Differential Telomere Processing by *Borrelia* Telomere Resolvases In Vitro but Not In Vivo

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Causative agents of Lyme disease and relapsing fever, including *Borrelia burgdorferi* and *Borrelia hermsii*, respectively, are unusual among bacteria in that they possess a segmented genome with linear DNA molecules terminated by hairpin ends, known as telomeres. During replication, these telomeres are processed by the essential telomere resolvase, ResT, in a unique biochemical reaction known as telomere resolution. In this study, we report the identification of the *B. hermsii* resT gene through cross-species hybridization. Sequence comparison of the *B. hermsii* protein with the *B. burgdorferi* orthologue revealed 67% identity, including all the regions currently known to be crucial for telomere resolution. In vitro studies, however, indicated that *B. hermsii* ResT was unable to process a replicated *B. burgdorferi* type 2 telomere substrate. In contrast, in vivo cross-species complementation in which the native resT gene of *B. burgdorferi* was replaced with *B. hermsii* resT had no discernible effect, even though *B. burgdorferi* strain B31 carries at least two type 2 telomere ends. The *B. burgdorferi* ResT protein was also able to process two telomere spacing mutants in vivo that were unresolvable in vitro. The unexpected differential telomere processing in vivo versus in vitro by the two telomere resolvases suggests the presence of one or more accessory factors in vivo that are normally involved in the reaction. Our current results are also expected to facilitate further studies into ResT structure and function, including possible interaction with other *Borrelia* proteins.

Spirochetes from the genus *Borrelia*, including *Borrelia burgdorferi* and *Borrelia hermsii*, are significant human pathogens, causing Lyme disease (33, 37) and relapsing fever (4, 18), respectively. Lyme disease is transmitted by slow-feeding, hard-bodied ixodid ticks, producing a gradually developing disease that can affect the skin, nervous system, heart, and joints, with symptoms that may take months or years to manifest. Tick-borne relapsing fever, by contrast, is transmitted by fast-feeding, soft-bodied argasid ticks, with disease characterized by cycles of recurring fever and bacteremia that resolve within weeks.

Despite the distinct clinical pathologies of the two diseases they cause, *B. burgdorferi* and *B. hermsii* share a number of similarities, including an obligate parasitic lifestyle, alternating between an arthropod vector and mammalian hosts. The genome of *B. burgdorferi* prototype strain B31 consists of a nearly 1-Mb linear chromosome, as well as 12 linear and 10 circular extrachromosomal elements (12, 21, 31). *B. hermsii*, like *B. burgdorferi*, has a linear chromosome, both linear and circular plasmids, and a G+C content of approximately 30%. Cross-species hybridization studies with *B. hermsii* genomic DNA on a *B. burgdorferi* DNA array indicated that the two species share at least 81% of the chromosomal genes and 43% of the plasmid genes (46).

A hallmark of all *Borrelia* species is a segmented genome with multiple linear DNA molecules terminated by covalently closed hairpin ends (5, 23) or telomeres (see also reference 14). Studies in *B. burgdorferi* indicate that replication of these molecules initiates at an internal origin and proceeds bidirectionally (6, 32). The inverted repeat dimer junctions generated during replication are processed to generate hairpin ends by telomere resolution, a unique DNA breakage and reunion event mediated by the telomere resolvase, ResT (15, 28). ResT exhibits a catalytic center similar to that of the type IB topoisomerases and tyrosine recombinases (17, 27, 28). In addition, ResT appears to have a hairpin binding module similar to that found in cut-and-paste transposases (2). The combination of these two active-site components contributes to the unique activity of ResT in generating covalently closed hairpin telomeres (13). While plasmid loss is a feature associated with in vitro propagation of *B. burgdorferi* (22, 38), the plasmid that encodes the resT gene, circular plasmid cp26, cannot be lost or displaced, and attempts to eliminate resT activity in vivo have been unsuccessful (10); ResT, therefore, appears to perform an essential function and telomere resolution is required for survival.

We report here the identification, sequencing, and in vitro activity of the *B. hermsii* ResT protein on *B. burgdorferi* telomeres. We demonstrate that *B. hermsii* ResT does not process a *B. burgdorferi* type 2 telomere in vitro but can, surprisingly,
substitute for the B. burgdorferi enzyme in vivo and process all telomere types without limits. We also report that B. burgdorferi ResT can process two spacing variants in vivo that cannot be processed in vitro. Our results suggest that the telomere resolution reaction in vivo may normally involve participation of Borellia accessory factor(s) that can modulate or stimulate the reaction.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All B. burgdorferi strains were cultivated in liquid Barbour-Stoener-Kelly II (BSK-II) medium (3, 34) supplemented with 6% rabbit serum (Cedarlane Laboratories) at 35°C in a 1.5% CO2 environment. B31-A is a high-passage noninfectious clone of strain B31 (9), while B31-SAa and B31-A3 are infectious clones derived from B31 and B31 ML, respectively (20, 35).

Isolation and sequencing of the B. hermsii resT gene. The resT gene from B. hermsii was recovered from a genomic library of B. hermsii strain HS1 in pUC18 (36) using colony hybridization at reduced stringency (46) with a 32P-labeled B. burgdorferi resT probe. The probe was produced by random priming of a PCR product generated with primers B52 and B53 (28). A clone (P7) containing the full-length resT gene was sequenced using Big Dye terminator chemistry at automated facilities at the University of California Irvine and the University of Calgary.

Plasmid constructs. pBSV2B3resT was used to provide B. hermsii ResT in cross-species complementation experiments. For its construction, B. hermsii resT, including 350 bp of sequence upstream and 42 bp of sequence downstream from the gene, was amplified from plasmid P7 using the primers B322 (CGACCTGCAGGATAATGATACATGTCTC) and B323 (CTCCGTCGGGAT ATGCAAAATTAGGAC; Sall sites are indicated in boldface) and cloned into pCR2.1-TOPO. The gene was excised from the TOPO vector with Sall and inserted into the Borrelia shuttle vector pBSV2 (40). The construct used for the studies reported here carried the B. hermsii resT gene in the reverse orientation, relative to open reading frames (ORFs) 1, 2, and 3, of the shuttle vector. The resT inactivation plasmid, pXL-resTΔA, which has a 375-bp internal deletion along with insertion of a gentamicin resistance gene (aacC1), was previously reported (10), as was the CHB inactivation plasmid, pKK82, which has a 389-bp deletion of the gene.

Enzymes, substrates, and in vitro reaction conditions. The B. hermsii resT gene was cloned into the plasmid vector pET15b (Novagen), carrying a six-His gene tag at the N-terminal end as previously described for the B. burgdorferi protein (2). Both ResT proteins were expressed and purified as described previously (2). Plasmid substrates carrying replicated type 1 (0/0, where the first value indicates the number of base pair insertions to the left of the homology box 1 and the second indicates the number of base pair deletions between boxes 2 and 3; pYT1) or type 2 (+3/-3; pYT11) telomeres or +4/-4 (pYT15) and +1 (pYT9) type telomere substrates have been previously described (42). The type 3 telomere substrate, pTB3 carried a 50-bp replicated telomere of the sequence AAATTAGTTTTT (36) using colony hybridization at reduced stringency (46) with a 32P-labeled B. burgdorferi resT probe. The probe was produced by random priming of a PCR product generated with primers B52 and B53 (28). A clone (P7) containing the full-length resT gene was sequenced using Big Dye terminator chemistry at automated facilities at the University of California Irvine and the University of Calgary.

PCR screening of B. burgdorferi transformants. Bacteria from B. burgdorferi colonies that grew in solid medium were added to 20-μl PCR mixtures via sterile toothpicks, while cultures that arose in selective medium in 96-well plates were diluted 1:100 to 1:5 in 5 ml of BSK-II and grown to mid-log phase prior to PCR screening of 1 μl of culture. Transformants were screened with primers specific for the kanamycin resistance gene (B70, CATATGACACATATTACACGGG AGAAC; B71, AAAGCGTTCTCGTAAATGAAGG; resT (B533, CTCGCC AGAGAATCTCAAAAGC; B346, CAAACTCTTCAACCTATGTTGCC) or the gentamicin cassette, aacC1 (B348, CCGAGCGCGACAGATGTTAAC; B349, CTGG CACCTAGTCGACATAGAC). PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems) with primers at a 0.3 pmol/ml final concentration in a 20-μl final volume. The PCR conditions were 94°C for 15 min, followed by 25 cycles of 94°C for 30 s, 50°C for 30 s, 68°C for 1 min, and 68°C for 5 min following the last cycle. Total reactions were run on 1.5% agarose and visualized by ethidium bromide staining.

Southern blot hybridization. B. burgdorferi plasmid DNA was prepared from 250-ml cultures using a QIAGEN Plasmid Midi Kit. Approximately 500 ng of DNA was digested for 20 h with Bgl II or Not I and separated by field inversion gel electrophoresis along with 500 ng of uncut DNA on a 0.8% Bio-Rad agarose gel. The probe was produced by random priming with a Random Primers DNA Labeling System Kit (Gibco-BRL) using the DNA membranes (ICN). DNA was cross-linked to the membranes with a UV Stratalinker 1800 (Stratagene). The aacC1 and resT probes were prepared from PCR using the primers listed above and labeled with [-32P]dCTP by random priming with a Random Primers DNA Labeling System Kit (Gibco-BRL). DNA membranes were prehybridized, hybridized, and washed by standard procedures, exposed to phosphor screens, and analyzed with a Cyclone Phosphorimage (Packard).

PCR detection of plasmids. Plasmid content was analyzed by PCR, with primers specific for regions unique to each plasmid, as previously described (35). Approximately 50 ng of purified DNA prepared from either Wizard genomic DNA purification (Promega), QIAGEN Midi-Kit (QIAGEN), or small-scale DNA extraction (6) was used as a template. PCR was performed as described above (see “PCR screening of B. burgdorferi transformants”) Total reactions were run on 1.5% agarose and visualized by ethidium bromide staining.

Field inversion gel electrophoresis. For plasmid analysis, approximately 250 ng of B. burgdorferi plasmid DNA was separated on a 0.65% Seakem agarose gel at 80 V for 40 min, followed by 21 h with Program 0, using an MJ Research PPI-200 programmable power inverter. DNA was depurinated, denatured, and neutralized and then transferred bidirectionally onto two Bio- trans nylon membranes (ICN). DNA was cross-linked to the membranes with a UV Stratalinker 1800 (Stratagene). The aacC1 and resT probes were prepared from PCR using the primers listed above and labeled with [-32P]dCTP by random priming with a Random Primers DNA Labeling System Kit (Gibco-BRL). DNA membranes were prehybridized, hybridized, and washed by standard procedures, exposed to phosphor screens, and analyzed with a Cyclone Phosphoimage (Packard).

Nucleotide accession number. The sequence of the full-length B. hermsii resT gene has been deposited in the GenBank repository under accession number D3938555.

RESULTS

Recovery and sequencing of the B. hermsii resT gene. The telomere resolvase encoded by the relapsing fever B. hermsii strain HS1 was recovered from a B. hermsii genomic library in pUC18. Clones carrying the resT gene were identified by low-stringency colony hybridization with a radioactive B. burgdorferi resT probe. A clone carrying the entire B. hermsii resT ORF was sequenced and found to share 71% identity with its B. burgdorferi orthologue. The B. hermsii resT gene encodes a 449-amino-acid protein as does B. burgdorferi resT; however, the use of a possible TTG start codon three amino acids prior to the ATG cannot be ruled out. As was noted for the B. burgdorferi gene (13), a degenerate telomere was found adjacent to the coding region: TATAATTTGTATTAGTA, where the underlined positions indicate matches to boxes 1, 2, and 3, respectively, of a type 1 (0/0) or type 2 (+3/-3) B. burgdorferi telomere (Fig. 1). The B. hermsii sequence displays a better match to functional telomerases than the B. burgdorferi sequence. The last A in the underlined sequence is the first position of the putative ATG start codon. The spacing between boxes 2 and 3 corresponds to a 1-bp insertion in a type 2 telomere or a 2-bp deletion in a type 1 telomere. The presence of a degenerate telomere directly adjacent to the resT coding region sug-
gests that the gene was previously located at the end of a linear replicon (13).

Alignment of the translated ORF with the *B. burgdorferi* ResT protein revealed a protein with 67% identity, as shown in Fig. 2. The predicted isoelectric points of the *B. burgdorferi* and *B. hermsii* proteins are 9.68 and 9.21, respectively. The two signature motifs (GRRX_{2}EX_{2}F and LGHX_{4–6}TX_{3}Y) near the catalytic residues of *B. burgdorferi* ResT and other telomere resolvases (17) were present in the *B. hermsii* orthologue (Fig. 2, gray boxes). The residues corresponding to the catalytic pentad (white asterisks in black boxes) of the tyrosine recombinases and type IB topoisomerases as well as the tyrosine nucleophile at position 335 were also present, as were other possible catalytic residues (17). The hairpin binding module motif PXW-(linker)-YXXK (2) was also present at position 139 to 148, with a conservative substitution of a histidine in *B. hermsii* for the lysine normally found at the last position. Our current understanding of the functional regions of the *B. burgdorferi* telomere resolvase and the sequence correspondence of the *B. hermsii* orthologue suggested proteins that are functionally related to a high degree.

### In vitro telomere resolution by *B. hermsii* ResT

Recombinant *B. hermsii* ResT protein was purified from *E. coli*, and its activity was compared with that of *B. burgdorferi* ResT on three replicated telomere types, as shown in Fig. 3. Similar activity levels were observed for both enzymes on a replicated type 1 (0/0) telomere (Fig. 3A). This was expected since the only telomere sequence reported to date from *B. hermsii* is a type 1 telomere on linear plasmid lp28-1 (26). In contrast, under conditions where the *B. hermsii* enzyme converted \( \% \) of a replicated type 1 telomere substrate, no activity was observed on a replicated type 2 \( (+/–) \) telomere (Fig. 3B). Use of additional *B. hermsii* ResT protein up to 25 \( \mu \)g/ml did not result in significant substrate conversion (data not shown), while the *B. burgdorferi* ResT protein effectively utilized a type 2 substrate with an initial reaction rate equal to 75% of that displayed on the replicated type 1 telomere (42). The *B. hermsii* ResT protein converted about 70% of the type 3 telomere substrate but was somewhat less effective than *B. burgdorferi* ResT (Fig. 3C). Finally, it is important to note that under identical reaction conditions to those used in the experiment.

### FIG. 1

Three types of telomeres in *B. burgdorferi*. Three telomere types have been found thus far in *B. burgdorferi* (11, 24, 42). A representative example of each telomere type is shown. Telomeres are aligned with their hairpin ends to the left. Conserved regions among the sequenced telomeres are boxed in color with numbers above the top sequence. Positions of known sequence variability in or to the left of box 1 in the type 2 telomere family are indicated by lowercase letters. Red arrows indicate the site of cleavage of ResT. (Reprinted from reference 13 with permission of the publisher.)

### FIG. 2

Amino acid sequence alignment of *B. burgdorferi* and *B. hermsii* ResT. A Clustal alignment visualized with Jalview (16) is shown. The hairpin binding motif found in cut-and-paste transposases is indicated by a dark gray box. The positions corresponding to the catalytic residues in tyrosine recombinases and type IB topoisomerases are indicated by asterisks with the active-site tyrosine at position 335 marked by a white box (see reference 17). The amino acids highlighted in the light gray regions denote conserved residues found in all telomere resolvases (17). The two proteins display 67% identity, with identical residues colored according to amino acid type.
shown in Fig. 3, B. hermsii ResT is more active on a B. hermsii telomere than the B. burgdorferi ResT protein (data not shown), indicating species specificity for substrate usage rather than partially active enzymes.

Cross-species complementation of the B. burgdorferi resT gene in vivo by the B. hermsii orthologue. The sequence similarity of B. hermsii ResT compared to B. burgdorferi ResT (Fig. 2), in combination with the limited functionality of B. hermsii ResT in vitro (Fig. 3), prompted us to assess the ability of B. hermsii ResT to substitute for B. burgdorferi ResT in vivo. A previous attempt to inactivate resT in B. burgdorferi B31-A by allelic exchange was unsuccessful, producing only merodiploid transformants containing both a wild-type and a mutant copy of resT (10). Telomere resolution appears to be an essential function, making it impossible to displace cp26 in its entirety or to inactivate the resT gene. To circumvent this difficulty, we first transformed B31-A with the shuttle vector pBSV2, into which the B. hermsii resT gene had been cloned (pBSV2BhResT, Km*) to create an intermediate strain carrying both the B. burgdorferi and B. hermsii resT genes (resT<sub>Bb</sub>/resT<sub>Bh</sub>). We then inactivated the native resT gene by allelic exchange in the intermediate strain using a resT<sub>Bb</sub> inactivation plasmid (XL-resTΔ391–764::flgb<sub>B</sub>:aacC1::Gm<sup>n</sup>) previously described (10). Transformants were initially screened by PCR, with primers specific for the gentamicin resistance cassette, the B. burgdorferi resT gene, and the B. hermsii resT gene. PCR results indicated that resT was successfully inactivated and that the gene was replaced in trans by B. hermsii resT (data not shown). These results were confirmed by Southern blotting (described below).

We first attempted to replace resT in the high-passage, highly transformable strain B31-A; 13 transformants were recovered. We proceeded to duplicate the complementation in two different infectious strains of B. burgdorferi, which have a full complement of plasmids. In this case, only three transformants were recovered for B31-5A4 and two transformants for B31-A3. The integrity of the resT cross-species complementation strains was confirmed by Southern blot analysis (Fig. 4). Plasmid DNA from wild-type B. burgdorferi, an intermediate clone containing both the B. hermsii resT transgene and the native B. burgdorferi resT gene, and a B. hermsii resT (resT<sub>Bb</sub>) cross-species complementation strain was analyzed. DNA was left uncut, digested with BglII to linearize cp26, or cut with NcoI and SpeI to excise the resT gene and then probed with the gentamicin cassette, aacC1 (Fig. 4A) or B. burgdorferi resT (resT<sub>Bb</sub>) (Fig. 4B). Uncut or linearized cp26 plasmids were observed in the parent (Bb, lanes 1 and 2), intermediate (Bb/Bh, lanes 4 and 5) and cross-species complementation strains (Bh, lanes 7 and 8). As expected, an inactivated resT gene was observed in DNA from the cross-species complementation strain digested with NcoI and SpeI and probed with the gentamicin resistance cassette, aacC1 (Fig. 4A, lane 9). A wild-type resT was observed in both the parent (Fig. 4B, Bh, lane 3) and the intermediate strain (Fig. 4B, Bb/Bh, lane 6), while an inactivated resT gene was also observed in the cross-species complementation strain with DNA digested with NcoI and SpeI and then probed with B. burgdorferi resT (Fig. 4B, lane 9). Southern blot analysis was also performed on the noninfectious strain B31-A and the infectious strain A3 with identical results (data not shown). Finally, disruption of the B. burgdorferi resT gene was confirmed by a lack of expression of the ResT protein as demonstrated by Western blotting using antibody specific for the B. burgdorferi ResT protein (T. J. Moriarty and G. Chaconas, unpublished results).

Phenotype and plasmid content analysis. Our ability to substitute the B. hermsii resT for the B. burgdorferi resT was somewhat surprising since in vitro results suggested that the B. hermsii protein would be unable to process the resident B.
burgdorferi chromosome due to the presence of a type 2 telomere at the left end. To determine if replacement of B. burgdorferi resT with B. hermsii resT had any effect on the morphology or growth phenotype, the parental, intermediate, and cross-species complementation strains were cultured in the absence of antibiotic selection and monitored for growth rate. There were no discernible morphological differences between the wild-type, intermediate, and cross-species complementation strains, and all three strain backgrounds tested grew at indistinguishable rates during six passages over an 18-day period (data not shown).

We also compared the plasmid content and topological DNA form in the constructed strains by analysis of plasmid preparations on field inversion gels. Figure 5 shows such a comparison of the strains in B31-A constructs. No major differences in plasmid topology, such as conversion of linear to circular forms, were apparent. However, we did notice the loss of lp25 following the first transformation and the loss of lp21 after the second transformation in most cases. To more completely assess any plasmid differences, we subsequently screened plasmid DNA preparations from all strains with primers specific for regions unique to each plasmid, as previously described (35). No changes in plasmid content were seen in the cross-species complementation strain derived from B31-A, which lacks 8 out of 21 plasmids screened (Table 1). Not unexpectedly, changes in plasmid content were observed, however, in the strains derived from both infectious parents that originally carried a full collection of linear and circular plasmids. Following the first transformation with the B. hermsii resT shuttle vector, cp9 was displaced due to incompatibility (40), and lp25 was universally lost, presumably because of a transformation barrier imposed by the putative restriction-modification gene it carries (BBE02) (25, 30). During the second transformation lp56 was also lost, again most likely due to the putative restriction-modification system (BBQ67) carried by this plasmid (30). In addition, lp21 loss was observed in three out of the five transformants recovered but not in any of the four control transformants screened, where chbC, a gene directly adjacent to resT on cp26, was knocked out instead (Table 1, group C). lp28-1, which carries a type 2 telomere at its right end (45), was surprisingly retained in all but one instance. Loss of lp28-4 was also observed in one case. Finally, plasmid stability was assessed over 50 generations, with plasmid content remaining static for all strains surveyed, including lp28-1 and lp21 in those transformants that carried them.

**Differential telomere resolution by B. burgdorferi ResT.** Previous studies into the effect of homology box spacing on
FIG. 5. Analysis of B. burgdorferi B31-A3 cross-species resT complementation clones by field inversion gel electrophoresis. The clones are described in the legend of Fig. 4. DNA standards are indicated to the left, and the positions of lp25, lp21, and the ResT in vitro versus in vivo.

DISCUSSION

We have isolated the B. hermsii resT gene through cross-species hybridization with B. burgdorferi resT. Sequence comparison between the two proteins revealed that B. hermsii ResT shares 67% identity with B. burgdorferi ResT and contains all the regions currently known to be necessary for telomere resolution, including the hairpin binding module and the catalytic residues. When B. hermsii ResT was purified and its function compared to that of B. burgdorferi ResT in vitro, however, it was found that B. hermsii ResT was unable to process a replicated type 2 telomere (Fig. 3B). Type 2 telomeres in B. burgdorferi B31 have been reported at the left end of the chromosome (21) and at the right end of lp28-1 (45). We nonetheless proceeded to assess the activity of B. hermsii ResT in vivo, by first supplementing B. burgdorferi with the B. hermsii resT

TABLE 1. Plasmid profiles of B. burgdorferi strains used in this study

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<tr>
<th>Straina</th>
<th>Clone</th>
<th>ResT typeb</th>
<th>Plasmid contentc</th>
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</thead>
<tbody>
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<tr>
<td>GCB266 A3 Bh</td>
<td>+</td>
<td>+ + + + + + + + + + + + + + + + + +</td>
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</tr>
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</table>

a Each set of related proteins is grouped into one of four categories, A to D; group C is comprised of the chbc knockout controls.
b Bb, B. burgdorferi; Bh, B. hermsii.
c Plasmid content was determined by PCR amplifications with primer sets designed to detect the presence of each plasmid (35).
orthologue and subsequently inactivating the native \textit{B. burgdorferi} \textit{resT} gene. We anticipated that the \textit{B. burgdorferi} \textit{resT} knockout would result in a lethal phenotype, due to the inability of these knockouts to process the telomere at the chromosome left end, but, surprisingly, transformants were recovered. The frequency at which allelic exchange occurred to inactivate the \textit{B. burgdorferi} \textit{resT} gene was comparable to that observed for inactivation of other \textit{B. burgdorferi} genes, arguing that mutation of both type 2 telomeres to a type 1 telomere during transformation was exceedingly unlikely.

Further analysis of the cross-species complementation strains derived from the high-passage strain B31-A or from either of two low-passage infectious clones (B31-A3 or B31-5A4) revealed no discernible changes in growth or morphology. Field inversion gel analysis of the DNA content supported the results of PCR plasmid screening and indicated that there were no obvious changes in DNA topology, such as plasmid circularization to eliminate the need for telomere processing (Fig. 5). Loss of lp28-1, which carries a type 2 telomere at the right end (45), occurred in only one out of five cross-species complementation transformants derived from the infectious strains. We expected complete loss of lp28-1, due to its type 2 telomere; however, the results were consistent with the ability of the \textit{B. hermsii} \textit{ResT} protein to process the type 2 telomere at the chromosome left end in vivo. The single transformant lacking lp28-1 probably resulted from the propensity of this plasmid to be lost during growth in culture (29, 35). The loss of \textit{cp9}, lp25, and lp56 during the genetic construction process was expected, as noted in the Results. However, the loss of lp21 in three of the five cross-species complementation clones recovered is less easily explained. This plasmid was not lost in any of the four transformants recovered when the \textit{chbC} gene, directly adjacent to \textit{resT}, was knocked out. lp21 carries a type 3 telomere at the right end (24) and a type 1 telomere at the left end (J. Denek and G. Chaconas, unpublished results), so there is no obvious reason for its loss based upon the sequence of its telomeres. Other studies have shown that transformation can cause the loss of lp21 (41, 43) but do not explain why this might occur in a nonrandom manner as we have observed.

We also report here the differential processing in vitro and in vivo of a +1 and a +4/-4 telomere by the \textit{B. burgdorferi} \textit{ResT} protein (Fig. 6). In this case, the in vivo analysis of telomere resolution was performed using a previously developed assay (15), where a replicated telomere is inserted at an internal site in lp17, resulting in the deletion of a 3.3-kb fragment from one end. The unexpected disparity in telomere processing in vivo versus in vitro for both the \textit{B. hermsii} and \textit{B. burgdorferi} \textit{ResT} proteins may simply be due to an inability of the in vitro conditions to faithfully mirror those in vivo. However, a more attractive possibility suggested by our results is the presence of one or more accessory factors in vivo that rescue the processing deficiency. A protein worthy of consid-

\begin{figure}
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\includegraphics[width=\textwidth]{fig6.png}
\caption{Differential telomere resolution by \textit{B. burgdorferi} \textit{ResT} in vitro versus in vivo. (A) A schematic of three replicated telomere substrates is shown with substrate type on the left and the axis of symmetry indicated by a vertical black line. The size of the insertion (+) or deletion (−) between the axis and box 1 and between boxes 2 and 3 on the +4/-4 and +1 telomeres is indicated. (B) Kinetics of in vitro telomere resolution on substrates shown in panel A. PstI-linearized plasmid substrates (1.5 nM) were incubated at 30°C for 0.5, 1, 2, or 3 min with 2.5 μg/ml (46 nM) \textit{ResT} protein. Open squares denote the 0/0 (type 1) substrate, while closed triangles and diamonds denote +4/-4 and +1 type substrates, respectively. (C) Analysis of \textit{B. burgdorferi} transformants with replicated telomere insertions in lp17 by field inversion gel electrophoresis. Plasmid DNA from untransformed B31-A (lane 1), B31-A transformed with pKK81 (lane 2; no telomere), pYT1 (lane 3; type 1 or 0/0 telomere), pYT15 (lane 4; +4/-4 telomere), or pYT9 (lane 5; +1 telomere) was analyzed on a field inversion gel as described in Materials and Methods. The migration positions of wild-type lp17, intact integrants (I), and resolved integrants (R) are indicated on the left.}
\end{figure}
eration may be FtsK (7), which appears to have an orthologue in B. burgdorferi (39). FtsK in Escherichia coli is a multifunctional protein that couples cell division and chromosome segregation as well as directly stimulating recombination by XerCD at dif sites in chromosomal dimers (1, 8); the latter process is functionally analogous to telomere resolution in Borrelia to generate chromosomal and plasmid monomers. Further investigation into the differences in ResT activity in vivo versus in vitro may reveal connections between ResT and proteins involved in plasmid and chromosome segregation and cell division. Similarly, further study into the differences between the B. burgdorferi and other Borrelia ResT orthologues may provide a successful avenue for structure-function analysis of this unique class of enzyme.

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