#### Corso di Laurea in Medicina e Chirurgia 'A' II ANNO – II SEMESTRE

CORSO INTEGRATO MICROBIOLOGIA	CFU tot	DOCENTE	MODULO	CFU PARZIALI
COORDINATORE: G. ANTONELLI	7	G. ANTONELLI (PO) M. ARTINI (PA)	MICROBIOLOGIA MED/07 MICROBIOLOGIA MED/07	5
		S. D'AMELIO (PA)	PARASSITOLOGIA VET/06	2

#### **Obiettivi dell'insegnamento:**

Conoscenza degli organismi patogeni (batteri, funghi, virus, protozoi, elminti e artropodi) per l'uomo e loro classificazione. Conoscenza delle differenze tra i vari organismi in termini di: struttura e morfologia, metabolismo, meccanismi patogenetici, riproduzione e replicazione, epidemiologia.

Conoscenza dei rapporti che si stabiliscono tra ospite e patogeni nelle infezioni umane e delle possibilità di trattamento, controllo e diagnosi delle infezioni.

#### Libri di testo (consigliati):

- **G. Antonelli**, M. Clementi, G. Pozzi G. M. <u>Rossolini</u> Principi di Microbiologia Medica. Casa Editrice Ambrosiana (CEA) – edizione 2022
- M. La Placa Principi di microbiologia medica- 2014 Società Editrice Esculapio EdiSes
- **Patrick R**. Murray, <u>Ken S. Rosenthal</u>, Michael A. <u>Pfaller</u> Microbiologia medica Editore: Edra - A cura di: P. Di Francesco Data di Pubblicazione: febbraio 2021
- G. <u>Cancrini</u> Parassitologia medica illustrata. Casa editrice EDRA edizione 2017 Casa Editrice Ambrosiana CEA

Modalità di valutazione: ESAME ORALE Evaluation methods: ...ORAL EXAM

#### Prof. G. ANTONELLI - Modulo: VIROLOGIA

**Obiettivi del modulo:** Comprendere come vengono classificati i virus animali, come funziona il loro apparato genetico e come interagiscono e influenzano l'ospite. I suddetti meccanismi molecolari e cellulari devono combinarsi con il concetto di patogenesi virale, epidemiologia, evoluzione e controllo.

**Programma del modulo:** Struttura e classificazione dei virus animali - Replicazione dei virus - Effetto del virus sulla cellula ospite - Patogenesi delle infezioni virali - Oncogenesi da virus – Meccanismi difensivi dell'ospite alle infezioni virali - Controllo delle infezioni virali - Principi di diagnosi virologica - Farmaci antiretrovirali - Sistema interferon - Vaccini antivirali. Struttura e caratteristiche morfologiche, patogenesi, controllo, epidemiologia, e diagnosi dei principali virus e infezioni virali importanti in patologia umana\*\*.

#### \*\*

Specific virus families: **Picornaviridae**, Caliciviridae, Astroviridae, Bunyaviridae, Filoviridae, **Hepeviridae** (HEV), **Coronaviridae** (SARS), Togaviridae, Flaviridae (HCV), Rabdoviridae, Paramyxoviridae, **Orthomyxoviridae**, Arenaviridae, Reoviridae, **Retroviridae** (HIV, HTLV-1), Polyomaviridae, **Papillomaviridae**, Adenoviridae, Parvoviridae (B19), Herpesviridae, Hepadnaviridae (HBV), Viroids (HDV), Prions.



Biology of microrganisms



Organisms not visible to the naked eye



Microbiology of microorganisms responsible for some human pathologies

## <u>Medical microbiology</u>

- Virology
- Bacteria
   Bacteriology
- Fungi Mycology
- Protozoa
- Helminths and arthropods

Parasitology

# <u>VIROLOGY</u>

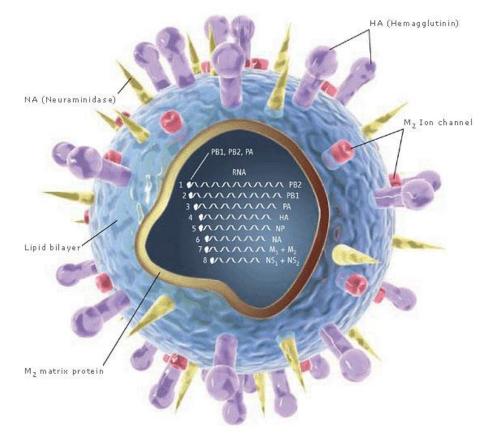


Illustration: Chris Bickel/Science. Reprinted with permission from Science Vol. 312, page 380 (21 April 2006) © 2006 by AAAS

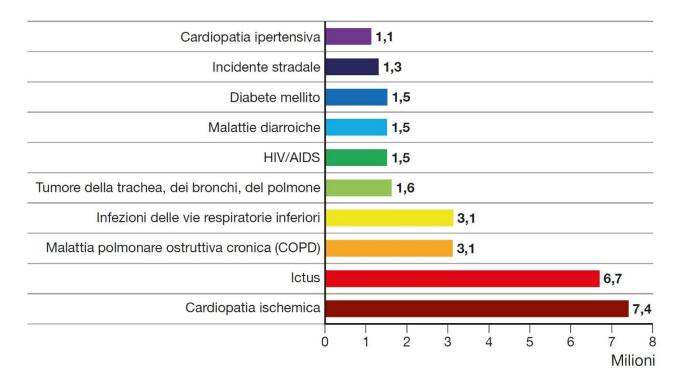


Figura 77.1 Le 10 principali cause di morte nel mondo nel 2012 (fonte OMS, 2016).

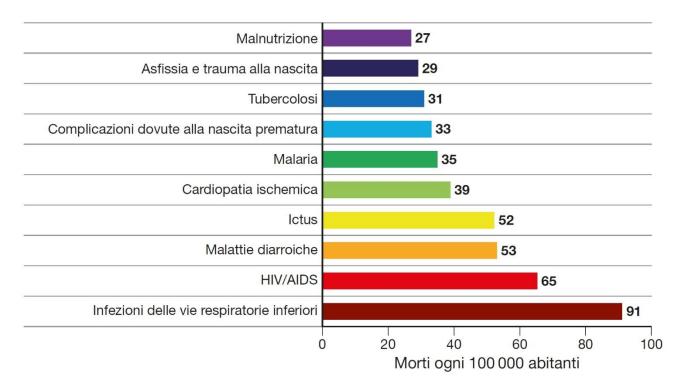


Figura 77.2 Principali cause di morte nel 2012 nei Paesi a basso reddito (fonte OMS, 2016).

JHU Has Stopped Collecting Data As Of **03 / 10 / 2023** 



# <u>VIROLOGY</u>

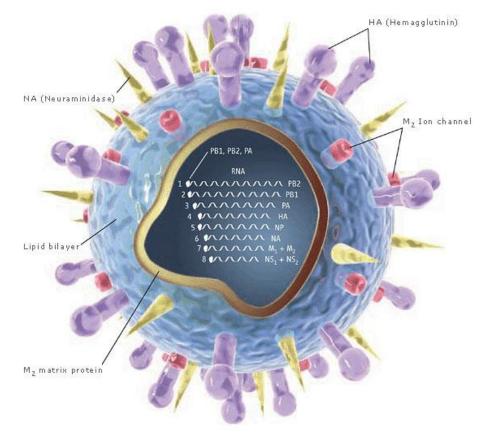


Illustration: Chris Bickel/Science. Reprinted with permission from Science Vol. 312, page 380 (21 April 2006) © 2006 by AAAS

Redifining determinants of viral complexity: lesson from RNA

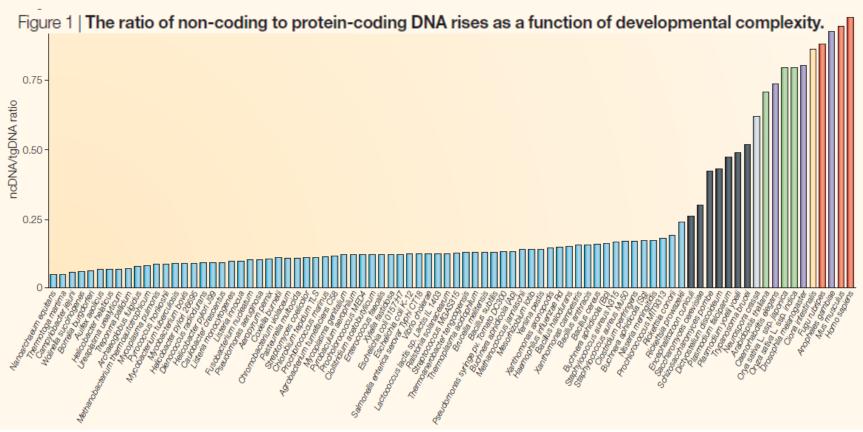
Organism	Genome size
Protopterus aethiopicus (lung fish)	139,000,000,000
Fritillaria assyriaca	124,900,000,000
Lilium longiflorum	90,000,000,000
Necturus maculosus (salamander)	50,000,000,000
Triturus cristatus (newt)	18,600,000,000
Zea mays	5,000,000,000
Xenopus laevis (frog)	3,000,000,000
Rattus norvegicus	3,000,000,000
Mus musculus	3,000,000,000
Homo sapiens	3,000,000,000
Bos Taurus	3,000,000,000
Gallus gallus	1,200,000,000
Oryza sativa	400,000,000
Fugu rubripes (puffer fish)	400,000,000
Drosophila melanogaster	165,000,000
Caenorhabditis elegans	100,000,000
Arabidopsis thaliana	100,000,000
Toxoplasma gondii	89,000,000
Plasmodium falciparum	25,000,000
Saccharomyces cerevisiae	12,067,280
Escherichia coli	4,639,221
Mycobacterium tuberculosis	4,397,000
Bacillus subtilis	4,170,000
Synechocystis sp. strain PCC6803	3,573,470
Haemophilus influenzae	1,830,137
Mycoplasma pneumoniae	816,394
Mycoplasma genitalium	580,000
Human immunodeficiency virus type 1	9,750

### NonCodingRNA/TotalGenomicDNA

OPINION

RNA regulation: a new genetics?

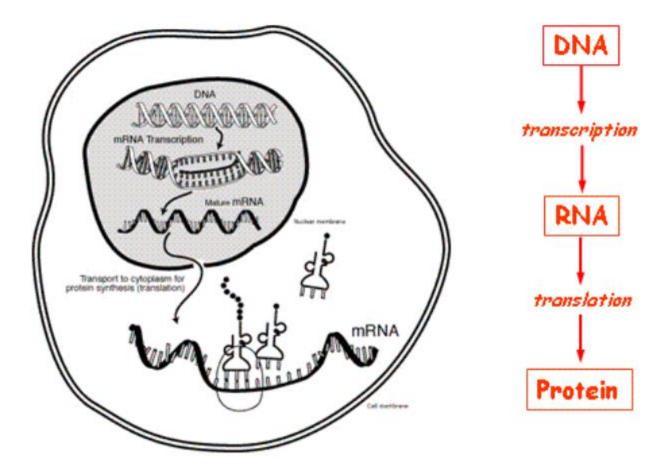
John S. Mattick

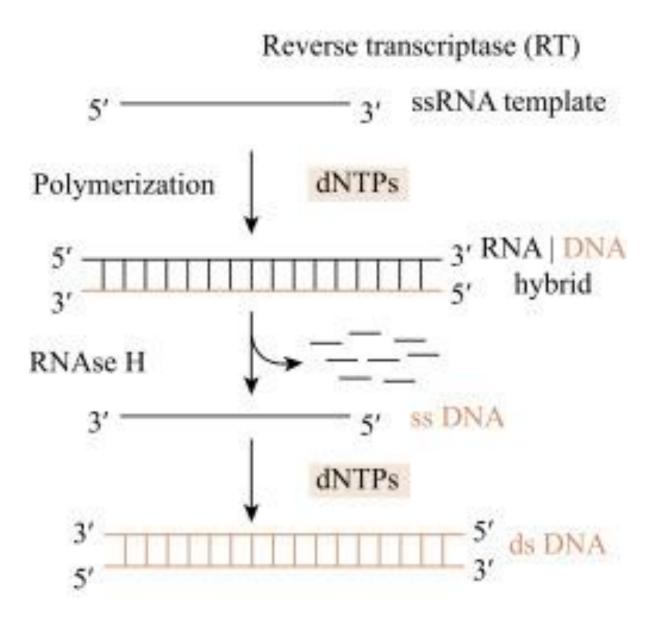


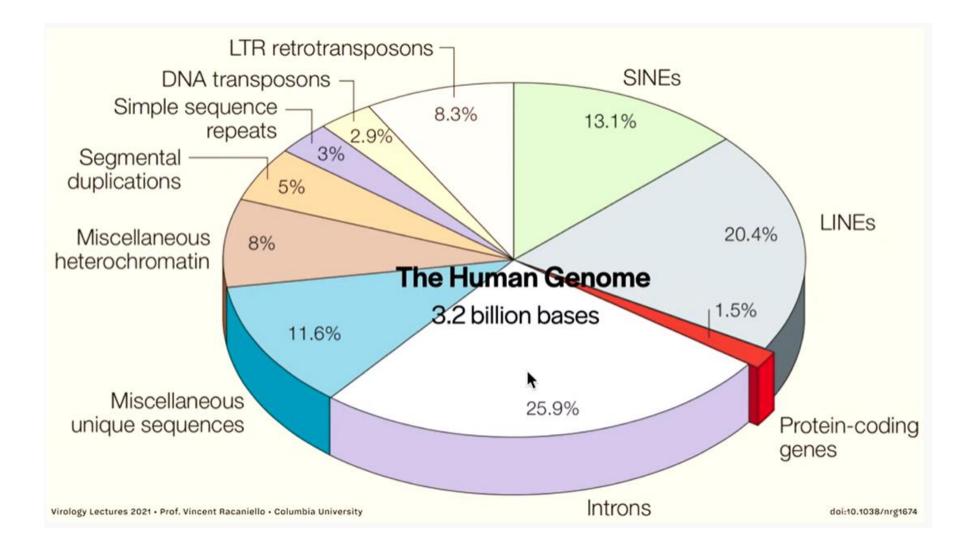
For 50 years the term 'gene' has been synonymous with regions of the genome encoding mRNAs that are translated into protein. However, recent genome-wide studies have shown that the <u>human genome is pervasively transcribed and produces many thousands</u> <u>of regulatory non-protein-coding RNAs (ncRNAs)</u>, including microRNAs, small interfering RNAs, and various classes of long ncRNAs.

It is now clear that these RNAs fulfil critical roles as transcriptional and post-transcriptional regulators and as guides of chromatinmodifying complexes. Redifining determinants of host-virus interaction complexity: lesson from human genome

### **Central Dogma of Biology: Classic View**









Liu et al., 2023, Cell 186, 287–304 January 19, 2023 © 2022 The Author(s). Published by Elsevier Inc. https://doi.org/10.1016/j.cell.2022.12.017

Young



Cell senescence



Younger endogenous retroviruses make us older

Eytan Zlotorynski 🖂

 Nature Reviews Molecular Cell Biology
 24, 165 (2023)
 Cite this article

 373
 Accesses
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 Altmetric
 Metrics

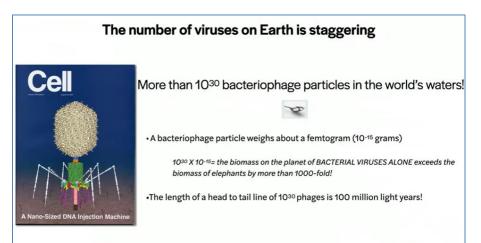
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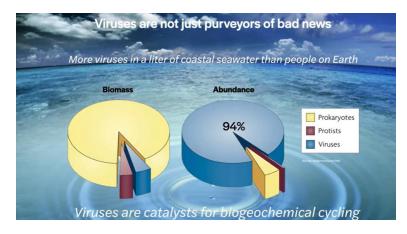
Old

### **Highlights**

- Derepression of the endogenous retrovirus contributes to programmed aging
- Upregulation of HERVK triggers the innate immune response and cellular senescence
- Extracellular HERVK retrovirus-like particles induce senescence in young cells
- Endogenous retrovirus serves as a potential target to alleviate aging

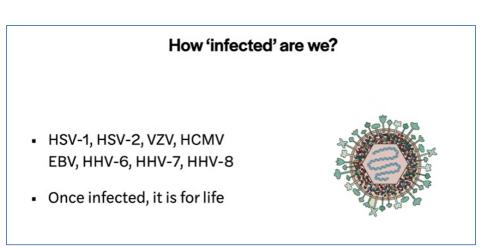
Redifining determinants of host-virus interaction complexity: lesson from virome

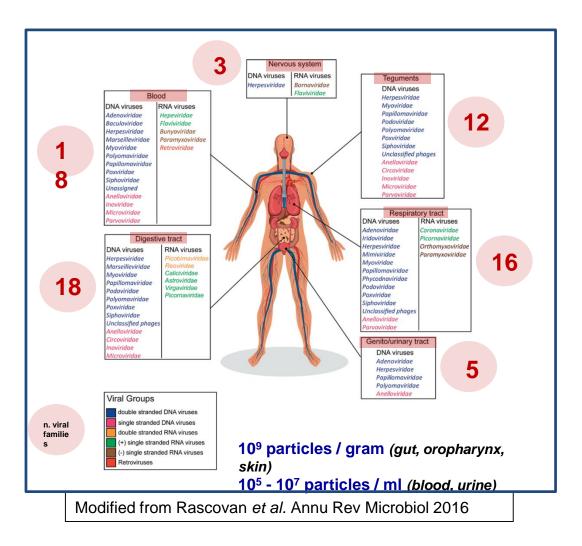


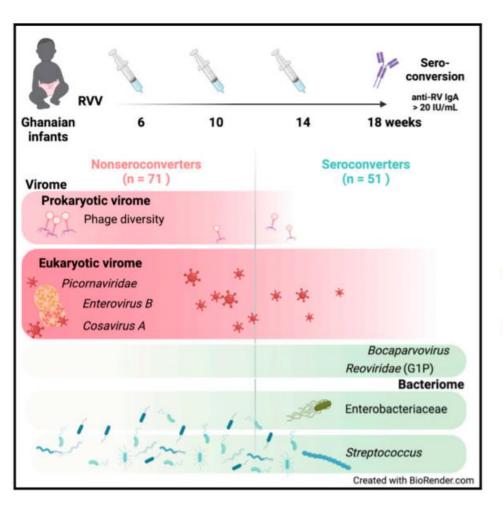


#### There are ~10 $^{16}$ HIV genomes on the planet today









**Clinical and Translational Report** 

### **Cell Host & Microbe**

#### Enteric virome negatively affects seroconversion following oral rotavirus vaccination in a longitudinally sampled cohort of Ghanaian infants

Kim et al., 2022, Cell Host & Microbe 30, 110–123 January 12, 2022 © 2021 The Authors. Published by Elsevier Inc. https://doi.org/10.1016/j.chom.2021.12.002

Longitudinal analysis of microbiota of Ghanaian infants receiving rotavirus vaccine

- Streptococcus and Enterobacteriaceae taxa positively associate with RVV seroconversion
- Enterovirus B, Cosavirus A, and phage richness negatively associate with RVV serostatus

# Virome composition may affect vaccine efficacy

Clinical Microbiology and Infection 25 (2019) 133-135





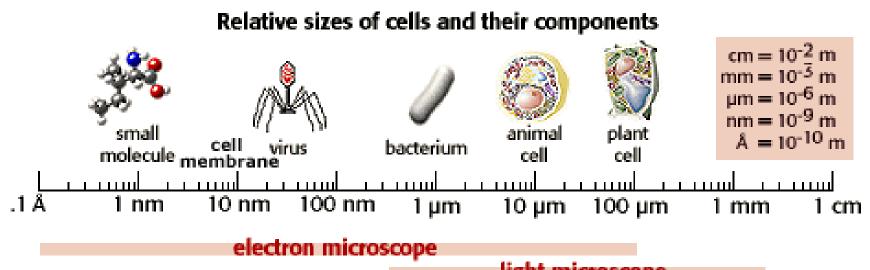
Virology: a scientific discipline facing new challenges

G. Antonelli<sup>\*</sup> M. Pistello

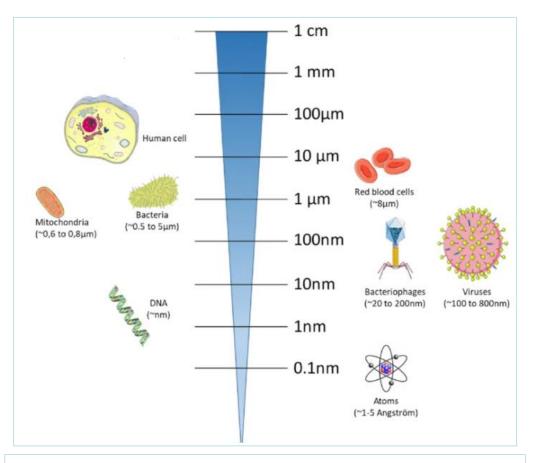
 Together with viral pathogens able to cause symptomatic chronic infections, there are viruses which constitute a stable (and little-recognized) part of our virome. Among the many advancements and the progress we have achieved in past decades, the findings that, to our opinion, have most blurred the fundamentals of traditional virology are the following:

• can be considered as the set of all viruses, eukaryotic and prokaryotic, present in the human body. The virome includes viruses that infect host cells, viruses that infect the majority of other types of microorganisms harboured by the body, and virusrelated genetic elements in our chromosomes [1]. Viruses, which can no longer be invariably considered pathogens, interact with the host and other members of the microbial communities (Archaea, bacteria and eukaryotes) in a variety of complex and meaningful ways. These complex interactions have just begun to be investigated but it is common opinion that they profoundly affect health status [2]. Due to the existence of commensal viruses, we should probably redefine chronic viral infections and focus our attention on the host rather than on infectious agents to dissect disease determinants. From a policy point of view, we must conclude that the integration of 'different' disciplines (virology, bacteriology, mycology, immunology, genetics, molecular biology) is essential for a more complete understanding of the role of the virome and its trans-kingdom interactions in the modulation of health and disease status.

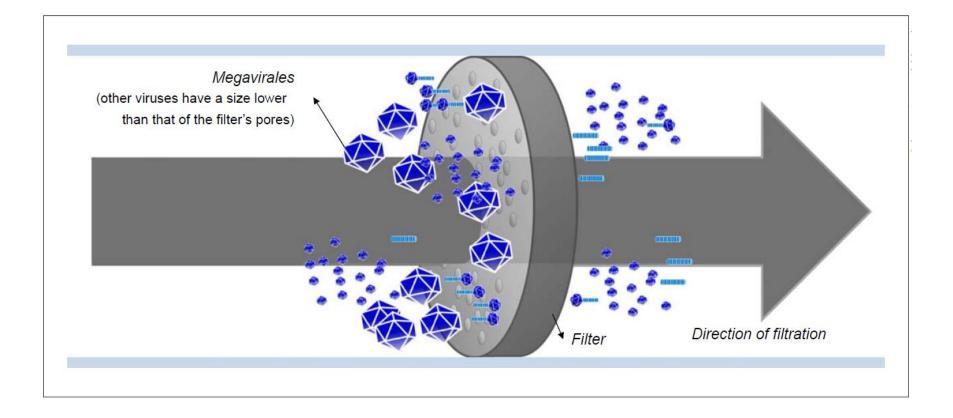
# Redifining the size of the viruses: lesson from Mimivirus



light microscope



The selection of filters for sterilization must account for the size range of the contaminants to be excluded. The most commonly used filter is composed of nitrocellulose and has a pore size of 0.22µm. The size of the bacteria ranges from 0.3 to 0.5 µm whereas the size of the viruses ranges from 20 nm to 0.36 µm. Thus a filter of 0.22µm retains all bacteria and spores but not all viruses.

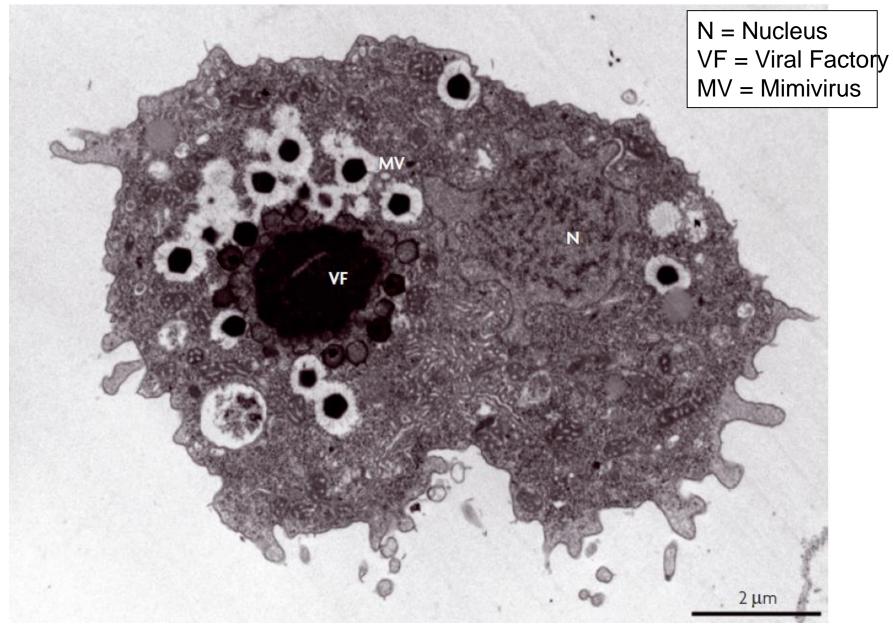


# A Giant Virus in Amoebae

Bernard La Scola,<sup>1</sup> Stéphane Audic,<sup>2</sup> Catherine Robert,<sup>1</sup> Liang Jungang,<sup>1</sup> Xavier de Lamballerie,<sup>3</sup> Michel Drancourt,<sup>1</sup> Richard Birtles,<sup>1</sup> Jean-Michel Claverie,<sup>2\*</sup> Didier Raoult<sup>1\*</sup>

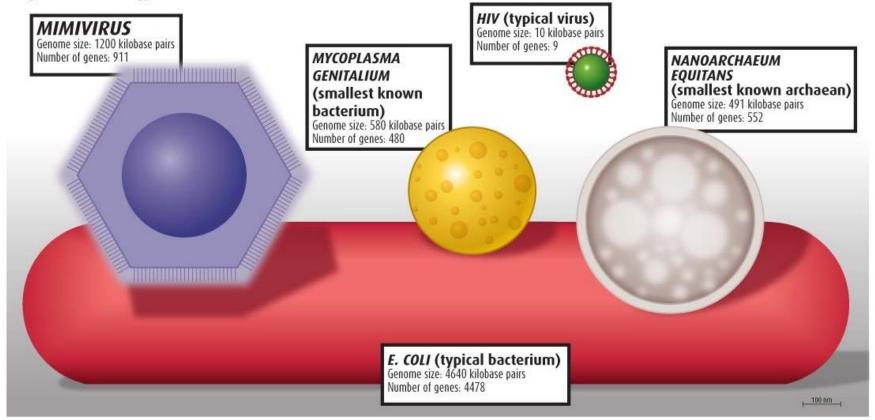
Study of this microorganism within Acanthamoeba polyphaga (2) revealed a characteristic viral morphology with mature particles of 400 nm in diameter and surrounded by an icosahedral capsid. This structure is consistent with the finding that Mimivirus is not filterable through 0.2- $\mu$ m pore size filters. No envelope was observed, but 80-nm fibrils attached to the capsid were visible (fig. S1). A typical virus developmental cycle, including an eclipse phase, was observed

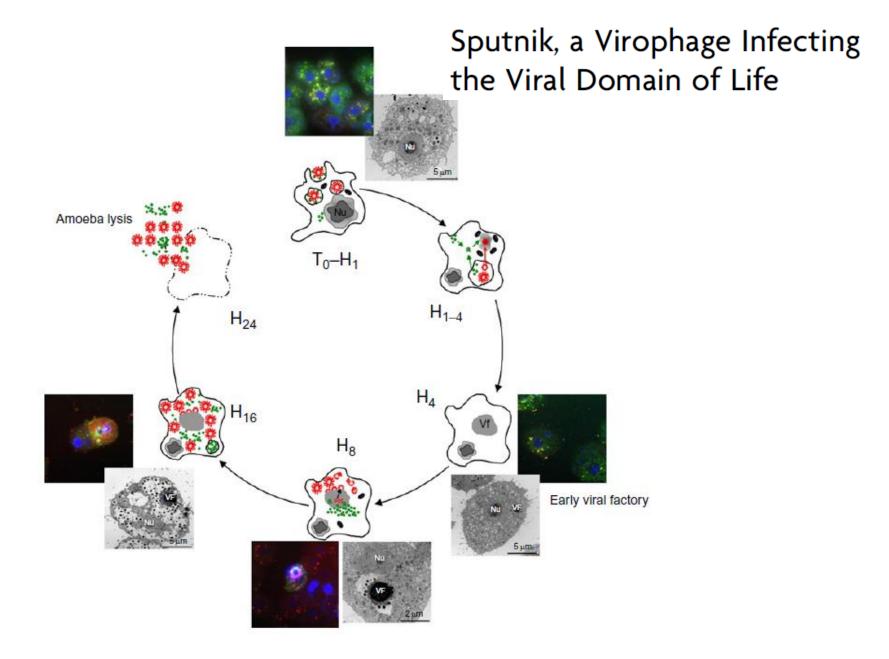
### Mimivirus - NCLDV (NucleoCytoplasmic Large DNA Virus)



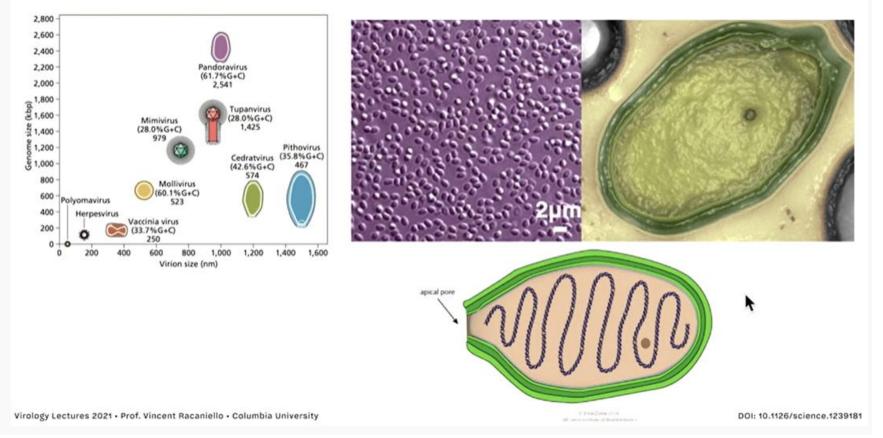
## NCLDV (NucleoCytoplasmic Large DNA Virus)

The giant virus Mimivirus is bigger than some bacteria and archaeans



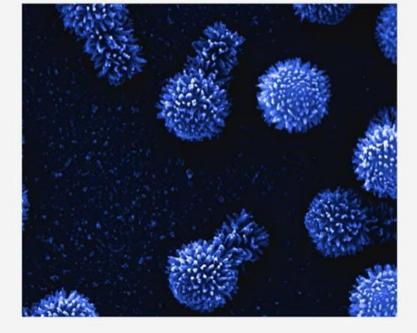


### Pandoravirus



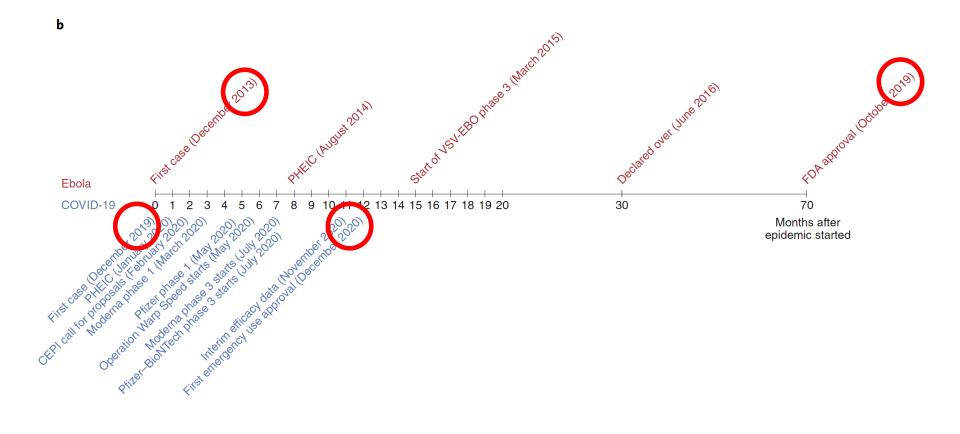
### Tupanvirus - Only the ribosome is lacking

- 20 aminoacyl tRNA syntheses
- 70 tRNAs
- Multiple translation initiation and elongation proteins
- Multiple translation related genes
   e.g. for tRNA, mRNA maturation
- Most complete translational apparatus of the virosphere



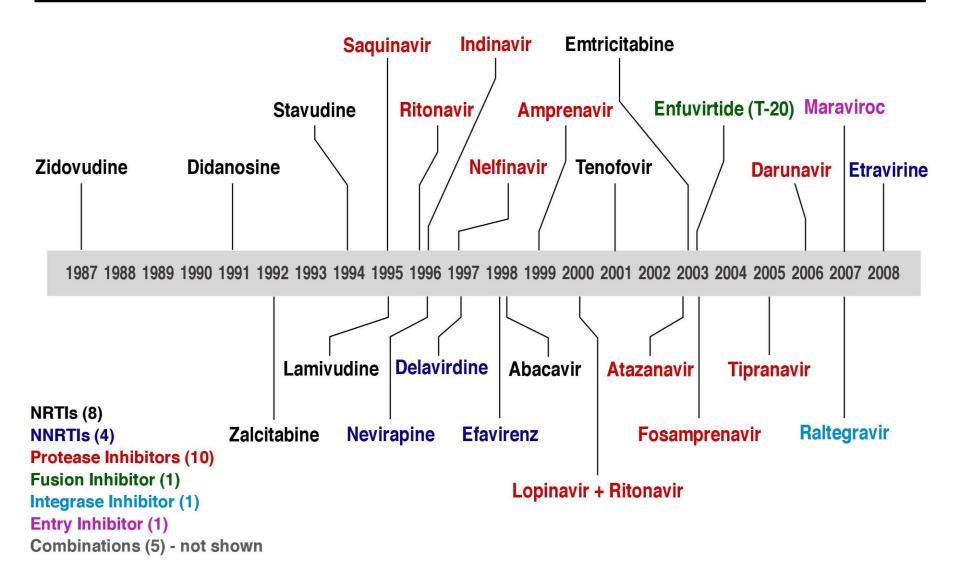
Virology Lectures 2021 • Prof. Vincent Racaniello • Columbia University

Redifining the medical intervention to fight viral diseases : lesson from HIV, HCV and SARS-CoV-2

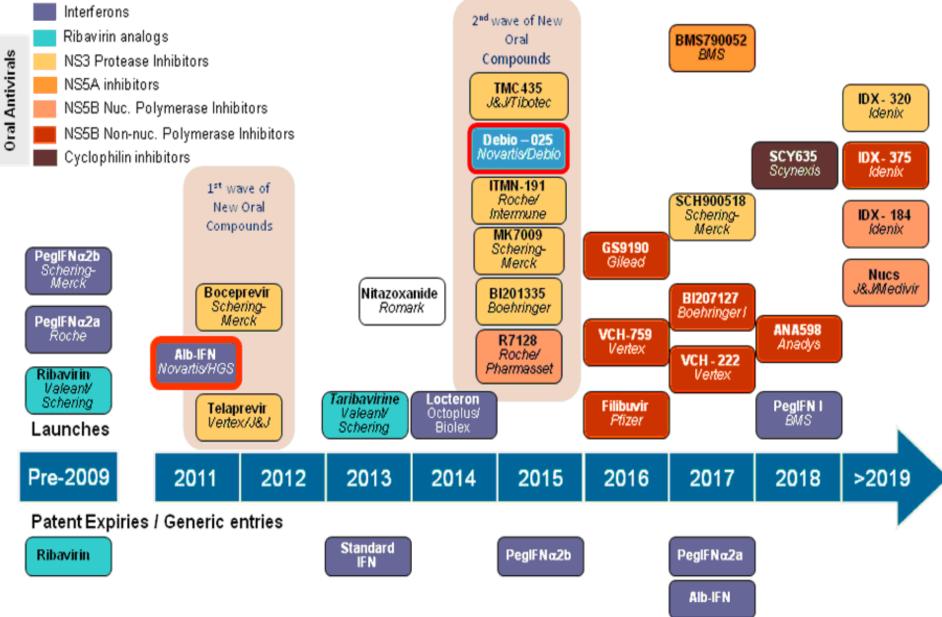


NATURE MEDICINE | VOL 27 | APRIL 2021 | 591-600 |

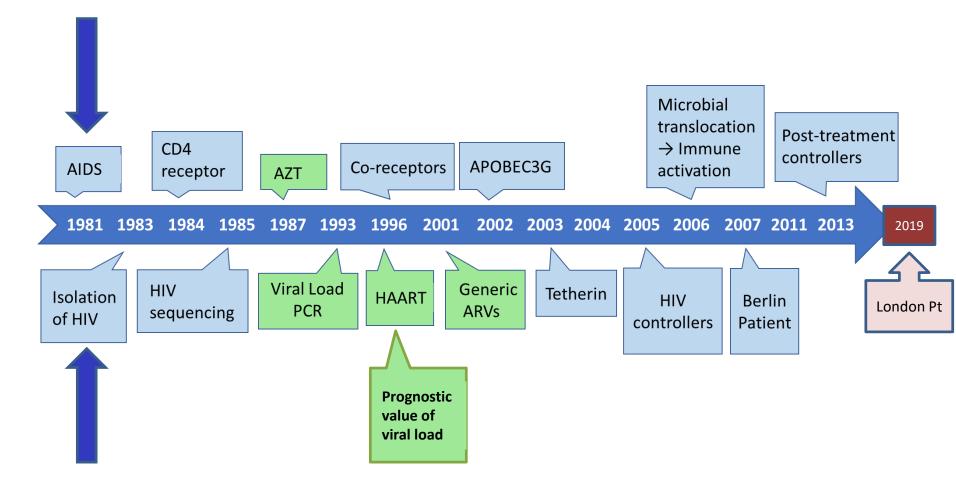
## **30 FDA-Approved Antiretroviral Drugs**







#### MILESTONES IN HIV-AIDS RESEARCH (VIROLOGICAL, DIAGNOSTIC AND PATHOGENETIC ISSUES)



# HIV Cure

Timothy Ray Brown (Berlin patient) and the "London patient" shared similar medical circumstances.

They were both HIV-1-positive and receiving ART therapy. They both eventually developed a blood cancer (acute myeloid leukemia and Hodgkin's lymphoma, respectively), which was treated with chemotherapy and various other therapeutics.

Both ultimately required a bone marrow transplant to replenish the blood stem cells that had been destroyed during chemotherapy.

In both cases, doctors used bone marrow cells from a donor who was homozygous for a mutation in the gene encoding the HIV co-receptor CCR5 (CCR5  $\Delta$ 32/ $\Delta$ 32), because this genotype confers resistance to HIV-1 infection. Both patients were cured of their cancer.

Both patients were also cured of their HIV infection, as evidenced by the absence of virus in their blood many months after termination of ART.

Brief Communication Open Access Published: 20 February 2023

### In-depth virological and immunological characterization of HIV-1 cure after CCR5 $\Delta$ 32/ $\Delta$ 32 allogeneic hematopoietic stem cell transplantation

Björn-Erik Ole Jensen <sup>⊠</sup>, Elena Knops, Leon Cords, Nadine Lübke, Maria Salgado, Kathleen Busman-Sahay, Jacob D. Estes, Laura E. P. Huyveneers, Federico Perdomo-Celis, Melanie Wittner, Cristina Gálvez, Christiane Mummert, Caroline Passaes, Johanna M. Eberhard, Carsten Münk, Ilona Hauber, Joachim Hauber, Eva Heger, Jozefien De Clercq, Linos Vandekerckhove, Silke Bergmann, Gábor A. Dunay, Florian Klein, Dieter Häussinger, … Guido Kobbe + Show authors

 Nature Medicine
 (2023)
 Cite this article

 8785
 Accesses
 1614
 Altmetric
 Metrics

#### Third person free of HIV after transplant

A 53-year-old man in Germany has become at least the third person with HIV to be declared cleared of the virus after undergoing a procedure that replaced his bone marrow cells with HIV-resistant stem cells from a donor. The man, who is being referred to as the 'Düsseldorf patient', received the treatment after being diagnosed with acute myeloid leukaemia. Stem-cell transplants are not suitable for people who don't need them to treat blood cancer because they are risky and difficult: the man called his treatment a "very rocky road".

## <u>VIROLOGY</u>

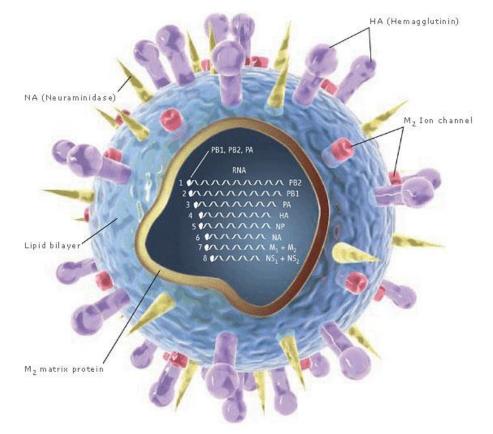
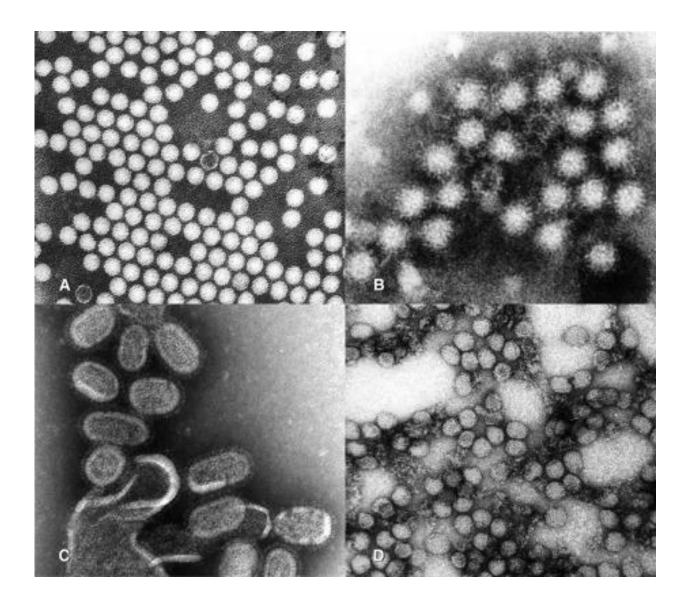


Illustration: Chris Bickel/Science. Reprinted with permission from Science Vol. 312, page 380 (21 April 2006) © 2006 by AAAS

# General characteristics of virus

- Viruses are unique in nature.
- They are the smallest of all self-replicating organisms, historically characterized by their ability to pass through filters that retain even the smallest bacteria.
- In their basic form, viruses consist solely of a small segment of nucleic acid encased in a simple protein shell.
- Viruses have no metabolism of their own, but rather are obliged to invade cells and parasitize subcellular machinery, subverting it to their own purposes.



**Definitions:** 

*Virion* - physical particle of the virus

*Core* - nucleic acid and tightly associated proteins within the virion *Capsid* - protein shell around NA or core

*Capsomere* - protein subunit making up the capsid

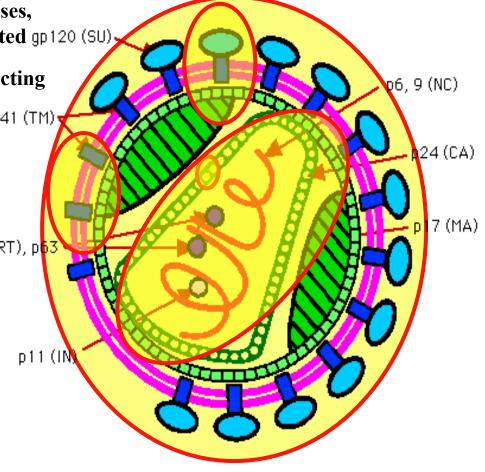
*Nucleocapsid* - core and capsid

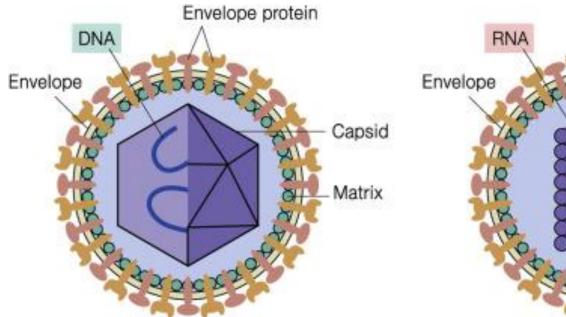
*Envelope* - lipid membrane found on some viruses, often derived by budding from infected gp120 (SU)

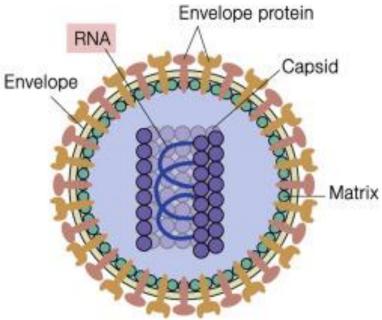
cells. *Peplomer* - ("spike")- morphological unit projecting from the envelope or surface of a naked virion gp41 (TM)

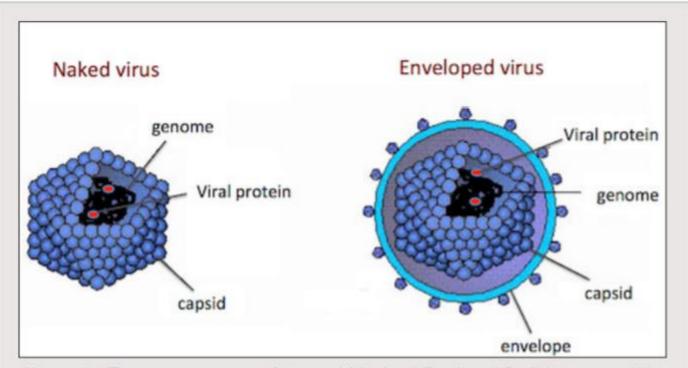
**Definition Usage:** <u>Viruses</u> outside of cells are usually metabolically inert. <u>Virions</u> consist of either DNA of RNA (constituting the genome) usually complexed with protein into a <u>core</u>, surrounded by a protein coat p55 (RT), p53 called the <u>capsid</u>, altogether called a <u>nucleocapsid</u>. The <u>capsid</u> is composed of identical subunits called <u>capsomeres</u>. It serves to protect and to ensure efficient delivery of the nucleic acid genome to new cells. Virally encoded <u>peplomer</u> spikes found protruding from the envelope or at the surface of a naked virion serve the critical function of receptor recognition necessary for binding and entry into susceptible cells. For many viruses, isolated viral nucleic acid is by itself infectious, albeit less so than when it is encapsidated.

#### VIRUS STRUCTURE



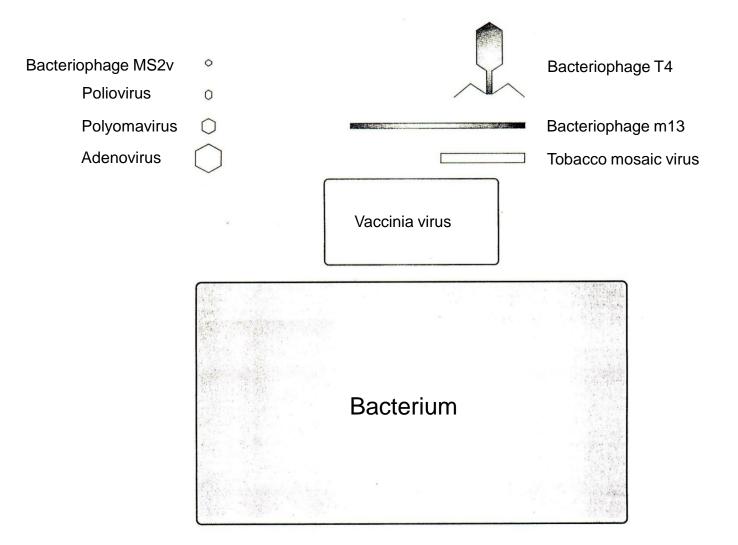


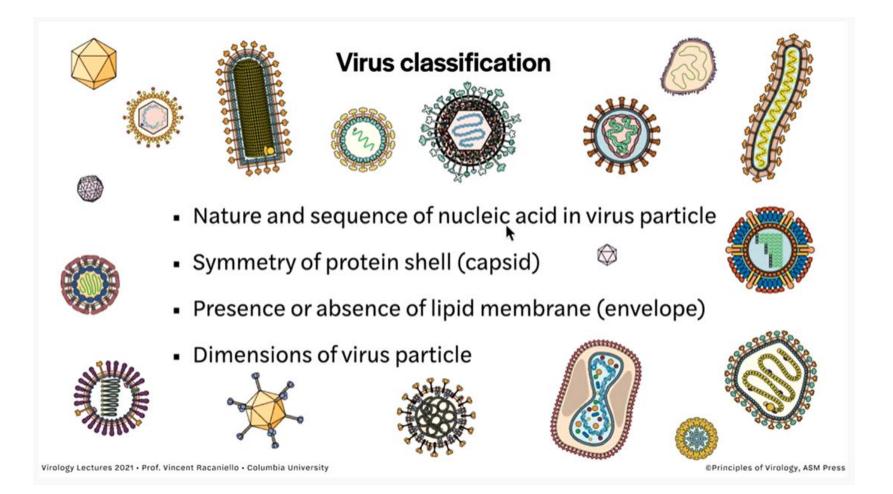




**Figure 1:** There are two types of viruses: Naked and Enveloped. Both have a capsid, but only the enveloped viruses have an envelope. All viruses also have a genome (RNA or DNA), and a few viral proteins that they need for replication — these are proteins that the host cells lack.

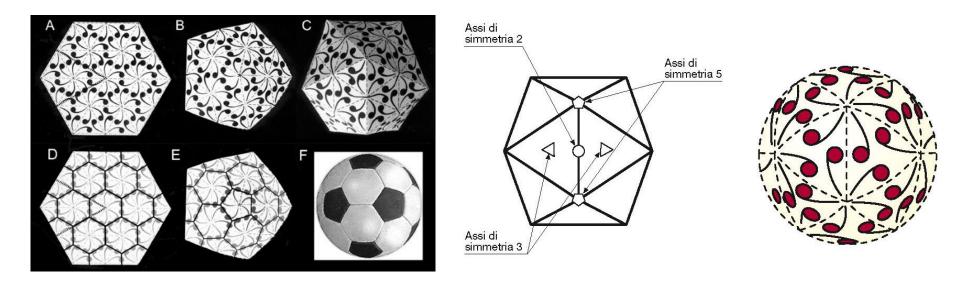
#### Viruses come in many shapes and sizes



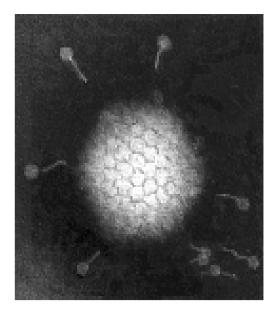


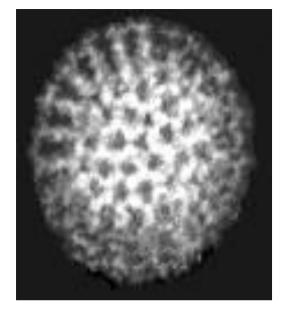
# **Icosahedral symmetry**

- An icosahedron is composed of 20 facets, each an equilateral triangle, and 12 vertices; because of the axes of rotational symmetry is said to have 5:3:2 symmetry.
- STRUCTURE UNITS are the smallest functional equivalent building units of the capsid, thus individual proteins.
- CAPSOMERS are morphological units seen on the surface of particles and represent clusters of structure units
- For icosahedral viruses, there are two kinds of capsomers called pentamers and hexamers



## **Electronmicrographs**





#### Adenovirus

Rotavirus

(courtesy of Linda Stannard, University of Cape Town, S.A.)

# Helical symmetry

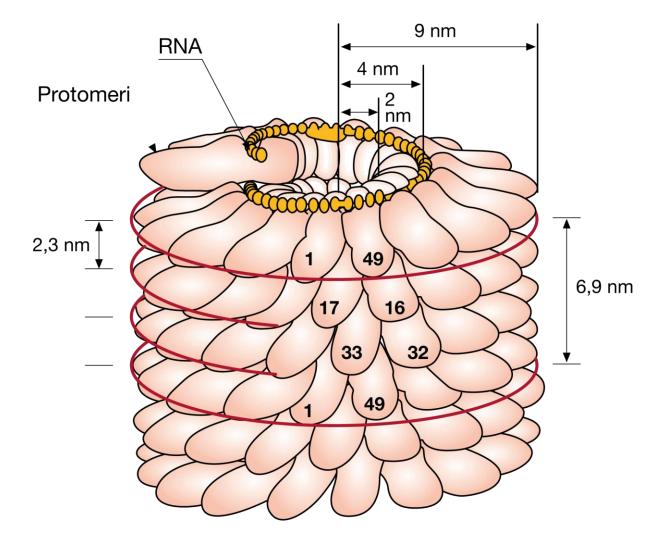


Figura 35.4 Struttura del virus del mosaico del tabacco.

# **Principles of Virus Structure**

- The broadest distinction is between the so-called "enveloped" and "nonenveloped" viruses that is, those that contain or do not contain, respectively, a lipid-bilayer membrane.
- Further categorization of virus structures depends on details of their molecular organization.
- Types of viral particles:
  - Icosahedral symmetry nonenveloped
  - Icosahedral symmetry enveloped
  - Helical symmetry nonenveloped
  - Helical symmetry enveloped

#### Structures and Organization of Viral Genomes

- Genomes of animal viruses differ greatly in size, from as small as 3 kb to as large as 250-280 kb and the consequent differences in genetic capacity mean that viruses from different families vary widely in terms of how many of the functions necessary for virus replication they can encode for themselves
- Types of viral genome:
  - double stranded DNA(dsDNA)
  - single stranded DNA(ssDNA)
  - ➤ single stranded positive-sense (+) RNA
  - ➤ single stranded negative-sense (-) RNA
  - double stranded RNA

## **DNA VIRUS**

- Single strand DNA (ssDNA)
- Double strand DNA (dsDNA):
  - linear
  - circular
  - partially double strand circular

## **RNA VIRUS**

#### •Single strand RNA (ssRNA):

- Nonsegmented Genomes
- Segmented
- •Double strand RNA (dsRNA):
  - Segmented

#### **Virus Properties and Their Use in Taxonomy**

- Genome structure
  - Nature of the viral genome (DNA or RNA)
  - Strandedness
  - Size kb/kbb
  - Conformation (linear, circular)
  - Polarity (positive sense, negative sense, ambisense)
  - Number of segments

#### **Virus Properties and Their Use in Taxonomy**

- Replication strategy
  - mechanisms of transcription
  - mechanisms of translation
  - post-transcriptional modifications
  - protein localization

•

- intracellular targeting and assembly of virion components
- post-assembly modifications and virus release

#### **Virus Properties and Their Use in Taxonomy**

- Virion morphology:
  - size
  - shape
  - capsid symmetry
  - presence or absence of an envelope
- Virion physical properties:
  - genome structure
  - sensitivity to physical or chemical insults
  - specific features of viral lipids, carbohydrates, and structural nonstructural proteins
- Antigenic properties
- Biologic properties:
  - replication strategy
  - host range
  - mode of transmission
  - pathogenicity
  - geographic distribution
  - tissue tropism
  - histology

# **ICTV Nomenclature**

**ORDERS (...-VIRALES)** HERPESVIRALES

FAMILIES (...-VIRIDAE) HERPESVIRIDAE

SUBFAMILIES (...-VIRINAE) BETAHERPESVIRINAE

**GENERA (.....VIRUS)** CYTOMEGALOVIRUS

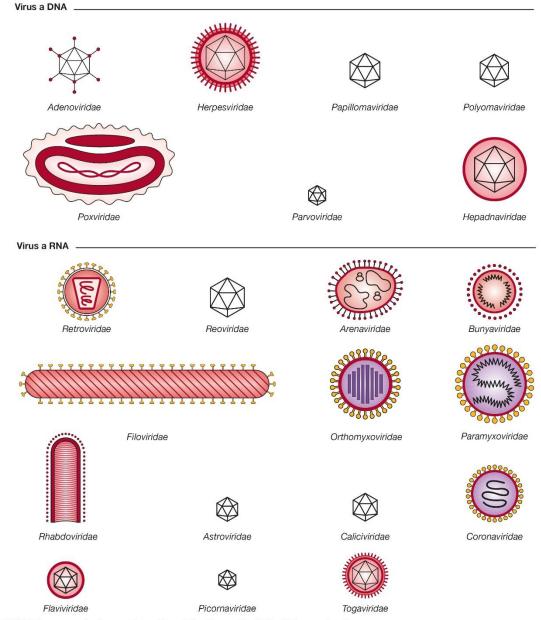


Figura 35.1 Rappresentazione schematica delle diverse famiglie di virus animali.

Gruppo	Nucleocapside	Pericapside	Virione	Genoma
dsDNA (I)				
Adenoviridae	Icosaedrico	No	Icosaedrico	1 lineare, 30-42 kb
Herpesviridae	Icosaedrico	Sì	Sferico con tegumento	1 lineare, 120-220 kb
Papillomaviridae	Icosaedrico	No	Icosaedrico	1 circolare, 8 kb
Polyomaviridae	Icosaedrico	No	Icosaedrico	1 circolare, 5 kb
Poxviridae	Complesso	Sì	Ovoidale	1 lineare, 130-375 kb
ssDNA (II)				
Parvoviridae	Icosaedrico	No	Icosaedrico	1 lineare, 130-375 kb
Anelloviridae	Icosaedrico	No	Icosaedrico	1 circolare, 3-4 kb
dsRNA (III)				
Reoviridae	Icosaedrico	No	Icosaedrico	10-12 lineare, 18-30 kb
ssRNA + (IV)				
Arteriviridae	Icosaedrico	Sì	Sferico	1 lineare, 13 kb
Astroviridae	Icosaedrico	No	Icosaedrico	1 lineare, 7-8 kb
Caliciviridae	Icosaedrico	No	Icosaedrico	1 lineare, 8 kb
Coronaviridae	Elicoidale	Sì	Pleiomorfo	1 lineare, 20-33 kb
Flaviviridae	Poliedrico	No	Sferico	1 lineare, 10-12 kb
Picornaviridae	Icosaedrico	No	Icosaedrico	1 lineare, 7-8 kb
Togaviridae	Icosaedrico	Sì	Sferico	1 lineare, 10-12 kb
Hepeviridae	Icosaedrico	No	Icosaedrico	
ssRNA – (V)				
Arenaviridae	Elicoidale	Sì	Sferico	2 lineare, 5-7 kb
Bornaviridae	n.d.	Sì	Sferico	1 lineare, 9 kb
Bunyaviridae	Elicoidale	Sì	Sferico	3 lineare, 10-23 kb
Filoviridae	Elicoidale	Sì	Pleiomorfo filamentoso	1 lineare, 19 kb
Orthomyxoviridae	Elicoidale	Sì	Pleiomorfo sferico	8 lineare, 12-15 kb
Paramyxoviridae	Elicoidale	Sì	Pleiomorfo sferico	1 lineare, 15-16 kb
Rhabdoviridae	Elicoidale	Sì	A proiettile	1 lineare, 11-15 kb
ssRNA RT (VI)				
Retroviridae	Sferico, troncoidale	Sì	Sferico	1 RNA dimerico, 7-11 kb
dsDNA RT (VII)				
Hepadnaviridae	Icosaedrico	Sì	Sferico	1 DNA parz. circolare, 3

Abbreviazione: n.d., non determinato.