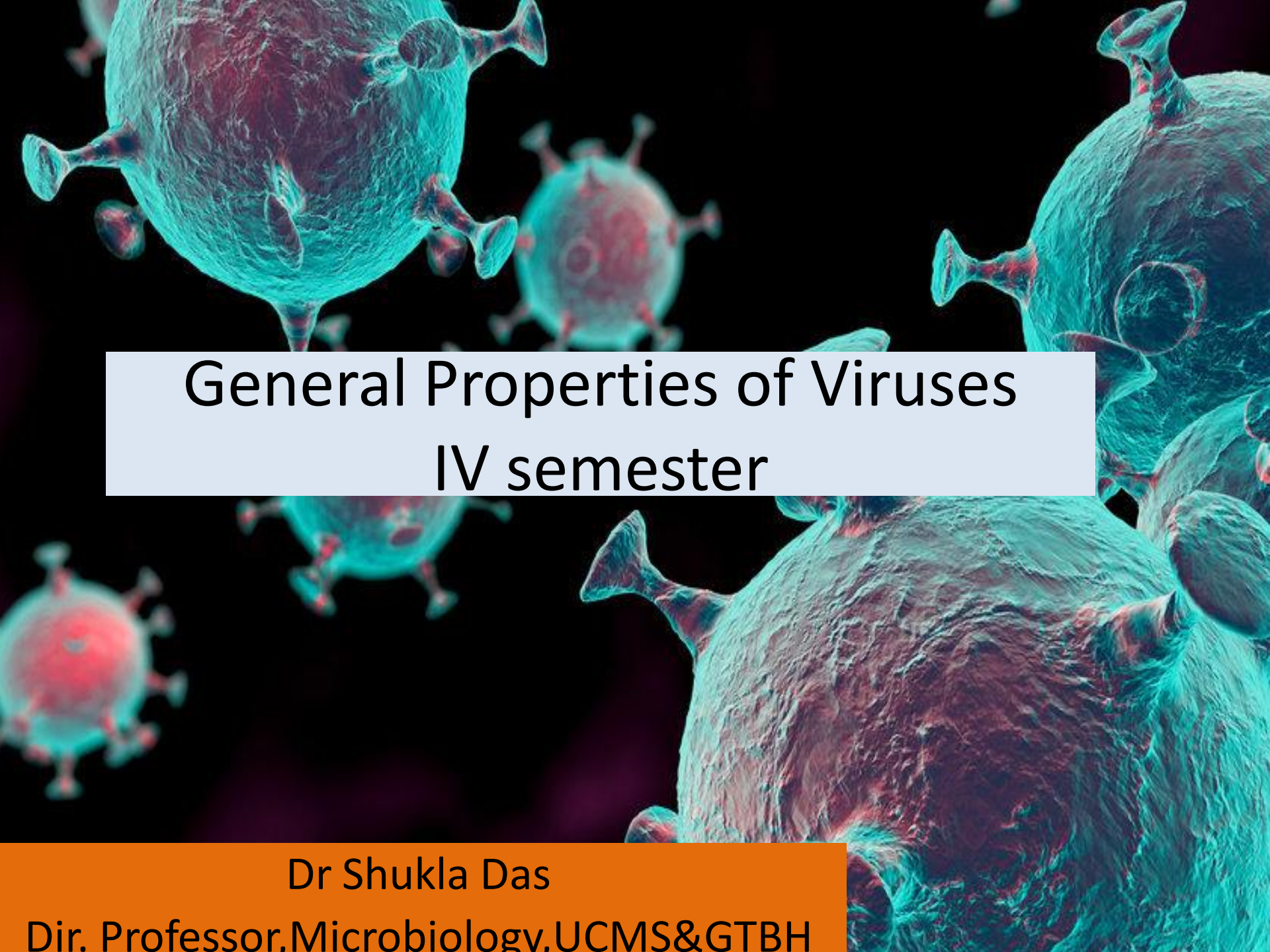


General Properties of Viruses
IV semester

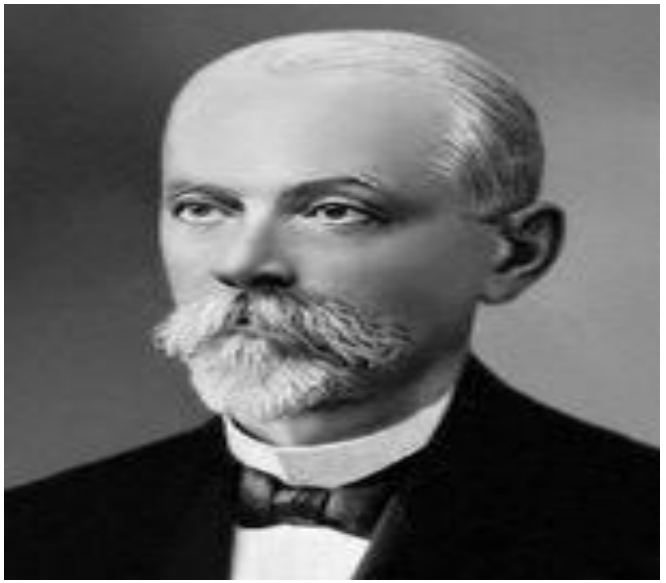
Dr Shukla Das
Dir. Professor,Microbiology,UCMS&GTBH

INTRODUCTION

- Viruses occupy the twilight zone that separates the 'living' from the 'non-living'.

- Recognizing the shape, size, and structure of different viruses is critical to the study of disease

- Viruses have an inner core of nucleic acid surrounded by protein coat known as capsid & maybe surrounded by an envelope



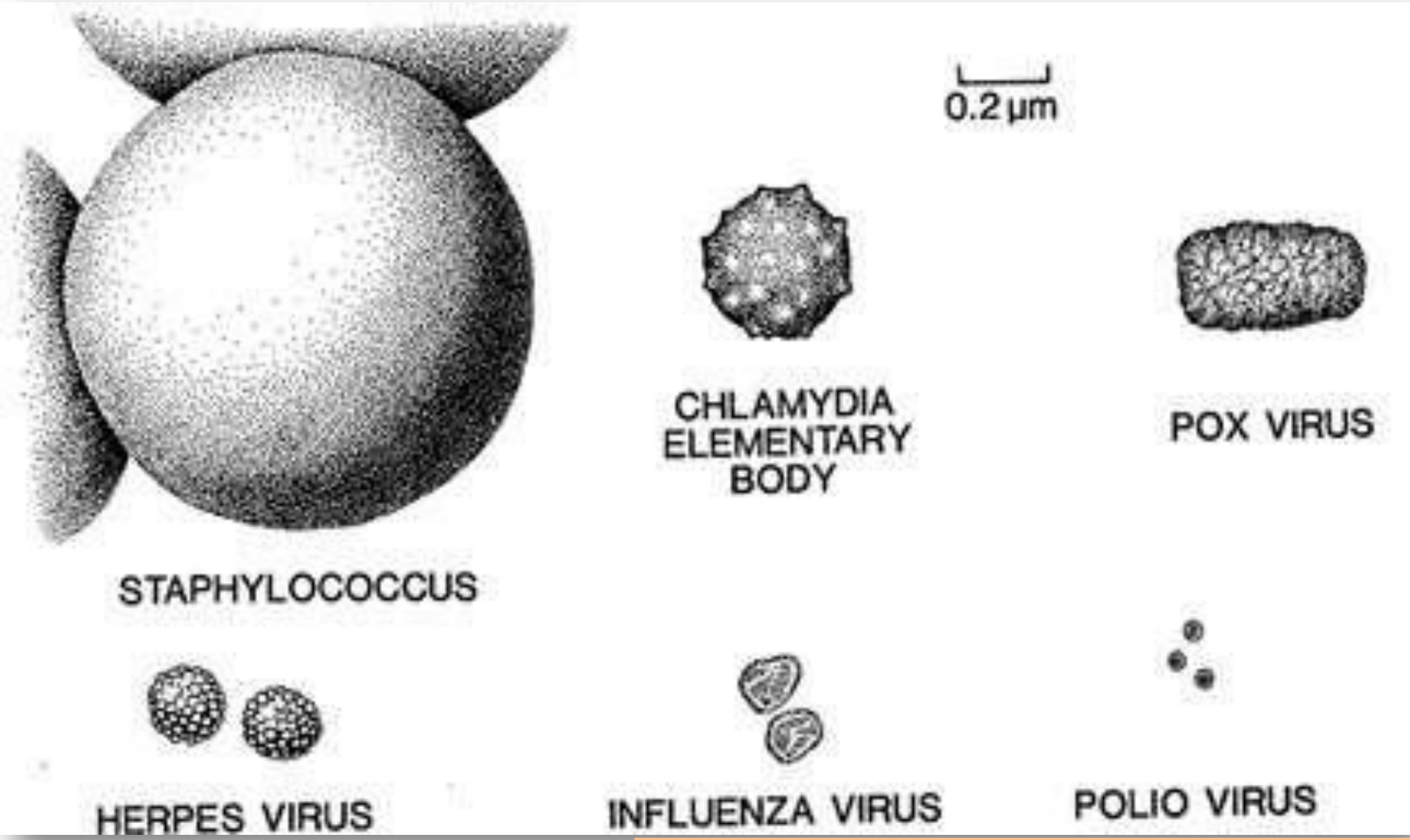
Dmitri Ivanovsky,

Martinus Beijerinck

General properties of viruses

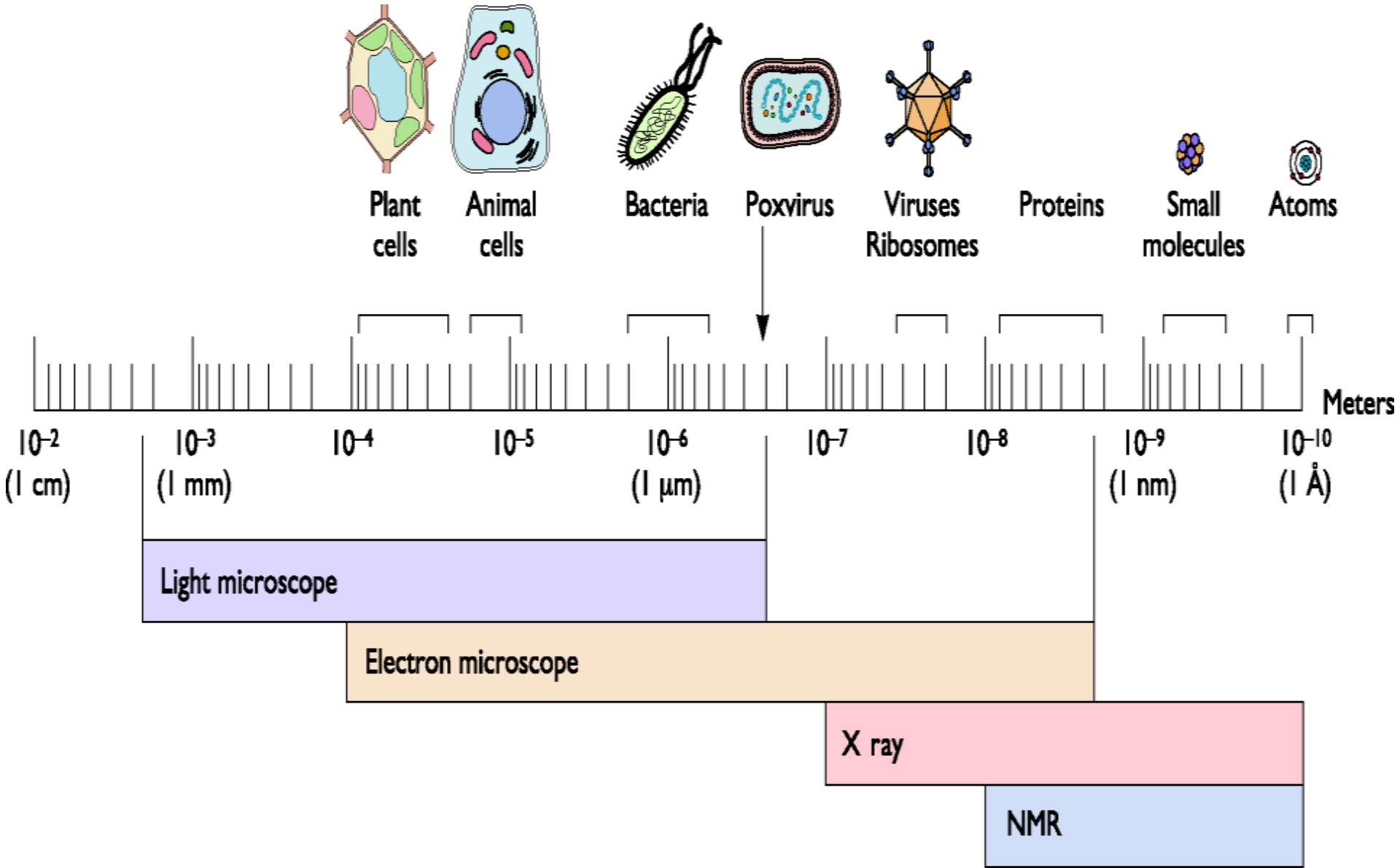
- Viruses do not have cellular organization.
- one type of nucleic acid, either DNA or RNA, but never both.
- obligate intracellular parasites.
- lack the enzymes necessary for protein and nucleic acid synthesis and are dependent for replication on the synthetic machinery of host cells.
- multiply by a complex process and not by binary fission
- Range in sizes from 20 – 250 nanometers
- Unaffected by antibiotics.

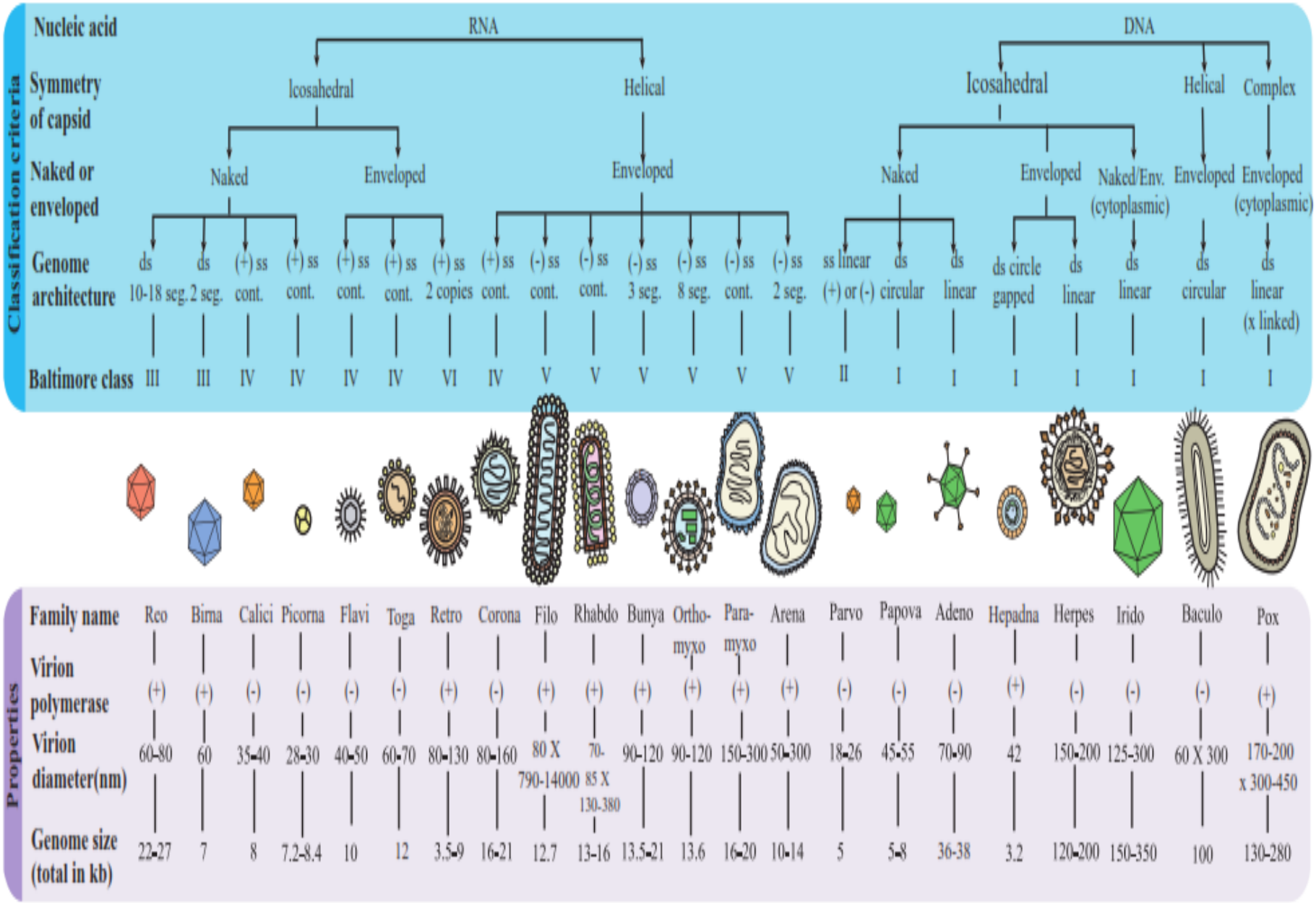
Viruses are Ultramicroscopic



Koneman et al. Color Atlas and Textbook of Microbiology 5th Ed. 1997

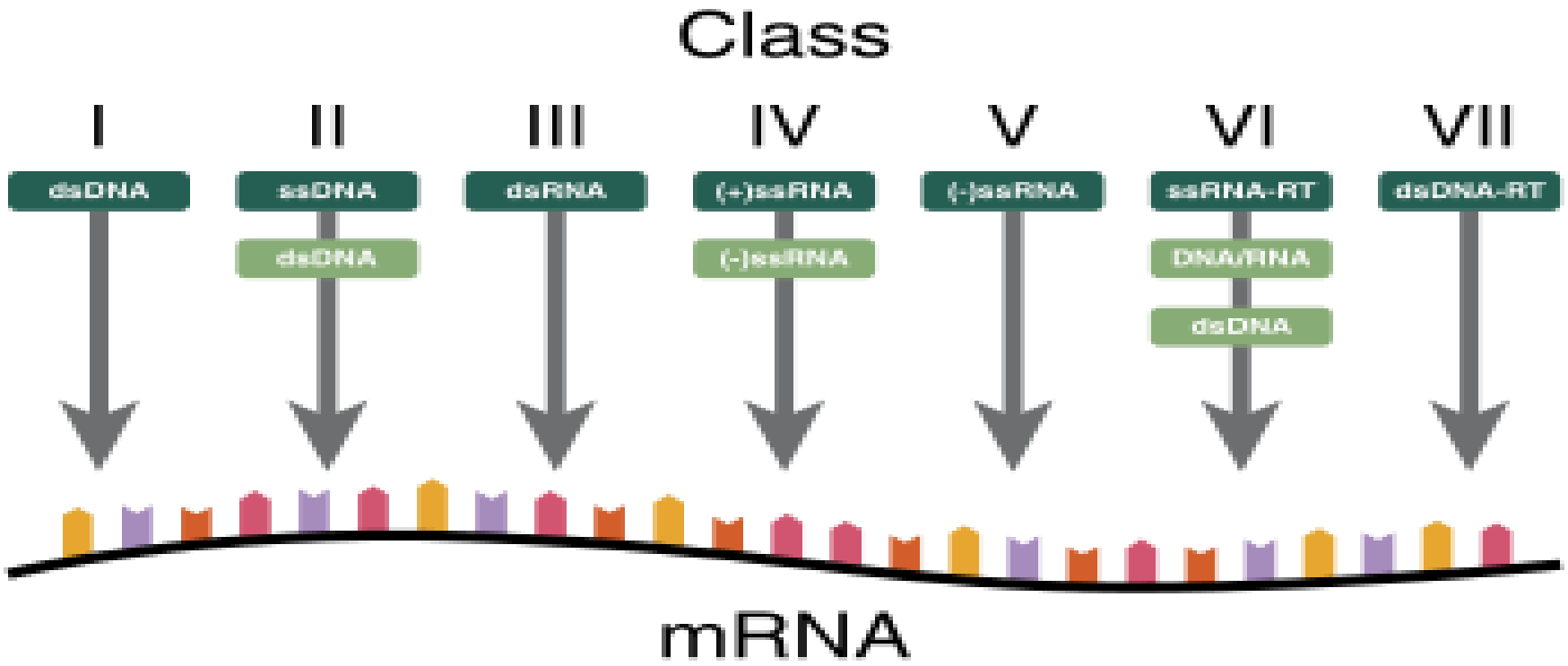
The size of viruses




Classification criteria
Nucleic acid
RNA
DNA
Symmetry of capsid
Icosahedral
Helical
Icosahedral
Helical
Complex
Naked or enveloped
Naked
Enveloped
Enveloped
Naked
Enveloped
Naked/Env. (cytoplasmic)
Enveloped
Enveloped (cytoplasmic)
Genome architecture
ds 10-18 seg.
ds 2 seg.
(+) ss cont.
(+) ss cont.
(+) ss cont.
(+) ss cont.
2 copies
(+) ss cont.
(-) ss cont.
(-) ss cont.
(-) ss 3 seg.
(-) ss 8 seg.
(-) ss cont.
(-) ss 2 seg.
ss linear (+) or (-)
ds circular
ds linear
ds circle gapped
ds linear
ds linear
ds circular
ds linear (x linked)
Baltimore class
III
III
IV
IV
IV
IV
VI
IV
V
V
V
V
V
V
II
I
I
I
I
I
I
I
Properties
Family name
Reo
Birna
Calici
Picorna
Flavi
Toga
Retro
Corona
Filo
Rhabdo
Bunya
Ortho-myxo
Para-myxo
Arena
Parvo
Papova
Adeno
Hepadna
Herpes
Irido
Baculo
Pox
Virion polymerase
(+)
(+)
(-)
(-)
(-)
(-)
(+)
(-)
(+)
(+)
(+)
(+)
(+)
(+)
(-)
(-)
(-)
(+)
(-)
(-)
(-)
(+)
Virion diameter(nm)
60-80
60
35-40
28-30
40-50
60-70
80-130
80-160
80 X 790-14000
70-85 X 130-380
90-120
90-120
150-300
50-300
18-26
45-55
70-90
42
150-200
125-300
60 X 300
170-200 x 300-450
Genome size (total in kb)
22-27
7
8
7.2-8.4
10
12
3.5-9
16-21
12.7
13-16
13.5-21
13.6
16-20
10-14
5
5-8
36-38
3.2
120-200
150-350
100
130-280

Baltimore classification

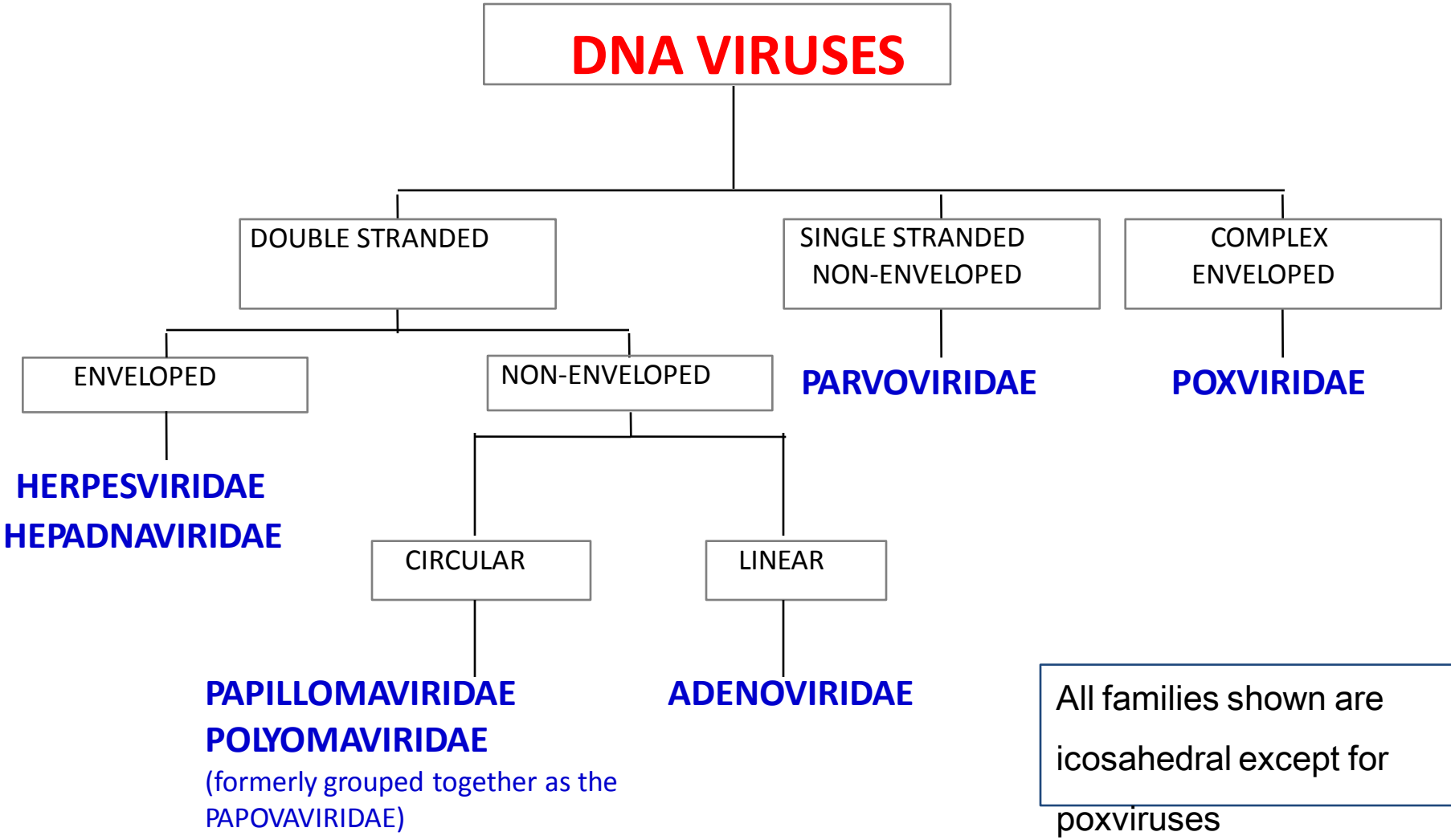
The Baltimore classification, developed by David Baltimore, is a virus classification system that groups viruses into families, depending on their type of genome (DNA, RNA, single-stranded (ss), double-stranded (ds), etc..) and their method of replication.

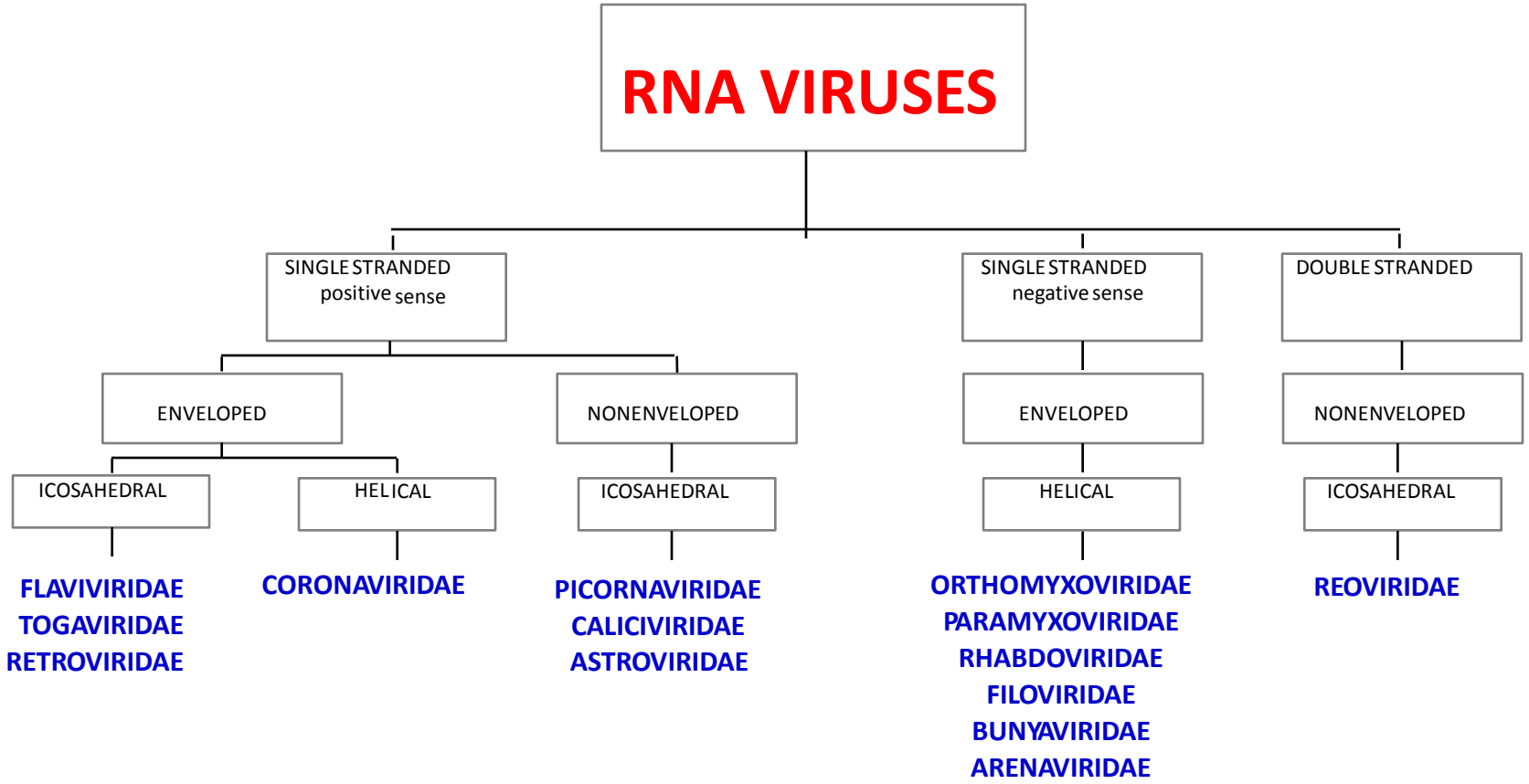


Baltimore Classification

Based on genetic contents and replication strategies of viruses. According to the Baltimore classification, viruses are divided into the following seven classes:

I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
II: **ssDNA viruses** (+ strand or "sense") DNA (e.g. Parvoviruses)
III: **dsRNA viruses** (e.g. Reoviruses)
IV: **(+)ssRNA viruses** (+ strand or sense) RNA
(e.g. Picornaviruses, Togaviruses,Flaviviridae, coronaviridae,calciviridae)
V: **(−)ssRNA viruses** (− strand or antisense) RNA
(e.g. Orthomyxoviruses, Rhabdoviruses,filoviridae,paramyxo,arenaviridae)
VI: **ssRNA-RT viruses** (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
VII: **dsDNA-RT viruses** DNA with RNA intermediate in life-cycle
(e.g. Hepadnaviruses)


DNA VIRUSES
DOUBLE STRANDED
SINGLE STRANDED
NON-ENVELOPED
COMPLEX
ENVELOPED
ENVELOPED
NON-ENVELOPED
PARVOVIRIDAE
POXVIRIDAE
HERPESVIRIDAE
HEPADNAVIRIDAE
CIRCULAR
LINEAR
PAPILLOMAVIRIDAE
POLYOMAVIRIDAE
(formerly grouped together as the PAPOVAVIRIDAE)
ADENOVIRIDAE
All families shown are icosahedral except for poxviruses


RNA VIRUSES
SINGLE STRANDED positive sense
SINGLE STRANDED negative sense
DOUBLE STRANDED
ENVELOPED
NONENVELOPED
ENVELOPED
NONENVELOPED
ICOSAHEDRAL
HELICAL
ICOSAHEDRAL
HELICAL
ICOSAHEDRAL
FLAVIVIRIDAE
TOGAVIRIDAE
RETROVIRIDAE
CORONAVIRIDAE
PICORNAVIRIDAE
CALICIVIRIDAE
ASTROVIRIDAE
ORTHOMYXOVIRIDAE
PARAMYXOVIRIDAE
RHABDOVIRIDAE
FILOVIRIDAE
BUNYAVIRIDAE
ARENAVIRIDAE
REOVIRIDAE

VIRAL STRUCTURE

- Virus particle = virion
- Protein which coats the genome = capsid
- Capsid usually symmetrical
- Capsid + genome = nucleocapsid
- May have an envelope
- Capsid protects NA. Its antigenic.
- In non-enveloped virus capsid initiates attachment to host cells for entry.

Viral structure

- Nucleic acid(ss or ds/circular or linear/segmented or non segmented) is within the protein coat (**capsid)**
- Subunits comprising the capsid are **capsomeres (polypeptides)**
- **Symmetry is based on capsomeres**

- Viral capsids are capable of self-assembly
- Rod-shaped viruses have **Helical symmetry** (rhabdo, Filo, Myxo)
- Spherical viruses have **Icosahedral (cubical) symmetry** Eg: all viruses except Pox
- **Complex Pox**

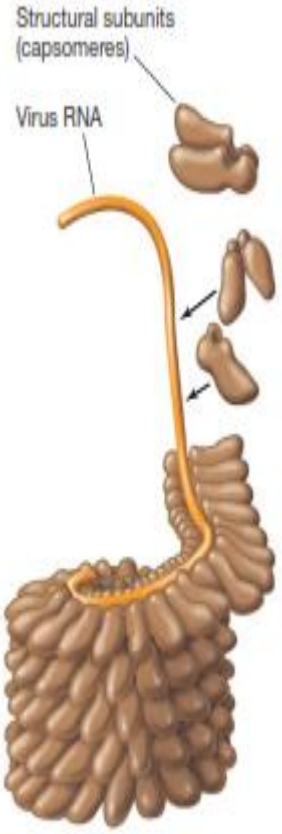


Fig: *Assembly of the tobacco* mosaic virus virion. The RNA assumes a helical configuration surrounded by the protein capsid. The center of the particle is hollow.

Fig: A model of an icosahedron

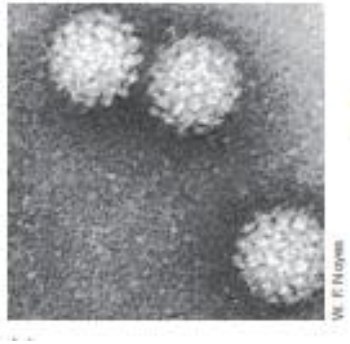
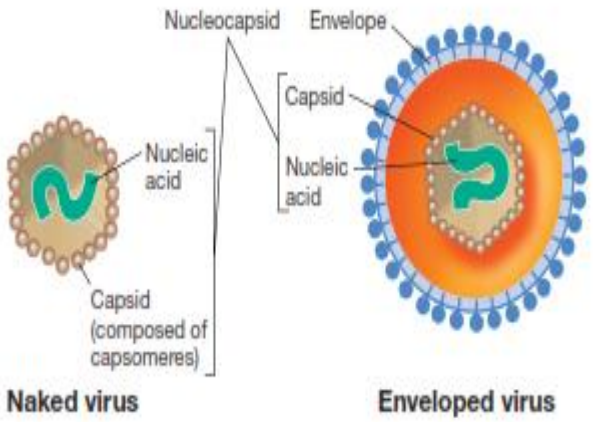


Fig: Electron micrograph of human papillomavirus, a virus with icosahedral symmetry. The virion is about 55 nm in diameter.

Additional viral structures

- ❑Some animal viruses are **enveloped**
- ❑Membrane is derived from host cell
- ❑Non-env DNA:(Parvo, papova, adeno)
- ❑**Non-env RNA:HepA, E,picorna**

- ❑Protein is viral encoded
- ❑Some bacterophage are **complex**
- ✓Icosahedral heads
- ✓Helical tails
- ✓Complex tail structure



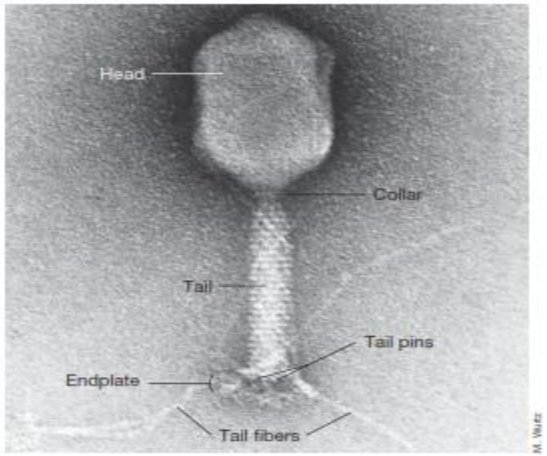
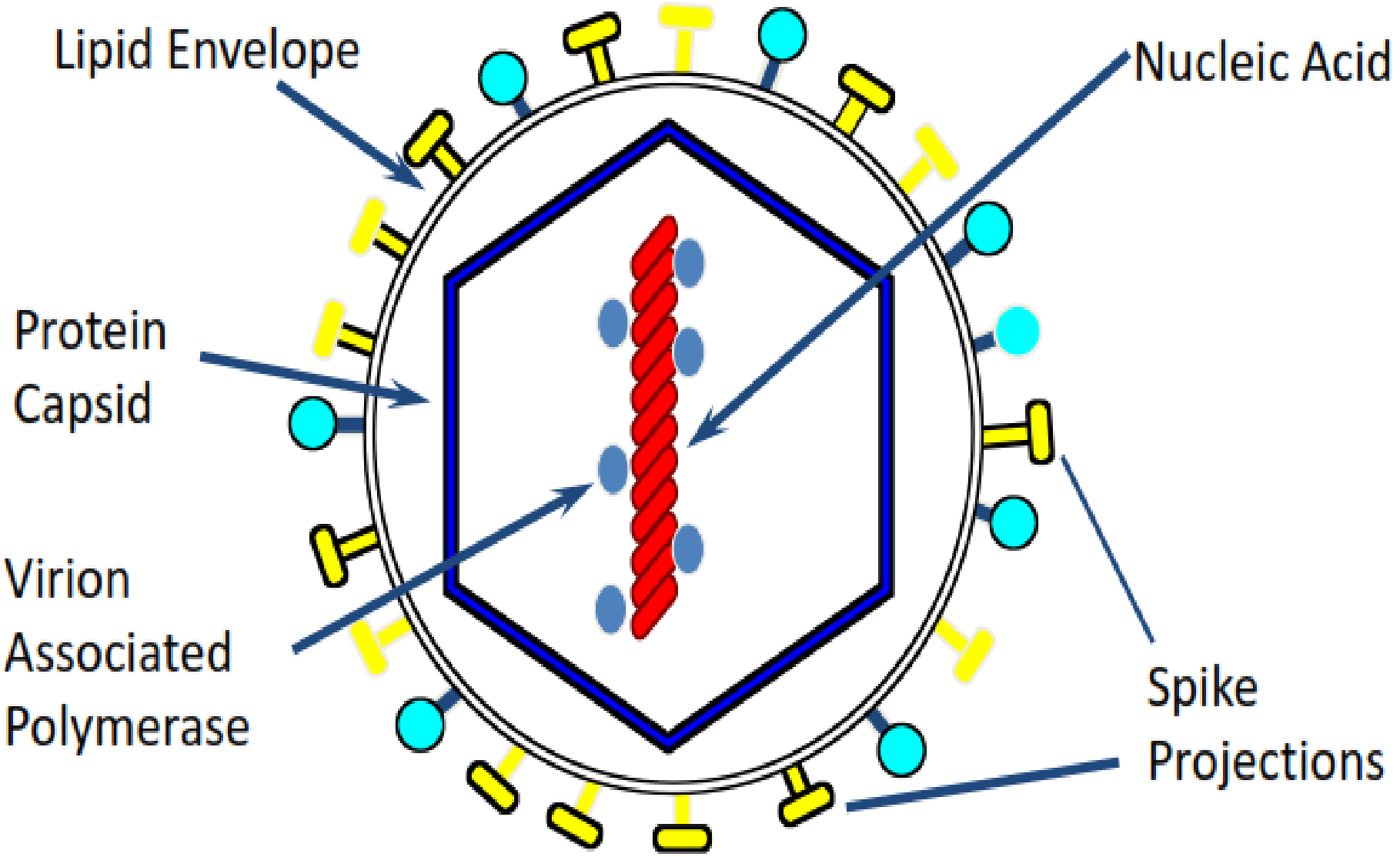
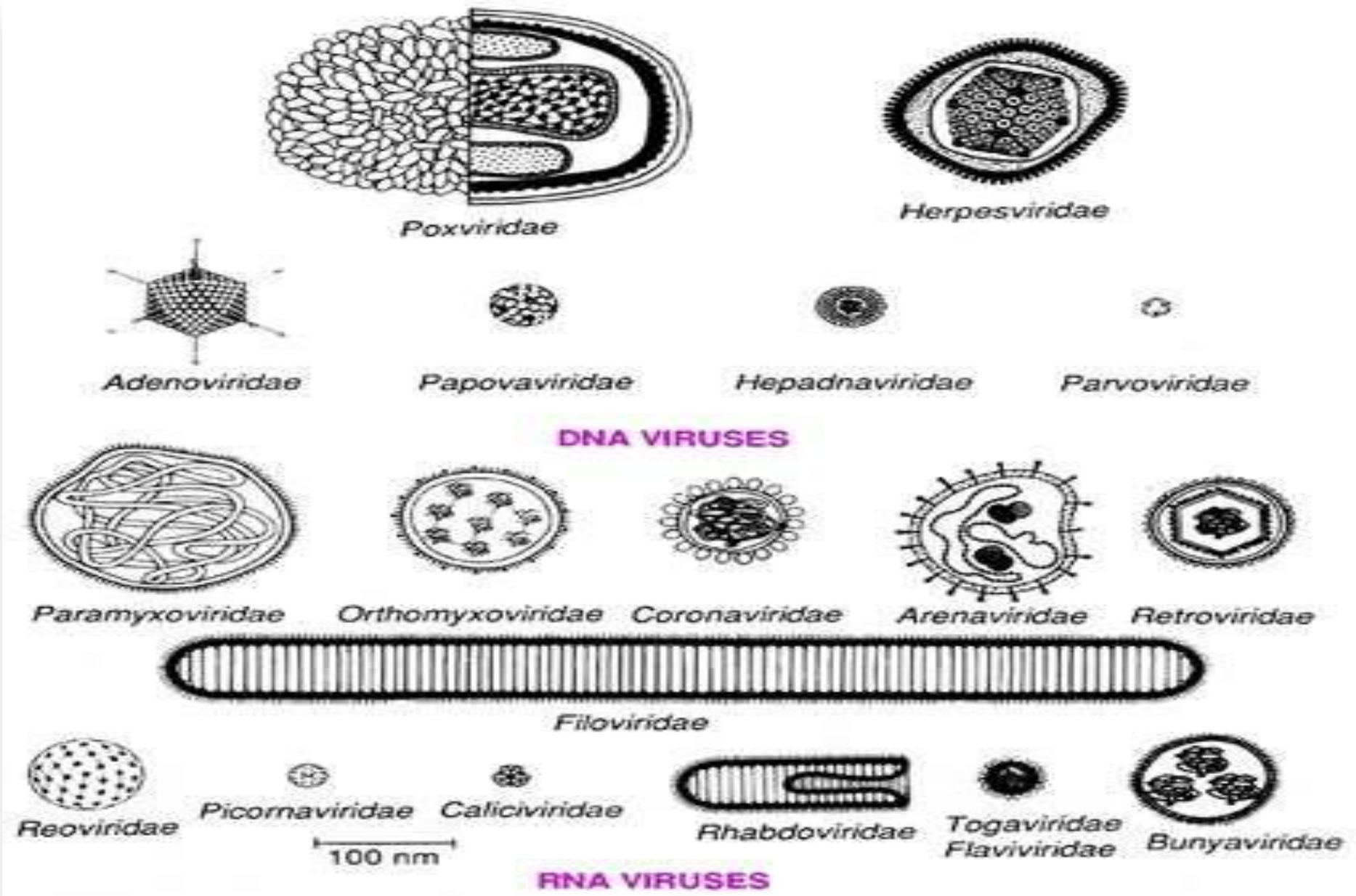


Fig: Bacteriophage T4 of *Escherichia coli.* The tail components function in attachment of the virion to the host and injection of the nucleic acid (Figure 9.10). The head is about 85 nm in diameter.

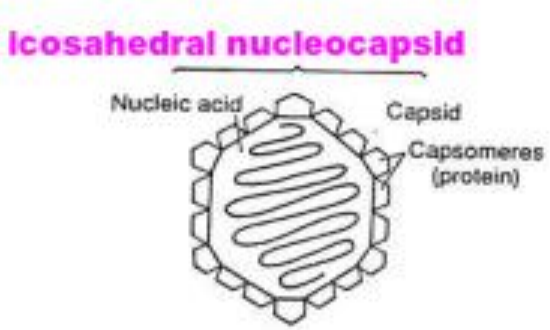
Virion Structure



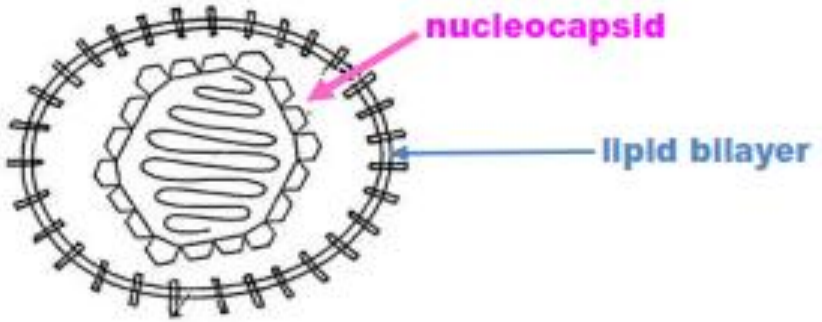
Virus particle = virion



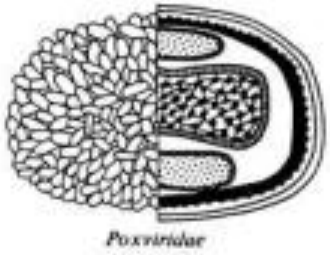
5 BASIC TYPES OF VIRAL STRUCTURE



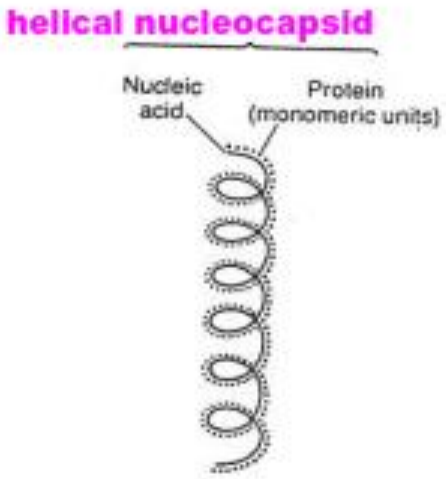
ICOSAHEDRAL



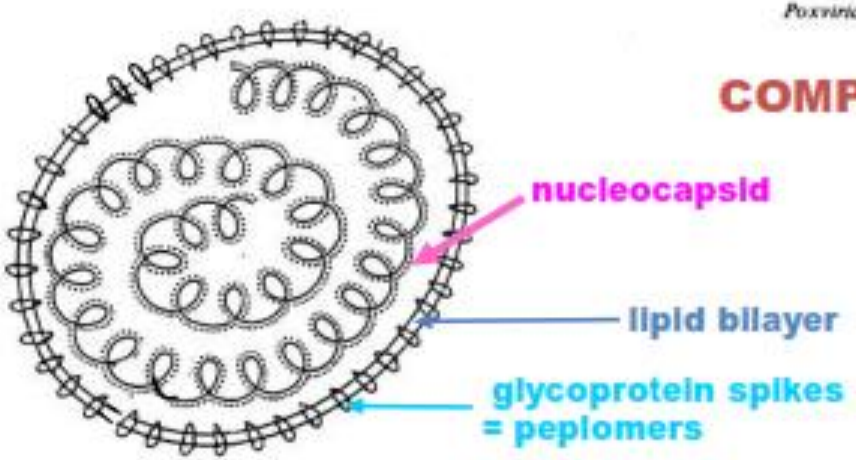
ENVELOPED ICOSAHEDRAL



COMPLEX



HELICAL



ENVELOPED HELICAL

How are viruses named?

- Based on:
  - The disease they cause

poliovirus, rabies virus

  - The type of disease

murine leukemia virus

  - Geographic locations

Sendai virus, Coxsackie virus

  - Their discovers

Epstein-Barr virus

  - How they were originally thought to be contracted

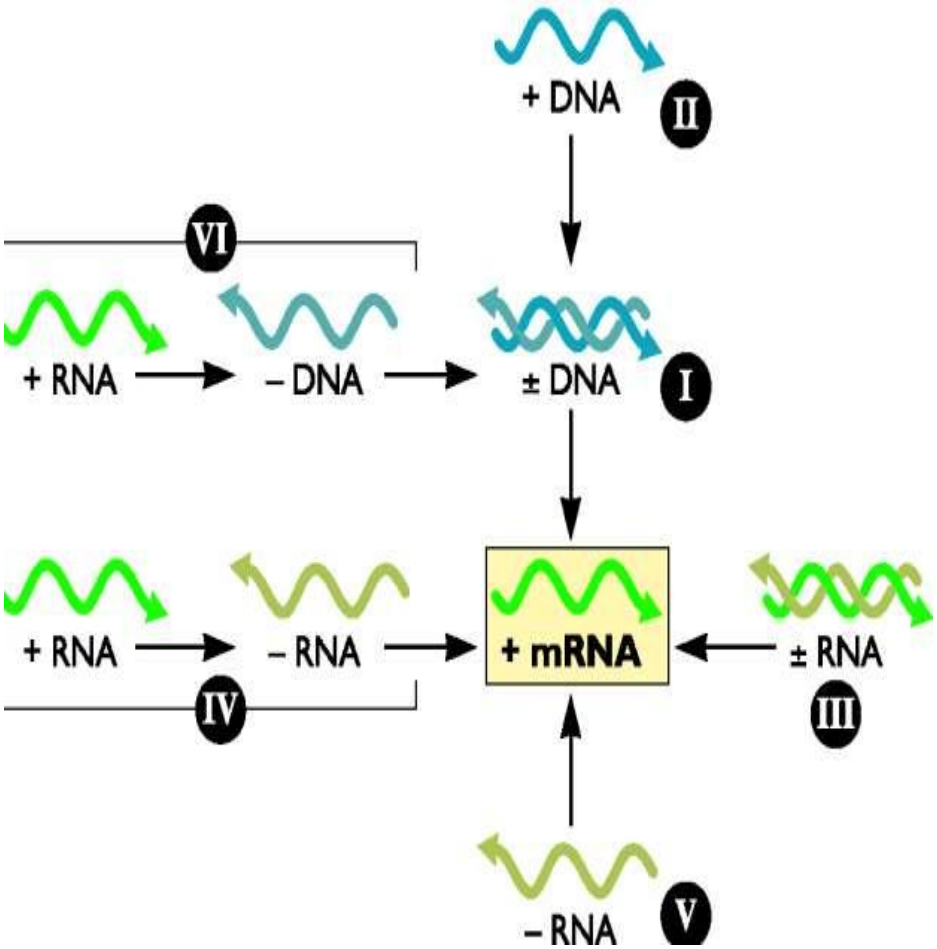
dengue virus (“evil spirit”), influenza virus (the “influence” of bad air)

  - Combinations of the above

Rous Sarcoma virus

Virus Classification I - the Baltimore classification

- All viruses must produce mRNA, or (+) sense RNA
- A complementary strand of nucleic acid is (–) sense
- Baltimore classification has + RNA as its central point



Virus classification II

- Based on 3 principles -
  - 1) classifying the virus itself, not the host
  - 2) the nucleic acid genome
  - 3) the shared physical properties of the infectious agent

(e.g capsid symmetry, dimensions, lipid envelope)

Virus classification III - the genomic system

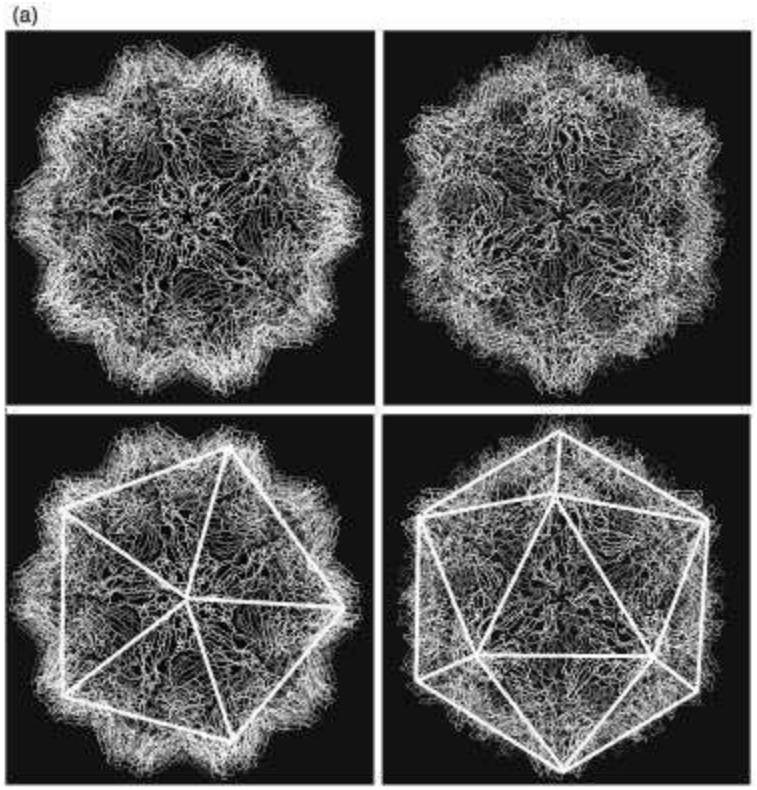
- More recently a precise ordering of viruses within families based on DNA/RNA **sequence**

In year 2000 there were >4000 viruses of plants,animals

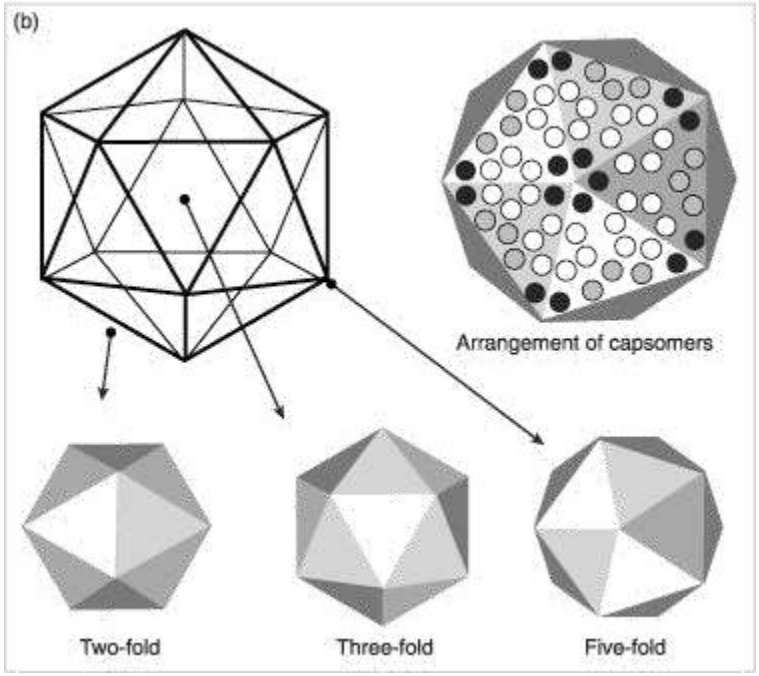
Family: “viridae” & genera

Human viruses :24 families

Icosahedral capsids

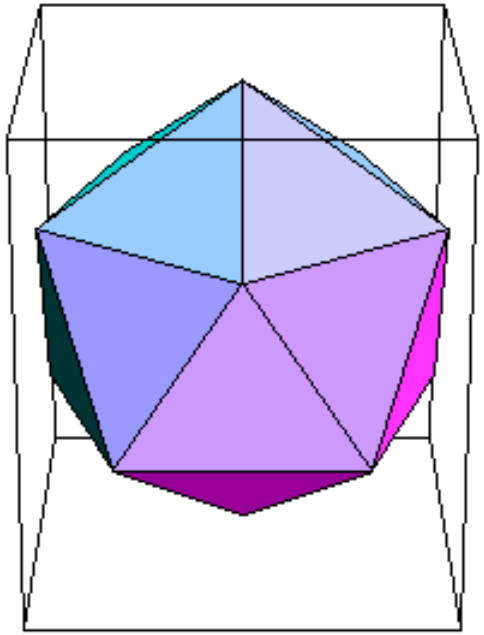


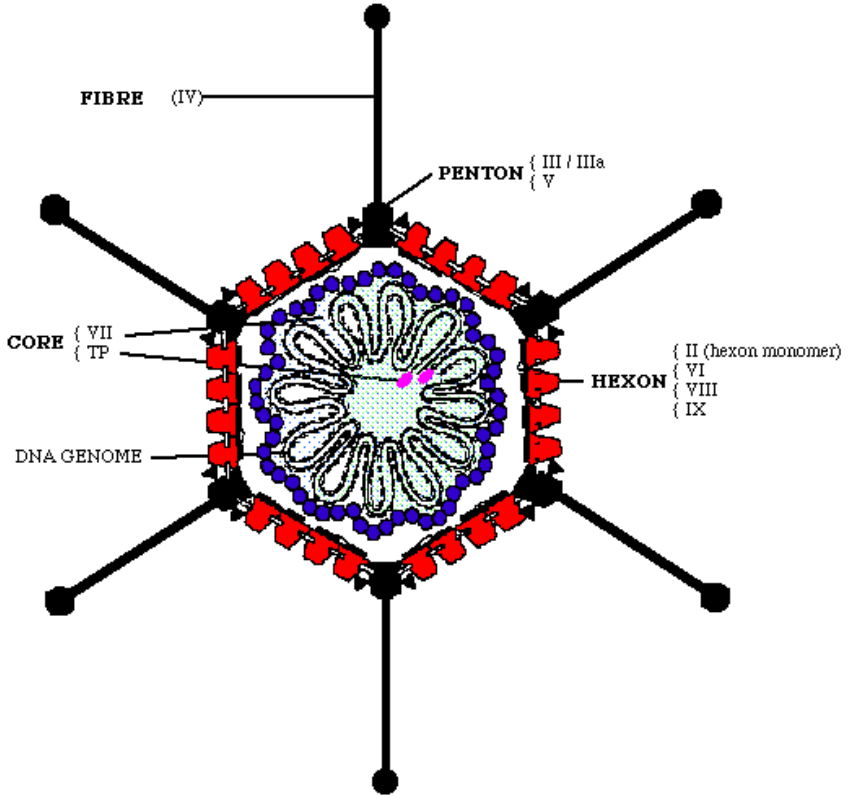
a) Crystallographic structure of a simple icosahedral virus.



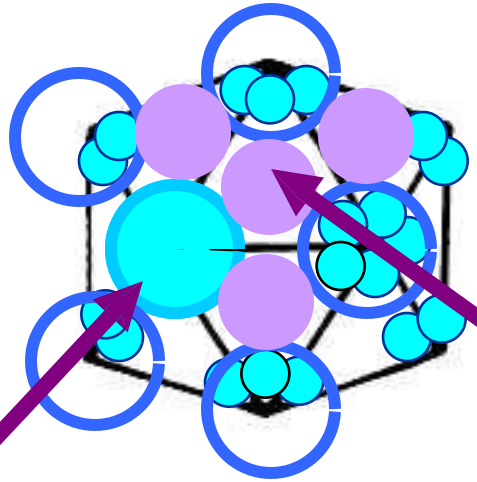
b) The axes of symmetry

Cubic or icosahedral symmetry





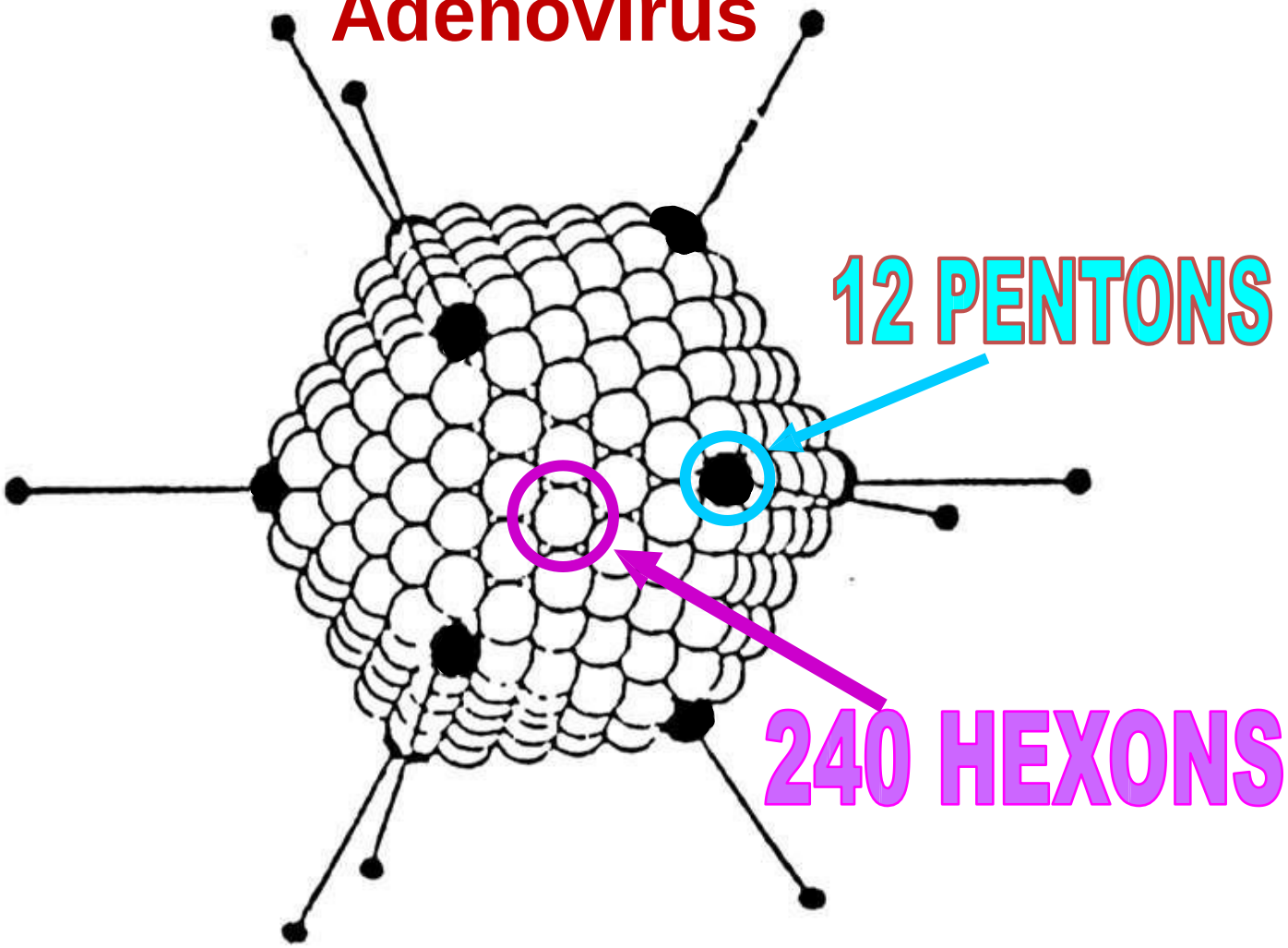
ICOSAHEDRAL SYMMETRY



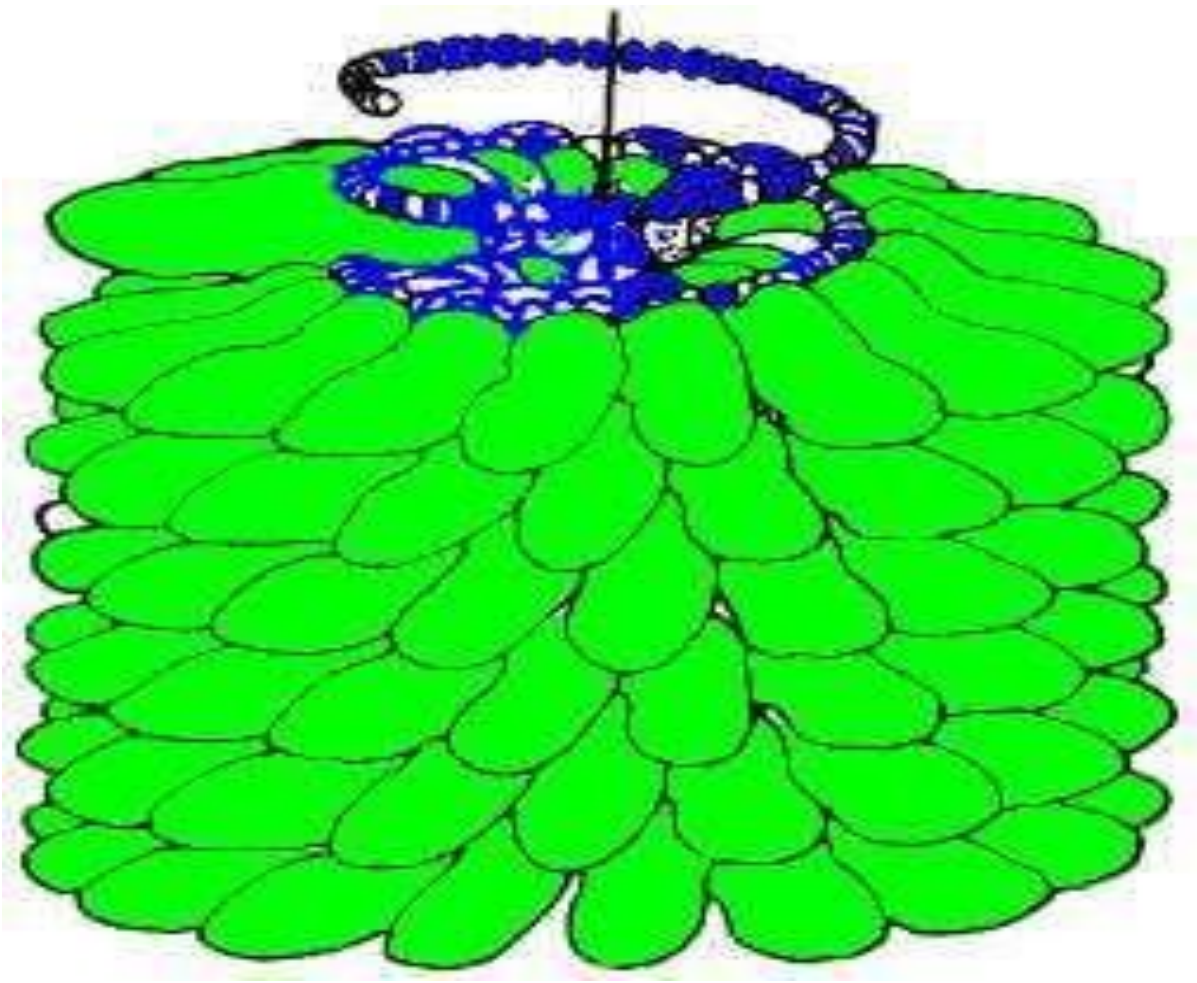
CAPSOMER = PENTON

CAPSOMER = HEXON

Adenovirus

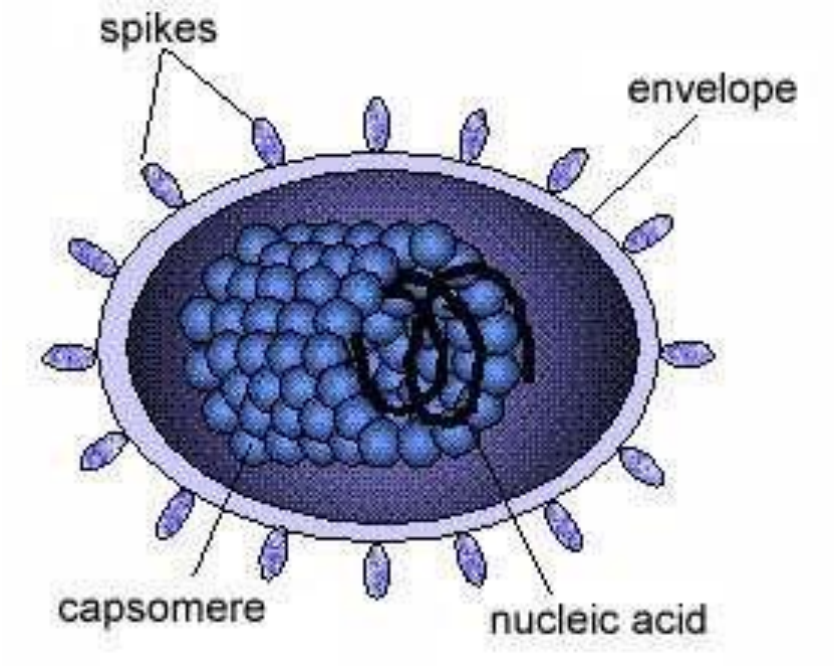


Helical symmetry



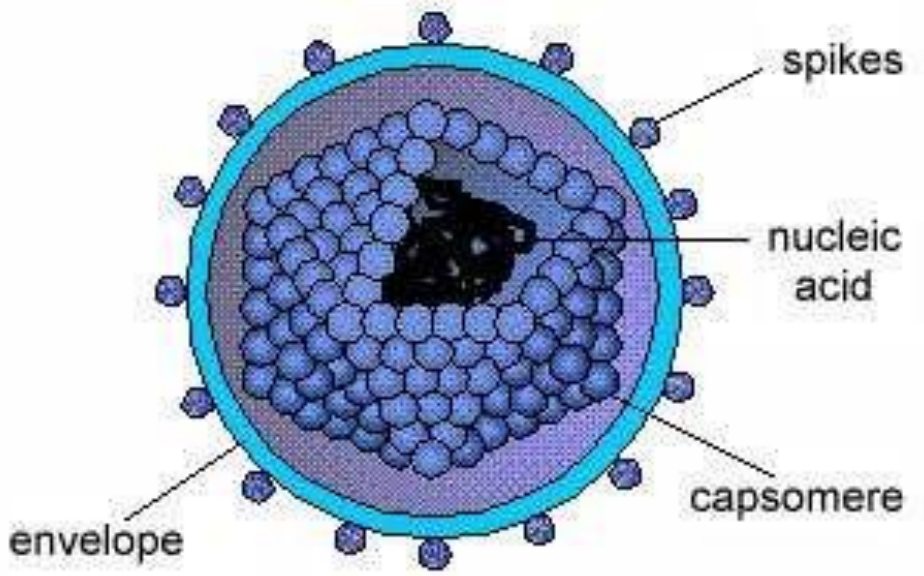
Tobacco mosaic virus

Enveloped helical virus



Corona
Rabdho,myxo,filov

Enveloped icosahedral virus



Herpes,hepdna,
toga,flavi

Properties of naked viruses

- Stable in hostile environment
- Not damaged by drying, acid, detergent, and heat
- Released by lysis of host cells
- Can sustain in dry environment
- Can infect the GI tract and survive the acid and bile
- Can spread easily via hands, dust, fomites, etc
- Neutralizing mucosal and systemic antibodies are needed to control the establishment of infection

Naked viruses(Non Enveloped)

Adeno-associated Virus (AAV)

Adenovirus

B19

Coxsackievirus - A

Coxsackievirus – B

Echovirus

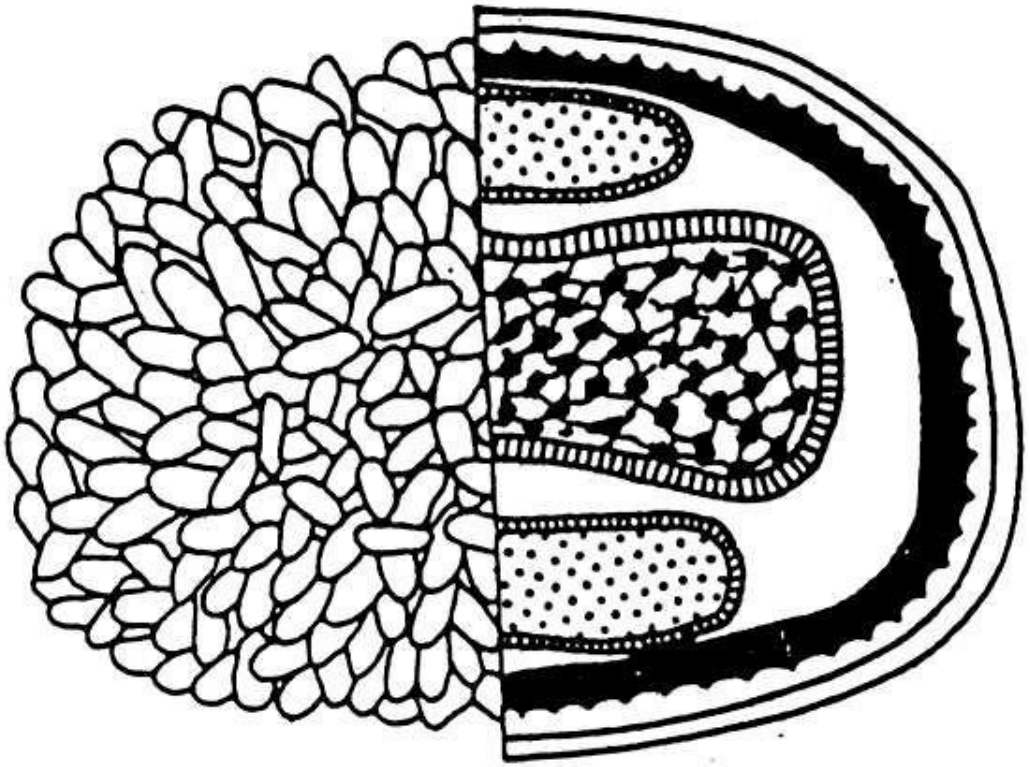
Hepatitis A Virus (HAV)

Hepatitis E Virus (HEV)

Norwalk Virus

COMPLEX SYMMETRY

surface view



cross section

POXVIRUS FAMILY

White, DO and Fenner, FJ.
Medical Virology, 4th Ed. 1994

ENVELOPE

Obtained by budding through a cellular membrane (except poxviruses) possibility of exiting cell without killing it contains at least one virally coded protein

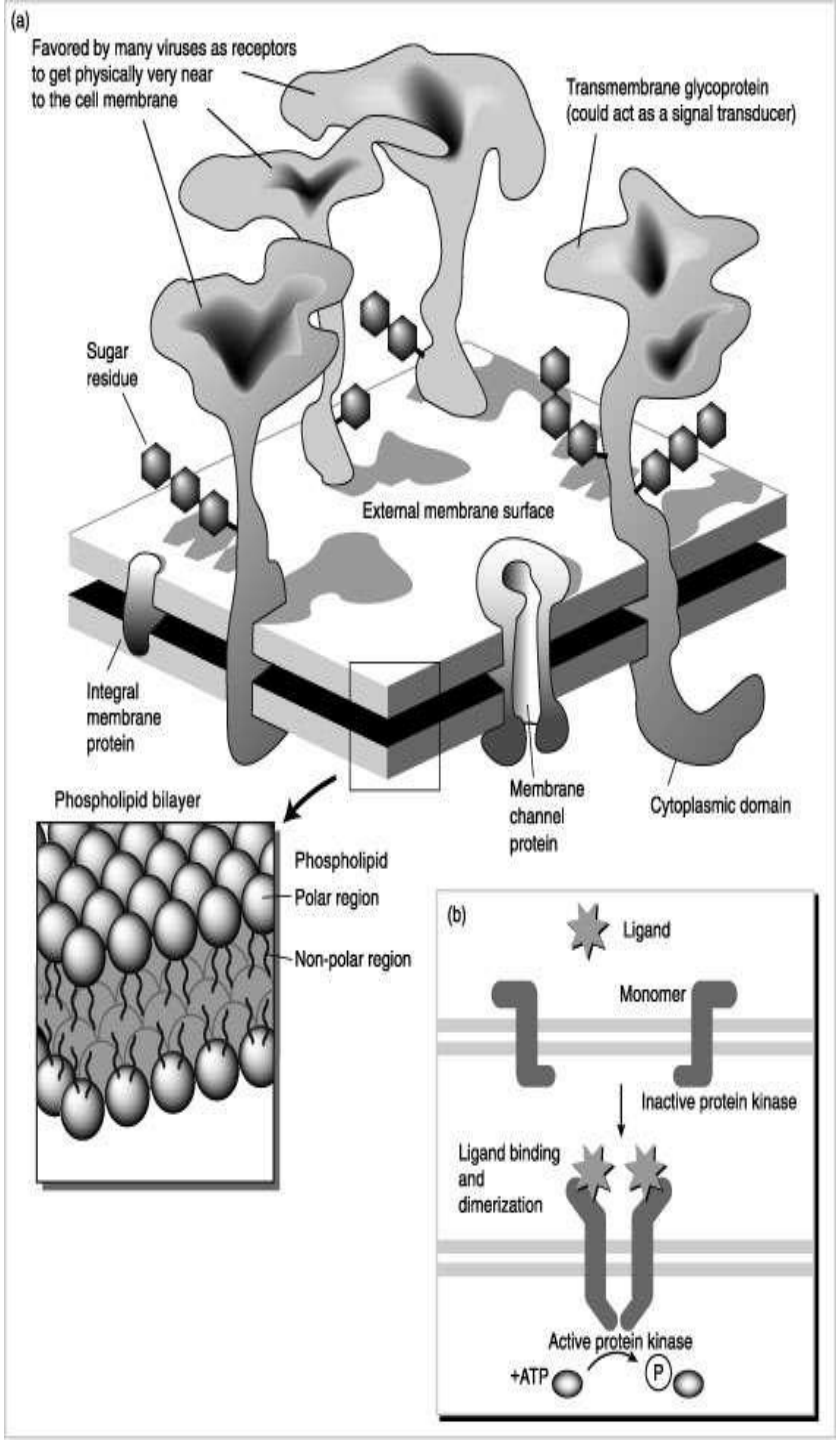
– attachment protein

loss of envelope results in loss of infectivity

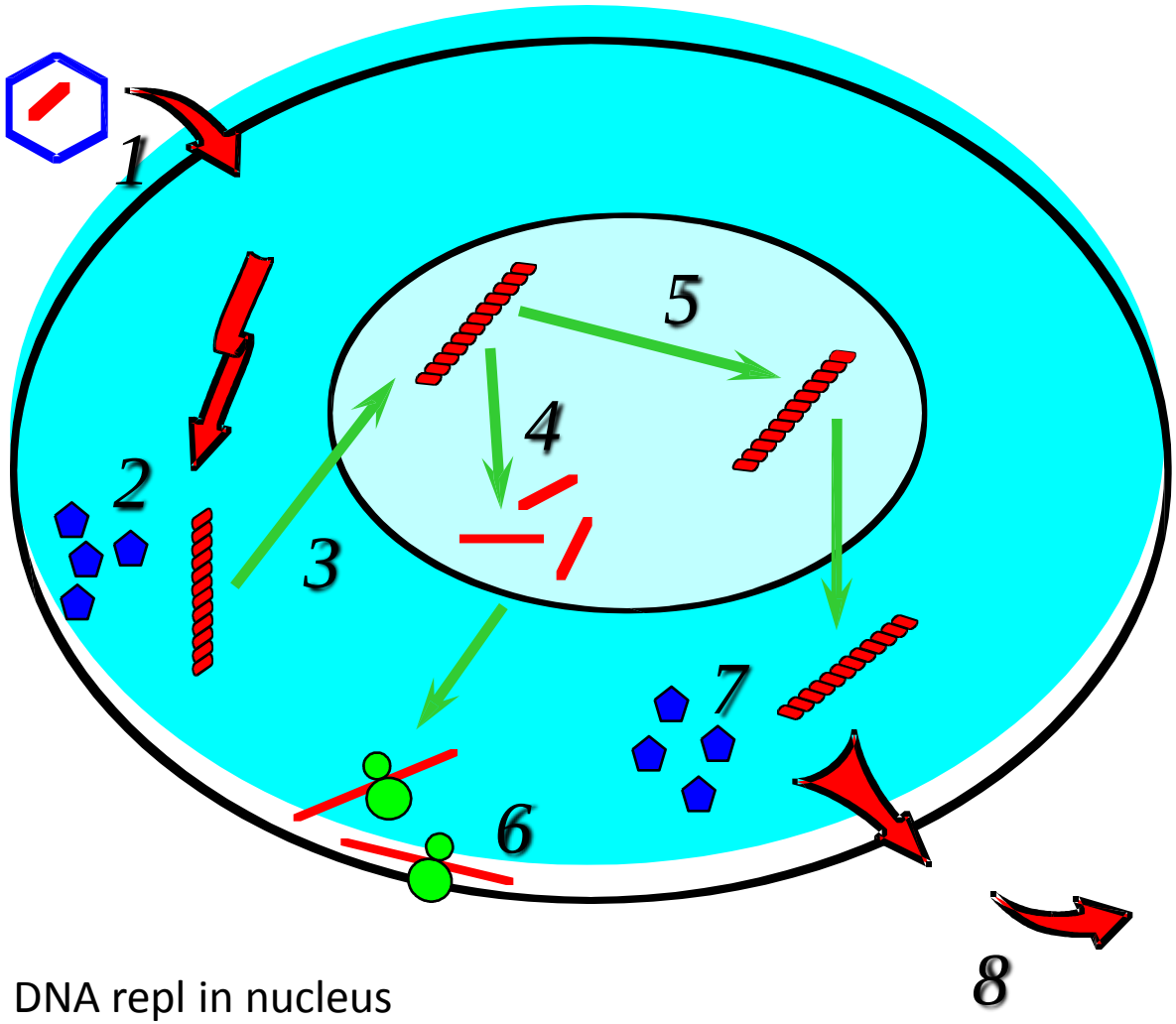
BASIC STEPS IN VIRAL LIFE CYCLE

- ADSORPTION
- PENETRATION
- UNCOATING AND ECLIPSE
- SYNTHESIS OF VIRAL NUCLEIC ACID AND PROTEIN
- ASSEMBLY (maturation)
- RELEASE

RECEPTOR	VIRUS
ICAM-1	polio
CD4	HIV
acetylcholine	rabies
EGF	vaccinia
CR2/CD21	Epstein-Barr
HVEM	herpes
Sialic acid	Influenza, reo, corona



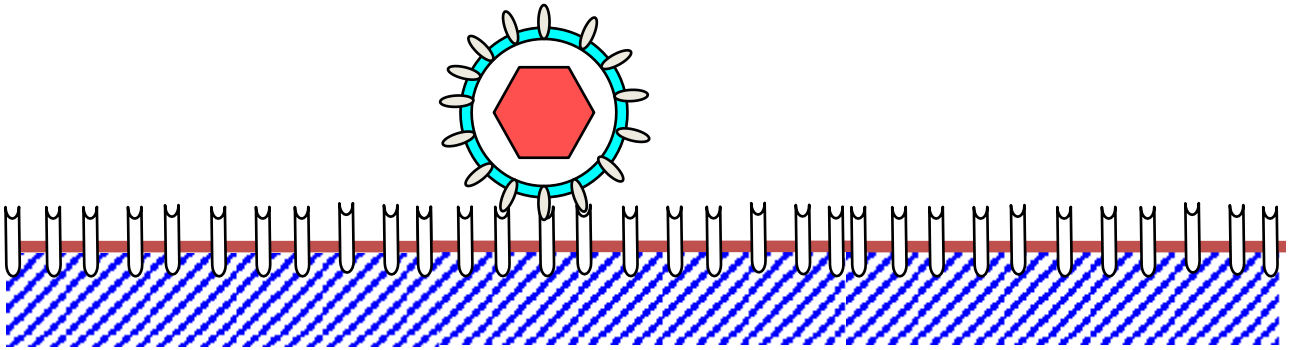
Virus Replication



1 Virus attachment and entry
2 Uncoating of virion
3 Migration of genome nucleic acid to nucleus
4 Transcription
5 Genome replication
6 Translation of virus mRNAs
7 Virion assembly
8 Release of new virus particles

DNA repl in nucleus
RNA in cytop,except RetroV&orthomyxo repl in nucleus

ADSORPTION



- TEMPERATURE INDEPENDENT
- REQUIRES VIRAL ATTACHMENT PROTEIN
- CELLULAR RECEPTORS

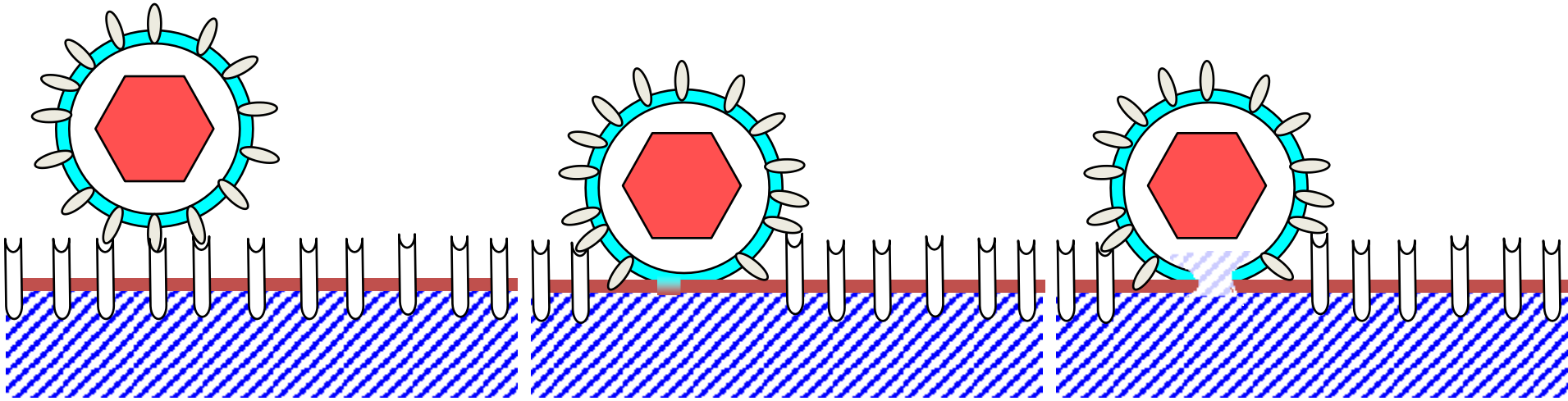
PENETRATION

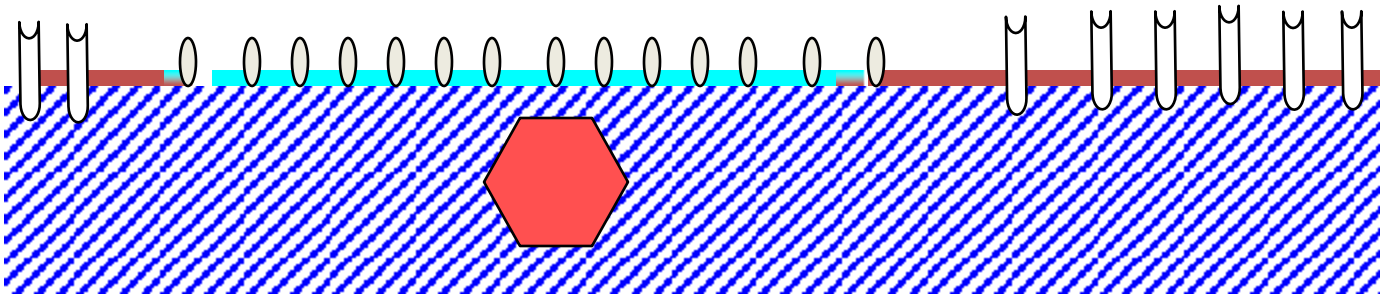
ENV VIRUS :FUSION WITH PLASMA MEMBRANE.EG:HIV ,ONLY NC ENTERS CYTOPLASM ,VIRAL ENVIS ATTACHED TO HOST CELL MEMBRANE

ENTRY VIA ENDOSOMES: VIROPEXIS OR PHAGOCYTOSIS

bacteriophage : capsid remains attached to cell wall only na enters

PENETRATION





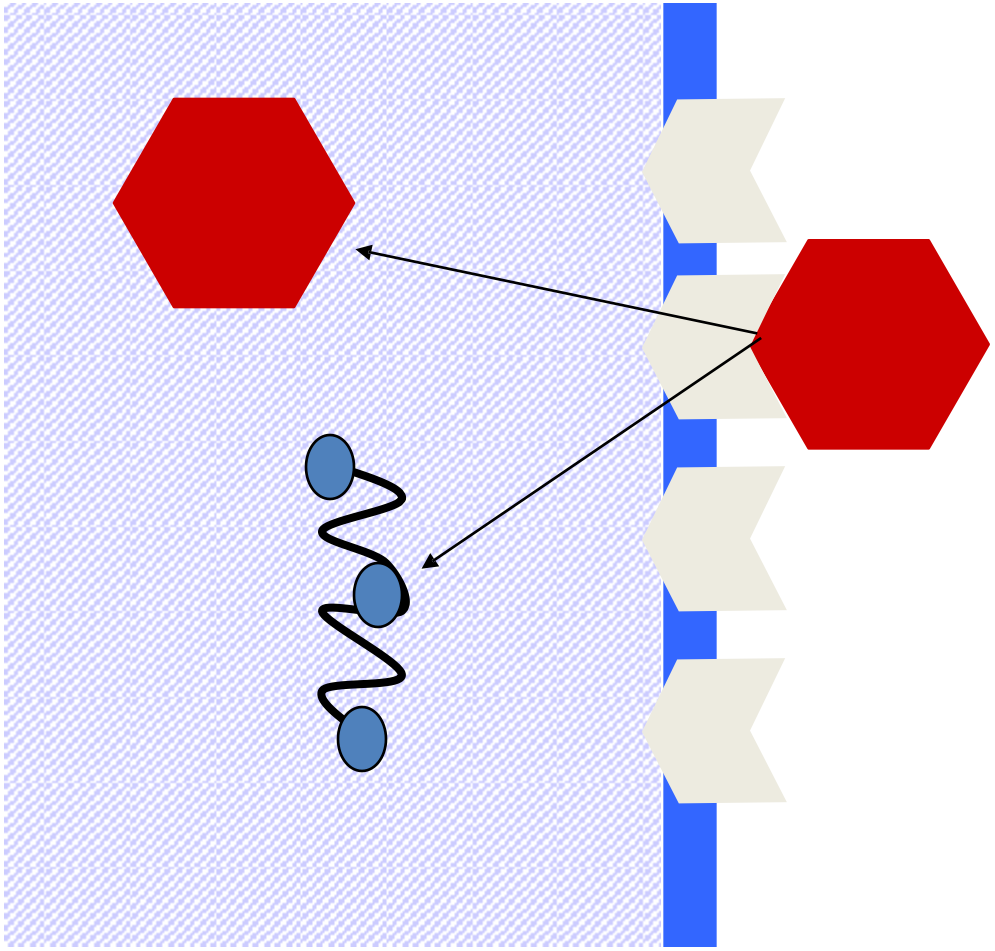
herpesviruses, paramyxoviruses, HIV

PENETRATION
- ENVELOPED VIRUSES

- FUSION WITH PLASMA MEMBRANE
- ENTRY VIA ENDOSOMES, FUSION WITH ACIDIC ENDOSOME MEMBRANE

PENETRATION
NON-ENVELOPED VIRUSES

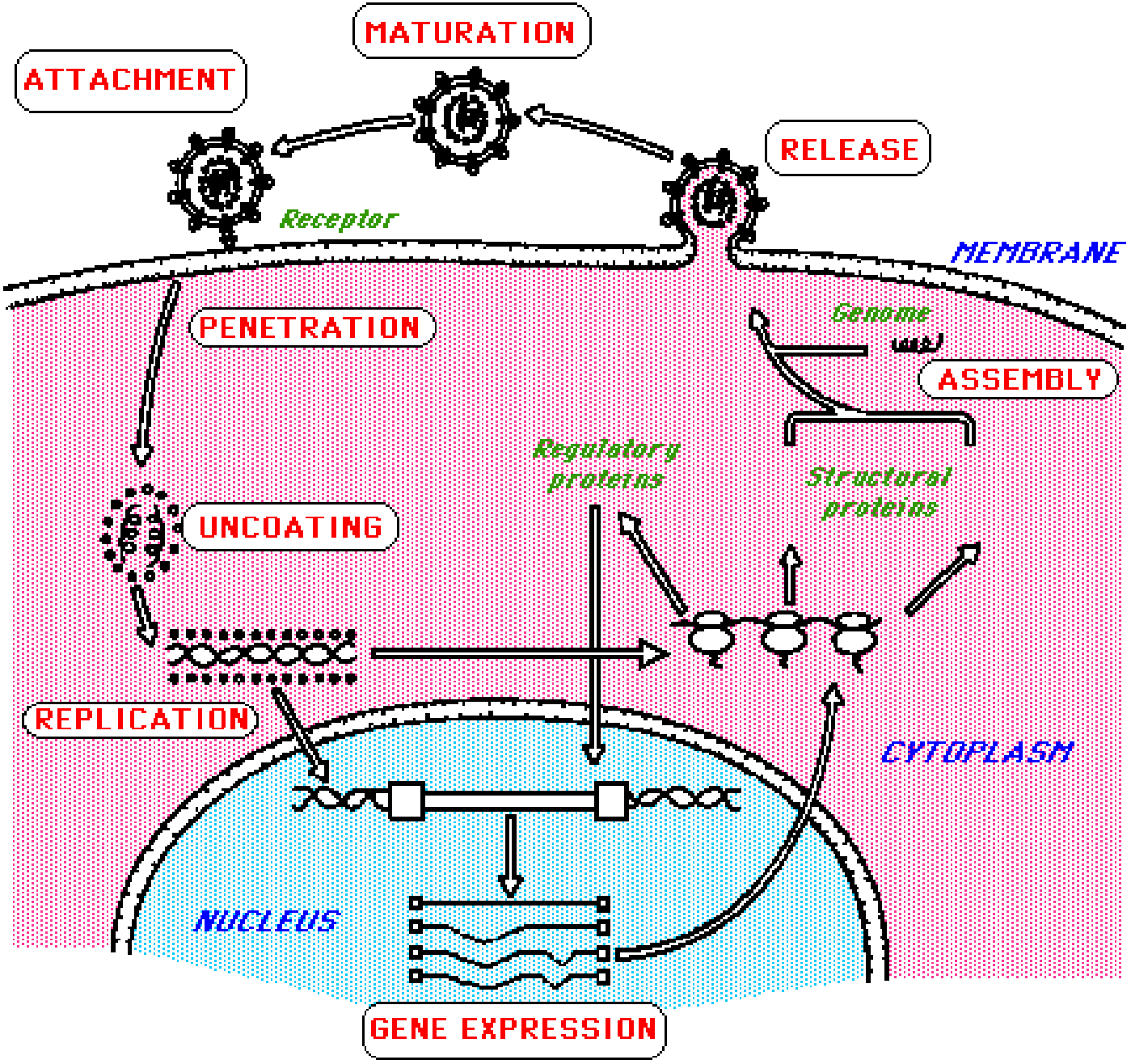
entry directly across plasma membrane:

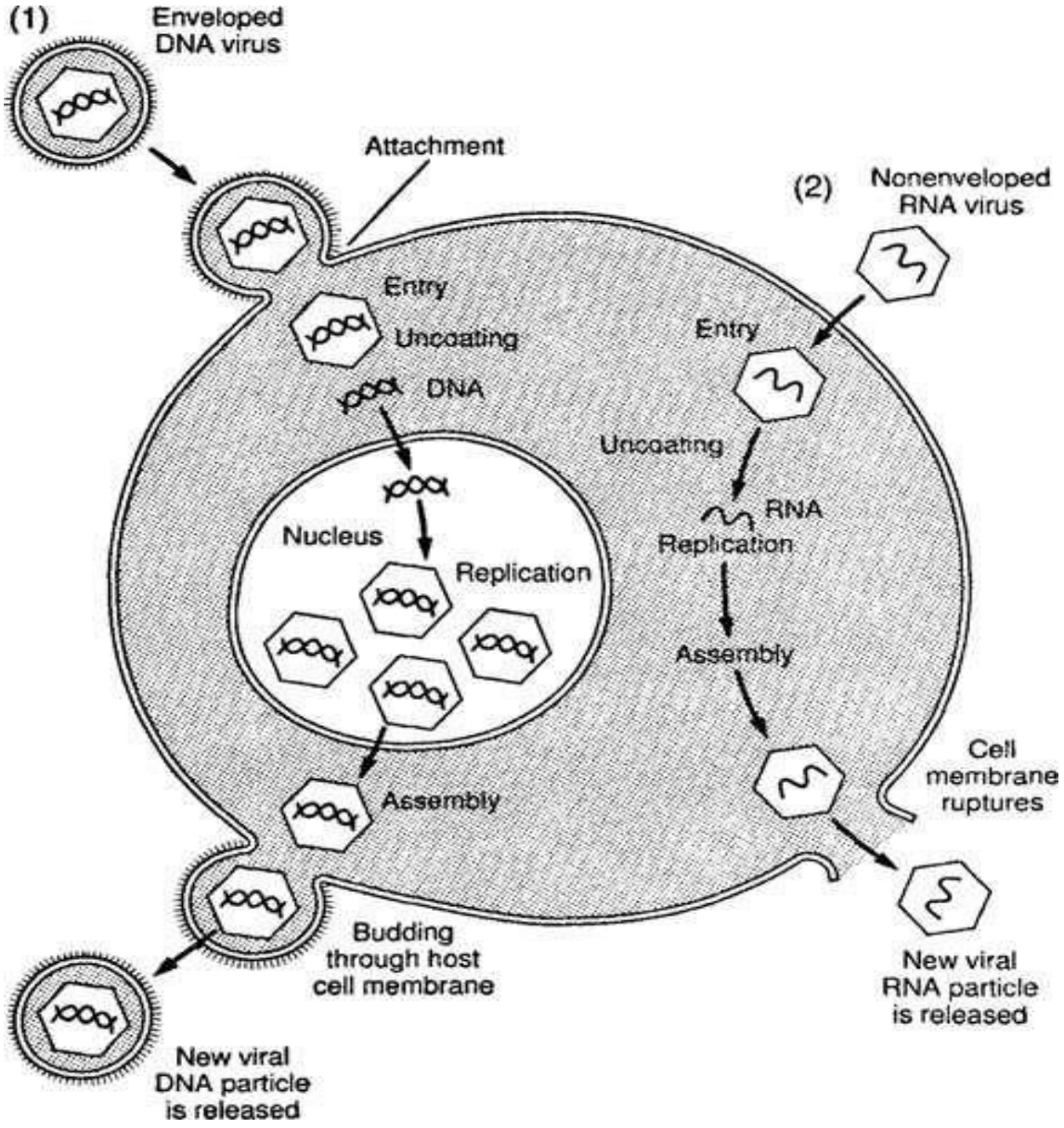


Replicative cycle

- "growth cycle" involves specific attachment of virus, penetration and uncoating, nucleic acid transcription, protein synthesis, maturation and assembly of the virions & release from the cell by budding or lysis.

DNA virus : replicate in nucleus except Pox
RNA virus: replication is in cytoplasm ex HIV &orthomyxo


MATURATION
ATTACHMENT
RELEASE
Receptor
MEMBRANE
PENETRATION
Genome
ASSEMBLY
Regulatory proteins
Structural proteins
UNCOATING
REPLICATION
CYTOPLASM
NUCLEUS
GENE EXPRESSION


(1)
Enveloped DNA virus
Attachment
(2)
Nonenveloped RNA virus
Entry
Uncoating
DNA
Nucleus
Replication
Assembly
Budding through host cell membrane
New viral DNA particle is released
Entry
Uncoating
RNA
Replication
Assembly
Cell membrane ruptures
New viral RNA particle is released

UNCOATING

- NEED TO MAKE GENOME AVAILABLE
- ONCE UNCOATING OCCURS, ENTER ECLIPSE PHASE
- ECLIPSE PHASE LASTS UNTIL FIRST NEW VIRUS PARTICLE FORMED

Lysosomal enzymes release NA from capsid.

SYNTHESIS OF VIRAL NUCLEIC ACID AND PROTEIN

- Nucleic acid may be made : nucleus or cytoplasm
- Protein synthesis is always in the cytoplasm

Type1: +ve ss RNA can directly translate to early proteins.
RNA polymerase can forms –ve ssRNA followed by
+ ssRNA & late proteins

Type II: -ve ss RNA transforms to +ve ssRNA which translates to form proteins& acts as template to form –ve ss RNA.

TypeIII: (ds RNA /Reovirus; segmented with +ve strand act as mRNA to form protein & forms a –Ve RNA.
The –ve strand will form +ve RNA .together dsRNA & assembly

TypeIV: Retrovirus- linear +ssRNA with RT & integrase enzyme form ssDNA forms DNA-RNA hybrid forms dsDNA attaches to host.

ASSEMBLY AND MATURATION

- NUCLEUS
- CYTOPLASM
- AT MEMBRANE

RELEASE

- LYSIS
- BUDDING THROUGH PLASMA MEMBRANE
- NOT EVERY RELEASED VIRION IS INFECTIOUS

Eclipse period: 30min to 30 hours
Env: acquired from plasma membr(inflz) or from nuclear mem(herpes)Viral glycoproteins are synthesised to saturate cell receptors

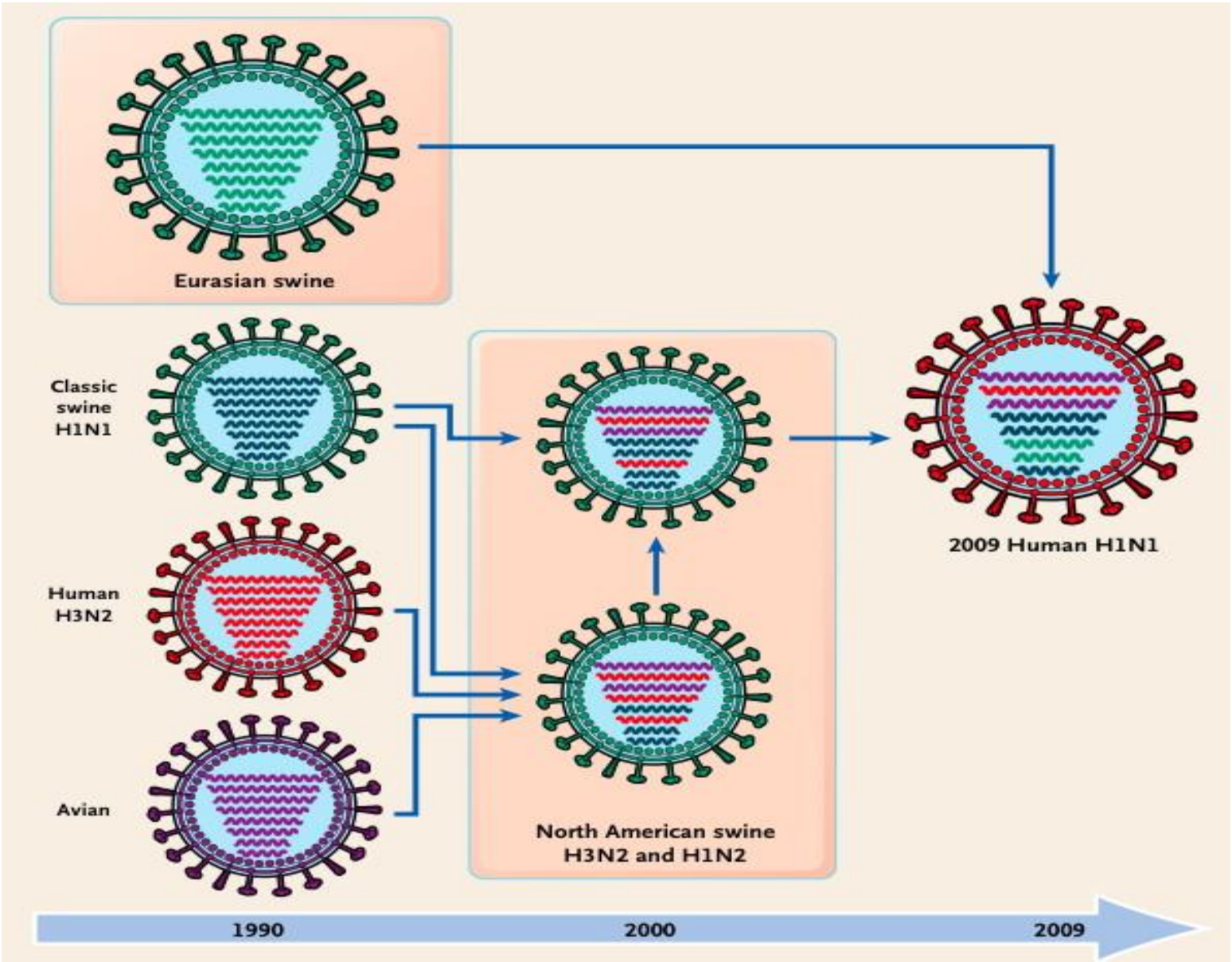
Abnormal Replication cycles:
Von Magnus phenomenon- incomplete virus with protein & no NA eg:Influz
Pseudovirions:virus encloses host cell NA so remain noninfective
Abortive infection: virus enter wrong host cell
Dependovirus: HepD

Viral Mutations: 10-4 to 10-8 mutations per bp per generation
Ts mutants used in vaccines
Genetic recombination:2 diff related virus infect same host cell can produce stable hybrids (COVID-19)
Reassortment: segmented virus: Rota,Reo,influz(2 strains of same virus)

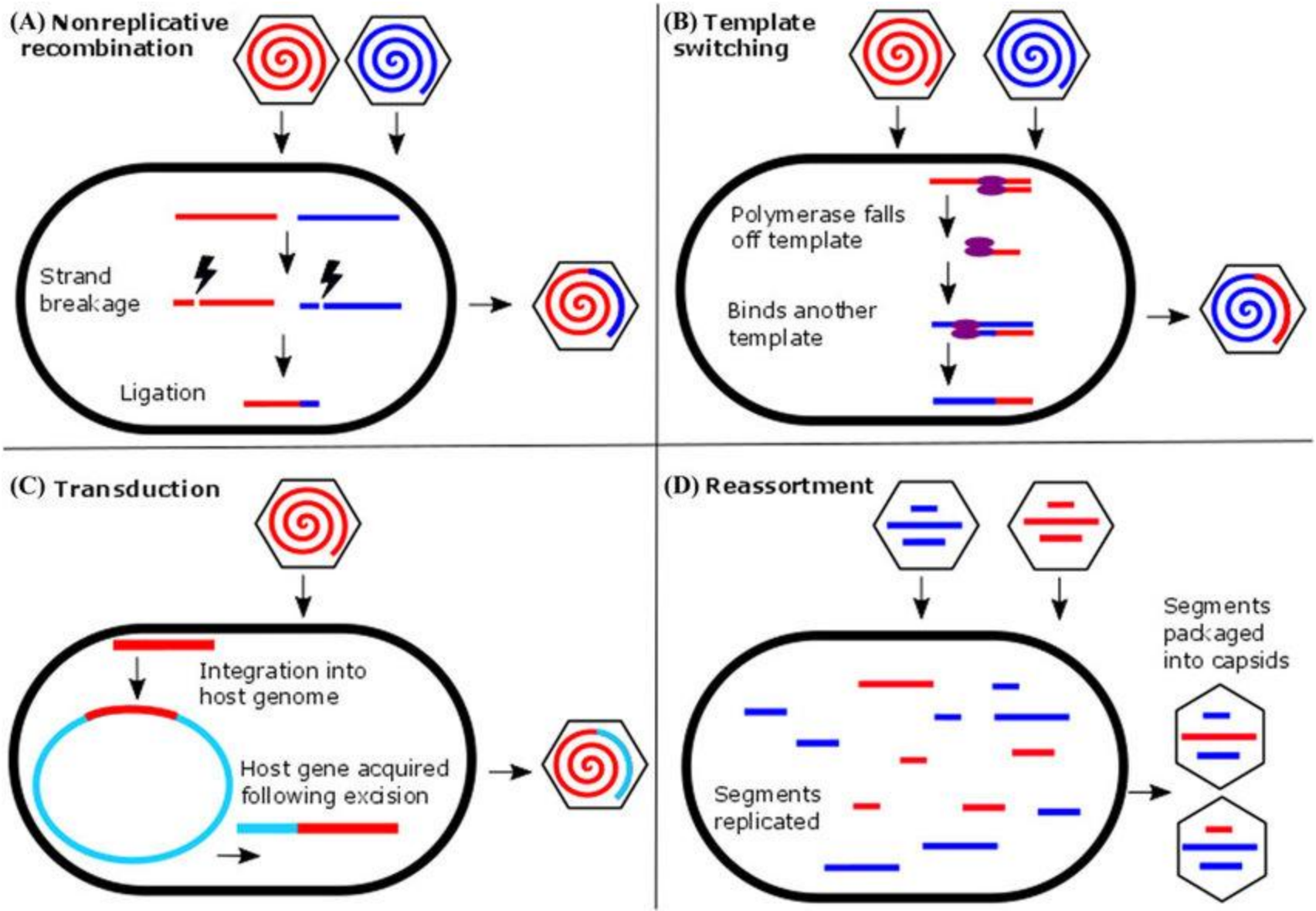
Genetic Reactivation:

- Marker Rescue: inactive & active virus(lab & virulent infz)
- Multiplicity Reactivation: eg in vaccine,inactive component gets activated (UV treated)
- Phenotypic mixing: genome surrounded by capsid of different but related virus(not stable)
- Pseudotype formation
- Genotypic mixing: diff virion surrounded by same.capsid..unstable.
- Complementation:host has 2 diff.virus(defective is rescued by stable virus)

Reassortment



Recombination



Transmission of Viruses

- Respiratory transmission:InfluenzA

  Faecal-oral transmission:Enterovirus

- Blood-borne transmission: HBV

- Sexual Transmission:HIV

- Animal or insect vectors
  - Rabies virus

- Transplacental route: Rubella, CMV, HSV, VZV, Parvo
- Conjunctival:adeno,entero70,cosacheA24,HSV
- Cutaneous: HSV, HPV, Molluscum contagiosum

Pathogenesis of viral infections

- viral infections are subclinical. It is not in the interest of the virus to severely harm or kill the host.
- The consequences of viral infections depend on the interplay between a number of viral and host factors.

Outcome

- **Acute Infection**
  - Recovery with no residue effects
  - Recovery with residue effects e.g. acute viral encephalitis leading to neurological sequale.
  - Death
  - Proceed to chronic infection
- **Chronic Infection**
  - Silent subclinical infection lifelong e.g. CMV, EBV
  - A long silent period before disease e.g. HIV, SSPE, PML
  - Reactivation to cause acute disease e.g. herpes and shingles.
  - Chronic disease with relapses and excerbations e.g. HBV, HCV.
  - Cancers e.g. EBV, HTLV-1, HPV, HBV, HCV, HHV-8

factors

- Effects of viral infection on cells (Cellular Pathogenesis)
- Entry into the Host
- Course of Infection (Primary Replication, Systemic Spread, Secondary Replication)
- Cell/Tissue Tropism
- Cell/Tissue Damage
- Host Immune Response
- Virus Clearance or Persistence

Spread of virus

- **Primary viremia**:virus spread to blood stream from pr. site or lymph node. virus remain free in plasma or cell associated .
- Secondary site replication:virus transported from blood to RES (BM,spleen,liver) with further multiplication
- **Secondary viremia**: from RES viruses spill over into blood.
- Target organs: from blood stream virus reach various organs or via neurons (rabies)
- Tropism

Tropism

- Lymphocytes: EBV,CMV,HBV,JC,BK virus

RNA: Mumps.Measles.rubella

- Monocyte/macrophage: CMV/ Polio.HIV.Measles
- Neutrophils: Influenza
- RBC: Parvo B19
- Free in plasma: polio,Toga virus

Virus shedding

- Portal of entry:local (influenza)
- Blood: vector bite or blood transfusion,needle pricks
- Target organ: skin(VZV) or salivary gland(mumps), kidney(CMV)
- No viral shedding:CNS(rabies)

Cellular pathogenesis

- Cellular response: (1) No apparent changeor abortive infection in non permissive cells, (2) Death, and (3) Transformation
- Direct cell damage (Cytocidal or Lytic):
  - shutoff of cell macromolecular synthesis
  - competition of viral mRNA for cellular ribosomes
  - competition of viral promoters and transcriptional enhancers for cellular transcriptional factors such as RNA polymerases, and inhibition of the interferon defense mechanisms.
  - Syncytia formation(glycoproteins fuse to neighbouring cells forming syncytia
- Indirect cell damage:
  - integration of the viral genome
  - induction of mutations in the host genome
  - inflammation
  - host immune response.
  - Immune mediated lysis

Cell tropism

Viral affinity for specific body tissues (tropism) is determined by

- Cell receptors for virus.
- Cell transcription factors that recognize viral promoters and enhancer sequences.
- Ability of the cell to support virus replication.
- Physical barriers.
- Local temperature, pH, and oxygen tension enzymes and non-specific factors in body secretions.
- Digestive enzymes and bile in the gastrointestinal tract that may inactivate some viruses.

Cell damage

- Viruses may replicate widely throughout the body without any disease symptoms if they do not cause significant cell damage or death.
- Retroviruses do not generally cause cell death, released from the cell by budding & noy cell lysis causing persistent infections.
- Picornaviruses cause lysis and death of the cells in which they replicate, leading to paralysis or death (usually due to respiratory failure)Eg Polio.
- Or fever and increased mucus secretion in the case of Rhinoviruses,

Immune response

- virus is cleared completely from the body and results in complete recovery.
- OR the immune response is unable to clear the virus completely and the virus persists.
- OR the immune response plays a major pathological role in the disease & tissue injury.
- CMI plays the major role in clearing virus infection & humoral immunity protects against reinfection.

Cytokines

- IFN α β: are protective produced by many cells
- IFNγ: mainly by T , NK, lymphocyes. Not antiviral but indirectly acts on macrophages and induces TIP(translation inhibitory protein) to stop viral replication
- Eg: protein kinase, NO synthetase, phosphodiesterase.
- RNA viruses are strong inducers of IFN& DNA kill cells.
- IFN α used treatment against(RhinoV, warts,HSV keratitis, HBV,HCV infections).

- Enhanced viral injury could be due to;-
  - Increased secondary response to cytotoxic T cells e.g. HBV
  - Specific ADCC or complement mediated cell lysis
  - Binding of un-neutralized virus-Ab complexes to cell surface Fc receptors, and thus increasing the number of cells infected e.g. Dengue haemorrhagic fever, HIV.:
  - Immune complex deposition in organs such as the skin, brain or kidney e.g. rash of rubella and measles.

Clearence or persistence

2 types of chronic persistent infections.

- True Latency - the virus remains latent following primary infection e.g. HSV, VZV. genome may be integrated into the cellular genome or exists as episomes.
- Persistence - the virus replicates continuously in the body at a very low level e.g. HIV, HBV, CMV, EBV.

Laboratory diagnosis

PURPOSE

- To start antiviral
- To isolate & identify virus in clinical samples.
- Screening blood donors
- Surveillance
- Outbreak investigations
- PEP
- Preventive measures in pregnancy
- Vaccine & Research

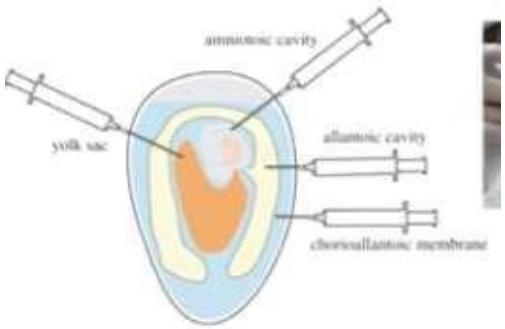
Direct demonstration

- EM
- IEM
- Fl M
- HPE ,giemsa stain for inclusion bodies
- Antigen/antibody detection :HAI,neutralization,CFT,ELISA,ICT,PCR
- Molecular tools

METHODS FOR CULTIVATION OF VIRUSES

Inoculation of virus into animals.

Inoculation of virus into embryonated eggs.



Tissue culture.

Cultivation of Viruses

Inoculation of Virus in Animals

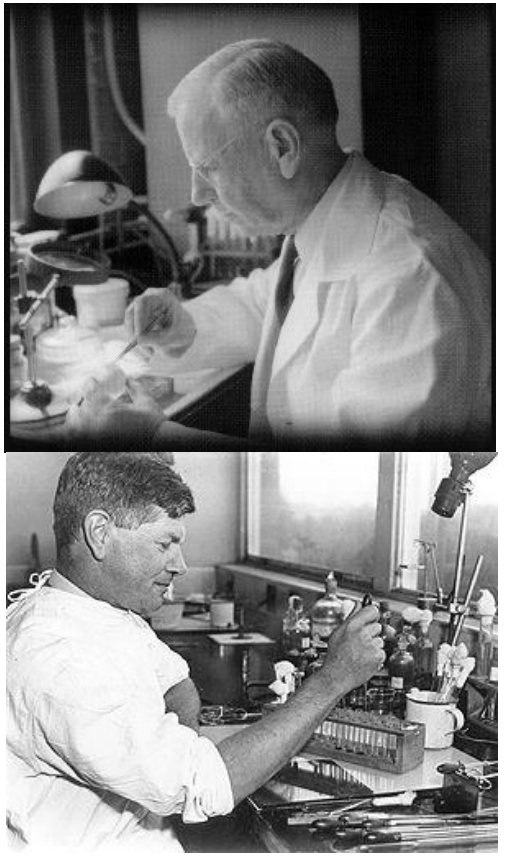
- For viral pathogenesis
- monkeys, mice, rabbits, guinea pigs for cultivating virus

Inoculation of Virus in Animals

- Different routes of inoculation in mice are:
  - intracerebral
  - subcutaneous
  - intraperitoneal
  - intranasal
- After the animal is inoculated with the virus suspension, the animal is:
  - observed for signs of disease
  - visible lesions
  - or is killed so that infected tissues can be examined for virus

Inoculation of Virus in Embryonated Eggs

- Goodpasture and Burnet in 1931 used the embryonated hen's egg for the cultivation of virus
- Eggs provide a suitable means for:
  - the primary isolation and identification of viruses
  - the maintenance of stock cultures
  - and the production of vaccines



Advantages

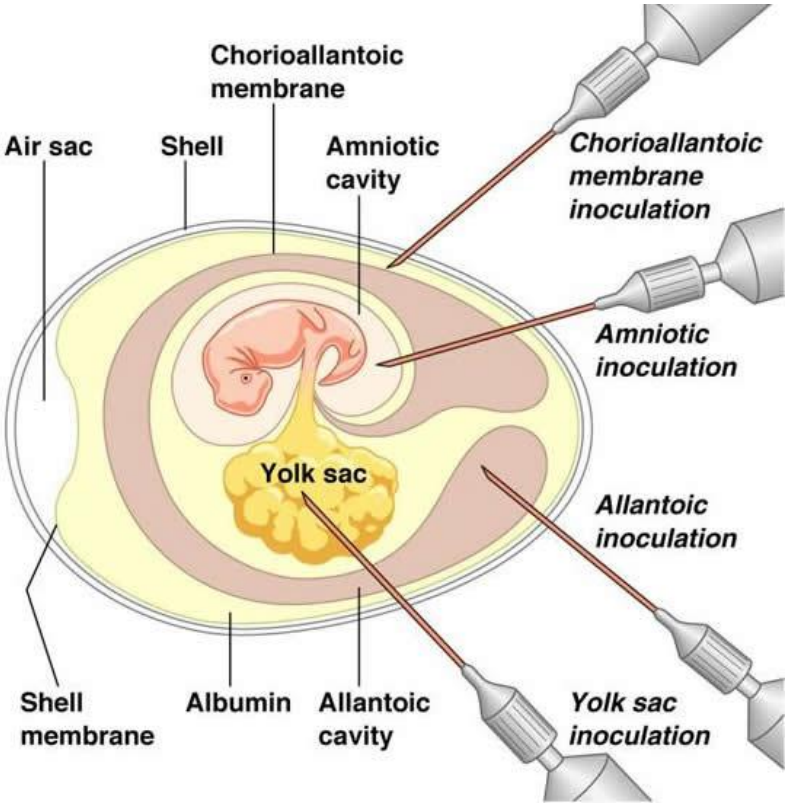
- eggs are simpler to handle than animals
- Defense mechanisms are not involved in embryonated eggs
- Eggs are economical and easily available
- They do not need feeding and caging
- Chick embryo offers many sites for cultivation of viruses

Inoculation of Virus

- The egg used for cultivation must be sterile and the shell should be intact and healthy.
- The egg must be injected through the shell, usually by drilling a hole or making a small window
- The viral suspension or suspected virus- containing fluid is injected into the fluid of the egg
- The exact tissue that is inoculated is guided by the type of virus being cultivated and the goals of the experiment

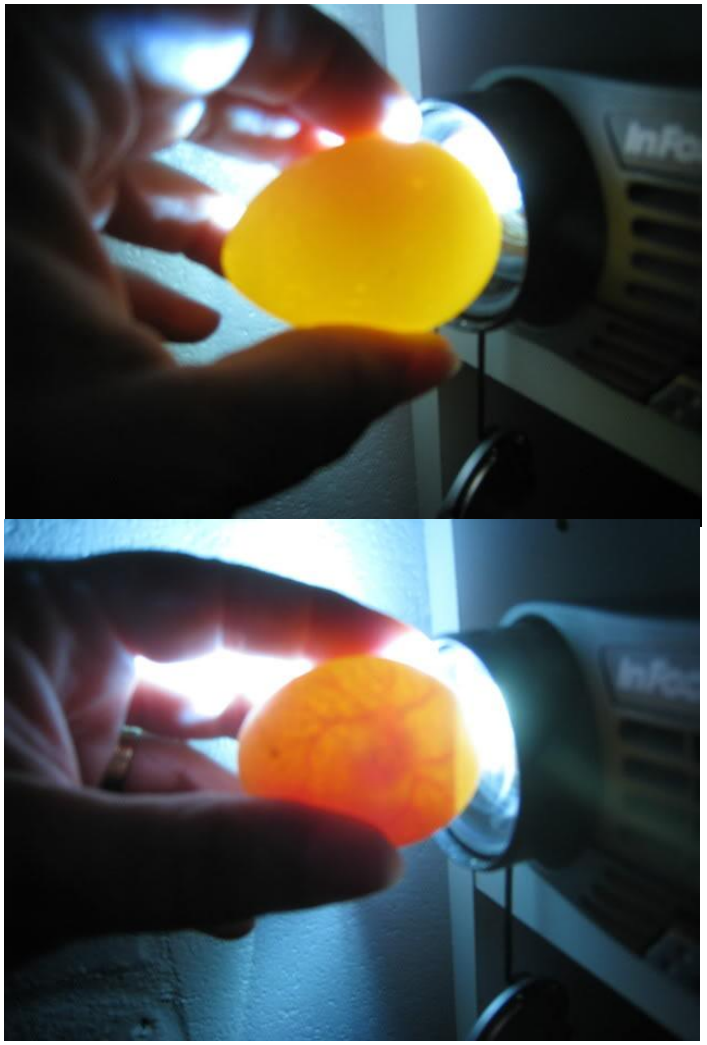
Routes of Viral Inoculation

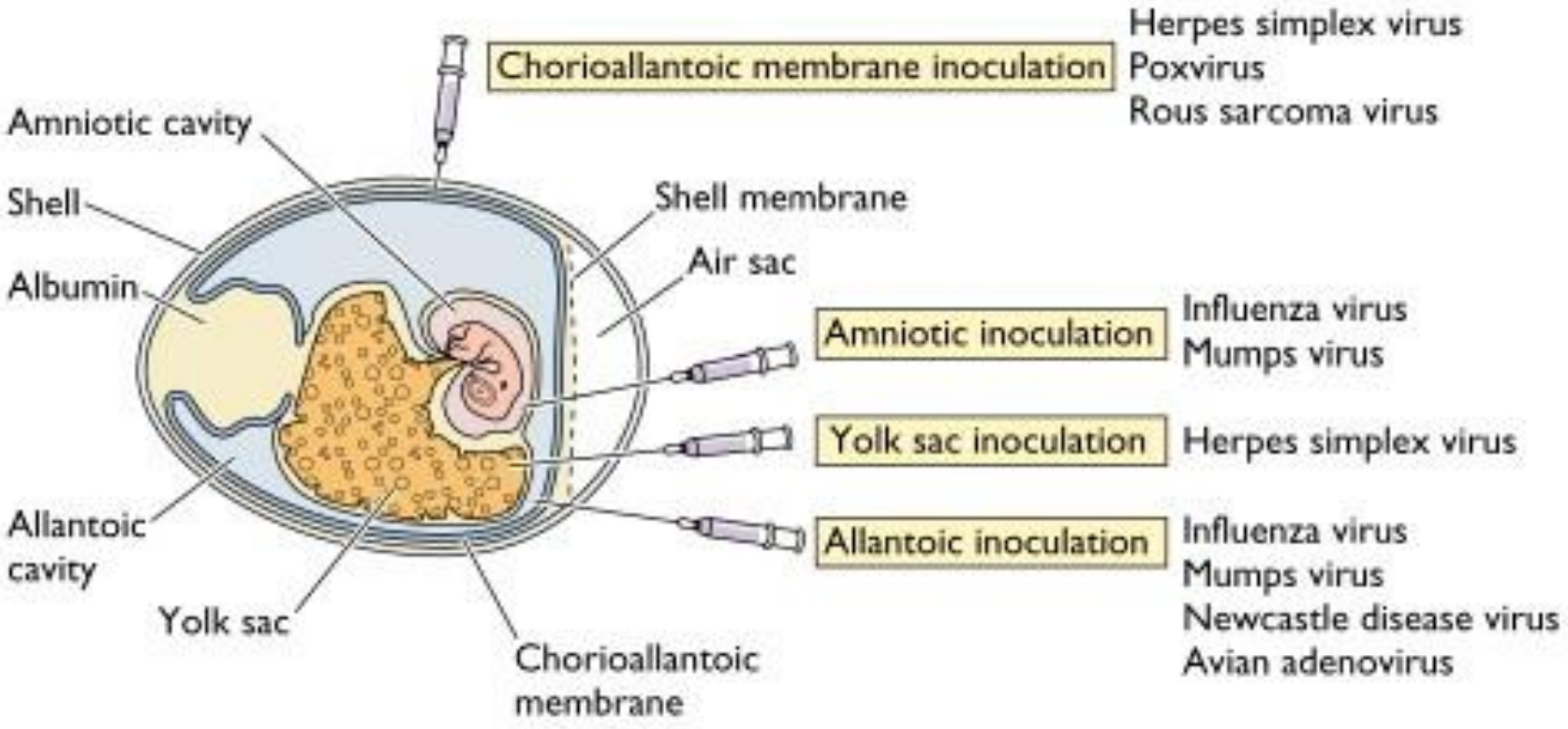
- sites of viral inoculation in embryonated eggs are:
  1. Chorioallantoic membrane (CAM)
  2. Amniotic Cavity
  3. Allantoic Cavity
  4. Yolk sac



Candling of Egg

- Candling is the process of holding a strong light above or below the egg to observe the embryo
- A candling lamp consists of a strong electric bulb covered by a plastic or aluminum container that has a handle and an aperture




Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Yolk sac
Chorioallantoic membrane

Tissue Culture

- Tissue culture is removal of cells, tissues or organs from an animal or plant & subsequent placement into artificial environment conductive to growth
- environment is of suitable glass or plastic culture vessel containing a liquid or semi-solid support medium with nutrients essential for survival and growth

Classes of Culture Cells

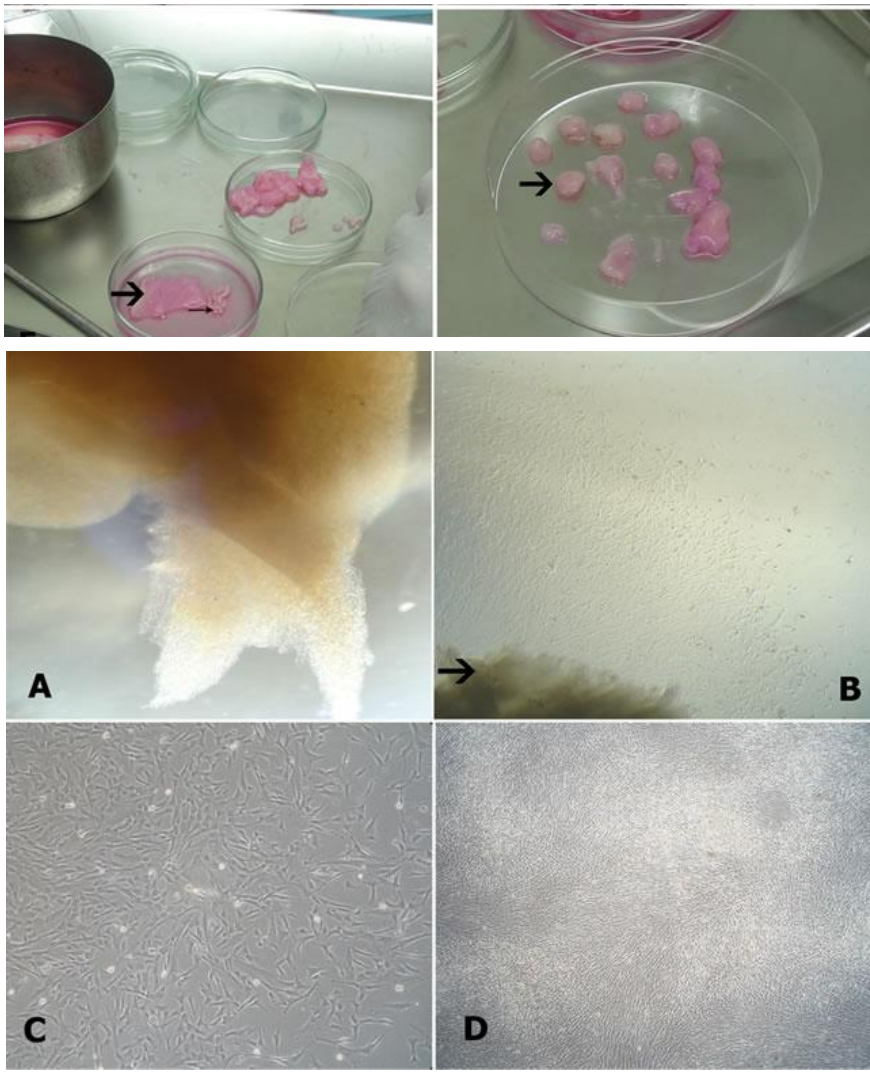
- Cultures of animal cells are usually divided into 3 classes:
  1. Primary cells
  2. Cell strains
  3. and cell lines

Primary Culture

- cells are surgically removed from an organism and placed into a suitable culture environment they will attach, divide and grow
- Most of the primary culture cells have a finite lifespan of 5-10 divisions in vitro
- Due to their limited lifespan, one cannot do long-term experiments with these cells

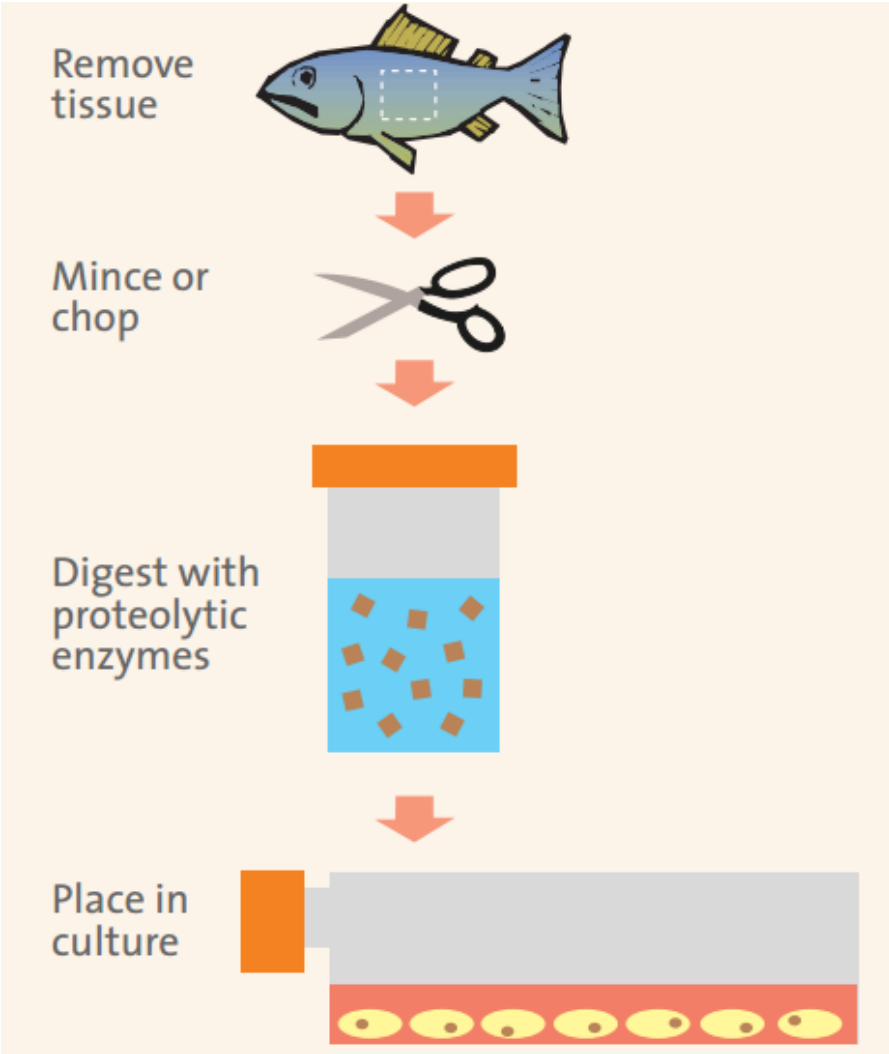
Primary Culture

- There are two basic methods for obtaining primary culture:
  1. **Explant cultures:**
     - Small pieces of tissue are attached (using plasma clots or fibrinogen) to a glass or treated plastic culture vessel and immersed in culture medium
     - After a few days individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow



Primary Culture

2. **Enzymatic dissociation:**
   - More widely used
   - speeds up the process by adding digesting (proteolytic) enzymes as trypsin or collagenase to the tissue fragments to dissolve the cement holding the cells together
   - creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide



Cell Strains

- Cell strains are cells that have been adapted to culture but have a finite division potential
- If these cells continue to grow at a constant rate over successive passages, these primary cells are referred to as a cell strain
- These cells have a finite lifespan of 40-60 divisions in vitro
- They are useful in vaccine production

Cell Lines

- If the cells in a cell strain undergo a transformation process (spontaneous or induced changes in morphology or growth properties) that makes them "immortal“ (able to divide indefinitely) they are called a cell line.

Culture Vessels

- **Flasks**

Culture Vessels

- **Cell culture dishes**

Culture Vessels

- **Multiwell plates**

Cell cultures in common use

a. Primary cell cultures	
1. Rhesus monkey kidney cell culture	
2. Human amnion cell culture	
3. Chick embryo fibroblast cell culture	
b. Diploid cell strains	
1. WI-38	Human embryonic lung cell strain
2. HL-8	Rhesus embryo cell strain
c. Continuous cell lines	
1. HeLa	Human carcinoma of cervix cell line
2. HEp-2	Human epithelioma of larynx cell line
3. KB	Human carcinoma of nasopharynx cell line
4. McCoy	Human synovial carcinoma cell line
5. Detroit 6	Sternal marrow cell line
6. Chang C/I/L/K	Human conjunctiva (C) Intestine (I), Liver (L) and Kidney (K) cell lines
7. Vero	Vervet monkey kidney cell line
8. BHK-21	Baby hamster kidney cell line

TABLE 10.2 Commonly Used Continuous Cell Cultures for Isolation and Detection of Enteric Viruses

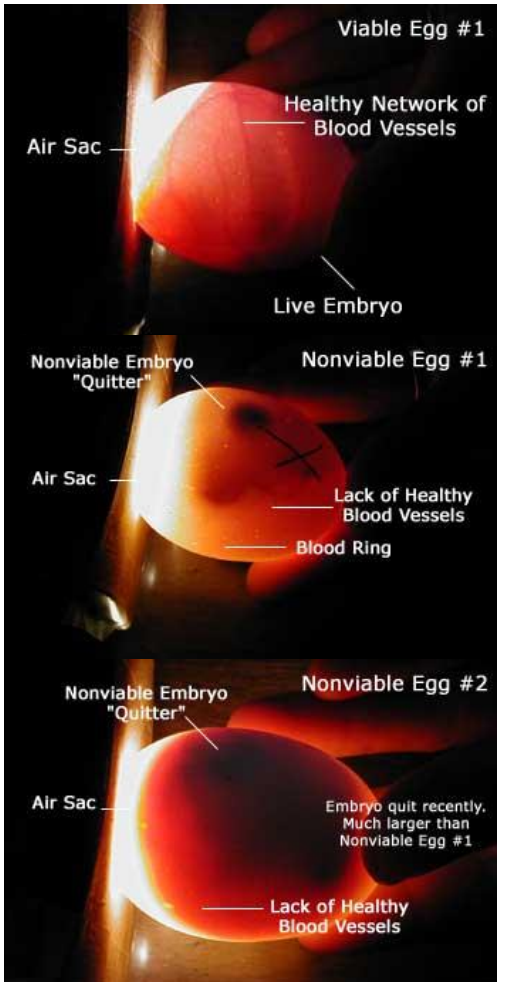
	Virus							
Cell culture line	Adeno	Astro	Coxsackie A	Coxsackie B	Echo	Polio	Rota	Hepatitis A
BGM	−[a]	−	−	+	+	+	−	−
BSC-1	−	*	−	*	*	+	*	*
CaCO2	+	+	*	+	+	+	+	+
Hep-2 (HeLa)	+	*		+	*	*	*	*
RD	*	*	+	−	+	*	*	
RfhK	*	*	*	*	*	*	*	+

[a] +, growth and/or production of cytopathogenic effect (CPE); −, no growth and/or production of CPE; *, no data.

Detection of virus growth

Detection of Viral Growth

- Viruses multiplying in embryos may or may not cause effects visible to the naked eye
- The signs of viral growth include:
  - Death of the embryo
  - Defects in embryonic development
  - and localized areas of damage in the membranes, resulting in discrete opaque spots called pocks



Detection of Viral Growth

- If a virus does not produce obvious changes in the developing embryonic tissue then:
- Embryonic fluids and tissues can be prepared for direct examination with an electron microscope
- Certain viruses can also be detected by:
  - their ability to agglutinate red blood cells
  - or by their reaction with an antibody of known specificity

Methods for detection of viruses in cell or Tissue culture

I. Cytopathic effect.

II. Haemadsorption.

III. Interference.

IV. Transformation.

V. Immunofluorescence.

VI. Metabolic inhibition.

1. Cytopathic effect

Many viruses cause morphological changes in cultured cells in which they grow. (CPE) .These changes can be readily observed by microscopic examination of the cultures

The CPE produced by different groups of viruses are characteristic and help in presumptive identification of virus isolate

- **enteroviruses** produce rapid CPE with crenation of cells and degeneration entire cell sheet ;

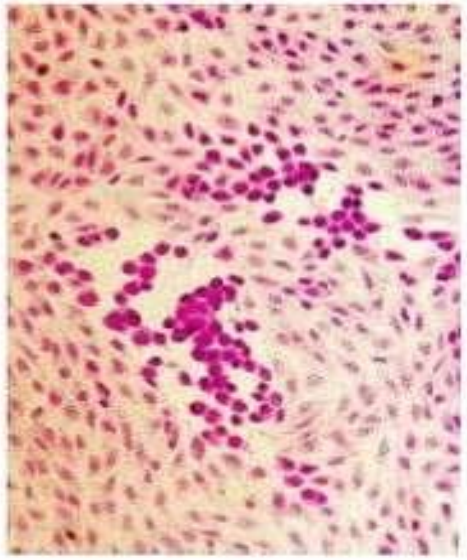
  measles virus produce syncytium formation;

  herpes virus causes discrete focal degeneration;

  adenovirus produce large granular clumps of grapes.

Cytopathic Effect (1)

Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells



Cytopathic effect of enterovirus 71 and HSV in cell culture: note the ballooning of cells.
(Virology Laboratory, Yale-New Haven Hospital, Linda Stannard, University of Cape Town)

2. Hemadsorption

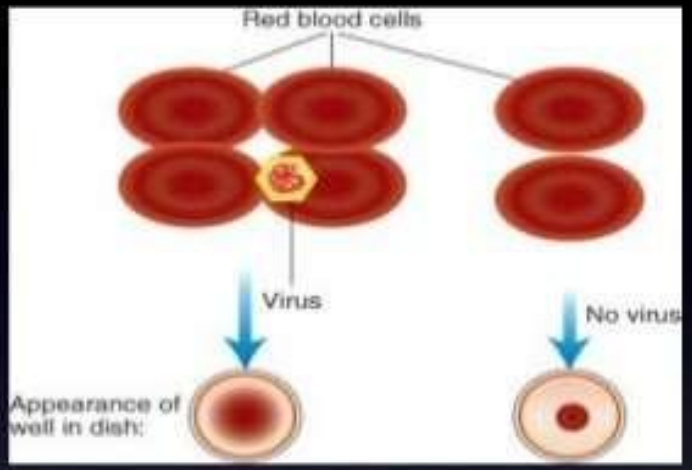
- When hemagglutinating viruses (influenza parainfluenza viruses) grow in cell cultures, their presence can be indicated by the addition of guinea pig erythrocytes to the cultures.

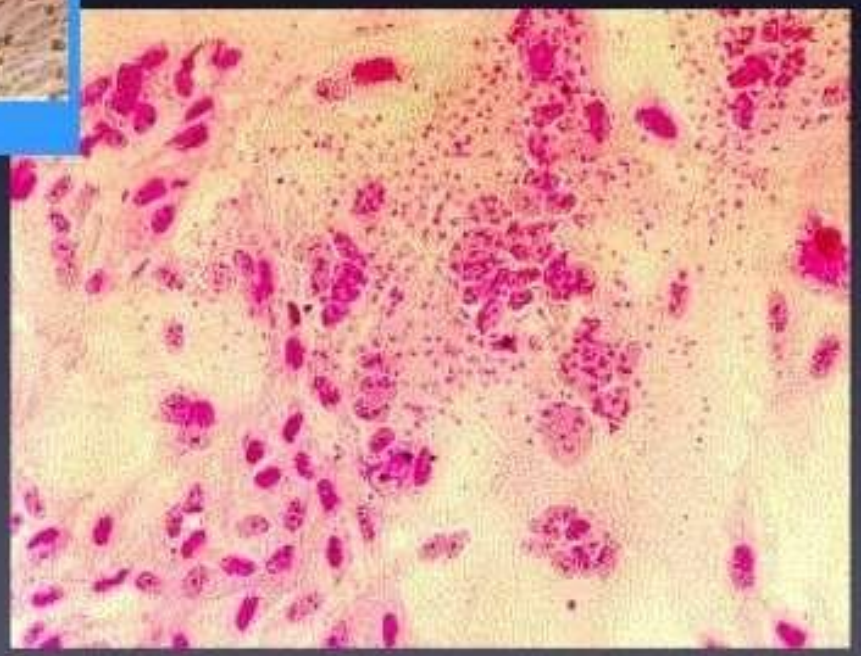
- If the viruses are multiplying in the cultures, the erythrocytes will adsorb onto the surface of cells. This is known as 'hemadsorption'.

HEMADSORPTION TEST

Hemadsorption - Influenza virus infected cells

MURRAY, 2nd Ed., Fig 53.5





Hemadsorption of red blood cells onto the surface of a cell sheet infected by mumps virus (Courtesy of Linda Stannard, University of Cape Town).

3. **Interference**

• The growth of first virus will inhibit second virus infection due to some inhibitory effect. This property of cell cultures is celled **interference. it is useful to detect** the growth of non-cytopathic viruses in cell cultures

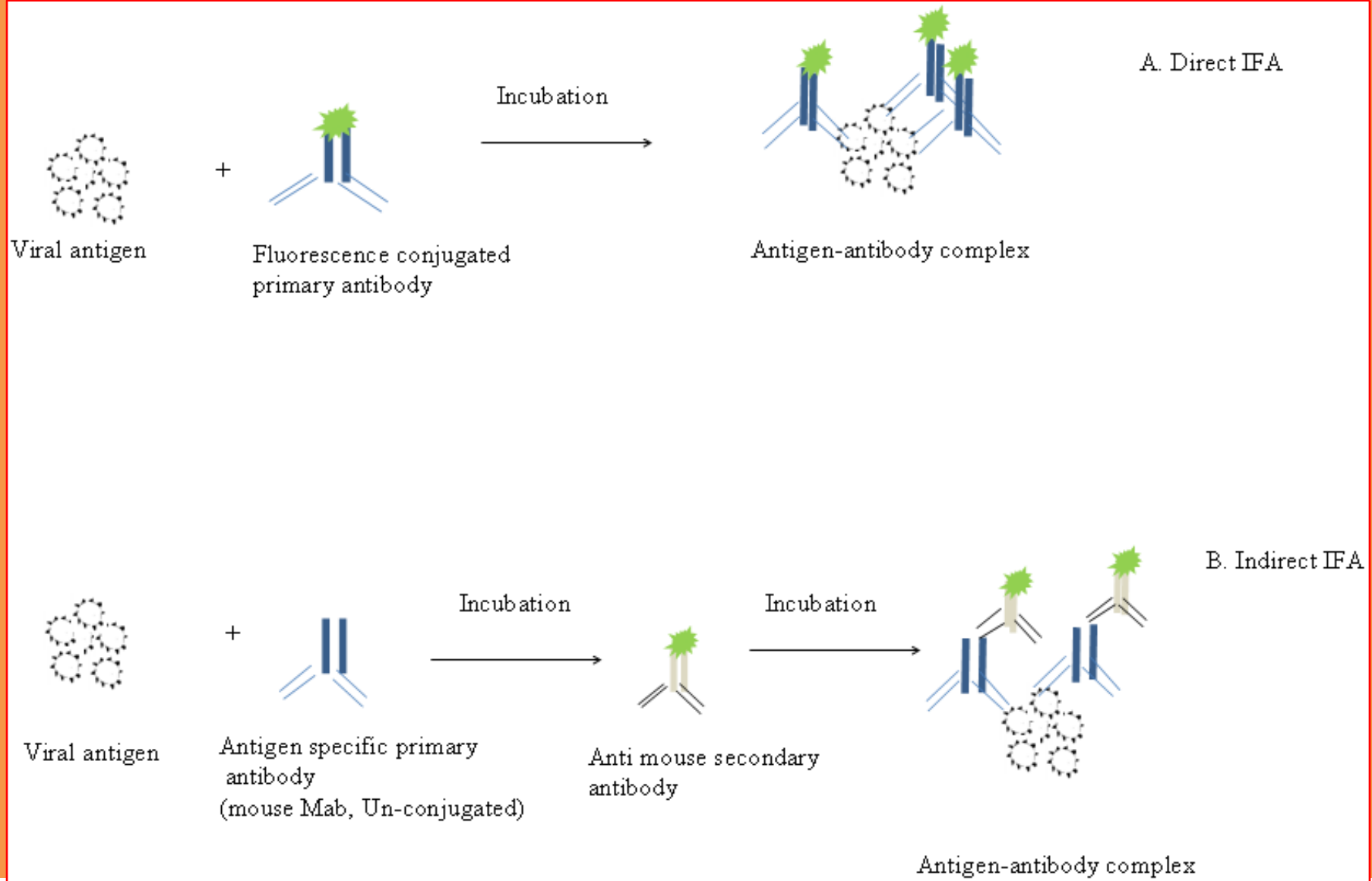
4. Transformation

If oncogenic viruses are inoculated into cell cultures, the infected cell grow fast and produce microtumours in the culture. This is called transformation. It indicated the presence of oncogenic viruses in the culture.

5. Immunofluorescence test

- Some cell from the cell culture are stained with a fluorescent dye conjugated antiserum and viewed under an UV microscope.
- Viral antigen present on the cell surface bind with the antiserum.
- Fluorescence from the cell is the positive indication for presence of virus in the cell. This is a widely used method in **diagnostic virology.**

Immunofluorescence Assay



6. Metabolic Inhibition

- In normal cell cultures, the medium turns acid due to the cellular metabolism.
- When viruses grow in cell cultures , the cell metabolism is inhibited and there is no acid production.
- This can be made out by the indicator (phenol red) incorporated in the medium.

Summary of Assay Methods for Viruses

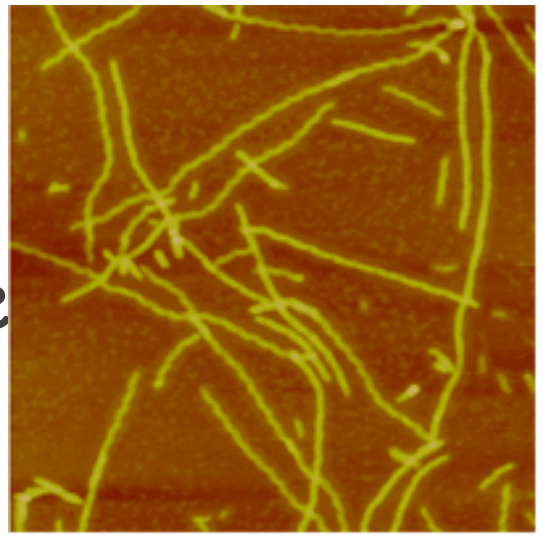
- Electron Microscopy (EM) and Immune EM
  - Insensitive (>1,000,000 particles/ml)
  - OK for clinical but not environmental virology
- Animal Infectivity
  - Slow, cumbersome, expensive, ethical considerations
- **Culture or infectivity**
  - **Now widely used in environmental virology**
  - **Cytopathogenic effects**
  - **Growth, but no cytopathogenic effects**
    - **detect viral antigens or nucleic acids**
- Immunoassays
  - insensitive for direct detection
  - Amplify viruses in cell cultures
- **Nucleic acid assays**
  - **insensitive for direct detection by hybridization**
  - **Amplify in cell culture or in vitro (PCR or RT-PCR)**

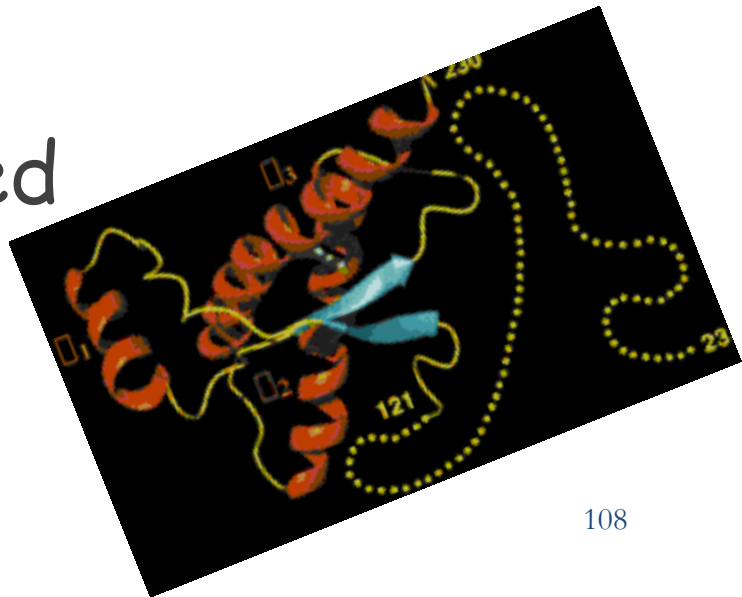
Viroids

- Small, circular RNA molecules without a protein coat
- Infect plants
- Potato famine in Ireland

Prions

- Prions are "infectious proteins"
- They are normal body proteins that get converted into an alternate configuration by contact with other prion proteins
- They have no DNA or RNA
- The main protein involved in human and mammalian prion diseases is called "PrP"





THANK YOU