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MicroReview

Hairpin telomeres and genome plasticity in *Borrelia*: all mixed up in the end

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Summary

Spirochetes of the genus *Borrelia* have a highly unusual genome structure composed of over 20 replicons. Most of these replicons are linear and terminated by covalently closed hairpin ends or telomeres. Moreover, the linear replicons are affected by extensive DNA rearrangements, including telomere exchanges, DNA duplications, and harbour a large number of pseudogenes. The mechanism for the unusual genome plasticity in the linear replicons has remained elusive. The enzymatic machinery (the telomere resolvase ResT) responsible for generating the hairpin ends from replicative intermediates has recently been shown to also perform a reverse reaction that fuses telomeres on unrelated replicons. Infrequent stabilization of such fusion events over evolutionary time provides the first proposed biochemical mechanism for the DNA rearrangements that are so prominent in the linear replicons of *B. burgdorferi*.

Introduction

Members of the genus *Borrelia* (Schwan *et al*., 1999; Barbour, 2001) display one of the most unusual genome structures in the bacterial world, if not in all creation. These obligate parasites, which cause Lyme disease (Burgdorfer *et al*., 1982; Benach *et al*., 1983; Steere *et al*., 1983; Stanek and Strle, 2003; Steere *et al*., 2004) and relapsing fever (Dworkin *et al*., 2002), have small segmented genomes made up of over 20 replicons (Fig. 1): a chromosome of about 900 kb accompanied by multiple plasmids (Fraser *et al*., 1997; Casjens *et al*., 2000; Glockner *et al*., 2004) that are also referred to as mini chromosomes (Barbour and Zuckert, 1997) or essential genetic elements (Stewart *et al*., 2005). Moreover, most of the replicons are linear and possess a rarely seen terminal structure of covalently closed hairpin ends (Barbour and Garon, 1987), a hallmark of the *Borrelia* species. Linear replicons with hairpin telomeres are unusual in the bacterial world (see Hinnebusch and Tilly, 1993; Casjens, 1999) and, thus far, have also been reported in one of the chromosomes of *Agrobacterium tumefacians* (Goodner *et al*., 2001) and in the phages N15 (Rybchin and Svarchevsky, 1999; Deneke *et al*., 2000; 2002) PY54 (Hertwig *et al*., 2003) and ϕKO2 (Casjens *et al*., 2004; Huang *et al*., 2004a) from *Escherichia coli, Yersinia enterocolitica* and *Klebsiella oxytoca* respectively.

In addition to the features noted above, the genomic sequence of *Borrelia burgdorferi* has revealed additional features uncommon in bacterial genomes. These include extensive DNA rearrangements including many duplications and pseudogenes (Casjens *et al*., 2000). DNA exchanges on the linear plasmids are a ubiquitous feature with some DNA sequences present in a variety of locations. Subtelomeric sequences are particularly vulnerable to DNA scrambling. Both ends of every linear plasmid, except the right end of lp54, share similarities with at least one other subtelomere. The sequence near the right-end telomere of the *B. burgdorferi* chromosome can also vary from strain to strain because of the presence of variable sequences that appear to be derived from linear plasmids (Casjens *et al*., 1997; Huang *et al*., 2004b). Furthermore, of the 167 pseudogenes in the *B. burgdorferi* genome, 87% are found on 10 linear plasmids. Recent sequence comparisons of the breakpoint of several DNA exchanges revealed only that these were non-homologous events (Huang *et al*., 2004b). Clearly, promiscuous DNA exchanges by linear replicons are an important feature in the evolution of the *B. burgdorferi* genome, but by what mechanism do such exchanges occur? Classical transposable elements that might be responsible for the state of flux of the *B. burgdorferi* genome have not been reported, and the mechanism for the extreme genome plasticity in *B. burgdorferi* has remained a mystery. However, a recent study suggests that the promiscuous scrambling of DNA in the linear replicons of *B. burgdorferi*

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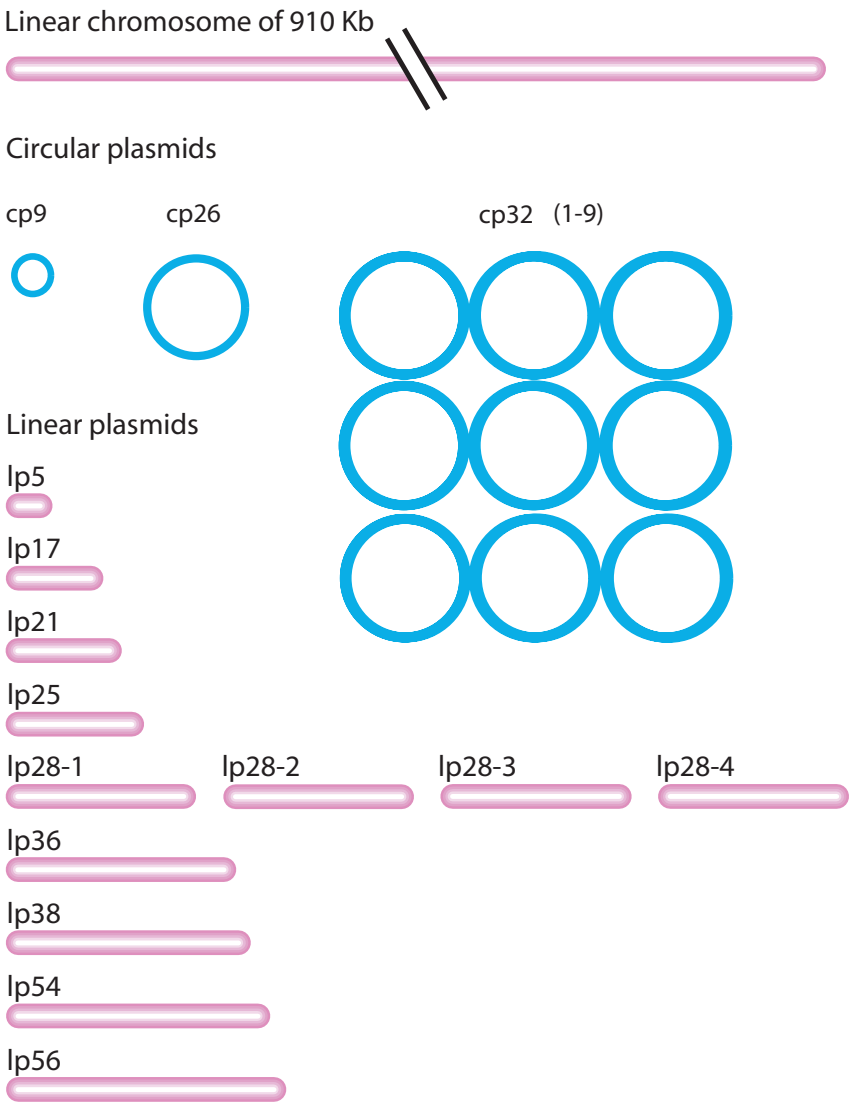

Fig. 1. The segmented and enigmatic genome of *B. burgdorferi*. Sizes are not drawn to scale. This figure is adapted from the study by Stewart *et al.* (2005) with permission from Elsevier.

is related to the unique replication mechanism that generates the covalently closed hairpin ends or telomeres (Kobryn and Chaconas, 2005).

Replication of linear *B. burgdorferi* DNA

Linear DNA molecules require specialized mechanisms to replicate their 5′ ends and the unusual covalently closed hairpin ends found in *Borrelia* species are a component of such a specialized adaptation (see Hinnebusch and Tilly, 1993; Casjens, 1999; Rybchin and Svarchevsky, 1999; Kobryn and Chaconas, 2001; Chaconas and Chen, 2005; Stewart *et al.*, 2005). A variety of possible molecular pathways for replication of this type of DNA have been hypothesized (Casjens, 1999). However, recent studies have revealed that *B. burgdorferi* uses the mechanism outlined in Fig. 2. The first important advance towards this understanding was the mapping of a bidirectional origin of replication to within a 2 kb region in the centre of the linear *B. burgdorferi* chromosome (Picardeau *et al.*, 1999). Sequence analysis also demonstrated that this region displays a profound switch in CG skew (Fraser *et al.*, 1997). A finer tuned analysis of CG skew predicted the origin to lie in the 240 bp *dnaA–dnaN* intergenic region (Picardeau *et al.*, 1999). This stretch of DNA also carries a binding site for the *Borrelia* DNA bending protein Hbb (Kobryn *et al.*, 2000) that, based on the replication of other bacterial chromosomes, is expected to play a role in replication. CG skew analysis of the linear plasmids also suggested that they replicate bidirectionally from an internal origin (Picardeau *et al.*, 2000). Recent work has localized the replication origins of lp25, lp28-1 and lp17 to internal regions of these plasmids (Stewart *et al.*, 2003; Beaurepaire and Chaconas, 2005).

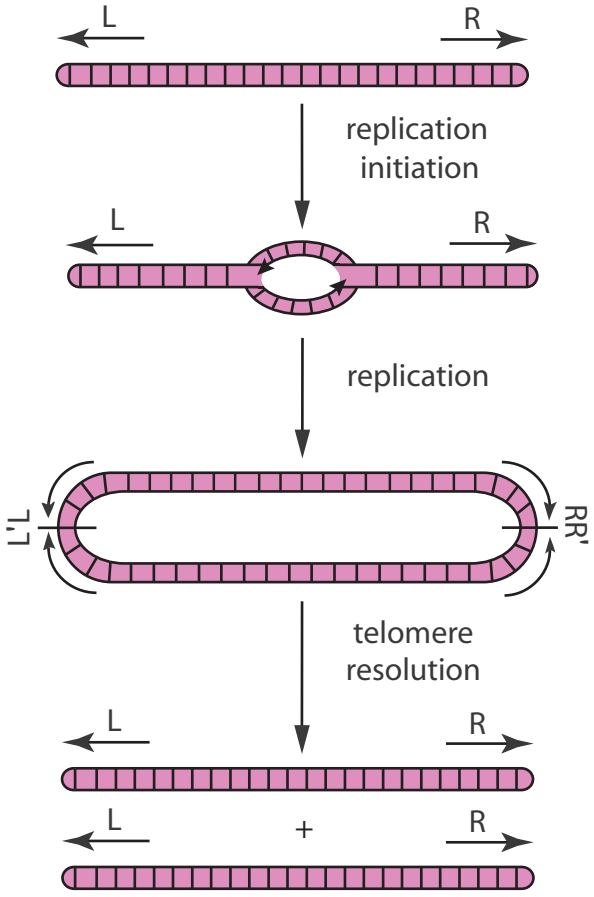

Fig. 2. The replication pathway for linear DNA replicons with covalently closed hairpin ends. The L and R arrows indicate the DNA hairpin telomeres at the left and right ends respectively. The lines bisecting the head-to-head (L′-L) and the tail-to-tail (R-R′) replicated telomere junctions are axes of 180° rotational symmetry. It is not yet known whether replication is completed before telomere resolution (as shown) or whether processing at either end can occur before replication is completed at the other. A similar replication strategy has been demonstrated for the *E. coli* phage N15 (Ravin, 2003; Ravin *et al.*, 2003). This figure has been adapted from the study by Kobryn and Chaconas (2005).

Another major advance in our understanding of *B. burgdorferi* replication was the demonstration that the replicated telomere, or dimer junction region (L′-L), in the replicative intermediate in Fig. 2, is a substrate for telomere resolution *in vivo* (Chaconas *et al.*, 2001). DNA breakage and reunion occurred at this site when it was placed at an internal position in lp17, resulting in deletion formation and generation of a hairpin telomere at the deletion site. The ability to generate deletions *in vivo* at desired locations has recently been used for 'targeted deletion walking' to map essential functions for lp17 replication (Beaurepaire and Chaconas, 2005) and will be useful for *in vivo* engineering of linear replicons in borreliae. The specialized enzyme responsible for the DNA breakage and reunion activity is ResT (Resolvase of Telomeres), which has been purified and shown to function *in vitro* (Kobryn and Chaconas, 2002), allowing detailed mechanistic studies of this reaction as summarized below.

The telomeres

The sequences of the prototype *B. burgdorferi* telomeres from the left and right ends of lp17 were determined by chemical sequencing around the covalently closed hairpin ends of this plasmid, an accomplishment not yet repeated for any other *Borrelia* plasmid (Hinnebusch and Barbour, 1991). The sequences of an additional nine telomeres, including one from a *B. hermsii* plasmid, were determined by S1 digestion of the hairpin loop followed by cloning and dideoxy sequencing (see Casjens *et al.*, 1997; Casjens, 1999; Huang *et al.*, 2004b). This facile approach provides reliable sequence information for most of the telomere but might not provide the complete sequence of the hairpin loop, where S1 nuclease might remove a nucleotide or two.

Until recently, the collection of telomere sequences was divided into two telomere types (see Fig. 3): five Type 1 or 0/0 telomeres typified by lp17, and four Type 2 or +3/–3 telomeres typified by the left end of the B31 chromosome. The numbering system attached to these two

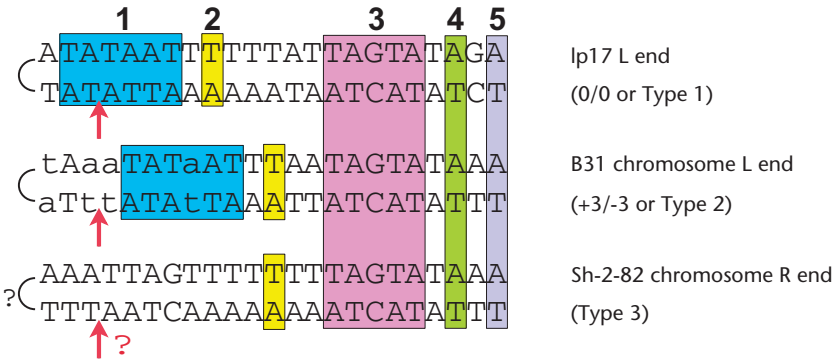

Fig. 3. Three types of hairpin telomeres in *B. burgdorferi*. Three telomere flavours have been found thus far in *B. burgdorferi* (Casjens *et al.*, 1997; Tourand *et al.*, 2003; Huang *et al.*, 2004b). A representative example of each telomere type is shown. The regions conserved among the sequenced telomeres are boxed and coloured 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 lower case letters.

telomere families (Tourand *et al*., 2003) denotes base pair insertions and deletions to the left of box 1 and between boxes 2 and 3 (Fig. 3). The five Type 1 telomeres do not have insertions or deletions (0/0), while the four Type 2 telomeres (+3/–3) have three extra base pairs to the left of box 1 and a three base pair deletion between boxes 2 and 3. Further study of these telomere types using synthetic oligonucleotides and *in vitro* telomere resolution reactions defined the left end of the Type 2 hairpin telomeres and showed that the Type 2 telomere is used with an initial rate of about 75% of the Type 1 telomere in the *in vitro* reaction (Tourand *et al*., 2003). Furthermore, these studies revealed that for both telomere types, DNA cleavage occurs at a fixed distance from the axis of symmetry in the replicated telomere (or from the box 3, 4, 5 region), rather than at a specific sequence within box 1.

More recently, a third type of telomere has been reported at the right end of the Sh-2-82 chromosome and the right end of lp21 (Fig. 3). Surprisingly, this telomere type (Type 3) lacks box 1 that is found in the Type 1 and 2 telomeres. The box 1 sequence, TATAAT, has also been found in a wide variety of diverse hairpin telomeres including those from *Escherichia coli* phage N15 (Rybchin and Svarchevsky, 1999), *Klebsiella* phage ϕKO2 (Huang *et al*., 2004a) and the mammalian African swine fever virus and Vaccinia virus (see Casjens *et al*., 1997). For this reason, it was inferred that this sequence is likely to favour the telomere resolution reaction. Sequencing of additional *Borrelia* telomeres and characterization of their biochemical properties, including interaction with ResT, will be required to elucidate the rules governing telomere structure and function.

The telomere resolvase ResT

Nomenclature

It seems appropriate to preface this section with some discussion of the varied nomenclature for the enzymes that promote telomere resolution. In 2000, before the purification of the first telomere resolving enzyme, there were already two different names for these enzymes in the literature. The putative N15 enzyme was first referred to as a 'telomerase' and subsequently as a 'protelomerase', signifying a prokaryotic telomerase (Rybchin and Svarchevsky, 1999). In contrast, the putative poxvirus enzyme was referred to as a telomere resolvase (Palaniyar *et al*., 1999; Sekiguchi *et al*., 2000). Not having a vested interest in either of these names, we have adopted the term telomere resolvase for the following reasons:

(i) The term telomere resolution has been universally accepted by all investigators and, thus, the term telomere resolvase accurately describes the reaction it performs.

(ii) Using the precedent of the resolvase enzymes that promote site-specific recombination to resolve cointegrate structures during the transposition process, 'telomere resolvase' is an accurate scientific name for an enzyme that resolves replicative intermediates into the final products containing hairpin telomeres.

(iii) Telomerases are a well-established family of enzymes involved in maintaining eukaryotic telomeres through a biochemical process totally unrelated to the telomere resolution process involved in the formation of hairpin telomeres. Thus, telomerase or protelomerase are confusing terms for the class of enzyme discussed here.

(iv) A proenzyme is an inactive enzyme precusrsor. One would therefore expect that a protelomerase is an inactive precursor of a telomerase, yet further confusing the use of such a term.

Adoption of a single, scientifically accurate name by all members of the various phage, bacterial and eukaryotic communities would certainly simplify matters and eliminate confusion; I therefore entreat my colleagues to help unify the field with a common nomenclature.

Identification of ResT

The first telomere resolvase to be putatively identified (Rybchin and Svarchevsky, 1999) and subsequently purified (Deneke *et al*., 2000) was TelN (Telomerase N15) from the *E. coli* phage N15. This work was a tremendous help for initiating studies in *Borrelia.* BLAST searches of this protein against the putative open reading frames from the *B. burgdorferi* genome revealed a predicted protein with very limited and localized amino acid homology to TelN, suggesting that it might be the *B. burgdorferi* enzyme (Rybchin and Svarchevsky, 1999). This turned out to be correct. Surprisingly, the reading frame, *BBB03*, now known as *resT*, is present on the circular plasmid cp26 rather than on a linear replicon. The encoded protein was subsequently purified and shown to be the telomere resolvase, ResT (Kobryn and Chaconas, 2002). This 449 amino acid protein efficiently promotes resolution of a replicated telomere or dimer junction (such as L′-L in Fig. 2) into two covalently closed hairpin telomeres. As expected, the *resT* gene is essential for *B. burgdorferi* (Byram *et al*., 2004).

Mechanism of action

The ResT protein appears to have a composite active site with components related to both cut-and-paste transposases (Bankhead and Chaconas, 2004), and to tyrosine recombinases and type IB topoisomerases (Kobryn and Chaconas, 2002; Deneke *et al*., 2004). The

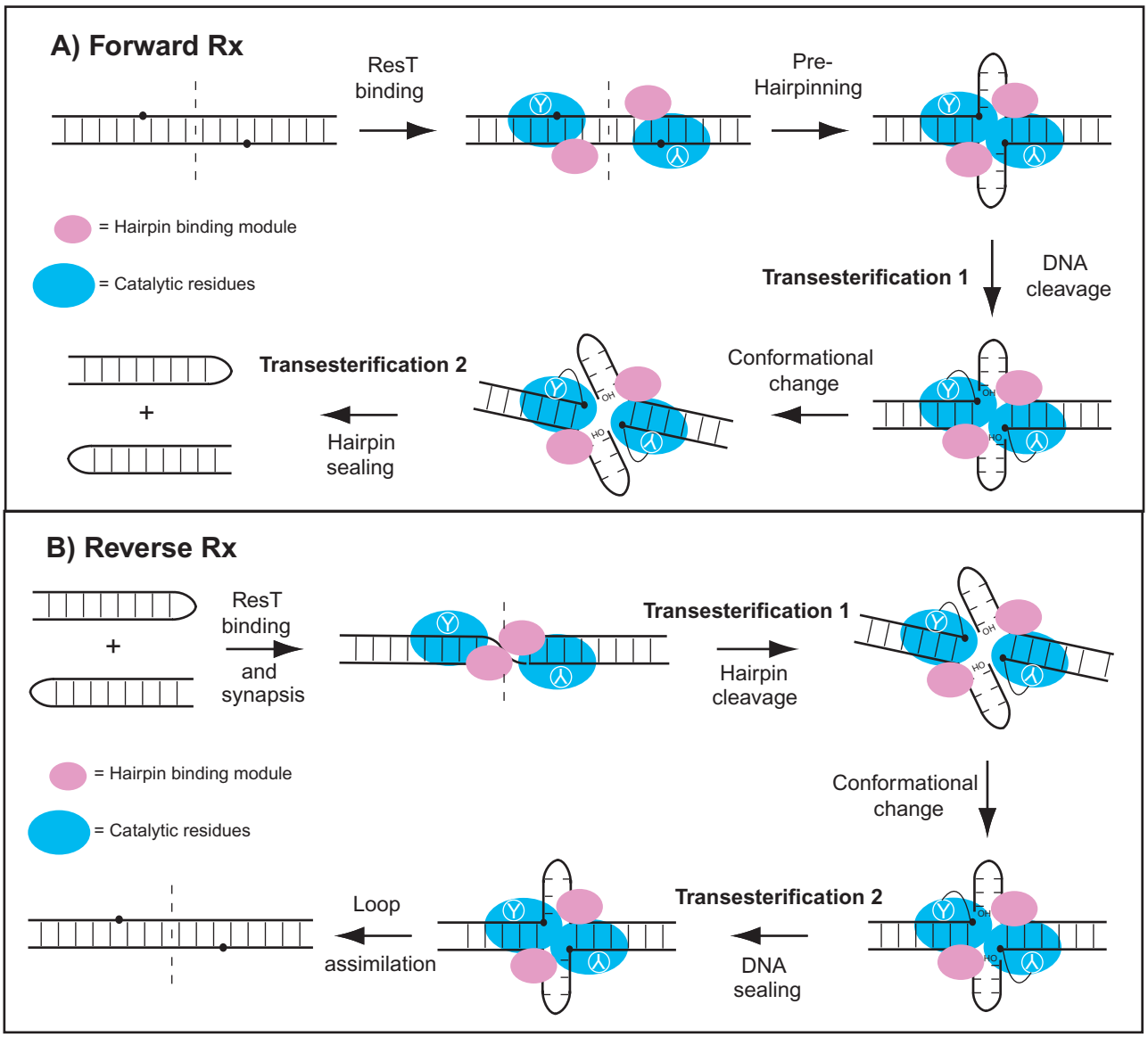

Fig. 4. Model of the composite active site mechanism of telomere resolution by ResT. The composite active site is represented by the large blue oval (catalytic residues with the active site tyrosine indicated in white) and the small pink oval (hairpin binding module). The axis of symmetry is indicated by the dashed line bisecting the replicated telomere substrate. Prehairpinning is predicted to result in the formation of two small non-base paired hairpin turnarounds.
A. The forward reaction (telomere resolution).
B. The reverse reaction (telomere fusion).
A has been adapted from the study by Bankhead and Chaconas (2004).

transposase-related part of the active site is a hairpin binding module that is believed to be involved in the distortion of DNA near the axis of symmetry to facilitate hairpin formation (Bankhead and Chaconas, 2004). The component with similarity to tyrosine recombinases or type IB topoisomerases includes a constellation of basic amino acids and an active site tyrosine nucleophile, which promote the chemical events through use of a covalent-3′phosphtyrosyl DNA-enzyme intermediate (Kobryn and Chaconas, 2002; Deneke *et al.*, 2004). The mixing of these active site components is a recipe for the unique biochemical activity of a telomere resolvase (Fig. 4A). The activity of the two active site components is choreographed such that DNA distortion, or what we refer to as prehairpin formation, must occur before DNA cleavage and subsequent completion of hairpin formation (Bankhead and Chaconas, 2004) and such that the cleavage and strand transfer reactions on the two sides of the

replicated telomere occur nearly simultaneously (Kobryn *et al*., 2005).

Reversal of ResT activity

Recent work (Kobryn and Chaconas, 2005) has shown that ResT can bind to the hairpin telomeres that comprise the normal telomere resolution end-products. Moreover, ResT can cleave and form covalent reaction intermediates with these hairpin end-products (Fig. 4B). Finally, ResT can fuse two hairpin telomeres on unrelated DNA molecules by running the telomere resolution reaction in reverse. This finding, which at first glance may appear to be esoteric and of interest only to a nucleic acid biochemist, likely provides the clue for the unusual genome plasticity found in *Borrelia* species. The remainder of this review will focus on the implications of ResT-mediated telomere fusion in genome plasticity and evolution of the *Borrelia* genomes. An interesting feature of the telomere fusion reaction mediated by ResT is that it is favoured at low temperatures (~8°C), leading us to previously predict (Kobryn and Chaconas, 2005) that ResT-mediated telomere fusions occur predominantly in the tick host [for details on the experiments demonstrating reversibility of telomere resolution by ResT and a discussion on control of reaction directionality, the reader is referred to the original report of this work (Kobryn and Chaconas, 2005)]. The *resT* gene does appear to have a 1.8-fold greater transcription at 23 versus 35°C (Ojaimi *et al*., 2003) consistent with proposed telomere fusions occurring predominantly at lower temperatures.

Reversal of ResT activity as the underlying force for genome plasticity in *Borrelia* species

The ability of ResT to fuse hairpin telomeres provides a hitherto unknown mechanism of genetic exchange in this or any bacterial genus, and one that may explain the origin of the vast number of genome duplications and telomere exchanges known to occur in the linear replicons of *Borrelia* species (Kobryn and Chaconas, 2005). As an example of how telomere fusions might promote genome plasticity, Fig. 5 shows how two successive rounds of stabilized telomere fusion can explain the recently described

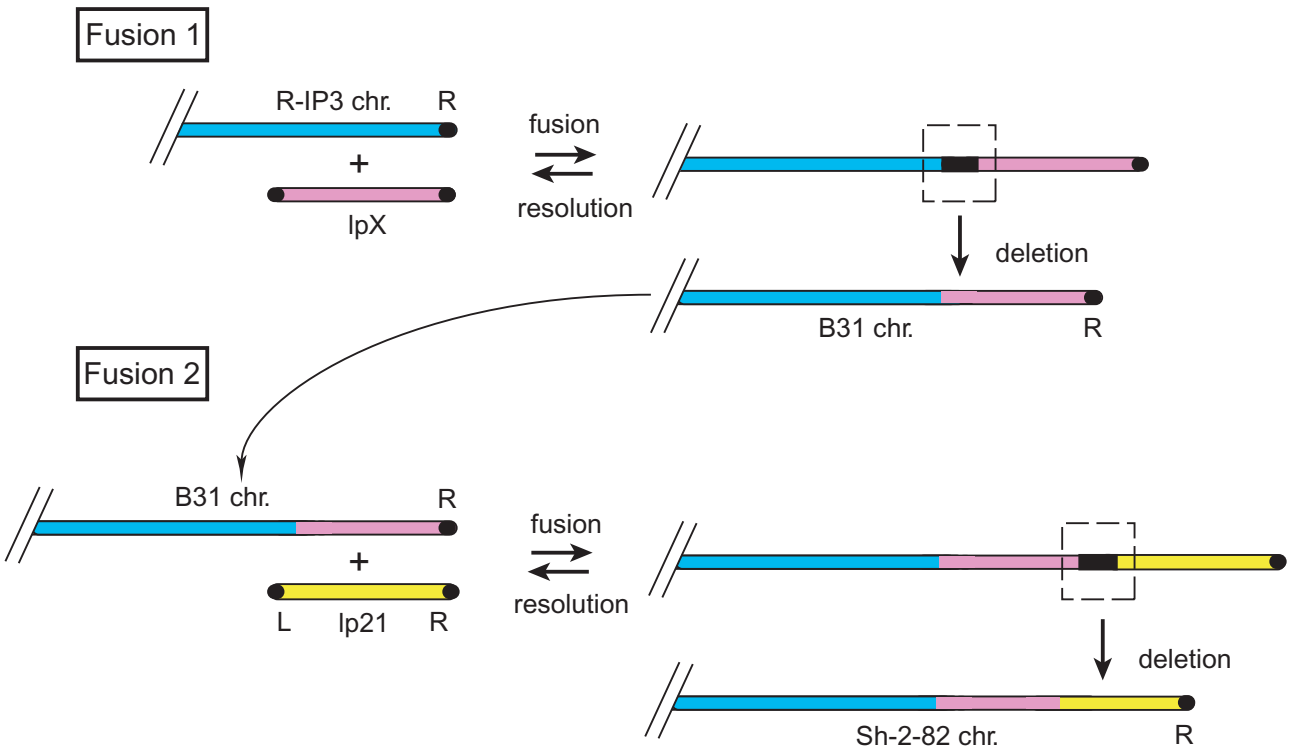

Fig. 5. Telomere exchange by ResT-mediated telomere fusion. Fusion 1 links an unknown linear plasmid (lpX) to the right end of the *B. burgdorferi* R-IP3 chromosome to generate the structure of the right-end telomere found in the B31 chromosome. The identity of lpX is not clearly discernible and the right end of the B31 chromosome shares homology with several linear plasmids (Casjens *et al*., 1997; 2000). Fusion 2 shows a telomere exchange that converts the right end of B31 to the right end observed for the Sh-2-82 chromosome through fusion with lp21 (see Casjens *et al*., 1997; Huang *et al*., 2004b). Successive rounds of telomere fusion with deletion formation can also explain the many examples of telomere exchange observed in the *B. burgdorferi* linear plasmids (Casjens *et al*., 2000). This figure has been adapted from the study by Kobryn and Chaconas (2005).

(Huang *et al*., 2004b) extensions of the right end of the R-IP3 chromosome to generate first the B31 chromosome and then the Sh-2-82 chromosome.

Each round of stabilized telomere fusion must have two steps: telomere fusion and stabilization. The fusion event joins two different linear replicons with similar telomeres by ResT-mediated reversal of the telomere resolution reaction. The product of this fusion is a new heterodimeric recombinant linear molecule carrying a replicated telomere at the joint. This replicated telomere would normally be resolved back into hairpin telomeres, thereby severing the link between the two replicons in the overwhelming majority of cases. However, an infrequent deletion or mutation involving the fused telomere would preclude telomere resolution and freeze the recombinant structure. A deletion removing the telomere resolution site might be specifically targeted to the fused telomere by incomplete joining in the reverse reaction, leaving a ResT molecule covalently linked at a nick in the telomere. Such covalent protein–DNA complexes are known to be foci for the formation of deletions and other chromosomal aberrations (Froelich-Ammon and Osheroff, 1995). Alternatively, a deletion could be derived from palindrome instability induced by passage of a replication fork through the inverted repeat of the fused telomere (Leach *et al*., 1997; Pinder *et al*., 1998). In some cases the presence of two origins and initiator proteins on a single plasmid may also destabilize the plasmid, resulting in deletion formation. Although the stabilizing events may be very infrequent, one needs very few such events over evolutionary time to have a large cumulative effect on progressive genome rearrangements.

The formation of stabilized telomere fusion events, as depicted in Fig. 5, can also explain the very large number of telomere exchanges observed in the linear *B. burgdorferi* plasmids. Multiple rounds of telomere fusion stabilized by deletions would produce the mosaic pattern of DNA duplications observed in these plasmids. Stabilized fusion of only one of several copies of a given plasmid in the cell would result in a partial plasmid duplication occurring for each such event, with a continuing dispersal and accumulation of DNA regions not prone to loss by deletion. The reversal of ResT activity also provides an alternative mechanism for the generation of linear plasmid dimers previously observed in *Borrelia* species (Marconi *et al*., 1996).

Although ResT from *B. burgdorferi* is the only telomere resolvase currently characterized from any *Borrelia* species, it is likely that ResT from other borreliae may display similar properties. The recent report of the genome sequence from *B. garinii*, a European agent of Lyme borreliosis, indicates that the linear plasmids in this species also display many genetic rearrangements (Glockner *et al*., 2004) and differences in gene order compared with *B. burgdorferi*. Once again, ResT-mediated telomere fusions may underlie these rearrangements and possibly played an underlying role in the evolution of all *Borrelia* species.

Predicted rules for telomere fusions

Fusion between two telomeres necessitates base pairing between the six nucleotide sticky ends that result from cleavage at each of the two hairpin telomeres (Kobryn and Chaconas, 2005). As noted earlier (see Fig. 3), *B. burgdorferi* has several related but distinct telomere types. Fusion does not occur between Type 1 and Type 2 telomeres (Kobryn and Chaconas, 2005) and probably does not occur between two Type 2 telomeres lacking a precise match in their sticky end sequence. Therefore, the difference in DNA sequence between telomere types is expected to establish rules defining which replicon can fuse with which. For example, the left end of lp17 (a Type 1 telomere) would be prohibited from fusing with the left end of the B31 chromosome (a Type 2 telomere). This limitation in fusion partners is consistent with the limited polarity of most, if not all of the recorded telomere exchanges. The requirement for a precise match in the sequences of the sticky ends at the two telomeres also provides an interesting possibility for limiting telomere fusions through the accumulation of mutations in the telomeres. We speculate that, at one point in time, all *Borrelia* telomeres may have been identical; over time mutations in the telomeres limiting the more deleterious stabilized telomere fusion events may have accumulated, giving rise to the different types of telomeres now known to exist.

Even with the sequence-imposed limitations on telomere fusion noted above, the accumulation of a small number of linear replicons in *Borrelia* species would be expected over time. However, this has not been observed, suggesting that a high degree of segmentation confers some selective advantage. What this advantage might be currently remains a mystery.

Linear and circular molecules

In addition to recombining unrelated linear DNA molecules through telomere fusion, ResT is also expected to circularize linear DNA molecules carrying two compatible telomeres. This begs the question of whether ResT has played a role in the generation of any of the circular molecules found in *Borrelia* species. For example, a 180 kb linear plasmid in *B. hermsii* was reported to exist as a circular form in the variant strain HS1 (Ferdows *et al*., 1996). A possible explanation for this conversion is that it occurred via a stabilized telomere fusion.

A recent report indicates that several *Borrelia* species (*B. parkeri*, *B. anserina and B. recurrentis*) harbour only

linear replicons (Schwan *et al*., 2005). In contrast, *B. burgdorferi* B31 carries 10 circular molecules (Casjens *et al*., 2000; Miller *et al*., 2000). Are these circles derived from stabilized telomere fusions of linear plasmids? Nine of these circles are cp32 plasmids or derivatives thereof. The cp32 family of plasmids appear to be circular prophage molecules (Eggers and Samuels, 1999; Eggers *et al*., 2001). The genes for three protein families encoded by these plasmids (PF-144, *bly* and *bdr*) have been detected in various linear plasmids in *B. parkeri*, *B. anserina and B. recurrentis* (Carlyon and Marconi, 1998; Carlyon *et al*., 2000a,b; Roberts *et al*., 2000; Stevenson *et al*., 2000; Schwan *et al*., 2005). Although not all three gene families are represented on the linear plasmids in all three species, it is clear that cp32 information can be found on linear plasmids, raising the possibility that the cp32 elements were generated by stabilized telomere fusion. However, linearization of a cp32 progenitor plasmid is an equally plausible explanation at this time for the lack of circles in some *Borrelia* species.

cp26, the one non-cp32 related circular plasmid in *B. burgdorferi* B31, is known to carry essential information (Byram *et al*., 2004), including the *resT* gene. *resT* is expected to be present in all *Borrelia* species because it is required for replication of the linear replicons and generation of the covalently closed hairpin ends. It must therefore be present on a linear DNA molecule in *B. parkeri, B anserina and B recurrentis.* It is tempting to speculate that a ResT-mediated telomere fusion was responsible for conversion of a linear *Borrelia* plasmid into a circular form, at some point in time, to generate cp26. Consistent with this proposal is the presence of a degenerate telomere at the upstream boundary of the *resT* gene on cp26, further suggesting that *resT* was adjacent to a telomere on a previous linear progenitor plasmid. The 5′ flanking sequence for the *B. burgdorgeri resT* gene (Fraser *et al*., 1997) is tataattgaattaatATG . . . where the underlined regions from left to right correspond to box 1 (Fig. 3), and to box 3 with a single mismatch at the central position. The upper case ATG is the *resT* start codon. The location of the *resT* gene directly adjacent to a hairpin telomere would also have interesting implications for its expression, because there would not be enough room for a promoter between the hairpin loop and the ATG start codon. However, replication of the hairpin telomere (L to L′L or R to RR′, Fig. 2) appears to reconstitute a promoter, at least *in silico* (data not shown), thereby providing a novel means of transcriptional regulation for a *resT* gene located directly adjacent to a hairpin telomere; transcription would occur when ResT is required to resolve the replicated telomere but not after telomere resolution has occurred. Studies on the linear plasmids carrying the *resT* gene in *B. parkeri*, *B. anserina and B. recurrentis* may help to confirm or negate these speculations.

Finally, why have more linear plasmids not been converted into circular form through stabilized telomere fusions? The answer to this question is not clear at present. Although both circular and linear plasmids appear to use the same paralogous families of replication and maintenance proteins (Stewart *et al*., 2003), differences that impede replication and/or maintenance in the alternative topological form appear to exist for at least cp9 (Chaconas *et al*., 2001) and lp17 (Beaurepaire and Chaconas, 2005), possibly prohibiting some such conversions.

Laying the groundwork for the formation of pseudogenes

One of the intriguing features of the *B. burgdorferi* strain B31 genome is the large number (167) of pseudogenes, 87% of which are located on the linear plasmids (Casjens *et al*., 2000). This is reflected by a high protein coding density (93%) for the chromosome compared with a very low density (53%) for the linear plasmids because of extensive DNA scrambling and the pseudogene load. Can ResT-mediated telomere fusions help explain the high abundance of pseudogenes on the linear *B. burgdorferi* plasmids? We believe that it can. Multiple rounds of telomere exchange can result in the accumulation of many gene duplications on the linear plasmids. Duplicated and therefore, non-essential gene copies can undergo continuous mutational decay without a selection process to maintain functionality.

But why do the pseudogenes accumulate in the *B. burgdorferi* plasmids rather than be sanitized from the genome? It has been proposed that bacterial species that are sheltered parasites (such as *B. burgdorferi*) have a higher propensity to accumulate pseudogenes; their lifestyles offer protection from horizontal gene transfer, including phage and transposons (see Lawrence *et al*., 2001). In the absence of genome invasion by such elements, it has been proposed that these bacteria have reduced deletion rates, allowing the accumulation of a high pseudogene load.

A cascade of recombinational promiscuity

It is noteworthy that the many DNA duplications resulting from stabilized telomere fusions are not expected to remain inert in their various and sundry locations. Host-mediated recombination between homologous regions on otherwise non-homologous plasmids would further serve to scramble the complement of linear plasmids through iterative rounds of recombination.

Unanswered questions

The work described above has added an important piece

of the puzzle to our understanding of genome plasticity in the segmented genome of *B. burgdorferi* and likely all *Borrelia* species. However, there are still a number of pivotal questions regarding the origins of these enigmatic, segmented genomes that remain unanswered. These include:

(i) *Why do borreliae carry a segmented genome composed of both linear and circular molecules?* A possible advantage of a linear DNA molecule in the gene conversions involved in the antigenic variation process (Zhang *et al.*, 1997; Barbour, 2002) may exist as a variety of such systems involving gene conversion events are located adjacent to telomeres (Barry *et al.*, 2003). An alternative hypothesis is that the presence of both circular and linear molecules provides possibilities for unique transcriptional control mechanisms based upon DNA topology. A segmented genome may also aid in the exchange of DNA (Qiu *et al.*, 2004) between different *Borrelia* strains and thereby provide a selective advantage. Finally, the least satisfying possibility is it is possible that a segmented genome of mixed toplological state provides no selective advantage but is a neutral change tolerated by the organism.

(ii) *What is the origin of* Borrelia *ResT?* Telomere resolvases have been identified in *Borrelia*, *Agrobacterium*, and phages from *Yersinia*, *Klebsiella* and *Escherichia.* There is little sequence identity between ResT from these diverse sources (Deneke *et al.*, 2004), thereby eliminating an easily recognizable source for the *Borrelia resT* gene from known othologues. However, the presence of a *resT* orthologue on several phages makes them possible ancestral sources of the *resT gene*. *resT* has not been identified on a transposon at this time; however, this would provide an alternative means of dissemination.

(iii) *What is the origin of the* Borrelia *telomeres?* One might expect that *resT* would have been initially associated with its cognate telomeric sequence to facilitate comigration, without which neither component of this enzyme-substrate pair would be of any use. The presence of a degenerate telomere immediately adjacent to the *resT* gene on the upstream side of cp26 is consistent with this idea; however, independent acquisition of both the *resT* gene and a replicated telomere substrate (L′L or RR′, see Fig. 2) cannot be ruled out.

(iv) *How did genome segmentation occur?* Once a *resT* gene was in place, genome segmentation may have occurred by continual introduction of replicated telomere sequences into a much larger DNA molecule(s). This may have occurred by transposition of a replicated telomere sequence to a variety of locations. Alternatively, telomeric sequences may have preexisted in the *Borrelia* genome or may have been generated by accumulated mutations in the A-T rich DNA. Finally, a host of independent circular plasmids may have arrived from another source and been converted to linear forms by one of the above scenarios.

A problem for the progressive segmentation theories is that segmentation would have required each new molecule to acquire a set of replication/maintenance functions in order for them to survive. The probability of both segmentation and concomitant acquisition of replication/maintenance functions would be extremely low.

Clearly there are a number of perplexing questions regarding the structure and function of *Borrelia* genomes that make the study of these important pathogens both fun and challenging. Hopefully the next few years will result in the addition of more pieces to this fascinating puzzle.

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