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

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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.

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Abbreviations

ASFV	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.

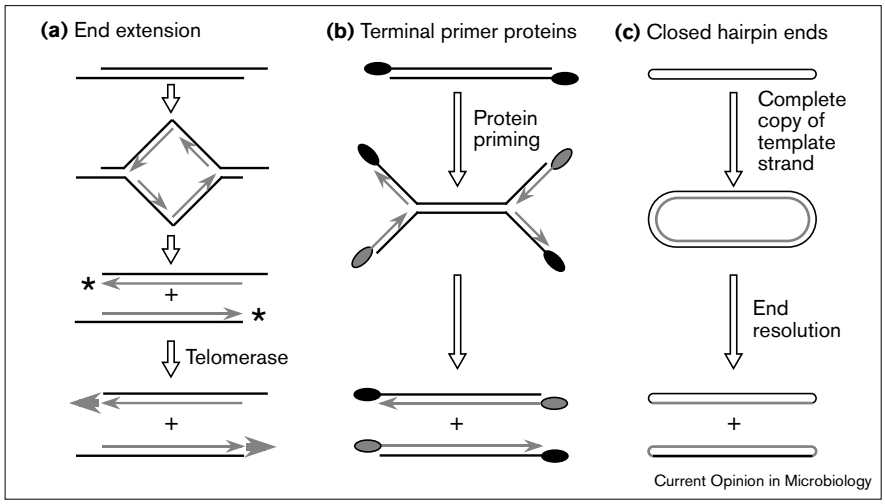
Replication of covalently closed hairpin telomeres

Covalently closed hairpin telomeres are not commonly found in nature. They are known on some fungal plastid 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 2c, 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.

Figure 1



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 parvoviruses [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 *Borrelias* from the other bacteria. End resolution has been shown to be necessary in the poxvirus and parvovirus 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?

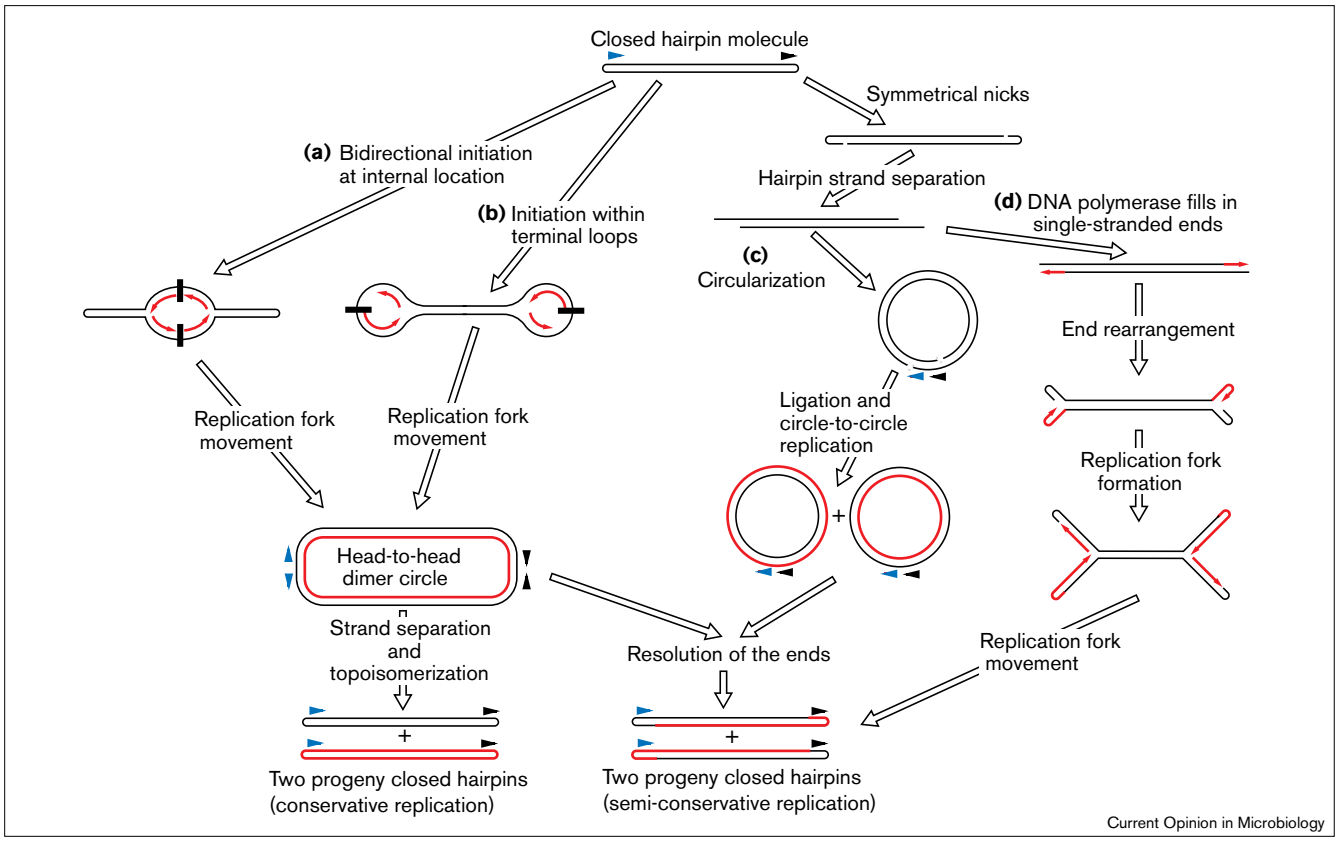
A common ~25 bp sequence that lies at the ends of all the *Borrelia* telomeres has been examined and in each case it has the same orientation relative to the DNA end (Figure 3) [11,15,17•,25,26]. A different but similar sized terminal inverted repeat has been determined for the bacteriophage N15 plasmid [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 iridopoxvirus 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*s, 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

Figure 2

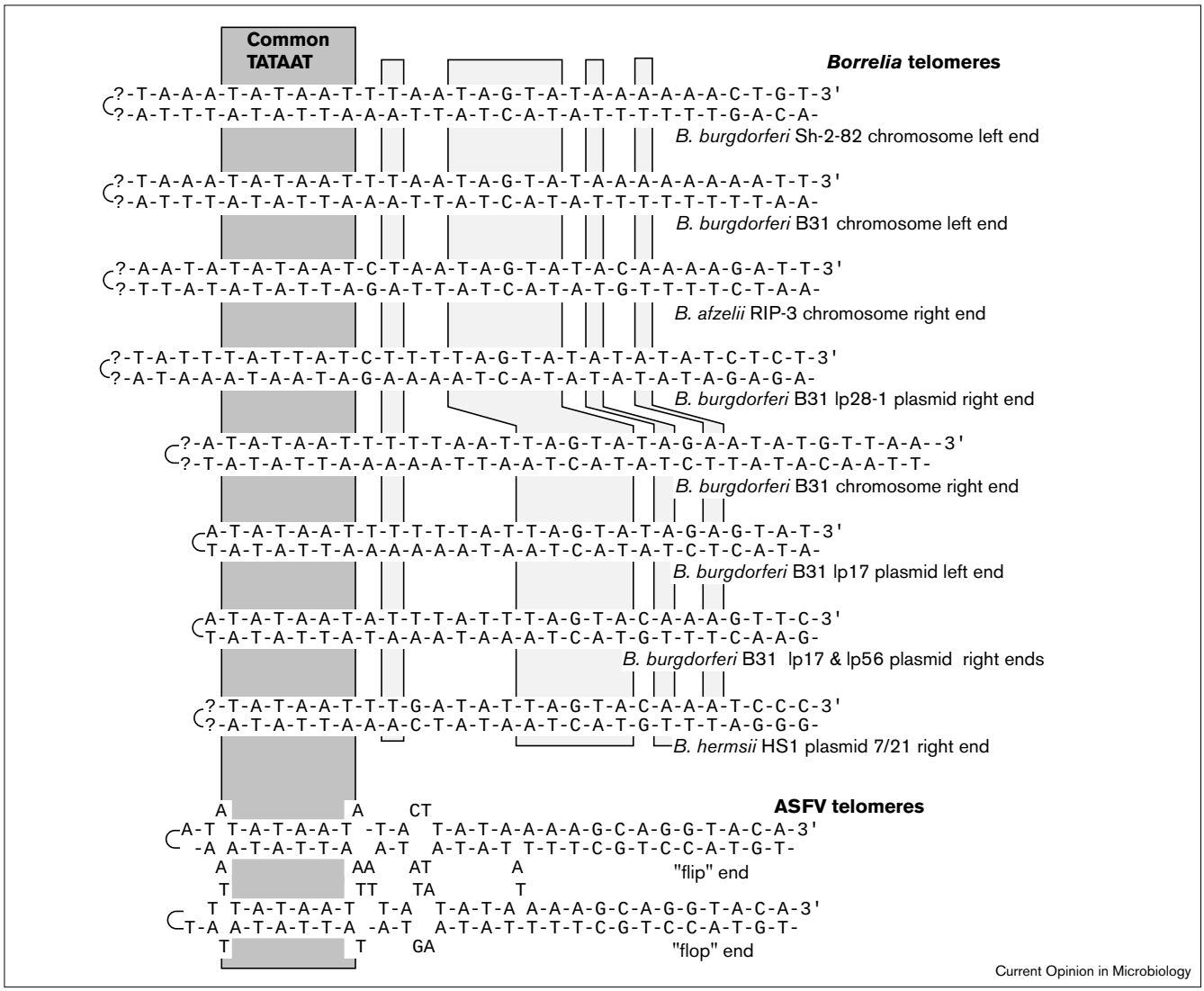


Four strategies to replicate linear DNAs with covalently closed hairpin telomeres. In scheme **(a)**, bidirection initiation of replication occurs near the center of the chromosome, whereas in scheme **(b)**, initiation occurs within the terminal loops. In both (a) and (b), replication is followed by strand nicking and reassortment during end resolution (semi-conservative replication) or strand separation and topoisomerization (conservative replication). In scheme **(c)**, strand nicking occurs both during circulization and after replication. In scheme **(d)**, strand nicking precedes duplication. Red lines indicate newly synthesized strands; black bars indicate replication origins on an unbroken template strand; the black and blue arrowheads indicate different sequences near the two telomeres to indicate the head-to-head and tail-to-tail joints in the possible dimer circle intermediate and the head-to-tail joints in the possible monomer circle intermediate. These strategies need not be mutually exclusive; for example, strategies (a) and (d) could combine to replicate the bulk and ends of the DNA, respectively.

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].

Figure 3



Borrelia and African swine fever virus (ASFV) telomere nucleotide sequences. The nine known *Borrelia* and two ASFV terminal sequences are aligned with their telomeres on the left (other alignments are possible that are nearly as good). Question marks in the terminal loops indicate that one or a few nucleotides may be missing from the sequence at that location. The dark gray box highlights a common sequence present in all of these telomeric regions, and the light gray boxes highlight additional sequence present in all of the *Borrelia* telomere sequences. The ASFV upper strand ATA immediately to the left of the dark box is the same as the *B. afzelii* right end, and five of the ASFV nucleotides immediately to the right are each the same as the nucleotide in the cognate position in at least one of the *Borrelia* ends. Note the unpaired nucleotides present in the ASFV telomeres; these are thought to arise by strand switching during its end resolution reaction. The 'flip' and 'flop' types of the two identical ends of ASFV are complements of one another that switch with each round of replication ([29] and references therein). There is no evidence for unpaired nucleotides in the *Borrelia* telomeres. Curiously, although it is expected that steric constraints would keep the leftmost 2–3 bp (hairpin loops) in the figure from physically forming, the three *Borrelia* ends whose sequences are known through the hairpin loops are nonetheless perfect palindromes.

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*s have opted to carry so many separate replicons.

Why be linear?

One can also wonder why the *Borrelia*s 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 free ends of linear DNAs are recombinogenic in many biological systems (e.g. see [52]). In addition, the protein-capped ends of the Streptomycete 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*s, 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

1. Watson J: **Origin of concatemeric T7 DNA.** *Nature New Biol* 1972, **239**:197-201.

2. Olovnikov A: **A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon.** *J Theoret Biol* 1973, **41**:181-190.

3. Lingner J, Cooper JP, Cech TR: **Telomerase and DNA end replication: no longer a lagging strand problem?** *Science* 1995, **269**:1533-1534.

4. Casjens S: **The diverse and dynamic structure of bacterial genomes.** *Annu Rev Genetics* 1998, **32**:339-377.

5. Salas M: **Protein-priming of DNA replication.** *Annu Rev Biochem* 1991, **60**:39-71.

6. •• Qin Z, Cohen SN: **Replication at the telomeres of the *Streptomyces* linear plasmid pSLA2.** *Mol Microbiol* 1998, **28**:893-903.
This paper provides evidence that the protein-bound ends of *Streptomyces* plasmid pSLA2 are generated by protein-priming.

7. Collins K: **Structure and function of telomerase.** *Curr Opin Cell Biol* 1996, **8**:374-380.

8. Biessmann H, Walter MF, Mason JM: ***Drosophila* telomere elongation.** *Ciba Found Symp* 1997, **211**:53-67.

9. Cavalier-Smith T: **Palindromic base sequences and replication of eukaryotic chromosome ends.** *Nature* 1974, **250**:467-470.

10. Bateman A: **Simplification of palindromic telomere theory.** *Nature* 1975, **253**:379.

11. Casjens S, Murphy M, DeLange M, Sampson L, van Vugt R, Huang WM: **Telomeres of the linear chromosomes of Lyme disease spirochaetes: nucleotide sequence and possible exchange with linear plasmid telomeres.** *Mol Microbiol* 1997, **26**:581-596.

12. Chen CW: **Complications and implications of linear bacterial chromosomes.** *Trends Genet* 1996, **12**:192-196.

13. • Huang CH, Lin YS, Yang YL, Huang SW, Chen CW: **The telomeres of *Streptomyces* chromosomes contain conserved palindromic sequences with potential to form complex secondary structures.** *Mol Microbiol* 1998, **28**:905-916.
This paper describes potential similarities between the replication of hairpin ends of parvovirus chromosomes and the protein-bound ends of *Streptomyces* plasmids.

14. Barbour AG, Garon CF: **Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends.** *Science* 1987, **237**:409-411.

15. Hinnebusch J, Bergstrom S, Barbour AG: **Cloning and sequence analysis of linear plasmid telomeres of the bacterium *Borrelia burgdorferi.*** *Mol Microbiol* 1990, **4**:811-820.

16. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK: **Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi.*** *Nature* 1997, **390**:580-586.

17. • Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson R: **A genome in transition: the twelve linear and nine circular extrachromosomal DNAs of an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi* contain many pseudogenes.** *Mol Microbiol* 1999: in press.
This paper analyzes the complete sequence of all twelve linear and nine circular plasmids present in *B. burgdorferi* isolate B31. A surprisingly large number of historical, apparently random rearrangements among the plasmids can be deduced from the sequences.

18. Svarchevsky AN, Rybchin VN: [Title translation: **Physical mapping of plasmid N15 DNA.**] *Mol Genet Microbiol Virusol* 1984, **N10**:16-22.

19. Stoppel RD, Meyer M, Schlegel HG: **The nickel resistance determinant cloned from the enterobacterium *Klebsiella oxytoca*: conjugational transfer, expression, regulation and DNA homologies to various nickel-resistant bacteria.** *Biometals* 1995, **8**:70-79.

20. Cotmore S, Tattersall P: **Parvovirus DNA replication.** In *DNA Replication in Eukaryotic Cells.* Edited by M DePamphilis. Cold

Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1996:799-813.

21. McLean MJ, Wolfe KH, Devine KM: **Base composition skews, replication orientation, and gene orientation in 12 prokaryote genomes.** *J Mol Evol* 1998, **47**:691-696.

22. Traktman P: **Poxvirus DNA replication.** In *DNA Replication in Eukaryotic Cells.* Edited by M DePamphilis. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1996:775-798.

23. Richet E, Abcarian P, Nash HA: **Synapsis of attachment sites during lambda integrative recombination involves capture of a naked DNA by a protein-DNA complex.** *Cell* 1988, **52**:9-17.

24. • Kennedy AK, Guhathakurta A, Kleckner N, Haniford DB: **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.

25. Kitten T, Barbour AG: **Juxtaposition of expressed variable antigen genes with a conserved telomere in the bacterium *Borrelia hermsii*.** *Proc Natl Acad Sci USA* 1990, **87**:6077-6081.

26. Zhang JR, Hardham JM, Barbour AG, Norris SJ: **Antigenic variation in Lyme disease *borreliae* by promiscuous recombination of VMP-like sequence cassettes.** *Cell* 1997, **89**:275-285.

27. Malinin A, Vostrov A, Rybchin V, Sverchevsky A: [Title translation: **Structure of the linear plasmid N15 ends.**] *Mol Genet Microbiol Virusol* 1992, **N5-6**:19-22.

28. Hinnebusch J, Barbour AG: **Linear plasmids of *Borrelia burgdorferi* have a telomeric structure and sequence similar to those of a eukaryotic virus.** *J Bacteriol* 1991, **173**:7233-7239.

29. Meireles M, Costa JV: **Nucleotide sequence of the telomeric region of the African swine fever virus genome.** *Virology* 1994, **203**:193-196.

30. Barbour AG: **Plasmid analysis of *Borrelia burgdorferi*, the Lyme disease agent.** *J Clin Microbiol* 1988, **26**:475-478.

31. Hyde FW, Johnson RC: **Characterization of a circular plasmid from *Borrelia burgdorferi*, etiologic agent of Lyme disease.** *J Clin Microbiol* 1988, **26**:2203-2205.

32. Schwan TG, Burgdorfer W, Garon CF: **Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation.** *Infect Immun* 1988, **56**:1831-1836.

33. Norris SJ, Carter CJ, Howell JK, Barbour AG: **Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein.** *Infect Immun* 1992, **60**:4662-4672.

34. Sadziene A, Barbour AG, Rosa PA, Thomas DD: **An OspB mutant of *Borrelia burgdorferi* has reduced invasiveness *in vitro* and reduced infectivity *in vivo*.** *Infect Immun* 1993, **61**:3590-3596.

35. Persing DH, Mathiesen D, Podzorski D, Barthold SW: **Genetic stability of *Borrelia burgdorferi* recovered from chronically infected immunocompetent mice.** *Infect Immun* 1994, **62**:3521-3527.

36. Xu Y, Kodner C, Coleman L, Johnson RC: **Correlation of plasmids with infectivity of *Borrelia burgdorferi* sensu stricto type strain B31.** *Infect Immun* 1996, **64**:3870-3876.

37. Samuels DS, Marconi RT, Garon CF: **Variation in the size of the *ospA*-containing linear plasmid, but not the linear chromosome, among the three *Borrelia* species associated with Lyme disease.** *J Gen Microbiol* 1993, **139**:2445-2449.

38. Casjens S, Delange M, Ley HL III, Rosa P, Huang WM: **Linear chromosomes of Lyme disease agent spirochetes: genetic diversity and conservation of gene order.** *J Bacteriol* 1995, **177**:2769-2780.

39. Anderson JF, Flavell RA, Magnarelli LA, Barthold SW, Kantor FS, Wallich R, Persing DH, Mathiesen D, Fikrig E: **Novel *Borrelia burgdorferi* isolates from *Ixodes scapularis* and *Ixodes dentatus* ticks feeding on humans.** *J Clin Microbiol* 1996, **34**:524-529.

40. Guttman DS, Wang PW, Wang IN, Bosler EM, Luft BJ, Dykhuizen DE: **Multiple infections of *Ixodes scapularis* ticks by *Borrelia burgdorferi* as revealed by single-strand conformation polymorphism analysis.** *J Clin Microbiol* 1996, **34**:652-656.

41. Mathiesen DA, Oliver JH Jr, Kolbert CP, Tullson ED, Johnson BJ, Campbell GL, Mitchell PD, Reed KD, Telford SR III, Anderson JF: **Genetic heterogeneity of *Borrelia burgdorferi* in the United States.** *J Infect Dis* 1997, **175**:98-107.

42. Tilly K, Casjens S, Stevenson B, Bono JL, Samuels DS, Hogan D, Rosa P: **The *Borrelia burgdorferi* circular plasmid cp26: conservation of plasmid structure and targeted inactivation of the *ospC* gene.** *Mol Microbiol* 1997, **25**:361-373.

43. Marconi RT, Casjens S, Munderloh UG, Samuels DS: **Analysis of linear plasmid dimers in *Borrelia burgdorferi* sensu lato isolates: implications concerning the potential mechanism of linear plasmid replication.** *J Bacteriol* 1996, **178**:3357-3361.

44. Barbour AG: **Linear DNA of *Borrelia* species and antigenic variation.** *Trends Microbiol* 1993, **1**:236-239.

45. Dykhuizen DE, Polin DS, Dunn JJ, Wilske B, Preac-Mursic V, Dattwyler RJ, Luft BJ: ***Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development.** *Proc Natl Acad Sci USA* 1993, **90**:10163-10167.

46. Marconi RT, Samuels DS, Landry RK, Garon CF: **Analysis of the distribution and molecular heterogeneity of the *ospD* gene among the Lyme disease spirochetes: evidence for lateral gene exchange.** *J Bacteriol* 1994, **176**:4572-4582.

47. Jauris-Heipke S, Liegl G, Preac-Mursic V, Rossler D, Schwab E, Soutschek E, Will G, Wilske B: **Molecular analysis of genes encoding outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato: relationship to *ospA* genotype and evidence of lateral gene exchange of *ospC*.** *J Clin Microbiol* 1995, **33**:1860-1866.

48. Livey I, Gibbs CP, Schuster R, Dorner F: **Evidence for lateral transfer and recombination in OspC variation in Lyme disease *Borrelia*.** *Mol Microbiol* 1995, **18**:257-269.

49. Will G, Jauris-Heipke S, Schwab E, Busch U, Rossler D, Soutschek E, Wilske B, Preac-Mursic V: **Sequence analysis of *ospA* genes shows homogeneity within *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* strains but reveals major subgroups within the *Borrelia garinii* species.** *Med Microbiol Immunol* 1995, **184**:73-80.

50. Qiu WG, Bosler EM, Campbell JR, Ugine GD, Wang IN, Luft BJ, Dykhuizen DE: **A population genetic study of *Borrelia burgdorferi* sensu stricto from eastern Long Island, New York, suggested frequency-dependent selection, gene flow and host adaptation.** *Hereditas* 1997, **127**:203-216.

51. Wang I, Dykhuizen D, Qiu W, Dunn J, Bosler E, Luft B: **Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto.** *Genetics* 1999, **151**:15-30.

52. Lloyd R, Low KB: **Homologous recombination.** In *Escherichia coli and Salmonella Cellular and Molecular Biology,* edn 3. Edited by F Neidhardt. Washington DC: ASM Press; 1996:2236-2255.

53. • Fischer G, Wenner T, Decaris B, Leblond P: **Chromosomal arm replacement generates a high level of intraspecific polymorphism in the terminal inverted repeats of the linear chromosomal DNA of *Streptomyces ambofaciens*.** *Proc Natl Acad Sci USA* 1998, **95**:14296-14301.
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 intraspecies polymorphism.

54. • Pandza S, Biukovic G, Paravic A, Dadbin A, Cullum J, Hranueli D: **Recombination between the linear plasmid pPZG101 and the linear chromosome of *Streptomyces rimosus* can lead to exchange of ends.** *Mol Microbiol* 1998, **28**:1165-1176.
This report demonstrates exchange of telomeric regions between *Streptomyces* linear plasmids and chromosome.

55. Volff JN, Altenbuchner J: **Genetic instability of the *Streptomyces* chromosome.** *Mol Microbiol* 1998, **27**:239-246.

56. Moyer R, Graves G, Rothe C: **The white pock mutants of rabbit poxvirus. I. Terminal DNA sequence publication and transposition in rabbit poxvirus.** *Cell* 1980, **22**:545-553.

57. Pryde FE, Gorham HC, Louis EJ: **Chromosome ends: all the same under their caps.** *Curr Opin Genet Dev* 1997, **7**:822-828.

58. Plasterk RH, Simon MI, Barbour AG: **Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*.** *Nature* 1985, **318**:257-263.

59. Barbour AG, Fish D: **The biological and social phenomenon of Lyme disease.** *Science* 1993, **260**:1610-1616.