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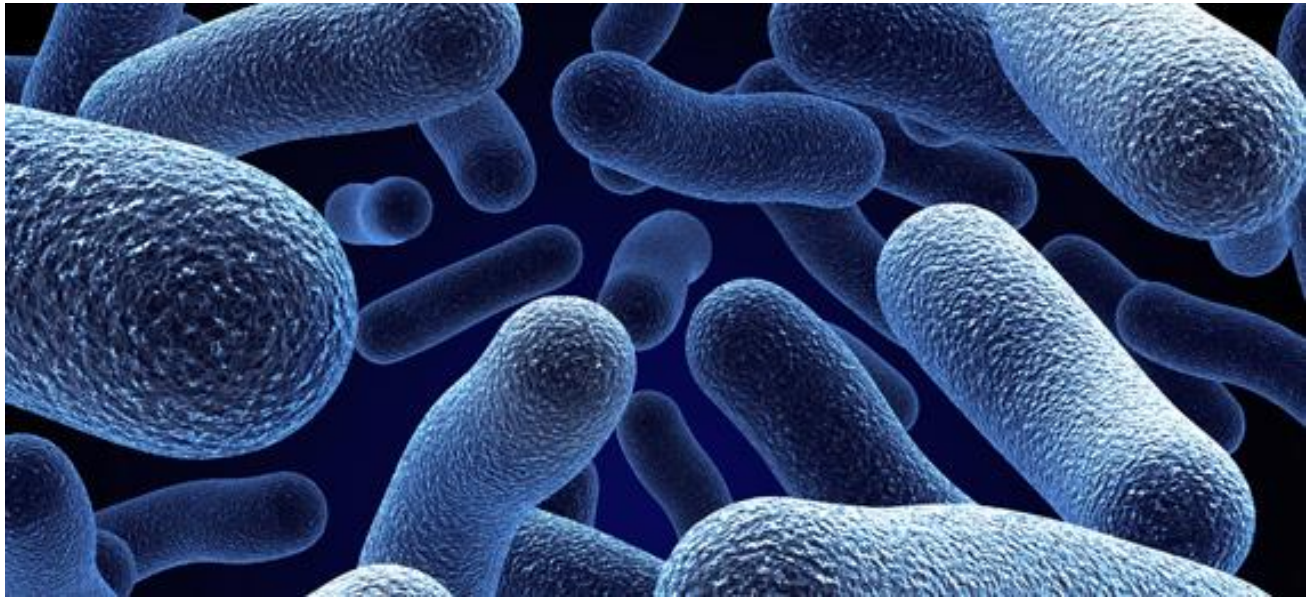
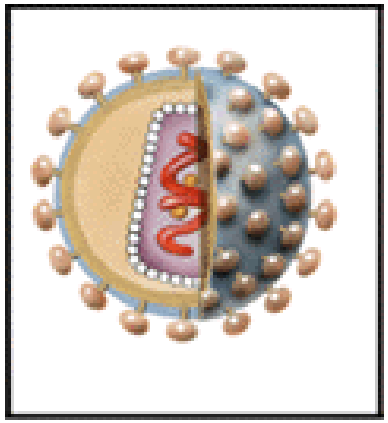
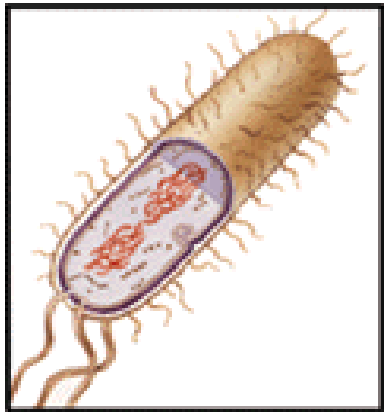
MICROBIOLOGY

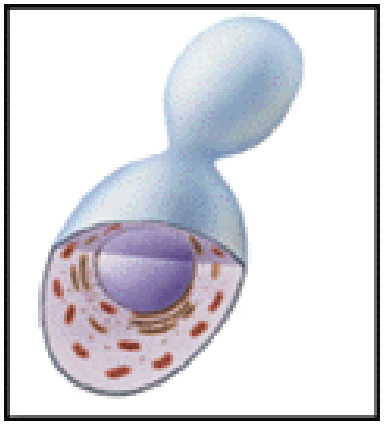


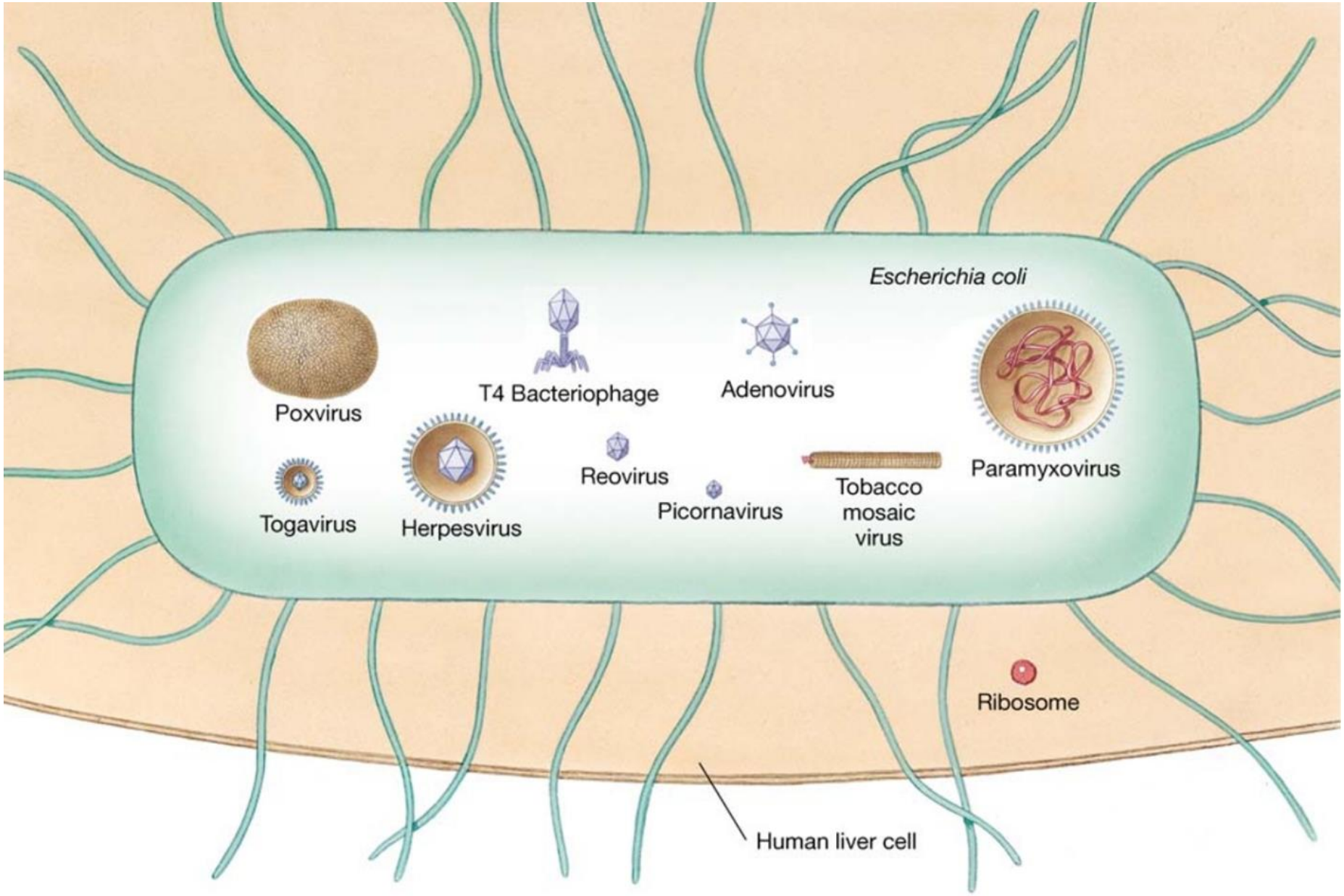
- Microbiology is the study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters
- It also includes viruses, which are microscopic but not cellular

Classic causative agents of infectious diseases








Escherichia coli
Poxvirus
T4 Bacteriophage
Adenovirus
Paramyxovirus
Togavirus
Herpesvirus
Reovirus
Picornavirus
Tobacco mosaic virus
Ribosome
Human liver cell

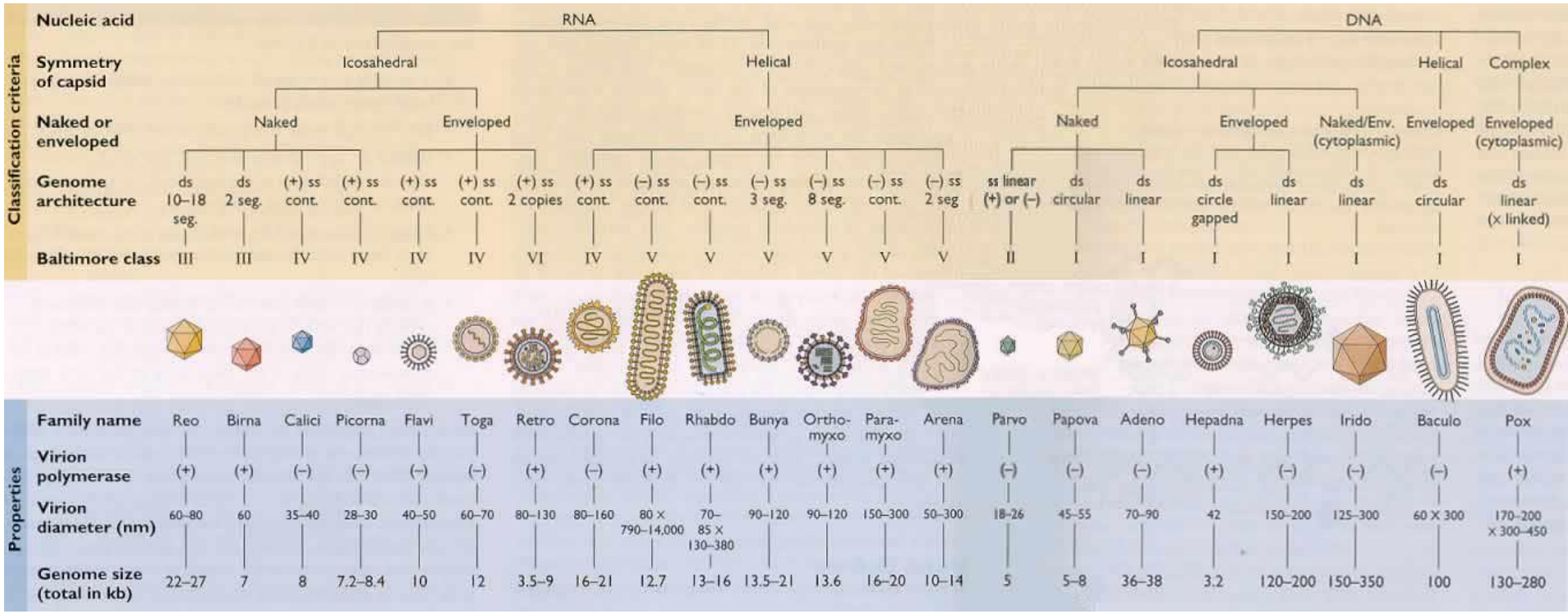
VIROLOGY

12th – 15th November 2013

School of Medicine, University of Split

International Committee on Taxonomy of Viruses

- The latest report by the International Committee on Taxonomy of Viruses (2012) lists **5450 viruses**, organized in over **2,000 species**, **287 genera**, **73 families** and **3 orders**.
- Virologists also study *subviral particles*, infectious entities notably smaller and simpler than viruses:
- viroids (naked circular RNA molecules infecting plants),
- satellites (nucleic acid molecules with or without a capsid that require a helper virus for infection and reproduction), and
- prions (proteins that can exist in a pathological conformation that induces other prion molecules to assume that same conformation).


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.
(+) ss 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 × 790–14,000
70–85 × 130–380
90–120
90–120
150–300
50–300
18–26
45–55
70–90
42
150–200
125–300
60 × 300
170–200 × 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

Epidemiology of AIDS

By the end of 2009

- more than 30 million people worldwide had died of AIDS
- over 16.6 million children had been orphaned
- a total of 33.3 million people worldwide were living with HIV/AIDS

Three Influenza Pandemics

Spanish flu	1918-1920	A/H1N1	Antigendrift	20-50 million deaths
Asian flu	1957-1958	A/H2N2	Antigenshift	1-1.5 million deaths
Hong Kong flu	1968-1969	A/H3N2	Antigenshift	0.75-1 million deaths

Cervical Cancer

- There were about **500‘000** incident cases of and **275‘000** deaths due to cervical cancer worldwide in 2002.
- Equivalent to about a tenth of all deaths in women due to cancer

Parkin DM et al. *CA Cancer J Clin* 2005; **55:** 74–108

Estimated hepatitis burden worldwide

		HAV	HBV	HCV	HDV	HEV
Total Infected	Worldwide	1.4 million	2 billion	~250–300 million	10–15 million	1.5%–26% Antibody prevalence
	US	3,579 (reported)	1.45 million[a]	4 million	NA	1.5%–3% Antibody prevalence
Chronic Infections	Worldwide	NA	350 million	170–200 million	200,000–300,000[b] 1–1.5 million[c]	NA
	US	NA	800,000–1.4 million	3.2 million	NA	NA
Newly Infected / Year	Worldwide	1.4 million	NA	3–4 million	NA	>50% of reported acute cases of hepatitis
	US	32,000 (estimated total)	46,000	19,000	NA	Usually travel related
Deaths / Year	Worldwide	0.1% <14 yrs 0.3% 15–39 yrs 2.1% 40 yrs	600,000	1.6%–2.5% of the carrier population	<10%,[b] <20%[c] Up to 80% in cases of fulminant hepatitis	0.5%–4.0%[d] 20% pregnant women in 3rd trimester
	US	5 (reported)	5,000	8,000–10,000	>1,000	NA

[a]Extrapolated from total chronic and new infections
[b]If coinfected
[c]If superinfected
[d]Depending on strain

Global HCV Infection

Total 170 m

Dengue

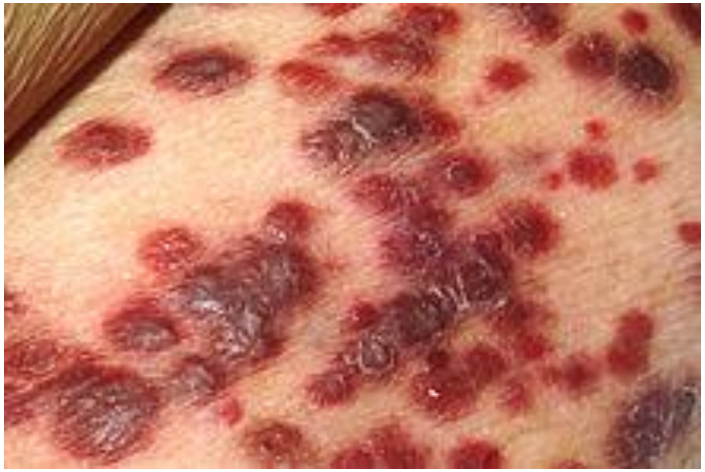
- Today about 2.5 billion people, or 40% of the world's population, live in areas with a risk of dengue transmission
- Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean.
- The World Health Organization (WHO) estimates that **50 to 100 million infections occur yearly, including 500,000 DHF cases and 22,000 deaths, mostly among children.**

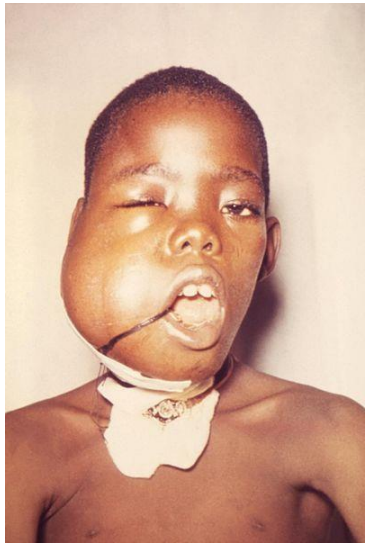
>60% of all human infections

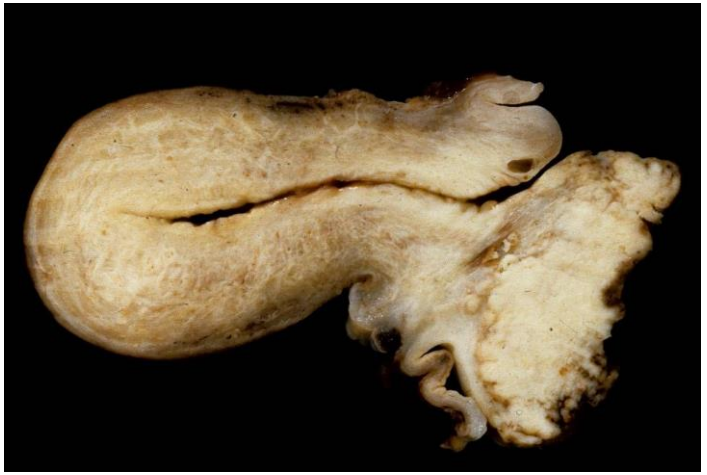
are caused by viruses

~15% are caused by bacteria

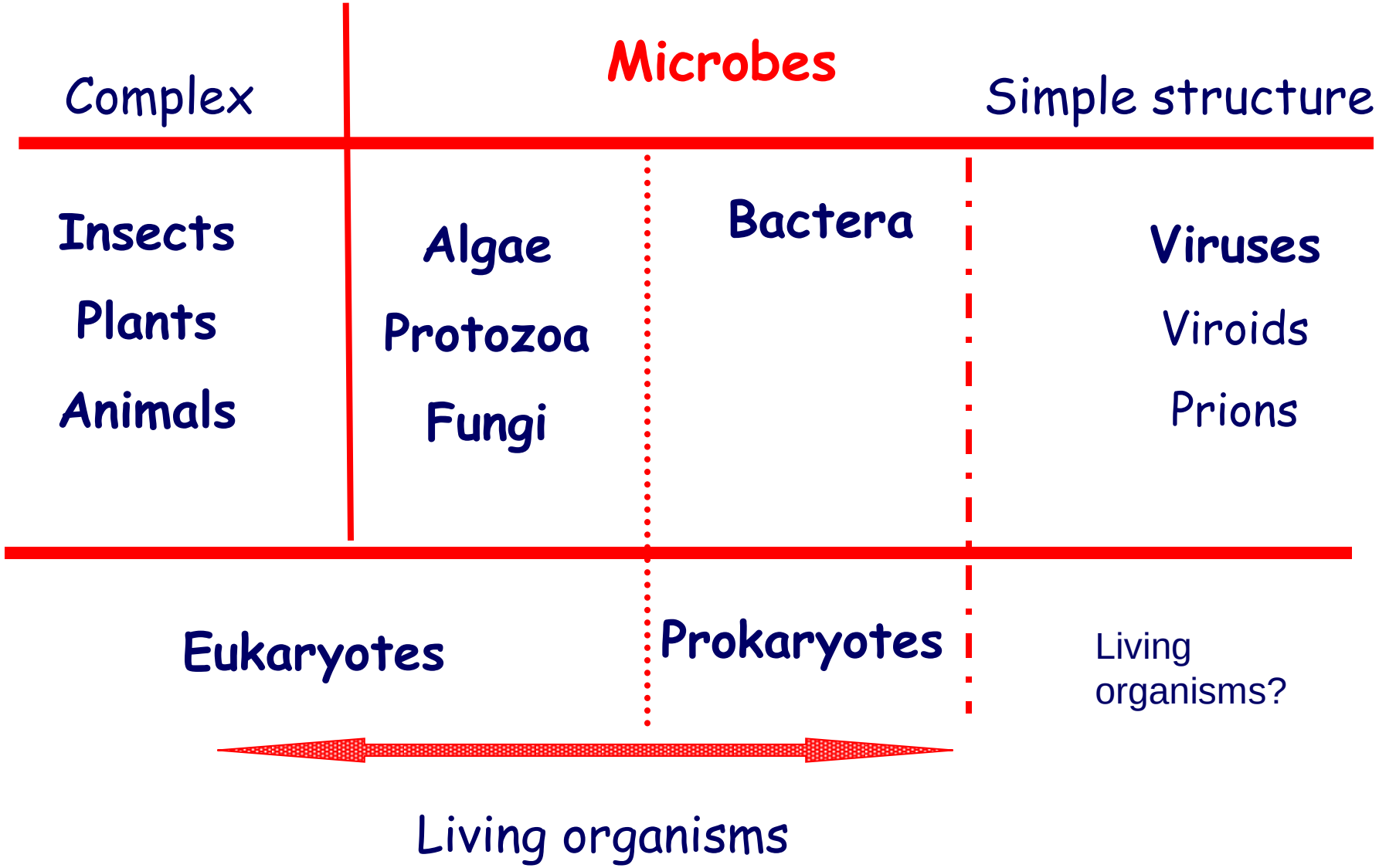
At least 15-20% of all human tumors worldwide have a viral cause







Organisms in nature



Characteristics Of Living Organisms

- Comprised one or more units called cells
- Reproduce (sexually or asexually)
- Grow and develop
- Obtain and use energy
- Respond to their environment (adaptation and evolution)

VIRUSES

- "FILTERABLE AGENTS"
- OBLIGATE INTRACELLULAR AGENTS

Year 1935 – Viruses can be crystallized

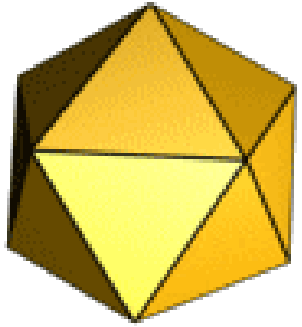
The first virus that could be crystalized and whose structure could therefore be elucidated in detail was tobacco mosaic virus (TMV), the virus that had been studied earlier by Ivanovski and Beijerink. In 1935, Wendell Stanley achieved its crystallization for electron microscopy and showed that it remains active even after crystallization.

Are viruses alive ??

VIRUSES

Viruses are both, dead AND alive

- Outside a living cell they are nothing but a large complex of organic chemicals
- Inside the cell they assume some properties of life (they can reproduce!)

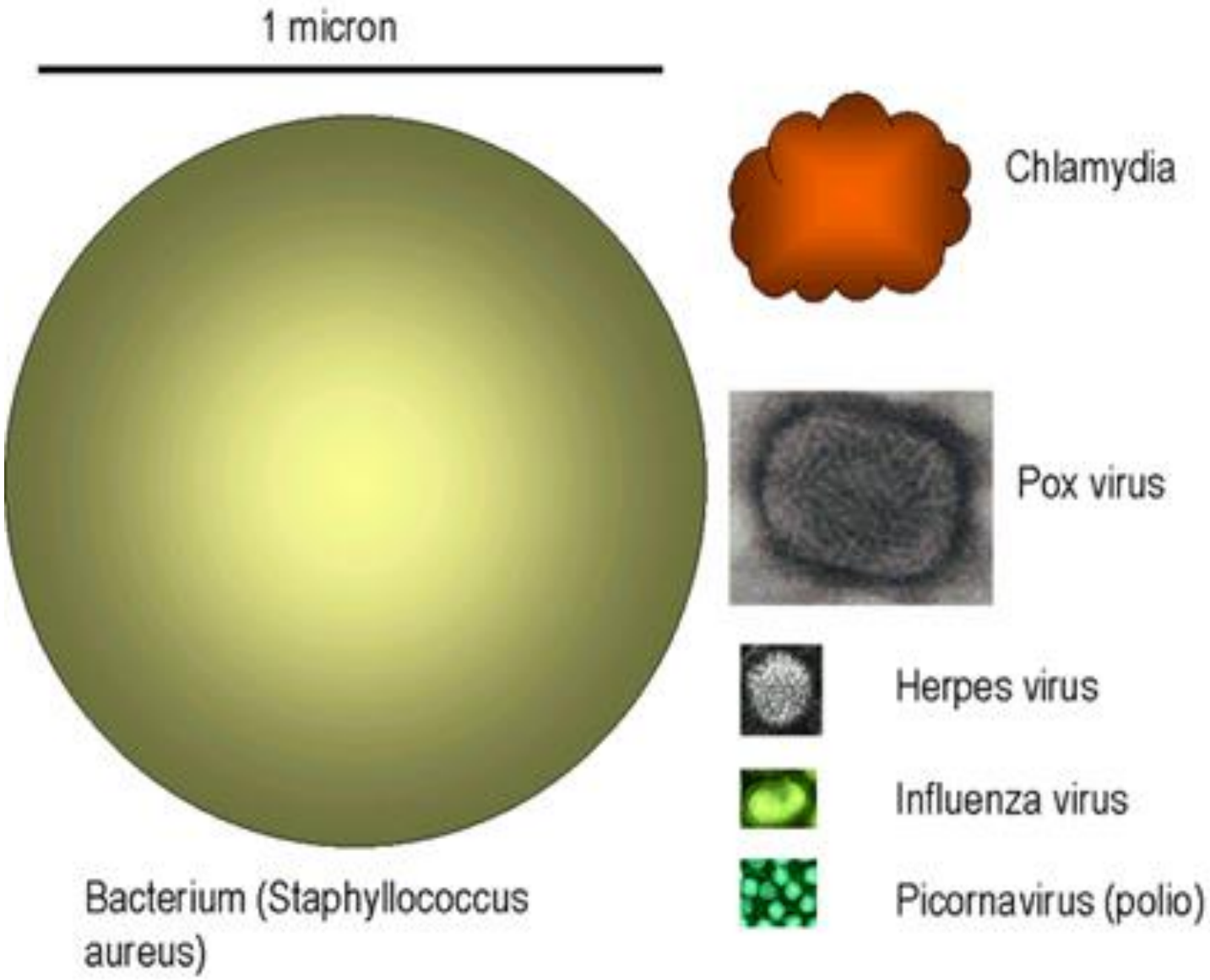


Virus Definition

Viruses are ultramicroscopic agents composed of an RNA or DNA core surrounded by a protein coat, that replicate only within the cells of living hosts, mainly bacteria, plants and animals.

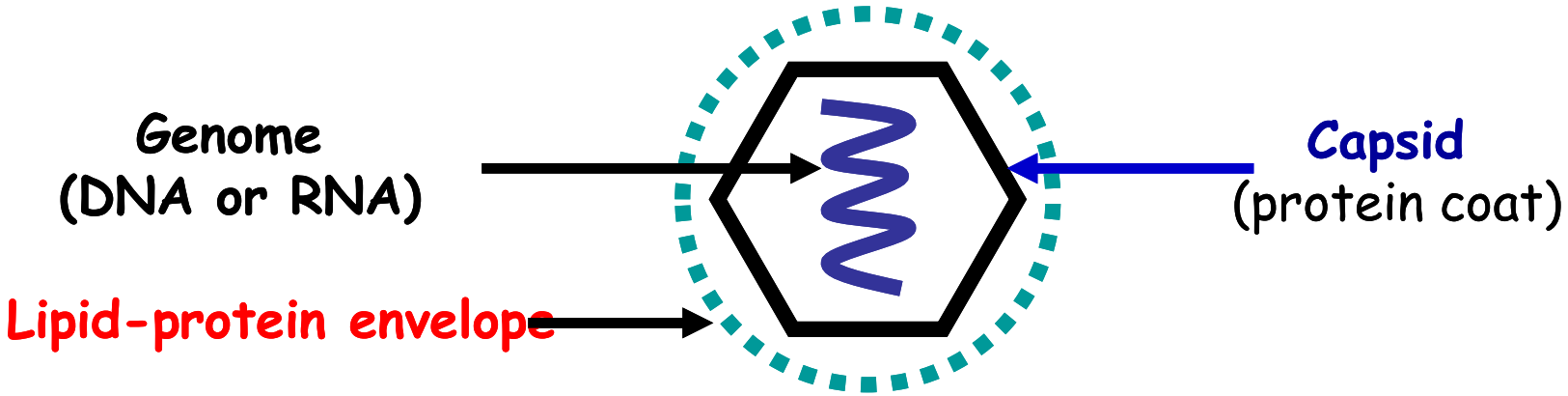
What are the differences between bacteria and viruses?

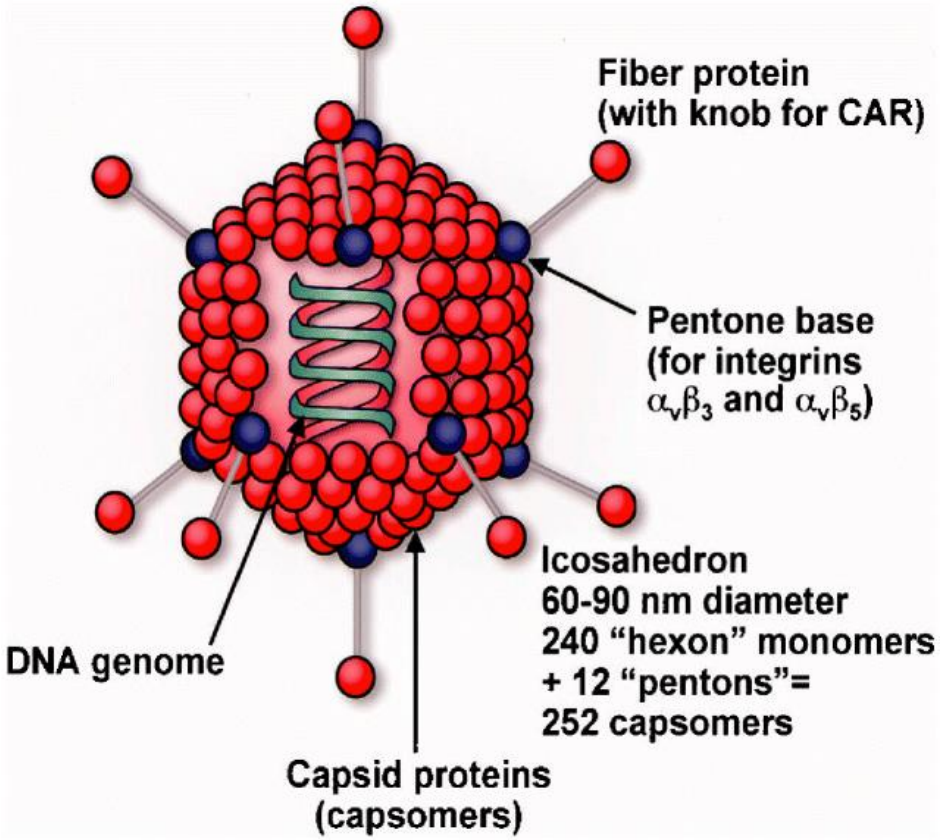
	Bacteria	Viruses
Growth in artificial media	Usually yes	never
Binary fission	yes	no
Contain DNA and RNA	yes	no
Protein synthesis	yes	no
Muramic acid	Usually yes	no
Antibiotic sensitivity	yes	no
Obligate intracellular parasites	some	all


1 micron
Chlamydia
Pox virus
Herpes virus
Influenza virus
Picornavirus (polio)
Bacterium (Staphyllococcus aureus)

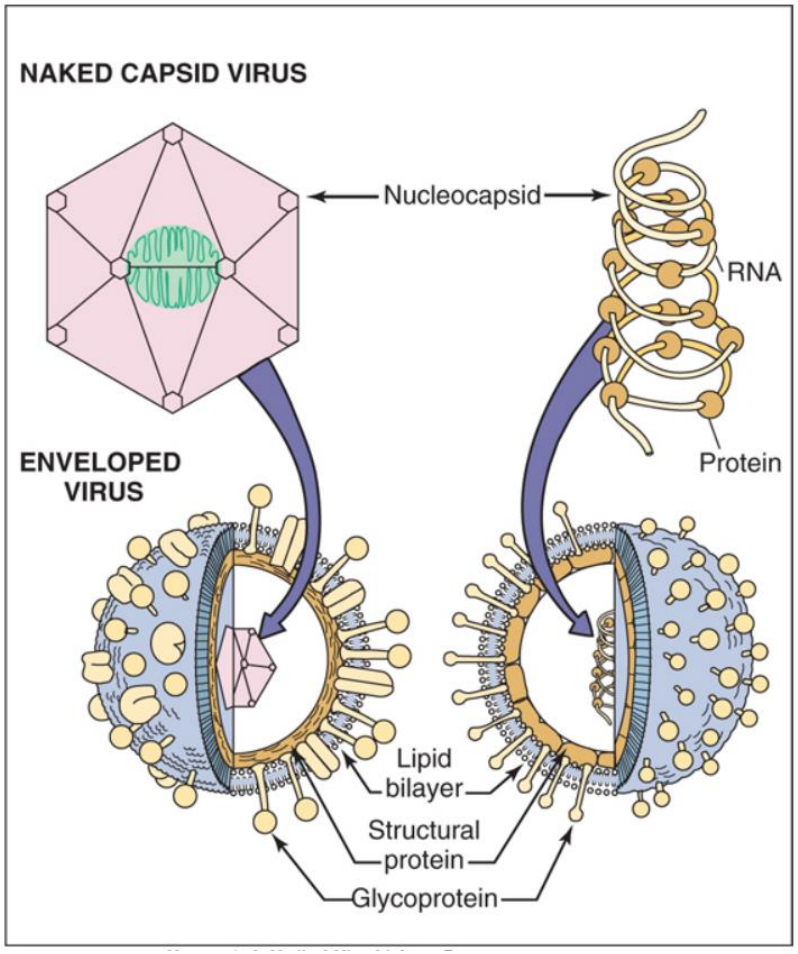
Viruses

- Obligate intracellular parasites
- They range in size from about 20 to 300 nanometres in diameter
- Relatively simple structure and composition
- With or without s ili bez lipoproteinske ovojnice

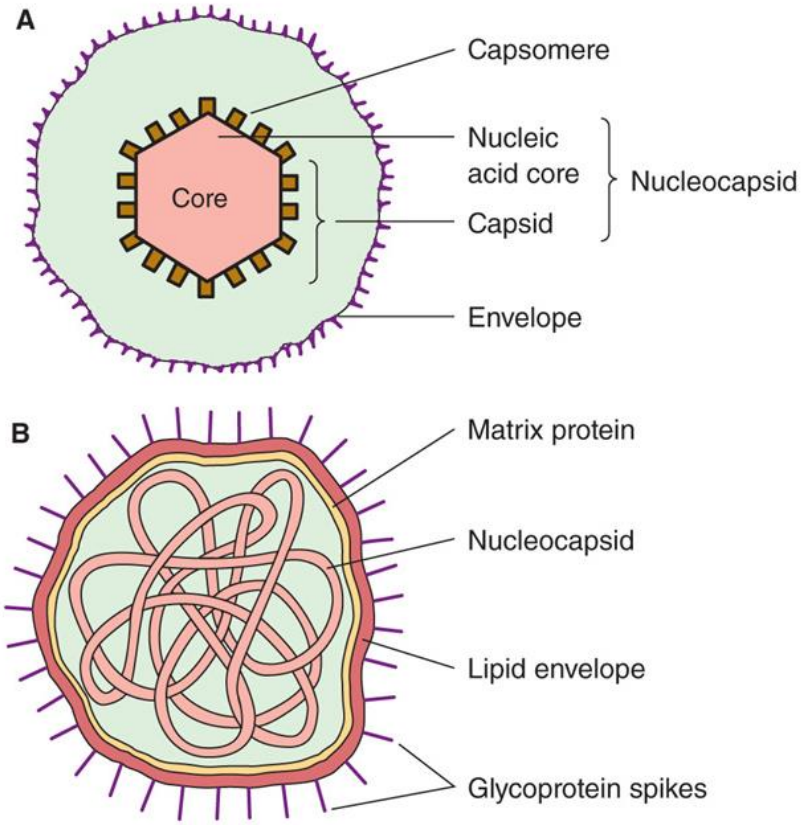




- Viral genetic material is packaged inside protein structures called capsids.



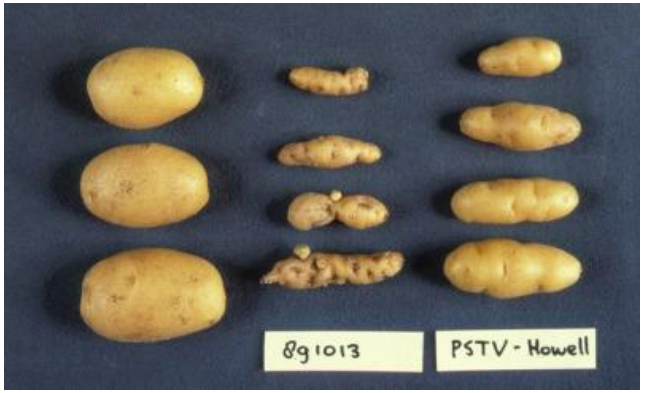
- Viruses are divided into two groups:
  - enveloped viruses are surrounded by an outer lipid membrane;
  - nonenveloped viruses lack this membrane.



- Where present, the envelope contains the viral proteins, which mediate binding to host cells.
- Where no envelope is present, this function is carried out by the outer capsid proteins.
- In general, nonenveloped viruses are more stable and can survive much longer in the environment.
- Capsids and envelopes determine the method of viral entry into and exit from host cells.

Viroids

- Particles smaller than viruses
- **Pure RNA** – ss linear or circular
- No protein-coding genes
- No protein coat
- Plant pathogens



- Viroids are not usually considered a form of life.

Prions

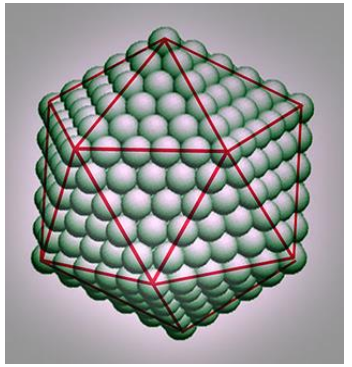
- Infectious proteins
- No nucleic acid
- Human and animal neurodegenerative diseases
- Long incubation period ("slow viruses")
- Prion diseases are always fatal

Viruses vs. prions

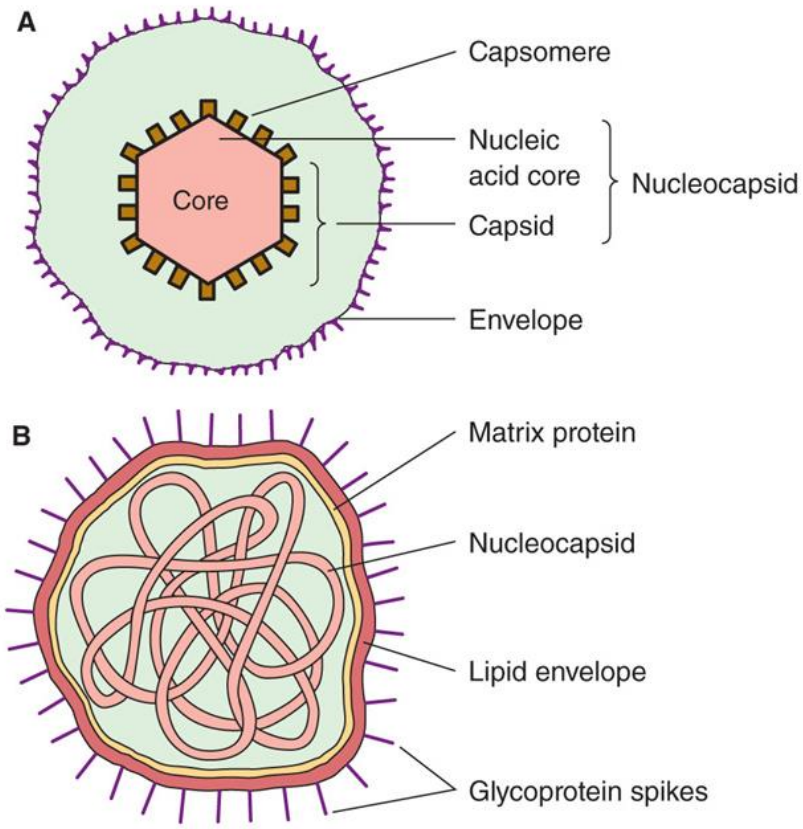
	VIRUSES	PRIONS
Filterability	yes	yes
Infectivity	yes	yes
Nucleic acid	yes	no
Recognizable shape (el. microscope)	yes	no
Proteins	yes	yes
Size	18-300 nm	4-6 nm

TERMINOLOGY

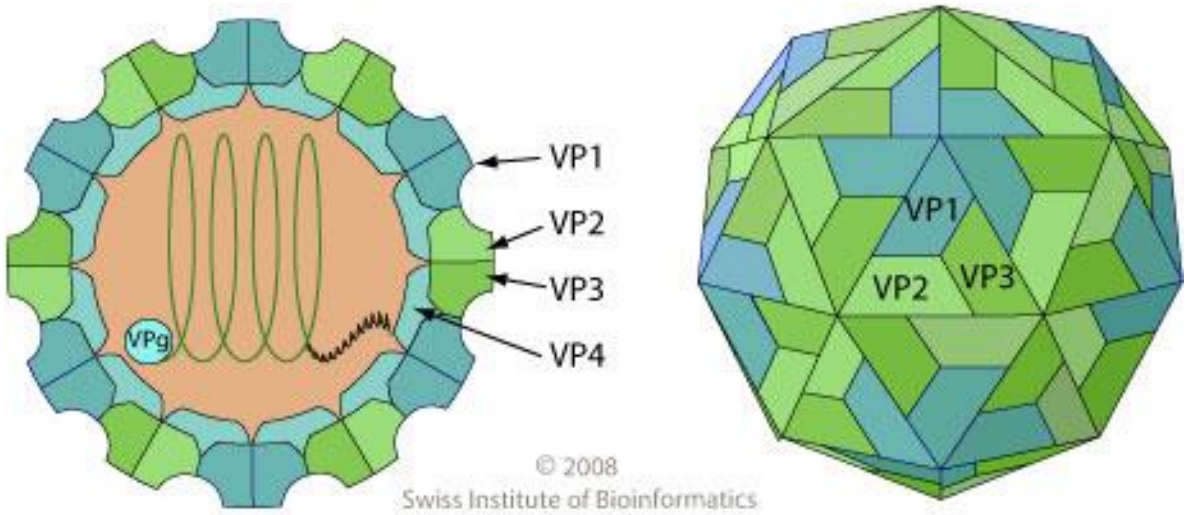
- Kompletna virusna čestica = **virion**
- **Virusni genom – RNK ili DNK**
- Proteinski omotač (ljuska) = **kapsida**
- Kapsida je obično **simetrična**
- Proteinske morfološke jedinice - **kapsomere**
- Kapsida + genom = **nukleokapsida**
- Vanjska ovojnica (staničnog porijekla)
- Glikoproteinski izdanci na ovojnici = **peplomere**



TERMINOLOGY



- **Capsid:** The protein shell, or coat, that encloses the nucleic acid genome.
- **Capsomeres:** Morphologic units seen in the electron microscope on the surface of icosahedral virus particles.
- **Envelope:** A lipid-containing membrane that surrounds some virus particles. These projections are called **peplomers**.
- **Nucleocapsid:** The protein–nucleic acid complex representing the packaged form of the viral genome.
- **Virion:** The complete virus particle (serves to transfer the viral nucleic acid from one cell to another).



- **Structural units:** The basic protein building blocks of the coat. They are usually a collection of more than one nonidentical protein subunit. The structural unit is often referred to as a **protomer**.

VIRAL GENOME

DNA VIRUSES

- **ds DNA**

  all except

  Parvoviridae

RNA VIRUSES

- **ss RNA**

  all except

  Reoviridae

  - Genome
    - segmented
    - non-segmented

VIRAL GENOME

DNA

Single-stranded

Double-stranded

DNA VIRUSES

Double-stranded

DNA

all except

Parvoviridae

VIRAL GENOME

RNA VIRUSES

- Single-stranded RNA
- All except *Reoviridae*
  - Genome
    - Non-segmented
    - Segmented

RNA

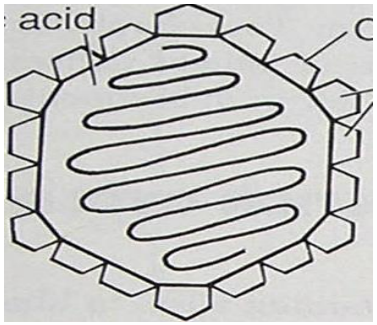
(+) pos. or (-) neg.

Segmented

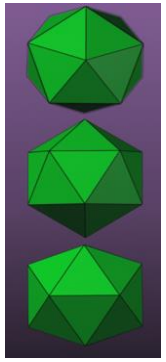
Double-stranded segmented

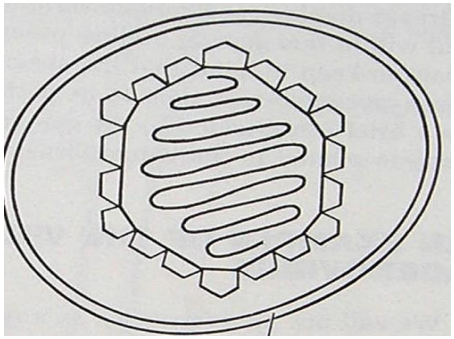
Types of Symmetry of Virus Particles

ICOSAHEDRAL SYMMETRY

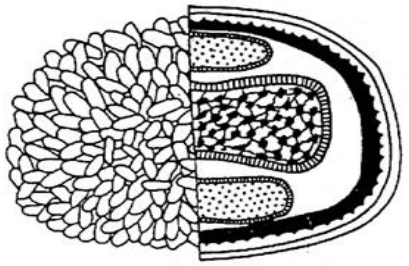


Icosahedron

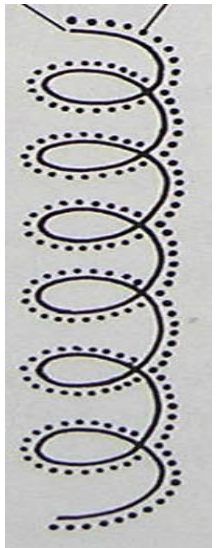


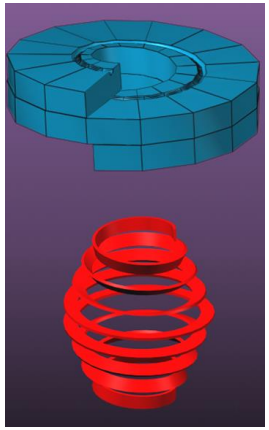


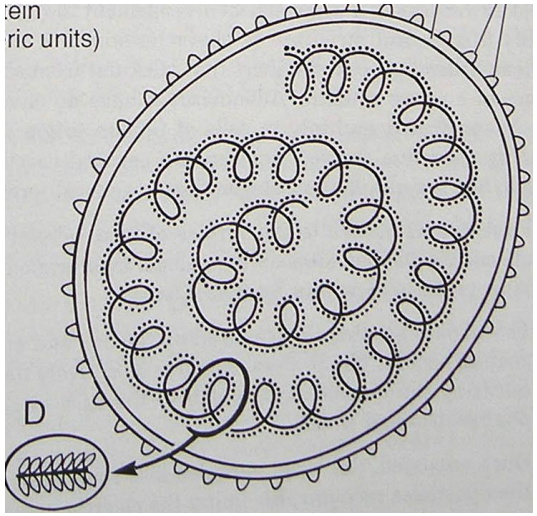
Enveloped icosahedron



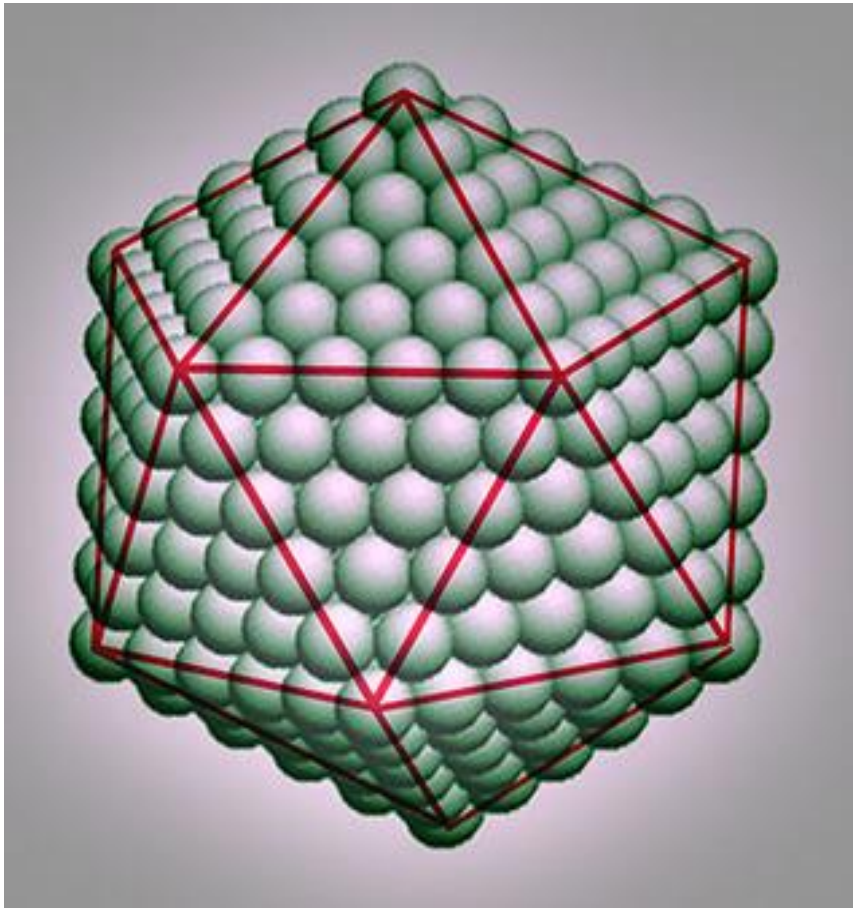
HELICAL (SPIRAL) SYMMETRY







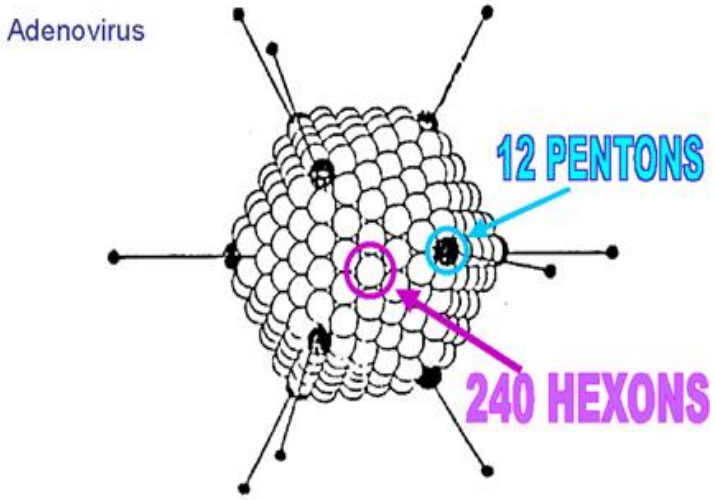
COMPLEX STRUCTURES



- In geometry, an **icosahedron** is a polyhedron with
  - 20 triangular faces
  - 30 edges and
  - 12 vertices

Name	Picture	Faces	Edges	Vertices	Edges per face	Faces meeting at each vertex
icosahedron	 (Animation)	20	30	12	3	5

Icosahedral Symmetry

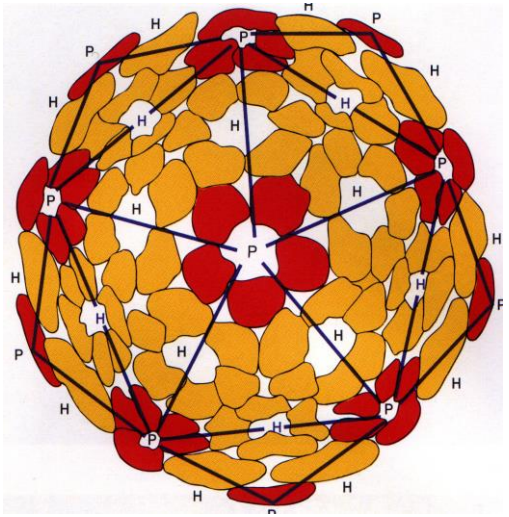


PENTONS

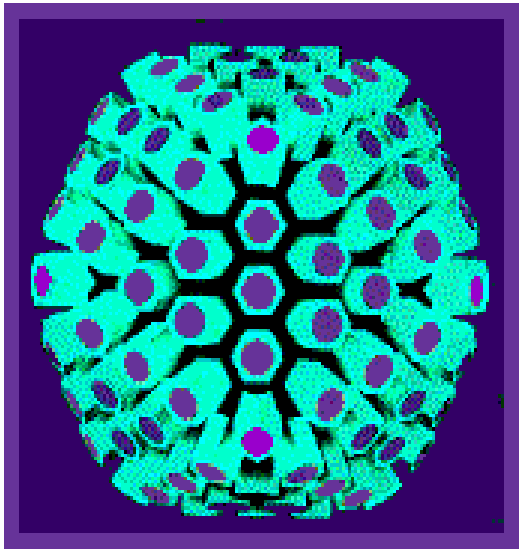
5 neighboring proteins

12 pentons

12 vertices



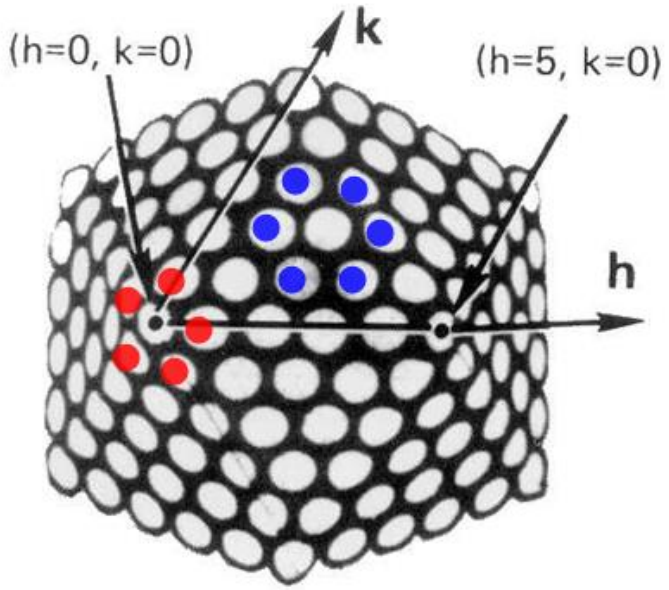
polipeptides → **capsomeres**



HEXSONS

6 neighboring proteins

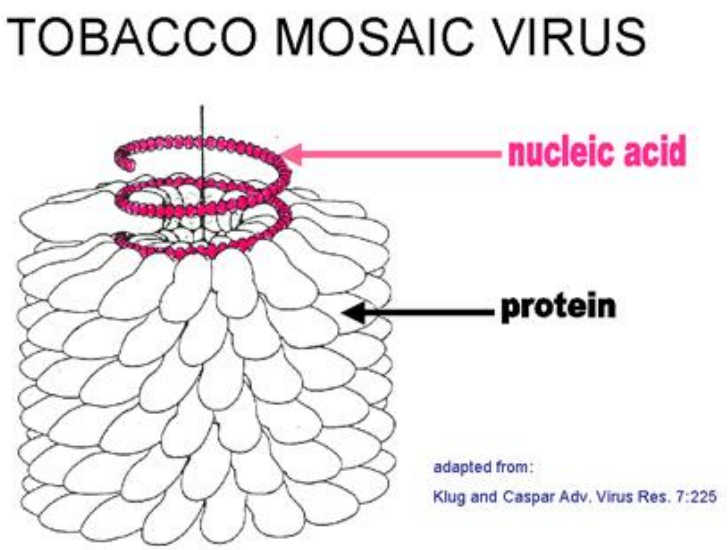
20 faces and 30 edges

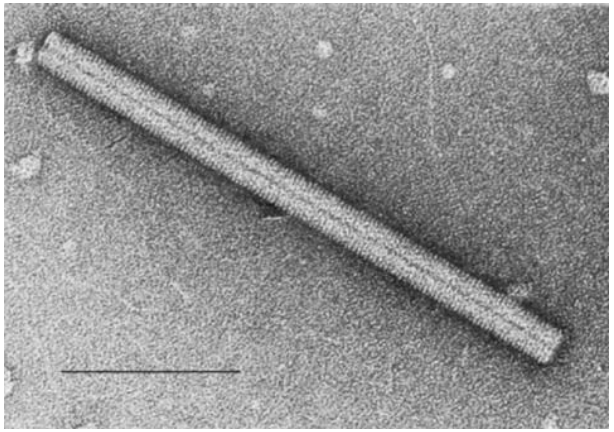


- Two types of capsomeres constitute the icosohedral capsid:
  - pentagonal (pentons) at the vertices and
  - hexagonal (hexons) at the faces

- There are always twelve pentons, but the number of hexons varies among virus groups.

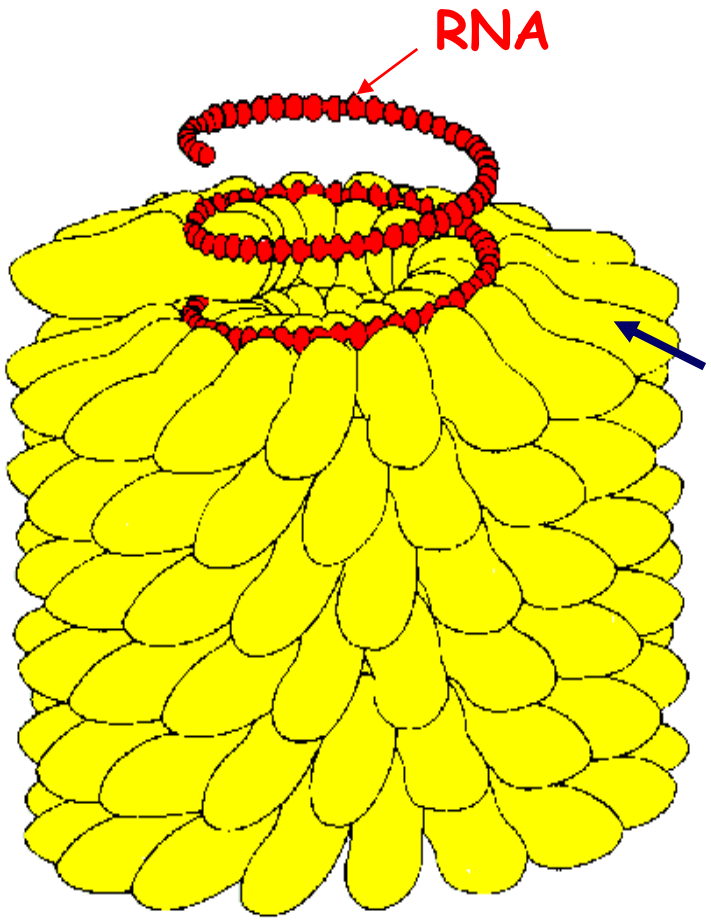
Helical Symmetry





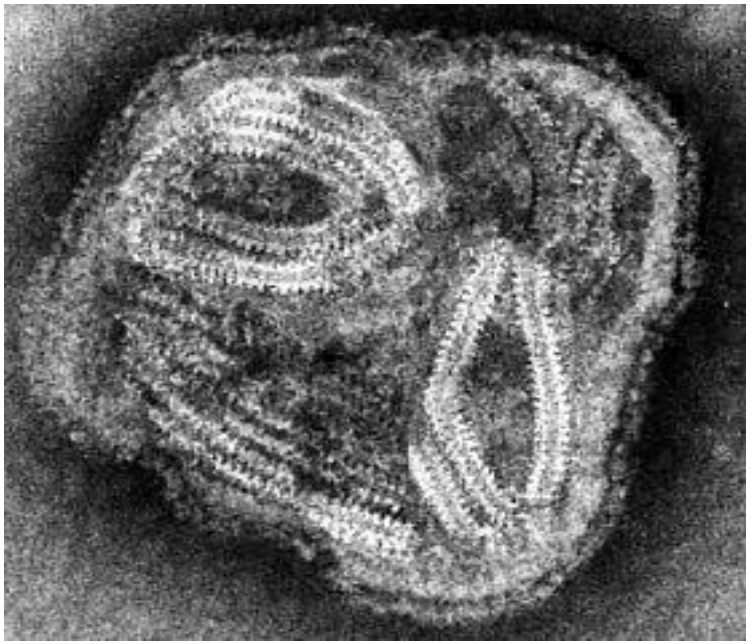
- The protomers are not grouped in capsomeres, but are bound to each other so as to form a ribbon-like structure.
- This structure folds into a helix because the protomers are thicker at one end than at the other.
- The diameter of the helical capsid is determined by characteristics of its protomers, while its length is determined by the length of the nucleic acid it encloses.

Helical Nucleocapsid Symmetry



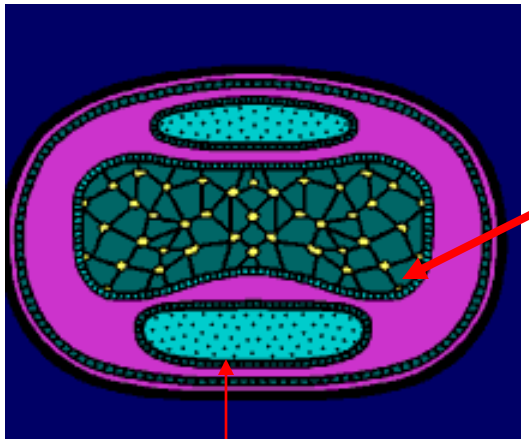
TMV

Parainfluenza 4 virus



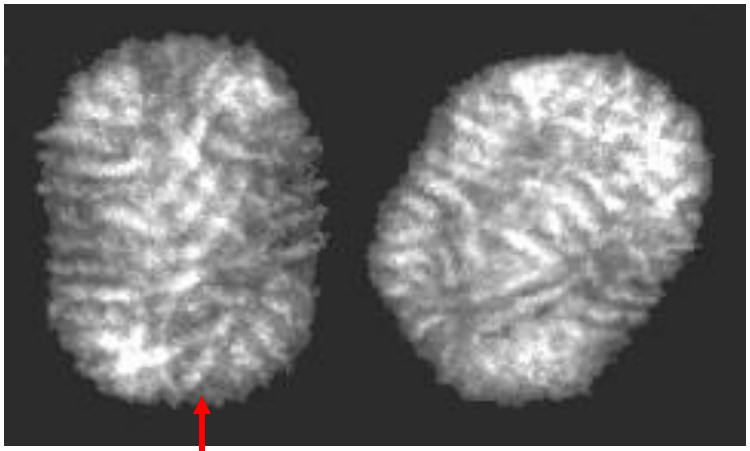
All animal RNA viruses with helical (spiral) nucleocapsid symmetry are enveloped

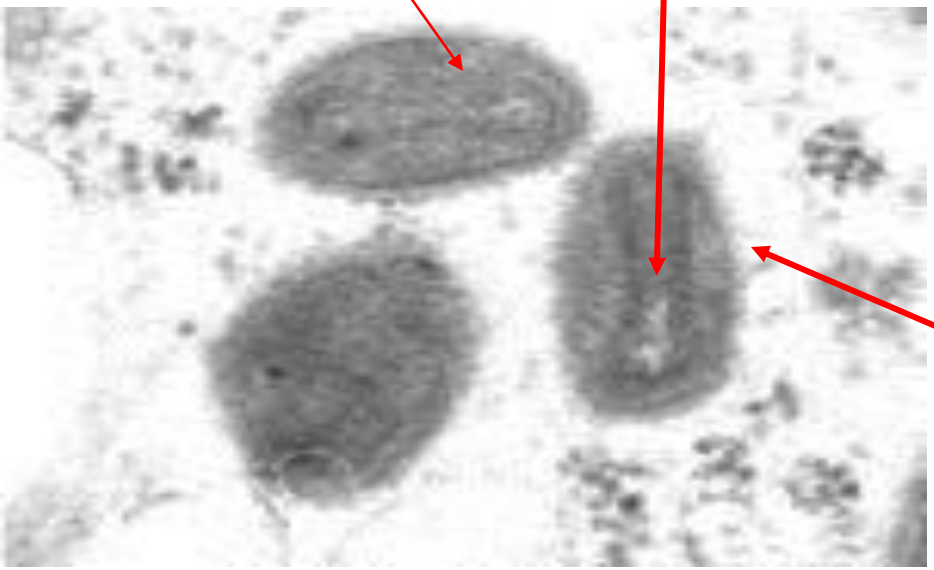
Poxviruses – Complex Capsid Symmetry



Nucleoid- ds DNA with proteins

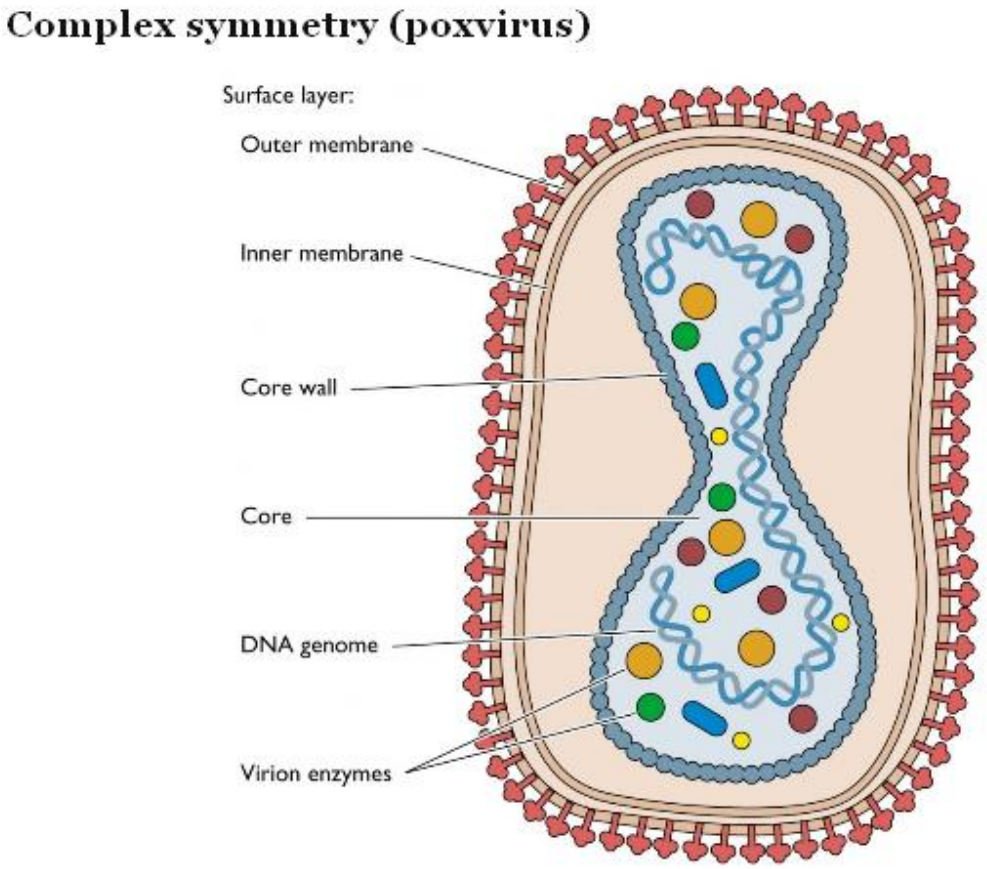
Lateral bodies





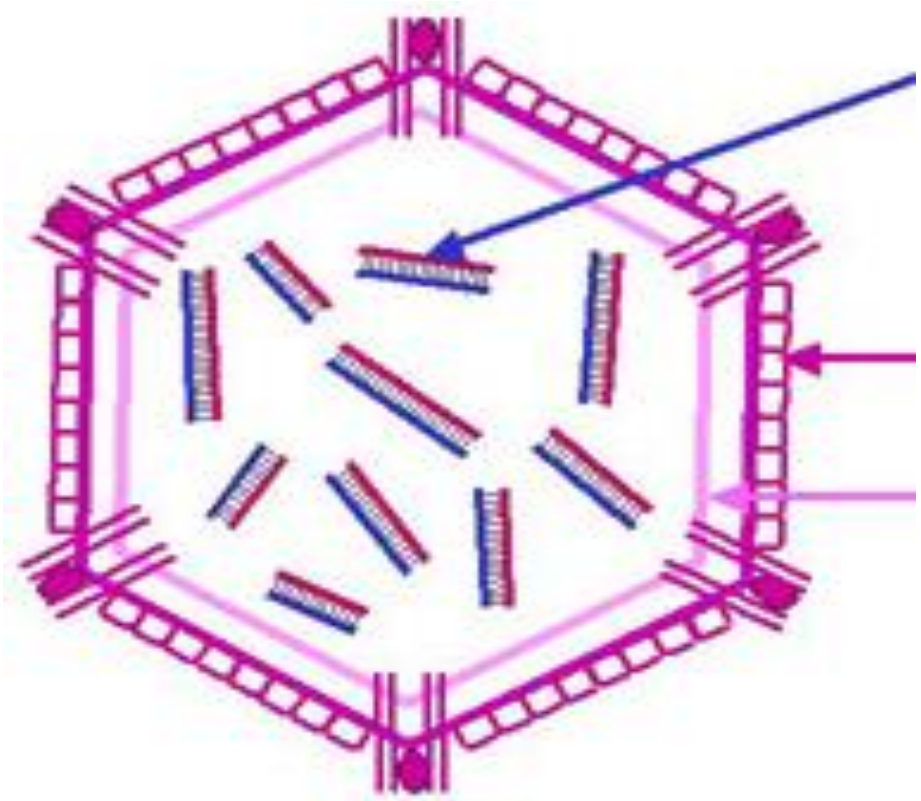
Outer membrane with tubular elements

Complex Symmetry

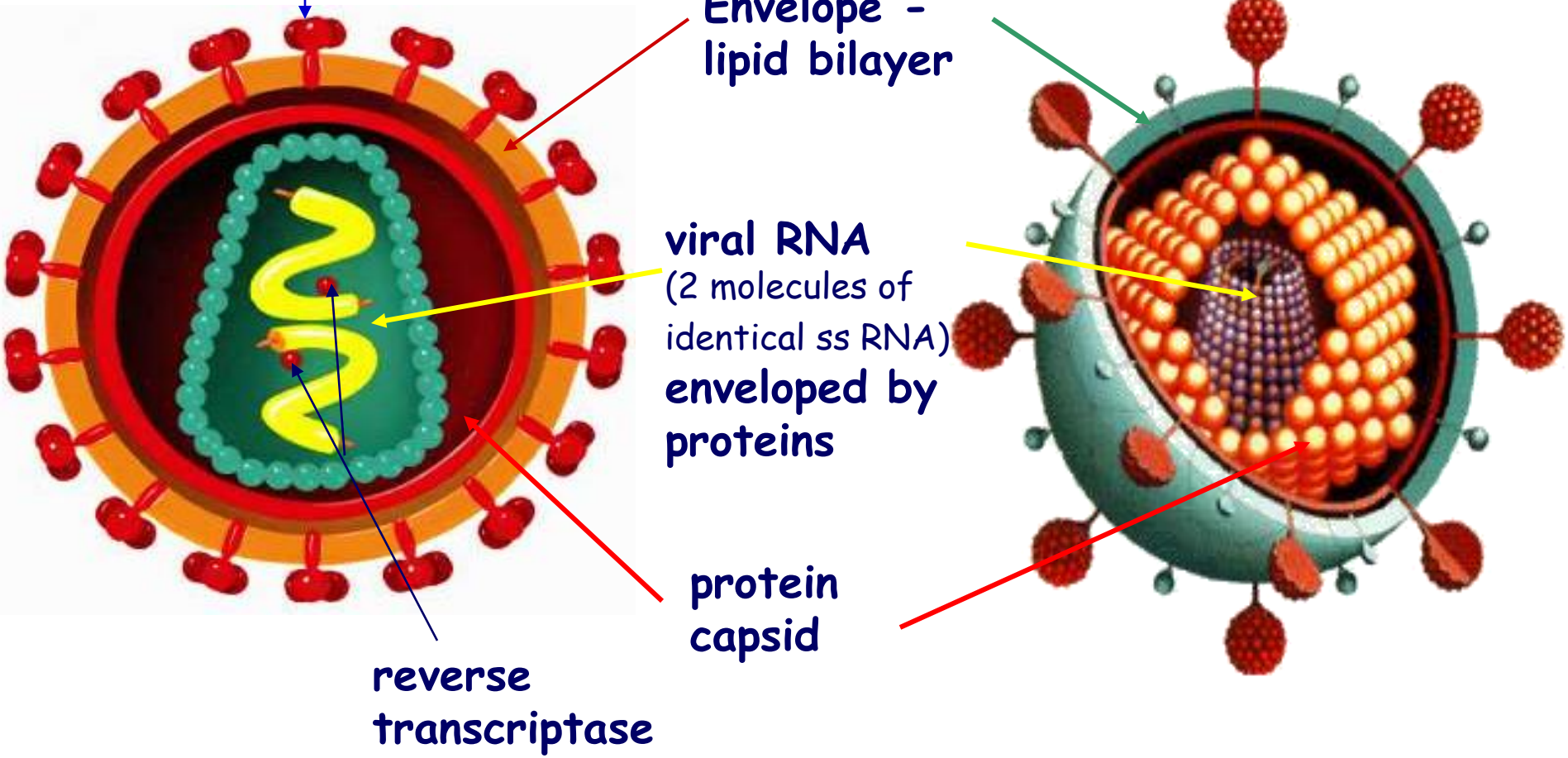


- This group comprises all those viruses which do not fit into either of the above two groups

REOVIRIDAE

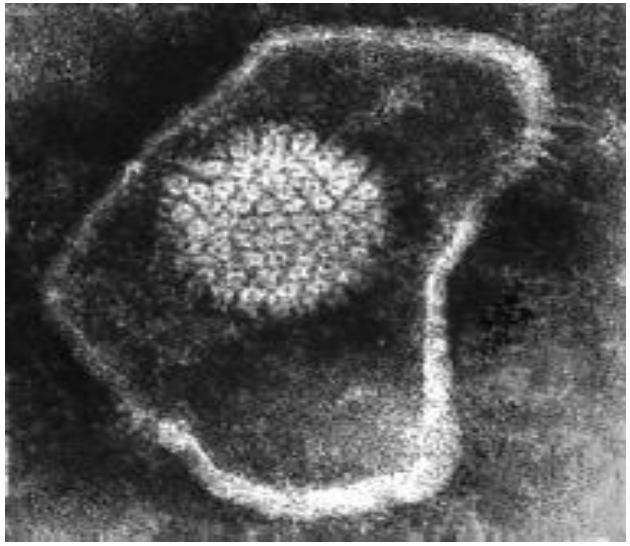

Genome = segmented double-stranded RNA
Outer capsid
Inner capsid

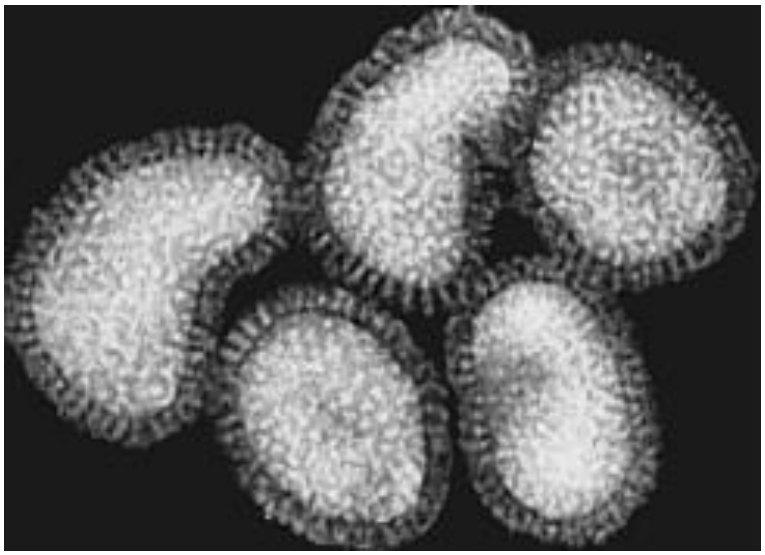
RETROVIRIDAE



Viral Lipid Envelopes

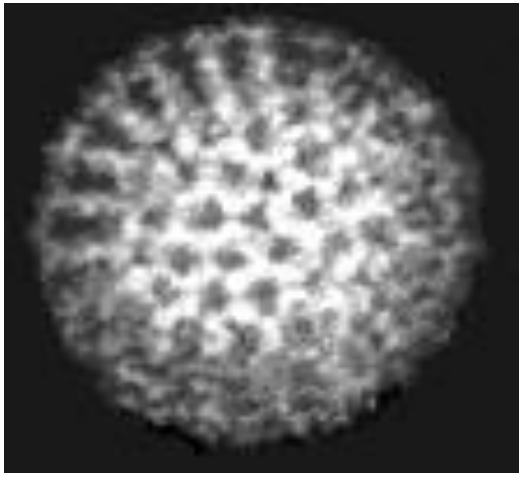
- The lipid is aquired when the viral nucleocapsid buds through a cellular membrane in the course of maturation
- There are always viral glycosylated proteins protruding from the envelope and exposed on the external surface of the viral particle



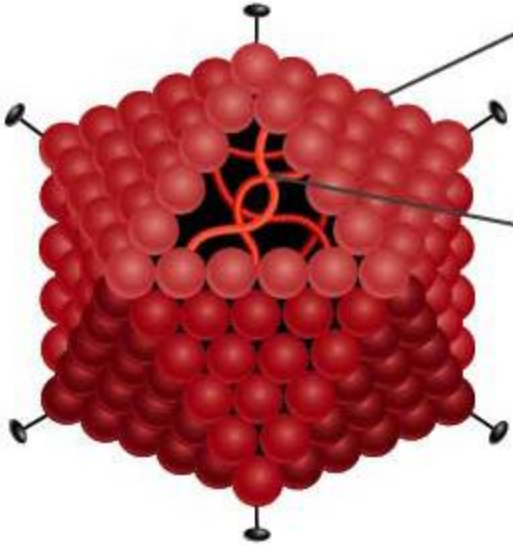


Non-enveloped viruses

- **Relatively stable in the environment** (heat, detergents, acid, organic solvents, dessication)



- **Non-enveloped *viruses* such as Norovirus, Rotavirus, Polio and Parvovirus are easily spread** (hands, dust, droplets)



- The new viruses then **leave the cell by lysis**

„Swimming Pool Conjunctivitis“

- Adenovirus tip 3 i 7

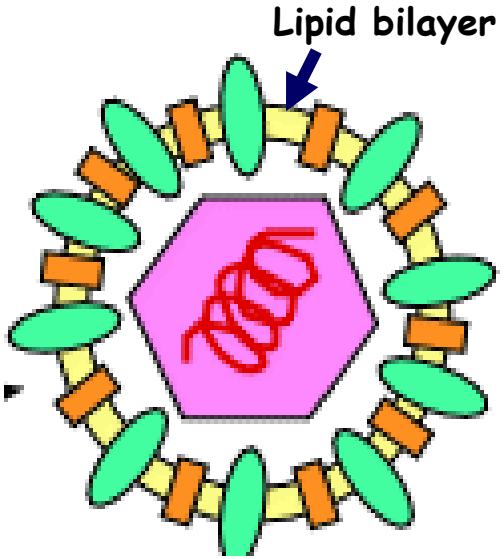
Epidemic keratoconjunctivitis

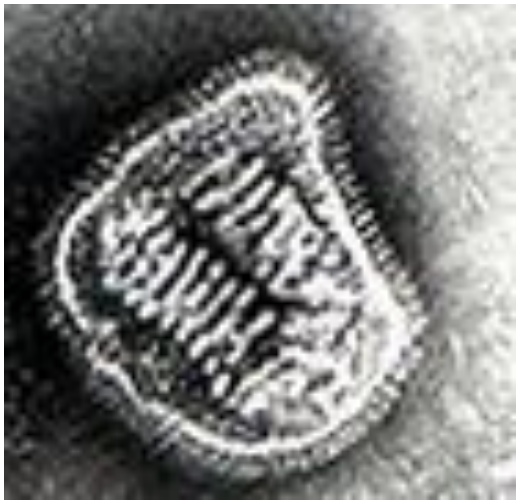
- Transmission of adenovirus can occur via aerosol droplets, the fecal-oral route, and by contact with contaminated fomites.
- Adenoviruses can survive **up to three weeks** at room temperature on environmental surfaces
- They are resistant to lipid disinfectants, but are inactivated by heat, formaldehyde, or bleach (sodium hypochlorite)

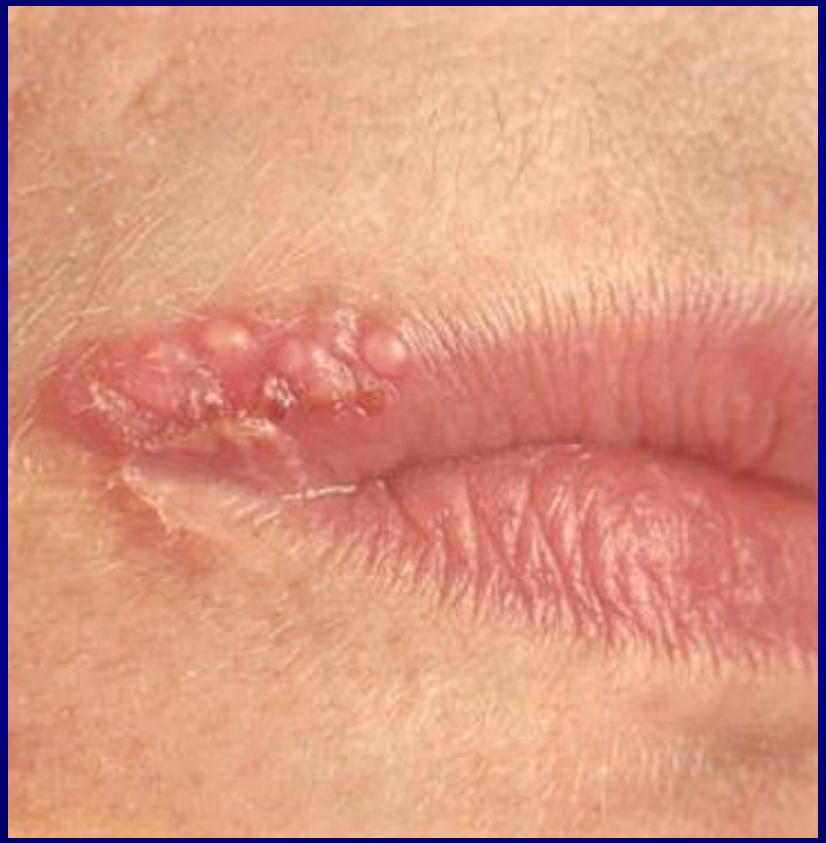
ENVELOPED VIRUSES

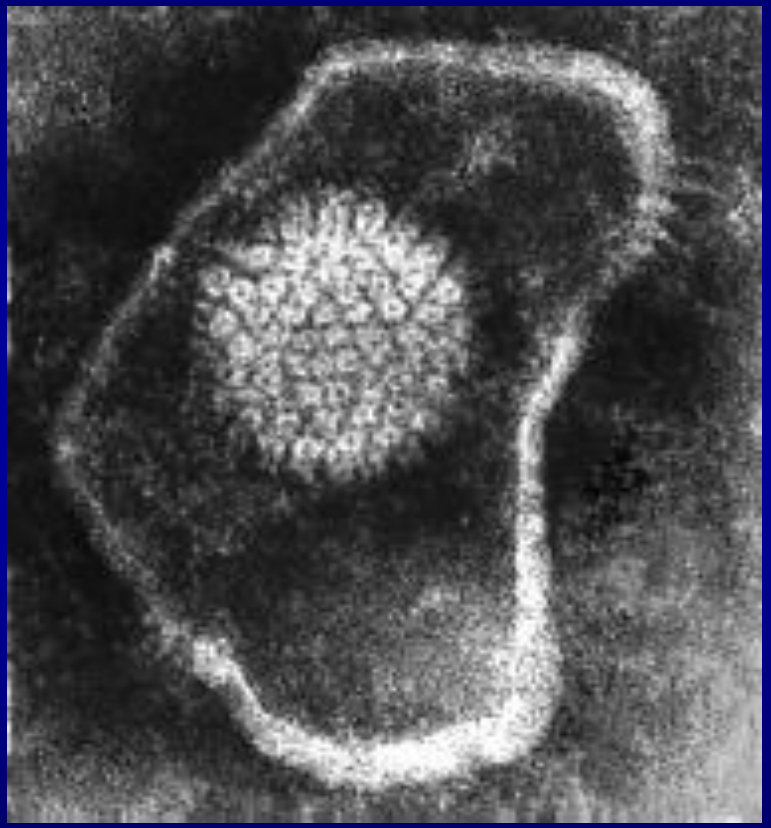
envelope = lipids, proteins, glycoproteins

- **Unstable in the environment**
- **Sensitive to heat, detergents, acid, organic solvents, dessication**
- Spread by droplets, secretions, blood, vectors
- No alimetray route of transmission
- Enveloped viruses are usually released from the host cell by a budding










100 ml
Alkohol
70 Vol.-%
HETTERICH
Lösung zur Anwendung auf der Haut
Für Säuglinge, Kinder und Erwachsene
Wirkstoff: Ethanol 70 % (V/V)
Hygienische Händedesinfektion
Kühlumschläge
100 ml
Alkohol
70 Vol.-%
HETTERICH
MADAUS, 51101 Köln

ENVELOPED VIRUSES

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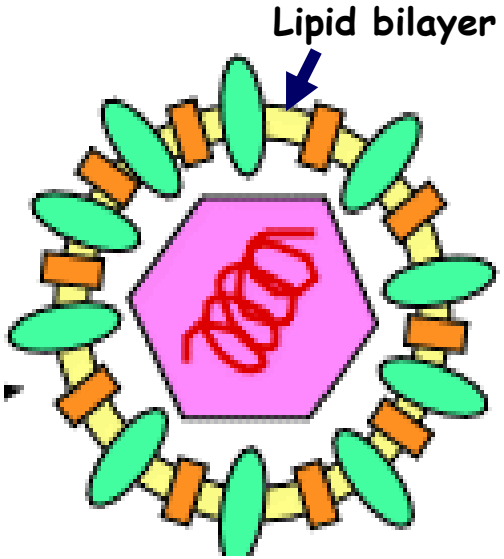
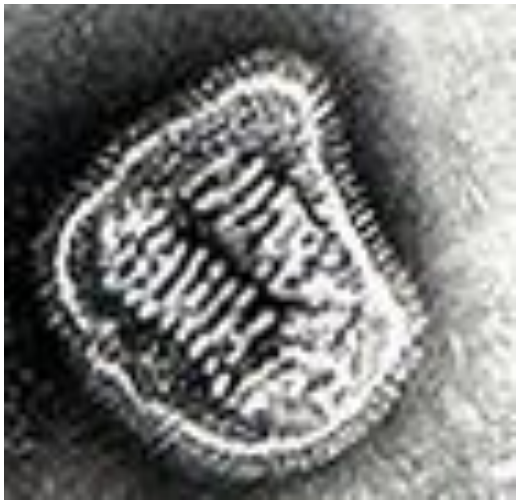
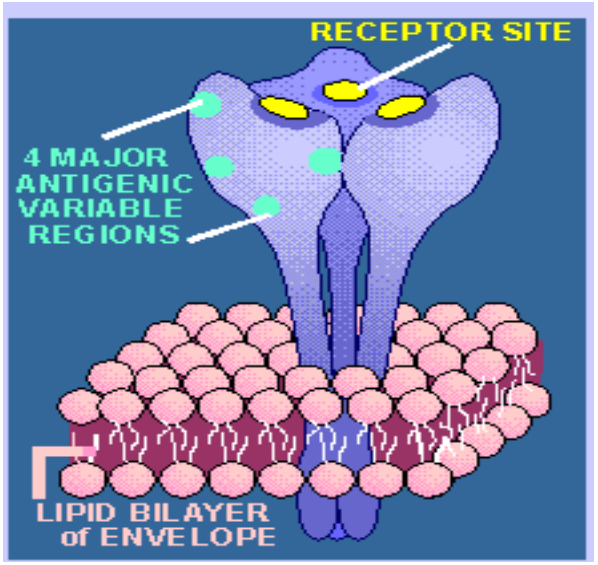
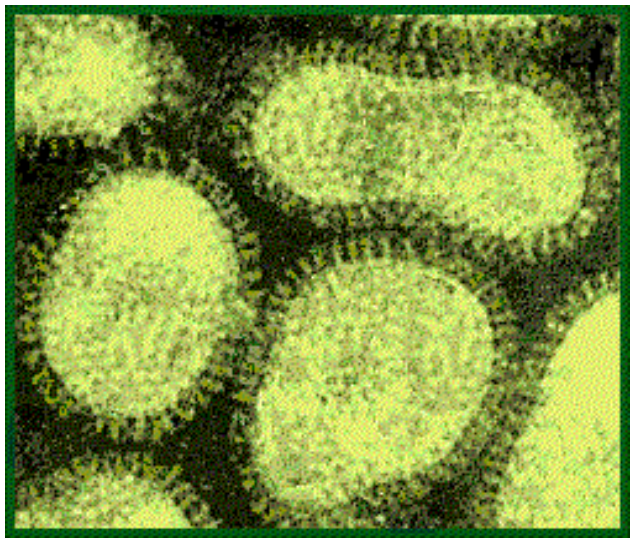




Peplomers





- A peplomer is a glycoprotein spike on a viral capsid or viral envelope
- These protrusions will only bind to certain receptors on the host cell
- They are essential for both host specificity and viral infectivity.
- Cause hemagglutination and hemolysis
- Important virus antigens
- Virus encoded
- Show enzymatic activity

Classification of Viruses

1. NUCLEIC ACID
2. CAPSID SYMMETRY
3. VIRAL ENVELOPEE
4. BIOLOGIC PROPERTIES (mode of transmission, natural host range)

Classification of viruses based on their nucleic acid

- RNA or DNA
- size
- segmented or non-segmented
- linear or circular
- Single- od double-stranded
- Single-stranded RNA
  - (+) infectious → genome equal to mRNK
  - (-) noninfectious - genome complementary to mRNK

DNA VIRUSES

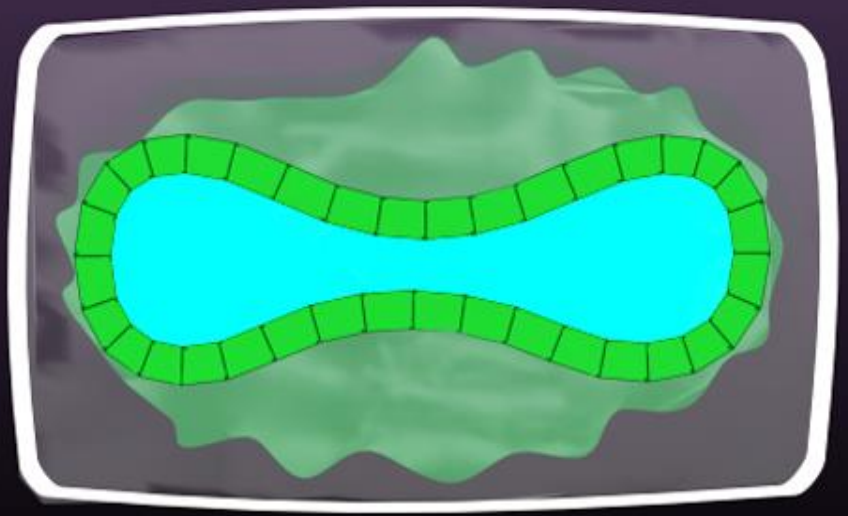
PARVOVIRIDAE

PAPOVAVIRIDAE

ADENOVIRIDAE

HERPESVIRIDAE

HEPADNAVIRIDAE



POXVIRIDAE

RNA VIRUSI

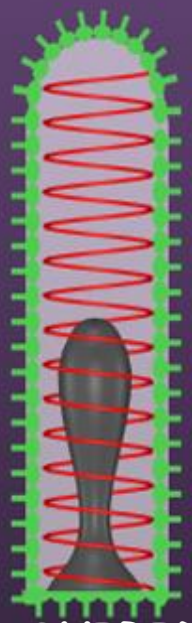
TOGAVIRIDAE
FLAVIVIRIDAE +

REOVIRIDAE

BUNYAVIRIDAE -

PICORNAVIRIDAE +

ARENAVIRIDAE ±



RHABDOVIRIDAE -

CORONAVIRIDAE +

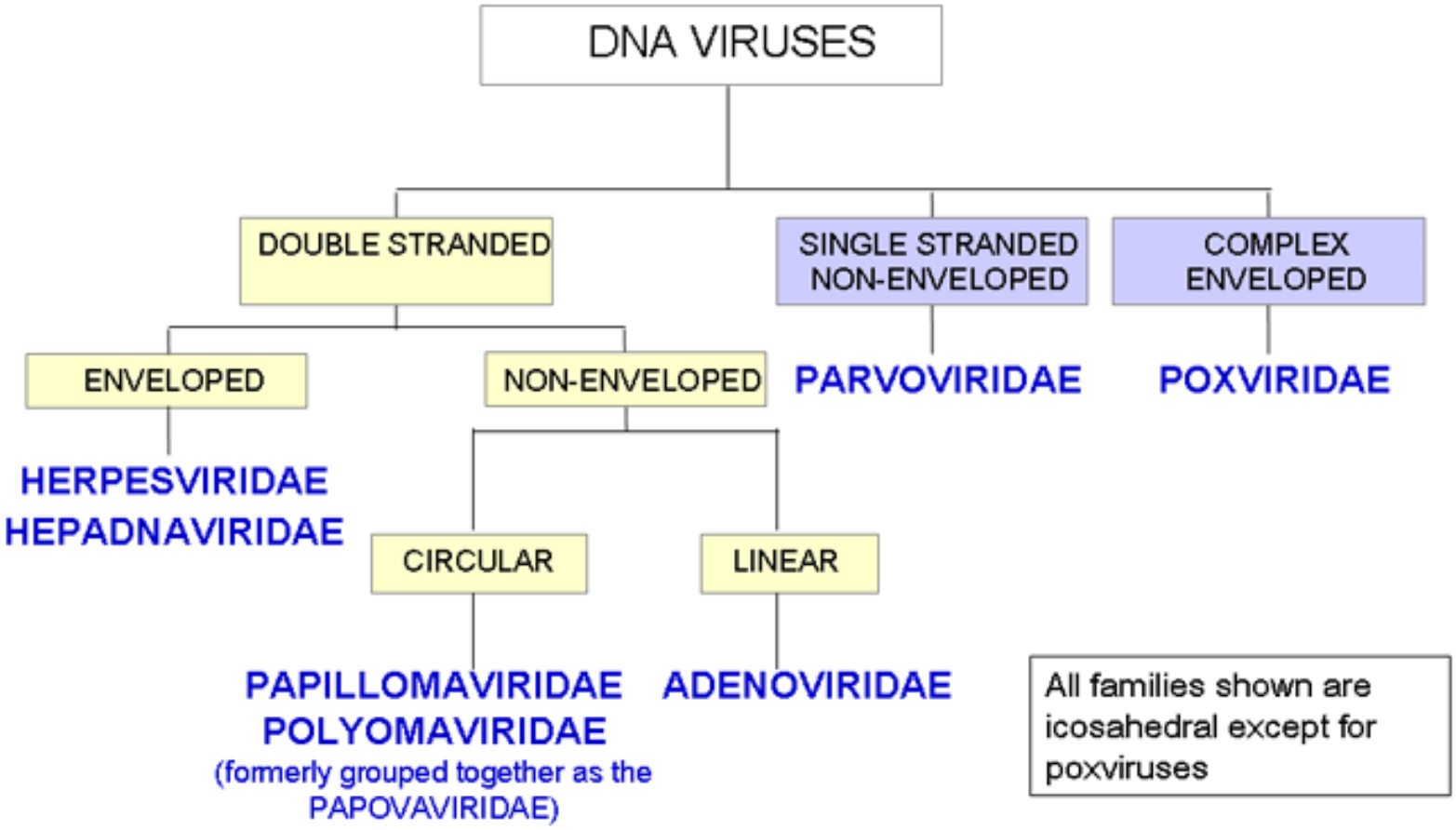
PARAMYXOVIRIDAE -

ORTHOMYXOVIRIDAE -

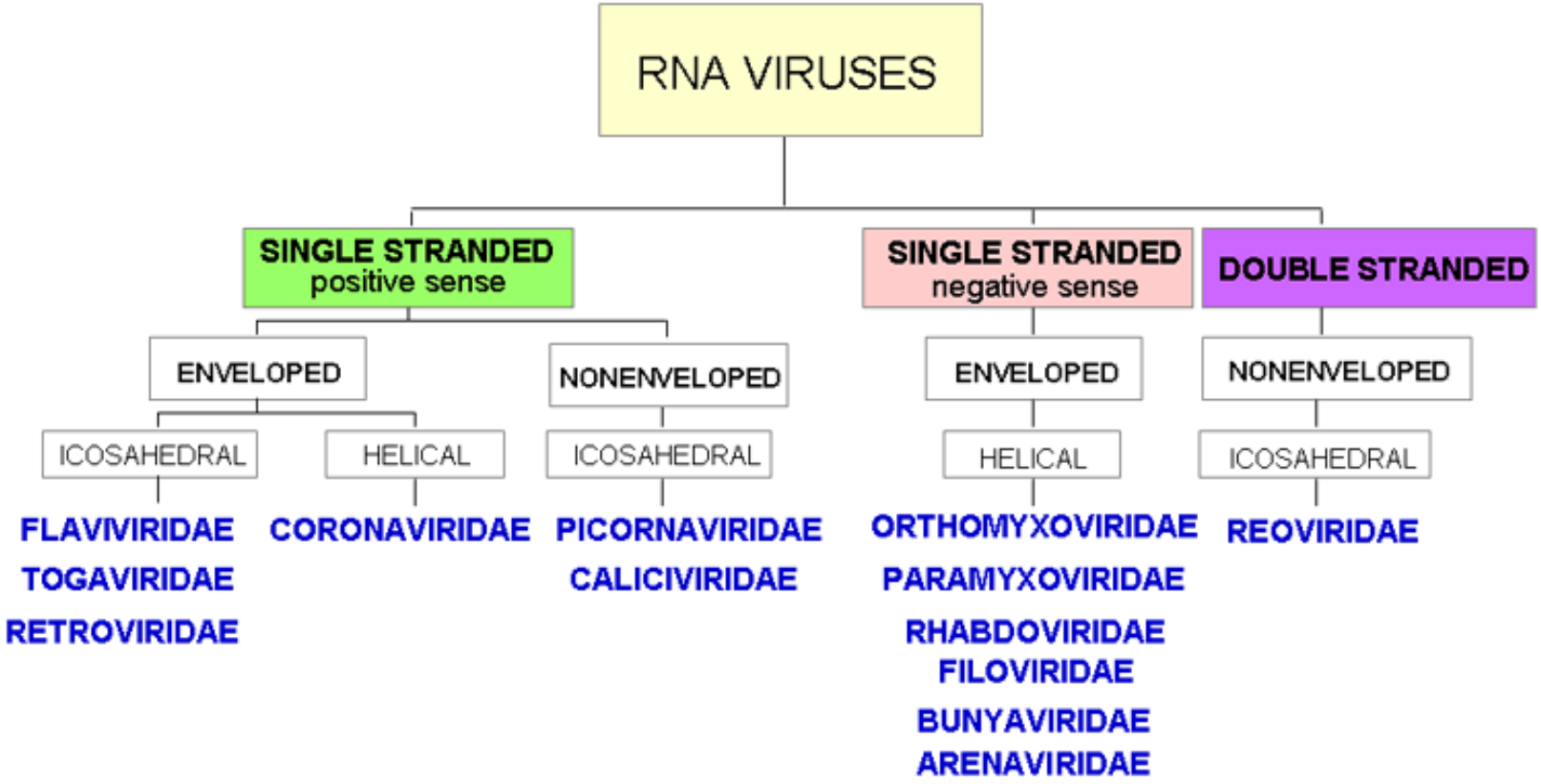
RETROVIRIDAE +

Classification based on viral capsid

- **Capsid symmetry**
  - icosahedral
  - helical
  - complex
- **Number of capomeres**



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



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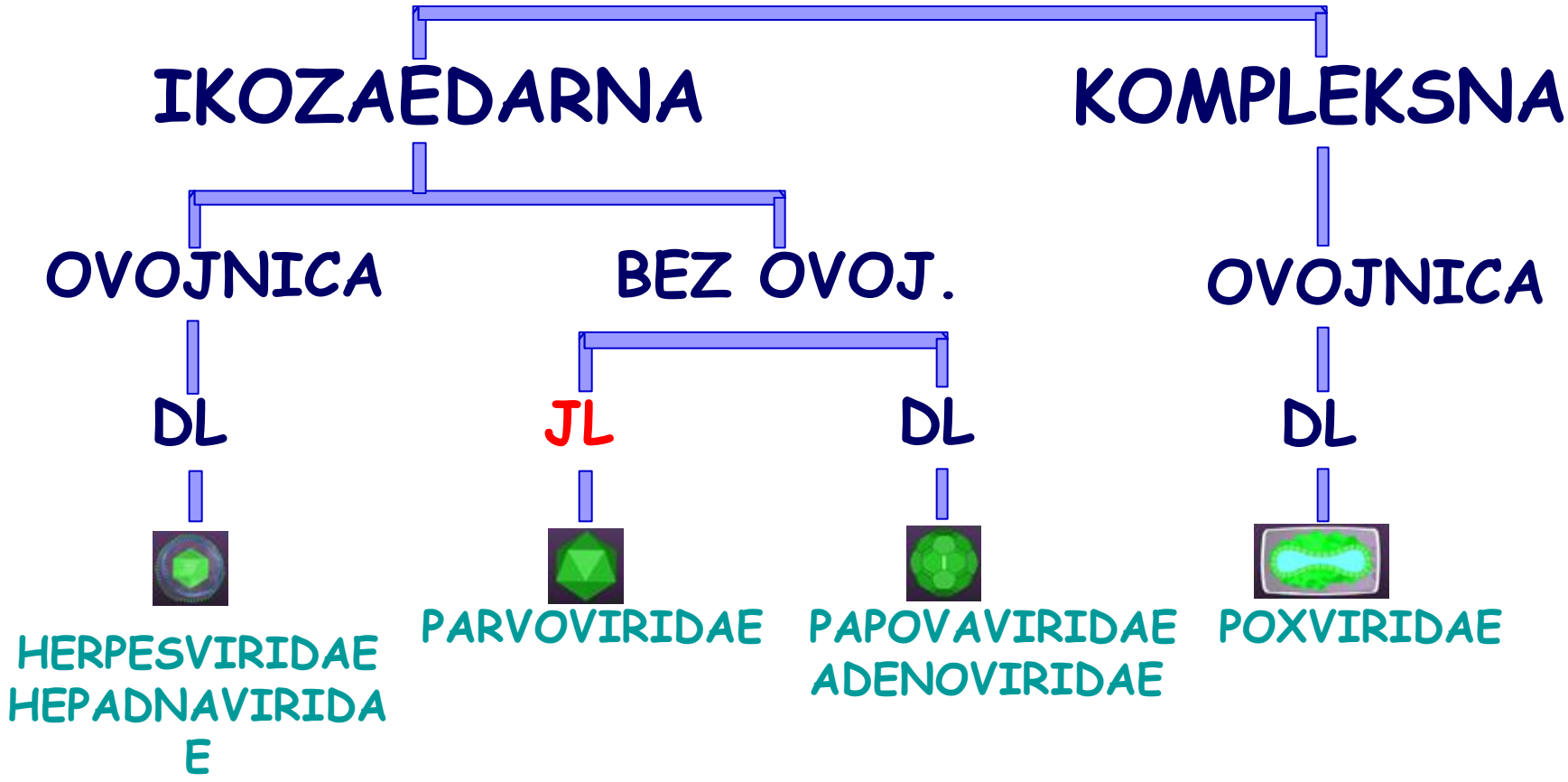
DNK VIRUSI- klasifikacija prema kapsidi



Svi osim Poxviridae imaju ikozaedarnu simetriju kapside

RNK VIRUSI - Klasifikacija prema kapsidi

- IKOZAEDARNA
  - OVOJNICA
    - JL
      - TOGAVIRIDAE
      - FLAVIVIRIDAE
      - RETROVIRIDAE
  - BEZ OVOJ.
    - JL
      - PICORNAVIRIDAE
      - CALICIVIRIDAE
    - DL
      - REOVIRIDAE
- SPIRALNA
  - OVOJNICA
    - JL
      - RHABDOVIRIDAE
      - PARAMYXOVIRIDAE
      - BUNYAVIRIDAE
      - ARENAVIRIDAE
      - ORTHOMYXOVIRIDAE
      - CORONAVIRIDAE
      - RETROVIRIDAE

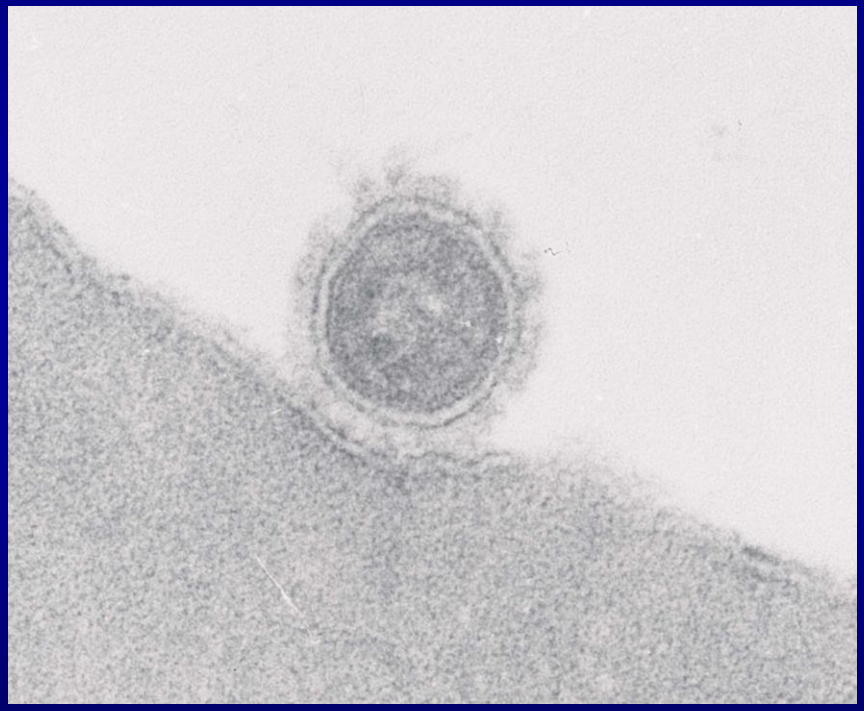
VIRUSES show TISSUE TROPISM

Hepatitis viruses → liver

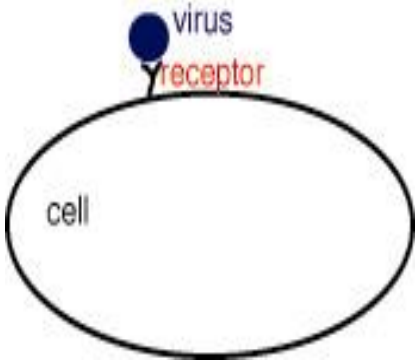
The rabies virus → CNS, salivary glands

To produce disease, viruses must enter a host, come in contact with susceptible cells, replicate, and produce cell injury

Interaction of a virion with a specific receptor site on the surface of a cell



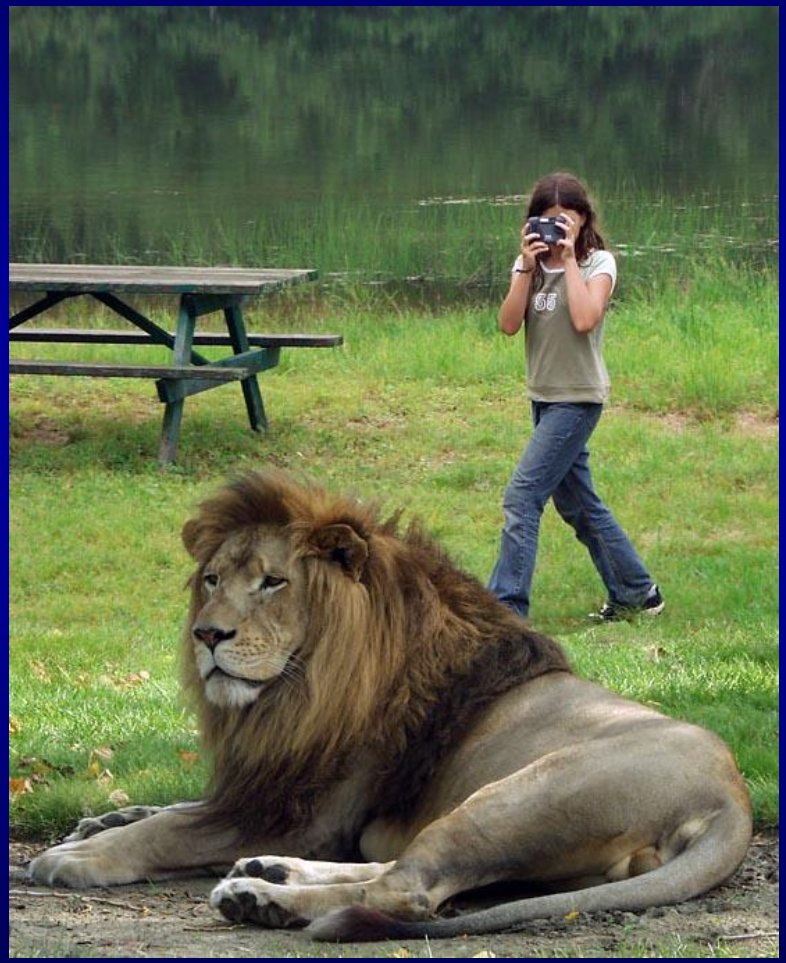
The lock and key principle



Viral antigens and cellular receptors

Virus	Viral antigen	Target cells	Cellular receptors
HIV	gp 120	T_H lymphocytes, macrophages, monocytes	CD_4 molecule, chemokine co-receptors
Rabies	G protein	neurons	acetylcholine receptor
Influenza A	hemagglutinin	epithelial cells	sialic acid

Susceptibility to animal lentiviruses



- Animal lentiviruses do not infect primates and himans

Lentiviruses of cats

- Feline immunodeficiency virus

Origin of HIV/AIDS

- Both HIV-1 and HIV-2 are believed to have originated in non-human primates in West-central Africa and were transferred to humans in the early 20th century.

Origin of HIV/AIDS

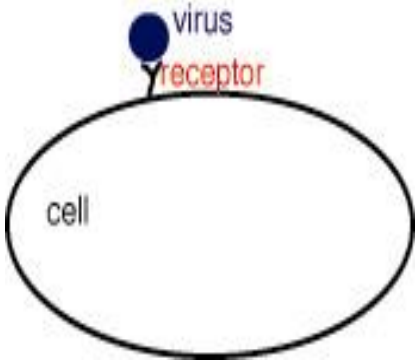
- Left to right: the African green monkey **source of SIV**, the sooty mangabey **source of HIV-2** and the chimpanzee **source of HIV-1**

Origin of HIV/AIDS

- HIV-1 appears to have originated in southern Cameroon through the evolution of SIV(cpz), a simian immunodeficiency virus (SIV) that infects wild chimpanzees
- HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N, and O.

Origin of HIV/AIDS

- Sooty mangabeys are naturally infected with a strain of Simian Immunodeficiency Virus (SIV), known as SIV_{sm}. Due to extensive human-mangabey contact in sub-Saharan Africa, SIV_{sm} has jumped from this species into humans on multiple occasions, resulting in HIV-2 virus.



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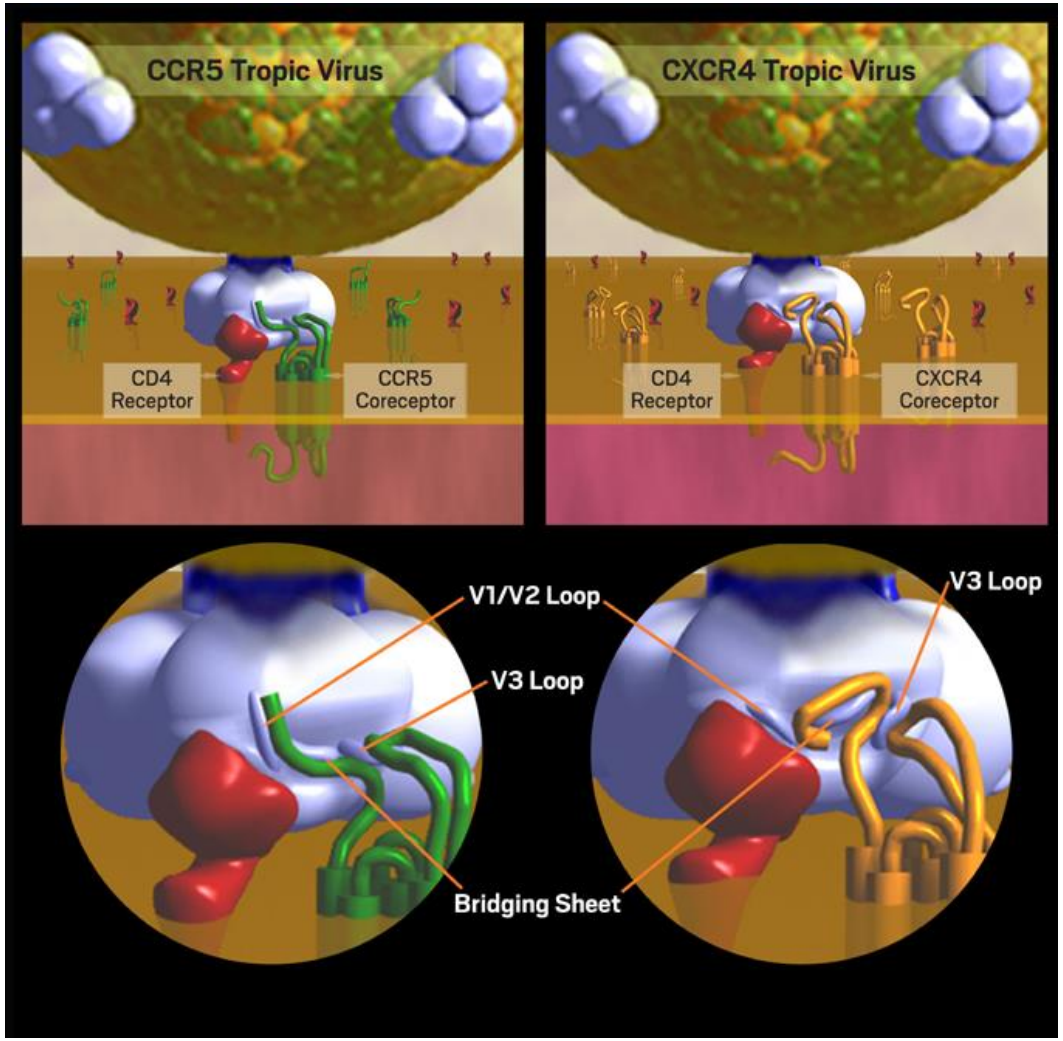
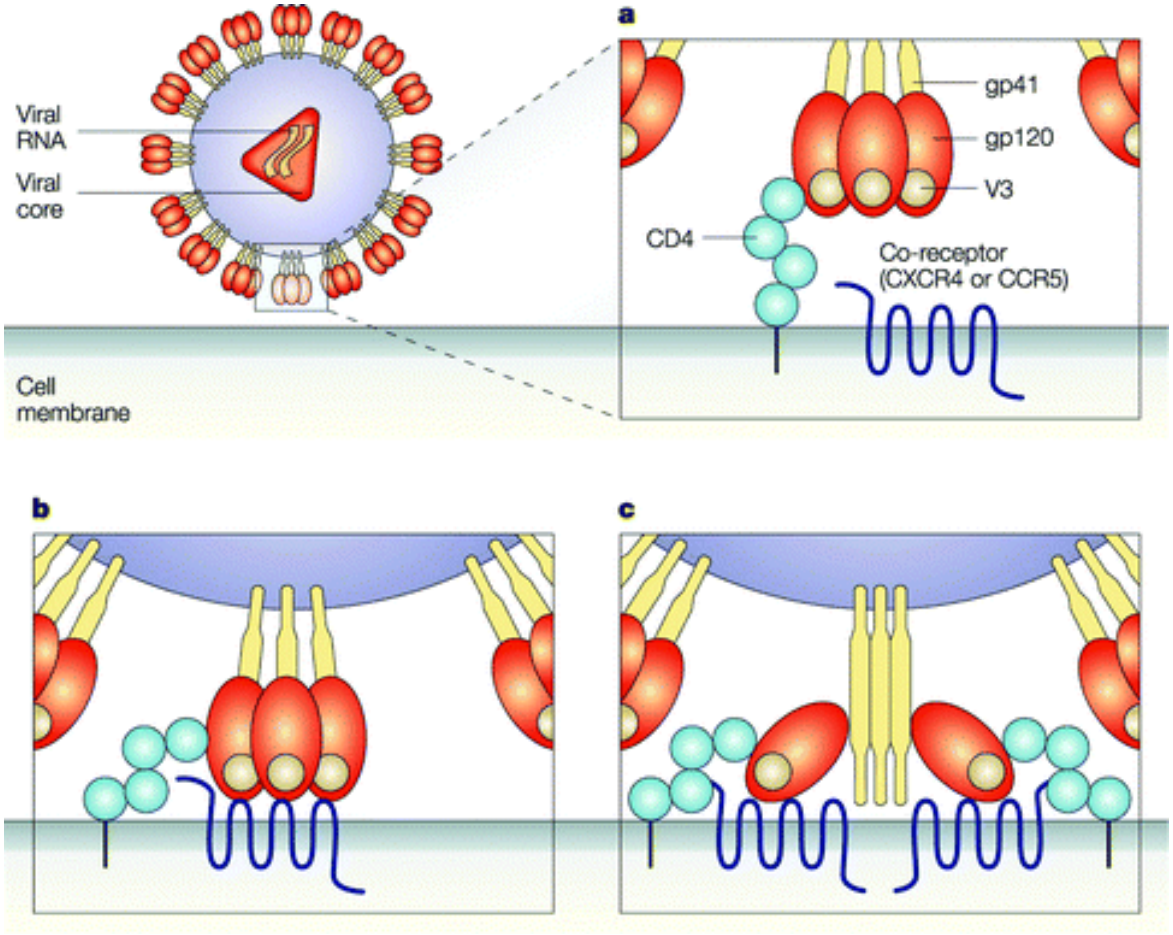
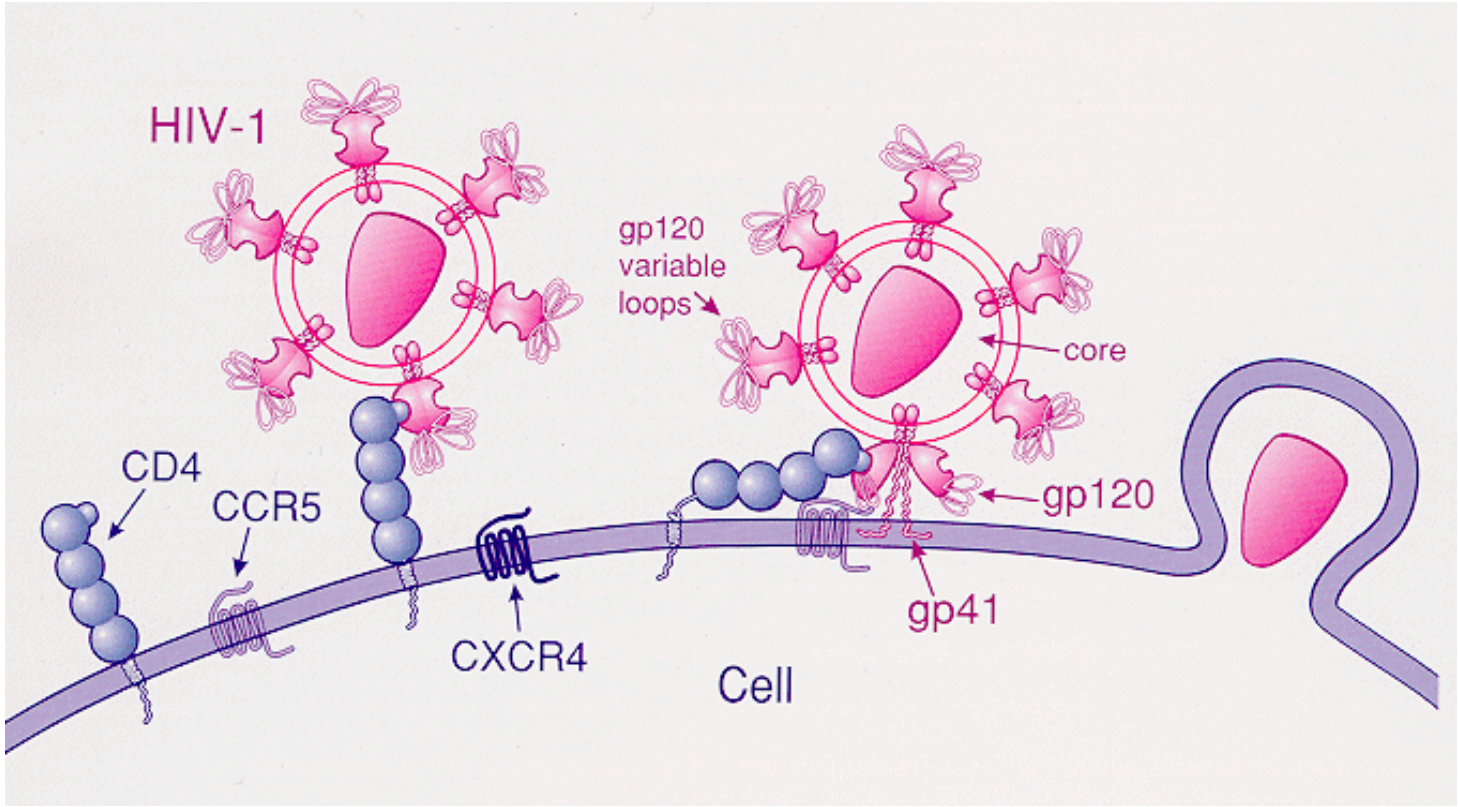
HIV receptor and co-receptor



HIV receptor and co-receptor



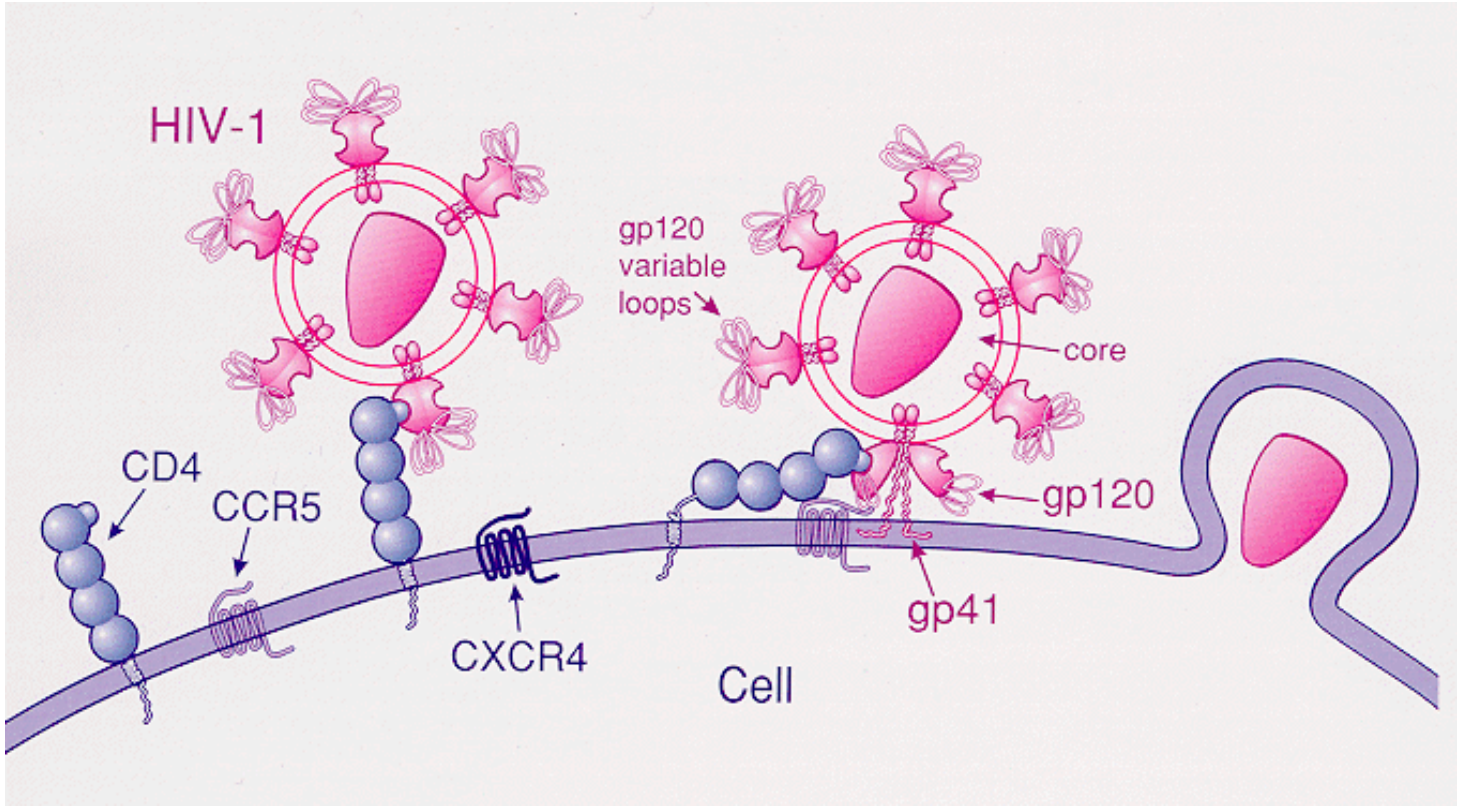
HIV receptor and co-receptor



Attachment of HIV to a CD4+ T-helper cell:

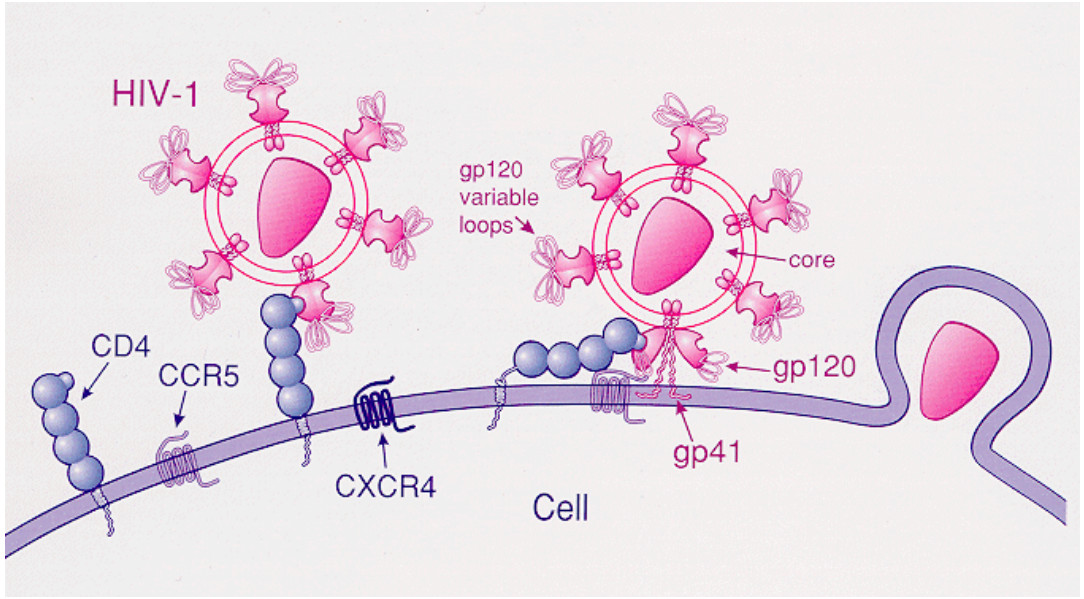
1) the gp120 viral protein attaches to CD4.
2) gp120 variable loop attaches to a coreceptor, either CCR5 or CXCR4.
3) HIV enters the cell.

HIV receptor and co-receptor



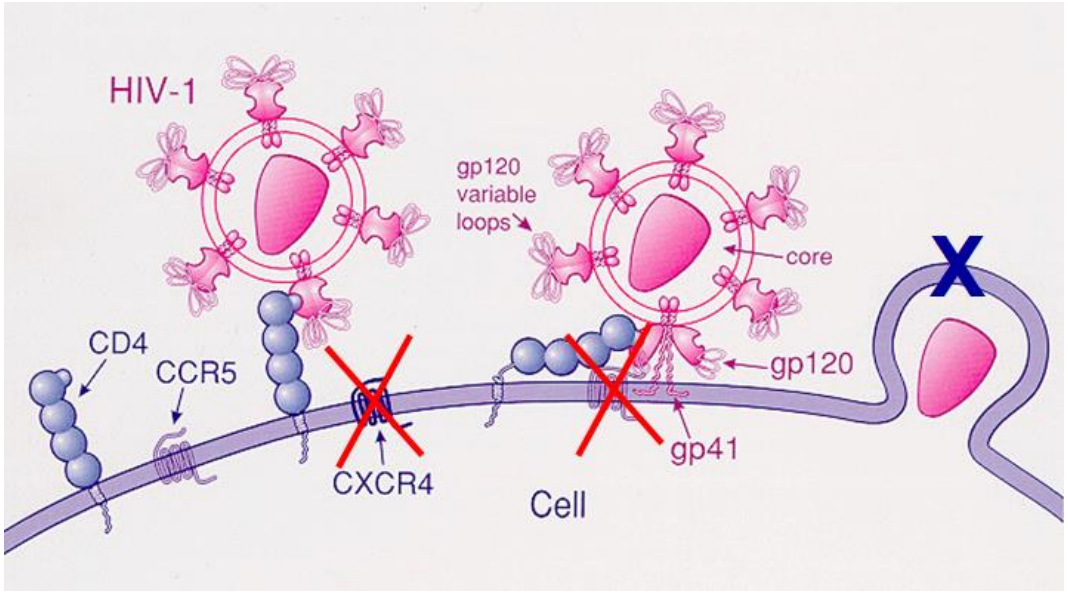
HIV most commonly uses CCR5 and/or CXCR4 as a co-receptor to enter its target cells

HIV receptor and co-receptor



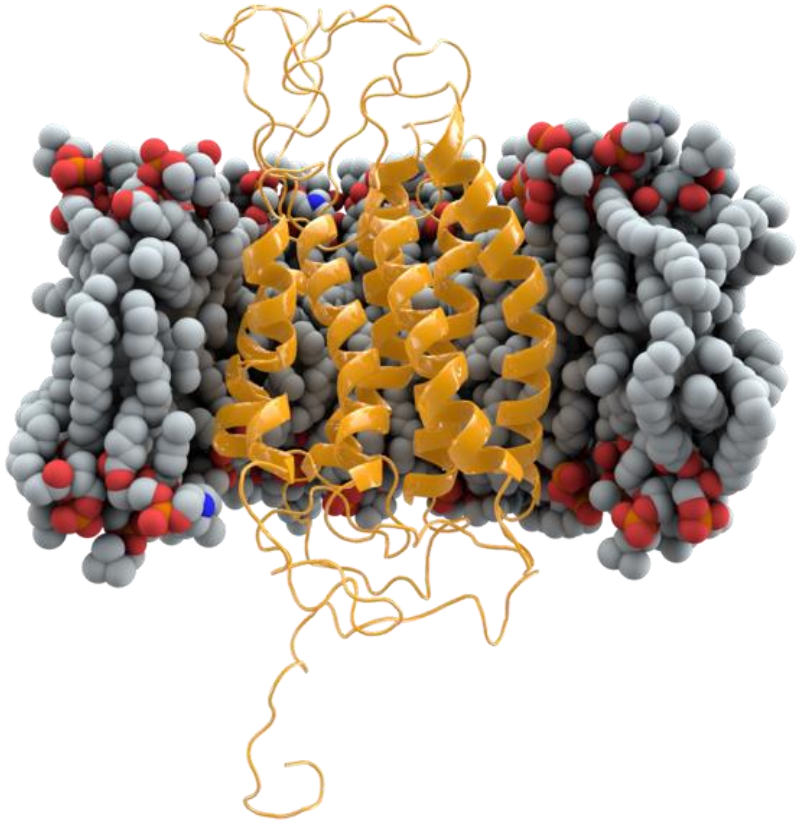
- *CCR5* is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines
- Macrophage (M-tropic) strains of HIV-1, or non-syncitia-inducing strains (NSI) use the beta-chemokine receptor CCR5 for entry and are thus able to replicate in macrophages and CD4+ T-cells

HIV receptor and co-receptor



- A few individuals carry a mutation known as CCR5-Δ32 in the CCR5 gene, protecting them against these strains of HIV.

Chemokine receptor type 5 (CCR5)



- **CCR5** or **CD195**, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines.
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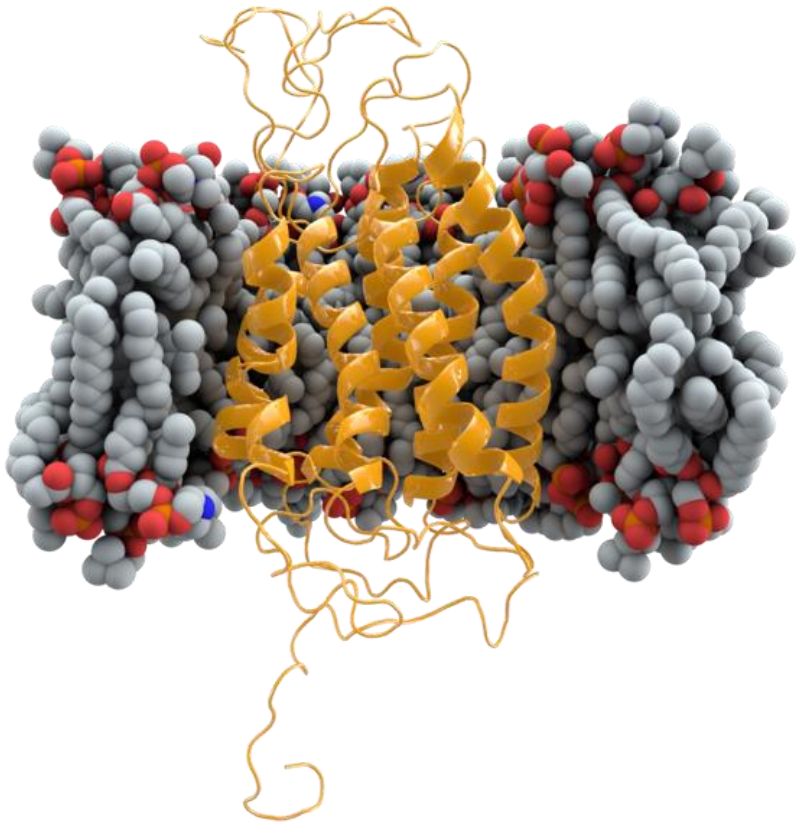
CCR5-Δ32 deletion mutation)



- In an AIDS patient who had also developed myeloid leukemia, and was treated with chemotherapy to suppress the cancer.
- A bone marrow transplant containing stem cells from a matched donor was then used to restore the immune system.
- However, the transplant was performed from a donor with CCR5-Δ32 mutation gene.
- After 600 days, the patient was healthy and had undetectable levels of HIV in the blood and in examined brain and rectal tissues.

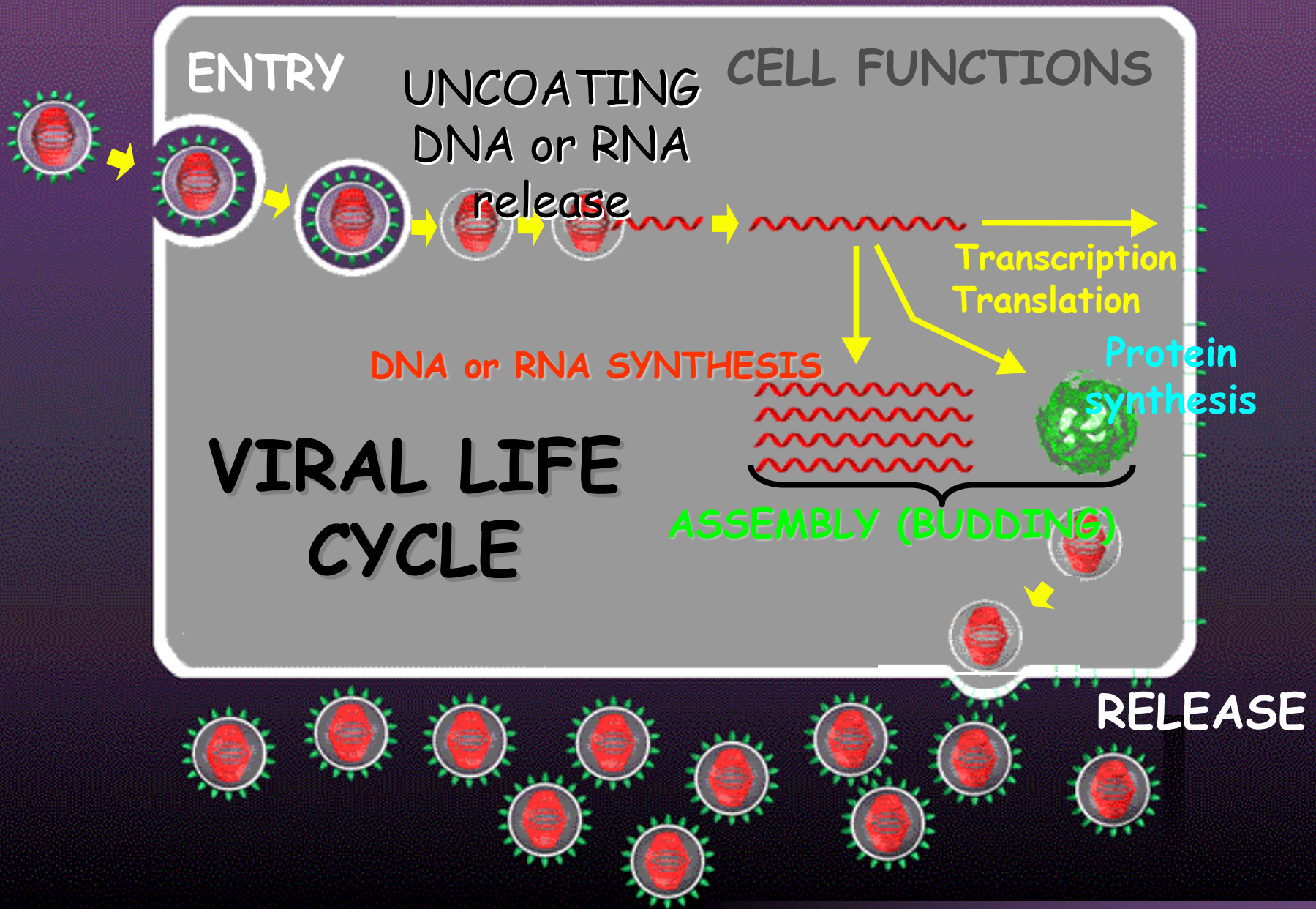
REPLICATION OF VIRUSES

- All viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.

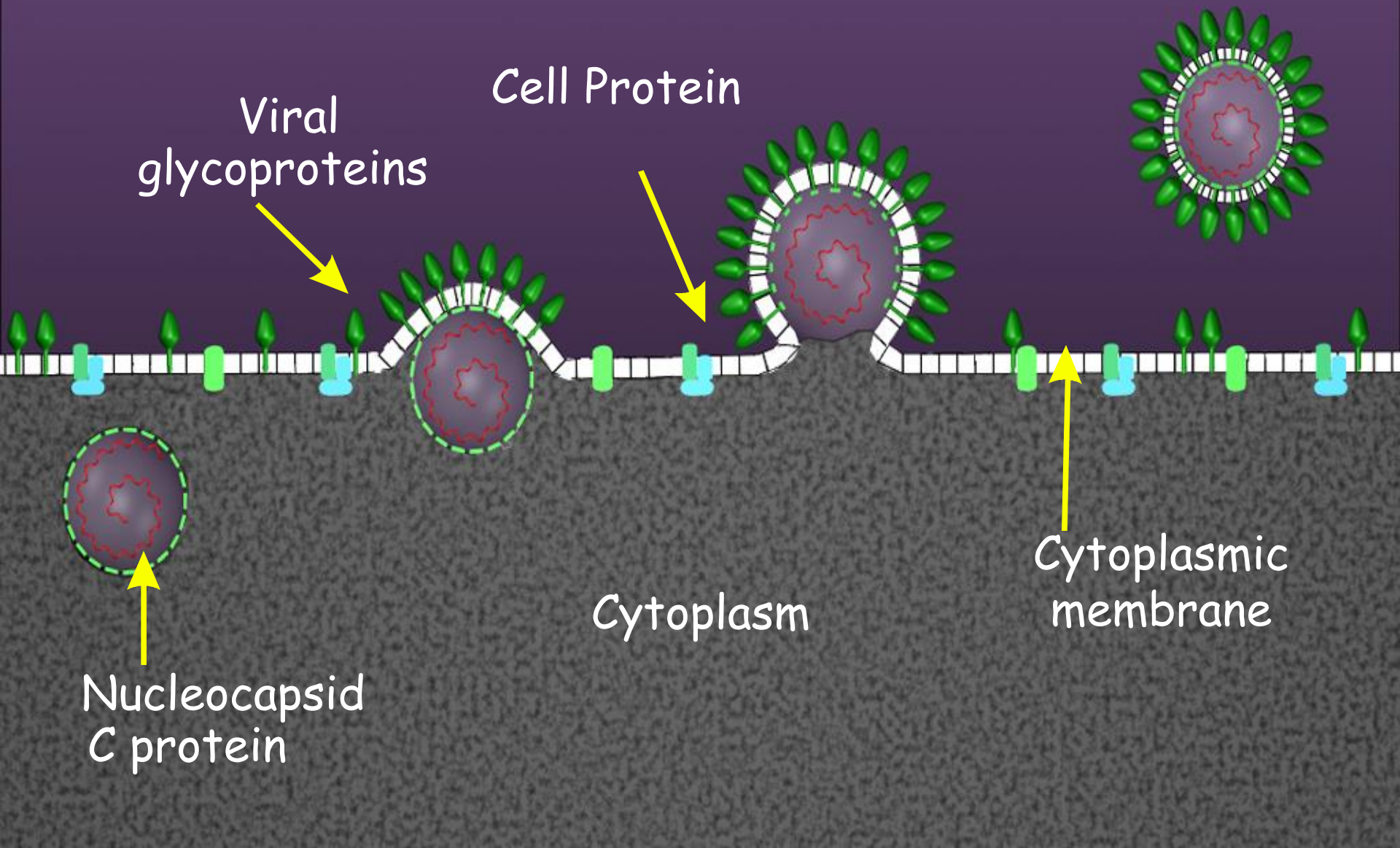
REPLICATION OF VIRUSES

- **Viruses**
  - For a virus to replicate, viral proteins must be synthesized by the host cell protein-synthesizing machinery
  - The virus genome must be able to produce a functional mRNA.
  - The viral nucleic acid carries the genetic specificity to code for all of the virus-specific macromolecules in a highly organized fashion.

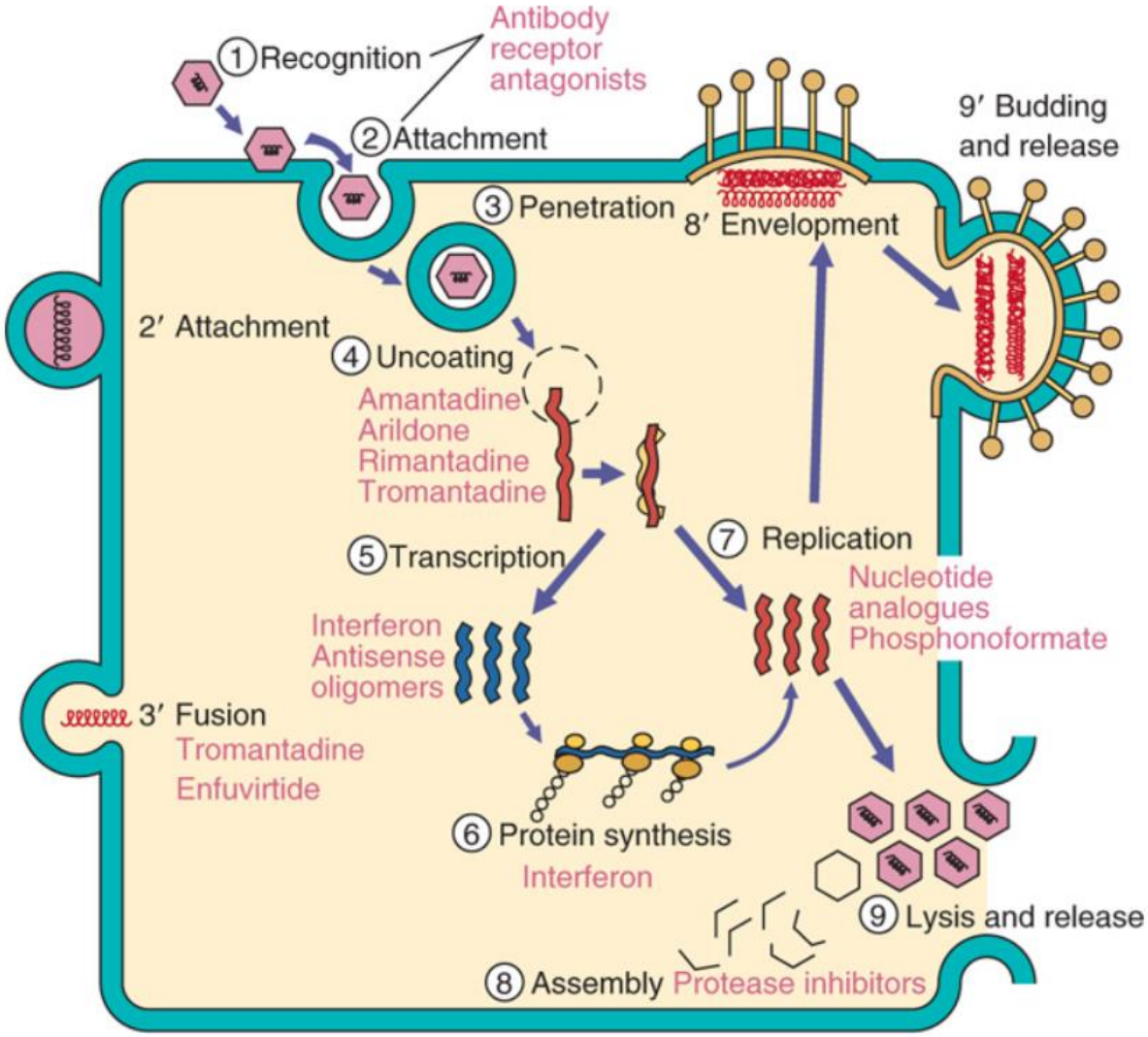
- **Infected cells**
  - The host cell must provide the energy ima ulogu tvornice koja daje
  - **substrates**
  - **energy**
  - **synthetic machinery** (enzimes)


ADSORPTION
ENTRY
UNCOATING
DNA or RNA
release
CELL FUNCTIONS
Transcription
Translation
DNA or RNA SYNTHESIS
Protein
synthesis
VIRAL LIFE
CYCLE
ASSEMBLY (BUDDING)
RELEASE
REPLICATION

ENVELOPED VIRUSES - MATURATION AND BUDDING

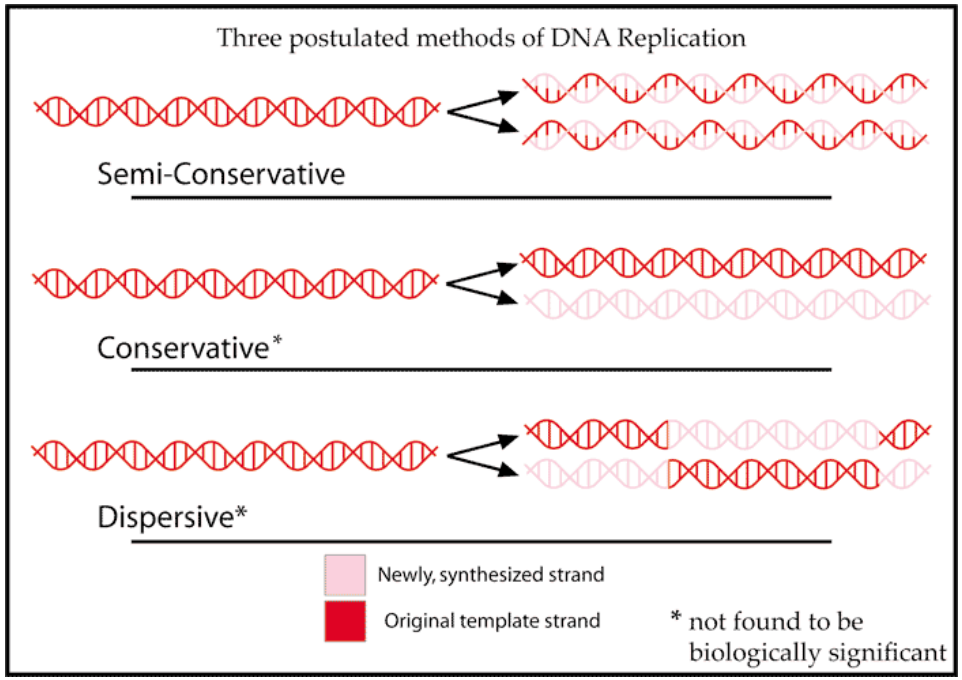


A general scheme of viral replication- each virus has to synthesize mRNK, proteins and a copy of ist genome



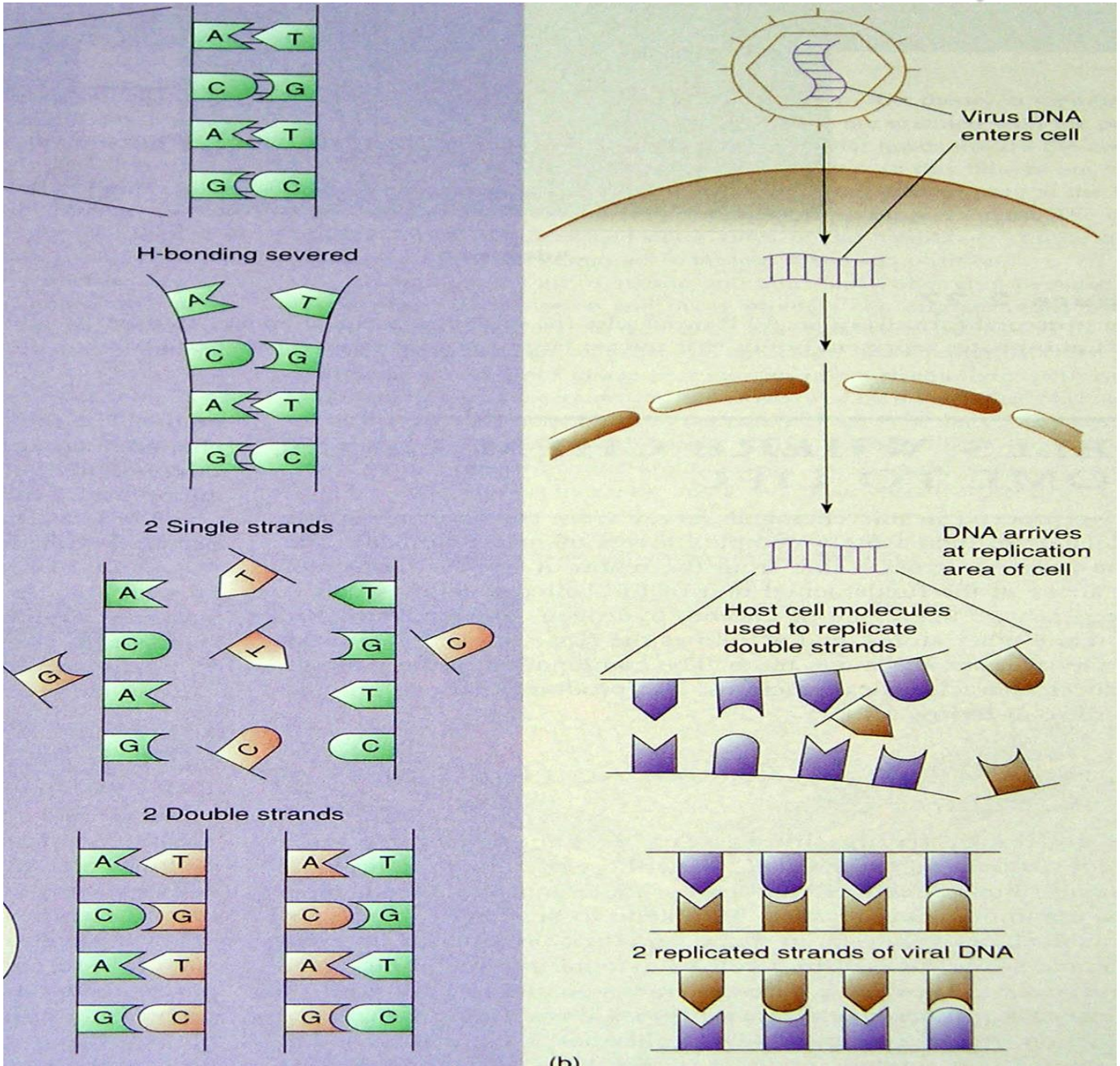
Viruses leave the cell by lysis or by budding (no binary division)

Semiconservative replication



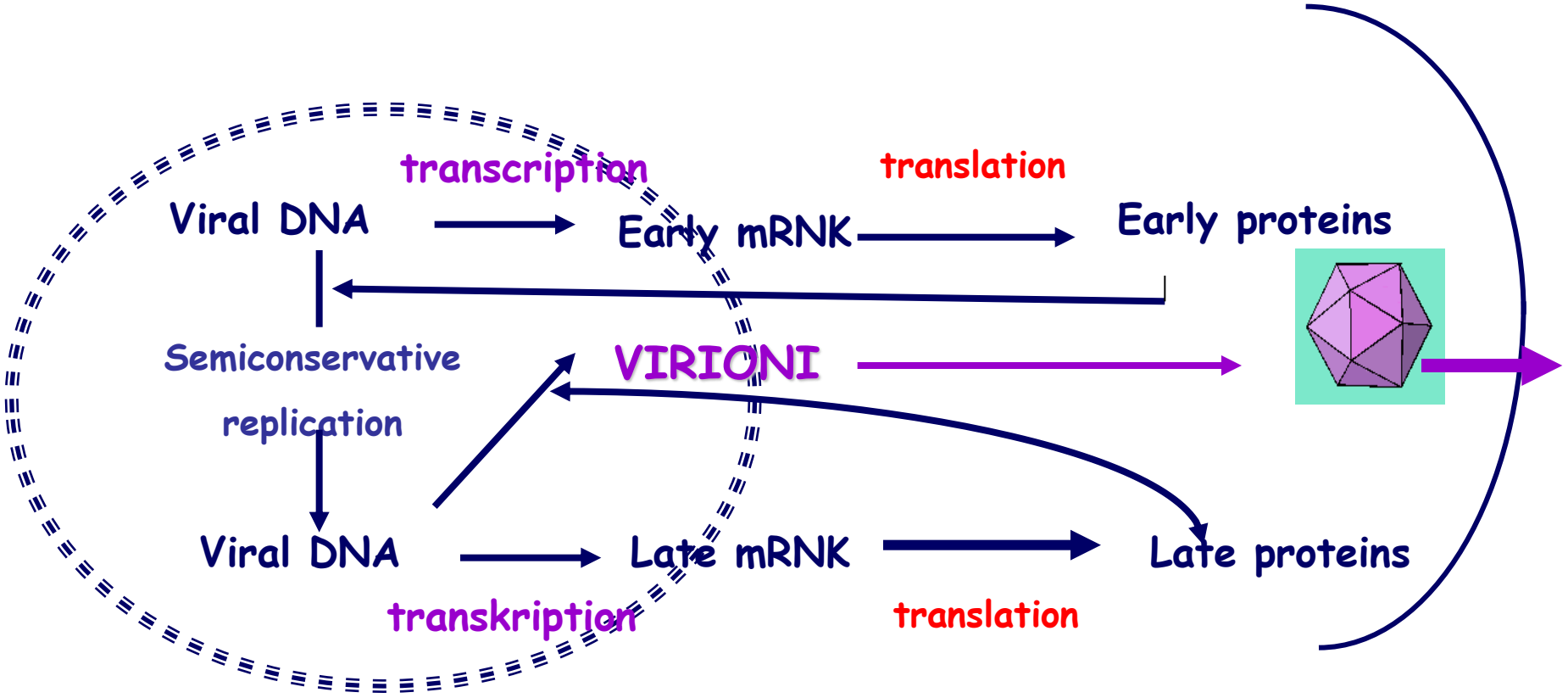
- **Semiconservative replication** describes the mechanism by which DNA is replicated in all known cells. This mechanism of replication was **one of three models originally proposed** for DNA replication:
  - **Semiconservative replication would produce two copies that each contained one of the original strands and one new strand.**
  - Conservative replication would leave the two original template DNA strands together in a double helix and would produce a copy composed of two new strands containing all of the new DNA base pairs.
  - Dispersive replication would produce two copies of the DNA, both containing distinct regions of DNA composed of either both original strands or both new strands.

DNA Viruses – Semiconservative Genome Replication



DNA VIRUSES

ALL EXCEPT POXVIRUSES REPLICATE IN THE NUCLEUS OF THE HOST CELL



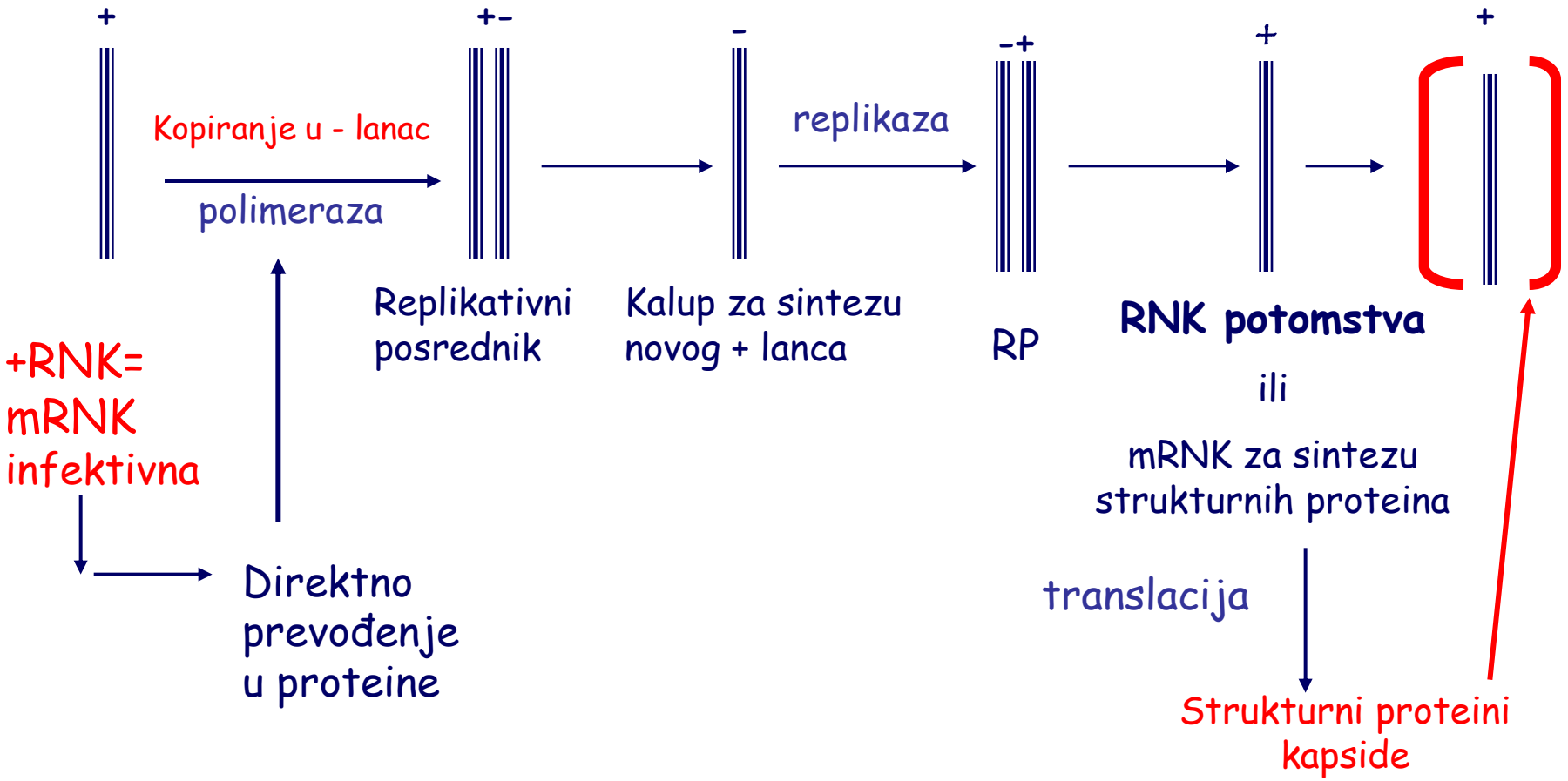
RNA VIRUSES

- All except orthomyxoviruses replicate in the cytoplasm of the infected cells

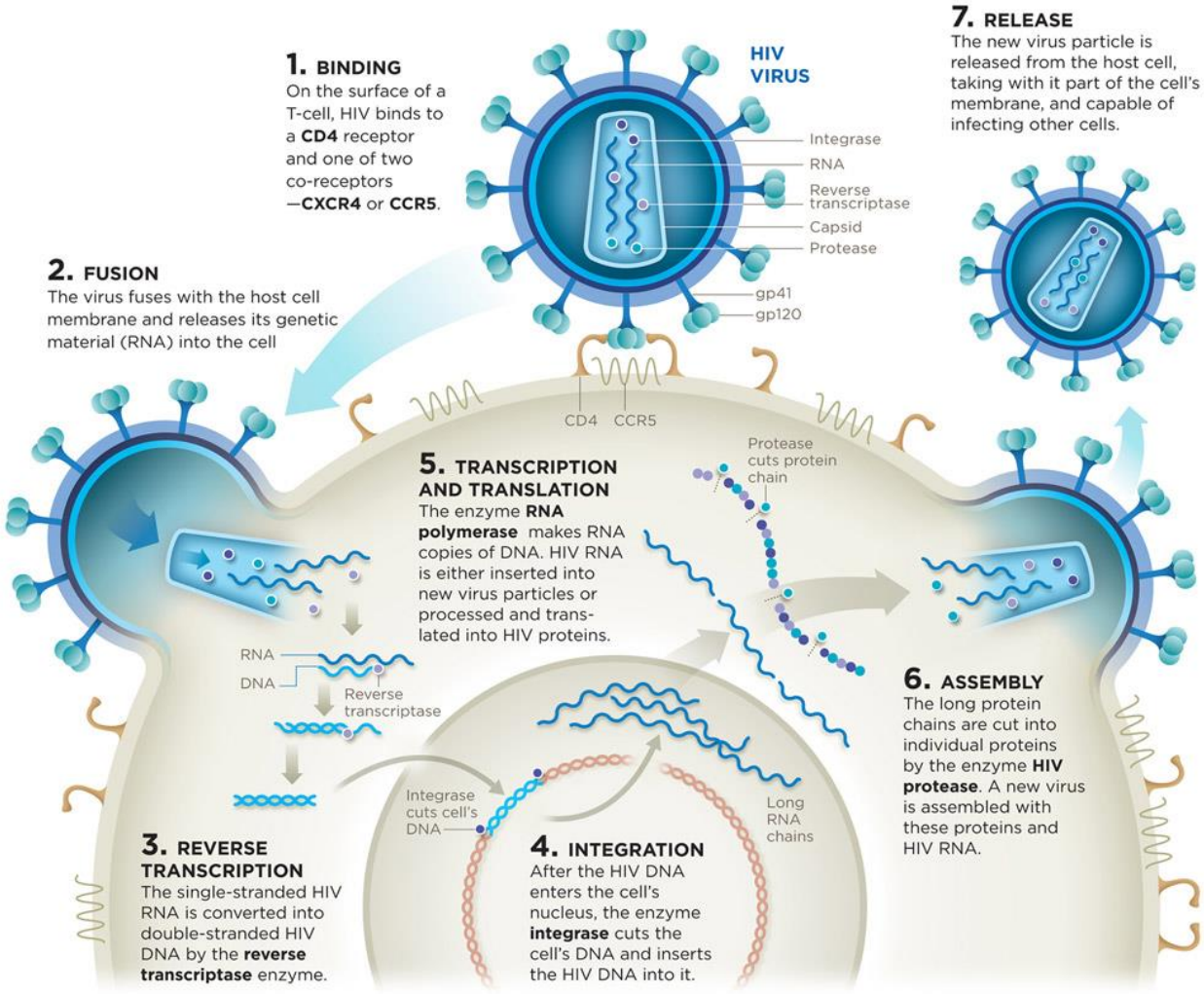
RNA VIRUSES

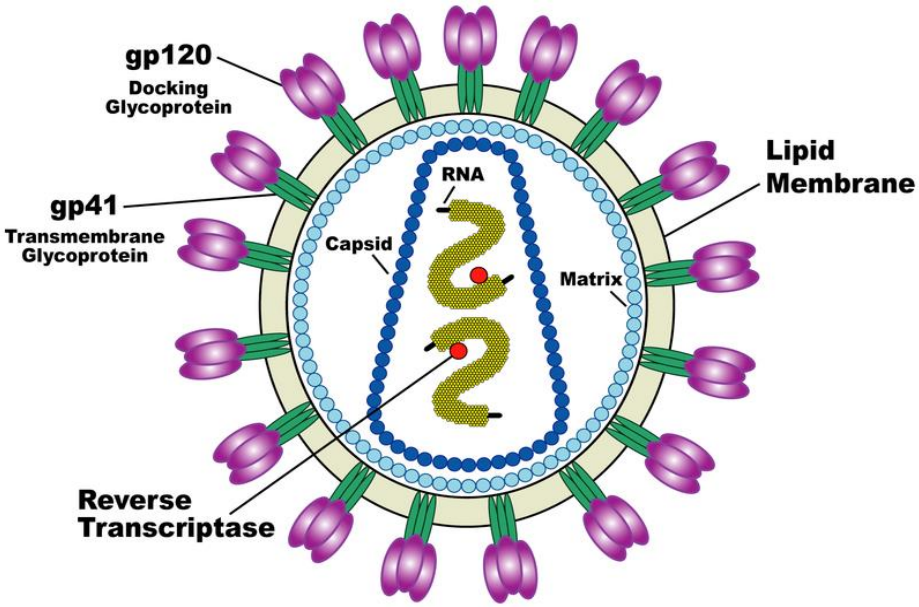
- **Transcription of RNA viruses**
  - ss (+) RNA genome = mRNA (RNA is infectious)
  - ss (-) RNA genome: RNA dependent RNA polimerase necessary for mRNA synthesis RNA
  - ds RNA genome – negative RNA-strain is copied by viral transcriptases for mRNA

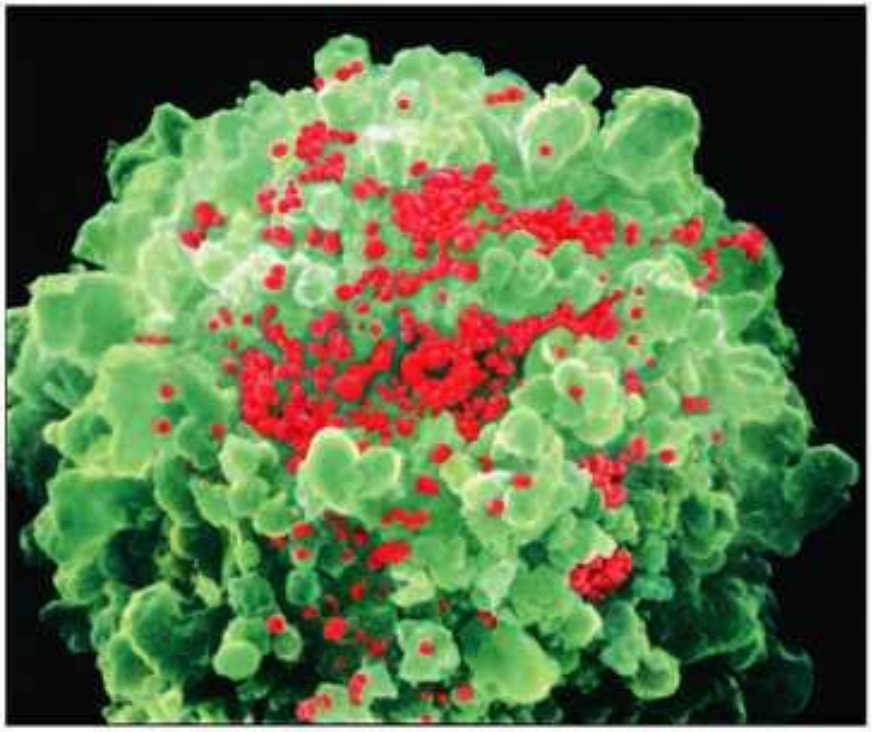
Replication of ss (+) RNA viruses

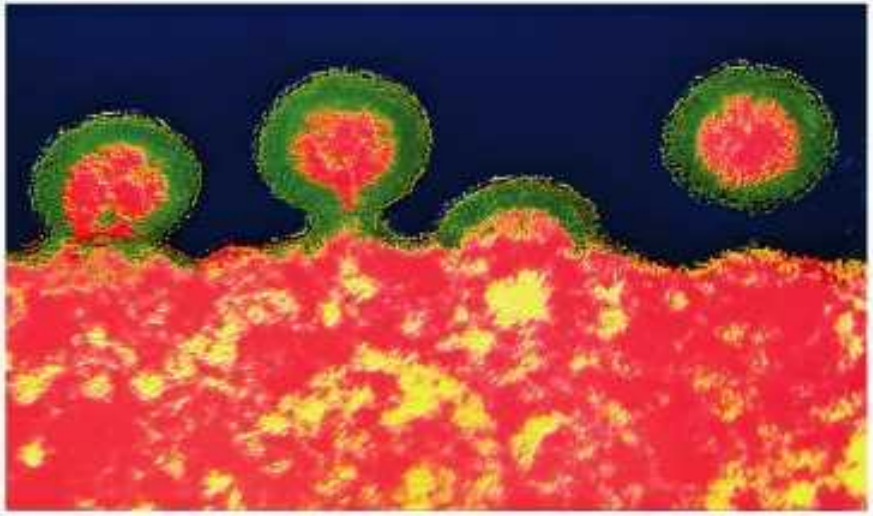


Replication of retroviruses



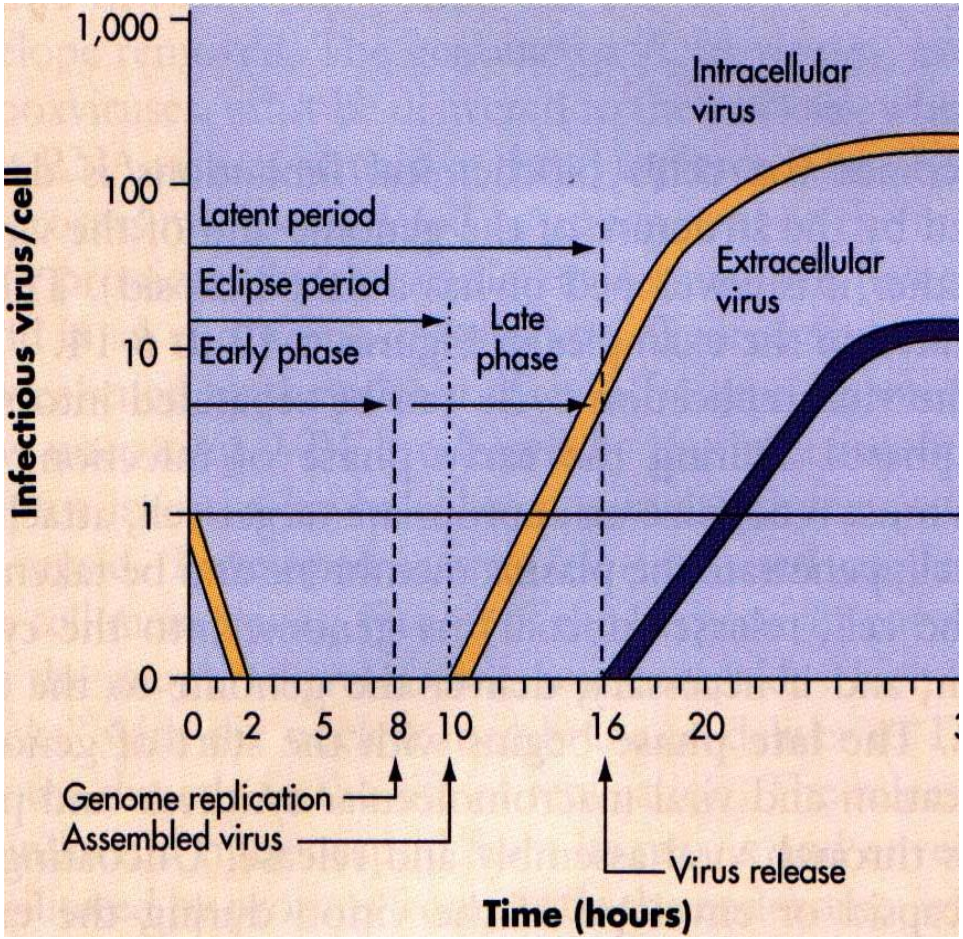




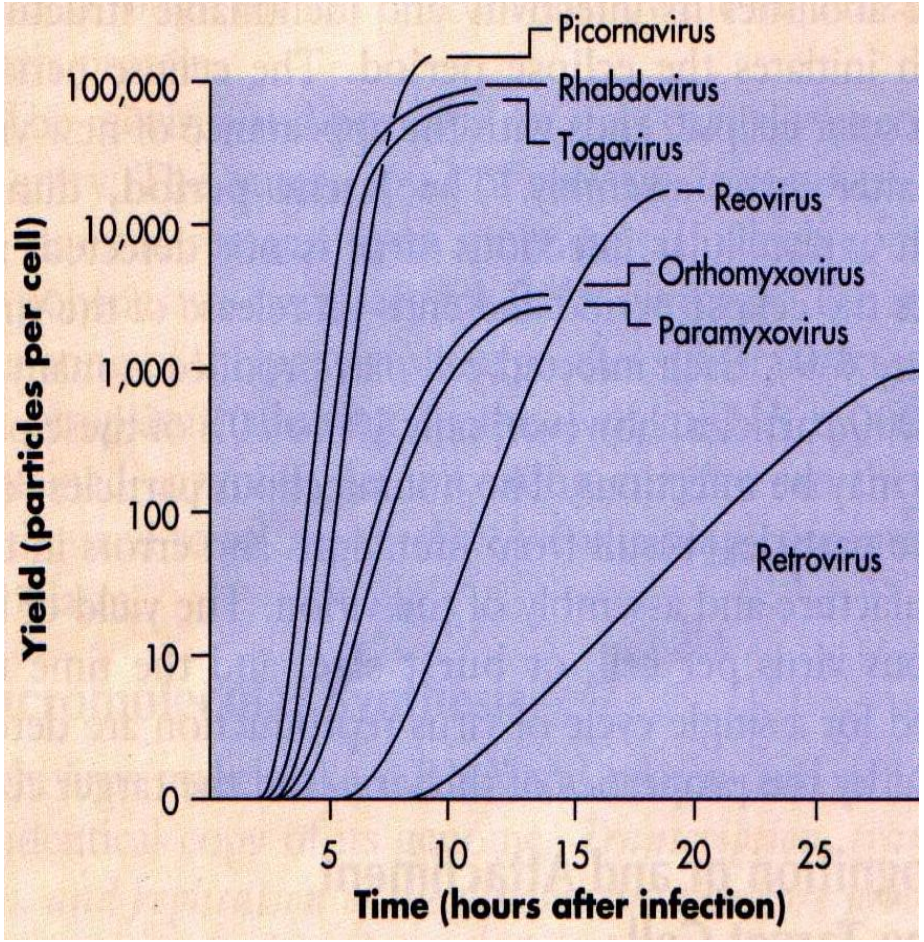


HIV budding from Th limfocita

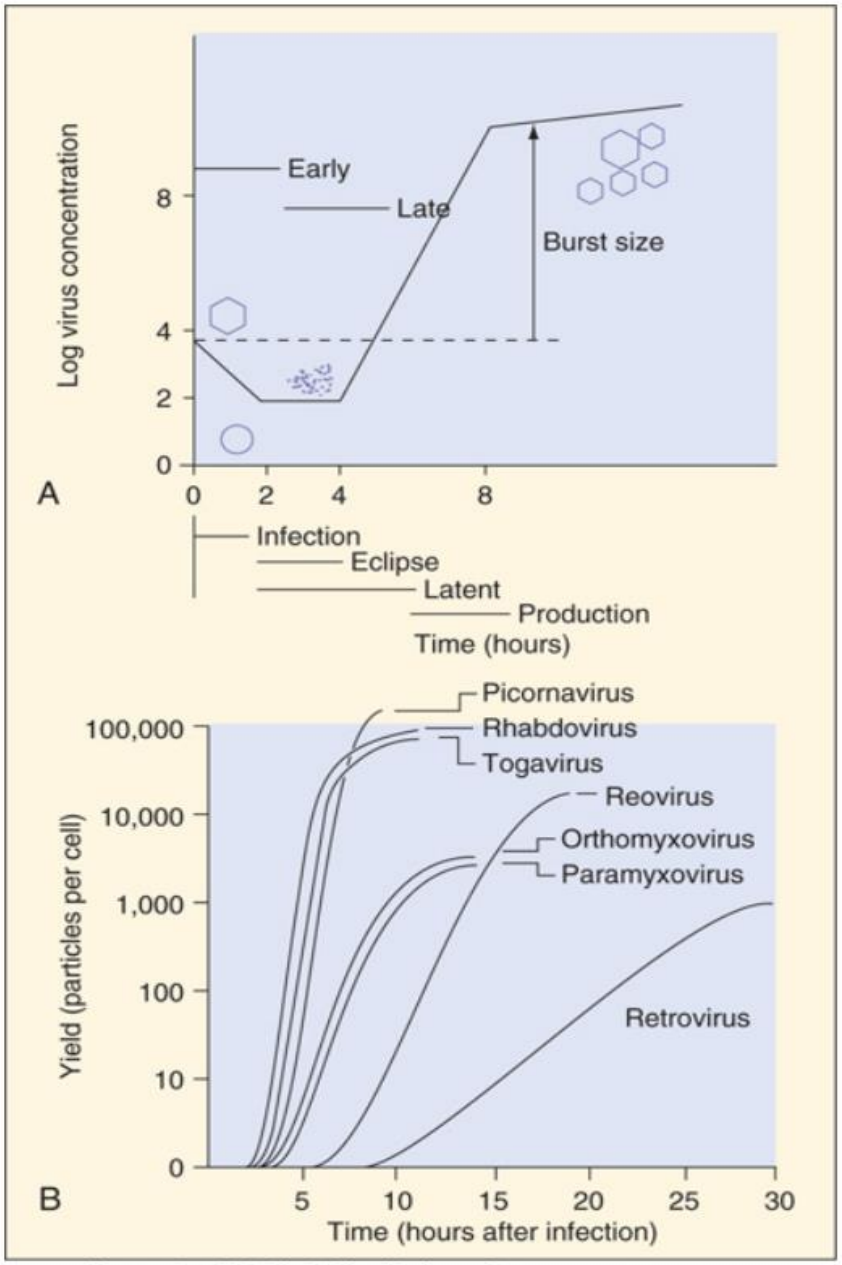
Viral Growth Curves



Single-cycle growth curve of a virus that is released on cell lysis



Growth curve and burst size of representative viruses



A: Single-cycle growth curve of a virus that is released on cell lysis. The different stages are defined by the absence of visible viral components (eclipse period), infectious virus in the media (latent period), or presence of macromolecular synthesis (early/late phases).

B: Growth curve and burst size of representative viruses.

Classification of viruses

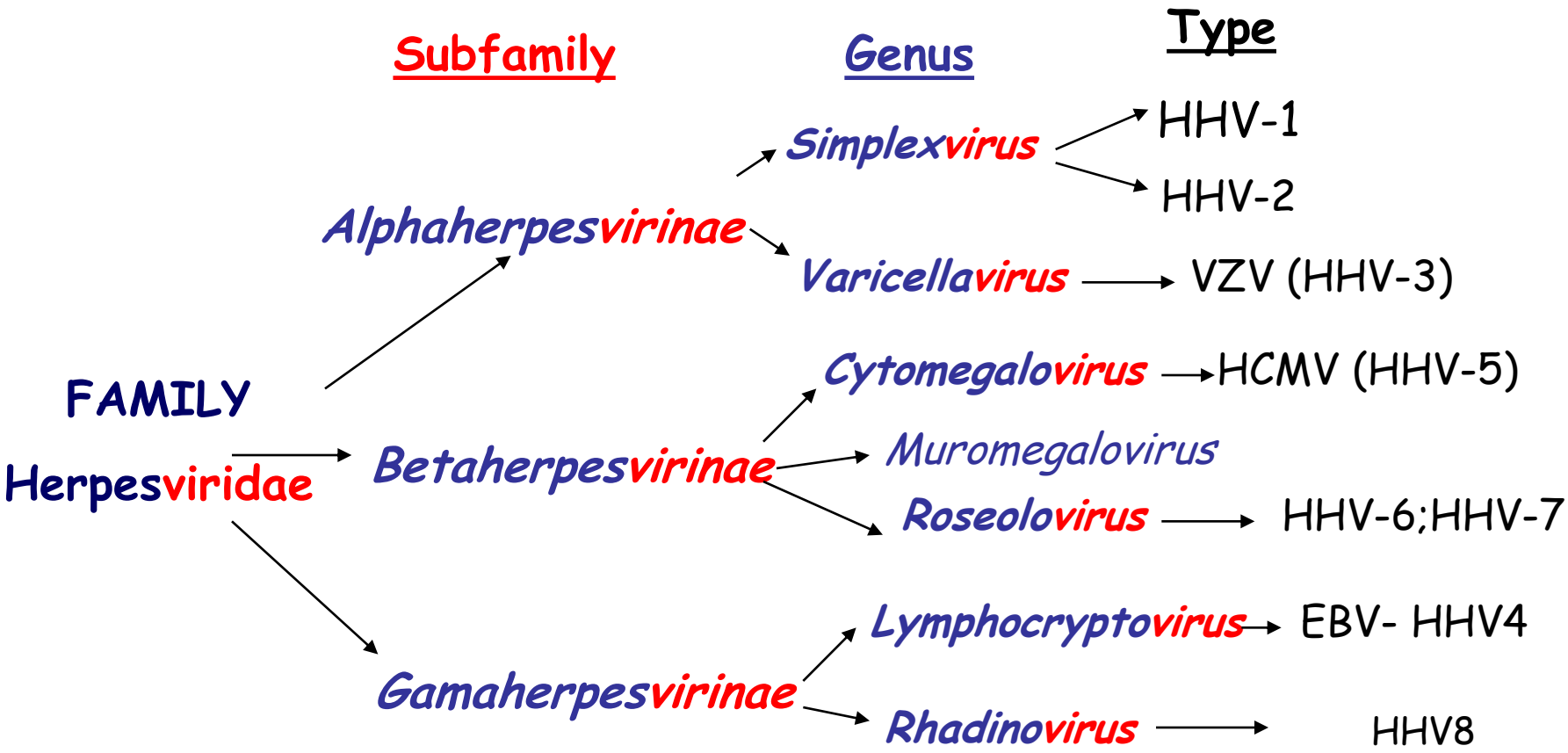
The Hierarchical virus classification system : family – subfamily - genus - species – strain/type

Virus family names have the suffix -viridae

* Poxviridae
* Herpesviridae
* Parvoviridae
* Retroviridae

- Subfamilies have the suffix -virinae.
- Genera have the suffix -virus.

Virus Taxonomy



The Baltimore system of virus classification

- Provides a useful guide with regard to the various mechanisms of viral genome replication.
- The central theme here is that all viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.

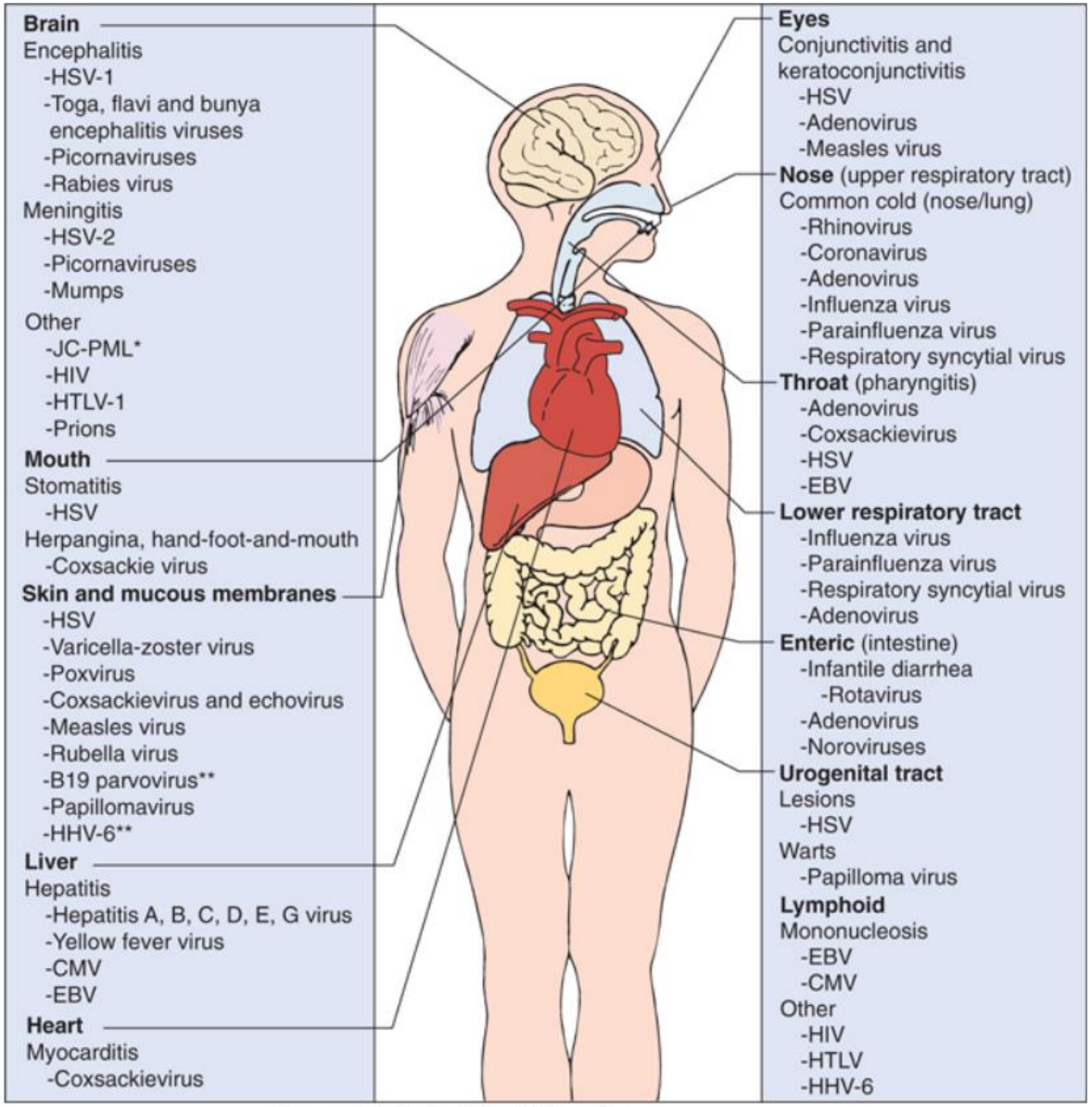
- **I: Double-stranded DNA** (Adenoviruses; Herpesviruses; Poxviruses, etc)
- **II: Single-stranded (+)sense DNA** (Parvoviruses)
- **III: Double-stranded RNA** (Reoviruses; Birnaviruses)
- **IV: Single-stranded (+)sense RNA** (Picornaviruses; Togaviruses, etc)
- **V: Single-stranded (-)sense RNA** (Orthomyxoviruses, Rhabdoviruses, etc)
- **VI: Single-stranded (+)sense RNA with DNA intermediate in life-cycle** (Retroviruses)
- **VII: Double-stranded DNA with RNA intermediate** (Hepadnaviruses)

>60% of all human infections

are caused by viruses

~15% are caused by bacteria

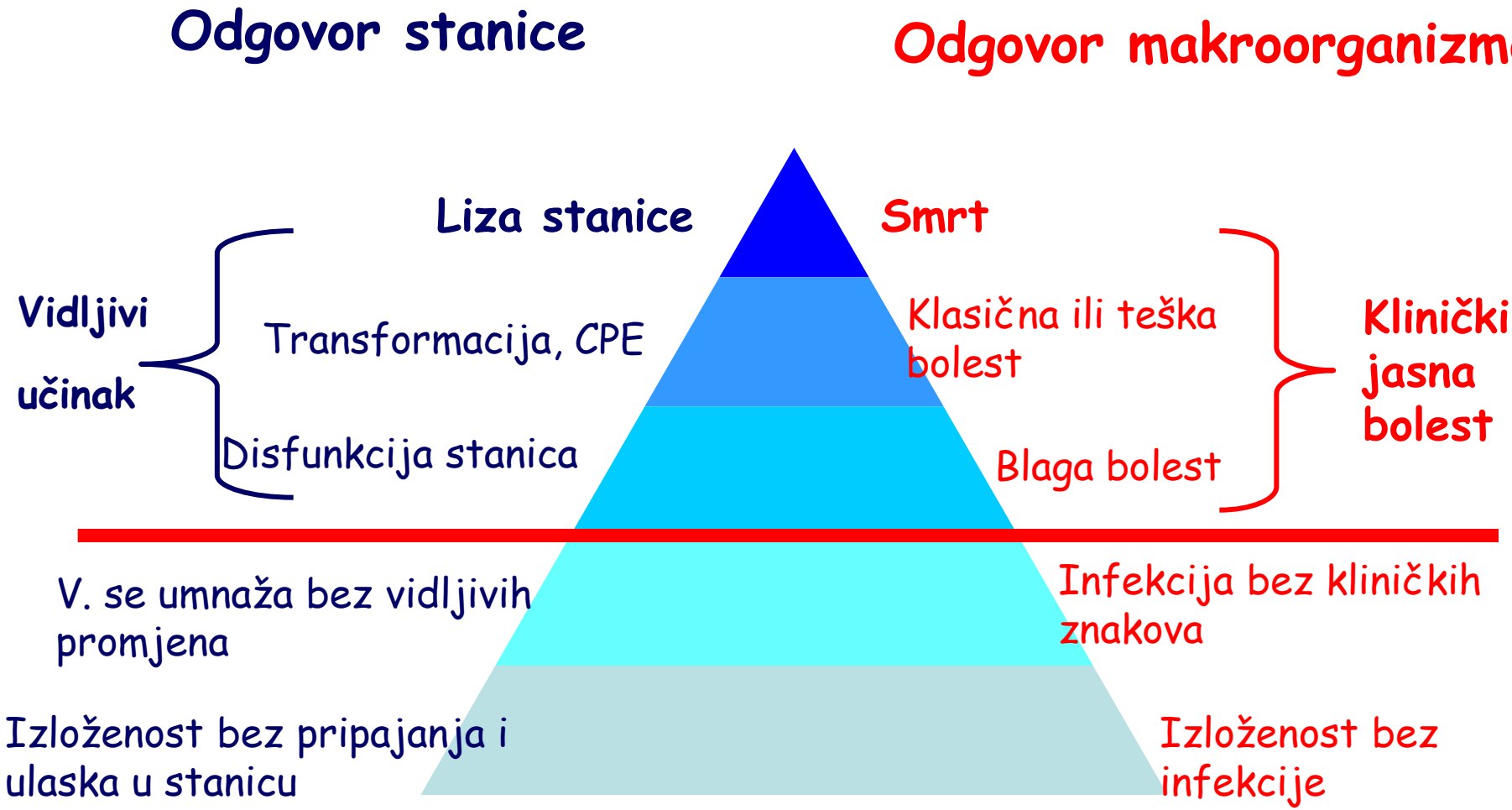
Major target tissues of viral disease



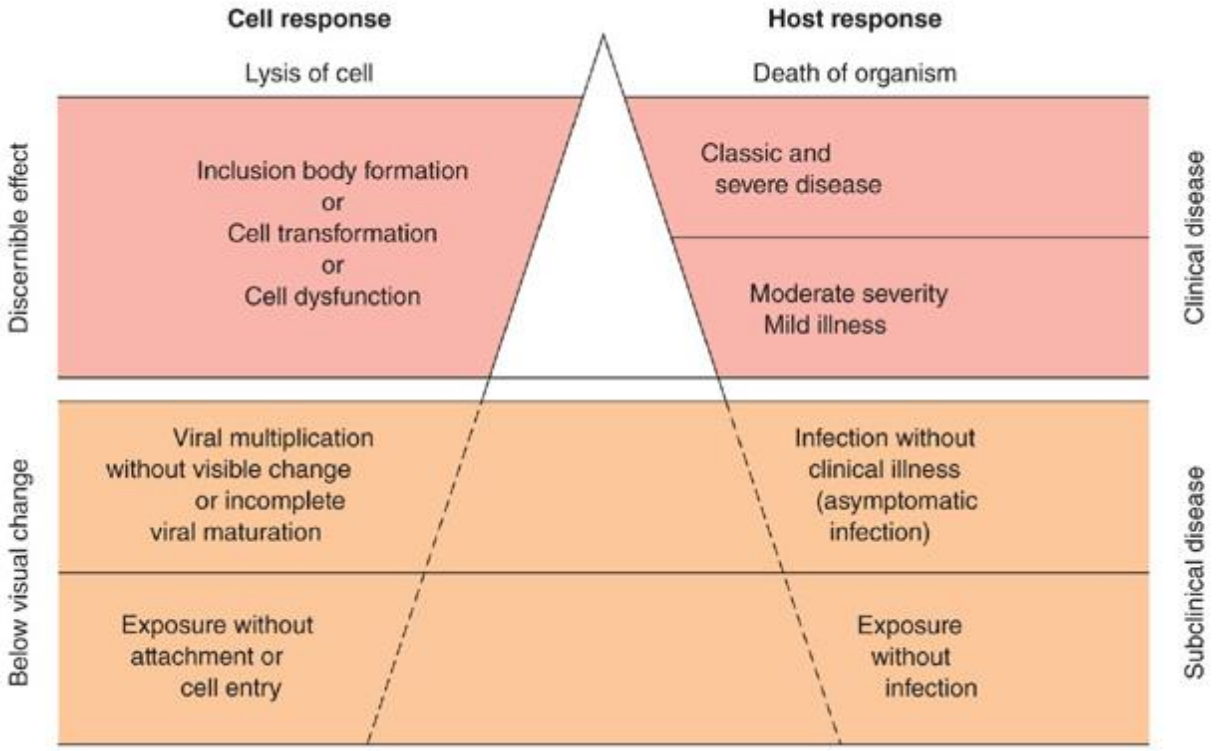
Modes of Transmission

- **Respiratory transmission**
  - Influenza viruses, Rhinoviruses
- **Fecal-oral route**
  - Enteroviruses, Rotaviruses, Noroviruses
- **Contaminated blood**
  - Hepatitis BV, HCV, HIV
- **Sexual contact**
  - HIV, HBV, HSV, HPV
- **Arthropod vectors**
  - arbo virusi
- **Transplacental transmission**
  - RV, CMV, HIV
- **Organ transplantation**
  - CMV,rabies

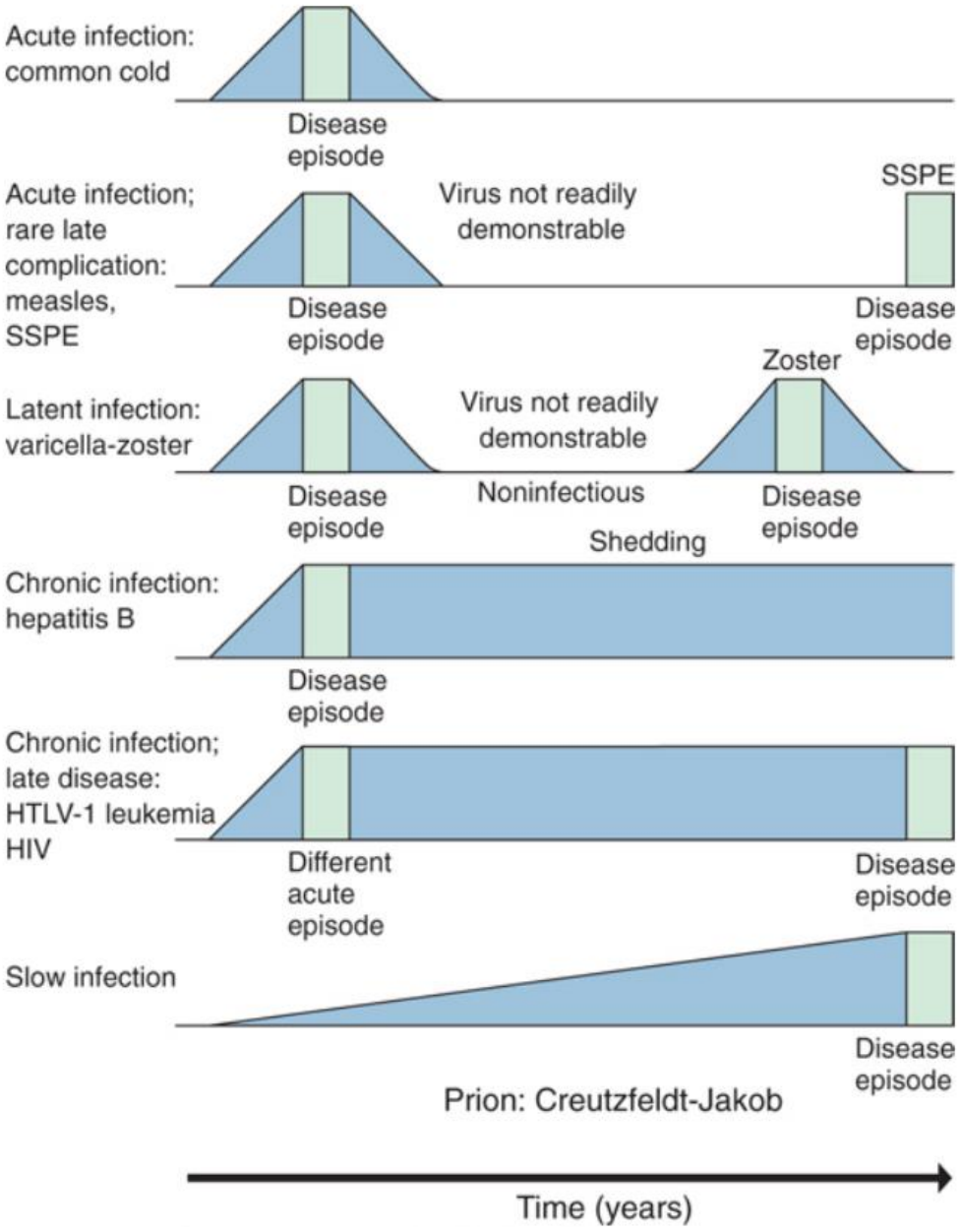
Tipovi odgovora makroorganizma i stanica na infekciju virusima



Iceberg concept of infection



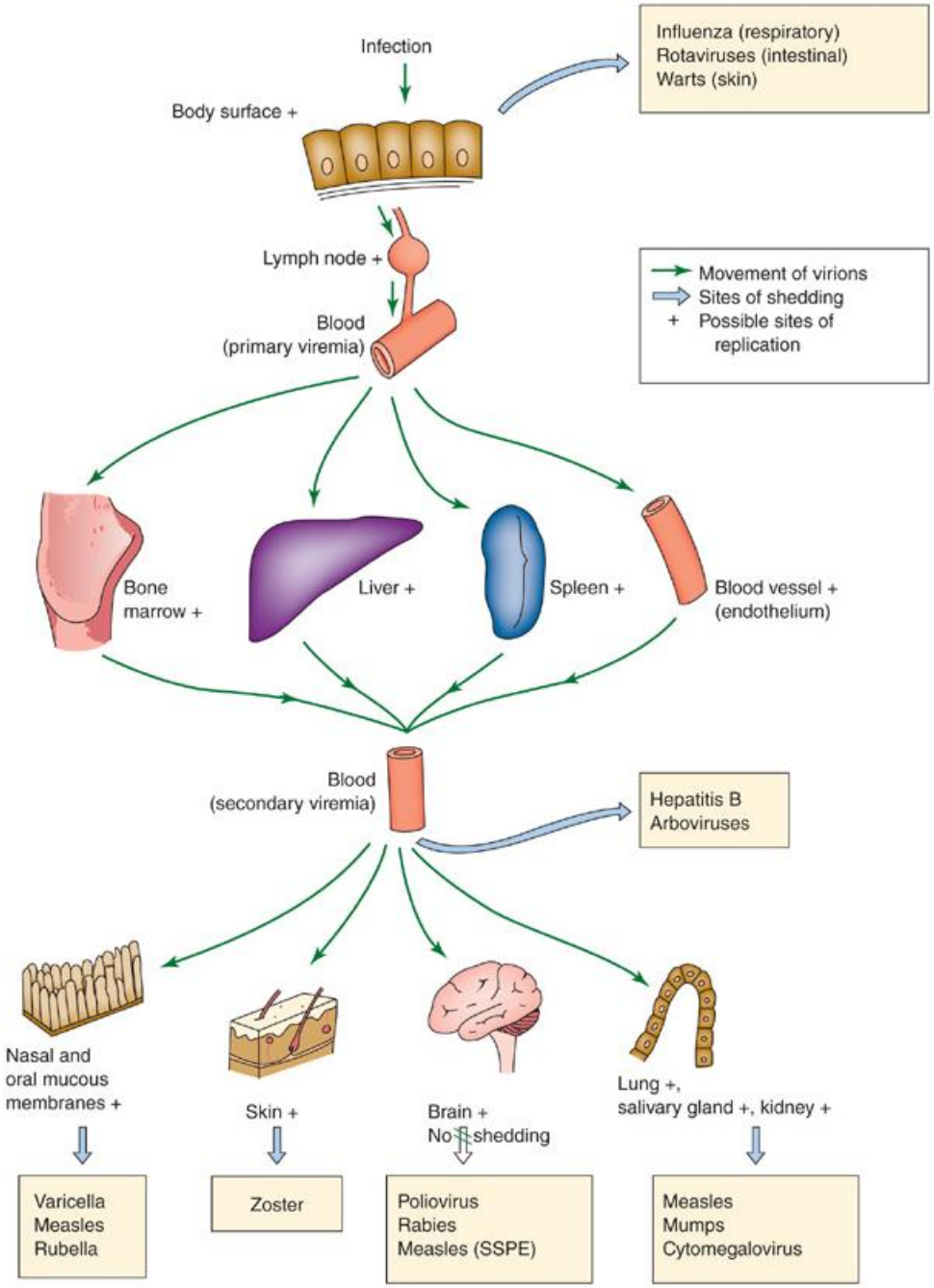
Types of host and cellular responses to virus infection



- Akute infection and various types of persistent infection
  - Blue represents presence of virus; green indicates episode of disease.

Acute and chronic viral infections

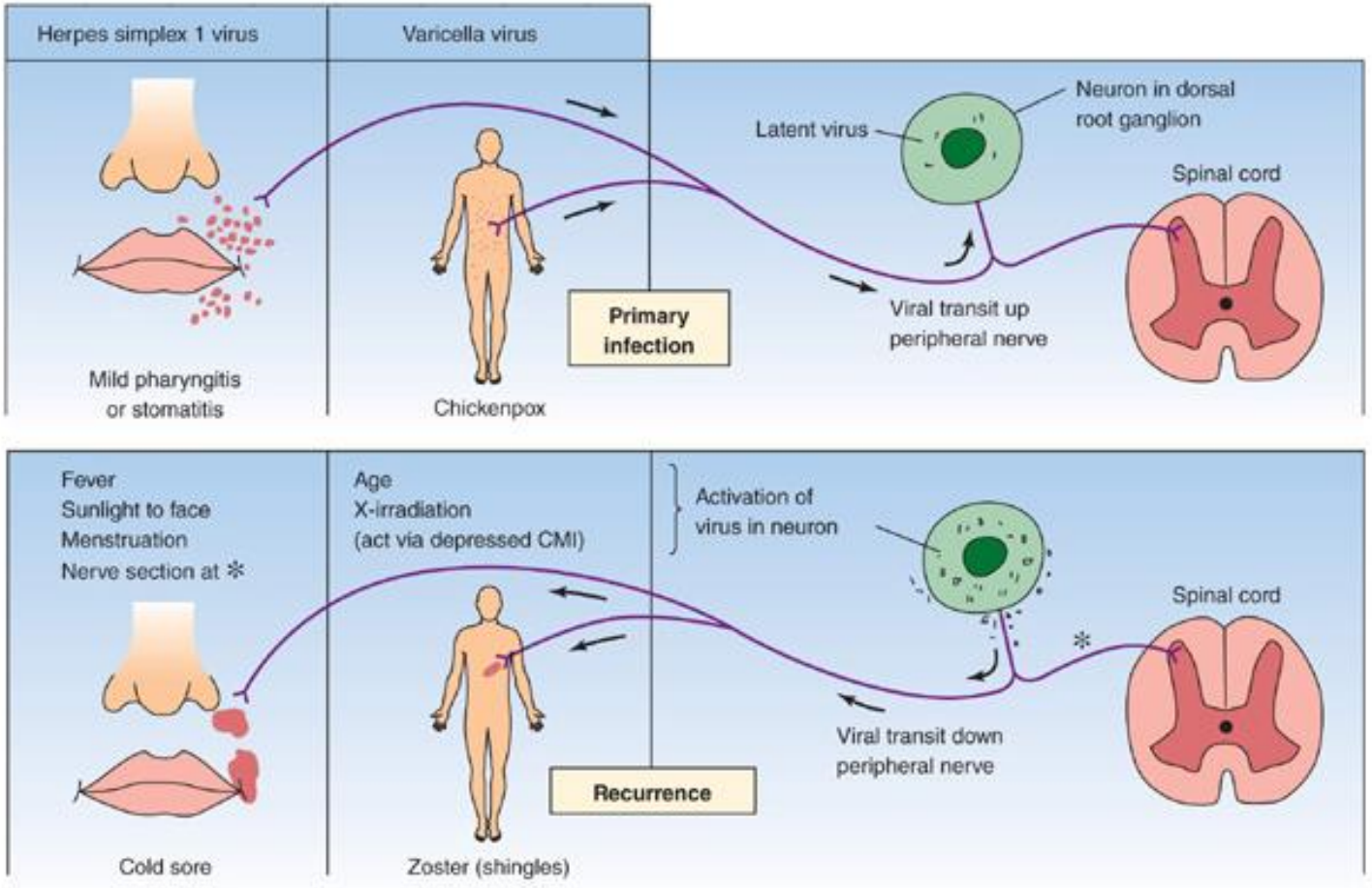
- Local infections
- Systemic infections (viremia)


Infection
Body surface +
Influenza (respiratory)
Rotaviruses (intestinal)
Warts (skin)
Lymph node +
Blood
(primary viremia)
Movement of virions
Sites of shedding
+ Possible sites of replication
Bone
marrow +
Liver +
Spleen +
Blood vessel +
(endothelium)
Blood
(secondary viremia)
Hepatitis B
Arboviruses
Nasal and
oral mucous
membranes +
Skin +
Brain +
No shedding
Lung +,
salivary gland +, kidney +
Varicella
Measles
Rubella
Zoster
Poliovirus
Rabies
Measles (SSPE)
Measles
Mumps
Cytomegalovirus

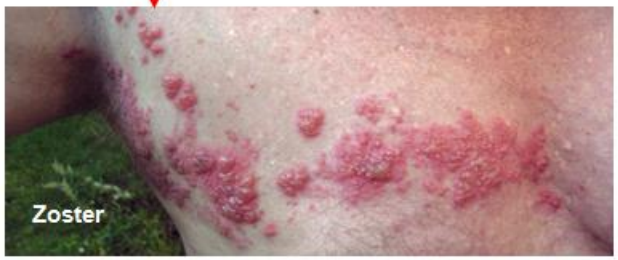
- **Latent Virus Infections**
  - The virus persists in an occult(hidden or cryptic) form, most of the time no new virus is produced; intermittent flare-ups
    - Herpes labialis, herpes zoster

- **Persistent (chronic) virus infections**
  - replicating virus can be continuously detected, often at low levels
    - Hepatitis B virus

Latent infections by herpesviruses



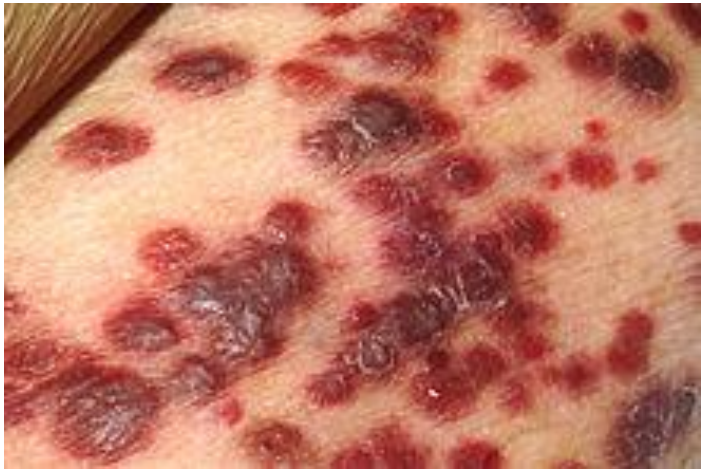

Varicella

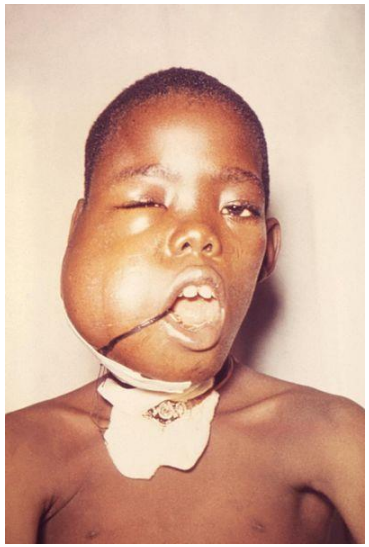

Zoster

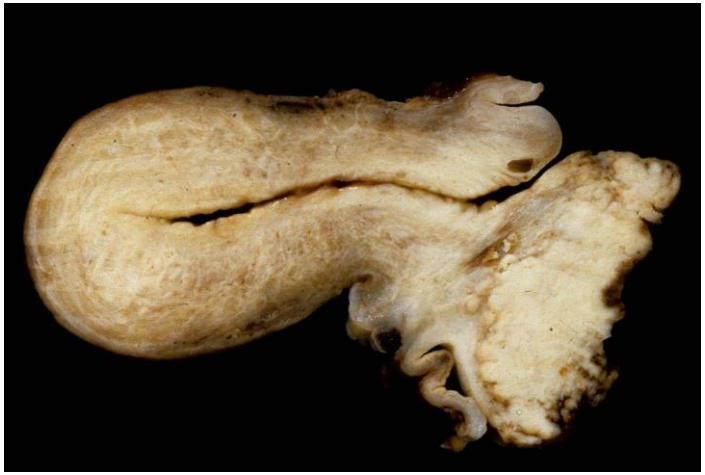
Incubation period of some frequent viral infections

Disease	Incubation
Influenza	1 - 2 days
Common cold	1 - 3 days
Measles	9 - 12 days
Inf. mononucleosis	30 - 50 days
Hepatitis B	50 - 150 days
Papilloma (warts)	50 - 150 days
HIV	1 - 12 years

At least 15-20% of all human tumors worldwide have a viral cause



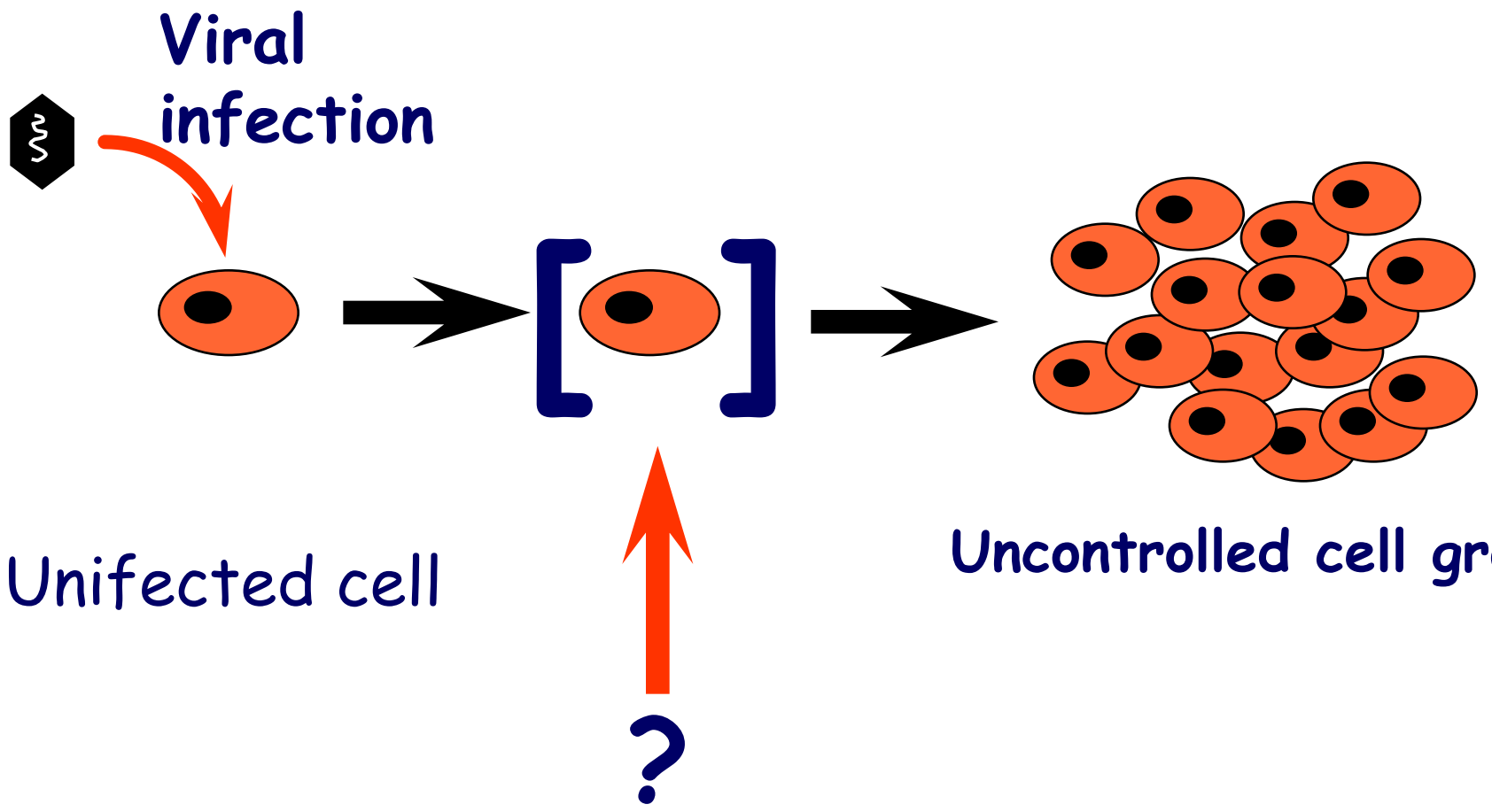




Tenets of Viral Carcinogenesis

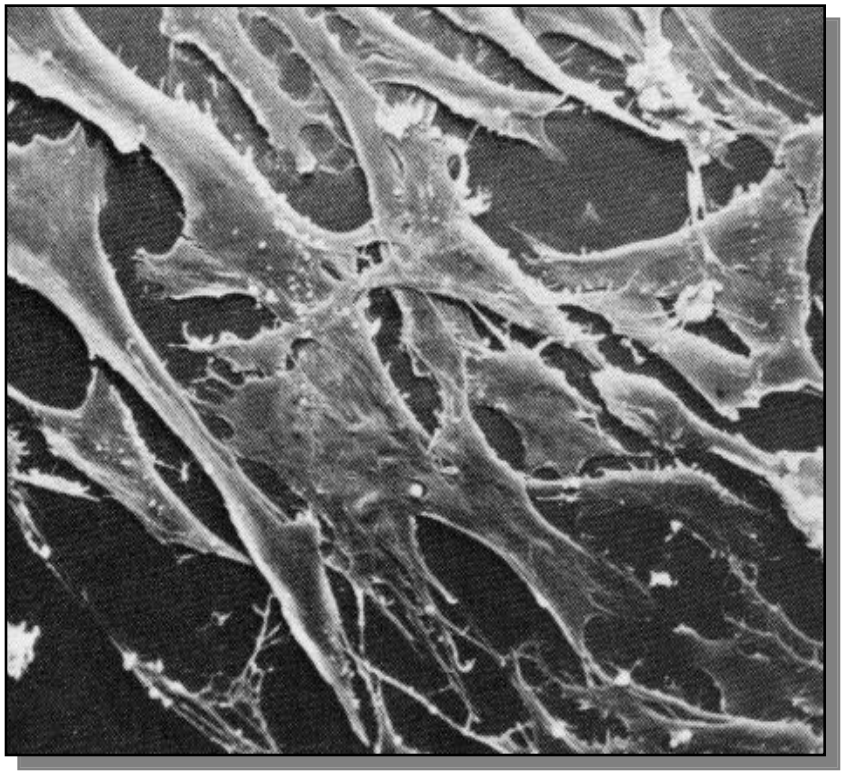
1. Viruses can cause cancer in animals and humans
2. Tumor viruses frequently establish persistent infections in natural hosts
3. Host factors are important determinants of virus-induced tumorigenesis
4. Viruses are seldom complete carcinogens
5. Virus infections are more common than virus-related tumor formation
6. Long latent periods usually elapse between initial virus infection and tumor appearance
7. Viral strains may differ in oncogenic potential
8. Viruses may be either direct- or indirect-acting carcinogenic agents
9. Oncogenic viruses modulate growth control pathways in cells
10. Animal models may reveal mechanisms of viral carcinogenesis
11. Viral markers are usually present in tumor cells
12. One virus may be associated with more than one type of tumor

Tumor Viruses

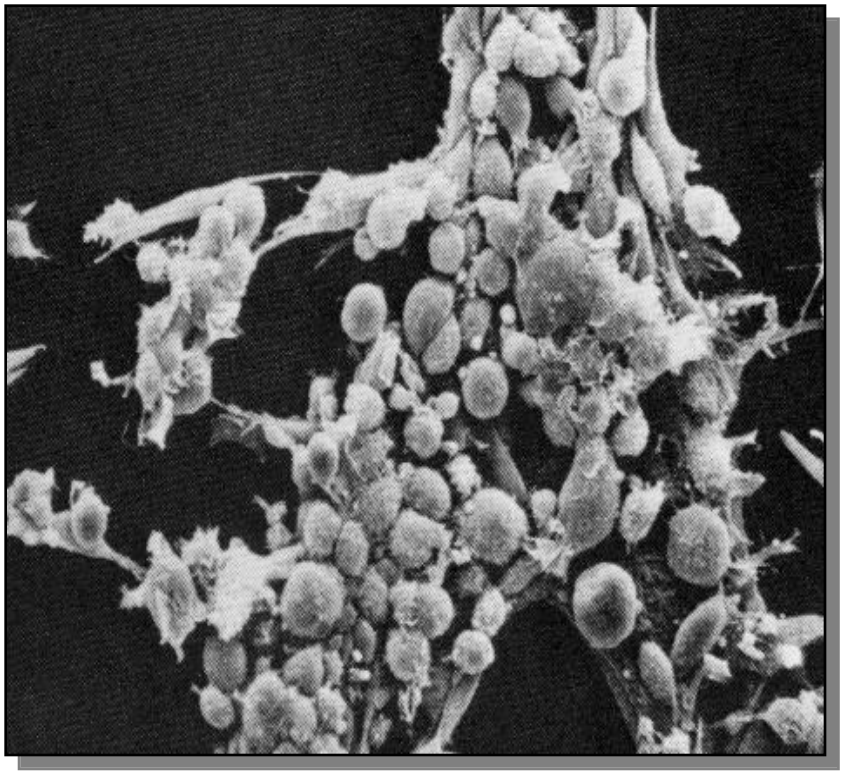


Virus-induced transformation

Normal cells



Transformed cells



Onkogenic viruses

- **Onkogenic DNA viruses**
  - *Adenoviridae*
  - *Herpesviridae*
  - *Poxviridae*
  - *Papovaviridae*
  - *Hepadnaviridae*

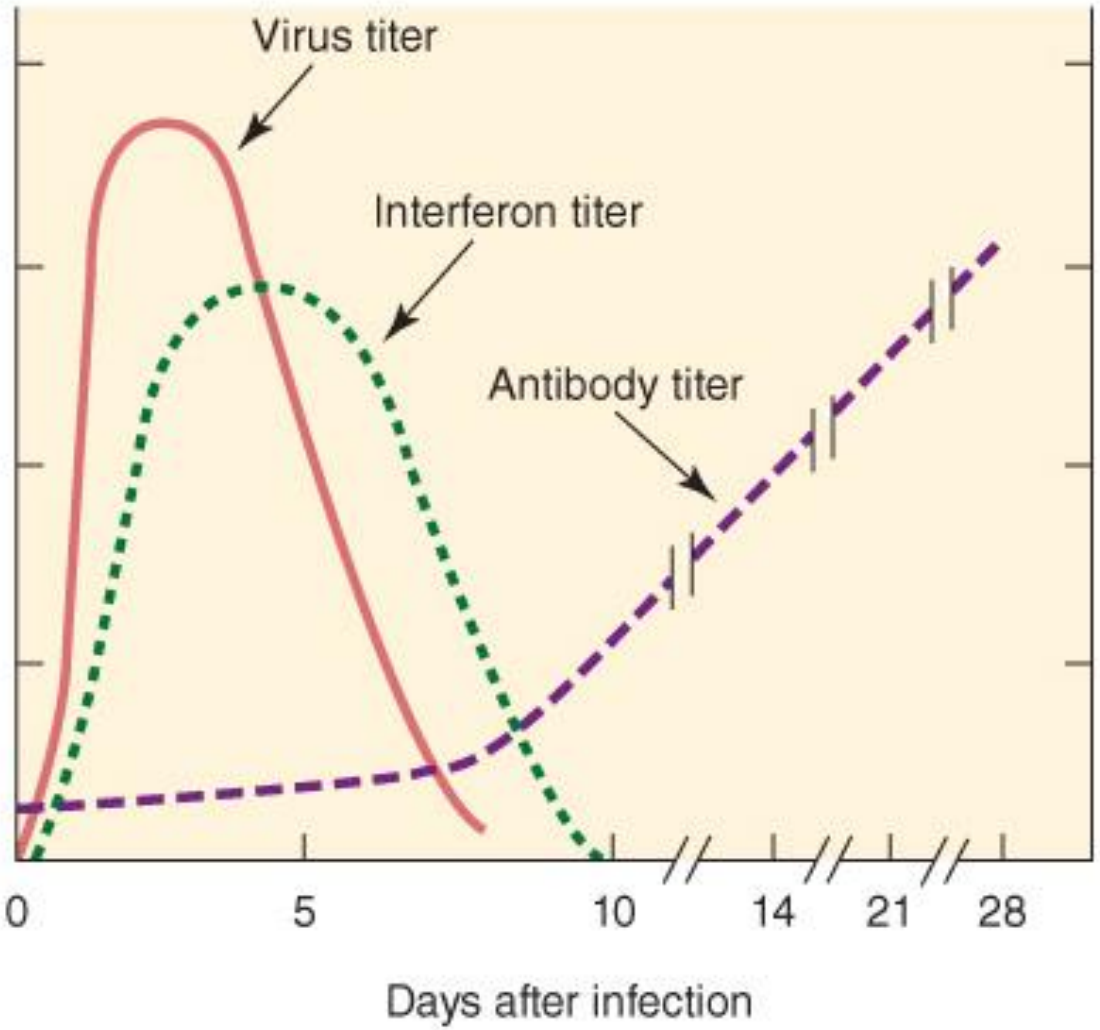
- **Onkogenic RNA viruses**
  - *Retroviridae*
    - Viral RNA transcribed in DNA (integrated in cellular DNA)
    - HTLV 1
    - HTLV 2

Association of Viruses With Human Cancers

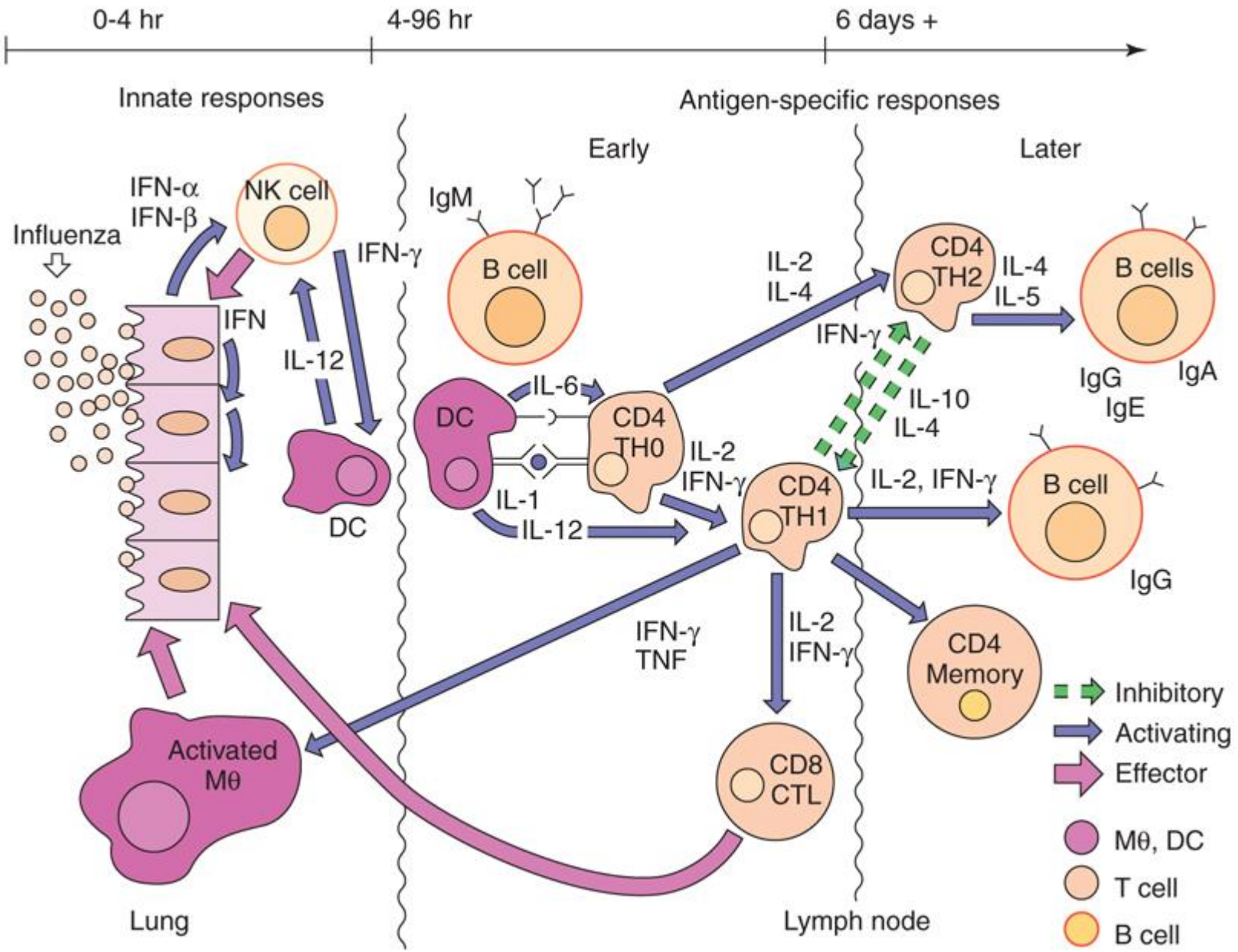
Virus Family	Virus	Human Cancer
Papillomaviridae	Human papillomaviruses	Genital tumors Squamous cell carcinoma Oropharyngeal carcinoma
Herpesviridae	Epstein-Barr virus Human herpesvirus 8	Nasopharyngeal carcinoma Burkitt lymphoma Hodgkin disease B-cell lymphoma Kaposi sarcoma
Hepadnaviridae	Hepatitis B virus	Hepatocellular carcinoma
Polyomaviridae	Merkel cell virus	Merkel cell carcinoma
Retroviridae	Human T-lymphotropic virus Human immunodeficiency virus	Adult T-cell leukemia AIDS-related malignancies
Flaviviridae	Hepatitis C virus	Hepatocellular carcinoma

INTERFERONS

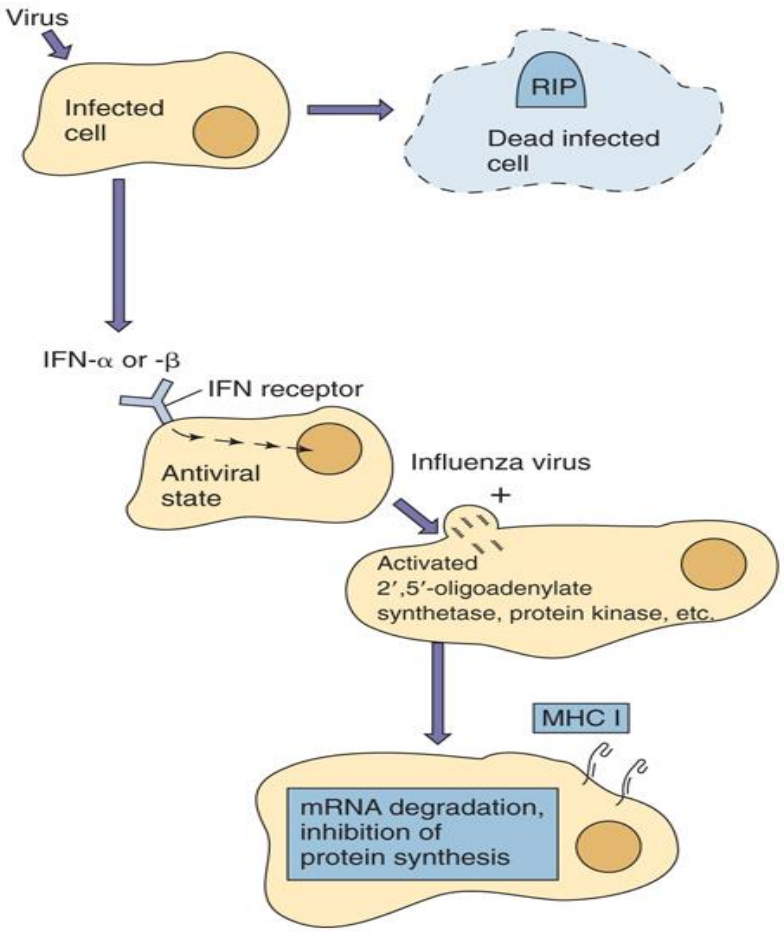
- Host-coded proteins that are memberes of the large cytokine family
- Produced by cells very quickly (within hours) in response to viral infection
- **Protect uninfected cells**
- **Infected cells are not protected**
- **Central to the innate antiviral immune response**
- **Produced by all vertebrate species**
- **Three general groups:**
    - **IFN-α, IFN-β, and IFN-γ**



Kinetics of interferon and antibody synthesis after respiratory viral infection. Interferons are involved in the host's early defense system against viral infections.


0-4 hr
4-96 hr
6 days +
Innate responses
Antigen-specific responses
Early
Later
Influenza
IFN-α
IFN-β
NK cell
IFN
IFN-γ
IL-12
DC
IgM
B cell
IL-6
DC
IL-1
IL-12
CD4
TH0
IL-2
IFN-γ
IL-2
IL-4
CD4
TH2
IL-4
IL-5
B cells
IgG
IgE
IgA
IFN-γ
IL-10
IL-4
CD4
TH1
IL-2, IFN-γ
B cell
IgG
IFN-γ
TNF
IL-2
IFN-γ
CD4
Memory
Activated
Mθ
CD8
CTL
Lung
Lymph node
Inhibitory
Activating
Effector
Mθ, DC
T cell
B cell

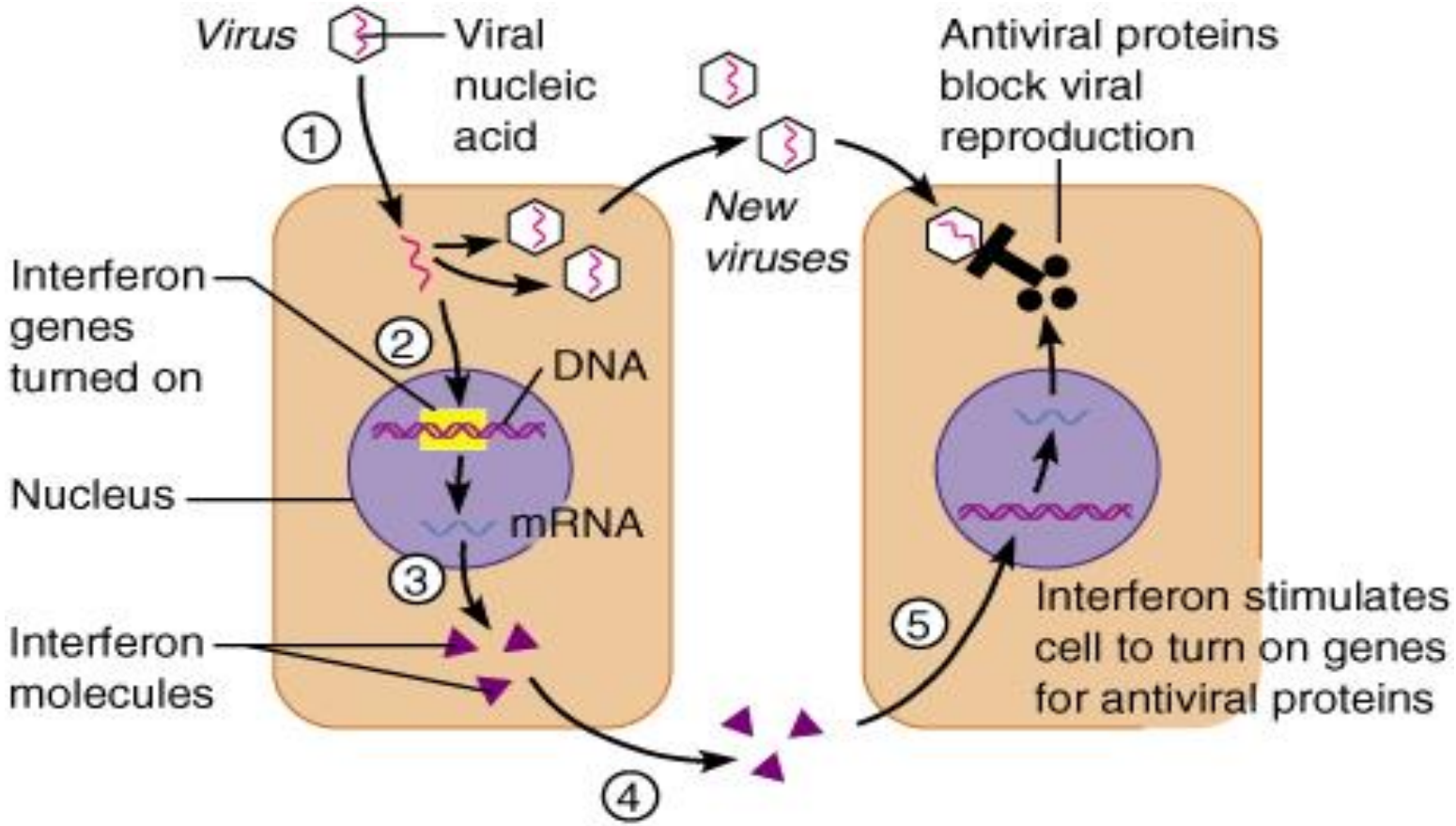
Induction of the antiviral state by interferon *(IFN)*-α or IFN-β



- Interferon is produced in response to viral infection but does not affect the initially infected cell.
- The interferon binds to a cell surface receptor on other cells and induces production of antiviral enzymes (antiviral state).
- The infection and production of double-stranded RNA activates the antiviral activity.

MHC I, Major histocompatibility antigen type 1.

Interferon



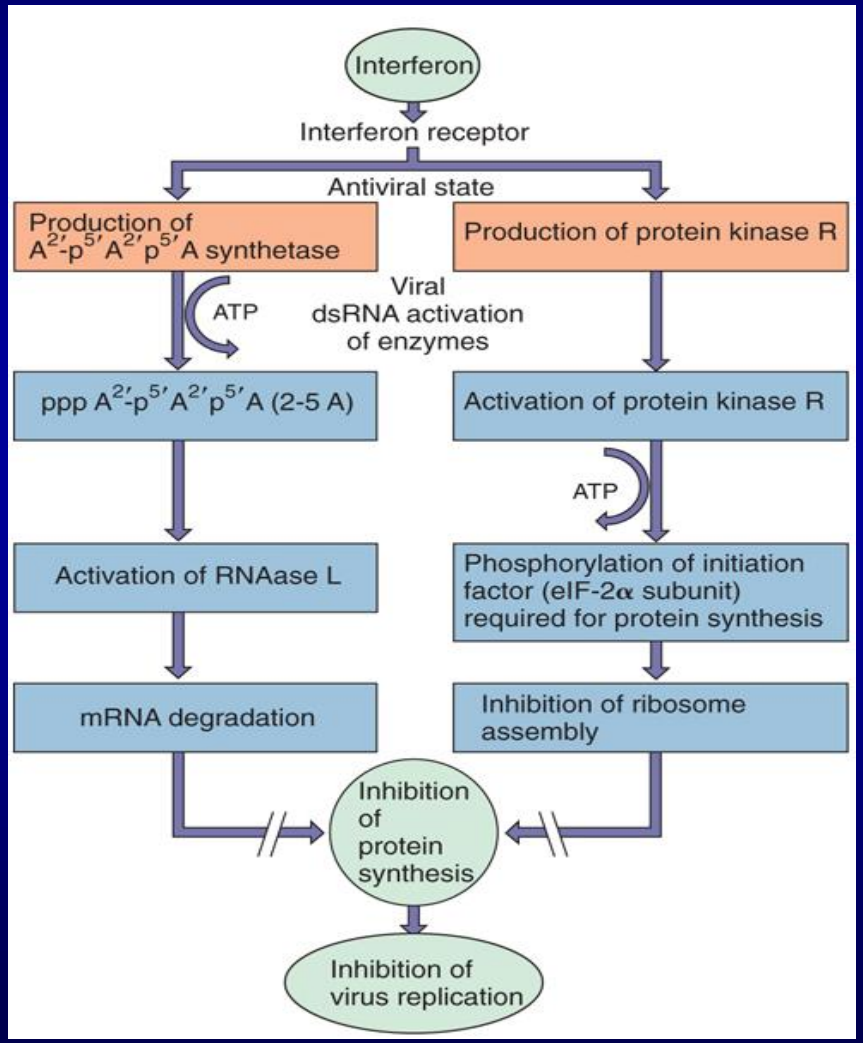
Host Cell 1

Infected by virus; makes interferon; is killed by virus

Host Cell 2

Entered by interferon from cell 1; interferon induces changes that protect it

The two major routes for interferon inhibition of viral protein synthesis



- One mechanism involves the induction of an unusual polymerase (2',5'-oligoadenylate synthetase [2-5A]) that is activated by double-stranded RNA *(dsRNA).*
  - The activated enzyme synthesizes an unusual adenine chain with a 2',5'-phosphodiester linkage.
  - The oligomer activates RNAase L that degrades messenger RNA *(mRNA)*.
- The other mechanism involves the induction of protein kinase R (PKR), which prevents assembly of the ribosome by phosphorylation of the elongation initiation factor (eIF-2α) to prevent initiation of protein synthesis from capped mRNAs.

ATP, Adenosine triphosphate.

Interferons

Interferon	Source	Induced by
Alpha	Most cell types	Viruses
Beta	Most cell types	Viruses
Gamma	Lymphocytes	Mitogens

Interferons

- Antiviral, anti-inflammatory (active against many viruses)
- Immunoregulatory - ↑ cellular immunity
- Antiproliferative – tumor growth inhibition (some tumors)

Nuspojave pri liječenju interferonima

- Temperatura
- Iscrpljenost, umor
- Bolovi u mišićima
- Mučnina
- Depresija…
- Itd.

Prevention and Treatment of Viral Infections

- Viral vaccines and immunisation

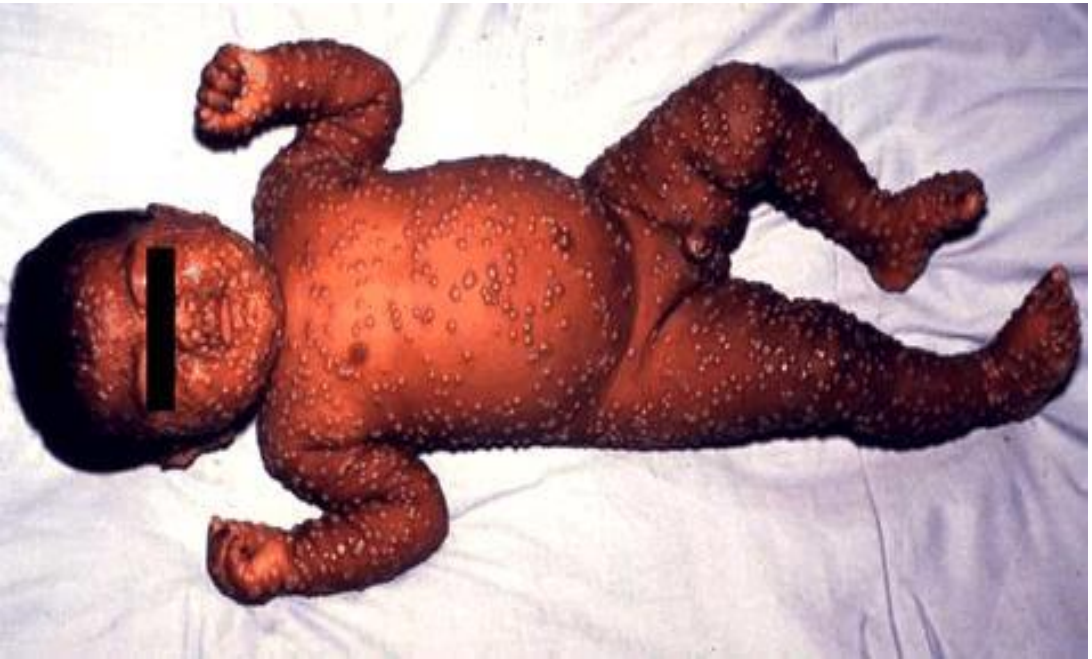
- Antiviral chemotherapy

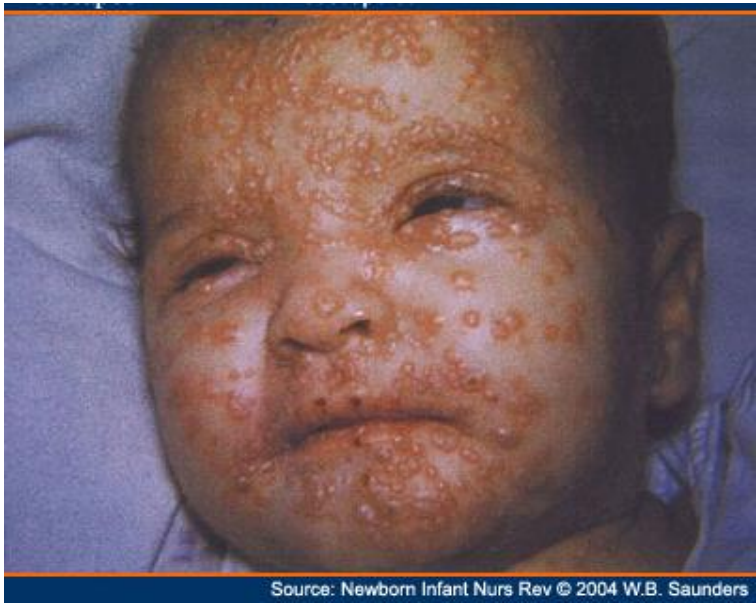
Viral Vaccines

- The purpose of viral vaccines is to use the immune response of the host to prevent viral disease
  - Killed-Virus Vaccines
  - Attenuated Live-Virus Vaccines

Smallpox

- Mummies
  - China, India
- Western Europe: mortality 25%





Smallpox

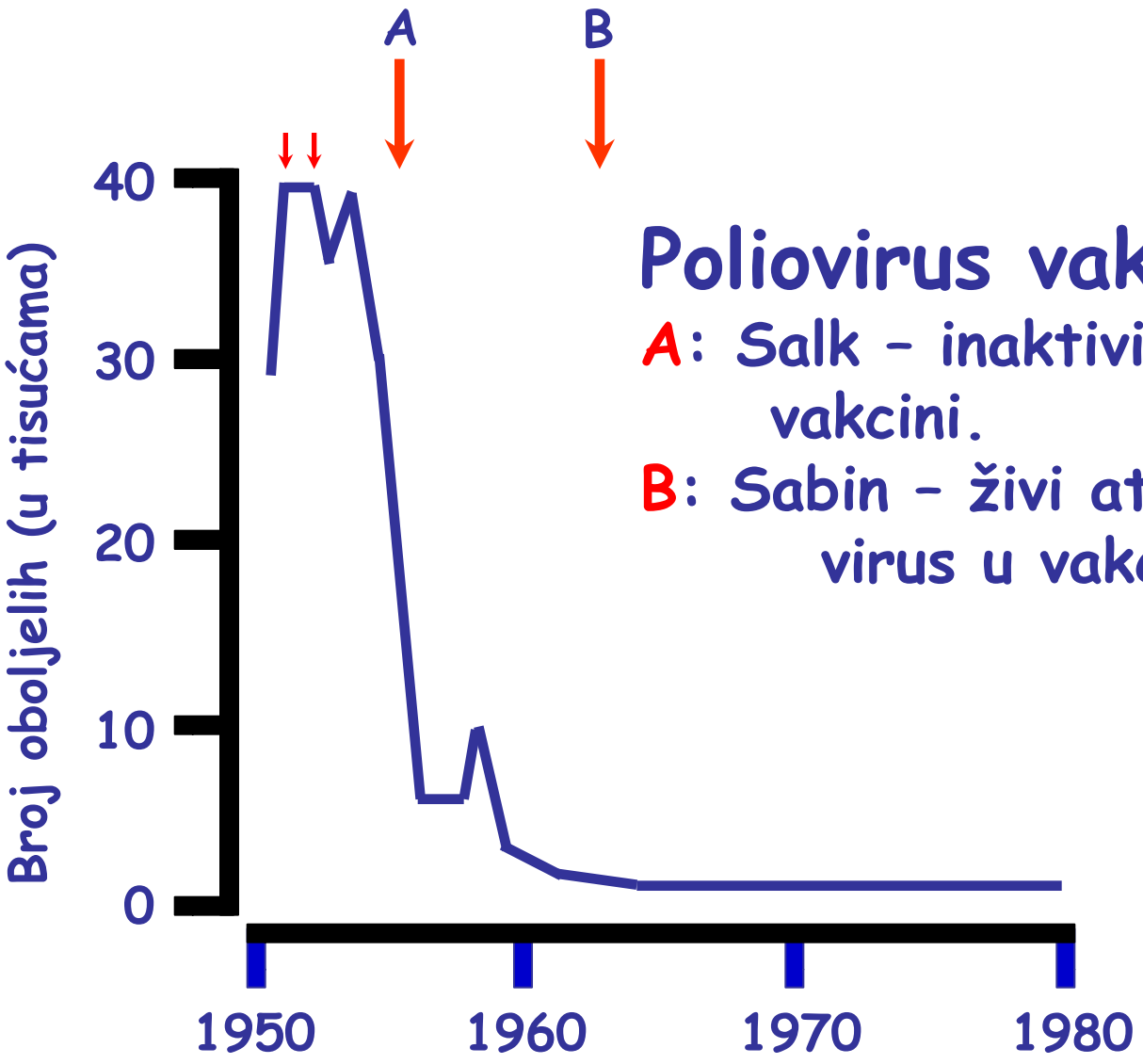
- Smallpox was endemic in China by 1000BC.
- In response, the practice of **variolation** was developed.
- Recognizing that survivors of smallpox outbreaks are protected from subsequent infection, variolation involves inhalation of the dried crusts from smallpox lesions like snuff, or in later modifications, inoculation of the pus from a lesion into a scratch on the forearm.

VIRAL VACCINES

1796.g. – E. Jenner –
Cowpox boginja →
Ø variola

1884.g. – L. Pasteur –
Rabies virus →
Ø rabies

Incidencija poliomijelitisa



Poliovirus vakcine

A: Salk – inaktivirani virus u vakcini.

B: Sabin – živi atenuirani virus u vakcini

Viral Vaccines

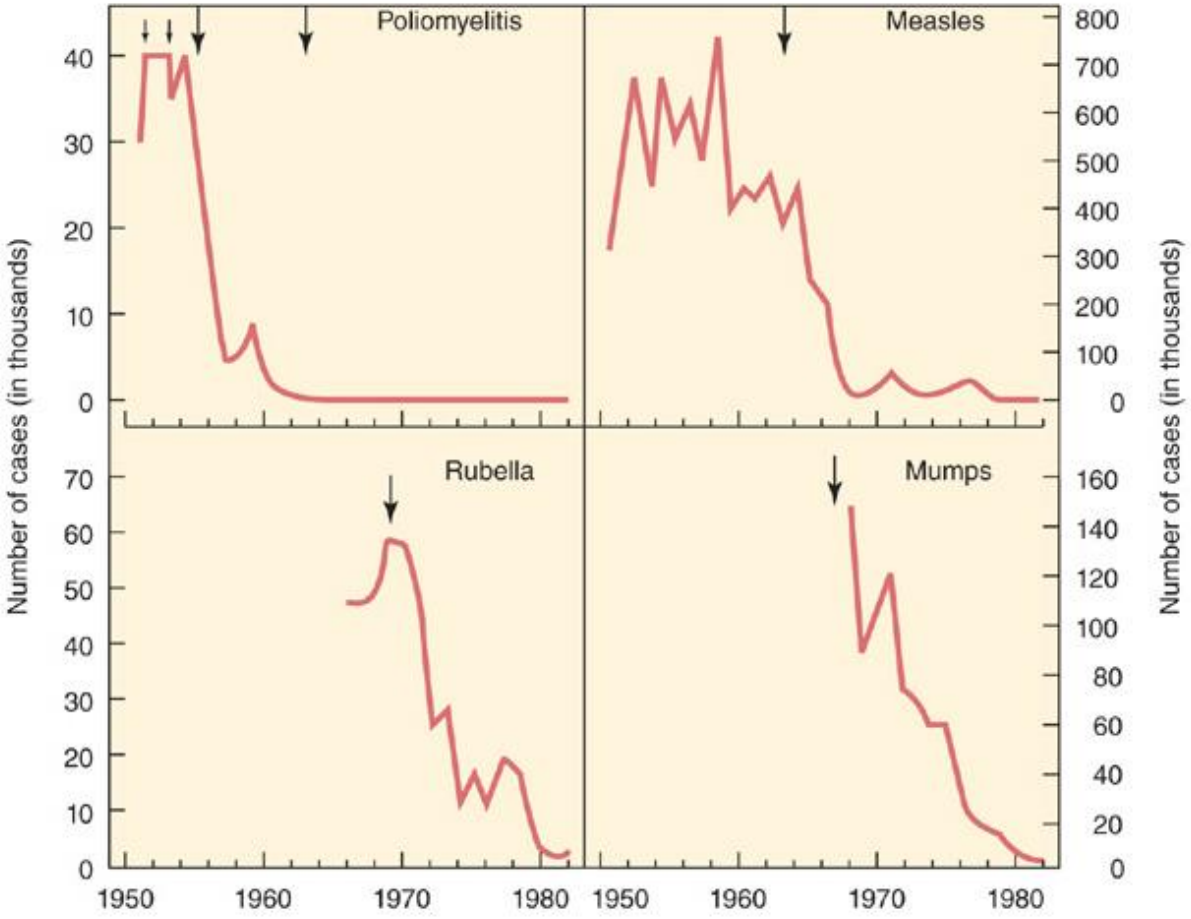
- Killed-Virus Vaccines
- Attenuated Live-Virus Vaccines

Comparison of Characteristics of Killed- and Live-Virus Vaccines

Characteristic	Killed Vaccine	Live Vaccine
Number of doses	Multiple	Single
Need for adjuvant	Yes	No
Duration of immunity	Shorter	Longer
Effectiveness of protection (more closely mimics natural infection)	Lower	Greater
Immunoglobulins produced	IgG	IgA and IgG
Mucosal immunity produced	Poor	Yes
Cell-mediated immunity produced	Poor	Yes
Residual virulent virus in vaccine	Possible	No
Reversion to virulence	No	Possible
Excretion of vaccine virus and transmission to nonimmune contacts	No	Possible
Interference by other viruses in host	No	Possible
Stability at room temperature	High	Low

Serum and secretory antibody response to orally administered, live attenuated polio vaccine and to intramuscular inoculation of killed polio vaccine.

Annual incidence of various viral diseases



Date of introduction of vaccine indicated by arrow

Virus Vaccines Approved in the United States (2011)

Use	Vaccine	Type	Cell Substrate
Common	Hepatitis A	Killed	Human diploid fibroblasts (MRC-5)
	Hepatitis B	Subunit (HBsAg)	Yeast (recombinant DNA)
	Influenza A and B	Killed	Embryonated chicken eggs
	Influenza A and B	Live (intranasal)	Embryonated chicken eggs
	Measles	Live	Chicken embryo fibroblasts
	Mumps	Live	Embryonated chicken eggs and chicken embryo fibroblasts
	Papilloma	Subunit (L1)	Yeast (recombinant DNA)
	Poliovirus (IPV)	Killed	Monkey kidney cells (Vero)
	Poliovirus (OPV)	Live	Monkey kidney cells
	Rabies	Killed	Human diploid fibroblasts (MRC-5) or rhesus fetal lung diploid cells or chicken fibroblasts
	Rotavirus[a]	Live	Monkey kidney cells (Vero)
	Rubella	Live	Human diploid fibroblasts (WI-38)
	Varicella	Live	Human diploid fibroblasts (MRC-5)
	Zoster	Live	Human diploid fibroblasts (MRC-5)
Special situations	Adenovirus[b]	Live	Human diploid fibroblasts (WI-38)
	Japanese encephalitis[c]	Killed	Mouse brain
	Smallpox	Live	Calf lymph
	Yellow fever[c]	Live	Embryonated chicken eggs

VIRUSNA CJEPIVA U PREVENCIJI BOLESTI

Bolest	Sastav cjepiva	Primjena
Poliomijelitis	Atenuirani živi virus (Sabin) Inaktivirani virus (Salk)	Per os s.c.
Ospice	Atenuirani živi virus	s.c.
Mumps	Atenuirani živi virus	s.c.
Rubela	Atenuirani živi virus	s.c.
Hepatitis B	Pročišćeni HBsAg Rekombinantni HBsAg	i.m.
Influenca	Inaktivirani virus	i.m.
Bjesnoća	Inaktivirani virus	i.m.
Žuta groznica	Atenuirani živi virus	s.c.
HPV 6,11,16,18	Rekombinirani kapsidni proteini	i.m.
V. boginje	Atenuirani živi virus	i.d.

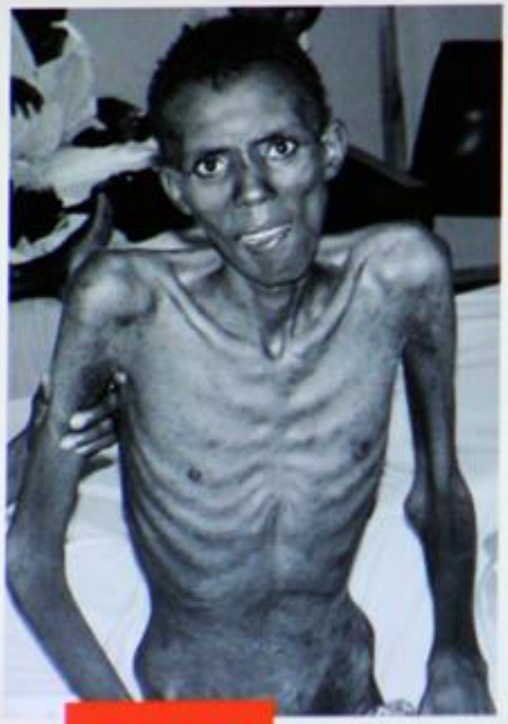
Antiviral Chemotherapy

Viruses

- Replicate in cells
- Use cellular metabolism
- Selectivity ??

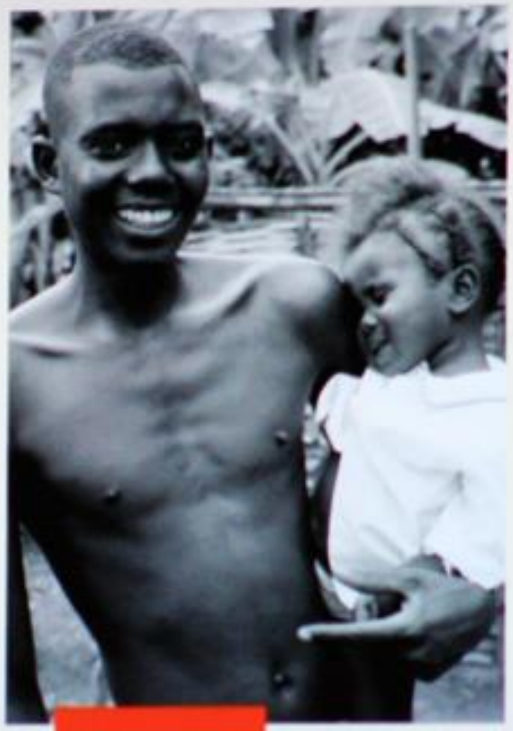
Antiviral Chemotherapy

- There is a need for antiviral drugs active against viruses for which vaccines are **not available** or **not highly effective** –
  - the latter perhaps because of a multiplicity of serotypes(eg, rhinoviruses) or
  - because of a constantly changing virus (eg, influenza, HIV).



Joseph

before treatment



Joseph

after 6 months of treatment

PHOTOS: Partners in Health, courtesy of Paul Farmer

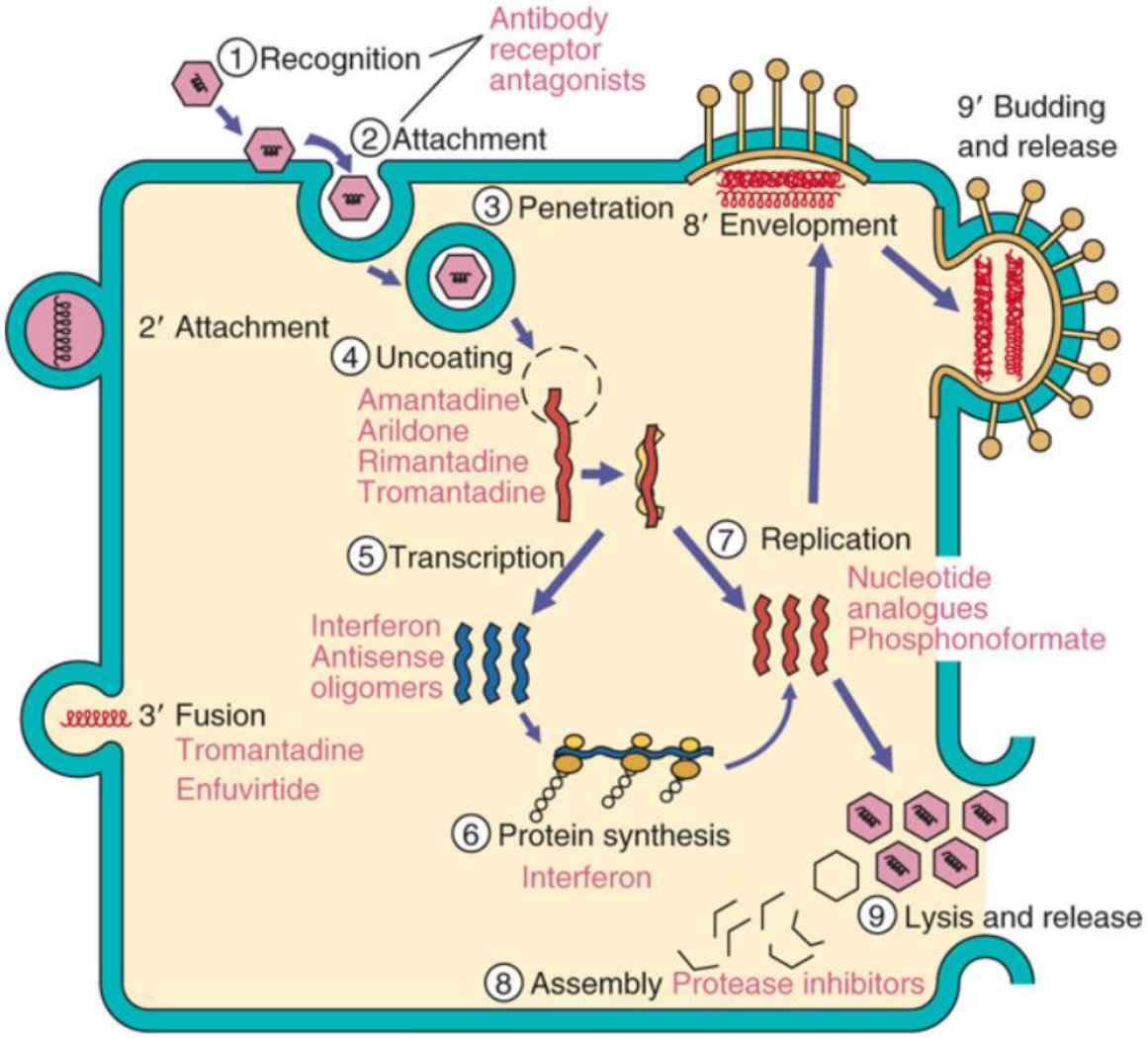
Antiviral Chemotherapy

- Because viruses are obligate intracellular parasites, antiviral agents must be capable of **selectively inhibiting** viral functions without damaging the host, making the development of such drugs very difficult.
- Another limitation is that many rounds of virus replication occur during the incubation period and the virus has spread before symptoms appear, making a drug relatively ineffective.

Antiviral targets

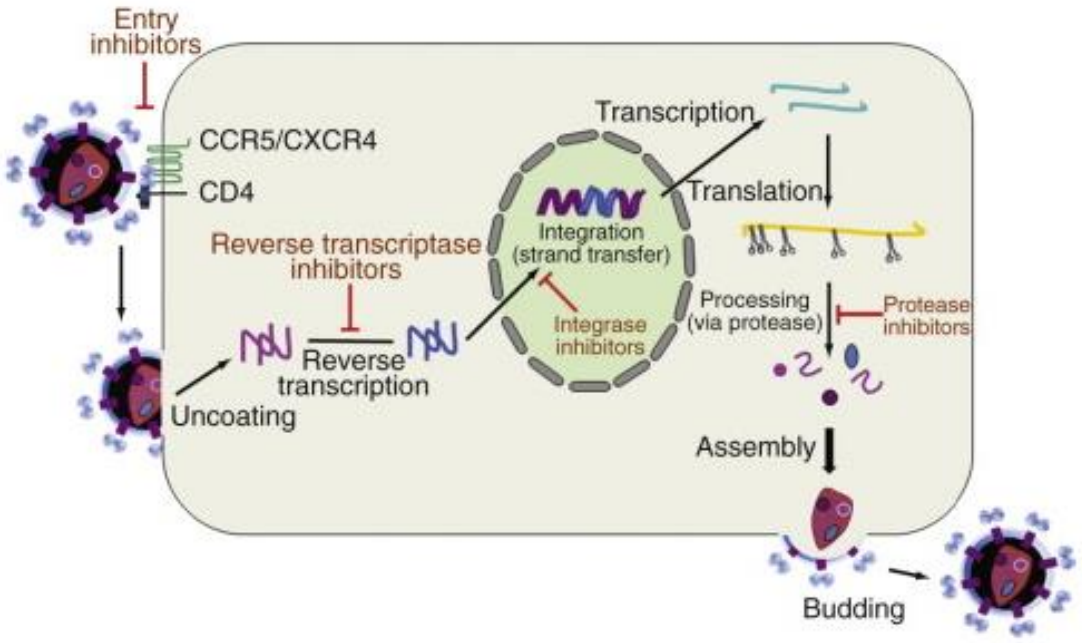
- Attachment
- Nucleic acid replication
- Protein synthesis
- Virus assembly and release

The antiviral drugs for susceptible steps



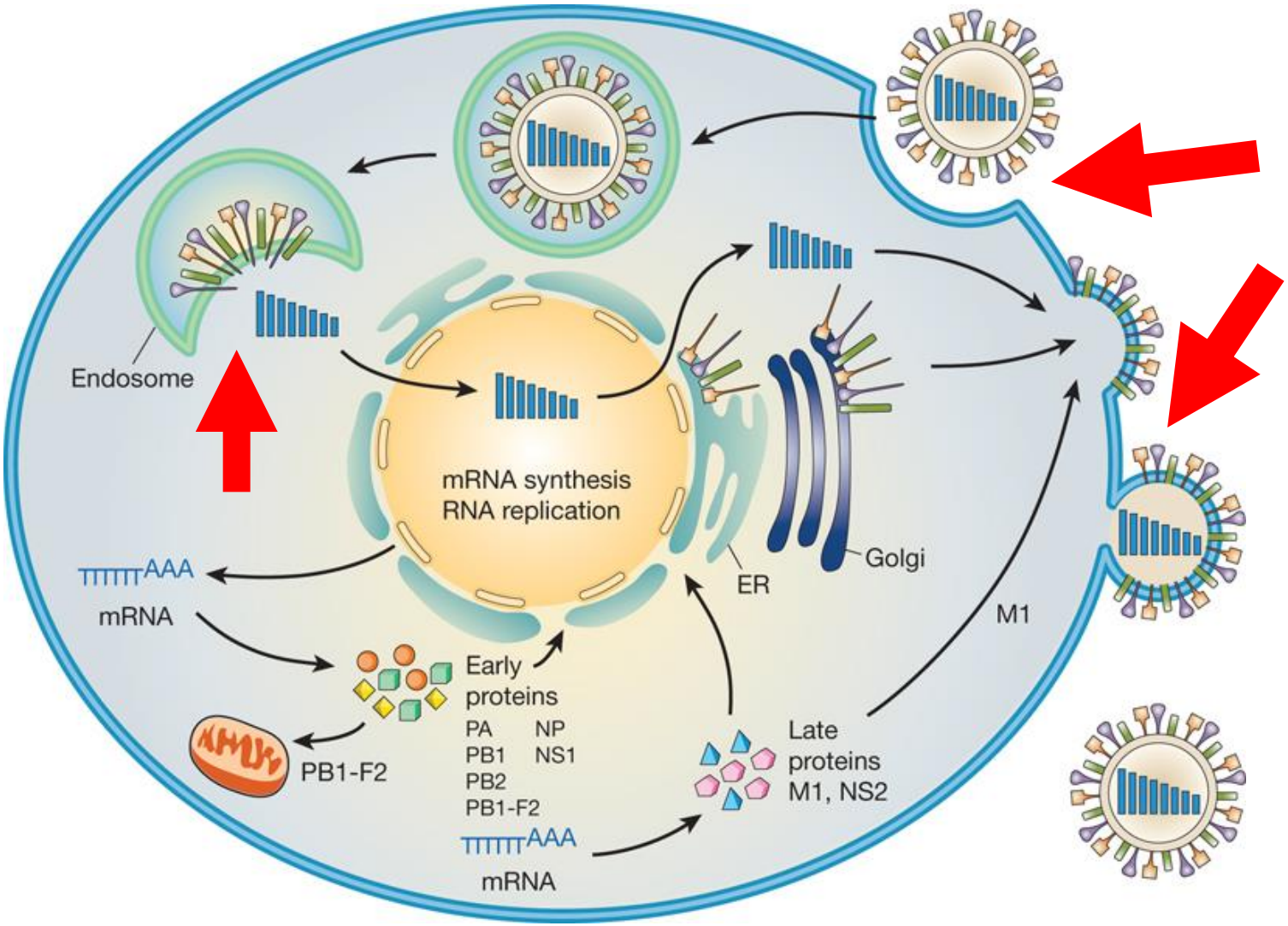
Other major targets:
Nucleotide biosynthesis and mutation: ribavirin
Thymidine kinase (drug activation): acyclovir, penciclovir
Neuraminidase: zanamivir, oseltamivir

Combination antiretroviral therapy

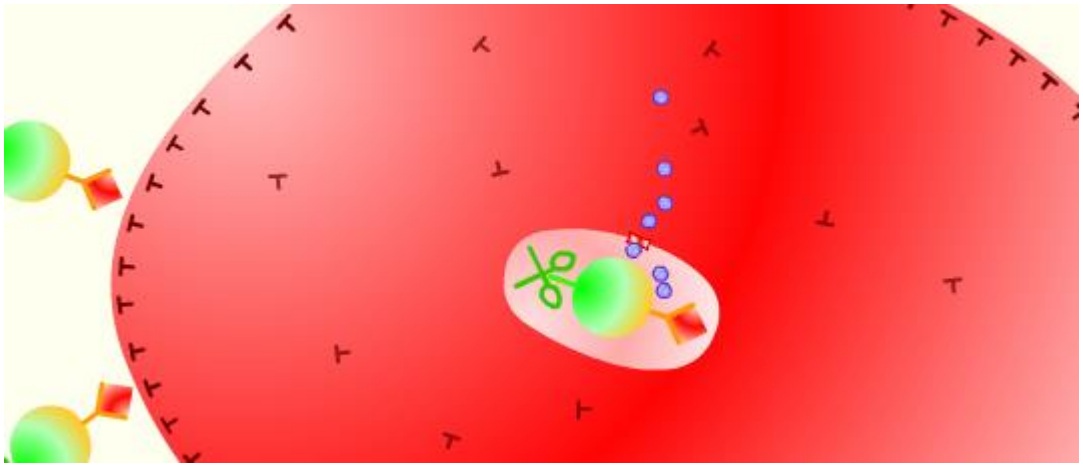


- Fusion inhibitors can block the virus–co-receptor interaction and late stages of virus-cellular membrane fusion.
- Reverse transcription in the cytoplasm is inhibited by structural analogues of native nucleotides/nucleosides.
- Newly formed viral cDNA is incorporated into preintegration complexes and actively transported into the nucleus. The first integrase inhibitor blocks strand transfer.
- Once viral DNA is transcribed and translated, homodimeric viral proteases cleave and process polypeptides into mature virions; **protease inhibitors** render these virions noninfectious.

Therapeutic possibilities



M2-inhibitors



- **Amantadin** i **Rimantadin** blokiraju “uncoating” virusa blokiranjem ionskog M2-kanala virusa

Viruses Treatable with Antiviral Drugs

- Herpes simplex virus
- Varicella-zoster virus
- Cytomegalovirus
- Human immunodeficiency virus
- Influenza A and B viruses
- Respiratory syncytial virus
- Hepatitis B and C viruses
- Papillomavirus
- Picornavirus

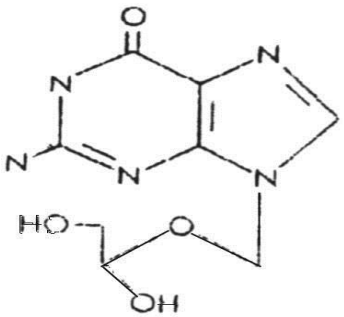
Antiviral Compounds

- Nucleoside and Nucleotide Analogs
- Reverse Transcriptase Inhibitors
- Protease Inhibitors
- Other Types of Antiviral Agents

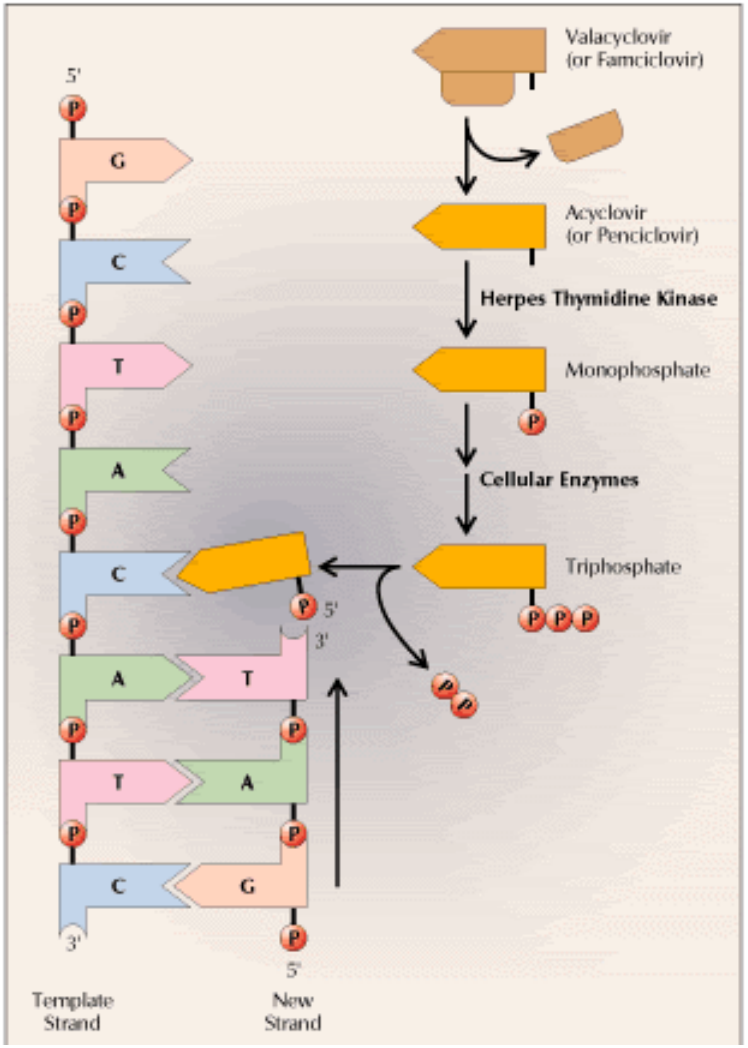
Nucleoside analogs

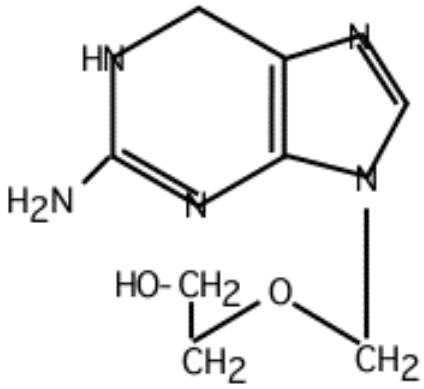
- The majority of available antiviral agents are nucleoside analogs.
- They inhibit nucleic acid replication by inhibition of polymerases essential for nucleic acid replication.
- In addition, some analogs can be incorporated into the nucleic acid and block further synthesis or alter its function.
- Analogs can inhibit cellular enzymes as well as virus-encoded enzymes.

GUANOSINE ANALOGS



Ganciklovir
(Citoven)
9-(1,3-dihydroxy-2-propoxy-methyl) guanin





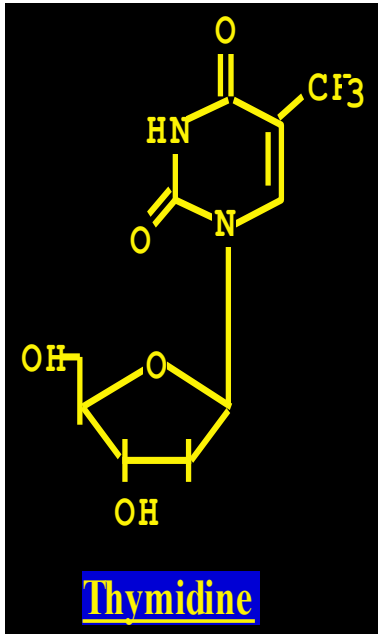
acycloguanosine (acyclovir)

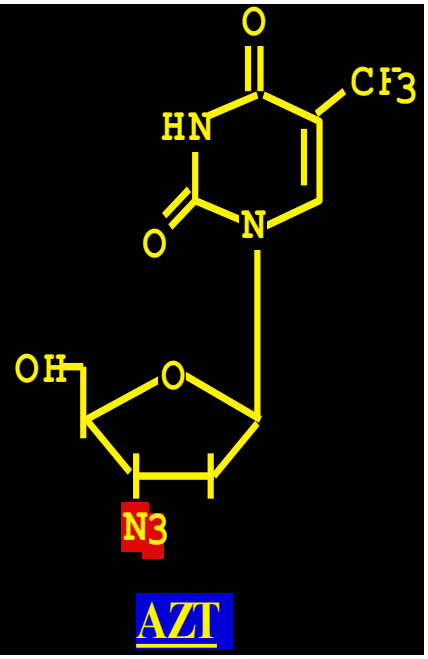
Aciklogvanozin
(Aciklovir, Zovirax)
9-(2hydroxyethoxymethyl) guanin

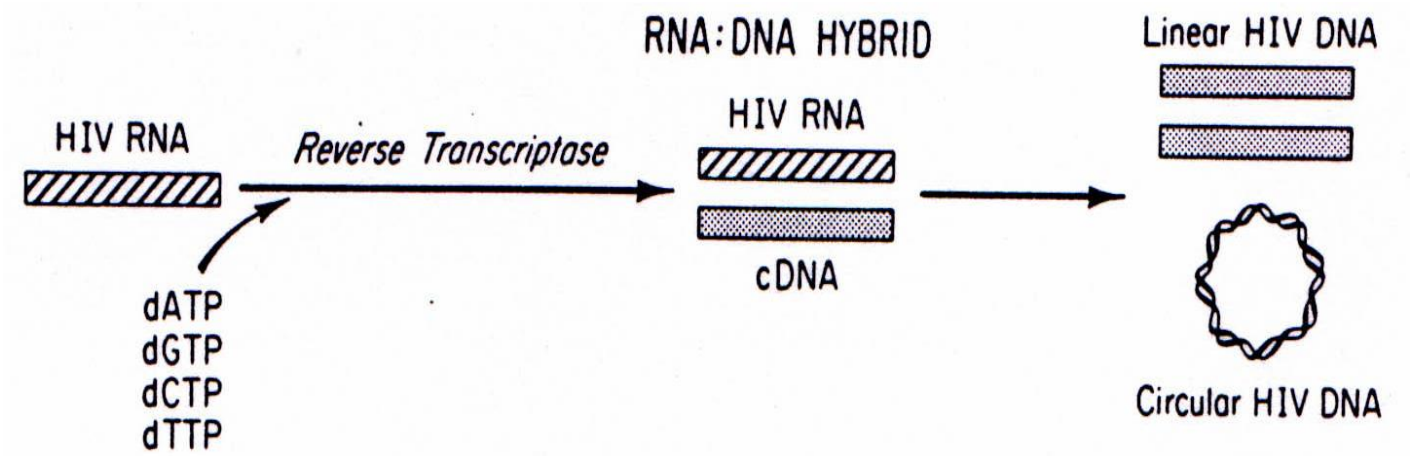
Azidothymidine

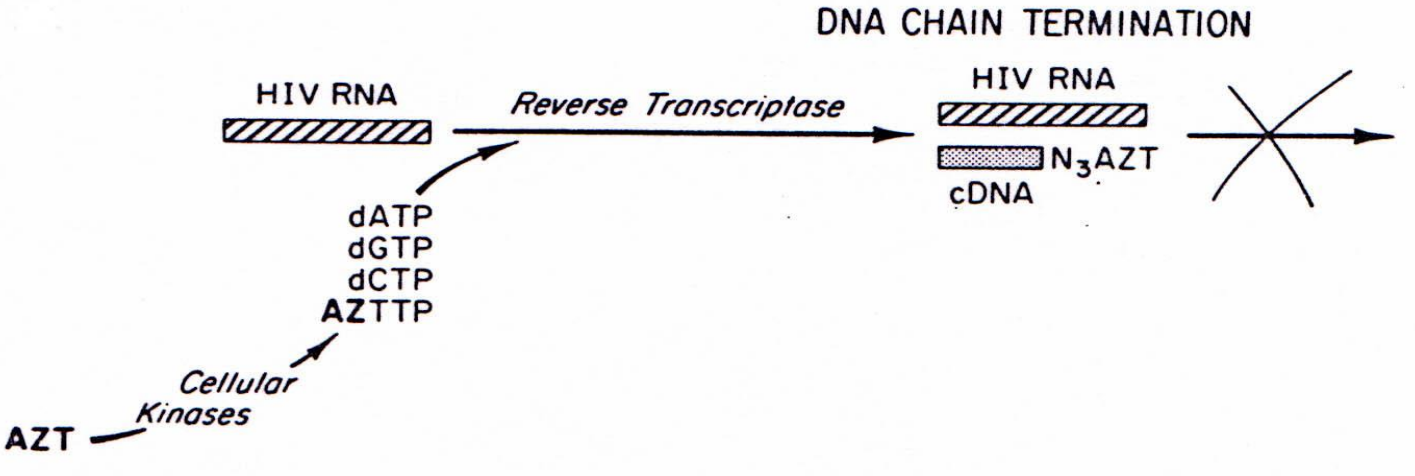
Thymidine Analog

(Retrovir, Zovirax, Zidovudin; AZT)



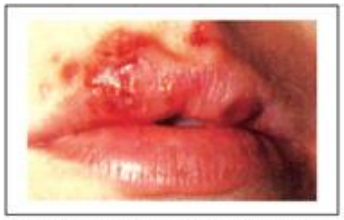




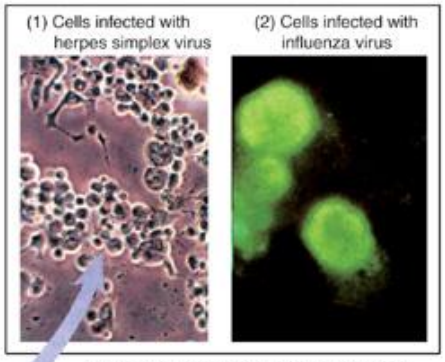


Examples of Antiviral Compounds Used for Treatment of Viral Infections

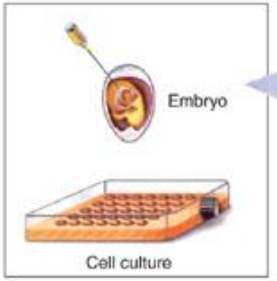
Drug	Nucleoside Analog	Mechanism of Action	Viral Spectrum
Acyclovir	Yes	Viral polymerase inhibitor	Herpes simplex, varicella-zoster
Amantadine	No	Blocks viral uncoating	Influenza A
Boceprevir	No	HCV protease inhibitor	HCV genotype 1
Cidofovir	No	Viral polymerase inhibitor	Cytomegalovirus, herpes simplex, polyomavirus
Didanosine (ddI)	Yes	Reverse transcriptase inhibitor	HIV-1, HIV-2
Entecavir	Yes	Reverse transcriptase inhibitor	HBV
Foscarnet	No	Viral polymerase inhibitor	Herpesviruses, HIV-1, HBV
Fuzeon	No	HIV fusion inhibitor (blocks viral entry)	HIV-1
Ganciclovir	Yes	Viral polymerase inhibitor	Cytomegalovirus
Indinavir	No	HIV protease inhibitor	HIV-1, HIV-2
Lamivudine (3TC)	Yes	Reverse transcriptase inhibitor	HIV-1, HIV-2, HBV
Lopinavir	No	HIV protease inhibitor	HIV-1
Maraviroc	No	Entry inhibitor (blocks binding to CCR5)	HIV-1
Nevirapine	No	Reverse transcriptase inhibitor	HIV-1
Oseltamivir	No	Viral neuraminidase inhibitor	Influenza A and B
Raltegravir	No	Integrase inhibitor	HIV-1
Ribavirin	Yes	Perhaps blocks capping of viral mRNA	Respiratory syncytial virus, influenza A and B, Lassa fever, hepatitis C, others
Ritonavir	No	HIV protease inhibitor	HIV-1, HIV-2
Saquinavir	No	HIV protease inhibitor	HIV-1, HIV-2
Stavudine (d4T)	Yes	Reverse transcriptase inhibitor	HIV-1, HIV-2
Trifluridine	Yes	Viral polymerase inhibitor	Herpes simplex, cytomegalovirus, vaccinia
Valacyclovir	Yes	Viral polymerase inhibitor	Herpesviruses
Vidarabine	Yes	Viral polymerase inhibitor	Herpesviruses, vaccinia, HBV
Zalcitabine (ddC)	Yes	Reverse transcriptase inhibitor	HIV-1, HIV-2, HBV
Zidovudine (AZT)	Yes	Reverse transcriptase inhibitor	HIV-1, HIV-2, HTLV-1



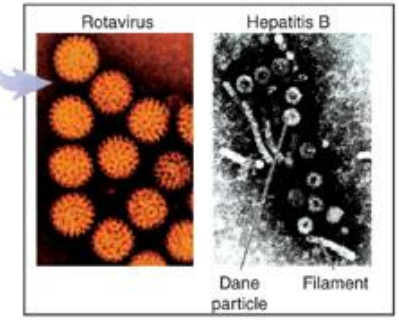
Signs and symptoms: Patient is observed for manifestations of typical virus infections. This is herpes simplex, type 1.



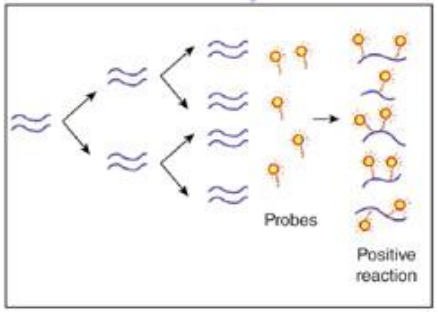
Cells taken from patient are examined for evidence of viral infection, such as cytopathic effects *(1)* or virus antigen detected by fluorescent staining *(2)*.



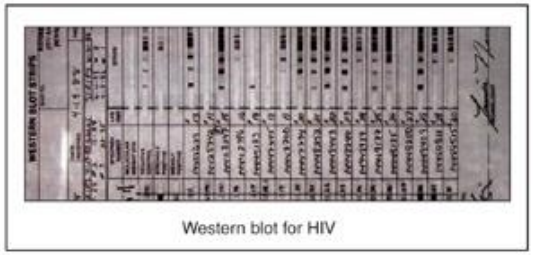
Culture techniques: Viruses require a living host to multiply.



Electron microscope is used to view virus directly. Viruses are sufficiently unique in structure that they can be differentiated to family or genus.



Genetic analysis (PCR): Detection of viral nucleic acid using specific probes.



Western blot for HIV

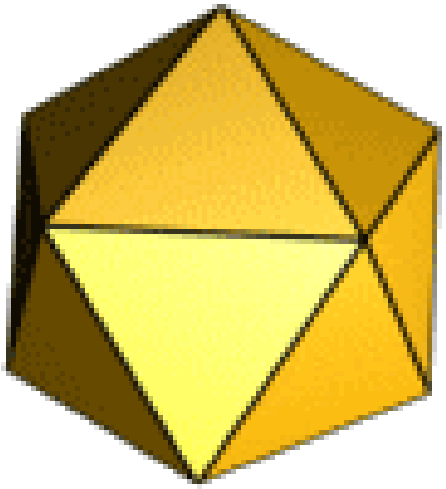
Serological testing for antibodies

- Summary of methods used to diagnose viral infections

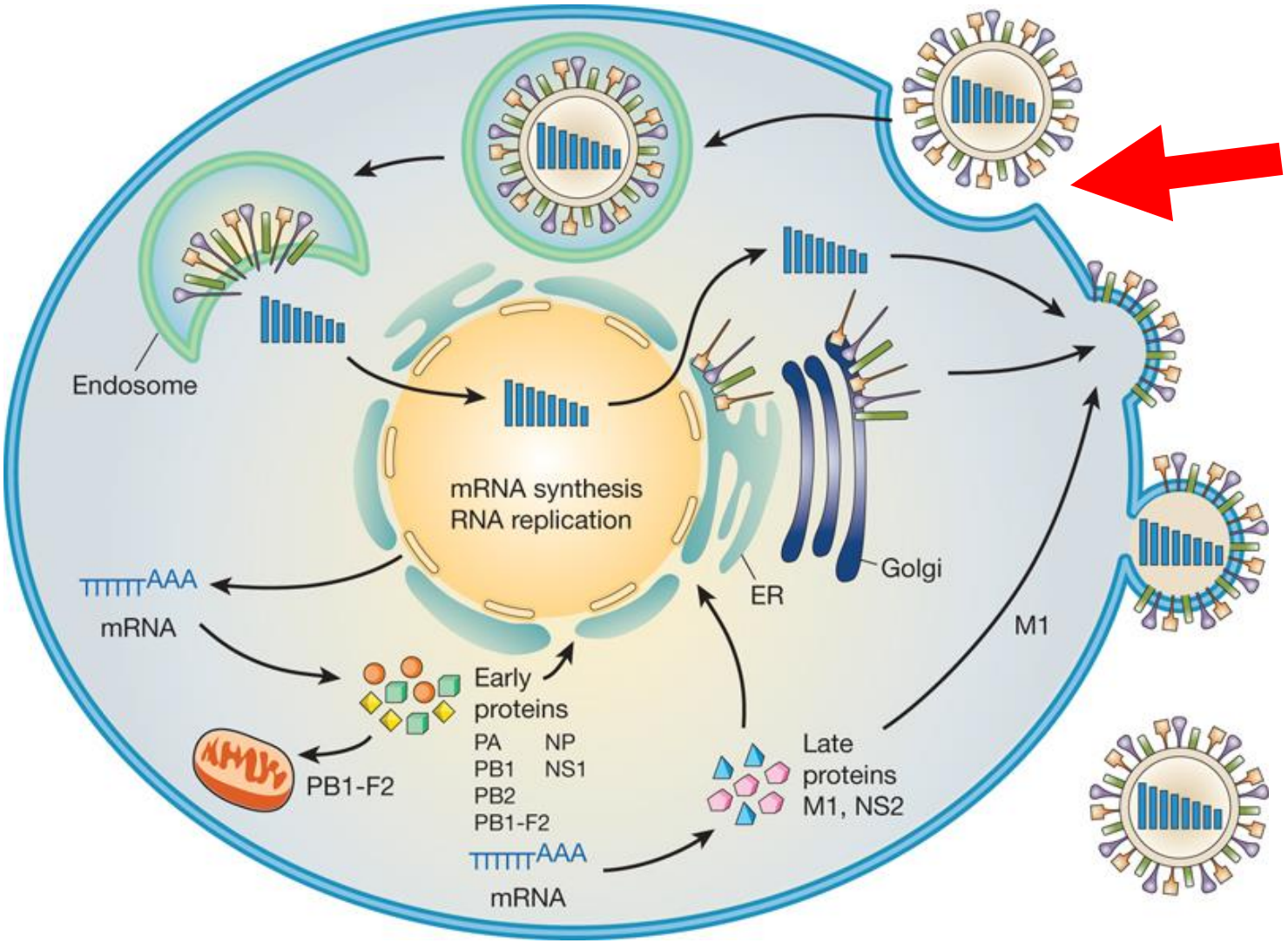
Borba protiv virusa

1918

2009



Vaccination



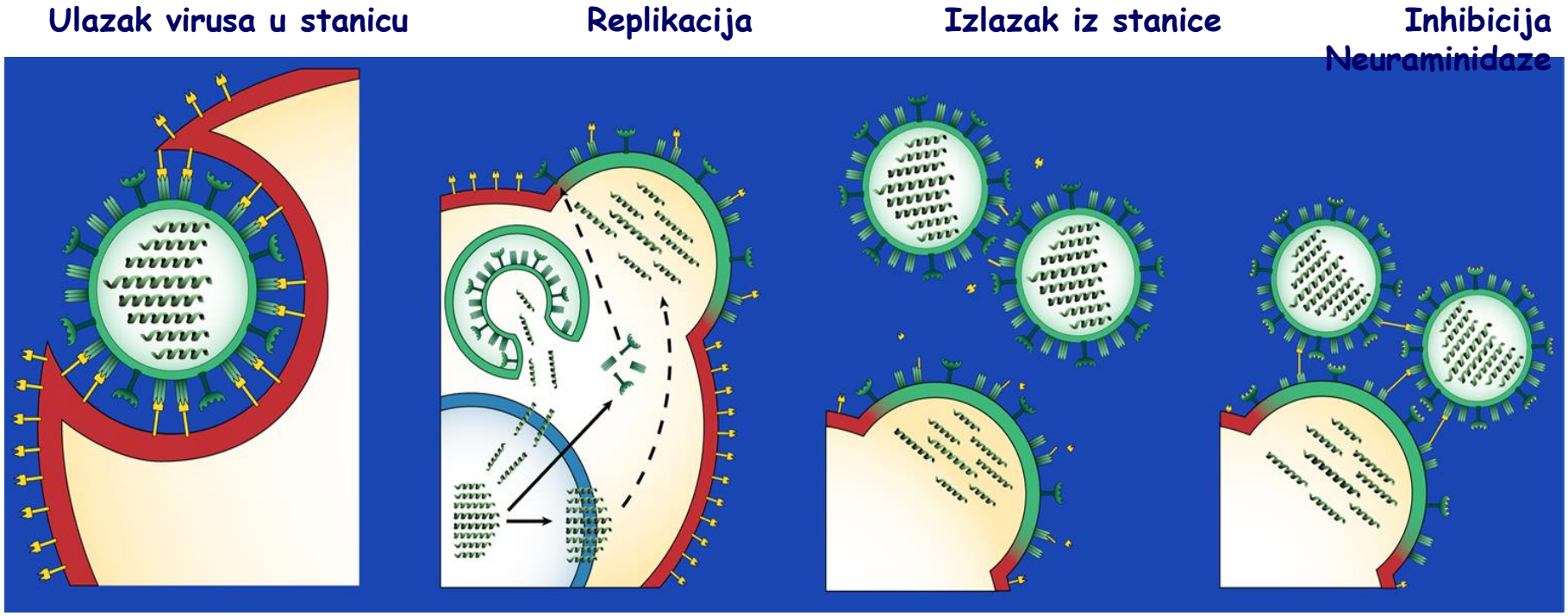
Mjesta djelovanja protuvirusnih lijekova

Ciljno mjesto	Agens	Virus
Adsorpcija	Peptidni analozi virus. receptora neutralizacijska protutijela dekstran sulfat, heparan	HIV gp120/CD4 većina virusa HIV, VHS
Prodor i razgrad. kapsida	Amantadin, rimantadin, tromantadin Arildon, dizoksaril	v. influence A VHS, pikornavirusi
Transkripcija	Interferon Oligonukleotidi	VHA,VHB,VHC,HPV HPV
Sinteza proteina	Interferon	VHB, VHC, HPV
Sinteza DNK (polimeraza)	Analozi nukleozida Fosfonomravlja kis. Fosfonooctena kis.	Herpesvirusi, HIV Herpesvirusi Herpesvirusi
Biosinteza nukleozida	Ribavirin	RSV
Djelovanje na timid.kinazu	Analozi nukleozida	VHS,VZV

Antiretrovirusna terapija

- **Analozi nukleozida**
  - kompetitivni inhibitori reverzne transkriptaze - kompeticija s prirodnim pirimidinima (AZT, d4T) ili purinima (ddI) i inhibicija elongacije lanca NK
- **Nenukleozidni analozi**
  - nekompetitivni inhibitori RT – vezanje na enzim RT i promjena njegove konformacije
- **Inhibitori proteaze**
  - sprječavaju proteolitičko cijepanje i finalno prevođenje (translaciju) produkta neophodnog za slaganje i oslobađanje virusa

INHIBICIJA NEURAMINIDAZE: Idealna terapijska strategija protiv influence



oseltamivir

- Većina protuvirusnih lijekova koji se primjenjuju u kliničkoj praksi su analozi nukleozida i inhibiraju virusne polimeraze
- Većina se tih preparata aktivira fosforilacijom pomoću staničnih ili virusnih kinaza

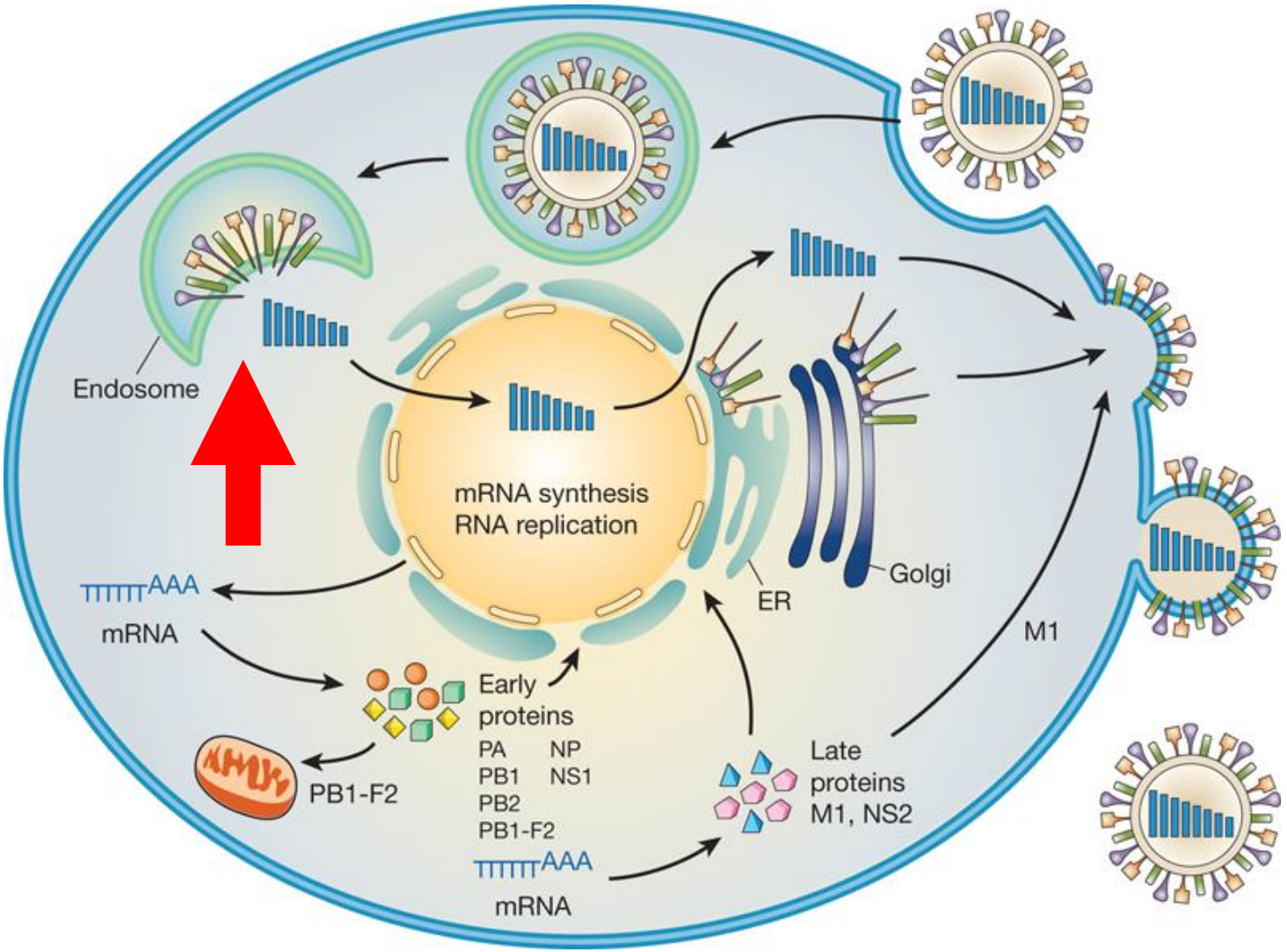
- Am 15.9.09 wurden nun in der USA 4 Impfstoffe gegen Influenza A(H1N1) durch die FDA (Food and Drug Administration) zugelassen. Jetzt, gut 3 Wochen später hat das CDC (Center of Diseases Controle) die zugehörigen Impfemfehlungen konkretisiert. Kurz zusammengefasst: bei den Zielpopulationen sind die Altersbeschränkungen der Impfstoffe zu berücksichtigen. Ab dem 10. Lebensjahr reicht eine Impfung!

Emea (Europa)			
Name	Pandemrix®	Focetria®	Celvapan®
Hersteller	GKS	Novartis	Baxter
Herstellung	§	§	Vero-Zellen
Antigendosis	3.75mcg/0.5ml	7.5mcg/0.5ml°	7.5mcg/0.5ml
Adjuvanz	AS03	MF59C.1	kein
Impfstofftyp	inaktiviert	inaktiviert	inaktiviert
Alter	ab 6. Monat#	ab 6. Monat	ab 6. Monat
Schema	2x*	2x*	2x*

* 0, 4 Wo, alle Alter
§ Hühner-Embryonen
# bis 10. LJ, 2x halbe Dosis (0.25ml)
° Hämagglutinin und Neuramindase

- Die europäische Arzneimittelbehörde EMEA hat inziwschen 3 Impfstoffe zugelassen. Dies ist Pandemrix(R), Celvapan(R) und Focetria(R). Alle Impfungen sind als 2-Dosen-Schema vorgesehen. Gemäss Angaben der EMEA haben gut 10% allgemeine Nebenwirkungen Lokalreaktionen, Kopfschmerzen oder Myalgien. Damit ist die Sicherheit ähnlich den Impfstoffen gegen die saisonale Influenza.

M2-inhibitori



Rezistencija na M2-inhibitore

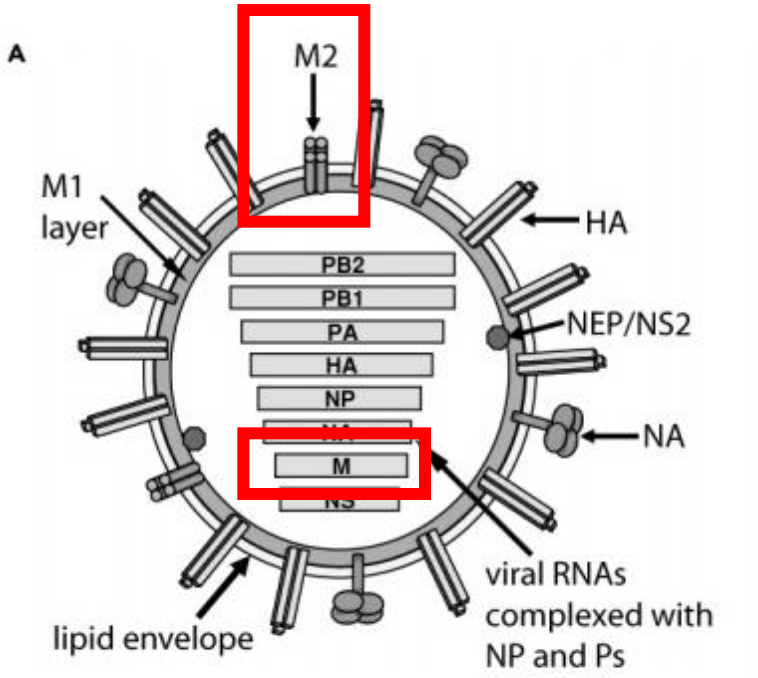
- 1994-1995

  0.4%

- 2003-2004

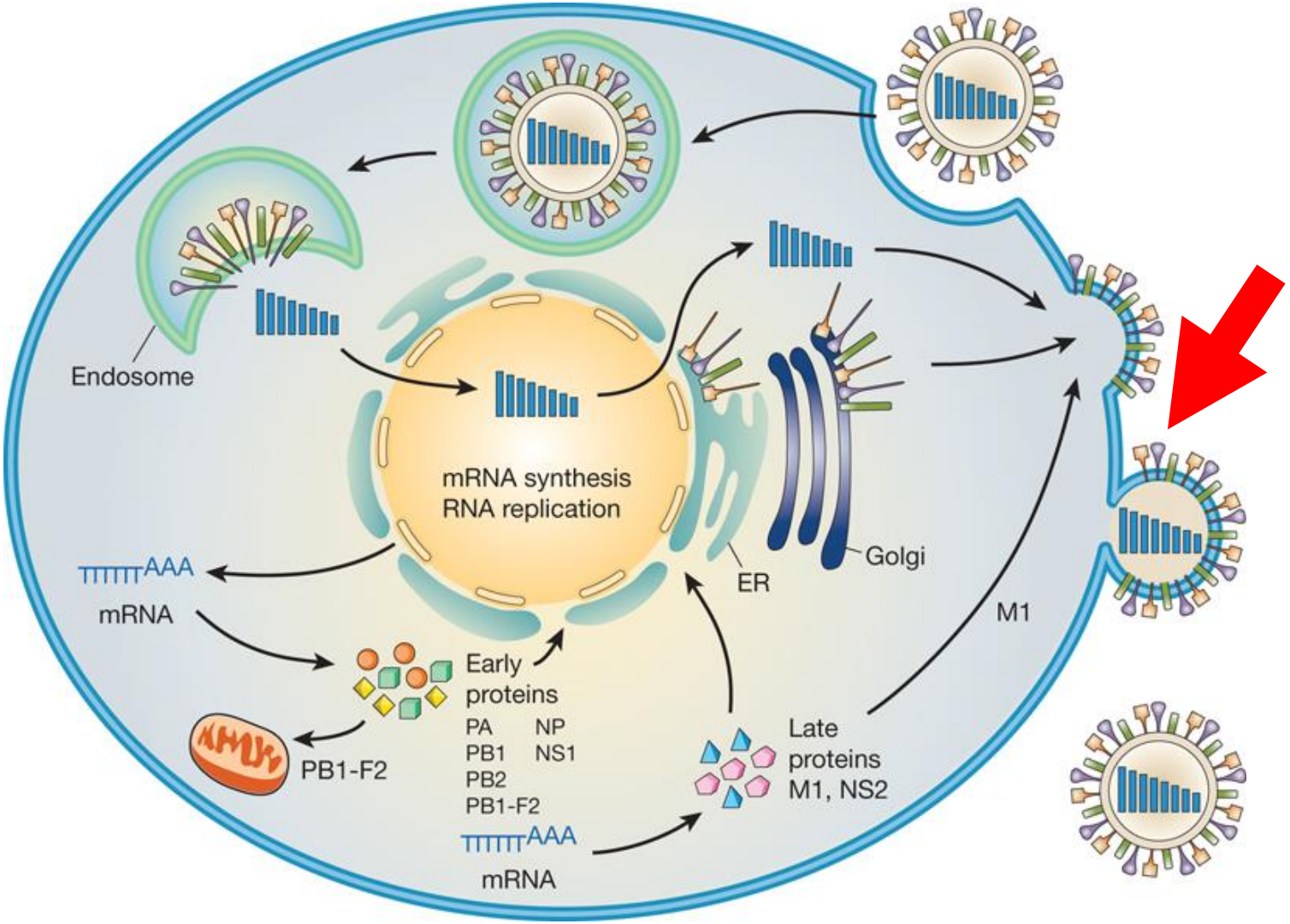
  12.3%

Rezistencija na M2-inhibitore

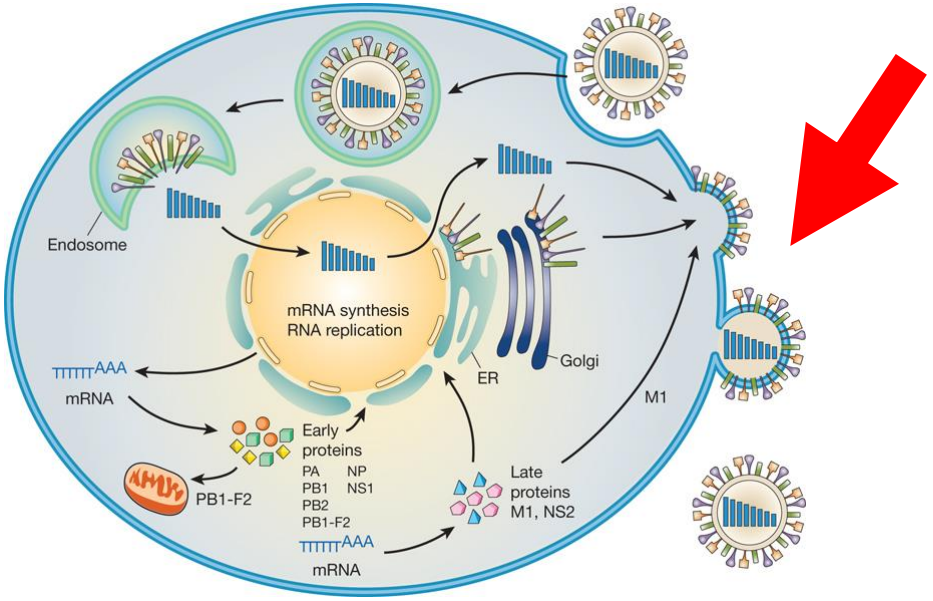


- **Mutacija u M2-genu**
- **Već nakon 2-3 dana od početka terapije 30% virusa je rezistentno**

Inhibitori neuraminidaze



Inhibitori neuraminidaze



- Oseltamivir = Tamiflu
- Zanamivir = Relenza

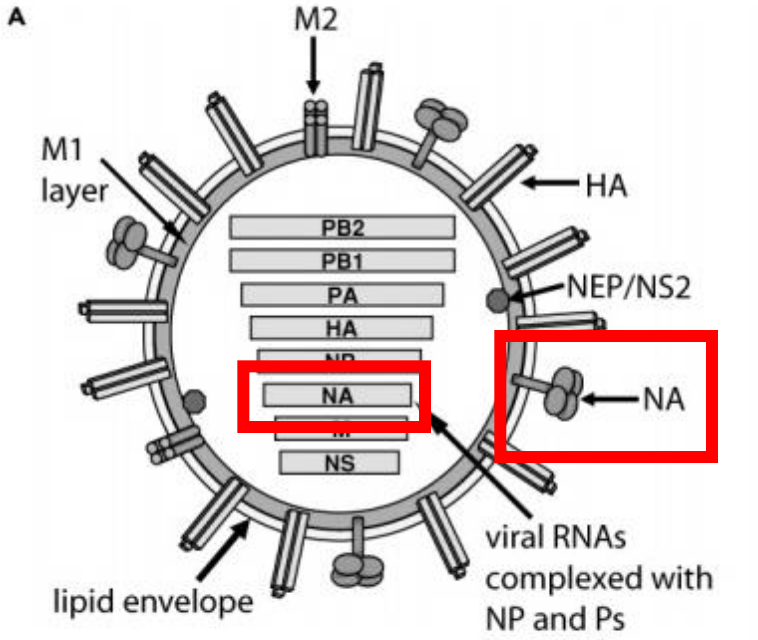
Rezistencija na Tamiflu

- Prirodna rezistencija
  - odrasli – 0.4%
  - djeca - 5.4%
- U 2007. i 2008.
  - **Rezistencija od 15% u neterapiranih osoba u cijelom svijetu**

Rezistencija na Tamiflu nakon terapije

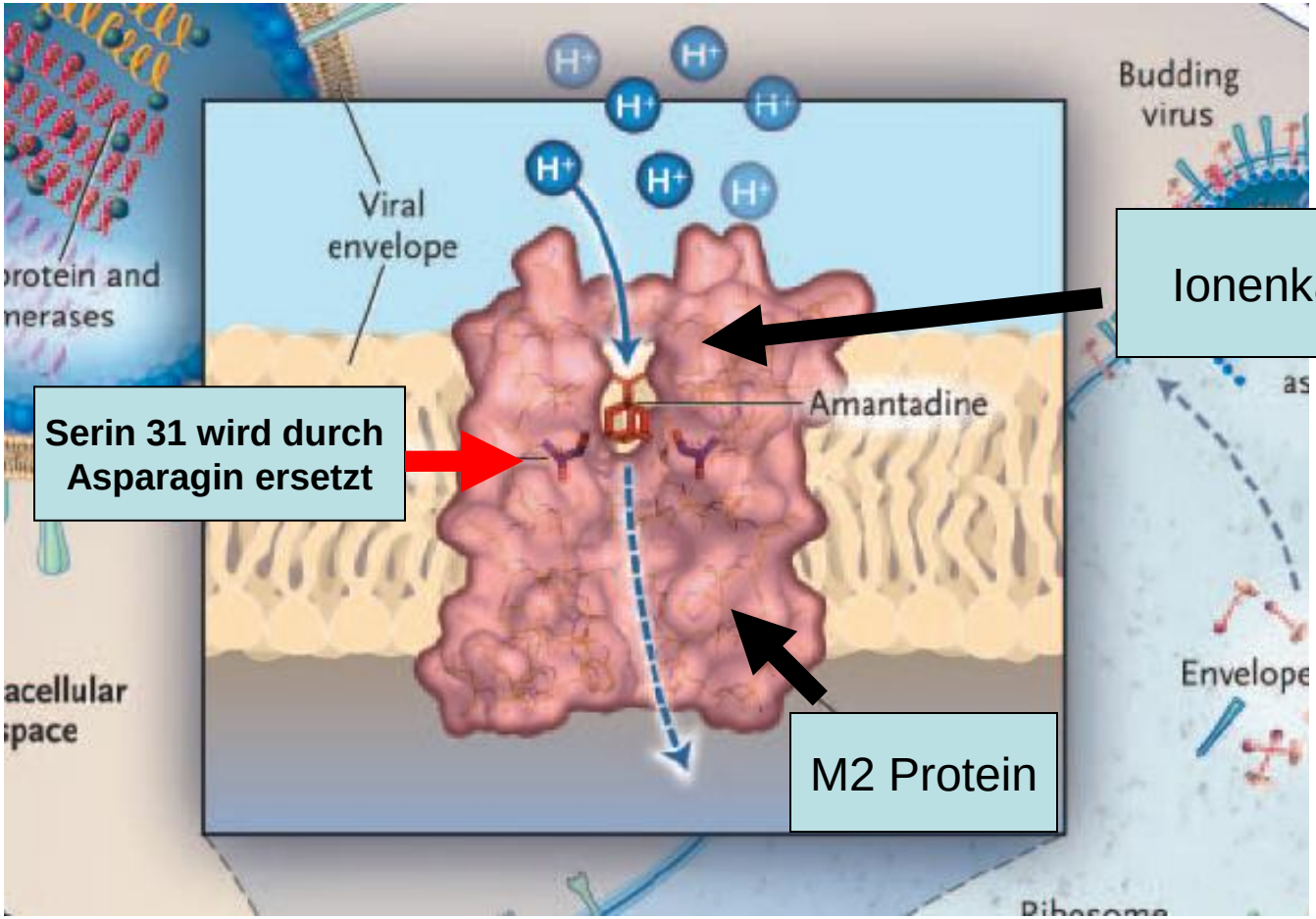
- „Outpatients“ - **1%**
- djeca - **4%**
- Hospitalizirana djeca - **18%**
- Osobe inficirane Influencom A (H5N1) neposredno nakon terapije - **25%**

Rezistencija na Tamiflu

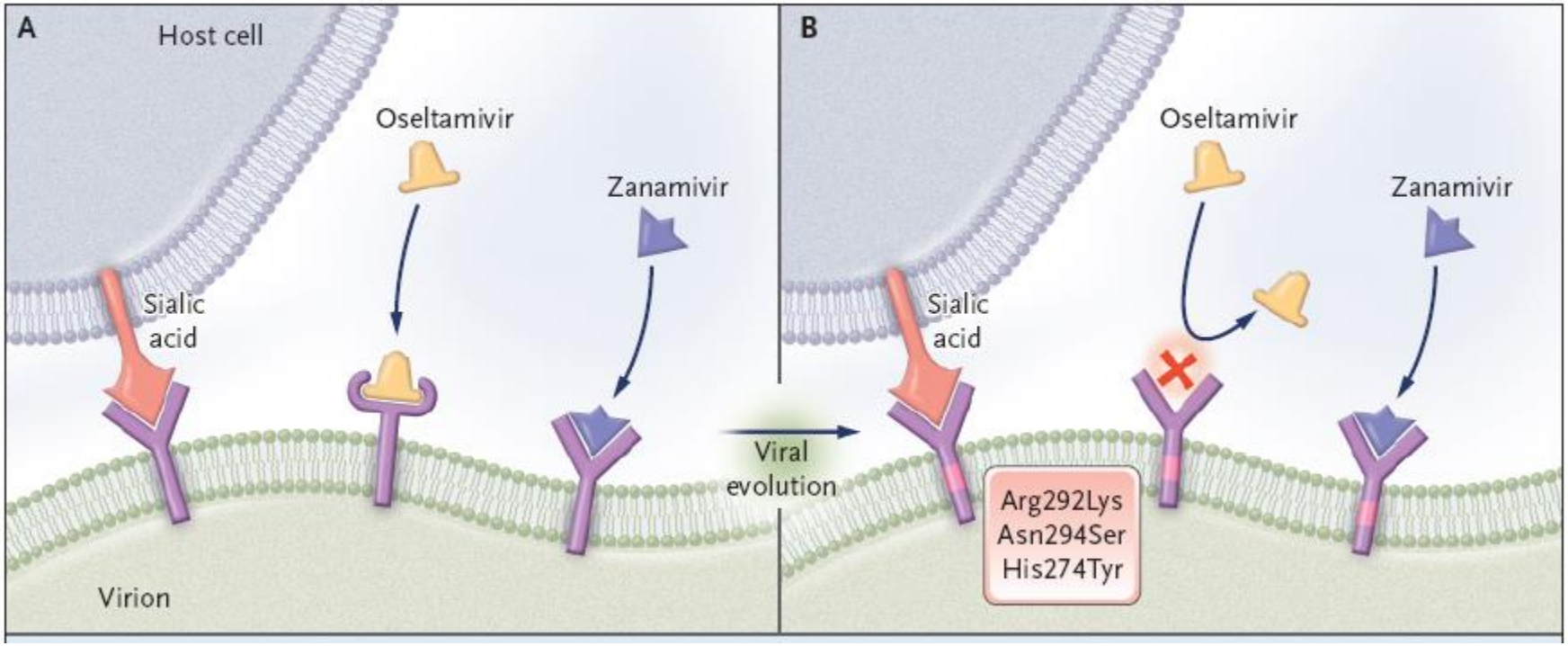


- **Mutacija u NA-genu**

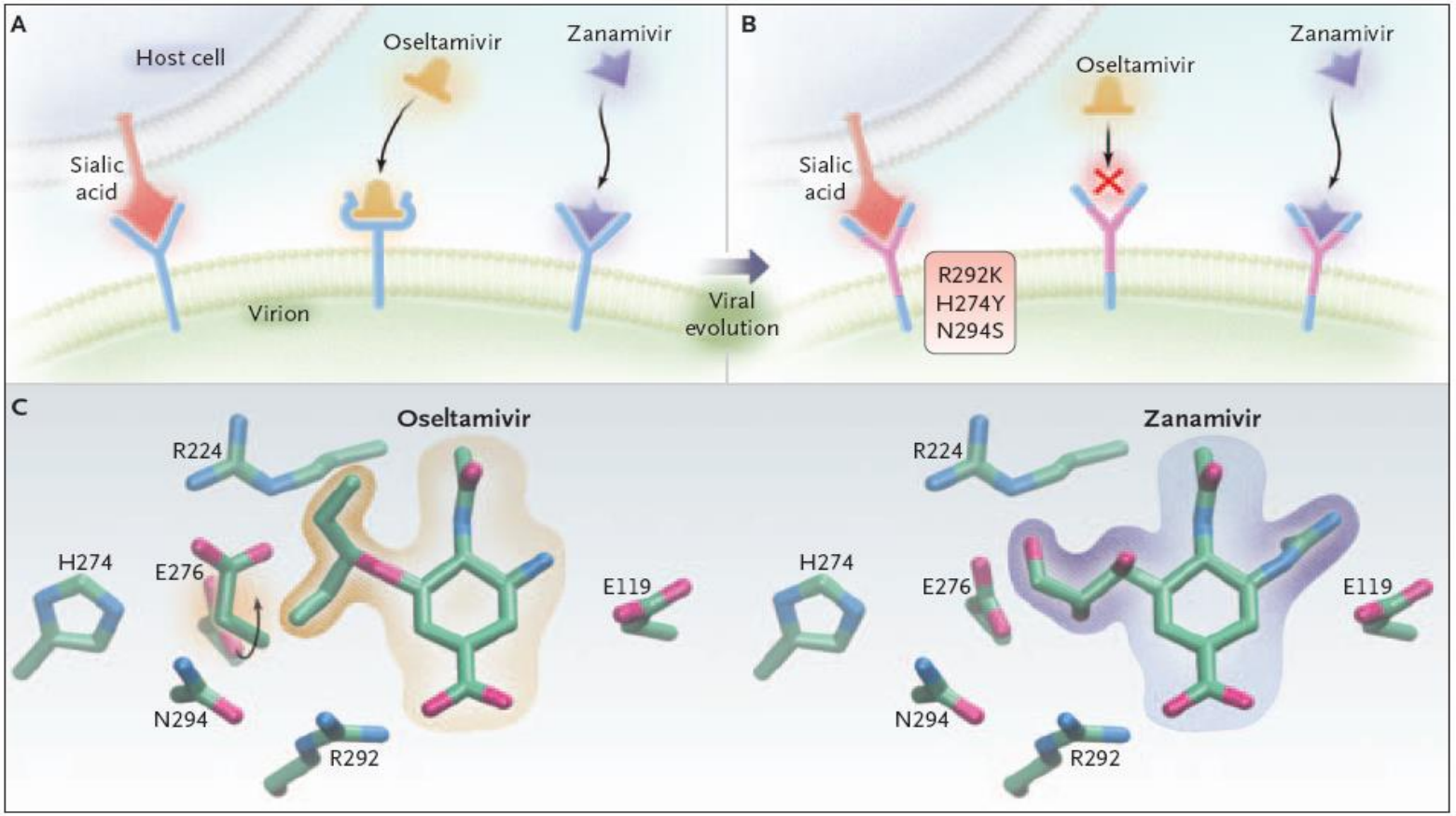
Rezistencija na Amantadin



Mutacija


A
Host cell
Oseltamivir
Zanamivir
Sialic acid
Virion
Viral evolution
B
Oseltamivir
Zanamivir
Sialic acid
Arg292Lys
Asn294Ser
His274Tyr

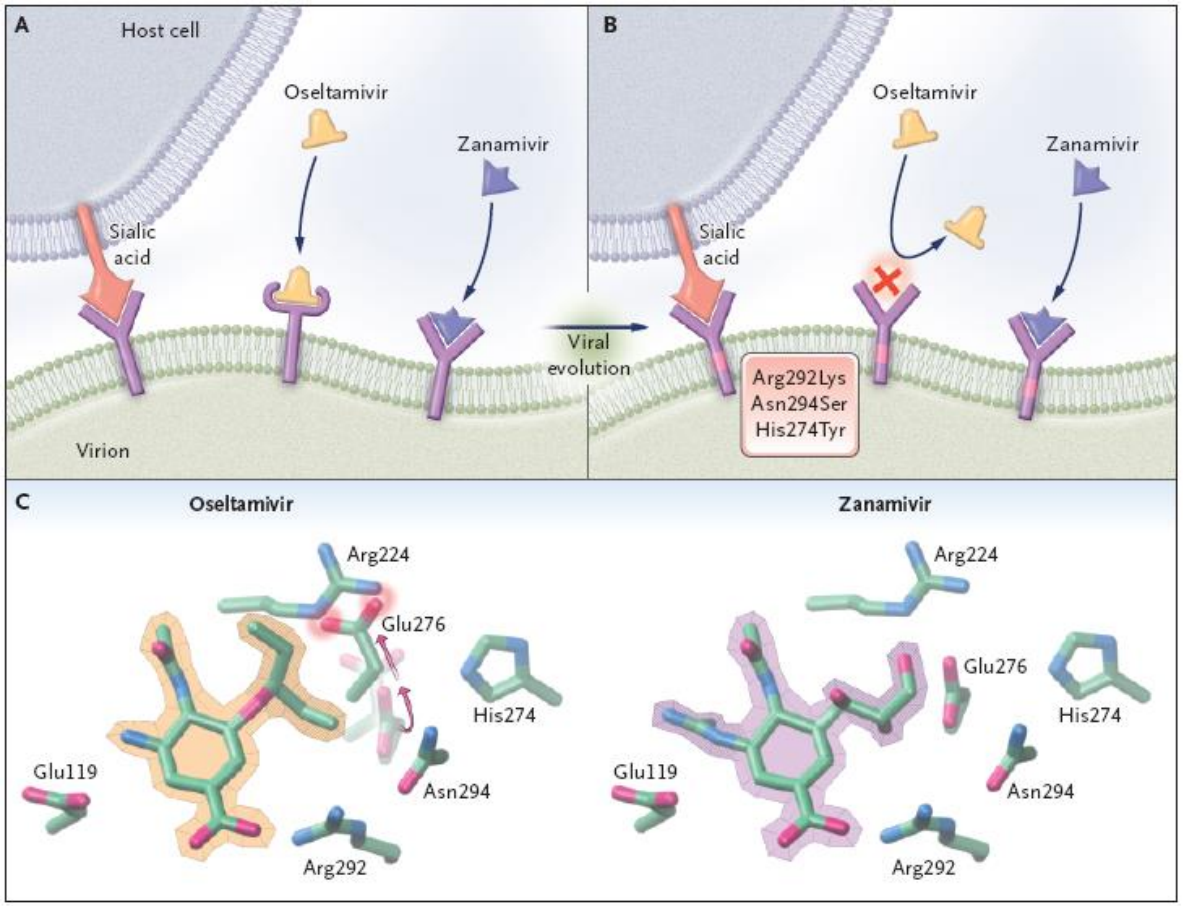
Oseltamivir (Tamiflu) - Rezistencija



Mechanism of Resistance to Oseltamivir.

The neuraminidase active site changes shape to create a pocket for oseltamivir, whereas it accommodates zanamivir without such a change (Panel A). Any of several mutations may prevent the binding of oseltamivir by preventing the formation of this pocket (Panel B); the oseltamivir-resistant virus can nonetheless bind to the host-cell sialic acid receptor and to zanamivir. The pocket for oseltamivir, illustrated by key amino acids in Panel C, is created by the rotation of E276 and bonding of the amino acid to R224 — events that are prevented by the mutations R292K, N294S, and H274Y and therefore result in resistance to oseltamivir. An E119V mutation may permit the binding of a water molecule in the space created by the smaller valine, also interfering with oseltamivir binding. None of these mutations prevent the binding of zanamivir or of the natural sialic acid substrate.

Oseltamivir (Tamiflu) - Rezistencija

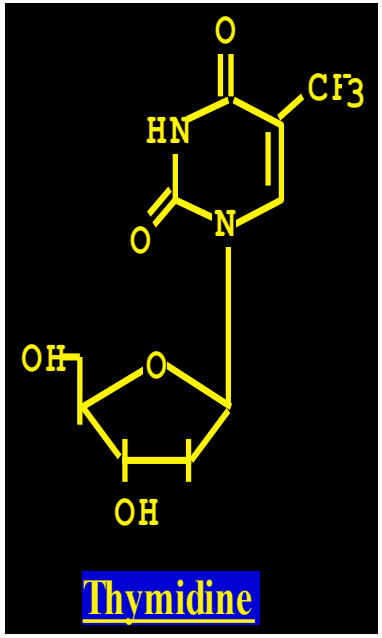


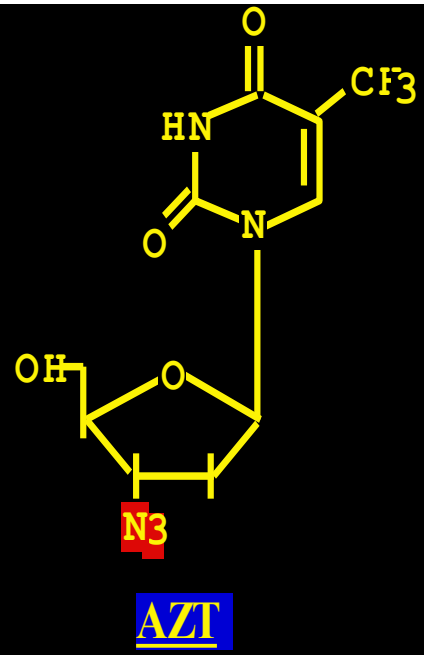
Mechanism of Development of Resistance to Oseltamivir.

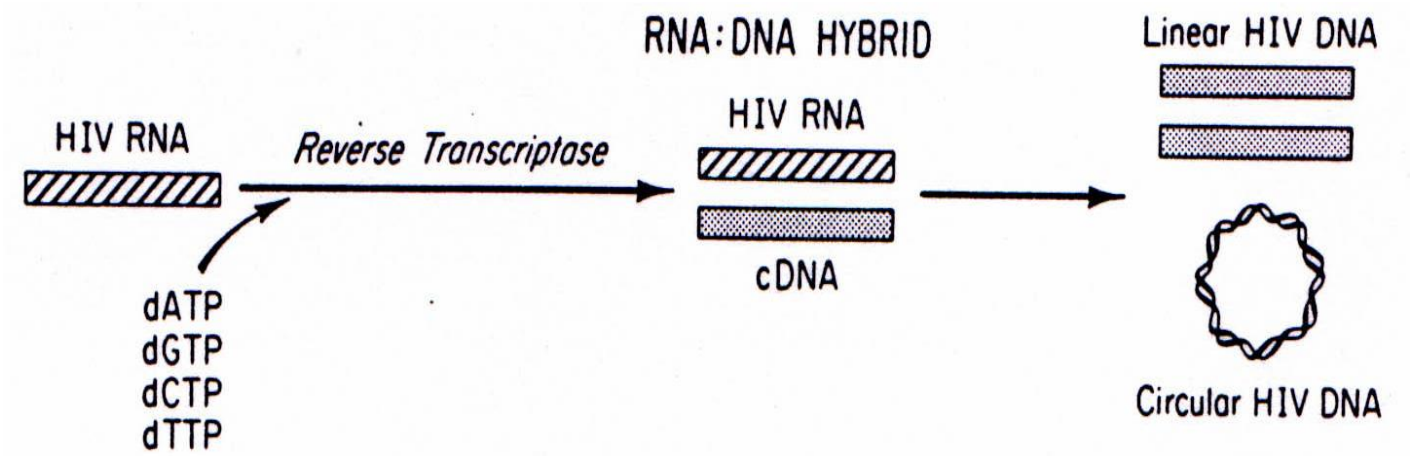
Binding of oseltamivir, but not zanamivir, to the neuraminidase active site requires a shape change that creates a pocket (Panel A), and mutations that prevent formation of this pocket may prevent binding of oseltamivir but permit binding of zanamivir (Panel B). Crystal structure analysis shows that binding of oseltamivir requires a rotation of the Glu276 residue away from the hydrophobic pentyloxy group of oseltamivir, which then makes hydrophobic contact with a methylene group on Glu276 (Panel C).[2] However, binding of either sialic acid (the natural substrate) or zanamivir does not require a change in the position of Glu276. The His274Tyr substitution in the neuraminidase active site pushes the Glu276 farther into the binding site and disrupts the hydrophobic pocket that is required only for oseltamivir binding.

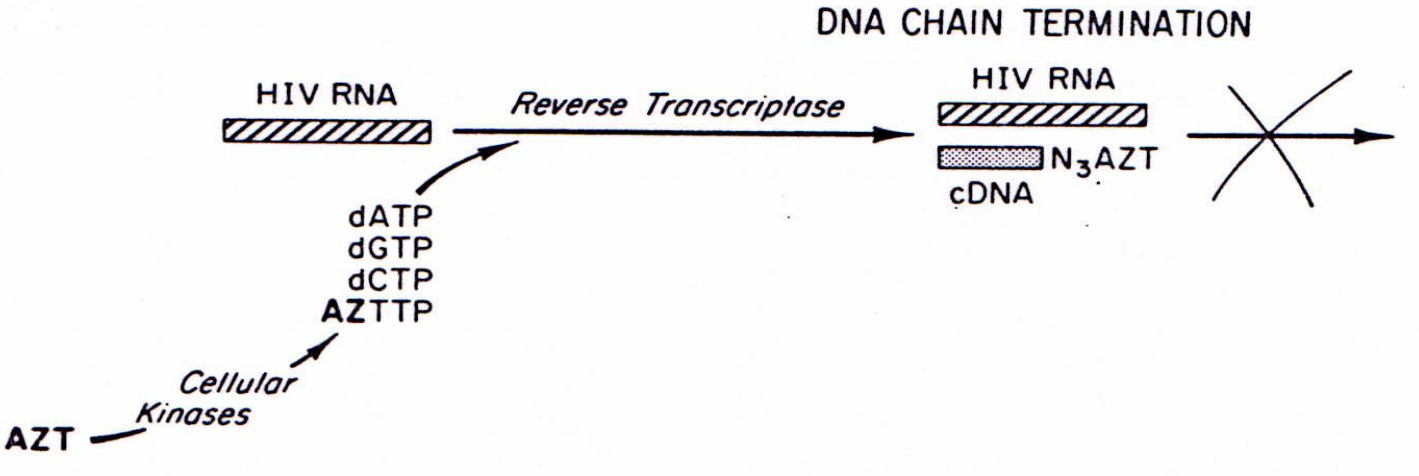
Azidotimidin

Analog nukleozida timidina (Retrovir, Zovirax, Zidovudin; AZT)





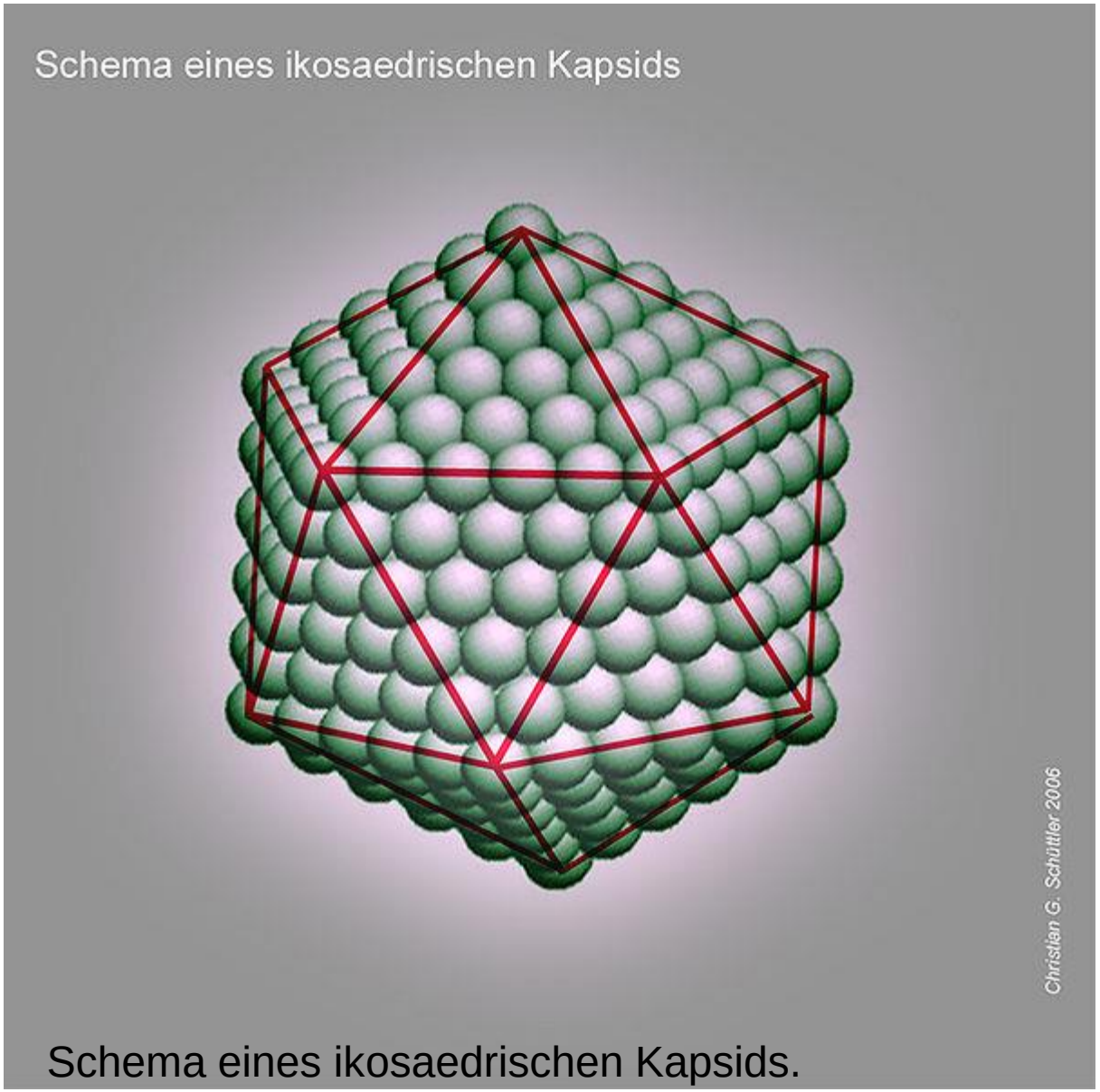




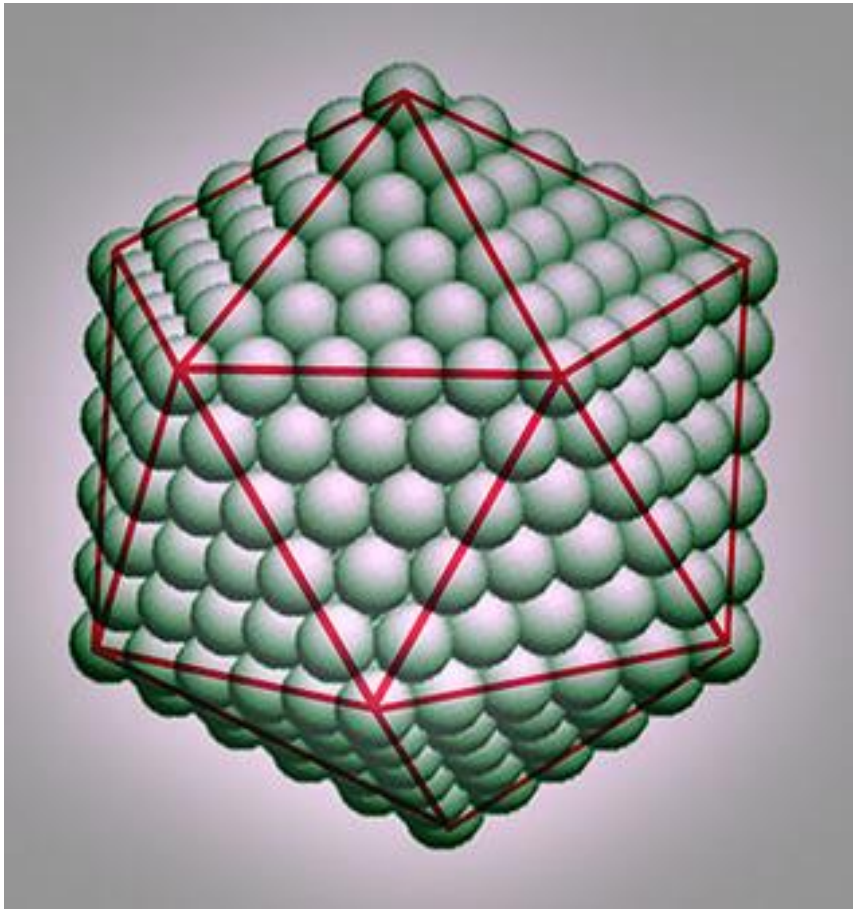
Antiretrovirusna terapija

- **Analozi nukleozida**
  - kompetitivni inhibitori reverzne transkriptaze - kompeticija s prirodnim pirimidinima (AZT, d4T) ili purinima (ddI) i inhibicija elongacije lanca NK
- **Nenukleozidni analozi**
  - nekompetitivni inhibitori RT – vezanje na enzim RT i promjena njegove konformacije
- **Inhibitori proteaze**
  - sprječavaju proteolitičko cijepanje i finalno prevođenje (translaciju) produkta neophodnog za slaganje i oslobađanje virusa

- Zur genaueren Beschreibung eines ikosaedrischen Kapsids, wurde 1962 von *Donald Caspar* und *Aaron Klug* eine geometrische Kennzahl eingeführt,[3] die sogenannte Triangulationszahl (T). Mit ihr kann man die Größe und die Komplexität eines Kapsids beschreiben.
  Durch Zusammenlagerung von drei identischen Molekülen eines beliebigen unregelmäßigen, nicht-symmetrischen Proteins kann ein gleichseitiges (rotationssymmetrisches) Dreieck gebildet werden. Diese Anordnung ist die kleinstmögliche symmetrische Einheit zur Ausbildung eines ikosaedrischen Kapsids. Da ein solches regelmäßiges Dreieck also aus mindestens drei Untereinheiten aufgebaut ist und ein Ikosaeder aus zwanzig solcher regelmäßiger Dreiecke besteht, sind mindestens 3x20=60 solcher Untereinheiten zur Ausbildung der einfachsten ikosaedrischen Symmetrie notwendig. Diese Mindestzahl von 60 wird durch die Triangulationszahl T=1 beschrieben. Größere und komplexere Kapside besitzen nur ganzzahlige Vielfache von 60, also z. B. häufig 180 (T=3), 240 (T=4), 960 (T=16). Die geometrisch möglichen Triangulationszahlen ergeben sich aus der Formel $T=h^2 + hk + k^2$, wobei h und k ganze Zahlen sind.



Schema eines ikosaedrischen Kapsids.
Die Kugeln entsprechen den einzelnen Kapsomeren



- Shema jedne ikozaedarne kapside
- Kugle odgovaraju pojedinačnim kapsomerama

- Als Kapsomer (pl. *Kapsomere*) bezeichnet man die kleinste regelmäßige Einheit, aus der ein Kapsid aufgebaut ist und die dessen Symmetrie bestimmt. Im einfachsten Fall besteht ein Kapsid aus identischen Kapsomeren, die wiederum nur aus einem Proteinmolekül bestehen. Sehr häufig jedoch besteht ein Kapsomer aus zwei bis fünf verschiedenen Proteinen, die sich zu einem regelmäßigen Kapsid zusammenlagern. Es kann auch aus verschiedenen Kapsomeren aufgebaut sein, z. B. bestehen Adenoviren aus zwei unterschiedlichen Kapsomeren (Pentone und Hexone), die wiederum selbst aus verschiedenen Virusproteinen bestehen.
- **Die einzelnen Untereinheiten, aus denen ein Kapsomer wiederum aufgebaut sein kann, werden gelegentlich auch als *Protomere* bezeichnet.**
- Bei einer vorgegebenen Genomsequenz eines Virus können jene Proteine, die das Kapsomer bilden, sehr leicht erkannt werden, da diese in bestimmten Abschnitten eine hohe Konzentration von positiv geladenen bzw. basischen Aminosäuren (Arginin, Lysin, Histidin) beinhalten. Diese basischen Proteindomänen der Kapsidproteine (Coreproteine) sind zur nicht-kovalenten Bindung an die negativ geladene virale Nukleinsäure notwendig, die verpackt werden soll.

Anordnung von drei unregelmäßigen Proteinen zu einer symmetrischen Untereinheit eines Viruskapsids

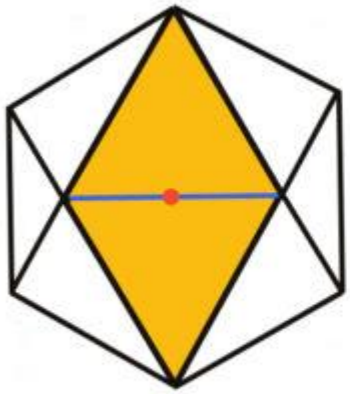
(Punkt ist die Achse der Rotationssymmetrie)

Drei asymmetrische, aber identische Objekte bilden eine Rotationssymmetrie

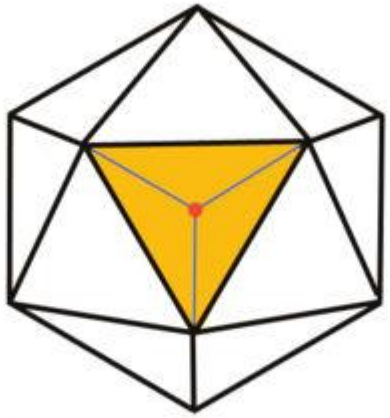
Ikosaedrisches Kapsid mit der Triangulationszahl T=1

Das Kapsid besteht aus 60 Exemplaren von "Felix the Cat" .
Mit dem Kopf bildet er eine 3fache-Symmetrie, mit der Pfote eine 5fache.

Symmetrieachsen bei ikosaedrischen Viruskapsiden

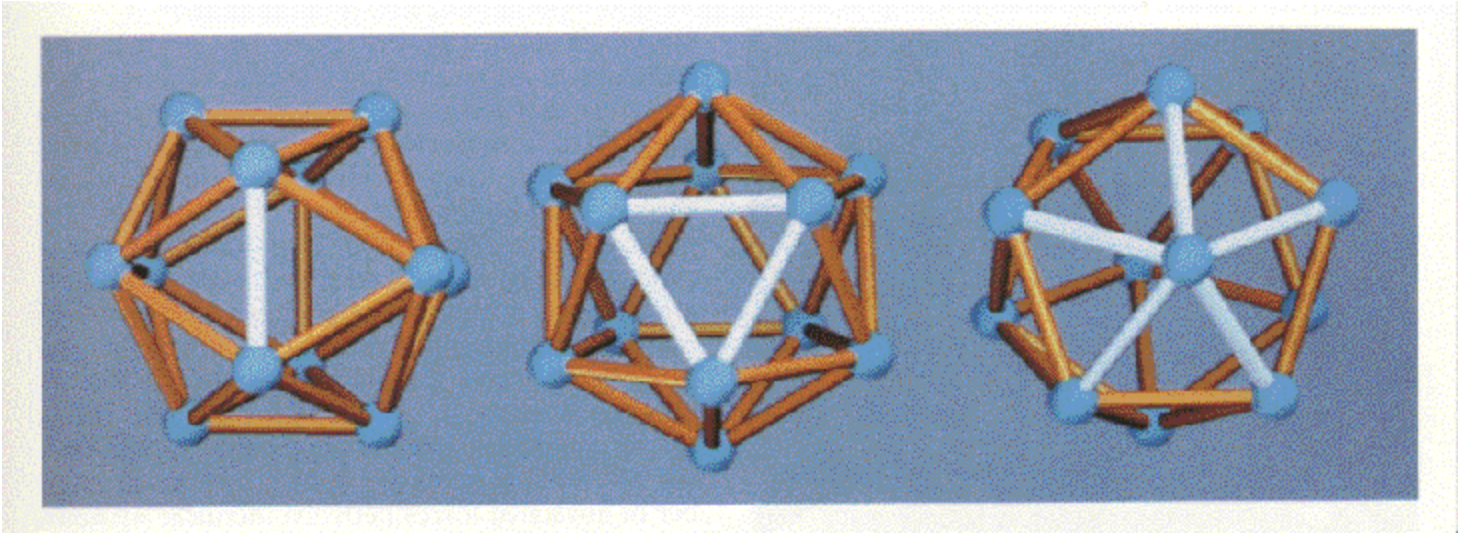


A



B

C



Naziv	Analog	Virus
Aciklovir	guanozina	
Ganciklovir	guanozina	
Adenin-arabinozid	purina	
Azidotimidin	timidina	
Dideoksiinozin i dideoksicitidin		
Ribavirin	guanozina	
Ostali analozi nukleozida		

Ostali antivirusni lijekovi

Naziv	Način djelovanja	Virus
Fosfonomravlja kiselina		
Fosfonooctena kiselina		
Amantadin i rimantadin		
Interferon		