JOURNAL OF BACTERIOLOGY, June 1986, p. 1106–1112
0021-9193/86/061106-07$02.00/0

Vol. 166, No. 3

Incompatibility Repressor in a RepA-like Replicon of the IncFI Plasmid ColV2-K94

PETER C. WEBER AND SUNIL PALCHAUDHURI*

Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Received 27 December 1985/Accepted 17 February 1986

The replication region Repl of the IncFI plasmid ColV2-K94 was cloned on self-replicating restriction fragments. Repl was structurally and functionally homologous to the RepA replicon of IncFII R plasmids. Despite this close relationship, these two replication systems were compatible with each other. The nucleotide sequence of the *copA* incompatibility-replication control gene of Repl was determined and compared with the *copA* sequence of RepA. Six base changes were found in a 24-base-pair span of the *copA* gene; these may result in the formation of a new, more stable, 49-base stem-loop structure in the potential CopA RNA repressor molecule. We postulate that these alterations weaken the interaction between RNA transcripts of the Repl and RepA replicons.

The RepA replicon of the IncFII R plasmids R1, R100, R6-5, and R538 is one of the best-studied plasmid replication systems (for reviews, see references 12 and 17). A 33-kilodalton protein, the product of the *repA* gene, is essential for the initiation of DNA synthesis at the origin of replication (*oriR*) of the RepA replicon (8, 9, 14). Stringent control over plasmid replication is effected by two repressors which regulate RepA protein expression at either the transcriptional or translational level.

An 11-kilodalton protein, the product of the *copB* gene, represses transcription of the *repA* gene by binding to its promotor region (5, 6). Deletion or mutation of the *copB* gene results in a 3.5-fold increase in copy number of plasmid R100 and an 8-fold increase in copy number of R1 (13). The second repressor is a 91-base untranslated RNA, the product of the *copA* gene (15, 18), which binds to the mRNA of the *repA* gene and prevents its translation into RepA protein (7, 23). The CopA RNA, and therefore its complementary sequence on the RepA mRNA, can form a 48-base stem-loop structure (15, 18). Mutations which not only increase copy number but also affect incompatibility map to the six-base loop of this structure (3, 12), so that this region is presumably the active site for the initiation of binding between RNA transcripts.

The 132-kilobase-pair (kb)-colicinogenic plasmid ColV2-K94, like the F plasmid, is a member of the IncFI incompatibility group. Recently, we reported that a replication region of ColV2-K94, Repl, showed homology to and was incompatible with the RepA replicon of IncFII R plasmids (21). In subsequent experiments, however, it was determined that Repl and RepA were indeed compatible replicons; our previous observations were probably due to the instability of ColV2-K94 derivatives in our C600 test strain (P. C. Weber and S. Palchaudhuri, unpublished observations). In this work we have cloned the Repl replicon and compared its properties with those of RepA; we then determined the nucleotide sequence of its replication control region. We offer here an explanation as to how these two very similar replication systems have evolved into compatible replicons.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* hosts and the plasmids used in this work are listed in Table 1. Recombinant plasmids containing Repl were derived from the 45-kb *Eco*RI-generated plasmid pWS17, on which Repl was originally isolated (21), and are illustrated in Fig. 1.

Cloning procedures and nucleotide sequence determination. Repl-containing restriction nuclease fragments of plasmid pWS17 were cloned into pBR322 or into self-replicating miniplasmids by the procedures described previously (22). DNA sequencing of restriction nuclease fragments cloned into the bacteriophage vector M13mp18 was performed by the dideoxy method (16). DNA was isolated on cesium chloride-ethidium bromide equilibrium density gradients as described previously (21), and restriction nuclease fragments were recovered from agarose gels for cloning by phenol extraction (1). The RNA folding program used was devised by B. Polisky and kindly provided by T. Dooley, and the free energy values for base pairing between RNA molecules were calculated by the rules of Tinoco et al. (20).

Incompatibility testing and copy number determinations. The procedures for incompatibility testing and copy number determinations by both dye-buoyant density centrifugation and gel densitometry have been described previously (10, 21).

RESULTS

Cloning the replication and incompatibility functions of Repl. The cloning and mapping of the Repl replicon was facilitated by a recently completed restriction map of the ColV2-K94::Tn*903* derivative pWS12 (22). Restriction nuclease fragments carrying Repl were isolated by two approaches: by cloning restriction fragments in pBR322 which rescue the replication of this ColE1-derived vector in the *polA* strain C-2110 and by generating miniplasmids in vitro which contain Repl and the adjacent kanamycin resistance marker of Tn*903*. Restriction nuclease fragments containing Repl from the *Eco*RI-generated pWS12 derivative pWS17 (21) are shown in Fig. 1.

The 4.0-kb *Hin*dIII fragment H9 contained Repl, as the pBR322 derivative carrying this restriction fragment

TABLE 1. Bacterial strains and plasmids

E. coli strain and plasmid	Genotype[a]	Reference or source
E. coli		
SP600	*hsdS thi-1 thr-1 leuB6 lacY1 fhuA21 supE44* (derived from C600)	21
HB101	*hsdS20 recA13 ara-14 proA2 leuB6 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44*	22
C-2110	*polA1 his rha*	22
JM103	Δ(*lac-pro*) *thi rpsL endA sbcB15 hsdR4 supE* (F′ *traD36 proAB lacI*^{q} Δ*lacZM15*)	24
Plasmid		
pWS12	ColV2-K94::Tn*903* (Km^r Tra$^+$ Rep1$^+$ Rep2$^+$)	10
pWS16	pWS12Δ107.0V-34.6V (Km^r Tra$^+$ Rep1$^+$, a self-replicating 72-kb *Bam*HI fragment containing Tn*903*)	21
pWS17	pWS16Δ110.8V-68.6V (Km^r Tra$^-$ Rep1$^+$, a self-replicating 42-kb *Eco*RI fragment containing Tn*903*)	21
pWS18	pWS12Δ127.7V-27.3V (Km^r Tra$^+$ Rep1$^+$, a spontaneous deletion mutant containing Tn*903*)	22
R538	Cm^r Sm^r Su^r	21
R100	Cm^r Sm^r Su^r Tc^r Hg^r	9
pRR933	R100 RepA: *Pst*I fragments P4 and P6 (Cm^r, self-replicating)	9
pRR935	R100 RepA: *Pst*I fragment P6 in pBR322 (Tc^r)	9
pWS109	H9 of pWS17 in pBR322 (Ap^r Rep1$^+$)	This work
pWS109Δ1	pWS109 deleted of *Pst*I fragment P19 of H9 (Ap^r Rep1$^+$)	This work
pWS109Δ2	pWS109 deleted of *Pst*I fragments P18 and P19 of H9 (Ap^r Rep1$^-$)	This work
pWS518	*Pst*I fragment P18 of H9 in pBR322 (Tc^r)	This work
pWS61	*Pst*I fragments P2 and P18 of pWS17 (Km^r, self-replicating Rep1 miniplasmid containing Tn*903*)	This work

[a] Abbreviations: Km, kanamycin; Cm, chloramphenicol; Su, sulfonamide; Sm, streptomycin; Tc, tetracycline; Ap, ampicillin; Hg, mercuric salts.

(pWS109) could replicate in the *polA* strain C-2110. *Pst*I digestion of pWS109 revealed two 0.55-kb *Pst*I fragments within H9, P18 and P19. Deletion of P19 (pWS109Δ1) still yielded a functional Rep1, although the copy number of this derivative was found to be increased approximately 10-fold. However, deletion of both P19 and P18 (pWS109Δ2) abolished Rep1 function, indicating that P18 was essential for replication.

Since the H9 fragment was adjacent to the Tn*903* transposon (22), an attempt was made to create a miniplasmid in vitro which contained both Rep1 and Tn*903*, whose kanamycin resistance gene would serve as a selection marker. Limited digestion of pWS17 DNA with *Pst*I and subsequent ligation yielded the miniplasmid pWS61 (Fig. 1), which consisted of the 6.0-kb *Pst*I fragment P2 (the 2.8-kb ColV2-K94 DNA plus the 3.2-kb Tn*903*) and the 0.55-kb P18 fragment. The overlapping portions of pWS61 and pWS109Δ1 (Fig. 1) indicated that the minimum essential region for Rep1 function comprises an area of 1.95 kb: a 1.4-kb *Pst*I-*Hin*dIII fragment shared by H9 and P2 and the 0.55-kb P18 fragment. The coordinates for Rep1 were 99.1V to 101.1V, a region which mapped between the Tn*903* transposon and the ColV2-K94 inverted repeat structure X_1 (22).

The P18 fragment was cloned into pBR322 to generate pWS518. P18 contained the incompatibility determinant of the Rep1 replicon, as evidenced by the ability of pWS518 to displace the resident ColV2-K94 derivatives pWS16 and pWS18 (Table 2). The remaining portion of the Rep1 replicon, cloned in pWS109Δ2, showed no such incompatibility behavior. It was interesting that the cloned P18 fragment exerted no incompatibility towards the intact ColV2-K94, pWS12, which is a cointegrate plasmid containing not only Rep1 but also a second replicon, Rep2 (21). Rep2 could replicate pWS12 when the incompatibility-replication control repressor from P18 turned off Rep1, since the pWS12 deletion derivatives pWS16 and pWS18, which lack Rep2, did not replicate in the presence of pW518 (Table 2).

A physical and genetic comparison of Rep1 and RepA. A comparison of the physical maps of Rep1 and RepA showed that both have homologous restriction sites (Fig. 1). When the *Pst*I fragments of pWS17 were blotted onto a nitrocellulose filter and probed with the R100 RepA miniplasmid pRR933, the Rep1 fragments P2, P18, and P19 showed homology to RepA (21; P. C. Weber and S. Palchaudhuri, unpublished observations). In addition to this structural homology between replicons, there was functional homology between the 0.55-kb P18 and P19 fragments of Rep1 and the 0.55-kb fragments termed F1 and F2, respectively, of the R1 RepA.

Deletion of the F2 fragment in the R1 RepA results in mutation of the *copB* gene and a subsequent 8-fold increase in copy number (13); as discussed above, deletion of P19 in Rep1 (pWS109Δ1) increased the plasmid copy number approximately 10-fold, suggesting that Rep1 also had a CopB-like function. Despite this relaxation in replication control, neither the F2 fragment of R1 nor the P19 fragment of pWS17 is essential for the operation of their respective replicons. When the F1 fragment of R1 is cloned into a vector, it can express incompatibility towards its parental replicon, as it contains the incompatibility-replication control gene *copA* (11); an identical behavior was observed with the cloned P18 fragment (Table 2).

Despite this strong evidence that Rep1 and RepA are closely related, their incompatibility functions did not cross-react: the cloned P18 fragment had no effect on the replication of the IncFII plasmid R538, and the cloned incompatibility gene of R100 (*Pst*I fragment P6) had no effect on ColV2-K94 replication (Table 2). As the nucleotide sequence of RepA has been reported previously for R1 and R100 (14, 19), we sequenced the P18 fragment in an attempt to explain the lack of incompatibility between these two replicons.

Nucleotide sequence of the *Pst*I P18 fragment of Rep1. The nucleotide sequence of P18 was determined. In addition to sequencing from the *Pst*I ends, use was made of an internal 263-base-pair (bp) *Sau*3A1 fragment of P18, which was cloned into the *Bam*HI site of M13mp18 and sequenced. This strategy and the complete DNA sequence of P18 are shown in Fig. 2.

When compared with the sequence of F1 of the R1 RepA

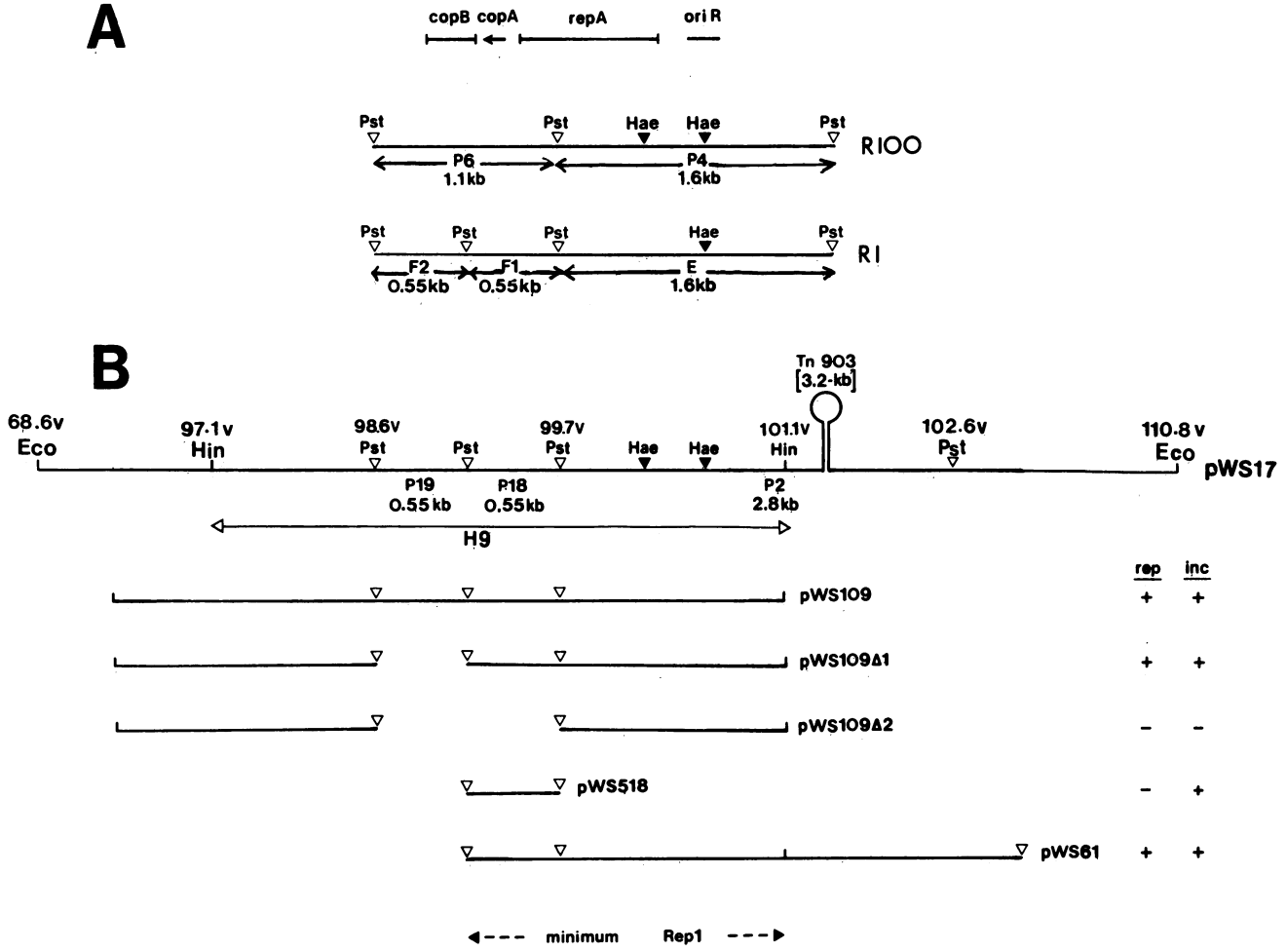

FIG. 1. (A) Genetic (top) and restriction (bottom) maps of the RepA replicons in the IncFII plasmids R100 and R1 (based on reference 14). (B) Restriction map of the Rep1 replicon in pWS17, oriented with respect to the RepA maps in panel A. All restriction fragments shown were cloned in pBR322 with the exception of pWS61, which is a self-replicating 6.55-kb miniplasmid. The ability of each recombinant plasmid to replicate (rep) itself in the *polA* strain C-2110 and to exert incompatibility (inc) against ColV2-K94 derivatives is shown at right; the minimum essential region for Rep1 function is delineated at the bottom. Restriction sites shown are for *Pst*I (Pst), *Hin*dIII (Hin), *Hae*II (Hae), and *Eco*RI (Eco).

replicon, P18 revealed 21 base changes. Regions corresponding to the *copB*, *copA*, and *repA* genes, as well as sequences controlling their expression, were all present on P18, suggesting that the genetic organization of Rep1 and RepA is identical. Although most of the 21 sequence alterations were scattered and did not appear to affect any important loci, there were six base changes (one of them being an insertion mutation) in only a 24-bp span of the *copA* incompatibility gene. As even single base changes in the *copA* gene of the RepA replicon can have profound effects on CopA RNA secondary structure, plasmid copy number, and incompatibility properties (3), these six base changes were suspected to be responsible for the altered incompatibility behavior of Rep1.

TABLE 2. Properties of cloned incompatibility genes of Rep1 and RepA

Plasmid	Cloned insert	% Loss of resident plasmids[a]			
		pWS16	pWS18	pWS12	R538
pBR322		0	0	0	0
pWS109	H9 (P18 + P19) of ColV2-K94[b]	90	90	0.5	0
pWS518	P18 of ColV2-K94	88	87	2.1	0
pWS109Δ2	H9ΔP18, ΔP19 of ColV2-K94	1.5	1.8	1.1	0
pRR935	P6 of R100	1.4	1.8	1.1	95

[a] Loss of resident plasmid indicates ability of cloned fragments to exert incompatibility towards resident plasmids by procedures described previously (10, 21).

[b] H9 is the minimum Rep1 replicon which includes P18 and P19; pWS109Δ2 is the deletion derivative of H9 which lacks both P18 and P19.

Potential secondary structure of the Rep1 CopA RNA. Potential secondary structures of the predicted Rep1 and RepA CopA RNA molecules were compared and are illustrated in Fig. 3. The six base changes in the ColV2-K94 CopA RNA resulted in a 49-bp stem-loop structure. Any possible destabilizing effects of the A-T insertion at position 190 have been offset by the change from A-T to G-C at 188, so that the resulting base pairing of the Rep1 CopA RNA stem-loop structure was actually more stable than that of the RepA (−25.95 kcal [−108.57 kJ] to −24.4 kcal [−102.09 kJ] per mole). Similarly, the change from C-G to G-C at 172 and the change from G-C to C-G at 181 were compensatory, as

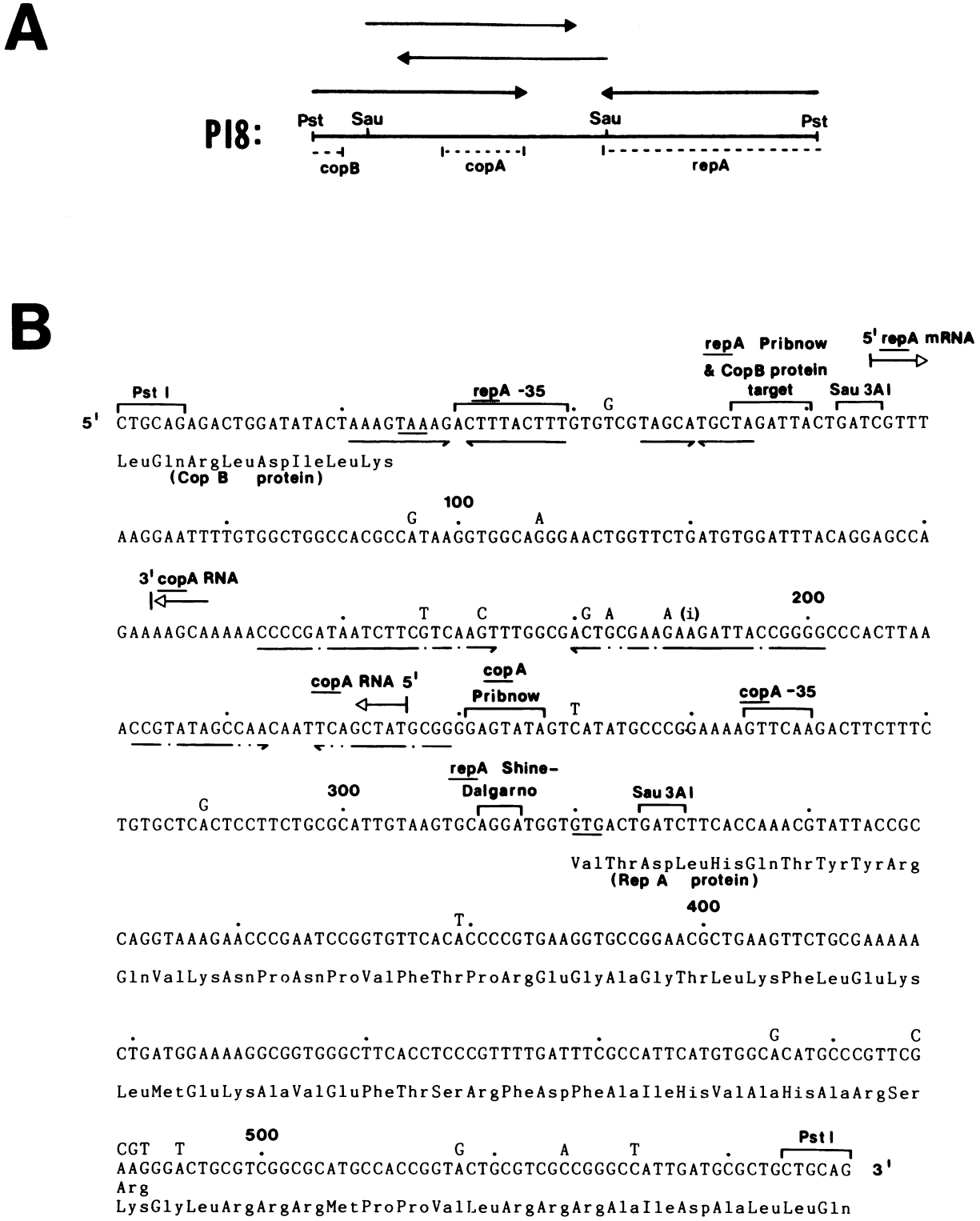

FIG. 2. (A) Strategy for sequencing the P18 fragment. The entire P18 fragment and an internal *Sau*3AI (Sau) fragment were cloned into the *Pst*I (Pst) and *Bam*HI sites, respectively, of the M13mp18 vector; each clone was sequenced a minimum of two times. (B) Complete nucleotide sequences of the 544-bp *Pst*I fragment P18. Listed above the ColV2-K94 sequence are differences found in the corresponding sequence for the R1 RepA (i.e., the 543-bp *Pst*I fragment F1 [14, 19]); a single base insertion of P18 not found in F1 is marked with an (i). Inverted repeat sequences are delineated by arrows under the DNA sequence. Assignment of protein-coding regions and transcription and translation signals is based on previous studies with RepA (13, 14, 18).

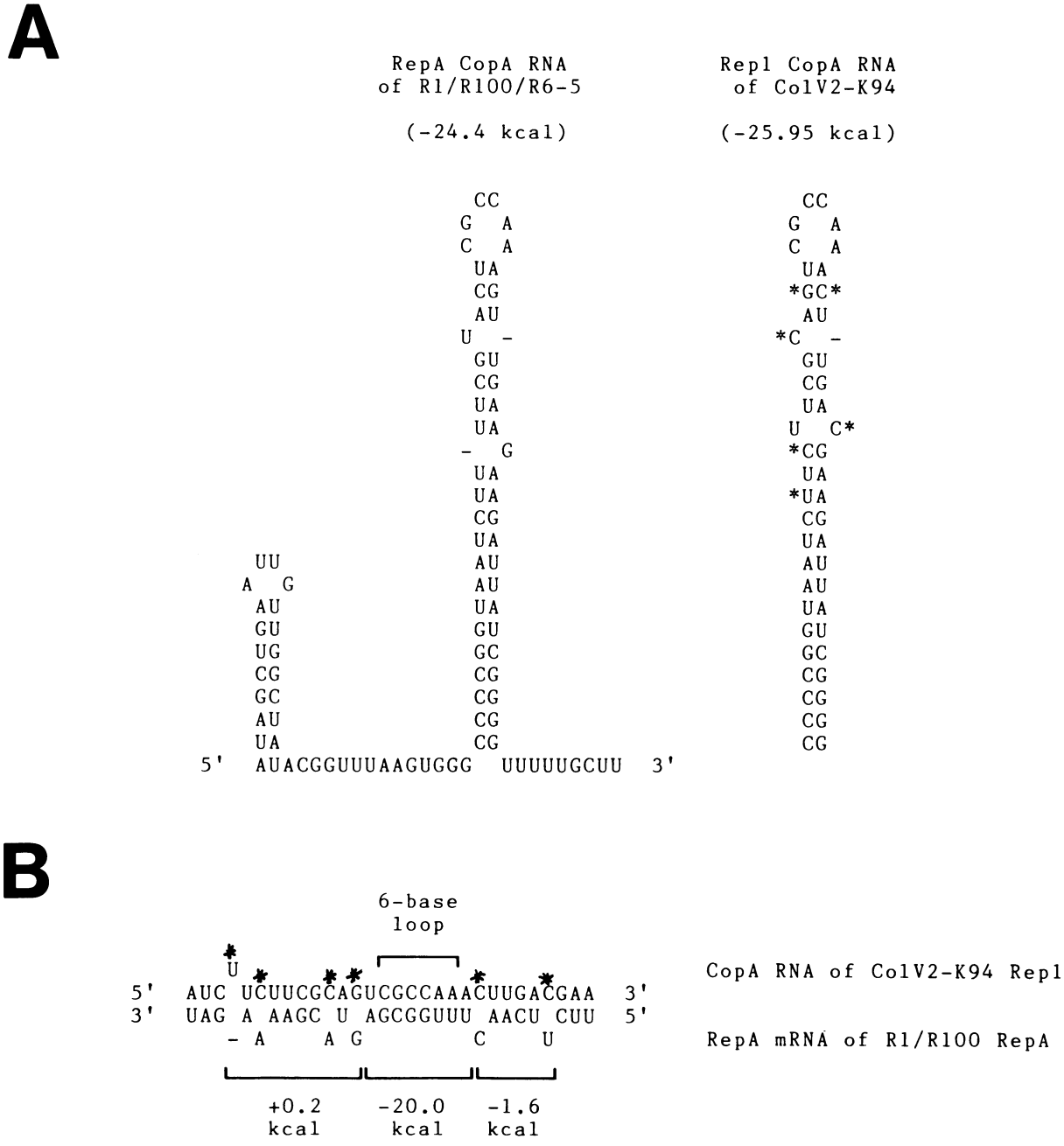

FIG. 3. (A) Comparison of potential secondary structure of Repl and RepA CopA RNA molecules. The complete structure of the 91-base CopA RNA of RepA (15, 18) is shown at the left; only the larger 49-bp stem-loop structure of the potential CopA RNA of Repl is shown at the right, with its six base alterations marked by asterisks. (B) Free energy of binding between the CopA RNA of Repl and the RepA mRNA of RepA, as calculated by the rules of Tinoco et al. (20). The binding energy is greatly reduced by base mismatches on either side of the six-base-loop region.

either of these mutations alone would have led to the formation of an unstable 15-base loop (see reference 3 for a characterization of such a *copA* mutant in RepA).

Perhaps of most interest was the fact that the six-base loop of the CopA RNA, the active site of the molecule and the site of most incompatibility mutations (12), remained identical in both Repl and RepA. Thus, the lack of incompatibility between these two replication systems had to be due to sequence differences in the stem structures. Figure 3B shows that the binding between the CopA RNAs and the RepA RNAs of the two replicons would be hindered by these six base changes. Strong binding would initially occur between the six-base loops of the two transcripts, but further binding between the regions flanking the loops would be greatly inhibited by the mismatches introduced by the six base differences between the R100 and ColV2-K94 sequences; thus, both RNA molecules would tend to remain in their hairpin conformations, rather than unfold to bind to complementary transcripts from the other replicon. The result of this lack of interaction is that the CopA RNAs of Repl and RepA are unable to repress the replication of each other, so that the two replicons are now rendered compatible.

DISCUSSION

Through cloning self-replicating restriction nuclease fragments, restriction enzyme analysis, and DNA sequencing, we have determined that the Rep1 replicon of the IncFI plasmid ColV2-K94 is structurally and functionally homologous to the RepA replicon of IncFII R plasmids. Despite the remarkable similarities between these two replication systems, they were mutually compatible with each other. Nucleotide sequence determination of the Rep1 *copA* incompatibility-replication control region revealed a cluster of six base changes which could sufficiently lessen the interaction between the *copA* transcript of one replicon and the *repA* transcripts of the other replicon; through this evolution of the *copA* gene, the two replication systems have now become compatible.

The Rep1 replicon differed from previously described *copA* mutants of RepA (3, 12), which have an elevated copy number, polar incompatibility (i.e., exerted in one direction only), and single-base changes which usually map to the six-base loop (not the stem) of the CopA RNA. In contrast, the Rep1 *copA* gene stringently regulated ColV2-K94 replication (copy number, 1 to 2 per chromosome) (10), had six base differences from the RepA *copA* gene which map to the stem (not the loop) of the CopA RNA, and caused Rep1 to behave as a member of a new incompatibility group which is distinct from that of the IncFII plasmids. For these reasons, the *copA* gene of Rep1 behaves as an allelic form of the RepA *copA* gene rather than as a mutant: both repressors appear to serve analogous functions but fail to cross-react. In the same way, allelic forms of the *copB* gene have been found in R1 and R100 (13).

Since Rep1 and RepA appeared to be functionally similar replication systems, these results suggest that the phenomenon of compatibility may not be a useful indicator of lack of relatedness between plasmids. Although ColV2-K94 is a member of the IncFI incompatibility group and contains the secondary replicon (Rep2) and partitioning genes of the F plasmid (10, 21), it also has a replicon (Rep1) homologous to the RepA replicon of IncFII plasmids and a transfer (*tra*) operon similar to that of the IncFII plasmid R538 (22). The relationship between ColV2-K94 and R538 is also evident from studies which produced a ColV2-K94-like plasmid through recombination between R538 and a small, nontransmissible IncFI ColV plasmid (4). These properties of ColV2-K94 suggest that its hybrid nature may be due to recombination across homologous *tra* operons of an IncFI and an IncFII plasmid; however, the proximity of the transposonlike structure X_1 to Rep1 indicates that a translocation event may have placed a RepA-like replicon on the ColV2-K94 plasmid.

It is not clear what stimulus, if any, triggered the six clustered base changes in the *copA* gene. Since each of these alterations alone would have little effect on the stability of the CopA RNA or on plasmid copy number (except for the changes at positions 172 and 181, which conveniently negate the deleterious effects of each other), it appears that the portion of the stem structure containing several bulge loops may be free to vary its nucleotide sequence. The abundance of variation elsewhere on the P18 fragment sequence supports this notion that the *copA* gene has been altered slowly with time. However, it is tempting to speculate that the *copA* gene changes were mediated through some outside selection pressure. For example, the Rep1 sequence evolution described here now enables the ColV2-K94 plasmid with its virulence properties (2) to coexist stably with IncFII antibiotic resistance plasmids, a situation which would certainly be of selective advantage to a pathogenic host.

ACKNOWLEDGMENTS

We thank Robert Rownd for his recombinant plasmids containing the RepA functions of R100, Tom Dooley of Barry Polisky's laboratory for the RNA folding program used, Vipul Patel for technical assistance in DNA sequencing, and Paul Chuba for stimulating discussions.

LITERATURE CITED

1. **Benson, A.** 1984. A rapid procedure for isolation of DNA fragments from agarose gels. BioTechniques **2**:66–67.
2. **Binns, M. M., D. L. Davis, and K. G. Hardy.** 1979. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. Nature (London) **279**:778–781.
3. **Brady, G., J. Frey, H. Danbara, and K. N. Timmis.** 1983. Replication control mutations of plasmid R6-5 and their effects on interactions of the RNA-I control element with its target. J. Bacteriol. **154**:429–436.
4. **Cooper, P.** 1971. Interaction of a colicinogenic factor with a resistance factor and with the fertility factor F in *Escherichia coli* K12. Genet. Res. **17**:151–159.
5. **Light, J., and S. Molin.** 1981. Replication control functions of plasmid R1 act as inhibitors of expression of a gene required for replication. Mol. Gen. Genet. **184**:56–61.
6. **Light, J., and S. Molin.** 1982. The sites of action of the two copy number control functions of plasmid R1. Mol. Gen. Genet. **187**:486–493.
7. **Light, J., and S. Molin.** 1983. Post-transcriptional control of expression of the *repA* gene of plasmid R1 mediated by a small RNA molecule. EMBO J. **2**:93–98.
8. **Masai, H., Y. Kaziro, and K. Arai.** 1983. Definition of *ori*R, the minimum DNA segment essential for initiation of R1 plasmid replication in vitro. Proc. Natl. Acad. Sci. USA **80**:6814–6818.
9. **Miki, T., A. M. Easton, and R. H. Rownd.** 1980. Cloning of replication, incompatibility, and stability functions of R plasmid NR1. J. Bacteriol. **141**:87–99.
10. **Mitra, G., and S. Palchaudhuri.** 1984. Genetic structure and stability of a copy number mutant of IncFI group plasmid ColV-K94 in *Escherichia coli* K12. Mol. Gen. Genet. **193**:349–357.
11. **Molin, S., and K. Nordström.** 1980. Control of plasmid R1 replication: functions involved in replication, copy number control, incompatibility, and switch-off of replication. J. Bacteriol. **141**:111–120.
12. **Nordstrom, K., S. Molin, and J. Light.** 1984. Control of replication of bacterial plasmids: genetics, molecular biology, and physiology of the plasmid R1 system. Plasmid **12**:71–90.
13. **Nordstrom, M., and K. Nordstrom.** 1985. Control of replication of FII plasmids: comparison of the basic replicons and of the *copB* systems of plasmids R100 and R1. Plasmid **13**:81–87.
14. **Rosen, J., T. Ryder, H. Inokuchi, H. Ohtsubo, and E. Ohtsubo.** 1980. Genes and sites involved in replication and incompatibility of an R100 plasmid derivative based on nucleotide sequence analysis. Mol. Gen. Genet. **179**:527–537.
15. **Rosen, J., T. Ryder, H. Ohtsubo, and E. Ohtsubo.** 1981. Role of RNA transcripts in replication, incompatibility, and copy number control in antibiotic resistance plasmid derivatives. Nature (London) **290**:794–799.
16. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
17. **Scott, J. R.** 1984. Regulation of plasmid replication. Microbiol. Rev. **48**:1–23.
18. **Stougaard, P., S. Molin, and K. Nordstrom.** 1981. RNAs involved in copy-number control and incompatibility of plasmid R1. Proc. Natl. Acad. Sci. USA **78**:6008–6012.
19. **Stougaard, P., S. Molin, K. Nordstrom, and F. G. Hansen.** 1981. The nucleotide sequence of the replication control region of the resistance plasmid R1*drd*-19. Mol. Gen. Genet. **181**:116–122.

20. **Tinoco, I., P. Borer, B. Dengler, B. Levine, O. Uhlenback, D. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246**:40–41.
21. **Weber, P. C., G. Mitra, and S. Palchaudhuri.** 1984. Second replicon in ColV2-K94 mediates the stable coexistence of two incompatible plasmids. J. Bacteriol. **160**:245–250.
22. **Weber, P. C., and S. Palchaudhuri.** 1985. A physical and genetic map of the IncFI plasmid ColV2-K94. Plasmid **13**:215–218.
23. **Womble, D. D., X. Dong, R. P. Wu, V. A. Luckow, A. F. Martinez, and R. H. Rownd.** 1984. IncFII plasmid incompatibility product and its target are both RNA transcripts. J. Bacteriol. **160**:28–35.
24. **Yanisch-Perron, C., J. Vierra, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strain. Nucleotide sequence of M13mp18 and pUC19 vectors. Gene **33**:103–119.