
COXIELLA BURNETII'S INFECTION-SPECIFIC SMALL RNA 12 (CBSR12) TARGETS CARA AND METK TRANSCRIPTS

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Coxiella burnetii is an obligate intracellular bacterial pathogen and the etiological agent of Q fever. Previous transcriptome analysis of *C. burnetii* by our group revealed several novel small RNAs (sRNAs) of varying sizes and expression patterns. Sequence alignments of these sRNA's across all strains of *C. burnetii* show strong conservation, indicating a functional role for these RNA's in *C. burnetii*'s intracellular lifestyle. Furthermore, during *C.*

C. burnetii's biphasic life cycle of metabolically active (LCV) and inactive (SCV) states, several of the sRNA's have shown differential expression in SCV and LCV cells via RNA-Seq and Northern blot analyses. One such sRNA, termed CbsR12, showed a marked upregulation in infected Vero host cells when compared to bacteria grown in axenic media. Additionally, RNA-Seq data and qRT-PCR analyses show a marked upregulation of CbsR12 in LCV cells compared to SCV cells. Here, we show that *C. burnetii* RNase III cleaves CbsR12 into two fragments, an observation supported by both in silico and 5' RACE analyses. In silico sRNA target prediction programs were used to determine possible mRNA targets of Cbsr12. We subsequently determined through in vitro electrophoretic mobility shift assays (EMSAs) and in vivo luciferase reporter assays, that CbsR12 binds *carA* transcripts, which codes for carbamoyl-phosphate synthase subunit A, and *metK* transcripts, which codes for S-adenosyl methionine synthetase. These genes code for essential enzymes involved in pyrimidine biosynthesis and the methionine cycle, respectively.