Attachment of wild-type *F* factor to a specific chromosomal region in a variant strain of *Escherichia coli* K12 : the phenomenon of episomic alternation

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INTRODUCTION

Sex in *Escherichia coli* is determined by a factor called *F*. Those individuals which possess the *F* factor have both donor and recipient potentiality with regard to the conjugal act. Those individuals which lack the *F* factor have recipient potentiality only. The presence of *F* thus determines donor potentiality. We shall refer to individuals which are potential donors of genetic material in conjugation as males and to the others as females or $F-$.

There are two kinds of males depending on the manner of presence of the *F* factor. $F+$ males possess the *F* factor in the *infectious* state characterized by two properties. First, the factor is readily transmissible to females upon cell contact as a result of which the females are converted to $F+$ males (Lederberg, Cavalli & Lederberg, 1952). Second, in the infectious state, the factor is susceptible to the disinfecting action of the dye acridine orange (Hirota, 1960).

The *F* factor of *Hfr* males is neither contagious (Cavalli, Lederberg & Lederberg, 1953; Hayes, 1953) nor disinfectible (Hirota, 1960). In fact, it has been shown in two cases tested to be located on the chromosome (Richter, 1957*a*, 1958). So, as the presence or absence of the factor distinguishes males and females, it is very likely its position in the cell which distinguishes the two kinds of males. This is summarized in Table 1.

Table 1. *Determination of mating type in* Escherichia coli

Mating type	Position of the *F* factor
Hfr ♂	chromosomal
$F+$ ♂	extrachromosomal
♀	absent

Hfr is the functional male state. $F+$ males are probably incapable of acting as donors except after a genetic change to *Hfr*. The evidence for this view is the finding that donor ability fluctuates among $F+$ cultures to a greater degree than can be accounted for by sampling error (Jacob & Wollman, 1956). The fertility of

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an $F+$ strain may thus be considered to depend on its rate of change to Hfr. For example, some $F+$ strains are male-sterile (i.e. they do not yield genetic recombinants in crosses with $F-$); some change to Hfr at rates about 10^{-6} to 10^{-5}; certain mutable strains, such as the strain to be discussed in this paper, change at much higher rates. We shall call those $F+$ males *standard* which change to Hfr at the same rate as the type strain K12, i.e. 10^{-6} to 10^{-5}.

Standard $F+$ strains change at a low rate to various kinds of Hfr in which the F factor is presumably fixed to different positions on the chromosome (Jacob & Wollman, 1958 a). The variant $F+$ strain to be described changes at a high rate to a specific kind of Hfr.

This paper discusses the genetic structure and behaviour of a strain of *Escherichia coli* which reversibly alternates between a specific Hfr state and the $F+$ state.

MATERIALS AND METHODS

Method of crossing

Most crosses were performed in liquid medium prior to plating. The standard cross procedure was as follows. Isolated single colonies of the parental strains were inoculated to tubes containing 10 ml. of 'Penassay' broth (Difco) and incubated without aeration for 15–18 hours at 37°C. After incubation the two cultures were mixed in the following amounts: 1 ml. each parent and 3 ml. fresh Penassay broth. The mixture was incubated without aeration for 2 or 3 hours at 37°C. It was then either plated on the appropriate selective medium or first washed before plating. Washing was carried out by centrifuging the mixed culture, decanting the supernatant, washing the pellet with 2 ml. water, and resuspending at the original concentration of cells in water or 1 % saline. Cross mixtures were plated at dilutions which would yield 50 to 100 recombinant clones per plate, except in the case of crosses plated on EMB medium where it was found necessary to plate more dilute suspensions. (In this way it was possible to avoid selective pressures against certain recombinants on EMB medium. The region of chromosome conferring selective disadvantage is linked to the locus determining the threonine(T) requirement of the T^- parent.) The cross plates were incubated at 37°C. for 30 to 40 hours at which time the recombinant colonies were picked and streaked on EMB medium. Isolated single colonies from these streak plates were used for the scoring of markers. Replica plating with cotton velvet was employed for scoring.

Some crosses were made directly on selective agar medium by plating the parental suspensions together on the same plates.

Use of media

Difco Penassay broth was used for the growing of cultures. *EMB complete medium* supplemented with 1 % sugar, or 1·5 % in the case of maltose and 2 % in the case of lactose, was used for scoring fermentation markers. EMB + sugar was also used as a selective medium for fermenters in conjunction with selection for

resistance to streptomycin or phage. Phage was added to the cross plates when the bacteria were plated, while streptomycin was incorporated into the medium at a concentration of 0·2 mg per ml. *EMS minimal indicator medium* was employed for certain crosses in order readily to ascertain the proportion of fermenters among the progeny. Most of the crosses, however, were plated on *Davis minimal medium* (DO medium) containing 0·5 % glucose. Supplements were added to minimal cross plates in these concentrations (gamma per ml.): leucine, threonine and arginine, 40; methionine, proline and histidine, 20; thiamine, 0·25. The same supplements were used at half-concentration for scoring nutritional markers. All these media are described by Lederberg (1950). In addition, *EM Galactose* is EMS medium minus succinate with 1 % galactose added. *EM Lactose* and *EM Maltose* are similar media, but with 2 % and 1·5 % concentration of sugar, respectively.

Tests for mating type

Segregants from crosses were tested for mating type by spot tests against female tester strains on appropriate selective media. For these tests the EM sugar media were particularly useful since the sugar supplement is the sole carbon source. This feature enabled selection for fermentation markers. Primary crosses were usually set up so that one marker was left available for progeny tests. For example, from the cross of $M^- Gal_2^- \times T^- L^- Gal_2^-$ on minimal medium, all progeny are $M^+ Gal_2^-$ and can therefore be tested against a female $M^- Gal^+$ tester on EM Galactose. The method of testing is as follows. Purified recombinants are inoculated to 1-ml. broths and grown up. A broth culture of the female tester is streaked across an EM Galactose plate. When the streak has dried, the recombinants are spotted on top of the streak by means of a loop. Control spots of the cultures to be typed may be placed on the same plate of EM Galactose. The plates are incubated and read at 24 and 48 hours. The occurrence of galactose-positive papillae is taken as an indication that recombination has occurred. Other useful spot tests are performed on EM sugar media supplemented with streptomycin. Direct spotting of S^s cultures against female S^r testers may be employed.

F transmission

Tests for the transmission of the F factor were performed according to methods described by Cavalli *et al.* (1953).

Markers and strains

A list of genetic markers is found in Table 2 and a list of bacterial strains in Table 3. All strains are derivatives of strain K12.

Efficiency of recombination

Efficiency of recombination (E.R.) is the reciprocal of the number of cells of a standard parental mixture required to give one recombinant, as determined from platings which yield 50–100 recombinants. Standard conditions have been defined above.

Table 2. *List of genetic markers*

Fermentation of sugars	Nutritional requirements	Drug resistance
+ = ferments,	+ = independent,	s = sensitive, r = resistant
− = does not	− = dependent	S streptomycin
Gal galactose	Th thiamine (B_1)	
Mal maltose	M methionine	Episomes
Xyl xylose	T threonine	♂ = present, ♀ = absent
	L leucine	F sex factor

Table 3. *List of bacterial strains, their designations and genotypes*

Designation	Genotype*
K12	$F+$ prototroph (λ)
W1321	♀ $M^- S^r Gal_4^-$
W1578	♀ $M^- Lac^- Gal_4^-$
W2580	♀ $T^- L^- Th^- Gal_2^- Xyl^-$ (λ)
W2660	♀ $T^- L^- Th^- S^r$
W2817	$F+ T^- L^- Th^- S^r$
W2924	♂₃ $T^- L^- Th^- S^r Mal_5^-$
W3062	♂₃ prototroph $Mal_5^- Gal_2^-$
W3135	♀ M^-
W3161	♀ prototroph $Gal_7^- Xyl^- S^r$
W3191	♀ $T^- L^- Th^- S^r Xyl^-$
W3234	♂₃ $M^- Gal_2^- Mal_5^-$ (λ)

* The genotype lists only the pertinent markers.

EXPERIMENTAL

The strain in question, ♂₃, was selected as a maltose-nonfermenting mutant (W2924) after ultra-violet irradiation of an $F+$ culture (W2817). The maltose marker was labelled Mal_5. Prototrophic and M^- derivatives of W2924 were obtained by crossing. As these always showed the same behaviour as the original mutant, they will also be referred to as ♂₃.

In early crosses ♂₃ behaved unusually in that (1) it was about 10,000 times as fertile as its $F+$ parent strain when the M^+ locus was selected, and (2) it was unstable, giving rise to a less fertile variant which was infectious for the F factor.

It was later found that the less fertile variant could in turn give rise to the more fertile variant and consequently the two variants will be referred to as the two states of ♂₃.

State I is the more fertile state and state II is the less fertile state. The test for state is thus a test for fertility in crosses.

As shown in Table 4, state I is 100 times more fertile than state II when the

Table 4. *Recombination efficiencies for ♂₃ × ♀ crosses*

	Recombination Efficiency	
State of ♂₃ culture (W3062)	× ♀ M^- (W1321)	× ♀ $T^- L^-$ (W2660)
I	$2 \cdot 2 \times 10^{-2}$	$6 \cdot 8 \times 10^{-6}$
II	$1 \cdot 4 \times 10^{-4}$	$6 \cdot 3 \times 10^{-7}$

Selection was imposed for $M^+ S^r$ and $T^+ L^+ S^r$ recombinants, respectively. The fraction of state I cells in the state I culture was $0 \cdot 87 \pm 0 \cdot 05$.

recovery of M^+S^r recombinants is taken as the test of fertility. Neither state is especially fertile when the yield of $T^+L^+S^r$ recombinants is taken as the measure of fertility. It should be noted that the recombination efficiency for a cross of standard $F+ \times$ female varies from 10^{-7} to 10^{-6} regardless of the selective markers employed.

Proof that the fertility of state II populations is due to the presence of state I variants

Cultures in state I transmit the gene M in crosses with an efficiency of 10^{-2}. This level of fertility is comparable to that of stable Hfr strains. Cultures in state II transmit M with an efficiency of 10^{-4}, a level intermediate between Hfr and standard $F+$ fertility. The transmission of the gene M by cultures in state II can be quantitatively accounted for by the presence of state I variants as shown by the following experiment.

$\male_3$ strain W3062 (prototrophic, S^s) was streaked out and one colony of each fertility level chosen. These were inoculated to tubes of broth. The broth cultures were plated on EMB Galactose (to determine viable cell counts and the proportion of state I cells in each culture) and also combined to yield mixtures ranging from whole state I culture to 1 part state I culture in 500 parts state II culture. These combinations were crossed by the standard procedure with the M^-S^r female strain W1321 and different dilutions plated on minimal medium + streptomycin. From the proportion of state I cells in the state I culture (0·85; all of 69 isolates of the state II culture were state II), the true proportion of state I cells in each mixture was calculated and plotted against the number of recombinants obtained per 10^{-5} ml. of cross mixture. The straight-line graph of Fig. 1 was obtained, indicating that the E.R. previously defined is a linear function of the concentration of donor cells in the range of efficiencies obtained.

The following counts of state I variants in state II cultures have been recorded in various experiments, including that detailed above: 2/80; 0/69; 1/150; 1/100. The expected recombination efficiency of a state II culture containing 1% state I cells was therefore calculated from the slope of the plot. That value, $1·8 \times 10^{-4}$, agrees well with the values ordinarily obtained (see Table 4).

Cultures in state II transmit the F factor efficiently as shown in Table 5. State II may therefore be equated with the infectious state of the F factor, that is the $F+$ state. Since there is no evidence that state II is intrinsically any more fertile than the standard $F+$ strain from which $\male_3$ arose, we may conclude that the distinction between state II and standard $F+$ is the rate of change to state I, i.e. the Hfr condition.

Cultures in state I transmit the F factor inefficiently. It is presumed that cells in state I, like cells of stable Hfr genotypes, are not infective for the F factor. This has not been proven. Presence of the $F+$ variants in the state I cultures renders such a proof difficult. In addition, it is likely that a lag occurs between the time of fixation of an F factor on the chromosome and the subsequent loss of all infectious F factors from the cell (see Cavalli-Sforza, 1959; Adelberg & Burns, 1960).
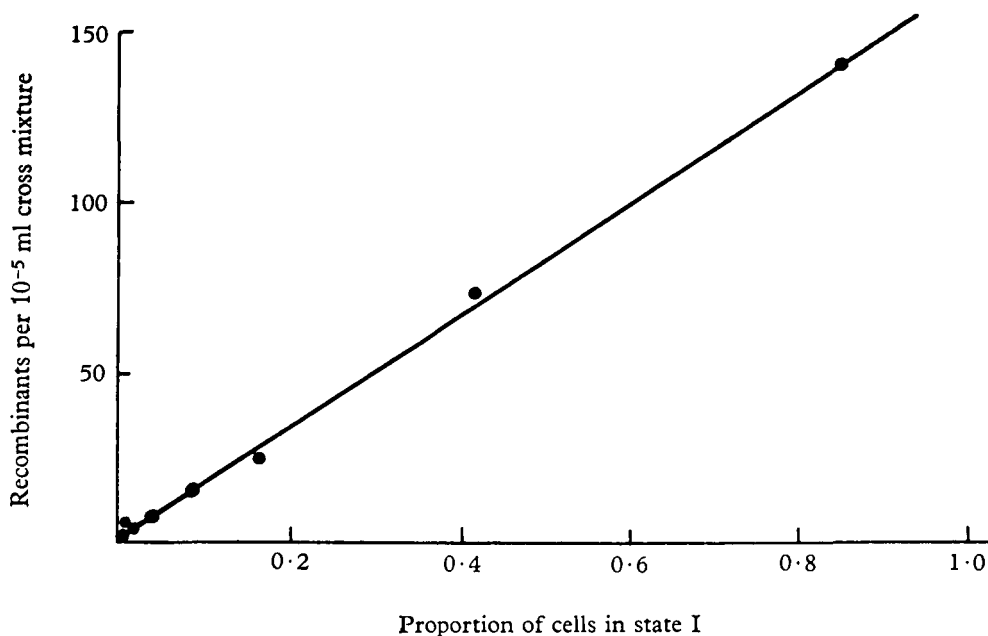
Fig. 1. Yield of recombinants versus cells in state I.

Table 5. F *status of progeny from matings of* $\male_3 \times \female$ *and standard* F $+ \times \female$

Type of donor parent	No. of isolates having mating type:			F status of recombinants*	
	I	II	std. $F+$	$F+$	$F-$
State I	98	2	0	10	90
State II	1	99	0	87	13
Standard $F+$	0	0	100	99	1

* Selected for prototrophy. For the first two crosses, W2924 ($\male_3$ $T^-L^-Th^-$) was mated with W1578 ($\female$ M^-). For the third cross, W2817 ($F+$ $T^-L^-Th^-$) was mated with W1578. For each cross, 100 single colony isolates of the broth culture of the donor parent were tested for mating type versus W3161 on EM Xylose, and 100 purified recombinants were tested versus W3135 on EM Galactose.

The functional male state is therefore inversely correlated with the infectious state of the F factor within the $\male_3$ system just as within *E. coli* K12 in general.

The behaviour of F − derivatives of $\male_3$

It is known that $F+$ strains may give rise to $F-$ variants, i.e. females, which may then be selectively enriched by passage through soft agar (Skaar, Richter & Lederberg, 1957). In addition, one may produce $F-$ variants at will by treatment of growing $F+$ cells with acridine orange (Hirota, 1960). The $F-$ variants obtained in both cases are reconvertible to $F+$ by growth in mixed culture with an $F+$ strain. The reconverted $F+$ display the same low fertility in crosses as the original $F+$ strains. When $\male_3$ is subjected to either of the above treatments,

one can also obtain $F-$ variants. However, such $F-$, when grown in mixed culture with standard $F+$, are always converted to state II, i.e. to $F+$ possessing high rate of change to state I in distinction from standard $F+$, i.e. $F+$ possessing low rate of change to state I. The $F-$ derivative of $\male_3$ is called $\female_3$ to distinguish it from the $F-$ derivative of standard $F+$ which is called standard $F-$ or female.

Two conclusions may be drawn from the above. First, the presence of F is a necessary condition to the expression of the $\male_3$ and therefore state I phenotype. Second, rate of change is a property of the bacterium which is conserved independently of the presence or absence of F.

Inheritance of the $\male_3$ property

Conjugation in *E. coli* involves the oriented transfer of the bacterial chromosome from the *Hfr* donor to the female recipient. Jacob & Wollman (1958a) have shown that the *Hfr* property itself is always the last marker transferred.

When one performs a cross by selecting recombinants which carry a given marker of the *Hfr* parent, markers which are transferred earlier than the selected marker appear in the progeny according to their linkage to the selected marker. Markers transferred later than the selected marker may not appear at all. According to current theory this is so because the transfer of the *Hfr* chromosome is usually incomplete, resulting in zygotes that are incomplete.

Since the *Hfr* property is the last marker to be transferred, it is not possible to ensure a set of complete zygotes for the genetic analysis of the *Hfr* property. One must therefore do the best one can, which is to select recombinants carrying a marker of the *Hfr* parent as near as possible to the *Hfr* marker. Table 6 presents

Table 6. *Linkage of the $\male_3$ character*

Cross	$\male_3$ character of progeny	No. of T^+L^+ recombinants of phenotype:				State of $\male_3$ progeny	
		Th		Mal$_5$		I	II
		+	−	−	+		
A. $\male_3$ state I × ♀	$\male_3$	*9*	8	*17*	0	13	4
	Non-$\male_3$*	19	*25*	0	*44*		
B. $\male_3$ state II × ♀	$\male_3$	5	*3*	*8*	0	1	7
	Non-$\male_3$*	1	*46*	0	*47*		

The crosses were W3062 ($\male_3$ $T^+L^+Th^+Mal_5^-$) × W3191 (♀ $T^-L^-Th^-Mal_5^+$). The italic figures represent the parental couplings.

* The non-$\male_3$ progeny include standard $F-$ and standard $F+$.

the data of such an experiment. W3062 was mated in each of its states with a female strain, W3191, and selection was imposed for $T^+L^+S^r$ recombinants. Both crosses demonstrate the tight linkage of the $\male_3$ property and Mal_5, the mutation which arose simultaneously with $\male_3$.

It is concluded that the $\male_3$ property is inherited as a single gene difference from standard female.

Now, these rare recombinant progeny which receive the $\male_3$ property apparently do not arise solely from matings of state I cells with female cells as is true for the M^+ recombinants. This is shown by the fact that the majority of the $\male_3$ progeny arising from a mating of state II × female are in state II, while the majority of the $\male_3$ progeny arising from a mating of state I × female are in state I. Since the linkage relation between state and Mal_5 is the same in the two states, the determinants of the two states may be regarded as alleles. The mating phenotypes in question preclude a direct test of allelism.

Although the two crosses agree in respect to linkage to Mal, they do not agree in respect to Th. The determinant of state II is linked to the marker Th (interaction = 77) while the determinant of state I is not (interaction = 1·5). Interaction equals the product of the two parental types divided by the product of the two non-parental types. These and other crosses have shown that the relationships of all other markers are the same in the two states and that these relationships are the same as found in all other strains of K12 (Richter, 1957 b). It is therefore concluded that the $\male_3$ property lies between Th and T in the sequence MTh $(\male_3\text{-}Mal_5)TL$ and that this block of markers is uncoupled in state I. The linkages found in state I are MTh and $(\male_3\text{-}Mal_5)TL$. The significance of this uncoupling will be discussed later.

Location of the F factor at the site of the determinant of rate of change

Since, as shown above, rate of change is a property of the bacterium *per se*, one ought to be able to follow it in crosses independently of the $\male_3$ character. One can then survey all progeny which have inherited high rate of change and ask how many are also $\male_3$ in phenotype.

To this end W3234 ($\male_3$ $M^-Gal_2^-$) in state I was mated with W2580 ($\female$ $T^-L^-Th^-$ Gal_2^-) on minimal (DO) agar. In order to minimize F transmission by cell contact, a low multiplicity of male cells was employed. Thus, 0·5 ml. of washed female culture was plated together with 10^{-2} ml. of washed male culture. The prototrophs obtained were necessarily Gal_2^-. Every isolated colony on one cross plate was picked, purified, checked for maltose fermentation and prototrophy, inoculated into a 1-ml. broth, and checked for mating type versus W3135 on EM Galactose. The results are presented in Table 7A(1). Of the thirty-two $\male_3$ progeny, there were thirty-one in state I and one in state II. Of the fifty non-$\male_3$, there were six Mal^+ standard $F+$ and forty-four females. Each of the forty-four females was grown in mixed culture with K12 and reisolated. The reisolated clones were then tested for mating type by crosses with W3135 on EM Galactose as before sex conversion. The results are shown in Table 7A(2). All forty-three Mal^+ females were converted to standard $F+$; the single Mal^- female was converted to state II. It must, then, have inherited high rate of change from its $\male_3$ parent. From the data, one can reconstruct the genotypes of all the progeny with respect to rate of change. This

is done in Table 8A. From the mating of state I × ♀, thirty-three progeny inherited high rate of change, of which thirty-two exhibited the $\male_3$ phenotype before sex conversion. Thus, almost all progeny which inherit high rate of change are $\male_3$. But, as we concluded earlier, the $\male_3$ phenotype depends on the presence of the F

Table 7. *Assortment of* Mal_5 *and mating type before and after sex conversion in crosses of* $\male_3 \times ♀$

		No. of $T^+L^+Th^+$ recombinants of mating type:							
		(1) before sex conversion of all female progeny				(2) after sex conversion of all female progeny			
		$\male_3$		Non-$\male_3$		$\male_3$		Non-$\male_3$	
		I	II	std. $F+$	♀*	I	II	std. $F+$	♀
A. State I × ♀	Mal_5 −	31	1	0	1	31	2	0	0
	+	0	0	6	43	0	0	49	0
B. State II × ♀	Mal_5 −	1	39	0	4	1	43	0	0
	+	0	0	22	1	0	0	23	0

* These include ♀$_3$. The crosses were W3234 ($\male_3$ $M^- Gal_2^- Mal_5^-$) × W2580 (♀ $T^- L^- Th^- Gal_2^-$).

factor. Therefore, F *is almost always inherited whenever high rate of change is inherited.* Now, in the case of state I × ♀, F is inherited by a minority of the wild-type progeny, i.e. progeny standard for rate of change (Table 8A as well as Table 5). Therefore, in state I the F factor must be located at the site of the determinant of rate of change.

Table 8. *Inheritance of* F *and rate of change in crosses of* $\male_3 \times ♀$ *(from data of Table 7)*

		No. of $T^+L^+Th^+$ recombinants of stated genotype		
		Total	$\male$	♀
A. State I × ♀	high rate of change	33	32	1
	standard rate of change	49	6	43
B. State II × ♀	high rate of change	44	40	4
	standard rate of change	23	22	1

The symbols $\male$ and ♀ are used to denote the presence and absence of F, respectively. Rate of change is determined after sex conversion as described in the text.

A similar cross was performed between W3234 in state II and W2580. The results are given in Tables 7B and 8B. In this case, F is inherited by a majority of the progeny whether they have inherited high rate of change or not. The cross yields no information.

The determinant of rate of change is given the locus symbol Hfr_3.

Y

DISCUSSION

These results cannot be discussed apart from the *episome* concept introduced by Jacob and Wollman (1958*b*). According to this view there are hereditary elements which can multiply either integrated with the chromosome, like prophage, or autonomously of the chromosome, like vegetative phage. These elements may be present in the cell in either of the above two states (integrated or autonomous), or absent from the cell entirely, as in a bacterium sensitive to phage.

The present work establishes the episomal nature of the F factor.

The F factor may be present (males) or absent (females) from the cell and, when present, may be either integrated (*Hfr* male) or autonomous ($F+$ male).

In the $\male_3$ strain there is an accelerated variation between the $F+$ state and the
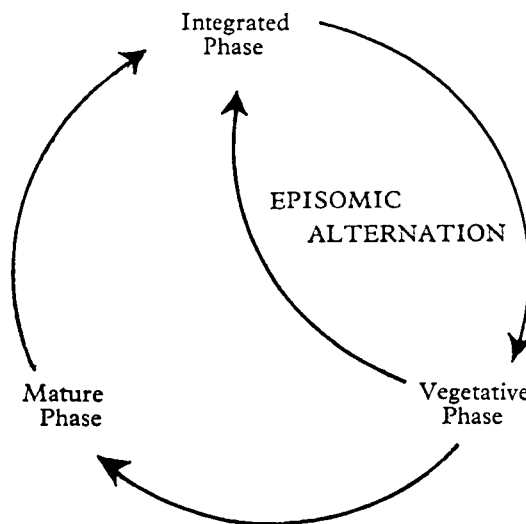

Fig. 2. Diagram of the lysogenic cycle showing the relation of episomic alternation.

Hfr state, involving always the same chromosomal site. This variation is the reflection of the *dance* of the F factor. In state II the F factor multiplies in the autonomous state ($F+ \rightarrow F+$). The attachment of an F factor to the Hfr_3 locus initiates state I ($F+ \rightarrow Hfr$). In state I the F factor multiplies in the integrated state ($Hfr \rightarrow Hfr$). The detachment of the F factor from the Hfr_3 locus initiates state II ($Hfr \rightarrow F+$) and the cycle of movements is completed. The back-and-forth shift of an episome between its integrated state of multiplication at a single locus and its autonomous state of multiplication is termed *episomic alternation*. Episomic alternation may be viewed in relation to the lysogenic cycle as shown in Fig. 2. The reader should refer to the article on lysogeny by Lwoff (1953).

The attachment of the F factor to the chromosome has as a consequence the disruption of linkages known to be present in the $F+$ state. In the $\male_3$ system, the $Th-Mal_5$ linkage found in state II is uncoupled in state I. This could be interpreted in one of three ways. (1) The change from $F+$ to *Hfr* involves the breaking of the chromosome. The break is perpetuated in vegetative divisions of the *Hfr*

bacteria. (2) F attaches to the chromosome at the change but the chromosome does not become broken until mating. (3) The change from $F+$ to Hfr involves the insertion of the episome into the chromosome. If the episome had an end which was unable to form an internucleotide linkage, the gap, so to speak, would be built into the structure of the episome-bearing chromosome. This gap could be in a single strand of deoxyribonucleic acid, rather than a gap in the duplex.

Episomic alternation and local change of state

The analysis of mutable loci in maize has shown that these loci are the sites of accessory elements. These are called controlling elements because they tend to suppress gene action at the loci where they reside (McClintock, 1956). A mutable allele, such as the variegated pericarp allele at the P locus, is considered to be a combination of an ordinary gene and one of these accessory elements (Brink, 1958). Change from variegated to red is explained by the removal of the element from the locus either by a process called transposition or by loss of the element from the genome. In transposition, the element moves from one locus to a second locus (McClintock, 1951). Transposition may lead to the origin of new mutable loci.

The Hfr_3 locus of *Escherichia coli* is similar to the P locus of maize. We may consider that the Hfr_3 locus has three alleles: *high rate of change*, F *present* (state I); *high rate of change*, F *absent* (state II); and *standard rate of change*. Like the variegated pericarp allele, the determinant of state I has a dual structure. One component is the gene for high rate of change. The other component is the F factor. Change from state I to state II may be explained by the removal of the F factor from the Hfr_3 locus. The consequence of this removal is the establishment of the infectious state in which the F factor multiplies autonomously.

The regular changes in state of $\male_3$ from Hfr to $F+$ and from $F+$ to Hfr parallel the variations in phase of certain *Salmonella* strains with regard to expression of flagellar antigens (Lederberg & Iino, 1956). In *Salmonella* this variation in phase depends on changes in state of a locus H_2 so that, when this locus turns 'on', the phase 2 antigen is expressed, while when this locus turns 'off', its epistatic action on another locus H_1 is thereby removed and the phase 1 antigen is expressed.

Episomic alternation offers a physical model for these changes in state at the H_2 locus.

The V locus of *Nicotiana* offers another example of reversible change of state at a single locus (Smith & Sand, 1957; Sand, 1957). v_S is an allele which promotes the synthesis of pigment in cells of the epidermis. v_s represents the inhibited condition of the same locus. Changes may occur from v_S to v_s and from v_s to v_S. These changes resemble the changes in state of the Hfr_3 locus from state II to state I and from state I to state II. The stable allele V may be compared to the allele for standard rate of change at the Hfr_3 locus.

Relation of the $\male_3$ system to the Lambda-K12 system

We have already considered that the Hfr_3 locus has three alleles. For certain purposes it is more instructive to consider that the Hfr_3 locus has two *stable*

alleles, high rate of change and standard rate of change, and to view the allele for high rate of change as a genetic system which exists in two states.

The Lambda-K12 system is another genetic system which exists in two alternative states. In the *lysogenic* state, the prophage is present on the chromosome near to *Gal*. In the *sensitive* state, it is not. Lysogenicity and sensitivity resemble states I and II of $\male_3$. The counterpart of standard rate of change is not known. These ideas are summarized in Table 9.

Table 9. *Relation of the $\male_3$ system to the Lambda-K12 system*

	$\male_3$	Lambda-K12
1. Gene relates to episome		
(*a*) episome integrated	state I	lysogenicity
(*b*) episome vegetative or absent	state II	sensitivity
2. Gene does not relate to episome	standard rate of change	—

One may thus think of lysogenicity-sensitivity as gene-determined in the same sense that change of state at the Hfr_3 locus is gene-determined. In this view, strain K12 would be a special strain of *Escherichia coli* which possesses a preferred chromosomal site for Lambda just as $\male_3$ is a special strain of K12 which possesses a preferred chromosomal site for *F*. Strains of *E. coli* lacking the preferred site for Lambda would appear not to be lysogenized by Lambda although lysogenization might be occurring with low frequency at a variety of sites.

SUMMARY

A variant strain of *Escherichia coli* K12 ($\male_3$) was obtained after ultra-violet irradiation. This strain differs from wild type in the possession of a preferred chromosomal site for the *F* factor.

When wild-type females are infected with the *F* factor they are converted to *F* + males which carry the *F* factor in the infectious state. These *F* + males (standard) change with low frequency to *Hfr* males which carry the *F* factor on the chromosome. When the variant female ($\female_3$) is infected with the *F* factor it is converted to an *F* + male (state II) which changes with high frequency to a specific *Hfr* male (state I) which carries the *F* factor at the preferred site (Hfr_3).

Male strains carrying the preferred site alternate between the specific *Hfr* state and the *F* + state. The mechanism of this alternation is the back-and-forth shift of the *F* factor between its integrated state of multiplication at the Hfr_3 locus and its autonomous state of multiplication. This process is termed *episomic alternation*.

Episomic alternation offers a physical model for regular changes in state of the gene.

The Hfr_3 locus has two allelic forms, high rate of change and standard rate of change. The allele for high rate of change may be considered as a genetic system which occurs in two possible states, state I in which the *F* factor is present at Hfr_3, and state II in which it is not.

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