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*EXCLUSION OF AN FLAC EPISOME BY AN HFR GENE**

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In a previous paper[1] we have described experiments which suggested that the presence of an Hfr gene excluded the acceptance of an Flac episome during conjugation. In these experiments a strain of *Escherichia coli* B which had been infected with Flac$^+$ was used as the chromosomal donor, and the Hfr strain AB 312, converted temporarily to the F$^-$ state (F$^-$ phenocopy), acted as the recipient. The offspring of such a cross were either Flac$^+$, F$^-$, or Hfr in mating type, but never Hfr-Flac$^+$.

A question that was not answered in the previous paper was whether or not the transfer of the chromosome during the mating prevented the simultaneous transfer of Flac$^+$; if this were the case, it would not be necessary to implicate a specific action of the Hfr gene present in the recipient. In this paper it will be demonstrated that simultaneous transfer of Flac$^+$ and chromosome can occur into an F$^-$ strain, as has already been suggested by the experiments of Adelberg and Burns,[2] and that therefore physical impedance by the chromosome is not responsible for the observed exclusion. This having been established, an experiment was carried out in which a mating between an Flac$^+$ donor and an Hfr recipient was interrupted after sufficient time to permit chromosomal transfer from the Flac$^+$ strain into an Hfr strain in F$^-$ phenocopy. Under these conditions, the Flac$^+$ factor, although able to be transferred, did not appear among the recombinants.

Another question that remained unanswered in the previous paper[1] was whether or not the Flac$^+$ episome ever entered the Hfr recipient. Flac$^+$ offspring were obtained, but no tests were carried out to ascertain that Flac$^+$ was actually transferred into the Hfr recipient. The Flac$^+$ offspring might, in fact, have resulted from the entrance of the Hfr chromosome into the Flac$^+$ strain. In the experi-

ment reported in this paper, reverse transfer from the Hfr into the Flac$^+$ parent was eliminated. Thus, all recombinants resulted from entrance of the chromosome of the Flac$^+$ parent into the Hfr parent. Under these conditions, transfer of the Flac$^+$ factor was found to be a rare event.

Materials and Methods.—The media, mating conditions, and tests used have been described.[1] The test for Flac$^+$ was simplified so that many strains could be tested with ease. Abbreviations used are: ade, adenine; arg, arginine; his, histidine; leu, leucine; met, methionine; pro, proline; thi, thiamine; thr, threonine; lac, lactose; sm^r, streptomycin-resistant; sm^s, streptomycin-sensitive; sf, sex factor.

Test for Flac$^+$: Presumptive Flac$^+$strains were crossed with lac$^-$ recipients which differed from them in being sm^r (see abbreviations above). Logarithmically growing cultures containing 10^9 cells per ml in broth were mixed in 25-mm diameter tubes in the proportion of 1.0 ml of the Flac$^+$ parent, 0.5 ml of the F$^-$ parent, and 1.5 ml of neopeptone broth. The suspension was incubated at 37° with gentle shaking. The conjugation was interrupted by rapid agitation of a 100-fold diluted aliquot at zero time and after 60 min. The treated samples were streaked directly on MacConkey lactose agar containing 100 γ of streptomycin per ml. Flac$^+$ strains gave rise to lac$^+$ colonies in the streaks of the 60-min samples, but not in the zero-time samples.

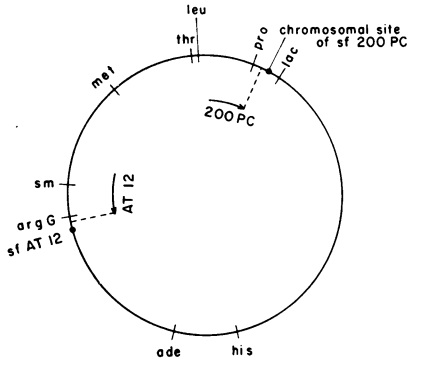

FIG. 1.—Linkage map of the pertinent genes.

Strains: Hfr strain AT 12 (see Fig. 1 for location of sex factor), which is met$^-$, ade$^-$, lac$^-$, T6^r, and sm^s, was obtained from Dr. A. J. Clark. Strain AB 1353 (thi$^-$, arg$^-$, pro$^-$, his$^-$, lac$^-$, T6^r, F$^-$) was obtained from Dr. A. Taylor. The Flac$^+$ donor, 200 PC (thi$^-$, thr$^-$, leu$^-$, T6^s, sm^r), was obtained from Dr. F. Jacob.

Results.—1. *Flac$^+$ transfer during chromosomal transfer:* This experiment was designed to test whether or not the chromosome and an Flac$^+$ episome can enter together from an Flac$^+$ donor into an F$^-$ recipient. Strain 200PC (thi$^-$, thr$^-$, leu$^-$, T6^s, Flac$^+$) was crossed with strain AB 1353 (thi$^-$, arg$^-$, his$^-$, pro$^-$, lac$^-$, T6^r, F$^-$). The mating was interrupted by killing 200PC, the minority parent, with phage T6 at times zero, 60 min, and 150 min. The following recombinant types were selected on minimal glucose agar: thr$^+$, leu$^+$, pro$^+$ at zero and 60 min, and thr$^+$, leu$^+$, his$^+$ at zero and 150 min. Colonies of the two types were picked and purified by two single colony isolations on the appropriate selective media. They were then tested for ability to ferment lactose on MacConkey agar, and for maleness by replica plating of a master plate onto two successive lawns of a suitably marked F$^-$ strain.[3] The results of this experiment are presented in Table 1. It can be seen that the majority of the chromosomal recombinants tested were lac$^+$ and males. Furthermore, most of the lac$^+$ strains segregated lac$^-$ strains with the same frequency as the Flac$^+$ parent. Thus, it can be concluded that most of the recombinants were Flac$^+$, and that therefore the chromosome

TABLE 1

FLAC+ TRANSFER DURING CHROMOSOMAL TRANSFER FROM FLAC+ STRAIN 200PC INTO F− STRAIN AB 1353

Type of selection	Time (min)	Frequency of recombinants (per cent male input)	Frequency of lac+ strains among chromosomal recombinants (per cent)	Frequency of males among lac+ strains (per cent)
thr+, leu+, pro+	60	4	68	86
thr+, leu+, his+	150	0.01	84	—

and the Flac+ episome can be transferred together.

About 4 per cent of the tested recombinants were of a peculiar lac type. They were lac− but gave rise to lac+ clones with relatively high frequency. This was a heritable characteristic, for reisolated lac− clones continued to give rise to lac+ clones with an approximate frequency of 1:100. Furthermore, they were "weak" males, i.e., weaker than males of the Flac+ type and rather resembling F+ males. No explanation is available for the origin of these strains.

2. *Flac transfer into Hfr strains:* Having determined that the chromosome and an episome can enter together into an F− recipient, a similar experiment was now carried out between an Flac+ donor and an Hfr recipient to see if the Hfr strain can at all receive the Flac+ episome. Strain 200PC (thi−, thr−, leu−, T6^s, sm^r, lac−, Flac+) was crossed with strain AT 12 (ade−, met−, lac−, T6^r, sm^s, Hfr) in F− aeration-phenocopy. The mating was interrupted by killing the donor, 200PC, at zero time and 150 min with phage T6. Two kinds of recombinants were selected for at these times: thr+, leu+, met+ and thr+, leu+, ade+. Of these, recombinants to be tested for other genetic characters were purified by two single colony isolations on the appropriate selective media. The results of this experiment are presented in Table 2. It can be seen that in contrast with the results obtained with an F− recipient, most of the recombinants were lac−. Therefore, there is a definite exclusion of the Flac+ episome by the Hfr gene in the recipient.

All the recombinants that had been purified were tested for the other, unselected auxotrophic character, i.e., all thr+, leu+, met+ recombinants were tested for adenine requirement, and all thr+, leu+, ade+ recombinants were tested for methionine requirement. When met+ was selected for from the donor, most of the colonies (47/48) were ade− as expected, since ade+ enters after met+ from 200PC. When ade+ was selected for from the donor, only 17 out of 64 colonies had also received met+, indicating a preferential inheritance of the Hfr chromosome of the recipient. In fact, out of 112 recombinants tested only three did not inherit the AT 12 sex factor A possible explanation for this unexpected preferential inheritance is that the sex factor of the recipient is a starting point for DNA replication, even in the state of F− phenocopy.

Of the lac+ recombinants, all but 2 out of 13 did not inject Flac+ and did not

TABLE 2

RECOMBINANT TYPES IN A CROSS BETWEEN FLAC+ DONOR 200PC AND HFR RECIPIENT AT 12

Type of selection	Frequency of recombinants (per cent male input)	Frequency of lac+ strains among recombinants (per cent)	Frequency of males among recombinants (per cent)
thr+, leu+, met+	0.15	8	97
thr+, leu+, ade+	0.02	14	97

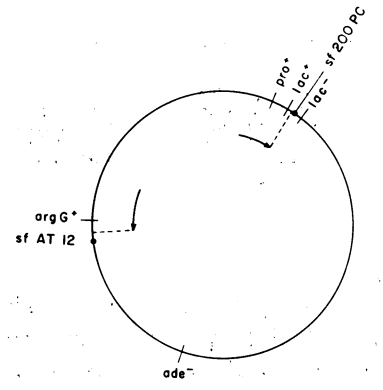

FIG. 2.—Proposed genetic structure of strain a30.

inject pro+ early. These 11 were Hfr offspring of the AT 12 type and not Flac+. The other two exceptional recombinants, a30 (ade−, sm^s) and m16 (met−, sm^s), segregated lac− clones, a30 with much higher frequency than m16. Therefore, they both appeared to be diploid for the lac region. Strain m16 injected lac+ with a frequency of about 50 per cent after 60 min, and pro+ early like the Flac+ parent. It did not inject arg G early. It can thus be concluded that m16 is an Flac+ strain, in which the sf AT 12 was crossed out.

Strain a30 injected lac+ and pro+ early and with about equal frequencies (1%), as if they were both chromosomal markers, and arg G early, like strain AT 12. Therefore, this strain appeared to be a double male harboring two chromosomal sex factors, one of them between two lac regions (Fig. 2). To test this hypothesis, strain a30 (ade−) was crossed with AB 1353 (pro−, thi−, his−, arg−, lac−, F−) and lac+, ade+ recombinants were selected after 60 min of mating. The mating was interrupted by agitation. The unselected marker pro+ was scored among the lac+ recombinants, and was found to occur among them with a frequency of 66 per cent. This supports the hypothesis that strain a30 injects the sequence lac-pro . . . . . sf AT 12, as well as the sequence arg G- - - - - pro-lac-sf 200PC. Furthermore, the lac+ recombinants were scored for maleness and only 11 per cent were males. All of the latter were of the Flac+ type. Therefore, it was confirmed that most of the time strain a30 injects lac+ as an early chromosomal marker and only in 11 per cent of the lac+ recombinants is the lactose region injected as an episome.

Discussion.—The experiments presented in this paper show that an Hfr recipient excludes Flac+ from an Flac+ donor. However, the lac+ allele can be recovered on the chromosome in about 10 per cent of the recombinants. Evidence for the mechanism of this chromosomal lac+ recovery has been obtained by Scaife and Gross.[4] They have shown that when an Flac+ episome activates injection of a chromosome carrying lac−, a recombination occurs between the episome and the chromosome in such a way that the chromosome is donated with the sequence lac+-pro . . . . . lac− sf 200PC (cf. Fig. 2). Thus, it is to be expected that in any cross involving an Flac+ donor, lac+ can be recovered as an unselected marker in a certain fraction of recombinants as a chromosomal gene.

Furthermore, the mechanism proposed by Scaife and Gross explains how the double male a30 was obtained in the cross between the Flac+ donor 200PC and the Hfr recipient AT 12. The lac+ allele entered early and on the chromosome, while the lac− allele and sex factor also entered with the donor chromosome at the very end of the injection. Such a strain, with the lac region in duplicate, is somewhat unstable and often loses one of the lac alleles during replication. This would explain why the strain segregates lac− clones. Strain a30 presumably gives rise also to Flac+ episomes since occasionally it donates the Flac+ episome to an F−

recipient during mating. However, it appears that in strain a30 these episomes are either not replicated or destroyed since Flac$^+$ is donated with low frequency. It is reasonable to attribute this loss of Flac$^+$ to the presence in strain a30 of the AT 12 sex factor. In fact, this phenomenon would parallel the observed exclusion of Flac$^+$ by the sex factor of Hfr recipients.

The author would also like to propose that the only true Flac$^+$ recombinant (m16) obtained arose exactly like a30. Lac$^+$ came in early on the donor chromosome, and lac$^-$ followed by the sex factor came in at the end. However, since the Hfr gene of the recipient was crossed out, this strain can harbor a replicating episome. We have preliminary evidence which suggests that strain m16 does have a duplicate lac region on its chromosome.

We thus arrive at the conclusion that an Hfr recipient completely excludes an incoming Flac$^+$ episome. A similar exclusion of Flac$^+$ by a strain carrying Fgal$^+$ has been observed by Echols.[5] He suggests that the excluded episome either does not enter or is destroyed after entrance. The present results favor the hypothesis of destruction. This is suggested by the behavior of strain a30 in which, because of the presence of Hfr, Flac$^+$ is expressed only rarely. In this strain absence of Flac$^+$ cannot be a case of nonentry. The phenomenon described here may be analogous to the restriction of phage λ by phage P_1, studied by Dussoix and Arber.[6] In this case Dussoix and Arber have shown that the incoming DNA is destroyed. It is interesting to speculate that destruction of "foreign" DNA is a general mechanism that plays a role in maintaining the integrity of the cell's genetic apparatus.

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*PHOTOREVERSIBILITY OF INDUCED MUTATIONS IN A NONPHOTOREACTIVABLE STRAIN OF ESCHERICHIA COLI**

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The photoreversal of mutations to prototrophy induced by ultraviolet light (UV) and the photoreversal of UV killing are differentially affected by acriflavine.[1] One possible explanation is that the lesions leading to photoreversible prototrophy differ from those leading to photoreversible death, and that they may be photoreversed by distinct mechanisms. The isolation of a mutant strain of *Escherichia coli* B that is unable to photoreverse UV killing[2] makes possible a direct test of this hypothesis. The "photoreactivating enzyme" normally produced by *E. coli*[3] is not obtainable from the mutant strain,[4] which suggests that this