose, where cross-reactivation may well have led to an overestimated value.

An average induced mutation rate per locus for bacteriophage T4 can be estimated as follows. The total *rII* mutation rate (Table 2) was 3.26×10^{-9} per rad, and represents the contributions of two cistrons. The *rII* cistrons are somewhat larger than the average T4 cistron, however, since the *rIIA* and *rIIB* polypeptides weigh 95,000 and 33,000 daltons, respectively (average = 64,000 daltons), whereas the average T4 early protein weighs only 45,500 daltons (O'FARRELL, GOLD and HUANG 1973). An average per-locus mutation rate is therefore $(3.26 \times 10^{-9}/2) \times (45{,}000/64{,}000) = 1.16 \times 10^{-9}$ per rad. Since 32.4% of the induced *rII* mutants were deletions, and most of these were probably multigenic, the average point mutation rate is therefore 0.78×10^{-9} per rad. This value is an underestimate, however, because the *rII* mutation rate in Table 2 is based only upon non-leaky mutants. Among many collections of misrepair-induced mutants studied in this laboratory, including the present collections, about equal numbers of leaky and nonleaky *rII* mutants were induced. A better estimate of the per-locus mutation rate is therefore 1.5×10^{-9} per rad; the significance of this value will be discussed in a forthcoming report. It should be noted, however, that even when leaky mutants are included, only some 5% to 10% of *rII* base pair substitutions are detected (DRAKE 1970). Compared to other loci which might be less able to tolerate missense mutations, therefore, the mutation rate of 1.5×10^{-9} per locus per rad may still be underestimated.

About half of the induced *r* mutants were *rI*. If the *rI* locus were composed of only one cistron, its mutability would therefore be about twice that calculated above; but the number of *rI* cistrons remains unknown.

Enough data have now accumulated to justify an initial comparison of radiation-induced mutation rates in diverse bacteriophage systems. Table 6 presents this compilation for those phage systems which display linear kinetics, the values being normalized (with reasonable assumptions) to mutations per rad per base pair. With the notable exception of certain host-range mutants in ϕX174, the values group fairly closely around 10^{-11} mutations per base pair per rad. There is a tendency for forward mutation rates per base pair to be somewhat lower than reversion rates, which may indicate that a fairly large fraction of base-pair substitutions typically go undetected in forward mutation measurements. However, the serratiaphage kappa may be considerably more mutable than are the coliphages.

The strong requirement for the px^+ and y^+ gene functions for gamma-ray mutability demonstrates a misrepair type of mechanism. The total *r* mutation rate of 5.9×10^{-4} per lethal hit is characteristic of misrepair mutagenesis in T4, similar rates being observed with ultraviolet and photodynamic white-light irradiation and with methyl methanesulfonate (DRAKE 1973; GREEN and DRAKE 1974).

Gamma irradiation also generates the wide spectrum of mutations which is typical of misrepair mutagenesis (DRAKE and BALTZ 1976), including both frameshift and base pair substitution mutations. The ratios of different muta-

TABLE 6

Ionizing-radiation-induced mutation rates in bacteriophage systems

Organism	Mutations scored	Mutations per rad	Ref.	Assumed target size (base pairs)	Mutations per base pair per 10^{11} rad
Coliphage T4	$r+ \rightarrow r$	1.5×10^{-8}	1		
		1.3×10^{-8}	2		
	$r+ \rightarrow rII$	5.5×10^{-9}	2	3500	0.16
	$rII \rightarrow r+$	1.6×10^{-11}	3		
		8.5×10^{-11}	4		
		5.0×10^{-11}	(average)	3	1.7
Coliphage T2	$h+ \rightarrow h$	8.9×10^{-12}	5	10	0.1
Coliphage S_D	p.m.m.	1.0×10^{-8}	6	2000	0.5
Coliphage ϕX174	host range	5.2×10^{-7}	7	10	5000
	CT reversion	1.1×10^{-11}	8	3	4
		(0.04–63)			(0.01–16)
Serratiaphage kappa	$c+ \rightarrow c$	4.1×10^{-8}	9	3000	3.4

All values are from systems displaying linear kinetics and refer to direct effects (irradiation in broth or its equivalent). 1 = BROWN (1966a). 2 = this report, the *rII* mutant rate being that for point mutants only but including leaky mutants, that is, 3.26×10^{-9} (2 — 0.324). 3 = BRIDGES, DENNIS and MUNSON (1970); 4 = BROWN (1966b); with the average taken and assuming that the *rII* mutants (of unknown type) might revert at three base pairs. 5 = ARDASHNIKOV, SOYFER and GOLDFARB (1964); note that T2*h* mutants arise at only a limited number of sites in the tail-fiber gene(s). 6 = KRIVISKY (1965); p.m.m. = plaque morphology mutants, it being arbitrarily assumed that two typical cistrons are involved. 7 = VAN DER ENT, BLOK and LINCKENS (1965). 8 = BLEICHRODT and VERHEIJ (1973, 1974), who studied the reversion of chain-terminating (amber, ochre, opal) mutants; values in parentheses provide the range observed with many different mutants. 9 = KAPLAN, WINKLER and WOLF-ELLMAUER (1960), and assuming that *c* mutants arise within three different cistrons (which is typical for lysogenic phages).

tional types vary, however, with different mutagens: frameshift mutations are somewhat more frequent with ultraviolet irradiation, and mutants revertible by hydroxylamine are markedly less frequent; and of the mutagens tested to date, only ionizing radiation efficiently induces deletions. Base-specific damages produced by different mutagens therefore appear to affect, to at least some extent, the resulting mutational pathway.

The induced deletions tended to be much larger than are deletions of spontaneous origin, and were probably almost always multigenic. Since only the region to the right of the *rII* cistrons is devoid of essential genes, and not that to the left, it is possible that only about half of the induced deletions were detected. It is notable that deletions not only arise by misrepair, but that they also arise with linear kinetics, even at the very high dose rate employed. Deletions also appear to arise with linear kinetics and by misrepair in *E. coli* (FRADKIN *et al.* 1969; KONDO *et al.* 1970), while in *Neurospora crassa* they arise with linear kinetics at very low dose rates, but with dose-squared kinetics at high dose rates (DE SERRES, MALLING and WEBBER 1967).

The induced deletions may arise from the gaps induced in replicating DNA by lesions which cannot act as templates (RUPP and HOWARD-FLANDERS 1968). Whereas point mutants may arise by untemplated DNA synthesis past such lesions (DRAKE and BALTZ 1976), deletions may arise by joining together the

ends of the gaps. If this supposition is correct, then rates of deletion induction by ionizing radiations may be very sensitive to the functional state of the DNA ligase.

None of the rapidly reverting mutants tested showed the behavior that would be expected of tandem duplications on the assumption that such mutants would behave like deletions in FUdR crosses. Tandem duplications might have gone undetected, however, either if the small sample size excluded such mutants because they are generated at low frequencies, or if tandem duplications revert at frequencies substantially greater than 10^{-2} and are therefore difficult to recognize as *rII* mutants in the first place. The extremely high reversion rates of the tested mutants makes their further characterization difficult, and presents an enigma concerning their nature. We believe that the most likely explanation is that they contain frame-shift mutations in regions of extensive local-base-sequence redundancy. When frameshift mutations arise in such regions, and particularly when they increase the amount of redundancy, they can revert at very high rates, so that stocks may contain revertant frequencies in excess of 10^{-3} (OKADA *et al.* 1972).

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LITERATURE CITED

ABRAHAMSON, S., M. A. BENDER, A. D. CONGER and S. WOLFF, 1973 Uniformity of radiation-induced mutation rates among different species. Nature **245**: 460–462.

ARDASHNIKOV, S. N., V. N. SOYFER and D. M. GOLDFARB, 1964 Induction of h-mutations in the extracellular phage T2 by γ-irradiation. Biochem. Biophys. Res. Commun. **16**: 455–459.

BALTZ, R. H., P. M. BINGHAM and J. W. DRAKE, 1976 Heat mutagenesis in bacteriophage T4: the transition pathway. Proc. Nat. Acad. Sci. U.S. **73**: (No. 4) (in press).

BENZER, S., 1957 The elementary units of heredity, pp. 70–93. In: *The Chemical Basis of Heredity*. Edited by W. D. MCELROY and B. GLASS. Johns Hopkins Press, Baltimore.

BLEICHRODT, J. F. and W. S. D. VERHEIJ, 1973 Reversion of cistron A *amber* mutants of bacteriophage φX174 by ionizing radiation. Mutation Res. **18**: 363–365. ——, 1974 Influence of oxygen on the induction of mutations in bacteriophage φX174 by ionizing radiation. Int. J. Rad. Biol. **25**: 505–512.

BRESLER, S. E., V. L. KALININ, YU. I. KOPYLOVA, A. S. KRIVISKY, V. N. RYBCHIN and V. N. SHELEGEDIN, 1975 Study of genetic effects of high energy radiations with different ionizing capacities on extracellular phages. Mutation Res. **29**: 1–20.

BRIDGES, B. A., R. E. DENNIS and R. J. MUNSON, 1970 Mutagenesis in *Escherichia coli*, III. Requirement for DNA synthesis in mutation by gamma rays of T4-phage complexed with *Escherichia coli*. Genet. Res. Camb. **15**: 147–156.

BRIDGES, B. A., J. LAW and R. J. MUNSON, 1968 Mutagenesis in *Escherichia coli*, II. Evidence for a common pathway for mutagenesis by ultraviolet light, ionizing radiation and thymine deprivation. Molec. Gen. Genet. **103**: 266–273.

BROWN, D. F., 1966a X-ray-induced mutation in extracellular bacteriophage T4. Mutation Res. **3**: 365–373. ——, 1966b X-ray-induced mutations in extracellular phages. Nature **212**: 1595–1596.

DE SERRES, F. J., H. V. MALLING and B. B. WEBBER, 1967 Dose-rate effects on inactivation and mutation induction in *Neurospora crassa*. Brookhaven Symp. Biol. **20**: 56–76.

DRAKE, J. W., 1963 Properties of ultraviolet-induced *rII* mutations of bacteriophage T4. J. Mol. Biol. **6**: 268–283. ——, 1966a Ultraviolet mutagenesis in bacteriophage T4. I. Irradiation of extracellular phage particles. J. Bacteriol. **91**: 1775–1780. ——, 1966b Heteroduplex heterozygotes in bacteriophage T4 involving mutations of various dimensions. Proc. Nat. Acad. Sci. U.S. **55**: 506–512. ——, 1970 *The Molecular Basis of Mutation*. Holden Day. San Francisco, 273 pp. ——, 1973 The genetic control of spontaneous and induced mutation rates in bacteriophage T4. Genetics Suppl. **73**: 45–64.

DRAKE, J. W. and R. H. BALTZ, 1976 The biochemistry of mutagenesis. Ann. Rev. Biochem. **45**: 11–37.

FRADKIN, G. E., 1964 The stability of functionally different regions of DNA to ionizing radiation. Dokl. Akad. Nauk SSSR **155**: 457–460.

FRADKIN, G. E., M. V. TOROSIAN, E. V. RABINKOVA and E. V. FILJKOVA, 1969 Induction of mutations in the lac-operon of *Escherichia coli* K12 chromosome by γ-irradiation. Genetika (USSR) **5**, No. 2: 125–132.

GREEN, R. R. and J. W. DRAKE, 1974 Misrepair mutagenesis in bacteriophage T4. Genetics **78**: 81–89.

KAPLAN, R. W., U WINKLER and H. WOLF-ELLMAUER, 1960 Induction and reversion of *c*-mutations by irradiation of the extracellular κ-phage of Serratia. Nature **186**: 330–331.

KONDO, S., H. ICHIKAWA, K. IWO and T. KATO, 1970 Base-charge mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics **66**: 187–217.

KRIVISKY, A. S., 1965 The mutagenic effect of ionizing radiation on extracellular bacteriophage. Dokl. Akad. Nauk SSSR **161**: 707–710.

KRIVISKY, A. S. and ZH. A. KZOJAN, 1970 The mutagenic action of X-rays on the extracellular phage lambda. Genetika (USSR) **6**, No. 3: 65–77.

LAWRENCE, C. W., J. W. STEWART, F. SHERMAN and R. CHRISTENSEN, 1974 The specificity and frequency of ultraviolet-induced reversion of an iso-1-cytochrome *c* ochre mutant in radiation-sensitive strains of yeast. J. Mol. Biol. **85**: 137–162.

MUNSON, R. J. and B. A. BRIDGES, 1973 The LET factor in mutagenesis by ionizing radiation, I. Reversion to wild type of a bacteriophage T4 amber mutant. Int. J. Rad. Biol. **24**: 257–273.

O'FARRELL, P. Z., L. M. GOLD and W. M. HUANG, 1973 The identification of prereplicative bacteriophage T4 proteins. J. Biol. Chem. **248**: 5499–5501.

OKADA, Y., G. STREISINGER, J. E. OWEN, J. NEWTON, A. TSUGUITA and M. INOUYE, 1972 Molecular basis of a mutational hot spot in the lysozyme gene of bacteriophage T4. Nature **236**: 338–341.

PARMA, D. H., L. J. INGRAHAM and M. SNYDER, 1972 Tandem duplications of the *rII* region of bacteriophage T4D. Genetics **71**: 319–355.

RIPLEY, L. S., 1975 Transversion mutagenesis in bacteriophage T4. Molec. Gen. Genet. **141**: 23–40.

RÜGER, W. and R. W. KAPLAN, 1966 Mutationauslösung durch Röntgenstrahlen bei Bestrahlung des freien Bacteriophagen Kappa unter verschiedene Bedingungen. Z. Allg. Mikrobiol. **6**: 253–269.

RUPP, W. D. and P. HOWARD-FLANDERS, 1968 Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**: 291–304.

SÉCHAUD, J., G. STREISINGER, J. EMRICH, J. NEWTON, H. LANFORD, H. REINHOLD and M. M. STAHL, 1965 Chromosome structure in phage T4. II. Terminal redundancy and heterozygosis. Proc. Natl. Acad. Sci. U.S. **54**: 1333–1339.

SHALITIN, C. and F. W. STAHL, 1965 Additional evidence for two kinds of heterozygotes in phage T4. Proc. Natl. Acad. Sci. U.S. **54**: 1340–1341.

TESSMAN, I., 1962 The induction of large deletions by nitrous acid. J. Mol. Biol. **5**: 442–445.

VAN DER ENT, G. M., J. BLOK and E. M. LINCKENS, 1965 The induction of mutations by ionizing radiation in bacteriophage ϕX174 and in its purified DNA. Mutation Res. **2**: 197–204.

WEIL, J., B. TERZAGHI and J. CRASEMANN, 1965 Partial diploidy in phage T4. Genetics **52**: 683–693.

WITKIN, E. M., 1969 Ultraviolet-induced mutation and DNA repair. Ann. Rev. Genet. **3**: 525–552.

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