# *THE ATTACHMENT OF THE MALE-SPECIFIC BACTERIOPHAGE F1 TO SENSITIVE STRAINS OF ESCHERICHIA COLI**

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The bacteriophage f1 infects specifically the strains of *Escherichia coli* which contain the sex factor F or similar genetic elements such as the colicinogenic factor V. It has an unusual structure, being filamentous with a length of 8500 Å. It contains DNA, unlike the better-studied RNA bacteriophages, which are also male-specific. It was isolated by Loeb[1] and described by Zinder *et al.*[2] A bacteriophage of this type was first described by Marvin and Hoffmann-Berling.[3] Others were described by Hofschneider,[4] Bradley,[5, 6] and Dettori and Neri.[7] These phages have many similar characteristics but can be distinguished serologically.[8]

F pili, described and characterized by Brinton *et al.*,[9, 10] are filamentous appendages of male strains of *E. coli*, which are consistently associated with the presence of the sex factor or of a similar genetic element. The F pili are defined morphologically by their specific attachment of the spherical RNA phages which adsorb laterally along the entire length of each pilus. They have a diameter of approximately 80 Å and a variable length which can be as much as 20 $\mu$ or longer. We have studied the attachment of the phage f1 to *E. coli* Hfr cells and *E. coli* cells containing the colicinogenic factor V. Our results show that the F pili are the site of attachment of f1.

*Materials and Methods.—Strains:* A concentrated stock of f1 phage was given to us by Dr. June Rothman, who had prepared and purified it. This stock was used for all our experiments.

The Hfr strain used (strain 5)[11] was *E. coli* K12 CR34 Hfr derived from[12] CR34 $F^-$ *thr*$^-$ *leu*$^-$ *lac*$^-$ *thy*$^-$ $Str^s$ (isolated by Okada, Yanugisawa and Ryan)[13] by David Pratt (personal communication). This Hfr has the same origin and direction of transfer as Hfr H.

The colicinogenic strain was[12] *E. coli* K12 C600 $colV^+$ $colE_1{}^R$ $colV^R$ $Az^R$ *thr*$^-$ *leu*$^-$ *thi*$^-$ obtained from Phyllis Kahn.[14]

*Media:* Cultures were grown in M9 (glucose-salts) medium prepared according to Adams,[15] and supplemented with 0.5% casamino-acids (Difco) and 5 $\mu$g/ml of thymine.

*Experimental procedure:* Phage attachment to sensitive cells was observed by infecting an exponential culture grown to approximately 2–4 $\times$ $10^8$ cells/ml with ~20–40 f1 phages per cell in presence of 0.025 *M* KCN. After 5 min the bacteriophage MS-2 (a male-specific RNA bacteriophage isolated by A. J. Clark) was added at a concentration of $10^{11}$ phages/ml. After another 5 min, drops of the mixture were placed on electron microscope specimen screens coated with a collodion film backed with a thin carbon layer. Five minutes were allowed for adsorption of the bacteria to the film, then the drops were sponged off with filter paper and replaced by drops of a 2% phosphotungstic acid (PTA) solution at pH 7.4. The PTA solution was removed immediately, and the specimens were examined in a Siemens-Elmiskop I.

To prepare F pili fragments, the cells were centrifuged; concentrated in phosphate buffer, pH 7.0, to ~$10^9$ cells/ml; and stirred violently in a Sorvall Omni-Mixer (Micro-attachment) for 10 min. The attachment of phages and the preparation of specimens were as described above without addition of KCN, but with the addition of a small amount of tryptone powder (Difco) to the solution before placing it on the screen. The use of tryptone results in a better distribution of pili fragments on the collodion film.

*Results.—Morphology of f1:* The dimensions of f1 measured in our preparations (Fig. 1) were 8500 Å in length and 60–70 Å in width; this is in agreement with a previous estimate (by Zinder *et al.*).[2] As noted by Bradley[6] in the phage ZJ/2, a

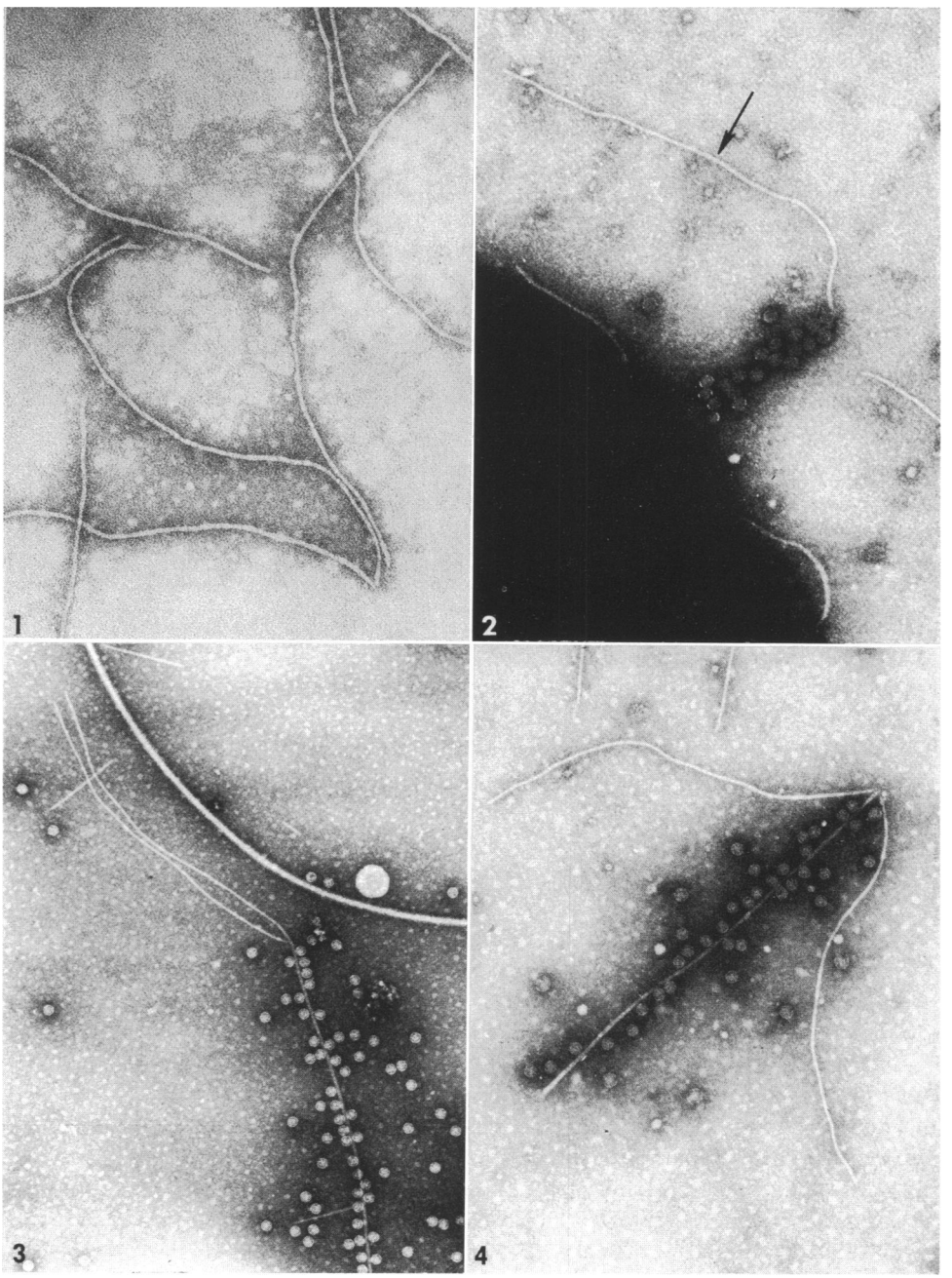



FIG. 1.—Several f1 bacteriophages. Negative staining with phosphotungstic acid. ×90,000.
FIG. 2.—An Hfr cell infected with an f1 phage. The phage (*arrow*) is attached to a short F pilus. Part of the cell appears as a dark mass at the bottom of the picture. The F pilus is covered with MS-2 phages which help to identify it. ×75,000.
FIG. 3.—Two f1 phages attached to the same F pilus. ×57,000.
FIG. 4.—An F pilus fragment with two attached f1 phages. ×64,000.

thin dark line appears often along the axis of the phage in PTA preparations. This can be interpreted as an axial hole. Marvin[16] has, however, shown electron micrographs which seem to indicate that the fd phage is composed of two units lying longitudinally side by side. Accordingly, Marvin has interpreted the black line as the separation between the two units. Our own pictures have not shown any formations favoring this latter interpretation.

*Attachment of f1 to Hfr cells:* In preparations made as described in *Materials and Methods*, the phage f1 was found frequently attached to the tip of F pili by one of its ends (Fig. 2). The F pili are clearly distinguishable from either f1 bacteriophages or type I pili[10] by their specific attachment of MS-2 bacteriophages. In some preparations nearly all F pili had f1 phage attached. Controls showed that the phage itself did not attach MS-2 phages and that broken-off type I pili did not attach to F pili. The f1 phage can be differentiated from type I pili fragments by its morphology: it is less rigid than the pili and has a constant length of ~8500 Å, whereas type I pili have variable lengths.

MS-2 does not interfere with the attachment of f1: nothing was changed in the final picture when the order in which the two phage types were attached was reversed. Neither did f1 ever attach along the length of F pili. It is clear therefore that the two phages have different attachment sites located on the same male-specific structure, the F pilus.

As shown in Figures 3 and 4, two f1 phages can attach to the same F pilus simultaneously, demonstrating that the tip of each F pilus contains two or more attachment sites.

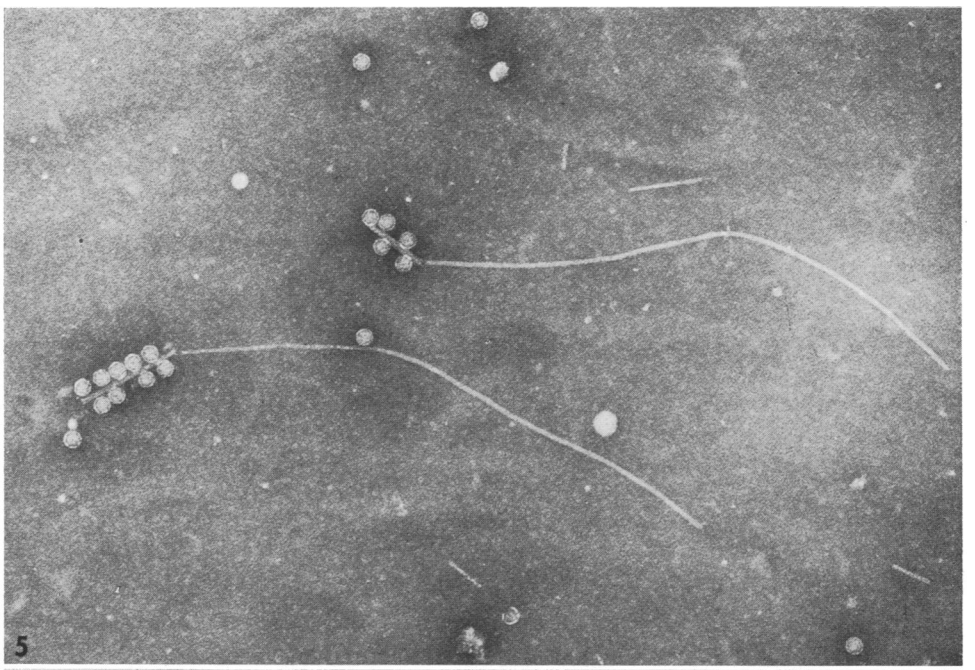
*Attachment of f1 to fragments of F pili:* When F pili fragments are prepared as described above, f1 can still attach to the isolated pieces (Fig. 4). In one preparation, 142 F pili fragments were examined. One hundred had one phage attached at one end, 18 had two phages attached at one end, only one fragment had one phage attached to each of its ends. In view of the high density of phages present on the screen, it is possible that the juxtaposition of phage end and pilus end was accidental in this last case. It seems clear that only one of the two free ends of an F pilus fragment can serve as an attachment site.

The attachment of two f1 phages to one end of the same F pilus occurs much less frequently than would be predicted if both phages had the same probability of attachment to many sites on the tip (Poisson distribution) or to two sites. It seems, therefore, that the attachment of a phage interferes to some extent with the attachment of a second phage. In one case only, we found three f1 phages attached to the same F pilus. This occurrence is therefore possible, but extremely rare.

After F pili are broken in the mixer, the fragments remaining connected with the cell can attach phages. Each of the many broken pieces obtained when cultures containing very long F pili are blended can attach phages (Fig. 5), presumably at the end that was distal to the cell. It appears, therefore, that attachment sites are disposed all along the length of the pilus, but that only those at one of the two ends are free for f1 attachment.

We have been unable to determine whether the phage can attach at either of its ends.

*Attachment of f1 to colicin V pili:* Cells containing the colicinogenic factor col V appear to possess a genetic determinant of fertility and are sensitive to male-specific



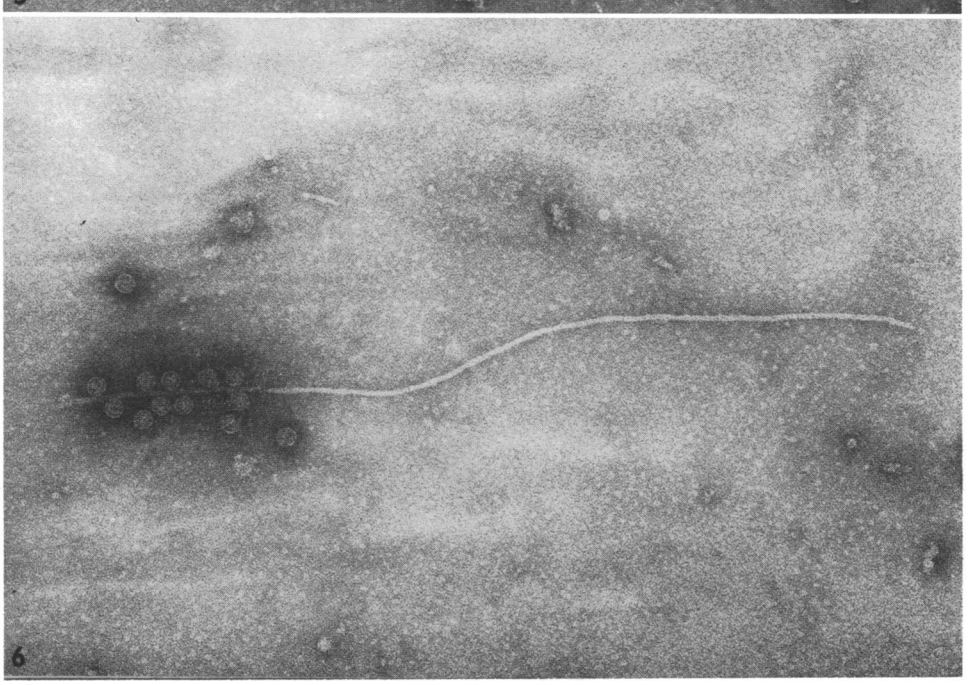




FIG. 5.—Small fragments from long F pili. In this preparation, as shown in the picture, almost all fragments had phages attached. ×90,000.

FIG. 6.—Attachment of f1 phage to a pilus fragment from *E. coli* C600 colV$^+$ cells. ×100,000.

bacteriophages.[14, 17] Cultures of C600 colV$^+$ contained pili capable of attaching the phage MS-2. The attachment of f1 to these pili was identical to that found for F pili of Hfr cells (Fig. 6).

*Polymers of f1 phage:* Bradley[6] noted that filamentous phages ZJ/2 could stick end to end to form longer filaments. We have found, in the lysate of f1 used in these experiments, particles which had twice, three times, and four times the normal length. Such particles showed no appearance of discontinuity at the point of junction. There is a chance that some of these longer particles may have been pieces of F pili or type I pili coming from the cells used for making the lysate. All doubt as to the phage origin of these particles is removed, however, by the fact that all three types have been found attached to F pili in a manner similar to that of the monomer units (Figs. 7 and 8).

*Discussion.*—Our results show that f1 attaches to the tip of F pili and that each break of the pilus, creating a new tip, creates also a new site of attachment. Furthermore, f1 and MS-2, which attach laterally along the pilus, do not compete for attachment sites. Should the pilus prove to have a simple structure and to be composed of a single protein, one would have to postulate that one face of that protein attaches f1, another face attaches MS-2, and still another face attaches neither.

Our results explain many of the results of Tzagoloff and Pratt[18] on the initial steps of infection with the filamentous phage M13. They found that each cell has two to three sites of attachment; most Hfr of F$^+$ strains contain only two to three F pili[10] (it should be noted that this is not universally true; the Hfr strain used here contains as many as 30 F pili per cell and has a correspondingly high number of f1 attachment sites). Tzagoloff and Pratt found that f1 DNA penetrates into the cell with a half time of 2 min, but a minimum time of less than 20 sec. This spread in penetration time could be due to the spread in length of F pili which can vary between a fraction of a micron and 20 $\mu$ or more. These authors also found that most of the protein of the phage remains outside the cell after injection of the DNA.

All of the above facts fit the notion that injection of f1 DNA takes place through the F pilus. This is in agreement with the hypothesis of Brinton[10] that the F pilus acts as a conductor of nucleic acids in various situations such as RNA or DNA male-specific phage infection and bacterial conjugation. Eileen Reizen has found (personal communication) that the DNA bacteriophage M13 interfered with mating of Hfr cells with F$^-$ cells, while the RNA phage M12 did not. This difference could have resulted from the difference in attachment site. The attachment of M13 at the tip of the F pili could impair their role in contact formation or their possible role in DNA transfer, or both.

The finding of dimers or polymers of the phage capable of attachment and perhaps of infection confirms the hypothesis that the heterozygous particles of the phage discovered by Notani and Zinder[19] are formed by two parental phages joined end to end (ref. 19; June Rothman, personal communication).

*Summary.*—In electron micrographs, f1 was seen to attach by one end to the tip of F pili (filamentous appendages of male strains of *E. coli* with a diameter similar to that of the phage). The F pili were identified by their specific attachment of the spherical RNA phage MS-2 which adsorbs laterally along the entire length of the pilus. Phage f1 can also attach to isolated F pili, but only at one of the two

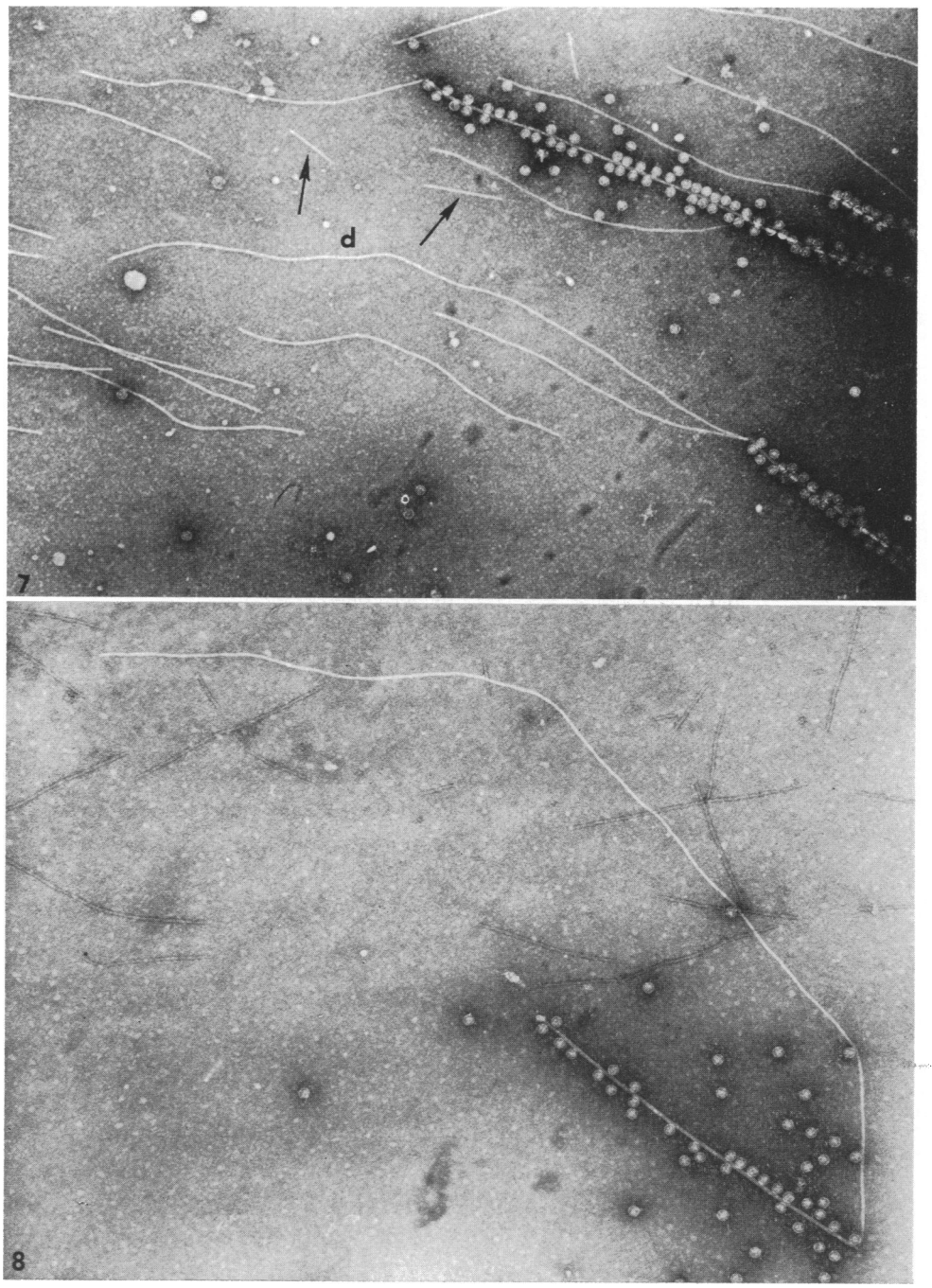



FIG. 7.—Several pili with phage attached. One phage (*d*) is a dimer with a length of ~18,000 A. A few fragments of type I pili or fimbriae (*arrows*) are seen. They are shorter, rigid, nonphage-specific pili, and not related to sexual type. ×49,000.

FIG. 8.—Trimer of f1 phage (length ~27,000 Å) attached to an F pilus fragment. ×55,000.

free ends of an F pilus fragment. That one end may contain several attachment sites, as shown by the attachment of two or even three f1 bacteriophages. The DNA and RNA male-specific phages have completely distinct attachment sites. Attachment sites for f1 are present along the entire length of an F pilus, but are only made available when the pilus is broken at any one point. Dimers and higher polymers of the phage attach in the same fashion.

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[12] Symbols used: *thr* = threonine; *leu* = leucine; *lac* = ability to utilize lactose; *thy* = thymine; Str$^s$ = sensitivity to streptomycin; col $E_1^R$ = resistance to colicine $E_1$; colV$^R$ = resistance to colicin V; Az$^R$ = resistance to sodium azide; *thi* = thiamine.

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