F-PILI REQUIREMENT FOR RNA BACTERIOPHAGE ADSORPTION*

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The discovery of Loeb and Zinder (1961) of a set of small "male-specific" bacteriophages which grow only on F^+, F' of Hfr mating types of E. coli raised several interesting questions regarding the mechanism of attachment of these phages and their association with the F (fertility) factor. A question asked by workers in this field was: "Do the tiny phages found by Loeb and Zinder (1961) inject their nucleic acid into the host bacterium with the aid of the conjugation or mating organ(s) already present on the male cells?" An interesting experiment by Crawford and Gesteland (1964) has shed light on this question. These workers observed in electron micrographs that RNA bacteriophages adsorbed to long filaments on the surface of male cells. Female cells showed no ability to adsorb phage and were resistant to infection. Brinton, Gemski and Carnahan (1964) confirmed these findings and named the thin filaments F-pili, to distinguish them from other more common forms of pili (fimbriae) synthesized by both male and female strains. Brinton et al. (1964) also showed that F-pili were genetically controlled by the F factor of E. coli. We have recently devised a rapid and quantitative assay method for F-pili which has allowed us to study in more detail their synthesis and biological function (Valentine and Strand, 1965). In this communication we show that F-pili are required for RNA-bacteriophage adsorption.

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Results and Experimental

The bacterial and bacteriophage strains and general experimental conditions were as described earlier (Valentine and Strand, 1965 and Ippen and Valentine, In Press). F-pili were assayed by the filtration method as modified by Ippen and Valentine, In Press. A suitable sample of male culture grown in Tryptone-Yeast Extract-Calcium broth (TYE) (Loeb and Zinder, 1961) at 37° to approximately 2 x 10^8 cells per ml was added to a test tube containing TYE broth, normally to a final volume of 1-8 ml depending on the experiment. Usually no more than 0.5 ml samples of culture were assayed because of the tendency of cells to clog the membrane filters. A sample of P^{32} labeled phage (usually 2-4 x 10^8 viable phage particles per ml reaction mixture; approximately 10,000 CPM per ml reaction mixture) was then added; again the quantity of radioactivity depended on the experiment. After 5 min. adsorption time at 0°C (or a suitable interval) the samples were quickly diluted to stop further interaction and filtered to remove unadsorbed phage. Radioactive particles which had become attached to F-pili remained on the filter pad and were washed and counted as before (Valentine and Strand, 1965). The assay appears to measure only simple phage adsorption to F-pili, subsequent "injection stages" being prevented by the incubation at 0°, while formation of the F-pili-phage complex occurs readily at this temperature. The filtration assay, then, provides a measure of the quantity of F-pili synthesized by a bacterial culture. In the experiments described below we have used the filtration assay with slight modifications to measure the F-pili levels and regeneration of F-pili by blended cultures.

The first experiment was designed to test whether or not F-pili were required for phage adsorption. First it was necessary to remove all the F-pili from the cell surface without damaging the cell. A clue as to how F-pili might be removed was provided by Brinton *et al.* (1964) who mentioned that F-pili appeared to be sheared from cells by mechanical

agitation (blending). We also treated male cells in the Waring blendor to obtain concentrates of free F-pili (Valentine and Strand, 1965). Blending was found to remove all F-pili from male cells as shown in Fig. 1.

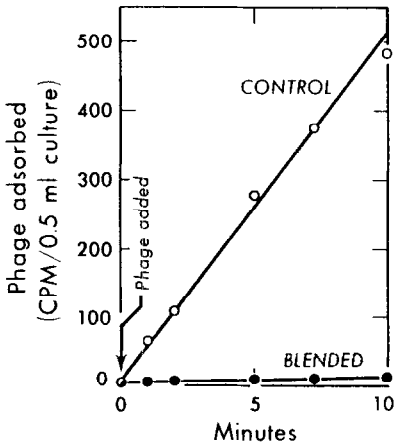

Fig. 1. F-pili requirement for RNA-bacteriophage adsorption. F-pili were removed by treating the cells in the Waring blendor for 2 min. at 0°C. The cells were collected by centrifugation and resuspended in 0.005 M Tris buffer pH 7.4 containing 0.85 per cent NaCl at 0°C. The blending cycle was repeated 3 times to remove all F-pili. The blended cells were suspended in Tryptone broth (4×10^8 cells/ml) at 0°C and radioactive phage were added to start the reaction. At the times indicated aliquots of 0.5 ml were assayed for adsorbed phage by the filtration assay described in the text. An unblended culture served as control.

For this experiment it was necessary to carry out the blending procedure at 0°C to prevent the rapid resynthesis of F-pili as shown below. Cells depilated by blending remained fully viable when stored in broth at 0°C for several hours. In Fig. 1 the control culture showing a high content of F-pili was a sample of the same culture used for blending. Note that radioactive phage was added to both the blended and control cultures at "0" time. The earlier observation (Ippen and Valentine, In Press) that F-pili may be conveniently assayed by the filtration method even at 0°C was of great importance for this experiment (Fig. 1) since the culture once freed of F-pili was never allowed to warm up thus totally preventing regrowth of F-pili. Our earlier attempts (Valentine and Strand, 1965) to carry out the blending experiment at 37°C were probably

not successful because of the rapid regeneration of F-pili at this temperature. The ease at which F-pili were removed by blending was not unexpected since, as we have shown earlier (Ippen and Valentine, In Press) even relatively mild procedures such as centrifugation of the male cells tends to break off as many as 50 per cent of the F-pili from the cell. Repeated cycles of washing and centrifugation of male cultures have been found to remove as many as 80 per cent of the F-pili; nearly all F-pili removed by such mechanical means may be recovered in the cell-free supernatant. Repeated cycles of blending as in Fig. 1 lowered the F-pili level of male cultures almost to that of female cultures; male cells then appeared to be phenotypically sterilized by blending. All mating types tested including F^+, F' and Hfr cultures showed similar behavior.

The rapid regeneration of F-pili was next investigated. For the regeneration experiment of Fig. 2 (3-hr. culture) a chilled suspension of male bacteria freed of F-pili by blending was placed in warm broth ($37^{\circ}C$) to allow regrowth of F-pili; samples were removed at different times and chilled at $0^{\circ}C$ to stop growth, and assayed by the filtration method to determine the amount of F-pili synthesized. As seen in Fig. 2 (3-hr. culture) synthesis of F-pili began after a short lag period of 1-2 min., which varied with different cultures; maximum pilation occurred after 8-10 min. incubation at $37^{\circ}C$.

The maximum F-pili concentration reached by blended cells was usually somewhat lower than the level of the untreated control sample. Regeneration of F-pili by a number of mating types was similar though the level of F-pili synthesized varied with different strains. However, the ability of a given culture to regenerate F-pili was found to be variable with different growth media and markedly affected by the age of the culture. As seen in Fig. 2 (24-hr. culture), blended cells prepared from a 24-hr. culture incubated at 37° with shaking showed a 20 min. lag period before detectable F-pili synthesis began and even after 60 min. only slight synthesis had occurred.

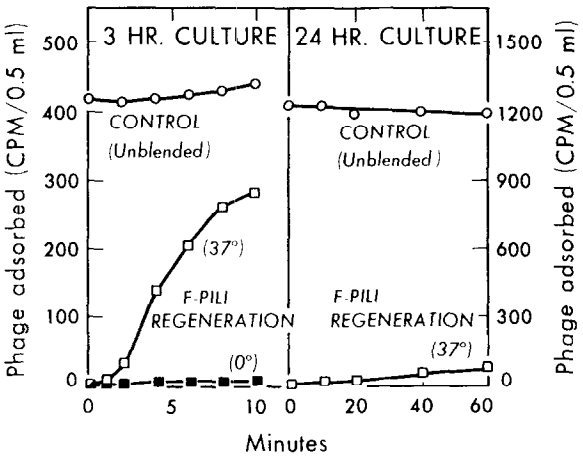

Fig. 2 (3-hr. culture). Resynthesis of F-pili by blended cells. Blended cells (E. coli K12 (K19)) prepared as in Fig. 1 were suspended in Tryptone broth (4×10^8 cells/ml); radioactive phage were added and the sample was incubated at 37°C. Resynthesis of F-pili was measured using 0.5 ml samples by the phage adsorption assay as in the text. A duplicate culture which was not blended served as control.

Fig. 2 (24-hr. culture). Resynthesis of F-pili by an aged culture. A culture of E. coli(K19) was grown for 24 hr. and blended and assayed as above.

In contrast using an actively growing culture F-pili synthesis began almost immediately (Fig. 2 (3-hr. culture)). We interpret the "lag" of the 24-hr. culture to be due to the generally poor physiological state of the aged cells and have no specific explanation. These findings might have bearing for conjugation experiments in which F-pili may play a role.

Discussion

The experiments presented above argue for the direct role of F-pili in RNA-phage infection. Firstly, blending of male cultures effectively removed all receptor sites for RNA phages. This was directly correlated with the presence or absence of F-pili in the culture. F-pili were found to regenerate rapidly though this ability was strongly affected by the physiological state of the cell. These findings have been supported by

electron micrographs (Brinton, Valentine and Carnahan, unpublished observations).

The requirement of F-pili for RNA bacteriophage adsorption raises many interesting questions regarding their mechanism of action. It is tempting to speculate that F-pili serve as hollow tubes for transport of phage RNA into the cell during infection. Experiments are under way to elucidate this point.

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