REPLICATION OF VIRUSES

LECTURE 2 DONE BY LAITH THEEB

LECTURE MAP, TOPIC AND SLIDE NO.

THE PATHOLOGICAL EFFECTS OF THE DISEASES CAUSED BY VIRUSES RESULT FROM THE INTERPLAY OF SEVERAL FACTORS:

- Toxic effects of viral gene products (proteins, enzymes) on the metabolism of infected cells.
- Reactions of the host to infected cells expressing viral genes, by inducing an immune response when Cytotoxic T lymphocytes and NKC sense MHC I molecules displaying viral foreign antigens.
- Modification of host gene expression by structural or functional interactions with the genetic material of the virus. Some viruses can induce carcinogenesis (oncoviruses) like: HPV (cervical cancer, oropharyngeal cancer); EPV; Herpes 8 (kaposi sarcoma); Hepatitis B&C. Also HTLV can be associated with leukemia.

HOST RANGE, SUSCEPTIBILITY AND PERMISSIVENESS

- **Susceptibility:** defines the capacity of a cell or an animal to become infected.
- The process of infection begins with the coming together of a virus particle and a susceptible host cell. Example: liver hepatocytes are **susceptible** to be infected by Hepatitis viruses because they have NTCP receptors for the virus. And here we can say that Hepatitis viruses have a **viral tropism** for hepatocytes. Susceptibility and tropism are determined by the host cells receptors and viral receptors.
- Viral tropism: is the ability of a virus to infect different and specific cell types (Nomaguchi et al. 2012)
- **Permissiveness: permissive** cell or host is one that allows a virus to circumvent its defenses and **replicate**. (Wikipedia)
- **Host range**: defines both the kinds of tissue cells and animal species that it can infect and in which it can multiply (wide Vs narrow), it is also determined by host and viral receptors.

- Viral replication is a complex process that involves multiple interactions at the molecular level.
- Discussion will concentrate on aspects relevant to understanding of viral pathogenesis at the molecular level.
- Important in the area of antiviral chemotherapy where it is needed to determine what stages are likely to be potential targets or susceptible to chemotherapeutic agents.

- **To infect a cell, the virion must:**

1. **Attach** to the cell surface by viral receptors and cell proteins.
2. **Penetrate** the cell.
3. **Become sufficiently uncoated** to make its genome accessible to viral or host machinery for transcription or translation. The translation of viral proteins mainly depend on cell machinery of replication (cell ribosomes).

- The cell acts as a factory providing the substrates, energy & machinery necessary for synthesis of viral proteins and replication of the genome.
- Each infected cell may produce (maximally) as many as 10^(5) particles (burst size), most of produced viral particles are defective, and only 1-10% which are infectious. This high ability for a virus to produce more viruses plays a role in the rapid evolution of viruses.

TYPES OF INFECTION

- **Infection of a cell may be:**
- **Productive (permissive):** There is penetration of the cell by the virus then replication and production of more infectious particles.
- **Abortive (non-permissive, defective):** There is penetration of the cell by the virus but no replication.
- **Stringent or restrictive (transient permissiveness):** The initial infected cell can support only few stages of virus replication, so the virus needs to transfer to another cell to complete its replication (HPV: infects basal cells the moves to keratinocytes.)
- **Transforming:** The virus transforms infected cells to cancer cells.

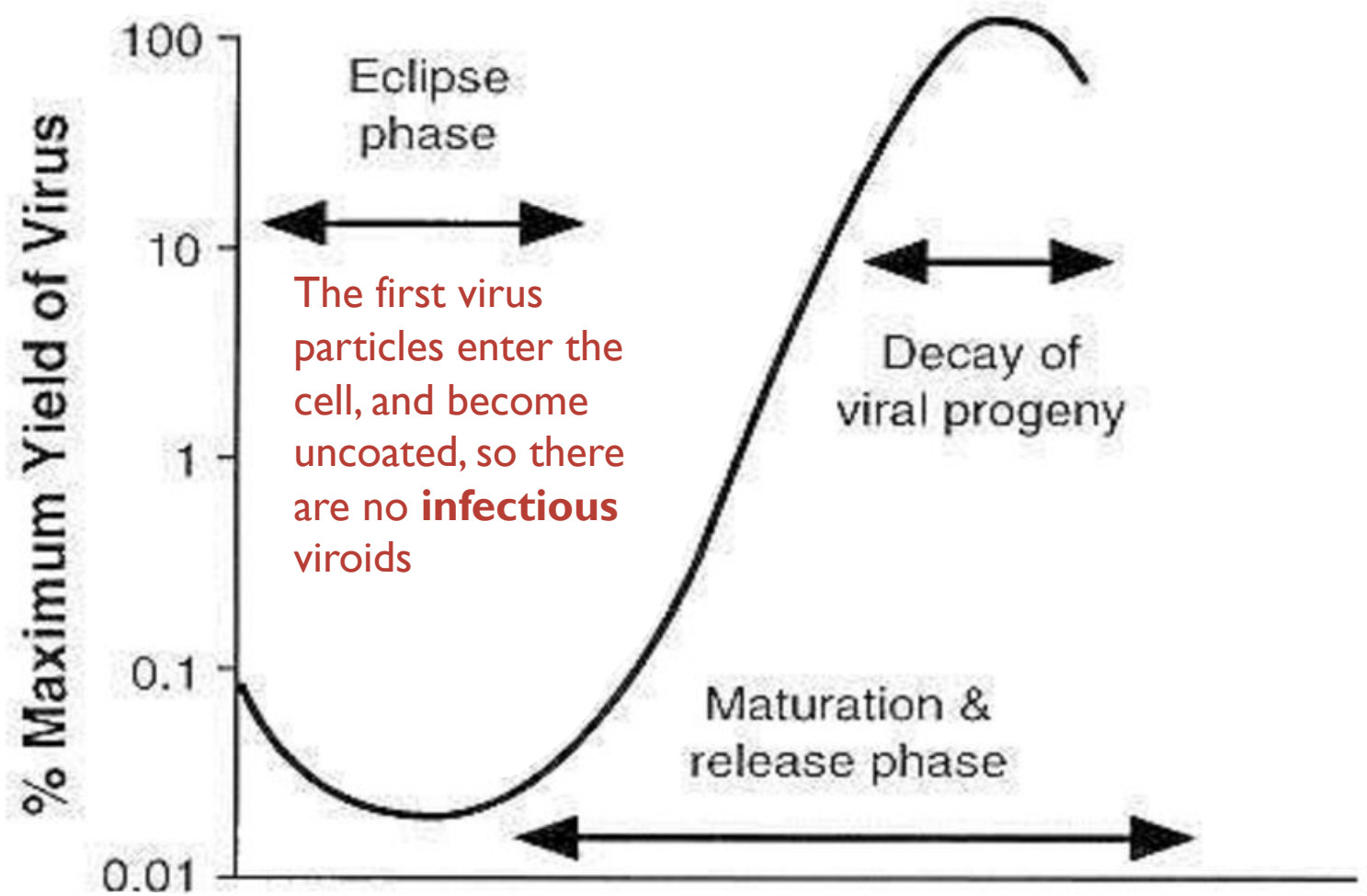
- **Virus replication can be divided into eight stages, namely:**
- **Attachment**, **penetration**, **uncoating** (uncoating means dissolving of caspid, release of genome & proteins or enzymes needes for the first steps of virus replication),
- **genome replication**, **gene expression**,
- **assembly**, **maturation** and **release**.
- **Note that:** These are purely arbitrary divisions, (not sharp divisions) used here for convenience in explaining the replication cycle of a non existing " typical " virus.
- Not all stages described here are detectable as distinct stages for all viruses, often they "blur" together and appear to occur almost simultaneously.

THESE STAGES CAN BE CLASSIFIED INTO THREE PHASES:

- **1.Initiation phase**
- Attachment
- Penetration
- Uncoating

- **2.Replication phase**
- DNA Synthesis
- RNA Synthesis
- Protein synthesis

- **3.Release phase**
- Assembly
- Maturation
- Exit from cell



The first virus particles enter the cell, and become uncoated, so there are no **infectious** viroids

Latent period

INITIATION PHASE: 1. ATTACHMENT:

- **Some notes about viral and cellular receptors/anti-receptors:**
- Attachment constitutes the specific binding of a viral protein VAP to a constituent of the cell surface (receptor/ anti-receptor). (**VAP:** VAMP-associated protein; **VAMP:** vesicle-associated membrane protein)
- Complex viruses may have **more than one species** of anti-receptor molecules.
- Anti-receptor molecules may have **several domains**, each of which may react with a different receptor.
- Mutations in the genes specifying anti-receptors may cause **loss of the capacity to interact with certain receptors.**
- Receptors identified thus far are largely **glycoproteins or glycolipids.**

ATTACHMENT

- **Some notes about viral and cellular binding:**
- Repulsion between virus and cell membrane impedes **attachment because both are negatively charged.** Although fusion of two lipid membranes is an energetically favorable process, there is a large activation barrier due to electrostatic repulsion between the polar head groups of the phospholipids (Chernomordik et al., 2006; Kozlov et al., 2010).
- Attachment results from random collision between virions and cell surface at a frequency of 10^(-3) to 10^(-4) leading to a physical complementary union.

- **Some notes about viral and cellular binding:**
- **Susceptibility** of a cell is limited by the availability of appropriate receptors, and not all cells in an otherwise susceptible organism express receptors.
- **Early binding is reversible** and firm binding requires specific receptor anti-receptor interaction.
- **Attachment** of viruses to cells in many instances leads to **irreversible** changes in the structure of the virion.
- In some instances, however, when penetration does not ensue, the virus can detach and elute from cell surface. Some viruses have specific mechanisms for detachment (neuraminidase in infuenza). enzymes are glycoside hydrolase enzymes that cleave (cut) the glycosidic linkages of neuraminic acids.
- Elution leads to changes in the virus VAP which decrease or eliminate the possibility of subsequent attachment to other cells

INITIATION PHASE: 2. PENETRATION:

- An energy dependent step that occurs almost instantaneously after attachmen and **it involves one of three mechanisms:**
- **Endocytosis (viriopexis)** of the virus particle resulting in accumulation of virus particles inside cytoplasmic vesicles. Most common.
- **Fusion of the virion envelope with the cellular membrane viral mambrane** (Requires fusion protein in viral envelope). Ex: HIV.
- **Translocation of the entire virus** across the plasma membrane. Rare and poorly understood.
- Penetration may be pH independent and it is usually immediately followed (inseparable) by uncoating.

INITIATION PHASE: 3. UNCOATING:

- It is poorly understood.
- The **virus capsid is completely or partially removed**, and the virus **genome exposed**, usually in the form of a **nucleoprotein complex.**
- Uncoating may be **initiated by attachment to the receptor** or prompted by the **acidic environment or proterases** found in an endosome or lysosome.

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES

- **1. DNA viruses:** (mechanism of replication will be discussed later)
- **All DNA viruses, except poxviurses, replicate in the nucleus**.
- **Transcription:** They utilize **cellular RNA polymerase** (DNA dependent RNA Polymerase) for transcription.
- **Replication**:
- In a few instances, it is **cellular enzymes** that replicate the viral genome assisted by viral proteins (**parvovirus**).
- In most cases the opposite is true, **viral enzymes** are responsible for genome replication although they utilize cellular proteins to aid this. Examples in next slide.

- **DNA viruses:**
- **Examples:**
- Simple DNA viruses (**parvo & papovaviruses**) utilize **host cell DNA-dependent DNA polymerase.**
- **Whereas** the larger more complex ones **(adeno, herpes, and poxviruses)** encode their **own polymerases**.
- **Fidelity of replication:**
- **Note that** viral polymerases are faster but **less precise (lower fidelity)** than cell polymerase causing a **higher mutation rate** and providing a target for antiviral drugs. Usually mutations are neither beneficial nor harmful for viruses, but sometimes they can cause drug resistance, vaccine escape or ability for the virus to adapt new species.

- **DNA viruses:**
- **Also**, in comparison to RNA replication, the fidelity of DNA replication is such that only one mistake is made in 10^(9) – 10^(10) base pair replication compared with one in 10^(3) – 10^(4) for RNA viruses.
- Error-free replication arises from the ability of DNA polymerase to proof-read the DNA which they have just synthesized.
- In contrast, RNA polymerases **need not be self- correcting** in as much as relatively high error rates can be tolerated.
- All DNA viruses known to infect vertebrates contain a monopartite genome. (One segment)

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **2. RNA viruses:**
- Most RNA viruses replicate in the cytoplasm **except (Retroviruses and orthomyxoviruses like influenza)**. part of the replicative cycle of which takes place in the nucleus.
- **Transcription:** they use their own transcriptase. (RNA dependent RNA polymerase)
- **Replication:**
- Virion associated RNA polymerases have the activities of RNA polymerase, 5' capping, and 3' polyadenylation.
- Host cells **can not replicate nucleic acid in the cytoplasm**, so viruses that replicate in the cytoplasm carry all enzymes necessary for their replication and this applies to **poxviruses and most RNA viruses.**

- **2. RNA viruses:**
- Replication and transcription of RNA viruses are similar processes as the template is RNA in both cases, and dsRNA intermediates are formed. dsRNAs are considered foreign molecules for the human body, so there are toll-like receptors (innate immune response) that sense the presence of dsRNA and promotes anti viral immune response.
- Since RNA is degraded relatively quickly, the RNA polymerase must be provided or synthesized soon after uncoating to generate more viral RNA, or the infection is aborted.

- **2. RNA viruses:**

- The genomes of ssRNA viruses are either:
- **1. Monopartite**: (picorna, toga, paramyxo, rhabdo, corona, and retroviruses).
- **2. Multipartite**: (orthomyxo, arena, and bunyaviruses).
- Most RNA genomes are linear

- **General notes about DNA and RNA viruses:**
- The virus **must be able** to interact with the cell biosynthetic machinery according to the biochemical rules of the cell.
- Transcription and hence translation usually proceed in **two phases, early and late:**
- 1. **The early phase** results in the synthesis of regulatory proteins and enzymes necessary for replication of viral nucleic acid.
- 2. **The late phase** leads to the synthesis of structural proteins which are usually made in excess.

- **General notes about transcription in DNA and RNA viruses:**
- **Transcription** of the viral genes is regulated by the interaction of specific **DNA- binding proteins** with promoter and enhancer elements in the viral genome.
- Cells from different tissues or species express **different DNA binding proteins.**
- Different DNA and RNA viruses **control** the duration, sequence and quantity of viral gene expression and protein synthesis in different ways.
- The more complex viruses encode their **own transcriptional** activators.

- **General notes about translation in DNA and RNA viruses:**
- Translation proceeds in essentially the same fashion as eukaryotic mRNA **utilizing cellular tRNA and initiation factors.**
- Posttranslational modification takes place **utilizing cellular pathways.**
- Structural proteins of the virus may act as **repressors** of transcription **by binding to viral DNA or RNA**.

- **General notes about translation in DNA and RNA viruses:**
- **Viruses employ different tactics to promote preferential translation of their viral mRNA: (how viruses let the cell translate viral mRNA instead of cellular mRNA):**
- 1. In many cases the **concentration of viral mRNA** in the cell is so large that it **occupies most of the ribosomes.**
- 2. **Block the egress** of cellular mRNA from the nucleus.
- 3. **Inhibit cellular macromolecular synthesis** and induce degradation of the cell's DNA and mRNA.
- 4. **Increase the permeability of the cell membranes** which decreases the ribosomal affinity for cellular mRNA.

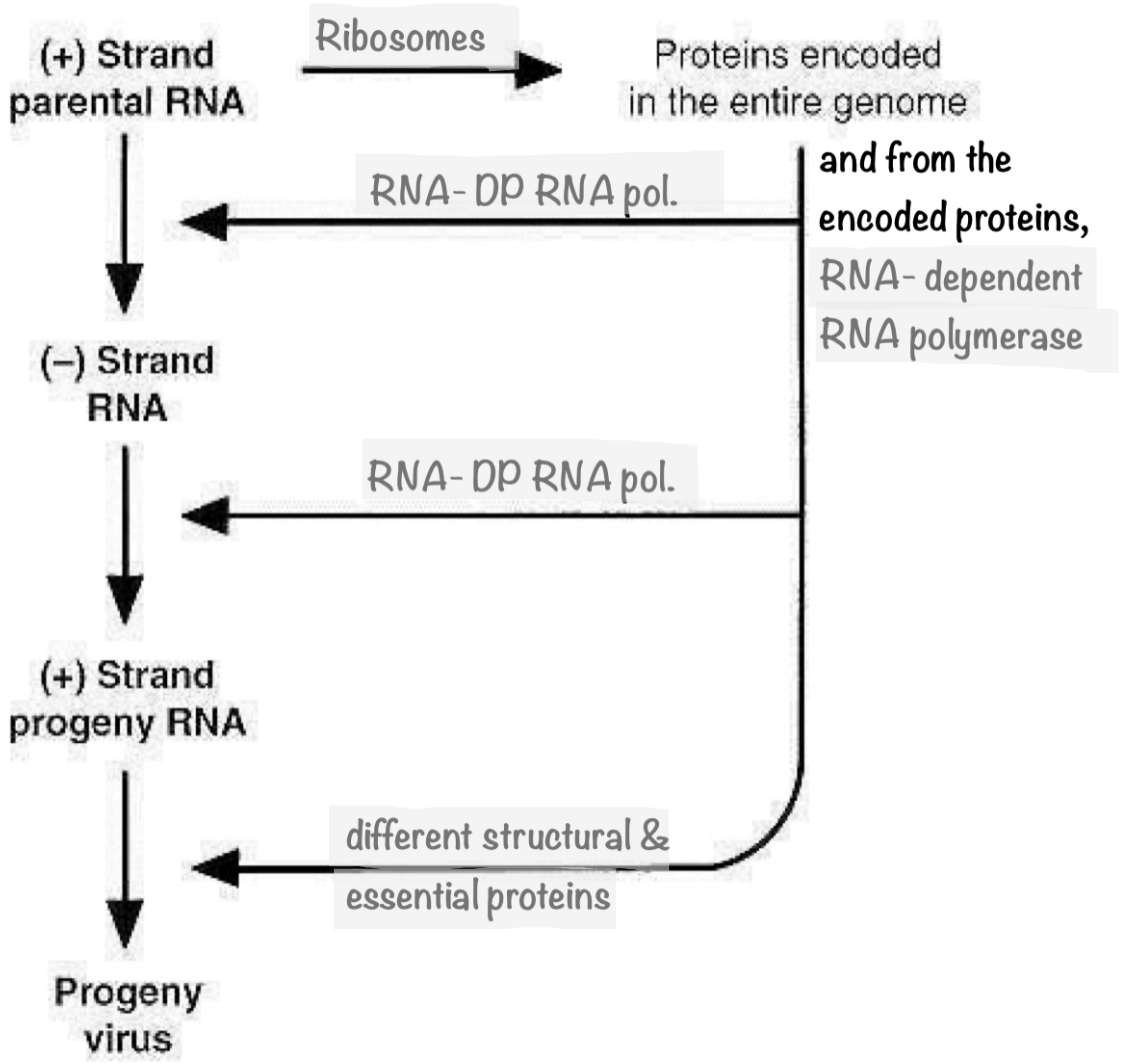
REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Positive sense:**
- **1. Positive (+) strand RNA viruses** coding for **one genome-sized mRNA** (polio from the Picornaviruses family, Flavi, HCV).
- Their coding domains are translated in their **entirety**.
- The product of translation, the polyprotein, is then **cleaved** by cellular or/and viral proteases.
- Synthesis of complementary full-length (-) strand RNA.
- The (-) strand RNA in turn serves as a template to make-more(+) strand RNAs

Flow of events during the replication of Picornaviruses (like polio):

Refer to this video for further explanation
https://youtu.be/THw34p66xlk

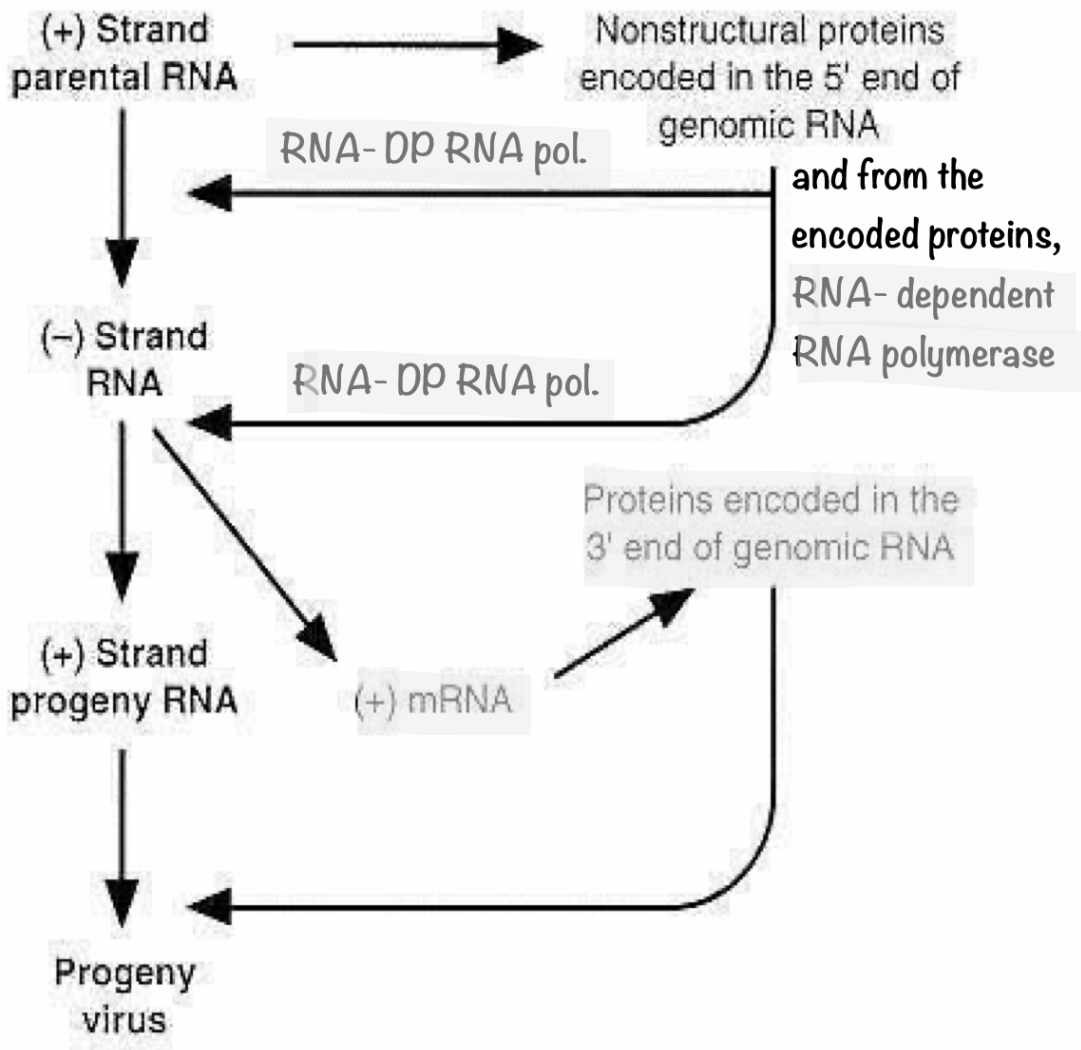


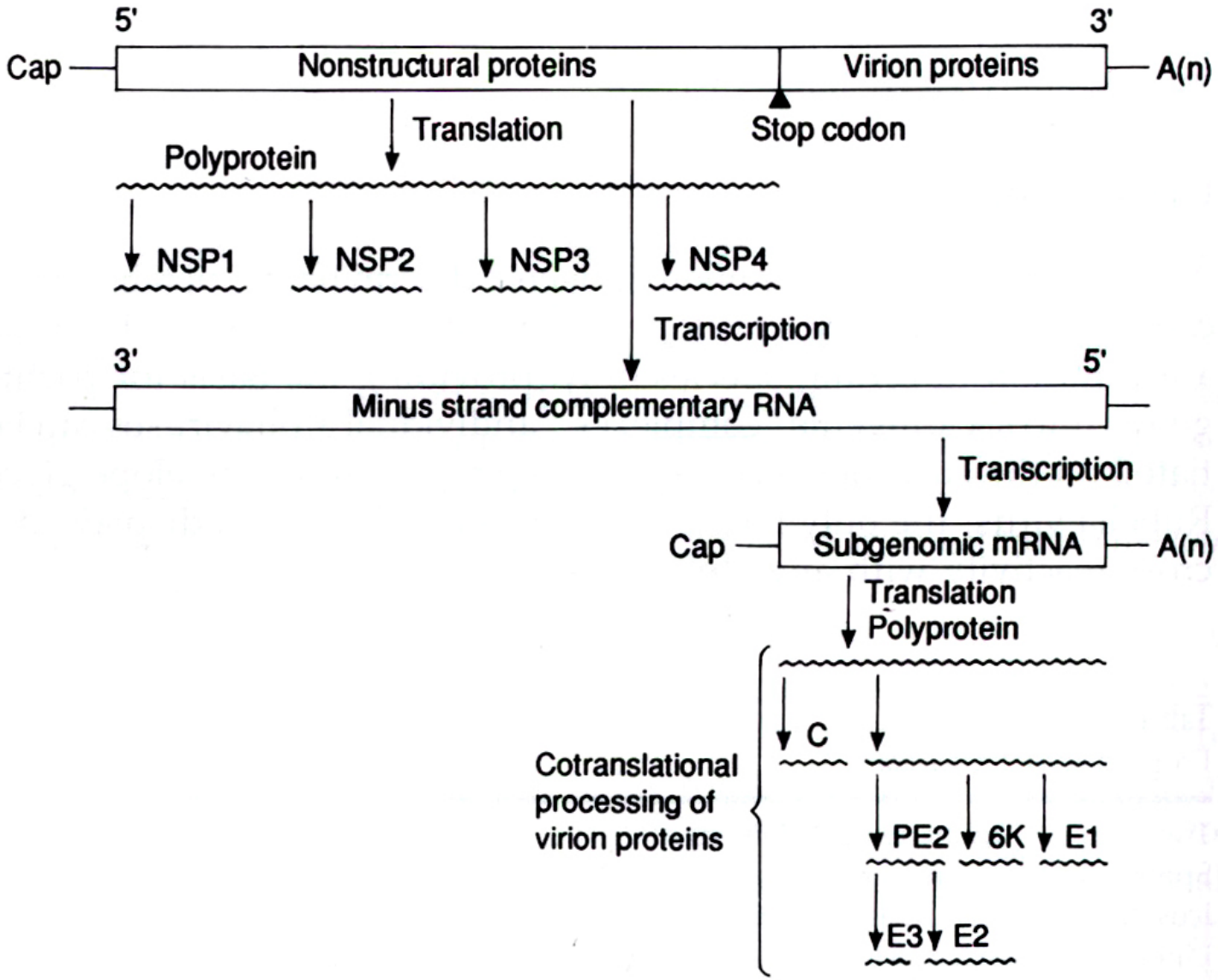
REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Positive sense:**
- **2. Positive (+) Strand RNA viruses** coding for **one or more subgenomic mRNAs** (Toga, corona, calici, HEV)
- Only a portion (the 5' end) of the genomic RNA is available for translation in the first round of protein synthesis
- A (-) strand is then synthesized, and this RNA in turn serves as a template for two size classes of (+) RNA molecules.
- Cleavage clearly involves virus-specified proteases, and the polyprotein itself is enzymatically active in trans.
- Two or more subgenomic mRNA species are made in cells infected with corona, calici or HE viruses.

Flow of events during the replication of Togaviruses:





FURTHER EXPLANATION FOR TOGAVIRUSES REPLICATION (ADDITIONAL)

Note that the negative sense strand serves as a template for two size classes of (+) RNA molecules: the progeny (+) genome (not shown), and subgenomic RNA (the small one).

REPLICATION PHASE:

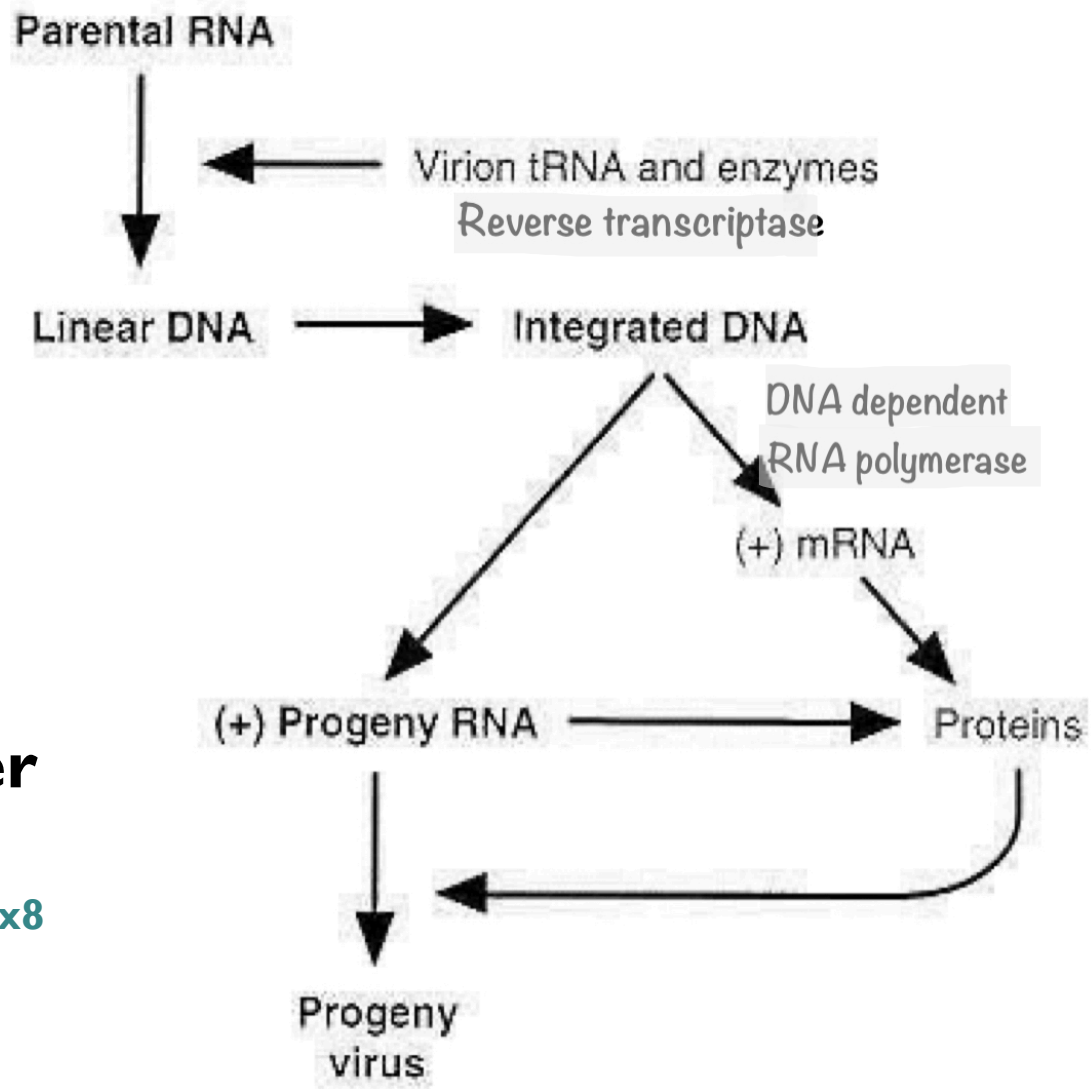
EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Positive sense:**
- **3. Retroviruses:**
- **First step** in replication is synthesis of a DNA strand complementary to the RNA genome using reverse transcriptase (RNA-dependent DNA polymerase), followed by digestion of RNA by a nuclease (ribonuclease H in the virion), and finally synthesis of a complementary DNA strand.
- The linear ds DNA translocated into the nucleus integrates into the host genome (Provirus).
- The products of transcription are genome-length RNA molecules (efficiently packaged into virions), and shorter, spliced mRNAs that are translated to yield polyproteins that are processed by cleavage to individual viral proteins.

Flow of events during the replication of Retroviruses:

Refer to this video for further explanation

https://www.youtube.com/watch?v=4_90RsgVsx8



REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

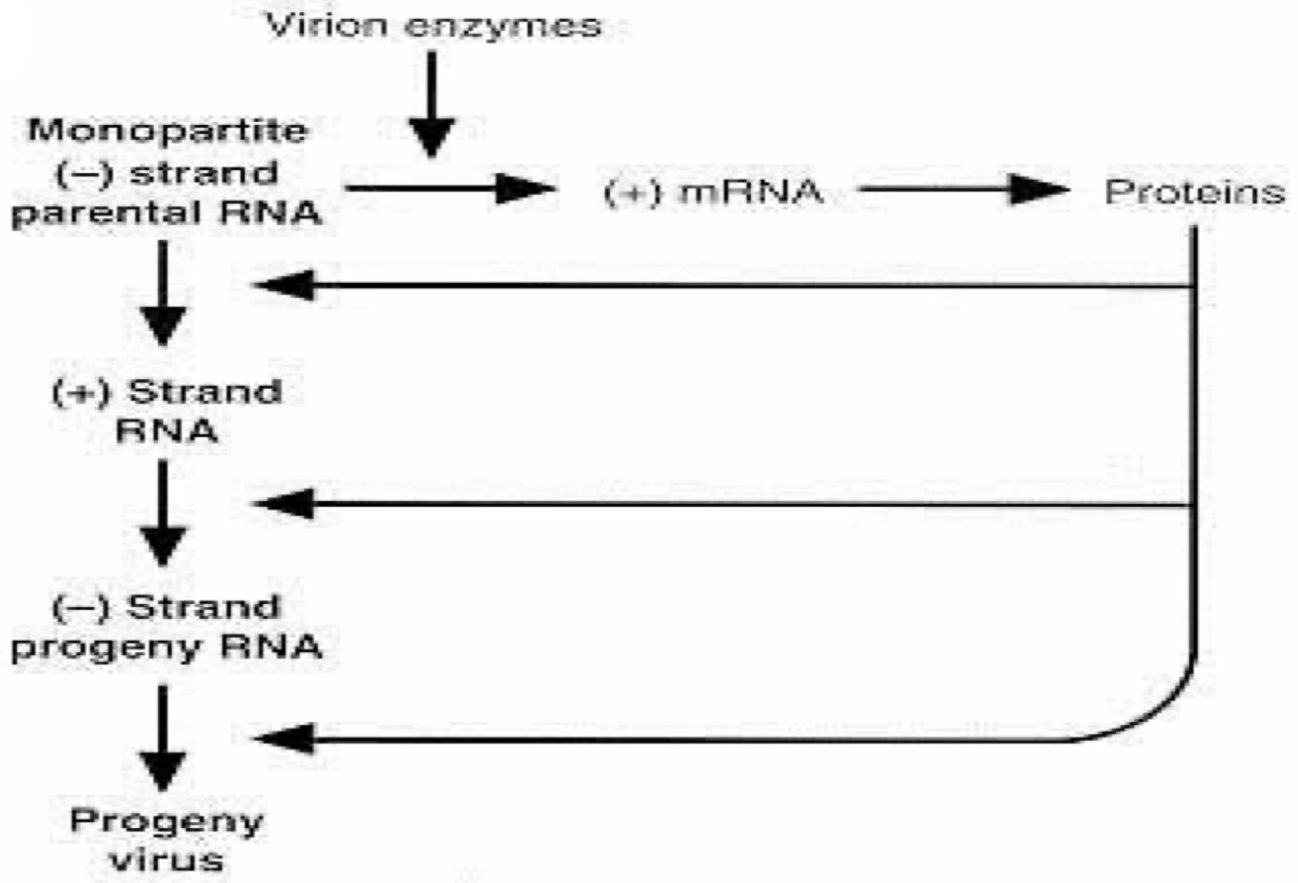
- **A. RNA viruses:**
- **I. Single Stranded RNA**: POSITIVE SENSE, **GENERAL NOTES:**
- Central to the replication of (+) strand viruses is **the capability of the genomic RNA to serve as mRNA after infection.**
- **The consequences are two fold:**
- **First,** enzymes responsible for the replication of the genome are made after infection
- **Second,** because all (+) strand genomes are monopartite, the initial products of translation of both genomic RNA and mRNA species are necessarily a single protein.

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Negative sense:**
- **1. Non segmented Negative (-) strand RNA viruses:**
- **They have their transcriptases** packaged in the virion.
- The transcription of the viral genome is the **first event after entry into cells** (multiple functional mRNAs are produced).
- Replication begins under the direction of newly synthesized viral proteins, a full length (+) strand is made and serves as a template for the synthesis of (-) strand genomic RNA.

Flow of events during the replication of Paramyxoviruses:

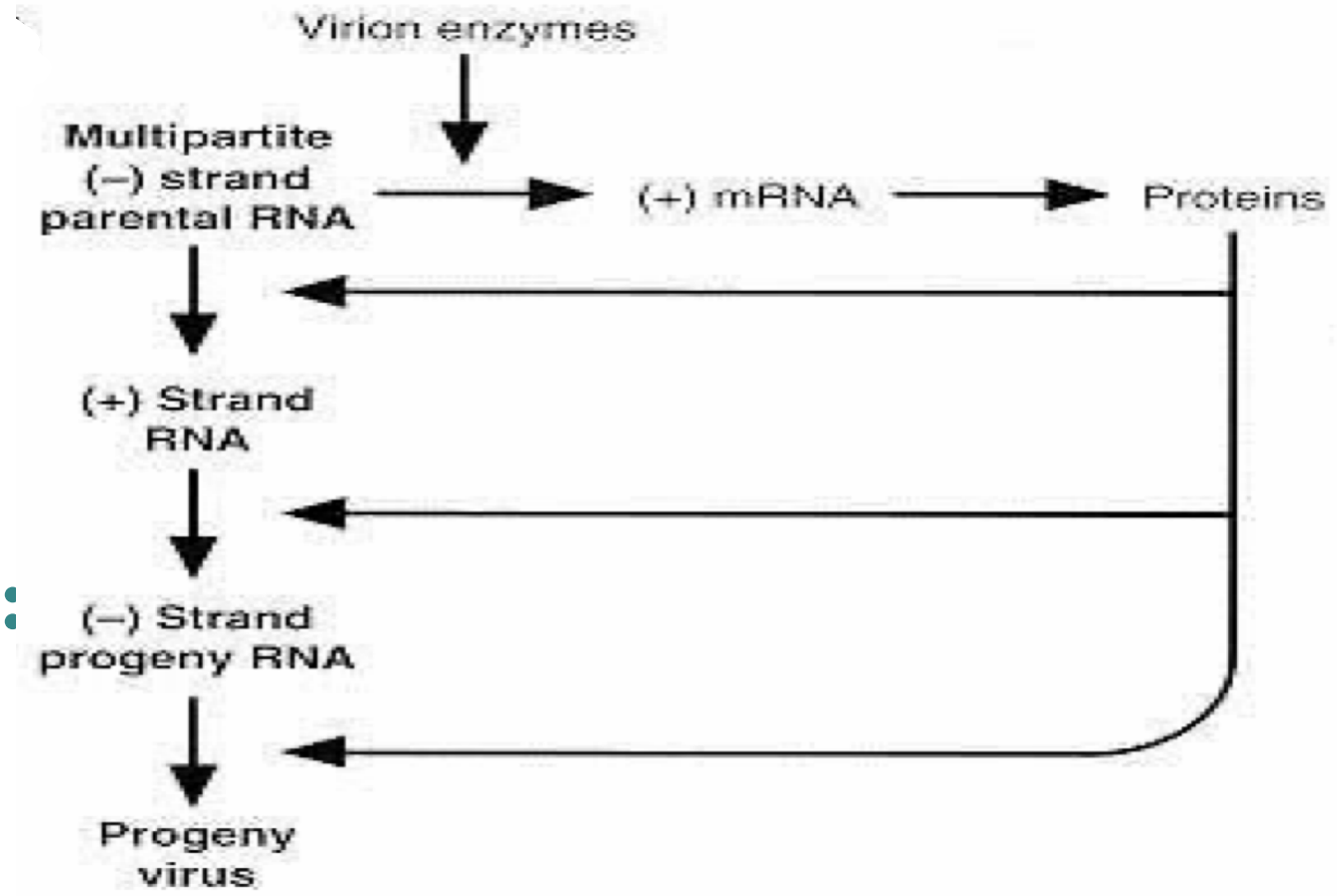


REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Negative sense:**
- **2. Segmented Negative strand RNA viruses:**
- **They have their transcriptases** packaged in the virion.
- The transcription of the viral genome is the **first event after entry into cells** (multiple functional mRNAs are produced).
- Replication begins under the direction of newly synthesized viral proteins, a full length (+) strand is made and serves as a template for the synthesis of (-) strand genomic RNA.

Flow of events during the replication of Orthomyxoviruses:



REPLICATION PHASE:

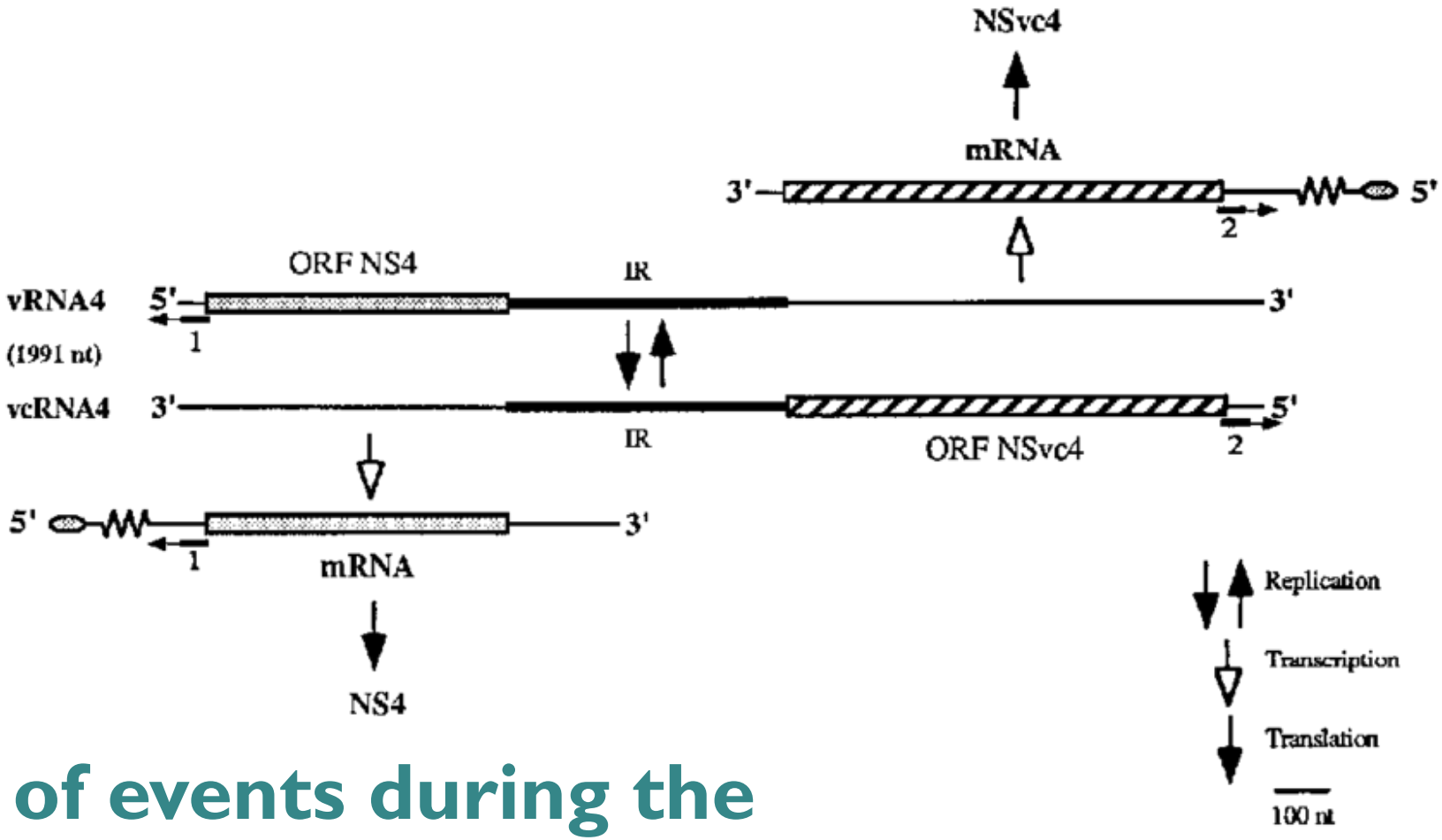
EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA**: NEGATIVE SENSE, **GENERAL NOTES:**
- The genes of (-) strand viruses serve as template for transcription **only**.
- **The consequences are three-fold:**
- **First**, the virus must bring into the infected cell the transcriptase to make its mRNAs.
- **Second**, naked RNA extracted from virions is not infectious.
- **Third**, mRNAs produced are gene unit length, they specify a single polypeptide.
- Consequently, the (+) transcript which functions as mRNA is **different** form the (+) strand RNA which serves as the template for progeny virus even though both are synthesized on the genomic RNA.

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **I. Single Stranded RNA: Ambisense:**
- Arenaviruses and Bunyaviruses
- The expression of this information takes place in **two stages**:
- A. The genomic RNA is transcribed to yield (+) strand subgenomic size mRNA.
- B. The appropriate full size complementary RNA is then transcribed to yield subgenomic size mRNA.
- Because the replicative cycles begin with the transcription of genomic RNA, the ambisense viruses must carry their own polymerase into the infected cell.



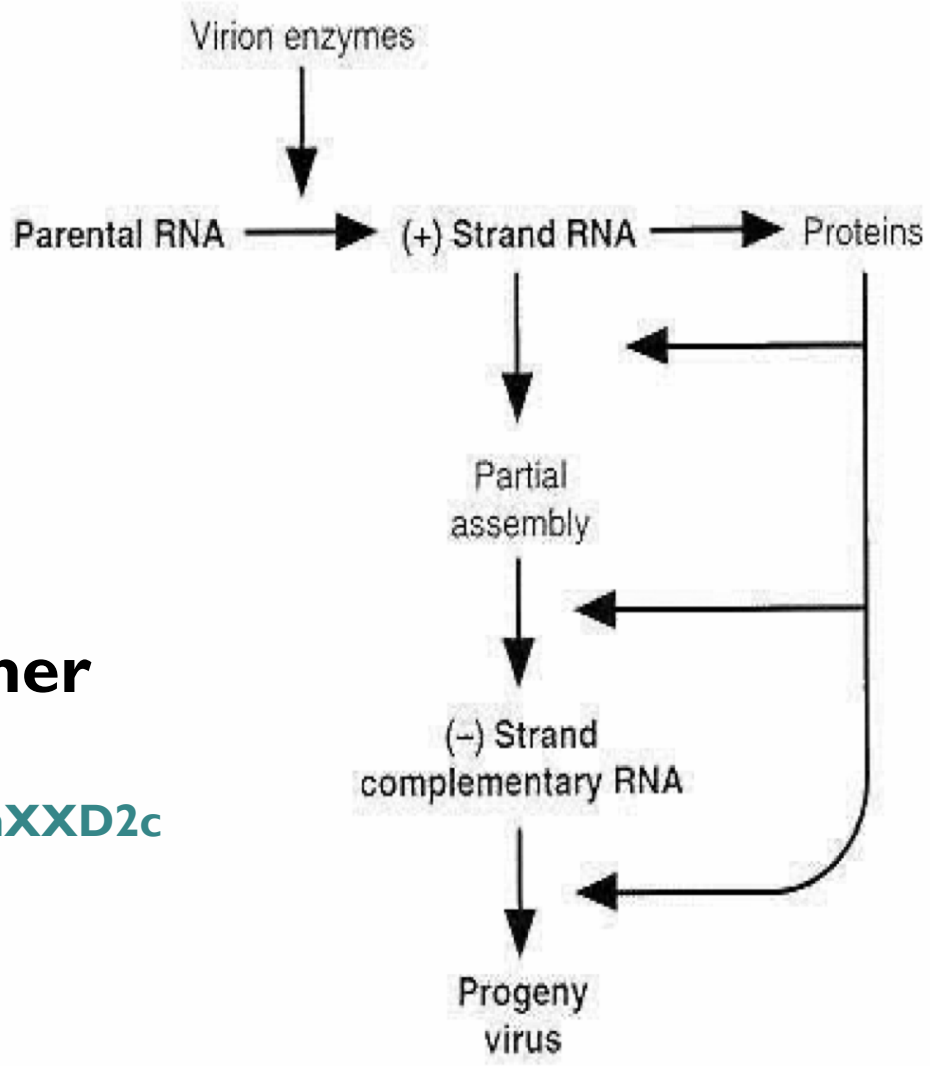
Flow of events during the replication of ambisense viruses:

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **A. RNA viruses:**
- **2. Double Stranded RNA:**
- The **multipartite** reovirus genome is transcribed within the partially opened capsid by a polymerase packaged into the virion.
- The 10 mRNA (+) strand species are extruded from the exposed vertices of the capsid.
- **The mRNA molecules have two functions:**
- **First**, they are translated as monocistronic messages to yield the viral proteins.
- **Second**, one RNA of each of the 10 species assemble within a precursor of particle in which it serves as a template for synthesis of the complementary strand, yielding ds genome segments.

Flow of events during the replication of Reoviruses:



Refer to this video for further explanation

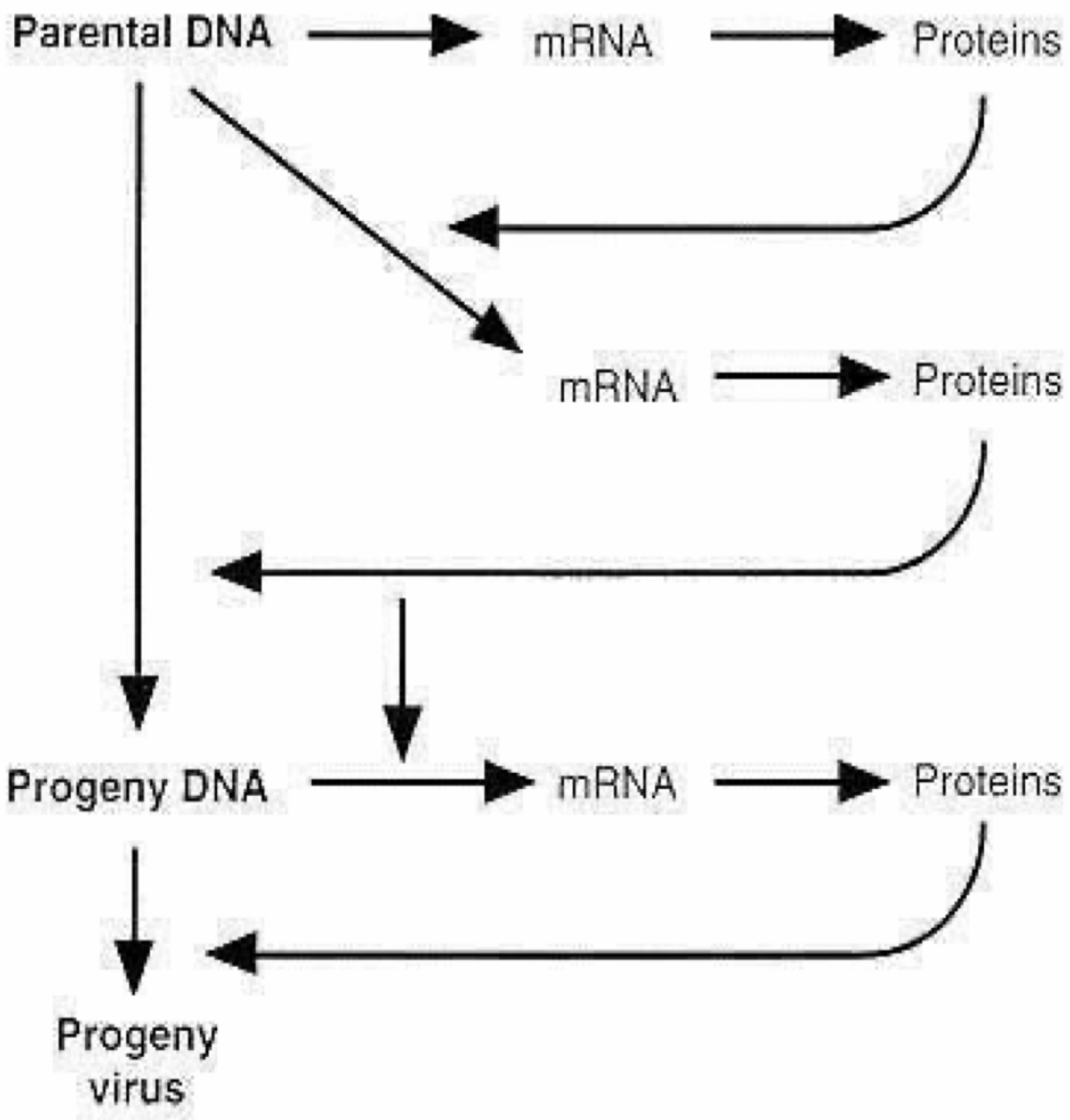
https://www.youtube.com/watch?v=03I_jhXXD2c

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **B. DNA viruses:**
- **1. Double Stranded DNA viruses that replicate in the nucleus:**
- **Significant differences** exist in the replication strategies of Nuclear viruses:
- **Papovaviruses (papilloma & polyomavirises)** encode a single protein that binds in close proximity to the origin of viral DNA synthesis, stimulates the cellular polymerase complex to replicate the viral DNA, and acts as a helicase.
- **Adenoviruses** encode a DNA polymerase but depend on the host cells for all other functions involved in the synthesis of their DNA.
- **At The other extreme are the herpesviruses; HSV** encodes numerous proteins involved in the pathway of the synthesis of DNA.

FLOW OF EVENTS DURING THE REPLICATION OF HERPESVIRUSES (HERPES SIMPLE VIRUSES):



REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **B. DNA viruses:**
- **2. Double Stranded DNA viruses that replicate in the cytoplasm:**
- Transcriptional events and most of the other events in the reproductive cycle seem to take place in the cytoplasm.
- Poxviruses have evolved all of the factors necessary for transcription and replication of their genome
- Because host transcriptional factors are not involved, the cis-acting sites for the synthesis and processing of the mRNA have diverged from those of the host.
- The initial transcription occurs in the core of the virion, the protein products of these transcripts function to release the viral genome from the core.

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

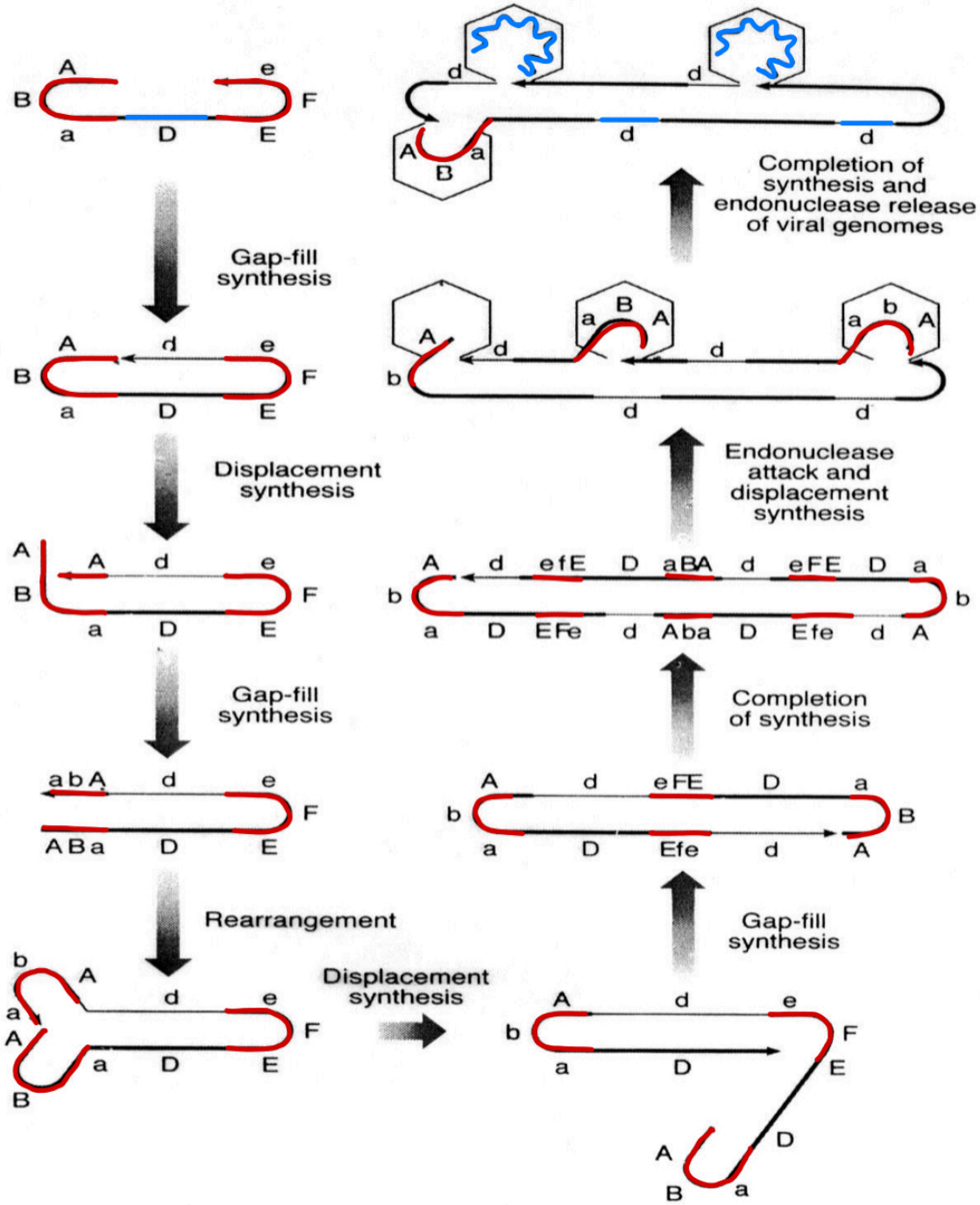
- **B. DNA viruses:**
- **3. Single Stranded DNA viruses (parvoviruses):**
- Multiplication requires the **synthesis of a DNA strand Single complementary to the ss genomic DNA** in the nucleus and transcription of the genome.
- The B19 virus replicates in **mitotically active cells** and prefers cells of the erythroid lineage.
- Factors available **only during the S phase of the cell's growth cycle** and cellular DNA polymerase are **required** to generate a complementary DNA strand.
- A ds DNA version of the virion genome is **required** for transcription and replication.

REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **B. DNA viruses:**
- **3. Single Stranded DNA viruses (parvoviruses):**
- Inverted repeat sequences of DNA at both ends of the genome **facilitate viral DNA synthesis**. It forms a ds molecule in the form of hairpin loops.
- The **palindromic sequence** (about 115 bases at both ends) can fold back on itself and forms ds sequences stabilized by hydrogen bonding in the form of hairpin Y or T shape.
- The ds DNA replicative intermediate is **transcribed by cellular RNA polymerases and replicated by DNA polymerase.**
- In the absence of a helper virus, the genomes of dependent parvovirus appear to integrate into a specific locus on a human chromosome.

FLOW OF EVENTS DURING THE REPLICATION OF PARVOVIRUSES:

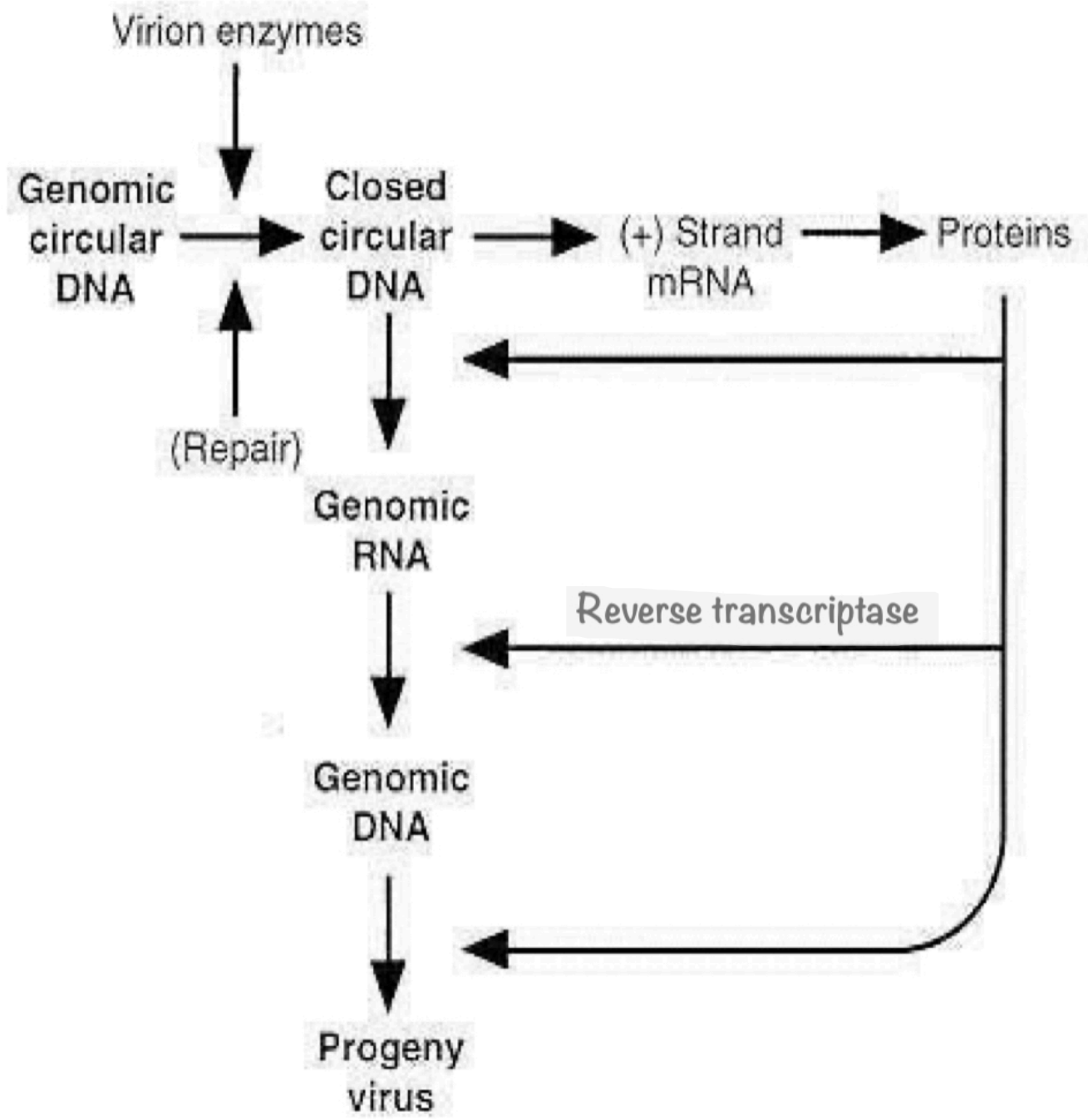


REPLICATION PHASE:

EXPRESSION AND REPLICATION OF VIRAL GENOMES:

- **B. DNA viruses:**
- **4. Hepadnaviruses:**
- Hepadnaviruses have a circular DNA genome. They replicate in the nucleus. They replicate using an mRNA intermediate.
- The gap in the DNA of the virus is repaired first by a **DNA polymerase packaged into virion.**
- the genome is then transcribed into **two classes of RNA molecules:**
- mRNAs specifying proteins and a full length RNA that serves as a template for the synthesis of genomic DNA by a virally encoded **reverse transcriptase.**

FLOW OF EVENTS DURING THE REPLICATION OF **HEPADNAVIRUSES** (HEPATITIS B VIRUS):



RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **A. Assembly:**
- **Assembly of DNA viruses, except poxviruses,** occurs in the nucleus and requires transport of the virion proteins into the nucleus.
- **Assembly of pox and RNA viruses** takes place in the cytoplasm.
- The assembly process begins when the concentration of structural proteins in the cell is **sufficient** to thermodynamically drive the process, much like a crystallization reaction.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **A. Assembly:**
- **Structural proteins of simple icosahedral viruses** can aggregate spontaneously to from structural units, which in turn assemble into empty capsids (procaspids).
- Somehow, the viral nucleic acid now enters this structure via a mechanism that seems to involve a nucleotide sequence known as the **"packing sequence".**
- **Helical viruses** assemble by adding blocks during coiling of the viral nucleic acid.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **B. Maturation:**
- Maturation and release are determined in part **by site of replication and the presence of an envelope.**
- Acquisition of an envelope occurs **after association of the nucleocapsid with regions of host cell membrane** modified with matrix proteins and glycoproteins.
- **Matrix proteins** line and promote the adhesion of nucleocapsids with the modified membrane.
- As more interactions occur, the membrane surrounds nucleocapsidand the virus buds from the from the membrane.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

•B. Maturation:

- Strategies for maturation:
- **Three** fundamental strategies for maturation have been described:
- **I. Non enveloped viruses,Intracellular assembly and maturation**: non enveloped viruses cause disintegration of the infected cell for their egress.
- **II. Strategy of enveloped viruses:** The last step in assembly of (-) strand RNA viruses is linked with their egress from infected cells by budding from the cytoplasmic or other membranes.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **B. Maturation:**
- **Viruses that mature and egress by budding vary considerably in their effects on host cell metabolism and integrity:**
- They range from **highly cytolytic** (toga, paramyxo), to viruses which are **frequently non cytolytic** (retroviruses).
- By virtue of the viral glycoprotein insertion into the cell surface, **however**, these viruses import upon the cell a new antigenic specificity and the infected cell can and does become a target for the immune mechanisms of the host.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **B. Maturation:**
- **III. Strategy for Herpesviruses:**
- They assemble their nucleocaspid in the nucleus.
- Envelopment and maturation occur at the inner lamella of the nuclear membrane.
- Herpesviruses are cytolytic and invariably destroy the cell in which the multiply.
- They also import new antigens on the infected cell.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **C. Glycosylstion, budding and egress:**
- In the glycosylation of their proteins, viruses use **existing pathways:**
- 1.This involves a " signal sequence " of 15- 30 hydrophobic amino acids that facilitate **binding** to a receptor on the **cytoplasmic side of the RER**.
- 2.It then passes through the lipid bilayer to the **luminal side** where the signal sequences are **removed** by a signal peptidase allowing the **addition** of oligosaccharides.

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **C. Glycosylstion, budding and egress:**
- 3. Glucose is then **removed** by glucosidase (trimming).
- 4. The viral glycoprotein is then transported to the **Golgi apparatus** probably inside a coated vesicle, where the core carbohydrate is **further modified and acylated** (addition of fatty acids).
- 5. Another coated vesicle now transports the acylated glycorprotein to the **plasma membrane**, probably with the help of a leading sequence that finds the destination (postal address or zip code).

RELEASE PHASE: ASSEMBLY, MATURATION, AND EGRESS OF VIRUSES FROM INFECTED CELLS:

- **C. Glycosylstion, budding and egress:**
- 5. Envelope glycoproteins are then **cleaved** into 2 polypeptide chains that remain covalently bound by S-S bonds.
- 6. Then the hydrophilic N-terminus of the glycoprotein finds itself **projecting from the external surface** of the membrane while the hydrophobic domain near the C-terminus **remains anchored in the lipid bilayer.**
- 7. Budding is a form of exocytosis (reversed endocytosis) and viruses **remain cell-associated** for few hours and large number of viruses are released in consecutive waves.

VARIABILITY IN VIRAL GENOMES AND VIRAL MULTIPLICATION

- On passage, viruses tend to yield defective mutants.
- **It is convenient to classify defective viruses into two groups:**
- **1. Viruses in the first group** lack one or more essential genes and therefore are incapable of independent replication without a helper virus.
- They can transform infected cells or transactivate oncogenic viruses in causing the cell to become malignant.

- **2. The second group** comprises viruses which contain mutations and deletions and therefore can not replicate in an efficient fashion.
- **Chronic debilitating infections of the CNS** might in some fashion be related to viruses that are sluggish in their replication, in their ability to destroy the infected cells, or in their ability to alter the infected cell sufficiently to make it a target for the immune system of the host.
- **Genetically engineered viruses** lacking one or several genes and which might be classified as defective may ultimately be greatest gift to mankind; the means for the introduction of genes to complement genetic deficits or to selectively destroy cancer cells.