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Interactions between the surface exclusion systems of some F-like plasmids

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SUMMARY

Four surface exclusion systems have been identified amongst a group of F-like plasmids in *E. coli*: Sfx_I(F), Sfx_{II} (ColV2 and R538-1*fin*$^-$), Sfx_{III} (ColVB*trp* and R1-19) and Sfx_{IV} (R100-1 and R136*fin*$^-$). None of these systems was expressed in stationary phase cells or, except for ColVB*trp*, during *fin*$^+$ transfer inhibition, showing that the surface exclusion gene(s) is usually co-controlled with the transfer genes.

Recipient cells carrying two plasmids specifying different surface exclusion systems did not always express both of these: the overall pattern suggested that the four systems and/or their sites of action are related. There was no surface exclusion between donor cells carrying two plasmids determining different surface exclusion systems and recipient cells carrying a plasmid determining either one of these. Our hypothesis to explain this and other results is that surface exclusion prevents interaction between the tip of the pilus on the donor cell and a receptor site on the recipient cell surface. Pili (probably mixed) with two types of tips would be present on cells carrying two different plasmids, the one unresponsive to the single surface exclusion system of the recipient cells allowing transfer of both plasmids.

1. INTRODUCTION

Surface exclusion is the process whereby plasmid transfer from one cell to another is reduced by the presence in the recipient cell of either the same, or a different, plasmid. Investigations of F, showing that surface exclusion prevents mating pair formation as measured by physical means (Achtman, Willetts & Clark, 1971; M. Achtman, personal communication), and transfer of donor DNA (Matsubara, 1968), suggest that it acts by reducing the interaction between donor and recipient cells required to initiate transfer.

Amongst F-like plasmids, four surface exclusion systems have so far been distinguished, those of F (Lederberg, Cavalli & Lederberg, 1952), of ColV2 and R538-1*drd* (Willetts & Maule, 1973; see below), of ColVB*trp* and R1-19 (Alfaro & Willetts, 1972) and of R100-1 and R136*fin*$^-$ (Watanabe *et al.* 1964; Alfaro & Willetts, 1972; Grindley *et al.* 1973; see below). The transfer systems of these F-like R factors are closely related to that of F (Willetts, 1971; Alfaro & Willetts, 1972; N. S. Willetts, unpublished data) and those of the Col factors are indis-

tinguishable from it (Alfaro & Willetts, 1972). Therefore, since the F gene *traS*, the product of which is directly required for surface exclusion, forms part of an operon including most of the transfer genes (Willetts, 1974), the diversity of surface exclusion systems is unexpected.

In the experiments described below, the surface exclusion properties of cells carrying pairs of plasmids specifying different surface exclusion systems were examined, in order to help define the roles played by the donor and recipient cells, and to determine if the four systems function separately from each other.

2. MATERIALS AND METHODS

(i) *Bacterial strains and plasmids*

Donor strains were derivatives of ED56 (Lac$^-$Trp$^-$Str^s), and recipient strains were derivatives of ED57 (Lac$^-$His$^-$Trp$^-$Lys$^-$Str^R). These strains are spontaneous ColVB^R mutants of JC6256 and JC3272 respectively (Achtman *et al.* 1972).

The sources of the plasmids F*lac*, F*his*, ColV2, ColVB*trp*, R1-19, R1-19K$^-$, R100-1, R1, R100 and R136 have been listed previously (Achtman *et al.* 1972; Alfaro & Willetts, 1972; Finnegan & Willetts, 1972). R538-1 was obtained from Dr N. Datta, and R136*fin*$^-$ ($\equiv$ 240*i*$^-$1; Grindley *et al.* 1973) from Dr N. D. F. Grindley. R538-1*fin*$^-$ was isolated as a high level donor after nitrosoguanidine mutagenesis of a strain carrying R538-1. It had little effect on F*lac* transfer (3-fold reduction), and its own transfer was reduced 12-fold by the compatible *fin*$^+$ plasmid R386. R538-1*fin*$^-$ was used rather than R538-1*drd-1* (Meynell & Cooke, 1969), since the latter is *fin*$^+$ and would have reduced transfer and surface exclusion by coexisting plasmids in the experiments to be described. Both R538-1 mutants surface-excluded, and were surface-excluded by, ColV2 to similar extents.

(ii) *Media and plasmid detection*

The formulae of L broth, and of M9 minimal medium, have been given previously (Willetts & Finnegan, 1970). Oxoid nutrient plates were used.

Transfer of plasmids to ED57 and its derivatives was measured using appropriately supplemented minimal medium plates: these always contained 200 μg/ml streptomycin to kill the ED56 donor cells. F*lac*, F*his* and ColVB*trp* were recognized by the Lac$^+$, His$^+$ and Trp$^+$ phenotypes they conferred. Progeny carrying R factors were selected by including an antibiotic as follows: R538-1 and R538-1*fin*$^-$, chloramphenicol (50 μg/ml) unless the recipient strain carried R1-19, when mercuric chloride (5×10^{-5} M) was used; R1 and R1-19, kanamycin (50 μg/ml); R1-19K$^-$, ampicillin (25 μg/ml); R100 and R100-1, tetracycline (10 μg/ml) unless the recipient strain carried R136*fin*$^-$, when chloramphenicol (50 μg/ml) was used; R136 and R136*fin*$^-$, tetracycline (10 μg/ml). R100 and R538-1, but not R1 or R136, determine resistance to mercury ions. ColV2 transfer was detected as described by Alfaro & Willetts (1972).

(iii) *Measurement of surface exclusion indices*

1 ml each of exponential-phase broth cultures of donor and recipient strains at 2×10^8 cells/ml were mixed and incubated at 37° for 60 min. Dilutions were then plated on the appropriate selective medium using the soft agar overlay procedure described previously (Alfaro & Willetts, 1972). Selection was applied only for acquisition of the plasmid present in the donor strain, not for retention of that (or those) originally present in the recipient strain. Both donor and recipient cultures were checked to confirm that > 96 % of the cells carried the relevant plasmid(s). Crosses using the plasmid-free host strain, ED57, as recipient were always performed in parallel, and the surface exclusion index was calculated as the ratio of the number of progeny obtained using ED57 as recipient to the number obtained using a plasmid-carrying derivative.

In many cases, surface exclusion was measured between donor and recipient cells carrying incompatible plasmids. Incompatibility acts after transfer to prevent inheritance of two incompatible plasmids by the same cell; it is unrelated to surface exclusion. Incompatibility should not affect the measurement of surface exclusion by the above technique if the incoming plasmid can displace the resident plasmid. The lack of apparent surface exclusion in many crosses between strains carrying incompatible plasmids (Alfaro & Willetts, 1972; see below) indicates that this is the case, and a reduction in the number of progeny found in other cases is therefore attributed to surface exclusion.

3. RESULTS

(i) *The four surface exclusion systems*

As detailed in the Introduction, four surface exclusion systems have been distinguished amongst F-like plasmids. For convenience, these are called Sfx_I (F), Sfx_{II} (ColV2 and R538-1*drd* or R538-1*fin*$^-$), Sfx_{III} (ColVB*trp* and R1-19) and Sfx_{IV} (R100-1 and R136*fin*$^-$). The results referring to surface exclusion between cells carrying single plasmids in Tables 2–7 (these Tables deal with the six pairs of surface exclusion systems in turn) summarize and extend the observations leading to this conclusion. In particular, results are presented for the first time showing that the surface exclusion systems of R538-1*fin*$^-$ and ColV2 are the same, as are those of R100-1 and R136*fin*$^-$ (although not measured reciprocally). It is apparent that there is almost no reduction in transfer between cells carrying plasmids determining different surface exclusion systems, and that reciprocality of surface exclusion is always observed between cells carrying plasmids determining the same surface exclusion system, even though the pili specified by these plasmids may be different (see below).

The surface exclusion systems of F (Sfx_I), ColV2 (Sfx_{II}) and R1-19 (Sfx_{III}) have been shown previously to be co-controlled with the plasmid transfer systems: all three surface exclusion systems are lost either in the presence of the appropriate transfer inhibitor, or after growth of the cells into stationary phase (Lederberg *et al.* 1952; Willetts & Finnegan, 1970; Alfaro & Willetts, 1972; Willetts &

Maule, 1973). These findings were extended to the other F-like plasmids under consideration here (Table 1). In all cases, surface exclusion was substantially reduced in stationary phase cultures, or, except for ColVB*trp*, during transfer inhibition. As in the case of F, then (Willetts, 1974), the genes determining the three other surface exclusion systems probably form part of an operon including the transfer genes. ColVB*trp*, which may be an exception, is being investigated further.

Table 1. *Inhibition of surface exclusion*

Plasmid in recipient	Surface exclusion indices: Exponential phase cells	Stationary phase cells[e]	Exponential phase cells during transfer inhibition[f]
F*lac*	300[a]	1[b]	6[a]
ColV2	540[b]	15[b]	6[b]
R538-1*fin*⁻	> 290[d]	25	6
R1-19	175[c]	2	17[c]
ColVB*trp*	100[d]	3	120[g]
R136*fin*⁻	(85)[d]	(2)	(14)

[a] These results are taken from Willetts & Finnegan (1970).

[b] These results are taken from Willetts & Maule (1973).

[c] These results are taken from Alfaro & Willetts (1972).

[d] These results are taken from Tables 2–7; indices for stationary phase cells and for exponential phase cells during transfer inhibition were measured using the same corresponding donor strain. Figures in parenthesis indicate that incompatible plasmids are involved.

[e] These were cultures shaken overnight in broth, diluted to 2×10^8 cells/ml immediately before use. They were compared to a similar culture of the plasmid-free host strain.

[f] For F*lac*, ColV2 and ColVB*trp*, the *fin*⁺ product was supplied by R100 (Finnegan & Willetts, 1972); for the R factors, the parental *fin*⁺ plasmid was used.

[g] Transfer of ColVB*trp* was reduced 70-fold by R100, even though surface exclusion was not affected.

(ii) *Recipient abilities of cells carrying two plasmids*

Although surface exclusion is probably determined by a gene(s) closely linked to the transfer genes, it should be emphasized that this gene is distinct from those required for formation of the pilus. Thus in the case of F, pilus-minus mutants frequently retain surface exclusion (Achtman, Willetts & Clark, 1971), and surface exclusion-minus mutants can retain pilus-forming ability (Willetts, 1974; N. S. Willetts & M. Achtman, unpublished data). Also, F (Sfx_I) and ColV2 (Sfx_{II}) specify pili indistinguishable on the basis of quantitative F-specific phage adsorption (MacFarren & Clowes, 1967; Alfaro & Willetts, 1972; Dennison & Hedges, 1972) and serological tests (Lawn & Meynell, 1970), whereas the pili of ColV2 and R538-1*drd* (both Sfx_{II}) are distinguishable by these tests, as are those of ColVB*trp* and R1-19 (both Sfx_{III}).

If the surface exclusion systems, like the transfer systems of these plasmids, are in some way related, interactions in the form of non-additivity might be expected. Derivatives of ED57 carrying two compatible plasmids specifying different surface

exclusion systems were therefore constructed, and their recipient abilities determined in matings with donor cells carrying a single plasmid specifying one of these systems (Tables 2–7, last lines). Comparative data for cells carrying single plasmids are given.

The results can be summarized as follows: (*a*) Sfx_I and Sfx_{II} are expressed in the presence of each other, but not in the presence of Sfx_{III} or Sfx_{IV}; (*b*) Sfx_{III} and Sfx_{IV} are expressed in the presence of Sfx_I or of Sfx_{II} or of each other. These observations show that the four surface exclusion systems do interact, and therefore suggest that they or their sites of action are related.

Table 2. *Sfx_I and Sfx_{II}*

Plasmid(s) in recipient	Plasmid(s) in donor			
	F*lac*	ColV2	R538-1*fin*$^-$	F*lac*, R538-1*fin*$^-$
F*his*	(310)	(3)[a]	2	(20), 3
ColV2	(2)[a]	540[b]	290	(2), 1
R538-1*fin*$^-$	10	> 290	—	12, —
F*his* R538-1*fin*$^-$	(200)	> 800	—	(310), —

Tables 2–7

The numbers represent surface exclusion indices: averages from two or more experiments are given. Figures in parentheses indicate that incompatible plasmids are involved. All strains carrying single plasmids or pairs of plasmids were stable except for those carrying R538-1*fin*$^-$. All donor strains carrying R538-1*fin*$^-$ were derivatives of JC6256 rather than the ColVB^R mutant ED56, since these proved to be more stable: trypsin (1 mg/ml) was therefore included when these were mated with recipient strains carrying a Col factor. Inocula of both JC6256 and ED57 derivatives carrying R538-1*fin*$^-$ were taken from single colonies on selective medium.

[a] Taken from Alfaro & Willetts (1972).
[b] Taken from Willetts & Maule (1973).
[c] The recipient carried R538-1*fin*$^-$, not ColV2.

In one case where it was possible to measure surface exclusion by cells containing two compatible plasmids determining the same surface exclusion system (R1-19K$^-$ and ColVB*trp*, both Sfx_{II}), no significant increase in surface exclusion towards donor cells carrying R1-19 was seen (Table 3).

(iii) *Donor abilities of cells carrying two plasmids*

Although the pilus is irrelevant to the surface exclusion properties of the recipient cells, it seems likely that the pilus type of the donor cells *is* important. This follows from the requirement for the pilus for mating pair formation (Novotny *et al.* 1969; Achtman *et al.* 1971; Ou, 1973*a*) and the observation that surface exclusion acts to prevent mating pair formation (see the Introduction). Furthermore, transfer of Tra$^+$ F*lac traS*$^-$ mutants was greatly reduced by the presence of an F*his* element in the recipient cell, so that the *traS* product does not seem to be required in the donor cell for surface exclusion to occur (N. S. Willetts & M. Achtman, unpublished data).

The role of the donor pilus in surface exclusion could be determined by (*a*) the

sequence and structure of the pilus subunit protein, (*b*) the nature of some later chemical modification of this or (*c*) the presence of a minor component in the pilus. Quite small differences may be involved since the pili of F and of ColV2, indistinguishable by quantitative F-specific phage adsorption and serological tests (Alfaro & Willetts, 1972; Lawn & Meynell, 1970) respond to different surface

Table 3. *Sfx*$_{I}$ *and* *Sfx*$_{III}$

Plasmid(s) in recipient	Plasmid(s) in donor		
	F*lac*	R1-19	F*lac*, R1-19
F*his*	(310)	4[a]	(10), 4
R1-19K$^-$	1[a]	(120)[a]	4, (3)
ColVB*trp*	1[a]	100	3, 1
R1-19K$^-$ ColVB*trp*	3	(150)	3, (3)
F*his* ColVB*trp*	(2)	55	(4), 2

Table 4. *Sfx*$_{I}$ *and* *Sfx*$_{IV}$

Plasmid(s) in recipient	Plasmid(s) in donor		
	F*lac*	R100-1	F*lac*, R100-1
F*his*	(310)	2[a]	(9), 3
R136*fin*$^-$	1	(85)	2, (1)
F*his* R136*fin*$^-$	(30)	(135)	(40), (20)

Table 5. *Sfx*$_{II}$ *and* *Sfx*$_{III}$

Plasmid(s) in recipient	Plasmid(s) in donor		
	R538-1*fin*$^-$	ColVB*trp*	R538-1*fin*$^-$, ColVB*trp*
ColV2	290	1[a]	5, 3
R1-19	(1)	175[a]	(1), 1
ColV2 R1-19	(1)	225	(1), 5

exclusion systems. Conversely, the pili of ColV2 and R538-1*drd*, and of ColVB*trp* and R1-19, although shown to be different by these tests, must have some similarity allowing them to recognize the same surface exclusion systems.

Cells carrying F and R1-19, or F and R136*drd* form 'mixed' pili (Lawn, Meynell & Cooke, 1971); this may be true for all pairs of F-like plasmids. Presumably mixing of pilin subunits, chemical modifications, and minor components might all occur, and this might change the response of the pilus to the surface exclusion system of the recipient. This possibility was investigated by constructing derivatives of ED56 carrying two compatible plasmids determining different surface

exclusion systems and different pili. The donor abilities of these derivatives were measured in crosses with recipient cells carrying a single plasmid determining one of the two surface exclusion systems (Tables 2–7, last columns).

In all these crosses, surface exclusion was effectively abolished. Similar results were obtained by Meynell & Ewins (1973) for transfer from an Hfr strain carrying R1-19 or R136*drd* to cells carrying either F or the R factor. One possible explanation for these observations is that the nature of the pilin subunit (or its chemical modification or any minor component) at the *tip* of the pilus is the important factor. This would presumably differ from pilus to pilus amongst the cells in the population, and those pili with tips not responsive to the surface exclusion system of the recipient cell would be able to form mating pairs and allow transfer of either of the two plasmids.

Table 6. *Sfx_{II} and Sfx_{IV}*

Plasmid(s) in recipient	Plasmid(s) in donor		
	R538-1*fin*⁻	R100-1	ColV2, R100-1
ColV2	290	1[a]	2, (1)[c]
R136*fin*⁻	(1)	(85)	5, (4)
ColV2 R136*fin*⁻	(5)	(75)	—, (20)

Table 7. *Sfx_{III} and Sfx_{IV}*

Plasmid(s) in recipient	Plasmid(s) in donor		
	R1-19	R100-1	ColVB*trp*, R100-1
R1-19K⁻	(120)[a]	(2)[a]	4, (2)
R136*fin*⁻	(2)	(85)	4, (15)
ColVB*trp* R136*fin*⁻	(100)	(40)	–, (40)

Although surface exclusion between cells carrying single plasmids determining the same surface exclusion system is always reciprocal, this does not hold for cells carrying two plasmids. For example, transfer from a cell carrying both F*lac* and R1-19 (Sfx_I and Sfx_{III}) to a cell carrying R1-19K⁻ (Sfx_{III}) is not subject to surface exclusion, whereas transfer of R1-19 to a cell carrying F*his* and ColVB*trp* (Sfx_I and Sfx_{III}), is (Table 3). Similar examples can be found in the other Tables. This non-reciprocality further emphasises the different roles of donor and recipient cells as well as the polarity of transfer.

Transfer from donor cells carrying two plasmids to recipient cells also carrying two plasmids always took place at frequencies consistent with the results in this and the preceding section (Tables 2–7). Thus surface exclusion was substantially reduced in all cases except two, presumably because mixed pili were formed by the donor cells, and the recipient cells expressed only one surface exclusion system.

The exceptions were Sfx_I and Sfx_{II}, and Sfx_{III} and Sfx_{IV}, where both systems are expressed in the same cell, and as expected surface exclusion was still observed.

Meynell & Ewins (1973) found that surface exclusion was present in crosses between Hfr donor cells carrying R1-19 (Sfx_I and Sfx_{III}) and recipient cells also carrying F and R1-19. However, we found that transfer from cells carrying F*lac* and R1-19 to cells carrying F*his* and ColVB*trp* (Table 3) or to cells carrying F*his* and R1-19K$^-$ (unpublished data) did not show surface exclusion. The reason for this discrepancy is not known: our results are consistent with the pilus type of the recipient cell being unimportant for surface exclusion.

4. DISCUSSION

The results presented above confirm the existence of at least four different surface exclusion systems amongst F-like plasmids. None of these systems was expressed after growth of the host cells into stationary phase, or, except for ColVB*trp*, during transfer inhibition. These properties of plasmid-specificity and inhibition during transfer inhibition, should serve as useful criteria for distinguishing surface exclusion from smaller 'non-specific' effects.

Although it is likely that surface exclusion leads to a reduction in DNA transfer, some of the published reports purporting to show this seem to be questionable. For example, the comparatively small (five-fold) reductions in R1-19 DNA labelling after transfer when the recipient strain carried F or R100 (Le Blanc & Falkow, 1973), cannot be due to surface exclusion since F and R100 specify surface exclusion systems not recognized by R1-19. Similarly, the small reductions in Hfr DNA transfer to minicells derived from strains carrying ColVB*trp*, R100 or R100-1 (Sheehy, Orr & Curtiss, 1972) cannot be ascribed to surface exclusion. In fact, the surface of minicells may not be comparable to that of whole cells Ou, 1973*b*, 1974).

Ou & Anderson (1972) and Ou (1973*a*) have proposed that the tip of the pilus attaches to a specific receptor site on the surface of the recipient cell to form a mating pair. Our results are consistent with this and the further hypothesis that surface exclusion prevents this attachment. The interactions between the four surface exclusion systems of the F-like plasmids tested, indicate that the receptor sites for transfer of these plasmids and/or their surface exclusion systems, may be biochemically related.

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