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Molecular Characterization of *Chlamydia trachomatis* and *Chlamydia psittaci* Plasmids

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Plasmids from *Chlamydia trachomatis* LGV-434 (serotype L2) and *Chlamydia psittaci* meningopneumonitis strain Cal-10 were cloned into the *Bam*HI and *Eco*RI sites of pBR322, respectively. The recombinant plasmids pCTL2 and pCPMn, each containing an entire respective chlamydial plasmid, were transformed into *Escherichia coli*. The sizes of the plasmids of *C. trachomatis* and *C. psittaci* were 7.3 and 6.2 kilobases, respectively. The two plasmids were found to be distinct by restriction endonuclease analysis, DNA-DNA hybridization, and electron microscopic heteroduplex analysis. However, partial homology was observed between restriction fragments of pCTL2 and pCPMn by Southern blot analysis. Polypeptide products encoded by these plasmids were synthesized in vitro by an *E. coli*-directed transcription-translation system and in vivo in *E. coli* maxicells and minicells. None of these polypeptides was immunoreactive with anti-chlamydial sera by immunoblotting or immunoprecipitation. Based on the comparative analysis data, the *C. trachomatis* and *C. psittaci* plasmids were found to share little genetic relatedness.

The genus *Chlamydia* consists of two species, *Chlamydia trachomatis* and *Chlamydia psittaci* (14). *C. trachomatis* is a specific pathogen of humans, whereas *C. psittaci* is primarily a pathogen of nonprimate species (28). Despite their diversity in adaptation to specific hosts, both species share a number of common biological properties that distinguish them from other obligate intracellular procaryotes. All chlamydiae undergo a complex and unique developmental growth cycle in eucaryotic cells that consists of two functionally distinct particle types (27). The elementary body (EB) is the infectious extracellular particle, whereas the reticulate body is the noninfectious, metabolically active intracellular form of the parasite. In addition to developmental cycle, chlamydial EBs share highly specialized surface structures that function in promoting uptake and internalization into cells (5) and inhibiting the fusion of host lysosomes with the chlamydia-laden endosome (13, 27). Despite these common biological properties, members of the two species share less than 10% chromosomal DNA homology (17) and are largely heterogenous antigenically (8). They do, however, possess a common antigenic determinant located on their lipopolysaccharide that is unique to the genus (6).

The presence of a small 7-kilobase (kb) plasmid has been demonstrated in strains from both chlamydial species (19, 22). Previous studies (19, 22) have shown similar but not identical restriction fragment profiles between plasmids of several strains of *C. trachomatis*. The *C. psittaci* plasmid restriction fragment profiles have been shown to be different from those of *C. trachomatis* strains. The function of the plasmid is unknown; however, its conservation throughout the evolution of the organism suggests its importance to the survival of the parasite. In this report, we have undertaken comparative characterization studies of the *C. trachomatis* and *C. psittaci* plasmids to determine if similarities in gene structure might help to understand some of the common biological properties shared by the two species.

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C. trachomatis strain LGV 434/Bu serotype L2 and *C. psittaci* meningopneumonitis strain Cal-10 were grown in

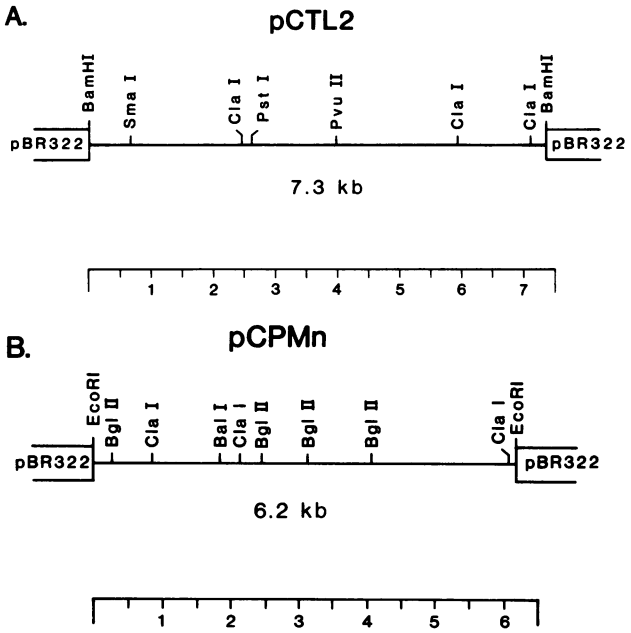

FIG. 1. Restriction endonuclease cleavage maps of (A) pCTL2 insert (7.3 kb) and (B) pCPMn insert (6.2 kb). The sizes of the inserts were determined by electron microscopic examination of their contour lengths. Single cleavage sites were observed for *Bam*HI, *Pst*I, *Pvu*II, and *Sma*I with the pCTL2 insert and with *Eco*RI and *Bal*I for the pCPMn insert.

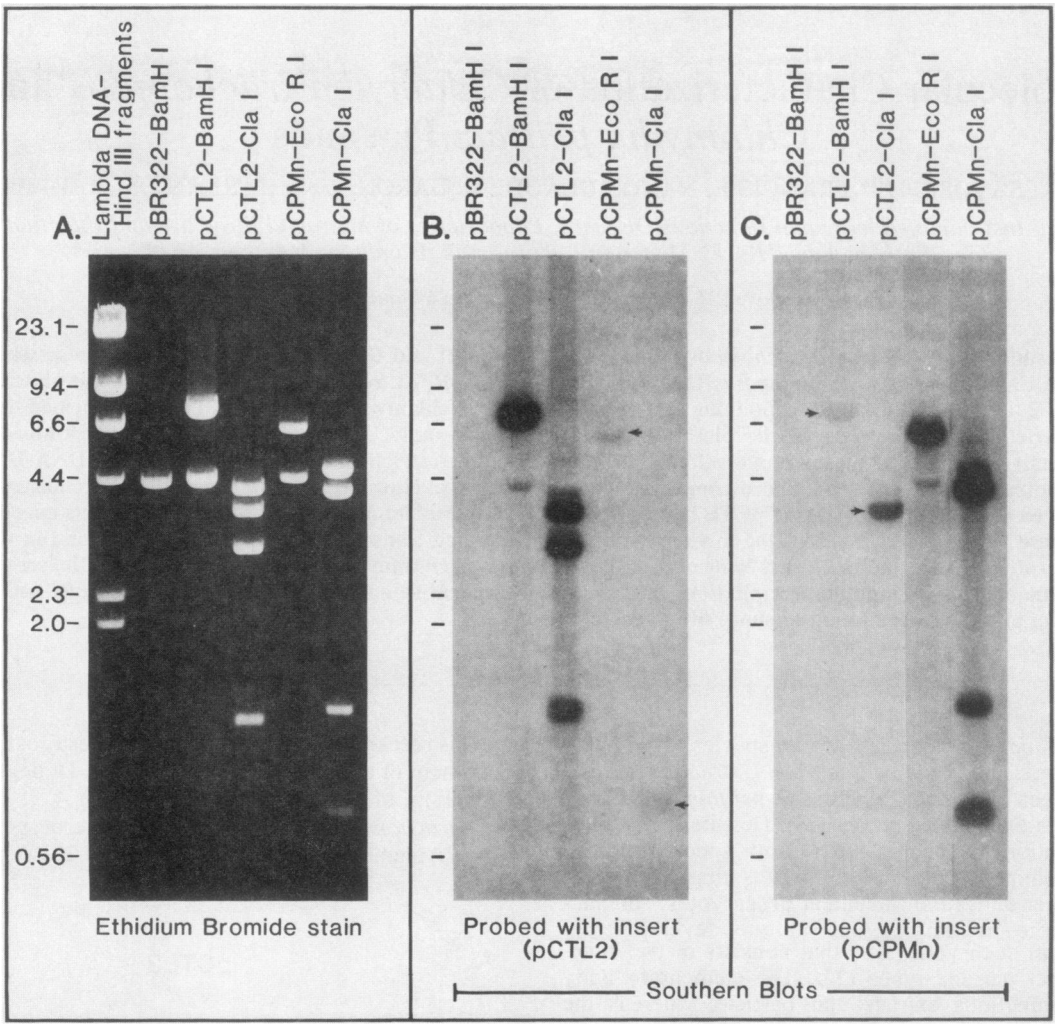

FIG. 2. Southern blot analysis of pCTL2 and pCPMn. Restriction endonuclease digests of pCTL2 and pCPMn with either *Bam*HI, *Eco*RI, or *Cla*I were electrophoresed on 0.7% agarose gel. (A) Ethidium bromide stain. (B and C) Parallel blots of the gel shown in panel A probed with ^{32}P-labeled pCTL2 and ^{32}P-labeled pCPMn inserts, respectively. Panel C is a composite of different exposures of the blot; this resulted in the observed exaggerated intensity of the *Cla*I fragment of pCTL2. Arrows indicate DNA fragments that hybridized with the heterologous probe. All hybridizations were carried out under high-stringency conditions (65°C and 2× SSC).

suspension cultures of L-929 cells, and EBs were purified on discontinuous Renografin density gradients (E. R. Squibb & Sons, Princeton, N.J.) as previously described (7). For transformation with recombinant plasmid DNA, *Escherichia coli* EM24 (*metB hsdR lacY supE supF galK galT trpR recA rpsL*) was used. *E. coli* maxicell mutant strain CSR603 (*uvrA recA*) and *E. coli* minicell-producing strain χ1488 (F^- *minA purE glnU* λ^- *pdxC minB his metC rpsL xyl ilv cysB cysA hsdR*) (10) were used for the synthesis of plasmid-encoded polypeptides.

C. trachomatis and *C. psittaci* EBs (6×10^{10} inclusion-forming units) were suspended in 20 ml of lysis buffer (50 mM Tris hydrochloride [pH 7.2], 25 mM dithiothreitol, 30 mM EDTA, 1% Sarkosyl [CIBA-GEIGY Corp., Summit, N.J.]). The suspension was held at 56°C, and proteinase K (200 μg/ml) was then added and incubation was continued until the solution cleared. Supercoiled plasmid DNA was purified by CsCl-ethidium bromide centrifugation (20, 23).

Plasmid DNA was precipitated as previously described (12), treated with ribonuclease (10 μg/ml), and then centrifuged through 1 M NaCl (20). The pellet containing plasmid DNA was suspended in 10 mM Tris hydrochloride–1 mM EDTA (pH 7.5). The sizes of the plasmids were determined by electrophoresis in 0.7% agarose gels and by electron microscopic examination of their contour lengths.

Single restriction sites have been reported with endonucleases *Bam*HI and *Eco*RI for *C. trachomatis* and *C. psittaci* plasmids, respectively (16, 19). Accordingly, plasmid DNA was digested with *Bam*HI or *Eco*RI and ligated to pBR322 cleaved at its single restriction sites. The resulting recombinant DNA pCTL2 (*C. trachomatis* L2 insert) and pCPMn (*C. psittaci* meningopneumonitis insert) were used for the transformation (15) of *E. coli*. The recombinant plasmids were isolated by the alkaline lysis method (3) followed by CsCl-ethidium bromide centrifugation.

The sizes of the inserts carried by pCTL2 and pCPMn were shown to contain the entire chlamydial plasmid (within the limits of our experimental procedures) by comparing

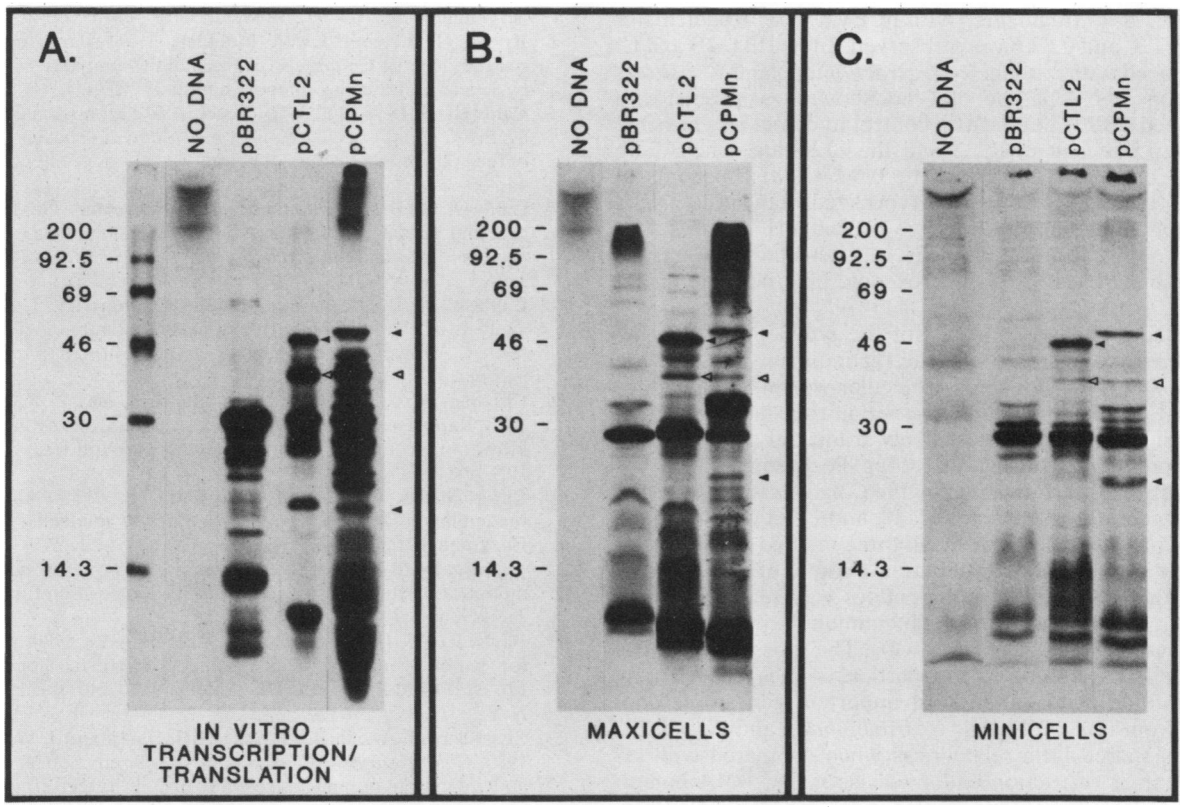

FIG. 3. Gel electrophoresis patterns of the polypeptides synthesized in vitro and in vivo by pCTL2 and pCPMn. Polypeptides synthesized by the three methods were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography to visualize [^{35}S]methionine-labeled products. (A) Synthesis by an *E. coli*-directed coupled in vitro transcription-translation system. (B and C) In vivo synthesis in maxicells and minicells, respectively. Polypeptide products observed in common with all three methods of synthesis are indicated by arrows; ◁, bands that comigrate; ◀, polypeptides of different M_rs.

restriction digests of the recombinant plasmids with those of the parent chlamydial plasmids (data not shown).

Restriction endonuclease mapping was done by single and mixed enzyme digests. The cleavage products were electrophoresed on 0.7% agarose gels (20).

For Southern blot analysis, the recombinant plasmids were digested with restriction endonucleases *Bam*HI or *Eco*RI and *Cla*I. The reaction mixtures were electrophoresed on 0.7% agarose gels and then transferred onto nitrocellulose membranes by Southern blot (29). The blots were probed with nick-translated plasmid DNA (24) that had incorporated [α-^{32}P]dCTP (New England Nuclear Corp., Boston, Mass.) to detect homologous and heterologous DNA-DNA hybridization (20). All hybridizations were carried out under high-stringency conditions (65°C and 0.15 M NaCl–0.015 M sodium citrate [SSC]). The nitrocellulose membranes were subjected to autoradiography to visualize DNA-DNA hybridization patterns.

In vitro detection of polypeptides coded by the plasmids was done by the method of Zubay (31) as modified by Collins (11) with an *E. coli*-directed, coupled in vitro transcription-translation kit (Amersham Corp., Arlington Heights, Ill.). In vivo synthesis of polypeptides encoded by the plasmids was done with maxicell strain CSR603 (25, 26) and minicell strain χ1488 (10, 21). The maxicell experiment was done by using standard procedures (25, 26). For the minicell experiment, the modified filtration procedure of Christen et al. (9) was followed. Standardized counts of each reaction mixture were solubilized in Laemmli solubilizing buffer and subjected to electrophoresis in 12.5% sodium dodecyl sulfate-polyacrylamide slab gels (18) and fluorography (4).

The antigenicity of the plasmid-encoded polypeptides was analyzed by immunoblotting and immunoprecipitation. Hyperimmune rabbit anti-L2 and meningopneumonitis EB sera were used for both immunoblotting and immunoprecipitation. The immunoblotting procedure described by Towbin et al. (30) as modified by Batteiger et al. (2) was used. Immunoprecipitation of the [^{35}S]methionine-labeled polypeptides was done essentially by the procedure of Anacker et al. (1).

The restriction maps of pCTL2 and pCPMn are shown (Fig. 1). The *C. trachomatis* plasmid had single cleavage sites for restriction endonucleases *Bam*HI, *Pst*I, *Pvu*II, and *Sma*I. The *C. psittaci* plasmid had single cleavage sites for restriction endonucleases *Eco*RI and *Bal*I. The *C. trachomatis* plasmid was not cleaved by restriction endonucleases *Sal*I and *Pvu*I. The *C. psittaci* plasmid was not cleaved by restriction endonucleases *Pvu*I, *Pvu*II, *Bam*HI, *Hpa*I, *Kpn*I, *Nde*I, *Nru*I, *Sal*I, *Sac*I, *Sph*I, *Tth*III, *Xba*I, *Xho*I, and *Xma*I. The plasmids of the two species appeared to be distinct on comparison of their restriction maps.

Southern blot analyses of the recombinant plasmids were done (Fig. 2). The recombinant plasmids were cleaved at single sites with either endonucleases *Bam*HI or *Eco*RI and also with *Cla*I. The restriction digests were electrophoresed in a 0.7% agarose gel and then subjected to Southern blotting. The *Cla*I digests of pCTL2 resulted in 4.1-, 3.5-,

2.8-, and 1.2-kb fragments. With pCPMn, *Cla*I fragments of 4.5, 3.9, 1.3, and 0.8 kb were observed. Blots (Fig. 2B and C) were probed with ^{32}P-labeled *C. trachomatis* and *C. psittaci* plasmid inserts. A parallel blot (not shown) was probed with ^{32}P-labeled pBR322 alone as a control to detect any possible homology between pBR322 and the plasmids. The 3.5-kb *Cla*I fragment of pCTL2 and the 0.8-kb *Cla*I fragments of pCPMn (Fig. 2B and C) cross hybridized, indicating partial DNA homology between the two plasmids (Fig. 2B and C, arrows). However, no homology was detected between the two plasmids by electron microscopic heteroduplex analysis.

Gel electrophoresis patterns of the polypeptides synthesized in vitro and in vivo by the recombinant plasmids are shown in Fig. 3. The small-molecular-weight polypeptides observed in the in vitro transcription-translation reaction containing pCPMn are most likely truncated products created by premature translational stops. Polypeptides with M_rs of 48 and 38 kd were encoded by the *C. trachomatis* plasmid, and polypeptides with M_rs of 52, 38, and 22 kd were encoded by the *C. psittaci* plasmids by all three methods of synthesis (polypeptides observed with parent plasmid pBR322 are not included). None of these polypeptides was immunoreactive with antisera of chlamydia EBs by immunoblotting or immunoprecipitation (data not shown). The possibility exists however, that cloning at the particular sites chosen may have resulted in inactivation of important genes. We concluded from this study that *C. trachomatis* and *C. psittaci* plasmids shared little relatedness when compared with respect to their restriction endonuclease maps, DNA homology, and plasmid-encoded polypeptides. The observed partial DNA homology between the *C. trachomatis* and *C. psittaci* plasmids is consistent with the reported lack of chromosomal DNA homology data between these two species (17). However, since partial DNA homology does exist between the two plasmids and since both plasmids express polypeptides of similar M_rs, a common function cannot be excluded.

To date, there are few reported studies on the plasmids of chlamydiae. Further studies might help to elucidate the role of these presently cryptic plasmids. Previous studies (16, 19, 22) have reported the presence of similarly sized plasmids in several strains of chlamydiae, suggesting their conserved nature. The distinct nature of these conserved plasmids between the two species of *Chlamydia* suggests their use as diagnostic probes. Additionally, the recombinant plasmids described here may serve as shuttle vectors between *E. coli* and the respective chlamydial species.

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