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A Developmental Stage-Specific Histone H1 Homolog of *Coxiella burnetii*

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Two DNA-binding proteins have been detected in *Coxiella burnetii* by Southwestern (DNA-protein) blotting. One of these, termed Hq1, is enriched in the small cell variant stage of the developmental cycle and displays compositional and primary amino acid sequence similarities to eukaryotic histone H1. *C. burnetii* appears to be another example of an intracellular parasite with morphologically distinct developmental forms whose nucleoid structure may be controlled by histone H1 homologs.

Coxiella burnetii, the etiologic agent of Q fever, undergoes an intracellular developmental cycle that involves morphologically distinct large and small cell types (12). Superficially, the *C. burnetii* developmental cycle is similar to that of an unrelated group of intracellular parasites, the chlamydiae. Both *C. burnetii* and chlamydiae have small, structurally stable, resistant cell types adapted for extracellular survival and larger more pleomorphic metabolically active cell types for intracellular multiplication (14). One of the prominent structural features distinguishing the extracellular cell types of both *Coxiella* species and chlamydiae is a highly condensed nucleoid structure. The larger metabolically active cell types have a more dispersed chromatin (11).

In *Chlamydia trachomatis*, the condensed nucleoid of elementary bodies is produced as a result of the activities of two proteins with primary amino acid sequence homology to eukaryotic histone H1 (7, 17, 20). These histone homologs are expressed late in the developmental cycle, concomitant with the condensation of chromatin that is characteristic of the differentiation of reticulate bodies back to infectious elementary bodies. The DNA-compacting activity of the chlamydial histone homologs is evident during expression in heterologous hosts (2, 3) and in in vitro assays (1, 4). In addition to a structural role, regulatory roles for the chlamydial histone homologs are also proposed. At maximal levels of expression of Hc1, there is a global inhibition of transcription as the chromatin becomes fully compacted. At substructural levels of expression, Hc1-mediated changes in DNA topology alter gene expression in recombinant hosts and Hc1 expression is correlated with a decrease in plasmid supercoiling levels late in the chlamydial developmental cycle (1). We have analyzed *C. burnetii* for possibly related histone-like proteins and describe here a histone H1 homolog that is unique to the small, metabolically dormant cell type. This protein likely plays a role in establishment of nucleoid structure and gene regulation in *C. burnetii.*

DNA-binding proteins of *C. burnetii.* A screening technique for DNA-binding proteins known as Southwestern (DNA-protein) blotting was used to identify potential *C. burnetii* histone-like proteins because it has been used to identify histone H1 homologs of *C. trachomatis* (8, 22). Figure 1 demonstrates DNA binding by the histone-like proteins Hc1 and Hc2 in whole-cell lysates of *C. trachomatis* serovars L2, D, and B and by *C. burnetii* strain Nine Mile phase I (9mi/I). *C. burnetii* proteins with sizes of 20 and 14 kDa display DNA-binding activity in this assay. *Rickettsia rickettsii*, an obligate intracellular parasite which does not undergo a life cycle with multiple cell types, does not exhibit DNA-binding proteins by this method.

Developmental stage specificity of Hq1. The large and small cell variants of *C. burnetii* 9mi/I were separated by isopycnic CsCl gradient centrifugation as described previously (23). En-

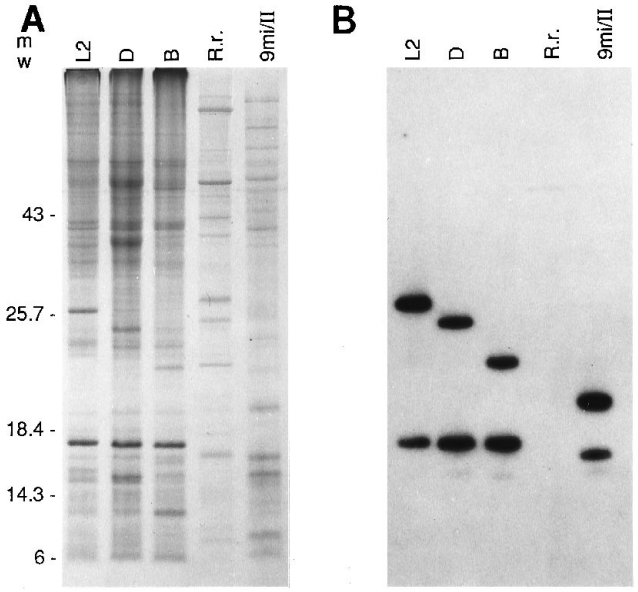

FIG. 1. DNA-binding proteins of *C. burnetii*. (A) Silver-stained SDS-PAGE profile. Whole-cell lysates of purified *C. trachomatis* serovars L2, D, and B; *R. rickettsii* (R.r.); and *C. burnetii* 9mi/II were solubilized and electrophoresed on 0.1% SDS–12.5% polyacrylamide gels (10) and silver stained by the method of Tsai and Frasch (21). (B) DNA-binding proteins. A parallel gel was electrophoretically transferred to a nitrocellulose membrane. The membrane was incubated with random primer ^{32}P-labeled pUC18 DNA in this Southwestern blot (8, 13, 19). The transfer was performed in 25 mM NaH_2PO_4 (pH 7.2) at 27 V for 1.5 h. The membrane was blocked with phosphate buffered saline-Tween 20 (PBST) prior to incubation for 16 h with 10 ng of random primer-labeled pUC18 in PBST. The membrane was then washed twice for 15 min in PBST and exposed to Kodak X-Omat AR15 film. MW, molecular mass (kilodaltons).

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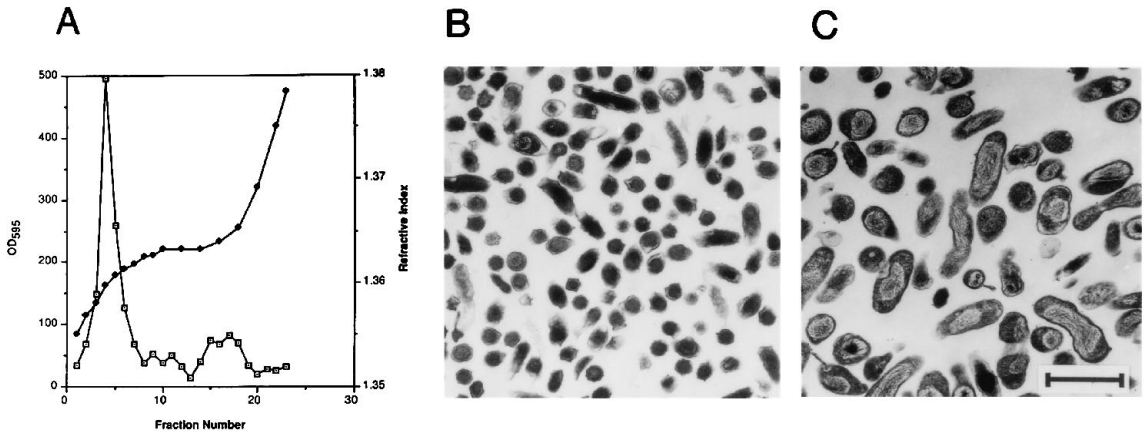

FIG. 2. Separation of large and small cell variants of *C. burnetii* by CsCl gradient centrifugation. Enrichment of the small cells and large cells by CsCl gradient centrifugation was confirmed by electron microscopy. (A) Optical density at 595 nm (OD_{595}) of fractions from an isopycnic CsCl gradient of phase I *C. burnetii* cells. The top of the gradient is on the left. The densities of the small and large cell types were estimated as 1.280 and 1.323, respectively. (B and C) Electron micrographs of enriched small cell (B) and large cell (C) fractions. Note the characteristic electron-dense nucleoid structure of the small cell variants as opposed to the dispersed chromatin of the large cell variant. Bar, 1 μm.

richment of the large cells and small cells was confirmed by electron microscopy (Fig. 2B and C). Note the characteristic electron-dense nucleoid structure of the small cell variants as opposed to the dispersed chromatin of the large cell variant. Enriched fractions of the two *C. burnetii* cell types were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with silver staining (Fig. 3A). In the absence of specific antibodies, the DNA-binding proteins were detected by Southwestern blotting (Fig. 3B). The 20-kDa DNA-binding protein, termed Hq1, is absent or is in reduced amounts in the large cell variants. The small amount of Hq1 detected in the large cell variants may be due to the presence of a few small cell variants or intermediate developmental forms which are apparent in the electron micrographs. The abundance of the 14-kDa DNA-binding protein did not appear to vary between the cell types.

Purification and N-terminal amino acid sequence of Hq1. Attempts to identify the *C. burnetii* histone-like proteins with polyclonal antisera to *C. trachomatis* Hc1 (7) or a monoclonal antibody to Hc2 (8) were negative. Similarly, *hctA* and *hctB*, encoding *C. trachomatis* Hc1 and Hc2, respectively, were not reactive in Southern blots against *C. burnetii* DNA (not shown). The affinity of the presumed histone-like proteins of *C. burnetii* for DNA suggested that they may be amenable to purification schemes developed for isolation of chlamydial histones. *C. burnetii* was extracted with a mixture of 100 mM sodium carbonate, 10 mM EDTA, and 1% octylglucopyranoside (pH 10.5), and Hq1 was purified by heparin-agarose affinity chromatography essentially as described previously (6). Hq1 bound heparin with high affinity and was eluted at a NaCl concentration of 1 M (Fig. 4). The 14-kDa DNA-binding protein was not purified to homogeneity by this procedure. The purified Hq1 fraction was subjected to N-terminal amino acid sequencing with an Applied Biosystems model 470A protein sequencer.

Cloning and sequencing. On the basis of the N-terminal amino acid sequence, degenerate oligonucleotide probes were designed and used as ^{32}P-labeled probes in filter hybridizations of endonuclease-digested *C. burnetii* genomic DNA. An *Eco*RI fragment with a size of approximately 2.2 kb was detected in genomic digests (not shown). This fragment was isolated from a lambda gt11 library of *C. burnetii* and subcloned in pUC18

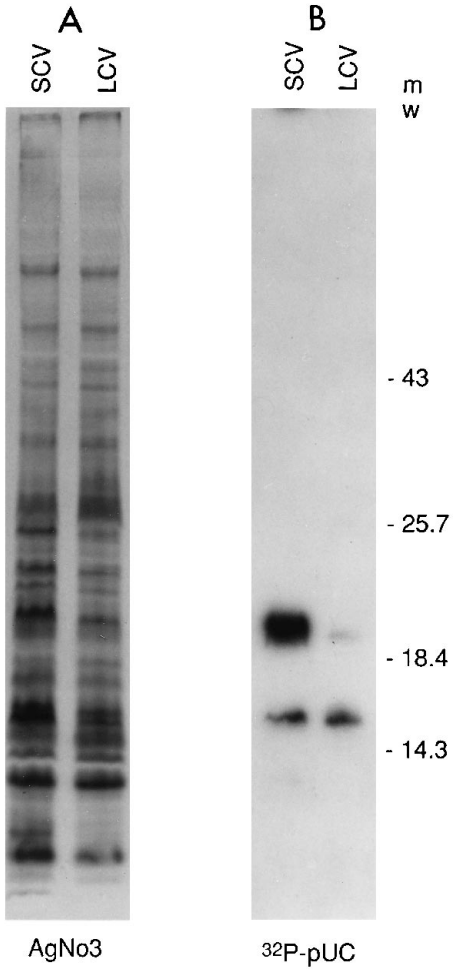

FIG. 3. Developmental stage specificity of Hq1. Enriched fractions of the two *C. burnetii* cell types described in the legend to Fig. 2 were subjected to SDS-PAGE with silver-staining (A), and the histone-like proteins Hq1 and Hq2 were detected by Southwestern blotting (B) by the methods described in the legend to Fig. 1. Hq1 is present in the small cell variants (SCV) but was absent or in reduced amounts in the large cell variants (LCV). The small amount of Hq1 detected in the large cell population may be due to the presence of a few small cell variants or intermediate developmental forms which are apparent in the electron micrographs. Hq2 concentration did not appear to vary between the cell types. MW, molecular mass (kilodaltons).

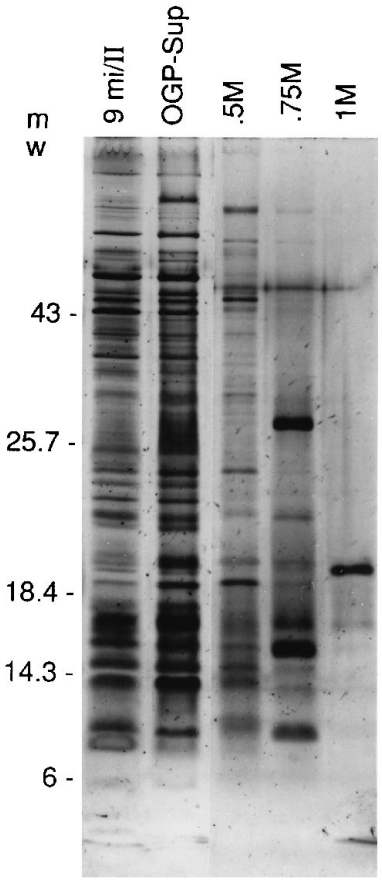

FIG. 4. Purification and N-terminal amino acid sequence of Hq1. Shown here is a silver-stained SDS-PAGE profile of fractions of a whole-cell lysate of *C. burnetii* (9mi/II), an octylglucopyranoside extract (OGP-Sup), and fractions eluted with increasing NaCl concentrations. *C. burnetii* was extracted with a mixture containing 100 mM sodium carbonate, 10 mM EDTA, and 1% octylglucopyranoside (pH 10.5), and Hq1 was purified by heparin-agarose affinity chromatography essentially as described previously (6). Hq1 bound heparin with high affinity and was eluted at a NaCl concentration of 1 M. The N-terminal amino acid sequence of the purified Hq1 was determined with an Applied Biosystems model 470A protein sequencer. MW, molecular mass (kilodaltons).

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                15                  30                  45                  60
AAAACACCAAAATTTTATCATTTTCAGCCGAATTTACTATTAAAAATCGCAATTTTCCCA

                75                  90                  105                 120
CGACTCTATAAGATCTCCGGGGGGCATGATTGGAATGAAAATGAGGATTAAATTGGAGATT

                135                 150                 165                 180
CCTCATTTTAATGAAAGGACCCTAGCAGTTTATTGCACTTAGTCGTTTAAACTTGTTGAT

                195                 210                 225                 240
TTTTTATGTAAAATATAATTGTCGTTTTTTATTAGCTAATCAGGGAGAAAAAGATGCCAG
                                                            M  [P  A

                255                 270                 285                 300
CAAAAAAACGAAAAACCACTCGTCAACGAAGACGTTCAAAAGCCCGATCTGCTTCGGCAA
  K  K  R  K  T  T  R  Q  R  R  R  S  K  A  R  S  A  S  A  N

                315                 330                 345                 360
ATACTGCGGCACTTAGAAAAGTTTCGAAAGAGCGAGATCAAGCCCGTAGGAAATTACGTG
  T  A  A] L  R  K  V  S  K  E  R  D  Q  A  R  R  K  L  R  A

                375                 390                 405                 420
CCGCTCAAAAGAAGCTAGCTAAAGCGAAGAAAGATGCAAGCCGTAAACTTGCTAAACTTC
  A  Q  K  K  L  A  K  A  K  K  D  A  S  R  K  L  A  K  L  R

                435                 450                 465                 480
GAAAAGAGGCCGCTCGAAAAGTAGCTGCTGCCAAAAAAACGAGAGCACCGTCTAAAAAGG
  K  E  A  A  R  K  V  A  A  A  K  K  T  R  A  P  S  K  K  G

                495                 510                 525                 540
GACGCAAAAAGGCTACTCGTAAAAAAGGAGGCGGCCGTTCACGTAAAACAGCACGTAAAG
  R  K  K  A  T  R  K  K  G  G  G  R  S  R  K  T  A  R  K  V

                555                 570                 585                 600
TATCTACGATGAAACGCGGACGCGGTCGTCCACGCAAAAAAGCGTAGTGCTTAGGCCGAT
  S  T  M  K  R  G  R  G  R  P  R  K  K  A  *

                615                 630                 645                 660
TCAAAAAGCCCGTTATTCGCGGGCTTTTTGTTTTTGTGCTTCCCTTAAGATATCTGTATT

                675                 690                 705                 720
CAGGTCTTTTGCCTTTTCGATTAACTCCCTTAAACCCTCCCTCGGGAGCGCTCCCGATTC

                735                 750                 765                 780
AGCAAAAACGACCACGCGATCACGCAAGATCATGACTGCCGGTATCGATTTCACTTAAAT

                795                 810                 825                 840
TCTTCCGCAAGTCTTTTTTCCTTTTCAATATTGACACTGCCGAAAACGACTTCAGGATAA

                855                 870                 885                 900
TCCTTTTCAACCTCTTCAATGATTTTTGTAAAAGTTAAGCAGGGCGCACACCAGTTAGCC
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FIG. 5. Cloning and sequencing of *C. burnetii* Hq1. On the basis of the N-terminal amino acid sequence, degenerate oligonucleotides were designed and used as hybridization probes against digested *C. burnetii* genomic DNA and a *C. burnetii* 9mi/I lambda gt11 library. A single *Eco*RI fragment with a size of approximately 2.2 kb was detected in genomic digests, and an insert of that size was isolated from a lambda gt11 library. This fragment was subcloned in pUC18 and sequenced with M13 and custom oligonucleotide primers. The complete coding sequence and deduced amino acid sequence are shown. A putative ribosome-binding site is underlined. The N-terminal amino acid sequence determined from the mature peptide is shown in brackets.

for nucleotide sequencing with M13 and custom oligonucleotide primers. The complete coding sequence and deduced amino acid sequence are shown in Fig. 5. The gene encoding Hq1 has been termed *hcbA*. The calculated molecular mass of the protein is 13,183 Da, and it has a predicted pI of 13.1. The strong basic charge of the protein likely contributes to the discrepancy between the predicted mass and that observed by SDS-PAGE, as is typical of H1 histones (15). Expression of *hcbA* in *Escherichia coli* resulted in a protein product that comigrated with authentic H1 from *C. burnetii* and displayed DNA-binding activity by Southwestern blotting (not shown).

Database searches (16) revealed a high degree of homology of Hq1 with the H1 family of eukaryotic histones, with the optimal alignment showing 34% identity over 119 amino acids of the gonadal histone H1 of the sea urchin *Parechinus angulosis*. This degree of similarity of Hq1 to eukaryotic histone H1 was comparable to that observed for chlamydial Hc1 or Hc2 with H1 (3, 7, 17, 20). Despite a number of histone-like proteins in bacteria, proteins with primary amino acid homology to eukaryotic histones remain relatively rare in prokaryotes. In addition to Hq1 and the chlamydial histone homologs, proteins with homology to H1 have been identified in *Pseudomonas aeruginosa* (5, 9) and *Bordetella pertussis* (18). Each of these proteins displays a level of homology comparable to that of eukaryotic H1 and Hq1, with the percent identities ranging between approximately 25 and 35%. It should be noted that the region of homology of each of the bacterial histone H1 homologs overlaps the C-terminal DNA-binding domain of eukaryotic H1, which is enriched in lysine and alanine residues. The organization of these amino acids is largely as tetrameric or pentameric repeats of two basic residues followed by two to three aliphatic residues; thus, the similarities observed between the various H1 homologs may actually represent a convergence of DNA binding structures. Whereas in eukaryotes, H1 interacts with the core histones (H2A, H2B, H3, and H4) to form a nucleosome complex, no equivalent core histone complex has been identified that associates stoichiometrically with the histone H1 homologs in prokaryotes. Instead, the prokaryotic H1 homologs are generally thought to act as tran-

scriptional regulators (1, 5, 9, 18), or, depending upon relative abundance, they may also play a structural role in compaction of the bacterial chromosome during periods of quiescence, as exemplified by chlamydial Hc1 and Hc2 association with the extracellular stage of the developmental cycle (2, 3). The association of Hq1 with only the small, metabolically inactive cell type of *C. burnetii* suggests that its expression is developmentally regulated. The Hq1 protein is likely to play a major role in both chromatin structure and transcriptional activity throughout the *C. burnetii* developmental cycle in a manner analogous to that of the chlamydial histone homologs.

Nucleotide sequence accession number. The nucleotide sequence of the *C. burnetii hcbA* gene has been submitted to GenBank and has been assigned accession number L79945.

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