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Original article

Modulation of the host transcriptome by *Coxiella burnetii* nuclear effector Cbu1314

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Abstract

Coxiella burnetii is a Gram-negative, obligate intracellular pathogen that directs the formation of a parasitophorous vacuole derived from the host lysosomal network. Biogenesis and maintenance of this replicative compartment is dependent on bacterial protein synthesis and results in differential expression of specific host genes. However, the mechanisms by which the pathogen induces changes in the host transcriptome is poorly understood. In the current study we identified a Dot/Icm secreted effector, Cbu1314, which encodes two nuclear localization signals that are required for nuclear localization and association with host chromatin. Chromatin immunoprecipitation (ChIP) and deep sequencing revealed that Cbu1314 associated with host genes involved in transcription, cell signaling, and the immune response. RNA sequencing of cells over-expressing Cbu1314 demonstrated that Cbu1314 modulates the host transcriptome and these transcriptional changes required a functional nuclear localization signal. Of the differentially expressed genes, sixteen were also identified as Cbu1314 targets using ChIP sequencing. Collectively these results suggest that Cbu1314 associates with host chromatin and plays a role in modulating the host transcriptome.

Keywords: *Coxiella burnetii*; Dot/Icm; T4SS; Nuclear effector

1. Introduction

Coxiella burnetii is a Gram-negative, obligate intracellular pathogen that is the causative agent of Q fever, a zoonotic disease with a nearly worldwide distribution [1]. In humans, infection predominately occurs through inhalation of contaminated aerosols. In most cases, Q fever results in an acute disease characterized by high fever and flu-like symptoms that typically resolves within 1–2 weeks and is readily mitigated by antibiotics. Chronic Q fever, however, is less common, occurring in 1–2% of cases, but results in a more severe disease associated with endocarditis and hepatitis [2]. In 1999 Q fever became a reportable disease, which coincided with a marked increase in the number of reported cases and recently an outbreak in the Netherlands resulted in over 4000 cases [3]. This increase in the number of Q fever cases suggests *C. burnetii* is an emerging pathogen and highlights our lack of understanding of this important pathogen.

Actin-dependent endocytosis of *C. burnetii* by alveolar macrophages leads to internalization in a tight-fitting phagosome that traffics through the default endocytic pathway to ultimately establish a *Coxiella*-containing vacuole (CCV)

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derived from the host lysosomal network [4]. Within 5 min of internalization, the nascent CCV is decorated with the early endosomal markers Rab5 and EEA1. However, in contrast to phagosomes, lysosomal fusion is delayed during which the CCV engages autophagosomes, presumably to deliver nutrients [5,6]. Following this initial stall, the CCV fuses with the lysosomes and the pH of the vacuole drops to about 4.5, triggering conversion from the environmentally stable small-cell variant (SCV) to the metabolically active large cell variant (LCV) [7]. Establishing this unique replicative compartment requires active bacterial protein synthesis and manipulation of numerous host processes including inhibition of apoptosis [8], induction of autophagy [9], recruitment of vesicles [10,11], and modulation of the host transcriptome [12].

Like its close relative, *Legionella pneumophila*, *C. burnetii* encodes a specialized Dot/Icm type IVb secretion system that is essential for intracellular replication, CCV formation, effector translocation, and inhibition of apoptosis [8,13,14]. To date, over a hundred T4SS substrates have been identified using bioinformatics [10,15–21], genomic assays [13], and bacterial-two hybrid approaches [15], however the function of most of these substrates remains unknown. Large-scale screening of these substrates has demonstrated that they localize to specific subcellular compartments, interfere with host cell processes, and are essential for intracellular replication and CCV formation [10,13,15,16,18,22]. Several substrates, including AnkG, CaeA, and CaeB promote host cell viability by inhibiting host apoptosis [23,24]. CvpA and CirA control membrane trafficking through manipulation of clathrin-coated vesicles [10] or Rho GTPases, respectively (Weber MM et al., manuscript submitted).

In order to establish a replicative niche, intracellular pathogens must first bypass host defense mechanisms. In most instances, manipulation of the host response requires release of bacterial effector proteins that trigger cytoskeleton rearrangements, alter vesicle trafficking, or disrupt cell signaling pathways [1]. Disruption of normal host cell processes are ultimately facilitated by changes in gene expression, however modulation of the host transcriptome can also occur through epigenetic modifications [25,26]. Numerous bacterial pathogens including *Anaplasma phagocytophilum* [27], *Ehrlichia chaffeensis* [28], *Chlamydia trachomatis* [29], *Shigella flexneri* [30], *L. pneumophila* (35) and *Listeria monocytogenes* [31] secrete effector proteins that modulate the host transcriptome, suggesting that this is a conserved mechanism among bacterial pathogens. *C. burnetii* secretes several effector proteins that localize to the nucleus [13,15,16], however the function of these nuclear effectors is ill-defined.

In the current study we preliminarily characterized a T4SS secreted substrate, Cbu1314 that localizes to the nucleus and associates with host chromatin. Nuclear import and association with host chromatin was dependent on two nuclear localization signals (NLS). Using chromatin immunoprecipitation and DNA sequencing (ChIP-Seq) we identified numerous host genes involved in transcription, signal transduction, and metabolism that were targeted by Cbu1314. Comparisons of transcriptional profiles from HEK293 cells overexpressing Cbu1314, Cbu1314ΔNLS, and empty vector revealed that overexpression of Cbu1314 resulted in differential expression of host genes associated with transcription, apoptosis, cell signaling, and immune response. Importantly sixteen target genes, identified using ChIP-seq, were differentially expressed. Collectively these results demonstrate that Cbu1314 associates with host chromatin and results in differential gene expression in the host.

2. Material and methods

2.1. Cell culture

HeLa and Hek293 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen), 4 mM L-glutamine, and 1% non-essential amino acids (Invitrogen) and were maintained at 37 °C with 5% CO_2.

2.2. Plasmid construction

To evaluate nuclear localization and association with host chromatin, Cbu1314 was expressed as a C-terminal fusion to Flag-tag by PCR amplifying the ORF and cloning it into the *SalI*/*KpnI* site of pCMV-DYKDDDDK-C (Clontech, Mountain View, CA). Alternatively, Cbu1314 was expressed as an N-terminal fusion to EGFP as previously described [15].

2.3. Dot/Icm-dependent translocation of Cbu1314

TEM translocation assays for *C. burnetii* in THP-1 cells were conducted as previously described [15,16]. *C. burnetii* 439 or *C. burnetii icmX::Tn* were transformed with pCC108 and individual colonies, expressing the β-lactamase fusion protein, were propagated in ACCM-2 broth. Host cells were seeded at 10^5/ml in 24-well plates and were infected with an MOI of 100 and incubated for 48 h. Infected cells were loaded with 6XCCF4/AM solution (LiveBLAzer-FRET B/G Loading Kit, Invitrogen) and incubated at room temperature for 4 h. The percentage of blue cells was quantified using a Nikon A1 microscope. Approximately 1000 cells were counted and results are presented as mean values from duplicate wells from two independent experiments.

2.4. QuikChange deletion of nuclear localization signals

Bioinformatic analysis of Cbu1314 using NLStradamus (http://www.moseslab.csb.utoronto.ca/NLStradamus) and cNLS mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) identified six potential nuclear localization signals (NLS). To delete each predicted NLS, the primers in Supplementary Table 1 were used with the QuikChange II site-directed mutagenesis kit (Agilent, Santa