HHS Public Access

Author manuscript

Cell Microbiol. Author manuscript; available in PMC 2016 October 17.

Published in final edited form as:

Cell Microbiol. 2016 July ; 18(7): 919–927. doi:10.1111/cmi.12609.

Interaction of the Lyme disease spirochete with its tick vector

Melissa J. Caimano[1], **Dan Drecktrah**[2], **Faith Kung**[3], and **D. Scott Samuels**[2,4,*]

[1]Departments of Medicine, Pediatrics, and Molecular Biology and Biophysics, UConn Health, Farmington, CT, USA

[2]Division of Biological Sciences, University of Montana, Missoula, MT, USA

[3]Department of Veterinary Medicine, University of Maryland–College Park and Virginia–Maryland Regional College of Veterinary Medicine, College Park, MD, USA

[4]Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT, USA

Summary

Borrelia burgdorferi , the causative agent of Lyme disease (along with closely related genospecies), is in the deeply branching spirochete phylum. The bacterium is maintained in nature in an enzootic cycle that involves transmission from a tick vector to a vertebrate host and acquisition from a vertebrate host to a tick vector. During its arthropod sojourn, *B. burgdorferi* faces a variety of stresses, including nutrient deprivation. Here, we review some of the spirochetal factors that promote persistence, maintenance and dissemination of *B. burgdorferi* in the tick, and then focus on the utilization of available carbohydrates as well as the exquisite regulatory systems invoked to adapt to the austere environment between blood meals and to signal species transitions as the bacteria traverse their enzootic cycle. The spirochetes shift their source of carbon and energy from glucose in the vertebrate to glycerol in the tick. Regulation of survival under limiting nutrients requires the classic stringent response in which Rel_{Bbu} controls the levels of the alarmones guanosine tetraphosphate and guanosine pentaphosphate (collectively termed (p)ppGpp), while regulation at the tick–vertebrate interface as well as regulation of protective responses to the blood meal require the two-component system Hk1/Rrp1 to activate production of the second messenger cyclic-dimeric-GMP (c-di-GMP).

Introduction

Several species of hard ticks in the genus *Ixodes* acquire and transmit the morphologically serpentine spirochetes *Borrelia burgdorferi* sensu lato as a crucial component of an enzootic cycle (Fig. 1) (Lane *et al.,* 1991; Piesman and Schwan, 2010; Radolf *et al.,* 2012). A few of these *Borrelia* genospecies, historically including *B. burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*, cause Lyme disease (Burgdorfer *et al.,* 1982; Benach *et al.,* 1983; Steere *et al.,* 1983), an emerging infection with a global distribution (Mead, 2015). *Ixodes* larvae acquire *B. burgdorferi* while feeding on infected vertebrates. Spirochetes reside in the midgut as larvae molt into nymphs, and then migrate to the salivary glands when the nymph feeds, at which point they transmit to the vertebrate host. Spirochetes migrate out of the

*For correspondence: scott.samuels@umontana.edu; Tel. (406) 243-6145; Fax (406) 243-4184.

midgut by, first, adhering to the midgut epithelium and, second, penetrating the intercellular junctions to access the hemocoel (Dunham-Ems *et al.,* 2009). *B. burgdorferi* must persist in the nutrient-limited tick as well as evade the vertebrate and tick immune systems; these host-specific responses are associated with a sea change of gene expression (Samuels, 2011; Radolf *et al.,* 2012; Iyer *et al.,* 2015).

B. burgdorferi has a sparse set of metabolic pathways, but is flush with transporters to obtain nutrients from its environment (Fig. 2) (Fraser *et al.,* 1997; Gherardini *et al.,* 2010; Corona and Schwartz, 2015). Spirochete burdens within the larval midgut substantially increase in response to the nutrient-rich, digested blood meal; however, these nutrients are drained in a few weeks and the spirochetes have to manage for months post-molt until the nymphs feed on a second vertebrate (Sonenshine, 1991; Kung *et al.,* 2013). The physiological capacity of *B. burgdorferi* is encoded on a relatively small, but exceedingly complex genome comprising a ~950-kb linear chromosome and a suite of ~20 to 25 unique linear and circular plasmids (lp's and cp's, respectively) ranging in size from 5 to 56 kb (Fraser *et al.,* 1997; Brisson *et al.,* 2012). The chromosome and plasmids carry several gene products required for persistence in the tick (Kenedy *et al.,* 2012; Kung *et al.,* 2013), including the outer membrane lipoproteins OspA, OspB and BptA as well as a transporter and enzymes involved in utilizing glycerol. The expression of the genes required during the tick phase of the enzootic cycle is predominantly controlled by three interacting regulatory systems: the RpoN–RpoS alternative sigma factor cascade (which was previously reviewed in Samuels, 2011; Radolf *et al.,* 2012), the second messenger cyclic-dimeric-GMP (c-di-GMP) and the alarmones guanosine pentaphosphate and guanosine tetraphosphate ((p)ppGpp).

Gene products required in the tick

Several *B. burgdorferi* plasmids, including lp54, lp25 and lp28-4, have been implicated in infection and/or persistence in ticks. lp54, the 54-kb linear plasmid, encodes numerous differentially expressed lipoproteins, many of which are known to support spirochete infection in the vector (see below). lp25 also carries genes required for mouse and tick infection (reviewed in Kung *et al.,* 2013): *pncA* (*bbe22*), which encodes a nicotinamidase that is essential for growth in mammals (Purser *et al.,* 2003), and *bptA* (*bbe16*), which encodes a putative lipoprotein of unknown function that is required for persistence in ticks (Revel *et al.,* 2005). Other genes on lp25 must be important, as lp25-deficient strains complemented with either *pncA* or both *pncA* and *bptA* are acquired by larvae at reduced levels and do not persist through intermolt (Gilmore *et al.,* 2014). *B. burgdorferi* lacking lp28-4 also display a reduced survival in ticks and diminished tick-to-mouse transmission (reviewed in Kung *et al.,* 2013).

Lipoproteins, referred to as outer surface proteins (Osps), are the most abundant component of the *B. burgdorferi* proteome (Kenedy *et al.,* 2012). Many of these *Borrelia*-specific proteins are vital for persistence in, and transmission through, ticks via interaction(s) with host or vector factors (Kenedy *et al.,* 2012; Kung *et al.,* 2013). OspA and OspB (BBA15 and BBA16, respectively) are encoded by an operon on lp54 and synthesized primarily during the vector phase *in vivo*: they are dominant lipoproteins that have crucial roles in persistence within the tick. OspA interacts with TROSPA, a tick protein required for spirochete

colonization of the gut epithelium (Pal *et al.,* 2004a), and binds plasminogen (Fuchs *et al.,* 1994). OspA is thought to protect spirochetes in the feeding tick gut from host-derived bactericidal antibodies (Battisti *et al.,* 2008).

Less abundant *B. burgdorferi* surface-associated proteins also have been implicated in supporting survival in the vector or transmission through feeding ticks; however, in most cases, their biological function(s) have yet to be determined. Many of the genes encoding these proteins are carried on lp54 along with the *ospAB* operon. Expression of *bba03* is increased in fed ticks, and BBA03-deficient spirochetes cannot compete with wild type to efficiently transmit to mice (Bestor *et al.,* 2012). BBA07 and BBA52 are exposed on the spirochete surface; a *bba07* mutant (Xu *et al.,* 2010b) and a *bba52* mutant (Kumar *et al.,* 2010) can infect mice and persist in ticks, but both mutants are defective in tick-mediated transmission. *bba57* encodes a putative outer membrane lipoprotein and is required for early murine infection and, potentially, transmission (Yang *et al.,* 2013b). Another lp54 gene product, Lp6.6 (BBA62), is required as pathogens enter and survive in the tick vector; *bba62* is one of the most highly expressed genes in engorged nymphs (with significantly higher expression in ticks than in the mammalian host-adapted state) (Iyer *et al.,* 2015) and *bba62* mutants are defective in transmitting to naïve hosts (Promnares *et al.,* 2009). BBA64 (P35) has also been shown to be important in persistence and transmission (reviewed in Kenedy *et al.,* 2012). Mutants in the gene encoding the surface-localized antigen BBA66 were infectious in mice by needle inoculation, but exhibited reduced spirochete burdens and pathology in joints; *bba66* mutants are acquired by larvae and persist through intermolt, but are impaired in their ability to be transmitted by nymphs (Patton *et al.,* 2013).

Other genomic elements carry genes involved in the tick phase of the enzootic cycle. OspC (BBB19) is a dominant lipoprotein encoded by the first borrelial gene mapped to a circular plasmid, cp26 (reviewed in Brisson *et al.,* 2012); expression of *ospC* is induced during nymphal transmission (Schwan *et al.,* 1995) and is required for early murine infection (Grimm *et al.,* 2004; Pal *et al.,* 2004b). Like OspA, it also binds plasminogen (Lagal *et al.,* 2006; Önder *et al.,* 2012), which could assist migration through the vector, although there is some controversy regarding the specific role of OspC in dissemination through tick tissues (Grimm *et al.,* 2004; Pal *et al.,* 2004b; Fingerle *et al.,* 2007; Dunham-Ems *et al.,* 2012). The outer surface gene product of *bbe31*, carried on lp25, binds to the tick protein TRE31 and promotes migration into the hemocoel during feeding (reviewed in Kung *et al.,* 2013). Arthritis-related protein (Arp), encoded by *bbf01* on lp28-1, is a target for antibody-mediated disease resolution in the mouse model and is antigenic in humans; mutants were compromised in transmission, suggesting that Arp supports survival or dissemination through ticks (Imai *et al.,* 2013).

Several chromosomal genes aid spirochete survival and their gene products function, albeit not always exclusively, in the tick phase of the enzootic cycle. BmtA, encoded by *bb0219*, is a putative manganese transporter that is essential in both ticks and mammals (reviewed in Kung *et al.,* 2013). Surface protein P66, encoded by *bb0603*, has integrin-binding and channel-forming activities (reviewed in Kenedy *et al.,* 2012); *p66* mutants survive through intermolt but are noninfectious in mice by tick transmission (Ristow *et al.,* 2012). *bb0405* encodes a surface-exposed transmembrane protein and mutants were unable to transmit from

ticks to mice (Kung *et al.,* 2016). The subsurface membrane protein LA7 (p22), encoded by *bb0365*, supports survival in the tick (Pal *et al.,* 2008); LA7-deficient *B. burgdorferi* were severely impaired in their ability to persist in feeding and quiescent ticks during transmission and intermolt (Yang *et al.,* 2013a). Dps/NapA/BicA (BB0690) is an ortholog of bacterioferritin that is uniquely fused to a copper-binding metallothionein-like domain (Wang *et al.,* 2012). In other bacterial species, Dps (DNA-binding protein from starved cells) is found at high levels during stationary phase, when it protects DNA as part of the cellular response to starvation. However, *in vitro* assays fail to demonstrate either DNA binding or protection against oxidative damage by Dps/NapA/BicA from *B. burgdorferi* (Li *et al.,* 2007). On the other hand, although *dps*/*napA*/*bicA* mutants are infectious in mammals and can colonize the tick midgut, they do not survive prolonged periods in unfed ticks (Li *et al.,* 2007). Wang *et al.* (2012) propose that Dps/NapA/BicA sequesters excess metals throughout the enzootic cycle, but *dps*/*napA*/*bicA* is highly expressed in fed nymphs, with significantly higher expression in ticks than in the mammalian host (Li *et al.,* 2007; Iyer *et al.,* 2015), and the gene is repressed during recovery from starvation *in vitro* (Drecktrah *et al.,* 2015).

Carbon utilization

B. burgdorferi, an extreme auxotroph, lacks genes encoding enzymes for the citric acid cycle and oxidative phosphorylation, deriving energy instead from the fermentation of sugars through glycolysis (Fraser *et al.,* 1997; Gherardini *et al.,* 2010). *In vitro*, the spirochetes are able to utilize a limited range of simple and complex carbohydrates for energy (von Lackum and Stevenson, 2005; Hoon-Hanks *et al.,* 2012). These substrates enter the cell primarily via the phosphotransferase system (PTS) (Fraser *et al.,* 1997; von Lackum and Stevenson, 2005; Corona and Schwartz, 2015), a multicomponent carbohydrate uptake system that couples sugar-specific transport across the cytoplasmic membrane with sugar phosphorylation. The borrelial genome encodes one Enzyme I (EI) component (*bb0558*) and two putative Histidine phosphocarrier protein (Hpr) paralogs (*bb0448*, *bb0557*) that form a phoshorelay system coupled to multiple membrane-associated, sugar-specific Enzyme II (EII) transporters (Fraser *et al.,* 1997; Corona and Schwartz, 2015). Glucose, the preferred carbon source, is predicted to be imported by two glucose-/maltose-specific (BB0116/MalX-1 and BBB29/MalX-2) and/or the glucose-specific (BB0645/PtsG) PTS transporters and a non-PTS ABC-type transporter with homology to the Mgl system in *Escherichia coli* (Death and Ferenci, 1993; von Lackum and Stevenson, 2005; Gherardini *et al.,* 2010) (Fig. 2). Glucose-6-phosphate is then funneled into glycolysis for energy generation or used as a substrate for synthesis of membrane phospholipids, lipoproteins and nucleic acids (Gherardini *et al.,* 2010). Recently, Khajanchi *et al.* (2016) demonstrated that *ptsG* mutants are avirulent in mice by syringe inoculation, suggesting that spirochetes rely heavily on this transporter *in vivo*. In ticks, spirochetes must compete for blood meal-derived glucose with rapidly differentiating midgut epithelial cells that are avidly engulfing the blood meal. Consequently, spirochetes must modify their metabolism to take advantage of alternate carbon sources available within the midgut. Consistent with this notion, *B. burgdorferi* possesses systems for uptake and utilization of a number of alternate carbon sources (von Lackum and Stevenson, 2005; Gherardini *et al.,* 2010; Corona and Schwartz, 2015), including glycerol (Glp) (Pappas *et al.,* 2011), glucose disaccharides, like trehalose (MalQ

and TreA) (Hoon-Hanks *et al.,* 2012), and chitobiose (Chb), an *N*-acetyl glucosamine (GlcNAc) dimer derived from chitin, the major component of tick cuticle (Tilly *et al.,* 2001; 2004; Rhodes *et al.,* 2009; Sze *et al.,* 2013). Glp-deficient organisms show significantly reduced survival within feeding ticks and, following the molt, strongly diminished capacity to transmit to mice via tick bite (He *et al.,* 2011; Pappas *et al.,* 2011; Caimano *et al.,* 2015). Surprisingly, neither the Chb system nor MalQ is required for survival during the blood meal or tick-to-mammal transmission (Tilly *et al.,* 2004; Hoon-Hanks *et al.,* 2012). Given that glucose and β-glucoside transporters share considerable sequence and structural similarity (Saier *et al.,* 1988; McCoy *et al.,* 2015), one or more of the glucose transporters likely promote uptake of GlcNAc (Sze *et al.,* 2013), an essential building block for peptidoglycan, or disaccharides. The genes encoding FruA2 (predicted to transport mannose), ChbC and MalX-2 are more highly expressed in ticks than in mammals (Iyer *et al.,* 2015).

B. burgdorferi does not contain a homolog of cAMP receptor protein (also called catabolite activator protein), which is used by gram-negative bacteria to couple PTS-mediated sugar transport to gene regulation (catabolite repression) (Fraser *et al.,* 1997). Instead, the spirochete has evolved novel mechanisms for regulating central carbohydrate metabolism through the enzootic cycle (Pappas *et al.,* 2011).

Regulation by (p)ppGpp and c-di-GMP

Although repression of the alternative sigma factor RpoS allows for induction of important tick phase genes, like *ospAB* (reviewed in Samuels, 2011; Radolf *et al.,* 2012), the purine signaling molecule c-di-GMP and stringent response alarmones (p)ppGpp are indispensible master regulators of *B. burgdorferi* biology in the tick (Fig. 1). Modulating the levels of these second messengers in response to external signals alters the transcriptional landscape, as well as many other cellular processes, adapting the spirochete to the challenges of a dynamic and hostile environment in the tick midgut (Caimano *et al.,* 2011; 2015; He *et al.,* 2011; Kostick *et al.,* 2011; Sultan *et al.,* 2011; Bugrysheva *et al.,* 2015; Drecktrah *et al.,* 2015). Classically, (p)ppGpp synthesis is triggered by an accumulation of uncharged tRNAs during nutrient stress, but a shortage of carbon, iron, phosphate or fatty acids can also induce the stringent response (reviewed in Potrykus and Cashel, 2008; Dalebroux and Swanson, 2012; Hauryliuk *et al.,* 2015). (p)ppGpp alters RNA polymerase activity both directly, by binding to the initiating holoenzyme, and indirectly, through sigma factor competition, to alter the transcriptome, including rRNA levels (reviewed in Potrykus and Cashel, 2008; Dalebroux and Swanson, 2012; Hauryliuk *et al.,* 2015). In *B. burgdorferi*, the bifunctional enzyme Rel_{Bbu} adjusts (p)ppGpp levels in response to nutrient stress, although the specific extracellular signals remain to be identified (Bugrysheva *et al.,* 2003; Concepcion and Nelson, 2003; Drecktrah *et al.,* 2015).

rel_{Bbu} mutants are unable to synthesize (p)ppGpp (Bugrysheva *et al.,* 2005; Drecktrah *et al.,* 2015) and are compromised for persistence in the tick between blood meals and/or during nymph feeding, but retain the ability to infect mice (Drecktrah *et al.,* 2015). The (p)ppGpp-dependent transcriptome, determined using a rel_{Bbu} mutant, included a number of persistence-related genes induced by the stringent response, such as the glycerol uptake and metabolism (*glp*) operon (Bugrysheva *et al.,* 2015; Drecktrah *et al.,* 2015). The *glp* operon is

induced in ticks and by glycerol *in vitro*; survival of *glp* mutants is compromised in the tick, highlighting the importance of glycerol as a signal and carbon source in this part of the enzootic cycle (He *et al.,* 2011; Pappas *et al.,* 2011). Glycerol presumably enters the cytoplasm through the glycerol uptake facilitator (GlpF) and is phosphorylated by a putative glycerol kinase (GlpK) to produce glycerol-3-phosphate. Glycerol-3-phosphate may be either used in the biosynthesis of phospholipids and lipoproteins or shuttled to glycolysis via conversion to dihydroxyacetone phosphate by a putative glycerol-3-phosphate dehydrogenase (GlpD) (Fraser *et al.,* 1997; Corona and Schwartz, 2015). A number of studies indicate that the *glp* genes (*bb0240–bb0243*) form an operon (He *et al.,* 2011; Pappas *et al.,* 2011; Bugrysheva *et al.,* 2015; Caimano *et al.,* 2015), although (p)ppGpp uniquely affects expression of the constituent genes: *glpF* and *glpK* are induced while *glpD* is repressed (Drecktrah *et al.,* 2015). Thus, the stringent response may direct glycerol toward production of phospholipids and lipoproteins instead of glycolysis, which would require GlpD. In addition, (p)ppGpp upregulates other genes encoding proteins required for persistence in the tick: OspA, Dps/NapA/BicA and PncA (Drecktrah *et al.,* 2015).

The Hk1/Rrp1 two-component system (TCS) consists of a membrane-bound hybrid histidine kinase and a cytoplasmic response regulator with diguanlyate cyclase activity (Caimano *et al.,* 2011; 2015; He *et al.,* 2011; Kostick *et al.,* 2011). The Hk1 periplasmic sensor consists of three ligand-binding domains, each with homology to ABC transporter periplasmic solute-binding proteins (Bauer *et al.,* 2015). Analyses of the Hk1 sensor point to amino acids and/or their derivatives as potential activating ligands (Bauer *et al.,* 2015). Upon ligand binding, Hk1 is thought to mediate a signal transduction cascade that culminates in phosphorylation of Rrp1. In turn, phosphorylated Rrp1 catalyzes the synthesis of c-di-GMP (Ryjenkov *et al.,* 2005), a ubiquitous bacterial second messenger associated with a wide range of lifestyle control networks, including the transition from planktonic to sessile states, biofilm formation, cell cycle progression, and virulence (reviewed in Römling *et al.,* 2013). In contrast to the RpoN/RpoS pathway, which activates genes required during the vertebrate host phase (Samuels, 2011; Radolf *et al.,* 2012), the Hk1/Rrp1 TCS functions exclusively during the tick phase of the enzootic cycle: spirochetes lacking either Hk1 or Rrp1 are virulent in mice but rapidly destroyed within feeding larvae and nymphs (Caimano *et al.,* 2011; He *et al.,* 2011; Kostick *et al.,* 2011).

Transcriptomic analyses of an *rrp1* mutant grown *in vitro* suggest that, during tick feeding, c-di-GMP promotes utilization of alternate carbon sources for glycolysis and biosynthesis of phospholipids and peptidoglycan (Rogers *et al.,* 2009; He *et al.,* 2011; Caimano *et al.,* 2015). Most notably, c-di-GMP is required for expression of the *glp* operon via PlzA (Caimano *et al.,* 2015), the only c-di-GMP effector protein identified to date in *B. burgdorferi* (Freedman *et al.,* 2010; Pitzer *et al.,* 2011; He *et al.,* 2014). In addition, c-di-GMP upregulates the expression of genes encoding known or putative PTS transporters for GlcNAc (MalX-2) and chitobiose (ChbC), presumably to take advantage of copious amounts of GlcNAc and chitobiose secreted by tick midgut epithelial cells for the formation of the peritrophic membrane and cuticle (Sonenshine, 1991). Loss of c-di-GMP also results in reduced expression of AckA, the acetate kinase required for generation of acetyl phosphate, which gives rise to acetyl-CoA, the starting point for synthesis of peptidoglycan by way of the mevalonate pathway (Xu *et al.,* 2010a; Van Laar *et al.,* 2012). Presumably, the dramatic

phenotypes displayed by *hk1* and *rrp1* mutants within feeding ticks stem, at least in part, from simultaneous dysregulation of both carbon metabolism and cell envelope biogenesis (Sze *et al.,* 2013; Caimano *et al.,* 2015).

In addition to its role in carbon metabolism, c-di-GMP also upregulates the expression of multiple genes encoding outer surface lipoproteins, including several OspE/BbCRASP paralogs, which have been shown to inhibit complement-mediated lysis by binding factor H and related proteins (reviewed in Kraiczy and Stevenson, 2013). Other surface-associated lipoproteins within the Hk1/Rrp1 regulon (such as the Mlps) may help to protect spirochetes from potentially borreliacidal molecules (including antimicrobial peptides and reactive oxygen species) encountered within the midgut during tick feeding (Caimano *et al.,* 2015).

These two nucleotide messengers converge on common pathways important for persistence, such as glycerol utilization, indicating there is likely some degree of coordination. While there is no known direct link between (p)ppGpp and c-di-GMP, a closer examination of their metabolic pathways indicates shared substrates and products. Both enzymes are in competition for the common substrate GTP; thus, an increase in the synthetic activity of Rel_{Bbu} or Rrp1 may decrease the ability of the other enzyme to produce its respective messenger. Alternatively, pyrophosphate (PP_i) released during c-di-GMP synthesis could potentially increase (p)ppGpp levels by product inhibition of Rel_{Bbu} activity, as PP_i is formed when (p)ppGpp is hydrolyzed. Thus, c-di-GMP and (p)ppGpp levels could vary inversely (or cooperatively) depending on the intracellular conditions.

Conclusions and perspectives

Borreliologists and medical entomologists have deciphered many of the molecular mechanisms wielded by the spirochete to adapt to its vector and host, as well as to be maintained in its natural enzootic cycle, which requires being transmitted from a feeding tick to a naïve host and being acquired from an infected vertebrate by a naïve vector. We have described some gene products, including those involved in carbon utilization, and how they are regulated so that *B. burgdorferi* can survive in the tick between blood meals. Perhaps surprisingly, as *Borrelia* is not known for doing business as usual in model organisms (Samuels and Radolf, 2009), the spirochete takes advantage of the stringent response, the classic bacterial response to nutrient deprivation.

Many, if not most, of the *B. burgdorferi* gene products involved in tick infectivity display little to no homology to proteins of known function. Therefore, the biological significance of the vast majority of these factors, especially their mechanistic role in spirochete survival in the vector or transmission between species, is enigmatic. There remains much to learn regarding the unexpected regulation of individual genes in the *glp* operon as well as the coordination of metabolic pathways as the spirochete cycles back and forth between vector and host. We sincerely hope that this Microreview provides the background, and a conceptual framework, for further research on the interaction of *B. burgdorferi* with its tick vector.

Acknowledgments

We apologize to authors whose studies were not cited in this review because of space limitations. We thank Ira Schwartz and Bob Gilmore for thoughtful review of the manuscript, and Utpal Pal, Justin Radolf and Ira Schwartz for useful discussions. Studies in our laboratories have been supported by National Institutes of Health grants AI051486 (to D.S.S.), AI88131 (to D.D. and D.S.S.), AI29735 (to Justin D. Radolf and M.J.C.), and AI080615 (to Utpal Pal) as well as an American Heart Association grant 16GRNT27740039 (to M. J.C.). The authors declare no conflict of interest.

References

Battisti JM, Bono JL, Rosa PA, Schrumpf ME, Schwan TG, Policastro PF. Outer surface protein A protects Lyme disease spirochetes from acquired host immunity in the tick vector. Infect Immun. 2008; 76:5228–5237. [PubMed: 18779341]

Bauer WJ, Luthra A, Zhu G, Radolf JD, Malkowski MG, Caimano MJ. Structural characterization and modeling of the *Borrelia burgdorferi* hybrid histidine kinase Hk1 periplasmic sensor: a system for sensing small molecules associated with tick feeding. J Struct Biol. 2015; 192:48–58. [PubMed: 26321039]

Benach JL, Bosler EM, Hanrahan JP, Coleman JL, Bast TF, Habicht GS, et al. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med. 1983; 308:740–742. [PubMed: 6828119]

Bestor A, Rego ROM, Tilly K, Rosa PA. Competitive advantage of *Borrelia burgdorferi* with outer surface protein BBA03 during tick-mediated infection of the mammalian host. Infect Immun. 2012; 80:3501–3511. [PubMed: 22851744]

Brisson D, Drecktrah D, Eggers CH, Samuels DS. Genetics of *Borrelia burgdorferi*. Annu Rev Genet. 2012; 46:515–536. [PubMed: 22974303]

Bugrysheva J, Dobrikova EY, Sartakova ML, Caimano MJ, Daniels TJ, Radolf JD, et al. Characterization of the stringent response and rel_{Bbu} expression in *Borrelia burgdorferi*. J Bacteriol. 2003; 185:957–965. [PubMed: 12533471]

Bugrysheva JV, Bryksin AV, Godfrey HP, Cabello FC. *Borrelia burgdorferi rel* is responsible for generation of guanosine-3′-diphosphate-5′-triphosphate and growth control. Infect Immun. 2005; 73:4972–4981. [PubMed: 16041012]

Bugrysheva JV, Pappas CJ, Terekhova DA, Iyer R, Godfrey HP, Schwartz I, Cabello FC. Characterization of the Rel_{Bbu} regulon in *Borrelia burgdorferi* reveals modulation of glycerol metabolism by (p)ppGpp. PLoS One. 2015; 10:e0118063. [PubMed: 25688856]

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease—a tick-borne spirochetosis? Science. 1982; 216:1317–1319. [PubMed: 7043737]

Caimano MJ, Kenedy MR, Kairu T, Desrosiers DC, Harman M, Dunham-Ems S, et al. The hybrid histidine kinase Hk1 is part of a two-component system that is essential for survival of *Borrelia burgdorferi* in feeding *Ixodes scapularis* ticks. Infect Immun. 2011; 79:3117–3130. [PubMed: 21606185]

Caimano MJ, Dunham-Ems S, Allard AM, Cassera MB, Kenedy M, Radolf JD. Cyclic di-GMP modulates gene expression in Lyme disease spirochetes at the tick-mammal interface to promote spirochete survival during the blood meal and tick-to-mammal transmission. Infect Immun. 2015; 83:3043–3060. [PubMed: 25987708]

Concepcion MB, Nelson DR. Expression of *spoT* in *Borrelia burgdorferi* during serum starvation. J Bacteriol. 2003; 185:444–452. [PubMed: 12511489]

Corona A, Schwartz I. *Borrelia burgdorferi*: carbon metabolism and the tick-mammal enzootic cycle. Microbiol Spectr. 2015; 3 MBP-0011-2014.

Dalebroux ZD, Swanson MS. ppGpp: magic beyond RNA polymerase. Nat Rev Microbiol. 2012; 10:203–212. [PubMed: 22337166]

Death A, Ferenci T. The importance of the binding-protein-dependent Mgl system to the transport of glucose in *Escherichia coli* growing on low sugar concentrations. Res Microbiol. 1993; 144:529–537. [PubMed: 8310178]

Drecktrah D, Lybecker M, Popitsch N, Rescheneder P, Hall LS, Samuels DS. The *Borrelia burgdorferi* RelA/SpoT homolog and stringent response regulate survival in the tick vector and global gene expression during starvation. PLoS Pathog. 2015; 11:e1005160. [PubMed: 26371761]

Dunham-Ems SM, Caimano MJ, Pal U, Wolgemuth CW, Eggers CH, Balic A, Radolf JD. Live imaging reveals a biphasic mode of dissemination of *Borrelia burgdorferi* within ticks. J Clin Invest. 2009; 119:3652–3665. [PubMed: 19920352]

Dunham-Ems SM, Caimano MJ, Eggers CH, Radolf JD. *Borrelia burgdorferi* requires the alternative sigma factor RpoS for dissemination within the vector during tick-to-mammal transmission. PLoS Pathog. 2012; 8:e1002532. [PubMed: 22359504]

Fingerle V, Goettner G, Gern L, Wilske B, Schulte-Spechtel U. Complementation of a *Borrelia afzelii* OspC mutant highlights the crucial role of OspC for dissemination of *Borrelia afzelii* in *Ixodes ricinus*. Int J Med Microbiol. 2007; 297:97–107. [PubMed: 17267282]

Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, et al. Genomic sequence of a Lyme disease spirochete, *Borrelia burgdorferi*. Nature. 1997; 390:580–586. [PubMed: 9403685]

Freedman JC, Rogers EA, Kostick JL, Zhang H, Iyer R, Schwartz I, Marconi RT. Identification and molecular characterization of a cyclic-di-GMP effector protein, PlzA (BB0733): additional evidence for the existence of a functional cyclic-di-GMP regulatory network in the Lyme disease spirochete, *Borrelia burgdorferi*. FEMS Immunol Med Microbiol. 2010; 58:285–294. [PubMed: 20030712]

Fuchs H, Wallich R, Simon MM, Kramer MD. The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor. Proc Natl Acad Sci U S A. 1994; 91:12594–12598. [PubMed: 7809084]

Gherardini, F.; Boylan, J.; Lawrence, K.; Skare, J. Metabolism and physiology of *Borrelia*. In: Samuels, DS.; Radolf, JD., editors. Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Norfolk, UK: Caister Academic Press; 2010. p. 103-138.

Gilmore RD, Brandt KS, Hyde JA. *pncA* and *bptA* are not sufficient to complement *Ixodes scapularis* colonization and persistence by *Borrelia burgdorferi* in a linear plasmid lp25-deficient background. Infect Immun. 2014; 82:5110–5116. [PubMed: 25245809]

Grimm D, Tilly K, Byram R, Stewart PE, Krum JG, Bueschel DM, et al. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc Natl Acad Sci U S A. 2004; 101:3142–3147. [PubMed: 14970347]

Hauryliuk V, Atkinson GC, Murakami KS, Tenson T, Gerdes K. Recent functional insights into the role of (p)ppGpp in bacterial physiology. Nat Rev Microbiol. 2015; 13:298–309. [PubMed: 25853779]

He M, Ouyang Z, Troxell B, Xu H, Moh A, Piesman J, et al. Cyclic di-GMP is essential for the survival of the Lyme disease spirochete in ticks. PLoS Pathog. 2011; 7:e1002133. [PubMed: 21738477]

He M, Zhang JJ, Ye M, Lou Y, Yang XF. Cyclic di-GMP receptor PlzA controls virulence gene expression through RpoS in *Borrelia burgdorferi*. Infect Immun. 2014; 82:445–452. [PubMed: 24218478]

Hoon-Hanks LL, Morton EA, Lybecker MC, Battisti JM, Samuels DS, Drecktrah D. *Borrelia burgdorferi malQ* mutants utilize disaccharides and traverse the enzootic cycle. FEMS Immunol Med Microbiol. 2012; 66:157–165. [PubMed: 22672337]

Imai D, Holden K, Velazquez EM, Feng S, Hodzic E, Barthold SW. Influence of arthritis-related protein (BBF01) on infectivity of *Borrelia burgdorferi* B31. BMC Microbiol. 2013; 13:100. [PubMed: 23651628]

Iyer R, Caimano MJ, Luthra A, Axline D Jr, Corona A, Iacobas DA, et al. Stage-specific global alterations in the transcriptomes of Lyme disease spirochetes during tick feeding and following mammalian host adaptation. Mol Microbiol. 2015; 95:509–538. [PubMed: 25425211]

Kenedy MR, Lenhart TR, Akins DR. The role of *Borrelia burgdorferi* outer surface proteins. FEMS Immunol Med Microbiol. 2012; 66:1–19. [PubMed: 22540535]

Khajanchi BK, Odeh E, Gao L, Jacobs MB, Philipp MT, Lin T, Norris SJ. Phosphoenolpyruvate phosphotransferase system components modulate gene transcription and virulence of *Borrelia burgdorferi*. Infect Immun. 2016; 84:754–764.

Kostick JL, Szkotnicki LT, Rogers EA, Bocci P, Raffaelli N, Marconi RT. The diguanylate cyclase, Rrp1, regulates critical steps in the enzootic cycle of the Lyme disease spirochetes. Mol Microbiol. 2011; 81:219–231. [PubMed: 21542866]

Kraiczy P, Stevenson B. Complement regulator-acquiring surface proteins of *Borrelia burgdorferi*: structure, function and regulation of gene expression. Ticks Tick Borne Dis. 2013; 4:26–34. [PubMed: 23219363]

Kumar M, Yang X, Coleman AS, Pal U. BBA52 facilitates *Borrelia burgdorferi* transmission from feeding ticks to murine hosts. J Infect Dis. 2010; 201:1084–1095. [PubMed: 20170377]

Kung F, Anguita J, Pal U. *Borrelia burgdorferi* and tick proteins supporting pathogen persistence in the vector. Future Microbiol. 2013; 8:41–56. [PubMed: 23252492]

Kung F, Kaur S, Smith AA, Yang X, Wilder CN, Sharma K, et al. A *Borrelia burgdorferi* surface-exposed transmembrane protein lacking detectable immune responses supports pathogen persistence and constitutes a vaccine target. J Infect Dis. 2016; 213:1786–1795. [PubMed: 26747708]

von Lackum K, Stevenson B. Carbohydrate utilization by the Lyme borreliosis spirochete, *Borrelia burgdorferi*. FEMS Microbiol Lett. 2005; 243:173–179. [PubMed: 15668016]

Lagal V, Portnoï D, Faure G, Postic D, Baranton G. *Borrelia burgdorferi* sensu stricto invasiveness is correlated with OspC-plasminogen affinity. Microbes Infect. 2006; 8:645–652. [PubMed: 16513394]

Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu Rev Entomol. 1991; 36:587–609. [PubMed: 2006870]

Li X, Pal U, Ramamoorthi N, Liu X, Desrosiers DC, Eggers CH, et al. The Lyme disease agent *Borrelia burgdorferi* requires BB0690, a Dps homologue, to persist within ticks. Mol Microbiol. 2007; 63:694–710. [PubMed: 17181780]

McCoy JG, Levin EJ, Zhou M. Structural insight into the PTS sugar transporter EIIC. Biochim Biophys Acta. 2015; 1850:577–585. [PubMed: 24657490]

Mead PS. Epidemiology of Lyme disease. Infect Dis Clin North Am. 2015; 29:187–210. [PubMed: 25999219]

Önder Ö, Humphrey PT, McOmber B, Korobova F, Francella N, Greenbaum DC, Brisson D. OspC is potent plasminogen receptor on surface of *Borrelia burgdorferi*. J Biol Chem. 2012; 287:16860–16868. [PubMed: 22433849]

Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, de Silva AM, et al. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. Cell. 2004a; 119:457–468. [PubMed: 15537536]

Pal U, Yang X, Chen M, Bockenstedt LK, Anderson JF, Flavell RA, et al. OspC facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. J Clin Invest. 2004b; 113:220–230. [PubMed: 14722614]

Pal U, Dai J, Li X, Neelakanta G, Luo P, Kumar M, et al. A differential role for BB0365 in the persistence of *Borrelia burgdorferi* in mice and ticks. J Infect Dis. 2008; 197:148–155. [PubMed: 18171298]

Pappas CJ, Iyer R, Petzke MM, Caimano MJ, Radolf JD, Schwartz I. *Borrelia burgdorferi* requires glycerol for maximum fitness during the tick phase of the enzootic cycle. PLoS Pathog. 2011; 7:e1002102. [PubMed: 21750672]

Patton TG, Brandt KS, Nolder C, Clifton DR, Carroll JA, Gilmore RD. *Borrelia burgdorferi bba66* gene inactivation results in attenuated mouse infection by tick transmission. Infect Immun. 2013; 81:2488–2498. [PubMed: 23630963]

Piesman, J.; Schwan, TG. Ecology of borreliae and their arthropod vectors. In: Samuels, DS.; Radolf, JD., editors. Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Norfolk, UK: Caister Academic Press; 2010. p. 251-278.

Pitzer JE, Sultan SZ, Hayakawa Y, Hobbs G, Miller MR, Motaleb MA. Analysis of the *Borrelia burgdorferi* cyclic-di-GMP-binding protein PlzA reveals a role in motility and virulence. Infect Immun. 2011; 79:1815–1825. [PubMed: 21357718]

Potrykus K, Cashel M. (p)ppGpp: still magical? Annu Rev Microbiol. 2008; 62:35–51. [PubMed: 18454629]

Promnares K, Kumar M, Shroder DY, Zhang X, Anderson JF, Pal U. *Borrelia burgdorferi* small lipoprotein Lp6.6 is a member of multiple protein complexes in the outer membrane and facilitates pathogen transmission from ticks to mice. Mol Microbiol. 2009; 74:112–125. [PubMed: 19703109]

Purser JE, Lawrenz MB, Caimano MJ, Howell JK, Radolf JD, Norris SJ. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. Mol Microbiol. 2003; 48:753–764. [PubMed: 12694619]

Radolf JD, Caimano MJ, Stevenson B, Hu LT. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat Rev Microbiol. 2012; 10:87–99. [PubMed: 22230951]

Revel AT, Blevins JS, Almazán C, Neil L, Kocan KM, de la Fuente J, et al. *bptA* (*bbe16*) is essential for the persistence of the Lyme disease spirochete, *Borrelia burgdorferi*, in its natural tick vector. Proc Natl Acad Sci U S A. 2005; 102:6972–6977. [PubMed: 15860579]

Rhodes RG, Coy W, Nelson DR. Chitobiose utilization in *Borrelia burgdorferi* is dually regulated by RpoD and RpoS. BMC Microbiol. 2009; 9:108. [PubMed: 19473539]

Ristow LC, Miller HE, Padmore LJ, Chettri R, Salzman N, Caimano MJ, et al. The β_3-integrin ligand of *Borrelia burgdorferi* is critical for infection of mice but not ticks. Mol Microbiol. 2012; 85:1105–1118. [PubMed: 22758390]

Rogers EA, Terekhova D, Zhang HM, Hovis KM, Schwartz I, Marconi RT. Rrp1, a cyclic-di-GMP-producing response regulator, is an important regulator of *Borrelia burgdorferi* core cellular functions. Mol Microbiol. 2009; 71:1551–1573. [PubMed: 19210621]

Römling U, Galperin MY, Gomelsky M. Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiol Mol Biol Rev. 2013; 77:1–52. [PubMed: 23471616]

Ryjenkov DA, Tarutina M, Moskvin OV, Gomelsky M. Cyclic diguanylate is a ubiquitous signaling molecule in bacteria: insights into biochemistry of the GGDEF protein domain. J Bacteriol. 2005; 187:1792–1798. [PubMed: 15716451]

Saier MH Jr, Yamada M, Erni B, Suda K, Lengeler J, Ebner R, et al. Sugar permeases of the bacterial phosphoenolpyruvate-dependent phosphotransferase system: sequence comparisons. FASEB J. 1988; 2:199–208. [PubMed: 2832233]

Samuels DS. Gene regulation in *Borrelia burgdorferi*. Annu Rev Microbiol. 2011; 65:479–499. [PubMed: 21801026]

Samuels DS, Radolf JD. Who is the BosR around here anyway? Mol Microbiol. 2009; 74:1295–1299. [PubMed: 19943896]

Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. Proc Natl Acad Sci U S A. 1995; 92:2909–2913. [PubMed: 7708747]

Sonenshine, DE. Biology of Ticks. New York: Oxford University Press; 1991. p. 447

Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, et al. The spirochetal etiology of Lyme disease. N Engl J Med. 1983; 308:733–740. [PubMed: 6828118]

Sultan SZ, Pitzer JE, Boquoi T, Hobbs G, Miller MR, Motaleb MA. Analysis of the HD-GYP domain cyclic-di-GMP phosphodiesterase reveals a role in motility and enzootic life cycle of *Borrelia burgdorferi*. Infect Immun. 2011; 79:3273–3283. [PubMed: 21670168]

Sze CW, Smith A, Choi YH, Yang X, Pal U, Yu A, Li C. Study of the response regulator Rrp1 reveals its regulatory role in chitobiose utilization and virulence of *Borrelia burgdorferi*. Infect Immun. 2013; 81:1775–1787. [PubMed: 23478317]

Tilly K, Elias AF, Errett J, Fischer E, Iyer R, Schwartz I, et al. Genetics and regulation of chitobiose utilization in *Borrelia burgdorferi*. J Bacteriol. 2001; 183:5544–5553. [PubMed: 11544216]

Tilly K, Grimm D, Bueschel DM, Krum JG, Rosa P. Infectious cycle analysis of a *Borrelia burgdorferi* mutant defective in transport of chitobiose, a tick cuticle component. Vector Borne Zoonotic Dis. 2004; 4:159–168. [PubMed: 15228817]

Van Laar TA, Lin YH, Miller CL, Karna SLR, Chambers JP, Seshu J. Effect of levels of acetate on the mevalonate pathway of *Borrelia burgdorferi*. PLoS One. 2012; 7:e38171. [PubMed: 22675445]

Wang P, Lutton A, Olesik J, Vali H, Li X. A novel iron- and copper-binding protein in the Lyme disease spirochaete. Mol Microbiol. 2012; 86:1441–1451. [PubMed: 23061404]

Xu H, Caimano MJ, Lin T, He M, Radolf JD, Norris SJ, et al. Role of acetylphosphate in activation of the Rrp2–RpoN–RpoS pathway in *Borrelia burgdorferi*. PLoS Pathog. 2010a; 6:e1001104. [PubMed: 20862323]

Xu H, He M, He JJ, Yang XF. Role of the surface lipoprotein BBA07 in the enzootic cycle of *Borrelia burgdorferi*. Infect Immun. 2010b; 78:2910–2918. [PubMed: 20421380]

Yang X, Hegde S, Shroder DY, Smith AA, Promnares K, Neelakanta G, et al. The lipoprotein La7 contributes to *Borrelia burgdorferi* persistence in ticks and their transmission to naïve hosts. Microbes Infect. 2013a; 15:729–737. [PubMed: 23774694]

Yang X, Qin J, Promnares K, Kariu T, Anderson JF, Pal U. Novel microbial virulence factor triggers murine Lyme arthritis. J Infect Dis. 2013b; 207:907–918. [PubMed: 23303811]

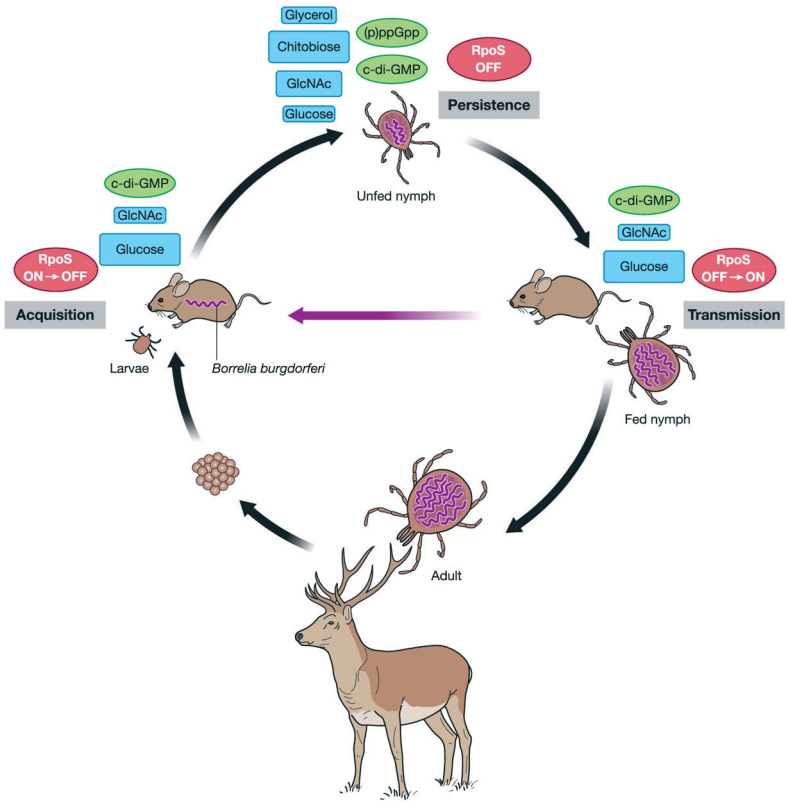

Fig. 1.

The enzootic cycle of *B. burgdorferi*. Acquisition: larval ticks must acquire *B. burgdorferi* by feeding on an infected vertebrate as the bacterium is not transovarially transmitted. Persistence: intracellular second messengers (p)ppGpp and c-di-GMP regulate persistence in the tick by various mechanisms, including modulation of carbohydrate preference, while the molecular gatekeeper RpoS is absent in unfed ticks. Transmission: following the molt into nymphs, infected ticks can transmit *B. burgdorferi* to uninfected hosts, completing the cycle. Adapted from Brisson *et al.* (2012). GlcNAc, *N*-acetyl glucosamine.

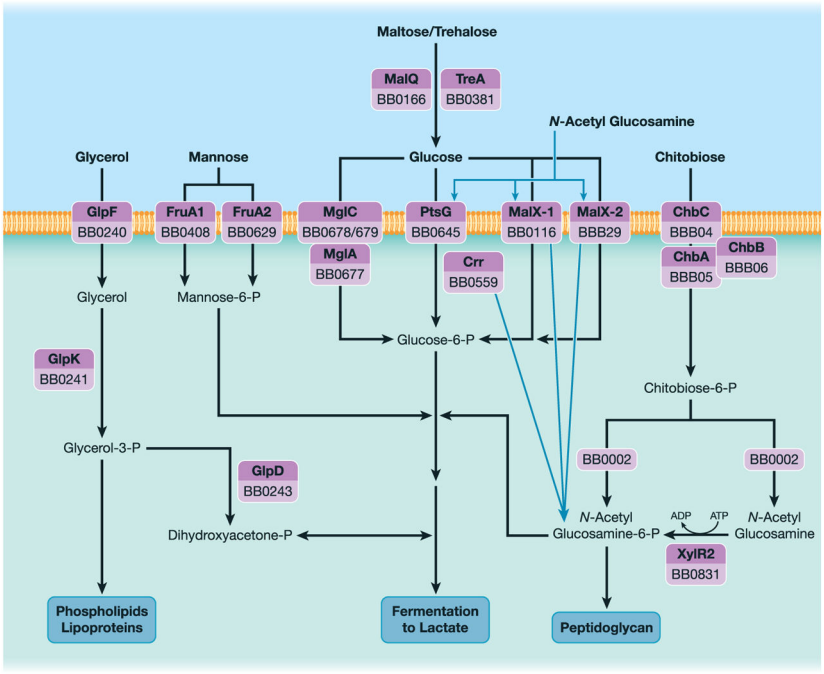

Fig. 2.

Schematic diagram of carbohydrate transport and metabolism based on functional studies and/or bioinformatics. Adapted from Corona and Schwartz (2015).