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Construction of Physical and Genetic Maps of *Chlamydia trachomatis* Serovar L2 by Pulsed-Field Gel Electrophoresis

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We constructed the physical map of *Chlamydia trachomatis* serovar L2 by using three restriction endonucleases, *Not*I (GC|GGCCGC), *Sgr*AI (C(A/G)|CCGG(T/G)G), and *Sse*8387I (CCTGCA|GG), and we analyzed the fragments by pulsed-field gel electrophoresis. A total of 25 restriction endonuclease sites and 13 genes and/or operons were located on the map. The genome size was determined to be 1,045 kb. Neither highly transcribed chlamydia genes nor developmental cycle-specific genes were clustered on the genome.

Chlamydia spp. are obligate intracellular eubacteria; because of their unique developmental cycle, they are classified in their own order, *Chlamydiales*, with one family, *Chlamydiaceae*. The family has only one genus, *Chlamydia*. The three species of *Chlamydia*, *C. trachomatis*, *C. pneumoniae*, and *C. psittaci*, are characterized by a common lipopolysaccharide epitope. *C. trachomatis* serovars A, B, Ba, and C are the agents of trachoma, which is endemic in many developing countries and is the leading cause of preventable blindness. Serovars D through K are a common cause of sexually transmitted genital infection worldwide. *C. trachomatis* serovars L1 through L3 are the agents of lymphogranuloma venereum, a sexually transmitted disease (26).

The *C. trachomatis* genome has a guanine-cytosine content of 45% (18). It consists of a single circular chromosome (34) and a 7.2-kb plasmid (28). The size of the chromosome has been estimated to be 1,000 kb by electron microscopy (34), 600 kb by reassociation rate (17), and 1,450 kb by pulsed-field gel electrophoresis of uncleaved genomic DNA (14). The DNAs of different serovars of *C. trachomatis* are more than 96% homologous (18), and the restriction length patterns obtained by cleavage with *Bam*HI of DNAs from the different serovars are nearly identical (7).

Information about chlamydial genetics has been difficult to obtain because of a lack of naturally occurring mutants. Likewise, mechanisms of genetic recombination, including transformation, conjugation, and transfer of transposons, have not been described for chlamydiae. Nevertheless, recombinant DNA technology offers ways to analyze individual chlamydial genes. Comparisons of chlamydial genes cloned in different laboratories have often been complicated by the fact that proteins of similar size have been considered identical. This was the case with the DnaK-like and S1 ribosomal protein gene products, both of which have molecular masses of 75 kDa (5, 16, 22, 32). A restriction map of the chlamydial genome would provide a framework on which known genes could be positioned. In this study we used pulsed-field gel electrophoresis for characterization of the chlamydial genome. We constructed a physical map of the *C. trachomatis* serovar L2 chromosome by using three restriction endonucleases with 8-bp recognition sequences (*Not*I, *Sgr*AI, and *Sse*8387I), and we mapped 13 genes or operons.

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MATERIALS AND METHODS

DNA preparation. *C. trachomatis* L_2/434/Bu elementary bodies were grown in L929 cells and purified as previously described (19). A pellet of 5×10^{10} purified elementary bodies was suspended in 1 ml of Hanks balanced salt solution containing 5 μg of RNase and 5 μg of DNase I (Worthington Biochemicals, Freehold, N.J.) and incubated for 30 min at room temperature. The elementary bodies were collected by centrifugation through 44% Renografin. The DNA extraction was performed by the method of Schwartz and Cantor (35). Briefly, the pellet of microorganisms was suspended in 0.5 ml of 0.05 M EDTA (pH 8.0) and mixed with an equal volume of 1.4% agarose (InCert; FMC Bio-Products, Rockland, Maine) at 42°C. The agarose blocks were cast in a size of 1 by 10 by 20 mm. The blocks were incubated for 24 h at 37°C in 0.5 M EDTA (pH 8.0)–0.01 M Tris containing 1% β-mercaptoethanol. The blocks were transferred to 0.1 M EDTA (pH 8.0)–0.01 M Tris–0.02 M NaCl containing 1 mg of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml and 1% Sarkosyl for 24 h at 50°C. The buffer was changed to 10 mM Tris (pH 8.0)–1 mM EDTA–40 μg of phenylmethylsulfonyl fluoride per ml for 1 h at 50°C. The blocks were stored in 0.05 M EDTA (pH 8.0) at 4°C.

Restriction endonuclease digestion and pulsed-field gel electrophoresis. Agarose blocks (1 by 5 by 5 mm) containing 1 μg of chlamydial DNA were subjected to single or double digestion with restriction endonucleases in 0.1 ml of the respective restriction endonuclease buffer containing 0.01% bovine serum albumin for 20 h at 37°C. For total digests of DNA, 20 U of restriction endonuclease *Not*I (Boehringer Mannheim Biochemicals), 10 U of *Sgr*AI (Boehringer), and 25 U of *Sse*8387I (Takara Biochemical Inc, Berkeley, Calif.) were used. For partial digestion with *Sse*8387I, 10 U was used. After restriction endonuclease digestion, blocks were dialyzed in buffer containing 10 mM Tris (pH 8.0)–1 mM EDTA and mounted on the teeth of an electrophoresis comb. The gel was cast with 1.0% agarose–NA at 55°C (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA [pH

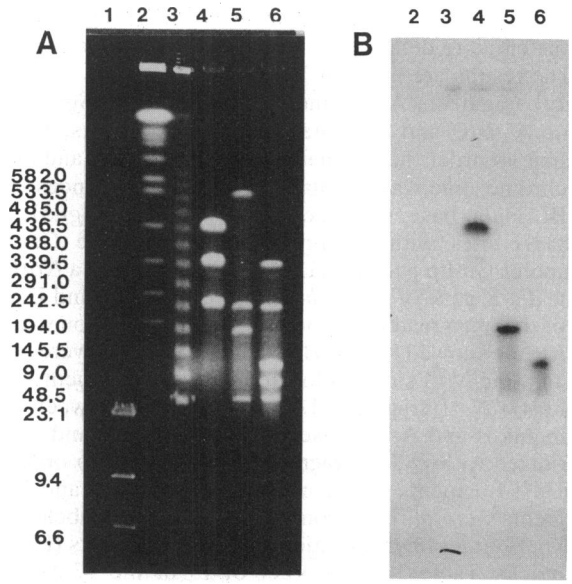

FIG. 1. (A) Pulsed-field electrophoresis gel. The switch time was a 15- to 75-s ramp for 20 h at 6 V/cm. Lanes: 1, lambda DNA digested with *Hin*dIII; 2, *S. cerevisiae* chromosomes; 3, ladder of annealed concatermers of bacteriophage lambda DNA; 4 through 6, *C. trachomatis* serovar L2 DNA digested with *Not*I (lane 4), *Sgr*AI (lane 5) or *Not*I-*Sgr*AI (lane 6). (B) Autoradiography of the same gel transferred to a nylon membrane and probed with ^{32}P-labeled pG83 encoding the *H1*-like gene from *C. trachomatis* MoPn (*H1-26*).

8]). The gel was electrophoresed at 14°C in a Chef II apparatus (Bio-Rad, Richmond, Calif.). For separation of fragment sizes between 4 and 200 kb, the gel was run for 20 h at 200 V with a ramp of the switch time from 1 to 15 s. For separation of the size range 6 to 600 kb, the same time and voltage were used but the ramp of the switch time was from 15 to 75 s. As size markers, 0.1 μg of *Hin*dIII-cut lambda DNA, a lambda ladder (Promega Corp., Madison, Wis.), and *Saccharomyces cerevisiae* chromosomes (Bio-Rad) were used. Gels were stained in 0.5× TBE containing 0.5 μg of ethidium bromide per ml and destained in water.

Southern hybridization. The agarose gel was soaked in 250 mM HCl for 3 min, the DNA was denatured for 30 min in 0.5 M NaOH–1.5 M NaCl, and the DNA fragments were transferred to Hybond-N nylon filters (Amersham Corp., South Clearbrook, Ill.) with 20× SSC (1× SSC is 150 mM NaCl plus 75 mM sodium citrate [pH 7.0]) (31). The filter was washed in 2× SSC and baked at 80°C for 2 h.

Genomic DNA probes. *Not*I and *Sgr*AI restriction enzyme DNA fragments were separated by gel electrophoresis and excised from the agarose gel. The DNA was purified by melting the agarose and then extracting sequentially with phenol, phenol-chloroform, chloroform, *n*-butanol, and ether. The DNA was precipitated with 0.3 M sodium acetate (pH 5.2) and 2 volumes of ethanol. The restriction endonuclease-generated DNA fragment (0.1 μg) was labeled with [^{32}P]dATP (Amersham) by the random oligonucleotides primer method (31). Hybridization was carried out in 6× SSC, 5× Denhardt reagent (50× Denhardt reagent, 10 g of Ficoll [type 400; Pharmacia] per liter, 10 g of polyvinylpyrrolidone per liter, 10 g of bovine serum albumin), 0.5% sodium dodecyl sulfate, and 100 μg of denatured salmon sperm DNA (Pharmacia) per ml at 68°C for 20 h. The filters were washed in 2× SSC containing 0.5% sodium dodecyl sulfate at 68°C for 2 h, 30 min, and 15 min. The filters were exposed to X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) for 24 h.

Probes for cloned genes of chlamydia were generously provided by the investigators listed in Table 3. The DNA was labeled and hybridized as described above, and the filters were exposed to X-Omat AR film for 2 h.

RESULTS

Digestion of *C. trachomatis* L2 DNA with restriction endonucleases and genome size estimation. The G+C content of *C. trachomatis* is 45%. To obtain relatively few fragments from restriction endonuclease cleavage of the chlamydial chromosome, it was necessary to use restriction endonucleases with 8-bp recognition sequences. *Not*I (GC|GGCCGC) and *Sgr*AI (C(A/G)|CCGG(T/G)G) each cleaved the chlamydial genome into four fragments. Double digestion with both enzymes revealed six fragments, two of which contained comigrating fragments (Fig. 1A, Tables 1 and 4). The bands varied in size from 555 to 10 kb. To obtain good separation and size determination in that size range, a ramp of the pulse time from 15 s to 75 s over 20 h was found to be optimal. The restriction endonuclease *Sse*8387I (CCTGCA|GG) cleaved the *C. trachomatis* genome into 17 fragments ranging in size from 220 to 9 kb; 10 bands ranged from 65 to 21 kb. To obtain separation of these latter bands, a ramp of the pulse time from 1 s to 15 s over 20 h was optimal (Fig. 2A). The estimated sizes of the fragments are listed in Table 1. The sums of the fragment sizes for each enzyme differed by less than 2%. The chromosome size was calculated as the mean of the values to be 1,045 kb (Table 1).

TABLE 1. Restriction fragments of *C. trachomatis* serovar L2[a]

Fragment	Length (kbp)	Fragment	Length (kbp)
*Not*I fragments		*Sse*8387I fragments	
A	456	A	220
B	340	B	200
C	250	C	102
D	10	D	83
		E	76
*Sgr*AI fragments		F	65
A	555	G	46
B	243	H	42
C	194	I	36
D	51	J	32
		K	28
*Sgr*AI-*Not*I fragments		L	24
1 (*Not*I-B)[b]	340	M	24
2 (*Sgr*AI-B)	243	N	21
3	116 × 2	O	21
4	82 × 2	P	15
5 (*Sgr*AI-D)	51	Q	9
6 (*Not*I-D)	10		

[a] The sizes of the fragments were calculated from the data in Fig. 1A and 2A. The bands in the double digest, present in either the *Sgr*AI or *Not*I digests, are indicated. Fragment 3 of the *Not*I-*Sgr*AI double digest contained two bands because the *H1-18* and *rRNA* genes both hybridized to *Not*I fragment C, *Sgr*AI fragment A, and fragment 3 of the double digest. The *H1-26* gene was also localized on fragment 3 but hybridized to *Not*I fragment A and *Sgr*AI fragment C (see Table 4). *Not*I-*Sgr*AI fragment 4 was composed of two comigrating bands. It contained a band that was generated from the overlapping part of *Not*I fragment A and *Sgr*AI fragment A (456 − 243 − 116 = 97 bp or 555 − 116 − 340 − 10 = 89 bp) and a band from the remaining part of *Not*I fragment C (250 − 116 − 51 = 83 kb) (see Fig. 4).

[b] Fragments given within parentheses are the same as the indicated double digestion fragments.

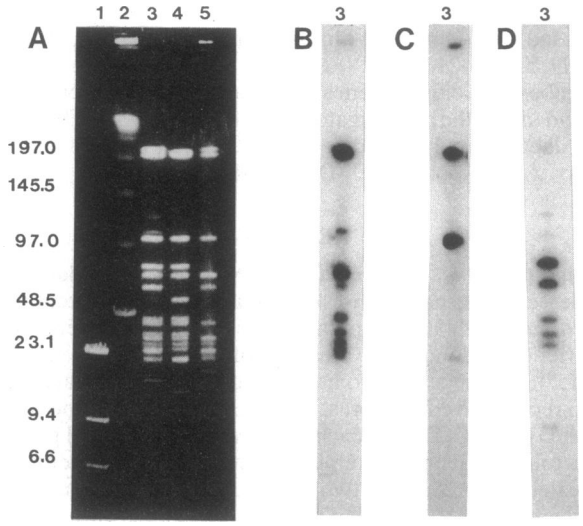

FIG. 2. (A) Pulsed-field electrophoresis gel. The switch time was a 1- to 15-s ramp for 20 h at 6 V/cm. Lanes: 1, lambda DNA digested with *Hin*dIII; 2, ladder of annealed concatermers of bacteriophage lambda DNA; 3 through 5, *C. trachomatis* serovar L2 DNA 3 digested with *Sse*8387I (lane 3), *Sse*8387I-*Not*I (lane 4), or *Sse*8387I-*Sgr*AI (lane 5). (B through D) *C. trachomatis* serovar L2 DNA digested with *Sse*8387I, transferred to a nylon membrane, and probed with the 456-kb *Not*I fragment of *C. trachomatis* serovar L2 genomic DNA labeled with [^{32}P]dATP by random priming (B), the 340-kb *Not*I fragment of L2 genomic DNA (C), or the 250-kb *Not*I fragment of L2 genomic DNA (D).

Physical map of *C. trachomatis* serovar L2 chromosome. To align the restriction enzyme fragments, we used five methods: (i) single digests, double digests, and comparison of the lengths of the fragments; (ii) Southern hybridization with known gene probes; (iii) cross-hybridization with purified fragments generated by restriction endonuclease digestion of the chlamydial genome; (iv) partial digestion and probing to one fragment to find the size of linked fragments; and (v) a linking clone to determine the localization of *Not*I fragment D. The results are listed in Tables 1, 2, and 4.

*Not*I fragments A, B, and Ç, together with *Sgr*AI fragments A to D and the data from double digests, made it possible to order the fragments in only one way, and only if the chromosome was circular. Chlamydial sequences in the EMBL data base were searched for *Not*I, *Sgr*AI, and *Sse*8387I sites with the program FIND in the Genetics Computer Group package (11). The search found a *Not*I site in the *dnaK* gene of *C. trachomatis* serovars L2 and D at bp 527 of the open reading frame (6, 10). Hybridization with the *dnaK* gene showed that *Not*I fragments D and B were linked and that the *Not*I site was located in *Sgr*AI fragment A (see Table 4). *Not*I fragment D was localized between *Not*I fragments B and A, because *Not*I fragments D and A both hybridized to *Sse*8387I fragment L (Table 2). To order the *Sse*8387I fragments on the map, *Not*I fragments and *Sgr*AI fragments were purified from pulsed-field gels, labeled, and used in Southern hybridization to *Sse*8387I digests (Fig. 2B, C, and D; Table 2). The order of 13 of the 17 *Sse*8387I fragments was determined by hybridization with the labeled *Not*I and *Sgr*AI fragments and by determination of localization of *Not*I and *Sgr*AI sites within the *Sse*8387I fragments. The order of *Sse*8387I fragments C and O within *Not*I fragment B and *Sgr*AI fragment A was not evident. By partial digestion with *Sse*8387I and additional total digestion with *Not*I followed by probing with the σ^{66} gene, it was determined that *Sse*8387I fragment C was adjacent to *Sse*8387I fragment P. Moreover, *Sse*8387I fragment P contained a *Not*I site (Fig. 3). A similar problem with *Sse*8387I fragments B and N was solved by digesting completely with *Sgr*AI and partially with *Sse*8387I and probing with the *S1* and β′ genes. The *Sse*8387I map was constructed in the context of *Not*I and *Sgr*AI (Fig. 4). If *Sse*8387I, *Not*I, or *Sgr*AI digestion produced fragments less than 6.4 kb in size, they may not have been detected in the gel system and not included in the map.

TABLE 2. Hybridization patterns of *Not*I and *Sgr*AI probes to *Sse*8387I digests[a]

*Sse*8387I		Hybridization of *Not*I probe (kb):				Hybridization of *Sgr*AI probe (kb):			
Fragment (kb)	Site (kb)	A (456)	B (340)	C (250)	D (10)	A (555)	B (243)	C (194)	D (51)
A (220)	*Not* I (214 + 6)		×		×	×			
B (200)		×					×		
C (102)			×			×			
D (83)	*Sgr*AI (76 + 7)			×		×			×
E (76)		×						×	
F (65)	*Not*I (57 + ?)	(×)[b]		×				×	
G (46)	*Sgr*AI (? + 17)	×				×	×		
H (42)				×		×			
I (36)	*Sgr*AI (32 + ?)	×						×	
J (32)	*Sgr*AI (24 + ?)			×					×
K (28)		×				×			
L (24)	*Not*I (21 + ?)				×[c]				
M (24)		×		×		×		×	
N (21)									
O (21)		×[d]	×			×	×[d]		
P (15)	*Not*I (13 + ?)		×	(×)		×			
Q (9)				×					×

[a] *Not*I and *Sgr*AI *C. trachomatis* DNA restriction endonuclease fragments were used as probes for hybridization to nylon membranes containing *C. trachomatis* serovar L2 DNA cleaved with *Sse*8387I.

[b] (×), weak hybridization.

[c] *Not*I fragment D was hybridized to both *Sse*8387I- and *Sse*8387I-*Not*I-digested L2 DNA.

[d] The hybridization to bands N and O was differentiated because the *groE* gene was localized to *Sse*8387I fragment N (see Table 4).

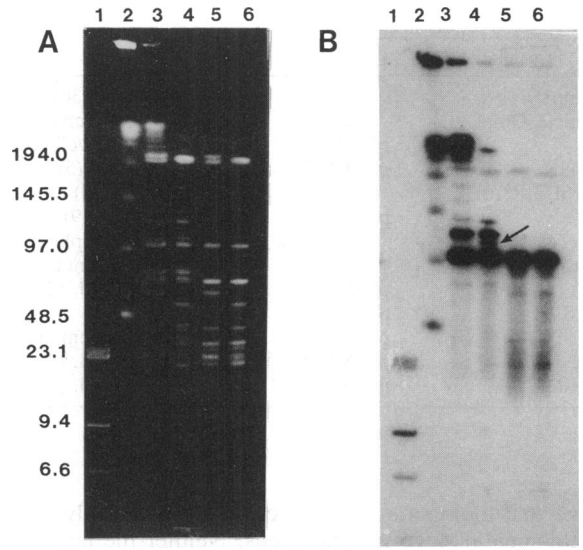

FIG. 3. (A) Pulsed-field electrophoresis gel. The switch time was a 1- to 15-s ramp for 20 h at 6 V/cm. Lanes: 1, lambda DNA digested with *Hin*dIII; 2, ladder of annealed concatermers of lambda bacteriophage DNA; 3 through 5, *C. trachomatis* L2 DNA digested with *Sse*8387I (lane 3), *Sse*8387I-*Not*I (lane 4), or *Sse*8387I-*Sgr*AI (lane 5); 6, triple digestion with *Sse*8387I, *Not*I, and *Sgr*AI. (B) Autoradiography of the gel transferred to a nylon membrane and probed with ^{32}P-labeled pKJ-4 encoding the σ^{66} subunit gene from *C. trachomatis* serovar L2. Samples in lanes are identical to those in panel A. The arrow indicates the band appearing after double digestion with *Not*I of the *Sse*8387I digest; fragment C is next to fragment P.

Mapping of *C. trachomatis* genes. The chromosomal locations of 13 genes were mapped. Ten probes were obtained for genes cloned from *C. trachomatis* serovar L2, and three genes were from the murine mouse pneumonitis strain (MoPn) *C. trachomatis* biovar (Table 3). Two of the 13 DNA probes represent operons. The cysteine-rich outer membrane proteins, Omp2 and Omp3, are encoded within the same operon with *omp3* located upstream of *omp2* (2, 3, 9). The *C. trachomatis* genes for the GroEL and GroES heat shock protein homologs are organized in a manner similar to those in *Escherichia coli* with *groES* located upstream of *groEL* (8, 25). The heat shock homolog *dnaK* is not linked to *dnaJ* in *C. trachomatis* as found in other microorganisms (6, 13).

The probe labeled *LPS* is the recombinant plasmid pFEN207 that, when transformed into *E. coli*, results in the expression of the chlamydial genus-specific epitope on bacterial lipopolysaccharide (27). The mechanism for the change of the bacterial lipopolysaccharide is not known.

C. trachomatis serovar L2 elementary bodies have two major cytoplasmic DNA binding proteins of 26 and 18 kDa as determined by polyacrylamide gel electrophoresis and transfer of the separated proteins to nitrocellulose membranes and probing with ^{32}P-labeled double-stranded chlamydial DNA (37). The N-terminal sequence of the 18-kDa protein was previously determined, and its gene was cloned from *C. trachomatis* serovar L2 (15). The protein has amino acid sequence homology to the C-terminal DNA-binding domain of eucaryotic histone H1. A monoclonal antibody to the 18-kDa DNA-binding protein recognizes both the chlamydial DNA-binding proteins and histone H1 in HeLa 229 cells (15). The 18-kDa H1-like gene was localized to *Not*I fragment C, *Sgr*AI fragment A, and *Sse*8387I fragment D (Table 4). Parara and Ganem (30) cloned a gene from murine *C. trachomatis* (MoPn) that is homologous to eucaryotic histone H1, but with a larger predicted size; the probe for this gene (*H1-26*) hybridized to *Not*I fragment A, *Sgr*AI fragment C, and *Sse*8387I fragment E (Fig. 1B; Table 4). The *dnaK* gene was localized to the *Not*I site between *Not*I fragments B and D. Figure 4 shows the minimal areas of the chromosome at which the genes are located. These are indicated by a bar; when more than one gene is present in the same region, they are marked above one another. The sizes of the mapped areas are from 4 kb for the *S1* gene up to 214 kb for the *mip* and *omp2-3* genes (Fig. 4).

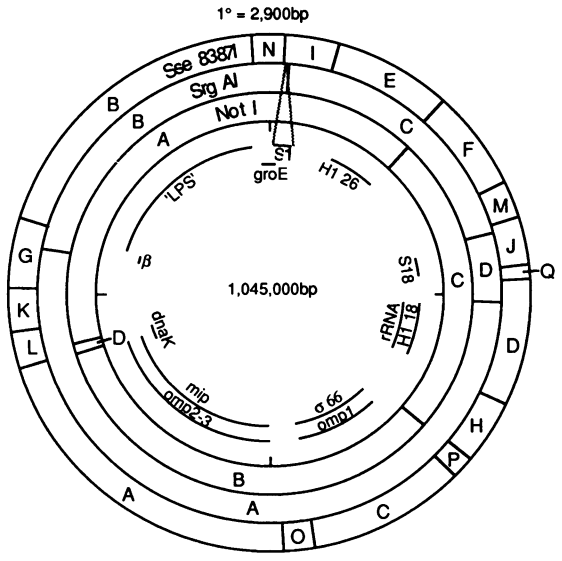

FIG. 4. Circular serovar L2 chromosome map. The arcs in the center indicate the areas in which the genes can be localized in the context of restriction endonucleases *Not*I, *Sgr*AI, and *Sse*8387I.

DISCUSSION

Chlamydiae have small genomes of approximately 10^6 bp and relatively low G+C contents (≈45%). The theoretical numbers of restriction endonuclease fragments for an 8-bp recognition sequence, a G+C content of 45%, and 10^6 bp of the chlamydial genome are 7 for *Not*I, 34 for *Sgr*AI, and 10 for *Sse*8387I. However, *Not*I and *Sgr*AI cleaved the genome only four times each, and *Sse*8387I cleaved 17 times. McCelland et al. (24) used pulsed-field gel electrophoresis of microbial genomes with different G+C contents and found that the trinucleotides CCG and CGG are rare in genomes with G+C contents of less than 45%. Furthermore, restriction endonucleases that recognize the tetranucleotide CTAG within the recognition sequence are rare cutters in microbial DNA with a G+C content above 45%. Both *Not*I and *Sgr*AI restriction endonucleases contain the trinucleotides CGG and CCG in their recognition sequences and cut less frequently than expected in the chlamydial genome, whereas *Sse*8387I, with none of the rare combinations in its recognition sequence, cleaved more frequently than expected.

Frutos et al. (14), using pulsed-field gel electrophoresis of uncut, circular DNA, estimated a genome size of 1,450 kb for *C. trachomatis* L2. However, circular DNA does not migrate predictably during pulsed-field gel electrophoresis. We compared the sizes of restriction endonuclease frag-

TABLE 3. Gene probes and sources

Type	Gene	Size (kb)	Enzymes	Protein(s)	Name	Source
L2	*omp1*	1.4	*Bam*HI-*Eco*RI	Omp1		Stephens et al. (36)
L2	*omp2-3*	3.5	*Sse*I-*Stt*I	Omp2, Omp3		Allen and Stephens (3)
L2	LPS gene			?	pFEN207	Nano and Caldwell (27)
L2	*dnaK*	4.0	*Sau*3A-*Sau*3A	DnaK	pCtX2-43	Birkelund et al. (5)
L2	*groE*	4.0	*Bam*HI-*Bam*HI	GroES, GroEL		Cerrone et al. (8)
L2	σ^{66}	2.1	*Sac*I-*Sac*I	σ^{66}	pJK-4	Koehler et al. (19)
L2	β′	0.4	PCR[a] products IV and V	β′		Koehler and Stephens (20)
MoPn	*rRNAB*	8.0	*Eco*RI-*Eco*RI			Engel and Ganem (12)
L2	*S1*	0.9	*Sau*3A-*Sau*3A	S1	pCtX2-3	Lundemose et al. (22)
MoPn	*S18*	1.5	*Eco*RI-*Eco*RI	S18	pG82	Engel et al. (13)
MoPn	*H1-26*	1.5	*Sac*I-*Sac*I	H1-26	pG83	Perara and Ganem (30)
L2	*H1-18*	5.2	*Sau*3A-*Sau*3A	H1-18	pCtX3-61	Lundemose et al. (23)
L2	*mip*-like	1.0	*Sau*3A-*Sau*3A	MIP	pCtX3-11	Lundemose et al. (21)

[a] PCR, polymerase chain reaction.

ments with those of a ladder of annealed concatermers of bacteriophage lambda DNA molecules. On the basis of the sum of restriction endonuclease fragments, the size of the *C. trachomatis* chromosome was estimated to be 1,045 kb. This size is in agreement with the 1,000 kb estimated by Sarov and Becker (34), who used electron microscopy and measured the contour length of the chlamydial chromosome.

The fragment hybridization results indicated that the *C. trachomatis* L2 genome does not have large duplications, as seen in other bacteria (1). The *rRNA* genes are known to be present in two copies (12, 29), and both were located within the 76-kb *Sse*8387I fragment D.

The chlamydial genes encoding proteins with known analogs in other microorganisms, such as DnaK, GroES, GroEL, the β′ subunit of RNA-polymerase, the σ^{66} subunit of RNA polymerase, ribosomal protein S1, and ribosomal protein S18 (Table 3), are all located close to the origin of replication in *E. coli* (90 to 21 min) except *S18*, which is positioned at 67 min (4). In contrast to their organization in *E. coli*, these genes in *C. trachomatis* were distributed over the entire chromosome. Thus the small *C. trachomatis* genome did not show clustering of these frequently transcribed genes.

One of the most crucial and fundamental elements of chlamydial biology is the transcription of a family of late developmental stage-specific genes. Neither the late developmental stage genes *omp2-3*, the two histone H1-like genes (2, 15, 33), nor the early genes *dnaK*, *groEL*, and *S1* (22) showed clustering. These data indicate that transcription of genes that are specific for the developmental cycle is regulated by global mechanisms.

The physical and genetic map of *C. trachomatis* provides an important foundation for genetic analysis of the chlamydial chromosome and will facilitate construction of an aligned cosmid or P1 library of the chromosome. The LGV biovar has nearly 100% DNA homology to the trachoma biovar, despite significant differences in virulence and pathogenicity. The comparison of the two groups by pulsed-field gel electrophoresis may provide new information about the similarities and differences between biovars. The homology of *C. trachomatis* to the other *Chlamydia* species, *C. psittaci* and *C. pneumoniae*, is low at the level of DNA homology, yet homologous gene products are highly conserved in structure and function. Chromosomal gene organization comparisons among the different species may also provide important information concerning their phylogenetic relationships and origins.

TABLE 4. Hybridization patterns of gene probes to restriction endonuclease fragments of *C. trachomatis* serovar L2 DNA[a]

Gene probe	Hybridization with fragment			
	*Not*I	*Sgr*AI	*Not*I-*Sgr*AI	*Sse*8387I
omp1	B	A	–[b]	C
omp2-3	B	A	–	A
LPS	A	B	–	B
dnaK	B, D	A	–	A
groE	A	B	–	N
σ^{66}	B	A	1	C
β′	A	B	2	G
H1-17	C	A	3	D
H1-26	A	C	3	E
rRNA	C	A	3	D
S1	A	B	2	I
S18	C	D	–	J
mip	B	A	–	A

[a] Band 3 of the *Not*I-*Sgr*AI double digest was composed of two comigrating fragments as indicated by the hybridization patterns of *H1-17* (17 kDa) and *H1-26* (26 kDa).
[b] –, not determined.

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REFERENCES

1. **Allardet-Servent, A., M.-J. Carles-Nurit, G. Bourg, S. Michaux, and M. Ramuz.** 1991. Physical map of the *Brucella melitensis* 16 M chromosome. J. Bacteriol. **173**:2219–2244.
2. **Allen, J. E., M. C. Cerrone, P. R. Beatty, and R. S. Stephens.** 1990. Cysteine-rich outer membrane proteins of *Chlamydia trachomatis* display compensatory sequence changes between biovariants. Mol. Microbiol. **4**:1543–1550.
3. **Allen, J. E., and R. S. Stephens.** 1989. Identification by sequence analysis of two-site posttranslational processing of the cysteine-rich outer membrane protein 2 of *Chlamydia trachomatis* serovar L2. J. Bacteriol. **171**:285–291.
4. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
5. **Birkelund, S., A. G. Lundemose, and G. Christiansen.** 1989. Characterization of native and recombinant 75-kilodalton immunogens from *Chlamydia trachomatis* serovar L2. Infect. Immun. **57**:2683–2690.
6. **Birkelund, S., A. G. Lundemose, and G. Christiansen.** 1990. The

75-kilodalton cytoplasmic *Chlamydia trachomatis* L2 polypeptide is a DnaK-like protein. Infect. Immun. **58:**2098–2104.
7. **Carlson, E. J., E. M. Peterson, and L. M. de la Maza.** 1989. Cloning and characterization of a *Chlamydia trachomatis* L3 DNA fragment that codes for an antigenic region of the major outer membrane protein and specifically hybridizes to the C- and C-related-complex serovars. Infect. Immun. **57:**487–494.
8. **Cerrone, M. C., J. J. Ma, and R. S. Stephens.** 1991. Cloning and sequence of the gene for heat shock protein 60 from *Chlamydia trachomatis* and immunological reactivity of the protein. Infect. Immun. **59:**79–90.
9. **Clarke, I. N., M. E. Ward, and P. R. Lambden.** 1990. Molecular cloning and sequence analysis of a developmentally regulated cysteine-rich outer membrane protein from *Chlamydia trachomatis*. Gene **71:**307–314.
10. **Danilition, S. L., I. W. Maclean, R. Peeling, S. Winston, and R. C. Brunham.** 1990. The 75-kilodalton protein of *Chlamydia trachomatis*: a member of the heat shock protein 70 family? Infect. Immun. **58:**189–196.
11. **Devereaux, J., P. Haeberil, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
12. **Engel, J. N., and D. Ganem.** 1987. Chlamydial rRNA operons: gene organization and identification of putative tandem promoters. J. Bacteriol. **169:**5678–5685.
13. **Engel, J. N., J. Pollack, E. Perara, and D. Ganem.** 1990. Heat shock response of murine *Chlamydia trachomatis*. J. Bacteriol. **172:**6959–6972.
14. **Frutos, R., M. Pages, M. Bellis, G. Roizes, and M. Bergoin.** 1989. Pulsed-field gel electrophoresis determination of the genome size of obligate intracellular bacteria belonging to the genera *Chlamydia*, *Rickettsiella*, and *Porochlamydia*. J. Bacteriol. **171:** 4511–4513.
15. **Hackstadt, T., W. Baehr, and Y. Ying.** 1991. *Chlamydia trachomatis* developmentally regulated protein is homologous to eukaryotic histone H1. Proc. Natl. Acad. Sci. USA **88:**3937–3941.
16. **Kaul, R., and W. M. Wenman.** 1985. Cloning and expression in *Escherichia coli* of species-specific *Chlamydia trachomatis* outer membrane antigen. FEMS Microbiol. Lett. **27:**7–12.
17. **Kingsbury, D. T.** 1969. Estimate of the genome size of various microorganisms. J. Bacteriol. **98:**1400–1401.
18. **Kingsbury, D. T., and E. Weiss.** 1968. Lack of deoxyribonucleic acid homology between species of the genus *Chlamydia*. J. Bacteriol. **96:**1421–1423.
19. **Koehler, J. E., R. B. Burgess, N. E. Thompson, and R. S. Stephens.** 1990. *Chlamydia trachomatis* RNA polymerase major sigma subunit. J. Biol. Chem. **265:**13206–13214.
20. **Koehler, J. E., and R. S. Stephens.** 1990. Characterization of *Chlamydia trachomatis* RNA polymerase beta′ subunit, p. 48–51. *In* W. R. Bowie, H. D. Caldwell, R. P. Jones, P.-A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), *Chlamydia* infections. Cambridge University Press, Cambridge.
21. **Lundemose, A. G., S. Birkelund, S. J. Fey, P. M. Larsen, and G. Christiansen.** 1991. *Chlamydia trachomatis* contains a protein similar to the *Legionella pneumophila mip* gene product. Mol. Microbiol. **5:**109–115.
22. **Lundemose, A. G., S. Birkelund, P. M. Larsen, S. J. Fey, and G. Christiansen.** 1990. Characterization and identification of early proteins in *Chlamydia trachomatis* serovar L2 by two-dimensional gel electrophoresis. Infect. Immun. **58:**2478–2486.
23. **Lundemose, A. G., S. Birkelund, H. Qvist, and G. Christiansen.** 1990. Characterization, cloning, and sequence of a 12.5 kDa membrane protein of *Chlamydia trachomatis* serovar L2, p. 129–132. *In* W. R. Bowie, H. D. Caldwell, R. P. Jones, P.-A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), *Chlamydia* infections. Cambridge University Press, Cambridge.
24. **McClelland, M., R. Jones, Y. Patel, and M. Nelson.** 1987. Restriction endonuclease for pulsed field mapping of bacterial genomes. Nucleic Acids Res. **15:**5985–6005.
25. **Morrison, R. P., H. Su, K. Lyng, and Y. Yuan.** 1990. The *Chlamydia trachomatis hyp* operon is homologous to the *groE* stress response operon of *Escherichia coli*. Infect. Immun. **58:**2701–2705.
26. **Moulder, J. W.** 1988. Characteristics of *Chlamydia*, p. 3–20. *In* A. L. Barron (ed.), Microbiology of *Chlamydia*. CRC Press, Inc., Baco Raton, Fla.
27. **Nano, F. E., and H. D. Caldwell.** 1985. Expression of the chlamydial genus-specific lipopolysaccharide epitope in *Escherichia coli*. Science **228:**742–744.
28. **Palmer, L., and S. Falkow.** 1986. A common plasmid of *Chlamydia trachomatis*. Plasmid **16:**52–62.
29. **Palmer, L., S. Falkow, and L. Klevan.** 1986. 16S ribosomal RNA genes of *Chlamydia trachomatis*, p. 89–92. *In* D. Oriel, G. Ridgway, J. Schachter, D. Taylor-Robinson, and M. Ward (ed.), *Chlamydia* infections. Cambridge University Press, Cambridge.
30. **Perara, E., and D. Ganem.** 1990. Late stage-specific gene from *Chlamydia* encodes protein homologous to eukaryotic histone H1, p. 44–47. *In* W. R. Bowie, H. D. Caldwell, R. P. Jones, P.-A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, M. E. Ward (ed.), *Chlamydia* infections. Cambridge University Press, Cambridge.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Sardinia, L. M., J. N. Engel, and D. Ganem.** 1989. Chlamydial gene encoding a 70 kilodalton antigen in *Escherichia coli*: analysis of expression signals and identification of the gene product. J. Bacteriol. **171:**335–341.
33. **Sardinia, L. M., E. Segal, and D. Ganem.** 1988. Developmental regulation of the cysteine-rich outer-membrane proteins of murine *Chlamydia trachomatis*. J. Gen. Microbiol. **134:**997–1004.
34. **Sarov, I., and Y. Becker.** 1969. Trachoma agent DNA. J. Mol. Biol. **42:**581–589.
35. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37:**67–75.
36. **Stephens, R. S., G. Mullenbach, R. Sanchez-Pescador, and N. Agabian.** 1986. Sequence analysis of the major outer membrane protein gene from *Chlamydia trachomatis* serovar L2. J. Bacteriol. **168:**1277–1282.
37. **Wagar, E. A., and R. S. Stephens.** 1988. Developmental-form-specific DNA-binding proteins in *Chlamydia* spp. Infect. Immun. **56:**1678–1684.