Participation of the Lytic Replicon in Bacteriophage P1 Plasmid Maintenance

MICHAEL B. YARMOLINSKY, EGON B. HANSEN, SAMINA JAFRI, AND DHRUBA K. CHATTORAJ

Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892 and Laboratory of Molecular Biology, LII Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701

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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 cI immunity repressor. Incomplete repression was particularly evident in lysogens of the thermoinducible P1 cl.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 cI.100 lysogen was elevated with respect to the copy number of P1 cI+. 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 cI 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 cI is antagonized by ant, normally controlled in P1 lysogens by the secondary immunity gene c4. The products of cI and c4 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 cI repression, so as to provide a stable source of excess incA. Replication from oriL, normally inhibited in P1 lysogens, was further restrained by additional c1 repressor supplied from a cloned P1 DNA fragment. Introduction of a cloned P1 DNA fragment bearing c4 also appeared to be effective. Definitive elimination of the lytic replicon function was achieved by use of a 2-kb deletion from EcoRI-17 into EcoRI-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 MgSO4 to induce receptor sites for and stabilize phage λ, respectively, or with 5 mM CaCl2 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

<table>
<thead>
<tr>
<th>Strain, phage, or plasmid</th>
<th>Relevant characteristic(s)*</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR157</td>
<td>supD43 supD74(Ts) recA56 srl::Tnl0</td>
<td>P1 transductant of MX397</td>
</tr>
<tr>
<td>BR469</td>
<td>sup journalistic, plates P1 well</td>
<td>17</td>
</tr>
<tr>
<td>BR1622</td>
<td>N14944(c1'-P1:5R BamHI-9)loxP</td>
<td>11</td>
</tr>
<tr>
<td>BR1623</td>
<td>N14944(pAL95)</td>
<td>See Materials and Methods</td>
</tr>
<tr>
<td>BR1813</td>
<td>recA (λ imm21) derivative of MC4100</td>
<td>46</td>
</tr>
<tr>
<td>BR1879</td>
<td>supF trp::Tnl0, plates P1 well</td>
<td>P1 transductant of BR469</td>
</tr>
<tr>
<td>BR1888</td>
<td>sup maliB[A'] Δlon cpsE3::Tnl0 Δlon plates P1 clear (41)</td>
<td>S. Gottesman</td>
</tr>
<tr>
<td>EH4132</td>
<td>CM1565/PlCm cl+</td>
<td>22</td>
</tr>
<tr>
<td>EH4133</td>
<td>CM1565/PlCm cl-100</td>
<td>22</td>
</tr>
<tr>
<td>MX397 (BR1867)</td>
<td>supD43 supD74(Ts)</td>
<td>28</td>
</tr>
<tr>
<td>N100 (152, D14)</td>
<td>recA56 sup maliB[A'] Δlon cpsE3::Tnl0</td>
<td>21</td>
</tr>
<tr>
<td>NS1494 (BR1739)</td>
<td>dnaA6(Ts) recA56</td>
<td>40</td>
</tr>
</tbody>
</table>

Phages and phage-plasmids

| P1Cm                     | P1 with Tn9 insert at IS1 site | 24 |
| pICm r m                 | Recombinant between P1Cm and P1 r m | 20 |
| pICm c1.100 r m          | Thermoducible P1Cm | 31 |
| P1Cm c1.100 r m          | Recombinant between above phages | 22 |
| P1Cm c1.100 >repL        | P1Cm r m with conditional (Am) mutation in repA | See Materials and Methods |
| λ·P1C4b                  | λ imm21 6515 b519 nin5 sr3α3- P1: EcoRI-9(c4)-22 | 37 |
| λ·P1:5R BamHI-9          | λ mini-P1 that, bearing loxP, can integrate via loxP × loxB recombination | 6 |
| λ·pBR322                 | (Ap) imm21 phage-plasmid | See Materials and Methods |
| λ·pBR322-incA            | (Ap) imm21 phage-plasmid incA clone | See Materials and Methods |

Plasmids

| pACYC184(Km)             | (Km) version of multicycop vector pACYC184 | See Materials and Methods |
| pACYC184(Km)-c1          | Multicyop (Km) c1 clone | See Materials and Methods |
| pAL12                    | 851-bp incA clone in pBR322 (Ap) | 4 |
| pAL18                    | 306-bp incA clone in pBR322 (Ap) | 2 |
| pAL95                    | pAL95 with 306-bp incA insert | See Materials and Methods |
| pAL95                    | 750-bp c4 sim clone in pBR322 (Ap) | See Materials and Methods |
| pBR322                   | Multicyop (Ap Tc) vector | 8 |
| pCM960                   | oriC plasmid | 44 |

*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 loxP by the process of prophage displacement as described by Sternberg et al. (40). The parental strains carried λ Δatt4im21-1: EcoRI-7 prophage (hereafter called λ-P1-7) integrated at loxP 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 c1.100 r m with P1vir 23.115(Am) (45) to yield, among the recombinants capable of lysogenization, P1Cm c1.100 r m 23.115. The 23.115 mutation, mapped to EcoRI-5 of P1 (35), results in a suppressible defect (46). A P1Cm c1.100 r m 23.115 lysogen was infected with λ imm21: P1:5R repA103 (7) and thermally induced. P1 23+ recombinants were selected as plaque formers on the nonsuppressing λ P1' 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 (pAL69 [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 c1.100 r m repA103. A c1' version of these phage, prepared by crossing in the wild-type allele from a λ imm34: P1:7 clone (37), was used in the subsequent construction of supD(Ts) lysogens (28).

Construction of vectors carrying c1, c4, or c4 and incA. pACYC184(Cm)-c1 is a clone of c1 without the neighboring loxP (which might otherwise allow site-specific recombination with P1 [39]) and was provided by Nat Sternberg. pACYC184(Km)-c1 was constructed by cleavage of pACYC184(Cm)-c1 at the unique EcoRI 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 c1.100. pACYC184(Km) is a kan variant of pACYC184 (40), constructed as above.

A pBR322 clone of c4, pAL95, was constructed from pBD101 (provided by June Scott), a pBR322 clone of a 0.75-kb fragment of P1 DNA that bears the c4 gene but not the closely linked sim (superimmunity) gene. The pBD101 plasmid was cleaved with BamHI and religated so as to eliminate about 4.0 kb from pBD101, including most of the Tn1000 (ph) transposon. The absence of the sim gene and the presence of the c4 gene were confirmed as described by Devlin et al. (16), using their pHBD6 as a sim + control (which prevents plaque formation by P1 c1.155) and using P1Cm c4.32 (32) to test for c4 complementation. A clone of c4 and incA, pAL99, was constructed from pAL95 by inserting the 306-bp BamHI incA fragment from pAL18 (2) into the BamHI site of P1Cm c4.32 (32) and similarly digested pAL62 (pBR322 carrying two copies of the BamHI incA fragment). The presence of the pBR322...
TABLE 2. Integrative suppression of a dnaA46(Ts) mutation by replicons of P1

<table>
<thead>
<tr>
<th>Integrated replicons*</th>
<th>Surviving fraction in the presence of b</th>
<th>c4 (pALA95 or ( \lambda)-P1-c4b)</th>
<th>c4 and incA (pALA93 or ( \lambda)-P1-c4b and pALA12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>(3 \times 10^{-6})</td>
<td>1</td>
<td>(2 \times 10^{-4})</td>
</tr>
<tr>
<td>(\lambda)-P1:5RBamH1-9</td>
<td>1</td>
<td>(1 \times 10^{-5})</td>
<td>1</td>
</tr>
<tr>
<td>P1.Cm</td>
<td>1</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

* The dnaA46 host was N1494. The strains of rows 2 and 3 are derivatives of BR1622 and BR1623, respectively, in which the P1 replicons were integrated at loxB.

* The surviving fraction is the ratio of bacterial colony formers under conditions selective for the carried plasmids at 42°C to those at 32°C. Carried plasmids or integrated \( \lambda\) prophages bearing regulatory elements are listed at the head of each column in parentheses. The \( \lambda\)-P1-c4b prophase was integrated at \( \lambda\) att.

* 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 \( inc\) 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 \( inc\) probes. This analysis revealed that the fragments are arranged such that the HindIII cleavage site within the pBR322 is towards the left arm of the \( \lambda\) in both the \( \lambda\)-pBR322 and \( \lambda\)-pBR322-\( inc\). The latter plasmid includes a single copy of \( inc\) inserted at the left end of the pBR322 insert.

The \( \lambda\)-pBR322 phases established themselves as stably maintained plasmids in \( \lambda\) 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 Tn5 insertional mutagenesis of P1.Cm. The P1 was chromosomally integrated at \( loxB\) by prophage displacement in N1494(\( \lambda\)-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-\( inc\) 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 Tn5 insert that interfered with the alternative replicon (data not shown). The insert is located within EcoR1-17, 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 EcoR1-17::Tn5 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 c1100 lysogens. Restriction mapping located the other Tn5 inserts at various distant sites. These results lead us to propose that the \( inc\)-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 \( cl\) insensitive or not fully repressed by \( cl\), possibly as a consequence of a low level of ant expression, the determinant of an antagonist of \( cl\) repression. We found that introduction of a source of additional c4 protein, the negative regulator of ant, eliminated integrative suppression by P1 in the presence of excess \( inc\). The c4 protein was supplied from a clone of c4 or from a considerably larger EcoR1-9 insert into a PBR325 plasmid or a \( \lambda\) prophage (Table 2, row 3). That this effect is due specifically to the modest increase in the gene dosage of c4 in the latter case was substantiated by additional experiments in which the wild-type c4 was replaced by a c4.32 allele (data not shown). Integrative suppression was largely eliminated by the presence of excess gene copies of both \( inc\) and c4. 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 \( \lambda\)-P1:5R to P1.Cm as described in Materials and Methods, and lysogens of repA103 and rep* versions of P1.Cm in the supD(Ts) host (MX397) were prepared. At the permissive temperature (32°C) the doubling time (1/2) of both lysogens was about 44 min. Upon shifting to the nonpermissive temperature in the presence of chlorampheni-
plasmid replicon primarily functions to control the P1 prophage. The ratio of cl-sensitive prophage was evident when the plasmid replicon is selectively inhibited by λ-plasmid replication. The colonies that arose from λ-plasmid-infected prophages seemed to distinguish between those that arose from the occasional uninfected bacterium and those that arose from bacteria that had been infected with λ-plasmid replicon.

We further conclude that the cl allele of P1, the primary replication origin of the plasmid can be shifted.

Relative copy numbers of P1 cl + at 32°C. A suggestion that the lytic replicon of P1 cl + lysogens can be the major contributor to prophage replication was made on the basis of measurements of the maximal antibiotic resistance conferred by P1 prophages bearing the cl + mutation [42]. We confirmed the validity of this suggestion on the basis of direct measurements of the relative copy numbers of P1 cl + and P1 cl - 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 cl + prophage in E. coli was found to be five times the copy number of P1 cl - prophage in E. coli. These results imply that in lysogens of P1 cl + 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 cl + lysogens when the plasmid replicon is blocked.

P1 cl + Δrepl 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-plasmid replicon 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 P1 cl - prophage.
c1.100 and of a P1cm c1.100 from which had been deleted a 2-kb segment between a Tn5 situated in EcoRI-17 and an IS50 situated at the proximal end of EcoRI-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 c1.100 ΔrepL lysogen that was infected with λpBR322-incA appeared to produce an Ap' colony containing bacteria cured of P1. This observation might seem to imply the absence from P1 c1.100 ΔrepL 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 c4 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 c1 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 PlkC). This is probably because P7 possesses a significantly different c4-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-
gential killing function, such as has been described for F (25). Unpublished evidence from this laboratory indicates that P1 possesses such an additive 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.

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LITERATURE CITED


