260g Directed Evolution of Aav to Generate Mutants with Enhanced Transport Properties

James T. Koerber, Narendra Maheshri, and David V. Schaffer

Successful gene delivery requires the efficient transport of a therapeutic gene to the nucleus of a specific cell type. Viral vectors have shown tremendous promise as gene delivery vehicles, but both cellular (e.g. attachment and trafficking) and systemic (e.g. neutralizing antibody responses and tissue transport) mass transfer barriers limit efficient gene delivery. For example, adeno-associated viral (AAV) vectors provide safe and efficient gene delivery and are being explored in clinical trials for cystic fibrosis, Parkinson's Disease, Alzheimer's Disease, and alpha-1-antitrypsin deficiency. However, many promising therapeutic cell types, including astrocytes and hematopoietic stem cells, remain refractory to AAV infection. In addition, almost two-thirds of humans harbor significant levels of neutralizing AAV antibodies, which further limits the large scale use of AAV as a gene therapy vector. Alternate AAV serotypes have shown only moderate abilities to address these issues. Furthermore, rational design approaches to enhance AAV, such as genetic peptide insertions or chemical modifications of AAV2, have met with limited success, and the absence of extensive structure/function relationships for other AAV serotypes further limits these approaches. Therefore, we have used molecular evolution techniques to generate several large randomly mutated AAV2 libraries and have selected for AAV variants with desirable, enhanced properties. First, we have selected for AAV virions that possess 100-fold improved resistance to rabbit antiserum against AAV2, yet retain AAV gene delivery efficiency. More importantly, these variants mediate sustained gene expression in a mouse model even in the presence of high levels of anti-AAV serum. Furthermore, we have made progress selecting AAV vectors with enhanced resistance to pooled human serum, as well as AAV mutants that exhibit novel cellular tropism. This high throughput approach can be employed to generate customized or ‘designer' gene delivery vectors with properties for numerous applications.