A Sequence That Affects the Copy Number and Stability of pSW200 and ColE1\textsuperscript{V}

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Pantoea stewartii SW2 contains 13 plasmids. One of these plasmids, pSW200, has a replicon that resembles that of ColE1. This study demonstrates that pSW200 contains a 9-bp UP element, 5'~AAGATCTTTC, which is located immediately upstream of the −35 box in the RNAII promoter. A transcriptional fusion study reveals that substituting this 9-bp sequence reduces the activity of the RNAII promoter by 78%. The same mutation also reduced the number of plasmid copies from 13 to 5, as well as the plasmid stability. When a similar sequence in a ColE1 derivative, pYC2301, is mutated, the copy number of the plasmid also declines from 34 to 16 per cell. Additionally, inserting this 9-bp sequence stabilizes an unstable pSW100 derivative, pSW142K, which also contains a replicon resembling that of ColE1, indicating the importance of this sequence in maintaining the stability of the plasmid. In conclusion, the 9-bp sequence upstream of the −35 box in the RNAII promoter is required for the efficient synthesis of RNAII and maintenance of the stability of the plasmids in the ColE1 family.

Plasmids of the ColE1 type are commonly found in the bacteria belonging to the family Enterobacteriaceae (2, 9, 10, 13, 14, 27, 37, 55). Rather than utilizing a replication initiation protein, these plasmids synthesize preprimer RNA (22, 31), which forms a DNA-RNA hybrid at oriV (48), which allows the RNA to be cleaved by RNase H (22, 41). The cleaved RNA, which is called RNAl, acts as a primer to facilitate the initiation of plasmid replication (22). Therefore, the capacity of the preprimer RNA to couple with oriV is critical to the initiation of plasmid replication (22, 48). The plasmid also tran

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FIG. 1. Identifying the region in pSW200 that affects plasmid stability. (A) Map of pSW201, pSW203, pSW240, and pSW249. An empty triangle represents a kanamycin resistance gene. (B) Sequence from nt 151 to 400 in pSW200. “−35,” −35 box of RNAIIp; sps, sequence for plasmid stability. (C) E. coli HB101, which contained pSW201, pSW203, pSW240, or pSW249, was cultured and replica plated to determine the numbers of colonies that did not contain a plasmid. The experiment was performed three times. Error bars represent standard deviations.

Pantoea stewartii subsp. stewartii, a member of the Enterobacteriaceae, is a corn pathogen that causes Stewart’s wilt (15). One of the strains, SW2, contains 13 plasmids (15). The two smallest plasmids in this strain, pSW100 and pSW200, contain a replicon that resembles that of ColE1 (18, 19). Although neither of these plasmids carries a gene that encodes the synthesis of bacteriocin, these two plasmids contain the smallest plasmids in this strain, pSW100 and pSW200, contain a replicon that resembles that of ColE1 (18, 19). Although neither of these plasmids carries a gene that encodes the synthesis of bacteriocin, these two plasmids contain mob genes and a bom region that are also present in ColE1 (19). The mob genes and bom enable pSW100 and pSW200 to be mobilized by the F plasmid (19). Plasmid pSW100 has about 10 copies per cell (18) and uses TraC in the sex pilus assembly as an apparatus for segregation to maintain the stability of the plasmid (25). This study finds that a 9-bp sequence region, which is located at −43 in RNAIIp in pSW200 and ColE1, is an UP element and important to the synthesis of RNAII and the stability of the plasmid.

MATERIALS AND METHODS

Strains and culture medium. Escherichia coli HB101 (8) was used as a host to determine the stability of the pSW200 replicon. E. coli CSH50 (33) was used in fusion studies to analyze the activity of RNAIIp. LB medium was utilized to culture E. coli. Ampicillin (Ap) at 100 μg/ml, kanamycin (Km) at 50 μg/ml, and chloramphenicol (Cm) at 25 μg/ml were used to culture the strains that were resistant to these antibiotics.

Plasmids. A Km resistance gene from pUC4-KISS (3) was isolated by PstI digestion and inserted into the PstI site in pSW200 to yield pSW201 (19). The Km resistance gene was ligated with a TaqI DNA fragment that contained the sequence from nucleotide (nt) 172 to 1473 in pSW200, and generated pSW203 (Fig. 1A) (19). DNA fragments that contained the regions in pSW200 from nt 334 to 998, 345 to 998, 354 to 998, 372 to 998, 375 to 998, 378 to 998, 379 to 998, 380 to 998, 381 to 998, and 389 to 998 in pSW200 (Fig. 2A) were amplified by PCR, using pSW201 as a template, and ligated with a Km resistance gene to generate pSW240, pSW241, pSW242, pSW243, pSW244, pSW245, pSW246, pSW247, pSW248, and pSW250, respectively. Plasmid pSW240M had a sequence that was identical to that of pSW240, except that the sequence from nt 380 to 386 (Fig. 1B) was mutated from 5′-AAGATCT to 5′-CCTCGAG. The sequence between nt 378 and 388 in pSW245 was mutated to generate pSW245-M1, pSW245-M2, pSW245-M3, pSW245-M4, pSW245-M5, pSW245-M6, pSW245-M7, pSW245-M8, pSW245-M9 and pSW245-M10 (Fig. 3A). 5′-CC, 5′-CCGGG, and 5′-CCCCCGGGGG were inserted at nt 389 in pSW245 and generated pSW245-I2, pSW245-I5, and pSW245-I10, respectively. Plasmid pSW250 was formed by inserting a PCR-amplified DNA fragment that contained the region from nt 380 to 439 into the XbaI site in a fusion vector, pKM005 (Table 1). Plasmid pSW241 to pSW250.

FIG. 2. Region upstream of −35 box in RNAIIp and stability of the pSW200 replicon. (A) The Region upstream of the −35 box in RNAIIp, from nt 334 to 388, in pSW240 was deleted to generate plasmids pSW241 to pSW250. sps, sequence for plasmid stability (gray box). (B) E. coli HB101, which contained one of these plasmids, was cultured and replica plated to determine the numbers of colonies that did not contain a plasmid. The experiment was performed three times. Error bars represent standard deviations.
FIG. 3. Sequence essential to the stability of pSW200. (A) The sps region in pSW245 was mutated by nucleotide substitution or insertion. “−35” represents the −35 box in RNAIIP. sps, sequence for plasmid stability. (B) and (C) Stability of plasmids was evaluated in E. coli HB101. Cells were cultured and replica plated to determine the numbers of colonies that did not contain a plasmid. The experiment was performed three times. Error bars represent standard deviations.

in pSW100 (25) with a Km resistance gene. 5′-AAGATCTTTC was inserted at the 5′ end of the pSW100 replicon in pSW142K to generate pSW142KWR. A DNA fragment that encoded the E. coli σCTD, from amino acid 236 to amino acid 329, was amplified using the primers 5′-CGATCTCGAGTTACTCGTCAGCGATGC and 5′-CGATCTCGAGTTACTCGTCAGCGATGC/H11032 and inserted into the EcoRI and XhoI sites in pET-32a(H11001)/H9251. Cells were cultured and replica plated to determine the numbers of colonies that did not contain a plasmid. The experiment was performed three times. Error bars represent standard deviations.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Fragment in pSW200 (nt)</th>
<th>Sequence in sps−35 region in RNAIIP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-Galactosidase activity (Miller units)</th>
<th>Plasmid stability&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>pSW261</td>
<td>360–439</td>
<td>5′-AAGATCTTTCCTTTGAGATC</td>
<td>1,932 ± 127.4</td>
<td>100</td>
</tr>
<tr>
<td>pSW262</td>
<td>360–439</td>
<td>5′-CCTCCGAGCTCTTTGAGATC</td>
<td>422 ± 67.6</td>
<td>33</td>
</tr>
<tr>
<td>pSW266</td>
<td>381–439</td>
<td>5′-AGGTTCTTTCCTTTGAGATC</td>
<td>1,360 ± 189.9</td>
<td>50</td>
</tr>
<tr>
<td>pSW267</td>
<td>382–439</td>
<td>5′-GGTTCTTTCCTTTGAGATC</td>
<td>908 ± 16.3</td>
<td>30</td>
</tr>
<tr>
<td>pSW268</td>
<td>387–439</td>
<td>5′-CTTTGAGATCTTTGAGATC</td>
<td>735 ± 53.7</td>
<td>10</td>
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<tr>
<td>pSW269</td>
<td>389–439</td>
<td>5′-TTTAGAGATCTTTGAGATC</td>
<td>659 ± 79.8</td>
<td>6.5</td>
</tr>
<tr>
<td>pSW263</td>
<td>380–439</td>
<td>5′-AAGATCTTTCCTTTGAGATC</td>
<td>2,321 ± 329.4</td>
<td>100</td>
</tr>
<tr>
<td>pSW264</td>
<td>380–439</td>
<td>5′-AAGATCTTTCCTTTGAGATC</td>
<td>2,384 ± 157.6</td>
<td>100</td>
</tr>
<tr>
<td>pKM005</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.8 ± 1</td>
<td>NA</td>
</tr>
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<sup>a</sup> Underlined sequence represents nucleotide substitution. The −35 box is boldfaced.

<sup>b</sup> Determined after 84 generations of culturing.

<sup>c</sup> NA, not applicable.
not alter the stability of the plasmid (Fig. 1C). However, deletion of the region from nt 334 to 381 (pSW249) (Fig. 1A and B) affected the stability of the plasmid. After 14 and 42 generations of culturing, 14.5% and 52% of the colonies had lost pSW249, respectively. At the 84th generation, the corresponding value was 89% (Fig. 1C). These results show that the region between nt 334 and 381 (Fig. 1A and B) contains the sequence that is critical to the stability of the pSW200 replicon.

Deletion analysis of sequence upstream of the $-35$ box in RNAIIp. Since the region between nt 334 and 381 contained a sequence that affected plasmid stability, this study deleted the region between nt 334 and 379 in pSW240 and generated pSW241, pSW242, pSW243, pSW244, pSW245, pSW246, and pSW247 to determine the sequence that is important to plasmid stability (Fig. 2A). These plasmids were stable in E. coli HB101 throughout 84 generations of culturing in LB broth (Fig. 2B). However, when the deletion was extended to nt 380, the plasmid, pSW248 (Fig. 2A), became unstable. After 14, 42, and 84 generations of culturing, 13.5%, 34.2%, and 50.4% of the cells, respectively, had lost the plasmid (Fig. 2B). Furthermore, plasmid pSW249, which contained a deletion from nt 334 to 381, was also unstable (Fig. 1C). When the deletion reached nt 388, the plasmid, pSW250 (Fig. 2A), was lost more quickly than pSW248. At the 14th generation, 39.7% of the cells had lost the plasmid; at the 42nd generation, the corresponding percentage was 83.4%, and at the 84th generation, it was 93.5% (Fig. 2B). The result indicated that the region between nt 380 and 388 is important to plasmid stability.

Mutational analysis of pSW245. In this study, pSW245 was mutated to elucidate how the sequence between nt 378 and 388 influenced the stability of the pSW200 replicon. Mutating nt 378 and 379 (pSW245-M1) (Fig. 3A) did not alter plasmid stability. The plasmid was stable in E. coli HB101 during 84 generations of culturing (Fig. 3B). However, a plasmid with a mutation in the region between nt 380 and 382 (pSW245-M2) (Fig. 3A) was unstable. At the 84th generation, the plasmid was absent from 95.2% of the colonies (Fig. 3B). Mutating the regions from nt 383 to 385 (pSW245-M3) (Fig. 3A) and nt 386 to 388 (pSW245-M4) (Fig. 3A) also destabilized the replicon. At the 84th generation, 74.4% and 95% of the colonies, respectively, had lost the plasmid (Fig. 3B). Additionally, mutating the nucleotide sequence immediately upstream of the $-35$ box, nt 387 or 388, only slightly affected the stability of the plasmid. A mutation from T to G at nt 387 (pSW245-M7) decreased the plasmid stability only 1.5% after 84 generations of culturing (Fig. 3A and B). On the other hand, a T-to-A (pSW245-M9) or T-to-C (pSW245-M8) mutation (Fig. 3A) did not influence plasmid stability, and the plasmids were stable over 84 generations of culturing (Fig. 3B). Similarly, a single nucleotide substitution from C to A at nt 388 (pSW245-M6) (Fig. 3A) reduced the stability by 1.5% after 84 generations of culturing. However, mutating nucleotides 387 and 388 simultaneously from TC to GA (pSW245-M5) markedly reduced the stability of the plasmid; by the 84th generation, 29.3% of the colonies had lost the plasmid (Fig. 3B). Mutating nt 386 to 388 from TTC to GGA (pSW245-M4) (Fig. 3A) further reduced stability. At the 84th generation, 95% of the colonies had lost the plasmid (Fig. 3B), indicating that the region from nt 380 to 388, which has a $-35$-AAGATCTTC sequence, is essential to maximal stability of pSW200. This segment of DNA is here called the sequence for plasmid stability or sps. Because the sps region contains a Dam methylation site, 5$'$-GATC, this study mutated this sequence to 5$'$-AATC (Fig. 3A) and found that the mutant, pSW245-M10, was stable during 84 generations of culturing (Fig. 3B). Additionally, 2, 5, and 10 nucleotides were separately inserted between sps and the $-35$ box of RNAIIp. These plasmids, pSW245-12, pSW245-15, and pSW245-110 (Fig. 3A), were unstable in E. coli HB101. At the 84th generation, 86%, 99%, and 73% of the colonies had lost the plasmids, respectively (Fig. 3C), suggesting that the distance between sps and the $-35$ box is also crucial to plasmid stability.

**sps and plasmid copy number.** Whether mutation of sps changed the copy number of pSW240 was studied. The first seven nucleotides in sps in pSW240 were changed from 5$'$-AGATCT to 5$'$-GATC, this study mutated this sequence to 5$'$-CCTCGAG to yield pSW240M. In E. coli HB101, the mutant plasmid was unstable. After culturing in LB broth for 84 generations, 67% of the colonies had lost the plasmid (data not shown). Meanwhile, E. coli HB101 was co-transformed with pACYC184 and pSW240. The transformants were cultured in LB broth that contained Cm and Km. The plasmids in the cells were purified, linearized by restriction digestion, and then separated by agarose gel electrophoresis (Fig. 4A, lane 1). Plasmid pACYC184 is known to have 18 copies per cell (10, 38). The intensity of the pSW240 band was compared with that of pACYC184, and the value was normalized to the size of the plasmid. Accordingly, pSW240 was estimated to have 13 copies per cell. A parallel experiment demonstrated that pSW240M had five copies per cell (Fig. 4A, lane 2), indicating that a mutation in sps reduces the copy number of the plasmid.

**sps and synthesis of RNAII.** To demonstrate that sps is important to the activity of RNAIIp, a transcriptional fusion between the region in pSW200 from nt 360 to 439 and lacZ was generated in a fusion vector, pKM005. This fusion plasmid, pSW261, in E. coli CSH50 exhibited 1,932 Miller units of $\beta$-galactosidase activity (Table 1). Another fusion plasmid in E. coli CSH50, pSW262, which had a sequence identical to that of pSW261 except that the first seven nucleotides in sps were mutated to 5$'$-CCTCGAG, exhibited 422 units of activity (Table 1), 78% less than that of pSW261. Deleting sps in pSW261, e.g., pSW266, pSW267, pSW268, and pSW269, also reduced promoter activity (Table 1), indicating that sps is critical to the
activity of RNAIIp. The instability of the plasmid was shown to be associated with reduced transcriptional activity (Table 1). The 5' -GATC sequence in sps in pSW263 was also mutated (Table 1). Changing 5' -GATC to 5' -AATC in pSW264 did not affect the activity of RNAIIp (Table 1).

Stabilization of pSW100 replicon by sps. The effect of sps on plasmid stability in a context other than pSW200 was examined. In addition to pSW200, P. stewartii SW2 has another ColE1-like plasmid, pSW100 (18). Unlike pSW200, pSW100 does not contain a sequence resembling sps. Our earlier work established that pSW100 utilizes a 38-bp TraC-binding sequence for segregation (Fig. 5A) and that this sequence is important to the stability of the plasmid (25). This work verified that a pSW100 derivative that contains a Km resistance gene, pSW100K (Fig. 5A), was stable (Fig. 5B). Decreasing the stability of the plasmid 75% over 84 generations of culturing in LB broth. However, inserting sps upstream of the −35 box in RNAIIp in pSW142K (pSW142KWR) (Fig. 5A) stabilized the plasmid. No plasmid loss was observed over 84 generations of culturing (Fig. 5B).

sps and copy number of ColE1 replicon. Plasmid pBR322 contains a sequence, 5'-AGGATCTTC, in RNAIIp which resembles sps in pSW200, 5'-AAGATCTTC. To study the function of this sequence, a DNA fragment that contained the region from nt 2436 to 3161 in pBR322 (38), including the entire replicon and sps, was ligated with a Km resistance gene to yield pYCW301. According to the analysis, a copy number of 34 copies per cell was estimated for pYCW301 (Fig. 4B, lane 1). When the first seven nucleotides in sps in pYCW301 were changed from 5'-AGGATCT to 5'-CCTCGAG, the copy number of the mutant plasmid, pYCW301M, decreased to 16 copies per cell (Fig. 4B, lane 2).

Binding of αCTD to sps. An earlier study demonstrated that the transcription in vitro of the RNAII promoter depends on αCTD, suggesting that the binding of αCTD to a region upstream of the −35 box is required to maximize RNAII synthesis (36). Therefore, a DNA affinity precipitation assay was performed to confirm the binding of His-αCTD to sps. Accordingly, His-αCTD (Fig. 6A, lane 1) and a biotinylated probe, p-sps, which contained RNAIIp and sps, were mixed. After the probe was captured using streptavidin magnetic beads, immunoblotting revealed the binding of His-αCTD to the probe (Fig. 6A, lane 2). A parallel experiment indicated that His-αCTD did not bind to a probe, p-msps, that lacks sps (Fig. 6A, lane 3), confirming the binding of His-αCTD to sps. Additionally, His-αCTD that was used herein had a segment of peptide in the N-terminal region that contained Trx, His, and S tags. This segment of peptide, which was purified from E. coli BL21[pET-32a(+)], (Fig. 6B, lane 1), did not bind to p-sps or p-msps (Fig. 6B, lanes 2 and 3), indicating that the binding of His-αCTD to p-sps was not attributable to the N-terminal tag region of the protein.

**DISCUSSION**

P. stewartii SW2 contains 13 plasmids. The two smallest plasmids in the strain, pSW100 and pSW200, are present in all P. stewartii strains that are isolated from nature (15), indicating that these two plasmids are stable throughout evolution. As is commonly known, plasmids of the ColE1 type usually do not use an active partition system for segregation to maintain their stability (16, 45). Rather, they segregate in random numbers into daughter cells during cell division (16, 45). Therefore, the goal of this work is to elucidate the mechanism by which pSW200 maintains its stability in the cell.

According to our results, pSW200 contains a 9-bp sequence, sps, that is located immediately upstream of the −35 box of RNAIIp and is critical to the stability of the plasmid. When this sequence is mutated, the pSW200 replicon becomes unstable and is rapidly lost during 84 generations of culturing (Fig. 1, 2, and 3). An analysis of the plasmid copy number after sps was mutated indicated that the number of copies of the pSW200 replicon declines from 13 to 5 per cell (Fig. 4A). An equation, $n_0 = 2^{1 - n}$ (44, 45), for the probability of the loss of ColE1
plasmids during random segregation, where $p_n$ and $n$ represent the probability that a cell does not receive a plasmid during segregation and the number of copies of the plasmid before cell division, respectively, is adopted to estimate the effect of the decline in the number of copies from 13 to 5 on plasmid stability. The calculation indicates that the probability of a cell not receiving a plasmid after cell division declines substantially, from $3.0 \times 10^{-3}$ to $2.0 \times 10^{-3}$, as the number of copies decreases from 13 to 5, respectively. Additionally, the drop in the number of copies is probably caused by a decrease in the synthesis of preprimer RNA, since a fusion study reveals that the activity of RNAIIp decreases 78% when $sp$s is mutated (Table 1), and an earlier study demonstrated that the level of preprimer RNA synthesis affects the number of copies of ColE1 (34). Moreover, a sequence that resembles that of $sp$s is present at the same location in both ColE1 and p15A. In a ColE1 derivative, pYCWW301, which lacks the rom gene, mutating this sequence reduces the number of copies from 34 to 17 bp that is located immediately upstream of the -APuPuATCTTC, which resembles that of $sp$s. The promoters of the ColE1 type commonly use $sp$s to promote the transcription of preprimer RNA to maintain a high number of copies, such that the plasmids can be stably maintained in their host. This study also analyzed the sequences of 15 plasmids of the ColE1 family in GenBank and found that 12 of them contain a consensus sequence, 5'-ApuPuATCTTC, which resembles that of $sp$s in pSW200, indicating that this sequence is present in many ColE1-type plasmids.

The UP element is a stretched A- and T-rich sequence of about 17 bp that is located immediately upstream of the −35 box and facilitates the efficient binding of RNA polymerase to a promoter through αCTD to enhance transcription (4, 17, 36). The promoters that are regulated by an UP element include E. coli rmB P1 and that of the Bacillus subtilis fengycin synthetase operon (24, 36). In fact, an UP element contains two subsites where the two αCTDs in RNA polymerase bind (17, 20). Furthermore, the presence of only the proximal subsite, where $sp$s is located, often suffices to activate transcription (17, 50). Ross et al. (36) demonstrated that deleting the C-terminal 73 amino acids in the RNA polymerase α subunit substantially reduced the capacity of RNA polymerase to transcribe the ColE1 preprimer RNA in vitro and proposed that the efficient synthesis of preprimer RNA depends on an UP element. This study shows that $sp$s is indeed an UP element, since αCTD binds to $sp$s (Fig. 6). Moreover, a transcriptional fusion that is generated with a fragment from nt 380 to 439 (pSW263), which contains an intact $sp$s but not the sequence upstream of $sp$s, yields LacZ activity to a degree that is similar to that of pSW261, which contains the region from nt 360 to 439 in pSW200 (Table 1), suggesting that the sequence upstream of nt 380 in pSW200 does not contain a distal UP subsite that promotes RNAII synthesis.

The ColE1 plasmid is known to contain three 5'-GATC methylation sites in RNAIIp, two of which, at −43 and −32, are also present in pSW200 (Fig. 1B). Patnaik et al. (34) demonstrated that mutations at both sites reduce the RNAIIp activity and plasmid stability. Our fusion study established that mutating the sequence at −43 in pSW200 to 5'-AATC does not affect the activity of RNAIIp or plasmid stability (Table 1), indicating that methylation at this site may not be important to RNAII synthesis. However, the possibility that methylation at −43 is important to RNAIIp activity and mutating the sequence to 5'-AATC strengthens the function of $sp$s as an UP element, potentially compensating for the mutation of the methylation site at −43, cannot be excluded. Additionally, 5'-AGATCT in sps is a DnaA-binding site (32, 42), but the binding of DnaA to $sp$s may not be important, since an earlier study revealed that ColE1 replication is independent of the dnaA gene (14).

Plasmid pSW200 may depend on additional mechanisms to maintain stability. In fact, pSW200 contains 41 15-bp repeats, from nt 3341 to 3955 (Fig. 1A) (19). This region confers a phenotype of plasmid exclusion (19); accordingly, a pSW200 derivative, pSW207, which does not contain the repeats cannot transform a host that contains a resident homoplasmid, pSW201, which itself contains the repeats (19), suggesting that the stability of pSW200 depends on these repeats. The function of these repeats is now being studied. In conclusion, this work identifies a sequence, $sp$s, that is important to the transcription of RNAII and to maintaining the copy number of plasmids of the ColE1 type.

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REFERENCES


