General Virology II

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Lecture Outline

- Host Range
- Replication Of Viruses
- Sterilization And Disinfection

Host Range

- **Viruses infect all major groups of organisms: vertebrates, invertebrates, plants, fungi, bacteria.**
- **Some viruses have a broader host range than others, but none can cross the eukaryotic/prokaryotic boundary.**

Factors which affect host range include;

i) whether the virus can get into the host cell

ii) if the virus can enter the cell, is the appropriate cellular machinery available for the virus to replicate?

iii) if the virus can replicate, can infectious virus get out of the cell and spread the infection?

Viral Replication

- **The major steps in viral replication are the same for all viruses.**
- **The cell acts as a factory, providing the substrates, energy, and machinery necessary for the synthesis of viral proteins and replication of the genome.**
- **Processes not provided by the cell must be encoded in the genome of the virus.**

One-Step Growth Curve

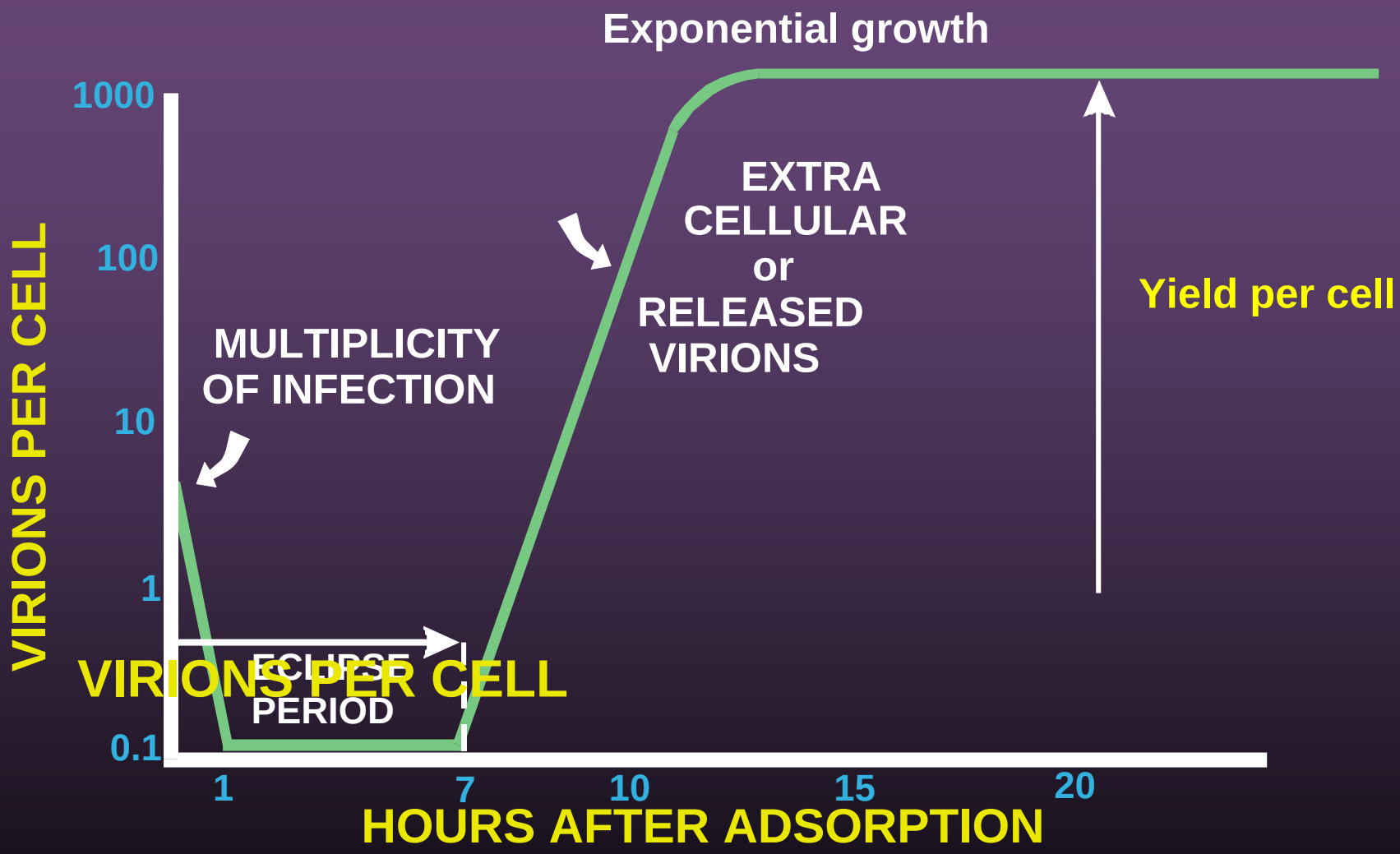
- Eclipse Period

  Following initial attachment of a virus to the host cell, the ability of that virus to infect other cells disappears. (1-20 hr)

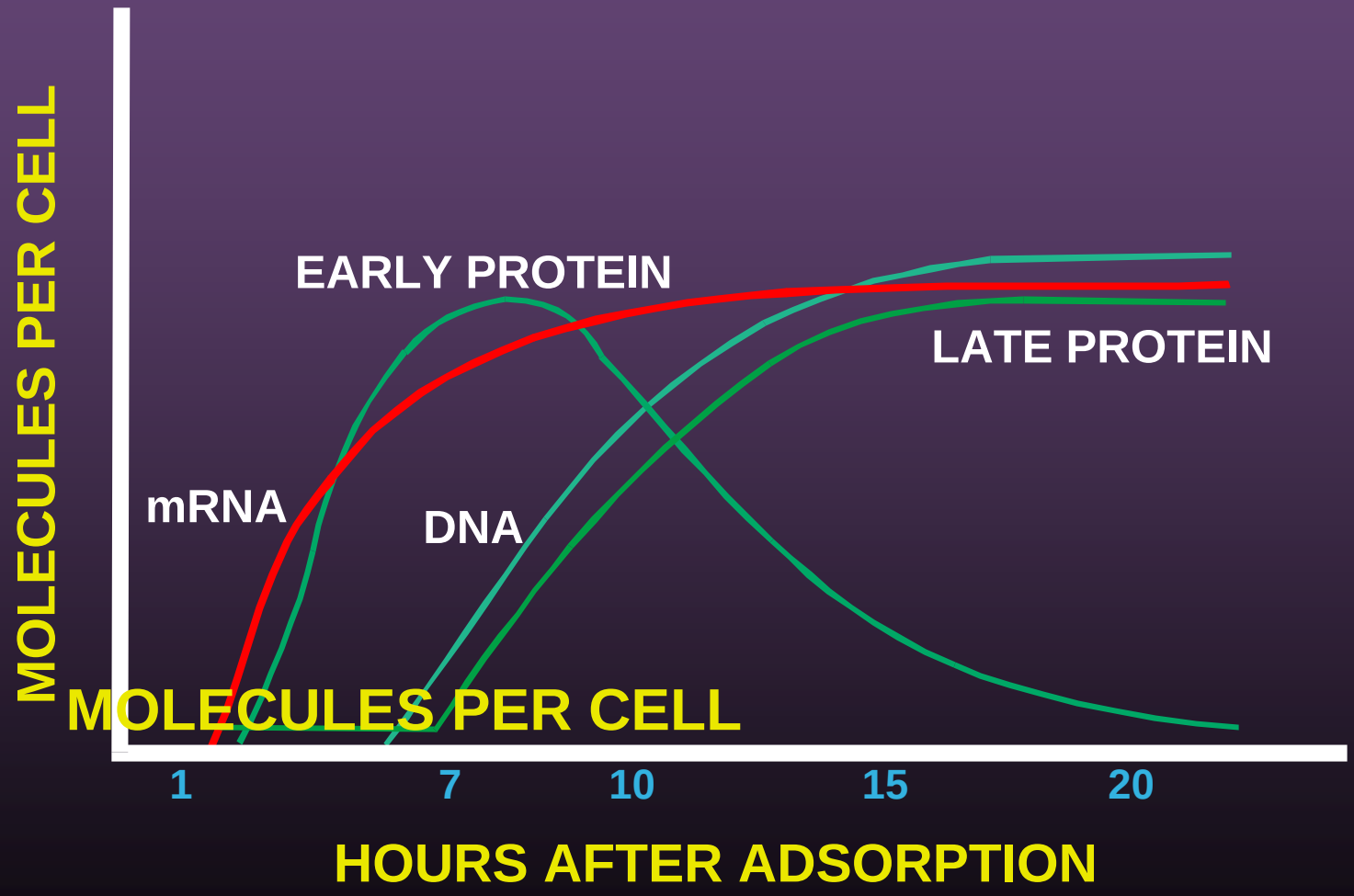
- Exponential Growth

  The number of progeny virus produced within the infected cell increases exponentially for a period of time, then reaches a plateau, after which no additional increase in virus yield occurs. (8 – 72 hr) → 100 to 10,000 virions/cell.

ONE STEP GROWTH CYCLE



ONE STEP GROWTH CYCLE



Replication Of Viruses

Principal Events Involved In Replication

1. ADSORPTION

- Virus attaches to the cell surface. Attachment is via ionic interactions which are temperature-independent.
- Viral attachment protein recognizes specific receptors on the cell surface (These may be protein or carbohydrate or lipid components of the cell surface).
- Cells without the appropriate receptors are not susceptible to the virus.

Replication Of Viruses

Principal Events Involved In Replication - cont.

1. ADSORPTION – Cont.

Example of cell receptors for viruses

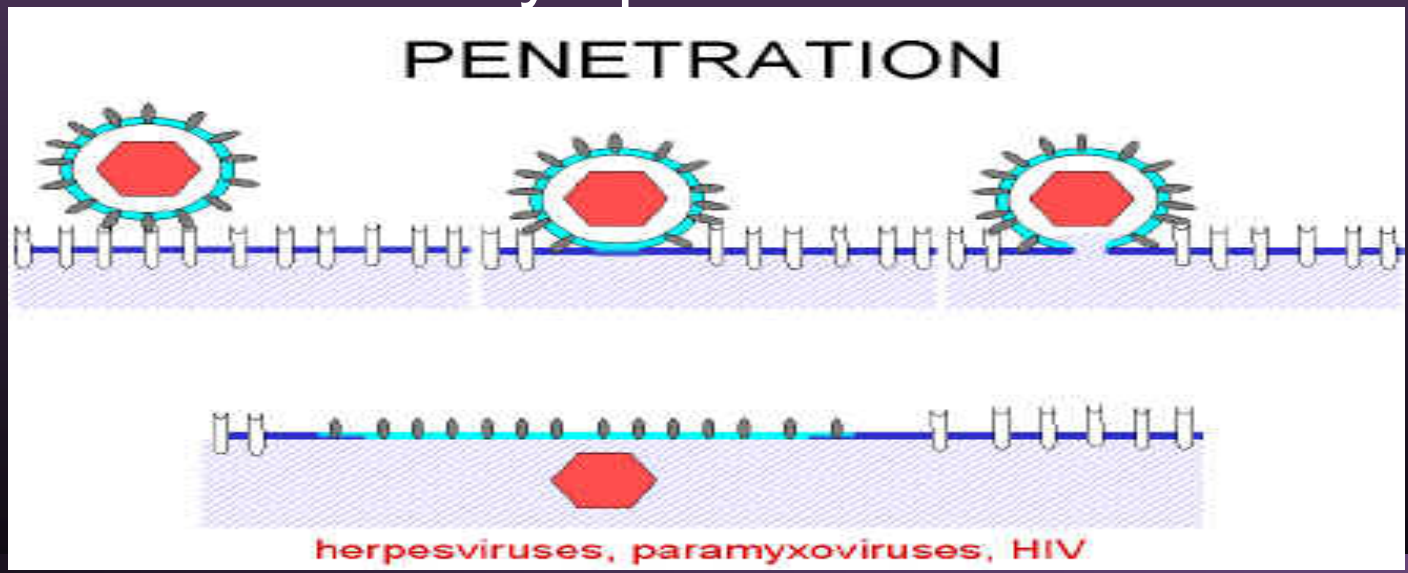
Family & Virus	Receptor
Herpesviridae **- HSV** **- CMV**	**Heparan sulphate of proteoglycans**
Retroviridae **- HIV**	**CD4 glycoprotein**

Replication Of Viruses

Principal Events Involved In Replication - cont

2. PENETRATION (Virus enters the cell)

(A) Entry by fusing with the plasma membrane. Some enveloped viruses fuse directly with the plasma membrane. Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell.

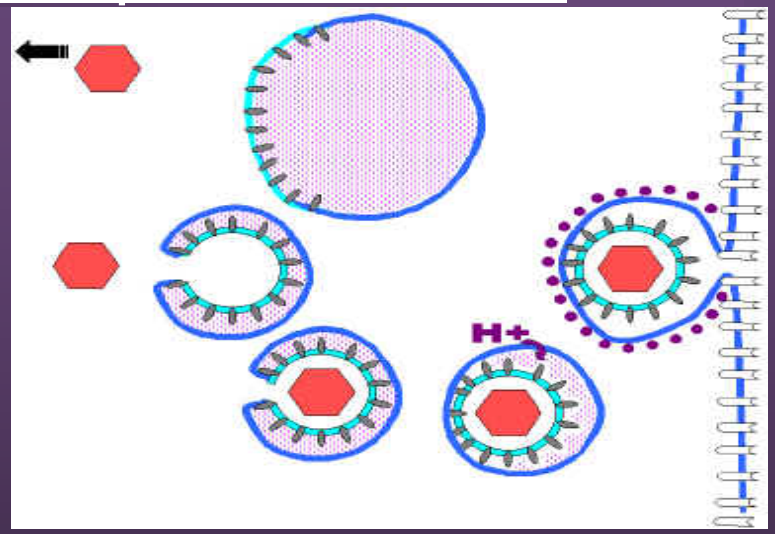


Replication Of Viruses

Principal Events Involved In Replication - cont

2. PENETRATION (cont.)

(B) Entry via endosomes at the cell surface.



•Some enveloped viruses require an acid pH for fusion to occur and are unable to fuse directly with the plasma membrane. These viruses are taken up by invagination of clathrin coated pits into endosomes. As the endosomes become acidified, the latent fusion activity of the virus proteins becomes activated by the fall in pH and the virion membrane fuses with the endosome membrane. This results in delivery of the internal components of the virus to the cytoplasm of the cell

Replication Of Viruses

Principal Events Involved In Replication - cont

2. PENETRATION (cont.)

Non-enveloped viruses may cross the plasma membrane directly or may be taken up via clathrin-coated pits into endosomes. They then cross (or destroy) the endosomal membrane.

Replication Of Viruses

Principal Events Involved In Replication - cont

3. UNCOATING

Nucleic acid has to be sufficiently uncoated that virus replication can begin at this stage. When the nucleic acid is uncoated, infectious virus particles cannot be recovered from the cell - this is the start of the **ECLIPSE phase** - which lasts until new infectious virions are made.

Virus macromolecular synthesis: viral genome strategies (1)

- Viruses have adopted a number of different strategies for the expression of their genes and the replication of their genomes.
- Certain cellular mechanisms and pathways are used by some but not by other viruses;
  - ✓ most DNA viruses use cell transcriptases located in the nucleus to generate mRNAs, and similarly situated DNA polymerases for genome replication, whereas other DNA viruses, notably the poxviruses, use a different strategy which includes mRNA synthesis by virion-bound transcriptases and polymerases, allowing them to synthesize both mRNAs and genomes in the cytoplasm.
  - ✓ Most RNA viruses, with the exception of retroviruses whose genomes can be copied into DNA, synthesize virus-specific mRNAs in the cytoplasm using virus-encoded enzymes.

Virus macromolecular synthesis: viral genome strategies (2)

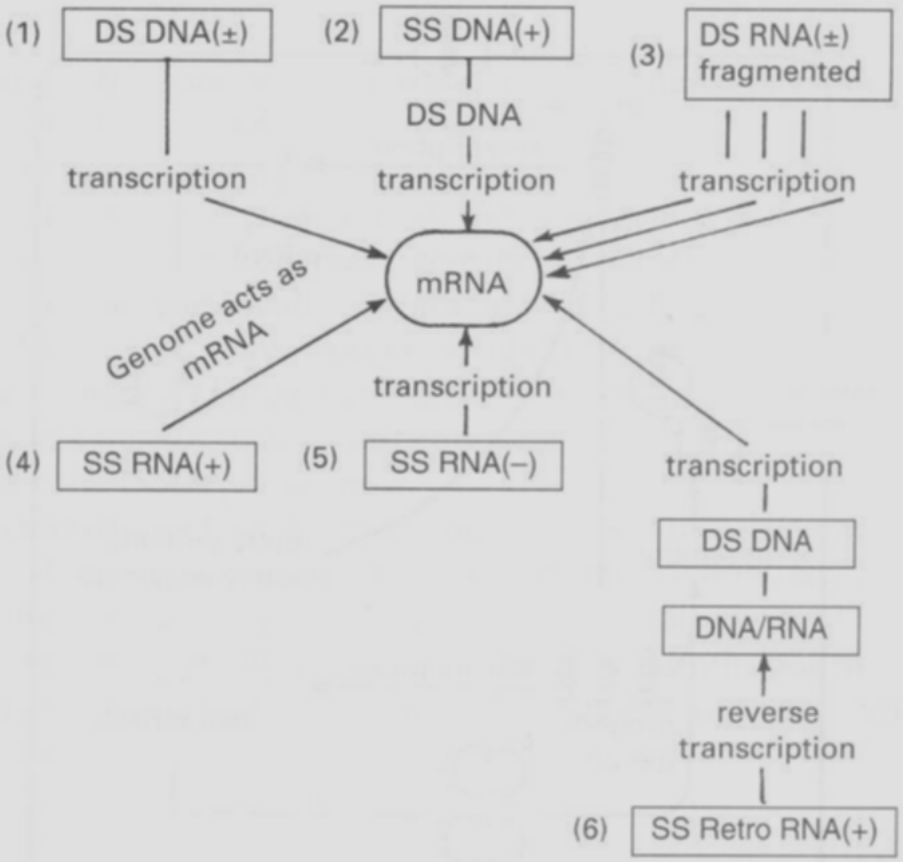


Fig. 2.1 Methods of transcription of the six different groups in the Baltimore classification of virus genomes. *Note*: (+) = positive-sense RNA, i.e. acts as a messenger; (–) = negative-sense RNA, i.e. transcribed to form complementary-sense strands, which then act as messengers.

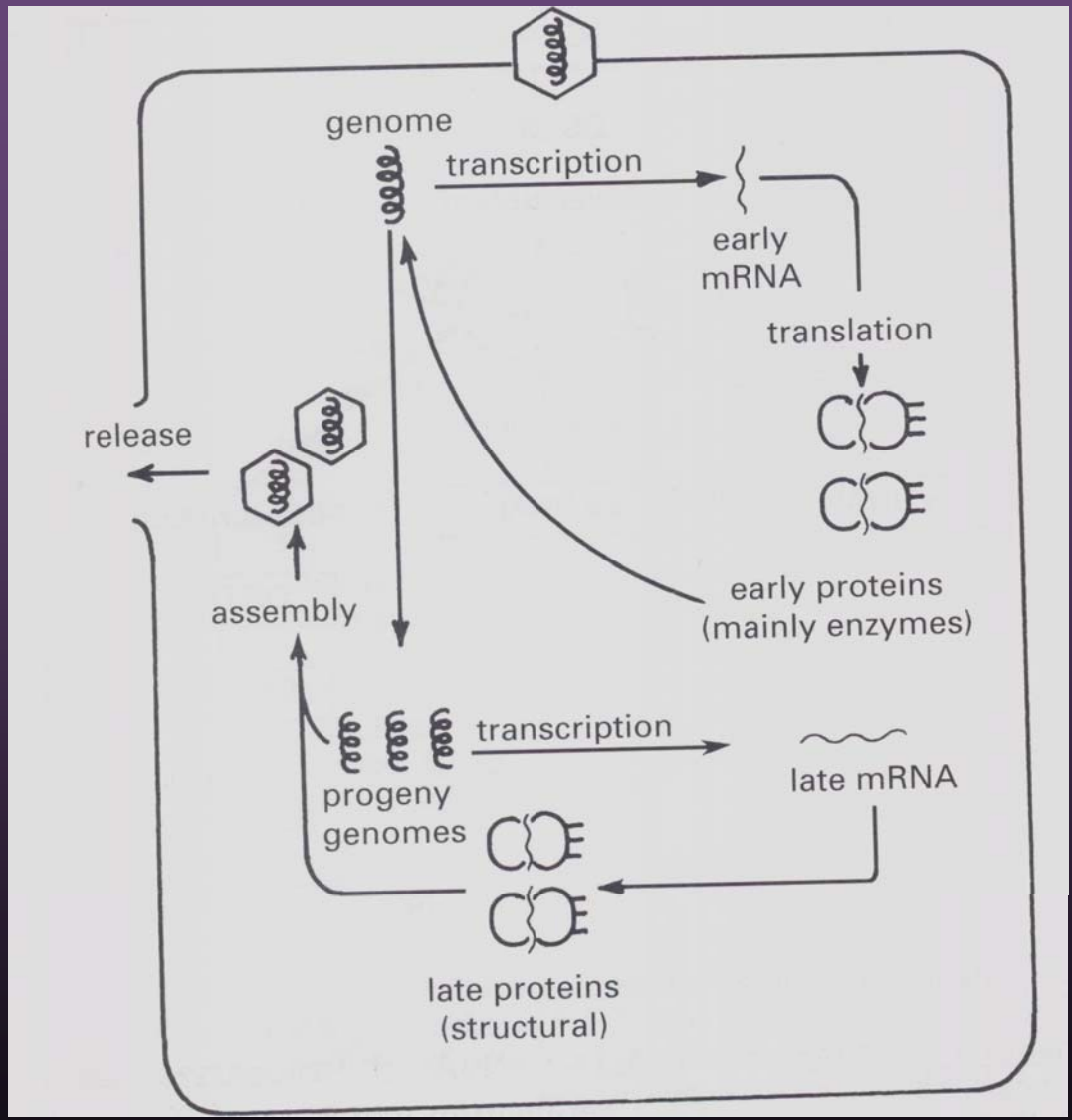
DNA viruses

- **The majority of viruses belong to families (Papovaviridae, Adenoviridae, Herpesviridae) whose members use the host nucleus for mRNA synthesis and genome replication, relying on host enzymes for transcription.**
  - **After entry of the virus and uncoating, virus DNA moves into the nucleus from the cytoplasm without modification.**
- **One family of viruses with complete ds DNA, the poxviruses, is distinct in that replication takes place in the cytoplasm.**
  - **The virus codes for its own RNA and DNA polymerases and produces functional mRNAs without requiring any cell nuclear functions.**
  - **RNA polymerase molecules are incorporated into virions as they are assembled and are used for early macromolecular synthesis during the next cycle of infection.**
  - **The presence of RNA polymerase in the virion is an obligatory requirement for the initiation of infection, and naked poxvirus DNA - unlike that of papovaviruses, adenoviruses and herpesviruses - is not infectious when extracted from virions.**

DNA viruses

- **The DNA of the hepadnaviruses is incorporated into virions in a partially ds form. Completion of the duplex molecule by a virion-associated DNA polymerase enzyme occurs in the cytoplasm before the mature DNA enters the nucleus to be transcribed by host transcriptase.**
- **Parvovirus virions contain a ss DNA molecule that moves directly into the nucleus after uncoating. It is then converted into duplex molecules and transcribed by host enzymes.**

DNA Viruses In General



Replication Of Viruses

Principal Events Involved In Replication - cont

4. SYNTHESIS OF VIRAL NUCLEIC ACID AND PROTEIN

A) DNA viruses

- **Transcription – 2 main types of mRNA are produced:**
  1. **Early mRNA – codes for enzyme required for DNA synthesis.**
  2. **Late mRNA – codes for structural proteins.**

- **Virus DNA synthesis (DNA-dependent DNA polymerase)**
  - **Larger viruses (HSV) code for their own enzyme.**
  - **Smaller viruses (adenovirus) use the host DNA polymerase.**

Replication Of Viruses

Synthesis Of Viral Nucleic Acid And Protein

A) DNA viruses – Cont.

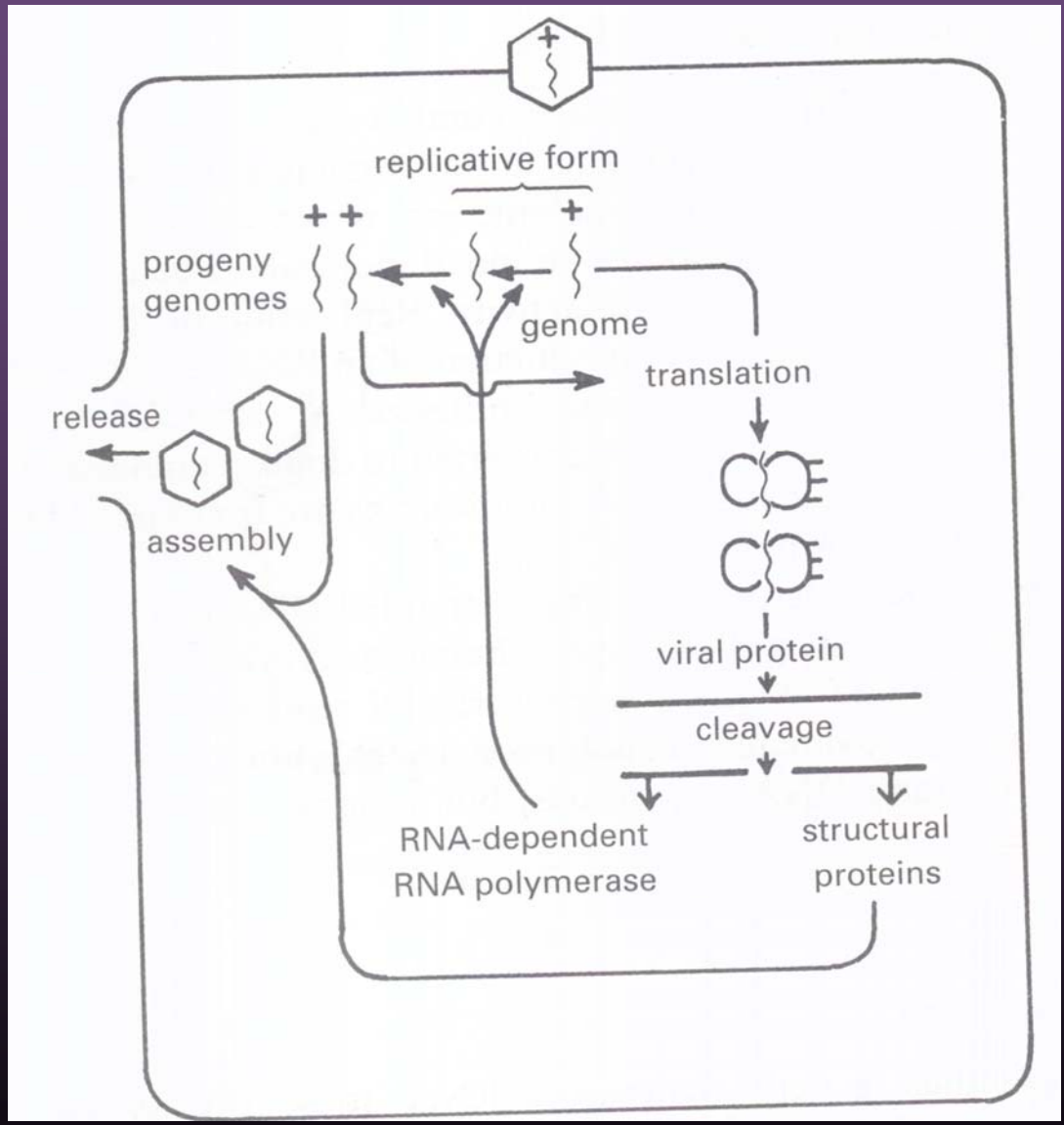
- **DNA synthesis of all DNA viruses take place in the nucleus (except poxviruses).**
- **Newly synthesised DNA act as a template for the transcription of the late mRNA.**

- **Virus protein synthesis (two stage process)**
  1. **Production of early proteins (non-structural protein)**
  2. **Production of late proteins (structural protein)**

ss (+) RNA viruses

- Picornaviruses, togaviruses and flaviviruses contain a ss of positive-strand RNA that has mRNA activity.
- This RNA is infectious by itself and is translated into protein immediately after uncoating in the cytoplasm.
- Picornaviruses use complete virus RNA as mRNA throughout the virus growth cycle, the individual protein being first synthesized as a single long polypeptide strand which is then cleaved to give the different functional proteins.
- Togaviruses synthesize at least 2 forms of mRNA, one being the same length as the virion RNA and the other being equivalent to the third of the virion RNA at the 3' end.

ss (+) RNA Viruses



Replication Of Viruses

Synthesis Of Viral Nucleic Acid And Protein

B) ss (+) RNA viruses (e.g. poliovirus)

- **No transcription - genomic RNA act as virus mRNA.**

- **Viral genome translated into very large polypeptide →cleaved into smaller proteins;**

  1. Structural proteins.

  2. RNA-dependent RNA polymerase for viral RNA replication.

  3. A protease for the polypeptide cleaving.

Replication Of Viruses

Synthesis Of Viral Nucleic Acid And Protein

B) ss (+) RNA viruses – Cont.

- **Virus RNA synthesis – (+) RNA strands synthesised off the template of the (-) RNA strand using RNA-dependent RNA polymerase.**

- **Progeny of (+) RNA functions as:**
  - **Genomes for new virus particles**
  - **Virus mRNA**

ss (-) RNA viruses

- Most RNA viruses with single-stranded negative-sense genomes replicate in the cytoplasm.

- The RNA of these viruses is incapable of initiating infection by itself and the virions contain a transcriptase that starts to synthesize functional mRNA soon after the virion envelope has fused with cell membranes, allowing the virus nucleocapsid to enter the cytoplasm.

ss (-) RNA viruses

- The genomes of these viruses may consist of a single molecule (paramyxoviruses, filoviruses and rhabdoviruses) or may be segmented (arenaviruses, bunyaviruses and influenza viruses).

- All viruses with segmented negative-strand genomes require a functional cell nucleus for replication, and initiate transcription by using capped oligonucleotide primers derived from the 5' ends of cell mRNA or mRNA precursors, which are spliced onto the 5' end of the virus mRNA. This phenomenon, known as 'cap-snatching', occurs also during the replication of certain plant viruses (e.g. the tenuiviruses, which have a segmented negative-strand RNA genome).

Replication Of Viruses

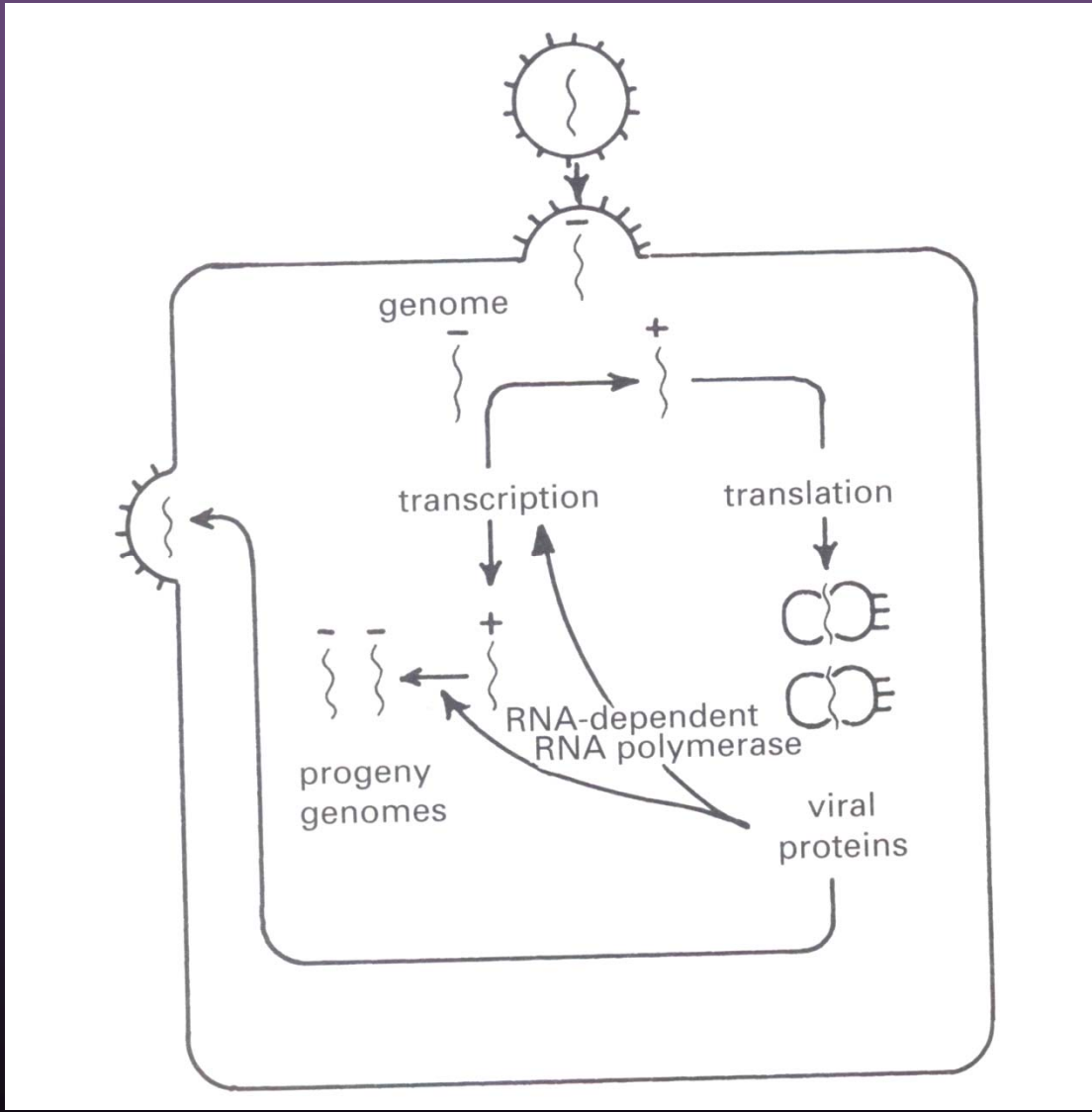
Synthesis Of Viral Nucleic Acid And Protein

C) ss (-) RNA viruses (e.g. Parainfluenza virus)

- **Transcription – mRNA synthesised off the parental ss (-) RNA using a viral transcriptase (RNA-dependent RNA polymerase).**
- **Virus progeny genomes produce as well by the transcriptase using (+) RNA strands complementary to the parental genome as a template.**

- **Virus protein synthesis**
  - **Non-structural proteins encode for transcriptase.**
  - **Structural proteins encode for envelope proteins & nucleocapsid proteins**

ss (-) RNA Viruses



Retroviruses

- Retroviruses resemble DNA viruses in that transcription takes place from ds virus DNA molecules situated in the nucleus and requires cellular DNA-dependent RNA polymerases.
- Virions possess a reverse transcriptase that copies the diploid RNA genome into a single DNA strand, which it then converts into a duplex molecule.
- These double-stranded DNA molecules then enter the nucleus and integrate into host chromosomes.
- Transcription by host enzymes then occurs.

Retroviruses

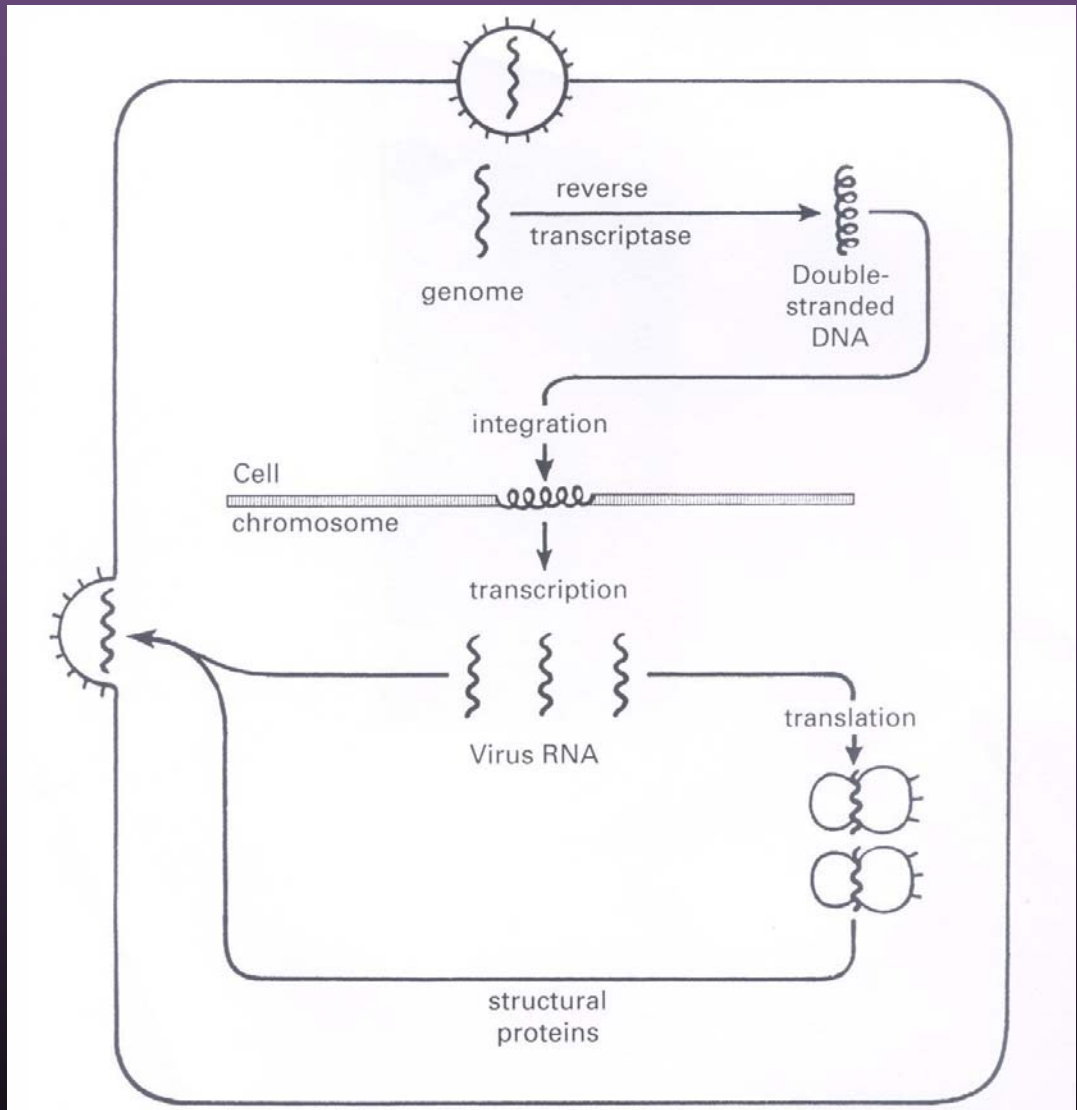


Fig. 2.5 Replicative cycle of retrovirus.

Replication Of Viruses

Principal Events Involved In Replication - cont

5. ASSEMBLY / MATURATION

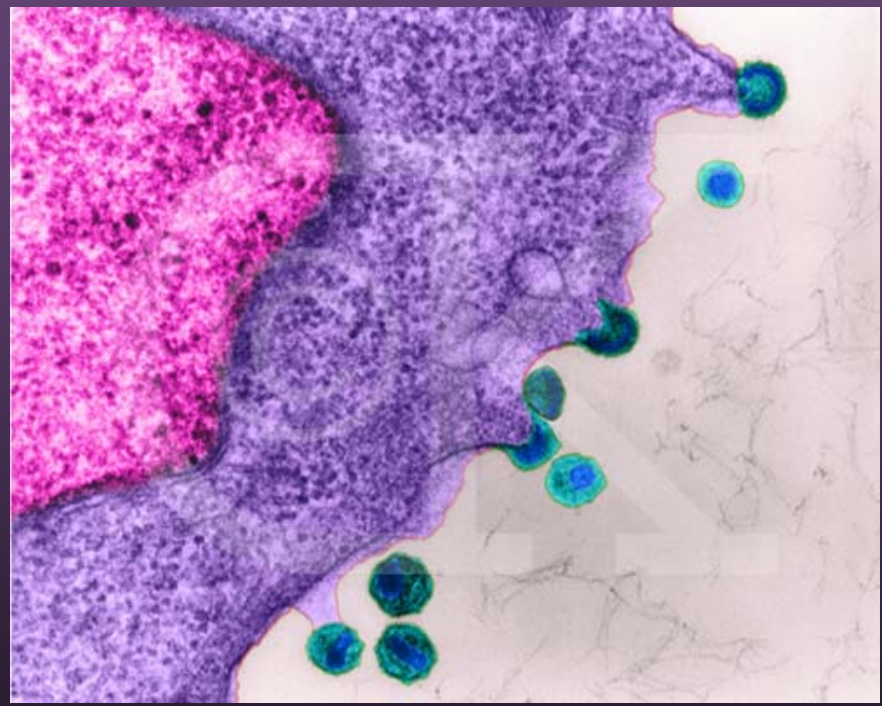
New virus particles are assembled. After assembly they may undergo a maturation process.

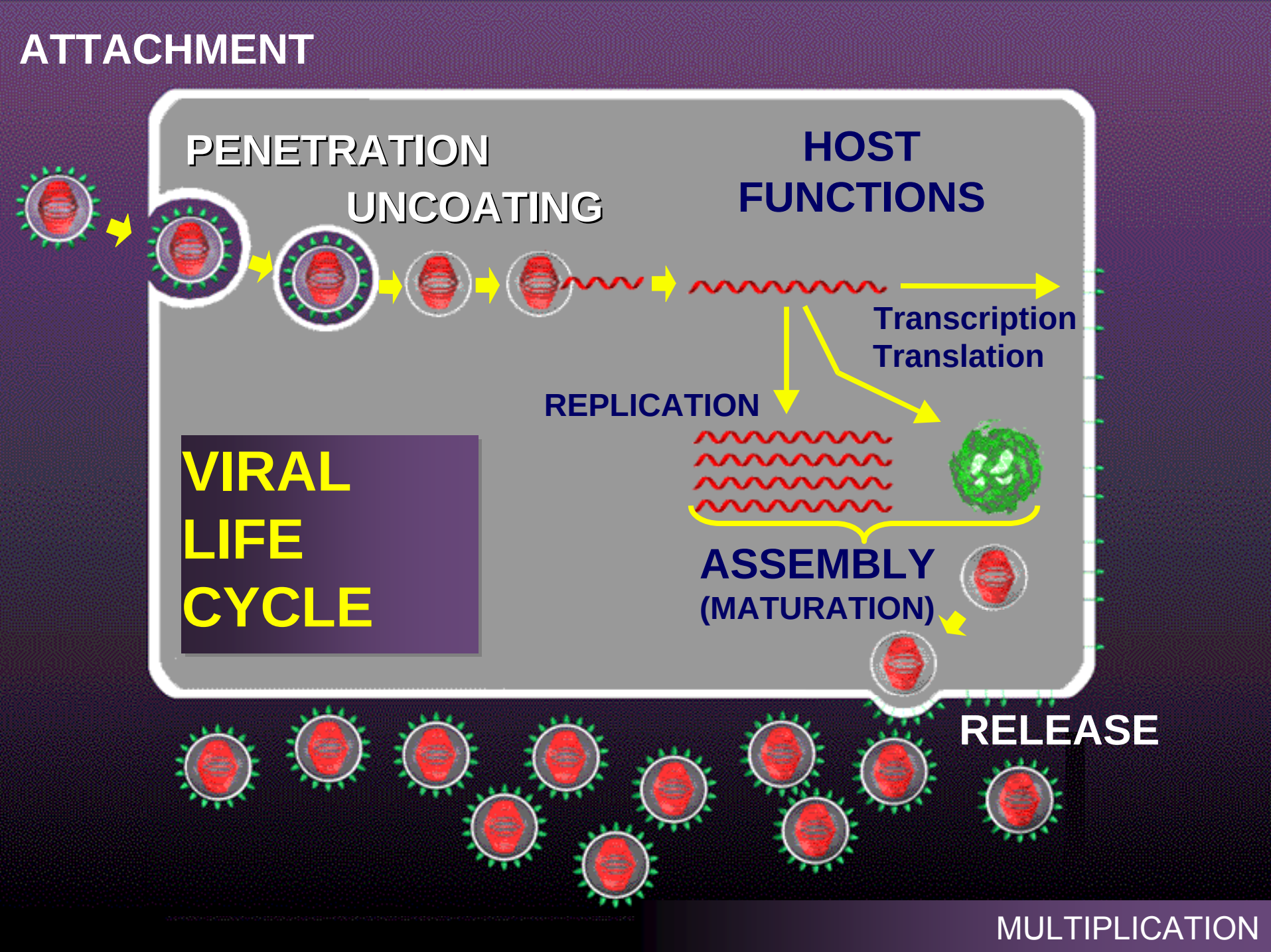
Replication Of Viruses

Principal Events Involved In Replication - cont

6. RELEASE

Virions may be released due to cell lysis, or, if enveloped, may bud from cell. Budding viruses do not necessarily kill the cell. Thus, some budding viruses may be able to set up persistence.




ATTACHMENT
PENETRATION
UNCOATING
HOST
FUNCTIONS
Transcription
Translation
REPLICATION
VIRAL
LIFE
CYCLE
ASSEMBLY
(MATURATION)
RELEASE
MULTIPLICATION

Sterilization and Disinfection

Sterilization and Disinfection (1)

- **Viruses, especially the enveloped viruses, are generally fairly labile and do not survive too well outside their host cells. However, some (e.g. hepatitis B virus) are very resistant to inactivation, and healthcare workers need to take special precautions to avoid transmitting such infections.**

- **Means of prevention of the spread of infection, and sterilization and disinfection of viruses, are very similar to those principles that are applied in bacteriology.**

Sterilization and Disinfection (2)

Spread may be by;

1) inhalation of aerosolized "droplets";

2) ingestion;

3) direct contact (skin/mucous membrane to skin/mucous membrane), or

4) indirect contact via intermediate "vomits".

Sterilization and Disinfection (3)

- **Moist heat (autoclaving 121°C x 20 minutes) or dry heat (oven, 180°C for 60 minutes) are effective against all viruses - lesser degrees of heat may inactivate many viruses (e.g. simple boiling) but may not reliably inactivate resistant viruses especially if times of exposure are short.**
- **Chemicals: halogens, especially chlorine as hypochlorite are effective against viruses but corrosive on instruments where activated glutaraldehyde ("Cidex") is preferred.**

  Detergents and lipid solvents inactivate readily the enveloped viruses which need an intact envelope for effective cell adsorption.
  Phenolic disinfectants damage proteins and thus inactivate bacteria but do not affect nucleic acids. Phenolics are not recommended for viral disinfection.