Tick-pathogen interactions: The metabolic perspective

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Abstract

The first tick genome published in 2016 provided an invaluable tool to study the molecular basis of tick-pathogen interactions. Metabolism is a key element in host-pathogen interactions. However, our knowledge of tick-pathogen metabolic interactions is very limited. Recently, a Systems Biology approach using omics datasets has revealed that tick-borne pathogen infection induces transcriptional reprograming affecting several metabolic pathways in ticks to facilitate infection, multiplication and transmission. Results suggest that the response of tick cells to tick-borne pathogens is associated with tolerance to infection. Here we review our current understanding of tick metabolism modulation by tick-borne pathogens with focus in the model intracellular bacterium *Anaplasma phagocytophilum*.

Keywords: *Ixodes*; *Anaplasma phagocytophilum*; *Borrelia* spp.; metabolism; Tick-pathogen interactions

Tick-pathogen metabolic interactions at the center of the infectious storm

Metabolism is key to cellular function [1,2]. In consequence, all major molecular pathways in living cells are interconnected to and regulated by the availability and levels of metabolites [1,2]. Recent studies in mammalian cells showed that interactions between intracellular bacterial pathogens and the host cells can lead to physiological changes in both interacting members [3]. The metabolic adaptations in mammalian cell-pathogen systems promote proliferation or elimination of the pathogen within the host cells [3]. Tick-pathogen interactions, however, involve a more complex array of outcomes which include conflict and cooperation that ultimately benefit both ticks and pathogens [4]. This is not surprising considering that in vector-borne pathogen systems the survival of the vector is essential for the completion of the life cycle of the pathogen. In addition, tick-borne pathogen infection increases tick performance in challenging environmental conditions [5,6]. Therefore, in some cases, vector tolerance to pathogen infection is an advantageous life trait in arthropod vectors [7].

Tick metabolism modulation by tick-borne pathogens is a result of coevolution and adaption to a considerable number of tick and reservoir host species [4,8,9]. The *Ixodes scapularis* genome, the first for a medically important chelicerate, is the only tick genome available so far [10]. The sequencing of the *I. scapularis* genome was an essential step towards the understanding of the molecular processes that support the parasitic lifestyle of the tick and its success as a vector of multiple pathogens including bacteria, viruses, protozoa, and helminths which constitute a growing burden for human and animal health worldwide [11]. This review focuses on recent research that provided insights on the finely tuned metabolic changes that the model pathogen *Anaplasma phagocytophilum* induces in its tick vector *I. scapularis*. For comparative purposes, tick metabolic changes induced by other pathogens including mainly *Borrelia* spp., flaviviruses and fungi will be also included (Table 1). Results suggest that tick-borne pathogens use tick metabolism as a hub to modulate tick physiology and tick cell processes including immunity and apoptosis among others.

Nutritional immunity and iron metabolism in ticks

Iron is essential for most organisms because it serves as an electron donor and acceptor in various metabolic processes. Therefore, iron holds a central position in host-pathogen interactions [12]. While host evolved mechanisms to deprive pathogens from iron, pathogens coevolved mechanisms to acquire host iron. Host mechanisms involved in limiting pathogens access to iron were termed 'nutritional immunity' [12]. During feeding, ticks are exposed to the large amounts of iron present in the blood meal. Excess iron can react with H_2O_2 which yields hydroxyl radical, a potent biological oxidant [12]. Therefore, a balanced regulation of iron levels is essential for ticks [13], and potentially, pathogen survival. Two ferritin (FER) proteins, FER1 and FER2, are the primary iron storage and transporter, respectively, in ticks [13]. In addition of controlling the production of reactive oxygen species from iron, like in other model organism [12, 13], ferritins may also contribute to nutritional immunity in ticks by depriving pathogens from essential iron supply. Interestingly, *Escherichia coli* infection induces the expression of a *fer* gene in *Dermacentor variabilis* and silencing of *fer* genes in *Haemaphysalis longicornis* decreases tick survival following a challenge with *E. coli* [13].

The tick-borne pathogen *Ehrlichia ruminantium* sense iron levels in mammalian cells and respond to iron starvation by upregulating virulence genes such as those involved in Type IV Secretion System (T4SS), which in turn may participate in iron acquisition by the pathogen [14]. A similar mechanism may be at play during pathogen infection in ticks. An iron-binding protein expressed in the infective stage of *A. phagocytophilum* [15], and hypothesized to be secreted by the T4SS, was found in tick salivary glands and tick cells infected with these bacteria [16]. Bacterial iron-binding proteins may sequester iron to overcome nutritional immunity in ticks. An interesting mechanism to scape nutritional immunity is that of *Borrelia burgdorferi* which requires none or very low amounts of iron to survive [17]. In addition, recent research provides evidence that *A. phagocytophilum* modulates tick heme metabolism. Host hemoglobin is cleavage to heme by three sequential set of reactions involving several hemoglobinolytic enzymes in ticks [18]. Due to the high redox potential of heme, this molecule is toxic and in excess can be bacteriostatic [19]. Quantitative proteomics analysis revealed that *A. phagocytophilum* modulates the levels of hemoglobinolytic enzymes which affects the levels of host hemoglobin in tick midguts and salivary glands [18].

This finding suggests that *A. phagocytophilum* infection may regulate the levels of heme in a tissue-specific manner with potential effects for pathogen and vector survival.

Tick hemocyte metabolism in response to tick-borne pathogen infection

Despite mammalian macrophages possess specialized effector functions to eliminate invading microorganism, many intracellular bacteria infect and replicate inside this immune cells [20]. Globally, macrophages can be divided into inflammatory (M1) and anti-inflammatory (M2) macrophages [20]. Both macrophage types M1 and M2 can be infected by intracellular bacteria; however, the metabolic profiles of these macrophages in response to pathogenic stimulus are substantially different [20]. After stimulation with lipopolysaccharide (LPS), macrophages M1 uptake more glucose via **glucose transporters** (GLUTs, see Glossary) and increase the glycolytic flux and the production of lactate, a process named **aerobic glycolysis**. In contrast, macrophages M2 have the metabolic profile comparable to that of unstimulated cells, but with higher TCA-cycle and oxidative activity [20].

Cellular immunity in ticks is mostly mediated by hemocytes, which are involved in the phagocytosis of different microbes [21]. After establishing a primary infection in tick midguts, *A. phagocytophilum* migrates to the salivary glands [11]. A secreted tick protein, P11, enables *A. phagocytophilum* to infect tick hemocytes, which are required for the migration of *A. phagocytophilum* from the gut to the salivary glands [22]. Isolation and *in vitro* culture of tick hemocytes has remained elusive. Therefore, the study of tick hemocytes metabolism in response to *A. phagocytophilum* infection has been based on the *I. scapularis* ISE6 cell line. Based on the transcriptional response to *A. phagocytophilum*, ISE6 cells were proposed to constitute a model of tick hemocytes involved in pathogen infection and immune response [23,24]. Comparison of the metabolic profiles of macrophages M1 with that of *A. phagocytophilum*-infected ISE6 cells suggests that the metabolic profile of tick hemocytes in response to pathogen infection is very different to that of inflammatory macrophages M1 (Fig. 1, Key Figure). Two major differences can be summarized: (i) ISE6 cells infected by *A. phagocytophilum* do not display the aerobic glycolysis observed in mammalian

macrophages M1 and (ii) **hypoxia-inducible factor 1-alpha (HIF-1*α*)** activation and stabilization in ISE6 cells is not dependent of succinate accumulation and aerobic glycolysis (Fig. 1). This suggests that the metabolic reprogramming associated with macrophage M1 activation does not occurs in tick hemocytes infected with *A. phagocytophilum*. Interestingly, while 2-deoxyglucose (2DG), an inhibitor of **glycolysis**, inhibits HIF-1α activation in macrophages M1 [25], this compound has no effect on HIF-1α activation in ISE6 cells [26]. Instead of aerobic glycolysis and succinate accumulation, HIF-1α activity in *A. phagocytophilum*-infected ISE6 cells appears to be mediated phosphoinositide 3-kinase (PI3K) pathway activation (Fig. 1). In agreement with this, the PI3K inhibitor LY294002 decreased HIF-1α DNA binding activity in infected ISE6 cells [26]. A metabolic property shared by macrophages M1 and ISE6 cells is, however, an overall decrease in **tricarboxylic acid cycle (TCA cycle)** activity upon bacterial infection [25,26].

The absence of immunogenic molecules such as LPS and diaminopimelic-type peptidoglycans (DAP-PGN) in *A. phagocytophilum* and other bacteria transmitted by *I. scapularis* was previously suggested to contribute to vector tolerance to tick-borne pathogens [7]. The lack of these immune activators in tick-borne pathogens may help these microbes colonize ticks without being eliminated [7]. Interestingly, lipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl diacylglycerol (PODAG) found in *A. phagocytophilum* activate the immune deficiency (IMD) pathway in ticks [27]. Immune priming with POPG and PODAG reduces the *A. phagocytophilum* burden in infected ISE6 cells [27]. This shows that tolerance to infection can be associated with immunity and resistance to tick-borne pathogens in ticks. This is expected considering that unchecked bacterial multiplication within host cells could affect vector, and therefore, tick-borne pathogen survival. Remarkably, recent research shows that IMD is involved in glucose and lipid metabolism regulation in *Drosophila* [28] and constitutive activation of IMD induces a significant reduction in the expression of genes involved in glycolysis, **gluconeogenesis** and TCA cycle in flies [29]. Whether IMD activation by *A. phagocytophilum* regulates tick hemocyte metabolism remains to be tested.

Molecular mechanisms involved in tick metabolism modulation by tick-borne pathogens

Tick metabolism modulation by tick-borne pathogens results mainly from the regulation of the levels of metabolic enzymes. Although it remains a challenge to explain how pathogen infection modify the level of thousands of tick genes and hundreds of tick proteins [30], the molecular mechanisms behind this have been partially characterized using the model bacteria *A. phagocytophilum*. To induce transcriptional reprograming in infected cells, *A. phagocytophilum* hijacks the transcriptional regulatory machinery of ticks by inducing the activation of signaling pathways [31] and the recruitment of transcription factors [32], metabolites [33], and histone modifying enzymes [34] (Fig. 2). *A. phagocytophilum* may also induce the degradation of tick proteins by tick proteasome which in turn decreases the levels of targeted metabolic enzymes [23]. In addition, *A. phagocytophilum* infection induces a high proportion of alternatively spliced transcript events (ASEs), which increases the predicted gene ontology processes related to lipids, lipoproteins, and cholesterol/sterol metabolism and other processes in human HL-60 cells infected by this pathogen [35]. The occurrence of ASEs has not been tested in *A. phagocytophilum*-infected ticks or tick cell lines yet. However, there is no reason to rule out the hypothesis that ASEs also occur in ticks in response to *A. phagocytophilum* infection.

Activation of tick signaling pathways by *A. phagocytophilum* involves G-protein-coupled-receptor (GPCR) that stimulates *Ixodes* p21-activated kinase (IPAK1) through the activation of PI3K which results in actin-mediated signaling [31]. Activation of IPAK1 results in actin phosphorylation and accumulation of actin in the nucleus which mediates binding of RNA polymerase II (RNAPII) and TATA box-binding protein (TBP) to the promoter of the gene *salp16* inducing its expression [31,36]. PI3K activation was also suggested to induce the expression of the genes *hif-1α*, *hif-1β* and acetyltransferase p300 upon *A. phagocytophilum* infection in tick cells [26] (Fig. 2). Mobilization of HIF-1α, HIF-1β and p300 at HIF target genes may induce HIF-mediated transcriptional activation of glycolytic genes [26].

Among the transcription factors (TFs) activated by *A. phagocytophilum* infection is the tick transcriptional activator protein-1 (AP-1) which induces the expression of the genes *iafgp* (*I. scapularis* antifreeze glycoprotein) and *kat* (kynurenine aminotransferase) in infected cells [32]. KAT is an enzyme involved in the production of the tryptophan metabolite xanthurenic acid [33]. Results suggests that xanthurenic acid acts as a co-factor of an uncharacterized TF

that regulates the expression of one organic anion transporting polypeptide (*oatp*) gene in *A. phagocytophilum*-infected ISE6 cells [33]. The upregulation of *oatp* and *kat* was proposed to be associated with increased levels of intracellular xanthurenic acid, which in turn favors *A. phagocytophilum* replication in tick salivary glands [33]. Conversely, RNAi-mediated silencing of *oatp* and *kat* expression decrease *A. phagocytophilum* levels in tick cells [33]. A general mechanism is then proposed by which *A. phagocytophilum* nucleomodulins recruit tick metabolites and TFs to activate target genes (Fig. 2).

The capacity of *A. phagocytophilum* to downregulate gene expression in mammalian neutrophils was associated with host histone deacetylase 1 (HDAC1) recruitment to the promoters of target genes by the bacterial nucleomodulin ankyrin repeat protein AnkA [37]. Upon *A. phagocytophilum* infection, AnkA is secreted through T4SS, enters the granulocyte nucleus, binds stretches of AT-rich DNA and recruits host HDAC1 to its binding sites. *A. phagocytophilum* infection also induces HDAC1 binding and deacetylation of histone H3 (H3) which in turn results in downregulation of many host defense genes including *CYBB*, which encodes a component (i.e. NOX2) of the NADPH oxidase [37]. Downregulation of *CYBB* reduces superoxide anion production by NADPH oxidase which is essential for *A. phagocytophilum* survival in neutrophils [37]. Homologs of mammalian HDAC1 and H3 were identified in the *I. scapularis* genome [34]. Whether *A. phagocytophilum* AnkA is secreted and recruits HDAC1 to regulate gene transcription in infected ticks is currently unknown. However, *Ixodes* HDAC1 is overrepresented in *A. phagocytophilum*-infected tick salivary glands and chemical inhibition of this protein decreases *A. phagocytophilum* burden in tick cells [34]. This shows that the expression and activity of HDAC1 is critical for *A. phagocytophilum* survival in both mammalian and tick cells. Notably, in mammalian cells, HDAC1 controls the expression of the metabolic enzyme fructose-1,6-bisphosphatase (FBP), a rate-limiting enzyme in gluconeogenesis [38]. Interestingly, high HDAC1 protein levels [34] were associated with low FBP protein levels in *A. phagocytophilum*-infected tick salivary glands [26]. Conversely, low HDAC1 protein levels [34] correlated with high mRNA and protein levels of *fbp* and FBP, respectively, in *A. phagocytophilum*-infected tick midguts [26]. The negative correlation between the protein levels of HDAC1 and that of *fbp* and FBP strongly suggests that HDAC1 may be recruited by *A. phagocytophilum* nucleomodulins to act as a repressor of *fbp* expression in tick salivary glands (Fig. 2). A decrease in FBP protein

levels may contribute to the inhibition of gluconeogenesis that *A. phagocytophilum* induces in infected tick cells (see below) [23].

Tissue-specific changes of tick carbohydrate metabolism in response to infection

Glucose is the most abundant carbohydrate in the hemolymph of adult ticks and it is required in tick embryonic development [39]. The regulation of glucose metabolic pathways in ticks is essential for *A. phagocytophilum* survival in the vector [23,26]. Notably, *A. phagocytophilum* infection inhibits gluconeogenesis by decreasing the protein levels of **phosphoenolpyruvate carboxykinase**-cytosolic (PEPCK-C) which results in lower levels of glucose in *A. phagocytophilum*-infected ISE6 cells [23,26]. PEPCK-C is also a key regulatory enzyme in **glyceroneogenesis** [40] and thus, low levels of PEPCK-C were associated with reduced glycerol 3-phosphate production in infected tick cells [41]. Low PEPCK-C activity and decreased glucose anabolism inhibits apoptosis which is essential for *A. phagocytophilum* survival in tick cells and tissues [23,30]. Interestingly, glycerol 3-phosphate levels were also reduced in tick nymphs infected by *Borrelia mayonii* (Table 1). Low levels of glycerol 3-phosphate may have a significant impact in fatty acids metabolism, a poorly explored facet in tick-pathogen interactions.

Tissue-specific differential regulation of apoptosis pathways was observed in adult female midguts and salivary glands in response to *A. phagocytophilum* infection [42]. Likewise, the transcriptional reprograming induced by *A. phagocytophilum* affects the levels of carbohydrate metabolism genes and proteins in a tissue-specific manner [26]. For example, the protein levels of all glycolytic enzymes increase in *A. phagocytophilum*-infected tick midguts, except for the cofactor-independent phosphoglycerate mutase (iPGM) and pyruvate kinase (PK) that despite gene up-regulation, the proteins were under-represented in this tissue [26]. In addition, gene and protein levels of phosphofructokinase (PFK) did not change in tick midguts in response to infection [26]. However, in salivary glands, the glycolysis enzymes fructose-bisphosphate aldolase A (ALDA), PFK and hexokinase (HXK) were under-represented while the rest of the glycolytic enzymes were over-represented. To accurately assess the metabolic impact of the differential protein representation in tick

midguts and salivary glands, a tissue-specific metabolomics approach is required. However, the lower levels of PK and HXK, key regulatory enzymes in glycolysis, in infected tick midguts and salivary glands, respectively, suggest that the production of glycolytic intermediates may be affected in a tissue-specific manner. It would be also important to assess the enzymatic activity of key enzymes which may vary in *A. phagocytophilum*-infected cells and tissues. For example, it was reported that the activity of HXK decreases more than two-folds in *A. phagocytophilum*-infected ISE6 cells [43]. The transcriptional activation of tick glycolytic genes was proposed to be mediated by tick HIF-1α [26], which contains structural domains conserved in model organisms and the gene *hif-1α* was significantly upregulated in infected tick midguts [26]. Another important gene targeted by HIF-1α is pyruvate dehydrogenase kinase 1 (*pdk1*) which encodes the protein PDK1 that controls the enzymatic activity of pyruvate dehydrogenase E1 (PDE1) which transforms pyruvate into **Acetyl-CoA**, the first step in the TCA cycle. PDK1 mRNA and protein levels were higher in *A. phagocytophilum*-infected tick salivary glands suggesting that this pathogen inhibits the TCA cycle in infected salivary glands [26].

Crosstalk between tick amino acid and carbohydrate metabolism during pathogen infection

Several recent studies have shown that amino acids are central to host-pathogen metabolic interaction [3,23,41,44]. When digested, the protein-rich diet of ticks generates large amounts of amino acids. Therefore, amino acid metabolism is of great interest in ticks. For example, tyrosine-associated toxicity is lethal for blood-sucking arthropods which points out the significance of tyrosine degradation pathways. Inhibition of tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD), the first two enzymes of the phenylalanine/tyrosine degradation pathway, caused the death of mosquitoes, kissing bugs and ticks after a blood meal [45].

Infection by *A. phagocytophilum* rewires the network of tick vector cell processes and changes the relative importance of some biological pathways including those involved in metabolism [46]. This is expected considering that *A. phagocytophilum* has a small genome

and cannot actively carry out several metabolic processes, and therefore requires and hijacks tick cell resources for survival within the vector [47]. For example, out of twenty amino acids, the *A. phagocytophilum* genome encodes only for the enzymes responsible of the biosynthesis of four amino acids (proline, glutamine, glycine and aspartate) and the glycolysis enzymes are reduced to those that produce glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (DHAP) from **phosphoenolpyruvate** (PEP) [47]. In consequence, *A. phagocytophilum* changes the levels of amino acid metabolism enzymes [41], which in turn affects the intracellular levels of several amino acids and metabolic intermediates [23,33]. Interestingly, tick cells use tyrosine as a fuel to synthetize PEP during early *A. phagocytophilum* infection [41]. This process is achieved by activating the tyrosine/**oxaloacetate** (OAA)/PEPCK-M/PEP node by which *A. phagocytophilum* infection may decrease the tyrosine pool, which in turn may protect ticks against tyrosine-induced toxicity *in vivo* [41].

Tick-borne flaviviruses are known to affect protein processing and amino acid metabolism in tick cells [48,49]. Particularly, infection by Langat virus (LGTV) increased protein levels of acetyl-CoA acetyltransferase 1 (ACAT1), aldehyde dehydrogenase (DP5CD), glutamate dehydrogenase (GLUD1) and fumarylacetoacetase (FAH) in ISE6 cells [49]. Knockdown of the gene *acat1* was associated with decreased LGTV genome replication and LGTV particle release, while knockdown of the gene *fah* decreased only LGTV particle release in ISE6 cells [48]. Interestingly, FAH catalyzes the last step in tyrosine degradation suggesting that flaviviruses infection may, similarly to *A. phagocytophilum*, contribute to tyrosine detoxification in ticks.

Tick lipid metabolism is exploited by different tick-associated microorganisms

Starvation and desiccating conditions that ticks have to endure during off-host periods affect the energy reserves of the tick [50]. For example, starvation of *Dermacentor variabilis* ticks in optimal humidity conditions is known to result in up to 60% losses in lipid reserves [50]. The parietal fat body, the main lipid reserve organ for triacylglycerol and cholesterol, is gaining

293 increasing interest for the study of infections in insects and ticks [51,52]. However, lipid
294 metabolism signaling pathways are still rarely studied in ticks.

295 Fatty acids play important roles as energy sources for several microorganisms including
296 pathogenic bacteria [53]. If a pathogen can prevent the use of energy reserves by the host,
297 this will affect its immune system, therefore promoting pathogen infection and persistence.
298 *B. burgdorferi* is not able to synthesize fatty acids and cholesterol, thus relying on the
299 vertebrate host cell or the blood meal of the tick for lipids supply [54]. Similarly, host
300 cholesterol and other lipids are required by *A. phagocytophilum* for infection and
301 multiplication in human cells [55]. Perilipin (PLIN), a major adipocyte lipid droplet-associated
302 phosphoprotein that plays a central role during lipolysis and cholesterol synthesis, is
303 differentially expressed in HL-60 cells in response to infection with *A. phagocytophilum* [56].
304 The bacteria are able to alter PLIN levels in infected cells, thus modifying the lipid
305 metabolism of the host to facilitate bacterial infection [56]. This phosphoprotein is likely to
306 play a similar role in the metabolism of the tick although its role has not been characterized
307 so far.

308 Other obligate intracellular pathogens such as viruses need to hijack the cellular machinery
309 for the synthesis of lipids to complete their replication. Flavivirus life cycle is very closely
310 associated to host cell lipids, and they can rearrange intracellular membranes and reorganize
311 the host cell lipid metabolism of infected cells to ensure viral replication [57]. Dengue virus
312 infection can alter metabolic pathways in humans such as fatty acid biosynthesis and **β-**
313 **oxidation** [58]. Exosomes are small extracellular vesicles that appear to play a central role in
314 cell-to-cell communication [58]. Exosomes transport bioactive molecules, such as messenger
315 RNA (mRNAs) and microRNA (miRNAs) that can be transferred in active form to adjacent
316 cells or to distant organs thus being important vehicles for metabolic cross-talk [59]. Recent
317 research has shown that Flaviviruses use arthropod-derived exosomes secreted by tick cells
318 as a mean for viral RNA and metabolite transmission from the vector, and the vertebrate
319 exosomes for dissemination within the host [60]. The knockdown of transcripts for a
320 glycosylphosphatidylinositol (GPI)-anchored protein (VNN) was associated with decreased
321 LGTV genome replication and LGTV release in ISE6 tick cells [48]. VNN is involved in
322 coenzyme-A (CoA) metabolism [61], which is critical for lipid metabolism.

Fungal infections can also modulate the lipid metabolism of the host [62]. Recent research with different isolates of *Metarhizium anisopliae* showed that infection of *Rhipicephalus microplus* ticks with entomopathogenic fungi can modulate the lipid content thus inducing changes in important metabolic pathways [62]. However, lipid metabolism modulation during *M. anisopliae* infection in ticks was independent of AMP-activated protein kinase (AMPK) and extracellular signal-regulated protein kinase (ERK) pathways [62] which are the major pathways involved in the breakdown of triacylglycerol into fatty acids in mammals [63, 64]. This suggested that tick lipid metabolism modulation by *M. anisopliae* occurs through alternative and uncharacterized lipolysis pathway.

Concluding Remarks

Interactions between ticks, hosts and pathogens involve many metabolic pathways such as carbohydrate, protein, lipid and redox that are affected to produce conflict and cooperation between them. These interactions at the metabolic level lead to mutual beneficial effects of the tick-hosts-pathogen molecular interactions. Among them, amino acids are central to the host-pathogen metabolic interaction. Nevertheless, synthesis and degradation of amino acids is narrowly connected to carbohydrate metabolism, and the later with the lipid metabolism.

Despite recent advances in the study of tick-host-pathogen molecular interactions, most of the pathways and molecules involved in these interactions are not fully characterized. Furthermore, the regulatory mechanisms affecting the metabolic pathways affected by these interactions are only partially characterized (see Outstanding Questions). Future directions should include the application of omics technologies together with data integration algorithms [46] to the investigation of tick interactome, regulome, miRNAome, epigenome and metabolome and their interactions to advance the metabolic perspective on tick-pathogen interactions.

The identification of the metabolic adaptations during tick-pathogen interactions that support tick survival, spread and pathogen infection and transmission could provide new therapeutic targets for the prevention and control of tick-borne diseases. Studies of the

interactions between the pathogen and the host lipid metabolism can help to identify potential targets for the development of vaccines or treatments against health threatening pathogens. These strategies could include the inhibition of lipid biosynthesis. However, manipulation of this major metabolic pathway can also be detrimental for the host. For instance, Lovastatin treatment, an inhibitor of cholesterol synthesis, appears to delay infection and increases survival rates in AG129 mice infected with Dengue Virus Serotype 2 while it also tends to increase viremia which could affect disease progression [65]. A better understanding of the interactions of pathogens with the host cell lipid metabolism and other metabolic pathways could provide new therapeutic targets to control infections by tick-borne pathogens. Furthermore, as recently proposed the application of machine learning and big data analysis to the tick-host-pathogen interactions datasets may also lead to the high throughput identification of candidate vaccine protective antigens [66].

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Glossary

Tricarboxylic acid cycle (TCA cycle): a central route for oxidative phosphorylation in cells. In the TCA cycle, acetyl-CoA is oxidized into carbon dioxide and reducing factors (NADH and $FADH_2$), that contribute to synthesis of ATP during oxidative phosphorylation, are produced. The TCA cycle is a crossroad of cellular metabolism in which carbohydrate, fatty acids and amino acid metabolisms are interconnected.

β-Oxidation: the major pathway for the degradation of fatty acids and is essential to produce energy from the generation of acetyl-CoA, NADH and $FADH_2$ when glucose supply is limiting.

Gluconeogenesis: a metabolic pathway that during a longer-term fast or starvation results in *de novo* glucose synthesis from non-carbohydrate carbon substrates, mainly lactate and glycerol.

Glyceroneogenesis: a metabolic pathway that is activated during glucose deprivation to synthesize glycerol 3-phosphate, a building block of triglyceride and fatty acid. In this pathway, glycerol 3-phosphate is synthetized from pyruvate, alanine, glutamine and TCA cycle intermediates.

Glycolysis: a major catabolic pathway in which one molecule of glucose is transformed into two molecules of pyruvate releasing in the process two net molecules of ATP and two of NADH. The expression of glycolytic genes is regulated by HIF-1α.

Aerobic glycolysis: A term frequently used to name a metabolic profile similar to that of the *Warburg effect* in which a cell, even under normoxic conditions, generates high amount of energy by using the glycolytic pathway, notably with high production of lactate.

Phosphoenolpyruvate carboxykinase (PEPCK): PEPCK catalyzes the first committed step in gluconeogenesis, in which OAA is converted into PEP. There are two isoforms of the enzyme: mitochondrial (PEPCK-M) and cytosolic (PEPCK-C). PEPCK-C is a key regulatory enzyme of glyceroneogenesis and gluconeogenesis.

Hypoxia-inducible factor 1-alpha (HIF-1α): a subunit of the heterodimeric transcription factor HIF-1 that together with HIF-1β regulates the cellular response to hypoxia by inducing the transcriptional activation of genes involve in glycolysis.

Acetyl-CoA: a central metabolic intermediate synthesized in the mitochondria. Acetyl-CoA is the molecule through which glycolytic pyruvate enters the TCA cycle to be oxidized for energy production. It can be derived from carbohydrates, fatty acids and amino acids.

Glucose transporters (GLUT): a wide group of membrane proteins that transport glucose across the cell membrane. The GLUT family is divided into three classes of which class I member GLUT1 is transcriptionally regulated by HIF-1α.

Oxaloacetate (OAA): a central metabolite produced in the TCA cycle that takes part in gluconeogenesis, amino acid metabolism and fatty acid synthesis among other pathways. In addition, OAA and PEP result from anaplerotic reactions that keep the metabolic flux of glycolysis and gluconeogenesis.

Phosphoenolpyruvate (PEP): a key metabolite that stands at the crossroad between gluconeogenesis, glycolysis and TCA cycle and can be produced by the activity of two enzymes enolase and PEPCK.

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583

584 **Figure legends**

585 **Figure 1. Metabolic profiles of tick hemocytes and mammalian pro-inflamatory**
586 **macrophages M1.** The figure displays the metabolic profile of *A. phagocytophilum*(bacteria
587 without LPS)-infected ISE6 cells, a model of tick hemocytes, compared to that of mammalian
588 macrophages M1 in response to other intracellular bacteria with LPS. High levels of
589 hexokinase (HXK, green) and phosphofructokinase (PFK, green) are associated with high
590 aerobic glycolysis and increased lactate production in mammalian macrophages M1 [25].
591 The levels and activity of tick HXK decreased (red), and the levels of tick PFK did not change
592 (black), in response to infection which was associated with lower levels of lactate in infected
593 ISE6 cells [23,26,43]. HIF-1α activation and stabilization (green), that enhances the
594 expression of glycolytic genes, is mediated by aerobic glycolysis and succinate accumulation
595 from glutamine in macrophages M1 [25]. In consequence, blocking aerobic glycolysis with 2-
596 deoxyglucose (2DG) inhibits HIF-1α activation in these macrophages [25]. In contrast, 2DG
597 does not inhibit HIF-1α activation (green) in ISE6 cells, an effect that is achieved by

LY294002, an inhibitor of the phosphoinositide 3-kinase (PI3K) pathway [26]. This suggests that HIF-1α activity in tick hemocytes infected with *A. phagocytophilum* is regulated by PI3K and not aerobic glycolysis. Macrophages M1 and ISE6 cells share a low TCA cycle activity [25, 26]. Vertical arrows represent decrease (red) and increase (green) in metabolite levels.

Figure 2. Molecular mechanisms potentially implicated in tick metabolism modulation by the model pathogen *A. phagocytophilum*. Molecular mechanisms proposed to participate in the transcriptional reprograming and proteome modulation induced by *A. phagocytophilum* infection in tick cells. Firstly, *A. phagocytophilum* activates the phosphoinositide 3-kinase (PI3K) signaling pathway in a G-protein-coupled-receptor (GPCR)-dependent manner to induce the expression of *salp16* [31], and hypoxia-inducible factor (HIF) *hif-1α* in ticks [26] (sequential steps and tick molecular factors colored in violet). Secondly, *A. phagocytophilum* recruits tick transcription factors (TF), such as transcriptional activator protein-1 (AP-1) [32] and metabolites, such as xanthurenic acid [33], to regulate the expression of the metabolic genes *oatp* [33] and *kat* [32] (sequential steps and tick molecular factors colored in blue). Other tick metabolic genes may be regulated by the same mechanism in which we propose that *A. phagocytophilum* uncharacterized nucleomodulins may participate in the recruitment of tick TFs. Thirdly, the recruitment of histone deacetylase 1 (HDAC1) to the promoters of target genes through the activity of nucleomodulins may contribute to the repression of tick genes, e.g. fructose-1,6-bisphosphatase (*fbp*) (sequential steps and tick molecular factors colored in green). Lastly, although the mechanism is unknown, *A. phagocytophilum* also targets tick proteins to the proteasome which in turn may result in the underrepresentation of targeted metabolic enzymes [23]. Abbreviations are as follow: *Ixodes* p21-activated kinase (IPAK1), RNA polymerase II (RNAPII), TATA box-binding protein (TBP), acetyltransferase p300 (p300). Closed and open chromatin were represented as red and green double-stranded DNA, respectively.

Table 1. Tick metabolic pathways affected by tick-borne pathogens

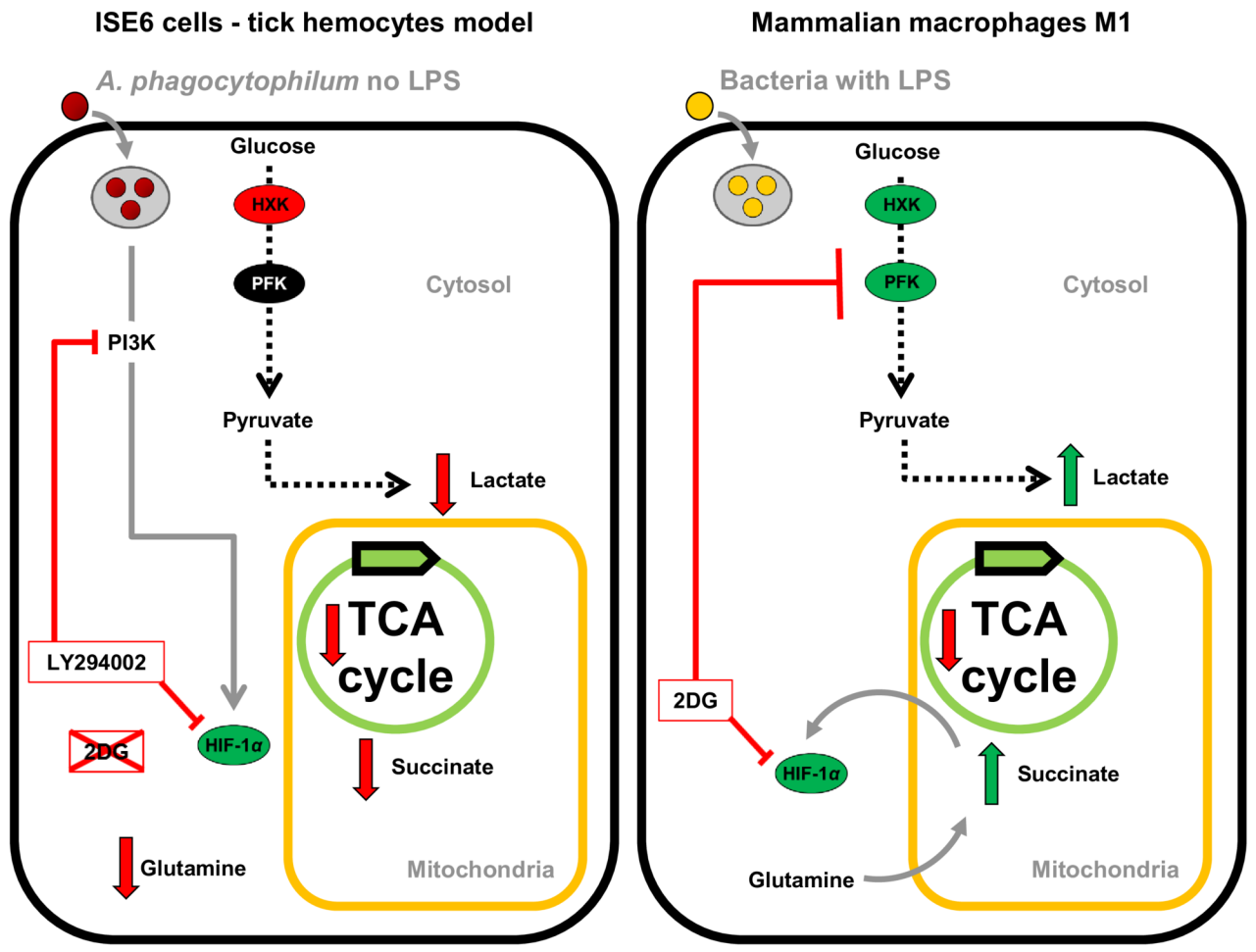
Metabolic pathways	Experimental system (*in vitro* vs. *in vivo*)	Affected by (name of pathogen species)	Effect on tick metabolism*	References
Glycolysis	ISE6 tick cells	***Anaplasma phagocytophilum***	Decreased levels and activity of HXK Increased levels of PEP Decreased levels of glucose and lactate	[26,43] [41] [23]
	Ixodes scapularis adults midguts		Upregulation of genes involved in glycolysis (except *hxk* and *pfk*) Upregulation of genes *hif-1α* and *hif-1β* Increased protein levels of glycolytic enzymes (except iPGM, PK and PFK) and glucose transporters GLUT1A and SGLT2	[26]
	Ixodes scapularis adults salivary glands		Upregulation of genes involved in glycolysis (except *hxk*, *pfk*, *alda*, *pgk1* and *enol*) Increased protein levels of glycolytic enzymes (except HXK, PFK and ALDA) and glucose transporter GLUT1A	[26]
Gluconeogenesis	ISE6 tick cells	***Anaplasma phagocytophilum***	Decreased levels of PEPCK-C and glucose Increased protein levels of PEPCK-M	[23] [26]
	Ixodes scapularis adults midguts		Upregulation of genes involved in gluconeogenesis Decreased levels of gluconeogenesis enzymes (except FBP)	[26]

	Ixodes scapularis adults salivary glands		Decreased levels of gluconeogenesis enzymes	
Glyceroneogenesis	ISE6 tick cells	***Anaplasma phagocytophilum***	Decreased levels of Glycerol 3-phosphate Increased levels of PEP which may inhibit the enzyme TPI	[26]
	Ixodes scapularis adults midguts		Upregulation of genes *tpi* and *gpdh* Increased and decreased protein levels of TPI and GPDH, respectively	
	Ixodes scapularis adults salivary glands		Upregulation of gene *tpi* Increased protein levels of TPI and GPDH	
	Ixodes scapularis nymphs**	***Borrelia mayonii***	Decreased levels of Glycerol 3-phosphate	[54]
Lipids	*Rhipicephalus microplus* adults fat body	***Metarhizium anisopliae***	Increased levels of cholesterol ester and triacylglycerol Decreased levels of free cholesterol	[51]
	Ixodes scapularis nymphs**	***Borrelia mayonii***	Decreased levels of cholesterol, ethanolamine phosphate and ethanolamine	[54]

Amino Acids	ISE6 tick cells	***Anaplasma phagocytophilum***	Decreased levels of alanine, glycine, glutamine, glutamate and methionine Increased levels of serine, proline, tyrosine and phenylalanine Tyrosine is used as fuel to synthetize PEP	[23,41]
			Upregulation of gene *oatp* Xanthurenic acid acts as a co-factor for unknown transcription factor(s) that control the expression of *oatp* KAT regulates the expression of *oatp*	[33]
	Ixodes scapularis adults midguts and salivary glands		Changes in the levels of genes and proteins involved in amino acid synthesis and degradation	[41]
	Ixodes scapularis nymphs salivary glands		Upregulation of genes *oatp* and *kat* Xanthurenic acid acts as a co-factor for unknown transcription factor(s) that control the expression of *oatp*	[33]
	Ixodes scapularis nymphs**	***Borrelia mayonii***	Decreased levels of alanine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, glutamate, glutamine and glycine	[54]
		Borrelia burgdorferi	Increased levels of tryptophan and glutamine Decreased levels of alanine, isoleucine, leucine, phenylalanine, proline, threonine, valine and glycine	

Redox	BME26 tick cells	***Anaplasma marginale***	Downregulation of pro-oxidant genes and upregulation of antioxidant genes	[67]
	Ixodes scapularis adults midguts and salivary glands	***Anaplasma phagocytophilum***	Significant changes in the levels of genes and proteins of hemoglobinolytic enzymes affecting host hemoglobin levels in tick tissues	[18]

* Full name of enzymes are in the main text except for phosphoglycerate kinase 1 (PGK1), enolase (ENOL), glycerol-3-phosphate dehydrogenase (GPDH), triosephosphate isomerase (TPI). ** Only the levels of tick metabolites in response to *Borrelia* spp. infection at day 4 of feeding were considered [54].


ISE6 cells - tick hemocytes model
Mammalian macrophages M1
A. phagocytophilum no LPS
Bacteria with LPS
Glucose
HXK
PFK
Cytosol
PI3K
Pyruvate
Lactate
TCA cycle
LY294002
2DG
HIF-1α
Succinate
Glutamine
Mitochondria

A. phagocytophilum

Tick cell

GPCR

Proteasome

PI3k

IPAK1

Actin

TF

AnkA

Actin

TF

Cytosol

Nucleus

HDAC1

RNAPII

Activation
(*oatp* and *kat*)

HDAC1

Repression
(*fbp*)

Actin

TBP

RNAPII

Activation
(*salp16* and *hif-1α*)

p300

HIF-1β

HIF-1α

RNAPII

Activation
(glycolytic genes)

Molecular factors

Bacterial nucleomodulins

Xanthurenic acid

Phosphate

Tick proteins

Molecular processes

Phosphorylation

Recruitment of tick proteins
and/or metabolites

Targeting tick proteins
to proteasome degradation