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Isolation and Molecular Characterization of the Ribosomal Protein L6 Homolog from *Chlamydia trachomatis*

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The cloning of a *Chlamydia trachomatis* eukaryotic cell-binding protein reported earlier from our laboratory (R. Kaul, K. L. Roy, and W. M. Wenman, J. Bacteriol. 169:5152–5156, 1987) represents an artifact generated by nonspecific recombination of chromosomal DNA fragments. However, the amino terminus of this plasmid-encoded fusion product demonstrated significant homology to *Escherichia coli* ribosomal protein L6. By using a 458-bp *PstI-HindIII* fragment of recombinant pCT161/18 (representing the 5′ end of the cloned gene), we isolated and characterized a *C. trachomatis* homolog of the ribosomal protein L6 gene of *E. coli*. Sequence analysis of an 1,194-bp *EcoRI-SacI* fragment that encodes chlamydial L6 (designated CtaL6e) revealed a 552-bp open reading frame comprising 183 amino acids and encodes a protein with a molecular weight of 19,839. Interestingly, complete gene homology between *C. trachomatis* serovars L2 and J, each of which exists as a single copy per genome, was observed. Expression of a plasmid-encoded gene product is dependent on the *lac* promoter, since no product was obtained if the open reading frame was oriented in opposition to the *lac* promoter. Immunblotting of purified ribosomes revealed functional, as well as antigenic, homology between the *E. coli* and *C. trachomatis* ribosomal L6 proteins.

The obligate intracellular parasite *Chlamydia trachomatis* is an important pathogen associated with a broad spectrum of human disease (29). The unique life cycle of this prokaryote features two distinct developmental forms: the extracellular, infectious elementary body (EB) and the intracellular, metabolically active reticulate body (36). Elucidation of the regulatory controls governing its developmental stages is pivotal to understanding the biology of *C. trachomatis*.

The initial EB attachment to the host cell represents a crucial step in this life cycle and appears to be necessary for successful invasion (36, 37). Attempts to study the attachment process have resulted in identification of two putative membrane protein adhesins of 18 and 31 kDa (9, 39). The cloning and sequencing of the 18-kDa surface-exposed adhesin has recently been reported from our laboratory (12). However, primary sequence comparisons of this protein with the SWISS-PROT protein data bank via BIONET have revealed significant homology of the N terminus of this binding protein with *Escherichia coli* L6 (EcoL6) and *Bacillus stearothermophilus* L10 (BstL6e) ribosomal proteins of the *spc* operon (13). In this report, we refer to the *C. trachomatis* L6 homolog or equivalent as CtaL6e on the basis of the nomenclature described by Shimmin and Dennis (30).

Investigations into ribosome structure and function in *Chlamydia* spp. have dealt with cloning and characterization of rRNA genes (23) and operons (5). Clustering of genes into operons is a characteristic feature of all bacteria. The 19 ribosomal protein operons of *E. coli* have been well characterized. Their organization represents a vivid example of tightly coordinated operon regulation. The nucleotide or amino acid (aa) sequences of all 52 ribosomal proteins in *E. coli* are known (3, 16). Homologies between these proteins and the ribosomal proteins of other prokaryotes (especially *Bacillus* spp.) (6) and eukaryotes (17) have been demonstrated. It is not known, however, whether *C. trachomatis* contains a full complement of 19 operons. Sardinia et al. (28) and, recently, Lundemose et al. (18) have characterized a chlamydial gene that encodes a protein homologous to ribosomal protein S1 in *E. coli*.

The work presented here concerns elucidation of the relationship of the cloned 18-kDa putative binding protein to ribosomal proteins EcoL6 and BstL6e. We report the cloning, sequencing, and partial characterization of a gene for a ribosomal protein from *C. trachomatis* that is structurally and functionally homologous to ribosomal protein EcoL6.

MATERIALS AND METHODS

Bacterial strains and media. *C. trachomatis* serovars L2 (L2/434/Bu), J (J/UW-36), D (D/UW-3), and K (K/UW-31) were grown in HeLa 229 cultures as described by Kuo et al. (14). EBs were harvested from cultures at 40 h and purified as described previously (39).

E. coli JM83 and DH5αF′ were used for propagation of plasmids and M13 bacteriophage, respectively (40). *E. coli* p678-54 was used for minicell preparation (1). These cells were made competent for transformation or transfection essentially by the method of Hanahan (10).

DNA isolation and manipulations. Chromosomal DNA was isolated from purified EBs as described previously (38). Both plasmid and bacteriophage replicative form DNAs were isolated by the alkaline lysis method of Birnboim and Doly (2) and purified by cesium chloride-ethidium bromide density gradient centrifugation.

For cloning studies, genomic DNAs from EBs of serovars D, J, K, and L2 were digested with selected restriction endonucleases and sized. Plasmid vector pUC18 was cleaved with the corresponding enzyme and treated with calf intestine alkaline phosphatase to minimize recircularization. Shotgun ligations of sized genomic DNAs to linearized pUC18 were done at 16°C overnight. These ligation mixtures were used to transform competent *E. coli* JM83. Ampicillin-

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resistant recombinant colonies were identified on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and isoproypl-β-D-thiogalactopyranoside plates. Further selection of positive clones from thousands of recombinants was accomplished by colony lifts (8) and hybridization (32) with a ^{32}P-labeled 458-bp *Pst*I-*Hin*dIII fragment from pCT161/18 (12).

Regions of interest in the inserts of these clones were subcloned into pUC and M13 vectors for DNA sequencing analysis as described by Messing and Yanisch-Perron et al. (19, 40).

DNA sequencing and sequence analysis. All DNA sequencing was performed by using the dideoxy chain termination method of Sanger et al. (27) with the Sequenase sequencing kit (United States Biochemical Corp., Cleveland, Ohio). Sequencing of inserts in both single-stranded M13 vectors and double-stranded pUC vectors has already been described (27, 35). For sequence extension in *Sac*I clones, a specific oligonucleotide primer, GGR#1 (5'-TTTGCGGCT TGTGTTCGT-3'), was synthesized at the DNA synthesis facility of the Department of Microbiology, University of Alberta, Edmonton, Alberta, Canada. Universal and 15-mer primers were purchased from Boehringer Mannheim Canada Ltd., Laval, Quebec. Sequence compilations, open reading frame (ORF) identification, and translation and restriction map construction were all performed with DNA Strider software for the Macintosh computer (C. Marck, Service de Biochimie, Centres d'Etudes Nucleaires de Saclay, Gif-sur-Yvette, France). Peptide alignments were determined with Microgenie (Beckman, Palo Alto, Calif.) on the IBM PC. Codon usage and pI determinations were done with IBI Pustell DNA sequence analysis software for the Macintosh computer.

Plasmid-directed synthesis of proteins. Recombinant expression products were analyzed by in vitro transcription-translation and minicell methods essentially as described previously (12). In vitro transcription-translation experiments were done as recommended by the manufacturer of the kit used (Amersham, Inc., Oakville, Ontario, Canada).

A mixture of polyclonal antibodies to EcoL3 and EcoL6 was a gift of M. Nomura (University of California, Irvine) and was reacted with the expression products after electrophoresis and transfer to nitrocellulose (34). Electrophoretic resolution of proteins was accomplished by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (15) on 12.5% acrylamide. For resolution of total proteins from whole ribosomes, 20% acrylamide gels were used.

Isolation of ribosomes. Whole ribosomes were isolated from JM83 cells harboring the pUC18 plasmid or the pCTJS8 plasmid essentially by the method of Held et al. (11). The 70S ribosomes were purified on a 15 to 30% sucrose gradient, identified in fractions by peak A_{260} and stored frozen for subsequent electrophoresis.

Autofluorography and radioanalytic imaging. All autofluorography was performed with Kodak X-OMAT AR X-ray film. Exposures were enhanced with intensifying screens. Radioanalytic imaging of immunoblots probed with [^{125}I] protein A was performed with the AMBIS radioanalytic imaging system to quantitate relative signal strengths.

Radiolabeling. Specific DNA fragments used as probes in clone selection were labeled with [α-^{32}P]dATP by nick translation (26), fill-in labeling (22), or random primer labeling (7). Protein A was labeled with ^{125}I by the lactoperoxidase method (20). All radioisotopes (^{32}P, ^{125}I, and ^{35}S) were purchased from Amersham.

Nucleotide sequence accession number. The GenBank accession number of the ribosomal protein L6 gene is M60652.

RESULTS

Identification of the gene for ribosomal protein L6. By following the strategy of Kaul et al. (12) in cloning the 18-kDa binding protein, we constructed recombinant DNA libraries of *Pst*I-digested genomic DNAs from serovars D, J, and K by using vector pUC18. Recombinants were screened by colony hybridization with the ^{32}P-labeled 458-bp *Pst*I-*Hin*dIII fragment of the pCT161/18 insert which encodes the putative *C. trachomatis* 18-kDa protein (12). One clone from each serovar was isolated. These clones were designated pCTDP1 (plasmid *C. trachomatis*, serovar D, *Pst*I cut, gene clone 1), pCTJP1 (serovar J), and pCTKP1 (serovar K). These clones each bear a 440-bp fragment of *C. trachomatis* DNA. Determination of the complete nucleotide sequences of these inserts on both the coding and noncoding strands revealed complete homology with the pCT161/18 insert over a 430-bp stretch from the 5' end. Beyond this point, the sequences of clones pCTDP1, pCTJP1, and pCTKP1 differed completely from that of pCT161/18.

Confirmation of this restriction fragment length polymorphism between serovars was attempted by probing a Southern transfer of genomic *Pst*I digests of serovars D, J, K, and L2 with an end-labeled *Pst*I-*Hin*dIII (458-bp) fragment. For all four of the serovars tested, the probe hybridized to a single band ~440 bp long (data not shown). All three 440-bp *Pst*I clones encode a portion of a putative ORF; that is, they encode a translational start codon but no translational stop codon in frame. To elucidate the entire ORF, we identified the presence of this 440-bp *Pst*I fragment within an 800-bp *Eco*RI fragment and an ~3.0-kb *Sac*I fragment in all four serovars by Southern hybridization of genomic digests. Recombinant libraries of genomic DNAs from serovars J and L2 cut with *Eco*RI and *Sac*I and sized on agarose gel were constructed with vector pUC18. Several hundred colonies were screened by colony hybridization with the ^{32}P-labeled 440-bp *Pst*I fragment. Three clones were identified, one in each of the serovar J *Eco*RI and *Sac*I libraries and one in the serovar L2 *Sac*I library. These clones were designated pCTJE1 (containing an 800-bp *Eco*RI fragment), pCTJS1, and pCTLS1 (containing 3.2-kb *Sac*I fragments from serovar J and L2 libraries, respectively).

Complete sequencing of the pCTJE1 insert on both strands revealed that the insert was 804 bp long. This clone allowed extension of the known sequence but did not contain the codon for termination of translation of the putative ORF. Further extension of this sequence in pCTJS1 was accomplished with specific synthetic oligonucleotide probe GGR#1. Subsequently, the known sequence was extended on both strands to a total of 1,194 bp. The complete sequence is shown in Fig. 1. The entire putative ORF and the deduced primary structure of the protein encoded by this gene are identified. The sequence of the putative ORF in serovar L2 (pCTLS1) was also determined, and it was exactly identical to that in serovar J.

Southern hybridization of *Xba*I-*Hin*dIII genomic digests of serovars D, J, K, and L2 (using the ^{32}P-labeled 440-bp *Pst*I fragment) revealed the existence of a single copy of this ORF in the genome (data not shown).

Analysis of the ORF. All analyses of the ORF product were done with assorted computer software for protein and DNA sequence analyses. *C. trachomatis* ribosomal protein Cta L6e comprises 183 aa residues with deduced and apparent molecular masses of 19,838 and 23,000 Da, respectively, and an estimated pI of 9.88. Homologies in primary protein structure are revealed by the aa alignments shown in Fig. 2.

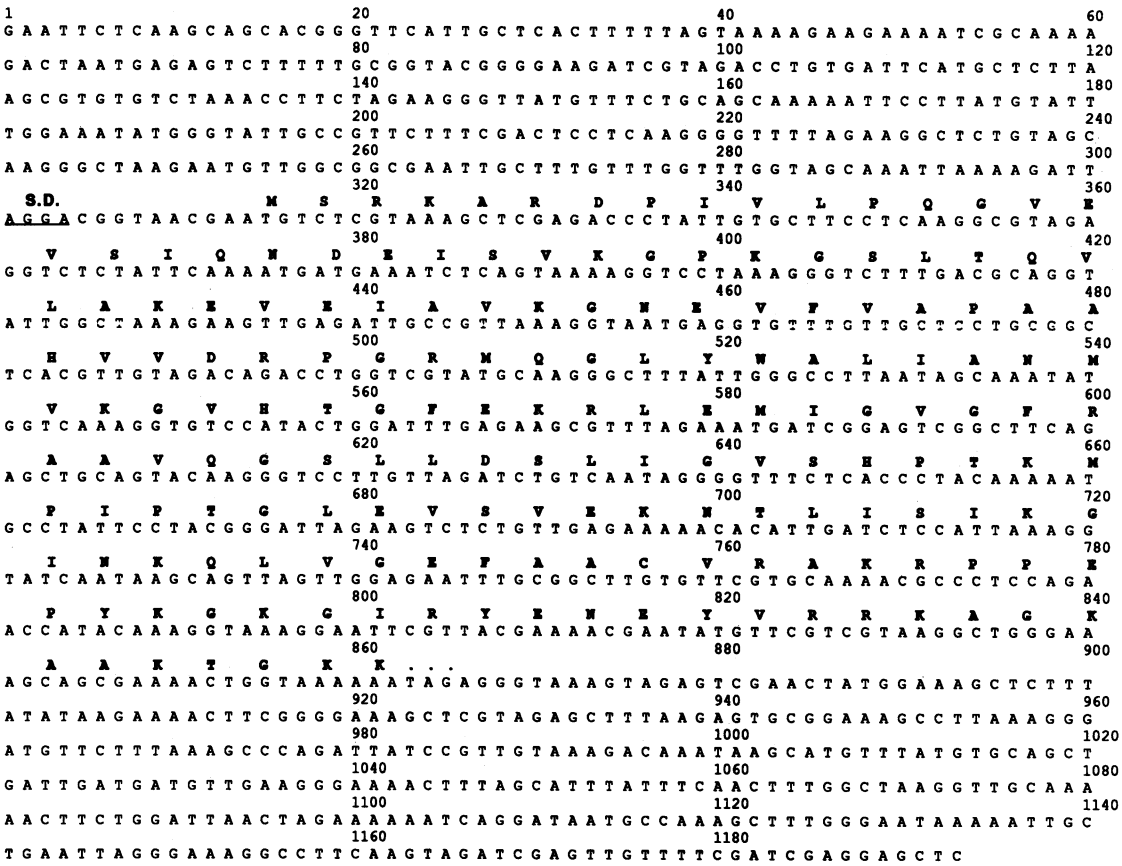

FIG. 1. Complete nucleotide sequence of the 1,194-bp chlamydial DNA that encodes putative chlamydial ribosomal protein CtaL6e. The ORF is translated into the single-letter aa code. The numbers above each line refer to nucleotide positions. The translation initiation start site is the methionine codon at position 314. The postulated Shine-Dalgarno (S.D.) region (ribosome-binding site) is underlined.

The CtaL6e primary structure is 56% homologous (counting 76 perfect matches and 26 conservative substitutions) to that of ribosomal protein EcoL6 and 62% homologous to that of ribosomal protein BstL6e (87 perfect matches and 26 conservative substitutions).

Expression of recombinant CtaL6e. Evidence that the recombinant 23,000-Da polypeptide encoded by pCTJS8 is equivalent to ribosomal protein L6 was derived from gene expression experiments. The 980-bp *Xba*I-*Hin*dIII fragment of pCTJS1 that encodes the whole ORF was subcloned into pUC18 and pUC19, generating plasmids pCTJS8 and pCTJS9, respectively, for use in plasmid-encoded protein expression experiments (Fig. 3). These plasmids carried the putative ORF in direct (pCTJS8) and inverse (pCTJS9) orientations with respect to the *lac* promoter of the pUC vector. The product of this ORF was expressed in large quantities in vivo by *E. coli* cells harboring plasmid pCTJS8 but not by those harboring plasmid pCTJS9 or pUC18, as documented by Coomassie blue-stained SDS-PAGE protein profiles of these cells (Fig. 3B). Immunoblots of the protein encoded by pCTJS8 (Fig. 3C) also revealed cross-reactivity with a mixture of polyclonal antibodies to ribosomal proteins EcoL3 and EcoL6 (seen as increased autoradiographic intensity of the lower [23-kDa] band with respect to pUC18- and pCTJS9-bearing cells). In addition, the cloned gene product was identical in size and mobility to the native 23-kDa chlamydial ribosomal protein as analyzed by immunoblotting (Fig. 3C).

Plasmids pCTJS8 and pUC18 were subsequently transformed into minicell-producing *E. coli* P678-54 to identify plasmid-encoded proteins. Recombinant plasmid pCTJS8 encodes a single protein with an apparent molecular weight of 23,000 as resolved by SDS-PAGE. This protein is not encoded by pUC18 (Fig. 4, lanes 1 and 2). The recombinant gene product was further identified by using an *E. coli*-derived coupled in vitro transcription-translation system with plasmids pCTJS8 and pUC18. A single protein with an apparent molecular weight of 23,000, not encoded by pUC18, was also produced from recombinant plasmid pCTJS8 (Fig. 4, lanes 3 and 4).

CtaL6e functional homology to EcoL6. To determine the degree of functional homology between ribosomal proteins CtaL6e and EcoL6, we attempted to quantitate the percent substitution of CtaL6e for EcoL6 in vivo. Whole ribosomes were purified from *E. coli* JM83 cells harboring pUC18 or pCTJS8. These ribosomes were resolved by one-dimensional SDS-PAGE and immunoblotted with a mixture of polyclonal antibodies to ribosomal proteins EcoL3 and EcoL6. An acrylamide concentration of 20% was used to maximize separation of proteins CtaL6e and EcoL6. After reaction with [^{125}I]protein A, the intensity of the label was quantitated by using autofluorography and the AMBIS radioanalytic imaging system. Ribosomal protein EcoL3 served as an internal standard for each sample. Immunoblotting and autofluorography demonstrated that both proteins CtaL6e and EcoL6 were present in small quantities (Fig. 5)

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Bst  MSRVGKKPIEIPAGVTVTVNGNTVTVKGPK   30
Cta  MSRKARDPIVLPQGVEVSIQNDEISVKGPK   30
Eco  MSRVAKAPVVVPAGVDVKINGQVITIKGKN   30

Bst  GELTRTFHPDMTITVEGNVITVTRPSDEKH   60
Cta  GSLTQVLAKEVEIAVKGNEVFVAPAAHVVD   60
Eco  GELTRTLNDAVEVKHADNTLTFGPRDGYAD   60

Bst  HRA LHGTTRSLLANMVEGVSKGYEKALEL   89
Cta  RPGRMQGLYWALIANMVKGVHTGFEKRLEM   90
Eco  GWA QAGTARALLNSMVIGVTEDFTKKLQL   89

Bst  VGVGYRASKQGKKLVLSVGYSHPVEIEPEE  119
Cta  IGVGFRAAVQGSLLDLSIGVSHPTKMPIPT  120
Eco  VGVGYRAAVKGNVINLSLGFSHPVDHQLPA  119

Bst  GLEIEVPSQTKIIVKGADKQRVGELAANIR  149
Cta  GLEVSVEKNTLISIKGINKQLVGEFAACVR  150
Eco  GITAECPTQTEIVLKGADKQVIGQVAADLR  149

Bst  AVRPPEPYKGKGIRYEGELVRLKEGKTGK   178
Cta  AKRPPEPYKGKGIRYENEYVRRKAGKAAKTGKK 183
Eco  AYRRPEPYKGKGVRYADEVVRTKEAKKK     177
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FIG. 2. Alignment of ribosomal protein aa sequences. Sequences for ribosomal proteins BstL6e from *B. stearothermophilus* (Bst) and EcoL6 from *E. coli* (Eco) are aligned with the deduced aa sequence for the putative *C. trachomatis* ribosomal protein (Cta). The single-letter aa code is used. Wide bars indicate complete homology, and narrow bars indicate a conservative substitution. The numbers of the amino acids are indicated at the far right.

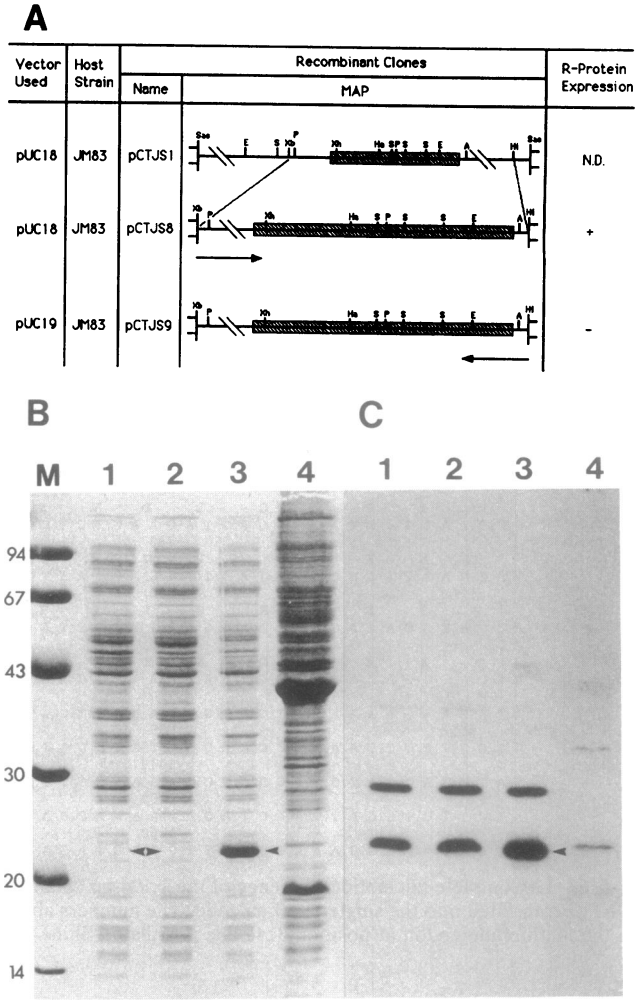

FIG. 3. (A) Construction of plasmids for expression of the putative ribosomal protein (R-protein). A restriction map of the constructs is shown. Hatched bars indicate the putative ORF. Arrows indicate directions of transcription from the *lac* promoter. Expression was determined in vivo by SDS-PAGE analysis of recombinant whole-cell lysates. Abbreviations: P, *Pst*I; Xh, *Xho*I; Ha, *Hae*III; S, *Sau*3A; Hi, *Hin*dIII; Xb, *Xba*I; E, *Eco*RI; Sac, *Sac*I; A, *Alu*I; N.D., Not done. (B and C) Identification of the putative ribosomal protein gene product in vivo. (B) Whole-cell lysates of pUC18 (lane 1)-pCTJS9 (lane 2)- and pCTJS8 (lane 3)-containing *E. coli* JM83 and serovar L2 EBs (lane 4) were resolved by SDS-PAGE and stained with Coomassie blue. Low-molecular-mass markers (lane M) are expressed in kilodaltons. (C) Western transfer of panel B immunoblotted with polyclonal antibodies to ribosomal proteins EcoL3 and EcoL6. The arrowheads indicate the 23-kDa gene product of pCTJS8, whereas the double arrow indicates host-synthesized EcoL6.

in whole-ribosome preparations from cells harboring pCTJS8 but not in the control harboring pUC19 alone.

By using radioanalytic imaging to provide counts per minute for each of these immune-labeled proteins and standardizing against the EcoL3 label intensity for each sample, we calculated a 45.4% decrease in the total EcoL6 protein in whole ribosomes isolated from cells bearing CtaL6e-expressing plasmid pCTJS8. This decrease is relative to the amount of EcoL6 protein detected in whole ribosomes from pUC18-bearing cells, which was taken as 100%.

DISCUSSION

The data presented here indicate that pCT161/18 (12) is a cloning artifact generated through a nonspecific recombinational event. Southern blot analysis of serovars L2, D, J, and K with a 458-bp *Pst*I-*Hin*dIII fragment representing the 5′ end of the pCT161/18 insert documented that all serovars possess the same 440-bp *Pst*I fragment rather than a 1,658-bp *Pst*I genomic DNA fragment, as reported for serovar L2 (12). Further nucleotide sequencing of *Pst*I clones from serovars L2, D, J, and K revealed exact identity among them. They also demonstrated identity with pCT161/18 through the first 430 bp; beyond that site, the sequences diverged completely. Our failure to observe any homology initially between pCT161/18 and other prokaryotic genes was due to the availability of an incomplete data bank. At least two unrelated DNA fragments from the *C. trachomatis* genome have ligated at the *Sau*3A site at position 430. Nonspecific ligation at this site may have been due to cross-contamination of our restriction endonucleases. Further, this recombination provided an ORF of sufficient length to encode a protein of 18 kDa possessing HeLa-binding ability. The binding characteristics of this gene product may be due to the presence of a highly charged C terminus (four of seven aa are lysine). These observations are also supported by our failure to obtain any inhibitory effect on cell adhesion in the presence of synthetic peptides directed to various epitopes of this molecule (unpublished data). However, we are unable to explain neutralization mediated by antibodies to the recombinant polypeptide. In any event, the

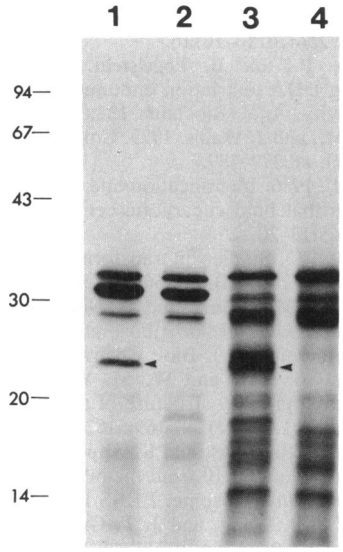

FIG. 4. Identification of the putative ribosomal gene product in vitro and in vivo. Gel electrophoresis pattern of ^{35}S-labeled gene products synthesized in minicells (lanes 1 and 2) and the in vitro transcription-translation system (lanes 3 and 4). Lanes 1 and 3 represent pCTJS8, and lanes 2 and 4 represent pUC18-directed protein synthesis. The arrowheads indicate the 23-kDa pCTJS8 product. Low-molecular-mass markers are expressed in kilodaltons.

product of pCT161/18 clearly represents a hybrid protein and not a cloned *C. trachomatis* cell-binding protein.

Since the N terminus of the pCT161/18-encoded protein bears homology to ribosomal proteins, extension of this sequence was undertaken for further analysis and comparison of the deduced ORF with ribosomal proteins EcoL6 and BstL6e. *Sac*I (pCTJS1 and pCTJL1) clones were selected from serovar J and L2 genomic DNA libraries by hybridization with the 440-bp *Pst*I fragment. The sequences of the ~3.0-kb *Sac*I inserts of pCTJS1 and pCTLS1 are identical in this region. The ORF is 552 nucleotides long. The postulated ribosome-binding site for translation of this ORF is the AGGA sequence 10 bases upstream of the start codon (12, 31). No [*rho*]-independent termination site for transcription is seen in the sequence (24). The lack of a strong transcriptional terminator suggests either the existence of a [*rho*]-dependent termination system for this gene or that it is transcribed as part of a polycistronic message.

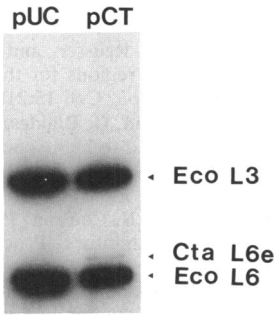

FIG. 5. Immunoblot analysis of whole ribosomal proteins resolved on SDS–20% PAGE and reacted with polyclonal antibodies to *E. coli* ribosomal proteins L3 and L6. Whole ribosomes were isolated from JM83 cells bearing a nonrecombinant (pUC) or recombinant (pCT) plasmid. Chlamydial L6 protein Cta L6e and *E. coli* proteins Eco L3 and Eco L6 are indicated.

Analysis of the 1,194-bp pCTJS1 sequence revealed portions of two ORFs flanking the 551-nucleotide chlamydial CtaL6e ORF. The upstream intergenic space is 27 nucleotides long, while the downstream intergenic space is 21 nucleotides long. Preliminary comparison of the deduced primary structures from the upstream and downstream ORFs that flank CtaL6e indicate approximately 30% homology with ribosomal proteins CtaS8e and CtaL18e. The L6-encoding gene (*rplF*) of *E. coli* is also flanked by EcoS8 and EcoL18 genes and represents a part of the *spc* operon which comprises 12 cotranscribed genes (3).

The CtaL6e ORF encodes a protein product 183 aa long. On this basis, the calculated molecular mass of protein CtaL6e is 19,838 Da. This value agrees closely with the molecular weights of 18,830 and 19,166 calculated for 176-aa protein EcoL6 (4) and 177-aa protein BstL6e (13), respectively, on the basis of aa sequencing data.

Alignment of the deduced CtaL6e primary structure with those of EcoL6 and BstL6e (Fig. 4) revealed considerable homology. The CtaL6e sequence matched at 87 positions (47.5%) over the length of the BstL6e sequence. In addition, there were 26 conservative substitutions, raising the amount of shared homology to 62%. CtaL6e had 76 matches (41.5%) over the length of EcoL6 and an additional 26 conservative substitutions. The resultant shared homology with EcoL6 was therefore 56%. These values compare favorably to homology comparisons between EcoL6 and BstL6e. EcoL6 and BstL6e share complete sequence matches at 88 positions (50%) and conservative substitutions at another 28 positions. This results in a shared homology between EcoL6 and BstL6e of 66%. There is a nearly 70% bias towards A or T in position 3 of codons in the gene for CtaL6e, compared with 51% in the gene for EcoL6. The overall calculated A+T contents of the genes for CtaL6e and EcoL6 are 56.7 and 48.0%, respectively. This is consistent with the calculated averages for whole chlamydial and *E. coli* genomes of 70 and 50% A+T, respectively (12, 21).

Southern blot hybridization analysis of serovar D, J, K, and L2 genomic DNAs cut with *Xba*I and *Hin*dIII revealed a common 980-bp single band (data not shown), indicating that the CtaL6e gene is present in a single copy in the *C. trachomatis* genome. Genes for the 52 ribosomal proteins in *E. coli* are also present in a single copy per haploid genome (25).

Expression of CtaL6e from plasmid pCTJS8 rather than pCTJS9 suggests that expression of this gene product originates from the *lac* promoter and not from an internal promoter on the 980-bp *Xba*I-*Hin*dIII fragment. The differences between the deduced and apparent molecular weights may be due to either the anomalous migration of this positively charged protein or posttranslational modifications.

Polyclonal antibodies to a mixture of EcoL3 and EcoL6 were used to evaluate antigenic homology between CtaL6e and EcoL6. These antibodies reacted to both the native chlamydial protein and the pCTJS8-directed gene product. Similar results obtained by immunochemical analysis of ribosomal proteins EcoL6 and BstL6e have been reported (6).

Takata (33) first demonstrated incorporation of *E. coli* ribosomal proteins into the ribosomes of *Serratia marcescens* by intergeneric mating experiments. Recently, Liu et al. (17) have shown the ability of a cloned chloroplast ribosomal protein to become incorporated into functional

ribosomes in place of the host homologous ribosomal protein. We used this approach to confirm the functional homologies of chlamydial and EcoL6 proteins. The CtaL6e and EcoL6 ribosomal proteins are sufficiently different in size that a 20% polyacrylamide gel can readily separate them. Once the two proteins were separated, their antigenic cross-reactivity was exploited to identify the presence of the larger CtaL6e protein in whole ribosomes from *E. coli* cells harboring the expressing plasmid (Fig. 5). The intensity of labeling on immunoblots was quantitated by computer-assisted radioanalytic imaging. The EcoL3 label intensity (the antiserum was a polyclonal mixture directed towards EcoL3 and EcoL6) was used to standardize the amount of protein loaded in each lane. By taking the counts per minute for EcoL6 in the control lane as 100%, we calculated that the relative amount of EcoL6 in whole ribosomes from recombinant cells was 55.6%. The assumption made by us and by Liu et al. (17) is that the stoichiometry of ribosome formation is maintained by substitution of recombinant protein for native protein in these cells. In our case, we calculated that CtaL6e makes up 45.4% of all of the L6 protein in the recombinant cell ribosomes. On the basis of this value, we determined that CtaL6e bears 7% antigenic cross-reactivity with protein EcoL6.

The nucleotide sequence, primary structure, and functionality of a putative ribosomal protein from *C. trachomatis* have been presented here. These data, along with the demonstrated immunochemical cross-reactivity of this protein with antibodies directed towards *E. coli* ribosomal protein EcoL6, support the nomination of CtaL6e as a structural and functional homolog of ribosomal proteins EcoL6 and BstL6e. Further support for this supposition is derived from the apparent position of this gene within an operon resembling the *spc* operon of *E. coli*. The sequence of the ~3.0-kb *Sac*I clone (pCTJS1) is being completed to establish homologies with the *spc* operon of *E. coli*. Promotion and regulation of this putative *C. trachomatis* ribosomal protein operon will also be examined.

ACKNOWLEDGMENTS

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