Borrelia burgdorferi: Carbon Metabolism and the Tick-Mammal Enzootic Cycle

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ABSTRACT Borrelia burgdorferi, the spirochetal agent of Lyme disease, is a zoonotic pathogen that is maintained in a natural cycle that typically involves mammalian reservoir hosts and a tick vector of the Ixodes species. During each stage of the enzootic cycle, B. burgdorferi is exposed to environments that differ in temperature, pH, small molecules, and most important, nutrient sources. B. burgdorferi has a highly restricted metabolic capacity because it does not contain a tricarboxylic acid cycle, oxidative phosphorylation, or any pathways for de novo biosynthesis of carbohydrates, amino acids, or lipids. Thus, B. burgdorferi relies solely on glycolysis for ATP production and is completely dependent on the transport of nutrients and cofactors from extracellular sources. Herein, pathways for carbohydrate uptake and utilization in B. burgdorferi are described. Regulation of these pathways during the different phases of the enzootic cycle is discussed. In addition, a model for differential control of nutrient flux through the glycolytic pathway as the spirochete transits through the enzootic cycle is presented.

THE ENZOOTIC CYCLE
Borrelia burgdorferi is the spirochetal agent of Lyme disease, the most commonly reported arthropod-borne disease in the United States (1–3). B. burgdorferi is a zoonotic pathogen that is maintained in a natural cycle involving mammalian reservoir hosts such as field mice, squirrels, and birds and an arthropod vector of the Ixodes species (4–6) (Fig. 1). In the United States, the principal vector is Ixodes scapularis, the common deer tick (5, 6). Because there is no transovarial transmission of B. burgdorferi, newly hatched larvae acquire the spirochete during their first blood meal on an infected mammalian host reservoir (7, 8). The spirochete is maintained in the midgut of the tick during molting to the nympha l stage. At this point, the spirochete is in a nonmotile state until the nymph begins to feed on the next mammalian host (9). The spirochete then begins rapidly replicating in the feeding nympha l midgut, leaves the midgut and enters the hemolymph, from which the bacteria migrate to the salivary glands and are transmitted to the next mammalian host (9–12) (Fig. 1).

During each stage of the enzootic cycle, B. burgdorferi is exposed to different environments. Each milieu varies by temperature, pH, small molecules, and most important, nutrient sources. The drastic changes in environmental attributes require the spirochete to not only modulate the expression of colonization factors necessary for persistence in either the vector or the mammalian host, but also to adjust its metabolic state to adapt to the changing nutrient profile. While in the mammalian host, B. burgdorferi can be found in the skin at the tick bite site, in the circulatory system, and at distal tissue sites such as joints and heart (13). During larval acquisition, spirochetes enter the midgut concurrent with the blood meal and its associated nutrients and host factors. After molting to the nympha l stage,
B. burgdorferi is confined to the midgut lumen of the unfed nymph. The lumen is a nutrient-poor environment consistent with the metabolically dormant state of the spirochetes at this stage (9, 14). When the nymph begins to feed, the incoming blood meal is surrounded by a peritrophic membrane that sequesters the blood meal and its accompanying nutrients away from the spirochetes within the midgut lumen (15). The ability to use a variety of available carbohydrates during the enzootic cycle is likely essential for the survival of B. burgdorferi during the tick phases and pathogenesis in mammalian hosts, as has been observed for other pathogenic bacteria (16).

B. BURGDORFERI GENOMICS

B. burgdorferi has a complex genome. The segmented genome of the B31 type strain consists of one large linear chromosome (about 910 Kb) and 21 circular (cp) and linear plasmids (lp) (17, 18). A substantial portion of the predicted open reading frames (ORFs) is annotated as hypothetical or as having homology to other spirochete genes without any functional annotation (17, 18). ORFs located on the linear chromosome encode primarily housekeeping functions, including most genes associated with metabolism (17, 19). Plasmid content in individual B. burgdorferi isolates is variable, although lp54 and cp26 are uniformly present in most characterized isolates (20–22). Plasmids can be lost during in vitro cultivation, which typically has no effect on in vitro growth; however, several plasmids play an essential role in vivo (22–30). cp26 is absolutely required for survival of the bacterium (25, 31); it encodes several proteins involved in purine biosynthesis, transport, telomere resolution, and outer surface protein C (OspC), an essential virulence factor (17, 25, 32–34). lp25 is required for mammalian infection (35). It has been shown that the critical gene on lp25 is pmcA, which encodes a nicotinamidase and is likely involved in NAD+ salvage (24, 32, 36). In addition, lp28-1 is required for persistence during mammalian infection, likely as the result of antigenic variation in vlsE (23, 35, 37–39).

REGULATION OF GENE EXPRESSION DURING THE ENZOOTIC CYCLE

RpoS

Bacteria typically regulate gene expression in response to environmental cues through processes that are often mediated by alternative sigma factors or two-component signaling systems (TCS) (40, 41). The B. burgdorferi genome encodes for only two alternative sigma factors and two TCS (17). Thus, to adapt to the different environments encountered during the enzootic cycle,
B. burgdorferi must rely on a limited repertoire of such components. Seminal studies by Norgard and colleagues demonstrated a link between one TCS, specifically Hk2-Rrp2, and the alternative sigma factors RpoN and RpoS (42, 43). The expression of several virulence genes is dependent on RpoS (42, 44). RpoS is also essential for repression of genes whose expression is required in the tick but not in the mammalian host (45). As a result, RpoS is absolutely essential for mammalian infection as well as migration through the tick during transmission (44, 46). Global transcriptome analyses of wild type and RpoS mutant strains under mammalian-like conditions defined the RpoS regulon and included both genes that are induced and repressed by RpoS. On this basis, RpoS has been referred to as a “gate-keeper” that controls the reciprocal expression of genes required for mammalian infection or maintenance in ticks (47).

In B. burgdorferi, RpoS expression is controlled by multiple layers of transcriptional and posttranscriptional regulation (48). The Rrp2-RpoN-RpoS pathway is required for transcription of rpoS (49–51). RpoN binds directly to a canonical -24/-12 sequence in the rpoS promoter to induce transcription (52). This interaction requires the activation of Rrp2, the encoded response regulator of the Hk2-Rrp2 two-component system (43, 50). It was assumed that Rrp2 would be phosphorylated and activated by Hk2; however, it was later demonstrated that Rrp2 could induce virulence gene expression independent of Hk2 (53). Xu et al subsequently showed that acetyl phosphate can function as the phosphate group donor for Rrp2 (54). RpoS expression is also modulated by several other effectors, including BosR (discussed later), DsrA (a small RNA), Hfq (an RNA chaperone), and CsrA (BB184) (55–58), although the role of CsrA has been recently challenged (59). It has also been suggested that BadR (BB693), a protein with sequence homology to the ROK family of proteins, represses the transcription of rpoS (60).

**BosR**

B. burgdorferi encodes a Fur/Per homolog, BosR (Borrelia oxidative stress regulator) (17, 61, 62). Its role in mediating the oxidative stress response is not clear, as B. burgdorferi BosR mutants are only slightly more sensitive to oxidative stress than wild-type spirochetes (63, 64). Several studies have demonstrated that BosR is a transcriptional activator of rpoS (63, 65, 66). Ouyang et al identified a “BosR box” to which the protein binds and demonstrated that the rpoS promoter contains three such binding sites; interestingly, bioinformatic analysis revealed the presence of 60 additional cis-acting “BosR boxes” in the B. burgdorferi genome, suggesting that BosR likely regulates the expression of additional genes in addition to its role as an activator of RpoS (67, 68). More recently, BosR was reported to be directly involved in repression of lipoprotein gene expression (69). The environmental signal or signals that control bosR expression have not been fully elucidated. It has been suggested that transition metals may be involved, because bosR expression is Zn2+-dependent and is posttranscriptionally inhibited by Mn2+ (61, 70).

**Hk1-Rrp1**

The second TCS in B. burgdorferi has been designated as Hk1-Rrp1. The response regulator Rrp1 contains a GGDEF motif characteristic of diguanylate cyclases that convert two GTP molecules to a molecule of c-di-GMP and is the sole protein in the genome containing this motif (17, 71). Ryjenkov et al demonstrated that Rrp1 functions as a diguanylate cyclase and its activity is dependent on phosphorylation of its receiver domain (72). The secondary messenger c-di-GMP has gained attention as a global regulator in bacteria that is associated with virulence, motility, and central metabolism (73, 74). Rrp1 receives its signal from the membrane-bound sensor histidine kinase Hk1 (75, 76). B. burgdorferi also encodes the other components of the c-di-GMP signaling system, including two phosphodiesterases (PdeA [BB363] and PdeB [BB374]) and a cyclic-di-GMP binding protein PlzA (BB733) (17, 77). The hkl1-rrp1 operon appears to be constitutively expressed (76), although rrp1 and plzA expression may be elevated during tick feeding (71, 77). Both Rrp1 and Hk1 deletion mutants are infectious in mice but are unable to survive in the tick vector (76, 78, 79); Rrp1 mutants also have defective motility (76, 79). Global transcriptome analyses of wild-type and Rrp1 mutant spirochetes revealed that c-di-GMP regulates the expression of a substantial number of genes (71, 78). Taken together, the data suggest that c-di-GMP signaling plays a critical role in tick colonization (80).

**CARBOHYDRATE METABOLISM**

B. burgdorferi has a very restricted metabolic capacity. Genes encoding functions related to carbohydrate transport and metabolism are listed in Table 1. The genome encodes enzymes of the glycolytic pathway but not of the tricarboxylic acid cycle or oxidative phosphorylation (17). The spirochete does encode the oxidative branch of the pentose phosphate pathway (17), but because
### TABLE 1  *Borrelia burgdorferi* genes encoding proteins involved in carbohydrate metabolism

<table>
<thead>
<tr>
<th>Gene Locus</th>
<th>Annotated Name*</th>
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<tbody>
<tr>
<td>Transporters</td>
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</tr>
<tr>
<td>bb448</td>
<td>PTS; phosphocarrier protein Hpr</td>
</tr>
<tr>
<td>bb557</td>
<td>PTS; phosphocarrier protein Hpr, ptsH</td>
</tr>
<tr>
<td>bb558</td>
<td>PTS; phosphoenolpyruvate protein phosphocarrier EI, pstE</td>
</tr>
<tr>
<td>bb559</td>
<td>PTS; glucose-specific EIIA, crr</td>
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<tr>
<td>bb645</td>
<td>PTS; glucose-specific EIIBC, fruA1</td>
</tr>
<tr>
<td>bb716</td>
<td>PTS; glucose-specific EIABC, malX1</td>
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<tr>
<td>bb729</td>
<td>PTS; fructose, mannose-specific EIIABC, fruA2</td>
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<tr>
<td>bb740</td>
<td>PTS; chitobiose-specific EIIC, chbC</td>
</tr>
<tr>
<td>bb741</td>
<td>PTS; chitobiose-specific EIIA, chbA</td>
</tr>
<tr>
<td>bb742</td>
<td>PTS; chitobiose-specific EIIB, chbB</td>
</tr>
<tr>
<td>bb743</td>
<td>Glycerol facilitator, glpF</td>
</tr>
<tr>
<td>bb744</td>
<td>ABC transporter (glucose, ribose, galactose), ATP-binding protein, mglA</td>
</tr>
<tr>
<td>bb745</td>
<td>ABC transporter (glucose, ribose, galactose), permease protein, mglC1</td>
</tr>
<tr>
<td>bb746</td>
<td>ABC transporter (glucose, ribose, galactose), permease protein, mglC2</td>
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<tr>
<td>bb747</td>
<td>Lactate permease, lctP</td>
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<td>Glycolysis</td>
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<tr>
<td>bb748</td>
<td>glucose-6-phosphate isomerase, pgi</td>
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<td>bb749</td>
<td>phosphofructokinase, pfk</td>
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<td>bb750</td>
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<td>bb754</td>
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<td>N-acetylmannosamine-6-phosphate epimerase</td>
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<tr>
<td>bb765</td>
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<td>N-acetylglucosamine-6-phosphate isomerase, nagA</td>
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<td>bb767</td>
<td>Glucosamine-6-phosphate isomerase, nagB</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>bb769</td>
<td>beta-glucosidase</td>
</tr>
<tr>
<td>bb770</td>
<td>Glucokinase</td>
</tr>
<tr>
<td>Maltose</td>
<td></td>
</tr>
<tr>
<td>bb771</td>
<td>4-alpha-glucanotransferase, malQ</td>
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</table>

(continued)
TABLE 1  B. burgdorferi genes encoding proteins involved in carbohydrate metabolism (continued)

<table>
<thead>
<tr>
<th>Gene Locus</th>
<th>Annotated Name*</th>
</tr>
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<tr>
<td>Trehalose</td>
<td>trehalase, treA</td>
</tr>
<tr>
<td>bb381</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>glycerol kinase, glpK</td>
</tr>
<tr>
<td>bb241</td>
<td>glycerol-3-phosphate dehydrogenase, glpD</td>
</tr>
<tr>
<td>bb243</td>
<td></td>
</tr>
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</table>

*Annotations based on Fraser et al (17) and NCBI B. burgdorferi B31 genome (NC_001318.1 and NC_001903.1).

ribose cannot support in vitro growth when supplied as the principal carbon source (81), this pathway is not likely to play a role in energy production. Thus, B. burgdorferi relies solely on glycolysis for ATP generation. The bacterium does not encode any complete pathways for de novo biosynthesis of fatty acids, amino acids, or nucleotides (17). For this reason, B. burgdorferi is completely dependent on the transport of nutrients and cofactors from extracellular sources and several salvage pathways (17, 25, 32, 33, 82–84). Consequently, B. burgdorferi has more than 50 genes encoding transporters for carbohydrates, oligopeptides, and amino acids (17, 32).

Typically, glycolysis yields a net of only two or three ATP molecules per glucose molecule; the reliance on only glycolysis for production of ATP could account for the spirochete’s slow growth, even under optimal in vitro cultivation conditions. B. burgdorferi is not only restricted to the carbohydrate sources available in the distinct environments it encounters during the enzootic cycle but also by the limited number of encoded carbohydrate uptake systems and catabolic pathways (17). von Lackum and Stevenson reported that B. burgdorferi can use six carbohydrates as the principal carbon source during in vitro growth in BSK medium—glucose, glycerol, maltose, mannose, N-acetylglucosamine (GlcNAc), and chitobiose (81). More recently, Hoon-Hanks et al demonstrated that trehalose can also support B. burgdorferi in vitro growth (85).

Glycolysis

B. burgdorferi encodes all enzymes of the glycolytic pathway (17). Glucose enters as either glucose (via a putative ABC transporter [discussed later]) or as glucose 6-phosphate (via a phosphoenolpyruvate-phosphotransferase system [PEP-PTS]) (Fig. 2). Acquired glucose would be converted to glucose 6-phosphate by the action of a putative glucokinase (BB831) and then to fructose 6-phosphate by glucose 6-phosphate isomerase (Pgi, BB730) (Fig. 3). B. burgdorferi encodes two enzymes with putative phosphofructokinase activity (BB020, BB727). Both genes have been expressed and characterized in vitro; BB020 is an active pyrophosphate-dependent phosphofructokinase that functions as a dimer (86, 87), whereas BB727 exists as a multimer in solution, with no measurable enzymatic activity in vitro (86). The fact that phosphofructokinase uses pyrophosphate as the phosphate donor rather than ATP is of particular interest because this would conserve intracellular ATP and increase the ATP yield per molecule of glucose. Cleavage of fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate and their ultimate conversion to pyruvate proceeds by the action of the glycolytic pathway, as expected. tpi, pgk, and gapdh comprise a single operon in B. burgdorferi (bb055-057) and are transcribed from a single promoter; this type of genetic organization has also been observed in other bacteria (88). B. burgdorferi does not encode for a pyruvate dehydrogenase or pyruvate oxidase (17); as a result, the only disposition of pyruvate is conversion to lactate by lactate dehydrogenase. This results in regeneration of NAD+ that is required for continued glycolysis (Fig. 3). The potential role of NADH/NAD+ ratio in regulating metabolite flux through the glycolytic pathway is discussed later in this chapter.

Other Carbohydrate Utilization Pathways

Glucose is the preferred carbohydrate for ATP generation, as in most bacteria. In addition to glycolysis, glucose-6-P can also enter the oxidative branch of the pentose phosphate pathway through conversion to 6-phosphogluconolactone through the action of glucose 6-phosphate dehydrogenase. As noted, several additional carbohydrates can support B. burgdorferi growth in vitro. Mannose can be taken up via a mannose-specific PTS, and the resultant mannose-6-phosphate is converted to fructose-6-phosphate by the action of ManA (BB407) and can be directed into glycolysis (17) (Table 1).

B. burgdorferi requires GlcNAc to reach high density during in vitro cultivation, making its import and use an absolute requirement for growth (81, 89, 90). It is assumed that GlcNAc is taken up through a glucose-
specific PTS. It is used for peptidoglycan biosynthesis, but it can also be converted to fructose-6-phosphate through the combined actions of NagA (BB151) and NagB (BB152) and thereby used as a substrate for glycolysis. Chitobiose, a dimer of GlcNAc, is a constituent of the tick cuticle and peritrophic membrane (15, 91). It has been demonstrated that chitobiose can substitute for the GlcNAc requirement during in vitro growth (90, 92). Studies have shown that a chitobiose-specific PTS is encoded on plasmid cp26 (bbb04, bbb05, bbb06) (Fig. 2) and that ChbC mutants cannot use chitobiose to support in vitro growth (90). After uptake via the chitobiose-specific PTS, chitobiose-6-P is cleaved into GlcNAc and GlcNAc-6-P by chitobiase (BB002), and the monomers enter the glycolytic pathway as fructose-6-P. The disaccharides maltose and trehalose can also be used after hydrolysis to glucose monomers by either amylomaltase (MalQ; BB166) or trehalase (TreA; BB381) (Fig. 2).

In addition to monosaccharides and disaccharides, B. burgdorferi also contains a pathway for uptake and use of glycerol. The genes comprise an operon (bb240-bb243), which encodes an uptake facilitator (GlpF), glycerol kinase (GlpK), and glycerol-3-phosphate dehydrogenase (GlpD) (17). Once glycerol has entered into the cytosol, it is phosphorylated by GlpK to yield glycerol-3-phosphate; GlpD converts glycerol-3-phosphate to dihydroxyacetone phosphate, which can enter the glycolytic pathway after conversion to glyceraldehyde 3-phosphate (81) (Fig. 2).

**Carbohydrate Transporters**

Phosphotransferase systems (PTS or PEP-PTS) simultaneously import and phosphorylate a sugar substrate by coupling phosphorelay of a phosphoryl group from phosphoenolpyruvate (PEP) to carbohydrate transport (93). PTS are composed of a number of proteins referred to as enzyme I (EI), enzyme II (EII), and histidine phosphocarrier protein (HPr). EII is composed of two cytoplasmic domains (EIIA, EIIB) and a transmembrane domain (EIIC). These domains can be located on a single polypeptide or separate protein molecules (94). PEP transfers a phosphoryl group to EI, which in turn transfers it to HPr and, ultimately, the phosphate is transferred to the sugar concomitant with its EIIC-mediated uptake (94, 95). Specificity of these systems is defined by the EII components downstream of the initial phosphorelay among PEP, EI, and HPr (93, 94).

Based on genome sequence, B. burgdorferi contains the complete PTS machinery (17) (Table 1). EI is encoded by bb558 and, interestingly, two genes are annotated as encoding HPr (bb557, bb448). It is important to note, however, that very few of the PTS components have been definitively shown to function in their putative roles by direct biochemical or genetic studies. A schematic diagram showing the specific carbohydrate transporters is presented in Fig. 2. Glucose import is mediated by the glucose-specific EII proteins (BB559, BB645, BB116, and BBB29); by homology, BB559 (EIIA) and BB645 (EIIBC) are annotated as glucose-specific, whereas BB116 and BBB29 (EIIBC) are annotated as maltose-specific.

Glucose could be transported into the cytosol by a putative ABC transporter MglAC1C2 (BB677-79) (17, 32, 97). This operon was initially annotated to encode a ribose/galactose transporter. It is unlikely that either sugar is used for ATP production, because neither can support spirochetal growth in vitro (81). B. burgdorferi membranes contain mongalactosyl diacylglycerol (98), and its synthesis would require a source of galactose. If galactose were imported through the MglAC1C2 transporter, a uridylyltransferase would be required for synthesis of UDP-galactose; such an activity has not been identified in the B. burgdorferi genome. Alternatively, UDP-galactose could be produced from UDP-glucose by chitobiose transport and is induced during the tick phase of the enzootic cycle (12, 90, 92). Although the genes for the three EII components are adjacent to cp26, the gene encoding the EIIC component, chbC, is divergently transcribed from those for chbA (bbb05) and chbB (bbb06); these latter genes are apparently not differentially expressed in ticks (92).

**FIGURE 3** The glycolytic pathway and control of glycolytic flux during the enzootic cycle. doi:10.1128/microbiolspec.MBP-0011-2014.f3
an epimerase (BB444) (32). If ribose were imported via this ABC transporter, it could be used for nucleotide biosynthesis. The route from ribose to ribose-5-phosphate is unclear, but bb545 is annotated as a xylulokinase, and it has been suggested that this enzyme could convert ribose to ribulose-5-phosphate and then ribose 5-phosphate through the action of ribose phosphate isomerase (BB657) (32). Ribose-5-phosphate could also be produced from glucose-6-phosphate via the oxidative branch of the pentose-phosphate pathway. Taken together, it seems unlikely that the MglAC1C2 transporter is used for galactose or ribose uptake; rather, it could serve as an additional route for glucose transport. 

*B. burgdorferi* encodes one carbon-specific major facilitator superfamily protein, GlpF (17). GlpF is a member of a family of conserved aquaglyceroporins that mediate diffusion of glycerol into the cytosol (99, 100). Interestingly, expression of the *glp* operon (bb240-243) is significantly induced during the tick phase of the spirochete enzootic cycle, and glycerol use is vital for maximal fitness of the spirochetes in the tick vector (12).

### NAD⁺/NADH BALANCE

The balance between NAD⁺ and NADH is an indicator of the intracellular redox state for both eukaryotic and prokaryotic cells, and NAD⁺ is a required cofactor for many cellular enzymes (101, 102). During glycolysis, NAD⁺ is reduced to NADH by GAPDH, and the cell must regenerate NAD⁺ to maintain a balanced redox state and allow glycolysis to continue (Fig. 3). NAD⁺ pools can be replenished by biosynthesis or oxidative metabolism of pyruvate. Many bacteria encode biosynthetic pathways that use tryptophan or aspartic acid to generate NAD⁺ (101, 102). *B. burgdorferi* does not encode this biosynthetic capacity and cannot metabolize pyruvate oxidatively (17). Instead, it must depend on alternative strategies to replenish NAD⁺. As noted, the only metabolic fate for pyruvate is its conversion to lactate by pyruvate dehydrogenase. During lactogenesis, NADH is oxidized, producing NAD⁺ that can be recycled back into the glycolytic pathway (103), which directly couples NAD⁺ regeneration to glycolysis.

In an oxidative environment, organisms must deal with the presence of reactive oxygen species. Among the consequences is formation of the disulphide form of coenzyme A (CoA). To deal with this, *B. burgdorferi* produces a CoA disulphide reductase (CoADR) (encoded by bb728) that uses NADH exclusively as a cofactor, producing reduced CoA and regenerating NAD⁺ (104, 105). A *B. burgdorferi* CoADR mutant was avirulent in mice and had reduced survival in feeding nymphs, suggesting an important role in maintaining an optimal redox state in the spirochete (105).

Microbes contain pathways for nicotinamide salvage that can be employed for NAD⁺ production (102, 106). *B. burgdorferi* pncA (bbe22) encodes a functional nicotinamidase that can complement *E. coli* and *S. typhimurium* pncA deletion strains (17, 36). *B. burgdorferi* pncA is absolutely required for both mammalian infection and persistence within the tick vector, suggesting the importance of this pathway for maintenance of the intracellular NAD⁺ pool in *B. burgdorferi* (24, 36). Although a nicotinamide uptake system has not yet been identified in *B. burgdorferi*, enzymes that would mediate the stepwise conversion of nicotinamide to NAD⁺ (pncB [bb635], nadD [bb782], nadE [bb311]) are apparently encoded in the *B. burgdorferi* genome (17).

### REGULATION OF GLYCEROL AND CHITOBIOSE UTILIZATION

Numerous global analyses of the wild-type *B. burgdorferi* transcriptome during *in vitro* growth in a variety of environmental conditions (e.g., temperature, pH, redox state) or in a mammalian host-adapted state have been reported. Similar studies have been performed to identify members of the RpoS, Rrp1, Rrp2, and BosR regulons (reviewed in 48, 107). A comprehensive survey of these studies revealed no definitive transcriptional control of carbohydrate uptake and utilization genes/pathways except for those involving glycerol and chitobiose (Fig. 4). Perhaps this can best be understood with the reasonable assumption that glucose is the preferred carbohydrate throughout the enzootic cycle and therefore constitutively used except for certain tick stages during which glucose availability would be limited.

### Glycerol Uptake and Utilization

Glycerol is a readily available carbohydrate in the tick vector and is produced by *Ixodes* spp. to serve as an antifreeze during overwintering (108, 109). The *B. burgdorferi* *glp* operon encodes the capacity for uptake of glycerol and its conversion to dihydroxyacetone phosphate (see above). Early global transcriptome studies showed that the *glp* operon is expressed at higher levels in cells grown at 23°C than at 35°C, suggesting a potential role in the vector (110). This was definitively confirmed by Pappas et al, who reported that *glpF* and *glpD* transcripts are substantially elevated during all tick stages compared with mouse joints (12). The *glp* operon is subject to RpoS-mediated repression (47), and
its expression is induced by Rrp1 (71, 78). The inability of Rrp1 mutants to survive in ticks was partially restored by complementation with the glp operon (78). Although a GlpD mutant could complete the experimental mouse-tick-mouse infection cycle, it had a fitness defect in ticks that was manifested in impaired growth and lower spirochete numbers in fed nymphs (12). Taken together, the findings demonstrate that the ability to use glycerol as a nutrient during the tick phase is critical and that modulation of glp operon expression is dependent, at least in part, on reciprocal regulation by RpoS and Rrp1.

**Chitobiose Uptake and Utilization**

Chitin, a polymer composed of chitobiose (glucosamine or GlcNAc dimer) units, is the primary component of the tick exoskeleton and the peritrophic membrane (15, 91). Chitobiose can substitute for GlcNAc, an essential requirement for *in vitro* cultivation, and can serve as the principal carbon source *in vitro* (81, 90). As described earlier, chitobiose can be taken up via a dedicated PTS, ChbABC, cleaved to GlcNAc and GlcNAc-6-P, and enter glycolysis after conversion to fructose-6-P. *chbC* is highly expressed during all tick stages and has little expression in mouse joints (12), an expected finding given that chitobiose is likely available as a nutrient only in the vector. However, the transcriptional regulators responsible for this differential expression are a matter of controversy. Rhodes et al suggested an RpoS involvement in *chbC* expression (111), a finding at odds with the global transcriptome analysis of an RpoS mutant that found no effect on *chbC* expression (47).

Recently, Sze et al reported that *chbC* transcript levels were significantly repressed in an Rrp1 mutant (112), but in this case as well, previous global transcriptome analyses of Rrp1 mutants did not reveal any differences in *chbC* expression (71, 78). Curiously, Sze et al suggested that *chbC* regulation by Rrp1 is mediated through BosR and RpoS, because expression of these transcriptional regulators was also repressed in their Rrp1 mutant. However, it must be noted that *chbC* expression is not altered in a BosR mutant, and a BosR binding site has not been identified upstream of *chbC* (65, 67, 113). BadR has also been implicated as a repressor of *chbC* expression. Based on current understanding of the roles for RpoS and Rrp1 (the former being responsible for the mammalian phase regulon and the latter a tick phase regulon) and the likelihood that chitobiose would only be available to the spirochete in the vector, it seems reasonable to conclude that *chbC* expression is regulated primarily by Rrp1 (and c-di-GMP), which functions during the vector phase of the enzootic cycle. Further studies to more clearly define the regulation of chitobiose uptake are warranted.

**FIGURE 4** Schematic diagram depicting reported regulatory circuits controlling glycerol and chitobiose utilization. Solid lines indicate interactions confirmed by *in vivo* studies; dashed lines indicate interactions observed *in vitro* only. Diagram is a summary of data from references 43, 47, 50, 54, 60, 71, 72, 78, 111, and 112. doi:10.1128/microbiolspec.MBP-0011-2014.f4
CONTROL OF CARBOHYDRATE UPTAKE AND GLYCOLYTIC FLUX DURING THE ENZOOTIC CYCLE

When multiple nutrient sources are available, bacteria initially use the preferred carbon source, typically glucose, and in an effort to conserve energy, also repress genes associated with the use of alternate nutrient sources; this process is referred to as carbon catabolite repression \((95, 114)\). Unlike free-living bacteria, \(B. burgdorferi\) is restricted during its enzootic cycle to using only nutrients found in the tick vector or the mammalian host. Concentrations of glucose and glycerol in mouse plasma are about 150 and 2.8 mg/100 mL, respectively \((115)\), and glycerol is abundantly present during all tick stages \((109)\). It may be assumed that \(B. burgdorferi\) uses glucose while in the mammal and at early stages of tick feeding. At later feeding stages or after cessation of vector feeding, glucose is depleted due to its uptake by tick midgut epithelial cells. This triggers a switch to glycerol utilization that is mediated by elevated levels of glycerol pathway proteins. Pappas et al proposed that this switch in carbohydrate utilization may represent the \(B. burgdorferi\) version of carbon catabolite repression but did not provide a mechanism for this process \((12)\).

\(B. burgdorferi\) carbohydrate metabolism has several unusual features \((Fig. 3)\). Among them are the sole reliance on glycolysis for ATP generation, a phosphofructokinase \((PFK)\) that uses pyrophosphate as the donor for conversion of fructose-6-P to fructose-1,6-bisphosphate, the inability to use pyruvate as an energy source, and the presence of lactate permease \((\text{LctP}; \text{BB604})\). The rate-determining step of glycolysis in most eubacteria and eukaryotes is catalyzed by PFK, an allosteric enzyme whose activity is typically controlled by the ADP/ATP ratio \((116)\). \(B. burgdorferi\) contains genes for two PFK enzymes \((\text{BB020}, \text{BB727})\). Interestingly, purified BB020 exists as a dimer in solution and, in comparison with other pyrophosphate-dependent PFKs \((\text{e.g., Treponema pallidum TP0542})\), is likely to be nonallosteric \((86, 87, 117)\). If PFK does not sense the ADP/ATP ratio, how is the flux of glycolytic intermediates through the pathway regulated in \(B. burgdorferi\)?

The end product of glycolysis is pyruvate, and continued function of the pathway requires regeneration of \(\text{NAD}^+\). In \(B. burgdorferi\), this can only be accomplished by conversion of pyruvate to lactate, and this reaction is the only metabolic fate for pyruvate, because the spirochete lacks pyruvate dehydrogenase \((\text{and a TCA cycle})\), pyruvate-formate lyase, or lactate oxidase \((17)\). Extracellular lactate could be re-acquired through the action of lactate permease, likely functioning as a lactate: \(\text{H}^+\) symporter \((118)\) \((\text{Fig. 3})\); interestingly, \(\text{lctP}\) transcription is significantly induced in feeding ticks \((\text{Schwartz et al.}, \text{unpublished observations})\). Typically, the acquired lactate would be used as an energy source by stepwise conversion to pyruvate \((\text{by lactate dehydrogenase [LDH]}\) and acetyl-CoA \((\text{via pyruvate dehydrogenase})\) and entry into the TCA cycle. However, the acquired lactate cannot be used in this manner in \(B. burgdorferi\), because pyruvate cannot be converted to any other metabolic intermediate. In addition, conversion of lactate to pyruvate by lactate dehydrogenase would generate \(\text{NADH}\), altering the \(\text{NAD}^+\)/\(\text{NADH}\) ratio in a manner unfavorable for continued glycolysis.

Taken together, these findings suggest a model for control of the glycolytic pathway in \(B. burgdorferi\), particularly during the tick phases of the enzootic cycle, by the intracellular pyruvate/lactate ratio \((\text{Fig. 3})\). When \(B. burgdorferi\) grows in an environment with abundant glucose \((\text{e.g., a mammal})\), the sugar would enter the glycolytic pathway by uptake through its PTS and would ultimately be converted to lactate and excreted. In an open system \((\text{mammalian tissue})\), excreted lactate would diffuse away, and glycolysis would continue to function normally as long as sufficient glucose is available. During tick feeding, glucose would also be available to \(B. burgdorferi\), and glycolysis would proceed initially as in the mammal. Furthermore, this metabolic state enables a process referred to as inducer exclusion, whereby phosphorylated \(\text{EIIAGlc}\) interacts directly with \(\text{GlpK}\) and prevents conversion of available glycerol to glycerol 3-phosphate \((119)\) \((\text{Fig. 3})\). At later stages of feeding, glucose becomes limiting \((\text{as a result of uptake by tick midgut epithelial cells})\). In addition, excreted lactate would accumulate in the tick midgut because this is essentially a physically closed environment, and \(\text{LctP}\) would be induced. As a result, \(B. burgdorferi\) would take up lactate, which would be converted to pyruvate by LDH. Elevated pyruvate levels would alter the PEP/pyruvate ratio, a key factor in controlling glucose uptake through its cognate PTS.

In \(E. coli\), when the PEP/pyruvate ratio is low \((\text{i.e., when pyruvate is high})\), the \(\text{EIIA}\) component is dephosphorylated and the import of glucose as glucose-6-P by action of the PTS is blocked; indeed, the PEP/pyruvate ratio is directly correlated with the phosphorylation state of \(\text{EIIA}^{\text{Glc}}\) \((119–121)\). Furthermore, \(\text{GlpK}\)-mediated conversion of glycerol to glycerol-3-phosphate would resume. Under these circumstances, glycerol would become an important carbohydrate source for continued energy generation via glycolysis. The significant increase in \(\text{glp}\) operon expression during the tick feeding stages...
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is consistent with this model. A second consequence of lactate uptake and conversion to pyruvate is the generation of an unfavorable NAD+/NADH ratio. This problem could be solved by the action of CoADR (see above), which would regenerate NAD+ and facilitate continued glycolysis (104, 105).

The precise details of the mechanism by which B. burgdorferi senses the levels of extracellular lactate, glucose, and glycerol and transmits signals to the transcription machinery remain elusive. The Rrp2/RpoN/RpoS and Hk1/Rrp1 (c-di-GMP) circuits regulate transcription of genes during the mammalian and vector phases of the enzootic cycle. The involvement of these transcriptional regulators in modulating the expression of genes whose products facilitate transport and utilization of the alternative carbon sources glycerol and citrate argues that they play an important role in these processes. Elucidation of the interactions between these two regulatory systems would help clarify how B. burgdorferi can alternate among different metabolic states in order to survive the drastically different environments encountered during its lifecycle.

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REFERENCES


