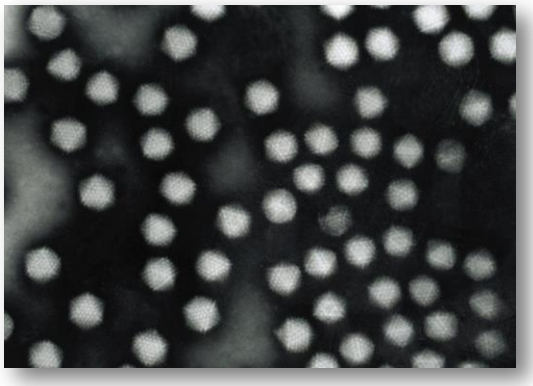
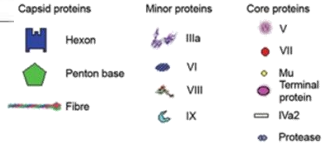
Vektor Adenovirus

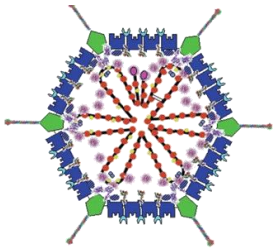


Adenovirus structure

TABLE 67-1 Human Adenovirus Types

Subgenus	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35
C	1, 2, 5, 6
D	8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-47
E	4
F	40, 41

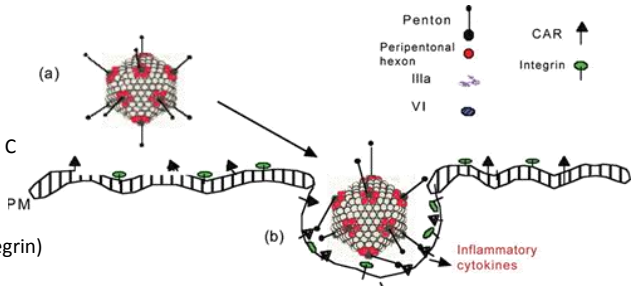


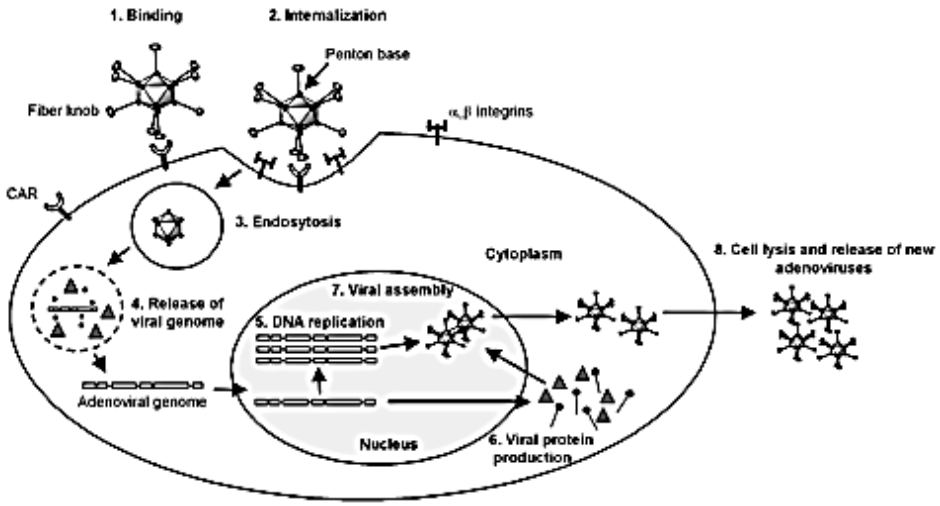


Russell W C J Gen Virol 2009;90:1-20

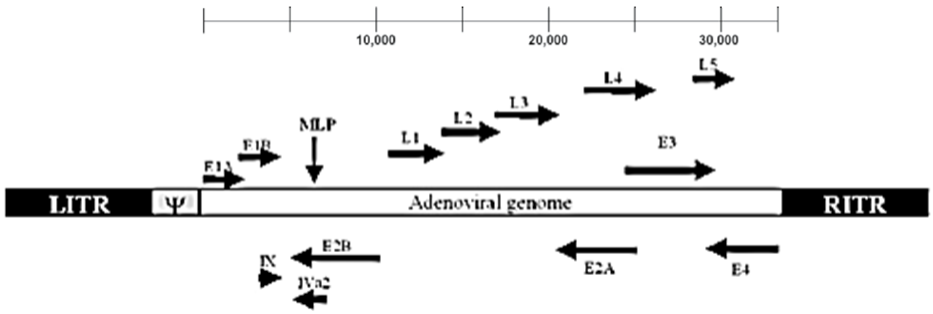
Adenovirus receptors

- Fiber
  - Spike of the virus
  - Binds coxsackie adenovirus receptor (CAR) in sub-group C
  - Binds CD46 in subgroup B
- Penton base
  - Co-receptor-binds RGD (integrin) for internalization of virion

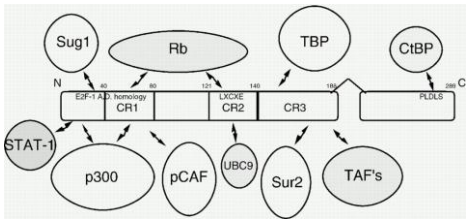




Adenoviral replication cycle. Viruses first attach to the coxsackie- adenovirus receptor (CAR) followed by interaction with cellular integrins resulting in internalization of the virus via receptor-mediated endocytosis. In the endosomes, the viral genome is released from the viral capsid and thereafter transported into the nucleus for DNA replication. Structural viral proteins assemble together with viral genomes in the nucleus followed by cell lysis and release of newly synthesized virion



Adenoviral genome. The genome contains early (E1-4), intermediate (IX and IVa2) and late (L1-5) genes flanked by left and right inverted terminal repeats (LITR and RITR, respectively) MLP: major late promoter, Y packaging signal.



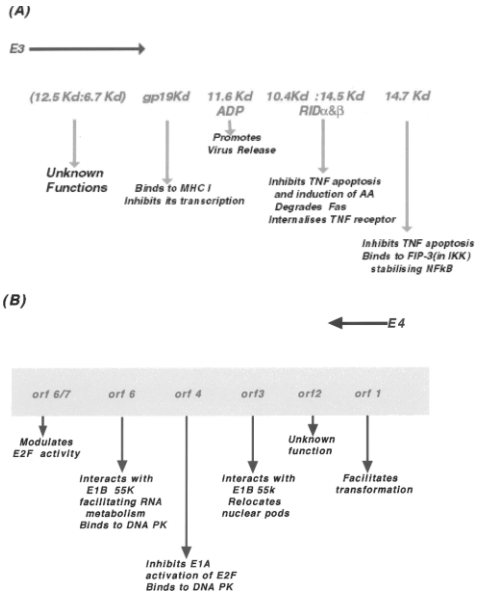
Binding map of proteins to E1A

E1, E2, E4

- Encode essential regulatory proteins involved in:
  - Transactivation of viral and cellular promoters
    - DNA replication
    - Cell cycle
    - Apoptosis
- Essential for replication in cell culture

E3

- Escaping host immune defenses
  - Preventing T cell cytotoxicity
  - Tumor necrosis factor (TNF) action
- Dispensable for replication in cell culture



Characteristics of E3 and E4 gene products. Horizontal arrows indicate the relative directions of transcription.

Russell W C J Gen Virol 2000;81:2573-2604

Transcription Units

Late genes

- Encodes proteins required for packaging the viral genome
- Essential for replication in cell culture

Table 2. Comparison of Common Gene Transfer Approaches

Features	Adenoviral	AAV	Retroviral	Lentiviral	HSV	Non-viral
Host range	broad	broad	restricted	broad*	restricted	broad
Transducing efficacy	very high	high/moderate	low	low	moderate	very low
Chromosomal integration	no	yes/no	yes	yes	no	no
Duration of expression	weeks-months	long-term	long-term	long-term	days	days
Construction procedure	easy	established	established	difficult	difficult	varied
Transgene size	5 - 36 kb	4 - 5 kb	4 - 5 kb	8 - 9 kb	large	unlimited
Vector yield (titer)	high ($>10^{11}$)	low ($<10^{9}$)	low ($<10^{7}$)	low ($<10^{6}$)	high (10^{10})	high
Host cell proliferation	not required	not required	required	not required	not required	not required
Regulatable expression	available	available	possible	possible	difficult	available
Immune responses	high/low**	low/rare	rare	rare	high	low

Notes *VSV-G pseudotyped HIV vectors
*reduced responses against gutless vectors

Breyer et al., 2001

Advantages of using Adenovirus for gene therapy

1. **Broad host range**
   - Can infect a broad range of mammalian cells
   - Allows for the expression of recombinant proteins in most mammalian cell lines and tissues
   - Have been used extensively to express human as well as non-human proteins

2. **Infection and expression of genes in replicative and non-replicative cells**
   - Retroviruses can only infect replicative cells
   - Transfection cannot be done in non-replicative cells
   - Best system to study gene expression in primary non-replicative cells
   - Allows for a direct comparison of results obtained with transformed cell lines and primary cells

Advantages of using Adenovirus for gene therapy

3. **Replicates efficiently to high titers**
- Production of 10^{10} to 10^{11} VP/mL
- Can be concentrated up to 10^{13} VP/mL

4. **Helper independent Ad can accommodate up to 7.5 kb of foreign DNA**
- Ad can normally encapsidate a viral DNA molecule slightly bigger than the normal DNA (105%)
- To provide additional cloning space, the E1 and E3 early regions of Ad have been deleted

Advantages of using Adenovirus for gene therapy

5. Homologous system for human genes
- Human virus as a vector and human cells as a host
- Proper folding and exact post-translational modifications of human proteins
- Most human proteins are expressed at high levels and are fully functional.

6. No insertional mutagenesis; remains epichromosomal
- Retroviruses integrate randomly into the host chromosome and can inactivate genes or activate oncogenes
- Ad remains epichromosomal and therefore does not interfere with other host genes.

Advantages of using Adenovirus for gene therapy

7. Propagation in suspension cultures

- 293 cells can be adapted to grow in suspension
- Allow production scale-up: > 20L

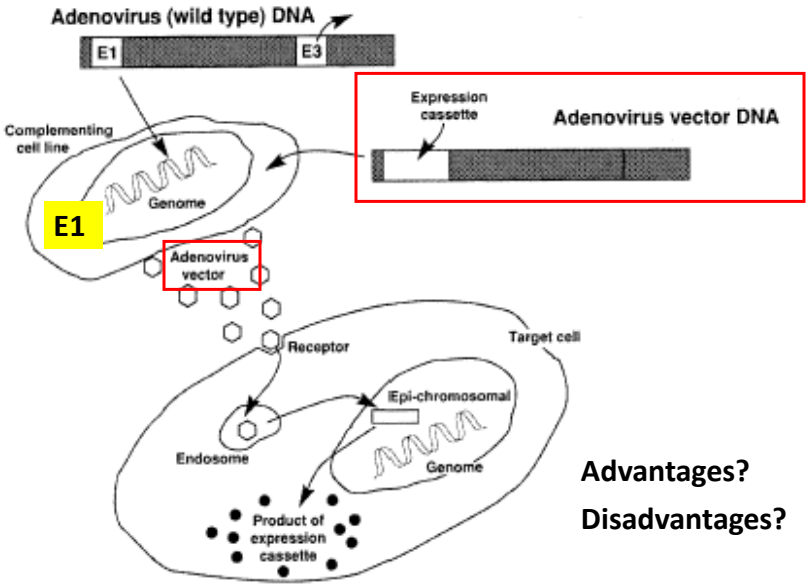
8. Simultaneous expression of multiple genes

- Insertion of two genes in a double expression cassette
- Co-infection using different recombinant viruses each expressing a different protein
- Modulation of the expression by changing the MOI

Construction of a Recombinant Adenovirus

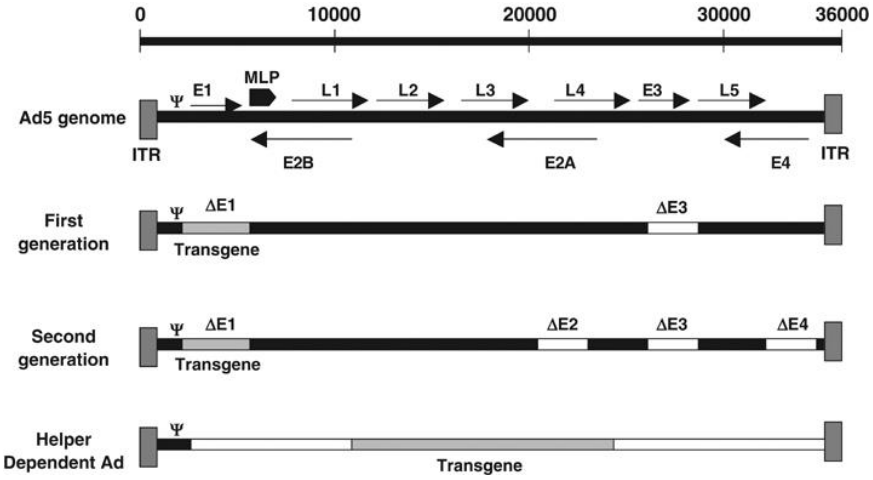
- Deletion of E1 and E3 regions
  - Renders the virus replication defective: E1 being essential, it is complemented in a specially designed packaging cell line (293)
  - Makes room for gene of interest
- Gene of interest is cloned into a transfer vector
- Gene of interest is transferred into the viral genome by homologous recombination

Generation of adenoviral vectors:



Advantages?

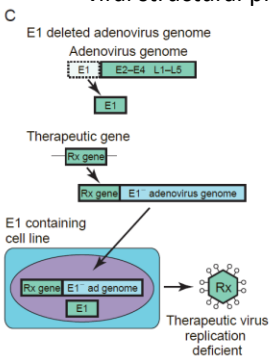
Disadvantages?



Map of adenovirus serotype 5 genome and different generations of adenoviral vectors. Early transcripts are represented by E1–E4 regions and late transcripts are represented by L1–L5 regions. MLP: major late promoter; : packaging signal.

Adenovirus vector

- **First generation,**
  - deletions in E1 ➔ insertion of expression cassettes of up to approximately 5 kb.
  - The limitation of first generation Ads was the discovery of leaky expression of viral genes ➔ transient transgene expression.
  - Transduced cells release cytokines and are recognized and destroyed by cytotoxic CD8+ and memory CD4+ T lymphocytes (CTL) via recognition of viral structural proteins presented on the cell surface by MHC class I.



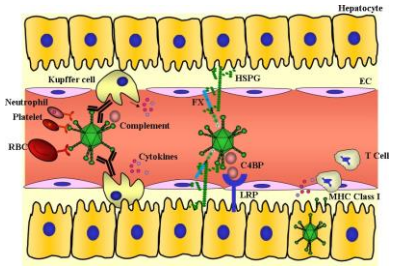
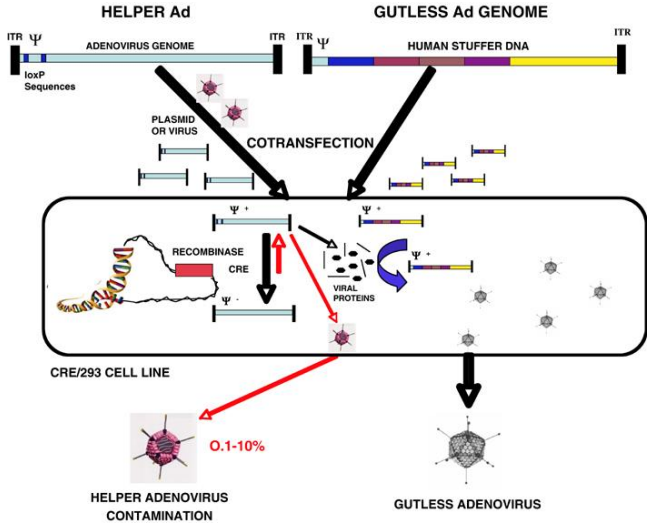


Fig. 2.3 Interactions of Ad with the host *in vivo*. Once in the bloodstream Ad5 is bound and/or phagocytosed by Kupffer cells, red blood cells (RBC), Platelets and neutrophils. Kupffer cell uptake is enhanced by antibody opsonization or complement proteins and leads to cytokine production. Ad complexes with FX or C4BP to transduce hepatocytes via HSPGs or LRP, respectively. Transduced hepatocytes present viral proteins via MHC and further release of cytokines leads to T cell mediated lysis.

Adenovirus vector

- Second generation
  - deletion of the E2 and/ or E4 regions potentially enabling cloning of 14 kb of foreign DNA into the vector.
  - The initial study mutated E2a leading to increased transgene expression and decreased inflammatory responses in mouse liver and lung. E4 has also been deleted to provide similar advantages
- Third generation /gutless or helper dependent Ad vectors (hdAd)]
  - devoid of all viral open reading frames,
  - vectors containing only essential *cis* elements (inverted terminal repeats and contiguous packaging sequences).
  - Deletion of all viral genes increases insert capacity to 36–38 kb and limits host immune responses by ablating leaky production of Ad genes, leading to persistent transgene expression
  - Scaling up problem



Generation of gutless adenovirus using the Cre/loxP system. Gutless and helper genomes are cotransfected in permissive 293 Cre-expressing cells, where both genomes are amplified and viral proteins produced. Then, packaging signal of the helper's genome is excised by Cre recombinase, preventing its packaging into the viral capsid, while gutless genome is still packageable. Efficiency of the excision process allows 90–99.9% purity of the gutless vector.

R Alba, A Bosch and M Chillon, 2005

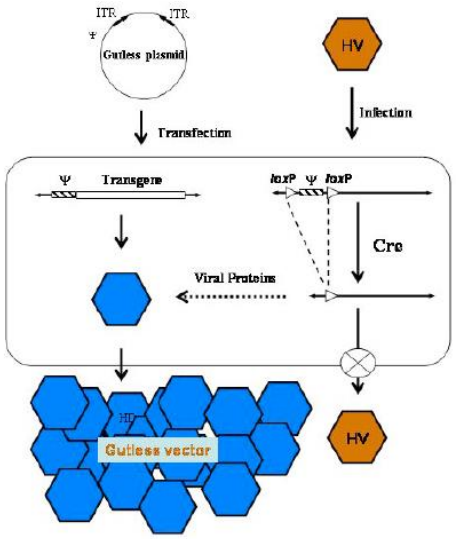


Figure 2. The gutless adenoviral vector system is dependent on Cre/*loxP* helper adenovirus. Only the inverted terminal repeats (ITR) and the viral DNA packaging signal (Ψ) regions of adenoviral DNA sequences are remained in the gutless vectors. A plasmid containing the entire gutless vector structure can be generated *in vitro*. The gutless DNA then is released from the circular plasmid with a restriction enzyme and transfected into cells that complement for E1 and provide expression of the Cre recombinase. When the helper virus is introduced into the Cre positive cells, the excision of the packaging signal through Cre/*loxP* in the helper virus renders its DNA unable to be packaged. However, the helper DNA, without its packaging signal region, still can replicate and provide transcripts for all of the necessary proteins for propagation of the gutless vector. The helper viruses (HV) are decreased, but cannot be completely eliminated from the gutless vector production.

Targeted Adenoviral Vectors

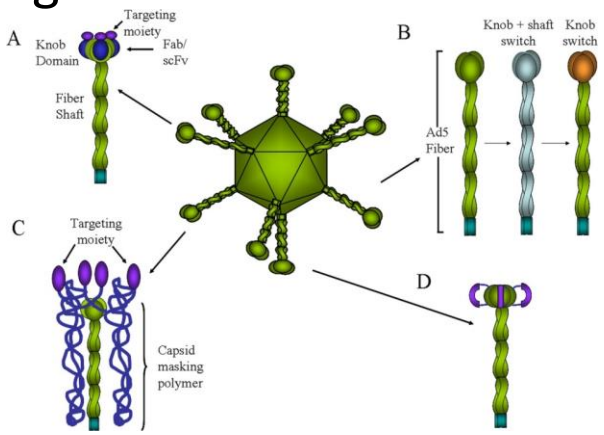


Fig. 2.2 Ad targeting. (A) Bispecific antibodies neutralize the Ad knob domain and retarget to novel/candidate receptors. (B) Serotype switching by exchanging either the Ad5 fiber knob or entire fiber for an alternative serotype fiber for new tropism. (C) Coating the Ad capsid with inert polymers covalently linked to targeting ligands. (D) Insertion of peptides into the surface exposed HI loop on the Ad fiber knob.

Gene Therapy Applications

- Cancer therapy : i.e. Ad expressing p53 (Gendicine)
- Cardiovascular disease
- vaccine for human immunodeficiency virus
- treatment of inherited disorders

Immune Responses to Ad Vectors

- Ads activate Response immune innate and adaptive
- The Ad capsid activates
  - the innate system independently of any viral gene expression
    - activation of
      - mitogen activated protein kinase (MAPK),
      - Jun N-terminal kinase (JNK) and
      - extracellular regulated kinase (ERK) pathways, resulting in nuclear translocation of nuclear factor kappa B (NFκB) and transcription of its target genes, including a number of cytokines.
    - i.v. Ad delivery,
      - thrombocytopenia, cytokine production, inflammation and a rise in liver enzymes.
  - Adaptive immune response

Safety and Regulatory Issues

- attenuated Ad as a vaccine in the American military without any reported side effects.
- Wild-type Ads → infect the upper respiratory tract, gastro-intestinal, or ocular epithelium.
- Jesse Gelsinger (18 year old participant in a phase I trial for ornithine transcarbamylase deficiency (a X-linked deficiency in urea synthesis)) → administered 3.8×10^{13} viral particles into the hepatic artery and died following systemic inflammatory syndrome, disseminated intravascular coagulation and multiple organ failure.
- none of the pre- clinical data in rodent models or small and large non-human primate studies revealed similar side effects