11-2011

Genetics of Coxiella burnetii: on the path of specialization

Michael F. Minnick
University of Montana, Missoula

Rahul Raghavan
Yale University

Published in final edited form as:
Future Microbiol. 2011 November ; 6(11): 1297–1314. doi:10.2217/fmb.11.116.

Genetics of *Coxiella burnetii*: on the path of specialization

Michael F Minnick[†,1] and **Rahul Raghavan**[2]

[1]Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA

[2]Department of Ecology & Evolutionary Biology, Yale University, New Haven, CT 06520, USA

Abstract

Coxiella burnetii is an extremely infectious, zoonotic agent that causes Q fever in humans. With the exception of New Zealand, the bacterium is distributed worldwide. *Coxiella* is classified as a select agent based on its past and potential use as a bioweapon and its threat to public health. Despite decades of research, we know relatively little regarding *Coxiella*'s molecular pathogenesis, and a vaccine is not widely available. This article briefly reviews the unusual genetics of *C. burnetii*; a pathogen that retains telltale genetic mementos collected over the course of its evolutionary path from a free-living bacterium to an obligate intracellular parasite of eukaryotic host cell phagosomes. Understanding why these genetic elements are maintained may help us better understand the biology of this fascinating pathogen.

Keywords

chromosome; *Coxiella*; genome reduction; genomics; genotype; plasmids; Q fever; selfish genetic elements

Background

History

Q fever is short for 'query' fever; a moniker first used by Edward Derrick to describe a 1935 outbreak of unknown etiology among abattoir workers in Brisbane, Australia. Although Derrick was unable to isolate the elusive microorganism, he was the first person to suspect a rickettsia and used guinea pigs as an animal model of infection by inoculating them with patient blood and urine [1]. Shortly thereafter, the actual pathogen was discovered independently and almost concurrently by two research groups working at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia and half way around the world at the Rocky Mountain Laboratories in Hamilton, Montana, USA. The Australians, Frank Burnet and Mavis Freeman, were the first to isolate the "virus" of Q fever from infected guinea pig liver samples provided by Derrick. The agent, on Derrick's suggestion,

[†]Author for correspondence: Tel.: +1 406 243 5972 Fax: +1 406 243 4184 mike.minnick@mso.umt.edu.

Financial & competing interests disclosure
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

became known as *Rickettsia burnetii*. Meanwhile, the American group, Harold Cox and Gordon Davis, were working with a "Nine Mile agent" found in *Dermacentor andersoni* wood ticks collected from the Nine Mile valley of Montana. Their work demonstrated the transmission of a nonfilterable agent from infected ticks to guinea pigs, and showed that the pathogen could be cultivated in embryonated chicken eggs and tissue culture. In an interesting twist of fate and medical detective work, Rolla Dyer, then Director of the NIH, visited the Rocky Mountain Laboratories for a few days in 1938; overlapping with Cox and Davis' research involving the Nine Mile agent. Within 10 days, Dyer came down with fever, sweats, chills and a severe retro-orbital headache; classical symptoms of Q fever, as described by Derrick. Subsequent work by Dyer revealed that the Nine Mile agent responsible for his acute illness was identical to *R. burnetii*, effectively bringing the story full circle. The new pathogen was renamed *Coxiella burnetii*, in 1948, to honor the two groups involved in its discovery.

Q fever

Q fever is categorized as acute or chronic in nature **(Box 1)**. Acute Q fever ranges from an asymptomatic state in roughly half of the cases, to an abrupt, flu-like illness that can be accompanied by high fever (~105°F), debilitating malaise, severe retro-orbital headache, atypical pneumonia, myalgia and hepatitis. Symptoms of acute Q fever occur about 2 weeks after exposure and can last for 1–2 weeks. Chronic Q fever (longer than 6 months) occurs in approximately 1–5% of cases and usually manifests as "culture-negative" endocarditis in patients with predisposing valvular defects [2].

Diagnosis of Q fever is typically confirmatory and relies heavily on serology to detect the presence of *Coxiella* antigens, or antibodies against them. Diagnostic tests normally involve indirect immunofluorescence assay, complement fixation or ELISA protocols, although PCR-based tests are becoming more common for early-stage detection. The titer and nature of the antibody response against *Coxiella* can be useful for the differential diagnosis of acute and chronic infection, as two antigenic forms of the bacterium, termed phase I and phase II, exist (see 'Bacteriology' section). During acute infection, the IgG antibody response against phase II antigens is first to appear, within 2 weeks of infection, and the titer is markedly higher than that of antibodies against phase I antigens, which arise later. By contrast, during chronic Q fever, titers against phase I antigens are much greater than those against phase II antigens, although both antibody types are typically elevated. In fact, phase I antibody (IgG) titers in excess of 1:800 are diagnostic for endocarditis. Finally, it is not uncommon for both phase I and II antibodies to remain elevated for months or even years without an underlying chronic infection.

Most cases of acute Q fever are self-resolving, but a 14-day course of doxycycline (100 mg/day) is highly effective at clearing the pathogen, when needed [3]. By contrast, chronic Q fever is very difficult to treat, and adults are typically given doxycycline (100 mg by mouth twice daily) in combination with hydroxychloroquine (600 mg by mouth once daily) for at least 18 months [3,4]. Even after therapeutic intervention with a combinational therapy, mortality rates of approximately 5% have been reported for chronic Q fever [3,4].

A vaccine against Q fever is not widely available. A formalin-inactivated, whole-cell preparation of the Henzerling strain (Q-Vax®; CSL Limited, Melbourne, Australia) is licensed for use in Australia, and the USA armed forces employ a similar preparation, called NDBR-105, for at-risk personnel. In addition, a soluble, trichloroacetic acid extract of phase I bacteria was used for experimental vaccination of humans in the former Czechoslovakia [5]. Although quite effective, these vaccines can only be administered after eliminating the possibility of a prior exposure to *C. burnetii* (by antibody and skin tests) in order to prevent a severe, delayed-type hypersensitivity reaction in the recipient. Moreover, these vaccines are not without potential side effects, including flulike symptoms, swelling, and erythema near the injection site.

Bacteriology

C. burnetii is a small (~0.2–0.4 μM wide by ~0.4–1.0 μM long), pleomorphic, Gram-negative, γ—proteobacterium that is most closely-related to *Legionella pneumophila* (Box 1). The bacterium is one of the most infectious agents known, with 1–10 viable bacteria able to cause an infection in the guinea pig model [6]. A generalist parasite of eukaryotic cells, *Coxiella* has been isolated from a diverse array of hosts, including arthropods, reptiles, birds and mammals. However, the most common reservoirs for transmission to humans are livestock animals, including goats, sheep and cattle, and the pathogen is shed in large quantities into their urine, milk and birth products. Although the number of reported cases would suggest otherwise (the highest was 171 cases in the USA in 2007 [7]), the pathogen is surprisingly common in the environment. For example, *Coxiella* DNA was detectable by PCR in nearly 24% of 1600 environmental samples collected from various regions of the USA between 2006 and 2008. Moreover, a PCR-based survey of American bulk-tank milk found that at least 94% of samples were positive for *Coxiella* over a 3-year period [8]. The actual number of Q fever cases is undoubtedly underreported and/or frequently misdiagnosed. An ongoing outbreak of Q fever in the Netherlands is the largest known, with over 4000 cases reported from 2007 to 2010 [9].

C burnetii is an obligate intracellular pathogen, which undergoes a developmental cycle that involves two distinct cellular forms (Figure 1). An infectious, small-cell variant (SCV) has been described as “spore-like”, and a “small-dense cell” subpopulation is extremely resistant to adverse conditions that may be encountered by the pathogen while in the extracellular environment, including heat, pressure, UV light, desiccation and certain disinfectants [10,11]. Shortly following passive entry into a host cell (typically and initially an alveolar monocyte/macrophage in humans), an SCV becomes located in an acidic (pH ~4.5), phagolysosome-like compartment called a parasitophorous vacuole (PV). At this point, interactions between the PV and autophagosomes provide nutrients, and fusion with lysosomes is delayed (i.e., a phagosome pause or stall) to enhance the pathogen’s survival [12]. The SCV then transforms into a metabolically-active, relatively fragile cell type, termed a large-cell variant (LCV) [13]. Over 5–6 days of slow intracellular replication with approximately 11 h generation time [14], the PV engages endolysosomal compartments to create a large vacuole filled with a high-density mixture of LCVs and SCVs (Figure 2). Eventually the bacterial population transforms into SCVs, which are released upon lysis of the host cell, to complete the life cycle [13,14].

Lipopolysaccharide (LPS) is the only *bona fide* virulence determinant of *C. burnetii* described to date [6]. In this role, LPS serves to mask bacterial surface antigens from pattern-recognition receptors of dendritic cells, thereby modulating the inflammatory cytokine response and allowing for infection of dendritic cells without their activation [15]. The net effect is to enhance persistence of the bacterial infection. LPS is also responsible for the phase I and II variation of the pathogen, as mentioned earlier. When *C. burnetii* is isolated from a natural, immunologically competent host, it is invariably a phase I phenotype, with LPS possessing full-length O-side chains in the outer membrane. By contrast, phase II bacteria possess LPS with truncated O-side chains and are only obtained in the laboratory following serial passage in embryonated chicken eggs or tissue-cultured host cells, undoubtedly because immunological selective pressure is absent. While phase I *Coxiella* cells are fully virulent for humans and require BSL3 containment, cloned phase II bacteria are avirulent and can be handled safely under BSL2 conditions. Phase I and II *C. burnetii* replicate with similar kinetics within indistinguishable PVs of human macrophages [16].

The type IV secretion system (T4SS) has recently attracted considerable attention as another potential virulence determinant of *C. burnetii* (see later). T4SSs are found in many proteobacteria that establish intimate symbiotic relationships with their hosts (e.g., mutualism, parasitism), where they serve to deliver bacterial effector molecules into host cells. In many respects, *Coxiella*'s T4SS resembles the type IVB (Dot/Icm) secretion system that is involved in subjugating phagosome maturation by *L. pneumophila* [17]. In fact, cloned *Coxiella* T4SS genes, such as *dotB, icmS* and *icmW,* are able to complement corresponding *L. pneumophila* mutants *in trans* [18,19]. The *Legionella* surrogate expression system has proven very useful not only for swapping out and analyzing secretion machinery components but also for analyzing effector molecules. For example, recent work has shown that the *Coxiella*'s ankyrin G protein (AnkG) can be delivered by *Legionella*'s T4SS and inhibit apoptosis of infected mammalian host cells [20]; an outcome that is characteristic of *Coxiella's* pathogenesis but not *Legionella*'s [21,22]. Moreover, transposon mutagenesis of *Coxiella*'s Dot/Icm system generates a mutant that is defective for intracellular replication [23], highlighting the potential role for T4SS in virulence.

Coxiella's genome

Coxiella genome groups

C. burnetii strains originating from disparate sources show considerable homogeneity in their 16S rRNA sequences, but have significant differences in their overall genome content. Restriction fragment-length polymorphism (RFLP) analyses that highlight these differences have been traditionally used to classify *C. burnetii* into six genomic groups [24]. More recently, a microarray based comparison of whole genomes has identified two additional genomic groups [25]. All *C. burnetii* isolates contain a related, autonomously replicating plasmid (QpH1, QpRS, QpDV and QpDG) or have plasmid-like sequences that are integrated into the chromosome. A correlation between plasmid content and disease presentation was noted in earlier studies involving a limited number of strains [26], but more extensive, recent surveys have raised questions regarding the relationship [27]. Nevertheless,

C. burnetii isolates from different genogroups have been shown to exhibit varying virulence in mouse and guinea pig models of acute Q fever, probably owing to differences in their gene repertoire [28]. Irrespective of the connection between plasmid-type and human disease, the absolute conservation of plasmid or integrated plasmid-like sequences in all isolates suggests an adaptive and essential role for these genetic elements in *Coxiella*'s biology. A recent report has confirmed this idea by demonstrating that plasmids are enriched in genes that encode Dot/Icm T4SS substrates that can potentially modify the host cell milieu to favor bacterial growth and survival [29].

Comparative genomics

C. burnetii is the only species in the *Coxiella* genus, but strains from different sources are known to be genetically diverse. The Nine Mile strain (NM), originally isolated by Cox and Davis and responsible for the first laboratory-acquired case of acute Q fever, is considered to be the reference strain [30]. The whole genome of the NM strain was sequenced in 2003 [31] followed by the sequences of two strains isolated from chronic human endocarditis cases [K (Q154) and G (Q212)], and an attenuated strain from rodents [Dugway (5J108–111)] in 2009 [32]. The genomes of all four strains are roughly 2 Mb in size with approximately 90% coding capacity (~2300 ORFs) and a G + C content of ~42.5%. The NM and K strains harbor QpH1 and QpRS plasmids, respectively, which are approximately 38 kb in size and have approximately 50 annotated ORFs. The G strain has a plasmid-like sequence integrated into its chromosome, and the Dugway strain has a larger, approximately 54 kb, QpDG plasmid with 66 annotated genes. Approximately 1500 (65%) chromosomal and 22 plasmid genes are shared among the four strains (Figure 3). A large portion (40%) of genes in *Coxiella* is of unknown function and approximately 10% of genes in all strains are pseudogenes, probably reflecting the pathogen's adaptation to its unique cellular compartment. The chromosome of *Coxiella* lacks bacteriophage or conjugation genes, and the genes associated with natural competence are pseudogenized in all strains, suggesting that the obligate intracellular parasite has limited avenues for horizontal gene transfer. However, there is intact recombination machinery, which is likely to be important in the insertion sequence (IS) element-mediated genome rearrangements observed in all four strains. Although the metabolic capability of *Coxiella* is fairly complex, deficiencies in a number of biochemical pathways are present and reflected in the pathogen's dependence on a eukaryotic host cell or a specialized medium for replication [33]. The genomes of all sequenced strains of *Coxiella* contain genes that encode proteins with eukaryotic domains, including ankyrin repeats, F boxes and tetratricopeptide repeats. These proteins may functionally mimic host cell proteins and aid in the pathogen's survival by modifying the intracellular environment. However, polymorphisms in the coding regions of these effector proteins have been observed and might contribute to strain-specific differences in virulence potential [23].

Consequences of the obligate intracellular lifestyle

The obligate dependence of *Coxiella* on eukaryotic host cells is reflected in its genome composition. Similar to other host-associated bacteria, *Coxiella* has a relatively small (~2 Mb) genome [32]. The recent availability of fully sequenced genomes from a large number of endosymbionts and parasites has increased our understanding of the phenomenon of

genome size reduction associated with host dependency. Contrary to previous speculation, there is no positive correlation between genome size and chromosome replication efficiency; in fact, many small-genomed obligate intracellular bacteria, like *Coxiella*, have unusually long generation times [34]. Studies in primary endosymbionts of insects have shown that the main driver of genome shrinkage is reduced selection pressure within the intracellular niche [35]. In addition, severe bottlenecks that occur between generations result in genetic drift, which can cause stochastic loss of advantageous genes and accumulation of slightly deleterious mutations in vital genes like ribosomal and heat-shock protein genes [36]. Another phenomenon observed in *C. burnetii* (and almost all host-associated bacteria) is an AT-rich genome, compared with related environmental bacteria. While the exact cause that underlies this skew is not understood, recent studies have shown that mutation in all bacteria is biased towards AT [37,38]. Hence, the high AT content of *Coxiella* and other obligate intracellular bacteria (under reduced selection pressure) might reflect the nucleotide composition at equilibrium, whereas strong natural selection to maintain higher GC content might underlie the observed lower AT levels in related free-living bacteria. Host cell-dependent bacteria generally have reduced opportunities for horizontal gene transfer, and consequently most ancient symbionts have minimal amounts of laterally-acquired DNA. However, recent endosymbionts and pathogens like *Coxiella* have a heavy load of selfish elements, especially ISs (see later). The relatively stable environs of the new niche render many genes superfluous, which provides IS elements with an increased array of genomic locations where their insertion causes little or no harm to the bacterium. Consequently, the number of IS elements increases in a bacterium immediately following a shift from a free-living to a host-associated lifestyle [39]. However, given enough time, most IS elements and nonfunctional genes interrupted by ISs are removed from the genome by the mutational force, which is biased towards deletion in bacteria. Although gene loss and the concomitant genome size reduction are characterized by stochastic events due to relaxed selection inside a host cell, comparative genomic approaches are starting to reveal some broad themes that are conserved across a large swath of bacteria. A recent study found that obligate intracellular bacteria from different phyla have lost the same 100 orthologous genes, suggesting a lifestyle-dependent, nonrandom pattern of gene loss [40]. Another interesting, recent observation is that the massive gene loss that accompanies the shift to a more predictable intracellular environment is tailored towards maintaining protein family diversity at the cost of protein family size (i.e., proteins with redundant functions are lost) [41]. A good example of this phenomenon is the set of genes that code for peptide chain release factors with partial overlap in codon specificity (e.g., UAG and UAA for PrfA; UGA and UAA for PrfB). *C. burnetii* has retained only one member of this family (*prfB*), while *L. pneumophila*, a related facultative intracellular parasite, possesses three.

Type IV secretion system

T4SSs are conjugation-related machines that facilitate the transport of effector molecules (nucleic acids or proteins) into host cells in order to enhance bacterial survival and virulence (reviewed in [42]). The first analysis of the *Coxiella* (NM) genome revealed sequences with considerable homology and conserved linkage to the type IVB secretion system regions 1 and 2 (R1 and R2, respectively) of *Legionella* [31]. In fact, *Coxiella*'s T4SS consists of 23 of the 26 Dot/Icm orthologs of *L. pneumophila* [31,43]. Recently, dozens of protein

substrates (effectors) of *C. burnetii*'s T4SS have been identified by two groups, using a combination of bioinformatics, bacterial two-hybrid screening using DotF (an inner membrane-associated T4SS component) as 'bait', the *Legionella* surrogate expression system, transposon mutagenesis and several innovative reporter-based screening techniques [23,44]. Interestingly, many of the identified protein substrates were previously annotated as hypothetical. A C-terminal signal domain typically required for T4SS-mediated translocation was previously shown in the Dot/Icm system of *Legionella* [45], and a similar motif was demonstrated in a *Coxiella* T4SS substrate by using a protein [CBU1825] that lacked the C-terminal 20 amino acids [44]. In addition to a signaling domain, T4SS effector proteins often contain eukaryotictype ankyrin (Ank) repeat domains [46], which are heterogeneous in nature and are known to enhance protein–protein interactions [47,48]. The original NM genome sequence analysis identified 13 genes encoding Ank domains [31], although subsequent analysis showed that many of the NM genes (e.g., AnkB) are disrupted [32]. Other strains of the bacterium (e.g., G, K, Dugway) possess intact orthologs of the Ank proteins as well as strain-specific Ank proteins, highlighting the heterogeneity of this protein family in *Coxiella* [48]. Recent work has also demonstrated that *Coxiella*'s T4SS complex is assembled at the pole(s) of the bacterium during infection of host cells, possibly to enhance interactions with the PV membrane [49]. Analysis of the virulence functions and differential targeting of effectors to specific locations within the host cell is currently of interest. As mentioned earlier, AnkG can be delivered by *Legionella*'s T4SS and inhibit apoptosis of infected mammalian host cells [20]. Recent work has also shown that a number of *Coxiella* T4SS effectors can inhibit replication of *Saccharomyces cereviseae* when overexpressed, and that ectopic overexpression of CBU0635 can interfere with host cell secretion [23]. Targeting of effectors to specific locations within the host cell (e.g., the nucleus, lysosomes, microtubules, mitochondria, cytosol and the PV membrane) has also been reported [23,48]. One interesting subset of T4SS substrates is the plasmid effector family, CpeA–CpeF, encoded on *Coxiella*'s plasmids [29]. While CpeA, B and F are encoded on the QpH1, QpRS and QpDG plasmids; CpeC, D and E are unique to QpH1. The utility and conservation of these sequences may help to explain the absolute maintenance of plasmids or integrated plasmid-like sequences by *Coxiella* [29].

Genetic elements & fossil genes

Insertion sequences

As a rule of thumb, ISs are rare in obligate intracellular bacteria, as there is little chance for genetic exchange to occur. In fact, a common notion is that genomes of obligate intracellular organisms are relatively static, with little genomic flux or plasticity. Thus, it was surprising when the first genome study discovered 19 copies of a highly-conserved IS*110*-like element of approximately 1450 bp in *Coxiella*'s NM strain, termed IS*1111* [50]. The IS*1111* element is delineated by 12 bp inverted repeats (IRs) and encodes an approximately 364 residue transposase whose expression is thought to be enhanced when the IRs align to form a circular intermediate. In the chromosome, the inner IRs of IS*1111* are flanked by 7 bp IRs that once served as a recognition site for insertion of the element. Sequence analysis of the NM strain's genome showed 21 copies of IS*1111* [31]. The study also revealed additional types of ISs, including five IS*30*-like and three IS*As1*-like elements, together with three

degenerate transposase genes of unknown phylogeny. Recent, comparative genomic work has shown that dozens of IS elements and additional IS types, including IS*652* and IS*4* families and three unknown transposases, are present in the genomes of K, G, and Dugway strains [32]. Collectively, *Coxiella* strains possess between 31 (NM) and 59 (K) IS elements belonging to eight IS families, plus three unknown transposases. Interestingly, IS elements were found to be absent in all *Coxiella* plasmids except QpDG, which had a single IS*4* element [31,32]. A high degree of sequence conservation within each IS family suggests that the elements were 'recently' acquired and then spread throughout the genome [31]. Work comparing the genetic diversity of several *Coxiella* genomic groups showed that nine out of 51 single-event polymorphisms occurred within five transposase genes or were found to contain a transposase gene [25]. In fact, differences in IS*1111* content can be used to assign isolates of *Coxiella* to specific genomic groups [51]. Recent work has conclusively shown that IS elements were involved in the chromosomal rearrangements observed between strains [32], enhancing *Coxiella*'s genetic diversity through their translocation, loss or the genomic rearrangement that occurs during transposition. IS-mediated rearrangements may also be involved in pseudogenization and the differential virulence potential of various strains.

Intervening sequence & putative S23 protein

The single-copy 23S rRNA gene of *C. burnetii* contains five selfish genetic elements, including an intervening sequence (IVS) and a nested ORF encoding a hypothetical protein. The IVS was first described by Afseth *et al*., who discovered a 444 bp element disrupting the sequence of *Coxiella*'s single-copy 23S rRNA gene [52]. The IVS is bordered by a set of 28 bp IRs and is responsible for fragmenting the 23S rRNA into approximately 1.7 and 1.2 kb segments. Like other IVSs, fragmentation is presumably caused by RNase III-mediated excision of the element during maturation of the 23S rRNA precursor, without subsequent religation of the daughter strands. Interestingly, the IVS element is also disrupted by a 106 bp ORF encoding a protein of approximately 14.2 kDa. Database searches with the predicted amino acid sequence suggest that the IVS ORF encodes a ribosomal S23p superfamily protein; a group of hypothetical polypeptides for which no function has been described to date, but whose unusual crystal structure consists of a homopentamer of four helix bundles that creates a doughnut-shaped structure with a tapered lumen [53]. The S23p protein is most closely related to orthologs encoded by the IVS ORFs of *Leptospira* spp. (~70% amino acid similarity), *Xanthomonas* and *Brucella*. Whether S23p is synthesized in *Coxiella* is unknown, and the absence of a ribosomal-binding site upstream of the ORF suggests that translation may be inefficient [52].

The roles that IVS and the S23 proteins play in *Coxiella*, or other pathogens, are mysteries. Several studies have shown that fragmented rRNAs are widespread in bacteria and eukaryotes, and the fragmentation does not interfere with ribosome function [54]. Conservation of the IVS and S23 protein sequences in all strains of *Coxiella* and across large phylogenetic distances suggests they provide a conserved function, play an adaptive role(s) and that lateral gene transfer may have been involved in their acquisition. Some studies have shown that IVS elements are more common in bacteria that associate closely with their eukaryotic hosts [55]. In this relationship, IVSs may serve as regulatory RNAs that promote

communication between bacteria and their host cells. This is conceivable, since IVSs are autonomous RNA elements derived from some of the most abundant transcripts in the bacterial cell, and their excision correlates with active protein production and exponential growth. A second possible role for IVSs is in regulating the rate of rRNA degradation during *Coxiella*'s developmental cycle. This possibility is supported by work showing that *Salmonella* strains that possessed fragmented 23S rRNAs degraded their rRNA at significantly higher rates as the cells entered stationary phase, relative to strains with comparatively less 23S rRNA fragmentation [56]. The difference undoubtedly reflects the fact that rRNA fragmentation provides more substrate for ribonucleases and RNA decay. Finally, a third possibility is that IVS splicing provides a point of regulation for maturation of the 23S rRNA, production of functional ribosomes, and fragmentation of the 23S rRNA in *Coxiella*.

Group I introns & their potential roles

Sequence analysis of the NM genome provided the first clues that a group I intron was present in *Coxiella*'s 23S rRNA gene [31]. Group I introns are selfish genetic elements that are widespread in mitochondria and chloroplasts of lower eukaryotes but are quite rare in bacteria. Group I introns encode autocatalytic RNAs; ribozymes that self-splice by means of a two-step transesterification reaction that uses guanosine as a cofactor [57]. Research examining splicing characteristics of the *Coxiella* intron resulted in the surprising discovery of two group I introns flanking an essential 34 bp exon of the 23S rRNA [58]. Both introns (Cbu.L1917 and Cbu.L1951) self-splice *in vivo* and *in vitro*, and L1917 is the only group I intron known to possess a G-to-A substitution at the 3′ (Ω) terminus. Prior to this discovery, Ω G was considered essential to group I intron splicing [59,60]. Subsequent work has shown that L1917 is a natural splice mutant with a decreased rate of self-splicing, and it is the only group I intron known to use both A and G cofactors [61]. The intron sequences of *C. burnetii* are nearly identical in all eight genomic groups [58]. This degree of conservation is highly unusual for introns, as they are normally found in various stages of degeneration (or loss) within distinct populations of an organism [62,63], and suggests that the elements confer a selective advantage upon *Coxiella*. Phylogenetic analyses suggest that the introns were acquired independently; L1917 was obtained from another bacterium such as *Thermotoga*, while L1951 was acquired from a lower eukaryote, like *Acanthamoeba* or *Chlorella* [58].

One potential role for the group I introns is to modulate *Coxiella*'s growth rate through inhibition of ribosome function, as previously shown in *E. coli* heterologously expressing the L1917 and L1951 introns and in translation reactions with a luciferase reporter *in vitro* [64]. An inverse correlation between quantities of *C. burnetii* genomic DNA and intron RNAs during early exponential phase in synchronized cultures certainly supports this possibility. Events surrounding intron splicing and religation of the resulting 23S rRNA could also conceivably serve as a point of regulating translation and growth of the bacterium, since the introns flank an essential 34 base exon, and proper splicing is required to produce a functional, mature 23S rRNA. This possibility is supported by work showing that exposure to RNA splice inhibitors, such as pentamidine, significantly impairs intron splicing and retards *Coxiella*'s growth rate in synchronized cultures [65].

Homing endonuclease

The presence of a putative LAGLIDADG homing endonuclease (HE; LAGLIDADG refers to a conserved protein motif) in *Coxiella*'s 23S rRNA intron was first discovered in the NM genome study, where the element was theorized to be involved in intron mobilization [31]. Homology searches with the HE's predicted amino acid sequence showed that the element was most closely related to intron-associated HEs of organelles and single-celled eukaryotes, such as *Acanthamoeba* and *Monomastix* spp. Since HEs are mobile, the authors suspected lateral gene transfer, from an unknown origin, in the acquisition of the element [31]. More recent work has shown that the HE is encoded entirely within the L1951 intron and that L1917 does not possess a HE [58].

Functional HEs can provide two useful functions to their host intron (reviewed in [57]). First, the HE's endonuclease activity can help to mobilize its host intron to a new palindromic target sequence in intronless alleles within a population; a process referred to as 'homing' [66]. Second, although group I introns, by definition, self-splice *in vitro*, the efficiency of RNA splicing is often enhanced *in vivo* by protein cofactors, like HE (a so-called 'maturase' activity). To what extent *Coxiella*'s HE is involved in either of these two activities has been difficult to analyze, as the gene is extremely toxic when heterologously expressed in *E. coli* [Raghavan R, Minnick MF, Unpublished Data]. Nevertheless, in consideration of the Goddard-Burt model of intron evolution [67], the existing data suggest that the L1917 intron has become fixed in all eight *Coxiella* genotypes and has therefore lost its HE. By contrast, although the L1951 intron is highly conserved in *Coxiella* it still possesses its HE. The L1951 HE's slight amino acid sequence divergence (≤1%) among *Coxiella* genotypes [58] suggests that the element is probably functional and just beginning to degenerate.

Intein

Like introns, internal proteins (inteins) are selfish genetic elements that invade coding sequences and typically employ an encoded HE for mobilization. However, unlike introns, the intein is transcribed and translated along with its flanking sequence, and self-splicing occurs at the protein level to release the intein/HE together with an intact, spliced host protein (exteins; reviewed in [57,68]). *C. burnetii* possesses a single intein in its replicative DNA helicase (DnaB), termed Cbu.DnaB [64]. This target locus is similar to other inteins, which frequently target enzymes involved in DNA replication and repair [68]. Not surprisingly, Cbu.DnaB was found to be functionally active (i.e., able to self-splice and generate the essential DNA helicase enzyme). The absence of an HE gene suggests that the Cbu.DnaB intein has been present for a long time, is fixed in *Coxiella* populations and may eventually be lost [69]. The G + C content and codon bias of the intein is remarkably similar to the rest of the *Coxiella* genome, and underscores the old age of the intein–host relationship. Interestingly, phylogenetic analyses revealed closest-neighbor, intein alleles (in the same position as their respective DnaB hosts) in two extremophile bacteria (*Alkalilimnicola ehrlichei* and *Halorhodospira halophila*), suggesting the possibility of vertical transfer from a common extremophilic ancestor [64].

Three lines of evidence suggest that the intein may play an adaptive role in *Coxiella*, including the high degree of intein amino acid sequence identity among genotypes (≥99%),

the element's G + C blending with the chromosome and the fact that the intein has not been lost after extinction of its HE gene. It is conceivable that the post-translational splicing step by the intein, required to obtain functional DnaB, could provide a point of regulation for some unknown mechanism. A reduction in levels of functional DnaB would undoubtedly slow the growth rate of the pathogen, conceivably promoting bacterial persistence and chronic infection of the host [70–72].

Fossil (pseudo) genes

The first genome analysis of the NM strain identified 83 pseudogenes [31]. Although this number is indicative of genome reduction, a majority of these fossil genes apparently arose from small indels or nonsense mutations, suggesting that they were relatively 'recent' events. Many of the NM pseudogenes also fall into certain functional categories, such as peptide synthetases, β-lactamases and constituents of a competence system [31]. A mutational bias for functional genetic groups indicates that the bacterium has been pseudogenizing genes that were once needed by a free-living ancestor, but which are no longer required for obligate intracellular parasitism. Work has also shown that 15.2% of *C. burnetii*'s pseudogenes are completely or partially deleted in one or more strains, whereas only 6.9% of coding ORFs are deleted. These results indicate that noncoding ORFs are being targeted for deletion at a much higher rate than functional, coding ORFs [25]. More recent, comparative genomics work has identified 125 additional pseudogenes in NM, K, G and Dugway strains. Over 75% of the 207 total NM pseudogenes were originally annotated as ORFs encoding hypothetical proteins [31]. The revised NM pseudogene count of 207 represents approximately 10% of all NM ORFs. Collectively, the percentage of pseudogenes ranges from 6.6% (Dugway) to 11.7% (K) of ORFs, and 65 of the pseudogenes are conserved among the four strains [32]. The large number of pseudogenes present in *Coxiella*'s genome, as compared to those of other obligate intracellular parasites indicates that the pathogen is at a relatively early stage of adaptation to its host cell niche [73]. Undoubtedly, pseudogenization is a major contributor to genetic variation among *Coxiella* strains.

Differential gene expression during the developmental cycle

One of the most striking differences between LCVs and SCVs is their metabolic state. Early work demonstrated *Coxiella*'s acid-dependent uptake of ^{14}C-labeled carbon sources, with a clear preference for glutamate over glucose [74]. More recent work has shown that extracellular SCVs are dormant, and they require an approximate 2-day long lag phase before attaining log-phase growth, as LCVs in synchronously infected cultures [14]. While acidic pH (pH 4.7–4.8), and possibly other cues, such as host cell nutrients or enzymes, trigger SCV-to-LCV metamorphosis and activate metabolism, the environmental signals that initiate LCV-to-SCV morphogenesis and dormancy are less clear. One possibility is the depletion of important cellular nutrients caused by a heavily infected host cell [75]. Physical constraints imparted by the PV to the host cell may also be involved in inducing morphogenesis and dormancy. For instance, *Coxiella* densities can reach thousands of bacteria per infected cell (Figure 2). As a result, the enlarged PV can literally push host cell organelles to the cytosolic membrane, where they may be functionally impaired. In support

of this notion is the observation that host cells with a high density of *C. burnetii* may have a reduced ability to traffic nutrient-laden vesicles to the PV [76]. Thus, organelle impairment may indirectly trigger morphogenesis through host cell stress and/ or nutrient deprivation.

There are a number of reports describing the types of gene products that are unique to SCVs or LCVs. However, since assigning a protein to a specific *Coxiella* cell type is predicated on the purity of the cell preparation and the sensitivity of the detection protocols employed, we will conservatively refer to 'stage-specifc' proteins as LCVHi/Lo or SCVHi/Lo.

SCV^{Hi}/LCV^{Lo} proteins

The highly-condensed nucleoid of SCVs, in contrast to the extended fibrillar nucleoid of LCVs, is an obvious structural difference in *Coxiella* cells [13]. The smaller cell size and metabolic dormancy of SCVs correlate with its compact chromosome, whereas the LCV's larger size and increased transcriptional activity are reflected in its greater volume and dispersed chromosome. Early work identified an approximately 13.2 kDa histone-like protein of SCVs, termed Hq1, that is homologous to H1 proteins of eukaryotes and encoded by the *hcbA* gene [77]. Like most histones, Hq1 is very basic (pI 13.1), owing to a high lysine content (29%). Hq1's homology and specificity for SCVs suggests that it may serve as a factor involved in chromatin structure and DNA condensation. Interestingly, the Hc1 ortholog of *Chlamydia* is also developmentally regulated with maximal expression in dormant, elementary bodies [78], and it has been shown to condense the nucleoid when heterologously expressed in *E. coli* [79]. A role for Hq1 in global gene regulation during LCV-to-SCV morphogenesis is conceivable, especially considering the marked change in DNA topology that occurs in the presence of these proteins [80]. Surprisingly, analysis of *hcbA* transcripts over the course of a synchronous infection showed that maximal expression occurs at 3 days postinfection [14], suggesting that Hq1 translation may be delayed until LCV-to-SCV morphogenesis occurs. Another potential effector of chromatin structure and/or function is ScvA, a small (~3.6 kDa), highly basic (pI ~11) protein that binds dsDNA and is unique to SCVs [75]. Transcript analyses over the course of a synchronously-infected culture showed that *scvA* mRNA is high (~10^3 copies per genome) and stable throughout a 0–8 days postinfection period [14]. Further analysis revealed that the ScvA protein is degraded during SCV-to-LCV transition and LCV replication, suggesting that regulation is post-transcriptional [14]. One intriguing hypothesis is that ScvA and Hq1 provide amino acid and nutrient sources during SCV-to-LCV metamorphosis, since both proteins are degraded during this phase of development [81].

Another $SCV^{Hi/}LCV^{Lo}$ protein is P1; an outer-membrane protein with a predicted β-barrel structure and porin activity [82,83]. Although originally thought to be a single protein, P1 is actually a constituent of a paralagous family of eight outer-membrane proteins, including P1A–P1D (CBU0311, CBU1414, CBU1413 and CBU1412) and OmpA1-OmpA4 (CBU0307, CBU1260, CBU1600 and CBU1814). Genes for each para-log are upregulated during log-phase growth in synchronously infected cultures [Battisti JM, Minnick MF, Unpublished Data] [14]. Although the substrate specificity of P1is unknown, an intriguing possibility is that each paralog has differential specificity as a porin. It is important to note that while transcript levels for the *p1*/*ompA* gene family uniformly increase during

exponential-phase growth, both OmpA1 and P1A proteins appear more prominent in SCVs as compared to LCVs [Battisti JM, Minnick MF, Unpublished Data].

Recent proteomic work using LCVs and SCVs has identified four additional SCV^{Hi}/ LCV^{Lo} proteins, including TolB (Involved in TonB-independent uptake of proteins), cystathionine γ-lyase (L-methionine biosynthesis), a hypothetical protein (CBU2079) and a GTP-binding Era-like protein (GTPase, cell growth) homolog [84]. Taken together, the data (Table 1) indicate that SCV^{Hi}/ LCV^{Lo} proteins are used to facilitate cell cycling and differentiation (Era homolog), promote uptake of unknown substrates (TolB, P1/OmpA), aid in nucleoid condensation during LCV-to-SCV morphogenesis (Hq1, ScvA), and provide for specialized biosynthetic processes (MetC).

LCV^{Hi}/SCV^{Lo} proteins

Not surprisingly, most LCV^{Hi}/SCV^{Lo} proteins reflect the enhanced metabolic state of LCVs. For example, elongation factors Tu and Ts (EF-Tu and EF-Ts, respectively) are essential cofactors for ribosome function and are markedly upregulated in LCVs [85]. EF genes are undoubtedly expressed to meet the increased demand for mRNA synthesis and translation during log-phase growth.

Recent work has also shown that *C. burnetii* maximally expresses a DNA-binding peroxiredoxin (bacterioferritin comigratory protein; BCP) during log-phase growth (2–3 days postinfection). BCP is a peroxidase that is dependent upon thioredoxin–thioredoxin reductase, and is likely involved in detoxifying endogenous hydroperoxide byproducts of metabolism [86]. The DNA-binding activity of *Coxiella*'s BCP has not been previously observed, and the protein may serve to protect supercoiled DNA from oxidative damage. BCP was also shown to be an LCV^{Hi}/SCV^{Lo} protein during a comparative proteomic analysis of LCVs and SCVs [84]. Work examining expression of T4SS genes in synchronously infected cultures showed that *icmQ*, *icmS*, *icmW* and *dotB* are all expressed during exponential-phase growth (24–96 h postinfection); a time period coinciding with enlargement of the PV [18]. More recent work has confirmed T4SS gene upregulation by quantifying similar and distinct mRNA transcripts, including *icmX*, *icmW*, *icmV*, *dotA*, *dotB* and *icmT* [87]. The correlation of T4SS/effector expression and growth is not surprising, considering that this determinant is undoubtedly involved in host cell subversion and biogenesis of the intracellular niche during host cell infection. To what extent T4SS genes are downregulated during LCV-to-SCV metamorphosis is unknown, but likely occurs since SCVs form late in PV maturation. Transcription of the T4SS gene, *dotA*, is also maximal during log-phase growth [14], in keeping with the other T4SS components [18,87].

Comparative proteomic work with purified LCVs and SCVs found 15 LCV^{Hi}/SCV^{Lo} proteins [84], including two proteins mentioned earlier (BCP and EF-Tu), two chaperonins (GroEL and HtpG), two ribosomal proteins (S1 and L9), FtsZ, ParB, the universal stress protein A (UspA), N utilization substance protein A, stringent starvation protein (SspA), segregation and condensation protein, DMRL synthase (RibH), and two hypothetical proteins (CBU0658 and CBU1754) (Table 2). Interestingly, the most abundant LCV^{Hi}/ SCV^{Lo} protein was a hypothetical protein, CBU0658. LCV^{Hi}/SCV^{Lo} proteins are apparently upregulated to facilitate a marked increase in translation (EF-Tu, N utilization substance

protein A, ribosomal proteins S1 and L9); counter stressful conditions of the PV or toxic byproducts of metabolism (BCP, GroEL, HtpG and UspA); help regulate transcription (RpoH, RpoS, RpoD and SspA); provide for biosynthesis (RibH); and assist in DNA replication and cell division (FtsZ, ParB and segregation and condensation protein).

How *Coxiella* regulates its genes

Coxiella's growth within the PV is controlled by complex, intertwined regulatory networks. The genome and gene-expression profiles of *C. burnetii* display hallmarks of a parasite that has adapted to life in a high-stress environment. However, unlike free-living bacteria that live in constantly changing environments, *Coxiella*'s specialized niche provides a much higher degree of predictability. Because of this, a large number of regulatory elements have become essentially superfluous; only 27 (1.5% of 1818 protein-coding genes) recognizable transcriptional regulators are present in *Coxiella* (Table 3) versus 207 (5% of protein-coding genes) in *E. coli* [88].

Unusual role for σ factors

The *C. burnetii* genome encodes three σ factors, including RpoD (σ^{70}), RpoH (σ^{32}) and RpoS (σ^{38}). These σ factors typically serve as global transcriptional regulators of genes that are expressed during normal growth or housekeeping activities (*rpoD*), response to heat/stress shock (RpoH) and starvation or stationary phase (RpoS). Although conditions inside a PV are predictable, the environment is extremely harsh and not conducive to bacterial growth. The low-nutrient and high-stress conditions of the PV actually upregulate expression of RpoS (σ^{38}) during exponential growth in *Coxiella* [14,89]; an observation that runs counter to RpoS' more typical role in regulating genes as a bacterium approaches stationary phase. However, RpoS is also known to accumulate during cellular starvation and modulates the expression of a large number of genes including those responsible for stress response. RpoS has also been shown to regulate genes that are required for the closely-related bacterium, *L. pneumophila*, to survive in protozoan hosts. Thus, it is plausible that σ^{38} regulates expression of genes that help *Coxiella* overcome stressful conditions in the PV [89]. The role of starvation in increasing RpoS levels is highlighted by elevated levels of SspA during *Coxiella*'s growth during exponential phase [14]. SspA is known to be induced by starvation for glucose, nitrogen, phosphate and amino acids, and in turn increases synthesis of RpoS. In most bacteria, RpoS function is influenced and controlled by at least four sRNAs, including *arcZ*, *dsrA*, *rprA* and *rydB*. To what extent sRNAs are involved in *Coxiella*'s unique RpoS transcriptional picture is unknown, as the sRNA profile in *Coxiella* has not been elucidated. Interestingly, previous work has shown that the *C. burnetti*'s *rpoS* ORF contains a region complementary to the first stem loop of *E. coli*'s *dsrA* sRNA [89].

Analysis of transcription by quantitative real-time-PCR has shown that all three *Coxiella* σ factors are rapidly expressed in synchronized infections of cultured host cells, with approximately 800 and 1000 *rpoH* transcripts per genome on days 1 and 2, respectively; approximately 60 *rpoD* mRNAs per genome on day 1 with a steady decline to <10 transcripts by day 4; and roughly 30 copies of *rpoS* mRNA on day 1 with a slight decrease in transcripts by day 4 [Coleman SA, Minnick MF, Unpublished Data]. These results suggest that RpoH may serve as a σ factor during SCV-to-LCV metamorphosis. Interestingly, heat shock

does not appear to significantly increase *rpoH* transcript levels in synchronized infections, suggesting that some other cue(s) is responsible for its induction.

Growth (slowly but surely)

Another adaptation that distinguishes *Coxiella* from free-living, γ-proteobacterial relatives is a markedly slow generation time of approximately 11 h [14]. While the exact cause is unclear, slow growth is a universal phenomenon observed in bacteria that reside in stable, nutrient-poor environments [90,91]. Like many slow-growing bacteria, *Coxiella* has only one rRNA operon, which may be an adaptation to the long generation time, since multiplicity of rRNA genes has been shown to be a burden during slow growth [92]. However, since the single rRNA operon in *Coxiella* is located near the chromosomal origin of replication (within the first 16% of the genome), an increased gene dosage during replication would effectively provide multiple copies of the rRNA genes during exponential-phase growth [91].

Dealing with stress in the PV

A high-stress, low-nutrient environment also activates the global stringent response. The stringent response employs a (p)ppGpp alarmone synthesized by SpoT and RelA, with the assistance of DksA, to help bacteria survive stressful conditions [93]. The RpoS regulon and stringent response pathway interact, with ppGpp inducing RpoS expression. In turn, this controls genes that participate in ppGpp metabolism [94]. *Coxiella* possesses a number of potential transcriptional regulators that might respond to levels of various nutrients like arginine, tryptophan and phosphonate (Table 3). The bacterium also possesses thiamine pyrophosphate and flavin mononucleotide riboswitches, which can regulate gene expression by sensing the levels of their cognate molecules [95]. Although iron is not believed to play an important role in virulence, a Fur-based regulatory system that detects and responds to iron concentrations is also present [96]. It has also been shown that *Coxiella* modulates the autophagic pathway in host cells, probably to compensate for a lack of nutrients within the PV [97].

In addition to a lack of nutrients, *C. burnetii* must deal with acid-induced stress in the context of the PV. Apparently in an effort to compensate for its acidic surroundings, over 60% of *Coxiella*'s proteome is basic, with more than 45% of proteins having pI values of at least 9.0. A good case in point for this alkaline shift is the RpoS protein itself, which has a pI of 9.6 in contrast to a pI of 4.6 for the *E. coli* ortholog. *Coxiella*'s basic proteins may act as a proton 'sink' to buffer protons that enter the cytoplasm [89]. *Coxiella* also has four predicted sodium ion/proton exchangers (CBU1590, CBU1259, CBU0459 and CBU1582-CBU1588) that may help maintain pH homeostasis [31]. SspA, in addition to its role in starvation, is also important for survival during acid-induced stress in *E. coli* [94], highlighting the interconnectivity of regulatory networks.

Another source of stress within PVs is a set of reactive oxygen species. *Coxiella* has a number of genes including *ahpC*, *bcp*, *sodB* and *sodC*, which are known to protect bacteria from both internal and external reactive oxygen species. *Coxiella* also possesses two universal stress proteins (Usp; CBU1916 and CBU1983) that are known to be upregulated in

response to environmental stress, and at least one of them (CBU1983; UspA) is expressed during intracellular growth [97]. It is important to note that UspA has been shown to protect *E. coli* from superoxide-generating agents [98] and is induced by ppGpp [99]; again underscoring the interconnectivity of regulatory networks. Another intriguing *Coxiella* stress-response protein is the 50S ribosomal protein L25/general stress protein, CTC (CBU1840). Two types of proteins constitute this family, including those that possess the N-terminal ribosomal protein L25 domain only (as in *E. coli*) and those that contain an additional C-terminal domain involved in stress response (as in *Bacillus subtilis*). Not surprisingly, *C. burnetii*'s CTC homolog is the second type, suggesting a role for this protein in controlling stress response.

Two-component regulatory systems

Two-component regulatory systems help bacteria detect and respond to various environmental stimuli. In *Coxiella*, only a few of these systems are apparent, perhaps reflecting the relative stability of the intracellular niche [32]. The PhoB–PhoR system of *Coxiella* may respond to P_i in the environment and modulate expression of phosphate regulon genes, akin to *E. coli*. Only an RstB-like protein of the RstB–RstA system is present in *Coxiella*. Mg^{2+} stimulates this signal transduction system in a PhoP-dependent manner in *E. coli*, however, since both a PhoP-PhoQ system and RstA are absent in *Coxiella*, it is not clear how the bacterium responds to magnesium levels in the environment. The GacA–GacS system is known to regulate virulence factors in bacteria that are pathogenic for plants [100] and also plays a role in inducing slow-growth variants under stress in *Pseudomonas aeruginosa* [101]. This two-component system interacts with the stringent response pathway and RpoS to initiate *Legionella*'s transmission phase [102]. Since SCVs are similar to *Legionella*'s transmission phase, it is possible that the GacA–GacS system plays a role in regulating biphasic development of *Coxiella* [32]. The quorum sensing regulators QseB–QseC, which respond to autoinducers and LuxR-family regulators that sense cell density, may control LCV growth within PVs. The QseB–QseC regulator has also been classified as a PmrA-PmrB two-component system in *Coxiella* and has been shown to regulate the Dot/Icm type IV secretion system [103]. Finally, *Coxiella* has two histidine kinases (CBU0789 and CBU1761) of unknown function that may serve as sensory components of two unidentified two-component systems.

Serendipity clusters

Recent transcriptome work examining *Coxiella*'s early response in gene expression during temperature stress revealed the presence of differentially expressed 'serendipity clusters' [104]. These intriguing transcriptional units are comprised of 5–11, closely-linked genes that are co-regulated irrespective of their promoters, membership in genetic networks (operons or regulons) and gene function. The ability of both cold and heat shock to cause the phenomenon, together with the activities of the genes identified (cell division, cell wall and membrane biosynthesis and [p]ppGpp degradation) suggests that stress-related cues may trigger a global regulatory event that dovetails with *Coxiella*'s developmental cycle. Interestingly, similar clusters of regulation were identified in *Rickettsia* spp., *Tropheryma whipplei* and *Listeria monocytogenes*. How these hot spots are co-regulated is unknown but

worth investigating, as it may represent a novel mechanism of global regulation in *Coxiella* and other intracellular bacterial pathogens.

A great deal remains to be done to fully understand *C. burnetii*'s regulatory machinery. However, the available evidence suggests the presence of a minimal regulatory apparatus that helps this obligate intracellular pathogen thrive in the very harsh, but relatively stable, phagolysosome.

Future perspective

The ability to conduct genetic research on *C. burnetii* has been greatly enhanced by several recent, landmark achievements. First, the obligate intracellular nature of *Coxiella* has historically required that the bacterium be grown in tissue culture, embryonated chicken eggs or animal models. Because of this, the most basic protocols, such as cultivation, isolation of genetic clones and purification of bacteria, have been cumbersome. The painstaking development of an axenic medium for growth of *Coxiella* (acidified citrate cysteine medium) has been a tremendous boon to the field [105]. For example, it is now possible to identify and isolate genetic clones as distinct colonies that can be picked from semi-solid acidified citrate cysteine medium plates. Second, genetic manipulation of *Coxiella* was recently achieved by *Himar1* transposon mutagenesis of *ftsZ* (a gene involved in septum formation during cell division) [106]. The *ftsZ* mutant displayed a slower growth rate compared with wild-type strains and showed a filamentous cell morphology. Although the *Himar1* system can only be used for generating random insertion mutants, it paves the way for the development of a system of site-specific mutagenesis; a goal that is likely to be accomplished in the very near future. Finally, a stably-maintained shuttle vector has recently been developed using the RSF1010 backbone. The resulting plasmid, pMK230, has been used to heterologously express β-lactamase fusion proteins in *Coxiella* [44], and provides a shuttle vector to conduct *trans*-complementation experiments following mutagenesis. This is an exciting time to work on *C. burnetii*, as many obstacles that have hampered our ability to conduct basic genetic experiments in the past are falling by the wayside; providing new opportunities for research and novel tools and approaches to accomplish them.

Acknowledgments

We thank L Hicks and I Warrier for helpful comments, and we are grateful to J Driver of the EMtrix Facility at The University of Montana (MT, USA) for electron microscopy and imaging.

Research in the MF Minnick laboratory was supported by NIH funding: R21 AI078125 and U54 AI065357 (Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Disease).

Bibliography

Papers of special note have been highlighted as: ▪ of interest

1. Mackerras IM. Edward Holbrook Derrick. Rec. Austral. Acad. Sci. 1979; 4:82–102.
2. Maurin M, Raoult D. Q fever. Clin. Microbiol. Rev. 1999; 12:518–553. [PubMed: 10515901]
3. Karakousis PC, Trucksis M, Dumler JS. Chronic Q fever in the United States. J. Clin. Microbiol. 2006; 44:2283–2287. [PubMed: 16757641]

4. Raoult D, Houpikian P, Dupont HT, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch. Intern. Med. 1999; 159:167–173. [PubMed: 9927100]
5. Kazar J, Brezina R, Palanova A, Tvrda B, Schramek S. Immunogenicity and reactogenicity of a Q fever chemovaccine in persons professionally exposed to Q fever in Czechoslovakia. Bull. World Health Organ. 1982; 60:389–394. [PubMed: 6982774]
6. Moos A, Hackstadt T. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. Infect. Immun. 1987; 55:1144–1150. [PubMed: 3570458]
7. Hall-Baker PA, Nieves J, Jajosky RA, et al. Summary of notifiable diseases. United States, 2007. MMWR Morb. Mortal. Wkly Rep. 2009; 56:1–94.
8. Kim SG, Kim EH, Lafferty CJ, Dubovi E. *Coxiella burnetii* in bulk tank milk samples, United States. Emerg. Infect. Dis. 2005; 11:619–621. [PubMed: 15829205]
9. Delsing CE, Kullberg BJ, Bleeker-Rovers CP. Q fever in the Netherlands from 2007 to 2010. Neth. J. Med. 2010; 68:382–387. [PubMed: 21209463]
10. McCaul, TF.; Hackstadt, T.; Williams, JC. Ultrastructural and biological aspects of *Coxiella burnetii* under physical disruptions. In: Burgdorfer, W.; Anacker, RL., editors. Rickettsiae and Rickettsial Diseases. Academic Press; NY, USA: 1981. p. 267-280.
11. Williams, JC. Infectivity, virulence, and pathogenicity of *Coxiella burnetii* for various hosts. In: Williams, JC.; Thompson, HA., editors. Q fever: The Biology of *Coxiella burnetii*. CRC Press; Boca Raton, FL, USA: 1991. p. 21-71.
12. Romano PS, Gutierrez MG, Beron W, Rabinovitch M, Colombo MI. The autophagic pathway is actively modulated by phase II *Coxiella burnetii* to efficiently replicate in the host cell. Cell Microbiol. 2007; 9:891–909. [PubMed: 17087732]
13. McCaul TF, Williams JC. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. J. Bacteriol. 1981; 147:1063–1076. [PubMed: 7275931]
14. Coleman SA, Fischer ER, Howe D, Mead DJ, Heinzen RA. Temporal analysis of *Coxiella burnetii* morphological differentiation. J. Bacteriol. 2004; 186:7344–7352. [PubMed: 15489446]
15. Shannon JG, Howe D, Heinzen RA. Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. Proc. Natl Acad. Sci. USA. 2005; 102:8722–8727. [PubMed: 15939879]
16. Howe D, Shannon JG, Winfree S, Dorward DW, Heinzen RA. *Coxiella burnetii* phase I and II variants replicate with similar kinetics in degradative phagolysosome-like compartments of human macrophages. Infect. Immun. 2010; 78:3465–3474. [PubMed: 20515926]
17. Shin S, Roy CR. Host cell processes that influence the intracellular survival of *Legionella pneumophila* . Cell Microbiol. 2008; 10:1209–1220. [PubMed: 18363881]
18. Zamboni DS, McGrath S, Rabinovitch M, Roy CR. *Coxiella burnetii* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophila* Dot/Icm system. Mol. Microbiol. 2003; 49:965–976. [PubMed: 12890021]
19. Zusman T, Yerushalmi G, Segal G. Functional similarities between the icm/dot pathogenesis systems of *Coxiella burnetii* and *Legionella pneumophila* . Infect. Immun. 2003; 71:3714–3723. [PubMed: 12819052]
20. Lührmann A, Nogueira CV, Carey KL, Roy CR. Inhibition of pathogen-induced apoptosis by a *Coxiella burnetii* type IV effector protein. Proc. Natl Acad. Sci. USA. 2010; 107:18997–19001. [PubMed: 20944063]
21. Lührmann A, Roy CR. *Coxiella burnetii* inhibits activation of host cell apoptosis through a mechanism that involves preventing cytochrome c release from mitochondria. Infect. Immun. 2007; 75:5285–5289.
22. Voth DE, Howe D, Heinzen RA. *Coxiella burnetii* inhibits apoptosis in human THP-1 cells and monkey primary alveolar macrophages. Infect. Immun. 2007; 75:4263–4271. [PubMed: 17606599]
23. 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. [PubMed: 21637816]

24. Hendrix LR, Samuel JE, Mallavia LP. Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE. J. Gen. Microbiol. 1991; 137:269–276. [PubMed: 1673152]
25. Beare PA, Samuel JE, Howe D, Virtaneva K, Porcella SF, Heinzen RA. Genetic diversity of the Q fever agent, *Coxiella burnetii*, assessed by microarray-based whole-genome comparisons. J. Bacteriol. 2006; 188:2309–2324. [PubMed: 16547017]
26. Samuel JE, Frazier ME, Mallavia LP. Correlation of plasmid type and disease caused by *Coxiella burnetii* . Infect. Immun. 1985; 49:775–779. [PubMed: 4030104]
27. Glazunova O, Roux V, Freylikman O, et al. *Coxiella burnetii* genotyping. Emerg. Infect. Dis. 2005; 11:1211–1217. [PubMed: 16102309]
28. Russell-Lodrigue KE, Andoh M, Poels MW, et al. *Coxiella burnetii* isolates cause genogroup-specific virulence in mouse and guinea pig models of acute Q fever. Infect. Immun. 2009; 77:5640–5650. [PubMed: 19786560]
29. Voth DE, Beare PA, Howe D, et al. The *Coxiella burnetii* cryptic plasmid is enriched in genes encoding type IV secretion system substrates. J. Bacteriol. 2011; 193:1493–1503. [PubMed: 21216993]
30. Davis GE, Cox HR. A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersonii*, reactions in animals, and filteration experiments. Pub. Health Rep. 1938; 53:2259–2276. [PubMed: 19315693]
31. Seshadri R, Paulsen IT, Eisen JA, et al. Complete sequence of the Q-fever pathogen *Coxiella burnetii* . Proc. Natl Acad. Sci. USA. 2003; 100:5455–5460. [PubMed: 12704232] ▪ First report of a complete *Coxiella burnetii* genome sequence.
32. Beare PA, Unsworth N, Andoh M, et al. Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus. Coxiella. Infect. Immun. 2009; 77:642–656. ▪ Manuscript describing genetic diversity and plasticity by comparing genomes of four *C. burnetii* strains.
33. Omsland A, Heinzen RA. Life on the outside: the rescue of *Coxiella burnetii* from its host *cell* . Annu. Rev. Microbiol. 2011; 65:111–128. [PubMed: 21639786]
34. Vieira-Silva S, Rocha EP. The systemic imprint of growth and its uses in ecological (meta)genomics. PLoS Genet. 2010; 6:e1000808. [PubMed: 20090831]
35. Moran NA. Microbial minimalism: genome reduction in bacterial pathogens. Cell. 2002; 108:583–586. [PubMed: 11893328]
36. Moran NA. Accelerated evolution and Muller's rachet in endosymbiotic bacteria. Proc. Natl Acad. Sci. USA. 1996; 93:2873–2878. [PubMed: 8610134]
37. Hershberg R, Petrov DA. Evidence that mutation is universally biased towards AT in bacteria. PLoS Genet. 2010; 6:e1001115. [PubMed: 20838599]
38. Hildebrand F, Meyer A, Eyre-Walker A. Evidence of selection upon genomic GC-content in bacteria. PLoS Genet. 2010; 6:e1001107. [PubMed: 20838593]
39. Moran NA, Plague GR. Genomic changes following host restriction in bacteria. Curr. Opin. Genet. Dev. 2004; 14:627–633. [PubMed: 15531157]
40. Merhej V, Royer-Carenzi M, Pontarotti P, Raoult D. Massive comparative genomic analysis reveals convergent evolution of specialized bacteria. Biol. Direct. 2009; 4:13. [PubMed: 19361336]
41. Mendonça AG, Alves RJ, Pereira-Leal JB. Loss of genetic redundancy in reductive genome evolution. PLoS Comput. Biol. 2011; 7:e1001082. [PubMed: 21379323]
42. Nagai H, Kubori T. Type IVB secretion systems of *Legionella* and other Gram-negative bacteria. Front. Microbiol. 2011; 2:136. [PubMed: 21743810]
43. Vogel JP. Turning a tiger into a house cat: using *Legionella pneumophila* to study *Coxiella burnetii* . Trends Microbiol. 2004; 12:103–105. [PubMed: 15058276]
44. Chen C, Banga S, Mertens K, et al. Large-scale identification and translocation of type IV secretion substrates by *Coxiella burnetii*. Proc. Natl Acad. Sci. USA. 2010; 107:21755–21760. [PubMed: 21098666] ▪ Describes the first stable shuttle vector for heterologous gene expression in *C. burnetii*.

45. Nagai H, Cambronne ED, Kagan JC, et al. A C-terminal translocation signal required for Dot/Icm-dependent delivery of the *Legionella* RalF protein to host cells. Proc. Natl Acad. Sci. USA. 2005; 102:826–831. [PubMed: 15613486]
46. Mosavi LK, Cammett TJ, Desrosiers DC, Peng ZY. The ankyrin repeat as molecular architecture for protein recognition. Protein Sci. 2004; 13:1435–1448. [PubMed: 15152081]
47. Pan X, Luhrmann A, Satoh A, Laskowski-Arce MA, Roy CR. Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. Science. 2008; 320:1651–1654. [PubMed: 18566289]
48. Voth DE, Howe D, Beare PA, et al. 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. [PubMed: 19411324]
49. Morgan JK, Luedtke BE, Shaw EI. Polar localization of the *Coxiella burnetii type* IVB secretion system. FEMS Microbiol. Lett. 2010; 305:177–183. [PubMed: 20199576]
50. Hoover TA, Vodkin MH, Williams JC. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. J. Bacteriol. 1992; 174:5540–5548. [PubMed: 1324903]
51. Denison AM, Thompson HA, Massung RF. *IS1111* insertion sequences of *Coxiella burnetii:* characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol. 2007; 7:91. [PubMed: 17949485]
52. Afseth G, Mo YY, Mallavia LP. Characterization of the 23S and 5S rRNA genes of *Coxiella burnetii* and identification of an intervening sequence within the 23S rRNA gene. J. Bacteriol. 1995; 177:2946–2949. [PubMed: 7751314]
53. Lin LY, Ching CL, Chin KH, Chou SH, Chan NL. Crystal structure of the conserved hypothetical cytosolic protein Xcc0516 from *Xanthomonas campestris* reveals a novel quaternary structure assembled by five four-helix bundles. Proteins. 2006; 65:783–786. [PubMed: 16948161]
54. Evguenieva-Hackenberg E. Bacterial ribosomal RNA in pieces. Mol. Microbiol. 2005; 57:318–325. [PubMed: 15978067]
55. Baker BJ, Hugenholtz P, Dawson SC, Banfield JF. Extremely acidophilic protists from acid mine drainage host Rickettiales-lineage endosymbionts that have intervening sequences in their 16S rRNA genes. Appl. Environ. Microbiol. 2003; 69:5512–5518. [PubMed: 12957940]
56. Hsu D, Shigh LM, Zee YC. Degradation of rRNA in *Salmonella* strains: a novel mechanism to regulate the concentrations of rRNA and ribosomes. J. Bacteriol. 1994; 176:4761–4765. [PubMed: 8045909]
57. Raghavan R, Minnick MF. Group I introns and inteins: disparate origins but convergent parasitic strategies. J. Bacteriol. 2009; 191:6193–6202. [PubMed: 19666710]
58. Raghavan R, Miller SR, Hicks LD, Minnick MF. The unusual 23S rRNA gene of *Coxiella burnetii*: two self-splicing group I introns flank a 34-base-pair exon and one element lacks the canonical Ω G. J. Bacteriol. 2007; 189:6572–6579. [PubMed: 17644584]
59. Vicens Q, Chech TR. Atomic level architecture of group I introns revealed. Trends Biochem. Sci. 2006; 31:41–51. [PubMed: 16356725]
60. Woodson SA. Structure and assembly of group I introns. Curr. Opin. Struct. Biol. 2005; 15:324–330. [PubMed: 15922592]
61. Raghavan R, Hicks LD, Minnick MF. A unique Group I intron in *Coxiella burnetii* is a natural splice mutant. J. Bacteriol. 2009; 191:4044–4046. [PubMed: 19376857]
62. Haugen P, Huss VA, Nielsen H, Johansen S. Complex group-I introns in nuclear SSU rDNA of red and green algae: evidence of homing-endonuclease pseudogenes in the *Bangiophyceae* . Curr. Genet. 1999; 36:345–353. [PubMed: 10654088]
63. Muller KM, Cannone JJ, Gutell RR, Sheath RG. A structural and phylogenetic analysis of the group IC1 introns in the order *Bangiales* (Rhodophyta). Mol. Biol. Evol. 2001; 18:1654–1667. [PubMed: 11504846]
64. Raghavan R, Hicks LD, Minnick MF. Toxic introns and parasitic intein in *Coxiella burnetii*: legacies of a promiscuous past. J. Bacteriol. 2008; 190:5934–5943. [PubMed: 18606739]
65. Minnick MF, Hicks LD, Battisti JM, Raghavan R. Pentamidine inhibits *Coxiella burnetii* growth and 23S rRNA intron splicing *in vitro* . Int. J. Antimicrob. Agents. 2010; 36:380–382. [PubMed: 20599360]

66. Ho Y, Kim SJ, Waring RB. A protein encoded by a group I intron in *Aspergillus nidulans* directly assists RNA splicing and is a DNA endonuclease. Proc. Natl Acad. Sci. USA. 1997; 94:8994–8999. [PubMed: 9256423]

67. Goddard MR, Burt A. Recurrent invasion and extinction of a selfish gene. Proc. Natl Acad. Sci. USA. 1999; 96:13880–13885. [PubMed: 10570167]

68. Gogarten JP, Senejani AG, Zhaxybayeva O, Olendzenski L, Hilario E. Inteins: structure, function, and evolution. Annu. Rev. Microbiol. 2002; 56:263–287. [PubMed: 12142479]

69. Pietrokovski S. Intein spread and extinction in evolution. Trends Genet. 2001; 17:465–472. [PubMed: 11485819]

70. Kussell E, Kishony R, Balaban NQ, Leibler S. Bacterial persistence: a model of survival in changing environments. Genetics. 2005; 169:1807–1814. [PubMed: 15687275]

71. Proctor RA, von Eiff C, Kahl BC, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat. Rev. Microbiol. 2006; 4:295–305. [PubMed: 16541137]

72. Tierrez A, Garcia-del Portillo F. New concepts in *Salmonella* virulence: the importance of reducing the intracellular growth rate in the host. Cell Microbiol. 2005; 7:901–909. [PubMed: 15953023]

73. Andersson JO, Andersson SG. Insights into the evolutionary process of genome degradation. Curr. Opin. Genet. Dev. 1999; 9:664–671. [PubMed: 10607609]

74. Hackstadt T, Williams JC. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii* . Proc. Natl Acad. Sci. USA. 1981; 78:3240–3244. [PubMed: 6942430]

75. Heinzen RA, Howe D, Mallavia LP, Rockey DD, Hackstadt T. Developmentally regulated synthesis of an unusually small, basic peptide by *Coxiella burnetii* . Mol. Microbiol. 1996; 22:9–19. [PubMed: 8899704]

76. Heinzen RA, Scidmore MA, Rockey DD, Hackstadt T. Differential interaction with endocytic and exocytic pathways distinguish parasitophorous vacuoles of *Coxiella burnetii* and *Chlamydia trachomatis*. Infect. Immun. 1996; 64:796–809. [PubMed: 8641784]

77. Heinzen RA, Hackstadt T. A developmental stage-specific histone H1 homolog of *Coxiella burnetii*. J. Bacteriol. 1996; 178:5049–5052. [PubMed: 8759877]

78. Hackstadt T, Baehr W, Ying Y. *Chlamydia trachomatis* developmentally regulated protein is homologous to eukaryotic histone H1. Proc. Natl Acad. Sci. USA. 1991; 88:3937–3941. [PubMed: 2023942]

79. Barry, CE3rd; Hayes, SF.; Hackstadt, T. Nucleoid condensation in *Escherichia coli* that express a chlamydial histone homolog. Science. 1992; 256:377–379. [PubMed: 1566085]

80. Barry, CE3rd; Brickman, TJ.; Hackstadt, T. Hc1-mediated effects on DNA structure: a potential regulator of chlamydial development. Mol. Microbiol. 1993; 9:273–283. [PubMed: 8412680]

81. Heinzen RA, Hackstadt T, Samuel JE. Developmental biology of *Coxiella burnetii* . Trends Microbiol. 1999; 7:149–154. [PubMed: 10217829]

82. McCaul TF, Banerjee-Bhatnagar N, Williams JC. Antigenic differences between *Coxiella burnetii* cells revealed by postembedding immunoelectron microscopy and immunoblotting. Infect. Immun. 1991; 59:3243–3253. [PubMed: 1715326]

83. Varghees S, Kiss K, Frans G, Braha O, Samuel JE. Cloning and porin activity of the major outer membrane protein P1 from *Coxiella burnetii* . Infect. Immun. 2002; 70:6741–6750. [PubMed: 12438349]

84. Coleman SA, Fischer ER, Cockrell DC, et al. Proteome and antigen profiling of *Coxiella burnetii* developmental forms. Infect. Immun. 2007; 75:290–298. [PubMed: 17088354]

85. Seshadri R, Hendrix LR, Samuel JE. Differential expression of translational elements by life cycle variants of *Coxiella burnetii* . Infect. Immun. 1999; 67:6026–6033. [PubMed: 10531263]

86. Hicks LD, Raghavan R, Battisti JM, Minnick MF. A DNA-binding peroxiredoxin of *Coxiella burnetii* is involved in countering oxidative stress during exponential-phase growth. J. Bacteriol. 2010; 192:2077–2084. [PubMed: 20173000]

87. Morgan JK, Luedtke BE, Thompson HA, Shaw EI. *Coxiella burnetii* type IVB secretion system region 1 genes are expressed early during the infection of host cells. FEMS Microbiol. Lett. 2010; 311:61–69. [PubMed: 20727011]

88. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering the genome. Nucleic Acids Res. 2004; 32(Database issue):D277–D280. [PubMed: 14681412]
89. Seshadri R, Samuel JE. Characterization of a stress-induced alternate s factor, RpoS, of *Coxiella burnetii* and its expression during the developmental cycle. Infect. Immun. 2001; 69:4874–4883. [PubMed: 11447163]
90. Merhej V, Royer-Carenzi M, Pontarotti P, Raoult D. Massive comparative genomic analysis reveals convergent evolution of specialized bacteria. Biol. Direct. 2009; 4:13. [PubMed: 19361336]
91. Vieira-Silva S, Rocha EP. The systemic imprint of growth and its uses in ecological (meta)genomics. PLoS Genet. 2010; 6:e1000808. [PubMed: 20090831]
92. Stevenson BS, Schmidt TM. Growth rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli* . J. Bacteriol. 1998; 180:1970–1972. [PubMed: 9537403]
93. Traxler MF, Summers SM, Nguyen HT, et al. The global, ppGpp-mediated stringent response to amino acid starvation in *Escherichia coli* . Mol. Microbiol. 2008; 68:1128–1148. [PubMed: 18430135]
94. Karp PD, Riley M, Saier M, et al. The EcoCyc database. Nucleic Acids Res. 2002; 30:56–58. [PubMed: 11752253]
95. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. Rfam: an RNA family database. Nucleic Acids Res. 2003; 31:439–441. [PubMed: 12520045]
96. Briggs HL, Pul N, Seshadri R, et al. Limited role for iron regulation in *Coxiella burnetii* pathogenesis. Infect. Immun. 2008; 76:2189–2201. [PubMed: 18316381]
97. Samoilis G, Psaroulaki A, Vougas K, Tselentis Y, Tsiotis G. Analysis of whole cell lysate from the intercellular bacterium *Coxiella burnetii* using two gel-based protein separation techniques. J. Proteome Res. 2007; 6:3032–3041. [PubMed: 17602512]
98. Nachin L, Nannmark U, Nyström T. Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion, and motility. J. Bacteriol. 2005; 187:6265–6272. [PubMed: 16159758]
99. Kvint K, Nachin L, Diez A, Nyström T. The bacterial universal stress protein: function and regulation. Curr. Opin. Microbiol. 2003; 6:140–145. [PubMed: 12732303]
100. Heeb S, Haas D. Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. Mol. Plant Microbe Interact. 2001; 14:1351–1363. [PubMed: 11768529]
101. Davies JA, Harrison JJ, Marques LL, et al. The GacS sensor kinase controls phenotypic reversion of small colony variants isolated from biofilms of *Pseudomonas aeruginosa* PA 14. FEMS Microbiol. Ecol. 2007; 59:32–46. [PubMed: 17233744]
102. Molofsky AB, Swanson MS. Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. Mol. Microbiol. 2004; 53:29–40. [PubMed: 15225301]
103. Zusman T, Aloni G, Halperin E, et al. The response regulator PmrA is a major regulator of the *icm/dot type* IV secretion system in *Legionella pneumophila* and *Coxiella burnetii* . Mol. Microbiol. 2007; 63:1508–1523. [PubMed: 17302824]
104. Leroy Q, Lebrigand K, Armougom F, Barbry P, Thiery R, Raoult D. *Coxiella burnetii* transcriptional analysis reveals serendipity clusters of regulation in intracellular bacteria. PLoS ONE. 2010; 5:e15321. [PubMed: 21203564] ▪ First report of gene clusters that are coregulated without regards to promoter type, membership in genetic networks or gene function, in intracellular bacterial pathogens.
105. Omsland A, Cockrell DC, Howe D, et al. Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. Proc. Natl Acad. Sci. USA. 2009; 106:4430–4434. [PubMed: 19246385] ▪ First report of host cell-free growth of *C. burnetii* using a complex, axenic growth medium.
106. Beare PA, Howe D, Cockrell DC, Omsland A, Hansen B, Heinzen RA. Characterization of a *Coxiella burnetii ftsZ* mutant generated by *Himar1* transposon mutagenesis. J. Bacteriol. 2009; 191:1369–1381. [PubMed: 19114492] ▪ First report of successful genetic manipulation of *Coxiella* by random mutagenesis with a mariner family transposon.

Box 1

Synopsis of *Coxiella burnetii*

- *Coxiella burnetii* is the zoonotic agent of Q fever which, with the exception of New Zealand, occurs worldwide
- Although once thought to be a rickettsia because of its size and obligate intracellular parasitism, the pathogen is an acidophilic, Gram-negative γ-proteobacterium most closely related to *Legionella pneumophila*
- *Coxiella* is one of the most infectious agents known. One to ten viable bacteria can cause an infection in guinea pigs.
- The primary reservoir for human infection is livestock including goats, sheep and cattle
- Transmission to humans mainly occurs through aerosols from infected animals or fomites. Sexually-transmitted, arthropod vector-borne and food-borne Q fever is rare
- Q fever can be acute (asymptomatic, atypical pneumonia, malaise, hepatitis) or chronic (endocarditis) in nature
- A biphasic life cycle involving small- and large-cell variants provides for dormancy/extracellular survival and intracellular growth in an acidic, phagolysosome-like vacuole, respectively

Executive summary

Coxiella's genome

- There are eight genome groups of *Coxiella* with considerable heterogeneity in genome content and virulence potential.
- Like many obligate intracellular bacteria, the genome is relatively small (~2 Mbp) and AT-rich (~42.5% G + C content). Approximately 1500 chromosomal genes (65% of total ORFs) are conserved between genome groups.
- A single type of plasmid (QpH1, QpDV, QpDG or QpRS) or integrated plasmid-like sequence is present in each genome group. The plasmids (and integrated plasmid sequences) share 22 genes; a degree of conservation that suggests they play adaptive and essential roles.

Genetic elements & fossil genes

- *Coxiella* possesses an unusually high number of selfish genetic elements, suggesting that its shift from a free-living bacterium to an obligate intracellular parasite was relatively 'recent'.
- *Coxiella* chromosomes (but not plasmids) possess dozens of insertion sequence elements belonging to eight IS families. IS elements have played a central role in generating genetic diversity in *C. burnetii* by enhancing chromosomal rearrangements.
- The 23S rRNA gene of *Coxiella* possesses five selfish genetic elements, including an IVS, S23p ORF, two group I introns and a LAGLIDADG homing endonuclease. In addition, *Coxiella's* replicative DNA helicase protein is disrupted by an intein. Phylogeny of these elements implicates other extremophilic organisms, *Leptospira*, and lower eukaryotes such as *Acanthamoeba*, as possible origins. Conservation and maintenance of selfish genetic elements in all genome groups and strains implies they play adaptive roles in *Coxiella*, such as modulating growth.
- Roughly 7–12% of the genome is pseudogenized. This large number of fossil genes suggests that *Coxiella* is at an early stage of adaptation to the intracellular niche.

Gene expression during a biphasic developmental cycle

- The biphasic lifecycle consists of dormant extracellular small-cell variants (SCVs) and metabolically active large-cell variants (LCVs).
- Gene products that are more abundant in SCVs correlate with: cell cycling and differentiation, uptake of unknown substrates, nucleoid condensation and specialized biosynthetic processes.

- Gene products that are dominant in LCVs correlate with an increase in: translation, stressors in the parasitophorous vacuole (PV)/toxic byproducts of metabolism, transcription, biosynthesis, DNA replication and cell division.

Gene regulation

- Although the PV is not conducive to growth, it is fairly stable and has allowed *Coxiella* to downsize its array of regulatory machinery. For instance, only 1.5% of its genes encode recognizable transcriptional regulators.
- Unlike most bacteria, RpoS (s^{38}) expression is maximal during exponential-growth phase, and undoubtedly reflects the high-stress, low-nutrient conditions encountered by *Coxiella* in the context of the PV.
- *Coxiella* is slow-growing, like many bacteria that live in stable, nutrient-poor habitats.
- Regulatory roles for sRNAs, stringent response, putative two-component regulatory systems and two universal stress proteins in dealing with stress encountered within PVs are likely, but have not been examined to date.
- Serendipity clusters of 5–11 closely linked genes may help co-regulate genes in response to stress without regards to their promoter types, operon/regulon membership or gene function.

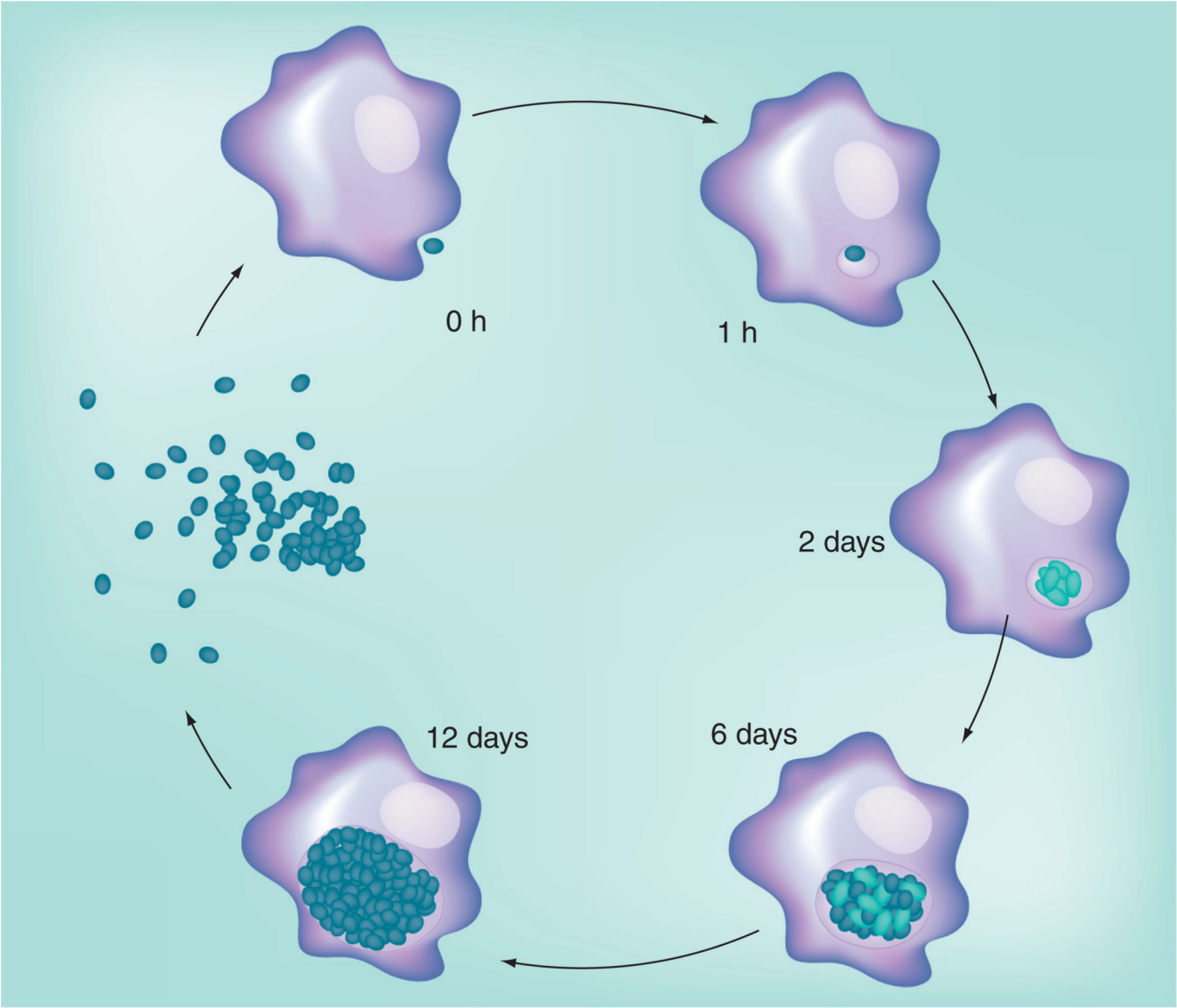

Figure 1. Developmental cycle of *Coxiella burnetii*

An alveolar monocyte/ macrophage internalizes a small-cell variant (SCV), which becomes closely associated with the membrane of an early endosome (0–1 h). Upon acidification of the vacuole, the SCV undergoes metamorphosis at ~8 h postinfection, followed by replication of the resulting large-cell variant [14]. By day 2, the parasitophorous vacuole (PV) is spacious and contains replicating large-cell variants. After a few days of replication (log-phase growth), SCVs begin to reappear at around day 6 (onset of stationary phase). The PV is now densely-packed and growing in size. On day 8, roughly half of the *Coxiella* within a PV are SCVs [14]. At day 12, the PV is packed with SCVs and the vacuole has, for all intents and purposes, consumed the host cell volume. Subsequent lysis of the host cell releases SCVs to repeat the cycle.

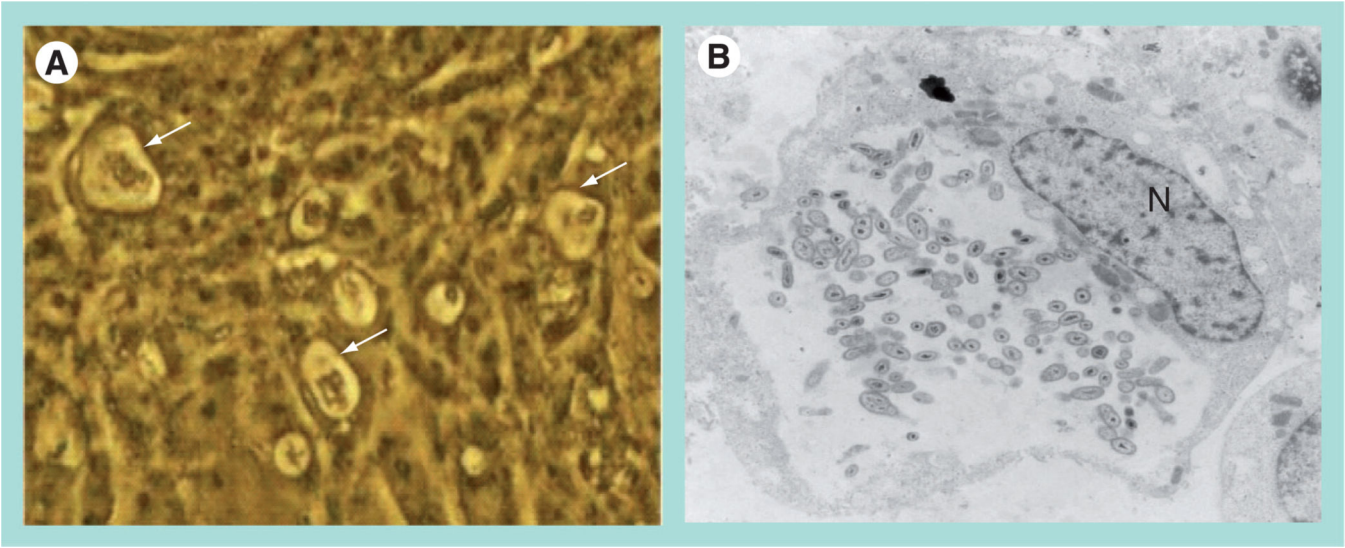

Figure 2. Parasitophorous vacuole of *Coxiella burnetii*

(A) Phase-contrast micrograph showing a synchronously-infected Vero cell monolayer at 4 days postinfection. Parasitophorous vacuoles appear as large vacuoles of varying sizes (examples arrowed). **(B)** Transmission electron micrograph showing a spacious parasitophorous vacuole in a synchronously-infected Vero cell (4 days postinfection). Note how the nucleus (N; upper right-hand corner of the Vero cell) is pushed to the periphery of the cell by the parasitophorous vacuole.

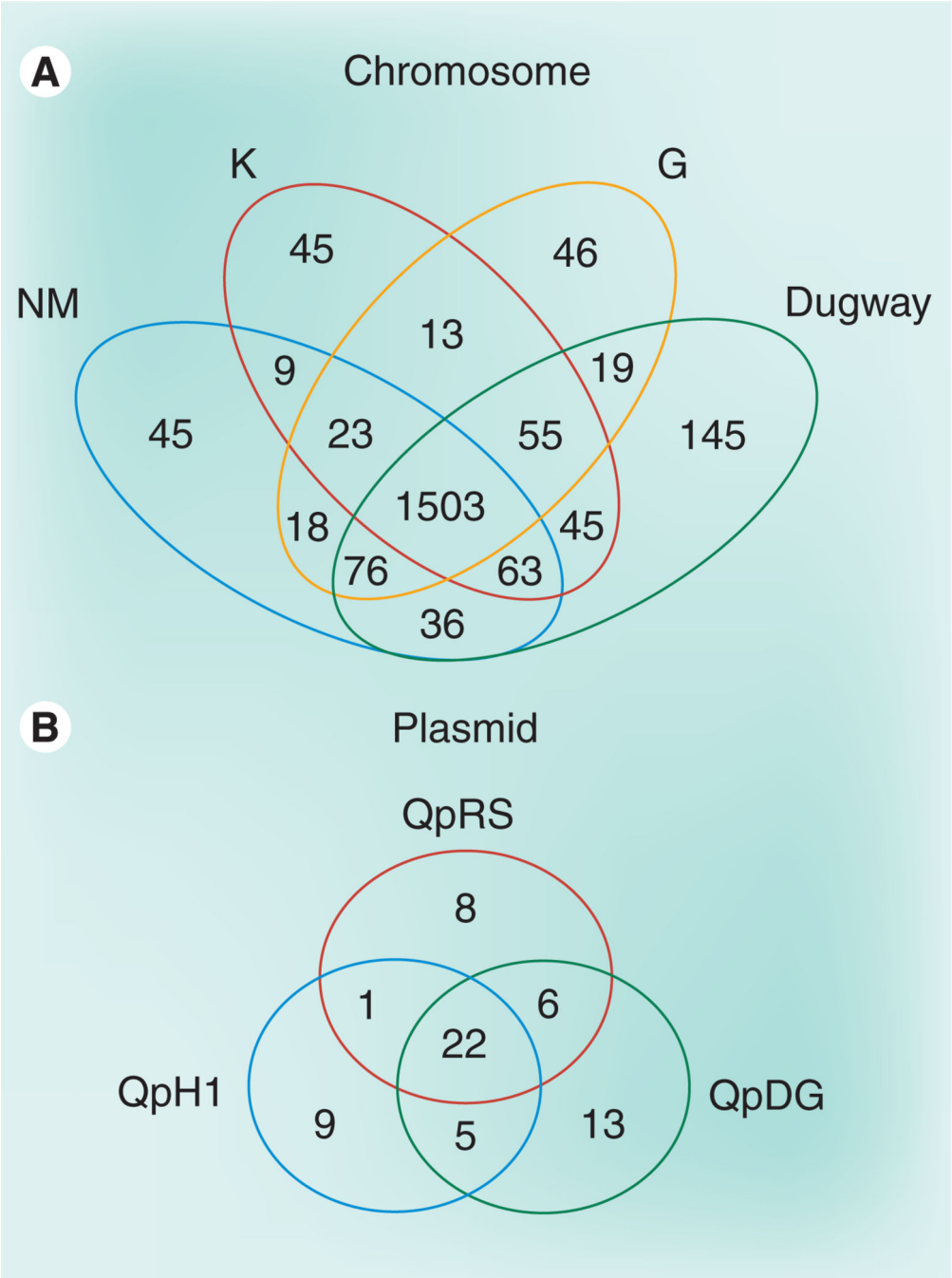

Figure 3.

Venn diagrams showing the number of unique and shared, full-length ORFs in the chromosomes (A) and plasmids (B) of four *Coxiella burnetii* strains, including the NM, G, Dugway and K strains.

Reproduced with permission from [32].

Table 1

SCV^{Hi}/ LCV^{Lo} gene products of *Coxiella burnetii* (in alphabetical order).

Gene product (description)	Locus tag†	Ref.
Era (GTP-binding Era-like protein)	CBU1502	[84]
Hq1 (histone H1-like protein, HcbA)	CBU0456	[14,77]
Hypothetical protein	CBU2079	[84]
MetC (cystathionine γ-lyase)	CBU2025	[84]
Omp34 (34 kDa Omp)		[81]
P1 (P1A Omp)	CBU0311	[14]
P1 paralogs:		[Battisti JM,
P1B–D, respectively	CBU1414, 1413, 1412	Minnick MF,
	1413, 1412	Unpublished Data]
OmpA1–4, respectively	0307, 1260	
	1600, 1814	
ScvA (Small cell variant protein A)	CBU1267a	[14,75]
TolB (TolB protein)	CBU0090	[84]

†Designations from GenBank accession number AE016828.

Table 2

LCV^{Hi}/SCV^{Lo} gene products of *Coxiella burnetii* (in alphabetical order).

Gene product (description)	Locus tag†	Ref.
BCP (bacterioferritin comigratory protein, peroxiredoxin)	CBU0963	[84,86]
DotA (T4SS component)	CBU1648	[14,87]
DotB (T4SS component)	CBU1645	[18,87]
EF-Ts (translation elongation factor-Ts)	CBU1385	[85]
EF-Tu (translation elongation factor-Tu)	CBU0236	[84,85]
FtsZ (cell division protein)	CBU0141	[84]
GroEL (heat shock protein, chaperone)	CBU1718	[84]
HtpG (heat shock protein, chaperone)	CBU0309	[84]
Hypothetical protein	CBU0658	[84]
Hypothetical protein	CBU1754	[84]
IcmQ (T4SS component)	CBU1634	[18]
IcmS (T4SS component)	CBU1642	[18]
IcmT (T4SS component	CBU1641	[87]
IcmV (T4SS component)	CBU1649	[87]
IcmW (T4SS component)	CBU1650	[18,87]
IcmX (T4SS component)	CBU1652	[87]
L9 (Ribosomal protein)	CBU0867	[84]
NusA (N utilization substance protein A)	CBU1433	[84]
ParB (chromosomal partitioning protein)	CBU1927	[84]
RibH (6,7, dimethyl-8-ribityllumazine synthase)	CBU0648	[84]
RpoD (σ^{70})	CBU1596	[Coleman SA, Minnick MF, Unpublished Data]
RpoH (σ^{32})	CBU1909	[Coleman SA, Minnick MF, Unpublished Data]
RpoS (σ^{38})	CBU1669	[14,89]
S1 (ribosomal protein)	CBU0528	[84]
ScpB (segregation/condensation protein)	CBU1060	[84]
SspA (stringent starvation protein A)	CBU1747	[84]
UspA (universal stress protein)	CBU1916	[84]

†Designations from GenBank accession number AE016828.

Table 3

Potential transcriptional regulators of *Coxiella burnetii* (in alphabetical order).

Gene	Product	Locus Tag[†]
argR	Arg repressor	CBU0480
bolA	BolA family protein	CBU0582
gacA.1	GacA family response regulator	CBU0712
gacA.2	LuxR family response regulator	CBU0780
gacA.3	LuxR family response regulator	CBU0955
gacA.4	LuxR family response regulator	CBU1043
oxyR	Hydrogen peroxide-inducible genes activator	CBU1476
phoB	P regulon transcriptional regulator	CBU0367
pspC	PspC domain containing protein	CBU0774
qseB	Transcriptional regulatory protein	CBU1227
relA	RelA/SpoT family protein	CBU1375
rpoA	RNA polymerase subunit α	CBU0263
rpoB	RNA polymerase subunit β	CBU0231
rpoC	RNA polymerase subunit β	CBU0232
rpoD	RNA polymerase σ^{70}	CBU1596
rpoH	RNA polymerase σ^{32}	CBU1909
rpoS	RNA polymerase σ^{38}	CBU1669
rpoZ	RNA polymerase subunit ω	CBU0302
spoT	GTP pyrophosphokinase	CBU0303
trpR	Trp operon repressor	CBU0509
	FeS assembly SUF system regulator	CBU1361
	GntR family transcriptional regulator	CBU0775
	LuxR family transcriptional regulator	CBU1804
	LuxR family transcriptional regulator	CBU1805
	LuxR family transcriptional regulator	CBU1972
	PAS domain-containing protein	CBU2041
	TetR family transcriptional regulator	CBU0818

[†]Designations from GenBank accession number AE016828.