

## Structural Studies Probe the Evolution of Spherical Viruses

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The similarities in the molecular structures and the packing arrangement of the major coat proteins in human adenovirus and bacteriophage PRD1 suggest an evolutionary link between the viruses [1]. Both viruses have icosahedral non-enveloped protein shells (capsids), vertex recognition fibers, and linear dsDNA genomes with terminal proteins. The capsids of both viruses are organized on a pseudo  $T = 25$  lattice, with pentameric proteins at the vertices and trimeric major coat proteins forming the facets.

Adenoviruses are responsible for a variety of human diseases including respiratory infections, conjunctivitis and enteric dysentery. There has been much recent interest in their practical use as vectors for human gene therapy. PRD1 is unusual in that it contains a lipid membrane beneath its protein capsid. Knowledge of their virion structures provides a framework from which to understand viral assembly, stability and disassembly.

The large size and complexity of both adenovirus (2700 polypeptides, 150 MDa, 1000 Å diameter) and PRD1 (66 Mda, 650 Å diameter) necessitate the combined use of X-ray crystallography (for atomic resolution data) and electron microscopy (EM) to obtain detailed images of the entire capsids. Docking methods [2] are used to computationally align the atomic resolution crystal structures of the capsid proteins to the positions observed in cryo-EM image reconstructions of the entire virions.

Hexon is the major protein component of the icosahedral adenovirus capsid. Its very long chain (ad2: 967aa; ad5: 951aa) forms two 8-stranded "viral" jelly-rolls at the subunit base that each have extensive projecting loops. In the trimer, these loops are highly convoluted and give rise to three tower domains visible by EM as "bumps" on the outer surface of the virus capsid. The recent 2.5 Å resolution crystal structure of adenovirus type 5 (ad5) hexon [3] has revealed the locations of the type specific hexon epitopes within the tower domains and will aid in the design of novel adenovirus-based vectors for gene therapy.

A structural alignment of the ad5 hexon model to a newly refined 2.3 Å resolution ad2 hexon model has been made and is being compared with optimized multiple-sequence alignments of all known hexon sequences to study the relationship of these hexons to those of other adenoviruses. Together, the alignments reveal the conserved structural elements of the protein. The sequences corresponding to these elements will be used to train a hidden-Markov model (HMM) which will be used to search for potential homologs. The ability of the HMM to locate remote homologs will be assessed by observing whether or not it can locate the PRD1 P3 protein which is known to have structural similarity with the adenovirus hexon. Supported by NIH (AI-17270).

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