

The Role of Genomics in Approaching the Study of *Borrelia* DNA Replication

Jorge García-Lara^{1*}, Mathieu Picardeau²,
B. Joseph Hinnebusch³, Wai Mun Huang⁴,
and Sherwood Casjens⁴

¹Department of Microbiology, 546 Biological Sciences Building, University of Georgia, Athens, GA 30602, USA

²Unité de Bactériologie Moléculaire et Médicale, Institut Pasteur, Paris, France

³National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Laboratory of Human Bacterial Pathogenesis, Hamilton, MT, USA

⁴Division of Molecular Biology and Genetics, Department of Oncological Sciences, University of Utah Medical School, Salt Lake City, UT 84132, USA

Abstract

The identification of chromosomal and episomal origins of replication in the genome of the causative agent of Lyme disease, the spirochete *Borrelia burgdorferi*, has been greatly facilitated by genomics. Analysis of genome features, including strand compositional asymmetries, organizational similarities to other bacterial origins of replication, and the presence of homologues of genes involved in replication and partitioning, have contributed to the identification of a collection of putative origins of replication within the *Borrelia* genome. This analysis has provided the basis for the experimental verification of origins in the linear chromosome and in the linear plasmid lp28-2. Information generated during the study of these origins will significantly contribute to the understanding of the mechanisms of replication and partitioning in *Borrelia*.

Introduction

The breadth of genomic information now becoming available greatly facilitates the development of genetic and molecular approaches to provide further insight into the biology of microorganisms. Its impact has been particularly remarkable for the genetically intractable bacterial pathogens, such as the causative agent of Lyme disease, the spirochete *Borrelia burgdorferi* (Fraser *et al.* 1997; Hardham and Rosey, 2000; Saint Girons *et al.*, 2000; Tilly *et al.*, 2000).

Genome analysis renders a prediction of gene function, and it may also be used to identify DNA elements that play roles in transcription, replication, recombination, and other cellular processes (Strauss and Falkow, 1997; Fraser *et al.*, 1997). Recently, the use of genomics has been

instrumental in predicting the putative location of origins of replication in the linear chromosome and linear plasmids of *Borrelia burgdorferi*, based on the i) strand compositional asymmetry; ii) homologies to the organization of other bacterial origins of replication; and, iii) the presence of open reading frames homologous to genes involved in replication and partition. The role of some of these candidate sequences as possible origins of replication has subsequently been demonstrated.

Analysis of DNA Strand Compositional Asymmetry to Localize Bacterial Origins of Replication

Recent analysis of the complete chromosomal sequences of the spirochetes *Borrelia burgdorferi* and *Treponema pallidum* showed strong strand-specific skews in nucleotide composition (Fraser *et al.*, 1997; Grigoriev, 1998; McLean *et al.*, 1998; Rocha *et al.*, 1999; Picardeau *et al.*, 1999). In bacteria, this strand compositional asymmetry is characterized by significant deviations from a random distribution (Lobry, 1995; Sueoka, 1995), with the leading strand of replication being richer in G and T than the lagging strand. In most of microbial genomes, base composition skew switches sign at the origin and terminus of replication, suggesting that replication forks diverge from and converge upon these two sites, respectively.

Several biochemical mechanisms have been proposed to be responsible for this strand compositional asymmetry (Frank and Lobry, 1999; Karlin, 1999; Mrazek and Karlin, 1998), one of which involves the replication process. Due to the antiparallel nature of DNA and the fact that the DNA polymerase involved in the replication of bacterial chromosomes (DNA polymerase III holoenzyme) only synthesizes DNA in the 5'→3' direction, the leading strand is made in a continuous fashion, while the lagging strand is made discontinuously in short Okazaki fragments (Figure

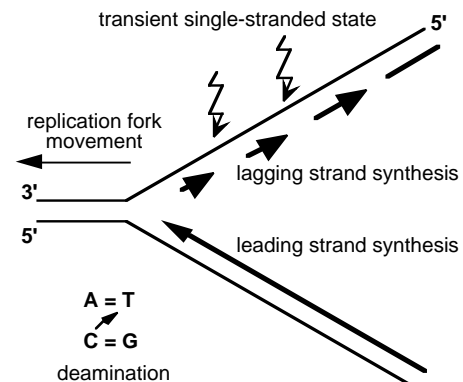


Figure 1. The DNA replication fork. During replication, the leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously in short Okazaki fragments. Exposure of single-stranded DNA increases the rate of spontaneous deaminations of C→T.

*For correspondence. Email jgarcial@panda.uchc.edu; Tel. (706) 542-4112; Fax. (706) 542-2674.

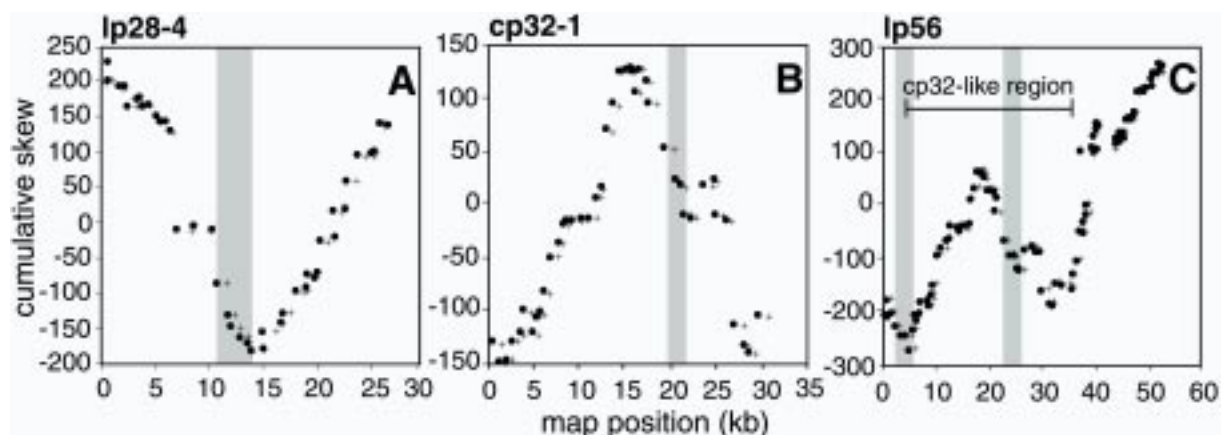


Figure 2. Cumulative CG skew diagrams of the *B. burgdorferi* plasmids: (A) linear plasmid lp28-4, (B) circular plasmid cp32-1, and (C) linear plasmid lp56. The region containing the putative “replication/partition gene cluster” (paralogous gene families 32, 49, 50, and 57/62) is indicated by a shaded box on the map. Fragment of lp56 with strong sequence homology with a cp32-like plasmid is enclosed within a doublehead arrow (Casjens *et al.*, 2000). Y axis shows the cumulative skew calculated as previously described in (Frank and Lobry, 2000). The map position of the beginning and the end of each ORF is represented by a pair of dots that are parallel to the x-axis.

1) (Kornberg and Baker, 1992). DNA polymerase III holoenzyme contains two polymerase core units, one for each strand, which have unequal processivity and replication fidelity on the two strands, and which therefore potentially bias mutations on each strand (Radman, 1998). It has also been suggested that the main feature responsible for the asymmetrical directional mutation pressure is the time spent in a single-stranded state (Francino and Ochman, 1997; Frank and Lobry, 1999; Grigoriev, 1998; Reyes *et al.*, 1998). During replication,

the lagging strand template is preferentially exposed to hydrolytic and oxidative damage and, therefore, is prone to mutation (Figure 1). Exposure of single-stranded sequences increases the rate of spontaneous deamination of methylated cytosines, which forms thymine (Frank and Lobry, 1999). Transcription may also participate in the base compositional asymmetry through transcription-coupled repair mechanisms and deamination events of the coding strand, which is the leading strand for most of the bacterial genes (Francino and Ochman, 1997). Therefore, both replication and transcription may be associated with preferential C->T deamination of the leading strand, which is in agreement with the observed GT richness of the leading strand of microbial genomes.

Table 1. “Replication/Partitioning Cluster” Genes on *B. burgdorferi* Plasmids

Plasmid*	Family 32†	Family 49†	Family 50†	Family 57/62†
lp5	0	0	0	1
lp17	1	0	0	1
lp21	1	0	0	1
lp25	1	1	1	1
lp28-1	2	2	2	1
lp28-2	1	1	1	2
lp28-3	1	1	1	1
lp28-4	1	1	1	1
lp36	1	1	1	1
lp38	1	1	1	1
lp54	1	1	1	1
lp56	2	2	2	1
cp9-1	0	1	1	1
cp26	1	1	1	1
cp32-1	1	1	1	1
cp32-3	1	1	1	1
cp32-4	1	1	1	1
cp32-6	1	1	1	1
cp32-7	1	1	1	1
cp32-8	1	1	1	1
cp32-9	1	1	1	1

* Plasmid number corresponds to an approximate indication of its size (see Casjens *et al.*, 2000 for their exact sizes).

† Only potentially functional genes are counted, although numerous obviously defective pseudogenes are also present on some of the plasmids (Casjens *et al.*, 2000).

AT and CG Skew Analysis as a Predictor of Replication Origins of *B. burgdorferi* Plasmids

In a previous study, Grigoriev (1998) showed a central strand switch of CG skew in linear plasmids lp17 and lp25 by using cumulative skew diagrams. In these diagrams, the putative origin and terminus of replication were predicted to be the loci that correspond to the global minimum and maximum values of cumulative skew (Grigoriev, 1998; Picardeau *et al.*, 1999). We, therefore, used cumulative skew analysis (combined AT and GC skew, [(A-T)/(A+T) and (C-G)/(C+G), respectively], cumulatively summed gene by gene (Frank and Lobry, 2000; Picardeau *et al.*, 2000) to identify possible candidate replication origins in 14 *Borrelia* plasmid sequences (linear plasmids lp17, lp25, lp28-1, lp28-2, lp28-3, lp28-4, lp36, lp38, lp54, lp56, and circular plasmids cp26, cp32-1, cp32-4, cp32-6) (Picardeau *et al.*, 2000; M. Picardeau, J. Lobry, and J. Hinnebusch, unpublished results; D. Ussery and S. Casjens, unpublished results). Because the *B. burgdorferi* plasmids are smaller and contain a lower density of ORFs (especially in linear plasmids) than the chromosome (Fraser *et al.*, 1997; Casjens *et al.*, 2000), patterns of plasmid cumulative skew diagrams are more distorted than those of the chromosome (Picardeau *et al.*, 2000). However, in all cases, the overall pattern of plasmids

analyzed (e.g., Figure 2A), like the linear chromosome, showed an obvious global maximum / minimum cumulative skew values (Picardeau *et al.*, 2000). For *B. burgdorferi* circular plasmid cp26, cumulative diagrams show two switchpoints that could correspond to the origin and terminus of replication (Picardeau *et al.*, 2000).

Patterns of cumulative skew diagrams can also indicate DNA rearrangements among plasmids. Unlike the *B. burgdorferi* chromosome, the minimum cumulative skew curves on the linear plasmids are eccentric, instead of symmetrical, which may be explained by DNA rearrangements among linear plasmids (Casjens *et al.*, 2000). For example, skew patterns of circular plasmid cp32-1 and linear plasmid lp56 is consistent with the suggestion from Casjens *et al.* (2000) that linear plasmid lp56 contains a copy of a cp32-like plasmid, integrated between nucleotides 6585 and 36935 (Figure 2C). In these two cases, cp32-1 and lp56, and those reported by Grigoriev (1998), lp17 and lp25, as well as those not shown here, the inflection points of the skew plots were very near genes in the "partition cluster" (see below). The finding that the "partition gene cluster" (Casjens *et al.*, 2000) maps near the global minimum/maximum cumulative skew values of the plasmids reinforces the conclusion that these regions contain the origin of replication (Picardeau *et al.*, 2000).

Prediction of Plasmid Origins of Replication in *Borrelia* by Organizational Homology

The structural arrangement of origins of replication of circular chromosomes and plasmids in Gram-negative and Gram-positive bacteria is highly conserved (Moriya *et al.*, 1988; Kornberg and Baker, 1992). A typical chromosomal origin contains two sequence elements within approximately 400-bp of DNA: i) 1 to 4 copies of a 9-mer sequence specific for the binding of the initiator of replication, DnaA (DnaA boxes); and ii) an AT-rich region normally containing reiterated sequences (2 to 5 copies) of 6 to 16 bp. In some cases, the plasmid origins contain DnaA boxes in addition to binding sites for a plasmid specific initiator protein, encoded in a gene normally located in the vicinity of the origin of replication. Even plasmids that replicate by DnaA-independent mechanisms (RNA- or iteron-regulated replication) often contain DnaA binding sites (Actis *et al.*, 1998; Seuffer and Messer, 1987) that provide either an alternative DnaA-dependent pathway for the initiation of replication or a role for DnaA as an accessory protein.

The DnaA box sequence is highly conserved in a variety of Gram-negative and Gram-positive bacteria (Messer and Weigel, 1996; Calcutt and Schmidt, 1992; Fujita *et al.*, 1992; Marczyński and Shapiro, 1992; Moriya *et al.*, 1992; Ogasawara and Yoshikawa, 1992; Salazar *et al.*, 1996; Yee and Smith, 1990; Zakrezwska-Czerwinska and Schrepf, 1992). Thus, the search for DnaA box-homologs has been used in *Borrelia burgdorferi* to aid in the identification of putative chromosomal origins of replication (Old *et al.*, 1993; Picardeau *et al.*, 1999; see below). However, the composition and stringency of the DnaA box consensus used by different authors as search criteria, and reports of new DnaA box sequences that diverge from the consensus TTAT(C,A)CA(C,A)A have complicated this type of analysis (Fuller *et al.*, 1984; Moriya *et al.*, 1988; Picardeau *et al.*, 1999; Singh *et al.*, 2000).

Moreover, the high A+T content of the *Borrelia* genome (71.4%) further affects the usefulness of identifying DnaA binding sites based on consensus alone. García-Lara *et al.* (2000) have recently successfully applied a similar approach to identify candidate episomal origins of replication in *Borrelia*. To select amongst the various DnaA box-like sequences generated by their analysis, these authors further scanned the plasmid sequences around these sites searching for iterated AT-rich sequences. Sequences containing a 9-mer AT-rich repeat in the vicinity of a putative DnaA box [TT(A,C,G)T(C,A)CA(A,C)A] were identified in two locations within the linear plasmid lp28-2. These repeats corresponded to the consensus AAA(T,G)AA(T,G)AA. The involvement of these sequences in replication remains to be demonstrated. Nevertheless, it is interesting that similar sequences can be found in many other circular and linear plasmids in *Borrelia burgdorferi*, e.g. cp9, cp32s, lp5, lp17, lp21, lp28-4 and lp56.

Potential Plasmid Partitioning and Replication Genes on *Borrelia* Plasmids

All *Borrelias* that have been examined carry a large number of linear and circular plasmids. *B. burgdorferi* isolate B31 culture MI, the only isolate whose complete complement of plasmids has been determined, carries twelve linear and nine circular plasmids ranging in size from 5 to 54 kbp (comprising a total of about 613 kbp) (Casjens *et al.*, 2000). These extrachromosomal sequences appear to have been built up by numerous DNA duplications and subsequent divergence such that there are many similarities among the plasmids (summarized in Casjens, 1999; Casjens, 2000; Casjens *et al.*, 2000). The molecular nature of these duplicative rearrangements could be attributed to: i) duplication and divergence of whole plasmids; ii) replacement of telomeric regions of one plasmid with a copy of a telomeric region from another plasmid; and, iii) random insertion of a copy of an internal region of one plasmid into another plasmid. One indication of the extent of past duplication is the observation that among the predicted 535 plasmid genes that are >300 bp in length, all but 63 have a paralog elsewhere on the B31 plasmids (Casjens *et al.*, 2000).

Because of the high sequence similarity among the B31 plasmids, it is conceivable that they use similar replication and partitioning mechanisms. On the other hand, if orderly partitioning of plasmids into daughter cells is to be achieved, plasmid specific proteins and cognate recognition sites must also exist. Combining these two concepts leads us to the prediction that similar but non-identical genes might be present on each of the plasmids to control these processes. There are in fact only a few families of paralogous genes whose members are widespread amongst all or nearly all of the 21 strain *B. burgdorferi* B31 plasmids. These are families 32, 49, 50 and the superfamily 57/62 (Casjens *et al.*, 2000). All 17 of the B31 plasmids larger than 21 kbp carry at least one member of each of these families; and in all cases these genes meet the non-identity requirement. In addition, on each of these plasmids genes from these four families are tightly clustered. The facts that plasmids < 21 Kbp in length do not have all four of these gene types suggests that a full set of all four types may not be essential for partitioning of small DNAs: lp5 has no family 32, 49 or 50 gene; cp9-1

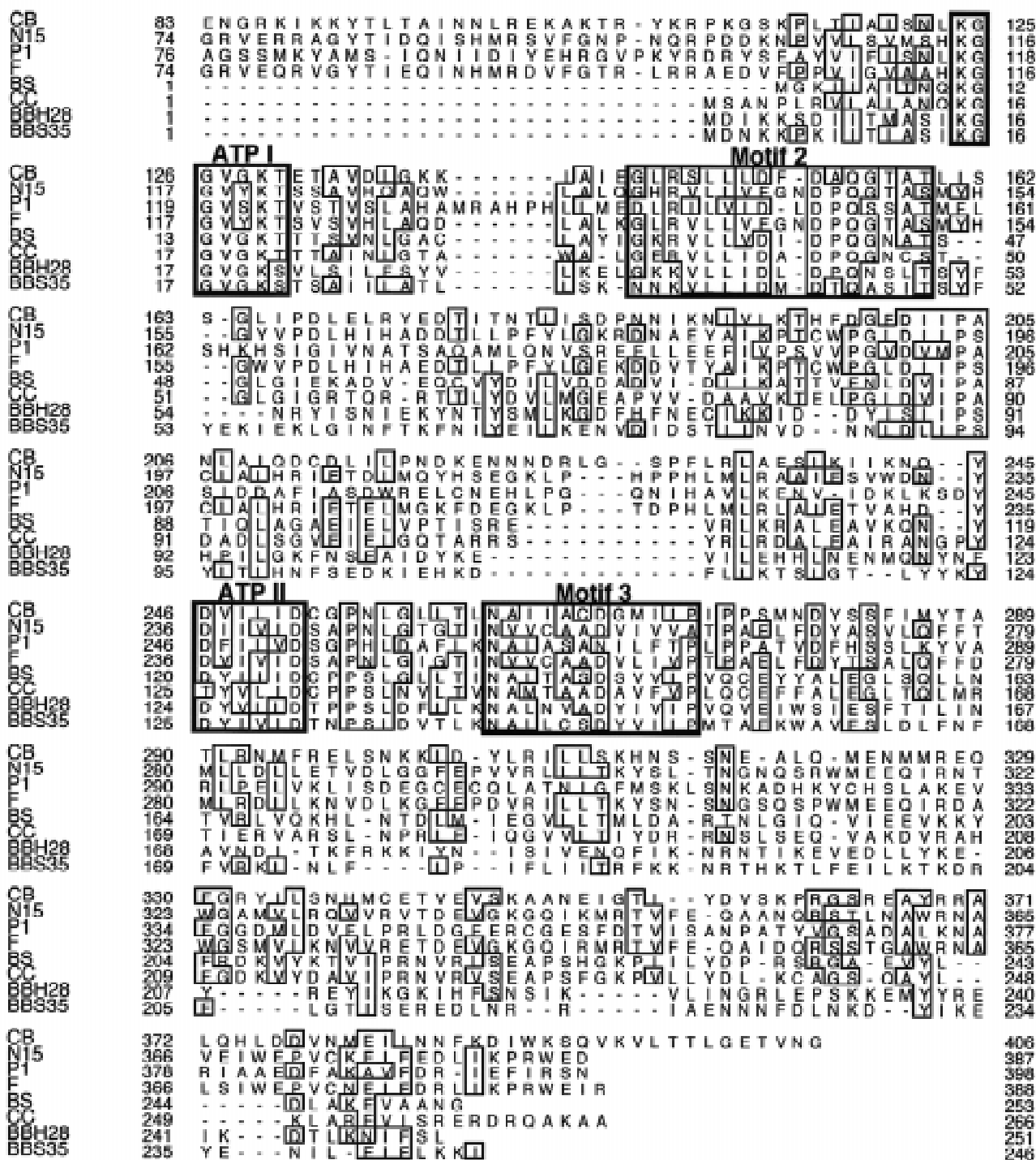


Figure 3. Alignment of *Borrelia* family 32 genes to known partitioning proteins. Eight homologous protein sequences, including two typical members of *Borrelia* gene family 32, were aligned with CLUSTAL V (Higgins et al., 1992), and positions where four or more of the sequences are identical are shaded and boxed with thin borders. Heavy boxes indicate the Walker A motif for nucleotide binding (ATP I) and the Mg²⁺ binding motif (ATP II) (Walker et al., 1982), and the motif 2 and 3 regions of higher conservation noted by Motallesi-Veshareh et al. (1990). Protein names are as follows: CB, *Coxiella burnetii* plasmid QpH1 QsopA (Lin & Mallavia, 1994); N15, phage N15 SopA (Ravin et al., 2000); P1, phage P1 ParA (Abeles et al., 1985); F, *E. coli* F plasmid SopA (Mori et al., 1986); BS, *Bacillus subtilis* chromosomal Soj (Ogasawara & Yoshikawa, 1992); CC, *Caulobacter crescentus* chromosomal ParA (Mohl & Gober, 1997); BBH28, *B. burgdorferi* linear plasmid lp28-3 protein BBH28 (Fraser et al., 1997); BBS35, *B. burgdorferi* circular plasmid cp32-3 protein BBS35 (Casjens et al., 2000). The N-terminal regions of the top four proteins in the figure are not shown.

has no family 32 gene; lp17 does not carry either a family 49 or 50 gene; and lp21 does not have a family 50 gene. Interestingly, superfamily 57/62 is the only paralogous gene family to have a member on every plasmid. Finally, homologs to these genes have also been found on all the plasmids from other *B. burgdorferi* isolates as well as from *B. garinii* and *B. hermsii* that have been completely sequenced (Dunn *et al.*, 1994; Caimano *et al.*, 2000; Stevenson *et al.*, 2000).

Might these genes be involved in plasmid replication and/or partitioning? Currently no experimental data are available. Members of families 49, 50 and 57/62 have no convincing database matches outside of *Borrelia* and their functions remain completely unknown. Some members of family 49 have been suggested to contain a motif present in RepC proteins (Dunn *et al.*, 1994), but such a motif is not present in all family members, and the role of this family of proteins remains uncertain. However, proteins encoded by family 32 genes are quite similar to the SopA/ParA/Soj family of proteins that are involved in bacterial replicon partitioning (Zuckert and Meyer, 1996; Casjens *et al.*, 1997; Stevenson *et al.*, 1998). These proteins have been studied in most detail in *Escherichia coli* F plasmid, P1 and N15 prophage plasmids, and *Bacillus subtilis* chromosome partitioning (Abeles *et al.*, 1985; Mori *et al.*, 1986; Ireton *et al.*, 1994; Ravin and Lane, 1999). This family of proteins will be referred to here as "SopA homologs." In the previously studied examples, the *sopA* genes form an operon with a *sopB*-like gene; no *sopB* homologs have been found on *Borrelia* plasmids, but other proteins could supply its function. In the F plasmid, N15 prophage plasmid and P1 prophage plasmid partitioning systems the SopB protein specifically binds a centromere-like site on the cognate plasmid to effect partitioning (Hayakawa *et al.*, 1985; Mori *et al.*, 1989). The SopA homologs play a role in replicon partitioning (Erdmann *et al.*, 1999; Bouet *et al.*, 2000), and with the help of SopB acts as a transcriptional autoregulator (Mori *et al.*, 1989; Watanabe *et al.*, 1989). Biochemically, it has been shown that SopA proteins can be membrane associated (Lin and Mallavia, 1998), have an ATPase activity that is activated by SopB (Hiraga, 1992), and that SopA and SopB interact to form hetero-oligomers (Hanai *et al.*, 1996; Kim and Shim, 1999). In *B. subtilis* the SopB homolog Spo0J is localized at the poles of growing cells, and requires the presence of the SopA homolog Soj for this localization (Lin *et al.*, 1997; Kim and Wang, 1998). Figure 3 shows an alignment of the SopA homologs on B31 plasmids cp32-3 and lp28-3 (for example) with the characterized F plasmid and P1 prophage SopA homologs, as well as several bacterial and archeal chromosomally-encoded homologs. The *Borrelia* plasmid-encoded SopA homologs are similar to the chromosomally-encoded Soj proteins in that they do not have the >100 aa N-terminal extensions present in the N15, F1- and P1-encoded proteins. Putative ATP and Mg⁺⁺ binding motifs (Walker *et al.*, 1982) are conserved in all the *Borrelia* SopA homologs, and in addition, conserved regions 2 and 3 of Motallebi-Veshareh *et al.* (1990) are present in the *Borrelia* proteins. Their high levels of similarity suggest that the *Borrelia* family 32 proteins might in fact have a role in plasmid partitioning. The family 32, 49, 50, 57/62 gene cluster may be a "putative partitioning gene cluster", since the genes in these clusters are very tightly packed, even slightly overlapping in some cases, and functionally related prokaryotic genes are often

clustered in the same manner. Specific genes for plasmid replication and partitioning are usually located adjacent to the plasmid origin of replication (Actis, *et al.*, 1998; Kornberg and Baker, 1992), so it is possible, or even perhaps likely, that the above genes are near the *Borrelia* plasmid origins.

Experimental Identification of the Replication Origin of the *B. burgdorferi* Chromosome

The linear chromosome of *Borrelia burgdorferi* has 910 kb and 853 predicted coding sequences (Fraser *et al.*, 1997). Among the possible coding sequences, replication proteins, including the initiator protein DnaA, have been mapped at the center of the linear chromosome (Old *et al.*, 1993; Casjens and Huang, 1993). The CG skew analysis of the chromosomal sequence of *B. burgdorferi* shows a switch of polarity at this centrally located putative origin of replication (Fraser *et al.*, 1997; Grigoriev, 1998). Strong base compositional asymmetry of the *B. burgdorferi* chromosome also acts at the level of the protein composition. Thus, the knowledge of a *B. burgdorferi* protein sequence should allow prediction of the orientation of the corresponding gene with respect to replication (Mackiewicz *et al.*, 1999; McInerney, 1998; Rocha *et al.*, 1999). A closer look at the putative origin locus indicates that the switch of the GC skew polarity occurs within the 240 bp intergenic sequence between *dnaA* and *dnaN* (Picardeau *et al.*, 1999). Similarly, an analysis of codon usage patterns is consistent with the location of the chromosomal replication origin between *dnaA* and *dnaN* (Lafay *et al.*, 1999). By using nascent DNA strand analysis, we experimentally demonstrated that the *B. burgdorferi* chromosome initiates replication bidirectionally from this origin, near *dnaA* and *dnaN* genes (Picardeau *et al.*, 1999).

Identification of Episomal Origins of Replication in *Borrelia burgdorferi*

Regions essential for replication of chromosomal and episomal DNA have been traditionally identified by cloning random DNA fragments into otherwise unstable heterologous DNA and selecting for its stable maintenance (Tumbula *et al.*, 1997). We have recently identified two fragments from lp28-2 that contain putative *Borrelia* replicons by the criteria described above. These fragments enable autonomous replication of *E. coli* plasmids in *Borrelia*, thereby confirming their activity as origins of replication in the spirochete (García-Lara *et al.*, 2000). Besides discriminating the minimal sequence within these fragments required for replication, and their interaction with the replication machinery, a number of additional questions remain to be addressed; *e.g.*, Do both of these putative origins of replication function in the lp28-2 plasmid?

DnaA box-like and iterative AT-rich elements included in both of these lp28-2 fragments lie near each of a "putative replication/partition gene cluster" (Figure 4). Moreover, one of these sets of DNA elements resides within gene BBG06, which belongs to the paralogous gene family 57. Interestingly, it has been reported that the replicative origins of some plasmids and bacteriophages in Gram-positive and Gram-negative bacteria are located within the coding sequence of genes involved in their replication (Bartosik *et al.*, 1998; Grado *et al.*, 1998; Hansen, 1989; Taghavi *et al.*, 1998).

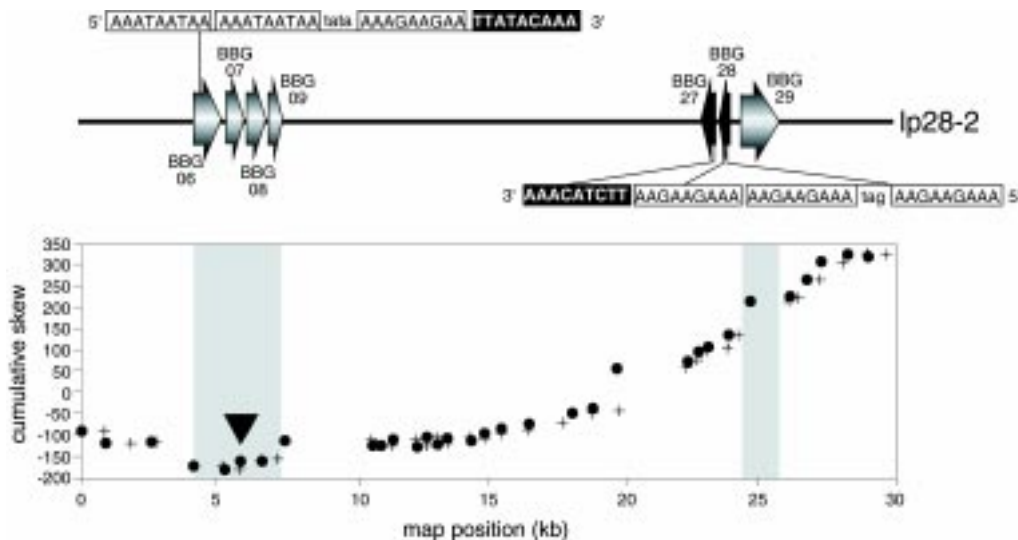


Figure 4. Diagram of plasmid lp28-2. Composition and location of putative DnaA boxes (black boxes), AT-rich iterated sequences (white boxes), genes belonging to the putative “partition/replication gene clusters” (gradient arrows), and the corresponding CG skew analysis. Black arrows correspond to genes unrelated to the putative “partition/replication gene clusters” which may contain a candidate for an origin of replication. The origin of replication suggested by the CG skew analysis is indicated with a black triangle. The map position of the beginning and the end of each ORF is represented by a pair of dots that are parallel to the x-axis.

al. 1996; Taylor and Wegrzyn, 1995). Hence, it is possible that genes in family 57 may participate in replication and/or partitioning of *Borrelia* plasmids.

Genes encoding partition proteins are adjacent to the origin of replication in various bacterial chromosomes and plasmids (Actis *et al.*, 1998; Kornberg and Baker, 1992), which, as mentioned above, coincides with minimum cumulative skew values. The provocative observation that the “putative replication/partitioning gene clusters” in the various plasmids, including lp28-2, have also been found at the inflection point in AT and CG skew is consistent with the suggestion that these regions contain origins of replication (Figures 2A-B, and Figure 4). It also reinforces the notion that these families of genes are good candidates to be involved in replication and/or partitioning. Nonetheless, these proposed roles remain to be demonstrated experimentally.

Like lp28-2, plasmids lp28-1 and lp56 also have two paralogous copies of at least one member of the family 32, 49, 50, 57/62 gene cluster, most likely due to duplicative rearrangements among the B31 plasmids (Casjens, 1999; Casjens, 2000; Casjens *et al.*, 2000). Thus, it is reasonable to consider that at least lp28-1 and lp56 may contain more than one origin of replication. Interestingly, lp56 shows two minimum cumulative skew values near the putative “replication/partitioning gene clusters” (Figure 2C).

In summary, organizational homologies, the presence of genes homologous to those involved in replication and/or partitioning, and locations of strand compositional asymmetries have contributed to the identification of chromosomal and lp28-2 origins and may point out replication origins on the other plasmids as well.

Genomics and the Mechanism of DNA Replication

The mechanism of replication of the linear chromosome and plasmids in *Borrelia burgdorferi* remains to be elucidated (Actis *et al.*, 1998; Casjens, 1999; Picardeau *et al.*, 1999).

Genome analysis and comparative genomics may provide some clues to this process.

Modes of bidirectional replication of bacterial chromosomes and plasmids from a single origin yield a common V-shaped cumulative skew pattern showing bipolar asymmetry (Fraser *et al.*, 1997; Grigoriev, 1998; Picardeau *et al.*, 2000; D. Ussery and S. Casjens, unpublished). Bidirectional replication of the *Borrelia* chromosome has been demonstrated by nascent DNA strand analysis (Picardeau *et al.*, 1999). In contrast, analyses of the skews of plasmids known to have replication mechanisms other than bidirectional do not yield a V-shaped pattern (Picardeau *et al.*, 2000). Since the *B. burgdorferi* plasmids show base compositional asymmetry with V-shaped cumulative diagrams similar to the *B. burgdorferi* linear chromosome, it suggests that they also replicate bidirectionally from an internal origin (Picardeau *et al.*, 2000).

The interplay between transcription and DNA-replication, previously reported for well characterized bacterial plasmids, bacteriophages and mitochondrial DNA (Bruand and Ehrlich, 1998) should also be considered as a feasible regulation for *Borrelia* plasmid replication. The “activation” of the origins of replication contained within the fragments isolated from plasmid lp28-2 appears to be growth regulated; it becomes more active as the cultures approach stationary phase (García-Lara *et al.*, 2000). Although various models could explain this phenomenon, it is reasonable to envision one that entails transcription-driven DNA replication mediated by promoters induced during stationary phase. More studies are needed to test this and other hypotheses.

The recent identification of origins of replication in *Borrelia burgdorferi* based on genomic analysis has provided a basis for finding additional origins of replication and the development of genetic tools. It will also provide the means for gaining insight into the mechanism of DNA replication and partitioning in *Borrelia*.

Acknowledgements

We thank Jean Lobry for useful comments to the manuscript.

References

- Abeles, A.L., Friedman, S.A., and Austin, S.J. 1985. Partition of unit-copy miniplasmids to daughter cells. III. The DNA sequence and functional organization of the P1 partition region. *J. Mol. Biol.* 185: 261-72.
- Actis, L., Tolmashy, M.E., and Crosa, J.H. 1998. Bacterial plasmids: Replication of extrachromosomal genetic elements encoding resistance to antimicrobial compounds. *Frontiers Biosci.* 3: d43-62.
- Bartosik, D., J. Baj, and Włodarczyk, M. 1998. Molecular and functional analysis of pTAV320, a repABC-type replicon of the *Paracoccus versutus* composite plasmid pTAV1. *Microbiol.* 144: 3149-3157.
- Bouet, J.Y., Surtees, J.A., and Funnell, B.E. 2000. Stoichiometry of P1 Plasmid Partition Complexes. *J. Biol. Chem.* 275: 8213-8219.
- Bruand, C., and Ehrlich, S.D. 1998. Transcription-driven DNA replication of plasmid pAM β 1 in *Bacillus subtilis*. *Mol. Microbiol.* 30: 135-145.
- Caimano, M.J., Yang, X., Popova, T.G., Clawson, M.L., Akins, D.R., Norgard, M.V., and Radolf, J.D. 2000. Molecular and evolutionary characterization of the cp32/18 family of supercoiled plasmids in *Borrelia burgdorferi* 297. *Infect. Immun.* 68: 1574-86.
- Calcutt, M.J., and Schmidt, F.J. 1992. Conserved gene arrangement in the origin region of the *Streptomyces coelicolor* chromosome. *J. Bacteriol.* 174: 3220-3226.
- Casjens, S. 1999. Evolution of the linear DNA replicons of the *Borrelia* spirochetes. *Curr. Op. Microbiol.* 2: 529-534.
- Casjens, S. 2000. *Borrelia* genomes in the year 2000. *J. Mol. Microbiol. Biotechnol.* 2: 401-410.
- Casjens, S., and Huang, W.M. 1993. Linear chromosomal physical and genetic map of *Borrelia burgdorferi*, the Lyme disease agent. *Mol. Microbiol.* 8: 967-80.
- Casjens, S., Palmer, N., van Vugt, R., Huang, W.M., Stevenson, B., Rosa, P., Lathigra, R., Sutton, G., Peterson, J., Dodson, R.J., Haft, D., Hickey, E., Gwinn, M., White, O., and Fraser, C.M. 2000. A bacterial genome in flux: The twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Mol. Microbiol.* 35: 490-516.
- Casjens, S., van Vugt, R., Tilly, K., Rosa, P.A., and Stevenson, B. 1997. Homology throughout the multiple 32-kilobase circular plasmids present in Lyme disease spirochetes. *J. Bacteriol.* 179: 217-27.
- Dunn, J.J., Buchstein, S.R., Butler, L. L., Fisenne, S., Polin, D.S., Lade, B.N., and Luft, B. J. 1994. Complete nucleotide sequence of a circular plasmid from the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Bacteriol.* 176: 2706-17.
- Erdmann, N., Petroff, T., and Funnell, B.E. 1999. Intracellular localization of P1 ParB protein depends on ParA and *parS*. *Proc. Natl. Acad. Sci. USA.* 96: 14905-10.
- Francino, M.P., and Ochman, H. 1997. Strand asymmetries in DNA evolution. *Trends Genet.* 13: 240-245.
- Frank, A.C., and Lobry, J.R. 1999. Asymmetric substitution patterns: a review of possible underlying mutational or selective mechanisms. *Gene* 238: 65-77.
- Frank, A.C., and Lobry, J.R. 2000. OriLoc: prediction of replication boundaries in unannotated bacterial chromosomes. *Bioinformatics.* in press.
- Fraser, C.M., Casjens, S., Huang, W.M., Sutton, G.G., Clayton, R., Lathigra, R., White, O., Ketchum, K.A., Dodson, R., Hickey, E.K., Gwinn, M., Dougherty, B., Tomb, J.F., Fleischmann, R.D., Richardson, D., Peterson, J., Kerlavage, A.R., Quackenbush, J., Salzberg, S., Hanson, M., van Vugt, R., Palmer, N., Adams, M.D., Gocayne, J., and Venter, J.C. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390: 580-6.
- Fujita, M.Q., Yoshikawa, H., and Ogasawara, N. 1992. Structure of the *dnaA* and DnaA-box region in the *Mycoplasma capricolum* chromosome: conservation and variations in the course of evolution. *Gene* 110: 17-23.
- Fuller, R.S., Funnell, B.E., and Kornberg, A. 1984. The *dnaA* protein complex with the *E. coli* chromosomal replication origin (*oriC*) and other DNA sites. *Cell.* 38: 889-900.
- García-Lara, J., Osborn, M.J., and Padula, S.J. 2000. Identification and cloning of an origin of replication from a linear plasmid of *Borrelia burgdorferi*. (submitted).
- Grado, M., Lasa, I., and Berenguer, J. 1998. Characterization of plasmid replicative origin from an extreme thermophile. *FEMS Microbiol. Lett.* 165: 51-57.
- Grigoriev, A. 1998. Analyzing genomes with cumulative skew diagrams. *Nucleic Acids Res.* 26: 2286-2290.
- Hanai, R., Liu, R., Benedetti, P., Caron, P.R., Lynch, A.S., and Wang, J.C. 1996. Molecular dissection of a protein SopB essential for *Escherichia coli* F plasmid partition. *J. Biol. Chem.* 271: 17469-75.
- Hardham, J., and Rosey, E. 2000. Antibiotic selective markers and spirochete genetics. *J. Mol. Microbiol. Biotechnol.* 2: 425-432.
- Hansen, E.B. 1989. Structure and regulation of the lytic replicon of phage P1. *J. Mol. Biol.* 207:135-149.
- Hayakawa, Y., Murotsu, T., and Matsubara, K. 1985. Mini-F protein that binds to a unique region for partition of mini-F plasmid DNA. *J. Bacteriol.* 163: 349-54.
- Higgins, D., Bleasby, A., and Fuchs, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. *Comput. Appl. Biosci.* 8: 189-191.
- Hiraga, S. 1992. Chromosome and plasmid partition in *Escherichia coli*. *Ann. Rev. Biochem.* 61: 283-306.
- Ireton, K., Gunther, N.W., and Grossman, A.D. 1994. *spoUJ* is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J. Bacteriol.* 176: 5320-9.
- Karlin, S. 1999. Bacterial DNA strand compositional asymmetry. *Trends Microbiol.* 8: 305-308.
- Kim, S.K., and Shim, J. 1999. Interaction between F plasmid partition proteins SopA and SopB. *Biochem. Biophys. Res. Commun.* 263: 113-7.
- Kim, S.K., and Wang, J.C. 1998. Localization of F plasmid SopB protein to positions near the poles of *Escherichia coli* cells. *Proc. Natl. Acad. Sci. USA* 95: 1523-7.
- Kornberg, A., and Baker, T. 1992. DNA replication, 2nd ed. W.H. Freeman and Co., New York.
- Lafay, B., Lloyd, T.L., McLean, M.J., Devine, K.M., Sharp, P.M., and Wolfe, K. 1999. Proteome composition and codon usage in spirochetes: Species-specific and DNA strand-specific mutational biases. *Nucleic Acids Res.* 27: 1642-1649.
- Lin, D.C.H., Levin, P.A., and Grossman, A.D. 1997. Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 94: 4721-6.
- Lin, Z., and Mallavia, L.P. 1994. Identification of a partition region carried by the plasmid QpH1 of *Coxiella burnetii*. *Mol. Microbiol.* 13: 513-23.
- Lin, Z., and Mallavia, L.P. 1998. Membrane association of active plasmid partitioning protein A in *Escherichia coli*. *J. Biol. Chem.* 273: 11302-12.
- Lobry, J.R. 1995. Properties of a general model of DNA evolution under no-strand bias conditions. *J. Mol. Evol.* 40: 326-330.
- Lobry, J.R. 1996. Asymmetric substitution patterns in the two DNA strands of bacteria. *Mol. Bio. Evol.* 13: 660-665.
- Mackiewicz, P., Gierlik, A., Kowalczyk, M., Dudek, M.R., and Cebrat, S. 1999. How does replication-associated mutational pressure influence amino acid composition of proteins? *Gen. Res.* 9: 409-416.
- Marczynski, G.T., and Shapiro, L. 1992. Cell-cycle control of a cloned chromosomal origin of replication from *Caulobacter crescentus*. *J. Mol. Biol.* 226: 959-977.
- McInerney, J.O. 1998. Replication and transcriptional selection on codon usage in *Borrelia burgdorferi*. *Proc. Natl. Acad. Sci. USA* 95: 10698-10703.
- McLean, M.J., Wolfe, K.H., and Devine, K.M. 1998. Base composition skews, replication orientation, and gene orientation in 12 prokaryote genomes. *J. Mol. Evol.* 47: 691-696.
- Messer, W., and Weigel, C. 1996. Initiation of chromosome replication. In: *Escherichia coli and Salmonella typhimurium: Cellular and molecular biology*, 2nd ed. F.C. Neidhart, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umberger, eds. ASM Press, Washington, D.C. p. 1579-1601.
- Mohl, D.A., and Gober, J.W. 1997. Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* 88: 675-84.
- Mori, H., Kondo, A., Ohshima, A., Ogura, T., and Hiraga, S. 1986. Structure and function of the F plasmid genes essential for partitioning. *J. Mol. Biol.* 192: 1-15.
- Mori, H., Mori, Y., Ichinose, C., Niki, H., Ogura, T., Kato, A., and Hiraga, S. 1989. Purification and characterization of SopA and SopB proteins essential for F plasmid partitioning. *J. Biol. Chem.* 264: 15535-41.
- Moriya, S., Atlung, T., Hansen, F.G., Yoshikawa, H., and Ogasawara, N. 1992. Cloning of an autonomously replicating sequence (*ars*) from the *Bacillus subtilis* chromosome. *Mol. Microbiol.* 6: 309-315.
- Moriya, S., Fukuoka, T., Ogasawara, N., and Yoshikawa, H. 1988. Regulation of initiation of the chromosomal replication by DnaA-boxes in the origin region of the *Bacillus subtilis* chromosome. *EMBO J.* 7: 2911.
- Motallebi-Veshareh, M., Rouch, D.A., and Thomas, C.M. 1990. A family of ATPases involved in active partitioning of diverse bacterial plasmids. *Mol. Microbiol.* 4: 1455-63.
- Mrazek, J., and Karlin, S. 1998. Strand compositional asymmetry in bacterial and large viral genomes. *Proc. Natl. Acad. Sci. USA* 95: 3720-3725.
- Ogasawara, N., and Yoshikawa, H. 1992. Genes and their organization in the replication origin region of the bacterial chromosome. *Mol. Microbiol.* 6: 629-34.
- Old, I.G., Margarita, D., and Saint-Girons, I. 1993. 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.
- Picardeau, M., Lobry, J.R., and Hinnebusch, B. J. 1999. Physical mapping of an origin of bidirectional replication at the centre of the *Borrelia burgdorferi* linear chromosome. Mol. Microbiol. 32: 437-445.
- Picardeau, M., Lobry, J.R., and Hinnebusch, B.J. 2000. Analyzing DNA strand compositional asymmetry to identify candidate replication origins of *Borrelia burgdorferi* linear and circular plasmids. Genome. Res. (in press).
- Radman, M. 1998. DNA replication: One strand may be more equal. Proc. Natl. Acad. Sci. USA 95: 9718-9719.
- Ravin, N., and Lane, D. 1999. Partition of the linear plasmid N15: interactions of N15 partition functions with the *sop* locus of the F plasmid. J. Bacteriol. 181: 6898-906.
- Ravin, V., Ravin, N., Casjens, S., Ford, M., Hatfull, G., and Hendrix, R. 2000. Genomic sequence and analysis of the atypical temperate bacteriophage N15. J. Mol. Biol.: in press.
- Reyes, A., Gissi, C., Pesole, G., and Saccone, C. 1998. Asymmetrical directional mutation pressure in the mitochondrial genome of mammals. Mol. Biol. Evol. 15: 957-966.
- Rocha, E.P.C., Danchin, A., and Viari, A. 1999. Universal replication biases in bacteria. Mol. Microbiol. 32: 11-16.
- Saint-Girons, I., Chi, B., and Kuramitsu, H. 2000. Development of shuttle vectors for spirochetes. J. Mol. Microbiol. Biotech.: in press, this volume.
- Salazar, L., Fsihi, H., de Rossi, E., Riccardi, G., Rios, C., Cole, S.T., and Takiff, H.E. 1996. Organization of the origins of replication of the chromosomes of *Mycobacterium smegmatis*, *Mycobacterium leprae* and *Mycobacterium tuberculosis* and isolation of a functional origin from *M. smegmatis*. Mol. Microbiol. 20: 283-293.
- Seufert, M., and Messer, W. 1987. DnaA protein binding to the plasmid origin region can substitute for primosome assembly during replication of pBR322 in vitro. Cell 48: 73-78.
- Singh, R.A., Choudhury, N.R., and Das, H.K. 2000. The replication origin of *Azotobacter vinelandii*. Mol. Gen. Genet. 262: 1070-1080.
- Stevenson, B., Casjens, S., and Rosa, P. 1998. Evidence of past recombination events among the genes encoding the Erp antigens of *Borrelia burgdorferi*. Microbiol. 144: 1869-79.
- Stevenson, B., Zückert, W., and Akins, D. 2000. Repetition, conservation, and variation: The multiple cp32 plasmids of *Borrelia* species. J. Mol. Microbiol. Biotech.: in press, this volume.
- Strauss, E.J., and Falkow, S. 1997. Microbial pathogenesis: Genomics and beyond. Science 276: 707-711.
- Sueoka, N. 1995. Intrastrand parity rules of DNA base composition and usage biases of synonymous codons. J. Mol. Evol. 37: 137-153.
- Taghavi, S., Provoost, A., Mergeay, M., and van der Lelie, D. 1996. Identification of a partition and replication region in the alcaligenes eutrophus megaplasmid pMOL28. Mol. Gen. Genet. 250: 169-179.
- Taylor, K., and Wegrzyn, G. 1995. Replication of coliphage lambda DNA. FEMS Microbiol. Rev. 17: 109-119.
- Tilly, K., Elias, A.E., Bono, J.L., Stewart, P., and P. Rosa. 2000. DNA exchange and insertional mutagenesis in spirochetes. J. Mol. Microbiol. Biotech.: in press, this volume.
- Tumbula, D.L., Bowen, T.L., and Whitman, W.B. 1997. Characterization of pURB500 from the Archaeon *Methanococcus maripaludis* and construction of a shuttle vector. J. Bacteriol. 179: 2976-2986.
- Walker, J., Saraste, M., Runswick, M., and Gay, N. 1982. Distantly related sequences in the α - and β -subunits of ATP synthetase, myosin, kinases and other ATP-requiring enzymes and common nucleotide binding fold. EMBO J. 1: 945-951.
- Watanabe, E., Inamoto, S., Lee, M.H., Kim, S. U., Oguwa, T., Mori, H., Hiraga, S., Yamasaki, M., and Nagai, K. 1989. Purification and characterization of the *sopB* gene product which is responsible for stable maintenance of mini-F plasmid. Mol. Gen. Genet. 218: 431-6.
- Yee, T. W., and Smith, D.W. (1990). *Pseudomonas* chromosomal replication origins: A bacterial class distinct from *Escherichia coli*-type origins. Proc. Natl. Acad. Sci. USA. 87: 1278-1282.
- Zakrzewska-Czerwinska, J., and Schrepf, H. 1992. Characterization of an autonomously replicating region from the *Streptomyces lividans* chromosome. J. Bacteriol. 174: 2688-2693.
- Zückert, W. R., and Meyer, J. 1996. Circular and linear plasmids of Lyme disease spirochetes have extensive homology: characterization of a repeated DNA element. J. Bacteriol. 178: 2287-98.