An I Pilus-determining R Factor with Anomalous Compatibility Properties, Mobilizing a Gentamicin-resistance Plasmid

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SUMMARY

An R factor complex, JR66, consisted of JR66a, a self-transmissible plasmid conferring resistance to streptomycin and kanamycin and capable of mobilizing JR66b: JR66b was a non-self-transmissible plasmid conferring resistance to gentamicin, streptomycin, tetracycline, chloramphenicol, kanamycin and sulphonamides. It was a member of compatibility group FII. JR66a, which determined I pili, was incompatible with members of two distinct groups, I and B. Plasmids of both these groups were shown to determine I pili.

INTRODUCTION

An R factor complex conferring resistance to multiple antibiotics, including gentamicin, was derived from a strain of *Klebsiella pneumoniae* isolated in Washington D.C. (Martin, Ikari, Zimmerman & Waitz, 1971). Benveniste & Davies (1971) showed that gentamicin resistance determined by this R factor complex (JR66) was mediated by a gentamicin-adenylating enzyme. The mechanism appeared to be the same as that determined by *fi*-R factors identified in France (J. Davies quoted by Witchitz & Chabbert, 1971; Witchitz & Chabbert, 1972).

Since we had recently investigated the compatibility properties of the French gentamicin-R factors (Datta & Hedges, 1972*b*) we decided to compare these plasmids with JR66.

Transfer of JR66 between strains of *Escherichia coli* K12 indicated that there were two R factors. One, JR66a, conferring resistance to streptomycin and kanamycin (SK), was efficiently transmitted. This R factor, which determined I pilus production, showed unique compatibility properties, described in this paper.

The other resistances were transmitted with markedly lower efficiency and never without JR66a (Martin *et al.* 1971; J. Davies, personal communication; and our own observations). We shall show that the gentamicin resistance was determined by a non-self-transmissible plasmid, JR66b, of the FII compatibility group (Hedges & Datta, 1972).

METHODS

Bacterial strains. All experiments were in strains of *Escherichia coli* K12; W677, J53, J62, HfrC (Clowes & Hayes, 1968) and *E. coli* C (Bertani & Weigle, 1953).

Plasmids. 'Standard' plasmids of various compatibility groups are listed in Table 1. In addition, R1 and R296 which specify F-like and I-like pili respectively (Lawn, Meynell, Meynell & Datta, 1967) were used as controls in experiments to show multiplication of sex-specific phages.

Table 1. *Standard plasmids of the compatibility groups studied*

No.	Compatibility group	Resistance*	Reference
R386	FI	T	Dennison, 1972
R1-1	FII	A, S, C, Su	Coetzee <i>et al.</i> 1972
R136	FII	T	Hedges & Datta, 1972
ColB-K98	FIII	—	Frydman & Meynell, 1969
R124	FIV	T	Hedges & Datta, 1972
R64	I	S, T	Lawn <i>et al.</i> 1967
R64-1	I	A, S, T	Datta <i>et al.</i> 1971
R46	N	A, S, T, Su	Datta & Hedges, 1971
RP4	P	A, T, K	Datta <i>et al.</i> 1971
R388	W	Su, Tp	Datta & Hedges, 1972a
R401	T	A, S	Coetzee <i>et al.</i> 1972
R40a	C	A, K, Su	Datta & Hedges, 1972b
R57b	C	A, C, Su, Gk	Witchitz & Chabbert, 1972
R483	B	S, Tp	Hedges, Datta & Fleming, 1972
R300	—	S, Su	Lawn <i>et al.</i> 1967
RA1	A	T, Su	Hedges & Datta, 1971
R387	—	S, C	Hedges & Datta, 1971
R6K	X	A, S	Kontomichalou, Mitani & Clowes, 1970

* A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Su, sulphonamides; Tp, trimethoprim; Gk, gentamicin with low-level kanamycin resistance.

Phages. MS2 (Davis, Strauss & Sinsheimer, 1961) which adsorbs to F pili; If1 (Meynell & Lawn, 1968) which adsorbs to I pili.

Media. Nutrient broth was Oxoid no. 2 (CM67); nutrient agar was the same, solidified with 1.2 % New Zealand agar. MacConkey agar was Oxoid (CM7b). Minimal salts agar was as described by Clowes & Hayes (1968).

Transfer of R factors was as described by Datta *et al.* (1971). Where no transfer was detected at 1 h after mixture, cultures were incubated overnight and retested.

Compatibility of R factors was tested as described by Coetzee, Datta & Hedges (1972).

fi character of plasmids was tested as described by Coetzee *et al.* (1972).

Phage increase was measured by methods described by Datta, Lawn & Meynell (1966) and Datta *et al.* (1971). Increase of phage MS2 in a bacterial culture indicated the presence of F pili; increase of phage If1 indicated the presence of I pili.

RESULTS

Separation of JR66a and JR66b

1. *Conjugal transfer.* When selection was made for resistance to either streptomycin (S) or kanamycin (K), resistances to both drugs (SK) were always co-transferred with an efficiency of about 5×10^{-3} . When selection was made for gentamicin (G), chloramphenicol (C) or tetracycline (T) resistance, all resistance markers [GSTCK and sulphonamide (Su)] were co-transferred with efficiency of about 10^{-5} . Every exconjugant clone carrying these resistances could, in turn, transfer SK resistance separately from, and at higher frequency than, the complete pattern. We inferred that SK resistance was determined by one plasmid (JR66a) whilst the other resistances were determined by another plasmid (JR66b), which might be non-self-transmissible.

2. *Transfection.* To isolate a culture carrying JR66b alone, DNA was extracted from w677 (JR66), and mixed with *Escherichia coli* C made competent for transfection by the

Table 2. *Compatibility of JR66a*

Mating mixtures were incubated for 1 h at 37 °C. Frequency of transfer was calculated per input donor cell.

Donor	Recipient	Selection	Frequency of transfer	Exconjugant clones tested for presence of each plasmid
w677 (JR66a)	J53	K	4×10^{-3}	—
	J53 (R64)	K	1×10^{-3}	18/20 JR66a only, 2/20 both present, unstable
	J53 (R64-1)	K	6×10^{-4}	14/20 JR66a only, 6/20 both present, unstable
	J53 (R483)	K	3×10^{-3}	16/20 JR66a only, 4/20 both present, stable*
J53 (R64)	w677	T	1×10^{-3}	—
	w677 (JR66a)	T	1×10^{-5}	20/20 R64 only
J53 (R64)	J53 (R483)	T	1×10^{-3}	20/20 both present, stable†
J53 (R483)	J62	Tp	1×10^{-3}	—
	w677 (JR66a)	Tp	1×10^{-3}	19/20 R483 only, 1/20 both present, unstable

* Trimethoprim resistance non-transmissible (see text).

† Each R factor separately transmissible.

JR66a co-existed stably with all other plasmids tested: R386, R136, ColB-K98, R124, R46, RP4, R388, R401, R40a, R57b, R300, RA1, R387, R6K.

method of Mandel & Higa (1970) modified by A. E. Jacob and S. J. Laver (to be published). Clones of *E. coli* C were isolated on plates containing gentamicin, chloramphenicol or tetracycline. They were resistant to the same drugs (GSTCKSu) as the donor. Since these resistances were not transmissible by conjugation to *E. coli* K12, we conclude that the transfectants carried JR66b only, which was non-self-transmissible.

Compatibility of JR66a

JR66a was compatible with R factors of all groups tested except with I plasmids R64 and R64-1 and with R483, the prototype of compatibility group B (Table 2). JR66a was susceptible to elimination by any of these three plasmids and capable of eliminating each of them. When JR66a was transferred to a recipient carrying both R64 and R483, both resident plasmids were eliminated. Thus JR66a belonged to two compatibility groups, I and B. Introduction of JR66a to J53 (R483) usually eliminated the latter, but in a minority of clones trimethoprim resistance was retained. From these clones trimethoprim resistance was not transmissible, although JR66a was transmissible with normal frequency.

From double R⁺ cultures, carrying JR66a and standard *fi*⁻R factors, transfer of each plasmid occurred separately and at normal rates, with one exception. J53 (JR66a)(RP4) transferred JR66a at normal frequency but RP4 at a frequency approximately 100-fold lower than normal. When selection was made for transfer of ampicillin resistance from this double, a minority of exconjugants carried recombinant plasmids with the resistance patterns ASK or AS.

Recombination between JR66a and the I-like R factor R64-1

When JR66a (resistance pattern SK) was transferred to a recipient carrying R64-1 (resistance pattern AST) the resident R factor was eliminated from the majority (14/20) of purified exconjugants. The minority (6/20) were unstable doubles which segregated not only the parental R factors but also plasmids conferring the following resistance patterns: TSK, ASK, AS (Table 2).

Table 3. *Compatibility of the gentamicin resistance determinant (JR66b)*

Donor	Recipient	Selection	Frequency of transfer	Exconjugant clones tested for presence of each plasmid
w677 (JR66)	J53	G	1×10^{-5}	—
	J53	T	3×10^{-5}	—
	J53 (R1-1)	G	5×10^{-6}	38/38 JR66 only
J53 (R1-1)	w677	A	1×10^{-3}	—
	w677 (JR66)	A	5×10^{-4}	32/32 R1-1 and JR66a (JR66b eliminated)

JR66b co-existed stably with all other plasmids tested: R64-1, R46, RP4, R388, R40a, R57b, R401, R6K, R483.

Table 4. *Transfer from doubles*

Donor	Recipient	Selection	Frequency of transfer	Exconjugants tested for the presence of each plasmid
J53 (JR66a) (R386)	J62	K	5×10^{-3}	20/20 JR66a only
		T	1×10^{-3}	20/20 R386 only
J53 (R386)	J62	T	> 1	—
J53 (JR66a) (R136)	J62	K	5×10^{-3}	20/20 JR66a only
		T	1×10^{-4}	19/20 R136 only, 1/20 both present
J53 (R136)	J62	T	1×10^{-3}	—
J53 (JR66a) (ColB-K98)	J62	K	8×10^{-3}	20/20 JR66a only
J53 (JR66a) (R124)	J62	K	5×10^{-3}	20/20 JR66a only
		T	2×10^{-5}	16/20 R124 only, 4/20 both present
J53 (R124)	J62	T	2×10^{-3}	—

Compatibility of JR66b

The efficiency of transfer of JR66b, mobilized by JR66a, was not reduced by any resident plasmid in the recipient. JR66b was compatible with all plasmids tested (including R64-1 and R483) except R1-1, a member of compatibility group FII. We conclude that JR66b belongs to group FII (Table 3).

f_I character of JR66

HfrC (JR66a) was not visibly lysed by male-specific phage MS2. The *f_I⁺* character of JR66a was retained after co-existence with plasmids of all four known F-like compatibility groups (R386: FI; R136: FII; ColB-K98: FIII; R124: FIV [Hedges & Datta, 1971]). The presence of JR66a in donors repressed the transfer rate of all the F-piliated R factors (i.e. R386, R136, R124) (Table 4).

Sex pili

w677 (JR66), w677 (JR66a) and J53 (R483) did not support multiplication of phage MS2. All these three cultures supported the multiplication of If1. When R483 was transferred to cultures of *Escherichia coli* K12 carrying I plasmids derepressed for pilus synthesis (R64*drd11*, R144*drd3*, R163*drd7*, ColI*drd* and I*drd16*) (Meynell, 1972) the incoming and resident plasmids co-existed stably. All four doubles were visibly lysed by phage If1, i.e. R483 did not repress I pilus synthesis *in trans*.

DISCUSSION

JR66 was of interest to us because it determines a gentamicin-adenylating enzyme analogous to that determined by R factors identified in France (J. Davies, personal communication; Witchitz & Chabbert, 1971, 1972). These latter R factors are *fi*⁻ and belong to compatibility group C (Datta & Hedges, 1972*b*). In fact, neither of the constituent plasmids of JR66 was a member of group C. The gentamicin-resistance determinant was carried by a non-self-transmissible FII plasmid. Thus what is occurring is not pandemic spread of a single plasmid clone. Genes determining gentamicin-inactivating enzymes (Benveniste & Davies, 1971) need not, however, have evolved separately in the two areas. We have observed recombination between C plasmids and the fertility factor, F, of *Escherichia coli* K12 (Datta & Hedges, unpublished): it is not unlikely that exchange of markers has occurred in nature between C and F-like plasmids.

The plasmid JR66a, which accompanied and mobilized the gentamicin resistance determinant, had unusual properties. It was *fi*⁺ but produced I pili, not F pili. In this it resembled R62 (Lawn *et al.* 1967) and several R factors described by Grindley & Anderson (1971). JR66a in donors repressed the transfer of all F-like R factors tested, even R386 which was not repressible by ordinary F-like *fi*⁺ plasmids (Dennison, 1972) (Table 4). The nature of the *fi*⁺ character of JR66a is thus different from that of ordinary F-like plasmids such as ColB-K98 (Frydman & Meynell, 1969) and R538-1 (Romero & Meynell, 1969; Dennison, 1972), and seems to resemble that of R62 (Meynell, 1973).

JR66a resembled plasmids of the I group (e.g. R64) in some respects but differed from them in others. Its similarities to R64 were: (i) pilus specificity – JR66a produced pili which served as receptors for phage If1 (as shown by multiplication of the phage in JR66a⁻ cultures); (ii) compatibility – the two plasmids were incompatible; (iii) genetic homology – this was indicated by the generation of recombinant plasmids after transfer of JR66a into a culture containing R64-1; (iv) interaction with RP4 – JR66a, like R64 (Datta *et al.* 1971) inhibited the transfer of RP4 from R⁺ strains and also like R64, it recombined with RP4.

On the other hand, JR66a differed from R64, and other members of the group, in the following ways: (i) unilateral exclusion – whereas the presence of a typical I-like plasmid in a recipient markedly reduces the rate of transfer of an incoming I plasmid, JR66a efficiently excluded R64 but was only slightly excluded by R64 (Table 2); (ii) incompatibility with R463 – typical I plasmids (exemplified by R64 and R144) co-exist stably with R483, a member of compatibility group B (Hedges, Datta & Fleming, 1972).

Since JR66a was incompatible with both R64 and R483, it belonged to two compatibility groups I and B. The group B plasmid R483, like JR66a and typical I group plasmids, determines I pili. Pilus synthesis by R483 appeared to be repressed, since transfer was at normal frequencies and R483⁺ cultures were not visibly lysed by phage If1, but there was no repression of pilus production *in trans* when R483 co-existed with derepressed mutant I plasmids. Introduction of JR66a usually eliminated R483 but in a minority of JR66a⁺ exconjugants, trimethoprim resistance was retained. This character was non-transmissible, indicating that R483 was not in its normal cytoplasmic state. Whether the trimethoprim-resistance determinants had become integrated into the chromosome or some other replicon, or whether R483 was still replicating autonomously but with defective compatibility and transfer functions, we have not discovered.

A plasmid incompatible with two groups of plasmids, themselves compatible, may be explained in either of two ways. It may have resulted from fusion of plasmids of two groups. An example is that produced by recombination between a ColV plasmid (compatibility

group FI) and an R factor, R538-1*drd* (compatibility group FII), which was incompatible with plasmids of both groups (Cooper, 1971). Alternatively, JR66a may represent an ancestral form of plasmids of groups I and B, which have diverged in evolution, becoming compatible with one another. Supporting the latter model is the fact that plasmids of both groups (I and B) determine I pili.

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