# A Small Rna that Regulates Pyrimidine and Methionine Metabolism is Necessary for Establishing Coxiella Burnetii's Intracellular Niche During Early Stages of Infection

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*Coxiella burnetii* is an obligate intracellular gammaproteobacterium and zoonotic agent of Q fever in humans. We previously identified 15 small RNAs (sRNAs) in C. burnetii with differential expression in the large and small cell developmental forms grown axenically and in infected host cells. Here, we describe the function of one of these sRNAs, termed Coxiella burnetii small RNA 12 (CbsR12). CbsR12 is highly expressed in both large- and small-cell variants in vitro, and is the dominant non-tRNA/rRNA/tmRNA transcript in both morphotypes during mammalian tissue culture infection. Through a combination of in vitro and in vivo assays, we have identified several targets of CbsR12. Of these, we have confirmed that CbsR12 binds to and upregulates translation of carA transcripts coding for carbamoyl phosphate synthetase A; an enzyme that catalyzes the first step of pyrimidine biosynthesis. In addition, Cbsr12 binds and downregulates translation of metK transcripts coding for S-adenosyl methionine (SAM) synthase, an essential component of the methionine cycle. Furthermore, we have established that CbsR12 is necessary for full expansion of Coxiella-containing vacuoles (CCVs) and is linked to growth rate in a dose-dependent manner in the early phase of infection of Vero and THP-1 cell lines. This is the first characterization of a trans-acting sRNA of C. burnetii and the first description of a bacterial sRNA that regulates carA and metK expression. This study also illustrates the utility of transposon insertion mutants in elucidating Coxiella's sRNAs and the importance of sRNA regulation in establishment of the intracellular CCV niche.