THE SEX HAIR OF E. COLI AS SENSORY FIBER, CONJUGATION TUBE, OR MATING ARM?*

Karin A. Ippen** and Raymond C. Valentine

Department of Biochemistry
University of California
Berkeley, California 94720

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Brinton (1965) has proposed that F-pili (sex hair) play an important role in the mating process of E. coli. However, the exact function of F-pili in the mating act is not clear. Brinton (1965) has suggested that F-pili might serve as conjugation bridges, or as arms to stabilize the mating pairs.

Our approach to the role of the sex hair has been to use the male-specific phages f1 and f2 as mating inhibitors. These viruses are known to attach to the F-pilus at different sites - f1 to the F-pilus tip and f2 to the side (Caro and Schnös, 1966). In our experiments we use f1 as a "tip inhibitor" and f2 as a "side inhibitor" as shown in the model below (Fig. 1). The results of our experiments using these two viruses are described.

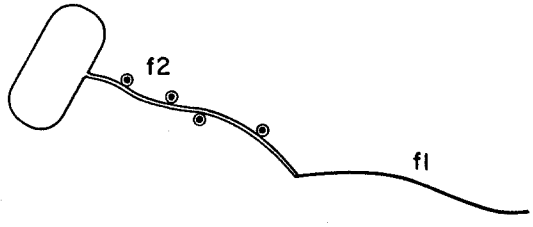

Fig. 1. Blocking the F-pilus with male-specific viruses.

Results and Experimental

Sex Hair Requirement for Mating. In our first experiment to correlate mating capacity with F-pilation, we measured episome (Flac') transfer after

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blending the male cells to remove F-pili. While this process effectively removed F-pili and simultaneously decreased donor capacity, the return of mating ability was highly variable - sometimes lag periods as long as 15 min were observed. The lag period was reduced considerably when the males were blended less vigorously in their own medium for shorter time periods still sufficient to shear off most of the F-pili. As observed by Brinton (1965), it was also most convenient to assay mating pair formation by the blended culture as distinguished from measuring completed transfer of Flac' into the acceptor cell. In agreement with Brinton's experiments we have observed a strong correlation between mating pair formation and the presence or absence of F-pili. A blending experiment is shown in Fig. 2. For this experiment E. coli C600 infected with a Flac' episome was grown to a cell density of 3×10^8 cells per ml in a glucose-salts medium supplemented with biotin and 0.1% casamino acids. The culture was cooled and F-pili were removed by blending for 1 min at 1/2 maximum speed using the Servall "Omni-mixer" (Brinton, 1965). Fragments of F-pili were removed by centrifugation and the blended male cells resuspended in fresh broth. The ratio of female to male cells in the mating mixture was about 10. A suitable lac^- and streptomycin resistant recipient strain was used. At 1 min intervals after mixing males and females, samples were removed and gently diluted 1:1000 into prewarmed broth at 37°C to prevent further pair formation. After 30-40 min for Flac' transfer to take place between the preformed mating pairs, samples of the culture were removed and plated on lactose, streptomycin medium to measure Flac' recombinants.

Regrowth of a new crop of F-pili by the blended cells was measured simultaneously with pair formation using the radioactive phage assay as described earlier (Ippen and Valentine, 1965). The lag period before F-pili synthesis began varied somewhat with different cultures but correlated well with pair-forming ability of the culture (Fig. 2). We conclude, in agreement with Brinton (1965), that F-pili are required for mating pair formation.

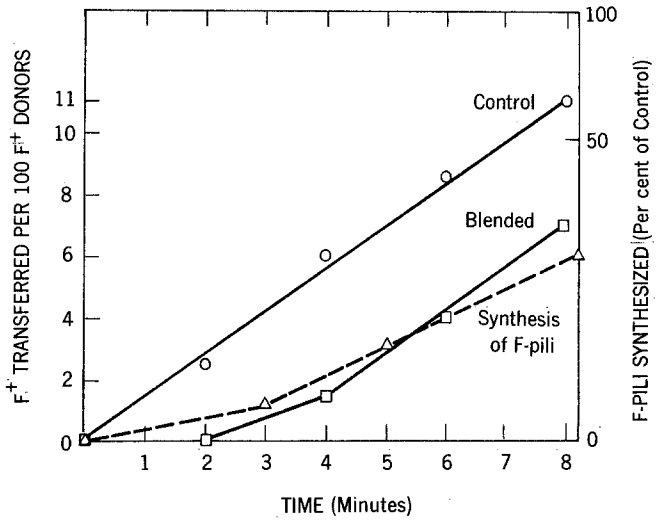

Fig. 2. F-pili requirement for mating pair formation. Male cells blended and mated as described in the text. Note parallel return of pair forming ability and F-pili regrowth.

Does fl Block the Tip? Caro and Schnös (1966) have shown by electron microscopy that male-specific phage fl adsorbs to the tip of the sex hair - it therefore seemed reasonable to use this virus as a "tip-blocking agent." First, it was necessary to destroy the infectivity of fl in order to rule out cell killing as a consequence of fl infection. Noninfectious segments of fl still containing an active adsorption end were easily prepared by sonication of the filamentous particles (Fareed et al., 1966). These tiny subviral segments readily adsorb to F-pili but do not kill cells - in fact the tiny DNA segments probably have no ability to initiate infection (Fareed and Valentine, in preparation). The result of an experiment using fl fragments as "tip-blocking agents" is shown in Fig. 3. It seems clear that fl fragments do inhibit mating pair formation, as would be expected if the F-pilus tip is vital. Moreover, exposure to the same concentration of fl fragments during the 30-minute incubation after pair formation had been stopped by dilution had no apparent effect on the number of Flac' recombinants formed. This lack of interference by the fragments once

mating pairs have been established indicates that the F-pilus tip is then no longer exposed to the "blocking power" of the fragments and again suggests its involvement in coupling. A control using whole f1 at the concentration of infective particles remaining in the sonicated preparation did not affect pair formation. The requirement of high concentrations of f1 fragments to inhibit mating (Fig. 3) is not unusual because of the poor adsorption of this virus.

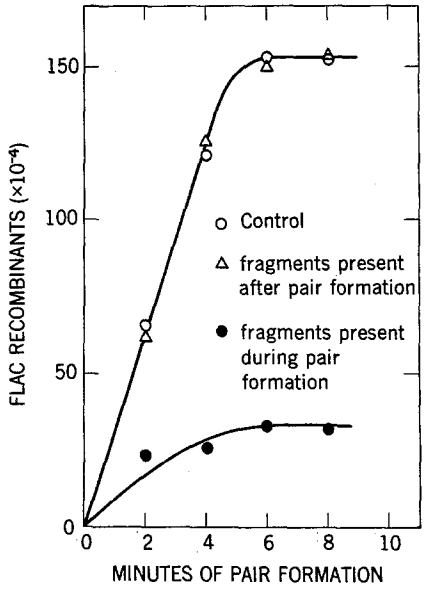

Fig. 3. Effect of f1 fragments on mating pair formation. f1 fragments prepared by sonication (4 min) of purified f1 as described earlier (Fareed et al., 1966). Approximately 3 x 10^{10} "active ends" were added per ml of mating mixture. Mating pairs were assayed as described for Fig. 2, except the entire procedure was carried out in nutrient broth. After pair formation the control was diluted into broth containing 3 x 10^{10} active fragments/ml as well as into broth alone. Note the inhibition caused by the presence of f1 fragments during the pair formation, and their lack of effect after dilution of the pairs.

f2 and the F-pilus Sides. Hundreds of particles of RNA phage can adsorb to the sides of a single sex hair - only a few, however, (perhaps only 1) manage to inject their RNA into the cell (Ippen, unpublished data). The effect of these tiny phages on the mating process was next studied (Fig. 4). For these experiments we used high concentrations of RN'ase in

the mating mixture to prevent phage RNA from entering the cell (Valentine and Wedel, 1965), hoping to localize the effect of f2 to the F-pilus. Some escape of phage RNA into the cell has been observed, however, so this technique is not completely successful. (It is of some interest that much of this RNA may be in the form of small, noninfectious pieces - Silverman and Ippen, in preparation).

It is obvious in Fig. 4 that the presence of f2, both during and after pair formation has an inhibitory effect on the mating process. It can be hypothesized that when phage are present during the formation of pairs, either a steric effect is caused by phage adsorption, and pairs cannot form, or the initial stages of injection of RNA prevent a mating contact from being effective. Unfortunately the effect of f2 on the different mating steps (pair formation versus transfer of Flac') cannot really be distinguished from these curves. However, when pairs formed in the absence of f2 are exposed to phage during the subsequent incubation at 37°C, inhibition is also observed. Since f2 adsorb to the sides of the F-pilus, it seems likely that this decrease is due to an interruption in the mating contact such that transfer of Flac' cannot be effected. Since Flac' transfer probably occurs within a few minutes after initiation the data are consistent with a model in which the adsorption (or injection) of f2 could prevent initiation of transfer from occurring, but could not interrrupt transfer once it had begun.

Although we have not entirely eliminated the possibility that some donor cells are affected by f2 other than on the pilus, our evidence so far suggests that these effects cannot be of the same magnitude as the interference with mating. The high multiplicity of f2 to male cells was used because not only was the male cell population less than 1/10 of the total number of cells present, the presence of RNase probably causes those particles attempting injection to fall off (Valentine and Wedel, 1965). Knolle (1967) has recently reported the results of similar experiments

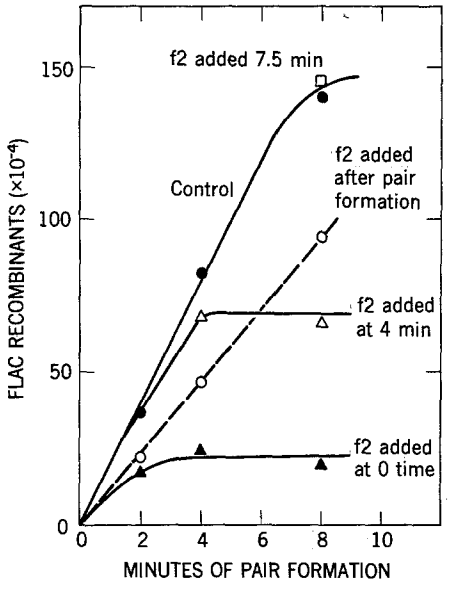

Fig. 4. Inhibition of mating by f2. Conditions for pair formation as previously described except that 100 μg/ml RNase was present in all media and plates. 1×10^{10} f2 per ml was added to the pair forming mixtures as indicated. The control (pairs formed in the absence of f2) was diluted into both broth, and broth containing this concentration of phage (dashed line). Note the effect of f2 both before and after pairs have formed.

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where UV inactivated RNA phage was used to interfere with the conjugation of Hfr strains, and his results, using a similarly high input multiplicity with respect to donor cells, correlate well with ours.

Discussion

Our aim was to use the male specific phages f1 and f2 as sex hair (F-pili) blocking agents - f1 for the tips and f2 for the sides. In this way we hoped to determine whether the F-pilus was necessary for mating (mating pair formation). These experiments are complicated somewhat because both with f1 fragments and RN'ase treated f2 some nucleic acid is transported into the cell; even though this "fragment" nucleic acid is probably not infectious, the possibility that their presence in the cell could interfere with mating cannot be eliminated.

It is still an interesting possibility that f1, in fact, blocks the tip of the F-pilus and prevents the male cell from contacting the female. Perhaps the tip-female interaction is the only mode of recognition between male and female cells. The male may "sense" the female through his pilus. If the F-pilus tip makes first contact then this information could be relayed to the male via the sides or center of the pilus. We have used f2 as a probe for this proposed "mating signal" (Brinton, 1965), and hypothesized that f2 might interfere with the female-male communication by injecting or starting to inject its RNA into the core of the pilus. The f2 inhibition of mating observed could be interpreted in terms of the mating signal model.

Although the experiments reported here are still indirect, we think they have led us to some interesting notions on the role of the F-pilus in mating. One of the most interesting of these is the possibility of a communications system (mating signal) between male and female (Jacob, 1966).

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