JOURNAL OF BACTERIOLOGY, Nov. 1969, p. 1118-1120
Vol. 100, No. 2

Dominance of Ultraviolet Radiation Resistance in Partial Diploids of *Escherichia coli* K-12

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Received for publication 15 September 1969

Although an F'13 *cap*R$^+$/*cap*R9 strain is nonmucoid and an F'13 *cap*R9/*cap*R$^+$ strain is mucoid, both strains are ultraviolet (UV)-resistant. In contrast, haploid *cap*R9 strains are UV-sensitive. Therefore, UV resistance is dominant to UV sensitivity, regardless of whether the *cap*R$^+$ allele is on the chromosome or on the F'13 episome.

Studies on the genetic control of radiation sensitivity in *Escherichia coli* have resulted in the mapping of a number of loci that affect the response to radiation damage (7). The locus that controls ultraviolet (UV) and X-ray sensitivity, designated *lon* by Howard-Flanders, Simson, and Theriot is also involved in filament formation (3, 8), and strains that are mutant at this locus have a mucoid clone morphology (8). One of the present authors showed that a gene mutation designated R_1 (10) and redesignated *cap*R (11), linked to a proline locus (*proC*) controls the synthesis of capsular polysaccharide, and strains that are mutant at this locus have a mucoid clone morphology. It is now well established that the phenotype of *cap*R and *lon* mutants are identical, i.e., mucoid and UV-sensitive (2-4, 8, 10, 12) and arising from the same mutational event (4, 12). The *lon* and *cap*R strains reactivate UV-treated bacteriophage T1 or T7 to the same extent as the wild types, *lon*$^+$ or *cap*R$^+$ (8; Uretz, *unpublished data*) and thus are not deficient in repair of UV-treated deoxyribonucleic acid (DNA). The mechanism by which *cap*R (*lon*) controls radiation sensitivity is unknown at the present time.

Previously it was shown that partial diploid strains of the type F'13 *cap*R$^+$/*cap*R9 were nonmucoid, whereas the partial diploid strain F'13 *cap*R9/*cap*R$^+$ was mucoid, indicating that the episomal (F'13) allele was dominant with respect to mucoidness (12). It therefore appeared worthwhile to determine the dominance situation with respect to UV radiation. In this note we show that UV resistance is dominant to UV sensitivity in both types of partial diploid containing *cap*R$^+$ and *cap*R9.

Methods and strains used have been described (10-13). Gene symbols are those of Taylor and Trotter (14), unless indicated otherwise. Yeast Extract-Nutrient Broth, used when growing strains to be UV irradiated, was chosen because strains with different *cap*R alleles produced the least amount of filaments when grown in this liquid medium at 37 C. The UV exposure was 450 ergs per cm^2 per sec, as determined by a YSI-Kettering, model 65 Radiometer. After irradiation, all procedures were carried out in semidark conditions (yellow or no light), including incubation of plated cells (37 C).

The F'13 episome contains *lac*$^+$ *proC*$^+$ and *pur*E in addition to the *cap*R allele indicated below. Partial diploids containing the *cap*R9 and *cap*R$^+$ alleles were constructed by performing the following matings. (All selective media contained 200 μg of streptomycin per ml).

MC140 (F'13 *cap*R9/*cap*R9 *leu*-1, *trp*-1) × MC 138 (*cap*R$^+$ *lac*-2, *pro*C1, *pur*E1)

↓ selection on minimal medium M-9 (1) with lactose

MC142 (F'13 *cap*R9/*cap*R$^+$); mucoid phenotype (37 C).

MC141 (F'13 *cap*R$^+$) × MC 139 (*cap*R9, *lac*-2, *pur*E, *leu*-1, *trp*-1, *str*-1)

↓ selection on minimal medium M-9 with lactose, leucine, and tryptophan

MC143 (F'13 *cap*R$^+$/*cap*R9); nonmucoid phenotype (37 C).

Similar matings, with other donors and recipients to give the same heterozygotes, yielded

similar results. The partial diploids were purified on media selective for retention of the episome. When mucoid strain MC142 (F'13 *cap*R9/*cap*R$^+$) was grown in Penassay medium containing 40 μg of acridine orange per ml [to cure cells of the F'13 episome (6)] and then plated on M-9 (1) medium not selective for episomal markers, it yielded nonmucoid clones that had lost *cap*R9 and, simultaneously, the other episomal markers (*lac*$^+$ *proC*$^+$ *purE*$^+$). When nonmucoid strain MC143 (F'13 *cap*R$^+$/*cap*R9) was grown in Penassay medium containing 40 μg of acridine orange per ml and then plated on M-9 medium not selective for episomal markers, it yielded mucoid clones that had lost *cap*R$^+$ and the other episomal markers *lac*$^+$ *proC*$^+$ *purE*$^+$. Strains MC142 and MC143 were tested for UV sensitivity, and both were found to be UV-R (Fig. 1). Samples of the same bacterial suspensions that were to be UV irradiated in the experiment of Fig. 1 were tested to prove that the cultures were still the proper partial diploid indicated. This was done by using acridine orange for curing, as outlined above. In some cases, with other similarly constructed mucoid strains, considerable difficulty was experienced in curing cultures. Even though the UV data obtained in such cases was always consistent with the results reported here, such data were not considered reliable in demonstrating these findings. In addition, because of the unexpected nature of the dominance found for MC142, unirradiated control dilution plates from the experiment reported in Fig. 1 for MC142 were grown, counted, and replica plated to two types of M-9 minimal media, one selective and the other nonselective for episomal markers. In this way it was shown that at least 93% of the partial diploids still retained the episomal markers at the time of irradiation. However, the slight flattening of the survival curve shoulder at high survivals (Fig. 1) appears to be real and reproducible in repeated experiments.

From previous results obtained with partial diploids (12) and from the present study, it is clear that the F'13 *cap*R$^+$/*cap*R9 strain is nonmucoid, whereas the F'13 *cap*R9/*cap*R$^+$ strain is mucoid. However, both partial diploids are resistant to UV. Thus, only the presence of a wild-type *cap*R$^+$ gene is necessary to produce a UV-resistant phenotype. The dominance of UV resistance in one type of partial diploid was first suggested by the findings of Greenberg and Woody-Karrer (5) that the radiation-resistant strain W3747 (an F'13 strain) transmitted radiation resistance to *E. coli* strain S1 when the F'

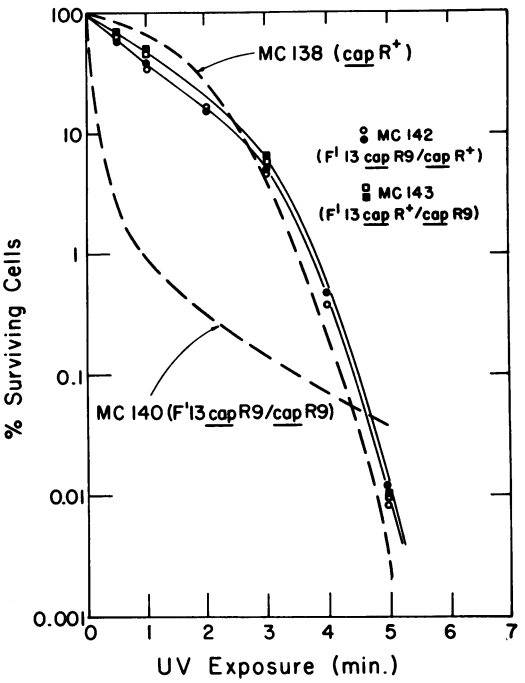

FIG. 1. *UV sensitivity of partial diploids heterozygous for the capR locus. Dotted curves indicate the sensitivity of the parental strains of MC142. A partial diploid, MC145, homozygous for capR$^+$ shows the same sensitivity as MC138. Similarly, haploid strains (e.g., MC128) containing the capR9 mutation show the same sensitivity as MC140.*

character and *lac*$^+$ were transferred. Walker and Pardee (15) conclusively demonstrated that the F'13 *lon*$^+$/*lon*$^-$ partial diploid was UV resistant, although tests for stability of the partial diploid were not reported. We also studied the response to UV of the homozygous partial diploids and found that strain MC140 (F'13 *cap*R9/*cap*R9) is no more sensitive to UV than the haploid *cap*R9 strains, whereas an F'13 *cap*R$^+$/*cap*R$^+$ strain is no more UV-resistant than the haploid *cap*R$^+$ strains (Fig. 1). Previously it was suggested that the dominance of the *cap*R9 allele on the episome in partial diploids, with respect to mucoid phenotype, could be explained if the *cap*R locus gene product were a protein composed of subunits (12) in which oligomers containing subunits produced by the *cap*R9 allele are defective and in which the episomal gene produces a greater number of subunits than the chromosomal gene. Evidence has been presented supporting the assumption that the *cap*R gene product is a protein (9, 11), but no further evidence on the presence of subunits is available.

The fact that UV resistance is dominant regardless of the position of the *cap*R$^+$ and *cap*R9 allele, whereas the episomal allele is dominant with respect to mucoid synthesis, does not seem to lend itself to easy interpretation. Either of the following two explanations are consistent with the data. (i) The active *cap*R gene product is a protein oligomer composed of subunits; oligomers containing both *cap*R$^+$ and *cap*R9 subunits are less effective in repressing enzymes necessary for polysaccharide synthesis (Lieberman, Buchanan, and Markovitz, *in preparation*), but mixed oligomers are still effective in performing their function to produce a UV-resistant phenotype. (ii) A small amount of *cap*R$^+$ oligomers is sufficient to confer UV resistance but not sufficient to repress enzymes for polysaccharide synthesis.

A third possibility must also be considered. The first cistron (*cap*R) controls capsular polysaccharide synthesis, and a second cistron (*lon*) in the same operon controls radiation sensitivity. However, several lines of evidence argue against this possibility. Despite many experiments in numerous laboratories, no UV-sensitive mutation that is nonmucoid and maps in the *cap*R or *lon* region has been described (if one carefully excludes strains that contain structural gene mutations in polysaccharide synthesis and ochre suppressors). Furthermore, data on differential suppression of polysaccharide synthesis and UV sensitivity by ochre suppressors (11) are not readily consistent with a two-cistron model.

This investigation was supported by Public Health Service grant A1-06966 from the National Institute of Allergy and Infectious Diseases and research grant CA 02739 from the National Cancer Institute.

We thank Aldona Rukuiza and Nancy Rosenbaum for their technical assistance.

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