Trends in Microbiology

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REVIEWS

Review

Nutrition and Bipartite Metabolism of Intracellular Pathogens

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The host is a nutrient-rich niche for microbial pathogens, but one that comes with obstacles and challenges. Many intracellular pathogens like *Legionella pneumophila, Coxiella burnetii, Listeria monocytogenes,* and *Chlamydia trachomatis* have developed bipartite metabolism within their hosts. This style of metabolic regulation enables pathogen sensing of specific nutrients to engage them into catabolic and anabolic processes, and contributes to temporal and spatial pathogen phenotypic modulation. Not only have intracellular pathogens adapted their metabolism to the host, they have also acquired idiosyncratic strategies to exploit host nutritional supplies and intercept metabolites. *Francisella tularensis* and *Anaplasma phagocytophilum* alter host autophagy, *Shigella flexneri* intercepts all host pyruvate, while *L. pneumophila* induces host protein degradation and blocks protein translation. Strategies of pathogen manipulation of host nutrients could serve as therapeutic targets.

Highlights

There is a general common theme of bipartite metabolism by intracellular pathogens.

Pathogens have evolved with mechanisms to override host-limited availability of nutrients.

Host nutrient supplies govern pathogen virulence properties.

Import of host nutrients occurs across the pathogen-containing vacuoles using host SLCs.

Nutritional Virulence

The biological instinct of microbes is to feed and proliferate, including within a nutrient-rich host. It is not surprising that restricting host nutrients, such as iron sequestration or tryptophan degradation, from pathogens is one of the most fundamental aspects of innate immunity against bacterial infections [1]. However, pathogens have evolved fascinating mechanisms to override host nutrient restrictions by increasing the levels of host nutrients and metabolites through manipulation of host cellular processes and metabolism [2–4]. This is becoming the hallmark of nutritional virulence of intracellular pathogens, to ensure availability of sufficient host metabolites for robust replication within the host cell [5,6]. These microbial strategies are potential targets for novel therapeutic approaches since microbial nutrition is indispensable for microbial growth and disease manifestation.

Nutritional based virulence is the microbial quest for food as the driving force for virulence in the host [5]. Nutrient acquisition is then one of the most fundamental aspects of bacterial pathogenesis. Colonization allows the organism to have continual access to a nutrient source, while immunoevasion and immunosuppression mechanisms allow the pathogen to continue to access the host's nutrient supply [7]; but host cell metabolism is also influenced by many intracellular bacterial pathogens, and the microbiome also plays a role in metabolic host–pathogen interaction [8,9].

Intracellular pathogens are diverse in the types of hosts they parasitize, the nutrients they utilize, and the host cellular processes they target. Some of the host cells for intracellular pathogens are the very cells designed to keep pathogens at bay, such as macrophages and neutrophils. In this review, we discuss nutritional based virulence of intracellular pathogens, with more emphasis on *L. pneumophila* since its metabolic regulation and its intersection with pathogenesis and disease manifestation *in vivo* has been extensively studied compared to other intracellular pathogens, and also closely related to *Coxiella*.

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Bipartite Metabolism of Intracellular Pathogens

Based on the sources of carbon and energy utilized at various stages of intracellular growth, metabolism of various intracellular pathogens such as *C. burnetii, L. pneumophila, L. monocytogenes,* and *C. trachomatis* has been described as bipartite (Figure 1A,B) [10–13]. The general aspect of a bipartite metabolism is two distinct networks for metabolite usage with different end goals. However, there is some level of substrate crossover in most, if not all, pathogens. Moreover, some of the metabolic preferences are regulated by growth phase, thus adding an extra level of complexity to the concept of a 'bipartite' metabolism. These complexities are discussed below.

The bipartite metabolism of *L. pneumophila* uses amino acids, with a preference for serine, for carbon and energy during exponential growth, and carbohydrates for anabolic processes at the postexponential phase (Figure 1A) [11,14–16]. Serine, cysteine, and alanine – in order of preference – are converted into pyruvate, which feeds the TCA cycle. Glutamate can be converted into the TCA cycle intermediate α-ketoglutarate, and aspartate can be converted into fumarate and oxaloacetate [14]. *L. pneumophila* can also take up host pyruvate to use directly in the TCA cycle [2]. Eylert *et al*. showed that the amino acids (alanine, glutamic

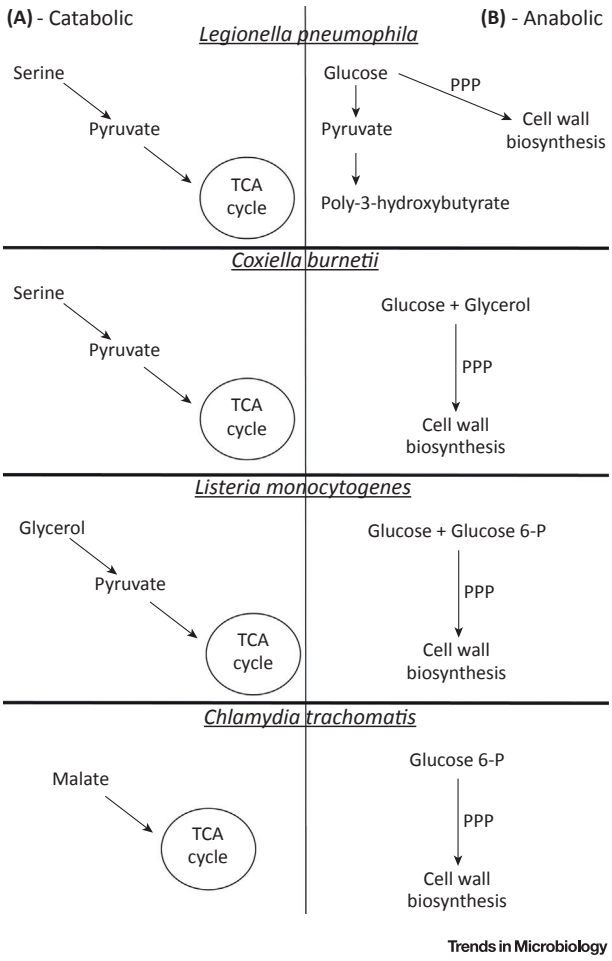

Figure 1. The Bipartite Metabolism of *Legionella pneumophila*, *Coxiella burnetii, Listeria monocytogenes,* and *Chlamydia trachomatis*. (A) Carbon and energy utilization of various intracellular pathogens relies on host metabolites to feed the TCA cycle. For *L. pneumophila* and *C. burnetii* host amino acids, particularly serine, are the primary sources of carbon and energy, whereas *L. monocytogenes* uses host glycerol, and *C. trachomatis* utilizes host malate to feed the TCA cycle. (B) Anabolic processes for these intracellular pathogens utilize host carbohydrates, primarily by the pentose phosphate pathway (PPP) for cell-wall biosynthesis. *L. pneumophila* also converts host glucose into the carbon storage molecule poly-3-hydroxybutyrate.

acid, glycine, asparagine, leucine, threonine, arginine, isoleucine, valine, and aspartic acid) are imported from the *Acanthamoeba castellanii* host cytosol into the *Legionella*-containing vacuole (LCV) and are converted by *L. pneumophila* to other metabolites [15].

Historically, *L. pneumophila* was described as being defective in glycolysis. However, it does have a functional glycolytic pathway, also referred to as the Embden–Meyerhof–Parnas (EMP) pathway, which is minimally utilized [11,15]. Instead, it favors the Entner-Doudoroff (ED) pathway for glucose catabolism [15]. The pentose phosphate pathway (PPP) functions only to generate mannose and histidine within *L. pneumophila* [11,17]. During exponential (E) phase, isotopolog labelling demonstrates that serine is the preferred amino acid for generating pyruvate to feed into the TCA cycle [11,15]. Some serine is diverted to the EMP and PPP pathways to generate mannose and histidine, and to generate the storage molecule poly-3-hydroxybutyrate (PHB) [15]. A shift into the postexponential (PE) phase of growth occurs when amino acids (and fatty acids) are low, when glucose becomes the predominant molecule metabolized [15,18]. Glucose is metabolized by the ED pathway to generate pyruvate then acetyl-CoA, used for the synthesis of large stores of PHB that are required for survival outside of the host [18].

Why *L. pneumophila* prefers amino acids over glucose, unlike most other genera of bacteria studied, is unknown. The answer likely lies within its genome and through better understanding of its evolution with protist hosts [19,20]. Like most driving factors for coevolution, the use of amino acids could keep it reliant on its host, limiting growth only when within a host, thus giving *L. pneumophila* an evolutionary advantage. Nutrition of *L. pneumophila* within protists has been likely a major driving force in its evolution as an intracellular pathogen [21].

C. burnetii, like its closest phylogenetic relative, *L. pneumophila*, has a similar bipartite metabolism (Figure 1B) [10]. It utilizes serine as the main source for carbon and energy, while glycerol is utilized preferentially in anabolic processes like cell wall biosynthesis [10]. Only recently has axenic culturing of *C. burnetii* been achieved [22]. Isotopic profiling has revealed that glucose is utilized by glycolysis as a source of carbon and energy, but it alone is not sufficient for extracellular growth [10], but that could be for a variety of reasons independent of the ability of *Coxiella* to use glucose for catabolic processes.

In contrast to the reliance of *L. pneumophila* and *C. burnetii* on host amino acids, *L. monocytogenes* and *C. trachomatis* do not utilize amino acids as sources of carbon and energy (Figure 1) [12,13,23]. Glycerol is primarily used by *L. monocytogenes* for carbon and energy, while utilizing glucose and glucose-6P primarily for biosynthesis of cell wall components and nucleotides (Figure 1) [13]. For *C. trachomatis*, host malate and other dicarboxylates feed into the TCA cycle for carbon and energy, and glucose-6P is utilized for cell wall biosynthesis while host malate is used as an energy source (Figure 1) [12]. The bipartite metabolism of these pathogens could be a reflection of the intracellular lifestyle. Having a bipartite metabolism could aid in sensing when nutrients are low in the host and directing a phase transition to prepare to find a new host.

Nutrient Supply Governs Regulation of Phase Variation in *Legionella*

Nutrient availability governs the biphasic lifestyle of *L. pneumophila*, and possibly *C. burnetii,* since it is closely related to *L. pneumophila* [24,25]. When nutrient levels are high, *L. pneumophila* is in the replicative phase [17,26]. When nutrient levels are low, the bacterium enters a transmissive phase. The intracellular lifecycle of *L. pneumophila* within the LCV also exhibits this biphasic regulation, as *L. pneumophila* is in the replicative phase within the LCV and upon escape into the cytosol transitions into the transmissive phase [27,28].

The biphasic lifestyle of *L. pneumophila* is characterized by dramatic changes in the transcriptome, which result in phenotypic modulations [24,29]. During the replicative phase, *L. pneumophila* undergoes exponential (E) growth; it is nonmotile, and it represses transmissive traits, such as lysosomal evasion [17]. The transmissive phase, during PE growth, prepares *L. pneumophila* for life outside the protective environment of the LCV. Traits expressed during the PE phase correspond with an increased virulence of *L. pneumophila*, which becomes cytotoxic, motile, sodium sensitive, and osmotically resistant [17]. These changes are necessary for *L. pneumophila* to invade a new host and start a second cycle of intracellular proliferation [17,28,30,31].

The transition between replicative and transmissive phenotypes of *L. pneumophila* is highly orchestrated and is governed by many regulators that are influenced by intracellular nutrient levels (Figure 2) [17,24,32,33]. Upon amino acid depletion, uncharged bacterial tRNAs activate RelA to synthesize the bacterial alarmone guanosine 3′-5′-bispyrophosphate (ppGpp) (Figure 2) [33]. SpoT, a bifunctional synthetase/hydrolase that responds to a variety of stimuli, such as fatty acid starvation, also synthesizes ppGpp, leading to increased levels of the alarmone (Figure 2) [17]. Accumulation of ppGGpp activates RpoS, an alternative sigma factor [17,34] which regulates the two-component systems CpxR/A and LqsR/S (Figure 2) [35].

In addition to triggering flagellation and various virulence-related traits, elevated ppGpp levels result in upregulation of the Dot/Icm effectors [29]. Complex cascades of regulatory networks

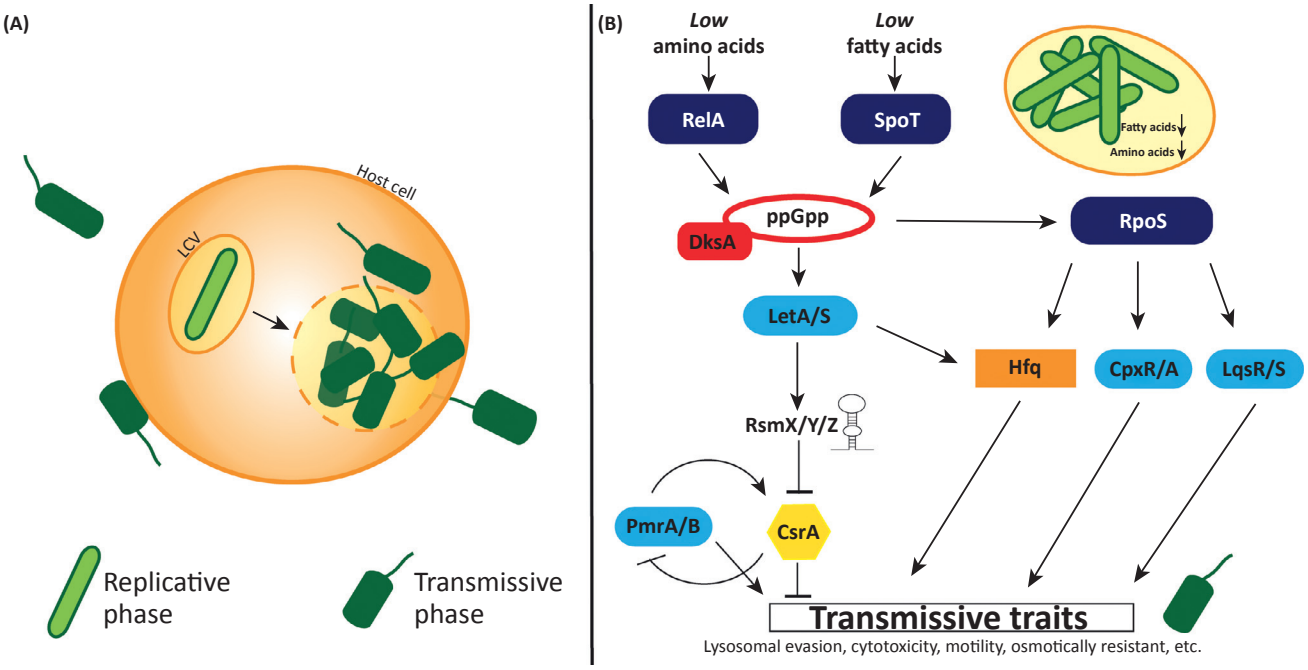

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Figure 2. Phase Variation and the Starvation Response in *Legionella pneumophila*. (A) Within the LCV, where nutrients are rich, *L. pneumophila* is in the replicative phase. Once nutrients are depleted, *L. pneumophila* enters the transmissive stage, to find another host. (B) Starvation is triggered upon sensing depletion in the amino acids and fatty acids of the intracellular environment; this triggers RelA and SpoT, leading to an increased level of the alarmone ppGpp. Accumulation of ppGpp is sensed by the two-component system LetA/S and the alternative sigma factor RpoS. LetA/S induces the small noncoding RNAs, RsmX/Y/Z, which block the global repressor of transmissive traits, CsrA. RpoS regulates the Hfq, CpxR/A, and LqsR/S regulators, leading to an increase in transmissive traits. PmrA/B activates 43 effectors and positively regulates CsrA, acting as a switch upon entry to the transmissive phase.

govern phenotypic transition at the PE phase, and most or all of these networks are under the direct or indirect control of ppGpp, which is triggered by nutritional availability [17]. Therefore, the virulence phenotypes of *L. pneumophila* are directly linked to nutrition.

C. burnetii also exhibits a biphasic lifestyle [25]. Inside a host, *C. burnetii* differentiates in a metabolically active large-cell variant [36]. Whereas, outside the host *C. burnetii* is metabolically quiescent and referred to as the small-cell variant that is environmentally resistant and less metabolically active [36]. Recent advances in culturing and genetic manipulations *in vitro* are starting to unravel biphasic regulation in *C. burnetii* [22]. Given the phylogenetic closeness of *C. burnetii* and *L. pneumophila*, it will be interesting to learn the similarities and differences between the biphasic lifestyles of these two pathogens and how they contribute to the varying pathogenesis.

The availability of nutrients in the host is most likely the strongest factor governing the survival of the pathogen. Therefore, it is not surprising that intracellular pathogens have evolved a mechanism to tie in sensing of host nutrient availability to growth phase regulation. Aberrant growth within a nutrient-depleted host could be detrimental to the pathogen.

How Intracellular Pathogens Overcome Host Nutrient Restriction

Recent studies have shown some common themes utilized by intracellular pathogens to overcome host restriction in order to satisfy their unique metabolic need within the host cell. Despite having diverse lifestyles, the obligate intracellular bacterial pathogens *Chlamydia, Coxiella, Anaplasma, Ehrlichia, and Rickettsia* all target cholesterol during host cell colonization as a potential source of membrane lipids, and to manipulate host cell signaling and trafficking [37,38]. To promote host cell entry, numerous pathogens utilize cholesterol-rich microdomains, known as lipid rafts, which serve as organizational and functional platforms for host signaling pathways involved in phagocytosis [37]. Although *Shigella* and *Listeria* are cytosolic pathogens, their nutritional adaptation to the host cytosol is distinct, and they manipulate unique host processes to obtain nutrients [39–42], and ABC transporters of nutrients are detrimental to disease manifestation indicating the importance of nutrient acquisition from the host cytosol [43]. *Coxiella* resides within phagolysosomes, a rich source of host nutrients [44,45]. The pathogen utilizes host autophagy to repair damage to the vacuolar membrane, but autophagy may also provide an additional source of nutrients [46]. A combination of physiochemical and nutritional growth requirements are strong indicators for why *C. burnetii* favors an acidified phagolysosome-derived vacuole in respiring tissue for replication [47].

Among the ~300 effectors injected by the *L. pneumophila* type IVb translocation system, AnkB is one of the few effectors known to be indispensable for the intracellular infection of both human macrophages and amoebae and for virulence in the A/J mouse model [2,20,48–52]. Recent studies on the AnkB effector have shown exploitation of multiple highly conserved eukaryotic processes with the ultimate goal of increasing amino acid availability in the host cell [2,48–52].

The LCV-anchored AnkB effector harbors multiple eukaryotic domains and motifs that enable this protein to hijack a number of evolutionarily conserved eukaryotic processes [51–55]. AnkB harbors an F-box domain and three Ankyrin (ANK) domains, 33-residue repeats involved in protein–protein interactions [56]. Interaction of AnkB with the host SCF1 ubiquitin ligase complex promotes K^{48}-linked polyubiquitination of host proteins, targeting the proteins for degradation by the proteasome [2,57]. Increased host protein degradation provides an abundance of amino acids in the cytosol for metabolism by *L. pneumophila* (Figure 3) [2].

The *ankB* mutant of *L. pneumophila* is severely defective in intracellular proliferation in amoebae and human macrophages due to the defect in assembly of K^{48}-linked polyubiquitinated

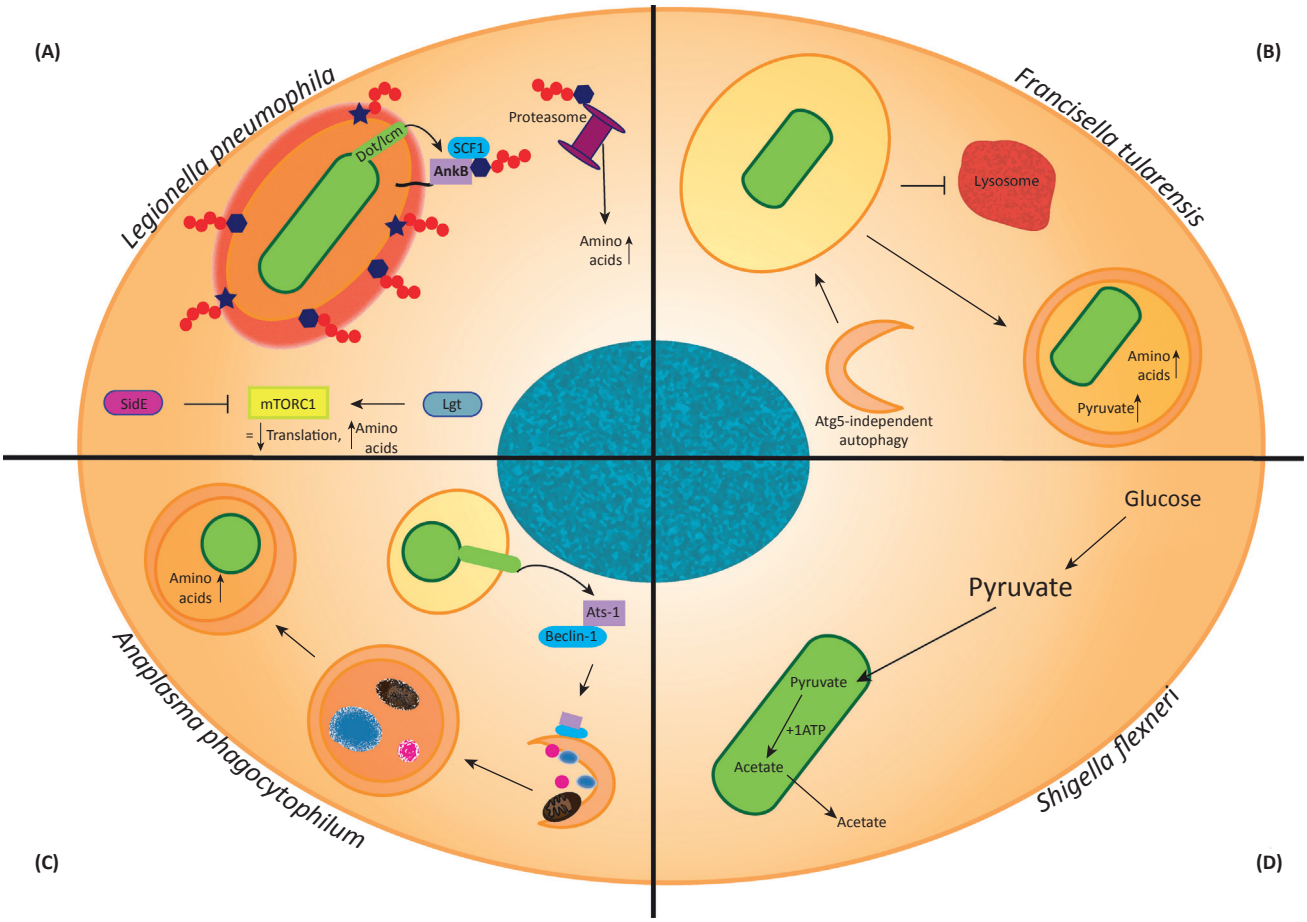

Figure 3. Exploiting Host Machineries to Boost Nutrient Availability by *Legionella pneumophila*, *Francisella tularensis, Shigella flexneri,* and *Anaplasma phagocytophilum*. (A) *L. pneumophila* increases host amino acid availability through two mechanisms. The type 4 secretion system (T4SS) effector AnkB induces ubiquitination and degradation of host proteins to increase the host amino acid levels in the cytosol. Additionally, the T4SS effectors of the SidE and Lgt families modulate mTORC1 to decrease host translation, increasing the availability of host amino acids. (B) *F. tularensis* induces autophagy in the host to acquire amino acids and pyruvate in a process that is not dependent on the canonical Atg5 autophagy pathway. (C) *A. phagocytophilum* induces host autophagy through the binding of its T4SS effector, Ats-1, to host Beclin-1. Autophagosomes are delivered to the pathogen-containing vacuole to increase amino acids for proliferation. (D) *S. flexneri* intercepts the total host output of pyruvate for rapid conversion into acetate, generating only 1 ATP but in a process that allows for rapid proliferation within the host.

proteins decorating the LCV and subsequent lack of increased levels of amino acids (Figure 3) [2,32,48]. This triggers a bacterial starvation response, mediated by the induced expression of RelA and SpoT, and results in elevated ppGpp levels (more on this is discussed in the next section) [2,32].

Intracellular growth can be restored to the *ankB* mutant within amoebae and human macrophages by supplementing excess amino acids, similar to genetic complementation [2,32]. Thus, it is clear that a threshold higher than endogenous levels of cellular amino acids is needed for intracellular replication of *L. pneumophila*. Remarkably, supplementation of infected cells with certain single amino acids, such as serine or cysteine, reverses the growth defect of the *ankB* mutant in amoebae and human macrophages [2]. Interestingly, in human cells, cysteine is semiessential and is the least abundant amino acid, but in amoebae cysteine is essential [2,32].

However, serine is not essential for either but is favored by *L. pneumophila* for use in the TCA cycle [15]. Similar to cysteine and serine, supplementation of infected cells with pyruvate or citrate, to feed the TCA cycle, rescues the *ankB* mutant for intracellular proliferation [2,32]. Therefore, by promoting proteasomal degradation of proteins in amoebae and human macrophages, *L. pneumophila* generates a gratuitous supply of cellular amino acids, which are the preferable source of carbon and energy for *L. pneumophila* to power intracellular growth within amoebae and human macrophages (Figure 3) [2]. However, the role of host ubiquitylation in *L. pneumophila*–host interaction is much more complex than just nutrients [58].

In contrast to the intracellular growth defects in human macrophages for two *ankB* mutants constructed in the AA100/130b strain and the Paris strain of *L. pneumophila*, the *ankB* mutant in the LP02 strain background does not exhibit a replication defect in the U937 human monocyte cell line [59]. However, the *L. pneumophila* LP02 strain has been shown to inhibit the mammalian target of the rapamycin complex 1 (mTORC1), a nutrient/energy sensor, to prevent protein synthesis, thus liberating more amino acids to drive intracellular replication (Figure 3) [59]. This process is driven by the SidE family of T4SS effectors (Figure 3) [59]. Conversely, the Lgt family of effectors activates mTORC1 [59]. Thus, major differences are exhibited between various strains of *L. pneumphila* in manipulating distinct host pathways but with the ultimate same outcome of increasing host cell levels of amino acids to power the TCA cycle of *L. pneumophila* [2,59]. Variation in mechanisms to increase host metabolites could be a reflection of the broad environmental host range that *L. pneumophila* encounters and the distinct evolution of different strains.

In contrast to *L. pneumophila, F. tularensis* and *A. phagocytophilum* manipulate the host autophagy by distinct mechanisms to increase the intracellular pool of host amino acids as the major sources of carbon and energy [3,4,60–63] and also to obtain iron [64]. In order to replicate in the host cytosol, *F. tularensis* induces ATG5-independent macroautophagy mediated by the type VI secretion system (Figure 3) [4,65,66]. This process is required for intracellular replication of *F. tularensis*, but failure to replicate due to inhibition of macroautophagy could be rescued with the addition of excess nonessential amino acids or pyruvate [4]. In addition, the pathogen also employs differential metabolite fluxes that vary between *Francisella* species, suggesting that the different utilization of substrates could be related to host specificity and virulence of *Francisella* [67,68]. In addition, the pathogen overrides host restriction of iron [69]. *A. phagocytophilum* is a tick-borne obligate intracellular pathogen that replicates within an autophagosome-like compartment [26,70]. Ats-1, a type IV effector of *A. phagocytophilum*, binds to host Beclin-1, inducing autophagosome formation (Figure 3) [3]. The autophagosomes are delivered to, and fuse with, the *A. phagocytophilum*-containing compartment, providing the amino acids needed for replication (Figure 3) [3], and host restriction of iron triggers virulence [71]. The pathogen subverts the carbohydrate metabolism of the tick host but it is not known whether similar process are exhibited in the mammalian host [72,73].

For *S. flexneri,* rather than altering host metabolism, the entire output of host glycolysis and PPPs is intercepted by the bacteria in the form of pyruvate (Figure 3) [74]. Pyruvate is converted into acetate, generating a single ATP. This energy-inefficient method is preferred due to the low enzyme costs, fast rate of conversion, the ability to handle high supply levels that would otherwise saturate the respiratory chain, and the possibility of extending host cell survival [74]. Metabolism of pyruvate is required for intracellular growth of *S. flexneri* [75]. Pyruvate is generated from glucose metabolism primarily by the EMP pathway, although the PPP and ED pathways are used but not essential [76]. Glycolysis mutants can be rescued with supplementation of pyruvate [76,77]. Thus, pyruvate is a major substrate to power metabolism

and proliferation of *S. flexneri* in HeLa cells. However, major metabolic sources can vary between *ex vivo* and *in vivo*, and the nutrient effects of *S. flexneri* have been observed in HeLa cells but not in macrophages [76,77].

Compared to other intracellular pathogens, *Chlamydia* accesses host amino acids and cholesterol through the lysosome, presumably via its close association with lysosomes and the Golgi apparatus throughout development [37,78]. *Chlamydia* likely accesses transferrin-bound iron in a similar fashion [37]. These observations point to general vesicular-dependent strategies for acquiring nutrients conserved across various pathogens. The key advantage afforded by these strategies may be to siphon host nutrients without gross disruption of nutrient trafficking throughout the host cell, thereby avoiding host nutrient restriction mechanisms.

Import of Nutrients into the Lumen of Pathogen-containing Vacuoles Using Human Solute Carriers

Growth within a vacuole limits the ease of access to the host's nutrient supply in the cytosol [79]. For organisms like *A. phagocytophilum*, direct delivery of nutrients, contained within autophagosomes, to the bacterial compartment overcomes this limitation [3]. Utilization of host human solute carrier (SLC) transporters is emerging as a new theme for importing nutrients into the pathogen-containing vacuole. The SLC transporters constitute a large superfamily of membrane-bound transporters encoded by 55 gene families present throughout the animal kingdom [80,81]. They include passive transporters, symporters, and antiporters that are located mostly in the plasma membrane, but some are also located in organelle membranes to import amino acids, glucose, lipids, and drugs [81].

C. trachomatis manipulates various host nutritional and inflammatory pathways to override host depletion of tryptophan and to obtain host nutrients such as cholesterol, iron, and glucose [78,82–84]. The host SLC35D2, a UDP-glucose transporter, is directed to the *C. trachomatis* inclusion [85]. Using this host transporter, *C. trachomatis* can acquire the precursor, glucose 1-phosphate, needed to generate large stores of glycogen within the inclusion [85]. SLC2a1 (also known as Glut1) is upregulated during *C. trachomatis* infection and can be found in close association with the inclusion [86]. As previously mentioned, *C. trachomatis* utilizes glycogen for anabolic processes as part of its bipartite metabolism.

Salmonella enterica serovar Typhimurium resides within a vacuole that is a unique intracellular replicative niche [87,88] of a highly complex and dynamic network of *Salmonella*-induced filaments [89], but it is also capable of reaching the host cell cytosol, which is a cell-specific process [90–92]. Formation of the intracellular niche is governed by multiple type III secretion systems [93]. Autophagy and ubiquitination may contribute to increased host cell amino acids, but may also constitute part of an innate host defense [94]. Arginine is an important regulator of the immune response and is used to generate reactive oxygen species, important for pathogen defense. *S. enterica* serovar Typhimurium recruits host SLC7a1, a transporter of arginine, to the *Salmonella*-containing vacuole [95]. The *S.* Typhimurium arginine transporter, ArgT, is required for intracellular replication in macrophages, working together with host SLC7a1 to sequester arginine and dampen the immune response [95]. The organism also requires inorganic polyP as an essential nutrient for virulence and survival in certain host cells, and this nutrient is likely imported by an SLC [96].

There is recent evidence for the role of human SLCs during infection of macrophages by *L. pneumophila* [97,98], which have been identified by mass-spectrometry to be present on the LCV membrane proteome [98–100]. Additionally, host SLC1a5 has been shown to be required

for replication of *L. pneumophila* in the MM6 monocytic cell line [97]. Homologs of these transporters can be found in protozoa by comparing the amino acid sequences of SLCs to the database of protozoan genomes in BLAST, but, to date, none have been functionally described [101]. They are members of an evolutionarily conserved family of transporters known as the major facilitator superfamily (MFS), which are present in all kingdoms of life.

Horizontal gene transfer has shaped the *L. pneumophila* genome; many eukaryotic-like genes have been acquired from evolution with protist hosts [32,102,103]. A pool of 11 eukaryotic SLC-like proteins in *L. pneumophila* with 42–56% amino acid similarity to eukaryotic SLCs have been recently identified [101]. These 11 proteins share strong three-dimensional homology with SLCs, supporting their role in nutrient transport [101].

Interestingly, two glucose SLC-like transporters were experimentally confirmed to contribute to intracellular infection by *L. pneumophila* [101]. Deletion of both of these transporters results in a growth defect in human macrophages and amoebae [101]. Considering that glucose is not utilized as a source of carbon and energy during early stages of growth, this finding highlights that there is still much to be discovered about nutritional virulence in *L. pneumophila*.

There is a functional redundancy within the SLC-like transporters identified, indicating the importance of nutrient transport in the evolution of *L. pneumophila*. Seven are predicted to be transporters of cationic amino acids [101]. Varying substrate specificity is likely to be the difference amongst these seven transporters. Like many of the >320 effectors in the *L. pneumophila* genome, this functional redundancy likely contributes to the ability of *L. pneumophila* to replicate within a variety of environmental hosts where individual SLC-like transporter function is specific for distinct environmental hosts, to ensure acquisition of amino acids needed for replication [20,21].

It is not unsurprising to find intracellular pathogens manipulating host nutrient transporters. Having control of the nutrient supply ensures that the pathogen receives the nutrients necessary for intracellular proliferation. Given that these transporters are already present in the host, and in some cases are important for immune modulation, they could serve as a target for bacterial manipulation.

Concluding Remarks

Survival within a host, and the requirement to feed and proliferate, can come with a unique set of challenges to the pathogen. Primarily, pathogens need to avoid host cell defense mechanisms and override host nutrient restriction, but the payout for the organism is high. Host cells are plentiful in nutrients, and their cellular processes can be exploited to further increase the availability of those nutrients. However, innate immunity limits pathogen access to host nutrients. Through nutritional virulence, pathogens use unique virulence mechanisms to overcome host nutritional immunity in order to meet their nutritional demands to proliferate.

Intracellular pathogens have varying nutritional requirements, from sugars to amino acids. Often, the select use of nutrients in metabolism is bipartite, with separate networks of nutrient usage for specific metabolic or anabolic pathways. Pathogens like *C. burnetii, L. monocytogenes, L. pneumophila,* and *C. trachomatis* have a bipartite metabolic profile. Regulating which nutrients are utilized for what purpose, and during what stage of infection, aids these intracellular pathogens in regulating virulence. Sensing when nutrients are depleted is crucial for preparing these pathogens for survival in the nutrient-poor extracellular environment. These pathogens represent a particular strategy to utilize nutrients within the host and represent a

Outstanding Questions

Is there a common theme of bipartite metabolic regulation for intracellular pathogens?

What are the evolutionary aspects of bipartite metabolism by intracellular pathogens?

How do other intracellular pathogens manipulate the host to generate additional nutrient supplies?

Are there unique nutritional virulence differences between cytosolic and intravacuolar pathogens?

Can we target pathogen nutrient acquisition processes for therapy?

How do intravacuolar pathogens import various nutrients across the pathogen-containing vacuolar membrane?

general theme of nutrient utilization in intracellular pathogens. Using nutritional virulence mechanisms, intracellular pathogens manipulate host cellular processes to increase the availability of nutrients, such as exploiting the host ubiquitin–proteasome system and blocking mTORC1-dependent protein synthesis, promoting host autophagy, or intercepting major host metabolites to acquire nutrients within the host [2–4,59].

Growth within a vacuole is beneficial in hiding from the host immune response, but it presents an additional difficulty in acquiring nutrients. Some organisms, such as *A. phagocytophilum*, overcome this by triggering a nutrient-rich autophagosome to fuse directly with the pathogen-containing vacuole, whereas *C. trachomatis* hijacks host SLC transporters to import nutrients into the vacuole. In *L. pneumophila,* host SLC-like transporters have been identified and are potential transporters on the LCV membrane to take up nutrients from the host cytosol.

Intracellular pathogens have found diverse mechanisms to overcome the host's nutrient-restriction defenses in order to generate a nutrient-rich environment. It is most likely that numerous pathogenic mechanisms have evolved to allow pathogen growth in the host just for the main purpose of feeding and proliferating with no intention of harming the host (see Outstanding Questions). Given the importance that nutrition has on virulence, these nutrient-acquisition mechanisms could serve as targets for drug intervention. Limiting access to important metabolites, or blocking intracellular pathogens from acquiring nutrients, could halt disease progression. Future studies on pathogen–host metabolic interaction is likely to reveal more nuanced mechanisms by which intracellular pathogens manipulate host metabolism.

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