JOURNAL OF VIROLOGY, July 1992, p. 3979–3985
0022-538X/92/073979-07$02.00/0

Vol. 66, No. 7

MINIREVIEW

Common and Unique Features of T Antigens Encoded by the Polyomavirus Group

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Simian virus 40 (SV40) and murine polyomavirus (PyV) have proved to be powerful models for understanding the molecular events involved in the regulation of eukaryotic genome expression and replication. In addition, the fact that these viruses are tumorigenic in animals and transform cells in culture has provided investigators with tools with which to dissect the mechanisms leading to tumorigenicity. Much of the progress made with these systems is due to their elegantly simple genome structure and strategy of productive infection. After infection, transcription initiating at the early promoter leads to synthesis of the early viral genes. SV40 encodes two early proteins, large T antigen and small t antigen, while PyV expresses three, the large, small, and middle T antigens. The action of one or more of these proteins results in replication of the viral genome, activation of the late transcription unit which encodes the viral capsid proteins, repression (autoregulation) of the early promoter, and efficient viral assembly. Remarkably, in addition to their roles in regulating viral transcription, DNA replication, and assembly, the T antigens are also responsible for tumor induction by these viruses.

Over the past few years, the complete genome sequences of several members of the polyomavirus group have become available. Twelve polyomaviruses have been identified, and the complete nucleotide sequences for nine of these have been reported (Table 1). Although these viruses exhibit a variety of host ranges and cell-type tropisms, they all share a common genome organization, consisting of an early coding block (the T antigens) and a late coding block (the capsid proteins VP1, VP2, and VP3) separated by a regulatory region that contains the viral origin of DNA replication (*ori*). This region also contains the promoters for both the early and late coding blocks, transcriptional enhancers, and multiple large T-antigen binding sites.

The genetics and biochemistry of the SV40 and PyV T antigens have been under intense study and are the subject of several excellent reviews (1, 9, 14, 28, 36). These studies have led to the realization that many T-antigen activities can be localized to discrete functional domains and/or require common structural motifs (9). We have used computer-assisted techniques to search for common features among proteins encoded by the polyomavirus group, to examine the conservation of the various motifs identified in genetic studies, and to assess sequence homology among functional domains (27a). In this article, I review common and unique features found in the large and small T antigens in relation to recent biochemical and genetic data on the structure and function of these viral proteins.

LARGE T ANTIGENS

Genetic and biochemical studies of the large T antigens encoded by SV40 and PyV have shown that they are multifunctional proteins composed of several functional domains. The mapping of individual biochemical activities to specific regions of the molecule and the role of phosphorylation and oligomerization in T-antigen function have been recently reviewed (9, 28). The organization of some of these activities and motifs for the SV40 T antigen is shown in Fig. 1A.

When the primary sequences of 10 large T antigens are aligned pairwise, overall amino acid identity varies from 15 to 80%. JC virus (JCV), BK virus (BKV), SA12 virus (SA12), and SV40 are closely related, with 75% identity. On the other hand, the avian budgerigar fledgling disease virus (BFDV) and bovine polyomavirus (BPyV) show the least identity (15 to 35%) with other members of the group. T antigens encoded by the remaining viruses, lymphotropic polyomavirus (LPV), hamster polyomavirus (HaPV), K virus (KV), and PyV, show an intermediate level of homology (45 to 55%) both with each other and with SV40 T antigen.

When the domain organization of the T antigens are compared, these proteins fall into two structural classes: one exemplified by SV40 (SV40 class) and the other by PyV (PyV class). There are two major differences between these classes. (i) The PyV prototype, which includes the T antigens encoded by PyV, HaPV, LPV, KV, BPyV, and BFDV, contains insertions within the amino-terminal domain relative to the SV40 class. (ii) The PyV-type T antigens lack a functional domain that maps to the carboxyl terminus of the SV40 class of proteins. In SV40, this domain has been shown to encode an activity that functions in viral assembly. To examine the relationships of the different T antigens in more detail, I have compared the sequences of each functional domain individually.

Amino-terminal domain. For the purposes of this discussion, the amino-terminal domain of the large T antigens is defined as extending from the initiation codon up to the nuclear localization signal (NLS). The assignment of this domain is based on the observations that (i) SV40 mutant T antigens that prematurely terminate just prior to the NLS retain certain biological activities, such as the ability to transform certain cell types and to transactivate viral promoters (34), and (ii) mild proteolysis of SV40 T antigen produces a metastable fragment extending from the amino terminus to the NLS (41). The size of this domain is the most variable portion (from 87 to 273 amino acids) among the different large T antigens. Because of the splicing pattern of early viral mRNA, in all cases the amino-terminal portion of

TABLE 1. The polyomavirus group

Virus	Host	Genome length (bp)	Virus-encoded proteins[a]						Reference
			LT	ST	MT	VP1	VP2	VP3	
JCV	Human	5,130	688	172		354	344	225	11
BKV	Human	5,153	695	172		362	351	232	33
SV40	Rhesus monkey	5,243	708	174		364	352	234	10, 30
LPV	African green monkey	5,270	679	189		368	356	237	22
BPyV	Bovine	4,967	586	124		365	353	232	32
HaPV	Hamster	5,366	751	194	401	373	346	221	7
PyV	Murine	5,392	785	195	432	385	319	204	12
KV	Murine	4,754	646	158		373	321	222	20
BFDV	Avian	4,980	554	145		343	341	235	31
SA12[b]	Chacma baboon	NR[c]	699	172		NR	NR	NR	6, 38
RKV	Rabbit	NR	NR	NR	NR	NR	NR	NR	13
RPV	Rat	NR	NR	NR	NR	NR	NR	NR	40

[a] Number indicates the number of amino acids in the primary sequence. Does not include agno- or SLP protein sequences. LT, large T antigen; ST, small t antigen; MT, middle T antigen.
[b] SA12 large and small T-antigen sequences are from Cunningham and Pipas (6a).
[c] NR, not reported.

this domain shares amino acid sequences with small t antigen.

In SV40, the amino-terminal domain plays an essential role in both viral replication and transformation. Several cellular proteins bind to this domain, including DNA polymerase α, retinoblastoma protein (Rb), and p107 (9). Three sequence elements within this domain are conserved among the T antigens. They include sequences similar to those of conserved region 1 (cr1) and conserved region 2 (cr2) of the adenovirus E1A proteins and the highly conserved hexapeptide HPDKGG (Fig. 2).

In E1A proteins, cr1 consists of the sequence (E/D) X_3LX(E/D)LX_2(L/I). In SV40 T antigen, this corresponds to amino acids 9 to 19, a region encoding a transformation function that is functionally analogous to the E1A 300,000-kDa binding function (25, 42). A sequence similar to this occurs in all T antigens (Fig. 2). In the T antigens, the LXXLL pattern is absolutely conserved within this region. The avian virus does not have the amino-terminal acidic residue but rather has a serine at that position.

The cr2 consensus sequence is (D/N)LXCXE. In SV40, this corresponds to residues 102 to 107 and forms part of the binding site for the cellular proteins Rb and p107. This sequence is present in the T antigens of all polyomaviruses with the exception of BFDV, which has a glycine at the first position and is missing the conserved cysteine. T antigens encoded by SV40, BKV, JCV, LPV, and PyV have been shown to complex with the Rb protein in vitro (8). The others have not yet been tested. In SV40 and PyV, mutations within this motif result in the inability to transform some cell types.

With the exception of the bovine virus, all large T antigens contain the absolutely conserved hexapeptide HPDKGG. This occurs at residues 42 to 47 in SV40. Amino acid substitution mutations within this sequence of the SV40 protein do not affect transformation but render the virus defective for viral DNA replication (24). All polyomaviruses, including BPyV, possess this sequence in small t antigen. Thus, this sequence represents an element distinct from cr1 and cr2 that may be involved in interacting with a cellular component essential for genome replication.

The amino-terminal domain varies in size among the different T antigens. This variability is largely due to the presence of extra sequences at two locations within the domain (Fig. 1). Following SV40 T antigen amino acid 66, the PyV, HaPV, and LPV T antigens have insertions ranging from 24 to 29 amino acids. There is no obvious relationship among the inserts of these different viruses. Following SV40 T antigen amino acid 113 which is just carboxyl terminal to the Rb-p107 binding site, PyV, HaPV, LPV, KV, and BPyV have inserts ranging in size from 6 to 115 residues. Again, there is little relation among the inserts. The variation in the structure of the amino-terminal domain, coupled with biochemical and genetic data, suggests that this domain is composed of multiple functional elements, perhaps each interacting with a different cellular target. Since this domain communicates with the viral DNA binding domain as a result of phosphorylation, it provides a way for T antigen both to control cellular functions and to communicate the state of cellular physiology to T-antigen replication functions.

In SV40, the amino-terminal domain contains at least one threonine and several serine residues that are phosphorylated (Fig. 2). Phosphorylation of some of these sites has been shown to have functional significance. Serines 120 and 123 are dephosphorylated in vitro by protein phosphatase 2A (9, 28). Treatment of T antigen with protein phosphatase 2A renders it more active in in vitro replication assays. Phosphorylation of SV40 T antigen at Thr-124 is essential for its ability to bind site II and stimulate DNA replication. This Thr is conserved in all but the BFDV T antigen. Two other residues that are phosphorylated in the SV40 T antigen are highly conserved. Ser-112 is conserved in all T antigens, and Ser-120 is present in all but the avian and bovine viral T antigens.

NLS. In SV40 T antigen the NLS, P(126)KKKRKV(132), is the minimal signal sufficient for nuclear transport. A similar sequence occurs at this position in nine of the sequenced T antigens (Fig. 2). Only the avian virus lacks this sequence.

DNA binding domain. The SV40 T antigen minimal DNA binding domain extends from amino acids 135 to 249. Amino acid identity among all sequenced T antigens in this domain is about 45%, with two exceptions. (i) The SV40, BKV, SA12, and JCV T antigens are more closely related to each other than to the rest, showing about 80% identity. (ii) The BFDV T antigen is only distantly related to the others, its maximal homology being with the LPV domain (26%).

Of the 115 amino acids in the SV40 DNA binding domain,

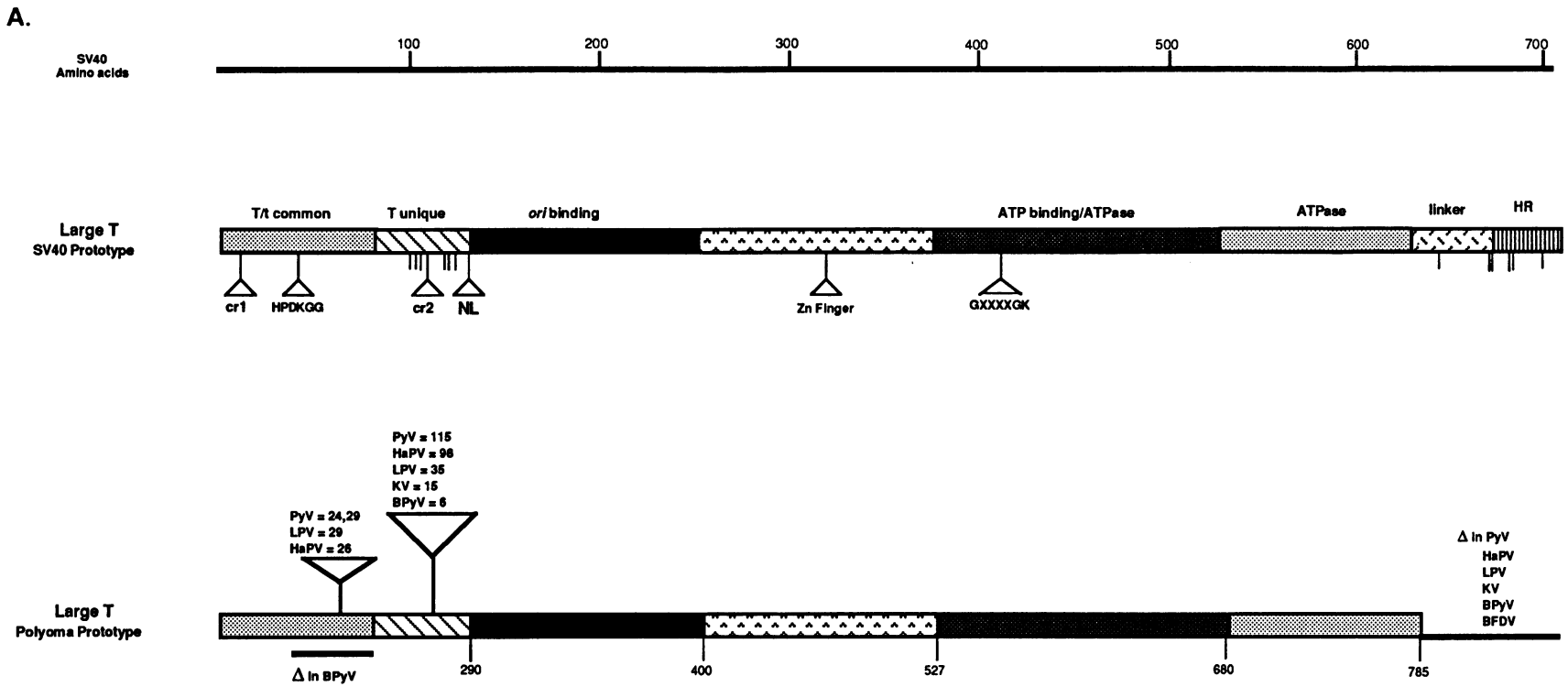

FIG. 1. Domains and motifs of large and small tumor antigens. (A) The arrangements of domains and sequence motifs for two prototype large T antigens (SV40 and PyV) are shown. Vertical lines indicate phosphorylated serine and threonine residues in SV40 T antigen. Note that all T antigens of the polyomavirus type lack a host range domain and contain inserts relative to SV40 T antigen within the amino-terminal domain. (B) The arrangements of motifs in small t antigens are shown. The small t antigens putatively encoded by BPyV and BFDV do not contain the cysteine repeats and bear little or no similarity to the small t proteins encoded by other polyomaviruses or to each other.

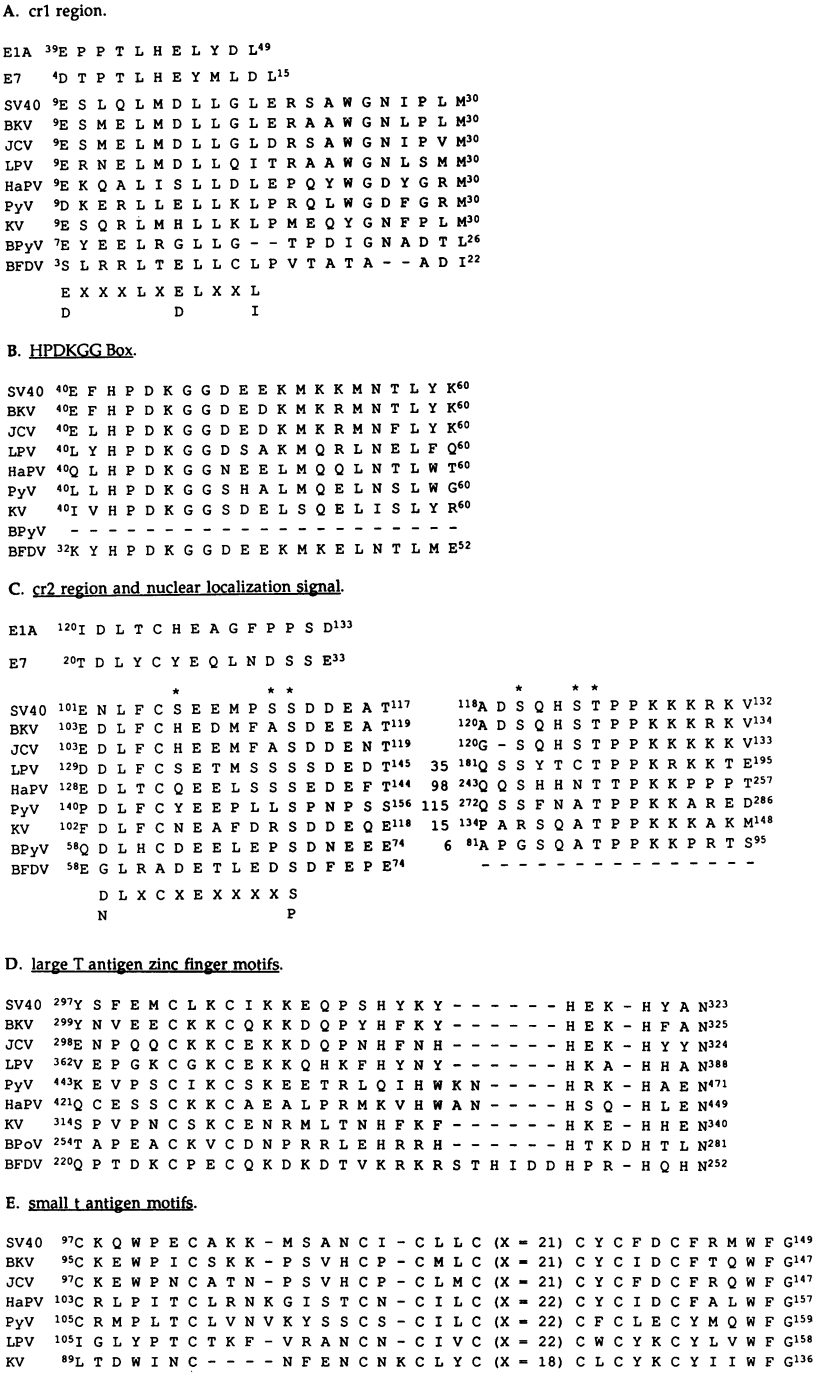

FIG. 2. Sequence motifs of large T and small t antigens. Sequence alignments of the cr1 motif (A), the HPDKGG box (B), cr2 (C), and the zinc finger motif (D) of large T antigen are shown. An asterisk indicates a residue phosphorylated in SV40 T antigen. (E) Cysteine repeats of small t antigens.

only 20 are absolutely conserved among all polyomaviruses, excluding BFDV. Among these is Glu-166, which when mutated in SV40 to a Gln or a Lys results in a T antigen with relaxed origin-binding specificity (19, 23). On the other hand, Ala-157, which yields a similar phenotype when changed to a Thr, is conserved only among SV40, JCV, and BKV.

The SV40 and PyV T antigens bind viral DNA within or near the origin of replication (*ori*) via the consensus pentanucleotide G(A/G)GGC. The *ori* regions of all members of the polyomavirus group, except BFDV, contain multiple copies of this sequence. Thus, it is likely that most T antigens bind viral DNA in a similar manner by recognizing this sequence. BFDV is the only member of the polyomavirus group that does not contain the GAGGC element in its regulatory region.

This fact, coupled with the observation that the BFDV T-antigen domain is only distantly related to the other T antigens, suggests that this T antigen might recognize *ori* DNA by a different mechanism. Interestingly, a possible zinc finger motif ($CX_2CX_{15}HX_3H$) is present near the amino-terminal boundary of the putative BFDV DNA binding domain. Perhaps this indicates that there are two ways to form a polyomavirus T-antigen binding domain, one involving a zinc finger and the other involving an alternative structure utilized by the other members of the group.

Zinc finger region. The zinc finger region of SV40 T antigen (approximately amino acids 250 to 370) lies between the *ori* binding and ATPase domains of the molecule. Amino acid identity among T antigens varies from 30 to 55% in this region. Of the 122 residues in SV40 T antigen, 17 are conserved among all T antigens. Although this region has not been shown to function as an independent domain, it possesses some common motifs and conserved sequences. Most prominent among these is a potential zinc-binding motif.

All T antigens have a C_2H_2 zinc finger motif (Fig. 2). In SV40 T antigen this sequence includes C-302, C-305, H-317, and H-320 and may play a role in oligomerization of the protein (16). There is little sequence conservation amino terminal to C-302. Y-292, D-284, and A-277 are conserved in all T antigens. There is also a leucine-rich hydrophobic sequence just C terminal to the conserved aspartic acid in all T antigens. The spacing between the conserved cysteine residues is always CXXC, with X = K in one of the positions in all but the avian virus T antigen. The size of the loop between the last conserved cysteine and first conserved histidine varies from 11 to 17 residues. There is little sequence conservation in this region. The loop between the conserved histidines is HXXH in eight of the nine sequenced T antigens. The bovine virus has three residues. There are several residues following the finger motif that are absolutely conserved in all T antigens. In SV40, these include N-323, A-324, Q-333, K-334, C-337, A-340, A-346, R-357, L-361, and R-364.

In SV40 T antigen, another leucine-rich stretch lies just C terminal to the zinc finger motif. Starting at L-345, there are four leucines with the spacing $LX_7LX_7LX_7L$. BKV has an identical arrangement of leucines. However, except for L-361, none of these residues is conserved among all T antigens. The functional significance of this pattern is still unclear.

ATPase-p53 binding domain. The most highly conserved domain among all polyomavirus T antigens is the ATPase domain. Sequences important for complex formation with the cellular p53 protein also lie within this domain. The consensus GXXXXGK motif, found in many nucleotide-binding proteins is present in all T antigens in the form $GPX_1X_2X_3GKT$, where the first X_1 = V or I and X_2 = N or D (39). The carboxyl-terminal portion of this region is very highly conserved among all T antigens, including the motif GXXXVNLE. Computer modeling has been used to generate a hypothetical structure for this domain on the basis of structural homologies with nucleotide-binding proteins whose structures have been solved (2). These models predict that the nucleotide-binding fold of T antigen lies between residues 418 and 530. For the purposes of this article, we consider this domain to extend from SV40 T antigen residue 371 to 530. Of these 130 residues, 33 are conserved among all polyomavirus T antigens. The T antigen of the avian polyomavirus BFDV, which shows little relation to most other T antigens throughout most of the molecule, nevertheless shows from 48 to 60% identity with other T antigens within the ATPase domain.

While computer modeling places the carboxyl-terminal boundary of the nucleotide-binding domain approximately at residue 530, many mutations that alter amino acids between residues 531 and 624 are ATPase defective (3, 5, 18, 37). When compared with the putative nucleotide-binding fold, the carboxyl-terminal region of this domain is relatively unconserved among the various T antigens. Amino acid identities range from around 20 to 40%. The SV40 region consists of 94 amino acids, of which three are absolutely conserved in all T antigens. All T antigens do have a stretch of hydrophobic residues (starting at SV40 amino acid 572) at a conserved position. Mutations in this region of the SV40 T antigen result in a protein that is defective for ATPase activity, p53 complex formation, and oligomerization (26, 37).

Host range domain. A functional domain located at the carboxyl terminus of the SV40 large T antigen has been shown to encode an activity, termed host range function, that is required late in viral productive infection, probably in viral assembly (15, 27, 35). This same function also allows human adenoviruses to grow in normally nonpermissive monkey cells and thus has also been called the adenovirus helping function (4). Sequence comparison analysis reveals that only the SV40, BKV, SA12, and JCV T antigens possess a host range domain. All other T antigens terminate at a position equivalent to SV40 T antigen residue 625, just after the sequences required for ATPase function.

The host range function has been shown to reside in the carboxyl-terminal 38 amino acids of SV40 T antigen. Sequences present in SV40 (residues 625 to 669) are not present in any other T antigen, and mutants with deletions in these sequences behave like wild type. This probably represents an interdomain linker, although it is possible that these sequences encode an as-yet unknown biochemical function. Of the 38 SV40 residues required for host range function, 22 are conserved in JCV and BKV. All polyomaviruses that lack a host range domain have insertions of extra amino acids within the amino-terminal domain of T antigen (see above). It is not clear whether these viruses also require a host range-like function for assembly. Such a function could be carried within these insertions or on another virus-encoded protein. Alternatively, they could assemble by a mechanism distinct from that of the SV40 class of viruses and thus not require this function. There is no obvious sequence identity between the amino-terminal inserts of the PyV class of viruses and the host range domain of the SV40 class.

SMALL t ANTIGENS

All polyomaviruses contain an open reading frame and splice signals consistent with their encoding a small t antigen (Fig. 1B). These small t antigens share an amino-terminal region with large T antigen, because both proteins initiate at the same ATG. The unique carboxyl termini of the small t antigens are created by differential splicing of a common precursor mRNA. The SV40 small t antigen has been reported to transactivate the adenovirus E2 promoter in some contexts (17) but not in others (29). In addition, the small t antigens from PyV and SV40 have been shown to form a complex with the catalytic subunit and one regulatory subunit of protein phosphatase 2A (21, 43). It is thought that by forming this complex small t antigen inhibits protein phosphatase 2A activity or alters its substrate specificity.

Large T-small t common region. The amino-terminal portion of all small t antigens has the same sequence as the corresponding large T antigen. Thus, features such as cr1 are present in all small t antigens. In addition, the conserved

HPDKGG box is present in all small t antigens. This includes the putative small t protein encoded by BPyV, although the large T antigen of this virus does not possess the HPDKGG box because the splicing pattern of the large T mRNA eliminates it.

Small t-antigen carboxyl-terminal domain. The carboxyl-terminal regions of the SV40-, BKV-, JCV-, HaPV-, and PyV-encoded small t antigens possess a stretch of cysteine residues with conserved spacing, followed by the conserved sequence Trp-Phe-Gly (Fig. 2). The consensus motif is $CX_5CX_{7-8}CXCX_2CX_{21-22}CXCX_2CX_3WFG$. The putative LPV small t antigen also has this motif but is missing the amino-terminal cysteine. In the KV small t antigen there is a slightly different spacing of the cysteines in the amino-terminal portion of the motif. The function of these sequences is not known.

The carboxyl-terminal regions of the putative small t antigens encoded by BPyV and BFDV do not have these cysteine repeats. In fact, both of these small t antigens have only a single cysteine in this region. There is little or no relation between the carboxyl-terminal domains of these small t antigens when compared with each other or with the small t antigens of the other polyomaviruses. This could indicate that this domain of the BPyV and BFDV proteins has a unique function. On the other hand, the coding sequences of the BFDV and BPyV small t antigens have been inferred from DNA sequence analyses. These proteins have not been observed nor have the splicing patterns of the early mRNAs of these viruses been analyzed. Thus, it is possible that these viruses do not express a small t antigen.

SUMMARY

Although 12 different members of the polyomavirus group have now been identified, only SV40 and PyV have been studied extensively. Whereas each member of the group shows a restricted host range, viruses infecting species from birds to humans have been reported. Although little is known concerning the biology of natural infections in the wild, it is apparent that these viruses exhibit various cell-type tropisms. Some viruses, such as LPV (B lymphocytes) or KV (pulmonary endothelium), are tightly restricted to specific cell types, while others, such as PyV, infect a variety of tissues in the animal. Despite these differences, all polyomaviruses share a common strategy of productive infection, expressing T antigens which act both on cellular targets, preparing cellular metabolism for supporting optimal viral replication, and then on targets within the viral genome, to regulate viral DNA replication, transcription, and assembly. Presumably, this common replication strategy restricts the degree to which the sequences of these viruses can diverge. Thus, sequence motifs conserved among these different viruses may indicate key structural elements essential for biochemical function.

In this article I have compared the sequences of all polyomavirus-encoded large and small T antigens sequenced to date. This has led to the following conclusions and speculations.

(i) Comparison of the domain organization of different large T antigens reveals that these proteins fall into two structural classes. Members of the SV40 class, which include SV40, JCV, BKV, and SA12, possess a carboxyl-terminal domain, which in SV40 has been shown to be dispensable for viral DNA replication but essential for virion assembly. The PyV class lacks the carboxyl-terminal domain and carries additional amino acids within the amino-terminal domain. When total amino acid identity is examined, members of the SV40 class show the highest degree of conservation (65 to 85%), while sequence identity among the remaining viruses varies from 18 to 55%.

(ii) The DNA binding domains of most large T antigens are closely related, with amino acid identities ranging from 35 to 86%. Several residues within this domain are invariant among all T antigens. All of these viruses have multiple copies of the consensus T-antigen-binding pentanucleotide (GAGGC) in their *ori* region, suggesting that all T antigens recognize this sequence. The single exception is the large T antigen encoded by the avian virus BFDV. The putative DNA binding domain of this protein shows little or no sequence relation to that of other T antigens. Furthermore, the GAGGC motif is not found in the *ori* region of this virus. Thus, the BFDV T antigen may bind DNA by a distinct mechanism.

(iii) Some sequence motifs, such as the zinc finger, nucleotide-binding fold, and cr1- and cr2-like sequences are conserved in all large T antigens. In addition, all large T antigens except the BFDV protein have an SV40-like NLS, and all except the BPyV protein have the hexapeptide HPDKGG.

(iv) All polyomaviruses have an open reading frame consistent with their encoding a small t antigen. All small t antigens have a cr1 motif and the HPDKGG box in the small t-large T common region. The small t unique regions of the BPyV- and BFDV-encoded proteins have little or no sequence relationship to each other or to the small t antigens encoded by the other viruses. All of the other viral small t antigens have cysteine-rich small t unique regions with a highly conserved spacing.

As indicated above, some of the biochemical activities of large and small T antigens act on cellular targets to alter cell regulation. One possible explanation for the variation observed among these proteins, for example the presence or absence of specific domains or sequence motifs, is that each viral T antigen has evolved to interact with targets, some of which are specific for the type of cells in which that virus normally grows. This is particularly relevant to the amino-terminal domains of the large T antigens which are known to interact with host proteins involved in regulating cell proliferation and gene expression and which show the greatest size variation of all the domains. Thus, even though the polyomaviruses may employ common strategies of replication and may interact with a common set of cellular proteins, some viral T antigens may target unique host systems specific for given cell types. This hypothesis predicts that more excitement and insight into cellular control functions await the further characterization of these complex multifunctional regulatory proteins.

ACKNOWLEDGMENTS

This work was supported by NIH grant CA40586 to J.M.P. and by a grant from the Pittsburgh Supercomputing Center through NIH Division of Research Resources cooperative agreement 1 P41 RR06009-01.

I thank Jailaxmi Vartikar for the computer-assisted sequence analyses. I also thank Brenda Collins, Alex Castellino, Ellen Fanning, Avril Arthur, Roger Hendrix, Keith Peden, Ashok Srinivasan, Terry Van Dyke, and Jailaxmi Vartikar for critical comments.

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