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Interactions between de-repressed F-like R factors and wild type Colicin B factors: superinfection immunity and repressor susceptibility

By ANNA FRYDMAN and ELINOR MEYNELL
Department of Microbiology, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1

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1. INTRODUCTION

Many types of transmissible plasmid can be defined according to the bacterial characters they determine, but the sex factors of the majority fall into only two classes, F-like and I-like, according to the structure of their sex pili (see Meynell, Meynell & Datta, 1968). The F-like class comprises F, ColV and ColB factors and about 50% of R factors (Lawn, Meynell, Meynell & Datta, 1967). Another criterion of relationship between two plasmids is their inability to replicate in the same bacterium, known as *superinfection immunity*, and, by this criterion, F and ColV are related to one another (Kahn & Helinski, 1964; Macfarren & Clowes, 1967) and differ both from F-like R factors (see Watanabe, 1963) and ColB factors (see Fredericq, 1957; Puig & Nagel de Zwaig, 1964; Hausmann & Clowes, 1968). If there were superinfection immunity between F-like R factors and ColB factors, this would indicate that they were related to one another, as is the case with I-like R factors and ColI (see Meynell *et al.* 1968).

F and ColV differ from other wild type plasmids in that their sex pili are formed continuously. R factors and ColB, however, determine the synthesis of a repressor which prevents pilus synthesis and limits the proportion of piliated cells to 1% or less of the population (see Meynell *et al.* 1968). This repressor is also active against F and ColV; thus, an F^+ culture carrying either an F-like R factor or ColB no longer has the high donor efficiency and F phage-sensitivity of one carrying F alone (the fi^+ character: see Watanabe, 1963; Puig & Nagel de Zwaig, 1964). Mutant R factors can be isolated in which production of the sex pilus is no longer repressed (Meynell & Datta, 1967). Theoretically, these should be of two kinds equivalent to the two recognized kinds of constitutive mutants in other systems, those not producing repressor and those insensitive to repression (see Beckwith, 1967). The two kinds may be distinguished by introducing the mutant factor into a bacterium together with a wild type sex factor producing repressor (cf. Jacob & Monod, 1961). Using a wild type R factor, it is possible to demonstrate the susceptibility of the F factor to repression, and thus the repressor-minus nature of its constitutive behaviour. However, tests with pairs of wild type and de-repressed R factors are hampered by superinfection immunity. If ColB factors and F-like R factors proved not to exhibit superinfection immunity, the mutations leading to

de-repression in de-repressed R factors could be examined by preparing R+ColB+ strains and seeing whether or not pilus synthesis directed by the R factor was repressed by ColB.

2. METHODS

Bacteria. These were derivatives of *Escherichia coli* K12: J53 (*pro⁻met⁻str*^{s}) was used as R factor donor; J62 (*pro⁻his⁻trp⁻lac⁻str*^{r}) with its ColB+ derivatives was used as recipient; and RC24 (*thr⁻leu⁻thi⁻lac⁻str*^{r}) made resistant to chloramphenicol, tetracycline or kanamycin by introduction of an appropriate wild type R factor, was used as colicin indicator.

ColB factors. ColB-K77, ColB-K98 and ColB-K166 (see Fredericq, 1957) were tested in J62 after transfer from their natural hosts, using streptomycin to eliminate the donor strain.

R factors. R1 (KmCmApSmSu), R136 (TcSu), R192 (TcCmSmSu) and R538-1 (CmSmSu) determine the production of an F-like sex pilus. Cultures carrying de-repressed (*drd*) mutants were lysed by phage MS2 on nutrient agar; the wild type factors were *fi*+.

Culture media. Nutrient broth was Oxoid broth no. 2 and nutrient agar was Oxoid blood agar base. Minimal salts medium appropriately supplemented with their individual requirements was used to distinguish the donor strain, J53, from the recipient, J62, in cases where this was necessary.

Transfer of de-repressed mutant R factors to ColB+ recipients. One part of the R+ donor, J53, freshly grown to 2–5 × 10^{8} bacteria/ml in broth at 37 °C, was mixed with nine parts of an overnight broth culture of the recipient, Col+ or Col⁻, J62. After 20 min at 37 °C, dilutions were plated in 2 ml volumes of 0·35 % nutrient agar overlay on nutrient agar plates containing 200 μg/ml streptomycin to eliminate the donor strain and either chloramphenicol 20 μg/ml, tetracycline 10 μg/ml or kanamycin 20 μg/ml for selection of recipients of R538-1*drd* or R192*drd*F7, R136*drd*H8 and R1*drd*16 or R1*drd*19, respectively.

The rate of transfer, expressed as number of R+ recipient colonies per R+ donor bacterium, was compared in parallel tests to Col+ and Col⁻ recipients.

Colicin production by R+ recipient colonies. Certain of the plates used for selection, as well as control plates of the recipient alone, were exposed to chloroform vapour to kill the bacterial colonies and then overlaid with a colicin B-sensitive indicator in a 0·35 % agar layer (see Fredericq, 1957). Since the plates for selection of the R factor contained antibiotic, it was necessary that the indicator bacteria should be antibiotic-resistant; thus, the streptomycin-resistant strain, 24, carrying the wild type R factor corresponding to the de-repressed mutant in the donor was used for this purpose.

Examination of clones derived from R+Col+ recipients. Colonies on plates which had not been chloroformed were inoculated as stabs into two nutrient agar plates, one to serve as a master and the other for colicinogeny testing after killing with chloroform. Clones which were thus seen to be colicinogenic were checked as being J62, and not J53, by their nutritional requirements; then subcultured in broth and streaked on nutrient agar plates without antibiotic to allow segregation of R factor

and Col factor. Generally, four or five but, in one case, 17, of the resultant colonies from each original R+Col+ clone were tested for four properties: antibiotic-resistance characteristic of the particular R factor and sensitivity to clearing by phage MS2; colicin production and phage restriction characteristic of the Col factor. The latter two were not observed to segregate from one another.

Table 1. *Col B factors and R factors*

Factors	Colicinogeny	Colicin B sensitivity	Restriction of: Phage BF23*	Restriction of: Phage W31†	Drug resistances conferred	Clearing by phage MS2
Col B-K77	+	−	−	0·5 with reduced plaque size	−	−
Col B-K98	+	−	10^{-7}	−	−	−
Col B-K166	+	−	10^{-6}	−	−	−
R1*drd*16	−	+	−	−	Km	+
R136*drd*H8	−	+	−	−	Tc Su	+
R192*drd*F7	−	+	−	−	Tc Cm Sm Su	+
R538-1*drd*	−	+	−	−	Cm Sm Su	+
R1*drd*19	−	+	−	−	Km Cm Ap Sm Su	+

* Strobel & Nomura (1966). † Watanabe & Okada (1964).

3. RESULTS AND DISCUSSION

(i) *Relationships between R factors and ColB factors*

When the four F-like R factors, R1*drd*16, R136*drd*H8, R192*drd*F7 and R538-1*drd*, were tested with the three ColB factors, B-K77, B-K98 and B-K166, it was apparent that neither the R factors nor the Col factors formed homogeneous groups. The rates of R factor transfer to Col+ relative to Col− recipients are shown in Table 2, together with the proportions of R+ recipient colonies which continued to produce colicin. Table 3 contains the results of tests for the continuing presence of both R and Col after a period of cultivation in antibiotic-free medium to allow segregation: the outcome of all these experiments is summarized in Table 4. All colonies on the primary selection plates were colicinogenic with the exception of R538-1*drd* transferred to a recipient with ColB-K77. None of the R factors was inherited stably with ColB-K77, to which they were therefore all related by superinfection immunity. A similar relationship between ColB-K77 and other F-like R factors has also been reported by Clowes, Hausmann, Nisioka & Mitani (1968). With R1*drd*16 and R538-1*drd*, and to a lesser extent with R192*drd*F7, the R factor tended to displace the ColB-K77 factor, but with R136*drd*H8 it was usually the R factor that was rejected; after transfer of R538-1*drd*, the few colonies that remained colicinogenic gave rise to about equal numbers of R+Col− and R−Col+ segregants. One stable R+Col+ clone was obtained; its stability did not appear to be due to the formation of a recombinant plasmid, for R and Col were not jointly

transduced by phage P1 (cf. Watanabe *et al.* 1964). The relationship between F-like R factors and ColB-K77 shows that these R factors do not constitute a single class of plasmid. The differing behaviour of the individual R factors with ColB-K77 is analogous to the patterns of segregation reported for ColV factors and F (MacFarren & Clowes, 1967).

Table 2. *Transfer of R factors to Col B⁺ strains*

	Col B factor in recipient					
	ColB-K77		ColB-K98		ColB-K166	
R factor in donor	Relative frequency*	Col⁺†	Relative frequency	Col⁺	Relative frequency	Col⁺
R1*drd*16	0·5–1 [4]	100 % (101/101)	0·05–0·3 [3]	100 % (112/112)	0·5–1·5 [4]	100 % (203/203)
R136*drd*H8	1·0–3·0 [5]	100 % (344/344)	0·5–1·0 [2]	100 % (105/105)	1·5–3·0 [4]	100 % (136/136)
R192*drd*F7	1·0 [3]	100 % (610/610)	0·7 [1]	100 % (350/350)	1·0–3·0 [3]	100 % (484/484)
R538-1*drd*	0·02–0·1 [8]	5·5 % (13/242)	0·0025–0·005 [2]	100 % (437/437)	0·5–2·0 [5]	100 % (851/851)

* Transfer to Col⁺ recipient/transfer to Col⁻ recipient.

† The proportions given are based on numbers of well-isolated colonies but larger numbers of bacteria plated from lower dilutions gave essentially similar results.

[ ] No. of experiments.

Table 3. *Segregation pattern of R⁺Col⁺ recipient clones*

	ColB factor in recipient															
	ColB-K77 segregant colonies					ColBK-98 segregant colonies					ColB-K166 segregant colonies					
R factor in donor	No. of clones tested	R Col	+ +	+ −	− +	− −	No. of clones tested	R Col	+ +	+ −	− +	− −	No. of clones tested	R Col	+ +	+ −	− +	− −
R1*drd*16	5		0	5	0	0	4		5	0	0	0	5		5	0	0	0
							1		4	0	1	0						
R136*drd*H8	7		0	0	4	0	5		5	0	0	0	3		4	0	0	0
	1		0	4	0	0							1		0	2	2	0
	1		0	3	1	0							1		1	3	0	0
	1		0	1	3	0							1		3	1	0	0
R192*drd*F7	5		0	5	0	0	5		5	0	0	0	6		5	0	0	0
	1		0	0	5	0							1		4	1	0	0
	1		0	4	1	0												
	1		0	2	3	0												
R538-1*drd*	1		0	17	0	0	4		5	0	0	0	7		5	0	0	0
	1		0	4	1	0	1		0	0	5	0						
	1		1	1	2	0	1		4	0	1	0						
	1		0	1	4	0												

Neither ColB-K98 nor ColB-K166 induced superinfection immunity to any of the four R factors and they therefore differed from ColB-K77 in this respect. A few Col$^-$ or R$^-$ segregants were produced with some combinations, although no R$^-$ or Col$^-$ colonies were observed when any of the parental strains were plated alone.

Table 4. *Transfer of R factors to ColB$^+$ recipients*

Col B factor in recipient	R factor in donor	Effect of ColB on frequency of R factor transfer	Colicinogeny of R$^+$ recipient colonies	Segregation of R and Col	Loss predominantly of
Col B-K77					
	R1*drd*16	Unchanged	+	+	Col
	R136*drd*H8	Unchanged	+	+	R
	R192*drd*F7	Unchanged	+	+	Col
	R538-1*drd*	Less	−*	+	Col
Col B-K98					
	R1*drd*16	Less	+	(±)†	(R)
	R136*drd*H8	Unchanged	+	−	.
	R192*drd*F7	Unchanged	+	−	.
	R538-1*drd*	Much less	+	(±)†	(R)
Col B-K166					
	R1*drd*16	Unchanged	+	−	.
	R136*drd*H8	Unchanged	+	(±)†	R or Col
	R192*drd*F7	Unchanged	+	(±)†	Col
	R538-1*drd*	Unchanged	+	−	.

* Predominantly. † Rare.

Table 5. *Susceptibility of purified stable R$^+$Col$^+$ clones to lysis by phage MS2*

	ColB-K77	ColB-K98	ColB-K166
R1-*drd*16	.	96 % (23/24)	100 % (25/25)
R136-*drd*H8	.	72 % (18/25)	100 % (16/16)
R192-*drd*F7	.	100 % (25/25)	98 % (34/35)
R538-1*drd*	.*	100 % (25/25)	100 % (35/35)

* One stable R$^+$Col$^+$ clone obtained, which was resistant to lysis.

Superinfection immunity, which leads to failure of two plasmids to replicate, may be accompanied by reduction in the frequency with which a bacterium carrying the one accepts the second initially. This is seen in *exclusion* of the F factor by a cell already carrying F (Lederberg, Cavalli & Lederberg, 1952; Scaife & Gross, 1962; Maas, 1963; Dubnau & Maas, 1968) or ColI and I-like R factors by one already with an I-like sex factor (Meynell, 1969). In the present experiments transfer of R538-1*drd* was decreased 10- to 50-fold to the ColB-K77$^+$ recipient, suggesting that exclusion operated here also. Nevertheless, in transfer of both R538-1*drd* and R1*drd*16, the numbers of R$^+$ recipient colonies were also reduced by the presence of ColB-K98, in the absence of any indication of superinfection immunity in the R$^+$Col$^+$ clones. Failure of a bacterium to accept foreign genetic material is often determined by the presence of a plasmid, as shown by the effects of F or R factors on sensitivity of the host to certain phages (Schell *et al.* 1963; Arber & Morse, 1965;

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Anderson & Lewis, 1965). When the incoming DNA is broken down, this failure is known as *restriction* (see Arber & Linn, 1969; Watanabe *et al.* 1966). However, until the mechanism is known, it is not possible to say whether the smaller frequencies of R factor transfer observed with ColB-K77 and ColB-K98 in the recipient were in any way related either to restriction or to one another. In neither case did transfer of the R factor lead to selection of a special fraction of the Col^+ recipient population, for a second transfer of the same R factor to an R^-Col^+ segregant occurred at no more than the original rate.

(ii) *Nature of the mutation in the de-repressed R factors*

All known F-like R factors are related to each other by the criterion of superinfection immunity, and after double infection with a de-repressed and a wild type R factor, the period of instability before one or other is rejected is marked by failure of the culture to lyse with phage MS2 (Meynell & Datta, 1969). This originally suggested that the de-repressed mutants were susceptible to repression by the wild type R factor, and thus that their de-repression, as with F, resulted from failure to produce repressor. The present tests of de-repressed R factors with ColB were begun because of doubt as to whether specific repression of sex pilus formation can satisfactorily be examined where superinfection immunity exists between the two plasmids. This was indeed justified, for, in contrast to the behaviour of the unstable clones with wild type R and R*drd* factors, almost all the clones which stably carried both ColB and R*drd* were lysed by phage MS2. The proportion of Col^+R^+ clones that failed to clear were no greater than in control transfers of the different R factors to a Col^- recipient. In the light of these results, the phage resistance of cultures carrying a de-repressed and a wild type R factor requires a fresh interpretation. Segregation of two R factors is prevented only by keeping the culture on a suitable combination of drugs, where it may depend on constant re-infection of one bacterium from another for growth. Thus, the observed absence of lysis may result from the mixed composition of the culture rather than from repression.

Since ColB-K98 represses fertility and pilus formation by F (Puig & Nagel de Zwaig, 1964), the clearing of the R^+Col^+ cultures by phage MS2 might signify either that the repressor determined by ColB-K98 differed in operator affinity from that of an fi^+ R factor, although both acted on F, or, perhaps, that it was produced in smaller amount, sufficient to reduce pilus production below the threshold for clearing of a culture with F but not with R*drd*. Alternatively, the mutant R factors might be repressor-insensitive. The last explanation was supported by their effects on strain HfrC as a chromosomal donor, for the mutations leading to the de-repressed states of these R factors were found not to allow high frequency polarized transfer of the Hfr chromosome, as would be expected if the integrated F factor were also liberated from repression (Meynell & Cooke, 1969).

The leading marker of the HfrC chromosome was transmitted at approaching the normal rate with a second de-repressed mutant of R1, R1*drd*19. If two kinds of de-repressed sex factor can arise by mutation, and if R1*drd*16, R136*drd*H8,

R192*drd*F7 and R5381*drd* are mutants which are repressor-insensitive while continuing to produce repressor, R1*drd*19 might thus be repressor-minus and still sensitive to repressor. When R1*drd*19 was tested with ColB-K98, it was seen to be transferred to the Col$^+$ recipient at the same relative rate (0·05) as R1*drd*16, but was, indeed, susceptible to repression as shown by the fact that none of 25 R$^+$Col$^+$ colonies, comprising five members of each of five recipient clones, were lysed by phage MS2.

It may be significant that those mutants, R1*drd*16, R136*drd*H8, R192*drd*F7 and R538-1*drd*, which were evidently repressor-insensitive, were originally selected for ability to bring about their own transfer at increased rate, while R1*drd*19 was differently selected by high frequency chromosome transfer from the *sfa* locus in the defective Hfr Richter $♀_3$ strain (Meynell & Datta, 1967). The repressor-minus mutant R factor, R100-1, isolated by Egawa & Hirota (1962), was also selected by high frequency chromosome transfer from an Hfr strain. However, until further de-repressed sex factors have been isolated, it is not possible to judge how far the kind of mutant obtained is influenced by the method of selection.

SUMMARY

F-like R factors are related to ColB-K77, but not to ColB-K98 or ColB-K166, by the criterion of superinfection immunity. Tests of de-repressed mutant R factors for susceptibility to repression by ColB-K98, known to produce repressor for F, showed that these could be either repressor-sensitive or insensitive. The former were independently shown not to produce repressor, which was thus, presumably the reason for their de-repressed state.

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