VIRUSES - Introduction, structure and classification

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History

- ## Watson and Crick, 1956

  easier to force cell to make large number of a few different small proteins rather than a few copies of a very large protein (information storage by RNA/DNA)

  stated: nucleic acid in virions was insufficient to code for more than a few protein molecules of limited size

  The only way to build a protein shell was to use the same type of molecule over and over again

  -hence their theory of identical subunits

History II

The introduction of NEGATIVE STAINING (Brenner and Horne, 1959) revolutionized the field of electron microscopy of viruses.
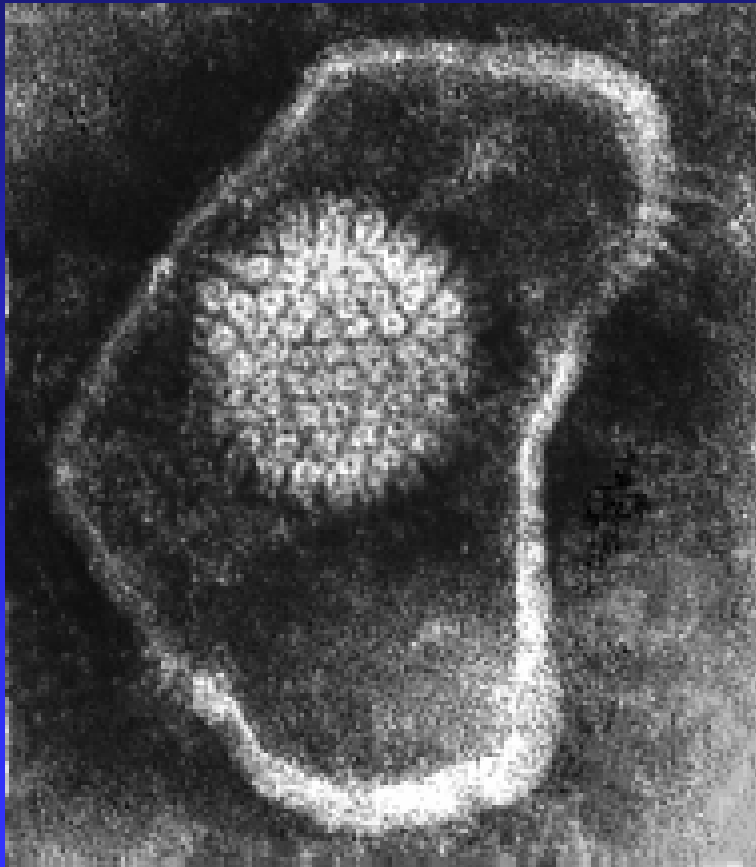
Potassium phosphotungstate- electron dense salt
Information about the architecture

First high resolution micrographs of negatively stained icosahedral viruses were:
Horne et al., 1959 - adenovirus
Huxley and Zubay, 1960 - turnip yellow mosaic virus

Herpesvirus Particle

HSV-2

All herpesviruses identical morphology

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

OUTLINE

1.What are they?
2. What do they look like?
3. How are they classified?
4. How important are they?

Viruses

- Virus comes from the Latin word for poison

- In two decades HIV has killed >18 million people

What is a virus?

- Viruses are cellular organisms whose genomes consist of nucleic acid, and which must replicate inside host cells using host metabolic machinery to form a pool of components which assemble into particles called VIRIONS, which serve to protect the genome and to transfer it to other cells.
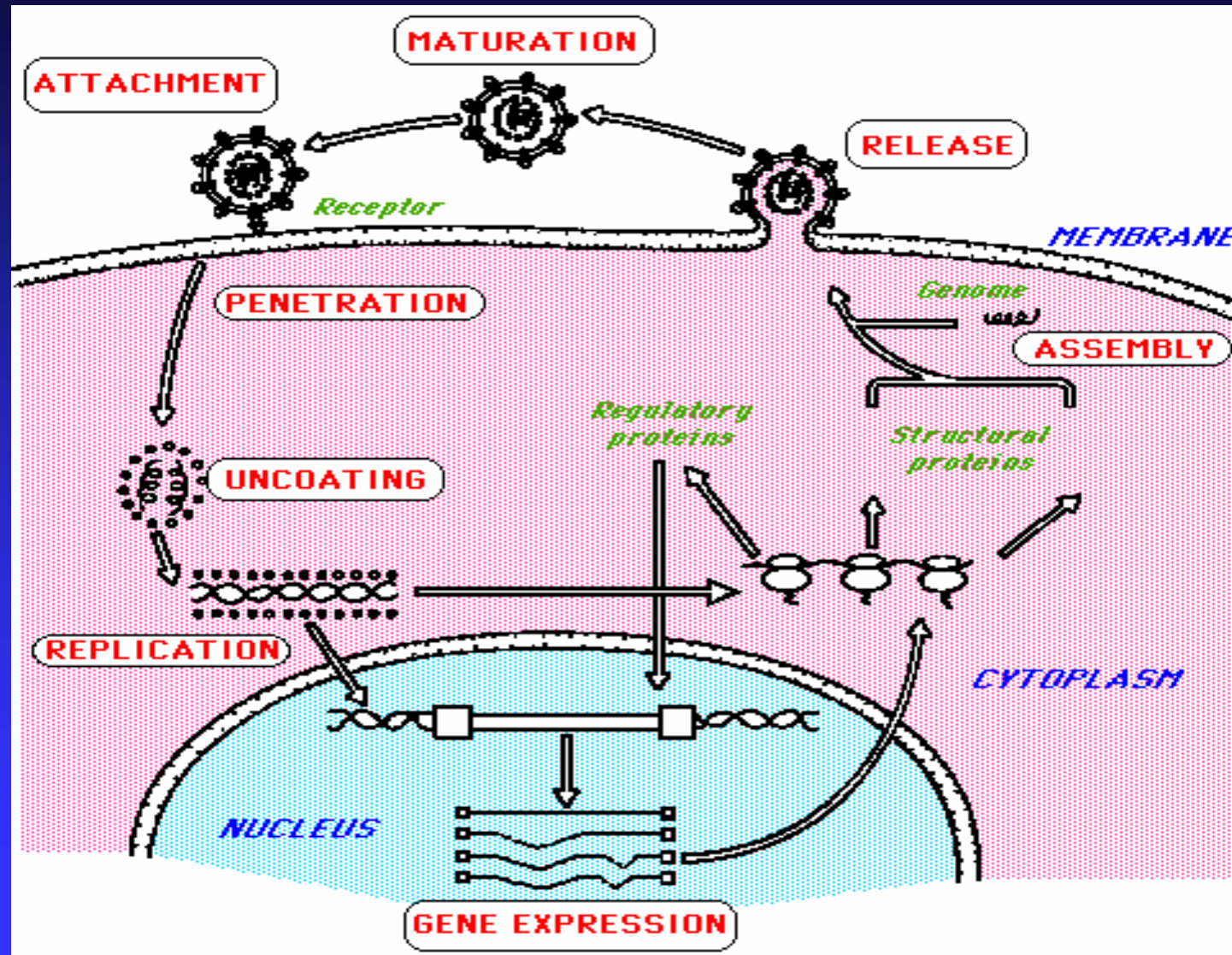
What is a virus?

- "DNA is just DNA. The only thing that distinguishes viral DNA from host DNA is its expected method of passing into future generations. ``Legitimate'' host DNA is just DNA that aspires to pass into the next generation via the orthodox route of sperm or egg. ``Outlaw'' or parasitic DNA is just DNA that looks to a quicker, less cooperative route to the future." Richard Dawkins, The selfish gene.

Is a virus living?

- viruses do not respire
- they do not move
- and nor do they grow
- however, they do most certainly reproduce

- By older, more zoologically biased criteria, then, viruses are not living. However, this results from a "top down" sort of definition, which has been modified over years to take account of smaller and smaller things.
- If one defines life from the bottom up – that is, from the simplest forms capable of displaying the most essential attributes of a living thing – one very quickly realises that the only real criterion for life is: – The ability to replicate

     – and that only systems that contain nucleic acids – in the natural world, at least – are capable of this phenomenon
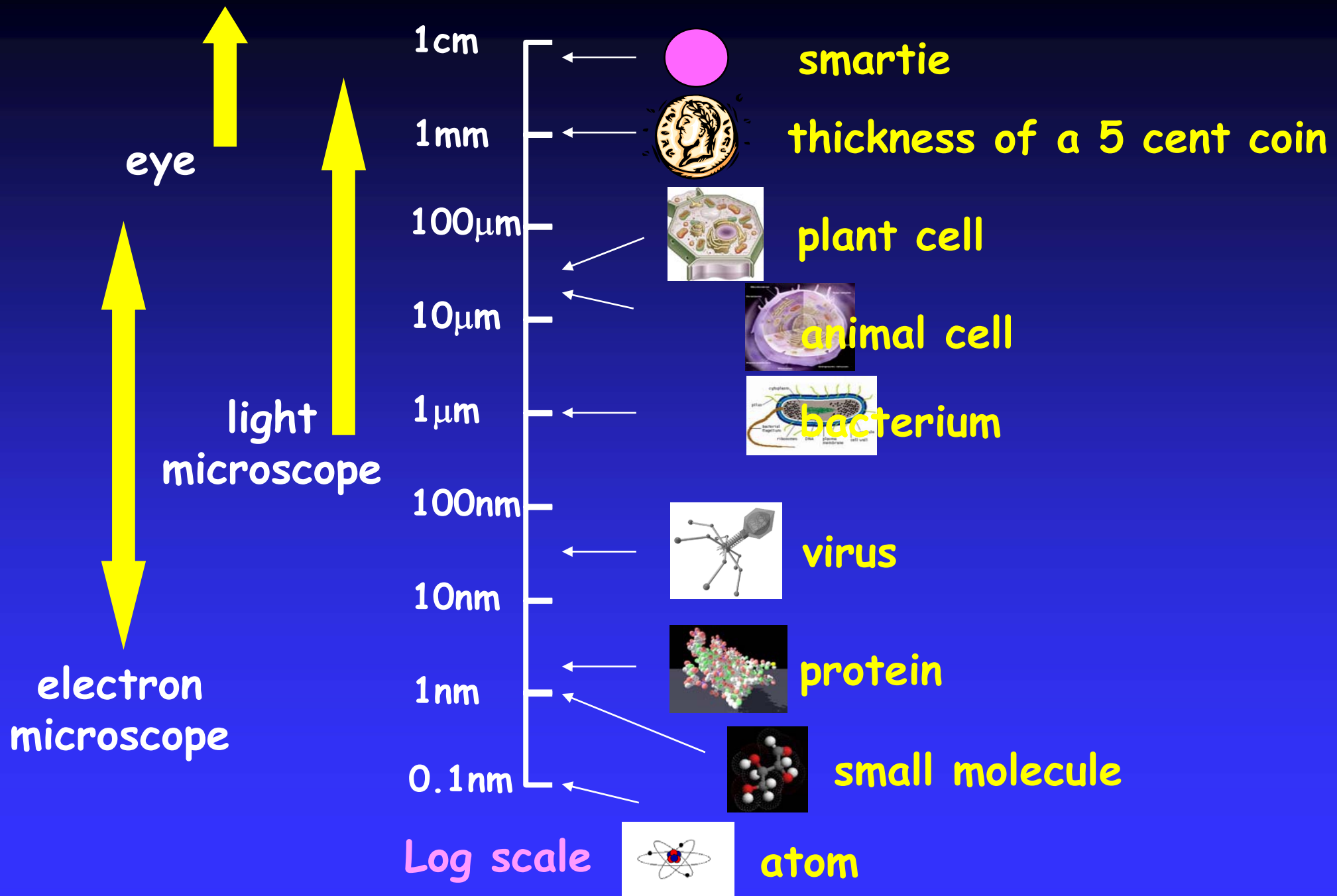
Virus replication

What is a virion?

- **VIRIONS** are **virus particles**:

  - **INERT CARRIERS of the genome**
  - **ASSEMBLED inside cells, from virus-specified components:**
  - **They do not GROW, and do not form by DIVISION.**

- **They may be regarded as the EXTRACELLULAR PHASE of the virus:**
- **they are exactly analogous to "spacecraft"**
- **in that they take viral genomes from cell to cell**
- **& they protect the genome in inhospitable environments.**

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eye

light microscope

electron microscope

1cm — smartie

1mm — thickness of a 5 cent coin

100μm — plant cell

10μm — animal cell

1μm — bacterium

100nm — virus

10nm

1nm — protein

0.1nm — small molecule

Log scale — atom

Definitions in viral structure

- The **CAPSID** denotes the protein shell that encloses the nucleic acid. It is built of structural units.
- **STRUCTURE UNITS** are the smallest functional equivalent building units of the capsid.
- **CAPSOMERS** are morphological units seen on the surface of particles and represent clusters of structure units.
- The capsid together with its enclosed nucleic acid is called the **NUCLEOCAPSID**.
- The nucleocapsid may be invested in an **ENVELOPE** which may contain material of host cell as well as viral origin.

- The VIRION is the complete infective virus particle
- INERT CARRIERS of the genome
  - Simplest form: Virion structure = nucleic acid contained within a protective protein coat
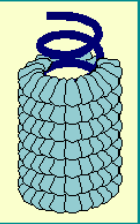
Virion Architecture

Architecture of virions regardless of host is based on two simple themes:

Sphere – normally in the form icosahedron (cubical)
- Best way of producing a shell of equivalently bonded identical structures
- Minimum free energy state
- Strong structure that can enclose a maximal volume

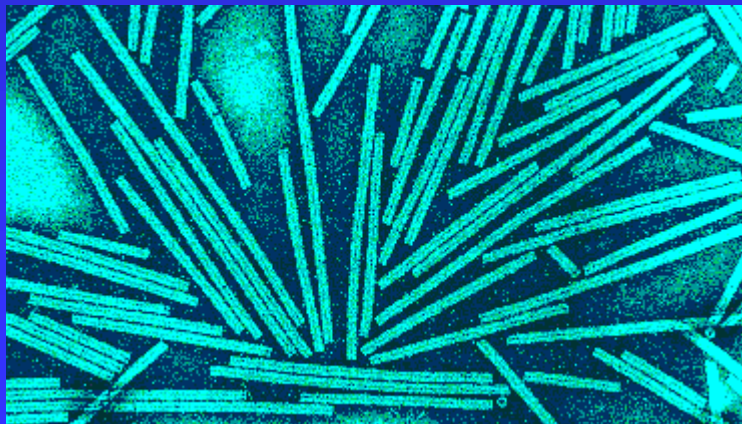**Helix** – cylindrical shape (spiral staircase)
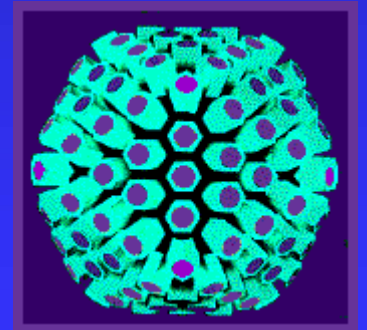
Helical viral structure II

• Plant virus with this structure are rod shaped and non-enveloped, while animal viruses tend to be long and flexible

• TMV, 2130 capsomers assemble into a helx, 6kb of RNA genome fits into a groove formed on assembly.

Examples
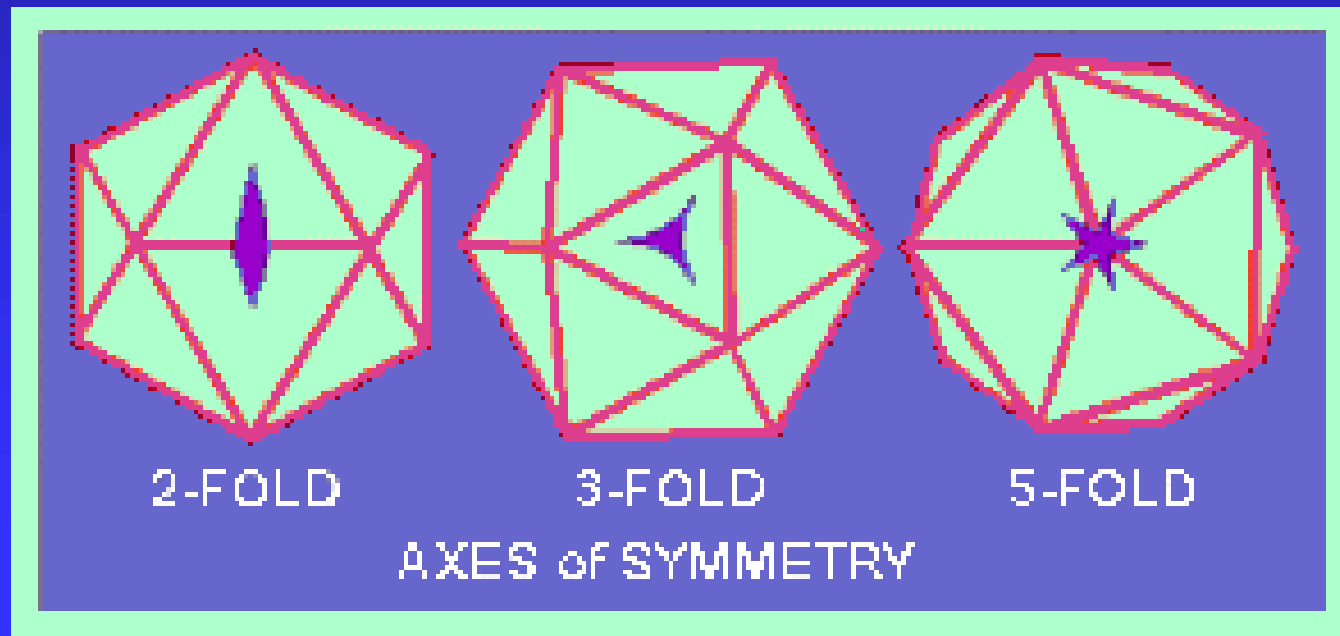Ebola
Lassa
Mumps/Measles
Rabies
SARS

Virion Architecture – icosahedron I

An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)

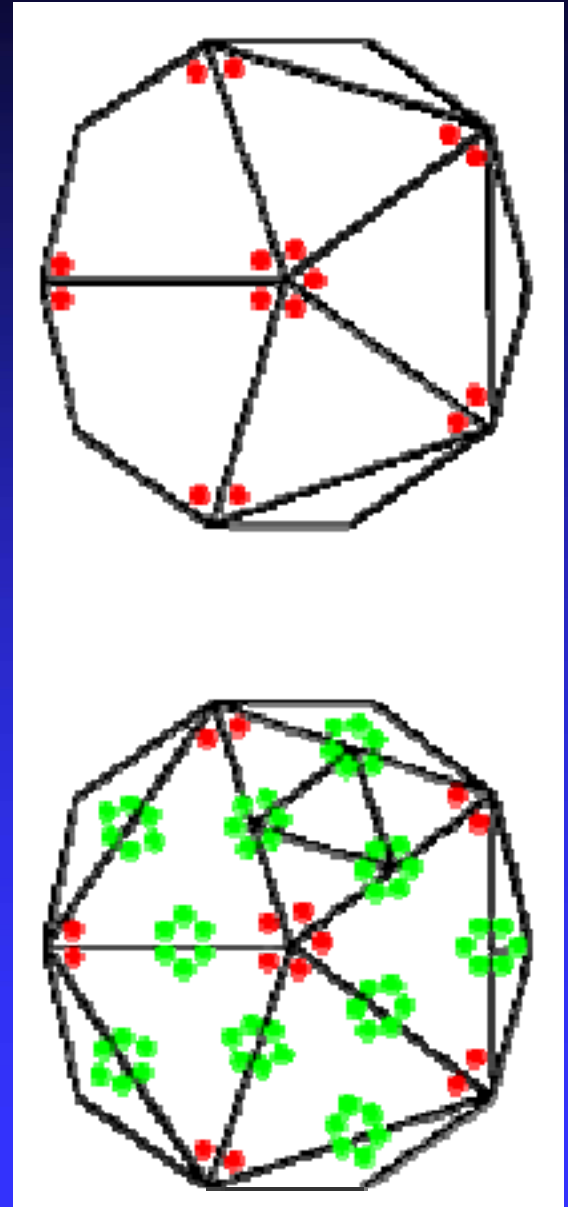Because of the axes of rotational symmetry is said to have 5:3:2 symmetry

2-FOLD   3-FOLD   5-FOLD

AXES of SYMMETRY
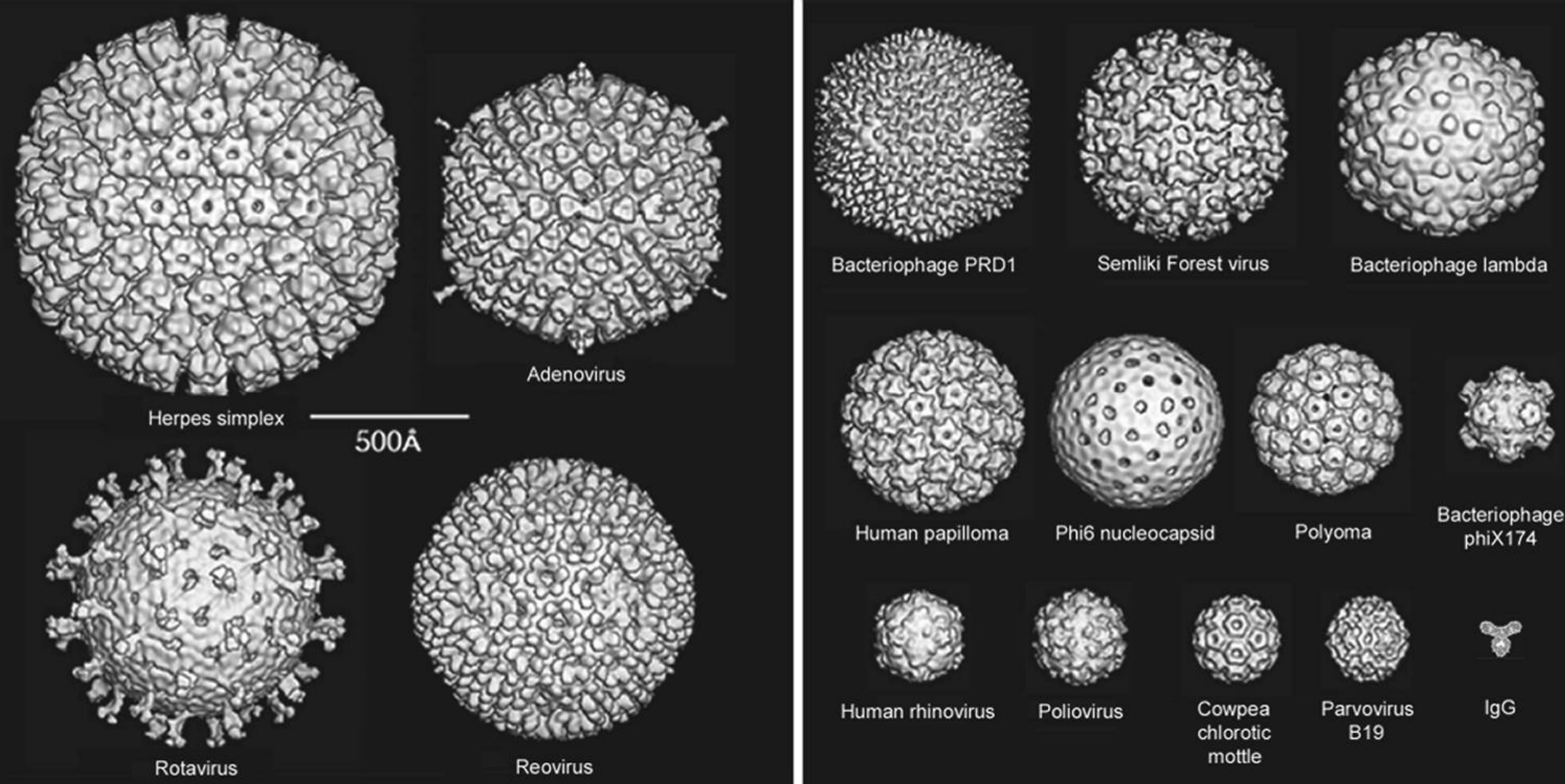
Larger virions II

Consider T=4:

The minimum free energy solution is to divide each triangle into 4 further triangles and place a subunit at each corner.

There will be 12 x 20 subunits, i.e. 240 with 12 pentamers and 30 hexamers. They are obviously not equivalent, but 180 are and the remaining 60 are making similar contacts. They are said to be quasi equivalent.

Examples of virus structure

Herpes simplex

Adenovirus

500Å

Rotavirus

Reovirus

Bacteriophage PRD1

Semliki Forest virus

Bacteriophage lambda

Human papilloma

Phi6 nucleocapsid

Polyoma

Bacteriophage phiX174

Human rhinovirus

Poliovirus

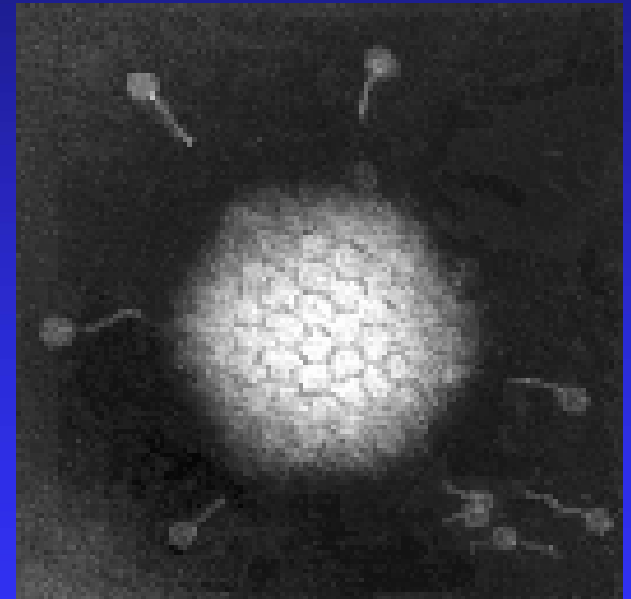Cowpea chlorotic mottle

Parvovirus B19

IgG

Spikes

Some animal viruses have special proteins in the penton position

Different to those at the hexon position.

These are frequently used as attachment points for long projections called spikes

e.g. adenovirus

Envelopes

• All animal viruses with helical symmetry have a lipid envelope

• Virions acquire an envelope during maturation through a process termed budding - from cellular membranes

• Proteins in the envelope are viral encoded. Two main types:
  • Glycoproteins that form the projections know as spikes
  • Matrix protein –layer on inside envelope-added rigidity

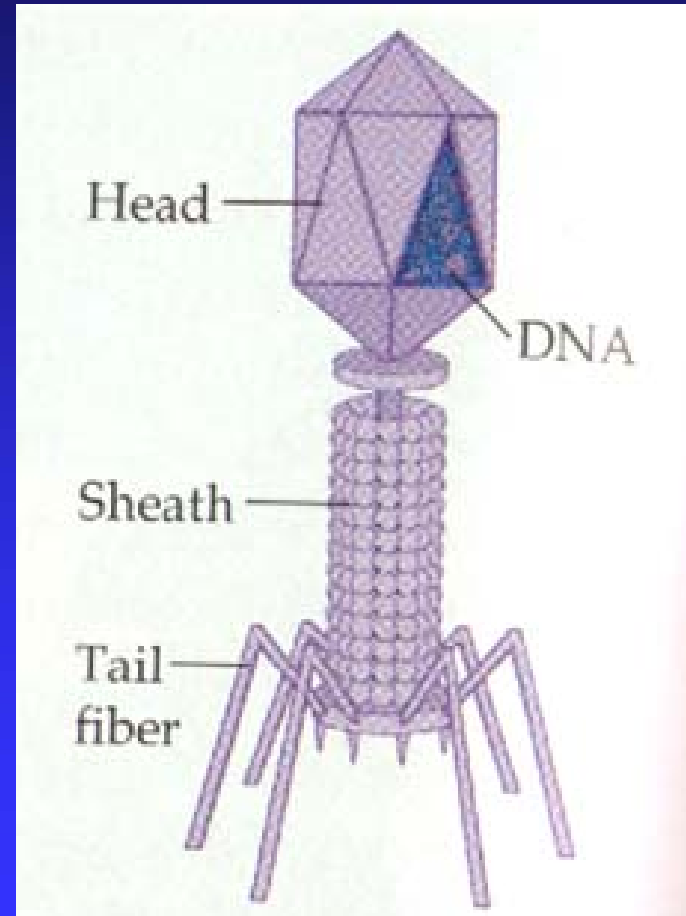• No envelope = naked virus, hydrophilic -so are protected from organic solvents

Bacteriophages

T2

bacteriophage T2 by H.-W. Ackermann

T4

Head

DNA

Sheath

Tail fiber
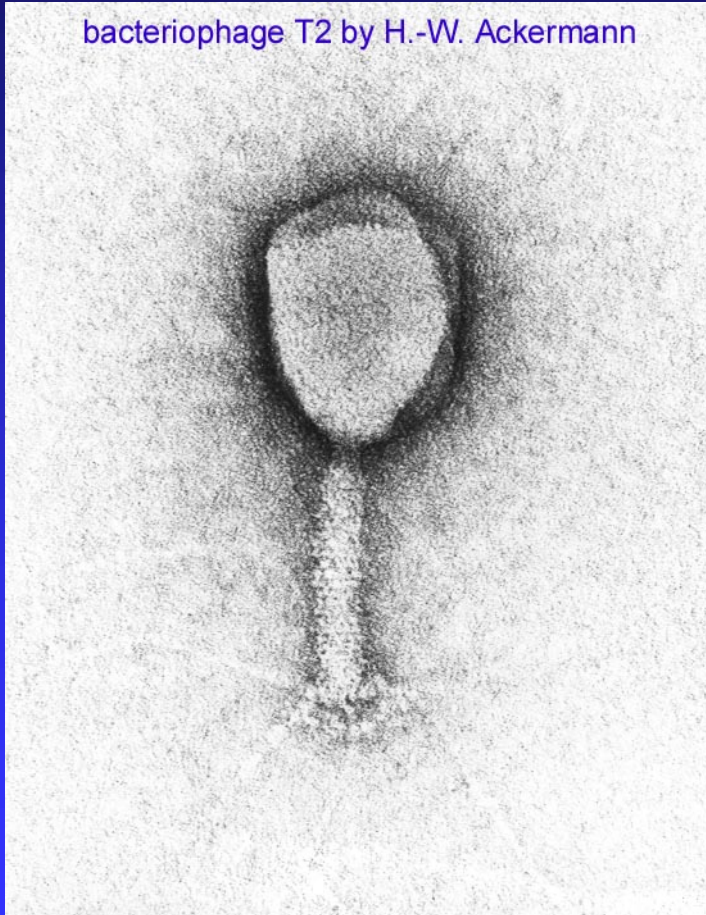
Properties of Viruses

- Small
    - can pass through 0.22μm filters
    - 20 - 350nm in size

- Totally dependent on living cells for replication & existence
- Possess only one species of nucleic acid, either DNA or RNA.   (Can be single stranded or double stranded)

- Have a component for attaching or docking to cells

- Able to take over the host cell to propagate themselves

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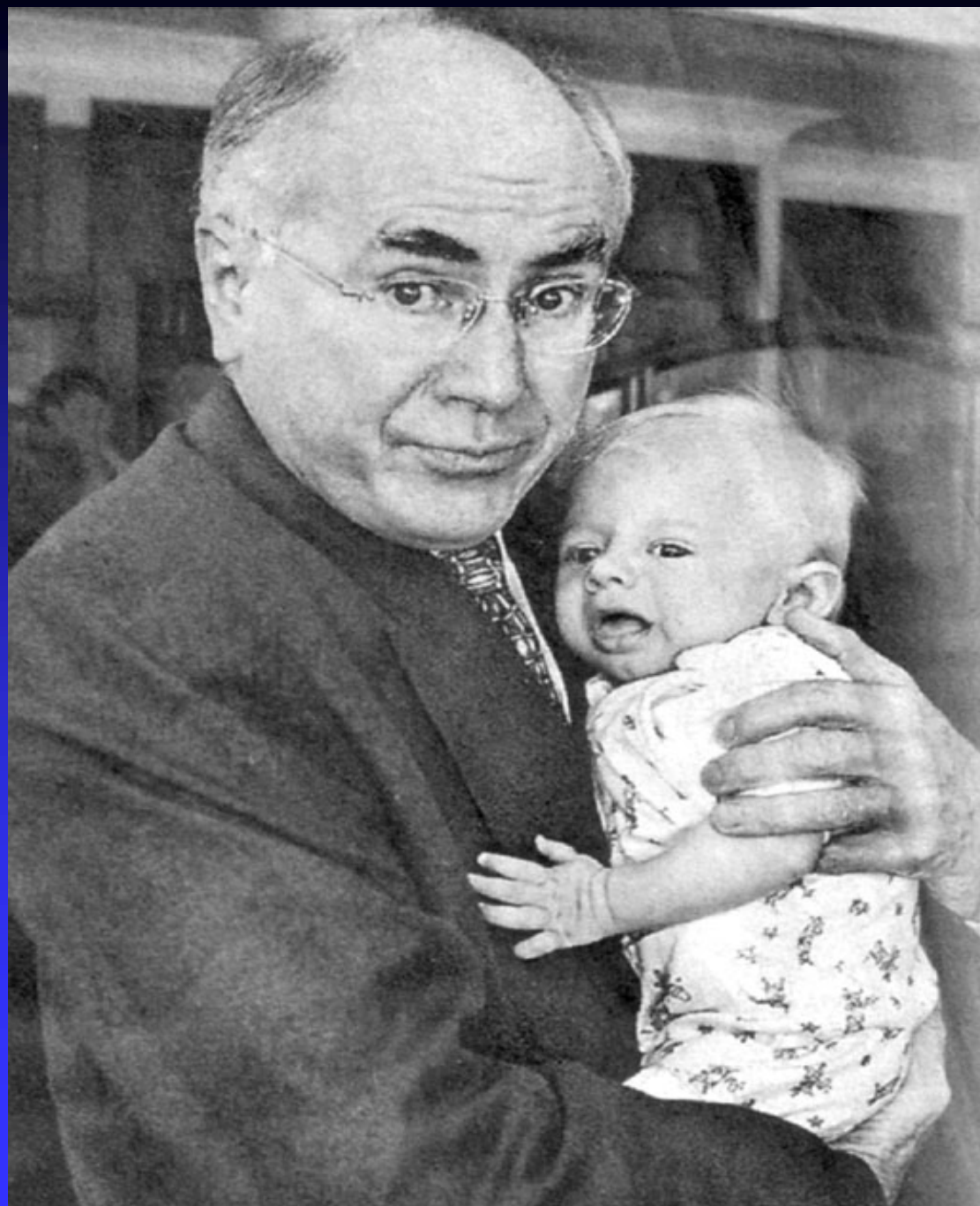
Classification

How are viruses named:

•   A universal system for classifying viruses, and a unified taxonomy, has been established by the International Committee on Taxonomy of Viruses (ICTV) since 1966.

•   Predictions about replication, pathogenesis

•   More than 1,550 virus species belonging to 3 orders, 56 families, 9 subfamilies and 233 genera are recognized by the ICTV

Virus classification

- Hierarchical
- Order
      Family
    -viridae
          Genus
        -virus
            Strain
                Quasispecies

Main criteria used for classification of Viruses

- The type of nucleic acid
- Number of strands of nucleic acid and their physical construction; ss or ds, linear, circular, circular with breaks, segmented.

- Polarity of the viral genome – viral genome acts as mRNA are termed "positive-stranded". Those where a transcript is made first are termed "negative-stranded"

- Replication strategy
- The symmetry of the nucleocapsid
- The presence or absence of a lipid envelope

Classification

The system makes use of a series of ranked taxons, with the:
-Order (*-virales*) being the highest currently recognised.
- then Family (*-viridae*)
        - Subfamily (*-virinae*)
            - Genus (*-virus*)
                - Species (eg: tobacco mosaic virus)

Order:	suffix - virales	eg	*Mononegavirales*
Families:	suffix – viridae	eg	*Paramyxoviridae*
Subfamilies:	suffix – virinae	eg	*Paramyxovirinae*
Genera:	suffix- virus	eg	*Rubulavirus*
Type species:		eg	Mumps virus

Other examples

Family: *Flaviviridae* (Flaviviruses)
        Genus: *Flavivirus* eg yellow fever virus
        Genus: *Hepacivirus* - hepatitis C virus

Family: *Paramyxoviridae* (Paramyxoviruses)
Subfamily: *Paramyxovirinae*
        Genus: *Paramyxovirus* (parainfluenzaviruses)
        Genus: *Morbillivirus* (measleslike viruses)
        Genus: *Rubulavirus* (mumps virus)
Subfamily: *Pneumovirinae*
        Genus: *Pneumovirus* (respiratory syncytial virus)
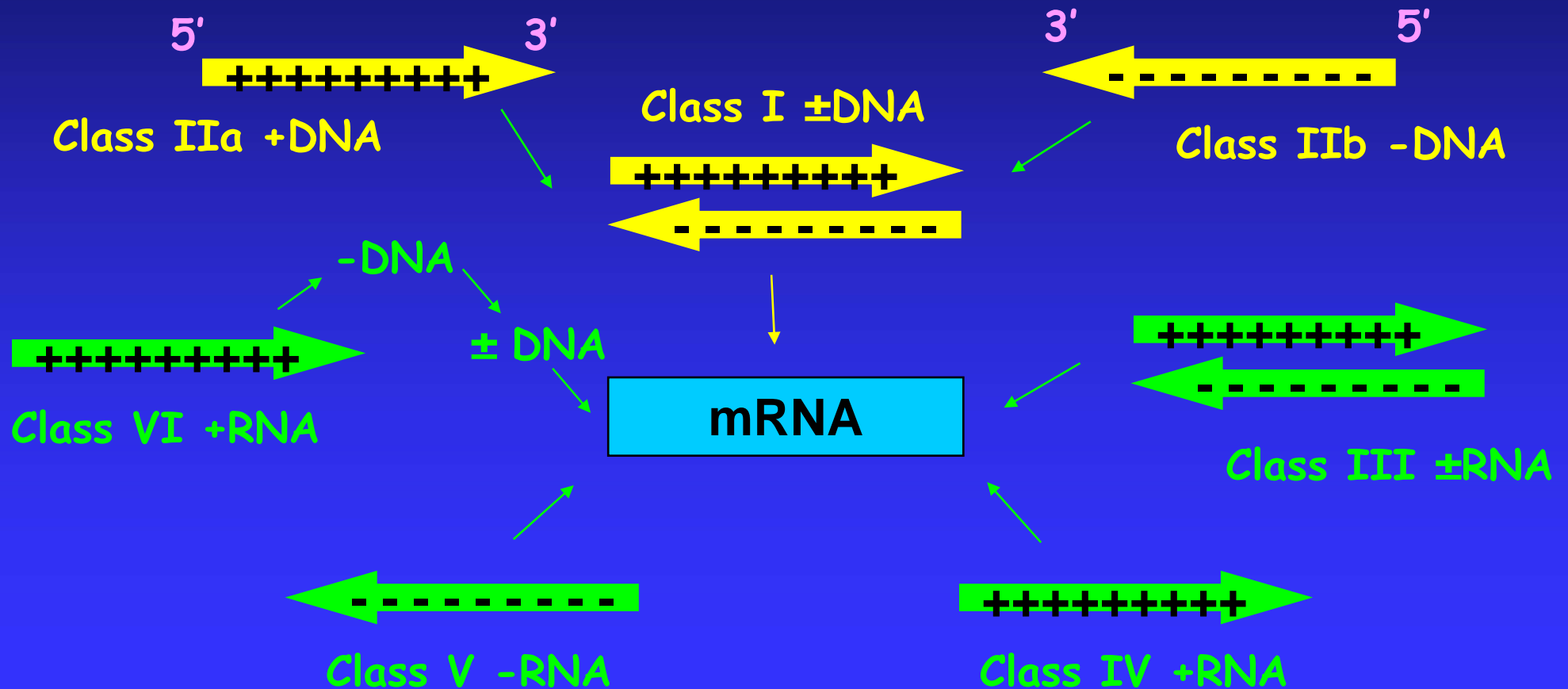
David Baltimore classification system

All viruses regardless of the nature of their genomes must use mRNA as the template for the synthesis of proteins

Baltimore system is based on the relationship between the viral genome and the mRNA used for translation.

- mRNA is defined as the positive strand
- Other molecules with the same sequence are also designated as positive strands (eg DNA)
- Nucleic acid molecules that are the complement of mRNA are designated as negative strands.

Baltimore classification system

The six classes of the Baltimore classification are organised as follows:

5'   3'   Class IIa +DNA

Class I ±DNA

3'   5'   Class IIb -DNA

-DNA

± DNA

Class VI +RNA

mRNA

Class III ±RNA

Class V -RNA

Class IV +RNA

Baltimore classification system

- **Poxviridae.** Class I ±DNA: mRNA synthesised in the normal fashion; -DNA as the template
- **Parvoviridae.** Class IIa +DNA: synthesis involves a dsDNA intermediate
- Class IIb –DNA: synthesis involves a dsDNA intermediate
- **Reoviridae.** Class III ±RNA: one strand acts as mRNA
- **Flaviviridae.** Class IV +RNA: can serve as mRNA directly (IVa/b)
- **Orthomyxoviridae.** Class V –RNA: acts as template for synthesis of mRNA (Va/b)
- **Retroviridae.** Class VI +RNA: dsDNA molecule needed for replication and expression

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Antiviral evasion mechanisms

- Escape interaction (VZV – CNS)
- Latency + restricted gene expression (HSV-LATs)
- Become invisible – immunosuppression (HBV - $\downarrow$ transcription)
  - ◆ $\downarrow$ Ag presentation (Vaccinia)
  - ◆ Down regulate host antiviral – associated genes (CMV – MHC Class II)
  - ◆ Continuous changing appearance (HIV quasispecies)
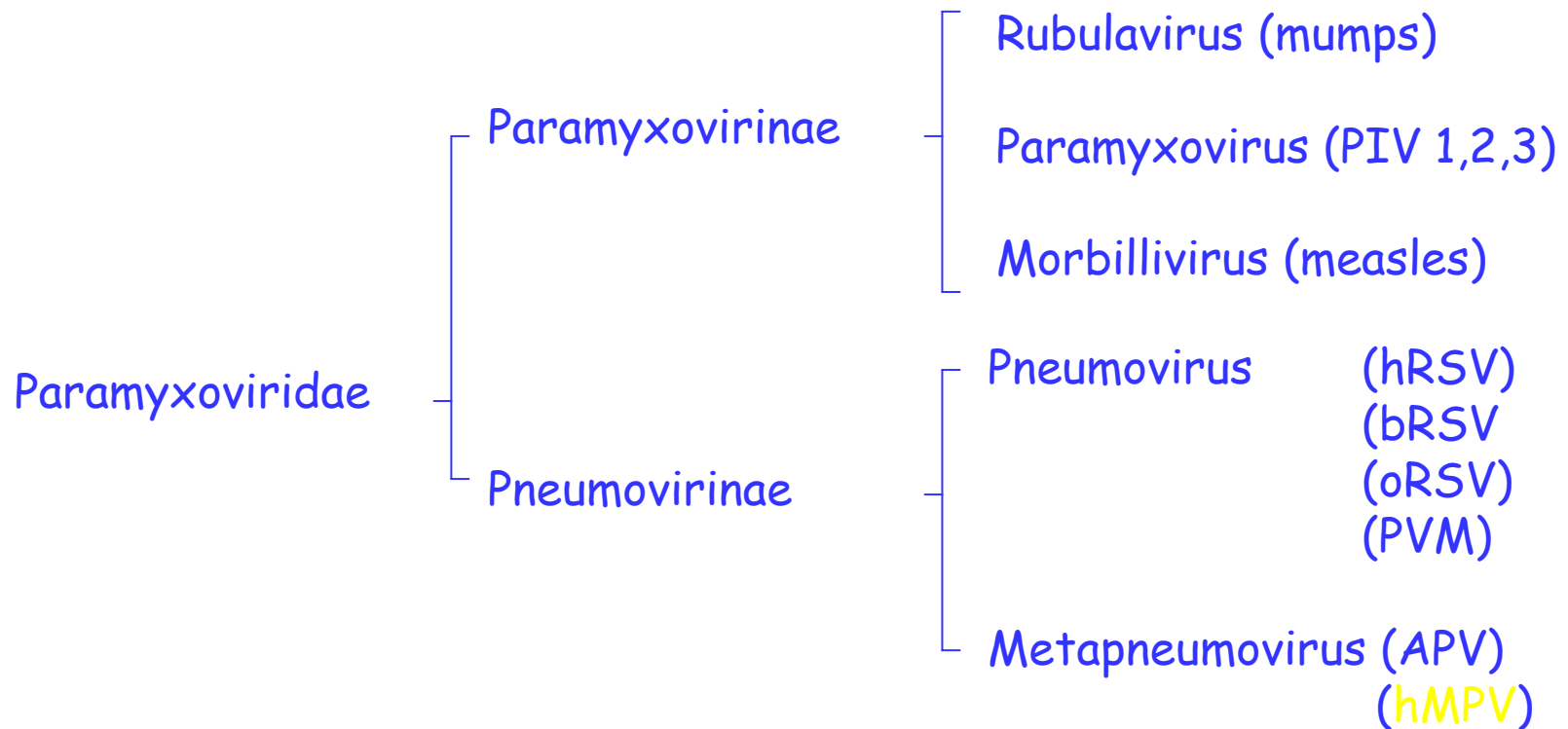- Destroy immune mechanisms (HIV – CD4)

Some Emerging Issues

- New respiratory virus – SARS
  - hMPV
  - Associations with chronic conditions
- Transfusion – HCV
  - Non A non B non C
  - New viruses (GBV, TTV, Sen V)
- Old viruses re-emerging
  - Smallpox
- Zoonoses
  - SARS
  - Rabies
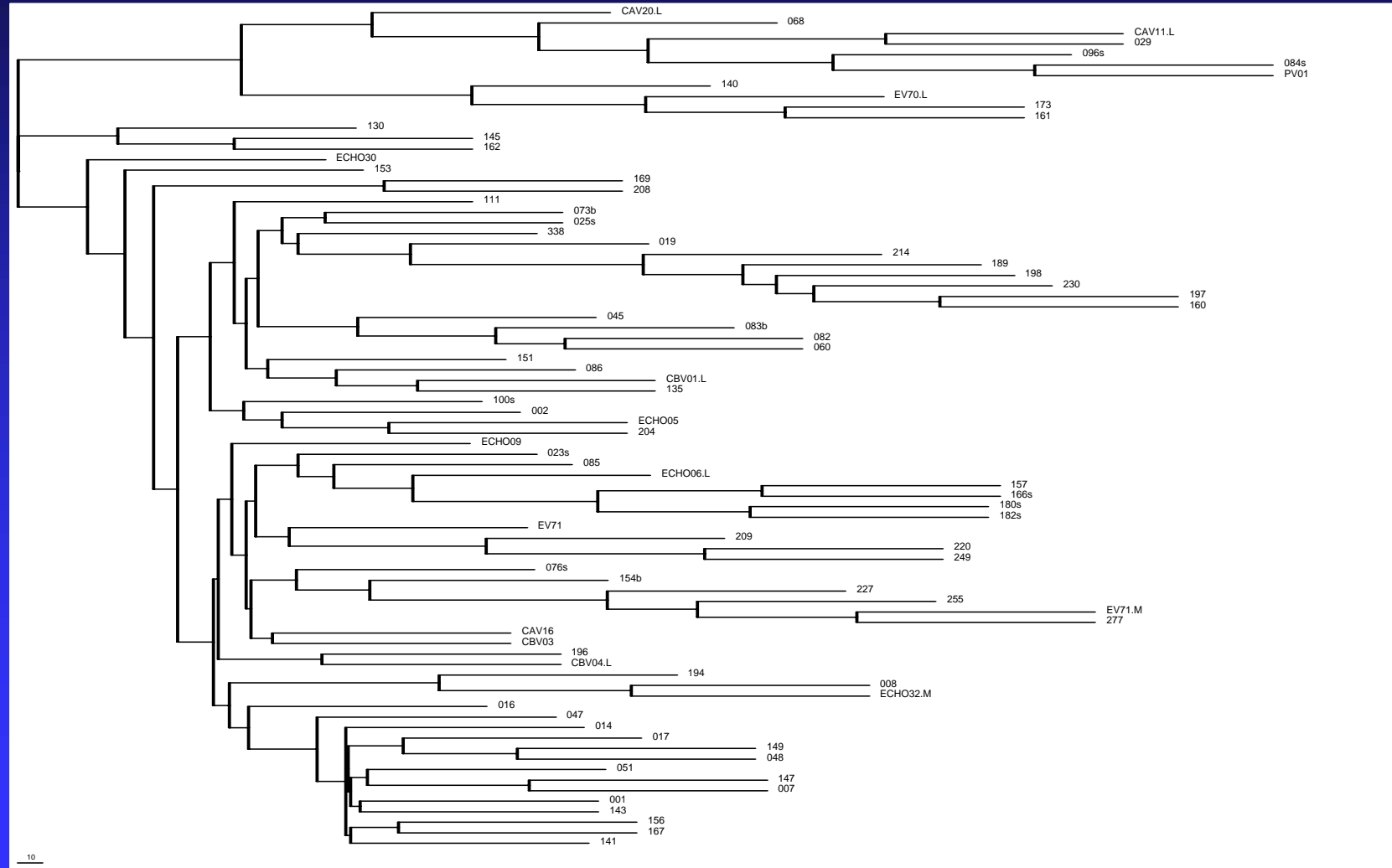 - Arenaviruses
  - Hantaviruses

Old viruses reemerging

SMALLPOX

Human metapneumovirus (hMPV)

Paramyxoviridae

- Paramyxovirinae
  - Rubulavirus (mumps)
  - Paramyxovirus (PIV 1,2,3)
  - Morbillivirus (measles)

- Pneumovirinae
  - Pneumovirus (hRSV)
    (bRSV
    (oRSV)
    (PVM)
  - Metapneumovirus (APV)
    (hMPV)

Phylogenetic relationship of hMPV isolates, based on N-gene sequence analysis

01-70

01-71/8

01-41

01-71/5

hMPV 00-1

hRSV #0018

hRSV B

hRSV #0017

hRSV RS

1

hRSV S2

1

1

hRSV mut c

1

1

1

bRSV

1

1

1

1

PVM

1

1

APV

APV N

1

APV Colora

1

1

1

APV MN10

1

APV MN8

APV MN11

1

APV MN9

ENTEROVIRUSES & DM

New viruses - Zoonoses

SARS CoV

Rabies

Arenaviruses

Hantaviruses

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

CORONAVIRUS
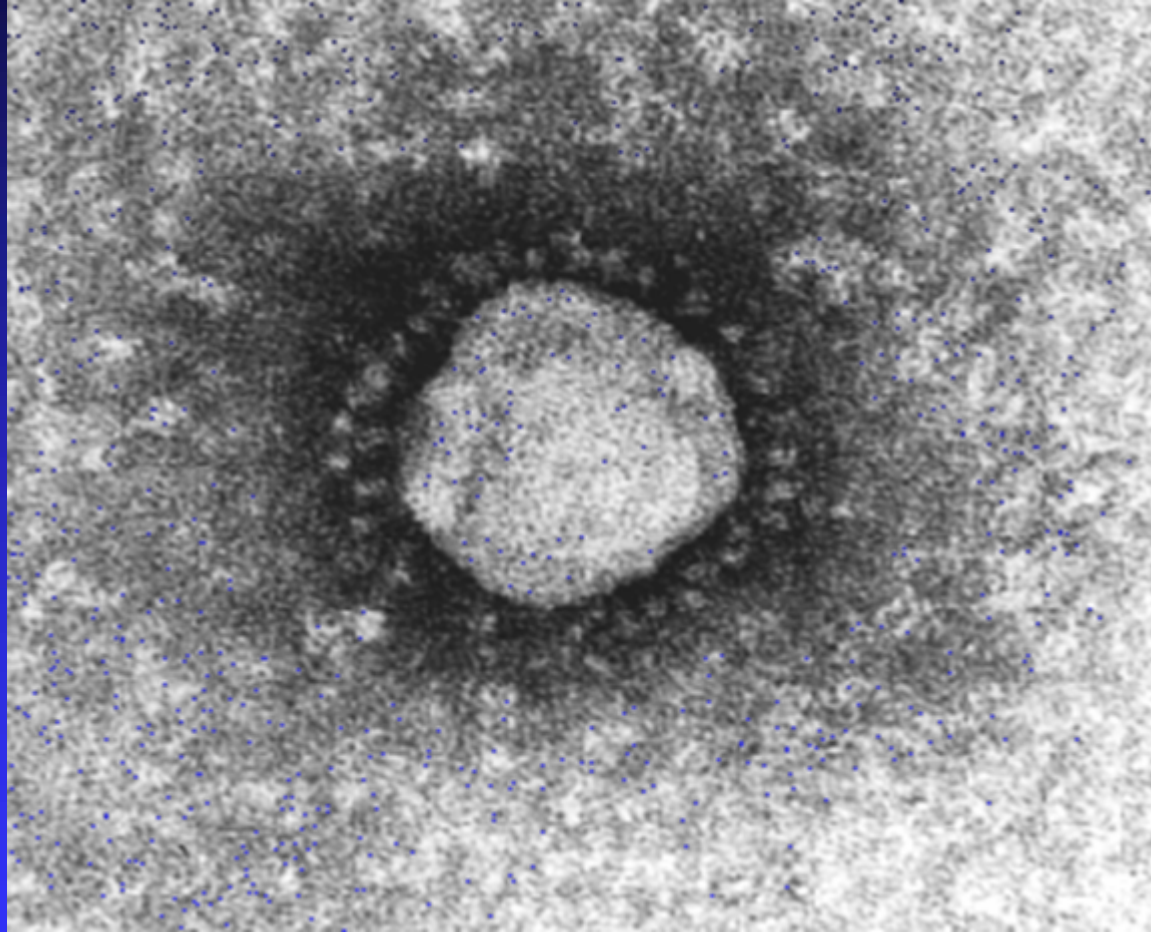
Probable cases of SARS by date of onset (n=5,923*)
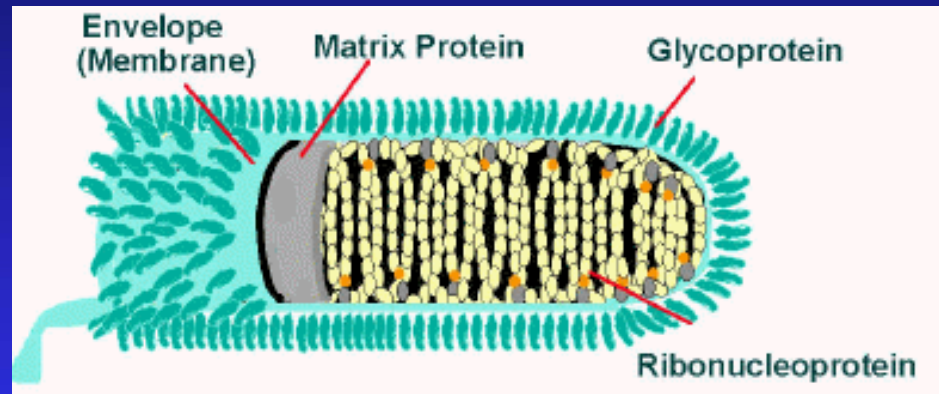Worldwide, 1 November 2002 - 16 June 2003
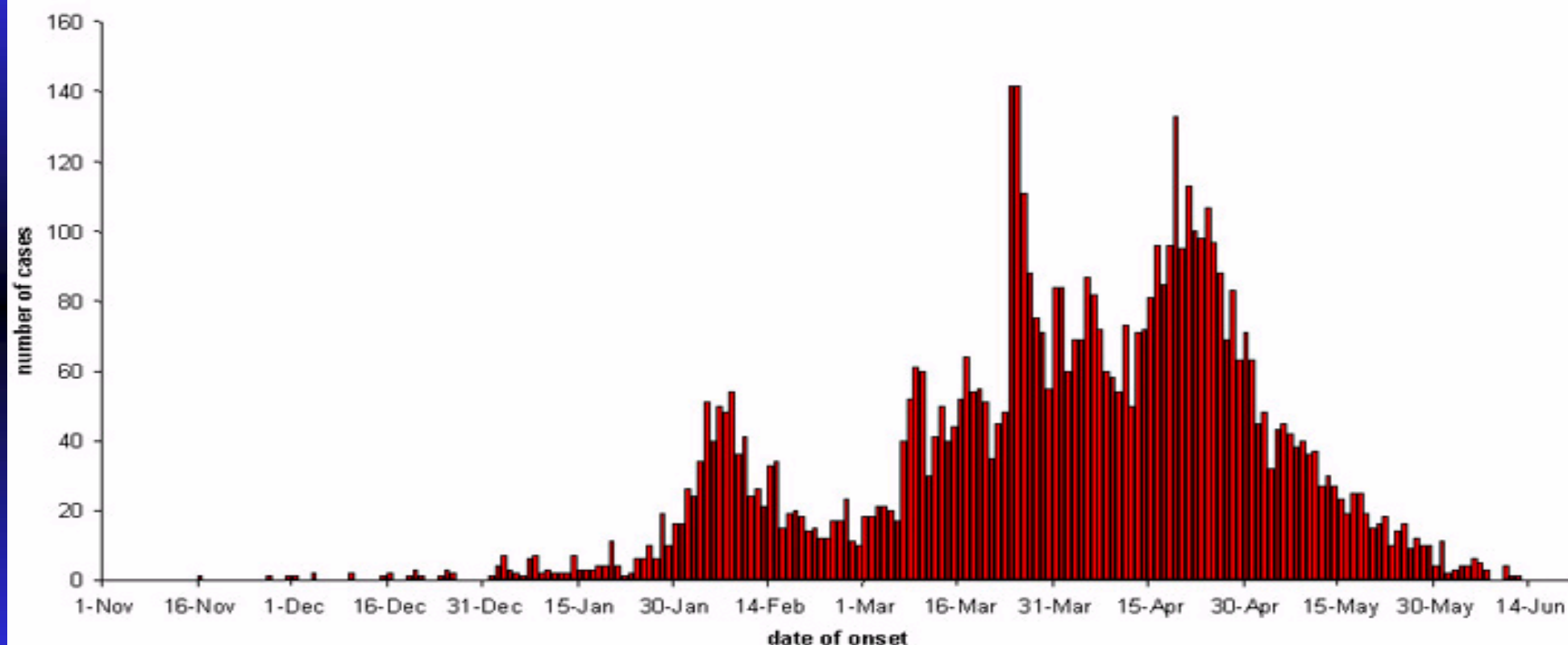
* This graph does not include 2537 probable cases (2522 from Beijing), for whom no dates of onset are currently available.
Source: Ministry of Health, China, WHO

Rabies Virus

Rabies virus particles

Structure of rabies virus (Source: CDC)

Epidemiology

Rabies is a zoonosis which is prevalent in wildlife. The main animals involved differs from continent to continent.

Europe	fox, bats
Middle East	wolf, dog
Asia	dog
Africa	dog, mongoose, antelope
N America	foxes, skunks, raccoons, insectivorous bats
S America	dog, vampire bats

Management and Prevention

- **Pre-exposure prophylaxis** - Inactivated rabies vaccine may be administered to persons at increased risk of being exposed to rabies e.g. vets, animal handlers, laboratory workers etc.

- **Post-exposure prophylaxis** - In cases of animal bites, dogs and cats in a rabies endemic area should be held for 10 days for observation. If signs develop, they should be killed and their tissue.

- Wild animals are not observed but if captured, the animal should be killed and examined. The essential components of postexposure prophylaxis are the local treatment of wounds and active and passive immunization.

- Once rabies is established, there is nothing much that could be done except intensive supportive care. To date, only 2 persons with proven rabies have survived.

Arenaviruses
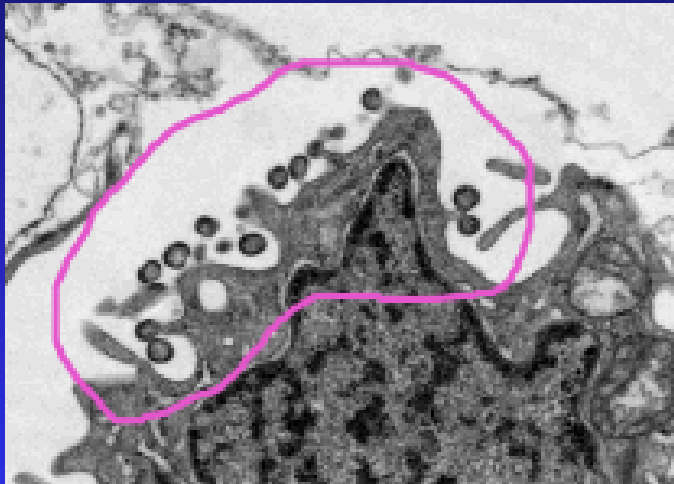
Lassa fever virus particles budding from the surface of an infected cell. (Source: CDC)

- Enveloped ssRNA viruses
- virions 80-150nm in diameter
- genome consists of 2 pieces of ambisense ssRNA.
- 7-8 nm spikes protrude from the envelope.
- host cell ribosomes are usually seen inside the outer membrane but play no part in replication.
- Members of arenaviruses include Lassa fever, Junin and Macupo viruses.

Lassa Fever

- Found predominantly in West Africa, in particular Nigeria, Sierra Leone and Liberia.

- The natural reservoir is multimammate rat (*Mastomys*)

- Man may get infected through contact with infected urine and faeces.

- Man to man transmission can occur through infected bodily fluids and Lassa fever had caused well-documented nosocomial outbreaks.


Mastomys

Clinical Manifestations

- Incubation period of 3-5 days.

- Insidious onset of non-specific symptoms such as fever, malaise, myalgia and a sore throat.

- Typical patchy or ulcerative pharyngeal lesions may be seen.

- Severe cases may develop the following:
  - Myocarditis
  - Pneumonia
  - Encephalopathy
  - Haemorrhagic manifestations
  - Shock

- The reported mortality rate for hospitalized cases of Lassa fever is 25%. It carries a higher mortality in pregnant women.

Rodent Carriers of Hantaviruses
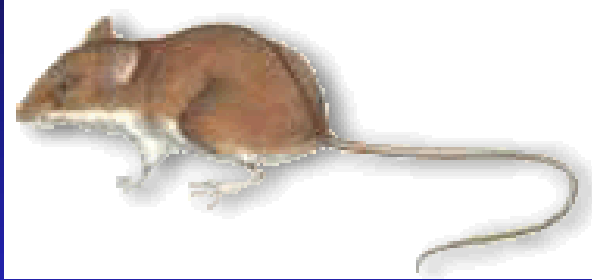
Striped field mouse (*Apodemus agrarius*)

Bank vole (*Clethrionomys glareolus*)

Deer Mouse (*Peromyscus maniculatus*)

Rat (Rattus)

Clinical Features of Hantavirus Disease

- The multisystem pathology of HVD is characterized by damage to capillaries and small vessel walls, resulting in vasodilation and congestion with hemorrhages.

- Classically, hantavirus disease consists of 5 distinct phases. These phases may be blurred in moderate or mild cases.

  - Febrile phase - abrupt onset of a severe flu-like illness with a erythematous rash after an incubation period of 2-3 days.

  - Hypotensive phase - begins at day 5 of illness

  - Oliguric phase - begins at day 9 of illness. The patient may develop acute renal failure and shock. Haemorrhages are usually confined to petechiae. The majority of deaths occur during the hypotensive and oliguric phases

  - Diuretic phase - this occurs between days 12-14 .

  - Convalescent phase - this may require up to 4 months.

Major Public Health Issues

Hepatitis C virus
HIV

Hepatitis viruses

- Very different viruses, similar presentations
- Different therapies
- Many other non viral causes
- Diagnosis highly focussed and closed
- Continually emerging agents (HCV SEN-V)

HEPATITIS VIRUS	ACUTE HEPATITIS	CHRONIC HEPATITIS	FULMINANT HEPATITIS	CIRRHOSIS	HCC
A	+	0	+	-	-
B	+	5-10%	+	+	+
C	+	75%	+	+	+
D	+	<5% 50%	+	+	+
E	+	0	+	-	-
G	+	20%	-	-	-
TTV	+	60%	-	-	-

Hepatitis - one syndrome many causes

Hepatitis Viruses

Hepatitis virus	Genomic material	Trans- mission	Incubation period (wks)	Chronic infection	Genus
A	RNA	Enteric	2-6	No	Picorna
B	DNA	Parenteral	4-24	Yes	Hepadna
C	RNA	Parenteral	4-20	Yes	Flavi
D	RNA	Parenteral	2-6	Yes	Unclassified satellite
E	RNA	Enteric	3-6	Yes	Calici -like
G	RNA	Parenteral	2-4	Yes	Flavi
TTV	DNA	?Enteric ?Parenteral	?	Yes	Circo

The incubation period is dose dependent, a larger dose results in a reduced incubation period

HCV genotypes in Australia

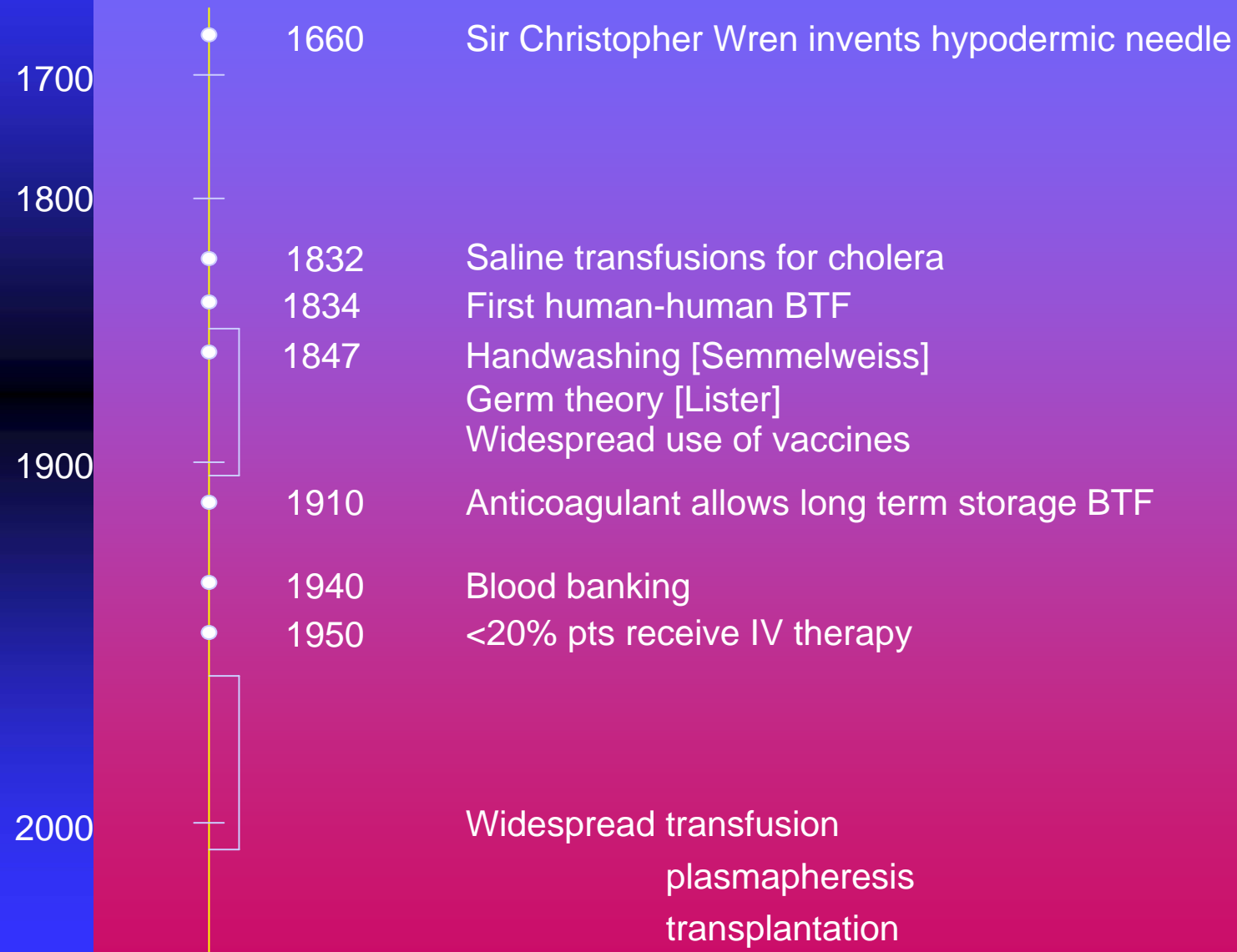**Distribution of genotypes in Australian patients**

- 3a 35%
- 4c/d 1%
- Co-infected 1%
- 1 3%
- 2b 5%
- 1a 40%
- 1b 15%
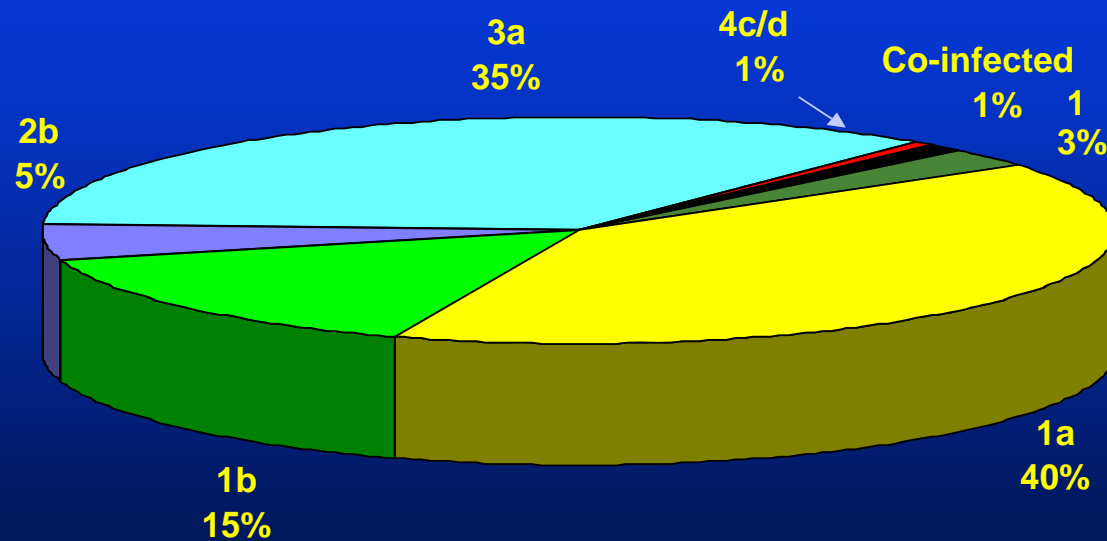
PCR products amplified from 296 HCV infected patients were sequenced and analysed to determine their genotype.
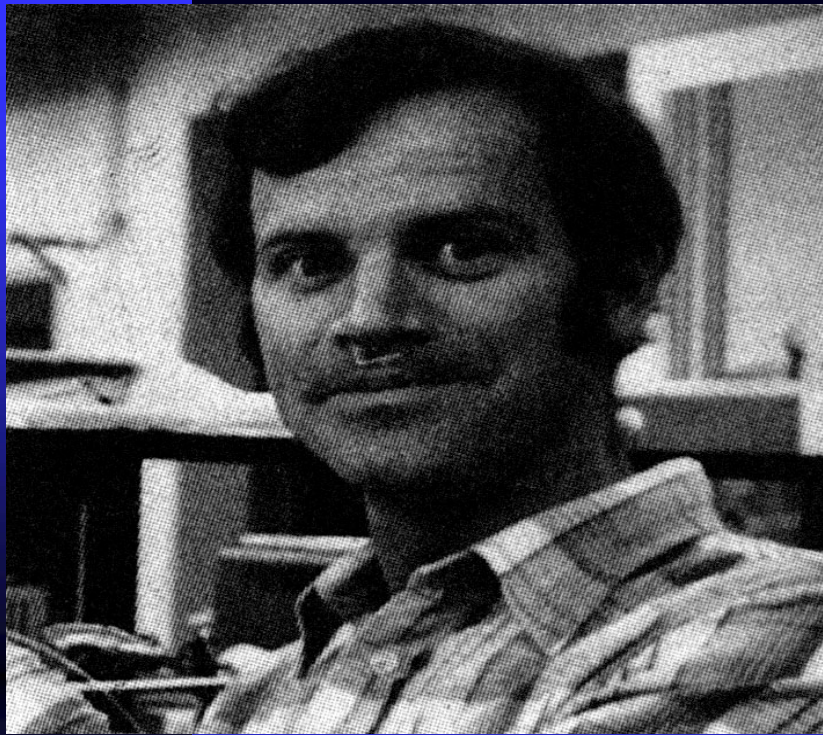
Legend: ■ 1 ■ 1a ■ 1b ■ 2b ■ 3a ■ 4c/d ■ Co-infected

Major Public Health Issues

SCREENING FOR BLOOD BORNE VIRUSES

Brief history of transfusion

•	1660	Sir Christopher Wren invents hypodermic needle
1700		
1800		
•	1832	Saline transfusions for cholera
•	1834	First human-human BTF
•	1847	Handwashing [Semmelweiss]
		Germ theory [Lister]
		Widespread use of vaccines
1900		
•	1910	Anticoagulant allows long term storage BTF
•	1940	Blood banking
•	1950	<20% pts receive IV therapy
2000		Widespread transfusion
		plasmapheresis
		transplantation

Randall Saiki

Kary Mullis