Requirement for Gene Therapy

1) Understanding of the disease process

2) Structure/function of gene to be introduced

3) Efficient delivery of gene

4) Control of gene expression

5) Prevention/control of immune responses

6) Animal model and assessment of function

7) Clinical trial

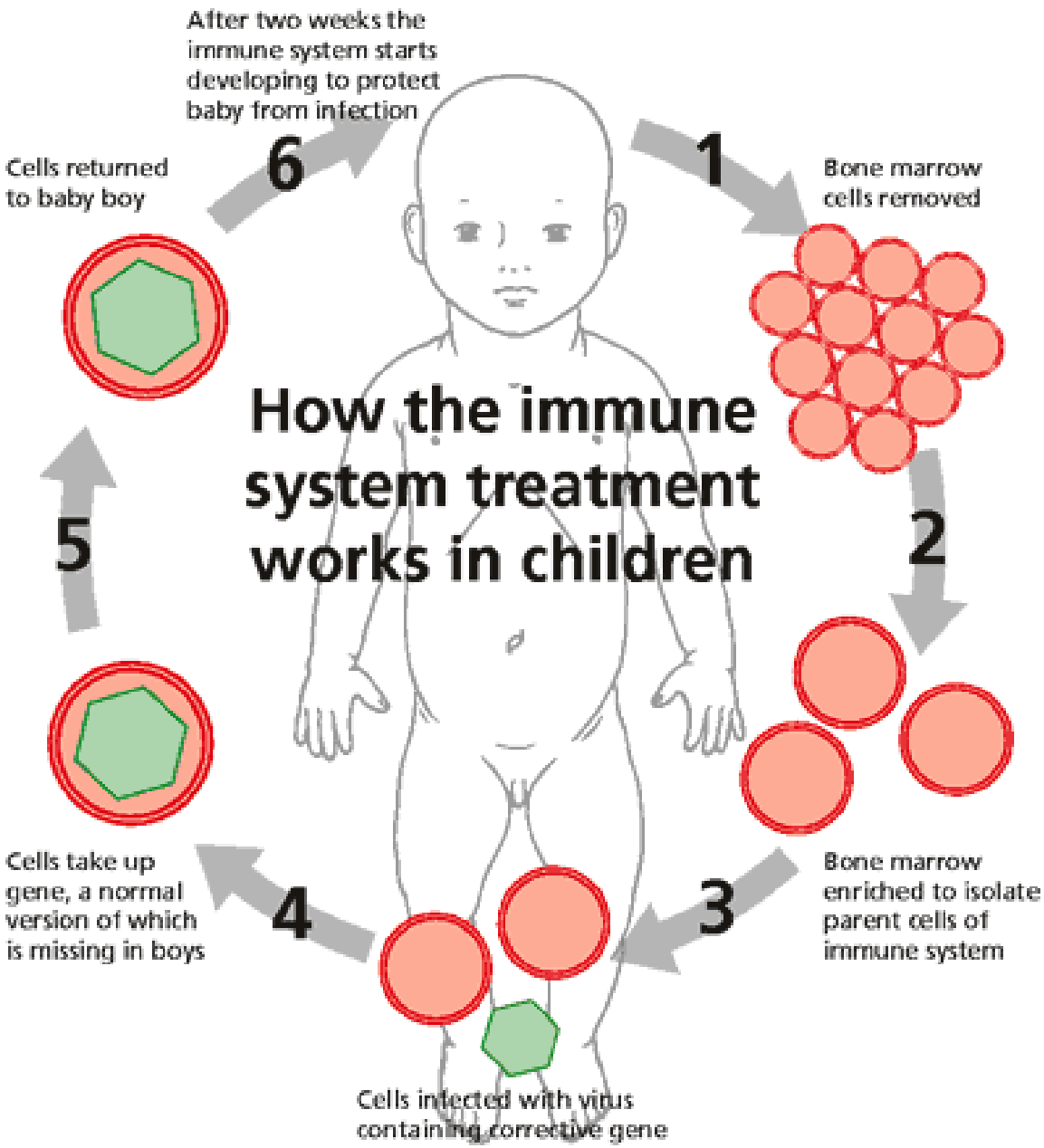
Gene Therapy Strategies

- Gene replacement
- Gene Augmentation Therapy (GAT)
- Gene Correction (Chimeraplasty)
- Targeted killing of specific cells
- Targeted inhibition of gene expression (Gene ablation)

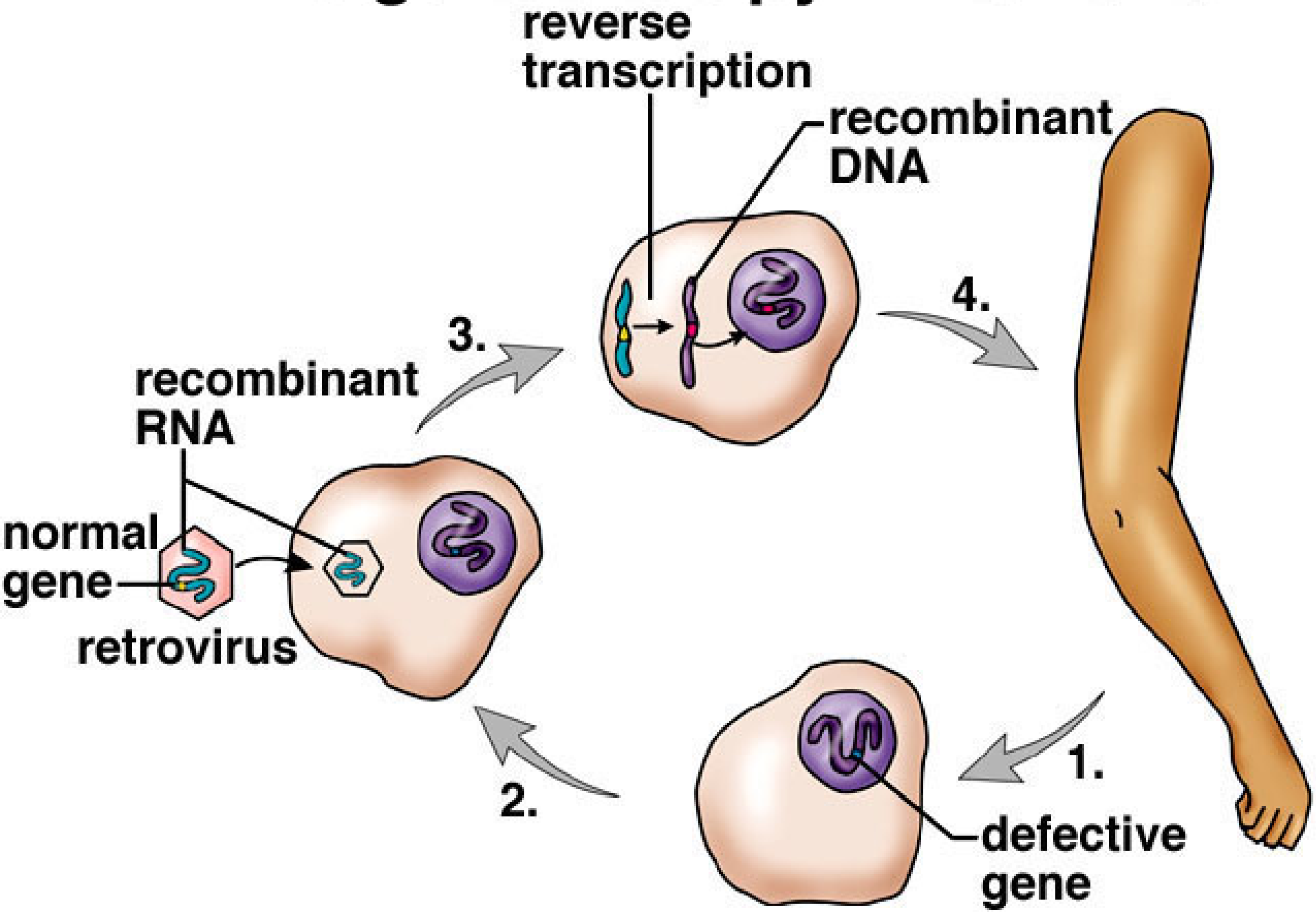
Gene Augmentation

- For diseases caused by **loss** of gene function
- **More copies** of normal gene
- raise levels of gene product
- **restore normal phenotype**
- **Apply to: Monogenic recessive diseases eg. cystic fibrosis, haemophilia, muscular dystrophy**

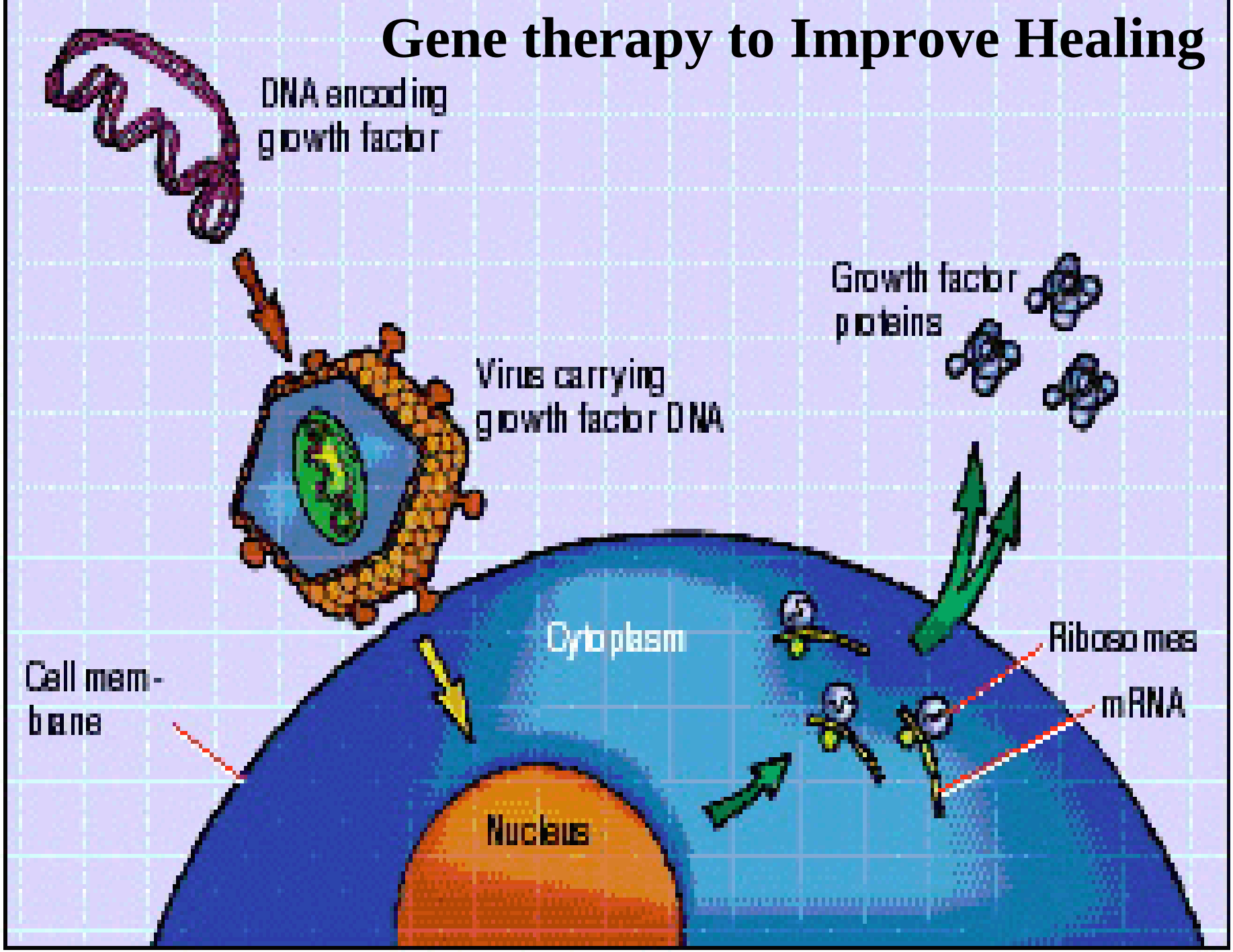
Gene Therapy



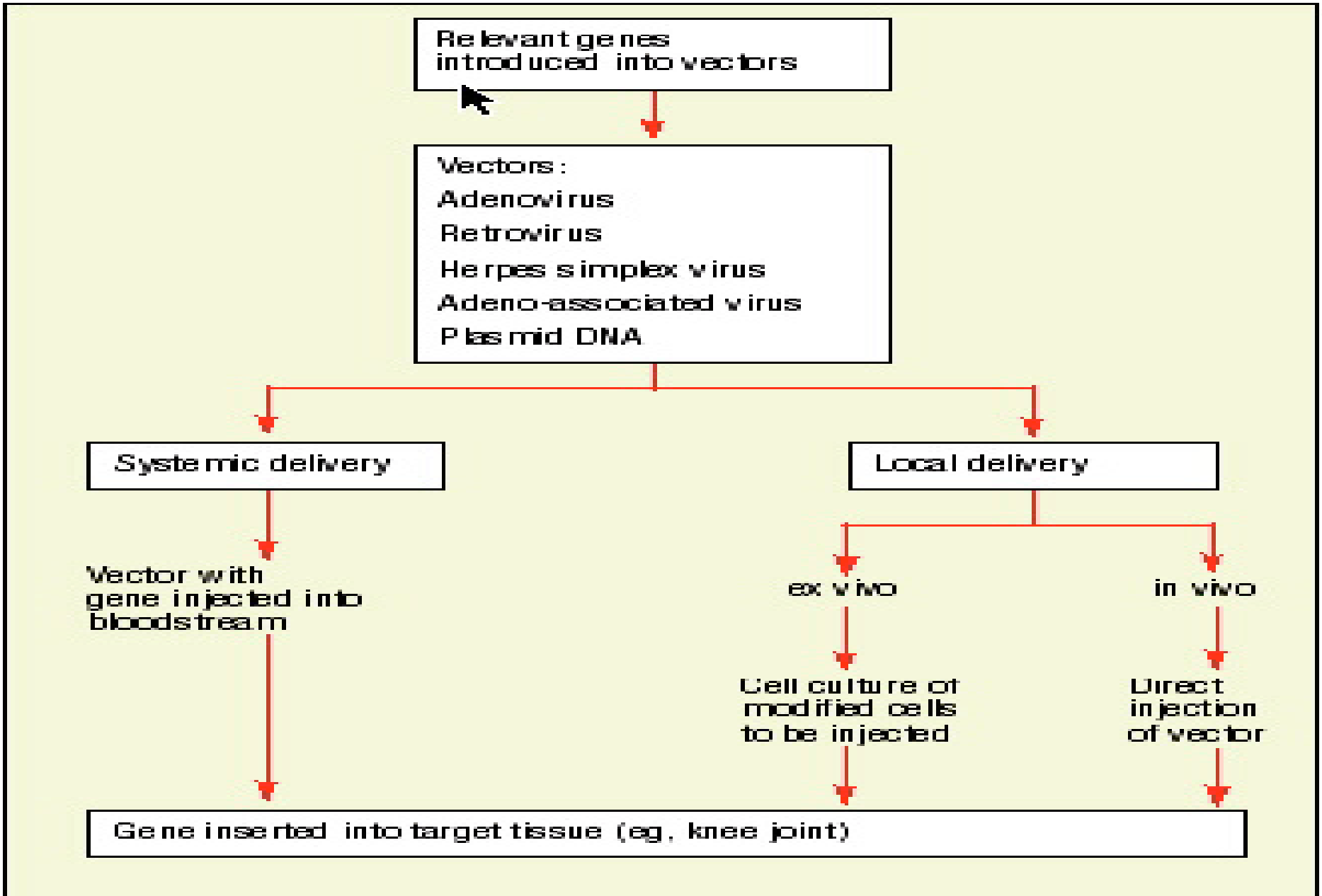
Ex viro gene therapy in humans



Gene therapy to Improve Healing



Gene Therapy- Strategies to deliver Gene to the target tissue



Targeted inhibition

- Ribozymes
  - can cleave (or repair) mRNA
- Triple helix oligonucleotides
  - block gene transcription
- Antisense oligos
  - block mRNA translation

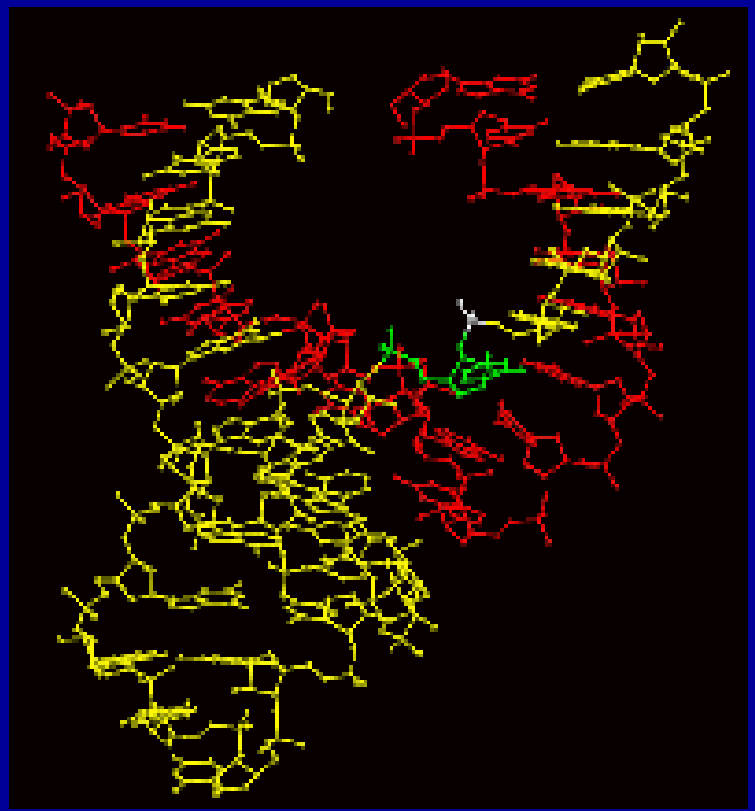
Ribozyme mechanism

Cleavage site

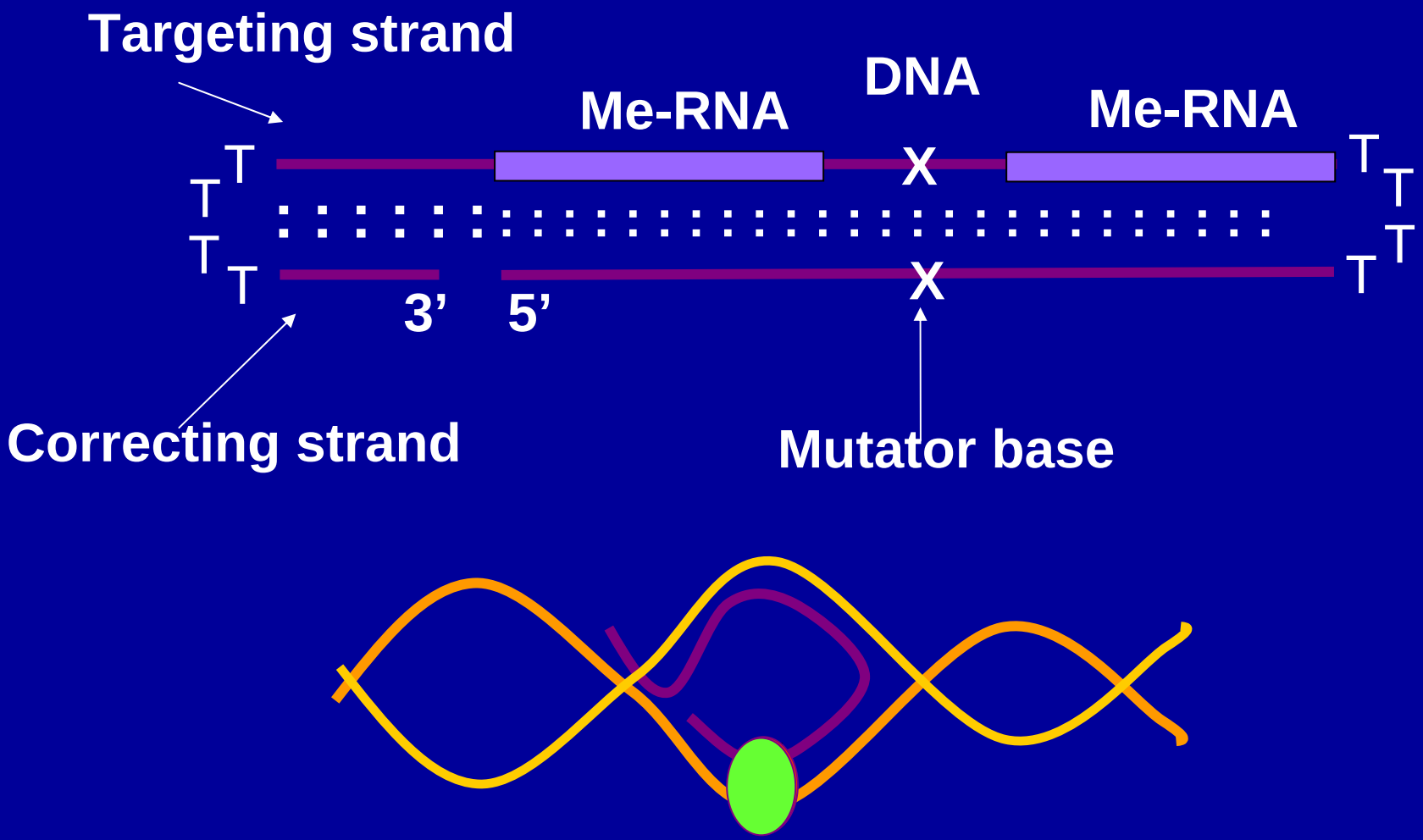
5’- - c g g a g u c a c u u c g - - 3’ mRNA

3’ G C C U C A U G A A G C 5’

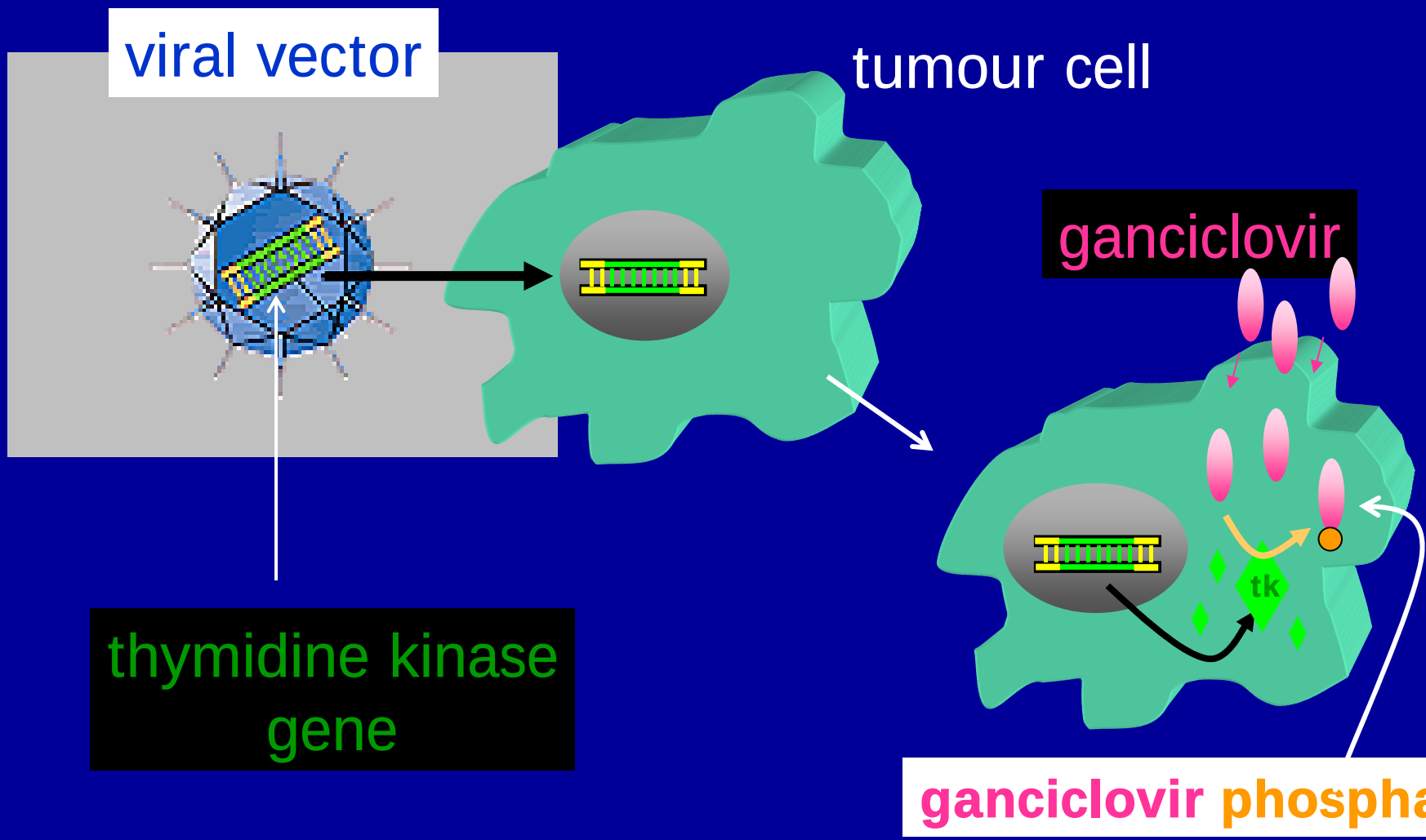
Ribozyme



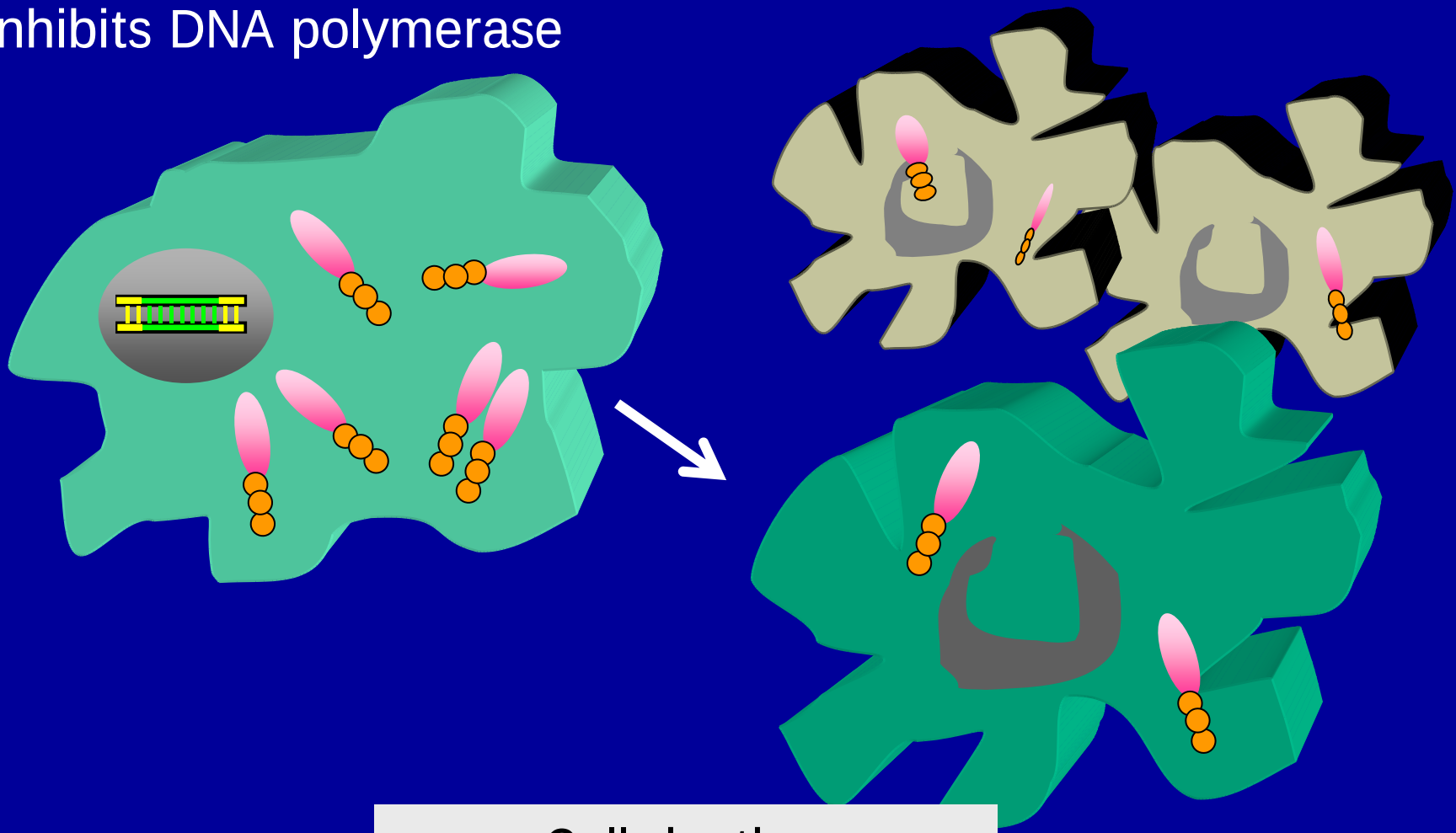
Gene Correction - Chimeraplasty



Targeted Killing - Genetic Pro-drug Activation Therapy



Ganciclovir phosphate inhibits DNA polymerase



Cell death –
Including bystander cells

Applications: Target tissues

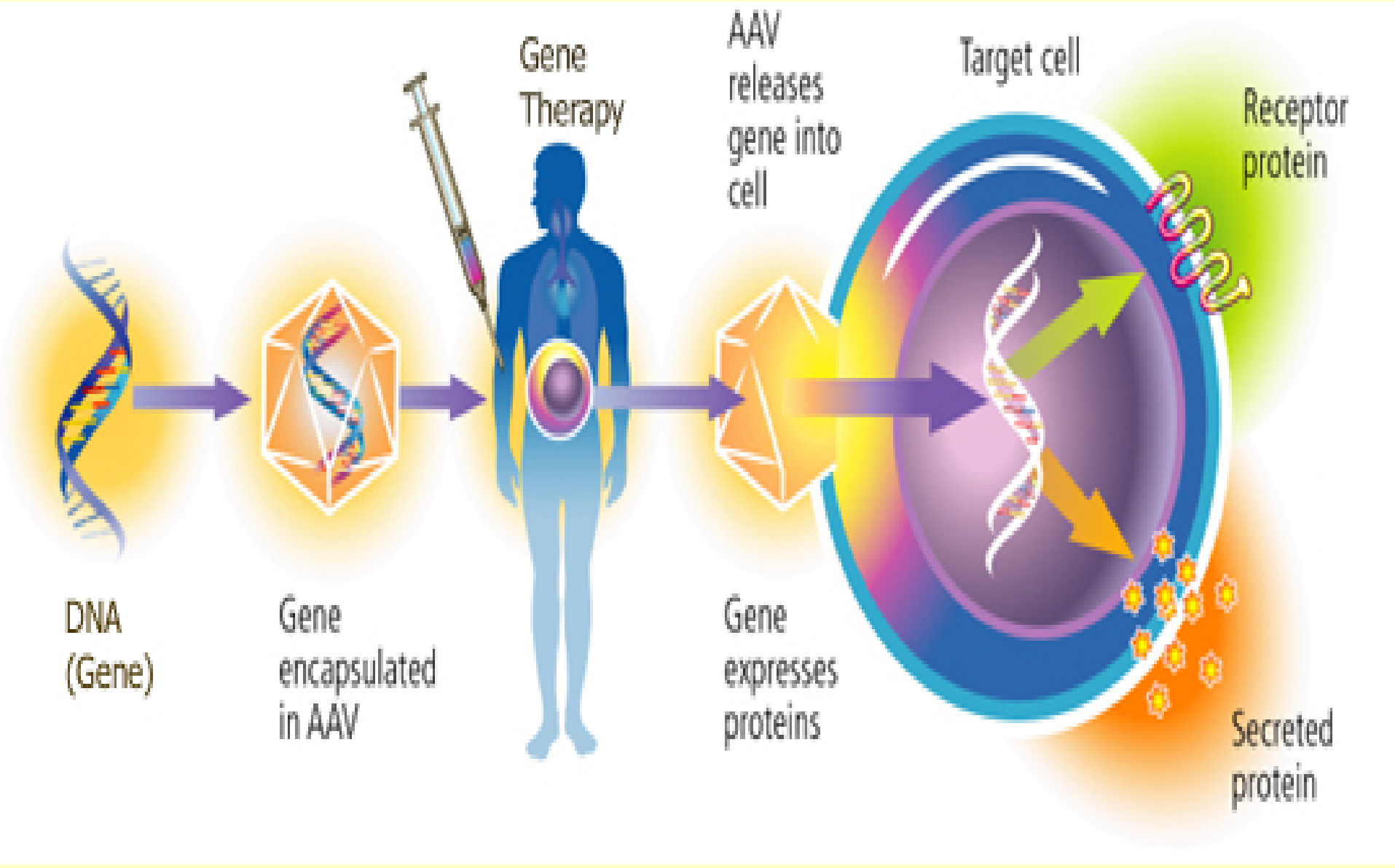
- Haematopoietic cells – easiest
- Muscle, Liver - good targets
- Eye retina – accessible but fragile
- Brain (neurons) – v. difficult
- Tumours – access to interior may be difficult

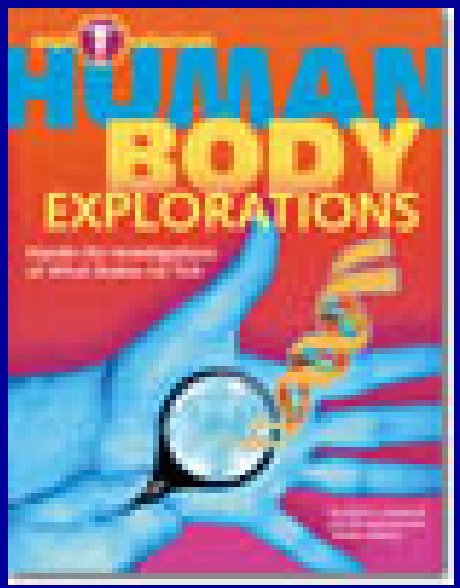
Problems with GT

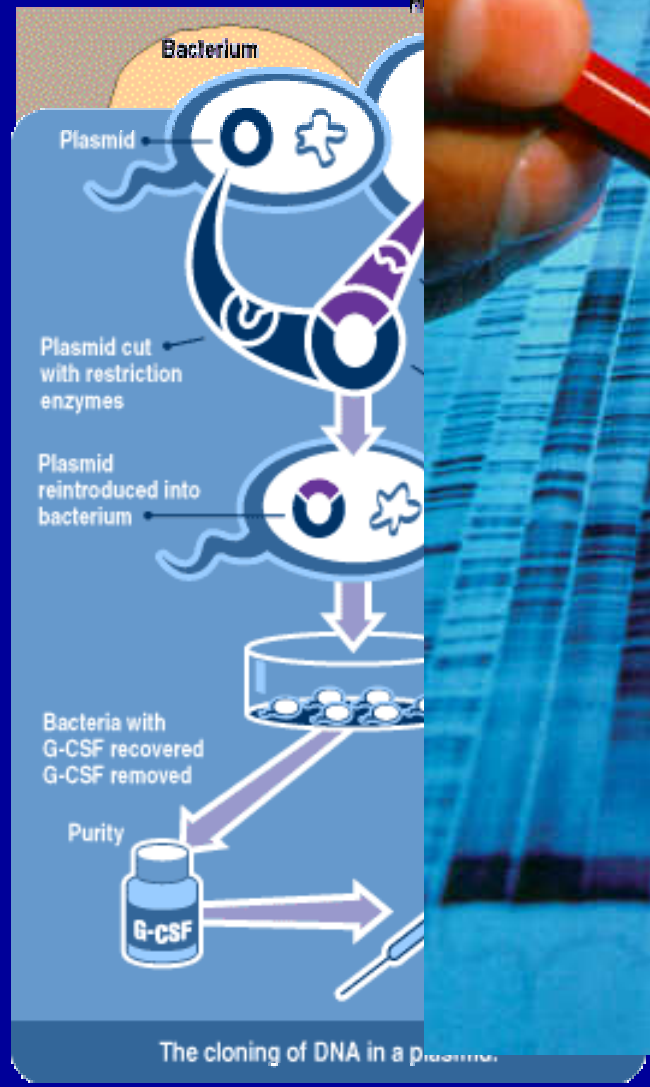
- **Safety:**
  - **Toxicity**
  - **Immune response**
  - **Integration**
    - **Malignant transformation**
    - **Germline integration**
  - **Viral replication through recombination**
- **Efficacy:**
  - **Target cell uptake**
  - **Control of gene expression**

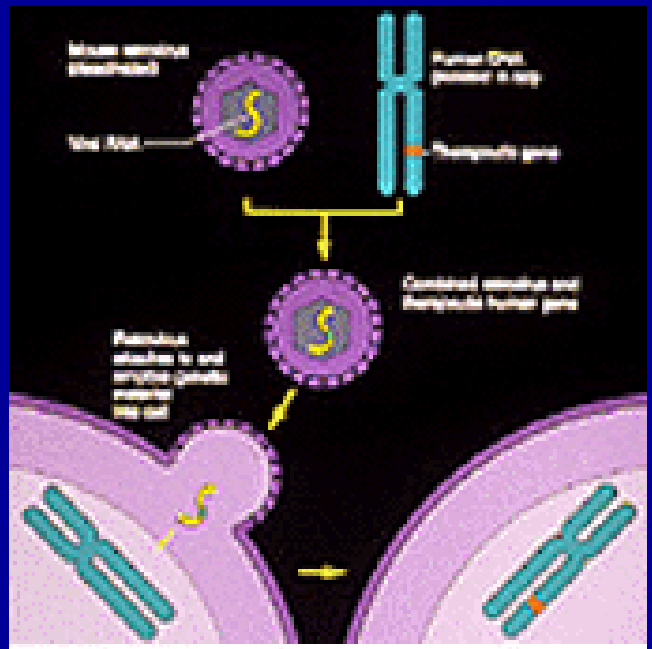
Conclusion

- Gene Therapy is still in its infancy
- Early promise and hope not fulfilled
- Risks, including death, seen as a major issue
- But, recent trials look more promising


DNA
(Gene)
Gene
encapsulated
in AAV
Gene
Therapy
AAV
releases
gene into
cell
Gene
expresses
proteins
Target cell
Receptor
protein
Secreted
protein


HUMAN
BODY
EXPLORATIONS


Bacterium
Plasmid
Plasmid cut with restriction enzymes
Plasmid reintroduced into bacterium
Bacteria with G-CSF recovered G-CSF removed
Purity
G-CSF



Gene therapy has been attempted to treat severe combined immunodeficiency caused by a missing enzyme, adenosine deaminase. [Image credit: National Cancer Institute.]

Goal of gene therapy:

1. **Management and correction of human diseases**
   - **a. Inherited and acquired disorders**
   - **b. cancer**
   - **c. AIDS/HIV**

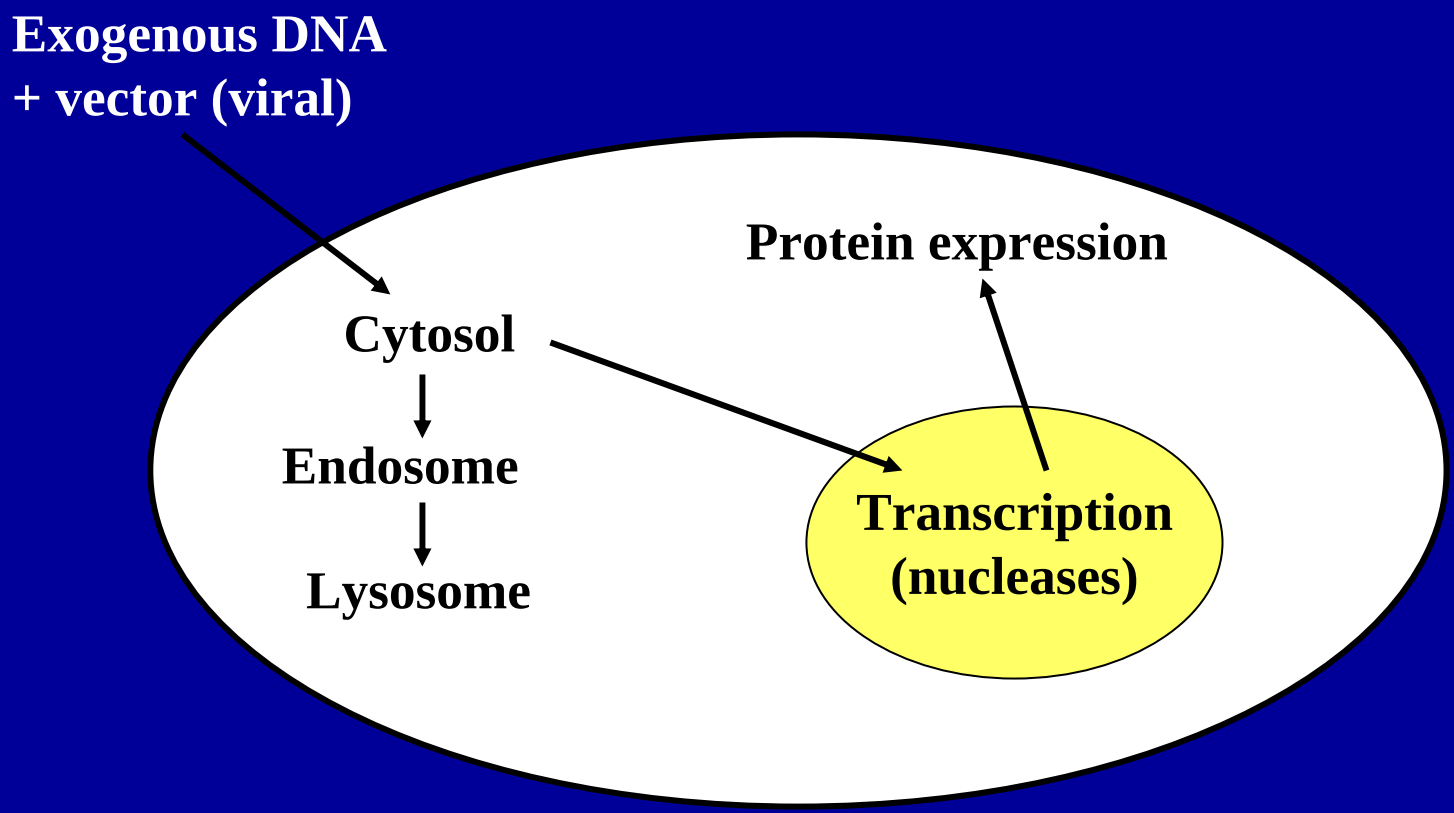
<u>Good news</u>: Promising advances during the last two decades in recombinant DNA technology.

1. **Recent success in treating SCID.**

<u>Bad news</u>: (Until recently) Efficacy in any gene therapy protocol not definitive.

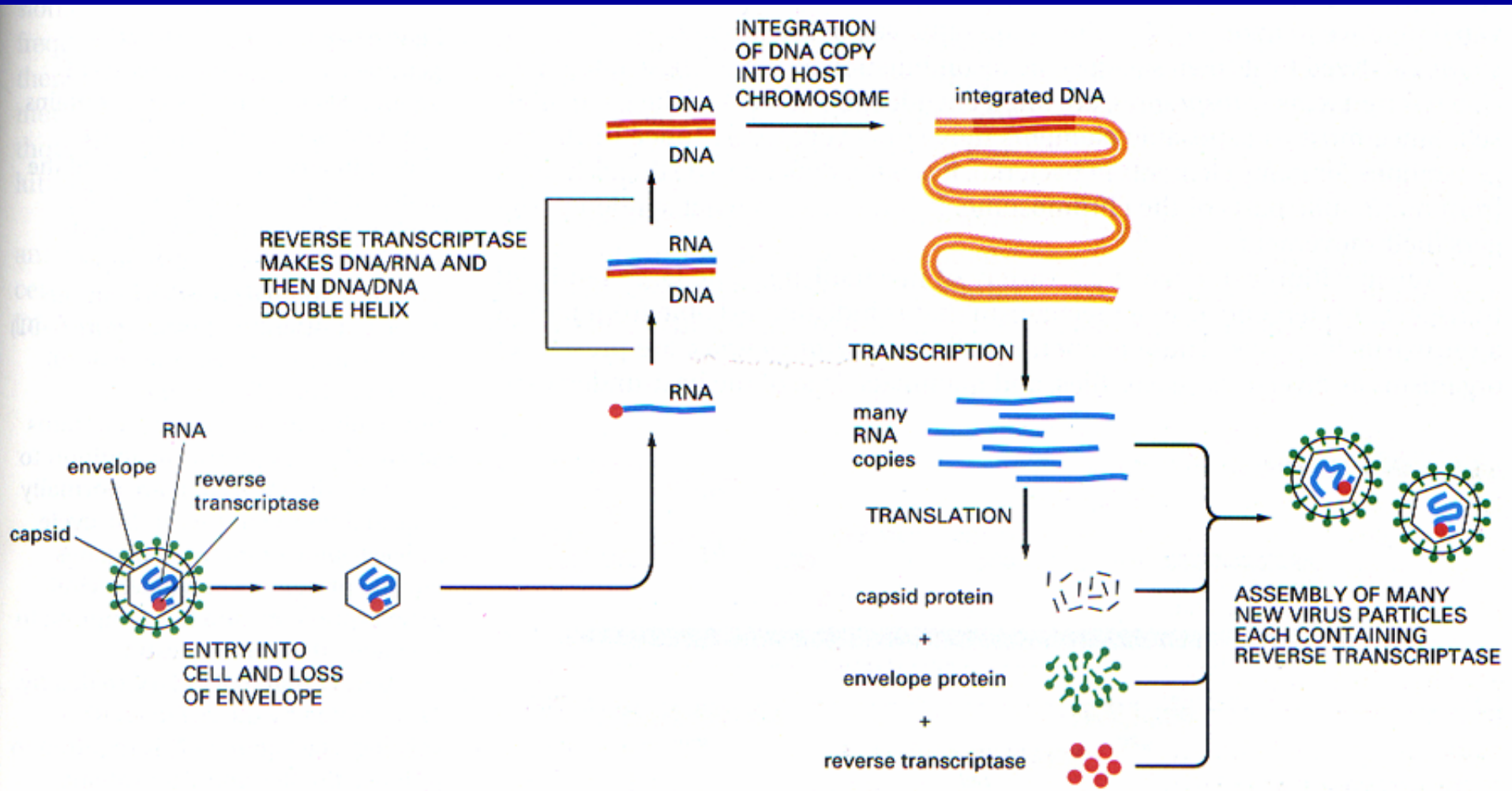
1. **Shortcomings in gene transfer vectors.**
2. **Inadequate understanding of biological interactions of vector and host. (Jesse Gelsinger case).**

Gene Transfer

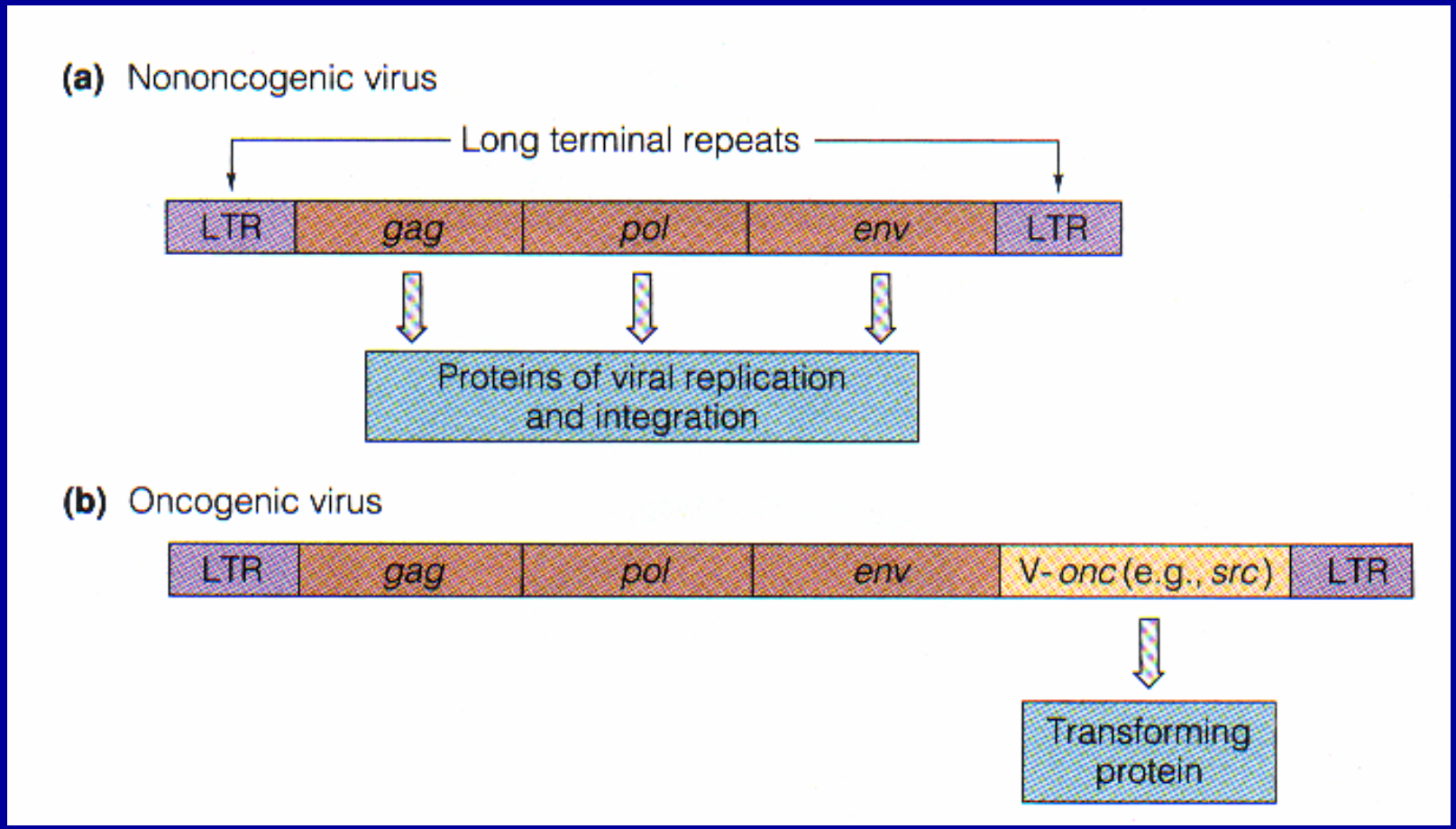


Barriers that prevent transfer of exogenous DNA

Retrovirus Life Cycle



Retrovirus Genome



Retrovirus vector construction for gene therapy

5' LTR	Packaging	Gene X	Neo^r	3' LTR

Retrovirus vector packaging for gene therapy

Packaging cell line express viral *gag* and *pol*
Produce high titer recombinant virus in 24-72 hours.

1. **Packaging cell line (293s)** **+** **Vector (transfection)**

2. **Collect virus after 24-72 hrs.**
3. **Concentrate virus / high titer.**
4. **Transduce host cell.**

Summary of commonly used vectors

	(63%) **Retrovirus**	(16%) **Adenovirus**	(2%) **Adeno-associated**	(13%) **Naked DNA/ Liposomes**
Insert size	8kb	~30kb	4kb	unlimited
Integration	yes	no	rare	rare
Production	$>10^6$cfu/ml	$>10^{11}$	$>10^{12}$	unlimited
Administration	*ex vivo*	*ex/in vivo*	*ex/in vivo*	*ex/in vivo*
Expression	long	transient	pot. good?	Transient
Express level	moderate	high	moderate	high
Immune	few	extensive	??	None
Safety concerns:	Insertional mutagenesis	Inflammatory response,	Inflammatory toxic response,	none toxic

Non-viral DNA carriers:

1. Cationic liposomes: Positively charged lipids interact with negatively charged DNA. (lipid-DNA complex).

-Transverses cell membranes

Advantages:

a. Stable complex
b. Can carry large sized DNA
c. Can target to specific cells
d. Does not induce immunological reactions.

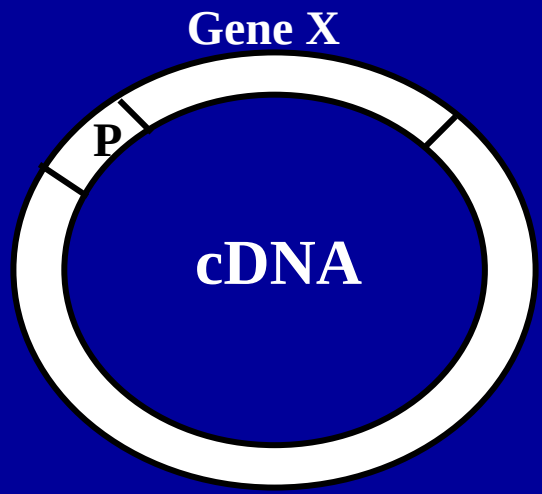
Disadvantages:

a. Low transfection efficiency
b. Transient expression
c. Inhibited by serum
d. Some cell toxicity

Non-viral DNA carriers:

2. Naked plasmid DNA injection

Promoter + gene of interest
(P) (gene X)



Expression observed in thymus, skeletal and cardiac muscle, skin.

Gene therapy to turn off genes:

Antisense approach:

***DNA makes mRNA, mRNA makes protein. antisense complements mRNA (sense) and prevents protein expression.**

***Small interfering RNA (siRNA) molecules.**

Categories of clinical gene transfer protocols.

1. **Inherited/monogenic disorders:**
   - ADA deficiency
   - Alpha-1 antitrypsin
   - Chronic granulomatous disease
   - Cystic fibrosis
   - Familial hypercholesterolemia
   - Fanconi Anemia
   - Gaucher Disease
   - Hunter syndrome
   - Parkinsons
2. **Infectious Diseases:**
   - HIV
3. **Acquired disorders:**
   - peripheral artery disease
   - Rheumatoid arthritis

contd…

Categories of clinical gene transfer protocols.

4. Cancer (by approach):

- Antisense
- Chemoprotection
- Immunotherapy: *ex vivo / in vivo*
- Thymidylate kinase
- Tumor suppressor genes