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REQUIREMENT OF BACTERIOPHAGE T4 GENE FUNCTION FOR SURVIVAL AFTER X-RAY IRRADIATION[1]

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Jen-Leih Wu and Yun-Chi Yeh (1981) Requirement of bacteriophage T4 gene function for survival after X-ray irradiation. *Bull. Inst. Zool., Academia Sinica* **20**(1): 49-55. The role of gene 59 function in DNA repair of bacteriophage T4 particles damaged by X-ray irradiation was studies *in vivo*. At a dosage of 13.5 Krads, 40% of the T4D phage particles were killed when plated on *E. coli* BB. However, only 7.7 Krads were required to kill 40% of T4*am*C5 phage particles in the nonpermissive host *E. coli* BB. Thus, the T4 mutant of gene 59 in nonpermissive host showed two times higher sensitivity of inactivation to X-ray irradiation than wild type T4D infected *E. coli* BB. The X-ray sensitivity of this mutant could be completely suppressed by plating on permissive *E. coli* CR63 which carried an amber suppressor gene. The bacteriophage T4 DNA biosynthesis in both mutant- and wild type-infected *E. coli* were not significantly affected by X-ray irradiation. The involvement of gene 59 function of bacteriophage T4 in DNA repair mechanism was discussed.

The structure of DNA is easily damaged by X-irradiation, ultraviolet light and alkylating agents[9]. Thus, these mutagens are the commonly used agents for the study of DNA repair mechanisms[13]. The two dark DNA repair mechanisms, excision repair and post-replication repair mechanisms, have been reported in *Escherichia coli*[5,12,17,24,32] and bacteriophage T4[8,11,15,16]. The gene function involved in post-replication repair mechanism is required for UV-, X-rayand alkylating agent damaged DNA repair[16,18,31]. However, the gene function for excision repair mechanism is only participated in UV-damaged DNA repair[14,19,26]. In *E. coli*, the excision repair process is initiated by the incision of the DNA strand at the UV-damaged region by a specific endonuclease coded from *uvr* A and *uvr* B genes of *E. coli*[5,6]. In bacteriophage T4, the excision repair is initiated by a thymine dimer-specific endonuclease coded for by the *v* gene product[11,26,37]. This process is followed by exonuclease degradation to produce a gap, reinsertion of new nucleotides by DNA polymerase I (gene *pol* A) and rejoining of the newly repaired DNA fragment to the parental strand by polynucleotide ligase (gene 30)[30]. Gene 1, 30, 42, 45 and 56 of bacteriophage T4 and gene *pol* A of *E. coli* are reported to be involved in excision repair in T4-infected *E. coli*[25]. Based on the enzymatic activity and different sensitivities to UV, X-ray and alkylating agent, *v* gene of phage T4 is similar to *uvr* gene of *E. coli*[16]. Post-replication repair is the recently described and the least understood of DNA repair mechanism. *E. coli uvr* mutants that are toally deficient in

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excision repair can still survive with a substantial number of pyrimidine dimers in their DNA[20]. The UV-damaged DNA in these mutants can still be used for normal replication even though the damaged bases are never removed from the parental DNA; however, the gaps are left in the daughter strands as replication proceeds past dimers[1,20,29]. These gaps are subsequently filled with correct base sequeces by recombination from the other parental strand[21]. By this way, the daughter strands are rendered complete. In bacteriophage T4, *x*, *y*, 41 and several early genes for phage replication are required for post-replication repair[8,15,25]. In this repair process, *x* and *y* genes of phage T4 are somewhat similar to *rec* A, *rec* B and *rec* C of *E. coli* in their sensitivities to UV, X-ray and alkylating agents[16].

Gene 59 of bacteriophage T4 belongs to the class of DNA-arrested gene and has been mapped between gene 32 and gene 33 in the T4 chromosome[10]. Gene 59 is an essential gene for phage DNA replication[34,36], recombination[23] and control the stability of its mRNA[33]. It has been shown that gene 59 is participated in the repair process for UV- and alkylating agent-damaged DNA[35]. However, the role of this gene product in repair of DNA lesions induced by X-irradiation is still unknown. The present study shows the requirement of gene 59 function for repair of X-ray damaged DNA. The involvement of gene 59 product in post-replication repair mechanism was discussed.

MATERIALS AND METHODS

(A) Bacteria and Bacteriophages

Escherichia coli K strain CR63 was used as a permissive host for T4 amber mutants. *E. coli* BB was used as a nonpermissive host for T4 amber mutants. *E. coli* B strain Tr201, a low thymine-requiring mutant, was also used as a nonpermissive host. Bacteriophage T4D was used as wild type. T4*am*C5 is an amber mutant of gene 59.

(B) Media

(i) *Minimal Salt Medium*

This medium was prepared by dissolving 7 g of Na_2HPO_4, 3 g of KH_2PO_4 and 4 g of NaCl per liter (pH 7.0) and used for preparing phage suspensions in X-ray irradiation experiment.

(ii) *1xCT⁻ and 1xCT⁺ media*[28]

$1xCT^-$ medium contained 0.2% glucose and 0.1% casein hydrolysate in 1x salt solution, which was prepared by 50-fold dilution of 50x salt solution. 50x salt solution contained 10 g of $MgSO_4 \cdot 7H_2O$, 100 g of citric acid$\cdot H_2O$, 500 g of K_2HPO_4 and 175 g of $NaNH_4HPO_4 \cdot 4H_2O$ in 670 ml of H_2O. $1xCT^-$ medium containing 5 μg/ml of thymine was named as $1xCT^+$ medium. These media were used for growing *E. coli* B Tr201 and also used for radioactive thymine-labeling experiment.

(iii) *X-ray Irradiation*

The T4 phage particles were suspended to a titer of 1×10^{10} PFU/ml (plaque forming unit/ml) in a minimal salt medium. Phage suspensions were maintained at a depth of less than 3.0 mm in petri dishes and irradiated with X-rays at a dose rate of 1,238 rad/min at room temperature. After exposure to various irradiation dosages, the phage solutions were withdrawn to determine survival of phage particles and phage DNA synthesis.

(iv) *Measurement of The Kinetics of Phage DNA Synthesis*

An overnight culture of *E. coli* B Tr201 was diluted 50-fold with $1xCT^+$ medium and grown to concentration of 5×10^8 cells/ml. These cells were centrifuged and the cell pellet was resuspended to a concentration of 1×10^9 cells/ml in $1xCT^-$ medium containing 40 μg/ml of D, L-tryptophan. An equal volume of phage suspension was added at a multiplicity of 5 phage particles per bacterium. Three minutes after infection, ^{3}H-thymine (10 μCi/ml, 0.42 Ci/m mole) was added to the infected culture. The amount of the isotope incorporated into DNA was determined by a slight modification of the procedure descirbed by Bollum[4]. Samples of 0.1 ml were taken at various times after infection and pipetted onto round Whatman filter paper (No. 3, 25 mm in diameter). The disks were washed for 10 min in three successive 200-

ml volumes of 5% trichloroacetic acid (TCA), followed by a wash in 200 ml of 95% ethanol. The filter papers were then dried and placed in vials containing 5 ml of toluene phosphor (4 g of 2, 5-diphenyloxazole and 50 mg of 1, 4-bis-2(5-phenyl-oxazolyl) benzene per liter of toluene) and counted in liquid scintillation counter.

RESULTS AND DISCUSSION

A. Effect of X-ray irradiation on extracellular phage particles

After various dosages of X-irradiation, the survival of phage particles was determined by plating on *E. coli* BB or *E. coli* CR63. The survival fraction as a function of radiation dosage was shown in Fig. 1. At a dosage of 13.5 Krads, 40% of the T4D phage particles were killed (or the survival fraction=0.60) when plated on *E. coli* BB. However, only 7.7 Krads were required to kill 40% of T4*am*C5 phage particles as they were plated on the same strain of *E. coli*. This result indicates that T4*am*C5 at the cellular level exhibits about two times higher susceptibility to inactivation caused by X-ray irradiation than T4D; this sensitivity is not as high as for MMS-treatment, which was three times more lethal T4*am*C5 than in T4D[35]. When the X-irradiated T4D and T4*am*C5 phage particles were plated on *E. coli* CR63, a strain carrying a suppressor gene for viral nonsense mutation, no difference was observed in the surviving fraction after irradiation between wildtype and mutant (Fig. 1). These results taken together suggest that T4 gene 59 function is required for repair of X-ray damages and the defect in repair of X-ray damages in T4*am*C5 is apparently suppressed by the amber suppressor gene in *E. coli* CR63.

B. Effect of X-ray irradiation on DNA replication

The effect of X-ray irradiation on the kinetics of T4 DNA synthesis was studies. T4*am*C5 or T4D phage particles were irradiated with different doses of X-rays, then infected *E. coli* B Tr201 at an MOI (multiplicity of infection) of 5. The incorporation of ^{3}H-thymine into the TCA-insoluble fraction was measured by liquid scintillation counter. As shown in Fig. 2, the total amount of DNA synthesis in T4*am*C5-infected cells after 35 minutes of infection was reduced to 83% of the controls (without X-ray irradiation) at 16 krads of X-ray dosage. In the case of X-ray irradiated T4D, DNA synthesis was reduced to 90% of the control

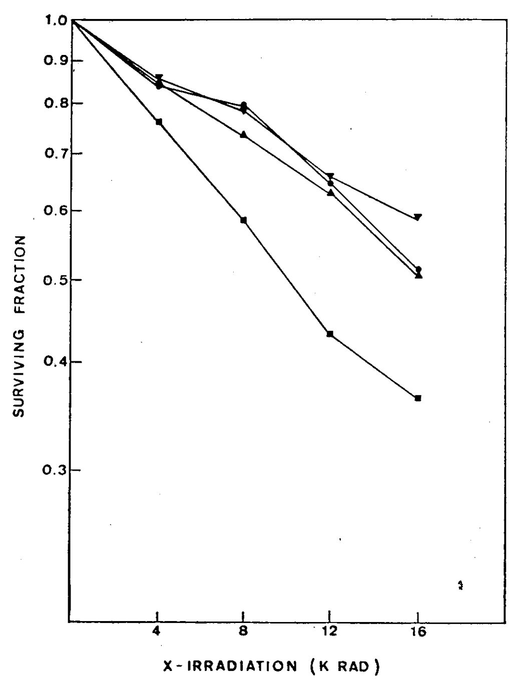

Fig. 1. Sensitivity of T4*am*C5 or T4D phage particles to X-ray irradiation. Phages T4*am*C5 or T4D were suspended in minimal salt medium at a concentration of 1×10^{10} PFU/ml and irradiated with X-rays at a dose rate of 1,238 rad/min at room temperature. After exposure to various X-ray dosages, the X-irradiated phages were plated on *E. coli* BB or *E. coli* CR63. Calculation of surviving fraction was:

Surviving fraction

$$=\frac{\text{PFU }(+\text{X-ray})\text{ in }E.\ coli}{\text{PFU }(-\text{X-ray})\text{ in }E.\ coli}$$

Symbols: •-•, T4D (*E. coli* BB);
▲-▲, T4D (*E. coli* CR63);
■-■, T4*am*C5 (*E. coli* BB);
▼-▼. T4*am* (*E. coli* CR63).

under the same condition (Fig. 3). This low sensitivity of DNA replication to X-ray irradiation can be explained as follows. The majority of the DNA strand breaks (94%) after X-ray irradiation are immediately repaired intracellular by polynucleotide ligase (Type I repair system), and DNA polymerase I with polynucleotide ligase (Type II repair system)[27]. The remaining strand breaks are repaired by the post-replication repair (Type III repair system), in which gene 59 is involved. Since most of the X-ray-induced strand breaks can be repaired without the participation of gene 59 function, the DNA replication of this mutant was not significantly affected by X-ray irradiation (Fig. 2.) However, the viability of mutant phage particles was sensitive to X-ray irradiation (Fig. 1). It is possible that the repair of certain critical strand breaks requires gene 59 function, and this determining the viability of mutant phage

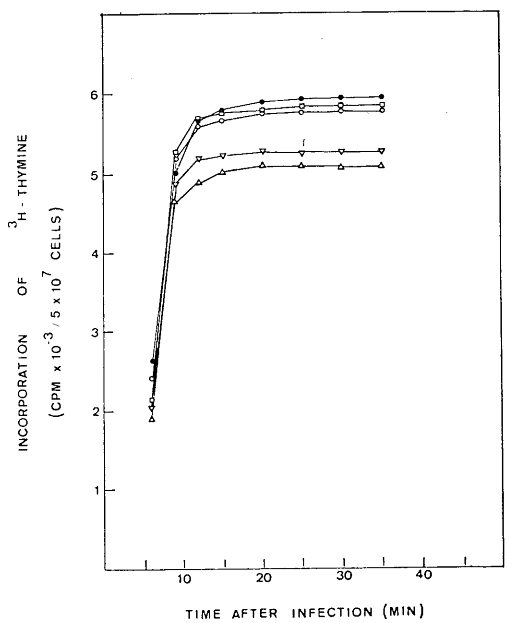

Fig. 2. Effect of X-ray irradiation on ^{3}H-thymine incorporation in T4*am*C5-infected cells. Phage particles of T4*am*C5 were suspended in minimal salt medium at a concentration of 1×10^{10} PFU/ml and irradiated with various X-ray dosages at a dose rate of 1,238 rad/min at room temperature. A culture of *E. coli* B Tr201 was infected with X-irradiated T4*am*C5 (M. O. I.=5) with aeration at 37°. Three min later, ^{3}H-thymine (10 μ Ci/ml, 0.42 Ci/m mole) was added. Samples of 0.1 ml were withdrawn at various intervals for counting radioactivity as described in Materials and Methods.

Symbols: ○-○, without X-irradiation;
□-□, 4 Krad;
•-•, 8 Krad;
▽-▽, 12 Krad;
△-△, 16 Krad.

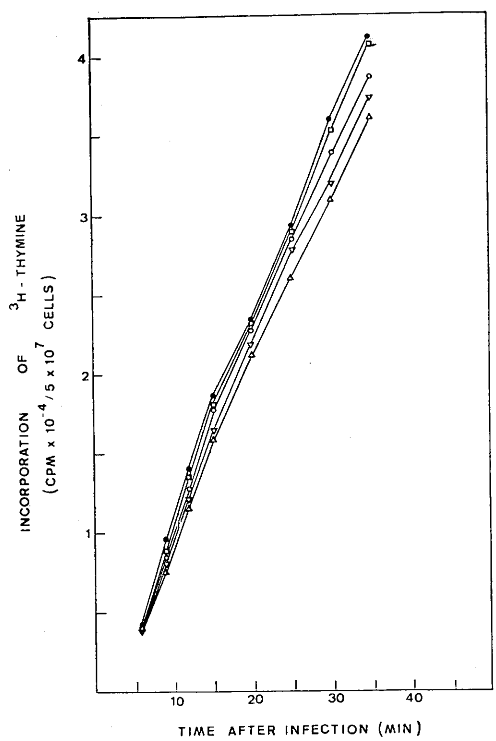

Fig. 3. Effect of X-ray irradiation on ^{3}H-thymine incorporation in T4D-infected cells. The experimental conditions were identical to those described in Fig. 2.

Symbols: ○-○, without X-irradiation;
□-□, 4 Krad;
•-•, 8 Krad;
▽-▽, 12 Krad;
△-△, 16 Krad.

particles after X-ray irradiation.

It is known that phage T4 controls the excision repair and post-replication repair processes for repairing its UV-damaged DNA[25]. T4 genes such as *v* gene, gene 30, gene 1 (hydroxymethyl-dCMP kinase), gene 42 (dCMP hydroxymethylase), gene 45 (unknown biochemical function) and gene 56 (dCTP pyrophosphatase) are involved in excision repair, which is initiated by the *v* gene product. The T4 genes such as genes *x*, *y*, 43 (DNA polymerase), 32 (DNA unwinding protein), 30, 1, 41, 42, 44 and 56 are reported to be involved in post-replication repair process[8,25]. Mutants of gene *x* are sensitive to UV, X-ray and alkylating agents[16]. Gene 30 is required for the repair of UV-damaged[2] and alkylated DNA molecules[7]. The requirement of gene 43 for the repair of UV-irradiated DNA molecules[22] and alkylated DNA molecules[3] is still uncertain. The studies of the function of gene 59 indicate that it is involved in the post-replication repair process, based on the following observations: (i) Gene 59 mutant is sensitive to UV-irradiation, MMS-treatment[35] and X-ray irradiation (Fig. 1). The sensitivities of this mutant to these three mutagens are similar to those of gene *x* mutants. (ii) The results of alkaline sucrose gradient sedimentation of intracellular mutant DNA, immediately after exposure to UV-irradiation, showed the DNA molecules were fragmented to the same size as those of wild-type[35]. This indicates that incision of the UV-irradiated DNA, presumably catalyzed by the *v* gene endonuclease, is normal in gene 59 mutant-infected cells. (iii) The MMS-induced DNA strand breaks, which are presumably repaired by the recombinational process, were not repaired in gene 59 mutant-infected cells[35]. (iv) Gene 59 function is involved in normal phage DNA replication[34].

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噬菌體 T4 之基因功能對 X-射線照射之生存需求性

吳金洌　　葉雲旗

利用噬菌體 T4 受到 X-射線照射後，來探討其基因功能在 DNA 修補工作中所佔的角色。以 *E. coli* BB 做爲寄主細菌來觀察 X-射線照射對噬菌體 T4 的致死情形；噬菌體野生型 T4D 經過 13.5 krads 之劑量照射後，有40%被殺死；而噬菌體變異株 T4 *am* C5 則僅需 7.7 krads 之照射劑量，即可使40%之變異株被殺死。換言之，噬菌體 T4 之 59 號基因變異株在不許可性寄主中，因 X-射線照射所引起的非活性化敏感性是兩倍於野生型之噬菌體。這種變異株之 X-射線敏感性可在許可性寄主細菌 *E. coli* CR63 中完全被恢復過來。不論是噬菌體 T4 之野生型或變異株，其 DNA 生合成之速率並不因 X-射線之照射而發生顯著影響。在本文中亦探討噬菌體 T4 之 59 號基因功能與 DNA 修補機制的關係。