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A Heat Shock Operon in *Coxiella burnetii* Produces a Major Antigen Homologous to a Protein in Both Mycobacteria and *Escherichia coli*

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A gene library from the DNA of *Coxiella burnetii* has been constructed in the cosmid vector pHC79. A particular clone, pJB196, reacted strongly with *Coxiella*-specific antibodies elicited in a number of different species of animals. This clone produced two abundant *C. burnetii*-specific polypeptides, a 14-kilodalton nonimmunoreactive protein and a 62-kilodalton immunoreactive protein. Sequencing identified two open reading frames, encoding polypeptides of 10.5 and 58.3 kilodaltons. The only transcriptional control element observed on the 5′ side of the initiation codon resembled a heat shock promoter. This heat shock promoter was functionally regulated in *Escherichia coli*, since both proteins were produced by growth conditions at 37°C and neither protein was detected at 23°C. There were four sequences from the literature that were highly homologous (>50%) to the 62-kilodalton protein from *C. burnetii*. Three were from *Mycobacterium* species and represent the immunodominant antigen of this genus. The other was from *E. coli*, detected as a gene that complements or suppresses a temperature-sensitive RNase activity. Since the recombinant protein was immunogenic, it may serve as an efficacious vaccine against *C. burnetii* and other pathogenic microorganisms that express the conserved antigen.

Q fever is caused by a gram-variable and weakly acid-fast-staining obligate intracellular bacterial parasite of eucaryotic cells (16). This bacterium, *Coxiella burnetii*, is unique among the pathogenic rickettsiae because it replicates in the microbicidal milieu of the phagolysosome (12), where it carries out a developmental cycle which consists of sporogenic differentiation with both binary and unequal cell division (16). Humans can be infected after exposure to only one virulent phase I microorganism through a bite from an infected tick or by aerosols. Acquisition of Q fever usually occurs after exposure to aerosols generated by infected, parturient animals (4). Microorganisms spread systemically from the lung and induce a primary, slowly resolved disease course which closely mimics influenza. In approximately 5% of infected individuals, the time course is extended thereby producing chronic hepatitis and occasionally endocarditis. The acute and chronic forms of the disease are treated with tetracycline (13). However, chronic endocarditis disease requires long-term (1 or 2 years) treatment with tetracycline or rifampin. Chemotherapy is successful only after surgery to replace the affected mitral or aortic valve.

The host immune response to infection by *C. burnetii* is first recognized by antibody formation to surface proteins of the avirulent phase II and the virulent phase I microorganisms (13, 36, 37). Antibodies directed against phase I (smooth-type) lipopolysaccharide (LPS) occur later in the infection (37), and high titers to phase I cells (20) and smooth-type LPS are diagnostic for chronic disease (Williams, unpublished data). However, most individuals with acute Q fever resolve the infection without the formation of antibody to the smooth-type LPS (Williams, unpublished data). Importantly, individuals vaccinated with inactivated phase I whole cells and considered to be immune usually do not develop titers to the smooth-type LPS (Williams, unpublished data).

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Recent studies indicate that the surface proteins of *C. burnetii* may be sufficiently immunogenic to be used as subunit vaccines (35). There are approximately 30 to 35 surface-exposed proteins of *C. burnetii* (31), with certain of these participating as primary antigens. High levels of antibodies and a cooperative cellular immune response against specific protein antigens are required for the protection against Q fever. The classical approach to vaccination has led to the production of a phase I whole cell preparation (26) and phase I subfractions (9, 10, 32, 33), which were efficacious but also caused undesirable side effects. However, the arduous task of growing and producing *C. burnetii* is quite expensive and hazardous, requiring special handling in containment facilities.

In theory, recombinant DNA technology would offer an advantage for a subunit-based vaccine. Once DNAs that encode protective immunogens are cloned, the immunizing antigen is purified, and humoral and cellular immune responses are elicited, cultivation of the parasite will not be necessary. A subunit vaccine may circumvent the pathogenic potential of the current investigational phase I whole cell vaccine (3). Furthermore, since classical genetic analysis of *C. burnetii* is difficult, recombinant DNA studies may also contribute to a basic understanding of the molecular biology of parasitism.

The present study was concerned with the cloning and sequencing of DNA encoding two tightly linked proteins from *C. burnetii*. Analysis of the primary structure of the operon revealed a heat shock promoter (HSP), two strong ribosomal binding sites, and a region of extensive dyad symmetry just distal to the second open reading frame. The proximal gene of the operon, *htpA*, starts 58 bases downstream from the common HSP and ends 27 bases upstream from *htpB*. The *C. burnetii htpB* gene product has a calculated molecular mass of 58.3 kilodaltons (kDa) and an apparent molecular mass of 62 kDa. The expression of both polypeptides is under the regulation of the endogenous HSP

because they accumulated in *Escherichia coli* at 37°C but not at 23°C. Regions of *C. burnetii htpB* are evolutionarily conserved because sequence homology was identified in an *E. coli* 17-kDa protein and a major antigen of the *mycobacteria*. Expression of *C. burnetii* antigens by recombinant DNA clones is an important first step in the development of unique skin test reagents, diagnostic antigens, and potential vaccines to combat Q fever.

MATERIALS AND METHODS

Construction of a gene library and antigen detection. The phase I clone 7 of the Nine Mile strain of *C. burnetii* (CB9MIC7) was grown in yolk sacs of embryonated chicken eggs and purified with a Renografin (E. R. Squibb & Sons, Princeton, N.J.) gradient procedure (34). DNA extraction and construction of the cosmid bank in pHC79 were as previously described (29). Colonies were screened for expression of antigens by modification of the Broome and Gilbert procedure (5). Lysates from *E. coli* were prepared by boiling the pelleted bacteria for 4 min in Laemmli sample buffer (15). Immunoprecipitations were done by incubating antigen and antibody for 30 min at room temperature, and the complex was collected batchwise with staphylococcal protein A immobilized on Sepharose beads (35). An in vitro transcription-translation system that utilized purified plasmid DNA was used according to the instructions of the manufacturer (Amersham Corp., Arlington Heights, Ill.).

Preparation of specific antibodies against *C. burnetii*. Six-week-old female C3H/HeN mice were inoculated intraperitoneally with 10^6 viable CB9MIC7. Seven weeks later blood was taken by exsanguination from the brachial artery, and serum was maintained at −70°C until use. Hyperimmune sera against *C. burnetii* were also produced in guinea pigs and rabbits for comparison. Human serum was obtained from a person with documented chronic Q fever (20) and from a person after vaccination and subsequent laboratory exposure. Serum was also obtained from a volunteer who had no known previous exposure and no detectable *Coxiella*-specific antibodies by an enzyme-linked immunosorbent assay (33, 36). Many of the immune sera cross-reacted with polypeptides of the *E. coli* host. These antibodies were removed from immune sera by adsorption with host antigen (19).

A peptidoglycan fraction (2) from recombinant *E. coli* was prepared and inoculated intraperitoneally into BALB/c mice three times at 2-week intervals. Sera were collected 2 weeks later, pooled, and maintained at −70°C until use.

Subcloning and sequencing. Restriction enzymes were obtained from Bethesda Research Laboratories (Gaithersburg, Md.), and the directions of the manufacturer were followed for their use. Subcloning was done either in the original vector pHC79, but without subsequent packaging, or in the pGEM1 vector. The sequence was determined by dideoxynucleotide chain termination (21) with primer extension.

Sequence analyses were performed with both the IntelliGenetics and Molgen Jr packages (J. Lowe, Fed. Proc. 45:1852, 1986). Hydropathy indices were plotted by the method of Kyte and Doolittle (14). These were computed by using the protein sequence management program from IntelliGenetics with an average window of nine amino acids.

RESULTS

Immunodetection of recombinant colonies for *C. burnetii*-specific antigens. The cosmid bank pJB series (30) derived from chromosomal DNA of CB9MIC7 was screened for the production of antigen by immunodetection at the colony level. All of the immune sera (see Materials and Methods) used at a 1:500 dilution detected a colony designated as pJB196, whereas normal sera used at the same dilution from animals and humans were not reactive with pJB196. A *C. burnetii*-specific polypeptide was detected by a Western blot (immunoblot) of crude whole cell lysates from pJB196 (data not shown). Immunoprecipitation of the lysate before Western blot analysis improved the resolution of the gel (Fig. 1) and revealed a polypeptide with a estimated molecular mass of 62 kDa. This antigenic polypeptide was visible only in pJB196 isolates.

The *C. burnetii*-specific polypeptide unique to pJB196 was verified by using purified recombinant DNA (from pJB196) and vector DNA (pHC79) in an in vitro transcription-translation assay. In addition to the documented gene products of pHC79 (28), a 62-kDa polypeptide common to pJB196 and some of its subclones was observed (Fig. 2). A broad band was present as well at 14 kDa. The 62- and 14-kDa polypeptides were also identified as prominent proteins in Coomassie blue-stained gels from crude *E. coli* lysates (Fig.

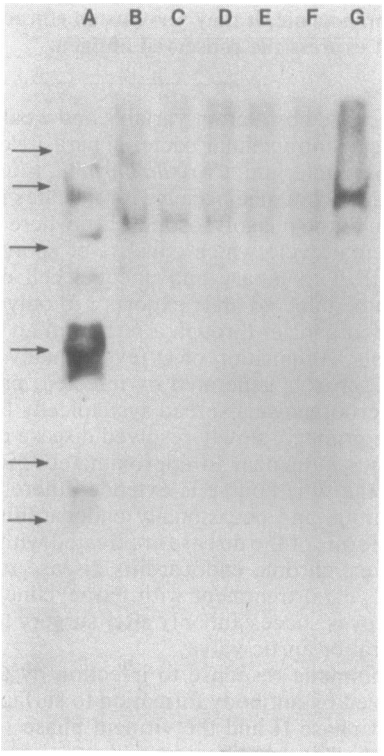

FIG. 1. Western blot of immunoprecipitated *E. coli* lysates and cell-free supernatants. Cultures of *E. coli* were grown to the midlog phase at 37°C, harvested by centrifugation, concentrated 20-fold in lysing buffer (15), and boiled for 4 min. Lysates were diluted 1:5, and cell-free supernatants were diluted 1:1. *C. burnetii*-specific antigens were immunoprecipitated with adsorbed convalescent human serum (1:10,000) and staphylococcal protein A-Sepharose beads. Samples were subjected to Western blot analysis and detected by the same serum as above and ^{125}I-labeled staphylococcal protein A. Lanes: A, lysate of *C. burnetii*; B, supernatant pJB25; C, supernatant pJB145; D, supernatant pJB196; E, lysate pJB25; F, lysate pJB145; G, lysate pJB196. The molecular weights of the protein markers (arrows, from bottom) are 14300, 18400, 25700, 43000, 68000, and 97000. pJB25 and pJB145 were independent clones from the same bank as pJB196.

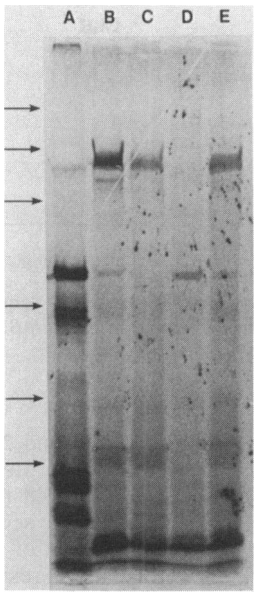

FIG. 2. In vitro transcription-translation analysis of purified plasmid DNAs. Each sample contained 1.5 μg of DNA, 0.7 μCi of [^{35}S]methionine, and components of a commercial kit (Amersham Corp.). The reaction proceeded at 37°C for 40 min and was followed by a 5-min chase with a nonradiolabeled methionine. The reaction was terminated with 2 volumes of chilled 75% acetone. The precipitated proteins were collected by centrifugation and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were fixed, dried, and subjected to autoradiography. Lanes: A, pHC79; B, pCS8; C, pCS10; D, pCS23; E, pCS26. The protein markers are the same as in Fig. 1. pCS8, pCS10, and pCS26 consisted of the 8-kb *Bam*HI fragment; pCS23 was a naturally occurring deletion resulting in a 2-kb *Bam*HI fragment.

3) of pCS46, pCS22, and pCS26C1 subclones of pJB196. The 62- and 14-kDa polypeptides appeared significantly and coordinately overproduced when compared with the other *E. coli* proteins. At 23°C, however, neither protein was detectable. Although subclone pCS36 carried an 8-kilobase (kb) *Bam*HI fragment, it did not express the two proteins. This was possibly due to an aberrant restriction profile (data not shown). The other subclones which did not express the complete polypeptides had deletions of the *Bam*HI fragment.

Subcloning and sequencing. Plasmid pJB196 contained two *Bam*HI fragments of 8 and 22 kb. After the *Bam*HI fragments of pJB196 were subcloned into the original vector, only the smaller fragment showed antigenic activity (pCS series). Further subcloned fragments localized the activity to a 5-kb *Bam*HI-*Cla*I fragment in a plasmid designated pCS26C1 (Fig. 4A). In vitro transcription-translation analyses and stained polyacrylamide gels confirmed the presence of both intact polypeptides and suggested the location of the N terminus between the *Bam*HI and *Hin*dIII sites (Fig. 4A).

A clone that contained a 1.8-kb *Bam*HI-*Hin*dIII fragment in the vector did not accumulate the 62-kDa product (Fig. 3F). The 14-kDa polypeptide was retained, and a novel 32-kDa polypeptide was suggested. An in vivo pulse with radiolabeled methionine yielded better evidence of the 32-kDa polypeptide (Fig. 5). These data suggested that the *Hin*dIII site bisects the gene for the 62-kDa polypeptide and that transcription proceedes from the *Bam*HI site to the *Hin*dIII site.

When the above fragment (1.8 kb) was inserted into the universal cloning site of M13 by directed cloning, only one orientation (of 20 clones sampled) was recovered. Because of this difficulty, the *Bam*HI-*Hin*dIII and *Bam*HI-*Cla*I fragments were recloned into the double-stranded DNA vector pGEM1 (Promega Biotec, Madison, Wis.) for sequencing. The sequencing strategy and restriction map are shown in Fig. 4B.

The sequence revealed five potential open reading frames. Only two of the reading frames had a sequence GAGAGGT or AGAGGGT, preceding the ATG initiation codons, which is consistent with the Shine-Dalgarno ribosomal binding site, AGGAGGT. One frame spanned 291 nucleotides and coded for a polypeptide of 10.5 kDa, and the other spanned 1,656 nucleotides and coded for a polypeptide of 58.3 kDa (Fig. 6). The distance between the two open reading frames was 27 nucleotides.

A region starting at position −92 resembled the HSPs of *E. coli* (−35, CTTGAA ... and −10, CCCCAT) (8). There is complete sequence agreement between the position −35 consensus box of *E. coli* and the analogous region of the presumptive *C. burnetii* HSP. Three differences were noted in the position −10 box; namely, TTA was substituted for CAT. Additionally, a region (position −107 to −97) proximal to the position −35 box contained an 11-base-pair AT stretch, a feature commonly associated with HSPs (18). Analysis of the sequence an additional 400 nucleotides upstream revealed no other type of known consensus promoter sequence. The presumptive TAA terminator codon of the proximal open reading frame, now designated as *htpA* is followed by a 27-base intergenic region. The distal open reading frame, designated *htpB*, was followed by sequences that resemble a rho-independent transcriptional termination

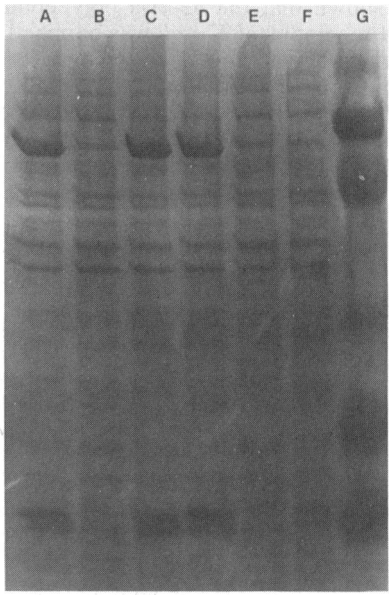

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of *E. coli* lysates. Cultures of *E. coli* grown at 37°C were harvested by centrifugation and suspended in 1:20 volume of Laemmli sample buffer. Samples representing 50 μl of the original culture were subjected to electrophoresis, and the gels were stained with Coomassie blue. Lanes: A, pCS46; B, pCS36; C, pCS22; D, pCS26C1; E, pCS26HI; F, pCS26H4; G, prestained molecular weight standards as in Fig. 1. pCS46, pCS36, and pCS22 consisted of an 8-kb *Bam*HI fragment subcloned into pHC79. pCS26H4 consisted of a 1.8-kb *Bam*HI-*Cla*I fragment, and pCS26H1 represented a deletion from that fragment.

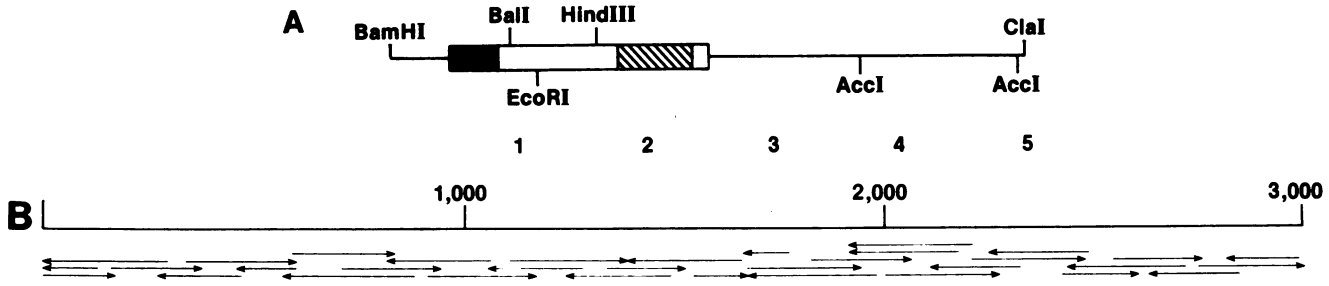

FIG. 4. (A) Restriction map (in kilobases) and (B) sequencing strategy of recombinant DNA insert in pCS26C1. Sequence data start at the *Bam*HI site and continue about 3 kb. The closed box is the locus for *C. burnetii htpA*. The open box delineates *htpB*, and the hatched box within it is the area homologous to the *E. coli ams* gene.

site. The overlapping regions of extensive dyad symmetry (positions 1982 to 2039 and 2027 to 2099) had a ΔG of -28.0 and -43.5 Kcal (ca. -117.2 and -182.0 kJ), respectively, and an A-rich stretch in the DNA-coding strand.

Polypeptide of *htpA*. The proximal open reading frame, now designated as *htpA*, coded for a hydrophilic polypeptide of 10.5 kDa (Fig. 6). Codon usage was not heavily biased for U-rich codons. A search for homologous nucleotide or amino acid sequences failed to uncover significant matches from several data bases.

Polypeptide of *htpB*. *htpB* coded for a polypeptide with an unusual sequence at the C terminus that had a repetitive structure of $(Met\text{-}Gly\text{-}Gly)_4$ followed by two Met residues. There was an Asn-Ser-Ser at positions 1920 to 1928, which is a potential site of N-glycosylation (27). Seven of 15 residues from positions 1092 to 1136 were either Ser or Thr, which is a potential site for O-glycosylation. Preliminary subfractionations had shown that the 62-kDa polypeptide in both *C. burnetii* and recombinant *E. coli* was enriched in a peptidoglycan fraction. This fraction from recombinant *E. coli* was inoculated into mice, and the polyclonal sera were used to probe whole cell lysates. The 62-kDa polypeptide of the recombinant *E. coli* was prominently recognized by the antiserum (Fig. 7). A faint band was evident at the same mobility in the nonrecombinant *E. coli* lysate. Several minor bands were stained in both *E. coli* lysates. By contrast, only one polypeptide of the *C. burnetii* lysate reacted with the antiserum (Fig. 7c), and it comigrated with the 62-kDa polypeptide from recombinant *E. coli*.

Homology of *C. burnetii htpB* to other bacterial sequences. Homology to polypeptides of *Mycobacterium leprae* and *M. tuberculosis* was based on published sequence data (17, 23). The 62-kDa polypeptides from *C. burnetii* and *M. leprae* can be aligned for their first 520 amino acids within one to four residues with an overall identity of about 55%. The C-terminal 40 residues show considerably more divergence and display homology for only limited small stretches. A repeating Met-Gly-Gly motif does occur in the C terminus of the *M. leprae* sequence.

An *E. coli ams* gene (7) encoding a 17-kDa polypeptide matched the sequence of the *C. burnetii htpB* gene. The *ams* sequence homology occupied approximately the last carboxyl third of the 62-kDa polypeptide (Fig. 4B). The nucleotide sequence homology of the corresponding *C. burnetii htpB* gene was 67%, and the amino acid homology was 63%. In fact, the nucleotide homology extended beyond the borders of the *ams* gene. There were two sites in the coding region of the *ams* gene where a small deletion or insertion can be postulated so as to align the deduced amino acid residues for the best fit. An alanine GCG codon in the *ams* sequence (between positions 2123 and 2124) had no corresponding analog in the *C. burnetii* protein. A valine codon and the next base, G (positions 2148 to 2151), of the *C. burnetii* gene do not correspond to the sequence of *ams*. This evolutionary alteration has the effect of shifting the reading frames of the two polypeptides for all residues on the 3′ side, even though the nucleotide homology is maintained. Thus, the overall amino acid homology is reduced. There is one stretch in the protein where 63 of 65 residues were identical.

Codon usage in *C. burnetii htpA* and *htpB*. Because the sequence data presented are the first published for this genus, it is interesting to note the codon usage frequencies and compare them with the corresponding mycobacterial codon frequencies (Table 1). Although the codon usage frequencies for *C. burnetii htpB* are biased for U-rich codons when redundancy allows choices, codon usage in *htpA* was unbiased. The U-rich bias involves the third position of most codons and the first two positions for Leu. By contrast, the codon usage for both mycobacterial species indicates that

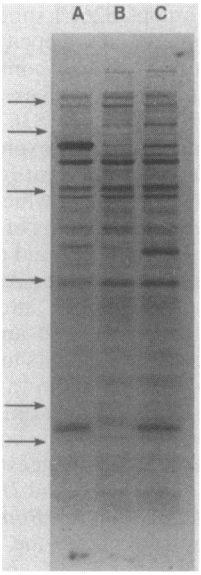

FIG. 5. Lysates from radiolabeled *E. coli* demonstrating full-length and truncated 62-kDa polypeptides. Cultures were grown to the midlog phase at 37°C and pulsed for 5 min with 20 μCi of [^{35}S]methionine per ml. The cultures were chilled, washed, and lysed in Laemmli sample buffer. The lysates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography. Lanes: A, pCS26C1; B, pHC79; C, pCS26H4. The prestained molecular weight markers (arrows) are as in Fig. 1.

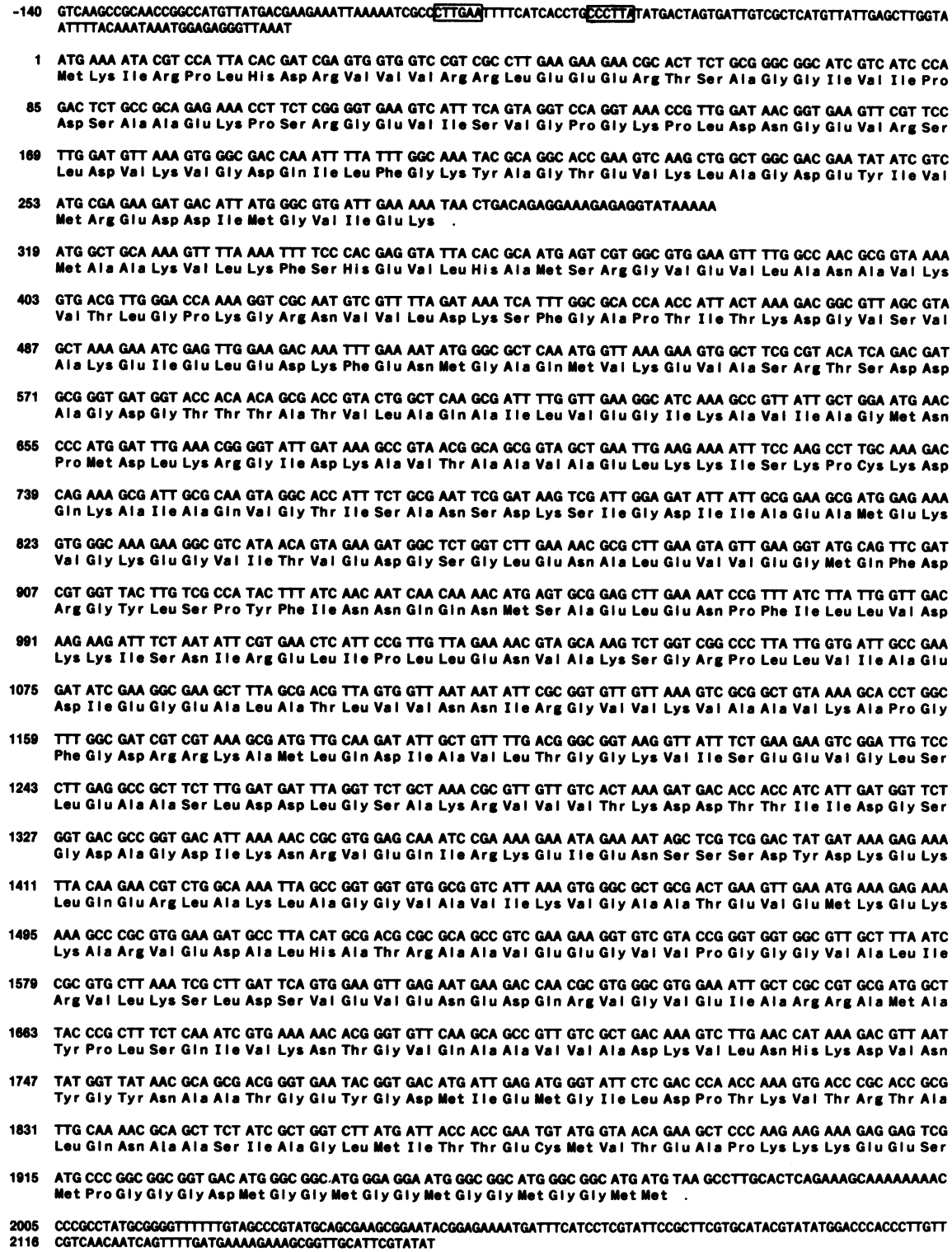

FIG. 6. Nucleotide sequence of *C. burnetii htpA* and *htpB* and surrounding regions. The deduced amino acid sequence is shown beneath the nucleotide sequence of the open reading frames. The promoter is boxed (positions −35 and −10).

sequences are biased for G+C-rich codons. This bias reflects the high G+C content (65%) in mycobacterial DNA (31).

Codon usage in the *C. burnetii* protein is distinguishable from that of endogenous proteins highly expressed in *E. coli*. Three codons, TCG (Ser), CCC (Pro), and GGA (Gly), that are very rarely found in highly expressed proteins of *E. coli* (22) are significantly employed in *C. burnetii htpB*. Two codons, CTG (Leu), and CAG (Gln), that are usually heavily biased for *E. coli* (22) are only minimally employed for *C. burnetii htpB*.

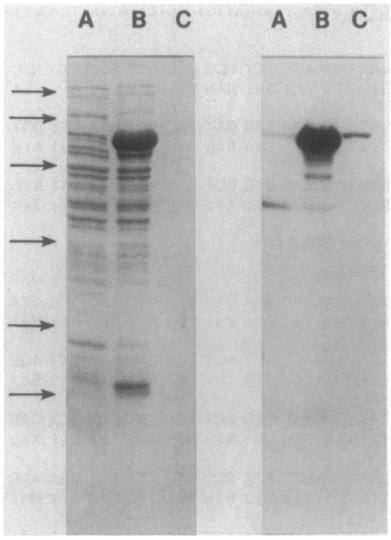

FIG. 7. Western blot with antiserum against peptiodoglycan fraction of recombinant *E. coli*. Lysates from *E. coli* grown at 37°C or *C. burnetii* grown at 35°C were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in duplicate. One set was stained with Coomassie blue (left panel). The other set was transferred to nitrocellulose and probed with antiserum from C3H/HeN mice immunized with peptidoglycan fraction of *E. coli*(pCS26C1) (right panel). Lanes: A, pHC79; B, pCS26C1; C, *C. burnetii* (CB9MIC7).

DISCUSSION

A recombinant *E. coli* strain carrying *C. burnetii* DNA in the cosmid vector pHC79 coordinately expresses two polypeptides. Although pJB196 was selected with immune sera from different sources, only one of the polypeptides is a major antigen of *C. burnetii*. The nonantigenic product is a 10.5-kDa polypeptide encoded by *C. burnetii htpA*. The other, encoded by *C. burnetti htpB*, is associated with a 58.3-kDa polypeptide, which has an apparent molecular mass of 62 kDa on denaturing polyacrylamide gels. Both polypeptides were highly expressed in *E. coli* under regulation of an endogenous promoter. A promoter with the properties of heat shock control was inferred from the sequence data. The promoter differs from the consensus heat shock element by 3 bases in the position −10 box. However, it differs by only 1 base in the position −10 box of the *dnaK* P2 gene (8). There is also a continuous stretch of AT just upstream of the −35 box, which often is associated with HSPs. Termination of polypeptide synthesis appears to be under the control of a rho-independent terminator with a dyad symmetry and an A-rich region. Further in vivo and in vitro studies of the putative regulatory elements should increase our knowledge of gene structure and function in *C. burnetii*.

The identification of a single HSP that controls both *C. burnetii htpA* and *htpB* suggests that we have discovered an operon involved in stress response. This is not surprising, since *C. burnetii* must cope with a wide range of environmental stimuli that include transmission to the eucaryotic host via aerosols and the poikilothermic tick vector. In addition, the mammalian host may trigger heat shock and other stress (i.e., microbicidal activities) responses as *C. burnetii* is engulfed into the phagolysosome. Such a defensive mechanism in combination with the acid activation of *C. burnetii* metabolism could play a critical role in pathogenesis. The high expression of the *C. burnetii* proteins in *E. coli* at 37°C may be due to the promoter configuration. Preliminary results indicate that *C. burnetii htpA* and *htpB* are poorly expressed at 23°C. However, at 37 or 42°C, the expression of both proteins is constitutive with marked accumulation. Although an HSP-like mechanism seems apparent, an unidentified regulatory element in the upstream sequences may also cause the overproduction. Alternatively, the 62-kDa polypeptide, which is homologous to a gene that may control RNase activity at high temperature in *E. coli* (7), may by mediating its own overexpression. In the in vitro coupled transcription-translation system, it is possible that σ^{70} and σ^{32} mediate the expression of the *C. burnetii htp* genes and the *bla* gene, respectively, under conditions when both promoters are present at the same concentration.

The *E. coli ams* gene, which represents the first identified

TABLE 1. Codon frequency for three homologous antigens[a]

Codon	Frequency in:			Codon	Frequency in:			Codon	Frequency in:			Codon	Frequency in:		
	A	B	C		A	B	C		A	B	C		A	B	C
TTT	1.1	0.2	0.2	TCT	1.8	0.7	0.0	TAT	0.5	0.2	0.0	TGT	0.2	0.0	0.0
TTC	0.2	1.3	1.1	TCC	0.5	0.9	1.9	TAC	0.7	1.1	1.3	TGC	0.2	0.0	0.0
TTA	2.4	0.0	0.0	TCA	0.5	0.4	0.0	TAA	0.2	0.0	0.0	TGA	0.0	0.2	0.0
TTG	2.9	1.8	0.8	TCG	1.4	0.9	0.9	TAG	0.0	0.0	0.0	TGG	0.0	0.2	0.2
CTT	1.4	0.6	0.0	CCT	0.4	0.2	0.0	CAT	0.4	0.0	0.0	CGT	1.4	1.3	0.8
CTC	0.4	2.0	2.3	CCC	0.7	0.6	0.9	CAC	0.4	0.4	0.4	CGC	1.8	2.0	2.6
CTA	0.0	1.1	0.2	CCA	0.7	0.0	0.0	CAA	2.2	0.4	0.2	CGA	0.2	0.2	0.2
CTG	0.4	4.4	7.1	CCG	0.7	1.8	2.1	CAG	0.4	2.0	2.4	CGG	0.4	0.4	0.6
ATT	4.3	1.5	0.8	ACT	0.5	1.3	0.2	AAT	2.0	0.4	0.4	AGT	0.4	0.2	0.0
ATC	1.8	3.9	4.7	ACC	2.0	3.9	4.9	AAC	2.0	2.6	2.6	AGC	0.4	0.7	0.6
ATA	0.4	0.0	0.0	ACA	0.9	0.7	0.4	AAA	6.7	0.9	0.4	AGA	0.0	0.0	0.0
ATG	4.2	1.7	0.9	ACG	1.3	1.1	0.9	AAG	1.6	7.4	7.7	AGG	0.0	0.0	0.0
GTT	4.0	1.3	0.9	GCT	3.6	3.0	1.1	GAT	3.4	1.3	1.5	GGT	4.7	4.4	3.4
GTC	1.8	4.8	5.3	GCC	2.0	6.8	8.1	GAC	2.7	4.6	4.1	GGC	4.3	4.1	5.3
GTA	2.4	0.4	0.4	GCA	2.0	1.1	0.2	GAA	6.3	1.8	1.3	GGA	1.1	0.4	0.6
GTG	2.9	3.1	3.6	GCG	3.8	2.8	3.9	GAG	2.2	7.9	9.0	GGG	0.0	0.7	0.9

[a] Codon frequency in percent is calculated from the open reading frame of the polypeptides from (A) *C. burnetii*, (B) *M. leprae*, and (C) *M. tuberculosis*.

member of a series of apparently homologous bacterial proteins, was selected on the basis of complementing a temperature-sensitive mutation. Coincidently, mRNA turnover was also restored at the high temperature. Transformation of the original host strain, HAK117, with pCS26C1 failed to suppress the temperature-sensitive defect (data not shown). Because the *C. burnetii htpB* nucleotide sequence homology extends beyond the borders of the deduced *E. coli ams* gene, a longer *ams* gene may exist in situ. The published *ams* gene sequence could have been artificially derived from a larger gene during restriction by exposing an internal AUG codon with a fortuitous Shine-Delgarno box.

The *C. burnetii htpB* gene was also homologous to a gene of *M. tuberculosis* and *M. leprae*. In fact, this polypeptide is found in most species of *Mycobacterium* and represents the dominant antigen of that genus (6). Thus, this gene or a portion thereof is apparently conserved in phylogenetically distant bacteria. *E. coli* is a gram-negative bacterium, whereas *Mycobacterium* species are weakly gram negative and strongly acid fast (30). *C. burnetii* is weakly acid fast, strongly gram negative, and weakly gram positive (16). Species of *Coxiella* and *Mycobacterium* are obligate intracellular parasites, and *Escherichia* species are classified as free living. Other bacteria also contain a homologous protein conserved between both gram-negative and -positive bacteria (25). This apparently common antigen of 65 kDa, described originally in *Pseudomonas aeruginosa*, would be a candidate for a larger set of conserved proteins that includes the homologous polypeptides from *Coxiella* and *Mycobacterium* species. The common antigen of *Pseudomonas* species has an amino acid composition and M_r resembling those of the antigen encoded by *C. burnetii htpB* (24).

The heat shock regulation exhibited by the operon encouraged us to search data bases for homologs of *C. burnetii htpA* and htpB. Based on molecular weights and functions, we determined that these proteins would be candidates for *groES* and *groEL*, respectively, of *E. coli*. As in *C. burnetii*, *E. coli groE* has been shown to exist as a bicistronic operon (8). A comparison of the deduced amino acid sequence between *E. coli groES* and *C. burnetii htpA* revealed 53% identity, and there was 75% identity between *E. coli groEL* and *C. burnetii htpB* (Roger Hendrix, personal communication). However, scanning the published mycobacterial sequences upstream from the 62-kDa coding region did not reveal coding sequences for the 14-kDa polypeptide. Therefore, the tight linkage between the 62- and 14-kDa polypeptides is not conserved in this genus. In another study (23a), the 62-kDa polypeptide in mycobacteria was found to be under control of an HSP.

The intracellular localization of the homologous 60- to 65-kDa polypeptide in *mycobacteria* has not been thoroughly investigated; in *M. tuberculosis* and *M. bovis* the location of the protein in the cell is not known. However, membrane association has been implicated in the case of *M. leprae* (11). *C. burnetii htpB* shows this same behavior both as a cloned product in *E. coli* and in its natural state in *C. burnetii*. A similar repeating motif occurs at the C terminus of *C. burnetii* and *M. leprae*. Perhaps these repeats serve as an anchor to the membrane or peptidoglycan fraction. Scanning the hydropathy profile of *C. burnetii htpB* revealed no further evidence for a membrane-spanning domain (1).

The purpose for cloning this polypeptide was to eventually assess its efficacy as a subunit vaccine against Q fever. The protein was immunogenic in mice, and polyclonal antibodies against it cross-react with *C. burnetii* and the recombinant *E. coli* clone from which it was extracted. Therefore, the antigen may be able to mediate protection against *C. burnetii* and other pathogens that express homologous sequences. The epitopes which specify the *Coxiella*-specific determinants of the polypeptide are currently being studied with monoclonal antibodies.

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