O-28

Mapping and Engineering Functional Domains of the Assembly Activating Protein of Adeno-Associated Viruses

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Adeno-associated viruses (AAV) encode a unique assembly activating protein (AAP) within their genome that is essential for capsid assembly. Studies to date have focused on establishing the role (or lack thereof) of AAP as a chaperone that mediates stability, nucleolar transport, and assembly of AAV capsid proteins. Here, we map structure-function correlates of AAP based on secondary structure and bioinformatics, followed by deletion and substitutional analysis of specific domains, namely, the hydrophobic N-terminal domain (HR), conserved core (CC), proline-rich region (PRR), threonine/serine rich region (T/S) and basic region (BR). First, we establish that the hydrophobic region (HR) and the conserved core (CC) in the AAP N-terminus are the sole determinants for viral protein (VP) recognition. However, VP recognition alone is not sufficient for capsid assembly or conferring serotype specificity. Enhancing the hydrophobicity and alpha-helical nature of the N-terminal AAP region through amino acid substitutions enabled assembly of previously unrecognized VPs into capsids. Interestingly, the adjacent PRR and T/S regions are flexible linker domains that can either be deleted completely or replaced by heterologous functional domains that enable ancillary functions such as fluorescent imaging and precise control over oligomerization. We also demonstrate that the C-terminal BR domains can be substituted with heterologous nuclear and nucleolar localization sequences that display varying efficiency or with IgG Fc domains for VP complexation and structural analysis. The newly engineered AAPs (eAAP) are more stable and require only about 20% of the original AAP sequence for efficiently supporting AAV capsid assembly. Our study sheds light on the structure-function correlates of AAP and provides multiple examples of engineered AAP that might prove useful for understanding and controlling AAV capsid assembly.