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Nutrition and bi-partite metabolism of intracellular pathogens

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Abstract

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.

Keywords

Intracellular pathogens; nutrition; virulence; host-pathogen interactions

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.

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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 will discuss nutritional based virulence of intracellular pathogens, with more emphasis on *Legionella 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*.

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 *Coxiella burnetii*, *L. pneumophila*, *Listeria monocytogenes*, and *Chlamydia trachomatis* has been described as bipartite (Fig. 1A-D) [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 will be 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 post-exponential phase (Fig. 1A) [11]. *L. pneumophila* relies solely on amino acids to generate carbon and energy during exponential growth (Fig. 1A) [14–16]. Serine, cysteine, and alanine – in order of preference – are converted into pyruvate, which feed the TCA cycle. Glutamate can be converted into the TCA cycle intermediate, α -ketoglutarate, and aspartate 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 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 Embden-Meyeroff-Parnas (EMP) pathway, which is minimally utilized [11, 15]. Instead, it favors the Enter-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, isotopologue labelling demonstrates that serine is the preferred amino acid to

generate pyruvate to feed into the TCA cycle [11, 15]. Some serine is diverted to the EMP and PPP to generate mannose and histidine, and to generate the storage molecule poly-3-hydroxybutyrate (PHB) [15]. A shift into the post-exponential (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 ED pathway to generate pyruvate then acetyl-CoA, used for the synthesis of large stores of PHB, which is required for survival outside of the host [18].

Why *L. pneumophila* prefers amino acids over glucose, like 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 co-evolution, 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 (Fig. 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 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 (Fig. 1C, D) [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 (Fig. 1C) [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 (Fig. 1D) [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 direct a phase transition to prepare to find a new host.

Nutrients supply govern regulation of phase variation of *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 growth (E); it is non-motile, and represses transmissive traits, such as lysosomal evasion [17]. The transmissive phase, during post-exponential (PE) growth prepares *L. pneumophila* for life outside of the protective environment of the LCV. Traits expressed during PE phase correspond with an increased virulence of *L. pneumophila*, which become 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 (Fig. 2) [17, 24, 32, 33]. Upon amino acid depletion, uncharged bacterial tRNAs activate RelA to synthesize the bacterial alarmone guanosine-3'-5'-bispyrophosphate (ppGpp) (Fig. 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 (Fig. 2) [17]. Accumulation of ppGpp, activates RpoS, an alternative sigma factor [17, 34], which regulates the two-component system CpxR/A and LqsR/S (Fig. 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 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 lifestyle 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 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, 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, 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 transporter are detrimental to disease manifestation [43]. *Coxiella* resides within phagolysosomes, which is a rich resource for 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 [51–56]. AnkB harbors an F-box domain and three Ankyrin domains (ANK), 33-residue repeats involved in protein-protein interactions [57]. Interaction of AnkB with the host SCF1 ubiquitin ligase complex promotes K⁴⁸-linked polyubiquitination of host proteins, targeting the proteins for degradation by the proteasome [2, 58]. Increased host protein degradation provides an abundance of amino acids in the cytosol for metabolism by *L. pneumophila* (Fig. 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 K⁴⁸-linked polyubiquitinated proteins decorating the LCV and subsequent lack of increased levels of amino acids (Fig. 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 will be 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 higher threshold 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 semi-essential 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 (Fig. 3) [2]. However, the role of host ubiquitylation in *L. pneumophila*-host interaction is much more complex than just nutrients [59].

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 [60]. However, the *L. pneumophila* LP02 strain has been shown to inhibit mammalian target of rapamycin complex 1 (mTORC1), a nutrient/energy sensor, to prevent protein synthesis, thus liberating more amino acids to drive intracellular replication (Fig. 3) [60]. This process is driven by the SidE family of T4SS effectors (Fig. 3) [60]. Conversely, the Lgt family of effectors activates mTORC1 [60]. Thus, major differences are exhibited between various strains of *L. pneumophila* 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, 60]. Variation in mechanisms to increase host metabolites could be a reflection of the broad environmental host range that *L. pneumophila* encounters and distinct evolution of different strains.

In contrast to *L. pneumophila*, *Francisella tularensis* and *Anaplasma phagocytophilum* manipulate the host autophagy by distinct mechanisms to increase the intracellular pool of host amino acids as the major sources for carbon and energy [3, 4, 61–64] and also to obtain iron [65]. In order to replicate in the host cytosol, *F. tularensis* induced ATG5-independent macroautophagy mediated by the type VI secretion system (Fig. 3) [4, 66, 67]. 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 varies between *Francisella* species, suggesting that the different utilization of substrates could be related to host specificity and virulence of *Francisella* [68, 69]. In addition, the pathogen overrides host restriction of iron [70]. *A. phagocytophilum* is a tick-borne an obligate intracellular pathogen that replicates within an autophagosome-like compartment [26, 71]. Ats-1, a type-IV effector of *A. phagocytophilum*, binds to host Beclin-1 inducing autophagosome formation (Fig. 3) [3]. The autophagosomes are delivered to and fuse with the *A. phagocytophilum*-containing compartment, providing the amino acids needed for replication (Fig. 3) [3], and host restriction of iron triggers virulence [72]. The pathogen subverts the tick host carbohydrate metabolism but it is not known whether similar process are exhibited in the mammalian host [73, 74].

For *Shigella flexneri*, rather than altering host metabolism, the entire output of the host glycolysis and pentose phosphate pathways are intercepted by the bacteria in the form of pyruvate (Fig. 3) [75]. 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 [75]. Metabolism of pyruvate is required

for intracellular growth of *S. flexneri* [76]. Pyruvate is generated from glucose metabolism primarily by the EMP pathway, although the PPP and ED pathways are used but not essential [77]. Glycolysis mutants can be rescued with supplementation of pyruvate [77, 78]. 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 macrophages [77, 78].

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, 79]. *Chlamydia* likely access 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 [80]. 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 [81, 82]. 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 [82].

C. trachomatis manipulate various host nutritional and inflammatory pathways to override host depletion of tryptophan and to obtain host nutrients such as cholesterol, iron, and glucose [79, 83–85]. The host SLC35D2, a UDP-glucose transporter, to the *C. trachomatis* inclusion [86]. Using this host transporter, *C. trachomatis* can acquire the precursor, glucose 1-phosphate, needed to generate large stores of glycogen within the inclusion [86]. SLC2a1 (also known as Glut1) is upregulated during *C. trachomatis* infection and can be found in close association with the inclusion [87]. 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 [88, 89] of a highly complex and dynamic network of *Salmonella*-induced filaments [90], but it is also capable of reaching the host cell cytosol, which is a cell-specific process [91–93]. Formation of the intracellular niche is governed by multiple type III secretion systems [94]. Autophagy and ubiquitination may contribute to increased host cell amino acids, but may also constitute part of an innate host defense [95]. Arginine is an important regulator of the immune response and is used to generate reactive

oxygen species, important for pathogen-defense. *Salmonella enterica* serovar Typhimurium recruits host SLC7a1, a transporter of arginine, to the Salmonella-containing vacuole [96]. *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 [96]. 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 [97].

There is recent evidence for the role of human SLCs during infection of macrophages by *L. pneumophila* [98, 99], which identified by mass-spectrometry to be present on the LCV membrane proteome [99–101]. Additionally, host SLC1a5 has been shown to be required for replication of *L. pneumophila* in the MM6 monocytic cell line [98]. 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 none to-date have been functionally described [102]. 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, 103, 104]. A pool of 11 eukaryotic SLC-like proteins in *L. pneumophila* with 42% - 56% amino acid similarity to eukaryotic SLCs have been recently identified [102]. These 11 proteins share strong 3-dimensional homology with SLCs, supporting their role in nutrient transport [102].

Interestingly, two glucose SLC-like transporters were experimentally confirmed to contribute to the intracellular infection of *L. pneumophila* [102]. Deletion of both of these transporters results in a growth defect in human macrophages and amoebae [102]. Considering glucose is not utilized as 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 evolution of *L. pneumophila*. Seven are predicted to be transporters of cationic amino acids [102]. Varying substrate specificity is likely to be the difference amongst these 7 transporters. Like much 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 specific SLCs function is specific for distinct environmental hosts to ensure acquisition of amino acids needed for replication in specific hosts [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 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 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 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, 60].

Growth within vacuole is beneficial in hiding from the host immune response, but presents an additional difficulty in acquiring nutrients. Some organisms, such as *A. phagocytophilum* overcome this by triggering 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 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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References

1. Hood MI and Skaar EP (2012) Nutritional immunity: transition metals at the pathogen-host interface. *Nature Reviews Microbiology* 10, 525. [PubMed: 22796883]
2. Price CT et al. (2011) Host proteasomal degradation generates amino acids essential for intracellular bacterial growth. *Science* 334 (6062), 1553–7. [PubMed: 22096100]
3. Niu H et al. (2012) Autophagosomes induced by a bacterial Beclin 1 binding protein facilitate obligatory intracellular infection. *Proc Natl Acad Sci U S A* 109, 20800–20807. [PubMed: 23197835]
4. Steele S et al. (2013) *Francisella tularensis* Harvests Nutrients Derived via ATG5-Independent Autophagy to Support Intracellular Growth. *PLoS Pathog* 9 (8), e1003562.
5. Abu Kwaik Y and Bumann D (2013) Microbial quest for food in vivo: ‘Nutritional virulence’ as an emerging paradigm. *Cell Microbiol* 15 (6), 882–90. [PubMed: 23490329]
6. Abu Kwaik Y (2015) Nutrition-based evolution of intracellular pathogens. *Environ Microbiol Rep* 7(1), 2–3. [PubMed: 25721587]
7. Brock SR and Parmely MJ (2017) *Francisella tularensis* Confronts the Complement System. *Front Cell Infect Microbiol* 7, 523. [PubMed: 29312899]
8. Kading N et al. (2017) Growth of *Chlamydia pneumoniae* Is Enhanced in Cells with Impaired Mitochondrial Function. *Front Cell Infect Microbiol* 7, 499. [PubMed: 29259924]
9. Gao J et al. (2018) Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. *Front Cell Infect Microbiol* 8, 13. [PubMed: 29468141]
10. Häuslein I et al. (2017) Multiple substrate usage of *Coxiella burnetii* to feed a bipartite-type metabolic network. *Frontiers in cellular and infection microbiology* 7, 285. [PubMed: 28706879]
11. Hauslein I et al. (2016) Pathway analysis using (13) C-glycerol and other carbon tracers reveals a bipartite metabolism of *Legionella pneumophila*. *Mol Microbiol* 100 (2), 229–46. [PubMed: 26691313]
12. Mehltitz A et al. (2017) Metabolic adaptation of *Chlamydia trachomatis* to mammalian host cells. *Molecular microbiology* 103 (6), 1004–1019. [PubMed: 27997721]
13. Grubmüller S et al. (2014) Analysis of carbon substrates used by *Listeria monocytogenes* during growth in J774A.1 macrophages suggests a bipartite intracellular metabolism. *Front Cell Infect Microbiol* 4, 156. [PubMed: 25405102]
14. George JR et al. (1980) Amino acid requirements of *Legionella pneumophila*. *J.Clin.Microbiol* 11,286–291. [PubMed: 6769947]
15. Eylert E et al. (2010) Isotopologue profiling of *Legionella pneumophila*: role of serine and glucose as carbon substrates. *J Biol Chem* 285 (29), 22232–43. [PubMed: 20442401]
16. Schunder E et al. (2014) Amino Acid Uptake and Metabolism of *Legionella pneumophila* Hosted by *Acanthamoeba castellanii*. *J Biol Chem* 289 (30), 21040–54. [PubMed: 24904060]
17. Oliva G et al. (2018) The Life Cycle of *L. pneumophila*: Cellular Differentiation Is Linked to Virulence and Metabolism. *Frontiers in Cellular and Infection Microbiology* 8 (3), doi: 10.3389/fcimb.2018.00003.
18. Gillmaier N et al. (2016) Growth-Related Metabolism of the Carbon Storage Poly-3-Hydroxybutyrate in *Legionella pneumophila*. *J Biol Chem* 291 (12), 6471–6482. doi: 10.1074/jbc.M115.693481. [PubMed: 26792862]
19. Best A et al. (2018) A *Legionella pneumophila* amylase is essential for intracellular replication in human macrophages and amoebae. *Sci Rep* 8 (1), 6340. [PubMed: 29679057]
20. Best AM and Abu Kwaik Y (2018) Evasion of phagotrophic predation by protist hosts and innate immunity of metazoan hosts by *Legionella pneumophila*. *Cell Microbiol*, e12971.
21. Best A and Abu Kwaik Y (2018) Evolution of the arsenal of *Legionella pneumophila* effectors to modulate protist hosts. *MBio* 10 9;9(5). pii: e01313–18. doi: 10.1128/mBio.01313-18. [PubMed: 30301851]
22. Omsland A et al. (2009) Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. *Proc Natl Acad Sci U S A* 106 (11), 4430–4. [PubMed: 19246385]

23. Nogueira AT et al. (2017) Characterization of the Growth of *Chlamydia trachomatis* in In Vitro-Generated Stratified Epithelium. *Front Cell Infect Microbiol* 7, 438. [PubMed: 29067282]
24. Al-Khodori S et al. (2009) The PmrA/PmrB two-component system of *Legionella pneumophila* is a global regulator required for intracellular replication within macrophages and protozoa. *Infect Immun* 77 (1), 374–86. [PubMed: 18936184]
25. McCaul TF and Williams J (1981) Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. *Journal of bacteriology* 147 (3), 1063–1076. [PubMed: 7275931]
26. Pruneau L et al. (2018) Comparative Transcriptome Profiling of Virulent and Attenuated *Ehrlichia ruminantium* Strains Highlighted Strong Regulation of *map1*- and Metabolism Related Genes. *Front Cell Infect Microbiol* 8, 153. [PubMed: 29868509]
27. Molmeret M et al. (2004) Disruption of the phagosomal membrane and egress of *Legionella pneumophila* into the cytoplasm during late stages of the intracellular infection of macrophages and *Acanthamoeba polyphaga*. *Infect Immun* 72, 4040–4051. [PubMed: 15213149]
28. Molmeret M et al. (2010) Temporal and spatial trigger of post-exponential virulence-associated regulatory cascades by *Legionella pneumophila* after bacterial escape into the host cell cytosol. *Environ Microbiol* 12 (3), 704–15. [PubMed: 19958381]
29. Bruggemann H et al. (2006) Virulence strategies for infecting phagocytes deduced from the in vivo transcriptional program of *Legionella pneumophila*. *Cell Microbiol* 8 (8), 1228–40. [PubMed: 16882028]
30. Alli OAT et al. (2000) Temporal pore formation-mediated egress from macrophages and alveolar epithelial cells by *Legionella pneumophila*. *Infect Immun* 68, 6431–6440. [PubMed: 11035756]
31. Gao L-Y and Abu Kwaik Y (2000) The mechanism of killing and exiting the protozoan host *Acanthamoeba polyphaga* by *Legionella pneumophila*. *Environ. Microbiol.* 2, 79–90. [PubMed: 11243265]
32. Al-Quadani T et al. (2012) Exploitation of evolutionarily conserved amoeba and mammalian processes by *Legionella*. *Trends Microbiol* 20, 299–306, DOI: 10.1016/j.tim.2012.03.005. [PubMed: 22494803]
33. Molofsky AB and Swanson MS (2004) Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. *Mol Microbiol* 53 (1), 29–40. [PubMed: 15225301]
34. Hales LM and Shuman HA (1999) The *Legionella pneumophila rpoS* gene is required for growth within *Acanthamoeba castellanii*. *J Bacteriol* 181 (16), 4879–89. [PubMed: 10438758]
35. Manske C and Hilbi H (2014) Metabolism of the vacuolar pathogen *Legionella* and implications for virulence. *Frontiers in Cellular and Infection Microbiology* 4 (125. doi: 10.3389/fcimb.2014.00125). [PubMed: 25250244]
36. Coleman SA et al. (2004) Temporal analysis of *Coxiella burnetii* morphological differentiation. *Journal of bacteriology* 186 (21), 7344–7352. [PubMed: 15489446]
37. Pokorzynski ND et al. (2017) Ironing Out the Unconventional Mechanisms of Iron Acquisition and Gene Regulation in *Chlamydia*. *Front Cell Infect Microbiol* 7, 394. [PubMed: 28951853]
38. Larson CL and Heinzen RA (2017) High-Content Imaging Reveals Expansion of the Endosomal Compartment during *Coxiella burnetii* Parasitophorous Vacuole Maturation. *Front Cell Infect Microbiol* 7, 48. [PubMed: 28293541]
39. Johnston DGW et al. (2017) MicroRNA-21 Limits Uptake of *Listeria monocytogenes* by Macrophages to Reduce the Intracellular Niche and Control Infection. *Front Cell Infect Microbiol* 7, 201. [PubMed: 28589100]
40. Eshwar AK et al. (2017) Cold-Shock Domain Family Proteins (Csps) Are Involved in Regulation of Virulence, Cellular Aggregation, and Flagella-Based Motility in *Listeria monocytogenes*. *Front Cell Infect Microbiol* 7, 453. [PubMed: 29124040]
41. Cheng C et al. (2017) Thioredoxin A Is Essential for Motility and Contributes to Host Infection of *Listeria monocytogenes* via Redox Interactions. *Front Cell Infect Microbiol* 7, 287. [PubMed: 28702378]
42. Marini E et al. (2018) Attenuation of *Listeria monocytogenes* Virulence by *Cannabis sativa* L. Essential Oil. *Front Cell Infect Microbiol* 8, 293. [PubMed: 30186775]

43. Aguilar-Bultet L et al. (2018) Genetic Separation of *Listeria monocytogenes* Causing Central Nervous System Infections in Animals. *Front Cell Infect Microbiol* 8, 20. [PubMed: 29459888]
44. Sobotta K et al. (2017) Interaction of *Coxiella burnetii* Strains of Different Sources and Genotypes with Bovine and Human Monocyte-Derived Macrophages. *Front Cell Infect Microbiol* 7, 543. [PubMed: 29379776]
45. Moses AS et al. (2017) Horizontally Acquired Biosynthesis Genes Boost *Coxiella burnetii*'s Physiology. *Front Cell Infect Microbiol* 7, 174. [PubMed: 28540258]
46. Mansilla Pareja ME et al. (2017) Alterations of the *Coxiella burnetii* Replicative Vacuole Membrane Integrity and Interplay with the Autophagy Pathway. *Front Cell Infect Microbiol* 7, 112. [PubMed: 28484683]
47. Vallejo Esquerre E et al. (2017) Physicochemical and Nutritional Requirements for Axenic Replication Suggest Physiological Basis for *Coxiella burnetii* Niche Restriction. *Front Cell Infect Microbiol* 7, 190. [PubMed: 28620582]
48. Lomma M et al. (2010) The *Legionella pneumophila* F-box protein Lpp2082 (AnkB) modulates ubiquitination of the host protein parvin B and promotes intracellular replication. *Cell Microbiol* 12 (9), 1272–91. [PubMed: 20345489]
49. Price CT et al. (2009) Molecular mimicry by an F-box effector of *Legionella pneumophila* hijacks a conserved polyubiquitination machinery within macrophages and protozoa. *PLoS Pathog* 5 (12), e1000704. [PubMed: 20041211]
50. Al-Khodori S et al. (2008) A Dot/Icm-translocated ankyrin protein of *Legionella pneumophila* is required for intracellular proliferation within human macrophages and protozoa. *Mol Microbiol* 70 (4), 908–23. [PubMed: 18811729]
51. Price CTD et al. (2010) Host-mediated post-translational prenylation of novel Dot/Icm-translocated effectors of *Legionella pneumophila*. *Front. Microbiol.* 1131.doi:10.3389/fmicb.2010.00131.
52. Price CT and Abu Kwaik Y (2010) Exploitation of Host Polyubiquitination Machinery through Molecular Mimicry by Eukaryotic-Like Bacterial F-Box Effectors. *Front Microbiol* 1, 122. [PubMed: 21687758]
53. Al-Khodori S et al. (2010) Functional diversity of ankyrin repeats in microbial proteins. *Trends Microbiol* 18 (3), 132–9. [PubMed: 19962898]
54. Price CT et al. (2010) Exploitation of conserved eukaryotic host cell farnesylation machinery by an F-box effector of *Legionella pneumophila*. *J Exp Med* 207 (8), 1713–26. [PubMed: 20660614]
55. Price CT et al. (2010) Indispensable role for the eukaryotic-like ankyrin domains of the ankyrin B effector of *Legionella pneumophila* within macrophages and amoebae. *Infect Immun* 78 (5), 2079–88. [PubMed: 20194593]
56. Price CT et al. (2010) Host-mediated post-translational prenylation of novel Dot/Icm-translocated effectors of *Legionella pneumophila*. *Front Microbiol* 1, 131. [PubMed: 21687755]
57. Wong K et al. (2017) Structural Mimicry by a Bacterial F Box Effector Hijacks the Host Ubiquitin-Proteasome System. *Structure* 25 (2), 376–383. [PubMed: 28111017]
58. Al-Quadan T and Kwaik YA (2011) Molecular Characterization of Exploitation of the Polyubiquitination and Farnesylation Machineries of Dictyostelium Discoideum by the AnkB F-Box Effector of *Legionella Pneumophila*. *Front Microbiol* 2, 23. [PubMed: 21687415]
59. Qiu J and Luo ZQ (2017) Hijacking of the Host Ubiquitin Network by *Legionella pneumophila*. *Front Cell Infect Microbiol* 7, 487. [PubMed: 29376029]
60. De Leon JA et al. (2017) Positive and Negative Regulation of the Master Metabolic Regulator mTORC1 by Two Families of *Legionella pneumophila* Effectors. *Cell Rep* 21 (8), 2031–2038. [PubMed: 29166595]
61. Ziveri J et al. (2017) Importance of Metabolic Adaptations in *Francisella* Pathogenesis. *Front Cell Infect Microbiol* 7, 96. [PubMed: 28401066]
62. Thelaus J et al. (2018) *Galleria mellonella* Reveals Niche Differences Between Highly Pathogenic and Closely Related Strains of Francisella spp. *Front Cell Infect Microbiol* 8, 188. [PubMed: 29922601]
63. Pavkova I et al. (2017) The Multiple Localized Glyceraldehyde-3-Phosphate Dehydrogenase Contributes to the Attenuation of the *Francisella tularensis dsbA* Deletion Mutant. *Front Cell Infect Microbiol* 7, 503. [PubMed: 29322032]

64. Barel M and Charbit A (2017) Role of Glycosylation/Deglycosylation Processes in *Francisella tularensis* Pathogenesis. *Front Cell Infect Microbiol* 7, 71. [PubMed: 28377902]
65. Ramakrishnan G (2017) Iron and Virulence in *Francisella tularensis*. *Front Cell Infect Microbiol* 7, 107. [PubMed: 28421167]
66. Clemens DL et al. (2018) The *Francisella* Type VI Secretion System. *Front Cell Infect Microbiol* 8, 121. [PubMed: 29740542]
67. Marecic V et al. (2017) Isolation of *F. novicida*-Containing Phagosome from Infected Human Monocyte Derived Macrophages. *Front Cell Infect Microbiol* 7, 303. [PubMed: 28725638]
68. Chen F et al. (2017) Differential Substrate Usage and Metabolic Fluxes in *Francisella tularensis* Subspecies *holarctica* and *Francisella novicida*. *Front Cell Infect Microbiol* 7, 275. [PubMed: 28680859]
69. Pilo P (2018) Phylogenetic Lineages of *Francisella tularensis* in Animals. *Front Cell Infect Microbiol* 8, 258. [PubMed: 30109216]
70. Brenz Y et al. (2017) Nramp1 and NrampB Contribute to Resistance against *Francisella* in Dictyostelium. *Front Cell Infect Microbiol* 7, 282. [PubMed: 28680861]
71. de la Fuente J et al. (2017) Tick-Pathogen Interactions and Vector Competence: Identification of Molecular Drivers for Tick-Borne Diseases. *Front Cell Infect Microbiol* 7, 114. [PubMed: 28439499]
72. Moumene A et al. (2017) Iron Starvation Conditions Upregulate *Ehrlichia ruminantium* Type IV Secretion System, *trJ* Transcription Factor and *mapI* Genes Family through the Master Regulatory Protein ErxR. *Front Cell Infect Microbiol* 7, 535. [PubMed: 29404278]
73. Cabezas-Cruz A et al. (2017) *Anaplasma phagocytophilum* Infection Subverts Carbohydrate Metabolic Pathways in the Tick Vector, *Ixodes scapularis*. *Front Cell Infect Microbiol* 7, 23. [PubMed: 28229048]
74. Cabezas-Cruz A et al. (2017) *Ixodes scapularis* Tick Cells Control *Anaplasma phagocytophilum* Infection by Increasing the Synthesis of Phosphoenolpyruvate from Tyrosine. *Front Cell Infect Microbiol* 7, 375. [PubMed: 28861402]
75. Kentner D et al. (2014) *Shigella* reroutes host cell central metabolism to obtain high-flux nutrient supply for vigorous intracellular growth. *Proc Natl Acad Sci U S A* 111 (27), 9929–34. [PubMed: 24958876]
76. Pieper R et al. (2013) Analysis of the proteome of intracellular *Shigella flexneri* reveals pathways important for intracellular growth. *Infect Immun* 81 (12), 4635–48. [PubMed: 24101689]
77. Waligora EA et al. (2014) Role of intracellular carbon metabolism pathways in *Shigella flexneri* virulence. *Infect Immun* 82 (7), 2746–55. [PubMed: 24733092]
78. Mattock E and Blocker AJ (2017) How Do the Virulence Factors of *Shigella* Work Together to Cause Disease? *Front Cell Infect Microbiol* 7, 64. [PubMed: 28393050]
79. Kintner J et al. (2017) Inhibition of Wnt Signaling Pathways Impairs *Chlamydia trachomatis* Infection in Endometrial Epithelial Cells. *Front Cell Infect Microbiol* 7, 501. [PubMed: 29322031]
80. Bierre H et al. (2018) To Be Cytosolic or Vacuolar: The Double Life of *Listeria monocytogenes*. *Front Cell Infect Microbiol* 8, 136. [PubMed: 29868493]
81. Cedernaes J et al. (2011) Comprehensive analysis of localization of 78 solute carrier genes throughout the subsections of the rat gastrointestinal tract. *Biochem Biophys Res Commun* 411 (4), 702–7. [PubMed: 21781957]
82. Schlessinger A et al. (2013) SLC classification: an update. *Clin Pharmacol Ther* 94 (1), 19–23. [PubMed: 23778706]
83. Leonard CA et al. (2017) Productive and Penicillin-Stressed *Chlamydia pecorum* Infection Induces Nuclear Factor Kappa B Activation and Interleukin-6 Secretion In Vitro. *Front Cell Infect Microbiol* 7, 180. [PubMed: 28553623]
84. Pan Q et al. (2017) *Chlamydia abortus* Pmp18.1 Induces IL-1 β Secretion by TLR4 Activation through the MyD88, NF- κ B, and Caspase-1 Signaling Pathways. *Front Cell Infect Microbiol* 7, 514. [PubMed: 29326885]
85. Ziklo N et al. (2018) Dysbiosis of the Vaginal Microbiota and Higher Vaginal Kynurenine/Tryptophan Ratio Reveals an Association with *Chlamydia trachomatis* Genital Infections. *Front Cell Infect Microbiol* 8, 1. [PubMed: 29404279]

86. Gehre L et al. (2016) Sequestration of host metabolism by an intracellular pathogen. *Elife* 5, e12552. [PubMed: 26981769]
87. Wang X et al. (2017) Orchestration of the mammalian host cell glucose transporter proteins-1 and 3 by *Chlamydia* contributes to intracellular growth and infectivity. *Pathog Dis* 75 (8), doi: 10.1093/femspd/ftx108.
88. Hume PJ et al. (2017) Swiss Army Pathogen: The *Salmonella* Entry Toolkit. *Front Cell Infect Microbiol* 7, 348. [PubMed: 28848711]
89. Young AM and Palmer AE (2017) Methods to Illuminate the Role of *Salmonella* Effector Proteins during Infection: A Review. *Front Cell Infect Microbiol* 7, 363. [PubMed: 28848721]
90. Knuff K and Finlay BB (2017) What the SIF Is Happening-The Role of Intracellular *Salmonella*-Induced Filaments. *Front Cell Infect Microbiol* 7, 335. [PubMed: 28791257]
91. Castanheira S and Garcia-Del Portillo F (2017) *Salmonella* Populations inside Host Cells. *Front Cell Infect Microbiol* 7, 432. [PubMed: 29046870]
92. Yin Y and Zhou D (2018) Organoid and Enteroid Modeling of *Salmonella* Infection. *Front Cell Infect Microbiol* 8, 102. [PubMed: 29670862]
93. Elfenbein JR et al. (2017) A *Salmonella* Regulator Modulates Intestinal Colonization and Use of Phosphonoacetic Acid. *Front Cell Infect Microbiol* 7, 69. [PubMed: 28361036]
94. Ilyas B et al. (2017) Evolution of *Salmonella*-Host Cell Interactions through a Dynamic Bacterial Genome. *Front Cell Infect Microbiol* 7, 428. [PubMed: 29034217]
95. Wang L et al. (2018) Autophagy and Ubiquitination in *Salmonella* Infection and the Related Inflammatory Responses. *Front Cell Infect Microbiol* 8, 78. [PubMed: 29594070]
96. Das P et al. (2010) Cationic amino acid transporters and *Salmonella* Typhimurium ArgT collectively regulate arginine availability towards intracellular *Salmonella* growth. *PLoS One* 5 (12), e15466. [PubMed: 21151933]
97. Varas MA et al. (2018) Inorganic Polyphosphate Is Essential for *Salmonella* Typhimurium Virulence and Survival in *Dictyostelium discoideum*. *Front Cell Infect Microbiol* 8, 8. [PubMed: 29441327]
98. Wieland H et al. (2005) Intracellular multiplication of *Legionella pneumophila* depends on host cell amino acid transporter SLC1A5. *Mol Microbiol* 55 (5), 1528–37. [PubMed: 15720558]
99. Bruckert WM and Abu Kwaik Y (2015) The complete and ubiquitinated proteome of the *Legionella*-containing vacuole within human macrophages. *Journal of Proteome Research* 14, 236–48. [PubMed: 25369898]
100. Naujoks J et al. (2016) IFNs Modify the Proteome of *Legionella*-Containing Vacuoles and Restrict Infection Via IRG1-Derived Itaconic Acid. *PLoS Pathog* 12 (2), e1005408. [PubMed: 26829557]
101. Hoffmann C et al. (2013) Functional analysis of novel Rab GTPases identified in the proteome of purified *Legionella*-containing vacuoles from macrophages. *Cell Microbiol* 16, 103452.
102. Best A et al. (2018) Mammalian Solute Carrier (SLC)-like transporters of *Legionella pneumophila*. *Scientific Reports* 8 (1), 8352. [PubMed: 29844490]
103. Gomez-Valero L et al. (2011) Comparative and functional genomics of *Legionella* identified eukaryotic like proteins as key players in host-pathogen interactions. *Front Microbiol* 2, 208. [PubMed: 22059087]
104. de Felipe KS et al. (2005) Evidence for acquisition of *Legionella* type IV secretion substrates via interdomain horizontal gene transfer. *J Bacteriol* 187 (22), 7716–26. [PubMed: 16267296]

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.

Outstanding Questions Box

1. Is there a common theme of bipartite metabolic regulation for intracellular pathogens?
2. What are the evolutionary aspects of bipartite metabolism by intracellular pathogens?
3. How do other intracellular pathogens manipulate the host to generate additional nutrient supplies?
4. Are there unique nutritional virulence differences between cytosolic and intra-vacuolar pathogens?
5. Can we target pathogen nutrient acquisition processes for therapy?
6. How do intra-vacuolar pathogens import various nutrients across the pathogen-containing vacuolar membrane?

Figure 1. The bipartite metabolism of *L. 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 source 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 for cell wall biosynthesis. *L. pneumophila* also converts host glucose into the carbon storage molecule, poly-3-hydroxybutyrate.

Figure 2. Phase variation and the starvation response in *L. 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, which triggers RelA and SpoT, leading to an increase 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 induce the small non-coding 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.

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 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) *Shigella 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.