An Inverted Repeat Sequence of the IncFI Plasmid ColV2-K94 Increases Multimerization-mediated Plasmid Instability

By PETER C. WEBER AND SUNIL PALCHAUDHURI*

Wayne State University School of Medicine, Department of Immunology and Microbiology,
540 E. Canfield Avenue, Detroit, Michigan 48201, USA

(Received 11 July 1985; revised 6 December 1985)

A detailed physical map of the region of the IncFI plasmid ColV2-K94 containing the Rep1 replicon, a Tn903 transposon, and an inverted repeat structure (X_1) with unknown properties was prepared by cloning restriction fragments into pBR325. Inserts carrying the 1.2 kb repeated sequence of X_1 , but not the IS903 sequence of Tn903, had a destabilizing effect on pBR325 and pBR322 plasmid maintenance. One of these derivatives, pWS139, was studied further and was shown to have elevated levels of multimeric DNA forms; this resulted in decreased copy number and plasmid instability, as multimerization reduces the effective number of randomly segregating plasmids per cell. A ColV2-K94 miniplasmid, which has a copy number much lower than that of ColE1-derived vectors, was also less stably inherited if it contained the X_1 structure. This destabilizing effect of the X_1 repeat sequence was dependent on the RecA function, but not the RecB or the RecC functions of the host. These results suggest that the inverted repeat sequence of the X_1 structure serves as a 'hot-spot' for generalized recombination. Thus, when present in *cis*, this sequence can generate plasmid instability because plasmid molecules are readily converted into multimeric forms through enhanced recombination at this site.

INTRODUCTION

Bacterial plasmids have evolved a number of mechanisms for ensuring their stable inheritance in a population of dividing host cells. Large, stringently-controlled plasmids are maintained by efficient membrane-associated partitioning systems (Ogura & Hiraga, 1983*a*; Austin & Abeles, 1983), or by gene products which couple cell division to plasmid replication (Ogura & Hiraga, 1983*b*). Small plasmids under relaxed replicational control tend to lack these elaborate partitioning mechanisms, as they can be stably maintained through their high copy numbers. Recently, Summers & Sherratt (1984) presented strong evidence that the multicopy plasmid ColE1 is randomly partitioned, and that multimerization of ColE1 derivative molecules in recombination-proficient strains leads to an increase in plasmid instability by reducing the number of segregating units per cell. These investigators found that instability through multimer formation is circumvented in the wild-type ColE1 plasmid through *recA*-independent, site-specific recombination at the *cer* (ColE1 resolution) locus, which resolves multimeric plasmid forms into monomers. As the *cer* locus has been deleted in the construction of ColE1-derived cloning vectors, these plasmids show varying degrees of instability.

The ColE1-derived cloning vector pBR325, which was constructed from the widely-used pBR322 vector (Bolivar *et al.*, 1977), was created to facilitate cloning of *EcoRI* restriction fragments through insertional inactivation of a chloramphenicol transacetylase gene (Bolivar, 1978; Prentki *et al.*, 1981). Recently, we have completed a detailed restriction map of the 130 kb IncFI plasmid ColV2-K94 (Weber & Palchaudhuri, 1985). We have used pBR325 to clone many of these restriction fragments in an attempt to study Rep1, which is one of two replicons in the ColV2-K94 cointegrate plasmid, and is homologous to the RepA replicon of IncFII plasmids (Weber *et al.*, 1984), and the three inverted repeat structures, X_1 , X_2 and X_3 , of ColV2-K94 (Mittra & Palchaudhuri, 1984).

Table 1. *E. coli* strains and plasmids

Strain or plasmid	Genotype and phenotype	Source or reference*
<i>E. coli</i>		
C600	HsdM ⁻ HsdR ⁻ <i>thi-1 thr-1 leuB6 lacY1 tonA21 supE44</i>	S. N. Cohen
C600 (RecA ⁻)	RecA ⁻ HsdM ⁻ HsdR ⁻ <i>thi-1 thr-1 leuB6 lacY1 tonA21 supE44</i>	This laboratory
HB101	<i>recA13 hsdS20 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44</i>	H. W. Boyer
JC5519	<i>recB21 recC22 thr-1 leuB6 proA2 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44</i>	B. Bachmann
C-2110	<i>polA1 His⁻ Rha⁻</i>	D. R. Helinski
Plasmids		
pWS12	ColV2-K94::Tn903 (Km ^r)	Mitra & Palchaudhuri (1984)
pWS15	pWS12Δ110:8V-68:6V (Km ^r)	Weber <i>et al.</i> (1984)
pWS62	pWS15Δ102:6V-99:2V (Km ^r)	This work
pWS63	pWS15Δ102:6V-93:6V (Km ^r)	This work
pWS109	pBR322 + H9 (Ap ^r)	This work
pWS139	pBR325 + H9 (Cm ^r Ap ^r)	This work
pWS139Δ1	pBR325 + H9AP19 (Cm ^r Ap ^r)	This work
pWS139Δ2	pBR325 + H9AP18,P19 (Cm ^r Ap ^r)	This work
pWS115	pBR325 + H15 (Cm ^r Ap ^r)	This work
pWS116	pBR325 + H16 (Cm ^r Ap ^r)	This work
pWS502	pBR325 + P2 (Km ^r Cm ^r Tc ^r)	This work
pWS503	pBR325 + P3 (Cm ^r Tc ^r)	This work
pWS202	pBR325 + 7.4 kb <i>Bam</i> HI/ <i>Hind</i> III fragment (Cm ^r Ap ^r)	This work
pWS212	pBR325 + 7.4 kb <i>Bam</i> HI/ <i>Hind</i> III fragment + H16 (Km ^r Cm ^r Ap ^r)	This work

* Addresses: B. Bachmann, *E. coli* Genetic Stock Center, Yale University, Conn., USA; H. W. Boyer, University of California at San Francisco, Calif., USA; S. N. Cohen, Stanford University School of Medicine, Stanford, Calif., USA; D. R. Helinski, University of California, San Diego, Calif., USA.

In this work, we provide evidence that a cloned inverted repeat sequence of the ColV2-K94 stem-loop structure X₁ (1.2 kb stem and 1.4 kb loop) destabilizes pBR325 as well as ColV2-K94 miniplasmids by increasing plasmid multimerization. As this phenomenon is *recA*-dependent, the X₁ repeat sequence appears to function as a 'hot-spot' for generalized recombination in generating elevated levels of multimeric plasmid forms. Since it converts monomeric plasmid molecules into multimers, this sequence behaves in a manner which is exactly opposite that of the *cer* locus carried on the wild-type ColE1 plasmid.

METHODS

Strains and media. The strains of *Escherichia coli* used are listed in Table 1. The media used included LB medium and M-9 medium. Antibiotics were added to give the following final concentrations: kanamycin at 70 µg ml⁻¹, chloramphenicol at 50 µg ml⁻¹, ampicillin at 50 µg ml⁻¹ and tetracycline at 20 µg ml⁻¹.

Recombinant plasmids and cloning procedures. The construction of the ColV2-K94 deletion derivative pWS15 (ColV2-K94::Tn903 Δ110:8V-68:6V) was described by Weber *et al.* (1984). Restriction fragments of pWS15 generated with *Pst*I, *Hind*III and *Bam*HI/*Hind*III were cloned into pBR325 according to the procedures of BRL. Self-replicating miniplasmids of pWS15 were constructed by digesting plasmid DNA with *Pst*I, ligating with T4 DNA ligase and transforming into *E. coli* C600 with selection for kanamycin resistance. All recombinant plasmids generated in this work are listed in Table 1 and illustrated in Fig. 1. Plasmid DNA was isolated on caesium chloride/ethidium bromide gradients (Palchaudhuri *et al.*, 1976) or by a rapid alkaline extraction procedure (Birnboim & Doly, 1979).

Plasmid stability testing. Stability of pBR325 clones was measured by growing an overnight culture of each strain with chloramphenicol selection, diluting to 10⁻⁷ in M-9 medium without antibiotic, and growing to stationary phase. This process of dilution and incubation was repeated three more times. Each cycle was found to consist of 20 generations, and the loss of chloramphenicol marker was determined after each cycle.

Stability of ColV2-K94 miniplasmids was measured over a much shorter time, due to their greater instability. An overnight culture of each strain was grown in kanamycin selection, diluted to 10⁻⁷ in LB medium without antibiotic, and grown to stationary phase. During this growth period, samples were diluted and plated at 0, 4, 7, and 10 h. These plates were used to determine the number of elapsed generations as well as the loss of the kanamycin resistance marker.

Fig. 1. (a) Physical and genetic map of pWS15 (Weber & Palchaudhuri, 1985), showing *Eco*RI (E), *Bam*HI (B), *Xho*I (X), and *Hind*III (H) restriction sites; inverted repeats (arrows) of Tn903 and the *X*₁ structure; and the location of the Rep1 replicon and distal portion of the transfer (*tra*) operon. (b) Fine structure map of the 'non-*tra*' region of pWS15. *Pst*I (P) restriction sites were mapped by *Pst*I/*Hind*III double digests of the pBR325 clones shown. Tn903 was cloned intact on two inserts which conferred kanamycin resistance in pWS212 and pWS502. Rep1 was functional in pWS139 and pWS139Δ1, based on the ability of these clones to rescue pBR325 replication in the PolA⁻ host C-2110. The two miniplasmids shown contained both Tn903 and Rep1 and were self-replicating (i.e. lacked the pBR325 vector). The coordinate system is that of Sharp *et al.* (1973).

RESULTS

Cloning and mapping the Rep1 replicon, Tn903 transposon and inverted repeat structure X₁ of ColV2-K94. The construction of pWS15, the largest *Eco*RI fragment of the ColV2-K94::Tn903 derivative pWS12 (Mitra & Palchaudhuri, 1984), was described by Weber *et al.* (1984). This deletion derivative extends from 68.6V to 110.8V and includes the Rep1 replicon, Tn903 transposon, inverted repeat structure *X*₁ and much of the distal portion of the ColV2-K94 *tra* operon (Fig. 1). The unique *Eco*RI, *Bam*HI and *Xho*I sites, and one of four *Hind*III sites, have already been mapped on pWS15 (Weber & Palchaudhuri, 1985). The remaining three *Hind*III sites (which generate the H9, H15 and H16 *Hind*III fragments) as well as 12 *Pst*I sites of the 'non-*tra*' region of pWS15 were mapped in this work through double digest analysis of restriction fragments cloned in pBR325 (Fig. 1).

Due to the unique *Hind*III and *Xho*I sites found in Tn903 (Grindley & Joyce, 1980), the insertion and orientation of this transposon was accurately determined (Weber & Palchaudhuri,

Table 2. *Stability of cloned restriction fragments and miniplasmids of ColV2-K94*

Host strain	Plasmid	Frequency of loss (% plasmidless cells per generation)	Increase in instability (fold)
<i>pBR325 and pBR322 clones</i>			
C600 Rec ⁺	pBR325	0.08	—
	pWS116	0.13	1.6
	pWS212	0.18	2.3
	pWS503	0.48	6.0
	pWS115	0.53	6.6
	pWS139	0.78	9.8
	pWS139Δ1	0.62	7.8
	pWS139Δ2	0.84	10.5
C600 RecA ⁻	pBR325	<0.01	—
	pWS139 monomer	<0.01	0
HB101 RecA ⁻	pBR325	0.01	—
	pWS139 monomer	0.03	3.0
	pWS139 dimer	0.34	34.0
JC5519 RecBC ⁻	pBR325	0.09	—
	pWS139	0.56	6.2
C600 Rec ⁺	pBR322	0.01	—
	pWS109	0.58	58.0
<i>ColV2-K94 miniplasmids</i>			
C600 Rec ⁺	pWS62	2.4	—
	pWS63	4.6	1.9

1985); Tn903 was cloned intact in this work in the pBR325 derivatives pWS212 and pWS502 (Fig. 1). Since electron microscopy of the pWS15 homoduplex molecule (not shown) revealed that the stem-loop structures Tn903 and X₁ were separated by a distance of 2.7 kb, the X₁ inverted repeat structure could also be positioned on the pWS15 restriction map. One of the X₁ repeat sequences was located on pWS139 and its deletion derivatives, and the entire structure was cloned on pWS503. The Rep1 replicon, located between X₁ and Tn903, was cloned in pWS139 and pWS139Δ1, as these plasmids could replicate in the PolA⁻ strain C-2110.

Implication of the cloned X₁ repeat in the instability of pBR325 clones. It was observed during the rapid isolation (Birnboim & Doly, 1979) screening of pBR325 clones of pWS15 that some recombinant plasmid restriction patterns were markedly fainter on ethidium bromide-stained agarose gels than others, suggesting a decrease in the stable inheritance of these clones due to copy number reduction. All of these clones (pWS139 and its derivatives, and pWS503) shared the 1.55 kb *Pst*I/*Hind*III fragment of pWS15, which contains a complete 1.2 kb repeat of the X₁ stem-loop structure (Fig. 1). Unfortunately, this restriction fragment could not be cloned directly into pBR325 as it would result in the loss of all three selection markers (ampicillin, tetracycline and chloramphenicol resistance) of this vector.

As ColE1 is randomly partitioned (Summers & Sherratt, 1984), if the copy numbers of these pBR325 clones were indeed reduced, the stability of these plasmids would be affected as well. To examine this effect, the stability of the pBR325 clones in the absence of antibiotic selection was measured over a period of 80 generations. The plasmids chosen for study were pBR325 without a cloned insert; pWS139, pWS139Δ1 and pWS139Δ2, which contain the first X₁ repeat on the 1.55 kb *Pst*I/*Hind*III fragment; pWS503, which contains the whole X₁ structure and thus both X₁ repeats; pWS115, which contains 0.75 kb of the second X₁ repeat; pWS116, which contains a complete IS903 sequence of Tn903; and pWS212, which contains the whole Tn903 transposon.

A comparison of the frequencies of loss of these plasmids (summarized in Table 2) indicated that one or both copies of the IS903 repeat of Tn903 had no effect on pBR325 stability when cloned, while the presence of both or one or even a portion of one of the X₁ repeats on the pBR325 vector increased plasmid instability six- to tenfold. This apparent effect of the X₁ repeat on pBR325 stability was *cis*- and not *trans*-acting, as the stability of the vector was unaffected by

Fig. 2. Rapid alkaline (Birnboim & Doly, 1979) isolations of pBR325 and pWS139, a pBR325 derivative carrying an X_1 repeat sequence. Lane A, C600 Rec^+ (pBR325); lane B, C600 Rec^+ (pWS139); lane C, HB101 RecA^- (pWS139 monomer); lane D, HB101 RecA^- (pWS139 dimer); lane E, JC5519 RecBC^- (pWS139). Monomeric (m), dimeric (d) and multimeric (n) plasmid forms and chromosomal DNA (c) are indicated. Note the elevated levels of dimers and multimers of pWS139 in C600 Rec^+ and JC5519.

a co-resident pWS15 plasmid (data not shown), and was independent of the vector used, as pWS109, the pBR322 analogue of pWS139, also showed a pronounced instability in C600 (Table 2).

Recent reports (Kasner *et al.*, 1985) have suggested that the stability of ColE1 vectors decreases as the size of their cloned inserts increases. This does not appear to be the case in the pWS15 clones, as the X_1 repeat inserts range in size from 1.8 kb (pWS115) to 5.0 kb (pWS503), and all confer instability on their vector. Also, the plasmid carrying the largest cloned fragment, pWS212 (9.05 kb insert), appears to be relatively stable when compared to pBR325 without an insert.

The X_1 repeat increases pBR325 instability through RecA-dependent multimerization. Summers & Sherratt (1984) demonstrated that ColE1 vector instability was due to an increase in multimeric forms of these plasmids. To see if increased multimerization was responsible for the instability of the X_1 repeat clones, rapid alkaline isolations (Birnboim & Doly, 1979) were done on these plasmids. All of the clones carrying the X_1 repeat showed a greater degree of multimerization than pBR325 or other pWS15 clones. The isolate of one such plasmid, pWS139, is compared with pBR325 in Fig. 2, demonstrating the abundance of dimeric and multimeric forms in the

plasmid population of pBR325 which carries an X_1 repeat sequence; pBR325 without an insert exists in predominantly monomeric form.

As these experiments had so far been done in a recombination-proficient strain, C600, the effects of the X_1 repeat on pBR325 maintenance were then studied in recombination-deficient strains. pWS139, a representative X_1 clone, was isolated from C600 and transformed into an isogenic C600 RecA⁻ strain, the RecA⁻ strain HB101 and the RecB⁻ RecC⁻ strain JC5519. This pBR325 derivative was stably inherited (Table 2) and exhibited reduced multimerization (Fig. 2) in the two RecA⁻ strains but not in the RecBC⁻ strain, so that instability due to X_1 -mediated multimerization was a RecA-dependent but RecBC-independent process. Interestingly, transformants in the RecA⁻ strains carried pWS139 in exclusively either a monomeric state or a dimeric state (Fig. 2). The latter was presumably due to the transformation of dimeric DNA molecules (prevalent in the C600 host) into a strain in which these plasmids cannot be resolved into monomeric forms due to the loss of RecA recombination functions.

As would be expected, the pWS139 monomer was quite stable in the RecA⁻ strain HB101, while the pWS139 dimer was relatively unstable: the monomeric clone was only three times as unstable as pBR325 without an insert, compared to 34 times the instability for the dimeric clone (Table 2). These results confirm that an increase in dimers and other multimeric forms in a pBR325 plasmid population, as mediated by enhanced RecA-dependent recombination occurring at a cloned X_1 sequence, would serve to reduce stable maintenance.

Effect of the X_1 repeat sequence on ColV2-K94 stability. ColV2-K94 is inherited quite stably in a RecA⁻ background (frequency of loss <0.05% per generation), but not in a RecA⁺ background (frequency of loss = 2–5% per generation; unpublished observations). This notable difference suggests that RecA-dependent multimerization may also have marked effects on ColV2-K94 stability. To test whether the X_1 repeat had any role in this instability, ColV2-K94 miniplasmids were constructed *in vitro* using *Pst*I; these derivatives, pWS63 and pWS62, consisted purely of Tn903 (used as a selection marker) and the minimal Rep1, with and without the X_1 structure, respectively (Fig. 1).

The stability of pWS62 and pWS63 was compared over a period of 20 generations in the recombination-proficient strain C600. The presence of the X_1 structure on the ColV2-K94 miniplasmid pWS63 caused it to have a twofold increase in instability, compared with the pWS62 miniplasmid (Table 2). Thus, even in ColV2-K94, a plasmid with a low copy number (about two copies per chromosome; Mitra & Palchaudhuri, 1984), the X_1 repeat sequence can enhance instability in a recombination-proficient strain.

DISCUSSION

The ColE1-derived vector pBR325 was used to clone restriction fragments of ColV2-K94 carrying the Rep1 replicon, Tn903 transposon and X_1 inverted repeat structure. In the process, a detailed physical map of the area of ColV2-K94 containing these functions (93.6V–107.0V) was also prepared (Fig. 1). Cloning these regions onto small restriction fragments has enabled us to study the functioning of Rep1, an IncFII-like replicon on an IncFI plasmid (Weber *et al.*, 1984), and the inverted repeat structure X_1 .

The evidence presented here suggests that the inverted repeat sequence of the X_1 structure is a 'hot-spot' for generalized, RecA-dependent recombination. When both (pWS503), one (pWS139 and its derivatives), or even part of one (pWS115) of the X_1 repeat sequences were cloned into pBR325, the resulting plasmids had increased instability due to elevated multimerization and reduced copy number of the vector molecules. A similar effect was also seen in a derivative of the stringently-controlled plasmid ColV2-K94 which contained the X_1 structure. This phenomenon was not observed in pBR325 derivatives containing one (pWS116) or two (pWS212) copies of the insertion sequence IS903 of the Tn903 transposon. The instability of the pBR325 clones was not due to insert molecular mass, as demonstrated by the assorted sizes of restriction fragments carrying the X_1 repeat which could cause instability, nor was it dependent on the RecB or RecC functions of the host.

Now that the X_1 inverted repeat structure has been cloned onto small restriction fragments, it

will be interesting to study its characteristics, as X_1 , whose stem-loop structure was reported long ago (Sharp *et al.*, 1973), may be a transposon with unknown phenotypic properties. This is suggested by the observation that ColV2-K94 can readily alter itself through rearrangement of its three inverted repeat structures (Davies *et al.*, 1982; Mitra & Palchaudhuri, 1984). The ability of an IS element to serve as a hot-spot for recombination is common in bacterial systems. RecA-dependent recombination at homologous IS sequences is commonly used in the integration of plasmids into host genomes (Davidson *et al.*, 1975), and the transitioning and amplification of R plasmid DNA is a RecA-dependent process known to occur at insertion elements such as IS1 (Rownd, 1982). It therefore seems feasible that an IS sequence, when cloned on a multicopy plasmid, could increase the number of unstable multimeric forms in the plasmid pool by simple homologous recombination between molecules.

REFERENCES

- AUSTIN, A. & ABELES, A. (1983). The partition of unit-copy miniplasmids to daughter cells. II. The partition region of miniplasmid P1 encodes an essential protein and a centromere-like site at which it acts. *Journal of Molecular Biology* **169**, 373–387.
- BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant DNA. *Nucleic Acids Research* **7**, 1513–1523.
- BOLIVAR, F. (1978). Construction and characterization of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique *EcoRI* sites for selection of *EcoRI*-generated recombinant molecules. *Gene* **4**, 121–136.
- BOLIVAR, F., RODRIGUEZ, R. L., GREEN, P. J., BETLACH, M. C., HEYNEKER, H. L., BOYER, H. W., CROSA, J. H. & FALKOW, S. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2**, 95–113.
- DAVIES, D. L., BINNS, M. M. & HARDY, K. G. (1982). Sequence rearrangements in the plasmid ColV-I-K94. *Plasmid* **8**, 55–72.
- DAVIDSON, N., DEONIER, R. C., HU, S. & OHTSUBO, E. (1975). Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. X. Deoxyribonucleic acid sequence organization of F and of F-primes, and the sequences involved in Hfr formation. In *Microbiology – 1974*, pp. 56–65. Edited by D. Schlessinger. Washington, DC: American Society for Microbiology.
- GRINDLEY, N. D. F. & JOYCE, C. M. (1980). Analysis of the structure and function of the kanamycin-resistance transposon Tn903. *Cold Spring Harbor Symposia on Quantitative Biology* **45**, 125–133.
- KASNER, J. P., WOMBLE, D. D. & ROWND, R. H. (1985). Relationship between plasmid copy number and molecular size of the plasmids ColE1 and NR1 (abstract). In *Plasmids in Bacteria*, p. 861. Edited by D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson & A. Hollaender. New York: Plenum Press.
- MITRA, G. & PALCHAUDHURI, S. (1984). Genetic structure and stability of a copy number mutant of IncFI group plasmid ColV2-K94 in *Escherichia coli* K12. *Molecular and General Genetics* **193**, 349–357.
- OGURA, T. & HIRAGA, S. (1983a). Partition mechanism of F plasmid: two plasmid gene-encoded products and a *cis*-acting region are involved in partition. *Cell* **32**, 351–360.
- OGURA, T. & HIRAGA, S. (1983b). Mini-F plasmid genes that couple host division to plasmid proliferation. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 4784–4788.
- PALCHAUDHURI, S., MAAS, W. K. & OHTSUBO, E. (1976). Fusion of two F-prime factors in *Escherichia coli* studied by electron microscope heteroduplex analysis. *Molecular and General Genetics* **146**, 215–231.
- PRENTKI, P., KARCH, F., IIDA, S. & MEYER, J. (1981). The plasmid cloning vector pBR325 contains a 482 base-pair-long inverted duplication. *Gene* **14**, 289–299.
- ROWND, R. H. (1982). R plasmid drug resistance gene amplification in bacteria. In *Gene Amplification*, pp. 273–279. Edited by R. T. Schimke. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- SHARP, P. A., COHEN, S. N. & DAVIDSON, N. (1973). Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. II. Structure of drug resistance (R) factors and F factors. *Journal of Molecular Biology* **75**, 235–255.
- SUMMERS, D. K. & SHERRATT, D. J. (1984). Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell* **36**, 1097–1103.
- WEBER, P. C. & PALCHAUDHURI, S. (1985). A physical and genetic map of the IncFI plasmid ColV2-K94. *Plasmid* **13**, 215–218.
- WEBER, P. C., MITRA, G. & PALCHAUDHURI, S. (1984). Second replicon in ColV2-K94 mediates the stable coexistence of two incompatible plasmids. *Journal of Bacteriology* **162**, 245–250.