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Differential Expression of Translational Elements by Life Cycle Variants of *Coxiella burnetii*

REKHA SESHADRI, LAURA R. HENDRIX, AND JAMES E. SAMUEL*

Department of Medical Microbiology and Immunology, Texas A & M University System Health Science Center, College Station, Texas 77843-1114

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Coxiella burnetii **replicates as distinct morphological forms, which may allow potential life cycle variants to survive the harsh environment of the phagolysosome. Monoclonal antibodies (MAbs) were compared by Western blotting for reactivity with large cell variant (LCV) and small cell variant (SCV) antigens to characterize proteins differentially expressed by *C. burnetii*. MAb NM7.3 reacted with a ∼32-kDa LCV-upregulated antigen, and MAb NM183 reacted with a ∼45-kDa LCV-specific antigen. MAb NM7.3 was used to screen a λZapII *C. burnetii* DNA expression library, and an immunoreactive clone was identified with sequence similarity to the *Escherichia coli tsf* gene, which encodes elongation factor Ts (EF-Ts). Since a similar screen with MAb NM183 did not identify immunoreactive clones, an alternate strategy was devised to clone the reactive antigen based on observations of cross-reactivity with the 45-kDa elongation factor Tu (EF-Tu) protein from *Chlamydia trachomatis*. The highly conserved nature of EF-Tu among eubacteria allowed PCR amplification of a *tuf* gene fragment (encoding ∼95% of the predicted EF-Tu open reading frame) from *C. burnetii* using degenerate primers. The product of the cloned *tuf* gene fragment reacted with MAb NM183 in Western blot analysis, confirming the identity of the 45-kDa LCV-specific antigen. Identification of two proteins differentially expressed by *C. burnetii*, EF-Tu and EF-Ts, both essential components of the translational machinery of the cell, supports the hypothesis that LCVs are metabolically more active than SCVs.**

Coxiella burnetii, the etiological agent of Q fever, is an obligate intracellular rickettsial pathogen with worldwide distribution and a broad host range that includes livestock and humans (3). Human disease is contracted mainly by inhaling aerosolized bacteria and is associated with domestic animal operations, putting workers at increased risk of infection (2). An acute Q fever typically presents as a flu-like illness, but occasional chronic infections occur which may manifest as endocarditis or hepatitis, resulting in significant mortality (4).

An extraordinary ability to persist in the environment is a hallmark of *C. burnetii*, and the basis for this stability may be the expression of resistant cell forms. *C. burnetii* appears to undergo an incompletely characterized life cycle within the phagolysosome of the eukaryotic host cell with at least two distinct morphological forms, designated large cell variant (LCV) and small cell variant (SCV) (7, 11, 17, 19, 21). These two forms have been characterized primarily by ultrastructural differences (17, 19): LCVs appear similar to typical gram-negative bacteria during exponential-phase growth with a clearly distinguishable outer membrane, periplasmic space, cytoplasmic membrane, and diffuse nucleoid, attaining lengths exceeding 1 μm. SCVs are 0.2 to 0.5 μm in diameter with electron-dense, condensed chromatin and condensed cytoplasm. SCVs are resistant to osmotic shock, oxidative stress, heat shock, sonication, and pressure, unlike the more fragile LCVs (1, 9, 18). Differences in resistance to breakage by osmotic and pressure stresses were employed to determine that LCVs may have greater metabolic activity than SCVs based on their ability to metabolize [^{14}C]glucose and [^{14}C]glutamate in axenic media (18). Limitations of this study included the facts that reduced activity may have been the consequence of the extreme osmotic shock conditions to which the cells were subjected and that the activity evaluated in axenic medium may not accurately reflect events in the intraphagolysosomal compartment. Therefore, these data did not conclusively establish a metabolic activity difference between SCVs and LCVs.

SCVs and LCVs can be separated to near homogeneity by gradient density centrifugation in 32% cesium chloride (10, 12, 25). Heinzen and coworkers demonstrated the abundance of a specific histone-like DNA binding protein (Hq-1) in SCV which may function in regulating gene expression by inducing topological changes in DNA (10, 14). This group also described a small, basic peptide (ScvA) only expressed by SCV, which might function in DNA binding or nutrient storage during extended periods of metabolic inactivity (12).

We speculated that characterizing additional differentially expressed proteins would provide insight into the function of the life cycle variants. A panel of monoclonal antibodies raised against formalin-killed *C. burnetii* was screened to identify proteins differentially expressed by LCV and SCV. This study presents the cloning and sequence analysis of two genes, designated *tsf* and *tufB*, encoding two immunoreactive antigens: elongation factor Ts (EF-Ts), that shows LCV-upregulated expression, and elongation factor Tu (EF-Tu), which appears to be LCV-specific and is not present at a detectable level in the SCV. These proteins are key components of the translational machinery of bacteria. Our findings, coupled with the SCV-specific expression of DNA-binding proteins, support the early observations regarding differences in the metabolic activities of LCV and SCV.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. Bacterial isolates (*C. burnetii*, *Escherichia coli*, and *Chlamydia trachomatis*) and plasmids used in this study are listed in Table 1. *E. coli* DH5α cultures were grown in Luria-Bertani medium at 37°C in a shaking water bath; *E. coli* MRF cells were infected with bacteriophage λZapII cloning vector (Stratagene, La Jolla, Calif.) and

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, 407 Reynolds Medical Building, Texas A & M University System Health Sciences Center, College Station, TX 77843-1114. Phone: (409) 862-1684. Fax: (409) 845-3479. E-mail: jsamuel@tamu.edu.

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TABLE 1.

Strain or plasmid	Relevent characteristics	Source or reference
Strains		
C. burnetii	Nine Mile, phase I, RSA 493	23
C. trachomatis	Biotype E	American Type Culture Collection
E. coli DH5α	F^- φ80d *lacZΔM15Δ(lacZYA-argF)U169 deoR recA endA1 phoA supE44* λ^- *thi-1 gyrA96 relA1*	GIBCO/BRL
E. coli JM109	F′ *traD* 36 *proAB lacIqZ Δ M15 recA1 endA1 gyrA96 thi-1 relA1 supE44* Δ(*lac-proAB*)	Promega
E. coli XL1-MRF′	*lac* (F′ *proAB lacIqZΔM15* Tn*10*) (Tet^r Δ*mcrA*) 183Δ(*mcrCB-hsdSMR-mrr*) 173 *endA1 supE44 thi-1 recA1 gyrA96 relA1*	Stratagene
E. coli SOLR	F′ *proAB lacIqZΔM15* Su$^-$(nonsupressing)*lac gyrA96 relA1 thi-1 endA1* λ^R *sbcC recB recJ umuC*::Tn5(Kan^r) *uvrC* Δ(*mcrCB-hsdSMR-mmr*)171	Stratagene
E. coli TOP10F′	F′[*lacIq* Tn*10* (Tet^r)] *lacZΔM15* Δ*deoR recA1 araD* 139 Δ(*ara-leu*)7679 *galU galK rpsL endA1 nupG mcrA* Δ(*mrr-hsdRMS-mcrBC*)φ80	Stratagene
Plasmids		
pSKII(−)	Cloning vector Ap^r	Stratagene
pB1-7	pSKII(−) with 9-kb *rpsB-tsf* insert	This work
pB1-7-L	pSKII(−) with 4-kb *Eco*RI insert from pB1-7	This work
pB1-7-B	pSKII(−) with 5-kb *Eco*RI insert from pB1-7	This work
pGEM-T	PCR cloning vector Ap^r	Promega
pROS100	pGEM-T with 900-bp *tuf* insert	This work
pGEX-4T-1	GST gene fusion vector Ap^r	Pharmacia
pROS101	pGEX4T-1 with 900-bp *tuf* insert	This work
pROS102	pGEX4t-1 with 900-bp tuf insert (opposite orientation)	This work
pCR2.1	TA cloning vector Ap^r	Invitrogen
pROS103	pCR2.1 with 1,100-bp *tuf* insert	This work

grown in top agar on NZY agar plates. Bacteriophage plaques were removed with sterile Pasteur pipettes and were transferred to phage dilution SM buffer, and plasmids were excised as described in the Stratagene λZapII-*Eco*RI-CIAP cloning kit instruction manual. In order to maintain plasmids in *E. coli*, antibiotics were incorporated into the media (100-μg of ampicillin ml^{-1} and 50-μg of kanamycin ml^{-1}). *C. burnetii* was grown in embryonated yolk sacs and was purified as previously described (23).

Preparation of MAb. Monoclonal antibodies (MAbs) were generated against formalin-inactivated *C. burnetii*. Hybridoma cell lines were created by using a standard mouse hybridoma fusion protocol (8). Briefly, three mice were immunized with 50 μg of inactivated bacteria four times at 14-day intervals. Two mouse spleens were used to isolate splenocytes which were then fused with Sp2/O-Ag-14 myelomas (American Type Culture Collection, Rockville, Md.), and hybridoma cultures which produced *C. burnetii*-reactive antibodies were detected with an enzyme-linked immunosorbent assay against whole organisms. Reactive clones were subcloned by limiting dilution, were tested for reactivity by Western blotting and by immunofluorescence assay using infected L929 cells, and were used to prepare ascitis fluid (8). Only MAbs which did not react with *E. coli* proteins were maintained.

Isolation of chromosomal and plasmid DNAs. *C. burnetii* Nine Mile phase I chromosomal DNA was extracted by a thermolysin-sodium dodecyl sulfate (SDS) procedure (22). Plasmid minipreps were prepared by an alkaline lysis procedure (Qiagen, Valencia, Calif.).

Screening of a *C. burnetii* genomic library. The MAbs designated NM7.3 and NM183 were used to screen a bacteriophage λZapII genomic DNA library of the *C. burnetii* Nine Mile isolate for clones expressing the immunoreactive antigen. The *C. burnetii* genomic DNA library was constructed with *Eco*RI partially digested chromosomal DNA fragments ligated with *Eco*RI-digested λZapII arms as described in the Stratagene λZapII cloning kit manual. Bacteriophage λZapII was mixed with *E. coli* MRF and was incubated on NZY agar plates to yield approximately 500 plaques per plate. Plates were incubated overnight at 37°C to obtain visible plaques. Isopropyl-β-D-thiogalactopyranoside (IPTG) induction was conducted by overlaying plates with nitrocellulose membranes soaked in 10 mM IPTG and incubating for 10 h at 37°C. The membranes were screened by Western blotting using either MAb NM7.3 or NM183. Once putative positive clones were identified, secondary retesting was conducted to confirm positive clones. These were subjected to in vivo excision to excise the pBluescript SK(−) phagemid containing the cloned insert from the λZapII vector. These clones were further characterized for production of the immunoreactive product by incubating *E. coli* harboring the plasmid in Luria-Bertani broth supplemented with 5 mM IPTG at 37°C overnight and then analyzing cell lysates by Western blotting.

Separation of LCV and SCV. LCV and SCV were separated essentially as described previously (10, 25). Nine Mile phase I bacteria were purified from infected yolk sacs and then resuspended in 32% cesium chloride. This was centrifuged at 27,000 rpm overnight and the resulting upper (SCV) and lower bands (LCV) were separated and pelleted by centrifugation. Both forms were resuspended in sucrose phosphate and were stored at −80°C until use.

Western blotting. *C. burnetii* or *E. coli* expressing cloned *C. burnetii* proteins was resuspended in sample buffer (4% SDS, 10% β-mercaptoethanol, 20% glycerol, and 0.25 M Tris, pH 8.0) and was boiled for 10 min, and solubilized protein was separated by SDS-polyacrylamide gel electrophoresis (PAGE). Proteins were transferred to nitrocellulose membranes and were reacted with antibodies as previously described (13). MAbs against *C. burnetii* were prepared as ascites and used at a 1:250 dilution. A horseradish-peroxidase-conjugated secondary antibody was used at a 1:1,000 dilution (Bio-Rad, Hercules, Calif.). Anti-glutathione *S*-transferase (GST) antibody (Sigma, St. Louis, Mo.) was used at a 1:1,000 dilution, and secondary anti-goat antibody (Sigma) was used at a 1:5,000 dilution. An estimation of the relative levels of EF-Ts expression by *C. burnetii* LCV and SCV was determined by separating equal amounts of organisms (as measured by optical density at 600 nm) and quantitating their Western blot reactivities by using densitometric analysis (AlphaEase 3.22, Alpha Innotech Corp., San Leandro, Calif.).

PCR amplification. All PCRs were carried out in a DNA thermo-cycler (Biometra, Tampa, Fla.) using a GenAmp kit (Perkin-Elmer, Branchburg, N.J.). One-hundred-microliter reactions were carried out with *Taq* DNA polymerase (Perkin-Elmer). Degenerate primers were ordered from Genosys Biotechnologies Inc., The Woodlands, Tex. Primers were designated P1 (5′GAYTAYGTNA ARAAYATG3′), P2 (5′RTCNCCNGGCATNACCAT3′), and P3 (5′TGGTAG AGCRCWYSCWTGGTAAG3′) and were used at final concentrations of 0.5 μmol per 100-μl reaction volume. The amplification procedure consisted of 30 cycles of 1 min at 95°C, 1 min at 45°C, and 1 min at 72°C. PCR products were separated in a 1% agarose gel and were purified using a Geneclean kit (Bio 101, Vista, Calif.). Desired PCR products were subsequently cloned into PCR cloning vectors pCR2.1 TOPO-TA (Invitrogen, Carlsbad, Calif.) or pGEM-T (Promega, Madison, Wis.).

Southern blotting. Genomic DNA from *C. burnetii* was digested with restriction enzyme according to the manufacturer's protocol (Boehringer Manheim, Indianapolis, Ind.). DNAs were then electrophoresed through 0.8% agarose gels and transferred to a nitrocellulose membrane (16a). Labeling of a DNA probe with [α-P^{32}]dCTP was carried out using a random DNA labeling Decaprime II kit (Ambion, Austin, Tex.). Blots were incubated with probe overnight at 65°C and then washed four times at high stringency for 30 min each at 65°C with 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS. Blots were analyzed for hybridization patterns on a phosphorimager image reproduction (Model SF, Molecular Dynamics, Sunnyvale, Calif.).

DNA sequence analysis. All DNAs were sequenced at Gene Technology Laboratories at Texas A & M University. Sequence homologies were compared using MacVector and BLAST programs.

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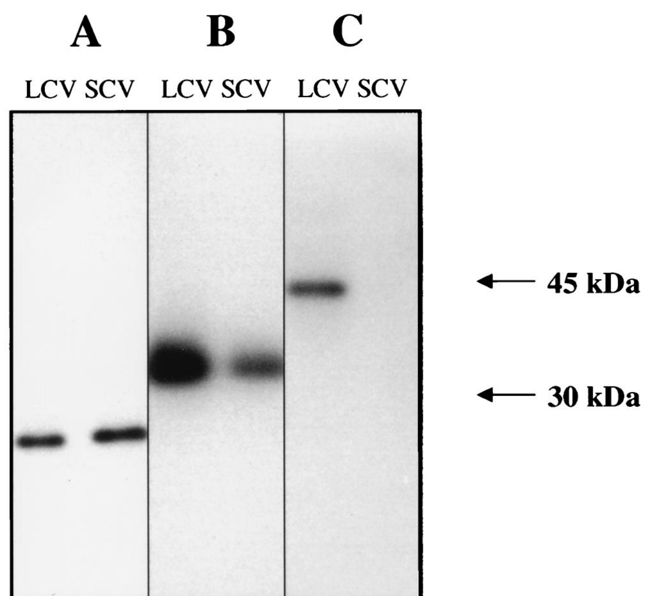

FIG. 1. Differential expression by LCV and SCV. LCVs and SCVs were separated by cesium chloride gradient centrifugation, and equal amounts (total protein) were separated by SDS-PAGE and were Western blotted. (A) Cb-Mip detected with MAb NM175. (B) A 32-kDa protein detected with MAb NM183. (C) A 45-kDa protein detected with MAb NM7.3.

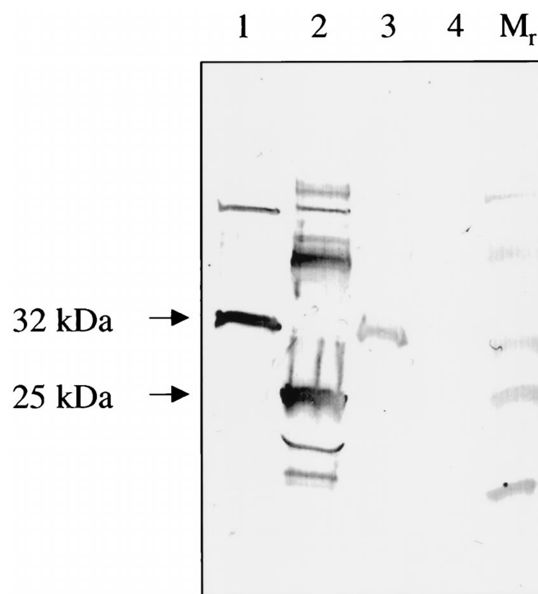

FIG. 2. Immunoreactivity of pB1-7 clones. Western blot detection of MAb NM7.3-reactive antigens. Lane 1, pB1-7-expressed 32-kDa immunoreactive product; lane 2, pB1-7-L-expressed major immunoreactive 25-kDa antigen and several additional reactive bands; lane 3, MAb NM7.3-detected 32-kDa product in *C. burnetii*; lane 4, pB1-7-B did not produce an immunoreactive product; lane M, molecular mass markers.

Nucleotide sequence accession numbers. The sequences of *rpsB-fts* and *tufB* have been deposited in GenBank under accession no. AF127534 and AF136604, respectively.

RESULTS

Screening of MAb. MAbs were generated against *C. burnetii* by immunizing mice with purified, formalin-killed organisms. The immunoreactivities of antibodies produced by hybridoma cell lines against *C. burnetii* proteins and absence of cross-reactivity with *E. coli* antigens on enzyme-linked immunosorbent assay was determined, and *C. burnetii*-specific hybridomas were cloned by limiting dilution. The antigen specificity of each MAb was determined by Western blotting using purified *C. burnetii*, and 10 MAbs were used in the survey to detect life cycle-specific antigens. To detect differentially expressed antigens, SCV and LCV were first separated by 32% cesium chloride isopycnic gradient centrifugation of purified organisms. Whole-cell lysates of each form were then separated by SDS-PAGE and were subsequently analyzed by immunoblotting with different MAbs. MAb NM7.3 detected a 32-kDa antigen that appeared to be produced by LCV at a level greater than fourfold higher than in SCV (Fig. 1B) when compared by scanning densitometry. MAb NM183 detected a 45-kDa antigen in LCV but not in SCV (Fig. 1C). The total protein applied to each lane of LCV and SCV was comparable, and several proteins were used as controls, including ScvA (SCV specific) (data not shown), Com-1 (not differentially expressed) (data not shown), and *C. burnetii* macrophage infectivity potentiator-like protein (Cb. Mip) (20) (Fig. 1A), which was expressed at comparable levels by LCV and SCV.

Cloning of the gene encoding the 35-kDa antigen. Since neither MAb cross-reacted with *E. coli* proteins, direct cloning of the gene encoding this antigen was performed using a *C. burnetii* gene bank expressed in *E. coli*. *Eco*RI-digested *C. burnetii* Nine Mile DNA was ligated into λZapII and then transfected into *E. coli* MRF cells. Plaques were screened for the production of MAb NM7.3-reactive protein. Immunoreactive plaques were purified, followed by in vivo plasmid excision. Reactive clone pB1-7 was analyzed by Western blotting and was confirmed to produce a 32-kDa antigen that reacted with MAb NM7.3 (Fig. 2, lane 1). pB1-7 was mapped by restriction enzymes and was found to have an internal *Eco*RI site. Two subclones of the 9-kb insert were constructed in pBluescript SKII(−) and were designated pB1-7-L (4-kb *Eco*R1 fragment) and pB1-7-B (5-kb *Eco*RI fragment) (Fig. 3A). Subclones were subjected to SDS-PAGE and were tested by Western blot analysis for expression of MAb NM7.3-reactive product. pB1-7-L expressed an approximately 25-kDa immunoreactive product, whereas pB1-7-B did not express an immunoreactive protein (Fig. 2, lanes 2 and 4, respectively).

DNA sequence analysis. To predict a function for the immunoreactive 32-kDa differentially expressed protein, the DNA sequence was determined for a region of pB1-7 that included the open reading frame (ORF) responsible for expression of this antigen (GenBank accession no. AF127534). BLAST comparison of the ENTREZ database to this sequence revealed 54% identity to the *E. coli rpsB-tsf* gene cluster which encodes 30S ribosomal subunit protein S2 and EF-Ts. Sequence analyses of subclones also revealed that pB1-7-L expressed an immunoreactive protein fused the β-galactosidase peptide and carried the majority of the *tsf* ORF while pB1-7-B contained the entire *rpsB* and the 5′ region of *tsf* (Fig. 3A and B).

Cloning of the gene expressing the 45-kDa antigen. The λZapII expression library was screened with MAb NM183, but no immunoreactive clones were identified (>10,000 plaques surveyed). An alternative strategy was devised to clone the gene encoding this antigen. MAb NM183 was found to cross-react strongly with a ~45-kDa protein from *C. trachomatis* (Fig. 4, lane 1). This molecular mass corresponds with the chlamydial EF-Tu protein previously identified (6). To test whether MAb NM183 reacted with this antigen, purified re-

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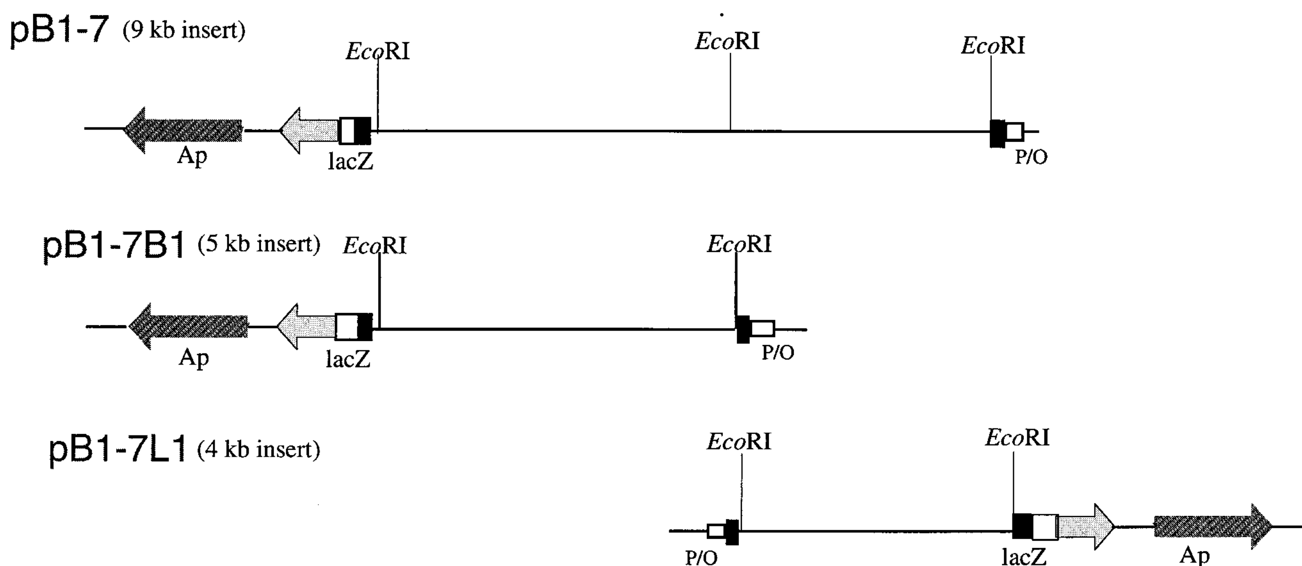

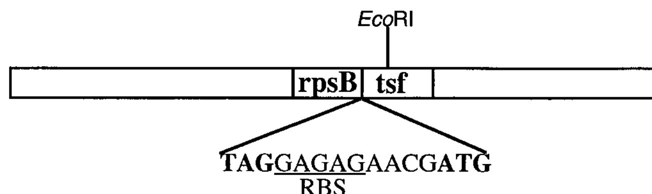

FIG. 3. Genetic map of the *C. burnetii rpsB-tsf* operon. (A) The major regions of the MAb NM7.3-reactive clone and subclones used for sequence analysis and ORF determination. (B) The genetic organization for the *rpsB-tsf* operon of *C. burnetii*, including the joint region between the two ORFs. A potential ribosomal binding site (RBS) is underlined and immediately follows the stop codon of *rpsB*.

combinant His-tagged-chlamydial EF-Tu was obtained from You-Xun Zhang (Boston University School of Medicine, Boston, Mass.) and was found to react strongly with MAb NM183 during Western blotting (Fig. 4, lane 2).

Therefore, the 45-kDa differentially expressed protein was likely to be an EF-Tu of *C. burnetii*. EF-Tus from other bacteria have been previously noted to have highly conserved regions (6). Low-degeneracy primers were developed to isolate these conserved regions from a *C. burnetii tuf* gene. The first set of PCR primers, P1 and P2, amplified a 900-bp internal *tuf*-like region (confirmed by sequence analysis) from *C. burnetii* template DNA (Fig. 5). To test the immunoreactivity of the predicted partial EF-Tu protein encoded by this PCR product (~85% of the predicted complete gene, based upon other bacterial EF-Tu sequences), the 900-bp PCR product was cloned into pGEM-T cloning vector. All the resultant clones (such as pROS100) had the 900-bp insert in a nonexpressing orientation with regards to the β-galactosidase promoter, suggesting a negative selection pressure for clones expressing this ORF. To overcome the orientation problem, clones were constructed in an alternate prokaryotic expression vector pGEX4T-1 GST fusion vector which also encodes the *lacI*^{q} suppressor, conferring stringent regulation of expression. This strategy yielded clones with inserts in the expressing orientation. To determine if a GST fusion product was expressed by these clones, proteins from IPTG-induced and uninduced clones were probed by Western blotting with an anti-GST antibody. Induced clones did produce a GST fusion product of the predicted size (~65 kDa) (Fig. 6, lanes 4 and 6). However, this protein failed to react with MAb NM183 (data not shown).

A larger clone that included the 5′ region of *tuf* was cloned. This strategy was based on evidence from Southern blot analysis of chromosomal digests probed with the 900-bp PCR-amplified *tuf* region that *C. burnetii* contain both *tufA* and *tufB* homologues. This probe hybridized with 6- and 10-kb *Eco*RI fragments of chromosomal DNA (Fig. 7, lane 2). Sequence information had revealed an internal *Pvu*I restriction site. Four fragments of hybridization were seen with DNA digested with *Pvu*I and *Eco*RI, suggesting two copies of the gene encoding EF-Tu. Organization of *tuf* loci and its flanking genes in other bacteria revealed conservation of tRNAs flanking the *tufB* genes in other organisms. This information was used to design an upstream primer, designated P3, based on the conserved threonine tRNA sequence. Primers P3 and P2 amplified an ~1,200-bp product. Sequence analysis (GenBank accession no. AF136604) confirmed this product encoded the entire N-ter-

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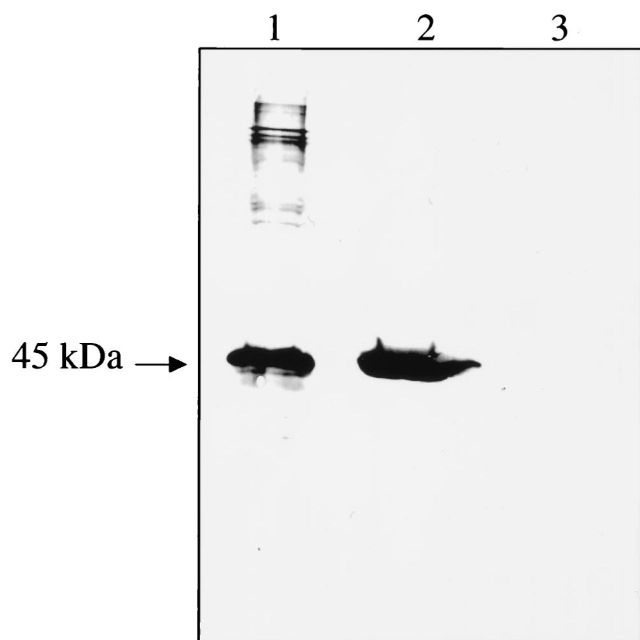

FIG. 4. Immunoreactivity with NM183. Lane 1, Western blot-detected 45-kDa antigen in *C. trachomati* bv. E; lane 2, NM183 reacted with purified *C. trachomatis* His-tagged recombinant EF-Tu; lane 3, no reactivity with *E. coli*.

minal region of EF-Tu as well as a weak promoter region (Fig. 5), similar to the previously characterized *tufB* promoter in *E. coli* (15). This product was cloned into pCR2.1 TOPO-TA (pROS103) and was shown to express a ~45-kDa antigen that reacted with MAb NM183 during Western blotting (Fig. 8, lane 4), confirming that EF-Tu is the immunoreactive 45-kDa LCV-specific protein.

DISCUSSION

This study presents the identification, cloning, and sequence analysis of two differentially expressed *C. burnetii* genes, designated *tsf* and *tufB*, which encode the elongation factors EF-Ts and EF-Tu, respectively. MAbs against *C. burnetii* were tested for reactivity against LCV and SCV lysates to identify differentially expressed proteins. A 32-kDa antigen that was upregulated more than fourfold by LCV compared to SCV and a 45-kDa antigen that was expressed by LCV and not detected in SCV were identified in this screen.

Cloning of the 32-kDa antigen was accomplished by the identification of an immunoreactive plaque from a *C. burnetii* genomic library with MAb NM7.3. Western blot analysis of the immunoreactive clone, designated pB1-7, confirmed the expression of a 32-kDa protein. Restriction enzyme mapping of pB1-7 indicated an internal *Eco*RI restriction site, and 4- and 5-kb fragments were subcloned (pB1-7-L and pB1-7-B1, respectively). pB1-7-L encoded a 25-kDa NM7.3-reactive protein, which resulted from the expression of a fusion protein consisting of β-galactosidase peptide and the 20-kDa 3′ end of EF-Ts, as revealed by sequence analysis. MAb NM7.3 also recognized a ~70- to 80-kDa immunoreactive product in *C. burnetii* and recombinant *E. coli* which is likely a oligomeric association of EF-Ts with the translational factor, EF-Tu. EF-Ts mediates reactivation of EF-Tu by displacing GDP and facilitating association of GTP. *Tsf* is found on an operon with *rpsB* (encoding small ribosomal protein S2) in *C. burnetii* as with other bacteria like *E. coli* and *Salmonella typhimurium*. The *C. burnetii* EF-Ts shows 53% identity and 67% similarity to the *E. coli* EF-Ts at the amino acid level. The promoter lying upstream of *rpsB* had typical RpoD-type -10 and -35 consensus sequences, and the spacing between these sigma factor recognition sites was characteristic of that reported in other *C. burnetii* genes (16).

Cloning of the gene encoding the NM183-reactive 45-kDa LCV-specific antigen was less direct because screening of several *C. burnetii* genomic libraries yielded no immunoreactive clones. However, MAb NM183 was found to cross-react strongly with a 45-kDa protein from *C. trachomatis*, although MAbs had been selected for their lack of cross-reactivity with *E. coli* proteins. This led to the speculation that EF-Tu was the 45-kDa immunoreactive protein because of its immunodominant nature, and this view was supported by MAb NM183 cross-reactivity with purified recombinant His-tagged EF-Tu from *C. trachomatis*. A *tuf* homologue gene encoding EF-Tu from *C. burnetii* was cloned by PCR amplification using degenerate primers, sequences which were obtained from the highly conserved regions of eubacterial EF-Tu sequences. A 900-bp internal region of the *tuf* homologue in *C. burnetii* was cloned and subcloned into the GST fusion vector pGEX4T-1. To ensure expression of the 900-bp ORF, the 900-bp fusion protein was tested by Western blotting for reactivity against anti-GST antibody and was found to express a fusion protein of the predicted size. However, the fusion protein did not react with NM183.

Our data also indicated that *C. burnetii* has two copies of the *tuf* gene, the occurrence of which has precedence in other bacteria like *E. coli* and *S. typhimurium*, as well as other enteric and selected gram-positive bacteria. To clone the entire region of the *tuf* homologues, the organization of various *tufA*- and *tufB*-containing operons were compared and found to show a conservation in the arrangement of tRNAs in the tRNA-*tufB* operon. Based upon the conservation of organization of the Thr-tRNA-*tufB*-Trp-tRNA sequence, primers were designed from conserved tRNA sequences for threonine and tryptophan. The Thr-tRNA-based primer combined with the P2 primer yielded a 1,200-bp amplified product which contained a region that encoded all but the 3′ end of the EF-Tu-B protein. The PCR product was cloned into pCR2.1 TOPO-TA cloning vector and was IPTG induced to express the EF-Tu-B product. This protein reacted with MAb NM183 during Western blotting, confirming that the immunoreactive LCV-specific 45-kDa antigen was EF-Tu.

Therefore, EF-Ts and EF-Tu, important components of the translational machinery of the cell, were found to be differentially regulated by the LCV and the SCV. EF-Tu is particularly interesting since it is believed to be a multifunctional protein which not only promotes binding of aminoacyl tRNA to the ribosome, but also plays a role in transcriptional activation and has been speculated to regulate synthesis of proteins which respond to stress at the level of transcription. The cellular content of EF-Tu, in *E. coli* for example, varies in direct proportion to the growth rate, and this results from a higher rate of *tuf* gene transcription in rapidly growing cells as well as a higher translational efficiency (judged by number of transcripts per mRNA) of *tuf* mRNA (27). EF-Tu has been shown to be methylated on amino acid residue lysine 56 in a nutrient-dependent manner, and the proposed role of this methylation is in triggering membrane dissociation in order to carry out an unknown intracellular regulatory function (26). In another study, methylation of EF-Tu has been reported to slow the rate of GTP hydrolysis in vitro (24). EF-Tu also has chaperone-like properties and has been shown to interact with unfolded and denatured proteins and to promote functional renaturation

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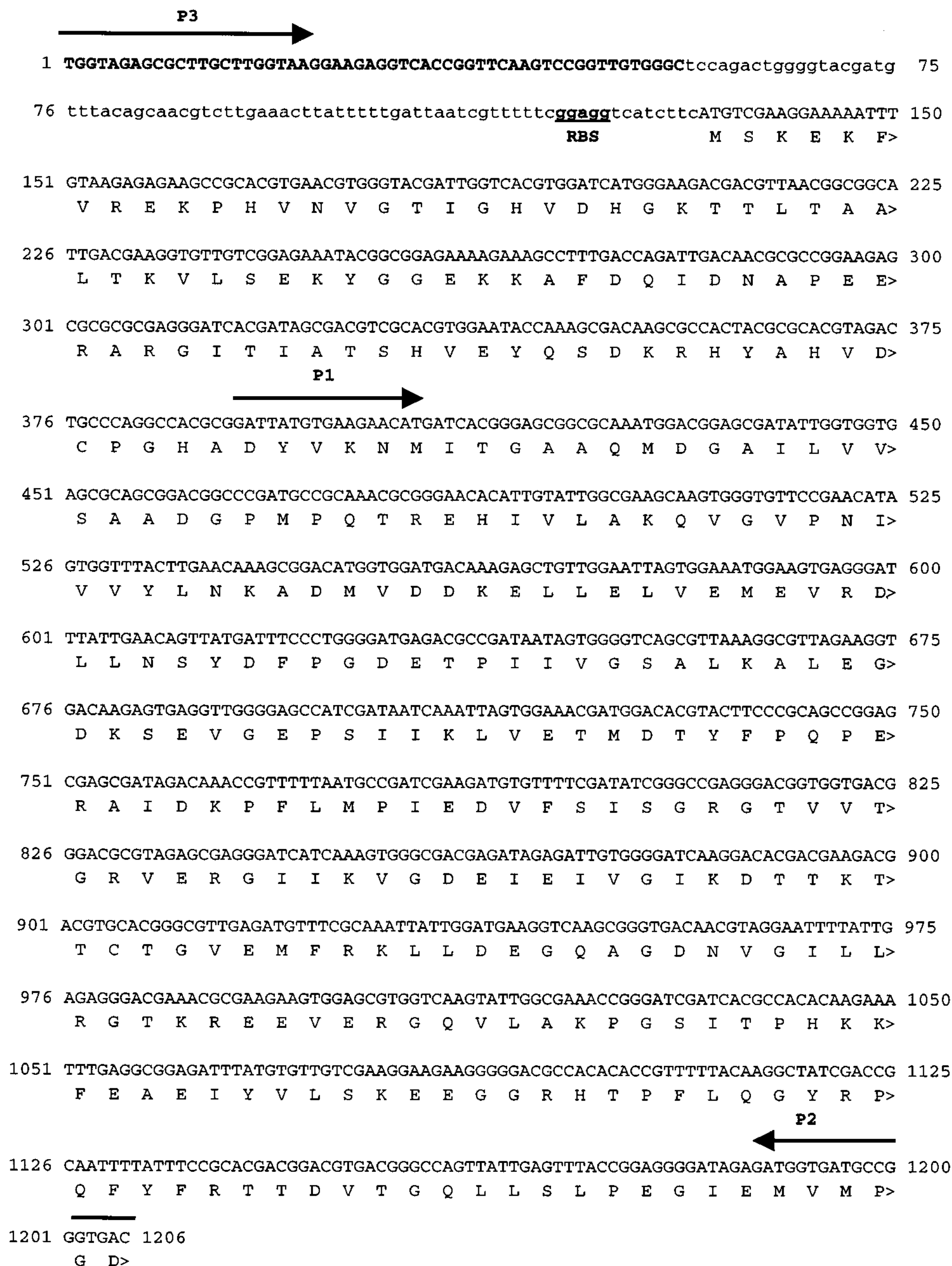

FIG. 5. DNA sequence of *C. burnetii* clone expressing EF-Tu peptide which is the MAb NM183-reactive antigen. Degenerate primers used in cloning this DNA from the *C. burnetii* chromosome are represented by bold arrows over the corresponding sequences. The predicted translation of this ORF is indicated. The potential ribosomal binding region (RBS) is underlined. The predicted threonine tRNA is noted in bold starting at bp 1. The GenBank accession no. of this sequence is AF136604.

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(5). The observation that EF-Tu remains undetected by Western blot analysis in the potentially less metabolically active, but not inert, SCV may be accounted for by several distinct mechanisms. For example, EF-Tu may be less stable in SCV, allowing a down regulation of vegetative protein synthesis while allowing the synthesis of stress- or starvation-induced proteins. The purification and separation of cell forms require several hours of preparation once bacteria are released from host tissues, and this may exaggerate the difference in EF-Tu. However, preliminary confocal microscopic evaluation of EF-Tu expression by *C. burnetii* in infected cells supports a radically reduced expression by many small cell forms (data not shown).

The observation that translational factors are either genetically down regulated or unstable in SCV has obvious implications in the development of a model of the functional roles of SCV and LCV and is consistent with earlier observations (11, 18). This hypothesis obviously raises questions regarding how these populations are maintained. For example, is there a central regulator of gene expression that mediates transcription of genes in SCV but remains relatively inactive or down

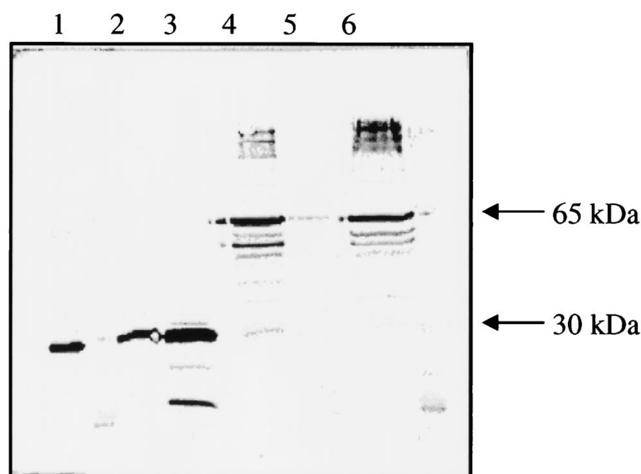

FIG. 6. Expression of GST fusion protein. Western blot of partial *tuf* clones in pGEX4T-1 was probed with anti-GST antibody to confirm expression of recombinant EF-Tu. Lane 1, purified GST; lane 2, uninduced pROS102 (gene inserted in nonexpressing orientation); lane 3, IPTG-induced clone pROS102 did not express a GST-fusion protein; lane 4, IPTG-induced clone pROS101 (gene inserted in correct orientation) expressed a ~65-kDa GST-EF-Tu fusion protein; lane 5, uninduced clone pROS101 did not express a significant amount of the fusion protein; lane 6, identically prepared sample as in lane 4.

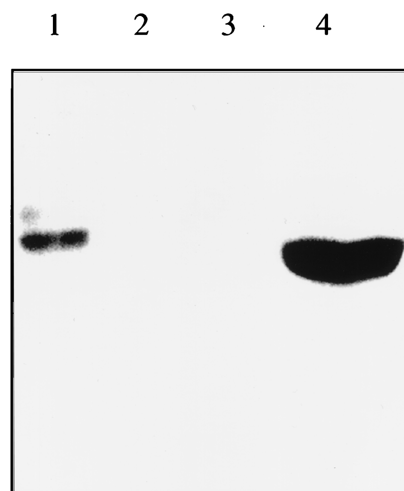

FIG. 8. Immunoreactivity of *C. burnetii tuf* clone with NM183. Lane 1, whole-cell lysate of *C. burnetii*; lane 2, pROS100; lane 3, pROS101; lane 4, pROS103.

regulated in LCV or vice versa (in other words, is there a RpoS homologue that is differentially expressed in *C. burnetii*)? Preliminary evidence suggests that *C. burnetii* may possess an *rpos* homologue, and work is underway to clone this gene and evaluate SCV versus LCV expression. However, the contradiction regarding the maintenance of two seemingly disparate populations of cells (in what seems like an equal proportion) within the same intraphagolysosomal compartment cannot be ignored. What is the trigger that induces the formation of one or the other cell type? What is the significance? Is the event programmed, or do random events lead to the coexistence of a similar number of LCVs and SCVs within the same microenvironment? Does each individual cell type secrete some unknown substance that alters its microenvironment and facilitates its own maintenance? These questions should be addressed in future investigations.

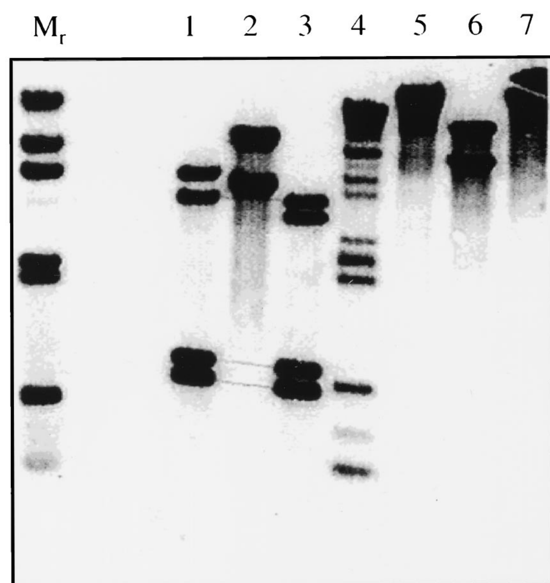

FIG. 7. Southern blot localization of putative *tufA* and *tufB* genes. The 900-bp PCR-amplified internal EF-Tu gene region of was used as a probe to hybridize with *C. burnetii* chromosomal DNA digests. Probes hybridized with *Pvu*I digest (lane 1), *Eco*RI (lane 2), *Eco*RI and *Pvu*I digest (lane 3), *Cla*I digest (lane 4), *Bam*HI digest (lane 5), *Hin*dIII digest (lane 6), and *Pst*I digest (lane 7). Molecular weight markers (lane M_r) are ^{32}P-labeled *Hin*dIII-digested Lambda DNA.

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REFERENCES

1. **Amano, K., J. C. Williams, T. E. McCaul, and M. G. Peacock.** 1984. Biochemical and immunological properties of *Coxiella burnetii* cell wall and peptidoglycan-protein complex fractions. J. Bacteriol. **160:**982–988.
2. **Babudieri, C.** 1959. Q fever: a zoonosis. Adv. Vet. Sci. **5:**81–84.
3. **Baca, O. G., and D. Paretsky.** 1983. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. Microbiol. Rev. **47:**127–149.
4. **Brouqui, P., and D. Raoult.** 1993. Chronic Q fever. Arch. Intern. Med. **153:**642–648.
5. **Caldas, T. D., A. E. Yaagoubi, and G. Richarme.** 1998. Chaperone properties of bacterial elongation factor EF-Tu. J. Biol. Chem. **273:**11478–11482.
6. **Cousineau, B., C. Cerpa, J. Lefebvre, and R. Cedergren.** 1992. The sequence of the gene encoding elongation factor Tu from *Chlamydia trachomatis* compared with those of other organisms. Gene **120:**33–41.
7. **Davis, G. E., and H. R. Cox.** 1938. A filter-passing infectious agent isolated from ticks. I. Isolation from Dermacentor andersonii, reactions in animals, and filtration. Public Health Rep. **53:**2259.
8. **Gooding, J. W.** 1988. Monoclonal antibodies: principles and practices. Academic Press, San Diego, Calif.
9. **Heinzen, R. A.** 1997. Intracellular development of *Coxiella burnetii*, p. 99–129. *In* B. Anderson (ed.), Rickettsial infection and immunity. Plenum Press, New York, N.Y.
10. **Heinzen, R. A., and T. Hackstadt.** 1996. A developmental stage-specific histone H1 homolog of *Coxiella burnetii*. J. Bacteriol. **178:**5049–5052.
11. **Heinzen, R. A., T. Hackstadt, and J. E. Samuel.** 1999. Developmental biology of *Coxiella burnetii*. TIMS **7:**149–154.
12. **Heinzen, R. A., D. Howe, L. P. Mallavia, D. D. Rockey, and T. Hackstadt.** 1996. Developmentally regulated synthesis of an unusally small, basic peptide by *Coxiella burnetii*. Mol. Microbiol. **22:**9–19.
13. **Hendrix, L. R., L. P. Mallavia, and J. E. Samuel.** 1993. Cloning and sequencing of *Coxiella burnetii* outer membrane protein gene *com1*. Infect. Immun. **61:**470–477.

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14. **Higgins, C. F., D. Hinton, C. S. J. Hulton, T. Owen-Hughes, G. D. Pavitt, and A. Seriafi.** 1990. Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? Mol. Microbiol. **4:**2007–2012.
15. **Lee, J. S., G. An, and J. D. Friesen.** 1981. Location of the *tufB* promoter of *E. coli*: cotranscription of *tufB* with four transfer RNA genes. Cell **25:**251–258.
16. **Mallavia, L. P.** 1991. Genetics of Rickettsiae. Eur. J. Epidemiol. **7:**213–221.
16a. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **McCaul, T. F.** 1991. The developmental cycle of *Coxiella burnetii*, p. 223–258. *In* J. C. Williams and H. A. Thompson (ed.), Q fever: the biology of *Coxiella burnetii*. CRC Press, Boca Raton, Fla.
18. **McCaul, T. F., T. Hackstadt, and J. C. Williams.** 1981. Ultrastructural and biological aspects of *Coxiella burnetii* under physical disruptions, p. 267. *In* W. Burgdorfer and R. L. Anacker (ed.), Rickettsiae and rickettsial diseases. Academic Press, New York, N.Y.
19. **McCaul, T. F., and J. C. Williams.** 1981. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. J. Bacteriol. **147:**1063–1076.
20. **Mo, Y. Y., N. P. Cianciotto, and L. P. Mallavia.** 1995. Molecular cloning of a Coxiella burnetii gene encoding a macrophage infectivity potentiator (Mip) analogue. Microbiology **141:**2861–2871.
21. **Rosenberg, M., and N. Kordova.** 1960. Study of intracellular forms of *Coxiella burnetii* in the electron microscope. Acta Virol. **4:**52–61.
22. **Samuel, J. E., M. E. Frazier, M. L. Kahn, L. S. Thomashow, and L. P. Mallavia.** 1983. Isolation and characterization of a plasmid from phase I *Coxiella burnetii*. Infect. Immun. **41:**488–493.
23. **Samuel, J. E., M. E. Frazier, and L. P. Mallavia.** 1985. Correlation of plasmid type and disease caused by *Coxiella burnetii*. Infect. Immun. **49:**775–779.
24. **Van Noort, J. M., B. Kraal, M. C. Sinjorgo, N. L. M. Persoon, E. S. D. Johanns, and L. Bosch.** 1986. Methylation in vivo of elongation factor EF-Tu at lysine-56 decreases the rate of tRNA-dependent GTP hydrolysis. Eur. J. Biochem. **160:**551–561.
25. **Wiebe, M. E., P. R. Burton, and D. M. Shankel.** 1972. Isolation and characterization of two cell types of *Coxiella burnetii*. J. Bacteriol. **110:**368–377.
26. **Young, C. C., and R. W. Bernlohr.** 1991. Elongation factor Tu is methylated in response to nutrient deprivation in *Escherichia coli*. J. Bacteriol. **173:**3096–3100.
27. **Young, F. S., and A. V. Furano.** 1981. Regulation of the synthesis of *E. coli* elongation factor Tu. Cell **24:**695–706.

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