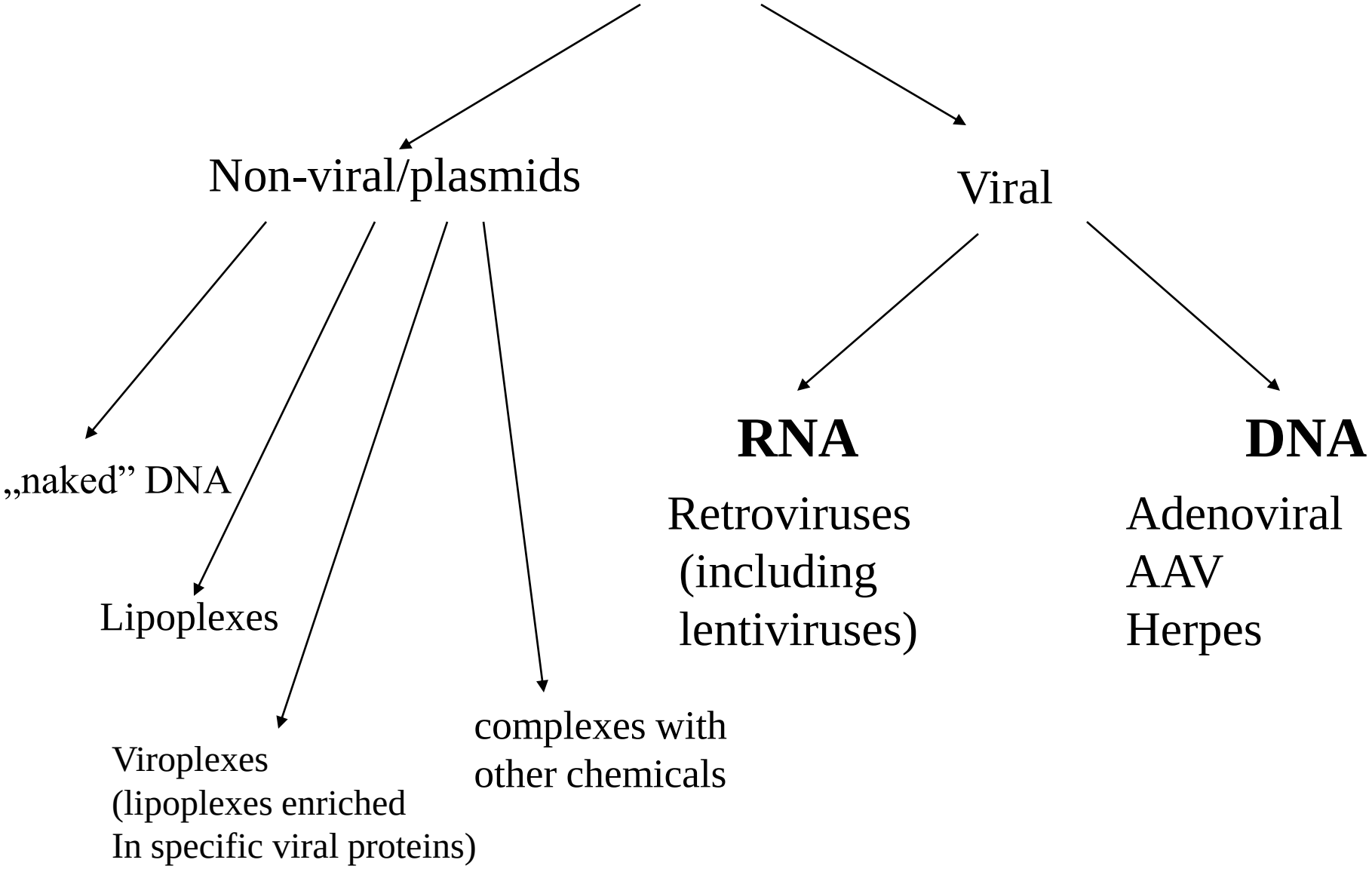
Lecture V

Viral vectors, cont.

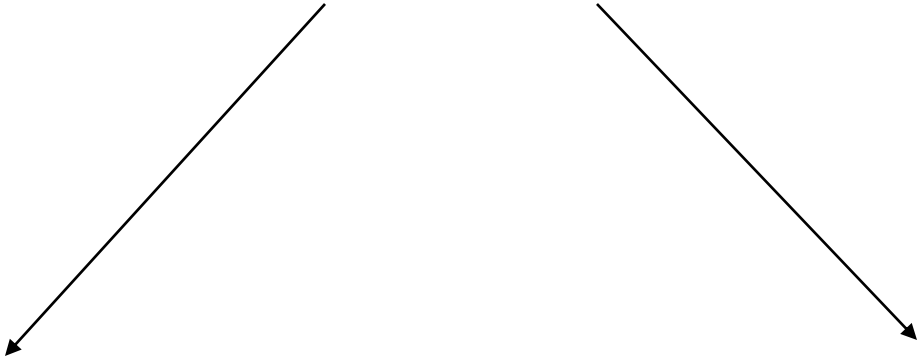
14 November 2011

Adenoviral vectors

Vectors


Non-viral/plasmids
Viral
„naked” DNA
Lipoplexes
Viroplexes
(lipoplexes enriched
In specific viral proteins)
complexes with
other chemicals
RNA
Retroviruses
(including
lentiviruses)
DNA
Adenoviral
AAV
Herpes

Viral vectors



Integrating

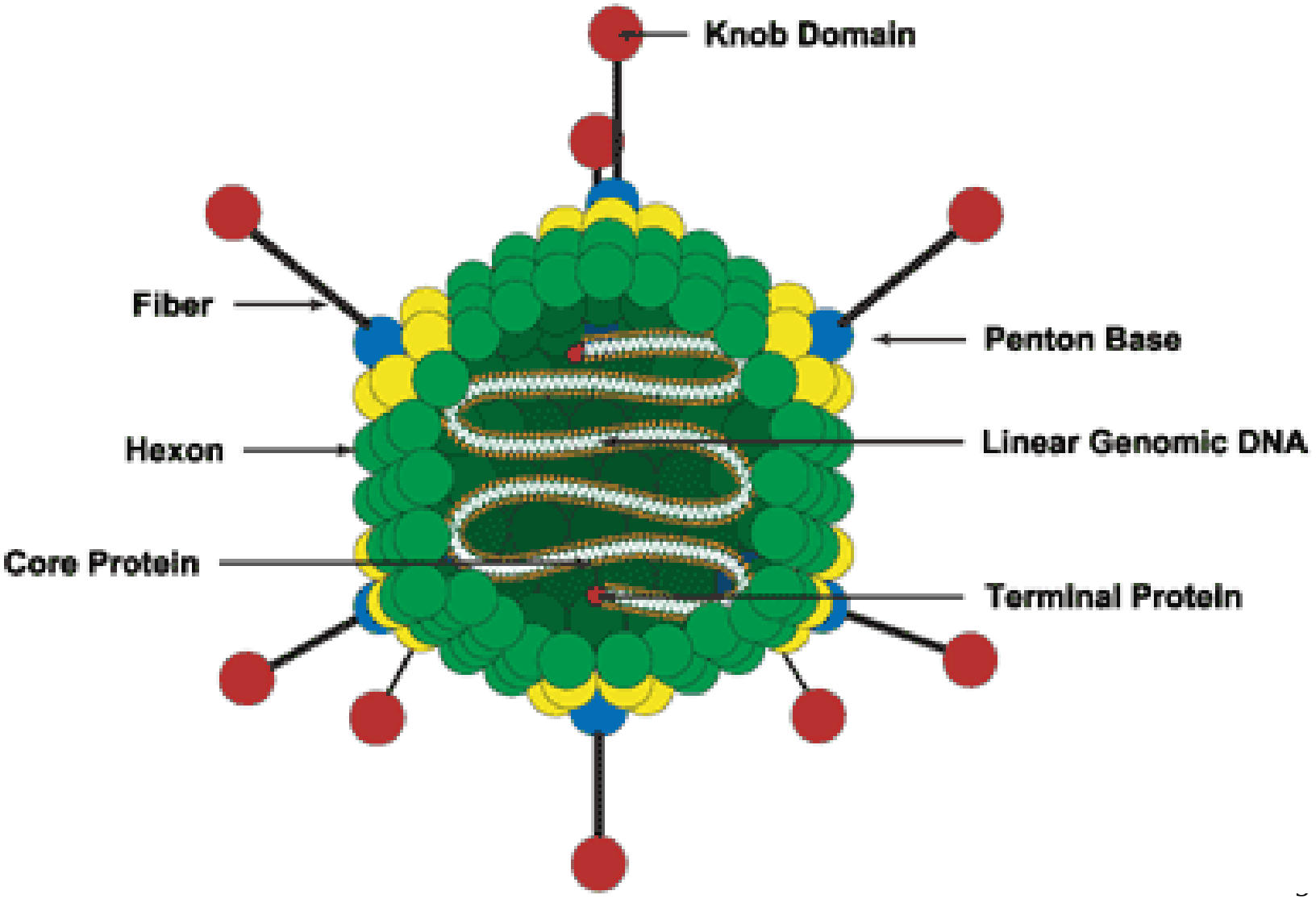
Lentiviral
-retroviral
-AAV (limited)

Non-integrating

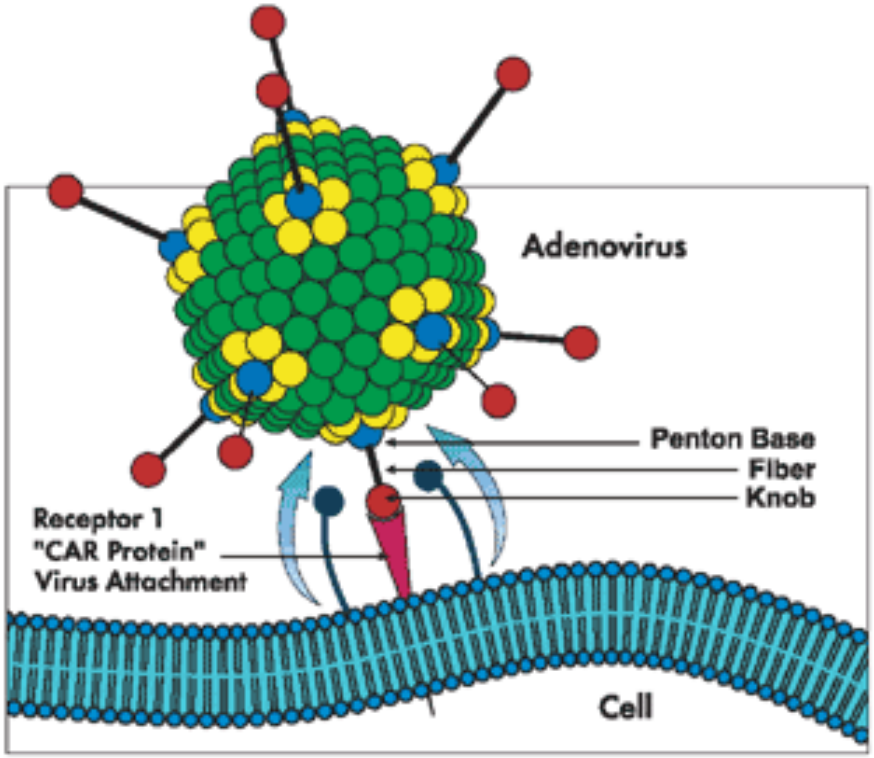
Adenoviral
HSV
Baculoviral

Integration depends on:
-LTR sequences and integrase (retroviruses)
- ITR seqeuences and rep proteins (AAV)

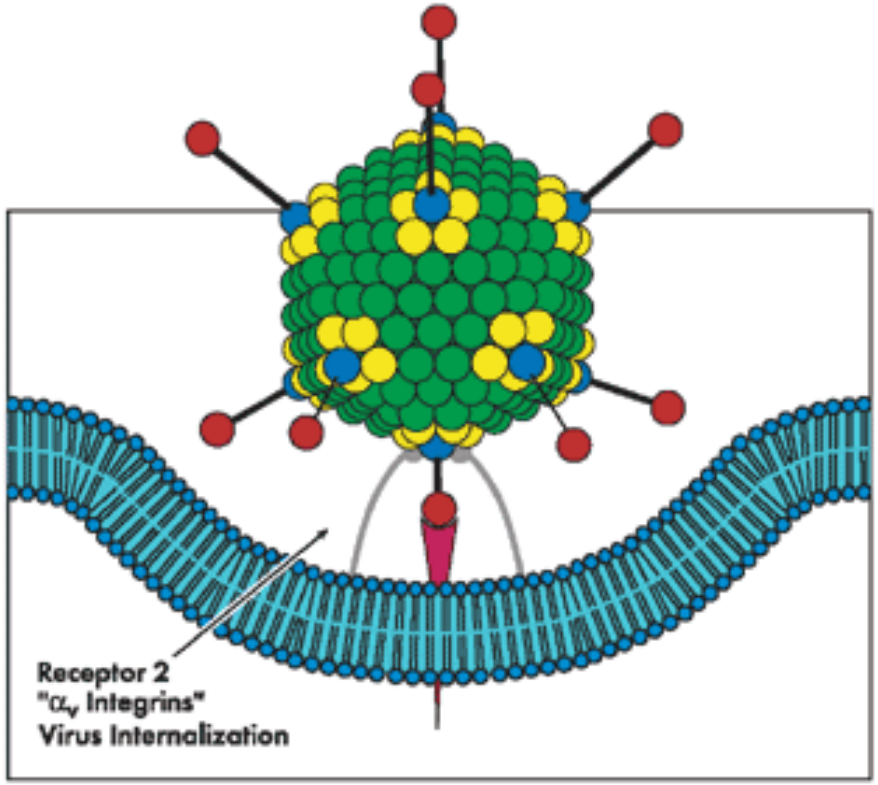
Adenoviruses



Binding and internalization of adenovirus



1) Attachment to cell surface receptor.



2) Receptor-mediated endocytosis

In vivo infection with adenoviruses

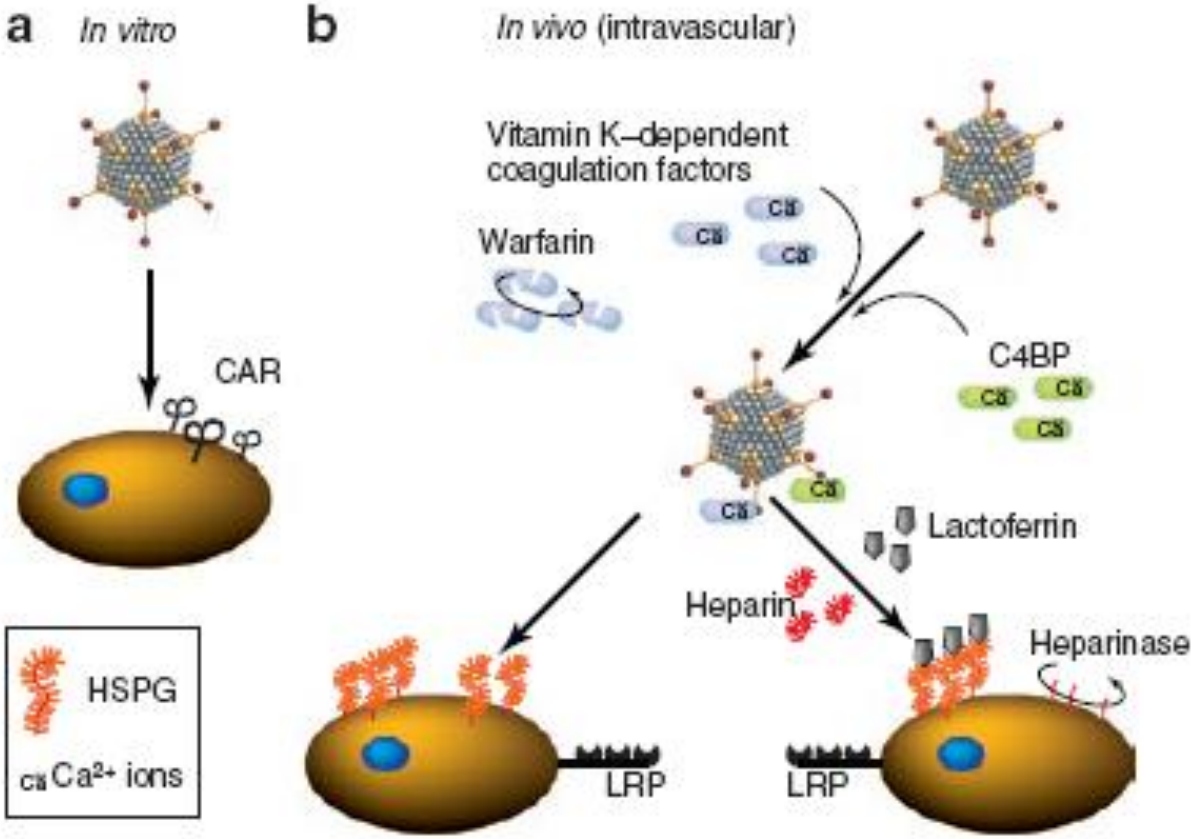


Figure 1 Pathways mediating adenovirus (Ad) infection of cells *in vitro* and liver cells *in vivo* following intravascular delivery. Following intravascular injection, plasma proteins bind Ad5; this leads to liver transduction through heparan sulfate proteoglycans (HSPG) and/or lipoprotein-related protein (LRP) binding, effects that can be blocked by pathway inhibitors including warfarin, heparin, heparinase, and lactoferrin.[31,35]

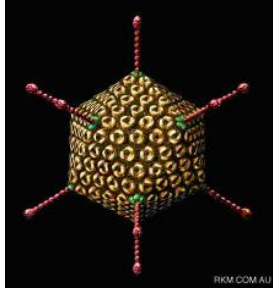
Waddington & Baker, 2008

Adenoviral serotypes and disorders caused by them

Table 1 Representative Serotypes and Pathology of Different Subgroups of Adenoviruses

	Subgroup					
	A	B	C	D	E	F
Representative serotypes	12,31	3,7	2,5	9,17,30	4	4,041
Cryptic enteritis	X					
Acute respiratory infections		X			X	
Hemorrhagic cystitis		X				
Pharyngitis		X	X			
Pneumonia		X	X			
Keratoconjunctivitis				X		
Diarrhea						X

Adenoviruses



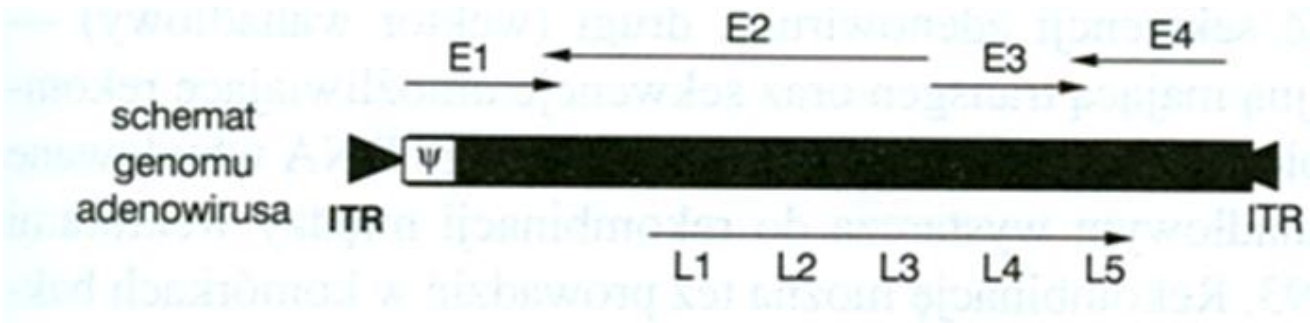
More than 50 serotypes – type 2 & 5 are used

Genom: 36 kbp, more than 50 proteins

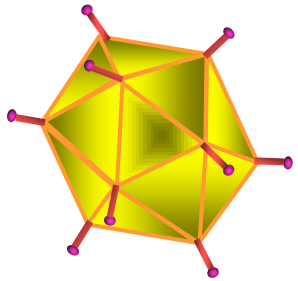
E1 region– contains genes regulating the expression of genes necessary for viral replication

E2 i E4 regions – together with E1 are required for viral replication

E3 region– is not required for replication, modulates response of cell to infections



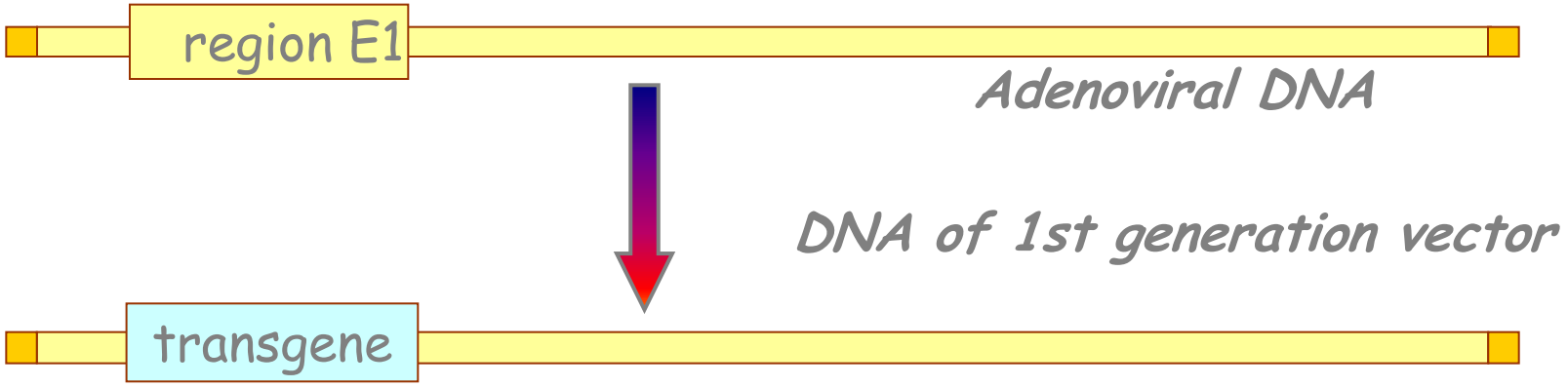
Adenoviruses and adenoviral vectors



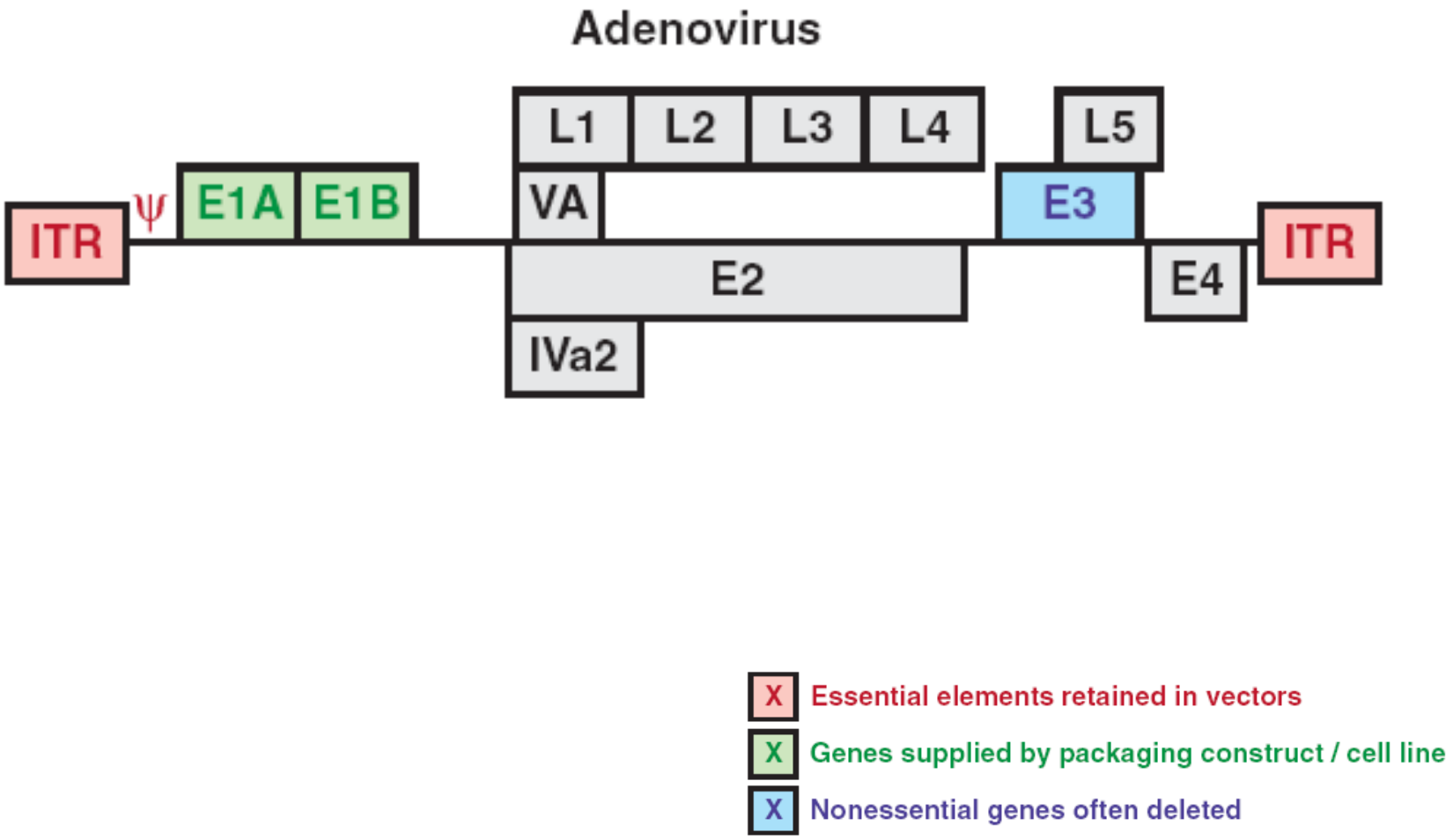
- ~50 serotypes of adenoviruses (for gene therapy type 2 and 5 were used),
  causing usually mild illness in humans

- Genome consists a 36 kb double-stranded linear DNA with ITR sequences at each end, with:
  Early genes (responsible for viral gene transcription, DNA replication, host immune suppression and host cell apoptosis
  Late genes (coding proteins required for virus assembly)

- E1 early gene is essential for the subsequent adenoviral gene expression

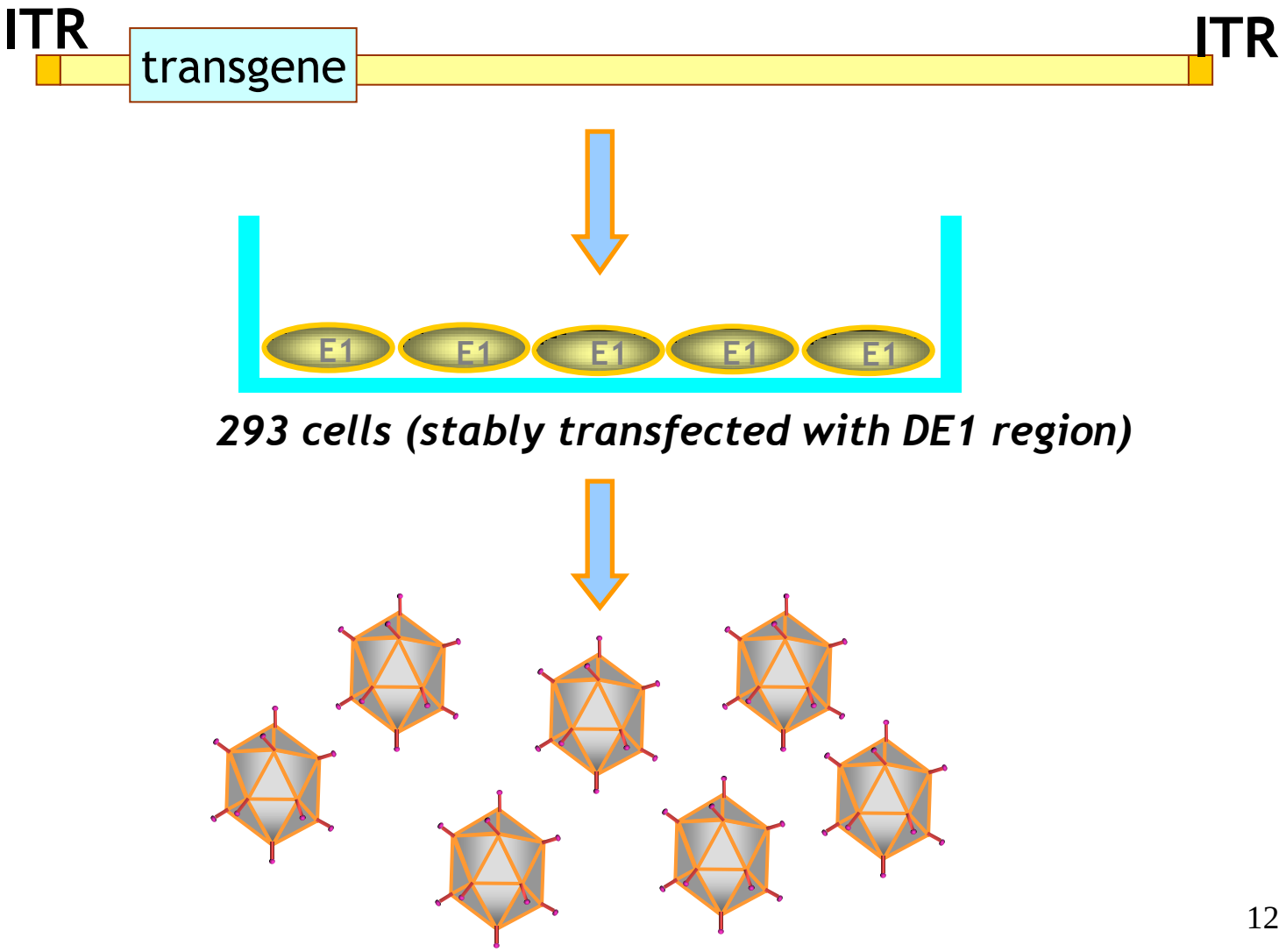


Essential and non-essential elements in different viral vectors

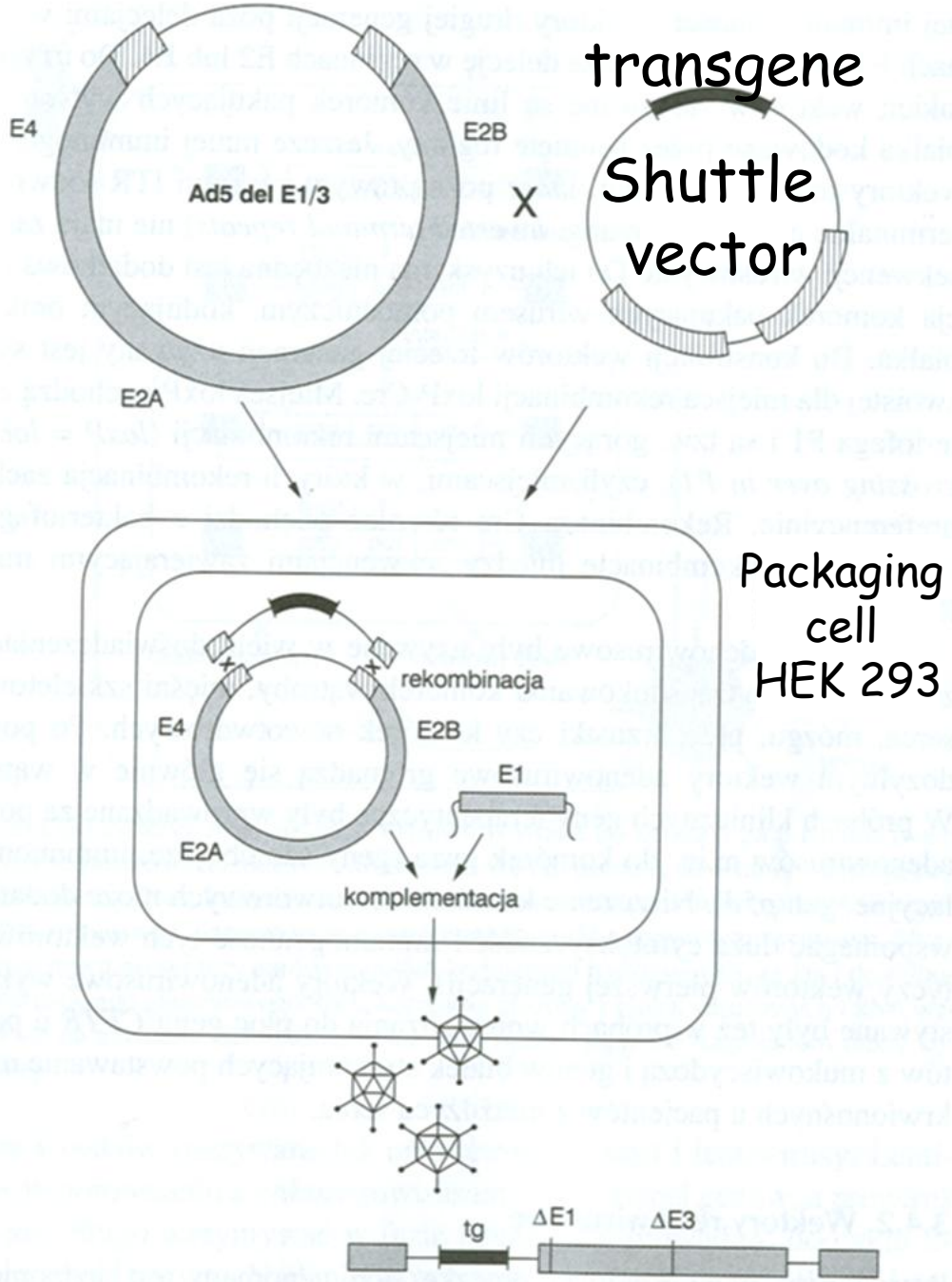


Verma & Weitzman, Ann Rev Biochem 2005

Production of DE1 adenoviral vectors



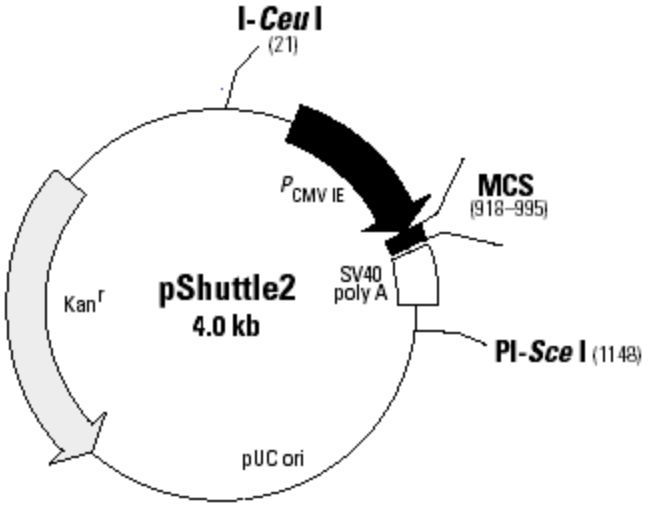
293 cells (stably transfected with DE1 region)

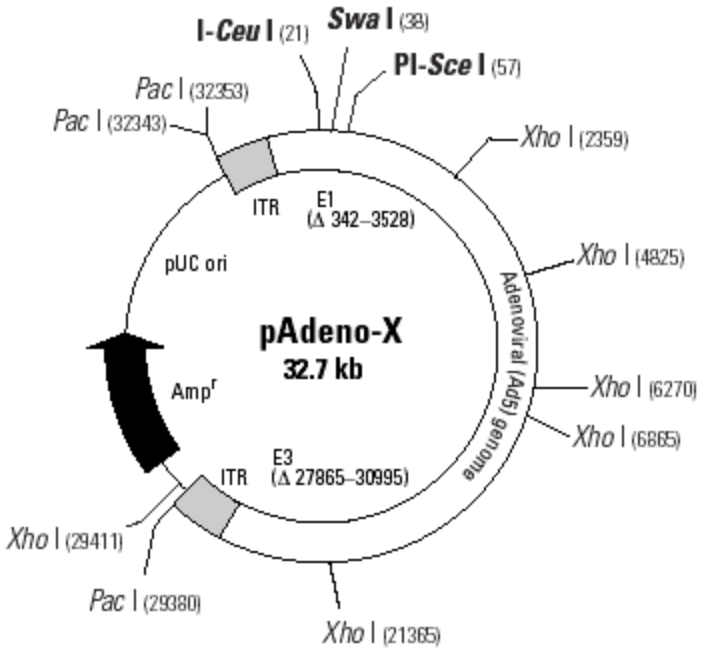


Construction of adenoviral vectors of 1st generation by homologous recombination

Homologic recombination in HEK 293 packaging cells

Production of adenoviral vectors without homologous recombination (1)





I-*Ceu* I Recognition Sequence

5'TAACTATAACGGTCCTAAGGTAGCGA3'
3'ATTGATATTGCCAGGATTCCATCGCT5'

PI-*Sce* I Recognition Sequence

5'ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGGCA3'
3'TAGATACAGCCCACGCCTCTTTCTCCATTACTTTACCGT5'

Pac I Recognition Sequence

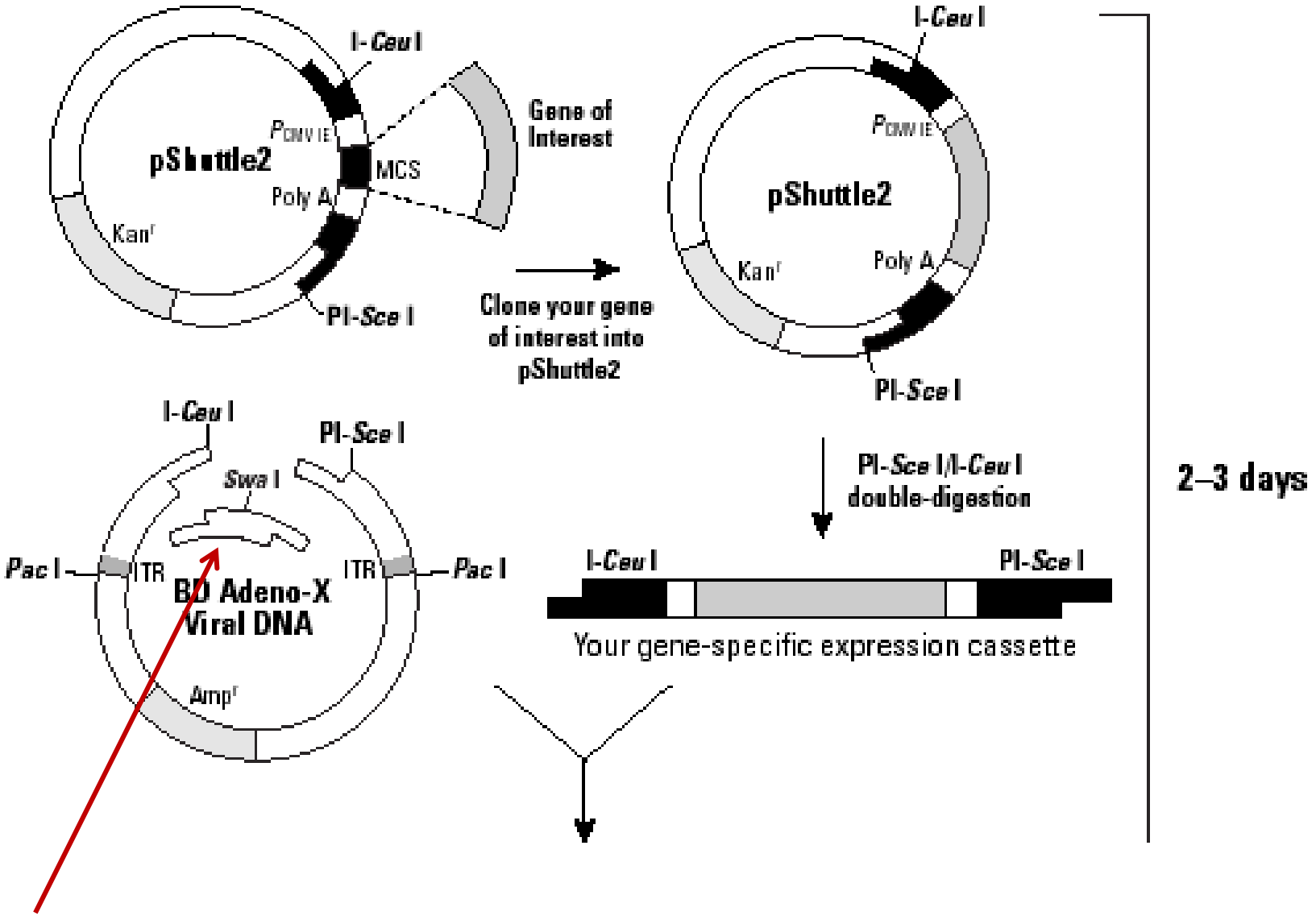
5'TTAATTAA3'
3'AATTAATT5'

Swa I Recognition Sequence

5'ATTTAAAT3'
3'TAAATTTA5'

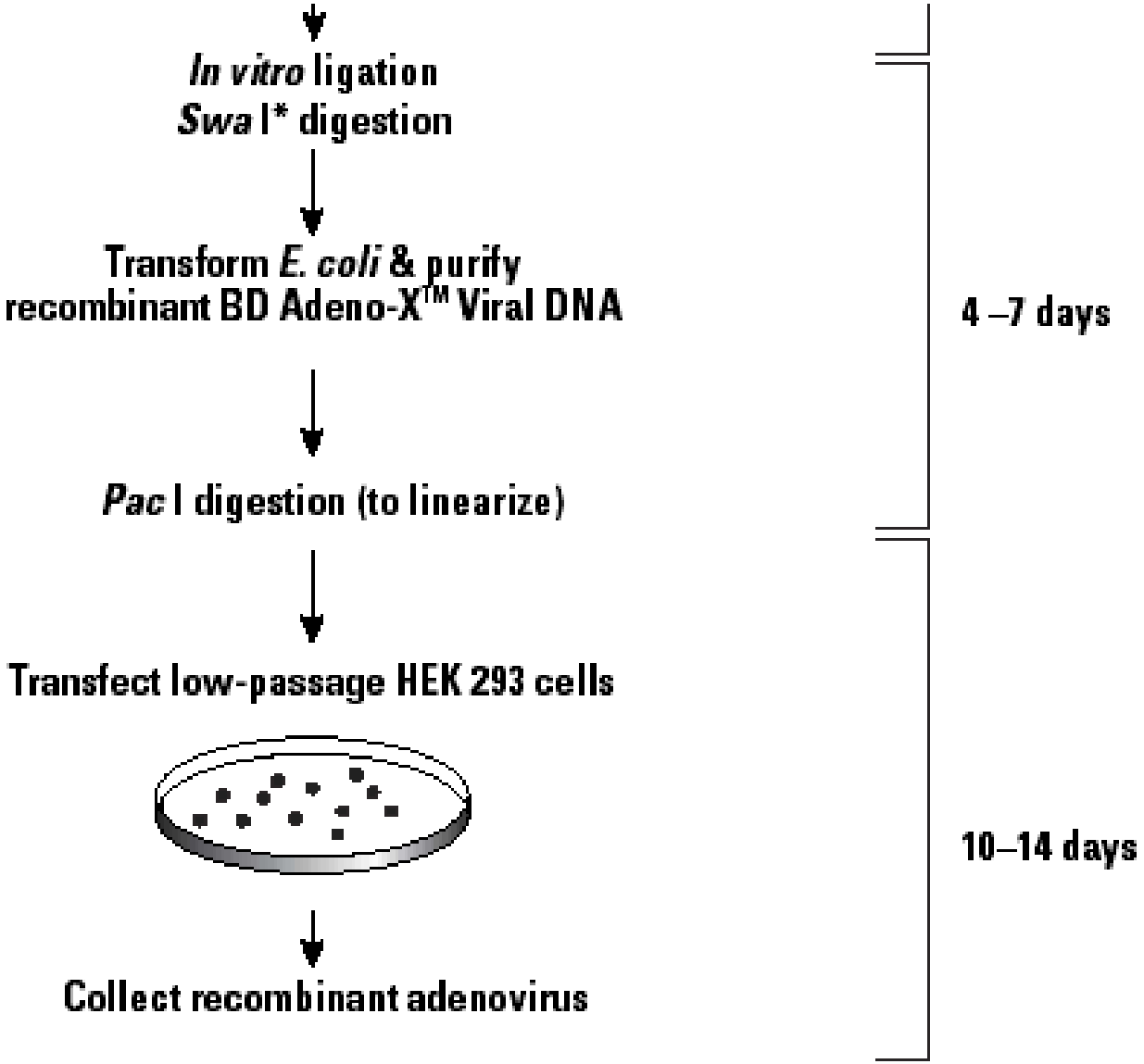
E1 genes are deleted from adenoviral genome
HEK 293 cells provide in trans the required E1 genes

Production of adenoviral vectors without homologous recombination (2)

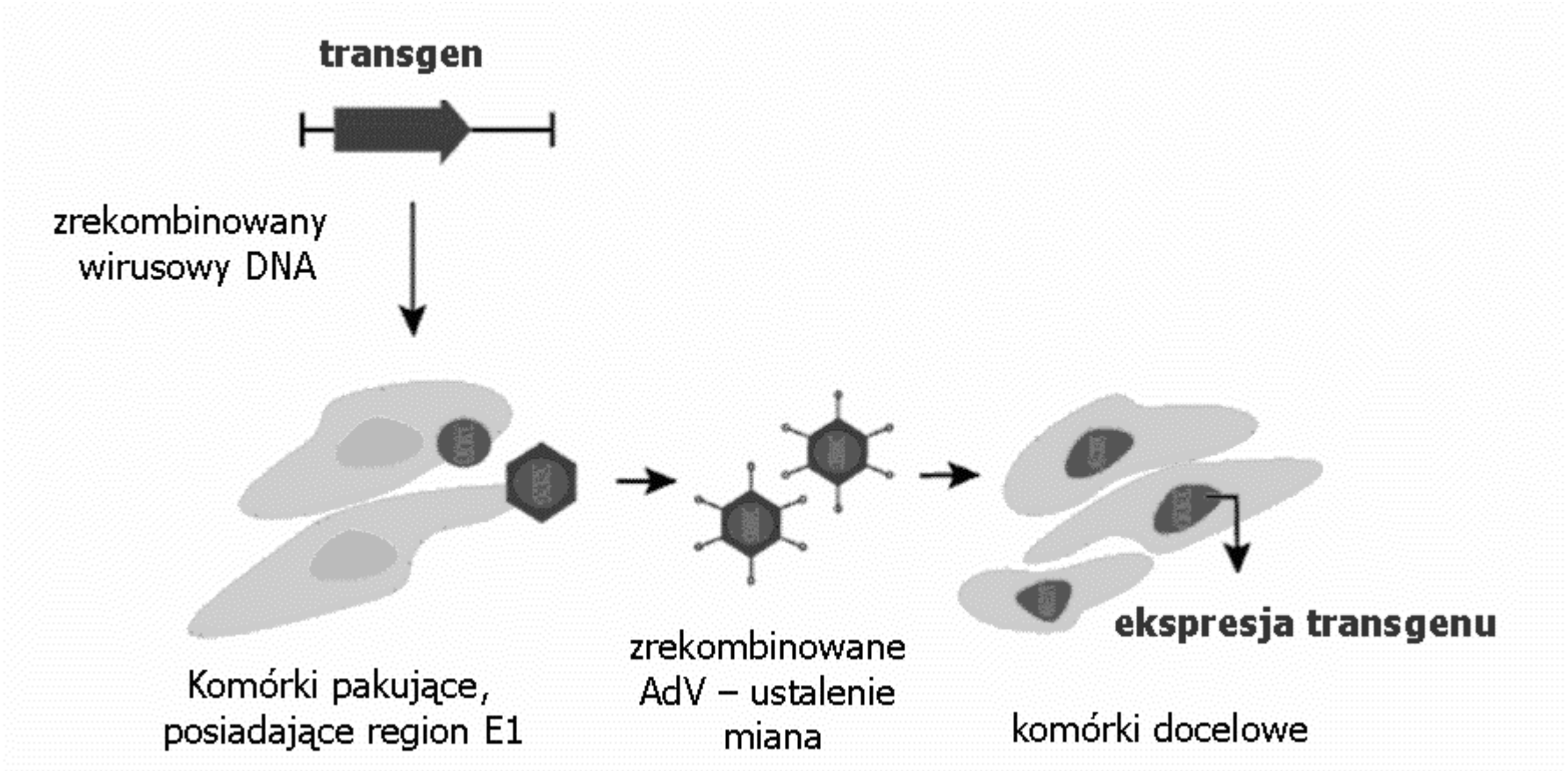


Swa I digestion – to reduce the frequency of non-recombinant clones

Production of adenoviral vectors without homologous recombination (3)

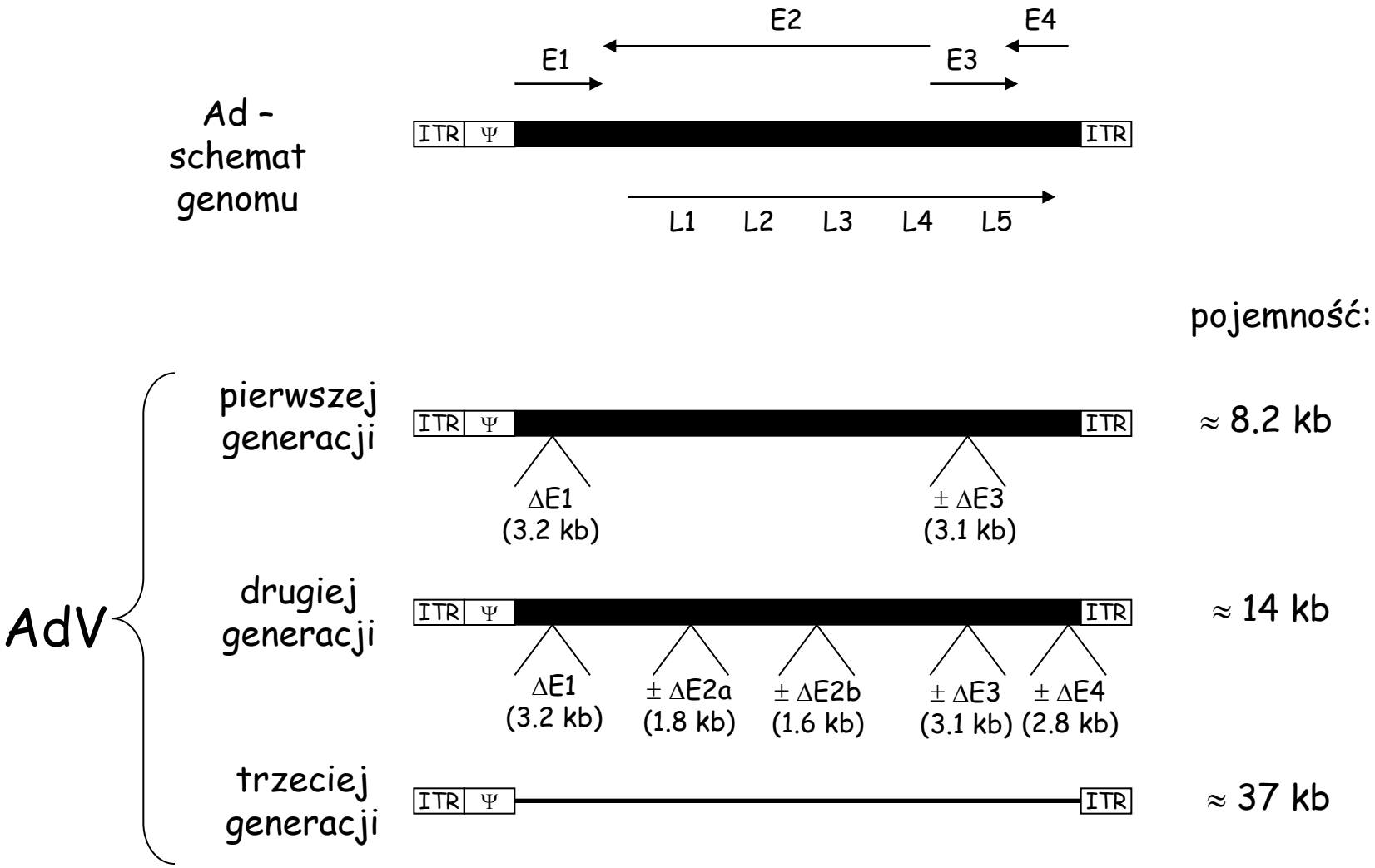


Scheme of production and application of adenoviral vectors



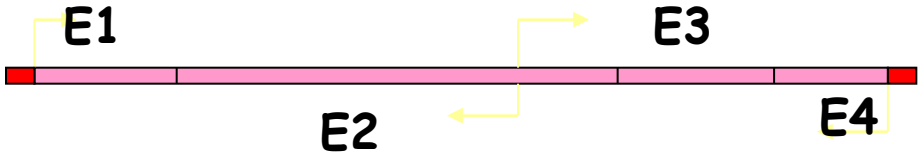
Stopa et al., Biotechnologia 2007

Generations of adenoviral vectors



Stopa et al., Biotechnologia 2007

Adenoviral vectors



Vector	Deletions	Production	Capacity	Features
ΔE1	E1 (and E3)	E1 complementing cells	7.5 kb	Viral protein neosynthesis, viral replication despite lack of E1
ΔE1E4	E1,E4 (and E3)	E1 and E4 complementing cells	10 kb	Reduced viral protein neosynthesis,
ΔE1E2	E1,E2A or E2B (and E3)	E1 and E2 complementing cells	9 kb	Block of viral replication sever inhibition of viral protein synthesis

Benihoud K. et al. 1999. Current Opinion in Biotechnology 10: 440.

Features of adenoviral vectors

Causes benign infection

Safety—lack of association with oncogenicity

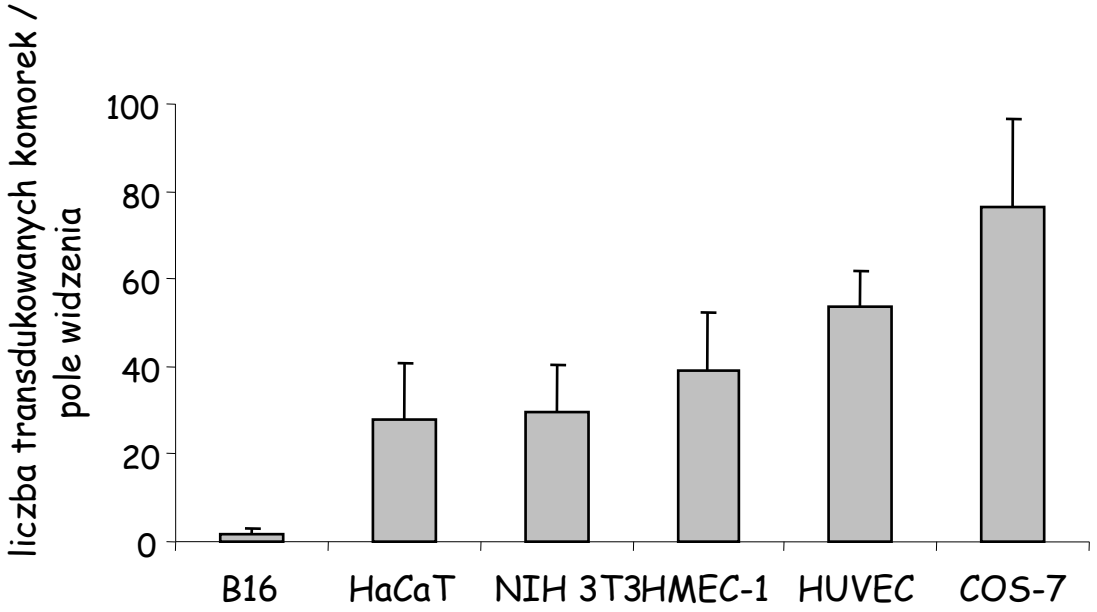
Well characterized and easily manipulated

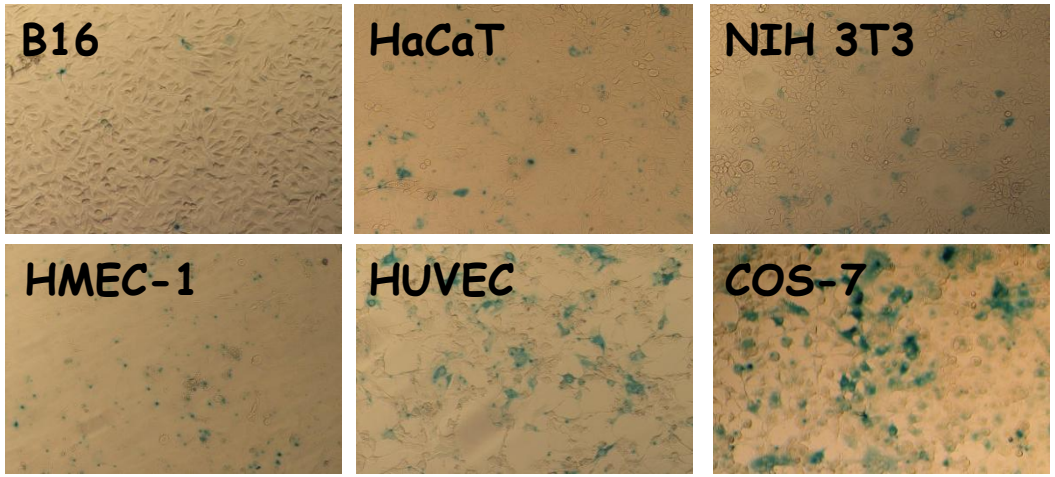
Stability and high titers of recombinant vectors

Ability to infect a broad range of cell types, including dividing and nondividing cells
High efficiency of cellular uptake of insert capacity (up to 37 kb)

Little risk of random chromosomal integration

Various cells are transduced with different efficacy with Ad





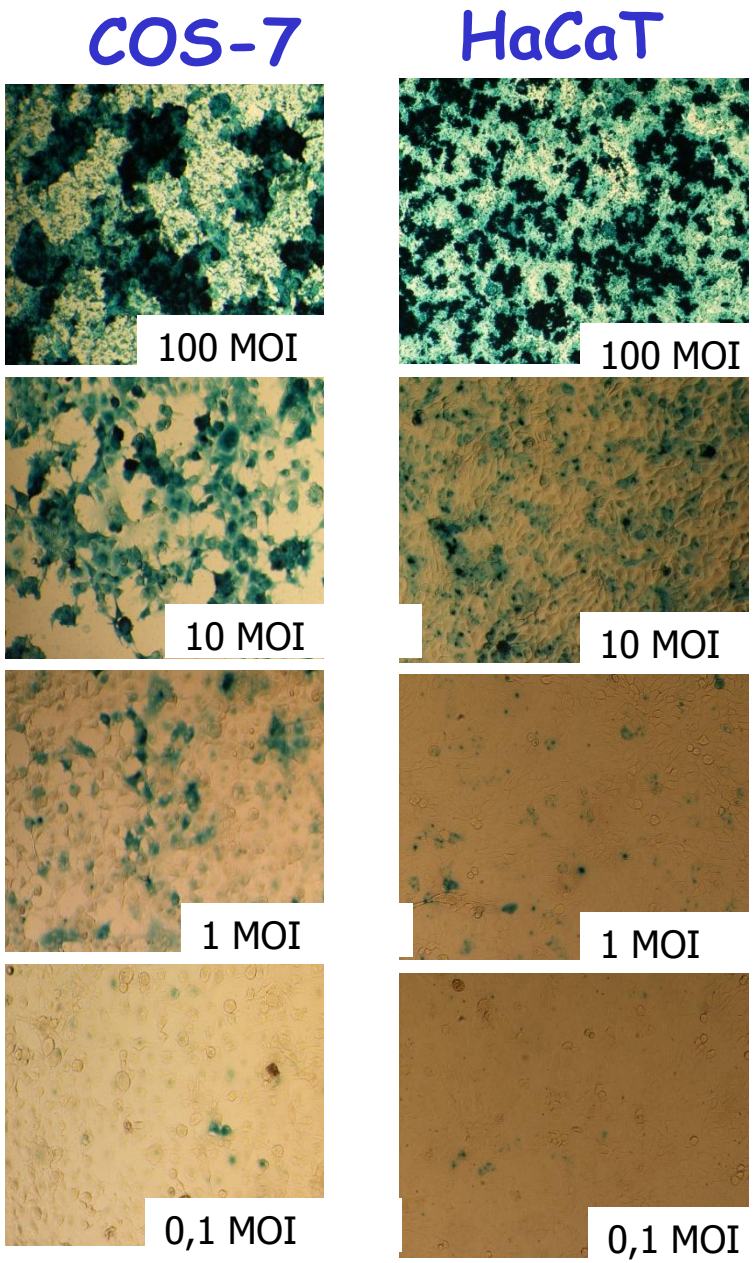
Stopa et al., Biotechnologia 2007

Increasing the titer of a vector improves the transduction efficacy

MOI – multiplicity of infection (infection units/ml)

$$\frac{\#\text{ of positive cells}}{D_{dilution} \times V} = \text{titer (IU/ml)}$$

V- volume of virus dilution added to cells



Stopa et al., Biotechnologia 2007

Features of Adenoviral Vectors

Adenoviral gene transfer is one of the most reliable methods for introducing genes into mammalian cells. Because infection by adenovirus is not cell-cycle dependent, you can deliver your gene to primary as well as transformed cell lines. Following infection, your target gene is transiently expressed at high levels since many cells receive multiple copies of the recombinant genome. Expression is transient because adenoviral DNA normally does not integrate into the cellular genome. However persistent expression in non-dividing cells has been observed *in vivo* (Chen, H. H. *et al*., (1999) *Human Gene Ther.* **10**:365–373.) Adenoviruses are capable of infecting a wide variety of proliferating and quiescent cell types from many different animal species including humans, non-human primates, pigs, rodents, mice, and rabbits (Table I). Published reports suggest that nearly all human cell types—including skin, muscle, bone, nerve, and liver cells—are susceptible to infection by adenovirus.

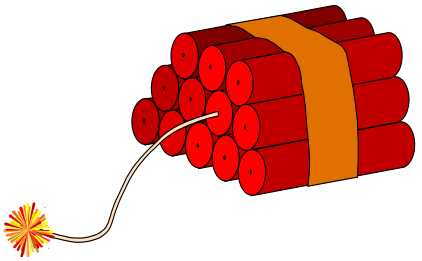
Application of adenoviral vectors in gene therapy

1. Gene therapy of inborn errors in metabolism – lack of OCT
2. Gene therapy of monogenic diseases – cystic fibrosis
3. Gene therapy of cardiovascular diseases – transfer of angiogenic genes
4. Gene therapy of cancer – it is possible that toxicity and immongenicity will enhance the therapeutic effectiveness

Adenoviral vectors of the first generation

Great:

- Very high transduction efficiency
- Broad host and cell type ranges
- Can be prepared in high titers
- Can transduce mitotic and post-mitotic cells
- Do not integrate with genome
- Can harbor ~ 7 kb of transgene



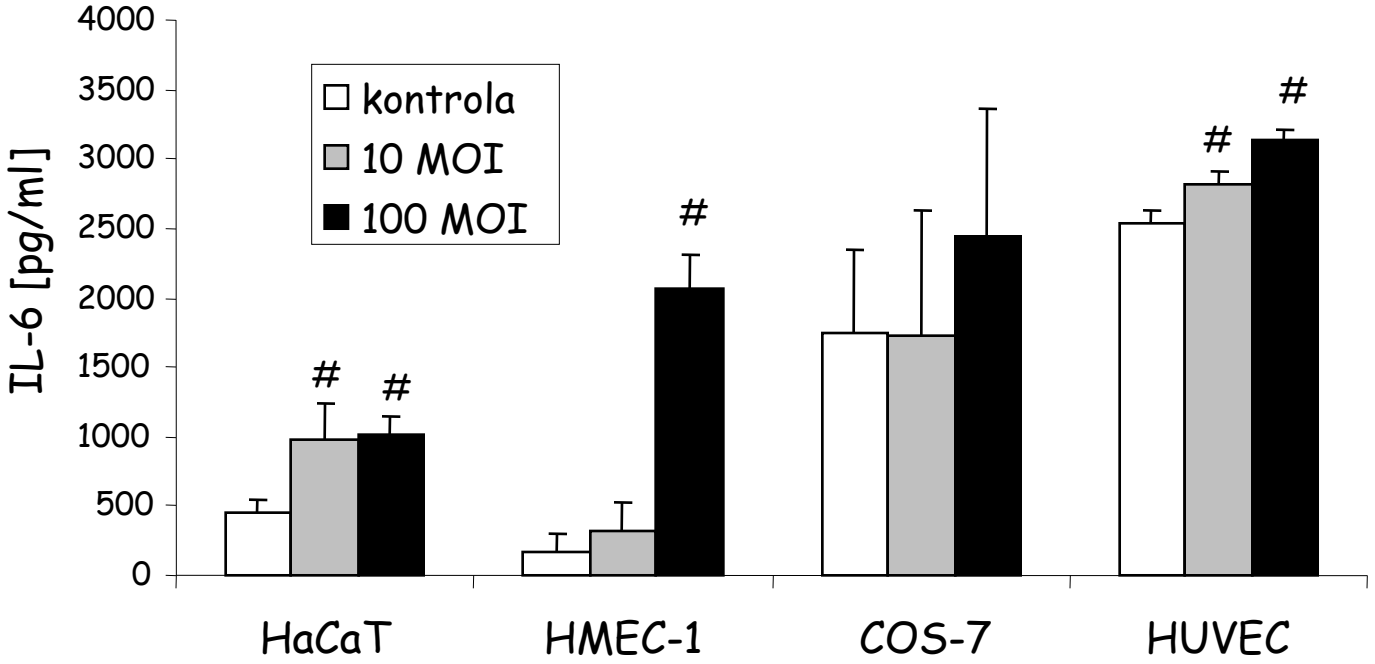
But:

- Strong immune response to viral proteins eliminate virally transduced cells within 30 days
- Neutralizing antibody response prevents readministration of adenovirus vector of the same serotype

Thus:

Adenoviral vectors provide the high but transient (<4 weeks) transgene expression

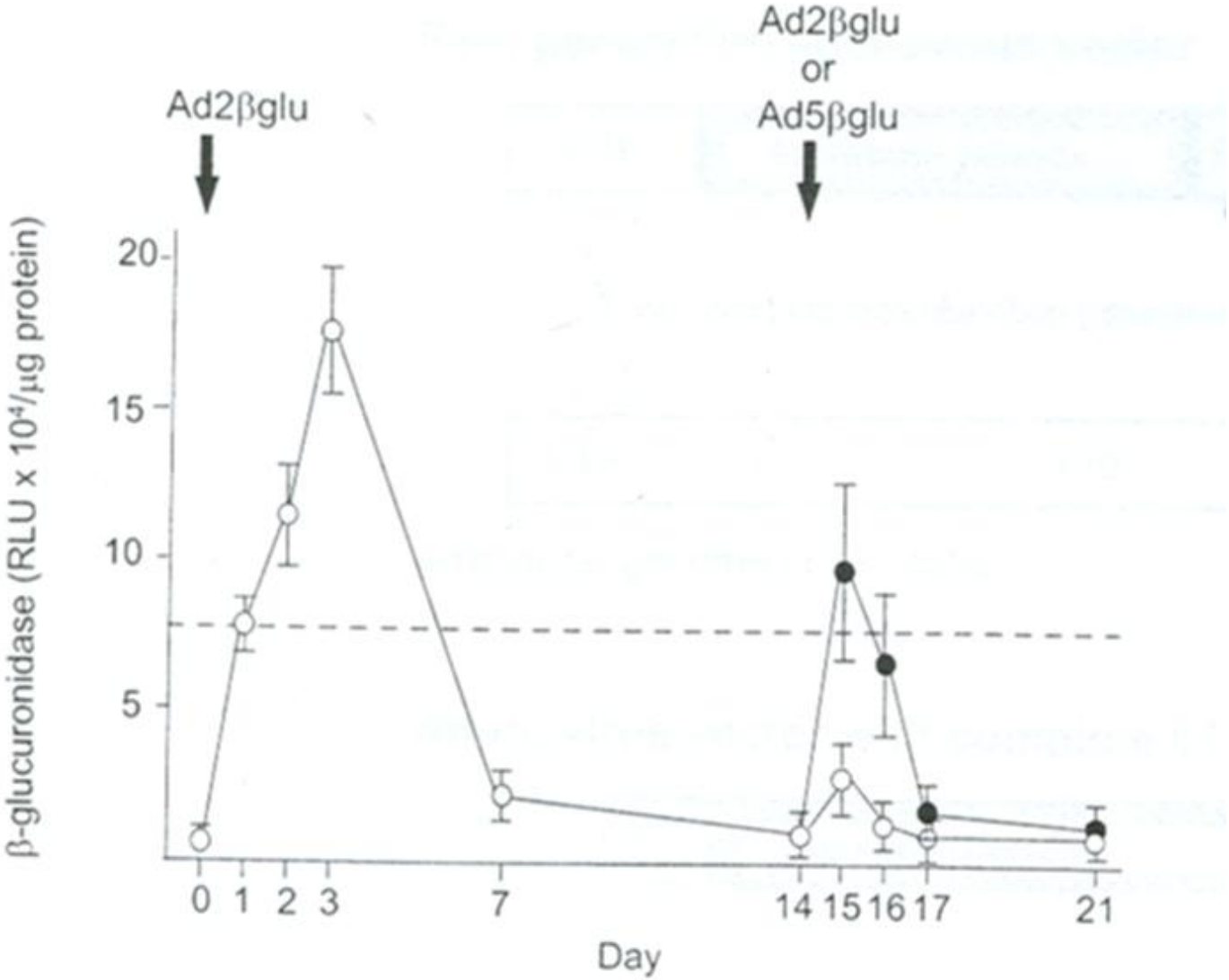
Proinflammatory effect of adenoviral vectors



Stopa et al., Biotechnologia 2007

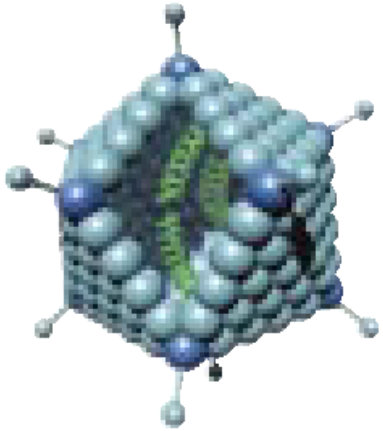
Short expression of transgene after adenoviral gene transfer

Serotype change – does not help much



Adenoviral vectors

Very efficient, transduce many cell types, provide high level of expression

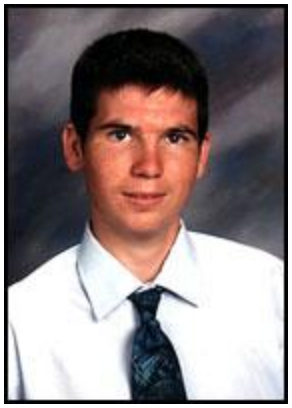


Very imunogenic

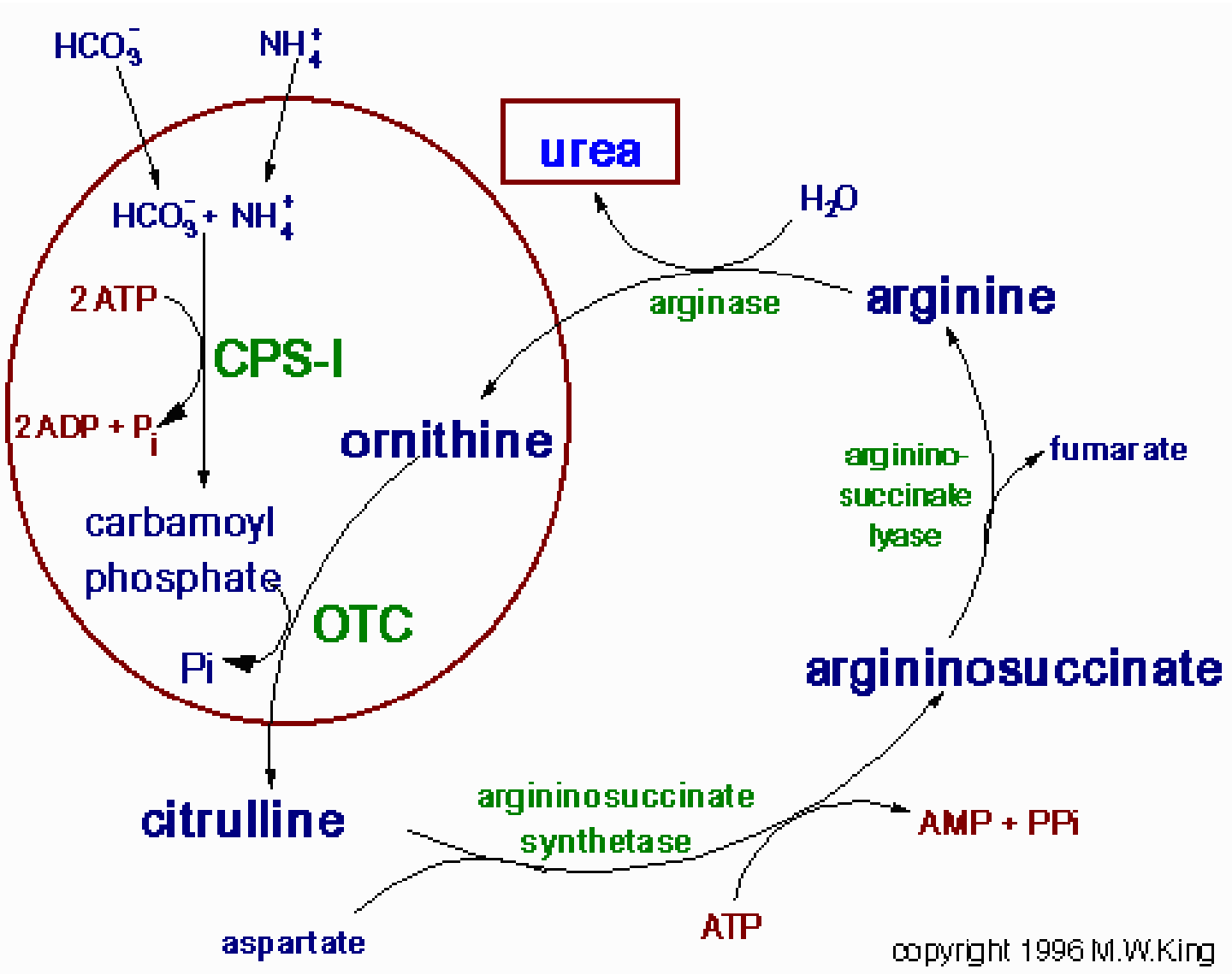
High dosage,
Multiple injections

systemic inflammatory response, death...

Ornithine transcarbamylase deficiency – gene thera

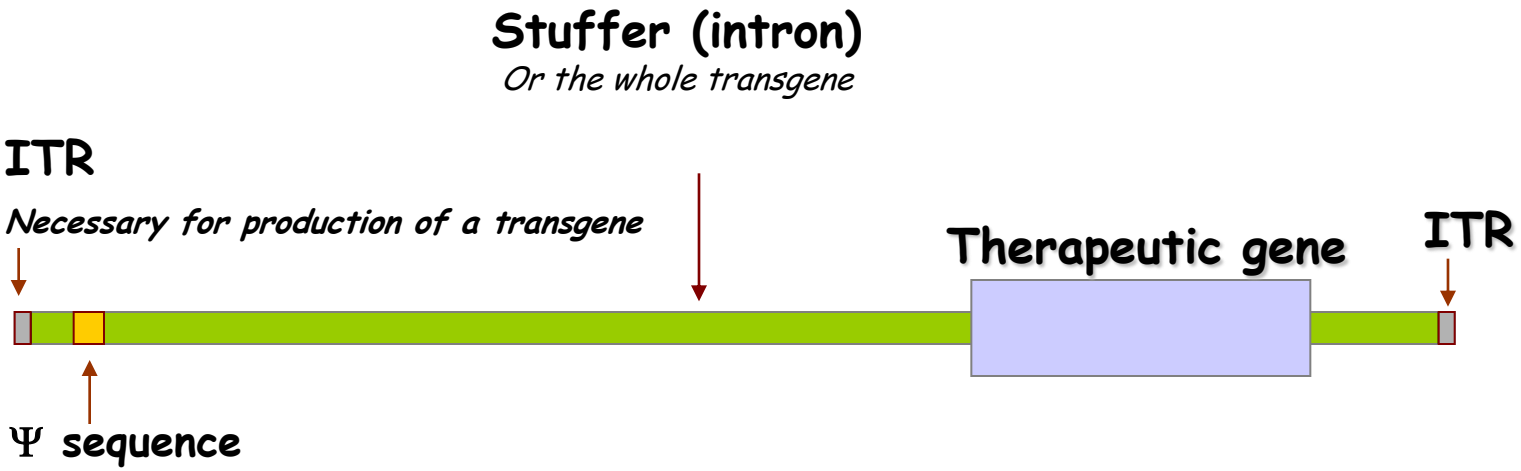


Jesse Gelsinger
1999

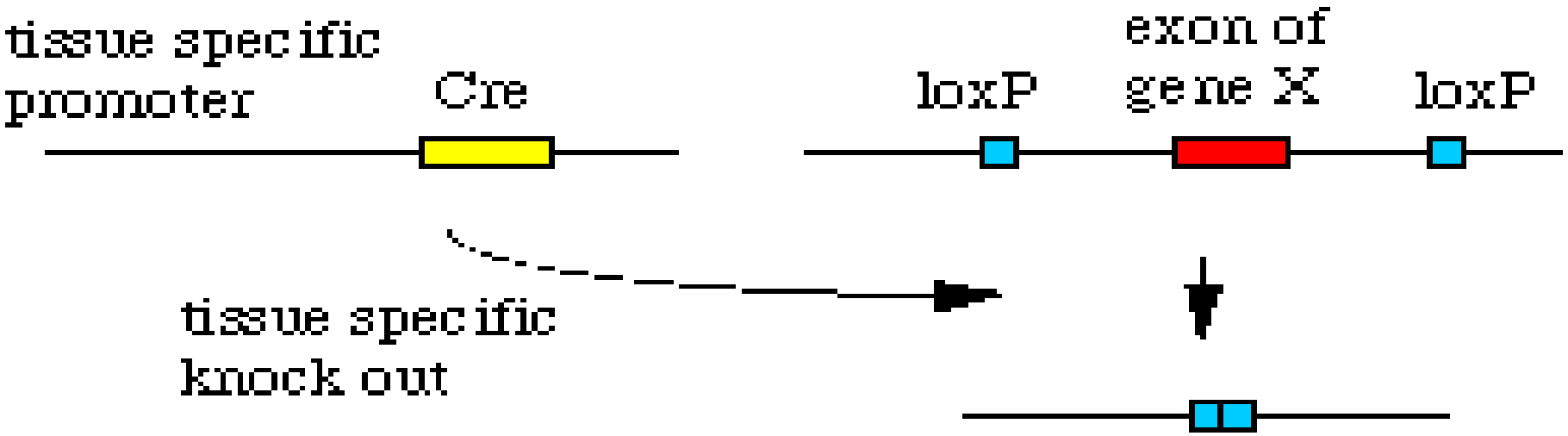


Gutless adenoviral vectors

Helper-dependent adenoviral vectors



Cre recombinase – to manipulate genes



ATAACTTCGTATA **ATGTATGC** TATACGAAGTTAT

inverted repeat — Spacer — inverted repeat

1st generation Ad vectors and helper vector

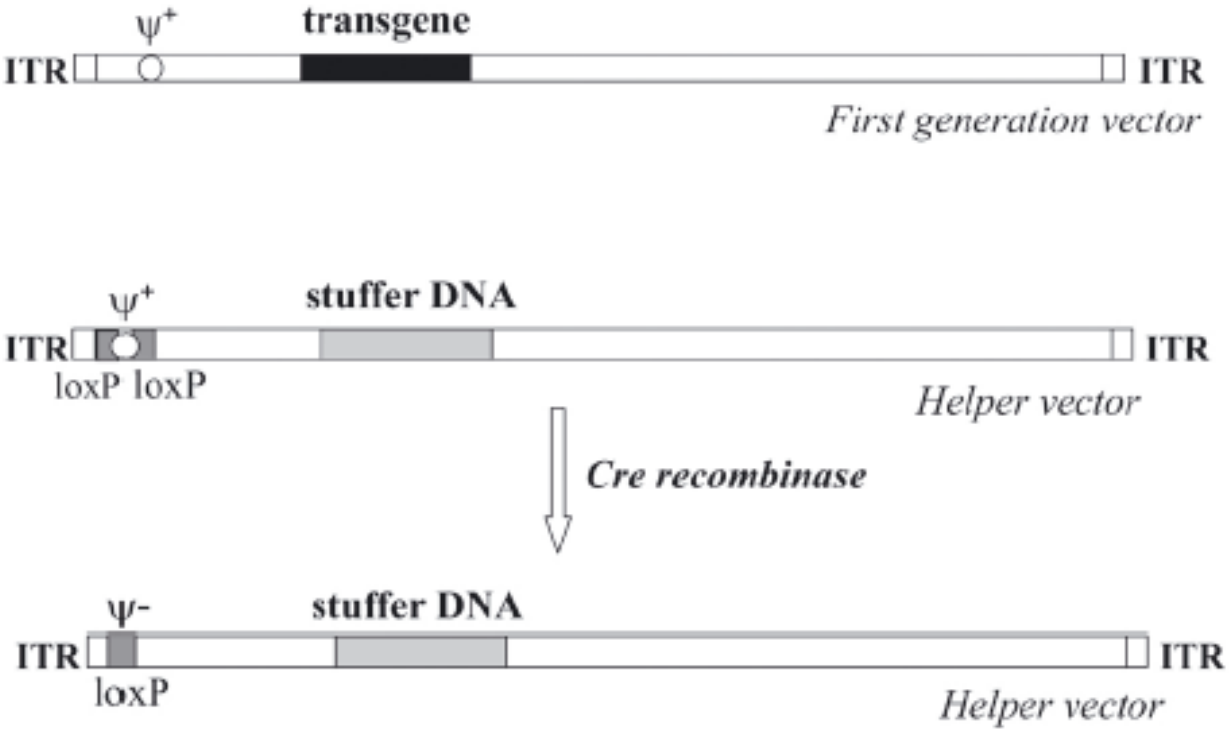
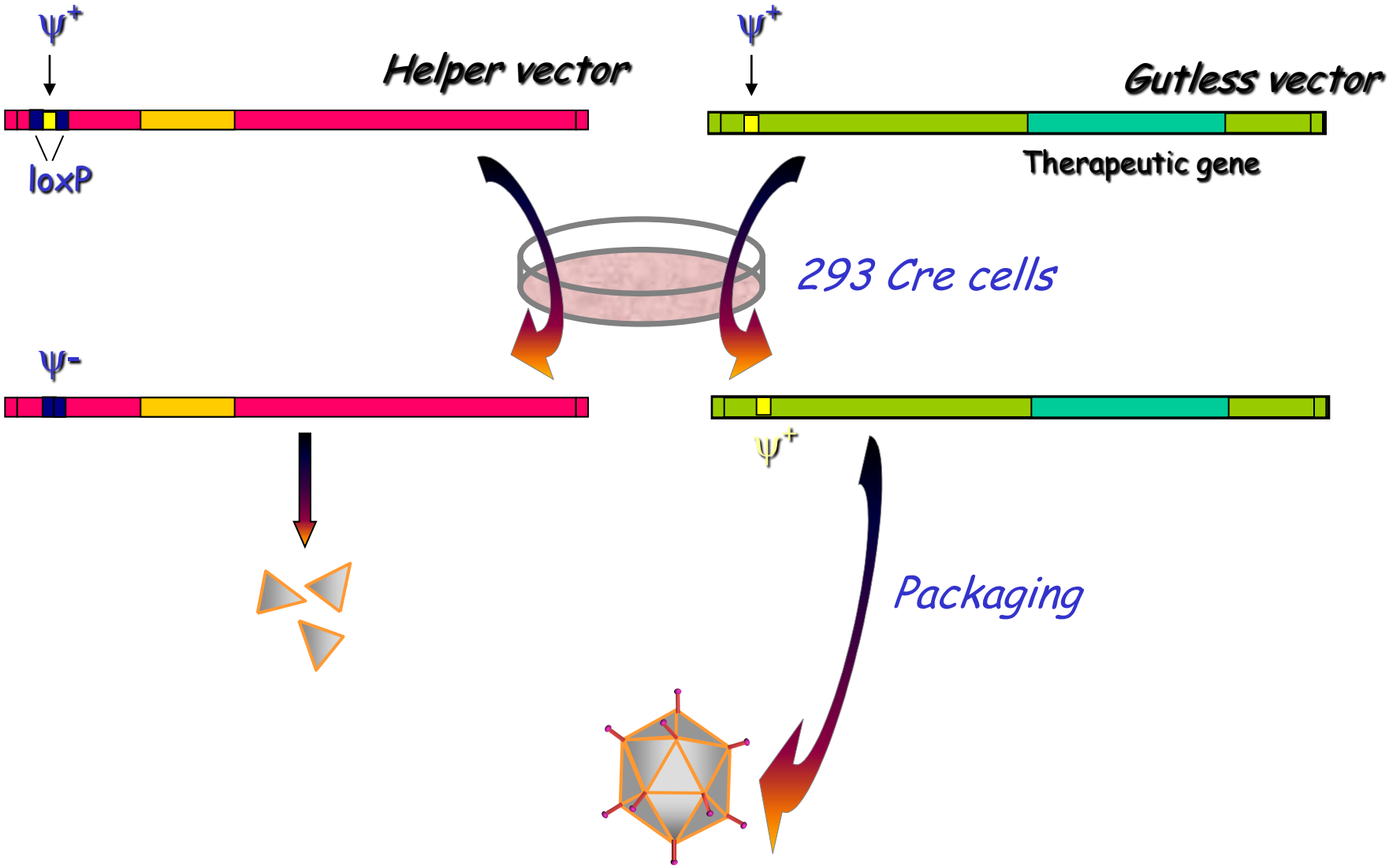
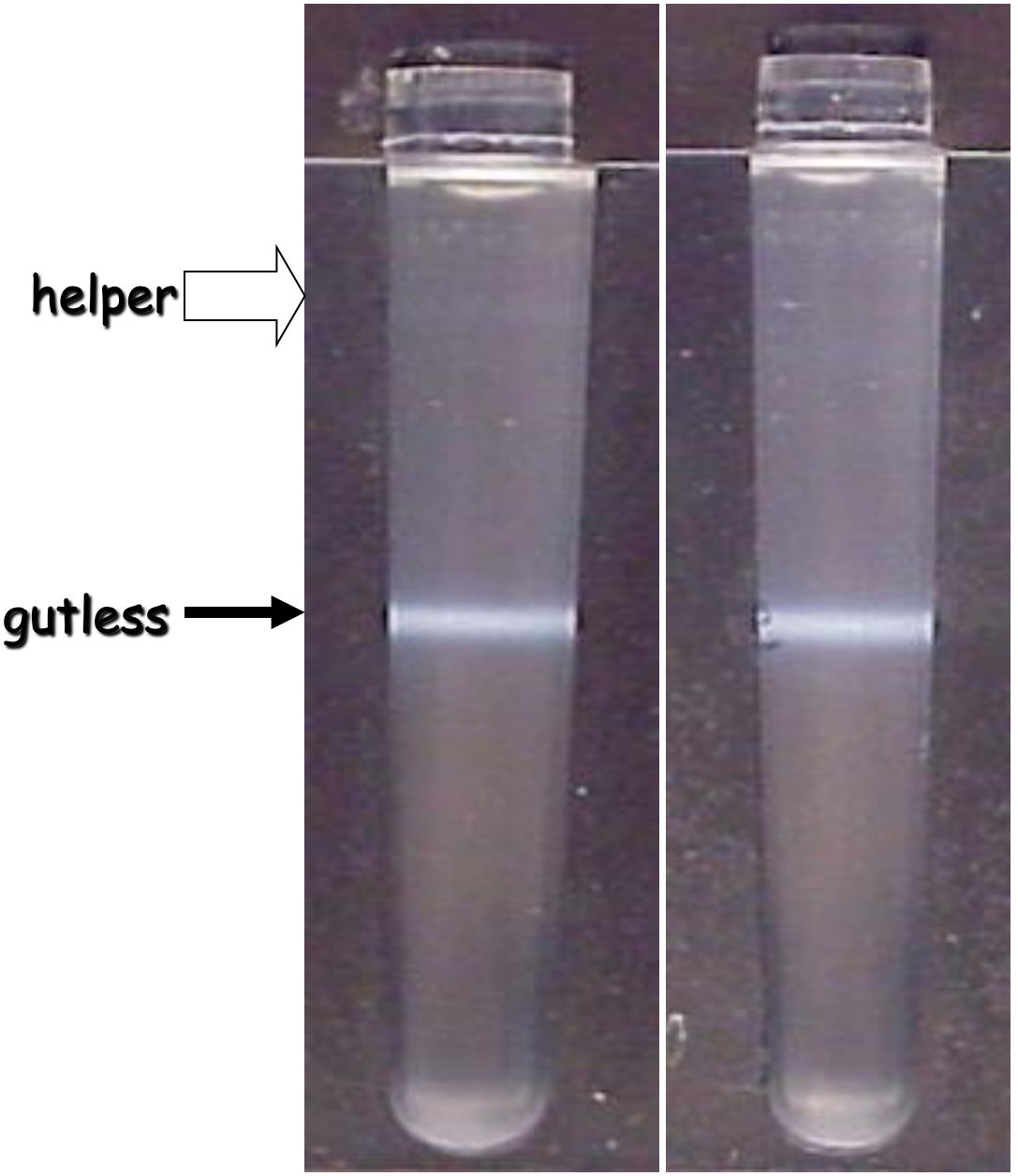


Figure 2. Schematic comparison of the first generation adenoviral vector and helper vector.
Note that in the helper genome the transgene is replaced by stuffer DNA, while the packaging sequence Ψ is flanked by loxP sites. In the presence of recombinase cre, Ψ is excised from the helper genome.

Packaging of gutless vectors



Purification of gutless vectors



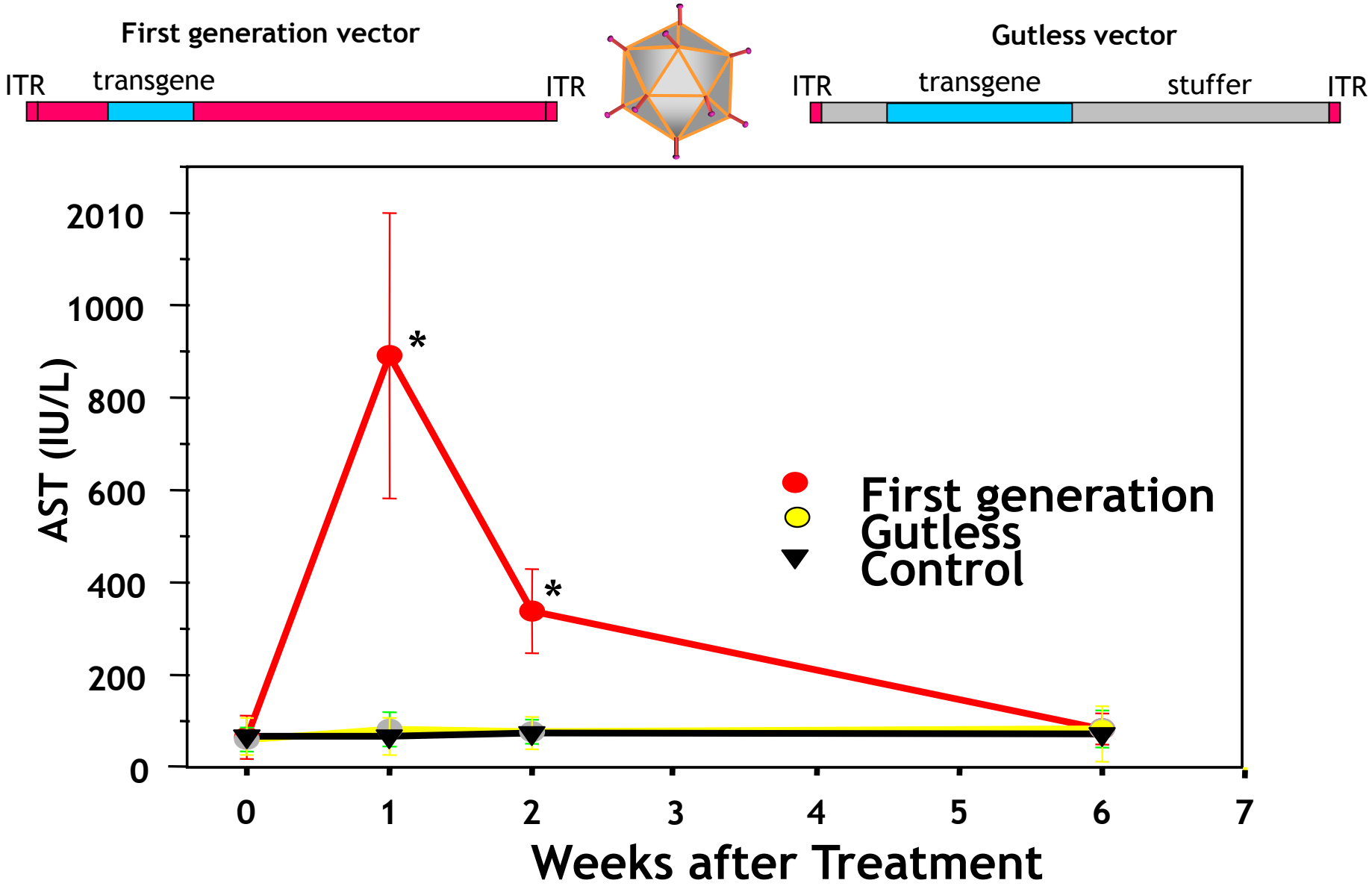
Helper-dependent adenoviral vectors

- Very high transduction efficiency
- Broad host species and cell type range
- Can transduce mitotic and post-mitotic cells
- Can harbor ~ 35 kb (!) of transgene
- Do not integrate with genome
- Do not produce any viral proteins
- Show significantly reduced immunogenicity in vivo

Drawback:

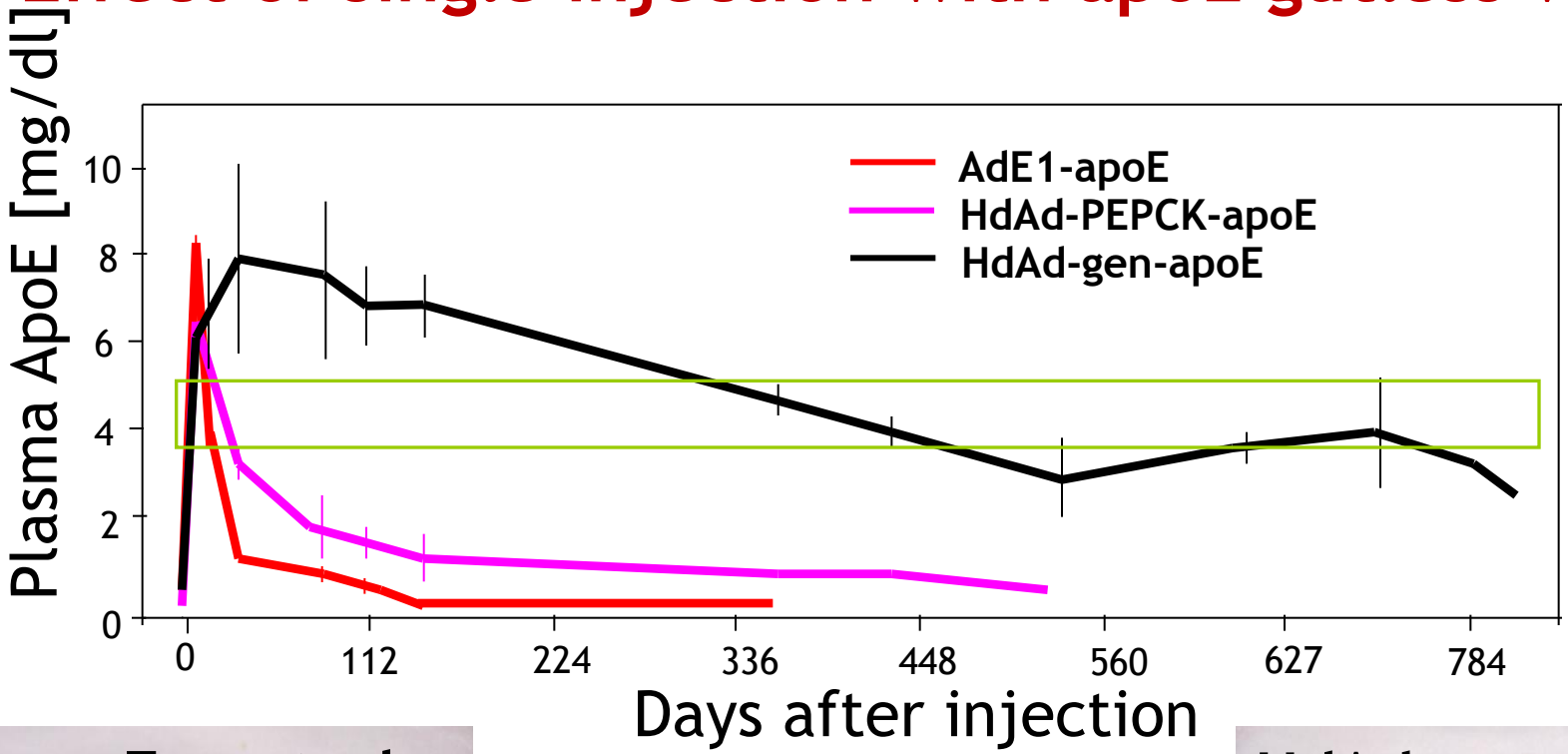
- Difficult for producing in high titers

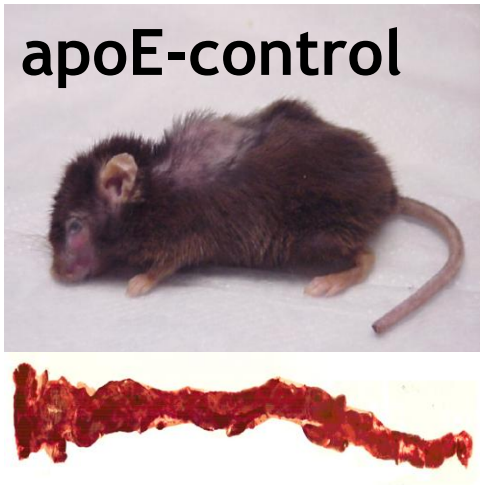
Aspartate aminotransferase (AST) after adenovirus injection

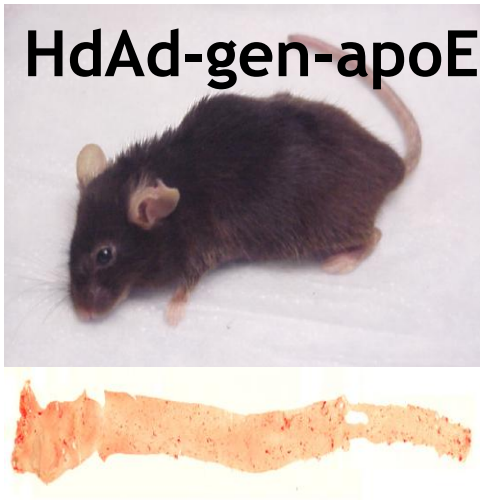


Kim et al. PNAS 2001

Effect of single injection with apoE gutless vector







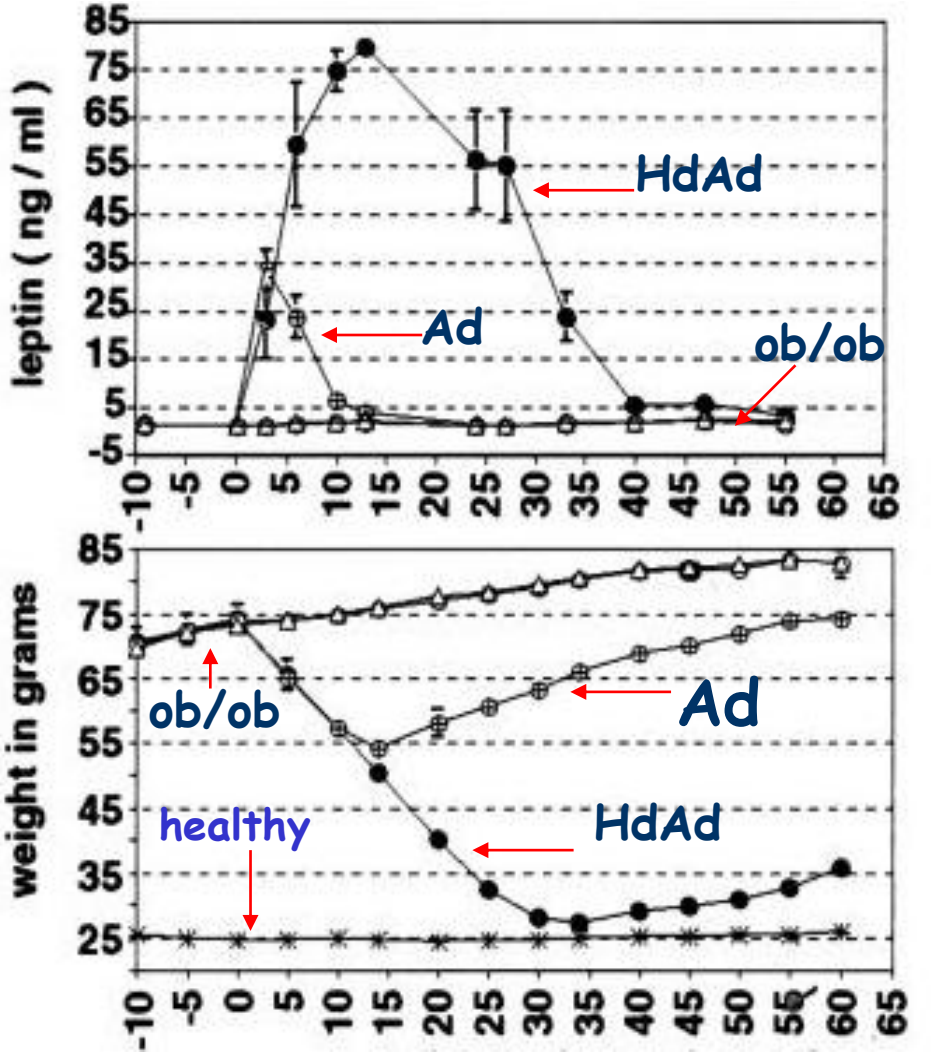
Kim et al. PNAS 2001

Treatment of obesity with HdAd vectors

Mice ob/ob

control mouse

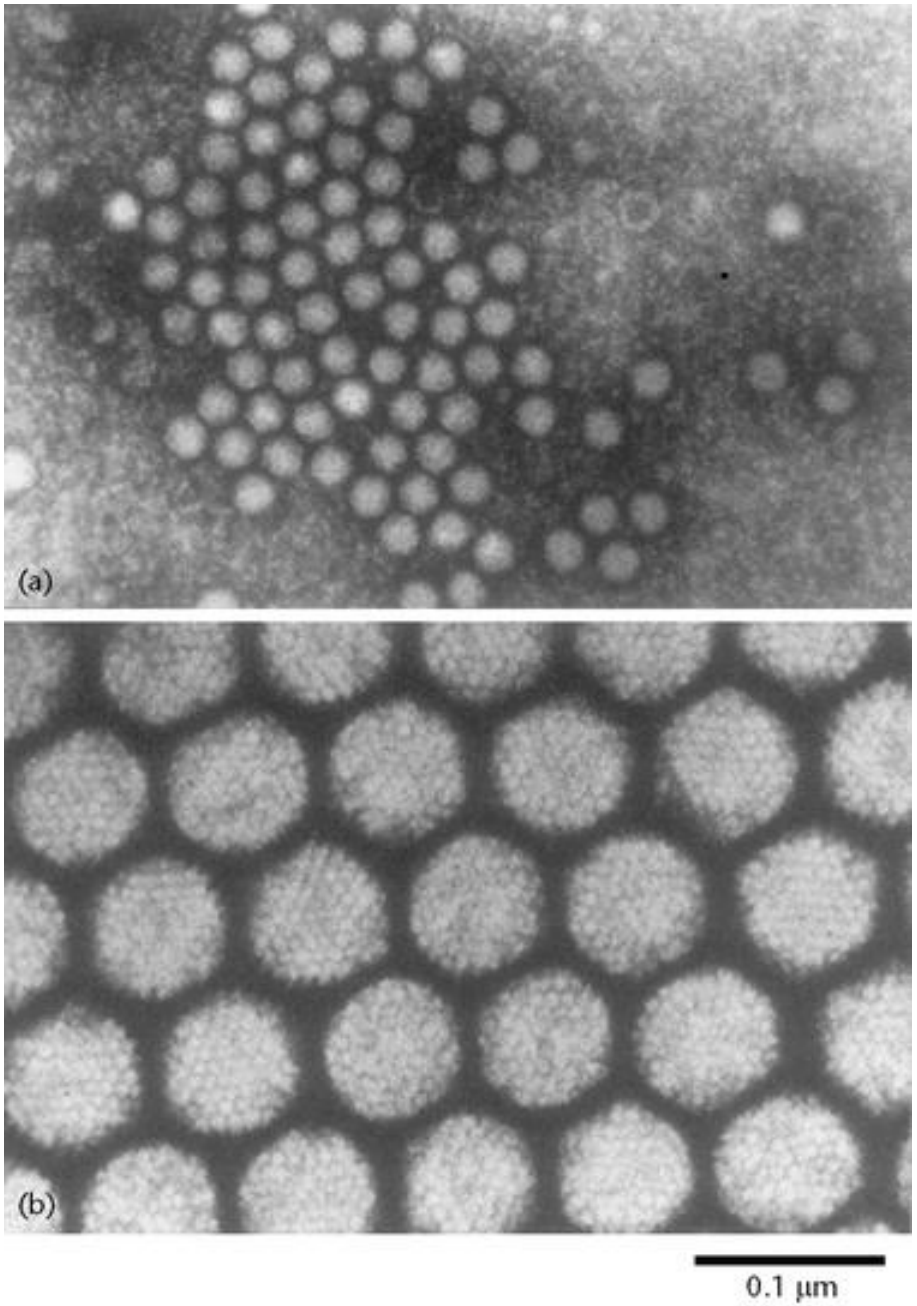
treated mouse



Morsy et al. 1998. Proc Natl Acad Sci USA 95:7866-7871.

AAV vectors

adeno-associated viral vectors



AAV

adenovirus

Adeno-associated viruses – AAV

Small, non-pathogenic single stranded DNA viruses

For replication require additional genes delivered by other viruses (adenoviruses or herpes simplex viruses)

Genome AAV – 4681 nucleotides, at both ends there are 145 nt-long ITR (*inverted terminal repeats*)

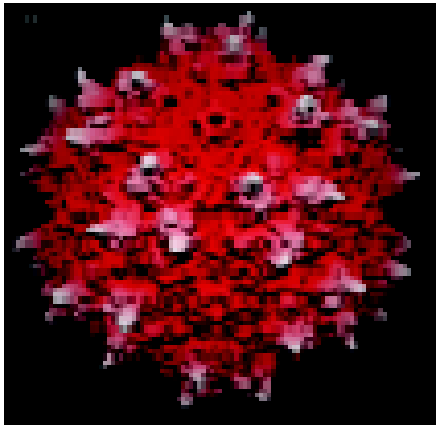
ITR – necessary *in cis* – initiation of replication
- packaging signal
- integration into genome

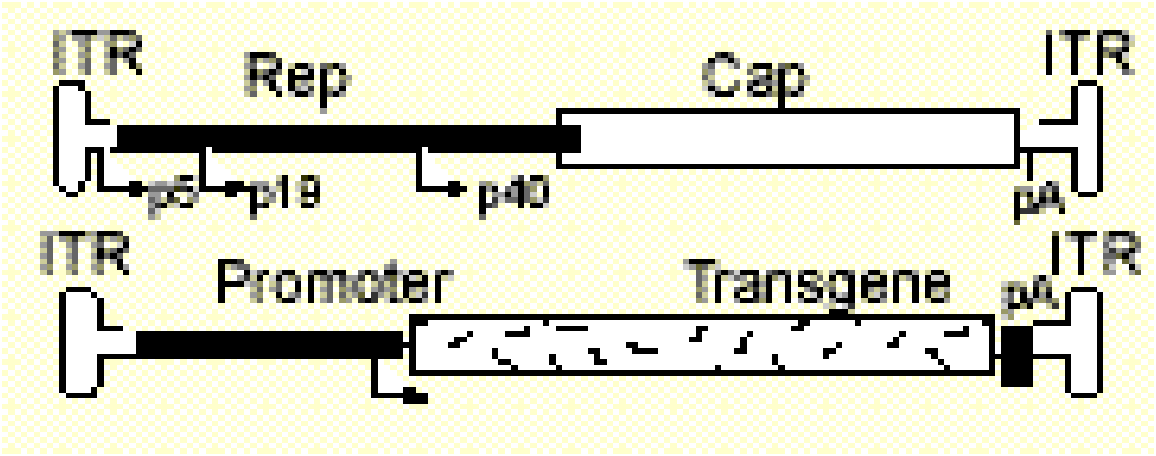
AAVs insigth

- AAV genome is a linear single stranded DNA flanked by inverted terminal repeats ITR(145nt);
- The genome has 2 genes:

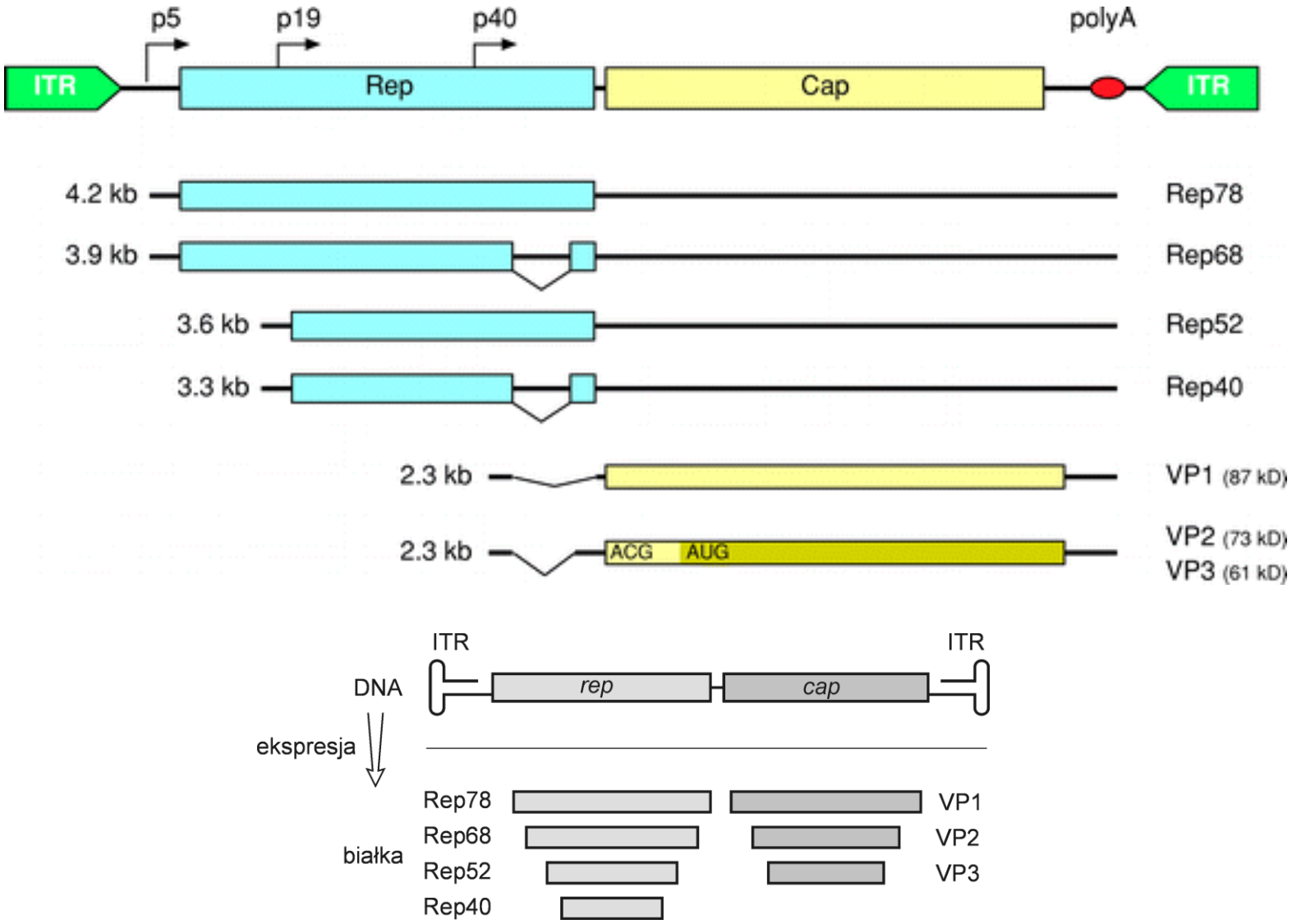
-cap (encodes viral capsid protein)

-rep (encodes 4 overlapping Rep proteins)



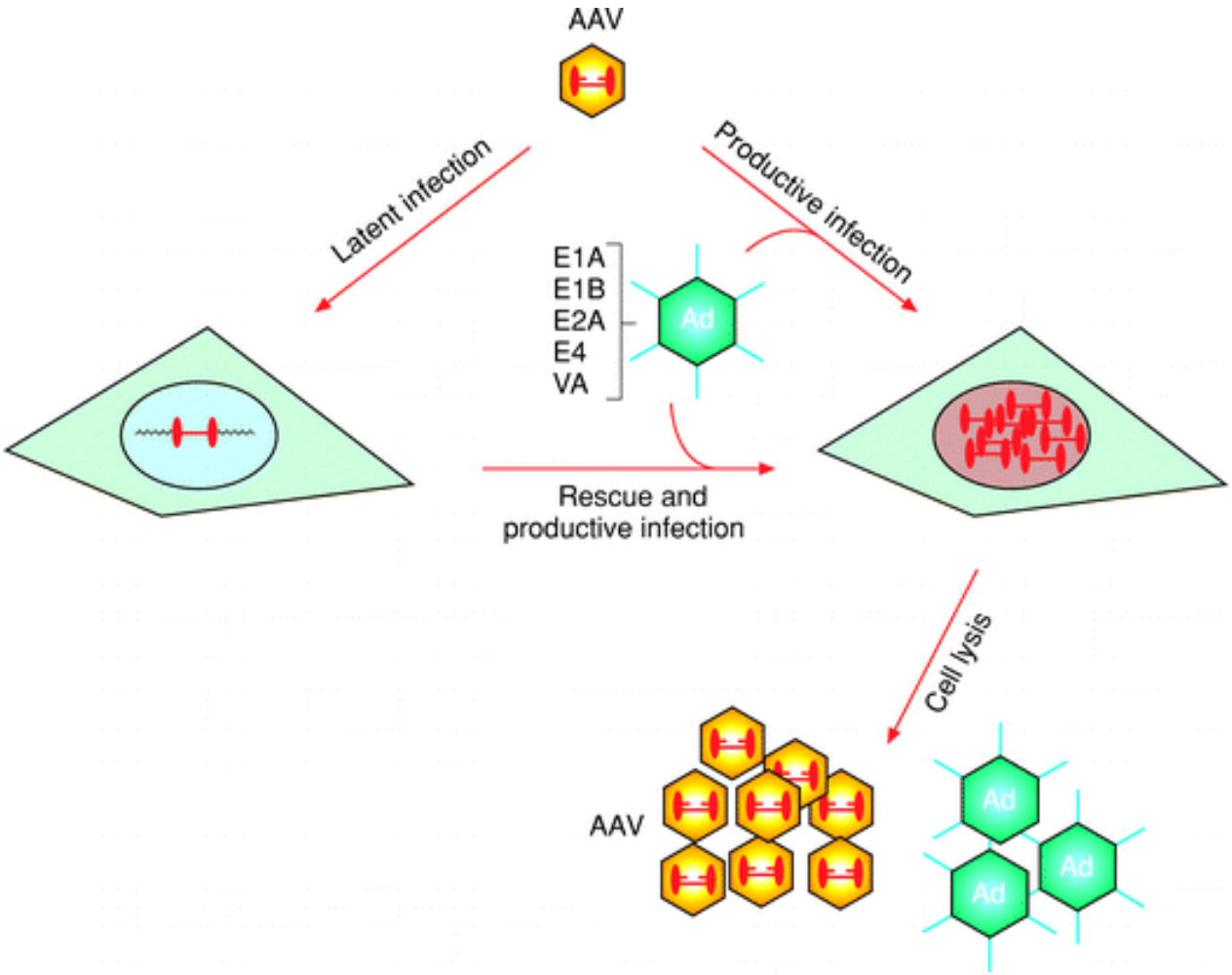


AAV genome organization



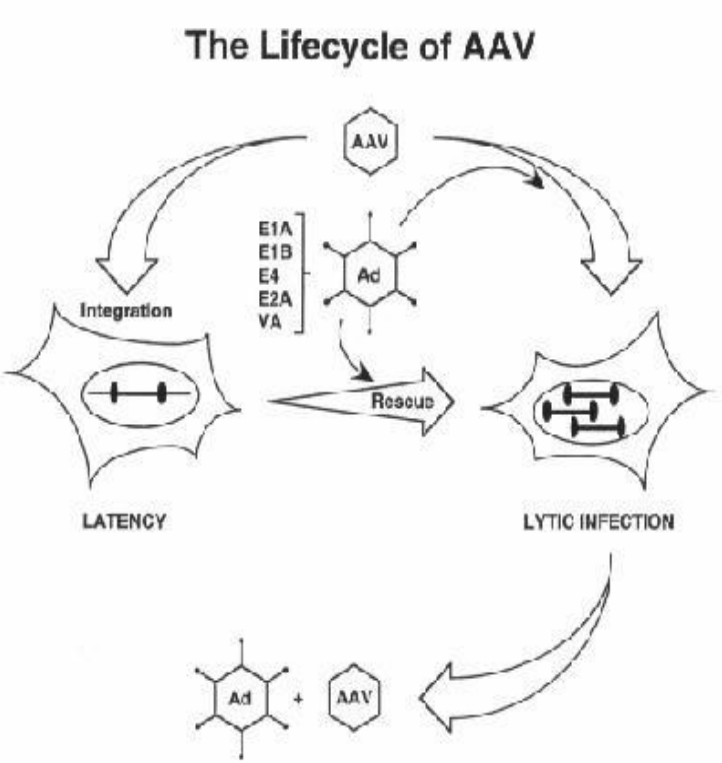
Rutkowski A et al., Biotechnologia 2007

Infectious cycle of AAV

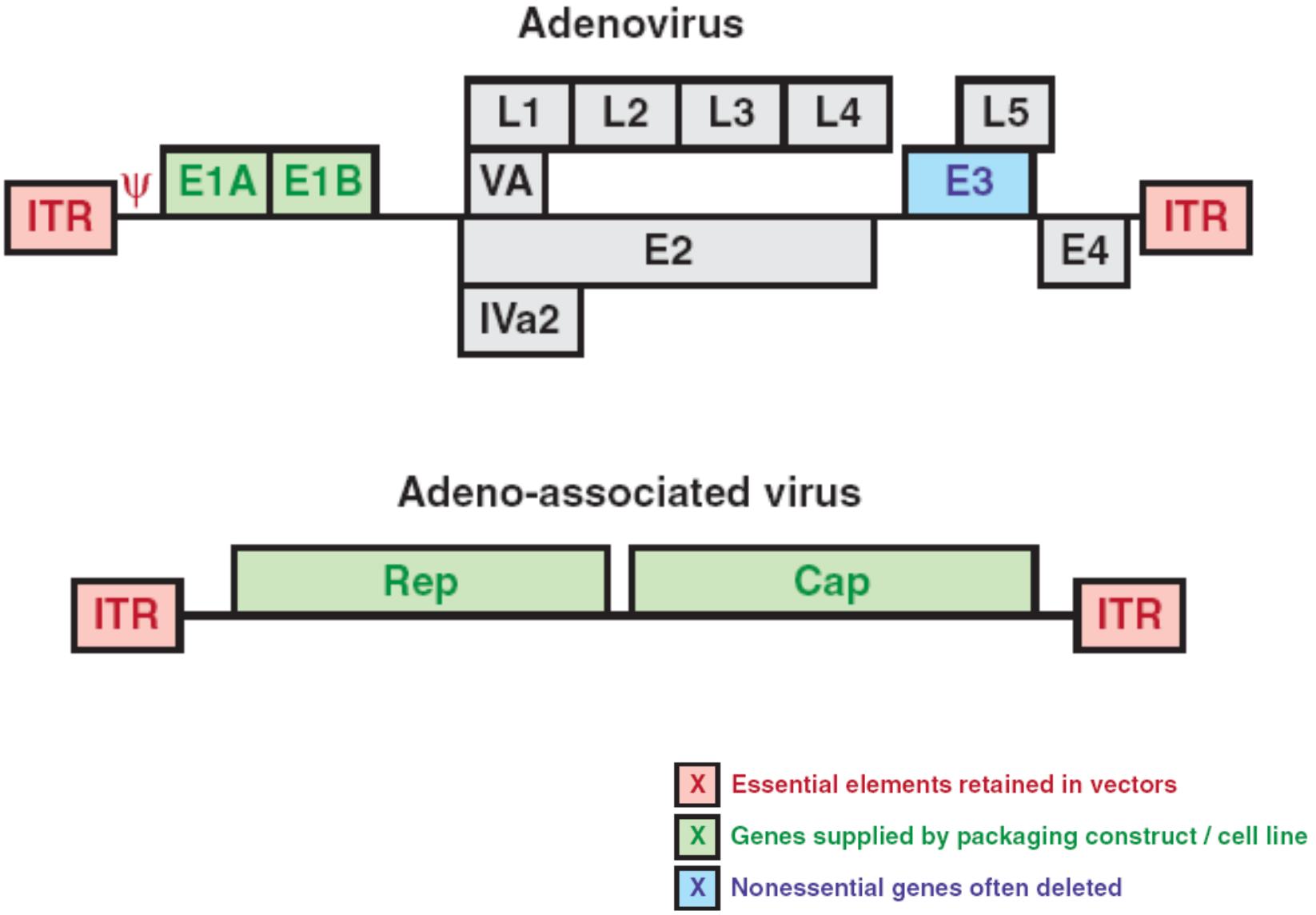


Site-specific integration

- AAV integrates usually stably into a specific site on chromosome 19q13.3 (AAVS1)
- Integration region- AAVS1 (RBS,TRS)
- Rep78 and Rep68 bind to a 109 bp DNA fragment near AAVS1 and can mediate complex formation (DNA of chromosome 19 and AAV harpin DNA)
- Viral DNA replication within AAVS1 are likely involved in site-specific integration;



Essential and non-essential elements in different viral vectors



Verma & Weitzman, Ann Rev Biochem 2005

AAV vectors

removal of rap and cap genes
transgene insertion

ITR

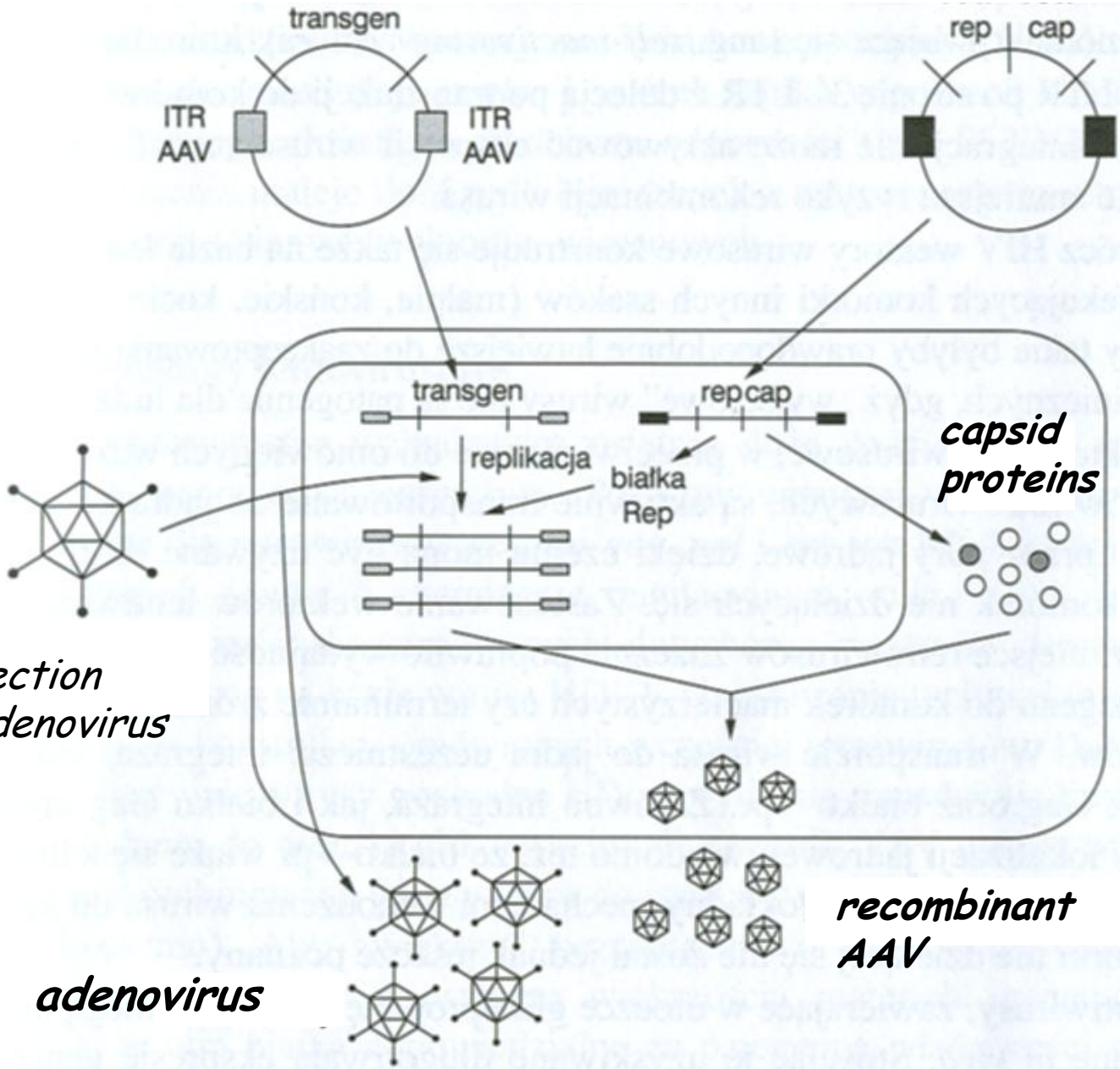
ITR

Ways of production of AAV vectors

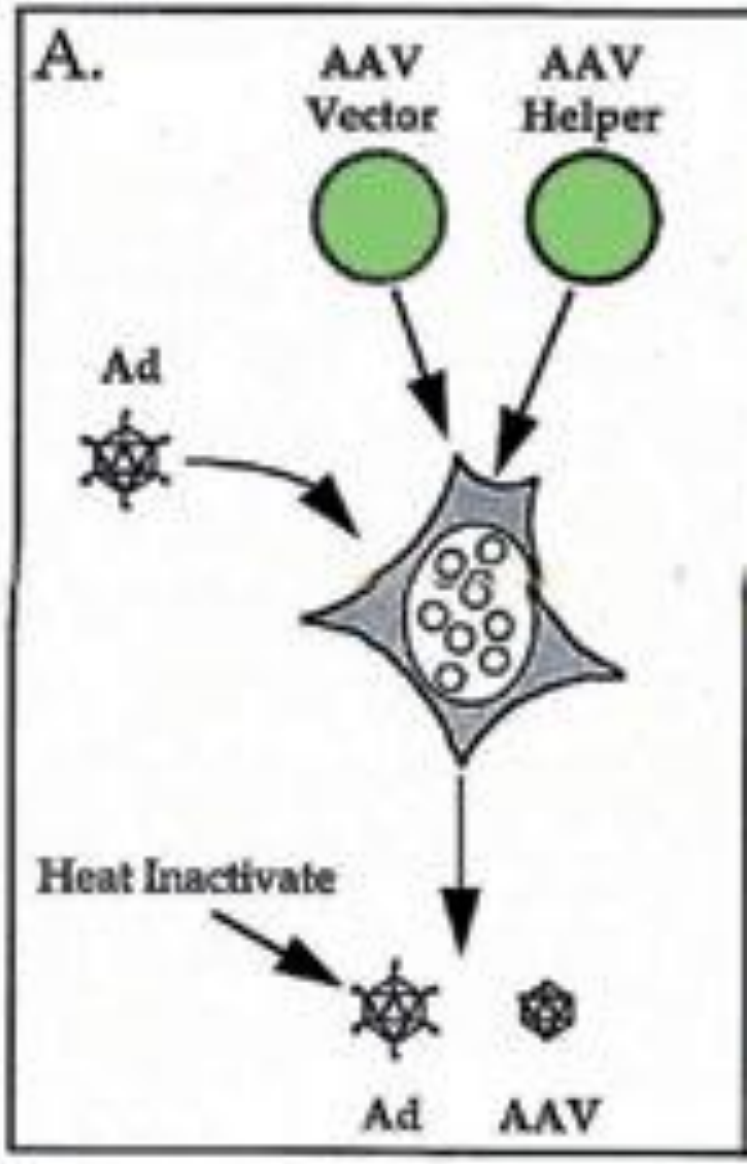
- dependent on helper vector

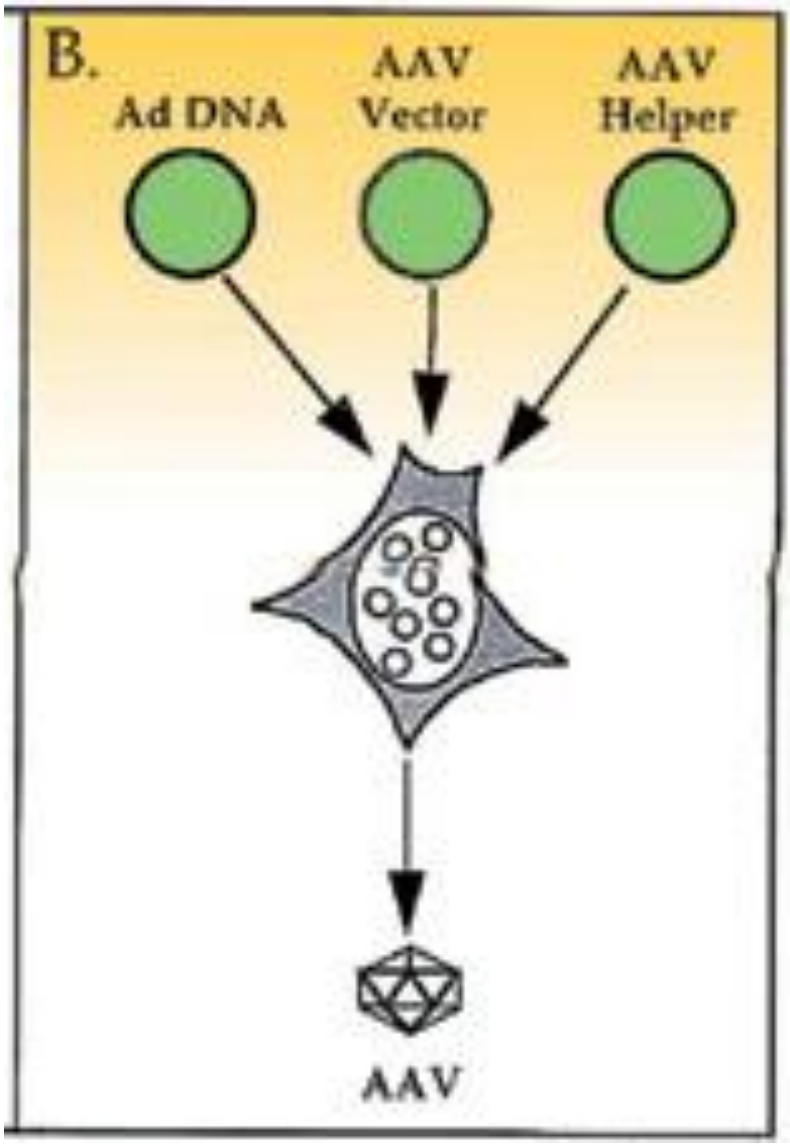
- helper-vectors independent

Construction of AAV vectors – system with helper adenoviral

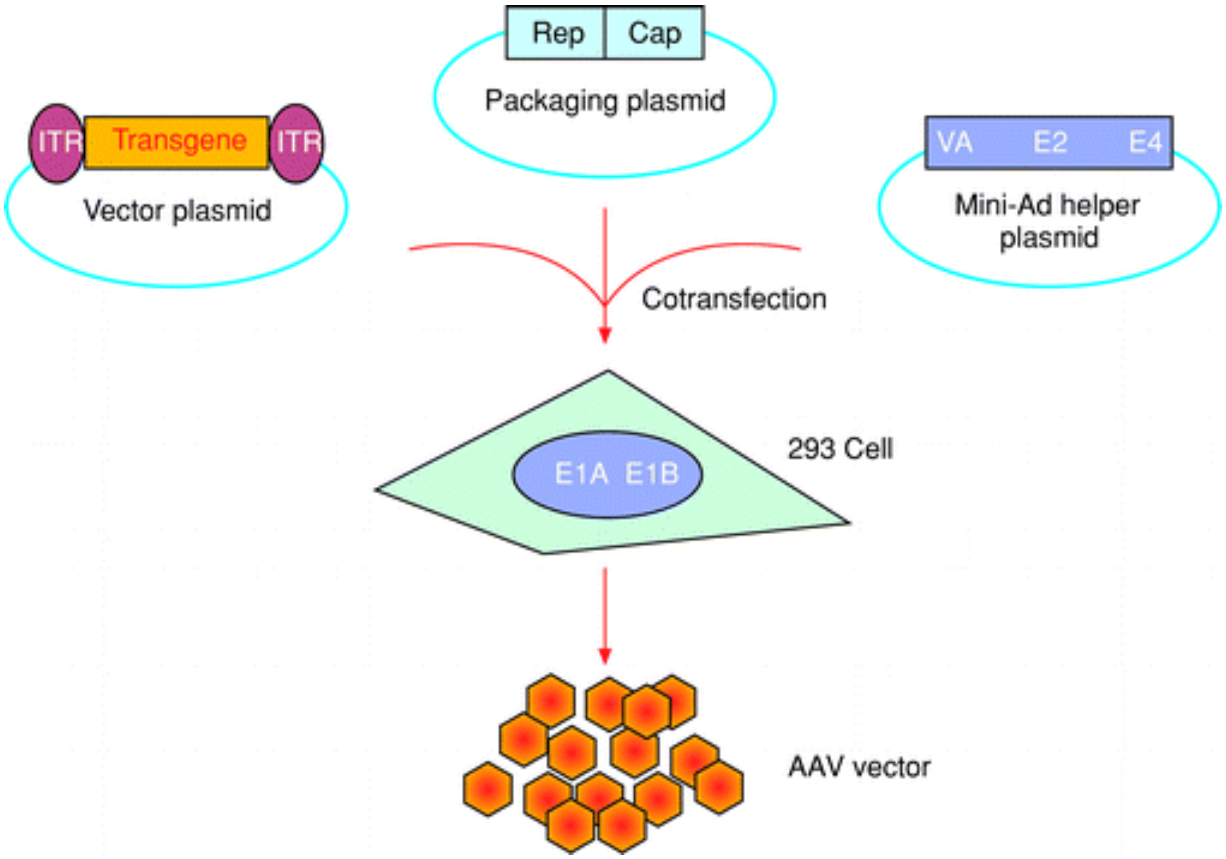


Production of AAV vectors – it is safer to omit helper adenovirus



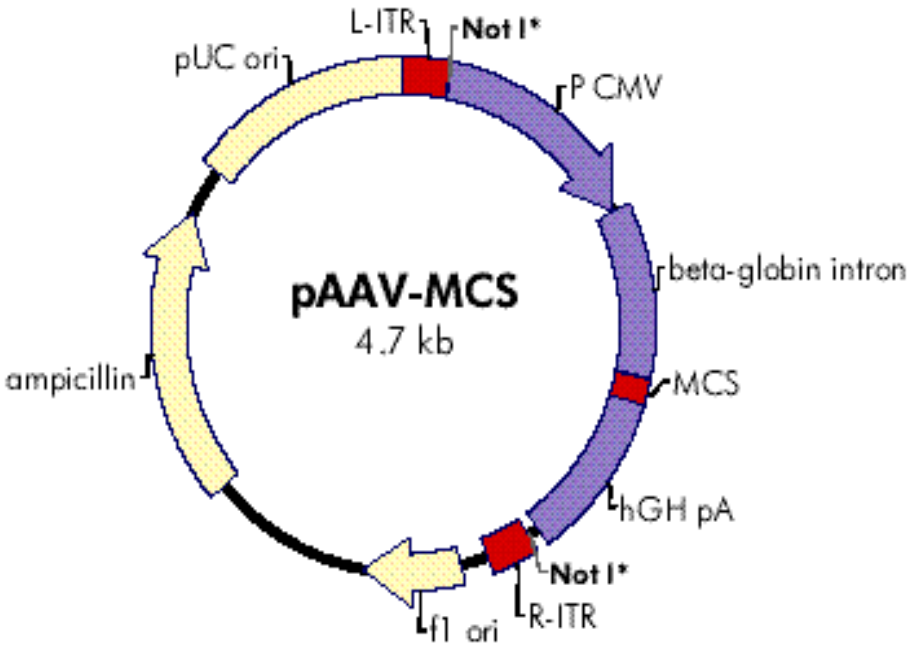


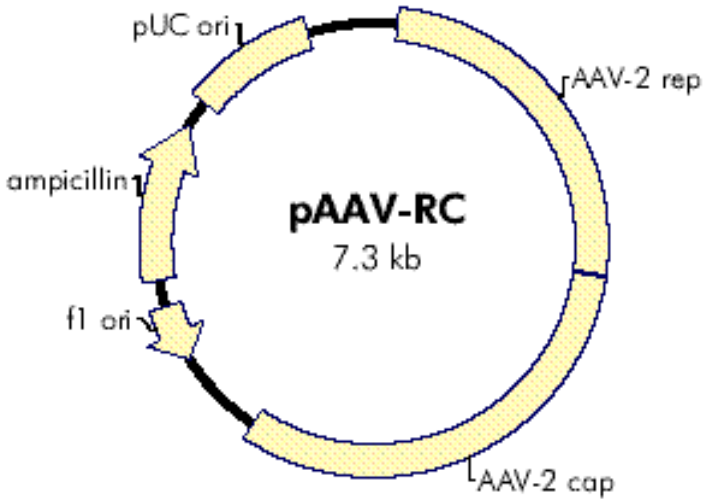
AAV Helper-Free System

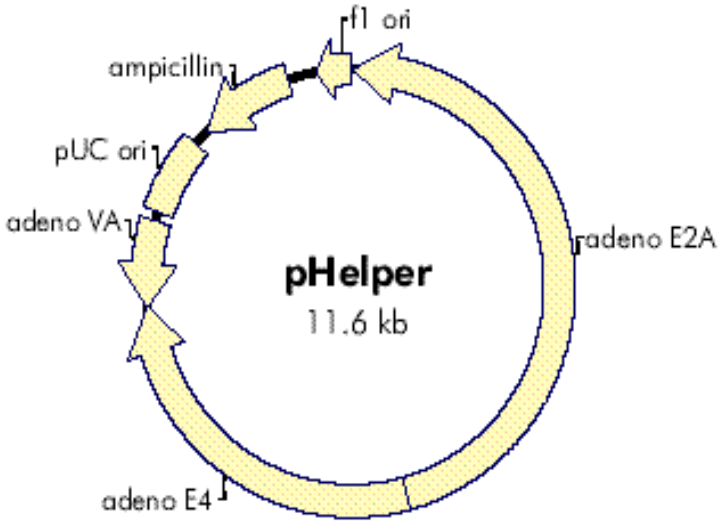


For production of AAV vectors only three sets of adenoviral genes are required: E1, E2A, E4 & VA

Vectors in AAV helper-free system

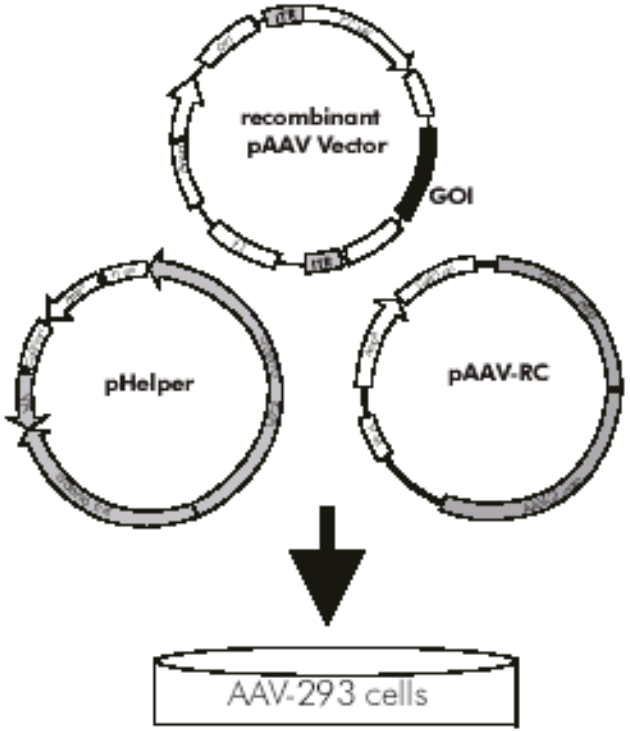






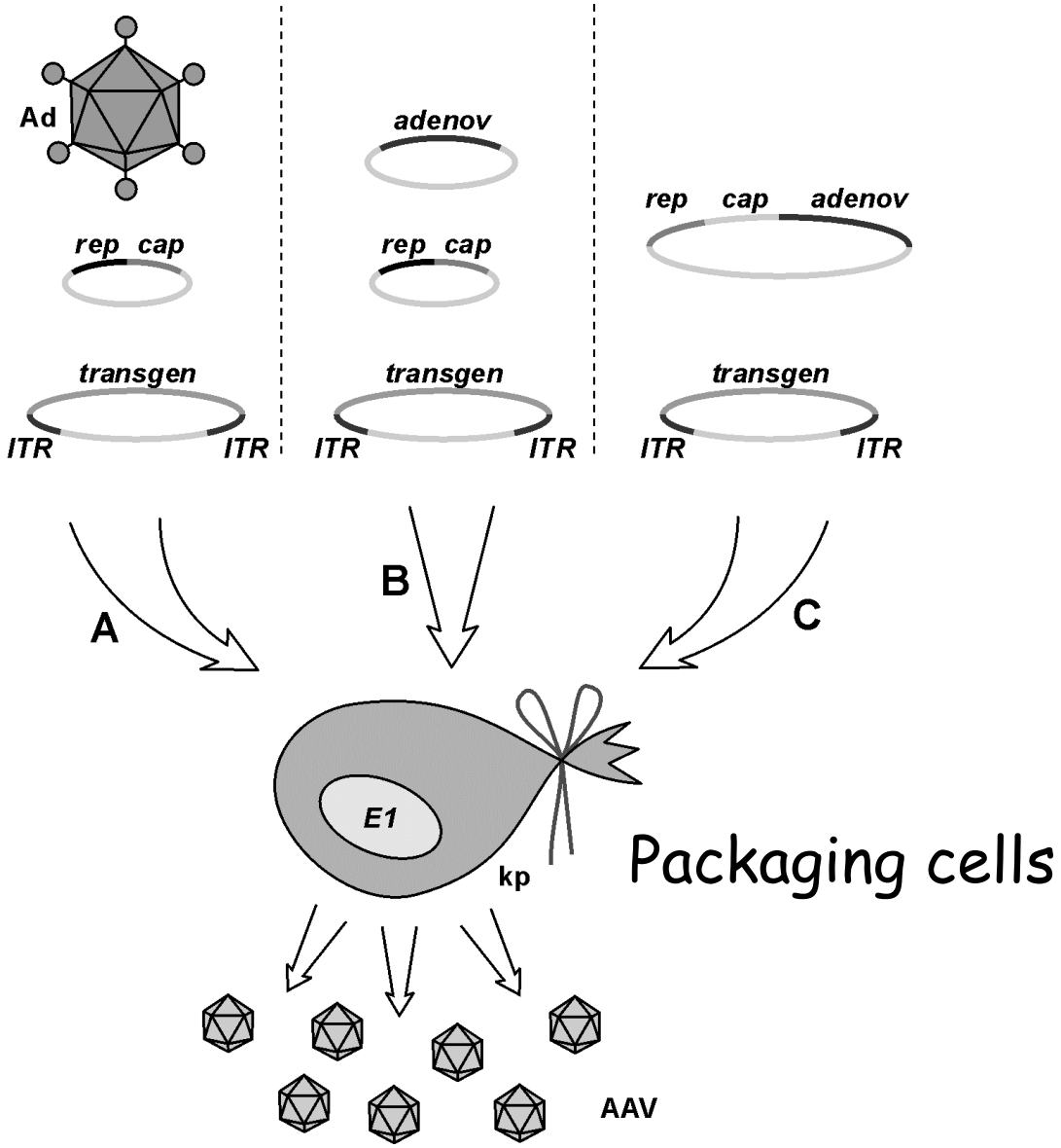
Helper-free production of AAV vectors (2)

Co-transfect
AAV-293 cells with:
Recombinant pAAV Vector
pAAV-RC
pHelper



Produce
AAV Particles
in AAV-293 cells

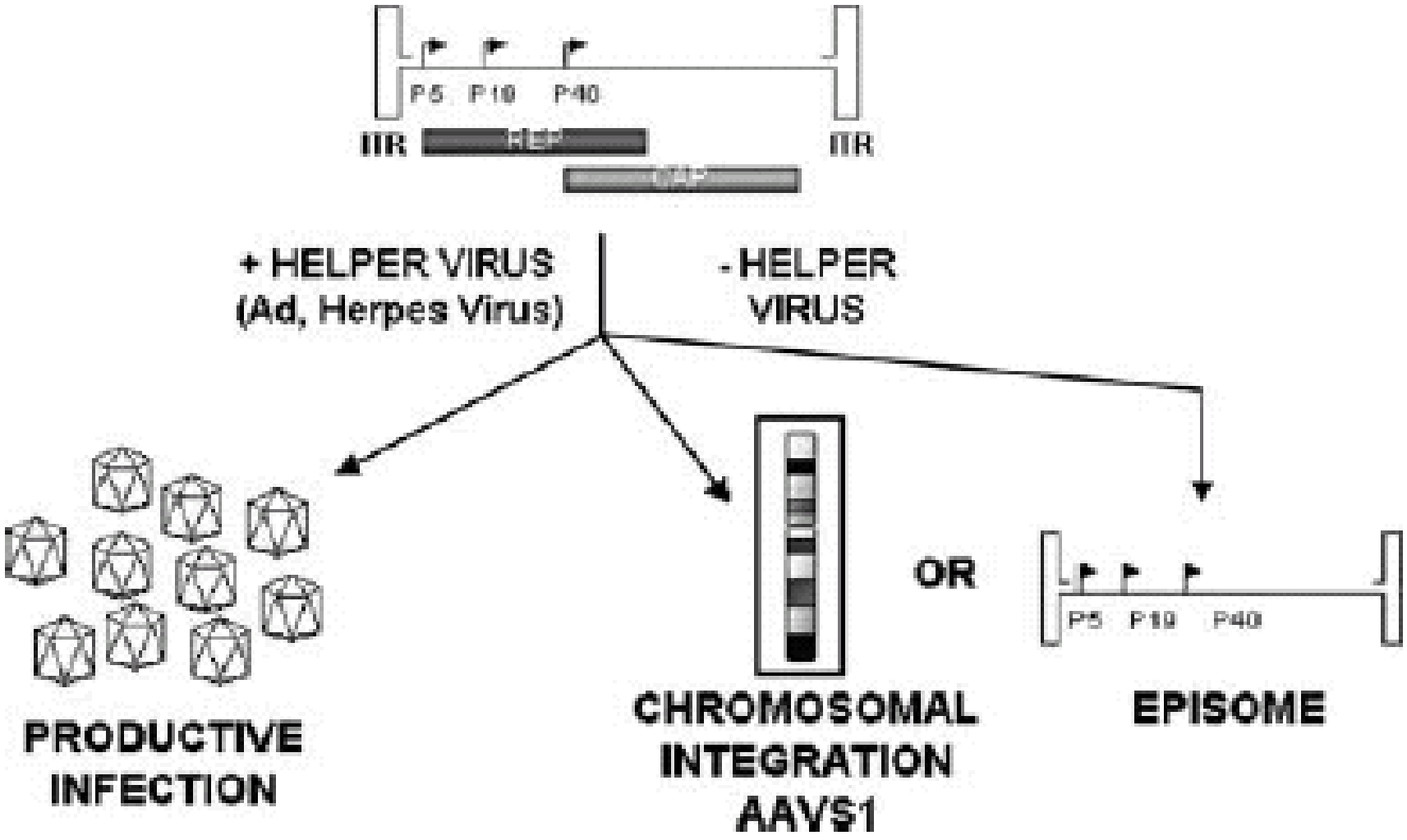
Strategies of production of AAV vectors



Rutkowski et al., Biotechnologia 2007

AAV and genomic integration

Infectious cycle of AAV



AAV vectors features

- due to the lack of Rep68 and Rep78 the specific integration into chromosome 19 is lost

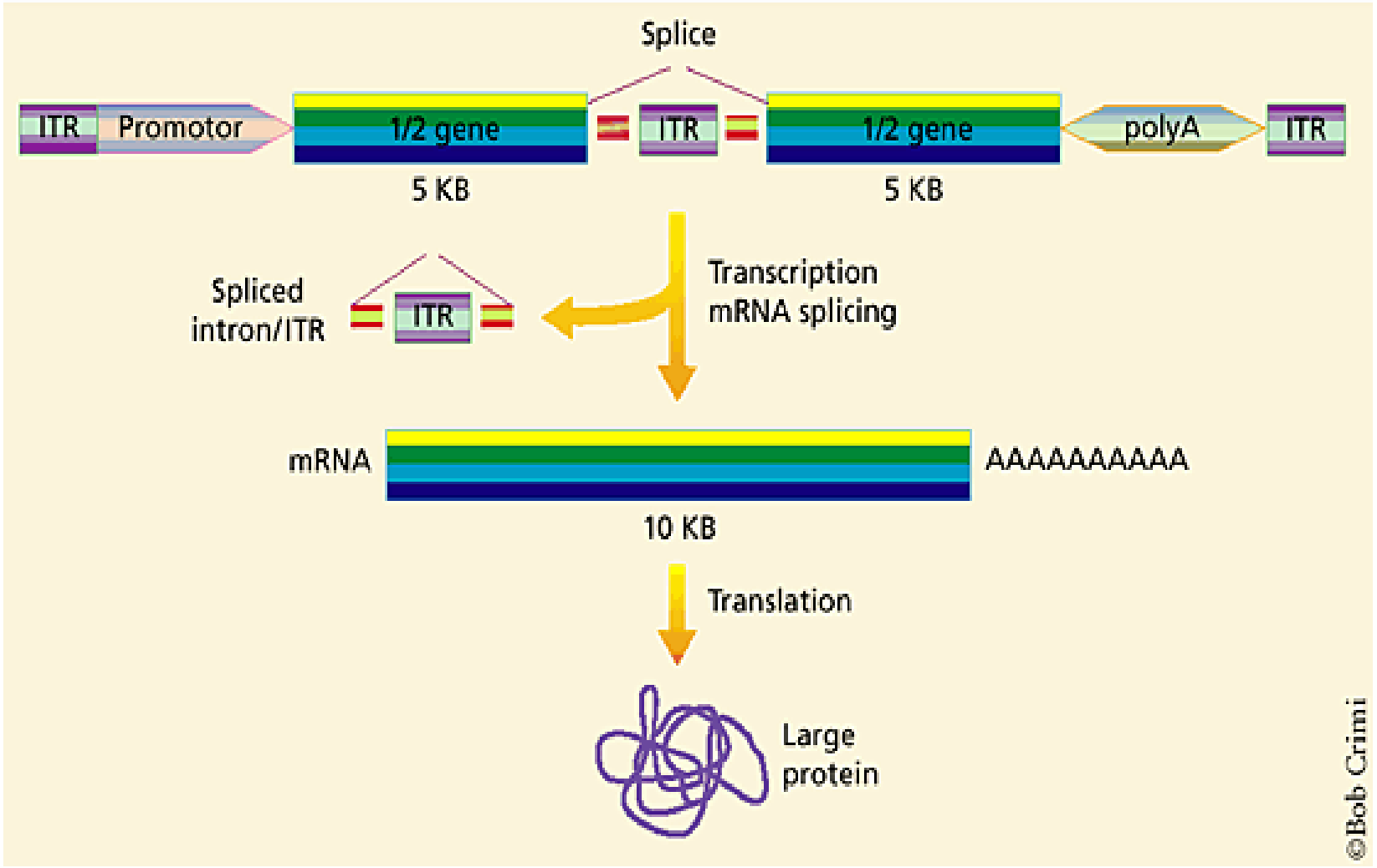
- **unspecific integration** (low efficacy, about 5-10%)

- **episomal expression**

- because of non-immunogenic nature the episomal expression in non-dividing cells can be long-term

How to deal with a small capacity of AAV vectors?

AAV- concatamerisation



AAV serotypes

11 serotypes are known

AAV-2 serotype is the most commonly used

Different serotypes can employ various receptors to enter the cells

- **AAV-2**: heparan sulphate
- **AAV-1** & **AAV-5** – sialic acid
- **AAV-5** co-receptor: PDGF-B receptor

Table 1 | **Key features of viral vectors**

Feature	Adenoviral vector	Helper-dependent adenoviral vector	AAV vector	Retroviral vector	Lentiviral vector
Particle size (nm)	70–100	70–100	20–25	100	100
Cloning capacity (kb)	8–10	~30	4.9 (10 after heterodimerization of two AAV virions)	8	9
Chromosomal integration	No	No	No (yes if *rep* gene is included)	Yes	Yes
Vector yield (transducing units/ml)	High (10^{12})	High (10^{12})	High (10^{12})	Moderate (10^{10})	Moderate (10^{10})
Entry mechanism	Receptor (CAR)-mediated endocytosis, endosomal escape and microtubule transport to the nucleus		Receptor-mediated endocytosis, endosomal escape and transport to the nucleus	Receptor binding, conformational change of Env, membrane fusion, internalization, uncoating, nuclear entry of reverse-transcribed DNA	
Transgene expression and practical application	Weeks to months; highly efficient short-term expression (e.g. for cancer or in acute cardiovascular diseases)	>1 year; highly efficient medium- to long-term expression	>1 year; medium- to long-term gene expression for non-acute diseases (onset of transgene expression after ~3 weeks)	Long-term correction of genetic defects	
Oncolytic potential?	Yes	No	No	No (but has potential to spread through the tumour without lysis, thereby spreading a suicide gene that encodes a pro-drug-converting enzyme)	
Emergence of replication-competent vector *in vivo*?	Possible but not a major concern	Negligible, low risk	Possible but not a major concern	Risk is a concern	Risk is a concern
Infects quiescent cells?	Yes	Yes	Yes	No	Yes
Transcriptional targeting affected by chromosomal integration site?	No	No	No	Yes	Yes
Risk of oncogene activation by the vector?	No	No	No	Yes	Yes

AAV, adeno-associated virus; CAR, coxsackie and adenovirus receptor; Env, viral envelope protein.