EVIDENCE FOR THE IDENTITY OF THE MATING-SPECIFIC SITE OF MALE CELLS OF ESCHERICHIA COLI WITH THE RECEPTOR SITE OF AN RNA PHAGE

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Since the discovery of male-specific bacterial filaments as receptors for RNA phages (Crawford and Gesteland, 1964) it has been tempting to associate such structures with the transport of genetic material (Brinton et al., 1964): As a working hypothesis it is conceivable that sex-specific fimbriae (pili) may provide an entry channel through the cell membrane for phage RNA (much as does the tail of, for example, T-phages for phage DNA); on the other hand such fimbriae may also serve as a channeling device for donor DNA during bacterial conjugation. If these fimbriae are involved in such dual functions, conjugation should interfere with phage invasion and phage invasion should disturb bacterial conjugation. Experiments to be described will provide supporting evidence for such hypothesis.

Materials and Methods — Bacterial and phage strains have been described (Marvin and Hoffmann-Berling, 1963; Knolle, 1966a, 1967d) as well as the general techniques of their handling (Knolle, 1967a, b), and their preparation for mating (Knolle and Ørskov, 1967). The preparation and the properties of UV-

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irradiated phage (UV-fr**) have been discussed before (Knolle, 1966b). Media were used as described elsewhere (Knolle, 1967a; Knolle and Ørskov, 1967).

Results

1. Reduction of recombination frequency after pre-treatment of donor cells with UV-fr — If fimbriae are involved in the mating process, coating of these structures with inactivated phage (i.e., UV-fr) should influence the efficiency of pairing; as a consequence, the recombination frequency should be reduced, depending on the amount of UV-fr used. That this is indeed the case is shown in Table 1.

Table 1

Recombinant formation after treatment of donor cells with UV-fr: Donor cells (strain AB261, Hfr, met$^-$, grown in CaYT to a titer of 2 x 10^8), were mixed with UV-fr (IM_{do} 60 and 600, in 2 experiments) for 10 min at 37°C. An equal volume of recipient cells (strain W677-41, F$^-$his$^-$pro$^-$thr$^-$leu$^-$ sm^r, grown exponentially to a titer of 2 x 10^8, concentrated by centrifugation and resuspension in CaYT to a titer of 2 x 10^9) was added. After 15 min at 37°C (to allow pairing) the mixture was diluted by the addition of a 10-fold volume of warm CaYT. After a total of 2 hours, samples were plated on sm-minimal agar, supplemented with his and pro, for scoring of recombinant colonies. Parallel samples were plated on minimal agar supplemented with met for the determination of the number of colony-forming donor cells. The number of colony-forming recipient cells was determined on sm-minimal agar supplemented with his, pro, thr, and leu.

Donor (AB261) pre-treated with	UV-fr	1/10 UV-fr	Control
Relative titer* of recombinant colonies	35 ± 3	120 ± 5	175 ± 6
Relative titer of donor input	51 ± 7	37 ± 6	47 ± 7

*Average counts from 5 plates.

**Abbreviations used: UV-fr, Ultraviolet-inactivated fr (from 6 x 10^{11} to 10^2 PFU); IM, input multiplicity (cf. Knolle, 1966a); IM_{do}, IM with regard to the concentration of donor cells; PFU, plaque-forming unit; SRP, serum-resistant PFU; YT, yeast tryptone medium; CaYT, YT supplemented with M/500 $CaCl_2$; sm, streptomycin.

The IM has been calculated on the basis of the number of PFU present in the freshly prepared lysate before UV-inactivation. In comparison with the control, the yield of recombinants is decreased to 69% with the low IM_{do} (60), and to 20% with the high IM_{do} (600) used. The viability of the donor cells has not been affected by coating of their fimbriae with inactivated phage.

2. Infection of conjugating cells with UV-fr: If sex-specific fimbriae serve as channels for chromosomal transfer as well as for RNA invasion (Knolle, 1967b, c; Knolle and Ørskov, 1967), mating cells should be refractory to RNA phage infection, while still permitting phages to adsorb.* Such effects are demonstrated in Table 2. In comparison with a control (B), pre-incubation of male with female bacteria (A) resulted in a reduction of the efficiency of fr-infection to 17%. The efficiency of phage-adsorption was much less reduced. (F^- cells could not be infected and did not precipitate phage (D); they did not interfere significantly with adsorption and infection, when added 5 min after the phage with diluent).

The effect of recombination on the sensitivity of male cells to fr is demonstrated in Table 3. Cells of two male strains were mated with recipients. After mixing with an excess of phage, more than 95% of the male cells in control tubes were infected by fr. In comparison with this control, with the F^+ strain 3300-141 the relative capacity of the cells to become infected is reduced to a final value of 20% as mating progresses. With the Hfr strain AB261, both the absolute and the relative capacity of cells to be infected by fr drop, the latter to a final value of less than 5%.

* Previous results had shown that in the presence of strong interference with fr-invasion by UV-fr (Knolle, 1966b) or by sodium azide (Knolle, 1967c), the rate of phage adsorption was only slightly reduced.

Table 2

Efficiency of adsorption and infection of fr with male cells, pre-incubated with female cells: Donor cells (AB261) and recipient cells (W677-10s, $F^-thr^-sm^r$) were prepared as described for Table 1. After pre-incubation, fr was added to the mixtures (IM 10^{-2}). After 5 min at 37°C, dilutions were made for the assay of SRP and of non-adsorbed phages.

	A	B	C	D
Pre-incubation a) 1.5 ml (10 min - 37°C) b) 1.5 ml Diluent 1.5 ml	AB261 F^- YT	AB261 YT YT	AB261 YT F^-	YT F^- YT
Infected cells (SRP)+ Infected cells (%)	13 ± 2 17	67 ± 4 88	65 ± 4 86	0 -
Non-adsorbed PFU+ Adsorbed PFU (calculated, %)	414 ± 9 71 ± 3	145 ± 5 90 ± 4	185 ± 6 87 ± 3	(144 ± 5) x 10 0

+ See Table 1.

Discussion — The physical properties of Type I pili have been studied extensively by Brinton et al. (1954, 1961, 1964). According to these studies such pili represent structural components of variable length, depending on the number of subunits per filament. These subunits are polymerized into right-handed helices with a diameter of 70 Å, with an axial hole of 20-25 Å diameter. F-fimbriae (or -pili) are indistinguishable from Type I pili in their physical appearance and may, therefore, be of similar composition and structure. The core of these fimbriae would, hence, have dimensions similar to the core of the tails of T-even phage, through which DNA injection is known to occur. There has been much correlative evidence for the dual involvement of F-fimbriae in bacterial mating and in fr-infection (Knolle, 1964; Knolle and Ørskov, 1967), and it has been proposed that these structures represent elements that are active in phage invasion as well as in chromosome transfer (Knolle, 1967c; Knolle and Ørskov, 1967).

Table 3

Sensitivity of recombining cells to infection by fr: Exponentially growing donor and recipient cells were mixed (5 ml 3300-141, $F^+leu^-his^-$, titer 4.2×10^7 and 5 ml W677-21, $F^-thr^-leu^-$ sm^r, titer 5.3×10^8; 5 ml AB261, titer 6×10^7 and 5 ml W677-41, titer 5×10^8; controls received YT in place of recipients). After various time intervals samples were withdrawn and infected with fr (IM_{do} 20). After 10 min at 37°C unadsorbed phage was inactivated with antiserum. Samples were plated for the determination of SRP. The relative rate of recombinant formation (with 3300-141 and W677-21, thr selection) was found to be 1, 1.75, 2.25 at 15, 40 and 90 min respectively, in a control incubation. The absolute rate of recombinant formation (with AB261 and W677-21) was found to be 2×10^{-4} with thr-leu selection after 2 hours of incubation in a second control.

Plaque yield	Time after mix. (min)	Mating mixtures, containing		Controls, containing	
		3300-141	AB261	3300-141	AB261
SRP*	15	49 ± 5	52 ± 5	76 ± 6	116 ± 7
	40	84 ± 6	44 ± 5	374 ± 12	183 ± 9
	90	120 ± 7	16 ± 3	584 ± 15	343 ± 12
%	15	65	45	100	100
	40	22	24	100	100
	90	20	4.7	100	100

*Average counts from 2 plates.

In the present experiments it has been observed that the ability of UV-fr to inhibit recombination is less than its ability to inhibit invasion of superinfectint RNA phage (cf. Knolle, 1966b). Since UV-fr (even with low IM) effectively inhibits the invasion of superinfecting viable fr, without significantly inhibiting its adsorption, it appears likely that UV-fr is capable of initiating invasion by entry of its RNA into F-fimbriae. In the present experiments, a significant reduction in the frequency of recombinant-formation is found only with a high input multiplicity of phage (IM_{do} 600). This inhibitory effect is, therefore, of

different nature and may not be due to blockage of the "DNA-transfer channel" by phage RNA: Chromosomal transfer seems to proceed with higher efficiency than phage RNA invasion.

The number of adsorption sites for fr (localized on F-fimbriae) on sensitive cells had been estimated to be of the order of several hundred per cell (Knolle, 1966b). Hence, it appears that the coating of F-fimbriae with UV-fr is correlated with the reduction in the efficiency of recombinant formation. This effect may be due to a reduction in the capacity of male cells to bind to female cells, or due to an interference with the function of F-fimbriae during the mating process.

On the other hand, recombination effectively reduces the efficiency of infection by fr while affecting adsorption to a much lesser extent. This differential effect of recombination on fr-infection is very similar to the effects of pre-infection by UV-fr, or of cell-starvation, on the efficiency of fr-infection and -invasion (Knolle, 1966b, 1967c). This finding is supplemented by the fact that recombination also reduces the probability of male cells to be infected by fr. Therefore, this may indeed be understood as a "crowding-out" effect, in which donor DNA blocks the core of an F-fimbrium for the passage of phage RNA.

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