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# Construction and Analysis of Viable Deletion Mutants of Polyoma Virus

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Viable mutants of polyoma with small deletions ranging in size from 2 to 75 base pairs were obtained by infecting 3T3 cells with polyoma DNA that had been cleaved once with *Hae*II endonuclease or with DNase-$Mn^{2+}$ digestion. The *Hae*II endonuclease-cleaved DNA yielded mutants with deletions at map position 72-73, whereas the mutants generated by DNase I-$Mn^{2+}$ digestion had deletions either at map position 72-73 or within the map coordinates 92 and 99. Both groups of mutants appeared to grow as well as wild-type virus in 3T3 cells. The deletions at map position 72-73 did not alter the virus's ability to transform rat cells. Hence, the region just to the early side of the origin of DNA replication is not essential for vegetative growth or transformation. But the mutants with deletions in the region between map coordinates 92 and 99, a segment thought to code for polyoma large and middle T antigens (Hutchinson et al., Cell **15**:65-77, 1978; Smart and Ito, Cell **15**:1427-1437, 1978; Soeda et al., Cell **17**:357-370, 1979), transformed rat cells at 0.2 to 0.05 the efficiency of wild-type virus.

To analyze viral genome function, it is important to know the organization of the protein coding regions as well as those needed for replication, transcription, processing, and translation. With simian virus 40 (SV40), these questions were approached by constructing deletion mutants in vitro (2, 3, 20, 28). The locations of the deletions were mapped with respect to known restriction endonuclease cleavage sites (3, 28) or by determining the precise nucleotide sequence alterations (4, 33-35); then, the phenotypic consequences of these genomic alterations were determined (3).

In this work, a similar strategy was applied to obtain a set of viable deletion mutants of polyoma virus, that is, mutants which can multiply vegetatively in the absence of helper virus in their permissive host, cultured mouse cells. The existence and phenotype of one such group of mutants indicates that there is a region on the early side of the origin of DNA replication (map position 72-73) which is dispensable for vegetative multiplication and cellular transformation. Another set of mutants with deletions within the region 92-99, a segment believed to code for amino acid sequences in polyoma large and middle T antigens (17, 29, 31), also failed to affect the virus's ability to multiply. However, these deletions markedly reduced the virus's capacity to transform cultured rat 1 cells.

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## MATERIALS AND METHODS

**Cells and virus.** All polyoma virus stocks were prepared in secondary mouse embryo cells (Flow Laboratories) grown in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum. After infection, cultures were incubated with DMEM containing 5% horse serum. Complete lysis of the infected cells generally occurred within 2 weeks. Virus was released from cell debris by sonication and chloroform treatment (24). Plaque assays with either polyoma virus or DNA were done on monolayers of 3T3 mouse cells as described previously (24). After infection, the monolayers were overlaid with DMEM containing 3% horse serum, 0.4 μg of dexamethasone per ml (26), and 0.75% agar (Bacto; Difco). Transformation assays were done by infecting subconfluent cultures of rat 1 cells (obtained from T. Ege); after 6 h the cultures were trypsinized, and $10^4$ or $10^5$ cells were seeded in DMEM containing 5% fetal bovine serum, 0.33% agar (Noble; Difco), and antipolyoma rabbit serum. Transformants were scored as colonies visible after 2 weeks. Under these conditions, uninfected rat 1 cells do not form any colonies. The wild-type parent of the polyoma deletion mutants described here was a plaque-purified derivative of the polyoma A-2 large-plaque strain (13).

**Preparation of polyoma DNA.** Covalently closed circular viral DNA was selectively extracted (15) from 3T6 cells that had been infected at a multiplicity of 5 to 10 PFU/cell. The DNA was purified further by centrifugation to equilibrium in CsCl-ethidium bromide gradients and sedimentation in 5 to 20% neutral sucrose gradients (22).

**Enzymes and their use.** Most restriction enzymes were purchased from New England BioLabs. *Mno*I, an isoschizomer of *Hpa*II, was obtained from Stephen Goff. Bacteriophage λ 5'-exonuclease, obtained ac-

cording to Little et al. (21), was used to digest the 5′ ends of linear polyoma DNA as previously described (2). Random cleavage across both strands of polyoma (I) DNA was achieved by digestion with DNase I under the following conditions: 100 μg of DNA per ml in 10 mM Tris-hydrochloride (pH 7.6), 1 mM $MnCl_2$, 100 μg of bovine serum albumin per ml, and 5 μg of DNase I per ml for 20 min at 25°C. The reaction was terminated by the addition of EDTA (to 20 mM) and sodium dodecyl sulfate (to 0.1%).

**Agarose and polyacrylamide gel electrophoresis.** Linear polyoma DNA was separated from circular forms by two cycles of electrophoresis in agarose slab gels (14 by 14 cm) (3). The first separation was performed in 1.0% agarose, and the region containing the linear DNA, localized by its fluorescence in the presence of ethidium bromide, was cut out of the gel and recast into a 1.5% agarose gel. After the second electrophoretic separation, the DNA was electrophoresed out of the agarose, purified by adsorption and elution from DEAE-cellulose (1 M NaCl), and concentrated by ethanol precipitation.

Restriction endonuclease cleavage digests were analyzed by electrophoresis in polyacrylamide gradient gels as previously described (3).

## RESULTS

**Construction of deletion mutants.** Linear SV40 DNA can be recircularized and subsequently replicated as circular DNA after transfection of monkey cells. In the absence of cohesive ends, a variable number of nucleotides are lost from the ends of the linear molecules during the circularization process, thereby creating mutant viral genomes with deletions ranging in size from about 5 to 200 base pairs (2, 3, 28).

The same approach was used to construct deletion mutants of polyoma virus. Covalently closed polyoma DNA was cleaved at map position 72 with *Hae*II endonuclease or at random sites with DNase I in the presence of $Mn^{2+}$ (23). The linear and uncleaved circular DNA molecules were separated from each other by two cycles of electrophoresis in agarose gels and treated with bacteriophage λ 5′-exonuclease to remove an average of 50 nucleotides from the 5′-phosphoryl termini (2). 3T3 cells were then transfected with the modified DNA to produce plaques.

**Deletions generated by *Hae*II endonuclease.** *Hae*II endonuclease cleaves polyoma DNA once at map coordinate 72.4, a site close to the origin of DNA replication but believed not to code for protein (11, 31). The specific infectivity of the linear DNA generated by *Hae*II endonuclease cleavage on 3T3 mouse cells was about $10^4$ PFU/μg, compared with $10^6$ PFU/μg with covalently closed polyoma DNA. Fifteen independently arising plaques were picked and purified once by plaque isolation; then, high-titer virus lysates and purified viral DNA were prepared from each.

Twelve of the DNA preparations were resistant to cleavage by *Hae*II endonuclease, presumably because they had lost the *Hae*II recognition site. *Hpa*II endonuclease cleaves polyoma DNA eight times (12, 13), and the *Hae*II cleavage site is contained in a 0.4-kilobase fragment, *Hpa*II-5 (Fig. 1); therefore, deletions at the *Hae*II endonuclease cleavage site should alter the electrophoretic mobility of the *Hpa*II-5 fragment. The *Hpa*II endonuclease digests of each of the *Hae*II endonuclease-resistant DNAs lacked fragment *Hpa*II-5. With mutants dl 1001 and dl 1004, the new fragment *Hpa*II-5 migrated faster than *Hpa*II-6; with a number of the other mutants, the modified *Hpa*II-5 migrated more slowly than *Hpa*II-6 (Fig. 2A). Table 1 summarizes the estimated sizes of the deletions calculated from the changed electrophoretic mobilities of fragment *Hpa*II-5.

The *Hae*III recognition site at map position 72.5 is only eight base pairs away from the *Hae*II cleavage site located at map position 72.4 (11, 31). Consequently, some information about the endpoints of the deletions could be obtained by cleaving the mutant DNAs with *Hae*III endonuclease, an enzyme that generates 24 fragments. Based on their *Hae*III endonuclease cleavage patterns, the deletion mutants could be divided into two classes: both dl 1001 and dl 1004 had lost fragment *Hae*III-14′ and had instead new fragments. Mutant dl 1001 had ap-

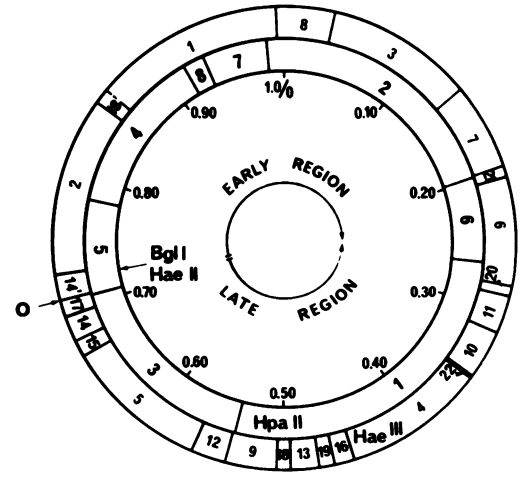


FIG. 1. *Physical map of polyoma virus DNA. The recognition sites for the endonucleases BglI, HaeII, HaeIII, and HpaII (12, 13) are indicated on the physical map. The regions transcribed early and late after infection (19) are indicated as well.*

parently lost the recognition site between *Hae*III-14′ and *Hae*III-2 and had a new fragment larger than the *Hae*III-2 (Fig. 2B; this new fragment is not clearly visible on a picture exposed to display the smaller fragments). Mutant dl 1004's deletion was probably wholly within the boundaries of *Hae*III-14′, yielding a fragment smaller than *Hae*III-18. The size of the deletions, as calculated from the shift in electrophoretic mobility of *Hae*III fragments, is also given in Table 1. The size of the deletions differed somewhat depending on whether the estimates were made from the *Hpa*II or *Hae*III endonuclease-generated fragments. This difference could be due to irregularities in mobility-versus-size relationships with fragments of this size or to differences in the base composition of various fragments (36).

**Deletions generated by DNase I-$Mn^{2+}$ digestion.** DNase I, in the presence of $Mn^{2+}$, makes random, double-stranded scissions in DNA (23). To construct mutants with deletions at sites other than those cleaved by restriction endonucleases, polyoma DNA was digested under conditions which cut only about 30% of the molecules; thus, only a few DNA molecules were cut more than once. Full-length linear DNA, isolated as described previously, was used to transfect 3T3 mouse cells to obtain plaques (specific infectivity of the order of $10^4$ to $10^5$ PFU/$\mu$g). Sixty-one independent plaques were picked and purified once by plaque isolation, and the virus was used to prepare viral DNA.

DNase I-generated deletion mutants would not be expected to be resistant to cleavage by any particular restriction enzyme, and the putative mutant DNAs were therefore screened by examining *Hpa*II and *Hae*III endonuclease digests. Of the 61 DNA preparations screened,

TABLE 1. *Characterization of deletion mutants lacking the HaeII endonuclease cleavage site at map position 0.724*[a]

| Mutant | Size of the deletion (base pairs) estimated from digestions with: | | Presence of *Hae*III recognition site at map position 0.725 |
|---|---|---|---|
| | *Hpa*II | *Hae*III | |
| dl 1001 | −75 | ND[b] | − |
| dl 1002 | −75 | ND | − |
| dl 1003 | −45 | ND | − |
| dl 1004 | −55 | −40 | + |
| dl 1005 | −45 | −23 | + |
| dl 1006 | −45 | −19 | + |
| dl 1007 | −20 | −18 | + |
| dl 1008 | −20 | −18 | + |
| dl 1009 | −20 | −15 | + |
| dl 1010 | −20 | −15 | + |
| dl 1011 | −20 | −13 | + |
| dl 1012 | −20 | −13 | + |

[a] Procedures used for the analyses are described in the legend to Fig. 2.
[b] ND, Not done.

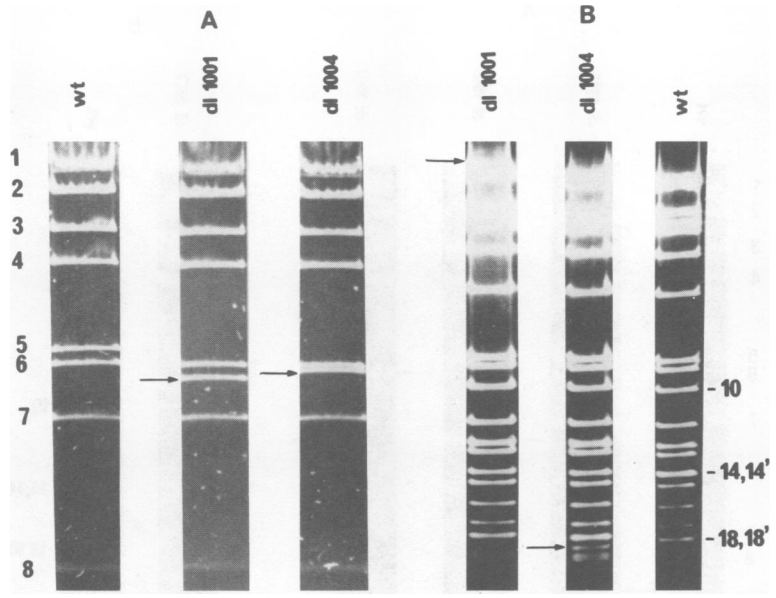


FIG. 2. *Polyacrylamide gel electrophoresis of DNA fragments produced by HpaII or HaeIII endonuclease digestion of representative mutant DNAs with deletions at the HaeII endonuclease cleavage site. Purified mutant and wild-type DNAs (0.2 to 0.5 μg) were digested with HpaII (A) or HaeIII (B) endonucleases and electrophoresed in linear 3 to 10% polyacrylamide slab gels.*

seven showed new cleavage patterns. Three (dl 1013, 1014, and 1015; Fig. 3A, Table 2) had alterations in fragment *Hpa* II-7 or -8. Three (dl 1017, 1018, and 1019; Fig. 3A, Table 2) contained deletions in fragment *Hpa*II-5 and one, dl 1016, had a deletion of less than five base pairs that was detected only as a shift in the mobility of fragment *Hae*III-17 (data not shown).

The deletions in fragment *Hpa*II-7 or -8 could be mapped more precisely by *Mbo*I endonuclease digestion (14). All three mutants mapped close to the boundary between *Hpa*II fragments 7 and 8 (see Fig. 1) in fragment *Mbo*I-7 covering this site (14). The mutants mapping in fragment *Hpa*II-5 were analyzed further by *Hae*III endonuclease digestion. Each of these three mutants was similar in that fragments *Hae*III-14′ and *Hae*III-2 were missing, and there was a new fragment larger than *Hae*III-1 (Fig. 3B illustrates that result with dl 1017 DNA). The deletions in dl 1017, 1018, and 1019 appeared to eliminate the *Hae*III cleavage site at 72.5 between fragments *Hae*III-2 and *Hae*III-14′. These three mutant DNAs were sensitive to cleavage by *Hae*II endonuclease, demonstrating that the deletions do not extend through the eight base pairs to map position 72.4. Polyoma DNA was also cleaved once by *Bgl*I endonuclease at map position 72.4. Double digestion of polyoma DNA with both *Bgl*I and *Hae*III endonucleases suggests that *Bgl*I endonuclease cleaves the DNA between the *Hae*III site at 72.5 and the *Hae*II site at 72.4. This observation was confirmed by sequencing data (11, 31). The DNA preparations of dl 1017, 1018, and 1019 were resistant to *Bgl*I endonuclease, and therefore each must have one endpoint of their deletion located within the few base pairs separating the *Hae*II and *Bgl*I cleavage sites. It is surprising that three independently derived mutants had deletions with such closely located endpoints.

**Phenotype of deletion mutants.** Each of the deletion mutants described here was isolated without a helper virus; therefore, they were viable. The presence of trace amounts of contaminating helper virus was unlikely for the following reasons: each of the virus isolates was plaque purified three times; when serially diluted, the infectivity of the virus decreased linearly, indicating that infection of cells with the mutants follows one-hit kinetics; finally, the specific infectivities of both DNA (PFU per microgram) and virions (PFU per hemagglutinating unit) were the same for the mutants and parent viruses.

The plaque morphology of all but one of the mutants was indistinguishable from that of the wild type. Mutant dl 1015 gave somewhat smaller plaques than did the wild-type virus. This experiment suggests that all the mutants

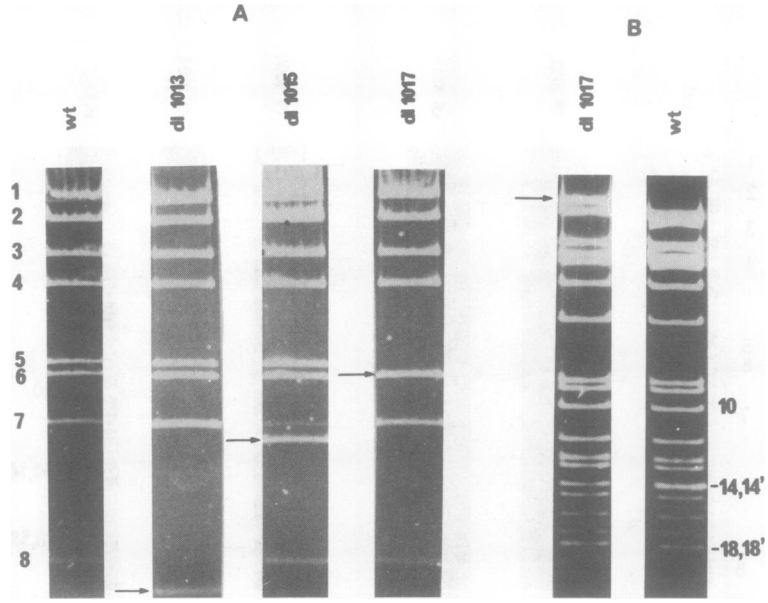


FIG. 3. *Polyacrylamide gel electrophoresis of DNA fragments produced by HpaII or HaeIII endonuclease digestion of representative DNase I-generated mutant DNAs. Purified mutant and wild-type DNAs (0.2 to 0.5 μg) were digested with HpaII (A) or HaeIII (B) endonucleases and electrophoresed in linear 3 to 10% polyacrylamide slab gels.*

TABLE 2. *Characterization of the deletion mutants generated by DNase I-$Mn^{2+}$ digestion of polyoma DNA*[a]

| Mutant | Altered *Hpa*II fragment | Net change (base pairs) |
|---|---|---|
| dl 1013 | 8 | −15 |
| dl 1014 | 8 | −5 |
| dl 1015 | 7 | −30 |
| dl 1016[b] | ND[c] | −2 |
| dl 1017 | 5 | −25 |
| dl 1018 | 5 | −45 |
| dl 1019 | 5 | −45 |

[a] Procedures used in the analyses are described in the legend to Fig. 3.

[b] Mutant dl 1016 could only be located by *Hae*III endonuclease digestion of the DNA. It maps in fragment *Hae*III-17, positioned between 0.695 and 0.708 map units.

[c] Not done.

except dl 1015 have a nearly normal rate of multiplication, but additional studies are needed to obtain more quantitative comparisons.

Representative deletion mutants were also tested for their ability to transform rat cells. Subconfluent cultures of rat 1 fibroblasts were infected at a multiplicity of about 5, and after 6 h the cells were seeded in soft-agar medium. After 2 weeks, the visible colonies were counted (Table 3). Wild-type virus had a transformation frequency of 0.2 to 0.3%. Mutants dl 1001, 1004, and 1017, whose deletions are located in noncoding regions (11, 31) close to the origin of replication, transformed with about the same frequency. The two mutants, dl 1013 and 1015, mapping in the middle of the early region had a significantly decreased transforming ability. Moreover, these two mutants, particularly dl 1015, produced smaller transformed colonies in the soft agar than did the wild-type virus. After these experiments were completed and submitted for publication, we learned that M. M. Bendig and W. R. Folk (private communication) and Griffin and Maddock (14) have also obtained mutants with deletions in the region of fragments *Hpa*II-7 and -8 which can still multiply as well as wild-type virus and which transform at reduced frequency.

## DISCUSSION

Viable deletion mutants of polyoma were isolated by infecting mouse 3T3 cells with two different preparations of linear viral DNA. One was made by cleavage of wild-type DNA with *Hae*II endonuclease, which cleaves polyoma DNA once at 72.4 map units on the physical map (12). The other contained circularly permuted linear DNA produced by cleavage with DNase I in the presence of $Mn^{2+}$.

The deletions cluster in two regions of the polyoma chromosome. One set of deletion mutants is represented by 12 *Hae*II endonuclease-generated deletions (dl 1001 through 1012), which center around the *Hae*II restriction site at 72.4, and three DNase I-generated mutants (dl 1017 through 1019), which lack between 20 and 75 base pairs between map positions 71.7 and 73.3. Another mutant (dl 1016) lacks only a few base pairs between map positions 69.5 and 70.8. These deletions map close to the origin of DNA replication at approximately 70.7 (Fig. 1), a site which also contains the 5′ end of early mRNA's (19); whether mutants 1001 through 1012 and dl 1017 through 1019 shorten the early RNAs remains to be determined. Judging from the polyoma nucleotide sequence (11, 31), this region of the DNA does not code for protein; specifically, no deletion is closer than about 30 base pairs to the first potential starting codon of the early proteins. These mutants grow normally in productive infections of mouse cells and transform rat cells at normal frequency.

The second region of the viral chromosome where deletions were found is between map position 92 and 99. These mutants (dl 1013 through 1015), which lack between 5 and 30 base pairs, were generated from DNase I-cleaved DNA. They map beyond the termination codon for small t antigen at map position 86 (28), in the putative coding sequences for middle and large T antigens (17, 29, 31). In productive infection, dl 1013 grows normally and dl 1015 grows at only a slightly reduced rate, and the final yields of both mutant DNA and virions are nearly the same as for wild-type virus. However, both mutants are defective in transformation of rat cells (Table 3). Mutant dl 1013, whose deletion maps in fragment *Hpa*II-8 (map position between 91.6 and 93.4), has a sevenfold-reduced transformation frequency, whereas dl 1015, whose deletion

TABLE 3. *Transformation of rat cells by viable deletion mutants*[a]

| Virus | Map location of deletion | No. of transformed colonies/$10^4$ cells[b] |
|---|---|---|
| Wild type | — | 32, 45 |
| dl 1001 | 0.73 | 33, 31 |
| dl 1004 | 0.73 | 20, 24 |
| dl 1013 | *Hpa*II-8 | 5.3, 5.7[c] |
| dl 1015 | *Hpa*II-7 | 1.4, 1.6[c] |
| dl 1017 | 0.73 | 34, 33 |

[a] Rat 1 fibroblasts were infected at 5 PFU/cell and then plated in soft-agar medium.

[b] Visible colonies were counted 2 weeks after seeding of the cells. The figures given represent counts from duplicate cultures seeded with $10^4$ cells.

[c] Counts from plates seeded with $10^5$ cells.

is in fragment *Hpa*II-7 (map position between 93.4 and 98.5), has a 25-fold reduction. Moreover, the cells transformed by dl 1013 and dl 1015 give smaller than wild-type transformed colonies in soft agar. Experiments are in progress to further characterize the cells transformed by mutants dl 1013 and dl 1015.

Generally, mutants in the early region that affect one or more of the T antigens influence both vegetative growth and transformation. The first mutants of polyoma virus isolated were temperature-sensitive point mutations, the *tsA* class (10). All *tsA* mutations map between 1 and 26 map units in the C-terminal portion of large T antigen (6, 25). At the nonpermissive temperature, these mutants are deficient in growth and transforming ability.

The *hr-t* mutant NG-18 (1), selected by its restricted host range in productive infection, maps between 80 and 84 map units (6, 32) and is transformation defective. Complementation between NG-18 and *tsA* mutants shows that the two classes of mutants define separate complementation groups (5, 8). Some *hr-t* mutants affect the structure and production of both small and middle T antigens (17, 18, 27). Our experiments show that certain alterations of the nucleotide sequence beyond the coding region of small t antigen (32) but within the putative coding region for large and middle T antigens (17, 29, 31, 32) do not prevent vegetative growth of the virus, although they do impair the ability of the virus to stably transform cells.

SV40 is closely related to polyoma virus in its genome organization (10). Viable deletion mutants of SV40 have been mapped to four different regions in the genome (3). One major group of deletions occurs in the same relative region of the early region as do the *hr-t* mutants of polyoma. A second large group has its deletions in the region proximal to the coding regions for late proteins (28). We had expected to find similar deletions among the DNase I-generated mutants of polyoma. But mutants in the *hr-t* region were conspicuous by their absence, possibly because our 3T3 mouse cells do not grow *hr-t* mutant virus. Why no mutants were obtained with deletions within the proximal portion of the late region is unclear. In polyoma DNA, noncoding sequences seem to occur between map position 67 to 71 (7, 16, 30). This target is similar in size to the corresponding region of the DNA on the early side of the origin of replication (71 to 73 map units), where several mutants were obtained. Perhaps, this region, though noncoding, serves some other essential function, and the actual target size for noninactivating deletions is much smaller than 4% of the genome.

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## LITERATURE CITED

1. **Benjamin, T. L.** 1970. Host range mutants of polyoma virus. Proc. Natl. Acad. Sci. U.S.A. **67**:394-399.
2. **Carbon, J., T. E. Shenk, and P. Berg.** 1975. Biochemical procedure for production of small deletions in simian virus 40 DNA. Proc. Natl. Acad. Sci. U.S.A. **72**:1392-1396.
3. **Cole, C. N., T. Landers, S. P. Goff, S. Manteuil-Brutlag, and P. Berg.** 1977. Physical and genetic characterization of deletion mutants of simian virus 40 constructed in vitro. J. Virol. **24**:277-294.
4. **Contreras, R., C. N. Cole, P. Berg, and W. Fiers.** 1979. Nucleotide sequence analysis of two simian virus 40 mutants with deletions in the late region of the genome. J. Virol. **29**:789-793.
5. **Eckhart, W.** 1977. Complementation between temperature-sensitive (ts) and host range nontransforming (hr-t) mutants of polyoma virus. Virology **77**:589-597.
6. **Feunteun, J., L. Sompayrac, M. Fluck, and T. Benjamin.** 1976. Localization of gene functions in polyoma virus DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:4169-4173.
7. **Flavell, A. J., A. Cowie, T. Legon, and R. Kamen.** 1979. Multiple 5' terminal cap structures in late polyoma virus RNA. Cell **16**:357-371.
8. **Fluck, M., R. Staneloni, and T. Benjamin.** 1977. Hr-t and ts-a: two early gene functions of polyoma virus. Virology **77**:610-624.
9. **Folk, W. R., and H.-C.E. Wang.** 1974. Closed circular DNAs with tandem repeats of a sequence from polyoma virus. Virology **61**:140-155.
10. **Fried, M., and B. E. Griffin.** 1977. Organizations of the genomes of polyoma virus and SV40. Adv. Cancer Res. **24**:67-113.
11. **Friedmann, T., P. La Porte, and A. Esty.** 1978. Nucleotide sequence studies of polyoma DNA. J. Biol. Chem. **253**:6561-6567.
12. **Griffin, B. E.** 1977. Fine structure of polyoma virus DNA. J. Mol. Biol. **117**:447-471.
13. **Griffin, B. E., M. Fried, and A. Cowie.** 1974. Polyoma DNA: a physical map. Proc. Natl. Acad. Sci. U.S.A. **71**: 2077-2081.
14. **Griffin, B. E., and C. Maddock.** 1979. New classes of viable deletion mutants in the early region of polyoma virus. J. Virol. **31**:645-656.
15. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365-369.
16. **Hunter, T., and W. Gibson.** 1978. Characterization of the mRNA's for polyoma virus capsid proteins VP1, VP2, and VP3. J. Virol. **28**:240-253.
17. **Hutchinson, M. A., T. Hunter, and W. Eckhart.** 1978. Characterization of T-antigens in polyoma-infected and transformed cells. Cell **15**:65-77.
18. **Ito, Y., J. R. Brocklehurst, and R. Dulbecco.** 1977. Virus-specific proteins in the plasma membrane of cells lytically infected or transformed by polyoma virus. Proc. Natl. Acad. Sci. U.S.A. **74**:4666-4670.
19. **Kamen, R., and H. Shure.** 1976. Topography of polyoma virus messenger RNA molecules. Cell **7**:361-371.

20. **Lai, C.-Y., and D. Nathans.** 1974. Deletion mutants of SV40 generated by enzymatic excision of DNA segments from the viral genome. J. Mol. Biol. **89**:170–193.
21. **Little, J. W., I. R. Lehman, and A. D. Kaiser.** 1967. An exonuclease induced by bacteriophage λ. I. Preparation of the crystalline enzyme. J. Biol. Chem. **242**:672–678.
22. **Magnusson, G., and M. G. Nilsson.** 1977. Multiple free viral DNA copies in polyoma virus-transformed mouse cells surviving productive infection. J. Virol. **22**:646–653.
23. **Melgar, E., and D. A. Goldthwait.** 1968. Deoxyribonucleic acid nucleases. II. The effect of metals on the mechanism of action of deoxyribonuclease I. J. Biol. Chem. **243**:4409–4416.
24. **Mertz, J. E., and P. Berg.** 1974. Defective simian virus 40 genomes: isolation and growth of individual clones. Virology **62**:112–124.
25. **Miller, L. K., and M. Fried.** 1976. Construction of the genetic map of the polyoma genome. J. Virol. **18**:824–832.
26. **Morhenn, V., Z. Rabinowitz, and G. M. Tomkins.** 1973. Effects of adrenal glucocorticoids on polyoma virus replication. Proc. Natl. Acad. Sci. U.S.A. **70**:1088–1089.
27. **Schaffhausen, B. S., J. E. Silver, and T. L. Benjamin.** 1978. Tumor antigen(s) in cells productively infected by wild type polyoma virus and mutant NG-18. Proc. Natl. Acad. Sci. U.S.A. **75**:79–83.
28. **Shenk, T. E., J. Carbon, and P. Berg.** 1976. Construction and analysis of viable deletion mutants of simian virus 40. J. Virol. **18**:664–671.
29. **Smart, J. E., and Y. Ito.** 1978. Three species of polyoma virus tumor antigens share common peptides probably near the amino termini of the proteins. Cell **15**:1427–1437.
30. **Smith, A. E., R. Kamen, N. F. Mangel, H. Shure, and T. Wheeler.** 1976. Location of the sequences coding for capsid proteins VP1 and VP2 on polyoma virus DNA. Cell **9**:481–487.
31. **Soeda, E., J. R. Arrand, A. Smolar, and B. E. Griffin.** 1979. Polyoma virus DNA I. Sequence from the early region that contains the viral origin of replication and codes for small, middle and part of large T-antigens. Cell **17**:357–370.
32. **Soeda, E., and B. E. Griffin.** 1978. Sequences from the genome of a non-transforming mutant of polyoma virus. Nature **276**:294–298.
33. **Thimmappaya, B., and T. Shenk.** 1979. Nucleotide sequence analysis of viable deletion mutants lacking segments of the simian virus 40 genome coding for small t antigen. J. Virol. **30**:668–673.
34. **Van Heuverswyn, H., C. Cole, P. Berg, and W. Fiers.** 1979. Nucleotide sequence analysis of two simian virus 40 mutants with deletions in the region coding for the carboxyl terminus of the T antigen. J. Virol. **30**:936–941.
35. **Volckaert, G., J. Feunteun, L. V. Crawford, P. Berg, and W. Fiers.** 1979. Nucleotide sequence deletions within the coding region for small-t antigen of simian virus 40. J. Virol. **30**:674–682.
36. **Ziegler, R. S., R. Salomon, C. W. Dingman, and A. C. Peacock.** 1972. Role of base composition in the electrophoresis of microbial and crab DNA in polyacrylamide gels. Nature (London), New Biol. **238**:65–69.