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Polyomavirus Tumor Induction in Mice: Effects of Polymorphisms of VP1 and Large T Antigen

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By testing recombinants between "high tumor" (inducing a high incidence of tumors) and "low tumor" (inducing a low incidence of tumors) strains of polyomavirus, we have previously shown that the key determinant(s) for induction of a high tumor profile resides in coding regions of the high tumor strain (R. Freund, G. Mandel, G. G. Carmichael, J. P. Barncastle, C. J. Dawe, and T. L. Benjamin, J. Virol. 61:2232–2239, 1987). Three single-amino-acid differences between the PTA (high tumor) and RA (low tumor) virus strains have now been identified by DNA sequencing, one each in the large T antigen, in the region common to the middle and small T antigens, and in the major capsid protein VP1. Further tests of appropriate recombinants and oligonucleotide-induced mutants show that VP1 of PTA is the major determinant for induction of a high tumor profile, including all tumors of epithelial origin. The differential effect of the VP1s of PTA and RA on the tumor profile is discussed in terms of a likely contribution of the polymorphic region of VP1 to binding of receptors and infection of different cell types in the animal. The polymorphism in the large T antigen has a more restricted action, which is seen only when tested in virus carrying the VP1 type of PTA; the PTA large T antigen then promotes more rapid growth of tumors of salivary gland and thymus than the RA large T antigen.

Different wild-type strains of mouse polyomavirus that are equally capable of transforming fibroblasts in culture may have sharply contrasting abilities to induce tumors following inoculation into newborn mice (4). Recent attempts to explore this unexpected finding have focused on two prototype strains: a "higher tumor" strain (inducing a high incidence of tumors) designated PTA and a "low tumor" strain (inducing a low incidence of tumors) designated RA. Virtually 100% of mice inoculated with PTA develop multiple tumors, which begin to appear as early as 5 to 6 weeks after inoculation. In contrast, only a small fraction of mice inoculated with RA ever develop tumors; affected animals usually bear only single tumors, and these develop after a much longer period, typically around 5 to 6 months of age.

Another remarkable and consistent difference between these virus strains concerns their tissue tropism. PTA induces a broad spectrum of tumor types. Tumors of epithelial origin predominate, particularly in the first 2 months; among the most common are tumors of the salivary gland, mammary gland, thymus, and hair follicle. PTA also induces tumors of mesenchymal origin, including those of the bone, kidney, subcutaneous connective tissues, and vascular endothelium. The RA strain, on the other hand, induces a much narrower array of tumors that includes only a part of the mesenchymal spectrum and is entirely lacking in any tumors of epithelial origin. The basis for these differences in tumorigenicity remains largely unknown.

A series of PTA-RA viral recombinants has been constructed and tested in order to identify viral genetic determinants necessary for a high tumor profile. Initial comparisons of the two viral DNAs by restriction enzyme analyses showed differences within the short (~450 bp) noncoding region but showed no difference in the approximately 4.8 kb of viral DNA comprising the early and late coding regions. Sequencing of the noncoding regions revealed multiple differences, lying both on the early side of the replication origin, affecting the number and array of large T-antigen-binding sites, and on the late side in the enhancer region, known to regulate early viral gene expression. Animal studies were carried out first with a series of viral recombinants in which all or parts of the noncoding region were exchanged between PTA and RA. Exchange in either direction did not alter the basic features of the tumor profiles. Thus, RA carrying the entire regulatory region of PTA remained a low tumor strain incapable of inducing epithelial tumors. PTA carrying the noncoding region of RA induced both epithelial and mesenchymal tumors, albeit at somewhat reduced frequencies and with some specific restrictions as to tumor type. These results pointed clearly to the existence of one or more structural determinants in PTA that enable this virus to induce a high frequency and broad spectrum of tumors, particularly those of epithelial origin (10).

Here we report results of further investigations into the nature of the structural determinants required for a high-tumor profile. The entire coding regions of PTA and RA have been sequenced. The data identify three structural polymorphisms consisting of single-amino-acid differences in VP1, in the large T antigen, and in the hr-t region shared in common by the small and middle T antigens. An earlier study showed that the difference in the small and middle T proteins of PTA and RA does not account for the differences in tumorigenicity between the two strains (7). Assessing the contributions of the polymorphisms in VP1 and large T, the present study clearly establishes that VP1 of PTA carries the structural determinant for epitheliotropism and overall induction of a high tumor profile. Parallel studies show that the same determinant also has a profound effect on the ability of the virus to replicate and spread in the animal (5), as well as on its hemagglutinating properties and the determination of plaque size on monolayers of cultured mouse fibroblasts (9).

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Taken together, these results strongly suggest that the region of difference in the VP1s of PTA and RA is involved in recognition of cell surface receptors in different tissues of the animal. The polymorphism in the large T antigen affects the tumor profile only in strains bearing the PTA-VP1 epitheliotropic determinant. The difference between the two large T antigens is manifested with respect to the size or growth rate of two particular epithelial tumor types, those of the salivary gland and the thymus.

MATERIALS AND METHODS

Subcloning and nucleotide sequence comparison of PTA and RA. Cloned viral DNAs from PTA and RA were subcloned into pUC18, M13mp18, and M13mp19 (10) by using standard recombinant DNA techniques (17, 19). Each viral genome was initially divided into the large and small *Bam*HI (nucleotide [nt] 4632)-to-*Eco*RI (nt 1560) DNA fragments. (For nucleotide numbering and restriction enzyme map of polyomavirus, see reference 12.) These subclones were further divided into the 1,144-bp *Bam*HI (nt 4632)-to-*Pst*I (nt 484) fragment, the 1,076-bp *Pst*I (nt 484)-to-*Eco*RI (nt 1560) fragment, the 1,402-bp *Eco*RI (nt 1560)-to-*Hin*cII (nt 2962) fragment, and the 1,620-bp *Hin*cII (nt 2962)-to-*Bam*HI (nt 4632) fragment.

By using the universal M13 primer or synthesized oligonucleotide primers, single-stranded template from these subclones was sequenced by the dideoxynucleotide sequencing method (21). Two loadings of the sequencing reactions from PTA and RA were analyzed in parallel by electrophoresis on 6% acrylamide gels, such that at least 400 nt of the two strains were discernible. Apparent differences were verified by reanalyzing the sequence such that the differences were well resolved and the sequence was clearly readable.

Construction of viruses. Two sets of viruses were constructed. The first set was based on the previously constructed low tumor strain PR-3, which contains the noncoding sequences from PTA and the coding sequences from RA (10). DNA fragments containing the PTA coding specificities were exchanged for corresponding fragments of RA. By using this approach, the coding sequences essential for a high tumor profile could be identified. The appropriate DNA fragments were gel purified and ligated to construct the large and small *Bam*HI-to-*Eco*RI fragments. These two fragments, when ligated together, reconstruct the viral genome. Genome structures of the recombinant viruses are illustrated schematically (see Fig. 2). PR/PTA-VP1 contains the *Bam*HI-to-*Hin*cII fragment from PTA (which encodes the glutamic acid at amino acid 92 of VP1) and the rest of the genome from PR-3. PR/PTA-VP1&LT contains the *Bam*HI-to-*Eco*RI fragment from PTA which encodes both the VP1 and the large T specificities of PTA. PR/PTA-LT contains the *Bst*XI (nt 173)-to-*Hin*cII fragment from PTA, which encodes the entire PTA early region and the remainder of the genome from PR-3, including the VP1 specificity of RA.

The second set of viruses, PTA/RA-LT and PTA/RA-VP1, was constructed by site-directed oligonucleotide mutagenesis as previously described (1) by using the single-stranded template from the subclone containing the large *Bam*HI-to-*Eco*RI fragment of PTA in M13mp19. PTA/RA-LT and PTA/RA-VP1 are constructed on the background of the high tumor strain PTA but encode the single-amino-acid substitution of RA large T antigen or VP1, respectively. In the case of PTA/RA-LT, the 20-mer CAGACGAAGCAGAAGAA CAG, encompassing nt 1783 to 1802 of the coding strand of large T antigen with mismatches at nt 1790 and 1791 (underlined), was used. In order to distinguish the mutant from the parental viruses, a silent change was introduced into the third position of codon 411. Large T-antigen codons 411 and 412 of PTA, encoding glutamic acid and proline, are GAGCCA; in RA, encoding glutamic acid and alanine, they are GAGGCA; and in PTA/RA-LT, also encoding glutamic acid and alanine, they are GAAGCA. In the case of PTA/RA-VP1, the oligonucliotide used for mutagenesis was GTGTATTATTCCCTGGGGAATCC; this sequence is complementary to nt 3802 to 3812 of the coding strand of VP1, with mismatches at nt 3802 and 3803 (underlined). To distinguish PTA/RA-VP1 from RA, codon 92 in VP1 in the mutant is GGG and in RA is GGA, both encoding glycine. Both oligonucleotide-induced mutants were verified in the fully reconstructed virus by sequence analysis.

To construct all of the viruses, the appropriate large *Bam*HI-to-*Eco*RI fragment was ligated to the appropriate small *Bam*HI-to-*Eco*RI fragment and transfected into NIH 3T3 cells by using the DEAE-dextran method (18). The resulting virus lysate was plaque-purified on NIH 3T3 cells, and a virus stock was propagated on baby mouse kidney cells. Titers were determined by plaque assay on NIH 3T3 cells.

Generation of tumor profiles. Tumor profiles were generated as previously described (4). Briefly, newborn C3H/BiDa mice (less than 18 h of age) were inoculated subcutaneously with 0.05 ml of crude virus suspension (2.5×10^6 to 1×10^7 PFU per animal). The mice were inspected twice weekly and were necropsied when moribund or at approximately 1 year of age. Tumors as well as apparently normal tissue were excised, and portions were either frozen or fixed in Bouin's fluid for subsequent histological analysis. Tumors scored as overt were visible at the time of necropsy and were generally greater than 2 mm. Tumors scored as occult were revealed by histological examination only. Bone tumors were scored on the basis of gross examination alone; this gives a minimum frequency, since occult bone tumors can be found in the absence of grossly visible ones.

Virus recovery from tumors, DNA isolation, and sequence analysis. A piece of frozen tumor was homogenized in a glass/glass homogenizer in phosphate-buffered saline. The homogenate was frozen and thawed three times and used to infect baby mouse kidney cells. The viral lysate from these cells was treated with 100 μg of DNase per ml for 1 h at room temperature and centrifuged for 45 min at 285,000 × *g* to pellet the virus particles. The pellet was resuspended in 0.4 ml of 0.3 M NaCl–0.05 M Tris [pH 8]–0.02 M EDTA–0.5% sodium dodecyl sulfate, treated with 100 μg of proteinase K per ml for 30 min at 65°C, and extracted twice with a phenol–chloroform (1:1) mixture. The nucleic acid, predominantly viral DNA, was precipitated with 70% ethanol and digested with *Bam*HI and *Eco*RI, and the appropriate DNA fragments were gel purified and cloned into M13mp19 or M13mp18. Relevant regions of the viral DNA were sequenced by using the dideoxynucleotide method (21) or the Sequenase method (United States Biochemical Corp.), with the universal M13 primer or synthesized oligonucleotide primers.

RESULTS

Comparison of sequences of PTA and RA coding regions. Figure 1 shows results of DNA sequencing and compares the entire coding regions of PTA and RA. Nucleotide sequences were identical throughout, except for eight single-base substitutions. Five of the eight changes are silent in terms of

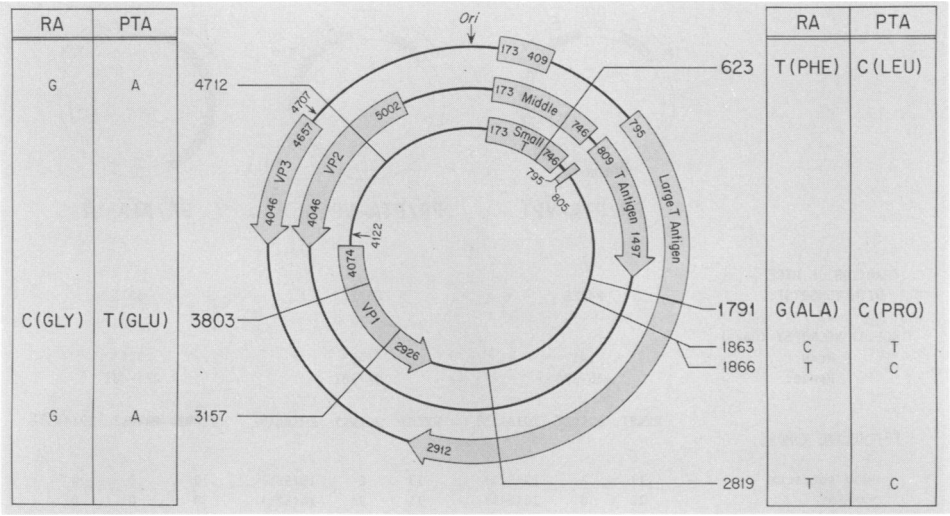


FIG. 1. Schematic diagram of the polyomavirus genome illustrating the nucleotide sequence differences in the coding regions between PTA and RA. The start sites, splice sites, and termination sites of the viral proteins and the sites where the nucleotide sequence differs between PTA and RA are indicated by using the numbering system of Griffin et al. (12). The amino acid changes which result from nucleotide differences in PTA and RA are shown in parentheses. The nucleotide difference at nt 623 affects amino acid position 151 of the middle T and small t antigens, the difference at nt 1791 affects amino acid position 412 of the large T antigen, and the difference at nt 3803 affects amino acid position 92 of VP1. Ori, Replication origin.

amino acid substitution. The other three introduce single-amino-acid differences, as shown. At position 151 of middle T and small t, RA encodes a phenylalanine and PTA encodes a leucine. At position 412 of large T, RA encodes an alanine and PTA encodes a proline. At position 92 of VP1, RA encodes a glycine and PTA encodes a glutamic acid. The differences at position 151 of the middle and small t antigens and the occurrence of an alanine at position 412 of the RA large T antigen have been reported previously (7, 13).

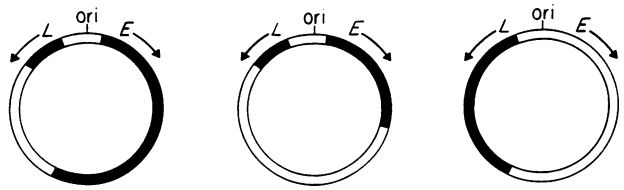
Tumor profiles of PTA-RA recombinant virus strains. To identify which coding region of PTA is essential for a high tumor profile, three recombinant viruses were constructed in which PTA-VP1 and PTA-LT sequences were present either singly or together. The starting point for constructing these recombinants was the previously described strain PR-3. This strain derives all its noncoding sequences from PTA and all its coding sequences from RA and induces a low tumor profile indistinguishable from that of RA (10). By exchanging various segments of PTA coding regions for homologous segments of RA in PR-3, the effects of PTA-VP1 and PTA-LT could be examined on a background containing PTA regulatory sequences that provide full potential for a high tumor profile. Details of construction of the three strains are given in Materials and Methods. Genome structures are shown schematically in Fig. 2, along with results of animal experiments establishing the tumor profiles of each recombinant.

The recombinant PR/PTA-VP1 contains the VP1 coding sequence of PTA and all other coding sequences of RA (for purposes of simplification, we refer to the derivations of genomic sequences only on the basis of changes affecting amino acid sequence [Fig. 1]). This recombinant virus induces a broad tumor profile in which virtually all of the major epithelial and mesenchymal tumor types are represented. This profile resembles that of PTA, with one exception: a much lower incidence of overt (i.e., grossly detectable) tumors of the salivary gland and thymus. Only single tumors of each type were found by gross examination of 25 animals inoculated. This contrasts with frequencies of 20 to 70% for overt salivary gland tumors and 50 to 100% for overt thymic tumors in previous experiments with PTA (4, 8). Microscopic examination, however, uncovered additional tumors of these two types in animals inoculated with PR/PTA-VP1. Scoring these occult tumors along with the single overt tumor brings the overall frequency of thymic and salivary gland tumors up to around 50%. These results show that VP1 of PTA enables the virus to initiate infection, leading to transformation of all major epithelial target cells. They suggest further that, in the salivary gland and thymus, the large T antigen of RA may not function as well as that of PTA in promoting rapid or persistent growth of tumor cells.

Results with the second recombinant, PR/PTA-VP1&LT, bear out this expectation concerning the large T antigens. This recombinant carries both large T antigen and VP1 specificities of PTA and induces a full PTA-like profile. In particular, tumors of the salivary gland and thymus are scored overwhelmingly as overt, in contrast to PR/PTA-VP1, in which they were almost exclusively occult.

To determine whether introduction of the PTA large T antigen by itself, i.e., linked to RA-VP1, would enable the virus to induce epithelial tumors, the recombinant PR/PTA-LT was tested. Eleven animals were inoculated, and none showed any epithelial tumors, either by gross or microscopic examination. This recombinant is clearly a low tumor strain, similar to RA and PR-3. These results confirm the requirement for PTA-VP1 as an essential determinant of epitheliotropism in tumor induction. They also demonstrate that the large T-antigen polymorphism has an effect only in conjunction with the appropriate VP1.

Tumor profiles of PTA mutant virus strains. As a further test of the significance of the structural variations of VP1 and the large T antigen, oligonucleotide mutagenesis was used to introduce the RA coding specificities into a PTA virus background. Figure 3 presents results with two such mutant viruses. PTA/RA-LT is identical to PTA except for 2 bases,



	PR/PTA-VP1			PR/PTA-VP1&LT			PR/PTA-LT		
FRACTION OF MICE WITH TUMOR(S):	24/25			26/27			5/11		
AGE AT NECROPSY (DAYS)									
MEAN:	141			148			399		
RANGE:	45-370			47-381			276-481		
	OVERT	OCCULT	TOTAL(%)	OVERT	OCCULT	TOTAL(%)	OVERT	OCCULT	TOTAL(%)
EPITHELIAL TUMORS									
HAIR FOLLICLE	11	2	13(52%)	13	2	15(56%)	0	0	0
MAMMARY	16	0	16(64%)	11	3	14(52%)	0	0	0
SALIVARY	1	11	12(48%)	19	2	21(78%)	0	0	0
THYMUS	1	11	12(48%)	15	3	18(67%)	0	0	0
MESENCHYMAL TUMORS									
BONE	11	-	11(44%)	3	-	3(11%)	0	-	0
KIDNEY	5	6	11(44%)	2	4	6(22%)	0	0	0
SUBCUTANEOUS									
CONNECTIVE TISSUE	4	5	9(36%)	4	4	8(30%)	4	1	5(45%)
VASCULAR ENDOTHELIUM	0	1	1(4%)	1	1	2(7%)	0	0	0

FIG. 2. Schematic diagrams and tumor profiles of recombinant viruses. White segments in the diagrams are from PTA, and the black segments are from RA. L and E indicate the late and early sides of the replication origin (Ori), respectively. Recombinant viruses are based on PR-3, which contains the noncoding sequences of PTA and the coding sequences of RA. PR/PTA-VP1 contains the PTA VP1 gene. PR/PTA-VP1&LT contains the PTA VP1 and large T-antigen genes. PR/PTA-LT contains the PTA large T-antigen gene. All the other coding sequences are derived from RA. The number of mice with a particular overt (grossly detected) or occult (microscopic) tumor type are indicated. Percentages reflect the number of mice with at least one of a particular type of tumor. Bone tumors were scored by gross examination only.

a GC-to-AG change at nts 1790 and 1791 that converts proline to alanine at position 412 of the large T antigen (see Materials and Methods) (Fig. 1). The tumor profile of PTA/RA-LT is broad in its representation of epithelial as well as mesenchymal tumors. It differs, however, from a typical PTA tumor profile in that it shows a shift from overt to occult tumors in both the salivary gland and thymus. This result with PTA/RA-LT is similar to that found with the recombinant strain PR/PTA-VP1, which has the identical structural determinants for VP1 and large T antigen (Fig. 2). The essential concordance of results with these two differently constructed viruses confirms the importance of PTA-VP1 in induction of epithelial tumors and also confirms the specific role of PTA large T antigen in promoting rapid or persistent tumor growth in the thymus and salivary gland.

Animals inoculated with the second mutant strain, PTA/RA-VP1, developed an array of tumors of both epithelial and mesenchymal origin. The tumor response was biphasic. Fourteen animals developed epithelial tumors, typical of PTA. These mice survived an average of 140 (range, 62 to 398) days at the time of necropsy. Another 15 animals developed tumors of subcutaneous connective tissue only, averaging 309 (range, 167 to 388) days of age, while the other 3 animals remained tumor-free until the experiment was terminated at about 1 year. Thus, the overall response was clearly mixed. The 18 animals that developed either no tumor or only fibrosarcomas (invasive but nonmetastasizing) were consistent with an RA-like tumor profile that would be expected for PTA/RA-VP1. However, the 14 mice that developed epithelial tumors would not be expected to do so on the basis of the RA-VP1 type of the mutant virus.

To explore this discrepancy, virus was isolated from several animals that bore epithelial tumors, and the viral DNA was sequenced in the region of the induced mutation to establish the likely origin of the virus. Codon 92 of VP1 is GAA (Glu) in PTA and GGA (Gly) in RA. The virus PTA/RA-VP1 was constructed with GGG (Gly) at codon 92 in order to distinguish this virus from RA and related recombinants. Virus isolated from the one thymic tumor tested had GAA at codon 92. This recovered virus could have resulted either from a low level of PTA contamination or from a back mutation (GGG to GAA). These two possibilities cannot be distinguished by further sequencing or other methods because of the PTA background used to construct the mutant. However, previous experiments showed that PTA can induce a high tumor profile at doses at least 1,000-fold less than those used here and also when mixed together with RA (4). The tumor profile obtained with PTA/RA-VP1 is therefore consistent with a very low level of PTA-like virus in the stock used for infection. The actual emergence in the tumor itself of such a low-level contaminant or revertant bearing PTA-VP1 suggests a strong selection for the latter VP1 type. Consistent with such selection is the recovery of virus of the input type GGG from kidneys of two of the animals that bore epithelial tumors. Virus was also recovered from two mammary tumors, and both were found to encode GGG (Gly) at codon 92, corresponding to the input PTA/RA-VP1. This finding raises several possibil-

	PTA/RA-LT			PTA/RA-VP1		
FRACTION OF MICE WITH TUMOR(S):	33/33			29/32		
AGE AT NECROPSY (DAYS)						
MEAN:	89			236		
RANGE:	58-371			62-398		
	OVERT	OCCULT	TOTAL(%)	OVERT	OCCULT	TOTAL(%)
EPITHELIAL TUMORS						
HAIR FOLLICLE	24	1	25(76%)	10	0	10(31%)
MAMMARY	28	1	29(88%)	7	1	8(25%)
SALIVARY	4	12	16(48%)	1	6	7(22%)
THYMUS	8	19	27(82%)	9	0	9(28%)
MESENCHYMAL TUMORS						
BONE	7	-	7(21%)	5	-	5(16%)
KIDNEY	18	11	29(88%)	1	6	7(22%)
SUBCUTANEOUS CONNECTIVE TISSUE	3	2	5(15%)	14	6	20(63%)
VASCULAR ENDOTHELIUM	1	0	1(3%)	0	2	2(6%)

FIG. 3. Tumor profiles of virus strains constructed by site-directed mutagenesis. PTA/RA-LT encodes an alanine at amino acid 412 of the large T antigen (RA-like), while all the other sequences are identical to those of PTA. PTA/RA-VP1 encodes glycine at amino acid 92 of VP1 (RA-like) while the rest of the sequences are identical to those of PTA. The number of mice with a particular type of tumor, either overt or occult, is indicated. Percentages reflect the number of mice with at least one of a particular tumor type. Bone tumors were scored by gross examination only.

ities. Virus carrying the RA-VP1 type may be able, at some low frequency, to infect and transform epithelial target cells, perhaps by direct interaction with receptors on mammary epithelial cells or possibility by clumping with virus particles bearing PTA VP1. Alternatively, the virus recovered from this tumor may contain a second site mutation in VP1 which, together with Gly at codon 92, renders it PTA-like.

DISCUSSION

Single-amino-acid differences in the large T antigen and VP1 of the PTA (high tumor) and RA (low tumor) strains of polyomavirus have been uncovered and evaluated for their effects on tumor induction in mice. Results point clearly to the importance of the VP1 polymorphism in PTA for inducing a high tumor profile and particularly for inducing tumors in epithelial target cells. That the single-amino-acid difference in VP1 should have such a profound effect on tumor profiles is borne out by sequencing the corresponding VP1 regions of two other independently isolated wild-type strains of polyomavirus which have been studied in parallel with PTA and RA (4). The high tumor strain A2, like PTA, was found to encode glutamic acid at position 92, while the low tumor strain A3 encodes glycine, as does RA (data not shown).

Studies comparing the abilities of polyomaviruses bearing the two different VP1 specificities to replicate and spread in neonatally infected mice and particularly to infect and amplify in the kidney also affirm the importance of glutamic acid at this position (5). Specification of host range or tissue tropism by this VP1 determinant falls neatly into epithelial versus mesenchymal cell types, with one exception. As noted here and in earlier reports (4, 10), renal mesenchyme is grouped along with epithelial targets as being susceptible to tumor induction only by viruses with the VP1 type of the high tumor strains. This epitheliumlike response of the renal mesenchyme may be related to its developmental origin and potential for epithelial expression in newborn mice. Developmentally, it derives from the same metanephrogenic blastema that, under the inductive influence of the ureteral bud, also gives rise to the cortical tubular epithelium; in fact, tubulogenesis continues during the first 2 weeks after birth.

The most likely explanation for the mode of action of this VP1 determinant is that it affects recognition of cell surface receptors by the virus. Subsequent events involving virus uptake, uncoating, and initiation of infection in the target tissue might also be involved. This interpretation implies that structural and functional differences exist between epithelial and mesenchymal target cells in their surface receptors and/or virus uptake mechanisms. Additional evidence for involvement of this region of VP1 in receptor recognition comes from studies of plaque size and hemagglutination (9), as well as from studies of the virus structure by X-ray diffraction (14).

The large T-antigen specificities encoded by PTA and RA produce differences in the tumor profile that are more limited and conditional than were found for VP1. Differential effects of the large T proteins are seen only with respect to epitheliomas of the thymus and salivary gland. At these sites, PTA large T antigen acts more effectively than RA large T antigen in promoting growth of tumors to a size that becomes detectable macroscopically. The effect of the antigen is therefore not at the level of initiation of transformation

as initially defined for large T antigens in cell culture systems (6, 11, 22), but rather suggests a persistent effect of the large T protein (20), perhaps in promoting a high level of virus transcription or viral DNA replication in tumor cells. With regard to the latter possibility, a comparison of A2 and RA DNA replication in established rat fibroblasts has shown that A2, which like PTA encodes proline at position 412, replicated better than RA (13).

The large T-antigen effect on tumor induction is conditional in the sense that it depends on linkage to the PTA-VP1 specificity. Thus, strains carrying RA-VP1 are virtually unable to induce tumors in either the salivary gland or the thymus, regardless of whether they encode PTA or RA large T antigens. This result is readily understood on the basis of the requirement for PTA-VP1 to successfully infect epithelial target cells. Although it has not been tested in this study, the effect of the PTA large T antigen may also depend on linkage to PTA's origin, with its particular array of large T-antigen-binding sites (3, 24). The binding of large T antigen as well as of tissue-specific cellular factors to origin sequences has been inferred and discussed previously with regard to high and low tumor strains (10) and also with respect to differences between A2 and PTA in induction of thymic tumors (8).

The finding that a single-amino-acid change in polyomavirus VP1 profoundly affects the ability of the virus to induce epithelial tumors in mice is not surprising in view of other well-documented cases in which variations in outer viral structural proteins clearly affect virus host range. Sequence variations in VP1 of the simian lymphotropic polyomavirus have been shown to underlie differences in host range for virus replication in B- and T-lymphoblastoid cells (15). Similarly, polymorphisms in the sigma-1 outer capsid protein of reoviruses dramatically affect tissue tropism and patterns of virus spread in mice (23, 25). Analogous observations have been made with regard to specificities in glycoproteins of enveloped RNA viruses, including human immunodeficiency virus and its interaction with CD4 receptor molecules (2).

Apart from mediating virus entry into cells, interactions of outer viral structural proteins with cell receptors can trigger physiological changes in the host cell that may be important for virus replication or for subsequent emergence of neoplasms, as suggested for the Friend erythroleukemia virus glycoprotein and its recognition of erythropoeitin receptors (16). While the cell receptor for polyomavirus has not been identified, purified VP1 can trigger transient expression of c-*myc* and c-*fos* and stimulate cell DNA synthesis following binding to quiescent mouse fibroblasts (26). Whether the particular VP1 polymorphism studied here affects simply virus attachment and uptake or affects inductive events relevant to tumor development as well remains to be investigated.

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REFERENCES

1. **Carmichael, G. G., B. S. Schaffhausen, D. I. Dorsky, D. B. Oliver, and T. L. Benjamin.** 1982. Carboxy terminus of polyoma middle-sized tumor antigen is required for attachment to membranes, associated protein kinase activities, and cell transformation. Proc. Natl. Acad. Sci. USA **79**:3579–3583.
2. **Cordonnier, A., L. Montagnier, and M. Emerman.** 1989. Single amino-acid changes in HIV envelope affect viral tropism and receptor binding. Nature (London) **340**:571–574.
3. **Cowie, A., and R. Kamen.** 1984. Multiple binding sites for polyomavirus large T antigen within regulatory sequences of polyomavirus DNA. J. Virol. **52**:750–760.
4. **Dawe, C. J., R. Freund, G. Mandel, K. Ballmer-Hoffer, D. A. Talmage, and T. L. Benjamin.** 1987. Variations in polyoma virus genotype in relation to tumor induction in mice: characterization of wild type strains with widely differing tumor profiles. Am. J. Pathol. **127**:243–261.
5. **Dubensky, T. W., R. Freund, C. J. Dawe, and T. L. Benjamin.** 1991. Polyomavirus replication in mice: influences of VP1 type and route of inoculation. J. Virol. **65**:342–349.
6. **Fluck, M. M., and T. L. Benjamin.** 1979. Comparisons of two early gene functions essential for transformation in polyoma virus and SV-40. Virology **96**:205–228.
7. **Freund, R., C. J. Dawe, and T. L. Benjamin.** 1988. The middle T proteins of high and low tumor strains of polyomavirus function equivalently in tumor induction. Virology **167**:657–659.
8. **Freund, R., C. J. Dawe, and T. L. Benjamin.** 1988. Duplication of noncoding sequences in polyomavirus specifically augments the development of thymic tumors in mice. J. Virol. **62**:3896–3899.
9. **Freund, R., R. L. Garcea, R. Sahli, and T. L. Benjamin.** 1991. A single-amino-acid substitution in polyomavirus VP1 correlates with plaque size and hemagglutination behavior. J. Virol. **65**: 350–355.
10. **Freund, R., G. Mandel, G. G. Carmichael, J. P. Barncastle, C. J. Dawe, and T. L. Benjamin.** 1987. Polyomavirus tumor induction in mice: influences of viral coding and noncoding sequences on tumor profiles. J. Virol. **61**:2232–2239.
11. **Fried, M.** 1965. Cell transforming ability of a temperature-sensitive mutant of polyoma virus. Proc. Natl. Acad. Sci. USA **53**:486–491.
12. **Griffin, B. E., E. Soeda, B. G. Barrell, and R. Staden.** 1981. Appendix B: sequence and analysis of polyoma virus DNA, p. 831–896. *In* J. Tooze (ed.), DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Hacker, D. L., K. Friderici, and M. M. Fluck.** 1989. A nonlethal mutation in large T antigen of polyomavirus which affects viral DNA synthesis. J. Virol. **63**:776–781.
14. **Harrison, S. C.** Personal communication.
15. **Kanda, T., A. Furuno, and K. Yoshiike.** 1986. Mutation in the VP-1 gene is responsible for the extended host range of a monkey B-lymphotropic papovavirus mutant capable of growing in T-lymphoblastoid cells. J. Virol. **59**:531–534.
16. **Li, J., A. D. D'Andrea, H. F. Lodish, and D. Baltimore.** 1990. Activation of cell growth by binding of Friend spleen focus-forming virus gp55 glycoprotein to the erythropoietin receptor. Nature (London) **343**:762–764.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **McCutchan, J. H., and J. S. Pagano.** 1966. Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethyaminoethyl-dextran. J. Natl. Cancer Inst. **41**:351–357.
19. **Messing, J.** 1983. New M13 vectors for cloning, p. 20–78. *In* R. Wu, L. Grossman, and K. Moldave (ed.), Methods in enzymology. Academic Press, Inc., New York.
20. **Rassoulzadegan, M., R. Seif, and F. Cuzin.** 1978. Conditions leading to the establishment of the N (α gene dependent) and A (α gene independent) transformed states after polyoma virus infection of rat fibroblasts. J. Virol. **28**:421–426.
21. **Sanger, F., S. Nicklen, and R. Coulson.** 1977. DNA sequences with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Schaffhausen, B. S.** 1982. Transforming genes and gene products of polyoma and SV40. Crit. Rev. Biochem. **13**:215–269.
23. **Tyler, K. L., D. A. McPhee, and B. N. Fields.** 1986. Distinct pathways of viral spread in the host determined by reovirus s1

gene segment. Science **233**:770–774.
24. **Weichselbraun, I., G. Haider, and E. Wintersberger.** 1989. Optimal replication of plasmids carrying polyomavirus origin regions requires two high-affinity binding sites for large T antigen. J. Virol. **63**:961–964.
25. **Weiner, H. L., D. Drayna, D. R. Averill, Jr., and B. N. Fields.** 1977. Molecular basis of reovirus virulence: role of the s1 gene. Proc. Natl. Acad. Sci. USA **74**:5744–5748.
26. **Zullo, J., C. D. Stiles, and R. L. Garcea.** 1986. Regulation of c-myc and c-fos mRNA levels by polyomavirus: distinct roles for the capsid protein VP1 and the viral early proteins. Proc. Natl. Acad. Sci. USA **84**:1210–1214.