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The effect of unbalanced growth on recombinant formation in *E. coli*

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1. INTRODUCTION

During conjugation between Hfr and F^- cells of *E. coli* K12 the Hfr transfers its chromosome in an oriented manner to the F^- cell. Transfer of the donor chromosome starts at the same extremity in all cells of a given Hfr type and requires approximately 120 min. for its completion (Wollman & Jacob, 1958). The conjugating cells generally separate before the entire chromosome has been transferred so that the zygotes produced carry only a fragment of the Hfr chromosome. These zygotes may give rise to stable genetic recombinants as a result of exchange between the donor fragment and the homologous region of the recipient chromosome.

The probability that a donor marker which has been transferred to an F^- cell will be incorporated into a genetic recombinant, has been shown to be approximately 0·5 under favourable conditions (Jacob & Wollman, 1961; de Haan & Gross, 1962). Several authors have observed that the yield of recombinants depends upon the medium upon which the zygotes are plated (Jacob & Wollman, 1961; Riley & Pardee, 1962). In the present paper it will be shown that the probability of genetic recombination is reduced if zygotes are transferred from a nutrient-rich to a synthetic medium. The results obtained indicate that recombination can only occur during a limited period after zygote formation. Under optimal conditions it is complete within an hour.

2. MATERIALS AND METHODS

The origins and direction of transfer of the various Hfr strains employed are indicated in Fig. 1 which also gives the symbols for the relevant genetic markers. The sources of these strains, and their genetic characters, as well as those of the F^- strains used, have been described previously (de Haan & Gross, 1962), with the exception of HfrR3:$TL^-B_1^-$ isolated by Reeves (personal communication). All Hfr strains were sensitive to streptomycin (S^s) and to phage T6 ($T6^s$), and all F^- strains were resistant to streptomycin (S^r) and to phage T6 ($T6^r$).

The culture media and methods of maintaining bacterial strains and preparing

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fresh cultures were described by de Haan & Gross (1962). A glucose-salts medium, M9, prepared according to Adams (1959, p. 446) was also employed.

For crosses, appropriate volumes of cultures of donor and recipient cells were mixed and incubated on an inclined turntable rotating at approximately 33 r.p.m. to allow formation of cellular union and the transfer of genetic material from donor to recipient cells. The number of recombinants formed from such mixtures was determined by plating duplicate 0·1 ml. samples, appropriately diluted, on to selective plates. Unless otherwise stated dilution was carried out with broth. All plates of selective medium contained 100 μg. per ml. of streptomycin to kill the Hfr cells. Viable counts were determined on nutrient plates with or without streptomycin.

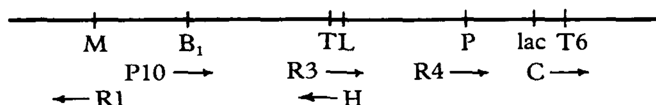

Fig. 1. The order of various loci on the chromosome of *Escherichia coli* K-12. The relative positions of the loci are only approximate, and the region shown constitutes about one-quarter of the chromosome. The position of the origins and the direction of transfer of the Hfr strains employed are indicated by the arrows. The symbols of the markers represent:

M = methionine, B_1 = vitamin B_1, T = threonine, L = leucine, P = proline } synthesis

T6 = phage T6: resistance (r) or sensitivity (s)
lac = lactose fermentation

The markers T and L are used throughout as one marker, TL.

The two methods used to interrupt genetic transfer have been described (de Haan & Gross, 1962).

Cell suspensions were filtered by means of 2 cm. diameter Oxoid membrane filters, employing negative pressure.

All experiments were performed at 37° C.

3. RESULTS

In the experiments reported in Table 1 donor and recipient cells were incubated together in broth for an hour, and treated with phage T6 to kill the Hfr cells. The mixtures were then diluted a thousand-fold in buffer and plated with the addition of small amounts of different supplements on medium selective for $TL+S^r$ recombinants. It may be seen that in both experiments cells plated without any supplements gave rise to fewer recombinants than those plated with broth supplement. Supplementation with low concentrations of threonine and leucine, the auxotrophic requirements of the recipient cells, did not increase the yield of recombinants. On the other hand vitamin-free casein hydrolysate, a mixture of free amino-acids, was as effective as broth in raising the yield of recombinants. The

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difference between the increments in the experiments may be due to variation in the level of impurities in the agar.

Table 1. *The effect of various supplements on the yield of recombinants from broth grown cells**

Samples plated with 0·1 ml. of:	No. of TL + S^r recombinants per plate: Expt. 1	Expt. 2
No addition	147; 137	65; 66
Threonine and leucine, 0·05 mg./ml. each	—	65; 74
Threonine and leucine, 1 mg./ml. each	134; 141	55; 58
Nutrient broth, 0·25 mg./ml.	150; 125	—
Nutrient broth, 5 mg./ml.	229; 235	—
Nutrient broth 25 mg./ml.	313; 348	402; 360
Vitamin-free casein hydrolysate, 10 mg./ml.	355; 336	—

* The parental strains employed were F^-W1:$TL^-B_1^-$ and HfrR4:M^-.

Since the Hfr cells were killed with phage T6 immediately before plating, supplementing the plates cannot lead to an increase in the number of zygotes. Instead it must affect either the efficiency of the recombination process or the expression of the recombinants after their formation. The latter possibility appears to be ruled out since the specific growth requirements of the recipient cells, viz. threonine and leucine, were entirely ineffective in raising the yield of recombinants (Table 1), despite the fact that the addition of these amino-acids permits all the F^- cells to undergo sufficient residual divisions on the selective medium to form visible microcolonies. It appears therefore that the failure of a sizeable fraction of the zygotes to yield recombinants on unsupplemented plates is due to inhibition of some step in recombinant formation as a result of the shift from an amino-acid rich medium to one lacking amino-acids.

This view is confirmed by the results in Table 2 which show that broth supplementation has little or no effect on the yield of recombinants when cells are grown

Table 2. *The effect of the medium in which the parental cells are grown and mated on the yield of recombinants on unsupplemented medium**

Medium in which cells were grown and mated	No. of TL + S^r recombinants per initial Hfr cell on plates with broth supplement	Ratio of numbers of TL + S^r recombinants on plates with and without broth supplement
Broth	0·41	2·38
M9 glucose-salts medium	0·51	1·25

* The parental strains employed were F^-W1:TL^-M^- and HfrH:B_1^-.

and mated in synthetic medium; under these conditions the yield of recombinants is as high as when cells are mated in broth and plated with broth supplement. The reduction in the probability of recombinant formation when zygotes which have

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been formed in broth are shifted to synthetic medium without supplement is thus associated specifically with the unbalanced growth (Kjeldgaard *et al.*, 1958) induced by the shift.

In the above experiments the effect of supplementation on the yield of recombinants was examined immediately after arresting transfer with phage T6. It appeared of interest to study the effect of shifting cells from a rich to a poor medium at intervals after formation of the zygotes. Hfr and F^- cells were therefore mixed in broth and incubated for 30 min. to allow zygote formation. Mating was then stopped by adding phage T6 to kill the Hfr cells and 10 min. later the mixture was diluted

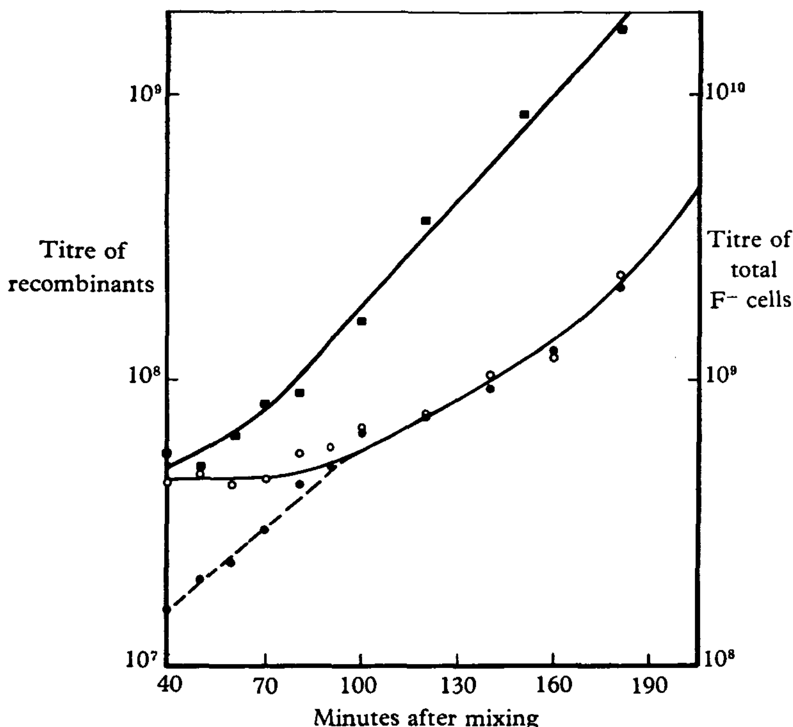

Fig. 2. The yield of TL + S^r recombinants from cells plated with and without broth at various times after zygote formation. Cultures of F^-W1:TL$^-$$B_1^-$ and HfrR4:M$^-$ grown in broth to *ca.* 5×10^8 cells/ml., were centrifuged, resuspended in the same volume of fresh broth and mixed. The mixture was incubated on a rotor for 30 min. and T6 added to stop further mating. Ten minutes later the mixture was diluted fifty-fold into warm broth and at intervals samples were withdrawn, diluted twenty-fold either with buffer or with broth and 0·1 ml. samples plated on selective medium containing methionine and B_1. F^- viable count was essayed on nutrient plates containing streptomycin.

○ Titre of TL + recombinants from samples diluted in broth
● Titre of TL + recombinants from samples diluted in buffer
■ Titre of total F^- cells

Yields of recombinants and total F^- viable counts are plotted from the time of dilution, i.e. 40 min. after mixing the Hfr and F^- cells.

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into fresh broth and incubated. Samples of the dilute suspension were withdrawn at intervals either into buffer or into broth and aliquots plated to determine the number of recombinants produced.

The results of such an experiment are shown in Fig. 2. It may be seen that the number of recombinants from samples plated with broth remains constant for almost an hour after dilution, i.e. 100 min. after mixing, and then begins to increase (see also Tomizawa, 1960). Samples diluted in buffer at first yield only one-third as many recombinants; however, the yield increases rapidly in successive samples and by about an hour after dilution exposure to the shift no longer has any effect.

4. DISCUSSION

We have shown that when zygotes are transferred from an amino-acid rich medium to one lacking amino-acids the probability that they will give rise to genetic recombinants is reduced. If the zygotes are first incubated in broth for an hour the yield of recombinants is no longer affected by the shift. Similar observations have been made by Witkin (1956) on the yield of ultraviolet induced prototrophic revertants.

In order to account for our observations it is not sufficient to suppose that the temporary imbalance induced by the shift results in inhibition of genetic recombination since recombination would then occur when the cells recovered from the shift and no reduction in the yield of recombinants would be observed. However, if one supposes in addition that the chromosome fragments transferred to the zygotes can only persist for a limited period, after which they are subject to some kind of destructive process then the effect of the shift is easily accounted for: by the time the cells recover their ability to bring about genetic recombination after the shift only a fraction of them will contain an intact donor fragment, and consequently fewer recombinants will be formed. Destruction of the donor fragment may also account for the fact that under optimal circumstances the probability of genetic recombinant formation does not appear to exceed 0·5 (Jacob & Wollman, 1961; de Haan & Gross, 1962), and for the fact that recombinant colonies generally contain only one recombinant type (Lederberg, 1957; Tomizawa, 1960).

5. SUMMARY

When zygotes formed by conjugation between Hfr and F^- cells of *E. coli* are transferred from an amino-acid rich medium to one lacking amino-acids the probability of genetic recombination is reduced. This result is interpreted as indicating that recombination can only occur during a limited period after zygote formation and that the metabolic imbalance caused by the change of medium leads to temporary inhibition of recombination. By an hour after zygote formation the yield of recombinants is no longer affected by transfer to amino-acid free medium.

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