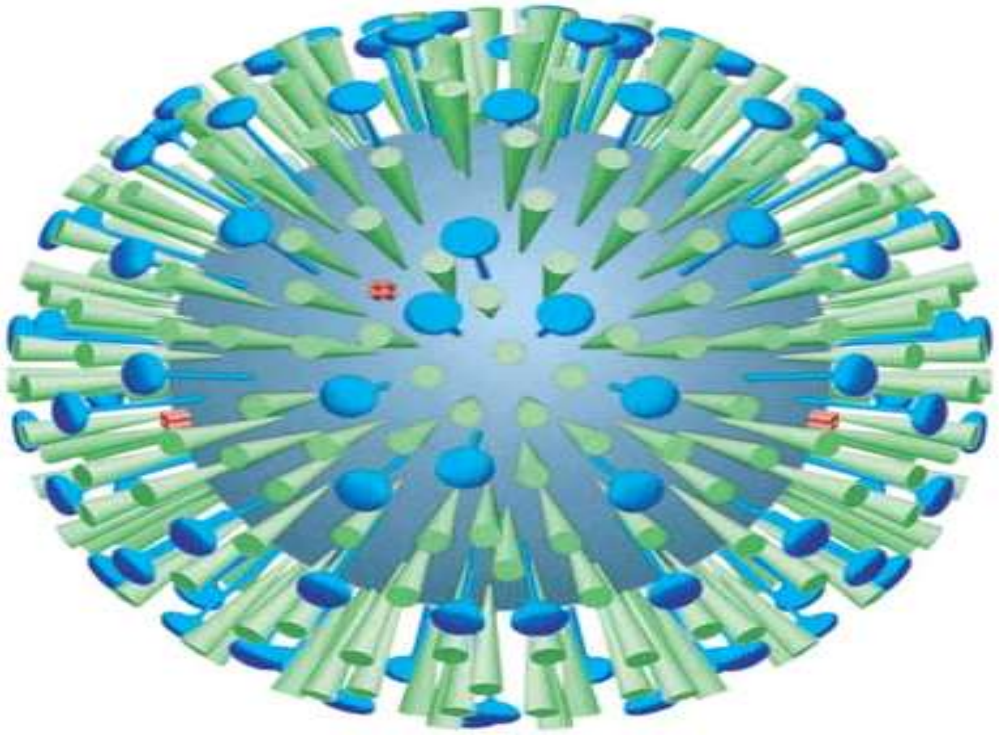
Ministry of higher Education and Scientific Research Southern Technical University

Institute of Medical Technology /AMARA

Theoretical Medical Viruses

Students of Second Class Medical Laboratory



Saedi ALsaedi

M. Sc. Biology

What are Viruses?

Definition-

- Viruses are non cellular particles made up of genetic material and protein that can invade living cells.
- Small, non-living, invades and reproduces inside a living cell .
- 1 virus- can produce thousands of new viruses .
- Viruses are very small – smaller than the smallest cell.

Characteristics

- Non living structures .
- Non-cellular .
- Contain a protein coat called the capsid .
- Have a nucleic acid core containing DNA or RNA (one or the other - not both) .
- Capable of reproducing only when inside .

- Outside of host cells, viruses are inactive .
- Lack ribosomes and enzymes needed for Metabolism .
- Use the raw materials and enzymes of the host cell to be able to .

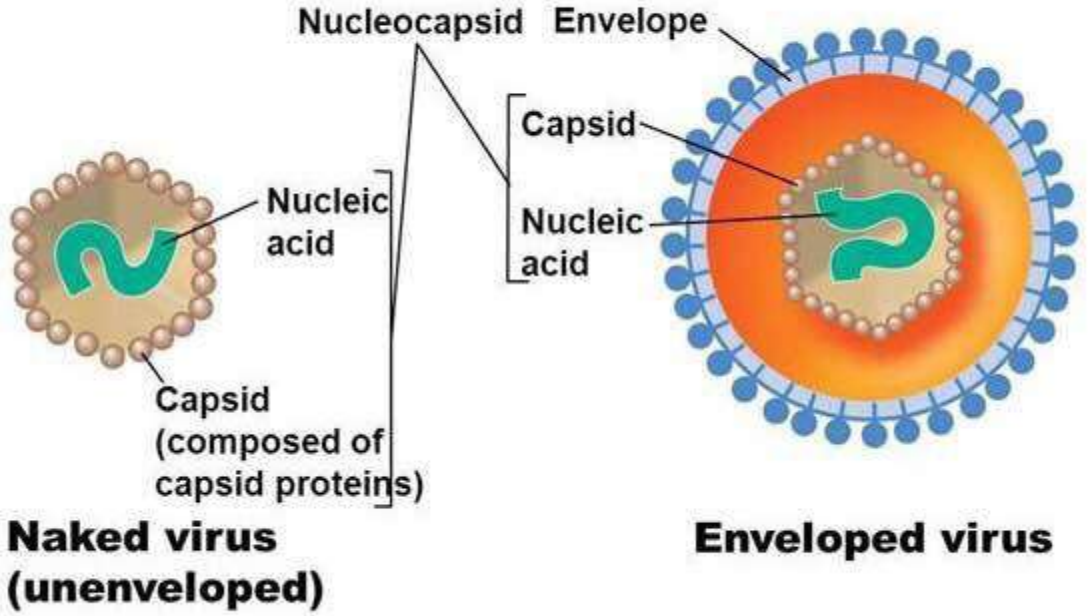
- Some viruses are enclosed in an protective Envelope .
- Some viruses may have spikes to help attach to the host cell .
- Most viruses infect only SPECIFIC host cells .

- Viruses cause many common illnesses Diseases .
- Smallpox, measles, mononucleosis, influenza, colds, warts, AIDS, Ebola, the “flu,” chicken pox, measles, polio, and hepatitis .
- Some viruses may cause some cancers like leukemia .
- Virus-free cells are rare .

Structure of viruses.

The structure which make up a virus particle are known as:

- Virion - the intact virus particle.
- Capsid - the protein coat.
- Capsomeres - the protein structure units of which the capsid is composed.
- Nucleic acid - Carrying genetic information.
- Envelope - the particles of many viruses are surrounded by a lipoprotein envelope containing viral antigens.



Viral Structure

- Varies in size, shape and symmetry
- VIP for classification
- 3 types of capsid symmetry:

1– Cubic (icosahedral)

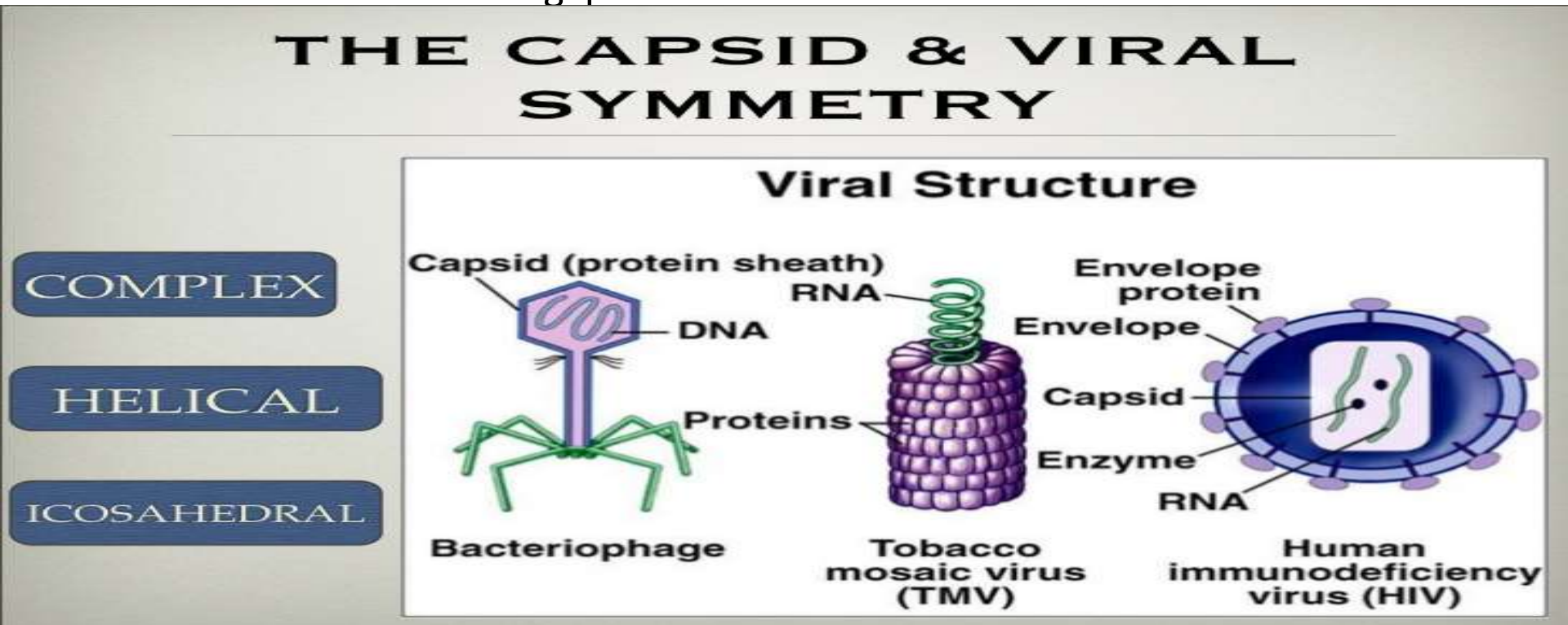
- Has 20 faces, each an equilateral triangle. Eg. adenovirus

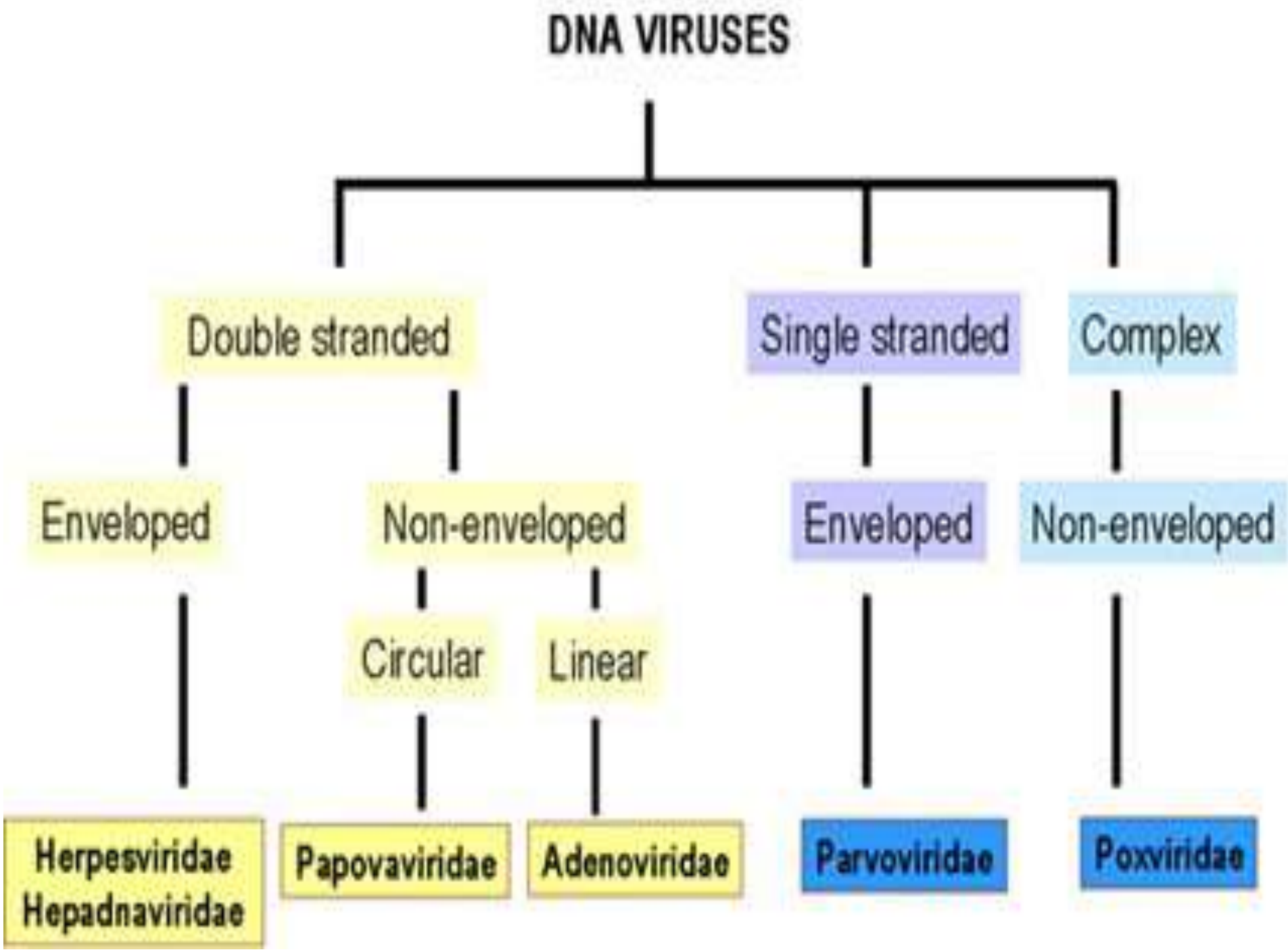
2– Helical

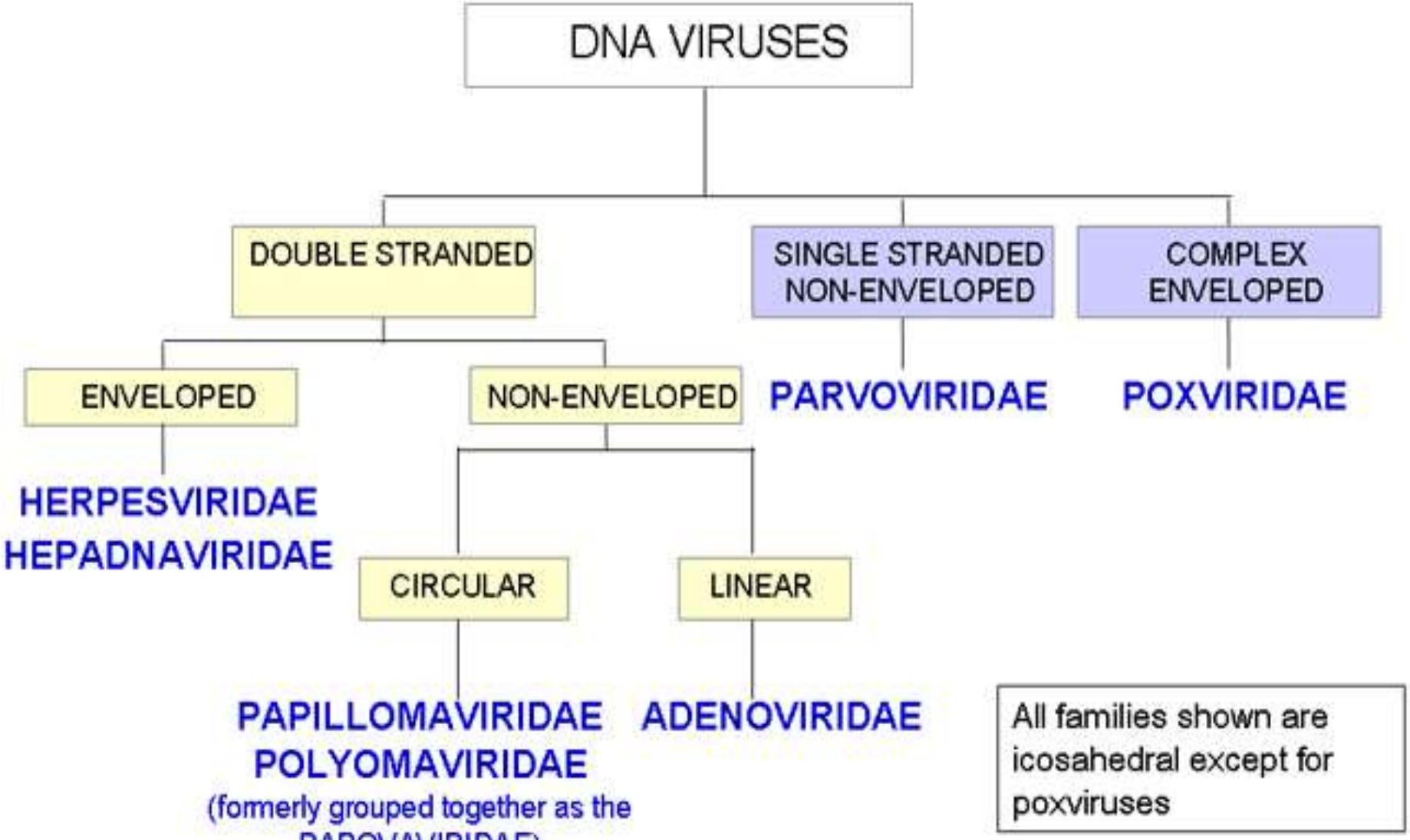
- Protein binds around DNA/RNA in a helical fashion eg. Coronavirus

3– Complex

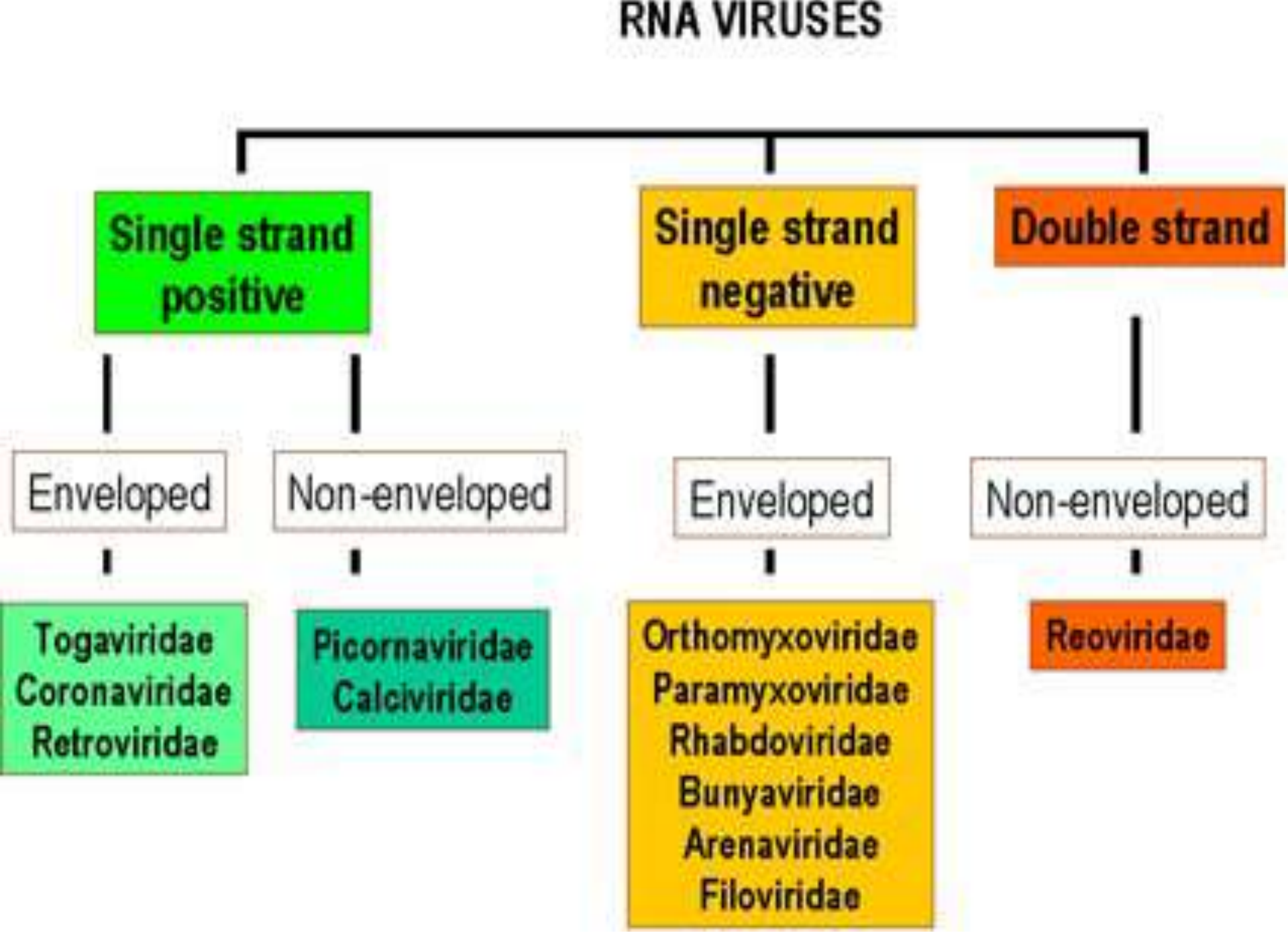
- Is neither cubic nor helical eg. poxvirus

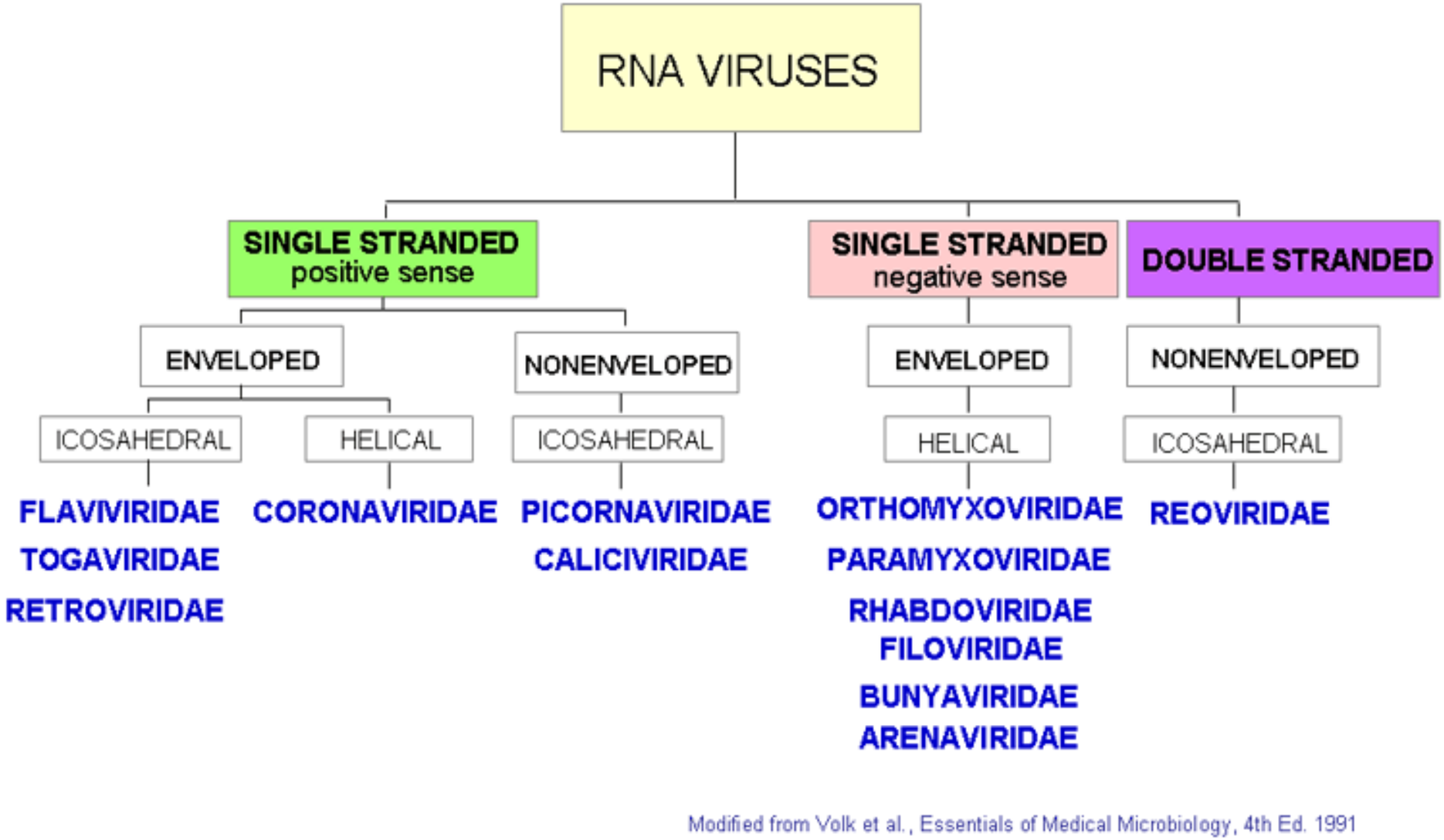



DNA VIRUSES
Double stranded
Single stranded
Complex
Enveloped
Non-enveloped
Enveloped
Non-enveloped
Circular
Linear
Herpesviridae
Hepadnaviridae
Papovaviridae
Adenoviridae
Parvoviridae
Poxviridae



Modified from Volk et al., Essentials of Medical Microbiology, 4th Ed. 1991


RNA VIRUSES
Single strand positive
Single strand negative
Double strand
Enveloped
Non-enveloped
Enveloped
Non-enveloped
Togaviridae
Coronaviridae
Retroviridae
Picornaviridae
Calciviridae
Orthomyxoviridae
Paramyxoviridae
Rhabdoviridae
Bunyaviridae
Arenaviridae
Filoviridae
Reoviridae



Modified from Volk et al., Essentials of Medical Microbiology, 4th Ed. 1991

STEPS IN VIRAL REPLICATION

1.Attachment & adsorption .

– specific binding of a Virion protein (the anti-receptor) to a constituent of the cell surface (the receptor) depend on the virus present and the availability of appropriate receptors host .

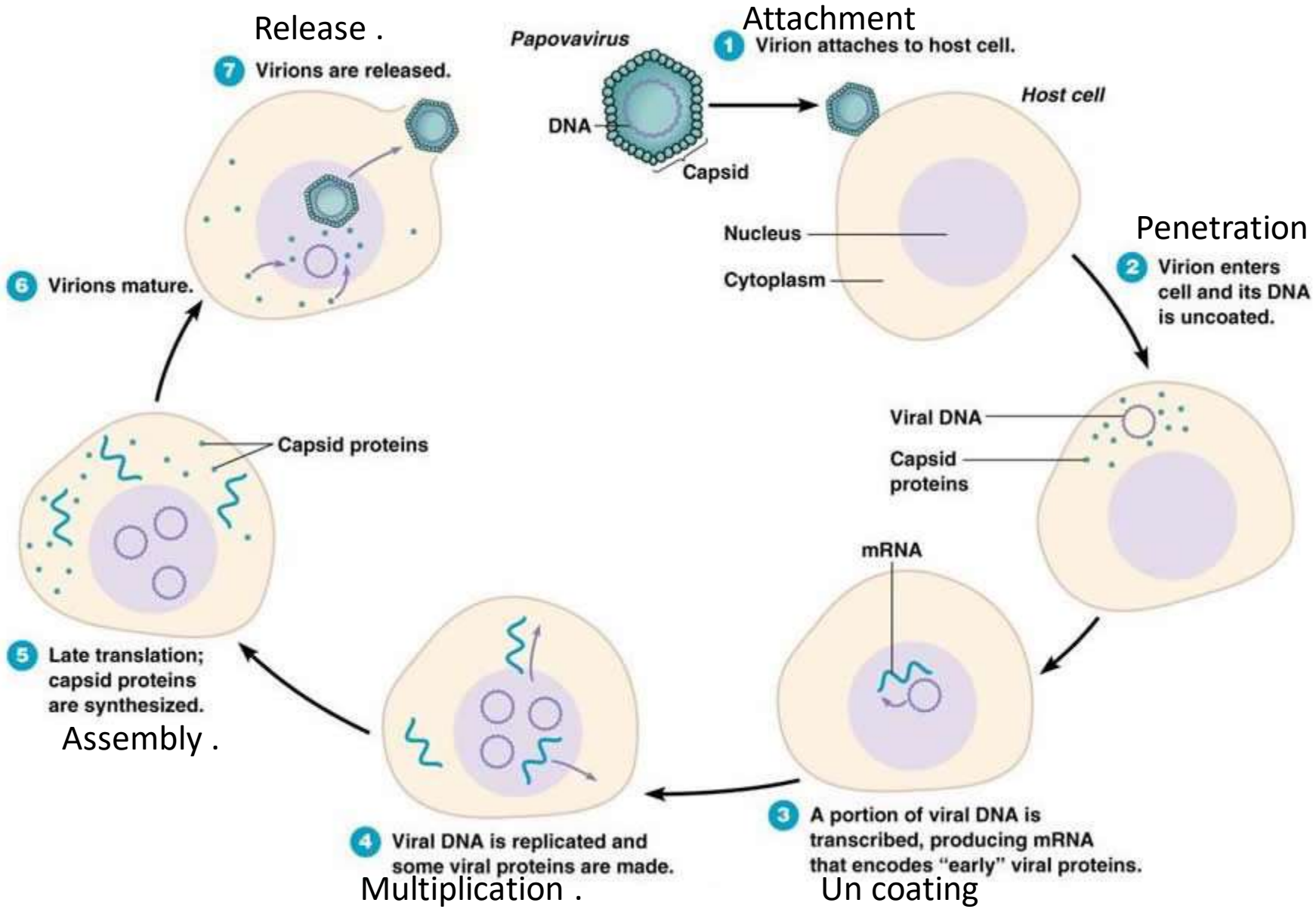
2-**Penetration** ; the virus taken up inside the cell membrane of the host is penetrated by the virus nucleic acid .Endocytosis entire virus engulfed by cell and enclosed in a vacuole .

3- **Un coating** ; is the physical separation of virus nucleic acid from structure virion .the genome may be releases as free nucleic acid .

4- **Multiplication ;**the specific mRNA must be transcribed from the virus nucleic acid for duplication of genetic information .some viruses carry RNA polymerase to synthesize mRNA .

5-Assembly ; Newly synthesized virus genomes and capsid polypeptides assemble together to from progeny viruses .

6-**Release** :non enveloped and complex viruses are released when the cell lysis . Enveloped viruses are liberated by budding or exocytosis .



Viral Infection of cell

Cultivation and identification of viruses

 What are viruses?

Viruses are infectious ,intracellular,obligate,parasites

 Methods to cultivate animal viruses:-

1) Embryonated Chick egg

2)Live animals

3)Tissue culture

 The primary purposes of viral cultivation are:

1. to isolate and identify viruses in clinical specimens
2. to prepare viruses for vaccines
3. to do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells.

METHODS OF CULTIVATION

- Various routes of inoculation

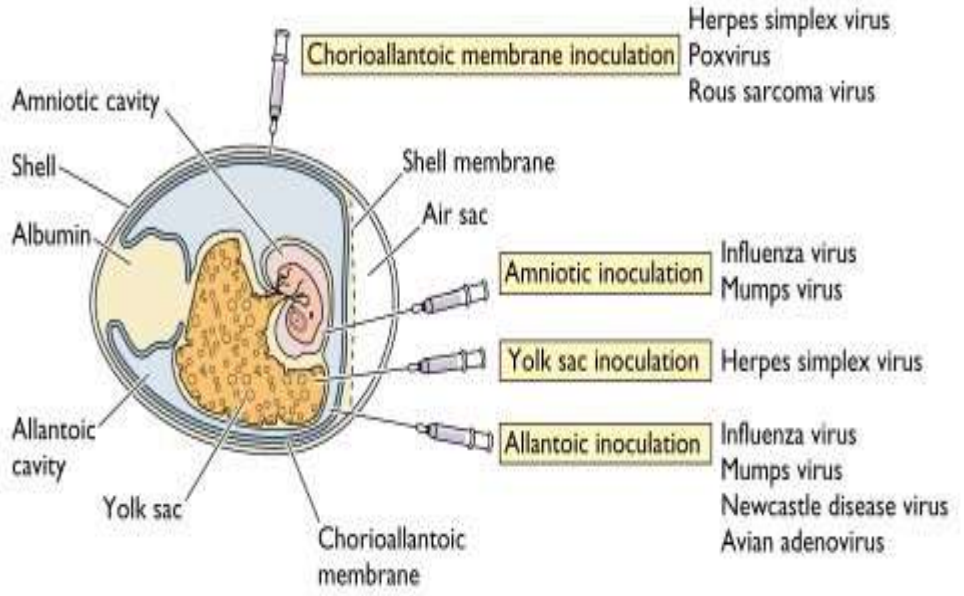
a)Yolk sac

b) Allantoic sac

c) Chorioallantoic membrane

d. Amniotic cavity

e. Intravenous



Isolation, Cultivation and Identification of animal viruses

1. In living animals

- using live animal eg.mice, rats, rabbits, guinea pigs, hamster, chickens, and monkey.
- the animal is exposed to the virus by injection of a viral preparation or specimen into the brain, blood, muscle, body cavity, skin, or footpads.
- use in example research to study the immune system's response to viral infections.
- HIV: immunodeficient mice grafted to produce human T cells and human gamma globulin. for examination with an electron microscope
- The signs of viral growth include death of the animal and defects in animal development. The infected animal tissue can be prepared for examination with an electron microscope .

2. In Embryonated egg

- use embryonated chicken, duck or turkey for inoculation of viral suspension .
- The signs of viral growth include death of the embryo, defects in embryonic development, and localized areas of damage in the membranes, resulting indiscrete, opaque spots called pocks (avariant of *pox*). The embryonic fluid and tissue can be prepared for examination with an electron microscope.
- Some can also be detected by their ability to agglutinate red blood cells or by their reaction with an antibody of known specificity that will affix to its corresponding virus, if it is present.

3. Using cell culture

- preferred type of growth medium for virus, more convenient than the previous two methods.
- use isolated cell from animal that are cultured *invitro*. Normal cells will form monolayer

present, the cells of monolayer will deteriorate as they multiply. Cell deterioration is called cytopathic effect (CPE).

CPE can be detected and counted = plaques by phages (plaque assay). Microscopic observation via electron microscope

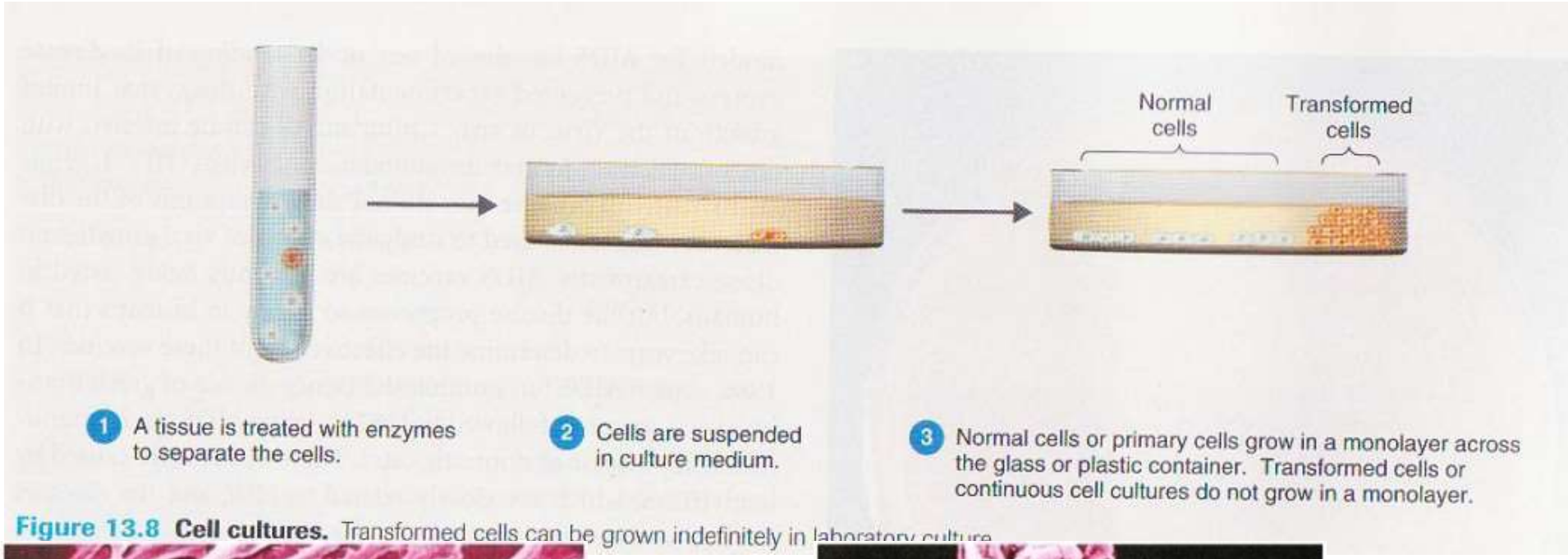
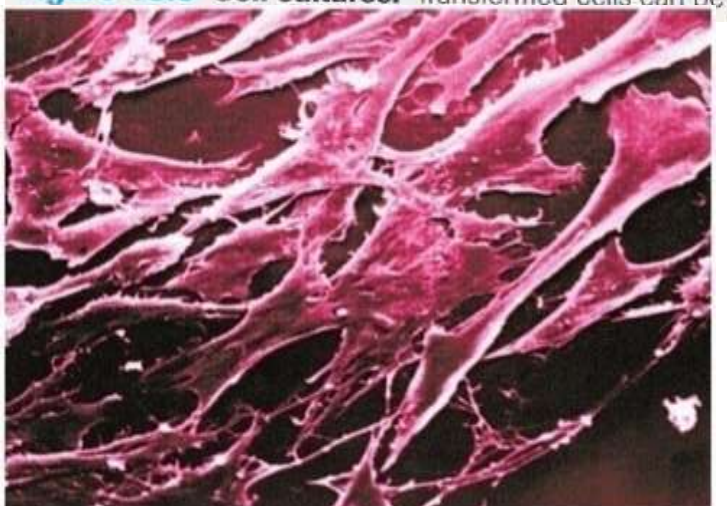
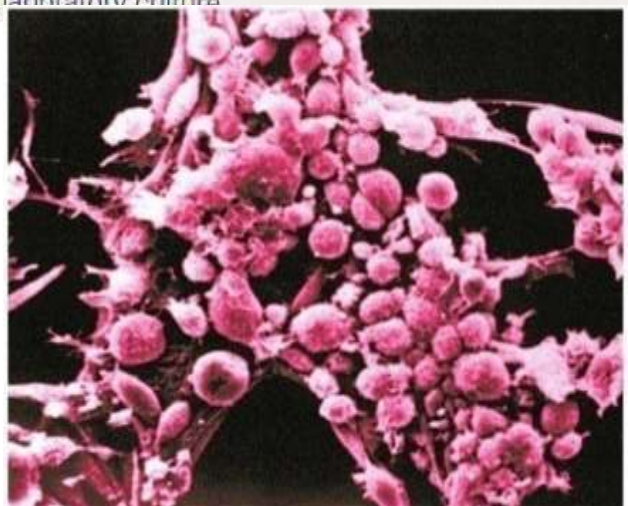


Figure 13.8 Cell cultures. Transformed cells can be grown indefinitely in laboratory culture



A. Normal



B. Transformed

ORTHOMYXOVIRUSES

Characteristics:

- Influenza A, B and C the only members

Segmented negative-sense RNA genome with eight nucleocapsid segments

Genetic instability responsible for annual epidemics (mutation:drift) and periodic pandemics (reassortment: shift)

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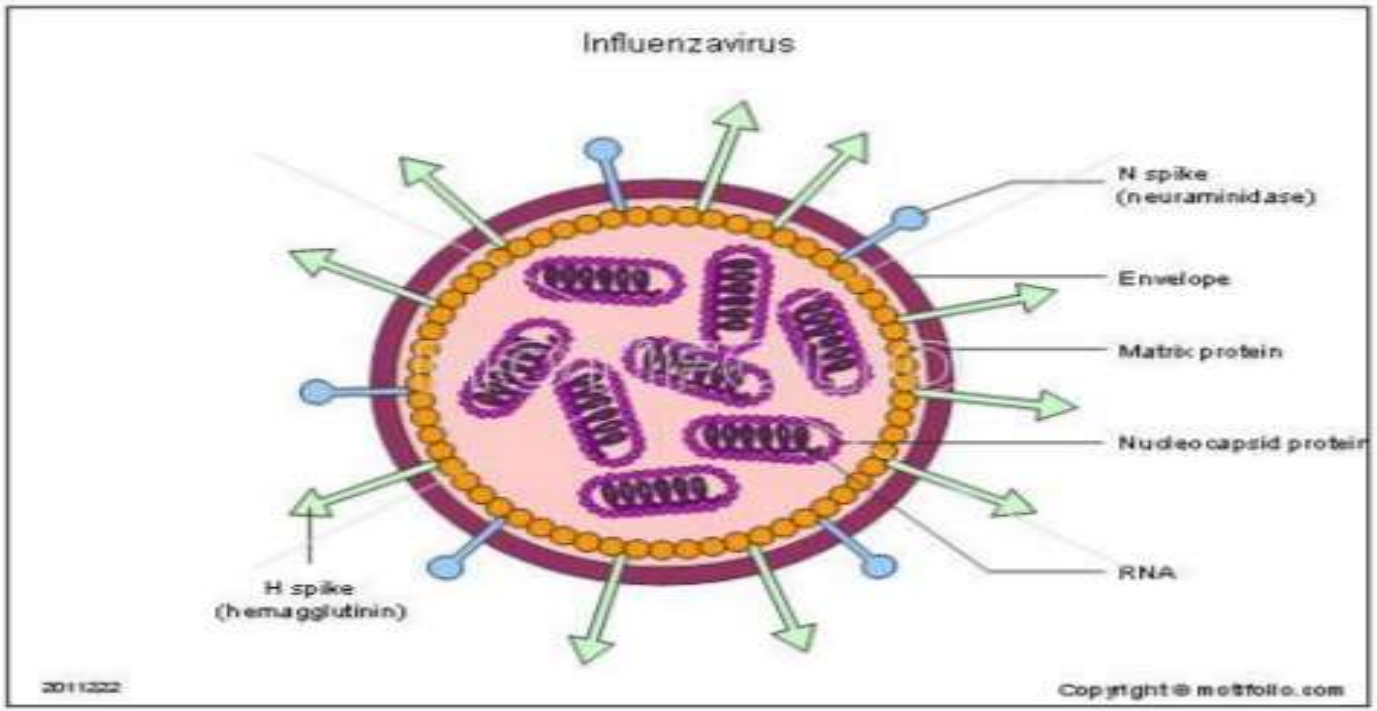
Characteristics



Structure & Replication:

Envelope with two group-specific glycoproteins:

1. Hemagglutinin (HA)

- **Functions:**
  - **a. Viral attachment protein – bind to sialic acid on**
    - **epithelial cell surface receptors**
  - **b. Promotes fusion of the envelope to the cell membrane**
  - **c. Hemagglutinates human, chicken and guinea pig rbc**
  - **d. Elicits protective neutralizing antibody response**

Envelope with two group-specific glycoproteins:

2. Neuraminidase (NA)

- **With enzyme activity**
- **Cleaves the sialic acid on glycoproteins, including the cell receptor → prevents clumping & facilitates release of virus from infected cells**
- **Target for two antiviral drugs: zanamivir (Relenza) and oseltamivir (Tamiflu)**

Structure & Replication:

Type-specific proteins: used to differentiate among influenza A, B, and C viruses

1. **Matrix protein (M_1)**
   - Viral structural protein
   - Interacts with nucleocapsid & envelope →promotes assembly
2. **Membrane protein (M_2)**
   - Forms membrane channel
   - Facilitates uncoating & HA production
   - Target for amantadine
3. **Nucleocapsid proteins (NP)**

Transcribes and replicates its genome in the target cell nucleus
Assembles and buds from the plasma membrane .
--Laboratory diagnosis : Detection of Antigen by ELISA .
--Isolation & identification of the virus : and inoculation in embryonated eggs & monkey kidney cells .
--Identified by CF ,FAT , Serology :HI, CF,ELISA .

Pathogenesis & Immunity:

- Virus first targets & kills mucus-secreting, ciliated, and other epithelial cells →loss of primary defense system

--Cleavage of sialic acid residues of mucus by NA →provide access to tissues

--Preferential release of the virus at the apical surface of epithelial cells and into the lungs →promote cell-to-cell spread & transmission to other hosts

--Spread to lower respiratory tract →shedding of bronchial or alveolar epithelium

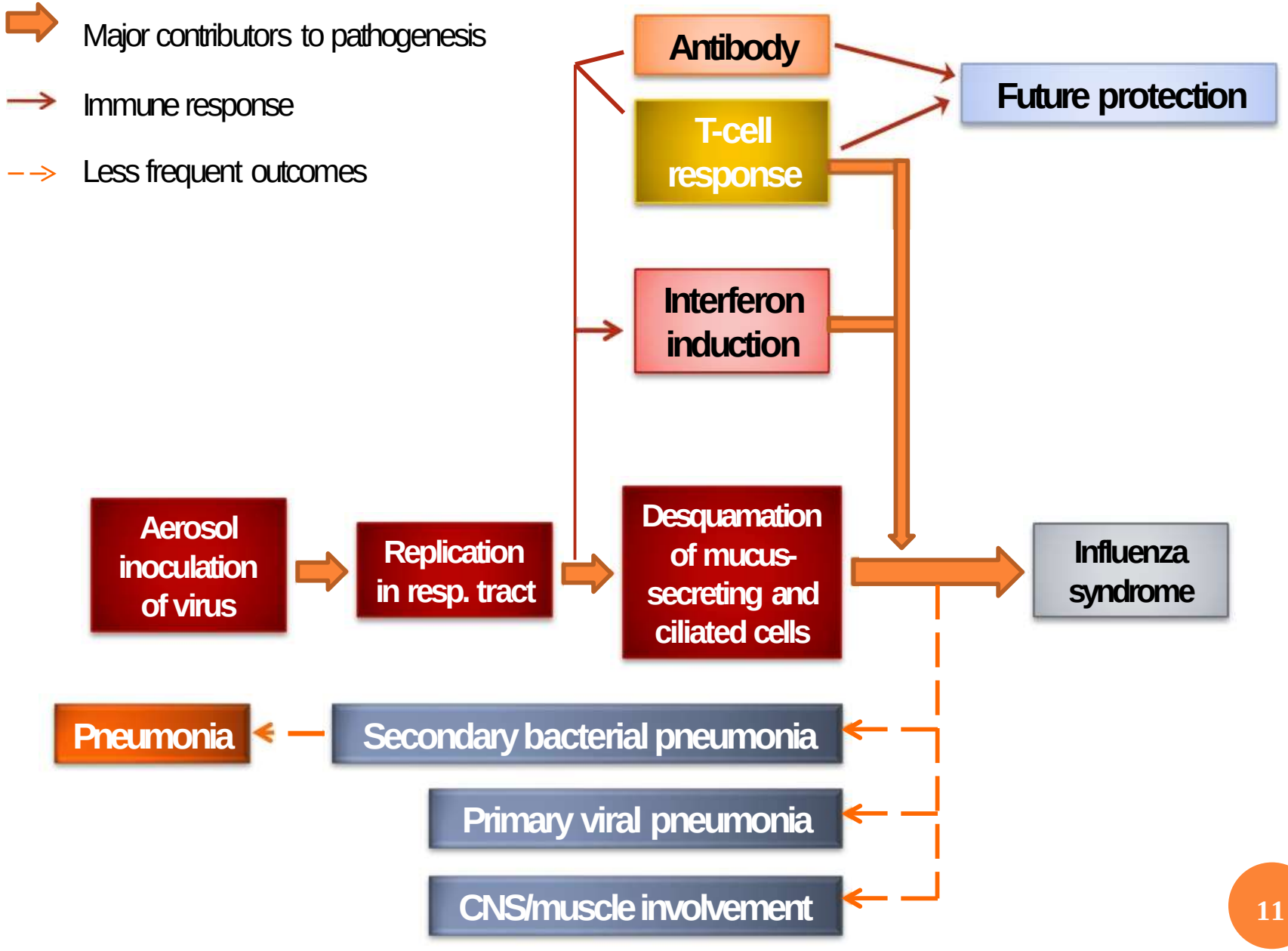
--Promotes bacterial adhesion to the epithelial cells →pneumonia

--Histologic: inflammatory response of mucosal membrane (primarily monocytes & lymphocytes) with submucosal edema

--Systemic symptoms due to the interferon and lymphokine response to the virus

- --Local symptoms due to epithelial cell damage

--Interferon & CMI responses (NK & T cell) important for immune resolution and immunopathogenesis →classic symptoms associated with interferon induction .


Major contributors to pathogenesis
Immune response
Less frequent outcomes
Antibody
T-cell response
Future protection
Interferon induction
Aerosol inoculation of virus
Replication in resp. tract
Desquamation of mucus-secreting and ciliated cells
Influenza syndrome
Pneumonia
Secondary bacterial pneumonia
Primary viral pneumonia
CNS/muscle involvement

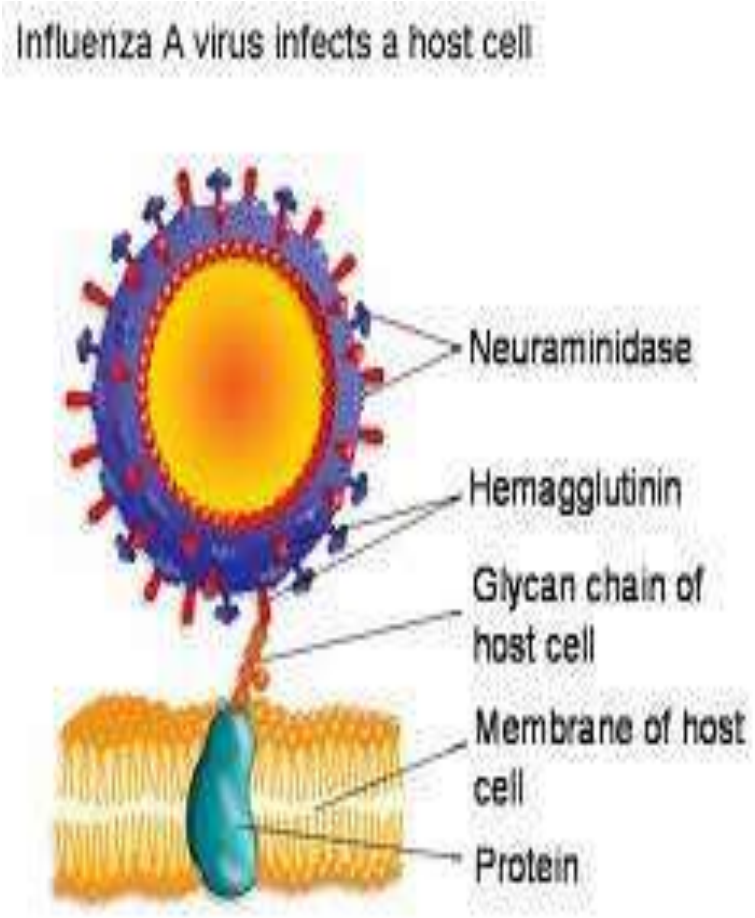
Why is influenza difficult to control even when there is vaccination available?

INFLUENZA A

- Influenza A
- Type A viruses are divided into types based on differences in two viral surface proteins called the **hemagglutinin (H)** and the **neuraminidase (N)**.
- Influenza type A viruses undergo two kinds of changes:
- **Antigenic drift.**
- **Antigenic shift.**

INFLUENZA B

- Influenza type B viruses change only by the more gradual process of antigenic drift.



INFLUENZA C

- Type C infection usually causes either a very mild respiratory illness or no symptoms at all;

Antigenic Changes:

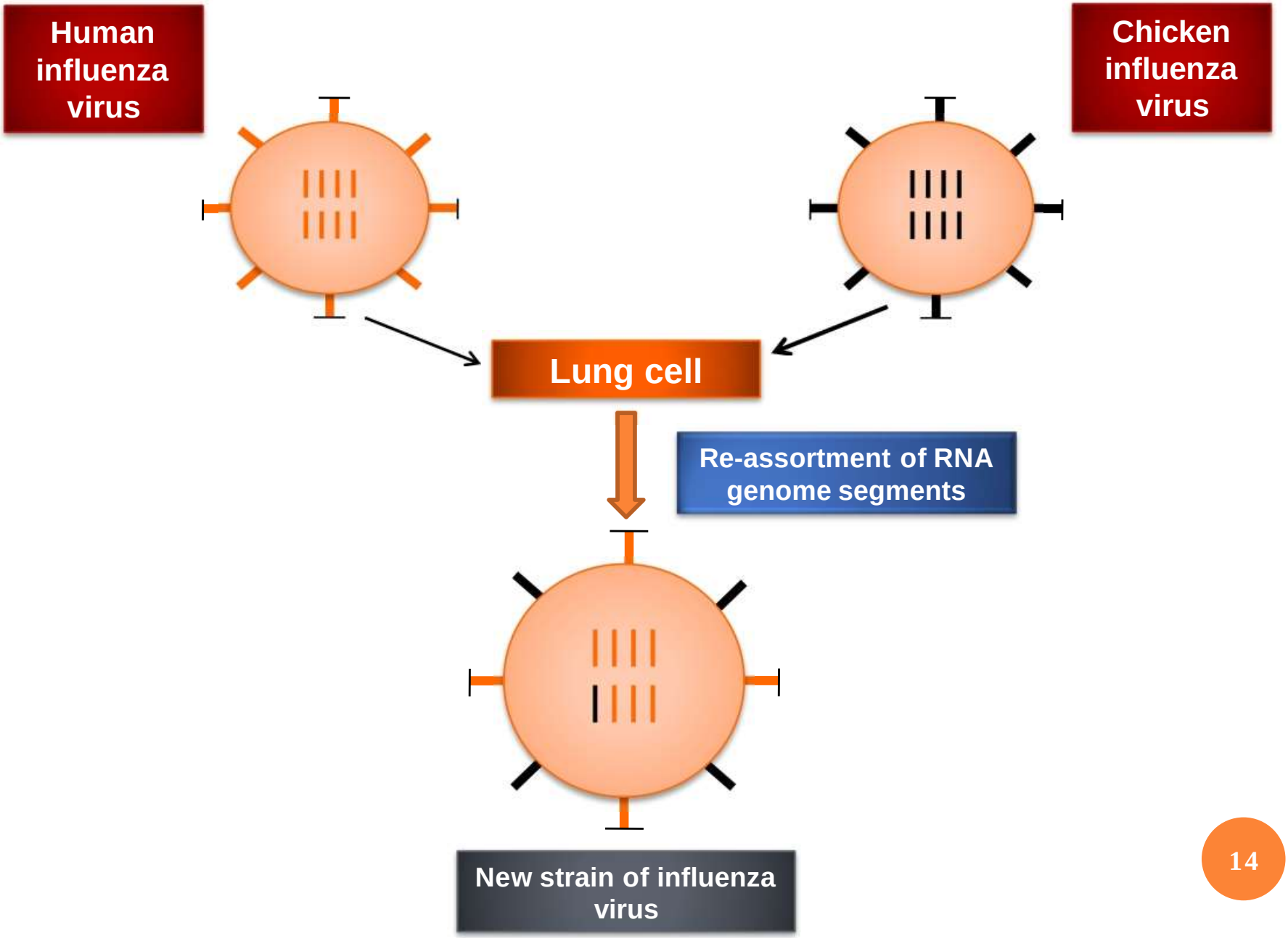
1. **Antigenic drift**
   - Minor change
   - Mutation of the HA and NA genes
   - Occurs every 2 to 3 years
   - Cause local outbreaks of influenza A & B
2. **Antigenic shift**
   - Major change

Result from re-assortment of genomes among different strains, including animal strains

- Associated with pandemics
- Qccurs only with influenza A

How is the virus transmitted?

- Virus is spread by inhalation of aerosol droplets expelled during talking, breathing, and coughing.
- Virus likes cool, less humid atmosphere
- Virus is extensively spread by school children.


Human influenza virus
Chicken influenza virus
Lung cell
Re-assortment of RNA genome segments
New strain of influenza virus

Who is at risk?

Seronegative people.

Adults: classic "flu" syndrome

Children: asymptomatic to severe respiratory tract infection

High-risk Groups:

- ✓ Elderly
- ✓ Immunocompromised people
- ✓ People with underlying cardiac or respiratory problems (including people with asthma and smokers)

complications

Diseases Associated with Influenza Virus Infections

Symptoms	Disorder
Acute infection in adults	Rapid onset of fever, malaise, myalgia, sore throat, and non-productive cough
Acute infection in children	Acute disease similar to that in adults but with higher fever, gastrointestinal tract symptoms (abdominal pain, vomiting), otitis media, myositis, and more frequent croup
Complications	Primary viral pneumonia Secondary bacterial pneumonia Myositis & cardiac involvement Neurologic syndromes: Guillain-Barre syndrome Encephalopathy Encephalitis Reye's syndrome

Laboratory Diagnosis of Influenza Virus Infection

Test	Detects
Cell culture	Presence of virus, limited cytopathologic effects
Hemadsorption to infected cells	Presence of HA protein on cell surface
Hemagglutination	Presence of virus in secretions
Hemagglutination inhibition	Type and strain of influenza virus or specificity of antibody
Antibody inhibition of hemadsorption	Identification of influenza type and strain
Immunofluorescence, ELISA	Influenza virus antigens in respiratory secretions or tissue culture
Serology: HI, headsorption inhibition, ELISA, immunofluorescence, complement fixation	Seroepidemiology

Paramyxovirus

1 Measles,
2 Mumps,
3 Respiratory Syncytial viruses.
4 Para inluenza viruses.

- **Structure**

– Negative sense ssRNA genome, helical nucleocapsid, envelope with attachment protein and F protein

- **Pathogenesis**

– Transmission in respiratory droplets and fusion of virus envelope via F protein with plasma membrane of cells in the respiratory tract
– Replication in cytoplasm, budding
– Viremia except for RSV and PIV
– Innate and antibody response important; many symptoms from immune response: rash in measles and swelling in mumps; PIV bronchitis and croup; RSV bronchiolitis and pneumonia in infants
– Squealed in CNS for measles and mumps

- **Diagnosis**

– Serology or nucleic acid
– Measles: Koplik spots; mumps: swelling of parotid gland

PARAMYXOVIRUSES

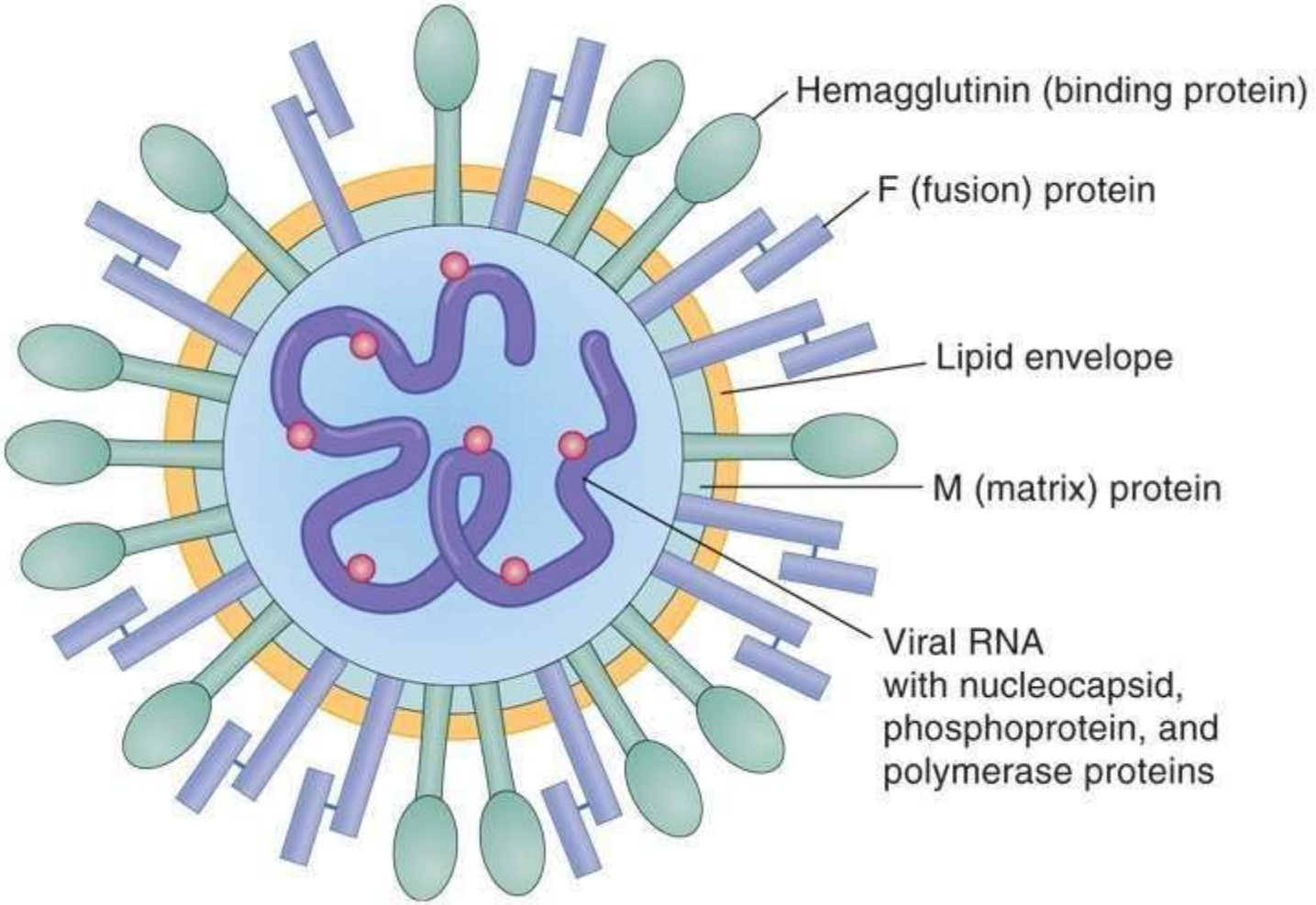
Properties of Orthomyxoviruses and Paramyxoviruses

Property	Orthomyxoviruses	Paramyxoviruses
Viruses	Influenza A, B, and C	Measles, mumps, RSV, and parainfluenza viruses
Genome	Segmented (8 pieces) ssRNA of negative polarity	Non-segmented ssRNA of negative polarity
Virion RNA polymerase	Yes	Yes
Capsid	Helical	Helical
Envelope	Yes	Yes
Size	Smaller (110 nm)	Larger (150 nm)
Surface spikes	HA and NA on different spikes	Hemagglutinin & neuraminidase on same spikes
Giant cell formation	No	Yes

Unique Features of the Paramyxoviridae

- Large virion with helical nucleocapsid
- Negative RNA genome
- Envelope containing viral attachment protein (HN, paramyxovirus and mumps virus; H, measles virus, and G, RSV) and a fusion protein (F)
  - HN with hemagglutinin & neuraminidase activity
  - H with hemagglutinin activity
  - G without hemagglutinin or neuraminidase acvitity
- Replicates in cytoplasm
- Penetrate the cell by fusion with and exit by
- Inducing cell-to-cell fusion → multinucleated giant cells

Paramyxoviridae structure



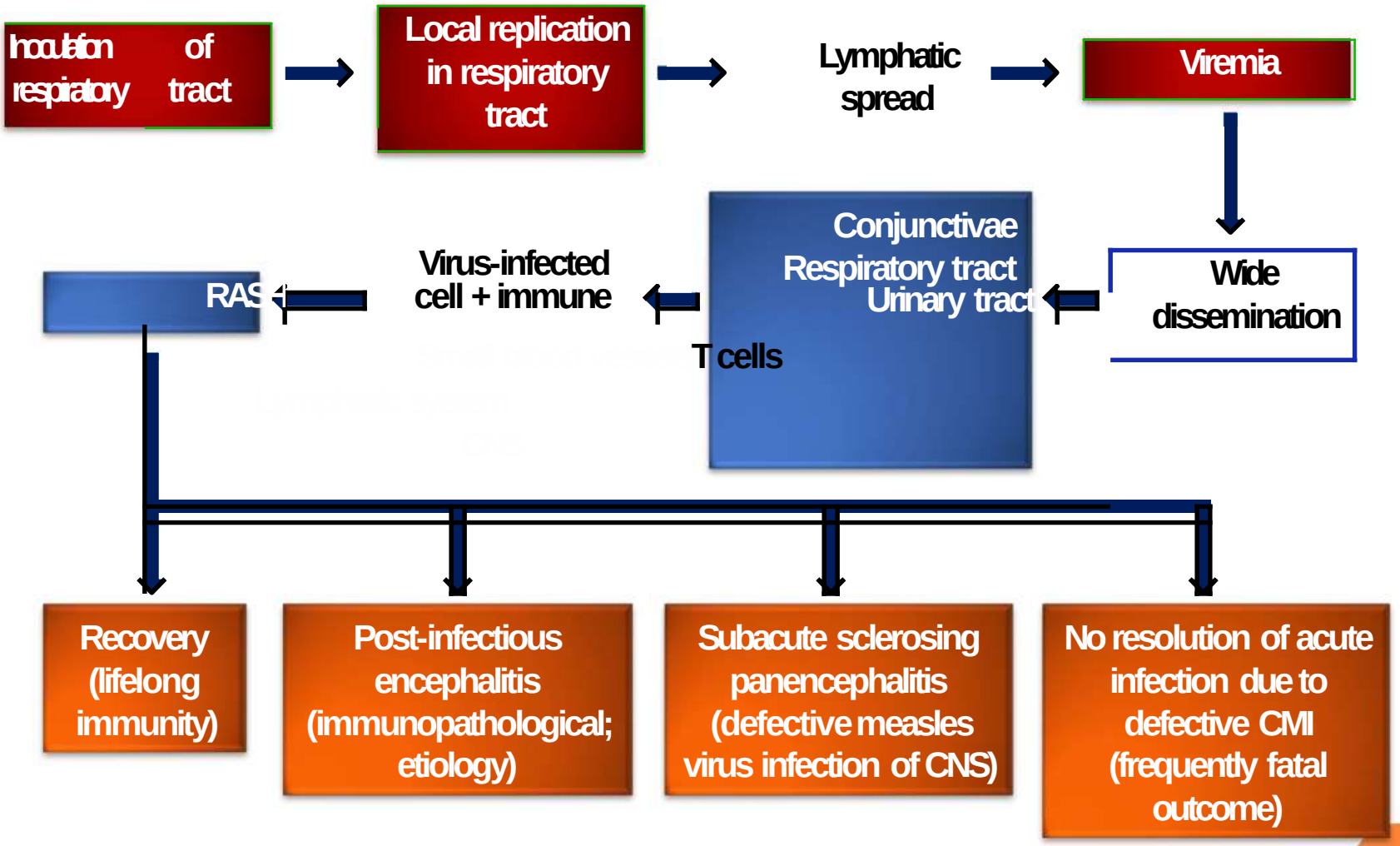
Transmission:

- Inhalation of large-droplet aerosols

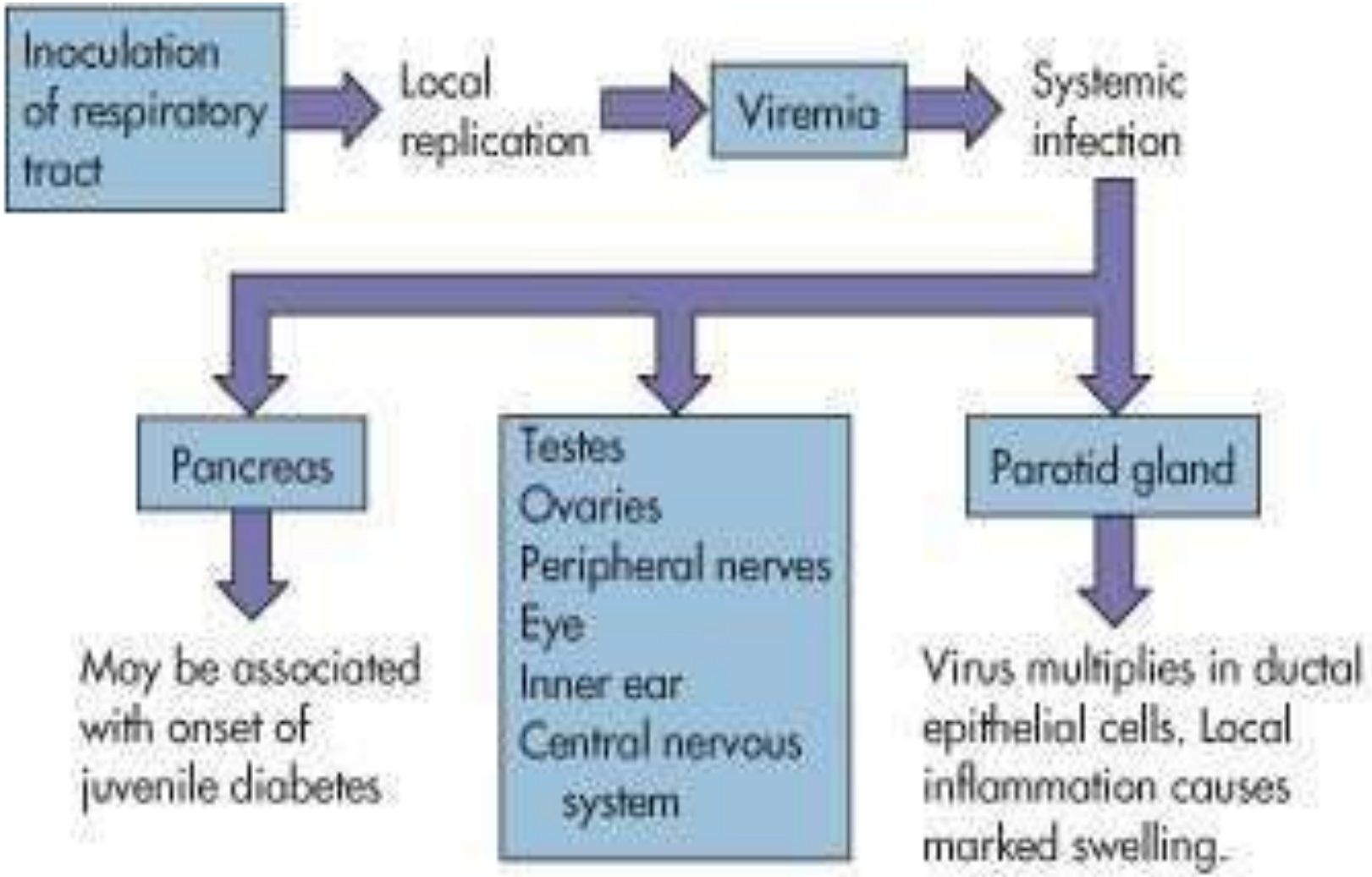
Disease Mechanisms:

- Infect epithelial cells of respiratory tract
- Spread systemically in lymphocytes and by viremia
- Replicate in cells of conjunctivae, respiratory tract, lymphatic system, blood vessels, and CNS
- Characteristicrashcausedbyimmune T cellstargetedtomeasles-infected endothelialcellslinings mallblood vessels

Mechanisms of spread and pathogenesis of measles



Mumps pathogenesis



Measles

Laboratory Diagnosis

Antigen

- Immunofluorescence

Cytopathologic effects

- Multinucleated Giant Cells with cytoplasmic inclusion bodies

Isolation and Identification of Virus

- Monkey or Human Kidney Cells
- Lymphoblastic cell line (B95-a)

Serology

- ELISA
- HI

– Virus culture
– Syncytia formation
– Hemadsorption
– Hemagglutination inhibition
– rtPCR

Transmission

- Airborne
- Direct contact with infected nasal or throat secretions.

Symptoms

•Fever
•Muscle Pain
•Rash (itchy, red areas that spread together)
•Redness and/or irritation of the eyes
•Sore throat
•White spots in the mouth (Koplik's spots

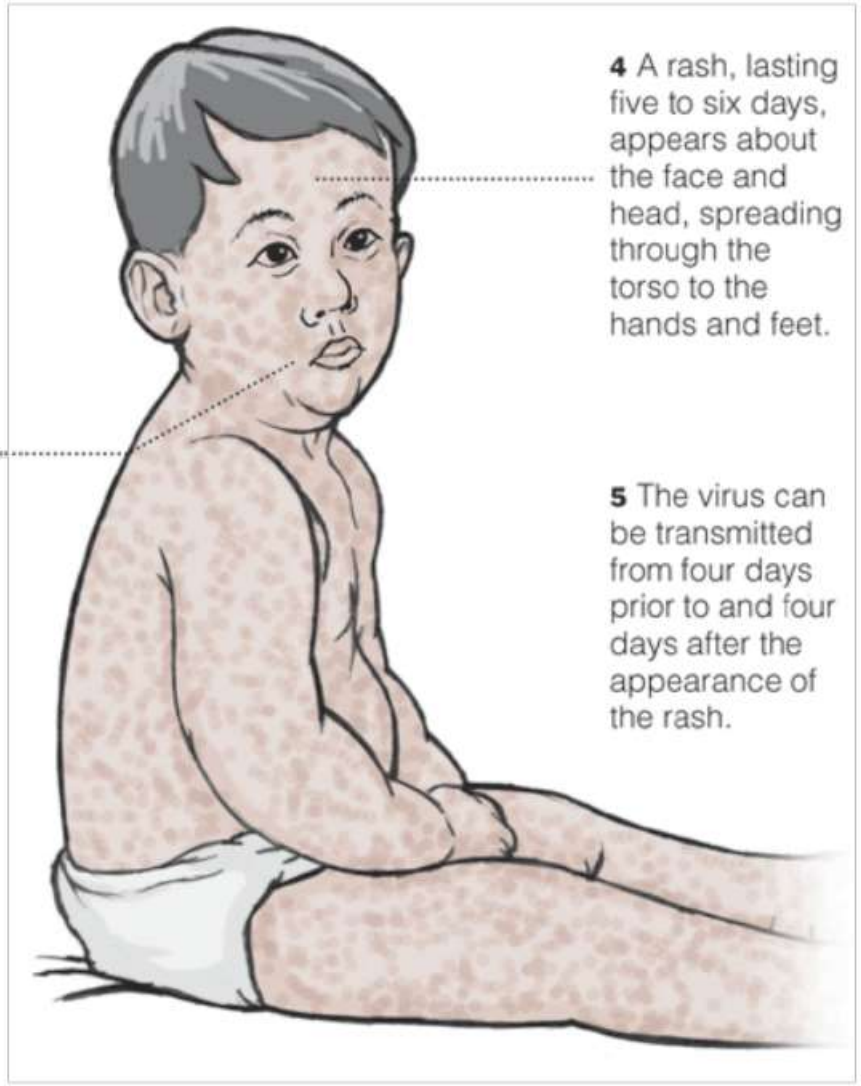
- **Incubation period: 7 to 13 days**
- **Prodrome: high fever + 3C's + P →most infectious**
- **Koplik's spots after 2 days of illness →last 24 to 48 hours**
- **Appearance of exanthem within 12 to 24 hours of the appearance of Koplik's spots**
- **Rashes undergo brawny desquamation**

Characteristics of Measles

1 The virus is spread by breathing in virus-containing droplets or by touching contaminated surfaces.

2 The virus grows in cells in the back of the throat and lungs. Symptoms appear after 10 to 12 days.

3 Infected person has a fever lasting two to four days, followed by a cough, runny nose and red, watery eyes.



4 A rash, lasting five to six days, appears about the face and head, spreading through the torso to the hands and feet.

5 The virus can be transmitted from four days prior to and four days after the appearance of the rash.

Sources: Centers for Disease Control; World Health Organization

The New York Times

Post-exposure: Immune serum globulin given within six days of exposure

Pre-exposure:

1. Live, attenuated vaccine
2. MMR
   - **Composition:**
     a. Measles – Schwartz or Moraten substrains of Edmonton B strain
     b. Mumps – Jeryl Lynn strain
     c. Rubella – RA/27-3 strain
   - **Schedule:** at 15-24 months and at 4-6 years
   - **Efficacy:** 95% lifelong immunization with a single dose

ParainfluenzaViruses

Characteristics:

- Four serotypes

Infection limited to upper respiratory tract

✓ Upper respiratory tract disease most common, but significant disease can occur with lower respiratory tract infection

- Not systemic and do not cause viremia .

-- Infection induces protective immunity of short duration

Respiratory Syncytial Virus

--Most important cause of pneumonia and bronchiolitis in infants

--Fusion protein causes formation of multinucleated giant cells →syncytia

--Humans and chimpanzees are the natural hosts

- Two serotypes – subgroup A and B

--Infection in infants more severe and usually involves lower respiratory tract than in older children and adults

- No viremia occurs

--Severe disease in infants with immunopathogenic mechanism

- Maternal antibody passed to infant →react with the virus →form immune complexes →damage respiratory tract cells

Mumps Virus

Two types of envelope spikes:

1. **With both hemagglutinin and neuraminidase activities**
2. **With cell-fusing and hemolytic activities**

- **Only one serotype**

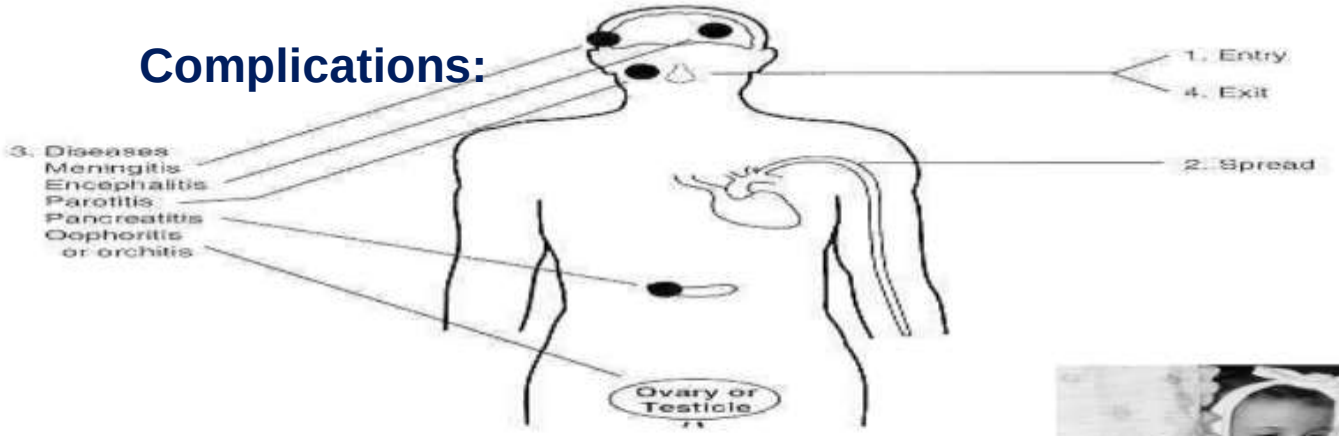
--Neutralizing antibodies directed against the hemagglutinin

- **Humans are natural hosts**

--Infects both upper and lower respiratory tracts →spread through blood →parotid glands, testes, ovaries, pancreas, and in some cases, meninges

Occurs only once →subsequent cases may be caused by parainfluenza viruses, bacteria, and by duct stones

Complications:



Pathogenicity of viral infection

Viral pathogenesis :The study of the Capability & manner of viruses to infect and cause disease.

Virulence **:** The degree to which a virus causes disease. Strains of virus differ greatly in their ability to cause disease

Mechanisms of Infection and Spread of Viruses through the Body

I. Routes of Entry

A. Respiratory Tract

B. Alimentary tract:

C. Skin, genital tract, and conjunctiva

Skin: Tough outer layer is nearly impenetrable - entry is through cuts, scrapes, bites (insect or animal), iatrogenic (human intervention - needles). Some viruses produce localized infection in skin (papilloma), but most move through the skin and into deeper layers and eventually into bloodstream (viremia).

Genital tract: genital tract is route of entry for important pathogens such as HSV, papilloma, HIV, HTLV, Hepatitis B and C. Sexual activity can cause minute drops in vagina and urethra, through which the virus may enter. Some virus stay local (papillomavirus), others spread systemically (HIV, Hep B and C, HTLV)

Eyes: Conjunctiva has protective mechanisms (lysozyme in tears, washing, eyelid wiping, etc) and is not usually a route of infection. A few viruses, however, can infect here. Usually the infectionis through a small tear in the conjunctiva and even then the infection is usually initiated by direct inoculationphysically touching something that has the virus on it).

II. Mechanisms of Spread in the Body

A. Viruses have the choice of setting up infection at the point they entered the body or entering the blood stream and setting up an infection at another point.

B. Local Spread on Epithelial Surfaces

In internal epithelium the surface is coated with water and this make spread much easier. Therefore these infections tend to have a shorter incubation time. In the case of influenza virus, paramyxoviruses, and rotavirus the epithelial tissue is infected but there is no invasion beyond this layer -- possibly because of lack of cellular receptors in the deeper tissue layers, or possibly due to higher temperatures inthe deeper cellular tissue. However, they can still be quite severe.

C. Subepithelial Invasion and Lymphatic Spread

D. Primary and Secondary Viremia

First entry of virus into the bloodstream is primary viremia (can be active or passive). This viremia may be subclinical and is the route by which viruses get to their sites of infection. After the infection occurs, then a secondary viremia can take place because of shedding of virus from the infected organ. This secodary viremia can then be the cause of infection at yet another site of the body. Viruses may circulate freely in the blood (hepadnaviruses, togaviruses, flaviviruses, and enteroviruses), or they may associate with leukocytes (WBC), platelets, or erythrocytes and be harbored by them (HIV, Rift Valley Fever, Colorado tick fever). The latter viral infections are more difficult to clear and tend to be more persistent infections.

E. Secondary sites of infection.

- **Skin** - this usually results in some sort of rash made up of macules, papules, vesicles or pustules.
- **CNS** - spread is usually from blood vessels in meninges and infection of neurons in cerebrospinal fluid, or directly from blood vessels of the brain and spinal cord.

- Another important route is travel of virus up neurons (rabies, varicella, herpes simplex)
- Meningitis is infection of lining of brain and CNS (meninges)
- Encephalitis is infection of brain
- Other organs - liver (hepatitis), heart (carditis), lungs (pneumonia), salivary glands (mumps), testes (orchitis)

III. Virus Shedding

Necessary for maintenance of infection in poulation.

A. Repiratory and oropharyngeal secreations: mucus or saliva from coughing sneezing and talking - measles. Chickenpox, rubella. Direct transmission of saliva or mucus - herpesviruses, CMV, EBV

B. Feces : Enteric viruses - can often persist for longer periods of time (nonenveloped)

C. Skin : Direct contact needed for transmission - molluscum contagiosum, warts, genital herpes, and poxviruses.

D. Urine : Viruria is principal mode of shedding in arenavirus infections of rodents. Mumps virus and CMV in humans

E. Milk : CMV in mother's milk

F. Genital secretions : HIV, HSV I, HSV II, papillomaviruses, hepatitis B and C, HTLV

G. Blood and body fluids : Heptitis B, C, D, HIV, HTLV. Luckily some of the more fatal hemorrhagic fevers can only be transmitted this way.

Pathogenic Properties of Viruses

1. Viruses avoid the host's immune response by growing inside cells.
2. Viruses gain access to host cells because they have attachment sites for receptors on the host cell.
3. Visible signs of viral infections are called cytopathic effects (CPE).
4. Some viruses cause cytocidal effects (cell death), and others cause noncytocidal effects (damage but not death).
5. Cytopathic effects include the stopping of mitosis, lysis, and the formation of inclusion bodies, cell fusion, antigenic changes, chromosomal changes, and transformation.

Control of Viral Infections and Diseases

1- Immunoprophylaxis : Immunoprophylaxis against viral illnesses includes the use of vaccines or antibody-containing preparations to provide immune protection against a specific disease.

2- Active Prophylaxis (Vaccines): Active immunization involves administering a virus preparation that stimulates the body's immune system to produce its own specific immunity.

Viral vaccines now available for use include the following types:

following types:

(1) Attenuated live viruses.

(2) Killed viruses.

(3) Recombinant produced antigens. A vaccinee is a person who has been vaccinated**.**

3- Immune Response to Vaccines: Vaccination evokes an antibody response and stimulates T lymphocytes. Vaccine effectiveness is assessed in terms of percentage of recipients protected and the duration and degree of protection.
Most effective viral vaccines protect more than 90 percent of recipients and produce fairly durable immunity.

4- Passive Prophylaxis : Passive immunity is conferred by administering antibodies formed in another host. Human immunoglobulins remain a mainstay of passive prophylaxis (and occasionally therapy) for viral illnesses; they are usually used to protect individuals who have been exposed to a disease and cannot be protected by vaccination.

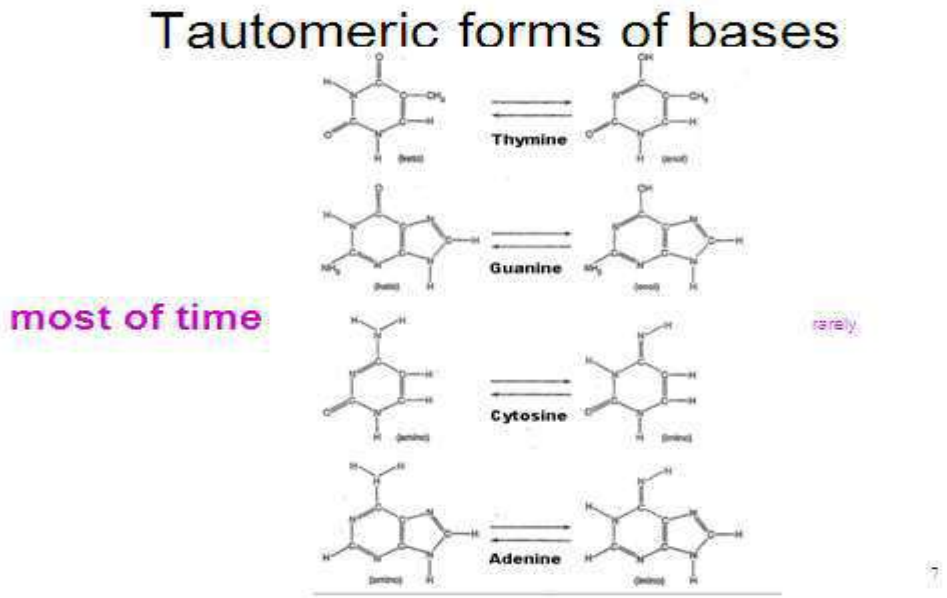
Sanitation and Vector Control

Many viral diseases are controlled by reducing exposure to the virus by:
(1) Eliminating nonhuman reservoirs.
(2) Eliminating the vector .
(3) Improving sanitation.

Antiviral Chemotherapy : There are three types of antiviral agents:
(1) Virucidal agents, which directly inactivate viruses.
(2) Antiviral agents, which inhibit viral replication.
(3) Immunomodulators, which boost the host immune response.

VIRAL GENETICS
MUTANTS
Spontaneous mutations
– tautomeric form of bases
– polymerase errors

These arise naturally during viral replication: e.g. due to errors by the genome replicating polymerase or a a result of the incorporation of tautomeric forms of the bases .



DNA viruses tend to more genetically stable than RNA viruses. There are error correction mechanisms in the host cell for DNA repair, but probably not for RNA.

Some RNA viruses are remarkably invariant in nature. Probably these viruses have the same high mutation rate as other RNA viruses, but are so precisely adapted for transmission and replication that fairly minor changes result in failure to compete successfully with parental (wild-type, wt) virus.

Mutations that are induced by physical or chemical means

Chemical: Agents acting directly on bases, e.g. nitrous acid
Agents acting indirectly, e.g. base analogs which mispair more frequently than normal bases thus generating mutations
Physical: Agents such as UV light or X-rays

Types of mutation

a) Point mutation
b) Insertion mutation
c) Deletion mutation

Examples of the kinds of phenotypic changes seen in virus mutants

2- **Temperature sensitive** (TS) mutants - These will grow at low temperature e.g. 31 degrees C but not at e.g. 39 degrees C, wild type grows at 31 and 39 degrees C. It appears that the reason for this is often that the altered protein cannot maintain a functional conformation at the elevated temperature.

2- **host range** - These mutants will only grow in a subset of the cell types in which the wild type virus will grow - such mutants provide a means toinvestigate the role of the host cell in viral infection .

3- Plaque size: Plaques may be larger or smaller than in the wild type virus, sometimes such mutants show altered pathogenicity.

4- Drug resistance: This is important in the development of antiviral agents - the possibility of drug resistant mutants arising must always be considered.

5- Enzyme-deficient mutants: Some viral enzymes are not always essential and so we can isolate viable enzyme-deficient mutants; e.g. herpes simplex virus thymidine kinase is usually not required in tissue culture but it is important in infection of neuronal cells

6- **"Hot" mutants:** These grow better at elevated temperatures than the wild type virus. They may be more virulent since host fever may have little effect on the mutants but may slow down the replication of wild type virions.

7- **Attenuated mutants:** Many viral mutants cause much milder symptoms (or no symptoms) compared to the parental virus - these are said to be attenuated. These have a potential role in vaccine development and they also are useful tools in determining why the parental virus is harmful .

EXCHANGE OF GENETIC MATERIAL

Recombination: Exchange of genetic information between two genomes.

"Classic" recombination: This involves breaking of covalent bonds within the nucleic acid, exchange of genetic information, and reforming of covalent bonds. This kind of break/join recombination is common in DNA viruses or those RNA viruses which have a DNA phase (retroviruses). The host cell has recombination systems for DNA.

Recombination of this type is very rare in RNA viruses (there are probably no host enzymes for RNA recombination). Picornaviruses show a form of very low efficiency recombination.

The mechanism is not identical to the standard DNA mechanism, and is probably a "copy choice“ kind of mechanism (figure 1) in which the polymerase switches templates while copying the RNA. Recombination is also common in the coronaviruses - again the mechanism is different from the situation with DNA and probably is a consequence of the unusual way in which RNA is synthesized in this virus.

So far, there is no evidence for recombination in the negative stranded RNA viruses giving rise to viable viruses (In these viruses, the genomic RNA is packaged in nucleocapsids and is not readily available for base pairing) .

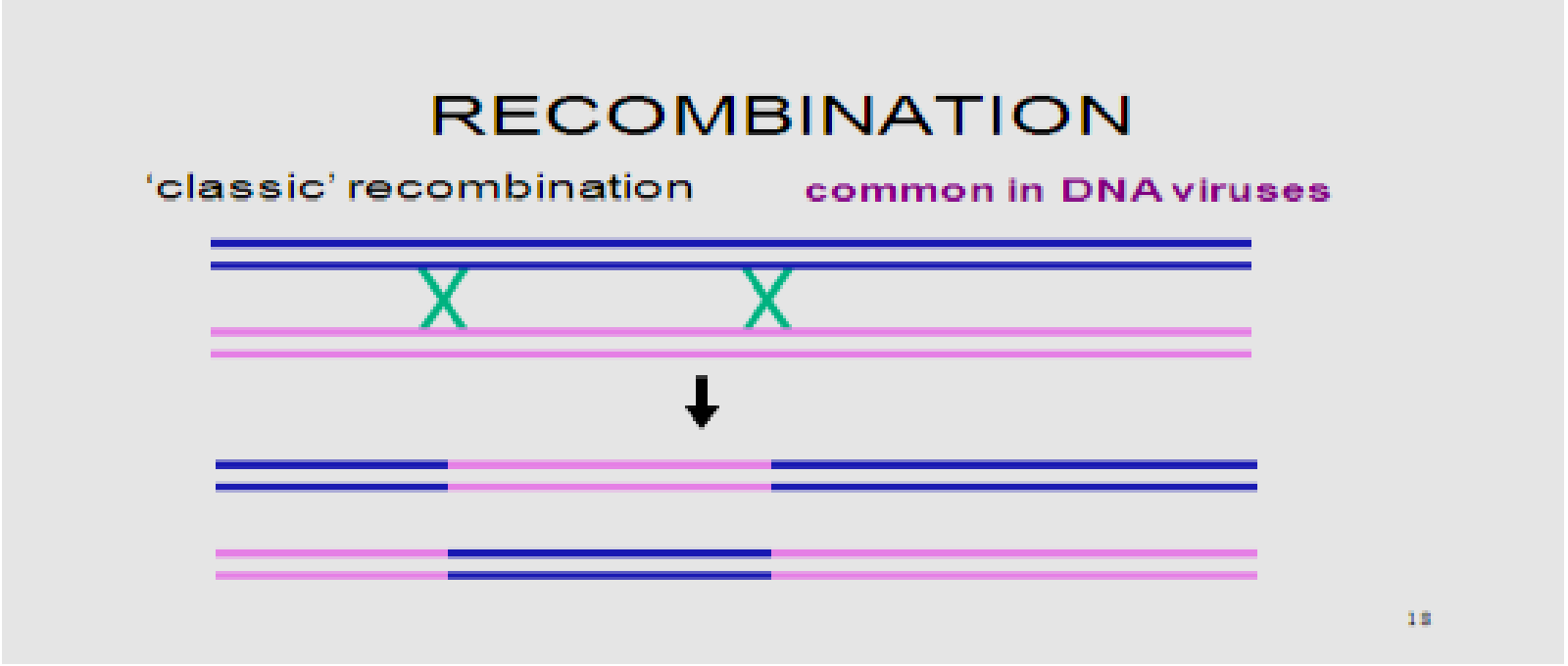


Figure 1: Copy choice recombination

Various uses for recombination techniques

a) Mapping genomes (the further apart two genes are, the more likely it is that there will be a recombination event between them).

b) Marker rescue - DNA fragments from wild type virus can recombine with mutant virus to generate wild type virus - this provides a means to assign a gene function to a particular region of the genome. This also provides a means to insert foreign material into a gene (figure 2).

Recombination enables a virus to pick up genetic information from viruses of the same type and occasionally from unrelated viruses or even the host genome (as occurs in some retroviruses).

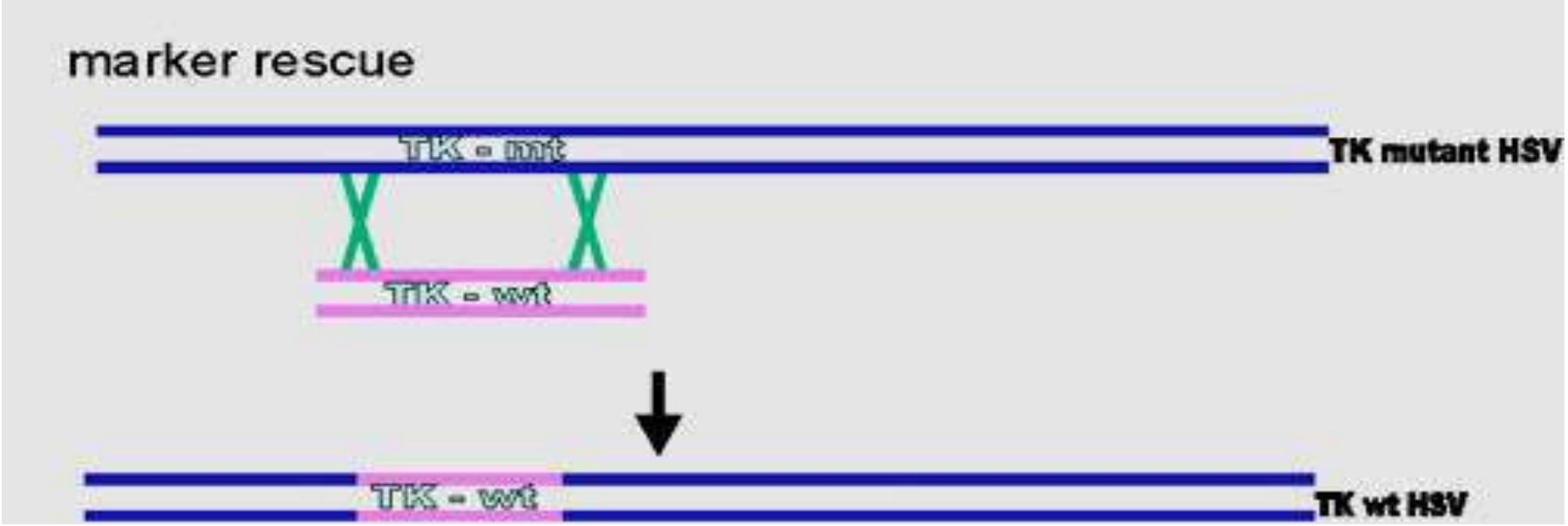


Figure2: Marker rescue

Reassortments

- Form of recombination (non classical)
- Very efficient
- Segmented viruses only
- Can occur naturally
- Used in some new vaccines : e.g for influenza and rotaviruses

If a virus has a segmented genome and if two variants of that virus infect a single cell, progeny virions can result with some segments from one parent, some from the other.

This is an efficient process - but is limited to viruses with segmented genomes - so far the only human viruses characterized with segmented genomes are RNA viruses e.g. orthomyxoviruses, reoviruses, arenaviruses, bunya viruses.

Reassortment may play an important role in nature in generating novel reassortants and has also been useful in laboratory experiments (figure 3,4). It has also been exploited in assigning functions to different segments of the genome. For example, in a reassorted virus if one segment comes from virus A and the rest from virus B, we can see which properties resemble virus A and which virus B. Reassortment is a non-classical kind of recombination

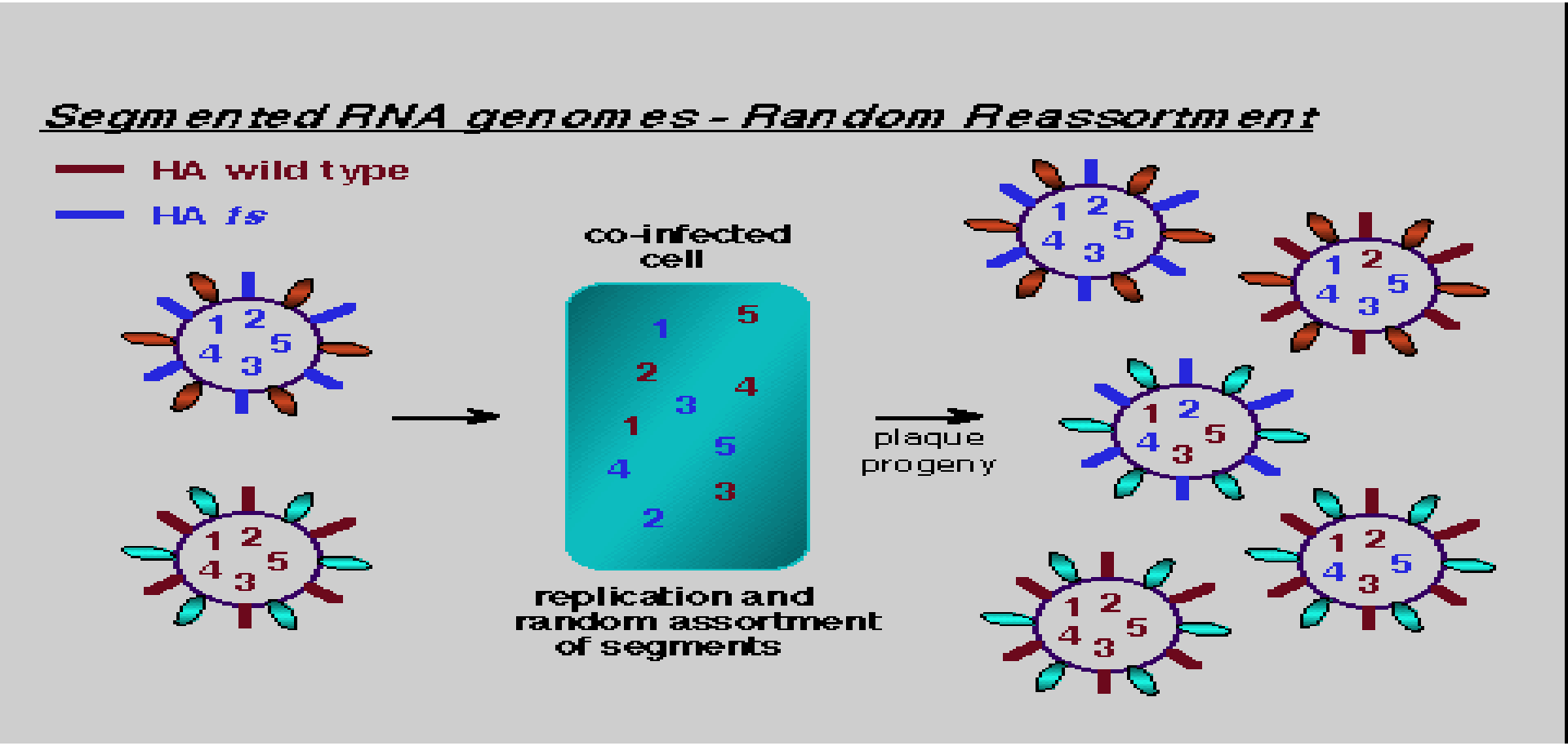


Figure 3: Reassortment of viral genome in segmented virus

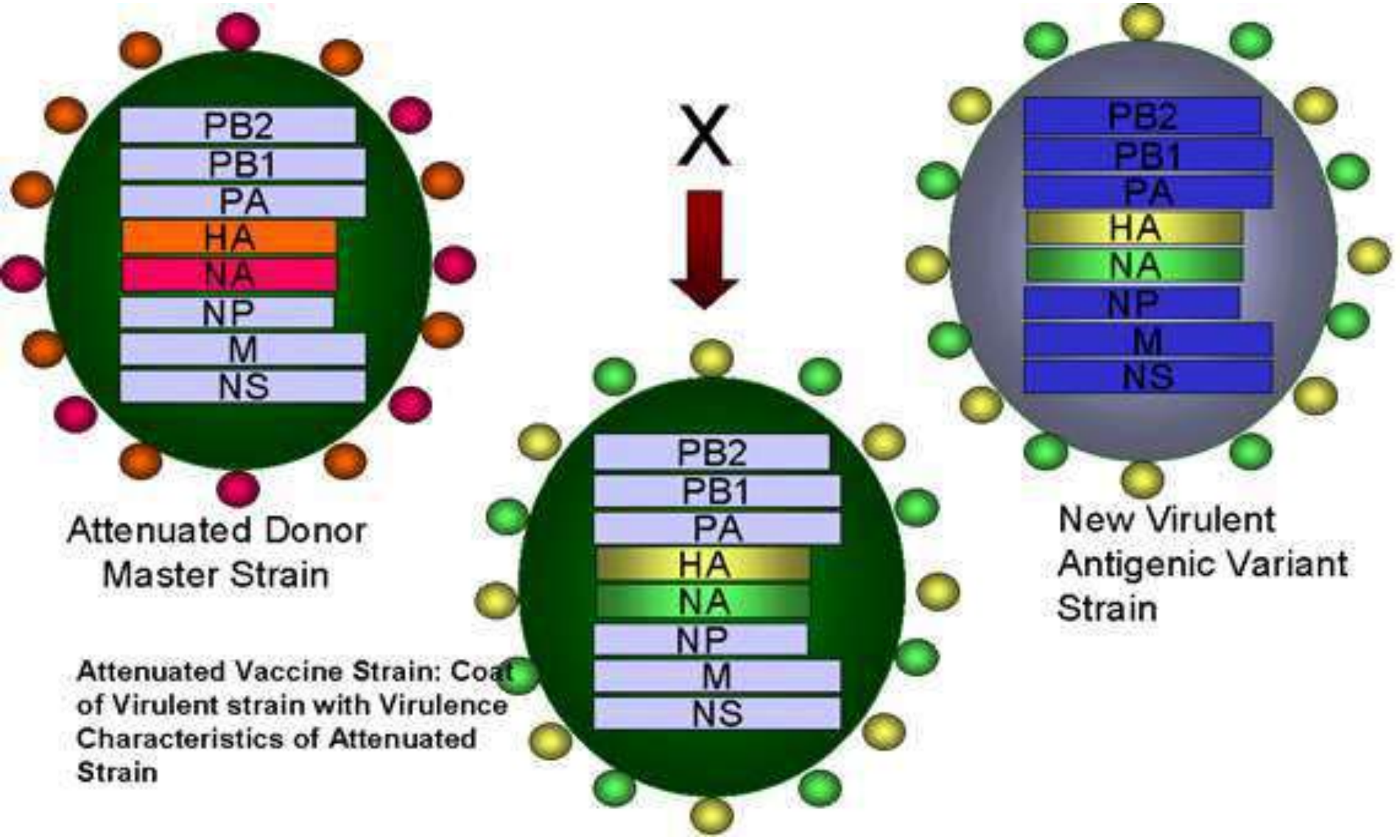


Figure 4: Reassortment of genes between the attenuated strain of influenza virus and a new virulent strain in the formation of an attenuated influenz avaccine

Applied genetics : There is vaccine called Flumist (LAIV, approved June 2003) for influenza virus which involves some of the principles discussed above. The vaccine is trivalent – it contains 3 strains of influenza virus:
The viruses are cold adapted strains which can grow well at 25 degrees C and so grow in the upper respiratory tract where it is cooler. The viruses are temperature-sensitive and grow poorly in the warmer lower respiratory tract.
The viruses are attenuated strains and much less pathogenic than wild-type virus. This is due to multiple changes in the various genome segments. Antibodies to the influenza virus surface proteins (HA - hemagglutinin and NA - neuraminidase) are important in protection against infection. The HA and NA change from year to year.
The vaccine technology uses reassortment to generate reassortant viruses which have six gene segments from the attenuated, coldadapted virus and the HA and NA coding segments from the virus which islikely to be a problem in the up-coming influenza season.
This vaccine is a live vaccine and is given intranasally as a spray and can induce mucosal and systemic immunity. A live, attenuated reassortant vaccine has recently (2006) been approved for rotaviruses (RotaTeq from Merke).
Another attenuated vaccine, Rotarix (Glaxo), is in development.

Phenotypic mixing

If two different viruses infect a cell, progeny viruses may contain coat components derived from both parents and so they will have coat properties of both parents. This is called phenotypic mixing (figure 5). **It involves no alteration in genetic material,** the progeny of such virions will be determined by which parental genome is packaged and not by the nature of the envelope.

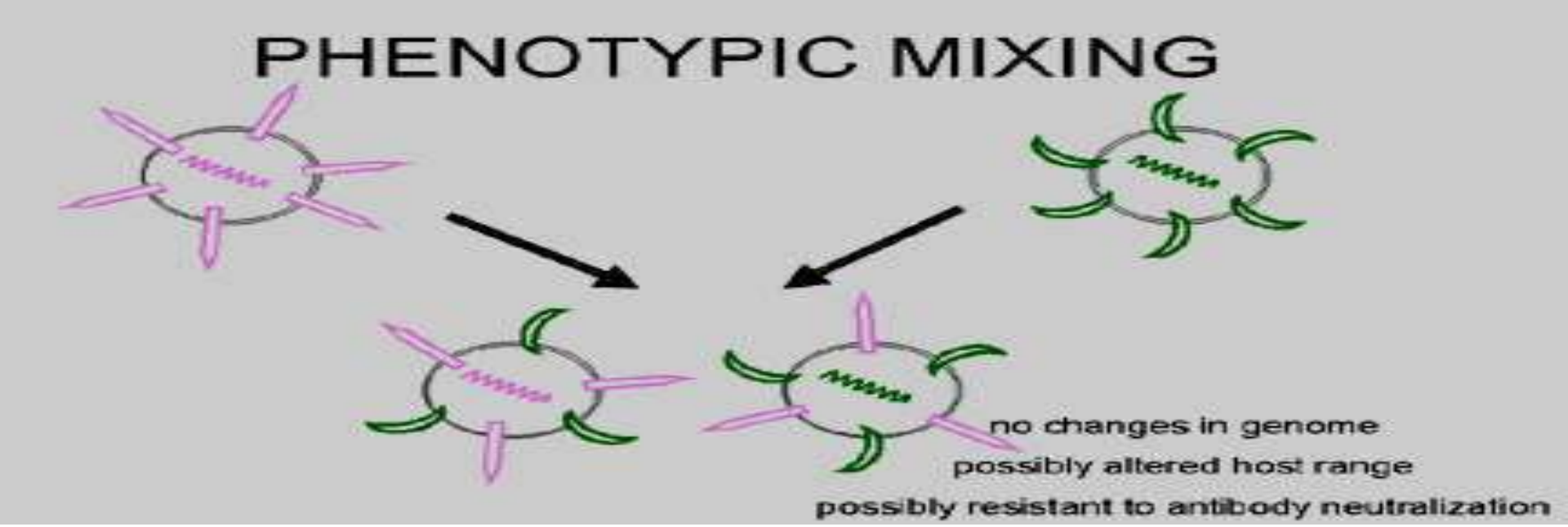


Figure 5: phenotype mixing between two different viruses infecting the same cell

Phenotypic mixing may occur between related viruses, e.g. different members of the Picornavirus family, or between genetically unrelated viruses, e.g. Rhabdo- and Paramyxo-viruses. In the latter case the two viruses involved are usually enveloped since it seems there are fewer restraints on packaging nucleocapsids in other viruses' envelopes than on packaging nucleic acids in other viruses' icosahedral capsids .

We can also get the situation where a coat is entirely that of another virus, e.g. a retrovirus nucleocapsid in a rhabdovirus envelope. This kind of phenotypic mixing is sometimes referred to as pseudotype (pseudovirion) formation (figure 6). The pseudotype described above will show the adsorption-penetration surface antigenicity characteristics of the rhabdovirus and will then, upon infection, behave as a retrovirus and produce progeny retroviruses. This results in pseudotypes having an altered host range/tissue tropism on a temporary basis

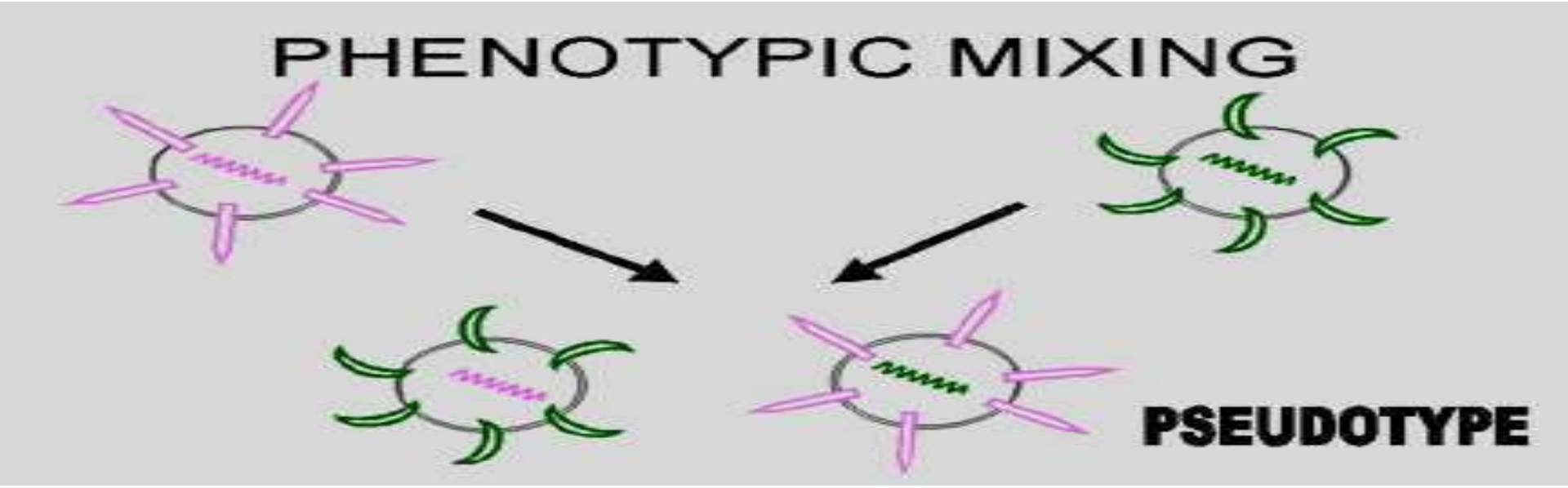


Figure 6:phenotype mixing to form pseudotype