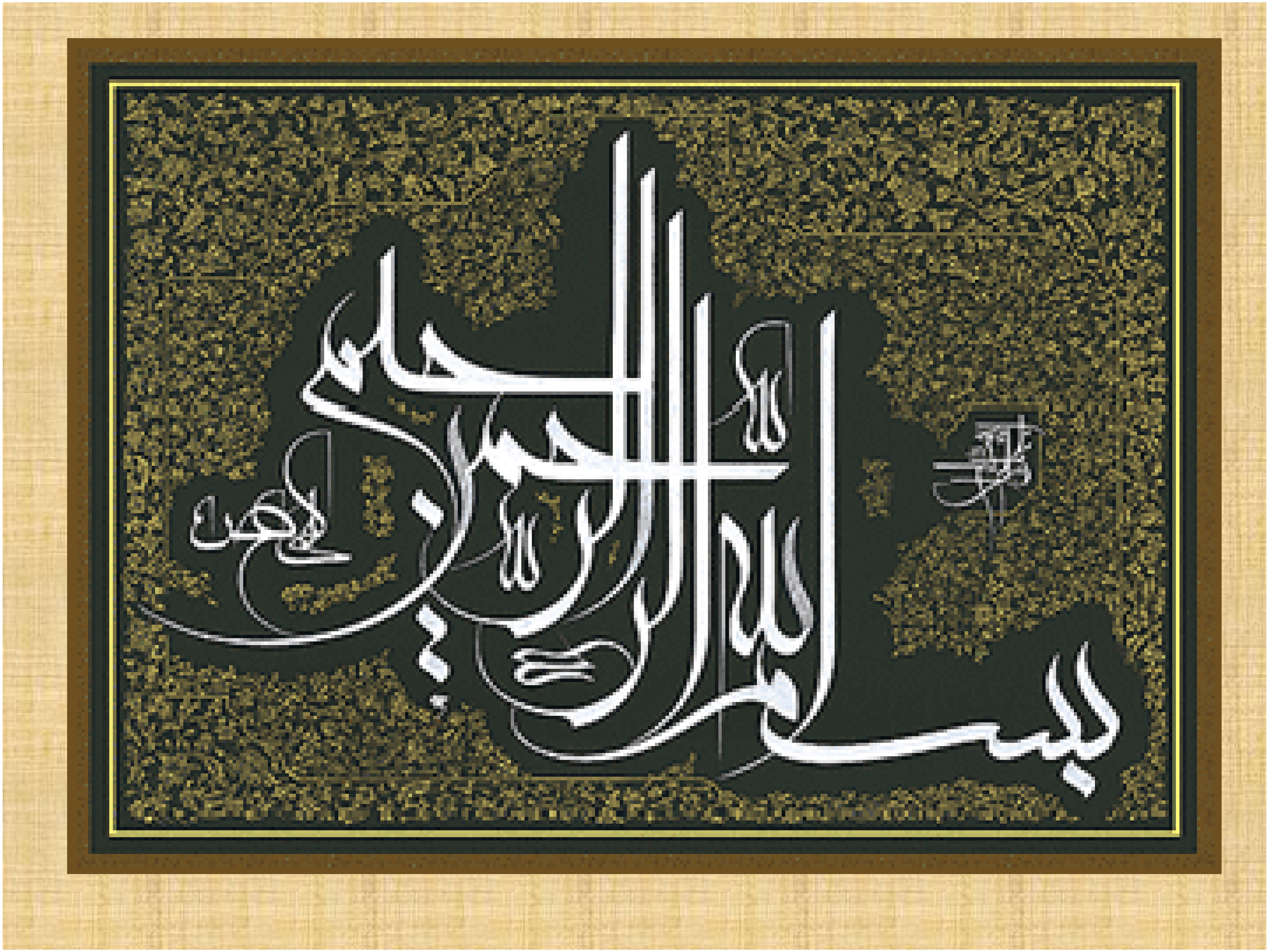
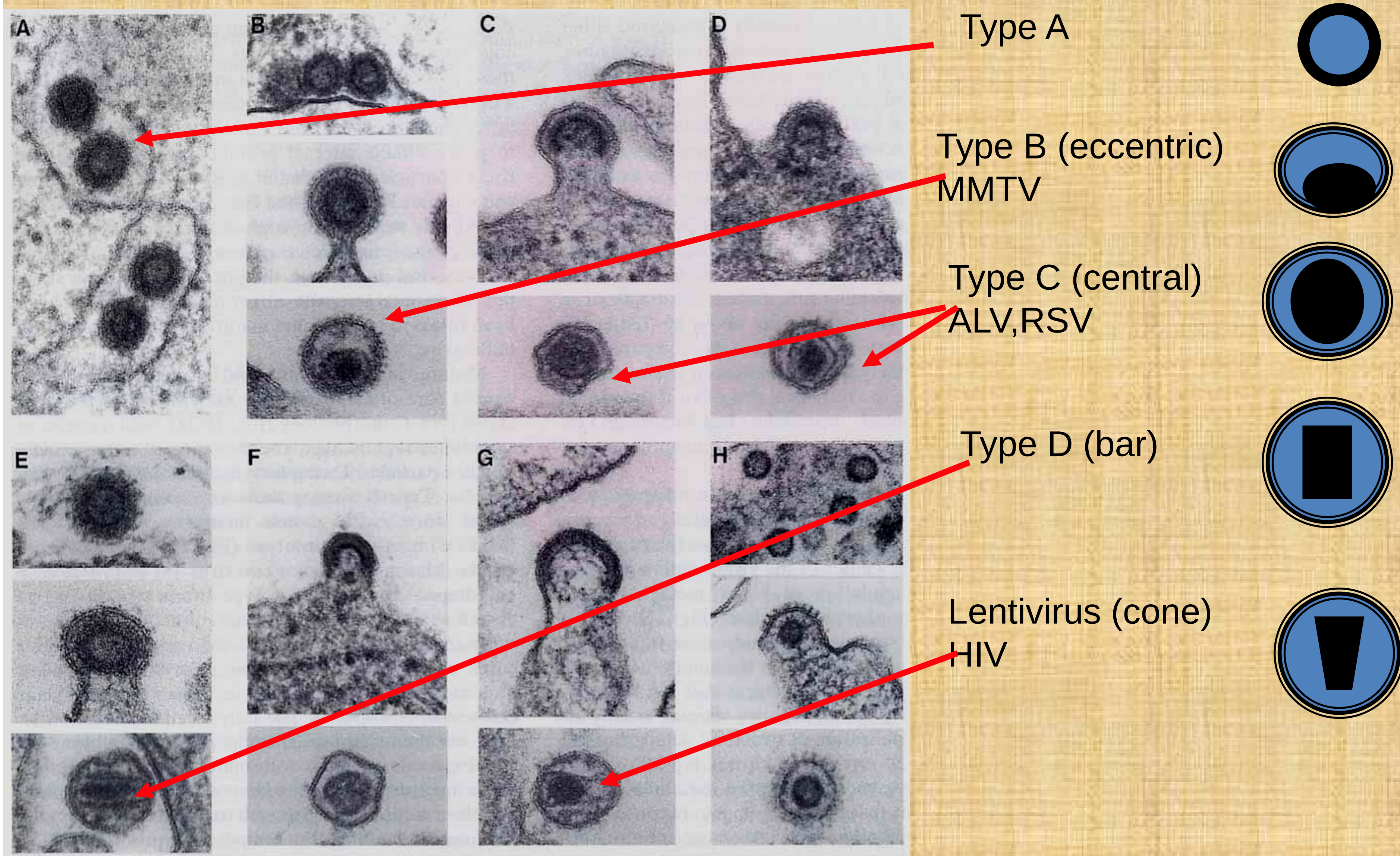

بسم الله الرحمن الرحيم

Retroviridae

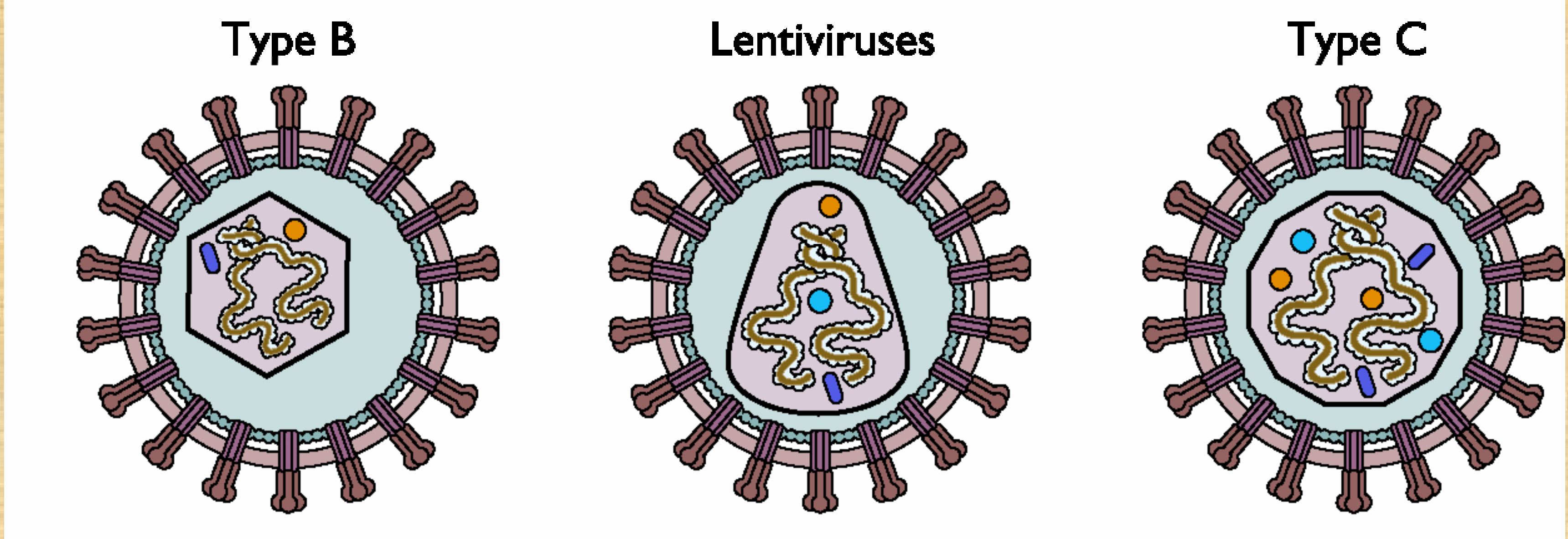
- Retroviruses (family *Retroviridae*) are enveloped, single stranded (+) RNA viruses that replicate through a DNA intermediate using reverse transcriptase.
- This large and diverse family includes members that are oncogenic, are associated with a variety of immune system disorders, and cause degenerative and neurological syndromes.

Retrovirus Virions

Thin Section EM of Some Retroviruses



Retrovirus structure



• Retrovirus virions are 80-120 nm in diameter, have spherical morphology, a phospholipid envelope with knobs

• Contain around 2000 molecules of nucleocapsid (NC) protein that bind to the two copies of (+) strand RNA genome

•Retroviral ribonucleoproteins are encased within a protein shell built from the capsid protein to form an internal core, which can have different shapes and has a conical shape in HIV

Retrovirus Structure and Function

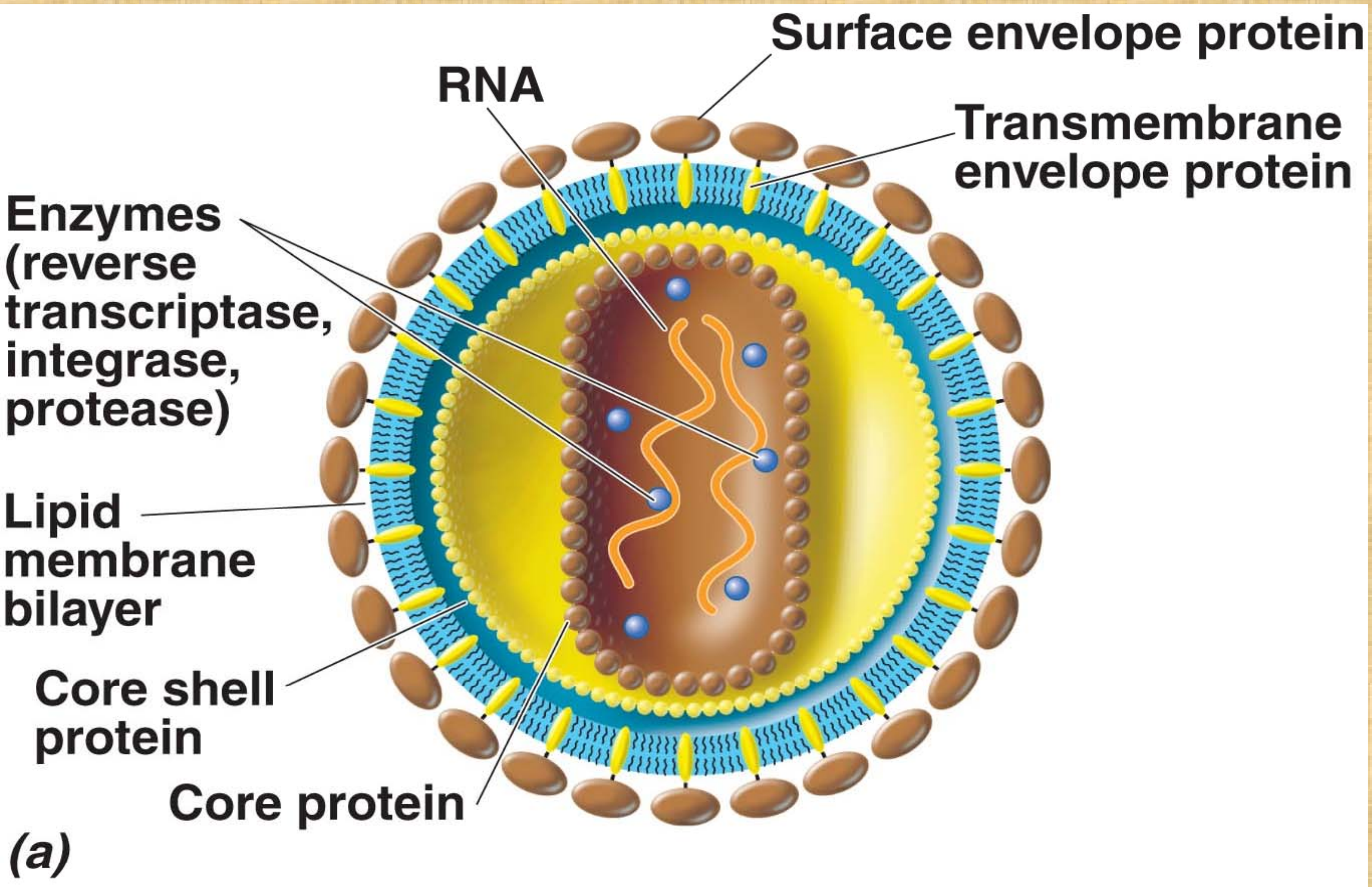


Figure 10.23a

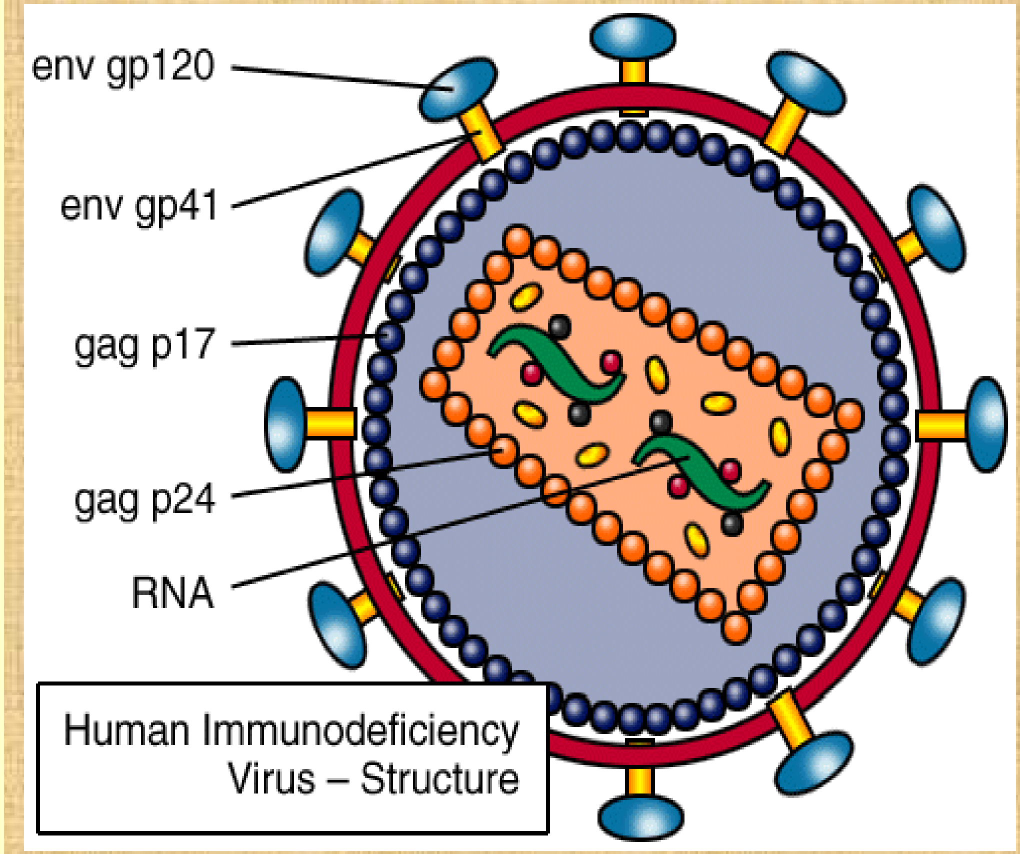
Retrovirus Classification
Family: *Retroviridae*

Genus	Features	Examples
1. *Alpharetrovirus*	Simple, Onco	Avian leucosis virus, RSV
2. *Betaretrovirus*	Simple, Onco	Mouse Mammary Tumor Virus
3. *Gammaretrovirus*	Simple, Onco	Murine leukemia virus (Moloney, Harvey)
4. *Deltaretrovirus*	Complex, Onco	Bovine Leukemia, Human T Cell Leukemia (HTLV)
5. *Epsilonretrovirus*	Complex, Onco	Walleye Dermal Sarcoma
6. *Lentivirus*	Complex	HIV
7. *Spumavirus*	Complex	Simian Foamy Virus

- **RNA virus, 120nm in diameter**
- **Envelope gp160; gp120 & gp41**
- **Icosahedral symmetry**
- **Nucleocapsid**
  - **Outer matrix protein (p17)**
  - **Major capsid protein (p24)**
  - **Nuclear protein (p7)**
- **Diploid RNA with several copies of reverse transcriptase**

Family : Retroviridae

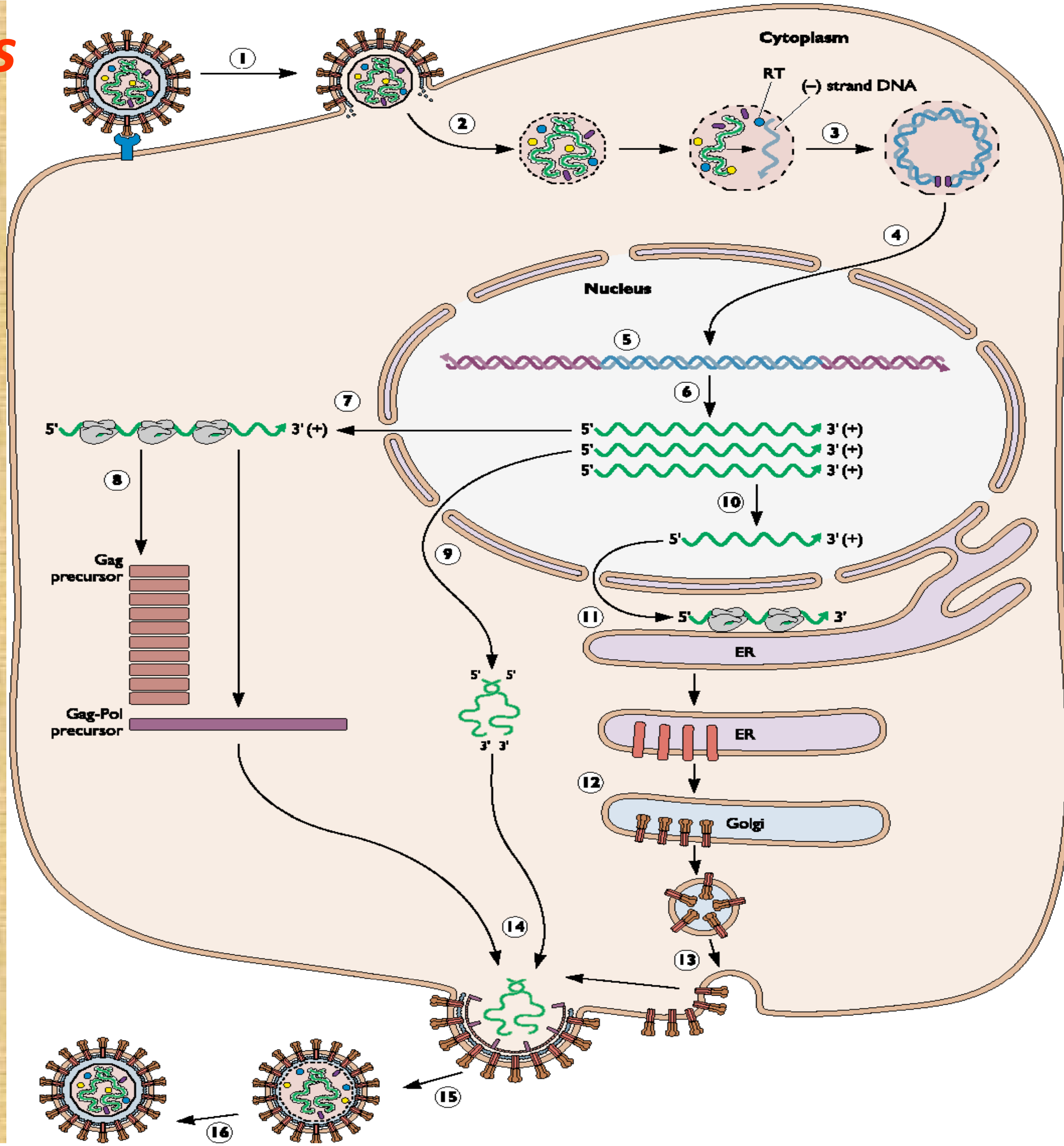
Subfamily : Lentivirus



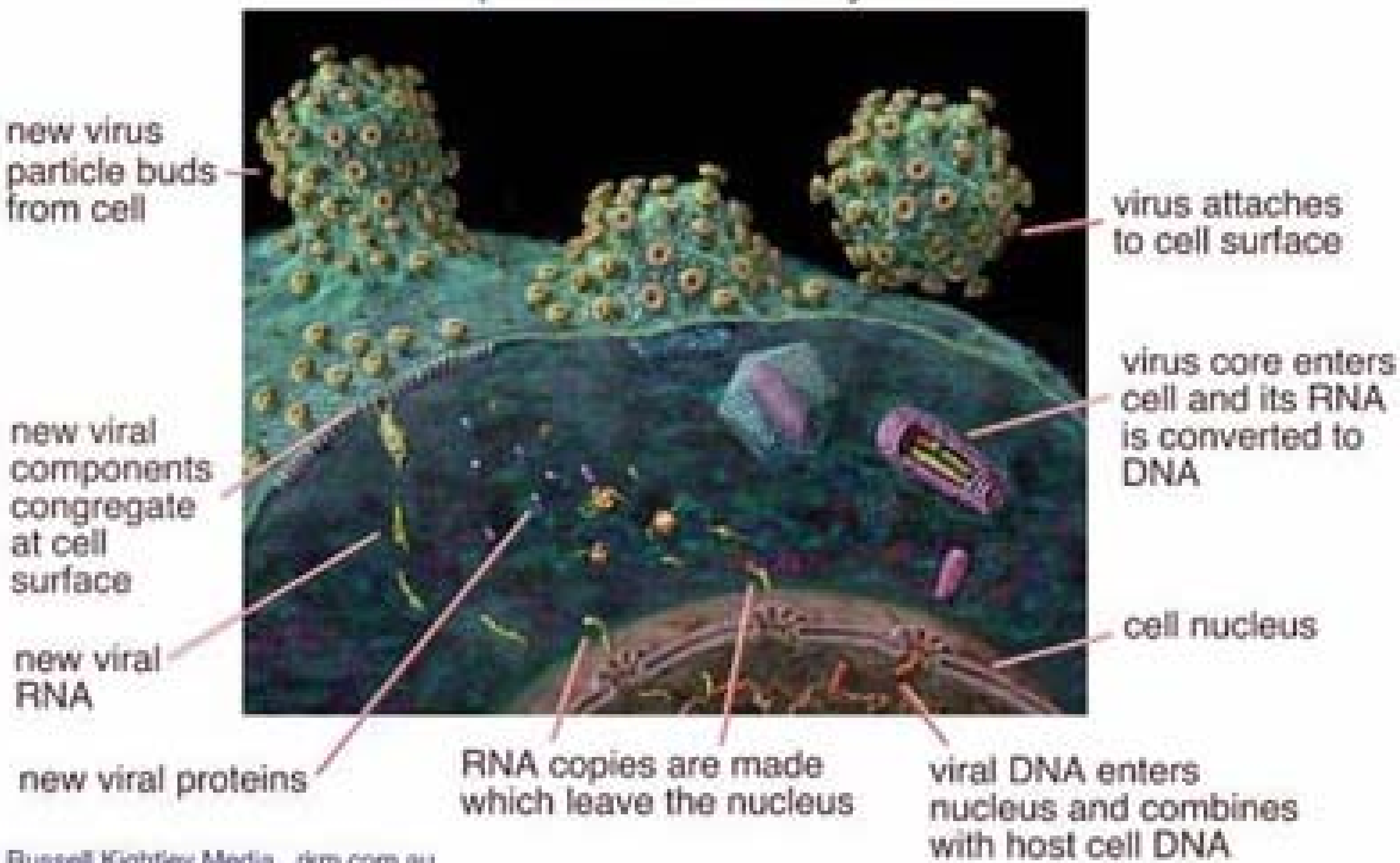
Retrovirus replication cycle

1. **Attachment of the virion to a specific cell surface receptor**
2. **Penetration of the virion core into the cell**
3. **Reverse transcription within the core structure to copy the genome RNA into DNA**
4. **Transit of the DNA to the nucleus**
5. **Integration of the viral DNA into random sites in cellular DNA to form the provirus**
6. **Synthesis of viral RNA by cellular RNA polymerase II using the integrated provirus as a template**
7. **Processing of the transcripts to genome and mRNAs**
8. **Synthesis of virion proteins**
9. **Assembly and budding of virions**
10. **Proteolytic processing of capsid proteins**

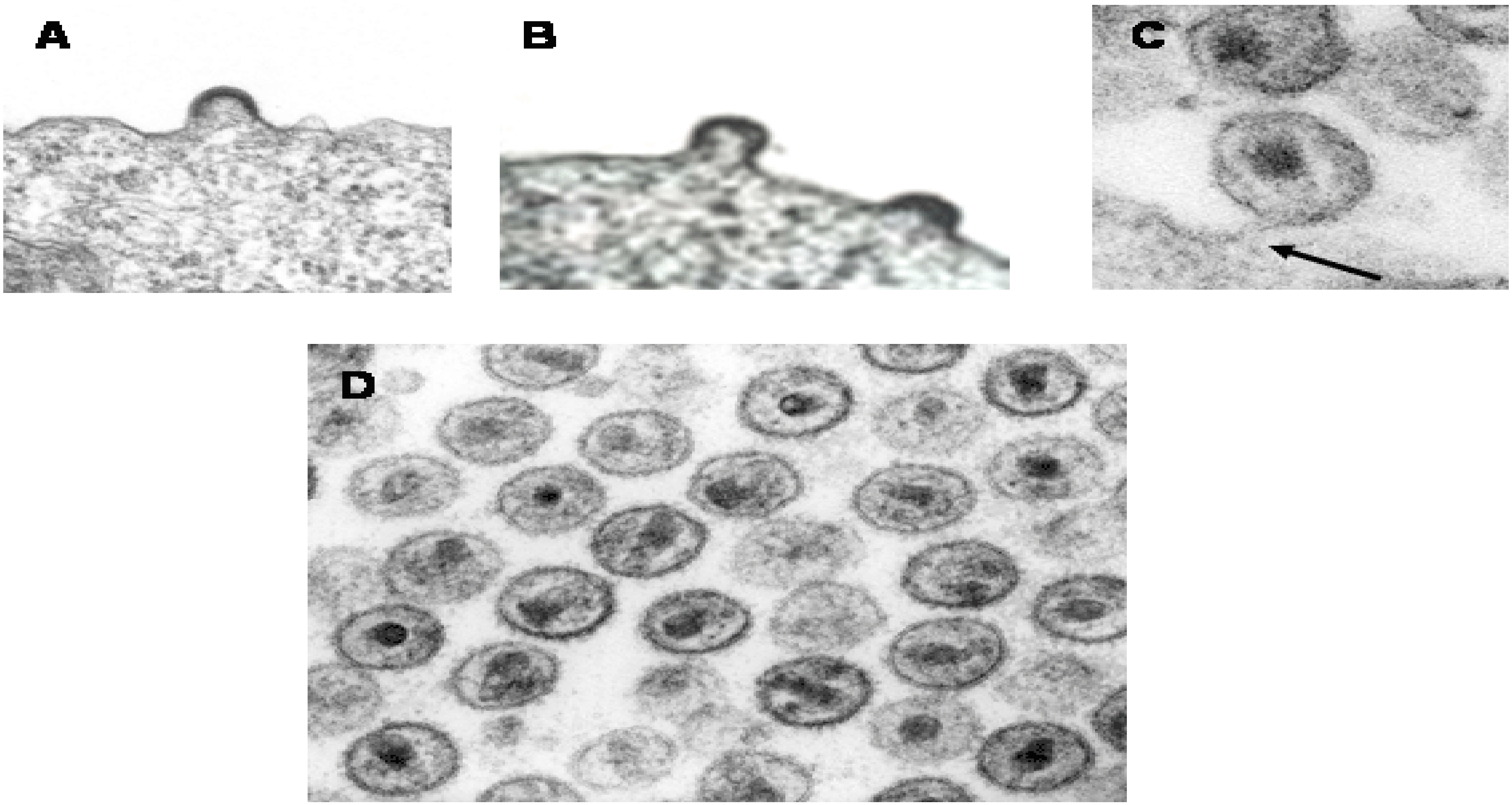
Retrovirus life cycle:



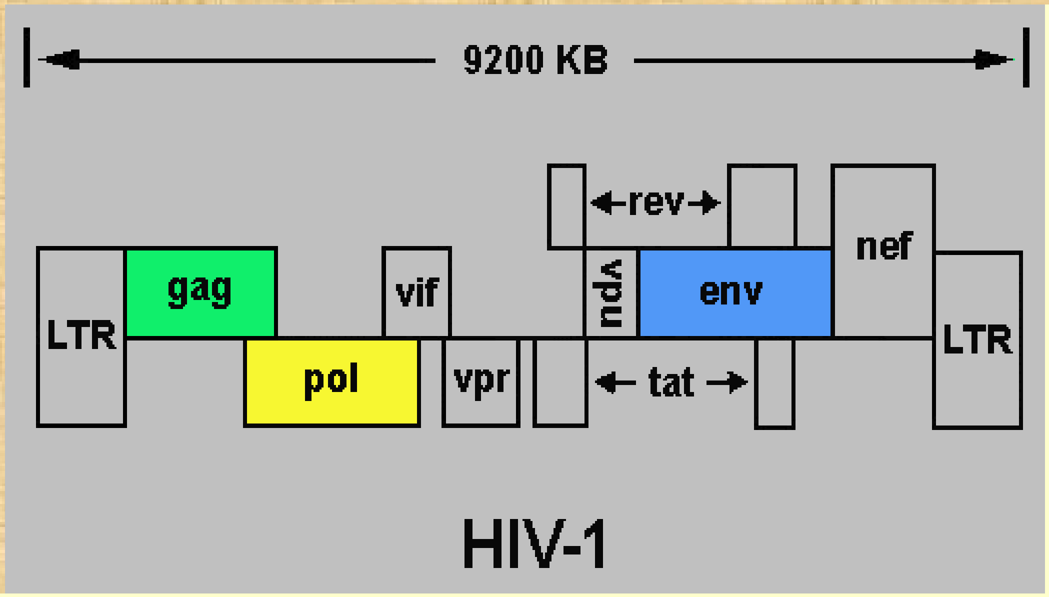
simplified HIV life-cycle



Electron micrograph of HIV budding



HIV Genome



- 9200 nucleotides (HIV-1):
- env - gp160 (gp120:outer membrane part, gp41: transmembrane part)
- gag core proteins – p55, p17 and p24
- pol – p66 (protease), p31,p51 (integrase/endonuclease)

EARLY **Accessory Genes** LATE

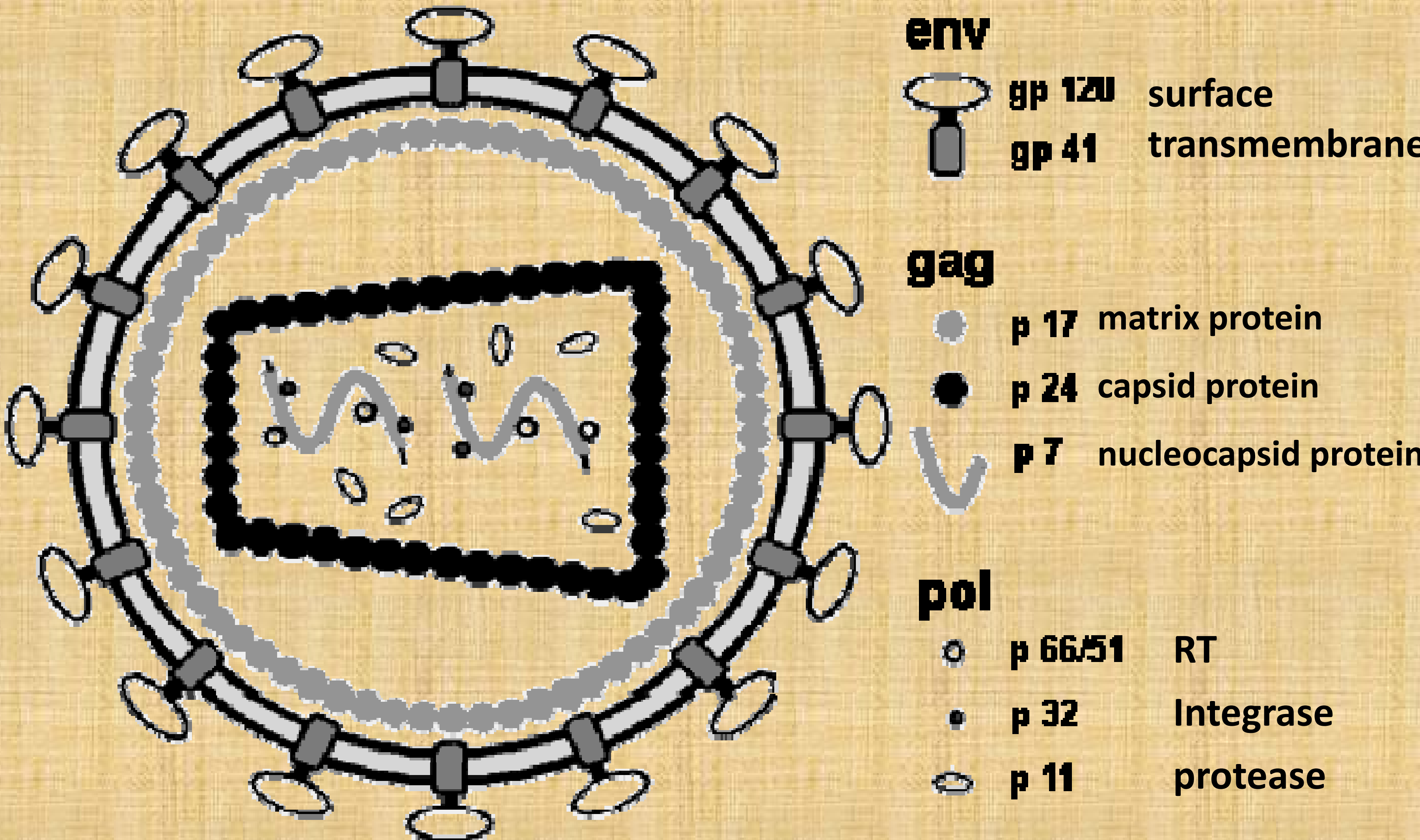
tat - trans activator of transcription **vif** - viral infectivity factor

vpr- viral protein R

rev- regulator of viral protein expression **vpu**- viral protein U

nef – negative regulatory factor **vpx** – HIV - 2 TAT and REV are essential for HIV replication

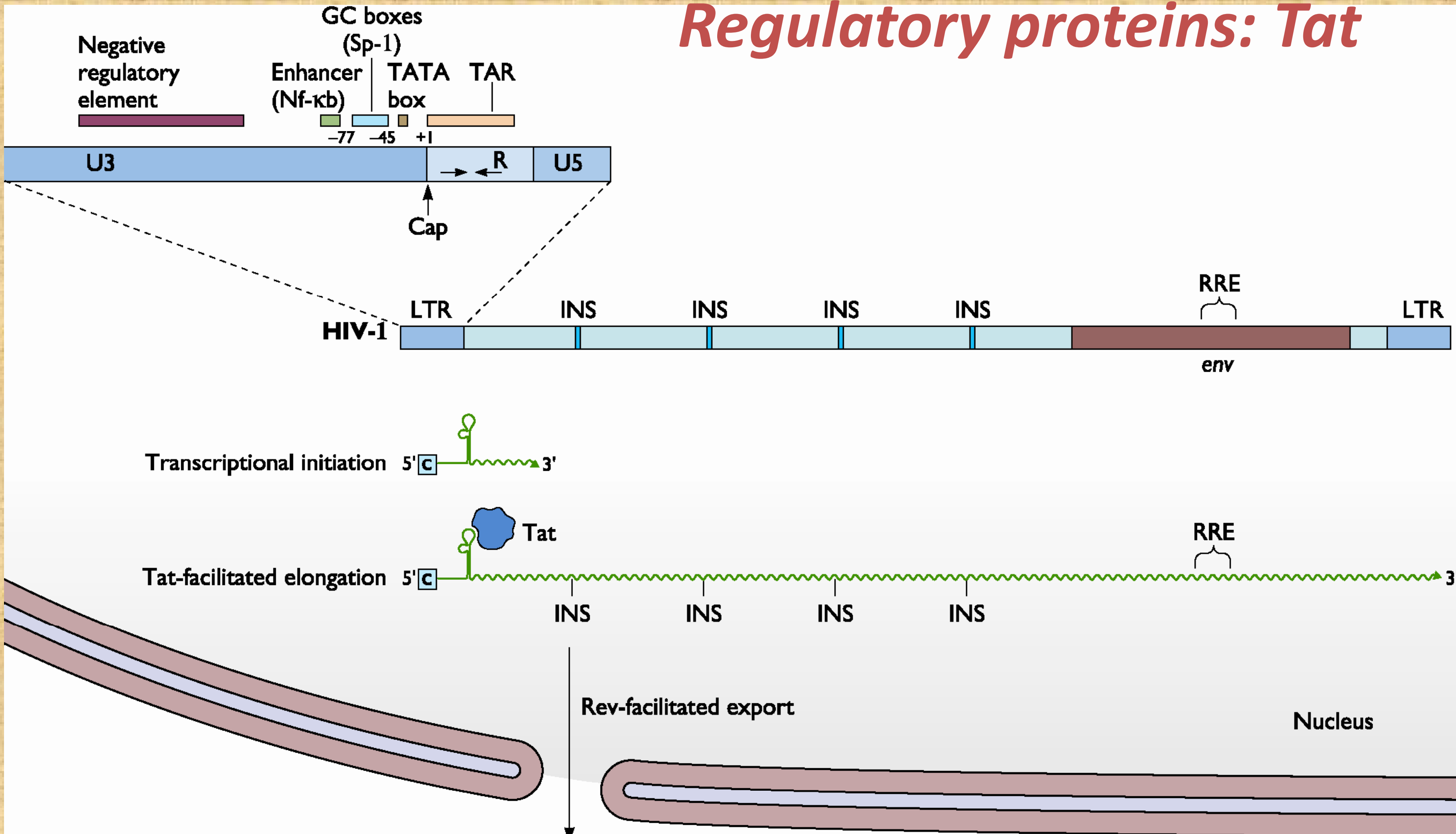
HIV Structure



Retroviral Proteins

- ***gag*, *pol*, *pro* and *env***
  - **Gag protein proteolytically processed into**
    - **MA (matrix)**
    - **CA (capsid)**
    - **NC (nucleocapsid)**
  - **Pol protein encodes enzymes**
    - **RT (Reverse Transcriptase which has both DNA polymerase and RNase H activities)**
    - **IN (Integrase)**
  - **Env protein encodes**
    - **SU surface glycoprotein**
    - **TM transmembrane protein**
  - **Pro (protease)**
- **“Accessory” genes (in Complex Retroviruses) - regulate and coordinate virus expression; function in immune escape**

Regulatory proteins: Tat

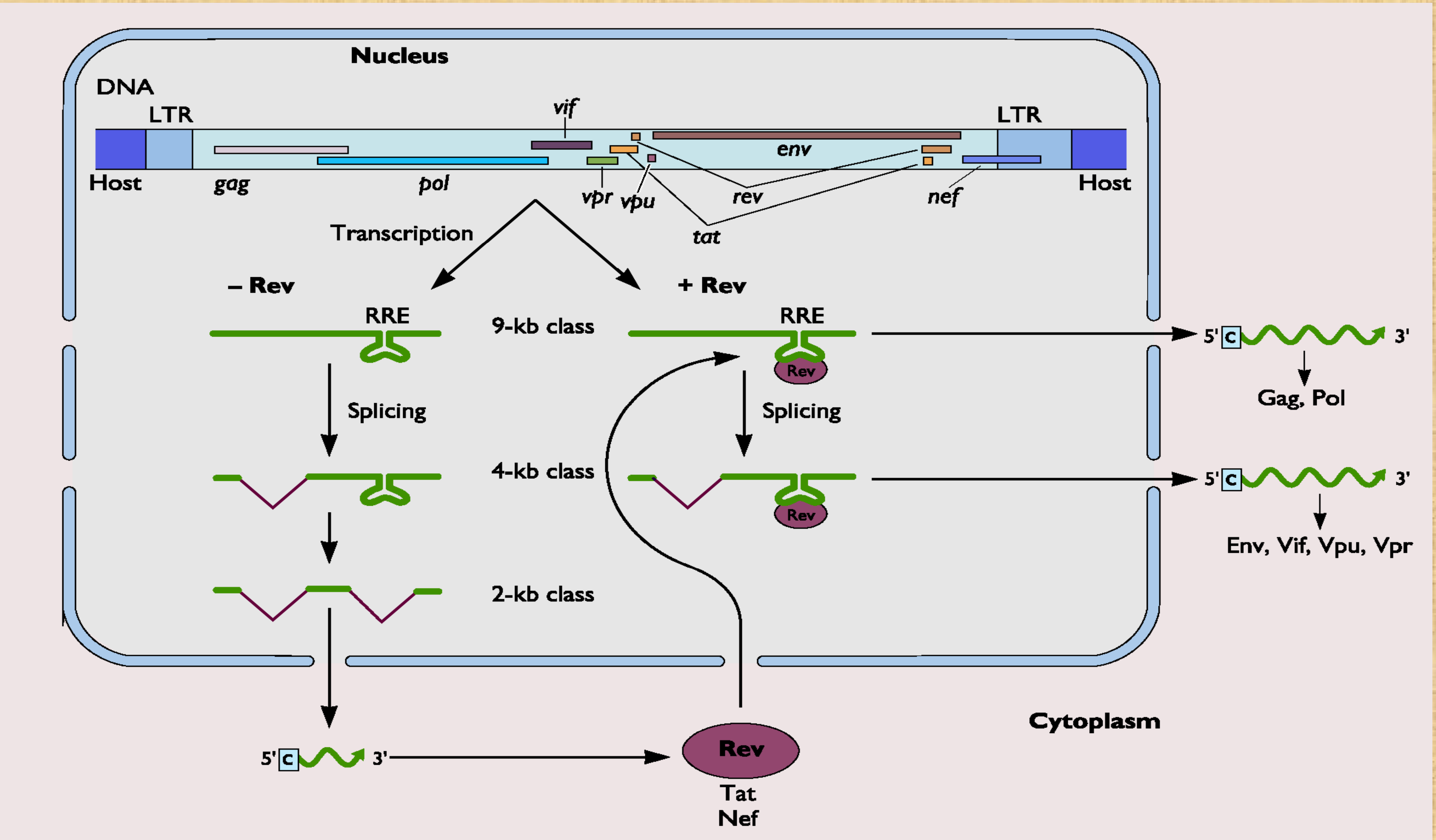


- **HIV LTR functions as a promoter in a variety of cell types in vitro**
- **It includes an enhancer sequence that binds a number of cell type specific transcription activators**

Mechanism of Tat activation

- Downstream of the site of initiation of transcription is the TAR RNA sequence which forms a stem loop that binds the 14 kd viral regulatory protein Tat
- In the absence of Tat, viral transcription terminates prematurely
- Tat facilitates efficient elongation
- Tat protein is cytotoxic to cells in culture
- Causes depolarization and degeneration of cell membranes
- in Transgenic expression mice causes a disease that resembles Kaposi’s sarcoma

Regulatory proteins: Rev



• **Rev Protein is an RNA binding protein that recognizes a specific sequence within the structural element in env called the Rev-responsive element (RRE)**

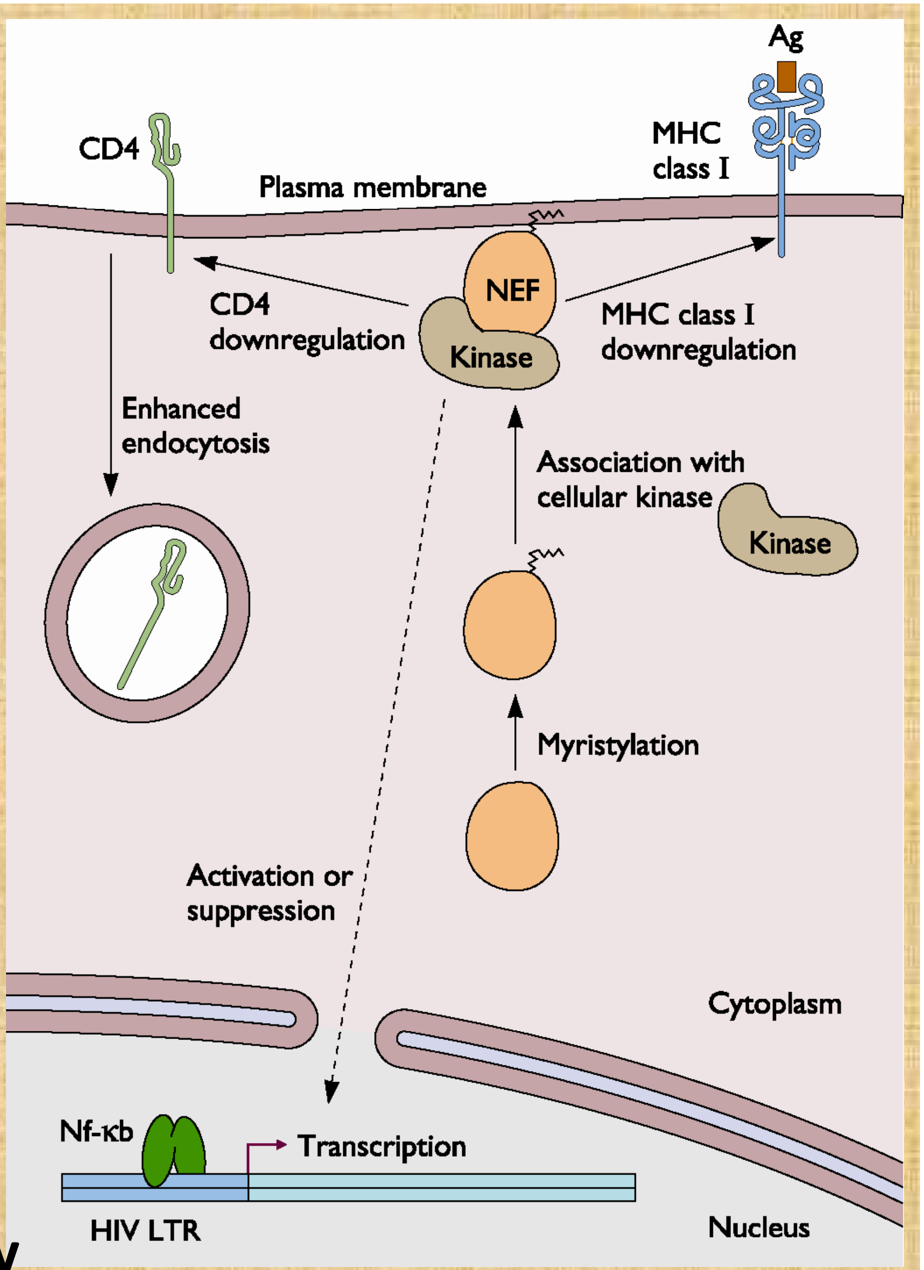
Rev protein:

- Rev activates the nuclear export of any RRE containing RNA
- As the Rev concentration increases, unspliced or singly spliced transcripts containing the RRE are exported from the nucleus
- Rev facilitates synthesis of the viral structural proteins and enzymes and ensures availability of full length genomic RNA to be incorporated into new virus particles
- The accessory proteins, Vif, Vpr and Vpu are also expressed later in infection from singly spliced mRNAs and their export to the cytoplasm is Rev dependent

HIV accessory proteins

Nef protein:

- Translated from multiply spliced early transcripts
- myristylated at its N-terminus and anchored to the inner surface of the plasma membrane
- Nef deleted HIV and SIV are much less pathogenic in vivo
- Nef downregulates expression of CD4 by enhancing endocytosis
- Can activate CD4+ T lymphocytes by modulating signaling pathways



HIV accessory proteins

Vif Protein:

- Viral infectivity factor
- Accumulates in the cytoplasm and at the plasma membrane of infected cells
- Mutant viruses lacking the *vif* gene were less infectious and defective in some way
- vif-defective virions enter cells, initiate reverse transcription, but do not produce full-length double stranded DNA
- vif inhibits antiviral action of a cytidine deaminase, which is synthesized in nonpermissive cells
- This enzyme deaminates deoxycytidine to deoxyuridine and leads to endonucleolytic digestion or G to A transitions

Subtyping of isolates

- RFLP Analysis
- Nucleotide sequence analysis
- Subtype specific genetic probes

Genetic variation in HIV

Mutations occur 65 times more than that observed in Influenza virus

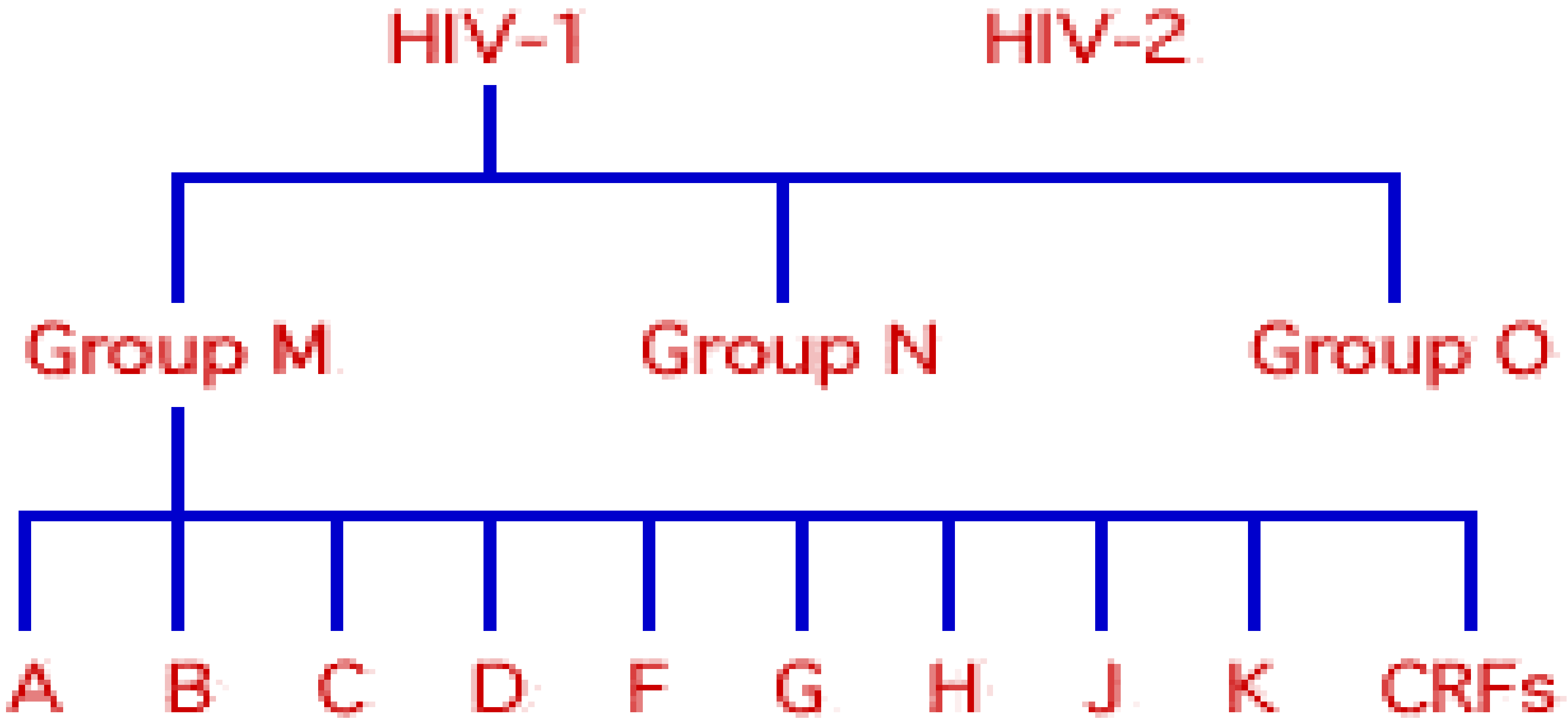
HIV-1 – Three groups **HIV- 2**

M (Major)

O (Outlier)

N (New)

Subtypes of HIV



Subtypes of HIV-1 – M

- **10 Subtypes/Clades/Genotypes Designated A-K Differ in Geographical distribution and major mode of Transmission**

Africa - A,C , D

US and Western Europe - B

Thailand - E, B

India - C

Distribution of HIV subtypes

Major subtypes in capitals

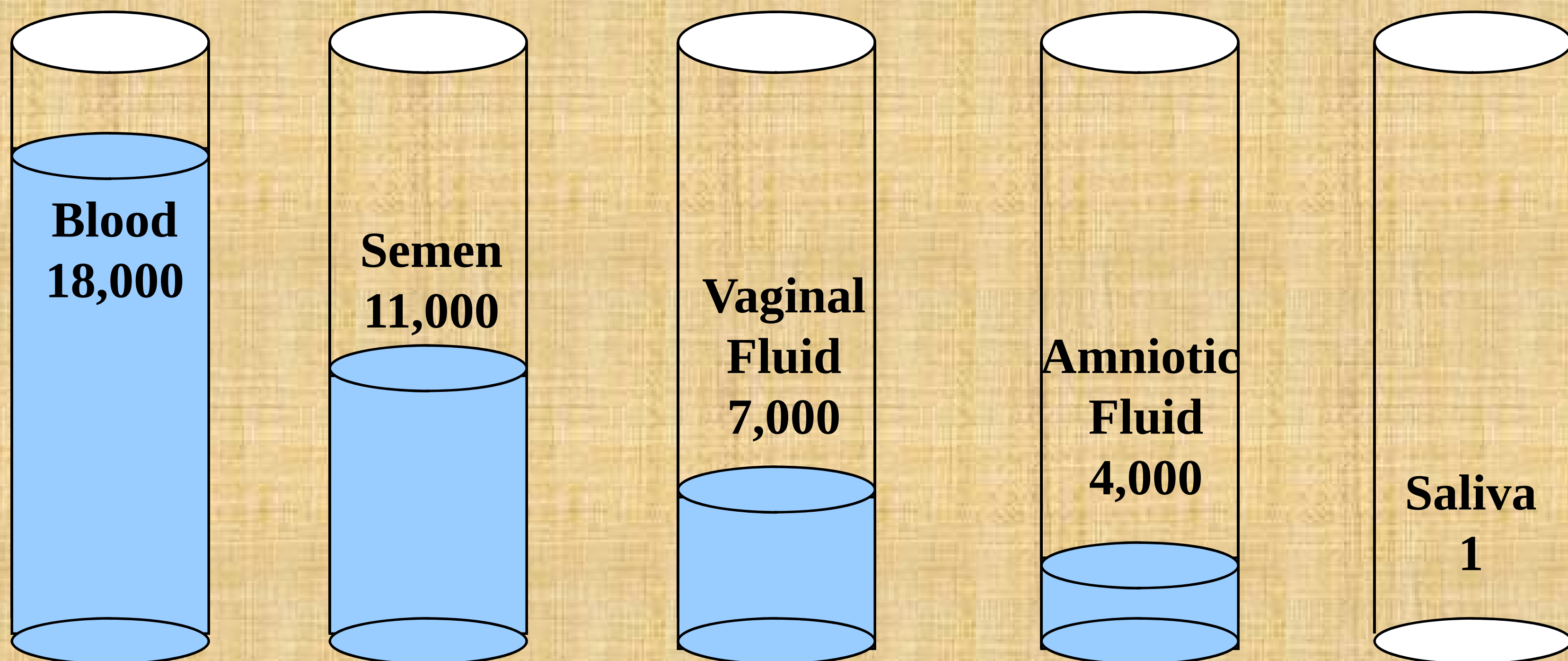
HIV Transmission

- HIV enters the bloodstream through:
  - Open Cuts
  - Breaks in the skin
  - Mucous membranes
  - Direct injection

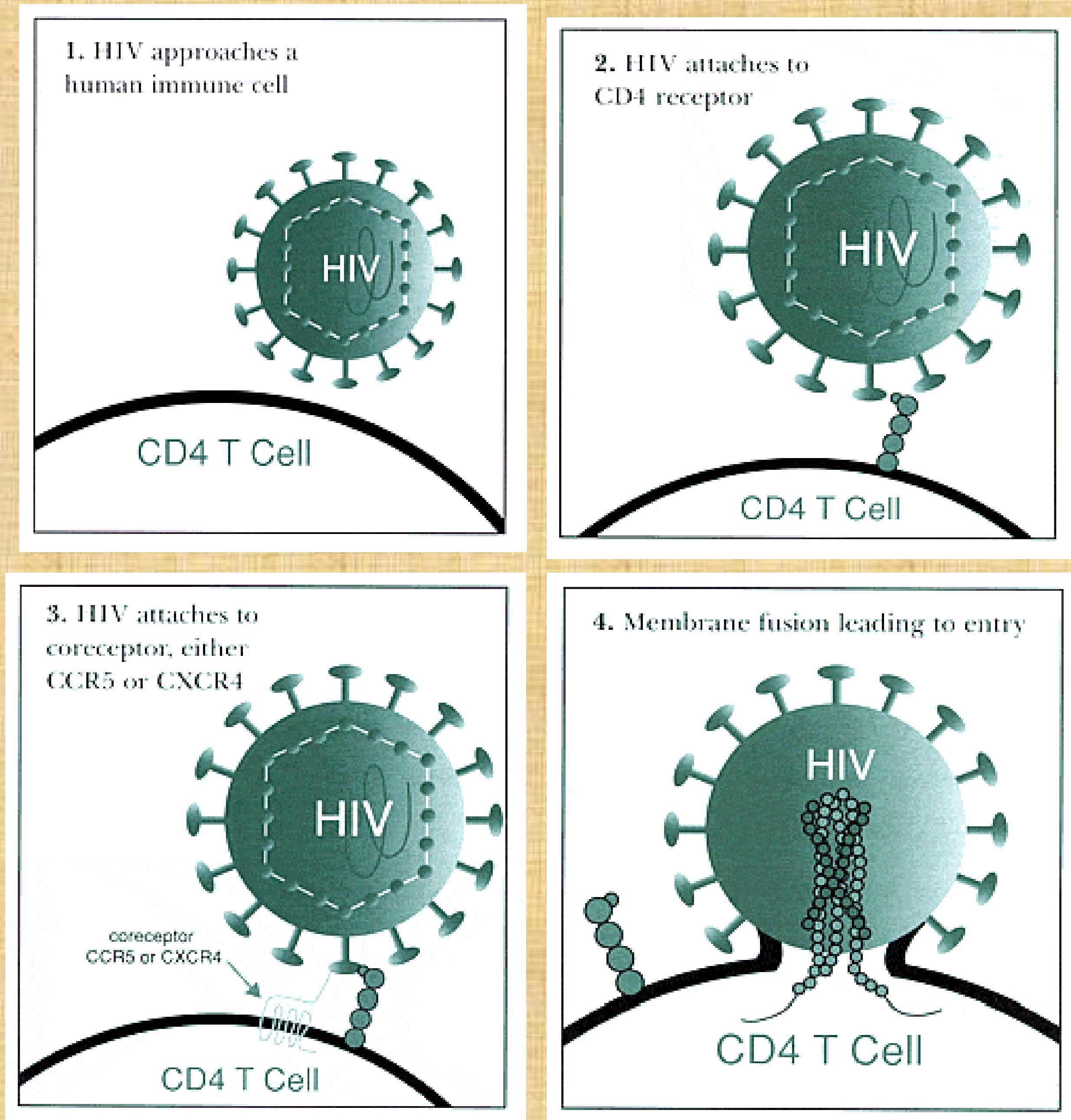
HIV Transmission

- Common fluids that are a means of transmission:
  - Blood
  - Semen
  - Vaginal Secretions
  - Breast Milk

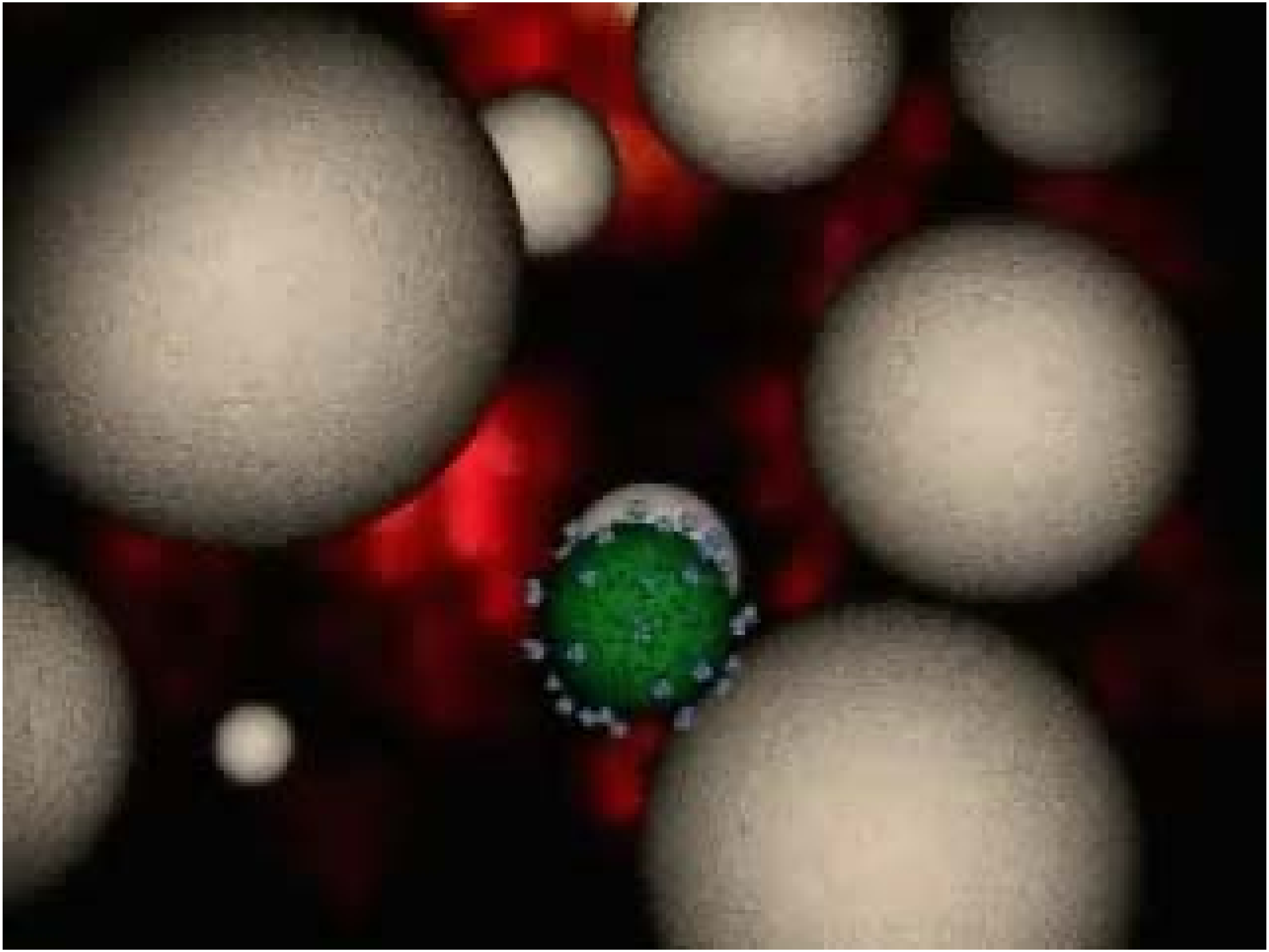
HIV in Body Fluids



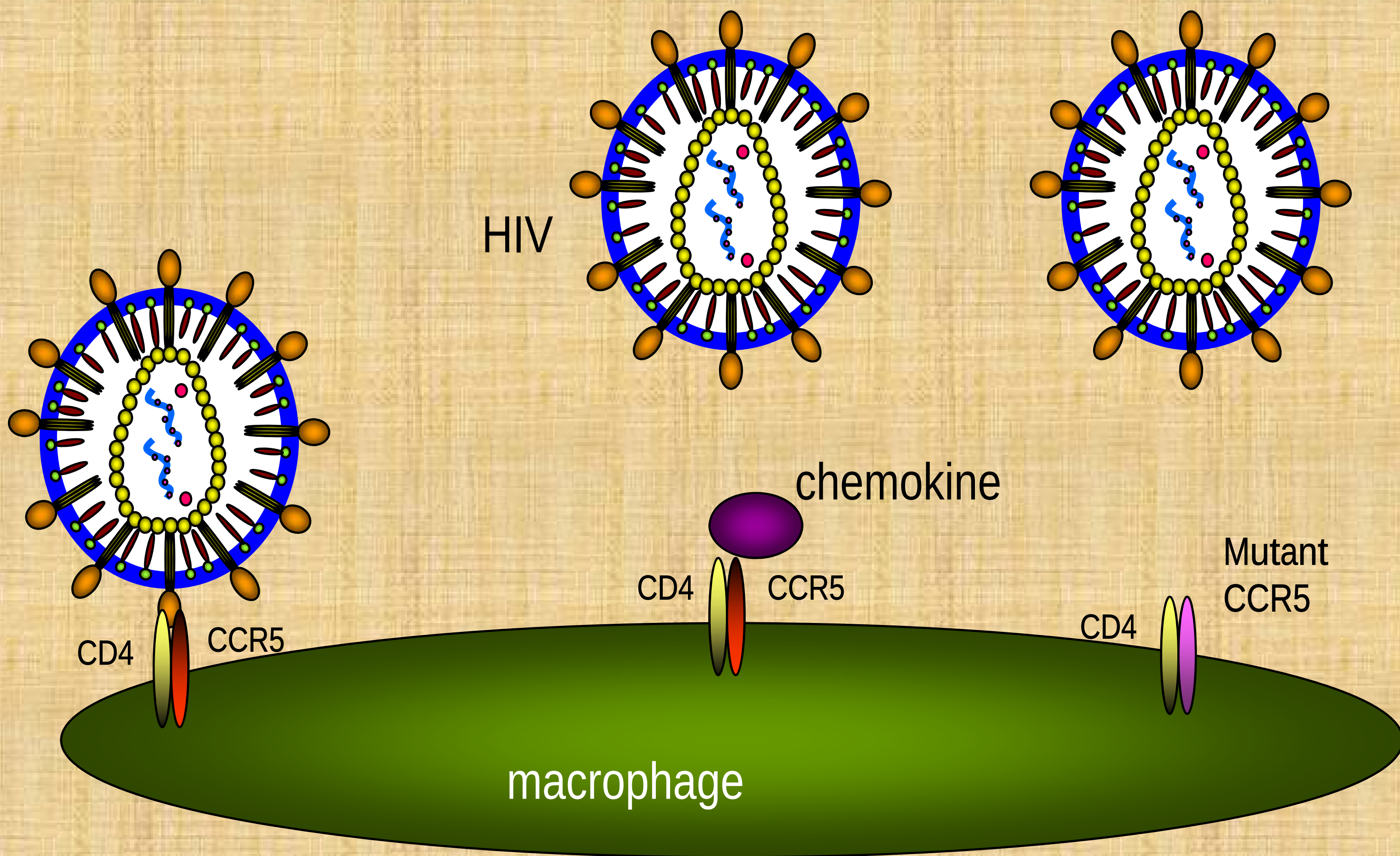
Average number of HIV particles in 1 ml of these body fluids



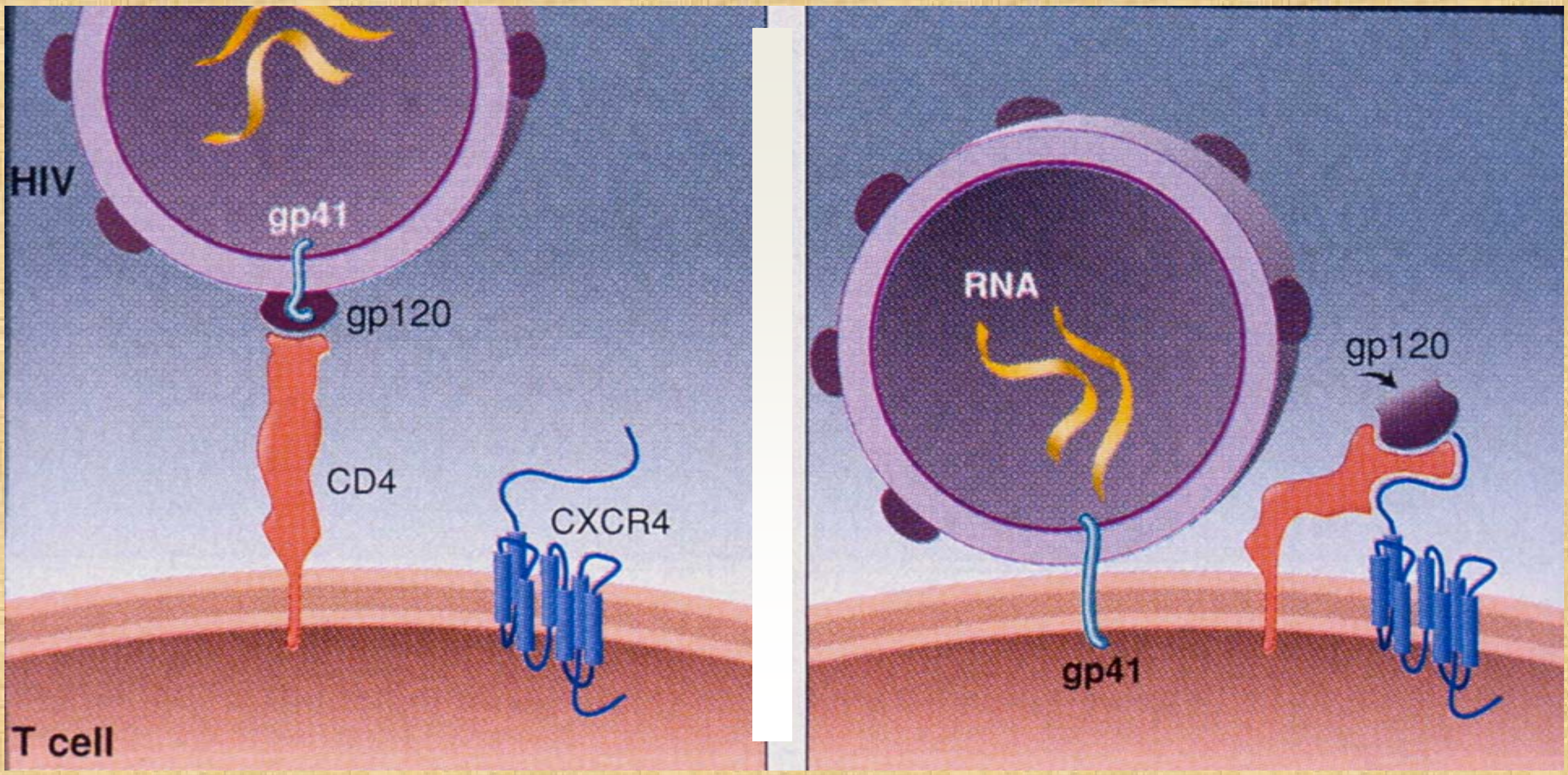
ENTRY OF HIV INSIDE T CELL

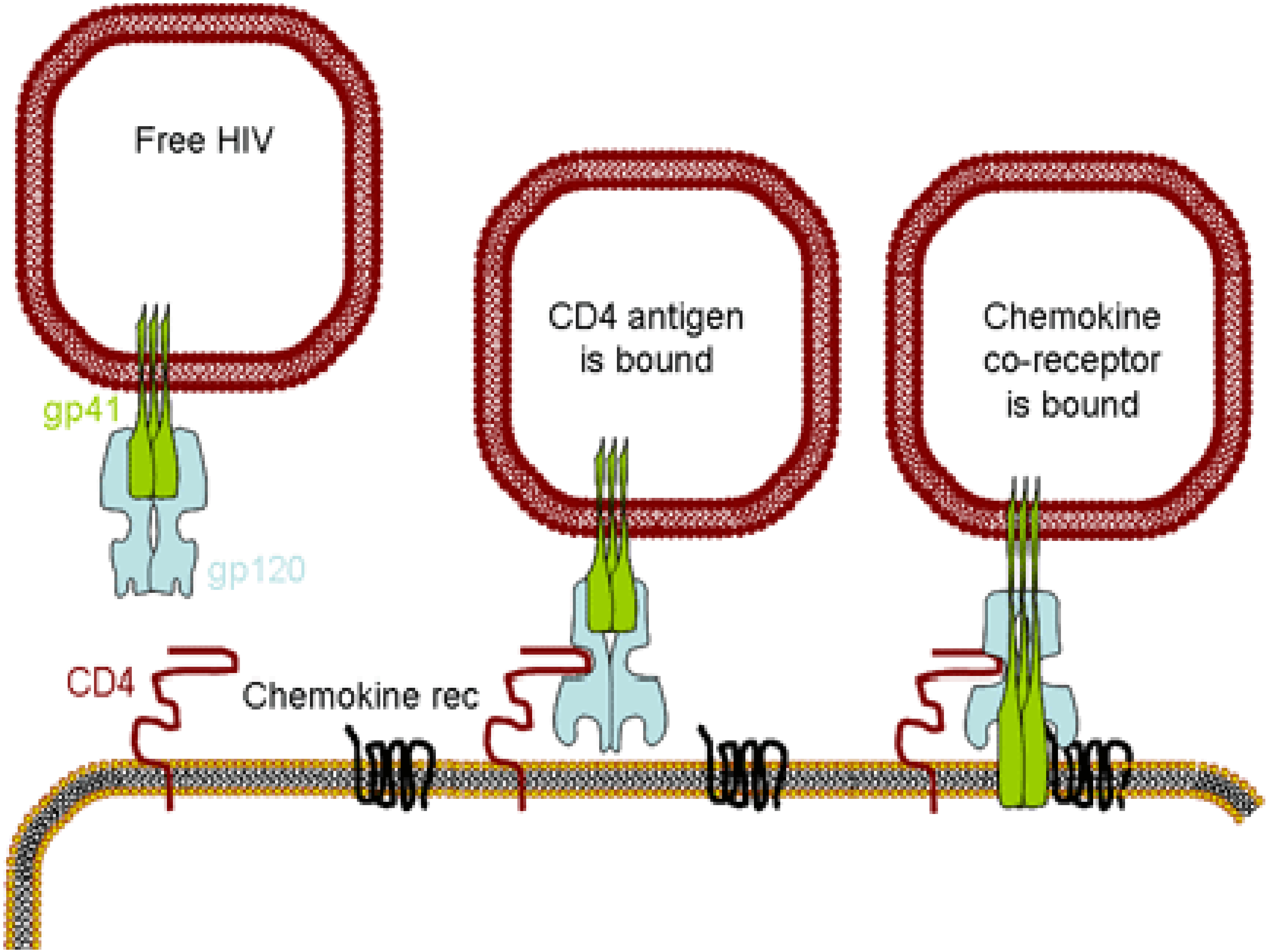


HIV - Life History
CCR5 - the co receptor



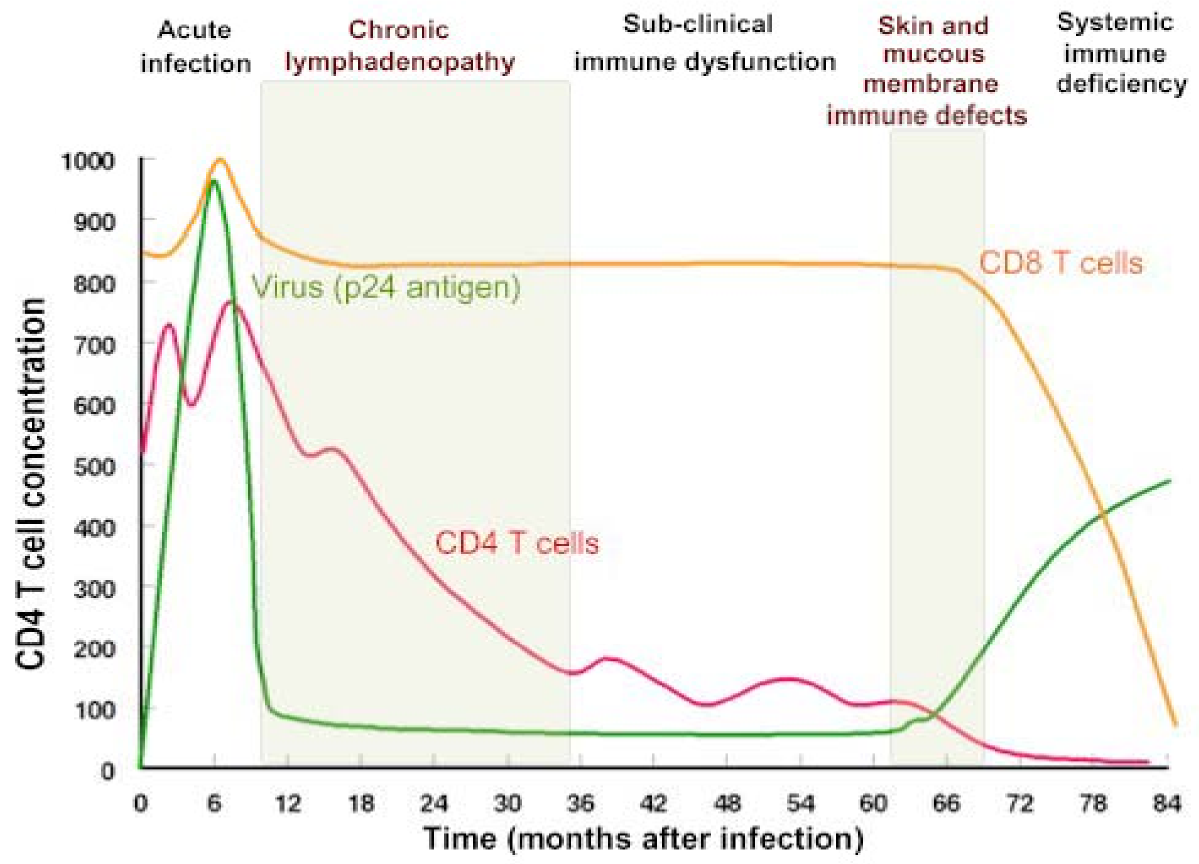
Virion interaction with CD4 receptor and CXCR4 co-receptor

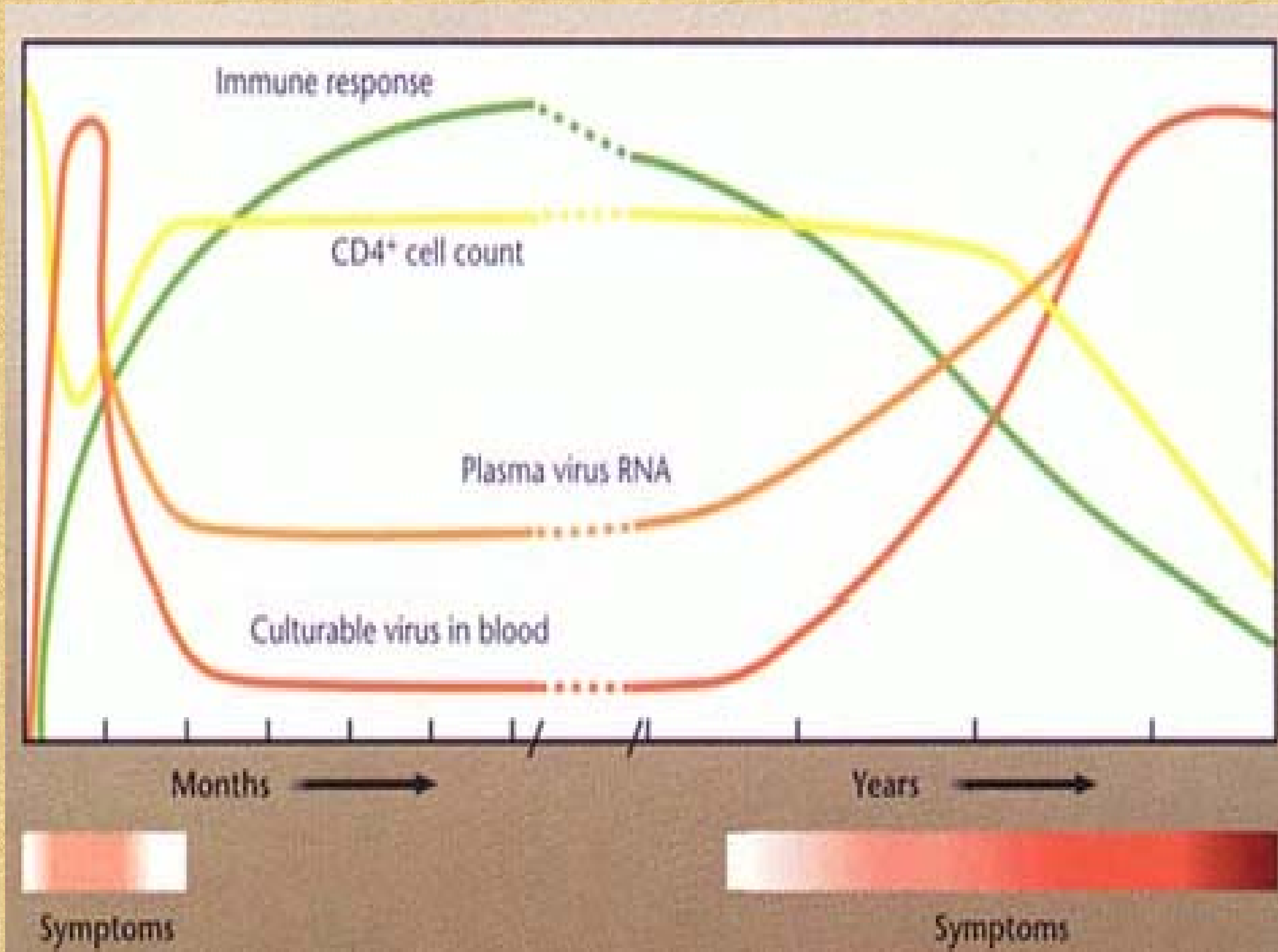



Free HIV
gp41
gp120
CD4 antigen
is bound
Chemokine
co-receptor
is bound
CD4
Chemokine rec

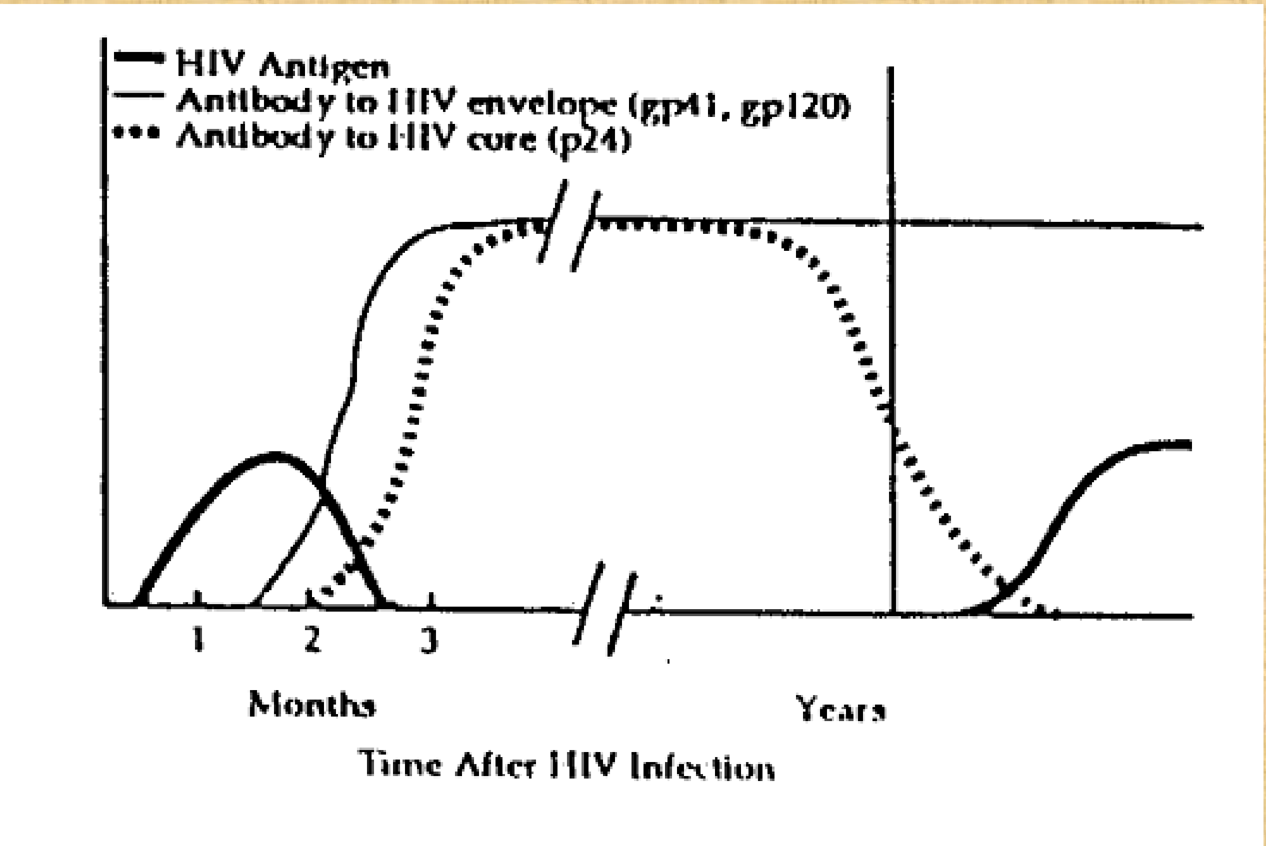
HIV and AIDS

The cellular and immunological picture - The course of the disease




Immune response
CD4⁺ cell count
Plasma virus RNA
Culturable virus in blood
Months
Years
Symptoms
Symptoms

Serology



HIV Antigen
Antibody to HIV envelope (gp41, gp120)
Antibody to HIV core (p24)
1
2
3
Months
Years
Time After HIV Infection

Disinfection & Inactivation

HIV is completely inactivated (10^5 units of infectivity) by treatment for 10 minutes at room temperature with any of the following:

- **10% household bleach**
- **50% ethanol**
- **35% isopropanol**
- **0.5% paraformaldehyde**
- **0.3% hydrogen peroxide**

The virus is also inactivated by extremes of pH (pH 1.0, pH 13.0).

However, when HIV is present in clotted or unclotted blood in a needle or syringe, exposure to undiluted bleach for at least 30 seconds is necessary for inactivation.

HIV is readily inactivated in liquids or 10% serum by heating at 56 °C for 10 minutes, but dried proteinaceous material affords marked protection. Lyophilized blood products would need to be heated at 68 °C for 72 hours to ensure inactivation of contaminating virus.