Structure, Function and Evolution of Linear Replicons in *Borrelia*

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Keywords: linear DNA, hairpin telomere, telomere resolvase,
protelomerase, telomere resolution, *Borrelia*

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Abstract

Spirochetes of the genus *Borrelia* include important human pathogens causing Lyme borreliosis and relapsing fever. The genomes of *Borrelia* species can be composed of up to twenty-four DNA molecules, most of which are linear. The plasmid content and linear replicon sequence arrangement varies widely between isolates. The linear replicons are terminated by covalently closed DNA hairpins or "hairpin telomeres." Replication of these elements involves a unique reaction, referred to as telomere resolution, to produce hairpin telomeres from replicative intermediates. The telomere resolvase, ResT, is thought to contribute to the genetic flux of the linear molecules by promoting stabilized telomere fusions. Telomere resolvases are related to the tyrosine recombinases and ResT can generate the crucial reaction intermediate of this class of enzyme, the Holliday junction. This observation has led to the proposal that telomere resolvases evolved from tyrosine recombinases inducing DNA linearization in the genomes that acquired them.

Terms definitions list

- 1) **hairpin telomere** = The linear replicons in *Borrelia* species are terminated by covalently closed DNA hairpin ends or hairpin telomeres.
- 2) **telomere resolution** = A reaction that processes dimer junctions (also known as replicated telomeres) in replication intermediates, resulting in the formation of covalently closed hairpin telomeres.
- 3) **telomere resolvase** = An enzyme that catalyzes telomere resolution through a unique two-step transesterification involving breakage and resealing of two phosphodiester bonds to generate hairpin telomeres.
- 4) **telomere fusion** = The covalent joining of two hairpin telomeres, either on the same or on different plasmids through reversal of the telomere resolution reaction by ResT.
- 5) **paralogous family** = a family of homologous genes that have duplicated and divergently evolved within a species.

Acronyms list (spell out up to 10 most important acronyms)

- 1) **cp** = circular plasmid
- 2) **lp** = linear plasmid
- 3) **PF** = paralogous family
- 4) **ResT** (or *resT* gene) = **Resolvase of Telomeres**
- 5) **rTel** = replicated telomere

Summary points list

- 1) Members of the genus *Borrelia* carry highly unusual segmented genomes, many of which contain an unprecedented number of DNA molecules.
- 2) With the exception of a few species that carry only linear DNA molecules, most *Borrelia* spp. carry a mix of circular and linear plasmids. The linear plasmids are terminated by covalently closed hairpin ends (hairpin telomeres).
- 3) A specialized enzyme (ResT, a telomere resolvase) is required to complete DNA replication and generate the covalently closed hairpin ends from replication intermediates (telomere resolution).
- 4) *Borrelia* genomes are in a state of flux, with continual rearrangement of DNA sequences carried on most of the linear replicons.
- 5) The hairpin telomeres are recombinogenic and are believed to serve as substrates for fusion of linear replicons, mediated by ResT.
- 6) Fusion of linear replicons appears to occur via reversal of the telomere resolution reaction. This is followed by deletion or mutation at the dimer junction to render the molecule inert to further action by the telomere resolvase, hence “freezing” the rearranged DNA structure.
- 7) Telomere resolvases likely evolved from tyrosine recombinases either within a *Borrelia* progenitor or outside borreliae and were transferred thereafter via horizontal gene transfer.

8) Linear genomic elements in *Borrelia* species may confer selective advantages. These include the stable maintenance of both inverted repeats involved in gene regulatory processes, and the *vls* locus involved in antigenic variation and persistent infection of Lyme spirochetes.

9) The sub-telomeric location of the expression sites for antigenic variation in Lyme and relapsing fever spirochetes may be required for, or stimulate recombination.

***Borrelia* Genome Structure**

Borrelia species are important both clinically, as causative agents of disease, and from a biological standpoint, as some of the most unique and fascinating microbes on the planet. Some pathogenic spirochetes in the genus *Borrelia* have long been known to cause louse-borne and tick-borne relapsing fever (7, 9, 34). More recently, other *Borrelia* species have been shown to cause Lyme disease, also referred to as Lyme borreliosis (6, 14, 15, 75-77). A hallmark of all *Borrelia* species is a segmented genome comprising anywhere from about half-a-dozen to two dozen DNA molecules (19, 26, 39, 40, 58). All species carry a linear chromosome of about 900 kb and an eclectic collection of up to 22 plasmids ranging between about 5 and 220 kb (20). The linear plasmids have also been referred to as mini-chromosomes (10) and essential genetic elements (80) because a number of them encode important metabolic functions or factors involved in host adaptation, adherence, immune evasion, or survival in either the tick vector, the host, or both (see (66)).

Besides carrying far more extrachromosomal elements than other bacteria, most *Borrelia* species harbor both circular and linear plasmids. Linear plasmids are, of course, uncommon in bacteria; those in *Borrelia* species are terminated by covalently closed DNA hairpin ends or hairpin telomeres. This type of unusual DNA structure is found only in *Borrelia*, on

one of the two *Agrobacterium tumefaciens* chromosomes (42), and in a small number of bacteriophages (see (21)).

The prototype Lyme disease species and the first member of the genus to be sequenced is *Borrelia burgdorferi* (19, 39). The genome of the type strain B31 is depicted in Fig. 1. Some of the most intriguing questions regarding this genome are:

- Why a segmented genome?
- Why linear replicons?
- How did this genome evolve?
- How do two dozen replicons peacefully coexist under one roof and how do they faithfully segregate to daughter cells?
- How are DNA molecules with covalently closed hairpin ends replicated?

In addition to these issues is the fascinating observation that the genome is in a state of constant flux. The plasmid content within individual isolates of *B. burgdorferi* is highly variable (84) and despite the absence of known active transposable elements, at least in *B. burgdorferi* B31 (28), exchange of DNA on the linear plasmids appears to be responsible for the apparent change in linear plasmid content and structure between strains (19, 20, 84). These observations beg the questions:

- How and why do the ongoing DNA exchanges between the linear replicons occur?

This review will focus on providing some background information on issues relevant to the questions above. Answers to questions for which solutions are available will be provided along with some proposals for answers to questions that remain enigmatic.

A Panoply of Plasmids

As noted earlier, *B. burgdorferi* B31 carries almost two dozen plasmids (12 linear and 10 circular), more than any other bacterium. The related species *B. afzelii* and *B. garinii* also harbor large numbers of circular and linear plasmids (40, 41). The issue of plasmid compatibility and how such large numbers of plasmids can be harmoniously maintained is not yet clear. Both circular and linear plasmids appear to be replicated using a similar set of plasmid maintenance functions (81) with the exception of the need for processing hairpin telomeres in the linear plasmids (discussed below). However, interconversion between circular and linear forms is not common and may be constrained by transcriptional variation of certain promoters on plasmids with differing topology (2, 13).

Each plasmid carries between one and four genes encoding members of five paralogous replication/maintenance protein families (19). These are paralogous families (PF) 32, 49, 50, and the related 57/62 families (Fig 1). These functions are known to confer the ability for autonomous plasmid replication (12, 35, 81, 83). PF57 and the related PF62 are believed to be

the replication initiator proteins (12, 35) and every plasmid carries either a PF57 or PF62 member (19). The PF32 family is also represented on almost every plasmid. This is the only paralogous family that has some sequence homology to known proteins: the ATPase portion of the ParA family of partition proteins (19, 91). The purified PF32 from Ip17 protein displays ATPase activity and a physical interaction with PF62, suggesting that it is not a strict ParA orthologue and that it may be involved in plasmid DNA replication (although this does not preclude a possible coincident role in plasmid partitioning) (30). The role of PF49 and PF50 members remains unknown at this time. Genetic study of the function of the paralogous families of replication/maintenance functions is difficult because inter-plasmidic complementation may obscure the presence of a mutation or the absence of a given family member. A set of bacterial plasmids each encoding related, but distinct, plasmid replication/maintenance functions provides a general explanation for non-competitive replication and partitioning strategies, resulting in many compatibility groups. However, the molecular mechanisms of plasmid replication and partitioning remain abstruse at this time and are an area for future research focus.

Circular and linear plasmids

There are both circular and linear plasmids in most *Borrelia* species. The cp32 family of circular plasmids are phage genomes (36, 37, 89), with a

very high degree of similarity to one another (see (20, 79)). They have been shown to carry surface-localized proteins that may be involved in adhesion and interaction with the host complement system (see (66)). Interestingly, *Borrelia* species that harbor cp32 plasmids carry a complex mixture of them. For example, *B. burgdorferi* B31 provides residence for seven cp32 plasmids, two cp32 deletion derivatives, cp9-1 and -2, and an integrated cp32 in lp56 (19, 62). Moreover, intact cp32 plasmids are rarely lost, suggesting that they carry important information for the tick-host cycle. Why multiple forms of the cp32s exist and are stably maintained remains a mystery. This phenomenon may simply be a result of a selfish phage propagation strategy. It will be of interest to determine the infectivity of strains lacking each and multiple cp32 plasmids when such strains can be constructed.

In contrast to other borreliae, the relapsing fever species *B. parkeri* and *B. recurrentis*, and the avian borreliosis species *B. anserina* have been reported to carry only linear plasmids (71). The relapsing fever species *B. duttonii* carries a single circular plasmid (58) and no cp32 plasmids. So at what point did the cp32 phage plasmids enter the *Borrelia* lineage? A query of the recently sequenced *B. duttonii* genome with cp32 sequences from *B. hermsii* and *B. burgdorferi* reveals multiple hits of small cp32-related sequences on a variety of *B. duttonii* plasmids. The widespread preponderance of cp32 plasmids in *Borrelia* species (78, 79), coupled with apparent remnants of cp32 sequences in one species lacking cp32 (*B.*

duttonii), suggests that cp32 phages entered the *Borrelia* lineage very early, before the split between Lyme and relapsing fever species. The apparent cp32 remnants in not only *B. duttonii*, but also linear plasmids from other *Borrelia* species raises the possibility that linear plasmids in the genus *Borrelia* may have arisen by linearization of cp32 plasmids followed by loss of phage functions resulting from DNA deletion and rearrangement associated with telomere fusion as described in a later section.

Replication of linear DNA in *Borrelia*: requirement for a telomere resolvase

Another unorthodox feature of *Borrelia* genomes is the presence of linear replicons terminated by covalently closed hairpin ends. The replication of such molecules represents a unique approach to solving the end-replication problem and requires a specialized enzyme. The overall scheme for duplication of linear *Borrelia* replicons is shown in Fig. 2. Replication is believed to occur bidirectionally from a central origin (12, 68). Replication through the hairpin ends results in the formation of a plasmid dimer junction. This replicated telomere (*rTel*) is specifically recognized by ResT, a telomere resolvase that resolves the dimer junction into two covalently closed hairpin telomeres (see (22, 23, 50)). This occurs through a DNA breakage and reunion reaction utilizing a 3'-phosphotyrosyl-enzyme intermediate with tyrosine 335 in a two-step transesterification (29, 53) similar to that for type IB topoisomerases and tyrosine recombinases (Fig. 3). A number of studies

probing the mechanism of action of ResT have been published in recent years (3, 5, 24, 29, 51-54, 57, 63, 81, 85-88), but this review will focus primarily on aspects of the enzyme related to evolution of the segmented genome, rather than the mechanistic biochemistry.

The nomenclature controversy: telomere resolvase versus protelomerase

The enzymes that catalyze telomere resolution have been referred to as both telomere resolvases and protelomerases. The two different names were coined before an actual enzyme had been identified or purified. An open reading frame that resided next to one of the hairpin telomeres of the *E. coli* phage N15 was first referred to as a “telomerase” (GenBank Accession U63086). Because of ambiguity with known eukaryotic telomerases, which employ a completely different mechanism, the hypothetical enzyme was subsequently referred to as a “protelomerase,” for prokaryotic telomerase (70). Around the same time, the putative poxvirus enzyme believed to perform telomere resolution was referred to as a telomere resolvase (67, 72). We have chosen to adapt the latter name for several reasons:

i) The term protelomerase is ambiguous both in terms of its possible relationship to eukaryotic telomerases and because a proenzyme is an inactive enzyme precursor. Technically, a protelomerase would be an inactive telomerase precursor, which is not the case. ii) The enzyme catalyzes the reaction universally referred to (by both camps) as telomere

resolution. Therefore, the label of telomere resolvase is scientifically correct.

iii) Resolvases are enzymes that promote site-specific recombination to resolve cointegrate structures and plasmid or chromosome dimers following DNA transposition and DNA replication. Telomere resolution is an analogous process and referring to the enzyme that performs this function as a telomere resolvase seems most appropriate. However, not all workers in the field agree with use of the term telomere resolvase and the non-uniform nomenclature will likely continue to be used.

***B. burgdorferi* telomeres**

The hallmark of *Borrelia* species is a genome of primarily, if not exclusively, linear DNA molecules terminated by covalently closed hairpin ends. Hairpin telomeres were first described in *B. burgdorferi* in 1987 (8). Obtaining DNA sequence information of the hairpin telomeres has not been a trivial undertaking. The telomeres of lp17 were sequenced directly using chemical sequencing methods in 1991 (44). Several telomeres were also sequenced after cloning, which involved treatment with a single-strand specific nuclease to open the hairpin ends (18, 39, 45). However, whether the entire hairpin sequence had been obtained, due to the necessity of cleaving with mung bean nuclease, was not certain. Recently, the sequence of the entire set of *B. burgdorferi* B31 telomeres has been reported using a direct genomic sequencing approach coupled with mung bean nuclease treatment of the

template DNA (86). The use of oligonucleotides, based upon the determined sequences, as substrates for *in vitro* telomere resolution reactions confirmed the integrity of the telomere sequences.

The hairpin telomere sequences from *B. burgdorferi* B31 are shown in Fig. 4. The covalent linkage to make the hairpin telomere occurs between the first nucleotide shown and the complementary nucleotide on the opposite DNA strand (not shown). The telomeres can be sorted into three distinct categories: Type 1, Type 2 and Type 3, based upon the presence, sequence and position of box 1 (86). Type 1 telomeres carry a box 1 sequence of TATTAT at position 5-10. Type 2 telomeres contain a modified box 1 (TATAAT) at position 2-7 and Type 3 telomeres do not carry a clearly defined box 1. A critical component of all telomeres is the well conserved box 3 sequence (TTAGTATA), which is found universally at position 14-21. This is the sequence that directs site-specific binding of ResT to the substrate (88) and positions the active site to cleave the *rTel* between nucleotides 3 and 4 on both strands (87) resulting in the six nucleotide sticky ends shown in Fig. 4.

Interestingly, the telomeres show a wide variation in their reactivity *in vitro* (with initial rates of reaction varying by at least 160-fold). Type 1 and Type 2 telomeres show no obvious difference in reactivity; however, Type 3 telomeres, which lack a clearly defined box 1, show the lowest efficiency of utilization. Some Type 3 telomeres display no detectable reactivity *in vitro*,

in spite of their confirmed reactivity *in vivo*. These results suggest that additional factors may be involved in promoting telomere resolution *in vivo*.

Telomere sequences from other *Borrelia* species have not been reported, with the exception of the right end of lp28-1 in *B. hermsii*, which carries a *B. burgdorferi* Type 1 telomere (49). The sequences of telomeres from other *Borrelia* species are of interest as the ResT proteins from different *Borrelia* species have demonstrated differential abilities to process *B. burgdorferi* Type 2 telomeres (5, 63, 85). Future research goals include the determination of telomere sequences in a variety of *Borrelia* species as well as an investigation of the possible interaction of ResT with other cellular proteins that might influence the reaction and possibly couple telomere resolution to cell division and DNA partitioning.

***B. burgdorferi* telomeres are recombinogenic**

One of the most intriguing properties of *Borrelia* genomes is the very fluid nature of the linear plasmids. Not only do these plasmids show dramatic differences between species, but also between independent natural isolates. Moreover, DNA sequence analysis of the linear plasmids of *B. burgdorferi* B31 has revealed that they are a patchwork of sequences that are represented many times on a variety of the linear plasmids, particularly near the telomeres (19) (see Fig. 5). What are the forces driving continual rearrangement of the linear plasmids? The answer appears to be that the

hairpin telomeres of *B. burgdorferi* are recombinogenic. We believe that this recombinogenicity is manifested primarily through reversal of the telomere resolution reaction (telomere fusion) and secondarily through frequent recombinational exchange of the many duplicated sequences resulting from telomere fusion.

Stabilized telomere fusions.

We have reported that ResT can catalyze the fusion of hairpin telomeres in a reversal of the telomere resolution reaction depicted in Fig. 3 (54). Most such reversal events would be rapidly resolved back into hairpin telomeres, but fusion events that become stabilized by deletion of the dimer junction formed would become permanent. Such hairpin telomere fusions would result in either the circularization of a linear plasmid or the transfer of at least part of one linear plasmid onto the end of another, creating a new hybrid linear plasmid. We believe that such events provide the driving force for continual diversification of the linear plasmids in *Borrelia* species.

Several candidate examples of stabilized telomere fusion events have been reported in the literature: i) there appear to have been at least two successive transfers of linear plasmids to the right end of the linear *B. burgdorferi* R-IP3 chromosome to produce the structure of the chromosome present in the Sh-2-82 strain (47). A schematic of these predicted fusion events are shown in Fig. 6. ii) lp54 has been found as a linear head-to-head

dimer fused at the right end in several *B. burgdorferi* strains (60). iii) a 170 kb linear plasmid of *B. hermseii* was found to be circularized in one variant strain (38). iv) The cp26 that carries the *resT* gene in *B. burgdorferi* may be another example of a linear plasmid that became circularized by telomere fusion. In the recently sequenced genomes of *B. recurrentis* and *B. duttonii*, the *resT* gene is carried on a 23 kb linear plasmid (lp23) that is mostly syntenic with *B. burgdorferi* cp26 (58).

The linear plasmids with the greatest sub-telomeric similarity are found next to hairpin telomeres with compatible sticky ends for telomere fusion (lp28-3L, lp56R, lp17L, lp28-4L, lp21L, chromosome R, lp28-1L, lp28-2R all have the sequence TATATA between the cleavage sites). Obversely, it is notable that the most recombinationally isolated and consequently the most stable linear plasmid is lp54. This plasmid appears to be present with an unperturbed structure in a variety of natural isolates (84), in five recently sequenced *B. burgdorferi* strains (S. Casjens, personal communication) and even in another species, the sequenced *B. garinii* strain PBi (40). The inert nature of lp54 in terms of DNA rearrangements can be explained by the DNA sequence of its telomeres (86). The left end telomere of lp54 has a sticky end sequence that does not match any other *B. burgdorferi* telomeres; hence, it cannot undergo ResT-mediated telomere fusion with any other plasmid, except itself (see point ii above). Similarly, the sticky end generated by cleavage of the right end of lp54 matches only the left end of

the chromosome where housekeeping genes are located (39). Fusions between lp54R and the chromosome left end are theoretically possible but may be prohibited for reasons other than sticky end complementarity. An area for future experimentation is to prove our contention that reversal of telomere resolution by ResT is the driving force for DNA rearrangements in the linear plasmids. Controlled overexpression of ResT *in vivo* in *B. burgdorferi* grown in culture would be expected to speed up the evolutionary clock and result in the formation of DNA rearrangements characteristic of those observed on the linear plasmids during the natural tick-host cycle.

Duplication and exchange of subtelomeric sequences.

One consequence of stabilized telomere fusions that create hybrid linear plasmids is that the sequences that are transferred to the new hybrid plasmid may become duplicated (*i.e.*, those sequences are still present on copies of the original plasmid that were not involved in the telomere fusion event). This creates substrates for genetic exchange by homologous recombination. Over time, subsequent rounds of stabilized fusions and homologous recombination would be expected to proliferate the duplications and create further mosaicism of sub-telomeric sequences (21, 54) as has been observed (Fig. 5) in the *B. burgdorferi* genome (19). Duplications generated by the processes outlined above are the logical starting points for

mutational decay to generate the plethora of pseudogenes observed on the linear plasmids (19).

Evolution of telomere resolvases

Telomere resolvases are a unique class of enzymes that promote the formation of covalently closed hairpin ends using a two-step transesterification. The first step involves cleavage of two DNA strands; this is followed by phosphodiester bond formation with the 5'-OH on the overhang of the opposing DNA strand (Fig. 3). The reaction performed is more complex than that of type IB topoisomerases but simpler than that of tyrosine recombinases, including the phage integrases and chromosome/plasmid dimer resolvases. The telomere resolvases and the tyrosine recombinases share a catalytic domain with a common protein fold and an almost identical constellation of active site residues, suggesting an evolutionary relationship (1, 29, 53). The structural and functional relatedness between the tyrosine recombinases and telomere resolvases is further underscored by the ability of certain tyrosine recombinases (λ integrase and the Flp recombinase) to be easily converted to enzymes that produce DNA hairpins by modification of the DNA substrates (56, 64). This highlights the relative ease with which a tyrosine recombinase may produce hairpin telomeres out of what was originally a recombination site.

More recently, *in vitro* studies have shown that *B. burgdorferi* ResT is able to synapse two *rTels* and promote strand transfer between them to form a Holliday junction (51); synapsis and Holliday junction formation are key features of the site-specific recombination reaction catalyzed by tyrosine recombinases (43) and the finding that ResT can perform these functions led to the proposal that ResT evolved from a tyrosine recombinase (51). In the case of ResT, the synapsis and strand transfer activity seem more likely to be an ancestral property of the enzyme rather than a necessary contemporary function. However, recent studies on the telomere resolvase (TelN) of phage N15 (a coliphage that possesses a linear prophage genome with hairpin telomeres) suggest a requirement for synapsis and strand exchange by this telomere resolvase to establish the substrate for lytic replication in the N15 life-cycle, again strengthening the link with tyrosine recombinases (61) and the proposal that telomere resolvases evolved from the recombinases (51).

Tyrosine recombinases are generally thought to have arisen by elaboration of an ancestral type IB topoisomerase (see (55)) and telomere resolvases appear to be a modification or elaboration of a tyrosine recombinase. One could, nonetheless, also propose that telomere resolvases evolved from the simpler type IB topoisomerases. These topoisomerases also show structural similarity to telomere resolvases and perform a similar two-step transesterification. Both type IB topoisomerases (74) and telomere

resolvases (1, 29, 31, 32, 46, 53) display the same reaction polarity and generate a 3'-phosphotyrosyl-enzyme intermediate. We do not favor this alternative, primarily because of the absence of type IB topoisomerases in 10 of the 11 bacterial genera where known or putative telomere resolvases are encoded (63), either by the bacteria themselves (*Borrelia*, *Agrobacterium*, *Acaryochloris*, *Cyanothece*, *Synechococcus*, *Crocospaera*) or their phages (*Yersinia*, *Klebsiella*, *Vibrio*, *Escherichia*, *Halomonas*). Moreover, eubacterial topoisomerase IB enzymes have not been reported to display sequence specificity (48, 55), which would be required for a telomere resolvase. Finally, type IB topoisomerases act as monomers and therefore are unable to synapse replicated telomeres; such *rTel* synapsis appears to be required by N15 TelN to produce the circular monomer form of the genome for lytic rolling circle replication (61). The evolution of telomere resolvases would be expected to occur with concomitant linearization of the DNA substrate (25). This would likely involve a rapid conversion of a tyrosine recombinase into a telomere resolvase, something that current data (56, 64) suggest may be relatively easy to achieve.

Linearization of *Borrelia* DNA

We propose two alternative models for the origin of genome linearity in *Borrelia*, starting with an ancestral organism carrying a circular chromosome. In model 1 (Fig. 7A) a phage tyrosine recombinase was

converted to a telomere resolvase through genetic drift. The tyrosine recombinase may have functioned as a phage integrase (e.g., λ Int) or a circular dimer resolvase (e.g., P1 Cre). Infection of the *Borrelia* ancestor with such a phage could have caused chromosome linearization when the newly evolved telomere resolvase recognized and acted at a site near the terminus of replication. Action at related sites in any co-resident plasmid prophages would also have resulted in linearization. Once linearized, reversal of this process would be expected to be kinetically, if not mechanistically disfavored, necessitating the action of the telomere resolvase at every subsequent cell cycle for complete replication of every linearized DNA molecule. The large number of highly similar cp32 plasmids, which are in fact plasmid prophage genomes, underscores the ability of *Borrelia* to accommodate a large number of closely related genetic elements lending credence to the idea that many replicons may have become linearized upon horizontal transfer of a nascent telomere resolvase activity. Subsequent decay or deletion of the now superfluous phage lytic functions could obscure the linear plasmids' original provenance as phage genomes.

An alternative mode of acquisition might also have been the transposition into the chromosome and plasmids of a phage telomere resolvase located next to a replicated telomere. The phage N15 genome displays a telomere resolvase juxtaposed with a replicated telomere during lytic replication (61). Such a phage gene arrangement flanked by the

inverted repeats of a mobile element also make a plausible system for dissemination of a telomere resolvase and its accompanying recognition site through horizontal gene transfer. Evidence for genetic exchange and plasmid transfers between *Borrelia* species has been presented (69), although the mechanism for the transfer of genetic information remains open to speculation. The only pathway for DNA transfer known at this time in *Borrelia* species is phage-mediated transduction (36).

Model 2 (Fig. 7B) is a variation of Model 1 in which an endogenous circular chromosome dimer resolving system is hypothesized to have acquired the ability to form DNA hairpins within the *Borrelia* ancestor. Bacteria that possess active homologous recombination machinery produce circular chromosome dimers due to replication restart accompanied by a crossover in about 10-15% of the population per cell cycle. Successful cell septation depends upon the resolution of the circular dimer into monomeric circles. This resolution is accomplished by the FtsK/*dif*/XerCD system (73). *dif* is the reaction site, Xer is the tyrosine recombinase that acts at the *dif* sites and FtsK is an integral component of the septum and a directional DNA translocase that brings *dif* sites together at the septum for resolution by XerCD. In *E. coli*, the resolution of plasmid dimers of a couple of classes of plasmids is catalyzed by Xer; different accessory proteins are used and the reaction sites are related to, but not exactly the same sequence as, *dif*. In this model, mutation or modification of the ancestral Xer recombinase

linearized the chromosome and also any plasmids whose segregation was aided by the ancestral recombinase (51). This model is consistent with the unique occurrence in *Borrelia* of an FtsK analogue (39, 82) without an accompanying Xer recombinase.

Direct experimental investigation into the evolution of ResT and the linearization of the *Borrelia* genome is not possible. Nonetheless, speculation into this area does allow one to make some predictions to aid future work on the structure, function and evolution of the fascinating, segmented and primarily linear replicons in *Borrelia* species. For instance, under Model 2, one might expect a continued critical role for FtsK in the ResT-mediated resolution of the linear chromosome and plasmids. The possibility may also exist to recapitulate chromosome linearization in another bacterial species by random mutagenesis of Xer.

Advantages of a linear segmented genome

The final question, which remains unanswered, is whether there is a selective advantage to having a segmented genome of primarily or exclusively linear DNA molecules. One possibility is that there is no selective advantage. Instead, DNA segmentation and linearization by a selfish DNA element resulted in a fully functional genome with a topology that differs from that of the bacterial status quo, but otherwise retains normal functionality. Alternatively, there may be distinct advantages resulting from

the unusual structure and function of the segmented *Borrelia* genomes. The first is that the continual scrambling of the linear plasmids through reverse telomere resolution may impart a distinct evolutionary advantage in terms of the reassortment of genetic material. This may generate new proteins by fusing similar, or unrelated genes to generate new alleles or new proteins. This type of protein fusion clearly occurs (19); however, no examples of new proteins that extend host range or offer other life cycle advantages have been reported at this time and an in depth *in silico* analysis will likely be required to uncover such proteins, if they exist. A detailed comparison of genes and sequences carried on linear plasmids within different strains of the same species may also be highly informative.

Another possible advantage of linear replicons is that they may provide a sub-telomeric recombination-enriched environment for the expression locus of the antigenic variation systems found in both relapsing fever (9, 26, 49) and Lyme spirochetes (65, 90). These recombination-driven antigenic variation systems are crucial for infection of and persistence in the vertebrate host. They function to vary surface antigens through gene conversion events that provide DNA sequence diversity at their respective expression sites that are located near linear plasmid telomeres. Interestingly, such antigenic variation systems are often associated with telomeres in a variety of organisms (11, 33). An increased frequency of recombination has been reported for *B. burgdorferi* telomeres (24), supporting of the proposal

that a telomeric expression site location may facilitate recombinational switching (16) and provide a selective advantage. A location of antigenic variation expression sites close to a telomere would also offer a distinct arrangement of expression sites as inverted repeats in close proximity to each other upon replication. This distinct organization resulting from a telomere-adjacent location on linear DNA might provide a required configuration for the gene conversion events that underlie antigenic variation. Determining whether the gene conversion events that underlie antigenic variation in borreliae can occur into an expression locus located on circular DNA molecules or at a large distance from a telomere will be of great interest.

Linear DNA molecules may also provide a stable residence for DNA sequences that would normally be unstable on supercoiled DNA molecules. An example of this may be the inverted repeat sequences found on the linear plasmids (27), which may play a regulatory role as non-translated RNA transcripts (59). Moreover, the crucial region required for antigenic variation of Lyme spirochetes, the *vls* locus, is unstable on circular cloning vectors in *E. coli* (19, 90) and is maintained on a linear plasmid in Lyme spirochetes. The presumed absence of DNA supercoiling on linear *Borrelia* plasmids (13) may allow stable maintenance and propagation of this critical region (4, 41, 90) involved in antigenic variation (see (65)), which is an essential component of its pathogenic lifestyle. The requirement of the *vls*

locus in *B. burgdorferi* may, therefore, provide a powerful genetic selection and distinct advantage for linearity of the DNA element that carries it.

The presence of both linear and circular replicons also offers an additional mechanism for gene regulation as to the topological state of a plasmid has been shown to have profound effect upon the transcription of some *B. burgdorferi* genes (2, 13).

Acknowledgements

We would like to thank Sherwood Casjens for communication of unpublished results, Alan Barbour, Richard Marconi, Scott Samuels and Brian Stevenson for helpful advice and discussions. Many thanks to Scott Samuels for critical review of the manuscript. Research in the authors' labs was supported by the Canadian Institutes of Health Research (GC, MOP-53086 and KK, MOP-79344). GC was supported by a Scientist Award from the Alberta Heritage Fund for Medical Research and holds a Canada Research Chair in the Molecular Biology of Lyme Borreliosis.

Figure Legends

Fig.1. A schematic of the segmented genome of *B. burgdorferi* B31 comprising a linear chromosome, 12 linear and 11 circular plasmids (17, 39, 62). Plasmids known at this time to carry important metabolic functions or virulence factors are indicated with asterisks (see (66)). The function of

many plasmid-encoded proteins remains undefined and the redundancy within the cp32 family of plasmids makes assessment of the importance of the individual family members difficult. The color-coded PF32, 49, 50, 57 and 62 genes are plasmid replication/maintenance functions. This figure is adapted from (19, 30), with permission.

Fig. 2. Replication of the linear chromosome and plasmids of *Borrelia*. The hairpin telomeres at the left (L) and right (R) ends of linear *Borrelia* DNA molecules are indicated by the arrows. Replication of the linear DNA results in the formation of dimer junctions, which contain an axis of 180 degree rotational symmetry (lines bisecting the junctions). Whether telomere resolution occurs in a concerted fashion at both ends (as shown), or whether it occurs independently at whichever end is replicated first, is not currently known. This figure is adapted from (53) and reprinted from (21), with permission.

Fig. 3. Telomere resolution by ResT. 1) The telomere resolvase, ResT, binds to a replicated telomere (*rTel*) substrate, which corresponds to the dimer junction L'L or RR' shown in Fig. 2 (88). The black dots denote the scissile phosphates and the vertical bar in the center of *rTel*/ the axis of 180 degree rotational symmetry. 2) Positive supercoiling is believed to facilitate the cooperative formation of a cross-axis complex where communication

between ResT protomers bound on both sides of the symmetry axis occurs (5, 52). 3) The action of the “hairpin binding module”, a region with sequence similarity to a motif found in cut-and-paste transposases, induces a DNA distortion that facilitates 4) DNA cleavage, the first transesterification event (3). 5) A conformational change then occurs to juxtapose the free 5'-OH groups to the 3-phosphotyrosyl enzyme intermediates on the opposite strands. 6) Nucleophilic attack of the phosphotyrosyl linkage by the 5'-OH groups, the second transesterification step, results in phosphodiester bond formation to stabilize the hairpins generated during step 5. This figure is adapted from (50) and (51), with permission.

Fig. 4. *B. burgdorferi* strain B31 hairpin telomere sequence alignment. The covalent linkage to form the hairpin occurs between the first nucleotide shown and the complementary nucleotide on the opposite DNA strand (not shown). The telomere Type (1,2, or 3) is shown in the left hand column. Telomere sequences are arranged in descending order, according to the initial rate of *in vitro* telomere resolution (86). The initial rate, expressed in fmol/min is shown in the right hand column. Telomere sequences are aligned with the hairpins (or symmetry axis in the replicated telomeres) to the left. The telomeres shown are half of the actual replicated telomere substrates used in the telomere resolution reactions. The colored boxes labeled 1 and 3 refer to previously identified regions of sequence homology (87), with some

modifications. The original box 1 sequence, TATAAT is indicated by a light blue box, while the newly identified box 1 sequence, TATTAT is shown in dark blue. The homology box 3 region has been expanded from the five nucleotide sequence TAGTA to the eight nucleotide sequence TTAGTATA. The telomere sequences of lp17L, lp17R, lp21R, lp28-1R, lp56R ChromL and ChromR have been previously reported (18, 39, 44, 45, 47, 90). This figure is reprinted from (86), with permission.

Fig. 5. The patchwork of repeated DNA sequences found in the sub-telomeric regions of a number of linear plasmids from *B. burgdorferi* B31. Each fill pattern represents a repeated sequence. Reprinted from (19), with permission.

Fig. 6. Telomere exchanges by ResT-mediated telomere fusions. In the first fusion an unknown linear plasmid was linked to the right end of the *B. burgdorferi* R-IP chromosome to generate the observed telomeric structure at the right end of the B31 chromosome. The second fusion shows further modification of the B31 chromosome right end by a stabilized fusion with lp21 to generate the reported structure (47) of the Sh-2-82 chromosome. This figure has been adapted from (54) and reprinted from (21), with permission.

Fig. 7. Models for possible modes of genome linearization.

A) Linearization by mutation of a λ -integrase type enzyme that integrates a phage genome into the chromosome to establish lysogeny and excises the phage genome out of the chromosome upon induction of the lytic cycle. Mutation of the integrase such that the enzyme can make DNA hairpins leads to linearization of the chromosome and phage genome. Mutation of the integrase could occur *in situ* or outside the *Borrelia* ancestor and then be transferred into the ancestor.

B) Linearization by mutation of an endogenous Xer-like circular dimer resolvase. The chromosome and multiple co-resident plasmids whose segregation is aided by the Xer circular dimer resolvase become simultaneously linearized.

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Annotated references

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Figure 1

Figure 2

Figure 3

Type	Telomere	Sequence						Initial Rate (fmol/min)
		1	5	10	15	20		
2	lp28-4R	A	A	T	T	A	T	48.8 ± 2.5
2	lp36R	T	A	T	T	A	T	43.0 ± 7.8
1	ChromR	A	T	A	A	T	T	41.7 ± 7.3
2	lp28-1R	T	A	T	T	A	T	41.4 ± 0.9
1	lp28-1L	A	T	A	A	T	T	40.4 ± 7.4
2	lp28-2L, 36L	A	A	T	T	A	T	39.3 ± 13.8
1	lp17L, 28-3L, 28-4L, 21L	A	T	A	A	T	T	33.7 ± 6.1
2	lp28-3R	A	T	A	T	T	A	29.2 ± 6.8
1	lp56R, lp17R	A	T	A	A	T	T	28.4 ± 2.8
2	lp38R	A	A	T	T	A	T	27.4 ± 0.1
2	lp56L	A	A	T	T	A	T	26.5 ± 5.4
3	lp21R	A	A	A	T	T	T	26.5 ± 6.1
2	ChromL	T	A	A	A	T	T	25.6 ± 0.4
3	lp25L	T	A	A	A	T	T	18.3 ± 3.1
3	lp25R	A	A	A	T	T	T	13.6 ± 2.1
3	lp28-2R	A	T	A	C	A	T	4.7 ± 2.2
3	lp38L	A	A	T	T	T	T	<0.3
3	lp54L	T	G	A	G	A	T	<0.3
3	lp54R	T	A	A	T	A	G	<0.3

Figure 4

Figure 5

Figure 6

Figure 7