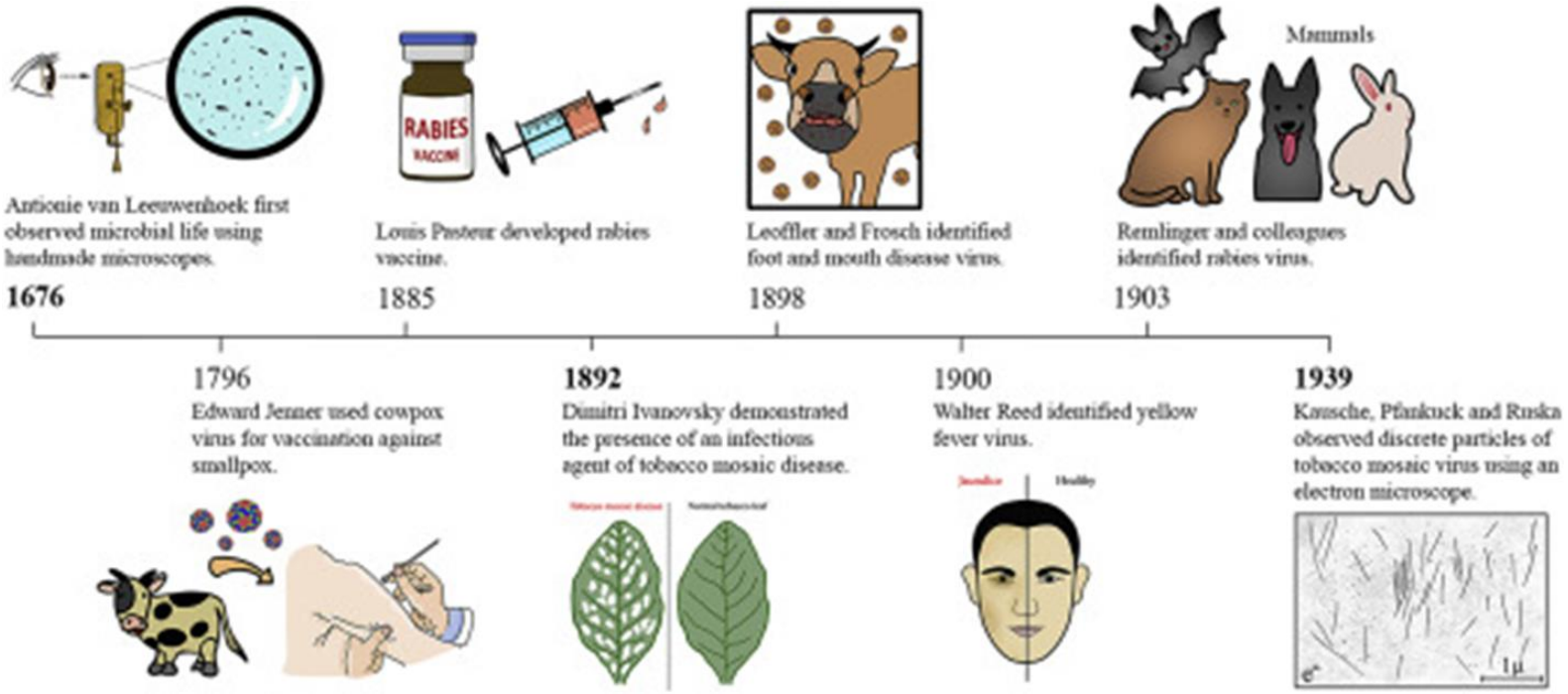
Why we study viruses?

- ✓Viruses Are Everywhere
- ✓Viruses Infect All Living Things
- ✓Viruses Can Cause Diseases
- ✓Viruses Can Cross Species Boundaries
- ✓Viruses Are Uniquely Valuable Tools to Study Biology
- ✓Viruses Can Be Used To Manipulate Biology
- ✓Virus ‘R’ Us (HERV endogenous viruses)

La storia della virologia



Prima descrizione di patologia di origine virale: Vaiolo in Cina nel X secolo a.C. ed alterazioni simil-vaiolose nella mummia del faraone Ramsete V (circa 2000a.C.)

Tentativi di prevenzione e cura di patologie di origine virale in atto prima della scoperta dei virus stessi: Jenner, vaccinazione antivaiolosa alla fine del ‘700 e Pasteur che lavorò sul vaccino per la rabbia alla fine del ‘800

A FEW DATES…

1798 : JENNER ➔ World's first vaccine : smallpox (variola) vaccine

1879-84 : PASTEUR ➔ Extension of the principle of vaccination + first preventive vaccines against different infectious agents (eg Rabies vaccine).

Microbes too small to be observed with light microscopy can cause diseases

1886 : MAYER : tobacco mosaic disease ➔ ***transmission from diseased plants to healthy plants***

1892 : IVANOVSKI ➔ tobacco mosaic disease transmitted by a substance that passed through a filter with pores small enough to retain most bacteria

1898 : BEIJERINCK ➔ tobacco mosaic disease infectious agent could reproduce and multiply in host cells of tobacco plants (➔ ***parasitism, no bacteria)***

Birth of a new life concept = the concept of virus : a novel organism smaller than bacteria and that could multiply only in living cells. (First exemple = **T**obacco **M**osaic **V**irus, **TMV**)

1901: Discovery of the first human virus ➔ **yellow fever virus**

1915-17 : TWORT & d'HERELLE ➔ Discovery of ***BACTERIOPHAGES*** (viruses of bacteria).

1935 : STANLEY ➔ Proteins purification and crystallization of TMV ➔ **TMV composed of proteins…**

1936-7 : BAWDEN et PIRIE ➔ TMV composed of nucleic acids (RNA)…

1939-60 : Electron microscopy, metal shadowing, negative staining… :
first observation of viral particles ➔ determination of the shape, morphology, structure…

etc.

DISCOVERY OF THE TOBACCO MOSAIC VIRUS

Adolf Mayer (1886)

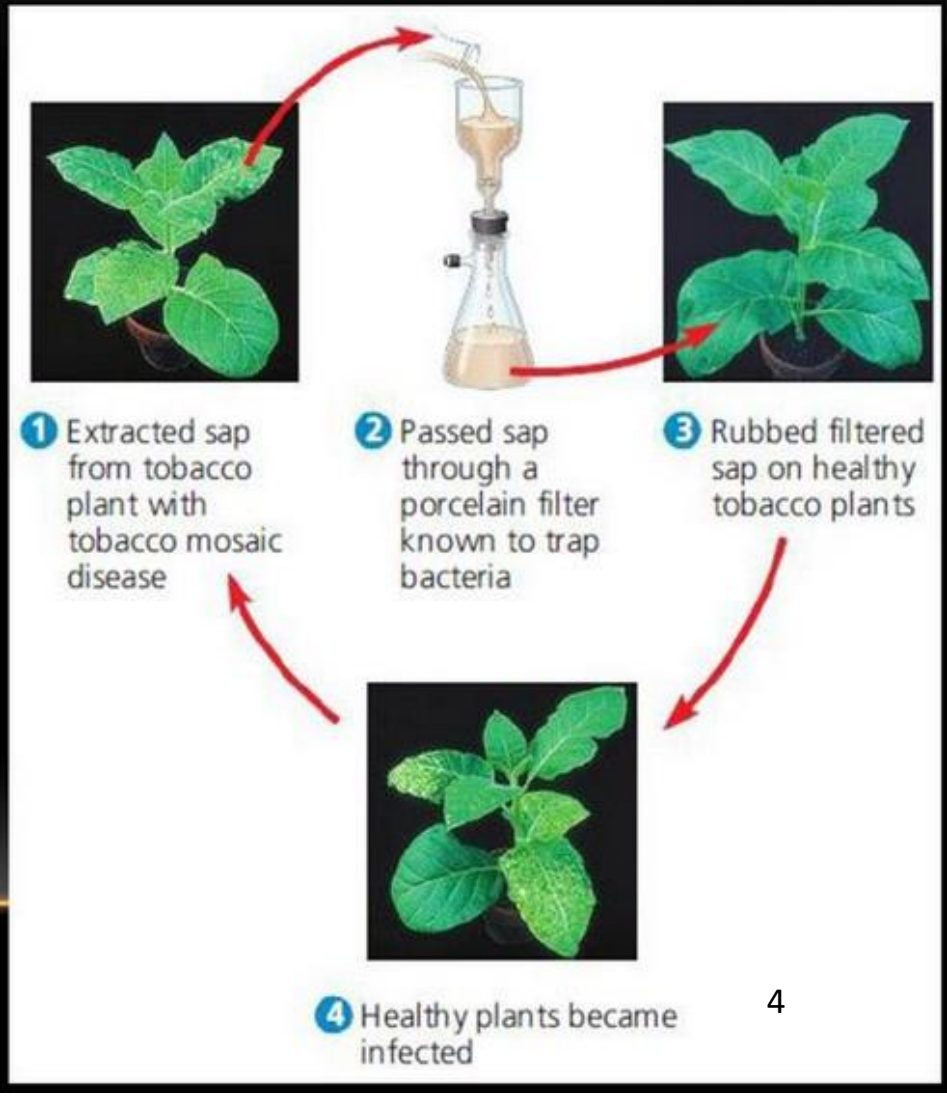
- Tobacco mosaic disease could be transferred between plants

Dmitri Ivanovsky (1892)

- Pathogen is non-bacterial and can pass through fine filters

Martinus Beijerinck (1898)

- Independently replicated Ivanovsky's experiments
- Agent was able to reproduce and multiply in host cells of tobacco plants
- Coined term "virus"

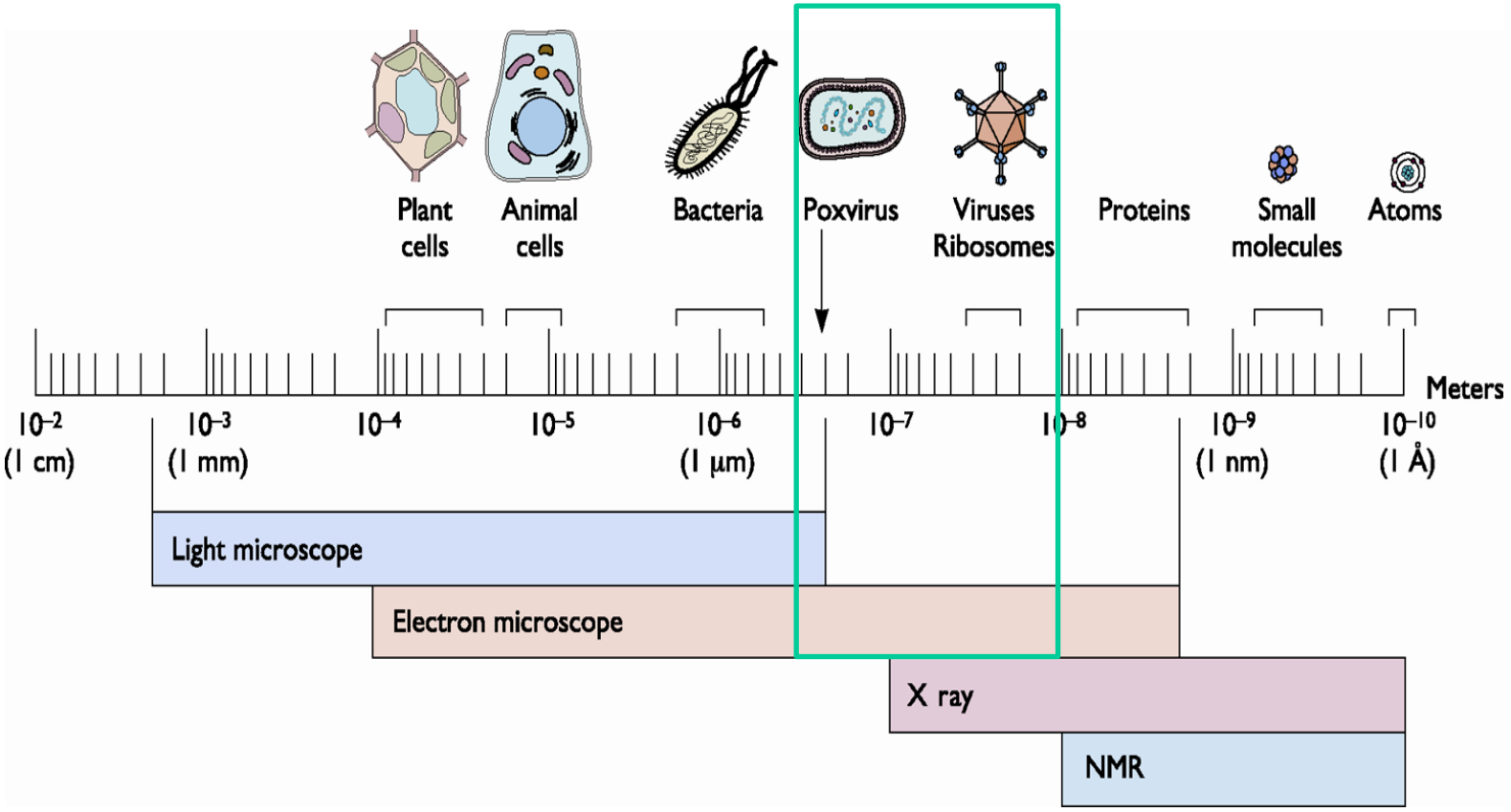


The nature of viruses

- Filterable agents (i.e., very small)
- Contain RNA or DNA but not both
- Obligate intracellular parasites
  - Cannot make energy or proteins independently of a host cell
  - Must encode any required process not provided by the cell
  - Must be infectious to endure in nature
- Viral components are assembled and do not replicate by division

Size of Viruses

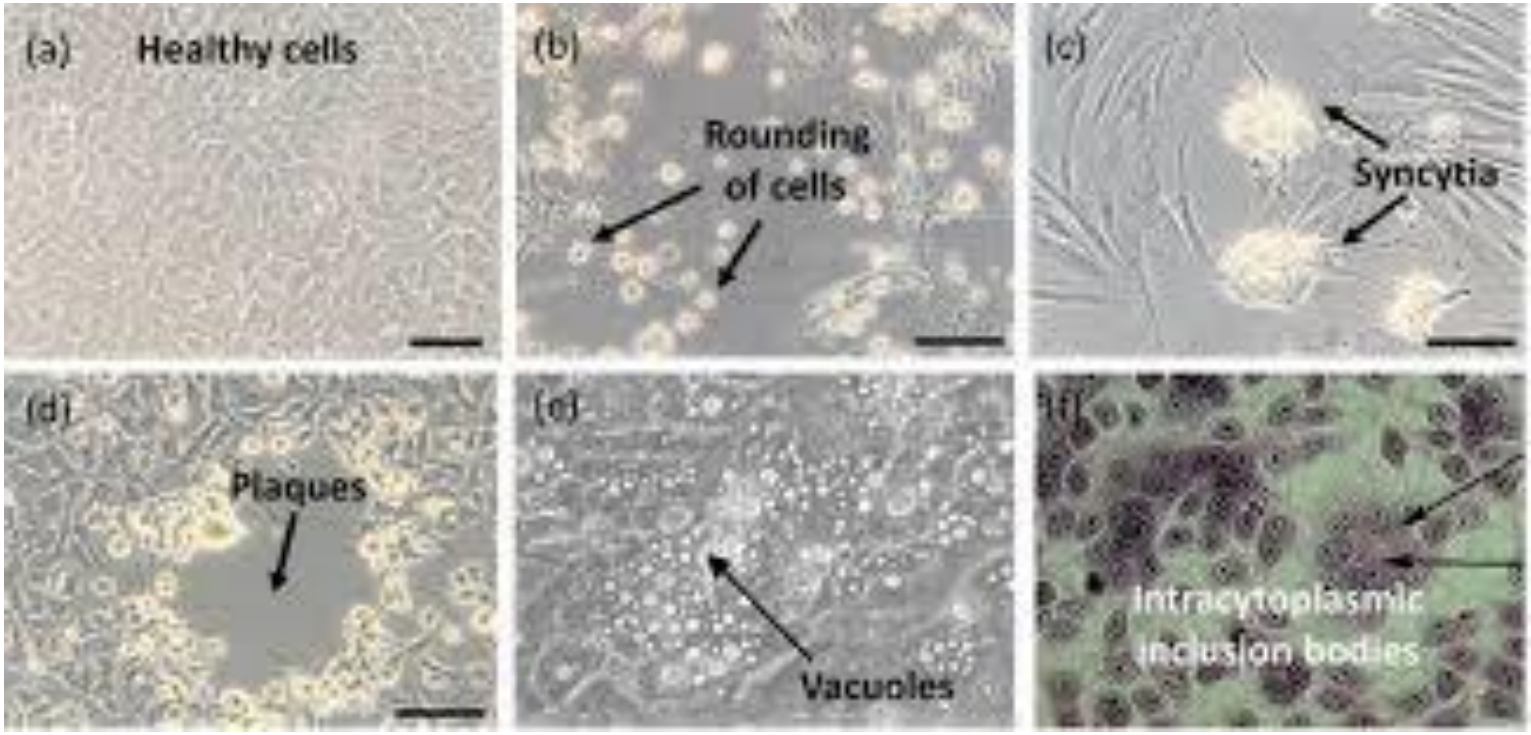
- Sub-microscopic size of 10-400 nm



From Flint et al. *Principles of Virology* (2000), ASM Press

Properties of Viruses

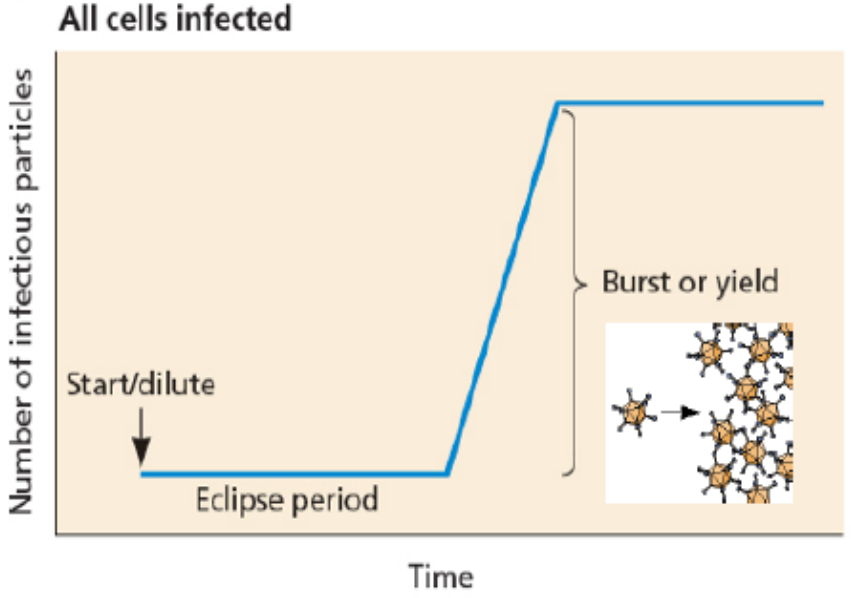
•They can be **cultured in vitro** using isolated eukaryotik cells or bacteria, depending on the virus type.

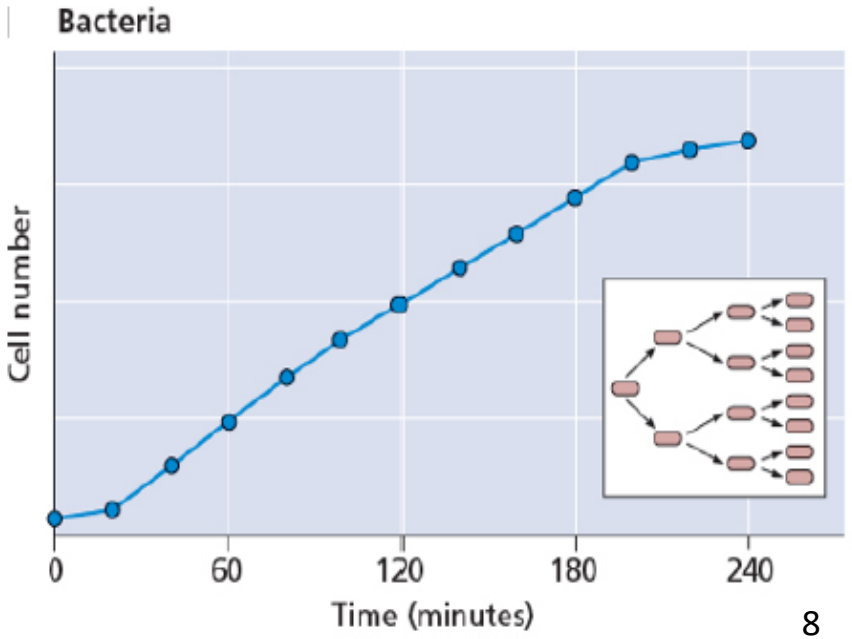


Viruses replicate by assembly of pre-formed components into many particles

Make the parts, assemble the final product

Not binary fission like cells



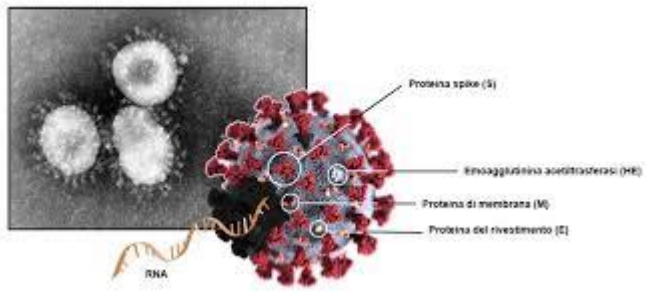


Distinctive Properties of Viruses

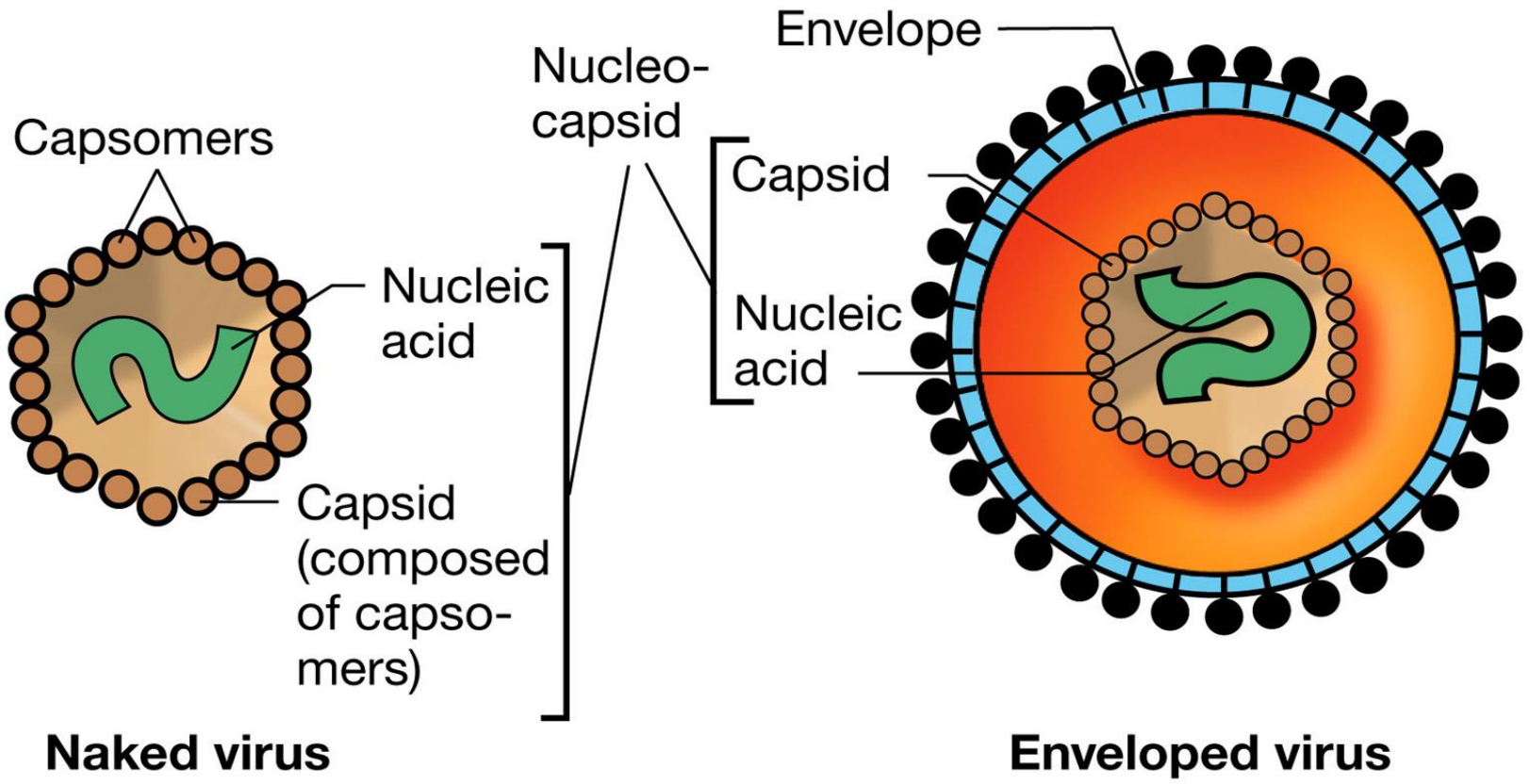
- Viruses are **absolutely incapable of autonomous replication**—they cannot replicate outside of host cells.
- They **survive for a limited time** (from minutes to a few days depending on the virus type) in the extracellular environment, but **never replicate outside cells**.
- As **obligate intracellular parasites**, viruses require not only nutrients produced by host cells, but especially the **transcription and translation systems** of those cells.
- They contain **essential enzymes** for their replication cycle (e.g., **polymerases**, **proteases**, etc.), while all other enzymes are provided by the host cell.

Viral components

- **Viral genome**: RNA or DNA
- **Capsid**: a protein coat that surrounds and protects the nucleic acid. It's made up of smaller protein subunits called **capsomers**.
- **Envelope**: an outer layer, present in some viruses, that is derived from the host cell's membrane. It often contains glycoproteins that help the virus attach to and enter new host cells.
- **Nucleocapsid:** consisting of the nucleic acid and capsid
- **Virion:** viral particle



The Virion = viral particle



The Virion = viral particle

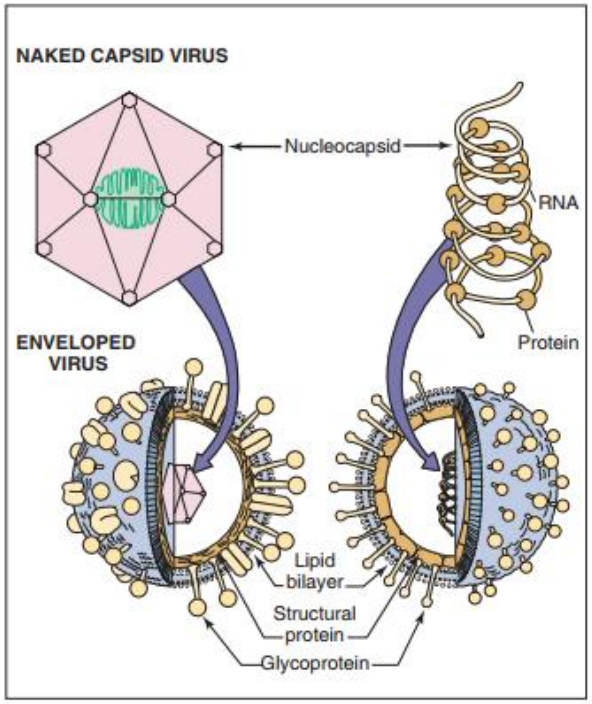


FIGURE 36-4 The structures of a naked icosahedral capsid virus *(top left)* and enveloped viruses *(bottom)* with an icosahedral *(left)* nucleocapsid or a helical *(right)* ribonucleocapsid. Helical nucleocapsids are always enveloped for human viruses.

Box 36-4 Virion Structure: Naked Capsid

Component
Protein

Properties*
Is environmentally stable to the following:
- Temperature
- Acid
- Proteases
- Detergents
- Drying

Is released from cell by lysis

Consequences*
Can be spread easily (on fomites, from hand to hand, by dust, by small droplets)
Can dry out and retain infectivity
Can survive the adverse conditions of the gut
Can be resistant to detergents and poor sewage treatment
Antibody may be sufficient for immunoprotection

*Exceptions exist.

Box 36-5 Virion Structure: Envelope

Components
Membrane
Lipids
Proteins
Glycoproteins

Properties*
Is environmentally labile—disrupted by the following:
- Acid
- Detergents
- Drying
- Heat

Modifies cell membrane during replication
Is released by budding and cell lysis

Consequences*
Must stay wet
Cannot survive the gastrointestinal tract
Spreads in large droplets, secretions, organ transplants, and blood transfusions
Does not need to kill the cell to spread
May need antibody and cell-mediated immune response for protection and control
Elicits hypersensitivity and inflammation to cause immunopathogenesis

*Exceptions exist.

The viral capsid: a protective coat

It protects nucleic acids (genome) against:

Physical damage – Mechanical protection, UV irradiation (from sunlight) leading to chemical modification and causing mutations, including lethal ones.

Enzymatic damage – Nucleases and esterases, derived from dead cells or deliberately secreted as a defense against infections

Protection from Temperature and pH variation

The viral capsid

- **Packaging** of the viral genome

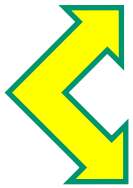
- **Interaction with host cells.** Capsid proteins of non-enveloped viruses mediate the binding and entry of the viruses.

- **Stimulation of the immune response.** The capsid proteins of non-enveloped viruses are often the major antigens of the virus.

The viral capsid

- Protein coat surrounding the viral genome
- Made up of proteins encoded by the viral genome
- Capsid proteins form capsomeres
- Each capsomere = 1 or several aggregated capsid proteins
- 1 capsomere = morphological unit of the capsid

Capsid of most viruses



Icosahedral symmetry

Helical symmetry

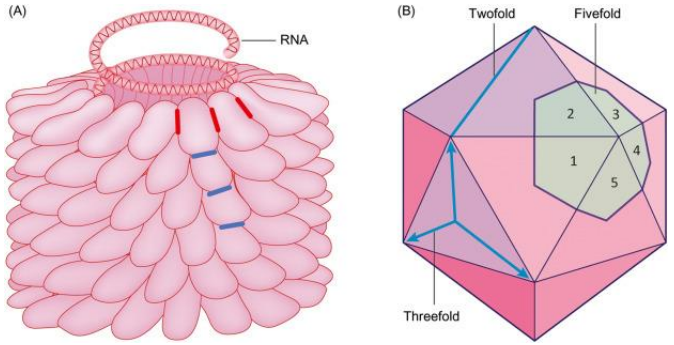
Viral Capsid Symmetry

Elicoidale: quando l'acido nucleico si avvolge sulla struttura proteica di base, determinando, al microscopio elettronico, una forma oblunga. Spesso le subunità proteiche sono identiche tra loro

Icosaedrica: quando i capsomeri (unità base dell'icosaedro costituente il capside) sono organizzati a costituire una struttura poliedrica regolare con 20 facce costituite da triangoli equilateri. All'interno è situato il nucleocapside, aderente in alcuni punti ai capsomeri. E' presente nella maggioranza dei virus

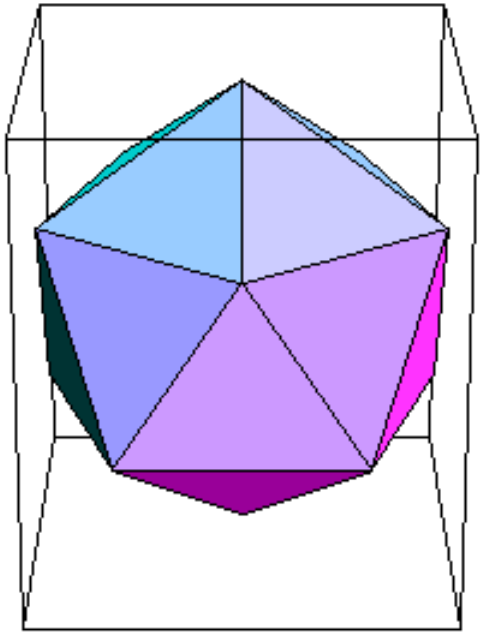
Complessa (complex): struttura non classificabile secondo canoni ristretti: batteriofagi, poxvirus, ecc.

Troncoconica (cone-shaped): tipica dei retrovirus



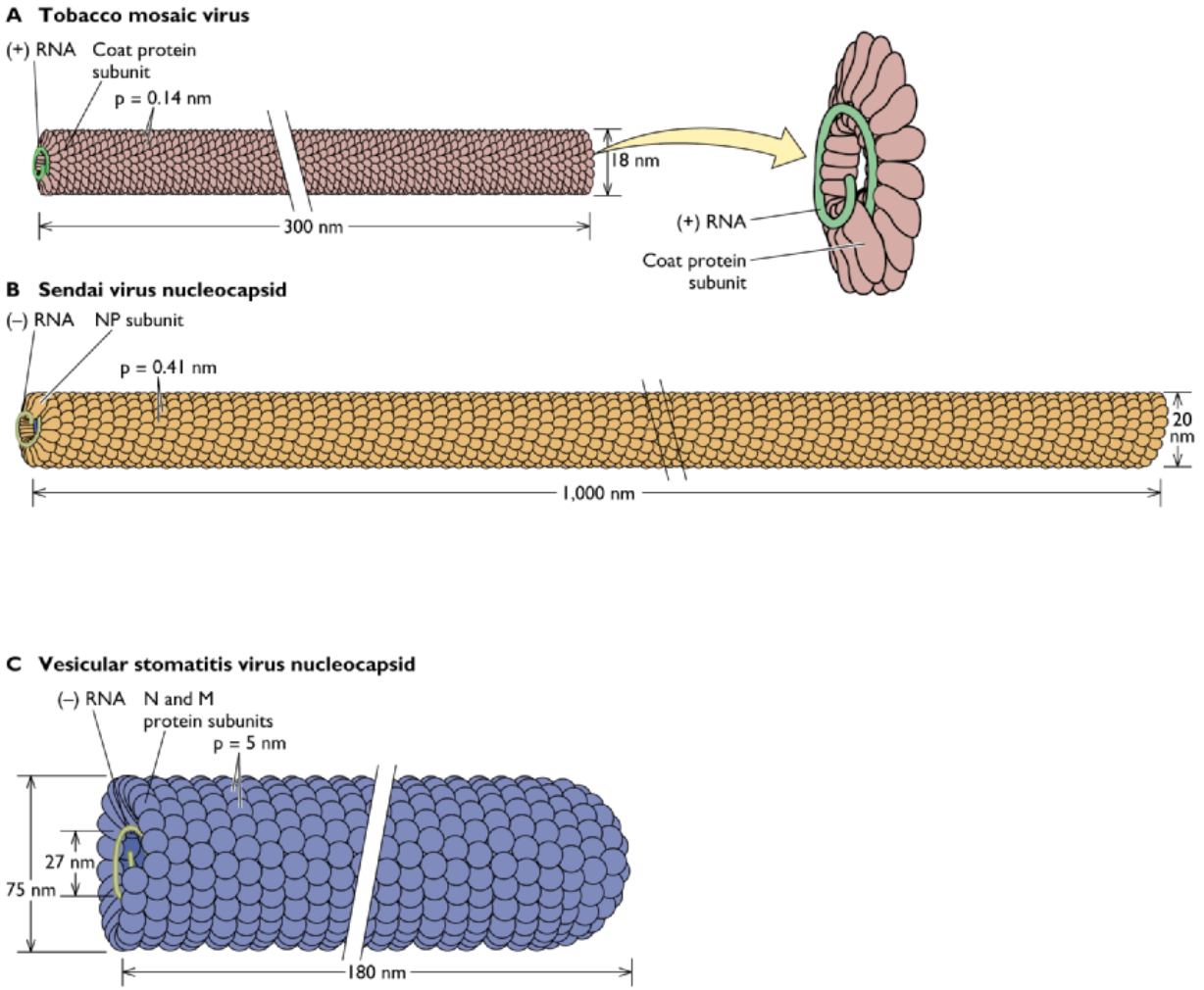
Struttura dei capsidi virali

Isometrici (quasi sferici)
Simmetria icosaedrica

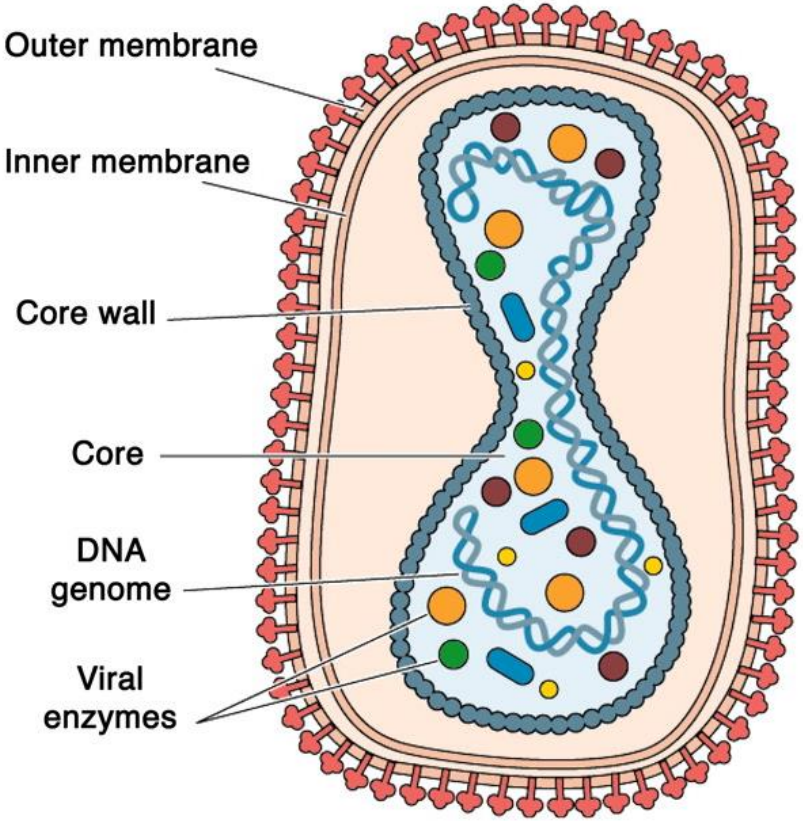


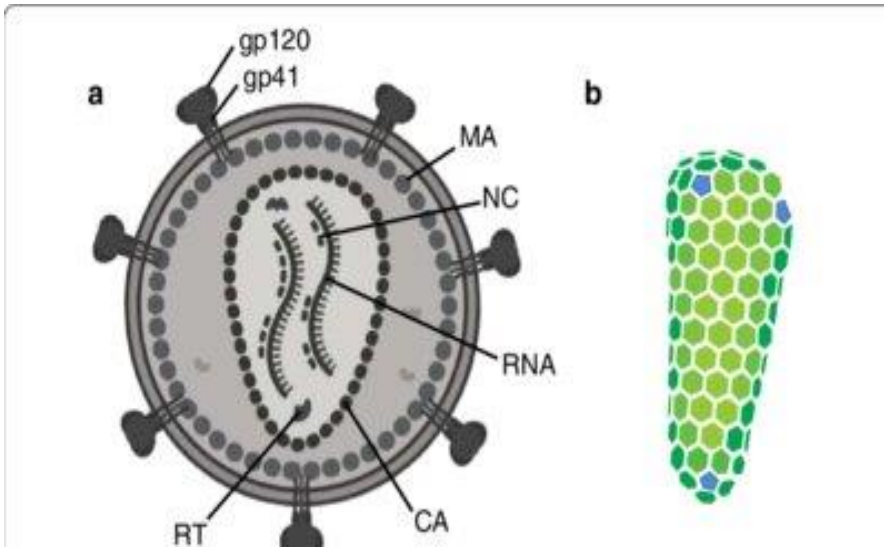
Struttura dei capsidi virali

Simmetria elicoidale



Struttura dei capsidi virali: Complessi (es. poxvirus e HIV)

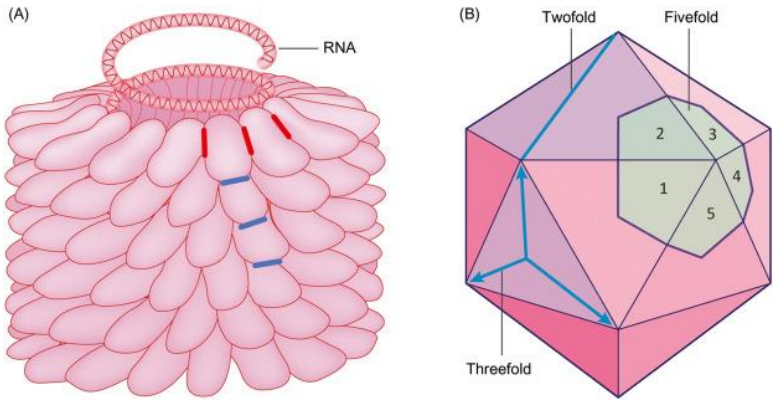




The viral capsid

E' proprio del virus, e' geneticamente determinato e costituito da subunità proteiche disposte in modo regolare, codificate dal genoma virale.

Dato il limitatissimo patrimonio genetico dei virus, le proteine costituenti i **capsomeri** (unità base del capside) sono molto poche, specifiche del virus, e, nei virus «nudi» in grado di interagire con i componenti sulla superficie cellulare fungendo da recettori.



Le dimensioni del capside determinano in qualche modo la quantità (quindi le dimensioni) del materiale genetico che può essere impacchettato nella particella virale.

Capside-Nomenclatura

Subunità strutturale (protomero): le singole proteine che costituiscono il capside

Unità morfologica (capsomero): la struttura più piccola visibile al microscopio elettronico, formata dall'interazione di più protomeri.

Nella simmetria icosaedrica

- **Pentoni (pentamer):** capsomeri formati da cinque protomeri
- **Esoni (hexamer):** capsomeri formati da sei protomeri

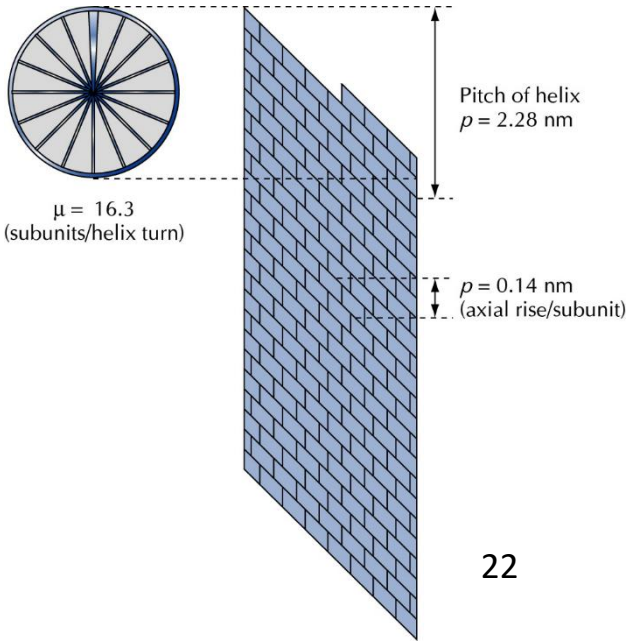
Capsidi virali: Simmetria elicoidale

Le dimensioni dei virioni con un capside a simmetria elicoidale sono date in termini di diametro, che dipende dalle caratteristiche dei protomeri, e di lunghezza, che dipende dalle dimensioni del genoma.

La simmetria elicoidale è definita da due parametri:
Ampiezza = diametro
Passo dell'elica (P) = distanza coperta da un intero giro di elica

$$P=\mu \times \rho$$

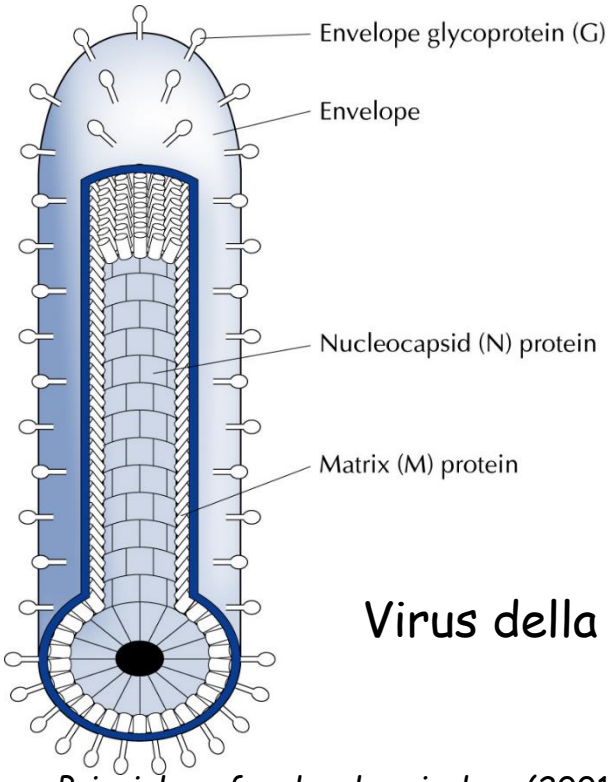
μ= numero di protomeri per giro d'elica
ρ= incremento assiale per subunità



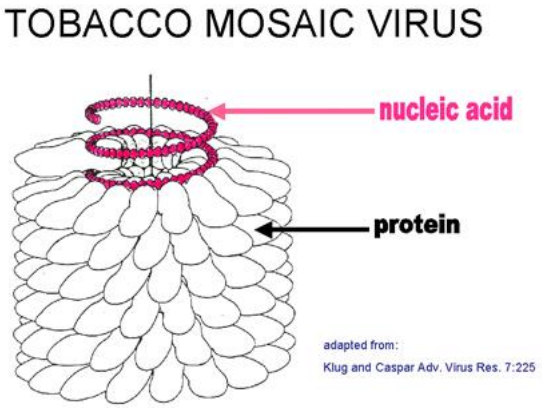
From Cann *Principles of molecular virology* (2001). Academic Press

Capsidi virali: Simmetria elicoidale

I virus animali a simmetria elicoidale sono tutti provvisti di envelope
Numerosi virus patogeni per l'uomo sono caratterizzati da questa struttura: virus dell'influenza (orthomyxovirus), i virus che causano la parotite epidemica e il morbillo (paramyxovirus), il virus della rabbia (rabdovirus), i coronavirus.



Virus della rabbia

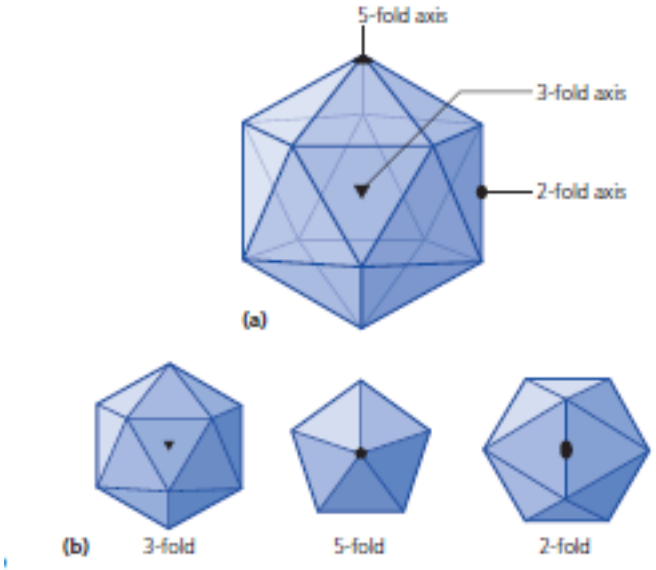


From Cann *Principles of molecular virology* (2001). Academic Press

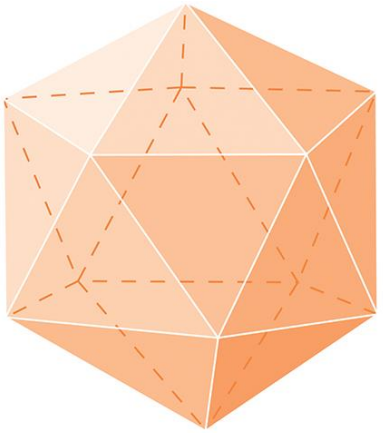
Capsidi virali: Simmetria icosaedrica

L'icosaedro è un solido con 20 facce triangolari e 12 vertici;
È un involucro **chiuso** (il capside elicoidale è una struttura **aperta**);
È caratterizzato da 3 assi di simmetria rotatoria:

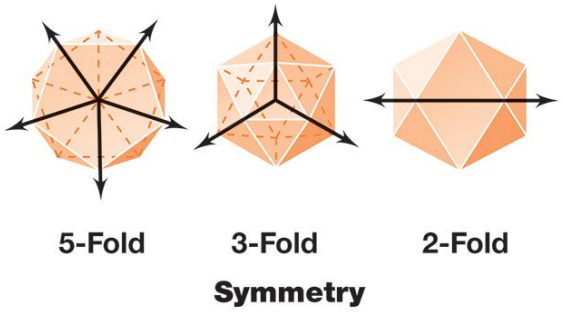
- asse di simmetria 5 che passa attraverso ognuno dei dodici vertici, sono possibili 5 rotazioni di 72°, ciascuna delle quali produce una configurazione identica
- asse di simmetria 3, che passa per il centro di ciascuna delle venti facce, sono possibili tre rotazioni di 120°, ciascuna delle quali produce una configurazione identica
- Asse di simmetria 2, che passa per ciascuno dei trenta spigoli dell'icosaedro, sono possibili 2 rotazioni di 180, ciascuna delle quali produce una configurazione identica



Icosahedral Symmetry



(a)



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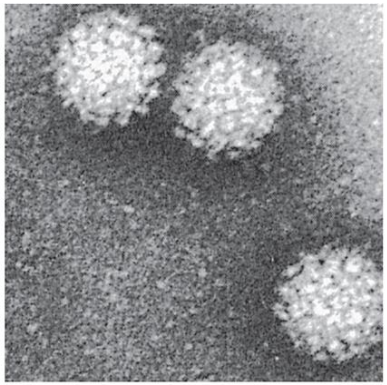
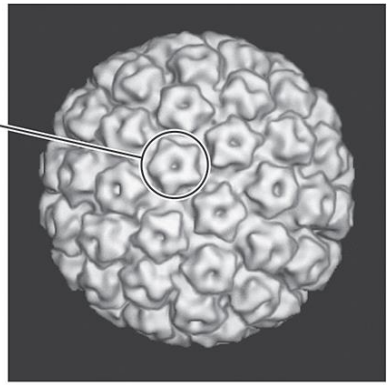


W.F. Noyes

(c)

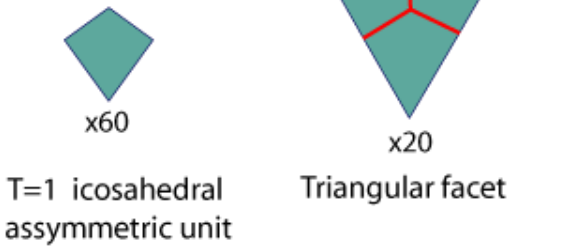


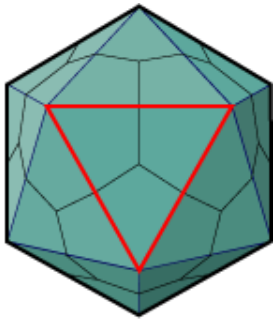
Tim Baker and Norm Olson

(d)

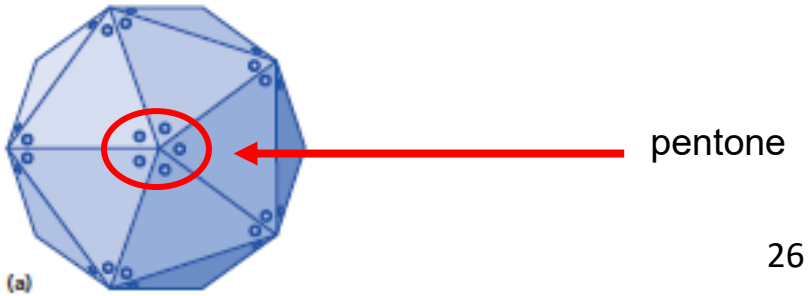
Capsidi virali: Simmetria icosaedrica

Gli involucri più semplici sono formati da 60 **protomeri**, tre per faccia, ognuno posto ad uno dei vertici.
L'insieme dei cinque protomeri attorno a ciascun vertice dell'icosaedro costituisce un **capsomero** (in questo caso un **pentone**)
Solo i virioni più piccoli e più semplici hanno un capside composto da 60 protomeri ed alcuni esempi si trovano tra i virus delle piante





Numero di triangolazione (T)
Numero di triangoli inscritti per faccia



Capsidi virali: Simmetria icosaedrica

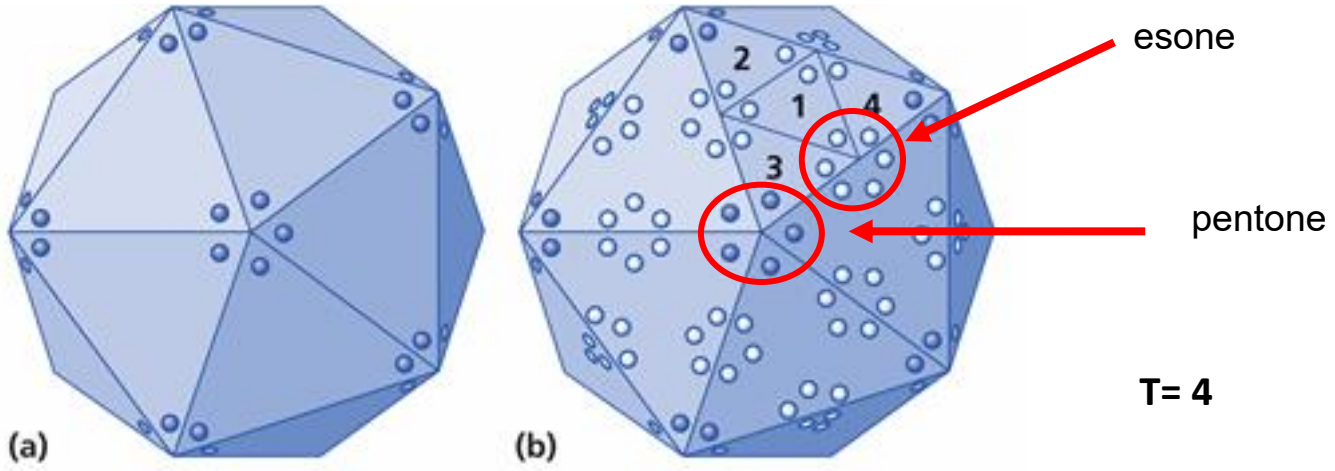
La maggior parte degli altri virus con capside a simmetria icosaedrica hanno più di 60 protomeri

Numero di triangolazione (T). Numero di triangoli inscritti per faccia. Il più piccolo numero possibile è1, poi 3, 4, 7, 9, 12 … quindi avremo

T=1 per l'icosaedro base (solo satelliti, dimensioni ridotte 18nm)

T=3 Tre triangoli inscritti per ciascuna faccia (sei emi-triangoli)

T=4 Quattro triangoli inscritti per ciascuna faccia etc…

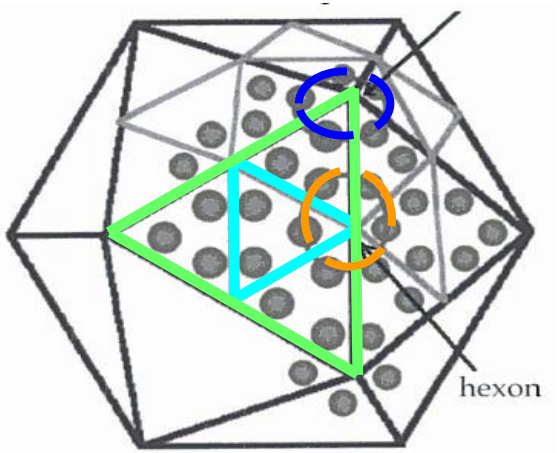


T= 1

T= 4

Introduction to Modern Virology, Seventh Edition. N. J. Dimmock, A. J. Easton and K. N. Leppard. *Principles of Virology* (2000), ASM Press

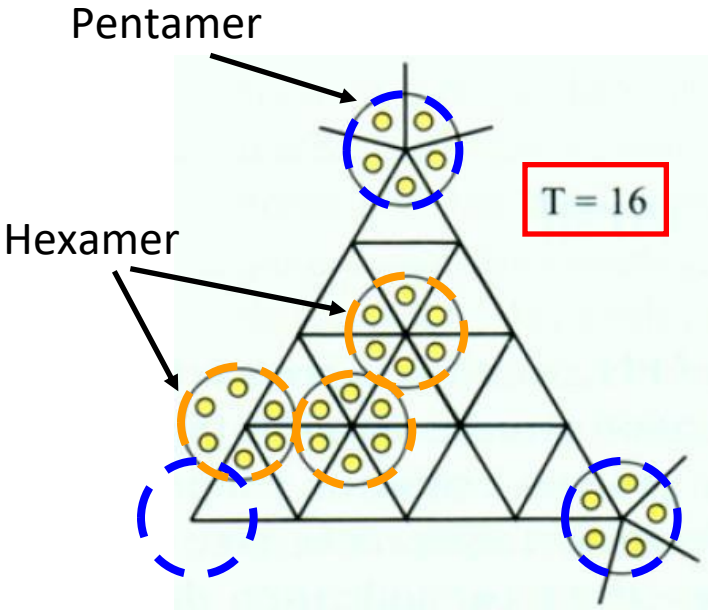
For **larger icosahedra capsids**, the **20 main faces** are larger and **subdivided in subtriangulations, containing each 3 capsid proteins**.



Triangulation number T = number of subtriangulations per main triangular face.

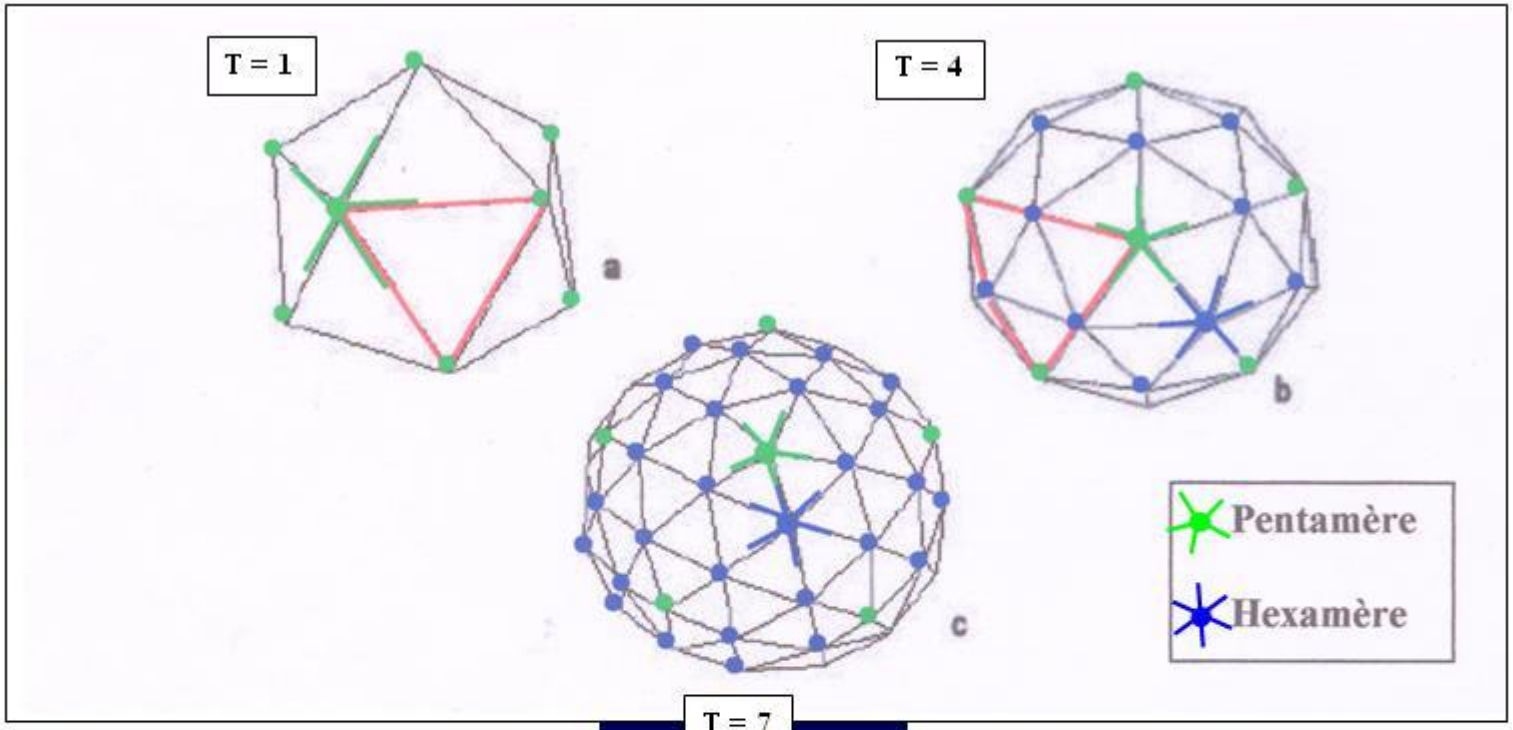
T = 4

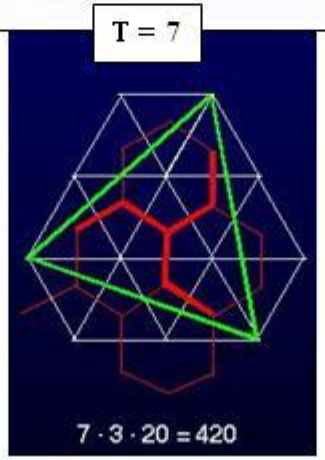
number of pentameres will always be 12, the number of hexameres depend on the size of the capsid, so from T



A capsid protein subunit is always engaged in a capsomere either pentamer or hexamer

Correlation between conformation and triangulation





The number of capsomeres is dependent on the size and the shape of the viral particle.
The more the capsid possess subunit, the more the capsid look spherical.

Struttura del virione

Pericapside (peplos, envelope)

Doppio strato lipidico che circonda il nucleocapside di molti virus animali. Deriva dalle membrane cellulari come risultato del processo di gemmazione della particella virale.

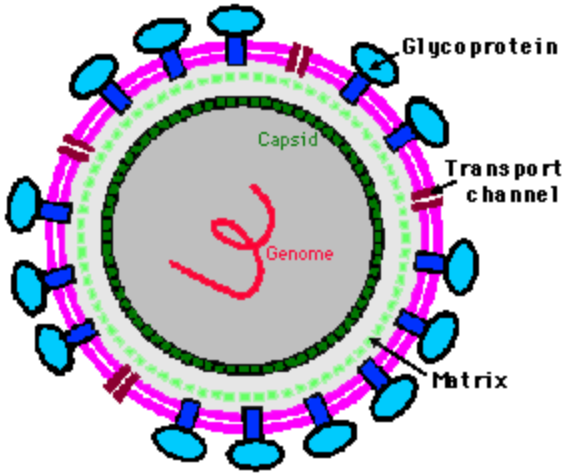
L'envelope contiene proteine, codificate dal genoma virale; spesso glicoproteine con un ruolo importante nel processo di attacco/adsorbimento e entrata del virus nella cellula ospite.

Pericapside (peplos, envelope)

Al doppio strato lipidico derivato dalle membrane cellulari sono associate proteine virus-specifiche quali:

•Glicoproteine, suddivise in base alla loro funzione in: glicoproteine esterne o di superficie, proteine transmembrana e canali di trasporto

•Proteine della matrice



ORIGIN OF THE VIRAL ENVELOPE

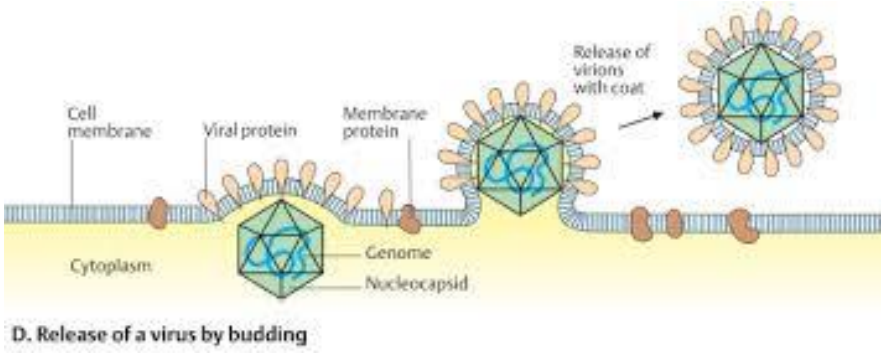
Derived from the host cell membranes

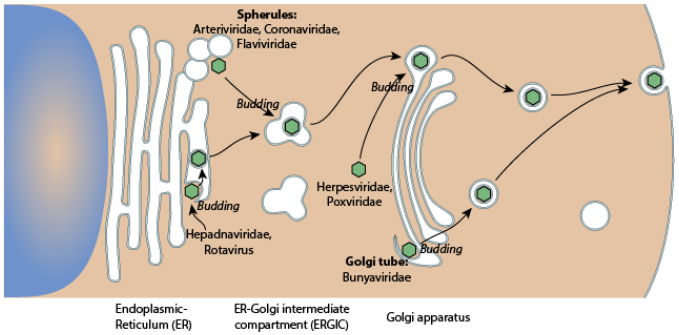
Plasma membrane (ORTHOMYXOVIRIDAE, RHABDOVIRIDAE, RETROVIRIDAE…)

Or

Endomembrane system (FLAVIVIRIDAE, HERPESVIRIDAE…)

The lipid composition of the viral envelope depends on the cell membrane from which it emanates





FRAGILITY OF THE VIRAL ENVELOPE

- !!! Enveloped viruses are more fragile than naked viruses
- **Lipid bilayer sensitive to :** organic solvents, detergents, biliary salts, pH variations, temperature, dessication…

Not anymore infectious without envelope

Consequences of this fragility :

- Limited survival in the external environment and in the digestive tract
- With exceptions… as usual

BENEFIT OF VIRAL ENVELOPE

- Contrary to naked viruses, virus release does not require cell lysis.
-Possibility of persistent infection

- Great adaptability, variability of the viral envelope proteins.

Variability ➔ Evolutionary advantage :

- help viruses to evade the host immune system
- to better adapt to a novel host (zoonose)

➔ numerous emerging virus in human

ex: HIV, SARS-CoV, Influenza virus, Ebola Virus, West Nile Virus, Zika virus, SARS-CoV2..

Viral Genome

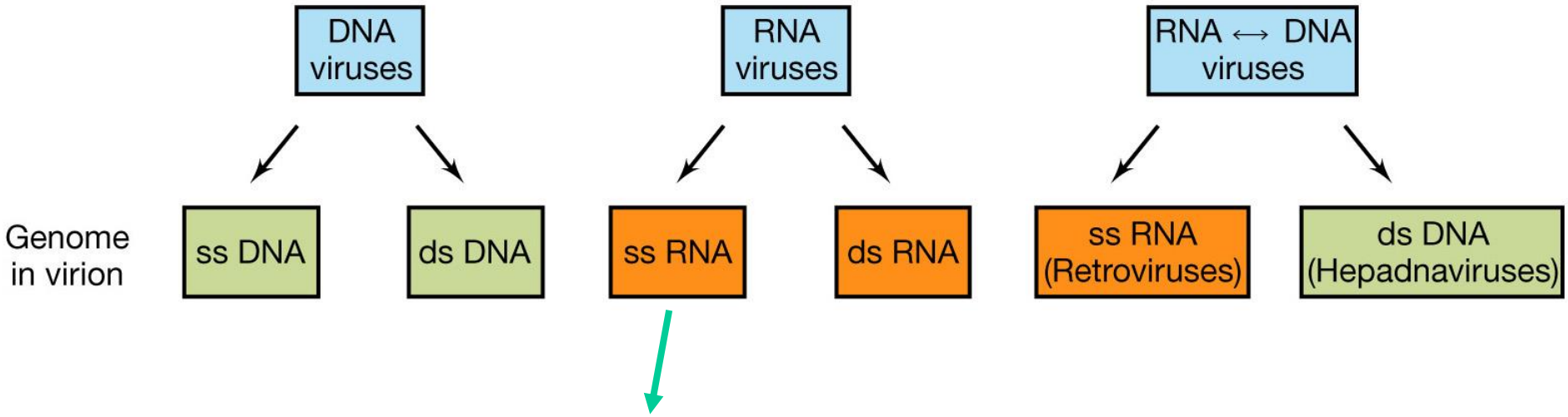
DNA Viruses: Among these, almost all animal viruses contain double-stranded DNA, with the exception of Parvoviridae, Circoviridae, and Anelloviridae.

RNA Viruses: Almost all RNA viruses contain single-stranded RNA, with the exception of the Reoviridae (e.g., rotavirus).

Cellular and Viral Polymerases

Type	Template → Product	Function
DNA-dependent DNA-Polymerase	DNA → DNA	Replication
DNA-dependent RNA-Polymerase	DNA → RNA	Transcription
RNA-dependent RNA-Polymerase	RNA → RNA	Replication and transcription of RNA-viruses
RNA-dependent DNA-Polymerase	RNA → DNA	Reverse transcription

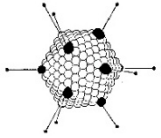
Genome architecture



Virus con RNA a **polarità positiva (+)** che può fungere nella cellula direttamente da RNA messaggero.

Virus con RNA a **polarità negativa (-)** che funge da stampo per la sintesi dell'RNA messaggero- questi virus hanno l'enzima RNA polimerasi RNA dipendente associato al virione.

I retrovirus hanno due molecole di RNA (+): sono diploidi



Genome architecture

Viruses with negative-sense RNA genomes: that is, genomes with polarity opposite to that of messenger RNA

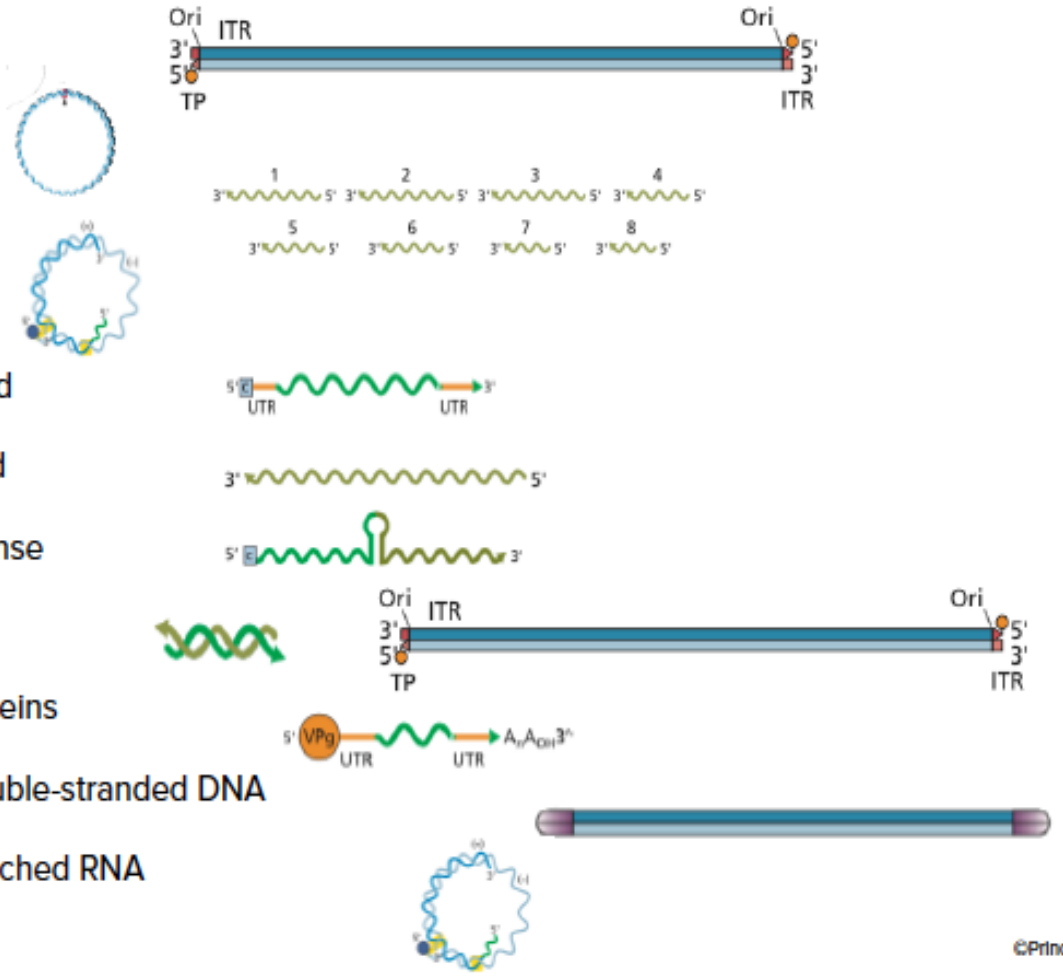
Some families have a **monopartite genome,** such as those belonging to the order **Mononegavirales;**

others have a **segmented genome (Orthomyxoviridae with 8 or 7 segments,** Arenaviridae with 2 segments, and Bunyaviridae with 3 segments).**The latter two families are unique because they possess a genome defined as ambisense (that is, a genome containing RNA of both positive and negative polarity).**

Viral genome

Viral DNA or RNA genomes are structurally diverse

- Linear
- Circular
- Segmented
- Gapped
- Single-stranded (+) strand
- Single-stranded (-) strand
- Single stranded, ambisense
- Double-stranded
- Covalently attached proteins
- Cross-linked ends of double-stranded DNA
- DNA with covalently attached RNA

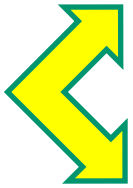


Viral characteristics and classification

- Nucleic acid nature: RNA vs DNA
- Capsid symmetry: Icosahedral, Helical, Complex
- Presence (enveloped) or absence (naked viruses) of the envelope
- Genome architecture: single strand or double strand, linear or circular, continous or segmented, genome polarity
- ICTV criteria: viral taxonomy
- mRNA production strategy: Baltimore classification
- Other (transmission route, pathology, tropism etc.)

Viral classification

2 main classifications for viruses



ICTV classification

Baltimore classification

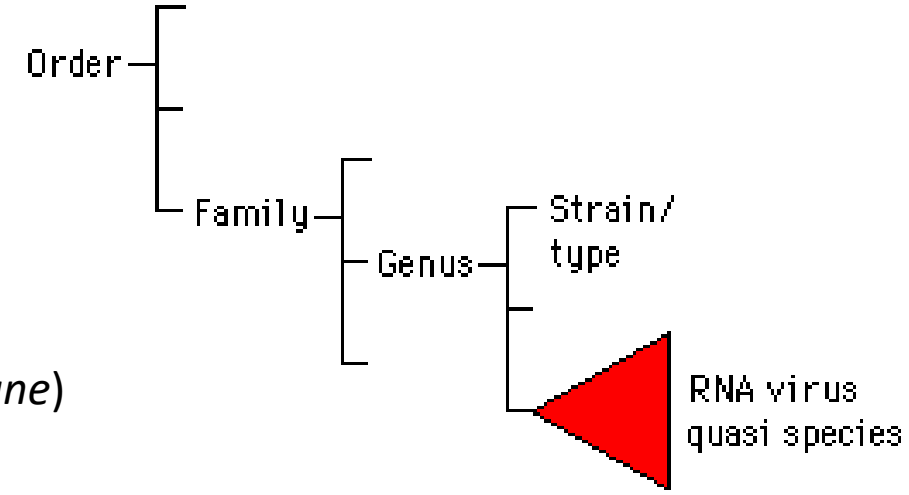
ICTV= International Committee on Taxonomy of Viruses since 1960s

Baltimore classification was created in 1971 by virologist David Baltimore

International Committee on Taxonomy of Viruses

VIROLOGY DIVISION - IUMS

- **Ordine** (- *virales*)
- **Famiglia** (- *viridae*)
- **Sottofamiglia** (-*virinae*)
- **Genere** (- *virus*)
- **Specie** e sottotipi/ceppi (*nome comune*)



The advent of **nucleotide sequence determination** has revolutionized biology and largely rationalized taxonomy, including that of viruses. The universal virus taxonomy provides a classification scheme that is supported by verifiable data and expert consensus. It is an indispensable framework both for further study of the currently recognized virus species and for the identification and characterization of newly emergent viruses, whether they result from natural, accidental, or deliberate dissemination.

ICTV TAXONOMY

International Committee on Taxonomy of Viruses : hierachical nomemclature, similarly to living beings

Order (-virales) → Family (-viridae) → Subfamily (-virinae) → Genus (-virus) → Type species → Strains

Order	Family	Subfamily	Genus	Type species
Mononegavirales	*Bornaviridae*		*Bornavirus*	*Borna disease virus*
	Rhabdoviridae		*Vesiculovirus*	*Vesicular stomatitis Indiana virus*
			Lyssavirus	*Rabies virus*
			Ephemerovirus	*Bovine ephemeral fever virus*
			Novirhabdovirus	*Infectious hematopoietic necrosis virus*
			Cytorhabdovirus	*Lettuce necrotic yellows virus*
			Nucleorhabdovirus	*Potato yellow dwarf virus*
	Filoviridae		*Marburgvirus*	*Lake Victoria marburgvirus*
			Ebolavirus	*Zaire ebolavirus*
	Paramyxoviridae	*Paramyxovirinae*	*Rubulavirus*	*Mumps virus*
			Avulavirus	*Newcastle disease virus*
			Respirovirus	*Sendai virus*
			Henipavirus	*Hendra virus*
			Morbillivirus	*Measles virus*
		Pneumovirinae	*Pneumovirus*	*Human respiratory syncytial virus*
			Metapneumovirus	*Avian metapneumovirus*

Replication and transcription in the nucleus

Rod-like shape

Linear, nonsegmented,
(-)ss RNA genome
6 to 10 genes
Enveloped viruses
Helical nucleocapsid
…

Filamentous virions
Highly contagious

F fusion protein

Baltimore Classification

(Division into replication classes)

All viruses must produce an mRNA that can be translated by the host cell's translational machinery.
In this classification system, the specific pathway from the viral genome to the production of mRNA defines a distinct viral class, based on the nature and polarity of the nucleic acid.

The New York Times

David Baltimore, Nobel-Winning Molecular Biologist, Dies at 87

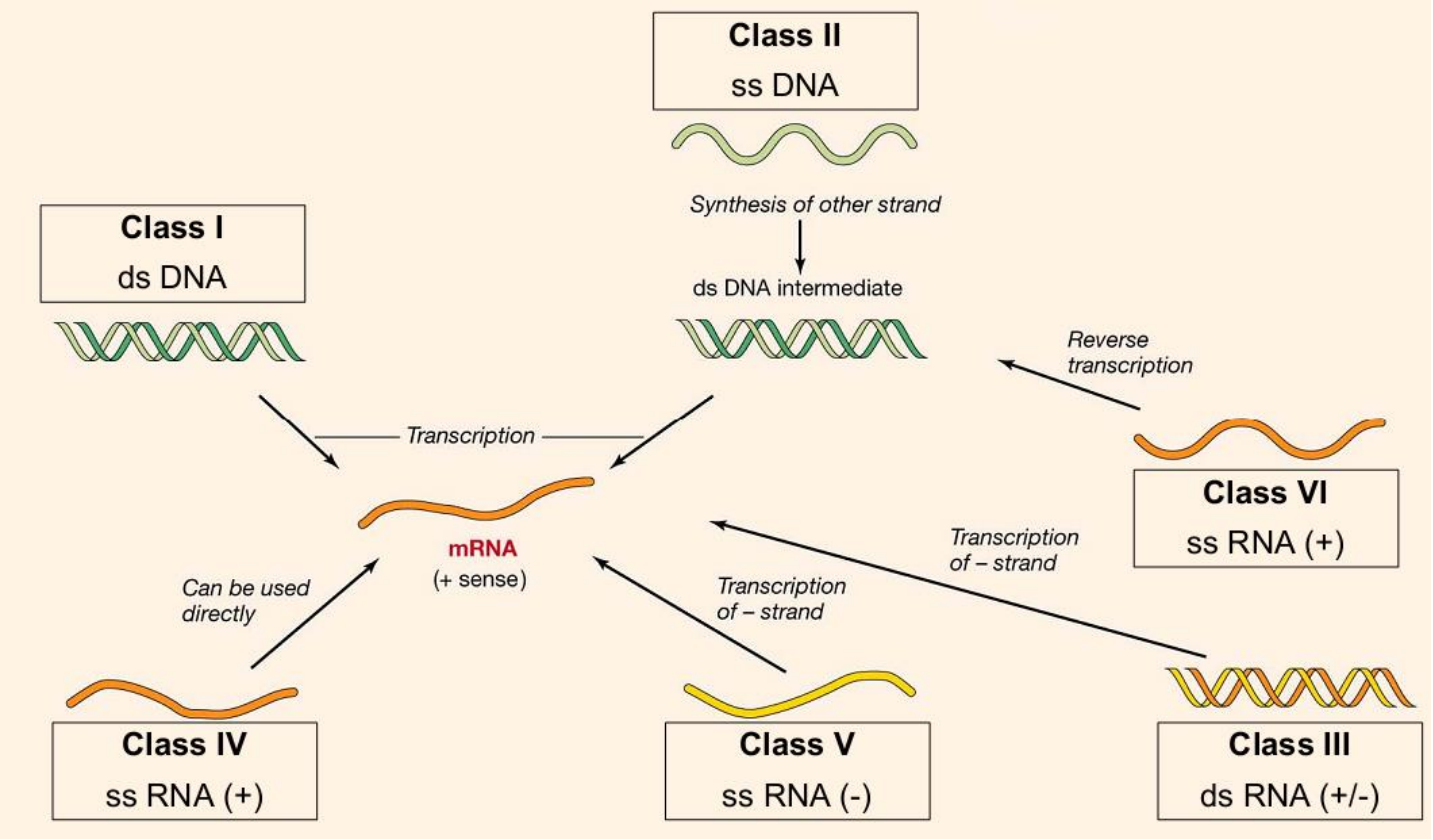
He was only 37 when he made a discovery that challenged the existing tenets of biology and led to an understanding of retroviruses and viruses, including H.I.V.

https://www.science.org/content/article/remembering-david-baltimore-titan-who-transformed-biology-and-spoke-bluntly

Baltimore classification

It is based on the nature of the viral genomes but specifically

It describes the obligatory relationships between the viral genome and its mRNA



Baltimore classification

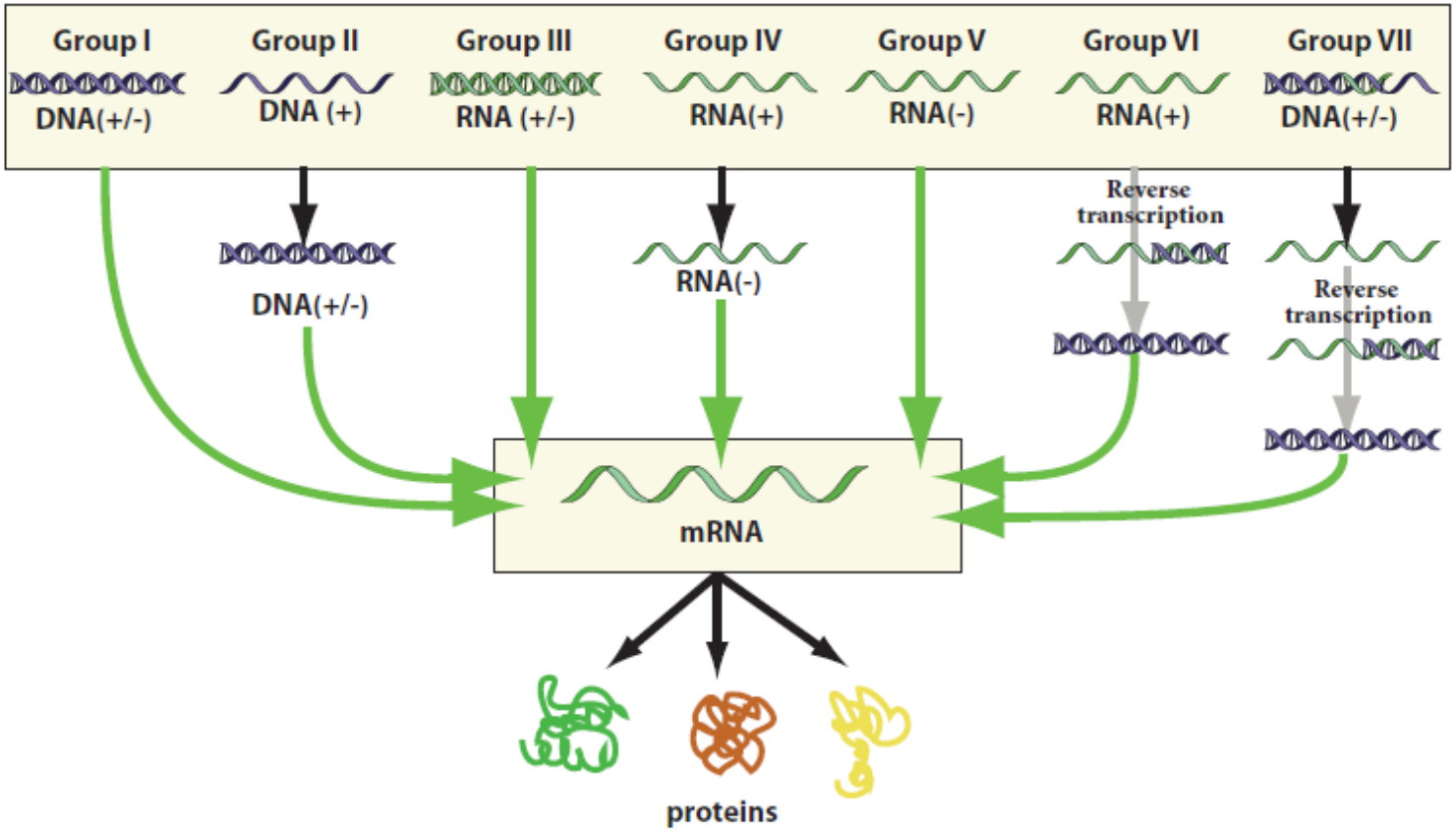
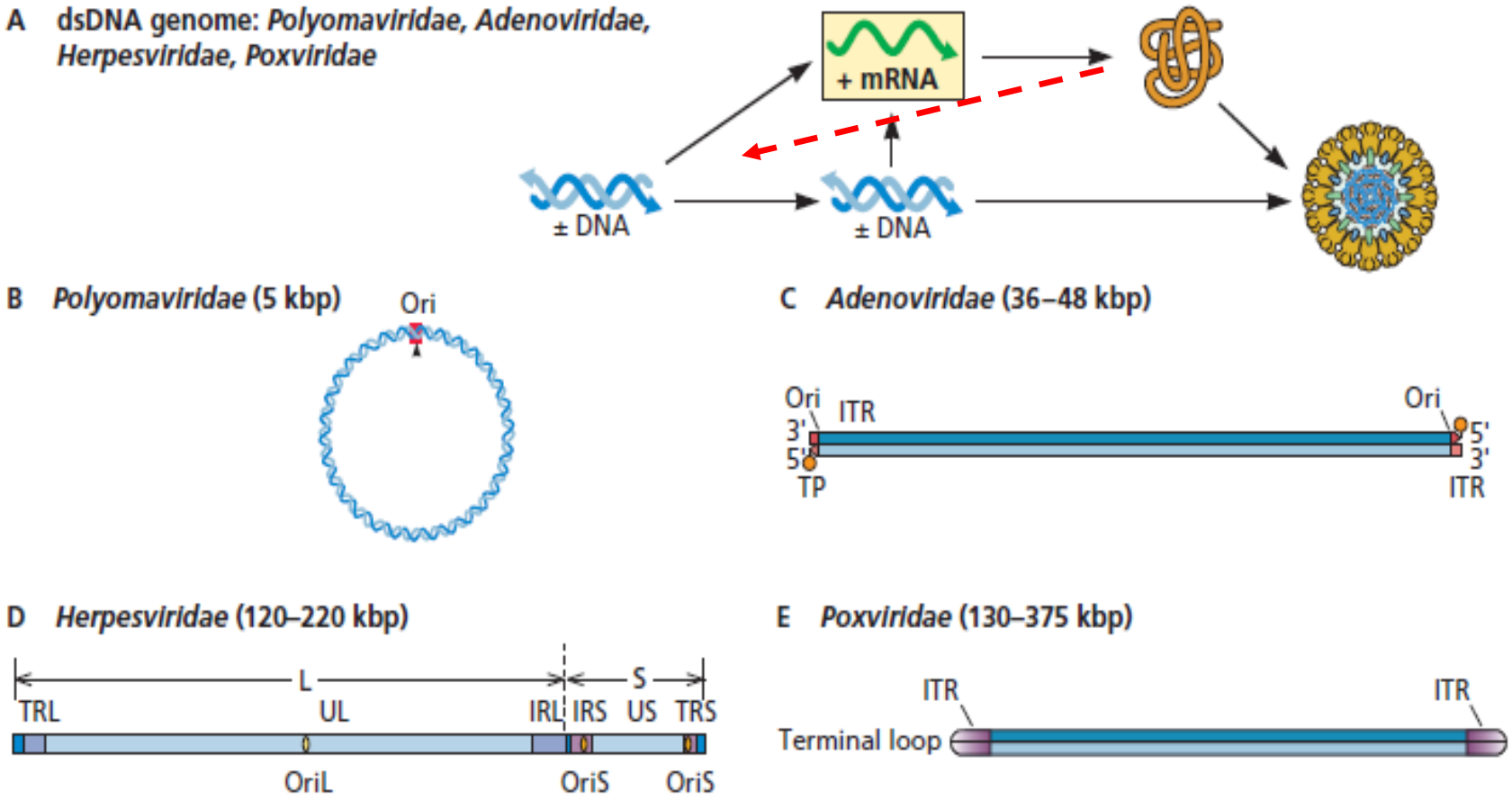


FIGURE 2.1. The Baltimore classification, a virus classification scheme based on the form of nucleic acid present in virion particles and the pathway for expression of the genetic material as messenger RNA.[1]

From Fields Virology

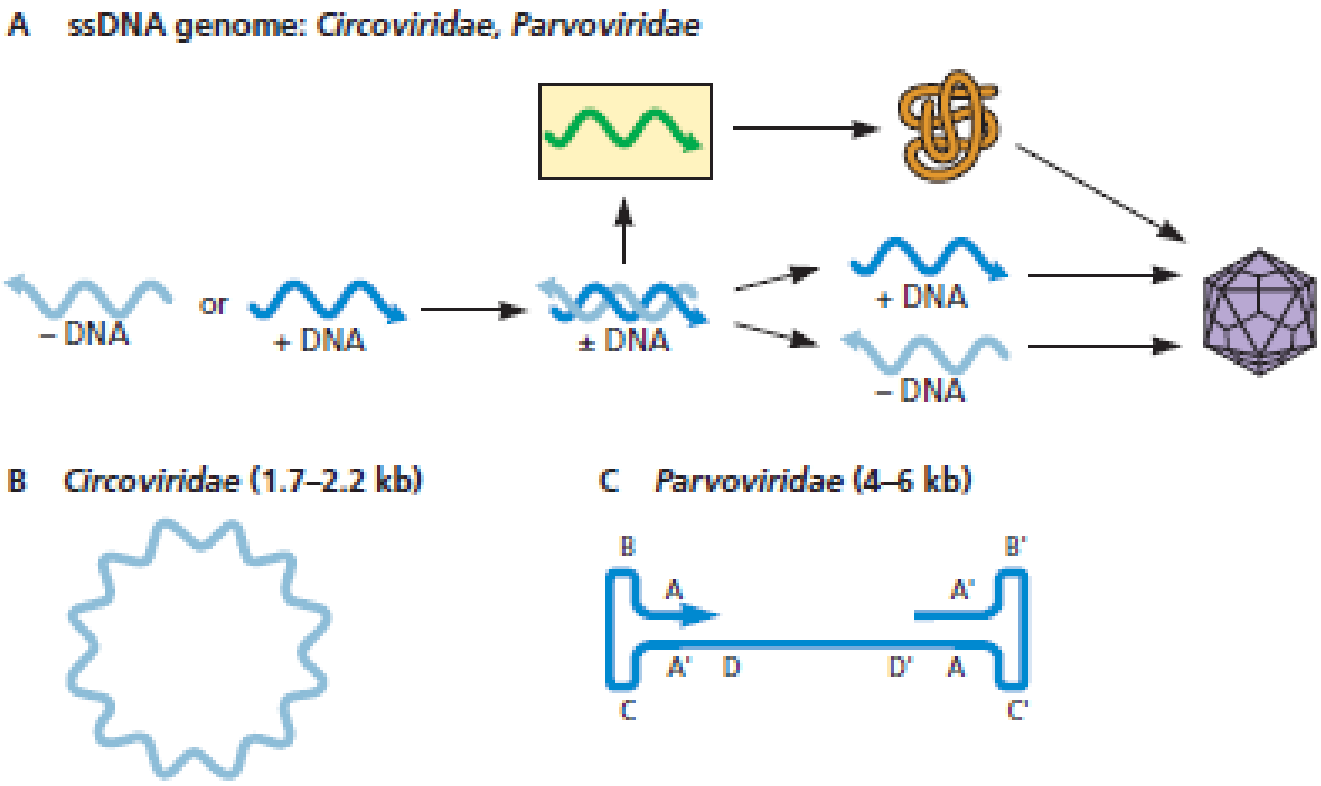
CLASSE 1 (dsDNA)



Schema di classificazione di Baltimore

From Flint et al. *Principles of Virology* (2015), ASM Press

CLASSE 2 (ssDNA)

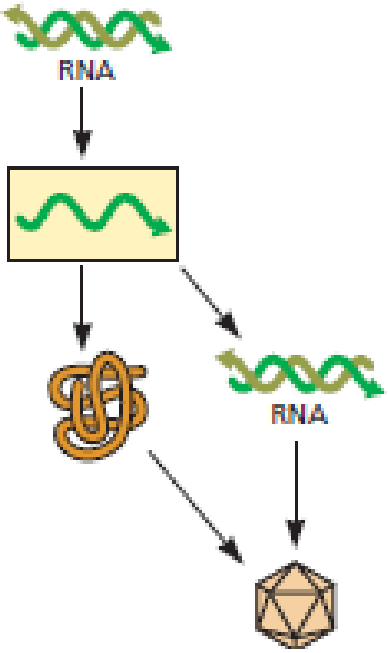


Schema di classificazione di Baltimore

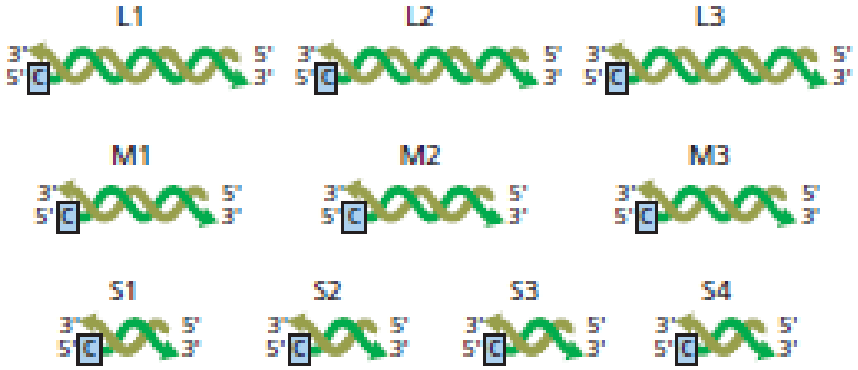
From Flint et al. *Principles of Virology* (2015), ASM Press

CLASSE 3 (dsRNA)

A dsRNA genome: *Reoviridae*



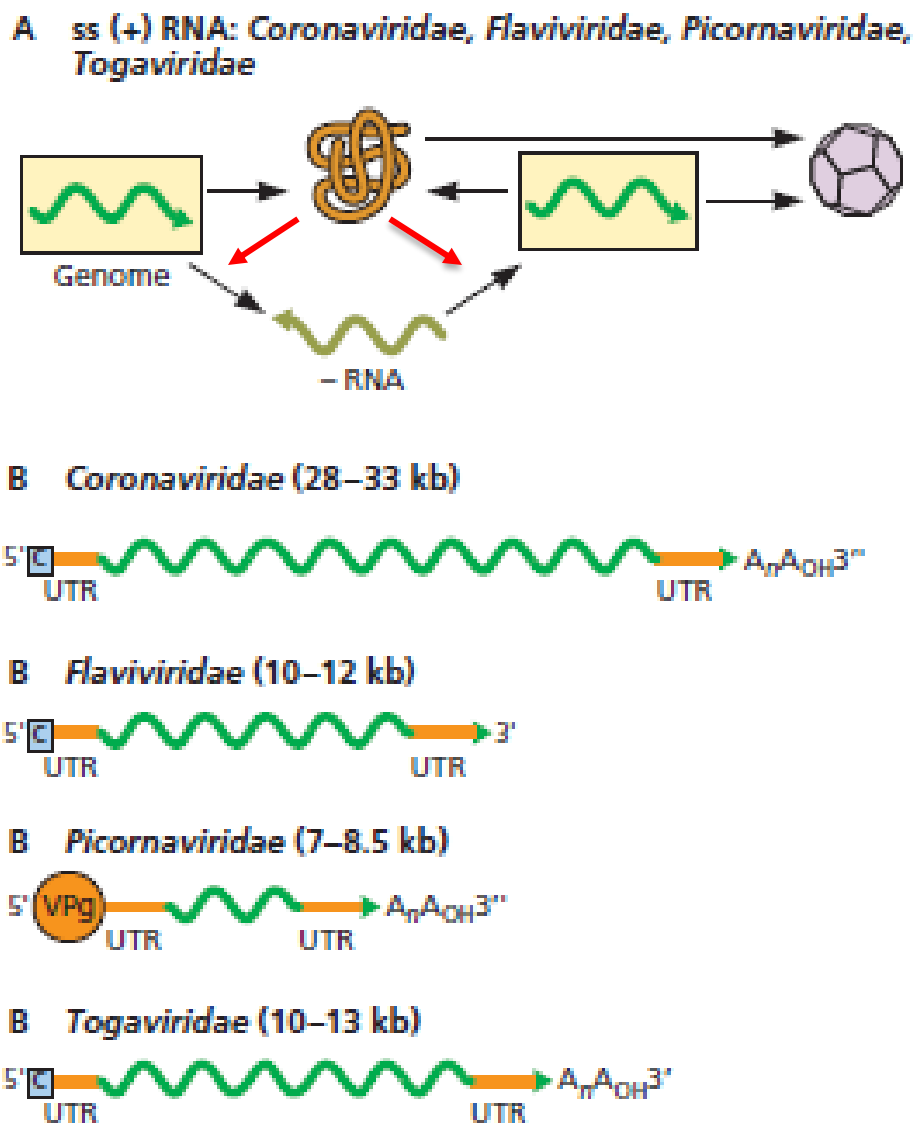
B *Reoviridae* (19–32 kbp in 10 dsRNA segments)



Schema di classificazione di Baltimore

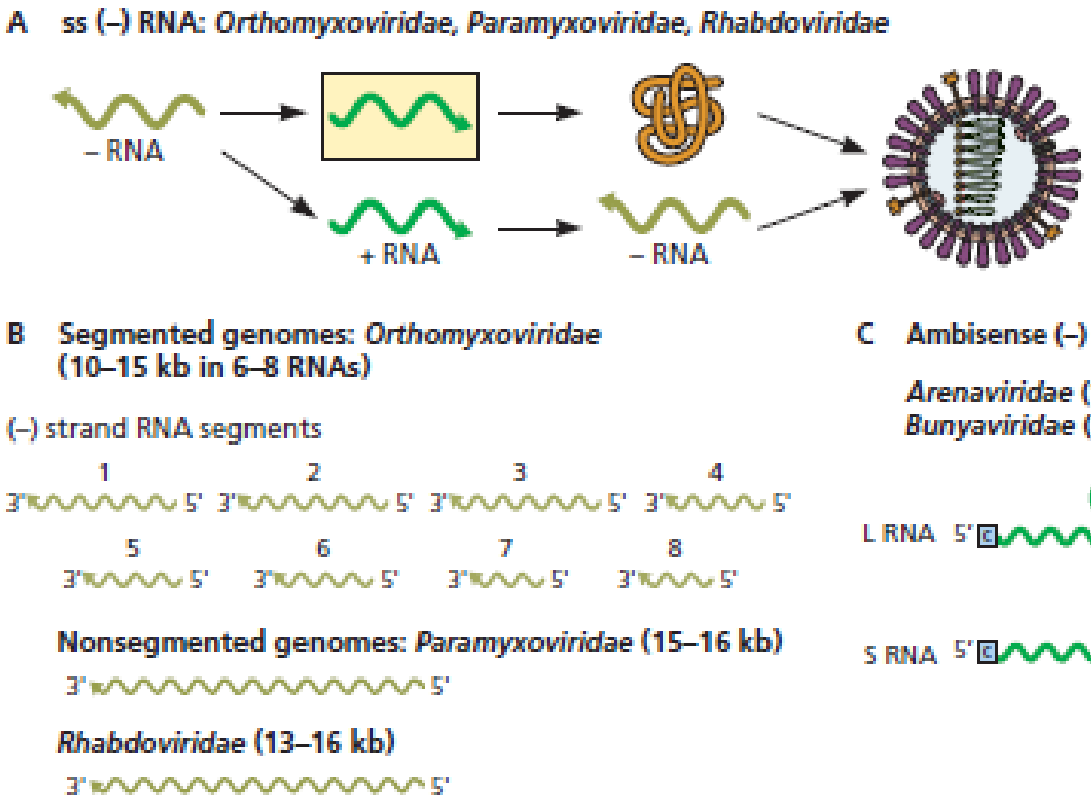
From Flint et al. *Principles of Virology* (2015), ASM Press

CLASSE 4 (ssRNA positive sense)



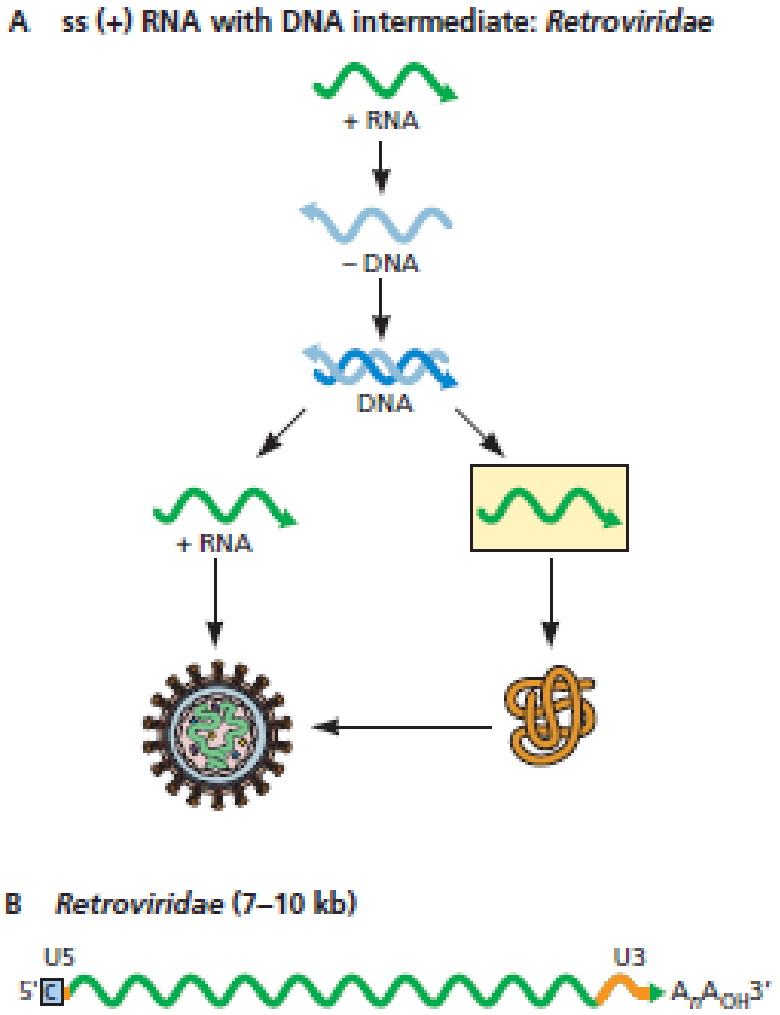
From Flint et al. *Principles of Virology* (2015), ASM Press

CLASSE 5 (ssRNA negative sense)



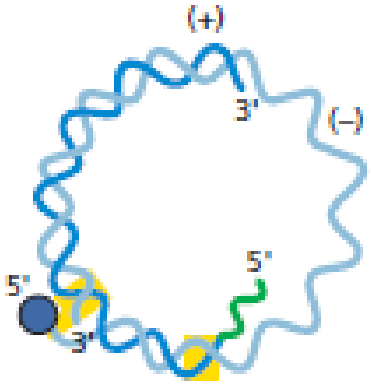
From Flint et al. *Principles of Virology* (2015), ASM Press

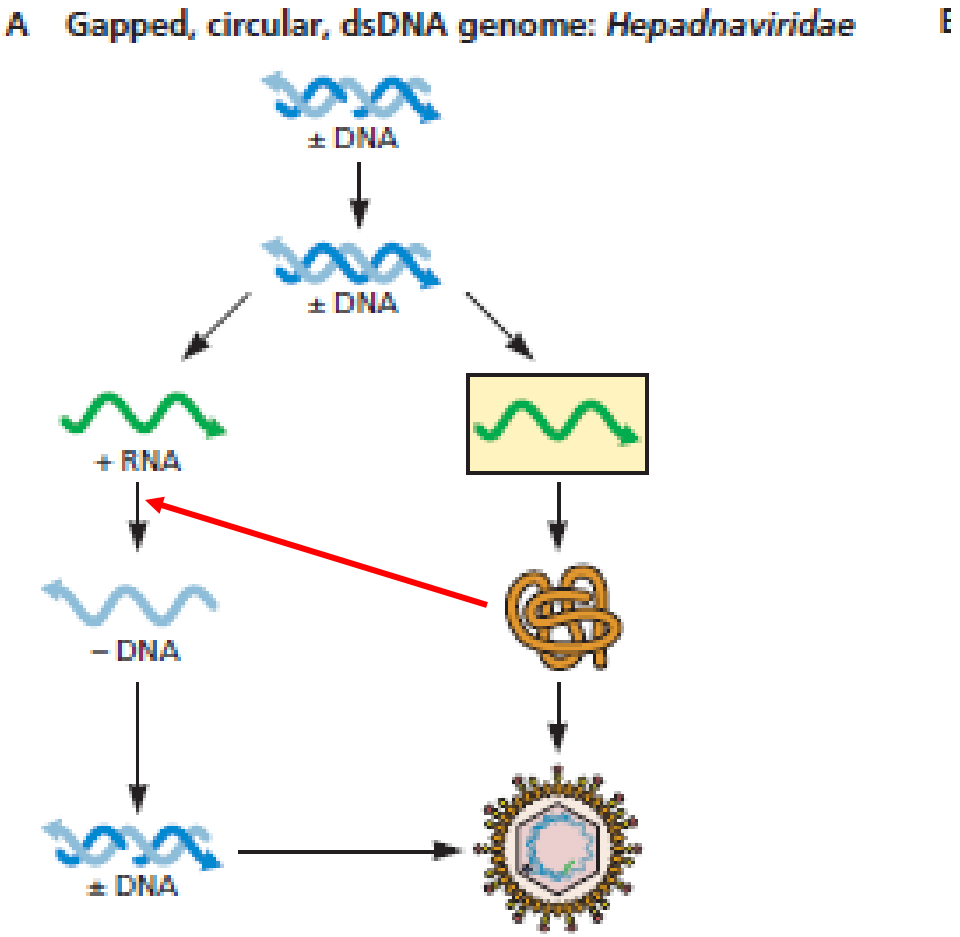
CLASSE 6 (ssRNA with DNA intermediate)



From Flint et al. *Principles of Virology* (2015), ASM Press

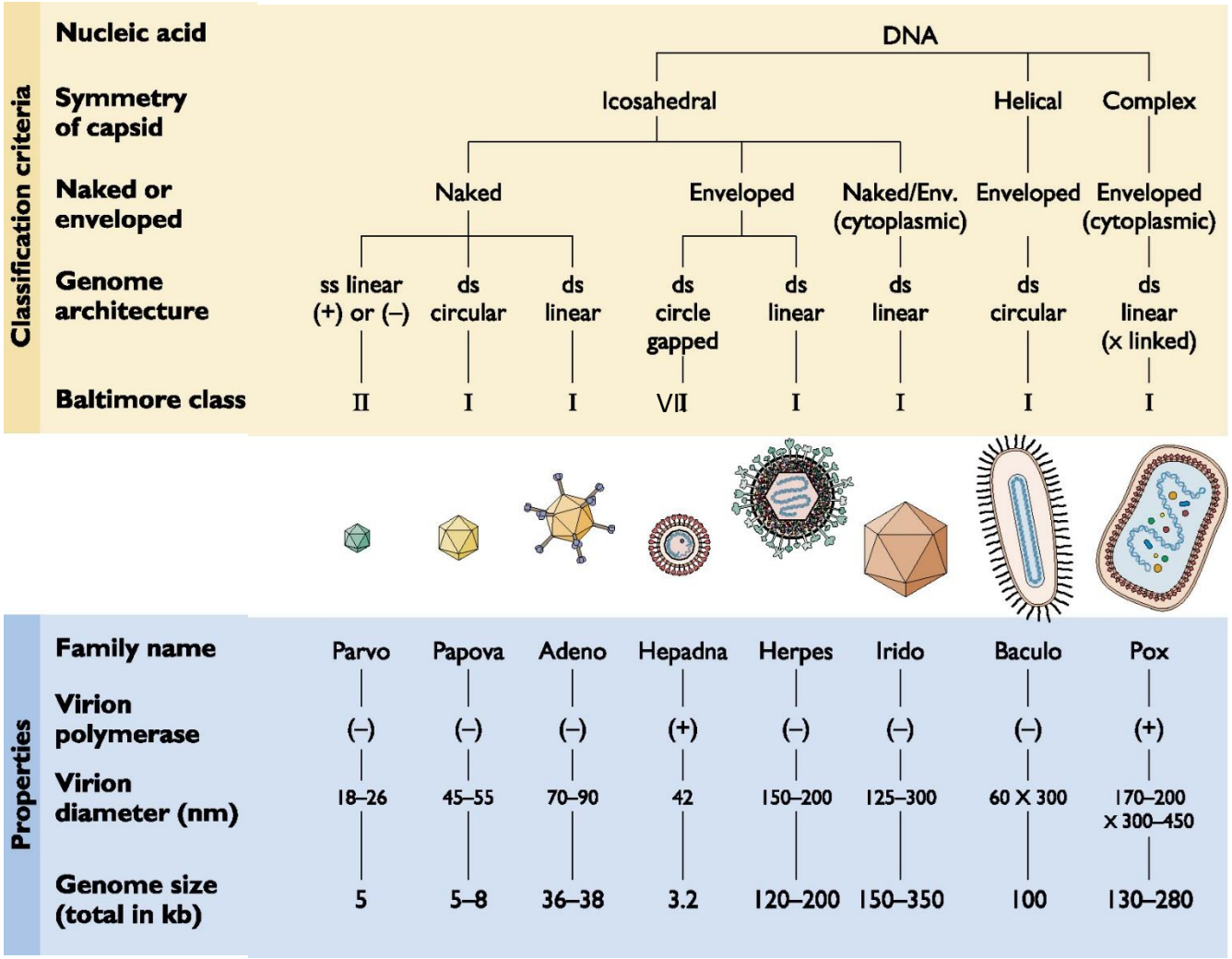
CLASSE 7 (dsDNA with RNA intermediate)





From Flint et al. *Principles of Virology* (2015), ASM Press

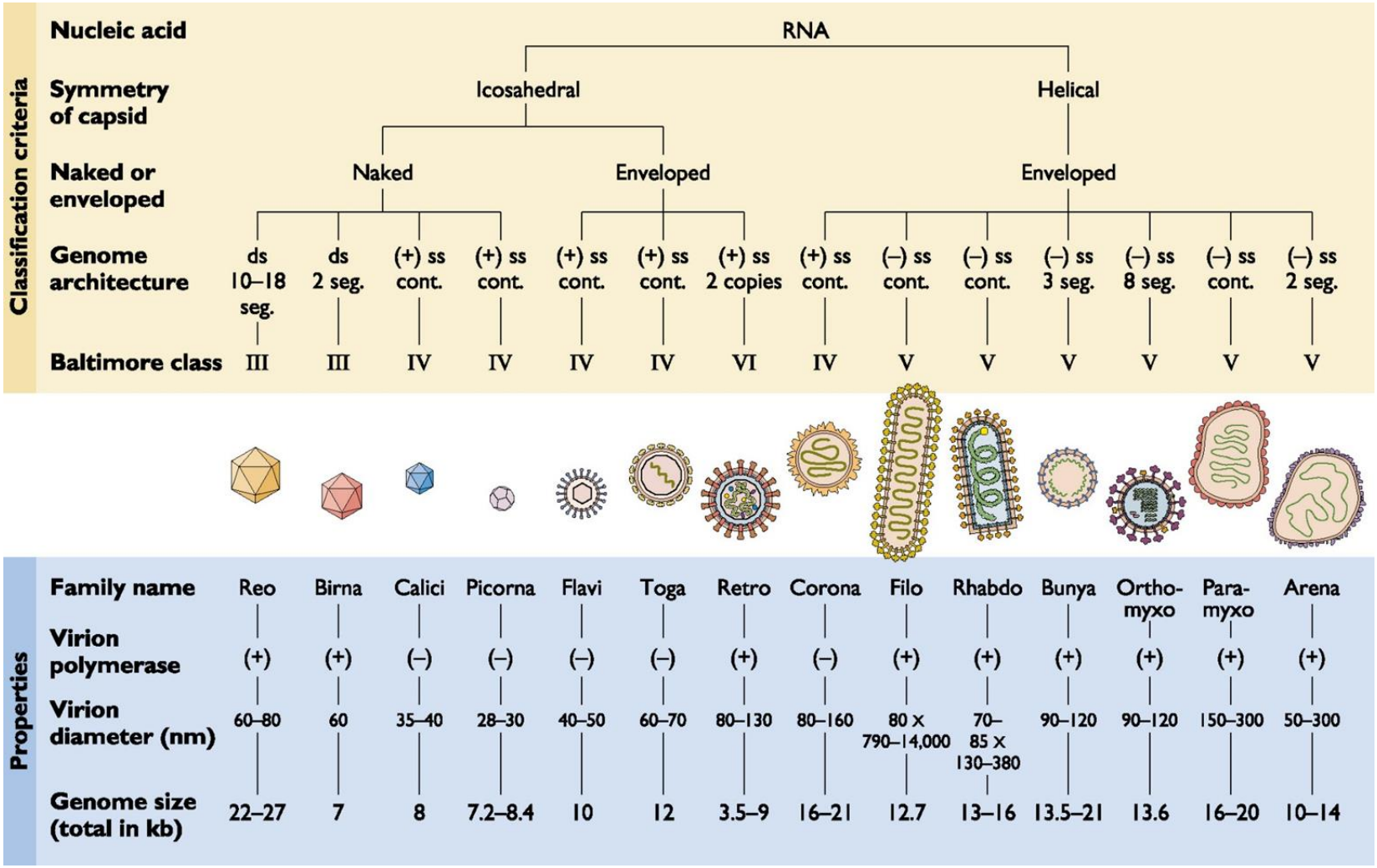
DNA viruses



The size of the genome reflects the complexity of the virus and its replication, which is more evident in DNA viruses than in RNA viruses.

From Flint et al. *Principles of Virology* (2000), ASM Press

RNA viruses



From Flint et al. *Principles of Virology* (2000), ASM Press