Commentary

Borrelia Outer Membrane Surface Proteins and Transmission Through the Tick

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Pathogens of the bacterial genus *Borrelia* differ from fellow spirochetes, *Treponema* and *Leptospira*, in their use of arthropod vectors for transmission between warm-blooded hosts. The agents of relapsing fever, *Borrelia recurrentis* and *Borrelia hermsii*, are transmitted by lice and fast-feeding soft ticks, respectively, whereas the Lyme disease spirochete, *Borrelia burgdorferi* (*Bb*), is transmitted to human hosts via the hard-shelled tick, *Ixodes* (1, 2). The evolutionary adaptation to both arthropod and warm-blooded hosts involved the invention of repertoires of outer membrane surface proteins, largely lipoproteins, conferring the ability to adhere, recognize, and respond to mammalian and arthropod tissues. As described in this issue by Yang et al., the most robust model to date for *Bb* lipoprotein function are two plasmid-encoded genes, paralog siblings *OspA* and *OspB*, encoding prominently expressed outer envelope lipoproteins (3), which function during the passage of borreliae within the tick vector midgut (4). In turn, a third gene encoding the lipoprotein OspC is essential for egress to the tick salivary glands (5). Here I will give a brief introduction to *Borrelia* biology, describe the tremendous capacity and complexity of borrelial lipoprotein repertoires, and then focus on new studies and methodologies being brought to bear on understanding *Bb* Osp lipoprotein function during tick transmission.

A Brief Natural History of Ticks and Borrelia. Ticks, like all biting (hematophagous) arthropods, routinely salivate into their food. Hematophagous arthropods possess pronounced salivary glands secreting numerous bioactive molecules that aid in blood pool formation and delivery, including anticoagulants, antiplatelet factors, vasodilators, histamine blockers, and in the case of the prolonged-feeding ticks, attachment cement (6, 7). As a consequence, and of concern to human and animal health, tick salivary secretion is also the route of transmission for pathogens such as the bacteria *Rickettsia* and *Borrelia*, apicomplexan protozoans *Theileria* and *Babesia*, as well as numerous viruses. *Ixodes* ticks take a blood meal, which can last several days, during each of their larval, nymph, and adult stages. *Bb* transmission is typically initiated by spirochete ingestion and midgut colonization during a larval stage feed on an infected mammalian host (such as the common *Bb* reservoir, the mouse *Peromyscus*). This is followed by a period of spirochete relative dormancy through the larvae-nymph molt. During a second nymphal feed on a new host, these borreliae proliferate, detach from the midgut epithelium, migrate to the salivary glands, and are transmitted to this same host. It is an infected nymphal feed on a human—the "accidental" nonreservoir host—that is the target for transmission-blocking vaccine development. Via vaccination of humans using borreliae midgut stage surface antigens it is sought to block human infection after tick ingestion of immune sera and thereby disruption of borreliae midgut stage development and egress to the salivary gland (8).

Unlike arthropod-born protozoan parasites, such as *Plasmodium* and *Leishmania*, borreliae do not undergo gross cellular transformations during their transit through the tick and maintain their spiroform shape composed of two membranes sandwiching periplasmic flagella. The transition from midgut to salivary glands predominantly involves changes in gene expression regulating the complement of surface proteins, which is likely accompanied by increased motility and capacity for tissue invasion. Expression of these genes may be triggered globally by a variety of factors, including an increase from ambient to warm-blooded host body temperature during the blood feed, a decrease in blood meal pH, tick factors secreted within the bloodmeal, CO_2 tension, spirochete density after proliferation within the midgut, and other factors related to the influx of host blood. A logical model (currently undergoing experimental scrutiny) distinguishes the reciprocal states "warm blood" versus "cold tick" and classifies as "group I" those *Bb* genes up-regulated in response to a temperature increase and pH decrease, with the remaining genes classified as "group II" (9).

The Borrelial Genomes and Encoded Lipoprotein Catalogs. The borrelial genome is perhaps the most structurally complex among bacteria. The 1.5-Mb *Bb* genome sequence is encoded in a single linear 0.91-Kb chromosome plus a highly dynamic complement of 12 linear and 9 circular extrachromosomal DNAs (10, 11). Approximately 5% of *Bb* chromosomal genes and 15% of plasmid genes are devoted to a catalog of >130 lipoproteins (11), which is larger and more complex than any encoded in a sequenced bacterial genome. The diversity in the lipoprotein repertoire is further enhanced by mechanisms of antigenic variation (in *Bb*, the *vlsE* alleles) (12, 13), plasmid recombination events mediating antigenic

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diversity among multigene families (e.g., the *Erp* and *Mlp* genes) (14), and acquisition of new genetic information via transduction of a population of phage-like plasmids (the cp32 plasmid family) (11). With the *Bb* genome template now in hand, it is important and straightforward to determine the complete genome sequences for the etiologic agents of tick-born and louse-born relapsing fevers, *Borrelia hermsii* and *B. recurrentis*. These genome sequences will allow comparison of the composition of the lipoprotein catalogs between *Borrelia* species, providing insights into differences in genome architecture arising from linear and circular DNAs, and an understanding of the evolutionary advantage of distributing lipoprotein genes between chromosomal and extrachromosomal DNAs. Some progress has been made; for example, it has been shown that the *Bb* cp32 plasmid family shares sequence similarities with plasmids in *B. hermsii* (15), including orthologs of two surface antigens, mlp and Bdr. *B. hermsii* and *Bb* also have similar mechanisms of antigenic variation via duplicative transposition (the *vmp* genes in *B. hermsii* and *vlsE* in *Bb*) (16).

Bb Outer Membrane Proteins. The majority of identified putative outer membrane *Bb* proteins are lipoproteins, typified by an NH_2-terminal type II signal sequence and a lipobox motif mediating NH_2-terminal lipidation on a conserved cysteine residue (10, 11). It is currently poorly understood and of interest to identify the structural motifs predicting localization of lipoproteins on the outer membrane surface versus orientation within the periplasmic space. The large catalog of *Borrelia* lipoproteins likely underpins immune evasion strategies, in remarkable contrast to the spirochete *Treponema*, which has relatively few lipoproteins (17) and is thought to be relatively invisible to the host with respect to outer surface antigens (18). The two spirochetes may have evolved distinct strategies for evasion of humoral immunity, with *Borrelia* relying on antigenic diversity and antigenic variation, whereas *Treponema* simply avoids presentation of surface antigens. The genome sequence for *Leptospira* is now available (19), and it is of interest to conduct whole genome comparisons of the three catalogs of putative surface proteins in conjunction with a meta-analysis of available protein expression and cellular localization studies in spirochetes and other bacterial genera. However, a general theme is clear: spirochetes share few orthologous membrane surface proteins and the majority of the *Bb* molecules are lineage-specific inventions, lacking orthologs outside of the *Borrelia* clade. BLAST analysis thus far identifies few "spirochete" lipoproteins, such as BB0155 that is found only in *Borrelia* and *Treponema* (TP0646). Proteins of wider phylogenetic representation in bacteria (e.g., TmpC, Tp92, Tpn50/OmpA, Tpn38b, and rlpA) might be involved in conserved cellular functions, such as transport or metabolic roles rather than interactions at the host interface. An additional class of outer membrane protein candidates contain domains of a wider phylogenetic representation that includes eukaryotes (20). This class includes proteins BB0172, BB0173, and BB0325, which contain vWA domain, a motif also represented in *Treponema* (e.g., GenBank/EMBL/DDBJ accession no. AAC65016), a putative surface membrane protein containing a PR1 domain (BB0689), and possible secreted proteins containing TPR repeats (e.g., LMP1, sequence data available from GenBank/EMBL/DDBJ under accession no. NP_212344).

Bb Outer Membrane Proteins OspA and OspB. The lipoproteins OspA and OspB are encoded on adjacent paralogous genes present on *Bb* linear plasmid lp54. *OspA/B* transcriptional regulation is considered to be linked via a single transcript, although there is preliminary evidence of differential transcript regulation (21). OspA and OspB share 53% amino acid identity and likely have a similar antiparallel "free-standing" β sheet protein structure associated with the outer membrane surface via a lipidated NH_2-terminal cysteine residue (22). Neither protein is thought to be expressed in the mammalian host, although OspA expression during persistent borreliosis has been implicated in human

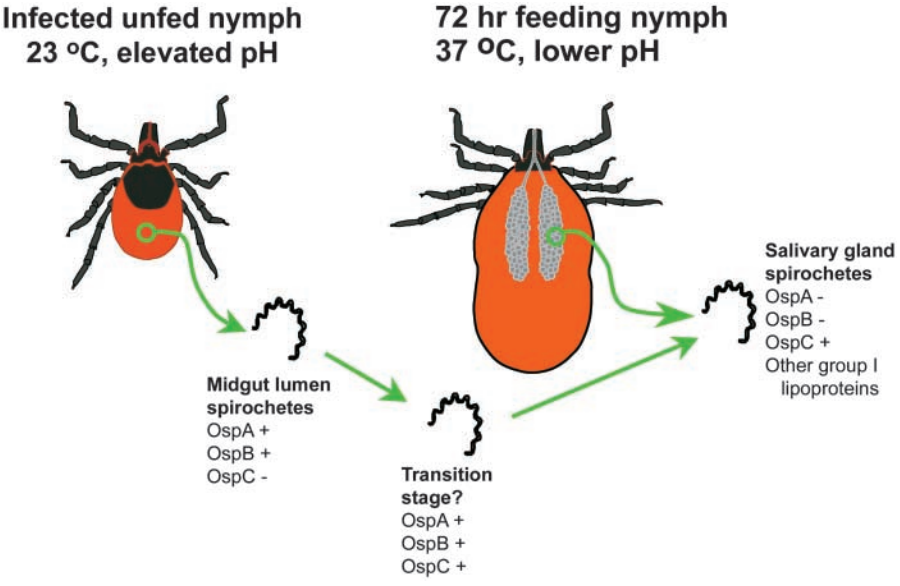

Figure 1. Maturation of borreliae within the tick midgut. Unfed infected nymphs acquire infection from a previous feed during the tick larval stage. Midgut borreliae are quiescent and express group II stage lipoproteins, predominantly OspA and OspB. During a nymphal stage feed on a new host in response to changes in temperature and pH the borreliae begin expressing OspC and other group I stage lipoproteins (e.g., DbA/B, Mlp, Erp, and vslE).

chronic Lyme arthritis (23). Recombination appears to play little or no role in generating OspA/B antigenic diversity, unlike plasmid-encoded multigene families such as the *Erp* and *Mlp* lipoproteins. This might be expected if OspA/B are expressed solely in the tick vector and do not encounter immune pressure driving antigenic diversity. Thus, the *Bb* lipoprotein catalog might be divided into two groups: those having greater antigenic diversity at a population level, driven by mammalian host humoral immune pressure, versus proteins functioning solely in the tick and encountering little immune pressure.

Dominant OspA/B expression occurs during midgut colonization after a larval feed on an infected host, likely in response to the ambient temperature and pH of the tick midgut (24). In recent years, an attractive model has been built: that OspA/B mediates adherence to larval midgut epithelium and perhaps additionally aids in survival during the larval-nymph molt and proliferation in response to a nymphal blood meal (Fig. 1). During the nymphal blood meal, OspA is down-regulated in proliferating borreliae in response to the temperature increase associated with the prolonged feeding period, freeing the spirochetes to migrate to the salivary glands. Competence to egress the midgut and invade salivary glands is correlated with expression of a third lipoprotein, OspC (described in the following section).

Targeted Gene Disruption and Complementation of OspA/B. The study by Yang et al. (4) demonstrates that targeted gene disruption of the extended *OspA/B* locus has no observable consequence on the ability of borreliae to establish an infection in mice, whereas the locus is critically essential for colonization of the tick midgut (4). In turn, complementation of either OspA or OspA/B expression restores the ability to colonize the midgut and invasion of salivary glands. These results greatly solidify a body of experimentation implicating OspA and OspB as midgut stage antigens (for review see reference 25) but more importantly set the stage for dissection of OspA/B function. Complementation of OspA alone indicates that OspB expression is not essential for tick transmission; however, the great plasticity of the *Bb* genome suggests that *OspB* evolutionary persistence is the result of positive selection pressures, and *OspB* must therefore be of intrinsic value to the organism. Therefore, it is of interest to complement OspB alone in order to determine if OspA and OspB have compensating or redundant functions. It is also of interest to express OspA (additionally, OspB and OspA/B) in trans in wild-type borreliae driven by an *OspC* or other group I gene promoter. If OspA is indeed a midgut lumen adhesion molecule then it might be expected that after a nymphal stage feed mature midgut stage spirochetes will express both introduced constitutive OspA and up-regulated OspC (as is known to occur during maturation of midgut borreliae) but be unable to release and exit the tick midgut. If this phenotype indeed results, then ultrastructural studies may reveal the mechanism of midgut wall transit; for instance, the resulting spirochetes may damage surrounding midgut epithelial tissues via secretion or recruitment of proteases and lipases but fail to exit the midgut due to their OspA-mediated adhesiveness.

Recently the outer membrane surface lipoprotein gene, *OspC*, was disrupted, resulting in a phenotype in which borreliae are capable of colonizing tick midguts but do not migrate to salivary glands (5). This phenotype correlates well with expression profiles showing OspC up-regulation after an infected tick nymphal feed (24). OspC shows little structural similarity with OspA (and presumably, OspB) and is composed of predominantly α-helical globular domains (26) rather than the extended antiparallel β-sheet structure of OspA (22, 27). In *OspC* disruptant borreliae, the spirochetes persist in late stage feeding midguts despite a presumed absence of both OspC and OspA/B. What are the roles of lipoproteins as adhesive proteins or involvement in tissue egress versus "surface coat" functions protecting against the potentially harsh environment of the midgut lumen? In *OspC* disrupted lines, it must be concluded that either a predominant coat lipoprotein is not necessary for protection against degradation or that other lipoproteins compensate for the lack of OspA/B/C. In response to nymphal feeding, other group I lipoproteins are up-regulated in addition to OspC, such as members of the Mlp, Erp, and Db lipoprotein families. However, these proteins apparently cannot compensate for loss of OspC function in egress to the salivary gland. This is a quandary to tease apart, determining the role of individual lipoproteins in protection from the environment, adhesion, tissue egress and invasion, and salivary gland recognition.

Conclusions. Many hurdles have been overcome recently in the ability to disrupt *Bb* genes without creating culture-mediated artifacts in phenotype, such as due to loss of *Bb* plasmid DNA, and targeted gene disruptions are increasingly contributing to understanding of *Bb* biology. Several gene disruptant lines have been generated aimed at determining the regulation mechanism of group I versus group II lipoprotein gene expression, including knockout of the response regulator Rrp2 (28) and alternative sigma factors, RpoN and RpoS (29). The gene knockout and complementation methodologies appear sufficiently facile and diverse to support a range of combinatoric gene disruption, complementation, and promoter-regulated trans expression experiments. These studies will illuminate the roles of additional tick stage outer membrane proteins and the panoply of lipoproteins involved in establishing and persisting infection in the warm-blooded host.

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