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Identification of novel small RNAs of *Coxiella burnetii*

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Identification of novel small RNAs of *Coxiella burnetii*

Coxiella burnetii is an obligate intracellular bacterial pathogen that causes Q fever in humans. *Coxiella* is one of the most infectious pathogens known due to its low infectious dose (ID₅₀: 1-10 bacteria), transmission through aerosols and incredible resistance to environmental factors. Thus, *Coxiella* is recognized as a potential bio-terrorism agent and a class B select agent. The bacterium undergoes a biphasic developmental cycle that alternates between a fragile, metabolically-active large-cell variant (LCV) and a durable, dormant endospore-like small-cell variant (SCV). As such, the bacterium undoubtedly employs complex modes of regulating its lifecycle, metabolism and pathogenesis. However, little is known about the mechanisms involved. Small RNAs (sRNAs) are a large, heterogeneous group of non-coding RNA molecules, usually 100-400 bases in length, that can act in *cis* or *trans* to regulate a variety of physiological processes at both transcriptional and post-transcriptional levels. sRNAs have also been shown to play an important regulatory role in controlling metabolism and virulence in several pathogenic bacteria. *Coxiella*'s developmental cycle and intracellular parasitism encompass several events and scenarios where sRNA-mediated regulation is likely involved and would be clearly adaptive. We hypothesize that sRNAs are employed in regulating the growth and development of *C. burnetii* and its infection of host cells. To address this hypothesis and identify potential sRNAs, we prepared total RNA samples from LCVs (3d) and SCVs (21d) cultured axenically and in infected Vero host cells. RNA samples were subsequently enriched and used to construct directional (strand-specific) cDNA libraries. These constructs were used to interrogate the transcriptome using RNA-seq (high throughput sequencing) technology. Using this approach, we identified fifteen novel *C. burnetii* sRNAs (CbSRs). Fourteen of the CbSRs were experimentally validated by Northern blotting. Transcript levels of most CbSRs were increased during the growth phase (LCV) of the organism indicating that they probably help regulate genes that are involved in metabolic functions. On the other hand, eight CbSRs were upregulated (≥ 2 -fold) during intracellular growth as compared to growth in axenic medium. This observation suggests that these CbSRs are involved in regulating the bacterium's stress response in the intracellular niche. By studying the specific role of each sRNA, we will be able to elucidate the molecular mechanisms involved in regulating the bacteria's life cycle and pathogenesis.