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Evolution

Coevolution of persistently infecting small DNA viruses and their hosts linked to host-interactive regulatory domains

(polyomavirus/papillomavirus/parvovirus/retinoblastoma protein/p53 protein)

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ABSTRACT **Although most RNA viral genomes (and related cellular retroposons) can evolve at rates a millionfold greater than that of their host genomes, some of the small DNA viruses (polyomaviruses and papillomaviruses) appear to evolve at much slower rates. These DNA viruses generally cause host species-specific inapparent primary infections followed by lifelong, benign persistent infections. Using global progressive sequence alignments for kidney-specific Polyomaviridae (mouse, hamster, primate, human), we have constructed parsimonious evolutionary trees for the viral capsid proteins (VP1, VP2/VP3) and the large tumor (T) antigen. We show that these three coding sequences can yield phylogenetic trees similar to each other and to that of their host species. Such virus–host "co-speciation" appears incongruent with some prevailing views of viral evolution, and we suggest that inapparent persistent infections may link virus and most host evolution. Similarity analysis identified three specific regions of polyoma regulatory gene products (T antigens) as highly conserved, and two of these regions correspond to binding sites for host regulatory proteins (p53, the retinoblastoma gene product p105, and the related protein p107). The p53 site overlaps with a conserved ATPase domain and the retinoblastoma site corresponds to conserved region 1 of E1A protein of adenovirus type 5. We examined the local conservation of these binding sequences and show that the conserved retinoblastoma binding domain is characteristic and inclusive of the entire polyomavirus family, but the conserved p53-like binding domain is characteristic and inclusive of three entire families of small DNA viruses: polyomaviruses, papillomaviruses, and parvoviruses. The evolution of small-DNA-virus families may thus be tightly linked to host evolution and speciation by interaction with a subset of host regulatory proteins.**

Disease-causing acute viral infections appear to affect host populations in a manner similar to predator effects on prey populations. This is seen with epidemic influenza, measles virus, poxvirus, and, more recently, human immunodeficiency virus (for early review see refs. 1 and 2). During early human evolution, however, tribal extinction, isolation, or immunity should eradicate most acute viral diseases but subacute or persisting viral infections could be maintained in small human populations (3). It has been observed that populations of RNA viral genomes (4) [or retroviruses (5)] can evolve at extremely high rates, generating diverse genetic compositions which may be considered as quasispecies (6, 7). In addition, some chronic viral infections of individual hosts show evolution of virus populations in apparent adaptation to host defenses, possibly leading to emergence of disease (8–10). The diversity, adaptability, and rapid evolution of these and other parasites has been proposed to be an important driving force in the evolution of the host, including the origin of sex (11). Such high evolutionary rates of viruses and reverse-transcriptase-mediated, vertically transmitted transposable elements (cellular retroposons) appear to result in a dislinkage from the much slower rates of host species evolution (for review see ref. 5). Yet high rates of evolution and adaptation to cause disease may not be a characteristic of all viral families.

Some virus families (especially the small DNA viruses, including the Polyomaviridae, Papillomaviridae, and some Parvoviridae), however, appear to be relatively stable genetically and also may not fit a predator–prey population model. In contrast to many RNA and some other viruses, the small DNA viruses generally cause inapparent primary infections followed by lifelong persistent infection with little disease (12–14). Also, unlike rapidly evolving RNA viruses, these DNA viruses replicate by using host error-correcting DNA replication processes which are thought to result in low error rates and account for much of their genetic stability. Yet genetic stability is not necessarily inherent to replication of small DNA viruses. The capsid gene of canine distemper virus (a parvovirus) can evolve at high rates, similar to the hemagglutinin (HA) gene of influenza (15). Nevertheless, the genetic stability of small DNA viruses seems well established, as exemplified by human papillomavirus type 16 (HPV16, specific to the genital epithelium). Worldwide isolates of this virus show remarkably little genetic variation during persistent infection (<5%) and are distributed in geographic and population patterns similar to the distribution and migration of major human racial populations (16).

However, the genetic stability of HPV16 and its characteristic host-dependent evolution become apparent only when the analysis is restricted to viruses that infect the same tissue. In contrast, mixing HPVs of different tissue specificity results in a complex but binary phylogenetic tree (17). These two major HPV phylogenetic subgroups seem to be due to selection for growth in specific mucosal or cutaneous epithelial tissues and diverge from a putative archetypical HPV (17, 18). In analogy to this observation of tissue-specific papillomavirus evolution, we have concentrated our analysis on polyomaviruses that are capable of propagation in a common host tissue, the kidney. Although most of the polyomavirus family members do replicate preferentially in kidneys [mouse polyomavirus, BK virus, JC virus, simian virus 40 (SV40), hamster polyomavirus], primary replication in other tissue is also known (i.e., K polyomavirus in lung). Alterations to regulatory DNA can also alter organ specificity of mouse polyomavirus replication (19), but this simple genetic adaption may be a biological dead end, as persistence and propagation of the infection does not occur (20). The human polyomaviruses (BK and JC viruses) have a highly variable regulatory sequence when grown *in vitro*, but this sequence is stably maintained by unknown mechanisms during persistent human infection (21, 22).

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Abbreviations: Ad, adenovirus; CR, conserved region; HPV, human papillomavirus; NCBI, National Center for Biotechnology Information; PIR, Protein Identification Resource; Rb, retinoblastoma protein; SV40, simian virus 40.

As a rule, polyomavirus and papillomavirus families are host species-specific for replication (23), ubiquitous [even in nongregarious hosts (24, 25)], and cause inapparent or mild primary infections in young animals, followed by lifelong, nonpathogenic, persistent maintenance of nonintegrated, nondefective, episomal viral DNA (26, 27). Some pathogenic versions [such as avian polyomavirus (28) and mouse K virus (29)] exist, but these are not characteristic of most infections (12). Even parvoviruses, which are most frequently associated with acute disease, most often cause inapparent and persistent infections (13, 30). Thus, silent persistent infections with little apparent affect on the host's health are more characteristic of these virus families.

The species-specific replication of small DNA viruses implies a possible linkage to the molecular processes of host speciation. The molecular basis of this species-specific replication has been examined only with mouse polyomavirus and monkey SV40 (23) and appears to be due to the binding of the viral (early protein) T-antigen/DNA complex to cellular p53 (31) regulatory protein and cellular DNA polymerase–primase complex, resulting in species-specific initiation of viral DNA synthesis (32, 33). In addition to p53, the regulatory proteins of many DNA viruses (e.g., adenovirus, SV40) also bind to the cellular retinoblastoma-associated gene product [Rb p105 regulatory protein (34, 35)], and as with p53, such binding may lead to cellular transformation. Thus these DNA viruses are intimately linked to cellular replication processes.

What are the expected rates of evolution for the small DNA viruses? Relative to the host genome, small viral DNA genomes are about a million times smaller, have very short replication times, and exhibit exponential growth and horizontal transmission modes. Such features should allow these viruses to evolve much faster than host evolution. The high rate of genetic drift seen with capsid genes (but not regulatory genes) of recently emerged acute lethal strains of canine parvovirus appear to support this expectation (15). Yet genetic stability appears commonly, especially during persistent infections with these (36) and other DNA (21, 22) viruses, including parvoviruses (13, 36).

Persistent infections might somehow allow the maintenance of stable viral genotypes in host populations, but how this could happen is not clear. Although symbiosis appears to be an important and stabilizing evolutionary mechanism, there is little evidence that virus–host symbiosis during persistence exists with these DNA virus families, as has been proposed for polydnaviruses such as campoletis sonorensis virus and its parasitic wasp host (37). Persistent parvovirus infection could in theory protect the host by suppressing both host tumor formation (for references see ref. 14) and/or superinfection with other viruses, but direct *in vivo* evidence for this protection is lacking. It remains to be determined whether the genetic stability seen during persistent infections of individual hosts is also seen on an evolutionary time scale. In addition, despite their established potential for rapid genetic change, the possibility that the small DNA viruses are slowly evolving needs further examination and comparison with the established rapid evolution of many other virus families. Because of the ability of polyomaviruses to infect diverse vertebrate species, we examined whether an analysis of the sequence data for the polyomaviruses would show evidence for a more intimate linkage between the evolution of the virus and its host vertebrate species and whether persistent infections might be involved. In this report we present evidence of virus–host coevolution linked to domains of viral regulatory proteins which interact with a restricted or crucial set of host regulatory proteins. We also suggest a link to persistent infections.

MATERIALS AND METHODS

Phylogenetic Analysis of Polyomavirus T Antigens and Viral Proteins VP1, VP2, and VP3. Sequences from the Protein Identification Resource (PIR) were analyzed by global progressive sequence alignment (38) to construct parsimonious evolutionary trees (Fig. 1). As described by the EUGENE manual (Molecular Biology Information Resource, Baylor College of Medicine), the program builds a preliminary phylogenetic tree, by the method of Klotz and Blanken (39), from distance measures calculated in pairwise comparisons of all sequences to be analyzed. A final tree is constructed from distances calculated from aligned sequences. All sequences are aligned pairwise using the SS2 algorithm of Altschul and Erickson (40), a method which finds the alignment having the minimum total cost from the Dayhoff cost matrix. The penalty for opening a gap was set to 2.5 and the incremental penalty for each space was set to 0.5.

Similarity Analysis of Putative Rb- and p53-Like Binding Regions (Figs. 2 and 3). The National Center for Biotechnology Information (NCBI), nonredundant protein database (PIR 31.0, January 26, 1992) was probed by the BLAST program, using the complete amino acid sequence (738 residues) of the mouse polyomavirus (A2) large T antigen. The neighborhood word score threshold (T) and the cutoff score were set at 12 and 56, respectively.

RESULTS AND DISCUSSION

By using the nonredundant amino acid sequence database of the NCBI (the PIR database along with published sequences), the sequence data for VP1 proteins, the combined VP2 and

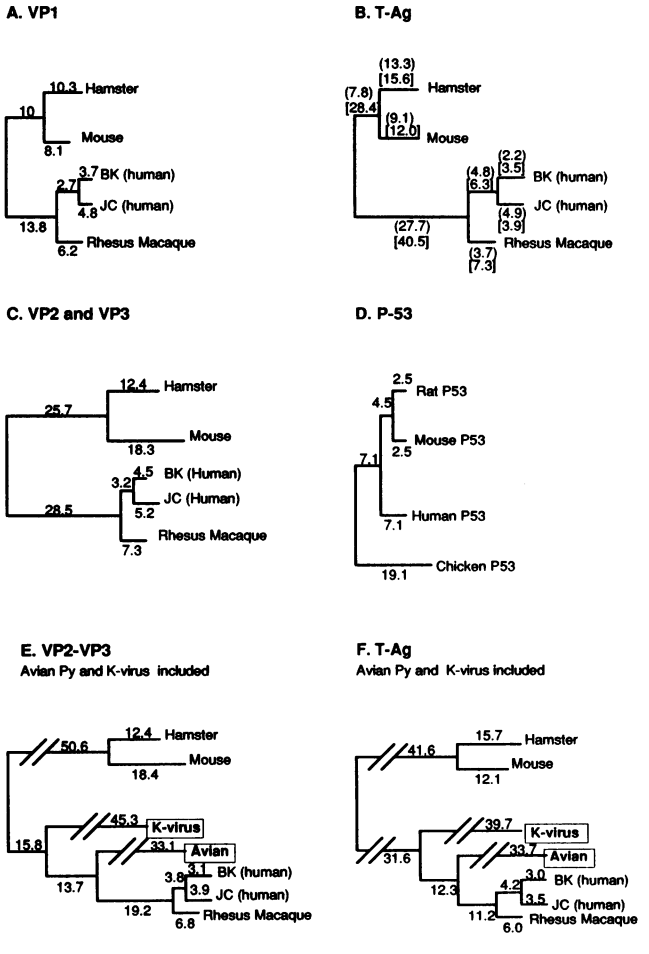

FIG. 1. Phylogenetic analysis of polyomavirus (Py) T antigens (T-Ag) and capsid proteins VP1, VP2, and VP3. In *B*, the p53-binding-domain distance measures are indicated in parentheses whereas the distance measures for the complete amino acid sequence of large T antigen are given in brackets.

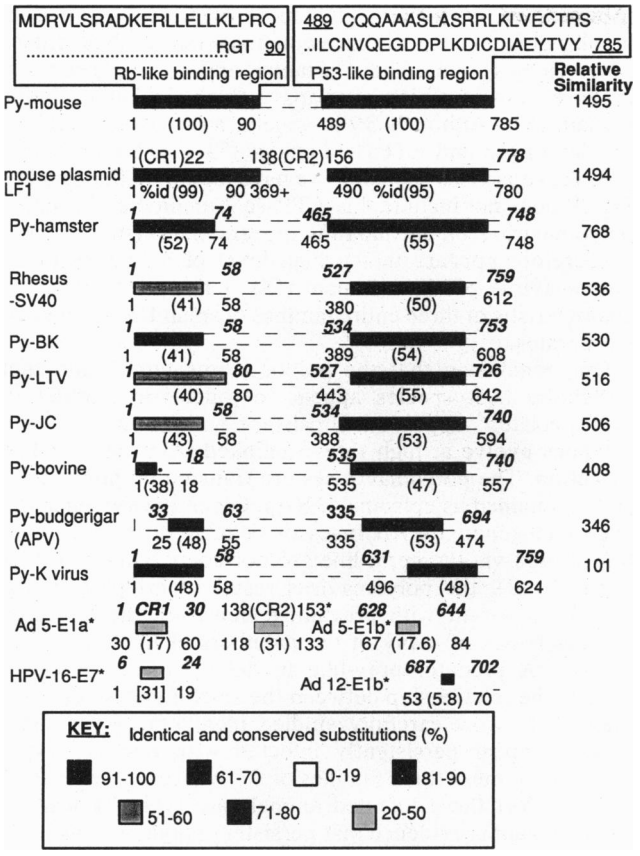

FIG. 2. Similarity analysis of putative Rb- and p53-like binding regions. The sequences retrieved are categorized into three predominant regions of the large T antigen, approximately within aa 1–90, 180–250 (not shown), and 490–778. The amino acid sequences of the two relevant regions are given at the top. Numbers below each box represent the amino acid numbers of the individual proteins corresponding to the mouse polyomavirus (Py) large-T-antigen amino acids indicated in italics above each box. Numbers in parentheses indicate percent amino acid identity (%id) to the corresponding region in polyomavirus large T antigen. The proportion of identical plus conserved amino acids is represented by the shaded areas according to the key. Adenovirus type 5 (Ad5) E1A and E1B, Ad12 E1B, and HPV16 E7, all have lower levels of amino acid sequence similarity to this region and are below the threshold of sensitivity of the program parameters (designated by stars). The approximately aligned regions as generated by the BLAST screen are displayed according to decreasing relative similarity (hit scores) in the p53-like binding region. LTV, lymphotropic virus; APV, avian papillomavirus.

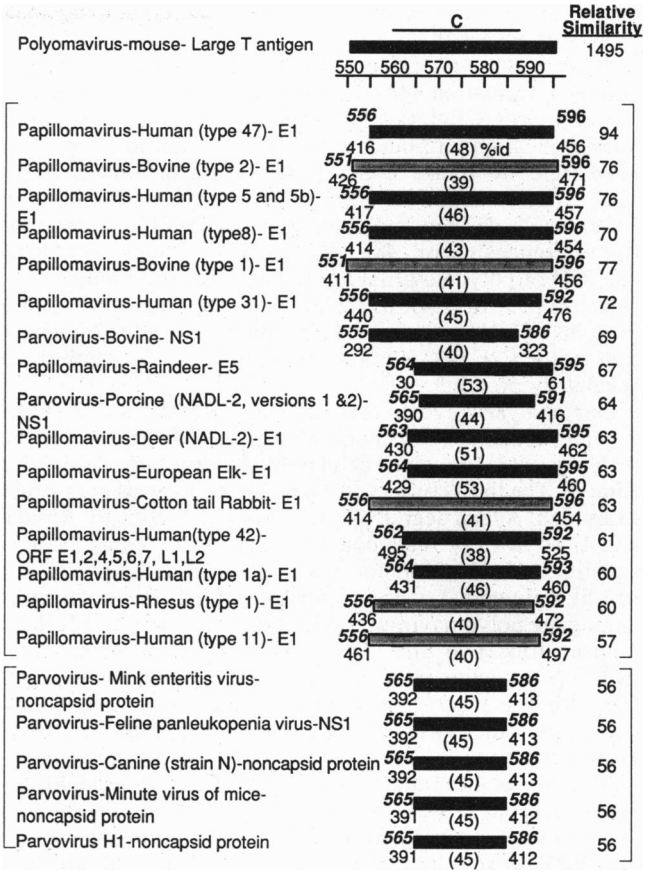

FIG. 3. Similarity analysis of putative p53-like binding region reveals corresponding sequences among regulatory proteins of Papillomaviridae and Parvoviridae. The key is as in Fig. 2. Bar C indicates the most frequently overlapping region. Complete translated cDNA sequences of various overlapping open reading frames (ORF) of E1–E7, L1, and L2 of human papillomavirus type 42 DNA are included in the analysis and represented in the figure.

VP3 proteins, and the large T antigens were analyzed for the clinically inapparent kidney-specific mouse, hamster, BK (human), JC (human), and SV40 (rhesus macaque) polyomaviruses. Parsimonious phylogenetic trees were generated by using a global progressive sequence alignment (38). The results of this analysis are shown in Fig. 1. With all three viral sequences (VP1, VP2/3, T antigen) analyzed, phylogenetic trees with the same root structure were generated. With all coding regions, mouse and hamster viral proteins appear to be derived from a common rodent ancestor, whereas the human BK and JC viruses originate from a common ancestor that is related to SV40. For comparison, a phylogenetic tree was also generated for the host p53 genes, and this tree is congruent to the polyomavirus trees. Thus these phylogenetic trees indicate that the polyomavirus capsid proteins VP1 and VP2/3 and the large T antigens diverge into sequences that are congruent with the accepted evolution of the host species. In addition, our phylogenetic patterns are consistent with early results using sequences from only three polyomaviruses and simple similarity comparison to the globin genes, which also implied virus–host coevolution (41). However, if the acute-disease-causing and/or non-kidney-specific types of polyomaviruses (avian polyomavirus and Kilham mouse polyomavirus) (28, 29, 42) are included into the phylogenetic analysis, a different root structure can be observed in that these two viruses now appear as distinct branches with no apparent relationship to the host phylogenetic tree (Fig. 1). This root difference might be due to a dislinkage of avian polyomavirus and Kilham mouse polyomavirus from host evolution, or perhaps these two viruses are a distinct lineage related possibly to a different tissue specificity, as seen with the HPVs.

Using the basic local alignment sequence analysis (BLAST) software and the entire NCBI database, we compared the sequence of the mouse polyomavirus large T antigen with all database entries. This analysis identified all of the polyomaviruses, in addition to a mouse plasmid sequence not yet characterized as viral, as highly similar to each other and also identified three domains within the large T antigens which were most highly conserved [aa 1–90, 180–250 (not shown), and 490–778]. Because the early regulatory proteins of many DNA viruses bind to (or interact with) regulatory proteins such as cellular p53 and/or Rb p105 or the related p107 (43) protein, we compared the conservation of these binding domains (34, 44, 45) among polyomavirus T antigens (Fig. 2). These common binding regions were first recognized as conserved regions 1 and 2 (CR-1 and CR-2) of the Ad5 E1A gene involved in cellular immortalization and have been

characterized by monoclonal antibody and mutational analyses. Ad5 E1A CR-1 (aa 37–54) binds Rb p105 and p107, as well as p300, and inhibits interferon response and E2F complex formation (46, 47). Comparison with SV40 and polyomavirus CR-1 (aa 1–22) indicates its importance for immortalization and binding to p107, but not to Rb p105 *in vitro* (refer to figure 1 in ref. 48). CR-2 of Ad5 E1A (aa 118–137) also binds Rb p105, as do the related regions of SV40 (aa 99–117) and polyomavirus (aa 138–156) T antigens (for references see ref. 48). SV40 aa 337–517 (equivalent to aa 489–664 of polyomavirus) are required for p53 binding (45). Because p53, Rb (34), p107, and p300 all interact with early proteins of DNA viruses and also interact with the transcription factor E2F (49, 50), a common function may link these regulatory proteins.

Our analysis reveals that the CR-1 Rb-like and p53-like binding domains correspond almost exactly to two of the highly conserved regions of all polyomavirus large T antigens (Fig. 2). The third conserved T-antigen region was not further examined. A high degree of both identity (38–99% for Rb-like and 47–95% for p53-like domains) and conserved amino acid substitutions (51–99% for CR-1 Rb-like and 61–97% for p53-like domains) were observed within these domains among the polyomaviruses. Avian polyomavirus and Kilham mouse virus (two unusually lethal polyomaviruses), however, had a significantly shorter region of similarity to either CR-1 Rb-like or p53-like domains. The BLAST screen did not detect any other viral or cellular genes with high similarity. However, the Ad5 and Ad12 E1B p53-binding regions exhibited about 18% and 6% sequence identity to SV40, respectively, but this was below the sensitivity for our analysis at a word score threshold of 12 (51). Also below our threshold, HPV16 E7 exhibited 31% and 33% identity to the CR-1 Rb and p53 binding sequences, respectively. Thus it appears that the presence of well-conserved CR-1 Rb-like and p53-like binding domains is exclusively characteristic of the entire family of polyomavirus members. Yet not all polyomavirus members are observed to interact with Rb p105 at CR-1 or with p53, although most appear to interact with Rb p105 at CR-2 (34, 48). It is possible that, as suggested by others, CR-1 interaction with the Rb-related p107 protein (52) may explain the importance of this region for cellular immortalization by SV40 or polyomavirus (48). SV40 and BK virus T antigens, but not mouse polyomavirus (51) large T antigen, stably bind p53 *in vitro*. The high conservation of the p53-like binding domain we observe in all polyomavirus T antigens, including those that do not bind p53, implies either that an overlapping function for this region is responsible for the conservation (such as ATPase; see below) or that the biochemical binding data do not accurately reflect the potential interaction of p53 with mouse polyomavirus T antigen. Stable binding might not be observed if p53 binding to T antigen is affected by p53 modifications (53, 54) or if formation of a stable complex is not necessary for a transient but biologically important interaction. Phosphorylation and localization analyses of p53 by Deppert *et al.* (55, 56) appear consistent with this view. Also, domain conservation may be a better predictor of function than biochemical analysis, as was seen with the homeobox domain.

A corresponding analysis for sequences similar to only the mouse p53-like (not CR-1 Rb-like) binding domain generated an expanded set of similar sequences. As seen in Fig. 3, this sequence set now includes all the members of three families of the small species-specific DNA viruses (polyomavirus, papillomavirus, and parvovirus). Similarity to the mouse p53-like binding region ranged from 38% to 53% in identity and from 51% to 77% in conserved amino acid substitutions. These relative similarities indicate that the papillomaviruses are generally more similar to the polyomaviruses than to the parvoviruses. Again, no cellular or other viral genes were identified as having a high degree of similarity to these binding domains. Others have noted that many DNA viruses, RNA viruses, and cellular proteins appear to conserve an ATPase domain which overlaps with the p53-like binding domain (57). Although SV40 genetic analysis supports the overlap of p53 and ATPase domains (58–60), the conserved p53-like consensus sequence we have identified (labeled C in Fig. 3) does not include this ATPase domain (aa 570–627 of polyomavirus; ref. 58) and thus appears to be distinct from it. It therefore appears that a high level of similarity to the polyomavirus p53-like domain (58) is the most conserved characteristic of three entire families of small DNA viruses of vertebrates.

Our conclusion that the polyomavirus family and their vertebrate host species appear to radiate in parallel, or "co-speciate," appears inconsistent with views that viral genomes evolve at high rates, unlinked from rates of host evolution. The polyomaviruses are transmitted horizontally and maintained as episomal DNAs. Given the evidence that even endogenous retroviruses or cellular retrotransposons transmitted via the germ line are not coevolving with their hosts (5, 61), the polyomavirus result seems paradoxical. Also inconsistent with prevailing views are the biological characteristics of many of the members of the small DNA viruses. A predator–prey-like model does not appear to explain the relationship between the small DNA viruses and their host. Most carefully studied members of vertebrate species appear persistently infected with one or several members of these three families of DNA viruses (see refs. 58 and 62). Yet these infected animals are typically healthy. Also relevant is evidence that persistent polyomavirus infections shed stable genotypes (21, 22, 62, 63) but *in vitro* growth of these strains rapidly selects for altered and variable regulatory sequences (64, 65). Thus both variability and stability of the viral genome appear to occur with the same virus host when subjected to different biological relationships. That avian and Kilham mouse polyomaviruses are both highly lethal in young animals (28, 29) and did not appear to fit well into a host coevolution scheme may indicate that acute disease favors less of a linkage to host evolution or that these non-kidney-infecting strains are a different lineage. We suggest that maintaining an inapparent persistent infection is a normal and important biological strategy for the small-DNA-virus families and that lethal disease may be an uncommon or aberrant situation. It therefore seems possible that a greater viral genetic diversity is associated with acute disease and that the genetic stability of small DNA viruses, even on an evolutionary time scale, is associated with inapparent viral persistence and is not in keeping with a predator–prey-like relationship. It is also possible that the hosts may have initially evolved some regulatory genes as intracellular forms of control over amplification of viral genomes. If so, aberrant p53, Rb p105, or p107 cellular expression may affect the ability of small DNA viruses to establish, maintain, or reactivate and amplify from persistent infections.

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