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Cellular damage associated with multiple mating in *E. coli*

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1. INTRODUCTION

In experiments in which the ratio of Hfr to F^- cells was varied systematically we have observed that the ratio of parental concentrations at which the maximum yield of recombinants is obtained depends on the Hfr strain employed. Moreover, in mixtures involving members of one group of Hfr strains and a particular F^- strain, the yield of recombinants actually decreases progressively as the Hfr concentration is raised above about one Hfr cell per five F^- cells. An analysis of these phenomena, which will be presented in this paper, indicates that simultaneous mating between an F^- cell and several Hfr cells may lead to death of the F^- cell.

2. MATERIALS AND METHODS

The materials and methods employed and the symbols for the genetic markers have been described previously (de Haan & Gross, 1962; Gross, 1963). In addition to the bacterial strains already described the following F^- strains have been used:

W1:M^-P^-, obtained as follows: W1:$TL^-B_1^-$ lac^- (Lederberg & Lederberg, 1952), was crossed with a P^- derivative of HfrC (Cavalli, 1950). A $TL^-P^-B_1^-lac^-$ derivative obtained from this cross was mated with HfrC:M^- and a $TL^+B_1^+lac^+$ recombinant which had acquired the M^- marker was isolated and purified.

W1:$M^-P^-lac^-$, isolated by Eggertson (personal communication) by the same procedure, except that in the second cross the lac^- marker was conserved.

3. RESULTS

(i) *The effect of donor:recipient ratio on the yield of recombinants*

In the experiments to be reported first we have examined the yield of recombinants from mixtures containing a constant number of F^- cells and different numbers of Hfr cells. The mixtures were incubated for one hour; mating was then stopped by the addition of phage T6 and the yields of recombinants determined. The results of

experiments of this type involving mixtures of HfrP10 or HfrR4 and two different F⁻ strains are presented in Fig. 1.

It may be seen that in mixtures containing a large excess of F⁻ cells the yield of recombinants increases linearly with Hfr concentration and is essentially the same for all four combinations of strains. However, when the Hfr concentration is increased beyond about one Hfr per five F⁻ cells marked deviations from linearity become apparent. In mixtures involving HfrR4 and F⁻W1:M⁻P⁻ the yield of recombinants falls progressively as the Hfr concentration is raised. With

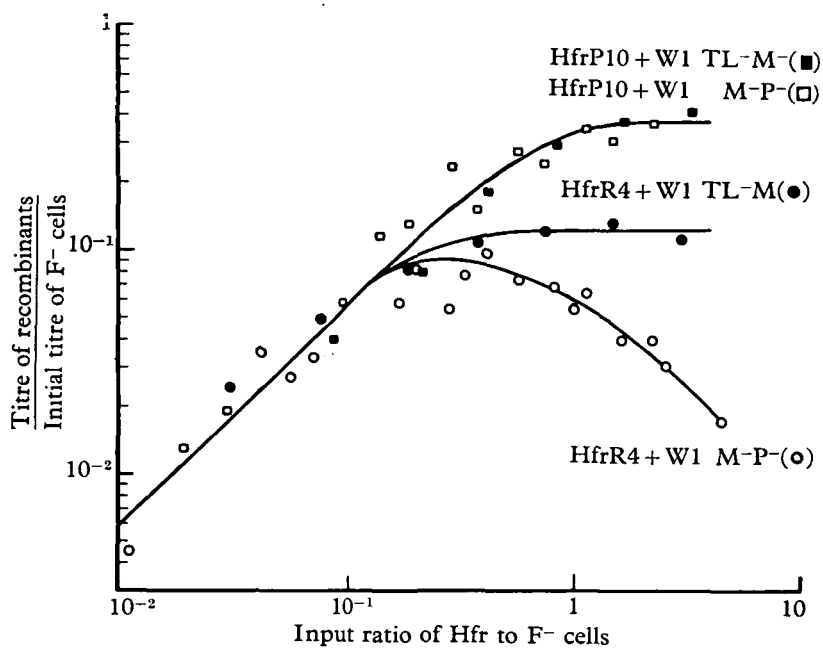

Fig. 1. The effect of Hfr concentration on the yield of recombinants. Mixtures containing approximately 2×10^8 F⁻ cells/ml. and different numbers of Hfr cells were incubated on a rotor for one hour; phage T6 was then added and after a further 10 min. the suspensions were assayed for recombinants. The data for each parental combination include the results of several independent experiments. In experiments with HfrP10:TL⁻B_1^-, M⁺S^r recombinants were selected. In those with HfrR4:M⁻, either P⁺S^r or TL⁺S^r recombinants were selected; the yield of TL⁺S^r recombinants has been multiplied by 2 for comparison with the other results since the TL⁺ marker is transferred half as frequently as is the P⁺ marker by HfrR4.

F⁻W1:TL⁻M⁻ and the same Hfr strain, the number of recombinants remains unchanged and the final yield corresponds to about one recombinant per 10 F⁻ cells. On the other hand, in combinations involving HfrP10 bacteria and either F⁻ strain, the yield of recombinants continues to rise linearly until the numbers of Hfr and F⁻ cells are about equal, and then levels off at a value corresponding to one recombinant for every two or three F⁻ cells.

Similar experiments have been performed with the same recipient strains and four other donor strains, and the results in each case were similar to those obtained

either with HfrP10 or with HfrR4. HfrR1 and HfrR3 behaved like P10, and HfrC and HfrH like HfrR4. All F⁻ strains which have been examined other than W1M⁻P⁻ have been found to behave like W1:TL⁻M⁻. The fall in yield of recombinants with increasing Hfr concentration in mixtures of F⁻ W1:M⁻P⁻ and donors of the R4 group is therefore dependent on some unusual sensitivity of this recipient strain.

Since the differences between the various parental combinations are only observed at high concentrations of donor cells, it is reasonable to suppose that they are associated with multiple mating, i.e. mating between F⁻ cells and several Hfr cells.

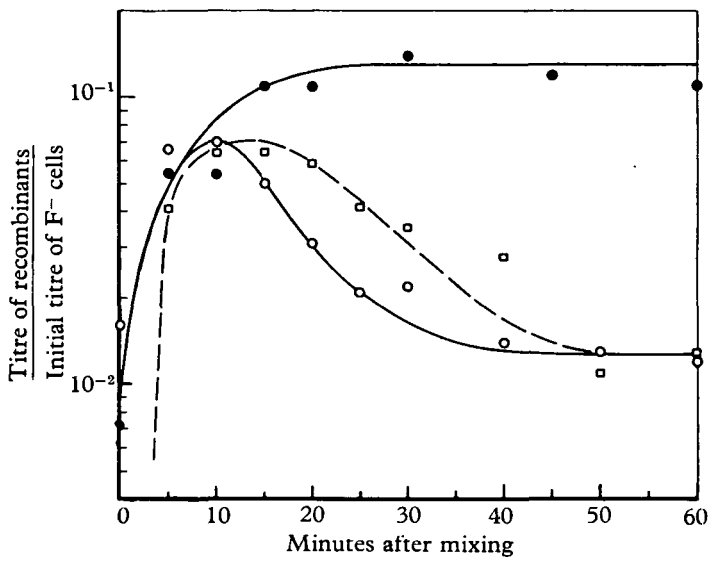

Fig. 2. The effect of duration of mating on the yield of recombinants. Mixtures containing $2{\cdot}0 \times 10^8$ cells/ml. of F⁻W1:M⁻P⁻ or F⁻W1:TL⁻M⁻ and a four-fold greater concentration of HfrR4 cells were incubated on a rotor; samples were withdrawn at intervals and diluted 1 in 5000 into warm broth. In the case of the mixture involving W1:M⁻P⁻ bacteria, an aliquot of each sample was withdrawn immediately after dilution and blended. The unblended and blended suspensions were assayed for recombinants one hour after the start of the experiment. The numbers of TL⁺Sʳ recombinants (W1:TL⁻M⁻ recipient) have been doubled for direct comparison with the numbers of P⁺Sʳ recombinants (W1:M⁻P⁻ recipient).

● W1:TL⁻M⁻ recipient
○ W1:M⁻P⁻ recipient; not blended
□ W1:M⁻P⁻ recipient; blended

Examination of such mixtures with the light microscope shows that clumps of donor and recipient cells occur in all the mixtures. The difference between the different combinations could be accounted for if some damage occurred to F⁻ cells, particularly to those of strain W1:M⁻P⁻, which mate with several cells of an Hfr strain of the R4 group but not of the P10 group. One would expect the yield of recombinants to deviate from linearity at donor:recipient ratios below unity since even when the F⁻ cells are more numerous than the Hfr cells many multiple mating complexes would be formed during the 60-min. mating period.

The role of multiple mating is confirmed by the experiment presented in Fig. 2. Cells of F⁻ W1:M⁻P⁻ or F⁻ W1:TL⁻M⁻ were mixed with a high concentration of HfrR4 cells, and samples diluted at intervals to prevent further contact formation. At the end of the experiment each of the diluted samples was assayed for recombinants. It may be seen that with W1:TL⁻M⁻ as recipient the number of recombinants in successive samples rises rapidly from the time of mixing and reaches a plateau after about 25 min. With W1:M⁻P⁻, on the other hand, the curve rises for the first 10 min. only; thereafter it falls progressively, indicating that the formation of additional contacts reduces rather than increases the probability of recombinant formation.

Additional information may be obtained by separating the mating cells in the successive samples from the mixture involving F⁻ W1:M⁻P⁻ by blending immediately after dilution (dashed curve, Fig. 2). The results show that the number of recombinants in the blended samples rises after a brief lag corresponding to the minimum time required to transfer the P⁺ marker, reaches a maximum after about 15 min., and thereafter decreases as in the unblended samples. The significant point is that from 15 min. until about 40 min. after mixing, samples which are blended immediately after dilution yield *more* recombinants than those left indisturbed. Thus the damage associated with multiple contact formation only occurs some time after dilution, i.e. after formation of the contacts.

The time required for irreversible damage to occur has been determined by diluting a sample of the mating mixture 20 min. after mixing and blending aliquots of the diluted suspension at intervals to ascertain the time when blending no longer 'rescues' any recombinants. It has been found that damage is complete by about 20 min. after dilution.

(ii) *The viability of F⁻ cells in mixtures with high concentrations of Hfr cells*

Hayes (personal communication) has observed a reduction of the viable count of cells of the F⁻ strain W677 to 1% of the initial value after 90 min. incubation with a 200-fold excess of HfrH bacteria. Some loss of F⁻ viability has also been reported recently by Zwaig *et al.* (1962) in crosses involving lower concentrations of the same Hfr strain, but not with other Hfr strains tested. It was noted in the present experiments that the number of non-recombinant F⁻ cells which form microcolonies on selective platings of mixtures of cells of F⁻W1:M⁻P⁻ and HfrR4 decreased progressively with increasing Hfr concentration. This suggested that the effects of multiple mating on recombinant formation were due to damage to the F⁻ cells.

Clowes (1963) has studied this question and has demonstrated a progressive loss of viability of cells of strain W1:M⁻P⁻ in mixtures with certain strains of the R4 group but not of the P10 group. In the experiment presented in Table 1 we have measured the F⁻ viable count and yield of recombinants in pairwise mixtures of Hfr strains P10 and R4 and F⁻ strains W1:M⁻P⁻ and W1:M⁻P⁻lac⁻. The latter strain behaves like F⁻W1:TL⁻M⁻ and was employed in its place so that identical types of recombinant could be selected with the two F⁻ strains.

It may be seen that there is a marked loss of F^- viability in the mixture of F^-W1:M$^-$P$^-$ and HfrR4 but not in any of the other combinations. The differences between the yields of recombinants in the other combinations is nonetheless reflected in differences in F^- viable count. Thus, in the mixture of F^-W1:M$^-$P$^-$lac$^-$ with HfrP10, the F^- count increases seven-fold during the period of incubation, whereas with HfrR4 it remains stationary. (The fact that the number of recombinants in the former mixture is greater than the initial number of F^- cells is probably

Table 1. *Yield of recombinants and F^- viable count in mixtures containing a high concentration of Hfr cells**

F^-	Hfr	No. of recombinants per initial F^- cell × 100	Viable F^- cells at 90 min. / Viable F^- cells at 0 min. × 100
W1:M$^-$P$^-$	R4	0·4	11
W1:M$^-$P$^-$	P10	29	113
W1:M$^-$P$^-$lac$^-$	R4	15	117
W1:M$^-$P$^-$lac$^-$	P10	165	706

* Mixtures containing $7{\cdot}0 \times 10^6$ F^- cells/ml. and $3{\cdot}0$–$5{\cdot}0 \times 10^8$ Hfr cells/ml. were incubated for 90 min. P^+S^r recombinants were selected in mixtures involving HfrR4 and M^+S^r recombinants in those involving HfrP10.

due to cell multiplication and continuous mating during the long period of incubation.) It may also be noted that at the high Hfr concentrations used in this experiment an F^- dependent difference in the yield of recombinants was observed in crosses involving HfrP10, and that here again the difference was reflected in the extent of growth of the F^- cells.

4. DISCUSSION

The efficiency of recombinant formation in mixtures containing relatively high concentrations of Hfr cells has been shown to depend on the combination of strains employed. In mixtures involving Hfr strains of one group (P10 type) the yield of recombinants increases linearly with Hfr concentration until the numbers of Hfr and F^- cells are roughly equal, whereas with the other group of Hfr strains (R4 type) the yield of recombinants ceases to increase once there are about one-fifth as many Hfr as F^- cells in the mixture; with most F^- strains it remains constant from then on, but with one particular strain, F^-W1:M$^-$P$^-$ it falls progressively.

We have confirmed the observation of Clowes (1963) that the progressive fall in yield of recombinants in the latter combination is due to loss of F^- viability, and have presented evidence that this results from mating of the F^- cells with more than one Hfr cell. The minimum number of Hfr cells required to kill an F^- cell is not known. The differences between the results with the other combinations of strains are also correlated with the extent of F^- growth, though no loss of viability has been detected.

At present little can be said concerning the nature of the damage to the F^- cells which results from multiple matings. It does not occur until about 20 min. after the

initiation of mating and is independent of the chromosomal segment transferred with high frequency by the Hfr cells. It may be caused either by the cumulative transfer of some material from the Hfr cells; or by some effect associated with the completion of cellular union between the F^- cell and the Hfr cells with which it is paired. In the latter case the effect of multiple contact formation may be analogous to the phenomenon of 'lysis from without' which occurs when numerous phage particles attach to the same bacterial cell (Delbrück, 1940). It is perhaps significant that stationary phase F^- cells are more sensitive to conjugal killing than log phase cells (Clowes, personal communication), a situation which also holds for killing of *E. coli* cells by the lytic enzyme extracted from phage T2 (Weidel & Primosigh, 1958).

The grouping of Hfr strains with respect to the extent of damage which they cause is not correlated with the position of the origin or direction of transfer of their chromosome during conjugation. Nor is it related to the stability of the mating pairs formed in broth (de Haan & Gross, 1962) or with their 'stringent' or 'relaxed' character (Stent & Brenner, 1961). The differences are stable, and in the few cases when this has been examined, different derivatives of the same Hfr have been found to show the same behaviour. If the analogy between the formation of cellular union in conjugation and phage attachment is valid, the grouping could reflect differences in the nature of the connection which the Hfr cells form with F^- cells. F^- strain W1:M^-P^- is a recombinant derived by successive crosses from the original W1 strain. Like W1, this strain is stringent in its requirement for amino-acids for RNA synthesis; its unusual sensitivity could be due to some peculiarity of its cell wall or membrane.

6. SUMMARY

In crosses in which the ratio of Hfr to F^- cells was varied it was found that with one group of Hfr strains the yield of recombinants increased linearly with donor concentration until the number of donor and recipient cells were approximately equal. With other Hfr strains marked deviations from linearity were observed at Hfr:F^- ratios greater than 1:5 and in crosses with one particular F^- strain the yield of recombinants actually decreased progressively as the Hfr concentration was raised beyond this value. The deviations from linearity observed with this group of strains have been shown to be due to damage to F^- cells which mate simultaneously with several Hfr cells.

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