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Construction of the Genetic Map of the Polyoma Genome

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Seven early mutants, three late mutants, and one plaque morphology mutant of polyoma have been mapped by marker rescue using wild-type restriction endonuclease fragments. The early mutants map between 1.0 and 26.4 units from the *Eco* RI site, a region previously shown to correspond to the 3'-OH terminal half of "early" RNA (Kamen et al., 1974). The late mutants as well as the plaque morphology mutant map between 26.6 and 45.4 map units, a region previously shown to correspond to the 3'-OH terminal half of "late" RNA (Kamen et al., 1974). Analysis of the genotype of rescued virus demonstrated that the modification of the mutant DNA during marker rescue was limited to the region of the genome covered by the wild-type restriction endonuclease fragment tested.

Temperature-sensitive mutants of the oncogenic papovavirus, polyoma, have been isolated and characterized (2-4, 7, 8). These mutants generally fall into two classes: (i) early mutants that are blocked in viral DNA synthesis (6, 7) at the restrictive temperature, and (ii) late mutants that do not form infectious particles although viral DNA is synthesized at the restrictive temperature (2-4). Genetic complementation analysis of the mutants has provided some further subdivisions in this classification scheme (2-4), but a detailed knowledge of the genetic organization of polyoma virus has remained elusive due to low recombination frequencies (14) and lack of sufficiently distinct conditional-lethal mutation types for genetic mapping by three-factor genetic crosses.

The genetic mapping technique of marker rescue is a novel one since it involves the biochemical manipulation of the viral DNA. The technique relies on the ability of a fragment of wild-type DNA, which has no infectivity by itself, to produce wild-type virus when annealed to a full-length mutant DNA and introduced to a cell. This will be referred to as a "rescue" event, but it is important to note that the original mutant DNA is converted to a wild-type DNA by cellular mechanisms.

The rescue of wild-type virus from transfections with heteroduplex DNA formed by annealing wild-type DNA fragments to conditional-lethal mutant viral DNA was first observed in ϕX174 studies (13, 28). The marker rescue technique has been shown to be an effective method for mapping mutants of simian virus 40 (SV40) (16-18). The marker rescue procedure relies on the ability to produce specific fragments of viral DNA by cleavage with restriction endonucleases, to purify the fragments by gel electrophoresis, and to know the physical order of the fragments within the genome.

A number of restriction endonucleases cleave polyoma DNA into discrete fragments, the physical order of which has been determined (10, 11; B. E. Griffin, unpublished data). In this report, individual restriction endonuclease fragments (RENFs) were tested for their ability to rescue a variety of early and late ts mutants. A genetic map of these mutations was constructed from the results. In addition, we tested the ability of fragments to convert a minute-plaque mutant of polyoma to the large-plaque type, and the results demonstrate that this mutation is located in the "late" region of the genome.

MATERIALS AND METHODS

Viruses. All virus stocks were prepared from single plaques and were grown at low multiplicities as previously described (9). The early mutant tsa and the late mutant tsC were derived from the Pasadena large-plaque strain by M. Fried (7; M. Fried, unpublished data). The CR variant was derived from the Pasadena large-plaque strain (Fried, unpublished data). The early mutants ts25, ts616, ts48, ts609, and ts697 and the late mutants ts10 and ts1260 were provided by W. Eckhart (4 and references therein). The ts697 mutant was derived from the P16 small-plaque strain, and the others were derived from the Pasadena large-plaque strain. The minute-plaque mutant, 208, was derived from the Pasadena large-plaque strain and provided by M. Vogt.

Cells and plaque assay. Monolayers of secondary whole mouse embryo cells were grown in Dulbecco modified Eagle medium supplemented with 5% fetal

calf serum for viral DNA preparation or 3% horse serum for virus stock production or plaque assay. Plaque assays were performed as previously described (8).

Preparation of polyoma DNA. All viral DNAs were prepared by Hirt extraction (12) of infected cells followed by CsCl-ethidium bromide equilibrium density centrifugation (23). The supercoiled form I (FI) polyoma DNA was further purified by neutral sucrose gradient centrifugation. Random, singly nicked form II (FII) DNA was prepared by X irradiation of FI to nick approximately 30% of the molecules. The FII singly nicked DNAs were purified from the remaining FI DNA by CsCl-ethidium bromide equilibrium density centrifugation.

Preparation of RENFs of DNA. RENFs were prepared from ^{3}H-labeled FI DNA except the *Hpa*II fragments, which were prepared from ^{32}P-labeled FI DNA. The ^{3}H-labeled fragments were purified by electrophoresis through 1.4% agarose tube gels, 20 cm in length. The DNA fragments were visualized by ethidium bromide staining (21, 24), cut individually from the gels, and removed from the agarose by electrophoresis. The fragments were judged by gel electrophoresis to be more than 90% pure. The ^{32}P-labeled *Hpa*II fragments (a gift of B. E. Griffin) were purified by electrophoresis on 3.3% polyacrylamide plus 0.5% agarose slab gels, located by autoradiography, sliced from the gels, and removed from the gel slices by electrophoresis. Each fragment was further purified by neutral sucrose gradient centrifugation and checked for purity by gel electrophoresis.

Restriction endonucleases and incubation conditions. The restriction endonuclease *Hind*III was prepared by procedures similar to those described previously (27). The *Hha*I enzyme was purified by the unpublished procedure of R. Roberts and P. Myers (personal communication). The *Hpa*II and *Eco* RI enzymes were kindly provided by B. Griffin and R. Kamen. The buffers used for enzyme digestions were: *Hha*I and *Hpa*II, 10 mM Tris-hydrochloride (pH 7.5)-10 mM $MgCl_2$-1 mM dithiothreitol; *Hind*III, same as *Hha*I and *Hpa*II + 50 mM NaCl; *Eco* RI, 10 mM Tris-hydrochloride (pH 7.5)-10 mM Mg_2Cl-100 mM NaCl. Incubations were at 37 C for a sufficient time and with sufficient enzyme for complete digestion. The cleavage sites of these endonucleases are shown in Fig. 2 (adapted from reference 11).

Transfection of cells with polyoma DNA. Near-confluent monolayers of secondary whole mouse embryo cells in 35-mm petri dishes were washed once with 2 ml of Dulbecco phosphate-buffered saline minus Mg^{2+} and Ca^{2+} (PBSA). A 0.4-ml aliquot of 1 mg of DEAE-dextran per ml of PBSA was distributed over the cell monolayer and incubated at 32 C for 20 min. The excess DEAE-dextran was removed, and a 0.3-ml solution of polyoma DNA in PBSA was distributed over the cell monolayer. The cells were incubated for approximately 30 min at 32 C, after which 1.5 ml of Dulbecco modified Eagle medium supplemented with 3% horse serum and receptor-destroying enzyme (8) was added. The cells were incubated at 32 C for approximately 6 days. Freeze-thawing followed by 0.5 min of gentle sonication was used to break open the infected cells.

Formation of heteroduplex DNAs. Heteroduplex DNAs were formed by combining random, singly nicked, FII mutant polyoma DNA, RENFs produced by cleavage of FI mutant DNA, and a single, purified RENF of wild-type polyoma DNA. In the first series of experiments (using *Hpa*II fragments), the molar ratios of the various DNA types were 1:10:approximately 250. In other experiments (using *Hind*III, *Hha*I, and *Hha*I + *Eco* RI fragments), the molar ratios of the DNAs were 1:4:40 (mutant FII DNA:RENFs containing sequences of the entire mutant genome:purified wild-type RENF). To a 40-μl volume containing 0.0125 μg of mutant FII DNA and the appropriate concentrations of mutant and wild-type fragments dissolved in 10 mM Tris-hydrochloride (pH 7.5) and 1 mM EDTA, 10 μl of 2 M NaOH was added. After standing at room temperature for 20 min, the solution was neutralized by the addition of 20 μl of a buffered HCl solution (1 volume of 2 M HCl, 1 volume of 1 M sodium phosphate, pH 7.0). The neutralized solution was incubated at 68 C for 30 min. Under these hybridization conditions, at least 80% of the full-length DNA molecules are in the double-stranded form; approximately four out of five full-length double-stranded DNA molecules contain one fragmented strand. After hybridization, 0.1 ml of distilled water and 0.45 ml of PBSA were added, and the solution was frozen until used for transfection of cells. The DNA hybridized in this manner showed a specific infectivity of 2×10^5 PFU per μg of full-length FII DNA by direct DEAE-dextran transfection assay at 32 C. Untreated FI or FII DNA has a specific infectivity of approximately 1×10^6 PFU per μg of DNA in DEAE-dextran transfection assays at 32 C.

RESULTS

Experimental design. The experimental design for genetic mapping of polyoma mutants by marker rescue is schematically presented in Fig. 1. Heteroduplexes were formed by combining, denaturing, and reannealing mutant (ts or minute plaque) FII DNA, a single purified wild-type RENF, and the full complement of mutant RENFs produced by restriction endonuclease cleavage of mutant FI DNA. The mutant RENFs were added for two reasons: (i) to prevent the reannealing of full-length mutant DNA strands of the added mutant FII DNA, and (ii) to minimize the enhancement of marker rescue by nonspecific fragments that Lai and Nathans observed (16). The results of Lai and Nathans demonstrated that a partial heteroduplex composed of a full-length mutant DNA and one wild-type restriction fragment was less effective in marker rescue than a partial heteroduplex with more than one wild-type restriction fragment. This resulted in an anomalous enhancement of infectivity by nonspecific fragments, leading to some confusion as to

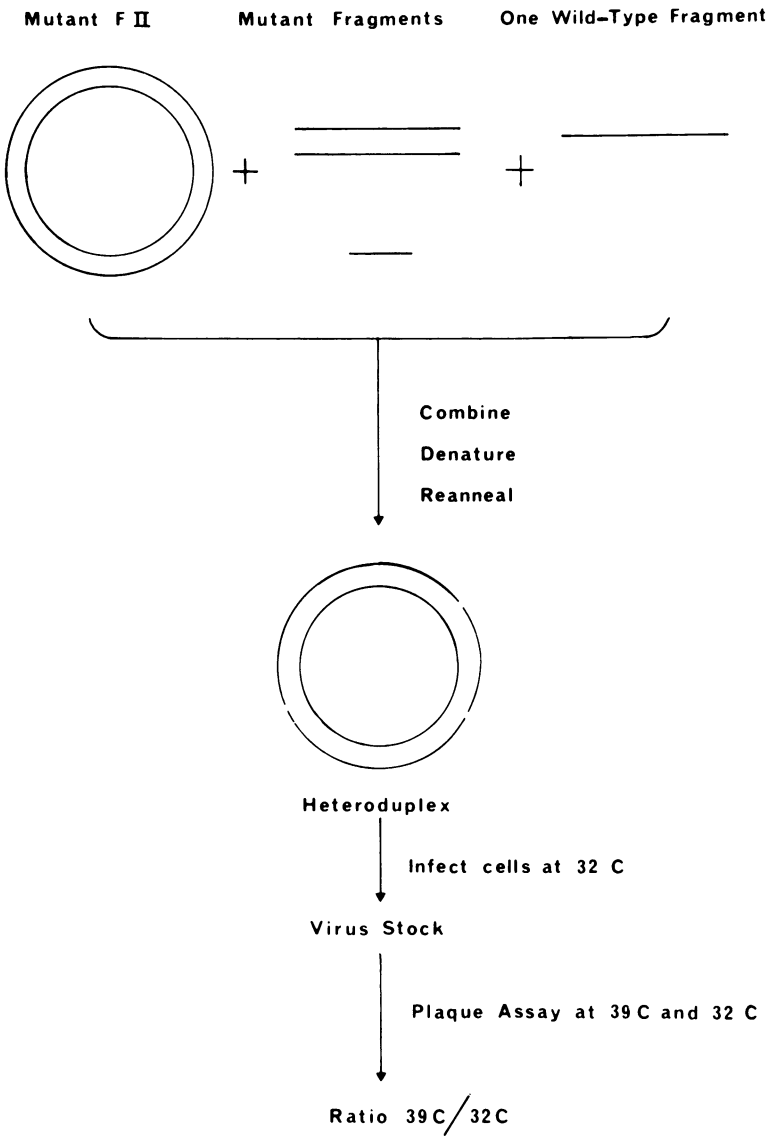

FIG. 1. *Experiment design for marker rescue. Mutant FII DNA was combined with the full complement of mutant restriction endonuclease fragments, produced by restriction nuclease digestion of FI mutant DNA, and a single, purified wild-type restriction nuclease fragment. The ratios of these three DNA components were adjusted to permit (i) the single wild-type fragment to compete successfully with a mutant fragment, and (ii) the fragments to compete successfully with the full-length mutant DNA during the reannealing reaction. The three DNA components were denatured and reannealed to allow the formation of heteroduplex circular DNA composed of one full-length mutant DNA and a complementary strand composed of one wild-type fragment and the remaining mutant fragments. Whole mouse embryo cells were infected with this DNA at 32 C in the presence of receptor-destroying enzyme to inhibit secondary infections. The virus stock obtained from this infection (see Materials and Methods) was assayed for plaque-forming ability at 39 and 32 C. The wild-type restriction endonuclease fragment resulting in the highest 39 C/32 C ratio corresponds to the mutated region of the mutant DNA tested.*

which wild-type fragment corresponded to the mutant region. We attempted to minimize this enhancement effect by starting with a full heteroduplex DNA.

The molar ratios of the three DNA types used to form the heteroduplex were balanced in a manner that (i) permitted the wild-type RENF to compete successfully with the corresponding

mutant RENF so that at least 9 out of 10 heteroduplexes contained the wild-type RENF, and (ii) permitted the RENFs to compete successfully with full-length DNA derived from the mutant FII DNA to enhance formation of heteroduplexes rather than homoduplexes.

Cells infected with the heteroduplex DNAs were incubated at 32 C in the presence of receptor-destroying enzyme, which inhibits reinfection by the newly formed virus (8). This provided a stock of mutant and wild-type virus, which was then plaque assayed at the permissive and nonpermissive temperatures (32 and 39 C). In the case of the minute-plaque virus, 208, the plaque assays were performed at 37 C, and the number of large-plaque viruses appearing in the presence of minute plaques was scored. The ratio of PFU assayed at 39 C to the PFU assayed at 32 C (referred to as the 39 C/32 C ratio) indicated which RENF corresponded to the ts region of the genome. Similarly, the ratio of large plaque to minute plaque at 37 C indicated which RENF corresponded to the region of the genome responsible for the plaque morphology of the mutant tested.

Marker rescue with *Hpa*II fragments. In the first experiment for genetically mapping polyoma mutants, the eight *Hpa*II RENFs of wild-type DNA were individually tested for their ability to "rescue" the early mutant tsa, the late mutants ts10, ts1260, tsC, and the plaque morphology mutant 208 (Table 1). For the early mutant, tsa, the most active fragment in marker rescue was *Hpa*II fragment 2 (*Hpa*II-2). For the late mutants ts10, ts1260 and tsC as well as the plaque morphology mutant 208, the most active fragment for marker rescue was *Hpa*II-1. These results, however, are not completely satisfactory since other fragments showed unusually high ratios (backgrounds). The results with tsC are particularly ambiguous. The fragments that showed high backgrounds were usually those that migrated just below the most positive fragment.

For instance, in the tsa case, fragment 3 gave a ratio approximatley one-third the ratio for the most positive fragment (*Hpa*II-2), whereas *Hpa*II fragments 2 through 4 gave high backgrounds when ts10 was tested. In the cases of ts10 and tsC, the *Hpa*II-4 fragment gave a high background. Although we have no complete explanation for this observation, our results using other RENFs demonstrate that the mutant region of these two mutants is not located in the *Hpa*II-4 region. The problem of high background was probably due to impurities of fragment preparations. Since the *Hpa*II fragments were greater than 90% pure as judged by gel electrophoresis in the presence of ethidium bromide and the method of heteroduplex formation used was designed to minimize the effect of nonspecific fragment enhancement of marker rescue, we considered that the effects of small contaminations in the fragment preparation were accentuated by the high ratios of fragments to mutant FII DNA used to form the heteroduplexes. In further experiments (Tables 2-5), we used molar ratios of mutant FII DNA to mutant RENFs to wild-type RENF of 1:4:40 in forming heteroduplex DNAs rather than ratios of 1:10:250 used in the *Hpa*II experiments of Table 1. This change generally improved the differences between the 39 C/32 C ratios of the various fragments (Tables 2-5). However, in some cases we still observed high backgrounds, and we suspect that this was due to the nonspecific fragment enhancement effect reported by Lai and Nathans (16), even though we attempted to minimize this effect in our experimental design.

TABLE 1. *HpaII*

Fragment	(39 C/32 C) or (LP/MP) × 100[a]				
	tsa	ts10	ts1260	tsC	208
1	1.0	33*[b]	17*	19*	6.9*
2	7.1*	2.5	6.7	12	2.5
3	2.2	6.7	3.3	2.3	0.5
4	<1.0	7.1	1.7	11.0	0.5
5	<0.5	0.6	0.3	2.0	<1.3
6	<0.1	0.6	<0.3	2.0	<1.3
7	<0.1	0.5	<0.3	<0.9	<1.6
8	<0.1	<0.7	<0.3	<1.0	<2.0
No	<0.1	<0.2	<0.3	<1.0	<0.8

[a] Values are averages of at least two plates for at least three dilutions over a range of two orders of magnitude for both 39 C and 32 C. LP, Large plaque; MP, minute plaque.
[b] Asterisk indicates the most positive response.

Marker rescue with *Hind*III fragments. The two fragments produced by cleavage of polyoma DNA with *Hind*III (11) were tested for their ability to rescue a variety of polyoma mutants. Every mutant tested was preferentially rescued by the smaller of the two fragments, which contained approximately 44% of the polyoma genome (Table 2). These results are consistent with the *Hpa*II results (Table 1) since the *Hind*III-44% fragment contained large segments of both the *Hpa*II-1 and *Hpa*II-2 fragments, and demonstrate a clustering of early and late mutations into a restricted region (44%) of the polyoma genome. These results have been further confirmed by testing fragments produced by cleavage with *Hha*I restriction endonuclease (see below).

Marker rescue with *Hha*I + *Eco* RI fragments. Digestion of polyoma wild-type DNA with a combination of *Hha*I and *Eco* RI restric-

TABLE 2. *HindIII*

(A)

Mutant	Fragment (%)	Avg PFU × 10^{-4}/ml		(39 C/32 C) × 100	44%/56%[a]
		39 C	32 C		
Early					
tsa	44	4.0	45	8.9*[b]	>44.5
	56	<0.05	25	<0.2	
ts25	44	3.2	20	16.0*	4.2
	56	0.80	21.5	3.8	
ts616	44	3.0	24	12.5*	4.8
	56	0.6	23	2.6	
ts48	44	0.25	6.5	3.8*	76.0
	56	0.005	10.5	0.05	
ts52	44	1.0	17.5	5.7*	35.6
	56	0.025	15.5	0.16	
ts609	44	1.20	12.5	9.6*	96.0
	56	0.005	4.0	0.1	
ts697	44	0.50	60.0	0.83*	>100
	56	0.005	60.0	<0.008	
Late					
ts10	44	11.5	70	16.4*	5.1
	56	0.8	25	3.2	
ts1260	44	2.2	25	8.8*	7.3
	56	0.3	25	1.2	
tsC	44	4.5	30	15.0*	4.8
	56	0.2	6.5	3.1	
Controls					
Wild-type 44% only		<0.005	<0.005		
Wild-type 56% only		<0.005	<0.005		
Mutant DNA, no wild-type *Hind*III RENF		<0.005	<0.005		

(B)

	Fragment	LP[c]	MP[d]	(LP/MP) × 100	44%/56%
Plaque morphology					
208	44	6.0	40	15.0*	10.7
	56	0.7	50	1.4	

[a] The wide variation in the 44%/56% ratios reflects, to some extent, variations in the purity of the different fragment preparations used to test each mutant. However, differences in virus strains and in the type or location of the mutation may also affect these ratios.

[b] Asterisk indicates most positive response.

[c] LP, Large plaque.

[d] MP, Minute plaque.

tion endonucleases produces four fragments approximately 46, 28, 14, and 12% (Fig. 2 and reference 10). These fragments are designated *Hha*I/*Eco* RI-1,-2,-3, and -4 in order of decreasing size.

The results of marker rescue using these *Hha*I + *Eco* RI fragments are shown in Table 3. Of the early mutants, tsa was rescued by fragment 3, whereas ts25 and ts616 were rescued almost equally well by fragments 3 and 4. Since it is relatively difficult to purify these two small fragments, another approach was used to demonstrate that the sequences in fragment 4 rather than sequences in fragment 3 correspond to the mutant regions of ts25 and ts616 (see below).

The late mutants ts10, ts1260, and tsC as well as the plaque morphology mutant 208 were rescued by fragment 1 preferentially. Combining the results that these late mutants can be rescued with the *Hind*III-44% fragment, the *Hpa*II-1 fragment, and the *Hha*I/*Eco* RI-1 fragment, the location of the mutant regions of the genome can be placed between approximately 26.6 and 45.4 map units from the *Eco* RI site.

Marker rescue with *Hha*I CR fragments. For further analysis of the early mutants, we measured the marker rescue ability of two fragments produced by *Hha*I cleavage of a polyoma variant, CR. This variant lacks the *Hha*I cleavage site at 26.4 map units from the *Eco* RI site (M. Griffiths and C. Barry, unpublished

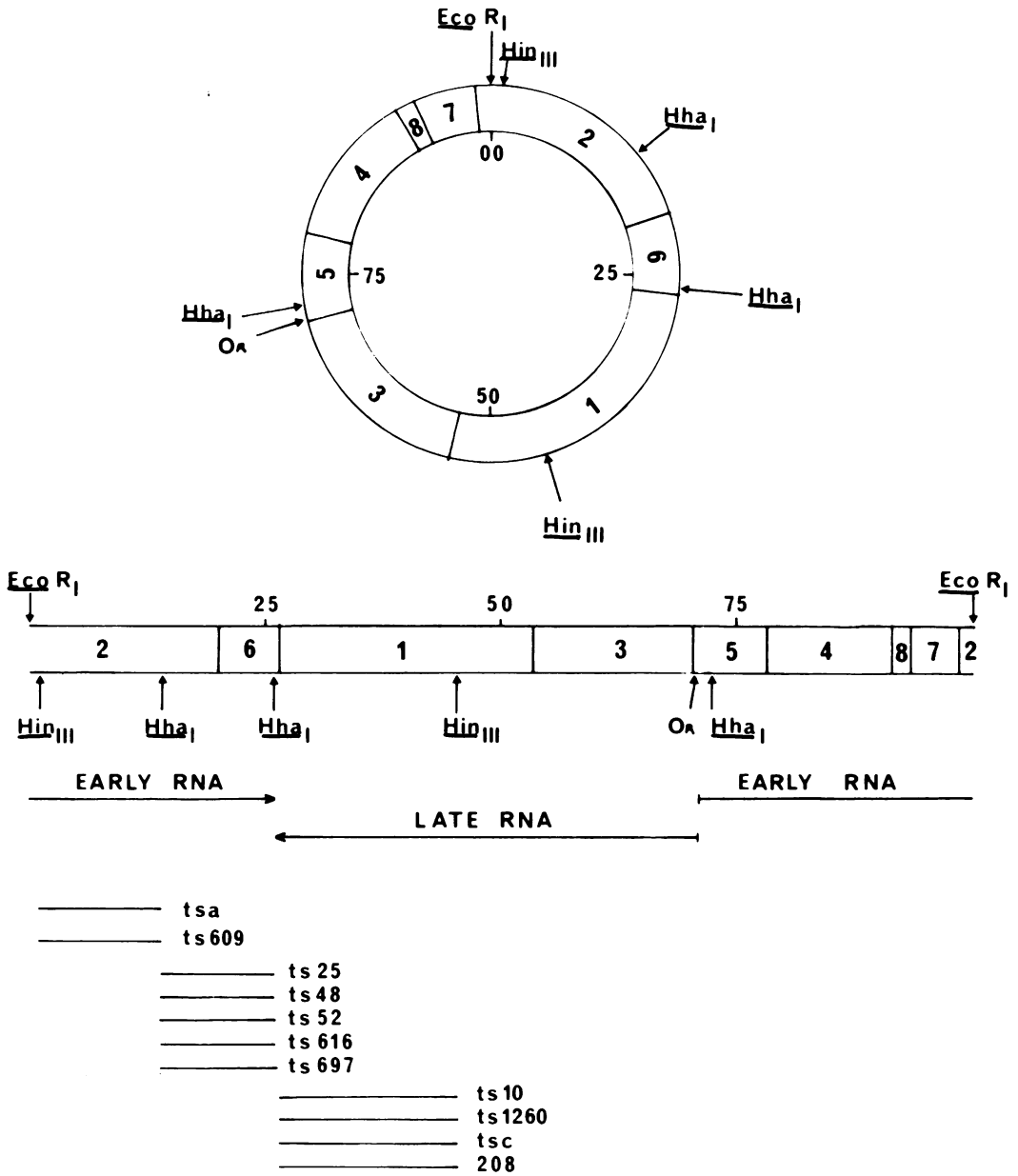

FIG. 2. *Physical and genetic map of the polyoma DNA genome. At the top is a circular physical map of the polyoma DNA genome (10, 11) showing the cleavage sites of certain restriction endonucleases from Escherichia coli (Eco RI), Haemophilus influenzae D (HinIII), and H. haemolyticus (HhaI). The position of the origin of DNA replication is denoted by (O_R). Within the concentric circles are the positions of the cleavage sites of a restriction endonuclease from H. parainfluenzae (HpaII). The arrangement of the eight HpaII fragments, numbered in order of decreasing size, is shown. The map units, with the Eco RI site at 00, are marked in the center of the circle; the 100 map units correspond to approximately 5,200 nucleotides. Below the circular map is a linear representation of the same physical map. The regions corresponding to early and late mRNA transcripts are designated by lines, the arrows designating the direction of transcription (15). Finally we have denoted the positions of the mutant sites of tsa, ts609, ts25, ts48, ts52, ts616, ts697, ts10, ts1260, tsC, and 208 with respect to the physical map.*

TABLE 3. *HhaI + Eco RI*

Mutant	(39 C/32 C) × 100			
	1	2	3	4
Early				
tsa	0.2	1.0	7.0*[a]	1.0
ts25	0.5	1.7	4.6*	6.7*
ts616	0.12	1.5	7.8*	5.0*
Late				
ts10	8.7*	2.4	1.4	1.1
ts1260	3.5*	0.3	0.1	<0.1
tsC	8.5*	3.7	1.4	1.0
Plaque morphology				
208	57*[b]	20	<7	<7

[a] Asterisk indicates the most positive response.
[b] Data for plaque morphology mutant 208 are expressed as (large plaque/small plaque) × 100.

data). Thus, *Hha*I cleavage of CR generates two fragments, one approximately 58% of the genome (designated *Hha*I CR-58%) and the other 42% of the genome (designated *Hha*I CR-42%). The results of marker rescue with the *Hha*I CR fragments are shown in Table 4. Some of the early mutants were rescued by *Hha*I CR-58% (e.g., ts25, ts48, ts52, ts697, and ts616), whereas others were rescued by *Hha*I CR-52% (e.g, tsa and ts609). It is therefore possible to distinguish different mapping regions for early mutants by using these fragments.

Marker rescue by *Hha*I fragments. To further locate those early mutants that mapped in the *Hha*I CR-58% fragment, the marker rescue ability of *Hha*I fragments derived from the A-2 strain of polyoma was tested. Three *Hha*I fragments are produced from this strain: (i) a 46% fragment, *Hha*I-46%; (ii) a 42% fragment, *Hha*I-42%; and (iii) a 12% fragment, *Hha*I-12% (10). To simplify the testing, we combined the 46 and 42% fragments and tested them collectively, since our purpose was primarily to distinguish which region of the *Hha*I CR-58% region was responsible for marker rescue of certain early mutants (see Table 4).

Table 5 presents the results of marker rescue of early mutants by either the combined *Hha*I-46% and *Hha*I-42% fragments or the *Hha*I-12% fragment. Those early mutants that previously were rescued by *Hha*I CR-58% (ts48, ts52, ts697, ts616, and ts25; see Table 4) were rescued by the *Hha*I-12% fragment of A-2. The early mutants that previously were rescued by *Hha*I CR-46% (tsa and ts609; see Table 4) were rescued by the combined *Hha*I-46% and *Hha*I-42% fragments as expected.

Genotypic analysis of the viral DNA produced by marker rescue. The marker rescue technique presumably involves only the conversion of the ts region of the mutant genome to the wild-type form and should not affect regions of the mutant genome outside the region of the wild-type fragment used to rescue the mutant DNA. This hypothesis is testable since tsa DNA is missing an *Hae*III cleavage site, which is present in the A-2 wild-type DNA. The tsa DNA lacks the *Hae*III cleavage site between *Hae*III-6 and *Hae*III-20 that is located at 28.0 map units (in *Hpa*II-1), resulting in a slower migration of tsa *Hae*III-6 and the absence of *Hae*III-20 when compared with the *Hae*III fragments of A-2 (Miller and Griffin, unpublished data).

Virus stocks produced from the experiment of wild-type *Hpa*II-2 fragment rescue of tsa DNA (see Table 1) were plaque assayed at 39 C, and nine plaques were picked. The plaques were grown at 39 C to produce virus stocks, and ^{32}P-labeled viral DNA was prepared from infections with each virus stock. The ^{32}P-labeled DNAs were digested with the *Hae*III and electrophoresed through 7% polyacrylamide gels. Nine out of nine plaques picked were shown to have the same *Hae*III cutting pattern as the original tsa DNA, and yet each plaque was no longer temperature sensitive (results not shown). This result demonstrates that marker rescue involves only a limited region of the genome and that those viruses plaquing at 39 C (see Table 1) were most likely derived from modification of the tsa genome within the *Hpa*II-2 region only.

DISCUSSION

A genetic map of the polyoma viral genome has been constructed by using a marker rescue

TABLE 4. *HhaI CR fragments*

Mutant	(39 C/32 C) × 100		Fold difference (58%/42% or 42%/58%)
	58%	42%	
tsa	0.7	3.1*	4.4
ts25	13.8*[a]	1.1	12.5
ts616	5.5*	1.3	4.2
ts48	36.0*	13.0	3.0
ts52	3.1*	0.67	4.6
ts609	0.1	8.5*	85.0
ts697	1.0*	0.27	3.7

[a] Asterisk indicates the most positive response.

TABLE 5. *HhaI fragments*

Mutant	(39 C/32 C) × 100		Fold difference [(46% + 42%)/12% or 12%/(46% + 42%)]
	46% + 42%	12%	
ts48	<0.014	6.15*[a]	439
ts52	0.75	10.6*	14.1
ts609	23.6*	2.5	9.4
ts697	<0.03	2.8*	93

[a] Asterisk indicates the most positive response.

technique. The map is diagrammatically presented in Fig. 2. Since high backgrounds were noted in certain sets of RENFs, we tested the ability of at least three different sets of RENFs to rescue each mutant. The results of each of these sets were consistent, and the results are summarized in Fig. 2.

Of the seven early mutants tested, two (tsa and ts609) are rescued by fragments containing the DNA region between the *Hind*III site at 1.0 map units and the *Hha*I site at 14.0 map units. The other five early mutants (ts25, ts616, ts48, ts52, and ts697) are rescued by fragments containing the DNA region between the *Hha*I site at 14.0 map units and the *Hha*I site at 26.4 map units. Thus the early mutants show a clustering effect in the DNA region between 1.0 and 26.4 map units, which corresponds to the 3'-OH terminal half of the "early" RNA (15). These mutants are of interest since most of them have been demonstrated to be defective in transformation at the restrictive temperature (4, 8).

Of the late mutants, ts10 and ts1260 have been classified into different groups by genetic complementation test (2–4). The third late mutant, tsC (Fried and Griffith, manuscript in preparation), efficiently complements early mutants but appears to show poor complementation with mutants of the other two late groups (W. Eckhart, personal communication) and is therefore reminiscent of the BC group of SV40. Our results indicate that ts10, ts1260, and tsC all map between 26.6 and 45.4 map units. This is the region specified by the 16S late stable mRNA that has been shown to encode the major capsid protein VP1 (26). Thus it would appear that the complementation observed between ts10 and ts1260 represents intragenic complementation and not intergenic complementation. This finding is similar to that observed for SV40, where the mutants from the two late complementing groups (B and C) and the third late group (BC, which complements early mutants but not other late mutants) all map in a region of the SV40 genome that apparently encodes the SV40 major capsid protein VP1 (17, 19, 22).

The plaque morphology characteristics of 208 are determined by the same region of DNA in which the late ts mutants ts10, ts1260, and tsC map (26.6 to 45.4 map units). Hence the minute-plaque morphology of 208 is determined by the major capsid protein VP1 rather than an early gene or altered sequence at the origin of DNA replication. Our results with *Hind*III fragments have been confirmed by an independent mapping technique (20) that involves the in vitro recombination of fragments from large-plaque A_2 and minute-plaque 208 parents (Miller and Fried, unpublished experiments). In these experiments, the hybrid DNA containing the 56% *Hind*III fragment from large-plaque viral DNA and the 44% *Hind*III fragment from 208 DNA produced minute-plaque virus, whereas the hybrid DNA containing the 56% *Hind*III fragment from 208 and the 44% *Hind*III fragment from large-plaque virus produced large-plaque virus upon infection of mouse cells with the hybrid DNAs.

The genetic organization of polyoma virus appears to be very similar to the genetic organization of SV40 determined by Lai and Nathans (17). Mutants of both viruses show a distinct clustering effect in the sense that no mutations have been mapped in approximately 55% of the genome, which comprises roughly half of the early region and half of the late region surrounding the origin of DNA replication. In the case of polyoma, we have yet to test two types of mutants, the host range mutants isolated by Benjamin (1) and the ts-3 mutant described by Eckhart and Dulbecco (5). This latter mutant has similar properties to the class D mutants that Lai and Nathans have mapped in SV40 (17), which lay towards the 5' terminus of the late RNA.

The marker rescue technique is a direct and relatively easy method for constructing a rough genetic map of small, DNA-containing animal viruses. Although more precise determinations of the genetic map positions can be made by using smaller restriction endonuclease fragments, such fragments are more difficult to purify and would be time consuming to test. More precise measurements of map locations of certain mutants of SV40 have been provided by the S_1 nuclease technique of Shenk et al. (25). The S_1 technique, however, can suffer from two major disadvantages: (i) it is purely biochemical and cannot relate base changes to phenotypic differences; and (ii) the mapping procedure apparently cannot distinguish all single-base mismatches and is therefore the method of choice for mapping deletion mutations only.

We have studied certain aspects of the marker rescue technique in greater detail. We have demonstrated that the region of the mutant genome covered by a wild-type restriction endonuclease fragment is converted to wild-type, whereas sequences in another region not covered by the wild-type fragment remain unchanged. The cellular events involved in the marker rescue technique are presently being studied.

Our results demonstrate not only the usefulness of marker rescue for the genetic mapping of ts and plaque morphology mutants of polyoma but also the usefulness of this method in

transferring genetic markers from one virus strain to another.

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