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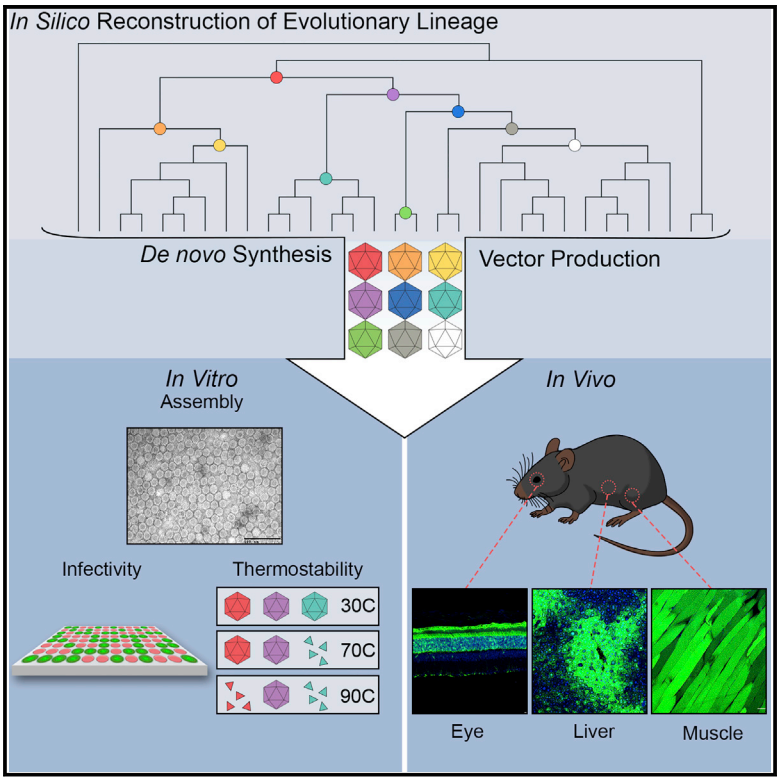
In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector

Graphical Abstract



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In Brief

Adeno-associated virus (AAV) vectors have enabled preclinical and, more recently, clinical therapeutic gene transfer. Zinn et al. reconstruct in silico approximations of evolutionary capsid intermediates yielding functional particles. Predicting the ancestor of the commonly used AAV serotypes 1, 2, 3, 7, 8, and 9 results in a potent gene therapy vector with broad applications.

Highlights

- In silico ancestral sequence reconstruction leads to infectious viral particles
- Anc80, an ancestor of AAV1, 2, 8, and 9, is a potent in vivo gene therapy vector
- The putative evolutionary lineage of AAV is functionally restored
- Ancestral sequence reconstruction elucidates complex structure-function relations

Zinn et al., 2015, Cell Reports *12*, 1056–1068
August 11, 2015
http://dx.doi.org/10.1016/j.celrep.2015.07.019

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In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector

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SUMMARY

Adeno-associated virus (AAV) vectors have emerged as a gene-delivery platform with demonstrated safety and efficacy in a handful of clinical trials for monogenic disorders. However, limitations of the current generation vectors often prevent broader application of AAV gene therapy. Efforts to engineer AAV vectors have been hampered by a limited understanding of the structure-function relationship of the complex multimeric icosahedral architecture of the particle. To develop additional reagents pertinent to further our insight into AAVs, we inferred evolutionary intermediates of the viral capsid using ancestral sequence reconstruction. In-silico-derived sequences were synthesized de novo and characterized for biological properties relevant to clinical applications. This effort led to the generation of nine functional putative ancestral AAVs and the identification of Anc80, the predicted ancestor of the widely studied AAV serotypes 1, 2, 8, and 9, as a highly potent in vivo gene therapy vector for targeting liver, muscle, and retina.

INTRODUCTION

Extensive preclinical studies have established a favorable safety profile for adeno-associated virus (AAV) vectors. In addition, AAV vectors have enabled demonstration of in vivo gene therapy efficacy in animal models of disease with etiologies ranging from inherited to infectious to common complex (Hastie and Samulski, 2015; Schnepp and Johnson, 2014). Furthermore, recent early-stage AAV clinical trials have led to the first demonstrations of clinical benefit in two forms of inherited blindness with AAV2 (Bainbridge et al., 2008; Jacobson et al., 2012; MacLaren et al., 2014; Maguire et al., 2008) and hemophilia B with AAV8 (Nathwani et al., 2011). One treatment based on AAV1 has been awarded a drug license by European regulators (Bryant et al., 2013). Based on these data, AAVs have been proposed as a platform technology for therapeutic in vivo gene delivery.

AAV is a 25-nm non-enveloped icosahedral capsid virus carrying a 4.7-kb single-stranded DNA genome flanked by inverted terminal repeats (ITRs). AAV classifies as a *Dependoparvovirus* genus within the *Parvoviridae* family. Its genome comprises genes encoding for replication (Rep), structural capsid (Cap), and assembly (AAP) proteins. AAV is a helper-dependent virus, requiring the heterologous cofactors to complete a replicative cycle that an adenovirus or herpesvirus can provide in the context of a co-infection. Replication-deficient AAV can be generated by eliminating all viral coding sequence in *cis* and providing those in *trans* during vector production. Particles generated in this manner can encode any type of transgene cassette that does not exceed the size of the genome of the wild-type virus (~4.7 kb) and enable gene transfer in vitro and in vivo to multiple cell and tissue targets (Knipe and Howley, 2013).

The AAV particle is composed of three C-terminally overlapping Cap proteins named VP1, 2, and 3. VP3, the smallest member of these structural proteins, is necessary and sufficient for full capsid assembly through multimerization. VP1, required for particle infectivity, and VP2, reported to be redundant structurally or functionally, are embedded in the wild-type T = 1 viral architecture at a VP1:2:3 ratio of ~1:1:10. A total of 60 VP monomers assemble into an icosahedral capsomer along 2-, 3-, and 5-fold axes of symmetry (Knipe and Howley, 2013). Every monomer within the 60-mer structure interfaces with seven neighboring capsid monomers. High-resolution crystallography studies identify a conserved core structure composed of an eight-stranded β-barrel motif and the αA helix as well as nine surface-exposed domains (VR-I to -IX), which can vary between the known primate AAV serotypes (DiMattia et al., 2012; Xie et al., 2002).

The structural diversity of natural AAVs is therefore largely contained within the nine surface regions of the capsid, which functionally results in divergent receptor-binding properties, post-entry trafficking, host response, and gene transfer efficiency to various cell and tissue targets. Structural determinants for many of these properties have largely remained elusive, with a few notable exceptions: receptor-binding motifs for certain serotypes (Bell et al., 2012; Kern et al., 2003; Wu et al., 2000), phospholipase activity and nuclear localization activity on VP1,2 unique domains (Girod et al., 2002; Grieger et al., 2006), a limited