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Review

The plasmids of *Borrelia burgdorferi*: essential genetic elements of a pathogen

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Abstract

The spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, has an unusual genome comprised of a linear chromosome and the largest plasmid complement of any characterized bacterium. Certain plasmid-encoded elements are required for virulence and viability, both in vitro and in vivo. The genetic tools to manipulate *B. burgdorferi* are sufficiently developed for precise molecular genetic investigations. *B. burgdorferi* now represents a prime system with which to address basic questions of plasmid biology and plasmid contributions to bacterial virulence and disease pathogenesis.
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1. Introduction

Perhaps nowhere else in the prokaryotic world have plasmids evolved to be as central to an organism's life cycle as they have in the spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease. The genomic sequence of the *B. burgdorferi* type strain identified 21 linear and circular plasmids, the largest number of any characterized bacterium (Casjens et al., 2000; Fraser et al., 1997). At least one plasmid has been demonstrated to be essential under all growth conditions tested, and others appear to be required for survival in

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the natural life cycle (Byram et al., 2004; Labandeira-Rey and Skare, 2001; Labandeira-Rey et al., 2003; Purser and Norris, 2000; Purser et al., 2003; Simpson et al., 1990b; Xu et al., 1996). The unique structure of the *B. burgdorferi* genome raises several interesting biological issues, including how an organism maintains and regulates 22 separate replicons, whether an essential plasmid is truly distinct from a chromosome, the selective advantages that may be gained by maintaining critical genes on extrachromosomal elements, and the evolution of linear plasmid forms. These issues have also been of central interest to plasmid biologists, although, overall, the two fields of study progressed independently of one another until quite recently. Partly, this was due to the initial interest in *B. burgdorferi* as a pathogen, and not for the unusual characteristics of its genome structure, and partly, it was due to the lack of genetic tools available to manipulate *B. burgdorferi*. However, recent advances have produced multiple selectable markers, hastening the development of targeted gene inactivation in infectious strains, multiple shuttle vectors, and a transposon mutagenesis system. The new genetic techniques, combined with the unusual plasmid system, position *B. burgdorferi* as a preeminent model for studying the vital functions plasmids contribute to the life cycle of an organism.

1.1. Borrelia burgdorferi life cycle

Borrelia burgdorferi has a complex life cycle with many of the factors needed for surviving in vivo encoded on its plasmids. The most direct line of evidence for this is the longstanding observation that many plasmids can be lost during serial liquid propagation of *B. burgdorferi* (Barbour, 1988; Grimm et al., 2003; Norris et al., 1995; Sadziene et al., 1993a; Schwan et al., 1988; Simpson et al., 1990b). The loss of these plasmids does not appear to affect the bacterium's ability to grow in vitro, but loss of certain plasmids correlates with lost or reduced infectivity in mice (Labandeira-Rey and Skare, 2001; Purser and Norris, 2000; Sadziene et al., 1993a; Schwan et al., 1988; Simpson et al., 1990b; Xu et al., 1996). The potential contributions of the plasmid genes to in vivo survival are best appreciated in the context of the natural environments *B. burgdorferi* must sense, adapt to, and replicate in.

An obligate parasite, the life cycle of *B. burgdorferi* parallels the life cycle of its hosts (Fig. 1). One of the major animal reservoirs of *B. burgdorferi* is the white-footed mouse, *Peromyscus leucopus*. However, other mammals as well as birds are competent hosts for the spirochete. Ticks of the *Ixodes* complex act as vectors to spread the spirochetes from one animal to another. A tick feeding on an infected animal ingests both the host's blood and the parasite. *B. burgdorferi* remains confined to the tick's midgut until the next bloodmeal, when the bacterium apparently senses the incoming blood and/or physiological changes in the tick. During the bloodmeal, *B. burgdorferi* migrates out of the tick midgut to the salivary glands, where it is spit back into the animal during tick feeding. Although humans are not natural hosts for *Ixodes*, these ticks are opportunistic and will feed upon humans, with the potential to transmit *B. burgdorferi* and produce Lyme

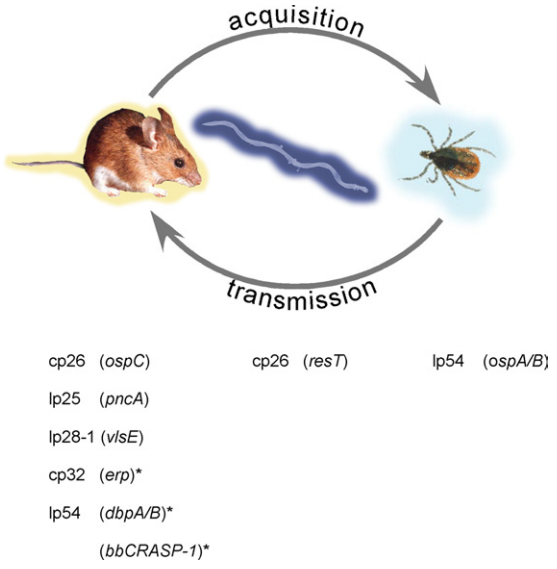

Fig. 1. Mouse–tick infectious cycle of *B. burgdorferi*. The primary reservoir of *B. burgdorferi* in the US is the white-footed mouse. Ticks acquire the spirochete by feeding on infected mammals and may later transmit the parasite during subsequent bloodmeals. Listed below the figure are the plasmid genes believed to be important for survival in each host; the plasmid shown in the center is probably essential in both. Asterisks denote genes presumed to be required in the mammal but for which this has not been demonstrated.