

# Virus Replication

*Flint et al. Principles of Virology (ASM), Chapters 2, 4 and 5*

*Wagner & Hewlett. Basic Virology (Blackwell). Chapter 6*

## The lifecycle of animal viruses

Our understanding of the lifecycle of animal viruses has been developed in large part by studying virus infections under conditions where cell cultures become infected synchronously with virus, in such a way that only a single cycle of infection can occur. Synchronous infection can be achieved by infecting cultures with a high amount of virus, such that all cells within the culture become infected rapidly. To do this, one typically infects cells at a **multiplicity of infection** of 5 to 10.

## Multiplicity of infection

Since virus infection of cells is a random event that follows a Poisson distribution, one can calculate the multiplicity of infection required to infect a given proportion of cells within a culture as follows:

$$P(0) = e^{-m} \text{ and } m = -\ln P(0)$$

### Definitions

P (k): fraction of cells infected by k virus particles

M: multiplicity of infection (MOI)

P(0): proportion of uninfected cells, P(0)

### Example

If one wants to infect 99% of cells in a culture dish, what MOI of input virus is needed?

$$P(0) = 1\% = 0.01$$

$$M = -\ln(0.01) = 4.6 \text{ plaque forming units per cell}$$

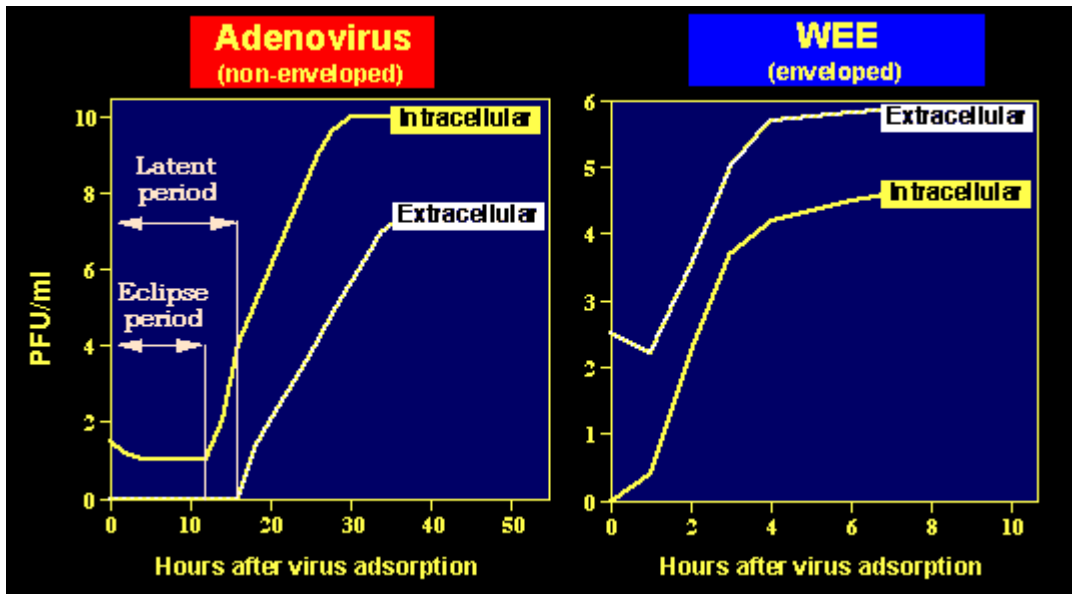
One-step growth analysis begins with the addition of virus to cells. After a brief (~1 hour) period of adsorption, the virus inoculum is washed away, and cells are cultured. Aliquots of the cells and the cell-culture fluid are then collected at various time points and analyzed for the presence of virus. A typical one-step growth analysis can be divided into several phases:

1. Adsorption of virus (initial phase).
2. **Eclipse phase.** This lasts for 10-12 hours, and it corresponds to the period during which the input virus becomes uncoated. As a result, no infectious virus can be detected during this time (any infectious virus detected is simply virus that is still stuck on the cell membrane).
3. **Synthetic phase.** This starts around 12 hours post-infection and corresponds to the time during which new virus particles are assembled.
4. **Latent period.** During this period, no extracellular virus can be detected. After ~18 hours, extracellular virus is detected. Ultimately, production will reach a maximum plateau level.

The virus **burst size** (amount of infectious virus produced, per infected cell) can be calculated on the basis of the results from a one-step growth experiment. Burst sizes for viruses typically vary between 10 and 10,000.

Viruses vary considerably in terms of the kinetics of their replication, and the specifics of their one-step growth curves. Two examples are shown below:

Representative mammalian virus growth curves



Differences between the two viruses shown include the fact that WEE replicates more rapidly than adenovirus, but that it does so to lower titers. In addition, WEE is an enveloped virus. This means that WEE must acquire its envelope from the host cell membrane during budding, in order for it to become infectious. Consequently, the level of intracellular infectious WEE is low. In contrast, adenovirus is non-enveloped, and therefore its infectivity is not dependent on budding from the host cell membrane. Consequently, the levels of infectious intracellular adenovirus can be very high. Indeed, they can be considerably higher than the levels of cell-free virus, suggesting that adenovirus is highly cell-associated.

## Steps in the Replicative Cycle of Viruses

**1. Attachment** - through a receptor.

**Specific:** CD4 on T-cells for HIV

ICAM on upper respiratory epithelial cells - Rhinoviruses (common cold)

Immunoglobulin-like receptors - polio virus

**2. Entry** - receptor-mediated endocytosis, e.g. Influenza and adenovirus  
- membrane fusion, e.g. herpesviruses and paramyxoviruses

**3. Uncoating** - triggered by pH changes in endosomes, e.g. Influenza A virus

**4. Replication & viral protein production**

- early proteins: control the next phase of replicative cycle, e.g. genome replication and late protein production.

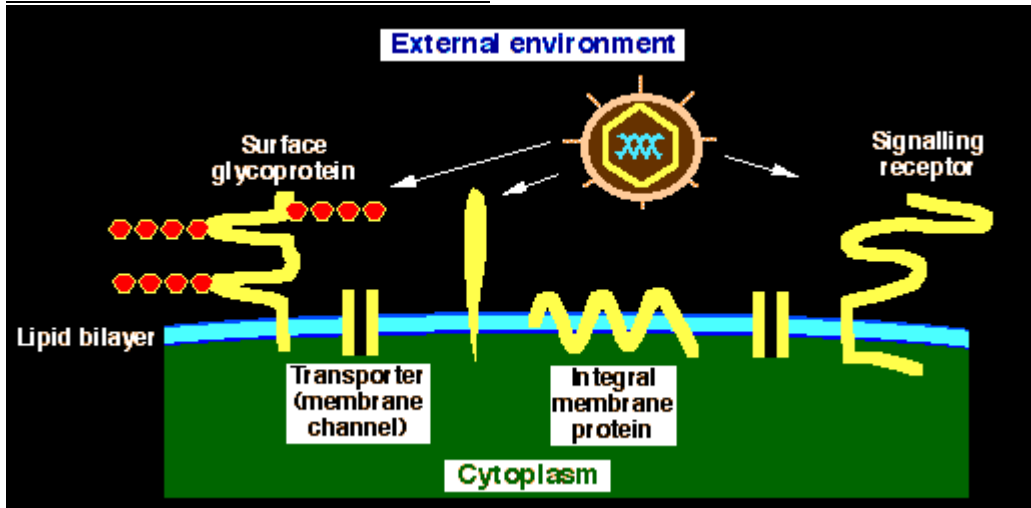
- late proteins: usually structural proteins

## 5. Assembly and release

### Virus attachment

Viruses typically attach to cells via specific cell surface receptors. Very often, virus receptors are molecules which project some distance away from the cell surface, allowing them to be contacted more easily by viruses.

#### General overview of virus attachment



Many different molecules are used as receptors by different viruses, including cell surface glycoproteins, components of the extracellular matrix and receptors involved in cell signalling and activation. Interestingly, certain receptors are targeted by several different viruses (e.g., sialic acid, heparan sulfate proteoglycans, ICAM-1, CAR, CD46, CD4 and certain integrins (see next page).

Furthermore, a number of viruses also use **coreceptors** to gain entry to cells – that is, these viruses require the presence of two essential molecules in order to infect their target cells. If either one of these receptors is absent, infection will not occur.

The distinction between a receptor and coreceptor is in large part a reflection of the sequential nature of virus attachment. Initial interactions with a primary receptor located some distance from the cell membrane may bring the virus into closer proximity with a secondary receptor (coreceptor) that is located much closer to the lipid bilayer. Binding of this coreceptor is often dependent on a conformational change in the virus, brought about by the initial interaction between the virus and its primary receptor.

A very well studied example of this phenomenon is the interaction between the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein, gp120, and the cell surface CD4 receptor. Binding of gp120 to CD4 results in a conformational change in

gp120, such that a binding site for the coreceptor is now exposed. HIV-1 coreceptors include chemokine receptors, most notably CCR5 and CXCR4.

Receptors for selected animal viruses

<b>Virus</b>	<b>Receptor</b>	<b>Biological Function or Type of Molecule</b>	<b>Coreceptor</b>
Adeno-associated virus	Heparan sulfate	Glycosaminoglycan; part of extracellular matrix	$\alpha_v\beta_5$ integrin
Adenovirus (subgroups A, C-F)	CAR ( <i>Coxsackie and adenovirus receptor</i> ); also used by several coxsackie viruses	Immunoglobulin (Ig)-like	$\alpha_v\beta_5$ , $\alpha_v\beta_3$ integrins. Also used by several picornaviruses; binds vitronectin ( $\alpha_v\beta_3$ )
Epstein-Barr virus	CD21 (aka CR2, complement receptor 2)	Ig-like; complement receptor	MHC class II (HLA-DR) in B cells; ? in epithelial cells
Herpes simplex virus	Heparan sulfate. Also used by AAV, Dengue, others	Glycosaminoglycan; part of extracellular matrix	HveA, Prr ( <i>Herpes virus entry mediator A</i> ; <i>Pvr-related protein 1</i> ).
HIV-1	CD4. Also used by human herpesvirus 7.	Ig-like; role in helper T cell function.	CCR5, CXCR4. Chemokine receptors.
Influenza virus	Sialic acid. Also used by reo-, corona- virus.	Carbohydrate	
Measles virus	SLAM. Some vaccine strains can use CD46; this is also used by human herpesvirus 6.	T/B cell surface protein; involved in cellular activation	
Poliovirus	Pvr ( <i>poliovirus receptor</i> , aka CD155)	Ig-like	
Rabies virus	Acetylcholine receptor, NCAM (CD56)	Neuronal receptor or adhesion molecule	
Rhinovirus (major subtypes)	ICAM-1 ( <i>intercellular adhesion molecule 1</i> ); also used by some coxsackieviruses	Ig-like; role in cell adhesion	

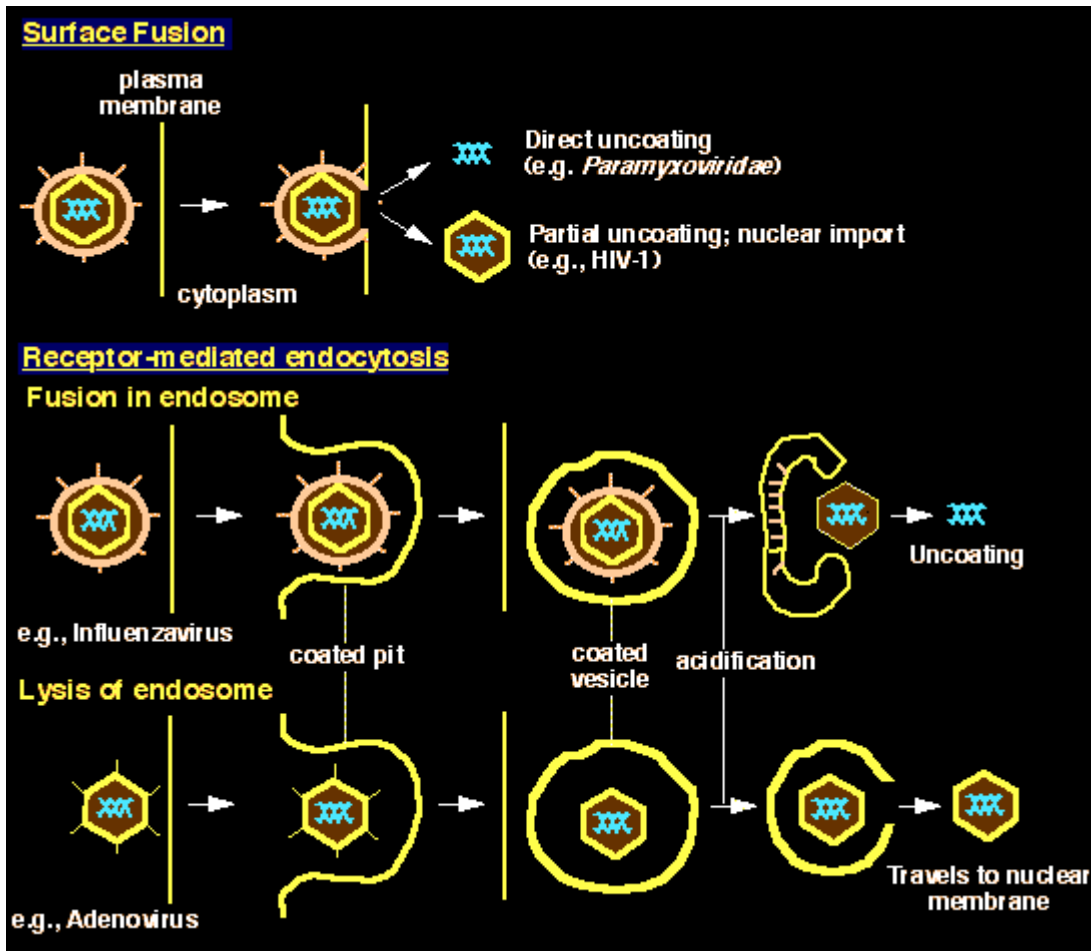
SV40	MHC-1 ( <i>major histocompatibility complex type 1</i> )	Antigen presentation	
Vaccinia virus	EGF receptor ( <i>epidermal growth factor</i> )	Growth factor receptor	

## **Virus Entry and Uncoating**

Following attachment, viruses must enter cells. This requires that the virus must traverse the lipid bilayer surrounding the cell, without killing the cell. Once inside the cell, the virus must disassemble itself in such a way that (1) its genetic information and any associated enzymes remain intact and (2) the viral nucleic acid and associated enzymes are directed to the appropriate cellular compartment (for DNA viruses, retroviruses, influenzaviruses, Borna disease virus and hepatitis delta virus, this is the nucleus).

The process of virus entry can proceed via several pathways. In the case of the *Paramyxoviridae*, the virus is able to fuse directly with the host cell membrane at neutral pH, via the action of the viral fusion protein (F). This results in uncoating of the viral genome at the cell membrane. Other viruses can also mediate membrane fusion at neutral pH, including retroviruses such as HIV-1. In the case of HIV-1, the virus particle does not fully disassemble following entry. Rather, a partially uncoated core particle remains. This then undergoes import into the nucleus, thereby allowing the virus life cycle to proceed.

### Overview of virus entry



**Receptor-mediated endocytosis.** Other viruses do not fuse directly with the host cell membrane, but instead undergo uptake into endocytotic vesicles.

**Endosome fusion.** In some cases, the virus takes advantage of the natural process of endosome acidification. One well studied example of this is the HA (hemagglutinin-neuraminidase) protein of **influenza virus**, which undergoes a low-pH induced shape change, such that the protein enters a fusion-competent state. This allows the virus to fuse with the host cell membrane, thereby bringing the virus particle into the cell.

Influenza also contains a second protein that is activated under conditions of low pH. This is the M2 protein, which has ion channel activity at low pH, thereby allowing protons to enter the virus. The entry of protons in turn disrupts electrostatic interactions between the viral ribonucleoprotein (RNP) and the major virion envelope protein, M1. This leads to virion disassembly while the virus is still in the endosome, and it is an essential part of the viral life cycle (so much so, that antiviral drugs such as amantadine target this step).

**Endosome lysis.** Non-enveloped viruses lack the lipid membranes found in enveloped viruses. As a result, these viruses cannot enter cells via a simple process of membrane fusion between the virus envelope and the host cell membrane. One strategy employed by

non-enveloped viruses is exemplified by adenovirus. This virus attaches initially to CAR, an immunoglobulin-like molecule, and it then binds to cellular integrins. Following binding, the integrin and virus are internalized, and the virus then begins to disassemble in the mildly acidic environment of the early endosome (~pH 6). As the endosome acidifies further, the penton base protein is released and this is thought to trigger endosome lysis, thereby mediating the escape of the partially disassembled core particle into the cytosol.

### **Other mechanisms of uptake and uncoating**

**Pore formation.** Some non-enveloped viruses, such as picornaviruses, form pores in the cell membrane. One example is poliovirus, which after binding to its receptor (Pvr) undergoes a conformational change that exposes a hydrophobic domain on the VP1 virion protein. This forms a pore in the cell membrane, through which the viral RNA is then released into the cytosol.

**Lysosomal uncoating.** Most viruses which enter the cell via endosomes escape before the endosomes arrive at the lysosome. However, the *Reoviridae* do not do so. These viruses rely on enzymes found in lysosomes (proteases) to carry out their uncoating. The lysosomal proteases convert the reovirus particle into an infectious subviral particle (ISVP) which then penetrates the cytosol.

### **Transport to the nucleus**

For those viruses which initiate their replication in the nucleus (DNA viruses, retroviruses, influenzaviruses, Borna disease virus and hepatitis delta virus), the viral genome and any essential enzymes or proteins must travel to, and enter, the nucleus. This process is dependent on the presence of **nuclear localization signals (NLS)** within the proteins associated with the viral genome. Such sequences are short and usually highly basic (positively charged); they act to permit large macromolecules or subviral particles to traverse the nuclear pore.

The nuclear import pathway requires an initial interaction between the NLS and a cytoplasmic NLS receptor – of which one example is karyopherin  $\alpha$  (or importin  $\alpha$ ). The complex between the NLS and importin  $\alpha$  then binds to importin- $\beta$  (karyopherin  $\beta$ ), which allows for docking to the nuclear pore complex, through binding to nucleoporins. Translocation across the pore then requires the action of additional proteins, including a small GTP-binding protein known as Ran.

The ability of HIV-1 to enter the nucleus of non-dividing cells is a unique and distinguishing feature of the lentiviruses, which differentiates them from the classical, transforming retroviruses such as Rous sarcoma virus. This property allows HIV-1 to infect terminally differentiated cells such as macrophages, and it also makes HIV-based vectors capable of transducing post-mitotic cells such as neurons.

The nuclear import of HIV-1 occurs as a result of the specific import of the viral preintegration complex (PIC) across the nuclear pore. The PIC contains the viral nucleic acid, with its closely associated proteins (including integrase and reverse transcriptase, as well as some of the protein components of the viral core particle). Contained within this complex are several nucleophilic viral proteins which contain NLS sequences – notably, the viral integrase, Vpr and matrix (MA) proteins. These proteins may all assist in the nuclear import of the viral preintegration complex (PIC).

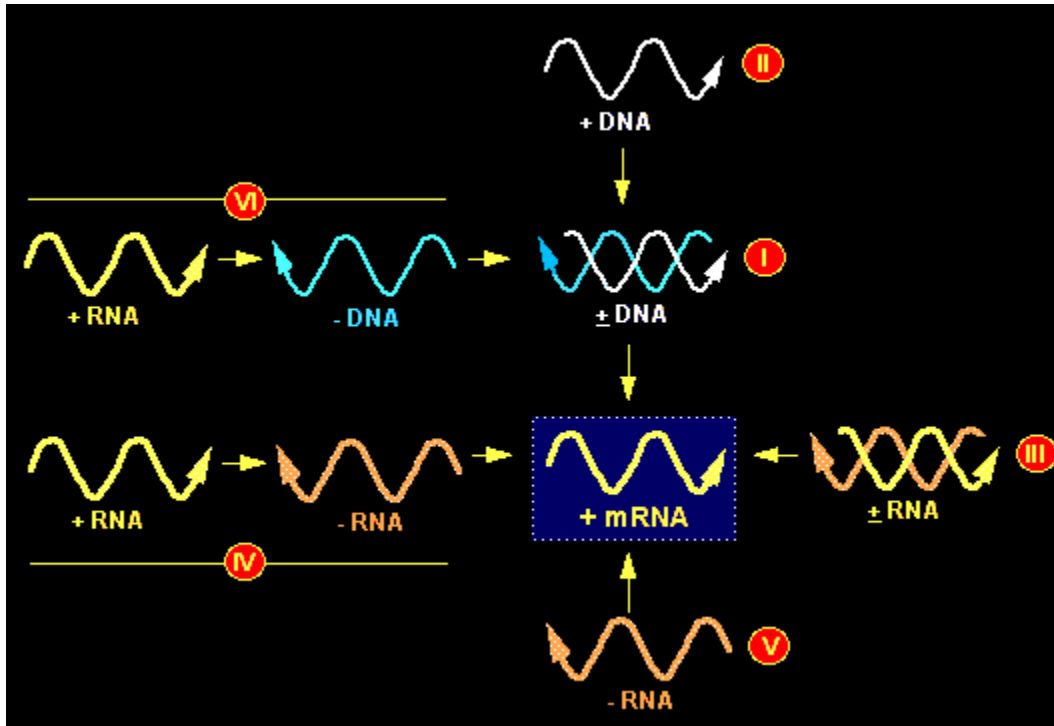
Nuclear import of the PIC is also enhanced by another factor, which is not a nucleophilic protein. Rather, this is a triple-stranded viral cDNA intermediate of reverse transcription, known as the “**central DNA flap**”. It is not completely clear how this unique DNA structure assists in viral nuclear import.

Finally, it should be noted that some viral proteins can cross the nuclear membrane by an NLS-independent pathway, including the VP22 protein of herpes simplex virus. Once again, the mechanistic basis for this remains uncertain.

## Virus Genome Replication and Gene Expression

**Baltimore Classification.** The Baltimore system of virus classification provides a useful guide with regard to the various mechanisms of viral genome replication. The central theme here is that **all viruses must generate positive strand mRNAs from their genomes**, in order to produce proteins and replicate themselves. The precise mechanisms whereby this is achieved differ for each virus family.

### Baltimore classification



**Replication of DNA viruses:** Most DNA viruses encode their own DNA polymerases. Exceptions are small DNA viruses -- parvoviruses and papillomaviruses. *Note that viruses with no DNA polymerase of their own require actively dividing cells to replicate, since they must use the cellular DNA polymerase (eg, HPVs and parvovirus B19; this has important consequences for pathogenesis).*

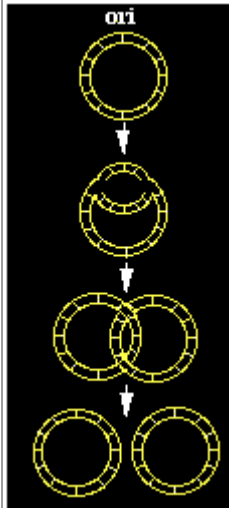
DNA viruses replicate their genetic material by one of three modes:

1. **Bidirectional replication from a circular substrate.** This process may proceed via a “theta-form” intermediate (eg, papillomaviruses), or in some cases via a “rolling circle” mechanism that results in the generation of concatemeric (head-to-tail) viral genomes. *More complex replication strategies may also exist, including recombination-dependent DNA replication.*
2. **Replication from a linear substrate.** In this case, synthesis of new DNA strands is not simultaneous. Rather, it occurs sequentially (ie, first one strand is made in its entirety & then the next strand is made). Examples include adenoviruses.
3. **Replication via an RNA intermediate.** **Hepadnaviruses** (hepatitis B virus) are unique since they contain a partially dsDNA genome that **must** be converted into an RNA form by the virion enzyme reverse transcriptase during the virus life cycle .

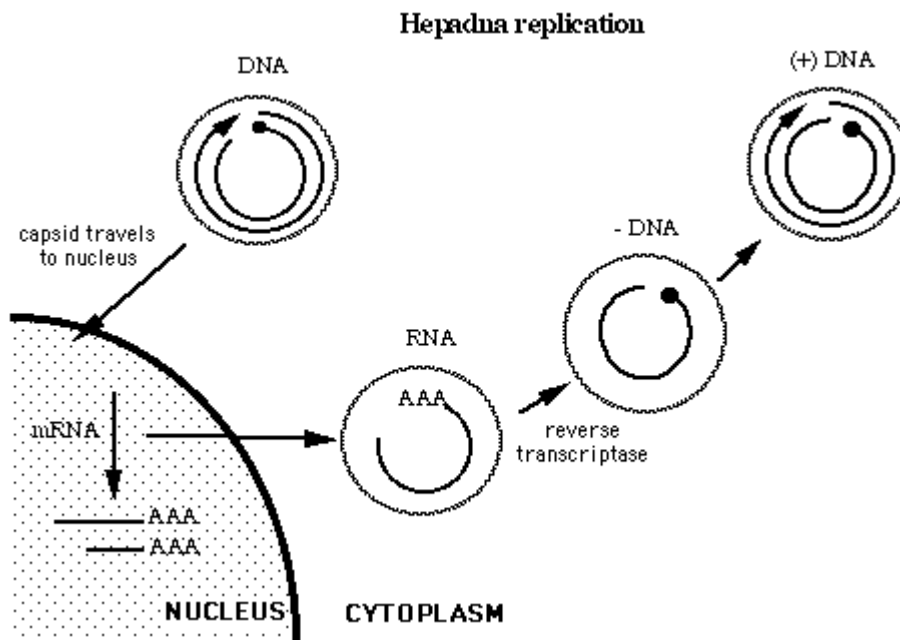
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### Example 1: Papovaviruses

Papovaviruses such as SV40 and human papillomaviruses are ds-DNA circles which replicate using a specific origin of DNA replication (ori) that serves as an initiation point for bidirectional DNA replication. Replication intermediates with a “theta-form” can be seen by electron microscopy.



**Example 2: Hepatitis B virus: Replication is thru a RNA intermediate & uses reverse transcriptase.** The virion DNA is incompletely double-stranded (ds). Once inside the cell, an intact dsDNA is made. The negative strand is then transcribed to RNA. This is used to make a complementary DNA strand (reverse transcription), prior to synthesis of the partial ds virion DNA.



### Replication of RNA Viruses.

RNA viruses have a variety of modes of replication. Three important points about their replication are as follows:

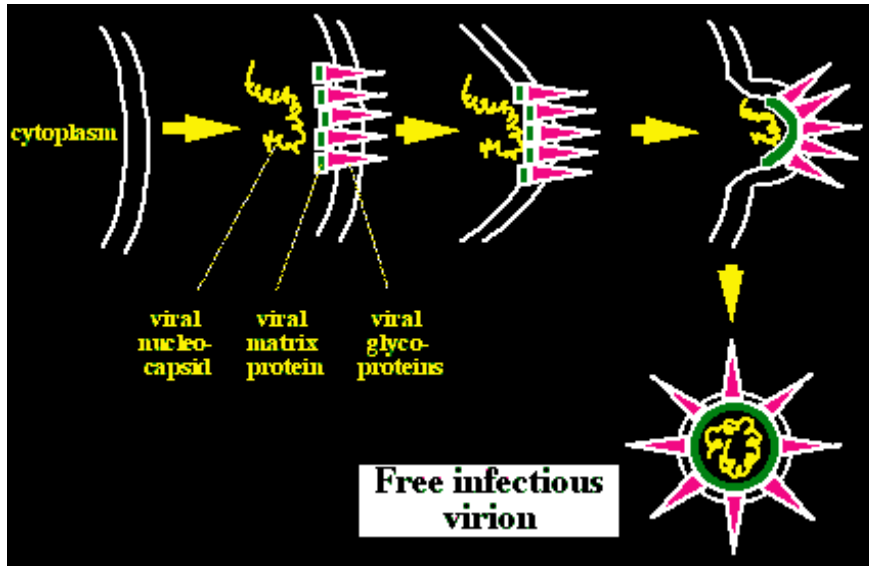
1. The viral RNA genome can act as its own message (positive strand viruses) OR the complementary strand can be the mRNA (negative strand viruses).
2. All RNA viruses except retroviruses encode an **RNA-dependent RNA polymerase**. In the negative strand RNA viruses this polymerase is part of the virion, and it must enter the cytosol along with the viral genome. This is necessary in order for the virus to generate mRNAs from its genome.
3. All RNA viruses **replicate in the cytoplasm** except orthomyxoviruses (influenza A & B), borna disease virus, hepatitis delta virus and retroviruses. *Additional feature of orthomyxoviruses: these viruses have segmented genomes (influenza A has 8 separate strands of genomic RNA)*
4. **Retroviruses are unique**. These viruses have a positive sense RNA genome which **must** be converted into a dsDNA form by the virion enzyme **reverse transcriptase** (an RNA-dependent DNA polymerase). This double-stranded DNA is then integrated (at random sites) into the host cell chromosome by the viral integrase enzyme. Upon integration into the host chromosome, the viral DNA can then be transcribed by cellular RNA polymerase II, to produce new genomic RNA molecules.

Replication of RNA Viruses						
Class	Genome	Virion RNA polymerase?	Genome infectious?	mRNA species	Primary gene product	Example
1	+ polarity (mRNA)	No	Yes	Genome size	Polyprotein	Picorna
2	+ polarity (mRNA)	No	Yes	Gene unit size	Single proteins	Corona
3	- polarity (anti-mRNA)	Yes	No	Gene unit size	Single proteins	Paramyxo
4	Segmented - polarity	Yes	No	Gene unit size	Single proteins	Orthomyxo
5	ds RNA	Yes	No	Gene unit size	Single proteins	Reo
6	Ambi-sense (+ & - polarity)	Yes	No	Gene unit size	Single proteins	Arena
7	DNA intermediate (+ polarity)	Yes	No	Genome length & gene unit size (spliced)	Polyprotein and single proteins	Retro (HIV, HTLV)

## Assembly, Release and Maturation

Virus particles are released from cells either by:- **lysis** of cells, or **budding** from cell membranes, usually the cytoplasmic membrane. Both processes lead to changes in cell membranes, which can often be observed *in vitro*, as part of the virally-induced cytopathic effect.

Virus assembly and budding (e.g., for paramyxoviruses)



*The viral matrix protein attaches to the inner surface of the plasma membrane & acts both as a stabilizing factor and as a recognition site for the nucleocapsid (genome + coating proteins). Viral envelopes contain both viral glycoproteins & host cell lipids (membrane-derived).*

**Receptor escape:** One central issue with which viruses must deal during their release from the host cell is how to escape from binding to their original receptor. Paramyxo- and orthomyxo- viruses have solved this problem by encoding **neuraminidase** enzymes (also known as sialidases) which cleave the sialic acid residues off the cell surface, allowing the virus to exit the cell. Inhibition of the neuraminidase enzyme has turned out to be a useful approach to antiviral therapy, as exemplified by drugs such as zanamavir (relenza) and oseltamavir (tamiflu). These drugs interfere with virus release from the infected cell, and result in the aggregation of virus particles at the cell surface.

**Virion maturation:** Many viruses are released from cells before they have undergone full maturation and conversion to an infectious form. One example is HIV-1. Maturation of the HIV-1 virion into its final, infectious state requires the action of the viral **protease** which trims and remodels many of the virion components, including the p55 Gag precursor protein. If the activity of protease is inhibited (for example, by antiviral drugs such as indinavir, ritonavir and the like), then immature, non-infectious virus particles will be released from the infected cell.