Function of the N-Terminal Half of RepA in Activation of Rts1 ori

YOSHIRO TERAWAKI,* YOSIFUMI ITOH,† HONG ZENG,‡ TETSUYA HAYASHI, and AKIRA TABUCHI

Department of Bacteriology, Shinshu University School of Medicine, Asa 3-1-1, Matsumoto 390, Japan

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The RepA protein of the Rts1 plasmid, consisting of 288 amino acids, is a trans-acting protein essential for replication. A mutant repA gene, repAΔC143, carrying a deletion that removed the 143 C-terminal amino acids of RepA, could transform, but at a low frequency, an Escherichia coli polA strain, JG112, when repAΔC143 was cloned into pBR322 with Rts1 ori in the natural configuration. The transformation was less efficient without the dyad DnaA box in the ori region, and no transformation occurred at 42°C, characteristic of Rts1 replication. A fusion of the 3'-terminal half of repA of the P1 plasmid to repAΔC143 yielded a pBR322 chimeric plasmid that contained Rts1 ori through hybrid (Rts1-P1) repA. This plasmid was maintained much more stably in JG112 at 37°C. At 42°C, however, it was quite unstable. The overproduced hybrid RepA protein showed interference with mini-Rts1 replication in trans and also exhibited an auto-repressor function, although both activities were decreased. These findings suggest that the N-terminal half of the RepA molecule of Rts1 is involved in the activation of the replication origin.

The essential replication regions of plasmids usually contain two important components: an origin sequence (ori) and a gene, rep, encoding a Rep protein (23). The Rep protein of various plasmids binds to ori, leading to the initiation of plasmid replication. It also binds to its promoter region, resulting in the autoregulation of Rep protein synthesis (2, 15, 21, 24, 30). P1 and Rts1 especially resemble each other in their basic replicon structures (2, 16). In addition, their Rep protein, RepA, shows high homology in amino acid sequence. P1 and Rts1, however, belong to different incompatibility groups.

To gain insight into the structure and function of the 288-amino-acid Rts1 RepA molecule, we constructed various repA mutants and examined their phenotypes with regard to Rts1 ori activation, autorepression, and inhibitory effect on mini-Rts1 replication (26, 27). Recently, we introduced site-directed mutations near the 3' terminus of repA and obtained mutant RepA proteins that had lost the ori activation function. One of these, RepAArg279 (Arg-279 to Gly), exhibited increased interference with mini-Rts1 replication (33). In this study, we determined that the RepA molecule of Rts1 with a deletion of the 143 C-terminal amino acids still retained the functions of Rts1 ori activation and autorepression, although at decreased levels.

MATERIALS AND METHODS

Bacterial strains and plasmids. Escherichia coli K-12 strains JC1569 (recA1 gal leu his arg met str) (5), JG112 (polA lac thy str) (20), JM109 [recA1 supE endA1 hsdR17 gyrA96 relA1 thl Δ(lac-proAB) F' (traD36 proAB + lacIq P15)] (32), and AB1157 (galK2 leu thr pro his thi lacY ara xyl supE44 str) (10) were used as host cells. The plasmids used are listed in Table 1. A mini-P1 plasmid, pALAI09, containing the rep and par regions of plasmid P1 (2) with the kanamycin resistance gene from Tn5 (18) and harbored in E. coli N100 (8), was kindly donated by A. Abeles.

Media and chemicals. Penassay broth (Difco Laboratories, Detroit, Mich.) was used for the cultivation of bacteria unless otherwise noted. L broth without glucose was used for transformation. MacConkey-Gal plates were prepared by adding galactose to a final concentration of 0.6% to MacConkey agar base (Difco) and used to examine the galK expression of AB1157 harboring the pFD51 chimeric plasmid. Restriction endonucleases, DNA polymerase I (Klenow fragment), EcoRI and Smal linkers, and T4 DNA ligase were purchased from Takara Shuzo, Kyoto, Japan.

Plasmid DNA preparation and transformation. Plasmid DNA was prepared by the method of Humphreys et al. (11), and transformation was carried out as described by Cohen et al. (6).

Construction of pTW547 derivatives and other recombinant plasmids. pTW547, described previously (13), is a PBR322 recombinant plasmid in which a mini-Rts1 subregion (coordinates 1441 to 697) is inserted as a HindIII-ClaI fragment (Fig. 1). In the fragment, Rts1 ori and repA, with a deletion of the 3'-terminal half, are present in the natural configuration. Hence, pTW547 encodes a mutant RepA protein, RepAΔC143, that lacks the 143 C-terminal amino acids. At the C terminus of RepAΔC143, six amino acids were added by read-through into the PBR322 sequence. A repA subregion corresponding to that in pTW547 was isolated from pTW100 (26) (Fig. 1) as a 0.5-kb HindIII-ClaI fragment and cloned between the HindIII and ClaI sites of pBR322, giving rise to pTWb:repAΔC143. The Rts1 ori sequence (coordinates 1441 to 1194) was isolated as a 0.25-kb HindIII-EcoRI fragment from pTW100. This 0.25-kb fragment was cloned between the HindIII and EcoRI sites of pBR322, giving rise to pTWb:Rts1 ori. The lac promoter, Plac, was isolated from pUC19 as a 181-bp PvuII-HindIII fragment or as a 232-bp PvuII-EcoRI fragment and cloned, respectively, into pTWb:repAΔC143 at the EcoRV and HindIII sites or into pBR322 at the EcoRV and EcoRI sites, giving rise to pTWb:
SmA1 linkers were ligated to the restricted ends after the ends were made blunt with the Klenow fragment. Then, the mini-Rts1 subregion (coordinates 1213 to 1010) in which the operator-promoter sequence of repA is accompanied by a very short stretch of the N-terminal portion of repA was isolated as a HindIII-SmA1 fragment and cloned in front of the promotorless galK gene of pFD51 (22), giving rise to pTW1213-S.

**Construction of repA fusion gene.** To construct a fusion of the Rts1 repA gene with the P1 repA gene, we converted the ClaI site of pTW547 to an EcoRI site by adding a 12-mer EcoRI linker after filling in the ClaI site with the Klenow fragment; this process yielded pTW547E. First, the 3' half of P1 repA (mini-P1 coordinates 1000 to 1852, encompassed by EcoRI and HindIII sites) was isolated from pALA109 as a 2.1-kb EcoRI-SmA1 fragment conferring kanamycin resistance. Then, this 2.1-kb fragment, in which the incA locus of P1 is also contained, was inserted between the EcoRI and ScaI sites of pTW547E, giving rise to pTW547-P1K. Thus, a hybrid (Rts1-P1) repA gene was generated. From this recombinant plasmid, Rts1 ori through Rts1-P1 repA, along with P1 incA, was isolated as a 1.6-kb HindIII fragment. This 1.6-kb fragment was cloned into the HindIII site of pBR322, giving rise to pTW547-P1A and pTW547-P1A' (Fig. 2). In the former, the cloned fragment is in an orientation such that the hybrid repA gene is transcribed towards the bla gene of pBR322, and in the latter the fragment is in the reverse orientation. pTW547-P1A1, which retains Rts1-P1 repA but lacks Rts1 ori, was constructed as follows. The smaller StyI fragment of pTW547-P1A, which contains the 3'-terminal region of Rts1 repA (coordinates 1191 to 1020), was ligated to the larger StyI fragment of pTW547-P1A, which contains the mini-Rts1 and mini-P1 regions (coordinates 1020 to 697 and 1000 to 1852), resulting in the generation of a 1.25-kb HindIII fragment (coordinates 1191 to 697 and 1000 to 1852) located in the HindIII site of pBR322. The hybrid repA gene carried on the 1.25-kb fragment also was cloned into the HindIII site of pACYC184, giving rise to pTW11:

**TABLE 1.** Plasmids used

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Mini-Rts1 composition or charactera</th>
<th>Reference or source</th>
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<tr>
<td>pBR322</td>
<td>Ap' Te'</td>
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<td>pACYC184</td>
<td>Cp' Te'</td>
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<td>A. Abeles</td>
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<td>This study</td>
</tr>
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<td>This study</td>
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<td>This study</td>
</tr>
<tr>
<td>pTW11:repA-A</td>
<td>pACYC184 (1191-216)</td>
<td>33 and this study</td>
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<td>pACYC184 (1191-697 and 1000-1852)</td>
<td>This study</td>
</tr>
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<td>pFD51 (1441-1194 and 1191-216)</td>
<td>26</td>
</tr>
<tr>
<td>pFD51:repA</td>
<td>pFD51 (1191-216)</td>
<td>26</td>
</tr>
<tr>
<td>pTW1213-S</td>
<td>pFD51 (1213-2020)</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Numbers in parentheses are mini-Rts1 coordinates (16) and mini-P1 coordinates (2) (two sets) and mini-Rts1 coordinates (one set). Ap, ampicillin; Tc, tetracycline; Cp, chloramphenicol; Km, kanamycin; Sp, spectinomycin.

**FIG. 1.** Maps of mini-Rts1 derivatives pTW547 and pTW100. (A) A mini-Rts1 subregion (coordinates 1441 to 697; see Fig. 2) that contains Rts1 ori and a repA deletion, repAΔC143, lacking the 3'-terminal half, was cloned into pBR322 between the HindIII and ClaI sites. Note that Rts1 ori and repAΔC143 are present in the natural configuration. repAΔ, repAΔC143. (B) Wild-type repA with its native promoter (coordinates 1191 to 216) is positioned upstream of Rts1 ori (coordinates 1441 to 1194), which is inserted separately into pFD51. The pTW100-type plasmid encoding repA (wild type) could replicate in JG112, but that encoding repAΔC143 could not (33). Numbers in parentheses are mini-Rts1 coordinates. Restriction sites: H, HindIII; E, EcoRI; C, ClaI; B, BamHI.
Rts1-P1 repA, which was used as an effector plasmid in the galK expression study. The orientation of the insertion was the same as that of repA (wild type) in pTW11:repA-A i.e., opposite to the tet gene of pACYC184.

Immunoblot analysis of RepA and its derivatives. JC1569 cells harboring pFD51 or pBR322 recombinant plasmids containing wild-type or mutant repA genes were grown in 1.5 ml of L broth at 37°C. The cell lysates, which were prepared as described previously (33), were adjusted to contain the same amount of total protein in each well and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Laemmli (17). Polypeptides in the gel were transferred to a nitrocellulose filter ( pore size, 0.45 μm) by the method of Towbin et al. (29). The filter was blocked with bovine serum albumin, treated with anti-RepA antibody, and treated with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G antibody (Promega Biotec, Madison, Wis.); color development was then done as recommended by the supplier.

Plasmid stability in single colonies. JG112 (polA) cells transformed with pTW547 or its derivatives formed small colonies on ampicillin (20 μg/ml)-containing plates after 30 h of incubation at 37°C. A single colony picked from each plate was suspended in Penassay broth and streaked onto an ampicillin-containing plate to yield a number of single colonies. Several of the single colonies were picked individually, suspended in Penassay broth, and then appropriately diluted. Twenty microliters of each suspension was spotted onto a plate with or without ampicillin (20 μg/ml). The number of colonies that developed on the plates was scored, and the ratio of plasmid-cured cells to total cells was determined.

Sensitivity of mini-Rts1 to overproduced RepA. The inhibitory effect of overproduced RepA on replication of a coexisting mini-Rts1 plasmid (pTW601) in trans was examined by introducing pBR322 recombinant plasmids carrying repA (wild type or hybrid) by transformation into JC1569 harboring pTW601. The transformants that developed on plates containing 20 μg of ampicillin per ml were picked individually and stabbed onto plates containing 30 μg of spectino-
The results of single colony isolates were tested by the addition of IPTG. The resulting colonies were tested by the addition of IPTG. The resulting colonies were tested in the orientation necessary to transcribe the bla gene of plasmid pBR322, and in the presence of chloramphenicol and ampicillin. When no repression occurred, the colonies on the plate were red after 16 h of incubation at 37°C.

**RESULTS**

**Rts1 ori activation by the N-terminal half of RepA.** pTW547 (13) is a pBR322 chimeric plasmid that contains Rts1 ori and a deletion derivative of the Rts1 repA gene in the natural configuration, i.e., spanning mini-Rts1 coordinates 1441 through 697 (unique ClaI site). The repA derivative encodes RepAAC143, which has a deletion of the 143 C-terminal amino acids of the wild-type RepA protein. Its synthesis in JC1569 was demonstrated in the immunoblotting profile (Fig. 3, lane 2). pTW547 could transform E. coli polA strain JG112. Although the transformants developed slowly on plates containing 20 μg of ampicillin per ml, they formed solid and isolated colonies after 16 h of incubation at 37°C. The transformants could subsequently form small colonies on serial purification. Single colonies purified on ampicillin-containing plates were shown to contain 0.1% or fewer drug-resistant cells, suggesting that pTW547 replicates inefficiently in the polA host. Since the percentages of single colonies harboring pTW547 did not increase on serial purification, it is unlikely that polA* revertants of JG112 were selected by the procedure. It is noteworthy that pTW550 (13), which lacks the dysad DNA boxes of pTW547, could also transform JG112. In this case, however, the number and the size of the transformants on the drug-containing plates were smaller than those with pTW547. Furthermore, no transformants of JG112 developed on plates incubated at 42°C with either pTW547 or pTW550. The temperature sensitivity of replication and the less efficient replication without the DNA boxes are characteristic of Rts1 replication (14, 28).

We then tested the individual effects of repAAC143 and Rts1 ori on the replication of pBR322 chimeric plasmids in JG112. repAAC143 with its native promoter (coordinates 1191 to 697) or the ori region (coordinates 1441 to 1194) was cloned into pBR322 at the site corresponding to that in pTW547; i.e., repAAC143 was inserted between the HindIII and ClaI sites, and Rts1 ori was inserted between the HindIII and EcoRI sites, giving rise to pTWb:repAAC143 and pTWb:Rts1 ori, respectively (see Materials and Methods). Unlike pTW547 and pTW550, neither of the pBR322 chimeric plasmids transformed JG112 (Table 2), indicating that both repAAC143 and Rts1 ori are required for transforming the polA host. Therefore, we concluded that the replication of pTW547 in JG112 was initiated at Rts1 ori and that ori activation was mediated by RepAAC143. The initiation, however, was very inefficient, as shown by the plasmid stability in single colonies.

**Insertion of the lac promoter upstream of repAAC143.** It may be argued that the pTW547 replication observed in...
TABLE 3. Stability of pTW547 and other plasmids in a pO4A host*

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Ratio of plasmid-carrying JG112 cells at:</th>
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<tr>
<td></td>
<td>37°C</td>
</tr>
<tr>
<td>pTW547</td>
<td>6.2 x 10^-4</td>
</tr>
<tr>
<td>pTW547-P1A</td>
<td>1.3 x 10^-2</td>
</tr>
<tr>
<td>pMY112S</td>
<td>1.1</td>
</tr>
<tr>
<td>pTW601</td>
<td>1.0</td>
</tr>
<tr>
<td>pALA109</td>
<td>1.0</td>
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</table>

* The number of colonies that developed on ampicillin (30 μg/ml)-containing plates was divided by the number of colonies that developed on plates not containing the drug (see Materials and Methods). The ratios are the averages of data from an analysis of five single colonies for each plasmid. The drugs used for pTW601 and pALA109 were spectinomycin (30 μg/ml) and kanamycin (10 μg/ml), respectively, instead of ampicillin. The temperatures indicated are for plating of cultures.

JG112 was not due to the RepAAC143-Rts1 ori interaction but that the promoter activity in the inactivated mini-Rts1 subregion activated the pBR322 replication origin, leading to replication of pTW547 and pTW550. To test this possibility, we inserted the lac promoter from pUC19 upstream of repAAC143 in pTWb:repAAC143 or at the EcoRI site of pBR322 in an orientation such that transcription occurred in the direction of the bla gene (and hence towards the pBR322 origin), giving rise to pTWb:repAAC143-Plac or pBR322: Plac, respectively. As expected, a large amount of RepAAC143 was visualized in the immunoblot profile of JM109 (lacI3) (with isopropyl-β-D-thiogalactopyranoside [IPTG]) cells containing pTWb:repAAC143-Plac (Fig. 3, lane 3). The Plac plasmids, however, did not transform JG112 under any culture conditions, even when IPTG was added to the L broth culture for transformation (Table 2). Thus, the possibility that some promoter activity occurring upstream of the bla gene may have activated ori in pBR322 was ruled out.

**Fusion of RepA of Rts1 with RepA of P1.** The inefficiency of pTW547 replication in the pO4A host may have been due to the unnatural C-terminal sequence of RepAAC143. Accordingly, we fused the 3'-terminal half of P1 repA (mini-P1 coordinates 1000 to 1852, contained in an EcoRI-HindIII fragment) (2) to the 5'-terminal half of Rts1 repA in pTW547 (see Materials and Methods). The recombinant plasmid obtained, pTW547-P1A, contains Rts1 ori through the hybrid repA gene, which is transcribed towards bla of pBR322. The hybrid protein, Rts1-P1 RepA, consists of 145 N-terminal amino acids from Rts1 RepA and 174 C-terminal amino acids from P1 RepA, and at the junction a proline residue is added by EcoRI linker insertion. Thus, Rts1-P1 RepA is composed of 320 amino acids. Its presence in JC1569 harboring pTW547-P1A was confirmed in the immunoblot analysis as a polypeptide slightly larger than wild-type Rts1 RepA (Fig. 3, lane 4).

pTW547-P1A transformed JG112 at 37°C but not at 42°C, as was the case with pTW547. The plasmid stability in single colonies of JG112 cells growing on ampicillin-containing plates revealed that pTW547-P1A was more stably maintained in the pO4A host than pTW547 at 37°C, while both plasmids were quite unstable at 42°C (Table 3). pMY1123, a mini-R100 plasmid with a low copy number, was used to examine the effect of temperature on bla gene expression. As shown in Table 3, JG112 carrying pMY1123 formed a large number of colonies at 42°C as well as at 37°C on ampicillin-containing plates, indicating that the expression of bla is not temperature sensitive. Since the bla genes in pTW547 and pTW547-P1A are derived from Tn3, as is that in pMY1123, we concluded that the temperature-sensitive growth of JG112 with pTW547 or pTW547-P1A on ampicillin-containing plates was due to the temperature sensitivity of plasmid replication. A mini-P1 plasmid, pALA109, was shown to be quite stable at both 37 and 42°C (Table 3). In contrast, mini-Rts1 plasmid pTW601 showed marked temperature sensitivity. Thus, Rts1 ori activation mediated by the hybrid RepA protein also exhibited the temperature sensitivity property of Rts1 replication, as was shown with RepAAC143. It should be mentioned, however, that pTW547-P1A', in which Rts1 ori through Rts1-P1 repA is inserted at the HindIII site of pBR322 in an orientation opposite to that of the bla gene, did not transform JG112 at any temperature. The amount of Rts1-P1 RepA synthesized in JC1569 with pTW547-P1A was approximately 1.6 times that synthesized in JC1569 with pTW547-P1A' (Fig. 3, lanes 4 and 5), a result that was consistently observed. Because Rts1-P1 repA in pTW547-P1A is positioned downstream of the P1 promoter of bla in pBR322 (25), transcription of the hybrid repA gene may have been enhanced by this P1 promoter. However, we are uncertain as to whether the smaller amount of hybrid RepA in the pTW547-P1A'-carrying cells caused deficient activation of Rts1 ori.

**Effect of RepA overproduction and autorepressor function.** Since the Rts1-P1 RepA hybrid protein activated Rts1 ori, it was expected that the hybrid RepA protein might interact in trans with a mini-Rts1 plasmid (pTW601) and have an inhibitory effect on the replication of pTW601. To study this possibility, we constructed pTW547-P1AΔ1, which contains the hybrid repA gene but has a deletion of the Rts1 ori sequence, by using pTW547-P1A (see Materials and Methods). Incompatibility between pTW601 and mini-P1 plasmid pALA109 was also examined. In the initial study, each of the transformant colonies growing on plates containing the donor marker drug (ampicillin) was examined for resistance to the resident marker drug (spectinomycin). Almost all colonies selected for pTW547-P1AΔ1 also were shown to be spectinomycin resistant (Table 4). However, when individual cells that composed each of the single ampicillin-resistant
transformant colonies were examined for spectinomycin resistance, only 58% were shown to retain pTW601 (Table 4, last column). Thus, the hybrid RepA protein, when overproduced, showed a decreased interference with mini-Rts1 plasmid replication in *trans*. Wild-type Rts1 RepA supplied in an excess amount had no inhibitory effect on mini-P1 plasmid replication (Table 4), indicating that Rts1 and P1 are functionally independent repilcoids. Indeed, pTW601 and pALA109 coexisted quite stably (Table 4, last row). Therefore, the ability of Rts1-P1 RepA to interfere with pTW601 replication could be ascribed to the possibly overproduced N-terminal portion of the molecule, which was derived from Rts1 RepA.

The autorepressor activity of the hybrid RepA protein was investigated with a *galk* expression system as described in Materials and Methods. AB1157 (*galk*) cells harboring pTW11:Rts1-P1 repA, when transformed with pTW1213-S, formed red colonies (having red centers with white margins) on MacConkey-Gal plates containing both chloramphenicol and ampicillin after 16 h of incubation at 37°C. In contrast, AB1157 (pTW11:repA-A) transformed with pTW1213-S formed white colonies on the plates, and cells harboring pACYC184 (without repA) and transformed with pTW1213-S formed red colonies. These findings suggest that the hybrid RepA protein retains autorepressor activity but at a decreased level.

**DISCUSSION**

Many plasmids encode their own initiator Rep proteins (23). Frequently, these proteins exist as dimers. One would expect that these proteins would contain regions specific for forming dimers as well as domains involved in DNA binding. Besides these, Rep proteins may have a sequence for interacting with host factors to activate the replication origin. Recently, it was clearly demonstrated that the binding of the P1 RepA protein to the origin sequence was greatly facilitated by the host factors DnaJ and DnaK, which serve to keep the protein in the monomeric form (31). Thus, the Rep protein displays various functions. However, studies of the functional domains of Rep molecules are scarce, except for the N-terminal region of P6K (9).

A most remarkable finding obtained in this study was that the N-terminal half of the Rts1 RepA molecule was able to activate Rts1 ori, although inefficiently. Hoping to obtain more efficient replication of a pBR322 recombinant plasmid in JC112 (pOL4), we fused the 174 C-terminal amino acids of P1 RepA to the N-terminal half of Rts1 RepA. We used P1 RepA because, although Rts1 is quite compatible with P1, their RepA proteins show a high homology (about 60%) in amino acid sequence (2, 16). As demonstrated by the plasmid stability in single colonies, pTW547-P1A, which encodes Rts1-P1 RepA, was more stably maintained in JC112 at 37°C than pTW547, which encodes RepAΔC143. The Rts1 ori activation mediated by Rts1-P1 RepA was temperature sensitive, as was the activation mediated by RepAΔC143, a result that is characteristic of Rts1 replication (28). The hybrid RepA protein, when supplied in *trans* in an excess amount, was inhibitory for mini-Rts1 replication and, in addition, showed an autorepressor function, although both activities were decreased in comparison with those of wild-type RepA protein. The low efficiency of ori activation by Rts1-P1 RepA and RepAΔC143 might be ascribed to lower DNA binding affinity of mutant RepA proteins, as suspected from the decreased autorepressor function of hybrid RepA protein. If we could measure the inhibition by RepA(Rts1-P1) on mini-P1 replication, the specificity of the hybrid protein could be analyzed easily. However, such an analysis is at present impossible, because pTW547-P1AΔ1, which encodes Rts1-P1 RepA, also contains a complete set of *incA* direct repeats of P1 at the 3' terminus of the cloned P1 repA fragment.

Our previous studies had suggested the importance of the C-terminal region of RepA for Rts1 replication and incompatibility functions (26, 33). One of the C-terminal mutant proteins, RepA*279* (Arg279 to Gly), showed increased interference with pTW601 replication in *trans* but could not induce replication from Rts1 ori (33), even when *repA*279 was positioned in the wild-type configuration, as in pTW547 and pTW547-P1A (unpublished data). Since RepA*279* has an intact N-terminal portion, the deficiency in Rts1 ori activation in cis is apparently inconsistent with the findings obtained in this study. One possible explanation is that the C-terminal region of Rts1 RepA is involved in the association of RepA molecules, which mediates the pairing of RepA DNA (*ori* or *inc* direct repeats) complexes and results in the inhibition of initiation, as proposed in the regulation of R6K (19), RK2 (7), and P1 (1) replication. RepA*279* might have an increased ability to form dimers and enhance the negative regulation of initiation. The increased inhibitory effect of RepA*279* on mini-P1 replication in *trans* could also be explained by enhanced heterodimer formation with wild-type RepA molecules. If this is the case, the C-terminal region of Rts1 RepA should mediate strong incompatibility, as observed with wild-type RepA in this study.

It was recently reported that the N-terminal protein of R6K, lacking the 141 C-terminal amino acids, retained the negative control function of initiation (corresponding to the incompatibility function) but lost the initiator function along with DNA binding affinity (9). It appears, therefore, that the functional domains for *ori* activation and incompatibility are oppositely located in Rts1 RepA. For determination of the functional domains of Rts1 RepA in more detail, construction of hybrid protein molecules with various combinations of RepA from Rts1 and P1 accompanied by Rts1 *ori* or P1 *ori* would be useful, since Rts1 and P1 have different specificities with regard to replication and its regulation.

**ACKNOWLEDGMENTS**

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