Viral Replication

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Viral Replication: Basic Concepts

- Viruses are obligate intracellular parasites
- Viruses carry their genome (RNA or DNA) and sometimes functional proteins required for early steps in replication cycle
- Viruses depend on host cell machinery to complete replication cycle and must commandeer that machinery to successfully replicate

Viral Replication: Basic Concepts

- Replication cycle produces
  - Functional RNA's and proteins
  - Genomic RNA or DNA and structural proteins
- 100's-1,000's new particles produced by each cycle
  - Referred to as burst size
  - Many are defective
  - End of 'eclipse' phase
- Replication may be cytolytic or non-cytolytic

Steps in Viral Replication: Attachment
(First Step)

- Surface protein on virus attaches to specific receptor(s) on cell surface
  - May be specialized proteins with limited tissue distribution or more widely distributed
  - Virus specific receptor is necessary but not sufficient for viruses to infect cells and complete replicative cycle

Selected Virus Receptors

Adenovirus	CAR
Coxsackievirus	CAR, CD55
Echovirus	Integrin VLA-2, CD55
Epstein-Barr Virus	CD21
HIV-1	CD4, CCR5, CXCR4
Measles virus	CD46
Parvovirus	Erythrocyte P Ag
Poliovirus	PVR
Rhinovirus	ICAM-1

Steps in Viral Replication: Penetration
(Second Step)

- Enveloped viruses penetrate cells through fusion of viral envelope with host cell membrane
  - May or may not involve receptor mediated endocytosis
- Non enveloped viruses penetrate by
  - Receptor mediated endocytosis
  - Translocation of the virion across the host cell membrane

Influenza Virus Replication Cycle

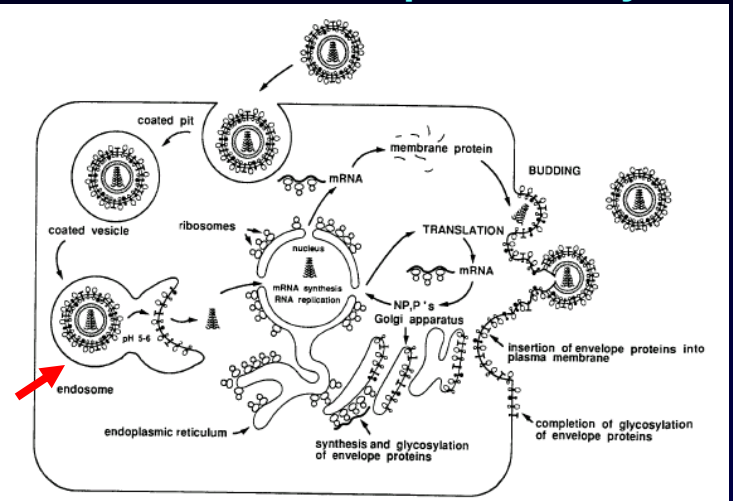

FIG. 13. Schematic diagram of the life cycle of influenza virus. See text for details of the model.

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Steps in Viral Replication: Uncoating
(Third Step)

- Makes viral nucleic acid available for transcription to permit multiplication to proceed
- Mechanism variably understood depending upon the virus

Uncoating of Influenza Virus

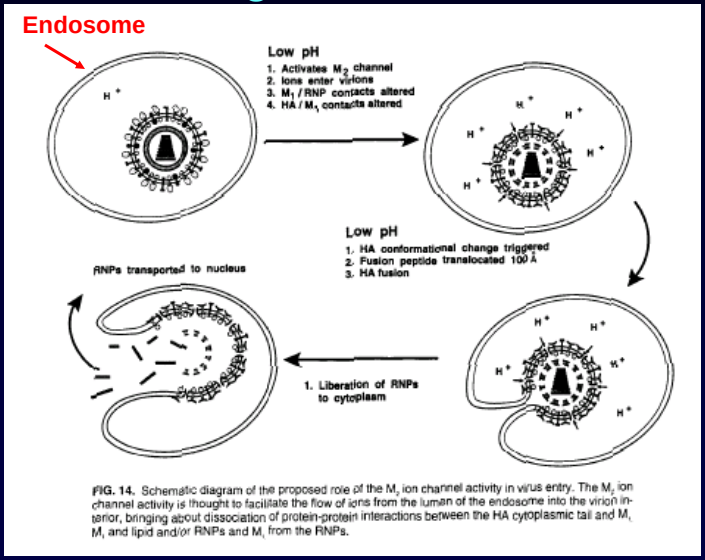

FIG. 14. Schematic diagram of the proposed role of the M_2 ion channel activity in virus entry. The M_2 ion channel activity is thought to facilitate the flow of ions from the lumen of the endosome into the virion interior, bringing about dissociation of protein-protein interactions between the HA cytoplasmic tail and M_1, M_1 and lipid and/or RNPs and M_1 from the RNPs.

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Steps in Viral Replication: Basic Strategies of Transcription and Translation
(Fourth and Fifth Steps)

- **(+) RNA → Proteins**
- **(-) RNA → (+) RNA → Proteins**
- **RNA → DNA → RNA → Proteins**
- **DNA → RNA → Proteins**

Steps in Viral Replication: Assembly and Release
(Sixth and Seventh Steps)

- Process involves bringing together newly formed genomic nucleic acid and structural proteins to form the nucleocapsid of the virus
- Nonenveloped viruses exhibit full maturation in the cytoplasm or nucleus with disintegration of cell

Steps in Viral Replication: Assembly and Release
(Sixth and Seventh Steps)

- Many enveloped viruses exhibit full maturation as the virion exits the cell
  - Viral proteins are inserted into the host cell membrane
  - Nucleocapsids bind to these regions and bud into the extracellular space
  - Further cleavage and maturation of proteins may occur after viral extrusion
  - Cytolytic activity of these viruses varies

Influenza Virus

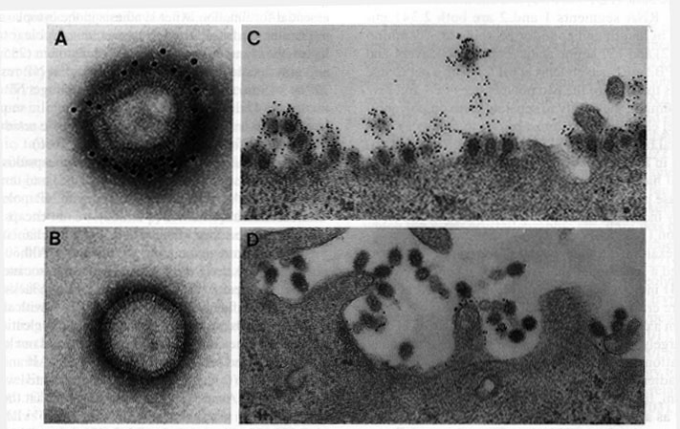

FIG. 2. Electron micrographs of purified influenza virus virions and virions budding from the surface of MDCK cells. **A:** Influenza A/Udorn/72 virus negatively stained with HA decorated with 10 nm gold (×159,250). **B:** Influenza A/Udorn/72 virus negatively stained with M_2 decorated with 10 nm gold (×159,250). **C:** Thin section of an influenza A/Udorn/72 virus-infected MDCK cell with HA decorated with 10 nm gold (H&E, ×40,600). **D:** Thin section of an influenza A/Udorn/72 virus-infected MDCK cell with M_2 decorated with 10 nm gold (×40,600). Courtesy of George Leser, Northwestern University, Evanston, IL.

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Retroviruses

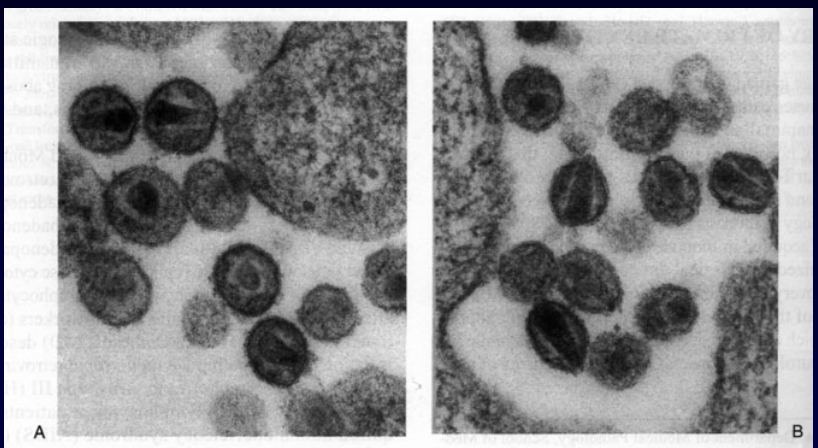

FIG. 1. Ultrastructure of primate lentiviruses. Electron microscopy of extracellular particles of HIV-1 (**A**) and SIV_{MAC} (**B**) reveals virions, about 110 nm in diameter, with a cone-shaped nucleoid surrounded by a lipid bilayer membrane, which contains envelope glycoprotein spikes (×100,000).

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Steps in Viral Replication: Assembly and Release
(Sixth and Seventh Steps)

- **Herpesviruses (enveloped) assemble nucleocapsids in the nuclei of infected cells and mature at the inner lamella of the nuclear membrane**
  - **Virions accumulate in this space, in the ER and in vesicles**
  - **Virion release is associated with cytolysis**

Herpes Simplex Virus

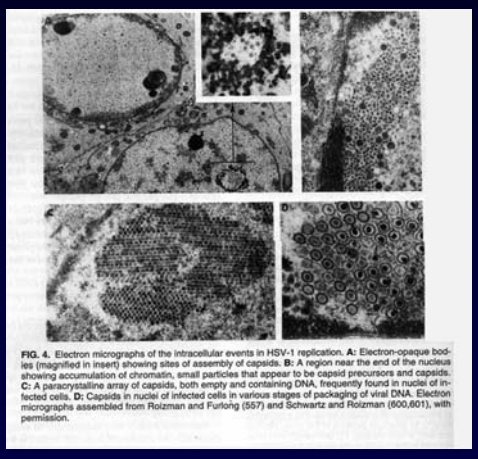

FIG. 4. Electron micrographs of the intracellular events in HSV-1 replication. **A:** Electron-opaque bodies (magnified in insert) showing sites of assembly of capsids. **B:** A region near the end of the nucleus showing accumulation of chromatin, small particles that appear to be capsid precursors and capsids. **C:** A paracrystalline array of capsids, both empty and containing DNA, frequently found in nuclei of infected cells. **D:** Capsids in nuclei of infected cells in various stages of packaging of viral DNA. Electron micrographs assembled from Roizman and Furlong (557) and Schwartz and Roizman (600,601), with permission.

FIG. 6. Electron micrographs of the envelopment and egress of virus from infected cells. **A:** Envelopment of virus from a protrusion of the nucleus. Note that the nucleus contains marginated chromatin. The inner lamellae of the nuclear membrane contain electron-dense, slightly curved patches representing regions of the membrane at which envelopment takes place. Note the spikes projecting from the surface of the membrane of the capsid being enveloped. **B:** An enveloped capsid and numerous unenveloped capsids found late in infection in the cytoplasm of infected cells. Some of the capsids appear to be in the process of being either enveloped or deenveloped. **C:** Micrograph showing an enveloped capsid in the space between the inner and outer lamellae of the nuclear membrane connecting with the cisternae of the endoplasmic reticulum. **D:** An unenveloped capsid in the nucleus and an enveloped particle bulging in the cisternae of the endoplasmic reticulum. **E:** Cytoplasmic enveloped particles enclosed in vesicles or cisternae of the endoplasmic reticulum. F: Modified nuclear membranes folded upon themselves, frequently seen in cells late in infection. The structures formed by such membranes have been designated as "reduplicated membranes." Electron micrographs assembled from Roizman and Furlong (557) and J. Schwartz and B. Roizman (unpublished micrographs).

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Schematic of Replication Cycle of (+) RNA Single Strand Viruses Coding for One Sized RNA

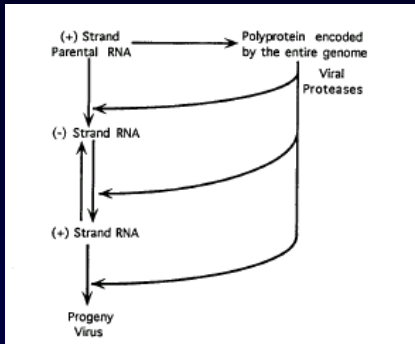

FIG. 2. Flow of events during the replication of positive strand RNA viruses that code for a single genome-sized (+) RNA. This RNA serves as their only mRNA species; it encodes a single polyprotein and is packaged into virions (e.g., picornaviruses, flaviviruses, hepatitis C viruses).

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Genomic RNA binds to ribosomes and is translated into polyprotein

Polyprotein is cleaved

Genomic RNA's serve as templates for synthesis of complementary full length (-) RNA's by viral polymerase

(-) strand RNA serves as template for (+) strand RNA's; these serve to produce more polyprotein, more (-) strand RNA's or become part of new virions

Schematic of Replication Cycle of (+) RNA Single Strand Viruses Coding for Genomic and Subgenomic RNA's

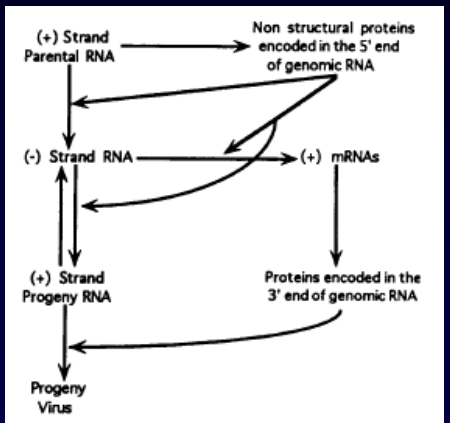

FIG. 3. Flow of events during the replication of positive strand RNA viruses that code for a genome-sized mRNA as well as for one or more subgenomic mRNAs (e.g., toga, corona, calici-, and hepatitis E viruses).

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Genomic RNA binds to ribosomes but only a portion of 5' end is translated into non-structural proteins

(-) strand RNA is synthesized. Different classes of (+) RNA's are produced. One is translated into a polyprotein which is cleaved to form structural proteins. Another is full length and serves as genomic RNA for new virions

Schematic of Nonsegmented (-) RNA Strand Virus Replication Cycle

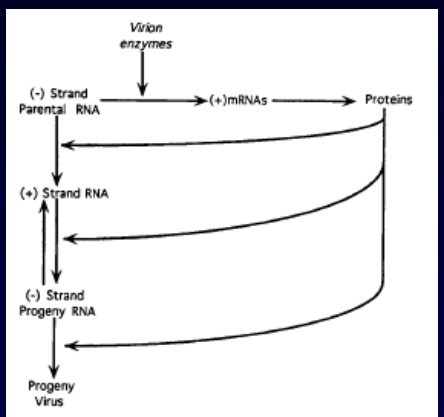

FIG. 5. Flow of events during the replication of nonsegmented negative strand RNA viruses (e.g., paramyxoviruses, rhabdoviruses, filoviruses).

Transcription of (-) strand occurs after entry and mediated by virion packaged transcriptase

(+) strand RNA's produced; proteins synthesized

Full length (-) strand RNA's produced and packaged into new virions

Transcription and translation take place entirely in cytoplasm

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Schematic of Segmented (-) RNA Strand Virus Replication Cycle

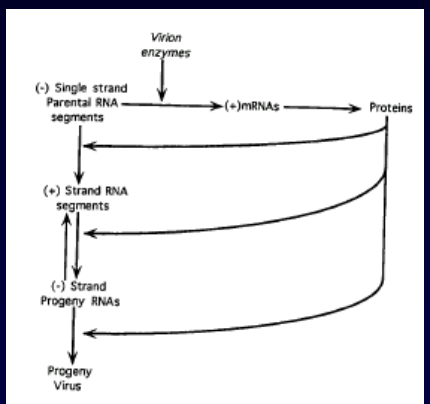

FIG. 6. Flow of events during the replication of segmented negative strand RNA viruses (e.g., orthomyxoviruses and bunyaviruses).

mRNA's are synthesized from each segment

Viral proteins are synthesized

(+) strand RNA's are synthesized and serve as templates for (-) strand genomic RNA's

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Schematic of Herpesvirus Replication Cycle (DS DNA Virus Which Replicates in Nucleus)

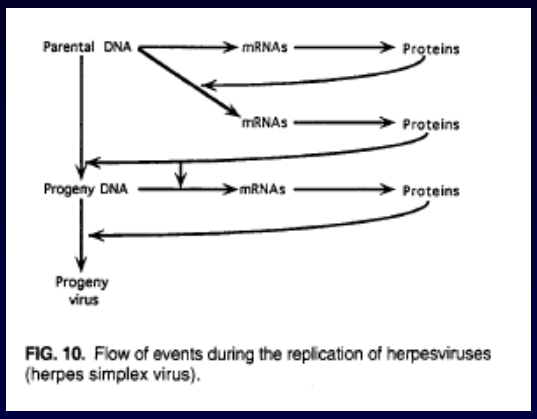

FIG. 10. Flow of events during the replication of herpesviruses (herpes simplex virus).

Sequential, ordered rounds of mRNA and protein production regulate replication

Structural proteins produced during last cycle of replication

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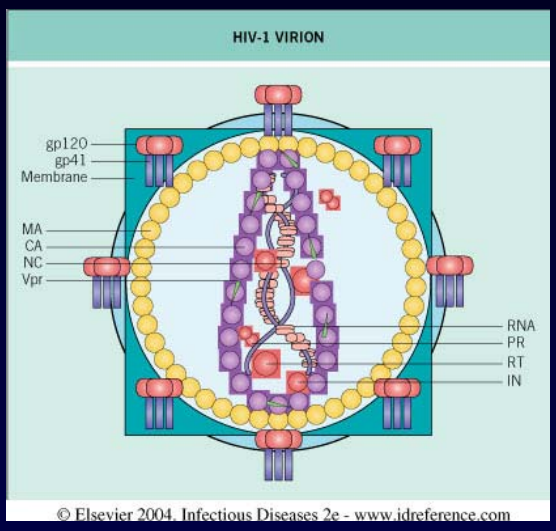
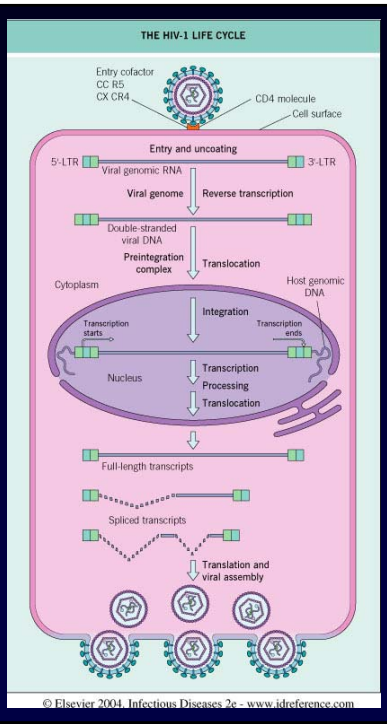
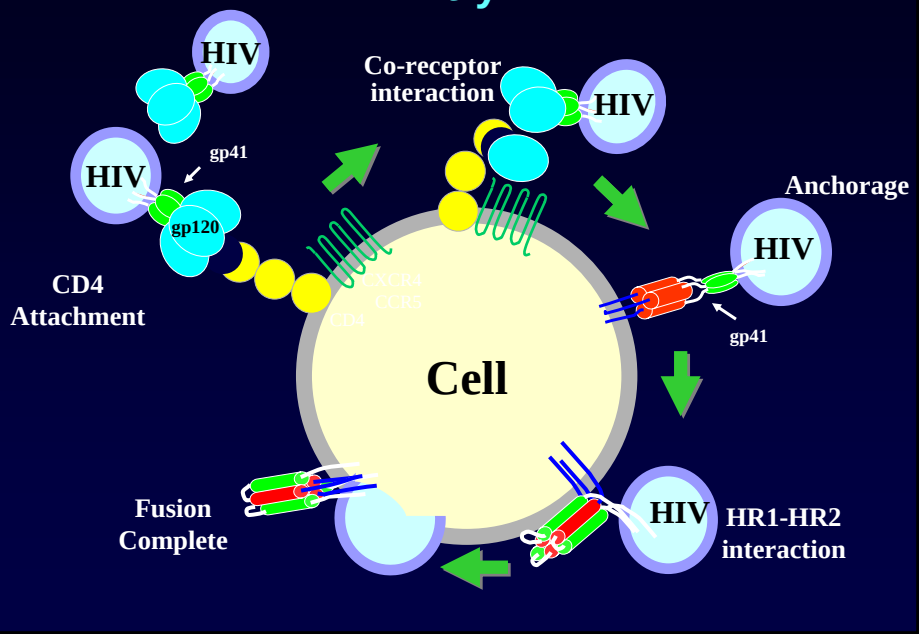
HIV-1 Virion



HIV Life Cycle



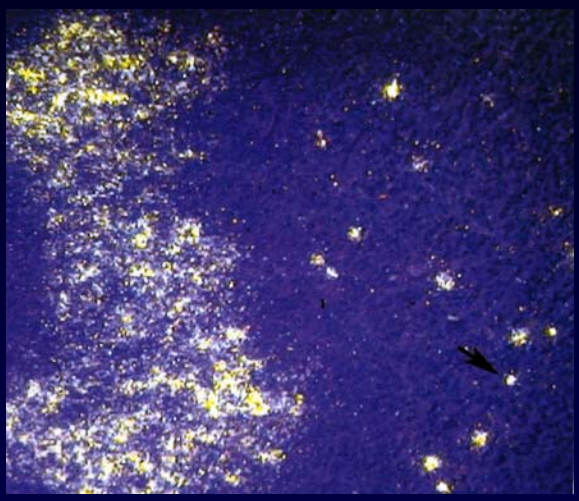
HIV Entry



Primary HIV Infection: Pathogenetic Steps

- Virus – dendritic cell interaction
  - Infection is typically with R5 (M-tropic) strains
  - Importance of DC-SIGN
- Delivery of virus to lymph nodes
- Active replication in lymphoid tissue
- High levels of viremia and dissemination
- Downregulation of virus replication by immune response
- Viral set point reached after approximately 6 months

PHI: Early Seeding of Lymphoid Tissue

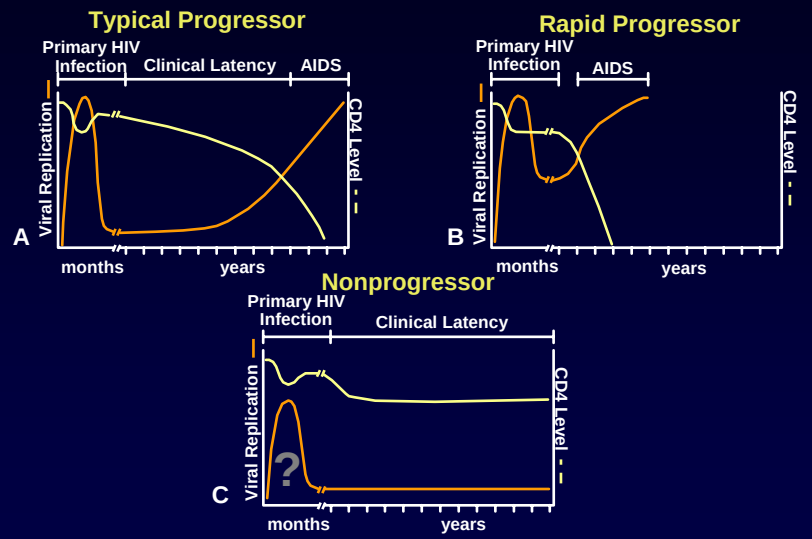


Schacker T et al: J Infect Dis 2000;181:354-357

Primary HIV Infection: Clinical Characteristics

- 50-90% of infections are symptomatic
- Symptoms generally occur 5-30 days after exposure
- Symptoms and signs
  - Fever, fatigue, myalgias, arthralgias, headache, nausea, vomiting, diarrhea
  - Adenopathy, pharyngitis, rash, weight loss, mucocutaneous ulcerations, aseptic meningitis, occas. oral/vaginal candidiasis
  - Leukopenia, thrombocytopenia, elevated liver enzymes
- Median duration of symptoms: 14 days

The Variable Course of HIV-1 Infection

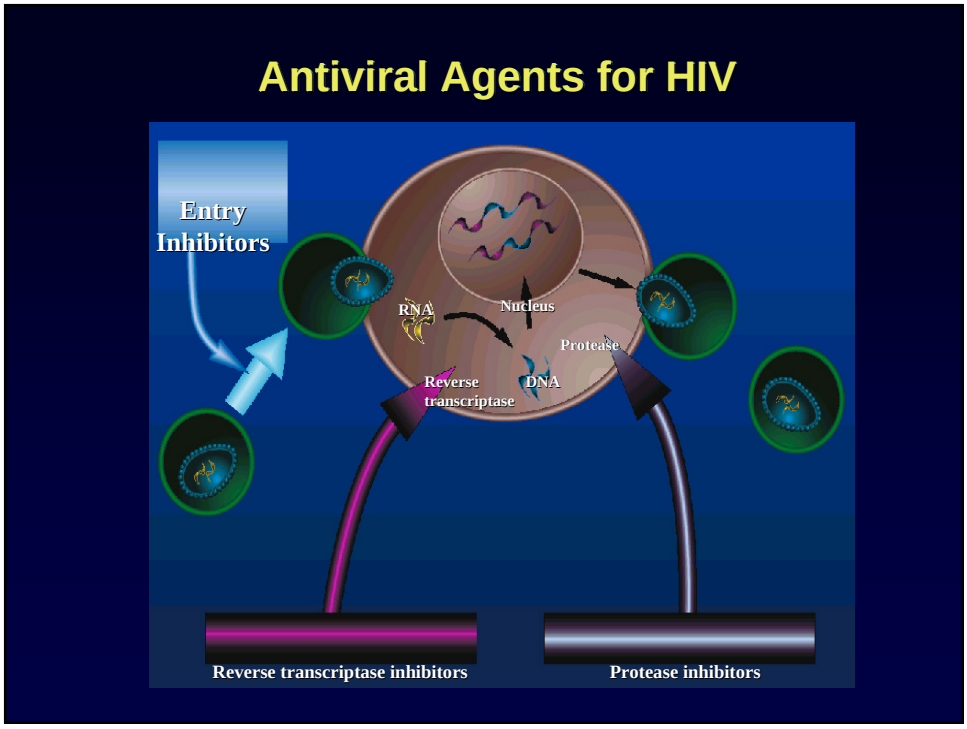


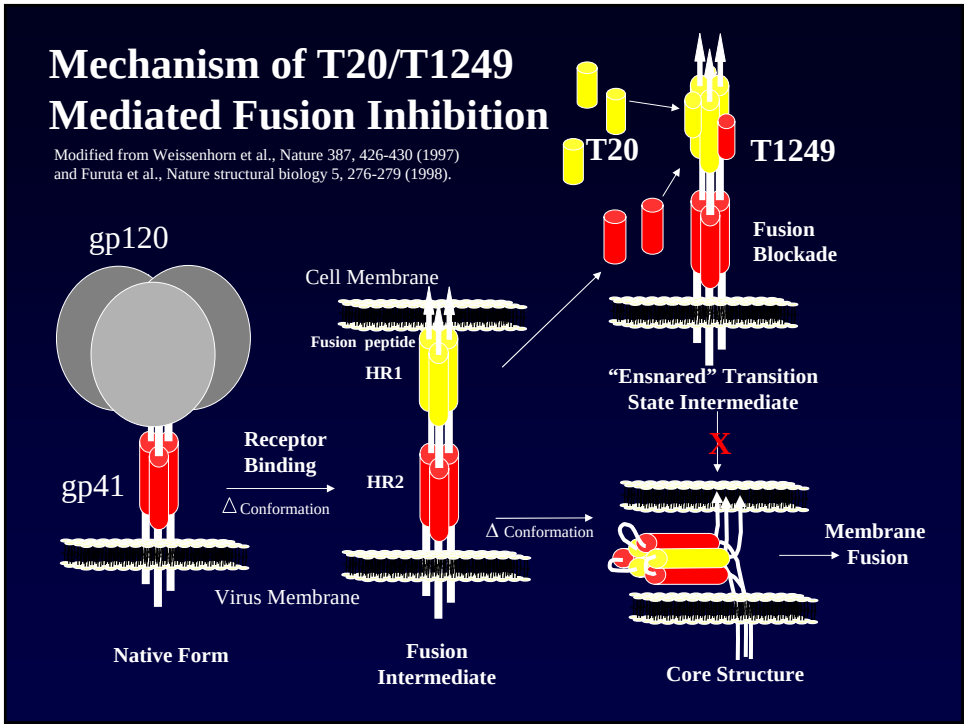
Reprinted with permission from Haynes. In: DeVita et al, eds. *AIDS: Etiology, Treatment and Prevention*. 4th ed. Lippincott-Raven Publishers; 1997:89-99.

Primary HIV Infection: Determinants of Outcome

- Severity of symptoms
- Viral strain
  - SI (X4) vs. NSI (R5) viruses
- Immune response
  - CTL response
  - Non-CTL CD8 responses
  - Humoral responses?
- Viral set point at 6-24 months post-infection
- Other host factors
  - Chemokine receptor and HLA genotype
- Gender and differences in viral diversity?
- Antiviral therapy
  - Near vs. long-term benefit?

Natural History of Untreated HIV-1 Infection


Antiviral Agents for HIV
Entry Inhibitors
RNA
Nucleus
Protease
Reverse transcriptase
DNA
Reverse transcriptase inhibitors
Protease inhibitors


Mechanism of T20/T1249 Mediated Fusion Inhibition
Modified from Weissenhorn et al., Nature 387, 426-430 (1997) and Furuta et al., Nature structural biology 5, 276-279 (1998).
T20
T1249
Fusion Blockade
gp120
Cell Membrane
Fusion peptide
HR1
"Ensnared" Transition State Intermediate
X
Receptor Binding
gp41
HR2
Δ Conformation
Δ Conformation
Membrane Fusion
Virus Membrane
Native Form
Fusion Intermediate
Core Structure