JOURNAL OF BACTERIOLOGY, June 1995, p. 3199–3204 Vol. 177, No. 11
0021-9193/95/$04.00+0

Genetic Map of the *Mycoplasma genitalium* Chromosome

SCOTT N. PETERSON,[1] THOMAS LUCIER,[2] KATHRYN HEITZMAN,[2] ELIZABETH A. SMITH,[3] KENNETH F. BOTT,[1,3] PING-CHUAN HU,[2,3]* AND CLYDE A. HUTCHISON III[1,3]

Curriculum in Genetics,[1] Department of Pediatrics,[2] and Department of Microbiology and Immunology,[3] University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Received 23 January 1995/Accepted 29 March 1995

At 600 kb, the genome of *Mycoplasma genitalium* is among the smallest known for cellular organisms capable of independent replication. As such, elucidation of the genetic makeup and chromosome architecture of this organism is of considerable interest. We have located 631 markers on the physical map of *M. genitalium*. The clones have been mapped by hybridizing 20 overlapping cosmid and lambda clones which encompass the entire *M. genitalium* chromosome to replica filters containing 856 genomic DNA clones. Three hundred fifty-six of these clones represent sequence tag sites, which were previously characterized by database searches. The remaining markers represent clones with an average size of 2.5 kb derived from *Sau*3A1 partial digestion of genomic DNA. The hybridization data can be divided into three classes: clones which hybridized to only one cosmid; clones which hybridized to two adjacent and overlapping cosmids; and clones which hybridized to several cosmids, which represent repetitive DNA. This rapid approach for placing clones on the physical map has allowed useful comparisons to be made with other bacterial chromosomes, especially that of the closely related organism *M. pneumoniae*, and has provided insight to the types of events which may have led to the reduction in size of this genome. Future use of these data is discussed.

Over 100 *Mycoplasma* species have been identified to date, most of which are pathogens of plants, animals, or humans (25, 30). They are typified by their small size, lack of a cell wall, and fastidious growth requirements, making their culture difficult. This group of microorganisms is believed to have evolved from a branch of gram-positive lactobacilli with considerable loss of genetic material (16, 36). The mechanism of genome reduction as well as its effect on chromosome architecture are not clear. Because most of the genetic systems established for other bacteria are not applicable to mycoplasmas, it is difficult to analyze their genome structure. For instance, expression of *Mycoplasma* genes in heterologous cells of organisms such as *Escherichia coli* is frequently problematic because of translation of the UGA stop codon as tryptophan (7, 37). Therefore, analysis of any particular aspect of physiology, pathogenicity, or evolution by conventional approaches in mycoplasmas is often not feasible.

Mycoplasma genitalium is a human pathogen which has been isolated from both the urogenital and respiratory tracts (2, 31). At 578 kb, its chromosome is the smallest of any self-replicating organism (14) and may be close to the hypothetical minimal genome size necessary for a cellular entity (17). By morphological, physiological, serological, and genetic criteria (3, 12, 16, 29, 31), it has been established that *M. genitalium* is more closely related phylogenetically to *M. pneumoniae* than to any other species. Yet at 800+ kb, the chromosome of *M. pneumoniae* is approximately 220 kb larger. How this divergence in genome size has influenced both the chromosome architecture and the phenotypic capabilities of these mycoplasmas will be of great interest from the standpoint of bacterial evolution. These two species could serve as a model system for studying the process of chromosome evolution in mycoplasmas if detailed genetic maps were available for both.

Recently, we conducted a survey of the *M. genitalium* genome by using random sequencing (21). This study was concurrent with the successful construction of a complete, overlapping, ordered library of this genome (14). We now report the use of information gained from these two studies to construct an extensive genetic map by using hybridization results rather than the more traditional method of performing genetic crosses, since the latter cannot be done with mycoplasmas. Furthermore, by obtaining markers which map to given regions of this chromosome, we now have the ability to characterize any given region in more detail.

MATERIALS AND METHODS

Libraries. *M. genitalium* G-37 (ATCC 33530) was grown in Hayflick's medium, and genomic DNA was prepared as described previously (21). Two micrograms of DNA was partially digested with the restriction enzyme *Sau*3A1, and digests were resolved on 1% low-melting-temperature agarose gels (SeaPlaque; FMC Bioproducts, Rockland, Maine). Fragments of between 2 and 4 kb were excised from the gel, ligated to pUC118, and transformed into competent *E. coli* DH5αF′ cells. Other libraries and partially sequenced clones (sequence tagged sites) were those described previously (21).

DNA and filter preparation. Plasmid DNA from all genomic clones was prepared as follows. Isolated colonies were inoculated into 600 μl of 2× YT (27) (containing 100 μg of ampicillin per ml) in 96-well microtiter plates (Biologics) and grown overnight. The plates were then spun at 2,500 rpm for 10 min in an IEC centrifuge, and the resulting pellets were resuspended in 150 μl of ice-cold TGE buffer (100 mM Tris-HCl [pH 9.0], 50 mM glucose, 10 mM EDTA). Three hundred microliters of lysis solution (1% sodium dodecyl sulfate [SDS], 0.2 M NaOH) was added to each suspension, mixed by inversion, and incubated on ice for 10 min. Samples were neutralized with 225 μl of 5 M potassium acetate (pH 4.8) and incubation for 20 min on ice. The plates were spun at 2,500 rpm for 10 min, and supernatants were transferred to fresh plates. DNA was then precipitated by the addition of 0.6 volume of 2-propanol and centrifugation for 10 min at 3,000 rpm. Pellets were resuspended in 500 μl of H_2O. DNA was denatured by the addition of 0.1 volume of 3 M NaOH and incubation at 65°C for 60 min. The samples were neutralized with 1 volume of 2 M ammonium acetate (pH 7.5) and then diluted in 1 M ammonium acetate for loading into a 96-well dot blotter (Schleicher & Schuell, Keene, N.H.). Samples were then drawn by vacuum onto Hybond N nylon membranes (Amersham, Arlington Heights, Ill.), and DNA was fixed to these filters by using a UV Stratalinker (Stratagene, La Jolla, Calif.).

DNA and probe preparation. Cosmid DNA was prepared by using Qiagen Plasmid Midi kits (Qiagen Inc., Chatsworth, Calif.), and lambda DNA was isolated by using Magic Lambda Prep kits (Promega Inc., Madison, Wis.). Cloned inserts were isolated from cosmids by *Not*I digestion followed by separation of the insert from the vector on 0.8% low-melting-temperature agarose gels (SeaPlaque). Gels were stained lightly in a solution of ethidium bromide (0.5 μg/ml), and bands of interest were excised. The agarose blocks contained DNA

* Corresponding author. Phone: (919) 966-2331. Fax: (919) 966-0135.

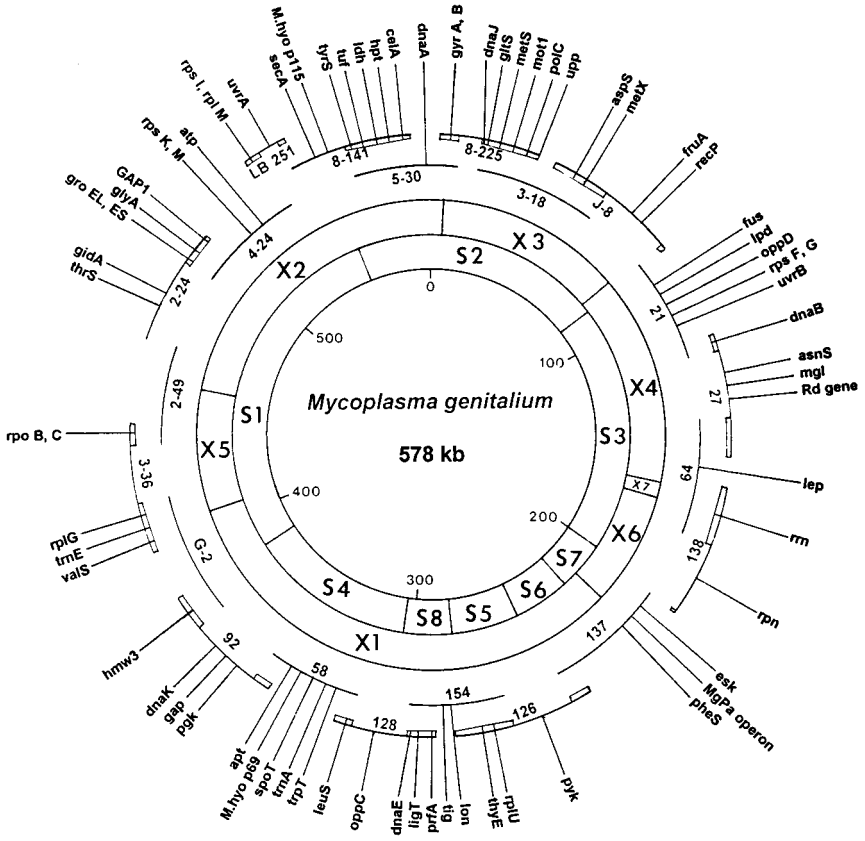

FIG. 1. Genetic map of *M. genitalium*. ×1 to ×7 are *Xho*I restriction fragments, and S1 to S8 are the *Sma*I fragments of the previously described physical maps (4). Fragment sizes are drawn to scale, assuming a 578-kb chromosome. Arcs around the outside of the physical maps are the clones comprising the ordered library (14). The genes were those identified on the randomly generated clones as previously described (21). Genes with indicator lines ending in boxes are in zones where neighboring clones from the ordered library overlap. Genes from random clones which hybridized with only one clone from the ordered library have indicator lines without boxes and are in zones where neighboring ordered clones do not overlap. If more than one gene is assigned to a zone, then they are listed alphabetically since their order within a zone is unknown.

at concentrations of at least 1 ng/μl and were equilibrated twice for 1 h in Tris-EDTA buffer.

Approximately 25 ng of DNA was used for labeling with [α-^{32}P]dCTP (800 Ci/mmol; New England Nuclear, Boston, Mass.) in a random priming reaction (Pharmacia Biosystems Inc., Piscataway, N.J.). Hybridizations were performed at 65°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1× Denhardt's solution (27)–0.1% SDS. Filters were washed once in 2× SSC–0.1% SDS at room temperature for 10 min and twice at 65°C for 10 min. This washing series was repeated with 1× SSC–0.1% SDS and finally 0.2× SSC–0.1% SDS. Probed filters were placed on Kodak X-Omat AR X-ray film at −70°C with two intensifying screens.

RESULTS

An ordered set of overlapping cosmids and a lambda clone representing the entire *M. genitalium* chromosome has recently been constructed (14). Each cloned insert from this ordered library was used individually to probe a bank of 856 clones randomly generated from *M. genitalium* genomic DNA (21). Three hundred eighty-six of these random clones were sequence tag sites for which an average of 300 nucleotides of sequence was determined from one end. The 20 overlapping cosmid clones and one lambda clone divide the chromosome of this organism into 42 zones alternating between regions where adjacent clones overlap and regions unique to a single clone (Fig. 1). The zones range in size from 0 to 30.3 kb (Table 1). We could assign 631 random clones to individual zones since they hybridized with either one or two overlapping clones from the ordered library of *M. genitalium*. Their frequency can be crudely estimated as 1.09 markers per kb, assuming a uniform distribution of markers (random clones) and a genome size of 578 kb. Table 1 shows the number of markers expected for each zone on the basis of this frequency and the size of each zone. While there was a reasonably close correspondence between observed and expected values in the majority of the zones, a few exhibited large deviations. Potential reasons for the apparent nonrandom distribution of the clones are considered in Discussion.

Figure 1 shows the distribution in the 42 zones of the ordered library of the 69 markers whose identities were previously determined by virtue of significant similarity to gene sequences in the GenEmbl database (21). The 80 genes identified on these markers are listed according to location on the chromosome in Table 2. A listing by their general functions along with details of their sequence comparisons and their accession numbers has been previously published (21). The exact location of each random clone within a zone is not known, and so the resolution of any marker is equal to the size of the zone in which it maps.

A partial *Eco*RI physical map of this genome was reported by Lucier et al. (14). Its completion could improve the resolution of our genetic map in most regions of the genome. To test its usefulness, three random clones with identified genes were labeled and hybridized to *Eco*RI digests of the ordered library. In each case, the position indicated in Table 1 and Fig. 1 was confirmed (data not shown). The *pgk* and *gap* genes hybridized

TABLE 1. Frequencies of ordered clones in map zones of the *M. genitalium* chromosome

Zone	Zone size (kb)	No. observed	No. expected	Frequency (no. observed/no. expected)
5-30	9.0	22	9.8	2.24
5-30/8-225	5.0	11	5.8	1.90
8-225	8.5	22	9.3	2.37
8-225/3-18	18.8	20	20.5	0.98
3-18	7.5	3	8.2	0.37
3-18/J-8	18.6	10	20.3	0.49
J-8	21.4	32	23.4	1.37
J-8/21	4.8	5	5.2	0.96
21	30.3	43	33.0	1.30
21/27	4.2	8	4.6	1.90
27	20.0	30	21.8	1.38
27/64	11.4	9	12.4	0.73
64	10.2	12	11.1	1.08
64/138	18.9	9	20.6	0.44
138	24.1	11	26.3	0.42
138/137	1.3	4	1.4	2.86
137	27.6	22	30.1	0.73
137/126	13.8	10	15.1	0.66
126	10.8	7	11.8	0.59
126/154	19.4	17	21.2	0.80
154	9.2	11	10.0	1.10
154/128	9.6	12	10.5	1.14
128	18.3	20	20.0	1.00
128/58	7.0	8	7.6	1.05
58	28.0	22	30.6	0.72
58/92	6.3	6	6.9	0.87
92	22.2	28	24.2	1.16
92/G-2	11.2	12	12.2	0.98
G-2	12.6	18	13.8	1.30
G-2/3-36	16.9	21	18.5	1.14
3-36	6.9	16	7.6	2.11
3-36/2-49	12.7	11	13.9	0.79
2-49	22.1	14	24.1	0.58
2-49/2-24	0[a]	3	0	
2.24	25.1	26	27.4	0.95
2-24/4-24	11.2	24	12.2	1.97
4-24	21.6	18	23.6	0.76
4-24/LB 251	3.4	9	3.7	2.43
LB 251	5.1	4	5.6	0.71
LB 251/8-141	3.1	5	3.4	1.47
8-141	16.6	15	18.1	0.83
8-141/5-30	21.3	22	23.3	0.94

[a] Cosmids 2-49 and 2-24 are adjacent but nonoverlapping. Clones mapped to this hypothetical zone extend across the *Eco*RI site shared by these cosmids.

to the 15.8-kb fragment in the 92 zone, and *rrn* hybridized to a 4.9-kb fragment in the 64/138 zone, which is consistent with a previous report of the *rrn* gene of *M. genitalium* being located on a 5-kb *Eco*RI fragment (39). The position of the *tuf* gene was determined by using a previously reported clone containing this gene (13) as a hybridization probe. It hybridized to a 13.0-kb fragment in the 8-141/5-30 zone.

Previous mapping data for the *M. genitalium* chromosome were limited in extent and of lower resolution, since the analysis was based on physical maps established by pulsed-field gel electrophoresis (4, 22). The locations reported for the *MgPa* operon and for the *rrn*, *tuf rpoC*, *uvrA*, *lep*, and *trpT* (UCA) genes in these earlier studies are consistent with their locations reported here.

Clones hybridizing to two overlapping cosmids can provide a check on the general accuracy of the remaining data as follows. The number of clones hybridizing to overlapping cosmids was 236, which translates to an average frequency (number observed/number expected) of 1.22. If the number of clones mapping to cosmids which do not overlap was artificially high because of improper data analysis, we might expect the frequency to be dramatically higher than 1.22. In actuality, it is 1.15.

Forty-two random clones hybridized with a group of cosmids (J-8, 21, 64, 138, 137, 126, 154, 128, 58, 3-36, and 2-49) which were previously shown to contain the *MgPa* operon or portions of the operon which exist as repeat sequences (14). The presence of *MgPa* repeat sequences in these clones is supported by the observation that the 12 clones in which sequencing data confirm the presence of *MgPa* repeats all show this hybridization pattern.

Another 42 random clones hybridized to more than one nonadjacent cosmid and therefore were not included in our analysis. While this could indicate the presence of previously unidentified repeat sequences in the genome, the inconsistent pattern of hybridizations within this group makes it unlikely. Inconsistent data of this type were recognizable and point out the potential for error in this method. Technical problems involving preparation of random clone DNA, preparation of filters, incomplete stripping of filters between hybridizations, or the hybridizations themselves are more likely. Technical problems of this kind are also likely to be responsible for the 141 random clones which failed to hybridize with any probe from the ordered library. These are considered in more detail in Discussion.

DISCUSSION

We have successfully located 78.5% of the randomly generated clones on the *M. genitalium* physical map. The failure of 16.5% (141) of the random clones to hybridize with any probes from the ordered library could reflect problems in the preparation of DNA from these clones or with their binding to membranes during the preparation of dot blots. Since 66 (47.5%) of the unassigned clones were located on two of the nine filters, we consider inconsistency of dot blot preparation to be the most likely cause. Careful analysis of the ordered library during its construction failed to find evidence for uncloned regions (14), and so lack of homologous probes for some unmapped clones due to gaps in the ordered library is unlikely. The 673 random clones which could be assigned to single zones or as *MgPa* repeats probably encompass nearly the entire genome, since this would be an average of one 2- to 4-kb clone for every 0.86 kb of the chromosome.

While the number of markers is quite large, for most of them we have no sequence information. Furthermore, no statement can be made concerning how individual markers physically relate to one another. Nevertheless, these markers do serve a useful purpose in that a chromosomal region of interest can be further characterized in a systematic manner at a higher resolution. Sequence data can readily be obtained from all clones mapping to any cosmid or zone. This fact combined with the size of the DNA insert provides information with regard to the distance between the two sequences. Specific oligonucleotides can then be synthesized for use in PCRs with cosmid DNA in which paired combinations of oligonucleotides from different clones are used to determine the orientation and distance of individual sequence tags. The oligonucleotides used for PCR can also be used to obtain more sequence information, which may facilitate further gene identifications.

The discrepancies between the number of clones mapping to some zones and the expected value determined from the size of that zone (Table 1) could be attributed to several factors. First, all regions of the chromosome may not be equally suitable for

TABLE 2. Mapped Genes of Mycoplasma genitalium

Gene	Zone	Function
dnaA	5-30	DNA replication initiation factor
gyrA	5-30/8-225	DNA gyrase
gyrB	5-30/8-225	DNA gyrase
dnaJ	8-225/3-18	DnaJ protein functions with DnaK protein
gltS	8-225/3-18	Glutamate tRNA synthetase
metS	8-225/3-18	Methionine tRNA synthetase
mot1	8-225/3-18	Helicase
polC	8-225/3-18	DNA polymerase III
upp	8-225/3-18	Uracil phosphoribosyltransferase (pyrimidine salvage)
aspS	3-18/J-8	Aspartate tRNA synthetase
metX	3-18/J-8	*S*-Adenosylmethionine synthetase
fruA	J-8	PTS enzyme II fructose permease
recP	J-8	Recombination
fus	21	Elongation factor G
lpd	21	Lipoamide dehydrogenase
oppD	21	Oligopeptide transport
rpsF	21	S6 ribosomal protein
rpsG	21	S7 ribosomal protein
uvrB	21	Excision repair
dnaB	21/27	Primosome protein-helicase
asnS	27	Asparagine tRNA synthetase
mgl	27	Galactose binding and transport operon
Rd gene	27	Rubredoxin
lep	64	Leader peptidase
rrn	64/138	rRNA genes
rpn[a]	138	Major ribosomal protein operon (*M. capricolum*)
rpsE, *rplO*		S5 and L12 ribosomal proteins
rplC		L3 ribosomal protein
esk	137	Protein kinase
MgPa operon	137	Operon encoding major adherence proteins of *M. genitalium*
pheS	137	Phenylalanine tRNA synthetase
pyk	126	Pyruvate kinase
rpIU	126/154	L21 ribosomal protein
thyE	126/154	Thymidylate synthetase
lon	154	Protease
tig	154	Trigger factor: chaperone
dnaE	154/128	DnaE primase
ligT	154/128	DNA ligase
prfA	154/128	Peptide chain release factor I
oppC	128	Oligopeptide transport
leuS	128/58	Leucine tRNA synthetase
apt	58	Adenine phosphoribosyltransferase (purine salvage)
M. hyorhinus p69	58	*M. hyorhinus* 69-kDa membrane protein: high-affinity transport
spoT	58	ppGpp phosphotransferase (stringent response)
trnA[b]	58	tRNA gene cluster
glnT, *tyrT*		Glutamine and tyrosine tRNAs
trpT	58	Tryptophan tRNA
dnaK	92	Chaperone
gap	92	Glyceraldehyde 3-phosphate dehydrogenase
pgk	92	Phosphoglycerate kinase
hmw3	92/G-2	Accessory adherence protein
rplG	G-2/3-36	L7 ribosomal protein
trnE[b]	G-2/3-36	tRNA gene cluster
leuT, *lysT*, *thrT*, *valT*		Leucine, lysine, threonine, and valine tRNAs
tuf	G-2/3-36	Elongation factor Tu
valS	G-2/3-36	Valine tRNA synthetase
rpoB	3-36/2-49	RNA polymerase subunit β
rpoC	3-36/2-49	RNA polymerase subunit β′
gidA	2-24	*gidA* DNA replication initiation
thrS	2-24	Threonine tRNA synthetase
GAP1	2-24/4-24	Amino acid permease
glyA	2-24/4-24	Glycine hydroxymethyltransferase
groEL	2-24/4-24	Chaperone
groES	2-24/4-24	Chaperone
atp	4-24	ATP synthetase
rpsK	4-24	S11 ribosomal protein
rpsM	4-24	S13 ribosomal protein
rpsI	4-24/LB 251	S9 ribosomal protein
rplM	4-24/LB 251	L13 ribosomal protein
uvrA	LB 251	Excision repair
M. hyorhinus p115	8-141	*M. hyorhinus* 115-kDa membrane protein
secA	8-141	Protein export (secretion)
celA	8-141/5-30	Uridine diphosphoglucose pyrophosphorylase
hpt	8-141/5-30	Hypoxanthine phosphotransferase (purine salvage)
ldh	8-141/5-30	Lactate dehydrogenase
tyrS	8-141/5-30	Tyrosine tRNA synthetase

[a] Two random clones with three identified genes homologous to those in the *rpn* operon of *M. capricolum* (20) were assigned to this operon.
[b] Clones containing tRNA genes in the same order as homologous genes identified in operons given these names in *M. pneumoniae* (28).

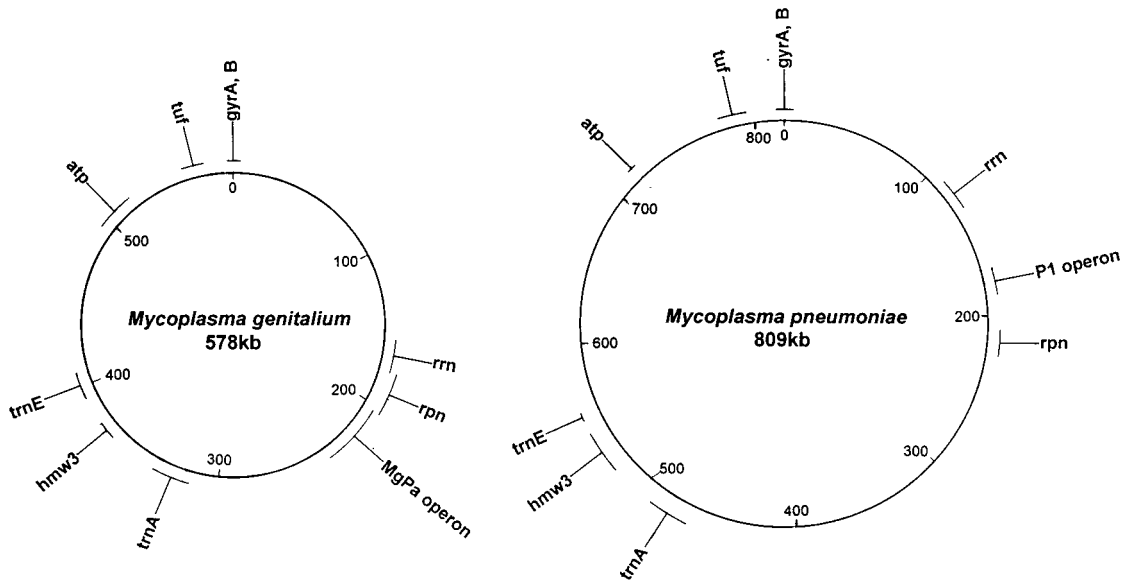

FIG. 2. Comparison of the locations of homologous genes which have been mapped on both the *M. genitalium* and *M. pneumoniae* chromosomes. The circles represent the chromosomes of these organisms, and they are drawn to scale, using the genome sizes determined by Lucier et al. (14) for *M. genitalium* and Wenzel et al. (35) for *M. pneumoniae*. The *gyrA* and *gyrB* genes are used as the starting point (0 kb) since they should be close to the origin of replication, and the numbers around the inside of each circle are the distance (kilobases) to that location on the chromosome from the starting point. The bar at the end of the indicator line for each gene shows the resolution with which its location can be mapped. The *M. genitalium* genes are mapped in their zones from the ordered library. The *M. pneumoniae* genes are mapped on an *Eco*RI physical map (35). Locations of the tRNA clusters (*trnA* and *trnE*) are from Simoneau et al. (28), and the *hmw3* gene was located as described by Krause and Lee (9) on the ordered genomic library for this species (34). All other genes were placed by Wenzel and Herrmann (34). Where the *rpn* genes are marked, both species have ribosomal protein genes mapped which are homologous to proteins in the large ribosomal protein operon of *M. capricolum* (*rpn*) [20]).

cloning in multicopy plasmids because of toxicity to host cells, instability of the DNA insert, or differences in the number of copies of some regions of the chromosome which are present in the cell. Commonly, eubacterial cells contain additional copies of the region of the chromosome close to the origin of replication (10). Thus, our presumption that the *M. genitalium* origin of replication is located near the zero point on our map (Fig. 1) is supported by the high frequency of clones from the 5-30, 5-30/8-225, and 8-225 zones and by the location of the *dnaA* and gyrase genes which are commonly found near the origin in eubacterial chromosomes (5, 18). Second, since clones containing *MgPa* repeat sequences could not be assigned to single locations, they were not included in the analysis shown in Table 1. If repeated sequences account for a significant proportion of the DNA in a zone, the number of clones hybridizing exclusively to this region could be well below expected. Nine of the ten zones believed to contain repetitive DNA have lower than expected frequencies, and one of these, 64/138, is the farthest below the expected for any of the large zones. Third, a very small zone would be likely to show considerable divergence from the expected frequency by chance alone. This would account for the large discrepancies in zones 3-18, 138/137, 4-24/LB 251, and LB 251/8-141. Finally, because most random clones are 2 to 4 kb in length, which is a significant percentage of the length of many zones, overlap regions may be overrepresented, particularly if they are adjacent to small, nonoverlapping zones.

The 5% (42) of the random clones which were not *MgPa* repeats yet hybridized to more than one nonoverlapping library clones are difficult to explain. Some may reflect the presence of previously undetected repetitive sequences; however, if this were true, we might expect the same conflicting hybridization pattern to occur in several clones as is true for *MgPa* repeats, and this was not observed. It is also possible that certain random clones may be more subject to nonspecific hybridization despite the stringent conditions used, i.e., tRNA clusters. We considered it safer under the circumstances not to include these in our map.

This genetic map of *M. genitalium* provides insight into changes in chromosome architecture that occurred during evolution of the mycoplasmas. rRNA sequencing suggests that mycoplasmas evolved from a branch of the lactobacillus group of gram-positive eubacteria, which contains members of the clostridia (15, 26, 36). Within the lactobacilli, extensive mapping data exist for only *Bacillus subtilis* (23). *Mycoplasma* genomes have undergone considerable reduction in size since divergence of this lineage from their gram-positive ancestors. The *M. genitalium* genome is only 12 to 14% the size of the *B. subtilis* genome (8, 32). As we might expect with such a great reduction in genome size, few similarities in gene arrangement are evident. One exception is the origin region (1), with the close association of *dnaA* and *gyrA* and *gyrB* and the higher occurrence of clones from this region, suggesting a close association of the origin of replication and the *dnaA* gene as is seen in *B. subtilis* (19).

Comparison of the *M. genitalium* chromosome structure with that of other mycoplasmas is hampered by the lack of adequate genetic maps for other species. Limited genetic maps of *Spiroplasma citrii* (38) and several strains of *M. mycoides* subsp. *mycoides* (24) have recently been published. These species are in a different mollicute lineage than *M. genitalium* (33) and have much larger genomes (1,780 and 1,200 to 1,280 kb, respectively), and the number of genes on their maps with homologs mapped on the *M. genitalium* chromosome is small. Yet in all three species, the *rpoC*, *glyA*, and *atp* genes are relatively close together, and in both *S. citrii* and *M. genitalium* they are not very far from gyrase genes (which were not mapped in *M. mycoides*).

Fortunately, significant genetic map data are available for *M. pneumoniae* (9, 11, 35). Figure 2 shows homologous genes with known locations in both genomes and suggests remarkable conservation of overall chromosome architecture even though they differ considerably in size. While resolution of these maps is somewhat limited, no major changes in gene order are evident, although the distances separating certain genes show substantial differences. The only apparent change in gene order is the reversal of the major attachment protein operon (*MgPa* and the corresponding P1) and the nearby ribosomal protein operon homologous to *rpn* of *M. capricolum* (20). This may reflect an inversion involving this region of the chromosome during the evolution of one of these species. Considerable similarity exists in the distances of the corresponding genes to the origin and between one gene and another (Fig. 2). Much of the difference in the sizes of these two genomes can be accounted for in the region between *trnA* and the *rpn* attachment protein operon region. This implies a gain or loss of DNA in a particular region of the chromosome as being primarily responsible for their difference in size. Experiments are currently ongoing to determine if this or other regions of the *M. pneumoniae* chromosome contain large regions of DNA lacking homology to anything in the *M. genitalium* genome.

ACKNOWLEDGMENTS

The first two authors contributed equally to this report.

This work was supported in part by NIH grants HL19171, AI33161, AI08998, and GM21313 and U.S. EPA Cooperative Agreement CR807392. T.L. and S.N.P. were supported by NIH training grants T32AI07151 and T32GM07092, respectively.

We acknowledge Shih-chin Hu for preparation of photographic material.

REFERENCES

1. **Bailey, C. C., and K. F. Bott.** An unusual gene containing a *dnaJ* N-terminal box flanks the putative origin of replication of *Mycoplasma genitalium*. 1994. J. Bacteriol. **176:**5814–5819.
2. **Baseman, J. B., S. F. Dallo, J. G. Tully, and D. L. Rose.** 1988. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. J. Clin. Microbiol. **26:**2266–2269.
3. **Clyde, W. A., Jr., and P.-C. Hu.** 1986. Antigenic determinants of the attachment protein of *Mycoplasma pneumoniae* shared by other pathogenic *Mycoplasma* species. Infect. Immun. **51:**690–692.
4. **Colman, S. D., P.-C. Hu, W. Litaker, and K. F. Bott.** 1990. A physical map of the *Mycoplasma genitalium* genome. Mol. Microbiol. **4:**683–687.
5. **Fujita, M. Q., H. Yoshikawa, and N. Ogasawara.** 1989. Structure of the *dnaA* region of *Pseudomonas putida*: conservation among three bacteria, *Bacillus subtilis*, *Escherichia coli* and *P. putida*. Mol. Gen. Genet. **215:**381–387.
6. **Hermann, R.** 1992. Genome structure and organization, p. 157–168. *In* J. Maniloff (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
7. **Inamine, J. M., K.-C. Ho, S. Loechel, and P.-C. Hu.** 1990. Evidence that UGA is read as a tryptophan codon rather than as a stop codon by *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, and *Mycoplasma gallisepticum*. J. Bacteriol. **172:**504–506.
8. **Itaya, M., and T. Tanaka.** 1991. Complete physical map of the *Bacillus subtilis* 168 chromosome constructed by a gene directed mutagenesis method. J. Mol. Biol. **220:**631–648.
9. **Krause, D. C., and K. K. Lee.** 1991. Juxtaposition of the genes encoding *Mycoplasma pneumoniae* cytadherence-accessory proteins HMW1 and HMW3. Gene **107:**83–89.
10. **Krause, D. C., and C. B. Nawn.** 1990. Physical analysis and mapping of the *Mycoplasma pneumoniae* chromosome. J. Bacteriol. **172:**4790–4797.
11. **Krawiec, S., and M. Riley.** 1990. Organization of the bacterial chromosome. Microbiol. Rev. **54:**502–539.
12. **Lind, K., B. O. Lindhardt, H. J. Schutten, J. Blum, and C. Christiansen.** 1984. Serological cross reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. J. Clin. Microbiol. **20:**1036–1043.
13. **Loechel, S., J. M. Inamine, and P.-C. Hu.** 1989. Nucleotide sequence of the *tuf* gene from *Mycoplasma genitalium*. Nucleic Acids Res. **17:**10127.
14. **Lucier, T. S., P.-Q. Hu, S. N. Peterson, X.-Y. Song, L. Miller, K. Heitzman, K. F. Bott, C. A. Hutchison III, and P.-C. Hu.** 1994. Construction of an ordered genomic library of *Mycoplasma genitalium*. Gene **150:**27–34.
15. **Maniloff, J.** 1983. Evolution of wall-less prokaryotes. Annu. Rev. Microbiol. **37:**477–499.
16. **Maniloff, J.** 1992. Phylogeny of mycoplasmas, p. 549–559. *In* J. Maniloff (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
17. **Morowitz, H. J.** 1984. The completeness of molecular biology. Is. J. Med. Sci. **20:**750–753.
18. **Ogasawara, N., M. Q. Fujita, S. Moriya, T. Fukuoka, M. Hirano, and H. Yosididawa.** 1990. Comparative anatomy of *oriC* of eubacteria, p. 287–295. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
19. **Ogasawara, N., S. Moriya, and H. Yoshikawa.** 1985. Structure and function of the region of the replication origin of the *Bacillus subtilis* chromosome. IV. Transcription of the *ori*C region. Nucleic Acids Res. **13:**2267–2279.
20. **Ohkuba, S., A. Muto, Y. Kawauchi, F. Yamao, and S. Osawa.** 1987. The ribosomal protein gene cluster of *Mycoplasma capricolum*. Mol. Gen. Genet. **210:**314–322.
21. **Peterson, S. N., P.-C. Hu, K. F. Bott, and C. A. Hutchison III.** 1993. A survey of the *Mycoplasma genitalium* genome by using random sequencing. J. Bacteriol. **175:**7918–7930.
22. **Peterson, S. N., N. Schramm, P.-C. Hu, K. F. Bott, and C. A. Hutchison III.** 1991. A random sequencing approach for placing markers on the physical map of *Mycoplasma genitalium*. Nucleic Acids Res. **19:**6027–6031.
23. **Piggot, P. J.** 1990. Genetic map of *Bacillus subtilis* 168, p 107–145. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
24. **Pyle, L. E., T. Taylor, and L. R. Finch.** 1990. Genomic maps of some strains within the *Mycoplasma mycoides* cluster. J. Bacteriol. **172:**7265–7268.
25. **Razin, S.** 1992. Mycoplasma taxonomy and ecology, p. 3–22. *In* J. Maniloff (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
26. **Rogers, M. J., J. Simmons, R. T. Walker, W. G. Weisburg, C. R. Woese, R. S. Tanner, I. M. Robinson, D. A. Stahl, G. Olsen, R. H. Leach, and J. Maniloff.** 1985. Construction of the mycoplasma evolutionary tree from 5S rRNA sequence data. Proc. Natl. Acad. Sci. USA **82:**1160–1164.
27. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Simoneau, P., C.-M. Li, S. Loechel, R. Wenzel, R. Herrmann, and P.-C. Hu.** 1993. Codon reading scheme in *Mycoplasma pneumoniae* revealed by the analysis of the complete set of tRNA genes. Nucleic Acids Res. **21:**4967–4974.
29. **Taylor-Robinson, D., P. M. Furr, and J. G. Tully.** 1983. Serological cross reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. Lancet **ii:**527.
30. **Tully, J. G.** 1989. Class Mollicutes: new perspectives from plant and arthropod studies, p. 1–31. *In* R. F. Whitcomb and J. G. Tully (ed.), The mycoplasmas, vol. V. Academic Press, New York.
31. **Tully, J. G., D. Taylor-Robinson, D. L. Rose, R. M. Cole, and J. M. Bove.** 1983. *Mycoplasma genitalium*, a new species from the human urogenital tract. Int. J. Syst. Bacteriol. **33:**387–396.
32. **Ventra, L., and A. S. Weiss.** 1989. Transposon-mediated restriction mapping of the *Bacillus subtilis* chromosome. Gene **78:**29–36.
33. **Weisburg, W. G., J. G. Tully, D. L. Rose, J. P. Petzel, H. Oyaizu, D. Yang, L. Mandelco, J. Sechrest, T. G. Lawrence, J. van Etten, J. Maniloff, and C. R. Woese.** 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. J. Bacteriol. **171:**6455–6467.
34. **Wenzel, R., and R. Hermann.** 1989. Cloning of the complete *Mycoplasma pneumoniae* genome. Nucleic Acids Res. **17:**7029–7043.
35. **Wenzel, R., E. Pirkl, and R. Herrmann.** 1992. Construction of an *Eco*RI restriction map of *Mycoplasma pneumoniae* and localization of selected genes. J. Bacteriol. **174:**7289–7296.
36. **Woese, C. R., J. Maniloff, and L. B. Zablen.** 1980. Phylogenetic analysis of the mycoplasmas. Proc. Natl. Acad. Sci. USA **77:**494–498.
37. **Yamao, F., A. Muto, Y. Kawauchi, M. Iwami, S. Iwagami, Y. Azumi, and S. Osawa.** 1985. UGA is read as tryptophan in *Mycoplasma capricolum*. Proc. Natl. Acad. Sci. USA **82:**2306–2309.
38. **Ye, F., F. Laigret, J. C. Whitley, C. Citti, L. R. Finch, P. Carle, J. Renaudin, and J.-M. Bove.** 1992. A physical and genetic map of the *Spiroplasma citri* genome. Nucleic Acids Res. **20:**1559–1565.
39. **Yogev, D., and S. Razin.** 1986. Common deoxyribonucleic acid sequences in *Mycoplasma genitalium* and *Mycoplasma pneumoniae* genomes. Int. J. Syst. Bacteriol. **36:**426–430.