Replication Origins of Single-Stranded-DNA Plasmid pUB110

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The two replication origins of plasmid pUB110 have been characterized. The site of initiation of DNA replication at the plus origin was mapped to within an 8-base-pair sequence. DNA synthesis initiated at the origin was made to terminate precociously in an inserted sequence of 18 base pairs that is homologous to a sequence in the origin. This suggests that pUB110 replicates as a rolling circle. The minus origin of plasmid pUB110 has been characterized, and the minimal sequence required for function has been determined. As with other minus origins, activity is orientation specific with respect to the direction of replication. Its activity is sensitive to rifampin in vivo, suggesting that RNA polymerase catalyzes single-strand to double-strand conversion. Unlike all other plasmids of gram-positive bacteria thus far described, the pUB110 minus origin is functional in more than one host.

Numerous plasmids have recently been isolated from gram-positive bacteria and analyzed in detail. Most of these plasmids have common characteristics, as inferred from homologies in DNA and protein sequences (14, 33) and, in some cases, demonstrated by interchangeable functions (12, 16, 32). Perhaps the most significant common feature is that they all replicate via a single-stranded DNA (ssDNA) intermediate (37, 38). The accumulation of ss circular DNA corresponding to one strand of the plasmid monomer was discovered for several plasmids in both *Bacillus subtilis* and *Staphylococcus aureus* (38), and more recently plasmid ssDNA was also identified in *Lactococcus lactis* (W. de Vos, personal communication), *Streptococcus pneumoniae* (7) and *Streptomyces lividans* (29). A rolling-circle mechanism of plasmid replication was proposed (21, 37) and demonstrated directly for pT181 (20, 21) and pC194 (14). The numerous homologies that these two plasmids share with others that accumulate ssDNA make it likely that they all replicate by a common mechanism. Rolling-circle replication was first shown for the ssDNA *Escherichia coli* bacteriophages (2), which have homologies with some of these plasmids in their origins and replication proteins (14). Because of their accumulation of ssDNA and significant similarities in both structure and mode of replication to the ssDNA phages (14), these plasmids are referred to as ssDNA plasmids (for a review, see A. Gruss and S. D. Ehrlich, Microbiol. Rev., in press).

A minus origin (M-O), which is distinct and separable from the plus origin, is the initiation site for conversion of ssDNA to double-stranded DNA (dsDNA) (16). Interestingly, although many ssDNA plasmids replicate in two or more hosts (7, 10, 13, 17), their M-Os are functional only in their native hosts. The *palA*-type M-Os of staphylococcal plasmids pT181, pC194, and pE194 (16) and the M-O of streptococcal plasmid pLS1 (7) are inactive in *B. subtilis*, and the pC194 *palA*-type M-O is inactive in *Streptococcus pneumoniae* and *E. coli* (7). This was determined by analyzing the proportion of plasmid ssDNA in different hosts, for plasmids with or

without their M-Os. In a foreign host, significant amounts of ssDNA are detected, indicating that the M-O is an inefficient or inactive signal for conversion of ssDNA to dsDNA; in these cases, conversion appears to initiate nonspecifically (16).

Analyses of *E. coli* ssDNA phages reveal three types of M-O recognition (for a review, see reference 2). For phage G4, an RNA primer is synthesized by the host primase, encoded by the *dnaG* gene, at a specific M-O. For ϕX174, formation of the RNA primer requires the primosome multiprotein complex, similar to the complex used for lagging-strand synthesis of the *E. coli* chromosome (11). For the filamentous phages, synthesis of an RNA primer at the M-O is effected by RNA polymerase (*rpo* gene products). In the absence of a specific signal, conversion is thought to occur by nonspecific primosome attachment to ssDNA (1).

We have analyzed the replication origins of plasmid pUB110. This plasmid was originally isolated from *S. aureus* (22) and was found to be replicative in *B. subtilis* (17). Unlike other plasmids isolated from *Staphylococcus* spp., pUB110 was well adjusted in *B. subtilis*, maintaining a high copy number and total segregational stability (30). DNA homology exists between plus origin sequences of pC194 and pUB110 (14); also, amino acid homology is observed in the two Rep proteins (14). We observed that, although these plasmids have related replication functions in the plus origin, their M-Os are completely unrelated; unlike all other plasmids described thus far, pUB110 M-O functions in two hosts. As in other cases, the plus origin and M-O functions are separable. We report here the 8-base-pair sequence containing the initiation site at the plus origin. The minimal DNA sequence and properties of the M-O are presented.

MATERIALS AND METHODS

Plasmids and strains. Plasmids were constructed in *E. coli* strains HVC45 (laboratory collection) and JM105 (41) and then were introduced into *B. subtilis* SB202. Strain RN450 (gift of R. Novick) was used for experiments in *S. aureus*. Standard transformation techniques were used in all cases. Plasmids pUC19 (41), pC194 (6, 19), and pUB110 (25) have all been sequenced; the published nucleotide numbering has been maintained in this work (pC194 as described in reference 6). Plasmid pHV1160 was derived from pHV653 (26), by inserting a polylinker present in pGC2 (28) upstream from

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the ampicillin resistance gene. The sequences T18 and T22 were synthesized in vitro and inserted in the polylinker. Plasmid constructions are described in the Results section and in Fig. 1 through 3.

Media and growth conditions. L broth was used for liquid cultures of all species. When required, chloramphenicol was added at 5 μg/ml, kanamycin was added at 10 μg/ml, and ampicillin was added at 50 μg/ml. In experiments testing the role of RNA polymerase in the conversion of ssDNA to dsDNA, protein synthesis was blocked by the addition of erythromycin at 100 μg/ml and RNA synthesis was blocked by addition of rifampin at 100 μg/ml. Cells were grown at 37°C for up to 2 h in these experiments.

Isolation and analysis of DNA. Preparation of plasmid DNA was performed as described by Maniatis et al. (23). Whole-cell DNA lysates were prepared and run according to the procedure of Projan et al. (31). Southern blot hybridization (SBH) analyses for identification of ssDNA were performed essentially as published (36, 38). Agarose gels, 0.7%, were prepared and run in buffer containing ethidium bromide (40 μg/ml) (38). Plasmid pC194 DNA was nick translated (Amersham kit; Amersham Corp., Arlington Heights, Ill.) by using the instructions of the supplier and was used as hybridization probe. Restriction enzymes were obtained commercially and used according to the instructions of the supplier. DNA fragments were isolated from agarose gels with the Gene-Clean kit (Bio 101, La Jolla, Calif.). DNA sequencing analyses were performed by standard procedures (34) and were performed directly on the plasmid constructs.

RESULTS

The plus origin. A well-known characteristic of rolling-circle replication is that it can terminate precociously at a sequence which is only partially homologous to the plus origin (9). This phenomenon had been previously used to demonstrate that plasmid pC194 generates circular ssDNA by a rolling-circle-type mechanism (14; M.-F. Gros and H. te Riele, unpublished data): replication initiated at the 55-base-pair (bp) plus origin (coordinates 1430 through 1485) on the plasmid can terminate at an 18-bp subsequence of the origin containing the nick site (Fig. 1A), generating a smaller replicative molecule.

The same 18-bp sequence is also present on pUB110 (coordinates 4309 through 4292; Fig. 1A), just upstream from the gene encoding the plasmid replication protein (18). As the replication proteins of pUB110 and pC194 are about 30% homologous, it was previously proposed that pUB110, like pC194, replicates by a rolling-circle mechanism, each initiated by a nick in the same position within the 18-bp sequence (14; Fig. 1A). We constructed pUB110 derivatives containing pUB110 sequences plus a direct duplication of the 18-bp sequence and asked whether a smaller molecule is generated.

Plasmid pHV1160 (Fig. 1B) consists of the Sau3A segments A, C, and D of pUB110 (coordinates 1659 through 4373) containing plus origin replication functions and the kanamycin resistance gene, almost all of pBR322, the chloramphenicol resistance gene of pC194, and a polylinker derived from plasmid pGC1 (28). Into the polylinker was cloned either the 18-bp sequence, to form pHV1161, or the 18-bp sequence plus 4 adjacent base pairs as present in the pUB110 sequence, forming pHV1162. Each sequence (T18 and T22 [Fig. 1A]) was inserted in direct orientation with the natural sequence present in pUB110, as indicated by the
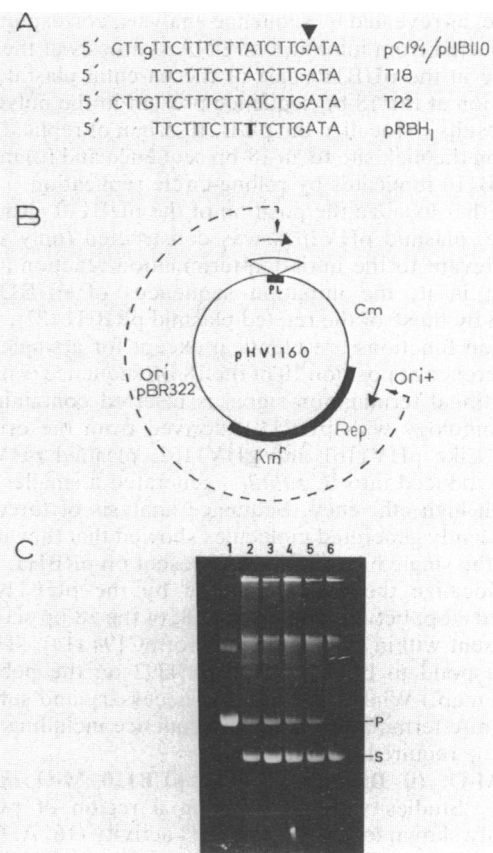

FIG. 1. Termination sequence of pUB110 replication. (A) The first sequence is the common sequence found in pC194 (bp 1428 through 1448) and pUB110 (bp 4313 through 4292). The small g indicates an extra base present in pUB110 but not in pC194. The second and third sequences show the termination signals T18 and T22. The fourth sequence is the 18-bp sequence found in pRBH1 containing a mismatch at position 10, shown in boldface. The triangle indicates the nick site found in pC194 (14). Replication proceeds in the rightward direction. (B) Plasmid pHV1160 containing pBR322 sequences, the chloramphenicol (Cm) resistance gene of pC194 (thin line), and the pUB110 sequence between positions 1659 and 4373 of the pUB110 map. The 18-bp sequence in pUB110 and either T18 or T22 (Fig. 1A) inserted in the polylinker (PL) in direct orientation are indicated by short arrows. Km^r, Kanamycin resistance gene; ori$^+$, plus origin replication functions. (C) 0.7% agarose gel with plasmid pHV1161 (containing T18) extracted from E. coli (lane 1), pHV1161 extracted from B. subtilis (lanes 2 and 3), pHV1162 (containing T22) extracted from B. subtilis (lanes 4 and 5), and a deletion derivative of pHV1161 extracted, retransformed, and reextracted from B. subtilis (lane 6). P, Parental plasmids; S, the smaller molecules generated by initiation-termination.

arrows in Fig. 1B; this also corresponds to the direction of plasmid replication (as determined from the polarity of the strand rendered single in the absence of an active M-O [H. te Riele, unpublished data]).

Plasmids pHV1161 and pHV1162 were constructed in E. coli and stably maintained in this host (Fig. 1C, lane 1). Unlike pC194, pUB110 does not replicate in E. coli (data not shown). However, upon introduction into B. subtilis, pHV1161 and pHV1162 were always accompanied by a smaller derivative (Fig. 1C, lanes 2 through 5). These smaller molecules could be easily purified by retransformation (Fig. 1C, lane 6, shows the smaller derivative of pHV1161). Their

structure, as revealed by sequence analysis, corresponded to that expected from initiation of DNA synthesis at the 18-bp sequence in the pUB110 part of the parental plasmids and termination at the 18-bp sequence present in the polylinker. These results (i) localize the pUB110 origin of replication by delimiting the nick site to an 18-bp sequence and (ii) indicate that pUB110 replicates by rolling-circle replication.

To further localize the position of the pUB110 plus origin nick site, plasmid pHV1098 was constructed (only the regions relevant to the initiation-termination reaction are described); in it, the initiation sequences of pUB110 are replaced by those of the related plasmid pRBH1 (27), whose replication functions are identical except for a single base-pair difference at position 10 of the 18-bp sequence (Fig. 1A). An additional termination signal is inserted containing the 18-bp homology with pUB110 (derived from the origin of pC194). Like pHV1161 and pHV1162, plasmid pHV1098, when introduced into *B. subtilis*, generated a smaller molecule with high efficiency. Sequence analysis of three such independently generated molecules showed that they did not contain the single base difference present on pRBH1. These results localize the nick generated by the pUB110 Rep protein to 8 bp, between bp 11 and 18, of the 18-bp sequence also present within the 55-bp origin of pC194 (14). (These 8 bp correspond to bp 4299 through 4292 on the published pUB110 map.) While the 18 bp are necessary and sufficient for accurate termination, a larger sequence including the 18 bp may be required for initiation.

The M-O. (i) Determination of pUB110 M-O minimal sequence. Studies were focused on a region of pUB110 previously shown to contain the M-O activity (16; A. Gruss, unpublished data), bp 1033-1545 on the pUB110 map (Fig. 2). Plasmid pUB110 was linearized at either the unique *Fnu*DII site or the unique *Pvu*II site, and *BAL*-31 deletions were initiated at those sites. In this way, sequences surrounding the M-O were reduced from either flanking side. Secondary cleavages at *Bam*HI and *Bgl*II, respectively, generated fragments that were subcloned onto shuttle plasmid pHV1610 (Fig. 3). This plasmid is comprised of pC194 and pUC19. The pC194 part is replicative in *B. subtilis* but lacks an M-O which is active in that host; it thus accumulates ssDNA (16). Forced cloning of the isolated pUB110 fragments was done into the polylinker region of the pUC19 segment of pHV1610. Initially, both orientations of two DNA fragments containing pUB110 M-O were examined for activity (results are summarized in Fig. 3). It was observed that M-O is active in just one orientation. Subsequently, M-O was cloned in its active orientation only. Plasmids were screened for production of ssDNA by SBH analysis of agarose gels containing total DNA lysates. The ssDNA migrates as a discrete band below dsDNA under the gel conditions used (38). The smallest plasmids which did not produce detectable amounts of ssDNA (i.e., with intact M-O) and the largest plasmids which did produce ssDNA (i.e., with impaired M-O) were selected for restriction mapping and plasmid sequencing of DNA boundaries. Figure 2 (lower part) presents the M-O activity of the segments of pUB110 present in the four plasmids just described, and that of a pUB110 deletant (as described in the Fig. 2 legend). The minimal pUB110 sequence required for M-O activity extends from bp 1522 to 1246 (Fig. 4). Only the plus strand (the strand utilizing the M-O) is shown. Significant palindromic sequences are indicated by arrows. A repeat heptamer, TTGCTGA (25), is present three times within the M-O and once just outside it (underlined in Fig. 4); the presence of TTG is in the highly
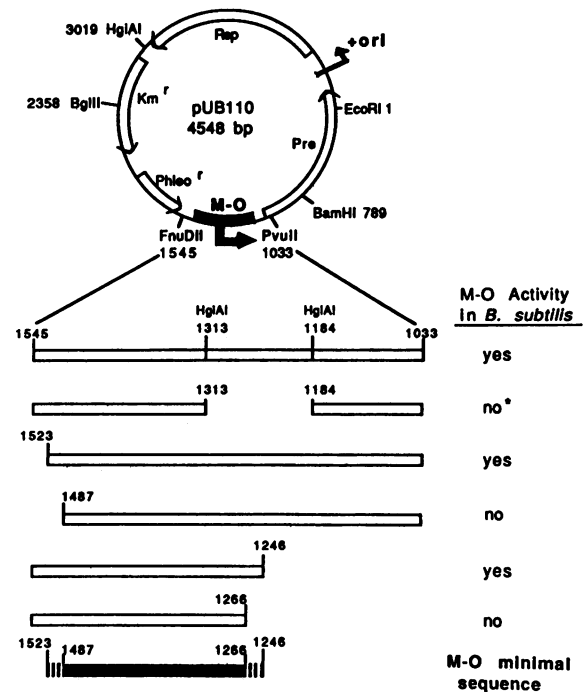

FIG. 2. Map of plasmid pUB110 and M-O localization. Above, map of pUB110, indicating certain unique restriction sites and their positions according to the published sequence. Open reading frames are indicated by open bars directly on the circles. Rep, Replication protein; Km^r, resistance to kanamycin; Phleo^r, resistance to phleomycin; Pre, protein mediating plasmid recombination (12). The plus origin (+ori) is shown, and the small bent arrow indicates the direction of replication. The region between the *Fnu*DII and *Pvu*II sites (positions 1545 and 1033, respectively) is expanded below to indicate more precisely the region required for M-O activity. The heavy bent arrow below orients the M-O with respect to the direction of plus origin replication. Deletions were generated in this region and tested for ssDNA production as described in Materials and Methods. The open bars indicate the intact remaining DNA of this region; M-O activities of these segments are given at the right. All deletions generated to delimit this region are as described in Results, except pUB110ΔHgiA1. The latter was obtained by partial digestion of pUB110 with restriction enzyme *Hgi*A1, resulting in deletion of 130 bp. The dark bar at the bottom gives the endpoints of the M-O obtained from these analyses.

conserved portion of the *B. subtilis* σ^{43} consensus sequence TTGACA (24) and may be involved in M-O recognition.

(ii) The pUB110 M-O function is RNA polymerase dependent in vivo in *B. subtilis*. It was of interest to know whether the pUB110 M-O is recognized by RNA polymerase. This was tested by determining whether conversion of ssDNA to dsDNA is inhibited by addition of rifampin in vivo; studies in *B. subtilis* and *E. coli* have shown that sensitivity to rifampin indicates a dependence on RNA polymerase activity (40). Two strains were tested, one containing pHV1611 (containing active M-O), and pHV1610 (no M-O). Cultures were grown to mid-log phase, and rifampin (100 μg/ml) was added to half of the cultures. Erythromycin (100 μg/ml) was added to all cultures to inhibit protein synthesis and thus prevent initiation of plasmid replication. Culture samples were removed at intervals during a period of 2 h, and whole-cell DNA lysates were prepared and run on an agarose gel. Gels were analyzed by SBH (Fig. 5A). Whereas the strain con-
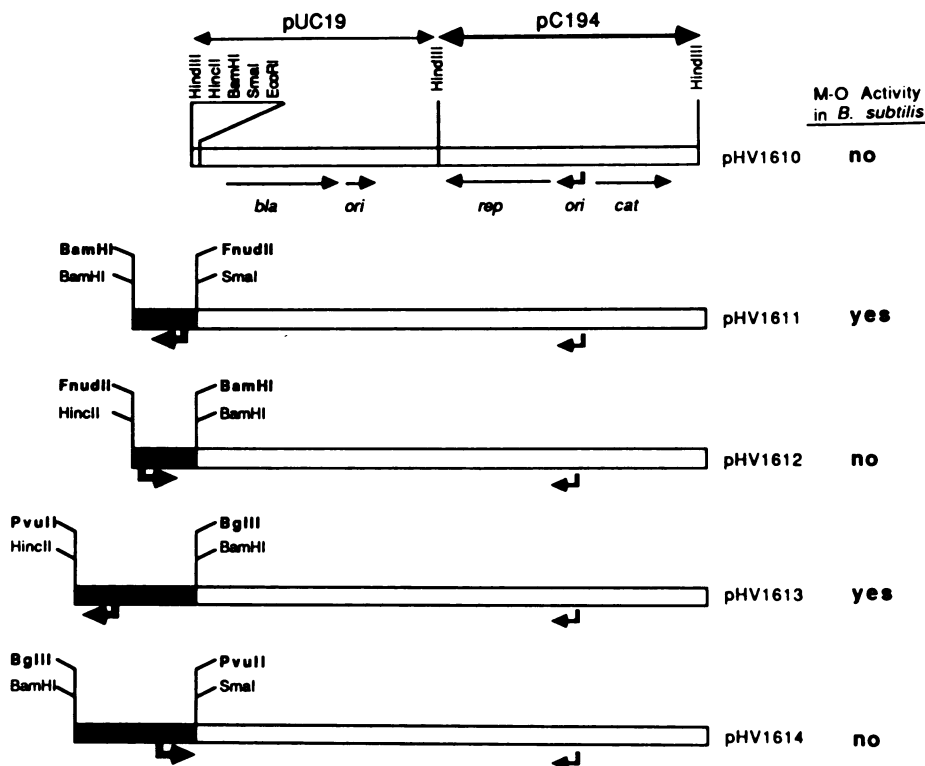
FIG. 3. Map of pHV1610, the test plasmid used for M-O minimal sequence determination, and initial insertions of pUB110 fragments containing M-O. Plasmid pHV1610 is comprised of pUC19 and pC194, joined at the *Hind*III sites. Genetic organization is indicated. In the pUC19 part, *bla* indicates the ampicillin resistance gene and *ori* indicates origin (showing direction of replication); in the pC194 part, *rep* indicates the region encoding Rep protein, *ori* indicates origin (bent arrow shows direction of replication), and *cat* indicates region encoding chloramphenicol resistance in pC194. Below, pUB110 fragments (closed box) containing the M-O were cloned at compatible sites into the polylinker region. Heavy bent arrows below pUB110 inserts indicate the orientation of the fragment with respect to the direction of pUB110 plus origin replication. The *Pvu*II-*Bgl*II segment and the *Bam*HI-*Fnu*DII segments of pUB110 (as shown in map in Fig. 2), both containing the M-O, were inserted into pHV1610 in both orientations (pUB110 sites indicated in boldface). These constructs were tested for M-O activity, and results are shown at right; only the orientation consistent with the direction of replication of pC194 was active. *Bal*-31-deleted fragments were subsequently cloned in the active orientation only.

taining pHV1611 (active M-O) without rifampin showed no detectable ssDNA (left panel), in the presence of rifampin (right panel), ss plasmid DNA was accumulated. This indicates that conversion of ssDNA to dsDNA is dependent on RNA polymerase activity and cannot occur if the action of RNA polymerase is blocked by rifampin. Interestingly, there is an increase in the amount of ssDNA in the presence of rifampin plus erythromycin. This could be caused by residual Rep protein activity, which may continue to initiate replication at the plus origin, or by the existence of replication intermediates which are slowly resolved by displacement of ssDNA from the double-stranded molecule.

Addition of rifampin to strains carrying plasmid pHV1610 (no M-O) also resulted in an inhibition of conversion of ssDNA to dsDNA (Fig. 5B). DNA lysates prepared from the erythromycin-treated culture (left panel) showed a slow disappearance of ssDNA, suggesting that conversion of ssDNA to dsDNA at nonspecific sites is occurring. In rifampin-treated cultures (right panel), ssDNA persisted at high levels, indicating that conversion was blocked. Thus, it appears that in *B. subtilis*, conversion of ssDNA to dsDNA initiated at either a specific site (M-O) or at random sites is mediated by RNA polymerase.

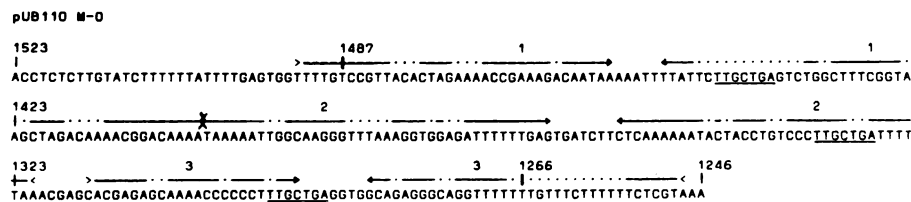**(iii) The M-Os of pUB110 and other staphylococcal plasmids**

FIG. 4. M-O sequence of plasmid pUB110. Only the plus strand, i.e., that which is recognized for activity, is shown. Presented is a composite sequence corresponding to the smallest active M-O that we have obtained. Positions 1487 and 1266 are indicated, as they are the endpoints of the largest sequence having lost activity (as detected by the appearance of ssDNA). Divergent arrows correspond to palindromic sequences, and dots indicate mismatches, loops, or both. Heptameric sequences (TTGCTGA) present as direct repeats (25) are underlined.
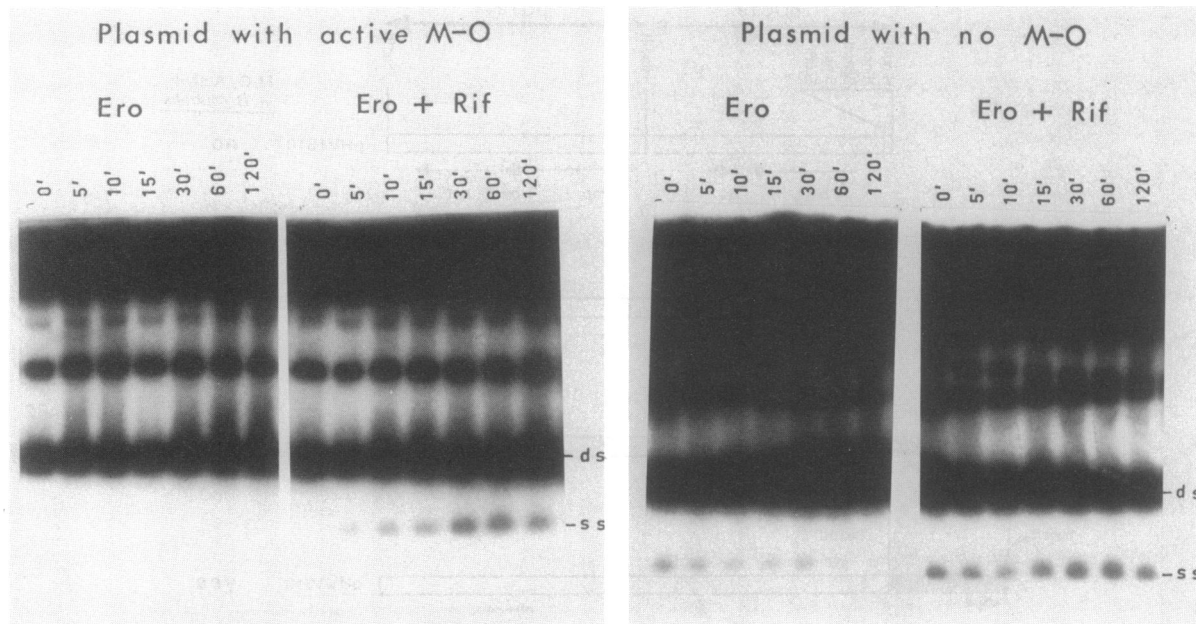
FIG. 5. Plasmid pUB110 M-O utilizes RNA polymerase in vivo in *B. subtilis*. Cultures of SB202 strains containing pHV1611 (active M-O) or pHV1610 (no M-O) were grown to mid-log phase, divided into halves, and incubated with either erythromycin alone (100 μg/ml) or erythromycin plus rifampin (100 μg/ml each) for 2 h. Samples were taken at time intervals indicated above wells (0 was taken with no drug addition); total cell DNA was prepared (31), and equivalent amounts of samples were run on a 0.7% agarose gel. Autoradiograph of SBH-treated gel (using as probe ^{32}P-labeled pC194) is presented. (A) Plasmid with active M-O, pHV1611, in SB202. (B) Plasmid with no M-O, pHV1610, in SB202. Abbreviations ss and ds represent ssDNA and dsDNA, respectively, of the corresponding plasmid. The high degree of hybridization at the chromosomal level corresponds to high-molecular-weight plasmid multimers, observed for ssDNA plasmids carrying foreign DNA insertions (15).

are RNA polymerase dependent in *S. aureus*. *S. aureus* strains harboring pC194, which has the *palA*-type M-O (active in *S. aureus* but not in *B. subtilis* [16]), or pUB110 were assayed as in the previous section for sensitivity of their plasmid M-Os to rifampin. If the same mechanism of conversion is used in any host, the pUB110 M-O should also be sensitive to rifampin in *S. aureus*. Results demonstrate (i) that the *palA*-type M-O of plasmid pC194 is RNA polymerase dependent in *S. aureus* (Fig. 6, lanes 1 through 3) (the high degree of homology among the *palA*-type M-Os leads us to propose that all M-Os of the *palA* type are recognized by RNA polymerase), (ii) that the M-O of pUB110 is functional in both *S. aureus* (Fig. 6, lane 4) and *B. subtilis* (Fig. 6, lane 7), as no ssDNA is accumulated in either host (this is the only plasmid thus far described that has a broad-range M-O), and (iii) that the mechanism for M-O activity of pUB110 is rifampin sensitive and is thus likely to be RNA polymerase dependent in both *S. aureus* and *B. subtilis* (compare untreated cultures in lanes 4 and 7 with rifampin-treated cultures in lanes 6 and 9, respectively, for both hosts).

DISCUSSION

The plus origin. Plasmid pC194 generates circular single-stranded replication intermediates via a rolling-circle mechanism, analagous to that described for the ssDNA phages of *E. coli* (14). Replication of pC194 is initiated with the introduction of a nick by the plasmid Rep protein between nucleotides 15 and 16 of an 18-bp sequence (between bp 1445 and 1446 on the pC194 sequence). Based on the findings (i) that this 18-bp sequence is also present in pUB110, (ii) that the replication proteins of pC194 and pUB110 show significant homology (about 30%), and (iii) that pUB110 accumu-

lates ssDNA in the absence of a functional M-O (this report), we hypothesized that pUB110 also replicates via a rolling-circle mechanism. This hypothesis was confirmed by demonstrating that pUB110 replication can initiate at its own origin and terminate at a duplication of the 18-bp sequence introduced elsewhere in the plasmid. When the initiation-termination reaction was performed on a plasmid in which the origin contained a single base-pair difference from the termination signal (Fig. 1A), deleted plasmids did not contain the base difference; this result localizes the nick site to 8 bp, between bp 11 and 18 of the 18-bp sequence (bp 4299 to 4292 on the pUB110 sequence). This localization is different from that reported previously, at about position 3550 (35); those results were deduced from electron microscopy analysis of theta-formed molecules, thought to be the replication intermediates.

The M-O. The M-Os of plasmids of gram-positive bacteria that have been thus far characterized are host specific. *palA*, an M-O present on numerous staphylococcal plasmids, is not recognized in *B. subtilis* (16). The M-O of pLS1 is only recognized in *S. pneumoniae* (7). We describe here an exception, the M-O of plasmid pUB110, which is derived from *S. aureus* but is functional both in its host of origin and in *B. subtilis*. We have shown that the M-O sequence of plasmid pUB110 is between position 1246 (1266 gives no activity) and 1522 (1487 gives no activity). The total sequence of the M-O is at least 226, and at most 276, bp in length. Results of similar analyses conducted during the course of this work by Viret and Alonso (39) describe the pUB110 M-O as smaller than that reported here (140 bp, coordinates 1380 to 1520). Results presented here show that the M-O cannot be as small as these authors had described.
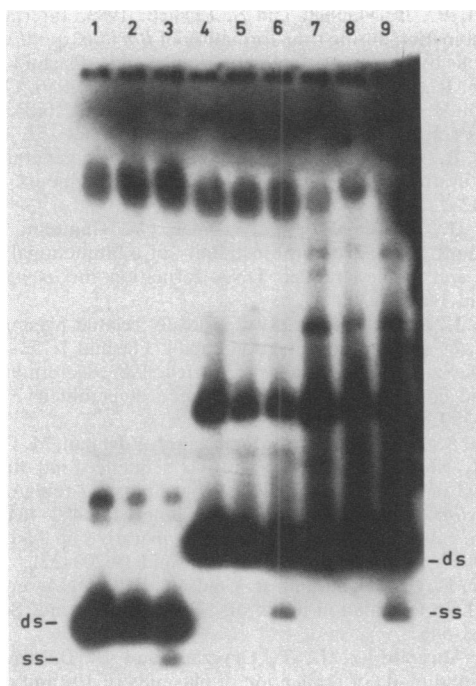
FIG. 6. Plasmid pUB110 M-O utilizes RNA polymerase in vivo in *S. aureus*, as does the staphylococcus-specific M-O (*palA*) of pC194. Cultures of staphylococcal strains containing either pUB110 or pC194 were grown to mid-logarithmic phase and treated for 2 h with (i) no additions, (ii) erythromycin (100 μg/ml), or (iii) erythromycin plus rifampin (100 μg/ml each). Total cell DNA was prepared (31) and run on a 0.7% agarose gel. Autoradiograph of SBH-treated gel (using ^{32}P-labeled pC194 and ^{32}P-labeled pUB110 mixed probe) is presented. Lanes: 1 through 3, pC194 in *S. aureus*: 1, no additions; 2, erythromycin added; 3, erythromycin and rifampin added; 4 through 6, pUB110 in *S. aureus*: 4, no additions; 5, erythromycin added; 6, erythromycin and rifampin added; 7 through 9, pUB110 in *B. subtilis*: 7, no additions; 8, erythromycin added; 9, erythromycin and rifampin added. Abbreviations ss and ds represent ssDNA and dsDNA, respectively, of the corresponding plasmid. Hybridization at the level of the chromosome is likely to be caused by chromosomal contamination of the probe.

The use of indirect assays which correlate M-O activity with transformability into a *dnaD23* mutant *B. subtilis* strain (39) rather than a direct measure of ssDNA of deletion derivatives as presented here may explain the discrepancy. Concomitant deletion of another locus on the plasmid, e.g., the *pre* locus which maps adjacent to M-O (12), may have affected the previously published results (39).

A potential secondary structure exists along part of this sequence (Fig. 4), as is also the case for all other M-Os, of replicons present in both *E. coli* and gram-positive hosts. However, there is no apparent sequence similarity between the pUB110 M-O and those of other plasmids. As mentioned, the M-O differs from others functionally, in that it is active in more than one host. Concerning ssDNA plasmids in various gram-positive hosts, the M-O seems to confer host specificity. Even where a plasmid can be established in a nonnative host, its M-O is inactive, and as a consequence (with the exception of *B. subtilis* [16]), plasmid copy numbers and stability functions are diminished (16, 7). It is, therefore, of interest that plasmid pUB110 is adapted to more than one host for both plus origin and M-O functions.

Activity of the pUB110 M-O is rifampin sensitive in both *B. subtilis* and *S. aureus*, suggesting that the conversion

from ssDNA to dsDNA is mediated by RNA polymerase and that the same mechanism of conversion is used in both hosts. Plasmid pC194, which has a *palA*-type M-O, was also tested in *S. aureus* and showed sensitivity to rifampin as well. A surprising feature of these results was the increased amounts of ssDNA observed in rifampin-treated cultures. Where did this new ssDNA come from? In these experiments, de novo synthesis of Rep protein was blocked by erythromycin. Residual Rep protein may allow further rounds of replication which would release ssDNA. Alternatively, replicative intermediates with partially displaced ssDNA may release the ss monomer after drugs are added. In addition, it appears that conversion initiated at nonspecific sites (i.e., M-O deleted) is rifampin sensitive. This would suggest that initiation of lagging-strand synthesis at nonspecific sites on ssDNA is mediated by RNA polymerase rather than a primosome complex, as has been suggested in *E. coli* (1).

The role of the M-Os in plasmid stability has recently been proposed, as the deletion of certain M-Os results in plasmid segregational instability (3, 4, 5, 8). In our hands, cloning of the pUB110 M-O onto an unstable vector, i.e., a pC194 derivative, does not result in its stabilization, nor does its deletion from pUB110 result in an increased rate of plasmid loss from *B. subtilis*. Neither is a significant change in copy number observed. Possibly, as proposed (4), the M-O has a synergistic role, in combination with other factors, to affect plasmid instability.

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