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Translational Control of Adeno-Associated Virus 2 by eIF5-Mimic Protein

Chingakham Ranjit Singh[1], Sarah Gillaspie[1], Mackenzie Thornton[1], Maureen Schick[1], Giovanni Di Pasquale[2], Sandra Afione[2], John A Chiorini[2], and **Katsura Asano**[1]

[1]Molecular Cellular and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS, USA; [2]Adeno-Associated Virus Biology Section, NIDCR, NIH, Bethesda, MD, USA

Adeno-associated virus 2 (AAV2) is a ssDNA virus and a member of the family Parvoviridae. AAV2 encodes capsid protein, DNA-binding SF3 helicase Rep, and Assembly-Activating Protein (AAP). Capsid and Rep proteins are produced as three (VP1, VP2 and VP3) and six isoforms, respectively, through alternative splicing and different transcriptional and translational start sites. VP2 and AAP translation is initiated by ACG and CUG codons, respectively, located within the transcript encoding VP2 and VP3. Here we report that the AAV2 Rep78 isoform interacts with a translational regulatory protein, eIF5-mimic protein 1 (5MP1). Since Rep78 and Rep68 bind 5MP1, but Rep52 does not, the 5MP1 binding to Rep78 requires its N-terminal DNA-binding domain missing in Rep52. 5MP1 binds Rep78 through the acidic surface of its C-terminal domain resembling the structure of double-stranded DNA by surface charge distribution. 5MP1 represses translation from non-AUG codons. Thus, we are currently testing the model that Rep78 binding to 5MP1 sequesters 5MP1 away from the ribosome and thereby decreases initiation accuracy, in favor of AAV2 production of VP2 and AAP initiated by non-AUG codons. The initiation frequencies from the firefly luciferase plasmids bearing VP2 ACG or AAP CUG start codon and the 24-nt region preceding each codon are ~10% compared to that from the AUG codon under a typical Kozak context (CCACCAUGG), and are indistinguishable from those from ACG or CUG codon under the same Kozak context. When measured using the luciferase plasmids with a full-length VP2-VP3 mRNA leader region prior to VP3 AUG codon and its luciferase gene in-frame to AAP, VP2 or VP2 and VP3, the initiation frequencies from AAP CUG and VP2 ACG codons are 6% and 19%, compared to combined frequencies from VP2 ACG and VP3 AUG start codons. Based on these and other results, we discuss translational control of AAV2 by 5MP.