REVIEW ARTICLE

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Linear plasmids and their replication

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It is still a common belief that plasmids are circular. However, linear plasmids have been reported to exist more than a decade ago. Two types of linear plasmids are known. One type contains covalently closed ends and are commonly found in *Borrelia*, the causative agent of tick fever. The other type is characterized by the covalent attachment of proteins at the 5' ends and exists in a number of bacterial genera including *Streptomyces, Rhodococcus, Mycobacterium* and *Planobispora*. Recently, a linear plasmid in *Salmonella enterica* serovar Typhi of the Enterobacteriaceae family have been reported for the first time. This paper reviews various postulated mechanisms of replication of linear plasmids and focuses on the components of the replication machinery of linear plasmids studied to date.

Key words: Linear plasmids; *Borrelia* spp.; *Streptomyces* spp.

Linear plasmids are commonly present in both pro- and eukaryotes, and belong to one of two types, those with covalently closed ends and those with proteins bound to their 5' termini. The linear plasmids of *Borrelia* contain short terminal inverted repeats (TIR) and covalently closed ends (1). *Borrelia burgdorferi*, the causative agent of Lyme disease, contains a linear chromosome, 12 linear plasmids and 9 circular plasmids (2). *Borrelia* linear plasmids contain the genes for surface proteins, viz. *ospA* and *ospB* of *B. burgdorferi* and the vmp gene family of *B. hermsii* (3). Linear plasmid 25 (lp25), lp28-1, and lp54 are required for persistent of mice, while lp54 is required for establishment of tick infection or movement within the tick gut (4-11).

Together with virus genomes having polypeptides attached at their 5' termini and transposons, linear plasmids with 5' attached proteins are known as invertrons (12). Such plasmids also contain TIRs, ranging in size from 44bp in SLP2 of *Streptomyces lividans* (13) to 95 kbp in pPZG 101 in *Streptomyces rimosus* (14). *Streptomyces* hosts carrying the linear plasmids pKSL, pSLA2-L, pSCL1 and SCP1 have been found to carry genes encoding echinomycin, lankacidins, clavulanic acid and methylenomycin, consecutively. Linear plasmids belonging to this class have also been reported in actinomycetes *Planobispora*, *Rhodococcus*, *Mycobacterium* and *Salmonella enterica* serovar Typhi (15-22).

REPLICATION OF LINEAR PLASMIDS

Replication of protein-capped linear plasmids occurs bidirectionally from an internal origin (23). The autonomously replicating sequence of *Streptomyces* linear plasmids generally contains an origin of replication and one or more genes required for replication (24). These genes are located near the origin and may be subsidiary in some cases (25). Replication generates a 3' leading-strand overhang at the telomeres (Figure 1). The lagging strand is about 280 bp short at its 5' terminus (23). It is proposed that end patching occurs by one of two mechanisms: the first (Figure 1A) assumes that pairing of palindromes in the 3' overhang causes it to fold back such that the terminal protein is anchored near the base of the overhang. The terminal protein (TP) acts as a primer and helps to fill the gap (26). In the second model (Figure 1B), TP acts as a nickase and nicks the template strand and attaches covalently to the 5' end. The gap is filled by DNA polymerase (26). *Streptomyces* chromosomes are linear (27) and contain telomeres similar to those present on *Streptomyces* linear plasmids. They replicate bidirectionally and it is suggested that telomeres are patched in similar manner as their linear plasmids (26, 28, 29). The product of the *tpg* gene is required for *Streptomyces rochei* chromosomal replication (30).

In linear plasmid pSLA2, the autonomously replicating sequence lies near the centre of the plasmid. It contains two 21-mer iterons followed by a series of CT and AG residues within the *rep1* gene that codes for the Rep1 DNA binding protein which promotes plasmid replication (24). Another gene downstream of *rep1*, *rep2*, encodes a protein with a helicase-like activity. Additional genes necessary for pSLA2 replication are the *tpg*s, terminal protein genes (30), *tap*, the gene for a terminus associated protein (31) loci required for linear replication (*rlrA/rorA*) (32). The presence of $rlrA^{pSLA2}$ in a circular derivative is inhibitory to its replication. The presence of $rlrA^{pSLA2}$ is not however inhibitory for replication in the linear form (32). The inhibitory effect of $rlrA^{pSLA2}$ in the circular derivative can be overcome by another locus, $rorA^{pSLA2}$ (33, 34).

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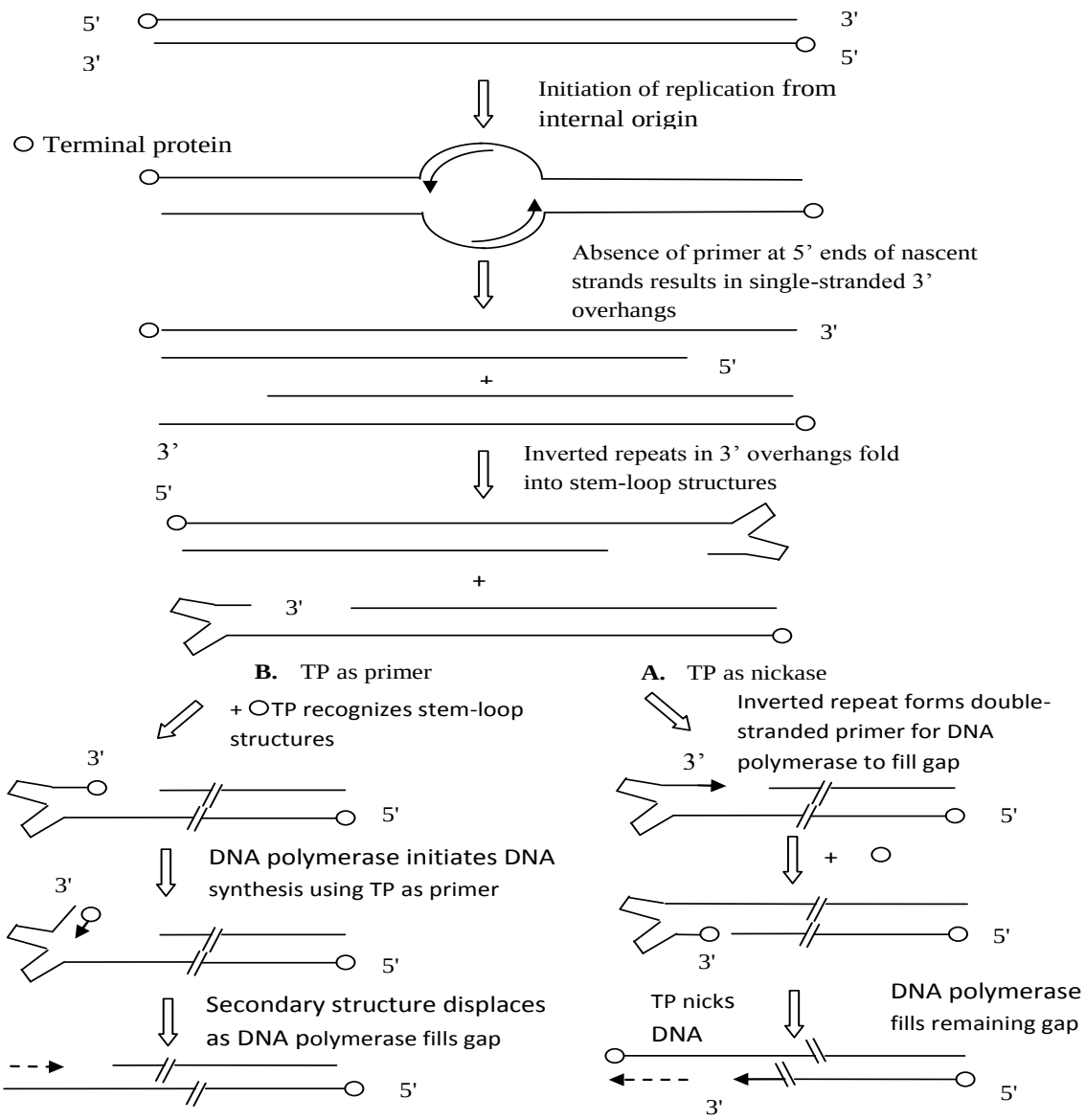

FIG. 1. Models for replication of linear plasmids containing 5' terminal proteins (37). Replication proceeds bidirectionally from the centre of the plasmid. Two methods of replication at the telomeres have been proposed. A. TP is used as a primer to complete replication at the end. B. TP acts as a nickase, nicking the DNA at the telomere. The gap generated is then filled up by DNA polymerase.

Copurification of DNA polymerase I (PolA) and a topoisomerase I with the Tap protein suggests a role in replication. However, DNA polymerase I has not been found to be essential for *Streptomyces* linear plasmid replication (35). Tap has been demonstrated to possess reverse transcriptase activity (36). The postulated mechanism of replication of linear plasmids with proteins attached covalently at their 5' ends is shown in Figure 1.

In the linear plasmid SCP1, the minimum replicon is within a 5.4 kb region that contains an A+T rich region and two open reading frames (ORFs). One of these codes for a protein that has the functional similarity to the primase *repA* gene of *Acidianus ambivalens* and the helicase-like protein of *Sulfolobus islandicus* (38). The A+T rich region may facilitate melting of the double-stranded DNA to initiate DNA replication. The autonomously replicating sequence (ARS) of linear plasmid SLP2 is located within approximately 5 to 10 kb sequence from the left end. The ARS contains the *tpg* gene, the *dnaB*-like gene and two DnaA boxes (T/C) (T/C) (A/G/C) TCCACA (39). One of these DnaA boxes is located within the *tpg* coding sequence and the other with the *dnaB*-like coding sequence. The minimal replicon of SCP1 contains an ORF (ORF SCP1.196) that codes a putative primase/helicase and a second overlapping ORF (ORF SCP1.197) that codes

for a hypothetical protein and direct repeats (38). SCP1 lacks *tpg* and *tap* homologues.

The basic replicon of plasmid pSCL1 is similar in configuration to that of pSLA2 (40). In the case of the linear plasmid SLP2, efficient replication as a circular derivative requires a 47 bp sequence upstream of 23-mer iterons. Inclusion of these sequences increased the transformation efficiency by about 1000 fold (41). This region is not transcribed as shown by RT PCR and hence may contain regulatory factor(s) that act *in cis* (41). SLP2 contains two *tpg* homologues – *tpg* SLP2 and a putative pseudogene on the right arm. SLP2 requires *tpgL* gene product for replication (42). SLP2 lacks a *rep* gene (24). It possibly requires a host DNA polymerase for DNA replication. It has been proposed (24) that the origin of replication of the plasmid lies close to the *tpg* gene, and the DnaA boxes.

A Rep-iteron structure is also found in the minimal replicon of plasmid pSHK1 of *Streptomyces* spp. HK1 (43). The gene for a putative Rep protein and adjacent noncoding sequences (*ncs*) form the basis of the basic replicon in the linear plasmids pSLA2-L from *Streptomyces rochei* (44), pSCL2 from *S. clavuligerus* (45), pRL1 from *Streptomyces* spp. 44030, pRL2 from *Streptomyces* spp. 44414, and pFRL2 from *Streptomyces* spp. FR1 (43).

Unlike any other linear plasmid reported so far, two origins of replication have been identified in the linear plasmid pFRL1 from *Streptomyces* spp. FR1 (46). One of these, *rep1A-ncs1*, lies adjacent to the telomere and the other, *rep2A-ncs2*, lies about 10 kb away from it. The first origin can propagate both in linear and circular modes, but the latter requires an additional locus, *rlrA-rorA*, for propagation in the linear mode. The Rep1A protein binds to the sequence *ncs1 in vitro*. The proteins Rep1A and Rep2A were transcribed at different levels at different times and each dominated at different points of time (46).

Rep-iteron structure is also found in *Mycobacterium celatum* linear plasmid pCLP. The basic replicon is a 2.8 kb fragment comprising of a putative Rep gene and a putative origin of replication consisting of 18 bp iterons and an AT-rich region (47). Linear plasmid pRHL3 from *Rhodococcus jostii* (48) minimal replicon is also similar to that of pCLP. It contains two ORFs: RHL3.237 encoding Rep1 (Rep protein) and RHL3.235 that codes for a protein homologous to a ParA protein from *R. erythropolis* (49). In this plasmid, iterons are present as control mechanisms. An approximately 40% AT-rich 600bp region is found upstream of *rep1* (50).

REPLICATION OF LINEAR PLASMIDS WITH CLOSED ENDS

Borrelia linear plasmids contain covalently closed ends. Replication of *Borrelia* linear plasmids initiates from an internal origin and proceeds bidirectionally (51) (Figure 1). A head-to-head dimer in which the telomere is duplicated is generated. The duplicated telomere is recognized by a telomere resolvase, ResT (52-54), which resolves the dimer into two covalently closed linear plasmids. ResT causes breakage of DNA, resulting in a conformational change in which the 5'-OH group of the DNA and the 3'-phosphate of a tyrosine residue in ResT are juxtaposed. The 5'-OH group exerts a nucleophilic attack on the 3'-phosphate group leading to the formation of a phosphodiester bond. This structure stabilizes the hairpin ends.

In *B. burgdorferi* linear plasmid, lp17, the basic replicon is 1.8kb in length and contains one ORF designated BBD14 (55) as well as adjacent non-coding sequences, 311 bp and 360 bp to the left and right, respectively. Expression of ORF BBD14 is required for replication (56). However, no iterons or DnaA boxes were detected in lp17 basic replicon. The exact location of the origin is not known. The gene for ResT in *B. burgdorferi* is not located on a linear plasmid but on a 26-kb circular plasmid (57). ResT recognizes the 25 kb inverted repeats for its function. It has been shown that circular plasmids of *B. burgdorferi* can replicate as linear derivatives when telomeres are added to the termini (58). Conversely, linear plasmids have also been demonstrated to replicate as circular derivatives in circular vectors (59).

The basic replicon of the linear prophage N15 is 5.2 kb and can drive replication of linear or circular derivatives. An ORF, *repA*, which codes for a protein with similarity to primase is required for replication (60). ResT of *E. coli* prophage N15 is encoded by the *telN* gene located adjacent to its recognition site (*telRL*) (61-63).

CONCLUSION

Linear plasmids and the mechanism by which they replicate are still mysteries. Extensive research needs to be undertaken to unravel such mysterious entities. In particular, the mechanism by which telomeres are replicated needs extensive investigation. Other avenues that might receive attention are the possible presence of more than one replicon in the same plasmid, components specifically involved in replication in different plasmids and in particular, the interaction among the different components of a replicon. Once investigated, the replication of linear plasmids may represent yet a new mode of DNA replication hitherto unknown to science.

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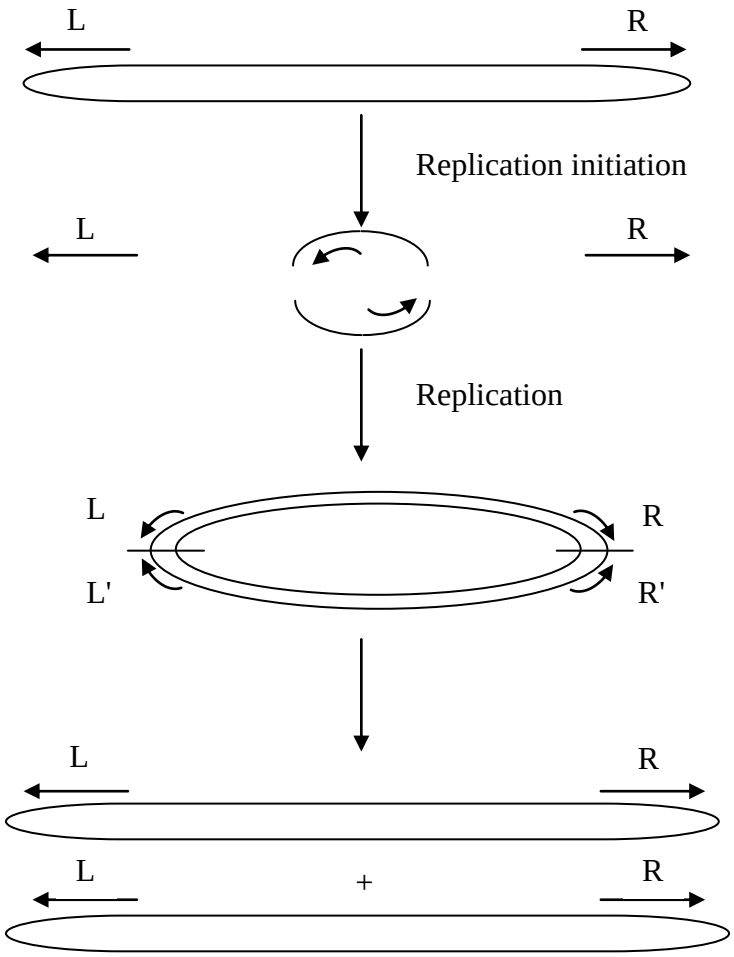

FIG. 2. Bidirectional replication of *Borrelia* linear plasmid from a central origin. L and R indicate left and right telomeres, respectively. L' and R' are replication-generated telomeres. ResT resolves the telomeres and generates two monomer plasmids (52).

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