Chapter 29

Viral Replication

Virus replication can be described in **two ways:**

-- The **first approach** is a **growth curve** which shows the amount of virus produced at different times after infection.

-- The **second approach** is a **stepwise description of the specific events** within the cell during virus growth.

Viral growth curve

-- When **a virion** infects a cell, it can replicate in **app. 10 hours to produce hundreds of virions** within that cell.

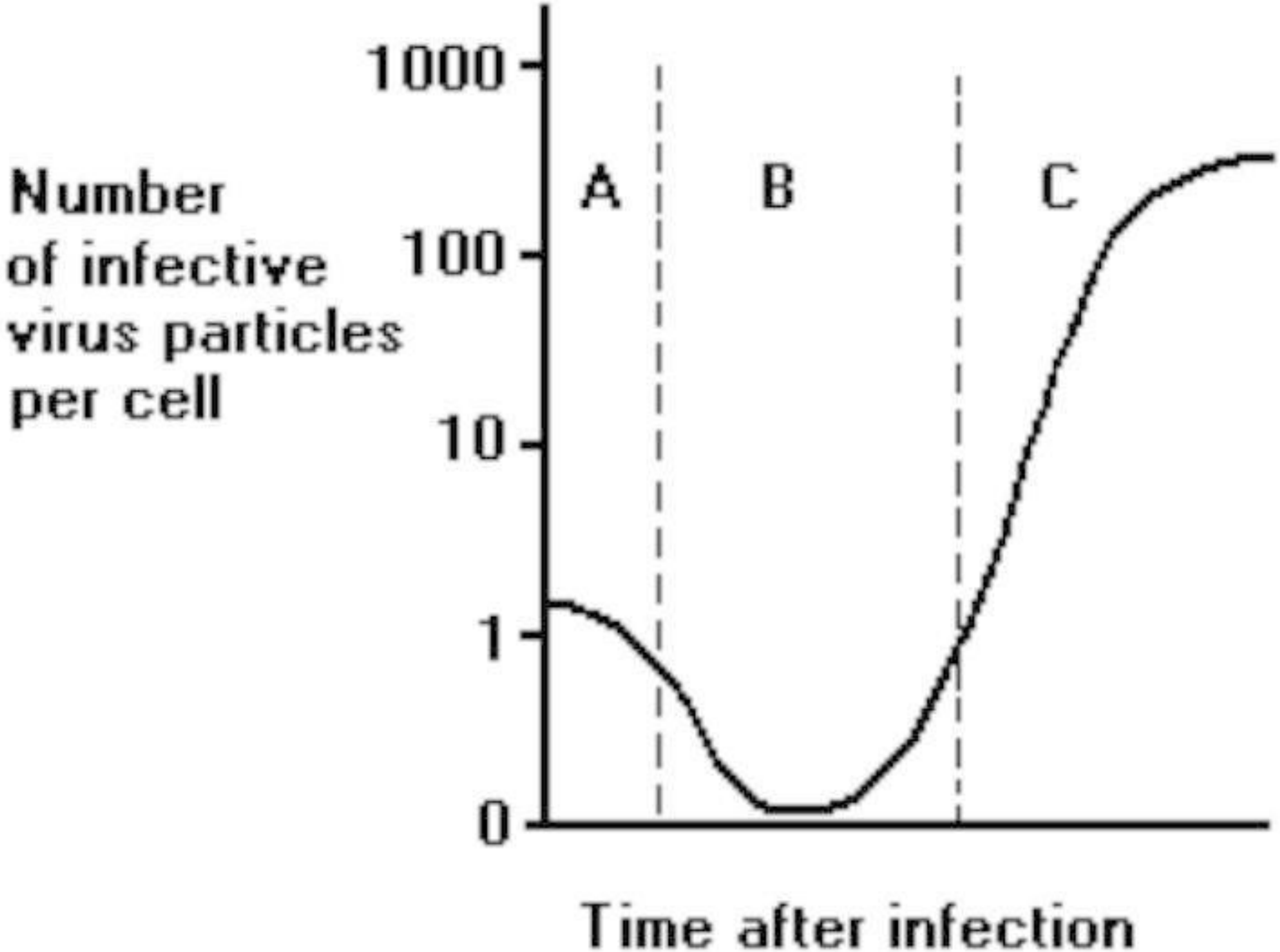
-- The time for **life cycle completion** is **minutes in some bacterial viruses**, but **hours for human viruses**.

-- The cycle **starts with disappearance of virus**.

-- Virus as such is not present but its **nucleic acid continues to function and accumulate.**

-- This is called **eclipse period**.

-- It ends with appearance of virus.



A. Infection **B. Eclipse phase** **C. Release of progeny**

-- The **latent period**, in contrast, is defined as the **time from onset of the infection to the appearance of virus extracellularly.**

-- **Alteration of cell morphology** accompanied by **marked derangement of cell function** begin toward the **end of the latent period.**

-- This **cytopathic effect (CPE)** culminates in the **lysis and death of cells.**

-- **CPE** can be **observed by microscopy** and is important for laboratory diagnosis.

-- **Not all viruses cause CPE**; some can replicate without causing much morphological or functional change in the cell.

Specific events during the growth cycle

-- Viral populations **do not grow through cell division** because they are acellular.

-- Instead, they **use the machinery and metabolism of a host cell** to produce **multiple copies** of themselves, and they ***assemble*** in the cell.

The life cycle of viruses differ greatly between species but there are six ***basic*** stages :

1. ***Attachment***

2. ***Penetration***

3. ***Uncoating***

4. ***Replication***

5. ***Protein modification***

6. ***Viral release***

Attachment

-- It is a **specific binding between viral capsid proteins and specific receptors on the host cellular surface**. This specificity determines the host range of a virus.

-- Attachment to the receptor can induce a **viral envelope protein** to undergo changes that results in the **fusion of viral and cellular membranes**, or **changes in *non-enveloped virus* surface proteins** that **allow the virus to enter**.

-- The receptors for viruses on the cell surface are proteins that have other functions in the life of the cell.

Penetration

-- It follows attachment: **virions enter the host cell through receptor mediated endocytosis or membrane fusion.**

-- This is often called **viral entry**.

-- **Bacteria**, like plants, have **strong cell walls that a virus must breach to infect the cell** but because of much less thickness some viruses can inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating

-- It is a process in which the **viral capsid is removed**.

-- This may be achieved by **degradation by viral enzymes or host enzymes** or by **simple dissociation**.

-- The end result is the **releasing of the viral genomic nucleic acid**.

Replication

-- It primarily involves **multiplication of the genome**.

-- It involves **synthesis of viral mRNA for all viruses except some positive sense RNA viruses.**

-- It is **followed by viral protein synthesis**, **assembly of viral proteins**, and **viral genome replication** mediated by early or regulatory protein expression.

-- This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis.

Protein modification

-- Following the structure-mediated self-assembly of the virus particles, **some modification of the proteins often occurs**.

-- In viruses such as HIV, this modification (sometimes called **maturation**) occurs *after* the virus has been released from the host cell.

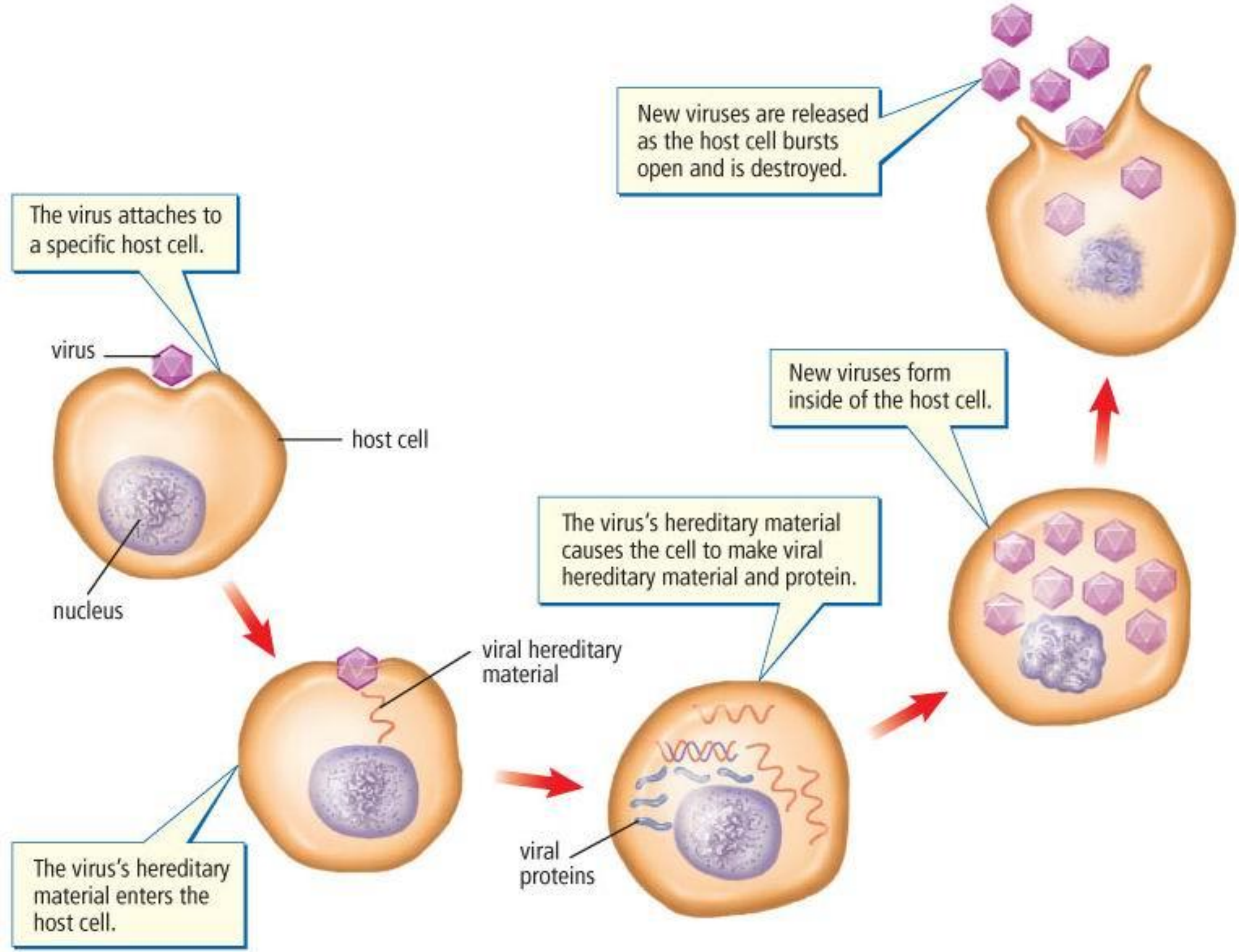
Viral release

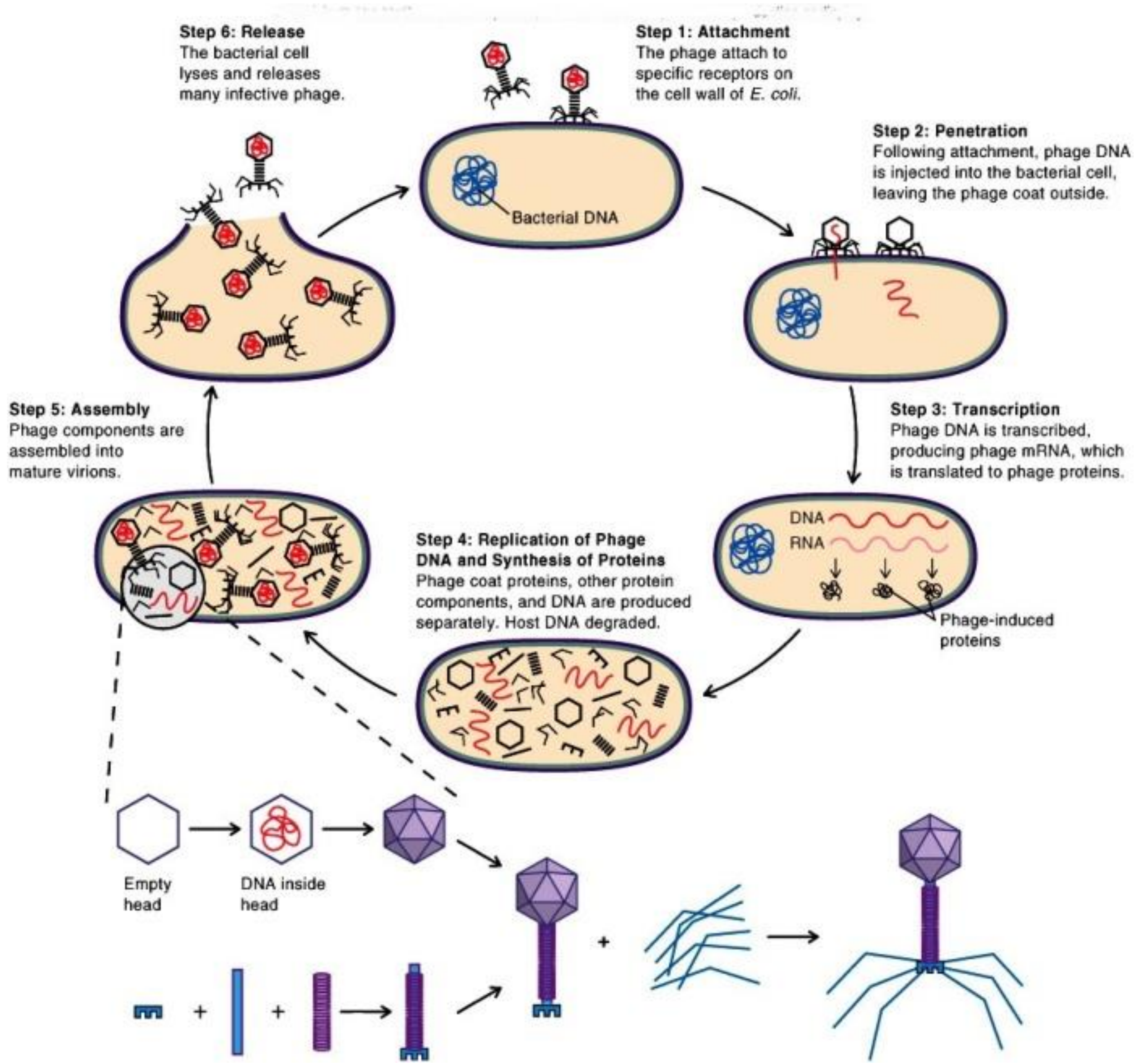
-- Viruses can be ***released* from the host cell by lysis**, a process that kills the cell by bursting its membrane and cell wall if present: this is a feature of many bacterial and some animal viruses.

-- Some viruses undergo a **lysogenic cycle** where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome.

-- **Enveloped viruses** (e.g., **HIV**) typically are released from the host cell by **budding**.

-- During this process the virus **acquires its envelope**, which is a modified piece of the host's plasma or other, internal membrane.


The virus attaches to a specific host cell.
virus
host cell
nucleus
viral hereditary material
The virus's hereditary material enters the host cell.
The virus's hereditary material causes the cell to make viral hereditary material and protein.
viral proteins
New viruses form inside of the host cell.
New viruses are released as the host cell bursts open and is destroyed.


Step 1: Attachment
The phage attach to specific receptors on the cell wall of E. coli.
Bacterial DNA
Step 2: Penetration
Following attachment, phage DNA is injected into the bacterial cell, leaving the phage coat outside.
Step 3: Transcription
Phage DNA is transcribed, producing phage mRNA, which is translated to phage proteins.
DNA
RNA
Phage-induced proteins
Step 4: Replication of Phage DNA and Synthesis of Proteins
Phage coat proteins, other protein components, and DNA are produced separately. Host DNA degraded.
Step 5: Assembly
Phage components are assembled into mature virions.
Step 6: Release
The bacterial cell lyses and releases many infective phage.
Empty head
DNA inside head

Genome expression and genome replication

-- The **first step** in viral gene expression is **mRNA synthesis**.

-- It is at this point that viruses follow **different pathways** depending on the **nature of their nucleic acid** and **part of the cell** in which they replicate.

-- **DNA viruses**, *except poxviruses* replicate in the nucleus by using **host cell DNA-dependent RNA polymerase** to synthesize their mRNA.

-- **Poxviruses replicate in cytoplasm** and thus have no access to the host cell RNA polymerase. Therefore, they **carry their own polymerase within the virus particle.**

-- The **genome of all DNA viruses consists of double stranded DNA**, **except for the parvoviruses** which have a **single stranded DNA genome.**

- **Most RNA viruses undergo their entire replicative cycle in the cytoplasm.**

- **Retroviruses** and **influenza viruses** have an **important replicative step in the nucleus.**

- **Retroviruses integrate a DNA copy of their genome into the host cell DNA,** and **influenza viruses synthesize their progeny genomes in the nucleus.**

- In addition, the **mRNA** of **hepatitis delta virus** is also **synthesized in the nucleus of hepatocytes.**

- **The genome of all RNA viruses consists of single stranded RNA**, *except* for **reoviruses** which have a **double-stranded RNA genome**. **Rotaviruses** are the important member.

RNA viruses fall into four groups according to strategies for synthesizing mRNA:

1. **Single stranded RNA of positive polarity** (e.g. **poliovirus**) which acts as mRNA.

2. **Single stranded RNA of negative polarity** (e.g. **measles, influenza** and **rabies** viruses) which **must first be transcribed into mRNA** before protein synthesis can begin.

   These viruses carry their own RNA-dependent RNA polymerase for this purpose.

   Certain viruses, .g., **arenaviruses** are **ambisense**, i.e., **they have some segments with positive and others with negative polarity.**

3. **Double stranded RNA**, e.g., **rotaviruses also carry their own polymerase** because cell has no enzyme which can dissociate positive strand from negative.

4. **Single stranded RNA of positive polarity,** that is **transcribed into double stranded DNA by RNA dependent DNA polymerase** (**reverse transcriptase**) carried by the virus.

   This **DNA copy is then transcribed into viral mRNA by the regular host cell RNA polymerase**.

   These are called **retroviruses.**

   These are **only diploid viruses**.

-- **Viruses that can utilize cell enzymes for replication produce infectious nucleic acid** where as **those which carry their own enzymes *cannot* produce infectious nucleic acid.**

-- The synthesized proteins are **early** (for replication of genome) or **late** (structural, i.e., after replication).

-- **Replicase is the most important early protein**.

-- Some viral mRNAs are translated into **precursor polypeptides** that **must be cleaved by proteases** to produce functional structural proteins, e.g., **picornaviruses** (such as **hepatitis A virus**), **flaviviruses** (e.g., **hepatitis C virus**) and retroviruses (e.g., **HIV**).

Lysogeny

-- **Lysogeny**, or the **lysogenic cycle**, is one of two methods of viral reproduction (the **lytic cycle** is the other).

-- **Lysogeny is characterized by integration of the bacteriophage nucleic acid into the host bacterium's genome** or **formation of a circular replicon in the bacterium's cytoplasm.**

-- In this condition the **bacterium continues to live** and reproduce normally.

-- The genetic material of the bacteriophage, called a **prophage**, can be **transmitted to daughter cells at each subsequent cell division**, and a **later event** (such as UV radiation or the presence of certain chemicals) can release it, causing **proliferation of new phages via the lytic cycle.**

- -- One of the **most important functions of lysogeny** from medical point of view is the **synthesis of several exotoxins** in bacteria such as diphtheria, botulinum, cholera, and erythrogenic toxins, encoded by the genes of integrated bacteriophage (prophage).

- -- **Lysogenic conversion** is the term applied to new properties that a bacterium acquires as a result of expression of the integrated prophage genes.

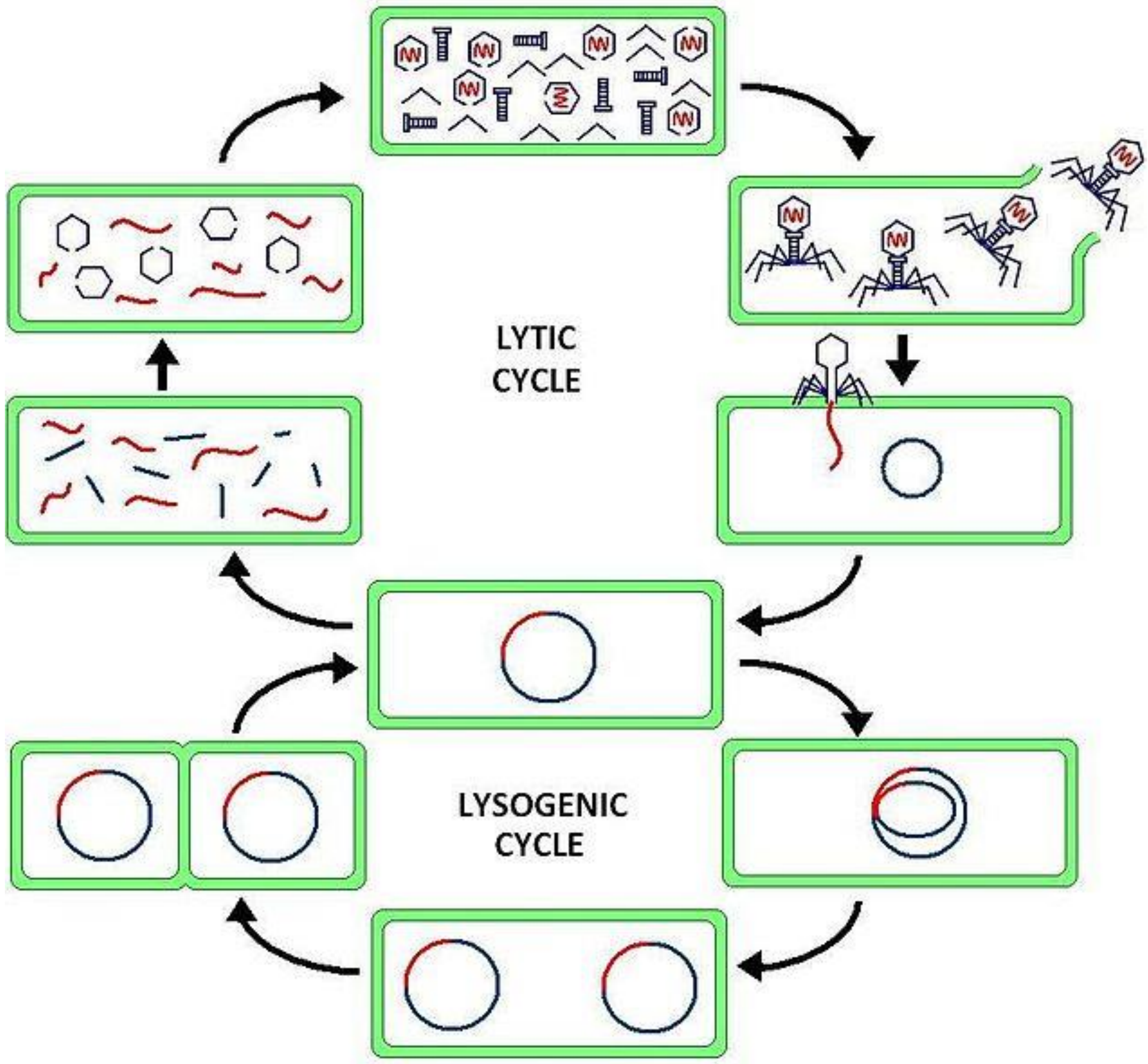
- -- It is mediated by bacteriophage through **transduction**.

- -- **Lambda (λ) phage** of ***E.coli*** is a **model system** as its several aspects are similar to **tumor viruses** and **herpesviruses**.

-- The **choice between lytic and lysogenic** cycle depends on balance of **repressor gene *c-I*** and its **antagonizer gene *cro***.

-- If the former predominates, lysogeny occurs, otherwise events progress to lytic cycle.

-- **UV light and certain chemicals can convert lysogenic cycle to lytic cycle.**


LYTIC
CYCLE
LYSOGENIC
CYCLE

Relationship of lysogeny in bacteria to latency in human cells

-- Some human viruses, e.g., **herpesviruses** and **Epstein Barr virus** exhibit **latency** which is almost similar to **lysogeny**.

-- When **HSV** infects neurons, a set of regulatory RNAs called **“latency associated transcripts” (LATS)** is produced which suppress viral replication.

-- If these genes are excised, viral replication starts.

-- **Cytomegalovirus (CMV)** encodes **microRNAs** that **inhibit the translation of mRNAs** required for viral replication.

-- They also code a protein that **prevents apoptosis** of infected cell.

Chapter 30

Genetics and Gene therapy

Viral genetics deals with:

1. **Mutations** and their effect on replication and pathogenesis

2. **Interaction between two genetically distant viruses** that infect the same cell.

In addition viruses serve as **vectors in gene therapy** and in **recombinant vaccines.**

Mutations

-- In virology, mutations have great role in production of **live attenuated vaccines.**

-- Mutations are induced so that **pathogenicity is lost but antigenicity is retained.**

-- **Natural mutations** play a great role in surface protein conversion, esp. in RNA viruses such as **influenza** and **hepatitis C**.

-- It makes effective vaccine development difficult due to this **instability.**

-- Same process leads to **drug resistance**.

Conditional lethal mutations

-- These mutations are extremely useful. They function **normally under permissive conditions** but **fail to do so under restrictive conditions**.

-- For example temperature sensitive mutants of **Rous sarcoma virus** can transform cells to malignancy at 37°C but when grown at 41°C, the normal phenotype is reverted.

-- These mutations have been clinically exploited to make an **influenza vaccine.**

-- The viruses reside and grow in cooler upper respiratory tract to produce antibodies but cannot cause disease (pneumonia) in lower warmer airways of lungs.

Defective interfering paricles

-- Some mutations result in **defective interfering particles** which can only replicate with the help of a **“helper virus”.**

-- If they infect a cell which is later infected by a normal virus, they **interfere** with its growth. So they can be used to help recovery from a viral infection.

Interactions between viruses

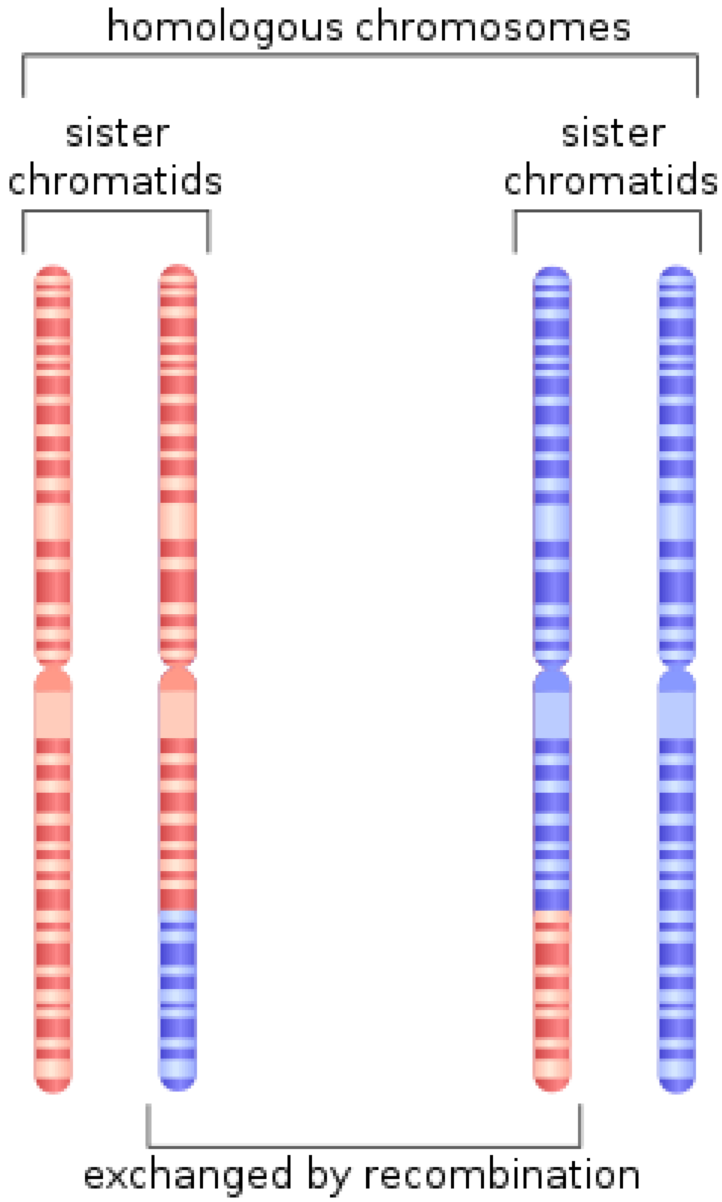
When **two genetically distinct viruses** infect a cell, three different phenomena can ensue:

Recombination

-- It is the **exchange of genes between two chromosomes** that is based on crossing over within regions of significant base sequence homology.

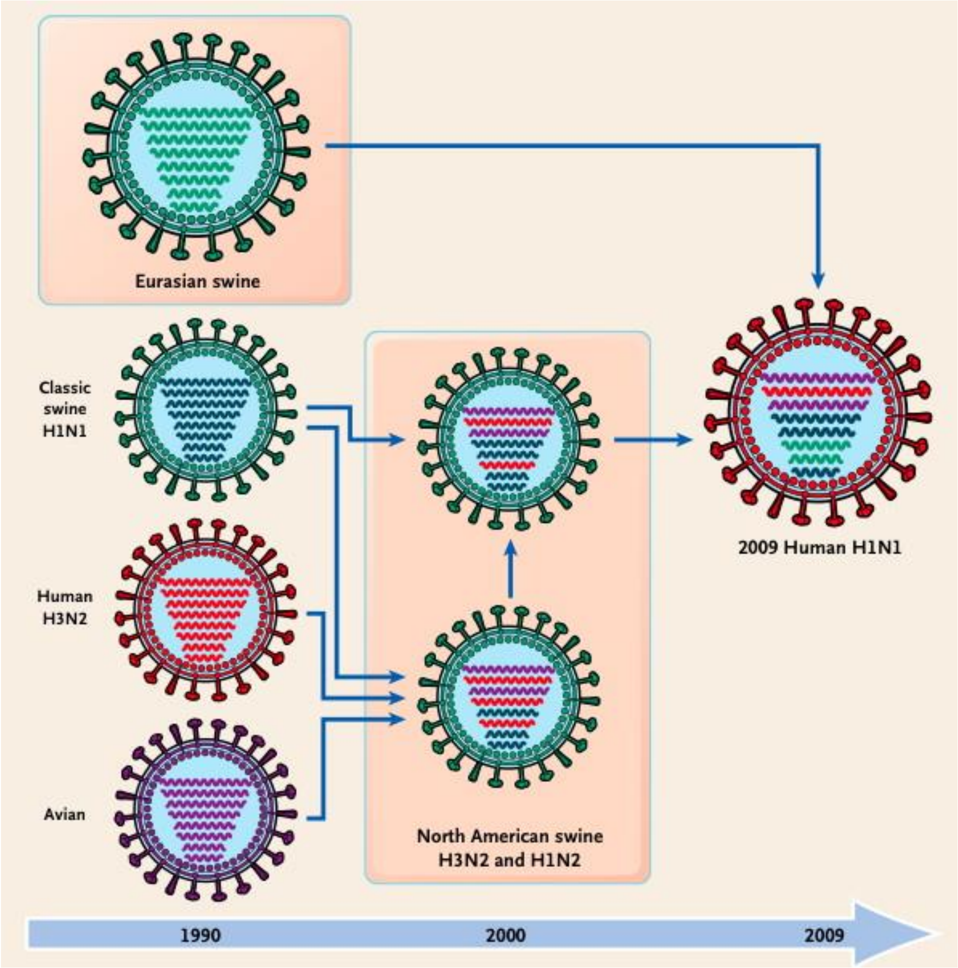
-- It occurs at **high frequency in DNA viruses**.

-- Frequency is **very low in RNA viruses**.


homologous chromosomes
sister chromatids
sister chromatids
exchanged by recombination

Reassortment is the term used when **viruses with segmented genomes**, such as influenza virus, **exchange segments**.

Frequency of gene exchange is much higher as compared with recombination.



Complementation

-- It occurs when either one or both viruses infecting a cell have a **mutation resulting in a non functional protein**.

-- The two viruses **complement each other to overcome the** deficiency.

-- This is the method by which “helper” viruses help defective viruses.

-- In clinical setting **hepatitis B** virus provides a **surface antigen** to **hepatitis delta virus**.

Phenotypic mixing

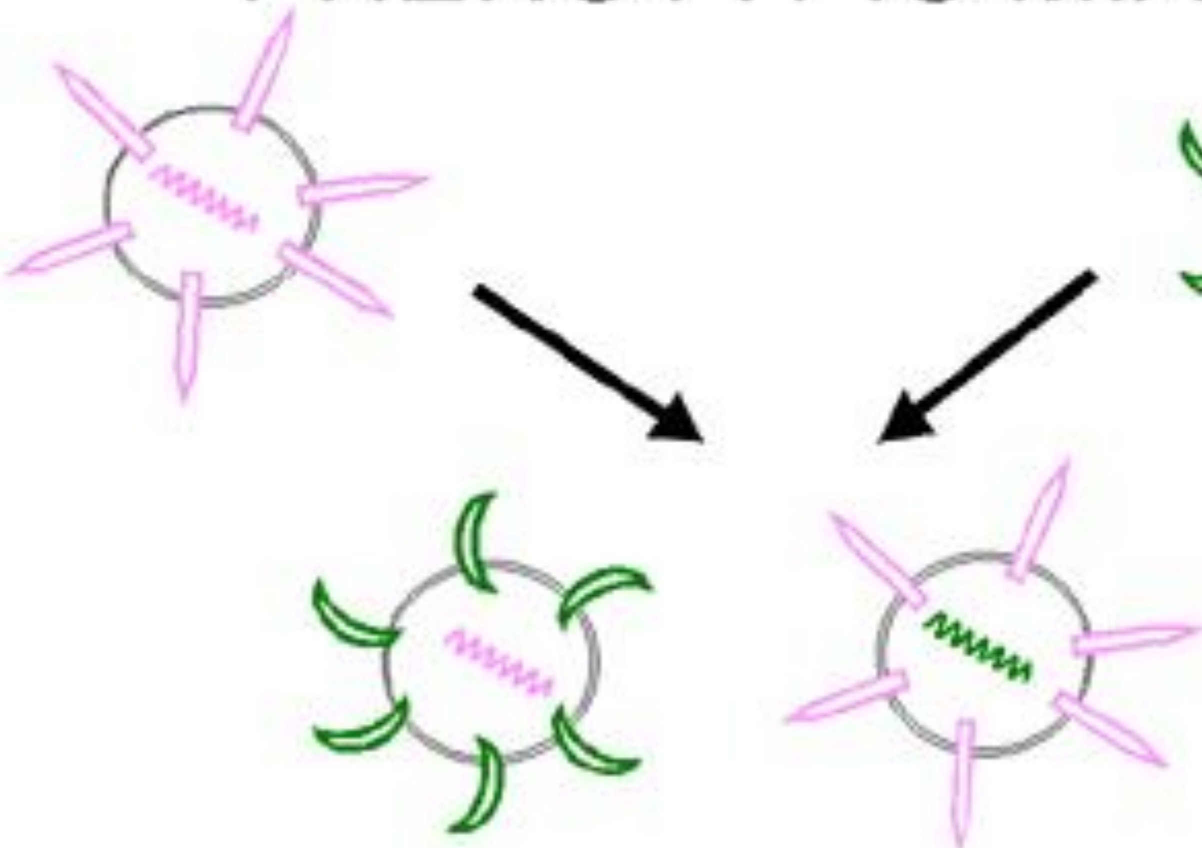
-- In phenotypic mixing, the **genome of virus type A can be coated with the surface proteins of virus type B.**

-- This phenotypically **mixed virus can infect cells as determined by its type B protein coat.**

-- However **the progeny virus will have a type A coat encoded by its genetic material.**

-- **Pseudotypes** is the name given to such viruses.

PHENOTYPIC MIXING



PSEUDOTYPE

Gene therapy and recombinant vaccines

Viruses are being used as **genetic vectors** in two novel ways:

1. **To deliver new functional genes** to patients with genetic diseases (**gene therapy**)

2. To produce **new viral vaccines that contain recombinant viruses** carrying the genes of several different viruses.

Gene therapy

-- **Retroviruses** are currently being used as vectors of **the gene encoding adenine deaminase (ADA**) in patients with immunodeficiencies resulting from a defective ADA gene.

-- These are excellent vectors because a **DNA copy of their RNA genome is stably integrated into the host DNA** and these genes are expressed efficiently.

-- **Retroviruses** are constructed by **removing several structural genes** and **replacing them with human genes** of interest.

Recombinant vaccines

-- Viruses with **large genomes** (e.g. **vaccinia virus**) are used.

-- **Non essential genes are removed and genes from other virus that encodes the antigen that elicits neutralizing antibody are introduced**.

-- Example is gene for surface antigen of **hepatitis B virus** introduced into **vaccinia virus** and expressed in infected cells.


Thank You!