Conservation of gene arrangement and an unusual organization of rRNA genes in the linear chromosomes of the Lyme disease spirochaetes *Borrelia burgdorferi*, *B. garinii* and *B. afzelii*

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Physical maps of the chromosomes of the Lyme disease spirochaetes *Borrelia garinii* and *Borrelia afzelii* have been elucidated for the enzymes *CspI*, *SgrAI*, *I-CeuI*, *SmaI*, *EagI*, *BssHII*, *MluI* and *Apal* by two-dimensional pulsed-field gel electrophoresis techniques. The maps contain 42 sites for *B. garinii* and 32 for *B. afzelii*. The mapping studies showed that the two chromosomes are linear DNA molecules of 953 and 948 kbp, respectively. A comparison of the physical maps of *B. garinii* and *B. afzelii* and the published map of the other Lyme disease spirochaete, *Borrelia burgdorferi* [Davidson, B. E., MacDougall, J. & Saint Girons, I. (1992) *J Bacteriol* 174, 3766–3774] revealed that the three chromosomes have few endonuclease sites in common, apart from a cluster in *rrl* (encoding 23S rRNA) and *rrs* (encoding 16S rRNA). Cloned borrelial genes were used as specific hybridization probes to construct genetic maps, using the physical maps as a basis. The resulting maps contain 41 genetic loci for *B. burgdorferi*, 39 for *B. garinii*, and 33 for *B. afzelii*. In contrast to the physical maps, the three genetic maps are closely related, with no detectable differences in gene order along the entire length of the chromosome. It is concluded that the chromosomes of these three borrelial species have undergone no major rearrangements, deletions or insertions during their evolution from a common ancestor. Detailed mapping of the region of the *B. garinii* and *B. afzelii* chromosomes that encodes rRNA revealed that each chromosome contains one copy of *rrs* separated by 5 kbp from two copies each of *rrl* and *rrf* (encoding 5S rRNA). The gene order is *rrs rrlA rrfA rrlB rrfB*. *B. burgdorferi* is the only other member of the eubacteria for which this particular rRNA gene arrangement has been observed. A DNA length polymorphism in the region of the borrelial rRNA genes was shown to be due to the presence of 2.2 kbp more DNA between *rrs* and *rrlA* in *B. garinii* and *B. afzelii* than in *B. burgdorferi*.

Keywords: *Borrelia*, linear bacterial chromosome, genetic map, rRNA operon, DNA length polymorphism

Abbreviations: *rrs*, 16S rRNA gene; *rrl*, 23S rRNA gene; *rrf*, 5S rRNA gene; PFGE, pulsed-field gel electrophoresis.

The GenBank accession numbers for sequences reported in this paper are L32145 (gb_ba:BORHTPG) for *htpG*; L32146 (gb_ba:BORMETG) for *metG*; L32595 (gb_ba:BORPGKTP1) for *pgk* and *tpi*; L32596 (gb_ba:BORLYME) for the PCR target; L32861 (gb_ba:BORPLSCTOP) for *plsC* and *parE*; L35050 (gb_ba:BORBMPA) for *tmpC*.

INTRODUCTION

The spirochaete *Borrelia burgdorferi*, first reported and described in 1984, is a causative agent of Lyme disease (Burgdorfer *et al.*, 1982; Johnson *et al.*, 1984). DNA–DNA hybridization of *Borrelia* isolates from

around the world has revealed a more complex taxonomy of the Lyme disease spirochaetes than was originally thought. At least two additional new species (*Borrelia garinii* and *Borrelia afzelii*) capable of causing the disease are now recognized (Baranton *et al.*, 1992; Canica *et al.*, 1993). All three species have been isolated in Europe whereas only *B. burgdorferi sensu stricto* is found in the USA. An understanding of the taxonomy of these spirochaetes is important since certain clinical aspects of Lyme disease are dependent on the identity of the infecting organism. Thus, arthritis is associated more commonly with *B. burgdorferi sensu stricto* infections, meningo-radculitis with *B. garinii* infections, and cutaneous manifestations with *B. afzelii* infections (Assous *et al.*, 1993). Genetic analyses of these different species therefore has the potential to provide important insights into the molecular basis of Lyme disease and its wide spectrum of symptoms.

Interest in the molecular genetics of *B. burgdorferi sensu lato* (for a review see Saint Girons *et al.*, 1994) was heightened by the discovery that its chromosome is linear and not circular like those of other characterized bacteria (Baril *et al.*, 1989; Ferdows & Barbour, 1989). The chromosome was also found to be small, around 1 Mbp (Baril *et al.*, 1989; Ferdows & Barbour, 1989). Physical mapping studies yielded results that were consistent with linearity and provided chromosome sizes of 946 and 952 kbp for *B. burgdorferi* strains 212 and Sh-2-82, respectively (Davidson *et al.*, 1992; Casjens & Huang, 1993). A comparison of the chromosomal maps of the two strains revealed that 33 out of the 41 cleavage sites of the six endonucleases used in both mapping studies were conserved (Casjens & Huang, 1993). Details of the molecular structure of the extremities of the chromosome have not been reported, although the telomeres of a 16 kbp linear plasmid also present in *B. burgdorferi* are covalently closed and their nucleotide sequences have been determined (Hinnebusch *et al.*, 1990; Hinnebusch & Barbour, 1991).

The rRNA gene organization in *B. burgdorferi* is also unusual. The chromosome carries a single gene encoding 16S rRNA (*rrs*) separated by 2.7 kbp from a tandemly repeated pair of 23S (*rrlA* and *rrlB*) and 5S rRNA (*rrfA* and *rrfB*) genes (Davidson *et al.*, 1992; Schwartz *et al.*, 1992; Fukunaga & Sohnaka, 1992; Gazumyan *et al.*, 1994). This gene organization contrasts with the situation in most bacteria, which have one or more rRNA operons each containing one copy of *rrs*, *rrl* and *rrf*.

The small size of the chromosome of Lyme disease spirochaetes makes them useful organisms for interspecies comparative studies aimed at providing insights into chromosome evolution. We have therefore commenced an investigation of the genetic relationship between *B. burgdorferi*, *B. garinii* and *B. afzelii*. In this paper we present data on the elucidation of physical and genetic maps of the *B. garinii* and *B. afzelii* chromosomes. The new maps have been compared with previously published physical and genetic maps of the *B. burgdorferi* chromosome (Davidson *et al.*, 1992; Old *et al.*, 1992a; Casjens & Huang, 1993).

The results of this investigation indicate that the physical maps of the *B. burgdorferi*, *B. garinii* and *B. afzelii* chromosomes have few common features, but the genetic maps are strikingly similar. We interpret these observations to indicate that the divergent evolution of the chromosomes of these three species involved changes in their nucleotide sequences but no major rearrangements or inversions.

METHODS

Bacterial strains, plasmids and gene probes. *B. burgdorferi* strain 212, *B. garinii* strains 20047, Ir210, NBS16, NT29, PD89 and Sika1, and *B. afzelii* strains VS461, BO23, ECM1 and IPer3 (Baranton *et al.*, 1992; Canica *et al.*, 1993; Fukunaga *et al.*, 1993), from the strain collection of the Institut Pasteur, were grown in BSKII medium (Barbour, 1984) as described previously for *B. burgdorferi* 212 (Davidson *et al.*, 1992). The strains used for mapping studies were *B. burgdorferi* 212, *B. garinii* 20047 and *B. afzelii* VS461, and unless specified otherwise the terms *B. burgdorferi*, *B. garinii* and *B. afzelii* have been used throughout to refer to these three strains. Plasmids used as hybridization probes for genetic mapping studies are listed in Table 1. The *pgk*, *tpi*, *plsC*, *parE*, *dnaB*, *gidB*, *moxR*, *tmpC*, *htpG*, *metG*, *miaA*, *pth* and *ftsH* genes were isolated and identified by sequencing of randomly chosen clones from a library of *B. burgdorferi* 212 DNA in pUC18 (Old *et al.*, 1992a). All other genes used (see Table 1) were also from *B. burgdorferi*, with the exception of the *p100* gene, which was from *B. afzelii*.

DNA preparations and mapping studies using pulsed-field gel electrophoresis (PFGE). The methods used for the preparation of high-molecular-mass genomic and chromosomal DNA in agarose blocks, DNA digestions, purification of DNA restriction fragments and two-dimensional PFGE experiments were those described previously (Davidson *et al.*, 1992; Tulloch *et al.*, 1991). The following techniques were used to elucidate physical maps of the chromosomes: double digests of high-molecular-mass chromosomal and genomic DNA, redigestion of PFGE-purified fragments with a second restriction endonuclease, and determination of the hybridization properties of fragments with a specific gene probe whose map location had been independently established. PFGE was performed in either a CHEF-DRII or a Chef Mapper apparatus (Bio-Rad). The borrelial DNA used as a template for the PCR was prepared from pelleted cells as described by Carniel *et al.* (1989).

Fragment nomenclature. Fragments produced by digestion of the chromosome by a single restriction endonuclease have been designated Sg, Ce, Sm, Ea, Bs, Ml, Ap, Cs, or Sc to identify the enzyme [*SgrAI*, *I-CeuI*, *SmaI*, *EagI*, *BssHII*, *MluI*, *ApaI*, *CspI*, or *SacII* (*KspI*), respectively], with a capital letter suffix, A or B, etc. in the order of decreasing fragment size (Table 2). For fragments indistinguishable in size, the suffix is numbered 1 or 2, etc., e.g. ApB1 and ApB2. Fragments produced by digestion with two restriction endonucleases have been designated by the two single-digest fragments from which they were derived, e.g. SmC–ApB1 contains DNA from the overlapping region of SmC and ApB1.

PCR. Specific primers (Table 3), designed to amplify part of the borrelial rRNA gene cluster, were synthesized on a model 381A DNA synthesizer (Applied Biosystems). The PCR was carried out in a model FTS-1C thermal cycler (Corbett-Research) with *Taq* DNA polymerase (Perkin Elmer-Cetus), using buffers supplied by Perkin Elmer-Cetus, 250 ng of each primer, and

Table 1. Hybridization of gene probes with *B. garinii* 20047 and *B. afzelii* VS461 chromosome fragments

Gene(s)	Plasmid/ probe	Gene product(s)	Reference/ source	<i>B. garinii</i>		<i>B. afzelii</i>	
				Hybridizing fragments*	Map location (kbp)	Hybridizing fragments*	Map location (kbp)
<i>pgk tpi</i>	pB45	Phosphoglycerate kinase, triose phosphate isomerase	GenBank L32595	BsD	0–90	ND	ND
<i>plsC parE</i>	pB38	1-Acyl- <i>sn</i> -glycerol-3-phosphate acetyltransferase, B-subunit of topoisomerase IV	GenBank L32861	BsD	0–90	SmD	0–118
<i>dnaB</i>	pB54	DnaB	D. Margarita & I. Old†	MIC	0–135	SmD, ApA	27–118
<i>fla</i>	pARA1	Flagellin	Gassmann <i>et al.</i> (1991)	ApB1	151–165	SmA, CsB	118–209
<i>gidA</i>	pB4	Glucose-inhibited division protein	Old <i>et al.</i> (1992a)	ApB1	184–241	SmA, CsB	118–209
<i>gidA</i> , <i>AB</i> <i>moxR</i>	pB43	Glucose-inhibited division proteins, regulator for methanol dehydrogenase	D. Margarita & I. Old†	ApB1	184–241	SmA, CsB	118–209
<i>rbo</i>	PCR product	Transcription termination factor rho	Tilly & Campbell (1993)	SmA, ApH2	241–264	SmA	118–269
<i>flgE</i>	M13DNAflgE	Flagellar hook protein	N. Charon, West Virginia University, USA	SgB–ApE2	264–316	ApB, SmC	278–398
<i>flhG</i>	pB30	Flagellar shift protein	D. Margarita & I. Old†	SgA, SgB, ApE2	315–317	ApB, SmC	278–398
<i>ftsA</i> , <i>Z</i> , p60 gene	pB29 pHMB35	Cell division proteins 60 kDa antigen	Old <i>et al.</i> (1992a) Howe <i>et al.</i> (1985)	ApE2 ApC2	316–326 326–408	ApB, SmC ND	278–398 ND
<i>tmpC</i>	pB46	Membrane lipoprotein	D. Margarita & I. Old†	SmA, ApC2	326–408	ND	ND
<i>rrlAB</i>	CO1–CO6 PCR product	3' end of 23S rRNA	This work	SmA, SmF, MIG	456–463	SmL, SmI or SmJ	461–468
<i>rrlAB</i>	CO8–CO9 PCR product	5' end of 23S rRNA	This work	SmF, SmG	456–463	SmL, SmK– MIA	461–468
<i>rrs</i>	CO4–CO5 PCR product	3' end of 16S rRNA	This work	SmD–MIG	468–469‡	SmK, MIA	473–474‡
<i>gyrB</i> , <i>B.1</i>	pB3	B- and A-subunits of DNA gyrase	Old <i>et al.</i> (1992a)	EaE, MIF	469–520	SmK, MID	482–488§
<i>gyrB</i> , <i>dnaAN</i> , <i>rpmH</i> , <i>rnpA</i>	pB22	B-subunit of DNA gyrase, DNA replication proteins, ribosomal protein L34, ribonuclease P (protein component)	Old <i>et al.</i> (1992b, 1993a, b); Saint Girons <i>et al.</i> (1994)	EaE, MIF	469–520	SmE, EaC	488–492§
<i>pheST</i>	pBDP218	α - and β -subunits of phenylalanyl-tRNA synthetase	J. Hinnebusch & A. Barbour, University of Texas Health Science Center, USA	MID, ApC1	542–616	SmE, MIC	529–566
<i>dnaJ</i> , <i>dnaK</i>	pKK11	Heat-shock proteins	Tilly <i>et al.</i> (1993)	MID, ApC1	542–616	MIC	529–613
<i>hspG</i> , PCR	pB25 pB2	Heat-shock protein PCR target	GenBank L32145 Old <i>et al.</i> (1992a); Rosa <i>et al.</i> (1991)	MID, ApC1 SmE, EaC	542–616 627–698	SmH ND	566–612 ND
<i>metG</i> , <i>groEL</i>	pB7 pKH317	Methionyl-tRNA synthetase Heat-shock proteins	GenBank L32146 Hansen <i>et al.</i> (1988)	SmE, EaC SgA, ApE1	627–698 706–728	ND SmB	ND 686–824
p100 gene	p100	100 kDa antigen	Jauris-Heipke <i>et al.</i> (1993)	MIb, ApA	823–843	SmF2	824–898
<i>ptb ftsH</i>	pB15	Peptidyl-tRNA hydrolase, cell division protein	D. Margarita & I. Old†	MIE	843–923	SmF2	824–898
<i>miaA</i>	pB5	2-Methylthio- <i>N</i> 6-isopentyladenosine hypermodification of tRNA	D. Margarita & I. Old†	MIE	843–923	SgB, SmG	898–936

ND, Not determined.

* Only the minimum number of fragments that define the gene location are listed.

† Unpublished results; *tmpC* corresponds to p39 (Casjens & Huang, 1993).‡ Precise location was established from the *MluI* site in the 16S rRNA sequence (Marconi & Garon, 1992a).§ Precise locations of *gyrBA* and *dnaAN rpmH rnpA* were established from a deduced *SmaI* site in *B. afzelii dnaA*.

Table 2. Endonuclease fragments of the chromosomes of *B. garinii* 20047 and *B. afzelii* VS461

The lower limit of fragment size detected was 1.4 kbp. Adjusted values (Adj) are the observed values after corrections to give chromosome sizes of 953 and 948 kbp for *B. garinii* and *B. afzelii*, respectively, and after minor adjustments to fit fragments into the map. ND, Not determined.

Fragment	Mean size (kb) of fragment generated by:																	
	<i>CspI</i>		<i>SgrAI</i>		<i>SacII</i>		<i>I-CeuI</i>		<i>SmaI</i>		<i>EagI</i>		<i>BssHII</i>		<i>MluI</i>		<i>ApaI*</i>	
	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj	Observed	Adj
<i>B. garinii</i> †																		
A			400	412	ND		493	493	275	274	460	457	278	278	280	278	130	130
B			310	316			458	457	255	251	190	185	260	255	230	219	90‡	90
C			220	225				3.2 3	182	184	180	178	184	192	140	135	80‡	82
D									153	164	70	70	90	90	85	82	70‡	70
E									75	75	60	60	75	75	80	80	62‡	62
F									3.2	3	3.2	3	37	37	60	59	55	55
G									1.8	2			23	23	54	56		
H													3.2	3	34	30	23	23
I															15	14		
Total			930	953			954	953	945	953	963	953	950	953	978	953		
<i>B. afzelii</i>																		
A	750	739	750	743	474	474	485	483	151	151	463	462	485	483	468	474	202	200
B	209	209	193	193	389	387	460	462	138	138	437	428	458	462	356	335	157	155
C			12	12	88	87		3.2 3	129	129	56	55	3.2	3	86	84	113	
D									118	118		3.2 3			56	55	104	
E									78	78							61	
F									74‡	74							57	
G									50	50							53	
H									46	46							51	51
I									34	34							43	
J									30	30							40	40
K									23	23							27	27
L									3.2	3							23	23
M																	15	
N																	10	
Total	959	948	955	948	951	948	948	948	948	948	959	948	946	948	966	948	956	

* List of fragments is incomplete for *B. garinii*.

† The *B. garinii* chromosome was not digested by *CspI*.

‡ Relative staining intensities indicated the presence of two of each of the *B. garinii* *ApaI* fragments of 90, 80, 70 and 62 kbp, and two *B. afzelii* *SmaI* fragments of 74 kbp.

30–100 ng template DNA in a final volume of 50 µl. The reactions were repeated for 35 cycles with a profile of 1 min at 95 °C, 1 min at 43 °C and 1 min at 72 °C. A 10 µl sample of the products was analysed by electrophoresis through agarose in

TBE (TBE is 89 mM Tris, 89 mM borate and 2 mM EDTA, pH 8.0).

Table 3. Oligonucleotides used as PCR primers for rRNA gene amplification

Oligonucleotide	Sequence	rRNA gene	Position in sequence*
CO1	AGGACCGAGATGGAC	<i>rrl</i>	2701–2716
CO2	CTTAAAGCTCCTAGGC	<i>rrl</i>	45–30
CO3	ATAACCTACTCTCCCG	<i>rrf</i>	104–89
CO4	CAAATCATCATGGCC	<i>rrs</i>	1188–1203
CO5	ATAGCTTCGGGATTCC	<i>rrs</i>	1432–1417
CO6	TGGCCAAAGATACGG	<i>rrl</i>	2925–2910
CO7	GGTTAAAGAAAAGAGG	<i>rrf</i>	5–20
CO8	GAAGGTGATAGTCCTGT	<i>rrl</i>	393–409
CO9	CTCTTCTCTATCGGTAG	<i>rrl</i>	531–515

* From Schwartz *et al.* (1992).

Southern blots and DNA hybridizations. Southern blots were prepared by the procedure of transfer with depurination (Reed & Mann, 1985), using nylon membranes (Hybond N+, Amersham). ³²P- and digoxigenin-labelled DNA probes were obtained by random priming (Feinberg & Vogelstein, 1984) using commercial kits (Boehringer Mannheim). Membranes were prehybridized with a solution containing 0.5% (w/v) SDS, 5× Denhardt's solution (1× Denhardt's solution is 0.02%, w/v, bovine serum albumin, 0.02%, w/v, Ficoll, and 0.02%, w/v, polyvinylpyrrolidone), and 5× SSPE (1× SSPE is 36 mM NaCl, 2 mM Na₂HPO₄ and 1 mM EDTA, pH 7.4). After hybridization with the probe at 60 °C for 18 h, the final stringent wash of the membrane was performed in 0.1× SSPE containing 0.1% (w/v) SDS for 15 min at 65 °C.

RESULTS

Restriction digestion and estimation of the sizes of the *B. garinii* and *B. afzelii* chromosomes

PFGE of undigested high-molecular-mass DNA from *B. garinii* or *B. afzelii* yielded a band with a mobility typical of

Fig. 1. Maps of the chromosomes of *B. garinii* 20047, *B. burgdorferi* 212 and *B. afzelii* VS461. (a) Physical maps. For each enzyme, the *B. garinii* map is represented at the top by open boxes, the *B. burgdorferi* map (Davidson *et al.*, 1992; *CspI* and *I-CeuI* sites mapped in this study) is represented in the middle by shaded boxes, and the *B. afzelii* map is represented at the bottom by open boxes. The three maps have been aligned by the centrally located *MluI* site in *rrs*. Fragments are designated A, B, etc. Only some of the *ApaI* sites and fragments are included in each map. (b) Genetic maps. Fill patterns for individual genes or gene clusters are the same in all three maps, with the *B. burgdorferi* map providing the key for the correspondence between specific fill pattern and gene/gene cluster. The genes *alaT* and *ileT* were not mapped for *B. garinii* and *B. afzelii*, while *pgk*, *tpi*, *tmpC*, *metG*, *PCR* and the *p60* gene were not mapped for *B. afzelii*.

a linear 1 Mbp DNA molecule, as reported previously (e.g. see Fig. 1a, lane 11 of Baril *et al.*, 1989). This 1 Mbp species was presumed to be the linear chromosome. Fragments of < 100 kbp, which were probably plasmids, were also seen. Endonucleases having GC-rich recognition sites (*SgrAI*, *I-CeuI*, *SmaI*, *MluI*, *BssHII*, *EagI* and *ApaI*) were found to cut the two chromosomes into a useful number of fragments for mapping studies (Table 2). *SacII* and *CspI* were also used for mapping *B. afzelii* (*CspI* did not digest the *B. garinii* chromosome). As was

found previously with *B. burgdorferi* (Davidson *et al.*, 1992), the presence of the plasmids complicated the digestion patterns obtained with high-molecular-mass genomic DNA from *B. garinii* and *B. afzelii*. The uncertainties in interpretation of the patterns were avoided by first isolating high-molecular-mass chromosomal DNA in agarose by PFGE, then digesting this DNA. The sum of the fragment sizes produced by each enzyme yielded estimates for the size of the two chromosomes (Table 2). For *B. garinii*, the mean value of

953 kbp for the first six enzymes was used as the chromosome size in the construction of the restriction map. For *B. afzelii*, each of the enzymes that was used for making a full map yielded only a few large fragments, with the exception of *Sma*I. Because of this it was considered most reliable to use the sum of sizes of the *Sma*I fragments only (948 kbp) for an estimate of the size of the *B. afzelii* chromosome.

Physical and genetic maps of the *B. garinii* and *B. afzelii* chromosomes

Physical maps of the *B. garinii* and *B. afzelii* chromosomes (Fig. 1a) were elucidated as described in Methods. The maps contain 42 digestion sites for *B. garinii* and 32 for *B. afzelii* (Table 4). A comparison of these maps with that of the *B. burgdorferi* chromosome (Fig. 1a) reveals few common sites in the three chromosomes, with the striking exception of a cluster of sites near the centre of each map. Analyses, presented below, indicated that these sites are located in the rRNA genes, loci that are both GC-rich and highly conserved. The similar location of certain other sites in two of the three species suggested limited conservation of these sites. Specifically, these were the *Bss*HII sites at map coordinates 921 and 930 in *B. burgdorferi* and *B. garinii*, respectively; the *Apa*I sites at map coordinates 523 and 534 in *B. burgdorferi* and *B. garinii*, respectively; and three pairs of *Sma*I sites (at map coordinates 689 and 702 in *B. burgdorferi* and *B. garinii*, map coordinates 669 and 686 in *B. burgdorferi* and *B. afzelii*, and map coordinates 592 and 612 in *B. burgdorferi* and *B. afzelii*).

The physical maps were used for constructing genetic maps. To do this, probes containing borrelial genes (Table 1) were hybridized with Southern blots of gel separations of restriction digests of *B. burgdorferi*, *B. garinii* and *B. afzelii* DNA to identify the restriction fragment that carried the gene. Using this approach, genetic maps were determined for the first time for *B. garinii* and *B. afzelii* and our previously reported genetic map of *B. burgdorferi* 212 (Old *et al.*, 1992a) was expanded from 14 to 41 loci (Fig. 1b). Nineteen of these 41 genes on the *B. burgdorferi* map (*fla*, *rbo*, *flgE*, *rrlA*, *rrlB*, *rrfA*, *rrfB*, *rrs*, *alaT*, *dnaN*, *dnaA*, *gyrB*, *gyrA*, *pheS*, *pheT*, *dnaJ*, *dnaK*, *groEL* and the p100 gene) have also been mapped on an American isolate of *B. burgdorferi*, Sh-2-82 (Casjens & Huang, 1993). Each of these genes was found to occupy the same position in the genome of both isolates, within the limits of precision of the mapping experiments.

The genetic maps of the chromosomes of the three different Lyme disease spirochaetes contain 41 loci for *B. burgdorferi*, 39 for *B. garinii* and 33 for *B. afzelii* (Fig. 1b). The uneven and different distributions of endonuclease sites along each of the chromosomes result in large dissimilarities in the resolution of the genetic maps for the same region of each chromosome. Although these dissimilarities complicate the comparison of the three maps, it is clear that the mapping studies provide no indication of any differences in the gene order of the chromosomes of the three species (Fig. 1b).

Table 4. Map locations of endonuclease sites in the *B. garinii* 20047 and *B. afzelii* VS461 chromosomes

The map coordinates are based on the sizes of single-digestion products (Table 2) and double-digestion products (data not shown).

<i>B. garinii</i>		<i>B. afzelii</i>	
Endonuclease	Map coordinate of site (kbp)	Endonuclease	Map coordinate of site (kbp)
<i>Bss</i> HII	90	<i>Apa</i> I	27
<i>Mlu</i> I	135	<i>Sac</i> II	87
<i>Apa</i> I	151	<i>Sma</i> I	118
<i>Bss</i> HII	165	<i>Csp</i> I	209
<i>Sma</i> I	184	<i>Apa</i> I	227
<i>Apa</i> I	241	<i>Sma</i> I	269
<i>Apa</i> I	264	<i>Apa</i> I	278
<i>Sgr</i> AI	316	<i>Sma</i> I	398
<i>Apa</i> I	326	<i>Apa</i> I	433
<i>Apa</i> I	408	<i>Apa</i> I	456
<i>Mlu</i> I	413	<i>Eag</i> I	462
<i>Bss</i> HII	420	<i>I-Ceu</i> I	463
<i>Apa</i> I	441	<i>Bss</i> HII	463
<i>Eag</i> I	457	<i>Sma</i> I	464
<i>I-Ceu</i> I	458	<i>Eag</i> I	465
<i>Bss</i> HII	458	<i>I-Ceu</i> I	466
<i>Sma</i> I	459	<i>Bss</i> HII	466
<i>Eag</i> I	460	<i>Sma</i> I	467
<i>I-Ceu</i> I	461	<i>Sac</i> II	474
<i>Bss</i> HII	461	<i>Mlu</i> I	474
<i>Sma</i> I	462	<i>Sma</i> I	488
<i>Sma</i> I	464	<i>Apa</i> I	496
<i>Apa</i> I	464	<i>Eag</i> I	520
<i>Mlu</i> I	469	<i>Mlu</i> I	529
<i>Eag</i> I	520	<i>Sma</i> I	566
<i>Mlu</i> I	528	<i>Sma</i> I	612
<i>Apa</i> I	534	<i>Mlu</i> I	613
<i>Mlu</i> I	542	<i>Sma</i> I	686
<i>Apa</i> I	616	<i>Sgr</i> AI	743
<i>Mlu</i> I	624	<i>Sma</i> I	824
<i>Sma</i> I	627	<i>Sma</i> I	898
<i>Eag</i> I	698	<i>Sgr</i> AI	936
<i>Sma</i> I	702		
<i>Apa</i> I	706		
<i>Sgr</i> AI	728		
<i>Bss</i> HII	738		
<i>Eag</i> I	768		
<i>Apa</i> I	768		
<i>Apa</i> I	823		
<i>Mlu</i> I	843		
<i>Mlu</i> I	923		
<i>Bss</i> HII	930		

High resolution mapping around the rRNA gene loci in *B. garinii* and *B. afzelii*

In view of the close similarity of the genetic maps it was

decided to use higher resolution mapping to analyse a short segment of the chromosome in more detail. The region containing the rRNA genes was chosen for this analysis since the number and arrangement of rRNA genes in *B. burgdorferi* is unique among eubacteria (Davidson *et al.*, 1992; Schwartz *et al.*, 1992; Fukunaga & Sohnaka, 1992). The *B. burgdorferi* chromosome has a single copy of *rrs*, separated by 2.7 kbp from a tandem duplication containing two copies each of *rrl* and *rrf* (Fig. 2a). Specific probes for *rrl*, *rrs* and *rrf* were made by PCR using CO1–CO6, CO4–CO5 and CO3–CO7, respectively, as amplicon pairs (Fig. 2a). *B. burgdorferi* DNA was used as the template DNA for the PCR.

rRNA gene copy numbers. The *rrs* probe hybridized with one *Mlu*I fragment (M1G for *B. garinii* and M1A for *B. afzelii*) and one *Hpa*I fragment (of 7 kbp for *B. garinii* and 2 kbp for *B. afzelii*) in Southern blots prepared from digests of *B. garinii* and *B. afzelii* DNA. Since there is only one site each for *Mlu*I and *Hpa*I in the reported *B. garinii* and *B. afzelii* 16S rRNA sequences [strains G1 (Marconi & Garon, 1992a) and R-IP3 (Marconi & Garon, 1992b), respectively], this observation established the presence of a single copy of *rrs* in both the *B. garinii* and *B. afzelii* chromosomes.

The *rrl* and *rrf* probes behaved identically when hybridized with Southern blots. With digests of the *B. garinii* chromosome, each probe hybridized to two *Hpa*I fragments (of 1.7 and 3.2 kbp) and two *Sma*I fragments (*Sma*A and *Sma*F). A similar result was obtained with

digests of *B. afzelii* DNA, where hybridization occurred with *Hpa*I fragments of 4.0 and 3.2 kbp and *Sma*I fragments *Sma*L and *Sma*I (or *Sma*J). These observations were consistent with the presence of two copies each of *rrl* and *rrf* in the chromosomes of both organisms.

Juxtaposition of *rrl* and *rrf*. The juxtaposition of the *rrl* and *rrf* genes in *B. garinii* and *B. afzelii* was determined by PCR with the amplicon pair CO1–CO3. PCR with these primers and *B. burgdorferi* DNA as template yielded a product of 350–355 bp, the size expected from the known juxtaposition of *rrl* and *rrf* in this organism (Fig. 2a). PCR products of a similar size were obtained with the same amplicon pair when either *B. garinii* or *B. afzelii* DNA was used as template. These observations indicated that the juxtaposition of *rrl* and *rrf* in *B. garinii* and *B. afzelii* was identical to that in *B. burgdorferi* (Fig. 2b).

Tandem duplication of *rrl*. The possibility that two copies of *rrl* were present in a tandem duplication in the *B. garinii* and *B. afzelii* chromosomes was explored by carrying out PCR with CO1–CO2 as amplicons. With *B. burgdorferi* DNA as template a single product of 586 bp was obtained. This product was of the size expected from the reported sequence (Schwartz *et al.*, 1992) for amplification from the 3' end of *rrlA* through *rrfA* into the 5' end of *rrlB* (Fig. 2a), and was indicative of one or more tandem duplications of *rrl* genes. Identically sized PCR products were obtained when either *B. garinii* or *B. afzelii* DNA was used as template with CO1–CO2, indicating that the rRNA genes were also tandemly

Fig. 2. Maps of the rRNA genes and intervening DNA of (a) *B. burgdorferi* and (b) *B. garinii* and *B. afzelii*. The *B. burgdorferi* map was derived from published data (Davidson *et al.*, 1992; Schwartz *et al.*, 1992; Gazumyan *et al.*, 1994). For the *B. garinii* and *B. afzelii* maps, sites were located as follows: *Sac*II sites, from the sequences of *B. garinii* G1 and *B. afzelii* R-IP3 16S rRNA (Marconi & Garon, 1992a, b); *Hpa*I sites, from Southern blotting data (this study) and the 16S rRNA sequences; *Eag*I, *Bss*HI and *Sma*I sites, from data in this study, assuming identity with the *B. burgdorferi* 23S rRNA gene when assigning precise locations. Sites marked (Bg) or (Ba) were only found in *B. garinii* or *B. afzelii*, respectively. The *Sma*I site marked (Bg*) was presented in *B. garinii* strains 20047, Ir210 and NB516 but not strains NT29, PD89 and Sika1. CO1, CO2, etc. indicate the location of PCR amplicons listed in Table 3. The 16 kbp mark on the scale corresponds to map locations 458 kbp (*B. burgdorferi*), 469 kbp (*B. garinii*) and 474 kbp (*B. afzelii*).

duplicated in these organisms. An *rrf* probe hybridized with these PCR products, confirming that in each case *rrf* was located alongside the 3' end of *rrl*. A 3.2 kbp fragment was seen in *I-CeuI*, *SmaI*, *EagI* and *BssHII* digests of *B. garinii* and *B. afzelii* DNA (Table 2). The observation that these fragments hybridized with an *rrl*-specific probe was consistent with the presence of a 3.2-kbp-long tandem duplication of *rrl* containing sites for each of these four enzymes (Fig. 2). PCR was carried out using CO8–CO6 as amplimers and a template of *B. burgdorferi*, *B. garinii* or *B. afzelii* DNA, then the reaction products were digested with *SmaI*, *EagI* or *BssHII* and the sizes of the digestion products were determined. For each enzyme the fragment sizes from the different templates were indistinguishable, indicating that the *SmaI*, *EagI* and *BssHII* sites in the *B. garinii* and *B. afzelii* *rrl* genes were in similar, if not identical, positions to those in the *B. burgdorferi* *rrl* genes (Fig. 2). The number of copies of the *rrl rrf* tandem repeat was calculated by using previously reported values for the sizes of *EcoRV* fragments that hybridized with a total rRNA probe (Baranton *et al.*, 1992). Summation of these sizes indicated that the rRNA genes were contained within 10.1 kbp for *B. garinii* and 11.5 kbp for *B. afzelii*. Given the sizes of *rrl*, *rrf*, *rrs* and the intervening DNA, it is not possible to contain more than two copies of the *rrl rrf* tandem repeat within these limits.

Juxtaposition of *rrs* and *rrl*. Digestion of *B. burgdorferi* DNA with *MluI* plus *BssHII* yielded a 6.2 kbp product that hybridized with the *rrs* probe (CO4–CO5) but not the *rrl* probe (CO1–CO6). By contrast, the corresponding fragment in digests of *B. garinii* and *B. afzelii* DNA was found to be 8.4 kbp. This and similar results with other enzyme digestions indicated the presence of an additional 2.2 kbp of DNA between *rrs* and *rrl* in *B. garinii* and *B. afzelii* compared with *B. burgdorferi* (Fig. 2b). This additional DNA contained an extra *SmaI* site (Fig. 2b) in *B. garinii* 20047, yielding a 1.8 kbp *SmaI* fragment (SmG) that hybridized to the *rrl* probe CO8–CO9. The *SmaI* site was not invariant in all *B. garinii* strains, being present in strains Ir210 and NBS16 but not NT29, PD89 and Sika1. It was absent from all the *B. afzelii* strains examined: VS461, BO23, ECM1 and IPer3.

DISCUSSION

Three species of spirochaete, *B. burgdorferi*, *B. garinii* and *B. afzelii*, have been implicated as aetiological agents of Lyme borreliosis (Burgdorfer *et al.*, 1982; Baranton *et al.*, 1992; Canica *et al.*, 1993). Of the three, *B. burgdorferi* has been studied most closely at the genomic level and was the first bacterium found to possess a linear chromosome (Baril *et al.*, 1989; Ferdows & Barbour, 1989; Davidson *et al.*, 1992). Since that discovery, linear chromosomes have been found in other bacteria, e.g. *Streptomyces lividans* (Lin *et al.*, 1993; Hinnebusch & Tilly, 1993). The physical mapping studies presented in this publication confirm that the chromosomes of *B. garinii* and *B. afzelii* share this unusual property of linearity. Another unusual feature of the *B. burgdorferi* genome is its complement and organization of rRNA genes. Most bacteria contain

equivalent numbers of each of the three rRNA genes grouped in rRNA operons (Krawiec & Riley, 1990). Every operon contains a single copy of each gene, and commonly the operons are present in the chromosome in multiple copies. *B. burgdorferi* differs from this pattern because it has only one copy of *rrs* and two copies of *rrl* and *rrf* (Fig. 2a) (Davidson *et al.*, 1992; Schwartz *et al.*, 1992; Fukunaga & Sohnaka, 1992). Furthermore, the presence of 2.7 kbp of DNA containing another gene in the opposite orientation between *rrs* and *rrlA* (*ileT*) suggests that *rrs* is transcribed independently of the other rRNA genes (Gazumyan *et al.*, 1994). Our PCR and hybridization analyses indicate that this unusual complement and organization of rRNA genes is also present in *B. garinii* and *B. afzelii*. This conclusion is in agreement with that reached by Schwartz *et al.* (1992) from an analysis of restriction fragment length polymorphisms in the *B. burgdorferi sensu lato* strains 20047 and IP3. Their strain 20047 has been reclassified as the *B. garinii* type strain and is the same as the strain used in this study, while their IP3, a Russian isolate also known as R-IP3 and IPer3, has been classified as *B. afzelii* (Canica *et al.*, 1993; Marconi & Garon, 1992b).

It had previously been observed that there is a high degree of conservation of cleavage site location in the chromosomes of two different isolates of the species *B. burgdorferi* (strains 212 and Sh-82-2) (Casjens & Huang, 1993). The present work indicates that this conservation does not extend to an interspecies comparison, since the chromosomes of *B. burgdorferi*, *B. garinii* and *B. afzelii* were found to have few conserved endonuclease sites. The conserved sites occur mostly in a cluster in the rRNA genes *rrl* and *rrs*, loci that are known to exhibit a high degree of sequence conservation (Marconi & Garon, 1992b). This level of dissimilarity in the maps is not unexpected since the three bacterial genospecies exhibit less than 70% DNA cross-hybridization (Baranton *et al.*, 1992; Canica *et al.*, 1993) and the endonucleases used for the mapping had recognition sequences of 6 or more bp.

In contrast to the physical maps, the genetic maps of the three different species are extremely closely related. No differences between the gene order of the three chromosomes were observed, although the existence of small differences cannot be unequivocally ruled out because the genetic mapping could only be carried out at low resolution. Nevertheless, it is clear that there has been no large inversion or translocation involving a significant proportion of either of the three chromosomes. Because the sizes of the three chromosomes do not differ significantly, the occurrence of large-scale deletions or insertions can also be ruled out, except for the unlikely situation of compensating insertions and deletions. We conclude from this analysis that the chromosomes of the Lyme disease spirochaetes have not undergone major genetic rearrangements during the divergent evolution of the three species from their common ancestor, and that differences between the physical maps most likely result from base substitutions. This genetic stability may be

related to the clonal properties of *Borrelia* (Dykhuizen *et al.*, 1993).

Detailed analyses of the region of the chromosome that contains the rRNA genes revealed the presence of a DNA length polymorphism in the Lyme disease spirochaetes. Both *B. garinii* and *B. afzelii* contain an additional 2.2 kbp of DNA between *rrs* and *rrlA*. The function of this DNA and its coding properties, if any, are not known. Interestingly, the existence of this polymorphism is partly responsible for the restriction fragment length polymorphism which facilitated the discovery that *B. burgdorferi sensu lato* consists of three different genospecies (Baranton *et al.*, 1992; Canica *et al.*, 1993; Fukunaga *et al.*, 1993). We have no data that shed light on the molecular nature of the genetic event responsible for the DNA length polymorphism. Its occurrence in the vicinity of rRNA genes may be significant, since the repeated sequences that result from the presence of multiple copies of these genes have been associated with the chromosomal rearrangement that characterizes the *Escherichia coli* K12 strain W3110 (Hill & Harnish, 1981). The presence of enzymes that mediate genetic recombination in the Lyme disease *Borrelia* can be inferred from the observation of recombination between two related *B. burgdorferi* genes, *ospA* and *ospB* (Rosa *et al.*, 1992). Determination of the nucleotide sequence between *rrs* and *rrlA* in *B. garinii* and *B. afzelii* and comparison of it with the known *B. burgdorferi* sequence (Gazumyan *et al.*, 1994) should provide insights into the mechanism by which the polymorphism arose.

The genetic stability seen in the chromosomes of these three borrelial species is in contrast to the situation in another spirochaete, *Leptospira interrogans*, where the chromosomes of two serovars exhibited large rearrangements (Zuerner *et al.*, 1993). Comparative genetic maps of four *Bacillus cereus* strains also indicate a significant degree of genomic plasticity in that organism (Carlson *et al.*, 1992). On the other hand, marked genetic stability has been found in the chromosomes of ten *Clostridium perfringens* strains (Canard *et al.*, 1992) and five *Mycoplasma hominis* strains (Ladefoged & Christiansen, 1992), while different strains of *Mycoplasma mycoides* subsp. *mycoides* have few differences in their chromosomal gene order (Pyle *et al.*, 1990). The well-characterized congruence between the genetic maps of *E. coli* and *Salmonella typhimurium* and their similarities with the *Shigella flexneri* map is another example of long-term retention of gene order (Riley & Krawiec, 1987; Okada *et al.*, 1991). In the future it is likely that the availability of a more extensive database on genetic variation in different bacterial species and genera will enable a meaningful evaluation to be made of the relative genetic stability in any one genus. At present the data are too limited to allow us to conclude whether or not the Lyme disease *Borrelia* are exceptional in terms of the stability of their chromosome.

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REFERENCES

- Assous, M. V., Postic, D., Paul, G., Névot, O. & Baranton, G. (1993). Western blot analysis of sera from Lyme borreliosis patients according to the genomic species of the *Borrelia* strains used as antigens. *Eur J Clin Microbiol & Infect Dis* **12**, 261–268.
- Baranton, G., Postic, D., Saint Girons, I., Boerlin, P., Piffaretti, J.-C., Assous, M. & Grimont, P. A. D. (1992). Delineation of *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* **42**, 378–383.
- Barbour, A. G. (1984). Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* **57**, 521–525.
- Baril, C., Richaud, C., Baranton, G. & Saint Girons, I. (1989). Linear chromosome of *Borrelia burgdorferi*. *Res Microbiol* **140**, 507–516.
- Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E. & Davis, J. P. (1982). Lyme disease – a tick-borne spirochetosis? *Science* **216**, 1317–1319.
- Canard, B., Saint-Joanis, B. & Cole, S. T. (1992). Genomic diversity and organization of virulence genes in the pathogenic anaerobe *Clostridium perfringens*. *Mol Microbiol* **6**, 1421–1429.
- Canica, M. M., Nato, F., du Merle, L., Mazie, J. C., Baranton, G. & Postic, D. (1993). Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. *Scand J Infect Dis* **25**, 441–448.
- Carlson, C. R., Grønstad, A. & Kolstø, A.-B. (1992). Physical maps of the genomes of three *Bacillus cereus* strains. *J Bacteriol* **174**, 3750–3756.
- Carniel, E., Mercereau-Puijalon, O. & Bonnefoy, S. (1989). The gene coding for the 190,000-dalton iron-regulated protein of *Yersinia* species is present only in the highly pathogenic strains. *Infect Immun* **57**, 1211–1217.
- Casjens, S. & Huang, W. M. (1993). Linear chromosomal physical and genetic map of *Borrelia burgdorferi*, the Lyme disease agent. *Mol Microbiol* **8**, 967–980.
- Davidson, B. E., MacDougall, J. & Saint Girons, I. (1992). Physical map of the linear chromosome of the bacterium *Borrelia burgdorferi* 212, a causative agent of Lyme disease, and localization of rRNA genes. *J Bacteriol* **174**, 3766–3774.
- Dykhuizen, D. E., Polin, D. S., Dunn, J. J., Wilske, B., Preac-Mursic, V., Dattwyler, R. J. & Luft, B. J. (1993). *Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development. *Proc Natl Acad Sci USA* **90**, 10163–10167.
- Feinberg, A. P. & Vogelstein, B. (1984). A technique for radio-labeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* **137**, 266–267.
- Ferdows, M. S. & Barbour, A. G. (1989). Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. *Proc Natl Acad Sci USA* **86**, 5969–5973.
- Fukunaga, M. & Sohnaka, M. (1992). Tandem repeat of the 23S and

5S ribosomal RNA genes in *Borrelia burgdorferi*, the etiological agent of Lyme disease. *Biochem Biophys Res Commun* **183**, 952–957.

Fukunaga, M., Sohnaka, M. & Yanagihara, Y. (1993). Analysis of *Borrelia* species associated with Lyme disease by rRNA gene restriction fragment length polymorphism. *J Gen Microbiol* **139**, 1141–1146.

Gassmann, G. S., Jacobs, E., Deutzmann, R. & Göbel, U. B. (1991). Analysis of the *Borrelia burgdorferi* GeHo *fla* gene and antigenic characterization of its gene product. *J Bacteriol* **173**, 1452–1459.

Gazumyan, A., Schwartz, J. J., Liveris, D. & Schwartz, I. (1994). Sequence analysis of the ribosomal RNA operon of the Lyme disease spirochete *Borrelia burgdorferi*. *Gene* (in press).

Hansen, K., Bangsberg, J. M., Fjordvang, H., Pedersen, N. S. & Hindersson, P. (1988). Immunochemical characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. *Infect Immun* **56**, 2047–2053.

Hill, C. W. & Harnish, B. W. (1981). Inversions between ribosomal RNA genes of *Escherichia coli*. *Proc Natl Acad Sci USA* **78**, 7069–7072.

Hinnebusch, J. & Barbour, A. G. (1991). Linear plasmids of *Borrelia burgdorferi* have a telomeric structure and sequence similar to those of a eukaryotic virus. *J Bacteriol* **173**, 7233–7239.

Hinnebusch, J. & Tilly, K. (1993). Linear plasmids and chromosomes in bacteria. *Mol Microbiol* **10**, 917–922.

Hinnebusch, J., Bergström, S. & Barbour, A. G. (1990). Cloning and sequence analysis of linear plasmid telomeres of the bacterium *Borrelia burgdorferi*. *Mol Microbiol* **4**, 811–820.

Howe, T. R., Mayer, L. W. & Barbour, A. G. (1985). A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. *Science* **227**, 645–646.

Jauris-Heipke, S., Fuchs, R., Hofmann, A., Lottspeich, F., Preac-Mursic, V., Soutschek, E., Will, G. & Wilske, B. (1993). Molecular characterization of the *p100* gene of *Borrelia burgdorferi* strain PKo. *FEMS Microbiol Lett* **114**, 235–242.

Johnson, R. C., Schmid, G. P., Hyde, F. W., Steigerwalt, A. G. & Brenner, D. J. (1984). *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int J Syst Bacteriol* **34**, 496–497.

Krawiec, S. & Riley, M. (1990). Organization of the bacterial chromosome. *Microbiol Rev* **54**, 502–539.

Ladefoged, S. A. & Christiansen, G. (1992). Physical and genetic mapping of the genomes of five *Mycoplasma hominis* strains by pulsed-field gel electrophoresis. *J Bacteriol* **174**, 2199–2207.

Lin, Y.-S., Kieser, H. M., Hopwood, D. A. & Chen, C. W. (1993). The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol Microbiol* **10**, 923–933.

Marconi, R. T. & Garon, C. F. (1992a). Phylogenetic analysis of the genus *Borrelia*: a comparison of North American and European isolates of *Borrelia burgdorferi*. *J Bacteriol* **174**, 241–244.

Marconi, R. T. & Garon, C. F. (1992b). Identification of a third genomic group of *Borrelia burgdorferi* through signature nucleotide analysis and 16S rRNA sequence determination. *J Gen Microbiol* **138**, 533–536.

Okada, N., Sasakawa, C., Tobe, T., Talukder, K. A., Komatsu, K. & Yoshikawa, M. (1991). Construction of a physical map of the

chromosome of *Shigella flexneri* 2a and the direct assignment of nine virulence-associated loci identified by Tn5 insertions. *Mol Microbiol* **5**, 2171–2180.

Old, I. G., MacDougall, J., Saint Girons, I. & Davidson, B. E. (1992a). Mapping of genes on the linear chromosome of the bacterium *Borrelia burgdorferi*: possible locations for its origin of replication. *FEMS Microbiol Lett* **99**, 245–250.

Old, I. G., Margarita, D. & Saint Girons, I. (1992b). Nucleotide sequence of the *Borrelia burgdorferi* *rpmH* gene encoding ribosomal protein L34. *Nucleic Acids Res* **20**, 6097.

Old, I. G., Margarita, D. & Saint Girons, I. (1993a). Unique genetic arrangement in the *dnaA* region of the *Borrelia burgdorferi* linear chromosome: nucleotide sequence of the *dnaA* gene. *FEMS Microbiol Lett* **111**, 109–114.

Old, I. G., Margarita, D. & Saint Girons, I. (1993b). Nucleotide sequence of the *Borrelia burgdorferi* *dnaN* gene encoding the β subunit of DNA polymerase III. *Nucleic Acids Res* **21**, 3323.

Pyle, L. E., Taylor, T. & Finch, L. R. (1990). Genomic maps of some strains within the *Mycoplasma mycoides* cluster. *J Bacteriol* **172**, 7265–7268.

Reed, K. C. & Mann, D. A. (1985). Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res* **13**, 7207–7221.

Riley, M. & Krawiec, S. (1987). Genome organization. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 967–981. Edited by F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.

Rosa, P. A., Hogan, D. & Schwan, T. G. (1991). Polymerase chain reaction analyses identify two distinct classes of *Borrelia burgdorferi*. *J Clin Microbiol* **29**, 524–532.

Rosa, P. A., Schwan, T. & Hogan, D. (1992). Recombination between genes encoding major outer surface proteins A and B of *Borrelia burgdorferi*. *Mol Microbiol* **6**, 3031–3040.

Saint Girons, I., Old, I. G. & Davidson, B. E. (1994). Molecular biology of the *Borrelia*, bacteria with linear replicons. *Microbiology* **140**, 1803–1816.

Schwartz, J. J., Gazumyan, A. & Schwartz, I. (1992). rRNA gene organization in the Lyme disease spirochaete, *Borrelia burgdorferi*. *J Bacteriol* **174**, 3757–3765.

Tilly, K. & Campbell, J. (1993). A *Borrelia burgdorferi* homolog of the *Escherichia coli* *rbo* gene. *Nucleic Acids Res* **21**, 1040.

Tilly, K., Hauser, R., Campbell, J. & Ostheimer, G. J. (1993). Isolation of *dnaJ*, *dnaK* and *grpE* homologues from *Borrelia burgdorferi* and complementation of *Escherichia coli* mutants. *Mol Microbiol* **7**, 359–369.

Tulloch, D. L., Finch, L. R., Hillier, A. J. & Davidson, B. E. (1991). Physical map of the chromosome of *Lactococcus lactis* subsp. *lactis* DL11 and localization of six putative rRNA operons. *J Bacteriol* **173**, 2768–2775.

Zuerner, R. L., Herrmann, J. L. & Saint Girons, I. (1993). Comparison of genetic maps for two *Leptospira interrogans* serovars provides evidence for two chromosomes and intraspecies heterogeneity. *J Bacteriol* **175**, 5445–5451.

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