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Effect on Exclusion of Alterations to the Sex Pilus

ELINOR MEYNELL[1] AND ANNA EWINS[2]

Department of Microbiology, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London SW1W 8RH, England

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Chromosomal genes from an Hfr donor, dependent for their transfer upon the integrated F factor, were not excluded by an F^+ recipient when the donor also carried an F-like R factor, and its sex pili contained, in addition to F pilin, another pilin of a different specificity.

Bacteria carrying the F sex factor are poor genetic recipients in matings with F^+ or Hfr donors, and the phenomenon is known as exclusion, entry exclusion, or surface exclusion. Exclusion applies to all genetic material of the donor cell, including chromosomal genes as well as genes forming part of the F factor itself, so that there is about a 100-fold difference between an F^- and an F^+ recipient in the numbers of genetic recombinants of every sort. Tests of the recipient immediately after mating with an F′ donor for production of enzymes determined by the F′ factor indicate that exclusion is due to failure of donor genes to be acquired rather than to their failure to be subsequently inherited (11). This conclusion is supported by physical evidence showing that F factor deoxyribonucleic acid (DNA) cannot be recovered from an F^+ recipient although it can from one that is F^- (7, 19, 29).

Other derepressed sex factors, including those of R factors and Col factors, bring about gene transfer by conjugation in the same general manner as autonomous F (8, 21). In particular, exclusion also occurs with these factors (22, 31) so that it is probably a general phenomenon.

One explanation for exclusion is that the presence of the donor's sex factor makes the donated DNA unacceptable to a recipient carrying the same sex factor. Against this, however, is the fact that genes excluded in conjugation are not excluded in transduction by phage (32). An alternative possibility, that exclusion operates during the process of mating by conjugation, may be tested with F^+R^+ donors. When the donor is an F^- bacterium carrying an F-like or an I-like R factor, the presence of F in the recipient does not lead to exclusion. Moreover, in matings between F^+R^+ donors and F^-R^- recipients, genetic tests show F and R transfer to occur separately, as occurs with other pairs of sex factors (12, 14, 20, 21, 27). Hence, it is possible to compare the individual rates of transfer of F and R from an F^+R^+ donor to F^+ and F^- recipients to see whether exclusion acts nonspecifically on both F and R or specifically on those genes whose transfer depends on F. The results reported here indicate that exclusion occurs only when the sex factors concerned in donor and recipient determine identical sex pili. This requirement appears fairly strict. Thus, when physically discrete F and R pili are produced with an I-like R factor (16) and each type is used in the corresponding type of genetic transfer (13, 28), R is transferred normally from the F^+R^+ donor to an F^+R^- recipient, whereas F is not. However, when the R factor is F-like and each of the donor's sex pili is a mixture of F and R pilin (16) and used for F as well as for R transfer (Ewins and Meynell, *unpublished data*), F exclusion is largely absent.

In practice, comparative estimates of the efficiencies of transfer of autonomous F or F′ factors to F^+ and F^- recipients are complicated by superinfection immunity which prevents the stable inheritance in the F^+ strain, as in an F^- strain, of those genetic markers which escape exclusion. However, these differences between F^+ and F^- can be eliminated by using an Hfr donor and measuring the frequency of recombinants for a proximal chromosomal gene which depends for its inheritance on recombination with the chromosome in both types of recipient.

[1] Present address: Biological Laboratories, University of Kent at Canterbury, England.
[2] Present address: The Royal Institute of Public Health and Hygiene, 28 Portland Place, London W1, England.

MATERIALS AND METHODS

Bacteria. The strain used as donor was HfrC *metB* which transfers pro^+ as a proximal and trp^+ as a distal marker (30). The recipient was strain J62 (*proA his trp lac strA*) (6).

R factors. The F-like R factors were R1*drd19* and R136*drdM1*, both derepressed mutants of the repressor-minus (i^-) type (20). The I-like R factor was R144*drd3* (21). None of these R factors inhibits expression of F.

R1*drd19* confers resistance to kanamycin, chloramphenicol, ampicillin, streptomycin, and sulfonamide. R136*drdM1* confers resistance to tetracycline and sulfonamide. R144*drd3* determines production of colicin Ib and resistance to kanamycin.

Culture media. Nutrient broth was Oxoid nutrient broth no. 2. Minimal agar was that of Tatum and Lederberg (24) and was supplemented as required for selection of the different types of bacteria in the mating mixtures.

Mating. Donor and recipient strains were grown overnight at 37 C in static broth culture. They were then diluted 1:100 in fresh broth and reincubated for a length of time which depended on the particular strain and the conditions of the experiment. The donors were incubated with gentle shaking until the bacterial concentration was judged by eye to have reached about 2×10^8/ml. To avoid going into F^- phenocopy (17), the recipients were incubated without shaking either in a 20 to 30 mm depth of medium until about 1 hr after maximum density was reached (experiments A, Table 1) or in a greater depth of medium for a shorter period (experiments B). Donor and recipient cultures were mixed to give 2 to 5×10^7 bacteria/ml of donors and 5×10^8 to 1×10^9 bacteria/ml of recipients; and the mixtures were incubated in a water bath at 37 C with shaking at 60 strokes/min for 1 hr (experiments A) or 30 min (experiments B). They were then centrifuged; the deposited bacteria were resuspended to one-half of the original volume in phosphate buffer, dispersed by a Rotamixer (Hook & Tucker Ltd.), diluted in buffer, and plated. Donors were selected on agar containing methionine, lactose, and 0.5% (v/v) broth. Recipients were selected on proline, histidine, tryptophan, glucose, and streptomycin (200 μg/ml), the medium also used for recombinants or R^+ transfer either with the omission of proline or tryptophan or with the addition of the appropriate antibiotic (chloramphenicol, 20 μg/ml, for R1*drd19;* tetracycline, 7.5 μg/ml, for R136*drdM1;* and kanamycin, 20 μg/ml, for R144*drd3*). A cross with HfrC R^- was included in every experiment as a check on the excluding ability of the F^+ recipient culture (17).

RESULTS

Table 1 shows the results of crossing strain HfrC, with and without an R factor as second sex factor, to recipients with and without F and R. The values shown include the frequencies of transfer of the R factor and of recombinants for the leading Hfr chromosomal marker, *pro*. Exclusion is expressed as the factor by which the frequency of transfer was decreased with reference to the F^- recipient when the recipient was F^+. Thus, the greater the value, the higher was the degree of exclusion, and a figure of one or less indicates no exclusion. In experiments A1 to A4, the recipient cultures were grown to a density at which exclusion had become less than maximal (17); this may be seen by comparing the values for the R^- HfrC donor, shown in the last column, with the corresponding values in experiments B1 and B2, where the recipient cultures were not incubated for so long.

The behavior of the Hfr donor with and without an I-like R factor, R144*drd3*, which determined sex pili physically discrete from the F pili responsible for Hfr transfer is illustrated in experiment A1. Transfer of R144*drd3* was unaffected by the presence of F in the recipient. The frequency of pro^+ recombinants was, however, decreased to the same extent as with the R^- donor; thus, the donor's I-like factor did not prevent exclusion of those donor genes depending on F for their transfer.

An Hfr donor carrying in addition to F an F-like sex factor, such as R1*drd19* or R136*drdM1*, produces not separate F and R pili but a mixed sex pilus with both F and R antigens (16). Nevertheless, high-frequency chromosome transfer requires that the integrated F be functional and that the pili contain F pilin, showing that Hfr transfer of leading genes, like pro^+, remains dependent on the F system of conjugation (9, 12, 14, 20, 21). Thus, in these R^+ Hfr strains Hfr transfer, although still brought about by F, uses a pilus that is partly R1 or R136 in structure. With these R^+ HfrC donors, experiments A2 to B2 of Table 1 show that: (i) with the F^+ recipient lacking an R factor, exclusion was either abolished (A2, A4) or greatly diminished (B1, B2) depending on the state of the recipient culture which determined its excluding capacity (17); and that (ii) with F^+ recipients carrying the same R factor as the Hfr donor, exclusion largely reappeared.

The frequencies of recombinants for the other chromosomal markers, *trp* and *his*, were higher for R^+ than for R^- HfrC and agreed with the values obtained with these R factors in an F^- donor (8). Frequencies were essentially equal with both F^+ and F^- recipients, as was expected if transfer of *trp* and *his* were due to the R factor. This is illustrated for *trp* with R144*drd3* in experiment A1. Values for trp^+ recombinants are also given for R1*drd19* because R1 itself preferentially transfers this region of the bacterial chromosome (26). R1*drd19* exclusion of trp^+ can be seen in experiment A3 with the F^+

TABLE 1. *Relation of exclusion to the structure of the sex pilus*

Expt	R⁺ HfrC									R⁻ HfrC[a]	
	R factor	Donor sex pili	Recipient strain (J62)	Recipient sex pili	Recipients[b]			Exclusion[c]		Recipients[d]	Exclusion[c]
					R⁺	pro⁺	trp⁺	R⁺	pro⁺	pro⁺	pro⁺
A1	R144*drd3*	F, I	F⁻	—	2×10^{0} [e]	1×10^{-2}	4×10^{-5} [f]	<1	63	9×10^{-3}	56
			F⁺	F	3×10^{0}	2×10^{-4}	4×10^{-5}			2×10^{-4}	
A2	R1*drd19*	Mixed F-R1	F⁻	—	2×10^{0} [g]	9×10^{-3}	4×10^{-3}	2	<1	8×10^{-3}	90
			F⁺	F	9×10^{-1}	1×10^{-2}	1×10^{-3}			9×10^{-5}	
A3	R1*drd19*	Mixed F-R1	F⁻	—	—	4×10^{-2}	8×10^{-3} [h]	—	70	2×10^{-2}	85
			F⁺(R1*drd19*)	Mixed F-R1	—	6×10^{-4}	7×10^{-5}			3×10^{-4}	
A4	R136*drdM1*	Mixed F-R136	F⁻	—	6×10^{0} [i]	8×10^{-3}	—	≈1	<1	1×10^{-2}	60
			F⁺	F	4×10^{0}	3×10^{-2}	—			3×10^{-4}	
B1	R1*drd19*	Mixed F-R1	F⁻	—	5×10^{-1} [j]	2×10^{-2}	4×10^{-4} [h]	2	21	2×10^{-2}	570
			F⁺	F	3×10^{-1}	1×10^{-3}	2×10^{-4} [h]			4×10^{-5}	
			F⁻(R1*drd19*)	R1	—	5×10^{-3}	1×10^{-4} [h]		4	—	
			F⁺(R1*drd19*)	Mixed F-R1	—	2×10^{-4}	1×10^{-5}		100	—	
B2	R136*drdM1*	Mixed F-R136	F⁻	—	4×10^{-1}	1×10^{-2}	—	2	10	5×10^{-3}	300
			F⁺	F	2×10^{-1}	1×10^{-3}	—			2×10^{-5}	
			F⁻(R136*drdM1*)	R136	—	6×10^{-3}	—		2		

[a] Control on the excluding ability of the F⁺ recipient culture.

[b] Frequencies expressed as the numbers of colonies of R⁺, *pro*⁺ or *trp*⁺ recipients divided by numbers of colonies of the donor strain produced by the mating mixture and corrected to the nearest whole number.

[c] Frequencies for F⁻ divided by frequencies for F⁺, R⁺, or F⁺R⁺ recipients by using the values before correction as given in [b].

[d] With HfrC R⁻ frequencies for *trp*⁺ recombinants were 400- to 1,000-fold lower than for *pro*⁺ with F⁺ as well as with F⁻ recipients.

[e] By kanamycin resistance.

[f] Corresponds to the frequency given by R144*drd3* when the donor is F⁻ (8).

[g] By chloramphenicol resistance.

[h] Corresponds to the frequencies given by R1*drd19* in an F⁻ donor with 1-hr and with 30-min mating periods (8, 26), equal to the times allowed in experiments A and B, respectively.

[i] By tetracycline resistance.

[j] The values for R transfer are lower in experiments B than in experiments A because of the shorter mating period.

(R1*drd19*) recipient where resistance transfer could not be measured because the recipient was already drug-resistant.

DISCUSSION

The contrasting behavior of an R^+ HfrC donor producing physically discrete F and I pili and one producing a single type of pilus in which the F pilin was mixed with pilin of a different specificity suggests that exclusion is governed directly by the sex pilus. With the R^+ donors producing mixed pili, *pro*$^+$ transfer nevertheless occurred through the activity of F and not of the R factor, as indicated by the high frequencies of *pro*$^+$ recombinants equal to the normal frequencies for HfrC (9, 12, 14, 20). Thus, exclusion seems to be avoided simply by altering the structure of the sex pili used in conjugation and without totally substituting the conjugation mechanism of a different sex factor.

The significance of the type of sex pilus is also suggested by naturally occurring F-like sex factors which are related by superinfection immunity but divided by antigenic differences in their sex pili (15). These factors exclude one another only when their sex pili are of the same serotype (A. Ewins and E. Meynell, *manuscript in preparation*). Recipient bacteria need not actually produce sex pili for exclusion to occur. Thus, exclusion is still present after mutation leading to loss of function in all but a few of the genes identified in pilus production (1, 25) and is not abolished when pilus production is repressed in wild-type sex factors (2, 22, 31). Furthermore, although exclusion of F may be absent when an F^+ recipient also carried certain species of repressor-positive R factor (2, 33; A. Ewins and E. Meynell, *manuscript in preparation*), absence of exclusion is unlikely to simply follow repression because not all species of R factor act in this way, except when a mutant is used which is able to produce its own type of pili in large amounts (A. Ewins and E. Meynell, *manuscript in preparation*).

The interpretation of an effect of the sex pili in exclusion is related to their role in conjugation. In our experience, provided the cultural conditions are optimal for mating (4, 10), pure cultures of the donor have shown as large a proportion of bacteria attached to one another as did mixtures with a non-excluding recipient. It may be that initial contacts depend on some general nonspecific property of the sex pili, such as their charge (18) or hydrophobic nature (5), and that mating specificity is introduced only at a later stage. There is no serological (A. M. Lawn and E. Meynell, *unpublished data*) or chemical (5) evidence of the sex factor determining a specific excluding substance in addition to sex pili as has been postulated (7, 31). If exclusion acts on gene transfer after the initial contacts have been made, there are broadly two different kinds of possibilities, depending on how transfer is thought to come about. Based on a proposal by Brinton (5) wherein the pilus, to which is bound the DNA to be transferred, is assembled from pilin molecules present in the membrane of the donor and moves across to be disassembled in the membrane of the recipient, the necessary equilibrium between polymerized and depolymerized pilin might be upset when the same type of pilin is already present in the recipient. Assuming, on the other hand, that the pilus simply acts as a channel for the donated DNA, the recipient's sex factor might recognize the donor's sex pilus as homologous and block it by attempting to reach the donor in the reverse direction (23). Both possibilities imply that exclusion is an integral feature of the mating process and is not independently determined. The second possibility was tested by making use of the known ability of nalidixic acid to prevent transfer (3) to see if exclusion could be abolished by mating a nalidixic acid-resistant Hfr donor with a sensitive F^+ recipient in the presence of nalidixic acid. However, the test provided no positive evidence in its favor because exclusion remained at its original high level, and transfer occurred normally to an F^- recipient under these conditions.

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