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I-like resistance factors with the *fi*$^+$ character

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SUMMARY

The *fi*$^+$ (fertility inhibition+) property was originally described in R factors related to F (F-like factors). The original *fi*$^-$ factors, in contrast, were related to the transfer factor of a colicin I factor (I-like factors). We describe here a group of R factors which are *fi*$^+$ but I-like. It is suggested that all the terms *fi*$^+$, *fi*$^-$, F-like and I-like be used in the description of transferable plasmids.

An early subdivision of transferable plasmids of the enterobacteria was based on that originally observed in resistance factors (R factors). According to this the plasmids could be classified into those that repressed the fertility of strains of *Escherichia coli* K12 (= K12) carrying the F factor, which were designated 'fertility inhibition+' (*fi*$^+$), and those without effect on F fertility, which were designated *fi*$^-$ factors (Egawa & Hirota, 1962; Watanabe & Fukasawa, 1962; Watanabe *et al.* 1964). Later work suggested that the *fi*$^+$ plasmids studied were related to the F factor of K12, so that they were designated 'F-like'; and that most *fi*$^-$ factors were related to the transfer factor associated with a classical determinant for colicinogeny, colicin Ib-P9 (Fredericq, 1956), so that they have been termed 'I-like' (Meynell, Meynell & Datta, 1968).

F-like factors code for the synthesis of sex fimbriae (or pili) known as F-fimbriae (Brinton, 1965), while the fimbriae promoted by I-like factors are designated 'I-like' (Meynell & Lawn, 1967). Sex fimbriae are necessary for bacterial conjugation and act as the receptors for donor-specific phages (Brinton, 1965; Meynell *et al.* 1968). The phages concerned are therefore divided into two groups: F-specific, of which the receptors are the F and F-like fimbriae; and I-specific, of which the I-like fimbriae are the receptors.

The *fi*$^+$ factors exert their repressing effect on F because they are themselves normally repressed, and owe this state to synthesis of a repressor to which the F factor is also sensitive (Meynell & Datta, 1965). In contrast, although *fi*$^-$ factors are also repressed in the wild state, the repressor concerned does not affect the F factor. It has been suggested that the terms *fi*$^+$ and *fi*$^-$ be abandoned on the grounds that F-like and I-like offer a more satisfactory subdivision of plasmids (Novick, 1969). In the present article we will present a case for the use of all four symbols in the description of plasmids.

We describe here a group of R factors which are both *fi*$^+$ and I-like. The nine factors studied were isolated from strains of *Salmonella typhimurium* and *S. enteritidis* phage-typed in the Enteric Reference Laboratory, and from wild strains of *E. coli*. All strains were isolated from man over a 3-year period and were of independent origin. They were initially selected for study because of their resistance to neomycin and kanamycin (K). Table 1 lists the factors investigated.

Although all the factors were in the repressed state, K could be transferred without difficulty from the original host strains to the lines of K12 used in these investigations. We have so far been unable to obtain the K determinant without its transfer factor by conjugation in any of these systems. The kanamycin R factors studied are therefore

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Class 1 factors (Anderson, 1968; Anderson & Threlfall, 1970), in which the resistance determinants and the transfer factors are covalently bonded to form a stable linkage group which is normally transferred intact to recipient cells. The prototypes of such factors are those originally discovered in Japan (Ochiai *et al.* 1959; Akiba *et al.* 1960). A Class 1 factor which has been closely studied is the tetracycline resistance factor T-Δ, identified in *S. typhimurium* (Anderson & Lewis, 1965; Anderson, 1968; Anderson, 1969).

Table 2 shows the results of experiments which demonstrate the *fi*+ character of the nine factors studied. Each factor was transferred both to K12F+ and to K12HfrH

Table 1. *Source of kanamycin resistance factors*

Original host strains		
Serotype	Phage type	Factor no.
Salmonella typhimurium	74	TP101
Salmonella typhimurium	Untypable	TP102
Salmonella typhimurium	11	TP103
Salmonella typhimurium	Untypable	TP104*
Salmonella typhimurium	89	TP105
Escherichia coli	.	TP106
Escherichia coli	.	TP107
Salmonella enteritidis	14	TP108
Salmonella typhimurium	141	TP109

* TP104 was referred to as factor 782 by Grindley *et al.* (1971).

Table 2. *Results of tests of* fi *character of the R factors investigated*

	Experiments in K12F+			Experiments in HfrH	
Factor no. TP	Reaction with phage μ_2 in spot tests	EOP of phage μ_2 in agar layer (K12F+ = 1)	Plaque morphology (agar layer)	Reaction with phage μ_2 in spot tests	Frequency of *pro* transfer as % of that of HfrH
101	VOL*	1	Opaque	VOL	1·5
102	VOL	1	Opaque	VOL	4·0
103	VOL	1	Opaque	VOL	2·5
104	VOL	1	Opaque	VOL	3·0
105	VOL	1	Opaque	VOL	1·0
106	VOL	1	Opaque	VOL	2·0
107	VOL	1	Opaque	VOL	3·0
108	VOL to −†	0	.	−	2·0
109	−	0	.	−	0·003

* Very opaque lysis. † No detectable lysis.

(= HfrH). Sensitivity to F-specific phage μ_2 in surface spot tests and in agar layer tests (Pitton & Anderson, 1970) was examined in all the resulting F+ strains; the HfrH strains were tested for sensitivity to μ_2 in spot tests, and for the frequency of *pro* transfer to K12F− in 1 h crosses.

It can be seen from this table that all the factors except TP109 reduced *pro* transfer from HfrH to K12F− to between 1 and 4 % of the usual Hfr frequency, and that TP109 reduced *pro* transfer to 0·003 % of its normal frequency.

Table 2 also shows the effect of each factor on the sensitivity of both F+ and HfrH to the phage μ_2. The lysis produced on both hosts was very opaque when the factors carried

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were TP101 to TP107 and is referred to in Table 2 as VOL. Although there was very faint and irregular lysis in spot tests with μ_2 on K12F$^+$(TP108), none could be found in agar layer tests on that strain, or in spot tests with HfrH(TP108). The efficiency of plating (EOP) tests with μ_2 were carried out only in agar layer, and when visible lysis occurred the EOP was 1. Factor TP109 abolished visible lysis by μ_2 in both K12F$^+$ and HfrH.

Each of the nine R factors was transferred to K12F$^-$ for the further characterization of the transfer factors. The resulting strains were examined for the synthesis of F-like or I-like fimbriae by testing their ability to support multiplication of the F-specific phage μ_2 and the I-specific phage If1 (Meynell & Lawn, 1968). A modification of the method of Easterling *et al.* (1969) was used. One ml of a broth culture of each strain, grown to a density of about 5×10^8 bacteria/ml, was diluted into 8 ml of broth. Sufficient phage was added in a volume of 1 ml to give a final concentration of *c.* 5×10^4 pfu/ml of either μ_2 or If1. The ratio of bacteria to phage was thus about 1000: 1. The mixture was incubated overnight at 37 °C without shaking, and was then centrifuged at 3000 r.p.m. for 15 min in an MSE Minor angle centrifuge. The phage content of the resulting supernatant was titrated in agar layer, using as the indicator strain, either K12F$^+$ (for μ_2), or K12F$^-$ carrying the derepressed I-like R factor T-Δ*drp*1 (for If1). (T-Δ*drp*1 was isolated by N. D. F. Grindley.)

Phage If1 multiplied about 1000-fold in strains carrying each of the nine factors under study, but no multiplication of μ_2 could be detected under these conditions. Control experiments with K12F$^-$ carrying the repressed F-like factor 334 (Pitton & Anderson, 1970; Smith *et al.* 1971) gave 100-fold multiplication of μ_2 and no multiplication of If1.

In the same way that two copies of the same transfer factor cannot coexist stably in the same cell (Scaife & Gross, 1962; Anderson, 1966), two R factors of homologous type – that is, both F-like, or both I-like – are usually incompatible with each other, although each is compatible with F and with factors of the heterologous type. It has been suggested that this incompatibility is due to the need for attachment of each plasmid to a unique specific cellular maintenance site which governs its replication and uniform segregation into daughter cells at cell division (Jacob, Brenner & Cuzin, 1963; Anderson, 1968; Anderson, 1969). Two homologous R factors in the same host cell will compete for this unique site, but only one can gain attachment, so that the other is lost.

We have already indicated that all the factors under investigation code for kanamycin resistance. K12F$^-$ lines carrying each factor were superinfected with an F-like or an I-like factor, and the progeny were examined for stability. T-Δ*drp*1 was used as the superinfecting I-like factor. For the F-like factor we used a segregant of R1*drd*19. R1*drd*19 (Meynell & Datta, 1967) is a repressor-minus (i^-) mutant of the R factor R1, which confers resistance to ampicillin, chloramphenicol, kanamycin, streptomycin and sulphonamides. The segregant used in these experiments had lost its determinant for kanamycin resistance, and was designated R1*drd*19K$^-$. It was thus possible to use the kanamycin-resistance marker of the nine resident R factors, as well as remaining resistance markers of R1, in testing for stability of the hybrids resulting from entry of R1*drd*19K$^-$.

These superinfection experiments showed that the resident K plasmid was always displaced by T-Δ*drp*1; that is, that none of the nine factors studied was compatible with the I-like superinfecting factor. In contrast, all the factors were compatible with the F-like factor R1-*drd*19K$^-$ so that the resulting hybrids were stable. In crosses from these hybrids the two R factors showed independent assortment, so that they had not recombined. It could therefore be concluded that the kanamycin R factors were I-like and not F-like in type, in spite of being *fi*$^+$. This conclusion is supported by the results of the multiplication experiments with phages If1 and μ_2, described earlier.

In order to establish that the *fi*$^+$ property was located on the I-like plasmid, HfrH lines carrying each plasmid were superinfected with T-Δ*drp*1. It was found that, when K was displaced, the resulting HfrH(T-Δ*drp*1) lines were fully sensitive to phage μ_2. This

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indicates that the normal derepressed synthesis of F-fimbriae by HfrH had been restored when each K resistance factor was lost. Moreover, tests of *pro* transfer from these lines showed that the frequency had reverted to the normal HfrH level. It could therefore be concluded that the *fi*$^+$ region had been displaced simultaneously with K by the I-like T-Δ factor.

The nine factors studied thus appear to constitute a group of transferable plasmids which are *fi*$^+$ and I-like. They all code for kanamycin resistance, and most (TP101 to TP108) inhibit F fertility to a similar extent. However, variations in their phage restriction properties in salmonellae (Anderson, 1966; Anderson, 1968), and the much greater inhibition of F fertility by TP109 (see Table 2), suggest that the factors are not homogeneous. The R factor R62 of Romero & Meynell (1969) may belong to this type of factor. Preliminary experiments suggest that the factors belonging to groups *fi*$^{+1}$ to *fi*$^{+3}$, described by Pitton & Anderson (1970), are also *fi*$^+$ and I-like.

There are two possibilities in relation to the origin of these plasmids. They may be recombinants between I-like and F-like factors. Or they may constitute a phylogenetically distinct group of factors that are *fi*$^+$ and I-like.

The question naturally arises of whether the *fi*$^+$ character is in any way connected with the normally repressed state of these factors; that is, whether the plasmids code for only one fertility inhibitor which affects both I-like and F-like factors. This would be expected if the factors constituted an independent group. Alternatively, the *fi*$^+$ character may be independent of the repression of the transfer factors carrying it, so that the synthesis of two repressors could be postulated, one affecting F-like and the other I-like factors. This would be predictable if the factors were recombinants between F-like and I-like factors. These aspects are being investigated.

This work shows that the terms *fi*$^+$ and *fi*$^-$, and F-like and I-like, can all be used with advantage. Transferable plasmids can thus be described as follows: *fi*$^+$, F-like; *fi*$^+$, I-like; and *fi*$^-$, I-like. Derepressed (*i*$^-$) mutants of *fi*$^+$, F-like factors are obviously *fi*$^-$ and F-like. In addition, we have *fi*$^-$ factors that are neither I-like nor F-like, and these await further characterization.

The *fi*$^+$ character is at present viewed in terms of effects on F and F-like factors. It was detected because the F factor is probably a repressor-minus (*i*$^-$) mutant of its wild ancestor, still sensitive to repression, and because the F factor can stably coexist with F-like as well as I-like factors. Because of the lack of a similar detection system, we cannot yet identify *i*$^-$ mutants of I-like factors. If such a system is found, however, it may be possible to extend the characterization of transfer factors in terms of their effect on *i*$^-$ mutants of I-like factors.

REFERENCES

AKIBA, T., KOYAMA, T., ISHIKI, T., KIMURA, S. & FUKUSHIMA, T. (1960). On the mechanism of development of multiple-drug-resistant clones of Shigella. *Japanese Journal of Microbiology* **4**, 219–227.

ANDERSON, E. S. (1966). Influence of the Δ transfer factor on the phage sensitivity of Salmonellae. *Nature, London* **212**, 795–799.

ANDERSON, E. S. (1968). The ecology of transferable drug resistance in the enterobacteria. *Annual Review of Microbiology* **22**, 131–180.

ANDERSON, E. S. (1969). The ecology and epidemiology of transferable drug resistance. In *Ciba Foundation Symposium on Bacterial Episomes and Plasmids*, ed. G. E. W. Wolstenholme and M. O'Connor. London: J. and A. Churchill Ltd.

ANDERSON, E. S. & LEWIS, M. J. (1965). Characterisation of a transfer factor associated with drug resistance in *Salmonella typhimurium*. *Nature, London* **208**, 843–849.

ANDERSON, E. S. & THRELFALL, E. J. (1970). Change of host range in a resistance factor. *Genetical Research* **16**, 207–214.

https://doi.org/10.1017/S0016672300012295 Published online by Cambridge University Press

BRINTON, C. C. (1965). The structure, function, synthesis and genetical control of bacterial pili and a molecular model for DNA and RNA transport in Gram-negative bacteria. *Transactions of the New York Academy of Sciences* **27**, 1003–1054.

EASTERLING, S. B., JOHNSON, E. M., WOHLHIETER, J. A. & BARON, L. S. (1969). Nature of lactose-fermenting salmonella strains obtained from clinical sources. *Journal of Bacteriology* **100**, 35–41.

EGAWA, R. & HIROTA, Y. (1962). Inhibition of fertility by multiple drug-resistance factor (R) in *Escherichia coli* K12. *Japanese Journal of Genetics* **37**, 66–69.

FREDERICQ, P. (1956). Recherches sur la fréquence des souches transductrices des propriétés colicinogènes. *Comptes rendus des séances de la Société de Biologie et de ses Filiales, Paris* **150**, 1036–1039.

GRINDLEY, N. D. F., ANDERSON, E. S., SMITH, H. R. & GRINDLEY, J. N. (1971). The effects of *Salmonella typhimurium* on derepressed mutants of F-like factors. *Genetical Research* **17**, 89–93.

JACOB, F., BRENNER, S. & CUZIN, F. (1963). On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symposia on Quantitative Biology* **28**, 329–348.

MEYNELL, E. & DATTA, N. (1965). Functional homology of the sex-factor and resistance transfer factors. *Nature, London* **207**, 884–885.

MEYNELL, E. & DATTA, N. (1967). Mutant drug resistant factors of high transmissibility. *Nature, London* **214**, 885–887.

MEYNELL, E., MEYNELL, G. G. & DATTA, N. (1968). Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. *Bacteriological Reviews* **32**, 55–83.

MEYNELL, G. G. & LAWN, A. M. (1967). Sex pili and common pili in the conjugational transfer of colicin factor Ib by *Salmonella typhimurium*. *Genetical Research* **9**, 359–367.

MEYNELL, G. G. & LAWN, A. M. (1968). Filamentous phages specific for the I sex factor. *Nature, London* **217**, 1184–1186.

NOVICK, R. P. (1969). In *Ciba Foundation Symposium on Bacterial Episomes and Plasmids* (ed. G. E. W. Wolstenholme and M. O'Connor), pp. 133–134. London: J. and A. Churchill Ltd.

OCHIAI, K., YAMANAKA, T., KIMURA, K. & SAWADA, O. (1959). Studies on transfer of drug resistance between Shigella strains and *Escherichia coli* strains. *Nippon-ijishimpo*, no. 1861, pp. 34–46. In Japanese (quoted by Akiba *et al.* 1960).

PITTON, J.-S. & ANDERSON, E. S. (1970). The inhibitory action of transfer factors on lysis of *Escherichia coli* K12 by phages μ_2 and ϕ_2. *Genetical Research* **16**, 215–224.

ROMERO, E. & MEYNELL, E. (1969). Covert *fi*$^-$ R factors in *fi*$^+$ R$^+$ strains of bacteria. *Journal of Bacteriology* **97**, 780–786.

SCAIFE, J. & GROSS, J. D. (1962). Inhibition of multiplication of an F-*lac* factor in Hfr cells of *Escherichia coli* K-12. *Biochemical and Biophysical Research Communications* **7**, 403–407.

SMITH, H. R., GRINDLEY, J. N., GRINDLEY, N. D. F. & ANDERSON, E. S. (1970). Derepression of F-*lac* in *Salmonella typhimurium* by a determinant for kanamycin resistance. *Genetical Research* **16**, 349–353.

WATANABE, T. & FUKASAWA, T. (1962). Episome-mediated transfer of drug resistance in Enterobacteriaceae. IV. Interactions between resistance transfer factor and F factor in *Escherichia coli* K12. *Journal of Bacteriology* **83**, 727–735.

WATANABE, T., NISHIDA, H., OGATA, C., ARAI, T. & SATO, S. (1964). Episome-mediated transfer of drug-resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. *Journal of Bacteriology* **88**, 716–726.

https://doi.org/10.1017/S0016672300012295 Published online by Cambridge University Press