NATURE OF Rec$^+$ REVERTANTS ISOLATED FROM *Escherichia coli* K-12 CULTURES WITH recA$^-$ MUTATIONS

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The nature of Rec$^+$ revertants isolated previously from cultures of recombinationally defective strain *Escherichia coli* K-12 AB 2463 recA13 was studied. With the aid of phage P1 vira the chromosome region of the recA gene in cells of strain JC2915F$^-$ were transduced, after which the recombination capacity of the transductants was determined by crossing with JC158Hfr cells and their resistance to ultraviolet radiation was established. Sensitivity of the transductants to suppressor phages was determined. The Rec$^+$ revertants were shown to differ with respect to the recA gene. In some Rec$^+$ revertants the Rec$^+$ phenotype appeared as the result of a back mutation in this gene from rec$^-$ to rec$^+$, whereas in other revertants the Rec$^+$ phenotype was due to indirect suppression.

KEY WORDS: *Escherichia coli*; Rec$^+$ revertants; transduction; genetic recombination; suppressor mutation.

The rec genes* controlling genetic recombination in *Escherichia coli* K-12 [4] are known to have pleiotropic effects. Besides recombinational effectiveness, cells carrying mutations for the rec genes have reduced viability, modified ability to divide and to repair DNA injuries induced by ultraviolet and ionizing radiations, and also reduced ability to induce phages λ, T4, and T7 [3, 6-9, 11-13].

The recA gene plays a key role in genetic recombinations of *E. coli* K-12. However, nothing is yet known about the genetic organization of this gene, the product synthesized under its control, or the mechanisms of its pleiotropic influences [4]. Nevertheless, previous investigations of strains of *E. coli* K-12 recA$^-$ led to the isolation of Rec$^+$ revertants, many of them with normal or almost normal ability to recombine and to repair DNA injuries induced by mutagens [1, 2].

The object of this investigation was to study the genetic nature of Rec$^+$ revertants.

EXPERIMENTAL METHOD

The test objects were 14 Rec$^+$ revertants isolated from *E. coli* AB 2463 recA$^-$ strains. To determine the genetic nature of the Rec$^+$ revertants, the chromosomal region of the recA genes was transferred from them by transduction with phage P1 vira to JC2915F$^-$ recipient cells with normal recombining capacity, after which the recombining capacity and other properties of the resulting transductants were investigated. Recombinationally defective strain *E. coli* AB 2463 recA13 was investigated in control tests.

The transducing lysates were obtained by repeated passage of phage P1 vira in cultures of Rec$^+$ revertants. Transduction was carried out by the standard method. For this purpose a culture of recipient strain *E. coli* JC2915F$^-$ CysC$^-$Thr$^-$Leu$^-$Pro$^-$His$^-$Arg$^-$Thi$^-$S^r was grown in L broth to a density of $2.0 \cdot 10^8$ cells/ml, centrifuged, and concentrated tenfold; after which 0.5 ml of the suspension of recipient cells was mixed with

*The nomenclature of Demerec et al. [5] is used in this paper and the abbreviations for the symbols for genetic markers are taken from Taylor and Trotter [10].

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TABLE 1. Properties of Transductants Obtained by Transfer of recA Chromosomal Region (gene)

Group of transductants	Frequency of $Thr^+Leu^+S^r$ recombinants per donor cell (crossed with E. coli (JC158)	Viability of cells after different doses of ultraviolet radiation, %					Sensitivity to phages		
		80	120	160	200	240	B17	B22	nj19
		erg/cm^2							
1	$4.0\cdot10^{-3}$—$7.0\cdot10^{-5}$	0.01	0.004	0.0007	0.0006	0.0001	—	—	—
2	0—$5.0\cdot10^{-7}$	0.02	0.005	0.0009	0.0002	0.0002	—	—	—
Control	$2.0\cdot10^{-6}$	0.0005	0.0004	0.0004	0.0001	0.000 01	—	—	—

0.5 ml of phage lysate, diluted to a concentration of $5.0\cdot10^7$ phage particles/ml, and the mixture was incubated for 20 min at 37°C; after which the cells were washed with phosphate buffer (pH 7.8) and seeded in a dose of 0.2 ml on dishes with selective medium for a selection of transductants for the $CysC^+$ marker, located on the chromosome next to the recA gene. After incubation for 48 h the seedings of transductants were purified by transfer to similar selective media. The ability of the purified transductants to undergo genetic recombination with donor cells of strain E. coli JC158Hfr $Thi^-SerA^-S^s$ was tested by selecting $Thr^+Leu^+S^r$ recombinants. The sensitivity of the transductants to ultraviolet radiation in doses of 0, 80, 120, 160, 200, and 240 ergs/cm^2 and to suppressor phases B17, B22, and nj19 also was determined.

EXPERIMENTAL RESULTS

By the use of P1 vira phage lysates obtained on 14 different cultures of Rec^+ revertants with titers of not less than $1.5\cdot10^9$ phage particles/ml, many $CysC^+$ transductants were obtained. For the subsequent work 50 $CysC^+$ transductants were selected from each experiment in which the donor was one of the Rec^+ revertants.

In experiments to study the recombining capacity of the $CysC^+$ transductants by crossing with JC158 donor cells, results were obtained to show that some transductants formed $Thr^+Leu^+S^r$ recombinants with the characteristically high frequency for Rec^+ wild-type strains, i.e., cells with normal ability to catalyze genetic recombination. Other transductants, on the other hand, were indistinguishable in their recombining power from cells carrying the rec^- allele. In experiments to determine the sensitivity of the $CysC^+$ transductants to ultraviolet radiation the degree of their resistance to this mutagen was shown to be the same as that of Rec^+ wild-type cells.

On the basis of these findings all the transductants were divided into two groups (Table 1). As this table shows, transductants characterized by normal recombining power and by resistance to ultraviolet radiation were placed in group 1. Phenotypically they corresponded to Rec^+Uvr^+ strains, whereas group 2 was composed of transductants which either did not take part in genetic recombination or which did recombine but formed recombinants with a very low frequency, although at the same time they remained resistant to ultraviolet radiation. The phenotype of these transductants was Rec^-Uvr^+.

Tests of the $CysC^+$ transductants for sensitivity to suppressor phages showed that, regardless of their phenotype, all were sensitive to phages B17, B22, and nj19.

When discussing the nature of the Rec^+ revertants isolated it might be assumed that the formation and, consequently, the genetic structure of these revertants would be determined by one of four possible mechanisms: 1) back mutation in the $recA^-$ gene leading to the $recA^+$ allele; 2) informational suppression of mutation of the $recA^-$ gene; 3) intragenic suppression in the $recA^-$ gene; 4) indirect suppression of mutation of the $recA^-$ gene.

The following conclusion can be drawn from the results of the experiments on transduction, crossing the transductant with Hfr donor cells, and determination of the phage sensitivity of the transductants. $CysC^+$ transductants, capable of forming recombinants when crossed with Hfr cells, inherited the rec^+ allele from the Rec^+ revertant when it arose as the result of a back mutation in the $recA^-$ gene. Consequently, the nature of these Rec^+ revertants was determined by the fact that they are rec^+ back mutants. The $CysC^+$ transductants which were unable to recombine inherited the rec^- allele from the Rec^+ revertants. Consequently, the restoration of the recombining power of these revertants was due to indirect suppression. Rec^+ revertants can thus be characterized by differences for the recA gene.

As regards resistance of transductants with no recombining power to ultraviolet radiation, this was probably attributable to the presence of phenotypically Rec^+, but genotypically rec^- additional mutations, responsible for the repair of DNA injuries, in the revertants.

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EFFECT OF HYPERTHERMIA ON SPERMATOGENESIS IN MICE AND THE ROLE OF HEAT TRAINING IN ADAPTATION OF THE SEX CELLS TO HIGH TEMPERATURES

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Keeping sexually mature albino mice in a hot and humid chamber at a temperature of 43°C and a relative humidity of 65% for a single exposure causes destruction of the spermatogenic epithelium, as reflected in degeneration and desquamation of the sex cells in 55% of the seminiferous tubules. Preliminary heat training for 10 days increased the physiological degeneration of the spermatogenic cells in only 16% of seminiferous tubules. This could be evidence of adaptation of the sex cells to the action of high temperatures.

KEY WORDS: adaptation; hyperthermia; spermatogenesis.

Hyperthermia in man and animals is known to cause damage to the cells of the spermatogenic epithelium. The degree of this damage depends on the height of the ambient temperature and the duration of exposure to it [2, 5, 6, 8, 9].

The problem of whether adaptation of sex cells to the action of heat takes place during daily exposures of increasing duration to high temperatures has not been discussed in the literature. The investigation described below was carried out for this purpose.

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