SEXUAL DIFFERENTIATION OF E. COLI*

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Received December 7, 1965

A remarkable form of differentiation is known to occur in E. coli (Cavalli, Lederberg, and Lederberg, 1953; Hayes, 1953); female cells are converted to male cells after infection with the fertility (F) factor. This genetic element alone is thought to be responsible for the female-male conversion. While sex conversion is a well-studied genetic phenomenon the biochemical events responsible for this conversion are almost totally unknown. The discovery (Crawford and Gesteland, 1964) that male cells synthesized thin filaments named F-pili (Brinton, Gemski, and Carnahan, 1964) provided one tool for biochemical studies. We presently assume that F-pili represent "gene products" of the fertility factor itself and that F-pilation is indicative of at least one stage of the sexual maturation process; see Brinton et al., 1964, for discussion of this point.

The experiments described below indicate that the female-male conversion is a relatively rapid process taking place in as little as 30 min after infection of the female cell with the fertility factor. The reverse conversion, male-female, is a relatively rare event which occurs occasionally in the male population apparently due to the spontaneous loss of the F-factor from a male cell.

*Supported by a grant from the National Science Foundation (NSF).

Results

Female-Male Conversion. The first experiments were designed to test the time requirement for sexual maturation of the newly infected female cell. It should be pointed out that the criterion of sexual maturity (maleness) used in the present study was not the ability of cells to mate, but was the development of an active penetration mechanism for phage RNA and of F-pili by the maturating male cell. In other words we say that the two systems - ability to conjugate and competence for phage infection - are completely interlocking and that a cell possessing an active penetration system for phage RNA and F-pili represents a sexually mature state. In view of the lack of direct experimental verification of this point, it might best be stated that maleness as measured by phage competence and development of F-pili represents at least a stable stage of the sexual maturation cycle of E. coli.

Sexual differentiation appears to occur rapidly in a female cell infected with the fertility factor - as shown in Fig. 1A and Fig. 1B some cells appear to be converted to males in as little as 30 min after infection. For this experiment a female strain resistant to streptomycin (SM) was grown in tryptone broth (Loeb and Zinder, 1961) to a density of approximately 3×10^8 cells/ml and infected with an F^+ strain (SM sensitive) grown as above to a density of 1.5×10^8 cells per ml. The ratio of male to female cells (1:2) varied slightly with different experiments. Infection was allowed to proceed in stationary culture at 37°C for 20 min before streptomycin (80 μg/ml) was added to prevent excessive growth of the male. Samples of 0.5-1.0 ml of cell culture were removed at the times shown in Fig. 1A and assayed for phage RNA penetration (Valentine and Wedel, 1965). The samples were diluted 1:10 in fresh broth at 37°C and infected with radioactive phage for 10 min to allow phage RNA penetration to occur. Penetration was terminated by rapid cooling of the samples to 0°C. The infected cells were next centrifuged to remove non-adsorbed phage, resuspended in saline solution (0.85%), and blended for

1 min to remove adsorbed phage which had not yet penetrated the cell. The blended cells were collected by centrifugation and filtered and counted as before (Valentine and Wedel, 1965). Various controls including male alone and female alone were run. The values of Fig. 1A were obtained using an F-infected female culture and a male alone as control. Note

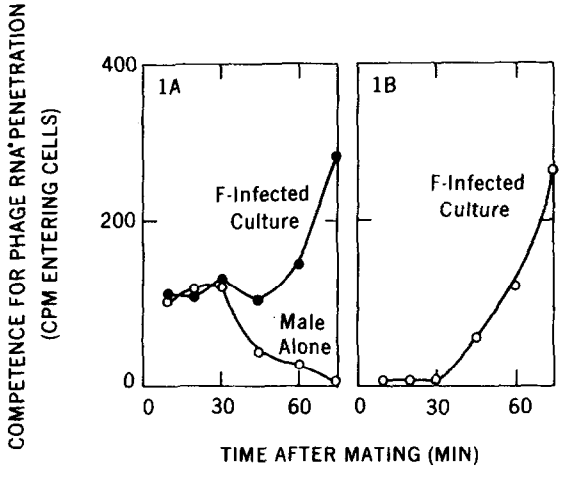

Fig. 1A. Sexual differentiation of E. coli - development of competence for male phages by an F-infected culture. Details as in text. Differentiation was measured by following the development of competence for RNA phage penetration. The male (F^+) strain was E. coli K12 (1485 of A. J. Clark) and the F^- strain was E. coli (C 600).

1B. Data of Fig. 1A replotted. Values for the male culture alone were subtracted from the values of the mated culture to obtain the net synthesis of phage competence by the F-infected culture. Note the 30-min "lag" period.

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that the background values for the male were significant especially for the early points and after addition of SM (20 min) a marked decay in competence set in. Edgell and Ginoza (1965) have explained this decay as due to the poor physiological state of the SM-treated cell. Various other procedures were used in an attempt to lower the background of the male culture, including killing of the donor males with phage T_6 and blending to remove F-pili, but none of these procedures gave significantly different

results from those above. The growth rate of the streptomycin-resistant female culture used for Fig. 1A and 1B was normal (cell density values not given in Fig. 1). Also, it was found in control experiments that SM at the level used did not alter F-pili synthesis or phage competence by SM-resistant strains.

Replotting the data of Fig. 1A leads to a clearer picture of the onset of sexual differentiation. Fig. 1B was obtained by subtracting the values of the male control from the values obtained with the mated culture. Note in Fig. 1B the "lag" period of approximately 30 min before phage competence begins and then the rapid rise ("burst") of competence. The asynchronous mating condition of the culture prevented a more precise temporal division of the maturation events; in other words the continuous mating occurring in the culture and asynchronous condition of the cells prevented, for example, any correlation between the state of cell-division and appearance of phage competence. After 90 min the fertility factor had spread to a large percentage of the population in some cases probably as the result of secondary infection by the newly "maled" cells.

It was of interest to determine the time after F-infection that F-pili biosynthesis began (Fig. 2). The protocol for this experiment was similar to Fig. 1 above except the samples were assayed for F-pili using the filtration procedure (Ippen and Valentine, 1965). Note in Fig. 2 that F-pili synthesis and competence for RNA-phage penetration were parallel events as expected if F-pili were required for RNA penetration. A lag period of about 30 min was also observed before F-pili synthesis commenced in the newly infected female. F-pilation was about 40-50% complete after 120 min when compared to a male culture of the same cell density. Nearly complete F-pilation occurred in cultures by the time they reached the early stationary phase of growth.

In summarizing the data of Figs. 1 and 2, it can be seen that sexual differentiation in E. coli is a rapid process with some cells exhibiting F-pili and phage competence as early as 30 min after infection with the sex

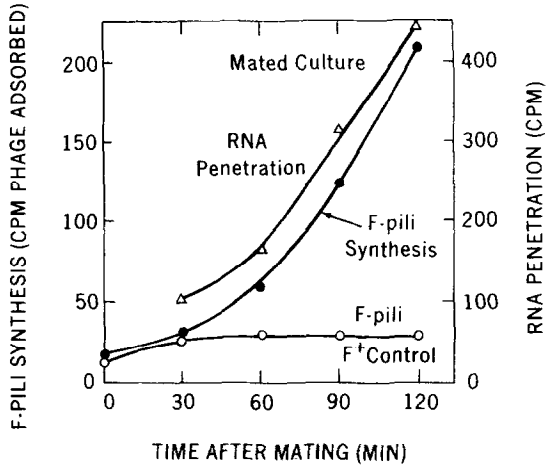

Fig. 2. Biosynthesis of F-pili by an F-infected culture. Conditions identical to Fig. 1 except 0.05 ml samples were removed for assaying F-pili by the filtration procedure; incubation was at 0°C to prevent RNA penetration. Note the parallel development of phage competence and F-pili by the mated culture.

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factor. Finer temporal division of the events of sexual differentiation have not as yet been achieved.

Male-Female Conversion. Male (F^+) cultures always contain female "revertants" which appear to have spontaneously lost their sex factor. When isolated such strains usually behave as typical females and do not synthesize F-pili. Fig. 3 shows the time course of development of the female revertants of a male culture after the male cells were killed by treatment with male-specific phage. As shown in Fig. 3, phage-resistant cells selected by this procedure proliferate rapidly and soon dominate the culture. The phage-resistant culture no longer synthesized detectable F-pili. Colonies obtained by subculturing the phage-resistant stock never regained the capacity to synthesize F-pili even after several transfers; when crossed with the male strain used in Fig. 1, they behaved as normal females, being readily converted to males on infection. In earlier experiments Brinton et al. (1964) were able to catalyze the male-female conversion of cultures

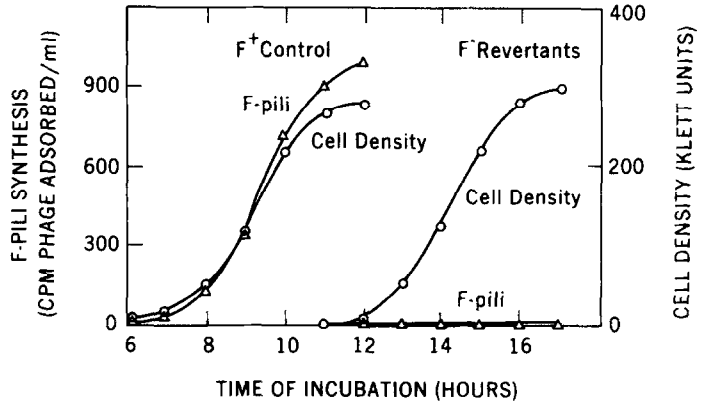

Fig. 3. Time course of development of female cells in an F^+ culture after killing of the males with "male" phage. Two identical broth cultures were inoculated with approximately 10^6 cells per ml. One culture was simultaneously infected with 2×10^{11} RNA phage. Samples were removed for optical density and F-pili determination; in order to remove excess "cold" phage which would interfere with the F-pili assay the samples were centrifuged and the cells resuspended in fresh broth after 11-hr growth. Note that the phage infected culture remained clear for 11 hrs due to phage lysis of the male cells before the F^- revertants began to dominate the population. The F^- revertants did not possess F-pili.

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by prolonged treatment of the male cells with acridine orange. Some acridine-treated cultures underwent almost complete sex conversion and likewise produced no F-pili. The above experiments help to solidify the correlation between maleness and F-pilation as first observed by Brinton et al. (1964). The interesting question of whether phage-resistant cells may still contain defective F-factors must await further investigation.

Discussion

In one sense the fertility factor of E. coli may be regarded as a special form of virus - its distinguishing feature being its unique mode of cellular infection or penetration. After entry into the female cell the virus-like genome of the F-factor directs the synthesis of "F"-induced enzymes and other structural products which ultimately lead to complete

sexual differentiation of the cell - the new male cell possesses an active transport system responsible for infectious transmission of the fertility factor itself. A secondary consequence of maleness appears to be the competence of the cells to infection by "male" phages - male cells possess phage receptor sites (F-pili) and an active transport system for phage RNA. It seems probable that one of the major biochemical events leading to maleness is concerned with alterations of the cell membrane - F-pili biosynthesis.

The present general state of the problem is similar to that of the T-phages before phage-induced enzymes were recognized. Combined genetical and biochemical approaches will surely shed light on this interesting and perhaps basic form of differentiation.

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