TRANSFER OF ULTRAVIOLET LIGHT INDUCED THYMINE DIMER FROM PARENTAL TO PROGENY DNA IN BACTERIOPHAGE T1 AND T4.*

Walter Sauerbier and Monica Hirsch-Kauffmann**
Department of Biophysics, University of Colorado Medical Center
Denver, Colorado

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In bacteriophages with double-stranded DNA, ultraviolet irradiation of 254 mμ forms thymine dimers ($\hat{\pi}$) (Beukers et al., 1960; Wacker et al., 1960) at approximately five times the rate of lethal hits (Wulff, 1963; Sauerbier, 1964). With the single-stranded DNA virus, ϕX 174, this ratio is 0.34 (David, 1963). In these experiments the assay for lethal hits is done with bacteriophage and with host bacteria in which no repair mechanisms are known to be active. Thus, the question arises as to why the majority of $\hat{\pi}$ is nonlethal in the double-stranded DNA bacteriophage. Possibly there exists an unknown cellular mechanism, different from host cell reactivation (HCR) (Sauerbier, 1961) and from v-gene reactivation (Streisinger, 1956; Harm, 1958) which removes $\hat{\pi}$ from DNA thereby raising the $\hat{\pi}$/lethal hit ratio to five.

The second obvious question whether $\hat{\pi}$ is ever lethal has not yet been satisfactorily answered. Thus far it is only known that not more than one out of three lethal hits in bacteriophage can be due to $\hat{\pi}$ (Sauerbier and Haug, 1964).

With respect to host cell reactivation the present concept calls for an excision of photoproducts and a resynthesis of excised regions. This is based upon the finding of $\hat{\pi}$ excision from the DNA of UV-irradiated bacteria by bacterial enzymes (Setlow and Carrier, 1964; Boyce and

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Howard-Flanders, 1964) and an increase in the number of DNA replication sites in the DNA of UV-irradiated bacteria (Pettijohn and Hanawalt, 1965). Since in these experiments the majority of cells are inactivated and not repaired the question arises whether the observations on excision and resynthesis are representative for the dead cells only.

In the case of T4 specific repairs, such as v-gene reactivation and possibly x-gene repair (Harm, 1963), the evidence for the removal of $\hat{T}$ by repair mechanisms is conflicting. Sauerbier (1964) reported no loss of $\hat{T}$ from UV-irradiated T4 DNA under conditions of repair. Setlow (1966), using slightly different methods, claimed removal of $\hat{T}$ from T4 DNA under repair conditions.

Answers to the preceding questions and to the controversy about T4 might be obtained by studying the transfer of $\hat{T}$ from parental DNA to progeny DNA of bacteriophage under conditions allowing, or not allowing, repair. As to the outcome of such experiments three predictions can be made: (1) If the majority of $\hat{T}$ were nonlethal because of removal by a hitherto unknown cellular mechanism no, or only a small fraction of, $\hat{T}$ could be transferred from parental to daughter DNA. (2) If $\hat{T}$ were never lethal, then $\hat{T}$ would be as efficiently transferred to daughter DNA as is thymine. (3) If $\hat{T}$ were indiscriminately excised from parental DNA by HCR or T4 specific repairs, no $\hat{T}$ should be transferred to daughter phage from parents which survived due to repair.

The results reported here disagree with the predictions 1 and 3. They show that the majority, and possibly all, of the $\hat{T}$ is transferred from parental to daughter DNA in T1 and T4.

Experiments: In labelling bacteriophage T1 and T4 with methyl-^{3}H-thymine and in assaying for $\hat{T}$ we followed in essence the procedures of Wulff (1963). There, $\hat{T}$ is characterized by its R_f in 86% butanol and subsequent reversion by 254 mμ irradiation into thymine. The purified ^{3}H-T1 and ^{3}H-T4 phage were UV-irradiated. Then part of them were analysed for $\hat{T}$ and the remaining

phage were adsorbed to HCR-deficient bacteria, E. coli B_{s-1}(Hill, 1960), or HCR-permitting B-3, at multiplicities of infection of 0.2-0.6. Progeny phage was harvested and treated with DNase. In the case of T4, multiplicities of infection were 0.1 and 10, and lysis was performed in the presence of high concentrations of T4 antiserum to avoid readsorption of phage. Lysates were purified from bacterial debris and low molecular weight material by several high and low speed sedimentations and a final CsCl density gradient centrifugation. Before acid hydrolysis CsCl was removed by dialysis.

The results with both T1 and T4 are given in Table I. In general it is seen that $\hat{\pi}$ is about as efficiently transferred from UV-irradiated parental phage to daughter phage as is thymine. This means that neither HCR of T1 nor T4 specific repairs and multiplicity reactivation (Luria and Dulbecco, 1949) of T4 efficiently prevent $\hat{\pi}$ from being transferred to progeny DNA. The experiments show large fluctuations in the amount of $\hat{\pi}$ transferred to progeny phage, (See Table I). It is difficult to avoid these fluctuations since the latent period of UV-irradiated T1 is dose dependent and since phage released by early lysis readsorb and are lost in the process of removing bacteria and bacterial debris from the lysate by centrifugation. However, phage having larger numbers of photoproducts have a longer latent period and a lesser chance of readsorption. A loss of early released phage will increase the average number of $\hat{\pi}$ observed in the progeny phage. On the other hand, an incomplete lysis of phage with photoproducts above the average number will reduce the $\hat{\pi}$ transferred to the recovered progeny phage. Both effects cannot be completely avoided in our experiments; e.g., in one experiment (Table I) more $\hat{\pi}$ are transferred per T1 equivalent of thymine to the recovered progeny phage than were contained in the parental phage on the average.

With T4 it appears that with conditions of multiple infection; i.e., when multiplicity reactivation does occur, the transfer of from parental to daughter DNA is less efficient than the thymine trans-

Table I. Analysis for thymine dimers in UV-irradiated bacteriophage T1 and T4: (A) In parental phage, (B) In progeny phage made of an aliquot of the same UV-irradiated parents as in (A).

	Phage	Host	MOI (1)	CPM T (2)	CPM T (3) Last but One Chromatography	CPM $\hat{\pi}\rightarrow T$ (4)	$\frac{\hat{\pi}\rightarrow T}{T}$ (5)	^{14}C-$\hat{\pi}$ (6) Input	^{14}C-$\hat{\pi}\rightarrow T$ (7) Recovered	Recovery Factor (8)	$\hat{\pi}/\phi$ Equivalent DNA (9)
A Parental	T1	B_{s-1}	--	3.8×10^5	--	376	9.9×10^{-4}	--	--	75%	16.80
B Progeny	T1	B_{s-1}	0.6	3.5×10^4	--	36	1.03×10^{-3}	--	--	75%	17.45
A Parental	T1	B-3	--	2.56×10^5	3.5	429	1.67×10^{-3}	713	693	97%	21.95
B Progeny	T1	B-3	0.3	1.84×10^5	12.9	204	1.11×10^{-3}	740	712	96%	14.69
A Parental	T1	B-3	--	6.94×10^4	26.0	126	1.82×10^{-3}	367	265	72%	32.10
B Progeny	T1	B-3	0.25	3.26×10^4	19.5	87	2.67×10^{-3}	182	136	75%	45.20
A Parental	T1	B-3	--	3.12×10^5	42.0	351	1.125×10^{-3}	737	479	65%	21.90
B Progeny	T1	B-3	0.3	1.97×10^5	34.3	131	6.65×10^{-4}	367	270	74%	11.40
A Parental	T4	B	--	9.7×10^4	47.8	29	2.95×10^{-4}	140	113	81%	24.10
B Progeny	T4	B	10	2.22×10^5	48.2	39.2	1.765×10^{-4}	490	375	77%	15.00
A Parental	T4	B	--	2.68×10^5	33.1	79.6	2.97×10^{-4}	359	208	58%	33.30
B Progeny	T4	B	0.15	5.69×10^4	30.8	16.6	2.92×10^{-4}	126	69	55%	34.50

(1) Multiplicity of infection = input ratio bacteriophage/bacteria.
(2) Counts per minute (CPM) in thymine of DNA hydrolysate.
(3) CPM in thymine position of chromatogram in the last butanol - H_2O chromatography before monomerizing $\hat{\pi}$ to T. In an 86% butanol chromatography run less than 10% of the total thymine remains in the position of the thymine dimer. Thus, not more than 10% of the CPM in (3) could have been transferred to the CPM in (4). The remainder of the counts has to come from the monomerization of $\hat{\pi}$.
(4) CPM of $\hat{\pi}$ which was monomerized to T by UV.
(5) Ratio of CPM of $\hat{\pi}$ monomerized to T (Column 4) vs. CPM in T (Column 2).
(6) Control ^{14}C-$\hat{\pi}$ added to paper strip.
(7) Control ^{14}C-$\hat{\pi}$ recovered after chromatography and monomerization.
(8) ^{14}C-$\hat{\pi}$ recovery factor = (7)/(6) X 100.
(9) Number of $\hat{\pi}$ per phage equivalent DNA in parental phage, and per phage equivalent parental thymine transferred to progeny DNA. Figures are corrected for $\hat{\pi}$ loss in chromatography and monomerization.

fer. With single infection both $\hat{\pi}$ and thymine are equally as well transferred (Table I).

Conclusions: The merit of the preceding experiments lies in their ability to physically separate dead and surviving organisms, thus allowing the chemical analysis of the surviving fraction only. When repair is allowed, as in the case of HCR, multiplicity reactivation, and v-gene repair, an analysis of the fraction surviving due to repair is possible. Thus we avoid pitfalls which may arise from making observations on the total population of UV-irradiated organisms.

The conclusions to be drawn from our observations are: (1) The ratio $\hat{\pi}$/lethal hit of 5 is not caused by a removal of $\hat{\pi}$ from DNA by a bacterial or a T^4 specific mechanism since the majority of $\hat{\pi}$ is handed down to daughter DNA. (2) As a rule, $\hat{\pi}$ does not interfere with DNA replication and presumably not with transcription since in single infection experiments $\hat{\pi}$ is transferred to daughter DNA and since the average burst size of the progeny lysates was around ten. (3) HCR and v-gene repair do not indiscriminately remove $\hat{\pi}$ from DNA. If they do remove $\hat{\pi}$ then they do it only in a highly selective fashion, by-passing the nonlethal $\hat{\pi}$.

Because of quantitative uncertainties, our experiments do not exclude the following possibilities: (1) That 1, or less, out of 5 $\hat{\pi}$ is lethal; (2) that HCR or v-gene repair selectively excises only lethal $\hat{\pi}$ without affecting the majority of $\hat{\pi}$ which are nonlethal per se.

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