Genet. Res., Camb. (1961), **2**, *pp.* 394–405
With 3 text-figures
Printed in Great Britain

The genetics of fimbriation in *Escherichia coli*

By G. A. MACCACARO and W. HAYES
Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W.12

(*Received* 22 *April* 1961)

INTRODUCTION

Fimbriae are filamentous, non-flagellar appendages present at the surface of a number of Gram-negative *Eubacteriales* (Anderson, 1949; Houwink & Van Iterson, 1950; Brinton, Buzzell & Lauffer, 1954; Duguid, Smith, Dempster & Edmunds, 1955; Constable, 1956; Duguid & Gillies, 1957, 1958) including *Escherichia coli K*-12 (Maccacaro, 1955; Maccacaro, Colombo & DiNardo, 1959; Brinton, 1959). This paper reports a genetic analysis of this character, which is of interest not only from a morphological point of view, but also because it affects some surface properties and basic physiological traits of the bacterial cell.

Types of fimbriation

Within the same species two main types of strain have been described (Brinton *et al.*, 1954; Duguid *et al.*, 1955; Maccacaro *et al.*, 1959; Maccacaro & Turri, 1959): (*a*) those which either possess fimbriae or can develop them under suitable environmental conditions, and (*b*) strains which lack fimbriae irrespective of the environmental conditions. The notations 'Fim$^+$' and 'Fim$^-$' have been applied to these two types; within a Fim$^+$ strain, the notation 'Fim$^+$' indicates expression, and 'Fim$^{(+)}$' lack of expression, of the character. A Fim$^{(+)}$ strain usually becomes Fim$^+$ if serially subcultured in nutrient broth, but reverts to the Fim$^{(+)}$ state after repeated subculture on nutrient agar. These Fim$^+ \rightleftharpoons$ Fim$^{(+)}$ conversions are always reversible and are not reflected in changes in colonial morphology. This is true for practically all species and genera which have so far been tested.

A rather different picture was presented by Brinton (1959), who reported that, in some strains of *E. coli B*, fimbriated cells (P$^+$) give rise to morphologically distinctive colonies, but mutate irreversibly to a non-fimbriated type (P$^-$) at a high rate (4×10^{-4}/cell/generation). The gap between these conflicting pictures was filled by the discovery that Fim$^+$ strains of *E. coli* K-12, if plated on nutrient agar after a long series of subcultures in fluid medium, give rise, among a majority of normal colonies, to a few small, smooth, compact colonies which are entirely composed of fimbriated cells. Occasionally some of these colonies produce a large rough outgrowth consisting of non-fimbriated cells. The former, colonially distinctive, type is insensitive to those environmental factors which controlled the Fim$^+ \rightleftharpoons$ Fim$^{(+)}$ conversion of the parental strain; the latter type is irreversibly non-fimbriated. These two types clearly correspond to Brinton's P$^+$ and P$^-$ types.

We have adopted the notation Fimσ^+ to indicate the stably fimbriated mutants which arise from Fim$^+$ strains, and the notation Fimσ^- to indicate the irreversibly non-fimbriated mutants which arise from Fimσ^+ strains. Our present knowledge about types of fimbriation is summarized in Fig. 1.

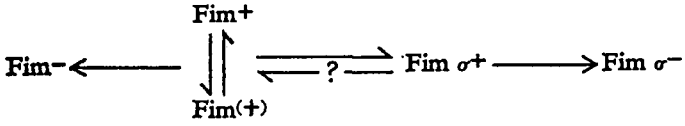


Fig. 1. Types of fimbriation and non-fimbriation in *Escherichia coli* K-12, and the relationship between them.

Detection of fimbriae and correlated properties

Fimbriae cannot be seen by light microscopy but are easily detectable by electron-microscopy, especially in shadowed preparations (Anderson, 1949; Houwink & Van Iterson, 1950; Brinton *et al.*, 1954; Duguid *et al.*, 1955; Constable, 1956; Duguid & Gillies, 1957, 1958; Maccacaro, 1955). Their presence on individual cells is marked by a sharp reduction in electrokinetic mobility (Brinton *et al.*, 1954; Maccacaro & Turri, 1959; Brinton, 1959). Fimbriated cells of both Fim$^+$ and Fimσ^+ types form a tenacious pellicle in broth culture and agglutinate red blood cells of the guinea-pig and other animals (Duguid *et al.*, 1955; Maccacaro *et al.*, 1959); in the case of Fimσ^+, however, this agglutination is more or less suppressed in the presence of peptone. Fimbriated cells are also agglutinated by a wide variety of dipolar molecules (dicarboxylic acids, diamines and monoamino-monocarboxylic acids) (Maccacaro & Dettori, 1960). In addition to the effect of environmental factors such as culture media and certain chemical agents (Table 1) on the expression of fimbriation, certain Fim types differ in their oxidative and glycolytic activity (Maccacaro & Dettori, 1959). When all these properties are considered together, it is possible to distinguish five types of cell with respect to fimbriation, of which two are fimbriated (Fim$^+$ and Fimσ^+) and three non-fimbriated (Fim$^-$, Fimσ^-, and Fim$^{(+)}$). These distinguishing properties are set out, with references, in Table 1.

Genetic mapping of the fim *locus*

A morphological character whose expression can be so easily elicited or repressed (Fim$^+ \rightleftharpoons$ Fim$^{(+)}$), as well as stabilized (Fimσ^+) and then irreversibly lost (Fimσ^-), seems to offer an unusual opportunity for genetic analysis of the control of bacterial morphogenesis. In the experiments reported here, haemagglutination of guinea-pig R.B.C. was the principal test used to discriminate between fimbriated and non-fimbriated recombinant types; Fim$^+$ and Fimσ^+ recombinants were distinguished by inhibition of this agglutination by peptone, as well as by colonial morphology.

Table 1. *Distinction between different types of fimbriation and non-fimbriation in* Escherichia coli *K*-12

Expression of fimbriation	Types of fimbriation: Fim⁻	Fim(+)	Fim⁺	Fimσ⁺	Fimσ⁻	References
Presence of fimbriae (electron microscopy)	− (not reversible)	− (reversible)	+	+	− (not reversible)	Anderson, 1949 Maccacaro, 1955
Sensitivity of fimbriation to cultural conditions	−	+	+	−	−	Duguid *et al.*, 1955 Maccacaro & Turri, 1959
Inhibition of expression of fimbriation by ethanol* & phenol†			+	−		
Agglutination of red blood cells	−	−	+	+ (Inhibited by peptone)	−	Duguid *et al.*, 1955 Maccacaro *et al.*, 1959
Agglutination by dipolar molecules	−	−	+	+	−	Maccacaro & Dettori, 1960
Electrophoretic mobility	fast	fast	slow	slow	fast	Brinton *et al.*, 1954 Maccacaro & Turri, 1959 Brinton, 1959
Q_{O_2}‡ *a*	26	95	90			Maccacaro & Dettori, 1959
Q_{O_2}‡ *b*		86	83	85	88	
Q_{CO_2}§ *a*	205	190	90			Maccacaro & Dettori, 1959
Q_{CO_2}§ *b*		172	69	85	78	

* = 4 per cent ethanol in nutrient broth.
† = 0·1 per cent phenol in nutrient broth.
‡ = substrate: sodium fumarate M/60.
§ = substrate: glucose M/240.
a = recombinants α_4 and β_1 (see Maccacaro & Dettori, 1959).
b = strain 58-161.

Earlier investigations (Maccacaro *et al.*, 1959), prior to the discovery of the σ types, had led to the following conclusions:

(*a*) In Fim⁺ F⁺ × Fim⁻F⁻ crosses, where the F⁺ donor loci *thr*⁺ and *leu*⁺ (ability to synthesize threonine and leucine) are selected, 70 to 80% of the prototrophic recombinants are fimbriated.

(*b*) The locus controlling fimbriation (*fim*) is rather closely linked to the *thr–leu* region.

(*c*) Clearcut mapping of the *fim* locus is perturbed by a marked, direct correlation between the length of Fim⁺ donor chromosome inherited by recombinant cells and the frequency of fimbriation among them.

(*d*) The rapidity with which fimbriation is expressed in Fim$^+$ recombinants is correlated with the degree of expression of the fimbriated parent.

Findings (*c*) and (*d*) suggest that Fim$^+$ inheritance might depend on a cytoplasmic as well as on a chromosomal contribution from the Fim$^+$ donor parent, but the possibility of negative interference was also considered as a possible explanation of finding (*c*). Furthermore, since F$^+$ × F$^-$ crosses were used, it was possible that the anomalies of recombination were due to heterogeneity among the F$^+$ chromosome fragments transferred to the zygotes.

EXPERIMENTAL METHODS AND RESULTS

Genetic analysis by means of Hfr × F$^-$ crosses

The validity and precision of genetic analysis employing Hfr × F$^-$ crosses is now well established (Wollman & Jacob, 1959). We therefore turned to the study of inheritance of fimbriation by means of such crosses. Strain Hfr*H* (Hayes, 1953)

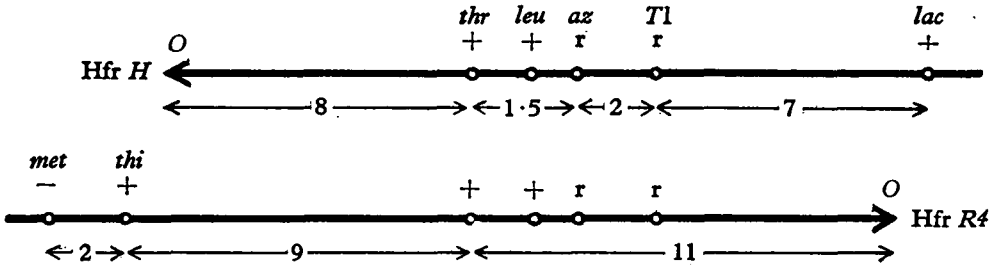


Fig. 2. Chromosome transfer by the Hfr strains *H* and *R4*. The diagram shows the leading chromosomal extremity, *O*, and the direction of chromosome transfer (indicated by arrow) by each strain. The distances between the relevant loci, in terms of times of transfer (minutes) in broth at 37°, are roughly to scale. Both strains are derived from the same *F*$^+$ strain and are identical in their nutritional requirements and other characters.

met = methionine
thi = thiamine
thr = threonine
leu = leucine
} synthesis

az^r = sodium azide resistance
*T*1^r = phage T1 resistance
lac$^+$ = lactose fermentation

Note: Hfr*H* transfers the segment *thr–lac* in less time than Hfr*R4* takes to transfer the shorter segment *O–thr*. This is accounted for by the time required by Hfr*R4* to *initiate* transfer (for other examples, see Taylor & Adelberg, 1960).

was used as Fim$^+$ donor; its relevant loci, the order of their arrangement on the chromosome and the approximate distances between them in terms of time of transfer in broth at 37° C. (Wollman, Jacob & Hayes, 1956), are given in Fig. 2. The F$^-$ strain (W945) was Fim$^-$ and complementary to Hfr*H* in all these characters. The segregation of unselected markers in prototrophic recombinants selected for *thr*$^+$ and *leu*$^+$ inheritance from the Hfr parent was orthodox, but the distribution of *fim*$^+$ within the different recombinant classes revealed the same anomaly observed in the Fim$^+$F$^+$ × Fim$^-$F$^-$ crosses in an even more striking way (see Table

https://doi.org/10.1017/S0016672300000872 Published online by Cambridge University Press

2A; also Maccacaro & Hayes, 1961). It therefore seemed that the *fim* locus could not be precisely located by means of linkage data. However, the unique features of conjugation in *E. coli* (Wollman, Jacob & Hayes, 1956) enable the position of a locus to be defined on a time scale which can be converted into a chromosome map. By periodic interruption of the mating process between conjugating cells it is possible to determine with accuracy the order and time of entry of donor genes into the recipient cells and, therefore, their sequence and distance from one another on the donor chromosome.

The Fim$^-$ streptomycin-resistant (S^r) strain W945 was used as recipient in interrupted mating experiments with two different Fim$^+$ streptomycin-sensitive (S^s) Hfr strains, *H* and *R4*. These Hfr donor strains are both derived from the same F$^+$ strain (58-161) and have the same genotype, but they differ in the position of the leading locus (*O*) and in the direction of entry of the chromosome into the recipient cells as shown in Fig. 2. The two crosses were carried out in the same way. Young broth cultures of the parental strains were mixed and kept in gentle

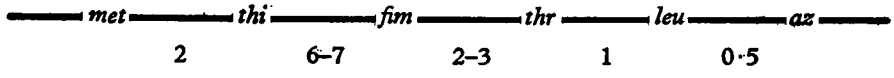


Fig. 3. Chromosomal location of the *fim* locus. Approximate time distances between the loci are given in minutes.

agitation at 37°. Samples of the mixture were removed at one-minute intervals, supplemented with streptomycin (100 μg./ml) and vigorously shaken in a blendor. The blended mixture was then diluted 10^{-3} and small loopfuls were plated on minimal agar + streptomycin to score for the appearance of *thr*$^+$*leu*$^+$ recombinants; these recombinants were later scored for inheritance of *fim*$^+$. Similar loopfuls were also inoculated into test-tubes containing nutrient broth + streptomycin to score for the appearance of *fim*$^+$ recombinants which were later tested for inheritance of *thr*$^+$ and *leu*$^+$; the pellicle-forming ability of Fim$^+$ cells confers upon them such a selective advantage that they overgrow Fim$^-$ cells even if present as a minute minority in the inoculum. However, the presence of fimbriae was always confirmed by haemagglutination tests.

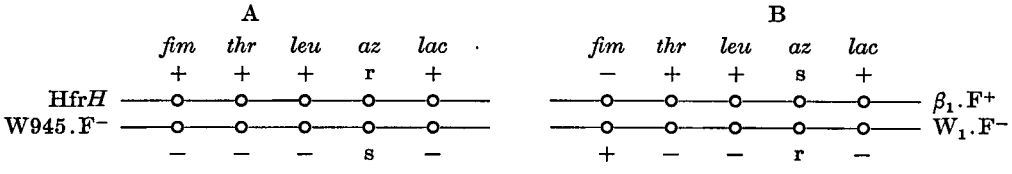
Results showed that when Hfr*H* is the donor, fimbriated recombinants begin to appear several minutes before *thr*$^+$*leu*$^+$ recombinants are detectable; conversely, when Hfr*R4* is the donor, the early *thr*$^+$*leu*$^+$ recombinants are non-fimbriated. More precisely, *fim*$^+$ enters recipient cells 2–3 minutes ahead of *thr*$^+$ in the Hfr*H* × W945 cross but 2–3 minutes after the same marker in the Hfr*R4* × W945 cross. These consistent results show that *fim* is located close to *thr*, at about one-quarter of its distance from *thi*, as shown in Fig. 3. This location for *fim* has been confirmed by the results of other crosses involving different donor and recipient strains and other selective and unselected markers. It accounts for previous difficulties in the mapping of *fim* which is thus the only known marker situated between the leading locus of the Hfr*H* chromsome and the *thr*—*leu* region.

Anomalies of fim *segregation*

On the basis of its location proximal to *thr* on the Hfr*H* chromosome, the exclusion of the *fim*$^+$ locus from the 20–30% of *thr*$^+$*leu*$^+$ recombinants could be interpreted formally in terms of the ratio of the probability of recombination in the short region *fim–thr* (2–3 minutes) to that of recombination in the much longer region *O–fim* (6 minutes). What the location of *fim* failed to explain was the fact (Table 2A) that the inheritance of *fim*$^+$ increases proportionately as the inherited length of chromosome distal to the selective markers *thr*$^+$*leu*$^+$ increases. The possibility that this anomaly might reflect peculiarities of the Fim$^+$ character and its expression, rather than location of the locus, was challenged by the analysis of reversed crosses between a Fim$^-$ donor and a Fim$^+$ recipient strain. Since a Fim$^-$ Hfr strain was not available, a Fim$^-$F$^+$ recombinant strain β_1 (Maccacaro & Dettori, 1959), derived from a cross between strains 58-161 Fim$^+$F$^+$ and W945 Fim$^-$F$^-$, was used as donor: the recipient strain was W_1 F$^-$ (see Table 2B for details) and *thr*$^+$*leu*$^+$ recombinants were selected. Table 2 compares the results

Table 2. *Percentage inheritance of the loci* fim$^+$ *and* fim$^-$ *among recombinant classes inheriting varying lengths of the donor chromosome to the right of the selective markers* thr$^+$leu$^+$

Distances between loci are not drawn to scale



thr$^+$ *leu*$^+$ recombinants	Per cent *fim*$^+$ inheritance	*thr*$^+$ *leu*$^+$ recombinants	Per cent *fim*$^+$ inheritance
az^s *lac*$^-$	50	*az*^{r} *lac*$^-$	47
az^r *lac*$^-$	68	*az*^{s} *lac*$^-$	29
az^r *lac*$^+$	89	*az*^{s} *lac*$^+$	15

of this cross (B) with those of the cross Hfr*H* × W945 (A). The same correlation is found between the frequency of inheritance of the *fim* locus and that of increasing lengths of chromosome to the right of *thr*$^+$*leu*$^+$, irrespective of whether the donor parent is Fim$^+$ or Fim$^-$. We therefore favour the view that the correlation is a consequence of the position of the locus rather than of the nature of its function, and should be interpreted as reflecting negative interference over large regions of the K-12 chromosome. Further data and a more expanded discussion of this problem are presented in another paper (Maccacaro & Hayes, 1961).

Transducibility of fim

Quantitative study of the genetics of fimbriation is limited by inability to select for, or even to recognize, colonies of Fim$^+$ cells within Fim$^-$ populations and vice versa. The only exception is the distinctive appearance of Fimσ^+ colonies

which can be distinguished from those of Fim$^+$, Fim$^-$ and Fimσ^- cells. Various attempts to devise a selective or differential technique based on the different oxidative ability of Fim$^+$ and Fim$^-$ cultures (Table 1) were not successful. This limitation becomes particularly stringent in transduction experiments, especially since the distance of the *fim* locus from the nearest selective marker (*thr*$^+$) is so great as to preclude joint transduction. Nevertheless, it has been possible to demonstrate the transducibility of fimbriation into non-fimbriated cells by making use of the great selective advantage conferred on fimbriated cells by their ability to form a tenacious pellicle on the surface of broth cultures (see above).

The following technique was used. Streptomycin-sensitive Fim$^+$ and Fimσ^+ variants of the same strain (58-161 F$^+$) were used as donors. Strain W945.S^r(Fim$^-$) was the usual recipient, but in some experiments a Fimσ^- variant of 58-161.F$^-$ was employed. Phage Plkc (kindly supplied by Dr S. W. Glover) was the transducing agent. Phage lysates (10^{10}–10^{11} plaque-forming units/ml.) from each of the two donors were added to young broth cultures of the recipient at multiplicities ranging from 10 to 10^{-4}. These adsorption mixtures were kept at room temperature for 90 minutes. One-millilitre samples were then distributed into a series of bottles containing 100 ml. broth + streptomycin, thus permitting at least six to eight generations for the expression of fimbriation by transduced cells. These broth cultures were incubated at 37° for 4 days, and then scored for pellicle formation and haemagglutinating activity. Samples were also subcultured to fresh broth to allow further time for the expression of fimbriation, and plated on nutrient agar for examination of single colonies.

The results of a number of such experiments support the following general conclusions.

(1) Fimbriated transductants inherit the Fim status (i.e. Fim$^+$ or Fimσ^+) of the donor parent, irrespective of whether the recipient is Fim$^-$ or Fimσ^-.

(2) The efficiency of transduction of fimbriation is higher with Fimσ^+ than with Fim$^+$ donors, with respect both to frequency and to the time required for pellicle formation. This was apparent despite the crudity of the selective method which blurs the determination of absolute transduction frequencies. On the other hand, substitution of Fim$^-$ by Fimσ^- as recipient did not alter the efficiency of transduction.

(3) Transduction of either the Fim$^+$ or the Fimσ^+ state is unlinked to that of any other marker tested. Similarly, transductants selected for *thr*$^+$ or *thr*$^+$*leu*$^+$ inheritance, from fimbriated donors, are non-fimbriated. It is worth emphasizing that more subtle features than are yet apparent in the transduction of fimbriation may well be masked by the coarseness of the present selective technique.

The relationship between Fim$^+$ and Fimσ^+ types

The main physiological feature characterizing the Fimσ^+ type is the stability of its fimbriation to environmental conditions, such as repeated subculture on solid medium or exposure to phenol or ethanol, which readily repress expression

in Fim$^+$ strains. This difference might be controlled, at the genetic level, by the interaction of a second chromosomal gene or of a cytoplasmic factor, or by stabilization of an episome (Jacob, Schaeffer & Wollman, 1960) in its chromosomal state. Fim$^+$ and Fimσ^+ variants of the donor strain 58-161 F$^+$ were therefore crossed, in parallel, with the Fim$^-$F$^-$ recipient strain W945.S^r in an attempt to analyse the situation. It was found that:

(1) Recombinants from Fim$^+$ × Fim$^-$ crosses are either Fim$^+$ or non-fimbriated.

(2) The frequency of Fim$^+$ recombinants from such crosses remains the same whether the fimbriation of the donor parent is expressed (Fim$^+$) or not (Fim$^{(+)}$).

(3) Recombinants from Fimσ^+ × Fim$^-$ crosses are either Fimσ^+ or non-fimbriated.

(4) The two types of cross do not differ significantly in the overall percentage of fimbriated recombinants.

These findings suggest that the Fimσ^+, as well as the Fim$^+$, state behaves as an integrated chromosomal function and that, if the Fim$^+$ → Fimσ^+ variation depends on mutation at a second, specific locus, this locus is likely to be very close to *fim*. These findings conform to those of the transduction experiments, but it is worth noting here that a few Fim$^+$ transductants were occasionally recovered from Fimσ^+ × Fim$^-$ transductions. These may represent exceptional Fimσ^+ → Fim$^+$ reversions, but the possibility that they arose from rare crossovers between *fim* and a very closely linked hypothetical σ locus cannot be excluded. It is known that closely linked loci are more often separable by recombination in crosses mediated by transduction than in conjugal crosses (see Maccacaro & Hayes, 1961). This view is supported by other findings reported in the next section.

The relationship between Fim$^-$ and Fimσ^- types

The two stably non-fimbriated types are distinguishable, at the biochemical level, by the greater oxidative activity of the Fimσ^- type and the greater glycolytic activity of the Fim$^-$ type (Table 1). We therefore tried to establish a genetic difference between them. Since, of the only two strains available for comparison, one (Fimσ^-; 58-161) required methionine (*met. thr$^+$leu$^+$*) and was S^s and F$^+$, while the other (Fim$^-$; W945) was *met$^+$thr.leu*, S^r and F$^-$, it was first necessary to make these strains identical except for their non-fimbriated status. This was done as follows:

(1) The Fimσ^- strain. An S^r mutant was selected and then converted to F$^-$ by acriflavine treatment (Hirota & Iijima, 1957) (= *met.thr$^+$leu$^+$*.S^r.F$^-$).

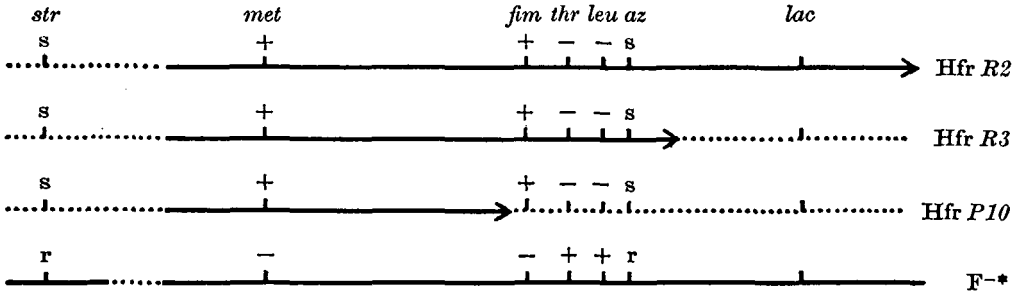
(2) The Fim$^-$ strain was first reverted to *thr$^+$leu$^+$* by a two-step selection of back-mutants; the absence of auxotrophic recombinants in crosses with other *thr$^+$leu$^+$* strains showed that these reversions were not due to suppressor mutations at other loci. The requirement for methionine was then introduced by means of interrupted mating with Hfr*R*1 which transfers *met* as an early marker (= *met. thr$^+$leu$^+$*.S^r.F$^-$).

https://doi.org/10.1017/S0016672300000872 Published online by Cambridge University Press

The Fim$^-$ and Fimσ^- strains so obtained were used as recipients in crosses with three different Fim$^+$ *met*$^+$*thr.leu* Hfr strains (*P*10, *R*2 and *R*3) which differed only in the position of their leading loci (Table 3). Selection was made for *met*$^+$*thr*$^+$*leu*$^+$ recombinants which were purified by single colony isolation and then scored for Fim status. Since the formation of these recombinants requires recombination in the *met–thr* region, which includes the *fim* locus, it was hoped that the resolution

Table 3. *Genetic crosses distinguishing the two non-fimbriated types, Fim$^-$ and Fimσ^-*

On the chromosome maps of the Hfr strains, the continuous line indicates the segment of chromosome transferred to the zygotes, and the arrow the direction of transfer



* For specifications of recipient strains used, see text.

Fim$^+$ Hfr donor	Per cent Fim types among *met*$^+$ *thr*$^+$ *leu*$^+$ recombinants					
	Fim$^-$ recipient			Fimσ^- recipient		
	Fim$^+$	Fimσ^+	non-fimbriated	Fim$^+$	Fimσ^+	non-fimbriated
*R*2	11	0 (180)†	89	18	4 (163)	78
*R*3	0	0 (241)	100	6	12 (123)	82
*P*10	0	0 (75)	100	0	0 (75)	100

† Figures in parentheses = total number recombinants tested from two or more separate crosses.

of analysis in the *fim* region might be increased. Details of these crosses and of their outcome are presented in Table 3. The appearance of an appreciable number of Fimσ^+ recombinants from the crosses between Fimσ^- and the two Fim$^+$ Hfr strains, *R*2 and *R*3, indicates rather strongly that Fimσ^- cells retain some part of the genetic determinant of the Fimσ^+ state which is absent from Fim$^-$ cells. The results of the crosses with strain Hfr*R*3 accentuate still more the genetic difference between Fim$^-$ and Fimσ^-, but raise the problem (which we have so far been

unable to solve) of why the Fim$^+$ character is not inherited at all by recombinants issuing from the Fim$^-$ recipient. However, this genetic difference was not apparent when the two non-fimbriated types (the Fim$^-$F$^+$ recombinant β_1—see above—and the Fimσ^- strain 58-161 F$^+$) were used as donors in crosses with a Fim$^+$F$^-$ recipient strain (W_1—see Table 2). Both crosses yielded the same proportions of Fim$^+$ and non-fimbriated recombinants, but no Fimσ^+ progeny: it is not known whether the non-fimbriated recombinants obtained from the Fimσ^- donor were Fim$^-$ or Fimσ^-.

DISCUSSION

Speculative models for fimbriation

The physiological and genetic differences between the two non-fimbriated types, Fim$^-$ and Fimσ^- (Tables 1 and 3), together support the idea that fimbriation is a function of the activity of at least two genetic determinants. If we call these determinants *A* and *B* and designate mutations in them, leading to loss or alteration of function, *a* and *b* respectively, we can construct three theoretical models for the determination of fimbriation (Table 4). In all models *A* and *B* would have to

Table 4. *Possible models of the genetic control of fimbriation*
At least two determinants *A* and *B*, are assumed

Model 1: *A* and *B* are chromosomal genes; *A* controls synthesis of fimbriae and *B* the type of fimbriation.

Model 2: *A* and *B* are chromosomal genes, together controlling synthesis of fimbriae; type of fimbriation is independently controlled.

Model 3: *A* is a chromosomal gene and *B* an episome; type of fimbriation is a function of the location of *B*.

Fimbriation status	Model 1	Model 2	Model 3
Fimσ^+	*AB*	*AB*	*AB*
Fim$^+$	*Ab*	*AB*	$A \rightleftharpoons B$
Fimσ^-	*aB*	*Ab*	*A*
Fim$^-$	*ab*	*aB*	*a*

be very closely linked in order to account for the mapping data which show that the loci for both types of fimbriation and of non-fimbriation are located within the same small region, as well as for the conformity of recombinants to the donor fimbriated type, irrespective of the type of the non-fimbriated recipient, in crosses mediated both by transduction and conjugation.

Model 1 supposes that *A* alone determines production of fimbriae, while the function of *B* is control of the type of fimbriation. This model fails to account for the irreversibility of the Fim$\sigma^+ \rightarrow$ Fimσ^- mutation and for the difference in oxidative capacity between Fim$^-$ and Fimσ^-; it is, of course, possible that the reduced Q_{O_2} of the Fim$^-$ strain W945 is due to mutation involving an oxidative function which only indirectly represses synthesis of fimbriae, but the location of the mutational site within the *fim* region makes this unlikely. In model 2, both

A and *B* are necessary for production of fimbriae, the Fim$^+$ → Fimσ^+ variation being controlled independently; the genetic data require, however, that the site of this independent control be closely linked to the *AB* system. This model explains the oxidative defect of the Fim$^-$ type which alone has a mutation in *A*, but not the irreversibility of the Fim$^-$ and Fimσ^- mutations, nor the fact that fimbriated recombinants have never been isolated from crosses, of reversed *F* polarity, between the two non-fimbriated types (i.e. by $Ab \times aB$) even when the sensitive method of selecting for fimbriated cells by pellicle formation in broth was used. Model 3 supposes that determinant *B* is an episome which, in co-operation with the chromosomal gene *A*, determines synthesis of fimbriae; its permanent fixation to the chromosome could account for stabilization of the character in Fimσ^+ cells while its spontaneous loss would explain the peculiar ability of Fimσ^+ cells to mutate irreversibly, at a relatively high rate, to the non-fimbriated Fimσ^- state. If maintainance of episome *B* depended on a functional gene *A*, then mutation in *A* would result in loss of *B* and thus also explain the irreversibility of the mutation to Fim$^-$. Certain Fim$^+$ cultures have shown some evidence of a diphasic variation between the Fim$^+$ and Fimσ^+ states, which could correspond to alternation of an episome between its non-chromosomal and chromosomal locations. However, there are two lines of evidence against this plausible model which we initially favoured. The first is that Fimσ^-.F$^+$ × Fim$^+$.F$^-$ crosses yield 20–30% of non-fimbriated recombinants, while model 3 predicts that all recombinants should be Fim$^+$. Secondly, all attempts to transfer fimbriation from Fim$^+$ to Fimσ^- cells, either by contact or conjugal infection which excluded transfer of the *fim* locus (see Hfr.*P*10 cross, Table 3), were entirely unsuccessful. These experiments also preclude models involving purely cytoplasmic determinants (plasmids). The one thing that is clear is that the physiological and genetic mechanisms of the control of fimbriation are likely to prove as complicated as Fig. 1 would suggest. It is important to point out, however, that we have so far examined only one strain of each of the types Fimσ^+, Fimσ^- and Fim$^-$; it is thus possible that one or more of these may have resulted from a deletion or be otherwise atypical.

SUMMARY

(1) Fimbriated cells of *E. coli K*-12 are of two types. In the Fim$^+$ type the expression of fimbriation is susceptible to reversible environmental suppression. This type gives rise to environment-stable mutants termed Fimσ^+.

(2) Fimbriated cells can yield two types of non-fimbriated mutant, Fim$^-$ and Fimσ^-, the latter arising from populations of Fimσ^+ cells. Neither type reverts to the fimbriated state.

(3) Both types of fimbriated and non-fimbriated cells can be distinguished by physiological and genetic criteria.

(4) Fimbriation of both types can be transferred to non-fimbriated cells of both types, and segregates among recombinants, in crosses mediated either by transduction or sexual conjugation.

(5) The genetic control of fimbriation involves at least two determinants, for one of which a chromosomal location (*fim*) has been mapped.

(6) Certain anomalies of *fim* segregation are interpreted in terms of negative interference over relatively large regions of the bacterial chromosome.

We are much indebted to Drs F. Jacob and E. L. Wollman for kindly supplying us with the Hfr strain *P*10, and to Dr P. Reeves for the gift of his Hfr strains *R*1, *R*2, *R*3 and *R*4.

One of us (G. A. M.) gratefully acknowledges the financial support given him by the Italian Consiglio Nazionale delle Ricerche.

REFERENCES

Anderson, T. F. (1949). In *The Nature of the Bacterial Surface* (eds. A. A. Miles & N. W. Pirie). Oxford: Blackwell. *Symp. Soc. gen. Microbiol.* **1**, 76–95.

Brinton, C. C. (1959). Non-flagellar appendages of bacteria. *Nature, Lond.*, **183**, 782–786.

Brinton, C. C., Buzzell, A. & Lauffer, M. A. (1954). Electrophoresis and phage susceptibility studies on a filament-producing variant of the *E. coli* B bacterium. *Biochim. Biophys. Acta*, **15**, 533–542.

Constable, F. D. (1956). Fimbriae and haemagglutinating activity in strains of *Bacterium cloacae*. *J. Path. Bact.* **72**, 133–136.

Duguid, J. P. & Gillies, R. R. (1957). Fimbriae and adhesive properties in dysentery bacilli. *J. Path. Bact.* **74**, 397–411.

Duguid, J. P. & Gillies, R. R. (1958). Fimbriae and haemagglutinating activity in *Salmonella, Klebsiella, Proteus* and *Chromobacterium*. *J. Path. Bact.* **75**, 519–520.

Duguid, J. P., Smith, I. W., Dempster, G. & Edmunds, P. N. (1955). Non-flagellar filamentous appendages ('fimbriae') and haemagglutinating activity in *Bacterium coli*. *J. Path. Bact.* **70**, 335–348.

Hayes, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75–93

Hirota, Y. & Iijima, T. (1957). Acriflavine as an effective agent for eliminating *F* factor in *Escherichia coli* K-12. *Nature, Lond.*, **180**, 655–656.

Houwink, A. L. & van Iterson, W. (1950). Electron microscopical observations on bacterial cytology: II. A study on flagellation. *Biochim. Biophys. Acta*, **5**, 10–44.

Jacob, F., Schaeffer, P. & Wollman, E. L. (1960). Episomic elements in bacteria. In *Microbial Genetics* (eds. Hayes, W. & Clowes, R. C.). Cambridge University Press. *Symp. Soc. gen. Microbiol.* **10**, 67–91.

Maccacaro, G. A. (1955). Cell surface and fertility in *Escherichia coli*. *Nature, Lond.*, **176**, 125–126.

Maccacaro, G. A., Colombo, C. & Dinardo, A. (1959). Studi sulle fimbrie batteriche: I. Lo studio genetico delle fimbrie. *Gior. Microbiol.* **7**, 1–20.

Maccacaro, G. A. & Dettori, R. (1959). Studi sulle fimbrie batteriche: IV. Metabolismo ossidativo e fermentativo in cellule fimbriate e sfimbriate. *Gior. Microbiol.* **7**, 52–68.

Maccacaro, G. A. & Dettori, R. (1960). *Gior. Microbiol.* **8**, 65.

Maccacaro, G. A. & Hayes, W. (1961). Pairing interaction as a basis for negative interference. *Genet. Res.* **2**, 406–413.

Maccacaro, G. A. & Turri, M. (1959). Studi sulle fimbrie batteriche: II. Osservazioni microelettroforetiche. *Gior. Microbiol.* **7**, 21–36.

Taylor, A. L. & Adelberg, E. A. (1960). Linkage analysis with very high frequency males of *Escherichia coli*. *Genetics*, **45**, 1233–1243.

Wollman, E. L. & Jacob, F. (1959). *La Sexualité des Bactéries*. Paris: Masson & Cie.

Wollman, E. L., Jacob, F. & Hayes, W. (1956). Conjugation and genetic recombination in *Escherichia coli* K-12. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 141–162.