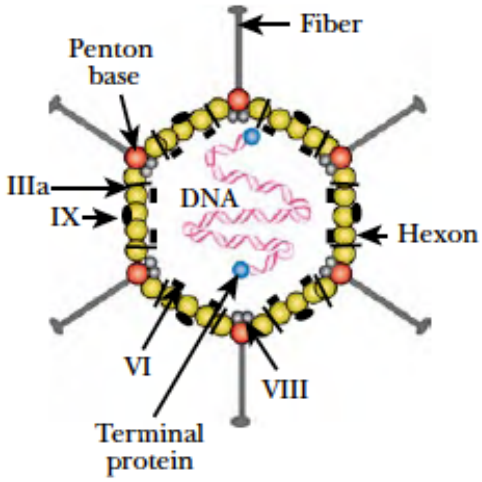
Animal virus

Adenovirus

Adenovirus are single linear Double stranded DNA human and vertebrate virus ,consisting of icosahedral capsid with 240 hexons.



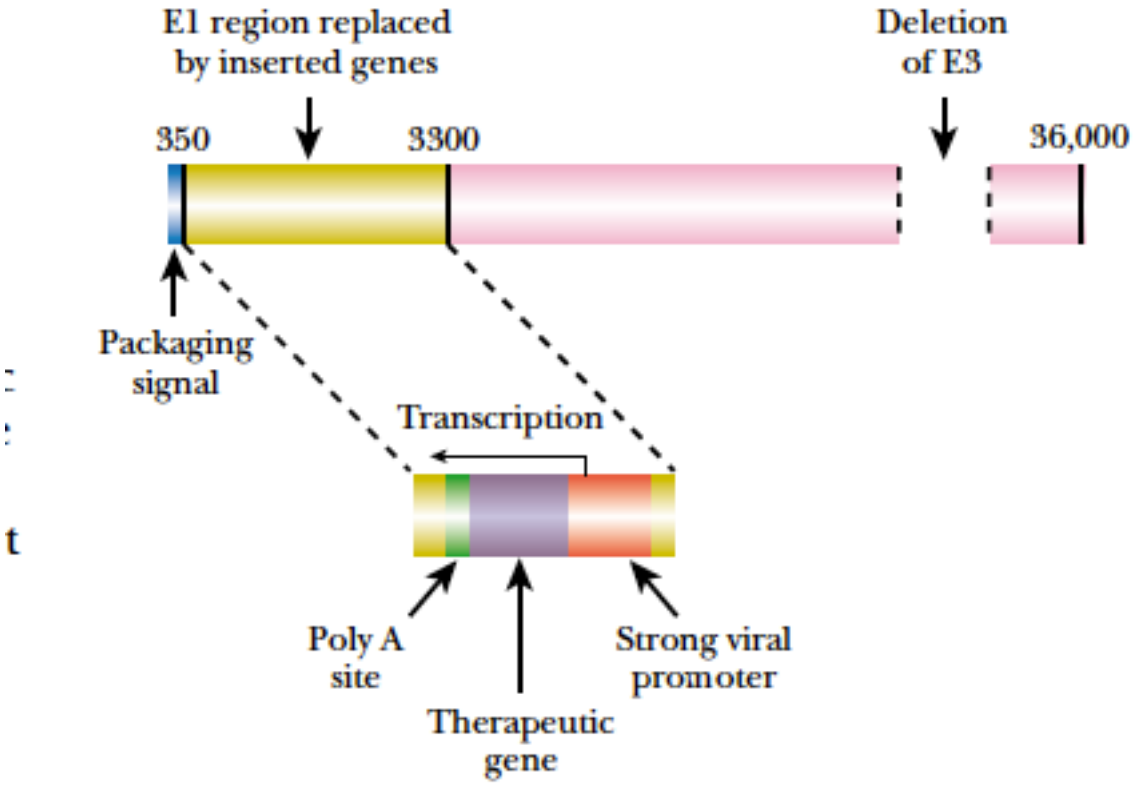
The hexon is trimer of hexon protein. The gene carried on adenovirus might be expressed efficiently in animal and humans but only for short duration because of host immune systems.The adenovirus enters human cells by recognizing and entering through CAR and Integrin receptors and inject iys DNA to nucleus through nuclear pore.

Adenovirus were choosen vectors for gene therapy becaus of thef ollowing reasons

1. They donot cause tumors
2. Easy to culture for propagation
3. Physiology is well understood
4. All Gene function are known
5. Genome sequence is known

Adeno virus vector is designed by disarming its deleting the early gene expressed after infection for E1A protein . E1A promotes transcription of other early virus genes and it binds to host cell Rb protein, which normally prevents the cell from entering S-phase. This prompts the host cell to express genes for DNA synthesis, which the virus utilizes for its own replication.

In the lab, crippled adenovirus is grown in genetically modified host cells that have the viral E1A gene integrated into host cell DNA. The virus particles generated by this approach cannot replicate in normal animal cells.



Genetically engineered adenovirus with natural **36,000 bp l**enth (wild type has approximately 36,000bp) for optimum pakaging . The therapeutic gene replaces usually E1 region but E3 may be replaced in case of larger insert.

In **cystic fibrosis,** Aerosols containing the engineered adenovirus with the cystic fibrosis gene have been sprayed into the noses and lungs, first of rats, and then of humans. In some instances the healthy cystic fibrosis gene was expressed and normal chloride ion movements were also restored.

Adeno-associated viruses (AAVs), members of the family *Parvoviridae* and the genus *Dependovirus*, are small, nonenveloped, single-stranded DNA viruses .AAV is a defective or "satellite" virus that depends on adenovirus (or some herpes viruses) to supply some necessary functions. It is usually found in cells that are infected with adenovirus. Unlike adenovirus, AAV seems to be entirely harmless.AAV genome is small (4681 nucleotides of single-strandedDNA) and the virus can carry only a relatively short segment of DNA. (AAV is unusual inpackaging both plus and minus strands into virus particles.Although each virus particlecontains only one ssDNA molecule, a virus preparation contains a mixture of particles, half with plus and half with minus strands.) On entering a host cell, the DNA is converted to the double-stranded replicative form.In the absence of helper virus, AAV integrates into the host chromosome and becomes dormant .

The permanantly integrated gene into host may be regulated by **double AAV system** as it has been done in monkey and rats for **erythropoietin protein required for RBC production. One AAV vector carries the gene for erythropoietin with a promoter that must be activated by a transcription factor. The second AAV vector carries an artificial regulatory system** . This

consists of two genes encoding hybrid proteins, each with one domain of the transcription factor. The other domain binds **rapamycin (used as an immunosuppressant).** In the presence of rapamycin, the two hybrid proteins associate via their **rapamycin binding domains** to form a functional transcription factor. This activates erythropoietin expression. After delivery of the two vectors to mice, there was no production of erythropoietin. But when the animals were injected with rapamycin, the transcription factor was assembled and the erythropoietin gene was activated. One of the major advantage of AAV is that it can be used again and again for treatment with out causing infection .