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Analysis of Linear Plasmid Dimers in *Borrelia burgdorferi* Sensu Lato Isolates: Implications Concerning the Potential Mechanism of Linear Plasmid Replication

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The *Borrelia* genome is composed of a linear chromosome and a number of variable circular and linear plasmids. Atypically large linear plasmids of 92 to 105 kb have been identified in several *Borrelia burgdorferi* sensu lato isolates and characterized. These plasmids carry the *p27* and *ospAB* genes, which in other isolates reside on a 50-kb plasmid. Here we demonstrate that these plasmids are dimers of the 50-kb *ospAB* plasmid (pAB50). The 94-kb plasmid from isolate VS116, pVS94, was an exception and did not hybridize with any plasmid gene probes. When this plasmid was used as a probe, homologous sequences in other isolates were not detected, suggesting that it is unique to isolate VS116. These analyses provide insight into the mechanism of linear plasmid replication and the mechanisms by which plasmid variability can arise.

Lyme disease, a tick-borne zoonosis (5, 7, 36, 37), is caused by *Borrelia burgdorferi*, *Borrelia garinii*, and *Borrelia afzelii* (1, 6, 19, 20, 38). Related species of uncertain pathogenic potential, i.e., *Borrelia japonica* (18) and *Borrelia andersonii* (21), have also been identified. These five species are collectively referred to as *B. burgdorferi* sensu lato (BBsl). The BBsl genome is composed of variable linear and circular plasmids (15, 30) and a linear chromosome (11). The *ospAB* operon is carried on the largest of the linear plasmids, which ranges from 48 to 60 kb (31). We refer to this variably sized plasmid as the 50-kb *ospAB* plasmid or pAB50. The importance of the BBsl plasmids is underscored by the fact that they are present in all isolates and that they carry genes encoding metabolic enzymes (24). In view of this, the extent of plasmid variability among isolates is surprising. The significance of and the mechanisms involved in generating plasmid variability are not completely understood. Plasmid loss (32), recombination (17, 22, 28), and lateral transfer of plasmids among BBsl species (22) have been demonstrated to be contributing processes. Here we demonstrate that linear plasmid dimer formation also contributes to plasmid

variability and discuss how these findings may provide information concerning the mechanisms of linear plasmid replication.

Bacterial isolates analyzed in this study were cultivated at 34°C in BSK-H (Sigma) supplemented with 6% rabbit serum. Pulsed-field gel electrophoresis (PFGE) was performed to analyze plasmid content as previously described (21). Several isolates carrying 92- to 105-kb plasmids, 40 to 50 kb larger than those typically observed (2–4, 8, 16, 22, 31–35, 39), were identified (Table 1). Only *B. burgdorferi* R100 had been previously shown to carry a plasmid in this size range (26). Southern blot analyses with an *ospA* probe (*ospA*-5'-GGCTGCTAACATTT-TGCTTACATGC) revealed that the *ospAB* operon was carried by the large plasmids in IKA2, HO14 (Fig. 1), BO23, and R100 and by a 50-kb plasmid in VS116 (data not shown). In IKA2, a second plasmid of 47 kb also bound the probe (Fig. 1); however, after continued in vitro cultivation, this plasmid was lost and an *ospAB*-hybridizing plasmid of 105 kb appeared (see plasmid profile in Fig. 3A, lane 3). In other isolates the large plasmids were stable with cultivation over 4 months.

TABLE 1. Description of BBsl isolates and sizes of their *ospAB*-carrying plasmids

Isolate	Geographic origin	Biological origin	Approximate <i>ospAB</i> plasmid size (kb)
<i>B. burgdorferi</i> Sh-2-82	United States	<i>Ixodes scapularis</i> tick	50
<i>B. burgdorferi</i> R100	United States	Hamster reisolate, originally from an <i>Ixodes scapularis</i> tick	92
<i>B. afzelii</i> BO23	Germany	Human skin isolate	98
<i>B. japonica</i> IKA2	Japan	<i>Ixodes ovatus</i> tick	47, 94, 105 ^a
<i>B. japonica</i> HO14	Japan	<i>Ixodes ovatus</i> tick	105
VS116 ^b	Switzerland	<i>Ixodes ricinus</i> tick	94

^a The species of *ospAB*-hybridizing plasmid present in this isolate changed with in vitro cultivation. For discussion see the text.

^b The species identity of this isolate remains unresolved. Recent data suggest that it may be a member of a newly described genospecies within the BBsl complex (27).

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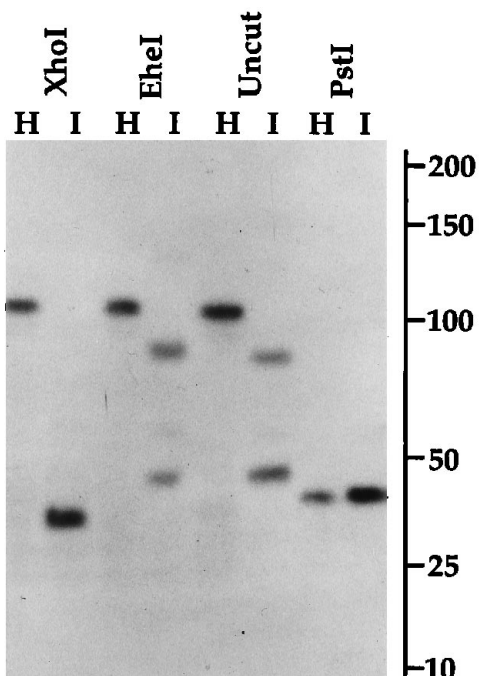


FIG. 1. Restriction and Southern blot analysis of plasmids in *B. japonica* isolates. H and I signify isolates HO14 and IKA2, respectively. The restriction enzymes used are indicated above the lanes, and molecular weight markers (in kilobases) are on the right. The blot was probed with an oligonucleotide directed at the *ospA* gene (see text). All methods were as previously described (23).

Probes to other targets on pAB50 were used in hybridization analyses to determine if an extended portion of this plasmid is carried by the large plasmids. A PCR-generated *p27* gene probe (amplified from *B. garinii* VSBP [GenBank accession

TABLE 2. Size and densitometry analysis of pLH1 restriction fragments

Fragment designation ^a	Restriction fragment sizes and relative densitometry values ^b					
	<i>Mlu</i> I	<i>Pst</i> I	<i>Pme</i> I	<i>Bam</i> HI	<i>Sal</i> I	<i>Sma</i> I
A	80	38 (2.1 ± 0.4)	32 (1.0)	79	45 (1.0)	78
B	12	27 (1.0)	26 (2.2 ± 0.2)	9.5	29 (1.8 ± 0.5)	14
C			3.5	3.0		
D			2.5			

^a The fragment designations (i.e., A to D) for each restriction enzyme are as indicated in Fig. 2.

^b Fragment sizes are in kilobases. Fragments smaller than 2.5 kb were not analyzed. The values in parentheses are the relative intensities (± the standard deviations) as described in the text. In each case, the central fragment of the plasmid obtained (refer to Fig. 2) was assigned a value of 1.0. Relative intensities were not determined for fragments obtained by digestion with *Mlu*I, *Bam*HI, or *Sma*I. The values with standard deviations are means of three independent determinations.

number M85216]) and p2H14, a cloned fragment of pAB50 (29) from *B. burgdorferi* Sh-2-82, were used as probes. These probes bound the large plasmids of all isolates except VS116, in which they hybridized to a 50-kb plasmid (data not shown). The map locations of *p27* and p2H14 on the 50-kb plasmid of Sh-2-82, pLS1, were determined (Fig. 2). Since these probe target sites are widely separated, it can be concluded that the large plasmids carry a complete copy of pAB50 and that the composition of pVS94 is distinct from that of the other large plasmids. We refer to the *p27* and *ospAB* ends of these plasmids as the tail and head ends, respectively.

To determine if the ~100-kb plasmids are dimers of pAB50, the large plasmid from HO14, pLH1, was excised from agarose gels, digested with various restriction enzymes, and analyzed by PFGE. Restriction digestions were performed as instructed by the supplier, and PFGE was performed in 1% agarose gels as previously described (8). With each enzyme tested, the sum of

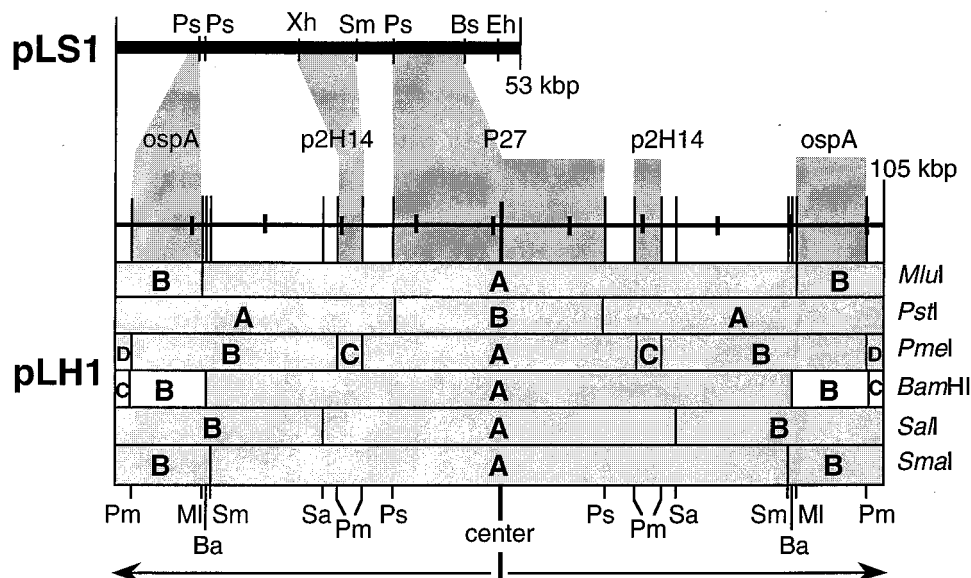


FIG. 2. Physical and genetic maps of pLH1 from *B. japonica* HO14. The physical map was constructed through single and double restriction digests as previously described (9). The black bar at the top of the figure shows the physical map of the 50-kb *ospAB* plasmid, pLS1, from *B. burgdorferi* Sh-2-82. The restriction maps of the *B. japonica* HO14 plasmid pLH1 are shown, with the enzymes used to generate them listed on the right. The lighter-gray and white rectangles indicate fragments that have been unequivocally ordered and that have not been ordered, respectively. The darker-gray regions are the map locations determined for the *ospAB* and P27 genes. Restriction site abbreviations are as follows: Ps, *Pst*I; Xh, *Xho*I; Sm, *Sma*I; Bs, *Bss*HI; Eh, *Ehe*I; Pm, *Pme*I; Ml, *Mlu*I; Ba, *Bam*HI; and Sa, *Sal*I. For each individual digestion, the fragments have been designated with letters in order to facilitate inspection of the data presented in Table 2.

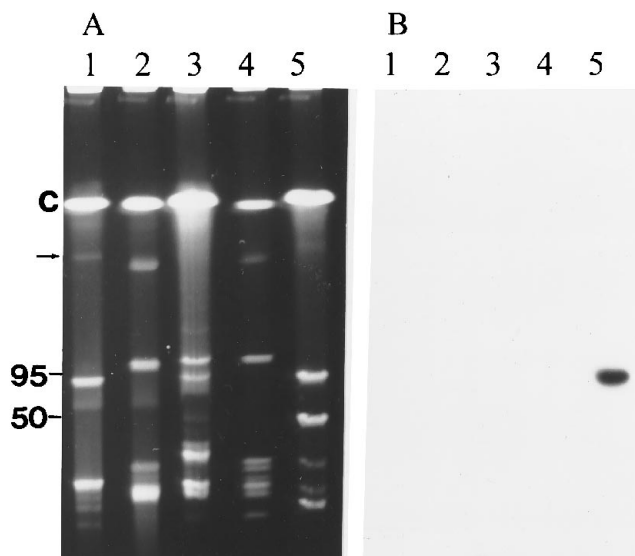


FIG. 3. Southern blot analysis of PFGE-fractionated DNA using pVS94 as the probe. The PFGE-fractionated DNA was stained with ethidium bromide (A) and then blotted and probed with random-primed labeled pVS94 (B). The isolates tested included *B. burgdorferi* R100, *B. afzelii* BO23, *B. japonica* IKA2, *B. japonica* HO14, and BBSI isolate VS116 (lanes 1 to 5, respectively). Molecular weight markers are indicated on the left (in kilobases). The position of the linear chromosome is indicated by a C, and the general region to which some of the circular plasmids migrate is indicated by the arrow.

the sizes of the restriction fragments was found to be less than the size of the undigested plasmid (Table 2). These results suggest that there are comigrating restriction fragments. Consistent with this, densitometric scanning of photographic negatives of the stained gels (Table 2) revealed that all fragments were not present in equimolar amounts. For example, the two *Pst*I fragments of 38 and 27 kb occur at a ratio of 2:1. If the 38-kb fragment is counted twice, the sum is close to the size of the uncut plasmid. In addition, only a single *ospAB*-hybridizing fragment was detected for each digestion. These observations are consistent with a dimeric plasmid with its monomers linked

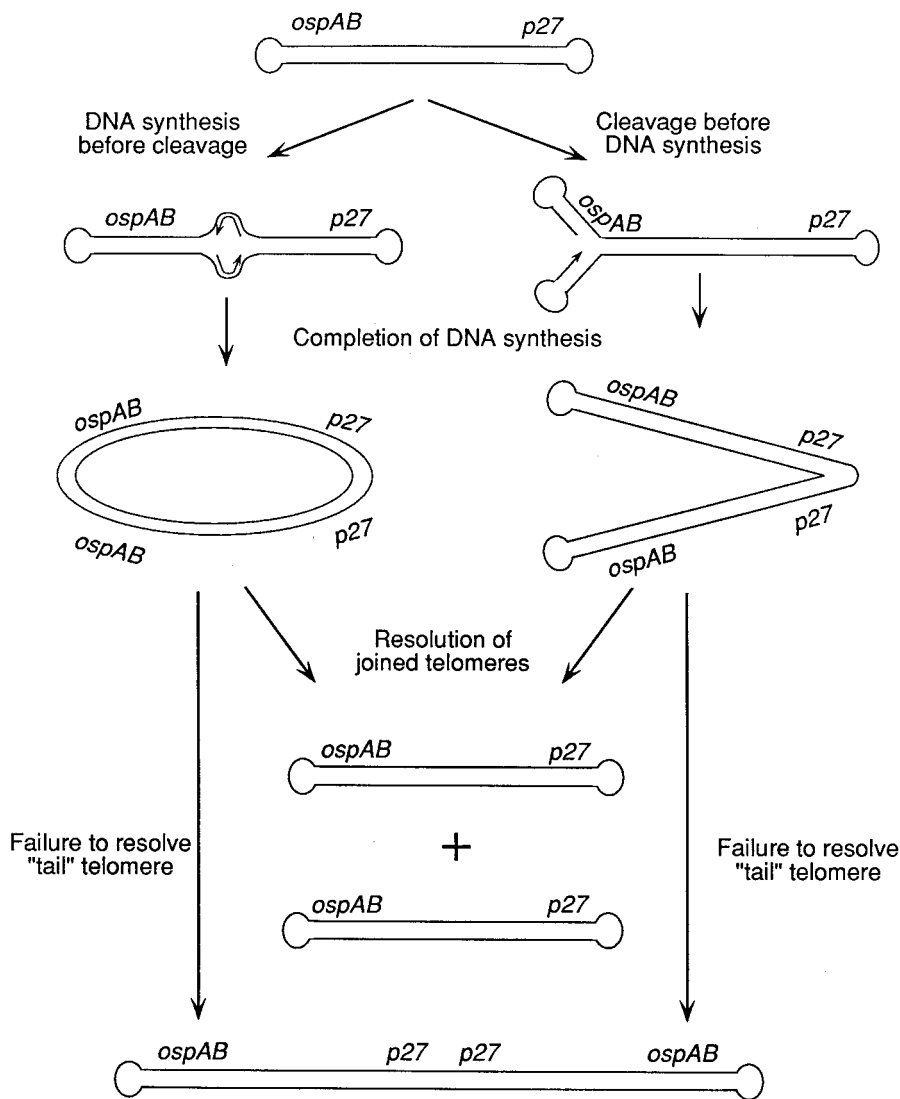


FIG. 4. Potential models for the mechanism of origin of the *ospAB* linear plasmid dimers. The model presented in the right half of the figure is similar to that proposed for vaccinia virus replication (25).

tail to tail. The validity of the tail-to-tail model was confirmed by restriction analysis and subsequent construction of a pLH1 physical map (Fig. 2). Restriction and Southern analyses suggest a similar structure for the large plasmids of IKA2 (Fig. 2) and BO23 (data not shown).

In contrast to the other large plasmids, pVS94 did not hybridize with any pAB50-targeting probes. pAB50 probes instead hybridized with a 50-kb plasmid in isolate VS116. To determine if pVS94 was a dimer of other BBsl plasmids, it was purified and used as a probe in Southern analysis. pVS94 hybridized only with itself, indicating that it is unique to isolate VS116 (Fig. 3). VS116 may belong to a previously unrecognized *Borrelia* species (27); hence, pVS94 may represent a species-specific plasmid. This analysis indicates that it cannot be inferred that all BBsl large plasmids have arisen via dimer formation.

The plasmid dimers may have arisen through recombination or from failed segregation after replication. While recombination-mediated dimer formation cannot be ruled out, it seems unlikely in view of the specific orientation of the monomers in these dimers. Dimers generated by recombination could be linked anywhere along the monomers since they are homologous throughout. Alternatively, the dimers may have arisen because of a replication error. It has been suggested that some BBsl plasmids may replicate by the rolling circle replication mechanism (10). However, dimers resulting from failed segregation after rolling circle replication could only be oriented head to tail. We found no evidence of head-to-tail structures. The BBsl plasmids may replicate by a mechanism like that proposed for the similarly structured vaccinia virus. Several models have been proposed (25) for vaccinia virus replication which differ with regard to the timing of telomeric cleavage or nicking and as to whether replication initiates from one or both telomeres. If the hairpin loop at one telomere is nicked and initiation proceeds from that end, then it is possible to form head-to-head or tail-to-tail junctional intermediates, depending on the end from which initiation occurs. By this model, if the pLH1 tail-to-tail dimer arose from failed segregation, then replication initiation would have to have occurred from the head telomere as depicted in Fig. 4 (right). However, it cannot be ruled out that tail-to-tail dimers could occur from a circular replication intermediate if segregation required independent cleavage events at each telomere junction but cleavage of the tail telomeres failed to occur because of an unidentified lesion (point mutation, etc.) (Fig. 4, left). On the basis of identification of circular-to-linear interconversion of a 180-kb plasmid, it has been proposed that replication of the *Borrelia hermsii* plasmids proceeds through a monomeric circular intermediate (12). The structures of the dimers presented here are not consistent with a replication model involving a monomeric circular intermediate. Any dimers that arose through this potential mechanism of replication would be oriented head to tail.

The telomeric sequences of some BBsl linear plasmids, including pAB50, and the African swine fever virus, an iridovirus, exhibit some homology (14, 15). Hinnebusch et al. (14) suggested the possibility of interkingdom genetic transfer between African swine fever virus and *Borrelia duttonii*, both of which are carried by the same arthropod vector, *Ornithodoros moubata*. Lateral plasmid exchange has been demonstrated among BBsl species, indicating that they can take up exogenous DNA (22). It is possible, as originally suggested by Hinnebusch and Barbour (13), that the *Borrelia* species may have stably incorporated linear viral DNA into the BBsl genome. While we can only speculate as to the molecular origins of these dimers and the linear plasmid component of the BBsl

genome, studies such as that presented here and the recent work of Ferdows et al. (12) are important because they increase our understanding of the molecular mechanisms that influence genome organization, plasmid stability, linear plasmid replication, and evolution in *Borrelia* species.

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