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Endo/Lysosomal pH-Induced Structural Changes are Conserved in Adeno-Associated Virus Capsids

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Adeno-associated virus (AAV) is a non-enveloped icosahedral virus packaging a single-stranded DNA genome, and in use as a human gene delivery vector due to its non-pathogenic nature and ability to package foreign genes. However, there is a need to understand the biology and biochemistry of how the virus trafficks through the cell after entry and how this can impact the efficiency of transgene expression. AAVs are assembled from three overlapping structural proteins: VP1, VP2, and VP3, at a 1:1:10 ratio, to form an oligomer of 60 viral proteins. The capsid interacts with cell surface receptors for virus attachment and entry, followed by internalization via clathrin-mediated endocytosis. The endosomal compartment gradually acidifies, changing in pH from 6.0 to 4.0, during which the unique region of VP1, VP1u, is proposed to become externalized outside the capsid, exposing a phospholipase domain that is critical for the escape from the endosome. To further elucidate the capsid structural dynamics required for this externalization and successful endo/lysosomal pathway trafficking, the structures of four AAV serotypes, AAV1, AAV2, AAV5, and AAV8, have been determined at pH conditions experienced in the extracellular space, late endosome, and lysosome – pH 7.4, 5.5, and 4.0, respectively. A combination of X-ray crystallography and cryo-electron microscopy and image reconstruction was used to obtain structures in the 3 to 3.5 Å resolution range. Localized capsid re-arrangements previously reported for AAV8 are conserved in AAV1, AAV2, and AAV5. The functional implications of these changes will be discussed, especially within the context of the AAV life cycle.