response against AAV-2, yet retain the high gene delivery efficiency inherent to AAV-2, could surmount the problem of pre-existing anti-AAV neutralizing antibodies in a significant fraction of the human population, and may present opportunities for readministration. Finally, not only do these libraries provide useful mutants directly applicable to gene therapy applications, but they also offer a way to continue to dissect AAV biology.

900. Packaging of Host Cell and Plasmid DNA into Recombinant Adeno-Associated Virus Particles Produced by Triple Transfection

Peter H. Smith,[1] J. Fraser Wright,[1] Guang Qu,[1] Susannah Patarroyo-White,[2] Amy Parker,[2] Jurg M. Sommer.[1]
[1]Development, Avigen, Inc., Alameda, CA; [2]Applications Research, Avigen, Inc., Alameda, CA.

In this study we characterized residual host cell and plasmid DNA impurities in preparations of highly purified AAV vectors. AAV-hFIX16, a recombinant AAV2 vector containing the coding sequence for human coagulation factor IX under the control of the human alpha-1 antitrypsin (hAAT) promoter, was produced by triple plasmid transfection of HEK293 cells and purified by sequential cation and anion exchange chromatography. Reverse packaging of the vector plasmid was eliminated by using an oversized plasmid backbone of approximately 7000 base pairs. As determined by real-time quantitative PCR (Q-PCR), residual HEK293 and plasmid DNA ranged from 1-5% of total vector DNA in preparations purified by chromatography. The levels of DNA impurities in the final product were not reduced by in-process or final treatment with Benzonase or DNase I. Column chromatography-purified vector was fractionated by CsCl density gradient centrifugation to further characterize the DNA impurities. Increasing amounts of HEK293 and plasmid DNA were observed in fractions ranging in density from 1.32 gm/mL (density of empty AAV capsids) to 1.38 gm/mL (density of vector particles). Southern blot analysis of gradient fractions demonstrated that the average size of residual DNA in each fraction ranged from 600 nucleotides near the empty capsid band to a maximal size of about 4,500 nucleotides in fractions containing intact vector particles. Negligible amounts of residual DNA were associated with fractions of a density higher than that of intact vector. These results suggest that AAV packages single-stranded HEK293 or plasmid DNA fragments of various sizes up to the packaging limit of AAV. Preferential (2 to 5-fold) packaging of vector plasmid sequences over Adenovirus helper plasmid or rep/cap-encoding plasmid sequences was observed. The data indicate that the amount of non-vector DNA in AAV vectors purified by column chromatography can be reduced three to five-fold by an additional gradient fractionation step, but that the remaining host cell and plasmid DNA fragments are approximately the same size as the vector genomes. Additional studies are required to more fully characterize packaged non-vector DNA, and to further optimize production methods to reduce residual DNA impurities in AAV vectors.

901. Evidence That Ionic Interactions Are Involved in Concentration-Induced Aggregation of Recombinant Adeno-Associated Virus

Guang Qu,[1] Christopher Connolly,[1] Alicia Koblansky,[1] Jurg M. Sommer,[1] Alan McClelland, J. Fraser Wright.[1]

Recombinant adeno-associated viruses (rAAV) are promising vectors for human gene therapy, and have demonstrated excellent safety and promising efficacy in pre-clinical and clinical studies. A key requirement for successful development of AAV vectors is to establish a reliable and cost-effective process to generate material of high purity and titer. Aggregation of vector particles may occur during purification, and result in reduced yield and deleterious effects following *in vivo* administration (eg reduced efficacy, increased immunogenicity). Huang *et al*. (*Mol Therapy* (2000) 1:S286) previously reported that AAV vector particles undergo concentration-dependent aggregation. In this study vector aggregation has been further characterized. We examined aggregation during tangential flow filtration, a process step used to concentrate and diafilter purified vectors at large-scale. Aggregation was assessed by dynamic light scattering, size-exclusion chromatography, and by quantification of loss following 0.2 µm filtration. We observed that aggregation was capsid particle (cp) concentration-dependent, and typically occurred when concentrations exceed the range 0.5-1.0 x 10^{14} cp/mL for column purified vectors. Considerable variability in the concentration at which aggregation occurred was observed, which may be attributable to variability in the levels of empty capsids, and in levels of DNA and/or protein impurities in the vector preparations. To investigate the mechanism(s) of AAV vector aggregation, we assessed the effect of surfactants and buffer pH on this phenomenon. Neither Polysorbate 80 (0.1%) nor Pluronic F68 (0.1%) added to vector in phosphate buffered saline, pH 7.2, affected the concentration at which aggregation was observed relative to control vector lacking surfactant. Adjusting the pH to values ≤4.5 or ≥10 resulted in reversal of concentration-induced vector aggregation, suggesting that ion bridges between charged amino acids (Glu, Asp, Lys) on the surface of vector particles contribute to inter-particle interactions. However, vector aggregates were also observed to be stable in approximately 3M CsCl (neutral pH), suggesting that other types of interactions play a role. In conjunction with further elucidation of the mechanism(s) of AAV vector aggregation, these observations will facilitate fomulation development for optimal large-scale vector purification and clinical use.

902. Construction and Analysis of Truncated Muscle-Specific Promoters (Muscle Creatine Kinase Promoter)

Bing Wang,[1] Liqiao Zhou,[1] Juan Li,[1] Xiao Xiao.[1,2]
[1]Dept. of Molecular Genetics and Biochemistry & Gene Therapy Center, University of Pittsburgh, Pittsburgh, PA, United States; [2]Dept. of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, United States.

Muscle is readily accessibly for direct injection of gene therapy vectors for the treatment of both muscle- and non-muscle diseases. The CMV promoter is not the ideal promoter for muscular dystrophy gene therapy, because it renders gene expression in non-muscle cells (such as APCs), which may potentially elicit an immune response against the transgene product. While tissue-specific promoters are highly desirable in gene therapy practice, those promoters are generally large in size and less active than viral promoters such as the CMV promoter. Large promotes are not well suited for AAV vectors. Our aim is to develop some highly compact, highly active yet highly tissue-specific promoters. The commonly used muscle-specific promoter (MCK) and its derivatives developed by Dr. Hauschka's lab are well known for the high tissue-specificity and moderate activities. The major regulatory regions include a muscle-specific enhancer and a 358-bp proximal promoter. Here we have constructed chimeric promoters containing one, two or three modified MCK enhancer with the minimal MCK promoter. We have compared the promoter activity and tissue-specificity of these promoters in differentiated and undifferentiated muscle cells in vitro and muscle tissues in vivo. Our results showed that the levels of of Luciferase activity achieved by the chimeric promoters, especially the modified construct dMCK (including two modified enhancers), tMCK (including three modified enhancers), were significantly higher (> 10 fold) than the original MCK promoter. We have also shown