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The survival of RP 1–1 in *Escherichia coli* and the influence of the *rec* and *polA* genes on the process

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SUMMARY

The survival of the plasmid RP 1–1 in *E. coli* depends on the presence of a functional *polA* gene. Unlike other plasmids that have this requirement, the replication of RP 1–1 is inhibited by chloramphenicol. RP 1–1 cannot be inherited by *E. coli recA* mutants, and inactivation of the *recA* gene product in a $recA_{ts}$ mutant leads to destruction of the plasmid. RP 1–1 cannot be inherited by *recB* or *recC E. coli* unless the strains also carry a suppressor of these genes, such as *sbcA* or *sbcB*. The pattern of replication of RP 1–1 in *E. coli* K12 and its mutants suggests that the survival of this plasmid in this species is the outcome of a balance between DNA polymerase I-specified replication and exonuclease destruction.

1. INTRODUCTION

RP 1–1 is a self-transmissible plasmid discovered in *Pseudomonas aeruginosa* strain 1822 (Ingram *et al.*, 1972). It specifies the production of Type IIIa β-lactamase, an enzyme that confers carbenicillin resistance on strains of *Pseudomonas aeruginosa* and ampicillin and carbenicillin resistance on *Escherichia coli* (Richmond & Sykes, 1973). So far all attempts to isolate it as covalently closed, circular DNA have failed (Ingram *et al.* 1972). RP 1–1 transfers at a frequency of between 10^{-1} and 10^{-2}/donor to plasmid-less strains of *Pseudomonas aeruginosa* PAO (Stanisich & Richmond, 1975) but at frequencies of 10^{-5} or less to *Escherichia coli* K12 (Richmond & Sykes, 1972). Moreover, the progeny of such intergeneric crosses is unstable. On prolonged incubation in the presence of carbenicillin or ampicillin, stable penicillin resistant colonies may be obtained, but in these the β-lactamase gene is integrated into the chromosome with concomitant loss of transfer ability (Richmond & Sykes, 1972).

This paper examines the behaviour of RP 1–1 following transfer to strains of *Escherichia coli* that carry mutations affecting DNA synthesis. It shows that DNA polymerase I is needed for replication of RP 1–1. In the absence of replication the plasmid is destroyed and one of the enzymes involved in this process is probably exonuclease V. The inheritance of RP 1–1 in the extra-chromosomal state in *E. coli* seems therefore to be a balance between the rates of replication by DNA polymerase I and destruction by nucleases. A preliminary version of this paper has already appeared (Richmond & Moillo-Batt, 1975).

2. METHODS AND MATERIALS

(i) *Strains and growth conditions*

The bacterial strains used in these experiments, together with their genetic constitution and source, are listed in Table 1. All experiments were carried out in nutrient broth at 37 °C unless stated to the contrary.

Table 1. *Strains used in these experiments and their sources*

	Designation	Markers	Source
Ps. aeruginosa	PAO12 (RP 1–1)	*ade-136 leu-8 chl-3* (RP 1–1)	Dr V. Stanisich
	PAO1670 (RP 1–1)	*ade-136 leu-8 rif-1 chl-3* (RP 1–1)	(this department)
E. coli	JC3272	*str*^{r} *his lys trp*	Dr A. J. Clark
	AB1157	*thr leu pro lac his str arg thi*	Dr A. J. Clark
	JC7620	*thr leu proA thi argE lacY galK xyl mtl ara tsx phx strA recB21 recC22 sbcB*	Dr A. J. Clark
	JC7741	*recA44 leu-5 lacY gal-6 his-1 str-104 xyl-7 mtl-2 argG5*	Dr A. J. Clark
	JC5743	*thr leu pro lac his arg thi recB21*	Dr A. J. Clark
	JC5489	*thr leu pro lac his arg thi recC22*	Dr A. J. Clark
	UB2627	JC3272 *recA*$_{ts}$*200*	This laboratory
	JG78	*Hfr rif metE polA1*	Dr J. Gross
	MM383	*thyA rha lac str*^{R} *polA12*	Dr P. Emmerson (University of Newcastle-upon-Tyne, England)
	PE169	*polA12 recC22 sbcA54*	Dr P. Emmerson
	KL398	F$^-$ *thi-1 metE70 leu-5 proC32 malA38 hisF860 thyA54 lacZ36 ara-14 mtl-1*	Dr R. Lloyd (University of Nottingham, England)
	KL399	As KL398 but *recA200*	Dr R. Lloyd

(ii) *Transfer experiments*

Basically two types of transfer experiments were carried out: one described as 'intergeneric' and the other as 'intraspecific'. In the first the donor was always a strain of *Pseudomonas aeruginosa* PAO carrying RP 1–1 and the recipient an *E. coli* K12 strain. In intraspecific crosses the transfer was between appropriate mutants of *Escherichia coli* K12. All conjugal transfer experiments were carried out for 3 h at 37 °C unless a temperature sensitive mutant was involved, in which case incubation was at 30 °C. Normally cultures containing about 10^8 bacteria/ml were used and equal amounts of the two suspensions mixed. After 3 h mating, (RP 1–1)$^+$ transconjugants were usually selected on agar containing 500 μg carbenicillin/ml and 200 μg/ml of either streptomycin or rifampicin (as counter-selective agent).

Wherever *E. coli* (RP 1–1) strains are used as donors in mating experiments it is difficult to measure accurate transfer frequencies since the proportion of Carb^R bacteria in the donor population is variable. In practice transfer frequencies in such experiments are quoted in terms of the total number of viable bacteria, whether Carb^R or not, which are mixed with the recipient.

(iii) *Temperature shift experiments*

A standard temperature shift experiment was carried out as follows. A colony growing on the surface of agar containing carbenicillin was picked into 10 ml prewarmed nutrient broth, and incubated with shaking at 30 °C. Samples removed at intervals were then plated on non-selective medium for viable count determinations and on selective agar to determine the proportion that was carbenicillin resistant. When the culture density had reached about 10^7 bacteria/ml (judged by the point at which turbidity first became visible), 5 ml culture was transferred to an empty, prewarmed tube at 42 °C, and a further 5 ml to another tube at 30 °C. Incubation was then continued at these temperatures and samples removed at intervals for plating at 30 °C on solid media as required. As long as the volume transferred did not exceed 5 ml the temperature shift to 42 °C took place within 2 min.

3. RESULTS

(i) *Survival of RP 1–1 in* E. coli

Although transfer of RP 1–1 between strains of *Pseudomonas aeruginosa* leads to stable inheritance of the plasmid by the recipients (Chandler & Krisnapillai, 1974; Stanisich & Richmond, 1975), this is not the case following transfer between *P. aeruginosa* and *E. coli* K12. Standard intergeneric crosses between *P. aeruginosa* PAO1670 (RP 1–1) and *E. coli* JC3272 produced $Carb^R[Str^R]$ colonies at a frequency of about 10^{-5}/donor. However, a large proportion of the bacteria in these colonies were dead and only about 1·0 % of the remaining viable organisms were penicillin resistant. Colonies derived in turn from these resistant bacteria were similarly composed of non-viable, viable and resistant bacteria, and this pattern of inheritance is very similar to that described previously for inheritance of RP 1–1 following transfer from *P. aeruginosa* to a different strain of *E. coli* K12 (Richmond & Sykes, 1972).

Although such *E. coli* (RP 1–1) derivatives are unstable with respect to the expression of drug resistance, they are capable of transferring RP 1–1, either to *E. coli* or to *Pseudomonas aeruginosa*. In *E. coli* recipients the plasmid is once more unstable, but carbenicillin resistant exconjugants of *P. aeruginosa* show stable inheritance. Further examination of these lines of *P. aeruginosa* showed that no change in the properties of RP 1–1 occurred during this period in *E. coli*.

In order to follow the replication of RP 1–1 in *E. coli* more accurately, a colony of *E. coli* JC3272 (RP 1–1) growing on agar containing carbenicillin was picked into antibiotic-free nutrient broth and the growth of the bacteria, and of the penicillin resistant component among them, was followed at 37 °C, by plating samples at intervals on unsupplemented nutrient agar and on agar containing carbenicillin. Fig. 1 shows the relative rates of growth of these two populations. The $Carb^R$ fraction of the culture amounted to about 0·4 % of the total at the beginning of the experiment and thereafter increased at about half the rate of the total population. Similar results were obtained when the growth of other colonies

picked from similar plates were examined in the same way (data not shown). A similar experiment carried out with *E. coli* AB1157 (RP 1–1) gave very similar results.

One possible explanation for the poor survival of RP 1–1 in *E. coli* is that the replication of this element is more sensitive to the temperature of incubation than is the host chromosome. To exclude this possibility the experiment was repeated

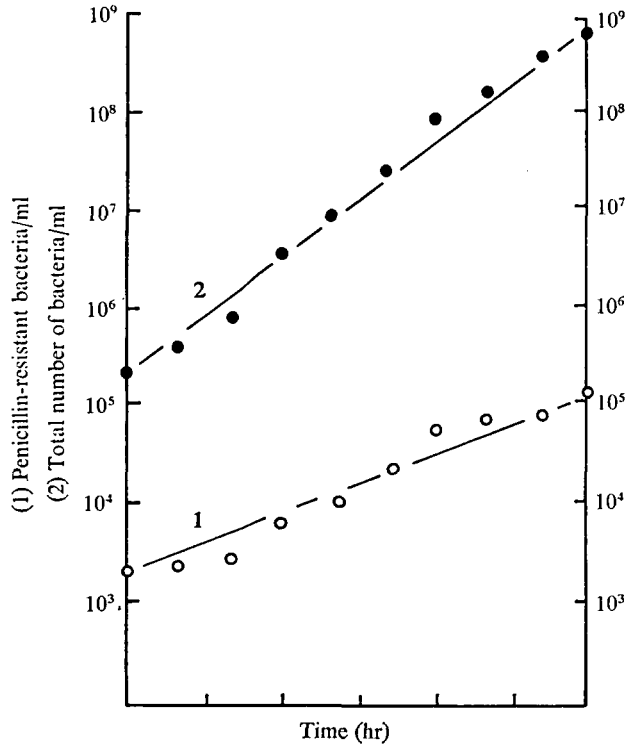

Fig. 1. Growth of *E. coli* JC3272 to which RP 1–1 has been transferred by conjugation from *Ps. aeruginosa* PA01670 (RP 1–1). Total culture (●); penicillin-resistant component (○).

and the rate of growth of the total population and of the $Carb^R$ bacteria in the culture was determined at 30 and 42 °C. In neither case was the rate of replication of the β-lactamase gene relative to the growth rate substantially altered, although the overall growth rate of the culture was, of course, influenced by the incubation temperature.

(ii) *DNA polymerase I and the replication of RP 1–1 in* E. coli

The poor survival of RP 1–1 in *E. coli* JC3272, and also in other lines of *E. coli* K12 (Richmond & Sykes, 1972), raises the possibility that this plasmid may use mechanisms of replication in *E. coli* other than those regarded as typical for self-transmissible plasmids.

Escherichia coli PE169 possesses a thermosensitive DNA polymerase I (Emmer-

son, McAthey & Strike, 1974), although the growth of this strain at permissive temperatures is normal. In addition to the *polA12* mutation it carries the markers *recC22*, *sbcA54* and *str-r*. This strain was chosen for these studies, rather than MM383 carrying *polA12* alone, since for reasons at present unclear, it gave a lower incidence of chromosomal integration of the *amp* gene from RP 1–1 (data not shown).

Pseudomonas aeruginosa PAO1670 (RP 1–1) was mated with *E. coli* PE169 under standard conditions for an intergeneric cross and $Carb^R[Str^R]$ progeny selected at 30 °C. Carbenicillin resistant colonies taken from the selection plates were then inoculated into nutrient broth without antibiotic at 30 °C and a standard temperature shift experiment carried out. At the time of the shift, the penicillin resistant proportion of the culture amounted to about 1·5 %. At the permissive temperature the penicillin resistant proportion of the culture remained approximately constant. At 42 °C, however, there was a decrease in the number of penicillin resistant bacteria (Fig. 2). RP 1–1 therefore seems to require a functional *polA* gene for replication and furthermore is actively destroyed in the absence of replication, since the number of $Carb^R$ bacteria decreased by almost half within one hour while the number of bacteria viable on non-selective agar (results not shown) more than doubled.

A similar experiment in which the survival of RP 1–1 transfer ability, rather than $Carb^R$, was measured in *E. coli* PE169 (RP 1–1) following a temperature shift to 42 °C showed that the transfer potential fell to about 1 % of its initial value within 5 h of the shift.

Two types of experiment support the view that this sharp decrease in the rate of replication of RP 1–1 at 42 °C in strain PE169 is due to a replication defect and not to some trivial cause, for example to a thermosensitivity of the β-lactamase specified by RP 1–1, or some other host-controlled defect. First, attempts to transfer RP 1–1 from *Pseudomonas aeruginosa* PAO1670 (RP 1–1) to non-conditional *polA* strains (e.g. *E. coli* JG112 and JG78) have always failed completely, as have attempted transfers to PE169 at 42 °C. Secondly, the replication of the $Carb^R$ portion of the *E. coli* (RP 1–1) culture is faster at 42 °C than at 30 °C in a pol^+ strain of *E. coli*, even though its proportion of the total culture does not change.

(iii) *The effect of chloramphenicol on the survival of RP 1–1 in* E. coli

To date the only class of plasmids whose replication has been shown to be dependent on a functional *polA* gene are small plasmids such as $ColE_1$ (Kingsbury & Helinski, 1970). Moreover, their replication differs from that of plasmids which are independent of *polA* since the process can continue in the presence of chloramphenicol (Clewell, 1972).

Chloramphenicol (50 μg/ml) was added to an exponentially-growing culture of *E. coli* JC3272 (RP 1–1) when the culture density had reached about 10^8 bacteria/ml. Samples taken at this point, and subsequently at 30 min intervals, were plated on unsupplemented nutrient agar to measure the total viable count and also on

nutrient agar containing carbenicillin to follow the ability of the cells to form carbenicillin resistant colonies. A portion of the culture incubated in the absence of chloramphenicol acted as control. Fig. 3 shows that addition of 50 μg chloramphenicol/ml halted the growth of JC3272 (RP 1–1) within 30 min and the viable count of the culture thereafter fell slowly as incubation in the presence of antibiotic continued. The Carb^R portion of the culture, which comprised about 0·5 %

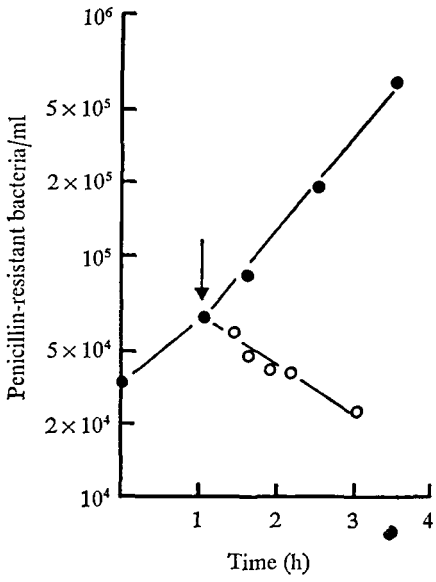

Fig. 2

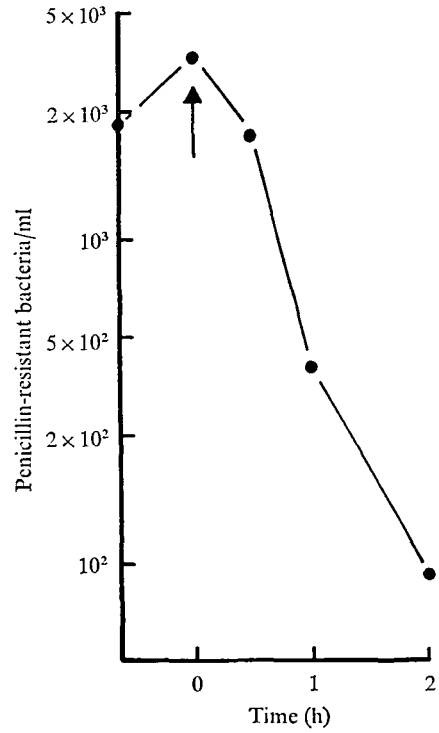

Fig. 3

Fig. 2. Survival of penicillin-resistant bacteria in cultures of *E. coli* PE169 (RP 1–1) at permissive and restrictive temperatures. Culture at permissive temperature (30 °C) (●); culture at restrictive temperature (42 °C) (○). The arrow indicates the point of the temperature shift.

Fig. 3. Effect of chloramphenicol on the survival of RP 1–1 in *E. coli* JC3272 (RP 1–1). Arrow indicates point of addition of chloramphenicol (50 μg/ml). In the absence of chloramphenicol the penicillin resistant portion of the cultures increased with a doubling time of about 35 min. In the presence of chloramphenicol the total number of bacteria/ml remained approximately constant for the 2 hr period of the experiment.

of the total when chloramphenicol was added, decreased much faster than the viability in the presence of the antibiotic. These results show therefore that despite the requirements of RP 1–1 replication in *E. coli* for DNA polymerase I, the process is nevertheless sensitive to inhibition by chloramphenicol and moreover (as in strain PE169) that RP 1–1 is broken down rapidly once replication ceases.

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(iv) *The effect of recA on the survival of RP 1–1 in* E. coli

E. coli colonies inheriting RP 1–1 are only recorded from matings of *Pseudomonas* donors and *E. coli* recipients if the latter are *recA*$^+$. In this respect, therefore, RP 1–1 differs from many R plasmids which transfer to *recA* recipients at frequencies close to those obtained with *rec*$^+$ strains (Low, 1968).

The role of the *recA* gene in the survival of RP 1–1 in *E. coli* has been studied using three temperature sensitive *recA* mutants, JC7741 and KL399 (Lloyd *et al.* 1974) and UB2627. With any of these three strains as recipient, no Carb^R progeny were obtained at 42 °C (limit of detection: 10^{-8}/donor) while at 30 °C transfer occurred at frequencies similar to that obtained with a *rec*$^+$ recipient.

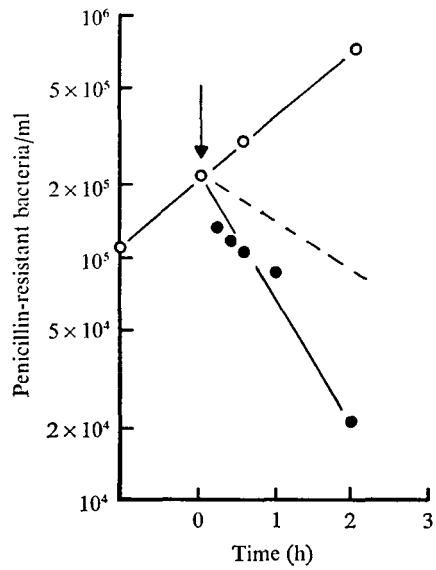

Fig. 4. Survival of RP 1–1 in *E. coli* 7741 (RP 1–1) at permissive (○) and restrictive (●) temperatures. The dashed line shows the survival of RP 1–1 in *E. coli* PE169 at 42 °C (data taken from Fig. 2).

The survival of RP 1–1 in a *recA*$_{ts}$ mutant at the permissive temperature occurred at about the same rate as in *rec*$^+$ strains (e.g. JC3272) whether survival was followed as the ability of the cells to form carbenicillin resistant colonies or by transfer ability. Transfer of the culture from permissive to restrictive conditions, however, resulted in rapid cessation of replication and disappearance of RP 1–1. A typical experiment involving *E. coli* JC7741 is shown in Fig. 4. *Pseudomonas aeruginosa* PAO1670 (RP 1–1) was mated with *E. coli* JC7741 and the Carb^R[Str^R] progeny selected at 30 °C. A single colony of *E. coli* JC7741 (RP 1–1) was picked from these plates into nutrient broth and the growth of the total population, and of the Carb^R component, was followed in a standard temperature shift experiment. At the point where the culture was divided the Carb^R bacteria comprised about

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0·1 % of the whole population. At 30 °C the replication of RP 1–1 continued at approximately the same rate as before the division of the culture into two parts, but at 42 °C the incidence of carbenicillin resistant bacteria fell rapidly, to reach about 30 % of the original value after an hour even though the whole population continued to grow at 42 °C with a doubling time of about 50 min. RP 1–1 therefore seems not only unable to replicate but to be unstable in *E. coli* $recA_{ts}$ at the restrictive temperature, and under these conditions the rate of destruction of the plasmid is even more rapid in this mutant than in *E. coli* $polA_{ts}$ after transfer to 42 °C (shown by dashed line in Fig. 4).

(v) *The effect of* recB *and* recC, *and of suppressors of these genes, on the survival of RP 1–1 in* E. coli

Mating experiments between *Pseudomonas aeruginosa* PAO1670 (RP 1–1) and either *E. coli recB* (strain JC5743) or *E. coli recC* (strain JC5489) always failed to give $Carb^R$ *E. coli*, but otherwise isogenic temperature sensitive mutants of *recB* and *recC* were not available to decide whether this result was due to a failure in the transfer process or in subsequent replication. The only *E. coli* strains carrying *recB* or *recC* that would allow survival of RP 1–1 after transfer from *Pseudomonas aeruginosa* were mutants which also carried *sbc* suppressor genes for *recB* or *recC* (e.g. strains JC7620 and PE169). The behaviour of RP 1–1 in *E. coli* PE169 (*recC sbcA*) has already been described (see Fig. 2). Following transfer to the restrictive temperature, RP 1–1 was lost, but nevertheless at a rate less than that found in *E. coli* $recA_{ts}$ at a similar temperature (Fig. 4). If the data in Figs. 2 and 4 are recalculated to exclude the effects of different growth rates, the disappearance of RP 1–1 at 42 °C was approximately five-fold faster in strain JC7741 than in PE169.

The effect of the *recB recC sbcB* genotype was examined by mating *P. aeruginosa* PAO1670 (RP 1–1) with *E. coli* JC7620 under standard conditions for an intergeneric cross and selecting $Carb^R[Str^R]$ progeny. Three individual colonies of JC7620 (RP 1–1) were picked from these plates and grown in nutrient broth to determine the survival of RP 1–1 in this strain when growing exponentially. The behaviour of cultures of *E. coli* AB1157 (RP 1–1) and *E. coli* JC3272 (RP 1–1) (a fresh isolate of each constructed as described earlier) were used as controls. In these experiments about 0·3 % of the bacteria in the control cultures were $Carb^R$ when tested at a cell density of 10^8 bacteria/ml. The three cultures of JC7620 (RP 1–1) grown under similar conditions gave equivalent values of about 2 % $Carb^R$ bacteria. RP 1–1 seems therefore to survive about ten-fold better in a *recB recC sbcB* background (and about six-fold better in a *recC sbcA* background) than it does in any *E. coli* strain so far examined, including strain AB1157, the immediate Rec^+ parent of JC7620 (see Table 2).

The poor net replication of RP 1–1 in *E. coli* AB1157 and JC3272 when compared with JC7620 was followed by comparing the proportion of $Carb^R$ bacteria in appropriate cultures growing in non-selective liquid medium. Fig. 5 shows that the proportion of $Carb^R$ bacteria in cultures of *E. coli* JC3272 (RP 1–1) decreased

at about three times the rate found in strain JC7620, and the behaviour of *E. coli* AB1157 (RP 1–1) was very similar to that of JC3272 (RP 1–1) (data not shown).

The relative stability of RP 1–1 in JC7620, when compared with JC3272 and with AB1157, could be due either to more effective replication or to reduced destruction in the *rec* mutant. In order to investigate this, chloramphenicol

Table 2. *Phenotypic characteristics of various strains of* E. coli *mutated in their Rec functions with respect to the survival and transfer of RP 1–1*

E. coli strain	Relevant genotype	Growth temperature (°C)	Exonuclease activity*			Frequency of RP 1–1 transfer to E. coli R^-†	Maintenance of RP 1–1‡ (%)
			I	V	VIII		
JC3272	*rec*$^+$	37	+	+	(±)	10^{-6}	0·3
JC7620	*recB21 recC22 sbcB12*	37	−	−	(±)	10^{-3}	2·0
JC5743	*recB21*	37	+	−	(±)	$< 10^{-8}$	0
JC5489	*recC22*	37	+	−	(±)	10^{-5}	0·1
JC7741	$recA_{ts}$	30	+	+	(±)	10^{-6}	0·03
JC7741	$recA_{ts}$	42	+	+ + +	(±)	$< 10^{-8}$	0
UB2627	$recA_{ts}$	30	+	+	(±)	10^{-6}	0·3
UB2627	$recA_{ts}$	42	+	+ + +	(±)	$< 10^{-8}$	0
KL399	$recA_{ts}$	30	+	+	(±)	10^{-6}	0·3
KL399	$recA_{ts}$	42	+	+ + +	(±)	$< 10^{-8}$	0
PE169	*recC22 sbcA54* $polA_{ts}$	30	+	−	+	10^{-3}	1·5
PE169	*recC22 sbcA54* $polA_{ts}$	42	+	−	+	$< 10^{-8}$	0

Abbreviations: +, activity detectable; −, no enzyme produced; (±), no detectable enzyme.

* Data derived from Clark, A. J. (1973*b*); and Emmerson *et al.* (1974).

† Quoted per donor organism using *E. coli* JC3272 Rif^R as recipient.

‡ Measured as the proportion (%) of the penicillin resistant bacteria in a colony picked directly from agar containing 500 μg carbenicillin/ml.

(50 μg/ml) was added to exponentially growing cultures of *E. coli* AB1157 (RP 1–1) JC3272 (RP 1–1) and JC7620 (RP 1–1) as described previously (see above). Samples were taken immediately following addition of the antibiotic, and subsequently at 30 min intervals, and plated both to determine the total viable count and the proportion of $Carb^R$ bacteria present. A portion of the cultures incubated without chloramphenicol acted as controls. The proportion of $Carb^R$ bacteria in the cultures of JC3272 (RP 1–1) and AB1157 (RP 1–1) fell to about 2% of their initial values within 2 h of the addition of chloramphenicol, while the equivalent decrease in JC620 (RP 1–1) was only about 50% during the same period (Fig. 6: data for AB1157 (RP 1–1) not shown). We conclude that RP 1–1 cannot replicate in the absence of protein synthesis.

4. DISCUSSION

The results obtained with the various *polA* mutants shows that DNA polymerase I is necessary for the survival of RP 1–1 in *E. coli*. In this respect, therefore, this plasmid is similar to the $colE_1$ plasmid (Kingsbury & Helinski, 1970) rather than to the commonly encountered self-transmissible R plasmids which rely on

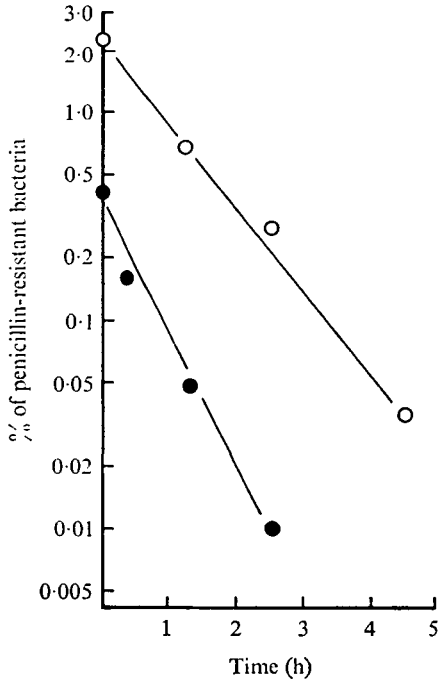

Fig. 5

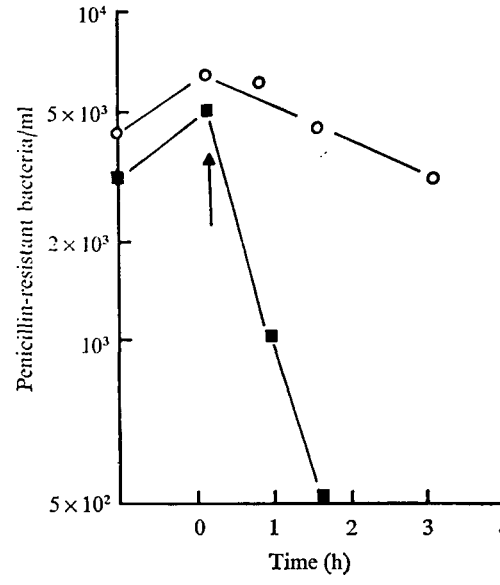

Fig. 6

Fig. 5. Survival of RP 1–1 in cultures of *E. coli* JC3272 (RP 1–1) (●) and JC7620 (RP 1–1) (○) growing in non-selective medium at 37 °C. The data are plotted as the proportion (%) of the total population that expresses penicillin resistance.

Fig. 6. Comparison of the survival of RP 1–1 in *E. coli* JC3272 (RP 1–1) (■) and JC7620 (RP 1–1) (○) following addition of chloramphenicol (50 μg/ml). The arrow indicates the point of addition of the antibiotic. In the absence of antibiotic both cultures grew with a doubling time of about 35 min. In the presence of chloramphenicol the total population of both cultures remained approximately constant over the 4 hr period following the addition of the antibiotic.

DNA polymerase III for their replication in *E. coli* and *P. aeruginosa*. Unlike the colE$_1$ plasmid, however, RP 1–1 replication is inhibited by chloramphenicol.

Inhibition of RP 1–1 replication, whether caused by inactivation of DNA polymerase I or by the action of chloramphenicol, leads to the disappearance of the plasmid in *E. coli*. Furthermore, the kinetics of plasmid loss argue that RP 1–1 is actively destroyed rather than merely diluted out as the culture grows.

Studies with *E. coli* mutated in the *recA*, *recB*, *recC*, *sbcA* and *sbcB* genes suggests that the destruction of RP 1–1 that is observed when plasmid replication ceases, is due to exonuclease action since the *rec* and *sbc* genes are known to influence the expression of these DNA degrading enzymes (Barbour *et al.* 1970; Kushner *et al.* 1971; 1972; Clark, 1973*a*). Table 2 correlates the expected levels of exonuclease I, V and VIII activity in the mutant strains with the observed survival properties of RP 1–1 in the same strains. Those mutants with high levels of exonuclease activity are those in which RP 1–1 is least stable, while the plasmid

survives best in *E. coli* JC7620, a mutant in which the level of exonuclease activity is likely to be the lowest of all the strains examined here (Kushner *et al.* 1972).

The sensitivity of RP 1–1 to exonucleases may explain why it has proved impossible so far to isolate this plasmid in cleared lysates of *E. coli* (Ingram *et al.* 1971). Cessation of plasmid replication leads to an increased net destruction of the plasmid; and there is no reason to expect that this hydrolysis would necessarily cease when the bacteria are disrupted to prepare DNA for centrifugation.

In view of the behaviour of RP 1–1 in the *E. coli pol* and *rec* mutants examined here, the simplest explanation of the observed instability of this plasmid in *E. coli* lines is that its survival is the outcome of a balance between DNA polymerase I dependent replication and exonuclease destruction. In wild type *E. coli* the net balance of the two processes appears to be insufficient to maintain RP 1–1 in the bacterial population for long periods in the absence of penicillin selection. Even in the presence of penicillin *E. coli* RP 1–1 clones contain many plasmid-less bacteria (Ingram *et al.* 1972).

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