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Effect of inhibition of DNA synthesis on mating in *Escherichia coli* K12

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1. INTRODUCTION

From studies involving inhibition of DNA synthesis in Hfr strains of *Escherichia coli* K12, either by thymine starvation (Pritchard, 1963) or amino-acid starvation (Suit, Matney, Doudney & Billen, 1964), during mating with F^- strains, it has been concluded that transfer of DNA from males to females can occur in the absence of DNA synthesis. This conclusion is at variance with the hypothesis (Jacob, Brenner & Cuzin, 1963) that the energy required for transfer is derived from the process of DNA replication. On the other hand, a second prediction from this hypothesis, that one polynucleotide chain of the DNA transferred during mating will have been synthesized during transfer, is strongly supported by recent experiments (Ptashne, 1965; Blinkova, Bresler & Lanzov, 1965; Gross & Caro, 1965).

The experiments involving inhibition of DNA synthesis must be regarded as inconclusive for reasons which have recently been enumerated (Gross & Caro, 1965). We have therefore thought it worth while to examine the effect of chromosome transfer of nalidixic acid (NAL) which appears to be a specific and very effective inhibitor of DNA synthesis in *E. coli*.

2. MATERIALS AND METHODS

Strains of *E. coli* K12 used were HfrH *thy*$^-$ *B1*$^-$ λ^- S^S and F^- *thy*$^-$ *thr*$^-$ *leu*$^-$ *met*$^-$ λ^+ S^r. Cultures were grown in synthetic medium (M9). Experiments were performed with log phase cultures washed by filtration immediately before use. All matings were done in M9 supplemented with thymine only.

3. RESULTS

Inhibition of DNA synthesis by NAL in our Hfr strain is specific and reversible as already reported for other strains of *E. coli* by Goss, Deitz & Cook (1965). As indicated (Fig. 1), incorporation of ^{14}C thymine is reduced to barely detectable levels immediately after the addition of NAL. There is an immediate resumption of DNA synthesis at an accelerated rate (cf. thymine starvation, Pritchard & Lark, 1964) if NAL is removed 40 min. later (P. Barth, personal communication). The rate of incorporation of ^3H uracil into RNA was found to be unaltered during 40 min. exposure to NAL at higher concentrations (250 μg./ml.) than those sufficient to stop DNA synthesis.

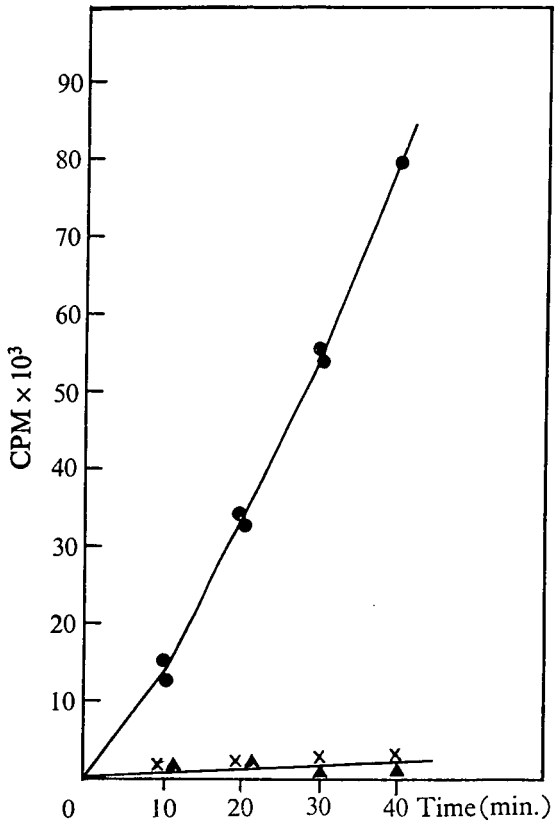

Fig. 1. Inhibition of DNA synthesis by NAL. Hfr (2×10^8/ml.) were suspended in M9 containing ^{14}C thymine (0·07 μC./ml.; 5·4 μg./ml.) and deoxyuridine (100 μg./ml.). Duplicate samples (1 ml.) were assayed at the times indicated by the method of Roodyn & Mandel (1960). ● Control, × 50 μg./ml. NAL, ▲ 100 μg./ml. NAL.

Formation of recombinants during mating was completely inhibited by NAL. A major contribution to this inhibition was found to be a reversible inhibition of chromosome transfer (see Fig. 2). This inhibition of transfer could be due to an effect either on transfer itself or on a hypothetical initiation step which may precede transfer. The following experiment was carried out in an attempt to distinguish between these alternatives (Fig. 3). NAL was added to a mating mixture after entry of *thr*$^+$*leu*$^+$, but before entry of *lac*$^+$, had commenced, and the ratio *lac*$^+$/*thr*$^+$*leu*$^+$, compared with the corresponding ratio in a control mating.

If NAL were to inhibit only initiation of transfer, then males which had already transferred *thr*$^+$*leu*$^+$ markers should continue to transfer the *lac*$^+$ marker with normal frequency; the above ratio should not be affected. This was not found, the ratio dropping to 0·07 compared with 0·5 in the corresponding control cross. This suggests that addition of NAL stops further transfer even in those pairs in which initiation of transfer has occurred. An alternative interpretation of this result, that addition of NAL irreversibly inhibits subsequent integration of donor markers into the recipient genome, cannot be ruled out. This seems unlikely, however, in view of the fact that prolonged exposure of donor and recipient to NAL before transfer does not appreciably alter the efficiency of subsequent transfer and integration (see Table 1).

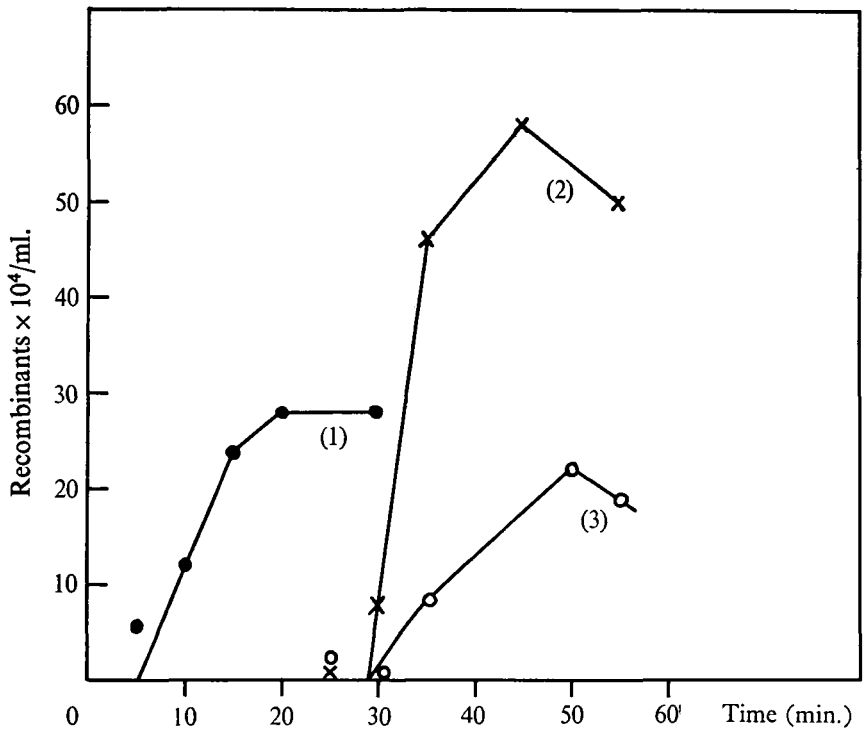

Fig. 2. Inhibition of chromosome transfer by NAL. Curve 1 (●); Hfr ($4{\cdot}5 \times 10^7$/ml.) and F^- ($6{\cdot}2 \times 10^7$/ml.) were mixed in equal volumes, diluted 2×10^{-2} 5 min. later. Curve 2 (×); parents mixed as before and NAL (50 μg./ml.) added 5 min. later. Mixture diluted 2×10^{-2} 25 min. after mixing. Curve 3 (○); parents mixed as before in presence of NAL, diluted 2×10^{-2} after 25 min. All cultures assayed for *thr*⁺*leu*⁺ recombinants after blending at times indicated.

Table 1. *Effect of NAL on pre-existing mating pairs*

	Time (min.)						
	0	10	15	20	25	30	35
Hfr	$2{\cdot}0 \times 10^8$	$2{\cdot}1 \times 10^8$	—	$2{\cdot}4 \times 10^8$	—	$2{\cdot}9 \times 10^8$	—
F^-	$2{\cdot}3 \times 10^8$	$2{\cdot}3 \times 10^8$	$2{\cdot}4 \times 10^8$	—	$1{\cdot}9 \times 10^8$	—	$1{\cdot}6 \times 10^8$
thr⁺*leu*⁺ (−NAL)	—	$2{\cdot}5 \times 10^4$	$4{\cdot}7 \times 10^4$	—	$7{\cdot}7 \times 10^4$	—	$8{\cdot}2 \times 10^4$
thr⁺*leu*⁺ (+NAL)	—	$3{\cdot}2 \times 10^4$	$3{\cdot}1 \times 10^4$	—	$3{\cdot}1 \times 10^4$	—	$1{\cdot}9 \times 10^4$

Hfr ($2{\cdot}2 \times 10^8$ ml.) and F^- ($4{\cdot}5 \times 10^8$ ml.) cells were mixed in equal volumes. After 5 min. NAL (50 μg./ml.) was added to half the culture. At the times indicated samples were diluted 10^{-2} into M9 + thymine, incubated for 60 min., blended and assayed for *thr*⁺*leu*⁺ recombinants and F^- viable titre. Viable titre of Hfr was obtained separately using the same starting culture as in the main experiment.

The principal result shown in Fig. 2 is a reversible inhibition of transfer. However another interesting feature of the experiments shown in Fig. 2 is the difference in the number of recombinants obtained in curves (2) and (3). In both cases the Hfr and F^- cells were exposed to each other at high cell concentrations for an equal length of time.

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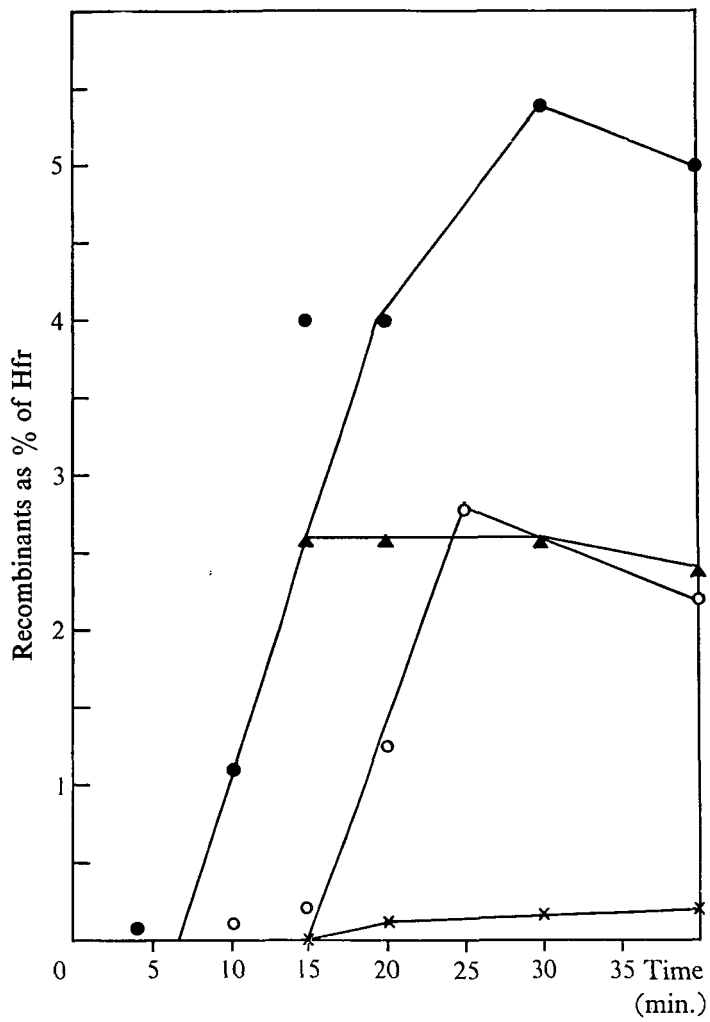

Fig. 3. Hfr ($1{\cdot}0 \times 10^8$/ml.) and F^- ($4{\cdot}7 \times 10^8$/ml.) cells were mixed in equal volumes for 5 min. The mixture was then diluted 10^{-2} (into M9 + thymine) and 50 μg./ml. NAL added to half of the culture at 13 min. At the times indicated, samples were removed, blended and plated for *thr*$^+$*leu*$^+$ and *lac*$^+$ recombinants. ● *thr*$^+$*leu*$^+$ (−NAL); ○ *lac*$^+$ (−NAL); ▲ *thr*$^+$*leu*$^+$ (+NAL); × *lac*$^+$ (+NAL).

In (3) NAL was added at the time of mixing whereas in (2) it was added 5 min. later. The difference in the number of recombinants obtained under the two conditions could be due to an effect of NAL either (*a*) on pair formation, (*b*) on the stability of pairs or (*c*) on integration. The data in Table 1 indicate that exposure to NAL for up to 30 min. has no marked effect on the stability of pre-formed pairs, or on subsequent integration. On the other hand, pre-treatment of both parents with NAL for longer than 10 min. was found to affect subsequent pair formation markedly (see Table 2).

We conclude that the major cause of the inhibition of recombinant formation by NAL is a reversible inhibition of chromosome transfer. Results in agreement with this conclusion have been obtained by Bouck & Adelberg (personal communication). In addition,

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Table 2. *Effect on mating of pretreatment with NAL*

	Time (min.)			
	0	5	10	20
F^-	$2{\cdot}5 \times 10^8$	$2{\cdot}2 \times 10^8$	$2{\cdot}2 \times 10^8$	$1{\cdot}7 \times 10^8$
thr$^+$*leu*$^+$	$8{\cdot}8 \times 10^5$	$9{\cdot}4 \times 10^5$	$8{\cdot}0 \times 10^5$	$4{\cdot}6 \times 10^4$

Hfr ($3{\cdot}2 \times 10^7$ ml.) and F^- ($4{\cdot}2 \times 10^8$ ml.) cultures were treated with NAL (50 μg./ml.) for times indicated before mixing in equal quantities. Six minutes after mixing the culture was diluted 10^{-2} into M9 + thymine, incubated for a further 40 min., blended and plated for *thr*$^+$*leu*$^+$ recombinants.

the data suggest that there is an inhibition of pair formation after prolonged exposure to NAL; an effect which is not associated with a corresponding loss in viability of either parent (see Table 1).

A reversible inhibition of chromosome transfer associated with a reversible inhibition of DNA synthesis is clearly in accord with the hypothesis of Jacob, Brenner & Cuzin (1963). Nevertheless, an unequivocal interpretation of our results cannot be made until the mechanism of inhibition of DNA synthesis by NAL is understood.

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