ROLE OF HOST-CELL REACTIVATION IN THE ENHANCEMENT OF COMPLETE TRANSDUCTION AFTER UV IRRADIATION OF P22 PHAGE

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Benzinger and Hartman (1962) reported that UV irradiation of transducing phage rapidly and exponentially inactivated abortive transductants (transduced segments inherited unilinearly), while the number of complete transductants (transduced segments integrated into the recipient chromosome) increases as previously shown by Garen and Zinder (1955). The present paper will show that the enhancement of complete transduction by UV is dependent upon host-cell reactivation since a mutant lacking host-cell-reactivation ability does not show enhancement when it is used as a recipient for transduction. The repair of UV inactivation of abortive transductants was less than that of complete transductants. Possible mechanisms for these phenomena will be discussed.

Materials and Methods

A UV-sensitive mutant was isolated from Salmonella typhimurium his-712 (a 7-gene multisite mutant, Ames and Hartman, 1961), by N-methyl-N'-nitro-N-nitrosoguanidine treatment, and 4 Nitroquinoline-1-Oxide (4NQO) screening. The latter compound, a strong carcinogen (Nakahara et al., 1957) is known to give effects qualitatively similar to UV (Takebe, 1966, Kondo and Kato, 1966). Therefore, UV-sensitive mutants were expected not to grow on the replica plates at the appropriate concentration (5×10^{-5}M) of 4NQO. Colonies failing to grow on the 4NQO plates were selected and tested for UV sensitivity. The mutant used in this study (his-712 hcr-1) was found to have a decreased (approximately 6.5 times less than the parent strain) ability to propagate UV-inactivated P22 and therefore is de-

ficient in the capacity for host-cell reactivation (HCR) (Fig. 1). Little change was noted in the number of recombinants produced when it was crosses with Hfr strains (kindly supplied by Dr. K.E. Sanderson), indicating that this strain continues to be Rec^+. The detailed characterization of the mutant will be published elsewhere (Yamamoto and Takebe, in preparation).

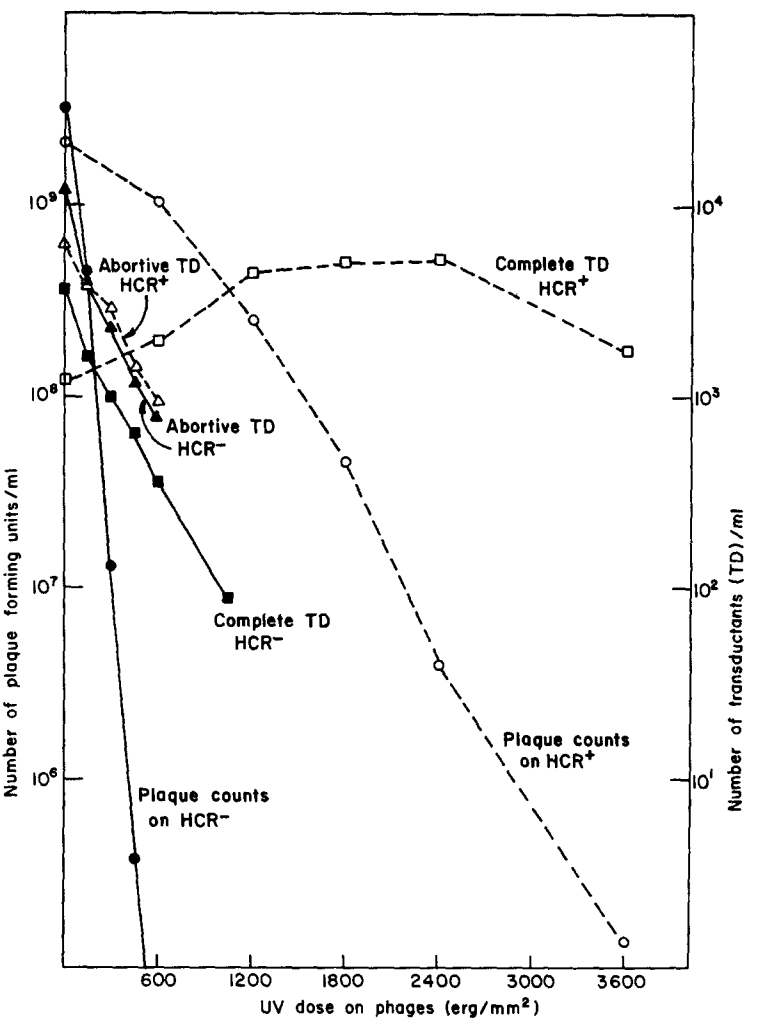

Fig. 1. Effect of UV on plaque-forming and transducing activities of P22 phage. Solid lines (closed symbols) represent experiments with Hcr^- recipient, and broken lines (open symbols) represent experiments with Hcr^+ recipient. The concentrations of bacterial suspension before phage infection were 1.3 x 10^9/ml for Hcr^+ and 3.0 x 10^9/ml for Hcr^- (same in Fig. 2).

The Hcr^- mutant (his-712 hcr-1) and the parent strain (his-712 hcr^+) were used as recipients in transduction, and P22(H1) phage grown on wild-type LT2 strain was used as the transducing phage. Other materials and methods concerning transduction were generally the same as described previously (Takebe and Hartman, 1962). The number of abortive transductants was scored over the entire surface of each plate, using a dissecting (x20) microscope.

P22 phage suspended in T2 buffer was irradiated by two GE germicidal lamps at a dose rate of 10.0 erg/mm^2/sec. The irradiated phage was immediately mixed with recipient cells in T2 buffer and kept at 37°C for 6 minutes for adsorption to occur. 0.1 ml of the mixture was spread on minimal agar supplemented by an amino acid pool lacking histidine. The multiplicity of infection was in the range of 0.8 to 1.6.

The source for photoreactivation (PR) was two 15 watt, black-light lamps (GE-F15T8BL) placed approximately 30 cm from the plates. After samples of infected bacteria had been spread on the surface of the agar, the plates were exposed to PR light for 30 minutes. For the PR of plaque-forming units, which cannot be done on the plate in soft agar overlay because of strong absorption by nutrient agar, indicator cells were infected by phages in T2 buffer and exposed to 4047 $\pm$ 50 Å light from a Bausch and Lomb grating monochromator for 10 minutes at 37°C. Both photoreactivating exposures were high enough to give the maximum PR.

Results

The effect of UV irradiation on transducing phage was compared using Hcr^+ and Hcr^- recipients (Fig. 1). The results with Hcr^+ showed essentially the same kinetics as described by Benzinger and Hartman (1962), except that the slope of the inactivation curve for abortive transductants relative to that for plaque-forming units is steeper than those reported by Benzinger and Hartman (1962). This might be due to the slightly higher multiplicity used, since some of the recipients may be killed by the infected phage (Benzinger, 1961). In the Hcr^- recipient, in addition, there was no enhancement of complete transduction by low doses of UV, and in fact the number of complete transductions was reduced, the curve being similar to that for abortive transduction. The difference between the curves

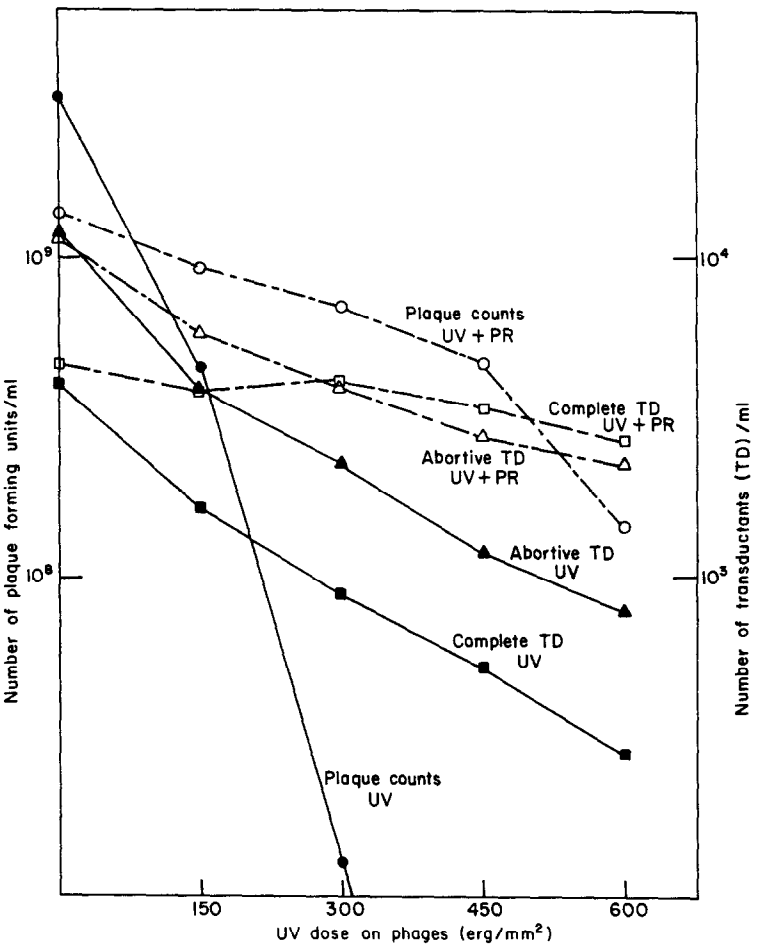

Fig. 2. Photoreactivation of UV effect on plaque-forming and transducing activities in Hcr^- recipient cells. Solid lines (closed symbols) represent control experiments with photoreactivation.

for abortive transduction in Hcr^+ and Hcr^- was small although it was reproducible. These curves suggest that complete transduction is susceptible to host-cell reactivation, whereas abortive transduction is much less susceptible.

The slope of the inactivation curve for plaque-forming units relative to that of abortive transductants was approximately 1 in the Hcr^+ recipient and approximately 0.2 (also to the slope of complete transductants) in the Hcr^- recipient.

Photoreactivation (PR) of the Hcr^- cell-phage complex after plating, (Fig. 2) indicated that cells giving rise to complete transductants are susceptible to photo-

reactivation. Abortive transduction showed a small effect of PR. Other experiments indicate very little or no PR takes place in Hcr^+ cells in the UV dose range of Figure 2. A small but positive PR (dose reduction of approximately 35%) was reported to occur in phages irradiated by high doses of UV (Benzinger, 1961).

Discussion

The enhancement of complete transduction by UV is dependent upon host-cell reactivation, since the Hcr^- strain does not show the enhancement, a behavior in contrast to that exhibited by Hcr^+ strains. Curtiss (1968) has proposed a model for UV-enhanced recombination, in which disruption of hydrogen bonding between complementary DNA strands by UV, followed by the breakage and pairing of strands participating in recombination, was assumed to be the initial step of the enhancement. HCR enzymes were not invoked in this model to explain the enhancement. If we use a model of recombination proposed by Howard-Flanders and Boyce (1966), HCR enzymes could take part in producing breaks of DNA strands. When UV-irradiated transducing segments are injected into Hcr^+ recipient cells, excision of pyrimidine dimers may take place and this would leave many single-strand breaks in the DNA of the segments. During the repair process after excision, the broken ends might unite with the DNA of the host chromosome, as proposed in the model, which may have breaks induced either spontaneously or by the pairing between the transducing segment and the host chromosome (although these authors did not indicate how these breaks might be made). Hcr^- recipients are assumed to lack the excision enzyme(s) (Howard-Flanders and Boyce, 1966), and therefore in the Hcr^- host, there would be no enhancement of recombination. It is reasonable to assume that HCR enzyme(s) play no role in normal (unirradiated) recombination since both Hcr^+ and Hcr^- strains used in this work show approximately the same frequency of transduction by unirradiated phage (Fig. 1) as well as in recombination with an Hfr strain. However, after UV-irradiation, HCR enzyme(s) play an important part in the events resulting in recombination.

Photoreactivation experiments support the above hypothesis. PR does not produce breaks since PR splits pyrimidine dimers into monomers without excision

(Cook, 1967). PR of UV-irradiated transducing phage increases the number of complete transductions to that level produced by unirradiated phages (Fig. 2). Therefore the increase does not represent an enhancement, but only a repair of the transducing segments.

Why do abortive transductants show only small recovery by PR and HCR? There are at least two possible explanations: abortive transducing segments might have a DNA configuration less susceptible to repair systems, or the repair process might require DNA replication, which does not occur for the abortive segments (Ozeki, 1956). Ozeki (1966) suggested that the DNA leading to abortive transductants might be in a circular form in the recipient cells since it is very stable. If abortive transducing segments are composed of double stranded circular DNA, it might be possible that they can be repaired only when they are in the "open" configuration and are less susceptible to repair if they have a closed structure which might lead to an unusual tertiary structure, such as supercoiling, or if they are bound with protein. Alternatively, abortive transducing segments might be single-stranded, since single-stranded phage is only slightly photoreactivable (Setlow, 1961) and HCR is not known to occur in single-stranded phages (Rörsch et al., 1963). This assumption is, however, difficult to reconcile with the observations that particles resulting in abortive transduction have the same mean buoyant density (Sheppard, 1962) and same sensitivity to P^{32} decay (Hartman and Kozinski, 1962) as infective particles. It is known that "repair DNA synthesis" is required in dark repair (Pettijohn and Hanawalt, 1964), and it is possible that DNA replication is required for complete in vivo photoreactivation. However, the unique characteristics of an abortive segment which are expressed only functionally, and which do not undergo replication, might be responsible for its low susceptibility to repair.

The slope of the inactivation curve for plaque-forming units relative to those for both complete and abortive transductants, in Hcr^- recipients (1/5), may represent the relative size of the phage genome and that of 7 genes in the his region. This value is in good agreement with the value of 0.20 to 0.25 proposed by Benzinger and Hartman (1962).

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