EVIDENCE THAT THE DARK REPAIR SYSTEM OF COLIPHAGE T4 IS CONTROLLED BY THE VIRAL GENOME.

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The U.V. sensitivity of T2 coliphages is greater than that of T4 coliphages (1,2). The level of this sensitivity is controlled by the v gene and behaves as a unit character (3,4,5). The v gene apparently controls reactions within the coli-coliphage system which repair the U.V. damage in the DNA of the coliphage. This repair does not require the presence of visible light, although the damage reparable by the v gene can also, for practically all inactivating U.V. wavelengths, be photoreactivated (6).

A major photobiochemical product of U.V. irradiation appears to be the formation of thymine dimers in DNA. This damage could theoretically be repaired either by degradation of the thymine dimer into two thymine residues in the intact DNA chain or by replication of the DNA after excision of the thymine dimer. The dimer excision repair process has been demonstrated for dark-reactivable strains of E. coli (7,8). Dimer excision has not been demonstrated for sensitive strains.

It would seem reasonable to assume tentatively that the v gene in T4 may be responsible for the production of a repair enzyme, e.g., an endonuclease capable of thymine dimer excision from T4 DNA. The question arises

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whether the v gene in T4 is merely parasitizing (inducing) the already existing excision repair mechanism of the bacterium or whether it is responsible for the de novo synthesis of a T4 dimer excision enzyme.

To distinguish between these two possibilities, post-U.V. irradiation survival curves were determined for T4 and T2 grown on a U.V.-resistant and on a U.V.-sensitive coli strain.

Materials and Methods

$T2^{2}$ and $T4^{2}$ bacteriophage were suspended in T broth (containing per liter of water: 3 g. NaCL and 10 g. tryptone). The suspensions were irradiated at a distance of 42 cm. from a G.E. G8T5 U.V. lamp (emitting chiefly at a wavelength of 2537Å) for variable amounts of time. E. coli B/r^{3} and E. coli $B_s{}^{4}$ were grown on T broth. Coli growing in log-phase were used to inoculate the top agar (containing per liter of water: 5 g. NaCL, 10 g. tryptone, and 7 g. Bacto agar) at 45° C. The phage suspensions, diluted in T broth, were quickly added to the fluid top agar and thoroughly mixed. The admixture was then poured onto the bottom Tryptone agar (containing per liter of water: 5 g. NaCL, 10 g. Bactotryptone, and 10 g. Bacto agar). After solidification of the top agar, the plates were incubated for 14 to 18 hours and plaque counts were determined (9).

Results and Discussion

If the phage is responsible for the synthesis of its own excision enzyme, then there should be no difference in the survival curves of T4 grown on the two bacterial strains. If, however, the phage utilizes the pre-existing

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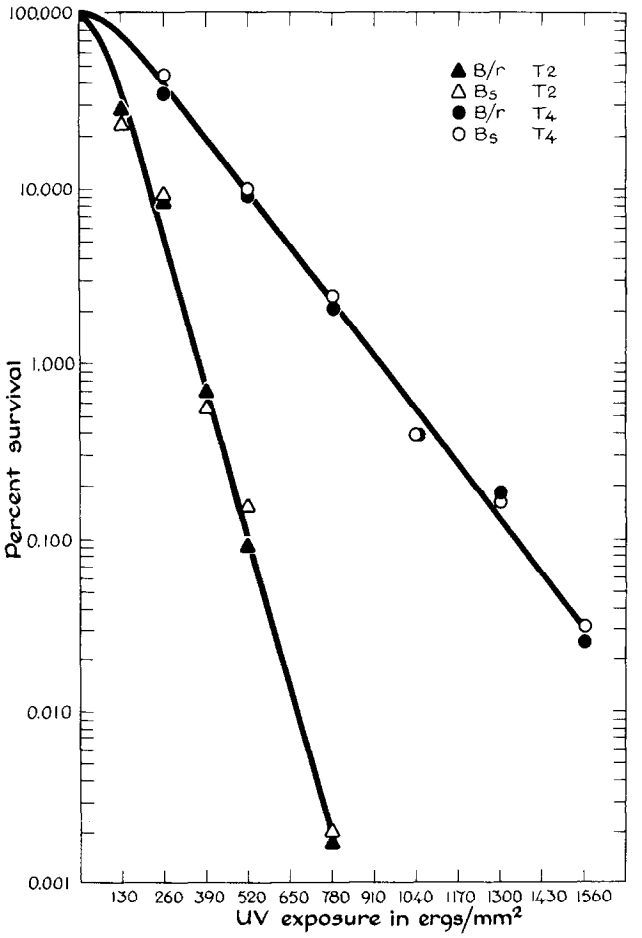
excision enzyme mechanism, there should be decreased T4 survival in the coli-coliphage system in which the host (E. coli B_s) lacks the excision enzyme.

The results (see Fig. 1) show that there is no detectable difference in the survival curves of T4 on E. coli B/r (which contains its own dimer excision enzyme) and on E. coli B_s (which lacks the dimer excision enzyme). The survival curves of T2 grown on each of the bacterial strains are presented as controls, since the T2 contains no v gene. Comparison of the T2 and T4 curves demonstrates that there is decreased survival in the absence of the v gene.



This method does not detect whether any of the other enzymes involved in repair of bacterial DNA are also used in the phage repair system.

It is concluded that the v locus of T4 is responsible for the de novo production of a component of a reactivation system for U.V. induced damage in the phage DNA.

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Note: After this work had been completed, it was noted that similar results were presented at the Biophysical Society Meeting (February, 1966) in abstract #TC2 by Setlow and Carrier.

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