Parvovirus Family Conundrum: What Makes a Killer?

Shweta Kailasan,[1] Mavis Agbandje-McKenna,[1] and Colin R. Parrish[2]

[1]Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, Florida 32610

[2]Baker Institute for Animal Health and Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853; email: crp3@cornell.edu

Annu. Rev. Virol. 2015. 2:425–50

First published online as a Review in Advance on August 26, 2015

The *Annual Review of Virology* is online at virology.annualreviews.org

This article's doi: 10.1146/annurev-virology-100114-055150

Keywords

Parvoviridae, dependoparvovirus, autonomous, pathogenicity

Abstract

Parvoviruses infect a wide variety of hosts, and their ancestors appear to have emerged tens to hundreds of millions of years ago and to have spread widely ever since. The diversity of parvoviruses is therefore extensive, and although they all appear to descend from a common ancestor and share common structures in their capsid and nonstructural proteins, there is often low homology at the DNA or protein level. The diversity of these viruses is also seen in the widely differing impacts they have on their hosts, which range from severe and even lethal disease to subclinical or nonpathogenic infections. In the past few years, deep sequencing of DNA samples from animals has shown just how widespread the parvoviruses are in nature, but most of the newly discovered viruses have not yet been associated with any disease. However, variants of some parvoviruses have altered their host ranges to create new epidemic or pandemic viruses. Here, we examine the properties of parvoviruses and their interactions with their hosts that are associated with these disparate pathogenic outcomes.

THE ANCIENT ORIGIN AND THE HOST RANGE OF PARVOVIRUSES

The Parvoviridae are an ancient family of viruses that package an ssDNA genome, and in recent years an extensive fossil record of the viruses has been identified through the recognition of viral DNA fragments integrated into the genomes of a variety of different animals, where they are found to be transmitted through the germline (1–3). The genomes in which parvovirus sequences have been found include a variety of invertebrates and mammals, and the sequences take various forms, from small fragmentary remnants or sequences with a small number of disruptions to long sequences that include intact genes. Dating the integration events can be difficult, but the degraded states of many of the sequences suggest that they were integrated millions of years ago. In addition, some integrated viral sequences appear to be syntenic and to have been integrated millions of years ago based on when the hosts diverged (1). Integrated viral sequences are found throughout the animal kingdom, and many have been found in hosts in which exogenous viruses have not been found, indicating either that the viruses have gone extinct or that we have not yet identified them. It is unclear at this time whether particular extant viruses coevolved with their hosts or have transferred from host to host, but it is likely that both mechanisms have occurred at different times, so that the current admixture of viruses and hosts has arisen over at least tens of millions of years (3). Despite their apparent great age and the wide range of hosts that they infect, all of the parvoviruses appear to share an ancient common ancestor.

The family Parvoviridae is divided into two subfamilies, the Densovirinae and the Parvovirinae, according to whether they infect invertebrates (including many arthropods and crustaceans) or vertebrates, respectively. Both subfamilies are further divided into several genera, mostly on the basis of genome organization (**Figure 1**) (4). For the purpose of study, the different members of the family Parvoviridae are roughly divided into three major groups: autonomous parvoviruses, dependoparvoviruses, and viruses of invertebrates. This review focuses on the viruses that infect vertebrates (the Parvovirinae), the autonomous parvoviruses and the dependoparvoviruses, which don't or do require a helper virus, respectively, for successful replication within cells. Members of these virus groups use related mechanisms for genome replication and control of tissue tropism, yet they differ dramatically in pathogenicity.

Where there has been deep sequencing of DNA from animal samples, including tissues or feces, many animals have been shown to carry the genomes of several different parvoviruses (in the case of feces these can also be potentially derived from the diet), and individual animals often show evidence of infection by multiple viruses (5–7). The dependoparvoviruses are not associated with any disease and replicate efficiently only in the presence of a helper virus such as an adenovirus (Ad) or herpes simplex virus (HSV) (8). This nonpathogenic property of the adeno-associated viruses (AAVs) has been exploited to deliver therapeutic genes to several mammals, including humans. In contrast, many of the autonomously replicating virus genera contain members that cause disease, and these can have highly divergent pathogenic outcomes, ranging from subclinical or nonpathogenic to severe and lethal symptoms. For example, *Erythroparvovirus* B19 virus (B19V) is the causative agent of fifth disease in children as well as various clinical diseases in adults. On the other hand, several other human parvoviruses include *Bocaparvovirus* human bocavirus (HBoV), *Tetraparvovirus* human parvovirus 4 (PARV4), and the protoparvoviruses bufavirus (BuPV) 1 and 2, recently isolated from nasopharyngeal aspirates, stool, and serum samples (9–11), but it is unclear whether the latter viruses are the causative agents of any of the disease symptoms they are associated with when infecting on their own, as they are often found as coinfections with other viruses. Several of the autonomous parvoviruses—including *Amdoparvovirus* Aleutian mink disease virus (AMDV or ADV), infecting minks; *Protoparvovirus* porcine parvovirus (PPV), infecting pigs; and *Protoparvovirus* minute virus of mice (MVM), infecting rodents—have both nonpathogenic

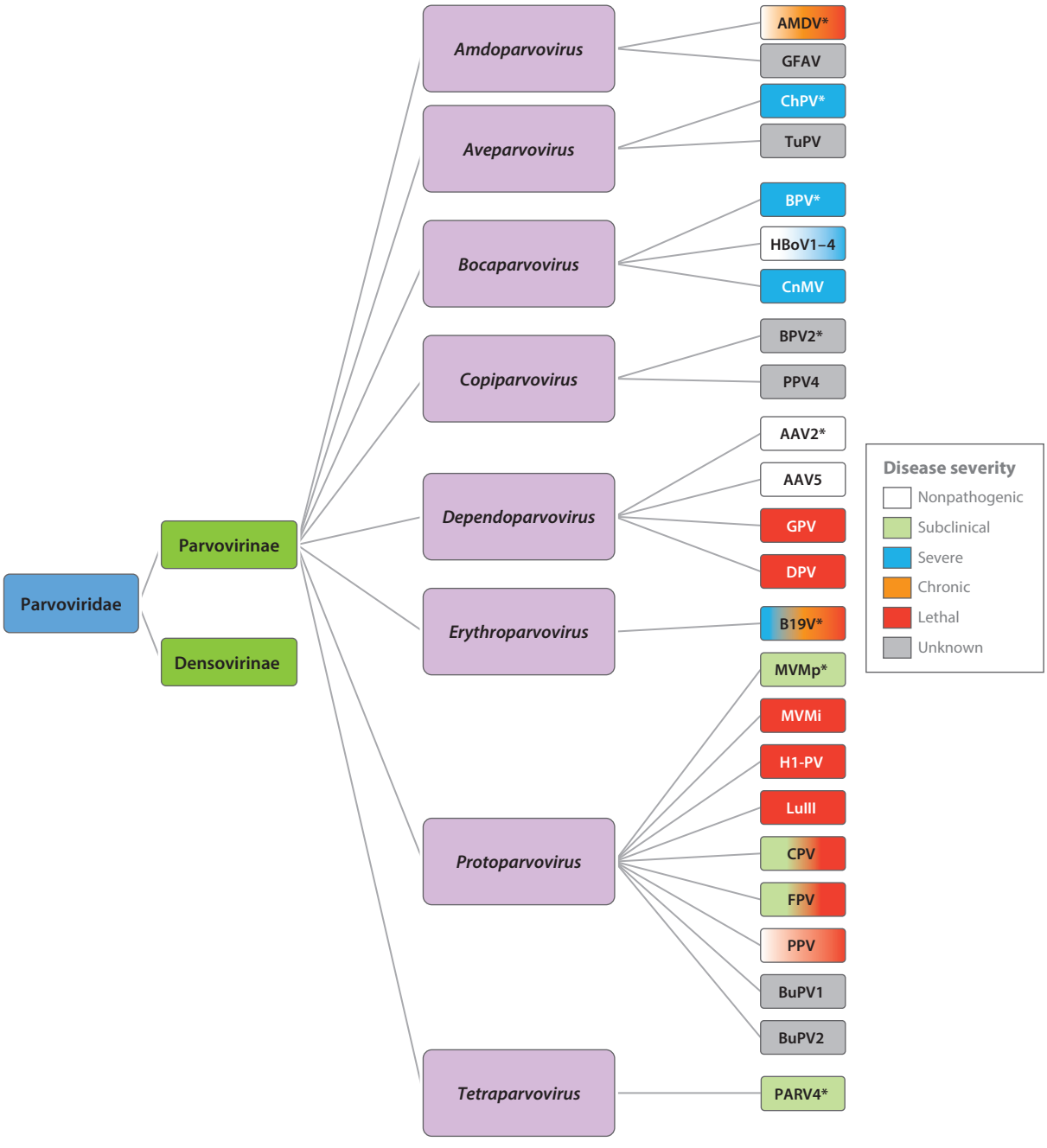


Figure 1

Classification of the family Parvoviridae. The family is divided into two subfamilies, Parvovirinae and Densovirinae. The eight genera under Parvovirinae are listed; some of their members that are discussed in this review are indicated. For the complete list of members under each genus, see Reference 4. Type members for each genus are indicated with an asterisk. The legend indicates the severity of disease caused by these viruses.

and pathogenic variants or are pathogenic only at certain host ages (often fetal or shortly after birth) (12). Furthermore, variants of other pathogenic autonomous parvoviruses, such as *Protoparvovirus* canine parvovirus (CPV), have evolved by an altered host range, in this case from *Protoparvovirus* feline panleukopenia virus (FPV), which moved from cats or related hosts among the carnivores to create a new pandemic virus that spread worldwide among dogs, coyotes, and wolves.

The linear ssDNA parvovirus genome is ~4,500 to ~5,500 bases in length, with terminal hairpins (ITRs) that are used in viral DNA replication. The genome is packaged into a nonenveloped capsid ~26 nm in diameter. The capsid is assembled from 60 copies of two or more forms of a single viral protein (VP), VP1–4, depending on the genus, encoded from the *cap* open reading frame (ORF). They are numbered in order of size, with VP1 being the largest form. These proteins share a common C-terminal sequence but have different N-terminal sequences as a result of either (*a*) alternative mRNA splicing and alternative translation initiation codon usage (in the dependoparvoviruses) or (*b*) alternative mRNA splicing, alternative translation initiation, or proteolytic cleavage following genome packaging (in the autonomously replicating parvoviruses) (13–17). A second major ORF, the *ns/rep* ORF, encodes nonstructural protein 1 and 2 (NS1 and NS2) (in autonomous parvoviruses) or Rep (in dependoparvoviruses). The dependoparvoviruses encode four gene products from the *rep* ORF: Rep78, Rep68, Rep52, and Rep40. Rep78 and NS1 have N-terminal sequences that confer DNA-binding and endonuclease functions, whereas their C-terminal domains have helicase function. The viral DNA is replicated by one or more of the host cell DNA polymerases, and the NS1/Rep protein is involved in the specific mechanisms of the processing steps in DNA replication, including introducing site-specific single-stranded nicks that allow polymerase extension, acting as a DNA helicase, and regulating gene expression (18). Due to the small size and limited genetic capacities of the viruses, each of the nonstructural and structural proteins serves many different functions during infection. The viruses appear to use a variety of common mechanisms to modulate interactions within the host cells and tissues and to mediate viral transmission in the environment, reflecting a highly economic usage of coding capacity.

THE NEED FOR DIVIDING CELLS IS A KEY DETERMINANT OF AUTONOMOUS PARVOVIRUS PATHOGENICITY

While the dependoparvoviruses can establish infection in both dividing and nondividing cells, where their DNA replication and gene expression are controlled by their helper viruses, all the autonomous parvoviruses replicate their DNA in mitotically active cells, where they can hijack components of the host cell DNA replication machinery to complete their replication. Diseases caused by autonomous parvoviruses therefore tend to be more severe in fetal or young animals, and the same viruses generally cause mild or subclinical infections in adults, in which there are fewer dividing cell populations. For example, AMDV causes acute interstitial pneumonia, killing alveolar cells and leading to fatal respiratory distress syndrome in newborn mink kits but resulting in a chronic disease in adult minks (19–21). Similarly, PPV causes severe disease in fetal piglets but little or no disease in older pigs, including the sow that is carrying the fetuses (22). In addition, several well-known pathogenic parvoviruses have tropisms for tissues that contain dividing cells in older animals, such as the developing erythroid precursor cells (B19V), lymphoid cells (mouse and carnivore parvoviruses), and regenerating epithelial cells in the small intestine (carnivore parvoviruses). Also, different disease syndromes may be seen in very young and older animals. Examples include infections by CPV and FPV: Neonatal infections result in myocarditis or cerebellar hypoplasia in dogs and cats, respectively, due to the presence of rapidly dividing cardiomyocytes or cerebellar germinal cells in very young animals (23). Adult dogs and cats do

not suffer myocardial or cerebellar diseases but instead experience subclinical infections or mild transient diarrhea.

The small parvoviral genome makes these viruses highly dependent on host cellular factors for successful infection. Hence, they have adopted unique ways to modulate their host cellular environment by exerting control over the cell cycle and regulating different cell signaling pathways to their benefit. In order to facilitate viral DNA replication, one of the early signs of infection is the hijacking of the cellular DNA replication machinery by arresting permissive cells in S phase, late S phase, intra-S phase, or G_2/M phase, depending on the virus (24). Some parvoviruses also elicit DNA damage response (DDR) pathways, which are naturally present in host cells to repair ssDNA breaks (25, 26). Activation of DDR pathways is triggered by the complex hairpin structures in the genome, which appear as ssDNA breaks resulting from the NS1 nicking step during replication of the ITRs. Instead of utilizing the DDR pathways to resolve these breaks, as would normally happen in the host cell genome, parvoviruses use sophisticated strategies to selectively activate or suppress the signaling molecules and modulate the cellular environment to establish infection and promote their own replication (27, 28). The interplay of the virus replication cycle and the DDR machinery not only is important for the virus but also may play a critical role in viral pathogenesis in a genus- or virus-specific manner (**Table 1**).

THE ROLE OF THE CONSERVED NS1 AND Rep PROTEINS AS DETERMINANTS OF PATHOGENICITY

The Parvovirinae major nonstructural proteins NS1 and Rep exhibit several conserved functions—nickase, helicase, and control of genome replication—despite evolutionary distance. NS1 and Rep proteins of various parvoviruses share an average sequence identity of ~30%, with 10–99.7% identity among the autonomous viruses and 33.3–99.6% identity among the dependoparvoviruses. Phylogenetic analysis shows that the dependoparvovirus Rep sequences are divergent from the autonomously replicating parvovirus NS1 protein sequences and cluster in their own group (**Figure 2**), reflecting the overall divergence of these different virus groups.

The NS1 proteins are able to cause direct cytopathic effects in permissive cells, characterized by necrosis and/or apoptosis, which can facilitate viral release and cell-to-cell spread (**Table 1**). For example, protoparvoviruses, which include the rodent parvoviruses (MVM, H-1PV, and LuIII), are known to induce cell death. In mouse A9 fibroblasts, prototype MVM (MVMp) NS1 interferes with CKIIα signaling, resulting in cell lysis by the destruction of cytoskeletal filaments and nuclear laminas and thereby facilitating viral spread (29). Similarly, rat parvovirus H-1PV, which is currently being developed as an anticancer gene therapy vector, arrests U937 promonocytic cells, human hepatocellular carcinoma cells, and rat glioma cells in the G_2/M phase of the cell cycle, leading to caspase-3 and caspase-9 activation by NS-mediated accumulation of reactive oxygen species and cell lysis (30–33).

In addition to the NS1 toxicity observed for the members of the *Protoparvovirus* genus, similar NS1-mediated host cell–specific toxicity has been reported for pathogenic erythroparvoviruses, bocaparvoviruses, and amdoparvoviruses in permissive cell lines or in their natural hosts. In B19V, which naturally infects cells in the erythroid lineage, NS1 lyses the cell and causes apoptosis through an unknown mechanism involving caspase-3, as observed in the human erythroleukemia cell line K562 and the erythropoietin-dependent megakaryocytic cell line UT-7/Epo (34). For this virus, NS1 also functions to mediate secretion of the AP-1 and AP-2 transcription factors as well as tumor necrosis factor α (TNF-α) and establishes pathogenesis by B19V-associated inflammation in acute and chronic arthritis patients (35). In nonpermissive cells (COS-7 monkey epithelial cells), transfected B19V NS1 can induce mitochondrion-mediated apoptosis (36). The NS1 of

Bocaparvovirus minute virus of canine (CnMV or MVC) causes apoptotic cell death specifically in permissive Walter Reed/3873D (WRD) canine cells by translocating Bax to the mitochondrial outer membrane, disrupting the mitochondrial outer membrane potential and thereby activating caspases (37). Another bocaparvovirus, *Bocaparvovirus* bovine parvovirus (BPV), initiates cell death by necrosis instead of apoptosis in host bovine tracheal cells (EBTr), mediated by the release of lactate dehydrogenase (38). AMDV is an unusual parvovirus in that it not only activates caspases to facilitate apoptosis and viral spread in permissive Crandell feline kidney (CrFK) cells but can also cleave sites on NS1 that are required for replication and translocation of the virus into the

Table 1 Cytopathic effects induced by nonstructural proteins during parvovirus infection

Genus	Virus	Virus factor(s) involved in DNA damage response	Cell cycle arrest phase	Induces apoptosis or cytotoxicity?	Cells	Reference(s)
Amdoparvovirus	AMDV	NS1	—	Caspase-mediated apoptosis	Crandell feline kidney (CrFK) cells	117
Bocaparvovirus	MVC	NS1 and DNA viral replication through ATM-SMC1 signaling	G_2/M or intra-S phase	Phosphorylation of p53 and mitochondrion-induced apoptosis	Walter Reed/3873D (WRD) canine cells	118
	BPV1	NS1 and/or NP1 (unclear)	—	Release of lactate dehydrogenase, mediating necrosis	Host bovine tracheal cells (EBTr)	38
	HBoV1	NP1	G_2/M phase	Mitochondrion-induced apoptosis through caspase-3 and caspase-9 activation	HeLa cells	50
Dependoparvovirus	AAV2	Helper virus proteins (Ad: E1b55K/E4orf6 complex; HSV: UL5, UL8, UL52, and the DNA-binding protein ICP8) through ATM signaling and DNA-PKcs	S phase	No; unclear cellular fate	—	—
		Rep78	—	p53-independent apoptosis activated by caspase-3	Human embryonal carcinoma cells (NT-2), p53-null promyelocytic human HL-60 cells	119

(*Continued*)

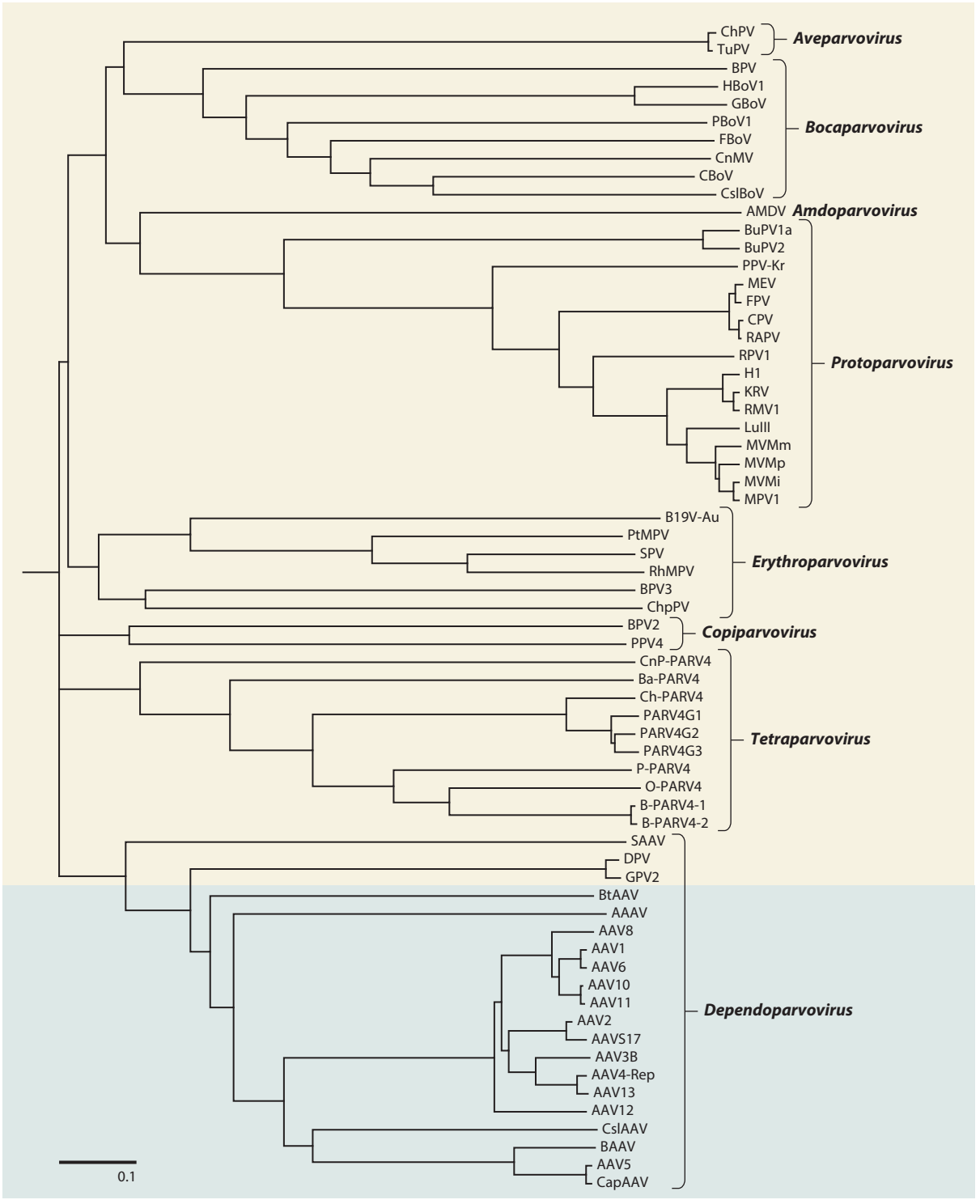
Table 1 (*Continued*)

Genus	Virus	Virus factor(s) involved in DNA damage response	Cell cycle arrest phase	Induces apoptosis or cytotoxicity?	Cells	Reference(s)
Erythroparvovirus	B19	Viral proteins (NS1, 11-kDa protein, 7.5-kDa protein, VP1, and VP2) and viral DNA replication through ATR signaling and DNA-PKcs	G_2/M or late S phase	—	—	—
		NS1	—	TNF-α-mediated apoptosis and caspase-3, caspase-6, and caspase-8 activation	Erythroid cells	34, 120
		11-kDa protein	—	Caspase-10-mediated apoptosis	Erythroid progenitor cells	49
Protoparvovirus	H-1PV	NS1 alone through reactive oxygen species accumulation	G_2/M phase	Caspase-3 and caspase-9 activation, inducing apoptosis; accumulation of cathepsin B and L in the cytosol	U937 promonocytic cells, human hepatocellular carcinoma cell line QGY-7703, rat glioma cells	31, 32, 121
	MVMp	NS1 and viral DNA replication through ATM signaling	G_2/M phase	Interference with CKIIα signaling	A9 fibroblasts	29

nucleus (39). The latter function seems to be an NS1-modified function to establish persistent infection in adult minks.

In contrast to the pathogenic parvoviruses, the nonpathogenic dependoparvoviruses show signs of efficient cytotoxicity only in the presence of helper proteins from Ad (E1b55K and E4orf6 complex) or HSV (UL5, UL8, UL52, and ICP8) (8). However, studies overexpressing Rep78 in human cell lines have shown that it can also directly regulate the cell cycle and induce apoptosis through caspase-3 activation (40). Regardless of this observation, these viruses have not been associated with any known disease. Thus, NS1 cytotoxicity is likely a significant contributor to parvovirus pathogenicity, although it is not a sole determinant, given the other effects of viral replication in the cells and the ranges of disease outcomes resulting from *cap* gene variants of the same virus with identical NS proteins.

The crystal structures reported for the N-terminal domains of the NS1 proteins of the autonomous viruses MVMp and HBoV1 and the Rep78 protein of the dependoparvovirus AAV5 show conservation of the active and catalytic site residues important for ssDNA binding and nicking incorporated onto a canonical fold of the histidine-hydrophobic-histidine (HUH) superfamily of


ChPV
TuPV
Aveparvovirus
BPV
HBoV1
GBoV
PBoV1
FBoV
CnMV
CBoV
CslBoV
Bocaparvovirus
AMDV
Amdoparvovirus
BuPV1a
BuPV2
PPV-Kr
MEV
FPV
CPV
RAPV
RPV1
H1
KRV
RMV1
LuIII
MVMm
MVMp
MVMi
MPV1
Protoparvovirus
B19V-Au
PtMPV
SPV
RhMPV
BPV3
ChpPV
Erythroparvovirus
BPV2
PPV4
Copiparvovirus
CnP-PARV4
Ba-PARV4
Ch-PARV4
PARV4G1
PARV4G2
PARV4G3
P-PARV4
O-PARV4
B-PARV4-1
B-PARV4-2
Tetraparvovirus
SAAV
DPV
GPV2
BtAAV
AAAV
AAV8
AAV1
AAV6
AAV10
AAV11
AAV2
AAVS17
AAV3B
AAV4-Rep
AAV13
AAV12
CslAAV
BAAV
AAV5
CapAAV
Dependoparvovirus
0.1

nucleases required for rolling-circle replication (**Figure 3**) (41–43). The only structure available for the helicase domain is for AAV2 Rep40 (44). The protein fold belongs to the SF3 DNA helicase family, and it has motifs for DNA binding and oligomerization. The Rep nickase domains are shorter in length than those of NS1 (**Figure 3*b*–*d***) (41–43). When projected onto the available 3D structures, amino acid sequence conservation varies along the length of the protein and differs among genera, suggesting that there may be other genus- and virus-specific functions in addition to the conserved nickase activity (**Figure 3*b*–*d***). For example, the following have been reported for MVMp NS1: functional domains of a predicted cyclin-dependent kinase (CDK) phosphorylation site (residues 219–223); conservation of an H-1PV NS1 acetylation site (residue Lys85) that is important for cytotoxicity, gene transcription, and viral replication; and an Akt/PKB phosphorylation site (Thr278) (**Figure 3*b***). These residues are located within NS1 regions that show different levels of conservation within each genus (**Figure 3*b*,*c***). Thus, differences in amino acid sequence conservation and overall protein length may contribute to the differences in the abilities of these proteins to function as effectors of parvovirus cytotoxicity, including targeting of host- and tissue-specific cellular factors, modulation of the cell cycle, initiation of necrosis/apoptosis or cell death, and establishment of cellular pathogenesis (**Table 1**).

ACCESSORY PROTEINS ARE INVOLVED IN PATHOGENESIS BUT ARE NOT COMMON DISEASE DETERMINANTS

A number of the parvovirus genera contain additional coding ORFs in their genomes that have been associated with virus pathogenesis or control of infection. The small alternatively translated (SAT) protein, encoded by a short ORF downstream of the VP2 initiation codon, is found only in the genomes of the *Protoparvovirus*, *Amdoparvovirus*, and *Tetraparvovirus* genera and is known to induce cell lysis and control viral spread between cultured cells (44, 45). For the *Erythroparvovirus* genus, two minor ORFs encoding small protein products (the 7.5- and 11-kDa proteins) dictate B19V's tropism for human leukemic bone marrow cells by blocking translation of downstream structural proteins in other hosts or cell types (46–48). The 11-kDa protein of B19V is a stronger inducer of apoptosis than B19V NS1, due to caspase-10 activation and targeted cell death of erythroid progenitor cells (49). For the *Bocaparvovirus* genus, although there is limited information available on the genus-specific NP1 encoded by ORF2 with respect to permissive cells, its function is analogous to that of NS1 in terms of inducing cell death. The HBoV1 NP1 has been reported to cause apoptosis in nonpermissive HeLa cells by the activation of downstream apoptosis activators such as caspase-3 and caspase-9 in the mitochondria (50). Furthermore, in addition to playing a role in viral replication and mediating cytotoxicity, NP1 upregulates expression of TNF-α mRNA. This elicits an inflammatory cytokine response that is indicative of its role in bocaparvovirus-specific pathogenesis. The protoparvoviruses also encode NS2, which is reported to complement the apoptotic function of NS1 and augment its cytopathic effect. For example, eliminating NS2 synthesis in a variant of the lethal lymphocyte-specific MVM strain MVMi (NS2-1990) resulted in a restricted and asymptomatic infection similar to that produced by the fibroblast-adapted MVMp strain (51). Additional studies have demonstrated that NS2 mutants are defective in capsid assembly in some host cells (52). For the nonpathogenic *Dependoparvovirus* AAV, the *cap* ORF2 encodes

Figure 2

Rooted phylogenetic tree for complete parvovirus NS1 and Rep sequences. The dependoparvoviruses and autonomously replicating parvoviruses are shown on blue and tan backgrounds, respectively. The sequence for at least one virus strain under each genus was used to generate the tree. The brackets on the right delineate the genera. The scale indicates the node length.

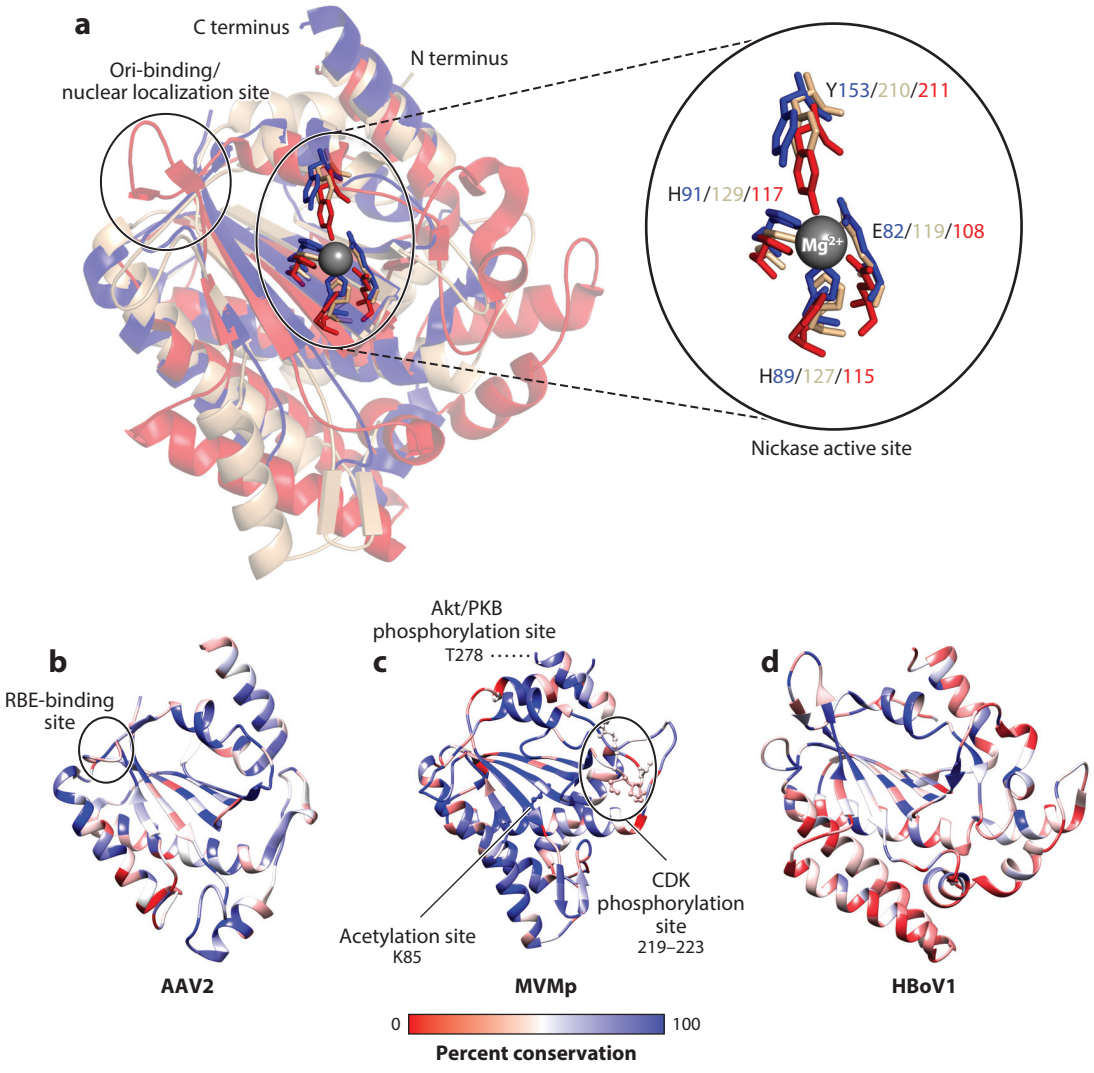


Figure 3

NS1 and Rep structures. (*a*) Cartoon representations of the superposed N-terminal structures of MVMp NS1 (*tan*), HBoV1 NS1 (*red*), and AAV5 Rep (*blue*). (*Inset*) The conserved nickase active site residues (shown in stick representation) and magnesium cation (shown as a gray sphere), with residue numbers corresponding to the three viruses, are listed in their respective colors. The N and C termini as well as the Ori-binding sites are indicated. (*b–d*) Maximum conservation percentages calculated using the NS1/Rep N terminus sequences for each available genus member for (*b*) the dependoparvoviruses, (*c*) the bocaparvoviruses, and (*d*) the protoparvoviruses are depicted on the structures of AAV2 Rep, MVMp NS1, and HBoV1 NS1, respectively. Minimum (*red*) to maximum (*blue*) conservation of residues at each position is shown. The known functional sites for the AAVs and protoparvoviruses are indicated, with the contributing residues listed on their respective structures. Structures were generated using PyMOL (123) and UCSF-Chimera (124).

a capsid assembly activating protein (AAP) in addition to the VP proteins (VP1–3) that is not found in the autonomous parvoviruses (53). This protein is essential for capsid assembly and AAV production but has no reported toxicity. Altogether, these examples highlight parvovirus proteins playing several vital roles in pathogenicity determination that appear to be genus specific and thus are not universal parvovirus strategies for disease etiology. The pathogenic parvovirus accessory

proteins serve to target cytopathic effects to host or specific tissues in order to establish a disease phenotype.

PERSISTENCE AND INTEGRATION OF VIRAL DNA ARE NOT SPECIFICALLY CONNECTED TO DISEASE

The presence of parvoviral genomes in host cells or tissues has not been connected to pathogenicity. *Bocaparvovirus* HBoV3 was isolated as an extrachromosomal closed circular DNA form (or episome) and is commonly detected in healthy adults as well as young children (54). Similar findings were reported for nonpathogenic AAV infections in young children, and a large number of genomic sequences have been isolated from human and nonhuman primate tissues (55, 56). However, in contrast to the AAV head-to-head or tail-to-tail ITR structures, bocaparvovirus genomes were found to exist as head-to-tail concatemers in infected tissues, suggesting an adapted mechanism of viral persistence different from that used by AAVs (57). Bocaparvoviruses infect immunocompromised individuals (e.g., infants below the age of 2 years or immunocompromised adults), but they are often found as coinfections with other respiratory pathogens such as respiratory syncytial virus, rhinovirus, and Ad. Therefore, sequencing and characterization of the ITRs of these newly emerging human pathogens and comparison with those of the AAVs may help us to elucidate the mechanism(s) for establishing persistent infections with or without disease phenotype, thus providing a deeper understanding of parvovirus pathogenicity.

THE DIVERSE ROLES OF THE CAPSID STRUCTURAL PROTEINS IN CONTROLLING VIRAL PROPERTIES AND PATHOGENICITY

The VP1 (and the smaller, overlapping VP) sequences of all the Parvovirinae members share sequence identities between 14% and 98%, with higher sequence identities for members of the same genus (**Figure 4**). The VP1 proteins for the *Dependoparvovirus* genus are divergent in sequence from all those of the autonomous pathogenic viruses, although, as expected, these also show genus-specific clustering (**Figure 4**). Additionally, the VP1 sequences from genera with human or other mammalian hosts, namely *Bocaparvovirus*, *Dependoparvovirus*, and *Erythroparvovirus* (with the exception of the bufaviruses), cluster more closely with one another than with the VP1 sequences from the other genera (**Figure 4**).

The largest viral capsid protein, VP1, contains an N-terminal peptide, the VP1 unique sequence (VP1u), that varies in length and sequence based on the genus; the amdoparvoviruses have the shortest VP1u sequence (43 aa) and the erythroparvoviruses have the longest (226 aa). VP1u has two significant functions. The first is a stretch of basic residues (arginines and lysines) that serve as nuclear localization signals (NLSs) (58–64); the second, which is absent from amdoparvoviruses, is a phospholipase A_2 (PLA_2) enzyme domain that enables virus escape from the late endosomal/lysosomal pathway during cell entry, allowing trafficking to the nucleus for replication (65, 66). Due to the absence of the multiple disulfide bonds that are found in the other secretary PLA_2s, the parvoviral enzymes have been classified as a separate group, the Class XIII PLA_2s (65). For the remaining VPs, the VP1/2 overlapping region contains the structural domain that assembles the capsid, additional NLS sequences used for nuclear entry, and, for the protoparvoviruses, a nuclear exit signal. The VP1/2/3 common region is required to perform the remaining infectious steps, including receptor attachment, endosomal/lysosomal trafficking, capsid assembly, and genome packaging, and is the target of the host immune response (12). Of these steps, the three that have been studied the most as potential determinants of host pathogenicity are (*a*) PLA_2

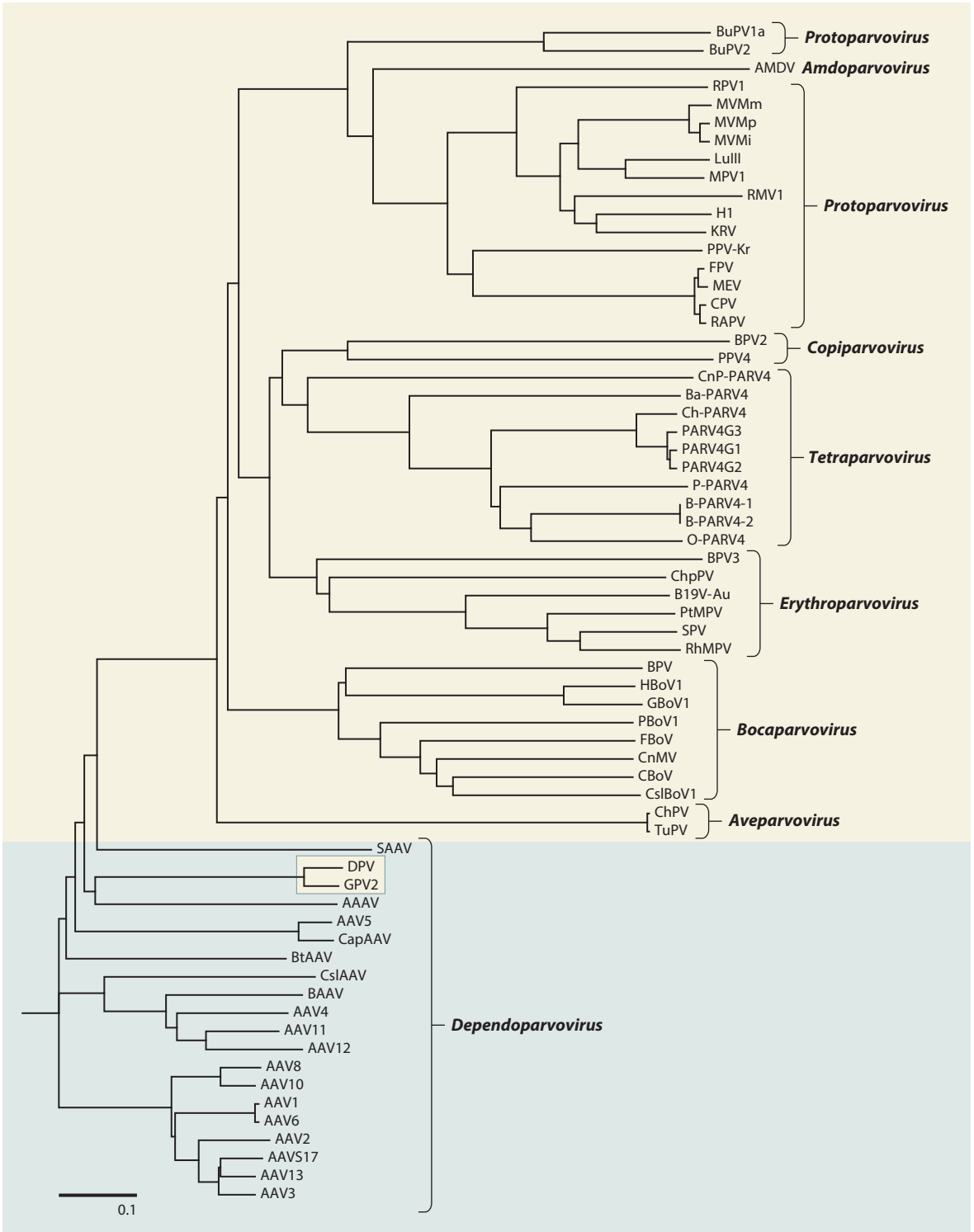

BuPV1a
BuPV2
Protoparvovirus
AMDV
Amdoparvovirus
RPV1
MVMm
MVMp
MVMi
LuIII
MPV1
RMV1
H1
KRV
PPV-Kr
FPV
MEV
CPV
RAPV
Protoparvovirus
BPV2
PPV4
Copiparvovirus
CnP-PARV4
Ba-PARV4
Ch-PARV4
PARV4G3
PARV4G1
PARV4G2
P-PARV4
B-PARV4-1
B-PARV4-2
O-PARV4
Tetraparvovirus
BPV3
ChpPV
B19V-Au
PtMPV
SPV
RhMPV
Erythroparvovirus
BPV
HBoV1
GBoV1
PBoV1
FBoV
CnMV
CBoV
CslBoV1
Bocaparvovirus
ChPV
TuPV
Aveparvovirus
SAAV
DPV
GPV2
AAAV
AAV5
CapAAV
BtAAV
CslAAV
BAAV
AAV4
AAV11
AAV12
AAV8
AAV10
AAV1
AAV6
AAV2
AAVS17
AAV13
AAV3
Dependoparvovirus
0.1

Table 2 Structural proteins known to control pathogenicity

Genus	Virus(es)	Determinant(s) of pathogenicity	Factor(s) contributing to disease	Reference(s)
Amdoparvovirus	AMDV	VP2	Antibody-dependent enhancement	93, 94, 96, 106
Erythroparvovirus	B19V	VP2, VP1u	Antibody-dependent enhancement	73, 107, 111, 112
Bocaparvovirus	HBoV1	VP1u	Disruption of tight junctions in human airway epithelial cells	75
Protoparvovirus	MVMp/i, F1 mutant, H-1PV, CPV, and FPV	VP2	Host tropism and tissue tropism	71, 85, 87–89
	PPV	VP1u, VP2	High PLA_2 activity and host tropism	65, 90, 122

enzyme activity, (*b*) host tissue tropism and receptor attachment, and (*c*) host antibody recognition (**Table 2**).

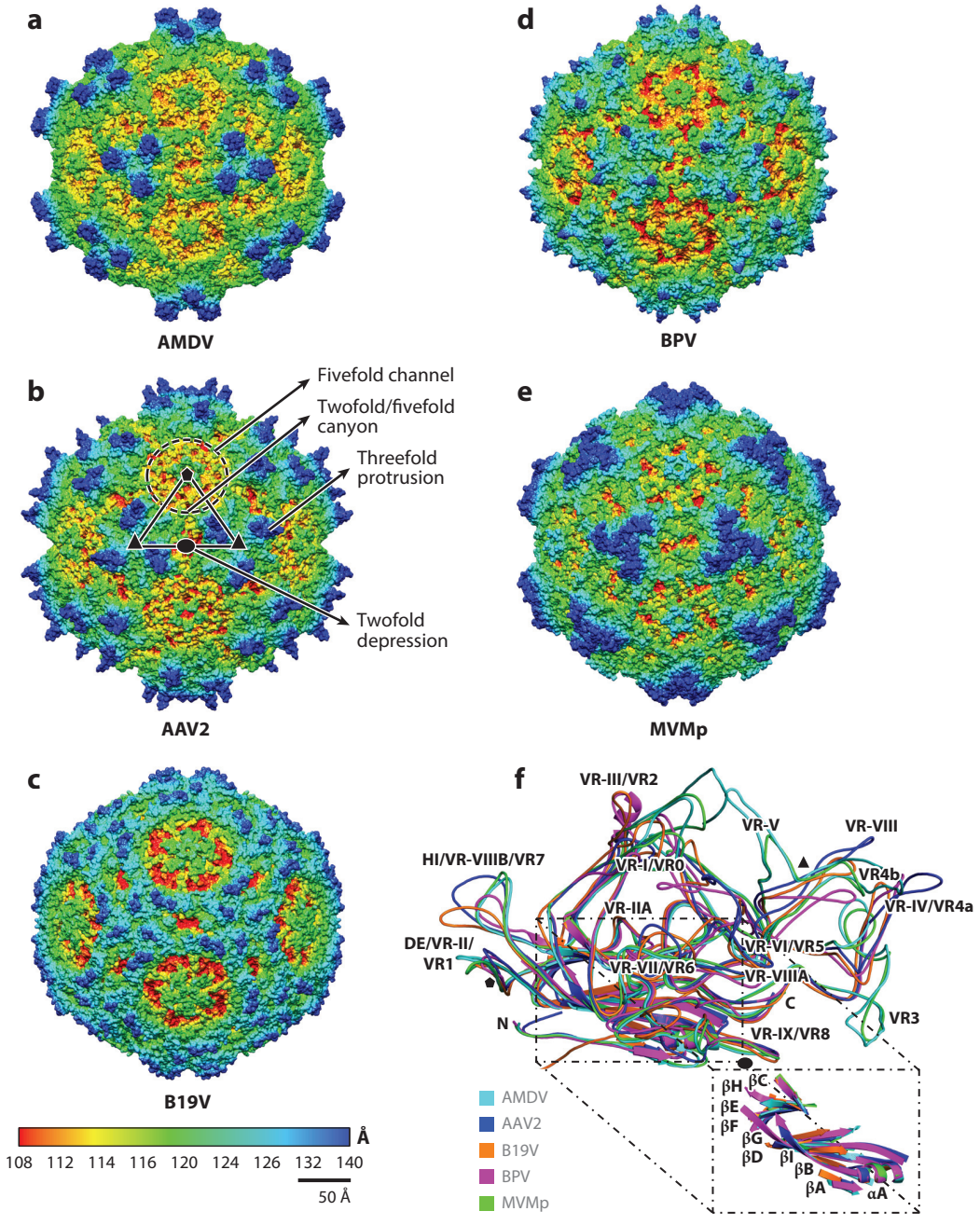
The structures for the type members for the original five of the eight now-known genera within the Parvovirinae subfamily have been determined by X-ray crystallography and/or cryoelectron microscopy and image reconstruction (cryo-reconstruction) (**Figures 1** and **5**). In addition, structures are available for several different members of the *Bocaparvovirus*, *Dependoparvovirus*, and *Protoparvovirus* genera. In all these structures—with the exception of that from B19V—VP1u, the VP1/2 common region, and the first ~15 aa of the VP1/2/3 common region are not observed. The lack of resolution of the VP1/2 overlap sequence (equivalent to the N-terminal sequence of VP2) is likely due to its predicted intrinsic disorder and low copy number. The lack of resolution of the N terminus of the VP1/2/3 common region is likely due to variation in its structural disposition. The ordered portion of the VP1/2/3 common region (~520 to ~550 aa) has conserved distinct capsid features: a depression at the twofold and surrounding the fivefold axis, protrusions at or surrounding the threefold axis, and a channel at the fivefold axis of symmetry (**Figure 5*a–e***). These conserved features exist despite low sequence identity, only 18–23%, between genera. The common architecture of the VP contributing to the parvovirus capsid structure consists of a conserved α-helix and a core eight-stranded β-barrel, linked by loops of varying lengths and conformations, which make up the majority of the viral capsid surface (**Figure 5*f***) (12, 67). The core features and the VP:VP interface regions on the interior of the capsid contain the residues with the highest sequence identity, whereas the surface loops show variability in sequence and morphology. Differences in surface loops give each genus a unique surface topology (**Figure 5**) (67).

The apexes of the loops flanking the conserved α-helix and β-strands have been defined as variable regions (VRs) for the dependoparvoviruses (VR-I to VR-IX), the protoparvoviruses (VR0 to VR8), and, recently, the bocaparvoviruses (VR-I to VR-IX) (**Figures 5*f*** and **6**) (68–71). Significantly, these VRs are seen in the different genera, and they cluster on the capsid surface to create subtle differences unique to each member of the different genera (12, 69). For example, VR-IX in BPV (equivalent to VR8 in MVM) has a raised conformation compared with those of the other type members, dividing the canonical twofold depression feature on the BPV capsid

Figure 4

Rooted phylogenetic tree for complete parvovirus VP1 sequences. The dependoparvoviruses and autonomously replicating parvoviruses are shown on blue and tan backgrounds, respectively. The sequence for at least one virus strain under each genus was used to generate the tree. The brackets on the right delineate the genera. The scale indicates the node length.

(**Figure 5**). Moreover, structural alignment of the VP structures for all the pathogenic and nonpathogenic members shows that elaborate insertions within the different VRs are present for pathogenic parvoviruses, whereas variability occurs only at the top of the VRs for nonpathogenic parvoviruses (**Figure 6**). A majority of these VRs are implicated in receptor binding, antigenicity, and genome-packaging functions for the parvoviruses in general (reviewed in 12, 69, 72). For



the pathogenic members, these VRs also control host- and tissue-specific functions, whereas for the nonpathogenic members, they serve to control transduction (packaged transgene expression) efficiency. Below we discuss the potential role of the VP regions in pathogenicity.

The VP1u PLA_2 Enzyme Activity as a Potential Determinant of Pathogenicity

Although much of the disease caused by parvoviruses results from cytotoxicity triggered by viral replication, recent studies have shown that PLA_2 can also contribute to disease in some cases. B19V PLA_2 activates inflammatory cardiomyopathy, which is accompanied by endothelial dysfunction and may contribute to pathogenesis (73). In addition, the PLA_2 enzymatic activity of HBoV1 was shown to be detrimental to tight junctions found in human airway epithelial cells, which normally act as barriers for pathogens and foreign entities and thereby prevent infection and tissue injury (74–76). AMDV does not contain this enzymatic domain and is still pathogenic (77). These observations suggest that there may not be a direct link between the presence of PLA_2 and the pathogenic outcome of parvoviral infection. However, when present, it maintains a conserved active site, its activity is essential for virus infection, and the level of activity differs among viruses (65). These observations suggest that genus-, strain-, or cell-specific interactions require further study to provide greater insight into the VP1u sequence and its PLA_2 functions.

Virus Attachment to Host Cell Receptors as a Determinant of Host Range, Tissue Tropism, and Pathogenicity

All parvoviruses utilize specific receptors for binding, cellular uptake, and infection, and a variety of different receptors have been identified (72). Depending on the virus and the host being examined, the receptors may include commonly expressed glycans as well as highly specific cell surface glycoproteins or glycolipids. In some cases, parvoviruses can bind to multiple receptors, often on different cell types, and use them individually or in combination for efficient cell binding and infection. Some receptor interactions can be modified by one or a few residue changes on the capsid. Sometimes critical residues are contributed by symmetry-related VPs. Other times, receptor-binding activity may be influenced by changes that are well separated on the capsid surface, suggesting that receptor binding may involve multiple sites on the capsid.

Viral adaptation to newer hosts can influence pathogenicity. An excellent example is provided by the phenomenon of host range jumping and host-specific evolution of CPV in the context of its transferrin receptor 1 (TfR) variation. Pathogenic CPV emerged in the late 1970s as a new virus infecting dogs. There is good evidence that dogs were not infected by a CPV-like virus prior to 1976, and the new dog-infecting virus spread worldwide among dogs, coyotes, and wolves

Figure 5

Parvovirinae capsid structures. (*a–e*) Surface diagrams of the available Parvovirinae type member structures, of AMDV, AAV2, MVMp, BPV, and B19V, radially color-cued according to the scale bar provided. The icosahedral asymmetric unit, represented by a triangle, is shown above the AAV2 structure, with the approximate icosahedral symmetry axes indicated as an oval, triangle, and pentagon for the twofold, threefold, and fivefold axes, respectively. Some of the conserved surface features are indicated on the AAV2 capsid structure: the twofold depression, threefold protrusions, twofold/fivefold canyon, and fivefold channel. (*f*) Cartoon representation of superposed VP2 structures. (*Inset*) Zoomed-in view of the conserved core, consisting of β-strands [β(A-BIDG-CHEF)] and an α-helix (αA). The variable regions (VRs) are labeled according to the nomenclature defined for the dependoparvoviruses, protoparvoviruses, and bocaparvoviruses. The N and C termini are marked, and the approximate icosahedral symmetry axes are indicated as an oval, triangle, and pentagon for the twofold, threefold, and fivefold axes, respectively. Structures were generated using PyMOL (123) and UCSF-Chimera (124).

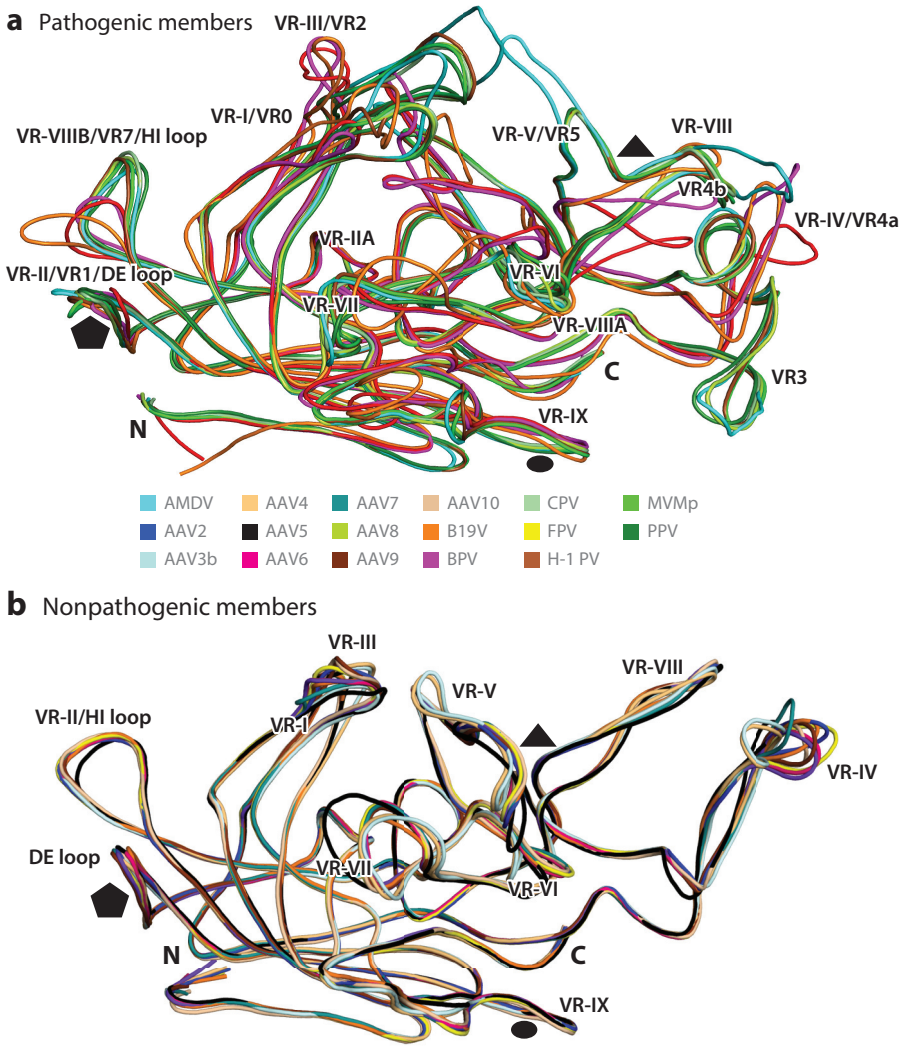


Figure 6

Superposed VP2 structures of (*a*) pathogenic and (*b*) nonpathogenic parvoviruses for which structures are available in the database. The approximate icosahedral symmetry axes are indicated with an oval, triangle, and pentagon for the twofold, threefold, and fivefold axes, respectively. The variable regions (VRs) defined for the autonomous parvoviruses (bocaparvoviruses and protoparvoviruses) and the dependoparvoviruses are also indicated on the structures. The N and C termini are labeled. Structures were generated using PyMOL (123).

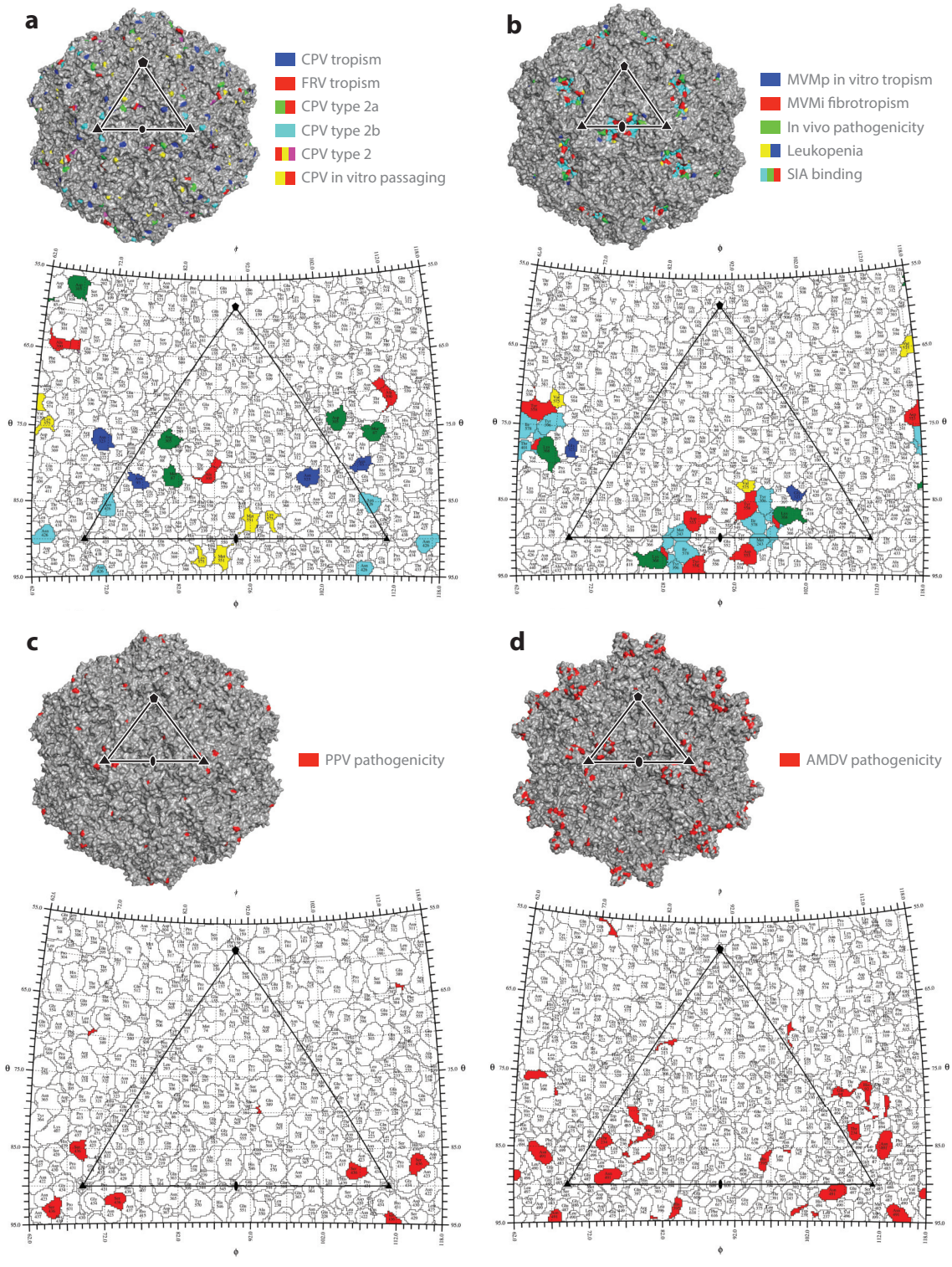
during a period of less than 6 months in 1978, likely from closely related viruses of cats and other carnivores (78). The host range of the emerging virus for canine cells or dogs was shown to be controlled by a combination of mutations in the VP gene that introduce changes in three regions in the capsid surface, defined originally by the mutations Lys93Asn and Asp323Asn as well as Gly299Glu or Ala300Asp (**Figure 7*a***) (79, 80). These residues are all on the surface of the capsid but are spaced 20–30 Å apart. During the course of passage of CPV in dogs the virus

acquired additional mutations that appear to further adapt it to the new host, including Arg80Lys, Ala300Gly, and Tyr305Met (**Figure 7*a***) (78, 81), the latter two of which are within the proposed TfR footprint. The canine TfR differs from the feline TfR by a number of mutations, but the one shown to affect binding is a single Lys383Asn (feline to canine) within the apical domain. Glycosylation of Asn383 in the canine TfR blocks binding by FPV-like viruses but allows binding by CPV-like viruses (82, 83).

Differences in host tissue tropism, controlled by the *cap* gene, have also been reported as a determinant of disease outcome for a number of closely related variants in the *Amdoparvovirus* and *Protoparvovirus* genera. For the protoparvoviruses, the studies conducted with MVMp and MVMi provide an example of the role of residues within VP1/2/3 in pathogenicity determination. The prototype MVMp strain is nonpathogenic in vivo and establishes an asymptomatic infection in permissive fibroblast cells in vitro (referred to as fibrotropism), whereas the immunosuppressive MVMi strain infects T lymphocytes in vitro, is neurotropic in mice, and establishes a lethal infection (84, 85). Both viruses require an interaction with cell surface sialic acid (SIA) for infection and propagate with a lytic infection only in their respective murine host cell types. The available data suggest that the host cell restriction results from intracellular host factors expressed in the respective cell types that facilitate viral replication (86). The MVM viruses are 97% identical in VP2 sequence. Two residues in VP2, 317 and 321, as well as the large-intron 3′ splice site, determine MVM fibrotropism (**Figure 7*b***) (87, 88). Substitution of the MVMi amino acids at these positions with those of MVMp (Ala317Thr and Glu321Gly) and four other mutations, at VP2 positions 399, 460, 553, and 558, conferred fibrotropism to MVMi (**Figure 7*b***). All of these residues are part of the shallow SIA-binding pocket on the MVMp/i capsid within the twofold depression (**Figure 7*b***). Residues in the analogous twofold depression for another *Protoparvovirus* member, H-1PV, are also implicated in host tissue tropism and pathogenicity as well as receptor attachment (71, 89). In contrast, for the PPV strains, differences in VP2 residues 381, 386, and 436, which are clustered around the threefold spike and the edge of the fivefold canyon region, dictate differences between the nonpathogenic NADL-2 strain and pathogenic Kresse strain (**Figures 1** and **7*c***) (90, 91).

In the *Amdoparvovirus* example, a small number of VP2 amino acid differences between two strains of AMDV (AMDV-G and AMDV-Utah 1) result in differences in replication in vitro and pathogenesis in vivo. The AMDV-G strain is nonpathogenic in minks but replicates efficiently in cell culture in susceptible cells, such as CrFK cells (**Table 1**). In contrast, the AMDV-Utah 1 strain is highly pathogenic but replicates poorly in cell culture. These AMDV strains, like MVMp and MVMi, are ∼97% identical in VP2 sequence. Studies using chimeras made with different sequence segments from both AMDV strains pinpointed the VP determinant for replication in cell culture and pathogenesis to residues located on the wall of the twofold depression and threefold protrusions (**Figure 7*d***) (92–94). These residues are analogous to those that control receptor attachment, tissue tropism, and pathogenicity for MVM and host range and receptor attachment for CPV, respectively (**Figure 7*a*,*b*,*d***).

Regardless of the type of parvovirus or consequence of infection, many of these viruses use the same glycans for cell recognition and use the same capsid sites for cellular recognition. These sites include the threefold depression (e.g., AAV2–heparan sulfate, AAV4–SIA, AAV5–SIA, and B19V–globoside), the base of the threefold protrusions (e.g., AAV1–SIA and AAV9–galactose), and the twofold depression (e.g., CPV–SIA, H-1PV–SIA, and MVMp/i–SIA). Therefore, these studies suggest that pathogenicity is not solely dependent on receptor interactions and may involve other virus-host interactions required for infection, including internalization, intracellular trafficking, nuclear entry, uncoating, assembly, and genome packaging (12).


a
CPV tropism
FRV tropism
CPV type 2a
CPV type 2b
CPV type 2
CPV in vitro passaging
b
MVMp in vitro tropism
MVMi fibrotropism
In vivo pathogenicity
Leukopenia
SIA binding
c
PPV pathogenicity
d
AMDV pathogenicity

Host Antibody Interaction as a Determinant of Pathogenicity

For a number of parvoviruses, antibodies play a variety of roles, including providing protection, causing antibody-dependent enhancement (ADE) of infection, establishing persistent infection, and promoting immunopathogenesis. As an example, antibodies against the capsid and NS proteins of AMDV are prominent features of the immunopathogenesis associated with Aleutian mink disease (95, 96). Minks with persistent infections develop hypergammaglobulinemia, plasmacytosis, and immune complex diseases characterized by high antibody titers (97, 98). Despite this heightened antibody response, the virus is not inactivated but retains infectivity in the host (97–99). Most of the AMDV particles are sequestered as immune complexes in lymphoid organs of infected adult minks, and viral replication is detected only within a small population of macrophages and follicular dendritic cells (100). Although anti-AMDV antibodies are capable of neutralizing AMDV infectivity in CrFK cells, these antibodies are required for persistent AMDV infection in macrophages using the Fc receptor–dependent mechanism of ADE (101–103). Thus, preexisting antiviral antibodies fail to protect the adult minks from AMDV infection and instead lead to an accelerated form of the disease (104). Furthermore, due to ADE, minks immunized with capsid-based AMDV vaccines were more susceptible upon virus challenge and developed acute inflammatory lesions (104).

Studies using peptides as antigens to probe the antibody responses to AMDV have indicated that the wall between the twofold and fivefold axes, as well as the shoulder and top of the threefold protrusions, are major immune-dominant sites on the capsid (**Figures 7*d*** and **8*a***) (94, 105, 106). A peptide antibody spanning VP2 residues 428–446 led to ADE-mediated AMDV pathogenesis with virus particles aggregating into immune complexes (**Figure 8*a***). This epitope, located on the twofold wall, is neutralizing when recognized by antibody in CrFK cells and includes VP2 residue 434, which is implicated in host tropism and pathogenicity.

For B19V, persistent infection leads to autoimmune-mediated destruction of granulocytes and thrombocytes (reviewed in 107). The majority of the antibody response against this virus is directed against the surface-exposed VP1u region and the VP2 residues that constitute the threefold protrusions (**Figure 8*b***) (108–111). In addition to these structural proteins, NS1 has also been shown to induce an antibody response and is an indicator of recent infection or exposure to B19V (112). The humoral response against B19V can also be protective (107). VP2 epitopes for neutralizing antibodies E, L, and 521-D include VP2 residue ranges 57–77, 345–365, and 446–466, respectively (**Figure 8*b***) (113). Another antigenic region on VP2, present between residues 253 and 515, can be localized to the twofold/fivefold wall and the base of the threefold protrusions (**Figure 8*b***) (110, 111, 113). Of these antigenic stretches, the epitope comprising VP2 residues 314–330 on the threefold protrusion is similar to the C37-B neutralizing antibody epitope in AAV2, which interferes with receptor binding (**Figure 8*b*,*c***) (114).

Figure 7

Host tropism and pathogenicity. Residues mediating host and tissue tropism in (*a*) CPV, (*b*) MVM, (*c*) PPV, and (*d*) AMDV are depicted on the capsid surface image (*top*) and a roadmap representation of the viral asymmetric unit (*bottom*). The colored residues show amino acids that are important determinants of host tropism and pathogenicity, as indicated on the right-hand side. The relevant residues are as follows. CPV tropism: 93, 323; FPV tropism: 299, 300; CPV type 2a: 87, 300, 305; CPV type 2b: 426; CPV type 2: 80, 564, 568, 300; CPV in vitro passaging: 80, 300, 305; MVMp in vitro tropism: 317, 321; MVMi fibrotropism: 399, 460, 553, 558; in vivo pathogenicity: 325, 362, 368; leukopenia: 321, 551, 575; sialic acid (SIA) binding: 241, 243, 362, 368, 396, 398, 399, 401, 403, 553, 558, 578; PPV pathogenicity: 436, 378, 383; AMDV pathogenicity: 92, 94, 115, 234, 238, 240, 241, 242, 352, 395, 434, 491, 534. Structures were generated using PyMOL (123) and RIVEM (125).

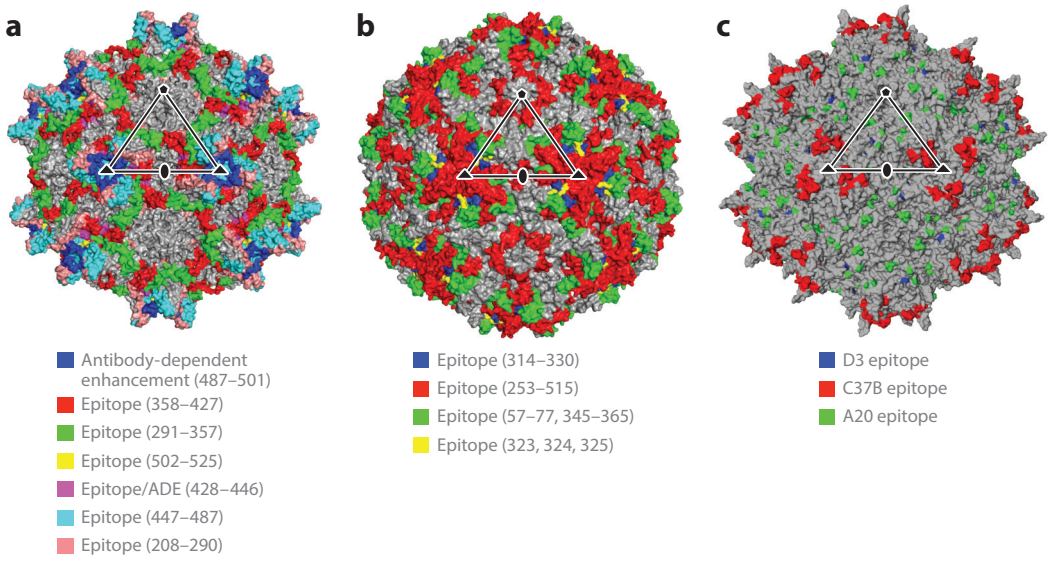


Figure 8

Antigenic epitopes of (*a*) AMDV, (*b*) B19V, and (*c*) AAV2 are depicted on the surface of the virus capsids. The colored residues show amino acids that are important as determinants of antigenicity, as indicated below each structure. Structures were generated using PyMOL (123).

Antibodies to many of the nonpathogenic AAVs are highly prevalent in human sera, as summarized in a review by Tseng et al. (**Figure 8*c***) (115). These neutralizing antibodies to AAV capsids may limit the efficacy of AAV gene delivery vectors. In contrast, Mori et al. (116) preincubated AAV vectors with anti-AAV antiserum at subneutralizing levels and observed enhancement of AAV infection in human monocytic cell lines. ADE may thus be able to improve the transduction levels achieved via AAV gene therapy in these cells.

CONCLUSIONS

Parvoviruses represent an ancient and widespread family of viruses that have successfully infected animals of all types for millions of years and in general appear to be highly host specific and display narrow tissue tropisms even within the animals that they do infect. Despite having DNA genomes, these viruses appear to evolve at rates similar to those seen for many RNA viruses, so that mutations are readily selected even during the course of very small numbers of host cell passages, allowing them (in some cases) to quickly change and adapt to new but related hosts as well as to alter their cell or tissue tropisms. As such, they represent a dynamic system with the potential for emergence of important new natural variants with altered properties. Although parvoviruses vary in tropism and lethality, they encode several conserved functions, including NS1-mediated toxicity; the ability, mediated by Rep/NS, to establish persistent infection; VP1u-related phospholipase function; and antigenicity of capsid proteins. The complex mechanisms involved in cellular entry into preferred cell or tissue types result in the varied level of disease outcome—from nonpathogenic to severe and lethal. For a complete understanding of what makes some of these viruses pathogenic and others nonpathogenic, we need to identify their cellular interacting partners and characterize the

interactions of those factors with all viral components, including the packaged genome. This is a complex challenge that can only be undertaken one viral protein at a time.

DISCLOSURE STATEMENT

M.A.M. is a Scientific Advisory Board member for Voyager Therapeutics, Inc., and has a sponsored research agreement with AGTC. Both companies have interest in the development of AAV for gene delivery applications. M.A.M. is also an inventor on AAV patents licensed to various pharmaceutical companies.

ACKNOWLEDGMENTS

The authors acknowledge support from the University of Florida College of Medicine, McKnight Brain Institute funds, National Institutes of Health (NIH) grants R01 GM082946 and R01 GM109524 to M.A.M., and NIH grants R01 AI092571 and R01 GM080533 to C.R.P.

LITERATURE CITED

1. Liu H, Fu Y, Xie J, Cheng J, Ghabrial SA, et al. 2011. Widespread endogenization of densoviruses and parvoviruses in animal and human genomes. *J. Virol.* 85:9863–76
2. Kapoor A, Simmonds P, Lipkin WI. 2010. Discovery and characterization of mammalian endogenous parvoviruses. *J. Virol.* 84:12628–35
3. Katzourakis A, Gifford RJ. 2010. Endogenous viral elements in animal genomes. *PLOS Genet.* 6:e1001191
4. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, et al. 2014. The family Parvoviridae. *Arch. Virol.* 159:1239–47
5. Canuti M, Eis-Huebinger AM, Deijs M, de Vries M, Drexler JF, et al. 2011. Two novel parvoviruses in frugivorous New and Old World bats. *PLOS ONE* 6:e29140
6. Lau SKP, Woo PCY, Tse H, Fu CTY, Au WK, et al. 2008. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *J. Gen. Virol.* 89:1840–48
7. Bodewes R, van der Giessen J, Haagmans BL, Osterhaus ADME, Smits SL. 2013. Identification of multiple novel viruses, including a parvovirus and a hepevirus, in feces of red foxes. *J. Virol.* 87:7758–64
8. Berns KI, Hauswirth WW, Fife KH, Lusby E. 1979. Adeno-associated virus DNA replication. *Cold Spring Harb. Symp. Quant. Biol.* 43:781–87
9. Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, et al. 2009. A newly identified bocavirus species in human stool. *J. Infect. Dis.* 199:196–200
10. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *PNAS* 102:12891–96
11. Phan TG, Vo NP, Bonkoungou IJO, Kapoor A, Barro N, et al. 2012. Acute diarrhea in West African children: diverse enteric viruses and a novel parvovirus genus. *J. Virol.* 86:11024–30
12. Halder S, Ng R, Agbandje-McKenna M. 2012. Parvoviruses: structure and infection. *Future Virol.* 7:253–78
13. Tattersall P, Cawte PJ, Shatkin AJ, Ward DC. 1976. Three structural polypeptides coded for by minute virus of mice, a parvovirus. *J. Virol.* 20:273–89
14. Becerra SP, Koczot F, Fabisch P, Rose JA. 1988. Synthesis of adeno-associated virus structural proteins requires both alternative mRNA splicing and alternative initiations from a single transcript. *J. Virol.* 62:2745–54
15. Johnson FB, Hoggan MD. 1973. Structural proteins of HADEN virus. *Virology* 51:129–37
16. Lederman M, Bates RC, Stout ER. 1983. In vitro and in vivo studies of bovine parvovirus proteins. *J. Virol.* 48:10–17
17. Chen KC, Shull BC, Moses EA, Lederman M, Stout ER, Bates RC. 1986. Complete nucleotide sequence and genome organization of bovine parvovirus. *J. Virol.* 60:1085–97

18. Cotmore SF, Tattersall P. 2006. Structure and organization of the viral genome. In *Parvoviruses*, ed. J Kerr, S Cotmore, ME Bloom, RM Linden, CR Parrish, pp. 73–94. London: Hodder Arnold
19. Bloom ME, Kanno H, Mori S, Wolfinbarger JB. 1994. Aleutian mink disease: puzzles and paradigms. *Infect. Agents Dis.* 3:279–301
20. Alexandersen S, Bloom ME, Wolfinbarger J. 1988. Evidence of restricted viral replication in adult mink infected with Aleutian disease of mink parvovirus. *J. Virol.* 62:1495–507
21. Viuff B, Aasted B, Alexandersen S. 1994. Role of alveolar type II cells and of surfactant-associated protein C mRNA levels in the pathogenesis of respiratory distress in mink kits infected with Aleutian mink disease parvovirus. *J. Virol.* 68:2720–25
22. Mengeling WL, Cutlip RC. 1976. Reproductive disease experimentally induced by exposing pregnant gilts to porcine parvovirus. *Am. J. Vet. Res.* 37:1393–400
23. Vihinen-Ranta M, Parrish CR. 2006. Cell infection processes of autonomous parvoviruses. In *Parvoviruses*, ed. J Kerr, S Cotmore, ME Bloom, RM Linden, CR Parrish, pp. 157–64. London: Hodder Arnold
24. Adeyemi RO, Pintel DJ. 2014. Parvovirus-induced depletion of cyclin B1 prevents mitotic entry of infected cells. *PLOS Pathog.* 10:e1003891
25. Cotmore SF, Tattersall P. 2013. Parvovirus diversity and DNA damage responses. *Cold Spring Harb. Perspect. Biol.* 5:a012989
26. Luo Y, Qiu J. 2013. Parvovirus infection-induced DNA damage response. *Future Virol.* 8:245–57
27. Vogel R, Seyffert M, Strasser R, de Oliveira AP, Dresch C, et al. 2012. Adeno-associated virus type 2 modulates the host DNA damage response induced by herpes simplex virus 1 during coinfection. *J. Virol.* 86:143–55
28. Adeyemi RO, Landry S, Davis ME, Weitzman MD, Pintel DJ. 2010. Parvovirus minute virus of mice induces a DNA damage response that facilitates viral replication. *PLOS Pathog.* 6:e1001141
29. Nüesch JPF, Rommelaere J. 2006. NS1 interaction with CKIIα: novel protein complex mediating parvovirus-induced cytotoxicity. *J. Virol.* 80:4729–39
30. Hristov G, Krämer M, Li J, El-Andaloussi N, Mora R, et al. 2010. Through its nonstructural protein NS1, parvovirus H-1 induces apoptosis via accumulation of reactive oxygen species. *J. Virol.* 84:5909–22
31. Rayet B, Lopez-Guerrero JA, Rommelaere J, Dinsart C. 1998. Induction of programmed cell death by parvovirus H-1 in U937 cells: connection with the tumor necrosis factor alpha signalling pathway. *J. Virol.* 72:8893–903
32. Di Piazza M, Mader C, Geletneky K, Herrero y Calle M, Weber E, et al. 2007. Cytosolic activation of cathepsins mediates parvovirus H-1-induced killing of cisplatin and TRAIL-resistant glioma cells. *J. Virol.* 81:4186–98
33. Ran Z, Rayet B, Rommelaere J, Faisst S. 1999. Parvovirus H-1-induced cell death: influence of intracellular NAD consumption on the regulation of necrosis and apoptosis. *Virus Res.* 65:161–74
34. Moffatt S, Yaegashi N, Tada K, Tanaka N, Sugamura K. 1998. Human parvovirus B19 nonstructural (NS1) protein induces apoptosis in erythroid lineage cells. *J. Virol.* 72:3018–28
35. Fu Y, Ishii KK, Munakata Y, Saitoh T, Kaku M, Sasaki T. 2002. Regulation of tumor necrosis factor alpha promoter by human parvovirus B19 NS1 through activation of AP-1 and AP-2. *J. Virol.* 76:5395–403
36. Hsu TC, Wu WJ, Chen MC, Tsay GJ. 2004. Human parvovirus B19 non-structural protein (NS1) induces apoptosis through mitochondria cell death pathway in COS-7 cells. *Scand. J. Infect. Dis.* 36:570–77
37. Chen AY, Luo Y, Cheng F, Sun Y, Qiu J. 2010. Bocavirus infection induces mitochondrion-mediated apoptosis and cell cycle arrest at G_2/M phase. *J. Virol.* 84:5615–26
38. Abdel-Latif L, Murray BK, Renberg RL, O'Neill KL, Porter H, et al. 2006. Cell death in bovine parvovirus-infected embryonic bovine tracheal cells is mediated by necrosis rather than apoptosis. *J. Gen. Virol.* 87:2539–48
39. Best SM, Wolfinbarger JB, Bloom ME. 2002. Caspase activation is required for permissive replication of Aleutian mink disease parvovirus in vitro. *Virology* 292:224–34
40. Hickman AB, Ronning DR, Kotin RM, Dyda F. 2002. Structural unity among viral origin binding proteins: crystal structure of the nuclease domain of adeno-associated virus rep. *Mol. Cell* 10:327–37

41. Tewary SK, Zhao H, Shen W, Qiu J, Tang L. 2013. Structure of the NS1 protein N-terminal origin recognition/nickase domain from the emerging human bocavirus. *J. Virol.* 87:11487–93
42. Tewary SK, Liang L, Lin Z, Lynn A, Cotmore SF, et al. 2014. Structures of minute virus of mice replication initiator protein N-terminal domain: insights into DNA nicking and origin binding. *Virology* 476:61–71
43. James JA, Escalante CR, Yoon-Robarts M, Edwards TA, Linden RM, Aggarwal AK. 2003. Crystal structure of the SF3 helicase from adeno-associated virus type 2. *Structure* 11:1025–35
44. Zádori Z, Szelei J, Tijssen P. 2005. SAT: a late NS protein of porcine parvovirus. *J. Virol.* 79:13129–38
45. Simmonds P, Douglas J, Bestetti G, Longhi E, Antinori S, et al. 2008. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa. *J. Gen. Virol.* 89:2299–302
46. Amand JS, Astell CR. 1993. Identification and characterization of a family of 11-kDa proteins encoded by the human parvovirus B19. *Virology*. 192:121–31
47. St. Amand J, Beard C, Humphries K, Astell CR. 1991. Analysis of splice junctions and in vitro and in vivo translation potential of the small, abundant B19 parvovirus RNAs. *Virology* 183:133–42
48. Brunstein J, Söderlund-Venermo M, Hedman K. 2000. Identification of a novel RNA splicing pattern as a basis of restricted cell tropism of erythrovirus B19. *Virology* 274:284–91
49. Chen AY, Zhang EY, Guan W, Cheng F, Kleiboeker S, et al. 2010. The small 11 kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. *Blood* 115:1070–80
50. Sun B, Cai Y, Li Y, Li J, Liu K, et al. 2013. The nonstructural protein NP1 of human bocavirus 1 induces cell cycle arrest and apoptosis in HeLa cells. *Virology* 440:75–83
51. Brownstein DG, Smith AL, Johnson EA, Pintel DJ, Naeger LK, Tattersall P. 1992. The pathogenesis of infection with minute virus of mice depends on expression of the small nonstructural protein NS2 and on the genotype of the allotropic determinants VP1 and VP2. *J. Virol.* 66:3118–24
52. Cotmore SF, D'Abramo AM, Carbonell LF, Bratton J, Tattersall P. 1997. The NS2 polypeptide of parvovirus MVM is required for capsid assembly in murine cells. *Virology* 231:267–80
53. Sonntag F, Schmidt K, Kleinschmidt JA. 2010. A viral assembly factor promotes AAV2 capsid formation in the nucleolus. *PNAS* 107:10220–25
54. Kapoor A, Hornig M, Asokan A, Williams B, Henriquez JA, Lipkin WI. 2011. Bocavirus episome in infected human tissue contains non-identical termini. *PLOS ONE* 6:e21362
55. Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. 2002. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *PNAS* 99:11854–59
56. Chen CL, Jensen RL, Schnepp BC, Connell MJ, Shell R, et al. 2005. Molecular characterization of adeno-associated viruses infecting children. *J. Virol.* 79:14781–92
57. Lüsebrink J, Schildgen V, Tillmann RL, Wittleben F, Böhmer A, et al. 2011. Detection of head-to-tail DNA sequences of human bocavirus in clinical samples. *PLOS ONE* 6:e19457
58. Vihinen-Ranta M, Wang D, Weichert WS, Parrish CR. 2002. The VP1 N-terminal sequence of canine parvovirus affects nuclear transport of capsids and efficient cell infection. *J. Virol.* 76:1884–91
59. Boisvert M, Bouchard-Lévesque V, Fernandes S, Tijssen P. 2014. Classic nuclear localization signals and a novel nuclear localization motif are required for nuclear transport of porcine parvovirus capsid proteins. *J. Virol.* 88:11748–59
60. Lombardo E, Ramírez JC, Garcia J, Almendral JM. 2002. Complementary roles of multiple nuclear targeting signals in the capsid proteins of the parvovirus minute virus of mice during assembly and onset of infection. *J. Virol.* 76:7049–59
61. Vihinen-Ranta M, Kakkola L, Kalela A, Vilja P, Vuento M. 1997. Characterization of a nuclear localization signal of canine parvovirus capsid proteins. *FEBS J.* 250:389–94
62. Grieger JC, Snowdy S, Samulski RJ. 2006. Separate basic region motifs within the adeno-associated virus capsid proteins are essential for infectivity and assembly. *J. Virol.* 80:5199–210
63. Wu P, Xiao W, Conlon T, Hughes J, Agbandje-McKenna M, et al. 2000. Mutational analysis of the adeno-associated virus type 2 (AAV2) capsid gene and construction of AAV2 vectors with altered tropism. *J. Virol.* 74:8635–47

64. Sonntag F, Bleker S, Leuchs B, Fischer R, Kleinschmidt JA. 2006. Adeno-associated virus type 2 capsids with externalized VP1/VP2 trafficking domains are generated prior to passage through the cytoplasm and are maintained until uncoating occurs in the nucleus. *J. Virol.* 80:11040–54
65. Zádori Z, Szelei J, Lacoste MC, Li Y, Gariépy S, et al. 2001. A viral phospholipase A_2 is required for parvovirus infectivity. *Dev. Cell* 1:291–302
66. Girod A, Wobus CE, Zádori Z, Ried M, Leike K, et al. 2002. The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity. *J. Gen. Virol.* 83:973–78
67. Chapman MS, Agbandje-McKenna M. 2006. Atomic structure of viral particles. In *Parvoviruses*, ed. J Kerr, S Cotmore, ME Bloom, RM Linden, CR Parrish, pp. 107–23. London: Hodder Arnold
68. Halder S, Nam HJ, Govindasamy L, Vogel M, Dinsart C, et al. 2013. Structural characterization of H-1 parvovirus: comparison of infectious virions to empty capsids. *J. Virol.* 87:5128–40
69. Kailasan S, Halder S, Gurda B, Bladek H, Chipman PR, et al. 2014. Structure of an enteric pathogen bovine parvovirus. *J. Virol.* 89:2603–14
70. Govindasamy L, Padron E, McKenna R, Muzyczka N, Kaludov N, et al. 2006. Structurally mapping the diverse phenotype of adeno-associated virus serotype 4. *J. Virol.* 80:11556–70
71. Kontou M, Govindasamy L, Nam HJ, Bryant N, Llamas-Saiz AL, et al. 2005. Structural determinants of tissue tropism and in vivo pathogenicity for the parvovirus minute virus of mice. *J. Virol.* 79:10931–43
72. Huang LY, Halder S, Agbandje-McKenna M. 2014. Parvovirus glycan interactions. *Curr. Opin. Virol.* 7:108–18
73. Lupescu A, Bock CT, Lang PA, Aberle S, Kaiser H, et al. 2006. Phospholipase A2 activity-dependent stimulation of Ca^{2+} entry by human parvovirus B19 capsid protein VP1. *J. Virol.* 80:11370–80
74. Chiu CC, Shi YF, Yang JJ, Hsiao YC, Tzang BS, Hsu TC. 2014. Effects of human parvovirus B19 and bocavirus VP1 unique region on tight junction of human airway epithelial A549 cells. *PLOS ONE* 9:e107970
75. Deng X, Yan Z, Luo Y, Xu J, Cheng F, et al. 2013. In vitro modeling of human bocavirus 1 infection of polarized primary human airway epithelia. *J. Virol.* 87:4097–102
76. Qu XW, Liu WP, Qi ZY, Duan ZJ, Zheng LS, et al. 2008. Phospholipase A_2-like activity of human bocavirus VP1 unique region. *Biochem. Biophys. Res. Commun.* 365:158–63
77. Willwand K, Kaaden OR. 1988. Capsid protein VP1 (p85) of Aleutian disease virus is a major DNA-binding protein. *Virology* 166:52–57
78. Allison AB, Kohler DJ, Fox KA, Brown JD, Gerhold RW, et al. 2013. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. *J. Virol.* 87:2342–47
79. Parker JS, Parrish CR. 1997. Canine parvovirus host range is determined by the specific conformation of an additional region of the capsid. *J. Virol.* 71:9214–22
80. Llamas-Saiz AL, Agbandje-McKenna M, Parker JS, Wahid AT, Parrish CR, Rossmann MG. 1996. Structural analysis of a mutation in canine parvovirus which controls antigenicity and host range. *Virology* 225:65–71
81. Shackelton LA, Parrish CR, Truyen U, Holmes EC. 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. *PNAS* 102:379–84
82. Palermo LM, Hueffer K, Parrish CR. 2003. Residues in the apical domain of the feline and canine transferrin receptors control host-specific binding and cell infection of canine and feline parvoviruses. *J. Virol.* 77:8915–23
83. Kaelber JT, Demogines A, Harbison CE, Allison AB, Goodman LB, et al. 2012. Evolutionary reconstructions of the transferrin receptor of caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLOS Pathog.* 8:e1002666
84. Itah R, Tal J, Davis C. 2004. Host cell specificity of minute virus of mice in the developing mouse embryo. *J. Virol.* 78:9474–86
85. Kimsey PB, Engers HD, Hirt B, Jongeneel CV. 1986. Pathogenicity of fibroblast- and lymphocyte-specific variants of minute virus of mice. *J. Virol.* 59:8–13
86. Spalholz BA, Tattersall P. 1983. Interaction of minute virus of mice with differentiated cells: Strain-dependent target cell specificity is mediated by intracellular factors. *J. Virol.* 46:937–43
87. Gardiner EM, Tattersall P. 1988. Mapping of the fibrotropic and lymphotropic host range determinants of the parvovirus minute virus of mice. *J. Virol.* 62:2605–13

88. Tattersall P, Bratton J. 1983. Reciprocal productive and restrictive virus-cell interactions of immunosuppressive and prototype strains of minute virus of mice. *J. Virol.* 46:944–55
89. Allaume X, El-Andaloussi N, Leuchs B, Bonifati S, Kulkarni A, et al. 2012. Retargeting of rat parvovirus H-1PV to cancer cells through genetic engineering of the viral capsid. *J. Virol.* 86:3452–65
90. Bergeron J, Menezes J, Tijssen P. 1993. Genomic organization and mapping of transcription and translation products of the NADL-2 strain of porcine parvovirus. *Virology* 197:86–98
91. Simpson AA, Hébert B, Sullivan GM, Parrish CR, Zádori Z, et al. 2002. The structure of porcine parvovirus: comparison with related viruses. *J. Mol. Biol.* 315:1189–98
92. Stevenson MA, Fox JM, Wolfinbarger JB, Bloom ME. 2001. Effect of a valine residue at codon 352 of the VP2 capsid protein on in vivo replication and pathogenesis of Aleutian disease parvovirus in mink. *Am. J. Vet. Res.* 62:1658–63
93. Bloom ME, Alexandersen S, Perryman S, Lechner D, Wolfinbarger JB. 1988. Nucleotide sequence and genomic organization of Aleutian mink disease parvovirus (ADV): sequence comparisons between a nonpathogenic and a pathogenic strain of ADV. *J. Virol.* 62:2903–15
94. McKenna R, Olson NH, Chipman PR, Baker TS, Booth TF, et al. 1999. Three-dimensional structure of Aleutian mink disease parvovirus: implications for disease pathogenicity. *J. Virol.* 73:6882–91
95. Bloom ME, Race RE, Wolfinbarger JB. 1982. Identification of a nonvirion protein of Aleutian disease virus: mink with Aleutian disease have antibody to both virion and nonvirion proteins. *J. Virol.* 43:608–16
96. Bloom ME, Race RE, Hadlow WJ, Chesebro B. 1975. Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. *J. Immunol.* 115:1034–37
97. Aasted B, Tierney GS, Bloom ME. 1984. Analysis of the quantity of antiviral antibodies from mink infected with different Aleutian disease virus strains. *Scand. J. Immunol.* 19:395–402
98. Aasted B, Bloom ME. 1984. Mink with Aleutian disease have high-affinity antiviral antibodies. *Scand. J. Immunol.* 19:411–18
99. Porter DD. 1986. Aleutian disease: a persistent parvovirus infection of mink with a maximal but ineffective host humoral immune response. *Prog. Med. Virol.* 33:42–60
100. Kanno H, Wolfinbarger JB, Bloom ME. 1993. Aleutian mink disease parvovirus infection of mink peritoneal macrophages and human macrophage cell lines. *J. Virol.* 67:2075–82
101. Stolze B, Kaaden OR. 1987. Apparent lack of neutralizing antibodies in Aleutian disease is due to masking of antigenic sites by phospholipids. *Virology* 158:174–80
102. Kanno H, Wolfinbarger JB, Bloom ME. 1993. Aleutian mink disease parvovirus infection of mink macrophages and human macrophage cell line U937: demonstration of antibody-dependent enhancement of infection. *J. Virol.* 67:7017–24
103. Aasted B, Alexandersen S, Christensen J. 1998. Vaccination with Aleutian mink disease parvovirus (AMDV) capsid proteins enhances disease, while vaccination with the major non-structural AMDV protein causes partial protection from disease. *Vaccine* 16:1158–65
104. Porter DD, Larsen AE, Porter HG. 1972. The pathogenesis of Aleutian disease of mink. II. Enhancement of tissue lesions following the administration of a killed virus vaccine or passive antibody. *J. Immunol.* 109:1–7
105. Bloom ME, Martin DA, Oie KL, Huhtanen ME, Costello F, et al. 1997. Expression of Aleutian mink disease parvovirus capsid proteins in defined segments: localization of immunoreactive sites and neutralizing epitopes to specific regions. *J. Virol.* 71:705–14
106. Bloom ME, Best SM, Hayes SF, Wells RD, Wolfinbarger JB, et al. 2001. Identification of Aleutian mink disease parvovirus capsid sequences mediating antibody-dependent enhancement of infection, virus neutralization, and immune complex formation. *J. Virol.* 75:11116–27
107. Lehmann HW, von Landenberg P, Modrow S. 2003. Parvovirus B19 infection and autoimmune disease. *Autoimmun. Rev.* 2:218–23
108. Kaufmann B, Simpson AA, Rossmann MG. 2004. The structure of human parvovirus B19. *PNAS* 101:11628–33
109. Rosenfeld SJ, Yoshimoto K, Kajigaya S, Anderson S, Young NS, et al. 1992. Unique region of the minor capsid protein of human parvovirus B19 is exposed on the virion surface. *J. Clin. Investig.* 89:2023–29
110. Sato H, Hirata J, Kuroda N, Shiraki H, Maeda Y, Okochi K. 1991. Identification and mapping of neutralizing epitopes of human parvovirus B19 by using human antibodies. *J. Virol.* 65:5485–90

111. Sato H, Hirata J, Furukawa M, Kuroda N, Shiraki H, et al. 1991. Identification of the region including the epitope for a monoclonal antibody which can neutralize human parvovirus B19. *J. Virol.* 65:1667–72
112. Mitchell LA, Leong R, Rosenke KA. 2001. Lymphocyte recognition of human parvovirus B19 nonstructural (NS1) protein: associations with occurrence of acute and chronic arthropathy? *J. Med. Microbiol.* 50:627–35
113. Saikawa T, Anderson S, Momoeda M, Kajigaya S, Young NS. 1993. Neutralizing linear epitopes of B19 parvovirus cluster in the VP1 unique and VP1-VP2 junction regions. *J. Virol.* 67:3004–9
114. Wobus CE, Hügle-Dörr B, Girod A, Petersen G, Hallek M, Kleinschmidt JA. 2000. Monoclonal antibodies against the adeno-associated virus type 2 (AAV-2) capsid: epitope mapping and identification of capsid domains involved in AAV-2-cell interaction and neutralization of AAV-2 infection. *J. Virol.* 74:9281–93
115. Tseng YS, Agbandje-McKenna M. 2014. Mapping the AAV capsid host antibody response toward the development of second generation gene delivery vectors. *Microb. Immunol.* 5:9
116. Mori S, Takeuchi T, Kanda T. 2008. Antibody-dependent enhancement of adeno-associated virus infection of human monocytic cell lines. *Virology* 375:141–47
117. Best SM, Shelton JF, Pompey JM, Wolfinbarger JB, Bloom ME. 2003. Caspase cleavage of the nonstructural protein NS1 mediates replication of Aleutian mink disease parvovirus. *J. Virol.* 77:5305–12
118. Luo Y, Deng X, Cheng F, Li Y, Qiu J. 2013. SMC1-mediated intra-S-phase arrest facilitates bocavirus DNA replication. *J. Virol.* 87:4017–32
119. Schmidt M, Afione S, Kotin RM. 2000. Adeno-associated virus type 2 Rep78 induces apoptosis through caspase activation independently of p53. *J. Virol.* 74:9441–50
120. Sol N, Le Junter J, Vassias I, Freyssinier JM, Thomas A, et al. 1999. Possible interactions between the NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. *J. Virol.* 73:8762–70
121. Li J, Werner E, Hergenhahn M, Poirey R, Luo Z, et al. 2005. Expression profiling of human hepatoma cells reveals global repression of genes involved in cell proliferation, growth, and apoptosis upon infection with parvovirus H-1. *J. Virol.* 79:2274–86
122. Van Leengoed LA, Vos J, Gruys E, Rondhuis P, Brand A. 1983. Porcine parvovirus infection: review and diagnosis in a sow herd with reproductive failure. *Vet. Q.* 5:131–41
123. DeLano WL. 2012. *The PyMOL Molecular Graphic System*. San Carlos, CA: DeLano Scientific
124. Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, et al. 2012. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J. Struct. Biol.* 179:269–78
125. Xiao C, Rossmann MG. 2007. Interpretation of electron density with stereographic roadmap projections. *J. Struct. Biol.* 158:182–87