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The nature and incidence of conjugation factors in *Escherichia coli*

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1. INTRODUCTION

The Resistance Factors (R factors) responsible for transmissible resistance to antibacterial drugs are able, like the sex-factor, F, to promote conjugation between bacterial cells. Some R factors are related to F, since under special conditions, R^+F^- bacteria produce the specific F pilus which acts as receptor for phages specific for F^+ bacteria (Brinton, Gemski & Carnahan, 1964), and thus become susceptible to infection by these phages (Meynell & Datta, 1966). The Resistance Transfer Factor (RTF), that part of the R factor concerned with conjugation, behaves as if it differed from F essentially only in producing a repressor which limits synthesis of the F pilus, whether by the RTF itself or by F when it is in the same cell. Thus, in established R^+F^- or R^+F^+ cultures, the proportion of piliated cells is much lower than it is in R^-F^+ cultures. Under special conditions where repression is lifted in R^+F^- cultures, bacteria with F pili and with conjugating ability increase in parallel, so that their proportion may equal that found in an R^-F^+ culture (Lawn, Meynell & Datta, in preparation).

The unrepressed behaviour of F itself might be exceptional among conjugation factors, while repression was the normal state. If so, F might revert to the repressed state by mutation, so that its host would now appear F^- by conventional methods of testing. We therefore looked for evidence of repressed conjugation factors related to F, using as criterion the presence of a small proportion of F phage-sensitive bacteria in an apparently phage-resistant culture.

2. MATERIAL AND METHODS

Bacterial strains. These are described in Table 1.

Clones resistant to phage MS2 were isolated from a non-lactose fermenting (*lac*$^-$) strain, RC711, carrying the plasmid F-*lac*$^+$ (Jacob & Adelberg, 1959). Cultures of RC711 F-*lac*$^+$ were grown in TYECa with phage MS2 for several days, with daily dilution 1/10 in fresh medium. Each day, a loopful of the culture was plated on McConkey lactose indicator medium, where it produced a mixture of *lac*$^+$ colonies, formed by cells still carrying the *lac*$^+$ of the F-*lac*$^+$, and therefore possibly still carrying F, and *lac*$^-$ colonies which presumably had lost F completely. About sixty *lac*$^+$ clones were purified and tested on plates with spots of concentrated phage MS2, but none were lysed. Six of these clones were then tested for ability to allow multiplication of the phage.

The frequency of transfer of F-*lac*$^+$ by RC711 F-*lac*$^+$ and its phage-resistant derivatives was measured using strain RC12 as recipient. Freshly-grown broth cultures were mixed in the proportion of 5×10^7/ml. donor to 5×10^8/ml. recipient and plated after incubation at 37°C. for 10, 30 and 60 min. Mixtures were plated on defined medium supplemented

with threonine, leucine and vitamin B1, and with lactose as carbon source, so that only bacteria which had received the *lac* gene could form colonies. Colony counts compared with the viable count of the donor gave a measure of transfer frequency.

Table 1. *Bacterial strains*

Strain	Relevant characters	Reference
SG.83	K12 58.161 (*met*$_1^-$.*lac*$^+$) S^r F$^-$ (acridine-cured)	
58.161/*sp*	K12 58.161 (*met*$_1^-$.*lac*$^+$) F$^-$ (spontaneous)	Hayes (1953)
RC.711		Meynell & Datta (1966)
RC.711(V2)	RC.711 with *col* V2 from K94	
RC.711(V3)	RC.711 with *col* V3 from K30	
RC.12	K12 W677 (*thr*$^-$.*leu*$^-$.*B*$_1^-$; F$^-$) S^r	
RC.20	RC.12 with *col* E1 from K30	
Hfr.C K1–5	F phage-resistant clones of K12 Hfr.C	Cavalli-Sforza (1950) Dr S. D. Silver (personal communication)
RC.49	K12 W1655 (*met*$^-$.λ^-.λ^r) F$^-$ (acridine-cured) I^r	
RC.59	RC.49 with *col* Ib from *Salmonella* SL.902	Ozeki, Stocker & Smith (1962)
RC.415	CA.18, natural host of *col* B	Fredericq (1965)
RC.416	K77, natural host of *col* B	Fredericq (1948)
RC.417	K166, natural host of *col* B	Fredericq (1948)
CA.7	Natural host of *col* V1	Fredericq (1965)
K.94	Natural host of *col* V2	Fredericq (1948) Clowes & Macfarren (in preparation)
K.30	Natural host of *col* V3	Fredericq (1948) Clowes & Macfarren (in preparation)
E. coli B		
WG.3	*E. coli* fertile with K12 F$^+$ and F$^-$. Transmitted fertility to 58.161/*sp*	Bernstein (1958, 1963)
WG.4	*E. coli* fertile with K12 F$^+$ and 58.161/*sp*, but not with F$^-$	Bernstein (1958, 1963)
F & R A-I$_4$	Strains transmitting fertility to K12 58.161/*sp*	Furness & Rowley (1957)
Freshly-isolated *E. coli*	Antibiotic-sensitive strains of *E. coli* isolated from clinical material in Hammersmith Hospital	

Culture media, and *Methods with F-specific phage*, MS2, were as in Meynell & Datta (1966).

Colicine production. This was tested by conventional techniques (Fredericq, 1958), using a universally sensitive indicator strain to detect the production of any colicine, and a set of specifically resistant indicator strains to identify the colicine produced.

3. RESULTS AND DISCUSSION

F-specific phage lyses only F$^+$ cultures, but different F$^+$ strains of *Escherichia coli* differ in their susceptibility, some showing more complete clearing, together with a higher efficiency of plating, than others (Dettori, Maccacaro & Turri, 1963). This was confirmed in the present experiments, and might reflect differences in the proportions of bacteria with the F phage receptor. The experimental findings shown in Table 2 indicate that the proportion of phenotypically F$^-$ bacteria in a culture need not be large for it to appear totally phage resistant by conventional methods of testing. Thus, even moderate repression would lower the proportion of phenotypically phage-sensitive bacteria to a point that made the culture appear phage resistant.

Table 2. *Visible lysis by phage MS2: effect of the proportion of sensitive to resistant bacteria*

Ratio of Hfr.H/RC709 F⁻ in bacterial inoculum	Phage dilutions plated in agar overlay		High titre phage (5×10^9 particles/ml.) spotted on surface inoculum
	10^{-8}	10^{-7}	
1:0	431 clear plaques	Semi-confluent lysis	Clear, with isolated secondary colonies
1:1	382 turbid plaques	Semi-confluent, incomplete lysis	Turbid
1:2	About 200 faint plaques	Bacterial growth uneven	Partial thinning seen by oblique light
1:4	About 24 faint plaques seen by oblique light	No visible lysis	No visible lysis
1:8	No visible lysis	No visible lysis	No visible lysis

Table 3 shows the results of testing bacterial cultures that appeared resistant to phage MS2. The first part of the table concerns the F factor itself in *E. coli* K12. Strain 58.161/*sp* has always been observed to behave differently from F⁻ strains: when used as recipient in crosses, not all the recombinants become F⁺ (Hayes, 1953); certain wild strains of *E. coli*, which do not react with true F⁻ lines of K12 give recombinants with 58.161/*sp*, or convert it to a fertile donor strain (Bernstein, 1958, 1963; Furness & Rowley, 1957); and it restricts the multiplication of phage T3 like an F⁺ strain (Schell *et al.*, 1963). Cultures of 58.161/*sp* contained a proportion of phage-sensitive bacteria, indicating that F must still be present, and so explaining these differences observed between it and a true F⁻ strain.

Table 3. *Sensitivity to phage MS2*

Strains			MS2 lysis eop*	No. of tests	Initial No. of plaques	Increase
Hfr.H			+1·0	6	$3{\cdot}5 \times 10^6$–$1{\cdot}5 \times 10^7$	×2000–50,000
Hfr.B1			+0·7	4	$8{\cdot}4 \times 10^5$–$2{\cdot}0 \times 10^6$	×20,000–100,000
RC.709 F⁻			—	18	$2{\cdot}4 \times 10^2$–$1{\cdot}0 \times 10^4$	None
SG.83 F⁻			—	1	$3{\cdot}15 \times 10^3$	None
58.161/*sp* "F⁻"			—	6	$3{\cdot}2 \times 10^2$–$4{\cdot}0 \times 10^3$	×77–500
MS2-resistant clones of RC.711 F-lac⁺:						
	TF_{30}**	*Ac-curing of lac⁺*				
Clone 1	$1{\cdot}0 \times 10^5$/ml.	+	—	1	$1{\cdot}2 \times 10^7$	×2000
2	$6{\cdot}0 \times 10^4$/ml.	+	—	1	$1{\cdot}4 \times 10^5$	×5800
3	$4{\cdot}5 \times 10^1$/ml.	+	—	1	$3{\cdot}7 \times 10^3$	×2·8
4	$5{\cdot}0 \times 10^2$/ml.	+	—	1	$1{\cdot}7 \times 10^3$	×6
5	<5/ml.	—	—	1	$1{\cdot}0 \times 10^3$	None
6	<5/ml.	—	—	1	$1{\cdot}8 \times 10^3$	None
RC.711 F-*lac⁺*	$2{\cdot}0 \times 10^5$/ml.	+	+1·0			
MS2-resistant clones of Hfr.C:						
	Recombination					
Clone K1	Undetectable		—	1	$2{\cdot}4 \times 10^3$	None
K2	,,		—	1	$1{\cdot}9 \times 10^3$	×15
K3	,,		—	1	$1{\cdot}6 \times 10^3$	None
K4	,,		—	1	$1{\cdot}2 \times 10^3$	None
K5	5% of normal rate		—	1	$1{\cdot}5 \times 10^6$	×14,000

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Table 3. *Sensitivity to phage MS2—continued*

Strains			MS2 lysis eop*	No. of tests	Initial No. of plaques	Increase
	Col	*Conjugation (Transmission of colicinogeny)*				
RC.415	B	+	—	1	$1{\cdot}9 \times 10^3$	None
RC.416	B	+	—	1	$1{\cdot}1 \times 10^5$	× 2000
RC.417	B	+	—	1	$3{\cdot}0 \times 10^3$	× 800
RC.20	E1	—	—	1	$5{\cdot}7 \times 10^2$	None
RC.49	None	·	—	1	$6{\cdot}0 \times 10^2$	None
RC.59 LFT***	Ib	+	—	1	$6{\cdot}6 \times 10^2$	None
RC.59 HFT***	Ib	+	—	1	$1{\cdot}1 \times 10^3$	None
CA.7	V1	—	—	1	$3{\cdot}2 \times 10^3$	None
K.94	V2	+	+0·15	1	$6{\cdot}5 \times 10^6$	× 15,000
K.30	V3	+	+0·05	1	$1{\cdot}38 \times 10^7$	× 6000
RC.711(V2)	V2	+	+0·15	1	$4{\cdot}8 \times 10^6$	× 20,000
RC.711(V3)	V3	+	+0·05	1	$8{\cdot}6 \times 10^6$	× 6000
RC.711	None	·	—	9	$3{\cdot}0 \times 10^2$–$9{\cdot}0 \times 10^3$	None
	Col	*Special characters*				
E. coli B	None		—	1	$5{\cdot}0 \times 10^2$	None
WG.3	I	Restores fertility to 58·161/*sp*	—	1	$6{\cdot}0 \times 10^5$	× 7000
WG.4	None	Gives recombinants with 58.161/*sp*, but not with F⁻	—	1	$5{\cdot}5 \times 10^5$	× 3400
F & R A	None	Restores fertility to 58/161/*sp* and to F⁻	—	1	$4{\cdot}4 \times 10^3$	× 2500
H	I+ another	Restores fertility to 58·161/*sp* only	—	1	$5{\cdot}4 \times 10^3$	× 900
J	V+B		—	2	$1{\cdot}8 \times 10^3$; $4{\cdot}0 \times 10^3$	None
L	None		—	2	$1{\cdot}3 \times 10^3$; $9{\cdot}0 \times 10^2$	None
O	B		—	1	$1{\cdot}3 \times 10^3$	None
R	I+B		—	1	$2{\cdot}0 \times 10^4$	× 1350
U	I+ others		—	1	$4{\cdot}0 \times 10^3$	× 165
I_4^+	I		—	1	$1{\cdot}3 \times 10^3$	None
Newly-isolated E. coli *strains:*						
	Col					
12 strains	None		—	1 each	$5{\cdot}0 \times 10^2$–10^4	None
3 ,,	B or H		—	1 ,,	$5{\cdot}6 \times 10^2$–10^4	None
2 ,,	K or A		—	1 ,,	$5{\cdot}6 \times 10^2$; $1{\cdot}4 \times 10^4$	None
2 ,,	+ unidentified		—	1 ,,	$8{\cdot}9 \times 10^2$; $1{\cdot}0 \times 10^4$	None
1 ,,	I		—	1	$1{\cdot}0 \times 10^3$	None
U.15486	B or H		—	2	$3{\cdot}7 \times 10^2$; $2{\cdot}8 \times 10^3$	× 500; 760
U.18416	B+another		—	1	$4{\cdot}0 \times 10^3$	× 54
U.18735	+(not V, I or E)		—	1	$2{\cdot}0 + 10^3$	× 25,000
F.18710	+(not V, I or E)		—	1	$5{\cdot}0 \times 10^3$	× 100
SG.18688	V		—	2	$3{\cdot}0 \times 10^3$; $2{\cdot}2 \times 10^3$	× 5·6; 5
P.20960	V+I		—	1	$1{\cdot}0 \times 10^4$	× 1000

* eop = efficiency of plating, compared with that on Hfr.H.

** Transfer frequency at 30 min.

*** The LFT (Low Frequency of Transfer) and HFT (High Frequency of Transfer) *col* I⁺ cultures were kindly supplied by Dr R. C. Clowes, who also measured the frequencies of transfer of the colicinogeny.

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Mutant strains resembling 58.161/*sp*, as well as genuine F⁻ strains, should emerge as secondary phage-resistant growth when F⁺ cultures are grown in the presence of F phage. Galucci & Sironi (1964), in reporting a probable mutation in the F factor, appear to have isolated such strains, and Zinder (1965) reports that a small percentage of phage-resistant mutants retain some donor capacity. Of five phage-resistant variants of strain Hfr.C of reduced fertility, kindly provided by Dr S. Silver, three supported no increase of phage MS2, but the other two contained a proportion of phage-sensitive cells which appeared to be higher with the variant which gave genetic recombinants at 5% the normal rate for Hfr.C than with the variant giving no detectable recombination.

When strain RC711 F-*lac*⁺ was grown with phage MS2, the phage-resistant growth consisted of a mixture of *lac*⁻ bacteria which had probably lost the F factor, and bacteria which were still *lac*⁺. Six *lac*⁺ clones which were not lysed by the phage were tested for the presence of phage-sensitive bacteria: two contained a high proportion, and transferred F-*lac*⁺ at nearly the same rate as RC711 F-*lac*⁺. Of the remaining four clones, two contained only a few phage-sensitive cells, and transferred F-*lac*⁺ at a correspondingly lower rate. No phage-sensitive bacteria, or transfer of the *lac*⁺ gene, could be detected with the last two isolates, which may thus possibly have been F⁻, but have integrated the *lac*⁺ gene into the chromosome. This view was supported by finding that growth in broth containing acridine, which eliminates the F factor (Hirota, 1960), resulted in loss of *lac*⁺ from clones 1, 2, 3 and 4, but not from 5 and 6. Growth in the presence of F phage should lead preferentially to the emergence of clones which are phenotypically F⁻, but in which F is nevertheless present, for while cells in which F is expressed are phage sensitive, and F⁻ cells can readily be reinfected with F, clones which carry an unexpressed F factor are resistant both to attack by the phage and to infection with the F factor which would restore their phage sensitivity.

An association between *col* V and F has been interpreted as possible co-transfer of the two factors (Fredericq, 1963), and strains carrying *col* V2, derived from *E. coli* K94, or *col* V3, derived from *E. coli* K30, are visibly lysed by F-specific phage (Kahn & Helinski, 1964; Clowes & Macfarren, in preparation). Our results confirm that strains with *col* V2 or *col* V3 contain a high proportion of phage-sensitive bacteria, and show that *col* V1, which does not promote its own transfer from *E. coli* CA7, likewise does not allow the phage to multiply.

Two *col* B factors carried conjugation factors which conferred sensitivity to F phage, although here the proportion of sensitive bacteria in the cultures was small. The third *col* B⁺ strain, 415, was totally insensitive, although it transmitted its colicinogeny as efficiently as strain 416. *Col* E1, which is unable to transfer itself, conferred no phage sensitivity.

Col I carries its own conjugation factor, but gave no F phage sensitivity. This was true even of an HFT system in which over 90% of the bacteria transferred their colicinogeny within 1 hour. Thus, the conjugation factor associated with *col* I, like the fi⁻ class of R factors, is an agent unrelated to F (Meynell & Datta, 1966).

Escherichia coli strain B behaves like an F⁻ strain which can only with difficulty be infected with F (de Haan, 1954), suggesting that it might carry an F-like agent. However, no evidence was found for this in the present experiments, for this strain did not support multiplication of phage MS2.

The wild strains of *E. coli*, WG3 and WG4, were amongst the 40/2000 strains found to show some fertility with K12F⁻ recipients (Lederberg, Cavalli & Lederberg, 1952), and were studied in detail by Bernstein (1958, 1963). She reported that strain WG3 gave recombinants with F⁺ or F⁻, and restored to 58.161/*sp* the ability to recombine with F⁻ strains and to transmit a fertility factor to them. The other strain, WG4, was said to have no transmissible F agent, but to give recombinants with F⁺ or with 58.161/*sp*, but not with F⁻. Table 3 shows that WG3 and WG4 both contained some phage-sensitive cells. To

see if the restoration of fertility to 58.161/*sp* reported to occur on incubation with WG3 involved the re-creation of a freely expressed F factor, the strains were grown together in broth overnight, and sixty colonies of 58.161/*sp* isolated from the mixture were tested for lysis by phage MS2. None were lysed. Bernstein reports that neither strain WG3 nor strain WG4 are colicinogenic, but in the present tests, WG3 produced a colicine, probably colicine I. In view of the colicinogeny of this strain, it now seems more likely that the phenomenon observed was similar to that reported by Clowes (1963) where variants of strain Hfr.C which had partially or totally lost their fertility upon becoming resistant to F phage, regained it on infection with *col* I. This was interpreted to mean that the F factor was still present in its original chromosomal site, but had lost the ability to promote conjugation, and that this ability was restored by the conjugation mechanism of *col* I.

Strains F & R, A to I_4 are wild strains of *E. coli* found by Furness & Rowley (1957) to restore to 58.161/*sp* the ability to produce recombinants with F^- recipients: in addition, strain A was reported to transfer fertility to true F^- strains. The remainder of the strains tested were freshly isolated *E. coli*, chosen for their sensitivity to all the antibacterial drugs in common clinical use, and thus their probable freedom from R factors. Many were colicinogenic, including all of those which permitted phage multiplication. The association between type of colicine produced and response to F phage was not analysed further amongst these strains, but there is no reason to suppose that the determinant for a particular sort of colicine is necessarily always associated with the same conjugation factor. Production of colicine V amongst these strains did not confer a degree of phage sensitivity sufficient to cause the culture to be visibly lysed.

A considerable number of the strains listed in Table 3 showed a proportion of F phage-sensitive bacteria. The only random sample of strains in this table is the set of twenty-six freshly isolated *E. coli*, but here it is striking that as many as six of the twenty-six were able to propagate the phage. If unexpressed F agents really are widespread amongst bacteria, this would explain how F-specific phage comes to be so abundant in nature, while appearing to have as host virtually only one exceptional bacterial strain (Zinder *et al.*, 1963). An F^+ strain would be expected to have a greater chance of survival if its F^+ character, which entails sensitivity to a phage abundantly present in its natural habitat, were largely unexpressed.

All evidence suggests that phage sensitivity and conjugating ability are dependent on the same surface structure, for it has never been possible to separate them in the individual bacterium. In reports of phage-resistant variants which could still conjugate, phage sensitivity was recorded only when cultures were visibly lysed, but Table 2 indicates that only a slight suppression of F function, probably insufficient to alter the observed frequency of recombination, would make the culture appear phage resistant. The results with RTF, which show a parallel increase of phage sensitivity and conjugation under conditions where repression is lifted (Meynell & Datta, 1966) lend further support to the view that the phage receptor is necessarily involved in conjugation, whether or not the F pilus actually constitutes the conjugation tube (see Brinton, 1965).

The F phage allows only one class of conjugation factor to be recognized. Other phages may well exist, using as their receptors the surface components determined by other conjugation factors, but until a strain in which the factor is fully expressed is available to serve as an indicator, the phage will remain undetected. Some plasmids, and the F factor itself, restrict the multiplication of certain phages (Schell *et al.*, 1963). Anderson & Lewis (1965) have made use of this phenomenon of restriction by a plasmid to detect the presence of a conjugating factor in *Salmonella typhimurium*. The typing scheme for *S. typhimurium* is partially based on restriction of the typing phages, and the presence of the conjugating factor was detected by an alteration in phage type. Anderson (1965) demonstrated that non-transmissible drug resistance determinants can become linked with such a conjugation factor in the same way as they can with F (Harada *et al.*, 1964).

SUMMARY

The sex factor F is itself exceptional among conjugation factors in being freely expressed in all the bacteria of a culture, while related factors which are largely unexpressed are widespread. These can be recognized by the presence of a small proportion of cells sensitive to phage specific for F^+ bacteria, and may sometimes constitute the conjugation factors associated with colicinogeny. Moreover, F itself can mutate to the repressed state so that derivatives of *E. coli* K12 which appear F^- include some in which F is still present but rarely expressed.

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