Evolution of the linear DNA replicons of the *Borrelia* spirochetes
Sherwood Casjens

Members of the spirochete genus *Borrelia* carry numerous linear DNA replicons with covalently closed hairpin telomeres. The genome of one member of this genus, *B. burgdorferi* B31, has now been completely characterized and contains a linear chromosome, twelve linear plasmids and nine circular extra-chromosomal elements. The phylogenetic position of the *Borrelia* spirochetes strongly suggests that a progenitor with circular replicons acquired the ability to replicate linear DNA molecules.

**Addresses**
Division of Molecular Biology and Genetics, Department of Oncological Sciences, University of Utah Medical School, Salt Lake City, UT 84321, USA; e-mail: sherwood.casjens@hci.utah.edu

**Current Opinion in Microbiology 1999, 2:529–534**
1369-5274/99/$ – see front matter © 1999 Elsevier Science Ltd. All rights reserved.

**Abbreviations**
ASBV African swine fever virus
ds double stranded

**Introduction**

All DNA polymerases require, in addition to the template strand, the 3′-end of a primer strand upon which to begin nucleotide polymerization (i.e. they cannot initiate de novo polymerization). Several decades ago, this fact was used to predict that even within living cells these enzymes alone would be insufficient to fully replicate ‘normal’ linear double stranded (ds) DNA molecules to the very ends [1–3]. Subsequent studies of the molecular genetics of many organisms has since shown that all ends (telomeres) of linear dsDNA molecules are indeed replicated by special mechanisms. Different organisms have independently solved the problem of replicating telomeres in several ways (Figure 1): first, by not having ends (i.e. circular DNA is common in bacteria; summarized in [4]); second, by priming synthesis at the extreme 5′-terminus with a special protein (i.e. in some viruses and bacteria [5,6**]); third, by having special mechanisms to elongate the number of terminal repeats after normal replication has failed to duplicate the extreme terminus (i.e. telomerase or other end extension mechanisms in eukaryotes [7,8]); or fourth, by taking the form of a closed hairpin DNA molecule (e.g. in some viruses and bacteria [9–11]). The large majority of studied bacteria carry only circular replicons, and no telomerase or other end-extension mechanism is known in bacteria; however, several taxa of bacteria utilize terminal proteins or hairpin ends to maintain linear DNA molecules. Among bacteria, members of the actinomycete family often carry linear DNAs whose terminal replication is thought to have been protein-primed [6**,12,13**], and members of the spirochete genus *Borrelia* carry DNAs with covalently closed hairpin ends [11,14,15]. In this review, I focus on the *Borrelia* replicons with hairpin telomeres.

**Replication of covalently closed hairpin telomeres**

Covalently closed hairpin telomeres are not commonly found in nature. They are known on some fungal plasmid DNAs as well as the poxvirus and parvovirus chromosomes, and they are common in the bacterial genus *Borrelia*. All *Borrelia* isolates examined to date have a linear chromosome (~900 kbp in length) and carry multiple linear plasmids; *B. burgdorferi* B31, the only isolate to have been studied exhaustively, carries a linear chromosome, 12 linear plasmids and nine circular plasmids [16,17*]. All of the *Borrelia* telomeres that have been examined have covalently closed hairpin ends [11,14,15]. Such replicons are very rare elsewhere in bacteria and only two other examples are known, both in Proteobacteria: the 50 kbp N15 prophage plasmid present in an *Escherichia coli* isolate [18], and a 45 kbp plasmid in a *Klebsiella oxytoca* isolate ([19]; E Gilcrease, S Casjens, unpublished data).

The replication of molecules with hairpin telomeres solves the replication problem described above, but presents a new dilemma: the two parental DNA strands, which are destined to end up in different daughter cells, are covalently joined at the telomeres. Figure 2 diagrams the major strategies by which such molecules might replicate. All strategies must utilize strand breakage to allow segregation of the two parental strands (only in the unlikely conservative replication branch of strategies depicted in Figure 2a,b are the ends thus created not rejoined to new partners). In Figure 2a,b, strand nicking and reassortment during end resolution follows duplication, whereas in Figure 2d, strand nicking precedes duplication, and in Figure 2c strand nicking is required both during the circularization and end-resolution steps. Possible variations on these themes include rolling circle instead of circle-to-circle replication in Figure 2e, and, if there is no lagging strand synthesis or initiation at only one end, the mechanism (Figure 2d) would be similar to a rolling hairpin mechanism [20]. Indeed, one can also imagine the scheme in Figure 2a or Figure 2b having no lagging strand synthesis, since it is possible to reach the dimer circle intermediate without it.

It seems most likely that the *Borrelia* chromosome initiates replication near the center (the Figure 2a scheme), because 66% of the genes are transcribed away from the center and there is a striking break in the GC skew (G–C/G+C) curve near the middle of the chromosome [17*]. Both of these observations are thought to be moderately reliable indicators of the location of origin of replication in bacterial chromosomes [21]. In addition, the complete genome sequence shows that *Borrelia* contains a full (if minimal) set of typical bacterial DNA replication functions, including the initiator protein DnaA [17*], suggesting that it initiates and assembles replication forks in a ‘standard’ way. Therefore, *Borrelia* is expected to utilize both leading and lagging strand synthesis.
Three ways to replicate linear DNA molecules. In each case, the upper strand 5′-end is on the left, and newly synthesized DNA is indicated by the gray arrows. (a) Linear molecules with free terminal 3′-overhangs, which most eukaryotic termini are thought to have, require extension of one end after (or before) complementary strand synthesis. Asterisks indicate regions not replicated by DNA polymerase. (b) Proteins (indicated by ovals) prime DNA polymerase action at the extreme 3′-end of the template strand. (c) A separate enzymatic mechanism exchanges strand connectivity to form the hairpin telomeres after (or before, see text and Figure 2) DNA polymerase synthesizes a complete complementary strand. Many variations in detail are possible with each of these strategies; for example, lagging strand synthesis need not occur to obtain complete replication in (b) and (c), and the nature of the terminal overhang will affect how terminal extension mechanisms might function.

The Figure 2d mechanism (with initiation at only one end) is currently favored for poxviruses [22], and similar rolling hairpin replication is fairly well understood in the paroviruses [20], but essentially nothing is known about the biochemistry of replication of the bacterial covalently closed hairpin replicons. If initiation and fork movement are similar to those processes in other bacteria, then one might expect an end-resolution mechanism is all that differentiates the Borrelia from the other bacteria. End resolution has been shown to be necessary in the poxvirus and parovirus systems, but is not well-understood in either system. In the bacterial replicons of this type, resolution could be mediated by specific proteins (e.g. transposases and integrases can create hairpin ends in some situations [23,24]), or it could be mediated by a replication fork initiated at the termini that would meet the ‘normal’ outwardly progressing fork (combination of mechanisms ‘a’ and ‘d’ in Figure 2) (a general topological strategy that appears to be used with terminal protein priming in a Streptomyces plasmid [6]). No clear-cut candidates for genes that might encode an end-resolution apparatus have emerged from the B. burgdorferi complete genome sequence, and more study will be required to understand even the overall strategy of replication of these molecules.

**Acquisition of hairpin telomeres?**

A common ~25 bp sequence that lies at the ends of all the Borrelia telomeres has been examined and in each case it has the same orientation relative to the DNA end (Figure 3) [11,15,17,25,26]. A different but similar sized terminal inverted repeat has been determined for the bacteriophage N15 plasmid [27]. Perhaps these common terminal sequences represent sites at which the hypothetical end-resolution apparatus functions?

The fact that this type of telomere is universally present in the genus Borrelia and is extremely rare in all other bacterial phyla (including other spirochetes; summarized in [4]), strongly suggests that a Borrelia ancestor’s circular chromosome became linear. How might this have happened? Hinnebusch and Barbour [28] noticed that the iridopoxivirus African swine fever virus (ASFV) has hairpin telomeres [29] that are somewhat similar in sequence to those of Borrelia (Figure 3). Thus, if a linear ASFV-like chromosome had integrated into the Borrelia progenitor’s circular chromosome and brought with it an end-resolution apparatus, it might have generated a linear molecule that could replicate in bacteria (it is interesting to note that the poxviruses, like the Borrelia, tend to have high A+T DNA, and that these viruses have few if any introns, so it is not too unlikely that virus genes might be expressed in such a situation). This scenario is made more plausible by the fact that both Borrelia and ASFV are transmitted by arthropods. In fact the African tick Ornithodoros moubata is known to transmit both B. duttoni and ASFV, so it is reasonable that members of these two very distant biological phyla could have come into genetic contact. To date no convincing overall similarities have been found between Borrelia and poxvirus DNA metabolism genes (or other genes), so if the above scenario were true, most of the poxvirus genome has subsequently been lost in Borrelia. It is of course possible that such short telomeric sequence similarities could have become similar through convergent evolution, but the above possibility remains a very intriguing hypothesis.

**Are the Borrelia plasmids ‘mini-chromosomes’?**

Are Borrelia’s multiple extra-chromosomal DNA elements in the plasmids or chromosomes? This may seem a purely semantic issue, but the word ‘plasmid’ conjures an image of
small non-essential DNAs present only in a small subset of individuals from a species, and ‘chromosomes’ are expected to be large, essential and present in all species members — but are these definitions sufficient to describe the *Borrelia* situation?

It was originally thought, largely by analogy to the most well understood bacteria *E. coli* and *Bacillus subtilis*, that all bacteria would carry a single chromosome, but recently a number of bacteria have been found to carry what appear to be essential genes on several DNA molecules and so can be thought of as having more than one chromosome (summarized in [4]). Only a small number of genes can be recognized as potentially essential on the *Borrelia* plasmids (a few genes for nucleotide metabolism and small molecule transport) [16,17*], and nearly all of the plasmids can be lost in culture without affecting growth there (e.g. [25,30–36]), indicating that they are not essential for cellular ‘housekeeping’ functions. A number of these elements, however, are universally (or nearly so) present in natural isolates. For example, a 54 kbp linear plasmid and a 27 kbp circular plasmid are present in essentially all of Lyme disease *Borrelia* isolates [37–42] (hundreds have been tested), and, in the few cases where it has been examined, the gene orders of cognate replicons are similar in different isolates [42,43]. Among the other less well studied *Borrelia* plasmids, at least four (and perhaps as many as 12) are present in all or nearly all of the isolates examined (N Palmer, S Casjens, unpublished data). These plasmids are often rapidly lost in culture, so these are minimum estimates of their universality. In addition, plasmid loss nearly always correlates with loss of infectivity in mice [34,35]. It seems clear that many of these extra-chromosomal elements are actually essential in nature, since all members of the *Borrelia* genus are obligate parasites in nature (although they can be propagated in complex medium in the laboratory), being found only within their arthropod and vertebrate hosts. Thus it does not seem unreasonable to think of these elements as mini-chromosomes [44].
Why should the *Borrelia* species carry so many different DNA molecules? Current theories for why bacteria carry genes on plasmids usually call upon the potentially increased genetic mobility of such replicons. In *Borrelia* there is some evidence for horizontal transfer of plasmids among individuals in the genus; however, the general finding is that although such transfer probably does occur, it does not appear to be extremely frequent [45–51]. Clearly a greater understanding of their biology will be required to develop convincing hypotheses for why the *Borrelia* species have opted to carry so many separate replicons.

**Why be linear?**

One can also wonder why the *Borrelia* species utilize linear DNA molecules. Are they linear simply because an ancestor stumbled upon this replication strategy and it happened to work, or is some specific advantage gained by being linear? We of course do not know the answer, but there is an abundance of evidence that *Borrelia* telomeres are not all linear.
evidence that free ends of linear DNAs are recombinogenic in many biological systems (e.g. see [52]). In addition, the protein-capped ends of the Streptomyces linear DNAs are apparently recombinogenic [53•,54•,55] as are the hairpin ends of poxviruses [56], so it is not true that only free, open DNA ends have this property. It has also been noted that sub-telomeric regions of eukaryotic chromosomes may also be more variable than the remainder of the genomes [57]. Could increased telomeric region plasticity be advantageous in Borrelia? It is interesting in this context to note that in at least the Lyme disease and relapsing fever Borrelia, a linear plasmid (lp28-1 in B. burgdorferi B31) carries a surface protein diversity generation system in which genetic information from a number of silent genes is moved, probably by homologous recombination, into an expression site that is adjacent to a telomere [25,26,58]. It seems possible that these bacteria use the recombinogenic property of the ends of linear DNA molecules to augment this process.

**Rapid Borrelia evolution?**

Most, but not all, of the linear *Borrelia* plasmids may be in the midst of a rapid evolutionary spurt, in that they contain evidence of numerous recent rearrangements that have left many genes in a state of serious mutational decay [17•]. On the 10 affected linear plasmids, more than half of the recognizable gene-like entities are likely to be nonfunctional. There are numerous cases of recent duplicative rearrangements, inversions, deletions, insertions and point mutations destroying reading frames. It is not known if this is an ongoing phenomenon, but it does appear to be largely (but not entirely) restricted to the linear plasmids and a small region adjacent to one chromosomal telomere. In several locations it can be deduced that the DNA rearrangement must have happened by a non-homologous mechanism [17•]. Perhaps the simplest explanation at present is that these linear plasmids are undergoing a period of rapid evolution that is manifested in numerous, apparently random rearrangements, which damage and duplicate genes, some of which are then no longer under selection and are allowed to decay. The ameliorative processes that presumably will eventually remove any unneeded DNA have not yet completed this ‘task’. Could this supposed rapid evolution be related to Lyme disease’s recent emergence as a widespread human disease? It seems more likely that other ecological factors have driven its rise [59], but this genetic volatility cannot be ruled out as a contributing factor.

**Prospects**

The complete genome sequence of a Borrelia isolate [16,17•] has moved the study of the biology and the evolution of this genus a very large step forward, but it has also created as many questions as it has answered. Some of these questions were discussed above. The deeply branched position of this genus in the ‘phylogenetic tree of life’ will no doubt make it fertile ground for the discovery of even more examples of the striking molecular diversity within the bacterial kingdom.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


534 Genomics


24. Kennedy AK, GuhaThakurta A, Kreek N, Hanfodd DD: Tn10 transposition via a DNA hairpin intermediate. Cell 1998, 95:125-134. This paper shows that Tn10 transposition occurs through intermediates with closed hairpin ends. This observation points out that such ends are likely to be less unusual than currently thought, and that known mechanisms can generate closed hairpin ends.


This report demonstrates genetic exchange between chromosomal telomeric regions in Streptomyces, and argues that this might be an important evolutionary mechanism that generates a high level of intraspecific polymorphism.


This report demonstrates exchange of telomeric regions between Streptomyces linear plasmids and chromosome.


