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Host- and Phage-Mediated Repair of Radiation Damage in Bacteriophage T4

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T4+ exhibits increased ultraviolet sensitivity on derivatives of *Escherichia coli* K12 or B lacking deoxyribonucleic acid (DNA) polymerase I. However, the sensitivity of T4*v* is not affected by the absence of host DNA polymerase. T4*x* and T4*y* also show increased sensitivity on DNA polymerase-deficient strains, but to a lesser extent than observed with wild-type T4. When T4*x* or T4*y*, but not T4+, are plated on a double mutant lacking both DNA polymerase and the *uvrA* gene product, a partial suppression of the polymerase effect is observed. Host ligase appears to be able to suppress to some extent the T4*y* phenotype but has no effect on wild-type T4 or other T4 mutants. T4*xv* incubated in *E. coli* B or B_{s-1} in the presence of chloramphenicol (50 μg/ml) shows increased resistance over directly plated irradiated phage. Increased survival under the same conditions was not observed with T4+ or other T4 mutants. The repair of X-ray-damaged T4 was investigated by examining survival curves of T4+, T4*x*, T4*y*, T4ts43, and T4ts30. The repair processes were further defined by observing the effects of plating irradiated phage on various hosts including strains lacking DNA polymerase I or polynucleotide ligase. Two classes of effects were observed. Firstly, the *x* and *y* gene products seem to be involved in a repair system utilizing host ligase. Secondly, in the absence of host DNA polymerase, phage sensitivity is increased in an unknown manner which is enhanced by the presence of host *uvrA* gene product.

Although T4 and its *Escherichia coli* host both code for appropriate enzymes necessary to repair ultraviolet damages to deoxyribonucleic acid (DNA), the relative role of these enzymes in repairing damage to the phage has not been fully assessed. There are three genes which directly affect T4 ultraviolet sensitivity: *v*, *x*, and *y* (8, 18). The *v*+ gene codes for a dimer-specific endonuclease (13, 36), but the functions of *x* and *y* are unknown. It is suspected, however, that they are involved in genetic recombination as well as repair (7, 19). It has also been reported (2, 3) that temperature-sensitive T4 mutants defective in DNA polymerase, ligase, nucleases, or gene 32 protein are ultraviolet sensitive.

It has been generally assumed that host enzymes play no role in the repair of ultraviolet-damaged T4 phage DNA, since survival curves of T4 directly plated on repair-positive (B) and repair-negative (B_{s-1}) hosts are identical (10, 17). Kozinski and Lorkiewicz (25) have shown, however, that both of these hosts are capable of physically nicking ultraviolet-irradiated T4, when phage enzyme synthesis is inhibited by chloramphenicol. This finding prompted us to investigate the effect of plating wild-type T4 and its ultraviolet-sensitive mutants on a variety of repair-deficient hosts. In some experiments, phage-infected complexes were held in chloramphenicol before plating, to bias in favor of host enzymatic repair.

It is clear that X-ray damages can also be repaired since a variety of bacterial mutants exist which have a wide range of sensitivities. Although the nature of the X-ray lesions and their subsequent repair is not well understood, there is a positive correlation between the ability to recombine and to survive ionizing radiation damage (21, 22). It also appears that recombinational ability is related to the repair of ionizing radiation damage to T4 bacteriophages, since T4*y* and T4*x* which are ultraviolet and gamma-ray sensitive also have impaired recombination ability (7, 19). It should be noted that there is a conflicting report as to the sensitivity of T4*x* since Freifelder (12) has stated that this mutant is not X-ray sensitive.

Despite the potential usefulness of genetically defined bacteriophages for investigating the repair of X-ray damage, little has been done due to the lack of sufficient mutants with altered survival properties. To further elucidate the possible repair mechanisms utilized by T4, temperature-sensitive mutants affected in the ligase or DNA

polymerase genes were examined for sensitivity to X rays. Both of these gene products have been implicated in the repair of ultraviolet damage by Baldy (2, 3), in the repair of ethyl methanesulfonate (EMS) damage by Ray et al. (29), and in genetic recombination by Bernstein (4). The X-ray sensitivities of the above phage mutants, as well as T4 wild-type, T4*x*, and T4*y*, were also examined in hosts lacking ligase or DNA polymerase I.

A preliminary account of these studies has been presented (S. Wallace and R. Melamede, Abstr. Biophys. Soc. **12**:153a, 1972; R. Melamede and S. Wallace, Abstr. Biophys. Soc. **12**:154a, 1972).

MATERIALS AND METHODS

Bacteria and bacteriophages. The properties of the bacterial and bacteriophage strains used in these studies are shown in Tables 1 and 2. The authors are grateful to the indicated investigators for supplying the bacterial and phage mutants, and to H. Rosenrkanz for giving us *E. coli* W3110 and W3478.

Media. The bottom and top layer agar, broth, gel buffer, and diluting fluid used were described previously (33).

Ultraviolet irradiation. The bacteriophages were irradiated with a Sylvania 15-w germicidal lamp powered through a Sola constant-voltage regulator. Phage samples, in a 3.5-cm diameter petri dish, were irradiated with stirring under conditions of negligible absorption. Radiation of wavelengths less than 250 nm was removed by filtration through glacial acetic acid contained in a 7.5-cm diameter quartz cell (S. Wallace, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1965). This procedure was utilized to eliminate wavelengths below 245 nm which damage the protein coat of the phage (35). T4 wild-type phage survival curves were originally calibrated by using uranyl oxalate actinometry (32). The D_{37} of T4+ obtained with the filter was 80 ergs/mm^2. Subsequently, T4 wild-type bacteriophage was used as a dosimeter, and the dose rate at 50 cm from the source was 3.5 ergs per sec per mm^2.

X irradiation. The X-ray source was a Picker unit with a beryllium window tube operated at 60 kvp

TABLE 1. *Escherichia coli strains*

Designation	Genotype with respect to repair	Phenotype with respect to repair	Source	References
B	Wild	Wild	Doermann	
B_{s-1}	*hcr⁻exr⁻*	UV[a], X-ray sensitive	Hill	20, 26
H/r30R	*uvrA⁺res⁺*	Radioresistant	Kondo	23
Hs30R	*uvrA⁻res⁺*	UV sensitive, slightly X-ray sensitive, does not excise thymine dimers	Kondo	23, 24
R15	*uvr⁺res-1*	UV, X-ray sensitive, deficient in DNA polymerase	Kondo	23, 24
OK2402	*uvrA⁻res-1*	Double mutant	Kondo	23
W3110	*pol+*	Wild	Cairns	
W3478	*polA1*	UV, X-ray sensitive, deficient in DNA polymerase I	Cairns	8, 16, 28
N1071	*lig+*	Wild	Gellert	15
N1072	*lop-8*	Overproduces ligase, UV sensitive	Gellert	15
N1252	*lop-8 lig-2*	Overproduces defective ligase, UV sensitive	Gellert	15

[a] UV, Ultraviolet.

TABLE 2. *Bacteriophage T4 mutants*

Designation	Phenotype with respect to repair	Source	References
T4+	Wild	Doermann	
T4*v*	UV[a] sensitive, deficient in a dimer excising endonuclease	Harm	13, 18, 36
T4*x*	UV, gamma-ray sensitive, recombination deficient	Harm	7, 19
T4*y*	UV, gamma-ray sensitive, recombination deficient	Boyle	7
T4tsB20	Temperature sensitive in gene 30-ligase, slightly UV sensitive	Edgar	2, 3, 11
T4tsL91	Temperature sensitive in gene 43-DNA polymerase, slightly UV sensitive	Edgar	2, 3, 9

[a] UV, Ultraviolet.

and 20 ma. Phage samples in 9X citrate broth were irradiated aerobically in 0.7-ml quantities with stirring. The dose rate was estimated by Fricke ferrous sulfate dosimetry (34) and was found to be approximately 70 krads/min.

Experimental procedure. Bacterial strains were routinely grown in broth at 37 C with aeration. Seed cultures were prepared from small inocula of fresh overnight stationary-phase cultures. Thiamine hydrochloride (0.2 mg/liter) was added to growth and plating media for *E. coli* strains N1071, N1072, and N1252 (15).

Lysates of T4*x* were prepared at 30 C as suggested by Boyle (7). T4ts43 was prepared at 25 C, whereas high-titer T4ts30 lysates were made at 30 C by using *E. coli* strain N1072. Partially purified phage lysates were diluted into citrate broth (9X) for X irradiation. For ultraviolet irradiation, phage lysates were partially purified by differential centrifugation and resuspended in gel buffer.

Chloramphenicol complexes were prepared by infecting washed, log-phase cells with bacteriophage at a multiplicity of infection of 0.05 to 0.1. At the time of infection, chloramphenicol was added to a final concentration of 50 μg/ml. If not otherwise indicated, the complexes were held for 30 min at 37 C with aeration and then plated and assayed for ultraviolet survival. Free phage were neutralized by anti-T4 serum 5 min after infection. Anti-serum was prepared as described by Adams (1), and the various preparations used had *K* values ranging between 200 and 250 min^{-1}. Phage adsorption was carried out in medium containing 20 μg of L-tryptophan per ml.

Survival curves were determined by diluting irradiated phage and plating on the indicated strains. Plates were usually incubated at 37 C. However, T4ts43 plates were incubated at 25 C and 43 C, whereas T4ts30 plates were incubated at 18 and 30 C. After ultraviolet irradiation, plating was done by using dim yellow light to prevent photoreactivation.

All procedures not specifically described here have been published by Adams (1). The data presented represent the average of at least three experiments, and, for the X-ray studies, generally five experiments. All the data were computer analyzed, and the final slopes were determined from the best fit line obtained by linear regression analysis. In all cases, the correlation coefficient, r, fell within the 1% level of significance. In addition, for the X-ray data, standard deviations were determined for each experimental point. In no case, even for the data representing partial suppression (see Fig. 10), do the points overlap by one standard deviation. Deviations are not presented on the figures to facilitate visualization of the data.

RESULTS

Repair of ultraviolet damages by host DNA polymerase I. Figure 1 shows the survival of T4+, T4*x*, T4*y*, and T4*xv* on *pol*+ and *polA1* cells. The latter strain is deficient in DNA polymerase I (8) and is ultraviolet (16) and X-ray sensitive (28). T4+ and T4*x* exhibit increased sensitivity on *polA1*, although T4*x* to a lesser extent

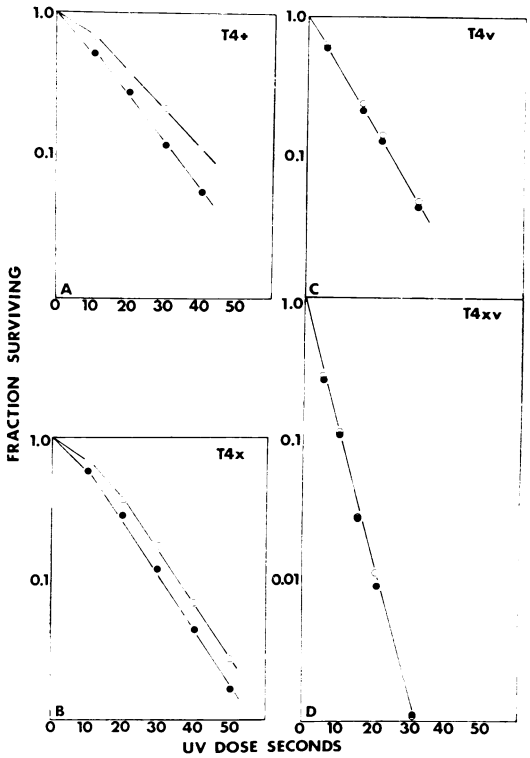

FIG. 1. *Ultraviolet survival of T4 mutants on pol+ (○) and polA1 (●) hosts. A, T4+; B, T4x; C, T4v; D, T4xv.*

than T4+. T4*v* and T4*xv* show no increased sensitivity on the DNA polymerase-deficient host. While these studies were in progress, Smith et al. (30) reported that wild-type T4 showed decreased ultraviolet survival in a DNA polymerase-negative strain of *E. coli* and T4*v* did not. Apparently, the *v* gene product must be present for increased sensitivity to be observed in *polA1*.

The survival of T4 temperature-sensitive gene 43 plated under permissive (25 C) or semi-restrictive (43 C) conditions on *pol*+ and *polA1* is shown in Fig. 2. The same slight increase in sensitivity exhibited by this mutant on wild-type cells is also observed in *polA1*. The increase in ultraviolet sensitivity exhibited by temperature-sensitive mutants on the T4 DNA polymerase gene (2, 3) has not been reproducible in our hands or those of Speyer (31). Apparently, the reported sensitivity increase is an artifact of plaque-counting technique (29). In any case, if T4 polymerase plays a role in ultraviolet repair, it does not seem to be affected by the absence of host DNA polymerase I.

The ultraviolet sensitivities of T4+, T4*y*, T4*x*, T4*v*, and T4*xv* on a series of suppressor-negative

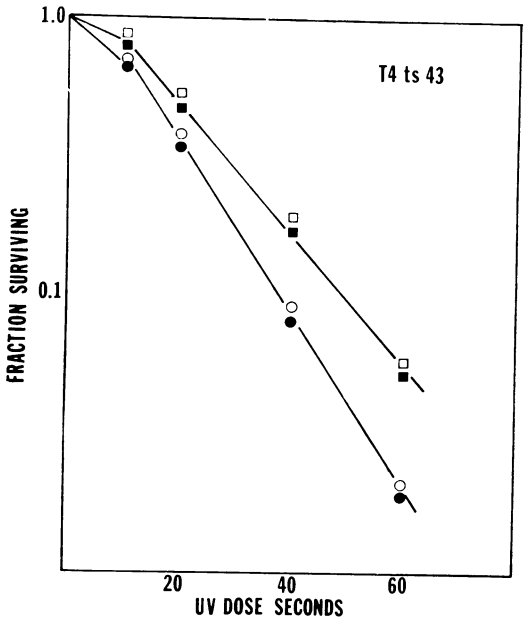

FIG. 2. *Ultraviolet survival of T4ts43 on pol+, 25 C (□); pol+, 43 C (■); polA1, 25 C (○); and polA1, 43 C (●).*

strains which lack DNA polymerase or ultraviolet-specific endonuclease function, or both, (22) are shown in Fig. 3. As can be seen, T4*v* and T4*xv* exhibit their mutant sensitivities independently of the strain they are plated on. T4+ shows the same increased sensitivity whether plated on *res-1*, lacking DNA polymerase, or *res-1 uvrA*, a double mutant which lacks both DNA polymerase and ultraviolet-specific endonuclease function. Both of these strains are ultraviolet and X-ray sensitive (23, 24). No increased sensitivity of T4+ or any of the mutants tested was observed when plated on the *uvrA* mutant. The behavior of T4*x* in the *res-1* strain mimics that observed when *polA1* served as host. On *res-1*, T4*y* shows increased sensitivity, the relative sensitivity being equal to that observed with T4*x* (Table 3). The similar response of T4*x* and T4*y* to the absence of host polymerase suggests their functioning in a common pathway distinct from that involving the *v* gene product and host polymerase. That T4*x* and T4*y* function in a common pathway is in agreement with Boyle's interpretation of his radiobiological and genetic data (5). Also, the sensitivity of T4*x* and T4*y* was decreased in *res-1* strains also lacking the *uvrA* gene product as shown in Fig. 3 and Table 3. This phenomenon was not observed with T4+.

It should be noted that both *polA1* and *res-1* were derived by mutagenesis of their respective parent strains, and that the effect measured here could be due to another mutation. We consider this possibility unlikely, since both of these DNA polymerase-minus mutants exhibit identical phenotypic responses with respect to repair.

Effect of host ligase on ultraviolet survival. Figure 4 shows the survival of T4+, *y*, *x*, *v*, and *xv* on *lig+*, *lop-8* and *lop-8 lig-2* bacterial hosts. The latter two strains overproduce active or defective ligase, respectively (15). Only T4*y* showed any difference when plated on these strains, that being increased resistance in the ligase overproducer. The response of T4 temperature-sensitive gene 30 in *lop-8* and *lop-8 lig-2* was no different than when plated on wild-type bacteria. The ultraviolet sensitivity of this mutant was not exaggerated in a ligase-defective bacterial strain.

Postirradiation incubation in chloramphenicol. Figure 5 shows the ultraviolet survival of T4 mutants directly plated on strains B and B_{s-1} or treated at time of infection with 50 μg of chloramphenicol per ml and held at 37 C prior to plating. T4*xv*, but no other mutant tested, exhibited increased resistance on both B and B_{s-1} chloramphenicol complexes. The time course of this resistance increase is shown in Fig. 6. As can be seen, the total increase appears to occur during the first 5 min of adsorption. Preliminary results indicate that T4*xv* exhibits the resistance increase in *pol+* chloramphenicol complexes but not in *polA1* chloramphenicol complexes. Thus, host DNA polymerase and host nucleases are implicated in the resistance increase of T4*xv* in the absence of phage-induced enzymes.

Phage-mediated repair of X-ray damages. Figure 7 shows the X-ray inactivation of temperature-sensitive T4 mutants in gene 43 (DNA polymerase) and gene 30 (ligase) at permissive and nonpermissive temperatures. As can be seen, there is no increased X-ray sensitivity of either of these mutants at the nonpermissive

TABLE 3. *Sensitivity comparison of T4 mutants on wild-type and mutant bacterial strains*

Bacteriophage mutant	Final slope in mutant bacteria/final slope in wild-type bacteria	
	res-1	*res-1 uvrA*
T4+	1.35	1.35
T4*v*	1.0	1.0
T4*xv*	1.0	1.0
T4*x*	1.22	1.08
T4*y*	1.22	1.11

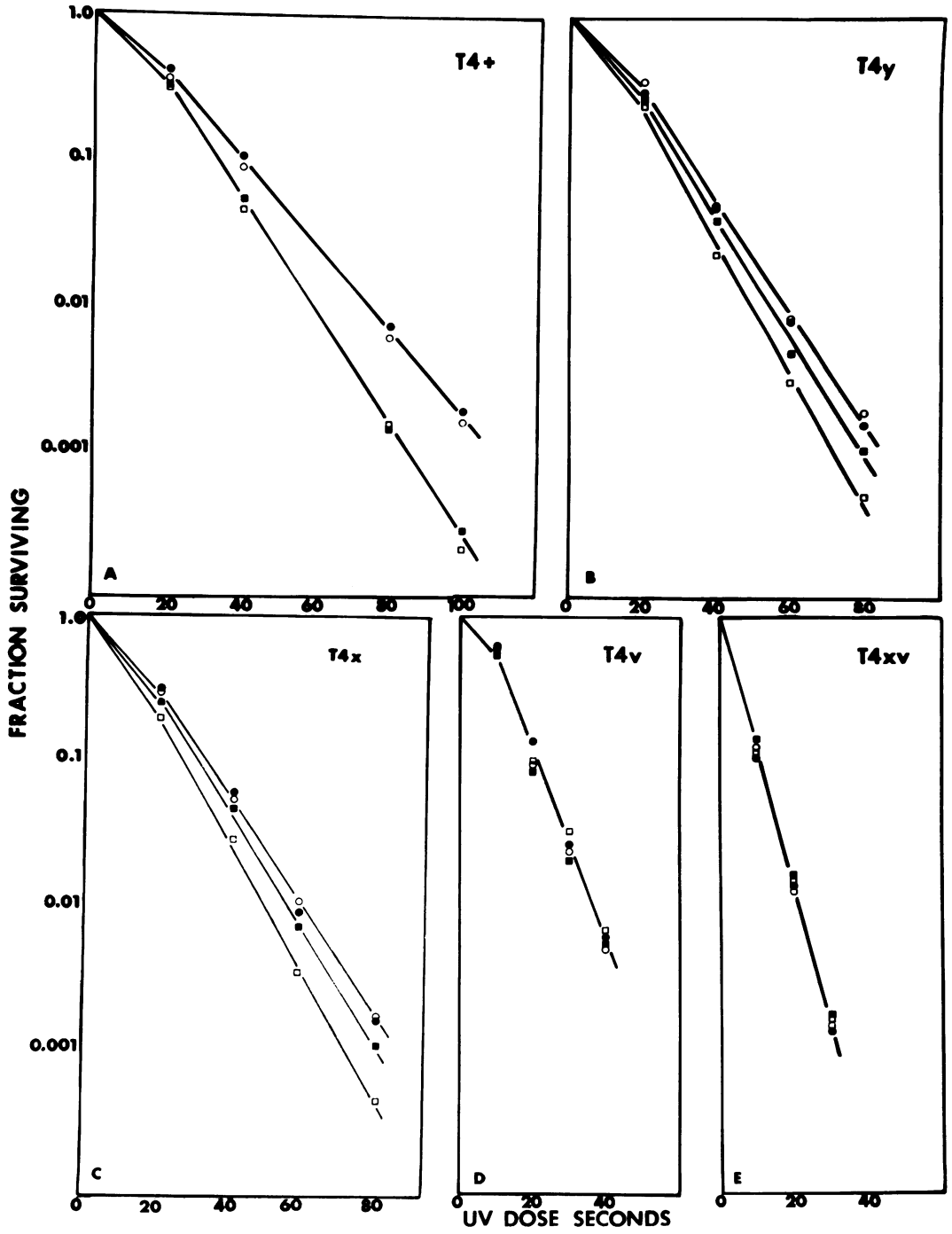

FIG. 3. *Ultraviolet survival of T4 mutants on uvrA⁺res⁺ (○); uvrA⁻res⁺ (●); uvrA⁺res-1 (□); and uvrA⁻res-1 (■). A, T4+; B, T4y; C, T4x; D, T4v; E, T4xv.*

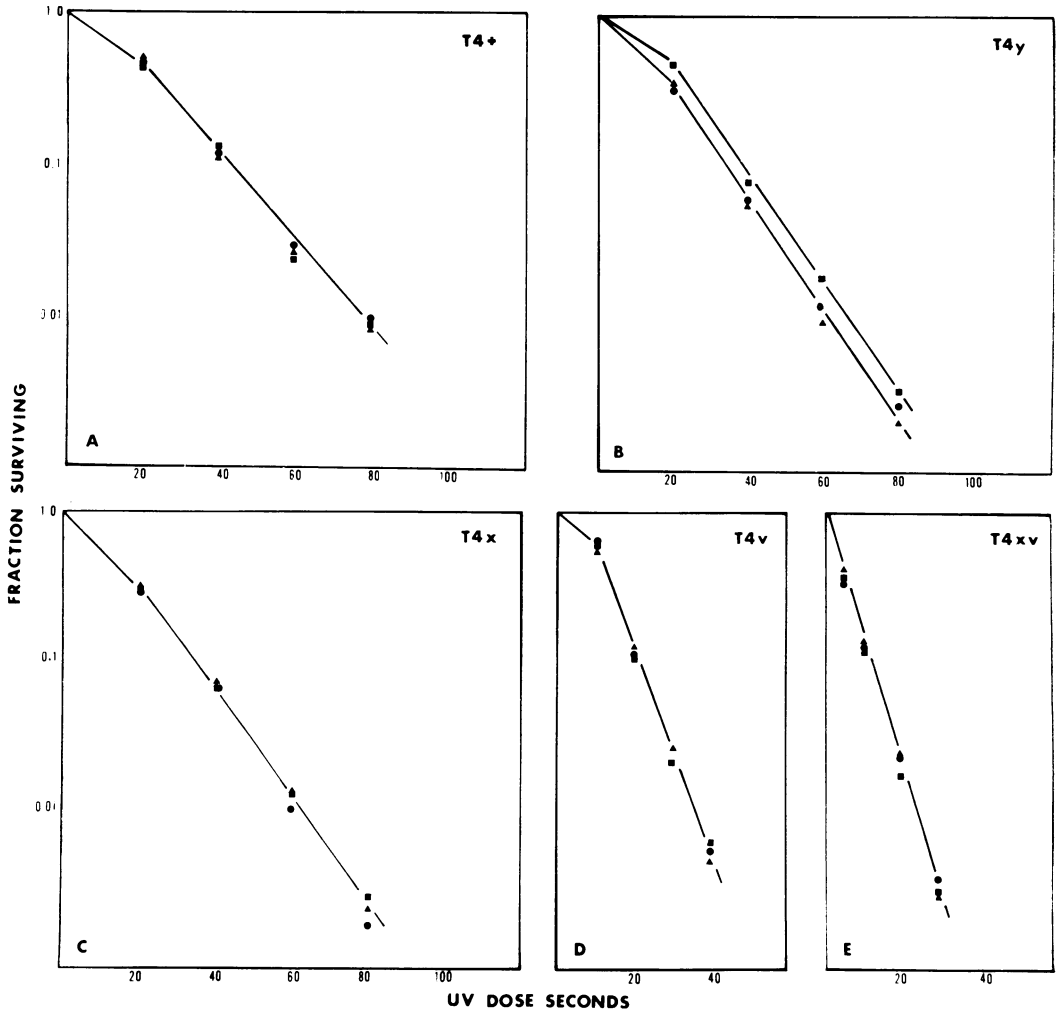

FIG. 4. *Ultraviolet survival of T4 mutants on lig+ (●); lop-8 (■); and lop-8 lig-2 (▲). A, T4+; B, T4y; C, T4x; D, T4v; E, T4xv.*

temperature tested, indicating the apparent lack of involvement of these mutants in repair of X-ray damage. The X-ray sensitivity of T4*x* is 11% more sensitive than wild-type phage (Fig. 8). T4*y* on the suppressor-negative host also exhibits 11% increase in sensitivity (Fig. 8). The sensitivity of T4*x* and T4*y* measured here with X rays is not as great as that reported by Boyle and Symonds (7) for gamma rays. It is theoretically possible that the gamma rays, which have a lower linear energy transfer than X rays, could inflict a preponderance of a different class of reparable damages than X rays.

Host-mediated repair of X-ray damages. The effect of host DNA polymerase I on the X-ray survival of T4+, T4*x*, and T4ts43 at permissive and nonpermissive temperatures is shown in Fig. 9. All three phage strains tested showed increased sensitivity on *polA1* to the same extent. The absence of phage DNA polymerase did not enhance the effect observed in the absence of host DNA polymerase I.

The X-ray sensitivities of T4+ and T4*y* on suppressor-negative strains lacking DNA polymerase or *uvrA* gene product are shown in Fig. 10. The results obtained on *res-1* with T4+ as well as T4*x* (not shown) are similar to those observed with *polA1*. Also, T4*y* exhibits increased sensitivity on DNA polymerase-negative *res-1*. Although there is no observable effect when T4 is plated on the *uvrA* mutant, the polymerase-minus

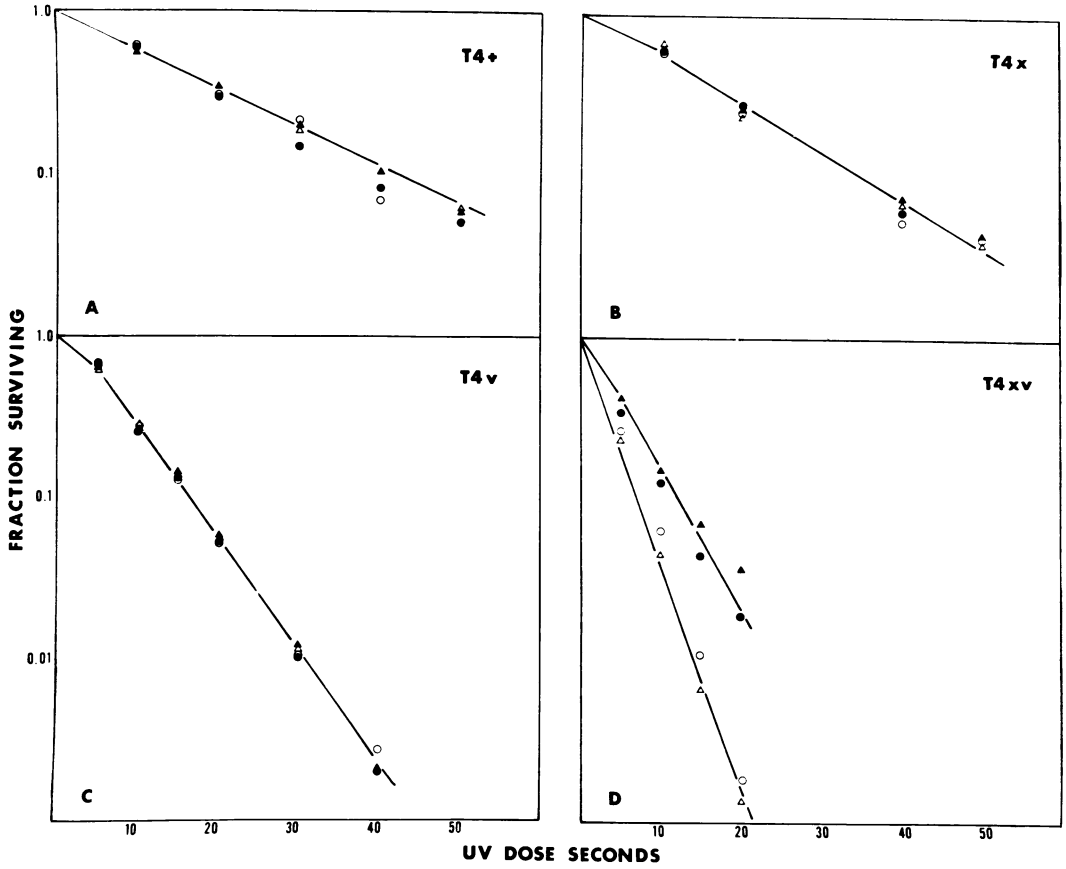

FIG. 5. *Ultraviolet survival of T4 mutants directly plated on B (○) or B_{s-1} (△) hosts; or plated after postir radiation incubation of B (●) or B_{s-1} (▲) phage complexes in chloramphenicol. A, T4+; B, T4x; C, T4v; D T4xv.*

sensitivity of all phage strains tested was partially suppressed on the *uvrA res-1* double mutant.

Figure 11 shows the X-ray survival of T4+ and T4*y* on wild-type, *lop-8*, and *lop-8 lig-2* strains. The latter two hosts overproduce active or defective ligase, respectively. Wild-type T4, but neither T4*y* nor T4*x* (not shown), exhibited increased sensitivity in the strain overproducing defective ligase.

The survival of the temperature-sensitive gene 30 mutant when plated on *lop-8* at permissive and nonpermissive temperatures is identical to that of the wild-type phage. However, when the gene 30 mutant was plated at nonpermissive temperatures on the *lop-8 lig-2* host, it did not exhibit the increased sensitivity of the wild-type phage. A likely explanation for the difference in sensitivity is that the nonpermissive temperature for the phage is in fact permissive for the temperature-sensitive *lig-2* mutation (27), thus rendering the host, in effect, *lop-8*.

DISCUSSION

Wild-type T4 showed a 35% increase in ultraviolet sensitivity in DNA polymerase-deficient strains of bacteria. This agrees closely with the 30% observed by Smith et al. (30) without filtering the germicidal lamp output. The increased sensitivity on *pol* minus hosts was not observed with T4*v* or T4*xv*, indicating that the v+ gene product may be functioning with DNA polymerase I, presumably during excision repair. In the absence of DNA polymerase I, nuclease action observed by Boyle et al. (6) in *polA1* cells might degrade at *v* gene product-induced nicks. It should be noted that the lack of bacterial polymerase affects *v* gene repair only to a minor extent since the increase in slope is 35% compared to about 120% in the absence of the *v* gene function.

Although v+ gene product appears to be responsible for nicking during ultraviolet excision repair in T4, under special circumstances host

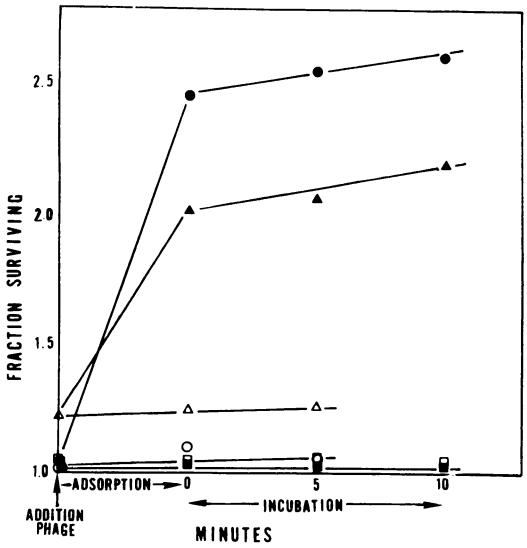

FIG. 6. *Time course of resistance increase of T4xv in chloramphenicol-treated complexes of B* (●) *and* B_{s-1} (▲); *and in untreated complexes of B* (○) *and* B_{s-1} (△). *Data are also shown for T4+ in chloramphenicol-treated complexes of B* (□) *and* B_{s-1} (■).

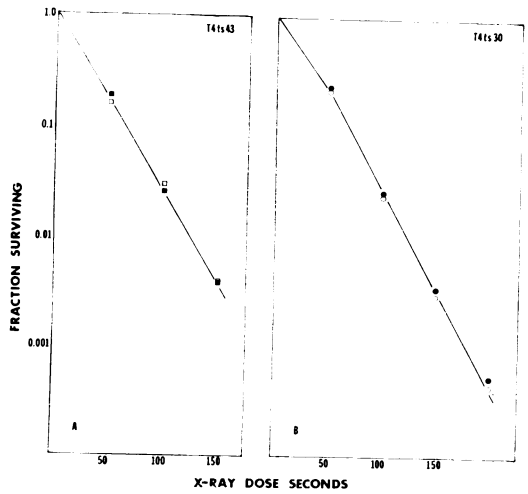

FIG. 7. *X-ray survival of temperature-sensitive mutants on E. coli B. A, T4ts43, 25 C* (□) *and 43 C* (■). *B, T4ts30, 18 C* (○) *and 30 C* (●).

nucleases seem to be able to perform this function. This is evidenced by increased ultraviolet survival of T4*xv* in B and B_{s-1} when incubated in chloramphenicol, in the absence of phage-induced biosynthesis. Kozinski and Lorkiewicz observed physical nicking and repair of ultraviolet-irradiated wild-type T4 in chloramphenicol complexes of both B and B_{s-1}; however, they did not observe biological repair. Similarly, host biological repair of wild-type T4, as well as single T4 mutants, was not observed in these studies presumably because phage repair systems mask the effect in a manner analogous to the masking of liquid holding recovery in certain strains of bacteria (14).

T4*x* and T4*y* showed a 22% increase in ultraviolet sensitivity in DNA polymerase-deficient bacterial strains. This effect was partially suppressed in DNA polymerase-deficient strains also lacking in the *uvrA* gene product. Suppression did not occur with wild-type T4. To account for this observation, we propose that, in the absence of *x-y* type repair, *v* type excision repair is extended. During extended excision repair, endogenous *uvrA* product, *v* gene product, host DNA polymerase, and other enzymes involved in excision repair could function to repair the damaged phage DNA. In the absence of host polymerase, removal of the *uvrA* product would increase

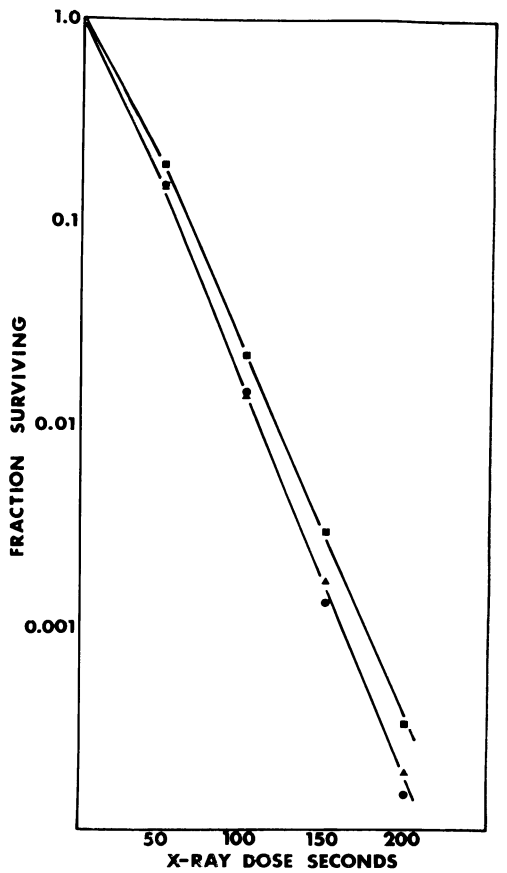

FIG. 8. *X-ray survival on E. coli B of T4+* (■), *T4x* (▲), *and T4y* (●).

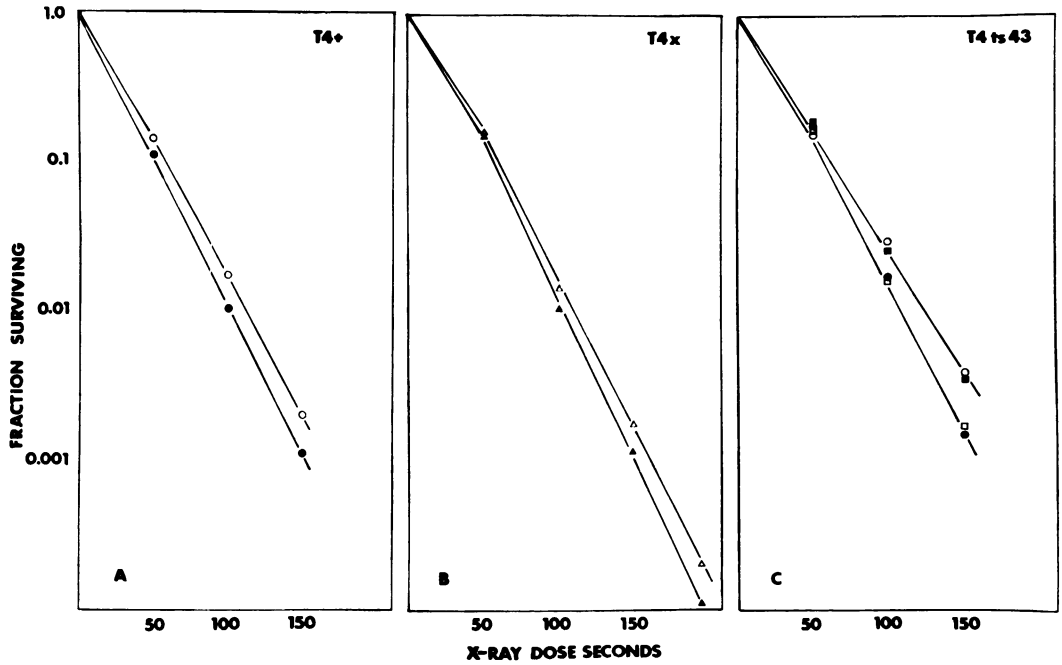

FIG. 9. *X-ray survival of T4 mutants on DNA polymerase-positive or -negative hosts. A, T4+ on pol+* (○) *and polA1* (●). *B, T4x on pol+* (△) *and polA1* (▲). *C, T4ts43 on pol+ 25 C* (○), *pol+ 43 C* (■); *polA1 25 C* (●), *polA1 43 C* (□).

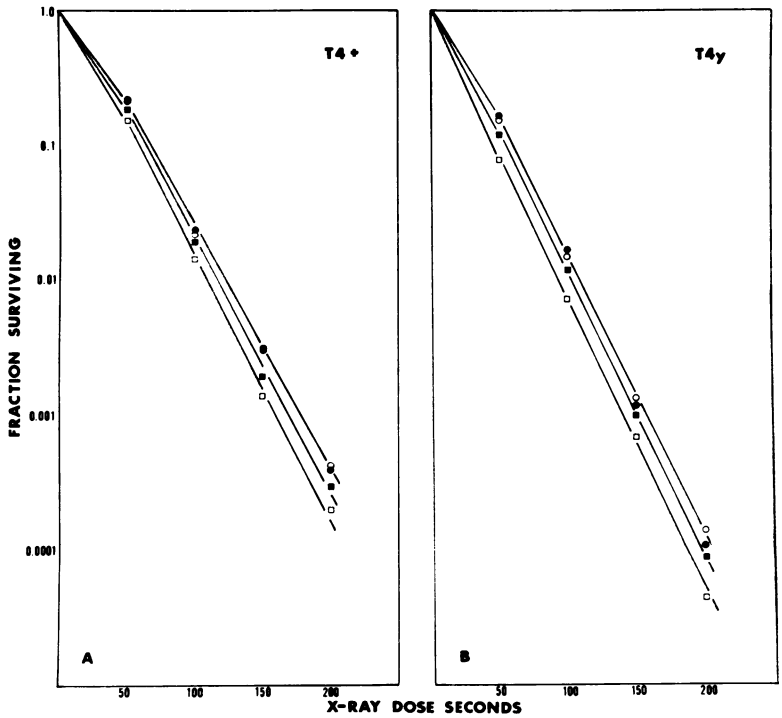

FIG. 10. *X-ray survival of T4+ (A) and T4y (B) on uvrA⁺res+* (○); *uvrA⁻res+* (●); *uvrA⁺res-1* (□); *and uvrA⁻res-1* (■).

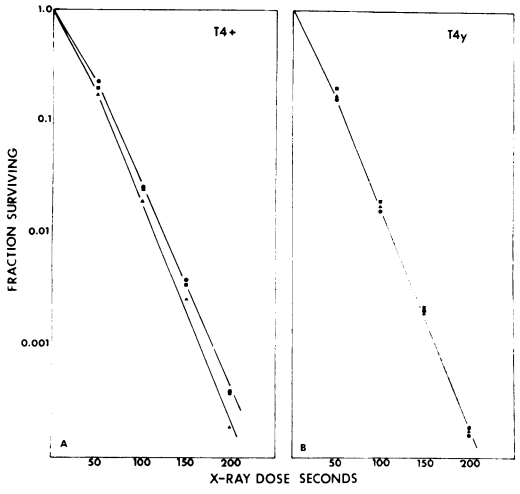

FIG. 11. *X-ray survival of T4+ (A) and T4y (B) on lig+ (●); lop-8 (■); and lop-8 lig-2 (▲).*

resistance though not necessarily back to the mutant phage phenotype. The implication is that nuclease action present in DNA polymerase minus hosts is making lethal damages out of *uvrA* nicks occurring at nonlethal ultraviolet damages or at nonspecific sites. Suppression of the polymerase effect would not occur with wild-type phage because the *x-y* pathway would be functioning and excision repair would not be extended. It should be noted that Kato and Kondo (24) found decreased breakdown of DNA after ultraviolet irradiation of the *uvrA res-1* double mutant compared to the *res-1* mutant alone. The response of T4*y* to the ligase overproducer is difficult to explain in the absence of further information.

We are presently studying functional survival by using T4 ultraviolet-sensitive mutants in a variety of mutant bacterial strains. Hopefully, we shall gain enough information from this type of approach to adequately assess the role of host enzymes in ultraviolet repair of T4 and to provide further insights into the nature of the phage-coded repair systems. Ultimately one should be able to provide a point of departure for elucidating the enzymatic nature of the *x* and *y* gene products.

The repair processes that occur following X irradiation are not well defined. Without appropriate mutants possessing altered sensitivities, there has been little benefit in using the relatively simple phage systems for studying X-ray repair. To investigate the possible involvement of specific gene products in X-ray repair, temperature-sensitive T4 mutants were examined for X-ray sensitivity under the same semirestrictive conditions which permit expression of ultraviolet sensitivity. The results indicate, at least to the limit of resolution of these measurements, that phage-coded DNA polymerase and ligase are not involved in the X-ray repair process. However, these same enzymes, when coded for by the host, appear to play a role in determining phage survival, since the absence of either of these products results in an increased sensitivity to X rays. The sensitivity is equal to that observed by us when using the T4*x* or T4*y* mutants. By plating X-irradiated T4*x* and T4*y*, both of which appear to be involved in the same repair pathway (5, 29), on various mutant hosts, it is possible to demonstrate two apparently distinct processes that affect phage survival. When both the *x* and *y* gene products are present, the lack of host ligase results in an 11% increase in phage sensitivity to X irradiation. When either of these phage gene products are lacking, there is no host-mediated sensitivity increase observed on the ligase minus host. It appears, therefore, that host ligase might function in conjunction with the *x* and *y* gene products in repairing X-ray-damaged phage DNA.

All phage strains tested showed increased sensitivity to X rays when plated on DNA polymerase-negative hosts. This is similar to the results obtained with ultraviolet-irradiated phage, with the exception of T4*v*, and for EMS-treated T4 (29). To evaluate the possible repair role of the host DNA polymerase I, one can look at the partial suppression of the polymerase minus sensitivity found when the host is also mutant at the *uvrA* locus. A possible explanation for the observed phage-host relationship involves the consideration of two types of single-stranded breaks: those that are directly caused by the X rays, and those that result from the *uvrA* gene product acting on some X-ray-induced helical distortions which could possibly result from base or sugar damages. When only polymerase is lacking, both types of damages could become sites for lethal degradation. When *uvrA* gene product and DNA polymerase are absent, only the single-stranded breaks that are directly caused by the X rays are lethal. A further complication, that results when trying to develop a working hypothesis regarding the repair of X-ray damage to T4, is the as yet unresolved question of whether the lack of host DNA polymerase prevents the repair of lethal damages or in fact allows for their creation. The increased DNA degradation observed when DNA polymerase minus bacteria are X irradiated (6), or when T4 is X-irradiated and plated on the polymerase-negative host (Marsden and Ginoza, *personal communication*), appears to be evidence for the latter phenomenon.

When one takes into account both systems which we have found to affect phage survival after X-irradiation, we can explain a 22% increase

in phage sensitivity. The calculation of Freifelder (12) indicates that there is substantially more repair occurring after X irradiation of T4. Hopefully, we shall be able to isolate an X-ray-sensitive mutant of T4, unless the repair of T4 X-ray damage is intimately linked to the replication of T4, and such a mutation would be lethal.

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ADDENDUM IN PROOF

A mutant deficient in the gene 32 product (DNA binding protein) has been shown to be as ultraviolet sensitive as T4*x* (3). We have not found this mutant (T4tsL67) to be X-ray sensitive when measured under similar conditions.

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