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Current Opinion in Virology

Adeno-associated virus: fit to serve

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Adeno-associated virus (AAV) is a helper-dependent parvovirus which has not been linked with human disease. This aspect, in combination with its broad cell and tissue tropism, and limited viral host response has made it an attractive vector system for gene therapy. The viral protein capsid, the primary interface with the host, is the main determinant for these phenotypes, is highly variable, and is most subject to pressures during replication. Here, we explore the evolutionary path of AAV and other parvoviruses in respect to these phenotypes, as well as directed evolution and engineering strategies that have exploited the lessons learned from natural selection in order to address remaining limitations of AAV as a therapeutic gene transfer platform.

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Current Opinion in Virology 2014, **8**:90–97

This review comes from a themed issue on **Virus evolution**

Edited by **Scott Hensley** and **Michael Brockhurst**

http://dx.doi.org/10.1016/j.coviro.2014.07.008

Introduction

Originally discovered as a contaminant in a preparation of simian adenovirus [1], adeno-associated virus (AAV) has come to prominence as an attractive candidate to serve as a vector system for therapeutic gene transfer. A member of the family Parvoviridae, AAVs carry their 4.7 kb single-stranded genomes within non-enveloped $T = 1$ icosahedral capsids [2]. AAVs have been isolated from a wide range of animal samples including human, non-human primate, caprine, bovine, and avian samples. However, a defining characteristic of AAV is its apparent dependence on a helper virus co-infection (such as adenoviruses or herpesviruses) for productive replication. Helper-dependent replication also distinguishes AAVs from other members of the family Parvoviridae and delineates the subfamily, dependoparvovirus (formerly known as dependovirus).

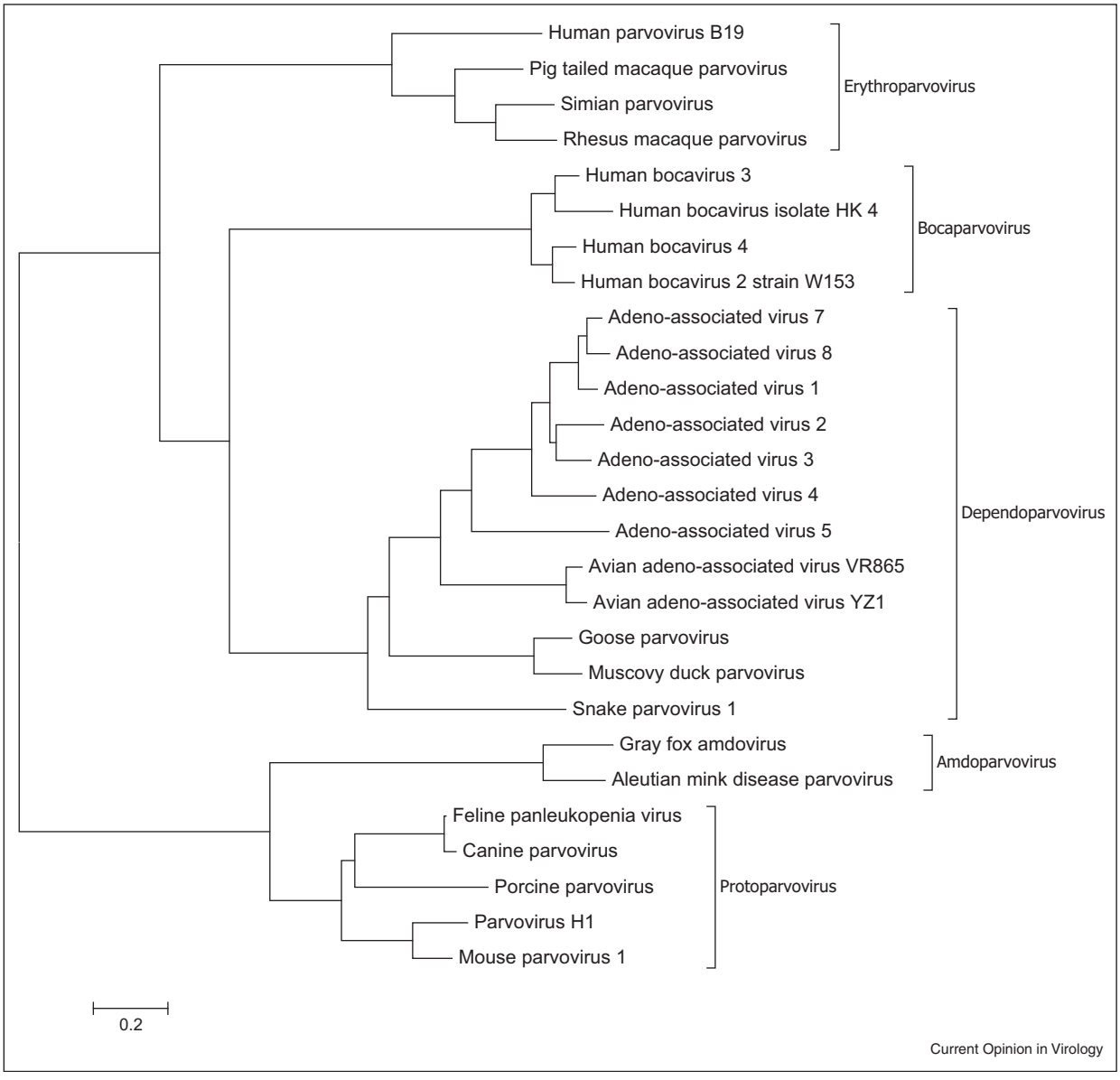
Due to a number of features inherent to their viral biology, AAVs have also become widely used as gene transfer vectors. AAV has not been associated with disease, has a wide and promiscuous tropism, is minimally immunogenic, and can achieve efficient and long-lived gene transfer. Eliminating all viral open-reading frames from the viral genome, replication defective AAV viral-like particles (also known as recombinant AAV or rAAV) containing heterologous genetic information can be assembled and packaged to high vector yields for gene transfer applications. When loaded with a therapeutic transgene, clinical safety and efficacy of AAV has been shown for inherited forms of blindness [3,4] and hemophilia B [5]. At the preclinical stage many approaches have been explored using AAV ranging from therapeutic paradigms for single gene inherited disorders, complex acquired disease (reviewed in Weinberg *et al.* [6]), and infectious disease (e.g. for influenza and HIV [7,8••,9,10]).

While AAV has evolved several desirable phenotypes for therapeutic gene transfer, its biology also poses certain important limitations to its application for gene therapy. AAV is only capable of efficiently packaging about 5 kb of DNA, excluding many therapeutic genes and approaches from development. Moreover, the fact that many AAV serotypes appear to be endemic results in extensive anti-viral immunity in human populations [11,12], complicating future AAV gene transfer in pre-immune subjects. Furthermore, although the natural diversity of AAVs is vast, and host tropism differs among AAV species, several important cell types and tissues for gene therapy remain to be unlocked for targeting. The study of AAVs' natural evolution and exploitation of laboratory based directed evolution and engineering has been creatively used to influence its biological properties and phenotypes to address some of these remaining limitations.

Parvoviridae evolution

A sister clade to dependoparvovirus, protoparvovirus (formerly parvovirinae) is a subfamily of Parvoviridae encompassing some of the first members to be isolated and characterized including Kilham rat virus and canine parvovirus [13] (Figure 1). These viruses bear many similarities to AAVs with a notable exception that they are all autonomous viruses capable of efficient replication without coinfection of a helper virus. An additional difference to keep in mind is that unlike AAV these viruses can also be pathogenic to their hosts. Despite these differences, interest surrounding the emergence of novel protoparvovirus members and the evolutionary drivers of other member viruses has motivated phylogenetic studies which may serve as a model for similar work in other Parvoviridae subfamilies including dependoparvovirus.

Figure 1



Phylogenetic relationship of representative members of the family Parvoviridae. Maximum likelihood phylogeny of viruses based on a full-genome alignment. Subfamilies are labeled with brackets.

One feature of protoparvoviruses which may apply to other subfamilies is the relatively high rate of observed nucleotide substitution relative to other DNA viruses. While studying canine parvovirus (CPV), it was observed that the rate of nucleotide substitutions in the CPV genome more closely resembled those of RNA viruses rather than those of double-stranded DNA viruses [14]. This high rate of variation has also been seen in another protoparvovirus, the minute virus of mice (MVM) where it has been attributed to being involved in immune escape [15]. Furthermore, similar substitution rates have also been observed in human parvovirus B19 [16], a member of another subfamily of Parvoviridae (erythroparvovirus) suggesting that this high rate of evolution may be a general characteristic of the Parvoviridae family. Additional studies have begun to explore the extent to which these high mutational rates affect evolution of parvoviruses.

Extensive phylogenetic studies on the emergence of CPV have helped to clarify and redefine a story of viral evolution. It had long been understood that CPV