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The Assembly Activating Protein Pleiotropically Supports Adeno-Associated Virus Production

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Although AAV is not considered pathogenic to an entire organism, viral production inundates the host cell, necessitating elimination of viral proteins preferably before the highly stable AAV capsid is assembled. In response, the virus evolved the Assembly Activating Protein (AAP), which functions both to stabilize the AAV viral capsid protein (VP) and to promote VP-VP interactions to nucleate capsid assembly. Through a novel phenotype-to-phylogeny mapping strategy that employs 12 natural serotypes and a set of 9 putative evolutionary intermediate AAV capsids (AncAAVs) we identified key residues involved in VP-VP interactions, that when strengthened reduce dependency on AAP. Here, we show a variable ability for AAP of AAV2 (AAP2) to rescue heterologous AAV and AncAAV assembly. Applying the phenotype-to-phylogeny mapping strategy to this variance identifies candidate residues involved in VP-AAP interaction, located on VP's beta barrel. Mutations neutralizing a negatively charged patch at this site robustly increase VP-AAP co-precipitation and also allows AAP2 to rescue heterologous virion assembly, suggesting this as the binding site for direct (or indirect) VP-AAP interaction. We demonstrate that VP is subject to both proteasomal and lysosomal/autophagosomal degradation, but rescuing VP protein by blocking degradation is not sufficient to rescue capsid assembly in the absence of AAP, highlighting an important scaffolding role for AAP. Motifs that potentially target VPs for degradation, adjacent to the identified AAP binding site and at the trimer interface, suggest that AAP blocks degradation both directly, by shielding targeting motifs, and indirectly, by promoting oligomerization which shields additional targeting motifs. The location and high level of conservation of these motifs may indicate that although they target the capsid proteins for degradation, they are critical for VP-VP interactions. AAP may have evolved to overcome the host's use of such critical residues to eliminate viral proteins, pleiotropically promoting stability, oligomerization, and transportation of capsid proteins.