Molecular and Genetic Properties of Plasmid R485 Conferring Resistance to Sulphonamides

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Plasmid R485 carrying a determinant of resistance to sulphonamides was isolated from *rec*⁺ and *recA* *Escherichia coli* hosts, and its molecular properties were examined and compared with the characteristics of an incompatible plasmid, R6K, a type plasmid of incompatibility group X (IncX). Examination of R485 DNA by electron microscopy revealed monomer, dimer and miniplasmid molecules in preparations isolated from the *rec*⁺ host, while only monomers were seen in preparations obtained from the *recA* host. The molecular sizes of the R485 monomers from the *rec*⁺ and the *recA* host were 34.9 ± 3.7 and 38.3 ± 0.8 megadaltons (Mdal), respectively. The dimer molecules of R485 (59.7 ± 2.9 Mdal) probably resulted from the recombination between one monomer molecule of normal size and another from which the miniplasmid (11.2 Mdal) was segregated. The G+C content of R485 DNA was 44.3 mol %. With a minimum copy number of approximately one per *E. coli* chromosome equivalent, plasmid R485 differs conspicuously from the multicopy plasmid R6K; this finding indicates that the replication of R485 and R6K is differently controlled.

The presence of plasmid R485 in a cell brought about a reduction of the frequency of conjugative transfer of an IncP plasmid, RP1, and an IncN plasmid, N3A, but it did not affect the transfer of IncW plasmid Sa. Plasmid R485 showed integrative suppression of the expression of a chromosomal temperature-sensitive *dnaA* mutation, indicating that its replication is independent of the *dnaA* gene product. The simultaneous presence of incompatible plasmids R485 and R6K in a cell resulted in the elimination of R6K in the absence of selection pressure, while under selective conditions only a decrease in the number of R6K copies was found. Inhibition of growth of *E. coli* E52int6K by plasmid R485 at elevated temperature also indicated the negative effect of R485 on R6K replication.

INTRODUCTION

Plasmid R485, determining resistance to sulphonamides (Hedges *et al.*, 1973), is incompatible with plasmid R6K (Kontomichalou *et al.*, 1970), the type plasmid of incompatibility (Inc) group X, which points to relatedness of these two extrachromosomal elements. On the other hand, some genetic properties of plasmid R485, e.g. its ability to inhibit the fertility of F-like plasmids (Hedges *et al.*, 1973; Gasson & Willetts, 1977) and to determine the formation of characteristic pili (Bradley, 1978), differ from those of plasmid R6K. While the IncX 'prototype' is well characterized and the mechanism of replication of R6K DNA is known (Kolter *et al.*, 1979), only the above information about plasmid R485, whose IncX status was discussed by Bradley (1980) was available until now.

In this work we provide both basic data on the molecular parameters and replication of R485 DNA and additional information about the genetic properties of plasmid R485, necessary for its overall comparison with plasmid R6K. Further, as the incompatibility of

related plasmids is functionally associated with the mechanisms of their DNA replication, especially with the control of the number of plasmid copies (Uhlín & Nordström, 1975; Terawaki & Kobayashi, 1978), the interactions of plasmids R485 and R6K coexisting in one cell line were also followed. The results obtained may contribute to existing knowledge about the replication of R plasmids.

METHODS

Bacterial strains and plasmids. Strains of bacterial hosts for R plasmids are listed in Table 1; plasmids used are shown in Table 2.

Drugs. The following antibiotics were used: ampicillin (Pentrexyl; Bristol Italiana), chloramphenicol (Spofa, Czechoslovakia), kanamycin and streptomycin (both as sulphates; Medexport, U.S.S.R.), spectinomycin (Trobicin; Upjohn, Belgium), sulphafurazol (Sulfasol; Pliva, Yugoslavia). Abbreviations for these drugs are listed in a footnote to Table 2.

Media. Penassay broth (PAB, Difco) or minimal medium M9 (Adams, 1959) supplemented with appropriate nutrients were used for bacterial growth. Transconjugant selection and Su resistance determination were carried out on supplemented M9 medium solidified with 1.5% (w/v) agar (Difco). Resistance to other antibiotics was determined on Tryptose Blood Agar Base (TBAB, Difco) to which the appropriate drug was added; the concentration of sulphafurazol was 1 mg ml⁻¹, and the concentrations of the other antibiotics ranged from 10 to 100 µg ml⁻¹.

Plasmid isolation. The procedure of Hansen & Olsen (1978) was used for the preparation of crude R-plasmid DNA. For analytical investigations, covalently closed circular (CCC) DNA forms of R plasmids were isolated either as described by Katz *et al.* (1973) or the crude plasmid preparations were purified by ultracentrifugation in a caesium chloride/ethidium bromide (CsCl/EB) gradient. ColE1 DNA was isolated according to Zasloff *et al.* (1978).

Agarose gel electrophoresis of plasmid DNA. Plasmid DNA samples were subjected to electrophoresis in the system described by Mickel, Arena & Bauer (1977), using 0.7% agarose (Bio-Rad), Ficoll 400000 (250 mg ml⁻¹; Pharmacia) and the vertical slab apparatus Pharmacia GE-4. The molecular size markers were plasmids ColE1 (4.2 Mdal; Staudenbauer, 1978), R6K (26 Mdal; Kontomichalou *et al.*, 1970) and R1*drd*19 (52 Mdal; Šrogl *et al.*, 1977). Electrophoretograms were photographed under short-wavelength ultraviolet light with a Pentacon Six camera through a green GR-1 filter using ORWO NP20 film (ASA 80).

Electron microscopy. Preparations of R485 DNA were spread by the aqueous protein film technique (Davis *et al.*, 1971) modified as described previously (Šrogl *et al.*, 1977). The molecules were observed in a Jeol 100B electron microscope at a voltage of 60 kV using a 50 µm objective aperture. Photographs were taken at a magnification of ×10000.

Contour length measurements of open circular (OC) DNA molecules were made on a Hewlett-Packard Digitizer 9864A from negatives enlarged six- to eightfold. The data were processed by a Hewlett-Packard 9830 calculator with ±0.3 µm accuracy. Lengths were converted to molecular weight assuming a linear mass density of 2.08 ± 0.03 Mdal µm⁻¹ (Stüber & Bujard, 1977).

G+C content of plasmid DNA. This was determined from the buoyant density (ρ) of the DNA in a gradient of CsCl (Suprapur grade; Merck), essentially as described by Schildkraut *et al.* (1962). [¹⁵N]DNA from *Pseudomonas aeruginosa* with the density $\rho_0 = 1.7350$ g cm⁻³ (acquired from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha) was used as a reference standard; this value was determined relative to the correct value of 1.7035 g cm⁻³ for *Escherichia coli* DNA given by Szybalski & Szybalski (1971). Centrifugation to equilibrium was performed in a Beckman An-H rotor at 44000 rev. min⁻¹ and 25 °C for 24 h; ρ was evaluated from the record obtained at 263 nm by a Beckman photoelectric scanner, and the molar percentage of G+C was calculated according to Woodward & Lebowitz (1980).

Determination of plasmid copy number. A method based on CsCl/EB analysis of crude bacterial lysates was used. Cells of *E. coli* J53-1 infected either with plasmid R485 or plasmid R6KΔ1 were cultured and total DNA was labelled with [6-³H]thymidine (Institute for Research, Production and Application of Radioisotopes, Praha) as described by Hochmannová & Nešvera (1982). Lysis of cells, pronase (Calbiochem) digestion and shearing of the lysates were performed according to Womble *et al.* (1977). Isopycnic centrifugation was done and distribution of radioactivity was followed as previously described (Nešvera *et al.*, 1978). The minimum number of plasmid copies per chromosome equivalent (n.p.c.) was calculated (Hochmannová & Nešvera, 1982) assuming values of 35 Mdal, 21 Mdal and 2500 Mdal for the molecular sizes of R485 (see Results), R6KΔ1 (Nešvera *et al.*, 1978) and the *E. coli* chromosome (Cooper & Helmstetter, 1968), respectively. In biplasmid strains R6K n.p.c. was estimated from the degree of Ap resistance expressed as LD₃₇ (the concentration of Ap at which only 37% of the cells formed colonies). The n.p.c. of plasmid R6KΔ1 in *E. coli* J53-1 determined by the direct radioisotopic method (Hochmannová & Nešvera, 1982) was used as standard in these estimations.

Table 1. *Bacterial strains*

<i>E. coli</i> K12 derivative	Genetic markers*	Source
J53-1	<i>rec⁺; pro met na^r</i>	N. Datta, London
IV-28-1	<i>recA56; leu thi rpsL</i>	J. Hubáček, Praha
C600	<i>thr leu thi lacY tonA supE</i>	A. I. Stepanov, Moscow
JC5455	<i>his trp tsx rpsE lacΔX74</i>	N. S. Willetts, Edinburgh
E52	<i>dnaA thy leu rpsL</i>	Department of Microbiology, Charles University, Praha
E52int485	<i>dnaA thy leu rpsL sul⁺</i>	This work
E52int6K	<i>dnaA thy leu rpsL bla⁺ aphC⁺</i>	This work

* The symbols for genetic markers are those of Bachmann & Low (1980) and Novick *et al.* (1976).

Table 2. *Plasmids*

Plasmid	Inc group	Drug resistance markers*	Source
R485	X	Su†	N. Datta, London
R6K	X	Ap Sm	A. I. Stepanov, Moscow
R6KΔ1	X	Ap Sm	Z. Lorkiewicz, Lublin
ColE1	—	—	Institute of Biophysics, Czechoslovak Academy of Sciences, Brno
R1drd19	FII	Ap Cm Sm/Sp Su (Km*)	J. Hubáček, Praha
N3A	N	Ap Sm/Sp Su	N. Datta, London
RP1	P	Ap Km Tc	H. Tschäpe, Wernigerode
Sa	W	Cm Km Sm Su	H. Tschäpe, Wernigerode

* Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulphonamides; Tc, tetracycline.

† A minimal inhibitory concentration of sulphafurazol of 1200 µg ml⁻¹ was estimated for *E. coli* J53-1(R485) in liquid M9 medium.

Determination of drug resistance. The minimal inhibitory concentration of Su was determined by the tube dilution method in liquid medium M9. The values of LD₅₀ were determined as described by Chiang & Clowes (1980), except that PAB with Ap (10 µg ml⁻¹) was used for growth of bacteria before plating to ensure the maintenance of plasmid R6K in host cells harbouring the incompatible plasmid R485.

Conjugation. This was done as described by Šrogl *et al.* (1977).

Testing of integrative suppression of *dnaA* ts mutation. Exponential phase cultures of *E. coli* E52 derivatives grown in PAB at 30 °C were plated on TBAB. Counts of viable cells at 42 °C divided by counts at 30 °C gave the frequency of reversion to temperature resistance. The transfer of chromosomal markers from selected revertants was detected by conjugation at 30 °C (Šrogl *et al.*, 1977); time of mating was prolonged to 140 min.

Incompatibility. This was tested as described by Coetzee *et al.* (1972).

RESULTS

Size of plasmid R485

The approximate size of plasmid R485 was estimated by agarose gel electrophoresis of crude DNA preparations from *E. coli* J53-1(R485). Based on the results of four independently performed analyses using three plasmid CCC DNAs as standards, a molecular size of 39.9 ± 2.6 Mdal was obtained for R485 DNA from the inverse linear relation between the logarithms of relative mobility and mass of the CCC plasmid (Meyers *et al.*, 1976). The plot of migration distances versus logarithm of mass of CCC plasmids using the decreasing sigmoid standard curve for plasmid size determination (Nass, 1978; O. Sova, personal communication) yielded the value of 35.9 ± 2.1 Mdal for R485 DNA.

Preparations of purified R485 DNA isolated from both *E. coli* J53-1 and *E. coli* IV-28-1 *recA* hosts were observed in an electron microscope. In addition to circular molecules of the

Fig. 1. Histograms showing the dispersion of contour lengths of R485 DNA isolated from two *E. coli* strains: (A) a *rec*⁺ host, J53-1; (B) a *recA* host, IV-28-1. 1 and 2 denote the size classes (see also Table 3).

Table 3. Dispersion of sizes of R485 DNA molecules within individual classes

Size class*	Mean length $\pm$ s.d. (μ m)	Molecular size $\pm$ s.d. (Mdal)	Percentage of total no. of molecules
A1	16.8 ± 1.8	34.9 ± 3.7	81.6
A2	28.7 ± 1.4	59.7 ± 2.9	16.3
Miniplasmid†	5.4	11.2	2.0
B1	18.4 ± 0.4	38.3 ± 0.8	100.0

* From Fig. 1; the A prefix denotes molecules from the *rec*⁺ host and the B prefix those from the *recA* host.

† Not shown in Fig. 1; from the *rec*⁺ host only.

Table 4. Buoyant density (ρ) and G + C content of R-plasmid DNA

DNA	ρ (g cm ⁻³)	G + C (mol %)
R485	1.6978	44.2
	1.6980	44.4
R6K	1.6972	43.7
	1.6975*	43.9†
R6KΔ1	1.6973	43.7
	1.6974	43.8

* Value determined by Kontomichalou *et al.* (1970); the published ρ value of 1.704 g cm⁻³ was corrected by subtraction of 0.0065 g cm⁻³ to relate it to the correct value for *E. coli* DNA (Szybalski & Szybalski, 1971).

† Calculated according to Woodward & Lebowitz (1980); by applying the classical equation of Schildkraut *et al.* (1962) to the original value of ρ , Kontomichalou *et al.* (1970) obtained 45 mol % G + C.

basic type, molecules of approximately double size and, occasionally, miniplasmids, were seen in R485 preparations obtained from the host with an intact recombination system. Neither dimers nor miniplasmids were observed in samples of R485 DNA isolated from the *recA* host. Contour length measurements revealed considerably greater size dispersion of monomer R485 molecules that originated from *E. coli* J53-1 (Fig. 1). The mean contour lengths of R485 DNA molecules belonging to individual size classes, and the corresponding molecular weights, are summarized in Table 3.

Fig. 2. CsCl/EB centrifugation analysis of [^3H]DNA of whole-cell lysates from *E. coli* J53-1(R485) (●) and *E. coli* J53-1(R6K Δ 1) (○). CCC DNA peak: fractions 8–12 for R485, fractions 8–13 for R6K Δ 1; (linear + OC) DNA peak: fractions 16–25 for R485, fractions 17–25 for R6K Δ 1.

Table 5. Results of fertility inhibition tests

Inhibited plasmid	Inhibition (transfer inhibition ratio)* by IncX plasmid:	
	R485	R6K Δ 1
N3A (IncN)	4.2	42.6
RP1 (IncP)	88.5	200.0
Sa (IncW)	1.4	67.2

* Transfer inhibition ratios were obtained by dividing the transfer frequency of inhibited plasmids from cells carrying no other plasmid by their transfer frequency from cells also carrying the indicated inhibiting IncX plasmid (Gasson & Willetts, 1977).

Base composition of R485 DNA

G+C contents of R485 and of R6K and R6K Δ 1 DNAs are presented in Table 4. No difference in base composition of R485 DNA was observed between preparations isolated from *rec*⁺ and *recA* hosts. Although the polynucleotide chain of R485 DNA is about 40% longer than that of IncX type plasmid R6K DNA, their base composition differs by 0.5% only.

Copy number of plasmid R485

A conspicuous difference between the quantity of CCC R485 DNA and CCC R6K Δ 1 DNA in the same host was found (Fig. 2). The minimum copy number of plasmid R485 in *E. coli* J53-1 was estimated to be 0.9 per chromosome equivalent (a molecular size of 35 Mdal for R485 was used; see Table 3, size class A1), while the value of 8.9 was calculated for plasmid R6K Δ 1 (both ratios are means from two independent experiments). Thus, in contrast to plasmid R6K Δ 1, whose replication is regulated by relaxed control (in a similar manner to the replication of the IncX type plasmid R6K — 11–13 copies per *E. coli* chromosome; Croso

Table 6. *Results of tests for integration of plasmid R485 into the E. coli chromosome*

Donor*	Frequency of recombinants		Su-resistant clones among recombinants	
	<i>trp</i> ⁺	<i>his</i> ⁺	<i>trp</i> ⁺	<i>his</i> ⁺
E52int485/1	1.3×10^{-6}	2.1×10^{-7}	149/152	10/10
E52int485/2	1.3×10^{-6}	5.3×10^{-7}	42/42	19/19
E52(R485)	$<2.9 \times 10^{-7}$	$<2.9 \times 10^{-7}$	0/0	0/0

* JC5455 was used as recipient strain in all matings.

et al., 1975; Chiang & Clowes, 1980), plasmid R485 seems to replicate under stringent control in *E. coli* J53-1.

Fertility inhibition

To test the influence of R485 on the conjugative transfer of IncN, IncP and IncW plasmids, biplasmid derivatives of *E. coli* J53-1 harbouring plasmid R485 together with one of the representative plasmids of the above incompatibility groups were constructed by the selection of transconjugants on M9 agar plates containing appropriate drugs. The frequencies of conjugative transfer of the tested plasmids from the biplasmid strains were compared with those from strains lacking plasmid R485. Plasmid R6K Δ 1 was examined in parallel fertility inhibition experiments (Table 5). The observed inhibition of fertility of IncN, IncP and IncW plasmids by plasmid R6K Δ 1 is in agreement with the results of Pinney & Smith (1974) and Olsen & Shipley (1975), proving the inhibition by plasmid R6K of fertility of other plasmids belonging to the incompatibility groups in question. On the other hand, plasmid R485 significantly inhibited only the transfer of plasmid RP1 (IncP). As full compatibility of both IncX plasmids with all the tested IncN, IncP and IncW plasmids was demonstrated (results not shown), wherever a decreased frequency of conjugative transfer of tested plasmids was observed it was undoubtedly due to inhibition of their fertility by IncX plasmids rather than to their elimination.

Integrative suppression

The IncX type plasmid R6K is known to suppress the phenotypic expression of chromosomal temperature-sensitive (*ts*) *dnaA* mutation via its integration into the chromosome (Sotomura & Yoshikawa, 1975). We found that the presence of plasmid R485 in cells of *ts* mutant E52 also caused a significant increase of reversion frequency to temperature resistance (1.9×10^{-4}) compared with the spontaneous frequency (3.8×10^{-5}); a reversion frequency of 1.6×10^{-3} was estimated for strain E52(R6K). To demonstrate that the phenotypic reversion of the *dnaA* *ts* mutation was really due to the integration of plasmid R485 into the *E. coli* chromosome, selected temperature-resistant revertants (designated E52int485/1 and E52int485/2) were examined for their ability to form *trp*⁺ and *his*⁺ recombinants by mating with JC5455 as recipient strain. It follows from the results presented in Table 6 that the frequency of *trp*⁺ recombinants was evidently higher in the mating crosses of both E52int485 strains than in the cross of E52(R485). Moreover, the majority of recombinants of both types exhibited sulphonamide resistance. These results reflect an actual integration of plasmid R485 into the *E. coli* chromosome. The ability of R485 to suppress the *dnaA* *ts* mutation via its integration into the chromosome indicates that the replication of plasmid R485 is independent of the *dnaA* gene product.

Incompatibility of plasmids R485 and R6K

Biplasmid transconjugants (derived from strains J53-1 and C600) harbouring R485 and R6K were obtained by selection on M9 agar plates containing Ap and Su. They were shown

to lose their ampicillin and streptomycin resistance after growth in PAB without drugs for 6 h or 18 h. All tested clones (60/60 for each strain) exhibited resistance to sulphonamides only. These results confirmed the incompatibility of plasmids R485 and R6K (Hedges *et al.*, 1973) and showed that in the absence of selection pressure plasmid R6K was eliminated preferentially from cells harbouring both incompatible plasmids. Under selective conditions Ap, Sm and Su resistances were preserved in the transconjugants but resistance to Ap decreased in strains harbouring both plasmids, compared with strains containing only R6K or R6K Δ 1. The following LD₅₀ values (μ g Ap ml⁻¹) for the tested strains were observed: J53-1(R6K Δ 1), 540; C600(R6K), 585; J53-1(R485,R6K), 150; C600(R6K,R485), 165. Uhlin & Nordström (1977) showed that the degree of Ap resistance of *E. coli* strains infected with R plasmids coding for TEM-1 β -lactamase was directly proportional to gene dosage. In the case of decreased Ap resistance, the resistance level is thus obviously proportional to the copy number of the R plasmid. The number of R6K copies in the tested strains could thus be estimated from the straight line constructed by plotting the value of 12 copies per chromosome for R6K Δ 1 as a standard (Hochmannová & Nešvera, 1982). As the number of 13 copies of plasmid R6K thus estimated in strain C600(R6K) was in exact agreement with the value reported for the *E. coli*(R6K) system in the literature (Chiang & Clowes, 1980), the estimates of 3.3 and 3.7 copies of plasmid R6K per chromosome in the respective biplasmid strains seem likely to correspond to the real values. The presence of plasmid R485 in an *E. coli* cell is therefore probably responsible for the decrease in the R6K copy number (to about a quarter of its normal value) even under selective conditions.

Inhibition of growth of strain E52int6K at elevated temperature by plasmid R485

A temperature-resistant revertant designated E52int6K was obtained and tested for the integration of plasmid R6K into the chromosome by the methods described for strain E52int485. Derivatives E52int6K(R485) and E52int485(R6K) were constructed by selection of transconjugants on M9 agar plates containing Ap and Su. To ensure the maintenance of incoming incompatible plasmids in these strains, media containing both drugs were used for all subsequent growth and tests. Comparison of growth of biplasmid and monoplasmid strains at 30 °C and 42 °C showed that 84% of E52int485(R6K) cells survived at 42 °C; this result was not very different from the survival of the temperature-resistant strains E52int485 and E52int6K used as controls. On the other hand, growth of E52int6K(R485) was almost totally inhibited at 42 °C (only 0.001% of the cells survived; this value was of the same order as the spontaneous frequency of temperature-resistant revertants of strain E52). These findings indicate the inhibitory effect of plasmid R485 on R6K replication.

DISCUSSION

The existence of R485 DNA as molecules of various lengths, namely as dimers, revealed in preparations from *E. coli* *rec*⁺ host (Fig. 1, A) is in agreement with the formation of plasmid pMB9 multimers observed in an *E. coli* *rec*⁺ host by Bedbrook & Ausubel (1976), and also shown by these authors for other plasmids harboured by *rec*⁺ cells. As the *recA* gene product is required for the formation of plasmid multimers (Bedbrook & Ausubel, 1976), dimers were not detected in preparations of R485 DNA isolated from an *E. coli* *recA* host (Fig. 1, B). The mean molecular size of the observed dimer forms of R485 DNA (59.7 ± 2.9 Mdal) is not exactly twice the mean molecular size of an R485 DNA monomer (69.8 Mdal); however, the difference of about 10 Mdal between these two values corresponds to the size of an observed miniplasmid (11.2 Mdal; Table 3). It seems that the monomers of R485 DNA in the *rec*⁺ host tend to segregate a plasmid of smaller size, as observed in the case of plasmid 222/R3 (68 Mdal; Nisioka *et al.*, 1969). Recombination of two R485 monomer molecules, one of them being of the basic size (35 Mdal), the other lacking the miniplasmid (24 Mdal) may then result in the formation of the observed dimers. It is of interest that the small segregant plasmid

from 222/R3 was about the same size (12 Mdal; Nisioka *et al.*, 1969) as the miniplasmid from R485. Such plasmid(s) may participate in the evolutionary development of R factors. The presented hypothesis explaining the formation of the 'non-exact' dimers of R485 DNA could be tested by analysing the sequence homology between monomers and 'dimers' of R485 DNA. The separation of these two molecular forms from the mixed population generated in an *E. coli* *rec*⁺ host (Table 3), necessary for such experiments, is in progress.

Differences in the ability of plasmids R485 and R6K to inhibit the conjugative transfer of some F-like plasmids were described by Hedges *et al.* (1973) and Gasson & Willetts (1977). Our observations also showed different influences of the incompatible, and thus probably related, plasmids R485 and R6K Δ 1 on the fertility of IncN and IncW plasmids (Table 5). One may thus conclude that the fertility inhibition (Fin) systems of the two IncX plasmids are distinct. As tested IncN, IncP and IncW plasmids varied in their susceptibility to Fin-system(s) of plasmid R485, they very probably differ in the sites at which the Fin-system acts.

The observed 'dominance' of oligocopy plasmid R485 over multicopy plasmid R6K in the incompatibility reaction is in agreement with results showing that some higher-copy-number mutants of IncFII plasmid R1 and IncT plasmid Rts1 exhibited decreased incompatibility towards plasmids from the IncFII and IncT groups when compared with their respective parent plasmids (Uhlén & Nordström, 1975; Terawaki & Kobayashi, 1978). Recently, Molin & Nordström (1980) demonstrated that the copy number control, incompatibility and regulation of initiation of plasmid DNA replication are different manifestations of the same trans-acting function of plasmid R1. Our results demonstrating the negative influence of plasmid R485 on the copy number of incompatible plasmid R6K, and its inhibitory action on the growth of E52int6K at elevated temperature, when replication of the *E. coli* chromosome was undoubtedly initiated from the origin(s) of the integrated plasmid (Chandler *et al.*, 1977), indicate that plasmid R485 may code for similar element(s) acting in trans on plasmid R6K.

The results presented in this paper represent a basis for further studies of replication of both plasmids R485 and R6K using mutants with altered numbers of their copies.

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