Published in final edited form as:

Microbiol Spectr. 2019 May ; 7(3): . doi:10.1128/microbiolspec.PSIB-0026-2019.

Protein Secretion in Spirochetes

Wolfram R. Zückert, Ph.D.

University of Kansas School of Medicine, Department of Microbiology, Molecular Genetics and Immunology, Kansas City, KS

Abstract

Spirochetes form a separate phylum of bacteria with two membranes but otherwise unusual morphologies and envelope structures. Distinctive common features of *Borrelia*, *Leptospira* and *Treponema* include the sequestration of flagella to the periplasm and thin peptidoglycan cell walls that are more closely associated with the inner membrane. Outer membrane compositions differ significantly between the genera. *Leptospira* most closely track gram-negative bacteria due to the incorporation of lipopolysaccharides. *Treponema* and *Borrelia* outer membranes lack lipopolysaccharide, with treponemes expressing only few outer membrane proteins, and *Borrelia* displaying a dizzying diversity of abundant surface lipoproteins instead. Phylogenetic and experimental evidence indicates that spirochetes have adapted various modules of bacterial export and secretion pathways to build and maintain their envelopes. Export and insertion pathways in the inner membrane appear conserved, while spirochetal experimentation with various envelope architectures over time has led to variations in secretion pathways in the periplasm and outer membrane. Classical type I-III secretion systems have been identified, with demonstrated roles in drug efflux and export of flagellar proteins only. Unique activities of periplasmic proteases, including a C-terminal protease, are involved in maturation of some periplasmic proteins. Proper lipoprotein sorting within the periplasm appears to depend on functional Lol pathways that lack the outer membrane lipoprotein insertase LolB. The abundance of surface lipoproteins in *Borrelia* and detailed protein sorting studies suggest a lipoprotein secretion pathway that either extends Lol through the outer membrane or bypasses it altogether. Proteins can be released from cells in outer membrane vesicles, or rarely as soluble proteins.

Introduction

Spirochetes form a distinct bacterial phylum of slender, diderm (dual-membrane) bacteria that exhibit either a coiled “corkscrew” or flat-wave “serpentine” morphology. These distinct phenotypes are at least partly due to various numbers of periplasmic flagella that are inserted sub-terminally at both poles of the bacteria, wrapping around the protoplasmic cylinder and often overlapping in the middle of the cell. Coordinated rotation of the flagellar bands or bundles, sometimes referred to as “axial filaments”, leads to rotation of the cell cylinder and cellular motility that is particularly prominent in viscous environments.

Spirochetes have evolved to occupy a variety of niches, from living freely in soil and marine sediments, to becoming fully host-adapted as commensals in the gut of termites and other arthropods, or as often pathogenic obligate parasites of vertebrates. Three spirochetal genera, *Borrelia*, *Leptospira* and *Treponema*, have garnered significant attention due to their medical importance and have emerged as unique spirochetal model systems in studies of microbial physiology and pathogenesis. *Borrelia burgdorferi* (recently reclassified as *Borrelia burgdorferi* (1)), together with other closely related species, causes tick-borne Lyme disease in temperate climates of the northern hemisphere; a distinct group of *Borrelia* species causes endemic tick-borne relapsing fever in the Western United States and Africa, or outbreaks of louse-borne relapsing fever among confined human populations. *Leptospira interrogans* and other pathogenic species are the causative agents of leptospirosis, a global zoonosis that is mainly transmitted through direct or indirect contact with soil or water contaminated with animal urine, fluids or tissues; other leptospires are either organisms with intermediate pathogenicity able to colonize animals or free-living saprophytes (e.g., *Leptospira biflexa*) growing in moist environmental settings. *Treponema pallidum* subspecies *pallidum* (hereafter referred to as *T. pallidum*) is the causative agent of venereal syphilis, an ancient sexually-transmitted human infection that has persisted in the human population to the present day; other non-venereal treponemes are also transmitted by direct human-to-human contact, and *Treponema denticola* is part of the complex human oral flora and common periodontal pathogen. Other treponemal species colonize vertebrates and insects, and can be found in aquatic and soil environments.

This chapter will review our current understanding of protein secretion mechanisms in *B. burgdorferi*, *L. interrogans* and *T. pallidum*. Despite their common phylogenetic classification as spirochetes, these three species differ significantly in their envelope structure, particularly when looking at the composition of the bacterial surface as the interface with the host. The generation and maintenance of these different interfaces throughout transmission and subsequent infection requires protein secretion pathways that have been evolutionarily adapted and fine-tuned for efficiency. Our current understanding of these mechanisms is most detailed for *B. burgdorferi*, in part due to the earlier availability of robust genetic tools. But recent technological advances are likely to further stimulate the analysis of leptospiral and treponemal protein secretion systems as well.

Spirochetal cell envelope ultrastructures

Cryo-electron tomography (cryo-ET) studies on *Borrelia*, *Leptospira* and *Treponema* have provided a comprehensive and remarkably detailed overview of the different envelope ultrastructures (2-6). The cross-referencing of these micrographic data with detailed protein localization studies now allow us to catalog the requirement for protein secretion pathways in the different systems.

Common to all three model spirochetes is a diderm envelope architecture similar to gram-negative bacteria, with an inner cytoplasmic membrane (IM), an outer membrane (OM) and a periplasmic space in between (Fig. 1). A thin periplasmic peptidoglycan cell wall layer appears to be more closely associated with the IM than the OM, as not to interfere with the movement of the periplasmic flagella that can be visualized between the cell wall and the

OM. The OM of both pathogenic and saprophytic *Leptospira* is considerably thicker than that of *Borrelia* or *Treponema*, mainly due to the presence of a protease-resistant layer of lipopolysaccharide (LPS) in the surface leaflet (6). In contrast, the *Borrelia* OM lacks LPS, but contains abundant OM proteins including a particularly large array of surface lipoproteins that can be removed by proteolytic surface shaving (3, 7). LPS is also missing from the envelopes of treponemes, with the *T. pallidum* OM containing only low-density integral membrane proteins and few surface lipoproteins (8), and *T. denticola* likewise displaying only distinct outer sheath surface proteins (9). Thus, the *Leptospira* OM most closely conforms to a typical gram-negative envelope structure, with LPS molecules serving as major and serotype-defining surface antigens. The envelope structures of *Borrelia* and *Treponema*, however, diverge significantly from those of more classical diderm bacteria, with consequences for bacterial virulence mechanisms and protein secretion pathway requirements. *T. pallidum*'s paucity of surface-exposed proteins contributes to it being a "stealth pathogen" that can disseminate and cause symptomatic tissue damage while evading host immune responses (8). The peculiar abundance of *Borrelia* surface lipoproteins (7), which serve as immunodominant antigens and drive transmission, colonization, dissemination and persistence (10, 11), puts the focus square on how this set of proteins reaches the outer leaflet of the OM.

Sec-mediated export and IM insertion

Bioinformatic evidence in the current inventory of clusters of orthologous groups (COGs) (12, 13) suggests that spirochetal protein export from the cytoplasm through the IM follows the general secretory pathway that has been described and amply investigated in other diderm bacterial models (Fig. 2). The determined *Borrelia*, *Treponema* and *Leptospira* genomes (14-18) encode for all the essential Sec proteins that are needed to form a functional translocon. The presence of homologs for the signal recognition particle Ffh and its receptor FtsY, but absence of the cytoplasmic SecB chaperone suggests that proteins can be exported co-translationally, i.e., upon docking of the translating ribosome to the Sec translocon via Ffh-Fts. SecB's function in post-translational export may be taken over by other cytoplasmic folding chaperones such as GroEL. There is no evidence for a functional twin-arginine transport (TAT) pathway in spirochetes that would allow for export of folded proteins through the IM; note that the described *T. pallidum* Tat proteins are unrelated to TAT, but rather part of a tripartite ATP-dependent periplasmic (TRAP)-type transporter system that seems to facilitate import of hydrophobic molecules through the periplasm and the IM (8, 19).

All three model spirochete genomes encode for homologs of the IM protein insertase YidC. Thus, the mechanism of release of integral IM proteins from the Sec translocon into the IM is likely similar to that of well-studied gram-negative model bacteria. At the same time, IM proteins with simple transmembrane (TM) topologies such as the abundant and highly immunogenic *B. burgdorferi* Bdr proteins with their single C-terminal TM domains (20-22), may be inserted into the IM by YidC in a Sec-independent manner (Fig. 2).

Signal peptide processing

Like in other bacterial systems, two distinct signal peptidases appear responsible for cleaving the N-terminal Sec-dependent signal peptides once exported spirochetal proteins have reached the periplasm (Fig. 2). Signal I peptidase LepB removes the signal peptides from soluble periplasmic or secreted proteins and integral OM proteins. Signal II peptidase Lsp removes signal peptides from lipoproteins that will ultimately remain associated with the IM or transported further to the periplasmic and surface leaflets of the OM (to be discussed below). Interestingly, *B. burgdorferi* encodes for three LepB homologs that – like sets of multiple LepBs in some gram-positive bacteria – may have different substrate specificities. *B. burgdorferi* LepB2, encoded by chromosomal ORF BB0031, is closest to a canonical LepB, with an N-terminal two-TM anchor domain and an appropriately localized periplasmic active site. LepB3 (BB0263) similarly is predicted to have all enzymatically relevant residues located in the periplasm, anchored by a single N-terminal TM domain. LepB1 (BB0030) immediately upstream of LepB2, however, appears to be a signal peptidase I-like protein with a four-TM domain topology that lacks the canonical active site residues. A conditional knockout of *lepB1* led to a marked growth defect with signs of significant envelope instability (M.K You, C.R. Whetstone and W.R. Zückert, unpublished results), but the protein's precise function remains unknown.

Sec-dependent and -independent secretion

Each of the three spirochetal model genera show *in silico* evidence of distinct subsets of Sec-dependent Type II (T2SS) or Sec-independent Type I (T1SS) and Type III (T3SS) secretion system homologs (12, 13) (Fig. 2). T2SSs span the periplasm and the OM as a major terminal branch of the general secretory pathway. There is no *in silico* evidence of T2SS components in either *Borrelia* or *Treponema* (12, 13). An identified T2SS in *Leptospira* appears to be incomplete, and its function in leptospiral protein secretion remains to be determined (23). Homologs of T1SS components, which together secrete ions, carbohydrates, drugs or proteins in other bacterial systems, have been identified in *Borrelia* and *Leptospira*, but not in *Treponema* (23). The *B. burgdorferi* T1SS is homologous to the Tol system in other diderm bacteria and consists of a trimeric outer membrane channel-tunnel protein (BesC), a periplasmic membrane fusion protein (BesA) and an inner membrane ATP-dependent export pump (BesB). A BesC-deficient mutant was non-infectious in a mouse model of infection and was more sensitive to several antibiotics, detergent, and DNA-intercalating agents. Recombinant BesC formed pores in lipid bilayers, and modeling of the *B. burgdorferi* Bes complex on the Tol structure suggested a less stable interaction of BesA with BesC due to a truncated α -helical interaction domain in BesA (24). Together, this suggests that the *B. burgdorferi* Bes system functions at least in part as an efflux pump for noxious compounds. No protein substrates for the *B. burgdorferi* Bes system have been identified. T3SS homologs have been identified in all three spirochetal model genera (12, 13), but appear limited to the IM protein complexes that export components of the periplasmic flagella, as shown for *B. burgdorferi* (25).

Lipoprotein modification

Lipoproteins can be found in all three possible compartments of the spirochetal envelope: in the periplasmic leaflet of the IM, the periplasmic leaflet of the OM, and in the surface leaflet of the OM. Based on *in silico* predictions, *T. pallidum* encodes for about 20 lipoproteins (26), while *L. interrogans* and *B. burgdorferi* encode for about 180 and 130 lipoproteins, respectively (15, 18, 27-29); in *B. burgdorferi*, this amounts to about 8% of the genome's coding capacity. *T. pallidum* lipoproteins have been localized to the periplasm, where they have been shown to function as part of nutrient import machineries (19). TP0453 is an OM low-abundance amphiphilic lipoprotein that is thought to additionally interact with the lipid bilayer through amphipathic helices (30). Another *T. pallidum* lipoprotein, encoded by ORF TP0435, has shown some very limited surface exposure when expressed heterologously in *B. burgdorferi* (31), but the biological significance of this finding remains to be determined. Similarly, only about half a dozen lipoproteins have been localized to the surface of *L. interrogans* (23), with an initial vaccine target, LipL32, recently being relegated to the periplasm (32). The most comprehensive localization study was carried out in *B. burgdorferi* by combining the analysis of an epitope-tagged lipoprotein expression library with quantitative proteomics. Of the 125 *B. burgdorferi* lipoproteins that were examined, 86 localized to the surface; only 8 of the 39 periplasmic lipoproteins were anchored in the OM (7). This unique abundance of surface-displayed *Borrelia* lipoproteins highlights their pivotal role in interactions with vector and host animals (10, 11), and also points to the requirement for an efficient lipoprotein secretion pathway that extends through the OM to the bacterial surface (discussed below).

All three model spirochetes encode for the known components of a diderm lipoprotein modification machinery (Fig. 2). In a first committing step, lipoprotein diacylglycerol transferase Lgt catalyzes the attachment of a diacylglycerol moiety to the side chain of a conserved cysteine within an internal N-terminal "lipobox" motif. Next, lipoprotein signal peptidase Lsp (a.k.a. signal peptidase II) recognizes the "lipobox" residues to remove the signal peptide. Finally, N-acyl transferase Lnt adds another acyl chain to the now available amino terminal cysteine, leading to N-terminally tri-acylated lipoproteins that are at least temporarily positioned in the periplasmic leaflet of the IM (33-36). Spirochetal Lsp appears to recognize "lipobox" motifs in lipoprotein signal peptides that are more relaxed than those found in other diderm systems (28, 36). Yet, as for other bacteria, spirochetal Lsp activity can be blocked by the antibiotic globomycin, a non-competitive Lsp inhibitor (37, 38) (39).

Lipoprotein periplasmic transport and secretion

One well-defined route for fully modified lipoproteins to the OM involves interactions with the Lol pathway. *B. burgdorferi*, *L. interrogans* and *T. pallidum* all encode for a partial Lol pathway that lacks the OM lipoprotein receptor/insertase LolB found only in β - and γ -proteobacteria (36) (Fig. 2). In *B. burgdorferi*, conditional mutants in the LolD ATPase and the periplasmic lipoprotein carrier LolA are lethal, indicating that the pathway is essential (K.M. Bridges, A.S. Dowdell, S. Chen, J.L. Kueker, J. Liu, J.S. Blevins & W.R. Zückert, unpublished results). Any direct or indirect involvement of the Lol pathway in surface lipoprotein secretion is pending experimental investigation. Several independent lines of

evidence point toward a periplasmic mechanism that maintains *B. burgdorferi* surface lipoproteins in a translocation-competent conformation by preventing them from folding prematurely in the periplasm (40-43). The commonly disordered N-terminal “tether” peptides of surface lipoproteins are intimately involved in this process, as tether deletion and substitution mutants of several surface lipoproteins were specifically deficient in OM translocation (43-45). This suggests a periplasmic “holding” mechanism that engages surface-targeted pro-lipoprotein peptides as they emerge tether-first from the IM Sec complex (Fig. 2). Surface lipoproteins are then thought to be delivered in an at least partially unfolded conformation to an OM lipoprotein “flippase”, which facilitates translocation through the OM and leads to the ultimate anchoring of surface lipoproteins in the surface leaflet of the OM (40, 41, 43) (Fig. 2). Intriguingly, *B. burgdorferi* encodes for distant homologs of five of the seven known LPS secretion pathway components – despite a lack of LPS. BB0838, a homolog of the OM LPS transporter LptD (46, 47) has been localized to the OM (48) and is likely essential due to a lack of Tn insertions (49, 50). BB0838 could be thus considered an orphan OM translocase in search of substrates, with the most likely substrates being either surface glycolipids found in the *B. burgdorferi* OM (51-53) or surface lipoproteins. If the latter is the case, this would represent a paradigm-shifting substrate expansion for the LptD family, and the main questions would turn to whether the Lol, Lpt or a yet to be discovered pathway feeds into this unique OM mechanism (Fig. 2).

Insertion and topology of integral outer membrane proteins (OMPs)

Spirochetal outer membranes contain varying sets of integral, membrane-spanning OMPs. Underscoring the gram-negative-like features of the leptospiral envelope, the *L. interrogans* OM is predicted to contain over 180 OMPs (29), including a leptospiral TolC homolog, an iron transporter FecA and the OmpL1 porin (23). In contrast, only 9 Tpr (*Treponema pallidum* repeat) proteins and BamA, a central component of the β -barrel assembly machinery (BAM) have been described in the OM of *T. pallidum*; nine additional hypothetical OMPs have been postulated (54). In *B. burgdorferi*, computational analysis predicts 41 outer membrane proteins (48), with OM localization confirmed experimentally for a subset of them, including the porins P13 and P66, BesC, BamA, and the LptD homolog mentioned above. P13 is an unusual OM porin in that it is predicted to assume an α -helical conformation (55-60) (Figs. 1 and 2). It is C-terminally processed by a periplasmic protease, CtpA (61) (Fig. 2), and found in multiprotein complexes with several surface lipoproteins (62). In purified form, P13 forms heterogeneous homooligomeric pores in lipid bilayers with no apparent gating mechanism or substrate specificity (57, 58, 63). Eight P13 paralogs are found on various *B. burgdorferi* plasmids, and one of them is upregulated in a P13 deletion mutant (57) suggesting that the expression of at least one P13 family protein is essential for cellular survival. A second well-studied porin, P66, assumes a more traditional β -barrel conformation, and in addition to forming a pore (56, 64-66), also functions as an adhesin that facilitates dissemination (67, 68).

The roles of some of the periplasmic and OM secretion pathway components for OMPs have been studied in *B. burgdorferi*. A Skp homolog is expressed downstream from BamA, but its function remains to be determined (69). As in some other bacteria, the *B. burgdorferi* HtrA chaperone/protease homolog (BB0104) has specific activity in the *Borrelia* periplasm, at the

OM and as a secreted protein (70). In concert with the above-mentioned CtpA, HtrA is involved in the proteolytic maturation of BB0323, a periplasmic OM lipoprotein that is required for envelope stability and contains a C-terminal peptidoglycan-binding LysM domain (71, 72); other identified HtrA substrates are involved in *Borrelia* chemotaxis and motility (73). At the same time, HtrA has been shown to degrade components of host extracellular matrix components (74).

Insertion of OMPs into the OM of diderm bacteria is generally mediated by the BAM complex, and Bam protein homologs are present in all three model spirochetes. Studies in *B. burgdorferi* have shown that its BamA homolog (BB0795) is associated with two periplasmic OM lipoproteins, BamB (BB0028) and BamD (BB0324) (75). Depletion of BamA led to a noticeable decrease of selected OMPs including P66 (76); the insertion mechanism of P13 remains unresolved and may bypass BAM like α -helical OMPs do in other systems (77) (Fig. 2). Together, this supports the fundamental conservation of OMP assembly mechanisms in diderm bacteria. In an apparent divergence, however, the usually essential BamD was dispensable for borrelial growth, while depletion of the generally auxiliary BamB led to a detectable growth defect (78-80). *B. burgdorferi* BamA also was shown to interact with a TamB (BB0794) homolog that in other bacteria is part of a parallel OMP Translocation and Assembly Module (TAM) pathway (69, 81). This indicates that *B. burgdorferi* has adapted modules of separate secretion machineries to function in a single hybrid pathway (82). Intriguingly, BamA depletion also led to a reduction of lipoproteins in the OM (76). Together, these data are compatible with a likely indirect BAM dependency of the surface lipoprotein flipping process (Fig. 2).

Release of outer membrane vesicles and interactions with host cells

In stark contrast to other bacterial systems, secretion and release of soluble spirochetal proteins into the milieu has been observed rather sporadically. This may be a function of the limited secretory capabilities of spirochetes, as detailed above, but requires further investigation. For example, the exoproteome of pathogenic *Leptospira* species includes proteases such as thermolysin, but it remains unclear how these proteins are secreted (83).

Borrelia outer membrane vesicles (OMV) have been shown to be released near the sites of cell division (2), and shedding can be observed both in culture or *in vivo*, e.g., within ticks taking a blood meal (84). OMVs, which primarily contain OM proteins but also periplasmic, IM and cytoplasmic components, are released under natural conditions from other diderm bacteria and have been shown to play roles in virulence and immunomodulation (85). The precise roles of OMVs in the pathogenesis of *Borrelia* infections remain to be defined. One surprising *B. burgdorferi* OMV-associated protein is enolase, a “moonlighting” signal peptide-less protein that is nevertheless exported and ultimately becomes accessible to the bacterial milieu. There, enolase is thought to work as one of the spirochete’s many plasminogen-binding proteins that facilitate pericellular proteolysis and thereby aid in nutrient acquisition and dissemination (86-88).

Low-level exchange of cholesterol lipids between *B. burgdorferi* and eukaryotic cells by either direct contact or via release and capture of OMVs has been observed in tissue culture

experiments (89). In one direction, this exchange allows for the acquisition of membrane lipids by a bacterium that is unable to synthesize its own. In the other direction, the transfer of bacterial lipids and glycolipids to host cells could result in antigen presentation and immune recognition that may contribute to the pathogenesis of Lyme disease through cell and tissue damage. Since at least a subset of *B. burgdorferi* surface lipoproteins are found associated with OM lipid rafts (90, 91), it is entirely conceivable that bacterial proteins are transferred to host cells via the same mechanism as well, thus extending spirochetal secretion pathways to eukaryotic cells.

Conclusions

Since the first description of “corkscrew-like animalcules in foul water” in 1773, the first definition of *spirochaeta* in 1838, and the ultimate discovery of *Spirochaeta pallida* (now *T. pallidum*) in syphilitic lesions in 1905 (92), spirochetes have become increasingly fascinating constituents of the global pan-microbiome. *B. burgdorferi* and *T. pallidum* were among the first fully sequenced bacterial pathogens (14, 16), and their genomes were as noteworthy for what was missing compared to other bacteria, e.g., an LPS biosynthesis pathway, as for the presence of numerous hypothetical and sometimes paralogous genes of unknown function. Since then, the development of robust genetic tools (93) has driven significant dissection of molecular mechanisms – including protein secretion – in *B. burgdorferi*, and by extension in some other *Borrelia* species. This has led to findings that have stimulated a re-evaluation of previously overgeneralized microbial processes. Studies of *Leptospira* are now catching up, due in no small part to the use of the genetically more amenable saprophytic *L. biflexa* system and development of improved genetic tools including *E. coli* to *L. interrogans* conjugation (94). At the same time, molecular investigations of *T. pallidum* that often had to resort to heterologous systems because of the inability to grow the spirochete *ex vivo*, are now bound to be transformed by a novel tissue culture-based culture system (95). Enteric spirochetes belonging to the genus *Brachyspira*, long recognized as veterinary pathogens, are now emerging as human pathogens in at-risk populations (96), beckoning further study of virulence mechanisms. As spirochetal research enters the post-genomic era and diversity in our understanding of both physiological and pathogenic microbial mechanisms is growing, we therefore would be wise to expect one or another paradigm-shifting discovery.

Acknowledgements

The author would like to thank all current and former laboratory members, mentors, collaborators and colleagues for their contributions to understanding spirochetal protein secretion. He is indebted to the editors for their infinite patience. This work was supported in part by unrestricted University of Kansas School of Medicine funds available to the author and by NIH Grants AI129522, AI133056, and AI139956.

References

1. Barbour AG. 2018 Borreliaceae In Whitman FR WB, Kämpfer P, Trujillo M, Chun J, DeVos P, Hedlund B, Dedys S (ed), Bergey's Manual of Systematics of Archaea and Bacteria doi: 10.1002/9781118960608.fbm00308.

2. Kudryashev M, Cyrklaff M, Baumeister W, Simon M, Wallich R, Frischknecht F. 2009 Comparative cryo-electron tomography of pathogenic Lyme disease spirochetes. *Mol Microbiol* 71:1415–1434. [PubMed: 19210619]
3. Liu J, Lin T, Botkin DJ, McCrum E, Winkler H, Norris SJ. 2009 Intact flagellar motor of *Borrelia burgdorferi* revealed by cryo-electron tomography: evidence for stator ring curvature and rotor/C-ring assembly flexion. *J Bacteriol* 191:5026–5036. [PubMed: 19429612]
4. Izard J, Renken C, Hsieh CE, Desrosiers DC, Dunham-Ems S, La Vake C, Gebhardt LL, Limberger RJ, Cox DL, Marko M, Radolf JD. 2009 Cryo-electron tomography elucidates the molecular architecture of *Treponema pallidum*, the syphilis spirochete. *J Bacteriol* 191:7566–7580. [PubMed: 19820083]
5. Liu J, Howell JK, Bradley SD, Zheng Y, Zhou ZH, Norris SJ. 2010 Cellular architecture of *Treponema pallidum*: novel flagellum, periplasmic cone, and cell envelope as revealed by cryo electron tomography. *J Mol Biol* 403:546–561. [PubMed: 20850455]
6. Raddi G, Morado DR, Yan J, Haake DA, Yang XF, Liu J. 2012 Three-dimensional structures of pathogenic and saprophytic *Leptospira* species revealed by cryo-electron tomography. *J Bacteriol* 194:1299–1306. [PubMed: 22228733]
7. Dowdell AS, Murphy MD, Azodi C, Swanson SK, Florens L, Chen S, Zückert WR. 2017 Comprehensive Spatial Analysis of the *Borrelia burgdorferi* Lipoproteome Reveals a Compartmentalization Bias toward the Bacterial Surface. *J Bacteriol* 199.
8. Radolf JD, Deka RK, Anand A, Smajs D, Norgard MV, Yang XF. 2016 *Treponema pallidum*, the syphilis spirochete: making a living as a stealth pathogen. *Nat Rev Microbiol* 14:744–759. [PubMed: 27721440]
9. Dashper SG, Seers CA, Tan KH, Reynolds EC. 2011 Virulence factors of the oral spirochete *Treponema denticola*. *J Dent Res* 90:691–703. [PubMed: 20940357]
10. Radolf J, Caimano M, Stevenson B, Hu L. 2012 Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol* 10:87–99. [PubMed: 22230951]
11. Steere AC, Strle F, Wormser GP, Hu LT, Brandt JA, Hovius JW, Li X, Mead PS. 2016 Lyme borreliosis. *Nat Rev Dis Primers* 2:16090. [PubMed: 27976670]
12. Natale DA, Galperin MY, Tatusov RL, Koonin EV. 2000 Using the COG database to improve gene recognition in complete genomes. *Genetica* 108:9–17. [PubMed: 11145426]
13. Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000 The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* 28:33–36. [PubMed: 10592175]
14. Fraser C, Casjens S, Huang W, Sutton G, Clayton R, Lathigra R, White O, Ketchum K, Dodson R, Hickey E, Gwinn M, Dougherty B, Tomb J, Fleischmann R, Richardson D, Peterson J, Kerlavage A, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams M, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton M, Horst K, Roberts K, Hatch B, Smith H, Venter J. 1997 Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390:580–586. [PubMed: 9403685]
15. Casjens S, Palmer N, van Vugt R, Huang W, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson R, Haft D, Hickey E, Gwinn M, White O, Fraser C. 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. [PubMed: 10672174]
16. Fraser C, Norris S, Weinstock G, White O, Sutton G, Dodson R, Gwinn M, Hickey E, Clayton R, Ketchum K, Sodergren E, Hardham J, McLeod M, Salzberg S, Peterson J, Khalak H, Richardson D, Howell J, Chidambaram M, Utterback T, McDonald L, Artiach P, Bowman C, Cotton M, Fujii C, Garland S, Hatch B, Horst K, Roberts K, Sandusky M, Weidman J, Smith H, Venter J. 1998 Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 281:375–388. [PubMed: 9665876]
17. Seshadri R, Myers G, Tettelin H, Eisen J, Heidelberg J, Dodson R, Davidsen T, DeBoy R, Fouts D, Haft D, Selengut J, Ren Q, Brinkac L, Madupu R, Kolonay J, Durkin S, Daugherty S, Shetty J, Shvartsbeyn A, Gebregeorgis E, Geer K, Tsegaye G, Malek J, Ayodeji B, Shatsman S, McLeod M, Smajs D, Howell J, Pal S, Amin A, Vashisth P, McNeill T, Xiang Q, Sodergren E, Baca E, Weinstock G, Norris S, Fraser C, Paulsen I. 2004 Comparison of the genome of the oral pathogen

Treponema denticola with other spirochete genomes. *Proc Natl Acad Sci U S A* 101:5646–5651. [PubMed: 15064399]

18. Nascimento A, Ko A, Martins E, Monteiro-Vitorello C, Ho P, Haake D, Verjovski-Almeida S, Hartskeerl R, Marques M, Oliveira M, Menck C, Leite L, Carrer H, Coutinho L, Degraeve W, Dellagostin O, El-Dorry H, Ferro E, Ferro M, Furlan L, Gamberini M, Giglioti E, Goes-Neto A, Goldman G, Goldman M, Harakava R, Jeronimo S, Junqueira-de-Azevedo I, Kimura E, Kuramae E, Lemos E, Lemos M, Marino C, Nunes L, de Oliveira R, Pereira G, Reis M, Schrieffer A, Siqueira W, Sommer P, Tsai S, Simpson A, Ferro J, Camargo L, Kitajima J, Setubal J, Van Sluys M. 2004 Comparative genomics of two *Leptospira interrogans* serovars reveals novel insights into physiology and pathogenesis. *J Bacteriol* 186:2164–2172. [PubMed: 15028702]
19. Deka RK, Brautigam CA, Goldberg M, Schuck P, Tomchick DR, Norgard MV. 2012 Structural, bioinformatic, and in vivo analyses of two *Treponema pallidum* lipoproteins reveal a unique TRAP transporter. *J Mol Biol* 416:678–696. [PubMed: 22306465]
20. Zückert W, Meyer J, Barbour A. 1999 Comparative analysis and immunological characterization of the *Borrelia Bdr* protein family. *Infect Immun* 67:3257–3266. [PubMed: 10377099]
21. Roberts DM, Theisen M, Marconi RT. 2000 Analysis of the cellular localization of Bdr paralogs in *Borrelia burgdorferi*, a causative agent of lyme disease: evidence for functional diversity. *J Bacteriol* 182:4222–4226. [PubMed: 10894730]
22. Barbour AG, Jasinskis A, Kayala MA, Davies DH, Steere AC, Baldi P, Felgner PL. 2008 A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with *Borrelia burgdorferi*. *Infect Immun* 76:3374–3389. [PubMed: 18474646]
23. Haake DA, Zückert WR. 2015 The leptospiral outer membrane. *Curr Top Microbiol Immunol* 387:187–221. [PubMed: 25388136]
24. Bunikis I, Denker K, Ostberg Y, Andersen C, Benz R, Bergström S. 2008 An RND-type efflux system in *Borrelia burgdorferi* is involved in virulence and resistance to antimicrobial compounds. *PLoS Pathog* 4:e1000009. [PubMed: 18389081]
25. Lin T, Gao L, Zhao X, Liu J, Norris SJ. 2015 Mutations in the *Borrelia burgdorferi* Flagellar Type III Secretion System Genes *fliH* and *fliI* Profoundly Affect Spirochete Flagellar Assembly, Morphology, Motility, Structure, and Cell Division. *MBio* 6:e00579–00515. [PubMed: 25968649]
26. Fraser CM, Norris SJ, Weinstock GM, White O, Sutton GG, Dodson R, Gwinn M, Hickey EK, Clayton R, Ketchum KA, Sodergren E, Hardham JM, McLeod MP, Salzberg S, Peterson J, Khalak H, Richardson D, Howell JK, Chidambaram M, Utterback T, McDonald L, Artiach P, Bowman C, Cotton MD, Fujii C, Garland S, Hatch B, Horst K, Roberts K, Sandusky M, Weidman J, Smith HO, Venter JC. 1998 Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 281:375–388. [PubMed: 9665876]
27. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Wattley L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC. 1997 Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390:580–586. [PubMed: 9403685]
28. Setubal J, Reis M, Matsunaga J, Haake D. 2006 Lipoprotein computational prediction in spirochaetal genomes. *Microbiology* 152:113–121. [PubMed: 16385121]
29. Pinne M, Matsunaga J, Haake DA. 2012 Leptospiral outer membrane protein microarray, a novel approach to identification of host ligand-binding proteins. *J Bacteriol* 194:6074–6087. [PubMed: 22961849]
30. Hazlett KR, Cox DL, Decaffmeyer M, Bennett MP, Desrosiers DC, La Vake CJ, La Vake ME, Bourell KW, Robinson EJ, Brasseur R, Radolf JD. 2005 TP0453, a concealed outer membrane protein of *Treponema pallidum*, enhances membrane permeability. *J Bacteriol* 187:6499–6508. [PubMed: 16159783]
31. Chan K, Nasereddin T, Alter L, Centurion-Lara A, Giacani L, Parveen N. 2016 *Treponema pallidum* Lipoprotein TP0435 Expressed in *Borrelia burgdorferi* Produces Multiple Surface/ Periplasmic Isoforms and mediates Adherence. *Sci Rep* 6:25593. [PubMed: 27161310]

32. Pinne M, Haake DA. 2013 LipL32 Is a Subsurface Lipoprotein of *Leptospira interrogans*: presentation of new data and reevaluation of previous studies. *PLoS One* 8:e51025. [PubMed: 23323152]
33. Belisle J, Brandt M, Radolf J, Norgard M. 1994 Fatty acids of *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins. *J Bacteriol* 176:2151–2157. [PubMed: 8157583]
34. Brandt M, Riley B, Radolf J, Norgard M. 1990 Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. *Infect Immun* 58:983–991. [PubMed: 2318538]
35. Beermann C, Lochnit G, Geyer R, Groscurth P, Filgueira L. 2000 The lipid component of lipoproteins from *Borrelia burgdorferi*: structural analysis, antigenicity, and presentation via human dendritic cells. *Biochem Biophys Res Commun* 267:897–905. [PubMed: 10673388]
36. Zückert WR. 2014 Secretion of bacterial lipoproteins: through the cytoplasmic membrane, the periplasm and beyond. *Biochim Biophys Acta* 1843:1509–1516. [PubMed: 24780125]
37. Erdile LF, Brandt MA, Warakowski DJ, Westrack GJ, Sadziene A, Barbour AG, Mays JP. 1993 Role of attached lipid in immunogenicity of *Borrelia burgdorferi* OspA. *Infect Immun* 61:81–90. [PubMed: 8418068]
38. Shang E, Summers T, Haake D. 1996 Molecular cloning and sequence analysis of the gene encoding LipL41, a surface-exposed lipoprotein of pathogenic *Leptospira* species. *Infect Immun* 64:2322–2330. [PubMed: 8675344]
39. Swancutt MA, Radolf JD, Norgard MV. 1990 The 34-kilodalton membrane immunogen of *Treponema pallidum* is a lipoprotein. *Infect Immun* 58:384–392. [PubMed: 2404869]
40. Chen S, Kumru O, Zückert W. 2011 Determination of *borrelia* surface lipoprotein anchor topology by surface proteolysis. *J Bacteriol* 193:6379–6383. [PubMed: 21908659]
41. Chen S, Zückert W. 2011 Probing the *Borrelia burgdorferi* surface lipoprotein secretion pathway using a conditionally folding protein domain. *J Bacteriol* 193:6724–6732. [PubMed: 21965569]
42. Kumru O, Schulze R, Rodnin M, Ladokhin A, Zückert W. 2011 Surface Localization Determinants of *Borrelia* OspC/Vsp Family Lipoproteins. *J Bacteriol* 193:2814–2825. [PubMed: 21441503]
43. Schulze R, Chen S, Kumru O, Zückert W. 2010 Translocation of *Borrelia burgdorferi* surface lipoprotein OspA through the outer membrane requires an unfolded conformation and can initiate at the C-terminus. *Mol Microbiol* 76:1266–1278. [PubMed: 20398211]
44. Kumru O, Schulze R, Slusser J, Zückert W. 2010 Development and validation of a FACS-based lipoprotein localization screen in the Lyme disease spirochete *Borrelia burgdorferi*. *BMC Microbiol* 10:277. [PubMed: 21047413]
45. Schulze R, Zückert W. 2006 *Borrelia burgdorferi* lipoproteins are secreted to the outer surface by default. *Mol Microbiol* 59:1473–1484. [PubMed: 16468989]
46. Li X, Gu Y, Dong H, Wang W, Dong C. 2015 Trapped lipopolysaccharide and LptD intermediates reveal lipopolysaccharide translocation steps across the *Escherichia coli* outer membrane. *Sci Rep* 5:11883. [PubMed: 26149544]
47. Konovalova A, Kahne DE, Silhavy TJ. 2017 Outer Membrane Biogenesis. *Annu Rev Microbiol* 71:539–556. [PubMed: 28886680]
48. Kenedy MR, Scott EJ 2nd, Shrestha B, Anand A, Iqbal H, Radolf JD, Dyer DW, Akins DR. 2016 Consensus computational network analysis for identifying candidate outer membrane proteins from *Borrelia* spirochetes. *BMC Microbiol* 16:141. [PubMed: 27400788]
49. Lin T, Gao L, Zhang C, Odeh E, Jacobs MB, Coutte L, Chaconas G, Philipp MT, Norris SJ. 2012 Analysis of an ordered, comprehensive STM mutant library in infectious *Borrelia burgdorferi*: insights into the genes required for mouse infectivity. *PLoS One* 7:e47532. [PubMed: 23133514]
50. Lin T, Troy EB, Hu LT, Gao L, Norris SJ. 2014 Transposon mutagenesis as an approach to improved understanding of *Borrelia* pathogenesis and biology. *Front Cell Infect Microbiol* 4:63. [PubMed: 24904839]
51. Hossain H, Wellensiek H, Geyer R, Lochnit G. 2001 Structural analysis of glycolipids from *Borrelia burgdorferi*. *Biochimie* 83:683–692. [PubMed: 11522398]
52. Ben-Menachem G, Kubler-Kielb J, Coxon B, Yergey A, Schneerson R. 2003 A newly discovered cholesteryl galactoside from *Borrelia burgdorferi*. *Proc Natl Acad Sci U S A* 100:7913–7918. [PubMed: 12799465]

53. Schroder N, Eckert J, Stubbs G, Schumann R. 2008 Immune responses induced by spirochetal outer membrane lipoproteins and glycolipids. *Immunobiology* 213:329–340. [PubMed: 18406378]
54. Cox DL, Luthra A, Dunham-Ems S, Desrosiers DC, Salazar JC, Caimano MJ, Radolf JD. 2010 Surface immunolabeling and consensus computational framework to identify candidate rare outer membrane proteins of *Treponema pallidum*. *Infect Immun* 78:5178–5194. [PubMed: 20876295]
55. Sadziene A, Thomas D, Barbour A. 1995 *Borrelia burgdorferi* mutant lacking Osp: biological and immunological characterization. *Infect Immun* 63:1573–1580. [PubMed: 7890424]
56. Pinne M, Thein M, Denker K, Benz R, Coburn J, Bergström S. 2007 Elimination of channel-forming activity by insertional inactivation of the p66 gene in *Borrelia burgdorferi*. *FEMS Microbiol Lett* 266:241–249. [PubMed: 17233736]
57. Pinne M, Östberg Y, Comstedt P, Bergström S. 2004 Molecular analysis of the channel-forming protein P13 and its paralogue family 48 from different Lyme disease *Borrelia* species. *Microbiology* 150:549–559. [PubMed: 14993304]
58. Östberg Y, Pinne M, Benz R, Rosa P, Bergström S. 2002 Elimination of channel-forming activity by insertional inactivation of the p13 gene in *Borrelia burgdorferi*. *J Bacteriol* 184:6811–6819. [PubMed: 12446631]
59. Nilsson C, Cooper H, Hakansson K, Marshall A, Ostberg Y, Lavrinovicha M, Bergström S. 2002 Characterization of the P13 membrane protein of *Borrelia burgdorferi* by mass spectrometry. *J Am Soc Mass Spectrom* 13:295–299. [PubMed: 11951966]
60. Noppa L, Östberg Y, Lavrinovicha M, Bergström S. 2001 P13, an integral membrane protein of *Borrelia burgdorferi*, is C-terminally processed and contains surface-exposed domains. *Infect Immun* 69:3323–3334. [PubMed: 11292755]
61. Östberg Y, Carroll J, Pinne M, Krum J, Rosa P, Bergström S. 2004 Pleiotropic effects of inactivating a carboxyl-terminal protease, CtpA, in *Borrelia burgdorferi*. *J Bacteriol* 186:2074–2084. [PubMed: 15028692]
62. Yang X, Promnares K, Qin J, He M, Shroder DY, Kariu T, Wang Y, Pal U. 2011 Characterization of multiprotein complexes of the *Borrelia burgdorferi* outer membrane vesicles. *J Proteome Res* 10:4556–4566. [PubMed: 21875077]
63. Barcena-Uribarri I, Thein M, Barbot M, Sans-Serramitjana E, Bonde M, Mentele R, Lottspeich F, Bergström S, Benz R. 2014 Study of the protein complex, pore diameter, and pore-forming activity of the *Borrelia burgdorferi* P13 porin. *J Biol Chem* 289:18614–18624. [PubMed: 24825899]
64. Bunikis J, Barbour A. 1999 Access of antibody or trypsin to an integral outer membrane protein (P66) of *Borrelia burgdorferi* is hindered by Osp lipoproteins. *Infect Immun* 67:2874–2883 [PubMed: 10338494]
65. Bunikis J, Noppa L, Ostberg Y, Barbour A, Bergström S. 1996 Surface exposure and species specificity of an immunoreactive domain of a 66-kilodalton outer membrane protein (P66) of the *Borrelia* spp. that cause Lyme disease. *Infect Immun* 64:5111–5116. [PubMed: 8945554]
66. Skare J, Mirzabekov T, Shang E, Blanco D, Erdjument-Bromage H, Bunikis J, Bergström S, Tempst P, Kagan B, Miller J, Lovett M. 1997 The Oms66 (p66) protein is a *Borrelia burgdorferi* porin. *Infect Immun* 65:3654–3661. [PubMed: 9284133]
67. Ristow LC, Bonde M, Lin YP, Sato H, Curtis M, Wesley E, Hahn BL, Fang J, Wilcox DA, Leong JM, Bergström S, Coburn J. 2015 Integrin binding by *Borrelia burgdorferi* P66 facilitates dissemination but is not required for infectivity. *Cell Microbiol* 17:1021–1036. [PubMed: 25604835]
68. Coburn J, Cugini C. 2003 Targeted mutation of the outer membrane protein P66 disrupts attachment of the Lyme disease agent, *Borrelia burgdorferi*, to integrin α v β 3. *Proc Natl Acad Sci U S A* 100:7301–7306. [PubMed: 12748384]
69. Iqbal H, Kenedy MR, Lybecker M, Akins DR. 2016 The TamB ortholog of *Borrelia burgdorferi* interacts with the beta-barrel assembly machine (BAM) complex protein BamA. *Mol Microbiol* 102:757–774. [PubMed: 27588694]
70. Gherardini FC. 2013 *Borrelia burgdorferi* HtrA may promote dissemination and irritation. *Mol Microbiol* 90:209–213. [PubMed: 23998919]

71. Stewart P, Hoff J, Fischer E, Krum J, Rosa P. 2004 Genome-wide transposon mutagenesis of *Borrelia burgdorferi* for identification of phenotypic mutants. *Appl Environ Microbiol* 70:5973–5979. [PubMed: 15466540]
72. Kariu T, Yang X, Marks CB, Zhang X, Pal U. 2013 Proteolysis of BB0323 results in two polypeptides that impact physiologic and infectious phenotypes in *Borrelia burgdorferi*. *Mol Microbiol* 88:510–522. [PubMed: 23489252]
73. Coleman J, Crowley J, Toledo A, Benach J. 2013 The HtrA protease of *Borrelia burgdorferi* degrades outer membrane protein BmpD and chemotaxis phosphatase CheX. *Mol Microbiol* 88:619–633. [PubMed: 23565798]
74. Russell TM, Delorey MJ, Johnson BJ. 2013 *Borrelia burgdorferi* BbHtrA degrades host ECM proteins and stimulates release of inflammatory cytokines in vitro. *Mol Microbiol* 90:241–251. [PubMed: 23980719]
75. Lenhart TR, Kenedy MR, Yang X, Pal U, Akins DR. 2012 BB0324 and BB0028 are constituents of the *Borrelia burgdorferi* beta-barrel assembly machine (BAM) complex. *BMC Microbiol* 12:60. [PubMed: 22519960]
76. Lenhart T, Akins D. 2010 *Borrelia burgdorferi* locus BB0795 encodes a BamA orthologue required for growth and efficient localization of outer membrane proteins. *Mol Microbiol* 75:692–709. [PubMed: 20025662]
77. Dunstan RA, Hay ID, Wilksch JJ, Schittenhelm RB, Purcell AW, Clark J, Costin A, Ramm G, Strugnell RA, Lithgow T. 2015 Assembly of the secretion pores GspD, Wza and CsgG into bacterial outer membranes does not require the Omp85 proteins BamA or TamA. *Mol Microbiol* 97:616–629. [PubMed: 25976323]
78. Misra R, Stikeleather R, Gabriele R. 2015 In vivo roles of BamA, BamB and BamD in the biogenesis of BamA, a core protein of the beta-barrel assembly machine of *Escherichia coli*. *J Mol Biol* 427:1061–1074. [PubMed: 24792419]
79. Gunasinghe SD, Shiota T, Stubenrauch CJ, Schulze KE, Webb CT, Fulcher AJ, Dunstan RA, Hay ID, Naderer T, Whelan DR, Bell TDM, Elgass KD, Strugnell RA, Lithgow T. 2018 The WD40 Protein BamB Mediates Coupling of BAM Complexes into Assembly Precincts in the Bacterial Outer Membrane. *Cell Rep* 23:2782–2794. [PubMed: 29847806]
80. Dunn JP, Kenedy MR, Iqbal H, Akins DR. 2015 Characterization of the beta-barrel assembly machine accessory lipoproteins from *Borrelia burgdorferi*. *BMC Microbiol* 15:70. [PubMed: 25887384]
81. Stubenrauch CJ, Lithgow T. 2019 The TAM: A Translocation and Assembly Module of the beta-Barrel Assembly Machinery in Bacterial Outer Membranes. *EcoSal Plus* 8.
82. Stubenrauch C, Grinter R, Lithgow T. 2016 The modular nature of the beta-barrel assembly machinery, illustrated in *Borrelia burgdorferi*. *Mol Microbiol* 102:753–756. [PubMed: 27618357]
83. Picardeau M. 2017 Virulence of the zoonotic agent of leptospirosis: still terra incognita? *Nat Rev Microbiol* 15:297–307. [PubMed: 28260786]
84. Dunham-Ems SM, Caimano MJ, Pal U, Wolgemuth CW, Eggers CH, Balic A, Radolf JD. 2009 Live imaging reveals a biphasic mode of dissemination of *Borrelia burgdorferi* within ticks. *J Clin Invest* 119:3652–3665. [PubMed: 19920352]
85. Ellis TN, Kuehn MJ. 2010 Virulence and immunomodulatory roles of bacterial outer membrane vesicles. *Microbiol Mol Biol Rev* 74:81–94. [PubMed: 20197500]
86. Floden A, Watt J, Brissette C. 2011 *Borrelia burgdorferi* enolase is a surface-exposed plasminogen binding protein. *PLoS One* 6:e27502. [PubMed: 22087329]
87. Nogueira S, Smith A, Qin J, Pal U. 2012 A surface enolase participates in *Borrelia burgdorferi*-plasminogen interaction and contributes to pathogen survival within feeding ticks. *Infect Immun* 80:82–90. [PubMed: 22025510]
88. Toledo A, Coleman J, Kuhlman C, Crowley J, Benach J. 2012 The enolase of *Borrelia burgdorferi* is a plasminogen receptor released in outer membrane vesicles. *Infect Immun* 80:359–368. [PubMed: 22083700]
89. Crowley JT, Toledo AM, LaRocca TJ, Coleman JL, London E, Benach JL. 2013 Lipid exchange between *Borrelia burgdorferi* and host cells. *PLoS Pathog* 9:e1003109. [PubMed: 23326230]

90. Toledo A, Crowley JT, Coleman JL, LaRocca TJ, Chiantia S, London E, Benach JL. 2014 Selective association of outer surface lipoproteins with the lipid rafts of *Borrelia burgdorferi*. *MBio* 5:e00899–00814. [PubMed: 24618252]
91. Toledo A, Perez A, Coleman JL, Benach JL. 2015 The lipid raft proteome of *Borrelia burgdorferi*. *Proteomics* 15:3662–3675. [PubMed: 26256460]
92. Ward HB. 1908 The Spirochetes and Their Relationship to Other Organisms. *The American Naturalist* 42:374–387.
93. Drecktrah D, Samuels DS. 2018 Genetic Manipulation of *Borrelia* Spp. *Curr Top Microbiol Immunol* 415:113–140. [PubMed: 28918538]
94. Picardeau M 2015 Genomics, proteomics, and genetics of leptospira. *Curr Top Microbiol Immunol* 387:43–63. [PubMed: 25388132]
95. Edmondson DG, Hu B, Norris SJ. 2018 Long-Term In Vitro Culture of the Syphilis Spirochete *Treponema pallidum* subsp. *pallidum*. *MBio* 9:e01153–01118;. [PubMed: 29946052]
96. Hampson DJ. 2018 The Spirochete *Brachyspira pilosicoli*, Enteric Pathogen of Animals and Humans. *Clin Microbiol Rev* 31.
97. Haake DA, Zückert WR. 2018 Spirochetal Lipoproteins in Pathogenesis and Immunity. *Curr Top Microbiol Immunol* 415:239–271. [PubMed: 29196824]

Figure 1. Envelope structures of model spirochetes *Borrelia*, *Leptospira* and *Treponema*. Spirochetes have a common diderm envelope structure with an inner membrane (IM) and outer membrane (OM) and a periplasmic space in between that contains a thin peptidoglycan cell wall and periplasmic flagella. A major difference between the three genera is seen in the OM: *Borrelia* display a limited set of integral OM proteins (OMPs), among them the unusual porin P13 with a predicted α -helical TM topology, but a large variable set of surface lipoproteins. The *Leptospira* OM most closely resembles a gram-negative OM, with lipopolysaccharide (LPS) being the major component of the surface leaflet, complemented by a large number of OMPs and a limited set of surface lipoproteins. *Treponema* only express rare OMPs with limited surface exposure. Some of the model proteins under study are labeled in italics. See text for details.

Figure 2. Generalized model of spirochetal protein secretion.

The left half of the figure shows Type I-III secretion systems (T1SS, T2SS, T3SS) as well as the pathways involved in the secretion of non-lipidated membrane and periplasmic proteins. The right half of the figure shows the pathways for lipoprotein (LP) modification, sorting and secretions. Pathways components or mechanisms that appear unique to a particular spirochetal genus are labeled by stippled red circles with the genus initial: *B*, *Borrelia*; *L*, *Leptospira*; *T*, *Treponema*. E.g., a potential T2SS has only been identified in *Leptospira*. The pathways in blue delineate the current alternative periplasmic and OM mechanisms that may be involved in the secretion of alpha-helical integral OMPs and surface lipoproteins in *Borrelia*; of note, *Leptospira* could also take advantage of its T2SS to secrete surface lipoproteins (23, 97). Release of outer membrane vesicles (OMV) has been observed and studied in *Borrelia*. See text for details.