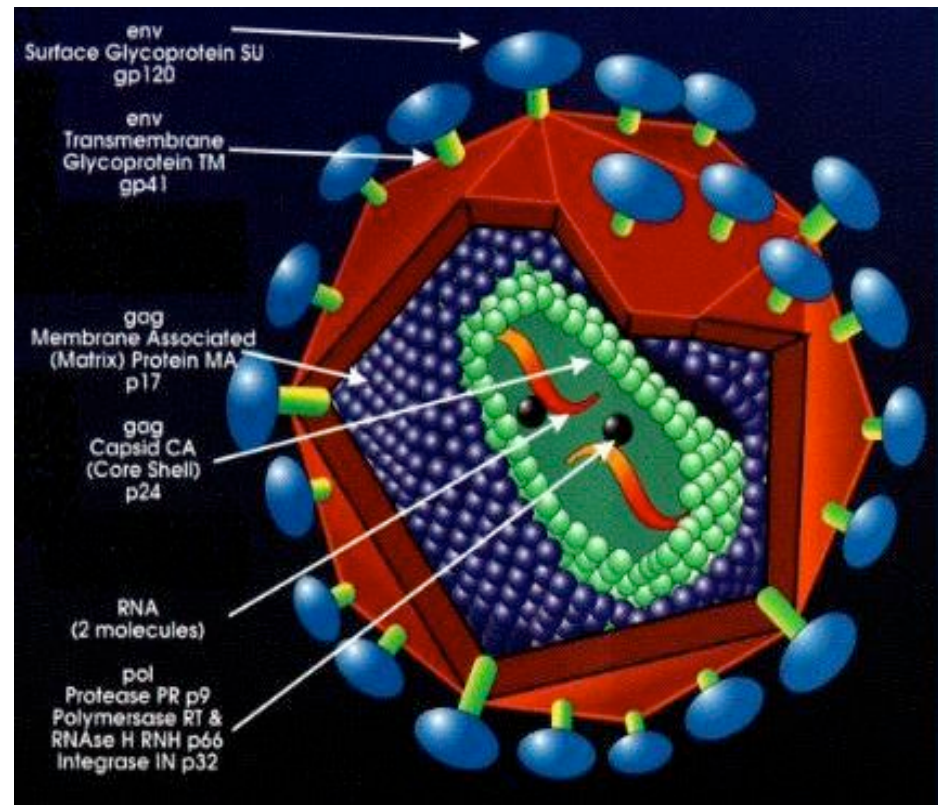


# Structural Biology of HIV

- Diameter of 100-120 nm with a spherical morphology
- Cone-shaped core surrounded by lipid matrix containing key surface antigens and glycoproteins
- Viral core contains 2 copies of genomic RNA, reverse transcriptase, integrase and protease

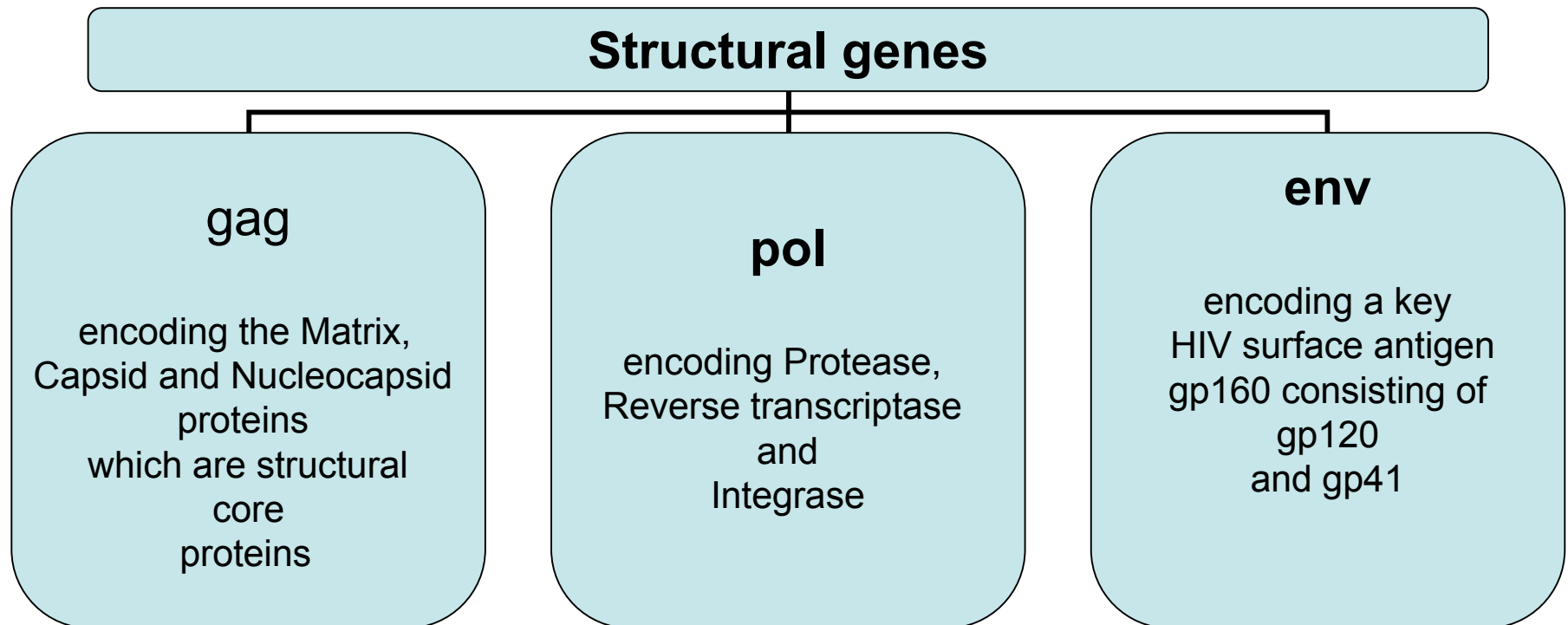




# HIV Genome Continued

- **Lysine tRNA** is bound to the viral RNA in the particle and serves as a primer for reverse transcription
- HIV genome also encodes several **cis-acting elements** (control elements lying adjacent to or near genes they influence) involved in virion packaging, RT, RNA retention in the nucleus, RNA export and processing, transcription, etc.
- No introns present in the genome

# Key Structural HIV Genes



# Regulatory Genes I

- Contain information required for the production of proteins that control HIV's ability to infect a cell, reproduce and cause disease.
- **tat** – transactivator of transcription encoded by 2 different exons from multiply spliced mRNA. The 102 aa Tat is responsible for activation of viral transcription through TAR binding, which creates binding sites for RNA pol II and other cellular proteins. It initiates synthesis of full-length transcripts. Tat is secreted into the circulation thus a possibility for inhibition by antibodies. It can also be a target for CTLs. Tat structure is rather conserved varying only slightly among different clades. Produced in excess in infected cells. Tat also induces apoptosis of T cells, even the uninfected ones. It has also been shown to act as a neurotoxin and give rise to cells causing Kaposi's carcinoma.
- **rev** – essential accessory protein whose function is to transport mRNA to the cytoplasm. Rev is a 117 aa phosphoprotein that binds to RRE (cis-acting element) within the env gene of all unspliced mRNAs. The N-terminal nuclear localization signal (NLS) directs its import back into the nucleus. Rev-dependent export of viral RNA distinguishes b/w early and late phase.

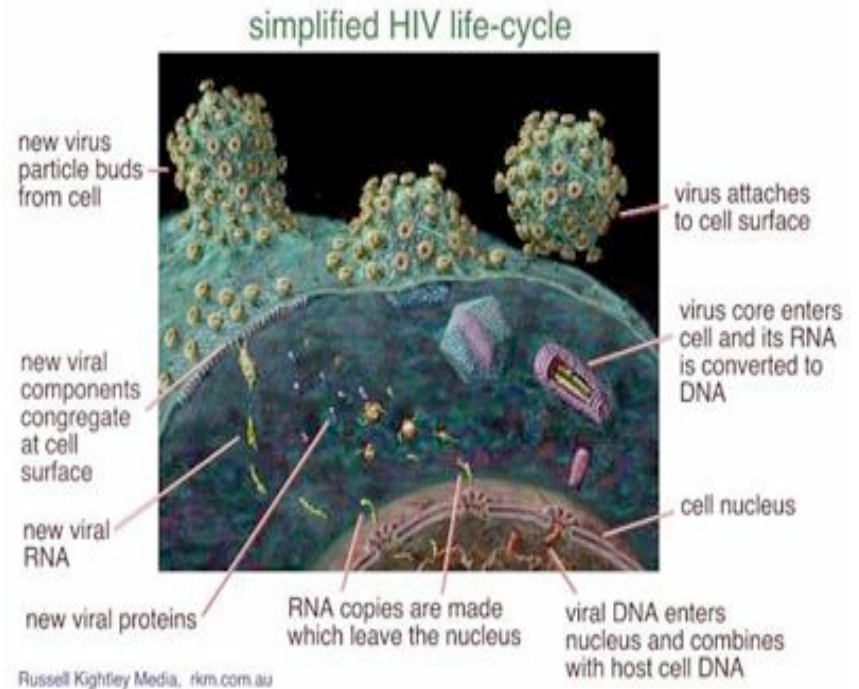
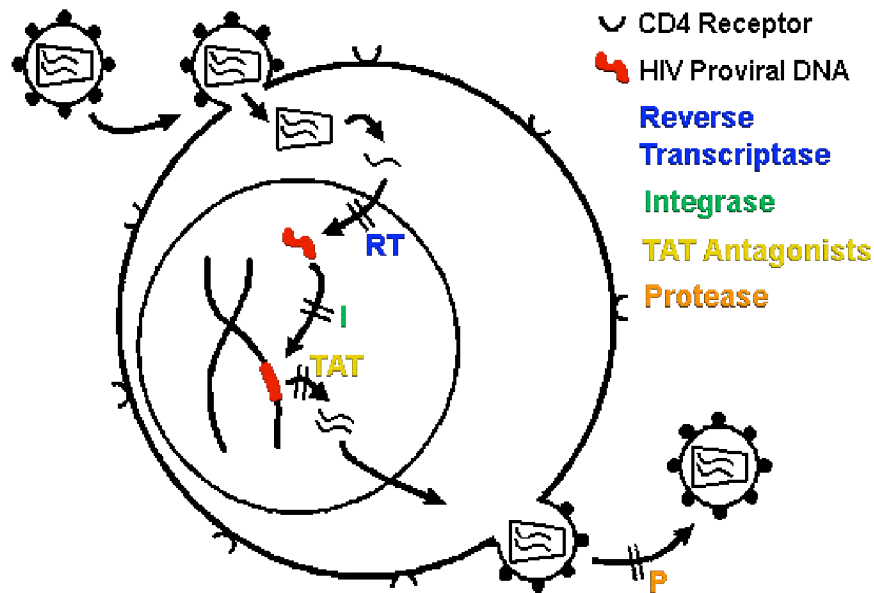
# Viral Regulatory Genes II

- **vif** – viral infectivity factor required for infection of human lymphocytes and some cell lines. Its ORF overlaps with the 3' end of pol. A 23 kDa protein found in the cytoplasm and cell membrane. The mechanism of action is not well understood but its importance in maturation process is recognized as the infectivity of Vif defective virions produced in non-permissive cells can be 25-100 times lower than wild type. Vif has been found in virus particles at levels similar to Pol but since it is also present in murine leukemia virus, possible significance of Vif incorporation is to be determined.
- **nef** – negative factor, a 27 kD determinant of progression to AIDS. It downregulates cell surface receptor expression, interferes with signal transduction pathways and enhances viral infectivity and production. Nef is post-translationally modified by phosphorylation and by the irreversible attachment of myristic acid to its N-terminus, which targets Nef to the cellular membrane. The most enigmatic HIV protein as its mechanisms of action are not well understood and many contradictory phenotypes have been associated with expression of Nef.

# Regulatory Genes III

- **vpu** – viral protein U, 81 aa membrane protein expressed as part of a bicistronic message also encoding Env and regulated by Rev. It promotes release of viral particles from plasma membrane of infected cell and degrades CD4 in the endoplasmic reticulum. The ability to form a cation-selective ion channel has also been described as another function of Vpu but its role is not know.
- **vpr** – viral protein R, 96 aa, 14 kDa protein responsible for G2 cell cycle arrest thought to indirectly enhance viral replication by increasing transcription from LTR. Vpr expression causes breaks in the nuclear lamin structure, which weakens nuclear envelope and interferes with DNA synthesis thus cycle arrest prior to mitosis. It is also implicated in facilitating infection of non-dividing cells, mostly macrophages. Vpr also functions to connect the pre-integration complex to the cellular nuclear import machinery.

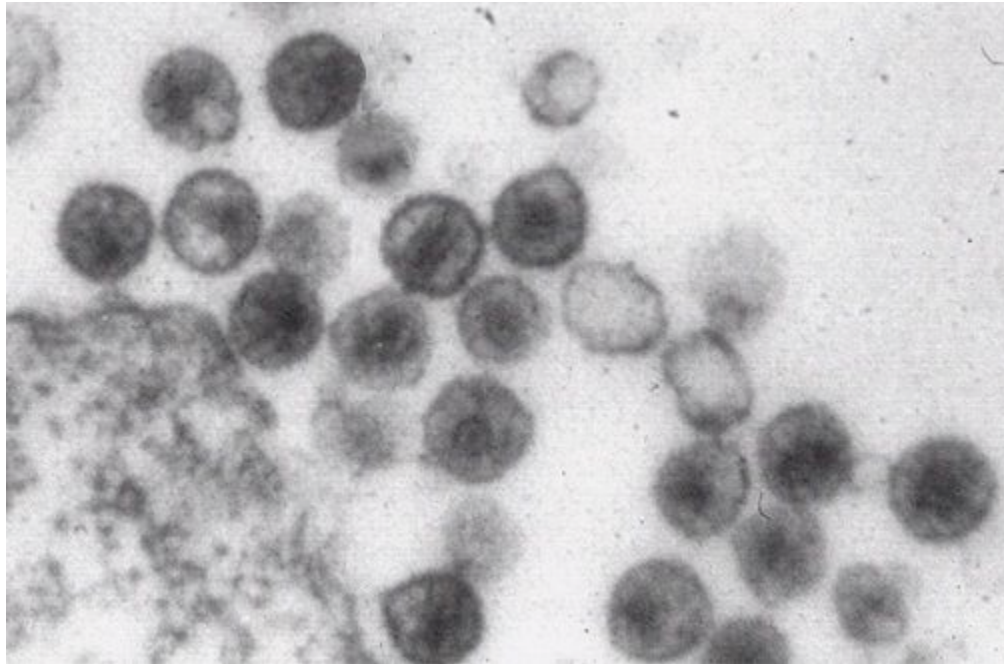
# Replication Cycle of HIV





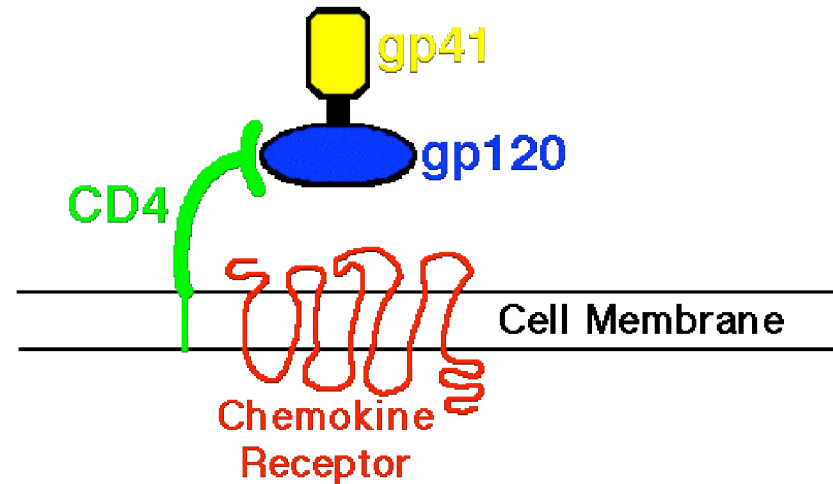
# HIV under electron microscope

- Note the central core and the viral envelope



# Viral Entry

- Binding of surface viral gp120 to cells bearing CD4 membrane receptor causing conformational change
- Co-receptor (chemokine receptor CXCR4/5) binding facilitates membrane fusion
- Deposition of pre-integration complex in the cytoplasm
- Reverse transcription takes place in the cytosol shortly after entry by HIV-1 Reverse transcriptase and is initiated by the binding of a cellular Lysine tRNA primer. The dsDNA product (provirus) is modified by the viral integrase in the cytoplasm

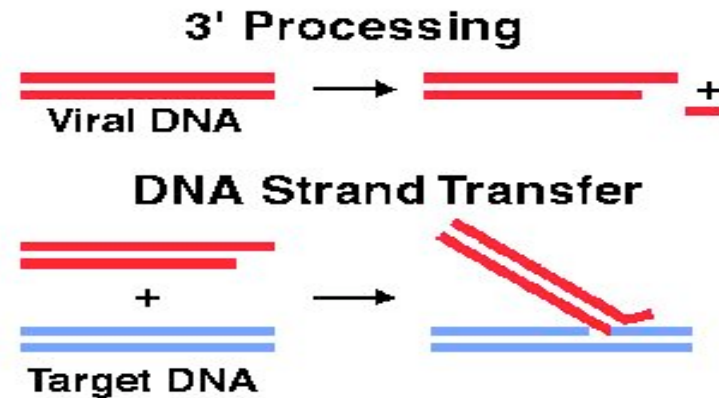


# Before integration into host DNA...

- **Vpr** (no NLS) activates cellular nuclear import machinery by connecting the pre-integration complex to proteins such as importin-alpha
- Matrix, Integrase and Vpr, all having nucleophilic characteristics important for active transport, bring the preintegration complex into the nucleus
- Now the complex is ready for integration
- Provirus preferentially integrates at sites of highly bent DNA, such as nucleosomes

# How does Integrase do it?

- It recognizes the LTRs at the 5' and 3' ends of the newly synthesized viral DNA duplex and cleaves 2-3 bases from the 3' ends.
- Trans-esterification reaction takes place joining proviral and cellular DNA ends
- 4-6bp gaps of mismatched ends are trimmed, filled and ligated



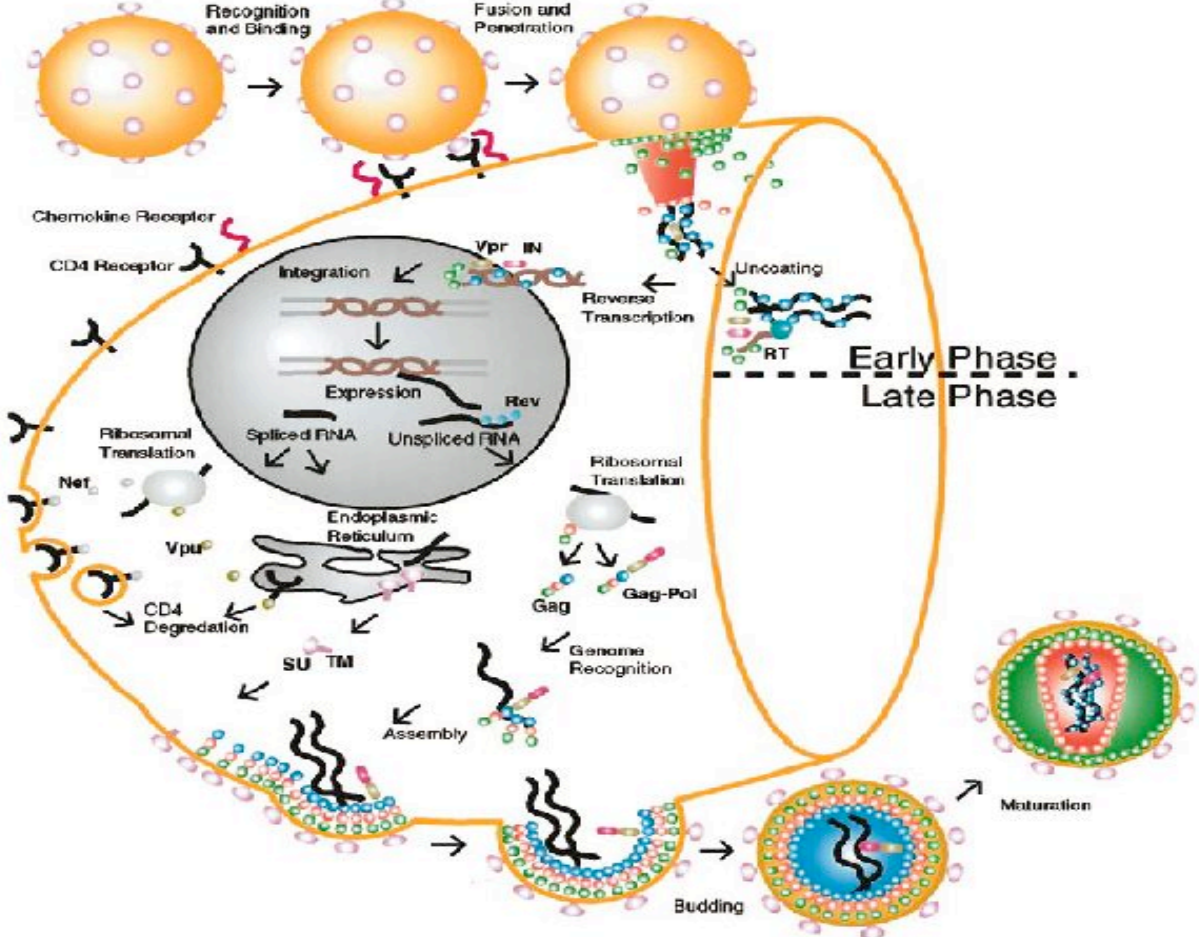
# Once permanently integrated...

- Early phase commences: only short spliced mRNA species encoding Tat, Rev and Nef are synthesized and transported out the nucleus and translated
- The amount of Rev (NLS) controls the level of singly and multiply spliced messages in the cytoplasm
- Differential distribution of viral RNAs is due to cis-acting nuclear retention sequences (CRS) in the gag, pol and env coding regions
- Transport to the cytoplasm is also dependent on Rev, which binds to the RRE of nascent unspliced mRNAs and recruits the cellular nuclear shuttling protein exportin-1. The complex is then transported through the nuclear pore to the cytosol where GTP is hydrolyzed to GDP, the complex dissociates and Rev goes back

# Late Phase

- Both Rev-independent (tat, rev and nef) and Rev-dependent (gag-pol, env, vif, vpr/vpx and vpu) messages are exported and translated
- The Gag polyprotein is synthesized in the ribosomes from the unspliced mRNA
- Ribosomal frameshift is involved in the generation of smaller amounts of Gag-Pol precursor proteins from the same mRNA.

# Overview of HIV-1 replication cycle



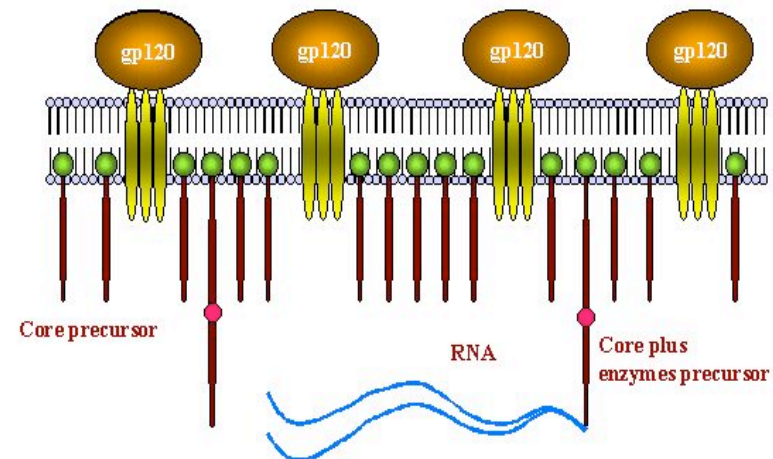
# Assembly and Budding

- Assembly of the virus particle takes place on the inner surface of the cell membrane, in macrophages also in vacuoles
- Gag precursor Pr55gag, major virion component, is cleaved into 4 major proteins: matrix, capsid, nucleocapsid, and p6gag in the viral particle and associates into virion spontaneously
- Gag is targeted to the cell membrane after being post-translationally modified, (addition of myristyl group)
- Pr55gag directs incorporation of Gag-Pol precursor, Pr160gag, the envelope protein, and Vpr



# More on assembly and budding

- Pr55gag of HIV-1, not HIV-2 also binds cyclophilin A, which is important because cyclosporin disrupts this association inhibiting HIV
- Env precursor, gp160 synthesized in the ER from the spliced env, is cleaved by a cellular protease into gp120 and gp41
- Vpr and Nef are also found in the virion
- The nucleocapsid domain of Gag interacts with the encapsidation sequence (psi) of the genomic RNA via its Zn finger domains
- At this point the lipid bilayer surrounds the core, budding occurs



Assembly of the new virion takes place at the cell membrane. Three types of protein make up the virion. These are the membrane protein (gp120 and gp41 complex) plus two precursor proteins of different sizes

# Maturation

- 1200-2000 copies of Gag bud to form an immature particle with 2 copies of unspliced viral genome
- Proteolytic processing mediated by the viral protease separates the domains of the different polyproteins and sets the conditions for RT
- Structural proteins rearrange
- 7-100 copies of Vif required for production of infectious virions in some cell lines are packaged although not known if essential
- **ONLY THE MATURE PARTICLES ARE COMPETENT FOR INFECTION**

# Important facts about HIV

- **No proofreading** mechanism leading to accumulation of mutations,  $10^4 - 10^6$  times the incidence of mutations in DNA counterparts
- 1 nt substitution per replication cycle leading to CD4<sup>+</sup> lymphocytes depletion at a rate of 2 billion per day
- The progeny of a single virus can differ greatly in antigenic configuration (gp120 and gp160) from the parent
- Extremely **rapid RNA virus replication**
- Half-life of free virus is 6-8 hours

# Facts on HIV continued

- Differential splicing of the viral genome controls in a temporal fashion the expression of structural and regulatory genes
- Replication cycle is similar but under much tighter control than for other retroviruses

# Infection

- After exposure to HIV, some people have a flu-like illness that lasts between a week to a month.
  - Fever
  - Headache
  - Enlarged lymph nodes
- Several symptoms of occur due to a decreasing CD4 T cell count including:
  - Fatigue, weight loss
  - Frequent fevers and sweats
  - Persistent skin rashes or yeast infections
  - Short-term memory loss

# HIV to AIDS

- Symptoms of opportunistic infections:
  - Coughing, shortness of breath
  - Fever
  - Lack of coordination, forgetfulness, vision loss
  - Persistent diarrhea
  - Severe headaches
  - Extreme fatigue
  - Nausea, abdominal cramps, vomiting
  - Conjunctivitis, ear infections, tonsillitis (children)

# Clinical Manifestations

- Kaposi's sarcoma
- Cervical cancer
- Pneumocystis carinii pneumonia
- Non-Hodgkin lymphoma
  - AIDS-related Burkitt lymphoma: chromosome-translocation
  - AIDS-related Large cell lymphoma: EBV infection
  - AIDS-related Primary effusion lymphoma: HHV-8 infection
- Pseudomonas
  - pseudomona aeruginosa
  - pseudomona mallei
  - pseudomona pseudomallei

# Diagnosis

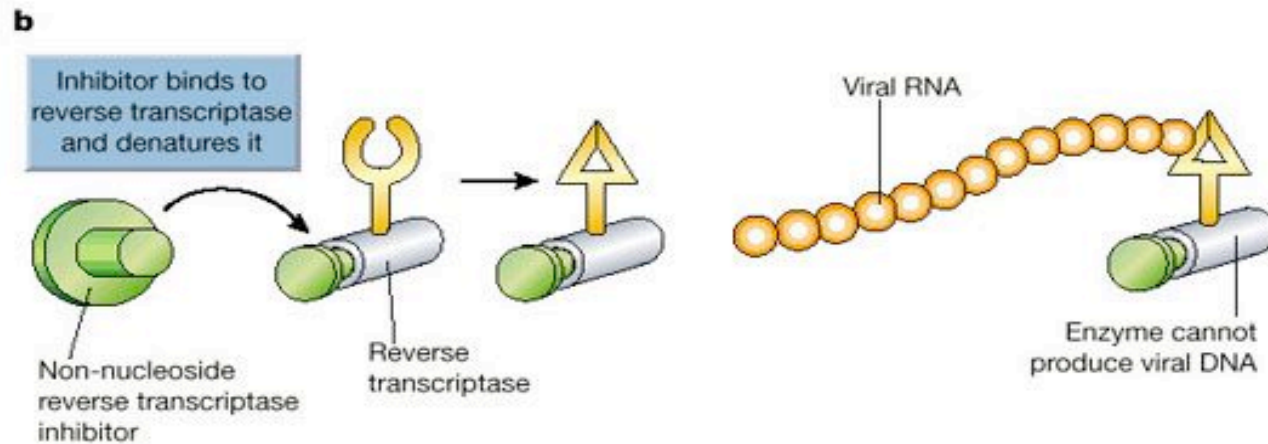
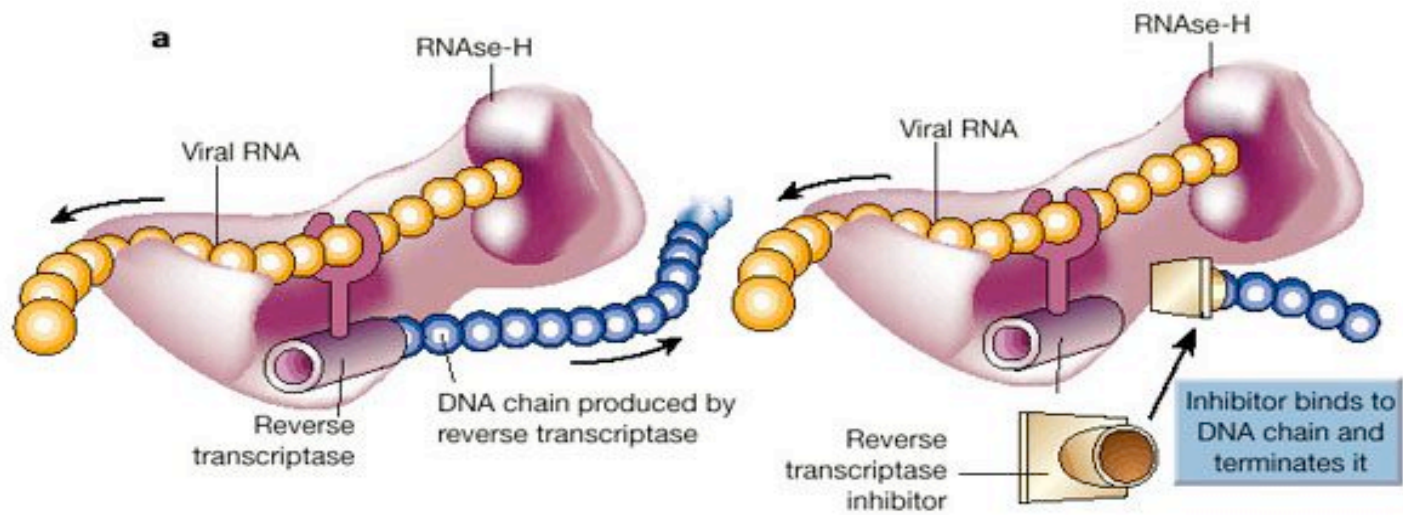
- Diagnosis is done by testing a person's blood for the presence of antibodies to HIV.
- Antibodies are generally not detectable until 3 to 6 months following infection.
- ELISA and Western Blots are generally used to test of HIV antibodies.
- Recently, the FDA approved the OraQuick Rapid HIV-1 Antibody Test.



# Current Treatments

- There is no known cure for HIV.
- HAART (highly active anti-retroviral therapy) drugs are used to reduce virus circulation.
- Effectiveness of treatment depends on genetic factors, viral strain, and drug mechanism.
- Three classes of HAART drugs exist
  - Nucleoside reverse-transcriptase inhibitors
  - Non-nucleoside reverse-transcriptase inhibitors
  - Protease inhibitors

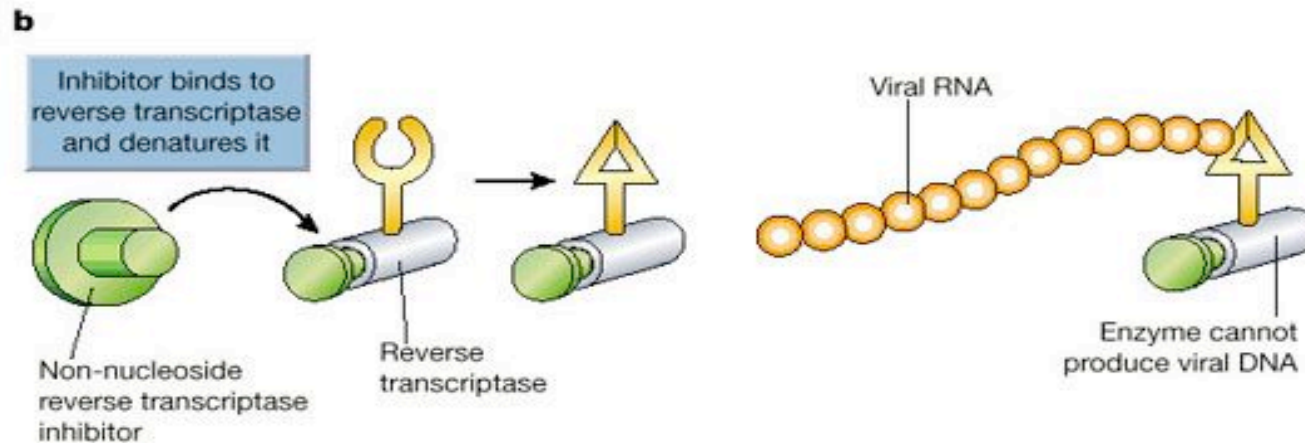
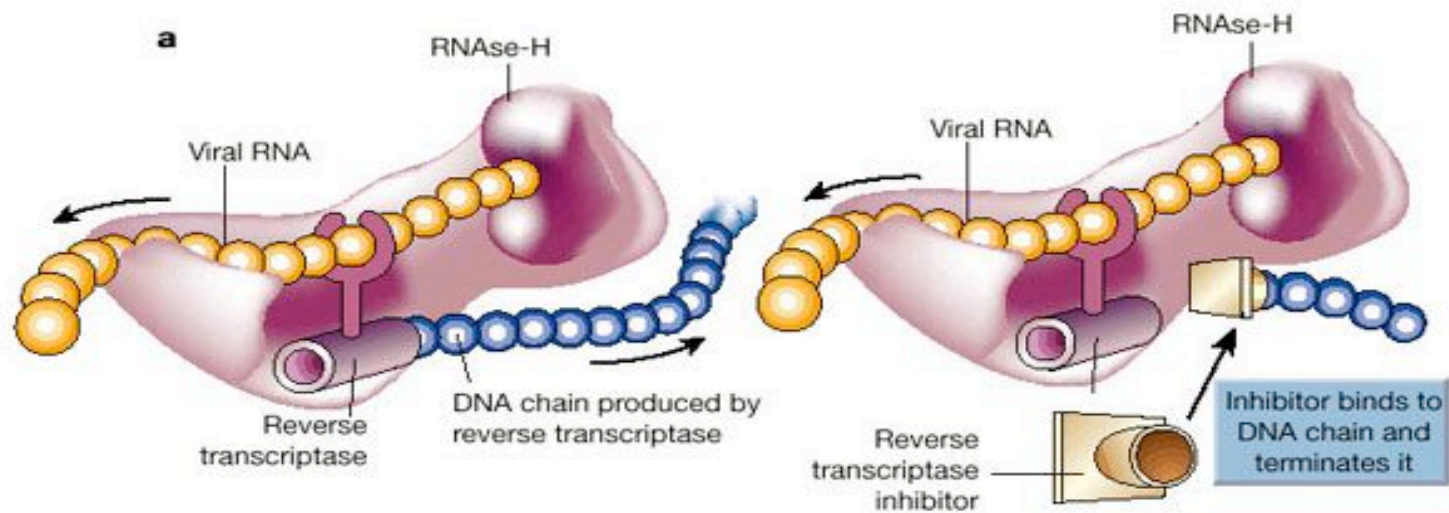
# Nucleoside Reverse-Transcriptase Inhibitors



# Nucleoside Reverse-Transcriptase Inhibitors

- Abacavir (ABC) – GlaxoSmithKline, 1998
  - 300mg 2x/day, rare side effects: pancreatitis, diabetes
  - Side effects: nausea, vomiting, diarrhea, anorexia, fatigue
- Lamivudine (3TC) – GlaxoSmithKline, 1995
  - 150mg 2x/day, rare side effects: fatality
  - Side effects: headache, chills, diarrhea, depression, rash
  - Trimethoprim/sulfamethoxazole (TMP/SMX) increases 3TC
- Zidovudine (AZT) – GlaxoSmithKline, 1987
  - 300mg 2x/day, rare side effects: lactic acidosis, hepatic stenosis
  - Side effects: hematologic toxicity, anemia, headache, myositis

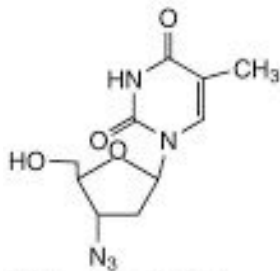
# Non-nucleoside Reverse-Transcriptase Inhibitors



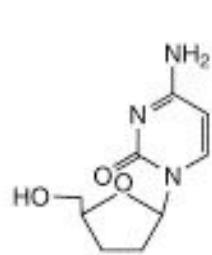
# Non-nucleoside Reverse-Transcriptase Inhibitors

- Delavirdine (DLV) – Pfizer, 1997
  - 400mg 3x/day
  - Not recommended with antihistamines and antacids
  - Side effects: headache, abdominal pain, fatigue, rash
  - Decreases drug metabolism, increases drug toxicity
- Nevirapine (NVP) – Boehringer-Ingelheim, 1996
  - 200mg 2x/day, rare side effects: hepatitis, facial edema
  - More rare side effects: oral lesions, blisters, conjunctivitis
  - Side effects: severe rash, fever, nausea, headache
  - Not recommended with oral contraceptives

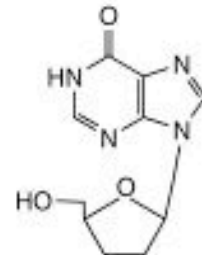
# Reverse-Transcriptase Inhibitors



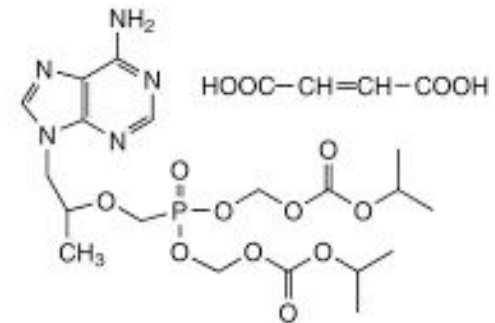
Zidovudine (AZT)



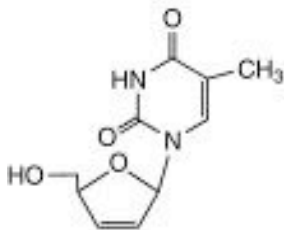
Zalcitabine (ddC)



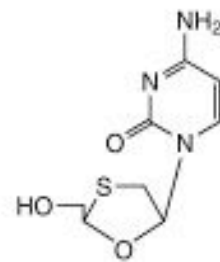
Didanosine (ddl)



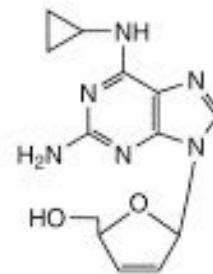
Tenofovir disoproxil fumarate



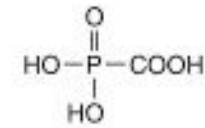
Stavudine (d4T)



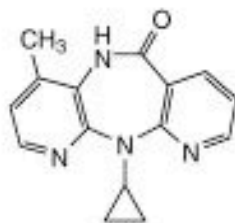
Lamivudine (3TC)



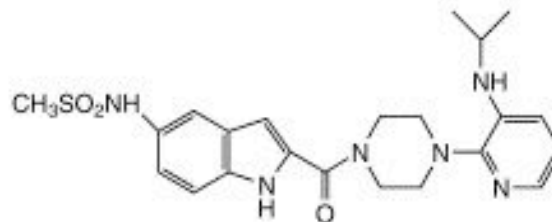
Abacavir



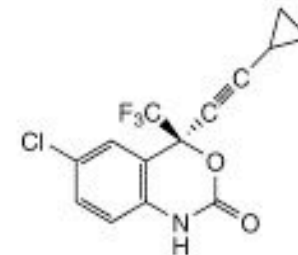
Foscarnet



Nevirapine



Delavirdine



Efavirenz

# Protease Inhibitors

