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Mutant Hfr strains defective in transfer: restoration by F-like and I-like de-repressed R factors

By MARYLYN COOKE, ELINOR MEYNELL and A. M. LAWN

*M.R.C. Microbial Genetics Research Unit, London, W.*12, *and Guinness–Lister Research Unit and Departments of Microbiology and Electron Microscopy, Lister Institute, Chelsea Bridge Road, London, S.W.*1*

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SUMMARY

Mutants of Hfr strains of *Escherichia coli* K12 defective in conjugation owing to failure to produce sex pili were isolated by resistance to F-specific phage. Tests of the ability of six de-repressed F-like R factors and four de-repressed I-like R factors to restore Hfr donor behaviour to these mutants indicated that there were several ways in which such restoration could occur. Of a total of 26 defective mutants, 12 were restored by F-like R factors. In these twelve, the function of the integrated F factor was evidently restored, because the sex pili contained F pilin subunits, distinguishable from the R pilin by serological tests. In contrast, among the four I-like R factors, only two were effective, but in all 26 defective mutants; the restored Hfr bacteria produced only I-like pili. The I-like sex factors, in restoring Hfr donor behaviour, did not therefore act by complementing the defective F.

1. INTRODUCTION

Every bacterial sex factor so far described determines the synthesis of a special appendage, a sex pilus, on which conjugation and gene transfer depend (see Brinton, 1965; see Meynell, Meynell & Datta, 1968; Novotny, Raizen, Knight & Brinton, 1969). Conversely, donor ability is absent when, as a result of either repression or a defect in pilus synthesis, sex pili are not formed. Wild-type F is de-repressed because, although sensitive to repression, it produces no repressor (i^-o^+), and therefore has maximum donor ability. Mutant F factors donating at far lower rates can be selected by isolating F^+ or Hfr bacteria resistant to donor-specific phage (Cuzin, 1962; Cuzin & Jacob, 1965, 1967; Hirota, Fujii & Nishimura, 1966; Nishimura, Ishibashi, Meynell & Hirota, 1967). All such mutants are apparently still i^-o^+, judging from their inability to repress i^-o^+ F-like R factors (Meynell & Aufreiter, 1969); since they are resistant to donor-specific phages of all types and no sex pili are seen by electron microscopy, they are believed to be defective in sex pilus synthesis and will be termed spi^-.

Conjugation involves the transfer of genes linked to the sex factor and, occasionally, of other structures for which linkage, if it occurs, is not sufficiently stable to be

* Present address.

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observed. Examples of the latter are the proportion of chromosome transfers by an F^+ donor not accountable for by Hfrs (Curtiss & Stallions, 1969) and the transfer of one plasmid mediated by another, like that of certain Col factors mediated by F or ColI (Fredericq, 1956; Smith, Ozeki & Stocker, 1963). One way of examining whether linkage to the sex factor is essential for transfer is to introduce a functioning sex factor into a strain with a defective sex factor and to look for the type of transfer characteristic of the defective factor. In this way, a functional autonomous F factor was shown to restore Hfr donor behaviour to a strain with a defective integrated F (Cuzin & Jacob, 1965). Recovery of Hfr transfer could mean either that function had been restored to the defective factor by a process like complementation, or that the second sex factor had substituted for the defective one in bringing about conjugation (Clowes, 1963). In general, superinfection immunity creates difficulties in introducing a second F factor to a strain which already carries a defective F (Maas, 1963), although attempts have been made to overcome this difficulty by examining bacteria which have just received the superinfecting F before segregation can occur (Achtman, Willetts & Clark, 1968). F does not induce superinfection immunity to an F-like R factor, and Hirota *et al.* (1966) found that when the de-repressed R factor, R100-1, was introduced into each of 54 defective mutant Hfr strains, Hfr behaviour reappeared in every case. Similarly, defective mutants of R100-1 restored one another's transfer without genetic recombination. Further experiments by Nishimura and his colleagues (Nishimura *et al.* 1967) and by Ohtsubo & Nishimura, described in outline in Ohtsubo & Nishimura (1968), led these authors to conclude that restoration is due to complementation of function and that at least seven complementation groups are involved.

In the present experiments the ability of six de-repressed F-like R factors and four de-repressed I-like R factors to restore Hfr donor behaviour to 12 defective mutants of HfrCavalli and to 14 of HfrHayes was examined. The results indicate that this can occur in several ways.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

Two Hfr strains were used as donors: HfrC *met*$^-$*str*-s, which transfers the bacterial chromosome with *pro*$^+$ as an early marker, and HfrH *thi*$^-$*str*-s, where the origin is near *thr*$^+$*leu*$^+$ (see Taylor & Trotter, 1967). The recipient strains used were J6-2 *pro*A$^-$*trp*$^-$*his*$^-$*lac*$^-$*str*-r and PA309 *thr*$^-$*leu*$^-$*thi*$^-$*trp*$^-$*his*$^-$*argH*$^-$*lac*$^-$*str*-r, both of which were F^-R^-. The chromosomal locations of the genetic markers are shown in Fig. 1. Mutants of the Hfr strains defective in F pilus formation (*spi*$^-$) were obtained by isolating colonies resistant to the donor-specific phage MS2; 12 mutants of HfrC and 14 of HfrH were individually derived from cultures inoculated with different phage-sensitive colonies of the parent Hfr strain to ensure that each resulted from a separate mutation. Stable phage-resistant clones were tested for the continued presence of the integrated F by their ability to give a low frequency (10^{-6}–10^{-7}) of recombinants for the leading marker, *pro* in the case of HfrC and

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thr leu in the case of HfrH. The more numerous phage-resistant clones with a higher recombination rate (*c.* 10^{-4}) were discarded.

(ii) *R factors*

(*a*) *F-like* (*fi*$^+$) *factors*

These were de-repressed mutants, R1*drd*19 and R1*drd*16 of R1 (KmCmApSmSu) R136*drd*M1 and R136*drd*H8 of R136 (TcSu); R192*drd*F7 of R192 (TcCmSmSu); and R538-1*drd* of 538-1 (CmSmSu).

(*b*) *I-like* (*fi*$^-$) *factors*

These were de-repressed mutants R64*drd*11 of R64 (TcSmSu); R144*drd*3 of R144 (KmcolI); R163*drd*E6 of R163 (TcKmcolI); and R538-2*drd* of R538-2 (TcSm). Each mutant R factor, which is referred to by its isolation number (see Meynell, Meynell & Datta, 1968; Romero & Meynell, 1969), determined the constitutive production of sex pili with consequent sensitization of the culture to lysis by the appropriate F or I donor-specific phage and a high rate of resistance transfer by conjugation ($> 10^{-1}$). It was introduced into the defective Hfr strains by conjugational transfer from a *thr*$^-$*leu*$^-$*thi*$^-$*lac*$^-$ donor strain, with selection for *thr*$^+$*leu*$^+$*lac*$^+$R$^+$ colonies.

(iii) *Donor-specific phages*

Phage MS2 attaches to F pili and to the pili of F-like R factors and produces visible lysis of cultures carrying de-repressed F or F-like sex factors. Phage If1 attaches to I pili and thus specifically lyses cultures with de-repressed I-like sex factors (see Meynell *et al.* 1968).

For mating, overnight cultures of the R$^+$ derivatives of the defective (*spi*$^-$) Hfr strains in Oxoid no. 2 broth were diluted 1/50 in the same medium and incubated at 37° with slow shaking for 2–3 hr until the bacterial concentration had reached about 2×10^8/ml. Cultures carrying de-repressed R factors tend to clump, but, when this occurred, the bacteria were dispersed by a short period in the refrigerator (T. Bickle, personal communication) or by vigorous agitation with a 'Rotamixer' (Hook & Tucker Ltd). The mating mixture consisted of 0·5 ml of donor culture and 4·5 ml of an 18–20 hr broth culture of the recipient which was slowly rotated on an inclined turntable at 37° for 30 min. At the end of the 30 min period the mixture was shaken on the 'Rotamixer' to separate mating pairs and 0·1 ml volumes of the undiluted mixture and of tenfold dilutions in phosphate buffer were plated in 2·5 ml volumes of 0·6 % water agar on minimal medium supplemented to select different classes of recombinants and containing 200 μg/ml streptomycin to kill the donor strain. Bacteria carrying an R factor conferring resistance to low concentrations of streptomycin frequently give rise to variants with a high level of resistance (Pearce & Meynell, 1968*a*), but control experiments with agar containing 2000–5000 μg/ml streptomycin showed that the routine use of 200 μg/ml was adequate for the present experiments. In every experiment, the donor culture was tested for susceptibility to the appropriate donor-specific

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phage on nutrient agar (Oxoid Blood Agar Base), as a control on the production of sex pili and their type, and to phage T3 to show that F was still present (Schell *et al.* 1963). In addition, in many experiments, the occurrence of a high rate of R factor transfer was established by plating on drug-containing medium fully supplemented for growth of the recipient strain. Most crosses were repeated at least three times with essentially similar results. In a number of cases a particular R factor was introduced several times into the same Hfr mutant, and in every case each R^+ isolate of the same mutant behaved in the same way.

3. RESULTS

Whether or not de-repressed R factors restore the function of an integrated but defective F, the R^+ strain will be a chromosomal donor, because a de-repressed R factor produces as many recombinants in an F^- strain as does the F factor in an

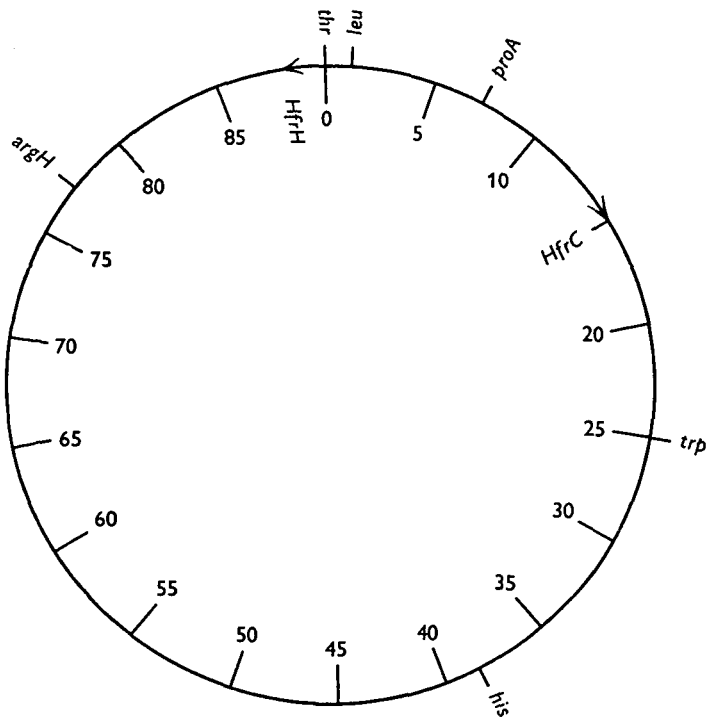

Fig. 1. Genetic map of *Escherichia coli* K12 with the positions of the genes referred to.

F^+ strain (Cooke & Meynell, 1969). But whereas the R factor, being autonomous, gives approximately the same number of recombinants for all genes wherever they are located on the bacterial chromosome, an integrated F factor produces oriented transfer of the chromosome starting at its site of integration, so that there are many more recombinants for proximal than for distal markers (see Jacob & Wollman, 1961). In examining the ability of an R factor to restore defective *spi*$^-$ Hfr

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strains, one must therefore look not only for a high frequency of recombinants for proximal genes (Tables 1 and 2, lines *a*) but also for a frequency greater than that of recombinants for genes located further from the origin (Tables 1 and 2, lines *b*). The latter will occur at the same frequency as given by an F^-R^+ donor, because the autonomous R factor itself brings about chromosome transfer. On the other hand, when there is no restoration of Hfr behaviour, recombinants for all markers, whether proximal or distal, should be produced at the lower frequency.

In the present experiments, the numbers of recombinants for the proximal Hfr markers, *pro* in the case of HfrC and *thr leu* in the case of HfrH, were compared with those for markers like *his* and *trp* distal from the origin (see Fig. 1). Only 30 min was allowed for mating in order to accentuate polarized transfer, when it occurred, by preventing the entry of distal markers. With HfrC, *his* and *trp* enter at 66 and 70 min, and with HfrH, *trp*, *his* and *arg*H at 25, 39 and 78 min, respectively (see Taylor & Trotter, 1967). Under these conditions, normal HfrC gave pro^+ recombinants at a frequency of 3×10^{-2} per donor cell and the frequencies for *trp* and *his* relative to *pro* were 0·0008 and 0·0006 respectively. Similarly, HfrH gave recombinants for *thr leu* at a frequency of 9×10^{-2}, with relative frequencies for *trp*, *his* and *arg*H of 0·07, 0·0095 and 0·0008 respectively. There was no obvious difference between individual defective spi^- mutants when they did not carry an R factor: these produced recombinants for *pro*, in the case of HfrC, or *thr leu* in the case of HfrH, at frequencies of around 10^{-6}. The frequencies for other markers were lower, but were not reliable since they approached the back-mutation rates to prototrophy of the recipient strains.

F-like R factors. The results with the 12 spi^- mutants of HfrC carrying either R1*drd*19 or R136*drd*M1 are shown in Table 1. Hfr behaviour was restored to a considerable extent in mutants *spi*1, *spi*4, *spi*5, *spi*8, *spi*9, *spi*10 and *spi*12, since the recombination rate for *pro* had risen from around 10^{-6} to a figure which was, on the average, about 10 % of that found for the original Hfr, with relatively far lower rates for the distal markers, *trp* and *his*. Thus, polarized transfer had been restored. By the same argument, *spi*3 was only slightly restored, and *spi*2, *spi*6, *spi*7 and *spi*11 not at all. The greater numbers of recombinants for distal markers, compared to R^- normal HfrC, represent the direct contribution of the R factor to chromosomal transfer (Cooke & Meynell, 1969). The results with R1*drd*19 in certain of the spi^- mutants are complicated by the fact that R1 preferentially gives recombinants for the *trp* region (Pearce & Meynell, 1968*b*). The numbers of *trp* recombinants thus exceeded the figure for *pro* in mutants *spi*2 and *spi*6, which did not recover Hfr donor behaviour, and approached it in the restored mutants, *spi*1, *spi*3, *spi*4, *spi*5 and *spi*10. In mutants *spi*7, *spi*8, *spi*9, *spi*11 and *spi*12, variant R1*drd*19 factors were used which had lost the ability to give an excess of this class of recombinant (Cooke, unpublished experiments).

None of the spi^- Hfr mutants was restored by the remaining de-repressed F-like R factors, R1*drd*16, R136*drd*H8, R538-1*drd* or R192*drd*F7 (see Meynell *et al.* 1968). The reason became clear when these R factor mutants were examined in normal, instead of spi^-, HfrC. Tests in these normal strains showed that, in becoming

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Table 1. *Frequencies of different classes of recombinants produced by defective mutants of HfrC carrying de-repressed R factors in crosses with J6-2*

R factors ...	F-like *i*⁻*o*⁺		I-like			
	R1*drd*19	R136*drd*M1	R64*drd*11	R144*drd*3	R163*drd*E6	R538-2*drd*
			HfrC*spi*1			
(*a*) *pro**	$2{\cdot}8\times10^{-3}$	$2{\cdot}9\times10^{-3}$	$8{\cdot}5\times10^{-3}$	$1{\cdot}7\times10^{-5}$	$1{\cdot}5\times10^{-5}$	$6{\cdot}1\times10^{-3}$
(*b*) *trp*†	0·25	0·012	0·012	0·41	1·3	0·01
(*b*) *his*†‡	< 0·001	0·007	0·004	1·0	0·2	0·0005
			HfrC*spi*2			
(*a*) *pro*	$3{\cdot}9\times10^{-5}$	$3{\cdot}8\times10^{-6}$	$1{\cdot}0\times10^{-2}$	$1{\cdot}5\times10^{-4}$	$5{\cdot}8\times10^{-5}$	$5{\cdot}3\times10^{-3}$
(*b*) *trp*	17·0	11·1	0·2	1·4	1·9	0·015
(*b*) *his*	< 0·04	1·7	0·008	0·19	0·22	0·002
			HfrC*spi*3			
(*a*) *pro*	$3{\cdot}3\times10^{-4}$	$4{\cdot}3\times10^{-5}$	$8{\cdot}3\times10^{-3}$	$2{\cdot}1\times10^{-4}$	$4{\cdot}2\times10^{-5}$	$6{\cdot}3\times10^{-3}$
(*b*) *trp*	0·8	0·91	0·017	0·81	1·6	0·016
(*b*) *his*	0·005	0·13	0·0028	0·83	0·1	0·0005
			HfrC*spi*4			
(*a*) *pro*	$2{\cdot}4\times10^{-2}$	$4{\cdot}2\times10^{-3}$	$6{\cdot}2\times10^{-3}$	$2{\cdot}2\times10^{-4}$	$4{\cdot}3\times10^{-5}$	$4{\cdot}5\times10^{-3}$
(*b*) *trp*	0·22	0·004	0·03	1·6	1·1	0·05
(*b*) *his*	0·014	0·003	0·005	0·14	0·47	0·05
			HfrC*spi*5			
(*a*) *pro*	$7{\cdot}6\times10^{-3}$	$2{\cdot}9\times10^{-3}$	$5{\cdot}6\times10^{-3}$	$2{\cdot}7\times10^{-4}$	$1{\cdot}2\times10^{-5}$	$5{\cdot}8\times10^{-3}$
(*b*) *trp*	0·14	0·012	0·025	0·93	1·2	0·014
(*b*) *his*	0·002	0·0045	0·006	0·02	0·64	0·002
			HfrC*spi*6			
(*a*) *pro*	$1{\cdot}3\times10^{-5}$	$7{\cdot}3\times10^{-6}$	$1{\cdot}3\times10^{-2}$	$4{\cdot}7\times10^{-4}$	$1{\cdot}1\times10^{-5}$	$6{\cdot}4\times10^{-3}$
(*b*) *trp*	17·7	4·1	0·1	0·8	0·9	0·01
(*b*) *his*	< 0·2	3·2	0·009	0·07	0·9	0·001
			HfrC*spi*7			
(*a*) *pro*	$2{\cdot}0\times10^{-5}$	$5{\cdot}0\times10^{-6}$	$2{\cdot}1\times10^{-2}$	—	—	$1{\cdot}2\times10^{-2}$
(*b*) *trp*	0·34	4·2	0·028	—	—	0·009
(*b*) *his*	0·024	0·92	0·0057	—	—	0·007
			HfrC*spi*8			
(*a*) *pro*	$1{\cdot}2\times10^{-3}$	$3{\cdot}1\times10^{-3}$	$2{\cdot}3\times10^{-2}$	—	—	$1{\cdot}2\times10^{-2}$
(*b*) *trp*	0·037	0·014	0·032	—	—	0·013
(*b*) *his*	0·026	0·003	0·005	—	—	0·005
			HfrC*spi*9			
(*a*) *pro*	$1{\cdot}4\times10^{-3}$	$4{\cdot}9\times10^{-4}$	$2{\cdot}0\times10^{-2}$	—	—	$1{\cdot}5\times10^{-2}$
(*b*) *trp*	0·02	0·065	0·038	—	—	0·016
(*b*) *his*	0·005	0·0073	0·008	—	—	0·009
			HfrC*spi*10			
(*a*) *pro*	$4{\cdot}6\times10^{-3}$	$1{\cdot}6\times10^{-3}$	$2{\cdot}6\times10^{-2}$	—	—	$1{\cdot}4\times10^{2}$
(*b*) *trp*	0·14	0·01	0·03	—	—	0·009
(*b*) *his*	0·012	0·005	0·004	—	—	0·006
			HfrC*spi*11			
(*a*) *pro*	$1{\cdot}7\times10^{-5}$	$6{\cdot}3\times10^{-6}$	$1{\cdot}6\times10^{-2}$	—	—	$1{\cdot}2\times10^{-2}$
(*b*) *trp*	0·37	3·7	0·03	—	—	0·013
(*b*) *his*	0·19	0·95	0·002	—	—	0·005
			HfrC*spi*12			
(*a*) *pro*	$2{\cdot}0\times10^{-3}$	$1{\cdot}9\times10^{-3}$	$1{\cdot}7\times10^{-2}$	—	—	$1{\cdot}4\times10^{-2}$
(*b*) *trp*	0·018	0·01	0·02	—	—	0·014
(*b*) *his*	0·004	0·005	0·005	—	—	0·007

* (*a*) *pro*: no. of *pro*⁺ recombinants/viable count of donor strain in mating mixture.

† (*b*) *trp*, *his*: no. of *trp*⁺ or *his*⁺ recombinants/no. of *pro*⁺ recombinants.

‡ Only a proportion of *his*⁺ recombinants produced with the recipient strain J6-2 developed into colonies when plated in an undiluted mating mixture; thus, when the number was low, it appeared to be even lower than it actually was. For this reason, only counts from dilutions which control experiments showed to be reliable have been recorded and where these produced no colonies, the frequency is expressed as < the figure for one colony.

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Table 2. *Frequencies of different classes of recombinants produced by defective mutants of HfrH carrying de-repressed R factors in crosses with PA 309*

R factors ...	F-like *i*⁻*o*⁺		I-like			
	R1*drd*19	R136*drd*M1	R64*drd*11	R144*drd*3	R163*drd*E6	R538-2*drd*
			HfrH*spi*1			
(*a*) *thr leu**	$3{\cdot}9\times10^{-5}$	$7{\cdot}8\times10^{-6}$	$2{\cdot}0\times10^{-2}$	$6{\cdot}1\times10^{-4}$	$3{\cdot}1\times10^{-5}$	$2{\cdot}1\times10^{-3}$
(*b*) *trp*†	11	0·5	0·006	0·1	0·84	0·006
(*b*) *his*†	< 0·01	0·46	0·002	0·03	0·06	< 0·001
(*b*) *arg*†	0·12	0·11	0·006	0·95	0·52	< 0·0001
			HfrH*spi*2			
(*a*) *thr leu*	$5{\cdot}5\times10^{-5}$	$1{\cdot}0\times10^{-5}$	$4{\cdot}0\times10^{-2}$	$8{\cdot}1\times10^{-4}$	$3{\cdot}6\times10^{-4}$	$1{\cdot}9\times10^{-3}$
(*b*) *trp*	7·1	1·25	0·002	0·06	0·14	0·02
(*b*) *his*	0·03	0·63	0·0008	0·02	0·04	0·004
(*b*) *arg*	0·14	0·04	0·003	0·93	0·67	0·004
			HfrH*spi*3			
(*a*) *thr leu*	$1{\cdot}1\times10^{-5}$	$9{\cdot}3\times10^{-6}$	$2{\cdot}0\times10^{-2}$	$3{\cdot}8\times10^{-4}$	$7{\cdot}6\times10^{-5}$	$1{\cdot}9\times10^{-3}$
(*b*) *trp*	18·2	0·77	0·007	0·11	0·14	0·014
(*b*) *his*	0·08	0·28	0·002	0·01	0·14	0·014
(*b*) *arg*	0·08	0·02	0·009	1·2	0·29	0·01
			HfrH*spi*4			
(*a*) *thr leu*	$2{\cdot}1\times10^{-5}$	$1{\cdot}3\times10^{-5}$	$2{\cdot}3\times10^{-2}$	$3{\cdot}1\times10^{-4}$	$2{\cdot}3\times10^{-4}$	$2{\cdot}3\times10^{-3}$
(*b*) *trp*	33	1·3	0·006	0·08	0·19	0·024
(*b*) *his*	0·05	0·41	0·002	0·32	0·07	0·005
(*b*) *arg*	0·25	< 0·02	0·004	1·6	0·52	0·005
			HfrH*spi*5			
(*a*) *thr leu*	$3{\cdot}4\times10^{-2}$	$8{\cdot}8\times10^{-3}$	$1{\cdot}0\times10^{-2}$	$1{\cdot}3\times10^{-4}$	$5{\cdot}0\times10^{-5}$	$2{\cdot}2\times10^{-3}$
(*b*) *trp*	0·04	0·04	0·01	0·3	0·34	0·02
(*b*) *his*	0·001	0·007	0·002	0·06	0·09	0·004
(*b*) *arg*	0·0006	< 0·0002	0·01	4·3	1·5	0·004
			HfrH*spi*6			
(*a*) *thr leu*	$3{\cdot}5\times10^{-2}$	$1{\cdot}7\times10^{-2}$	$1{\cdot}1\times10^{-2}$	$2{\cdot}5\times10^{-4}$	$8{\cdot}8\times10^{-5}$	$2{\cdot}3\times10^{-3}$
(*b*) *trp*	0·05	0·01	0·01	0·06	0·3	0·024
(*b*) *his*	0·002	0·004	0·003	0·01	0·07	< 0·002
(*b*) *arg*	0·0005	0·00005	0·009	2·0	1·5	0·002
			HfrH*spi*7			
(*a*) *thr leu*	$2{\cdot}3\times10^{-5}$	$1{\cdot}3\times10^{-5}$	$3{\cdot}9\times10^{-2}$	$4{\cdot}0\times10^{-4}$	$1{\cdot}3\times10^{-4}$	$1{\cdot}5\times10^{-3}$
(*b*) *trp*	6·1	1·6	0·08	0·21	0·45	0·18
(*b*) *his*	0·03	1·1	0·005	0·03	0·07	0·01
(*b*) *arg*	0·23	0·045	0·003	0·95	0·32	0·002
			HfrH*spi*8			
(*a*) *thr leu*	$1{\cdot}3\times10^{-5}$	$9{\cdot}5\times10^{-6}$	$1{\cdot}8\times10^{-2}$	$8{\cdot}0\times10^{-4}$	$1{\cdot}0\times10^{-4}$	$1{\cdot}9\times10^{-3}$
(*b*) *trp*	10·0	2·5	0·006	0·03	0·11	0·016
(*b*) *his*	0·1	0·55	0·0007	0·008	0·09	0·008
(*b*) *arg*	0·2	0·13	0·004	0·7	0·46	0·008

* (*a*) *thr leu*: no. of *thr*⁺ *leu*⁺ recombinants/viable count of donor strain in mating mixture.

† (*b*) *trp*, *his*, *arg*: no. of *trp*⁺, *his*⁺ or *arg*⁺ recombinants/no. of *thr*⁺*leu*⁺ recombinants.

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de-repressed, the R factors had mutated to insensitivity to repressor, although they continued to produce repressor acting on the integrated F factor, whose function was required for Hfr transfer to occur (Meynell & Cooke, 1969; Frydman & Meynell, 1969). Any restoration by these R factors would therefore be largely cancelled by concomitant repression of F. The subsequent isolation of repressor-insensitive (o^c) mutants of F (Frydman *et al.* 1970) now allows all de-repressed R factors, whether or not they still produce repressor, to be tested. In isolating de-repressed R factors, the type of mutant obtained evidently depends on the method of selection. Direct selection for high-frequency transfer of drug-resistance has so far yielded only mutants which are repressor-insensitive (o^c), whereas mutants (i^-) which no longer produce repressor have been found by selecting for expression of F in a bacterium carrying both F and R (Egawa & Hirota, 1962). R1*drd*19, which restored Hfr behaviour, is i^- (Meynell & Cooke, 1969; Frydman & Meynell, 1969) and was selected for promotion of chromosome transfer by the remnant of F in the Richter ♀$_3$ strain (Meynell & Datta, 1967). R136*drd*M1 was isolated in the same way and also confirmed as i^- by the tests used with R1*drd*19.

Recombinant frequencies for eight defective mutants of HfrH are shown in Table 2. The two i^- F-like factors, R1*drd*19 and R136*drd*M1, restored Hfr donor behaviour to *spi*5 and *spi*6 and to none of the others. The numbers of recombinants for *thr leu* in the restored mutants were about 30 % of those obtained with the original Hfr. Of the remaining mutants, HfrH *spi*9–*spi*14, Hfr behaviour was restored in *spi*10, *spi*13 and *spi*14, but not in *spi*9, *spi*11 or *spi*12. Crosses with the repressor-positive F-like R factors are omitted, for the same reason as with the defective mutants of HfrC. Except for certain features of R538-1*drd*, referred to in the Discussion, there was no esssential difference between the recombinant frequencies for these R factors in the Hfr mutants and in F^- strains (Cooke & Meynell, 1969). The frequencies for R192*drd*F7 were in general somewhat lower in the former, but this was almost certainly the result of allowing only 30 min for mating instead of the 1 hr used by Cooke & Meynell (1969).

I-like R factors. The striking features of the results with the four I-like R factors were: first, that R64*drd*11 and R538-2*drd*, but not R144*drd*3 and R163*drd*E6, restored Hfr behaviour; and, second, that restoration with R64*drd*11 and R538-2*drd* occurred with every one of the mutants, HfrC *spi*1–*spi*12 (Table 1); HfrH *spi*1–*spi*8 (Table 2); and HfrH *spi*9–*spi*14 (not included in the tables).

Nature of sex pili

(*a*) *The F-like R factors, R1*drd*19 and R136*drd*M1*

F pili can be distinguished from the F-like R pili determined by R1*drd*19 or R136*drd*M1 by the antigenic structure of the pilin molecules of which they are composed, using cross-absorbed antisera to label the pili, which are then examined by electron microscopy (Lawn & Meynell, 1970). When the R^+ derivatives of the *spi*$^-$ Hfr mutants were examined in this way, recovery of Hfr transfer was evidently correlated with reappearance of F antigen, for sex pili reacting with specific

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anti-F serum were present in all but one of the restored mutants and could not be detected in any of the mutants that were not restored. Bacteria in which both F and F-like R factor pilins are synthesized as a result of the presence of a functional F factor and of an R factor produce sex pili which are mixtures of the two kinds of pilin molecule (Lawn, Meynell & Cooke, 1970). The pili produced by most of the restored R$^+$ *spi*$^-$ Hfr mutants had essentially the same appearance as the pili of normal Hfr strains carrying the R factor; the proportions of F and R antigens also appeared the same although the method does not allow the proportions of F antigen to be compared with any precision when it may be scattered throughout the pili or concentrated in denser patches. There was one exceptional mutant, HfrH*spi*5, which after restoration by either R1*drd*19 or R136*drd*M1 produced sex pili reacting only with R, not with F, antiserum. In addition, the reaction with F antiserum of HfrC*spi*9 was significantly less than that of the other restored strains.

(*b*) *The I-like R factors, R64*drd*11 and R538-2*drd

F-like and I-like sex pili are distinct from one another as shown by serological reactions and reactions with donor-specific phages (Lawn *et al.* 1967). In contrast to normal Hfr strains carrying I-like sex factors, where the production of both F and I-like sex pili by the bacteria can be readily observed, the *spi*$^-$ Hfr mutants carrying R64*drd*11 of R538-2*drd* produced only I-like sex pili. Cultures were lysed by I-specific phage If1, but not by F-specific phage MS 2. Five mutants, HfrC*spi*4, HfrC*spi*10, HfrC*spi*12, HfrH*spi*13 and HfrH*spi*14, were examined by electron microscopy. Although they produced abundant I-like pili, no F pili were detectable by labelling with either F-specific antiserum or particles of F-specific isometric phage MS2.

4. DISCUSSION

It is evident that Hfr chromosome transfer cannot be simply restored in *spi*$^-$ Hfr mutants by any functional sex factor, since Hfr behaviour was restored with only certain of the R factors, and with some of these, only by certain of the mutants. Thus, the local change in the chromosome produced by integration of F does not automatically lead to Hfr transfer when conjugation occurs. This agrees with the observation that a normal Hfr strain does not act as an Hfr donor when the integrated F is repressed by an i^+o^c R factor which, being itself de-repressed, is highly efficient in bringing about conjugation (Meynell & Cooke, 1969).

The contrast between the two F-like R factors, R1*drd*19 and R136*drd*M1, which restored Hfr transfer in only 12 of the 26 *spi*$^-$ mutants, and the two I-like factors, R64*drd*11 and R538-2*drd*, which restored them all, suggests that restoration comes about in a fundamentally different way in each case.

With the F-like R factors, the function missing in the 12 mutants susceptible to restoration was proably one shared by F and the related R factor, so that the R factor could thus supply the product of the gene mutated in F, as occurs in complementation. The missing function was, indeed, restored, as shown by the presence of pili reacting with F-specific antiserum in all but one of the restored mutants.

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The absence of a reaction with this one mutant could have been due either to the total absence of F pilin, or its presence in amounts indetectable by the present method: a few discrete molecules of F antigen in predominantly R pili might fail to react to an appreciable extent. In contrast, the consistently negative reactions of the non-restored mutants indicated strongly that these belonged to a separate class in which F antigen was, in fact, absent.

There are two ways in which the R factors might have led to reappearance of sex pili containing F antigen: by restoring a defect either in the synthesis of F pilin or in the processes leading to the assembly of the pilus. F pilin molecules co-polymerize with pilin molecules determined by an F-like R factor to form mixed sex pili (Lawn *et al.* 1970). In this way they resemble two antigenic types of flagellin synthesized simultaneously, which give rise to antigenically mixed flagella (Pearce & Stocker, 1967). If sex pili are formed by spontaneous assembly of subunits after the provision of an initiation mechanism as occurs with flagella (see Iino, 1969; Kushner, 1969), it could be this mechanism that was missing in the mutated F factor and which the R factor could replace. In such cases, when the R factor was merely replacing a defect of this kind in pilus assembly, the sex pili of each mutant restored by the same R factor might be expected to contain the same amount of F pilin as when wild-type F was present. In contrast, defects at different stages in the synthesis of F pilin might be restored by a given R factor, but with variable efficiency, so that the F pilin in some mutants was less than in others.

The finding that R1*drd*19 and R136*drd*M1 did not restore 14 of the 26 mutants implies that some functions are not sufficiently alike in F and F-like R factors to substitute for one another. An alternative, but unlikely, possibility is that, although F was still present in these mutants, as shown by a low efficiency of plating of phage T3 (Schell *et al.* 1963), it had been lost from its original site of integration in the chromosome during infection either with R1*drd*19 or with R136*drd*M1. This was, however, directly disproved by making use of the fact that Hfr donor behaviour could always be restored upon infection with R64*drd*11. In effect, when HfrC*spi*3 (R136*drd*M1), HfrC*spi*2 (R1*drd*19) and HfrH*spi*1 (R1*drd*19) were tested by superinfecting with R64*drd*11 the result was the expected Hfr transfer.

The striking features of the I-like R factors, in contrast to the F-like, were, first, that a given R factor behaved in the same way with every one of the Hfr mutants. Thus, restoration of Hfr transfer did not depend on the particular mutational defect as it did with the F-like factors. Secondly, efficient recovery of Hfr donor behaviour occurred without the production of F pili. It therefore appeared that, in restoring Hfr donor behaviour, the I-like factors, R64*drd*11 and R538-2*drd*, did not act by complementing the defective F. A similar absence of complementation was shown for the ColI factor in another defective mutant of HfrC (Clowes, 1963). The explanation originally proposed for HfrC*spi*4 (R64*drd*11) (see Meynell *et al.* (1968), where it is referred to as HfrCdr) was that recovery of Hfr transfer was due to frequent recombination between this R factor and the integrated F, leading to insertion of the R factor within F at its chromosomal site. The origin of transfer

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belonging properly to R64*drd*11 would then bear essentially the same relation to the chromosome as that of the originally functional F and could substitute for it in bringing about Hfr transfer. Such an explanation, based on physical, rather than on functional, interaction between the two sex factors would presuppose that, provided there were no gross changes in the DNA, mutants in which a variety of different functions were affected would be equally likely to be restored. It could be proved by a direct demonstration of frequent physical union between the genomes of R64*drd*11, or R538-2*drd*, and F.

The gene *arg*H is close to the integrated F factor of HfrH in such a way that it is one of the last markers to be transferred. A curious feature of some of the R factors which did not restore Hfr transfer was that in many crosses there were nearly as many recombinants for this distal marker as for the proximal markers, *thr leu*, and many more than for *his* or *trp*, which are at intermediate positions in the HfrH chromosome. This was especially noticeable with the I-like factor, R144*drd*3 (Table 2) and the repressor-positive F-like factor, R538-1*drd*. If R64*drd*11 restores Hfr transfer by recombining with the integrated F factor, the other R factors may do the same, but with these differences: first, recombination evidently occurs less often; and secondly, when recombination occurs, the R factor can be inserted in either direction. Such a situation has already been recognized in a chromosomal *sfa* locus for ColV, consisting of a retained fragment of a once-integrated ColV factor. Here, the reintroduction of ColV into the strain gives two kinds of stable Hfr, which transfer the chromosome from this region in exactly opposite directions; or, when ColV is autonomous, to a relatively high frequency of recombinants on either side of the *sfa* region (Kahn, 1969).

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