Deoxyribonucleic Acid Repair in Bacteriophage T4: Observations on the Roles of the x and v Genes and of Host Factors

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Studies were carried out to determine the effect of mutation in the host *pol* I gene on survival of ultraviolet (UV)-irradiated bacteriophage T4. Whereas a slightly reduced survival was observed in *Escherichia coli* strain P-3478 (*pol* A₁) compared to strain W-3110 (*pol* A⁺), no such difference was observed in two strains isogenic except for the *pol* A gene. It was also shown that, whereas bacteriophage T4 x is sensitive to UV irradiation, X irradiation, and treatment with methylmethanesulfonate (MMS), phage T4 v_1 is sensitive only to UV irradiation. The survival of damaged phage T4 x is neither affected by the presence of the *rec* A, *rec* B, or *pol* A mutations in the host, nor is there evidence that phage T4 effects repair of *rec* A or *pol* A mutants previously treated with either UV or MMS.

Increased sensitivity to ultraviolet (UV) radiation in bacteriophage T4 has been described in association with a number of mutations. Harm (15) isolated two non-linked mutants defective in genes v and x , each of which is abnormally sensitive to UV. Boyle (3, 5) described the isolation of a new radiation-sensitive mutant of T4 called T4 y which phenotypically is very similar to T4 x but which is genetically distinct from both genes v and x by linkage data. In addition, Baldy (1) described UV sensitivity of phages mutant in genes 30, 43, or 47. The v gene has been shown to code for a UV-specific endonuclease that attacks deoxyribonucleic acid (DNA) containing pyrimidine dimers and presumably carries out the initial endonucleolytic incision step in dimer excision *in vivo* (12, 13, 32). The mechanism of post-endonucleolytic dimer excision in T4-infected cells is unknown. No mutants other than phage T4 v have been shown to be defective in excision of dimers specifically. After excision, repair synthesis and covalent joining might well be performed by the T4 DNA polymerase (gene 43; reference 9) and T4 ligase (gene 30; reference 10), respectively. This would account for the observations of Baldy, mentioned above. The product of gene 47 is unknown but is concerned with host DNA degradation after infection with T4 (29).

The x gene product has also not been identified. Harm (16) has shown that the UV sensitivity of

T4 x is not due to any intrinsic differences in the DNA of the phage. In addition, he has demonstrated that genetic recombination within the *rII* locus is significantly reduced by x mutations. The association of UV sensitivity and recombination deficiency in phage T4 is very reminiscent of a class of *Escherichia coli* mutants designated *rec* (7, 18). The best characterized of these fall into two phenotypic groups carrying either the *rec* A or *rec* B/C mutations. Both groups show a sensitivity to UV irradiation as well as to agents that cause phosphodiester bond breakage such as X irradiation and monofunctional alkylating agents. In contradistinction to the *rec* mutants, the excision-defective mutants *uvr* A, B, and C are sensitive to UV but not to X irradiation or methylmethanesulfonate (MMS).

The present studies demonstrate that bacteriophage T4 v_1 is sensitive only to UV irradiation, whereas phage T4 x is sensitive to X and UV irradiation and MMS, suggesting the existence of two distinct DNA repair modes coded by the T4 genome and formally analogous to the excision and post-replication repair modes in *E. coli*. In addition, we wish to report our observations on the role of DNA polymerase I of *E. coli* in the *in vivo* repair of damaged T4 DNA.

MATERIAL AND METHODS

Bacterial and phage strains. *E. coli* JC-2918 (*rec* A⁺) and JC-2926 (*rec* A) are isogenic except for the

rec A locus. Strains JC-5744 (*rec B*⁺) and JC-5743 (*rec B*) are similarly related. These strains were originally isolated in the laboratory of Alvin Clark and were kindly supplied to us by Kendrick C. Smith. *E. coli* JG-139 (*pol A*⁺) and JG-138 (*pol A*₁) are an otherwise isogenic pair. Strain JG-138 was originally isolated by Julian Gross by transduction of the *pol A* mutation from strain P-3478. They were donated by Kendrick C. Smith. Strains W-3110 and P-3478 (*pol A*₁) originally obtained by J. Cairns were provided by David Korn. Strain N-14-4 (*uvr D*) was originally isolated by I. Tomizawa and was supplied to us by Ann Ganesan. *E. coli* AB-1157 is a *rec*⁺, *uvr*⁺ derivative of strain K-12 obtained from Paul Howard-Flanders (18).

Bacteriophages T4D, T4x, and T4v₁ were originally obtained from Walter Harm.

Bacteriophage survival experiments. Bacteriophages were exposed to UV irradiation at 2,540 nm at doses between 0 and 400 ergs/mm², using a 15-watt low-pressure mercury-vapor germicidal lamp (General Electric). Treatment with MMS (Aldrich Chemical Co., Inc.) was carried out by incubating the phage at a concentration of 1.0×10^9 /ml in 10^{-2} M MMS in 0.005 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer at pH 8.0. Incubation was carried out for varying periods of time at 37 C. X irradiation was performed with a 50 kilovolt peak X-ray unit (21a) at a mean dose rate of 8.8 krad/min. Both UV and X irradiation were carried out with the phage suspended in a minimal salt medium consisting of 7 g of Na₂HPO₄, 3 g of KH₂PO₄ and 4 g of NaCl per liter, pH 7.0. Phage suspensions for irradiation were maintained at a thickness of less than 3.0 mm. Prior to plating, phage were diluted in tryptone broth (1% tryptone [Difco]; 0.5% NaCl) at 4 C. Phage titers were measured by using the standard soft-agar overlay method. Plates and soft agar contained tryptone-yeast extract (1% tryptone [Difco]; 1% yeast extract [Difco]; 1% NaCl).

Repair of host cells by phage T4. Host cell repair experiments were carried out as previously described for *v* gene repair of *E. coli* Bs-1 (13, 17). *E. coli* JC-2926 (*rec A*) was exposed to UV irradiation at 100 ergs/mm² or incubated with 8×10^{-2} M MMS for 30 min at 37 C. The treated cells were incubated for 15 min with phage previously irradiated at 3,600 ergs/mm² to reduce the host killing capacity of the phage (30) (UV-inactivated phage). Incubation of bacteria with phage was at a multiplicity of 3 to 5 phage/cell in yeast-tryptone broth at 37 C, after which appropriate dilutions were plated, and the number of survivors was determined by colony count. The phage used in the experiments involving UV-irradiation damage was T4v₁ in order to eliminate any possible *v* gene-mediated repair. When MMS was the DNA-damaging agent, phage T4D was used.

Enzymological studies. All crude extracts were prepared by sonic treatment of cells in 0.05 M Tris-hydrochloride, pH 8.0, using a Biosonik III sonicator. DNA polymerase I was assayed by the procedure of Richardson et al. (26). Strains P-3478 and JG-138 were found to have less than 1% of the wild type level of DNA polymerase I activity. Adenosine triphos-

phate (ATP)-dependent nuclease activity of *E. coli* was assayed by the method of Tanner and Oishi (28) using sonically treated ³H-labeled T4 DNA as substrate. The activity of this enzyme was also measured in cells infected with phage T4D or T4x, in which case phage infection was allowed to proceed for 17 min before addition of chloramphenicol at a final concentration of 150 µg/ml. The infected cells were then harvested by centrifugation. In some experiments, chloramphenicol was added at the time of phage infection.

RESULTS

Sensitivity of T4x and T4v₁ to UV, MMS, and X rays. Both bacteriophages T4x and T4v₁ show an increased sensitivity to UV irradiation, confirming the observations of Harm (15; Fig. 1). In addition, phage T4x shows increased sensitivity to both MMS treatment and X irradiation, whereas T4v₁ does not (Fig. 2 and 3). Comparisons of phage survival after DNA damage indicates that the genetic constitution of the host cell makes no difference. A single exception was the observation that after UV irradiation bacteriophage T4D shows a slight but significant increase in UV sensitivity in strain P-3478 (*pol A*) as compared to strain W-3110 (*pol A*⁺) (Fig. 4). However the survival curves, obtained when the isogenic pair JG-138 (*pol A*) and JG-139 (*pol A*⁺) were used as hosts, were identical to that usually observed for T4D (Fig. 5). UV irradiation of phage TVv₁ resulted in an equally decreased survival in both *pol A*⁺ and *pol A* strains (Fig. 4 and

FIG. 1. UV sensitivity of bacteriophages T4D, T4x and T4v₁. Experimental details are given in the text.

5). Numerous experiments failed to demonstrate a statistically significant difference between the survival of UV-irradiated phage $T4v_1$ in strain P-3478 as compared to W-3110 (Fig. 4). No increased UV sensitivity of phage T4 was detected in strain N-14-4 (*uvr D*).

FIG. 2. MMS sensitivity of bacteriophages T4D, T4x and T4v₁. Phages were incubated with 1×10^{-2} M methylmethanesulfonate in 0.05 M Tris-hydrochloride, pH 8.0, at 37 C.

FIG. 3. X-ray sensitivity of bacteriophages T4D, T4x and T4v₁. Experimental details are given in the text.

FIG. 4. UV sensitivity of bacteriophages T4D and T4v₁ when plated on *E. coli* W-3110 (▲, ●) and P-3478 (△, ○).

FIG. 5. UV sensitivity of bacteriophages T4D and T4v₁ when plated on *E. coli* JG-138 (▲, ●) and JG-139 (△, ○).

Host cell repair by bacteriophage. No significantly increased survival of *rec A* or *pol A* cells treated with UV radiation or MMS was observed after infection with UV-inactivated phage T4 or T4v₁ in the several combinations of these variables that were tested (Table 1).

Enzymological studies. Normal levels of ATP-

TABLE 1. *Effect of phage on survival of damaged hosts^a*

Expt no.	Treatment	Host cell	Phage	Survival after treatment alone N/No	Surviving untreated cells ^b	Surviving treated cells ^b		Ratio observed/expected
						Observed	Expected	
1	UV	JC-2926 <i>rec A</i>	T4 _{v1}	6.1×10^{-4}	1.1×10^6	7.0×10^2	6.7×10^2	1.0
2	UV	JC-2926 <i>rec A</i>	T4 _{v1}	1.2×10^{-4}	3.1×10^6	4.0×10^2	3.7×10^2	1.1
3	MMS	JG-138 <i>pol A</i> ₁	T4	4.3×10^{-2}	1.4×10^7	1.1×10^6	6.0×10^5	1.8
4	MMS	JG-138 <i>pol A</i> ₁	T4	2.6×10^{-3}	1.4×10^7	4.0×10^4	3.6×10^4	1.1
5	MMS	JC-2926 <i>rec A</i>	T4	5.5×10^{-5}	1.0×10^7	1.3×10^2	5.5×10^2	0.24
6	MMS	JC-2926 <i>rec A</i>	T4	1.8×10^{-5}	6.3×10^6	2.0×10^2	1.1×10^2	1.8

^a Experiments with UV-irradiated *E. coli* Bs-1 as the host cell and phages T4 and T4_{v1} as the inactivated phages were previously carried out in an identical manner (11). The ratios of observed/expected number of survivors in those experiments ranged from 9.2 to 21.0 for T4_{v1} and 0.4 to 2.0 for T4. Thus, the ratios seen in the present experiments are not considered as evidence of significant phage-mediated repair.

^b After infection with UV-inactivated phage.

dependent nuclease activity were demonstrated in uninfected cells and in cells infected with phage in the presence of chloramphenicol. Infection with either T4 or T4x under conditions allowing synthesis of phage-coded proteins resulted in a complete inhibition of ATP-dependent nuclease activity in each case (Fig. 6).

DISCUSSION

Excision repair in T4. In vitro studies on the *v* gene-coded endonuclease have shown that the purified enzyme alone does not cause excision of dimers (13). Since the endonuclease makes single-stranded nicks on the 5' side of pyrimidine dimers in double-stranded DNA (31), it is necessary that an enzyme with a 5' nuclease activity carry out excision in vivo. Such an activity could conceivably be coded for by the phage, but it would necessarily have to be distinct from phage T4 DNA polymerase since the latter enzyme does not possess a 5' nuclease activity (8). However, to date, no mutants of T4 other than T4_{v1} have been identified as defective in dimer excision. In addition, it has been shown that crude extracts of uninfected cells promote excision of dimers in vitro from UV-irradiated T4 DNA preincubated with dimer-specific endonuclease, suggesting that excision of dimers may be carried out by host rather than phage-coded enzymes (13). A specific host enzyme that possesses a 5' nuclease activity and is known to carry out dimer excision in vitro is DNA polymerase I of *E. coli* (20). Whether or not this function of DNA polymerase I is subverted in vivo remains to be clearly established.

FIG. 6. Assay of ATP-dependent nuclease activity. Preparation of crude extracts of uninfected and phage-infected cells is described in the text. The protein concentrations of the crude extracts were adjusted to a common final concentration of 5.0 mg/ml. ATP was present at a concentration of 0.5 mM. Symbols: ●, uninfected *E. coli* B-3 with ATP; □, uninfected *E. coli* B-3 with no added ATP; △, T4-infected cells with ATP; ○, T4-infected cells with no added ATP.

Significant in this respect is the observation that a *pol A* mutant, although sensitive to UV, does excise dimers to the same extent as wild-type strains (4).

Another way of assessing the role of DNA polymerase I in excision repair would be to compare the survival of UV-irradiated phage T4

in wild-type and *pol A* mutants of *E. coli*. A number of authors have reported a slight increase in UV sensitivity of T4 in the Cairns mutant *E. coli* P-3478 (27; S. Wallace and R. Melamede, *Biophys. Soc. Abstr.*, p. 153a, 1972). Using P-3478 and the wild-type strain W-3110 from which it was derived, we have confirmed this result. However when using two otherwise isogenic strains created by transduction, this differential is completely abolished, and the slope of the survival curves in each case is identical to that usually obtained for UV-irradiated T4. This result implies that the reduced survival noted in P-3478 is not a function of the *pol A* mutation but of some other defect in this strain. Recent studies (14) indicate that *N*-methyl-*N*-nitro-*N*-nitrosoguanidine produces mutations selectively at the replicating region, resulting in a high frequency of comutation at closely linked sites. A gene known to map very close to *pol A* which also affects UV sensitivity in *E. coli* is *uvr D* (23). Mutants in this gene have normal DNA polymerase I activity (22). Another recently discovered gene designated *rep* (22) maps at the same place as *uvr D* but differs from it by being unable to support the growth of phage P2 and having a somewhat reduced UV sensitivity. Our studies on strain N-14-4 (*uvr D*) have failed to reveal any significantly increased UV sensitivity of phage T4. The defective function(s) in strain P-3478 that increases the sensitivity of UV-irradiated T4 does require a functional *v* gene since the sensitivity of T4_{v1} is not significantly different in Strains P-3478 and W-3110. It should also be noted that the ratio of the slope of the T4 survival curves in strain P-3478 relative to that in W-3110 is only 1.3, whereas for T4_{v1} it is about 2.5. Thus the defective function(s) in strain P-3478 is not *essential* for excision repair *in vivo*, or else this mutation is quite leaky.

If indeed DNA polymerase I of *E. coli* does not affect T4 DNA repair *in vivo*, how does dimer excision in T4-infected cells occur? Grossman et al. (19, 21) have purified an exonuclease from *M. luteus* that *in vitro* causes excision of dimers from UV-irradiated double-stranded DNA previously nicked by a UV-specific endonuclease. This enzyme was recognized by its ability to exonucleolytically degrade either unirradiated or UV-irradiated single-stranded DNA at the same rate *in vitro*. Preliminary studies in our laboratory have indicated the presence in uninfected *E. coli pol A* of a deoxyribonuclease activity that degrades UV-irradiated heat-denatured DNA at the same rate as unirradiated DNA. In addition, extracts of T4-infected cells possess a second activity with the same capability. These enzymes are currently being investigated with respect to their role in dimer excision.

Recombinational repair in T4. The object of this aspect of our studies has been to develop a formal analogy between the excision and recombinational repair systems in *E. coli* and bacteriophage T4. This has been attempted (i) by comparing the phenotypes of various repaired-deficient mutants of T4 with those reported in *E. coli* and (ii) by searching for a functional relationship between the *x* gene product and the *rec A* or *B/C* gene products.

Our results demonstrate that, in addition to UV irradiation, bacteriophage T4_x is abnormally sensitive to treatment with MMS and to the effects of ionizing radiation. At the time of writing Ray et al. (25) reported an identical observation using ethylmethanesulfonate. In contradistinction to T4_x, phage T4_{v1} is sensitive only to UV irradiation and not to MMS or ionizing radiation. In addition, Boyle (3, 5) has shown the existence of a gene in T4 called *y*, mutation in which confers a phenotype almost identical with T4_x, i.e., increased sensitivity to UV and X irradiation and lowered recombination frequency. The *y* gene however is unlinked to either *v* or *x*. These general observations are consistent with the notion that in bacteriophage T4 as in *E. coli* there exists both an excision and a recombinational mode of DNA repair (Table 2).

The *rec* phenotype in *E. coli* that is associated with UV, X-ray, and MMS sensitivity is a feature of both the *rec A* and *rec B/C* mutants although there are very distinct quantitative differences between them. Recently a large number of other *rec* mutants have been identified, but little has yet been reported concerning their relative capacities for DNA repair (7). The *rec B/C* genes are known to code for an ATP-dependent nuclease activity that is readily detectable in extracts of appropriate cells (2, 6, 24). Tanner and Oishi (28) made the observation that infection of *E. coli* B with phage T4 resulted in the disappearance of this nuclease activity and that this effect required expression of phage protein synthesis. We reasoned that the turning off of the host recombination enzyme may be a requirement for normal phage recombination to occur and that the inability to carry out this function may be a feature of a recombination-defective mutant. Our results show that the loss of host deoxyribonuclease activity is not controlled by the *x* gene. Nor is there evidence for a new ATP-dependent nuclease induced by phage infection, although it is possible that a similar activity was not identified because of its requirement for a different cofactor.

The *rec A* gene product has not yet been identified. Thus, in an effort to discern a functional relationship between the *x* gene of T4 and the *rec A* gene of *E. coli* we have attempted to demon-

TABLE 2. Comparisons between bacterial and bacteriophage DNA repair genes

Determinations	<i>Escherichia coli</i>		Bacteriophage T4		
	Excision repair	Recombination repair	Excision repair	Recombination repair	
Specific genes involved	<i>uvr</i> A, B, and C	Multiple genes designated <i>rec</i>	<i>v</i>	<i>x</i>	<i>y</i>
Mutants sensitive to:					
UV	+	+	+	+	+
MMS	—	+	—	+	?
X rays	—	+	—	+	+
Recombination deficiency present	—	+	—	+	+
Gene product	? Endonuclease	?	Endonuclease	?	?

strate host-cell reactivation of UV- and MMS-treated phage T4 x . In addition, the reverse situation was tested, i.e., repair of treated *rec* A (and also *pol* A) cells by wild-type phage. Host cell reactivation was tested by observing the sensitivity of chemically or physically damaged T4 x phage when plated on host cells differing only in the *rec* A function (isogenic by transduction). The results indicate that the presence or absence of the *rec* A gene does not influence the repair of damage in phage T4 x . A possible effect of the *x* gene on the UV and MMS sensitivity of *rec* A and *pol* A cells was tested by carrying out experiments similar to the *v* gene-mediated repair observed by Harm (17) and Friedberg (11). In these experiments, it has been shown that infection of UV-irradiated excision-defective strains of *E. coli* with phage T4 under conditions which permit expression of some phage functions but which do not result in cell killing results in a significant increase in host cell survival. This effect is not observed when the infecting phage is defective in the *v* gene. *v* gene-mediated repair presumably results from expression of the phage excision endonuclease which facilitates excision repair of the host DNA. In the present experiments, we were unable to show any *x* gene-mediated repair of *rec* A bacteria which had been treated with either UV radiation or MMS.

Although the most reasonable conclusion from these experiments is that the *x* gene and *rec* A gene products are not analogous, it is conceivable that there is a stringent species specificity that precludes activity of one gene product on the DNA of the other. Further evidence for the postulated role of the *x* and *y* genes of T4 would be provided by the demonstration that in cells infected with UV-irradiated bacteriophage T4, a recombinational mode of repair exists which is

dependent on these genes. If so, the identification of the *x* gene product may provide some insights into the nature of the *rec* A gene product and vice versa.

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