MICROBIOLOGY
(Virology)
DOCTOR 2019 | MEDICINE | JU

Slide words are in **black** and Doctor words are in **green**

Replication of viruses

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*** The pathological effects of the diseases caused by viruses result from several factors:**

(مش شرط كل الفيروسات تأثر بكل العوامل الموجودة)

1)Toxic effects of viral gene products (direct toxic effect from e.g. proteins, enzymes..) **on the metabolism of infected cells.**

2)Immune **reactions of the host on the infected cells expressing viral genes** as the body will attack these infected cells(the foreign bodies will cause an innate/adaptive immune response to start)

3)Modification of host gene expression by structural or functional interactions with the genetic material of the virus, the viruses that make this are called oncoviruses, and this process causes cancer(e.g EPV , human herpesvirus 8, hepatitis b &c)

Host Range, Susceptibility, and Permissiveness

→What determine if the virus can enter a particular cell?

Answer: If proteins on the virus surface associate with a receptor on the host cell (e.g hepatitis virus infects hepatocytes mainly because its receptors are present on these cells surfaces)

*** The process of infection begins with the coming together of a virus particle and a susceptible host cell.**

*** The host range of a virus (tropism)**: (الكائنات المستهدفة للإصابة من الفايروس)

both the kinds of tissue cells and animal species that it can infect and in which it can multiply (wide(many types of tissues/species) **narrow**(specific types) **).**

*** Susceptibility :**(معدل قابلية التأثر)

the capacity of a cell or an animal to become infected.

* 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.

اختصار اللي فوق:

Viruses replicates, and we will focus in our studying on the replication's stages that are relevant to viral pathogenetic effect, because it helps us in antiviral chemotherapy as we can determine stages that can be a target to the chemotherapeutic agents.

Antiviral Chemotherapy: علاج كيميائي من الفيروسات

* **To infect a cell, the virion must 1)attach to the cell surface, 2)penetrate the cell, and 3)become sufficiently uncoated to make its genome accessible to viral or host machinery for transcription or translation.**
* **The cell acts as a factory providing the substrates, energy, and machinery necessary for synthesis of viral proteins and replication of the genome,** although there are some proteins that help in viral replication.
* **Each infected cell may produce as many as 10^5 viral particles (**the number called **burst size** as it's the maximum number that cell burst when it's reached)**, only 1-10% of** these viruses **are infectious**

→Although the percentage of infectious viruses is relatively small, the number of these viruses is high 10^{3-} 10^4 and this is one of the reasons of the rapid evolution of viruses.

Types of Infection

*** Infection of a cell may be:-**

1)Productive (permissive): the cell releases infectious virion.

2)Abortive (non-permissive, defective): the virion enter the cell but the cell doesn't support releasing infectious virions.

3)**Stringent** or **restrictive**(transient permissiveness): certain layers in the cell support only one stage of viral replication .

4)**Transforming:** convert the cell to a cancerous cell**.**

* **Virus replication can be divided into eight stages, namely: Attachment, penetration, uncoating** (dissolving of the capsid , release of the genome and proteins necessary for replication)**, genome replication, gene expression, assembly, maturation and release.**
* **These are purely arbitrary divisions** (not sharp divisions , some steps may occur in sync with other steps) **, 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-:

I. – Initiation phase

1)Attachment 2)Penetration 3)Uncoating

II- Replication phase

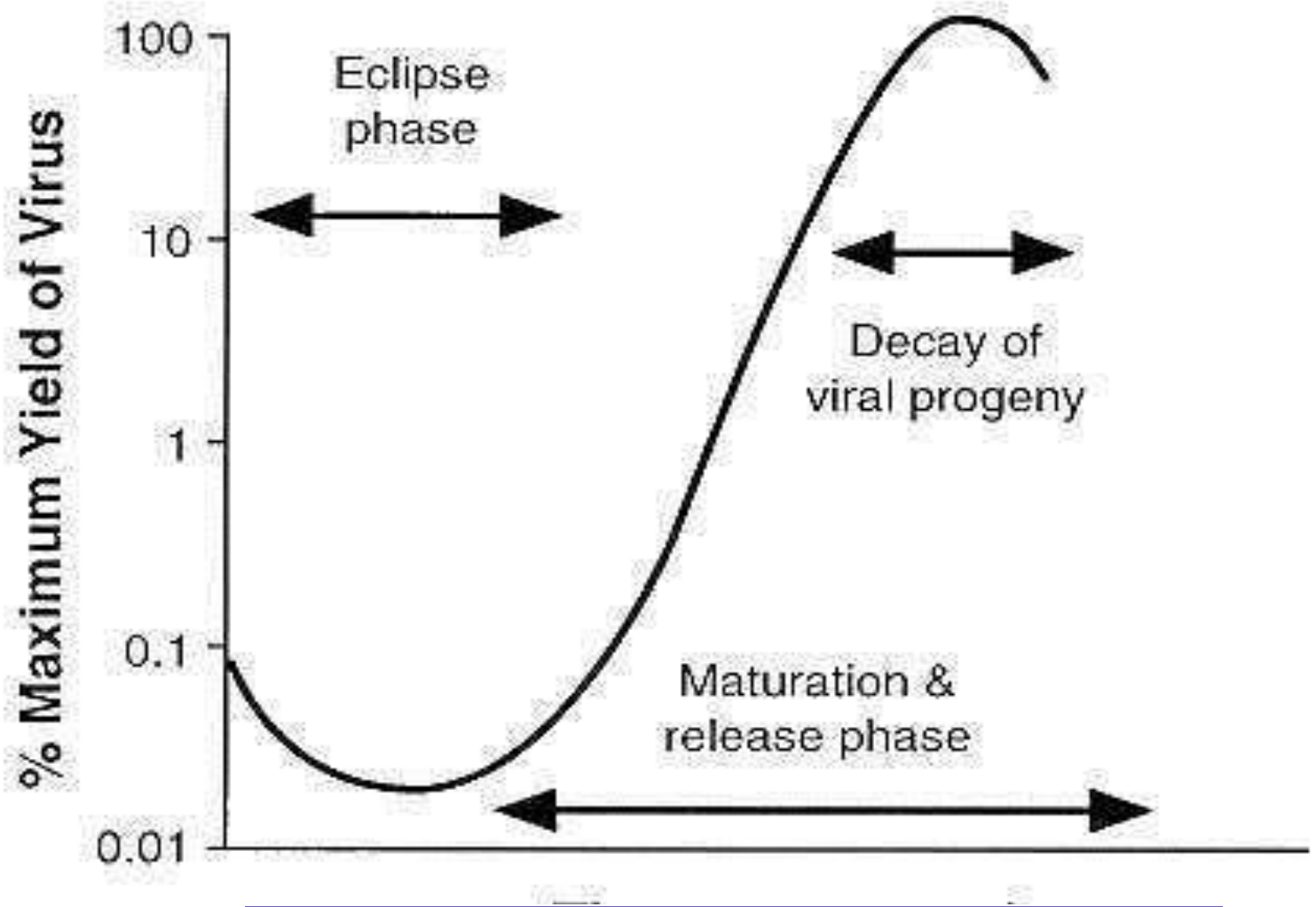
1)DNA Synthesis 2)RNA Synthesis 3)Protein synthesis

III.- **Release phase**

1)Assembly 2)Maturation 3)Exit from cell

→The difference between assembly and maturation:

Assembled viruses have multiprotein on their envelope that need to be cleaved by protease enzymes so the virous become mature (it becomes mature when the protein is cleaved because certain parts will be exposed and then interact with certain receptors of the cell



Latent Period

شرح للمنحنى السابق:

المنحنى يمثل عدد الفيروسات التي يمكن مشاهدتها مع الزمن

1)مرحلةEclipse phase تمثل مرحلة دخول الفيروسات للخلية ويكون عددها قليل (يمكن لأكثر من فيروس دخول نفس الخلية) ويبدأ عدد الفايروسات المشاهد يقل أكثر لأن أغشية الفيروسات التي دخلت تبدأ بالتّحلل لذلك ينخفض المنحنى في هذه المرحلة

2)مرحلة maturation & release يزداد عدد الفايروسات بشكل كبير عن طريق ال Replication لذلك يصعد المنحنى

3)مرحلةDecay of viral progeny تصنيع الفايروسات يوقف لسببين:

أ- الخلية لا تتحمل تجّمع الفيروسات

ب- تعطّل وظائف الخلية

Attachment (Adsorption)

* **Attachment :a specific binding of a viral protein (VAP) (anti-receptor) to a receptor of the cell surface.**

(anti-receptor: the protein on the virus surface that will interact with the cell receptor)

* **Complex viruses may have more than one type of anti-receptor molecules.**
* **Anti-receptor molecules may have several domains, each of which may react with a different receptor.**

على قولة الدكتور: مش مهم نحفظ تفصيل الخطوات هلأ لأنه رح نيجيها بعدين

* **Mutations in the genes may cause loss of the capacity for the anti-receptors to interact with certain receptors.**

→Most of Receptors are glycoproteins or glycolipids.

→both virus and cell membrane are negatively charged, **that's why there is repulsion between them and attachment is quite impeded because of this.**

* **Because of electrostatic repulsion, Attachment requires ions to reduce it, but it is largely independent of temperature and energy.**

* **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.**

* **Early binding is reversible and firm binding requires specific receptor anti- receptor interaction.**

معنى الجملتين:

← ارتباط الفيروس بالخلية ينتج عن تصادم عشوائي بينهم، بمعدل 10^{-4} إلى 10^{-3}

مرة في الثانية، وينتج عن هذا التصادم اتحاد فيزيائي بين الفيروس والخلية

←هناك نوعين من الارتباط بين الفيروس والخلية

Early binding وهو قابل للانفكاك

firm binding غير قابل للانفكاك ويتطلب ارتباطًا قويًّا

*** The susceptibility of a cell is limited by the availability of appropriate receptors, and not all cells in an otherwise susceptible organism express receptors.**

*** Attachment of viruses to cells in many instances leads to irreversible changes in the structure of the virion,** in order the virus can enter the cell.

*** In some instances, however, when penetration does not ensue, the virus can leave cell surface,** and go randomly to other cell.

*** Some viruses have specific mechanisms for detachment (e.g. neuraminidase).**

*** Elution leads to changes in the virus (anti-receptor) VAP which decrease or eliminate the possibility of attachment to other cells.**

Elution: استخراج مادة من مادة أخرى عن طريق الغسيل بسائل مذيب

Penetration

*** An energy dependent step that occurs immediately after attachment and it involves one of three mechanisms:**

1)Endocytosis (viriopexis) of the virus particle resulting in accumulation of virus particles inside cytoplasmic vesicles. Most common.

2)Fusion of the virion envelope with the cellular membrane (Requires fusion protein in viral envelope) (e.g. HIV)

3) **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**.

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** → (the genome and it's associated proteins) .

***Uncoating may be initiated by attachment to the receptor or prompted by the acidic environment or proterases found in an endosome or lysosome.**

Expression and Replication of viral Genomes

DNA Viruses

→All DNA viruses, replicate in the nucleus except poxviruses (the most complex, it occurs in cytoplasm)**.**

→For transcription: They need cellular RNA polymerase (DNA –dependent RNA Polymerase) for transcription.

→For DNA Replication: simple DNA viruse (Parvo and Papovaviruses (that are polyoma viruses)**) need host cell DNA – dependent DNA polymerase, whereas the larger more complex ones (adeno, herpes, and poxviruses) encode their own polymerases**

→Viral polymerases are faster but less precise than cell polymerase causing a higher mutation rate and providing a target for antiviral drugs.

→ most mutations are neutral, they don't give any advantages or disadvantages for the virus, some gives resistance for the viruses to chemotherapies, and some gives the virus the ability to live in new species such as covid-19)

→ Usually viral enzymes are included in genome replication with assistance of cellular proteins, but some of viruses use cellular enzymes assisted by viral proteins in replication (e.g. parvovirus). (point 2&3 in the slides rephrased)

*** All DNA viruses known to infect vertebrates contain a monopartite genome.**

monopartite genome: (جينوم عبارة عن قطعة واحدة)

*** The fidelity of DNA replication is such that only one mistake is made in $10^9 - 10^{10}$ base pair replications compared with one in 10^3-10^4 for RNA viruses.**

(عشان هيك مع انه RNA
بكون حجمها صغير إلا إنه في كل Replication بصير في طفرات)

*** Error – free replication arises from the ability of DNA polymerase to proof-read (إعادة تدقيق)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.**

(الطفرات RNAمش كتير مهمة فما بحتاج إعادة تدقيق)
(صحيح بعض الطفرات بتخرب الفيروس، لكن بعضها بقويه وبطوره)

RNA Viruses

→Most RNA viruses replicate in the cytoplasm using their own transcriptase, exceptions to this being influenza (Orthomyxovirus) and retroviruses, part of the replicative cycle of which take place in the nucleus.

→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. (They have the machinery to replicate by their themselves)

*** Replication and transcription of RNA viruses are similar processes as the template is RNA in both cases, and dsRNA intermediates are formed.**

(تشكل سلسلة ثنائية من RNA هو اللي بصير غالبًا+ الجسم ما بصنع هاد الشيء بشكل طبيعي فبعتبر هاي السلسلة المزدوجة اشي غريب وبهاجمها)

*** Since RNA is degraded relatively quickly** (very unstable)**, the RNA polymerase must be provided or synthesized soon after uncoating to generate more viral RNA, or the infection is aborted.**

→**The genomes of ssRNA viruses are either**:

Monopartite (one segment) (picorna, toga, paramyxo, rhabdo, corona, and retroviruses) or

Multipartite (multisegmented) (orthomyxo, arena, and bunyaviruses)

→**Most RNA genomes are linear**

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.

→The early phase results in the synthesis of regulatory proteins and enzymes necessary for replication of viral nucleic acid.

→The late phase leads to the synthesis of structural proteins which are usually made in excess.

الفايروس بكون خلص بده يتصنع فبكون البروتينات متأخرة

*** 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.**

* 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.

*** Viruses employ different tactics to promote preferential translation of their viral mRNA:-**

-In many cases the concentration of viral mRNA in the cell is so large that it occupies most of the ribosomes.

-Block the egress of cellular mRNA from the nucleus. (so there is no production of the cellular protein in this case)

- **Inhibit cellular macromolecular synthesis and induce degradation of the cell's DNA and** messenger **RNA.**

-Increase the permeability of the cell membranes which decreases the ribosomal affinity for cellular mRNA.

Expression and Replication of viral Genomes

I- RNA Viruses

1- Positive (+) strand RNA viruses coding for one

Genome – sized m RNA (polio, Flavi, HCV)

(سلسلةRNA)

(polio الموجبة بتتصرف أحيانًا على انها mRNAوبتترجم عطول لبروتينات زي فيروس)

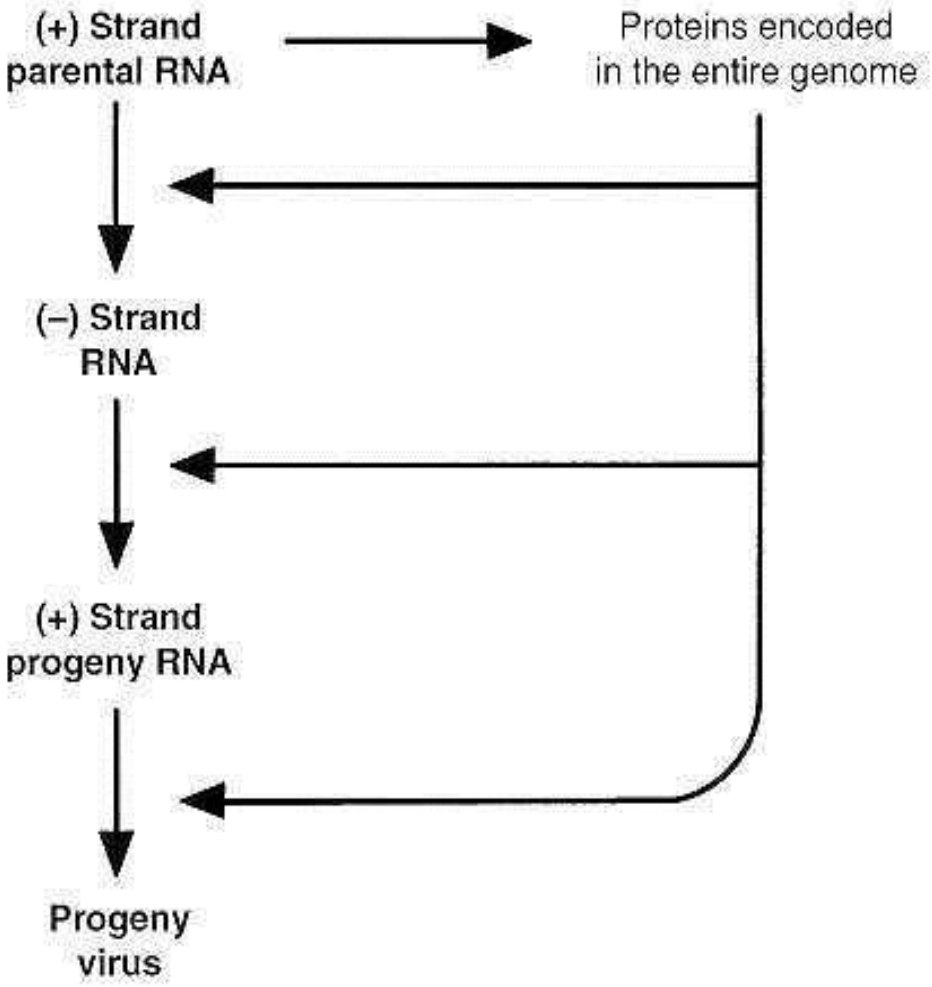
* Their coding domains are translated in their entirety.

* The product of translation, the polyprotein, is then cleaved by viral and cellular protease to give functional protein .

* 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



2- Positive (+) Strand RNA viruses coding for one or more subgenomic mRNAs (Toga, corona, calici, HEV).

(corona gives sub genomic mRNA)

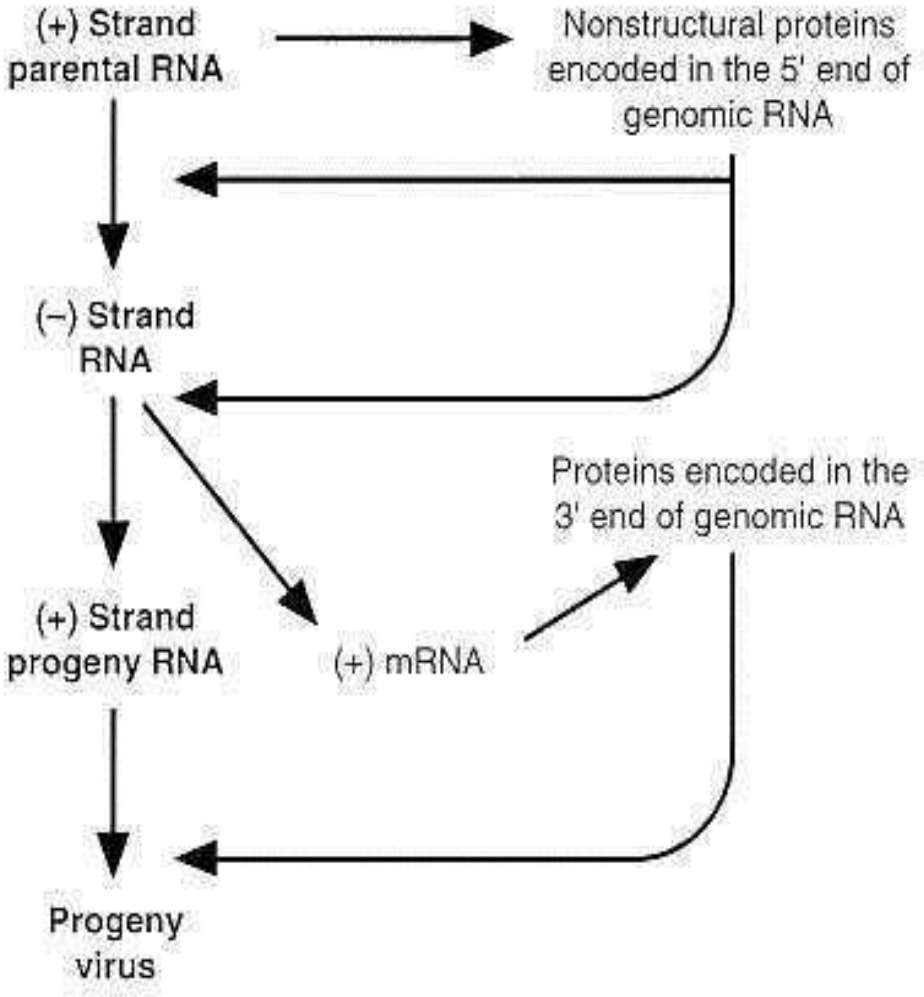
Only a portion (the 5' end) of the genomic RNA is available for translation in the first round of protein synthesis.

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.



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:

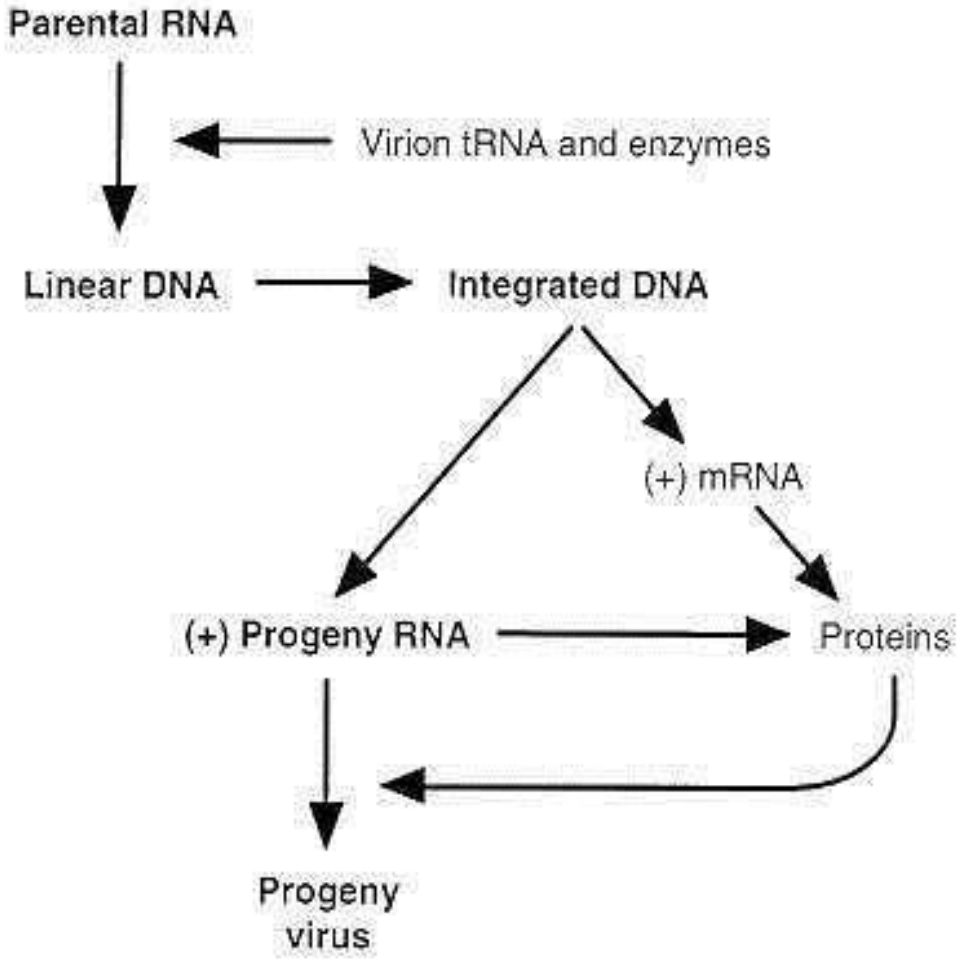
First, enzymes responsible for the replication of the genome are made after infection

Second, because all (+) strand genomes are monopartite, the initial products of translation of both genomic RNA and mRNA species are necessarily a single protein.

3- Retroviruses

* First step in replication is synthesis of a DNA strand complementary to the RNA genome (reverse transcription by reverse transcriptase), followed by digestion of RNA by a nuclease (ribonuclease H in the virion), and finally synthesis of a complementary DNA strand that will affect the host.
* 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.



4- Non segmented Negative (-) strand RNA viruses

* They have their transcriptases packaged in the virion.

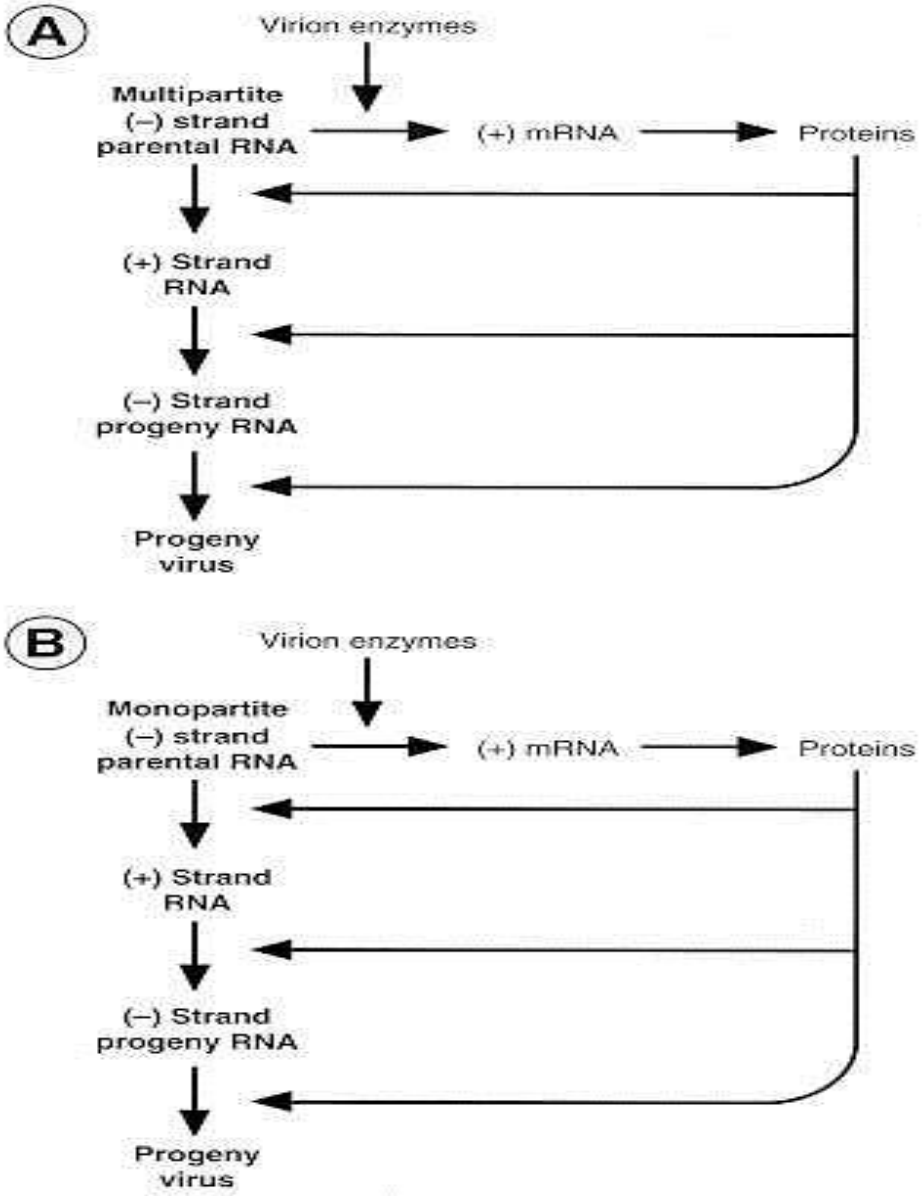
* The transcription of the viral genome is the first event after entry into cells (multiple functional mRNAs are produced).

* 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

5- Segmented Negative strand RNA viruses

- The first step involves the synthesis of mRNAs from each segment of the genomic RNA.
- The mRNAs of influenza virus have heterogeneous nonviral 5′ end sequences (8 – 18 nucleotides) that are stolen" from the host cell mRNA molecules by viral proteins.
- The newly synthesized viral proteins replicate the genomic RNA segments to yield precise (+) strand copies of the virion RNAs
- A unique characteristic of them is reassortment of their genes in cells infected by more than one virion of the same group introducing new genotypes.

Flow of events during the replication of Orthomyxoviruses and Paramyxoviruses.



The genes of (-) strand viruses serves 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. (so we need to bring the enzyme)

Second, naked RNA extracted from virions is not infectious .

(يعني لما نطلع الجينوم لحاله وندخله الخلية بطريقة أو بأخرى)

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.

6-Ambisenes RNA Viruses (part of RNA is + and the other is -)

(e.g.Arenaviruses and Bunyaviruses)

* The expression of this information takes place in two stages.

* The genomic RNA is transcribed to yield (+) strand subgenomic size mRNA.

* 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.

7- Double Stranded RNA viurses

* The multipartite reovirus genome is transcribed within the partially opened capsid by a polymerase packaged into the virion

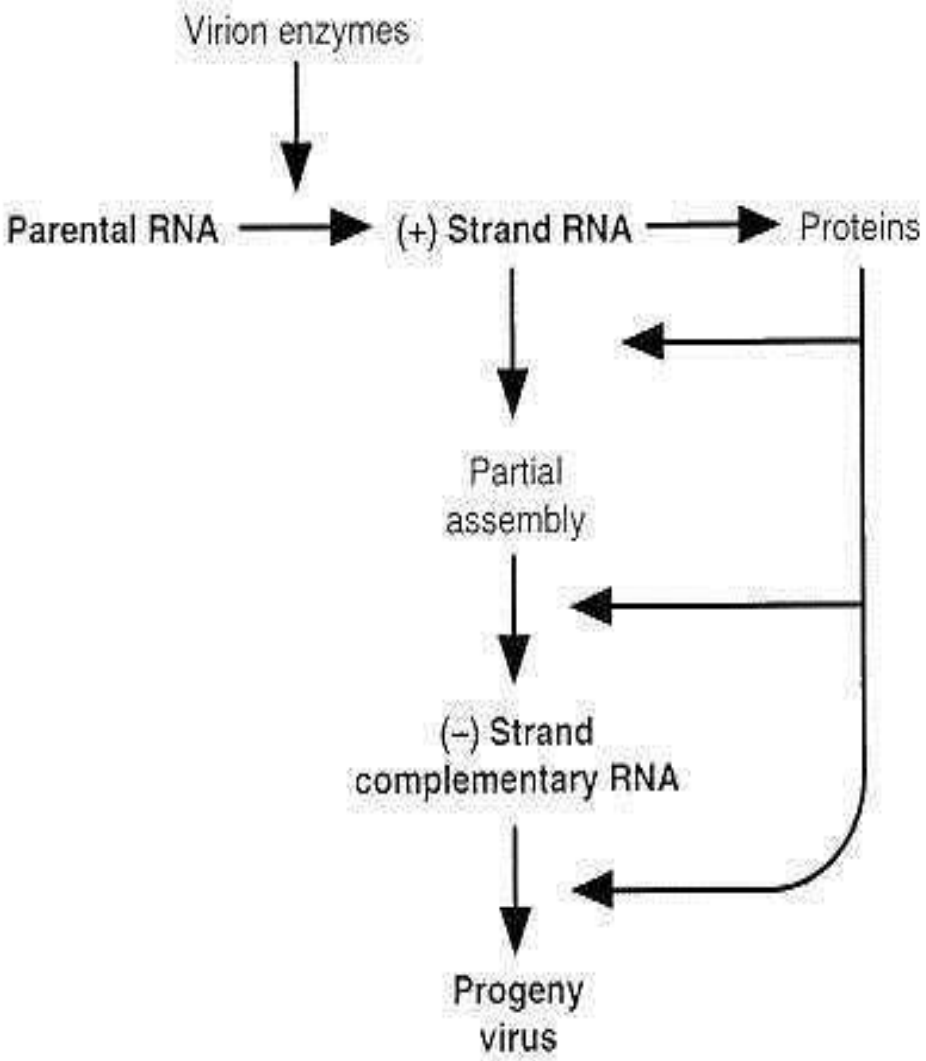
* The 10 mRNA (+) strand species are extruded from the exposed vertices of the capsid.

* 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 servers as a template for synthesis of the complementary strand, yielding ds genome segments.

Flow of events during the replication of Reoviruses



*** II- DNA Viruses**

1- Double – Stranded DNA Viruses that Replicate in the Nucleus

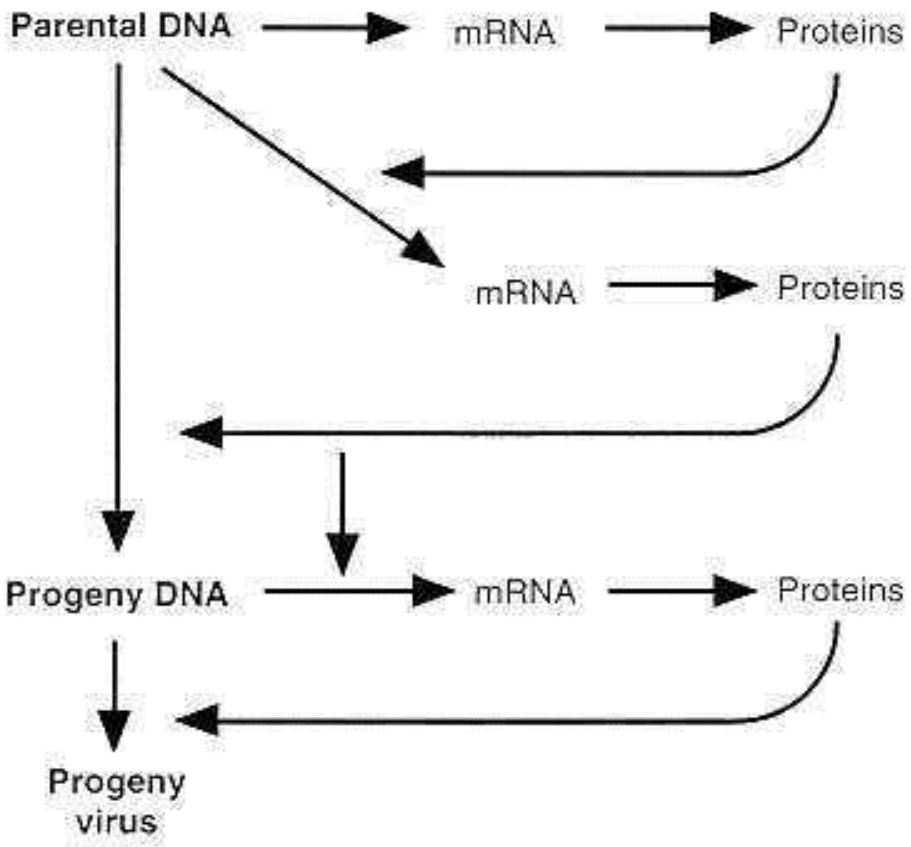
* Significant differences exist in the replication strategies of Nuclear viruses.

* Papovaviruses 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 simplex 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.
* Poxviurses 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.

3- Single- stranded DNA viruses (Parvoviruses)

*** Multiplication requires the synthesis of a DNA strand complementary to the ss gnomic DNA in the nucleus and transcription of the genome.**

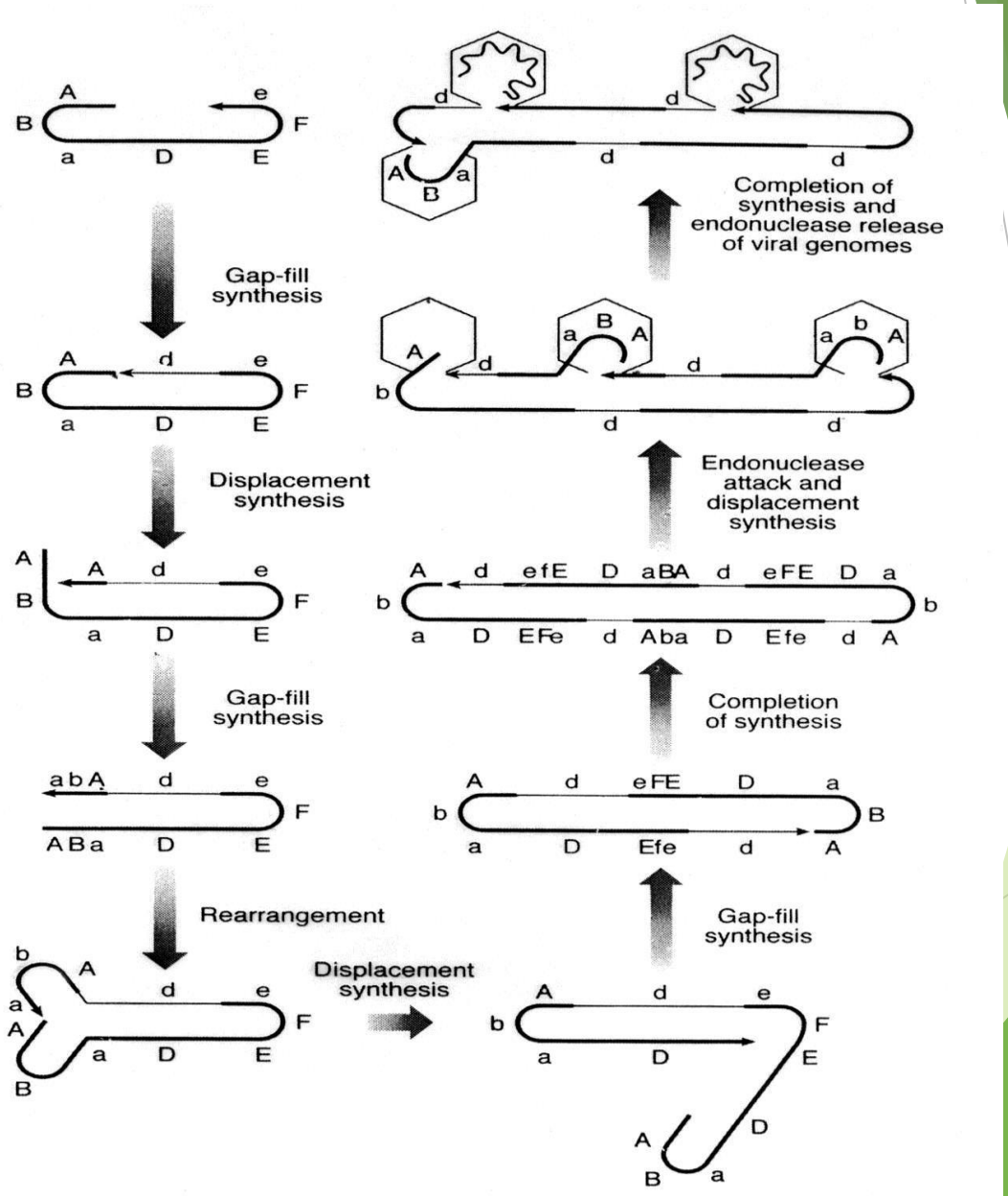
*** The B19 virus replicates in mitotically active cells and prefers cells of the erythroid lineage,** not in the mature RBCs.

*** 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.**

* 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 it self 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



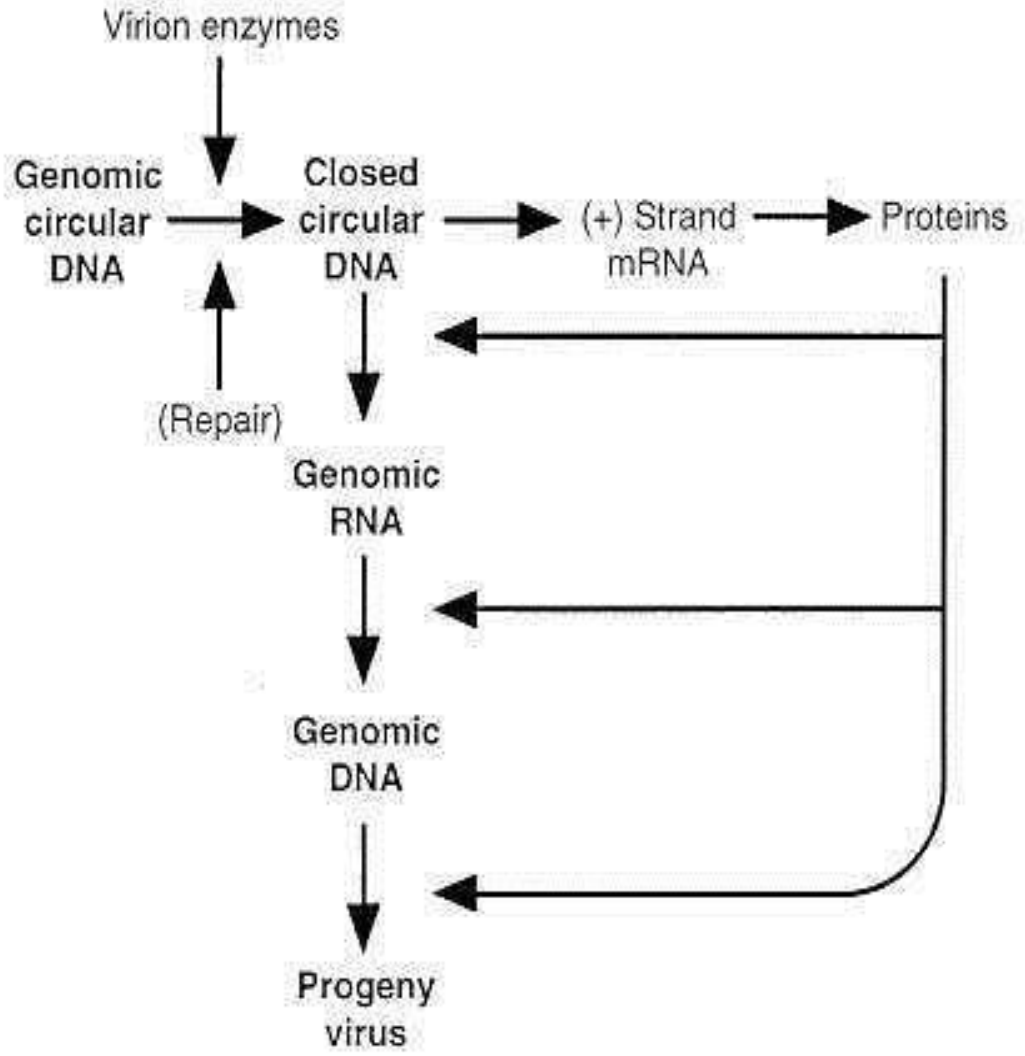
4- Hepadnaviruses

*** Hepadnaviruses have a circular partially ds DNA genome. They replicate in the nucleus.**

*** 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).



Assembly, Maturation, and Egress of viruses from infected cells

* 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 crystalization reaction.

*** Structural proteins of simple icosahedral viruses can aggregate spontaneously to from structural units, which in turn assemble into empty capsids (procasids).**

*** 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.**

* 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 by matrix protein and glycoproteins.

* Matrix proteins line and promote the adhesion of nuclecocapsids with the modified membrane.

* As more interactions occur, the membrane surrounds the nucleocapsid and the virus buds from the membrane.

strategies for maturation

*** Three fundamental strategies for maturation have been described:-**

I. Intracellular assembly and Maturation

- Nonenveloped 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.

* 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 noncytolytic

(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.

III- Strategy for Herpesviruses

- They assemble their nucleocapsid in the nucleus.

  -Envelopment and maturation occur atthe inner lamella of the nuclear membrane

  - Herpesvirurses are cytolytic and invariably destroy the cell in which they multiply.

- They also import new antigens on the infected cell.

Glycosylation and Budding

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

* Glucose is then removed by glucosidase (trimming).

* 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).

*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.

* Envelope glycoproteins are then cleaved into 2 poly-peptide chains that remain covalently bound by S-S bonds.

* Then the hydrophilic N-terminus of the glycoprotein finds itself projecting from the external surface of the membrane while the hydropobic domain near the c-terminus remains anchored in the lipid bilayer.

* Budding is a form of exocytosis (reversed endocytosis) and viruses remain cell- associated for few hours and large numbers 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.

* 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.

* 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 viruses greatest gift to mankind; the means for the introduction of genes to complement genetic deficits or to selectively destroy cancer cells.