Gene Localization, Size, and Physical Map of the Chromosome of *Streptococcus pneumoniae*

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A physical map of the *Streptococcus (Diplococcus) pneumoniae* chromosome, which is circular and 2,270 kbp in circumference, has been constructed. The restriction enzymes *Apa*I, *Sma*I, and *Sac*II were used to digest intact chromosomes, and the fragments were resolved by field inversion gel electrophoresis (FIGE). The digests produced 22, 20, and 29 fragments, respectively. The order of the fragments was deduced from Southern blot hybridization of isolated labeled fragments to separated fragments of the various restriction digests. Genetic markers were correlated with the physical map by transformation of recipient cells with FIGE-isolated DNA fragments derived from genetically marked *S. pneumoniae* strains. In addition, markers were mapped by the hybridization of cloned genes to FIGE-separated restriction fragments. Six rRNA gene (*rrn*) clusters were mapped by hybridization to *rrn*-containing fragments of *Haemophilus influenzae*.

Transformation of *Streptococcus (Diplococcus) pneumoniae* was the first method found to transfer genetic information in bacteria (1, 14), and it has had a monumental impact on the development of molecular biology. Although the pneumococcal transformation system has been extensively investigated for the past 45 years (6, 17, 40, 41), understanding of its genetics and biochemistry, compared with that of *Escherichia coli* (2, 44) and/or *Bacillus subtilis* (16), is still relatively primitive. The absence of a genetic map has been a major impediment to the investigation of the molecular genetics of *S. pneumoniae*.

The ability to separate large fragments of DNA by pulsed-field gel electrophoresis has provided the technology to map the chromosomes of bacteria (10, 19, 20, 25-27, 43, 44), and this technology has gained wide acceptance for analyzing the chromosomes of more complex organisms as well. The map of the *S. pneumoniae* chromosome should provide a useful framework for further molecular and genetic investigations.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All bacteria used in this study are derived from pneumococcus strain R6. A subculture of *S. pneumoniae* 800 or 801 was used in this study (28), along with a multiply marked strain (strain 119) resistant to 200 µg of streptomycin (*str-41*) per ml, 75 µg of fusidic acid (*fus-rA*) per ml, 4 µg of novobiocin (*nov-r1*) per ml, 2 µg of optochin (*opt-r2*) per ml, 1 µg of rifampin (*rif-rF*) per ml, and 5 µg of streptolydigin (*stg-rF*) per ml (47). The *uvr* resistance gene (48) was located with the use of the cloned gene (42). To map the *amiA* gene, strain 801 bearing *amiA3* was used (12). The methotrexate-sensitive bacteria in the resistant population were selected in synthetic medium containing an excess of isoleucine (40). The strain resistant to 0.1 µg of cefotaxime per ml was obtained by transforma-

tion of strain 801 with DNA extracted from strain C506, which is resistant to a higher level of cefotaxime (1 µg/ml) (23). A second level of resistance (0.4 µg/ml) was obtained by transformation of a strain resistant to 0.1 µg of cefotaxime per ml with the same C506 DNA (23). The DNAs of the two new strains, designated Cef0.1 and Cef0.4, were used to localize the mutations.

The culture media and transformation procedures have been described elsewhere (7). For several experiments, pneumococci were grown in brain heart infusion broth (Difco Laboratories Inc.) supplemented with 40 µg of thymidine per ml and 150 µg of glutamine per ml. Cultures were incubated at 37°C without aeration and were maintained during growth at pH 7.8 with the addition of 1 N NaOH.

DNA preparation and digestion with restriction enzymes. *S. pneumoniae* DNA embedded in agarose beads was prepared by the method described previously for *Haemophilus influenzae* (20), with the exception that lysozyme was omitted in the lysis buffer. A number of *S. pneumoniae* genes have been cloned in plasmids and used for genome mapping (for the clones used, see Table 3). Restriction enzymes were obtained from New England Biolabs, Pharmacia (Uppsala, Sweden), and Bethesda Research Laboratories. The reaction conditions were those recommended by Bethesda Research Laboratories.

Electrophoresis and hybridization. Southern blot hybridization and nick translation were performed essentially as described by Maniatis et al. (30). The field inversion gel electrophoresis (FIGE) has been described in detail elsewhere (20). Two electrophoresis programs were used to separate fragments. Program A (0.3- to 60-s forward range and 0.1- to 20-s reverse range in 200 steps) exhibits maximal separation of the largest fragments, of more than 150 kbp, whereas program B (0.3- to 30-s forward range and 0.1- to 10-s reverse range in 45 steps) yields the best separation of fragments of less than 150 kbp.

Gene mapping. In order to correlate genetic markers with the physical map, two procedures were utilized: Southern blot hybridization of cloned genes and transformation of fragments separated by FIGE. The cloned genes were la-

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beled with ^{32}P by nick translation and were hybridized to fragments of restriction digests transferred to nylon membranes. To test for transforming activity, the restriction digests were separated by FIGE in 1% low-melting-point agarose (FMC Corp.). Following electrophoresis, a portion of the gel was stained with ethidium bromide and DNA bands were detected with the UV transilluminator. The gel was sliced, individual bands were melted at 68°C for 10 min and equilibrated at 37°C with 5 mM EDTA–100 mM NaCl (pH 8.0), and agarase (Calbiochem) was added to 40 U/ml (3). After overnight digestion, the DNAs were ethanol precipitated and dissolved in 35 μl of 30 mM sodium citrate (pH 7.0). An aliquot of 5 μl from each sample was used to transform competent pneumococcal cultures, which were selected for donor markers. In some experiments, the individual bands were extracted by electroelution. For equivalent amounts of DNA in the gels, electroeluted DNA gave lower levels of biological activity than agarase-digested DNA did.

RESULTS

The basic strategy for chromosomal mapping consists of immobilizing cells in a solid matrix, such as in low-melting-temperature agarose, and subsequently removing cellular components by treatment with agents that dissolve the outer surface and proteins. The intact chromosome(s) embedded in agarose can then be directly cut with restriction enzymes, producing fragments separable in an agarose gel. The restriction enzymes are chosen on the basis of the number of fragments produced per chromosome.

Restriction enzyme digests and size of the chromosome of *S. pneumoniae*. The DNA of *S. pneumoniae* has a G+C content of 39% (4) and would be expected to be cut less frequently by the restriction enzymes with recognition sites consisting of only G and C. A number of restriction enzymes were tested for their abilities to produce a reasonable number of fragments after FIGE: *NotI*, *SfiI*, *RsrII*, *EagI*, *NaeI*, *ApaI*, *SmaI*, *BglI*, *NarI*, *SacII*, and *BssHII*, as well as *MluI*, *KpnI*, *SalI*, *SacI*, *PstI*, and *PvuII*. The restriction enzymes *NotI* (GCGGCCGC), *SfiI* (GGCCNNNNNGGCC), and *RsrII* [CGG(A/T)CCG] did not cut the *S. pneumoniae* chromosome, and *EagI*, *NaeI*, *BglI*, *NarI*, *BssHII*, *MluI*, *KpnI*, *SalI*, *SacI*, *PstI*, and *PvuII* yielded too many fragments to obtain an accurate estimate of their number and size directly from gels stained with ethidium bromide. *ApaI* (GGGCCC), *SmaI* (CCCGGG), and *SacII* (CCGCGG) each gave less than 30 fragments that could be conveniently separated by FIGE, and they were chosen for further study.

Analysis of these digestions is shown in Fig. 1. The largest fragments, of more than 150 kbp, were separated by program A, and those of less than 150 kbp were separated by program B (see Materials and Methods). The smallest fragments were separated in 1% agarose gels in the presence of 0.1 μg of ethidium bromide per ml, either by standard electrophoresis or by FIGE. A summary diagram of all gel patterns is shown in Fig. 2. For size determinations, lambda multimeric DNA (λ ladder) and lambda DNA digested with *HindIII* enzyme were used as molecular weight markers. DNA fragment sizes determined from agarose gels are listed in Table 1. The estimated genome sizes ranged from 2,240 to 2,272 kbp.

Construction of a physical map. Restriction enzymes *SmaI*, *ApaI*, and *SacII* were found to produce distributions of DNA fragment sizes useful for construction of the *S. pneumoniae* genome map. The approach used was to separate individual restriction enzyme fragments, label them with

FIG. 1. *SmaI*, *ApaI*, and *SacII* restriction enzyme digests of the chromosome of *S. pneumoniae*. Lanes A through E, program B; lanes F through I, program A (see Materials and Methods). Lane A, lambda DNA digested with *HindIII*; lane B, lambda DNA ladder; lanes C and F, *SmaI* digests; lanes D and H, *ApaI* digests; lanes E and I, *SacII* digests; lane G, *SmaI*-*ApaI* double digests.

^{32}P -labeled deoxyribonucleoside triphosphate, and hybridize them to separated, restriction enzyme-digested chromosomal DNA fragments. A single DNA fragment should produce a single band of hybridization to DNA digested with the same enzyme, but it may produce one or several bands hybridizing with the DNA fragments from other restriction enzyme digests. Hybridization with two or more fragments of another restriction enzyme digest was evidence for an overlap and was used to construct the map. By this procedure, almost all *SmaI* and *ApaI* fragments could be located unambiguously on the *S. pneumoniae* map (see Fig. 3). However, *ApaI* fragments 20 and 22 hybridized to the same *SmaI* fragment (fragment 4), and their positions relative to each other are assigned arbitrarily. In a like manner, *ApaI* fragments 16, 17, 18, 19, and 21 and *SmaI* fragments 16 and 19 could not be precisely ordered within the physical map.

The order of some *ApaI* and *SmaI* fragments was deduced by hybridization with fragments obtained by digestion of *S. pneumoniae* DNA with *SacII*, *EagI*, and *NaeI*. Although a large number of fragments were produced with *EagI* and *NaeI* and most of them could not be separated in an agarose gel, some individual fragments could be used as linkers for construction of the map. Fragments produced with *EagI* and *NaeI* were particularly useful for the assignment of *SmaI* fragments 4 and 5, since the junction between these fragments maps in the same position as the junction between corresponding *ApaI* fragments 5 and 4. *SmaI* fragment order 5-14-7 and *ApaI* fragment order 4-14-3 have been confirmed by hybridization with one *Sse8387I* (CCTGAGG) fragment. Some *SacII* fragments were used for map construction, but only a partial *SacII* map was obtained (Fig. 3).

rRNA repeats in the *S. pneumoniae* genome. The DNAs of

FIG. 2. Logarithmic gel band plot of *S. pneumoniae* 119 DNA restriction fragments, based on estimated sizes.

H. influenzae and *S. pneumoniae* exhibit low levels of cross-hybridization. In the process of testing for possible specific homologies between these organisms, it was found that cross-hybridization was confined in large measure to the rRNA genes (*rrn*) that are repeated six times in the *S. pneumoniae* genome and six times in the *H. influenzae* genome (27). Hybridization of ^{32}P -labeled *H. influenzae* DNA to a *SmaI* digest of *S. pneumoniae* DNA resulted in the detection of six different labeled bands. *S. pneumoniae* total DNA labeled and hybridized to *H. influenzae* DNA digested with the *SmaI* or *ApaI* enzyme revealed six bands that correspond to the fragments containing *rrn* genes (27). This limited DNA homology was also confirmed by hybridization of individual *SmaI* or *ApaI* fragments of *S. pneumoniae* DNA to *SmaI*- and *ApaI*-digested *H. influenzae* DNA. Electrophoresis (not FIGE) of a *SmaI* digest of *S. pneumoniae* DNA revealed the presence of a 900-bp multicopy fragment that hybridized to itself, four *ApaI* fragments, and four *SacII* fragments. From these data, we concluded that there are at least six *rrn* clusters in the *S. pneumoniae* chromosome and that they exhibit strong similarities to the *rrn* genes of *H. influenzae*. However, the restriction map of *S. pneumoniae* and the hybridization data indicate that the genetic loci are not completely homologous, since there are no small *ApaI* *rrn* fragments similar to the 1.6-kbp *ApaI* fragment of *H. influenzae* (27). Indeed, there appear to be no *ApaI* sites in at least some of the *rrn* gene clusters of *S. pneumoniae*.

Assignment of genetic markers. The ability to transform chromosomal markers is a unique property of naturally competent bacteria such as *S. pneumoniae*. We have used this property to map genetic markers to specific restriction fragments. DNA from multiply marked strain 119 was digested by the three restriction enzymes *ApaI*, *SacII*, and *SmaI*, separated by FIGE, and used for mapping. Whenever

TABLE 1. *S. pneumoniae* R6 DNA fragment sizes as determined by FIGE

Fragment	Fragment size (kbp) after digestion with:		
	<i>SmaI</i>	<i>ApaI</i>	<i>SacII</i>
1	380	330	310
2	340	330	290
3	290	255	185
4	260	235	177
5	235	205	160
6	150	127	118
7	110	118	110
8	104	98	94
9	95	94	72
10	90	88	65
11	60	65	63
12	52	60	60
13	26	59	54
14	25	56	52
15	19	51	52
16	19	26	52
17	7.6	25	50
18	5.8	19	45
19	3.2	15	44
20	0.9	8.5	40
21		2.6	26
22		2.3	26
23			25
24			21
25			21
26			14
27			8
28			6.1
29			0.87
Total	2,271.6	2,272.0	2,240.1

possible, the DNA from each individual band was extracted and used to transform competent recipient cells. The results are summarized in Table 2. Biological activity was often found in a single band only when this band could be isolated without contamination from adjacent or similar-size fragments. Cloned genes obtained from various sources were labeled and hybridized to separated *SmaI*, *ApaI*, and *SacII* restriction digest fragments. These genes and the hybridization results are listed in Table 3.

DISCUSSION

The intact *S. pneumoniae* DNA does not migrate into an agarose gel, and it does not contain restriction sites for *NotI* and *SfiI* enzymes. A physical map of the chromosome of *S. pneumoniae* that is circular and 2,270 kbp in length, with an error of less than 5%, has been generated. The ability of a restriction enzyme fragment to hybridize to two or more fragments of a digest with another restriction enzyme was evidence for an overlap and provided the basis for the map construction. This technique helped us to deduce the complete *ApaI* and *SmaI* restriction maps, since all DNA fragments generated by these enzymes could be readily separated. The large number of *SacII* fragments with closely similar sizes proved too difficult to isolate; therefore, only a partial *SacII* map is presented. Nonetheless, some of the *SacII* DNA fragments, as well as some of the *EagI* and *NaeI* fragments, were particularly useful in resolving *SmaI* and *ApaI* DNA fragment orders. However, it was still necessary to assign a few *ApaI* and *SmaI* DNA fragments to the map

FIG. 3. Physical map of *S. pneumoniae* R6 genome, made by using *ApaI*, *SmaI*, and *SacII* restriction enzymes. Some restriction sites for *SacII* are unknown. Small *SmaI* fragments (14 to 19) are indicated close to the largest circle. Small *ApaI* fragments (16 to 22) are drawn outside the largest circle.

arbitrarily (e.g., *SmaI* fragments 16 and 19 and fragments 12 and 15 and *ApaI* fragments 16, 17, 18, 19 and 21, and 20 and 22). One of these arbitrary assignments, *SmaI* fragments 16 and 19, may be resolved, because *SmaI* separated genetically linked antibiotic resistance markers in fragments 16 and 13 (see below).

The results presented above strongly suggest the presence of at least six rRNA operons on the *S. pneumoniae* genome. From the results of the hybridization between labeled *S. pneumoniae* and *H. influenzae* DNAs and individually labeled *SmaI* fragments of *S. pneumoniae* DNA, it appears that the genomes of these two bacteria have retained the highly conserved *rrn* gene regions that share a great deal of DNA homology. However, these regions presumably have

TABLE 3. *S. pneumoniae* genes and plasmids^a

Gene cloned	Plasmid	Restriction fragment(s)			Reference
		<i>SmaI</i>	<i>ApaI</i>	<i>SacII</i>	
<i>hexA</i>	pSP11	5	4	13–17	31
<i>hexB</i>	pSP41	11	3	3, 4	35
<i>uvs-402</i>	pSP2-800	2	1	1	42
<i>comA</i>	pXF211	7, 8	14, 15	3	5
<i>malM</i>	pLS70	5	4	12	45
<i>recP</i>	pXF402	12	11	13–17	38
<i>pbp-2x</i>	pCG11	3	12, 13	6, 7	24
<i>pbp-1a</i>	R7	3	13	13–17	15
<i>pbp-2b</i>	pCGD4	7–9	9	2	9
<i>polA</i>	pSM23	13, 14	14	24–26	32
<i>sul-d</i>	pLS80	3	3	6	29
<i>pbp-3</i>	pCC1	1	2	4, 5	39
<i>lytA</i>	pGL80	8–10	8, 9	9	11
<i>ung</i>	pSP61	2	1	1	34
<i>pspA</i>	pPSPA	7, 8	3	3, 4	33
ΩBM4200	pVJ413	2	10	18–20	49
ΩBM6001	pVJ187	2	1	1, 2	50

^a Labeled plasmid DNA was hybridized to FIGE-separated fragments of *S. pneumoniae* DNA previously digested with *SmaI*, *ApaI*, or *SacII*.

diverged, as indicated by (i) the presence of *ApaI* sites in the *rrn* regions of the *H. influenzae* Rd genome and the absence of *ApaI* sites in the *rrn* regions of the *S. pneumoniae* genome and (ii) the existence of 900-bp *SmaI* *rrn* fragments, indicating two *SmaI* sites in *S. pneumoniae* *rrn* operons, compared with a single site in the *SmaI* *rrn* operons of *H. influenzae*. The *rrn* genes of *S. pneumoniae* have been mapped to fragments, but their more precise locations have not been determined. No bands that cross-hybridized with *H. influenzae* DNA other than those of the *rrn* genes were detected.

We have assigned 26 genetic markers to specific locations in the *S. pneumoniae* genome (Fig. 4). The linkage relationship between some of these genes can be summarized as follows. (i) Genes known to be genetically linked, such as rifampin resistance and streptolydigin resistance genes (*rif-r23* and *stg-rF*), were located on corresponding fragments of three restriction enzyme digests. (ii) The two linked streptomycin resistance and fusidic acid resistance markers (*str-41* and *fus-rA*) are on the same *ApaI* or *SacII* digestion fragment, but *str-41* is located on *SmaI* fragment 13 and *fus-rA* is located on *SmaI* fragment 16. This result suggests that *SmaI* fragment 19 (3.2 kbp) is not likely to be between *SmaI*

TABLE 2. Transformation ability of the DNAs extracted from FIGE-separated restriction fragments^a

Marker	No of background transformants/ml ^b	10 ³ transformants/ml with strain 119 DNA	<i>SmaI</i>		<i>ApaI</i>		<i>SacII</i>	
			Fragment(s)	10 ³ transformants/ml	Fragment(s)	10 ³ transformants/ml	Fragment(s)	10 ³ transformants/ml
<i>nov-r1</i>	10	150	1	15	1, 2	9.5	4, 5	6.2
<i>str-41</i>	10	200	13	2.4	3	9.4	6	6
<i>fus-rA</i>	8	100	15, 16	0.8	3	9.4	6	12
<i>rif-r23</i>	430	150	8–10	20	8–10	20	9–12	30
<i>stg-rF</i>	20	200	8–10	16	8–10	7.7	9–12	20
<i>amiA</i>	500	200	6	20	16–18	9.6	2	10
<i>opt-r2</i>	420	200			5	7.5	8	4
<i>cef0.1</i>	110	40	1–3	20	11–15	12	6	30
<i>cef0.4</i>	1,600		1, 2	60	1, 2	10		

^a DNAs isolated from strains carrying several markers were digested with restriction enzyme *SmaI*, *ApaI*, or *SacII*. A *hex* mutant was transformed with the DNA extracted from bands separated after FIGE.

^b The background activity represents the average number of transformants obtained after transformation with DNA fragments other than the fragment that contained the major biological activity.

FIG. 4. Physical and genetic map of *S. pneumoniae* R6, made by using *Apa*I, *Sma*I, and *Sac*II restriction enzymes. The genes were mapped after transformation of recipient cells with FIGE-isolated DNA fragments derived from genetically marked *S. pneumoniae* strains or hybridization of available cloned genes.

fragments 13 and 16 but is clockwise to *Sma*I fragment 16. (iii) The genes *hexA* and *hexB* are on different separated fragments; therefore, they do not belong to the same operon. The *mutL* and *mutS* genes of *E. coli* are homologous to *hexB* and *hexA*, respectively (36, 37), and are also found in different operons. (iv) The penicillin-binding protein (*pbp*) genes are also dispersed on different fragments of the chromosome, as they are in *E. coli*. However, *pbp-2x* and *pbp-1a* may be linked, since both *pbp-2x* located by hybridization and the *cef-1* marker located by transformation map to the same chromosomal region. It is worth noting that mutations of the *pbp-2x* gene confer resistance to 0.1 µg of cefotaxime (24). It is also possible that *pbp-3* and *cef-4* are the same gene. (v) The *lytA* gene and the *stg-r* and *rif-r* genes are on the same fragment. The *stg-r* and *rif-r* genes are genetically linked (46). To test linkage between the *lyt* gene and the other two, we have transformed the *lyt-4* mutant with DNA from strain 119 and have isolated colonies for their rifampin and streptolydigin resistance. We found a high frequency of cotransformation (60 to 78%) for doubly resistant clones that are also *lyt*⁺.

The size of the *S. pneumoniae* genome (2,240 to 2,270 kbp) is in reasonable agreement with the size estimated by viscoelastic measurements (1,700 + 500 kbp) (18, 21). The fact that the *S. pneumoniae* chromosome is smaller than that of *E. coli* (22, 44) may be related to its many requirements for growth, i.e., 12 amino acids and six vitamins (40). The genome sizes (1,980 and 2,340 kbp) and growth requirements of *H. influenzae* and *H. parainfluenzae* (19, 20, 26, 27) support this notion. It is likely that many metabolic pathways present in *E. coli* do not exist in *S. pneumoniae*; e.g., the DNA repair pathways for photoreactivation and SOS-like systems are not found in *S. pneumoniae* (13). The reduced size of the pneumococcal chromosome may have resulted from the evolutionary addition of genetic information to a smaller genome or from the elimination of DNA from a more complex organism. This model has been sug-

gested to explain the recent acquisition of new sequences of penicillin-binding proteins (8). Another observation favors the selective-loss hypothesis. A strain of pneumococcus frequently subcultured in complete medium to maintain a competent cell population changed from uracil prototrophy to auxotrophy (40). This spontaneous uracil requirement could not be transformed back to uracil independence, indicating that an irreversible change in one or several genes had occurred. It is not impossible that both addition and loss of genetic information can occur in the same system.

With the addition of more genes from cloned DNA and transformation assays, the utility of the map will be greatly expanded. The map will help to define functional relationships, especially in groups of genes coding for sets of proteins with related functions, such as penicillin-binding proteins or enzymes involved in capsular polysaccharide synthesis. The poorly understood mechanism of pneumococcal pathogenicity should be amenable to investigation by this enhanced genetic approach.

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REFERENCES

1. Avery, O. T., C. M. McLeod, and M. McCarty. 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by deoxyri-

- bonucleic acid fraction isolated from pneumococcus type III. *J. Exp. Med.* **79**:137–158.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. *Microbiol. Rev.* **47**:180–230.
 3. **Bucan, M., T. Yang-Feng, A. M. Colberg-Poley, D. J. Wolgemuth, J. L. Guenet, U. Francke, and H. Lehrack.** 1986. Genetic and cytogenetic localisation of the homeo box containing genes on mouse chromosome 6 and human chromosome 7. *EMBO J.* **5**:2899–2905.
 4. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. *Bergey's manual of determinative bacteriology*, 8th ed. Williams & Wilkins, Baltimore.
 5. **Chandler, M. S., and D. A. Morrison.** 1987. Competence for genetic transformation in *Streptococcus pneumoniae*: molecular cloning of *com*, a competence control locus. *J. Bacteriol.* **169**:2005–2011.
 6. **Claverys, J.-P., and S. A. Lacks.** 1986. Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria. *Microbiol. Rev.* **50**:133–165.
 7. **Claverys, J. P., M. Roger, and A. M. Sicard.** 1980. Excision and repair of mismatched base pairs in transformation of *Streptococcus pneumoniae*. *Mol. Gen. Genet.* **178**:191–201.
 8. **Dowson, C. G., A. Hutchison, J. A. Brannigan, R. C. George, D. Hansman, J. Linares, A. Tomasz, J. M. Smith, and B. G. Spratt.** 1989. Horizontal transfer of penicillin binding protein genes in penicillin resistant clinical isolates of *Streptococcus pneumoniae*. *Proc. Natl. Acad. Sci. USA* **86**:8842–8846.
 9. **Dowson, C. G., A. Hutchison, and B. G. Spratt.** 1989. Nucleotide sequence of the penicillin-binding protein 2B gene of *Streptococcus pneumoniae* strain R6. *Nucleic Acids Res.* **17**:7518.
 10. **Ely, B., and C. J. Gerardot.** 1988. Use of pulsed field gradient gel electrophoresis to construct a physical map of the *Caulobacter crescentus* genome. *Gene* **68**:323–333.
 11. **Garcia, P., J. L. Garcia, E. Garcia, and R. Lopez.** 1986. Nucleotide sequence and expression of the pneumococcal autolysin gene from its own promoter in *Escherichia coli*. *Gene* **43**:265–272.
 12. **Gasc, A. M., A. M. Sicard, and J. P. Claverys.** 1989. Repair of single and multiple substitution mismatches during recombination in *Streptococcus pneumoniae*. *Genetics* **120**:29–36.
 13. **Gasc, A. M., N. Sicard, J. P. Claverys, and A. M. Sicard.** 1980. Lack of SOS repair in *Streptococcus pneumoniae*. *Mutat. Res.* **70**:157–165.
 14. **Griffith, F.** 1928. The significance of pneumococcal types. *J. Hyg.* **27**:113–159.
 15. **Hakenbeck, R.** Unpublished data.
 16. **Henner, D. J., and J. A. Hoch.** 1980. The *Bacillus subtilis* chromosome. *Microbiol. Rev.* **44**:57–82.
 17. **Hotchkiss, R. D.** 1957. Isolation of sodium deoxyribonucleate in biologically active form from bacteria. *Methods Enzymol.* **3**:692–696.
 18. **Karcher, S., and A. M. Sicard.** Unpublished data.
 19. **Kauc, L., and S. H. Goodgal.** 1989. The size and physical map of the chromosome of *Haemophilus parainfluenzae*. *Gene* **83**:377–380.
 20. **Kauc, L., M. Mitchell, and S. H. Goodgal.** 1989. Size and physical map of the chromosome of *Haemophilus influenzae*. *J. Bacteriol.* **171**:2474–2479.
 21. **Klotz, L., and B. H. Zimm.** 1972. Size of DNA determined by viscoelastic measurements: results on bacteriophages, *Bacillus subtilis* and *Escherichia coli*. *J. Mol. Biol.* **72**:779–800.
 22. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a genomic library. *Cell* **50**:495–508.
 23. **Laible, G., and R. Hakenbeck.** 1987. Penicillin-binding proteins in β lactam-resistant laboratory mutants of *Streptococcus pneumoniae*. *Mol. Microbiol.* **1**:355–363.
 24. **Laible, G., R. Hakenbeck, A. M. Sicard, B. Joris, and J. M. Ghuysen.** 1989. Nucleotide sequences of the *pbp X* genes encoding the penicillin-binding proteins 2X from *Streptococcus pneumoniae* R6 and a cefotaxime-resistant mutant C506. *Mol. Microbiol.* **3**:1337–1348.
 25. **Le Bourgeois, P., M. Mata, and P. Rhizenthaler.** 1989. Genome comparison of *Lactococcus* strains by pulsed-field gel electrophoresis. *FEMS Microbiol. Lett.* **59**:65–70.
 26. **Lee, J. J., and H. O. Smith.** 1988. Sizing of the *Haemophilus influenzae* Rd genome by pulsed-field agarose gel electrophoresis. *J. Bacteriol.* **170**:4402–4405.
 27. **Lee, J. J., H. O. Smith, and R. J. Redfield.** 1989. Organization of the *Haemophilus influenzae* Rd genome. *J. Bacteriol.* **171**:3016–3024.
 28. **Lefevre, J. C., J. P. Claverys, and A. M. Sicard.** 1979. Donor deoxyribonucleic acid length and marker effect in pneumococcal transformation. *J. Bacteriol.* **138**:80–86.
 29. **Lopez, P., M. Espinosa, and S. A. Lacks.** 1984. Physical structure and genetic expression of sulfonamide-resistance plasmid pLS80 and its derivatives in *Streptococcus pneumoniae* and *Bacillus subtilis*. *Mol. Gen. Genet.* **195**:402–410.
 30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 31. **Martin, B., H. Prats, and J. P. Claverys.** 1985. Cloning of the *hexA* mismatch repair gene of *Streptococcus pneumoniae* and identification of the product. *Gene* **34**:293–303.
 32. **Martinez, S., P. Lopez, M. Espinosa, and S. A. Lacks.** 1986. Cloning of a gene encoding a DNA polymerase exonuclease of *Streptococcus pneumoniae*. *Gene* **44**:79–88.
 33. **McDaniel, L. S., J. Yother, M. Vijayakumar, L. McGarry, W. R. Guild, and D. E. Briles.** 1987. Use of insertional inactivation to facilitate studies of biological properties of pneumococcal surface protein A (PspA). *J. Exp. Med.* **165**:381–394.
 34. **Méjean, V., J. Rives, and J. P. Claverys.** 1990. Nucleotide sequence of *Streptococcus pneumoniae ung* gene encoding uracil-DNA glycosylase. *Nucleic Acids Res.* **18**:6693.
 35. **Prats, H., B. Martin, and J. P. Claverys.** 1985. The *HexB* mismatch repair gene of *Streptococcus pneumoniae*. Characterization, cloning and identification of the product. *Mol. Gen. Genet.* **200**:482–489.
 36. **Priebe, S. D., S. M. Hadi, B. Greenberg, and S. A. Lacks.** 1988. Nucleotide sequence of the *hexA* gene for DNA mismatch repair in *Streptococcus pneumoniae* and homology of *hexA* to *mutS* of *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **170**:190–196.
 37. **Prudhomme, M., B. Martin, V. Mejean, and J.-P. Claverys.** 1989. Nucleotide sequence of the *Streptococcus pneumoniae hexB* mismatch repair gene: homology of HexB to MutL of *Salmonella typhimurium* and to PMS1 of *Saccharomyces cerevisiae*. *J. Bacteriol.* **171**:5332–5338.
 38. **Rhee, D.-K., and D. A. Morrison.** 1988. Genetic transformation in *Streptococcus pneumoniae*: molecular cloning and characterization of *recP*, a gene required for genetic recombination. *J. Bacteriol.* **170**:630–637.
 39. **Schuster, C., B. Dobrinski, and R. Hakenbeck.** 1990. Unusual septum formation in *Streptococcus pneumoniae* mutants with an alteration in the D,D-carboxypeptidase penicillin-binding protein 3. *J. Bacteriol.* **172**:6499–6505.
 40. **Sicard, A. M.** 1964. A new synthetic medium for *Diplococcus pneumoniae* and its use for the study of reciprocal transformations at the *amiA* locus. *Genetics* **50**:31–44.
 41. **Sicard, A. M.** 1987. Gene conversion in *Streptococcus pneumoniae*. *Microbiologia SEM* **3**:5–12.
 42. **Sicard, N., and A. M. Estevenon.** 1990. Excision-repair in *S. pneumoniae*: cloning and expression of an *uvr*-like gene. *Mutat. Res.* **235**:195–201.
 43. **Smith, C. L., and C. R. Cantor.** 1987. Purification, specific fragmentation and separation of large DNA molecules. *Methods Enzymol.* **151**:483.
 44. **Smith, C. L., J. G. Economa, A. Schutt, S. Klco, and C. R. Cantor.** 1987. A physical map of the *Escherichia coli* K12 genome. *Science* **236**:1448–1453.
 45. **Stassi, D. L., P. Lopez, M. Espinosa, and S. A. Lacks.** 1981. Cloning of chromosomal genes in *Streptococcus pneumoniae*. *Proc. Natl. Acad. Sci. USA* **78**:7028–7032.
 46. **Tiraby, G.** Personal communication.

47. Tiraby, G., and M. S. Fox. 1973. Marker discrimination in transformation and mutation of pneumococcus. *Proc. Natl. Acad. Sci. USA* **70**:3541–3545.
48. Tiraby, G., and A. M. Sicard. 1973. Integration efficiency in DNA-induced transformation of pneumococcus. II. Genetic studies of mutant integrating all the markers with a higher efficiency. *Genetics* **75**:35–48.
49. Vijayakumar, M. Unpublished data.
50. Vijayakumar, M., S. D. Priebe, and W. R. Guild. 1986. Structure of a conjugative element in *Streptococcus pneumoniae*. *J. Bacteriol.* **166**:978–984.