INDUCTION BY RADIATION OF A NEW DIRECTION OF CHROMOSOME TRANSFER DURING CONJUGATION IN AN HFR STRAIN OF ESCHERICHIA COLI[1]

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In E. coli K-12 sexual recombination results from the transfer of male (Hfr) genetic material in a sequential manner into the female (F^-) cell. The order of transfer for the various male markers is determined, for a given Hfr strain, by the location of the origin and the direction of transfer (Jacob and Wollman, 1961). Transfer usually terminates before the entire chromosome enters the female cell, presumably due to random breakage of the male chromosome. This results in a gradient of transfer, i.e., a higher probability of transfer for those genetic markers near the origin than for those markers located more distally.

When the Hayes Hfr strain (Hfr H) is subjected to either P^{32} decay or X-irradiation before mating, the number of recombinants carrying a given male marker decreases approximately exponentially with the product of dose and distance from the origin (Fuerst, Jacob, and Wollman, 1956 and Marcovich, 1961). The results are consistent with the idea that X-irradiation or P^{32} decay produces randomly distributed chromosomal lesions which result in a shorter average length of transferred male chromosome. Similar results have been found using α-rays and UV irradiation (Joset and Wood, unpublished).

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We have been able to verify these earlier studies on Hfr H (to be published), but have unexpectedly found an Hfr strain (Hfr Ral) which acts quite differently. We describe here some experiments which indicate that radiation treatment of the Ral strain before mating induces chromosomal transfer proceeding in the opposite direction from normal Ral transfer.

Materials and Methods

Hfr Ral was obtained as a UV mutant of *E. coli* (K-12) W1485 F^+ (source: Pasteur Institute, Paris). It is prototrophic for the markers studied here and *str*^{S}. The F^- strain used was PA-309 (*arg*$^-$, *thr*$^-$, *leu*$^-$, *try*$^-$, *his*$^-$, *lac*$^-$, *gal*$^-$, *str*^{r}), (source: Dr. H. Marcovich). The media have been described elsewhere (Stent and Fuerst, 1955; Marcovich, 1961). Matings were carried out by mixing male and female cells in exponential growth (about 2 x 10^8 cells/ml) in a 1:10 ratio, and shaking gently at 37°C for 90 minutes; aliquots were subsequently plated out on recombinant-selective media. Interrupted mating experiments were performed using an electro-mechanical shaker in which 3 ml of soft agar medium plus the proper aliquot of mating mixture were shaken violently for 3 seconds, then poured immediately onto a recombinant-selective plate.

Radiation experiments were carried out using X-rays, UV, P^{32} decay, and α-rays (Po^{210} source). P^{32} techniques were similar to those of Fuerst et al. (1956).

Results

The results of an interrupted mating experiment using unirradiated cells are shown in Fig. 1A. The entry times are taken as the times when the linear portions of the recombinant curves extrapolate to zero. The average entry times for four such experiments are: *his* 23 min., *try* 36 min., *thr* 65 min., *arg* 78 min. These marker intervals have been confirmed by work on other strains, both in this laboratory and by A. L. Taylor (Genetics, in press).

Figure 2 shows the effect of X-irradiation on the frequency of appearance of various recombinant types in crosses involving Hfr Ral. In this so-called gradient plot, recombinant frequencies for selected X-ray doses are plotted against marker entry times and the points corresponding to a common dose are joined.

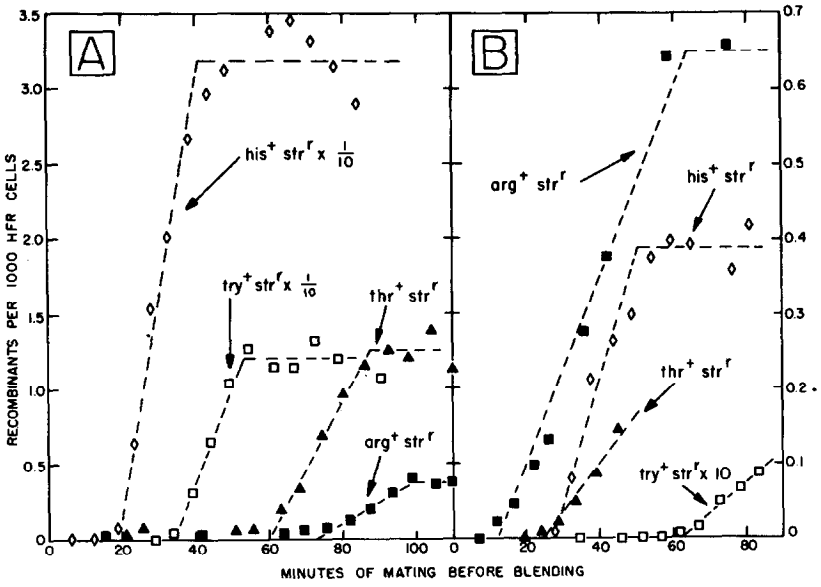

Figure 1. Interrupted mating experiments using Ral cells.
Part A - Exponentially growing Ral and PA309 cells were mixed in a 1:100 ratio and shaken gently at 37°C. Samples for blending were taken at approximately one minute intervals, and the numbers of recombinants of the various types were determined as a function of the time between the start of mating and blending. These numbers were all normalized to the female titre at the time of blending.
Part B - Same as Part A except that the Ral cells were given a 500 ergs/mm^2 dose of 2537Å UV (41% survival) just prior to mating. The number of recombinants is plotted per 1000 Hfr cells as measured before irradiation.

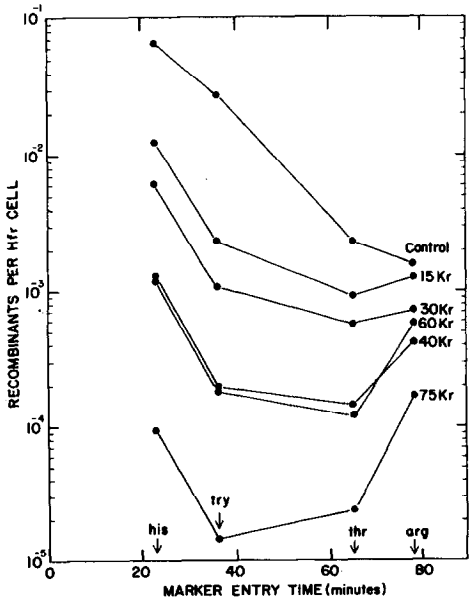

Figure 2. Effects of X-irradiation of Hfr Ral on genetic recombination. An exponential culture of Hfr Ral in broth was irradiated with 37kV X-rays at a cell concentration of 2.10^8 cells/ml. The dose rate was 30 Kiloroentgens/mn. The cells were then diluted ten times in an exponential suspension of female cells, mated at 37°C for 90 min. and plated out on selective media.

As the X-ray dose to the male cell is increased, the gradient curves become concave upward, in marked contrast to the linear gradient curves derived from previous work on Hfr H at comparable radiation doses. (Marcovich, 1961). It is evident from Fig. 2 that at high doses there are actually more arg recombinants than his recombinants. To a lesser extent, the anomalous X-ray resistance is also seen for thr and try. Similar results are obtained when Hfr Ral cells are subjected to decay of incorporated P^{32} before mating. The ability to form arginine recombinants, involving the transmission of the presumably distalmost Hfr character, shows the least sensitivity to P^{32} decay; and the ability to form threonine recombinants is also more resistant than is the case for the two proximal markers. As in the X-ray case, this is in marked contrast to the results obtained with Hfr H, where the greatest P^{32} sensitivity was associated with recombinants selected for the most distal characters (Fuerst et al., 1956). Entirely comparable results are obtained using either α-rays or UV to irradiate the Ral cells before mating.

From interrupted mating experiments with freshly irradiated Hfr Ral cells, curves such as shown in Fig. 1B are obtained. Here a low dose of UV (Surviving fraction 41%) was used to irradiate the cells. The most striking features of these curves are the early entry times for arginine and threonine which are approximately 11 and 24 minutes respectively, in contrast to values of 78 and 65 min. for the unirradiated case (Fig. 1A).

When arginine recombinants obtained from irradiation blendor experiments (such as that of Fig. 1B) are analyzed for histidine, it is found that essentially none of these recombinants obtained from samples blended less than 60 minutes after the start of mating are also prototrophic for histidine, in spite of the fact that the entry time for histidine itself is about 28 minutes. (With no irradiation, unselected proximal markers generally appear with a frequency of 50% or more.) This indicates that the early arginine recombinants observed in Fig. 1B arise from a mode of transfer different from the normal one seen in the unirradiated case (Fig. 1A). This new mode of transfer, as deduced by genetic analysis, has the arginine character near the origin, followed by threonine, lactose, galactose, and tryptophan in that order.

We have been able to isolate stable Hfr "invertants" from irradiated Hfr Ral cells. Interrupted mating experiments using these invertants give the following marker entry times: arg 7 min., thr 14 min., try 41 min., his 56 min. Preliminary experiments indicate that the str^S marker is transferred as a distal marker by both the Ral and invertant strains. This means that the origin for transfer by invertants is separated on the circular chromosome from the normal Ral origin, with the str^S marker located between the two. The number of stable invertant Hfr's obtained by re-isolating and testing irradiated Hfr Ral survivors is considerably less than that predicted from the high frequency of appearance of arg recombinants in radiation experiments, indicating a transient change in some of the cells.

Discussion

The data described above are consistent with the idea that irradiation prior to mating induces a fraction of the Hfr Ral population to transfer the bacterial chromosome in the reverse direction during mating. It is reasonable to expect that the fraction of cells so induced increases with dose, and in fact at high doses the predominant mode of transfer appears to be reversed with respect to normal Ral transfer (Fig. 2). The results are undoubtedly greatly complicated by other effects of irradiation (e.g. lethality, loss of ability to mate, interruption of chromosomal transfer, etc.).

It is found that a low UV dose induces an absolute increase in the final number of arginine recombinants, in comparison with control matings. Fig. 1 shows an increase by a factor of about 1.7 and in other experiments a factor as large as 8 has been obtained. This indicates that the invertants under study are actually induced by irradiation and are not merely due to selection.

Two general classes of mechanism for the induction by irradiation of a new mode of transfer appear consistent with the data. Irradiation might liberate the F factor from its original location, with subsequent re-attachment at another specific location on the chromosome, in keeping with our finding that all of the stable invertants so far tested have had their origins at the same location, - i.e. with arg entering at about 7 minutes, followed by thr, etc. Preliminary experiments on

F^+ cells arising spontaneously from either Ral or Ral invertants indicate that they too are induced by irradiation to transfer arginine first. This occurs to a smaller extent than in the Hfr Ral case, but may nevertheless be significant in relation to a possible preferential site for the sex factor in the Ral Hfr-F^+ system, similar to that observed in the F' strains discussed by Adelberg and Burns (1960) and Scaife and Gross (1963). All of the Hfr's which we have been able to isolate, either from the F^+ strains discussed above or directly from the parent W1485 F^+, appear identical with either the Ral or Ral-invertant Hfr strains.

Another possibility, with less supporting evidence, is that the Hfr Ral is a peculiar type of "double male" similar to that reported by Clark (1963). Normally the invertant origin would be suppressed but would become active or dominant as a result of radiation treatment. In this proposal the invertant origin might play an important part in the replication of DNA, assuming that replication is necessary for transfer (Jacob, et al., 1963; Roeser and Konetzka, 1964). This origin might be an initiator of vegetative replication by the male chromosome, and be occasionally used as an origin of transfer.

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