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Amino acid deprivation and its effect on mating ability in *Escherichia coli* K12

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1. INTRODUCTION

Two principal mechanisms have been proposed to account for chromosome transfer in *Escherichia coli* K12. Chromosome replication in *E. coli* is initiated from a fixed point, and normally proceeds by means of a single replication point in a predictable order around the circular chromosome (Lark, Repko & Hoffman, 1963; Pritchard & Lark, 1964). One mechanism for transfer, proposed by Bouck & Adelberg (1963), suggests that only chromosomes which have terminated replication can be transferred by a mechanism independent of DNA synthesis. The other, suggested by Jacob & Brenner (1963), implicates DNA synthesis as the motor by which one daughter chromosome is transferred to the recipient cell. Most of the accumulated evidence predominantly favours the Jacob and Brenner model (see Ptashne, 1965; Fulton, 1965; Hollom & Pritchard, 1965, and Gross & Caro, 1966). However, Suit, Matney, Doudney & Billen (1964) have presented evidence claiming to show that DNA synthesis is unnecessary for genetic transfer in *E. coli* K12. Two experimental approaches were used by them. It was shown that donor cells irradiated with ultra-violet light can still transfer markers with the same kinetics as unirradiated donor cells, and second they found that donor cells starved of amino acids transfer markers with essentially the same kinetics as unstarved cells.

It is known from the work of Lark, Repko & Hoffman (1963) that cells of *E. coli* (strain 15T$^-$) starved for amino acids, although they can complete the current round of DNA synthesis, are unable to initiate another. Suit *et al.* (1964) were able to show that the Hfr donor cells used in their experiments behaved similarly and synthesized only 10–15% more DNA after removal of their required amino acid, histidine. The starved cells were found to exhibit transfer kinetics similar to unstarved control suspensions when mated with either an F$^-$*arg*$^-$ or an F$^-$*pro*$^-$ strain. In both cases mating took place on membrane filters using a multiplicity of *c.* 10F$^-$ cells to each Hfr cell. In this way mating cells were maintained in close contact and at high concentrations; however, under these conditions considerable opportunities exist for cross-feeding of the donor cells by the recipient cells.

We have repeated the experiment of Suit *et al.* (1964) by starving donor cells for methionine and found much the same result except when the experiment was performed under conditions where cross-feeding could not take place. Krisch & Kvetkas (1966) have recently reported the same findings using the bacterial strains originally employed by Suit *et al.* (1964).

2. MATERIALS AND METHODS

All media used have been described previously (Fisher, 1966) except that amino-acid supplements have been varied as necessary.

Total cell counts were performed by means of a Coulter Counter, Model A, fitted with a 30 μ aperture.

Hfr strains: 55, Hfr *met*$^-$ (Hayes, 1953); 146, *met*$^-$*his*$^-$ obtained from 55 by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) after the method described by Adelberg, Mandel & Chen (1965).

F$^-$ *strains:* 132 (*thr*$^-$*leu*$^-$*his*$^-$*B*$_1^-$*str-r*) (Fisher, 1966); 141 (*thr*$^-$*leu*$^-$*met*$^-$*pro*$^-$*thy*$_2^-$) was kindly supplied by Professor R. H. Pritchard.

3. RESULTS

Logarithmically growing cells of strain Hfr 55 were washed on a membrane filter, resuspended in unsupplemented M9 medium and starved of amino acids by incubation at 37°C. for various periods of time. Duplicate samples were mixed with washed cells of 132 F$^-$ and the cells of one sample were collected on a membrane which was incubated for 30 min. at 37°C. The other sample was held at 37°C. for 30 min. Dilutions were plated on supplemented minimal medium to count the number of recombinant colonies. The results are shown in Table 1. There was a small increase (*c.* 20%) in cell number during

Table 1. *Mating between amino-acid starved cells of Hfr 55 and unstarved cells of 132 F*$^-$

Period of starvation (min.)	*thr*$^+$*leu*$^+$ recombinants per 10^3 Hfr cells	
	On filter	In buffer
0	100	84
20	133	74
60	126	44
90	39	20

Bacteria were grown aerobically in complete M9 medium (Fisher, 1966), harvested by filtration (Schleicher & Schüll, type B6, unbacked) and resuspended in unsupplemented M9 medium. The suspension of Hfr 55 was held at 37°C. with aeration. The suspension of 132 F$^-$ was kept in an ice bath until required. At the times indicated the bacteria were counted in a sample removed from the suspension of Hfr 55, and duplicate mixtures consisting of 0·1 ml. Hfr 55 and 1·0 ml. 132 F$^-$ were prepared. The bacteria in one of each pair were collected on a membrane filter (Millipore HAWP) which was incubated in a water-saturated atmosphere at 37°C. for 30 min. The unfiltered mixture was held at 37°C. for 30 min. After incubation the cells on the membrane were resuspended in 1·1 ml. of buffer and dilutions were plated on selective medium. Recombinants colonies were counted after 24 hours' incubation at 37°C.

the course of the starvation treatment. With cells mated on membranes no appreciable decrease could be observed in the frequency of recombinants per Hfr cell up to 60 min. One can suggest that although the cells used in the experiment were nominally starved, since they were placed in high concentrations on the membrane filter they were in a position to obtain nutrient by cross-feeding. The contrast between data (Table 1) for crosses performed on membranes and in buffer supports this idea.

To test the possibility that the results which had been obtained might be due to cross-feeding, the same experiment was repeated using an F$^-$ strain unable to cross-feed the donor cells, viz. 141 F$^-$. Table 2 shows the number of *thr*$^+$*leu*$^+$ recombinants observed. It is evident from these results that where cross-feeding cannot take place, amino-acid starved donor cells cannot form zygotes. The fact that cells used in the first experiment were able to mate indicates that recovery from the amino-acid starved infertile condition

Table 2. *Mating between amino-acid starved cells of strain Hfr 146 and unstarved cells of 141 F⁻*

Period of starvation (min.)	*thr*⁺*leu*⁺ recombinants per 10^3 Hfr cells	
	On filter	In buffer
0	84	2
45	4·5	3·2
90	< 1·0	< 1·0

See Table 1 for experimental procedure.

can take place within 30 min., because of cross-feeding. We believe that the explanation of the results obtained by Suit *et al.* (1964) lies in their failure to take precautions to prevent cross-feeding.

4. DISCUSSION

The results described here are important for a number of reasons. There have been two proposals made for the mechanism by which the chromosome of *Escherichia coli* K12 is transferred during mating, the DNA synthesis-dependent model of Jacob & Brenner (1963) and the model of Bouck & Adelberg (1963) which postulates transfer of completed chromosomes by an independent mechanism. It is known from the work of Lark, Repko & Hoffman (1963) that in the absence of amino acids, cells complete the current round of DNA replication but do not initiate a new cycle. Amino-acid starved cells would thus have completed a round of replication but be unable to initiate a new round of DNA replication when mated. Thus if initiation of DNA synthesis is required for transfer of genetic material between mating cells one would not expect such transfer to occur with cells effectively starved of amino acids.

If it can be proved that the Jacob & Brenner model (1963) correctly describes the mechanism of transfer, another interesting question is raised. The work of Nagata (1963) suggested that replication of the chromosome of *E. coli* K12 was initiated from the sex-factor, F, and proceeded in an order opposed to that of transfer of markers during mating. Thus to reconcile Nagata's result with the conclusion which logically follows about direction of replication during mating it was necessary to postulate that 'mating-induced replication' operated differently from the replication involved in cell growth. However, it appears from the results presented here and by Hollom and Pritchard (1965) that certain characteristics are the same, viz. both types of replication are sensitive to nalidixic acid and withdrawal of amino acids for initiation. It would therefore appear necessary to re-examine Nagata's conclusion. One must still explain why mating induced replication requires *c.* 90 min. for completion whereas normal replication for cell division takes only about 40 min. It may be that the cell possesses a mechanism which can distinguish replication induced for transfer from that induced for cell division and impose a restraint on the rate of completion of the transfer process.

SUMMARY

The conclusion by Suit, Matney, Doudney & Billen (1964) that Hfr donor cells of *Escherichia coli* K12, starved of required amino acids can mate, has been re-examined. It appears that their conclusion is not valid and that apparent fertility of amino-acid starved cells is due to cross-feeding by the F⁻ cells. The relationship of this result to the alternative mechanisms for chromosome transfer in *E. coli* is discussed.

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