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Loss of Hfr DNA from *Escherichia coli* merozygotes during inhibition of conjugation by nalidixic acid

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SUMMARY

The effect of nalidixic acid on conjugal recombination was studied in matings with recipient strains carrying *recA200*, a mutation which confers a thermosensitive Rec⁻ phenotype. Addition of nalidixic acid to Hfr $Nal^S \times F^-Nal^R$ *recA200* matings at low temperature (35 °C) caused a sharp 10- to 20-fold decline in the yield of recombinants if plating on selective agar was delayed. Two separate processes were identified as being responsible for this decline. Those merozygotes in which the transferred DNA was free of the donor cell lost the ability to form recombinants through inactivation of this DNA, an effect which could be prevented by using exonuclease deficient (*recB sbcB*) recipients or by prior growth of exonuclease proficient recipients in medium containing 0·25 M sodium chloride. No more than 50% of the observed loss of recombinants could be attributed to this effect. The remaining merozygotes lost their ability for recombinant formation provided mating pairs, and presumably the displaced donor DNA strand, remained intact. This process was thought to involve withdrawal of transferred DNA (DeHaan & Gross, 1962) and was studied in isolation in matings with *recA*⁺, or *recA200 recB sbcB* recipients. A mechanism involving re-annealing of the displaced Hfr DNA to the donor molecule as a result of nalidixic inhibition of gyrase activity in the donor causing relaxation of DNA supercoils is proposed to account for this withdrawal event.

1. INTRODUCTION

Although F-mediated bacterial conjugation is normally associated with new DNA synthesis this is not essential for efficient transfer of a pre-existing single strand of DNA from donor to recipient cell (Sarathy & Siddiqi, 1973; Kingsman & Willetts, 1978). Nevertheless, donor strains very rapidly cease transfer of DNA when matings are exposed to nalidixic acid, a potent inhibitor of bacterial DNA synthesis (Barbour, 1967; Bouck & Adelberg, 1970; Hane, 1971). Recent studies (Gellert *et al.* 1977; Sugino *et al.* 1977) have shown that the cellular target for nalidixic acid is DNA gyrase, an enzyme which catalyses the negative supercoiling of DNA (Mizuuchi, O'Dea & Gellert, 1978). The implication is that gyrase activity is essential during conjugation if the donor strain is to achieve transfer of DNA, but whether it is directly involved in catalysing transfer replication (Kingsman

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& Willetts, 1978), or is simply needed to maintain the donor molecule in a supercoiled form, remains to be established.

During a study of the kinetics of conjugal recombination we discovered that the merozygote population present in Hfr matings after exposure to nalidixic acid is not stable with respect to subsequent recombinant formation. This was particularly noticeable if the recipient strain carried *recA200*, a mutation which confers a thermosensitive recombination-deficient phenotype (Lloyd *et al.* 1974), when it was found that the yield of recombinants declined sharply if plating on agar medium was delayed. We report the investigation of this effect here since the results provide an explanation for the irreversible inactivation of transfer replication by nalidixic acid (Bouck & Adelberg, 1970; Hane, 1971) which is consistent with inhibition of transfer being a reflection of the relaxation of supercoils in the donor molecule following gyrase inhibition (Drlica & Snyder, 1978).

2. MATERIALS AND METHODS

(*a*) *Strains.* The bacterial strains used are listed in Table 1. Genotypic and phenotypic designations follow the recommendations of Bachmann, Low & Taylor (1976) and Demerec *et al.* (1966). The abbreviations Str and Nal, with superscript S for sensitive or R for resistant, describe the response to streptomycin and nalidixic acid, respectively. Strain N1353 was derived from Hfr KL226 by selecting for resistance to 40 μg nalidixic acid (Boehringer) per ml of growth medium (Hane & Wood, 1969).

(*b*) *Media.* LB broth and agar, 56/2 minimal salts agar and dilution buffer, soft agar for overlays, and the supplementation of selective agar plates have been described previously (Lloyd *et al.* 1974; Lloyd & Low, 1976; Lloyd & Johnson, 1979; Low, 1973). Unless specified otherwise, the LB media contained 0·01 M sodium chloride.

(*c*) *Matings.* Liquid matings were conducted in LB broth medium at 35 °C, a temperature at which *recA200* strains exhibit a recombination-proficient phenotype (Lloyd & Johnson, 1979). Exponential cultures (*c.* 2×10^8 cell per ml) grown at 37 °C (donors) or 35 °C (recipients) were mixed in conical flasks in a ratio of 1 donor to 10 recipients, and incubated at 35 °C. Mating was usually terminated after 40 min incubation either by adding nalidixic acid to a final concentration of 5 μg per ml, taking care to avoid disruption of mating aggregates, or by violently agitating the mating mixture in a blending device (Low & Wood, 1965). In the latter case, a sample of the blended mating was immediately diluted 100- to 200-fold into warm (35 °C) fresh medium containing 150 μg streptomycin sulphate (Sigma) per ml to limit further mating. In both cases, incubation was continued for a further 40–80 min. To assay for recombinants or F-prime exconjugants at any time before or after mating had been terminated, samples were removed and, after suitable dilution, pipetted into 3 ml molten overlay agar kept at 42 °C. The overlays were immediately blended before pouring on to suitably selective minimal agar plates. The number of viable recipient cells in mating mixtures was estimated

by plating samples on LB agar containing 150 μg streptomycin per ml, or on minimal agar selective for recipients. Colonies were scored after 24 (LB agar) or 48 h (minimal agar) incubation.

Table 1. Escherichia coli *K-12 strains*

Strain	Genotype*	Source or reference
KL399	F$^-$ *thi-1 metE70 leuB6 proC32 hisF860 thyA54 malA38 lacZ36 ara-14 mtl-1 xyl-5 rpsL109 rpsE2015 sup-78 recA200*	Lloyd *et al.* (1974)
KL425	F$^-$ *thi-1 metE70 leuB6 proC32 thyA54 lacI22 lacZ118 malA38 ara-14 mtl-1 xyl-5 rpsL109 rpsE2015 sup-78 supD$^-$ nalA$^-$*	Lloyd & Low (1976)
KL426	F$^-$, as KL425 but *recA200*	Lloyd & Low (1976)
N1331	F$^-$ *lacI22 lacZ118 proB48 metE90 trpA9605 nalA19 rpsL171*	Lloyd & Johnson (1979)
N1332	F$^-$, as N1331 but *recA200*	Lloyd & Johnson (1979)
N1438	F$^-$ *thi-1 metE70 leuB6 proC32 malA38 lacZ36 ara-14 mtl-1 xyl-5 rpsL109 rpsE2015 nalA302 recB21 sbcB15*	Lloyd & Johnson (1979)
N1439	F$^-$, as N1438 but *recA200*	Lloyd & Johnson (1979)
KL226	Hfr (Cavalli) *relA1 tonA22* T2^R	Low (1973)
N1353	Hfr, as KL226 but *nalA$^-$*	This work
NH4104	F′ *lac$^+$* (F42)/*uvrA6 proA2 thr-1 leuB6 his-4 thi-1 ara-14 lacY1 supE44*?	B. Wilkins

* All loci are assumed to be wild type except those listed here. The *sup-78* allele in certain of the strains is an uncharacterized mutation near *his* which confers a Mal$^+$ phenotype on these strains.

3. RESULTS

Nalidixic acid prevents conjugal transfer of DNA through its effect on the donor strain alone (Bouck & Adelberg, 1970; Hane, 1971). Consequently, its addition to Hfr matings should generate a finite population of merozygotes from which it should be possible to recover a constant number of recombinant progeny, at least until the time these begin to replicate. The results shown in Fig. 1 illustrate that this is not the case. Since the recipient strains used were resistant to nalidixic acid, the observed decline in the recombinant yield indicated that nalidixic acid might have some effect on the Hfr which could subsequently reduce the number of merozygotes that retain a selected donor allele. We therefore initiated a more detailed study of the effect of nalidixic acid on Hfr $\times$ F$^-$ *recA200* matings to determine whether the factor, or factors, responsible for the particularly rapid and extensive reduction in the yield of recombinants could provide further insight into why inhibition of gyrase activity in the donor strain should so effectively prevent transfer of DNA.

In several experiments of the type illustrated in Fig. 1 we routinely observed a 10- to 20-fold reduction in the recovery of recombinants provided the recipient carried *recA200* (Table 2, see also Fig. 4). By comparison, a more stable recovery

of recombinants was obtained when the recipient carried *recA*+ although, depending on the strain used, this never exceeded 60–80% of the number of recombinants obtained by plating immediately after the exposure to nalidixic acid (Fig. 1*b*, Table 2). Nevertheless, we could not trace any direct action of nalidixic acid on the recipient. There was no reduction in the number of viable F⁻ cells during the exposure to nalidixic acid, even if a Nal^S recipient was used (Table 2). Mating did not appear to alter the sensitivity of the recipient since no decline in the

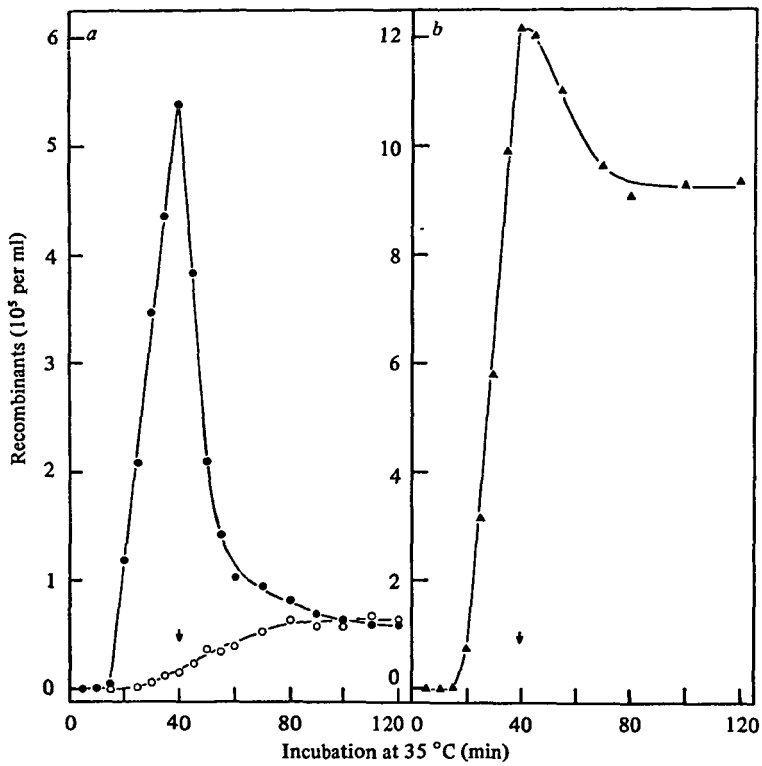

Fig. 1. Effect of nalidixic acid on the recovery of recombinant colonies from Hfr × F⁻ matings. The procedure was as described in Materials and Methods. The time of adding nalidixic acid is indicated by the arrow. Selection was for Pro+(Str^R) recombinants, either at 35 °C (closed symbols) or 42 °C (open symbols). Recipient strains were KL426 (*recA200*) for (*a*) and KL425 (recA+) for (*b*), while the donor strain in both cases was KL226.

number of Lac+ (Str^R) F-prime exconjugants could be detected in crosses with the F-*lac*+ donor strain, NH4104 (Table 2). The possibility that nalidixic acid has some specific effect on exconjugants harbouring linear fragments of Hfr DNA is unlikely in view of the fact that *recA200* merozygotes which had completed *recA*-dependent functions, and were therefore able to form recombinant colonies at 42 °C, were unharmed (Fig. 1*a*). Finally, nalidixic acid appeared to have no effect on matings when the Hfr donor strain carried a *nalA* mutation and was therefore resistant to nalidixic acid action (Fig. 2*a*). We concluded that the decline in the

yield of recombinants from matings with a Nal^S Hfr was a reflection of the termination of DNA transfer by nalidixic acid action on the donor cells.

Previous studies (Lloyd & Johnson, 1979) revealed that Hfr DNA is prone to inactivation in *recA200* merozygotes, but not until transfer of DNA has been terminated by mating pair separation, an effect which probably reflects exonuclease attack on the exposed distal end of the transferred DNA (Itoh &

Table 2. *Recovery of haploid recombinants, F′ exconjugants, and recipients from matings after exposure to nalidixic acid*

Type of cross and progeny selected	Time (min) after exposure to nalidixic acid							
	0	5	10	15	20	30	40	50
KL226 × KL426 (*recA200 nal*⁻)								
Pro⁺ (Str^R) recombinants	4·1	2·78	1·82	1·08	0·73	0·40	0·16	
Str^R recipient cells	1·20	1·22	1·44	1·42	1·45	1·72	1·70	
KL226 × KL399 (*recA200 nal*⁺)								
Pro⁺ (Str^R) recombinants	10·6	8·0	5·6	4·0	3·1	1·8	1·3	1·1
Str^R recipient cells	1·50	1·60	1·46	1·85	1·96	2·24	2·36	2·57
KL226 × N1331 (*recA*⁺ *nal*⁻)								
Pro⁺ (Str^R) recombinants	8·9	8·8	7·5	6·9	6·3	6·3	5·6	5·7
KL226 × N1332 (*recA200 nal*⁻)								
Pro⁺ (Str^R) recombinants	6·55	5·75	2·80	1·55	1·0	0·65	0·50	0·45
NH4104 × N1331 (*recA*⁺ *nal*⁻)								
Lac⁺ (Str^R) exconjugants	4·80	4·84	5·41	5·50	5·80	5·50	5·50	5·90
NH4104 × N1332 (*recA200 nal*⁻)								
Lac⁺ (Str^R) exconjugants	3·74	3·40	3·73	3·92	3·78	3·84	4·33	4·20

The experimental procedure was as described in the legend to Fig. 1. The numbers given refer to the yield of the selected progeny per ml of the mating mixture and are in units of 10^5 for recombinants and exconjugants, and 10^8 for recipient cells.

Tomizawa, 1971). If the majority of *recA200* merozygotes present in mating mixtures after the termination of transfer by nalidixic acid contained Hfr DNA which was free of the donor cell, the rapid decline in the yield of recombinants could therefore be explained by the inactivation of this DNA through exonuclease attack on the free end. To test this possibility, we made use of the fact that inactivation of Hfr DNA in *recA200* merozygotes (after mating pair separation) can be prevented by limiting exonuclease activity through mutation of both *recB* and *sbcB* (Kushner, Nagaishi & Clark, 1972), or by growing the recipient in medium containing a high concentration of sodium chloride (Lloyd & Johnson, 1979; see also Fig. 3*a*). The results indicate that inactivation of transferred Hfr DNA is at least partly responsible for the rapid decline in the yield of recombinants from *recB*⁺*sbcB*⁺*recA200* merozygotes, since both the rate and extent of this decline were considerably reduced when exonuclease activity was limited by either of these methods (Figs. 2*b*, 3*b*). However, it could not be the only factor involved since some decline in the yield of recombinants was still evident, though this was no longer dependent on the recipient carrying the *recA200* allele.

A clue as to the nature of the process responsible for reducing the yield of recombinants even when exonuclease inactivation of transferred DNA was prevented came from control experiments in which mating mixtures were blended prior to adding nalidixic acid. Under these conditions we observed a very stable recovery of recombinants both from *recA*$^+$ and *recA200* merozygotes, though in the latter case this depended on matings being conducted in high salt medium

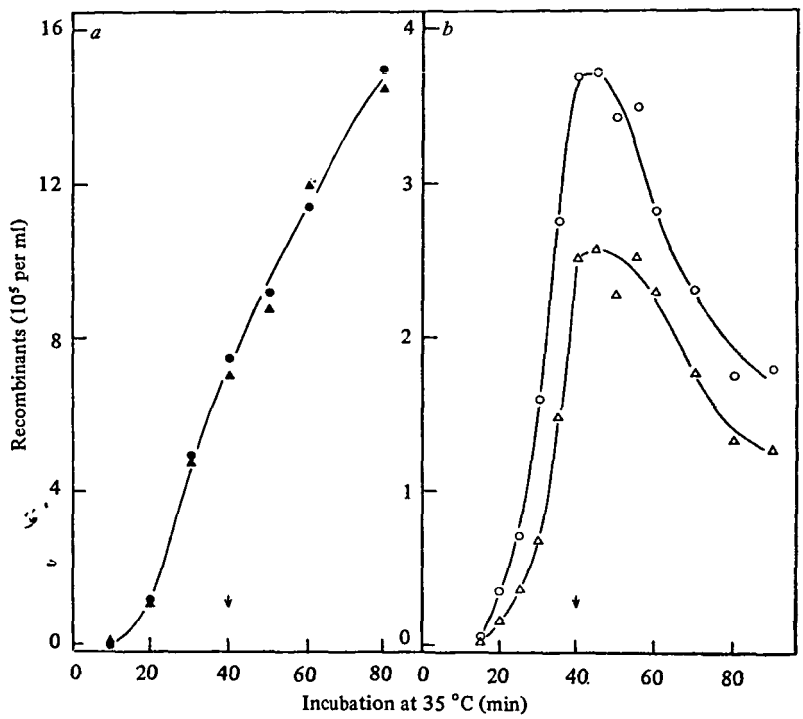

Fig. 2. Effect of nalidixic acid on the recovery of recombinants from matings with a Nal^R Hfr (*a*) or exonuclease deficient (*recB sbcB*) recipients (*b*). When added, nalidixic acid was present from 40 min onwards, and selection was for Pro$^+$(Str^R) recombinants at 35 °C. (*a*) Hfr Nal^R(N1353) × KL426(*recA200* Nal^R) with (●) and without (▲) exposure to nalidixic acid. (*b*) Hfr Nal^S(KL226) mated with *recA*$^+$ strain N1438 (○) or *recA200* strain N1439 (△).

(Fig. 3*a*). Since this indicated that the exonuclease-independent decline in the yield of recombinants (Figs. 2*b*, 3*b*) relied on mating pairs remaining united, we suspected that merozygotes might lose transferred Hfr DNA by its withdrawal from the cell. The existence of such a DNA withdrawal process was previously reported by DeHaan & Gross (1962). However, we have no evidence to indicate that transferred DNA is actually withdrawn from the merozygote other than that it fails to be incorporated into stable recombinant progeny.

The extensive decline in the yield of recombinants observed after the addition of nalidixic acid to Hfr × F$^-$ *recA200* matings in low salt medium must therefore reflect the combined effect of two separate processes. To determine what proportion

of the net effect could be due to inactivation of transferred Hfr DNA, as opposed to its withdrawal, we carefully monitored the rate at which the yield of recombinants declined after exposure of matings to nalidixic acid and compared this with the decline observed in parallel matings which had been blended to expose the transferred DNA immediately to exonuclease attack. The results (Fig. 4) revealed that nalidixic acid was only slightly less effective than blending in reducing the yield of recombinants. By subtracting the DNA withdrawal component (data from

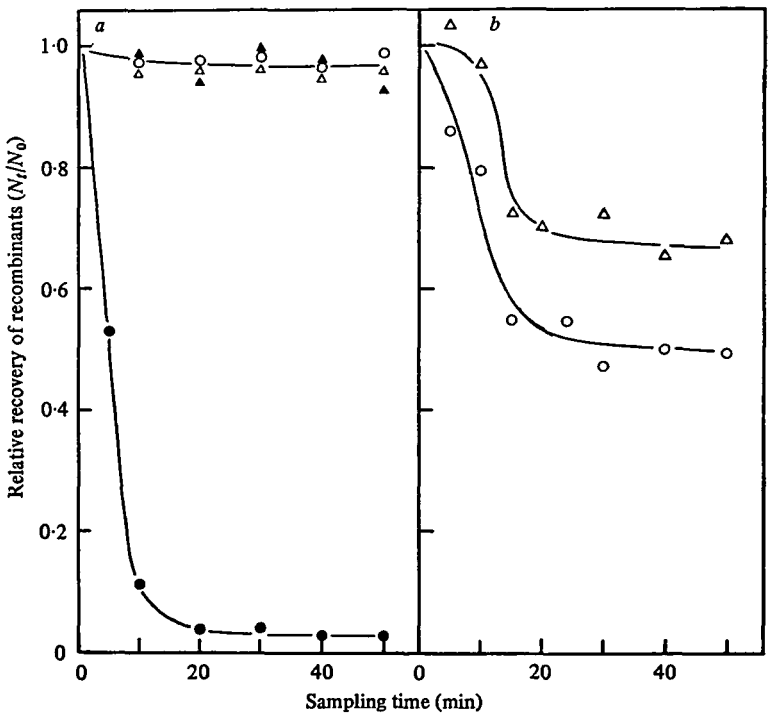

Fig. 3. Recovery of recombinants from Hfr matings conducted in high salt medium with (*a*) or without (*b*) separation of the mating pairs prior to the addition of nalidixic acid. Donor and recipient cultures were grown in LB broth containing either 0·01 M (closed symbols) or 0·25 M (open symbols) sodium chloride and mated for 40 min at 35 °C before blending and diluting 100-fold (*recA200* recipient) or 200-fold (*recA*+ recipient) into warm LB broth of the same salt concentration but which contained 5 μg nalidixic acid per ml (*a*), or simply adding nalidixic acid to a final concentration of 5 μg per ml (*b*). The donor strain was KL226 and the recipients N1331 (▲△) or N1332 (●○). Selection was for Pro+(Str^R) recombinants at 35 °C, and the first sample was removed immediately after exposure to nalidixic acid.

Fig. 3*b*) from the net effect observed after exposure to nalidixic acid, it is possible to calculate that no more than about 50% of the merozygotes lost the ability to form recombinants through inactivation of transferred DNA (Fig. 4, dotted line). This also reveals that there is no delay before the onset of this inactivation, which implies that the distal end of the transferred DNA is exposed to exonuclease attack in these merozygotes either before or very shortly after the addition of nalidixic acid to the mating mixtures. Furthermore, it suggests that the majority of

merozygotes which are still united with the donor cell when transfer is terminated can lose transferred DNA by the process of withdrawal.

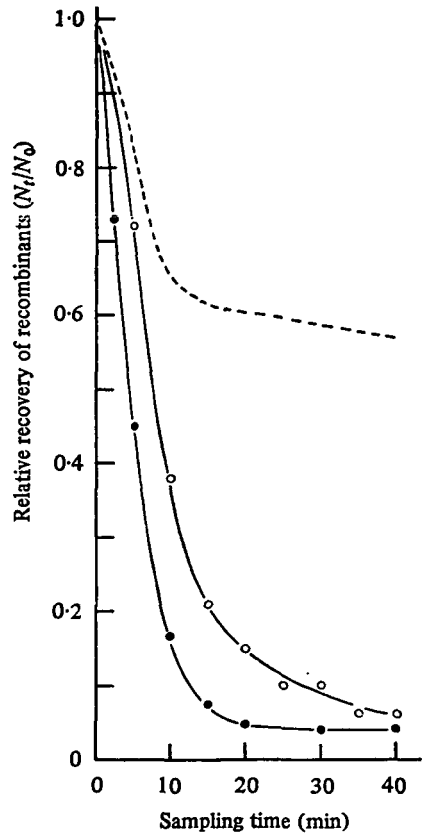

Fig. 4. Recovery of recombinants from Hfr KL226 × F⁻ N1332(*recA200*) matings after exposure to nalidixic acid (open symbols) or after separation of mating pairs (closed symbols). Selection was for Pro⁺(Str)^R recombinants at 35 °C and each point is the mean of at least five independent experiments. The dotted line is based on the values obtained by subtracting the loss of recombinants seen in the KL226 × N1332 mating in Fig. 3*b* from that seen in this case after exposure to nalidixic acid.

4. DISCUSSION

While transfer is in progress during bacterial conjugation, a single strand of DNA is being displaced from donor to recipient cell (Rupp & Ihler, 1968; Siddiqi & Fox, 1973). What happens to this strand in Hfr matings when transfer is abruptly terminated through the action of nalidixic acid on the donor cells has never been made clear. Because transfer does not resume immediately when nalidixic acid is removed, but instead appears to re-initiate from the Hfr origin, it has been suggested that the displaced strand is broken at the site of transfer replication (Bouck & Adelberg, 1970; Hane, 1971).

Our approach to this problem was to re-examine the effect of nalidixic acid on the recovery of recombinant progeny from Hfr matings. By making use of the

specific properties of recipient strains carrying *recA200* we were able to demonstrate that transferred Hfr DNA is indeed free of the donor cell in many of the merozygotes present after the termination of transfer. This is consistent with the rapid decline in the number of recombinants which was observed when plating on agar medium was delayed (Fig. 1*a*) and which could be limited by preventing exonuclease inactivation of the Hfr DNA in the merozygote (Figs. 2*b*, 3*b*). Previous studies had revealed that Hfr DNA becomes prone to exonuclease attack in *recA200* merozygotes only when its distal end is exposed (Lloyd & Johnson, 1979). However, breakage of the displaced donor strand does not necessarily follow the interruption of DNA transfer by nalidixic acid. This was indicated by the discovery of an exonuclease–independent process which could reduce the number of merozygotes capable of forming recombinant progeny by as much as 50% (Figs. 1*a*, 2*b*, 3*b*). The simplest explanation for this is that the affected merozygotes retained contact with the donor through the transferred DNA strand, and that once transfer had been inhibited the displaced strand was withdrawn from the recipient (DeHaan & Gross, 1962). This conclusion was supported by our finding that blending mating mixtures prior to the addition of nalidixic acid could lead to a stable recovery of recombinant progeny provided exonuclease activity in the recipient was limited (Fig. 3*a*).

The rate and extent of the decline in the number of recombinants recovered from crosses with *recA200* recipients (Figs. 1*a*, 4) indicate that the vast majority of mating events between Hfr and F^- cells are resolved within a relatively short period after exposure to nalidixic acid, either through breakage of the displaced donor strand or the withdrawal of transferred DNA from the merozygote. This provides a simple explanation for the observation by Bouck & Adelberg (1970) and Hane (1971) that transfer of a selected donor allele does not resume after transient exposure to nalidixic acid until a period of time has elapsed which equals the normal entry time for that allele. Most of the matings between Hfr and F^- cells would be quickly resolved one way or another during the transient exposure to nalidixic acid, so that effectively the only way to resume transfer would be from the Hfr origin.

A question that remains to be answered is whether breakage of the donor strand displaced to the recipient and withdrawal of transferred DNA from the merozygote can be related to a common mechanism. Since these events could be detected only when the donor was sensitive to nalidixic acid (see Fig. 2*a*), the possibility arises that they may be directly related to the inhibition of gyrase activity in the Hfr cell. Although gyrase inhibition promotes a number of responses in sensitive cells (for a review see Kreuzer *et al.* 1978), one of the more immediate effects is the rapid relaxation of supercoils in both plasmid and chromosomal DNA (Gellert *et al.* 1977; Drlica & Snyder, 1978). Relaxation of supercoils would not only counter the unwinding of the donor DNA needed for strand transfer but might also promote re-annealing of the displaced strand to the donor molecule, particularly if gyrase inhibition also provoked synthesis of *recA* protein (Shibata *et al.* 1979). The net effect of this could be the withdrawal of the displaced strand

or, if sufficient strain was imposed, its breakage, leaving the transferred strand in the recipient. However, our experiments cannot rule out the possibility that breakage of the donor DNA strand may simply be a reflection of spontaneous mating pair separation.

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