JOURNAL OF BACTERIOLOGY, Sept. 1989, p. 4785–4791
0021-9193/89/094785-07$02.00/0

Vol. 171, No. 9

# Participation of the Lytic Replicon in Bacteriophage P1 Plasmid Maintenance

MICHAEL B. YARMOLINSKY,[1]* EGON B. HANSEN,[1]† SAMINA JAFRI,[1]‡ AND DHRUBA K. CHATTORAJ[2]§

*Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892[1] and Laboratory of Molecular Biology, LBI Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701[2]*

Received 7 February 1989/Accepted 15 June 1989

**P1 bacteriophage carries at least two replicons: a plasmid replicon and a viral lytic replicon. Since the isolated plasmid replicon can maintain itself stably at the low copy number characteristic of intact P1 prophage, it has been assumed that this replicon is responsible for driving prophage replication. We provide evidence that when replication from the plasmid replicon is prevented, prophage replication continues, albeit at a reduced rate. The residual plasmid replication is due to incomplete repression of the lytic replicon by the *c*1 immunity repressor. Incomplete repression was particularly evident in lysogens of the thermoinducible P1 *c*1.100 prophage, whose replication at 32°C remained almost unaffected when use of the plasmid replicon was prevented. Moreover, the average plasmid copy number of P1 in a P1 *c*1.100 lysogen was elevated with respect to the copy number of P1 *c*1$^+$. The capacity of the lytic replicon to act as an auxiliary in plasmid maintenance may contribute to the extraordinary stability of P1 plasmid prophage.**

P1 prophage replicates in *Escherichia coli* as a stable, low-copy-number, 90-kilobase (kb) plasmid. (See reference 47 for all but selected references to primary sources concerning P1.) A 4-kb segment of P1 DNA, the plasmid replicon, suffices to support stable plasmid maintenance at the low copy number. This segment consists of a 1.5-kb module, responsible for controlled replication from an origin known as *oriR*, and an adjacent 2.5-kb module, responsible for partitioning of the daughter plasmids at cell division.

Replication from *oriR* requires the *oriR*-specific initiator, the product of the adjacent *repA* gene. A regulatory element, *incA*, is situated immediately downstream of *repA* and consists of a set of sites that, like *oriR* itself, bind initiator and interfere with *oriR* function. Also required for replication from *oriR* is the bacterial *dnaA* gene product, for which presumptive binding sites in *oriR* are present.

P1 lytic replication initiates at an origin known as *oriL* (14, 22, 38). The lytic replicon appears not to require *dnaA* function (23). It does depend on the integrity of an open reading frame (*repL*) within which *oriL* is probably embedded (22, 38). Control of the lytic replicon is exerted by the *c*1 immunity repressor acting at an operator (designated Op53) to repress an operon that includes *repL* and an additional gene, not essential for replication, upstream of it (22, 38). Repression by *c*1 is antagonized by *ant*, normally controlled in P1 lysogens by the secondary immunity gene *c*4. The products of *c*1 and *c*4 do not inhibit replication from the plasmid replicon (5), nor does the control element of the plasmid replicon (*incA*) inhibit the lytic replicon (unpublished results).

We have examined the growth of P1 lysogens when either or both replicons are inhibited. Replication from *oriR* was inhibited in one of three ways: (i) mutational inactivation of the bacterial *dnaA* function in a host whose own *dnaA* requirement for DNA replication is bypassed by the use of a *dnaA*-independent replicon (23); (ii) raising the temperature of a P1 *repA*(Am) lysogen bearing a thermosensitive nonsense suppressor; (iii) infection with a λ-pBR322-*incA* chimera under conditions of λ repression, so as to provide a stable source of excess *incA*. Replication from *oriL*, normally inhibited in P1 lysogens, was further restrained by additional *c*1 repressor supplied from a cloned P1 DNA fragment. Introduction of a cloned P1 DNA fragment bearing *c*4 also appeared to be effective. Definitive elimination of lytic replicon function was achieved by use of a 2-kb deletion from *Eco*RI-17 into *Eco*RI-14 (22). This deletion eliminates the entire *repL* open reading frame.

The results reported here provide evidence for a modest contribution of the lytic replicon to normal P1 prophage replication and indicate conditions under which the lytic replicon is permitted a major contribution.

## MATERIALS AND METHODS

**Strains and microbiological methods.** *E. coli* K-12 strains, phages, and plasmids that were used in the experiments are listed in Table 1. Bacteria were generally grown with agitation in LB broth containing, per liter, 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl. Broth was supplemented, when appropriate, with 0.2% maltose and with 10 mM $MgSO_4$ to induce receptor sites for and stabilize phage λ, respectively, or with 5 mM $CaCl_2$ to stabilize and promote adsorption of phage P1. Antibiotics were used at the following concentrations: chloramphenicol, 12.5 μg/ml; kanamycin, 25 μg/ml; and ampicillin, 50 μg/ml. P1 modification of λ phages was accomplished by growing plate stocks on hosts that carry P1Cm *cry* (33). Transformations, phage crosses, and in vitro packaging were performed by standard procedures (34).

Viable bacteria in unchilled samples of broth culture were counted as colony formers by the pour plate method. It was found that the viabilities of various strains of *E. coli* K-12 in suspensions at 43°C were reduced as much as 100-fold by sudden chilling on ice; the loss of viability depended on the duration of the growth period at the elevated temperature.

* Corresponding author.
† Present address: Chr. Hansen's Laboratorium A/S, Genetisk Afdeling, DK2970 Hørsholm, Denmark.
‡ Present address: Genetics Ph.D. Program, University of Iowa, Iowa City, IA 52242.
§ Present address: Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD 20892.

TABLE 1. Bacterial strains, phages, and plasmids

| Strain, phage, or plasmid | Relevant characteristic(s)[a] | Reference or source |
|---|---|---|
| *E. coli* K-12 | | |
| BR157 | *supD43 supD74*(Ts) *recA56 srl*::Tn*10* | P1 transductant of MX397 |
| BR469 | *sup*$^+$, plates P1 well | 17 |
| BR1622 | N1494(λ *c*I$^+$-P1:5R *Bam*HI-9)$_{loxB}$ | 11 |
| BR1623 | N1494(P1Cm)$_{loxB}$ | See Materials and Methods |
| BR1813 | *recA* (λ *imm*$^{21}$) derivative of MC4100 | G. Weinstock (34) |
| BR1879 | *supF trp*::Tn*10*, plates P1 well | P1 transductant of BR469 |
| BR1888 | *sup*$^+$ *malB*[λ$^r$] Δ*lon cpsE*3::Tn*10* (Δ*lon* plates P1 clear [41]) | S. Gottesman |
| EH4132 | CM1565/P1Cm *c*1$^+$ | 22 |
| EH4133 | CM1565/P1Cm *c*1.100 | 22 |
| MX397 (BR1867) | *supD43 supD74*(Ts) | 28 |
| N100 (152, DJ14) | *recA3 sup*$^+$ | 21 |
| NS1494 (BR1739) | *dna46*(Ts) *recA56* | 40 |
| Phages and phage-plasmids | | |
| P1Cm | P1 with Tn*9* insert at IS*1* site | 24 |
| P1Cm r$^-$ m$^-$ | Recombinant between P1Cm and P1 r$^-$ m$^-$ | 20 |
| p1Cm *c*1.100 | Thermoinducible P1Cm | 31 |
| P1Cm *c*1.100 r$^-$ m$^-$ | Recombinant between above phages | |
| P1Cm *c*1.100 Δ*repL* | | 22 |
| P1Cm r$^-$ m$^-$ *repA103* | P1Cm r$^-$ m$^-$ with conditional (Am) mutation in *repA* | See Materials and Methods |
| λ-P1*c*4*b* | λ *imm*$^{21}$ *b*515 *b*519 *nin*5 srlλ3- P1:*Eco*RI-9[*c*4], -22 | 37 |
| λ-P1:5R*Bam*HI-9 | λ-mini-P1 that, bearing *loxP*, can integrate via *loxP* × *loxB* recombination | 6 |
| λ-pBR322 | (Ap) *imm*$^{21}$ phage-plasmid | See Materials and Methods |
| λ-pBR322-*incA* | (Ap) *imm*$^{21}$ phage-plasmid *incA* clone | See Materials and Methods |
| Plasmids | | |
| pACYC184(Km) | (Km) version of multicopy vector pACYC184 | See Materials and Methods |
| pACYC184(Km)-*c*1 | Multicopy (Km) *c*1 clone | See Materials and Methods |
| pALA12 | 851-bp *incA* clone in pBR322 (Ap) | 4 |
| pALA18 | 306-bp *incA* clone in pBR322 (Ap) | 2 |
| pALA93 | pALA95 with 306-bp *incA* insert | See Materials and Methods |
| pALA95 | 750-bp *c*4 Δ*sim* clone in pBR322 (Ap) | |
| pBR322 | Multicopy (Ap Tc) vector | 8 |
| pCM960 | *oriC* plasmid | 44 |

[a] The names of integrated prophages and of antibiotics to which resistance is conferred (Cm, chloramphenicol; Km, kanamycin; Ap, ampicillin; Tc, tetracycline) are in parentheses.

**Construction of *dnaA*(Ts) *E. coli* integratively suppressed by P1.** We integrated P1Cm into the *E. coli* chromosome in both orientations at *loxB* by the process of prophage displacement as described by Sternberg et al. (40). The parental strains carried λ Δ(*att*)*imm*$^{21}$-P1:*Eco*RI-7 prophage (hereafter called λ-P1:7) integrated at *loxB* in orientations determined by transductional analysis according to Sternberg et al. (40).

**Construction of P1 mutants.** P1Cm r$^-$ m$^-$ *repA103*(Am) was constructed by first crossing P1Cm *c*1.100 r$^-$ m$^-$ with P1*vir* 23.115(Am) (45) to yield, among the recombinants capable of lysogenization, P1Cm *c*1.100 r$^-$ m$^-$ 23.115. The 23.115 mutation, mapped to *Eco*RI-5 of P1 (35), results in a suppressible defect (46). A P1Cm *c*1.100 r$^-$ m$^-$ 23.115 lysogen was infected with λ *imm*$^{21}$-P1:5R *repA103* (7) and thermally induced. P1 23$^+$ recombinants were selected as plaque formers on the nonsuppressing λ$^r$ P1$^s$ strain BR1888. The phage in individual plaques were tested for their capacity to lysogenize stably a pair of isogenic suppressing and nonsuppressing strains (BR469 and BR1879) and a *recA* nonsuppressing strain (N100) that carried either a pBR322 vector or pBR322 bearing a functional *repA* gene (pALA69 [7]). Phage that lysogenized *E. coli* more efficiently if the bacteria carried either an amber suppressor or a source of RepA protein were assumed to be P1Cm *c*1.100 r$^-$ m$^-$ *repA103*. A *c*1$^+$ version of these phage, prepared by crossing in the wild-type allele from a λ *imm*$^{434}$-P1:7 clone (37), was used in the subsequent construction of *supD*(Ts) lysogens (28).

**Construction of vectors carrying *c*1, *c*4, or *c*4 and *incA*.** pACYC184(Cm)-*c*1 is a clone of *c*1 without the neighboring *loxP* (which might otherwise allow site-specific recombination with P1 [39]) and was provided by Nat Sternberg. pACYC184(Km)-*c*1 was constructed by cleavage of pACYC184(Cm)-*c*1 at the unique *Eco*RI site within the *cat* (chloramphenicol acetyl transferase) gene and insertion of a 0.8-kb *kan* cassette from pUC-4K (Pharmacia, Inc.). These plasmids confer thermoresistance on a *recA* host carrying P1 *c*1.100. pACYC184(Km) is a *kan* variant of pACYC184 (10), constructed as above.

A pBR322 clone of *c*4, pALA95, was constructed from pBD101 (provided by June Scott), a pBR322 clone of a 0.75-kb fragment of P1 DNA that bears the *c*4 gene but not the closely linked *sim* (superimmunity) gene. The pBD101 plasmid was cleaved with *Bam*HI and religated so as to eliminate about 4.0 kb from pBD101, including most of the Tn*1000* (γδ) transposon. The absence of the *sim* gene and the presence of the *c*4 gene were confirmed as described by Devlin et al. (16), using their pBHD6 as a *sim*$^+$ control (which prevents plaque formation by P1 *c*1.55) and using P1Cm *c*4.32 (32) to test for *c*4 complementation. A clone of *c*4 and *incA*, pALA93, was constructed from pALA95 by inserting the 306-bp *Bam*HI *incA* fragment from pALA18 (2) into the *Bam*HI site of pALA95.

**Construction and use of λ-pBR322-*incA* and λ-pBR322 chimeras.** Lambda-pBR322-*incA* and λ-pBR322 were constructed from the *Bam*HI-cleaved *imm*$^{21}$ vector λD69 (27) and similarly digested pALA62 (pBR322 carrying two copies of the *Bam*HI *incA* fragment). The presence of the pBR322

TABLE 2. Integrative suppression of a *dnaA46*(Ts) mutation by replicons of P1

| Integrated replicons[a] | Surviving fraction in the presence of[b]: | | | |
|---|---|---|---|---|
| | Vector (pBR322) | *incA* (pALA12) | *c4* (pALA95 or λ-P1:*c4b*) | *c4* and *incA* (pALA93 or λ-P1:*c4b* and pALA12) |
| None | $3 \times 10^{-6}$ | | | |
| λ-P1:5R*Bam*HI-9 | 1 | $1 \times 10^{-5}$ | | |
| P1Cm | 1 | 0.4[c] | 1 | $2 \times 10^{-4}$–$9 \times 10^{-4}$ |

[a] The *dnaA46* host was NS1494. The strains of rows 2 and 3 are derivatives of BR1622 and BR1623, respectively, in which the P1 replicons were integrated at *loxB*.

[b] The surviving fraction is the ratio of bacterial colony formers grown under conditions selective for the carried plasmids at 42°C to those at 32°C. Carried plasmids or integrated λ prophages bearing regulatory elements are listed at the head of each column in parentheses. The λ-P1:*c4b* prophage was integrated at λ *att*.

[c] Colony size at 42°C much reduced relative to colony size at 30°C.

insert within the vector results in a relative clarity of the plaques made by the phage and the loss of *int* gene function (as shown by the red plaque test of Enquist and Weisberg [18]). The additional presence of *incA* in certain of the phages was detected by a test for incompatibility exerted towards a mini-P1 bearing a *cat* gene, pALA318 (1), and verified by restriction analysis and Southern hybridization with *incA* probes. This analysis revealed that the fragments are arranged such that the *Hin*dIII cleavage site within the pBR322 is towards the left arm of the λ in both the λ-pBR322 and λ-pBR322-*incA*. The latter plasmid includes a single copy of *incA* inserted at the left end of the pBR322 insert.

The λ-pBR322 phages established themselves as stably maintained plasmids in λ lysogens; the loss rate, as measured by segregation of ampicillin-sensitive colony formers following growth on nonselective media, was no more than 0.1% per bacterial generation.

**Plasmid copy number determinations.** The ratio of P1 DNA to DNA of the *E. coli* chromosomal origin, *oriC*, was determined by blot hybridization as described in detail by Hansen (22). Nitrocellulose filters bearing DNA of the lysogenic CM1565 (for the genotype, see reference 22) were prepared in duplicate. One filter was hybridized to $^{32}$P-labeled P1 DNA, the other was hybridized to $^{32}$P-labeled *oriC* (pCM960) DNA, and the radioactivities retained after washing at high stringency were compared.

## RESULTS

**Functioning of the lytic replicon of an integrated P1 prophage.** A previous report from this laboratory established that the plasmid replicon of P1 requires the bacterial *dnaA* function, whereas the replicon responsible for the lytic replication of P1 appears to be independent of *dnaA* (23). It was also found that a complete P1 prophage, when chromosomally integrated at a site called *loxB*, can support the replication of a *dnaA*-deficient *E. coli*, suggesting that bacterial survival in this case is due to incomplete repression of the lytic replicon in the prophage state. Although the lytic replicon during vegetative growth generates rolling circles (9, 13), which would be lethal if initiated within the bacterial chromosome, theta forms of replication intermediates are present during the early stages of the lytic cycle (13). Alternatively, survival could result from expression of a third P1 replicon (19).

Preliminary evidence that the relevant P1 replicon is the lytic replicon was obtained by the Tn*5* insertional mutagenesis of P1Cm. The P1 was chromosomally integrated at *loxB* by prophage displacement in NS1494(λ-P1:7) of Sternberg et al. (40), a *recA56* strain of *E. coli* bearing the *dnaA46* mutation. Lysogens of unmutagenized P1 grow at 42°C, a temperature nonpermissive for growth of the unlysogenized parent. Introduction of a pBR322-*incA* plasmid (pALA12) was required to render *oriR* inactive (Table 2, compare rows 1, 2, and 3), presumably owing to residual *dnaA* gene function at 42°C. Among five lysogens screened, one was severely growth impaired at 42°C, which suggested that it had acquired a Tn*5* insert that interfered with the alternative replicon (data not shown). The insert is located within *Eco*RI-17, adjacent to the essential portion of the lytic replicon, between positions 3600 and 3900 in the numbering system of Hansen (22). It is similarly located to a P1 *Eco*RI-17::Tn*5* insert obtained by Windle. The latter was shown by Hansen (28) to cause a delay of 2 to 4 min in the induction of lytic replication after thermal derepression of P1 *c*1.100 lysogens. Restriction mapping located the other Tn*5* inserts at various distant sites. These results lead us to propose that the *incA*-insensitive replicon that is weakly expressed in an integrated (unmutagenized) P1 prophage is the lytic replicon.

Partial expression of the lytic replicon could be the consequence of readthrough from an upstream promoter that is either *c*1 insensitive or not fully repressed by *c*1, possibly as a consequence of a low level of *ant* expression, the determinant of an antagonist of *c*1 repression. We found that introduction of a source of additional *c*4 protein, the negative regulator of *ant*, eliminated integrative suppression by P1 in the presence of excess *incA*. The *c*4 protein was supplied from a clone of *c*4 or from a considerably larger *Eco*RI-9 insert into a pBR325 plasmid or a λ prophage (Table 2, row 3). That this effect is due specifically to the modest increase in the gene dosage of *c*4 in the latter case was substantiated by additional experiments in which the wild-type *c*4 was replaced by a *c*4.32 allele (data not shown). Integrative suppression was largely eliminated by the presence of excess gene copies of both *incA* and *c*4. Individually, they were relatively ineffective.

**Functioning of the lytic replicon in P1 plasmid replication at 43°C.** To determine whether the lytic replicon can participate in the replication of P1 plasmid, it was necessary to inhibit *oriR* function conditionally. We used the amber mutation *repA103* (7) and a mutant strain of *E. coli* in which the *supD* suppressor is thermosensitive. The mutation *repA103* was transferred from λ-P1:5R to P1Cm as described in Materials and Methods, and lysogens of *repA103* and *rep*$^+$ versions of P1Cm in the *supD*(Ts) host (MX397) were prepared. At the permissive temperature (32°C) the doubling time ($t_{1/2}$) of both lysogens was about 44 min. Upon shifting to the nonpermissive temperature in the presence of chloramphen-

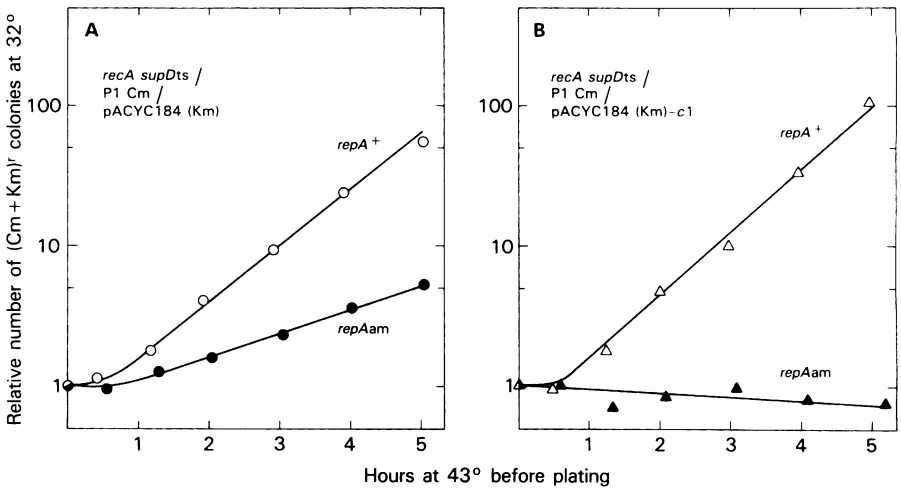


FIG. 1. Effect of additional copies of immunity gene *c*1 on the growth of P1 lysogens when the plasmid replicon is selectively inhibited at 43°C. P1Cm lysogens of *supD*(Ts) *recA* strain BR157 carrying either pACYC184(Km) (A) or pACYC184(Km)-*c*1 (B) were grown to log phase at 32°C in LB supplemented with chloramphenicol and kanamycin. Inhibition of the plasmid replicon was initiated by dilution into fresh medium at 43°C without chloramphenicol. Samples were plated at intervals on LB agar supplemented with chloramphenicol and kanamycin, and colonies were counted after ca. 40 h at 32°C. Open symbols, Derivatives of BR157/P1Cm; closed symbols, derivatives of BR157/P1Cm $r^- m^-$ *repA103*.

icol (to select the bacteria that retain P1Cm), there was an increase in the growth rate of the P1Cm *repA*$^+$ lysogens to a $t_{1/2}$ of 34 min, whereas the growth rate of the P1Cm *repA103* lysogens was diminished to a $t_{1/2}$ of 110 min.

The residual growth of the P1Cm *repA103* lysogens might result from incomplete loss of suppressor activity or partial derepression of the lytic replicon. In the latter case, the presence of additional *c*1 immunity repressor would be expected to cause a complete cessation of growth of the P1Cm *repA103* lysogen at 43°C, since the lytic replicon is under direct *c*1 control. We observed a complete cessation of growth of a *recA supD*(Ts) strain carrying both P1Cm *repA103* and a source of additional *c*1 repressor when the culture was shifted to 43°C (cf. Fig. 1A and B). This result supports the hypothesis that the lytic replicon can function in the replication of P1 plasmid as it does in the replication of integrated P1.

**Functioning of the lytic replicon in P1 plasmid replication at 32°C and influence of the *c*1 allele.** To confirm and extend these results, we determined the extent to which the lytic replicon functions at 32°C, after blocking the P1 plasmid replicon by homoimmune infection with a λ-pBR322-*incA* plasmid chimera. In this kind of experiment, the growth inhibition of the *recA* P1 lysogens is not conditional because the inhibitory chimera λ-pBR322-*incA* establishes itself as a stable plasmid.

Experiments were performed in which the prophage was either P1 *c*1$^+$ or an otherwise isogenic, thermoinducible P1 *c*1.100. Growth curves for a P1 *c*1.100 lysogen are shown in Fig. 2A. Following infection, samples of cultures grown with selection for the P1 prophage were plated with selection for only the λ-pBR322-*incA* or for the control λ-pBR322. Colonies that arose from λ-pBR322-*incA*-infected lysogens were composed primarily of segregants that had lost P1. The λ-pBR322-infected controls retained P1. The growth rate of P1 lysogens in which the plasmid replicon had been blocked by λ-pBR322-*incA* infection at 32°C appeared no less than the growth rate of the *supD*(Ts)/P1 *repA103* lysogens tested at 43°C. Moreover, when the prophage of the λ-pBR322-*incA*-infected lysogens carried the *c*1.100 allele, they grew at a rate that closely approached the growth rate of the λ-pBR322-infected control. This diminished inhibition was made particularly evident by plating the culture samples with selection for the P1 prophage. The colonies that appeared varied somewhat in size, but it was not possible on the basis of colony size (as had been the case with lysogens of P1 *c*1$^+$) to distinguish between those that arose from the occasional uninfected bacterium and those that arose from bacteria that had been infected with λ-pBR322-*incA*. We conclude that the *c*1-sensitive replicon can drive P1 plasmid replication at both 32 and 43°C, irrespective of the technique used to eliminate replication from *oriR*. We further conclude that by varying the *c*1 allele of P1, the primary replication origin of the plasmid can be shifted.

**Relative copy numbers of P1 *c*1.100 and P1 *c*1$^+$ at 32°C.** A suggestion that the lytic replicon of P1 *c*1.100 lysogens can be the major contributor to prophage replication was made on the basis of measurements of the maximal antibiotic resistance conferred by P1Cm and P1Km prophages bearing the *c*1.100 mutation (42). We confirmed the validity of this suggestion on the basis of direct measurements of the relative copy numbers of P1 *c*1.100 and P1 *c*1$^+$ plasmids. The ratio of P1 DNA to DNA of the *E. coli* chromosomal origin *oriC* was determined by slot blot hybridization. On this basis, the copy number of P1 *c*1.100 prophage in EH4133 was found to be five times the copy number of P1 *c*1$^+$ prophage in EH4132. These results imply that in lysogens of P1 *c*1.100 at 32°C, the lytic replicon is on the average considerably more active than the plasmid replicon. It is for this reason that there is little interference with the growth at 32°C of P1 *c*1.100 lysogens when the plasmid replicon is blocked.

**P1 *c*1.100 Δ*repL* prophage lacked the capacity for *incA*-resistant replication.** To establish unequivocally that the alternative replicon responsible for residual plasmid replication in the presence of λ-pBR322-*incA* is the lytic replicon and not another immunity-sensitive P1 replicon that is as yet uncharacterized, experiments were undertaken with a known lytic replicon deletion. A comparison was made between the growth rates of infected lysogens of P1Cm

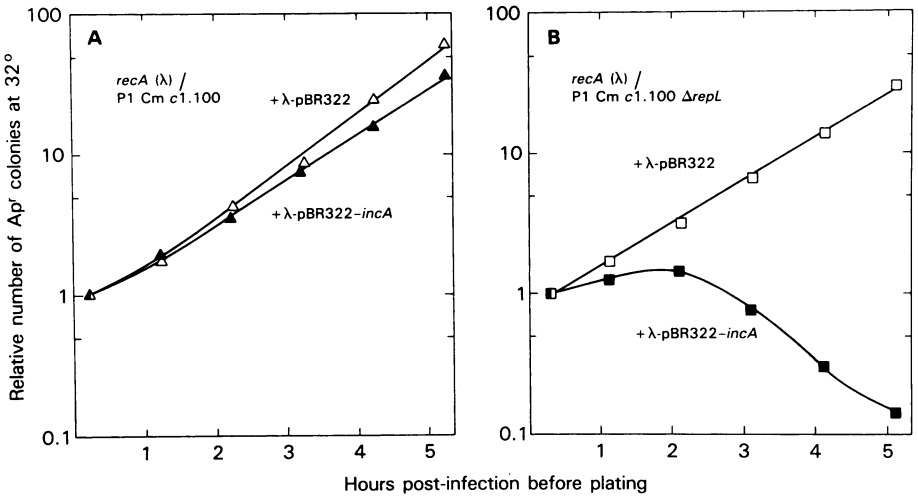


FIG. 2. Effect of selective inhibition of the plasmid replicon on the growth of a P1 *c*1.100 Δ*repL* lysogen at 32°C. P1Cm lysogens of BR1813, either *rep*$^+$ (A) or Δ*repL* (B) were infected with λ *imm*$^{21}$-pBR322 (open symbols) or with λ *imm*$^{21}$-pBR322-*incA* (closed symbols). BR1813 harbors a λ *imm*$^{21}$ prophage which assures repression of λ functions of the infecting phages. Bacteria were grown to log phase at 32°C in LB supplemented with chloramphenicol, concentrated by low-speed centrifugation at room temperature, suspended in fresh broth, and exposed to phage for a 10-min period without agitation at a multiplicity of exposure of ca. 20. They were then diluted to a density of about 500 viable cells per ml in LB supplemented with ampicillin and chloramphenicol. Samples were taken for the assay of colony formers on LB agar supplemented with ampicillin. Colonies were counted after ca. 24 h at 32°C.

*c*1.100 and of a P1Cm *c*1.100 from which had been deleted a 2-kb segment between a Tn*5* situated in *Eco*RI-17 and an IS*50* situated at the proximal end of *Eco*RI-14 (22). As before, selection for the presence of the P1 plasmids was maintained during the postinfection incubation in broth. A deletion of the *repL* open reading frame eliminated the residual *incA*-resistant replication (Fig. 2). This experiment provides definitive evidence that such residual replication is due entirely to the lytic replicon.

Every viable P1 *c*1.100 Δ*repL* lysogen that was infected with λpBR322-*incA* appeared to produce an Ap$^r$ colony containing bacteria cured of P1. This observation might seem to imply the absence from P1 *c*1.100 Δ*repL* prophage of an addictive system, such as the *ccd* functions of F (25, 29), that kills cells cured of plasmid. However, the existence of an addictive function is revealed by experiments similar to those presented here but performed without selection for retention of the P1 plasmid during the period of inhibition of the plasmid replicon. A dramatic growth inhibition of cured cells was observed (unpublished data). We interpret the high efficiency of plating of P1 lysogens that were infected with λ-pBR322-*incA* as owing to the infrequent segregation at cell division of a P1-free cell that escapes death, a segregation that cumulatively becomes highly probable. Prolonged incubation of the infected cells in the presence of 20 μg of chloramphenicol per ml appears to diminish the frequency of such escapes.

## DISCUSSION

Two differently controlled replicons function in P1 prophage. One has been designated the plasmid replicon, the other is the lytic replicon. These names imply a division of labor that appears not to be strictly followed. Among P1 strains with an altered immunity repressor, both functions may be assumed by the lytic replicon. The capacity of a lytic replicon to support replication in the plasmid mode is not unique to P1. It has also been reported in λ (26) and P4 (30).

Indirect evidence has been supplied previously for the existence of P1 plasmid replication under control of an immunity-sensitive replicon (42). The experiments reported here provide direct support for that hypothesis and identify the replicon responsible with the recently described lytic replicon.

How, if at all, do the two replicons of P1 communicate with each other? If *oriR* functioning is controlled in P1 as it is in the similar RepFIA replicon of F (43) and RepFIB replicon of pCG86 (R. Maas, S. Saadi, and W. K. Maas, Mol. Gen. Genet., in press), then under conditions that permit the lytic replicon to drive the average plasmid copy number higher than the number set by the *incA* control circuit, *oriR* would be prohibited from functioning. It would fire only during the rare intervals when the plasmid copy number fell to its normal value. In other words, we expect the plasmid replicon to be inhibited by replication initiated at the lytic origin. Is there a signal produced by a failure of the plasmid replicon that activates the lytic replicon? The observation that the basal expression of the lytic replicon in a P1 prophage appears sensitive to even a modest increase in *c*4 gene dosage (Table 2, row 3) suggests that the activity of the lytic replicon will be increased when a failure of the plasmid replicon lowers the dosage of immunity genes. The contribution of the lytic replicon can also be affected by other influences that separately dictate the efficacy of the *c*1 repressor. Unpublished experiments of one of us (D.K.C.) suggest that a close relative of P1, P7, more efficiently represses its lytic replicon that does our "wild-type" P1 (from Plk*c*). This is probably because P7 possesses a significantly different *c*4-*ant* region.

The functioning of a second replicon in P1 prophage has been suggested on the basis of plasmid stability considerations. Mini-P1 λ-P1:5R is appreciably less stable than is P1 itself. One explanation that has been offered for this difference is that the lytic replicon of P1 can serve as a reserve replication system that is turned on when the plasmid replicon fails to fire (36). It is unlikely that a reserve replicon (whether available continuously or only on demand) can by itself account for the extraordinary stability of P1 plasmid. A larger contribution may come from a P1-specified postsegre-

gational killing function, such as has been described for F (25). Unpublished evidence from this laboratory indicates that P1 possesses such an addictive function.

The different guarantees of P1 plasmid stability can now be seen to include (i) replication from both the plasmid and lytic replicons, each of which exerts control on the other; (ii) a large reserve of replication potential in the plasmid replicon (11, 12) as in the lytic replicon; (iii) active partitioning (3); (iv) active equilibration of plasmid dimers with monomers (6); and (v) killing of cured segregants. Whatever their relative importance, the multiplicity and variety of these guarantees are striking. An increasingly appreciated feature of many low-copy-number plasmids, the possession of more than one functional replication origin (15), appears not to be shared by their bacterial hosts. This seeming extravagance on the part of relatively small genomes may be a consequence of a way of life that involves frequent horizontal transmission to a variety of intracellular environments.

## ACKNOWLEDGMENTS

M.B.Y. thanks Tapio Palva for hospitality during the summer of 1985, when a portion of the work was undertaken in the Molecular Genetics Laboratory, Department of Genetics, University of Helsinki, Finland. We are indebted to Susan Gottesman, June Scott, and Nat Sternberg for providing certain of the phage, plasmid, and bacterial strains used; to Kit Tilly and Michael Lichten for constructive criticism of the manuscript; and to Beverly Miller, May Liu, and Edna Wyatt for its preparation.

E.B.H. was a Fogarty Visiting Fellow, and S.J. was a predoctoral Fulbright Scholar, who subsequently received additional support from Becton Dickinson and Co., administered by the American Society for Cell Biology.

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