Public Abstract

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Title:Protein processing strategies by adeno-associated virus type 5 and the effects of the adenovirus E4orf6/E1b-55k E3 ubiquitin ligase complex on AAV protein stability

We report the initial identification of the ubiquitination of a parvovirus non-structural protein by the adenovirus E4orf6/E1b-55k/Cullin 5 E3 ubiquitin ligase complex. The small Rep and capsid proteins of adeno-associated virus type 5 (AAV5) were found to be specifically targeted for ubiquitination and degradation in a proteasome-dependent manner by the adenovirus E3 ubiquitin ligase complex. The AAV5 small Rep proteins were also found to be ubiquitinated in 293 cells during transient transfection in the absence of E4orf6. This is the first example of poly-ubiquitination of a parvovirus non-structural protein.

We also report that unlike AAV2, AAV5 likely utilizes an additional internal methionine translation start site within the Rep52 reading frame to encode Rep40, a start site that also is within AAV2, but is not used. Therefore AAV5, and the other animal AAVs, have likely evolved an alternate strategy from that of the AAV2-like AAVs to encode this potentially critical protein.

Finally, we report that significant increases in the production of recombinant AAV (rAAV) for gene therapy applications can be accomplished as a result of enhancing the splicing of the capsid-encoding transcripts. By improvement of the non-consensus AAV splice donor sequence to that of the native U1 snRNP binding site, and by utilizing alternate promoters to optimize the levels of splicing of these RNAs, we are able to generate levels of rAAV that are ten to fifteen fold greater than those generated by traditional methods. The enhancement of splicing reveals to be a simple method by which to enhance the production of rAAV during transient transfection.