Parvovirus host range, cell tropism and evolution

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The past few years have seen major advances in our understanding of the controls of evolution, host range and cell tropism of parvoviruses. Notable findings have included the identification of the transferrin receptor TfR as the cell surface receptor for canine parvovirus and feline panleukopenia virus, and also the finding that specific binding to the canine TfR led to the emergence of canine parvovirus as a new pathogen in dogs. The structures of the adeno-associated virus-2 and porcine parvovirus capsids, along with those of the minute virus of mice, have also advanced our understanding of parvovirus biology. Structure–function studies have shown that in several different parvoviruses the threefold spikes or peaks of the capsid control several aspects of cell tropism and host range, and that those are subject to selective pressures leading to viral evolution. The cell and tissue tropisms of different adeno-associated virus serotypes were demonstrated to be due, in part, to specific receptor binding.

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Abbreviations

AAV	adeno-associated virus
ADV	aleutian mink disease virus
CPV	canine parvovirus
FPV	feline panleukopenia virus
HSPG	heparan sulfate proteoglycan
MVM	minute virus of mice
PPV	porcine parvovirus
SCID	severe combined immunodeficient
TfR	transferrin receptor

Introduction

Members of the family *Parvoviridae* infect a wide variety of hosts, ranging from insects to primates. In vertebrate hosts they cause disease mainly in the young. In the recent past, some canine parvoviruses (CPV) and feline panleukopenia viruses (FPV) have shifted their host range and can therefore serve as models for emerging diseases, allowing us to follow the evolution of a virus during and after its emergence as a new pathogen. Adeno-associated viruses (AAVs) are a family of non-pathogenic viruses that also contain very promising gene-therapy vectors.

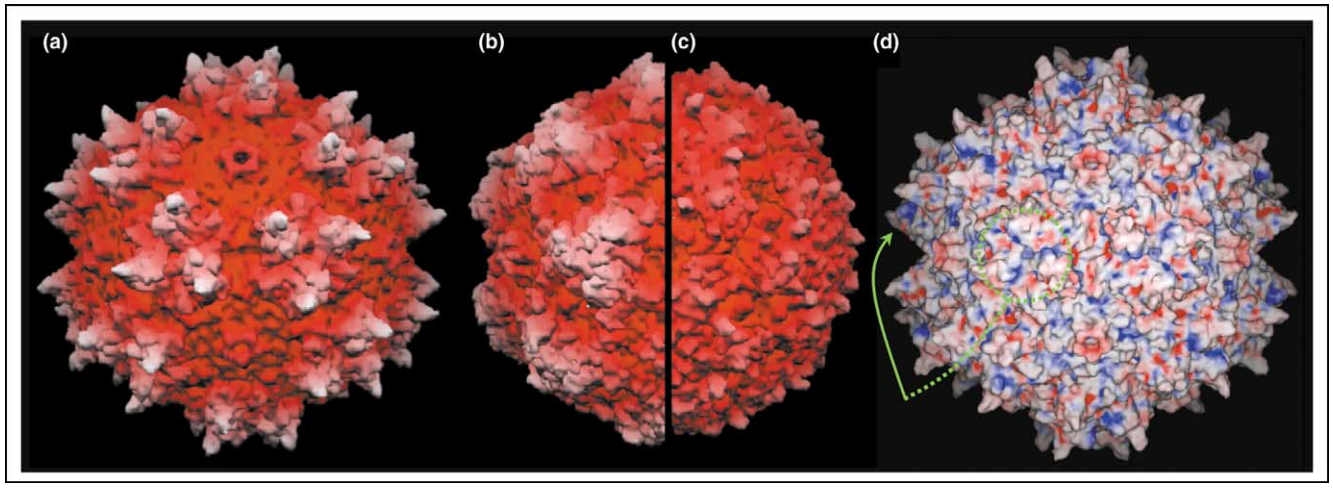
Parvovirus capsids are non-enveloped, ~260 Å in diameter and display a T=1 icosahedral symmetry. They are made up of 60 copies of between two and four overlapping capsid proteins variously designated VP1–VP4. For each virus there is one capsid protein that forms the majority of the capsid structure, as well as minor proteins that form the same core structure, but with additional sequences on their amino-termini. Despite the differences in protein forms and the low homology between some of the viruses, it is now clear that several prominent structural elements on the capsid surface are common to most parvoviruses. These include raised regions at the fivefold axes of symmetry, which in some viruses might form a pore into the capsid, depressed regions (canyons) surrounding the fivefold axes, one or three protrusions at or surrounding the threefold axes of symmetry (threefold spikes or peaks) and depressed regions (dimples) at the twofold axes of symmetry (Figures 1 and 2) [1–6,7•,8••].

Many parvoviruses have tightly controlled host ranges and tissue tropisms, whereas others infect many hosts and a variety of tissues. Understanding controls of these properties for AAV or some autonomous parvoviruses might allow the therapeutic delivery to be controlled or modified in gene therapy applications. Determining the origins and evolutionary processes that gave rise to CPV as a new virus of dogs would increase our understanding of disease emergence, as it is one of only a few examples of a virus that has extended its host range by mutation to infect and become established in a previously resistant host. The recent high-resolution structure of the AAV-2 capsid, recent findings about cell surface receptors and their roles in controlling CPV host range, and information about tropism of several other parvoviruses, are giving a clearer picture of the tropism and host-range control of parvoviruses. Here we review the recent findings in these areas and connect them to earlier studies.

CPV and FPV: acquisition of extended host range by multi-step adaptation and binding to an additional receptor

Despite the fact that the DNA sequence of CPV is > 99% similar to the DNA sequence of FPV the properties of these viruses differ in a variety of ways, including the host range *in vivo* and *in vitro*. Both viruses replicate in feline cells, but only CPV isolates can replicate efficiently in dogs and in cultured dog cells [9]. The *in vivo* host range

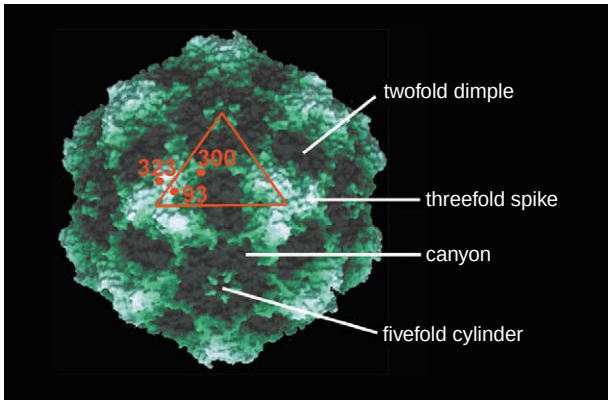
Figure 1



Surface renderings of the capsids of **(a)** AAV-2, **(b)** CPV and **(c)** insect densovirus drawn to scale. The figure shows that most structure elements are shared between CPV and AAV-2, although the threefold proximal peaks are more prominent in AAV-2 than the spikes in CPV. By contrast the surface of the insect densovirus capsid appears smoother with no distinct spikes at the threefold axes of symmetry. **(d)** The electrostatic surface potential of AAV with blue representing positive charge and red showing areas of negative electrostatic potential. The putative receptor binding sites are positively charged patches on the sides of the threefold proximal peaks and are shown head on (green circle) or edge on (green arrow). From Xie *et al.* with permission. Copyright © 2002 by The National Academy of Sciences of the United States of America.

of the viruses show some variations, as the FPV clearly infects the thymic cells of dogs, even after oral inoculation [9]. In addition, the earliest strains of CPV (designated CPV type-2 to distinguish them from a distantly related parvovirus of dogs, also known as the minute virus of canines [10]) do not replicate efficiently in cats, the natural host of FPV, whereas more recent strains have gained this ability [11]. Residues in three separate structural regions of the capsids on the threefold spike are important for determining the host range differences between CPV and FPV. Structures around VP2 residues 93, 300 and 323 are all involved in controlling canine host range (Figure 2) [12–16]. The region around residue 300 is also involved in controlling the *in vivo* feline host range of CPV [17]. When the capsid structure was determined under different pH conditions and divalent ion concentration [5], variation or flexibility of the protein was detected in only a small number of positions, suggesting that the CPV capsid has a very stable structure. The sequence differences that determine the canine host range result in only small changes in the structures of the viruses [1]. Naturally occurring variants of CPV (designated CPV type-2a and CPV type-2b), which replaced the CPV type-2 strain and regained the ability to infect cats, also have mutations in this region [11,18]. Evolution has continued for at least two of these regions in the capsid protein, with recent isolates of CPV showing substitutions at VP2 residues 426 and 297 (near residues 93 and 300 on the threefold spike, respectively) [19–21]. Only a small number of additional changes in the CPV have been detected, and although some of these changes have been found in viruses all over the world, they are found in only a minority of viruses [21]. Interestingly, some of the changes that became widespread were synonymous codon (non-coding) changes, suggesting that there was selection for DNA sequence, or that those changes hitchhiked along with other changes in the genome.

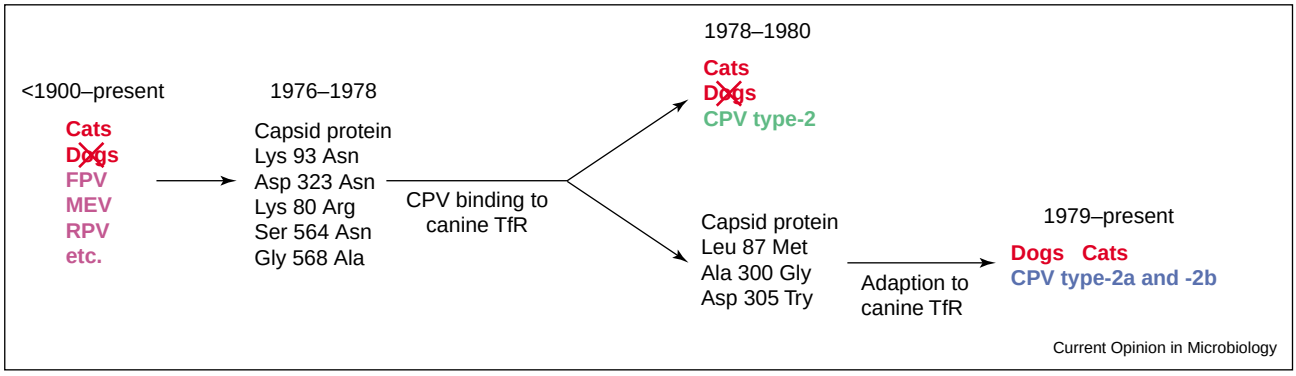
Figure 2



Surface rendered model of the CPV capsid. The structural elements of the twofold dimple, the threefold spike, the canyon and the fivefold cylinder are indicated. One asymmetric unit of the capsid is shown by the red triangle. Areas on the threefold spike that influence the canine host range of CPV and FPV (VP2 residues 93, 300 and 323) are indicated in red. The threefold related structures are involved in cell tropism and antigenicity in several different parvovirus models.

Some of the host ranges of CPV and FPV in animals are recapitulated in tissue culture cells, and it is now clear

Figure 3



Model of CPV evolution. CPV evolved from FPV or a closely related parvovirus. FPV-like viruses cause disease in cats but not dogs (indicated in red), and do not replicate in cultured dog cells. FPV infects feline cells by binding the feline TfR, but is unable to bind the canine TfR. Changes in the capsid protein gave rise to an ancestral CPV between 1976 and 1978 that could bind the canine TfR and subsequently infect canine cells and cause disease in dogs. Although it could bind the feline TfR and infect feline cells *in vitro*, the original CPV virus (termed CPV type-2) could no longer infect cats. Around 1980, CPV type-2 was replaced by a more evolved virus (CPV type-2a), and that virus had accumulated several changes in its capsid gene, which led to a more efficient infection of canine cells. CPV type-2a and subsequent viruses had regained the ability to efficiently infect cats and they now cause disease in both dogs and cats. Evolution is still occurring in these viruses as can be seen by the global emergence of a new antigenic variant termed CPV type-2b, as well as several other substitutions in the genome.

that the block to infection by FPV in cultured dog cells is primarily due to the lack of a functional cell-surface receptor for that virus. This deficiency could be overcome by expression of the feline TfR in the canine cells, where those cells became susceptible to FPV binding and infection [22•,23••]. A difference in the binding of CPV and FPV capsids to the canine TfR controls the canine host range. This specific binding can be affected by each of the three regions of the capsid that control host range [23••,24]. Substitution of both residues 93 and 323 in FPV with the CPV sequences allow the virus to bind to the canine TfR and to infect canine cells, whereas changes in the vicinity of residue 300 could greatly reduce binding to the canine TfR. The further evolution of CPV in dogs after 1978 led to the acquisition of mutations in the capsid protein in the host-range-determining region around residue 300. This group of changes appears to result in the more efficient use of the canine TfR for cell infection.

The current model of CPV evolution is summarized in Figure 3. These findings all indicate that TfR binding and efficient usage of the canine TfR are central events in the evolution and pathogenesis of CPV [23••]. Use of the TfR as the cellular receptor for these viruses on feline and canine cells is also closely correlated with their pathogenicity in nature, as the TfR is highly expressed on crypt cells in the intestinal epithelium and on hematopoetic cells, which are the main target cells of CPV and FPV in animals [25].

Controls of cell and tissue tropism

Minute virus of mice

Differences in the cell tropism between two different strains of minute virus of mice (MVM) have also been mapped to the capsid gene [26]. The VP2 residues 317 and 321 are both important in controlling the cell tropism of allotropic MVM strains and are at the base of the threefold spike of the virion. In addition, residue 321 aligns with the host-range-controlling residue 323 of CPV [3]. Other sequences influencing cell tropism (VP2 residues 399, 553 and 558) were selected by passage of single mutants of MVM in the resistant cells, and these changes are located within the dimple of the capsid some distance from the original mutations [3].

Porcine parvovirus

In porcine parvovirus (PPV), determinants of the different tissue tropisms of the nonpathogenic NADL-2 strain and the pathogenic Kresse strain have been mapped to a region within the capsid gene. One amino acid difference between the two strains in this region (residue 436) is on the threefold spike of the virus capsid, while two other residues (378 and 383) are on the edge of the canyon surrounding the fivefold cylinder [7•,27].

Aleutian mink disease virus

Wild-type aleutian mink disease virus (ADV) isolates do not generally replicate in cultured cells but readily infect mink. The ADV-G strain replicates in cultured cells but not in mink, and the altered tropism was mapped to a sequence within the capsid gene. Coding differences between the two strains map to a region predicted to be on the threefold related structures of the capsid [28–30].

Adeno-associated viruses

For the dependovirus adeno-associated viruses (AAV), several serotypes have been defined that differ in their host-cell tropisms [31,32]. This tropism is determined,

at least in part, by their preference for different receptors [33]. AAV-2 and AAV-3 use heparan sulfate proteoglycans (HSPGs) as a low affinity primary receptor, in addition to αVβ5 integrins and/or human fibroblast growth factor receptor [34–37]. AAV-4 and AAV-5 both require sialic acid for cell attachment and infection, but AAV-4 transduction was only blocked with soluble α2-3 sialic acid, whereas AAV-5 could be blocked with either α2-3 or α2-6 sialic acid [38•]. Determinants for HSPG-binding to AAV-2 capsid are located at several positions in the primary structure [39–41]. Insertions at VP1 residue 522 cause the loss of infectivity and binding to heparin [39] and two clusters of positively charged amino acids on the capsid surface that are involved in heparin binding (amino acids 509–522 and 561–591) were also identified [40]. Using epitope mapping of monoclonal antibodies that inhibit cell attachment, a putative receptor binding site on the capsid of AAV-2 was identified within the regions between residues 493 and 502 and between residues 601 and 610, whereas insertions at residues 534, 573 and 587 prevented only binding of the antibody [41]. In the three-dimensional structure of the assembled particle these determinants for receptor binding and tissue tropism cluster within the peaks that surround the threefold axes of the capsid (Figure 1) [8••].

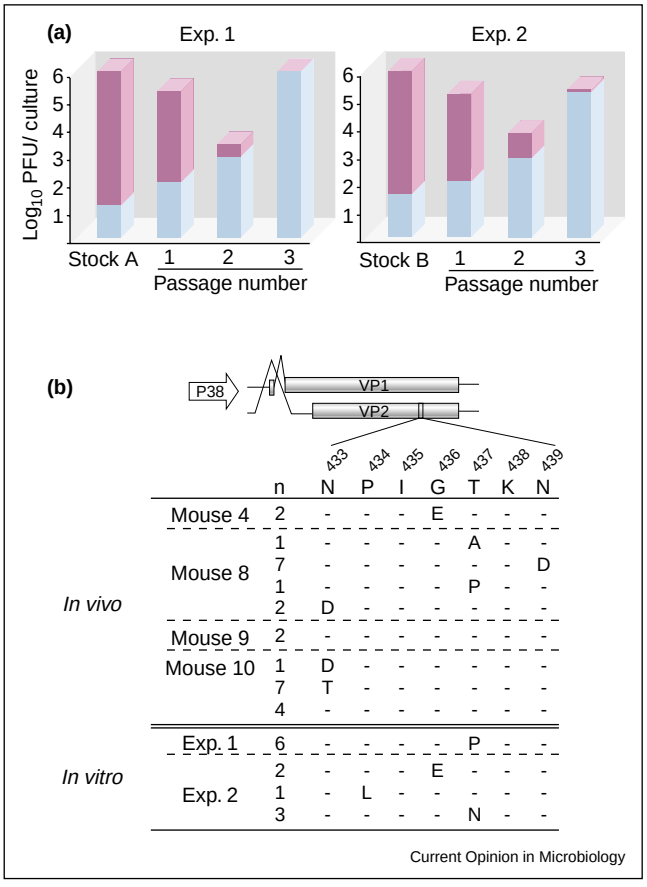
The host range of AAV-2 was extended by inserting either the L14 epitope [42] or a single chain variable fragment directed against human CD34 [43] into the capsid protein. However, these redirected AAV-2 particles retained the ability to infect or transduce cells susceptible to the wild-type virus. The host range of AAV was also expanded by using bispecific F(ab′γ)2 antibodies directed against AAV and cell surface integrin [44]. These results suggest that AAV-2 has a broad host range and tissue tropism and that it is able to use a variety of different receptors to mediate cell uptake and infection or transduction. Although infections using the different receptors or inserted ligands occurred, it is not yet clear just how the receptors affect efficiency of virus infection. The results reported for AAV-4 and AAV-5 indicate that these viruses are receptor specific, due mostly to differences in the sialic acid binding, rather than to receptor effects on the later steps of infection [38•].

Experimental studies of virus sequence variation and evolution

The evolution of CPV during the process of serial passage leading to attenuation of a vaccine strain showed a pattern of change consistent with mutation followed by recombination, rather than sequential selective sweeps of individual mutations. It was also surprising to find that several of the changes observed during the attenuation process were identical to, or at the same positions as, changes observed during the natural evolution of CPV and FPV [45•].

During a study investigating the emergence of antibody escape mutants of MVM *in vitro* and in severe combined immunodeficient (SCID) mice it was seen that even without selection a high degree of heterogeneity develops in MVM populations under both conditions (Figure 4) [46••]. As soon as antibody selection was applied, neutralization escape mutants were rapidly selected and dominated the culture. These findings have broad consequences for the development and safety of live attenuated viral vaccines

Figure 4



Mutant frequency and antibody evasion of MVM after growth from a cloned stock and during *in vitro* or *in vivo* selection with antibodies. **(a)** In a clonal population of MVM the frequency of monoclonal antibody-resistant mutants was examined. The graph shows the high frequency of resistant mutants (blue part of the bar) in the clonal population (Stock) and their increase during *in vitro* selection for antibody escape mutants in two independent experiments. These data illustrate the adaptability of parvoviruses to selection pressure *in vitro*. **(b)** Amino acid differences in clones obtained during selection for antibody escape mutants in SCID mice or *in vitro*. The transcriptional scheme of the gene giving VP1 and VP2 is shown on the top. Changes in the amino acid sequence are shown below for clones obtained from selection experiments either *in vitro* (see **(a)**) or from monoclonal antibody therapy in immune deficient severe combined immunodeficiency mice infected with MVM. The number (n) represents the number of clones from each population showing the corresponding amino acid substitution. The data show that multiple escape mutants arose simultaneously in the same mouse or *in vitro* experiment, also indicating a high mutation frequency of this virus.

in general and indicate that the adaptability, heterogenicity and recombination potential of these DNA viruses may generally be underestimated.

Antigenic structure and variation

Apart from being important in determining tropism of parvoviruses the threefold spike or peaks also contain major antigenic sites. Neutralizing antibodies against this region in the capsid have been described for CPV and FPV [14,47], as well as for MVM [46••]. Antibodies also bind structures of ADV, although these can lead to immune enhancement of the disease, an important feature of ADV infection and pathogenesis [48•].

Conclusions

The recent results discussed above, together with earlier findings, indicate that parvovirus cell tropisms and host range, as well as evolution, are controlled mainly by raised structural elements around the threefold axes of symmetry. The atomic model of AAV-2 indicates that this structural element is also present although composed of different parts of the primary sequence compared with other parvoviruses [8••], showing that high-resolution models are indispensable for explaining the molecular processes controlling these features of viral biology. Although the same structural element of viruses is involved in several host and tissue tropisms, each appears to be affecting a different mechanism. Host ranges of CPV and FPV are controlled by receptor binding, whereas the cell tropisms of MVM appear to be due to restrictions of intracellular steps. Many of the AAVs have a broad host range and the capsid can infect cells using a variety of different receptors; the alteration of cell tropisms of AAV vectors to give enhanced gene therapy treatments appears possible. Parvoviruses can also serve as models to better understand the general features of evolutionary and biological events that lead to host-range and other changes of viruses, which is important information in a world where long distance air travel and human interactions with new environments brings us into contact with novel viruses with increasing frequency. The emergence of CPV as a well-documented case of host-range shifts will be better understood when the results from related parvoviruses allow a more general understanding of host range control and evolution of these small non-enveloped DNA viruses.

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- •• of outstanding interest

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