Molecular Homology and Incompatibility Relationships between F and IncH1 Plasmids

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IncH1 plasmids and the F plasmid of *Escherichia coli* display one-way compatibility. An entering IncH1 plasmid is incompatible with a resident F plasmid, but is compatible when it is the resident plasmid. There is little molecular homology between IncH1 plasmids and the F plasmid. A single 5 MDal *Eco*RI restriction enzyme fragment from digests of several IncH1 plasmids hybridizes with probes constructed from the primary replication region of F. Homology can be demonstrated only with the gene for the essential replication protein of F (gene E), but the expression of incompatibility behaviour appears to be associated with the presence of the secondary replicon of the F plasmid. Thus R27 and F are compatible under growth conditions allowing replication and maintenance of F by the secondary replicon. However, a mutant F plasmid which lacks the secondary replicon of F is incompatible with R27 in both directions, irrespective of the growth conditions used.

INTRODUCTION

Smith *et al.* (1973) demonstrated one-way incompatibility between the F plasmid and plasmids of the IncH1 group. They showed that when an IncH1 plasmid entered a cell carrying F as the resident plasmid, the latter segregated. However, when F entered a cell carrying an IncH1 plasmid, the two plasmids were compatible. In some cases, segregants of IncH1 plasmids which had lost resistance to tetracycline also lost their one-way incompatibility with F: the two plasmids were compatible irrespective of which plasmid was used as the donor.

In general, it has been shown that closely related plasmids of the same incompatibility group exhibit significant homology, as judged by DNA–DNA hybridization (Falkow *et al.*, 1974). However, it has also been shown that there is little homology between F and four IncH1 plasmids (Smith *et al.*, 1973; Roussel & Chabbert, 1978). The incompatibility relationship observed for F and IncH1 plasmids could be explained if the homology resided in a replication region (replicon) shared by the two plasmid groups.

We showed that incompatible IncFI plasmids share regions of specific DNA homology that are able to express incompatibility. Individual IncFI plasmids possessed combinations of these loci and we suggested that the presence of multiple *inc* determinants on a single plasmid may lead to cross-reactions between members of different incompatibility groups (Lane, 1981; Bergquist *et al.*, 1982). We examined the DNA homology between the F plasmid and several plasmids of the IncH1 group by hybridization of radioactive fragments of the F incompatibility regions to restriction enzyme digests of the IncH1 plasmids. The aims of this investigation were to examine the extent of molecular homology between the F plasmid and IncH1 plasmids and to establish if this homology could be related to the observed incompatibility behaviour of the plasmids.

Table 1. *Plasmids*

Plasmid	Selective markers*	Inc group	Source	References
R27	Tc	H1	N. Datta	Grindley <i>et al.</i> (1972)
R726	TcCmSmSu	H1	N. Datta	Datta & Olarte (1974)
R726-1	CmSmSu	H1	N. Datta	
pIP166	TcCmSmSu	H1	Y. Chabbert	Chabbert & Gerbaud (1974)
TP123	TcCmSmSu	H1	N. Datta	Grindley <i>et al.</i> (1972)
pIP522	CmSmSu	H2	Y. Chabbert	Chabbert & Gerbaud (1974)
TP116	CmSmSu	H2	N. Datta	Grindley <i>et al.</i> (1972)
pNZ130	SmSu		L. Watson	Watson <i>et al.</i> (1982)
F and F'- <i>lac</i> ⁺	-; Lac ⁺	FI	P. L. Bergquist	Bergquist <i>et al.</i> (1982)
F::Tn9	Cm	FI	P. L. Bergquist	This paper
F(Δ33-43)::Tn9	Cm	FI	P. L. Bergquist	This paper

* Abbreviations: Tc, tetracycline resistance; Cm, chloramphenicol resistance; Sm, streptomycin resistance; Su, sulphonamide resistance; Lac, lactose.

METHODS

Plasmids. The plasmids used are listed in Table 1. Plasmid DNA was prepared by a minor modification of the method of Currier & Nester (1976). *Eco*RI digests of IncH1 plasmid DNAs were electrophoresed on a 0.7% acetate agarose gel and were coupled to diazobenzyloxymethyl (DBM) paper together with an *Eco*RI digest of F. The DBM paper was hybridized with radioactive probes constructed by nick translation of the primary (f5) and secondary (f7, *incE*) replicon regions of F (Bergquist *et al.*, 1982) under conditions that allowed detection of sequences having 20% base mismatch or less (Hyman *et al.*, 1973).

Molecular probes. The *incB* sequence and the E gene sequences used as probes were, respectively, 240 and 380 bp fragments that were isolated from 8% acrylamide gels after digestion of pNZ130 with *Sau*3A (Watson *et al.*, 1982). The E gene is located between F co-ordinates 42.251 and 46.004. The *Sau*3A fragments used are entirely internal to the coding sequence (Murotsu *et al.*, 1981). The *incC*-E gene sequence was a *Pst*I-*Alu*I fragment of 470 bp which was isolated from an appropriate digest of pNZ130. This fragment carries 100 bp of the coding sequence for the carboxy-terminal portion of the E gene as well as the five 19 bp repeated sequences of the *incC* region. There were no suitable restriction enzyme cleavage sites that allowed removal of the E gene sequence from this fragment. Nick translation of these fragments was done by the method of Rigby *et al.* (1977).

Assessment of homology between the E gene-*IncC* probe and the *Eco*RI fragment from IncH1 plasmids. An approximation to the relative amount of homology to the probe was obtained by densitometry of the bands that hybridized in the gel and the radioautograph, to correct for the different amounts of DNA present (Fig. 2). Confirmation of the limited amount of homology found in this way was provided by hybridization of the E gene probe at low stringency (40% mismatch) to IncH1 and F plasmid DNAs coupled to DBM paper. The paper was washed under conditions of increasing stringency up to 10% mismatch, and radioautographed after each wash. The relative amount of radioactivity hybridized remained roughly constant at 20% mismatch.

Incompatibility. Incompatibility testing was performed using *E. coli* strain PB1150 (His⁻, *rpoB*, *recA1*) carrying either R27 or one of the F plasmids as the recipient bacterium. In experiments on the effect of growth conditions on segregation of F, incompatibility testing was performed by the method of Gardner *et al.* (1982) because of the observed segregation of Lac⁻ in colonies on the selective plates. After about 22 generations growth in either L-broth or minimal salts medium plus amino acid supplements, the colonies were spread either to L plates or to minimal agar plates. The colonies that appeared on these plates were then replicated to suitable broth or minimal plates containing tetracycline plus 5-bromo-4-chloro-3-indolyl β-D-galactoside (Xgal) and isopropylthio-β-D-galactoside (IPTG) or lactose for the determination of the presence or absence of the plasmid(s) they carried (see Table 2). Incompatibility testing in experiments with F(Δ33-43)::Tn9 followed the same procedure and the results were confirmed using the maintenance test as described by Bergquist *et al.* (1982).

RESULTS AND DISCUSSION

Hybridization of probes

Preliminary experiments demonstrated that only the f5 fragment (*incB*, C, D) showed hybridization to the 5 MDal fragment present in digests of all of the IncH1 plasmids. Other experiments showed that the only homology between F and the IncH1 plasmids resided in this fragment. Probes containing the *incB*-E gene-*incC* region were originally broadly defined by cleavage sites for *Pst*I at F44.1, 45.88 and 47.3 kb co-ordinates (Bergquist *et al.*, 1982 and Fig. 1).

Fig. 1. Restriction map of F showing the replication regions and probes used. The top portion of the figure shows the *EcoRI* cleavage sites and the fragment numbers of the F plasmid on the outside of the circle; F kb co-ordinates are shown on the inside. The replication regions are shown on a larger scale in the middle portions of the figure. The origin and extent of the DNA fragments used for probes are indicated in the lower portion of the figure. Filled triangles indicate the repeat sequences of *incB* and *incC*. Restriction enzyme sites: RI, *EcoRI*; S3, *Sau3A*; PI, *PstI*; AI, *AluI*. Other abbreviations (see Lane, 1981): pif, interference with phage T7 multiplication; 1° rep, primary replicon (f5); 2° rep, secondary replicon (f7). The cloning and isolation of restriction enzyme fragments carrying the *incB*, C, D and E regions and the DNA sequence of this region have been described (Lovett & Helinski, 1976; Murotsu *et al.*, 1981; Bergquist *et al.*, 1982).

Probes for both the *incB* and the *incC* regions hybridized to the 5 MDal *EcoRI* band found in digests of the IncH plasmid DNAs. The nucleotide sequence of this region revealed that it included two sets of repeated sequences which flanked a gene coding for a protein essential for the replication of miniF (Murotsu *et al.*, 1981; Watson *et al.*, 1982). *IncB* consisted of four 19 bp repeats in one orientation while *incC* had five 19 bp repeats in the opposite orientation. The sequence of the nine repeats is highly conserved (Murotsu *et al.*, 1981).

The 240 bp *Sau3A* fragment which included the secondary origin of replication and the *incB* sequence did not hybridize with any fragment from the IncH1 plasmids. A second *Sau3A* fragment of 380 bp, which included most of the gene for the E protein, hybridized to the 5 MDal restriction fragment, as did a *PstI*-*AluI* fragment of 470 bp which includes the portion of the gene coding for the carboxy-terminal fragment of the E protein and the *incC* repeat sequences (Fig. 2). Since the repeated sequences were common to the *incB* and *incC* probes, it appeared

Fig. 2. Hybridization of replication region probes to IncH1 and IncF1 plasmids. Lanes: 1, R726; 2, R726-1; 3, R27; 4, pIP166; 5, F. (a) Ethidium bromide-stained gel; (b, c, d) radioautographs of the DBM paper after hybridization. The position of the 5 MDal fragment of the IncH1 plasmids that hybridizes to the E gene probe is indicated by the arrowheads. Molecular weights (MDal) of some *Eco*RI fragments of F are shown to the right of (a); (b) *incB* probe; (c) E gene-*incC* probe; (d) *incC* probe.

likely that the hybridization of the *incC* probe to the 5 MDal fragment represents homology with the coding sequences for the E protein. A less likely possibility was that the homology shown by R27 was with the F spacer sequences between the 19 bp repeats of the *incC* probe.

The extent of hybridization of the E gene-*incC* probe to the 5 MDal *EcoRI* fragment of the *IncH1* plasmids was approximately 20% of the value for the f5 fragment of F. This result suggests that there may be only limited homology between the E gene sequence of the F plasmid and the analogous sequence in the *IncH1* plasmids.

In separate experiments we examined the hybridization of the *incB*, E gene and *incC* probes to restriction enzyme digests of the plasmids TP123 (displaces F), TP116 (compatible with F) and pIP522. The latter plasmid is a tetracycline-sensitive segregant of pIP166 (Chabbert & Gerbaud, 1974). The E gene probe did not hybridize to any *EcoRI* fragment of either TP116 or pIP522 but hybridized to a 5 MDal fragment of TP123 (data not shown). TP116 is classified as an *IncH2* plasmid on the basis of its compatibility with F and thus absence of hybridization was in accordance with these results.

Smith *et al.* (1973) first described tetracycline-sensitive segregants of TP124 which were compatible with F, unlike the parental plasmid. pIP522, a tetracycline-sensitive segregant of the *IncH1* plasmid pIP166, showed the same phenotypic behaviour in incompatibility tests (data not shown). In addition, the 5 MDal restriction enzyme fragment of the parental plasmid that hybridized with the E gene probe was lost in the segregant. However, with other plasmid segregants that were tetracycline-sensitive there was no obligatory loss of the restriction enzyme fragment that hybridized to the E gene probe. For example, R726-1 is a tetracycline-sensitive segregant of R726 (Datta & Olarte, 1974) that retained its homology with F and was incompatible with F as an entering plasmid (see Fig. 2, incompatibility data not shown). In addition, Taylor (1983) has shown that tetracycline-sensitive derivatives of R27 resulting from transposition of Tn7 still retained their incompatibility with the F factor.

Incompatibility between F and R27

What is the explanation for the one-way incompatibility shown by F and *IncH1* plasmids? We examined the possibility that it was related to the presence of two functional replicons in F (Lane & Gardner, 1979), so that this plasmid was able to replicate from either its primary (f5) or secondary (f7) replicon. The deletion mutant F(Δ 33-43) lacked the f7 region (Anthony *et al.*, 1974) and it is assumed to replicate only from the primary replicon. Thus F(Δ 33-43) should be incompatible with R27 if this plasmid interferes with replication from the primary replicon of F. This suggestion was tested in the following way. The secondary replicon of F is partially defective and cannot support efficient replication of F at fast growth rates (Lane & Gardner, 1979; Gardner *et al.*, 1980). When F'-*lac*⁺ was present as the resident plasmid in incompatibility tests with R27, the Lac⁺ character could be seen to segregate if the transconjugants were plated on enriched medium containing lactose and an indicator dye. After non-selective growth for approximately 40 generations in broth medium, few cells contained the resident plasmid (Table 2). On the other hand, if the initial plating of the transconjugants was onto minimal medium with the appropriate supplements and Xgal plus IPTG, segregation was not observed on the plate and most cells contained both the resident and the entering plasmid on testing after non-selective growth (Table 2).

The results in Table 2 suggested that the incompatibility function of the *IncH1* plasmid is expressed when it enters a cell containing a resident F plasmid, and that it shuts off replication from the primary replicon of F. Replication of the F plasmid is then assumed by the secondary replicon of F, which can support plasmid replication best at low growth rates. If this assumption is correct, the deletion mutant F(Δ 33-43) would be expected to be incompatible with R27 in either broth or minimal medium.

The results of similar experiments to those in Table 2 with a chloramphenicol-resistant derivative of F(Δ 33-43) were unexpected since only the selected plasmid was maintained (Table 2), although it should be noted that the segregation test used favours situations in which the plasmids tested are weakly incompatible (discussed in Bergquist *et al.*, 1982; Gardner *et al.*,

Table 2. *Effect of secondary replicon of F on incompatibility with R27*

Medium	Incoming plasmid	Resident plasmid	Selection*	No. of trans-conjugant colonies tested	Segregation test†				Maintenance test‡ No. of colonies containing both plasmids
					Percentage of colonies containing:				
					Resident only	Incoming only	Both	Neither	
<i>Effect of growth medium</i>									
Broth	R27	F'-lac ⁺	Tc	214	0	99.5	0	0	0/24
	F'-lac ⁺	R27	Lac	482	2.1	0.9	100	0	24/24
Minimal	R27	F'-lac ⁺	Tc	759	2.1	0.9	97	0	24/24
	F'-lac ⁺	R27	Lac	977	4.1	0	95.9	0	24/24
<i>Effect of presence or absence of secondary replicon§</i>									
Broth	R27	F::Tn9	Tc	554	5.5	94.5	0	0	0/24
	R27	F(A33-43)::Tn9	Tc	869	43.2	56.8	0	0	0/24
	F::Tn9	R27	Cm	325	0.3	1.2	98.5	0	24/24
	F(A33-43)::Tn9	R27	Cm	483	5.4	91.5	3.1	0	0/24

* Tc, tetracycline; Cm, chloramphenicol; Lac, lactose.

† Incompatibility testing was done by the method of Gardner *et al.* (1982) because of the observed segregation of Lac⁻ in colonies on the selective plates. The results are the totals from two separate experiments.‡ Maintenance test for the presence of the two plasmids was done as described by Bergquist *et al.* (1982).§ An experiment using minimal medium gave similar results to those reported above for F'-lac⁺ when F::Tn9 was used as donor or recipient. F(Δ33-43)::Tn9 was incompatible with R27 either as donor or recipient in minimal medium.

1982). R27 and $F(\Delta 33-43)$: : Tn9 were completely incompatible in both directions in the plasmid maintenance test, and hence $F(\Delta 33-43)$ was indistinguishable from an *IncH1* plasmid in this test.

That the *f5* and *f7* regions of *F* were the only ones involved in this incompatibility behaviour has been demonstrated by experiments in which cells carrying cloned *f5* (pML31; Lovett & Helinski, 1976) and *f5f7* (Lane & Gardner, 1979) fragments were used in similar incompatibility tests with R27. The incompatibility behaviour of the miniplasmids was identical with that of the entire plasmids (data not shown).

Interpretation of incompatibility tests

The incompatibility results reported above suggested that when *F* was the resident plasmid, entry of R27 resulted in a shift to replication from the secondary replicon. The two plasmids appeared to be incompatible unless minimal medium was used for growth. $F(\Delta 33-43)$ did not have the option of using the secondary replicon and segregated when R27 entered. We suggest that the secondary replicon was used for replication by wild-type *F* when it was the entering plasmid, so that replication of R27 was not prevented, but $F(\Delta 33-43)$ must use the primary replicon. It appeared that the region of homology in R27 had an essential role in the replication of that plasmid even though the fragment, on its own, was not a replicon (P. L. Bergquist, unpublished observation).

The one-way incompatibility behaviour of *F* and *IncH1* plasmids has similarities to the phenomenon of dislodgement described for R394 (*IncT*) and *Plac*-R447b with plasmids from incompatibility group N (Coetzee *et al.*, 1972, 1975). Dislodgement may result in either the complete elimination of the resident plasmid, loss of some of the phenotypic characters of the resident, or recombination between the two plasmids. Thus far, all plasmids susceptible to dislodgement carry DNA sequences homologous to N plasmids. Coetzee *et al.* (1975) concluded that N group plasmids produced a nuclease which recognized N DNA exclusively. On entry of the plasmid into a cell, the target for the nuclease was a fragment of DNA carrying kanamycin resistance from R447b inserted into the resident A-C group plasmid, *Plac*. The N-group specific nuclease was postulated to be repressed after the establishment of the *IncN* plasmid in the cell.

An interaction of this nature, involving the degradation of a plasmid carrying a specific fragment of DNA, would be difficult to distinguish from an incompatibility reaction. We believe that *IncN*-plasmid type dislodgement was not involved in the phenomenon we have described. *F* and the *IncN* plasmid R447b are completely compatible and no homology could be detected between the *f5* and *f7* regions of *F* and R447b (P. L. Bergquist, unpublished observation). It is more difficult to exclude the possibility of a nuclease coded for by the region of the *IncH* plasmids that is homologous with *F*. However, if this type of dislodgement reaction was occurring, it is difficult to explain the results showing that R27 and *F'*-*lac*⁺ are compatible at slow growth rates (Table 2). If a transiently expressed nuclease was involved, the *F* DNA should have been attacked irrespective of the growth conditions.

The expression of the *E* gene of *F* appears to be subject to autoregulatory control (Kline & Trawick, 1983; Sogaard-Andersen *et al.*, 1984). It is tempting to speculate that the presumed protein from the homologous region of R27 could have a negative regulatory effect on *E* gene expression by binding to the putative operator sequence between *incB* and the *E* gene (Sogaard-Andersen *et al.*, 1984). However, the extent of the homology between the gene *E* coding sequence of *F* and the homologous region of the *IncH1* plasmids is not known. Heteroduplex analysis to define the extent of homology and examination of this homology by sequence analysis are obvious ways to resolve this question.

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