INHIBITION OF BACTERIAL MATING BY AMINO ACID DEPRIVATION *

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A variety of recent experiments indicate that the DNA transferred during bacterial mating by Hfr and F' donor strains of Escherichia coli K12 replicates before entering the recipient cell. Gross and Caro (1965) have recently presented an excellent review of this subject. The published work is generally consistent with the model of Jacob, Brenner, and Cuzin (1963) for chromosome transfer, in which the donor chromosome is replicated as it is transferred.

However, the results of experiments involving the effect of inhibitors of DNA synthesis on bacterial conjugation have not been consistent. Two such inhibitors, phenethyl alcohol (Roeser and Konetzka, 1964) and nalidixic acid (Hollom and Pritchard, 1965), have been reported to markedly inhibit chromosome transfer by Hfr strains, as demanded by the model of Jacob, Brenner, and Cuzin (1963). On the other hand Suit, Matney, Doudney, and Billen (1964) have reported that chromosome transfer by one histidine deficient strain, Hfr G6, is completely unaffected by deprivation of histidine, with or without ultraviolet irradiation. Under these conditions, the synthesis of DNA by this Hfr strain is severely inhibited.

Evidence is presented here that the results obtained by Suit et al. (1964) arose from the fact that their mating conditions did not completely deprive the Hfr cells of histidine. The present data indicate that the ability of Hfr G6 to act as a genetic donor is greatly reduced by strict amino acid deprivation.

MATERIALS AND METHODS

The strains of E. coli K12 used in matings were Hfr G6 his^-_{323} $DNA\text{-}C^-$ str^s and F^- 1021 arg^-_6 thi^- str^r, both kindly supplied by Dr. Thomas S. Matney, and F^- 1021 arg^-_6 thi^- str^r his^-, a UV-induced mutant prepared in our laboratory from the above recipient strain. Hfr H arg was used in comparative measurements of DNA synthesis.

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Cultures were grown in synthetic M9 medium (Anderson, 1946) supplemented with 1 μg/ml of thiamine and with 20 μg/ml of L-histidine and/or 70 μg/ml of L-arginine as required. All matings were performed with log phase cultures (about 10^8 cells/ml) washed by membrane filtration and resuspended in refrigerated M9 medium supplemented only with thiamine. Hfr cells were starved for histidine by incubating a resuspended culture for one hour at 37° C. Viable counts were made on nutrient agar plates. All plate assays were performed using the soft agar overlay technique.

For liquid matings, resuspended refrigerated cultures were mixed to give a tenfold excess of recipient cells, supplemented with desired amino acids, rapidly warmed to 37° C, and incubated for 60 minutes. Following dilution, the mating was interrupted by mechanical agitation and aliquots were plated onto M9 agar (1.5%) plates supplemented with thiamine, histidine, and streptomycin, to select for *arg*$^+$ *str*^{r} recombinants.

For membrane filter matings, approximately 7×10^7 resuspended donor cells and 7×10^8 resuspended recipient cells were mixed at 4° C and brought down on a 25 mm filter (0.45 μ pore size, Millipore Filter Corp.). After two washings the filter was transferred to a prewarmed M9 agar plate supplemented with thiamine and any desired amino acids at the above concentrations. After 60 minutes the cells were suspended, diluted, blended and plated, as outlined by Matney and Achenbach (1962).

The rate of DNA synthesis was measured by pulse labelling with tritiated thymidine (Thymidine-Methyl-H^3, 30 μc/ml, 360 mc/mM, New England Nuclear Corp.) following the general procedure outlined by Ecker (1965).

RESULTS AND DISCUSSION

Suit *et al.* (1964) have reported that Hfr G6 *his*$^-$ increases in total DNA by 10-15% during the first 30 minutes with no further increase, when incubated in the absence of histidine. Suit, Goldschmidt, and Matney (1965) postulate that this property is due to a specific mutation which prevents the completion of the DNA replication cycle in the absence of a required amino acid. We have confirmed this property of Hfr G6 by following the rate of incorporation of tritiated thymidine as a function of the duration of prior histidine starvation Figure 1 shows that the rate rapidly decreases to low levels during the first half hour of histidine starvation. For comparison, Fig. 1 shows the much more gradual decline in uptake of tritiated thymidine by Hfr H *arg*$^-$, a typical *DNA-C*$^+$ strain, during arginine starvation. In other experiments we have found that adding histidine to a culture of Hfr G6 after one hour of histidine starvation brings about an immediate resumption in the uptake of H^3-thymidine. The prestarvation rate of uptake is achieved within 20 minutes.

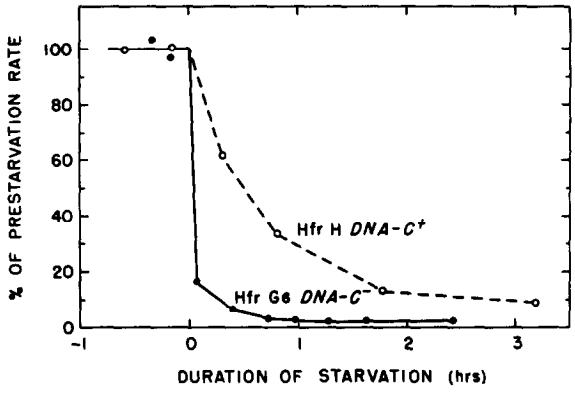

Fig. 1. Rate of incorporation of H^3-thymidine by each of two auxotrophic Hfr strains of E. coli K12 as a function of duration of amino acid starvation. Exponentially growing cultures in amino acid-supplemented M9 medium were rapidly resuspended at time 0, by membrane filtration, into prewarmed unsupplemented M9 medium. Samples were removed periodically, incubated for 5 minutes at 37° C with tritiated thymidine, and extracted with cold 5% trichloracetic acid. Rate is defined as cpm incorporated per min exposure to the isotope per unit optical density and expressed as % of the prestarvation rate. ●——●, Hfr G6 his⁻ DNA-C⁻; O----O, Hfr H arg⁻ DNA-C⁺.

In attempting to repeat and extend the mating experiments of Suit et al. (1964) we first compared matings of Hfr G6 his⁻ and F⁻ 1021 arg⁻ in liquid medium with matings on membrane filters. The liquid matings gave very consistent results, indicating that:

1. If histidine is present in the mating mixture normal mating efficiencies (25-40 arg⁺ str^r recombinants per 100 input Hfr cells) are obtained regardless of whether or not the Hfr cells have been prestarved for histidine.

2. If histidine is absent from the mating mixture much lower mating efficiencies (0-2 recombinants per 100 Hfr cells) are obtained whether or not the Hfr cells have been prestarved for histidine.

When matings were performed on membrane filters the results were variable. In some experiments very high mating efficiencies were obtained for both starved and unstarved Hfr cells with no histidine present in the mating plate, as reported by Suit et al. (1964). Other apparently identical experiments gave results similar to those from our liquid matings; recombinant formation was severely inhibited when histidine was absent during mating.

These discrepancies suggested the possibility that in the intimate association of a membrane filter mating the his⁺ recipient cells (present in extremely high local concentration) can, in some circumstances, supply enough histidine to satisfy the requirement of the his⁻ Hfr donors. To reduce the likelihood of such cross-feeding in filter matings a mutant was isolated from F⁻ 1021

which was auxotrophic for histidine as well as arginine. When Hfr G6 was mated with this strain similar results were obtained from both liquid and membrane filter matings. These results, shown in Table I, are consistent with the results obtained from liquid matings involving the his^+ recipient.

TABLE I

Histidine starvation of Hfr before mating	Amino acid supplementation of mating medium	Recombinants per 100 Hfr cells, liquid mating	Recombinants per 100 Hfr cells, filter mating
None	None	0.9	1.1
None	Histidine	27.3	39.4
None	Histidine + arginine	26.3	32.4
1.0 hour	None	0.3	1.6
1.0 hour	Histidine	36.9	33.0

Matings involve Hfr G6 his^- str^s and F^- 1021 arg_6^- str^r his^-. This Hfr transfers the arg_6 marker at about 10 minutes. All matings were mechanically interrupted after 60 minutes and samples assayed for arg^+ str^r recombinants after dilution.

The data of Table I show that under our experimental conditions recombinant formation is severely depressed when histidine is not present in the mating medium. In the presence of histidine normal numbers of recombinants are formed even when the Hfr cells have been incubated without histidine for one hour prior to mating. Supplementation of both amino acids (arginine and histidine) required by the F^- strain is not necessary for normal recombinant formation as long as the requirement (histidine) of the Hfr donor is met. This makes it unlikely that histidine deprivation has any effect on genetic integration in the recipient cell.

We conclude that the ability of Hfr G6 his^- $DNA\text{-}C^-$ to act as a genetic donor is inhibited in a reversible manner by the removal of histidine from the medium. Since DNA synthesis by this Hfr is rapidly and reversibly inhibited by the removal of histidine, these results are consistent with the predictions of the model of Jacob, Brenner, and Cuzin (1963). However, our experiments do not directly relate chromosome transfer to DNA synthesis. The possibility remains that histidine deprivation inhibits a step in mating prior to chromosome transfer.

SUMMARY

The rapid cessation of DNA synthesis induced in E. coli K12 Hfr G6 his$^-$ DNA-C$^-$ by histidine deprivation, reported by Suit, Matney, Doudney, and Billen (1964), has been confirmed. Evidence is prevented that the ability of this Hfr to participate as a genetic donor in bacterial mating is drastically reduced by histidine deprivation during mating. The conflicting results previously reported by Suit et al. are accounted for in terms of histidine supplementation of donor cells by his$^+$ recipient cells.

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