

A Physical Gene Map of the Bacteriophage P22 Late Region: Genetic Analysis of Cloned Fragments of P22 DNA

REX L. CHISHOLM, ROBERT J. DEANS, ETHEL NOLAND JACKSON,¹
DAVID A. JACKSON, AND JOAN E. RUTILA

Department of Microbiology, University of Michigan, Ann Arbor, Michigan 48109

Accepted December 21, 1979

A physical gene map of the late region of the P22 chromosome has been constructed by genetic analysis of restriction enzyme fragments of P22 DNA cloned in a plasmid vector. Cleavage sites for restriction endonucleases *SalI*, *SstI*, *SmaI*, *XhoI*, and *BglI* were mapped on P22 DNA to provide physical reference points in addition to the *EcoRI*, *HindIII*, and *BamHI* cleavage sites previously mapped. Restriction enzymes *KpnI*, *BglII*, and *XbaI* were found to have no cleavage sites on P22 DNA. Fragments of P22 DNA produced by cleavage with *EcoRI*, *BamHI*, or *EcoRI* plus *BamHI* were cloned in *Escherichia coli* using the plasmid vector pBR322, and the resulting recombinant plasmids were introduced into *Salmonella typhimurium*. The genes present on a cloned fragment were identified by the ability of the hybrid plasmid to complement or recombine with P22 amber mutations in known genes when mutant phage were used to infect *S. typhimurium* strains carrying the recombinant plasmids. These experiments place all phage genes required for P22 head morphogenesis except gene 3 on the physical map between coordinates 0.000 and 0.318. The coding capacity of this interval is in close agreement with the molecular weights of the proteins assigned to it. The single gene for the P22 base plate protein is placed between coordinates 0.376 and 0.420 on the physical map. These results also show that distances on the recombination frequency map are significantly distorted relative to the physical gene map of the late region. The recombination frequency map is expanded in the region of the physical gene map where terminally redundant ends of the circularly permuted mature chromosomes fall.

INTRODUCTION

A chromosome map which shows the physical location of genes on the DNA molecule is an invaluable aid to the study of genetic organization and function. However, the circularly permuted and terminally redundant form of the mature linear P22 chromosome poses some difficulties for biochemical and physical studies of P22 chromosome structure, since the ends of the molecules are not convenient physical reference points. Restriction endonuclease cleavage sites do provide physical markers independent of chromosome permutation, but construction of restriction fragment maps is complicated by the appearance of minor fragments derived from chromosome ends (Jackson *et al.*, 1978a). Permuted genomes also present

special problems in the isolation of specific chromosome fragments. While restriction enzyme cleavage generates specific fragments which can be separated by gel electrophoresis, preparations of such fragments are often contaminated by heterogeneous-sized pieces derived from permuted chromosome ends. In addition, identification of the genes present on a particular fragment is impeded by the fact that the recombination frequency map is distorted in the region of molecular ends (Botstein *et al.*, 1972; Jackson *et al.*, 1978b). Thus genes cannot be assigned to restriction fragments by simply overlaying the genetic map on the physical map of restriction sites.

Our investigations of regulatory mechanisms controlling bacteriophage P22 late gene expression required construction of the P22 physical gene map presented here.

¹ To whom reprint requests should be addressed.

In earlier work, cleavage sites of restriction endonucleases *EcoRI* (Jackson *et al.*, 1978b) and *HindIII* (Deans and Jackson, 1979) were located to provide physical reference points on P22 DNA. The physical map was initially oriented with the recombination frequency map by use of early region substitutions or deletions which were genetically defined and could be related to the cleavage site map (Jackson *et al.*, 1978b). Subsequently, *Tn1* insertions located in late region genes 20, 16, *ant*, and 9 were assigned to individual *EcoRI* or *BamHI* fragments (Weinstock, 1977). No substitutions, deletions or insertions in the remaining late region genes are available, and the recombination frequency map is significantly distorted with respect to gene size in this region. In order to locate late genes more precisely on the P22 physical map, we have cloned fragments of P22 DNA in the plasmid vector pBR322, and then have identified the genes on the cloned P22 fragments by their ability to complement or recombine with known P22 amber mutations when mutant phage are used to infect plasmid-bearing cells. These experiments have produced a physical gene map of the late region of the P22 chromosome. The physical gene map and the cloned P22 DNA fragments described here will be valuable tools in future genetic and biochemical studies of P22 gene regulation and P22 chromosome maturation.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. P22 cI-7 obtained from M. Levine was the source of P22 DNA for all experiments. P22 amber mutants used are from the collections of D. Botstein or M. Levine. *Escherichia coli* K12 strain ED8654 *met*⁻ *r_k*⁻ *m_k*⁺ *supE* *tyr* *TtrpR*⁻, obtained from N. Murray, was used as the host in the cloning experiments. *E. coli* strain RR1 carrying pBR322 (Bolivar *et al.*, 1977) was obtained from H. Boyer. *Salmonella typhimurium* LT2 strains DB5289 *r*⁻ *m*⁻ *cysA1348am hisC527am* and DB5297 *r*⁻ *m*⁻ *suppE suII cysA1348am hisC527am* were obtained from D. Botstein.

DNA preparations. Bacteriophage P22 DNA was prepared from virus particles as previously described (Jackson *et al.*, 1978b).

Cells containing pBR322 (Bolivar *et al.*, 1977) or recombinant plasmids were grown in M9-casamino acids medium (Miller, 1972) to midlog phase ($A_{650} = 0.75-0.9$), at which point chloramphenicol was added to 150 $\mu\text{g/ml}$ to amplify the plasmid (Clewell, 1972). Plasmid DNA was isolated from cleared lysates prepared as described by Clewell (1972) followed by phenol extraction and equilibrium centrifugation in CsCl-ethidium bromide density gradients. Ethidium bromide was removed by extraction with NaCl-saturated isopropanol, and the DNA was extensively dialyzed against 10 mM Tris-HCl, pH 8.1, 1 mM EDTA, 10 mM NaCl.

Restriction endonuclease cleavage analysis. Restriction endonuclease *EcoRI* was prepared according to the procedure of Thomas and Davis (1975). *EcoRI* digestions were carried out in 6 mM Tris-HCl (pH 7.4), 6 mM MgCl₂, 50 mM NaCl, and 50 $\mu\text{g/ml}$ gelatin. *BamHI* and *SalI* were purified by an unpublished procedure of R. Roberts. *BamHI* cleavage reactions were performed in 6 mM Tris-HCl (pH 7.4), 6 mM 2-mercaptoethanol, 6 mM MgCl₂, as were *BamHI/EcoRI* double digestions. The reaction conditions for *SalI* cleavage were 6 mM Tris-HCl (pH 7.4), 6 mM MgCl₂, 0.2 mM EDTA, 150 mM NaCl, and 50 $\mu\text{g/ml}$ gelatin. *XhoI* and *SmaI* were purchased from New England Biolabs. *XhoI* digestions were performed in reaction conditions described for *BamHI*. *SmaI* reaction conditions were 20 mM KCl, 6 mM Tris-HCl (pH 8.1), 6 mM MgCl₂, 6 mM 2-mercaptoethanol, 100 $\mu\text{g/ml}$ gelatin. *KpnI*, *XbaI*, *BglI*, and *BglII* were purchased from Bethesda Research Laboratories and used in the reaction mixtures specified by the supplier. All reactions were incubated for 1 hr at 37°, then terminated by the addition of 0.10 vol of 25% Ficoll, 100 mM EDTA, 2% SDS, and 0.0025% bromophenol blue. Products of the restriction reactions were analyzed by agarose and polyacrylamide gel electrophoresis as previously described (DeLeys and Jackson, 1976; Deans and Jackson, 1979). DNA in gels was visualized by staining in 1 $\mu\text{g/ml}$ ethidium bromide for 30 min, then destaining in distilled water for 30 min. Fragment sizes were estimated from comparisons of their electrophoretic

mobilities with those of standard DNA molecules of known size.

Construction of hybrid plasmids. Fragments of P22 DNA produced by digestion with *Eco*RI, *Bam*HI, or *Eco*RI plus *Bam*HI were inserted into the plasmid vector pBR322 (Bolivar *et al.*, 1977) by ligation of the cohesive restriction enzyme termini. One microgram appropriately digested pBR322 DNA was mixed with a 5- to 10-fold molar excess of P22 restriction fragments in a 100- μ l reaction mixture consisting of 66 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 66 μ M ATP, 10 mM dithiothreitol, 50 μ g/ml autoclaved gelatin. T4 polynucleotide ligase (0.1 unit) (purchased from Bethesda Research Laboratories) was added and the mixture incubated 4 hr at 16°. In some experiments the restricted pBR322 DNA was first treated with bacterial alkaline phosphatase to prevent ligation of recircularized pBR322 molecules (Ullrich *et al.*, 1977). DNA in 25 mM Tris-HCl (pH 8.1) was incubated at 65° for 10 min with 0.1 units bacterial alkaline phosphatase/ μ g DNA. The phosphatase reaction was terminated by phenol extraction.

Transformation of host cells. The products of the ligation reactions were introduced into *E. coli* K12 strain ED8654 by a modification of the procedure of Cameron *et al.* (1975). Cells were grown in L-broth (Miller, 1972) to an A_{650} of 0.7, recovered by centrifugation, and then resuspended in half the original volume of ice-cold CTG (50 mM CaCl₂, 50 μ g/ml thymidine, 10% glycerol). Following a 20-min incubation on ice, cells were pelleted and resuspended in 0.05 vol cold CTG and kept on ice until used (a maximum of 24 hr). Recombinant plasmid DNAs were introduced into bacterial hosts by mixing 0.2 ml CaCl₂-shocked cells and 0.1 ml ligation mixture. This mixture was held 5 min on ice, incubated at 45° for 2 min, then returned to ice for an additional 5 min. One milliliter of L broth was then added and the mixture incubated at 37° for 45 min to permit expression of the plasmid encoded antibiotic resistance. Aliquots (0.1 ml) of this culture were then plated onto tryptone plates containing 50 μ g/ml ampicillin.

In situ colony hybridization. ³²P-Labeled P22 DNA, prepared by nick translation

(Rigby *et al.*, 1977) to a specific activity of 5.0 to 50 $\times 10^6$ cpm/ μ g, was hybridized to colonies grown on nitrocellulose disks (Schleicher and Schuell BA85) and lysed *in situ* as described by Grunstein and Hogness (1975), except that filters were processed by placing them on 9-cm Whatman No. 1 circles saturated with the appropriate solutions. The filters were baked at 80° a minimum of 2 hr *in vacuo* then incubated in 6 \times SSC (1 \times SSC is 0.15 M NaCl, 15 mM sodium citrate), 0.02% polyvinyl-pyrrolidone, 0.02% Ficoll-400, 0.02% bovine serum albumin (Denhardt, 1966). Up to six filters were placed in a 9-cm petri dish and covered with 30 ml of hybridization solution consisting of 1.5 $\times 10^5$ cpm/filter of P22 [³²P]-DNA in 6 \times SSC, 0.5% SDS, and 0.02% each polyvinyl-pyrrolidone, Ficoll-400, and bovine serum albumin. Mineral oil was layered on top of the hybridization solution to prevent evaporation. Hybridization was carried out for 18 hr at 65°. Following hybridization, the filters were subjected to five sequential 30-min washes with 2 \times SSC, 0.5% SDS, 10 mM glycine (pH 9.5) at 65°. Finally, the nitro-cellulose filters were rinsed in 2 \times SSC and air dried. Autoradiograms of the filters were prepared by exposing them to Kodak X-Omat X-ray film in Dupont Cronex intensifier screens for 18 hr at -70°.

Electron microscopy. Length measurement of recombinant plasmid molecules was performed using a Zeiss EM-10A electron microscope and the aqueous procedure of Davis *et al.* (1979). Either SV40 form II DNA (5.22 kb; Fiers *et al.*, 1978) or pBR322 form II dimer (8.72 kb; Sutcliffe, 1978) DNA was present in all samples as an internal length standard.

Recombination and complementation of P22 mutants with cloned fragments of P22 DNA. Plasmid DNA containing cloned P22 fragments was isolated from *E. coli* and introduced into *S. typhimurium* LT2 strain DB5289 as described above except that thymidine (50 μ g/ml) was added to the growth medium of cultures at $A_{650} = 0.3$. About 1 μ g plasmid DNA in 0.1 ml 0.1 M Tris-HCl (pH 7.4) 50 μ g/ml thymidine, was incubated with 0.2 ml prepared cells, and ampicillin resistant transformants selected as described above.

The efficiency of transformation by this procedure is much lower for *S. typhimurium* than for *E. coli* (Lederberg and Cohen, 1974). Only 30–60 ampicillin resistant colonies are recovered from transformation of *S. typhimurium* DB5289 by 1 μ g pBR322 DNA, while transformation of *E. coli* ED-8654 by the same DNA yields $5\text{--}20 \times 10^4$ ampicillin resistant colonies.

S. typhimurium strains containing the recombinant plasmids were grown overnight in L broth + 50 μ g/ml ampicillin, diluted 10-fold with fresh L broth and grown 2 hr with shaking at 37°C. These cultures were used as lawns for measuring the plating efficiency of P22 amber mutants.

To test the ability of plasmid-containing strains to complement P22 amber mutants, cultures were grown in L broth at 37°C with shaking to about 3×10^8 cells/ml, and then P22 particles were added to a multiplicity of infection of 3 phage/cell. Incubation and

aeration continued at 37°C for 40 min, at which point CHCl_3 was added to lyse the culture. Progeny phage were titered on DB5289 (Su^-) and on DB5297 (Su^+), and the burst sizes of wild-type plus amber progeny and of wild-type progeny only were calculated.

RESULTS

Location of SalI, SstI, SmaI, XhoI, and BglI Cleavage Sites in P22 DNA

The cleavage sites for several restriction enzymes which make a small number of cuts in P22 DNA have been located relative to the previously identified *EcoRI* and *HindIII* targets in the P22 chromosome (Jackson *et al.*, 1978b; Jackson and Deans, 1979). When P22 DNA is digested with *SalI*, two bands are obtained (see Fig. 1). The smaller fragment is present in low molar yield relative to the larger fragment. A similar low molar yield band present following digestion

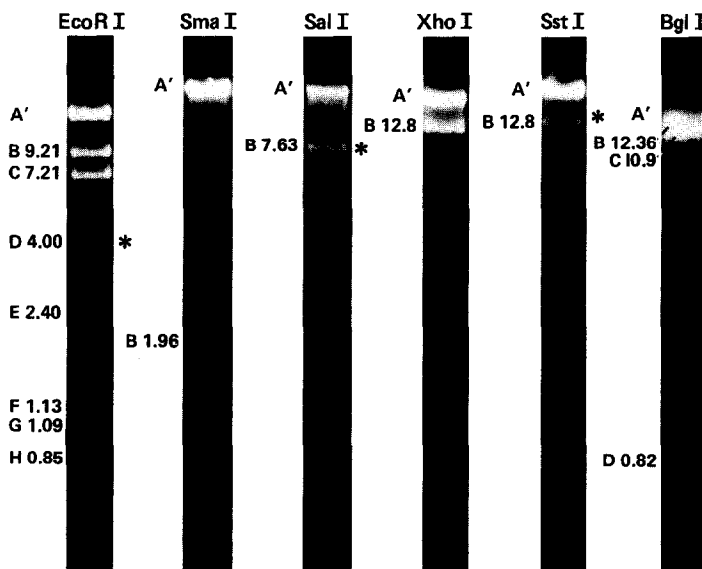


FIG. 1. Agarose gel electrophoresis of P22 DNA fragments produced by digestion with various restriction endonucleases. The letter designation for each fragment and its size (in kb) determined from electrophoretic mobility is shown to the left of each track. Bands are designated by capital letters in order of increasing electrophoretic mobility. Fragments in bands marked with an asterisk are derived from a single restriction enzyme cleavage and a mature chromosome end at *pac* (see text for explanation). These fragments are present in less than equimolar yield (see Jackson *et al.* (1978) for details). Fragments labeled A' are heterogeneous shortened derivatives of intact fragments designated A in Fig. 2 which are produced by headful packaging. Restriction endonucleases *KpnI*, *BglII*, and *XbaI* did not cleave P22 DNA when tested in parallel with digestions of λ DNA which produced the λ cleavage fragments expected for each of these enzymes (gels not shown).

of P22 DNA by *EcoRI* has been shown to be a consequence of P22 DNA packaging (Jackson *et al.* 1978a). Because mature P22 DNA molecules are linear and circularly permuted, the cleavage map is circular (Jackson *et al.*, 1978b). These mature chromosomes are generated by sequential headful packaging from a unique initiation site called *pac* (Jackson *et al.*, 1978a; Tye *et al.*, 1974). The fragment present in low molar yield in the *EcoRI* digest has been shown to arise from a chromosome end at *pac* and *EcoRI* cleavage at the first site counter-clockwise from *pac* (Jackson *et al.*, 1978a). The smaller *SalI* fragment (*SalI* B) (see Fig. 1) is derived in an analogous fashion from a molecular end at *pac* plus a single *SalI* cleavage. Double digestion with *EcoRI* plus *SalI* generates two new fragments (3.6 and 5.6 kb) which do not correspond to any *EcoRI* or *SalI* digestion product. The *EcoRI* B fragment (9.2 kb) is lost, while the other *EcoRI* fragments are not cleaved by *SalI*. The sum of the sizes of the two new double digestion fragments equals the size of *EcoRI* B, indicating a single *SalI* site is located in *EcoRI* B. Because the *SalI* B fragment represents sequences from *pac* counter-clockwise to the *SalI* site, the size of *SalI* B, together with the sizes of the *EcoRI/SalI* double digestion fragments, locate the *SalI* cleavage site at coordinate 0.183 on the P22 map (see Fig. 2).

Digestion of P22 DNA by *SstI* is analogous to *SalI* digestion. Two fragments, one a low molar yield packaging fragment, are produced upon *SstI* digestion (see Fig. 1). *EcoRI/SstI* double digestion places the *SstI* cleavage site in the *EcoRI* B fragment. As with *SalI*, the size of the packaging fragment (12.8 kb) locates the *SstI* site at 0.309 P22 map units (see Fig. 2).

As shown in Fig. 1, *SmaI* digestion produces two fragments. *SmaI* A' is heterogeneous in length as a result of the counter-clockwise movement of packaging cleavages relative to the genetic map, as successive terminally redundant headfuls are cut from the concatemer (Jackson *et al.*, 1978a). *SmaI* B is 2.0 kb long. In an *EcoRI/SmaI* double digestion the only *EcoRI* fragments altered are *EcoRI* A and the packaging fragment *EcoRI* D (which is a seg-

ment of *EcoRI* A). *SmaI* B remains intact. Two double digestion fragments, 13.9 and 3.8 kb, are seen. The two *SmaI* sites are therefore located 13.9 and 3.8 kb into *EcoRI* A from the ends. Because the 4.0-kb *EcoRI* D fragment is cleaved by *SmaI*, the 3.8-kb *EcoRI/SmaI* fragment must originate at *EcoRI* site 1, placing *SmaI* site 1 at 0.005. Therefore *SmaI* site 2 is located at the other end of the 2.0-kb *SmaI* fragment at P22 map coordinate 0.958. A packaging fragment extending from *pac* to *SmaI* site 1 should be present. However, due to the small size (less than 200 base pairs) and low yield of this fragment, it has not been detected.

XhoI digestion of P22 DNA also produces two bands (Fig. 1). Again, the upper band contains fragments which are heterogeneous in length. The size of the other fragment (*XhoI* B) is 12.8 kb. Upon *XhoI/EcoRI* double digestion only the *EcoRI* A (and the *EcoRI* D segment of *EcoRI* A) are cleaved, resulting in the 12.8-kb *XhoI* fragment and two double digestion products, 3.9 and 2.9 kb in length. Consequently the two *XhoI* sites are located 3.9 and 2.9 kb from the ends of *EcoRI* A which are defined by *EcoRI* sites 1 and 7. Placement of an *XhoI* cleavage site 2.9 kb from *EcoRI* site 7 would cause *XhoI* cleavage of P22 *HindIII* fragment C (see Fig. 2) whereas an *XhoI* site at 3.9 kb into *EcoRI* A from *EcoRI* site 7 would not. Analysis of the *HindIII/XhoI* double digestion products of P22 DNA indicates *HindIII* C is absent (data not shown). The two *XhoI* cleavage sites are thus located at map coordinates 0.002 and 0.692, placing *XhoI* cleavage site 1 less than 100 base pairs from *pac*.

Four *BglI* cleavage sites have been placed on the P22 chromosome by digestion of P22 DNA in combination with *EcoRI*, *HindIII*, *BamHI*, *XhoI*, and *SstI*. The products of *BglI* cleavage of P22 DNA are shown in Fig. 1. In a *BamHI/BglI* double digestion, the *BglI* C fragment is lost, and the *BamHI* B fragment (see Fig. 2) is intact (data not shown). Therefore, *BglI* C must overlap both *BamHI* cleavage sites. Double digestion with *HindIII* places one terminus of *BglI* C in the *HindIII* A fragment at coordinate 0.335. *EcoRI* fragments F and H are both cleaved by *BglI*, while *EcoRI* G

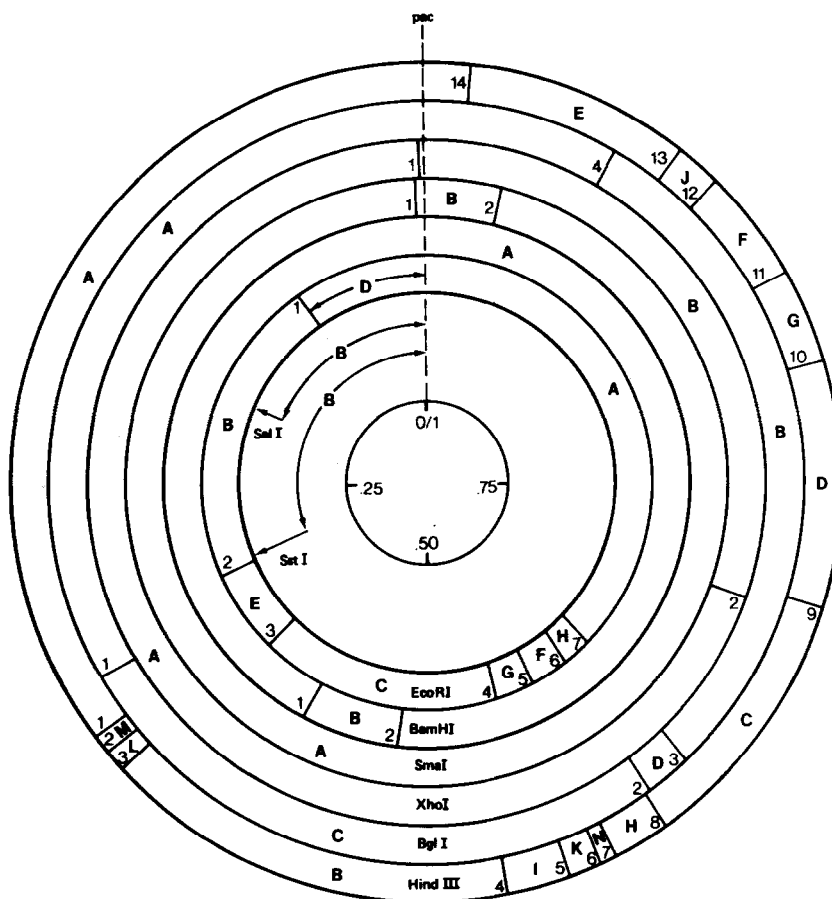


FIG. 2. Restriction endonuclease cleavage site map of P22 DNA. *EcoRI*, *BamHI*, *SmaI*, *XhoI*, *BglI*, and *HindIII* cleavage sites are shown to scale in six concentric rings. Positions of the single cleavage sites for *SalI* and *SstI* are indicated by arrows. *KpnI*, *BglII*, and *XbaI* do not cleave P22 DNA. The cleavage sites for each restriction endonuclease are numbered sequentially in a counter-clockwise direction starting with the first site counterclockwise from *pac*. The *EcoRI* and *HindIII* cleavage site maps have been reported previously (Jackson *et al.*, 1978b; Deans and Jackson, 1979a), except that Youderian and Susskind (manuscript in preparation) have shown that the order of *HindIII* fragments H and I were reversed and the correct order is shown here. The *BamHI* sites were mapped by Weinstock (1977). Physical map coordinates are fractions of the length of the P22 genome (41.6 kb; Jackson *et al.*, 1978a). The coordinate system has been reoriented, with the origin now placed at *pac*. Previously the origin of the coordinate system was defined at *EcoRI* site 1 (Jackson *et al.*, 1978b). The coordinates of each cleavage site are reported in Table 1.

is not. The size and map positions of these fragments (Fig. 2) demand that *BglI* fragment D must be generated by cleavage at *BglI* sites 2 and 3 in *EcoRI* F and H, respectively (Fig. 2). Map coordinates of *BglI* site 2 (0.598) and *BglI* site 3 (0.618) were determined by *BglI/HindIII* double digestion. *BglI/SstI* double digestion indicates *BglI* A overlaps the *SstI* site as shown in Fig. 2 and is generated by cleavage at *BglI*

sites 1 and 4. The exact location of *BglI* site 4 (0.916) was determined from the sizes of *BglI/HindIII* double digestion products. As can be seen in Fig. 1, the *BglI* A' fragment appears heterogeneous and smaller in size than predicted. Since the *pac* site is located internal to *BglI* A, this fragment is never intact, and it appears heterogeneous in size due to the varying positions of chromosome ends.

The location of the cleavage sites of the five restriction endonucleases used here are shown in Fig. 2 in relation to the positions of *EcoRI*, *HindIII* and *BamHI* sites previously mapped (Jackson *et al.*, 1978a; Deans and Jackson, 1979; Weinstock, 1977). The locations of the restriction sites displayed in Fig. 2 provide useful physical markers in all regions of the P22 genome, and in particular facilitate the physical subdivision of the genome as described below.

Cloning of P22 EcoRI Fragments

The utility of the physical map shown in Fig. 2 can be increased by location of specific P22 genes relative to the known restriction targets. In previous work, some early P22 genes (Jackson *et al.*, 1978b) and genes 20, 16, *ant*, and 9 (Weinstock, 1977) have been located with respect to particular restriction fragments. To facilitate the ordering of additional P22 genes, and particularly late genes, with respect to the physical map, we have cloned fragments of P22 DNA produced by cleavage with *EcoRI* or *BamHI* or *BamHI* plus *EcoRI*. In our initial cloning experiments, unfractionated *EcoRI* limit digest fragments of P22 DNA were inserted by ligation into the *EcoRI* site of the plasmid vector pBR322, and the products introduced into *E. coli* by transformation. Because the pBR322 vector *EcoRI* linear DNA was treated with bacterial alkaline phosphatase to eliminate ligation of recircularized pBR322 linears (Ullrich *et al.*, 1977), all Ap^r and Tc^r clones obtained were expected to contain inserted P22 *EcoRI* fragments. Comparison of *EcoRI* digests of plasmid DNA prepared from these clones and P22 DNA allowed initial identification of the cloned P22 *EcoRI* fragments. Clones pP22-1, pP22-2, pP22-3, pP22-4, pP22-6, and pP22-7 which contain P22 *EcoRI* fragments H, F, D, B, E and G respectively were obtained in this manner (see Figs. 3 and 4). The identity of the cloned fragments was confirmed in two ways. First, each recombinant plasmid was digested with *EcoRI* and the resulting fragments separated by electrophoresis in agarose, then transferred to nitrocellulose by the method of Southern (1975) and hybridized with ³²P-

TABLE 1
PHYSICAL MAP COORDINATES OF RESTRICTION
ENDONUCLEASE CLEAVAGE SITES ON P22
DNA^a

Restriction endonuclease cleavage site	Map position	Restriction endonuclease cleavage site	Map position		
<i>EcoRI</i>	1	<i>BamHI</i>	1	0.420	
	2		2	0.481	
	3	<i>SalI</i>	1	0.183	
	4				
	5				
	6				
	7				
<i>HindIII</i>	1	<i>SmaI</i>	1	0.005	
	2		2	0.958	
	3	<i>XhoI</i>	1	0.002	
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
		13	<i>BglI</i>	1	0.335
				2	0.598
	14		3	0.618	
			4	0.916	
				0.982	

^a Physical map coordinates are fractions of the P22 genome (41.6 kb; Jackson *et al.*, 1978b). Coordinates of *EcoRI* cleavage sites were determined previously (Jackson *et al.*, 1978b) but values given here reflect realignment of the coordinate system to originate at *pac*. *HindIII* site coordinates were reported previously (Deans and Jackson, 1979), and *BamHI* site coordinates were calculated from the data of Weinstock and Botstein (Weinstock, 1977). Coordinates for cleavage sites for the other enzymes in the table were determined as described in the text.

labeled P22 DNA. As shown in Fig. 3, the fragments derived from the recombinant plasmids hybridize with P22 DNA and migrate at the positions characteristic of P22 *EcoRI* fragments, supporting the initial identification of the cloned P22 DNA fragments. Digestion with additional restriction enzymes was used to further characterize each of the recombinant plasmids. Beside providing further evidence for the identity of the cloned P22 segment, this analysis defines the orientation of the

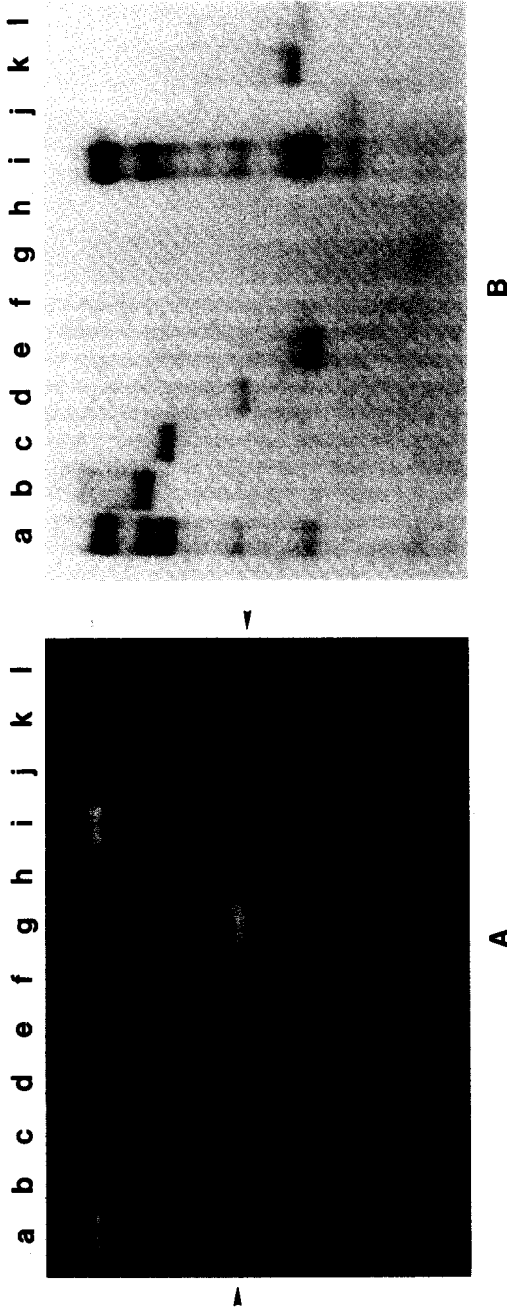


FIG. 3. *Eco*RI or *Eco*RI plus *Bam*HI cleavage patterns of P22 plasmid clones. P22 DNA isolated from virus particles or recombinant plasmid DNAs bearing P22 fragment inserts were digested with *Eco*RI (tracks a-h) or *Eco*RI plus *Bam*HI (i-l), fractionated on a 0.8% agarose gel, transferred to a nitrocellulose filter (Southern, 1975), and hybridized to P22 [³²P]DNA (8 × 10⁵ cpm/μg). Preparation of P22 [³²P]DNA and hybridization conditions were the same as described under Materials and Methods for colony hybridization, with the addition of 50 μg/ml salmon sperm DNA to the prehybridization and hybridization mixtures. (A) Ethidium bromide stained gel (a) P22, (b) pP22-4, (c) pP22-10, (d) pP22-3, (e) pP22-6, (f) pP22-2, (g) pP22-7, (h) pP22-1, (i) P22 (digestion with *Bam*HI was not complete) (j) pP22-201, (k) pP22-203, (l) pP22-250. The positions of the pBR322 band are indicated by an arrow at the left (*Eco*RI) or right (*Eco*RI plus *Bam*HI). (B) Autoradiogram of the nitrocellulose filter prepared from the gel in (A) and hybridized with P22 [³²P]DNA.

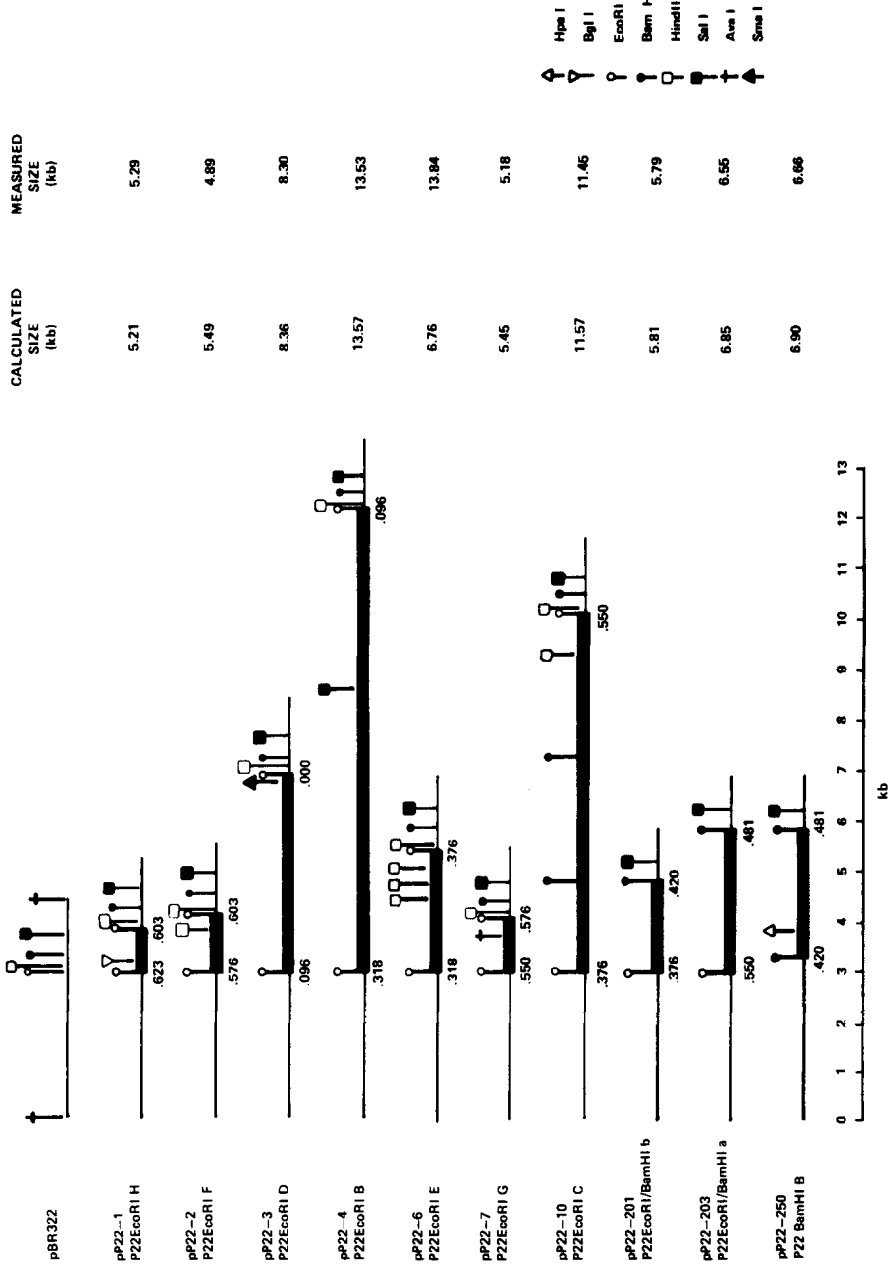


FIG. 4. Recombinant plasmids containing P22 restriction endonuclease fragments. The cloned P22 sequences are shown as a bar, and the pBR322 sequences as a line. The P22 physical map coordinates of the cleavage sites which generate the cloned fragment are shown immediately below the junction of P22 and pBR322 sequences in each plasmid. The restriction enzyme cleavage sites which were used to determine the orientation of each insert are shown above the diagram of each plasmid. For purposes of this diagram, the circular plasmids have been drawn as linear molecules opened at the *Ava*I cleavage site in pBR322. Plasmid pP22-6 is drawn as a monomer but is isolated from cells as a dimer. The *Hpa*I site used to orient *Bam*HI B in pP22-250 was placed on the P22 map by R. J. Deans (1979). The contour length of each plasmid was determined by electron microscopy. The number of DNA molecules measured for each species ranged from 25 to 94. The standard deviation of the measured lengths varied from ± 4.6 to $\pm 8.3\%$ of the mean length.

inserted P22 fragments relative to pBR322. Figure 4 illustrates the structure and orientation determined for each recombinant plasmid.

The contour length of each recombinant plasmid was measured by electron microscopy, and the molecular weights calculated from these results compared with the molecular weights predicted from the known sizes of the vector and inserts (Fig. 4). As can be seen from the data in Fig. 4, the calculated and measured sizes of the recombinant plasmids are in good agreement, except in the case of pP22-6, for which the calculated size is only one half that determined by electron microscopy. *EcoRI* digestion of pP22-6 yields only two fragments, the pBR322 *EcoRI* linear and the cloned P22 *EcoRI* E fragment. This result indicates that pP22-6 is a dimer consisting of two copies each of the pBR322 *EcoRI* linear and the P22 *EcoRI* E fragment. *HindIII* digestion shows this plasmid to consist of two monomeric units (pBR322 linked to P22 *EcoRI* E) in a head to tail orientation.

Neither the *EcoRI* A nor *EcoRI* C fragments of P22 were obtained in the shotgun experiment described above. This result may suggest that these segments of P22 encode functions lethal to the *E. coli* host. Attempts to clone these fragments from DNA enriched for these specific fragments were therefore undertaken. The P22 *EcoRI* C fragment has been cloned (J. Yoder, E. Jackson, and D. Jackson, in preparation) in a λ gt phage vector (Murray and Murray, 1974; Thomas *et al.*, 1974). *EcoRI* digestion of this recombinant phage produces three fragments, only one of which—P22 *EcoRI* C—has two *EcoRI* termini. Following ligation and transformation into *E. coli*, all plasmids carrying P22 sequences should have *EcoRI* C inserts. A 10-fold molar excess of *EcoRI* digested λ gt·P22 *EcoRI* C hybrid phage was mixed with *EcoRI* digested pBR322, ligated, and introduced into *E. coli*. Colonies carrying P22 sequences were then identified by colony hybridization using P22 [³²P]DNA as the probe. A single P22 positive colony was obtained from this experiment. The plasmid obtained from these cells, pP22-10, contains an *EcoRI* insert which hybridizes with P22 DNA (see

Fig. 3), comigrates in gel electrophoresis with *EcoRI* C, and yields a *Bam*HI digestion pattern indistinguishable from that of *EcoRI* C. Because of the very low yield of plasmids containing *EcoRI* C in this experiment and because of our inability to recover *EcoRI* C plasmids (but not plasmids containing other P22 *EcoRI* fragments) in other clonings from DNA fragment pools enriched for *EcoRI* C, there may be some question as to whether the *EcoRI* C insert in pP22-10 is the wild-type sequence.

Several attempts to clone the P22 *EcoRI* A fragment have been unsuccessful despite the use of DNA pools enriched for P22 *EcoRI* A DNA. P22 DNA consisting of 65 mol% P22 *EcoRI* A DNA was obtained by preparative sucrose gradient centrifugation of *EcoRI* digested P22 DNA and ligated to *EcoRI* digested pBR322 DNA. Since the *EcoRI* A fragment carries the P22 early promoters controlled by phage repressor, the ligation mixture was also introduced into an *E. coli* host (ED8654) lysogenized with a λ imm21 phage which encodes a repressor able to repress P22 early functions (Botstein and Herskowitz, 1974). No recombinant plasmids containing *EcoRI* A were obtained from these experiments. Other P22 *EcoRI* fragments, present as minor components of the enriched *EcoRI* A preparation, have been cloned from these pools, suggesting that a function or functions coded for by P22 *EcoRI* A may be lethal for the host cells, even in a λ imm21 lysogen.

Cloning of P22 BamHI and BamHI/EcoRI Double Digestion Products

As can be seen from Fig. 2, the *EcoRI* C fragment of P22 is cleaved at two sites by *Bam*HI, to produce three new fragments, one having two *Bam*HI termini, and the other two having one *EcoRI* terminus and one *Bam*HI end each (Weinstock, 1977). The *Bam*HI site in pBR322 is located in the tetracycline resistance gene, 375 bases from the *EcoRI* site (Sutcliffe, 1978). Insertion of a DNA fragment at the *Bam*HI site of pBR322 therefore inactivates the tetracycline resistance phenotype. Recombinant plasmids containing either *Bam*HI or *Bam*HI/*EcoRI* fragments can thus be readily

TABLE 2
PLATING EFFICIENCY OF P22 AMBER MUTANTS ON *S. typhimurium* Su⁻ STRAINS
CONTAINING CLONED FRAGMENTS OF P22 DNA^a

P22 strain	Su ⁻ lawns				
	5289/pBR322	5289/pP22-3	5289/pP22-4	5289/pP22-201	5289/pP22-250
wild type	1.0	1.0	1.0	1.0	1.0
19 ⁻ amN11	8.2 × 10 ⁻⁶	10 × 10 ⁻⁶	21 × 10 ⁻⁶		
3 ⁻ amH314	84 × 10 ⁻⁶	4.3 × 10 ⁻⁶	27 × 10 ⁻⁶		
3 ⁻ amH317	21 × 10 ⁻⁶	2.5 × 10 ⁻⁶	7.7 × 10 ⁻⁶		
2 ⁻ amH303	0.3 × 10 ⁻⁶	1.1	0.2 × 10 ⁻⁶		
1 ⁻ amN109	2.6 × 10 ⁻⁶	1.1	4.7 × 10 ⁻⁶		
8 ⁻ amN123	16 × 10 ⁻⁶	0.044	76 × 10 ⁻⁶		
8 ⁻ amH304	5.8 × 10 ⁻⁶	7.3 × 10 ⁻⁶	0.0019		
8 ⁻ amH202	1.1 × 10 ⁻⁶	1.5 × 10 ⁻⁶	0.0045		
8 ⁻ amN125	55 × 10 ⁻⁶	56 × 10 ⁻⁶	0.0065		
5 ⁻ amN8	24 × 10 ⁻⁶	21 × 10 ⁻⁶	1.1		
5 ⁻ amN114	4.2 × 10 ⁻⁶	3.6 × 10 ⁻⁶	0.84		
5 ⁻ amN3	3.1 × 10 ⁻⁶	2.5 × 10 ⁻⁶	0.91		
7 ⁻ amN1375	15 × 10 ⁻⁶	11 × 10 ⁻⁶	0.1		
16 ⁻ amN121	2.3 × 10 ⁻⁶	1.4 × 10 ⁻⁶	0.31		
9 ⁻ amH1272	87 × 10 ⁻⁶			0.005	67 × 10 ⁻⁶
9 ⁻ amH840	0.7 × 10 ⁻⁶			0.004	10 × 10 ⁻⁶
9 ⁻ amH1014	0.4 × 10 ⁻⁶			0.002	22 × 10 ⁻⁶

^a The plating efficiency is the ratio of the titer on the plasmid-containing strain to the titer on DB5297 (Su⁺). When several different amber alleles of a gene are used, the order in the table corresponds to the genetic map order determined by tests for recombination of the markers with prophage deletions of varying lengths within the gene. Gene 8 and gene 5 mutations were ordered by F. Winston and D. Botstein (unpublished experiments) and gene 9 mutations were ordered by P. Berget (unpublished experiments). Gene 8 amber alleles H202 and N125 fall in the same group. Gene 8⁻amN123 lies nearest the end of the gene encoding the amino terminus of the protein (King *et al.*, 1978). Gene 9⁻amH1272 represents the promoter-proximal and 9⁻amH1014 the promoter-distal groups of gene 9 amber mutations (Berget, unpublished experiments).

identified by screening Ap^r colonies for tetracycline sensitivity (Bolivar *et al.*, 1977). Using this method, the *Bam*HI B fragment and both *Bam*HI/*Eco*RI double digestion fragments were cloned. Figure 4 shows the characteristics of the three plasmids obtained. Plasmids pP22-201 and pP22-203 contain the large and small *Bam*HI/*Eco*RI fragments, respectively, and pP22-250 contains the *Bam*HI-B fragment. Each of these plasmids was analyzed as described above for the *Eco*RI inserts (see Figs. 3 and 4).

Physical Location of P22 Late Genes

Genes required for morphogenesis of the P22 particle are expected to lie from about coordinate 0.9 counterclockwise to about coordinate 0.4 of the P22 physical map

(Jackson *et al.*, 1978b; Deans and Jackson, 1979; Weinstock, 1977). In order to define further the physical location of P22 genes in this region, we have mapped known P22 late genes to individual cloned fragments. Plasmids containing P22 DNA inserts were introduced into *S. typhimurium* DB5289 (see Materials and Methods). P22 amber mutants defective in known genes were then plated on these Su⁻ strains carrying the recombinant plasmids. The P22 inserts in the plasmids were derived from wild-type DNA. Therefore recombination between an infecting amber mutant phage and the chimeric plasmid will produce phage able to grow in the Su⁻ host if the cloned P22 DNA fragment carries the wild-type allele of the defective gene. Recombination between P22 amber mutants and cloned fragments

TABLE 3

BURST SIZE OF P22 AMBER MUTANTS IN *S. typhimurium* Su⁻ STRAINS CARRYING CLONED FRAGMENTS OF P22 WILD-TYPE DNA^a

Host	P22 Strain	Burst size on indicator	
		Su ⁻	Su ⁺
5289/pBR322	Wild type	500	682
	1 ⁻ amN109	0.015	0.075
	2 ⁻ amH303	0.018	0.056
	8 ⁻ amN123	0.024	0.059
	8 ⁻ amH202	0.008	0.059
	5 ⁻ amN8	0.015	0.074
	5 ⁻ amN114	0.0005	0.006
	7 ⁻ amN1375	0.031	2.58
	16 ⁻ amN121	0.011	0.079
	9 ⁻ amH1014	0.0004	0.128
5289/pP22-3 (<i>Eco</i> RI D)	Wild type	328	352
	1 ⁻ amN109	4.0	131
	2 ⁻ amH303	3.7	122
	8 ⁻ amN123	0.7	4.5
5289/pP22-4 (<i>Eco</i> RI B)	Wild type	466	403
	8 ⁻ amH202	0.13	0.25
	5 ⁻ amN8	2.3	18.5
	5 ⁻ amN114	0.84	10.1
	7 ⁻ amN1375	2.24	11.2
	16 ⁻ amN121	1.89	14.1
5289/pP22-201 (<i>Eco</i> RI/ <i>Bam</i> HI b)	Wild type	439	453
	9 ⁻ amH1014	0.0046	0.204

^a The ability of plasmid-carrying Su⁻ strains to complement P22 amber mutations was measured as described under Materials and Methods.

should be detected by an increase in plating efficiency on the Su⁻ host carrying the plasmid (Campbell *et al.*, 1978). Table 2 shows that plating efficiencies of amber mutants are increased two to six orders of magnitude by the presence in the Su⁻ host of a cloned fragment carrying the wild-type allele. The data of Table 2 indicate that P22 *Eco*RI fragment D (plasmid pP22-3) contains wild-type alleles of 2⁻amH303, 1⁻amN109, and 8⁻amN123, while P22 *Eco*RI fragment B (plasmid pP22-4) carries the wild-type alleles of the other 8⁻ amber alleles tested, as well as the 5⁻, 7⁻, and 16⁻ amber alleles used. All three 9⁻ amber mutants plate with increased efficiency on the Su⁻ host containing plasmid pP22-201, and therefore these markers lie in the P22 *Bam*HI/*Eco*RI fragment between *Bam*HI site 1 and *Eco*RI site 3.

Certain of the amber mutants plate on a particular plasmid-containing Su⁻ host as effi-

ciently as on the Su⁺ host (see Table 2). Such high plating efficiency would be expected if the plasmid produced the phage protein defective in the amber mutant. To determine whether these plasmids can complement P22 amber mutants, the burst size of amber and wild-type progeny produced by infection of the plasmid-bearing Su⁻ cell was measured (Table 3). A sizeable burst of phage which are still genetically amber indicates complementation is occurring. Table 3 shows that pP22-3 provides the gene 1 and gene 2 proteins. (The efficient complementation observed is not due to production of p2 by the few wild-type recombinant phages produced since another fragment containing gene 2 inserted at the *Pst*I site of pBR322 does not allow growth of P22 gene 2 amber mutants even though the number of wild-type recombinants is as high as in infection of the pP22-3 strain; J. Ruttila, unpublished data.) The results of Table 3 therefore suggest that gene 2 as well as gene 1 is wholly contained in P22 *Eco*RI fragment D. Gene 8 protein is not produced by either pP22-3 or pP22-4, as expected since gene 8 alleles are rescued from both fragments D and B (Tables 2 and 3). Gene 9 protein is not produced by pP22-201. The data of Table 3 suggest that pP22-4 may provide genes 5, 7, and 16 polypeptides, since significant numbers of amber mutant progeny are produced. The burst sizes of the 5, 7, or 16 amber progeny are low, perhaps because the plasmid does not produce the required large amounts of these phage structural proteins. However, we cannot exclude the possibility that the low levels of complementation observed are due to production of functional proteins 5, 7, or 16 by the small number of wild-type recombinant phage produced in the infection.

DISCUSSION

Restriction Endonuclease Cleavage Site Map of P22 DNA

Seven *Eco*RI cleavage sites and 14 *Hind*-III cleavage sites were mapped on P22 DNA by previous work in this laboratory (Jackson *et al.*, 1978b; Deans and Jackson, 1979). These 21 sites define a map of the

P22 chromosome having precisely located physical markers in most regions of the genome. In order to provide additional physical markers in some regions of special interest, we have determined the locations of cleavage sites for five additional restriction enzymes. Certain of these deserve comment.

Both *Sst*I and *Sal*I cleave P22 DNA at separate, single sites within the *Eco*RI B fragment, a segment about 20% the size of intact P22 which was not previously subdivided (Fig. 2). The *Sal*I site was independently mapped by Weinstock and Botstein (Weinstock, 1977).

The *Sal*I site proves useful in the analysis of P22 DNA packaging. We have proposed that P22 initiates headful packaging of phage DNA at *pac* and that headfuls are packaged sequentially in a counterclockwise direction from that site (see Fig. 2). *Eco*RI cleavage of a linear P22 chromosome encapsulated at *pac* therefore yields a fragment having one end at *pac* and one end at *Eco*RI site 1 (coordinates 0.000–0.096 in Fig. 2). By this scheme, a *Sal*I digest of P22 DNA should contain an analogous fragment having one end at *pac* and one at the *Sal*I site. The fact that such a fragment (*Sal*I B) is found and is present in low molar yield provides additional evidence for the position of *pac* and the counterclockwise direction of sequential packaging proposed earlier (Jackson *et al.*, 1978a).

*Xho*I and *Sma*I each cleave P22 DNA close to *pac* and should be useful aids to further study of the phage DNA packaging initiation region. *Bgl*I provides an additional cleavage site in *Eco*RI E, and so further subdivides the interesting regulatory region *imm*1 (Botstein *et al.*, 1975; Levine *et al.*, 1975; Susskind and Botstein, 1975). Three restriction endonucleases which do not cleave P22 DNA have been identified: *Xba*I, *Bgl*II, and *Kpn*I. Weinstock and Botstein (Weinstock, 1977) report *Xba*I, *Bgl*II, *Kpn*I, and *Xho*I do not cleave P22 DNA. Although our results are in agreement for the first three enzymes, we find that *Xho*I cleaves P22 as shown in Fig. 1. Our results are summarized in the physical map of P22 DNA diagrammed in Fig. 2.

Restriction Fragments of P22 DNA Cloned in Plasmid Vector pBR322

The original physical map of *Eco*RI sites could be aligned approximately with the P22 genetic map, but the method by which some P22 early genes were assigned to individual restriction fragments (Jackson *et al.*, 1978b) could not be extended to map late genes relative to particular cleavage sites. Our approach to placing P22 late genes on the P22 physical map was to clone individual P22 restriction fragments in a plasmid vector, and then to determine the P22 genetic markers present on the resulting recombinant plasmid by a marker rescue procedure. Fragments which together represent 60% of the P22 chromosome, including the entire late region, have been cloned in pBR322. Shotgun cloning of a P22 *Eco*RI digest yielded each of the *Eco*RI fragments B, D, E, F, G, and H as individual inserts in pBR322. Cleavage of each recombinant plasmid with appropriate restriction endonucleases served to identify the orientation of each insert in the vector.

Two P22 *Eco*RI fragments, A and C, were not recovered as inserts in the plasmid from the shotgun cloning experiment. Attempts to clone *Eco*RI A from DNA preparations enriched for this fragment were also unsuccessful, although other P22 *Eco*RI fragments present as minor contaminants in this preparation were cloned in these experiments. This result is not surprising, as the P22 early promoters are located on *Eco*RI A (Botstein and Herskowitz, 1974; Jackson *et al.*, 1978b), and expression of P22 genes on the fragment might interfere with plasmid replication or cell growth. The amount of phage repressor in the λ *imm*21 lysogenic host might be insufficient to repress multiple copies of the cloned fragment. Isolation of P22 *Eco*RI A clones may also be hindered by both the large size of the fragment and the reduced proportion of intact *Eco*RI A resulting from circular permutation.

One plasmid containing P22 *Eco*RI C was obtained from an experiment utilizing a DNA preparation in which the only DNA fragment having two *Eco*RI termini was

fragment C. A single recombinant plasmid was isolated in this experiment. The low frequency at which P22 *EcoRI* C is recovered in a plasmid raises the possibility that this fragment may also encode functions deleterious to the host. If so, the single *EcoRI* C clone obtained may carry a mutation inactivating this function. All of the sequences of *EcoRI* C were readily cloned in three separate segments as fragments *Bam*HI B (pP22-250), *EcoRI/Bam*HI a (pP22-203), and *EcoRI/Bam*HI b (pP22-201). Therefore, if a lethal function is expressed when *EcoRI* C is inserted at the *EcoRI* site of pBR322, it is not expressed from any of these three subsegments of *EcoRI* C.

The most surprising result of the shotgun cloning experiments was the recovery of P22 *EcoRI* D from a recombinant plasmid, since previous results predict that fragment D has only one *EcoRI* terminus and that the other end derives from the *pac* site (Jackson *et al.*, 1979). However, *EcoRI* cleavage of chimeric plasmid pP22-3 (Fig. 3) generates a P22 fragment which comigrates with fragment D in an *EcoRI* digest of P22, and which has the expected restriction pattern when digested with *Sma*I and *Xho*I. This result led us to consider whether some of the fragment D formed in a P22 *EcoRI* digest might be generated by *EcoRI*-specific cleavage at a site very near *pac*. Since D is found in low yield in a wild-type P22 *EcoRI* digest, the postulated *EcoRI* site near *pac* would have to be partially blocked. To test this model we have measured the molar yield of the packaging fragment B observed in a *Sal*I digest of wild type P22 DNA (Figs. 1 and 2). The yield of the *pac-Sal*I site 1 fragment is in good agreement with the yield of the *pac-EcoRI* site 1 fragment (*EcoRI* D) (R. J. Deans, 1979). Therefore *EcoRI* fragment D is not generated by *EcoRI*-specific cleavage near *pac*. The cloning of P22 *EcoRI* D thus suggests that the *pac* terminus has one or more nucleotides in common with the *EcoRI* recognition sequence. Experiments to define the *pac* terminus further are in progress.

Physical Gene Map of P22

In order to place late genes on the P22 physical map, restriction fragments of wild-type P22 DNA were tested for ability to recombine with or to complement P22 mutants defective in known late genes. When a *S. typhimurium* Su⁻ strain carrying a chimeric plasmid is used as a host for plating a P22 amber mutant, an increase in plating efficiency on this host over the Su⁻ control indicates the plasmid carries the wild type allele of the amber lesion (Table 2). Some P22 late genes were found to be expressed when carried on a plasmid insert, since Su⁻ strains containing the plasmid pP22-3 complemented P22 amber mutants defective in genes 1 or 2 (see Table 3). The plating efficiency of these P22 amber mutants on the complementing host (1⁻ or 2⁻ *am* on DB5289/pP22-3) was equal to the plating efficiency on the Su⁺ host (Table 2). The ability of P22 amber mutants to plate on noncomplementing hosts (e.g., 8⁻ *am* on DB5289/pP22-3 or DB5289/pP22-4; 9⁻ *am* on DB5289/pP22-201) must be due to recombination, and the plating efficiencies are lower. It is not yet clear whether the high plating efficiencies and low burst sizes of P22 late mutants on the Su⁻ strain carrying pP22-4 are due to complementation or recombination of cloned *EcoRI* B with the infecting amber mutant phage.

From the data of Tables 2 and 3, all P22 morphogenetic genes except gene 3 can be placed on the physical map of *EcoRI*, *Hind*III, and *Bam*HI sites. Assignment of a mutational allele to a restriction fragment is based on the plating efficiency test (Table 2). Furthermore, complementation for a particular gene indicates the gene is intact on the cloned fragment. P22 *EcoRI* D therefore contains intact genes 1 and 2 as well as a small portion of the amino-terminal end of gene 8. The coding capacity of fragment D is estimated as 1.5×10^5 daltons of protein, and the sums of the molecular weights of the gene 1 and 2 proteins is 1.6×10^5 (Botstein *et al.*, 1973). Although most of the coding capacity of *EcoRI* D has been accounted for, gene 3 protein (0.1×10^5 daltons) which is en-

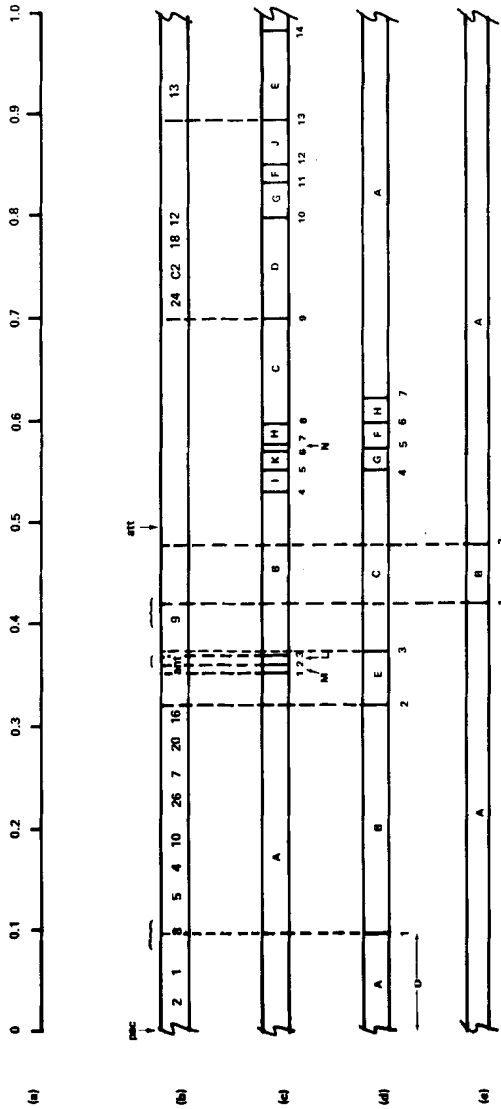


Fig. 5. Physical gene map of P22 DNA. The circular map of restriction endonuclease cleavage sites on P22 DNA (Fig. 2) is broken at *pac* to generate the linear representations here. (a) Physical map coordinates of P22 DNA. (b) Physical gene map of P22 DNA. The P22 genes shown have been located on the restriction fragments defined by the dotted lines as described in the text. A bracket signifies the gene overlaps the cleavage sites indicated. Positions of genes within cleavage fragments are not to scale. (c) *Hind*III cleavage site map of P22 DNA (redrawn from Deans and Jackson (1979) with the order of H and I corrected (Youderian and Susskind, manuscript in preparation)). (d) *Eco*RI cleavage site map of P22 DNA (redrawn from Jackson *et al.* (1978b) with physical map coordinates realigned to originate at *pac*). (e) *Bam*HI cleavage site map of P22 DNA (redrawn from Weinstock, 1977). Genes were placed relative to the cleavage sites shown in (b) as follows. Jackson *et al.* (1978) assigned genes 24-13 to *Eco*RI A and located *pac* at 0 and *att* at 0.496. This work (see text) locates intact genes 2 and 1, and the gene 8 mutant allele 8-*am*N123 on *Eco*RI D; 8-*am*H304 through gene 16 to *Eco*RI B; and 9⁻ alleles *am*H1272 to *am*H1014 (encompassing all 9⁻ amber alleles ordered by genetic mapping; Berget, personal communication) between *Eco*RI site 3 and *Bam*HI site 1 (*Eco*RI/*Bam*HI fragment b). More extensive physical mapping of gene 9 by a different approach (Berget, unpublished experiments) has also shown that most of gene 9 lies in *Eco*RI/*Bam*HI fragment b. However, some Tn1 insertions in *Bam*HI B have been found to inactivate gene 9, indicating that gene 9 spans *Bam*HI site 1 (P. Berget, personal communication; Weinstock, 1977). Weinstock (1977) also mapped Tn1 insertion sites in genes 20 and 16 to *Eco*RI B, and in gene *ant* to *Eco*RI E. Deans and Jackson (1979) mapped the *ant*⁻ Tn1 insertions (Weinstock, 1977) relative to *Hind*III sites, and also determined the approximate locations of genes 24-13 as shown (exact positions of genes 18 and 12 relative to *Hind*III sites 10 and 11 unknown).

coded adjacent to gene 2 in the clockwise direction, could be accommodated. Gene 3 amber mutants do not plate with increased efficiency on Su^- strains carrying pP22-3 (*EcoRI* D insert). However, any gene 3 sequences would be quite close to the junction of P22 and vector sequences, and the frequency of the recombination required to rescue the markers might be very low. The possibility that some gene 3 sequences might be on *EcoRI* D cannot therefore be excluded.

The plating efficiency tests (Table 2) shows that *EcoRI* site 1 is located in gene 8 with most of gene 8 included on *EcoRI* fragment B. Gene 16 is on *EcoRI* B (Table 3), so that all genes which map between 8 and 16 (genes 5, 4, 10, 26, 7, 20; Poteete and King, 1977; Botstein *et al.*, 1972) lie on *EcoRI* B. Weinstock and Botstein (Weinstock, 1977) have isolated Tn1 insertions in genes 20 and 16 and have shown that these insertions are located in P22 *EcoRI* B in agreement with our results.

EcoRI fragment B can code for about 3.4×10^5 daltons of protein, and the sum of the molecular weights of the proteins now known to be encoded on B is 3.3×10^5 . We have no information to indicate whether any sequences corresponding to *sieA*, the next known gene counterclockwise from gene 16 (Susskind *et al.*, 1974), are present on fragment B, but *sieA* is not expressed by the plasmid clone since P22 plates on strains containing pP22-4.

Amber mutants in gene 9 have been placed in six ordered groups by deletion mapping (P. Berget, personal communication). A mutant from the most N-terminal group (9^{-am} H1272) and another mutant from the most C-terminal group (9^{-am} H1014) plate on an Su^- strain carrying pP22-201. Therefore, both these alleles are located on *EcoRI/BamHI* fragment b (Table 2). The coding capacity of this fragment is about 6.7×10^4 daltons, while the molecular weight of the gene 9 protein has been measured as 7.6×10^4 (Botstein *et al.*, 1973). Thus gene 9 is unlikely to be wholly contained on *EcoRI/BamHI* b. Weinstock and Botstein (Weinstock, 1977) and Berget (personal communication) found that four of the Tn1 insertions which inactivate gene 9 are lo-

cated in the *BamHI* B fragment. Berget (unpublished results) has examined deletions extending from gene 20 to various sites in gene 9, which localize most of gene 9 to *EcoRI/BamHI* fragment b, and a small segment of gene 9 to the *BamHI* B fragment. These results, together with the data of Table 2, indicate that *BamHI* site 1 lies in gene 9, probably near the C-terminal end of the gene. The distance between the end of gene 9 and the *att* site is therefore less than 10% of the P22 genome, or a maximum of 4.2 kb, about half the size of the analogous nonessential region of the λ chromosome (Davidson and Szybalski, 1971).

The physical gene map of the P22 chromosome in Fig. 5 summarizes the positions of late genes relative to *EcoRI*, *HindIII*, and *BamHI* sites. Comparison of this map with the recombination frequency map of P22 (Botstein *et al.*, 1972) shows that distances in the late region of the recombination frequency map are distorted relative to the physical gene map. Genes 2 through 16 represent about 50% of the recombination frequency map, but only about 30% of the physical gene map (Fig. 5). Most of the expansion of the recombination frequency map occurs in genes 3, 2, 1, 8, and 5. Since the molecular ends of mature P22 chromosomes fall in this portion of the map (Jackson *et al.*, 1978), these genes are often located in a terminally redundant region of the mature chromosome. In a cross between phages mutant in this region, a significant proportion of the progeny will be heterozygotes which will score as wild-type recombinants in a standard cross, thus increasing the apparent recombination frequency.

Genetic recombination and complementation between cloned fragments of P22 DNA and phage mutants is a simple and powerful way to correlate the physical and genetic maps of the P22 chromosome. As demonstrated here for the case of gene 8, this method can be used to map individual mutational alleles in a single gene relative to restriction sites, thereby generating a physical map of a single gene. Mutations in many P22 genes are available, and 33 restriction targets have already been mapped on P22 DNA (Fig. 2). The avail-

ability of fragments cloned in pBR322, a vector for which the nucleotide sequence is known (Sutcliffe, 1978), should facilitate mapping many additional restriction sites in specific regions of P22. Furthermore, the plating efficiency test can be used as a simple method to detect and characterize additional plasmid clones. Thus the methods described here can be applied to facilitate P22 physical gene mapping to a high degree of resolution.

The availability of cloned fragments of P22 DNA will be important to a wide variety of future studies of P22. The use of strains carrying cloned fragments as permissive hosts should be a useful preliminary step in characterization and mapping of new mutants. Appropriate sets of overlapping cloned fragments can be used to quickly screen for mutations in a specific region. Plasmid bearing strains which complement P22 mutants can also serve as permissive hosts for isolation and propagation of classes of phage mutants not previously accessible, such as deletions in essential genes. Complementing strains will also be useful sources for phage protein isolation. Cloned fragments are currently serving in this laboratory as valuable sources of specific DNA segments for use as probes in nucleic acid hybridization experiments. Use of the clones and physical map information described here to generate transcriptional maps of P22, together with measurements of P22 gene function on cloned fragments, will be powerful approaches to studying control of P22 gene function.

ACKNOWLEDGMENTS

We thank David Botstein and Myron Levine who supplied the P22 mutant strains used here, and Peter Berget, Fred Winston and David Botstein who provided access to unpublished data. This work was supported by Grant AI-12369 from the National Institutes of Health. R.J.D. was supported in part by Institutional Research Grant 1N-40Q to the University of Michigan from the American Cancer Society. R.L.C. was supported by Training Grant T32-GM-07544 from the National Institutes of Health.

REFERENCES

- BOLIVAR, R., RODRIGUEZ, R. L., GREENE, P. J., BETLACH, M. C., HEYNEKER, H. L., and BOYER, H. W. (1979). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* 2, 95-113.
- BOTSTEIN, D., CHAN, R. K., and WADDELL, C. H. (1972). Genetics of bacteriophage P22. II. Gene order and gene function. *Virology* 49, 268-282.
- BOTSTEIN, D., and HERSKOWITZ, I. (1974). Properties of hybrids between *Salmonella* phage P22 and coliphage λ . *Nature (London)* 251, 584-589.
- BOTSTEIN, D., LEW, K. K., JARVIK, V., and SWANSON, C. A., JR. (1975). Role of antirepressor in the bipartite control of repression and immunity by bacteriophage P22. *J. Mol. Biol.* 91, 439-462.
- BOTSTEIN, D., WADDELL, C. H., and KING, J. (1973). Mechanism of head assembly and DNA encapsulation in *Salmonella* phage P22. I. Genes, proteins, structures and DNA maturation. *J. Mol. Biol.* 80, 669-695.
- CAMERON, J. R., PANASENKO, S. M., LEHMAN, I. R., and DAVIS, R. W. (1975). *Proc. Nat. Acad. Sci. USA* 72, 3416-3420.
- CAMPBELL, J. L., RICHARDSON, C. C., and STUDIER, F. W. (1978). Genetic recombination and complementation between bacteriophage T7 and cloned fragments of T7 DNA. *Proc. Nat. Acad. Sci. USA* 75, 2276-2280.
- CLEWELL, D. B. (1972). Nature of ColE1 plasmid replication in the presence of chloramphenicol. *J. Bacteriol.* 110, 667-676.
- DAVIS, R. W., SIMON, M., and DAVIDSON, N. (1971). Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. In "Methods in Enzymology" (L. Grossman and K. Moldave, eds.), vol. 21, pp. 413-428. Academic Press, New York.
- DAVIDSON, N. and SZYBALSKI, W. (1971). Physical and chemical characteristics of lambda DNA. In "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 45-82. Cold Spring Harbor Press, Cold Spring Harbor, N. Y.
- DEANS, R. J. (1979). "Physical Map of Phage P22: Generation and Characterization of Specific Fragments of the P22 Chromosome." Ph.D. thesis, The University of Michigan.
- DEANS, R. J., and JACKSON, E. N. (1979). Restriction endonuclease *Hind*III cleavage site map of bacteriophage P22. *Virology* 95, 359-372.
- DELEYS, R. J., and JACKSON, D. A. (1976). Electrophoretic analysis of covalently closed SV40 DNA: Boltzman distribution of DNA species. *Nucleic Acids Res.* 3, 641-652.
- DENHARDT, D. T. (1966). A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* 23, 641-646.
- FIERS, W., CONTRERAS, R., HAEGEMAN, A., ROGIER, R., VANDE VOORDE, A., VAN HEUVERSWYN, H., VAN HERREWEGHE, J., VOLCKAERT, A., and YSEBAERT, M. (1978). Complete nucleotide sequence of SV40 DNA. *Nature (London)* 273, 113-120.

- GRUNSTEIN, M., and HOGNESS, D. S. (1975). Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene. *Proc. Nat. Acad. Sci. USA* **72**, 3961-3965.
- JACKSON, E. N., JACKSON, D. A., and DEANS, R. J. (1978a). *EcoRI* analysis of bacteriophage P22 DNA packaging. *J. Mol. Biol.* **118**, 365-388.
- JACKSON, E. N., MILLER, H. I., and ADAMS, M. L. (1978b). *EcoRI* restriction endonuclease cleavage site map of bacteriophage P22 DNA. *J. Mol. Biol.* **118**, 347-363.
- KING, J., HALL, C., and CASJENS, S. (1978). Control of the synthesis of phage P22 scaffolding protein is coupled to capsid assembly. *Cell* **15**, 551-560.
- LEDERBERG, E. M., and COHEN, S. N. (1974). Transformation of *S. typhimurium* by plasmid deoxyribonucleic acid. *J. Bacteriol.* **119**, 1072-1074.
- LEVINE, M., TRUESDELL, S., RAMAKRISHNAN, T., and BRONSON, M. J. (1975). Dual control of lysogeny by bacteriophage P22: An antirepressor locus and its controlling elements. *J. Mol. Biol.* **91**, 421-438.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor Press, Cold Spring Harbor, N. Y.
- MURRAY, N. E., and MURRAY, K. (1974). Manipulation of restriction targets in phage λ to form receptor chromosomes for DNA fragments. *Nature (London)* **251**, 476-481.
- POTEETE, A. R., and KING, J. (1977). Functions of two new genes in *Salmonella* phage P22 assembly. *Virology* **76**, 725-739.
- RIGBY, P. W., DIECKMANN, M., RHODES, C., and BERG, P. (1977). Labelling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**, 237-251.
- SOUTHERN, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 503-517.
- SUSSKIND, M. M., and BOTSTEIN, D. (1975). Mechanism of action of *Salmonella* phage P22 antirepressor. *J. Mol. Biol.* **98**, 413-424.
- SUSSKIND, M. M., BOTSTEIN, D., and WRIGHT, A. (1974). Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. III. Failure of superinfecting phage DNA to enter *stx* A⁺ lysogens. *Virology* **62**, 350-366.
- SUTCLIFFE, J. A. (1978). pBR322 restriction map derived from the DNA sequence: Accurate size markers up to 4361 nucleotide pairs. *Nucleic Acids Res.* **5**, 2721-2728.
- THOMAS, M., CAMERON, J. R., and DAVIS, R. W. (1974). Viable molecular hybrids of bacteriophage λ and eukaryotic DNA. *Proc. Nat. Acad. Sci. USA* **71**, 4579-4583.
- THOMAS, M., and DAVIS, R. W. (1975). Studies on the cleavage of bacteriophage lambda DNA with *EcoRI* restriction endonuclease. *J. Mol. Biol.* **91**, 315-328.
- TYE, B. K., HUBERMAN, J. A., and BOTSTEIN, D. (1974). Non-random circular permutation of phage P22 DNA. *J. Mol. Biol.* **85**, 501-532.
- ULLRICH, A., SHINE, J., CHIRGWIN, J., PICTET, R., TISCHER, E., RUTTER, W. J., and GOODMAN, H. M. (1977). Rat insulin genes: Construction of plasmids containing the coding sequences. *Science* **196**, 1313-1319.
- WEINSTOCK, G. M. (1977). "Genetic and Physical Studies of Bacteriophage P22 Genomes Containing Translocatable Drug Resistance Elements." Ph.D. thesis, Massachusetts Institute of Technology.