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Repressor-minus and operator-constitutive de-repressed mutants of F-like R factors: their effect on chromosomal transfer by HfrC

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1. INTRODUCTION

Those R factors classed as *fi*$^+$ for 'fertility inhibition', because they reduce genetic transfer mediated by F (see Watanabe, 1963), repress the formation of F pili necessary for conjugation. An F$^+$ donor carrying such an R factor no longer transfers its autonomous F factor nor produces genetic recombinants at its accustomed rate; with an Hfr strain, recombinants for the leading chromosomal markers are reduced 100-fold or more (Egawa & Hirota, 1962; Watanabe & Fukasawa, 1962). These R factors themselves determine sex pili structurally resembling the F pilus, and their effect on F is probably due to the repressor responsible for the low frequency with which they themselves bring about conjugation (see Meynell, Meynell & Datta, 1968). De-repressed mutants of R factors can be isolated with which conjugation and pilus production are expressed in virtually every cell (Meynell & Datta, 1967); if these mutants had lost the ability to produce repressor, then F should function normally in an F$^+$R$^+$ cell, just as in the absence of the R factor (Egawa & Hirota, 1962). Thus, in an Hfr strain, where F and chromosome form a single continuous structure, a normal rate of transfer of the leading chromosomal markers might imply that, on de-repression of the R factor, recombination mediated by F was once more in operation and that F was therefore no longer repressed. On the other hand, a continuing low rate of transfer for the leading chromosomal marker would indicate that F was still repressed and that despite the high-frequency conjugation brought about by the de-repressed R factor, the oriented transfer characteristic of the Hfr strain was still impaired. That is, in chromosomal transfer by an Hfr donor, conjugation and gene transfer form a co-ordinated system. The effects of de-repressed mutant R factors on strain HfrC have accordingly been examined, by comparing the numbers of recombinants for markers near the Hfr origin and their frequencies relative to other markers generally.

2. METHODS

R$^+$HfrC donor strains. Wild type and de-repressed mutants of the *fi*$^+$R factors, R1 (KmCmApSmSu), R136(TcSu), R192(TcCmSmSu) and R538–1(CmSmSu), and *fi*$^-$ R factors, R64(TcSm), R144(Km*col*I), R163(TcKm*col*I) and R538-2(TcSm),

were transferred from an F⁻ host requiring threonine, leucine and thiamine to strain HfrC*met*⁻*str*ˢ, using for selection a minimal salts glucose medium supplemented with methionine and containing chloramphenicol (20 μg/ml), kanamycin (20 μg/ml) or tetracycline (10 μg/ml), depending on the particular R factor.

The media, conditions of mating and recipient strains, J62 *pro*⁻*his*⁻*trp*⁻*str*ʳ and PA309*thr*⁻*leu*⁻*thi*⁻*arg*⁻*his*⁻*trp*⁻*xyl*⁻*str*ʳ, were the same as used in examining R⁺F⁻ donor strains (Cooke & Meynell, 1969), with a similar mating period of 1 h to allow comparisons to be made between the two kinds of donor. Each cross was repeated several times and experiments typical of the behaviour of each R factor are given in Table 1.

3. RESULTS AND DISCUSSION

The Hfr strain, HfrC, transfers the bacterial chromosome clockwise from a point about 2 min before *tsx*, resulting in the production of many recombinants for *pro* or for *thr leu*, with relatively fewer for genes situated further from the origin (Taylor & Trotter, 1967). This is illustrated in the first two lines of Table 1, which give figures for the frequency of *pro*⁺ and *thr*⁺*leu*⁺ recombinants together with the relative frequencies of those for other markers. When strain HfrC carries a wild-type *fi*⁻R factor, its characteristic donor behaviour is not appreciably altered, as is seen with R64, R144, R163 and R538-2. However, when the *fi*⁻ factor is a de-repressed mutant, such as R64*drd*11, R144*drd*3, R163*drd*E6 or R538-2*drd*, the R factor itself determines the production of recombinants at about the same rate and of the same variety as in an F⁻ donor strain; thus contributing sufficient recombinants for all chromosomal regions to increase the ratio for distal to proximal markers (Table 1).

It is against the background of these results with *fi*⁻R factors that the behaviour of the *fi*⁺ factors and their de-repressed mutants can be analysed (Table 1). When a wild-type *fi*⁺R factor is carried by HfrC, the total number of recombinants for all markers is reduced but the ratios of one class to another are largely unchanged. The general effect is simply that of a depressed rate of transfer of the HfrC chromosome, whose polarity is preserved (Egawa & Hirota, 1962). R1*drd*14, R1*drd*19, R1*drd*10 and R1*drd*16 are independent de-repressed mutants of the *fi*⁺ factor, R1, and it may be seen that, like the derepressed mutant, R100-1 reported by Sugino & Hirota (1962), the presence of R1*drd*19 in HfrC did not significantly decrease the frequency of *pro*⁺ recombinants. The relatively large numbers of recombinants for *trp* are due to R1 preferentially transferring this region of the chromosome (Pearce & Meynell, 1968). R1*drd*14 behaved in the same way. However, the characteristic features of an Hfr donor were lost when HfrC carried either R1*drd*10, R1*drd*16, R136*drd*H8, R192*drd*F7 or R538-1*drd*. The numbers of recombinants for the leading HfrC genes, *pro* or *thr leu*, were reduced to about the same level as with the wild-type R factors, while recombinants for other markers were substantially increased, just as with a de-repressed *fi*⁻ factor. Polarity of transfer was thus largely undetectable and the strain superficially resembled an F⁻R⁺ donor (Cooke & Meynell, 1969). This suggested that polarized transfer de-

pended directly on the functioning of the integrated F, which continued to be repressed with R1*drd*10, R1*drd*16, R136*drd*H8, R192*drd*F7 and R538-1*drd*.

The difference between these mutants and R1*drd*14 or R1*drd*19 in their action on F was also apparent on examining the sex pili. Pili determined by R1, R136 and R192 can be distinguished from F pili by differences in antigenic structure, observed in the electron microscope (Lawn, 1967; Lawn & Meynell, in preparation). When the R+ derivatives of HfrC were examined, R pili were present in all the strains, but significant amounts of F antigen were present only in HfrC(R1*drd*14) or HfrC(R1*drd*19).

Table 1. *Effects of wild type and de-repressed R factors on polarity of chromosome transfer by HfrC*

R factor	Recipient	Frequency of recombinants* for *pro*	Frequency of recombinants* for *thr leu*	Recombinant ratios relative to *pro* or *thr leu* for *arg*	*xyl*	*his*	*trp*	HfrC chromosome: Transfer rate maintained	HfrC chromosome: Effect on polarity
None	J62	$2{\cdot}8 \times 10^{-2}$	—	—	—	0·00021	0·00021	—	—
	PA309	—	$1{\cdot}2 \times 10^{-2}$	0·033	0·0065	0·0023	0·0033	—	—
				fi− R factors					
R64	J62	$9{\cdot}5 \times 10^{-3}$	—	—	—	0·0008	0·0013	Yes	None
R64*drd*11	J62	$1{\cdot}6 \times 10^{-2}$	—	—	—	0·0078	0·007	Yes	Minimal
R144	J62	$1{\cdot}1 \times 10^{-2}$	—	—	—	0·00005	0·00069	Yes	None
R144*drd*3	J62	$8{\cdot}5 \times 10^{-3}$	—	—	—	0·0025	0·0087	Yes	Minimal
R163	J62	$1{\cdot}4 \times 10^{-2}$	—	—	—	0·0002	0·0007	Yes	None
R163*drd*E6	J62	$8{\cdot}3 \times 10^{-3}$	—	—	—	0·0016	0·0094	Yes	Minimal
	PA309	—	$4{\cdot}9 \times 10^{-3}$	0·037	0·024	0·00061	0·031	Yes	Minimal
R538-2	J62	$3{\cdot}9 \times 10^{-3}$	—	—	—	0·00015	0·00097	Yes	None
R538-2*drd*	J62	$2{\cdot}1 \times 10^{-2}$	—	—	—	0·019	0·021	Yes	Minimal
				fi+ R factors					
R1	J62	$2{\cdot}1 \times 10^{-6}$	—	—	—	< 0·15	0·35	No	None
R1*drd*19	J62	$1{\cdot}0 \times 10^{-2}$	—	—	—	0·0066	0·19	Yes	None
R1*drd*16	J62	$1{\cdot}5 \times 10^{-5}$	—	—	—	< 0·07	56·0	No	Extreme
R136	J62	$5{\cdot}4 \times 10^{-6}$	—	—	—	< 0·07	< 0·07	No	None
R136*drd*H8	J62	$4{\cdot}5 \times 10^{-5}$	—	—	—	0·17	0·29	No	Extreme
	PA309	—	$1{\cdot}8 \times 10^{-5}$	0·16	—	0·16	1·7	No	Extreme
R192	J62	$3{\cdot}6 \times 10^{-4}$	—	—	—	0·23	0·061	No	None
	PA309	—	$2{\cdot}1 \times 10^{-4}$	0·14	0·034	0·034	0·38	No	None
R192*drd*F7†	J62	$5{\cdot}1 \times 10^{-5}$	—	—	—	0·25	1·5	No	Extreme
	PA309	—	5×10^{-5}	0·22	0·13	0·14	7·6	No	Extreme
R538-1	J62	$1{\cdot}7 \times 10^{-4}$	—	—	—	0·0029	0·0029	No	None
R538-1*drd*	J62	$1{\cdot}9 \times 10^{-4}$	—	—	—	0·03	0·46	No	Extreme
	PA309	—	$8{\cdot}3 \times 10^{-6}$	0·35	—	0·42	1·6	No	Extreme

* Expressed as proportion of donor cells in mating mixture.

† The frequency of *pro*+ recombinants was consistently lower with the de-repressed mutant than with the wild type.

There are thus two categories of de-repressed mutant R factors: those which have also ceased, and those which continue, to repress F. The de-repressed mutant, R100-1, reported by Sugino & Hirota (1962), belongs, with R1*drd*14 and R1*drd*19, to the first category in allowing high-frequency polarized transfer of an Hfr chromosome; and F$^+$R100-1$^+$ bacteria produce F pili, distinguishable from R100-1 pili by their greater affinity for F-specific RNA phages (Nishimura, Ishibashi, Meynell & Hirota, 1967). One explanation for the continuing repression of F with R1*drd*10, R1*drd*16, R136*drd*H8, R192*drd*F7 and R538-1*drd* would be that gene expression in F and R factors was not, after all, controlled by the same repressor produced by the R factor. It is unlikely, however, because of the simultaneous de-repression of F and R with mutants such as R1*drd*14, R1*drd*19 and R100-1. Moreover, differences in rate of transfer between different wild type R factors are reflected in the extent to which each R factor is able to depress polarized transfer of the HfrC chromosome. For instance, HfrC produces more *pro*$^+$ recombinants with R538-1, which is itself transferred at a frequency of 10^{-2} (Romero & Meynell, 1969), than with R1, whose rate of transfer is nearer 10^{-4} (Datta, Lawn & Meynell, 1966). Similar differences exist between R136 and R192.

The most probable explanation is therefore that, with gene expression in R and F under the same control, repressor-minus (i^-) and operator-constitutive (o^c) mutations analogous to those in other systems (see Beckwith, 1967) lead to de-repression of the sex factor genes by affecting, in the one case, production of, and, in the other, susceptibility to, repressor. Thus, R1*drd*14 and R1*drd*19 would fail to produce repressor, while perhaps remaining sensitive to repression, and the remainder would be resistant, and continue to produce repressor as shown by their action on F. The absence of superinfection immunity between F-like R factors and the colicin factor, ColB-K98, allowed the two kinds of mutants to be tested for repression by ColB-K98, which produces repressor acting on F. In effect, when R1*drd*16, R136*drd*H8, R192*drd*F7, R538-1*drd* and R1*drd*19 were tested in this way (Frydman & Meynell, 1969), only R1*drd*19 was sensitive, as expected from the present findings.

SUMMARY

Certain *drd* mutants of *fi*$^+$R factors, when carried by strain HfrC, allowed polarized transfer of the Hfr chromosome to occur at the normal rate. These mutants were independently shown to be repressor-sensitive and so owed their de-repression to failure to produce repressor (i^-). With other *drd* mutants, independently shown to be repressor-insensitive (o^c), the rate of polarized chromosome transfer was as low as with the wild type and only R pili were produced by the HfrC$^+$ bacteria. These R factors, therefore, continued to produce repressor and the donor behaviour of an Hfr strain depends on functioning of the integrated F.

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