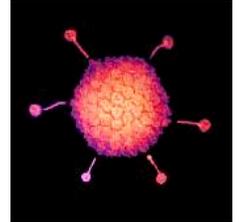
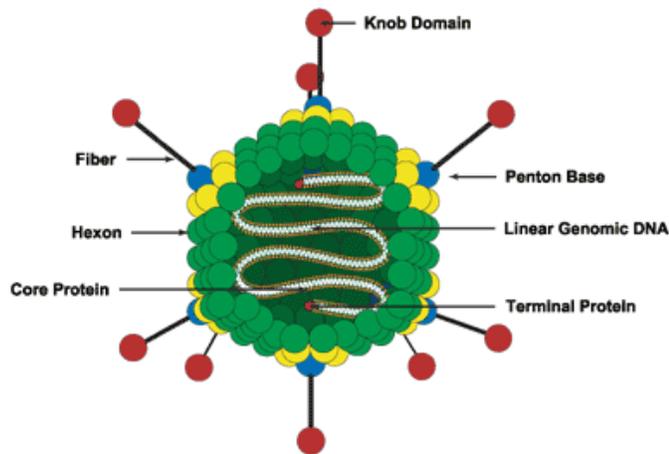


Adenovirus and Adenoviral Vectors

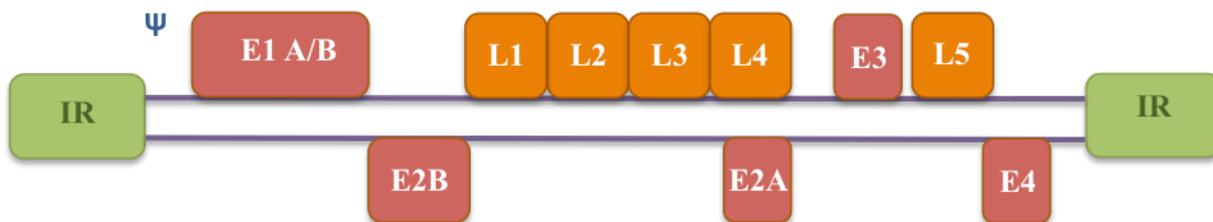


Family: *Adenoviridae*
Genus: *Mastadenovirus*
Naked with icosahedral capsid
57 adenoviral serotypes in humans
Size: ~ 70 - 90 nm in diameter
Genome: Linear, ~ 26 - 48 Kb dsDNA
Risk Group: 2

Adenovirus Capsid and Genome Structure and Life Cycle



The viral capsid contains 252 proteins mainly of 3 different types: fiber, penton base, and hexon. The fiber and penton base proteins are key in receptor binding and cell internalization, while the hexon proteins compose most of the viral capsid. The fibers or “spikes” are associated with each penton base and facilitate attachment to host cell receptors. The capsid harbors a linear, ~ 36 Kb dsDNA. The genome, which consists of early (E) and late (L) transcription units and a packaging sequence (ψ), is flanked by two inverted terminal repeats (IR) that act as origins of replication.

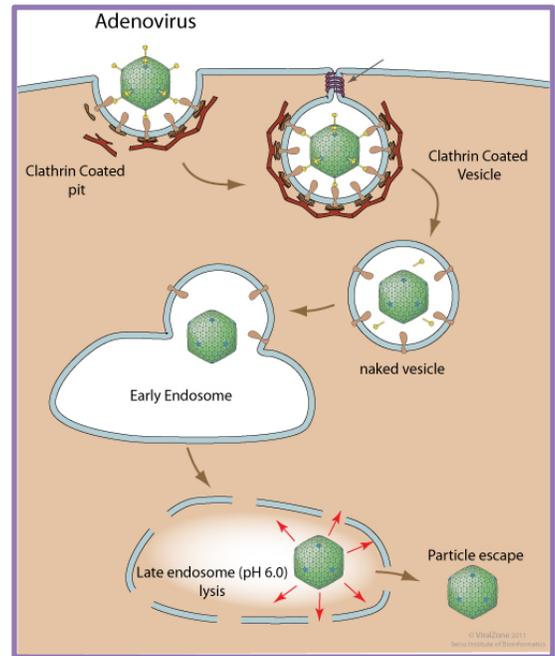


Entry of adenoviruses into the host cells involves two sequences of interactions between the virus and the host cell:

1. Binding of the knob domain of the fiber protein to the cell receptor initiates entry into the host cell. There are two types of receptors known:
 - ❖ CD46 for the group B human adenovirus serotypes
 - ❖ The coxsackievirus adenovirus receptor (CAR) for all other serotypes
2. A specialized motif in the penton base protein then interacts with an integrin molecule, α integrin, stimulating internalization of the adenovirus - binding to the integrin results in endocytosis of the virus via clathrin-coated pits, stimulating cell signaling and inducing actin polymerization, which leads to entry of the virion into the host cell within an

endosome. The endosome then acidifies, causing capsid proteins to disassociate. The virus disassembles in the endosome and the viral DNA is transported to the nucleus, resulting in transient expression of viral genes since adenoviral DNA is not integrated in the host genome (it remains in an episomal state).

The life cycle of adenovirus is separated by the DNA replication process in two distinct phases: the early and late phase. Early genes are responsible for the expression of regulatory proteins, while late phase genes code for structural proteins necessary to pack all the genetic material resulting from DNA replication. Once the viral components have been expressed, the virus is assembled into its capsid and released from the host cell as a result of virally induced cell lysis.



Recombinant Adenoviral Vectors

There are several different adenovirus serotypes, with human serotypes 2 and 5 from group C being the most frequently used to construct viral vectors. They belong to a group of adenoviruses involved in acute respiratory disease (ARD) and pharyngoconjunctival fever.

Adenoviruses used for the construction of adenoviral vectors can be either replication-competent or replication-deficient. The table below highlights some of the differences between both types of viruses when used as live viral vectors.

Replication-Competent Ad	Replication-Deficient Ad
High-level expression of recombinant proteins	High-level expression of recombinant proteins
LYTIC – NO long-term protein expression	Long-term protein expression
Expression of host viral proteins	NO Expression of host viral proteins

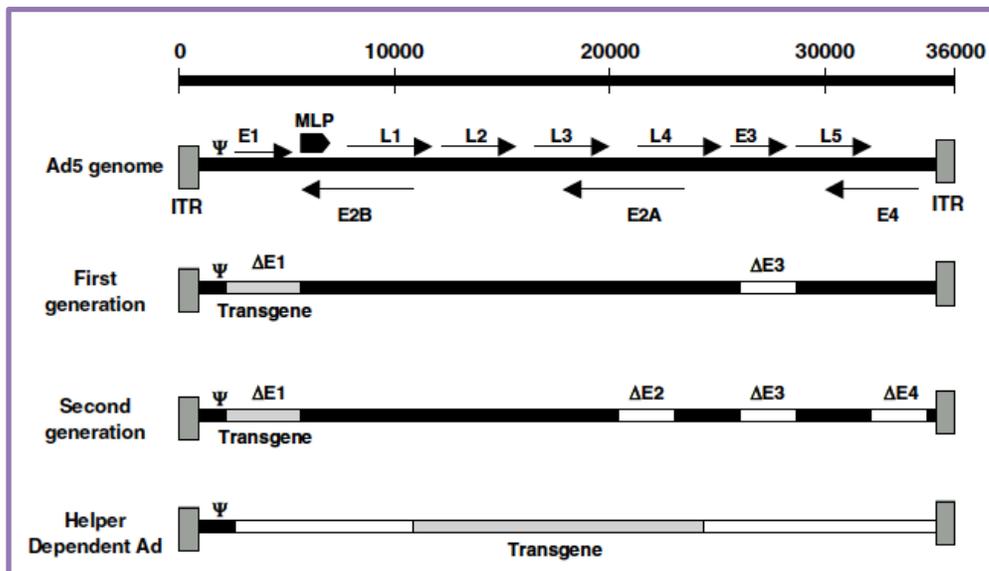
Different generations of adenoviral vectors have been constructed in order to improve their safety.

- *First-generation adenoviral vectors* contain the viral genome except for the E1 region. To propagate these vectors, an E1-expressing helper cell line must be used, such as HEK-293, 911, N52.E6 or PER.C6. Though E1-deleted vectors cannot replicate *in vivo*, residual expression from adenoviral genes triggers a cytotoxic T lymphocyte (CTL)

immune response against infected cells, which leads to the elimination of transduced cells and the loss of transgene expression.

- *Second-generation adenoviral vectors* are generated by combining deletions of different early regions - E1 + E3 and E2/E4 - which increases the vector cloning capacity up to ~ 14 Kb. However, second-generation Ad vectors are still immunogenic and toxic *in vivo*.
- *Third-generation or gutless adenoviral vectors* are devoid of all coding viral regions. They are also called *helper-dependent adenoviruses* because of the need of a helper virus that carries all coding regions, and *high-capacity adenoviruses* because they can accommodate up to 36 Kb of DNA. Gutless adenoviruses only keep the 5' and 3' inverted terminal repeats (ITRs) and the packaging signal (Ψ) from the wild-type adenovirus.

The figure below shows the genomic map of adenovirus serotype 5 wild-type virus and different generations of adenoviral vectors.



Adenovirus Infection in Humans and Routes of Exposure in the Lab



The adenovirus serotypes classically used in the construction of adenoviral vectors, Ad 2 and Ad 5, are usually associated with infection of the respiratory tract and conjunctivitis. Exposure can occur directly by oral contact, through contaminated fomites, and possibly the fecal-oral route. Care should be taken when conducting procedures that may lead to the generation of aerosols and droplets, such as when performing intranasal inoculation of adenoviral vectors in rodents. A biosafety cabinet should be used for work with wild-type adenovirus

and adenoviral vectors. The infectious dose has been estimated in > 150 p.f.u. (plaque forming units) when given intranasally, and the incubation period ranges from 1 to 10 days.

Cell Tropism of Adenovirus

Adenoviruses attach to host cells that express either the coxsackievirus adenovirus receptor (CAR) or CD46. The tropism of adenoviral vectors can be modified via pseudotyping.

Resources

- <http://researchcompliance.uc.edu/training/adenovirus/story.html>
- Alba R, Bosch A, and Chillon M. Gutless adenovirus: last-generation adenovirus for gene therapy. *Gene Therapy* 2005;12:S18-S27.
- Live Viral Vectors - Construction of Replication-Deficient Recombinant Adenovirus. From: *Methods in Molecular Medicine, Vol. 87: Vaccine Protocols, 2nd ed.* Editors: A Robinson, MJ Hudson, and MP Cranage. Humana Press Inc., Totowa, NJ.
- <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds3e-eng.php> -Pathogen Safety Data Sheet for Adenovirus Types 1, 2, 3, 4, 5 & 7