Proc. Nat. Acad. Sci. USA
Vol. 72, No. 9, pp. 3721–3725, September 1975
Microbiology

Mating signal and DNA penetration deficiency in conjugation between male *Escherichia coli* and minicells*

(mating signal/F pili/DNA synthesis/surface exclusion)

JONATHAN T. OU

The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Communicated by Thomas F. Anderson, July 7, 1975

ABSTRACT **Strain OU122, a *dnaB* mutant of HfrH which stops vegetative DNA synthesis immediately when the temperature is shifted to 43°, was mated at 43° with minicells with and without 1 mM Zn^{2+}. Synthesis of DNA was detected in the mating mixture containing minicells derived from either $F^-\chi925$ or $F^+\chi1115$ cells, but only a small amount was detected in the mixture containing 1 mM Zn^{2+} which inhibits the formation of mates (previously called mating pairs). The supernatant liquid from cell cultures did not induce DNA synthesis, suggesting that DNA synthesis was not stimulated by diffusible molecules. Additional experiments showed that Zn^{2+} inhibited DNA synthesis associated with DNA transfer but did not inhibit the DNA transfer that had already been initiated. Thus, the stimulation of DNA synthesis observed required physical interaction of cells and F pili. Reisolated minicell exconjugants derived from the cross OU122 × $F^-\chi925$ minicells were shown to contain transferred DNA in contrast to either the cross OU122 × $F^-\chi925$ minicells in 1 mM Zn^{2+}, or the cross OU122 × $F^+\chi1115$ minicells. Thus, $F^+\chi1115$ minicells retained the property of surface exclusion at 43°.**

As it begins to mate with a recipient bacterium, a male *Escherichia coli* cell prepares to transfer DNA in response to some kind of a signal from it. Such signals might be transmitted through F pili, which are hair-like appendages produced by the sex factor F (1). Alternatively, wall-to-wall contacts (2, 3), which might be formed by retraction of the connecting F pili (4, 5), might be required in order to result in a signal for DNA transfer.

One effect of such a signal is to lead to the synthesis of DNA associated with DNA transfer (transfer replication). Jacob *et al.* (6) suggested that concurrent DNA synthesis in the donor is necessary during DNA transfer, and arguments for and against this hypothesis have been made in recent years (for a review, see ref. 7). One approach to this problem is to use temperature-sensitive DNA mutants. Using such mutants, the *dnaB*$^-$ mutants of HfrH and F^- which are unable to synthesize DNA vegetatively at the restrictive temperature (42°), Bonhoeffer (8) observed that recombinants were produced even when matings were performed at 42°. This was taken to mean that DNA synthesis in the donor is not required for DNA transfer. On the other hand, when Bresler *et al.* (9) directly measured DNA synthesis in the mating mixtures of the thermally sensitive Hfr and F^- strains isolated by Bonhoeffer, they found that DNA was synthesized in the mating mixture at 43°. By crossing donors and recipients that were identical temperature-sensitive strains except for the F'*lac*$^+$ factor harbored in the donor, Marinus and Adelberg (10) confirmed the observation of Bresler *et al.* that DNA transfer did occur at the restrictive temperature and that DNA synthesis occurred during mating. Subsequently, Vapnek and Rupp (11), who analyzed sex factor DNA after mating with heat-stable recipients, and Curtiss *et al.* (12), who used minicells derived from thermosensitive strains for mating, showed that DNA synthesis occurred in the heat-sensitive male cells during mating. Vapnek and Rupp (11) further showed that DNA was synthesized in the male to replace the heavy strand of sex factor DNA that had been transferred to the recipient. These experiments all suggested that DNA transfer is accompanied by DNA synthesis in the donor. Recently, Sarathy and Siddiqi (13), using a *dnaB* mutant of HfrH (*dnaB*$^-$, *thy*$^-$), showed that DNA synthesis did occur in the donor in the mating mixture containing thymine, whereas when the donor was starved for thymine, DNA was transferred without apparent DNA synthesis in the donor. Their finding strongly suggests that DNA synthesis induced by mating is not required for DNA transfer and merely occurs in order to form a complementary strand of donor DNA to replace the strand that has been transferred to the recipient. In other words, transfer of DNA can be independent of the DNA synthesis (transfer replication) that is normally triggered by mating. Under conditions suitable for DNA synthesis, the detection of transfer replication can be used as a sign of mating, i.e., an indication of whether or not the donor receives a mating signal. In this way, we wish to discover whether a mating signal is transmitted to donor cells under various conditions of culture and in mixed cell cultures with various types of potential recipient strains (F^- and F^+).

It is known that male cells growing exponentially are poor recipients of DNA transferred from donor cells. This phenomenon has been termed surface exclusion (or entry exclusion) (14, 15). It has been shown that the presence of F pili on the recipient is not the cause of surface exclusion (16). One possible mechanism for surface exclusion is that male cells may fail to interact with each other to form mates (14). (The term "mate" was used instead of "mating pair," since examinations with a Coulter Counter have shown that in addition to pairs, many aggregates of cells were formed) (ref. 17; Ou, unpublished results). However, using the Coulter Counter, it has been shown that the formation of mates between male cells does occur but is prevented by the presence of 10^{-3} M Zn^{2+} (17) or of the filamentous DNA phage, f1 (18). It therefore seemed possible that surface exclusion might result from the failure of the signal for transfer replication to be generated by the contact between a donor and an F^+ recipient. This can now be determined by measuring the occurrence or nonoccurrence of transfer replication in a

* This paper was presented in part at the 13th International Congress of Genetics, The University of California, Berkeley, California, 20–29 August 1973.

cross evincing surface exclusion, such as the cross of an Hfr by minicells from an F^+ strain.

To study these phenomena, the mating system involving temperature-sensitive donor cells and minicells used by Curtiss *et al.* (12) was used. Minicells derived from either F^+ or F^- cells were used as recipients in crosses with the HfrH strain that is temperature-sensitive in DNA synthesis (*dnaB*$^-$) at the restrictive temperature, 43°. Both the synthesis and the transfer of DNA to recipients were measured. Minicells are produced by an abnormal cell division and contain no detectable DNA or F pili (19). Since it has been well established that a single strand of preexisting DNA is transferred to receptor cells (16, 20–22), Hfr cells whose DNA had been labeled with [^{3}H]thymine were used as donors in measurements of the amount of DNA transferred into minicells. Such experiments suggest that: (*a*) a mating signal is generated by a cell to cell contact and seems to be transmitted through an F pilus, (*b*) DNA synthesis associated with DNA transfer occurs in the male when mated with either (F^-) or (F^+)-minicells, (*c*) after mating, (F^-)-minicells contain transferred DNA, but (F^+)-minicells do not (surface exclusion), (*d*) 1 mM Zn^{2+} inhibits transfer replication but not DNA transfer, and thus (*e*) DNA synthesis and DNA transfer are independent events.

MATERIALS AND METHODS

All bacterial strains are derivatives of *E. coli* K12. Strain OU122 (supplied by F. Bonhoeffer) is a temperature-sensitive DNA mutant of HfrH (8) which stops DNA synthesis immediately when the temperature is raised to 43°. Its genotype is *thy*$^-$ *thi*$^-$ and *dnaB*$^-$*70* (23). Strains $F^+\chi$1115 and $F^-\chi$925 (supplied by R. Curtiss) are minicell-producing bacteria and both have the following chromosomal genotypes: *thr*$^-$ *ara*$^-$ *leu*$^-$ *azi*$^-$ *ton*$^-$ *lac*$^-$ *minA*$^-$ *gal*$^-$ λ$^-$ *thi*$^-$ *minB*$^-$ *mal*$^-$ *xyl*$^-$ *mtl*$^-$ *str*$^-$. The genotype and phenotype of strain F^-W1-3 have been described (17).

Nutrient broth of composition previously described (24) was routinely used for growing cells and matings. M9 synthetic medium has also been described (24). Sucrose solution was made in M9 medium.

Minicells were prepared essentially by the method of Sheehy *et al.* (25). Briefly, exponentially-growing minicell-producing bacteria (about 4×10^8 colony-forming cells per ml) were centrifuged at 3000 rpm in a Sorvall GS-3 rotor for 5 min to eliminate most large cells in the culture. The supernatant was then centrifuged in the same rotor at 9000 rpm for 20 min, and the pellets (mostly minicells) were resuspended in 6 ml of broth. This minicell suspension was layered on a 5–20% linear sucrose gradient and centrifuged at 5000 rpm for 20 min in a Beckman SW27 rotor in a Beckman L3-50 ultracentrifuge. The minicell suspension was collected with a pasteur pipet and pelleted in a Sorvall SS-34 rotor at 15,000 rpm for 20 min. The pellets were again run through the above purification procedure, beginning from sucrose gradient centrifugation, and then finally suspended in broth and used for mating. The isolation procedures were carried out at 4°. The final concentration of minicells was 1 to 5×10^{10} minicells per ml, measured in a Petroff-Hausser bacteria counting chamber in a phase microscope. The concentration of contaminating viable cells was only 1 to 10×10^3 cells per ml, as measured by colony forming ability on nutrient plates.

Matings at a ratio of 1 male to 100 minicells were performed for 90 min in a water bath with gentle shaking at 43°. For the determination of DNA synthesis during mating, the following procedure was used. Strain OU122 was grown to about 4×10^8 cells per ml in broth with unlabeled thymine (40 μg/ml) at 30°. The culture was then washed to eliminate thymine and resuspended in warmed broth (43°) without added thymine. [^{3}H]Thymine (53.2 Ci/mmol, New England Nuclear) was then added to both Ou122 and minicell cultures to a final concentration of 1 μCi/ml. Twenty minutes later, male cells and minicells were mixed. Samples were withdrawn periodically, and the incorporation of [^{3}H]thymine into cold trichloroacetic acid-insoluble material was determined.

Our determination of the transfer of the preexisting strand of DNA into minicells was carried out as follows. OU122 cells grown to about 4×10^8 cells per ml at 30° in broth which contained [^{3}H]thymine (1 μCi/ml) were washed to get rid of any remaining free [^{3}H]thymine. The cells were then resuspended in warmed broth (43°) and immediately mated with minicells in broth which contained 40 μg/ml of unlabeled thymine or 0.5 μg/ml of [^{14}C]thymine (47 μCi/mmol, New England Nuclear) in some cases. After 90 min of gentle shaking, the mixture was mechanically blended to interrupt mating and immediately subjected to two centrifugations in a 5–20% linear sucrose gradient as described above to purify the minicell fraction. Its content of [^{3}H]thymine (and [^{14}C]thymine) was then measured as cold trichloroacetic acid (10%)-precipitable material.

To measure the [^{3}H]thymine incorporation, samples (0.1 ml) were added into cold (0°) 10% trichloroacetic acid containing 100 μg/ml of unlabeled thymine. After 15–20 min, the samples were collected on 0.45 μm pore size Millipore filters and washed five times with cold 10% trichloroacetic acid which contained 100 μg/ml of thymine, five times with 95% ethanol, and five times with cold 10% trichloroacetic acid. The filter was thoroughly dried and placed in a vial containing 5 ml of toluene/Liquifluor. Radioactivity was determined in a scintillation counter.

RESULTS

Stimulation of DNA synthesis at 43° in the cross OU122 × $F^-\chi$925 minicells

The matings listed in Fig. 1 were carried out to see whether contact between OU122 cells and minicells induces DNA synthesis in the donor, and if it does, what kind of contact is necessary for the initiation of DNA synthesis. Twenty minutes before mating, 1 μCi/ml of [^{3}H]thymine (final concentration) was added to the OU122 culture. The warmed (43°) minicell suspension containing 1 μCi/ml of [^{3}H]thymine was then mixed with OU122 cells with and without the addition of 1 mM Zn^{2+}, and samples were withdrawn periodically to measure the incorporation of [^{3}H]thymine into trichloroacetic acid-insoluble material. As shown in Fig. 1, as soon as minicells and OU122 cells were mixed without 1 mM Zn^{2+}, ^{3}H counts in the cold trichloroacetic acid-insoluble fraction began to increase, whereas the controls, minicells and OU122 alone, showed little [^{3}H]thymine incorporation. The mating mixture, which contained 1 mM Zn^{2+}, showed a small increase initially and quickly leveled off in the incorporation of [^{3}H]thymine into cold trichloroacetic acid-insoluble fraction.

The above results could be obtained if the recipient cells produce and release into the medium some diffusible molecule(s) that may stimulate DNA synthesis in the donor. This possibility was tested by adding OU122 cells to the supernatant of the recipient culture and measuring DNA synthesis.

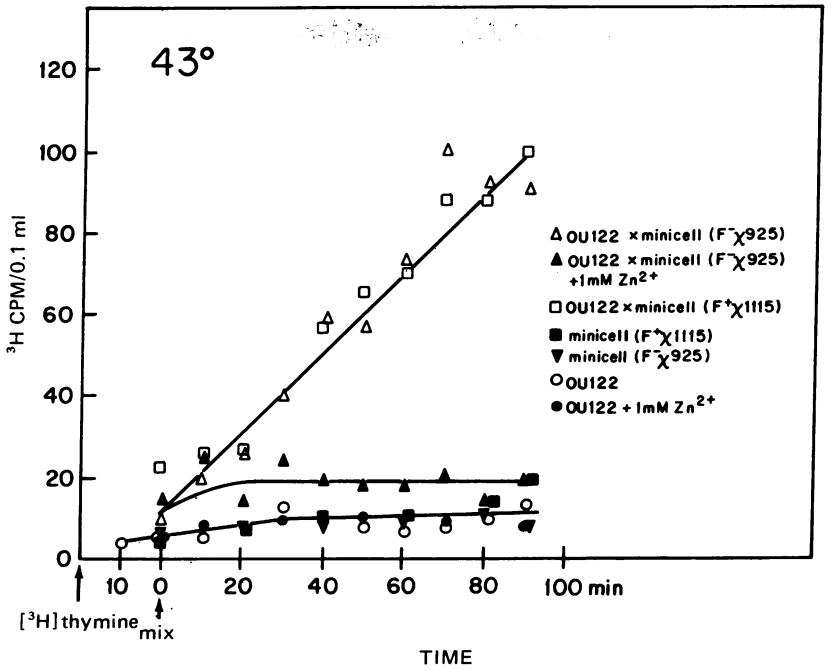

FIG. 1. Initiation of DNA synthesis in the mating mixture of OU122 cells and minicells. [^{3}H]Thymine (1 μCi/ml) was added to broth cultures of OU122 cells and minicells as soon as the temperature was raised to 43°. Twenty minutes later they were then mixed with or without the addition of 1 mM Zn^{2+} at a ratio of 1 male to 100 minicells, and the mixtures were gently shaken. Periodically, samples (0.1 ml) were taken and mixed with 1 ml of cold 10% trichloroacetic acid. After 15–20 min the samples in trichloroacetic acid were collected on 0.45 μm Millipore filters, washed, and measured for the incorporation of [^{3}H]thymine, as described in *Materials and Methods*.

No detectable DNA synthesis was observed from this mixture.

Another question is whether the DNA synthesis indicated by Fig. 1 might have occurred in the minicells to form the complementary strand to the transferred single strand of donor DNA. Cohen *et al.* (16, 20) have shown that DNA isolated from minicells immediately after mating contained both single- and double-stranded DNA, and that the fraction of double-stranded DNA increased upon further incubation after mating. To check whether or not DNA synthesis occurred in the minicells during mating, a double labeling experiment was performed. OU122 cells were grown in broth containing [^{3}H]thymine as described above. The culture was then washed twice and the cells were resuspended in fresh medium. Just before mixing at 43°, [^{14}C]thymine (final concentration, 0.5 μCi/ml) was added to all cultures to be used in mating. In addition to the experimental procedures described above, the mating mixtures were vigorously blended at the end of 90 min of mating and the minicell exconjugants were immediately isolated and purified. For the control minicell portions, immediately after mixing of minicells and OU122 cells, the mixture was blended and subjected to the isolation and purification procedures. The concentration of purified minicells were then measured with a Petroff-Hausser counting chamber, and the amounts of trichloroacetic acid-precipitable ^{14}C and ^{3}H materials were also determined. The kinetics of [^{14}C]thymine incorporation (DNA synthesis during mating) into trichloroacetic acid-precipitable material were similar to the ones shown in Fig. 1. On the other hand, a substantial ^{3}H count (360 cpm/2.2 × 10^9 minicells) was detected from minicell exconjugants with very few ^{14}C counts (11 cpm/2.2 × 10^9 minicells), whereas few counts of both ^{14}C (10 cpm/2.0 × 10^9 minicells) and ^{3}H (60 cpm/2.0 × 10^9 minicells) were detected in the control minicells.

The minicell exconjugants mated in 1 mM Zn^{2+} were also analyzed for transferred DNA. As in the control minicells, few counts were detected for both ^{14}C (9 cpm/2.8 × 10^9 minicells) and ^{3}H (80 cpm/2.8 × 10^9 minicells). This eliminates the possibility that there was DNA transfer, while DNA synthesis was inhibited in the mating mixture with 1 mM Zn^{2+} in Fig. 1. However, the possibility still remains that 1 mM Zn^{2+} might inhibit DNA synthesis, and by so doing, it inhibits the subsequent DNA transfer. To test the effect of Zn^{2+} on DNA synthesis, similar experiments to

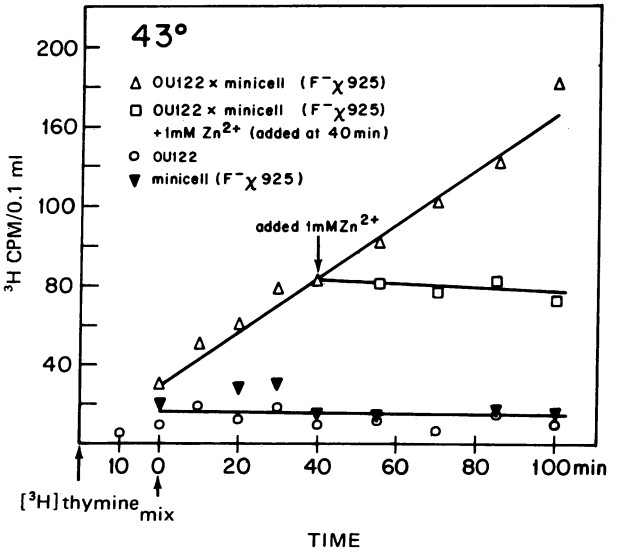

FIG. 2. Immediate inhibition of DNA synthesis stimulated by mating by the addition of 1 mM Zn^{2+} in the mating mixture of OU122 cells and minicells. Experimental procedures were similar to those described in the legend of Fig. 1, except that 1 mM Zn^{2+} was added to one of the mating mixtures at 40 min after mixing donor and recipient cells.

those in Fig. 1 were performed, except that 1 mM Zn^{2+} was added after mating was allowed to proceed for a period of time after mixing. DNA synthesis was stopped immediately upon addition of 1 mM Zn^{2+} (Fig. 2). It can also be shown that chromosome transfer is not dependent on transfer replication. We have previously shown that upon addition of 1 mM Zn^{2+} to preformed mates DNA is transferred normally (17). This observation was also found to be true when the formation of recombinants was checked in mating at 43° between the OU122 strain and thermally stable strain, F^-W1-3 (data not shown). Thus, although Zn^{2+} inhibits transfer replication (Fig. 2), it does not interfere with the process of chromosome transfer itself. These results confirm those of Sarathy and Siddiqi (13).

Stimulation of DNA synthesis at 43° in the cross OU122 × $F^+\chi$1115 minicells

In the experiments described in Fig. 1, OU122 cells were also mated with minicells derived from strain $F^+\chi$1115 to see whether these minicells can also induce DNA synthesis in the donor. Initiation of DNA synthesis is seen as soon as they were mixed. The rate of DNA synthesis is similar to that of the mixture of OU122 cells and minicells derived from $F^-\chi$925 (Fig. 1). The control, $F^+\chi$1115 minicell culture alone, showed little incorporation of [^{3}H]thymine. With 10^{-3} M Zn^{2+} in the mating mixture, DNA synthesis was not stimulated, as in the case of $F^-\chi$925 minicells (data not shown).

Transfer of male DNA into minicells at 43°

Since, as shown above, (F^+)-minicells seem to behave as F^- minicells do in terms of stimulation of DNA synthesis, one must ask whether at 43° they have lost the property of surface exclusion which (F^+)-minicells retained at 37° as Cohen *et al.* (18) and Sheehy *et al.* (25) previously demonstrated. To test this possibility, [^{3}H]thymine-labeled OU122 cells were mated to minicells at 43° in broth which contained unlabeled thymine, and the amount of DNA in minicells was determined kinetically. As seen in Table 1, only $F^-\chi$925 minicell exconjugants received DNA, whereas negligible amounts of DNA were transferred: (*a*) to $F^-\chi$925 minicells mated in the presence of 1 mM Zn^{2+}; (*b*) to $F^+\chi$1115 minicell exconjugants; or (*c*) to control minicells isolated from mating mixtures that had been blended immediately after mixing to prevent mating. The small initial increase in [^{3}H]thymine incorporation shown in the mating mixture of OU122 and F^- minicells containing 1 mM Zn^{2+} may be the result of a small fraction of mates formed initially by competition with Zn^{2+} for the tips of F pili (Fig. 1). The results show that minicells derived from $F^+\chi$1115 retain their surface exclusion character at 43°.

Table 1. Amount of DNA (^{3}H cpm) detected in 5 × 10^8 minicell exconjugants

Crosses	30 min	60 min	90 min
OU122 × F^- χ925 minicells	45	450	400
OU122 × F^- χ925 minicells + 1 mM Zn^{2+}	40	60	30
OU122 × F^+ χ1115 minicells	25	10	15
F^- χ925 minicells*	10	25	35
F^+ χ1115 minicells*	10	35	15

[^{3}H]Thymine-labeled OU122 cells were mixed at 43° with minicells in broth containing unlabeled thymine (40 μg/ml), at a ratio of 1 to 100, and the mixtures were gently shaken. Samples were withdrawn at 30 min, 60 min, and 90 min after mixing, mechanically shaken vigorously, and submitted to the isolation procedures as described in *Materials and Methods*. The amount of DNA in terms of cold 10% trichloroacetic acid-insoluble material in minicells was then measured. OU122 cells (8×10^6) contained 1.65×10^5 cpm.

* These cells were treated and isolated the same way as the mating mixtures, except that [^{3}H]thymine-labeled OU122 cells were added just before the vigorous mechanical shaking.

DISCUSSION

The results of the experiments described here indicate that (*i*) DNA transfer does not require transfer replication, confirming the finding of Sarathy and Siddiqi (13), and (*ii*) a cell to cell contact is required for the generation of a mating signal, which is responsible for transfer replication and DNA transfer. The following observations suggest that the F pilus may be the medium for transmission of this signal. (*i*) We have previously shown that DNA can be transferred between "separate" mates of a mating pair connected by an invisible thread, presumably an F pilus (24). It seems unlikely, although not unequivocally ruled out, that this separate mating pair transmitted a mating signal by a cell wall to cell wall contact, and then separated to transfer DNA. (*ii*) Zn^{2+} was shown to inhibit mate formation by acting on the tip of the F pilus (17, 26). It is not clear whether or not a direct cell wall to cell wall contact may also be necessary. If the signal is mediated by F pilus, it seems that these molecules must then exist on the surface of a female cell required to generate a mating signal.

The transfer of DNA requires physical contact of the mating partners, as seen by the greatly reduced amount of DNA transferred in the (F^-)-minicell exconjugants derived from the mating mixture that contained 1 mM Zn^{2+}. The small amount of transferred DNA (Table 1) correlates with the small amount of DNA synthesis observed in the mating mixture with 1 mM Zn^{2+} (Fig. 1). The amount of DNA received by the (F^-)-minicell exconjugants correlates well with the amount of DNA synthesized in the donor (Fig. 1 and Table 1). In contrast, minicells derived from F^+ cells, even though they stimulate DNA synthesis (Fig. 1), are unable to receive transfer DNA. The failure to detect transferred DNA in (F^+)-minicell exconjugants may be due to a rapid degradation of DNA as the DNA enters minicells. However, this does not seem to be the case since Khachatourians *et al.* (27) and Sheehy *et al.* (25) found that: (*i*) the degradation of transferred DNA in minicells does not exceed 40–50% in 3 hr after mating, (*ii*) the nature of DNA degradation is apparently the same in all minicells regardless of the source (whether from F^- cells or cells containing plasmids), and (*iii*) DNA degradation is an all or none phenomenon. Our results, therefore, suggest that the surface exclusion displayed by minicells derived from F^+ cells is due to the inability of these cells to receive transferred DNA although they are capable of sending a mating signal to the donor upon mating, as measured by the stimulation of DNA synthesis. In other words, a conjugal mate is required to reach a stage beyond mating signal generation to become a fully effective mate.

As we now know, 30–40% of male cells in dense cultures form clumps in which the cells are connected to each other

by F pili (17, 18). Yet, as seen in Fig. 1, there is no detectable DNA synthesis in the male culture at 43°. These observations on whole male cells differ from those using F^+ derived minicells. In the former case, both the generation of mating signal and DNA transfer are blocked, while in the latter case, only the transfer of DNA is inhibited. It is possible that the male recipient cell may contain other factors, absent from minicells, which prevent transmission of the mating signal. However, it is not clear if the parent cells of (F^+)-minicells can also stimulate transfer replication in the donor when mated. If such factors on whole male cells exist, they may explain the results obtained by Fenwick and Curtiss (28), who showed that no DNA synthesis was detected in the mating mixture of *dnaB*$^-$ donor cells and minicells, both of which contain plasmids (R64-11, a drug resistant factor). Their mating system seems to mimic the mating within the donor cell culture so far as the presence of sex factors in both the donors and the recipients is concerned. It seems, therefore, that a male cell practices its surface exclusion at two levels: the first is characterized by the deficiency in the generation of a mating signal and the second is characterized by DNA penetration deficiency.

I thank Thomas F. Anderson for his constructive criticism and helpful comments during the course of this study. This investigation was supported by Grant GB-29291X from the National Science Foundation, by Grant CA-06927 from the National Institute of Health, by Public Health Service Grant RR-05539 from the Division of Research and Facilities and Resources, respectively, and by an appropriation from the Commonwealth of Pennsylvania to The Institute for Cancer Research.

1. Brinton, C. C., Gemski, P. & Carnahan, J. (1964) *Proc. Nat. Acad. Sci. USA* **52,** 126–132.
2. Anderson, T. F. (1958) *Cold Spring Harbor Symp. Quant. Biol.* **23,** 47–58.
3. Anderson, T. F., Wollman, E. L. & Jacob, F. (1957) *Ann. Inst. Pasteur* **93,** 450–455.
4. Novotny, C. P. & Fives-Taylor, P. (1974) *J. Bacteriol.* **117,** 1306–1311.
5. Jacobson, A. (1972) *J. Virol.* **10,** 835–843.
6. Jacob, F., Brenner, S. & Cuzin, F. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28,** 329–348.
7. Curtiss, R., III (1969) *Annu. Rev. Microbiol.* **23,** 69–136.
8. Bonhoeffer, F. (1966) *Z. Vererbungl.* **98,** 141–149.
9. Bresler, S. E. Lanzov, V. A. & Lukjaniec-Blinkova, A. (1968) *Mol. Gen. Genet.* **102,** 269–284.
10. Marinus, M. G. & Adelberg, E. A. (1970) *J. Bacteriol.* **104,** 1266–1272.
11. Vapnek, D. & Rupp, W. D. (1971) *J. Mol. Biol.* **60,** 413–424.
12. Curtiss, R., III, Seigel, R. L., Stallions, D. R. & Denbos, G. V. (1970) *Bacteriol. Proc.*, 35.
13. Sarathy, P. V. & Siddiqi, O. (1973) *J. Mol. Biol.* **78,** 443–451.
14. Achtman, M. (1973) "Genetics of the F sex factor in *Enterobacteriaceae*," in *Current Topics in Microbiology and Immunology* (Springer-Verlag, Berlin), pp. 79–123.
15. Novick, R. P. (1969) *Bacteriol. Rev.* **33,** 210–263.
16. Cohen, A., Fisher, W. D., Curtiss, R., III & Adler, H. I. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33,** 210–263.
17. Ou, J. T. & Anderson, T. F. (1972) *J. Bacteriol.* **111,** 177–185.
18. Ou, J. T. (1973) *J. Bacteriol.* **114,** 1108–1115.
19. Adler, H. I., Fisher, W. D., Cohen, A. & Hardigree, A. A. (1966) *Proc. Nat. Acad. Sci. USA* **57,** 321–326.
20. Cohen, A., Fisher, W. D., Curtiss, R., III & Adler, H. I. (1968) *Proc. Nat. Acad. Sci. USA* **61,** 61–68.
21. Ohki, M. & Tomizawa, J. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33,** 651–658.
22. Rupp, W. D. & Ihler, G. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33,** 647–650.
23. Wechsler, J. A. & Gross, J. D. (1971) *Mol. Gen. Genet.* **113,** 273–284.
24. Ou, J. T. & Anderson, T. F. (1970) *J. Bacteriol.* **117,** 1306–1311.
25. Sheehy, R. J., Orr, C. & Curtiss, R., III. (1972) *J. Bacteriol.* **112,** 861–869.
26. Ou, J. T. & Anderson, T. F. (1972) *J. Virol.* **10,** 869–871.
27. Khachatourians, G. G., Sheehy, R. J. & Curtiss, R., III (1974) *Mol. Gen. Genet.* **128,** 23–42.
28. Fenwick, R. G. & Curtiss, R., III. (1973) *J. Bacteriol.* **116,** 1212–1223.