TRANSFER AND INTEGRATION OF MARKERS OF CHROMOSOMAL SEGMENT Try-His OF Escherichia coli K-12 AFTER X-RAY IRRADIATION

A. P. Pekhov and E. V. Yudin

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Investigations have shown that treatment of donor cells of Escherichia coli K-12 with ionizing radiation leads to a decrease in the frequency of genetic recombination due to conjugation and measurable by estimating the results of transmission of selective and unselective signs from donor to recipient. The radiosensitivity of the loci, moreover, is proportional to their distance from the initial point on the transmitted segment of the chromosome [1,9,10].

In accordance with these results, which were mainly obtained during the study of the proximal chromosome segment O-T-L-Lac-Gal, ionizing radiation lowers the frequency of linkages between the threonine and lactose loci, and also between loci controlling the synthesis of threonine and leucine.

In the present investigation, the effect of x-ray irradiation of the donor was studied on the genetic recombination of E. coli K-12 during the transfer of the more distal chromosomal segment Try-Pro-Lac-His from donor to recipient.*

EXPERIMENTAL METHOD

Strain E. coli Hfr B_1-S^s was used as donor, transmitting genes in the following order: O-T-L-Try-Pro-Lac-His. . . . . F [7]. The recipient was E. coli strain J62E-Pro-Try-His-Lac-S^r [4].

Eight-hour broth cultures were used in the experiment, and before crossing took place they were centrifuged at 3000 rpm for 5 min, after which the residue of bacteria was transferred to fresh broth, to give a concentration of 10^9 cells/ml.

The donor cells were irradiated with the RUM-7 x-ray apparatus (aluminum filter 0.1 mm, distance 5 cm). The donors' cells, irradiated with different doses, were mixed with unirradiated recipients' cells in the proportion of 1:10. The mixtures of bacteria were incubated for 100 min at 37°, after which samples were collected, seeded on dishes with selective media, and the seedings were again incubated at 37° for 72 h. For selection of recombinants Try^+S^r, a minimal medium was used, to which were added glucose (1%), proline (60 μg/ml), histidine (60 μg/ml), and streptomycin (200 units/ml). Selection of the recombinants Pro^+S^r took place on a minimal medium with glucose (1%), tryptophan (60 μg/ml), histidine (60 μg/ml), and streptomycin (200 units/ml). Recombinants His^+S^r were selected on a minimal medium with glucose (1%), tryptophan (60 μg/ml), proline (60 μg/ml), and streptomycin (200 units/ml). The recombinants were purified by reseeding on similar media. The genetic structure of the recombinants was studied by determining the results of transfer by means of a technique of replicas of the corresponding recombinant colonies on dishes with media used to determine nonselective signs. Analogous crosses in which the donor cells were not irradiated were used as controls.

EXPERIMENTAL RESULTS

The effect of x-ray irradiation of the donors' cells in various doses on the frequency of recombination was determined from the number of recombinant colonies Try^+S^r, Pro^+S^r, and His^+S^r, appearing on the dishes with the corresponding selective media.

*The symbols used in this paper are as follows: B_1, thiamine; T, threonine; L, leucine; Try, tryptophan; Pro, proline; His, histidine; Gal, galactose; Lac, lactose; S^s/S^r, sensitivity to streptomycin/resistance to streptomycin.

Laboratory of Genetics of Microorganisms, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR V. D. Timakov).
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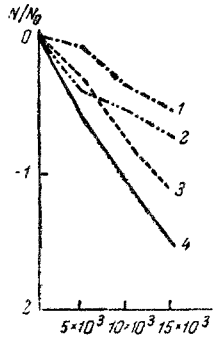

Effect of x-ray irradiation on transmission of selective signs. Along the axis of ordinates — ratio between number of recombinants formed after irradiation and number of recombinants without irradiation (logarithms); along the axis of abscissas — doses of irradiation (in R): 1) Try^+S^r; 2) rate of survival; 3) Pro^+S^r; 4) His^+S^r.

Genetic Structure of Recombinants Try^+S^r, Pro^+S^r, and His^+S^r (Inheritance of Genes Controlling Nonselective Signs, in Percent of Number of Recombinants)

Dose of irradiation (in R)	Try^+S^r			Pro^+S^r			His^+S^r		
	Phenotype of colonies								
	Pro^+	Lac^+	His^+	Try^+	Lac^+	His^+	Try^+	Pro^+	Lac^+
0	38,8	37,8	2,5	100	95,7	1,5	100	31,0	29,7
5×10^3	26,2	23,4	2,1	100	93,5	1,2	100	13,5	20,0
10×10^3	22,9	22,9	2,0	100	93,7	1,0	100	7,5	7,5
15×10^3	14,0	13,1	4,4	100	81,9	0,5	100	—	—

It is clear from the corresponding curves (see figure), in which the ratio between the number of recombinants in crosses with the irradiated donor and the number of recombinants selected from crosses in which the donor cells were not irradiated is plotted, the number of these recombinants depended on the dose of irradiation.

Remembering the order of transmission of the genes by the donor strain used, it could also be seen that the radiosensitivity of the loci Try, Pro, and His, measured by the number of analogous recombinants, was associated with their distance from the point 0 of the chromosome transmitted initially by the donor cells. The farther the investigated loci from the point 0 of the transmitted segment of the chromosome, the greater their sensitivity to irradiation.

To discover whether x-ray irradiation affects the frequency of linking of the loci, the genetic structure of recombinants Try^+S^r, Pro^+S^r, and His^+S^r was studied. The results are given in the table.

The table shows that the percentage of recombinants Try^+S^r inheriting the ability to synthesize proline and ferment lactose gradually diminished with an increase in the dose of irradiation, whereas the number of analogous recombinants synthesizing histidine was not appreciably changed. In the case of the proline recombinant, the frequency of inheritance of lactose and histidine loci also diminished, and all these recombinants were capable of synthesizing tryptophan. So far as the recombinants His^+S^r are concerned, being tryptophan-independent, many of them were characterized by loss of the ability to synthesize choline and to ferment lactose, while the number of these recombinants also depended on the dose of irradiation.

It may be concluded from the analysis of these results and their comparison with the authors' earlier results [1] of a study of the frequency of recombination after irradiation of a donor strain of E. coli and subsequent transfer of the segment O-Gal, that the distal loci are more sensitive to radiation than the proximal. The results obtained also indicate that the radiation sensitivity of the chromosomal segment Try-His is not homogeneous throughout its extent. The greatest sensitivity is found characteristically in the region Pro-Lac. This is in agreement with results showing that the genes controlling radiation sensitivity of E. coli are situated between the Pro and His loci [4].

The decrease in the frequency of linkage between the loci depending on the dose of radiation may be explained by the fact that this agent causes breaks in the DNA of the donor, as a result of which the possibility of simultaneous transfer and integration of two or more linked genes of the donor E. coli cells which, as we know, have one linkage group [8], is reduced. It may also be postulated that local breaks in the DNA lead either to a reduction in genetic exchange or to the formation of nonviable products of recombination. The probability of this explanation is confirmed by many results indicating that x-ray irradiation can produce chromosome breaks, and also analyzable injuries (breaks) in DNA [2,5,6]. The possibility is not thereby ruled out that, besides this type of action on the genetic recombination of E. coli K-12, x-rays may also have an effect on the sex factor causing the formation of mutants with a lower capacity for chromosome transfers. This hypothesis requires experimental verification.

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