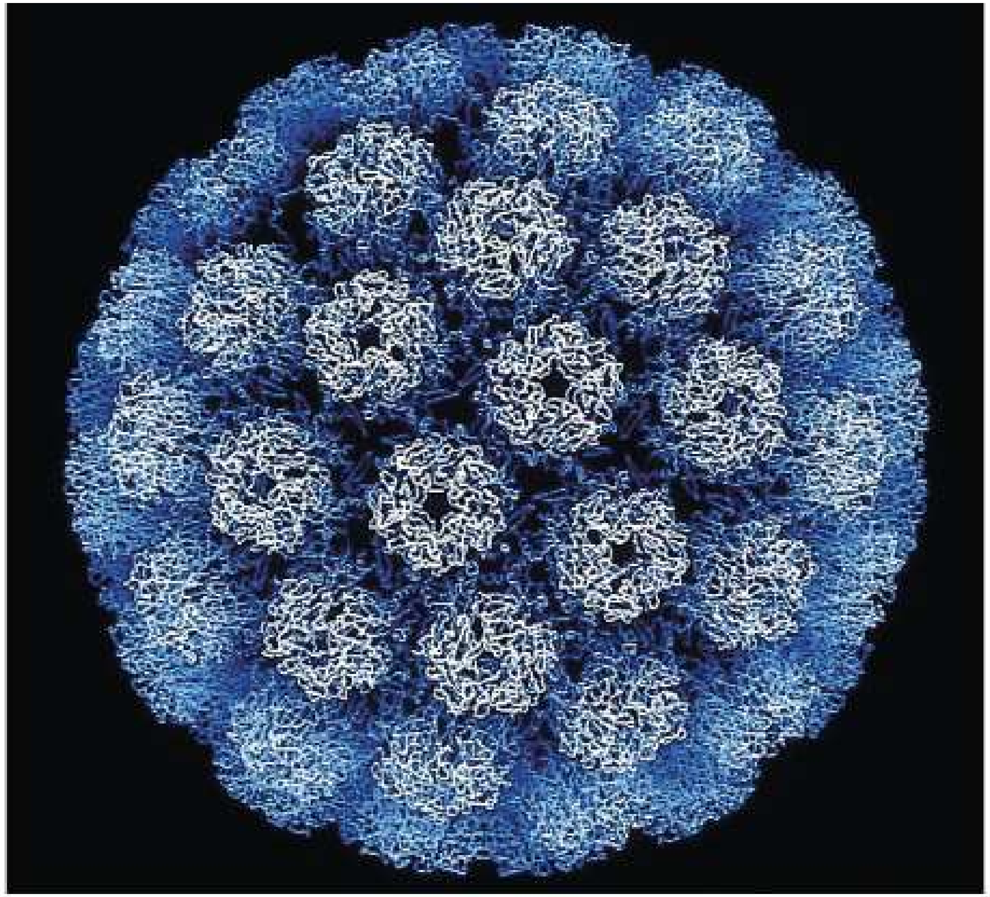
Perhaps the first written record of a virus infection consists of a heiroglyph from Memphis, drawn in approximately **1400 BC**, which depicts a temple priest called **Siptah** showing typical clinical signs of paralytic

B

MICROBIOLOGIA GENERALE

Virus structure, classification and cultivation

What is a virus?



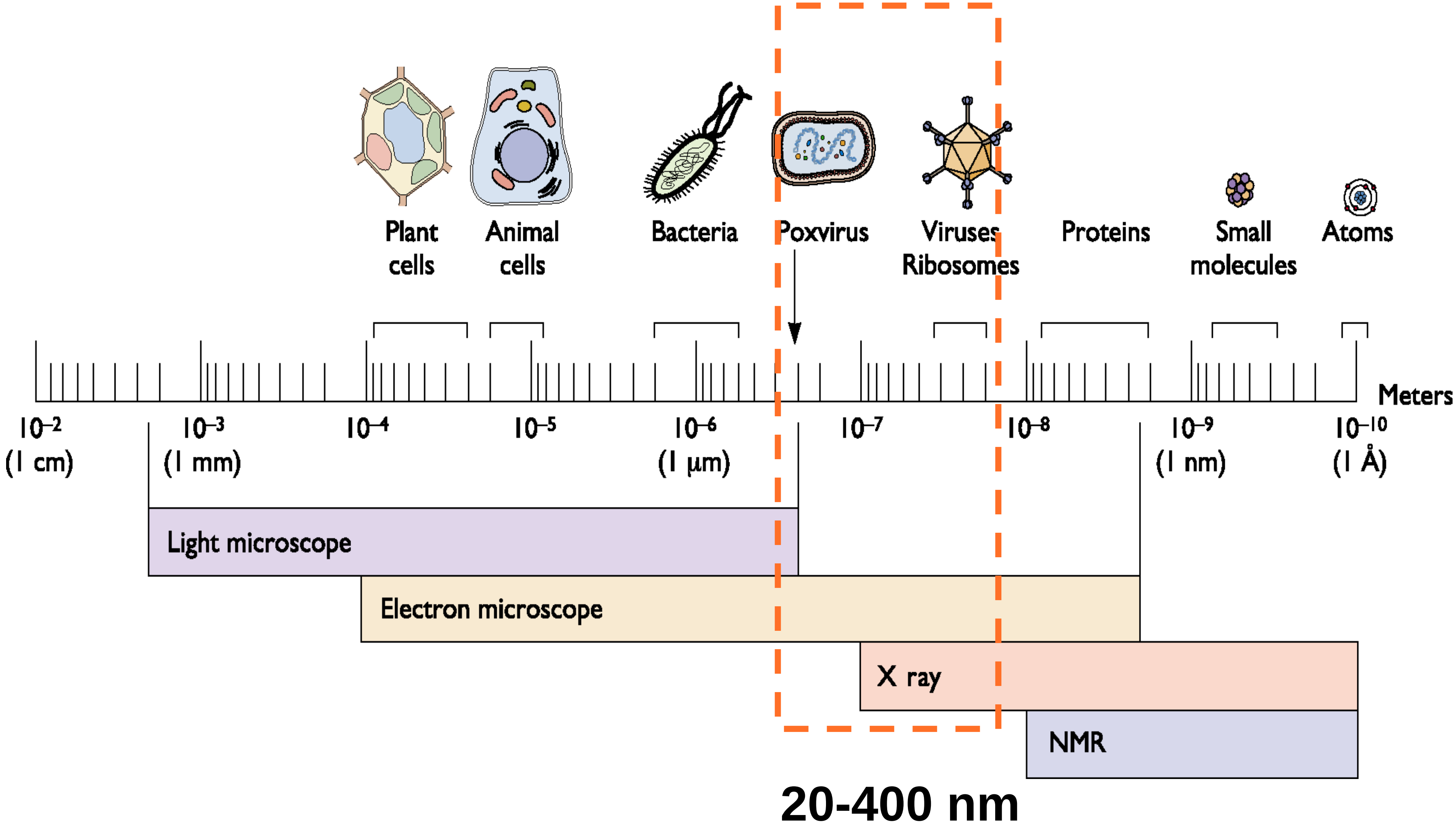
What is a virus?

- A virus is a very small, infectious, obligate intracellular (genetic) parasite.

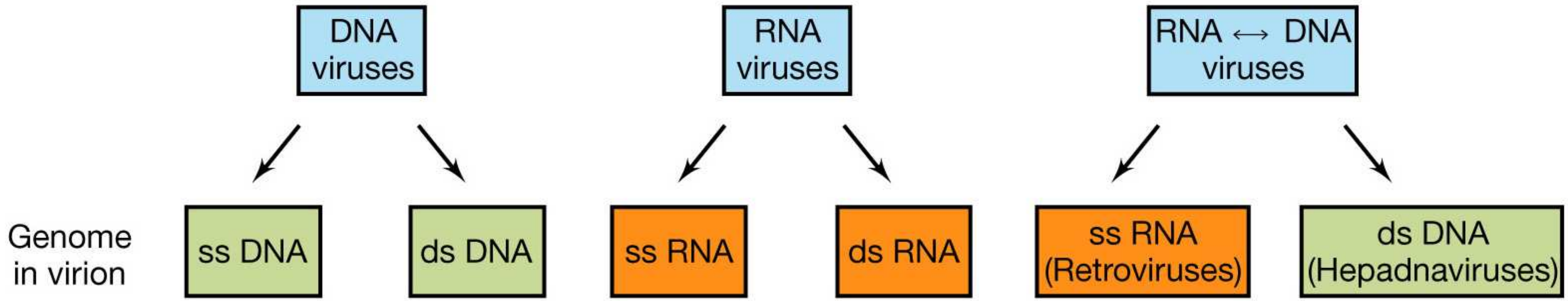
- Size typically 20-400 nM: rod shaped or spherical

- The virus genome comprise either DNA or RNA.

The small size of viruses

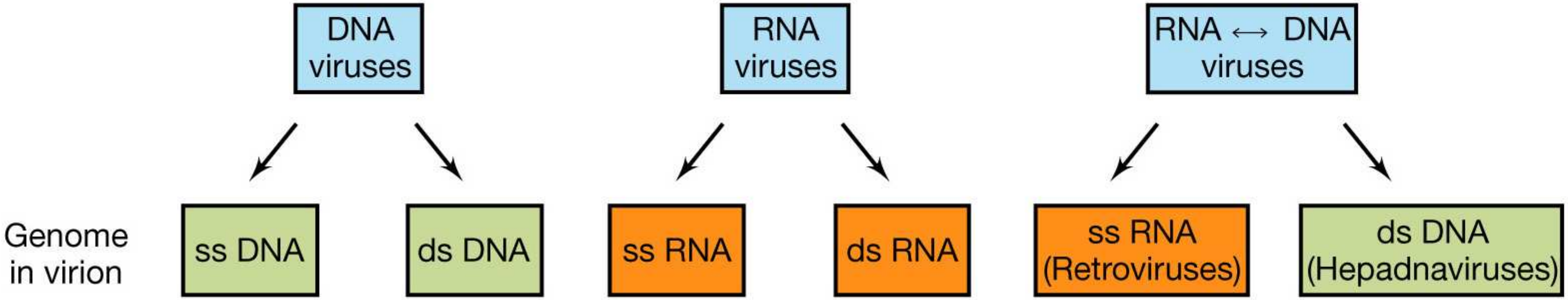


Virus genomes



- The **genomes of viruses** can be composed of either DNA or RNA, and some use both as their genomic material at different stages in their life cycle.
- However, only **one type** of nucleic acid is found in the virion of any particular type of virus.
- This can be **single-stranded (ss)**, **double-stranded (ds)**, or in the hepadnaviruses, partially double-stranded.

Virus genomes



MWs: ranging from 1.5 to 240 x10^6

Coding capacity: ranging from few proteins to >200 proteins

SS RNA genomes

- +ve (sense) and -ve (anti-sense) RNA genomes

Positive (sense) **AUG GCA CGA** → **met ala arg**

Negative (anti-sense) **UAC CGU GCU** ↑

What is a virus?

- **Viruses** lack the genetic information which encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (ribosomes)

- Within an appropriate host cell, the **viral genome** is replicated and directs the synthesis, by cellular systems, of other virion components

- A **progeny virion** assembled during the infectious cycle is the vehicle for transmission to the next host cell or organism where its disassembly leads to the beginning of the next infectious cycle

What is a virus?

•Unlike all living organisms, **viruses** do not 'grow' or undergo division but are produced from the assembly of pre-formed newly synthesized components within the host cell.

•Particles are produced from the assembly of pre-formed components; other agents 'grow' from an increase in the integrated sum of their components and reproduce by division. **Virus particles (virions)** themselves do not 'grow' or undergo division

• **Viruses** infect all types of living cells - animals, plants & bacteria.

Viruses follow a simple common three part general strategy to ensure their survival:

1 - All viruses package their genomes inside a **particle** that mediates transmission of the viral genome from host to host

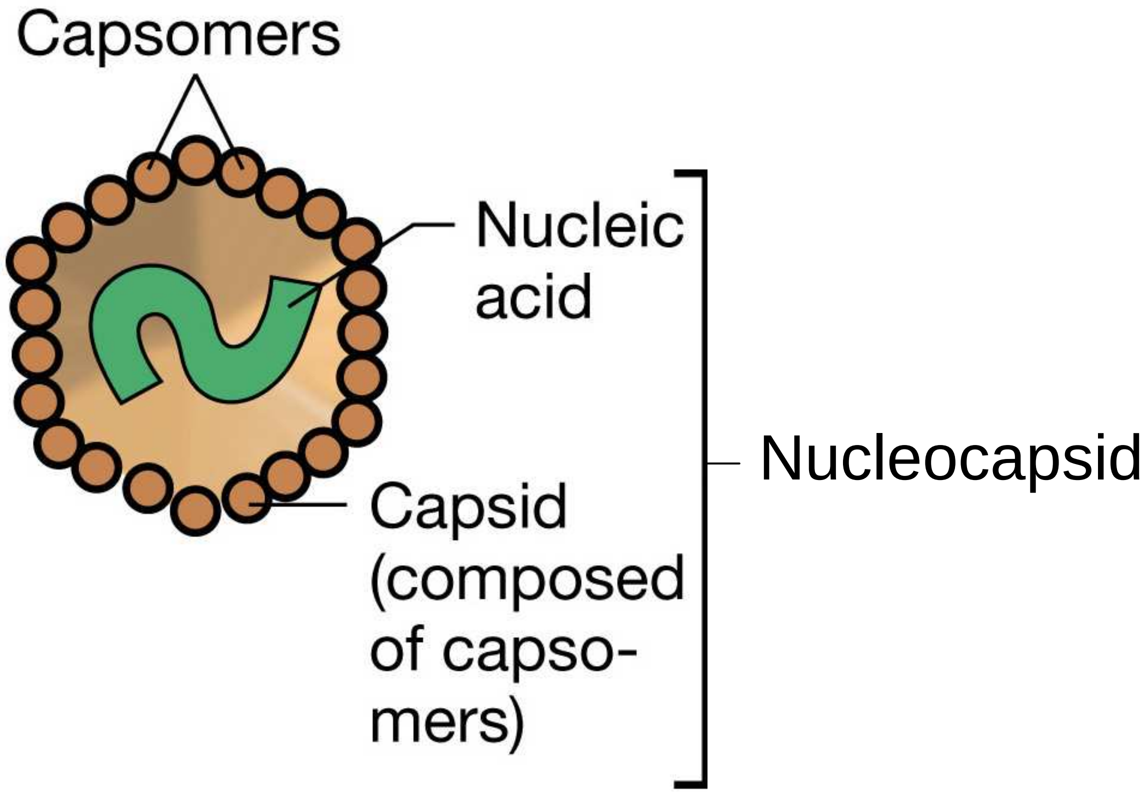
2 - The viral genome contains the informations for initiating and completing an **infectious cycle** within a susceptible, permissive cell

3 - All viruses are able to **establish themselves in a host population** so that virus survival is ensured.

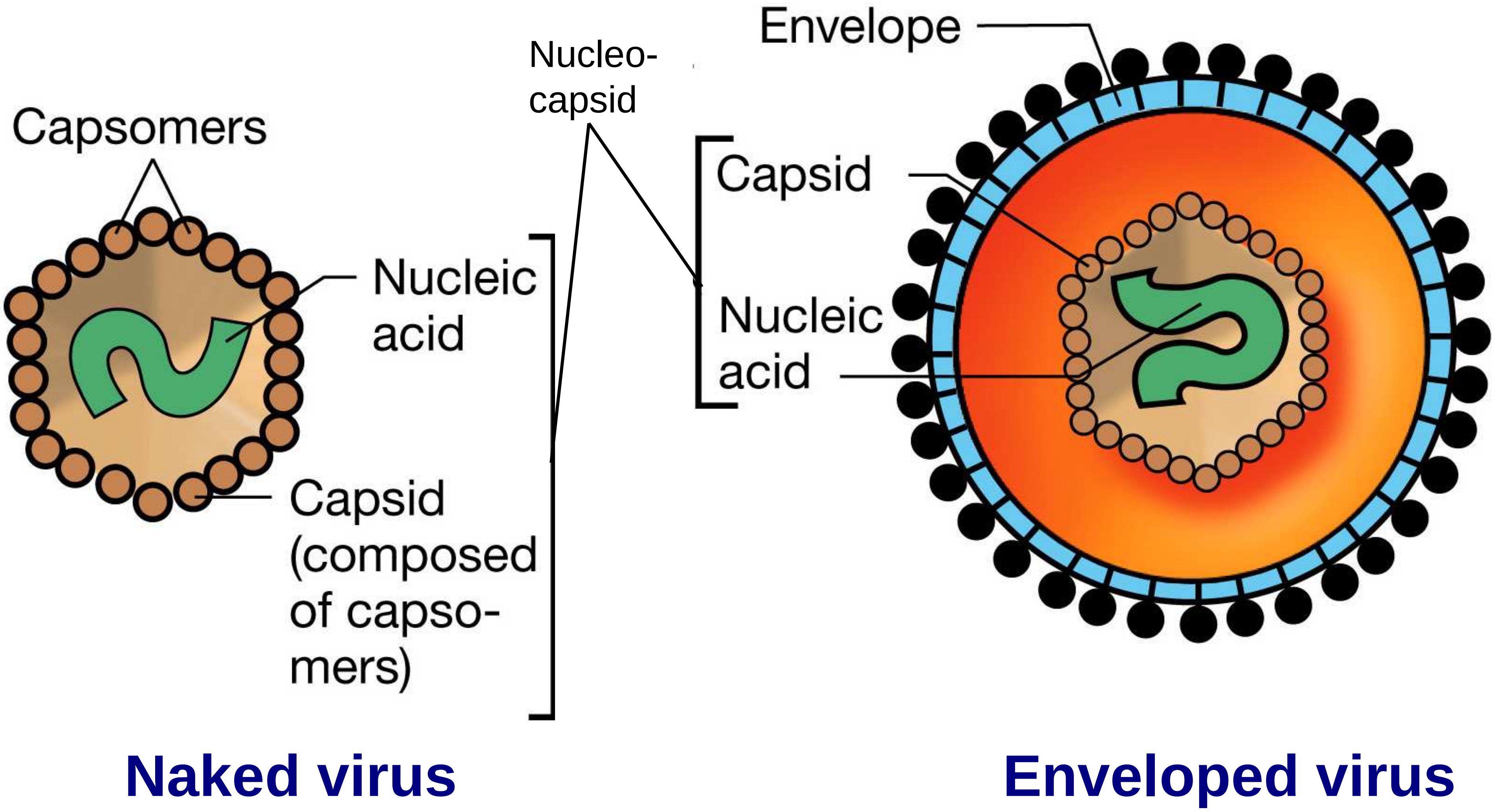
Introduction to Virology:

nature of the virion

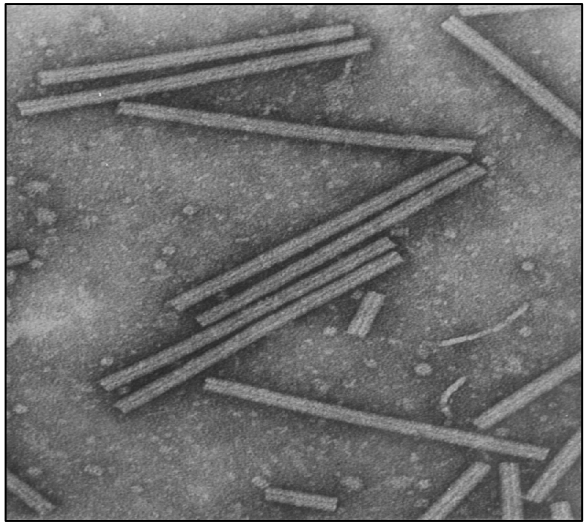
Virus structure: a schematic representation

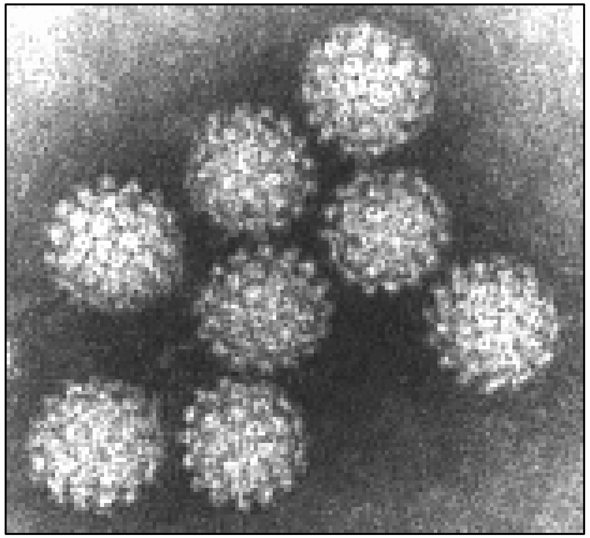


Virus structure: a schematic representation

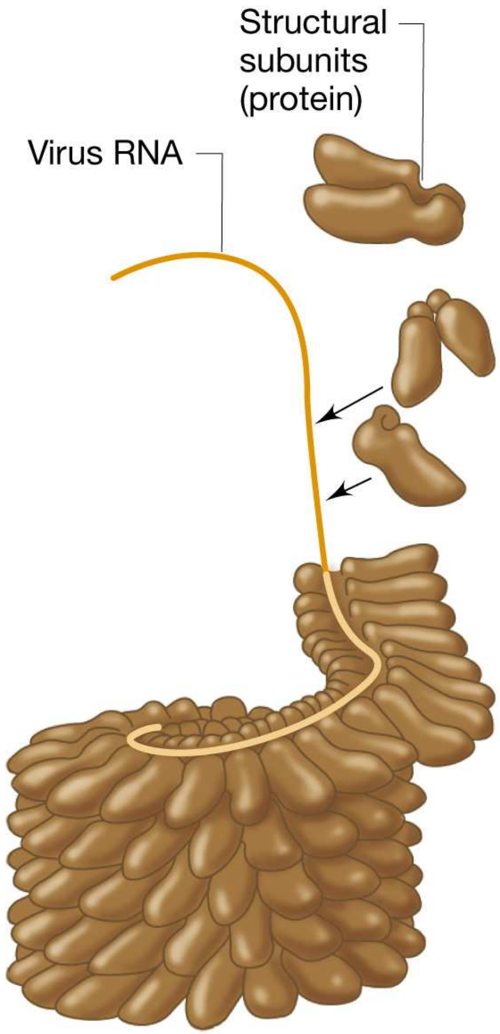


Structure of virus capsids





Structure of virus capsids: Helical symmetry

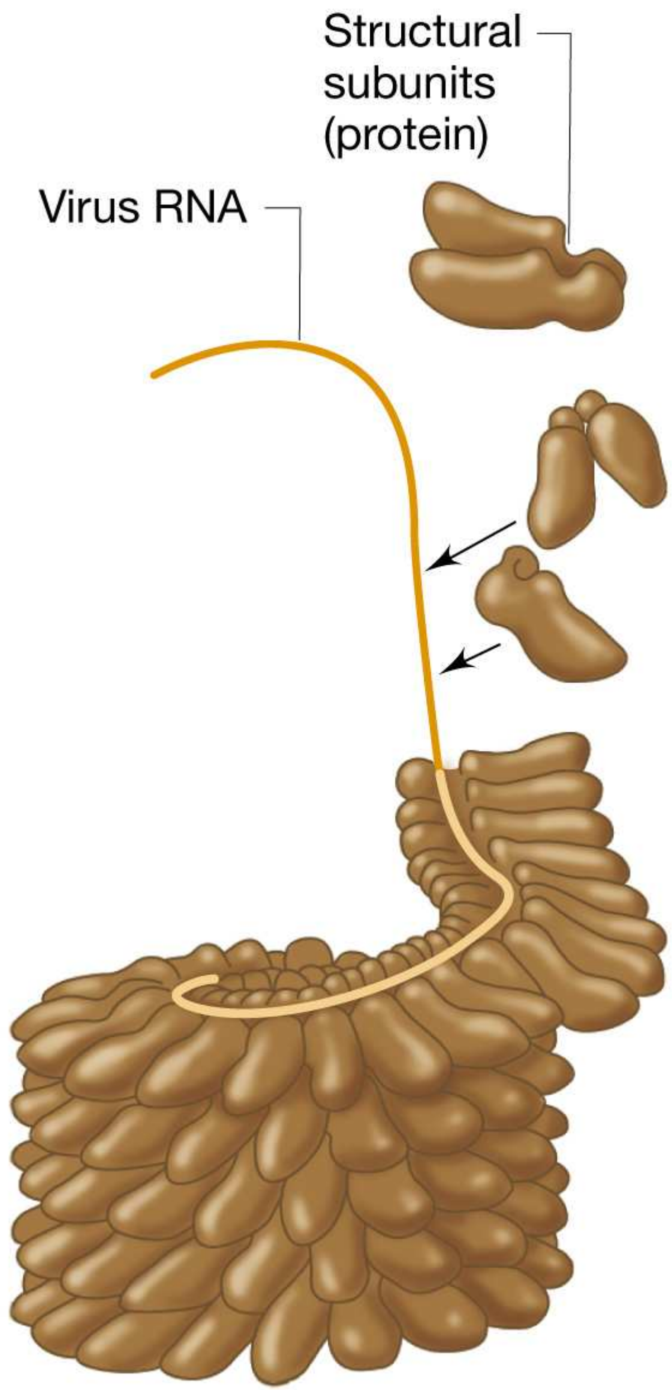


- The simplest way to arrange multiple, identical protein subunits is to use rotational symmetry and to arrange the irregularly shaped proteins around the circumference of a circle.
- 20-30nm wide, length variable, 300nm.
- One coat protein suffices.
- Sometimes flexible.

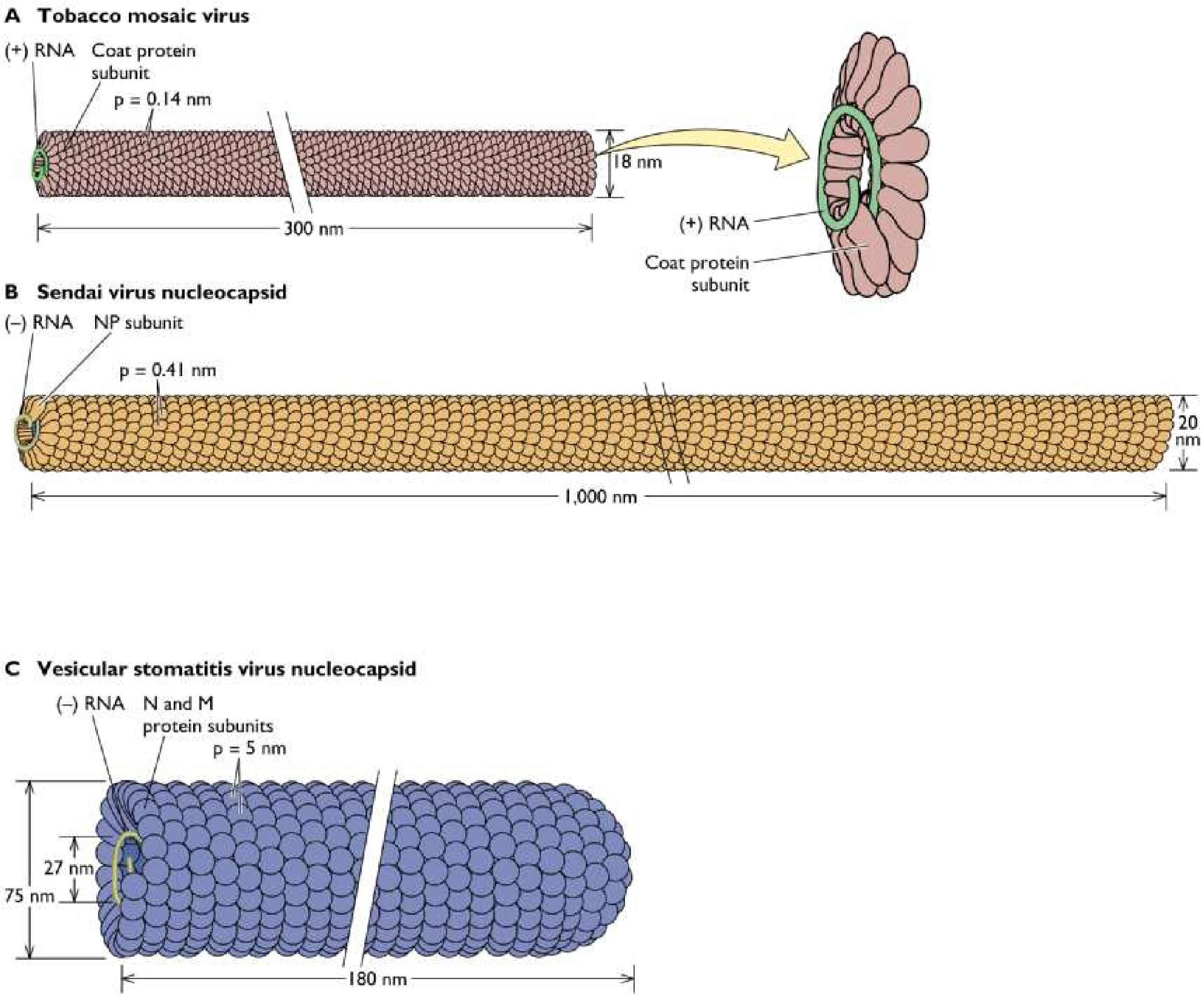
- Arrangement of virus nucleic acid and protein coat in a tobacco mosaic virus **(TMV).**

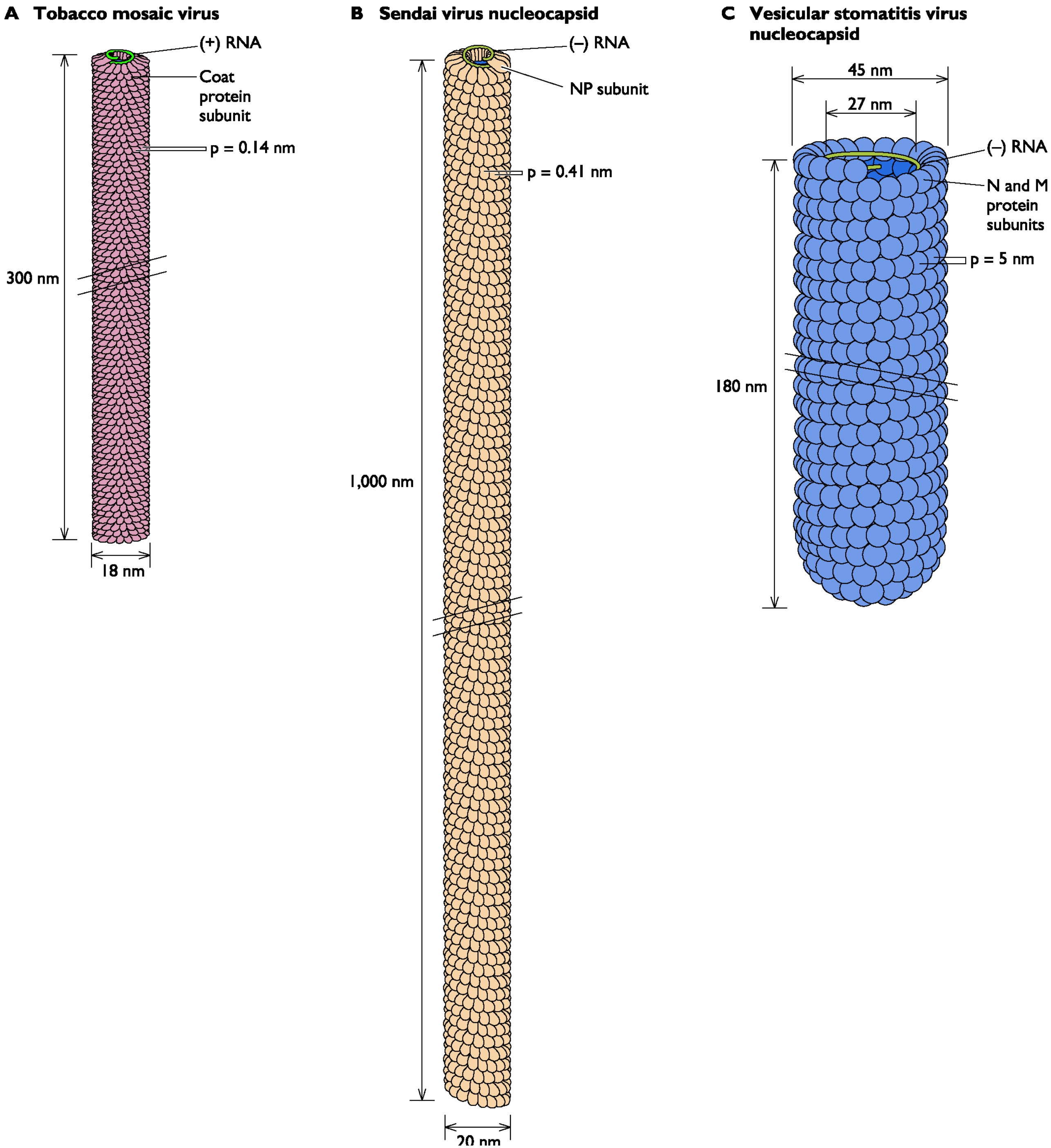
- The RNA assumes a helical configuration surrounded by the protein capsid.

- The center of the particle is hollow.

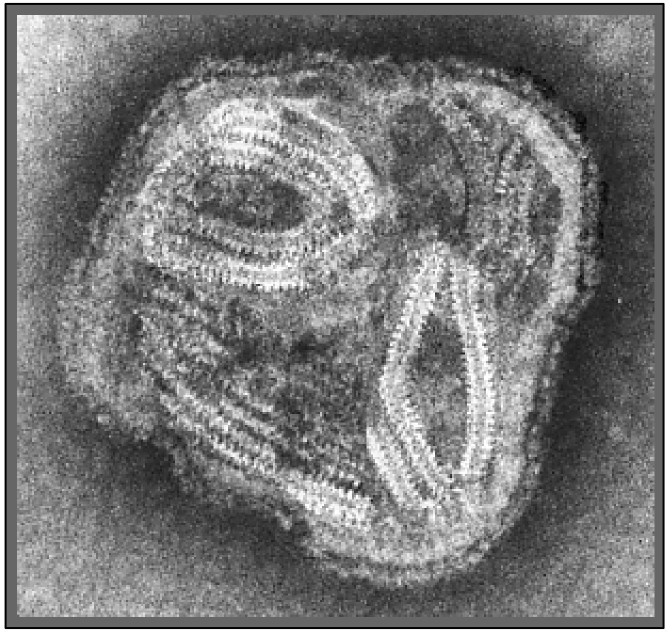


Structure of virus capsids: Helical symmetry

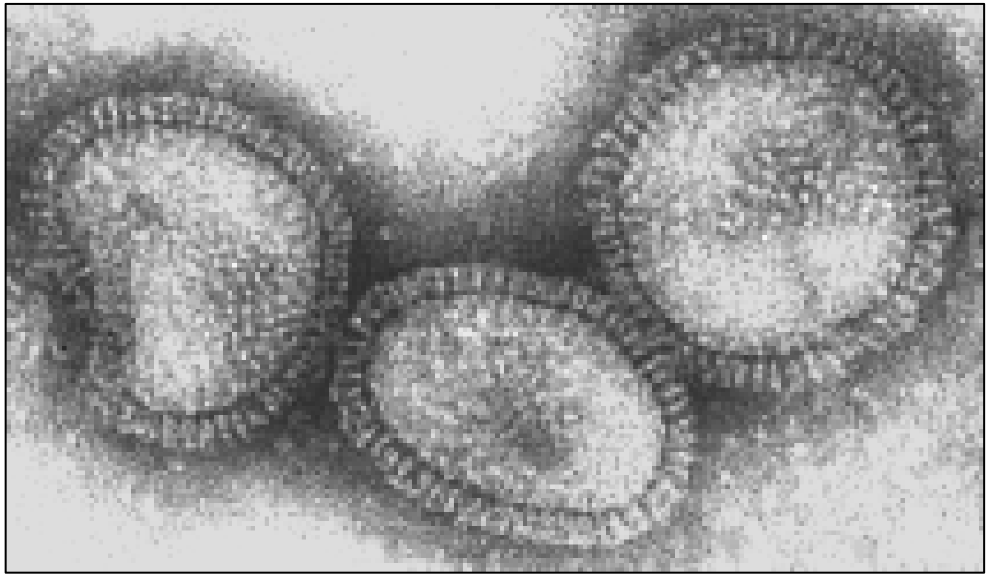



A Tobacco mosaic virus
(+) RNA
Coat protein subunit
p = 0.14 nm
300 nm
18 nm
B Sendai virus nucleocapsid
(–) RNA
NP subunit
p = 0.41 nm
1,000 nm
20 nm
C Vesicular stomatitis virus nucleocapsid
45 nm
27 nm
(–) RNA
N and M protein subunits
p = 5 nm
180 nm

Structure of virus capsids: Helical symmetry

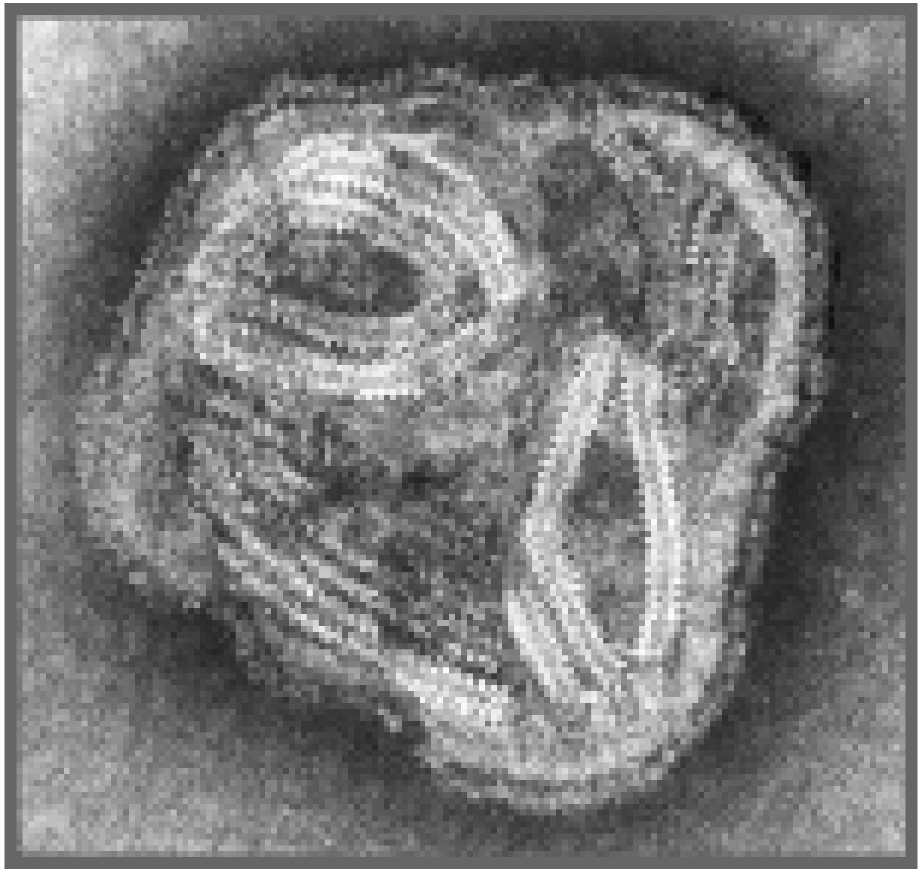


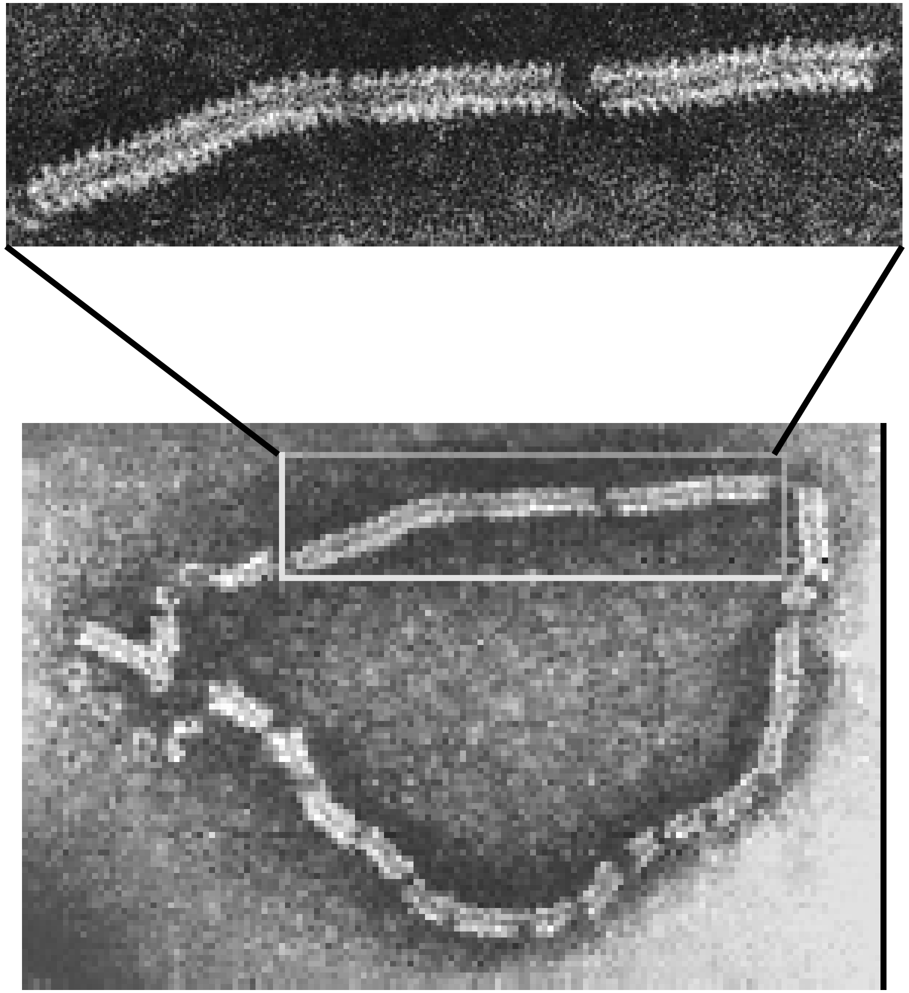
Paramyxovirus



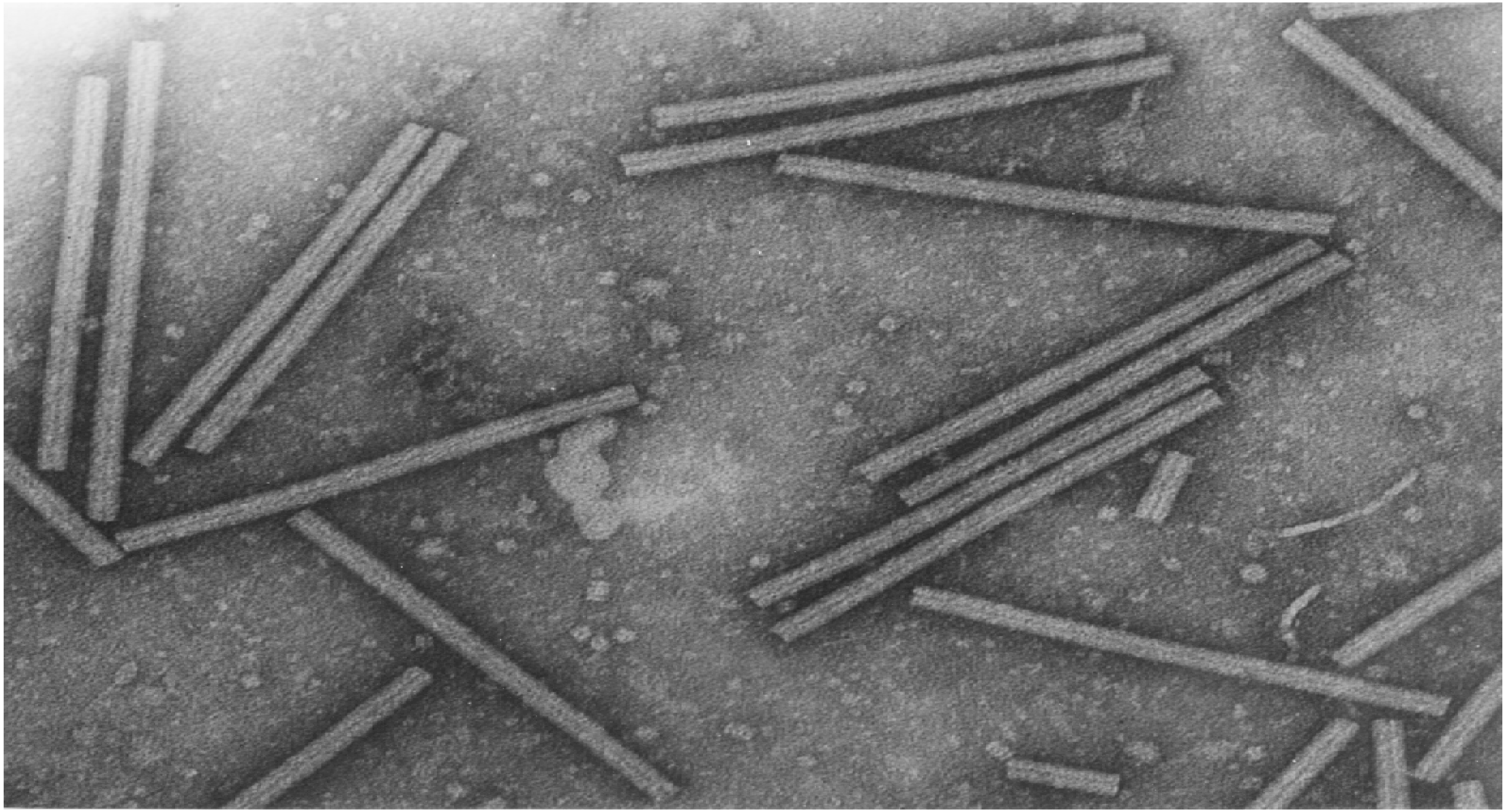
Orthomyxovirus

Measle virus: an example of virus structure with helical simmetry

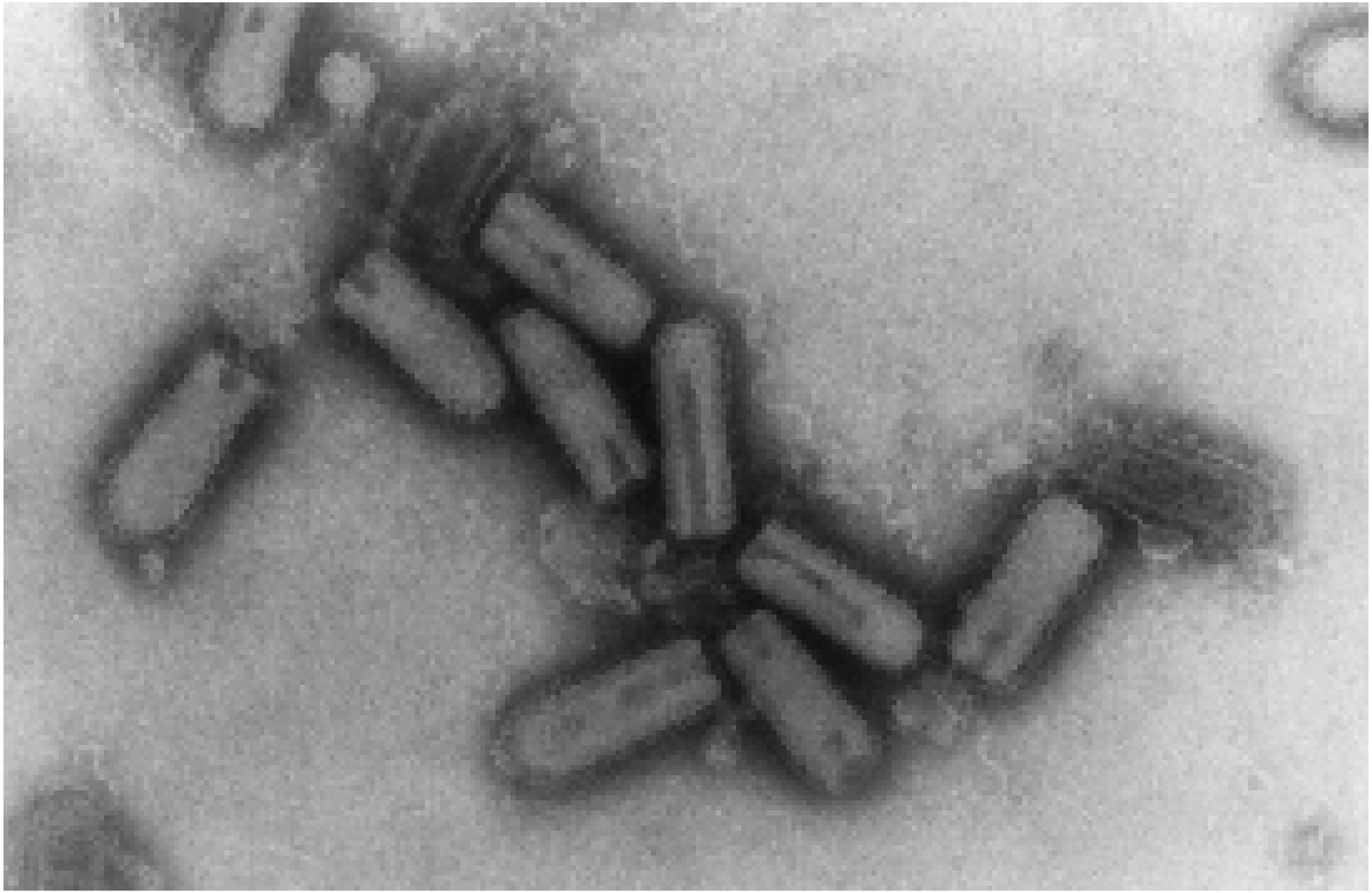




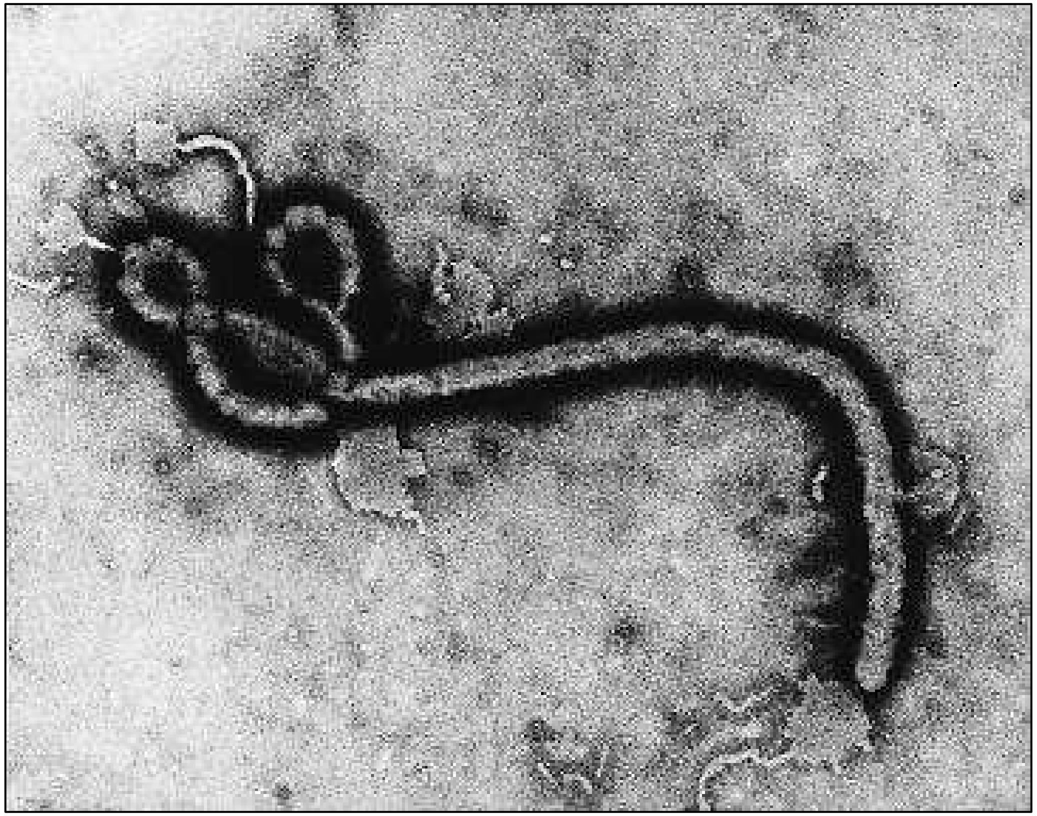
Tobacco mosaic virus (TMV)



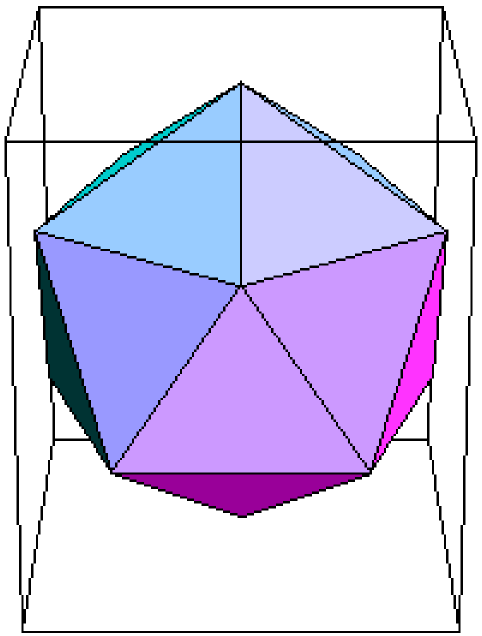
Vesicular stomatitis virus (VSV)



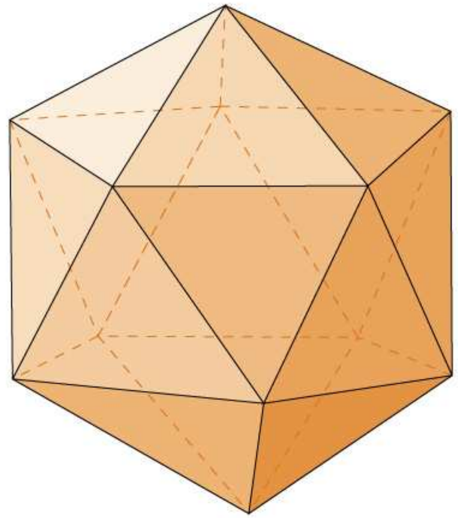
Ebola virus



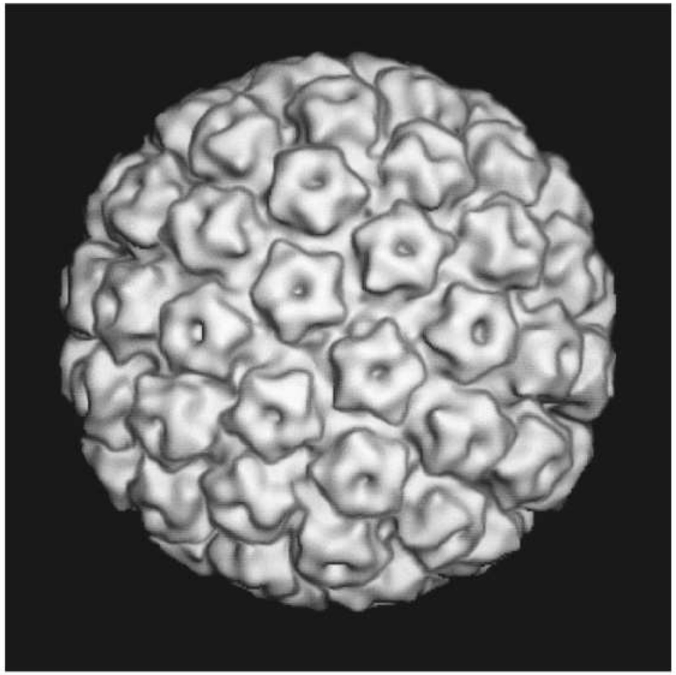
Structure of virus capsids: Icosahedral symmetry



Icosahedral symmetry: a model of an icosahedron and three-dimensional reconstruction of human papilloma virus calculated from images of frozen hydrated virions.



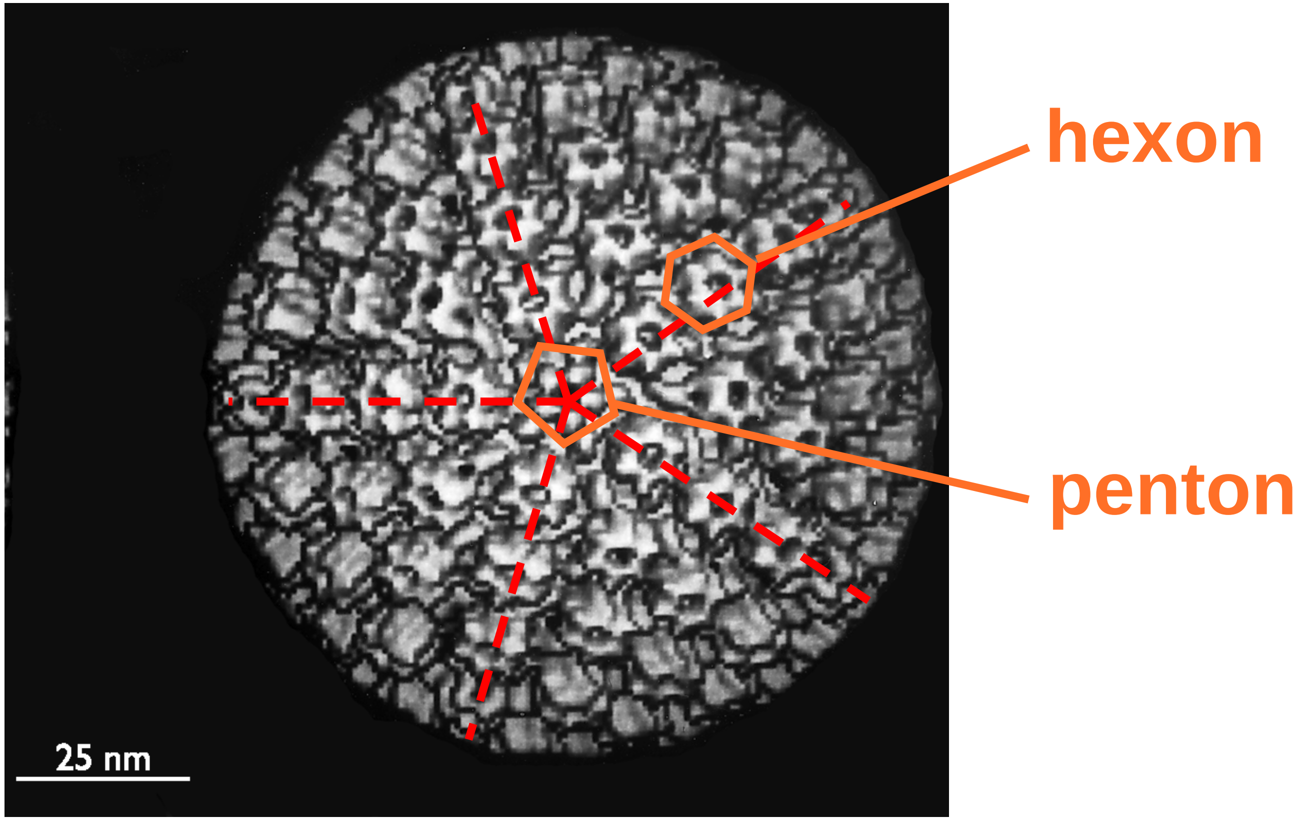
(a)



Tim Baker and Norm Olson

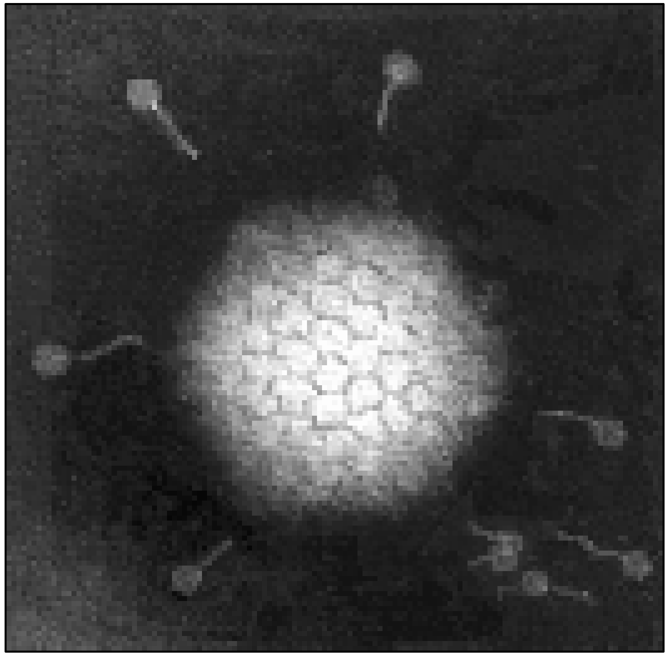
(c)

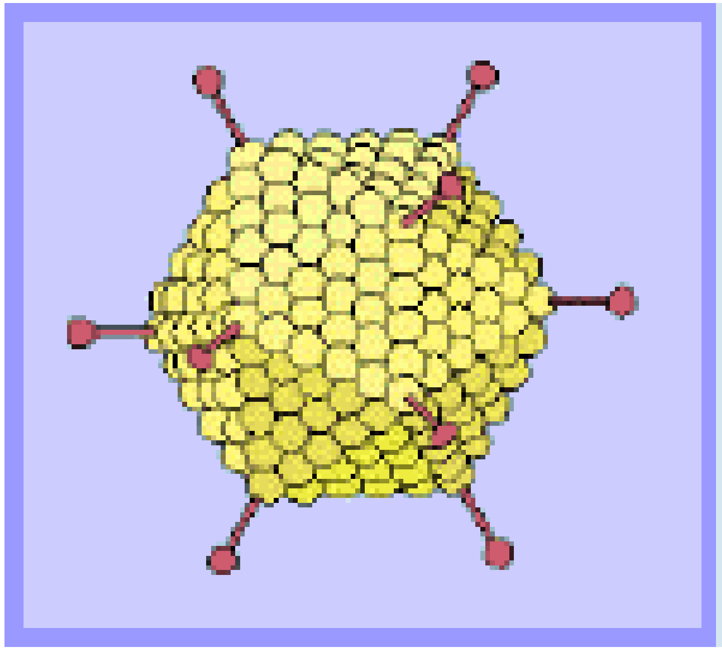
Structure of virus capsids: Icosahedral symmetry



HSV-1 capsid

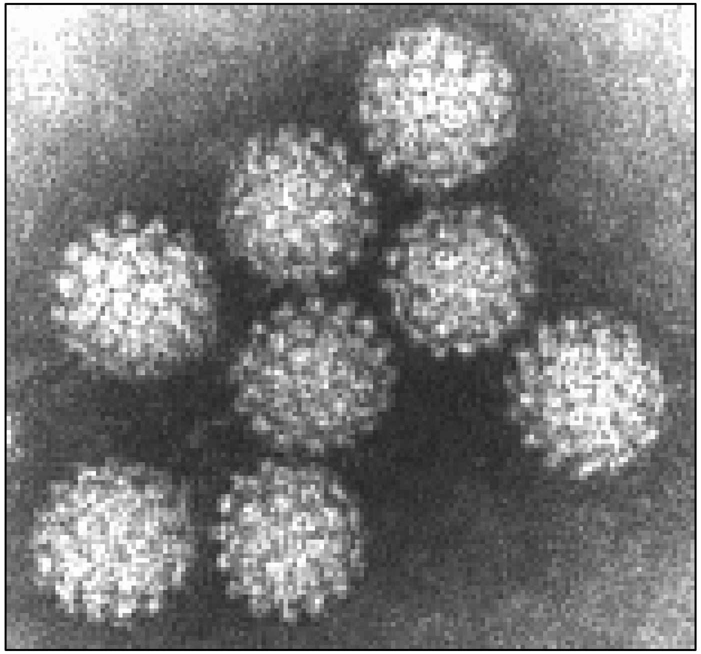
Structure of virus capsids: Icosahedral symmetry

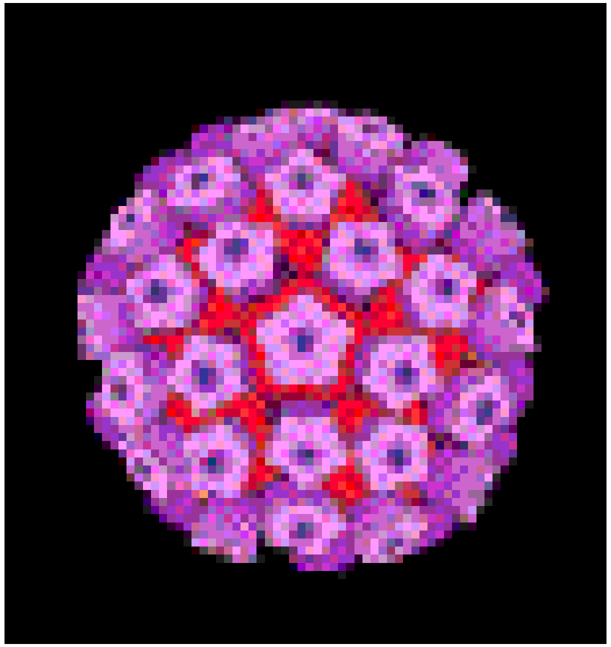




Adenovirus

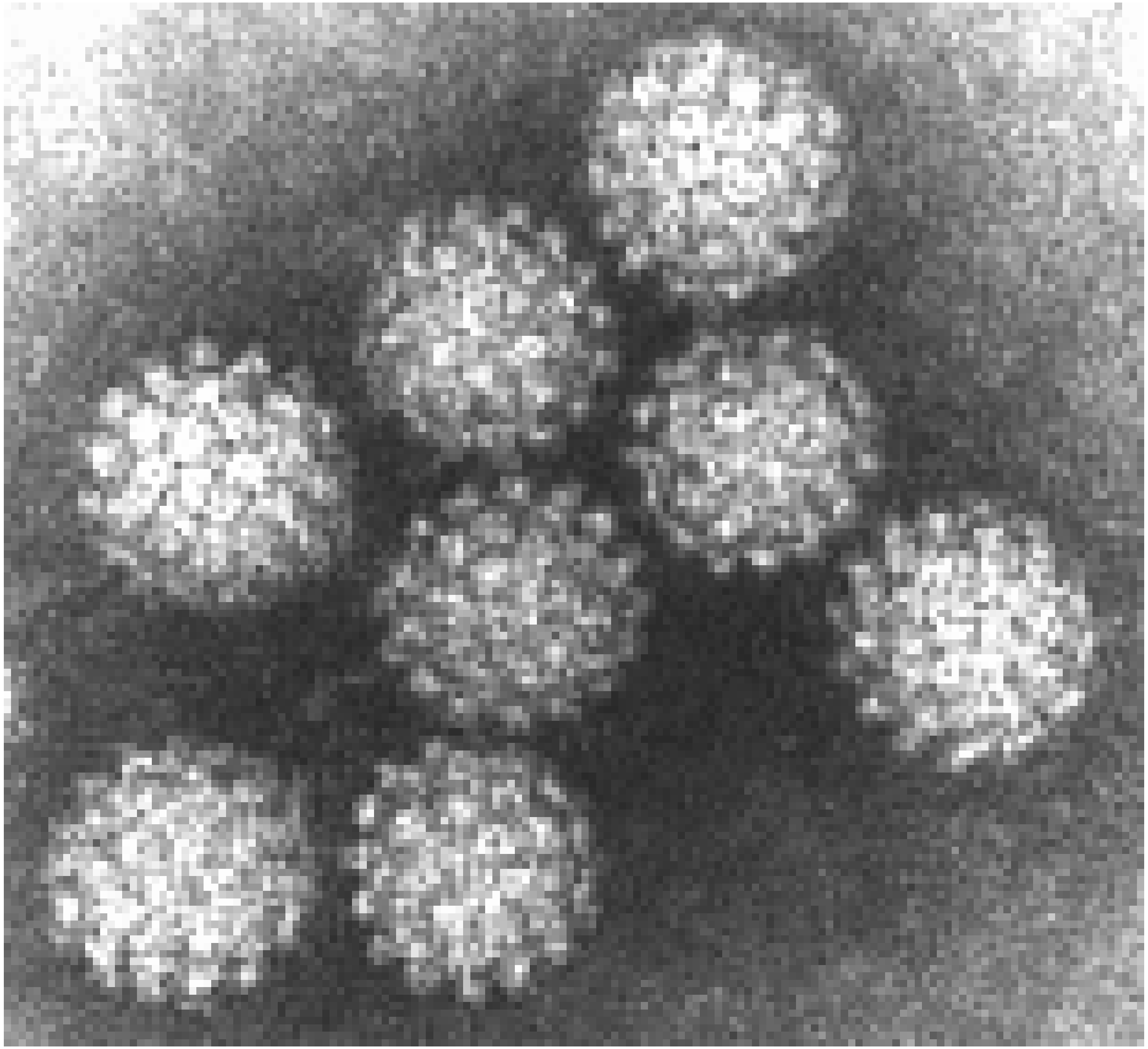
Structure of virus capsids: Icosahedral symmetry



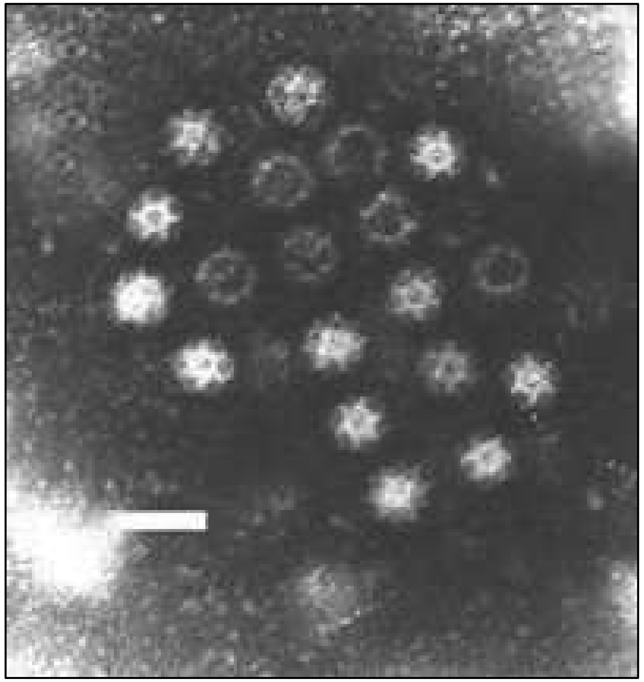


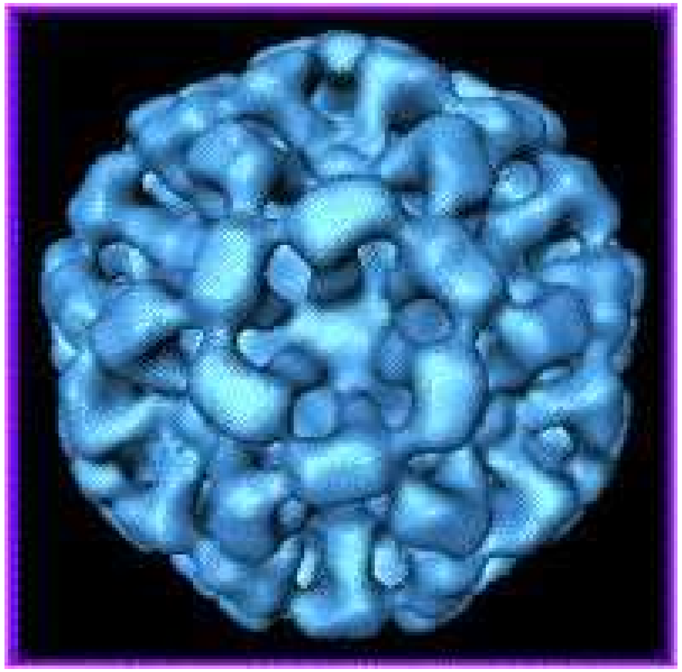
Papillomavirus

Human papillomavirus (HPV)



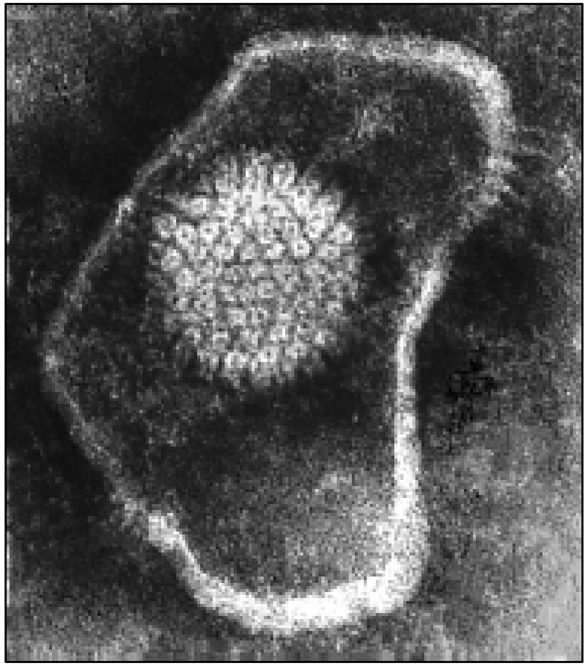
Structure of virus capsids: Icosahedral symmetry



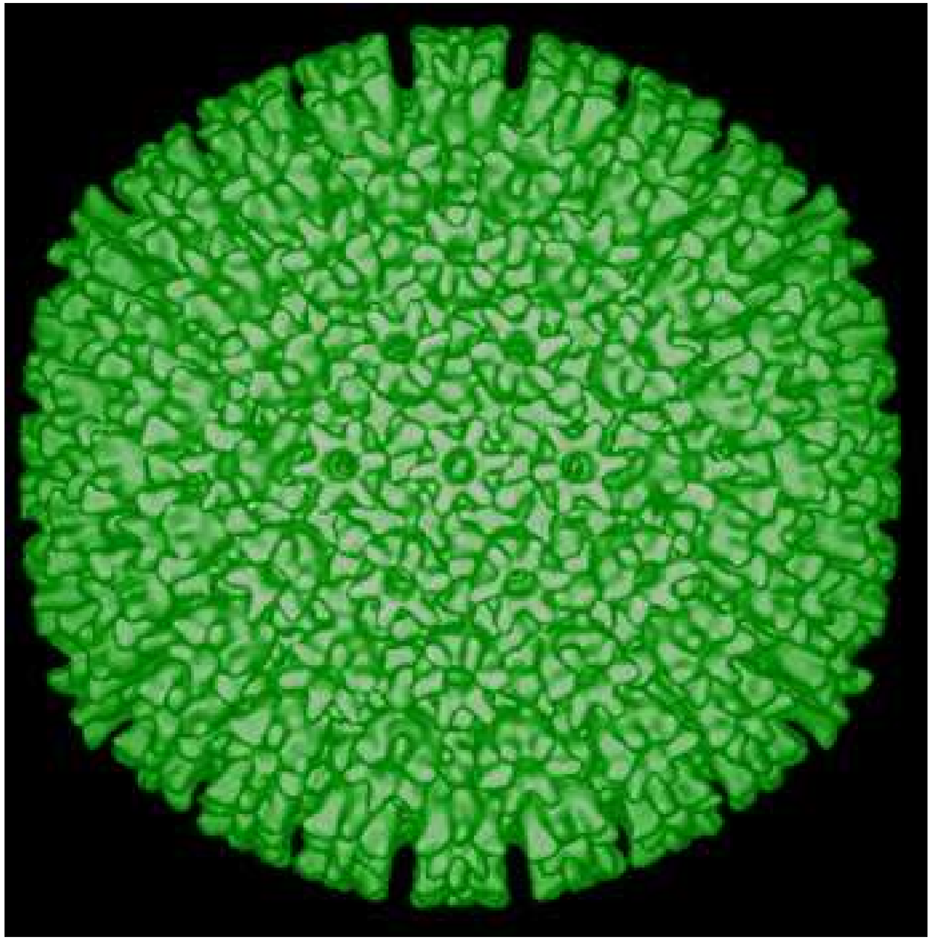


Calicivirus

Structure of herpesvirus capsids: Icosahedral symmetry

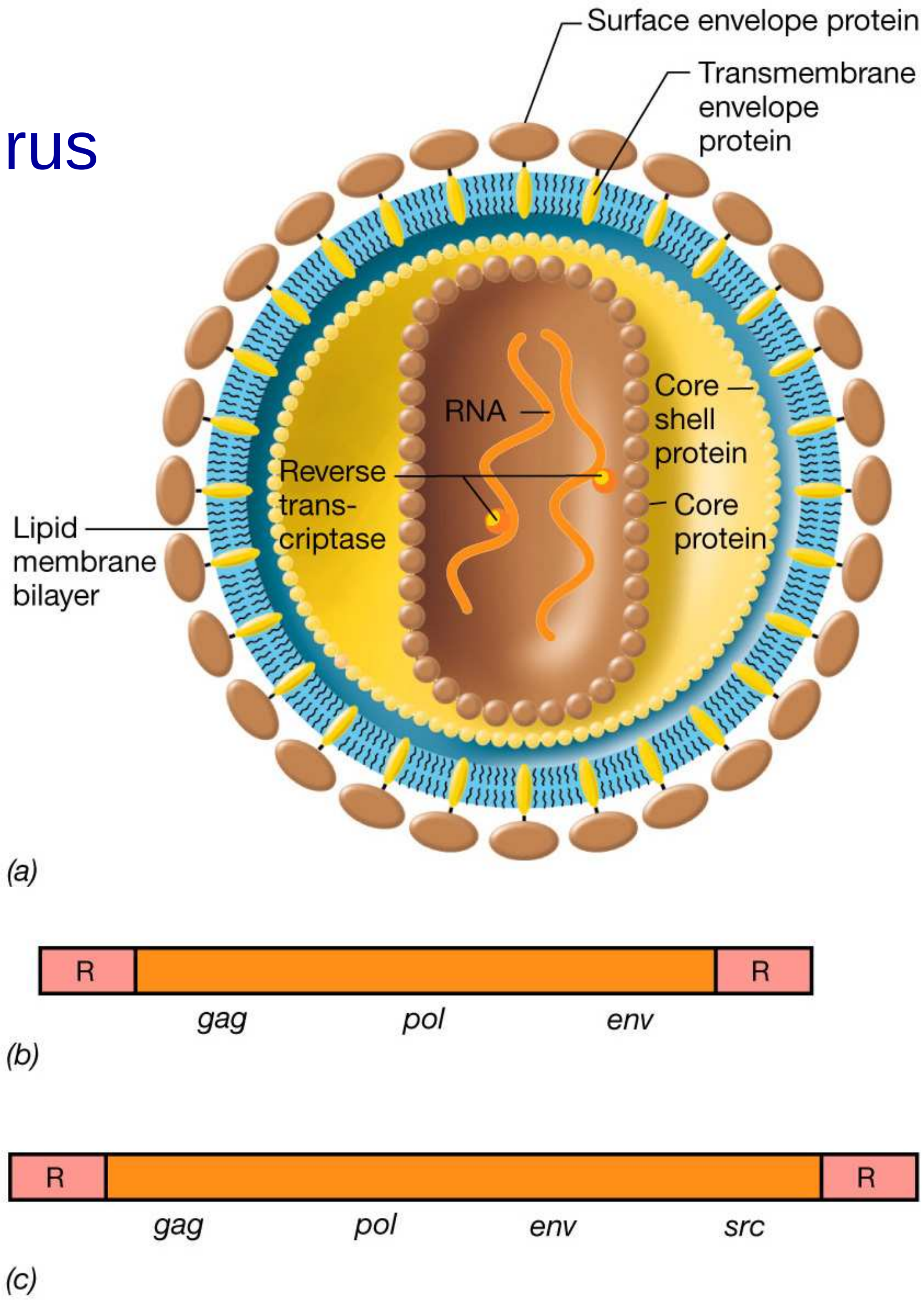


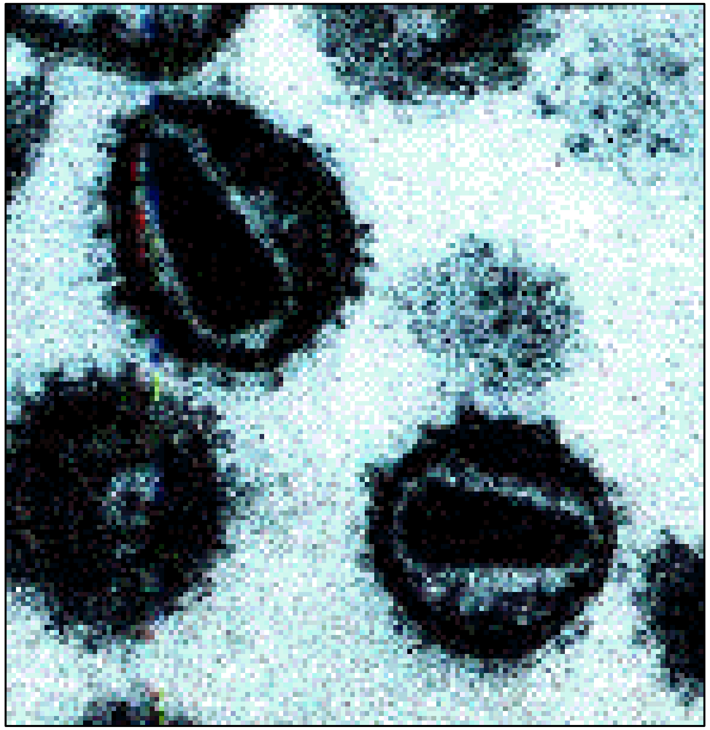
HSV-1

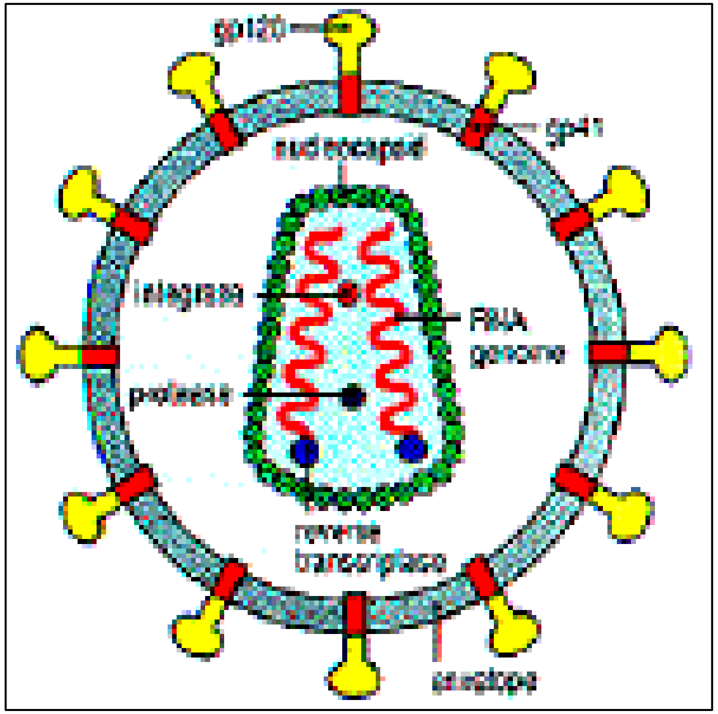


CMV

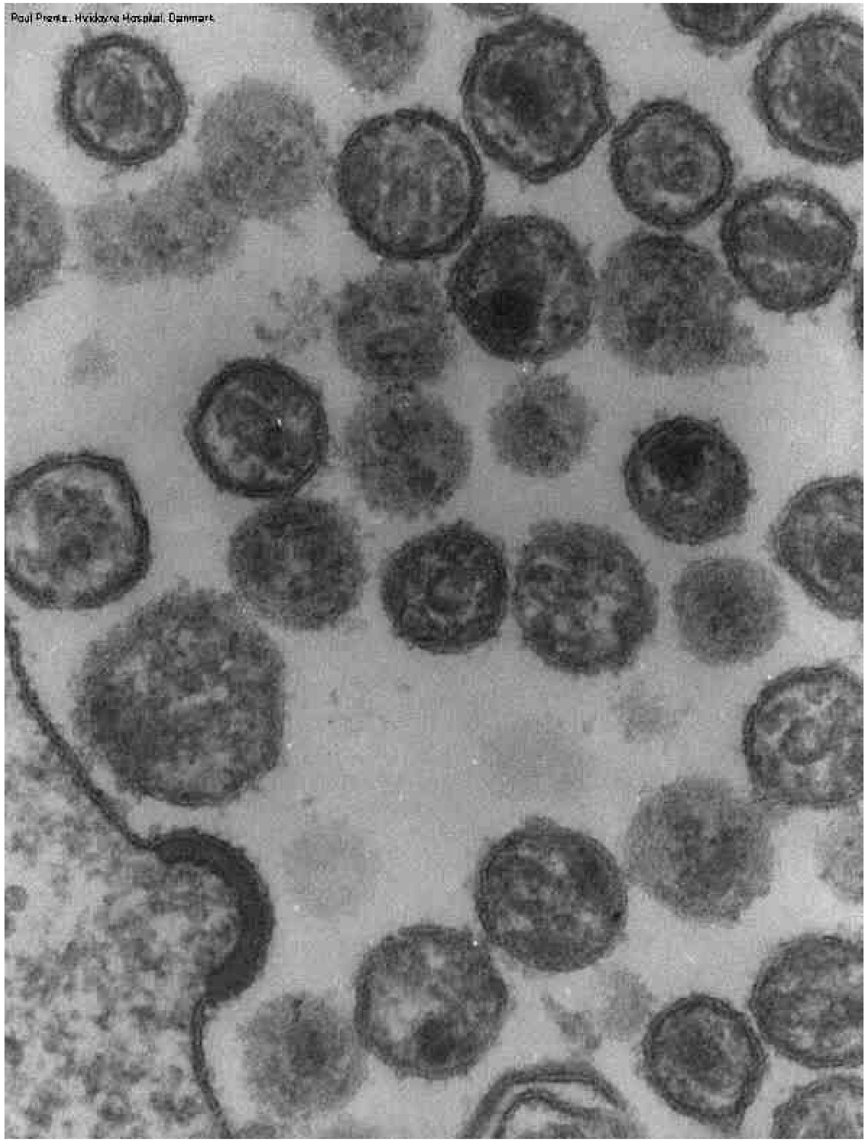
Structure of a retrovirus





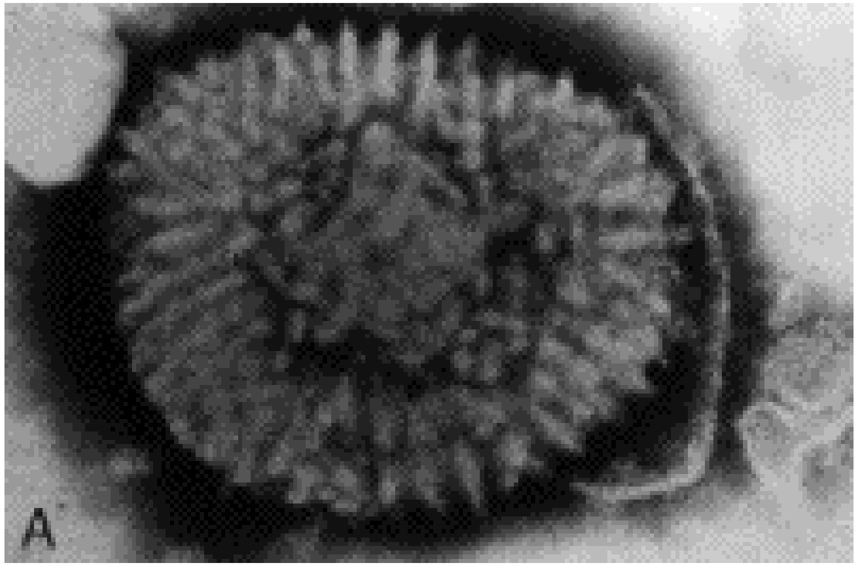


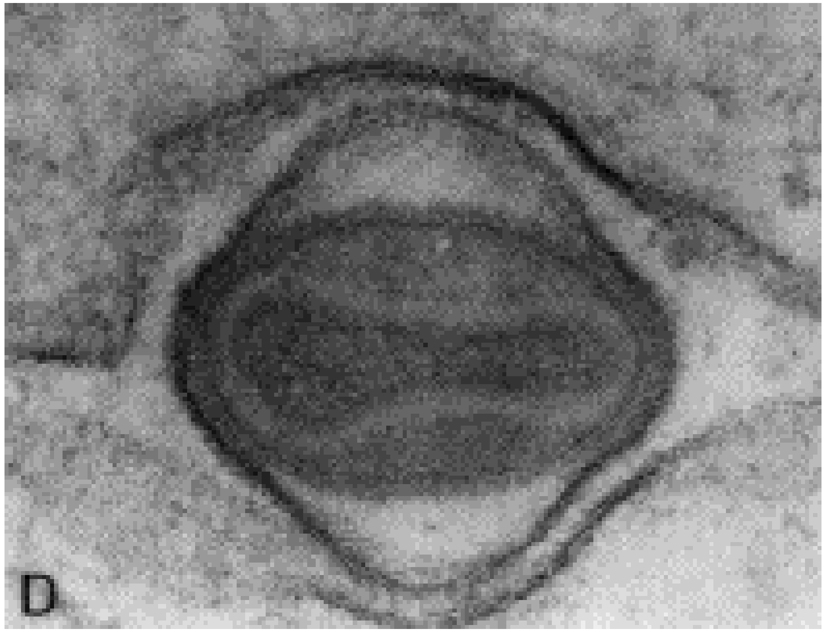
Human immunodeficiency virus

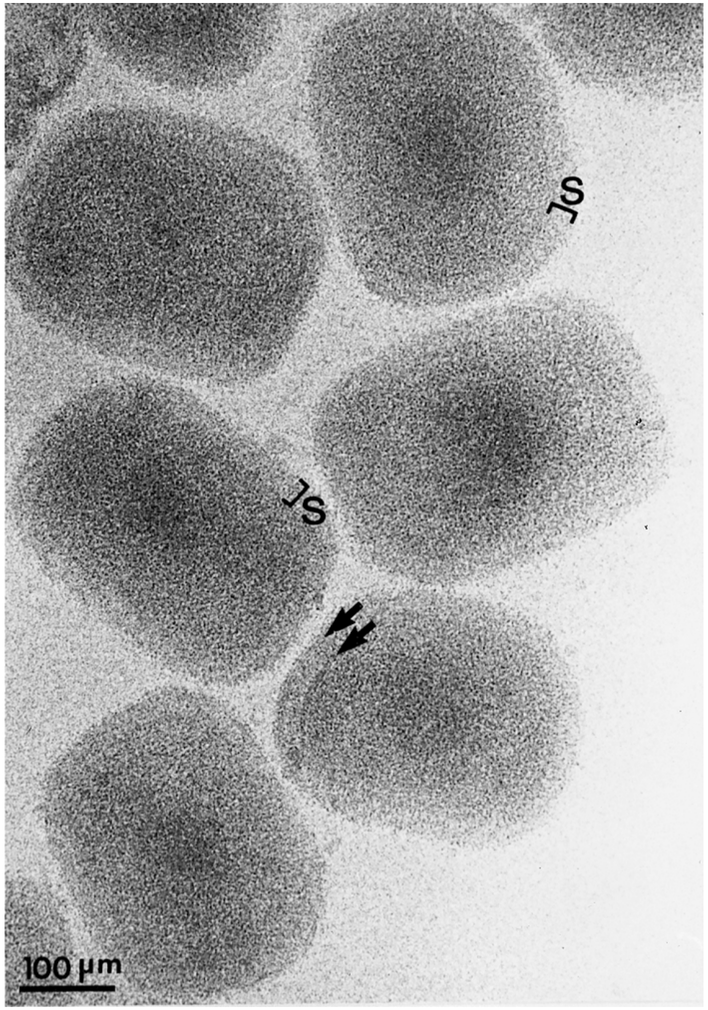


Human Immunodeficiency virus (HIV)

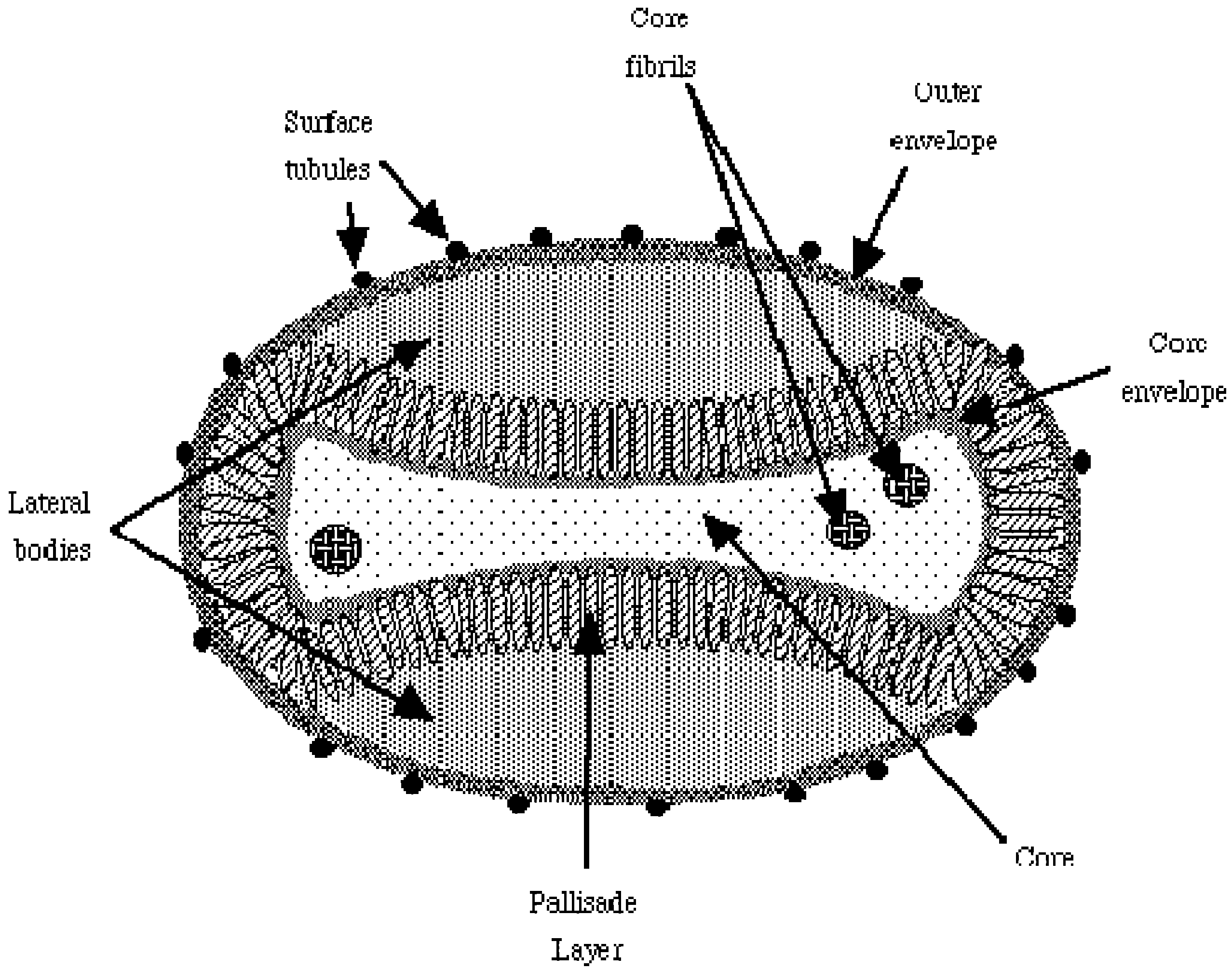
The **complex simmetry** of **Poxviruses**



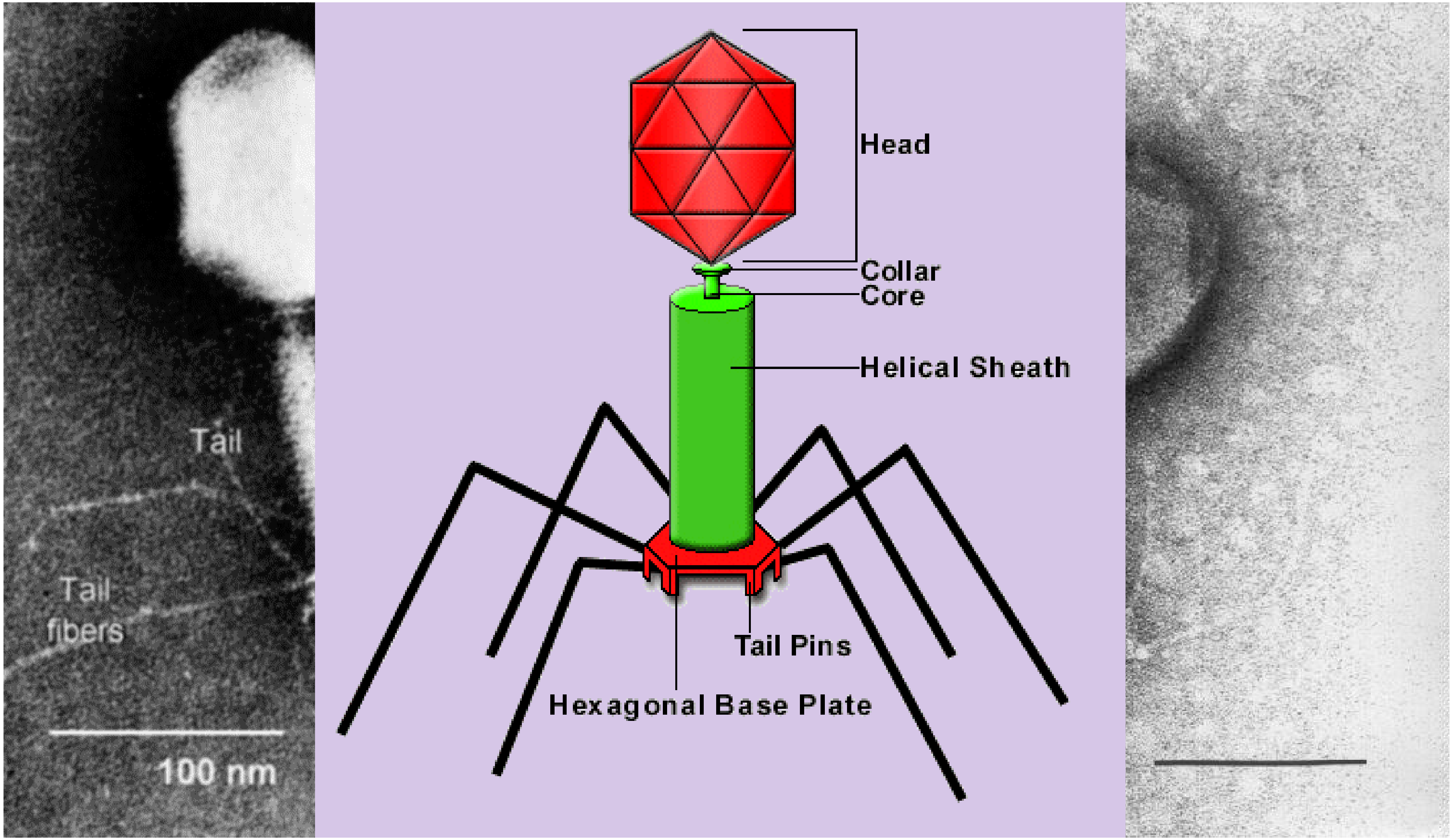




Structure of virus capsids: complex symmetry



Bacteriophage T4: an example of complex simmetry



Virus capsid: functions

To protect the fragile nucleic acid genome from:

- **Physical damage** - Shearing of the nucleic acid by mechanical forces.
- **Chemical damage** - UV irradiation (from sunlight) leading to chemical modification causing mutations.
- **Enzyme damage** - Nucleases derived from dead or leaky cells or deliberately secreted as a defense against infection.

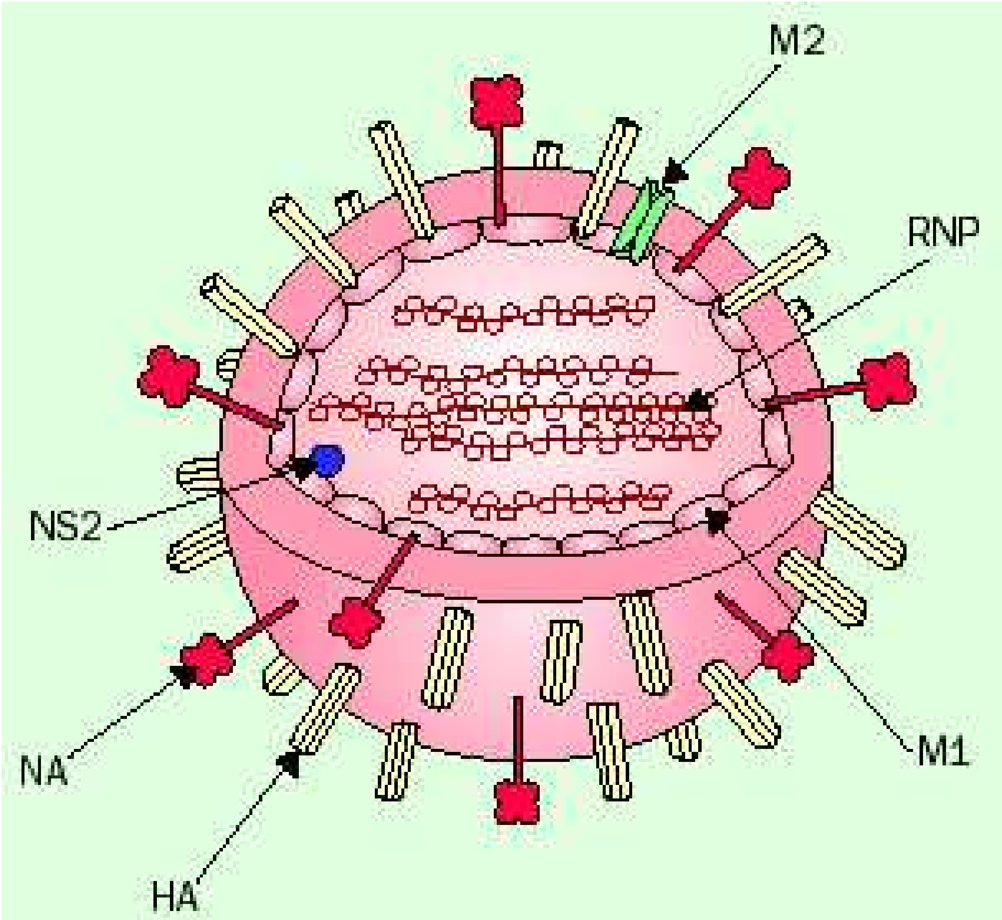
Virus capsid: functions

- **Packaging of the viral genome**

- **Interactions with the host cell.** Capsid proteins of naked viruses mediates attachment and virus entry.

- **Stimulation of the host immune system.** Capsid proteins of naked viruses are often the majors virus antigens

Functions of envelope proteins



the Orthomyxoviruses

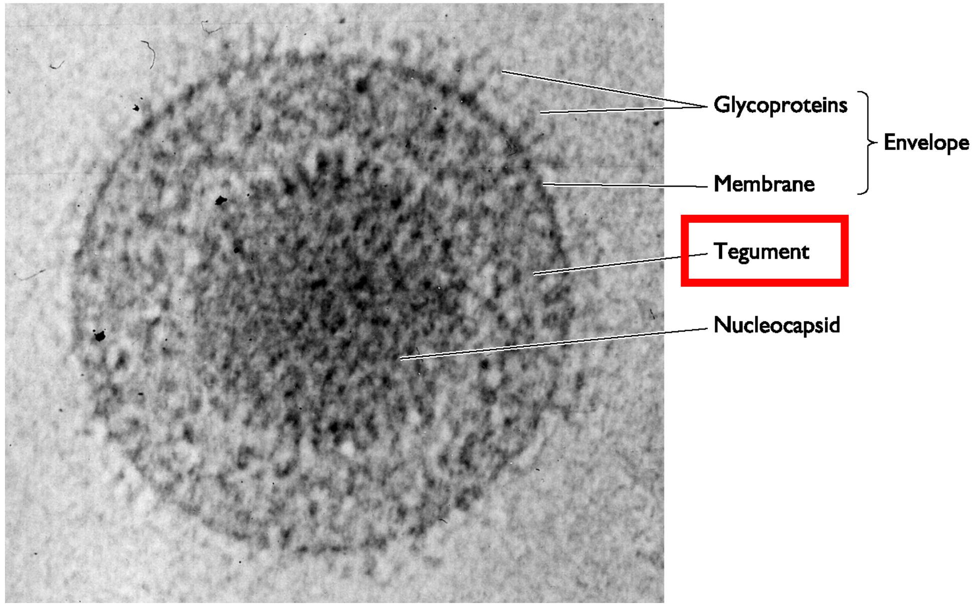
Attachment, fusion and entry
(e.g. HA)

Enzymatic activity
(e.g. NA)

Ion channel
(e.g. M2)

Major antigens
(e.g. HA, NA)

Enveloped virus with additional protein layers



The structure of an herpesvirus

Introduction to Virology:

virus classification

Criteria of classification

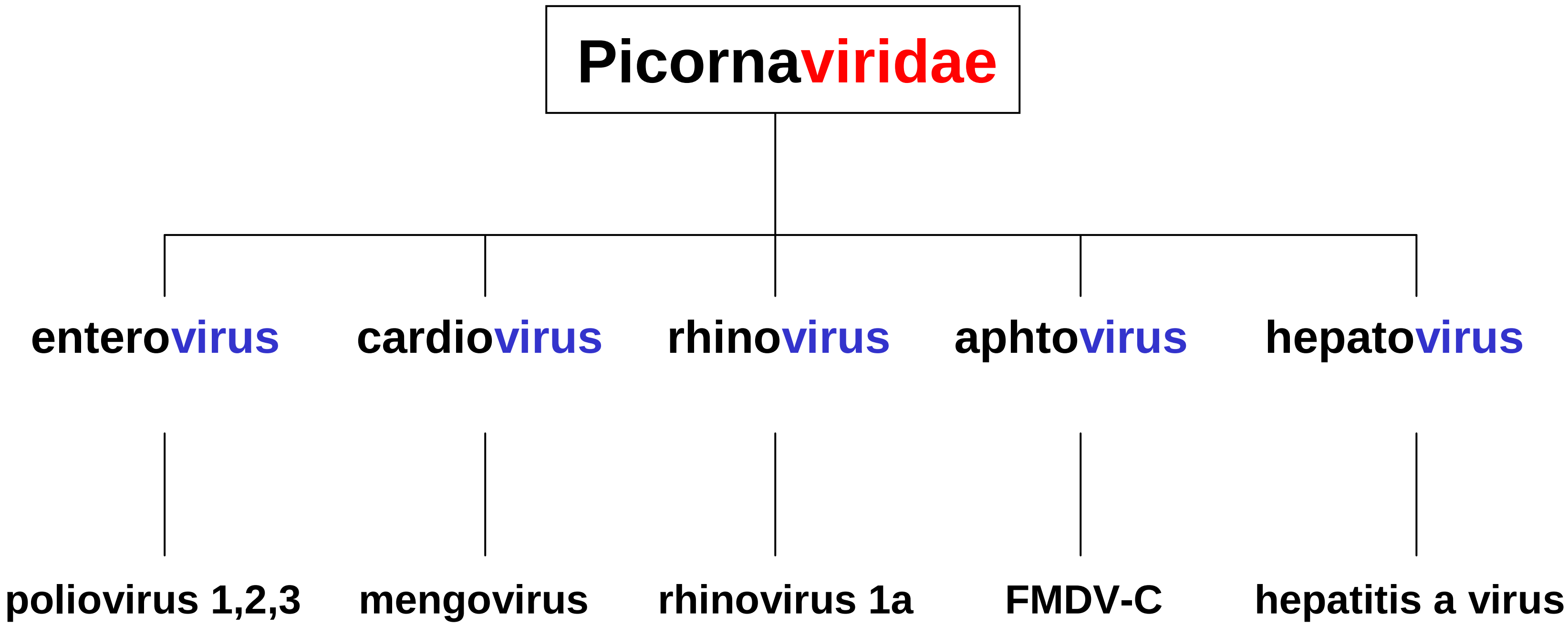
International Committee on Taxonomy of Viruses

- Host: animals, plant, bacteria.
- Nature of the nucleic acid in the virion : RNA or DNA
- Simmetry of the capsid: icosahedral, helical or complex
- Presence or absence of an envelope
- Genome architecture: ds, ss, fragmented, size
- sequence homology
- replication strategies

Nomenclature: some basic rules

- **Order** (- *virales*)
- **Family** (- *viridae*)
- **Sub-family** (-*virinae*)
- **Genera** (- *virus*)
- **Species** (*common names*)

Nomenclature: some basic rules



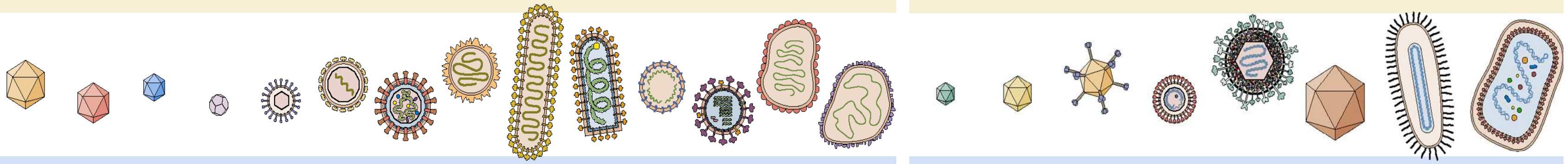
Classification schemes for animal viruses

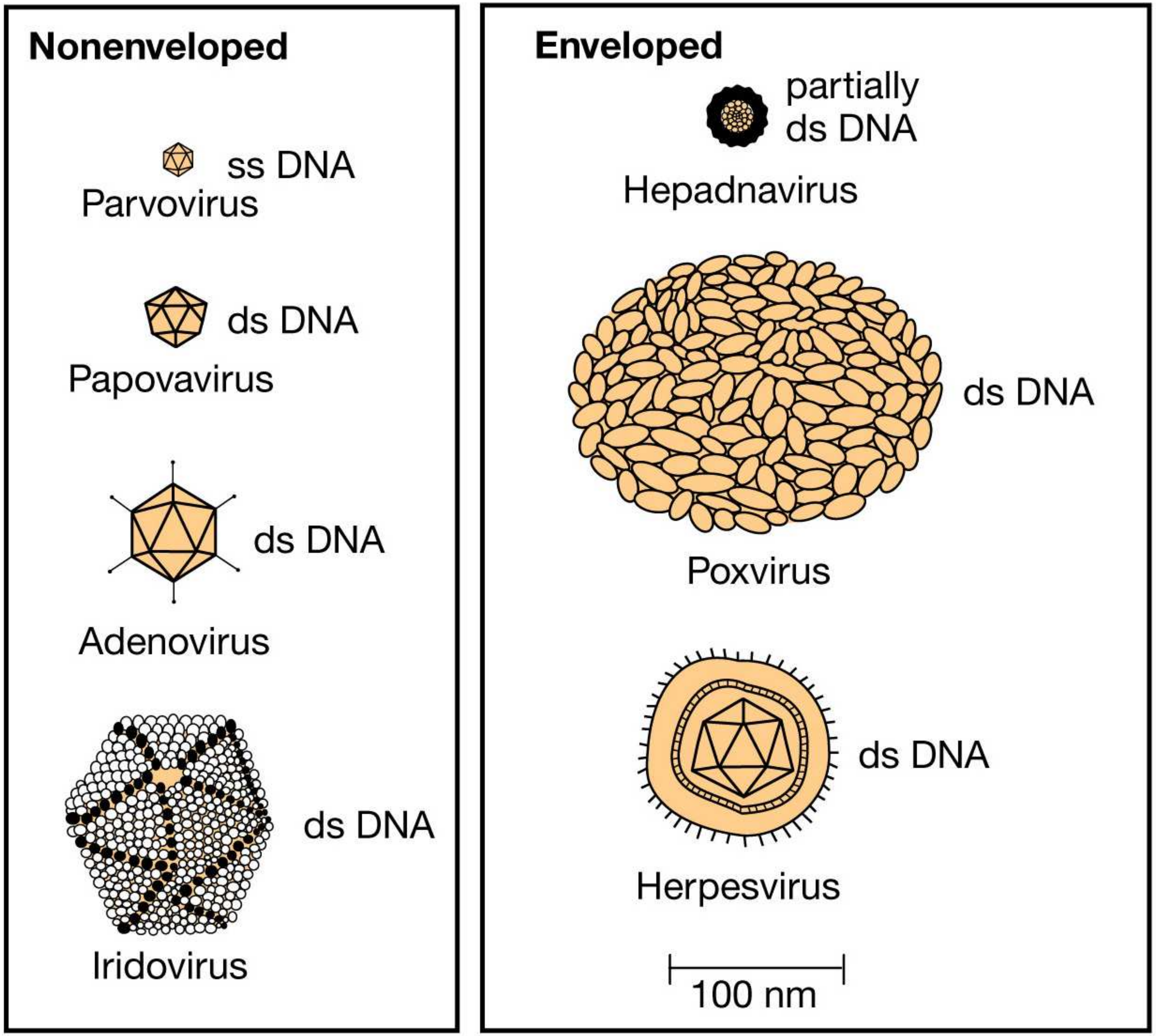
Classification criteria / **Properties** — RNA viruses

Nucleic acid	RNA													
Symmetry of capsid	Icosahedral							Helical						
Naked or enveloped	Naked				Enveloped			Enveloped						
Genome architecture	ds 10–18 seg.	ds 2 seg.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss 2 copies	(+) ss cont.	(–) ss cont.	(–) ss cont.	(–) ss 3 seg.	(–) ss 8 seg.	(–) ss cont.	(–) ss 2 seg.
Baltimore class	III	III	IV	IV	IV	IV	VI	IV	V	V	V	V	V	V
Family name	Reo	Birna	Calici	Picorna	Flavi	Toga	Retro	Corona	Filo	Rhabdo	Bunya	Ortho-myxo	Para-myxo	Arena
Virion polymerase	(+)	(+)	(–)	(–)	(–)	(–)	(+)	(–)	(+)	(+)	(+)	(+)	(+)	(+)
Virion diameter (nm)	60–80	60	35–40	28–30	40–50	60–70	80–130	80–160	80 X 790–14,000	70–85 X 130–380	90–120	90–120	150–300	50–300
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

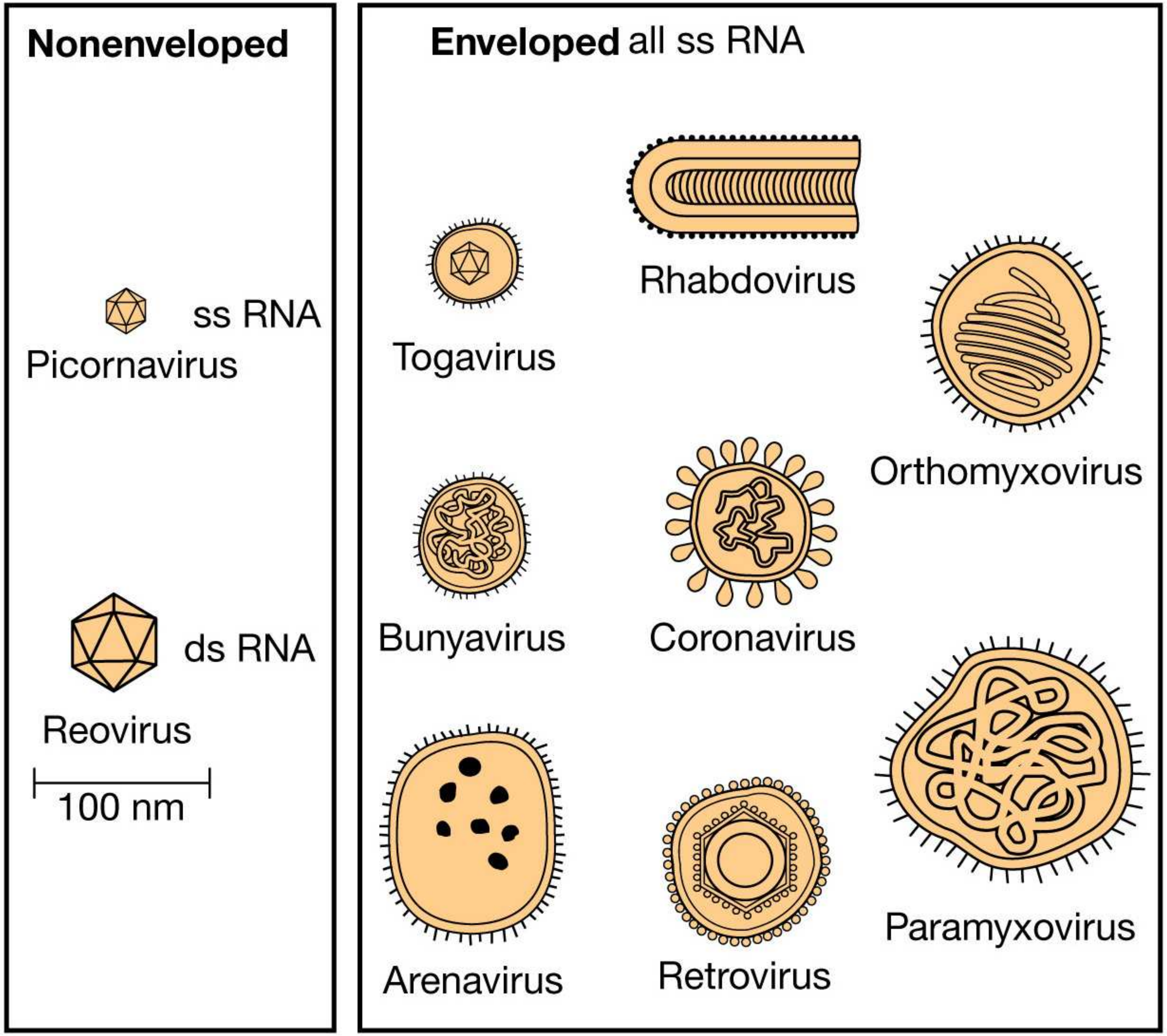
Classification criteria / **Properties** — DNA viruses

Nucleic acid	DNA							
Symmetry of capsid	Icosahedral						Helical	Complex
Naked or enveloped	Naked			Enveloped		Naked/Env. (cytoplasmic)	Enveloped	Enveloped (cytoplasmic)
Genome architecture	ss linear (+) or (–)	ds circular	ds linear	ds circle gapped	ds linear	ds linear	ds circular	ds linear (x linked)
Baltimore class	II	I	I	I	I	I	I	I
Family name	Parvo	Papova	Adeno	Hepadna	Herpes	Irido	Baculo	Pox
Virion polymerase	(–)	(–)	(–)	(+)	(–)	(–)	(–)	(+)
Virion diameter (nm)	18–26	45–55	70–90	42	150–200	125–300	60 X 300	170–200 x 300–450
Genome size (total in kb)	5	5–8	36–38	3.2	120–200	150–350	100	130–280





(a) DNA viruses

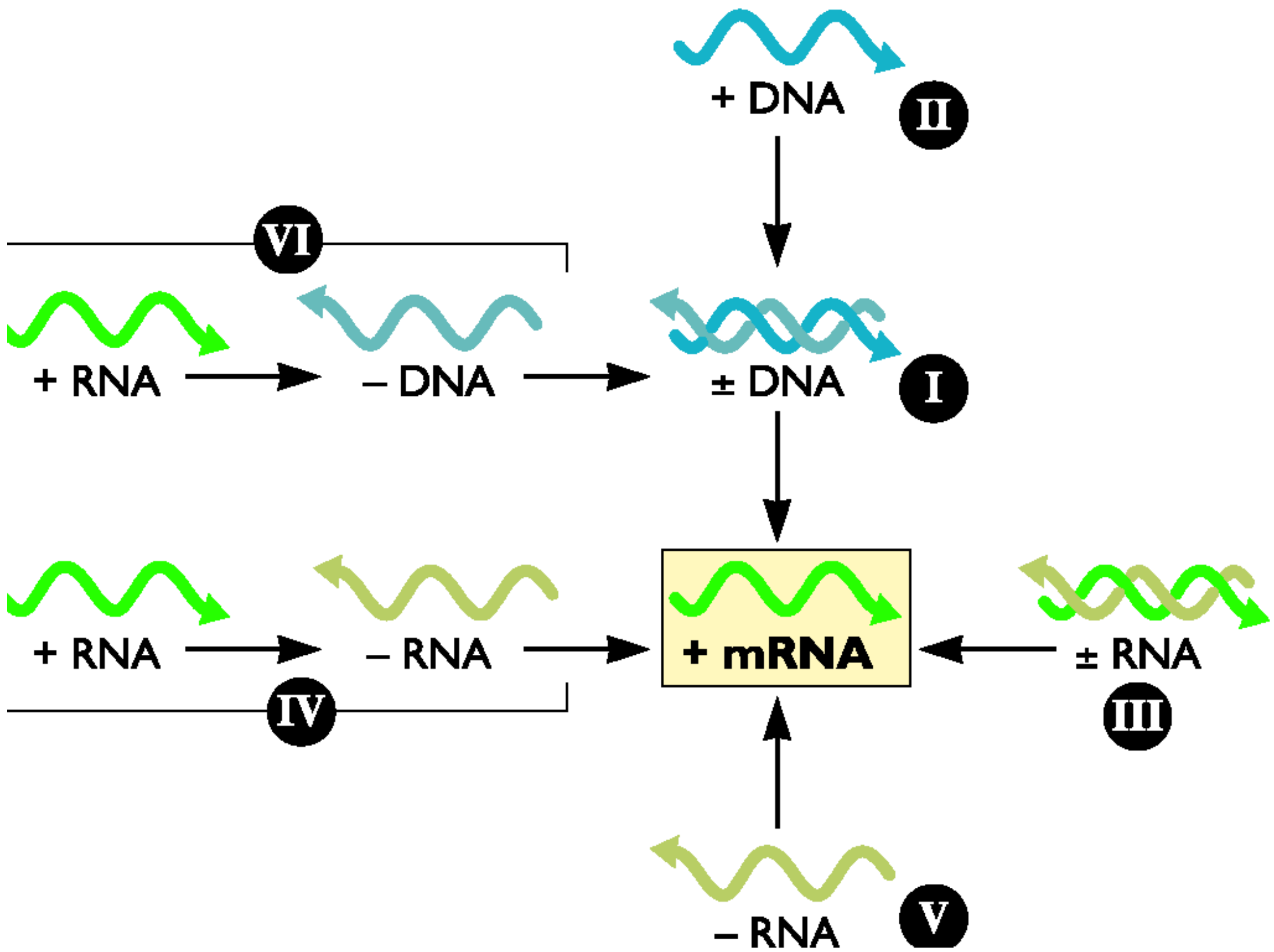


(b) RNA viruses

The Baltimore classification system

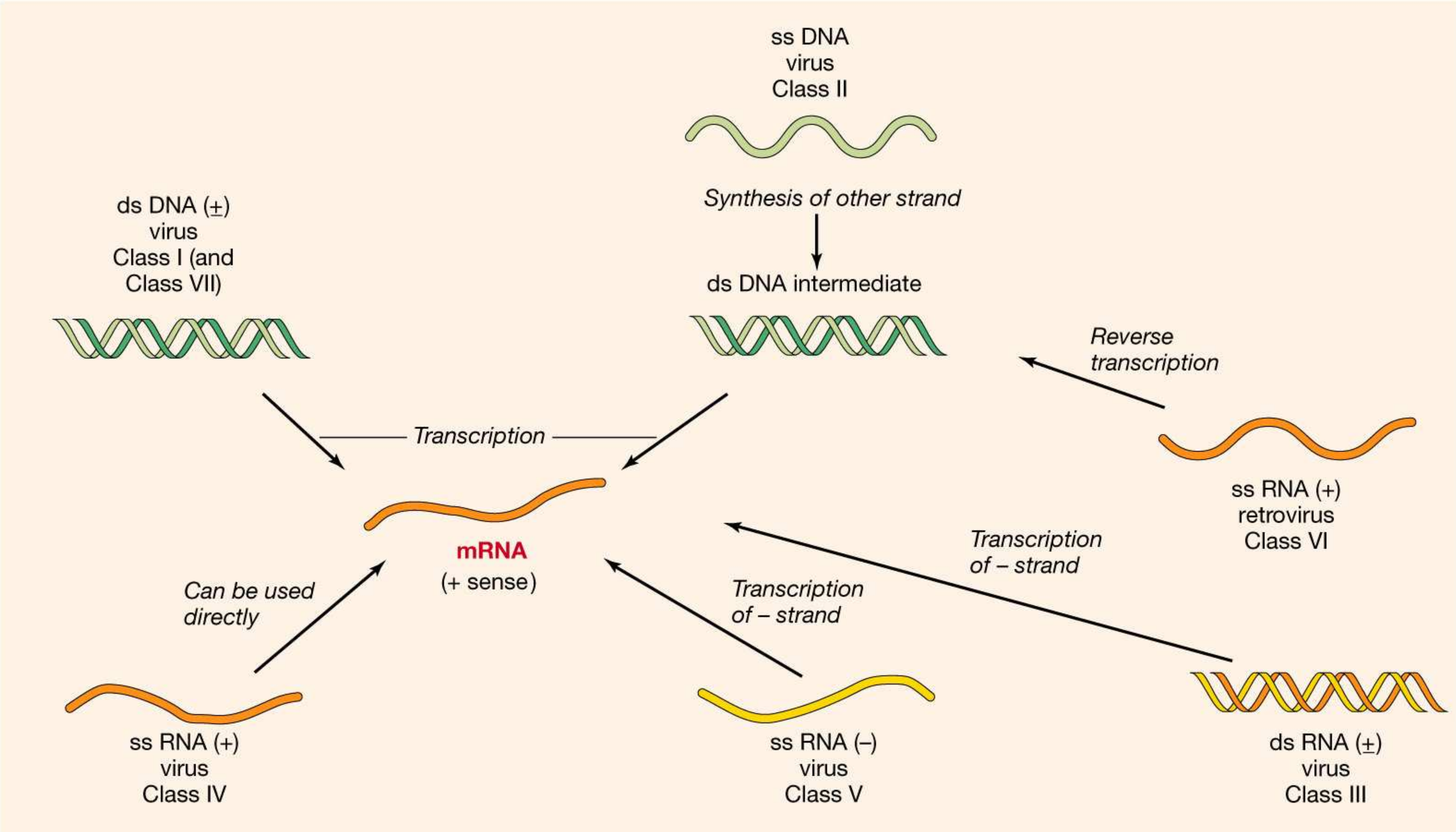
- **Is based on the nature and polarity of the virus genomes**

- **Describes the obligatory relationships between the viral genome and its mRNA**



Baltimore classification: the unique pathways from various viral genomes to mRNA define specific virus classes on the basis of the nature and polarity of their genomes

Synthesis of mRNA after infection of cells by viruses of different types



Introduction to Virology:

origin of viruses

Origin of Viruses

- **Regressive theory:** viruses are degenerate forms of intracellular parasites. The leprosy bacillus, rickettsiae and chlamydia have all evolved in this direction. Begs the question of RNA virus evolution ?

- **Progressive theory:** Normal cellular nucleic acids that gained the ability to replicate autonomously and therefore to evolve. DNA viruses came from plasmids or transposable elements. Retroviruses derived from retrotransposons and RNA virus from mRNA.

- **Co evolution theory:** Viruses coevolved with life.

Introduction to Virology:

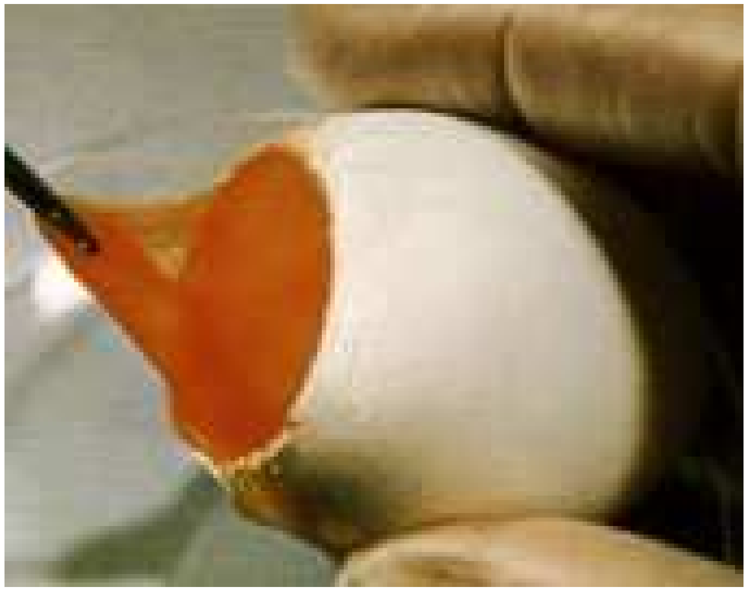
techniques to study viruses

Techniques used to study viruses

Living hosts:

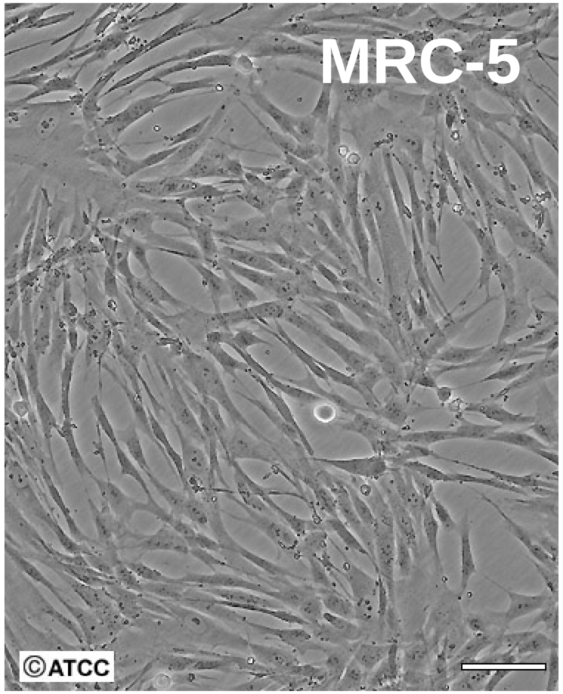
- cultured cells
- embryonated eggs
- laboratory animals

•Embryonated eggs used to propagate viruses in the early decades of this century. Effective for the isolation and culture of many viruses e.g.influenza.

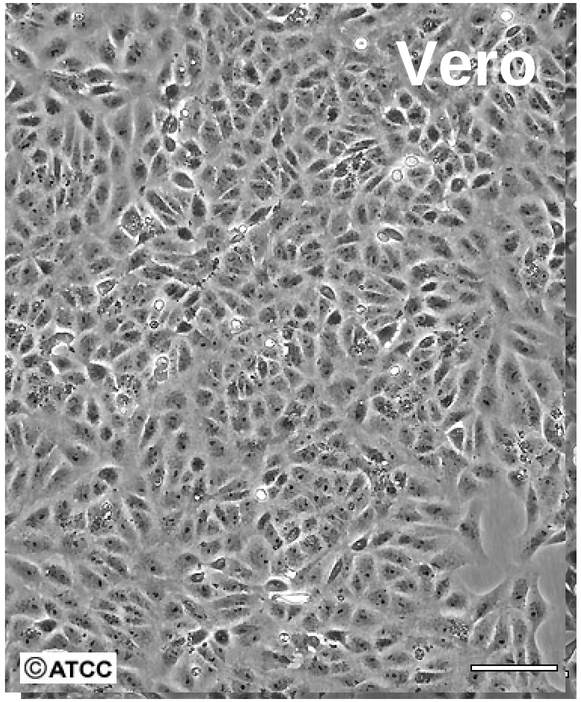


Cell cultures in Virology

Primary cell cultures

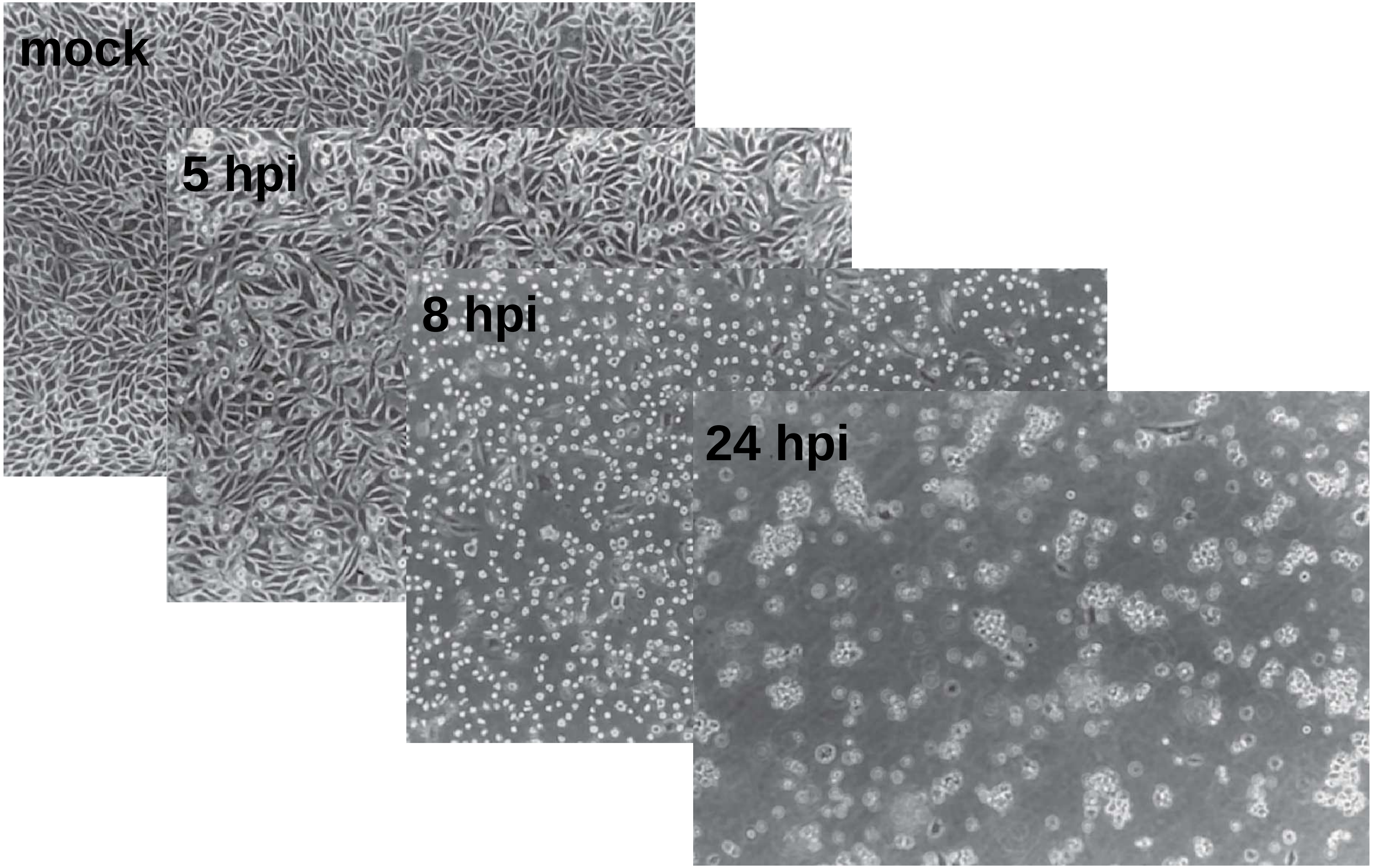


Diploid cell strain



Continuous cell lines

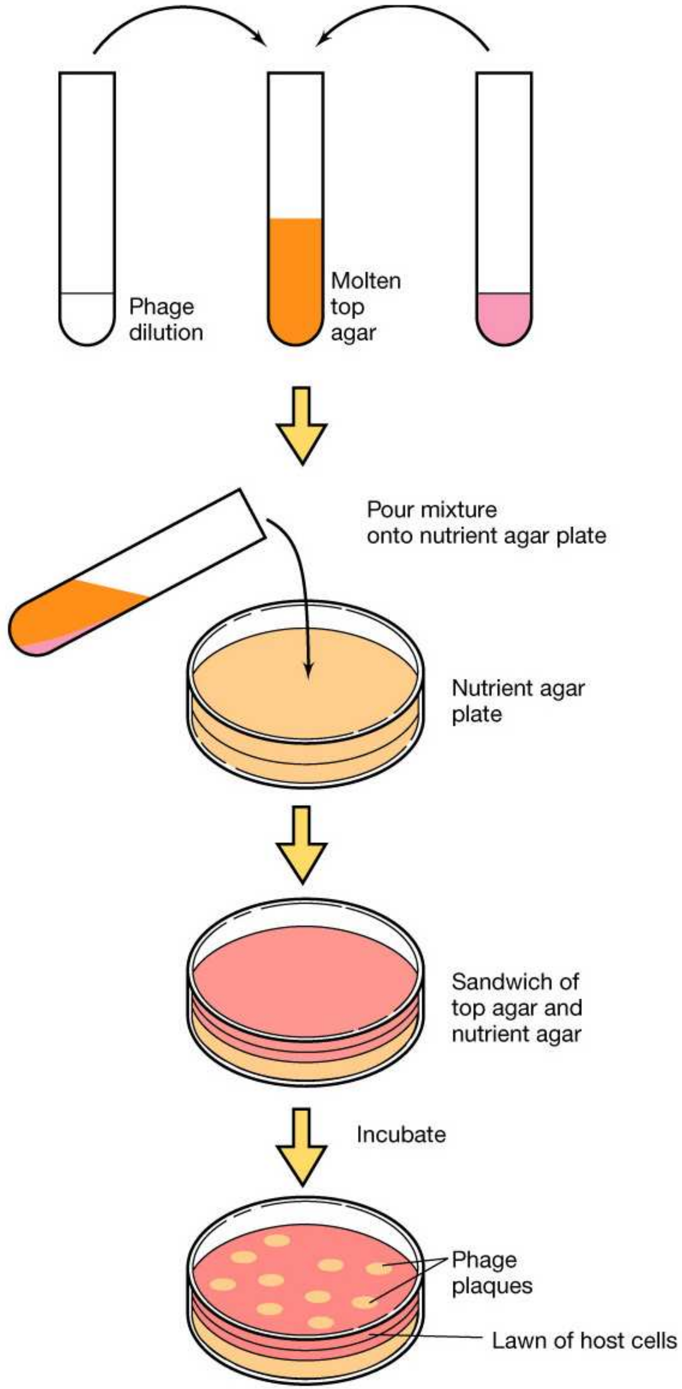
Poliovirus' CPE



Some examples of cytophatic effects of viral infection of animal viruses

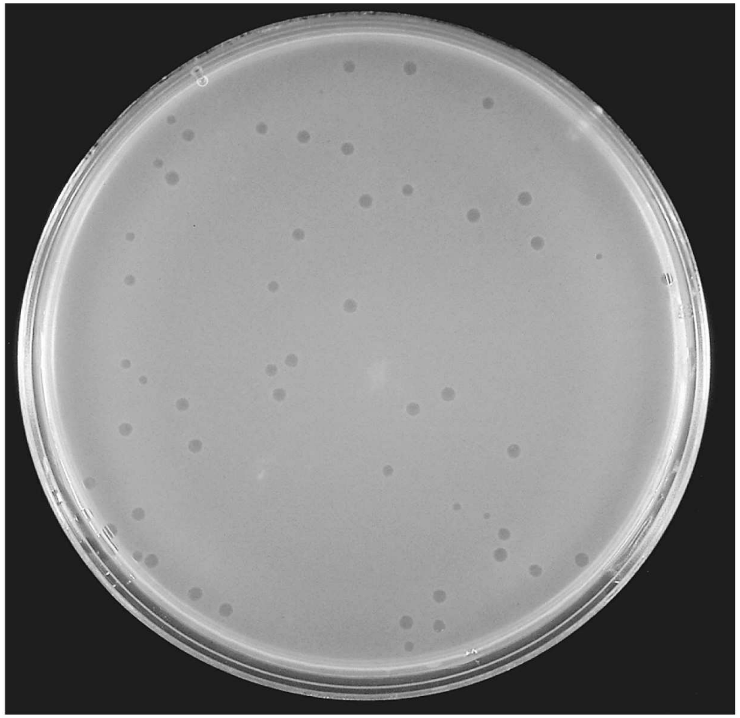
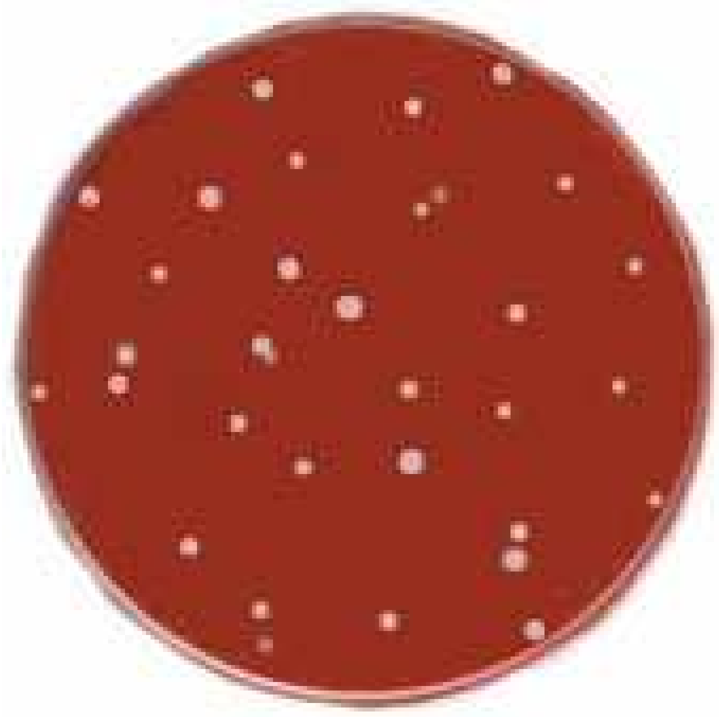
Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Papovaviruses
Syncytia (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of tissue culture cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses
Inclusion bodies	
Virions in nucleus	Adenoviruses
Virions in the cytoplasm (Negri bodies)	Rabies virus
"Factories" in the cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesvir

Quantification of bacterial virus by plaque assay using the agar overlay technique.



(a)

Quantifying viruses

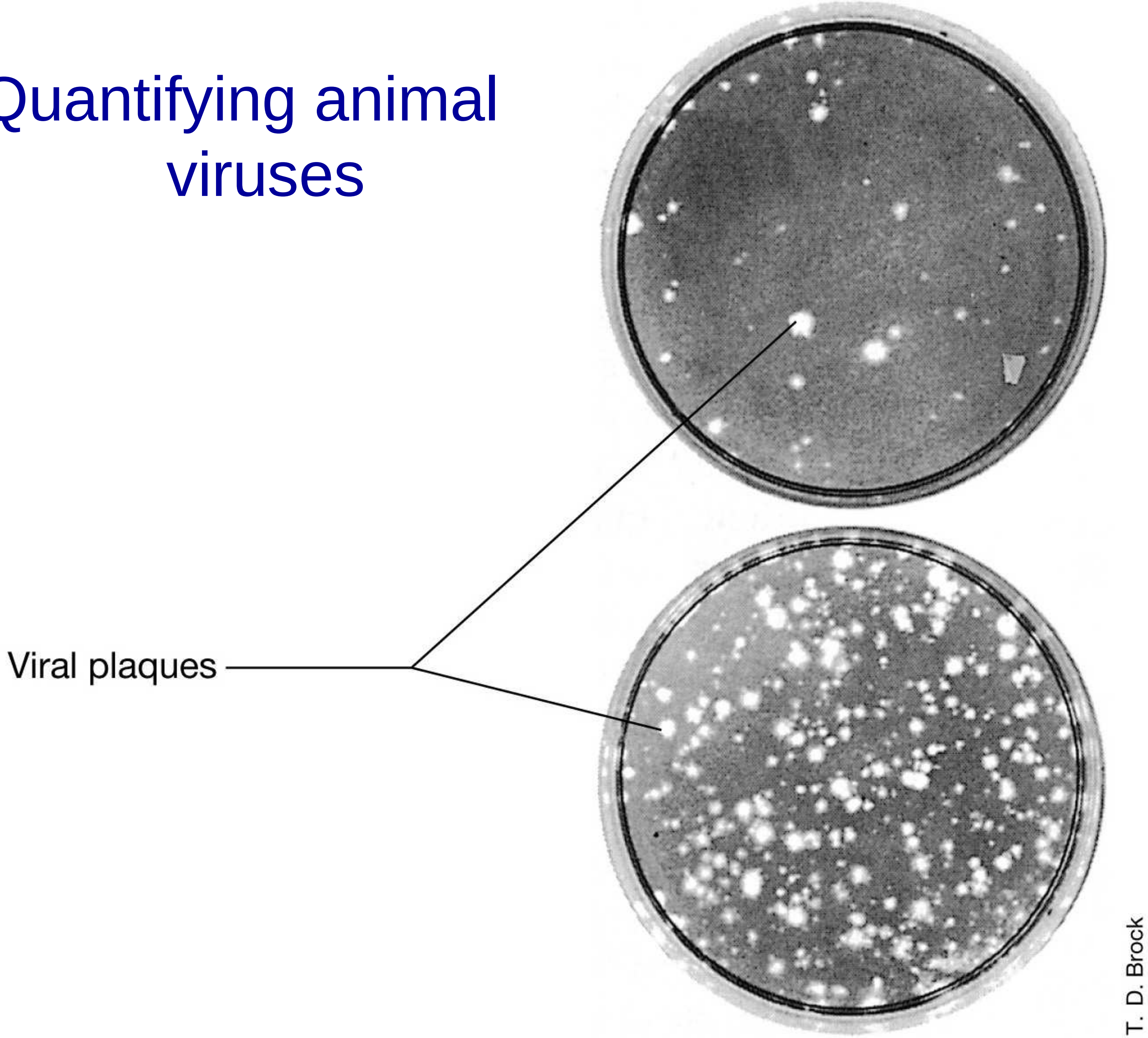
 

•**The plaque assay** - dilutions of the virus are used to infect a cultured cell monolayer, covered with agar to restrict virus diffusion virus.

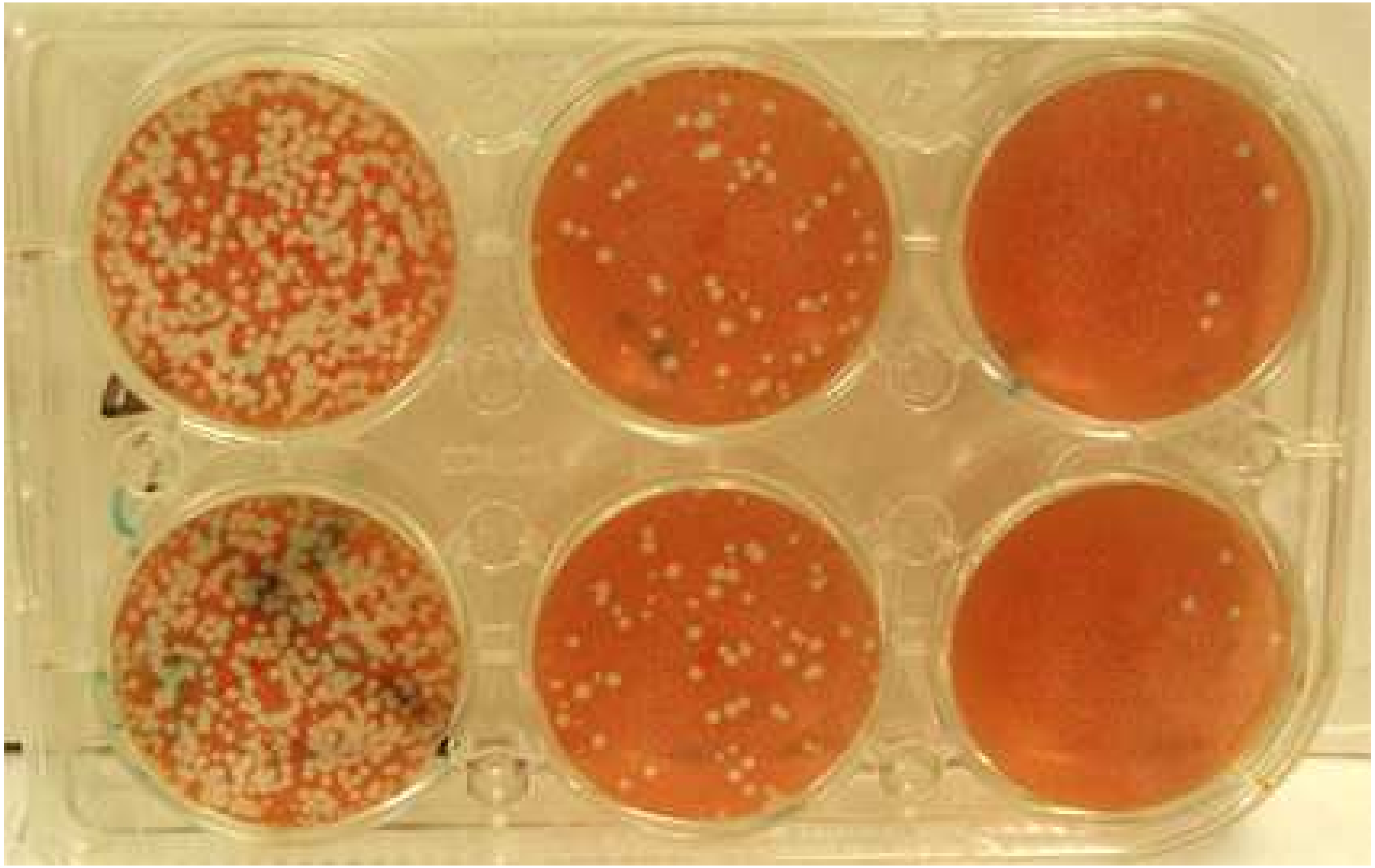
•Results in localized cell killing and the appearance of plaques.

•The number of plaques directly relates to numbers of infectious virus particles applied to the plate.

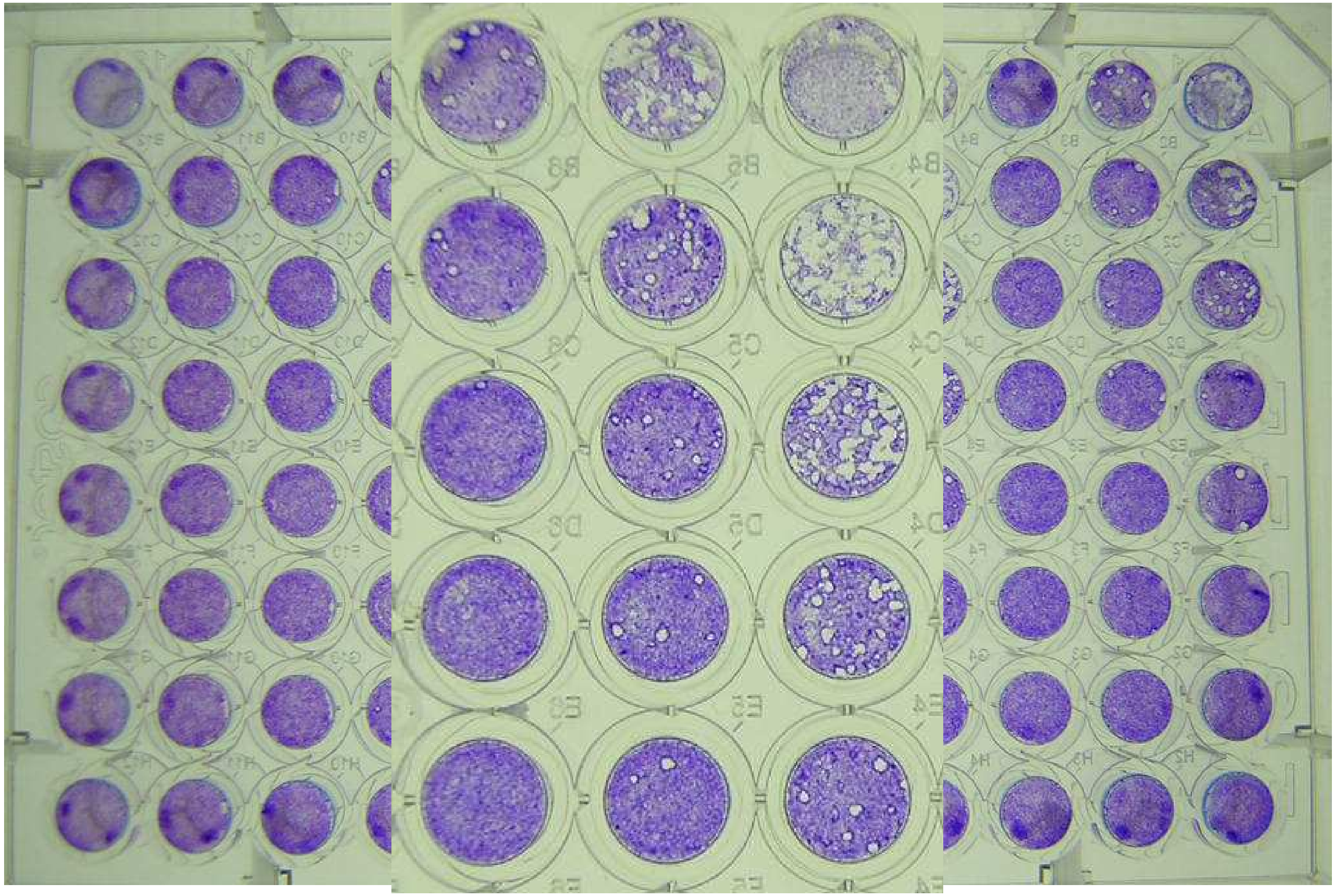
Quantifying animal viruses



An example of Plaque Assay



Titration of MCMV infectivity by plaque assay (7 dpi)



Other practical approaches

- Serology.
- Structural studies, purification, EM, X-ray.
- Biochemical analysis, electrophoresis
- Genetic
- Molecular biology, nucleic acid sequencing.