**REVIEW
ARTICLE****Molecular biology of the *Borrelia*, bacteria with linear replicons**I. Saint Girons,¹ I. G. Old¹ and B. E. Davidson²

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¹Unité de Bactériologie Moléculaire et Médicale, Institut Pasteur, 75724 Paris cedex 15, France²Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia**Keywords:** Lyme disease, spirochaetes, *Borrelia*, linear chromosome, linear plasmids***Borrelia* and Lyme disease**

The genus *Borrelia* was known more than a century ago following the discovery of *Borrelia recurrentis* as the causative agent of epidemic relapsing fever. Interest in *Borrelia* increased dramatically after the recent discovery of Lyme disease and the observation that it is caused by a novel member of the genus. *Borrelia* are spirochaetes, organisms that comprise a separate phylum in the kingdom of eubacteria (Woese, 1987). They differ from other bacteria in having a helical shape with multiple waves and endoflagella which are located between the inner and outer membrane. In this review, we concentrate on the molecular biology of the causative agents of Lyme disease and North American relapsing fever (*Borrelia burgdorferi sensu lato* and *Borrelia hermsii*, respectively) because they have received the most research attention. To contain the review within reasonable limits we have not attempted to cover the taxonomy of the *Borrelia* or the widespread literature on antigenic variation of *B. hermsii*. Interested readers are referred to recent reviews on these topics by Barbour (1990) and Wilske *et al.* (1992).

Lyme disease is a multisystemic disorder which involves the joints, heart and nervous system (for a review see Steere, 1989). The disease was shown to be transmitted by ticks of the genus *Ixodes* and caused by a spirochaete (Burgdorfer *et al.*, 1982). Analysis of these spirochaetes revealed that they were from a new species of *Borrelia* (Hyde & Johnson, 1984), which was named *B. burgdorferi* in honour of its discoverer, W. Burgdorfer (Johnson *et al.*, 1984). The delineation of a single species was based on DNA/DNA hybridization studies performed on a small number of strains that originated from the USA (Hyde & Johnson, 1984). Subsequent DNA/DNA hybridization, 16S rRNA sequencing and ribotyping of strains from

different geographical origins in Europe and the USA led to the realization that at least three different species are responsible for Lyme disease: *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii* (Postic *et al.*, 1990; Baranton *et al.*, 1992; Marconi & Garon, 1992; Canica *et al.*, 1993). The existence of several different aetiological agents for Lyme disease is correlated with different patterns of symptoms seen in Europe and the USA (Assous *et al.*, 1993; Van Dam *et al.*, 1993). We use the term *B. burgdorferi* to refer to *B. burgdorferi sensu stricto* and Lyme disease spirochaetes to cover *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii* (formerly group VS461). A fourth species, *B. japonica*, was recently isolated from Japanese ticks (Kawabata *et al.*, 1993; Postic *et al.*, 1993).

Initially, studies on *Borrelia* spp. proceeded slowly, due to the lack of a suitable medium for cultivating the organism. Even now, *Borrelia* spp. such as *B. recurrentis* cannot be cultivated *in vitro*. The liquid medium defined by Kelly for the growth of *B. hermsii* and found suitable for the growth of *B. burgdorferi* was improved by Barbour, Stoenner and Kelly to give BSKII medium, which is now used universally to culture Lyme disease *Borrelia* and *B. hermsii* (Barbour, 1984). In particular, BSKII medium contains N-acetylglucosamine, an essential component of the chitin in arthropod cuticles. Some other components, for example gelatin, are not essential for growth but their presence improves the cell yield to modest but usable levels (up to 2×10^8 bacteria per ml). The doubling time of *B. burgdorferi* is between 6 and 12 h (Barbour & Hayes, 1986). Colonies were obtained for the first time on solid BSKII medium a few years ago (Kurtii *et al.*, 1987).

Investigations of the molecular biology of *Borrelia* spp. have been hampered by the unavailability of tools for genetic transfer. Consequently, both classical and reverse genetics have not been possible. Methods enabling the genetic manipulation of spirochaetes have so far remained elusive. Indeed, the only published report describes electroporation of *Serpulina hyodysenteriae* (Ter Huurne *et al.*, 1992).

The GenBank accession number for the sequence reported in this paper is U04527 (gb-ba: BBU04527).

The *Borrelia* genome

Lyme disease spirochaetal DNA has a low G + C content of 28.0–30.5 mol% (Johnson *et al.*, 1984; Baranton *et al.*, 1992). This value is comparable with that found in other members of the genus and is significantly less than that of other spirochaetes such as *Treponema denticola* (36.0 mol%), *Leptospira interrogans sensu lato* (35–40 mol%) (Haapala, 1969; Hyde & Johnson, 1984) and *Treponema pallidum* (53 mol%) (Miao & Fieldsteel, 1980).

As with many bacteria, the genome of *B. burgdorferi* consists of a chromosome and a number of different plasmids. The feature that distinguishes the borrelial genome from most other bacterial genomes is that the chromosome and some of the plasmids are linear DNA molecules (Fig. 1). This unusual property has enhanced interest in the molecular genetics of *B. burgdorferi* and related species. Up to now, linear chromosomes have also been described for *Streptomyces lividans* (Lin *et al.*, 1993) and *Agrobacterium tumefaciens* (Allardet-Servent *et al.*, 1993), while linear plasmids have been observed in *Streptomyces* spp. (Kinashi *et al.*, 1987).

The linear chromosome of Lyme disease spirochaetes

Discovery of the linearity by pulsed field gel electrophoresis (PFGE). The discovery that the chromosome is linear was made concurrently in two laboratories when high-molecular-mass *B. burgdorferi* DNA was subjected

to analysis by PFGE (Baril *et al.*, 1989; Ferdows & Barbour, 1989). The prior discovery of linear plasmids in this organism was helpful in interpreting these analyses (Barbour & Garon, 1987). The DNA for the PFGE experiments was prepared *in situ* in agarose blocks by procedures that have been established with other organisms to minimize shearing. Large open circular DNA molecules such as bacterial chromosomes do not migrate from the origin during PFGE, presumably because the looped molecules become impaled on spurs of agarose (Serwer & Hayes, 1987). Surprisingly, the majority of the *B. burgdorferi* chromosomal DNA was not retained at the origin but migrated as a band with mobility approximately equal to that of a linear duplex molecule of 1 Mbp. Digestion of the DNA with different endonucleases before electrophoresis yielded in each case a number of fragments of total molecular mass between 900–1000 kbp. The possibility that the 1 Mbp DNA was composed of supercoiled circular molecules was eliminated because its electrophoretic mobility was unaffected by the introduction of supercoil-relaxing single-strand breaks. The conclusion from these experiments was that the *B. burgdorferi* chromosome is a linear molecule of between 900 and 1000 kbp.

Chromosome copy number has not yet been reported for a Lyme disease spirochaete, but a detailed investigation with *B. hermsii*, which also has a 1 Mbp linear chromosome, revealed a relatively high value of 16 copies of chromosome per cell for *B. hermsii* grown in mice (Kitten & Barbour, 1992). By contrast, actively growing *Escherichia coli* has on average 6.5 chromosomal origins and 1.9 termini per cell, while *Azotobacter vinelandii* contains up to 40 chromosomal copies per cell (Bremer & Dennis, 1987; Punita *et al.*, 1989).

Physical and genetic maps of the linear chromosome.

A physical map of the chromosome of *B. burgdorferi* was first reported for strain 212 (Davidson *et al.*, 1992). In determining the map extensive use was made of digestions in agarose blocks of chromosomal DNA or restriction fragments that had been purified by PFGE. The use of chromosomal DNA, free of the linear plasmids, eliminated uncertainties in the analysis of the digestion products. A total of 47 digestion sites were located for the enzymes *SgrAI*, *SacII*, *MluI*, *BssHII*, *EagI*, *SmaI*, *NaeI* and *ApaI*. In most regions of the map the gap between restriction sites was 50 kbp or less, enabling good resolution for the mapping of genetic loci. A number of important conclusions were reached from the mapping studies. First, by summing the sizes of the different digestion products an accurate value of 946 kbp was obtained for the size of the *B. burgdorferi* chromosome. This value agreed well with that reported in the initial PFGE studies described above. Second, the data obtained in the mapping studies were consistent with the chromosome being linear. The alternative explanation that the chromosome is circular would require the presence of a < 1.5 kbp region of the chromosome which contained at least one site each for *SgrAI*, *SacII*, *BssHII*, *MluI* and *SmaI*. This explanation is unlikely given the low G + C content of the *B. burgdorferi*

Fig. 1. Schematic scale representation of the genome of *B. burgdorferi* B31 (the type strain). The depiction of the ends of linear replicons as single-strand loops is speculative, except for the 16 kbp plasmid (Hinnebusch & Barbour, 1991). The complement of replicons shown is that reported by Sadziene *et al.* (1993b).

Fig. 2. PFGE separation of self-ligated *MluI* fragments of the *B. burgdorferi* 212 chromosome. Fragments MIB and MIC were purified by PFGE, and the agarose blocks containing them were excised, washed and then incubated with DNA ligase for 36 h at 10 °C. The blocks were embedded in a second agarose gel and the ligation products were resolved by further PFGE. Lanes: 1, MIB; 2, MIC. The *MluI* map of *B. burgdorferi* 212 is shown at the bottom of the figure (Davidson *et al.*, 1992).

chromosome. One possibility that cannot be eliminated by any of the experiments that have used PFGE is that the *B. burgdorferi* chromosome contains an extremely fragile locus which is broken during the preparation of high-molecular-mass DNA. It may require the determination of the structure of the chromosomal extremities to be able to exclude this possibility unequivocally.

The molecular structures of the chromosomal telomeres are currently unknown, and constitute an important area of investigation. The following experiment provides some relevant information. *MluI* fragments of the *B. burgdorferi* 212 chromosome, purified by PFGE electrophoresis, were self-ligated *in situ* in the agarose and then resubjected to PFGE (Fig. 2). The internal fragment, MIC, yielded a ladder of bands corresponding to monomer, dimer, trimer, tetramer and pentamer as well as a significant quantity of presumably open circular polymeric DNA which remained in the well. By contrast, the end fragment, MIB, yielded only the monomeric and dimeric species, indicating that the telomeric ends were not ligatable. Attempted self-ligation of undigested chromosomal DNA gave no higher polymers (data not shown), again indicating non-ligatable telomeres.

Determination of the restriction map of a second *B. burgdorferi* strain (Sh-2-82, isolated in New York) enabled interesting conclusions to be reached about the stability of the chromosomes within the species (Casjens & Huang, 1993). The size of the Sh-2-82 chromosome, 952 ± 12 kbp, was not significantly different from that of the 212 chromosome, but 8 of the 41 sites for the restriction enzymes *SgrAI*, *SacII*, *MluI*, *BssHII*, *EagI* and *SmaI* were

Fig. 3. Genetic map of the *B. burgdorferi* 212 chromosome. Details of the individual genes can be obtained from the GenBank or EMBL databases, with the exception of *pheST* (A. Barbour, unpublished), *fliG* and *flgE* (N. Charon, unpublished), *ftsA*, *gidB*, *htpG*, *metG*, *miaA*, *moxR*, *parE*, *pgk*, *plsC*, *pth*, *tmhC* and *tpi* (I. G. Old, unpublished).

in dissimilar locations. This means that the nucleotide sequences of the chromosomes of the two strains differ by at least 3.2% in the 248 bp that make up the relevant sites.

The unavailability of gene transfer systems in Lyme disease spirochaetes has prevented the use of classical genetic procedures to construct a genetic map for this organism. The availability of a physical map of the *B. burgdorferi* chromosome made it possible to commence genetic mapping of the chromosome by other means (Davidson *et al.*, 1992; Old *et al.*, 1992b; Casjens & Huang, 1993). The procedures used were simple: Southern blots of PFGE separations of restriction digests

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ACGAGCAGCAGCAGCTTTTATGCTTTTCCAAGAAATAGTGCTATCTCTAAAGGATTTAAATTAATAATTTCCAATAAATGTTTCATATACAACAACCTCAACTATTTTCTTATCTCAGA 120
R A R A A K I A K G L I T D I E L P N L N I I E L L H E Y V V V E V I K R I E S
ATTACCAAGCTTACTTTTGTGACCTTCAAATGAGGTCTGGTACTTTGACAGAAATACAGCCTGTAAGCCCTCTTTAAATCATCTCCTGTAAGATTTGGAATATCTTTTACT 240
N G L K S K T Q E F Q P E P V K V S I V A T L G E K F D D G T L N P I D K K S
TATTTTGAATTTCTAAAGCTTCATTCATGGCCTTAGTAGTCCACTTCTAAATCCCATAACATGAGTTCCTCTCTGTTATTAATATTATTAACAAAAGAAGAATGTTGTCAGA 360
I K S N K F A E N M A K T L G S R F G M V H T G G E R T N I N N V F S L I N D S
ATAGCTTTCAGTCCATTTAAGCCCCACATTAACAATAACATCATTATAAAACCAATCAATATAAAGGTTCTGATTGAAAAGCTTTGCTGTCATTAGTTAAATAATCTACAAAAGATTT 480
Y S E T W K L G V N V I V D N I F G D I Y P E S Q F A K S D N T L Y D V F S K
TATCCACCCTCAAATAAAATTTGAAGATTTTCTTTACCAATCTTTTATCTCAATTTGAAATGTATTTTATCGTTTAAAAAGCAAGCTTTTAAGCCTTTTCAAGAACATC 600
I G G E F Y F K S S K E K G I R K D E I S I Y I K D N L F A L E K L R K E L V D
GAAATATAATCTAAAGTTTCAAATAATCCGCCAAAAGTAACTTAGTCCCGTAAACAGAGATTTCCCCACAACCTTCTACTTTAGAAGTCGGAATACCTTTTGAAGAAAGT 720
F N Y D L T E F I E S D A L F T V K T G T V S S E G V V E V K S T P I G K S F T
TTGCTTAAAAATTTTCCATCTCTATTAACATAAAGCTCTAAAAACGAAGATAGAGCATTTACAACCGAAATTCACAGCCATGAAGTCCCCAGAACTTTATACGTGCCTTTATATAA 840
Q R F I K G D R N V Y V E L F S S L A N V V S I G V G H L G G S V K Y T G K N F
CTTACCACCAAGTGAATTTTGTAAAAACAGTTCAAGGGTGACACTAATACCTCTCTCATGAATTCGGTAGGAATACCTCTCCCATTAATACAGTTATAGTATTATCTAA 960
K G G S H L K T L V L E L T V S I G E E E H I D T P I G R G N D I V T I T N D L
ATTGATAATACATCTATTCTATCACAACCGCAGCTAAAGCCTCATCAATGCTATTGTCACACCTCATATAACCAATGGTCAATCCATTATAGAACTGAGCCTATATACATGCC 1080
N I I V D I R D C F A A L A E D I S N D V V E Y V L H H L G N I S V S G I Y M G
AGGCCTTTTCTAACAGCCTCAAGTCTTTTAAGACCTGAATGTTACGACCAACATAATTCAGTAAACCTTCCATTCTTTAATTAATCTAGACACAGATAAATCTACATTAATAAAA 1200
P R K R V A E L G K L V Q I N S A V Y N L L G E M <-- gyrB
AAAAATATCTATTACATAGAAATACCAAAAACCTTTTAAAAATTCAGCAACAAATTTAGATAGAATCTTAATTTAATATATACTATATAATGTTAAAGTATAGTGCTAAACCAACA 1320
dnaA ---> M E K S K N I W S L I L T E I A K K E L S E E E F Y V V F F E N L C F L S
AGGACAAAAGATGAAATCAAAAATATATAGGAGCTTGATTTAAACAGAAATAAAAAAGAACTATCAGAAGAAGATTTTATGTTTGTGTTGAAATTTGTGCTTTTGAATCAAA 1440
rbs
I G D N I K I S T P N L F H K N Q I E K R F T K K I K E I L I K N G Y N N I V I
TAGTGACAATATTAATAATCTACTCCAAATTTATTTTCATAAAAAATCAAAATAGAAAAAGATTACAAAAAAATTAAGAAATCCTTATAAAAAATGGCTACAATAACATAGTCATTG 1560
V F T N Q P P K T H S N K Q E T K N P A L N E T F S K F D K L K E K T T S K E A
TATTTACAAATCAACCCAAAACCTATTCTAACAAACAAGAACTAAACCCGGCTCTTAACGAAATCTTTCAAAGTTTCGACAAGCTCAAAGAAAAACAACTTCCAAAGAAGCAA 1680
I Q N I Q D R I K M Y I K K E E E E P T K N P F L K K R Y T F E N F I G P
TCCAAAATATTCAAGATCGTATAAAAAATGATATCAAAAAAGAAGAAGAGCCCAAAATTTTAAAAACCCCTTCTTAAAAAAGATATACATTTGAAAAATTTATCATCGGGCCAA 1800
N N K L A Y N A S L S I S K N P G K K Y N P C L I Y G G V G L G K T H L L Q S I
ATAATAAATCTGCTTACAATGCCAGCTTGCTCAATCTCAAAAAATCCTGGGAAAAATATAATCCGTTGTTAATTTATGGTGGAGTTGGACTTGGAAAAACACATTTGCTTCAAGCATAG 1920
S N K T E R S P S E L T K I L Y V T A E N F L N E F V E S I K T H E S I K T K F K K
GAAACAAAACAGAAGATTACATCATAACCTTAAAAATATATGTTACTGCTGAAAAATTTTAAATGAATTTGTAGAAAGCATAAAGACACAGAAACAAAAAATTTAAAAAAT 2040
Y R Y L D M L L I D D I H D L Q K K E G I Q E E L F H T F N A L Y E D N K Q L V
ACAGATCTTAGACATGCTACTTATAGCATATCCAGGACTTACAAAAAAGAAGGTATACAAGAAGAGCTTTTACACATTTAATGCCCTTGTGAAGACAATAAACACTAGTAT 2160
F T C D R S P S E L T N F T D R L K E N R F T R G F L N V D I S K P N F E L R A A I
TCACATGTGACCGATCTCCTTCTGAATTTACAAATTTTACAGATCGATTAAGAGCAGATTCACAGGGGATTAATGTTGATATATCAAGCCCAATTTTGAAGTCAAGAGCAGCTATTG 2280
V E K K A E E D G I N V P K N I L N L V A Q K V T T N V R D L E A A V T K L K A
TCCAAAAAAGCAGAAGAAGATGCGCTAAATGTCCTTAAATATACTAAATCTGGTTGCTCAAAAAGTTACAACCAACGTAAGAGACCTTGAAGCTGCTGAACAAAATCAAGAGAT 2400
Y I D L D N I E I D I E I V E K I I K E I I I Y E K E T T N E P N N K I N I E N
ATATAGATTTAGACAATATAGAAATGACATTGAAATTTGTTAAAAATATCAAGAAATATAATTTACGAAAAAGAAACAACCAATGAGCCAAACAATAAATTAATATCGAAAAATA 2520
I K K I L L R E L K I T H K D I E G H S K K P E I T K A R H I Y A Y L L R N F T
TAAAAAATACTCTTAAGAGAGCTTAAAAATCACACAAAGATATTGAGGGGCATAGTAAAAAACAGAGATAACAAAAGCTAGACATATTTATGCGTACCTTTTGAGAAATTTTACAG 2640

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Fig. 4. For legend see facing page.

were hybridized with probes of characterized genes, thereby revealing the identity of the fragments carrying the genes. The resolution of the physical map determines the resolution that can be obtained by this approach. Since certain areas of the chromosome are richer than others in sites for the endonucleases used for mapping, there is considerable variation in the degree of precision with which different genes can be mapped by this process. Thus the chromosomal locations of genes such as *gidA* and *fla* were determined only approximately, whereas it was possible to map the locations of the rRNA genes and *pheS* precisely because they contained sites for one of the endonucleases used. The rDNA cluster, *fla*, *flgE*, *rbo*, and the *dnaA gyrBA* cluster, *pheS*, the *grpE dnaKJ* cluster, and *groEL* have been located on both the *B. burgdorferi* 212 and Sh-2-82 maps and all loci mapped in identical positions of the genome, within the limits of precision of the mapping procedure. Our current map of the *B. burgdorferi* 212 chromosome has a total of 39 genetic loci (Fig. 3).

Few comparisons of chromosomal gene organization within a bacterial species have been performed. In six

strains of *Mycoplasma mycoides* subsp. *mycoides* the relative positions of nine loci were found to be conserved while one gene had undergone a translocation (Pyle *et al.*, 1990). Analysis of different strains of *Clostridium perfringens* belonging to different biotypes showed that the overall organization of the chromosome in that bacterium is well-conserved (Canard *et al.*, 1992). By contrast, the chromosomes of two strains of *L. interrogans* exhibit several major DNA rearrangements (Zuerner *et al.*, 1993) and detailed comparison of six commonly used *E. coli* K12 strains revealed insertions and deletions ranging in size from 1 to 86 kbp (Perkins *et al.*, 1993).

We have recently extended our mapping studies to include the chromosomes of *B. garinii* and *B. afzelii* (C. Ojaimi, B. Davidson, I. Saint Girons & I. Old, unpublished). Interestingly, all of the 30 genetic loci examined have been found to have the same relative positions in the *B. burgdorferi*, *B. garinii* and *B. afzelii* chromosomes. Since these loci are dispersed throughout the entire chromosome this observation indicates the absence of major chromosomal translocation events during speciation and

E L S T V E I G K I I G K G K T H S T V L Y S I N K M I D R R N D R N D K E I I N N L I I
AGCTATCAACAGTTGAAATTGGAAAAAATTATTGGAGGGAAAAACCATTCAACAGTGCCTTTATTTCGATAAAATAAATAGATAGAGACAGAAAATATGACAAAGAAATTAAACAATTTAATCA 2760

T E L M N K I K K N *
CAGAATTTATGAACAAAATAAAAAAAATTAACCTTAATTGAAGAATCAAAAATTTATTTTAAACAATTGAACCAAATAATTTATCAAAAAACAAAAAACCTACAATTAAACA 2880

AGATATTTACTTGTCATCATATAGTATTAAATACAATTAACAATCAACAGTAGCTTACTACTATTACTATATATCTATAAAAAATAAAAAATACAAAATCCACAAAATCTTTAAG 3000

dnaN --> M L H N T T F F I C E T N Q I M N E I E K A K G I I L N R N
ATAAAATGTAATAGAATATATAGAGGCATCATGCTACACAACACATTTTTATTTCGGAACAAATCAAATTATGAATGAGATAGAAAAGCAAAGGGAATAATATTAACAGAAATA 3120

rbs

M N D I W S A L L I E V K K S N L I I K S T D R N I F F E S T I S I V S E T D F
TGAATGATATTTGGAGTGCAATGTTAATAGAAGTAAAAAATCTAATCTCATAATCAAATCAACAGACAGAAATATATTTTTTGAAAGCACAAATTTCAATTGTTTCAGAAACAGATTTTA 3240

K V L I A N S N F Y D A V K A F N F Y K K I K I V F N E N N S K L E I M G A L T N
AGGTTAATAATGACTTCAAATTTTATGATGCGAGTAAAGCAATCAATTTTTATAAGAAAAATCAAAATAGTTTAAATGAAAATAATAGTAATAGAAATTAAGGAGAGTTAAATG 3360

D E K E E Y E D H L K E P T F S Y E E I E N Y N Y D M V N E D Y T F G G I E I K Q
ATGAGAAAGAGGAATATGAAGATCATTAAAAGAGCCCACCTTTTCATACGAAGAAATAGAAAAATTACAATTTATGATGCGTAAATAGAGATTATACCTTTTGGAAATTGAAATAAAACAAA 3480

K S F K K V I N R I A F T S A H L D E S K N V L N G V Y F S K D E S K L L L V S
AATCTTTTAAAAAGTAATAATAGAAATGCTTTTCAGCACATCTTGATGAATCTAAGAATGATTAATAGTGGTGTAITTTTTTCAAAGAGTAGAAGATTCTAAATTTACTACTTGTTTCTA 3600

T N G H R M S I C K I T A G T I V E E D V N F I V P V K I F N F L K H L S A G E G M
CTAATGGTCACAGAATGCTATTTGTAAACACAGAAGTCATAGTTGAAGAAGATGTGAATTTTATAGTTCCTGTAATAATTTAATTTCTTAAACACATCTTATGTCGGGAGAGGTTATGG 3720

V K I K F S D K K F Y V E F G D N Y K I A C S L I N G N Y P D Y K S I I P K E Q K
TTAAATTAAGGTTTCGGATAAAAAATTTTATGTGAATTTGATAATTATAAAATAGCTTGTAGCTTAATTAATGGTAATTTATCCGGATTATAAAAGCATTATACCTAAAGAACAGAAAA 3840

N K S L V S L G I L K D R L G A R N V N L Y V D K S R K L V L T F S E L K L L K L L G
ATAAATCTTTAGSTTCGCTAGGCATTTAAAAGATAGACTTCAAGAGTCAATTTTATAGTTGATAAAATCAAGAAAGTGGTTTTAACTTTCTCTGAGTTACAATTA AAACTTCTGGAG 3960

E D L I T G R K G E F F I K D P N Y L Y D G A D E V M A I N I S Y F V E A I S V
AAGATTTGATTACTGGAAGAAAAGGTGAATTTTTCATTAAGATCCAATTTATCTGTATGACGGAGCAGATGAGGTATGGCTATTAATATTTTCATATTTTGTGGAAGCTATTAGTGTT 4080

F E T S K I E I Q F N S G N V L K L S E P E N F N F T H L I M P M S L G *
TTGAAACTTCAAAGATAGAAATACAATTTAATTCGGGCAATGATTAATAATTGAGCGAACCTGAAAATTTTAATTTTACGCACCTTGATAATGCCGATGCTTTTGGGTTAAAGTGAGTTAT 4200

ORF -->

M N D S A F K K I G N V L
TAATGCTATTTGAAATATAAAAAAGCTTTTCTAGGTTTATTCATATTTGTTTTTTCGATTGCGGTATAAAAAATTGATATGAATGATCTGCTTTTAAAAAATAGGCAATGTTCTTA 4320

K D Y L E S N L L V K K I S S K L L I A D K W N Q I F E A L S D D V L T D D F
AGGATTATTTAGAAATCCAATTTGCTTGTTAAATAAAAAATAGTTCGAATTAATTGATTGCCGATAAATGGAATCAGATATTTGAGGCTTTATCAGATGATGAAGTTTGTAGATTTTA 4440

K N E Q I L F L E V S N S I L C S I A I N K S K I I N S V K E L T G I K I I D
AAAATGACAGATCCTTTTCTTGAGTGAAGCAATTCAGTATTTTGTGTAGTAGCAATCAATAAGTCGAAGATAATAAATTCAGTAAAGAAATTAACAGGTATTAAAAATATAGATA 4560

I K V L V R *
TAAGGTTTGGTAAGGTAATTAATTGACCAATTCGATTTATATGCTATACCATGTTAGTGTTTAAATTTAGTCTTGGCAGCATAAAAATGTTTTTAAATGAGGAGCGGTTTTGAAAGA 4680

rpsH --> M K R

rbs
T Y Q P S R V K R N R K F G F R A R M K T K G G R L I L S R R R R A K G R M K L T
ACTTATCAACCCGATCGTGTTAAAGAGAAATAGAAAATTTGGGTTTAGAGCTAGAATGAAAATCAAAAGCGGAAGACTTATTCTTCAAGGCGAAGGGCAAAAGGAAGAAATGAAATTAAC 4800

rnpA --> M R K K R N I S L K S K I E I Q K I F K E G K L I R F S N L N L K M F Y K S N
V S D E K K K Y *
GTTTCTGATGAGAAAAGAAATATAGTTTAAATCAAAAATAGAAATCAAAAAATTTTCAAGAAGAGCGAAGCTGATTAGATTAGCAATCTTAATCTTAAATGTTCTATAAATCAAA 4920

H L V Y S R I
TCATTTGGTTTATTCTAGAATTC 4943

Fig. 4. Nucleotide sequence of 4943 bp in the region of the *B. burgdorferi* *gyrBA*, *dnaAN*, *rpmH*, *rnpA* gene cluster (Old et al., 1992a, b, 1993a, b). The noteworthy factors are the inversion of *dnaA* and *dnaN* (see Fig. 5) and the absence of *recF* in the region, compared to the organization in *E. coli*, *B. subtilis* and *P. putida*. The coding strand is presented (except for *gyrB*). Possible 'Pribnow boxes' of promoters are underlined, while potential ribosome-binding sites with identity with the 3' end of the *B. burgdorferi* 16S RNA (5'-CACCUCCUUU-3') are underlined and marked 'rbs'. Putative DnaA boxes are marked by '*****'. The primary structure of the corresponding polypeptides, as deduced from the nucleotide sequence, is indicated in the one letter code. This sequence, which has been assigned the GenBank accession number U04527, includes the sequences previously designated L16681, L14948, L04647 and Z12166.

a significant degree of chromosomal stability. Whether stability of this type has also occurred at the micro level will not be known until high resolution maps, possibly generated by DNA sequencing, are available for comparison.

The origin of replication – a novel arrangement? The

circular chromosomes found in bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* have specific regions where replication of the chromosome is initiated (for a review see Smith *et al.*, 1991). These origins of replication (*ori*) contain at least four characteristically arranged 9 bp nucleotide repeats (DnaA boxes) which are sites for the binding of the protein DnaA, the initial step in replication (Holz *et al.*, 1992). Subsequently, DnaB and DnaC bind to the DnaA–DNA complex, replication

commences and proceeds bidirectionally around the chromosome. Termination of replication also takes place at a specific site on the chromosome, *ter*, which is located opposite *ori* on the chromosomal map. It is of some interest to determine how this process has been adapted to achieve the replication of the linear borrelial chromosomes.

One strategy used was to isolate *B. burgdorferi* homologues of genes that have a chromosomal location near to *ori* in better studied bacteria (Old *et al.*, 1992a, b, 1993a, b). The identification of genes under these circumstances rested upon sequence homology, not functional properties. Use of this approach led to the discovery of a gene cluster in *B. burgdorferi* which contains the homologues of *rnpA*, *rpmH*, *dnaN*, *dnaA* and *gyrBA* in that order (Fig. 4). This gene order differs from the normally highly conserved

Fig. 5. Genetic organization in the *dnaA* regions of three typical (*Bacillus subtilis*, *P. putida* and *E. coli*) and three atypical eubacteria (*Buchnera aphidicola*, *Mycoplasma capricolum* and *Borrelia burgdorferi*). The figure is aligned with respect to the *dnaA* gene. Open reading frames (boxed regions) and intergenic regions (gaps) are drawn approximately to scale. The direction of transcription is shown by single-headed horizontal arrows while origin regions are marked with vertical arrows (in *Bacillus subtilis* a fourth origin is located between *gyrA* and *rrnO*). The genes shown are: *gldA*, *gldB* (glucose inhibited division proteins); 50K, 60K (proteins of undetermined function); *rnpA* (ribonuclease P, protein component); *rpmH* (ribosomal protein L34); *dnaA* (chromosome replication initiation protein); *dnaN* (DNA polymerase III, β -subunit); *recF* (inducer of SOS DNA repair); *gyrB*, *gyrA* (DNA gyrase, B- and A-subunits); *ksgA*, [S-adenosyl methionine-6- N' , N' -adenosyl (rRNA) dimethyltransferase]; *lica*, (outer membrane oligosaccharide biosynthesis); *orf* (open reading frames). For *Borrelia burgdorferi* 212 the direction of transcription of *gldAB* relative to *dnaA* is not known, and its placement on the downstream side of *gyrA* is inferred from the genetic arrangement in *B. afzelii*.

regions found in other eubacteria in a manner suggestive of a rearrangement during the evolution of the *B. burgdorferi* chromosome (Fig. 5). The *dnaA* gene cluster was found to be located almost precisely in the centre of the *B. burgdorferi* chromosome, close to the rDNA genes (Old *et al.*, 1992b; Casjens & Huang, 1993).

While the *dnaA* region of eubacteria is normally highly conserved, the number of origins of replication is not, varying from one for *E. coli* to four for *Bacillus subtilis* (Fig. 5). In *E. coli*, *P. putida* and *B. subtilis*, characteristic groups of DnaA boxes are located either adjacent to or near the gene *gldA*. In *P. putida* and *B. subtilis*, a second origin is located between *dnaA* and *rpmH*, while in *B. subtilis* there is a third origin between *dnaA* and *dnaN* and a fourth between *gyrA* and *rrnO*.

The *dnaA* region is considered to contain the ancestral origin of replication. A search for DnaA boxes (5'-TTATCCACA-3') in the *B. burgdorferi* *dnaA* region revealed none in the intergenic DNA region surrounding *dnaA* and two upstream of *rpmH* (Old *et al.*, 1993a). In *E. coli*, *oriC* is located upstream of *gldA*, approximately 40 kbp from *dnaA* (Fig. 5), while there is a single DnaA box in the *dnaA* regulatory region, essential for auto-regulation of the gene. The *B. burgdorferi* *gldA* homologue

has been cloned and mapped (Old *et al.*, 1992b). As in *E. coli*, the *B. burgdorferi* gene has been translocated relative to *dnaA* and *gldA* lies over 250 kbp from the central *dnaA* cluster. Determination of the nucleotide sequence around *gldA* should provide useful information on this point.

It is possible that the nucleotide sequences and/or arrangement of *B. burgdorferi* DnaA boxes are atypical. In this respect, it is interesting to note that *B. burgdorferi* DnaA differs in the normally highly conserved region which may be involved in DNA binding (Old *et al.*, 1993a).

Thus at this stage, the available data on the location of *ori* in the *B. burgdorferi* chromosome are inconclusive. A central location near *dnaA* has the appeal of symmetry, since bidirectional replication would proceed divergently to each telomere, where termination should occur more or less concurrently. As has been suggested previously (Old *et al.*, 1992b), this arrangement could have evolved from a circular chromosome by deletion of the DNA opposite the origin which normally encodes information for replication termination. Termination at the telomeres of the linear chromosome would not require this information. If *ori* is located near *gldA*, a different pathway for the evolution of the linear chromosome must have been

followed, e.g. deletion of the terminus region followed by translocation of *gidA*.

An unusual organization of rRNA genes and ribosomal protein operons. The arrangement and organization of the rRNA genes in the Lyme disease spirochaetes is unusual among bacteria in that there are two copies each of *rrl* (23S) and *rrf* (5S) but only one copy of *rrs* (16S) (Davidson *et al.*, 1992; Fukunaga *et al.*, 1992; Schwartz *et al.*, 1992). *rrl* and *rrf* are tandemly duplicated in the order *rrlA rrfA rrlB rrfB*, while *rrs* is located more than 2 kbp upstream of *rrlA*. Genes encoding tRNA^{Ala} and tRNA^{Ile} are present in this intervening DNA (Schwartz *et al.*, 1992; Gazumyan *et al.*, 1994). Normally, rRNA genes are present in operons that have the order: promoter, *rrs*, *rrl*, *rrf*. This arrangement results in the synthesis of equimolar amounts of the three rRNA species. The pattern of transcription of the rRNA genes in these *Borrelia* species has not been reported, so the manner in which it is regulated (presumably to achieve equimolar amounts of the three rRNA species) is not clear. It is likely that the genes of each *rrl rrf* pair are cotranscribed in a single transcript, since there is little DNA between them which could specify a promoter (Schwartz *et al.*, 1992). On the other hand, the presence of a relatively good *E. coli*-like promoter sequence in the 182 bp spacer between *rrfA* and *rrlB* raises the possibility of independent transcription of each *rrl rrf* pair (Schwartz *et al.*, 1992). This unusual rRNA gene arrangement is typical of Lyme disease spirochaetes since *B. anserina*, *B. turicatae* and *B. hermsii* each have only a single copy of both *rrl* and *rrf* (Schwartz *et al.*, 1992). Thus, the duplication of *rrl* and *rrf* has evolved recently in the Lyme disease spirochaetes. The nature of the mechanism whereby co-ordinate synthesis of the 16S and other rRNAs is achieved, assuming that one exists, is an interesting topic for further investigation.

Recent work by Schwartz's group indicates that in *B. burgdorferi* the gene encoding elongation factor Tu (*tuf*) is not part of the *str* operon, which usually comprises the genes encoding S12 and S7 ribosomal proteins and elongation factors EfG and EfTu in other bacteria. Instead, it is located only 50 bp upstream of *rpsJ*, which encodes the ribosomal protein S10 (Gazumyan *et al.*, 1994). The gene order of the operon which encodes the S10 ribosomal protein matches that found in *E. coli*. The implications of this unusual arrangement for regulation of ribosomal protein and elongation factor Tu synthesis in *B. burgdorferi* are being determined.

Linear and circular plasmids

Plasmids constitute a significant proportion of the *Borrelia* genome both quantitatively and genetically. For example, 17% of the coding capacity of a low passage infectious isolate of *B. burgdorferi* B31 is in plasmid DNA (Marconi *et al.*, 1993a; Sadziene *et al.*, 1993b). Both linear and circular plasmids have been observed.

Linear plasmids. Linear *Borrelia* plasmids were first observed in *B. hermsii* (Plasterk *et al.*, 1985) and subse-

quently found to be a feature of all Lyme disease spirochaetes. Their role in antigenic variation has been the subject of a recent review (Barbour, 1993). In *B. hermsii* linear plasmids harbour the genes for the important antigens known as variable major proteins (Vmps) and provide the vehicle for the translocation of these genes that is responsible for antigenic variation (see section on antigenic differences). In Lyme disease spirochaetes, *ospAB* and *ospD*, which encode outer-surface proteins (Osps), are located on linear plasmids. Other linear-plasmid-encoded functions remain to be elucidated.

The linear *B. hermsii* plasmid bp7E, which carries the expressed version of the *vmp7* allele, has a copy number of 14 per cell (0.9 per chromosome) in cells grown in mice and one-quarter to one-half this number in broth-cultured organisms (Kitten & Barbour, 1992). The type strain *B. burgdorferi* B31 has four different linear plasmids (Fig. 1). The 16 and 49 kbp plasmids of *B. burgdorferi* B31 also have a copy number of approximately one per chromosome (Hinnebusch & Barbour, 1992). These copy number values for both *B. hermsii* and *B. burgdorferi* suggest that replication and partitioning of plasmids and chromosome are tightly coupled in these organisms. Such tight coupling may be achieved genetically and/or by the organization of the individual borrelial genomic elements (chromosome and plasmids) into nucleoids which are distributed along the length of the cell and which replicate and segregate as discrete units (Kitten & Barbour, 1992).

The telomeres of the linear plasmids. Two questions of major interest follow from the discovery of linear plasmids in *Borrelia*. What are the structures of the telomeres and how are they replicated? A definitive answer to the first question has been provided by the work of Barbour and his associates (Hinnebusch & Barbour, 1991). After initially establishing that the linear plasmids have covalently closed ends that are disrupted by S1 nuclease but not protease treatment, they isolated terminal fragments from the 16 kbp linear plasmid of *B. burgdorferi* B31, and directly determined their nucleotide sequences. Noteworthy was the discovery of a palindrome that constituted an inverted terminal repeat for the plasmid (Fig. 6). At each telomere the two strands of DNA are covalently connected by four, unpaired nucleotides that form a hairpin loop. Significant homology, indicative of a common evolutionary origin, exists between the nucleotide sequences at the left ends of the 16 kbp plasmid, the 49 kbp *B. burgdorferi* B31 linear plasmid, and a linear *B. hermsii* plasmid. Another prokaryote replicon, that of the lysogenic coliphage N15, exists as a linear double-stranded DNA molecule with palindromic terminal hairpin loops (Svarchevsky & Rybchin, 1984). However, the non-borrelial telomere which most clearly resembles the 16 kbp *B. burgdorferi* plasmid telomere is that of a eukaryotic virus, African swine fever virus. This close resemblance led Hinnebusch and Barbour to propose the intriguing possibility that the linear *Borrelia* plasmids arose by a horizontal genetic transfer between kingdoms (Hinnebusch & Barbour, 1991). The fact that African swine fever virus and *B. duttoni*, an aetiological agent of

Fig. 6. Nucleotide sequences of the telomeres of the 16 kbp linear plasmid from *B. burgdorferi* B31. Nucleotides shown in bold type comprise an indirect repeat sequence. The data are from Hinnebusch & Barbour (1991).

relapsing fever, share the same tick host adds credence to this proposal.

Circular plasmids. Circular plasmids carried by bacterial pathogens frequently confer virulence properties and other desirable characteristics such as antibiotic resistance. The number of circular plasmids in *B. burgdorferi* varies between strains and within a given isolate since some are lost during *in vitro* cultivation. For example, the complement of circular plasmids in *B. burgdorferi* Sh-2-82 declined from six to four in the course of 2 years of *in vitro* passaging from its initial low-passage state (less than 10 generation times) (Simpson *et al.*, 1990a). The lost plasmids were found to be non-essential for infection of *Peromyscus leucopus*. In the Lyme disease spirochaetes, circular plasmids have been shown to carry the following loci: *ospC* (see section on *OspC*), homologues of *guaA* and *guaB*, which encode the enzymes that convert XMP to GMP and IMP to XMP, respectively (N. Margolis, D. Hogan & P. Rosa, unpublished), and a class of species-specific repetitive DNA found in a number of circular plasmids but nowhere else in the genome (Simpson *et al.*, 1990b). While it has been suggested that this repetitive DNA may encode functionally similar proteins such as related antigens, further work is needed before any firm conclusions about its function are reached.

Major outer-membrane proteins of *Borrelia*

Proteins of high abundance that are found in the outer membrane of borreliae have been studied extensively because they could be major antigens and play an important role in pathogenesis. In Lyme disease spirochaetes, the relevant proteins are referred to as *OspA* and *OspB* while in *B. hermsii* they are the *Vmps*. Despite considerable effort, the functions of *OspA* and *OspB* remain poorly defined, although a recent report (Sadziene *et al.*, 1993a) showed that a mutant strain of *B. burgdorferi* in which *OspB* was truncated and under-expressed had diminished penetration capability and infectivity. Importantly, this report emphasized the value of studying antibody-resistant mutants of infectious isolates as a means of determining the role of the *Osp*s in pathogenesis.

The *ospAB* operon in Lyme disease spirochaetes

The determination that *ospA* and *ospB* are organized in an operon was made by deletion analysis and transposon Tn5 mutagenesis of a plasmid which expressed both *OspA* and *OspB* in *E. coli* (Howe *et al.*, 1986). The insertion of Tn5 within *ospA* abolished expression of *ospB*, suggesting transcription of both genes from a single promoter. This

was confirmed by the isolation of a 2.2 kbp transcript that hybridized with *ospA*- and *ospB*-specific probes. In *B. burgdorferi*, *ospAB* is carried on a 49 kbp linear plasmid (Barbour & Garon, 1987) while in *B. garinii* and *B. afzelii* it is present on 55 and 56 kbp linear plasmids, respectively (Samuels *et al.*, 1993). Determination of the nucleotide sequence of *ospAB* from *B. burgdorferi* B31 was notable since it provided the first nucleotide sequence of borrelial DNA (Bergström *et al.*, 1989). The deduced translation products from *ospA* and *ospB* were 273 and 296 amino acids long, respectively, and contained signal sequences with sites (Leu-X-Y-Cys) for processing by signal peptidase II. A combination of results confirmed that *OspA* and *OspB* are lipoproteins (Brandt *et al.*, 1990). In particular, it was shown that fatty acids are covalently linked to these two peptides in a way typical of most bacterial lipoproteins.

OspA and *OspB* differ in both their molecular masses and the relative amounts of their expression (for a review see Wilske *et al.*, 1992). As early as 1983 a panel of monoclonal antibodies recognizing *OspA* and which discriminate between strains was developed (Barbour *et al.*, 1983). Within the *B. burgdorferi* species, *OspA* was found to be 99% identical in three different strains (Bergström *et al.*, 1989; Wallich *et al.*, 1989; Fikrig *et al.*, 1990). In contrast, there were significant inter-species differences with 73–86% identity for *OspA* throughout the Lyme disease spirochaetes (Eiffert *et al.*, 1992; Jonsson *et al.*, 1992; Zumstein *et al.*, 1992). A similar degree of divergence between the three species was seen in *OspB* (Jonsson *et al.*, 1992). The most highly conserved region of *OspA* is the NH₂-terminus. The two *Osp* proteins show 53% overall sequence identity, indicating a recent evolutionary duplication of an ancestral *osp* gene. It has been suggested that this duplication occurred before the geographical dispersion of the strains (Bergström *et al.*, 1989).

Antigenic differences or true antigenic variation?

Borrelia are effective at evading the host immune response. The mechanisms by which they do this are better understood for *B. hermsii*, where true antigenic variation is well-documented, than they are for the Lyme disease spirochaetes.

Infection of a human (or experimental animal model) by a relapsing fever spirochaete such as *B. hermsii* leads to a characteristic pattern of periodic fevers recurring at intervals of 4–7 d (for a review of antigenic variation in relapsing fever *Borrelia* species, see Barbour, 1990). Blood levels of borreliae rise and fall coincidentally with the fever. Analysis of this phenomenon was aided by the

observation that inoculation of a mouse with a single *B. hermsii* HS1 cell yielded progeny spirochaetes with 25 different serotypes. This experiment demonstrated significant capacity for antigenic variation in *B. hermsii*. The variation, which is called multiphasic antigenic variation, is reversible.

At the molecular level, serotype specificity in *B. hermsii* is determined by the abundant membrane lipoproteins referred to as Vmps, which vary from 21 to 39 kDa in molecular mass. The genes encoding these proteins are located on linear plasmids and are silent except when they are present in a site immediately adjacent to one of the linear plasmid telomeres. Molecular analysis of the *vmp* locus in the 25 *B. hermsii* HS1 serotypes referred to above indicated that the different *vmp* genes have related sequences (39–78%) and are flanked on each side by identical nucleotide sequences (Restrepo *et al.*, 1992). These latter sequences are presumed to act as sites for homologous, inter-plasmidic recombinations which enable different *vmp* alleles to be translocated between silent and active (or expression) sites, thereby bringing about a change in the nature of the expressed Vmp with consequent antigenic variation. The translocation to the expression site, which has a subtelomeric location, is predominantly unidirectional and is either a form of gene conversion or a double crossover in which the previously active allele is lost.

Burman *et al.* (1990) noted that of the known mechanisms used by pathogens to achieve antigenic variation, the one that most closely resembles the *B. hermsii* mechanism is that used by a eukaryote, the African trypanosome. In the trypanosome, DNA rearrangements are used to create a telomeric environment for the expression of hitherto silent genes which encode different forms of the abundant surface protein (Vsg). The secondary structures of Vmps and VsGs are similar although there is no sequence similarity between the *vmp* and *vsg* genes (Burman *et al.*, 1990). In the absence of additional experimental data we can do no more at present than draw attention to this surprising similarity between these two phylogenetically quite distant organisms.

Following infection by Lyme disease spirochaetes, the pathogen evades the immune response and then establishes a chronic infection. One possibility is that this state is achieved through changes in the outer-surface proteins such as OspA, OspB and OspC, but current evidence for a mechanism involving true antigenic variation is not convincing. Different strategies have been used in an attempt to identify the mechanisms of cell-surface changes in *B. burgdorferi*. In one approach *B. burgdorferi* was examined to determine whether bona fide antigenic variation in OspB had occurred. The progeny of single organisms grown *in vitro* were examined using several clones obtained from HB19, a human isolate (Bundoc & Barbour, 1989). Variants in which OspB differed in molecular mass and its reactivity with monoclonal antibodies were found, but none of the variations were caused by major DNA rearrangements or the non-transcription of *ospB*.

In a second approach, escape variants were selected by obtaining growth of *B. burgdorferi* in the presence of monoclonal antibodies directed against OspA or OspB or both (Sadziene *et al.*, 1992, 1993a; Coleman *et al.*, 1994). Three kinds of variants were obtained. The first had OspA and OspB proteins of the same electrophoretic mobilities as the wild-type but were no longer recognized by selecting antibodies. The second had truncated derivatives of either OspA, OspB or both. The third expressed neither OspA nor OspB and had lost the 49 kbp linear plasmid carrying *ospAB*. Two major conclusions concerning *B. burgdorferi* arose from these studies: (a) OspA and OspB are not essential for *in vitro* growth and (b) reduced size and expression of OspB are associated with lower virulence. A third analysis by Rosa *et al.* (1992) yielded data suggesting that recombination between *ospA* and *ospB* could be a means of generating antigenic diversity.

In conclusion, despite concentrated efforts directed towards the analysis of variability in the major outer-surface proteins of Lyme disease spirochaetes, a mechanism of antigenic variation similar to that found in *B. hermsii* has not been demonstrated.

OspA and OspB recombinant proteins are being evaluated for the development of a vaccine against Lyme disease. Immunization of mice was obtained after syringe injection or infected tick bites (Fikrig *et al.*, 1990, 1992). The mode of immunization is relevant since the *Borrelia* load delivered through a syringe can be up to 10^8 organisms whereas tick bites deliver at most 10^6 (Gern *et al.*, 1993). In addition, tick saliva possesses antiinflammatory properties which have been shown to lower host immune defences. The only vaccine commercialized in the USA is for dogs. This procedure has a 40% efficiency (for a review, see Barbour & Fish, 1993). It is not yet clear whether a human vaccine will provide a realistic way of combating Lyme disease.

OspC, a member of the Vmp family

No function had been ascribed to a circular *Borrelia* plasmid until recently when *ospC* was localized to the 27 kbp plasmid of Lyme disease spirochaetes (Marconi *et al.*, 1993a; Sadziene *et al.*, 1993b). *ospC* encodes the immunodominant major protein formerly known as pC (Fuchs *et al.*, 1992), an outer-membrane protein, which exhibits 70–74% identity across the three species of Lyme disease spirochaetes (Jauris-Heipke *et al.*, 1993; Wilske *et al.*, 1993). Interestingly, all the other analysed *Borrelia* species carry *ospC* on one or more linear plasmids (Marconi *et al.*, 1993b). OspC belongs to a family of surface-exposed proteins which comprise the Vmp of *B. hermsii* (Carter *et al.*, 1994) and has been proposed as a candidate for a vaccine against Lyme disease (Preac-Mursic *et al.*, 1992).

The 27 kbp plasmid of Lyme disease spirochaetes is either very stable or essential for *in vitro* growth because it has never been cured. Sadziene *et al.* (1993b) analysed derivatives of the *B. burgdorferi* B31 lineage for plasmid

content and the expression of *ospC* and found that although *ospC* was present throughout, some derivatives did not produce OspC. In particular, OspC was produced by two high-passage isolates, *B. burgdorferi* B312 and B314, but not by the parental strain, B31, or another high-passage derivative, B313. The plasmid complement of these four strains differed in a way that suggested that a product of the 16 kbp linear plasmid, lp16, specifically caused negative regulation of *ospC*. This hypothesis remains to be tested, since the obvious way of achieving it, by introducing lp16 back into strain B314, is not possible until genetic transfer is available for *B. burgdorferi*.

The regulation of *ospC* expression appears to be interdependent with that of *ospAB*, since low-passaged strain CA-11 2A does not express *ospAB*, but expresses *ospC* (Wilske *et al.*, 1993). After 40 passages, the situation was reversed (Margolis & Rosa, 1993). There is a clear need for further analyses of this area to determine if a regulatory circuit exists that controls the levels of the various Osps.

OspD, a putative virulence factor

An interesting search for proteins important for pathogenesis and immunogenicity was undertaken by Norris *et al.* (1992) using the rationale that proteins present in a low-passage isolate and absent from the corresponding high-passage derivative are potential candidates. Two-dimensional gel electrophoresis was used to identify four proteins associated exclusively with low-passage isolates. The proteins were isolated and found to be lipoproteins. The gene for the most abundant, OspD, a 28 kDa protein, was cloned and located on a 38 kbp linear plasmid. A similar gene is present in plasmids in the 35–40 kbp range in other low-passage strains from *B. burgdorferi* and *B. garinii*. The predicted amino acid sequence of OspD had no significant similarity with the sequences of any proteins in the databases. An intriguing glimpse into the regulation of *ospD* was provided by the discovery of seven 17 bp direct repeats, each containing a –35 and a –10 element of a σ^{70} -like promoter sequence, in its 5' flanking DNA. Transcription initiation was mapped to the closest promoter to the start codon. It was proposed that the six upstream promoter sequences could serve as stacking sites for RNA polymerase molecules awaiting a free promoter and/or sites which undergo recombinational events affecting promoter function. The OspD protein was expressed by low-passage strains but its presence is not required for infectivity. It therefore cannot be a virulence factor.

Endoflagella, characteristic of spirochaetes

Spirochaetes have endoflagella, which are the functional equivalents of the external flagella found in other bacteria. A unique feature of spirochaetal endoflagella is that they are contained within the periplasm (for a review see Charon *et al.*, 1992). One or several flagella, depending on the spirochaete genus, are inserted subterminally at each end of the cell cylinder and provide an efficient motility system for the organism.

In contrast to most spirochaetal endoflagellar filaments, which are composed of a number of polypeptides, the *B. burgdorferi* endoflagellum consists of only one type of subunit, the 41 kDa protein Fla. Analysis of *fla* predicts that the protein is comprised of 336 amino acids and has a molecular mass of 35.8 kDa. The discrepancy between the measured and predicted molecular masses of Fla may result from glycosylation of the gene product (Luft *et al.*, 1989). The sequence of Fla is highly conserved (96–97% identity) within the Lyme disease spirochaetes (Wallich *et al.*, 1990; Gassmann *et al.*, 1991; Jauris-Heipke *et al.*, 1993) and also has a significant degree of homology with the analogous flagellin protein in other spirochaetes (*B. hermsii* and *T. pallidum*) and in phylogenetically distant bacteria (*Bacillus subtilis*, *Serratia marcescens*, *E. coli* and *Salmonella typhimurium*).

A flagella-less *B. burgdorferi* mutant, characterized by a reduced ability to penetrate human endothelial cell layers, has been reported (Sadziene *et al.*, 1991). The mutation involved neither a major DNA rearrangement nor the failure of transcription and was therefore probably a nonsense or frameshift mutation.

Recently, two other flagellin genes, *fliG*, encoding flagellar shift protein, and *flgE*, encoding the hook protein, have also been isolated (N. Charon, unpublished results). The three flagellin genes are not arranged in a single operon but are scattered on the chromosome (Fig. 3).

Potential involvement of heat-shock proteins in autoimmune reactions

It has been suggested that many late symptoms of Lyme borreliosis are caused by autoimmune reactions resulting from structural similarities between borrelial antigens and host proteins. Heat-shock proteins (Hsps), which are highly conserved and present in all cellular organisms (Lindquist, 1986), may be important in the development of these reactions through molecular mimicry by the pathogen. In this regard, it is of interest that a monoclonal antibody specific for the *B. burgdorferi* flagellin detects the human chaperonin HSP60 (Dai *et al.*, 1993). *Borrelia* must survive in the two different environments of an arthropod vector at ambient temperature and a warm-blooded host. The transmission from vector to host by the bite of a tick will cause a sudden increase in temperature similar to the heat stress normally responsible for the heat-shock response. For these two reasons, the molecular biology of borrelial Hsps has attracted attention (Carreiro *et al.*, 1990; Cluss & Boothby, 1990).

The first Hsp that was characterized at the molecular level was the so-called 'common antigen' (Hansen *et al.*, 1988), which is homologous to GroEL from other bacteria. Subsequently, *dnaK*, *dnaJ* and *grpE* were isolated and analysed (Anzola *et al.*, 1992a, b; Tilly *et al.*, 1993). *B. burgdorferi* DnaJ contains four cysteine-rich repeats, each with the Cys-X-X-Cys-X-Gly-X-Gly motif characteristic of DnaJ proteins. *B. burgdorferi* *dnaJ* and *grpE* complement the corresponding *E. coli* mutations while *B. burgdorferi* *dnaK* does not complement *E. coli* *dnaK* mutations. The

grpE, *dnaK* and *dnaJ* genes may form an operon with the promoter upstream of *grpE*.

Genetic transfer and genetic tools

As mentioned in the first section, the genetic analysis of *B. burgdorferi* has been hampered by the absence of any exchange mechanism, such as transduction or transformation, that allows the introduction of genes into *Borrelia* cells. Bacteriophage particles have been visualized by electron microscopy in the culture of a Lyme disease spirochaete but have not been physically isolated (Hayes *et al.*, 1983; Neubert *et al.*, 1993). As yet, none of the linear and circular plasmids have been developed as a tool for gene delivery. Electroporation has resulted in homologous recombination (D. S. Samuels, personal communication) within *Borrelia* wild-type cells transformed with a linear fragment of *Borrelia* DNA carrying a mutation in *gyrB* (Samuels *et al.*, 1994).

Undoubtedly, the development of a transformation system for *Borrelia* is essential for sophisticated genetic analysis in this genus and would be much welcomed by investigators in the field. Similarly, a method for conjugal transfer between a well-studied bacterium such as *E. coli* or *B. subtilis* and *Borrelia* would provide major opportunities for studying the pathogenic and antigenic properties of these spirochaetes.

For editorial reasons, we have carefully limited the number of references cited, using reviews which can lead interested readers back to the original paper wherever possible. We hope that our colleagues will excuse us for not citing all their papers and understand the dilemma we faced.

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