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Modulation of host cell metabolism by T4SS-encoding intracellular pathogens

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Intracellular bacterial pathogens intimately interact with the infected host cell to prevent elimination and to ensure survival. One group of intracellular pathogens, including *Coxiella burnetii, Legionella pneumophila*, *Brucella spp., Anaplasma phagocytophilum,* and *Ehrlichia chaffeensis*, utilizes a type IV secretion system (T4SS) that injects effectors to modulate host cell signalling, vesicular trafficking, autophagy, cell death and transcription to ensure survival [1]. So far, little emphasis has been directed towards understanding how these bacteria manipulate host cell metabolism. This manipulation is not only important for gaining access to nutrients, but also for regulating specific virulence programs [2,3]. Here, we will summarize recent progress made in characterizing the manipulation of host cell metabolism by *C. burnetii* and other intracellular pathogens utilizing a T4SS.

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Introduction

In its natural habitat, *Coxiella burnetii* is an obligate intracellular zoonotic pathogen with a broad host range, which reaches from arthropods, through birds, pets and ruminants to humans [4]. Humans are mainly infected by *C. burnetii* through inhalation of contaminated dust from infected sheep and goats, which shed the bacteria in milk, feces and birthing products [5,6]. Infection with *C. burnetii* causes Q fever in approximately 40% of human cases, with the majority experiencing flu-like symptoms. However, acute Q fever can also present as atypical pneumonia or hepatitis. Importantly, a small proportion of 3–5% of the infected individuals will develop chronic Q fever, which mainly presents as endocarditis and for which efficient treatment options are missing [7]. Essential for its ability to establish a productive infection is the expression of the type IV secretion system (T4SS) [8,9]. *C. burnetii* uses its T4SS to inject roughly 150 effector proteins into the host cell cytoplasm [10]. Other important pathogens also rely on a T4SS to establish a productive infection. One of them is *Legionella pneumophila*, which can cause severe pneumonia, called Legionnaires' disease [11]. Essential for its intracellular survival is the ability to create a replicative niche, the *Legionella*-containing vacuole (LCV). The development of the LCV depends on T4SS-mediated translocation of over 300 effector proteins into the host cell cytoplasm [12]. *Brucella abortus* is the causative agent of the zoonosis brucellosis [13]. This pathogen replicates in the *Brucella*-containing vacuole (BCV), which is derived from the ER. Essential for the establishment of the BCV is the T4SS and, so far, roughly 15 effector proteins have been identified [1]. *Anaplasma phagocytophilum* is the major cause of human granulocytic anaplasmosis, while *Ehrlichia chaffeensis* is the causative agent of human monocytotrophic ehrlichiosis [14]. To date, only 7 effector proteins have been identified for these two species [15,16]. In general, the T4SS effector proteins of these diverse bacteria have been shown to function as virulence factors as they modulate signaling pathways and processes in the host cell, such as vesicular trafficking, autophagy, apoptosis, pyroptosis, transcription and metabolism. However, many of them remain to be characterized and likely function in other pathways, including the modulation of host metabolism, as described below.

T4SS-mediated modulation of host cell metabolism

Metabolism is a series of chemical transformations that regulate cell survival, proliferation and fate, among others. Metabolic pathways, such as the tricarboxylic acid cycle, glycolysis or lipogenesis either generate or degrade basic nutrients, provide building blocks, maintain homeostasis and produce energy. Intracellular pathogens have adapted a mesophilic and heterotrophic life style to survive and proliferate in the microenvironment of eukaryotic cells, including immune cells, by exploiting metabolic pathways and intermediates provided by the host [17,18]. Thus, multiple substrates are used by *C. burnetii* in catabolic and anabolic process and thus, in a bipartite-type metabolic network [19]. Similarly, *L. pneumophila* exhibits a bipartite metabolic network. Depending on the stage of infection, *L. pneumophila* uses either, amino acids, carbohydrates or glycerol as major substrates [20].

The role of autophagy and amino acids for pathogenicity

Several pathogens described above interfere with autophagy, a lysosomal degradative pathway that ensures cellular homeostasis by elimination of defective organelles, proteins, aggregates or intracellular bacteria [21]. Some bacteria circumvent autophagy at different steps of the process, while others exploit autophagy to acquire nutrients, specific autophagic proteins or membranes [22]. *C. burnetii* requires the interaction of the *C. burnetii*-containing vacuole (CCV) with the autophagic pathway for intracellular survival and replication [10,23–25]. While it is clear that the T4SS effector protein Cig2/CvpB contributes to the fusion of the CCV with autophagosomes [26,27,28•,29•,30], the role of autophagy for *C. burnetii* replication is still not resolved. It might be essential for homotypic fusion of CCVs [10,28•] or for providing an ample supply of nutrients [31•]. *A. phagocytophilum* manipulates the host autophagy degradation pathway by secreting the T4SS effector protein Ats-1 which binds directly to Beclin 1, a central regulator of autophagy [32]. *E. chaffeensis* ejects the T4SS effector protein Etf-1, which interacts with the RAB5-BECN1-PIK3C3 autophagy master regulator complex via GTP-bound RAB5, to induce autophagy [33•]. *A. phagocytophilum*, as well as *E. chaffeensis*, have limited capacity for biosynthesis and metabolism and, thus, strongly depend on the host cell in order to acquire nutrients needed for survival and replication [34]. However, whether these nutrients are provided by the autophagic pathway still awaits verification.

C. burnetii is auxotrophic for 11 amino acids [35•] and therefore only proliferates in an environment containing them in sufficient amounts [19,36]. The autophagic machinery might be important for the delivery of these essential amino acids to the CCV, but other pathways might be also involved. In addition, it remains unclear which role-specific amino acids have for *C. burnetii* physiology and how the uptake of specific amino acids is spatially and temporally regulated. *C. burnetii* encodes several predicted amino acid and peptide transporters [37], but it is not yet known if they are required for intracellular growth. For *L. pneumophila* it was demonstrated that several putative transporters ensure the uptake of essential nutrients into the *L. pneumophila*-containing vacuole (LCV) [38–40]. Furthermore, the bacterium is able to boost the levels of nutrients, such as amino acids, in the host cell cytosol. The T4SS effector protein AnkB increases the amount of free amino acids by inducing proteolysis by the proteasome [41]. Recently, a study identified two T4SS effector proteins that modulate the activity of the master regulator of host amino acid metabolism, the mechanistic target of rapamycin complex 1 (mTORC1). SidE and Lgt work in concert to provide host cell amino acids for bacterial use [42•]. This is important for the *L. pneumophila* infection as the presence of amino acids was shown to lead to bacterial replication [39]. In contrast, nutrient limitation initiated an egress mechanism in which *L. pneumophila* differentiated into a transmissive form and then a spore-like form, which is well equipped for persisting in the environment or for infecting new hosts [20]. Whether *C. burnetii* also encodes T4SS effector proteins that regulate the availability of amino acids is currently unknown. However, because amino acids can be utilized by *C. burnetii* as the only macronutrients [31•], studying if and which role-specific amino acids play in the context of the *C. burnetii* developmental cycle will greatly increase our understanding about bacterial pathogenicity and/or persistence. That certain amino acids are indispensable for *C. burnetii* survival is highlighted by the paper of Sandoz *et al.* [35•], in which the authors utilize auxotrophy complementation as selection for genetic manipulation of *C. burnetii*.

L. pneumonia also modulates mitochondrial dynamics in both T4SS-dependent and T4SS-independent manners to induce a switch from mitochondrial respiration to a Warburg-like phenotype, which enables bacterial replication [43••]. Whether *C. burnetii* also influences the mitochondria to alter host cell metabolism is unknown. However, it is tempting to speculate that this might indeed be the case, as mitochondrial localization has been demonstrated for several T4SS effector proteins, including AnkJ [44], CBU1825 [9], CbuA0020 [45] and MceA [46].

The role of carbohydrate metabolism for pathogenicity

In addition to amino acids, *C. burnetii* utilizes carbohydrates for growth in axenic medium. Glucose serves for the biosynthesis of cell wall compounds and for generating energy via glycolysis and the TCA cycle. The gluconeogenic substrate glycerol is taken up and predominantly used in the synthesis of cell wall components [19]. In contrast, *L. pneumophila* channels both glucose and glycerol primarily into anabolic reactions, such as gluconeogenesis and the pentose phosphate pathway [47]. *L. pneumophila* also metabolizes *myo*-inositol to contribute to intracellular proliferation and virulence [48]. It is not clear if the bacteria use T4SS effector molecules to actively manipulate host carbohydrate anabolic or catabolic pathways for provision of these substrates. The *B. abortus* T4SS effector protein BPE123 suggests that such a scenario is possible [49]. BPE123 interacts with the key glycolytic/gluconeogenic host enzyme α-enolase, leads to its association with the *Brucella*-containing vacuole, and induces structural and/or functional changes resulting in α-enolase activation. Enzyme depletion by RNA interference impairs *B. abortus* intracellular replication in HeLa cells, confirming the protein's role during the infection process [49]. If this interaction serves to provide a nutrient source for the bacteria or if it affects another biological activity of the multifunctional protein α-enolase, remains an exciting and open question.

The role of lipids in pathogenesis

In addition to amino acids, glucose or glycerol, intracellular pathogens seem to rely on the lipid metabolism of the host. Accordingly, *L. pneumophila* manipulates components of host cell lipid metabolism. The pathogen secretes T4SS effector proteins, such as RidL, SidP, SidF, LidA and LpnE, which interact with host phosphatidylinositols to either evade the endocytic pathway or to nurture interactions between the *L. pneumophila* vacuole, the endoplasmic reticulum or other secretory pathways [50]. LpdA is another T4SS effector protein with phospholipase activity, which was shown to contribute to virulence of *L. pneumophila* in the murine lung [51]. In addition, host-cell endosomal Rab GTPases bind and activate the T4SS effector protein VipD, which then exhibits phospholipase A1 activity, and modulates the lipid composition of endosomal membranes [52]. Moreover, *L. pneumophila* also modulates host sphingolipid metabolism via the T4SS effector protein sphingosine-1 phosphate lyase (*Lp*Spl) [53]. Mechanistically, *Lp*Spl depletes host S1P and inhibits autophagy in the host cell, facilitating intracellular replication of *L. pneumophila* [53].

Furthermore, *C. burnetii* also exploits host cell lipids for its own benefit. The *C. burnetii* T4SS effector protein Cig2/CvpB interacts with phosphatidylinositol 3-phosphate (PI(3)P) and phosphatidylserine on both CCV and early endosomes. Cig2/CvpB then perturbs the activity of PI(5)P, which increases the levels of PI (3)P on the CCV membrane, leading to enhanced delivery of membrane material, proteins and lipids to the CCV [28•]. Moreover, the oxysterol-binding protein-related protein 1 long (ORP1L) was shown to localize to the CCV in a T4SS-dependent manner. In fact, ORP1L can simultaneously bind to the CCV and the endoplasmic reticulum, suggesting that ORP1L is a component of CCV–ER membrane contact sites [54]. The assumption, that cholesterol is then transported to lipid droplets (LDs), is similar to the adenovirus model, in which the RIDα protein was demonstrated to mediate host LDL-cholesterol transport through ORP1L for esterification and storage in LDs [55]. In fact, one rapidly growing field of research relating pathogen interactions with lipid metabolism is the study of lipid droplets.

Lipid droplets

LDs are highly dynamic organelles present in virtually all eukaryotic cell types and also in several prokaryotes [56]. These organelles are composed of a hydrophobic core of neutral lipids, such as triglycerides and cholesterol esters, with relative amounts varying between cell types. This core is surrounded by a phospholipid monolayer and a set of associated ubiquitous proteins [57,58]. LDs not only regulate intracellular lipid storage and lipid metabolism, providing energy depots and cellular building blocks, they also contribute to adaptive stress responses, cell survival and immune response signaling [56,59,60]. More importantly, LDs play a major role in host–pathogen interactions [61]. They are hijacked by intracellular pathogens, such as *Chlamydia trachomatis*, to capture triglycerides and cholesterol esters for bacterial replication, while *Mycobacterium tuberculosis* uses triglycerides in LDs as an energy source and *Mycobacterium leprae* induces LD biogenesis as part of the bacterial immune evasion strategy required for persistent infection [62]. LDs have also been associated with *C. burnetii* infections [63]. In 1994, Brouqui *et al.* showed an immunohistologic image of *C. burnetii*-infected and fat-loaded macrophages in resected heart valves of an infected patient. Moreover, in 2012, Graham *et al.* observed that LDs are present in intact CCVs in primary human alveolar macrophages [64]. Perilipin-2 (PLIN2), also called adipose differentiation-related protein, a protein that surrounds the LD, was shown through comparative microarray analysis to be upregulated in the human monocytic cell line THP-1 after infection with *C. burnetii* [65]. Recently, Mulye *et al.* observed that, after treating *C. burnetii*-infected mouse alveolar macrophages (MH-S) with chloramphenicol, less LDs were counted per cell indicating that *C. burnetii* protein synthesis is indeed essential for LD accumulation [66]. They also observed a reduced number of host LDs after infecting MH-S and THP-1 cells with the *C. burnetii* T4SS *dotA* mutant as compared to wild type *C. burnetii*. This clearly demonstrates that induction of host LD accumulation is dependent on *C. burnetii* effector protein(s). The authors hypothesized that the increased number of LDs in infected macrophages could be due to fission of already existing LDs rather than the formation of new ones [67]. This was supported by the observation of smaller sized LDs in *C. burnetii*-infected cells as opposed to non-infected cells. Their data also revealed that blocking LD formation or breakdown either increased or suppressed *C. burnetii* growth, respectively [66]. Thus, *C. burnetii* growth is altered when LD homeostasis is manipulated. Yet, it is still unknown whether *C. burnetii* utilizes fatty acids or sterols released by the breakdown of LDs as nutrient depots or for producing lipid immune mediators such as prostaglandin E2.

Cholesterol

(3β)-Cholest-5-en-3-ol, i.e. or cholesterol, is a sterol and, in its non-esterified form, an important lipid component of eukaryotic membranes. Its presence in a lipid bilayer strongly influences membrane structure by increasing rigidity and hydrophobicity, thereby decreasing membrane permeability (reviewed in Ref. [68]). Cholesterol is also an essential element of nanoscale membrane microdomains known as 'lipid rafts'. They serve as spatial organizers in the membrane to compartmentalize events, such as signaling and trafficking in immune receptor activation and phospholipase D activation in mechanotransduction, or single-pass transmembrane protein sorting to the plasma membrane (for a review, see Ref. [69]). Pathogens frequently use these lipid raft domains as entry sites into their target cells. Like other intracellular pathogens, *C. burnetii* interacts with host cell cholesterol at many different levels. This was thoroughly reviewed recently for obligate intracellular pathogens [70]. We will, therefore, focus solely on *Coxiella*–cholesterol interactions.

The entry of *C. burnetii* into non-phagocytic murine embryonic fibroblasts (MEFs) is strongly impaired when genetically engineered MEFs are used that are only able to synthesize desmosterol, the immediate precursor of cholesterol. These cells are also defective in lipid raft formation. Invasion is restored if the MEFs are cultured in cholesterol-containing medium. Intracellular replication of bacteria, that had already invaded the cells, is not affected by the absence of cholesterol [71].

Cholesterol has opposing effects on the CCV. During the early stages of CCV formation, increased concentrations of cholesterol lead to increased acidification of the CCV, resulting in *C. burnetii* death [72]. A caveat, which should be taken into account here, is that it is not clear if and how the presence of desmosterol affects the impact of cholesterol on membrane structure. Even though desmosterol cannot functionally replace cholesterol in lipid raft formation, it can substitute for cholesterol in cellular sterol homeostasis and in proliferation, and their lipid–lipid interaction determining properties in biological membranes are very similar [73]. Hence, one cannot exclude that the presence of desmosterol in the cholesterol-deficient MEFs might contribute to the bacteriolytic effect of the externally added cholesterol. The combined desmosterol and cholesterol concentrations could probably lead to an unnaturally rigid and impermeable CCV membrane, resulting in the observed lysis of the bacteria. Between four and six days post-infection, cholesterol biosynthesis and uptake is transiently upregulated in *C. burnetii*-infected cells [74]. Accordingly, the CCV membrane is sterol-rich. However, it is still not clear if this is a feature of importance for the bacterial infectious cycle or rather a consequence of manipulating intracellular vesicle transport processes. The possibility that bacterial effectors contribute to this process is based on the observation that the host protein ORP1L is recruited in a T4SS-dependent manner at early time-points to the CCV ([54]; see the section on metabolic pathways). Additional experiments will be required not only to clarify the role of ORP1L in CCV–ER interactions and cholesterol trafficking, but also to identify the effector molecule(s) involved.

C. burnetii contains two eukaryote-like sterol reductase homologs, Cbu_1158 and Cbu_1206. Sterol reductases reduce double bonds during sterol biosynthesis. The bacterium itself does not synthesize detectable amounts of cholesterol [71], even though Cbu_1206 can functionally complement a Δ24 sterol reductase deletion mutant in yeast. Thus, Cbu_1206 enables the *Sacchromyces cerevisiae* Δ24 sterol reductase deletion mutant *erg4* to generate the end-product ergosterol [75]. The Cbu_1158 amino acid sequence groups with proteins described to be sterol-Δ7-reductases [76], but has not yet been characterized biochemically. Both proteins are not classified as T4SS effectors and most likely locate to the bacterial cytoplasmic membrane. In addition, they are not fully conserved in published *Coxiella* genomes. When compared with the genome sequences of the *C. burnetii* Nine Mile Phase I [37] and phase II [77] isolates, several strains, for example CbuG_Q212, Cb175_Guyana or 701CbB1, contain different frameshift mutations in Cbu_1158, leading to truncated and, most likely, non-functional proteins. Cbu_1206, in contrast, possesses only different combinations of up to three synonymous and/or four non-synonymous codon exchanges in other isolates (see Supplementary Table 1 for details). It will be interesting to see if a knock-out of both genes has an effect on infectivity and pathogenicity. Taken together, the interactions of cholesterol with *C. burnetii* are complex, time-dependent and still far from being fully understood.

Conclusions

For obligate intracellular pathogens, a coordinated interaction with the host cell is essential for establishing a niche permissive for intracellular replication. The interactions between host and *C. burnetii* can be considered to represent co-existence. The metabolic activities of both partners are formed and modified by this intimate interaction. Current data indicate that the T4SS and its effector proteins modulate host cell metabolic pathways for bacterial benefit. However, how the secretion of specific effector proteins is regulated is unknown. The bacteria might sense certain metabolic states to initiate the secretion of effector proteins that alter host cell metabolism. How the pathogens profit from this is also largely unknown. They might use metabolites as nutrients, might need them for the establishment and maintenance of the replicative vacuole or use them to alter anti-microbial host cell responses. A more intensive characterization of these interactions will not only provide new insights into pathogenesis and host cell physiology, it might even open up avenues for drug discovery to treat the respective disease.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mib.2018.11.010.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Grohmann E, Christie PJ, Waksman G, Backert S: **Type IV secretion in Gram-negative and Gram-positive bacteria**. *Mol Microbiol* 2018, **107**:455-471.
2. Oliva G, Sahr T, Buchrieser C: **The life cycle of *L. pneumophila*: cellular differentiation is linked to virulence and metabolism**. *Front Cell Infect Microbiol* 2018, **8**:3.
3. Eisenreich W, Heesemann J, Rudel T, Goebel W: **Metabolic host responses to infection by intracellular bacterial pathogens**. *Front Cell Infect Microbiol* 2013, **3**:24.
4. Cutler SJ, Bouzid M, Cutler RR: **Q fever**. *J Infect* 2007, **54**:313-318.
5. Mori M, Roest HJ: **Farming, Q fever and public health: agricultural practices and beyond**. *Arch Public Health* 2018, **76**:2.
6. Delsing CE, Warris A, Bleeker-Rovers CP: **Q fever: still more queries than answers**. *Adv Exp Med Biol* 2011, **719**:133-143.
7. Maurin M, Raoult D: **Q fever**. *Clin Microbiol Rev* 1999, **12**:518-553.
8. Beare PA, Gilk SD, Larson CL, Hill J, Stead CM, Omsland A, Cockrell DC, Howe D, Voth DE, Heinzen RA: **Dot/Icm type IVB secretion system requirements for *Coxiella burnetii* growth in human macrophages**. *MBio* 2011, **2**:e00175-00111.
9. Carey KL, Newton HJ, Lührmann A, Roy CR: **The *Coxiella burnetii* Dot/Icm system delivers a unique repertoire of type IV effectors into host cells and is required for intracellular replication**. *PLoS Pathog* 2011, **7**:e1002056.
10. Lührmann A, Newton HJ, Bonazzi M: **Beginning to understand the role of the type iv secretion system effector proteins in *Coxiella burnetii* pathogenesis**. *Curr Top Microbiol Immunol* 2017, **413**:243-268.
11. Vogel JP, Isberg RR: **Cell biology of *Legionella pneumophila***. *Curr Opin Microbiol* 1999, **2**:30-34.
12. Sherwood RK, Roy CR: **Autophagy evasion and endoplasmic reticulum subversion: the Yin and Yang of *Legionella* intracellular infection**. *Annu Rev Microbiol* 2016, **70**:413-433.
13. Celli J: **The changing nature of the *Brucella*-containing vacuole**. *Cell Microbiol* 2015, **17**:951-958.
14. Ismail N, McBride JW: **Tick-borne emerging infections: ehrlichiosis and anaplasmosis**. *Clin Lab Med* 2017, **37**:317-340.
15. Rikihisa Y: **Role and function of the type IV secretion system in *Anaplasma* and *Ehrlichia* species**. *Curr Top Microbiol Immunol* 2017, **413**:297-321.
16. Yan Q, Lin M, Huang W, Teymournejad O, Johnson JM, Hays FA, Liang Z, Li G, Rikihisa Y: ***Ehrlichia* type IV secretion system effector Etf-2 binds to active RAB5 and delays endosome maturation**. *Proc Natl Acad Sci U S A* 2018, **115**:E8977-E8986.
17. Sprenger M, Kasper L, Hensel M, Hube B: **Metabolic adaptation of intracellular bacteria and fungi to macrophages**. *Int J Med Microbiol* 2018, **308**:215-227.
18. Eisenreich W, Rudel T, Heesemann J, Goebel W: **To eat and to be eaten: mutual metabolic adaptations of immune cells and intracellular bacterial pathogens upon infection**. *Front Cell Infect Microbiol* 2017, **7**:316.
19. Häuslein I, Cantet F, Reschke S, Chen F, Bonazzi M, Eisenreich W: **Multiple substrate usage of *Coxiella burnetii* to feed a bipartite metabolic network**. *Front Cell Infect Microbiol* 2017, **7**:285.
20. Eisenreich W, Heuner K: **The life stage-specific pathometabolism of *Legionella pneumophila***. *FEBS Lett* 2016, **590**:3868-3886.
21. Glick D, Barth S, Macleod KF: **Autophagy: cellular and molecular mechanisms**. *J Pathol* 2010, **221**:3-12.
22. Bah A, Vergne I: **Macrophage autophagy and bacterial infection**. *Front Immunol* 2017, **8**:1483.
23. Gutierrez MG, Vazquez CL, Munafo DB, Zoppino FC, Beron W, Rabinovitch M, Colombo MI: **Autophagy induction favours the generation and maturation of the *Coxiella*-replicative vacuoles**. *Cell Microbiol* 2005, **7**:981-993.
24. Winchell CG, Steele S, Kawula T, Voth DE: **Dining in: intracellular bacterial pathogen interplay with autophagy**. *Curr Opin Microbiol* 2016, **29**:9-14.
25. Larson CL, Martinez E, Beare PA, Jeffrey B, Heinzen RA, Bonazzi M: **Right on Q: genetics begin to unravel *Coxiella burnetii* host cell interactions**. *Future Microbiol* 2016, **11**:919-939.
26. Maturana P, Graham JG, Sharma UM, Voth DE: **Refining the plasmid-encoded type IV secretion system substrate repertoire of *Coxiella burnetii***. *J Bacteriol* 2013, **195**:3269-3276.
27. Voth DE, Beare PA, Howe D, Sharma UM, Samoilis G, Cockrell DC, Omsland A, Heinzen RA: **The *Coxiella burnetii* cryptic plasmid is enriched in genes encoding type IV secretion system substrates**. *J Bacteriol* 2011, **193**:1493-1503.
28. • Martinez E, Allombert J, Cantet F, Lakhani A, Yandrapalli N, Neyret A, Norville IH, Favard C, Muriaux D, Bonazzi M: ***Coxiella burnetii* effector CvpB modulates phosphoinositide metabolism for optimal vacuole development**. *Proc Natl Acad Sci U S A* 2016, **113**:E3260-3269.

The authors mechanistically link the activity of CvpB (Cig2) to the fusion of autophagic vacuoles with the CCV. This step in replicative vacuole biogenesis is important for Coxiella virulence in an*in vivo* wax moth insect model.

29. • Kohler LJ, Reed Sh C, Sarraf SA, Arteaga DD, Newton HJ, Roy CR: **Effector protein Cig2 decreases host tolerance of infection by directing constitutive fusion of autophagosomes with the *Coxiella*-containing vacuole**. *MBio* 2016, **7**.

The authors study the effect of a*cig2* (*cvpB*) inactivating transposon mutation on CCV biogenesis. They demonstrated that Cig2 function is needed for fusion of the CCV with autophagosomes and for virulence in the wax moth disease model.

30. Winchell CG, Graham JG, Kurten RC, Voth DE: ***Coxiella burnetii* type IV secretion-dependent recruitment of macrophage autophagosomes**. *Infect Immun* 2014, **82**:2229-2238.
31. • Vallejo Esquerra E, Yang H, Sanchez SE, Omsland A: **Physicochemical and nutritional requirements for axenic replication suggest physiological basis for *Coxiella burnetii* niche restriction**. *Front Cell Infect Microbiol* 2017, **7**:190.

This paper contributes to a better understanding of*C. burnetii* intracellular niche restrictions, demonstrating that solely host amino acids are essential macronutrients for bacterial replication, although glycolytic and gluconeogenic substrates can also be used for generating biomass.

32. Niu H, Xiong Q, Yamamoto A, Hayashi-Nishino M, Rikihisa Y: **Autophagosomes induced by a bacterial Beclin 1 binding protein facilitate obligatory intracellular infection**. *Proc Natl Acad Sci U S A* 2012, **109**:20800-20807.
33. • Lin M, Liu H, Xiong Q, Niu H, Cheng Z, Yamamoto A, Rikihisa Y: ***Ehrlichia* secretes Etf-1 to induce autophagy and capture nutrients for its growth through RAB5 and class III phosphatidylinositol 3-kinase**. *Autophagy* 2016, **12**:2145-2166.

This paper describes for the first time the activity of an*Ehrlichia chaffeensis* T4SS effector protein. Etf-1 induces autophagy to increase the pool of available nutrients for bacterial replication.

34. Dunning Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen JA, Seshadri R, Ren Q, Wu M, Utterback TR *et al.*: **Comparative genomics of emerging human ehrlichiosis agents**. *PLoS Genet* 2006, **2**:e21.
35. • Sandoz KM, Beare PA, Cockrell DC, Heinzen RA: **Complementation of arginine auxotrophy for genetic transformation of *Coxiella burnetii* by use of a defined axenic medium**. *Appl Environ Microbiol* 2016, **82**:3042-3051.

In this study the authors not only examined the amino acid auxotrophies of*C. burnetii*, but more importantly developed a non-antibiotic tool for genetic manipulation of *C. burnetii*.

36. Hackstadt T, Williams JC: **Stability of the adenosine 5'-triphosphate pool in *Coxiella burnetii*: influence of pH and substrate**. *J Bacteriol* 1981, **148**:419-425.
37. Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, Ward NL, Tettelin H, Davidsen TM, Beanan MJ *et al.*: **Complete genome sequence of the Q-fever pathogen *Coxiella burnetii***. *Proc Natl Acad Sci U S A* 2003, **100**:5455-5460.
38. Jones SC, Price CT, Santic M, Abu Kwaik Y: **Selective requirement of the shikimate pathway of *Legionella pneumophila* for intravacuolar growth within human macrophages but not within Acanthamoeba**. *Infect Immun* 2015, **83**:2487-2495.
39. Sauer JD, Bachman MA, Swanson MS: **The phagosomal transporter A couples threonine acquisition to differentiation and replication of *Legionella pneumophila* in macrophages**. *Proc Natl Acad Sci U S A* 2005, **102**:9924-9929.
40. Wieland H, Ullrich S, Lang F, Neumeister B: **Intracellular multiplication of *Legionella pneumophila* depends on host cell amino acid transporter SLC1A5**. *Mol Microbiol* 2005, **55**:1528-1537.
41. Price CT, Al-Quadan T, Santic M, Rosenshine I, Abu Kwaik Y: **Host proteasomal degradation generates amino acids essential for intracellular bacterial growth**. *Science* 2011, **334**:1553-1557.
42. • De Leon JA, Qiu J, Nicolai CJ, Counihan JL, Barry KC, Xu L, Lawrence RE, Castellano BM, Zoncu R, Nomura DK *et al.*: **Positive and negative regulation of the master metabolic regulator mTORC1 by two families of *Legionella pneumophila* effectors**. *Cell Rep* 2017, **21**:2031-2038.

The authors describe two*L. pneumophila* T4SS effector proteins that modulate the activity of mTORC1, the master regulator of host amino acid metabolism.

43. •• Escoll P, Song OR, Viana F, Steiner B, Lagache T, Olivo-Marin JC, Impens F, Brodin P, Hilbi H, Buchrieser C: ***Legionella pneumophila* modulates mitochondrial dynamics to trigger metabolic repurposing of infected macrophages**. *Cell Host Microbe* 2017, **22**:302-316 e307.

In this study the authors identified with MitF for the first time a translocated effector protein that induces mitochondrial fragmentation. The*L. pneumophila*-induced modulation of mitochondrial dynamics promotes a metabolic switch. Thus, the T4SS-induced Warburg-like effect favors bacterial replication.

44. Voth DE, Howe D, Beare PA, Vogel JP, Unsworth N, Samuel JE, Heinzen RA: **The *Coxiella burnetii* ankyrin repeat domain-containing protein family is heterogeneous, with C-terminal truncations that influence Dot/Icm-mediated secretion**. *J Bacteriol* 2009, **191**:4232-4242.
45. Weber MM, Chen C, Rowin K, Mertens K, Galvan G, Zhi H, Dealing CM, Roman VA, Banga S, Tan Y *et al.*: **Identification of *Coxiella burnetii* type IV secretion substrates required for intracellular replication and *Coxiella*-containing vacuole formation**. *J Bacteriol* 2013, **195**:3914-3924.
46. Fielden LF, Moffatt JH, Kang Y, Baker MJ, Khoo CA, Roy CR, Stojanovski D, Newton HJ: **A farnesylated *Coxiella burnetii* effector forms a multimeric complex at the mitochondrial outer membrane during infection**. *Infect Immun* 2017, **85**.
47. Hauslein I, Manske C, Goebel W, Eisenreich W, Hilbi H: **Pathway analysis using (13) C-glycerol and other carbon tracers reveals a bipartite metabolism of *Legionella pneumophila***. *Mol Microbiol* 2016, **100**:229-246.
48. Manske C, Schell U, Hilbi H: **Metabolism of myo-Inositol by *Legionella pneumophila* promotes infection of amoebae and macrophages**. *Appl Environ Microbiol* 2016, **82**:5000-5014.
49. Marchesini MI, Morrone Seijo SM, Guaimas FF, Comerci DJ: **A T4SS effector targets host cell alpha-enolase contributing to *Brucella abortus* intracellular lifestyle**. *Front Cell Infect Microbiol* 2016, **6**:153.
50. Pizarro-Cerda J, Kuhbacher A, Cossart P: **Phosphoinositides and host-pathogen interactions**. *Biochim Biophys Acta* 2015, **1851**:911-918.
51. Schroeder GN, Aurass P, Oates CV, Tate EW, Hartland EL, Flieger A, Frankel G: ***Legionella pneumophila* effector LpdA Is a palmitoylated phospholipase D virulence factor**. *Infect Immun* 2015, **83**:3989-4002.
52. Gaspar AH, Machner MP: **VipD is a Rab5-activated phospholipase A1 that protects *Legionella pneumophila* from endosomal fusion**. *Proc Natl Acad Sci U S A* 2014, **111**:4560-4565.
53. Rolando M, Escoll P, Nora T, Botti J, Boitez V, Bedia C, Daniels C, Abraham G, Stogios PJ, Skarina T *et al.*: ***Legionella pneumophila* S1P-lyase targets host sphingolipid metabolism and restrains autophagy**. *Proc Natl Acad Sci U S A* 2016, **113**:1901-1906.
54. Justis AV, Hansen B, Beare PA, King KB, Heinzen RA, Gilk SD: **Interactions between the *Coxiella burnetii* parasitophorous vacuole and the endoplasmic reticulum involve the host protein ORP1L**. *Cell Microbiol* 2017, **19**.
55. Cianciola NL, Greene DJ, Morton RE, Carlin CR: **Adenovirus RIDalpha uncovers a novel pathway requiring ORP1L for lipid droplet formation independent of NPC1**. *Mol Biol Cell* 2013, **24**:3309-3325.
56. Walther TC, Farese RV Jr: **Lipid droplets and cellular lipid metabolism**. *Annu Rev Biochem* 2012, **81**:687-714.
57. Tauchi-Sato K, Ozeki S, Houjou T, Taguchi R, Fujimoto T: **The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition**. *J Biol Chem* 2002, **277**:44507-44512.
58. Kory N, Thiam AR, Farese RV Jr, Walther TC: **Protein crowding is a determinant of lipid droplet protein composition**. *Dev Cell* 2015, **34**:351-363.
59. Farese RV Jr, Walther TC: **Lipid droplets finally get a little R-E-S-P-E-C-T**. *Cell* 2009, **139**:855-860.
60. Henne WM, Reese ML, Goodman JM: **The assembly of lipid droplets and their roles in challenged cells**. *EMBO J* 2018, **37**.
61. Melo RC, Weller PF: **Lipid droplets in leukocytes: organelles linked to inflammatory responses**. *Exp Cell Res* 2016, **340**:193-197.
62. Herker E, Ott M: **Emerging role of lipid droplets in host/pathogen interactions**. *J Biol Chem* 2012, **287**:2280-2287.
63. Brouqui P, Dumler JS, Raoult D: **Immunohistologic demonstration of *Coxiella burnetii* in the valves of patients with Q fever endocarditis**. *Am J Med* 1994, **97**:451-458.
64. Graham JG, MacDonald LJ, Hussain SK, Sharma UM, Kurten RC, Voth DE: **Virulent *Coxiella burnetii* pathotypes productively infect primary human alveolar macrophages**. *Cell Microbiol* 2013, **15**:1012-1025.
65. Ren Q, Robertson SJ, Howe D, Barrows LF, Heinzen RA: **Comparative DNA microarray analysis of host cell transcriptional responses to infection by *Coxiella burnetii* or *Chlamydia trachomatis***. *Ann N Y Acad Sci* 2003, **990**:701-713.
66. Mulye M, Zapata B, Gilk SD: **Altering lipid droplet homeostasis affects *Coxiella burnetii* intracellular growth**. *PLoS One* 2018, **13**:e0192215.
67. Long AP, Manneschmidt AK, VerBrugge B, Dortch MR, Minkin SC, Prater KE, Biggerstaff JP, Dunlap JR, Dalhaimer P: **Lipid droplet de novo formation and fission are linked to the cell cycle in fission yeast**. *Traffic* 2012, **13**:705-714.
68. Subczynski WK, Pasenkiewicz-Gierula M, Widomska J, Mainali L, Raguz M: **High cholesterol/low cholesterol: effects in biological membranes: a review**. *Cell Biochem Biophys* 2017, **75**:369-385.
69. Raghunathan K, Kenworthy AK: **Dynamic pattern generation in cell membranes: current insights into membrane organization**. *Biochim Biophys Acta* 2018.
70. Samanta D, Mulye M, Clemente TM, Justis AV, Gilk SD: **Manipulation of host cholesterol by obligate intracellular bacteria**. *Front Cell Infect Microbiol* 2017, **7**:165.
71. Gilk SD, Cockrell DC, Luterbach C, Hansen B, Knodler LA, Ibarra JA, Steele-Mortimer O, Heinzen RA: **Bacterial colonization of host cells in the absence of cholesterol**. *PLoS Pathog* 2013, **9**:e1003107.

72. Mulye M, Samanta D, Winfree S, Heinzen RA, Gilk SD: **Elevated cholesterol in the *Coxiella burnetii* intracellular niche is bacteriolytic**. *MBio* 2017, **8**.

73. Huster D, Scheidt HA, Arnold K, Herrmann A, Muller P: **Desmosterol may replace cholesterol in lipid membranes**. *Biophys J* 2005, **88**:1838-1844.

74. Howe D, Heinzen RA: ***Coxiella burnetii* inhabits a cholesterol-rich vacuole and influences cellular cholesterol metabolism**. *Cell Microbiol* 2006, **8**:496-507.

75. Gilk SD, Beare PA, Heinzen RA: ***Coxiella burnetii* expresses a functional Delta24 sterol reductase**. *J Bacteriol* 2010, **192**:6154-6159.

76. Moliner C, Raoult D, Fournier P-E: **Evidence that the intra-amoebal *Legionella drancourtii* acquired a sterol reductase gene from eukaryotes**. *BMC Res Notes* 2009, **2**:51.

77. Millar JA, Beare PA, Moses AS, Martens CA, Heinzen RA, Raghavan R: **Whole-genome sequence of *Coxiella burnSetii* nine mile RSA439 (phase ii, clone 4), a laboratory workhorse strain**. *Genome Announc* 2017, **5**:e00471-17.