Properties of Viruses, Viral Replication and Evolution

Medical Microbiology (BMS 4510)

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Introduction to Virology

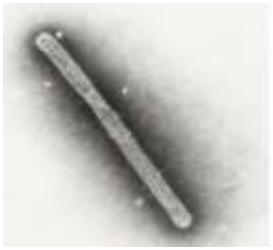
- Viruses are obligate intracellular parasites composed of genetic material (DNA or RNA) enclosed in a protein coat (**capsid**)
  - Nucleic acid + capsid protein = **nucleocapsid/core**
- Cannot survive outside host (obligate intracellular parasites)
- Very small (20 to 300nm)
- Viruses use host biochemical machinery for reproduction (have no machinery of their own)
  - Have no functional organelles
  - Metabolically inert when outside host

Introduction to Virology

- **Historical Perspective**
  - 1892: Dmitri Iwanowski filtered sap from diseased tobacco plants hoping to isolate causative bacteria
  - No bacteria was isolated
  - Filtrate caused tobacco mosaic disease when injected into healthy plants
  - Early 1900: viruses (*virus*-poison) where described
  - Electron microscopy made is possible for visualization of viruses

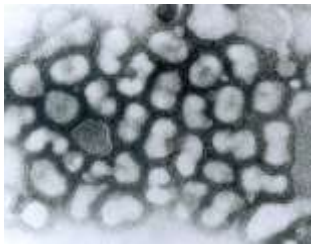
Viral Structure

- **Enveloped viruses** have a lipid membrane around the capsid.
  - Protective and aids in entry



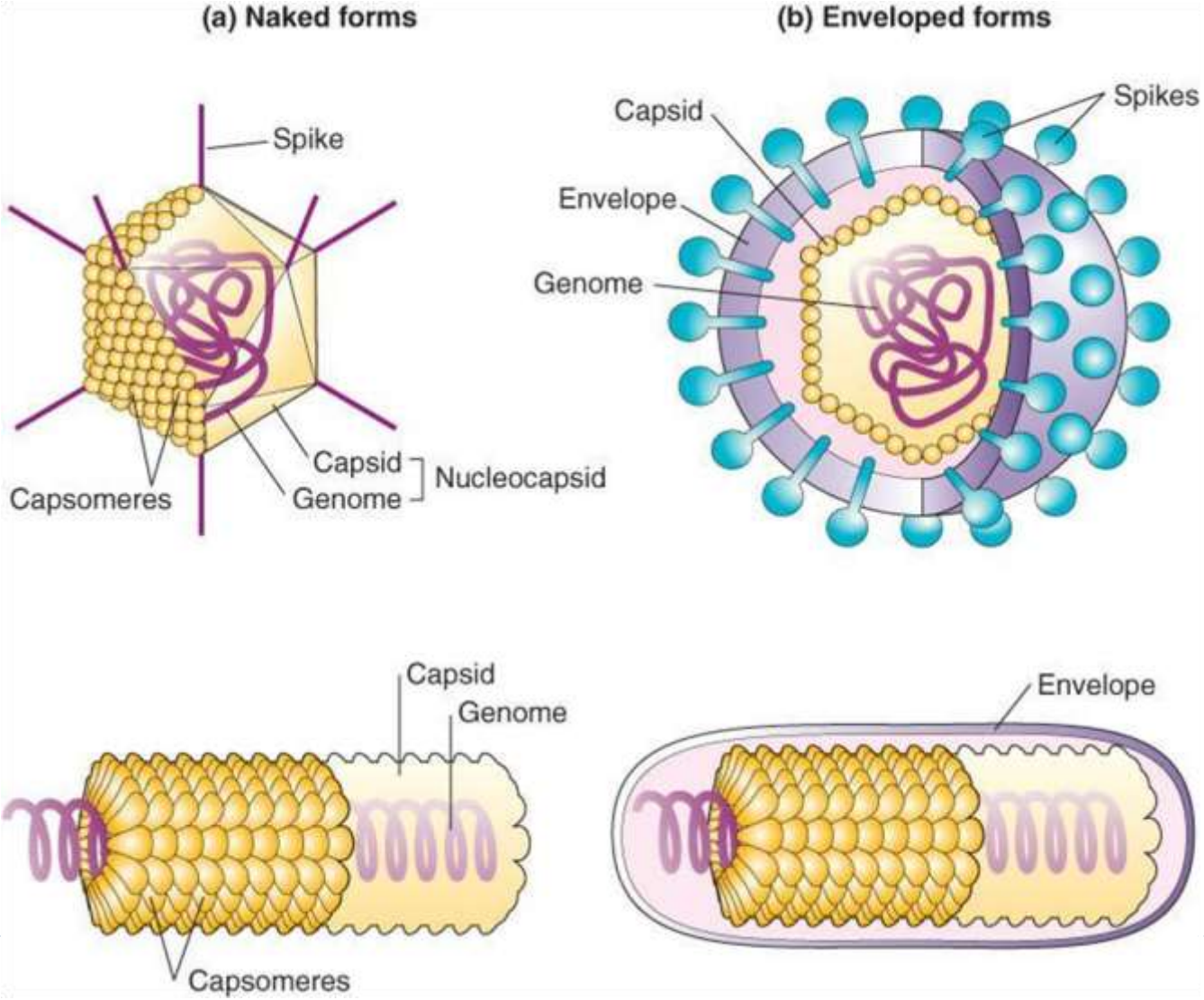
- **Naked viruses** do not have an outer membrane

- Viruses often have surface protrusions (**spikes**)
  - Used for attachment, very immunogenetic

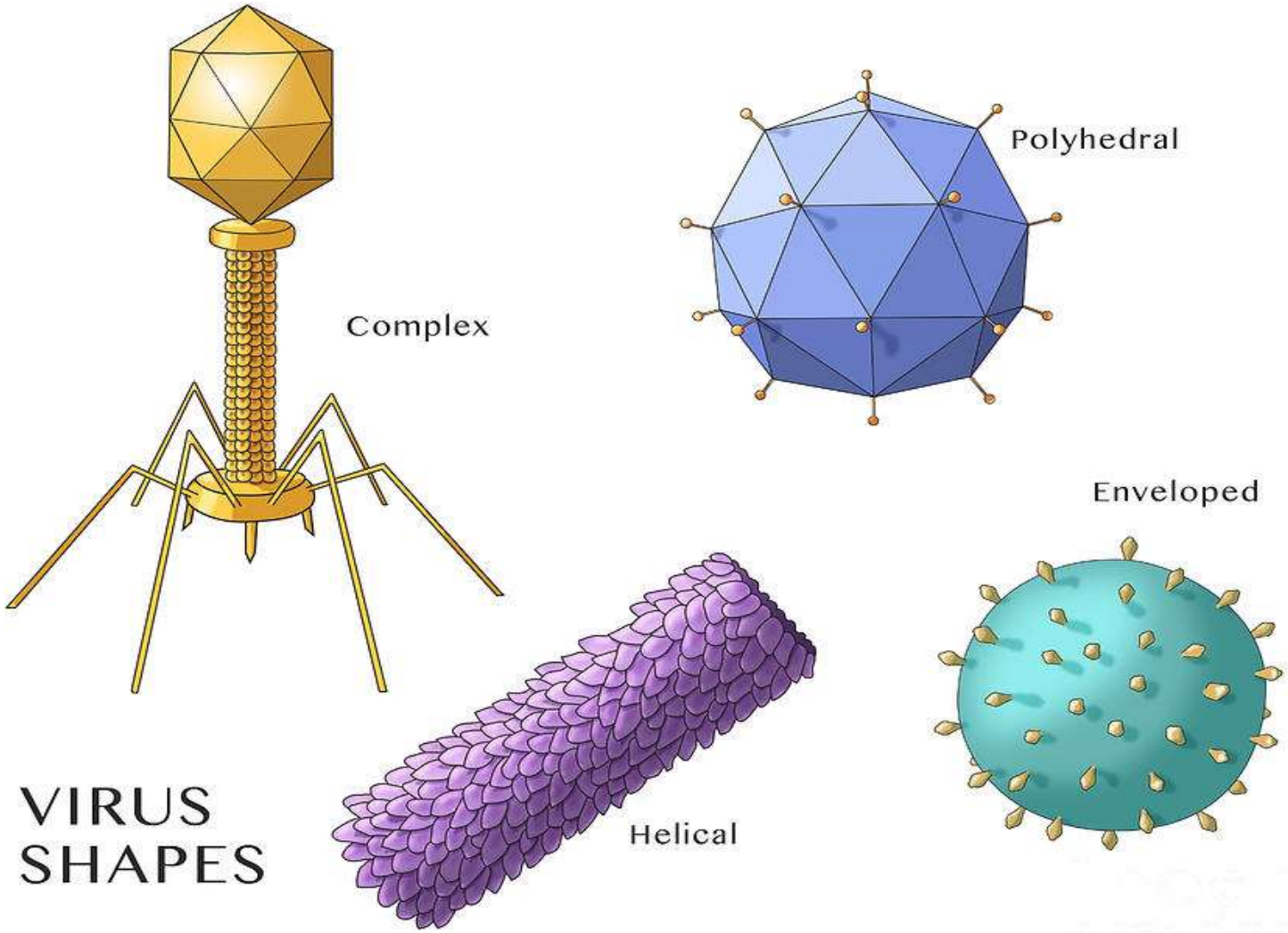


- Basic shapes
  - **Spherical/polyhedral** (icosahedral symmetry has 12 faces and
  - **cylindrical/helical** (rod shaped)

VIRAL STRUCTURE

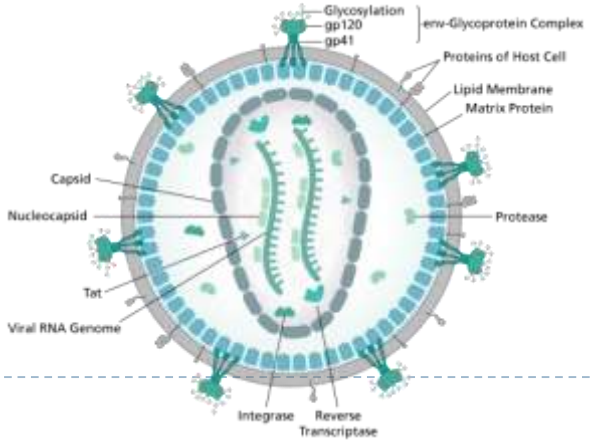


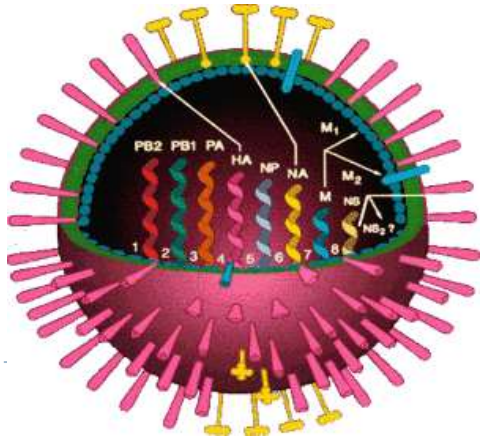
VIRAL STRUCTURE



Genome structure

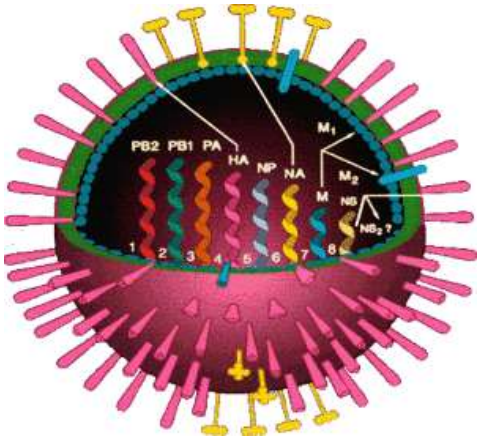
- DNA or RNA (Single or double stranded)
- Single stranded genomes can either have + sense or – sense genomes (with respect to mRNA)
- Viral genomes are either linear or circular
  - Some genomes are segmented e.g. Influenza viruses





Viral Proteins

- **Structural proteins**
  - Coded by viral genomes
  - Form part of the structure of the virion
  - Form the envelope and or core
  - Receptors: Involved in ligand binding



- **Non-structural proteins**
  - Perform enzymatic roles or are involved in viral replication
  - E.g. transcriptases (dsDNA or dsRNA to mRNA) and reverse transcriptases (RNA to DNA), integrases,
  - Neuraminidase a glycoprotein spike of Ortho- and paramyxoviruses has enzymatic activity

Classification of viruses

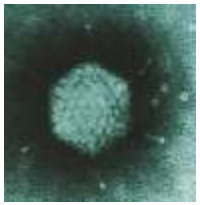
- **Family:** ends in **–dae**
- **Subfamily:** ends in **–nae**
- **Genus: has virus name-** ends in **virus**
- **E.g..**
  - Parvoviridae
    - Parvovirinae
      - *Parvovirus*

Classification criteria

- Nucleic acid/replication
- Host range
- Diseases caused/organs affected
- morphology, (shape, size, structure, presence of envelope etc,)

Classification of viruses

- **Single stranded DNA viruses**
  - **Parvoviruses (**Family**: Parvoviridae)**
  - Naked polyhedral (18-25nm)
  - Very small viruses; infect rats, mice and harmsters
  - *Dependovirus:* adeno-associated virus occur in humans
    - Cause no known disease



- **Double stranded DNA viruses**
  - **Papoviridae (Papoviruses)** (papilloma, Polyoma viruses)
  - Naked polyhendral (40-57nm)
  - Small viruses, induce tumors and warts (papillomavirus), reactivated in immunocompromised individuals (Polyomavirus JC virus causes progressive multifocal leukoencephalopathy (PML) in AIDS patients)

  - **Adenoviridae (Adenoviruses)**
  - Naked polyhedral (70-80nm)
  - Medium sized viruses that cause respiratory infections in humans

Classification of viruses

- **Double stranded DNA viruses cont'd**

  - **Herpesviridae (Herpesviruses) (**herpes simplex, varicela-zoster, cytomegalovirus (CMV), Epstein-Barr virus (EBV), Roseolovirus)
  - Enveloped polyhedral viruses (150-250nm)
  - Medium sized viruses, cause chicken pox, shingles, Burkitt's lymphoma, infectious mononucleosis, CMV causes pneumonia and brain lesions in immunocompromised individuals, EBV causes Burkett's lymphoma.

  - **Poxviridae (Poxviruses)** (variola, cowpox, vaccinia)
  - Enveloped complex viruses (20-350nm)
  - Very large complex viruses, cause smallpox (variola), and cowpox, immunity to small pox (vaccinia)

  - **Hepadnaviridae (Hepadnaviruses)** (Hepatitis B like virus)
  - Enveloped polyhedral virus (42nm)
  - Double stranded DNA virus, cause hepatitis and liver cancers

Classification of viruses

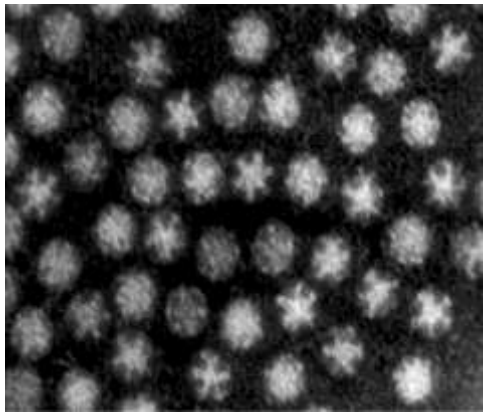
- **Single stranded positive sense RNA viruses**
  - **Picornaviruses (Picornaviridae)** (polioviruses, coxsackievirus, rhinoviruses, hepatitis A virus)
  - naked polyhedral viruses (18-38nm)
  - Smallest RNA viruses
  - Enteric viruses (polioviruses), common cold viruses (rhinoviruses)

  - **Togaviruses (Togaviridae)** (arboviruses, alphaviruses)
  - Enveloped polyhedral viruses (40-60nm)
  - Transmitted by arthropods (arboviruses)
    - Cause chikungunya virus infection
    - Rubella is not an arbovirus; transmitted via respiratory route or congenitally

Classification of viruses

- **Single stranded positive sense RNA viruses**
  - Caliciviruses **(Caliciviridae)** Icosahedral viruses (3
    40nm)
  - Norwalk viruses cause gastroenteritic, Hepatitis E virus cause hepatitis, *norovirus* also causes gastroenteritis.

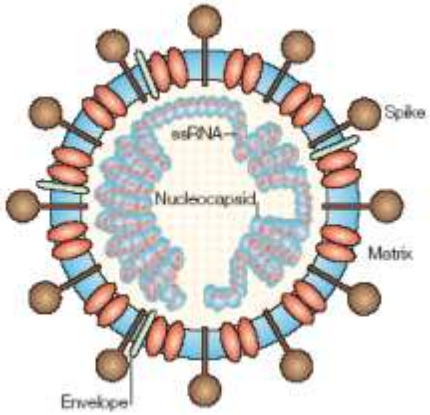
  - **Astroviridae** (Astroviruses)
  - Star shaped viruses
  - Have two capsid proteins
  - Have been found in feces of gastroenteritis patients
  - **Astrovirus**



Classification of viruses

- **Flaviviridae** (flaviviruses)
  - Enveloped icosahendral viruses (40-50nm)
  - Hepatitic C (blood borne), arboviruses (yellow fever, dengue fever and hemorrhagic fever , West Nile, St Louis encephalitis, Japanese encephalitis, Zika virus etc

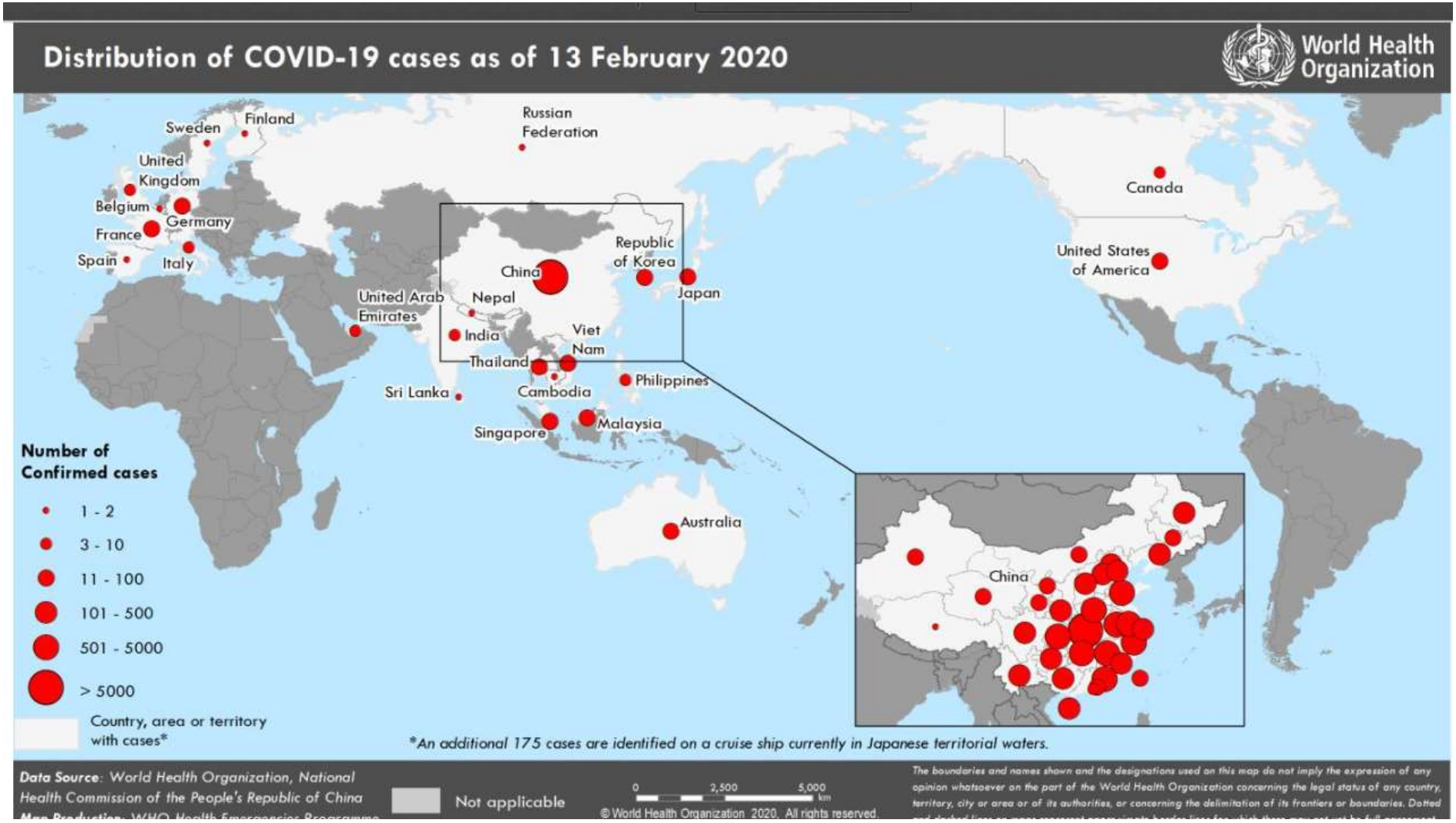
- **Coronaviruses (Coronaviridae)**
  - Enveloped helical viruses (80-130nm)
  - Associated with upper respiratory tract infections and common cold
  - Severe acute respiratory syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS)
  - Current Wuhan, China outbreak (Novel COVID-19)



Latin *corona*, meaning "crown"

Wuhan Coronavirus

- COVID-19 outbreak in Wuhan, China had some link to a large seafood and live animal market, suggesting animal-to-person spread
- Later, a growing number of patients reportedly did not have exposure to animal markets, indicating person-to-person spread.
- As of 10 February 2020, there have been 1,015 confirmed deaths and more than 42,850 confirmed cases in the coronavirus pneumonia outbreak.


Distribution of COVID-19 cases as of 13 February 2020
World Health Organization
Sweden
Finland
Russian Federation
United Kingdom
Belgium
Germany
France
Spain
Italy
China
Republic of Korea
Japan
United Arab Emirates
Nepal
India
Viet Nam
Thailand
Cambodia
Philippines
Sri Lanka
Singapore
Malaysia
Australia
Canada
United States of America
China
Number of Confirmed cases
1 - 2
3 - 10
11 - 100
101 - 500
501 - 5000
> 5000
Country, area or territory with cases*
*An additional 175 cases are identified on a cruise ship currently in Japanese territorial waters.
Data Source: World Health Organization, National Health Commission of the People's Republic of China
Not applicable
0 2,500 5,000 km
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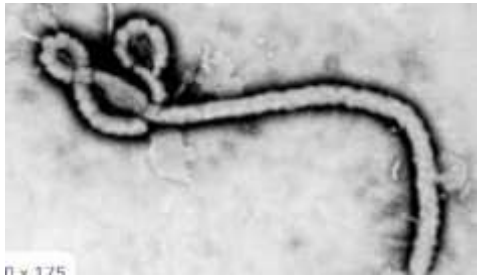
Classification of viruses

- **Single stranded negative sense RNA viruses**
  - **Orthomyxoviruses** (Influenza A, B & C,)
  - Enveloped helical viruses (80-200nm)
  - Medium sized viruses
  - Have ability to agglutinate red blood cells

  - **Paramyxoviruses** (measles and mumps viruses)
  - Enveloped helical virus (150-300nm)
  - similar to Orthomyxoviruses but larger
  - Cause measles and mumps
  - *Pneumovirus* causes respiratory syncycial virus infection.

Classification of viruses

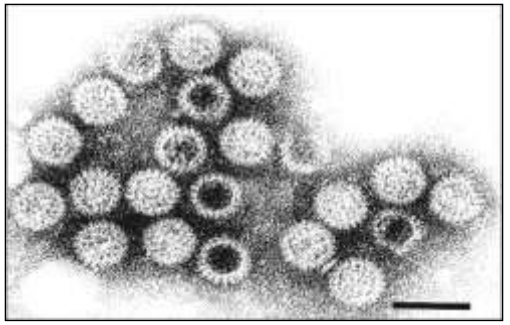
- **Single stranded negative sense RNA viruses**
  - **Rhabdoviridae** (Rhabdoviruses) (rabies virus)
    - Enveloped helical viruses (70x180nm)
    - Bullet shaped viruses with spiked envelop
    - Cause rabies and Newcastle disease in chickens
  - **Arenaviridae** (Arenaviruses)
    - Enveloped helical viruses (50-200nm)
    - Cause slow infections and hemorrhagic fevers
  - **Filoviridae** (Filoviruses) (Ebola and Marburg)
    - Enveloped helical viruses (80x14000)
    - Ebola and Marburg viruses cause hemorrhagic fevers



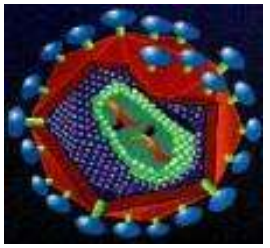
  - **Bunyaviridae** (Bunyaviruses)
    - Enveloped viruses (90-120nm)
    - Arboviruses (*Bunyavirus*-cause encephalitis, *Nairovirus*-cause Congo hemorrhagic fever)
    - Zoonotic (*Hantavirus*-cause hemorrhagic fevers in humans)

Classification of viruses

- **Double stranded RNA viruses**
  - **Reoviruses (Reoviridae)**
  - Naked polyhedral viruses (60-80nm)
  - Involved in upper respiratory tract infections
  - Rotaviruses cause diarrhea in humans



- **Reverse transcription viruses (Retroviridae)**
  - **Retroviruses** (lentiviruses/slow viruses; HTLV I, II, HIV-1, HIV-2)
  - Enveloped helical viruses (100-120nm)
  - Include tumor viruses, cause leukemia and AIDS



  - **Hepadnaviruses** (Hepadnaviridae) (Hepatitis B virus)
  - Enveloped polyhedral virus (42nm)
  - Double stranded DNA virus, cause hepatitis and liver cancers
  - Replication proceeds via an RNA intermediate (coded for by an in-house reverse transcriptase)

- Other viruses (**Deltaviridae**-Delta viruses)
  - Hepatitis D virus
  - Replication depended on co-infection with hepadnavirus
  - Cause chronic hepatitis and cirrhosis

Epidemiologic classification

- **Enteric viruses**
  - Replicate in the GI tract
  - Cause gastroenteritis
  - E.g. Rotaviruses, caliciviruses, astroviruses, some adenoviruses and coronaviruses, other viruses like polioviruses reolicate in the GI tract but do not case gastroenteritis
  - Mostly transmitted via fecal-oral route

- **Respiratory viruses**
  - Acquired by inhalation of droplets
  - Replicate in the respiratory tract
  - Orthomyxoviruses, rhinoviruses, coronaviruses, paramyxoviruses, adenoviruses etc

- **Arboviruses**
  - Transmitted by arthropods (**ar**thropod **bo**rne = arbo)
  - Reoviruses, Bunyaviruses, flaviviruses and togaviruses and some rhabdoviruses

Epidemiologic classification

- **Sexually transmitted viruses**
  - Herpes viruses, papilloma viruses, certain retroviruses (HIV) and Hepatitis viruses
- **Zoonotic viruses**: Filoviruses (Ebola/monkeys), Bunyaviruses (Hantaviruses/rodents)
- **Hepatitis viruses** (the hepatitis alphabet)
  - Targets the liver
  - Yellow fever, rift valley fever, Hepatitis A, B, C, D , and E.
  - Hepatitis A and E- enteric, Hepatitis B, C and D are blood borne or sexually transmitted

End Here

4th Year

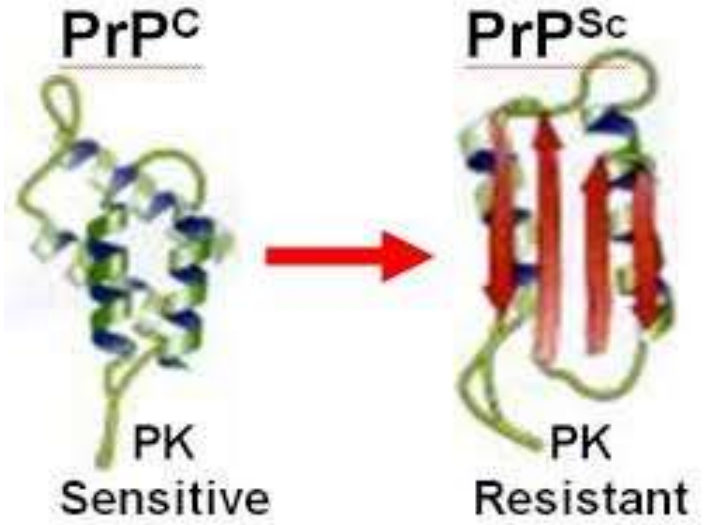
Prions

- Causative agents of bovine spongiform encephalopathy ('mad cow disease'), scrapie in Sheep and Kuru (Creutzfeldt-Jakob syndrome) in humans
- Proteinaceous infectious particles (prion)
- Composed of prion protein (PrP^{sc}) and devoid of nucleic acid (particular protein conformation causes disease)
  - Same amino acid sequence but different conformation (alpha helix to beta pleated sheets)
- Highly resistant to physical and chemical agents
- Nonimmunogenic
- Produce slow infections
- **Viroids**
  - Infectious nucleic acids (mostly RNA)
  - Infect plants (no known animal pathogens)

Prions

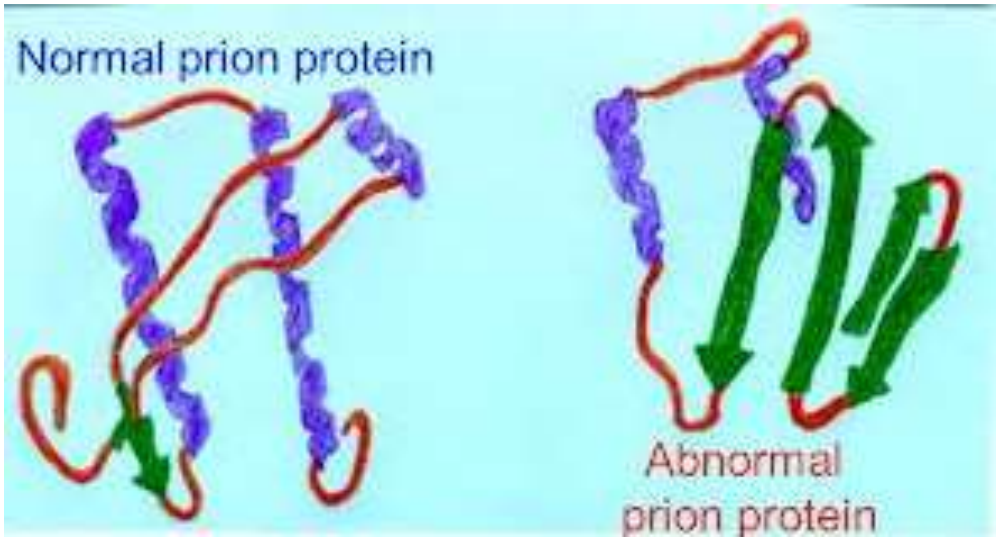
- Prions are oligomers of 33-35kDa proteins (PrP^{sc})
- PrP^{sc} have same amino acid sequence as PrP^{C} found in membranes of neurons but differs in protein conformation.
  - Normal protein has a-helices and scrapie protein has B-pleated sheets
- Spontaneous generation of PrP^{sc} from PrP^{C} occurs by a stochastic event with an incident of 1 per million persons per year.
- PrP^{sc} replicates by forming heterodimers with normal prion proteins, serving as a template for folding of normal prion protein to scrapie conformation.
  - Scrapie protein is highly resistant to proteolysis
  - Reaction proceeds spontaneously and exponentionally (like replication of an infectious agent)
  - Scrapie isoforms accumulate due to resistance to digestion.
- The genes for prion proteins are found in chromosome 20.

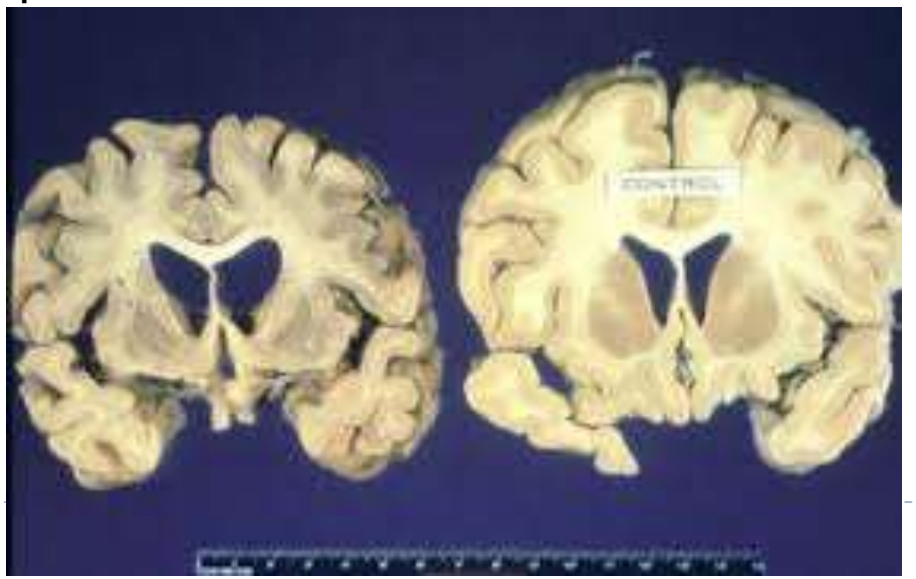
PRIONS



A-helical structure

B-pleated structure





Papua New Guinea Highlanders

Subacute spongiform encephalopathies

- Lethal neurodegenerative diseases in human
  - Similar to scapie of sheep and mad cow disease of cows
  - Human diseases: Kuru and Creutzfeldt-Jakob disease

- **Pathology**: basic lesion is a progressive vacuolation in neurons, astrocytes and oligodendrocytes, astroglial hypertrophy and proliferation and spongiform change in grey matter.

- Incubation period of about 3 years
  - Slow disease progression

Kuru

- First described in 1957 in a tribal group of New Guinea Highlands
  - Women and children practiced ritualistic cannibalism of their own deceased relatives
  - Disease declined after custom was stopped but because of the long incubation period, sporadic cases do occur

Creutzfeldt-Jakob disease

- Similar to scrapie of sheep
- Occurs 1 in a million people per year
- Not due to infection; iatogenic transmission
  - Neurosurgery eg cornea or dura matter transplants
- Pathogenesis: Spontaneous generation of PrP^{sc} from PrP^{C}

Summary

- Viruses are small obligate intracellular parasites
  - Biochemically inert outside host
  - Use host cellular machinery for reproduction/replication
- Have DNA or RNA (ss or ds) enclosed in a protein core (capsid)
  - Can be enveloped or non-enveloped (naked)
- Code for structural (form virions) and non-structural proteins (enzymes)
- Classification schemes are based on host range, type of genetic material, replication, disease type etc

Mechanisms of Infection and Spread of Viruses

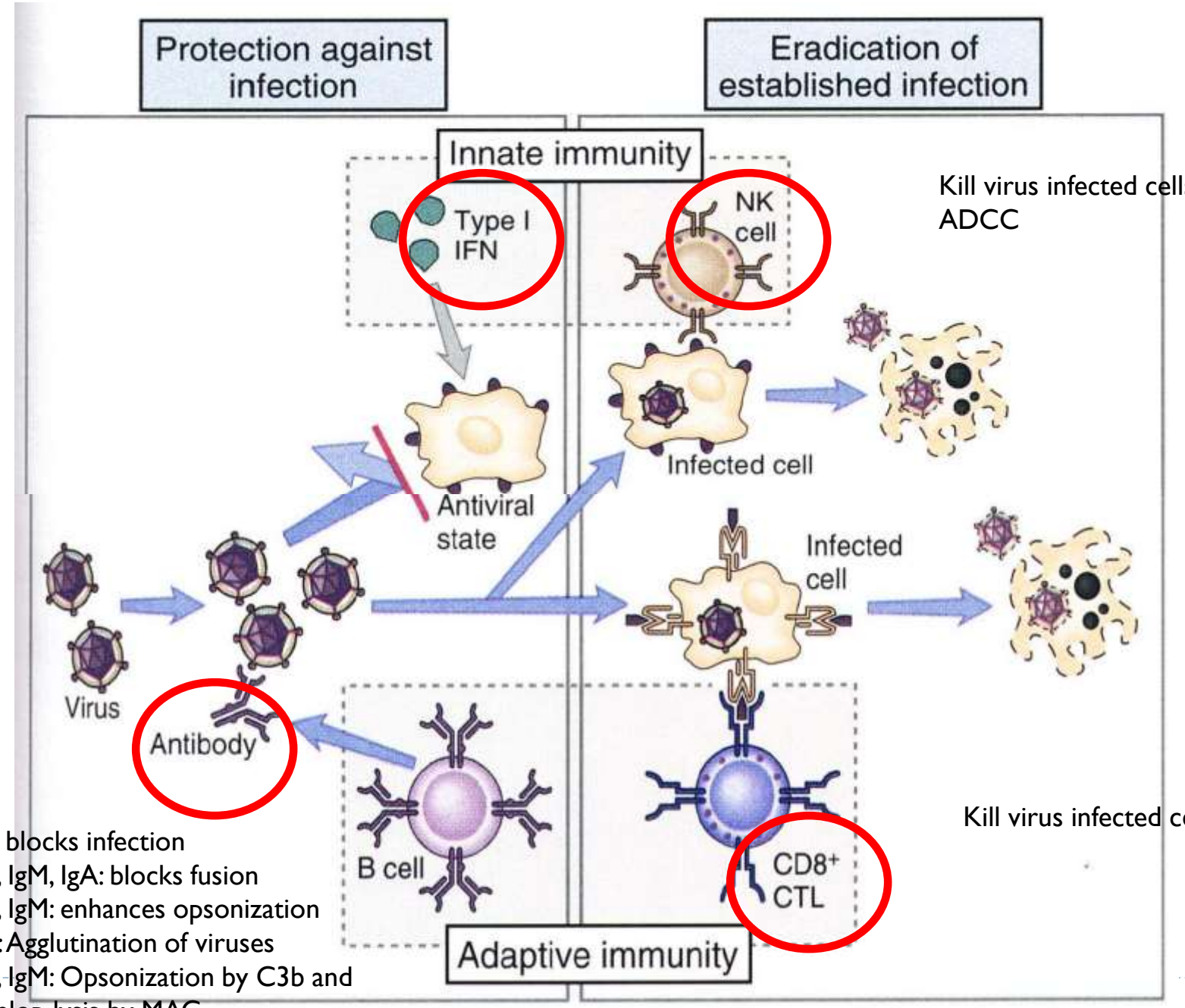
- To cause infection and disease, viruses must enter, replicate, and spread in target organs or systemically
- The body has mechanical, biochemical and immune defenses
- Routes of infection
  - Skin
  - Alimentary canal
  - Urogenital stract
  - Respiratory tract

Lines of defense

- First line: Anatomical barriers
  - Mechanical barriers (Skin and mucous membranes)
  - Chemical barriers (acid and antibacterial peptides)
    - Complement proteins and defensins
  - Physiological barriers (temperature, pH)
- Second line: innate immune cells
  - Phagocytes (monocytes/macrophages, neutrophils and dendritic cells)
  - Natural killer cells (NKCs)
  - Inflammatory cells (mast cells, basophils, eosinophils)
- Third line: adaptive immune cells
  - Lymphocytes (T cells and B cells)
  - **Humoral immunity**: Immunoglobulins (antibodies)
  - **Cell-medicated immunity**: T cells
    - Effector and memory responses

Innate immune responses to viral infections

- Inhibition of virus infection by Type I interferons
  - Production by infected cells
    - Best inducers – RNA viruses
    - Other inductive stimuli
    - Double stranded RNA
  - Induce "antiviral state state"
  - Inhibit viral replication in both infected cells
  - Bystander cells activate NK cells
  - Activate immune responses & enhance T-cell recognition of infected cells
- NK cell cell-mediated killing of infected cells
  - Lyse infected cells
  - Cells infected with many different viruses have reduced levels of class I MHC expression



IgA: blocks infection
IgG, IgM, IgA: blocks fusion
IgG, IgM: enhances opsonization
IgM: Agglutination of viruses
IgG, IgM: Opsonization by C3b and envelop lysis by MAC

Summary

TABLE 17-1 Mechanisms of humoral and cell-mediated immune responses to viruses

Response type	Effector molecule or cell	Activity
Humoral	Antibody (especially, secretory IgA)	Blocks binding of virus to host cells, thus preventing infection or reinfection
	IgG, IgM, and IgA antibody	Blocks fusion of viral envelope with host-cells plasma membrane
	IgG and IgM antibody	Enhances phagocytosis of viral particles (opsonization)
	IgM antibody	Agglutinates viral particles
	Complement activated by IgG or IgM antibody	Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex
Cell-mediated	IFN-γ secreted by T_H or T_C cells	Has direct antiviral activity
	Cytotoxic T lymphocytes (CTLs)	Kill virus-infected self-cells
	NK cells and macrophages	Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC)

Mechanisms of Infection and Spread of Viruses

- The skin
  - Surface contains keratinized cells provides an impermeable layer to viruses
  - Small cuts and abrasions can cause viruses to enter and replicate (papillomaviruses, poxviruses)
  - Arboviruses are introduced through bites (eg mosquitoes, ticks, sand flies)
  - Zoonotic viruses are introduced by animal bites e.g. Rabies
  - Blood borne viruses are introduced by punctures e.g. HIV, Hep B, C

Mechanisms of Infection and Spread of Viruses

- The Gastrointestinal tract
  - Many viruses are acquired by ingestion
  - Protected by squamous epithelium, mucus, acids, bile, proteolysis enzymes ,IgA
  - Viruses are taken up by M cells and are transported to local lymph nodes (Peyer's patches) where they replicate in mononuclear phagocytes
  - Eg enteroviruses, coronaviruses, caliciviruses, rotaviruses

Mechanisms of Infection and Spread of Viruses

- The respiratory tract
  - Protected cleansing mechanisms (mucus, ciliated cells)
  - Viruses attach to specific receptors on epithelial cells
  - E.g. rhinoviruses, orthomyxoviruses, systemic: measles, rubella, chicken pox)

Mechanisms of spread

- Local spread on epithelial surfaces
- Subepithelial invasion and lymphatic spread
- Spread by the blood stream: viremia

Local spread on epithelial surfaces

- Many viruses can replicate in epithelial cells and can be shed into the environment
  - Papillomaviruses infect basal layers of the skin
  - Poxviruses also infect via the skin
- Viruses that infect via the respiratory or GI tract enter via epithelial cell linings
  - eg Paramyxoviruses, influenza viruses, rotaviruses
- Restriction of viral infection to epithelial cells cannot be equated to lack of severity of clinical disease

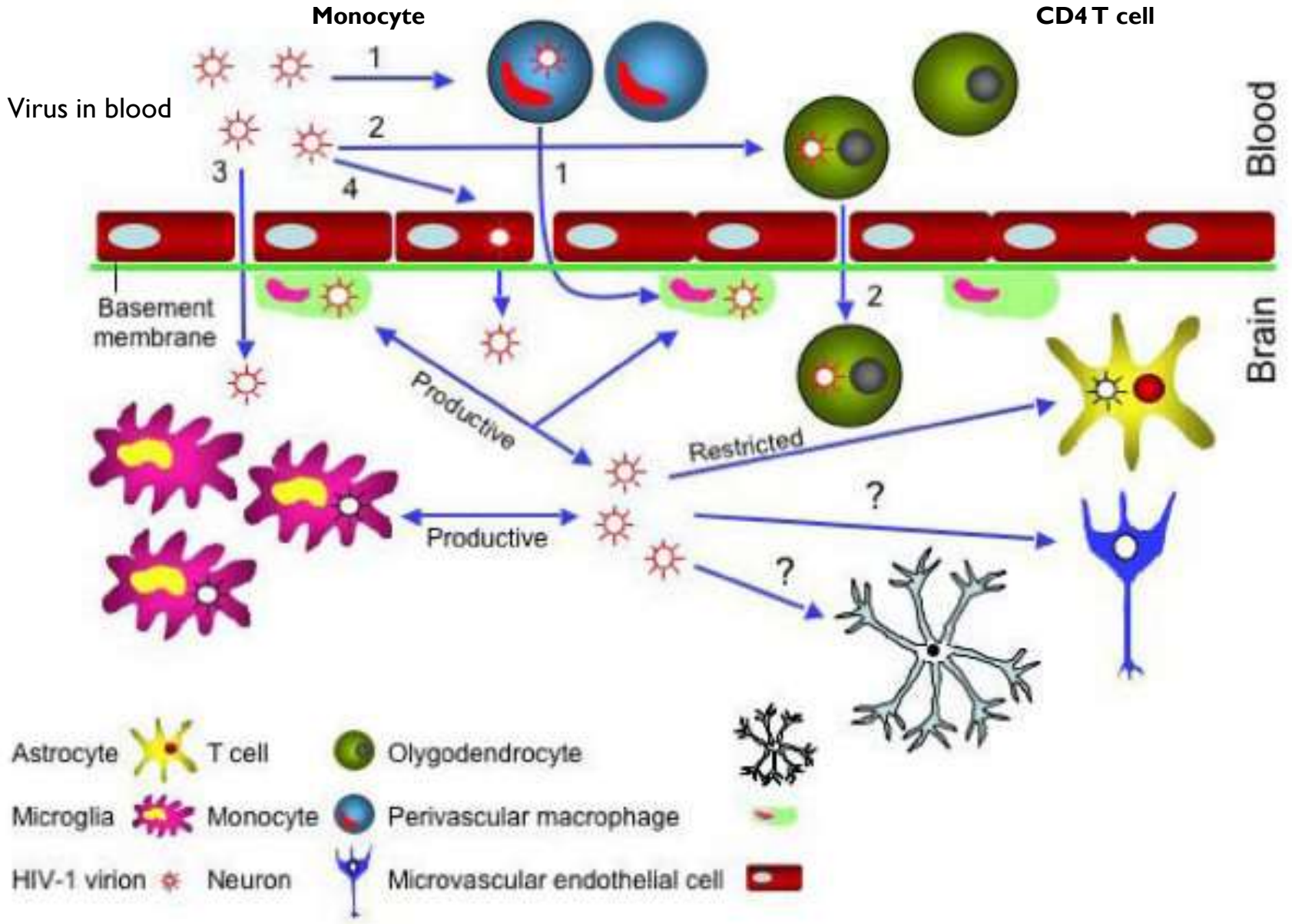
Subepithelial invasion and lymphatic spread

- Viruses via the epithelial surfaces can reach subepithelial tissues and be taken by dendritic cells and tissue macrophages or enter lymphatics to local lymph nodes
- The mononuclear cells process viruses (innate immunity) or present viral antigens to lymphocytes (induction of adaptive immunity)
- However, some virus escape immune defenses and replicate in mononuclear phagocytes
- Some viruses escape lymphatic and enter the blood stream

Spread by the blood stream: viremia

- Most effective and rapid vehicle for viral spread
- Viruses are spread as free virions or associated with lymphocytes/phagocytes ('Trojan horses')
- Vascular endothelial cells (with tight junctions) restrict viral spread to tissues including the brain
  - Some viruses are able to infect endothelial cells and enter tissues/organs from the blood (e.g. WNV)
  - Cell free virus had been shown to pass though tight junctions
  - Lymphocyte/phagocyte associated virus traverse tissues by virtual of circulating lymphocytes/phagocytes trafficking across endothelial cells (Trojan horse hypothesis for viral entry into the CNS e.g. HIV, JC virus, WNV etc
  - Retrograde axonal transport: neural tropic virus e.g. polio viruses can infect peripheral nerves and traffic into the CNS via axons

Entry of viruses into the CNS

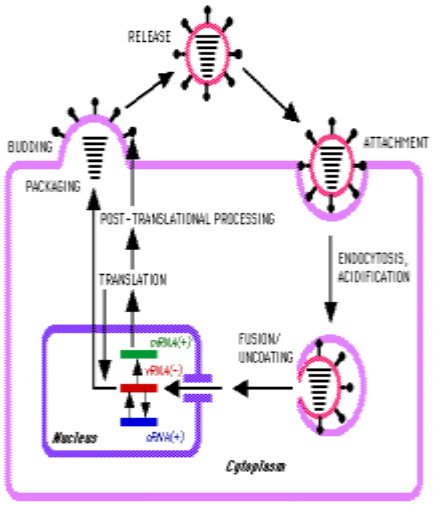


Virus Shedding

- Respiratory or oropharyngeal secretion
  - Measles, chickenpox, rubella,
- Feaces
  - Enteric viruses
- Skin
  - Poxviruses, herpesviruses
- Urine (viruria)
  - Mumps, CMV, JC virus
- Milk
  - CMV
- Blood
  - HIV, Hepatitis, B, C, D, HTLV
- Genital secretions
  - Hepersviruses, HIV, HTLV, Papillomaviruses, Hepatitis B, C,

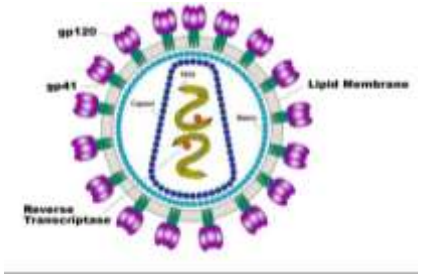
Viral Replication

- General features of viral life cycle
  - Attachment
  - Penetration
  - Uncoating
  - Transcription of early mRNA
  - Translation of early proteins (non-structural proteins)
  - Viral DNA/RNA replication
  - Transcription of late mRNA
  - Translation of late proteins (structural proteins)
  - Assembly of virions
  - Release



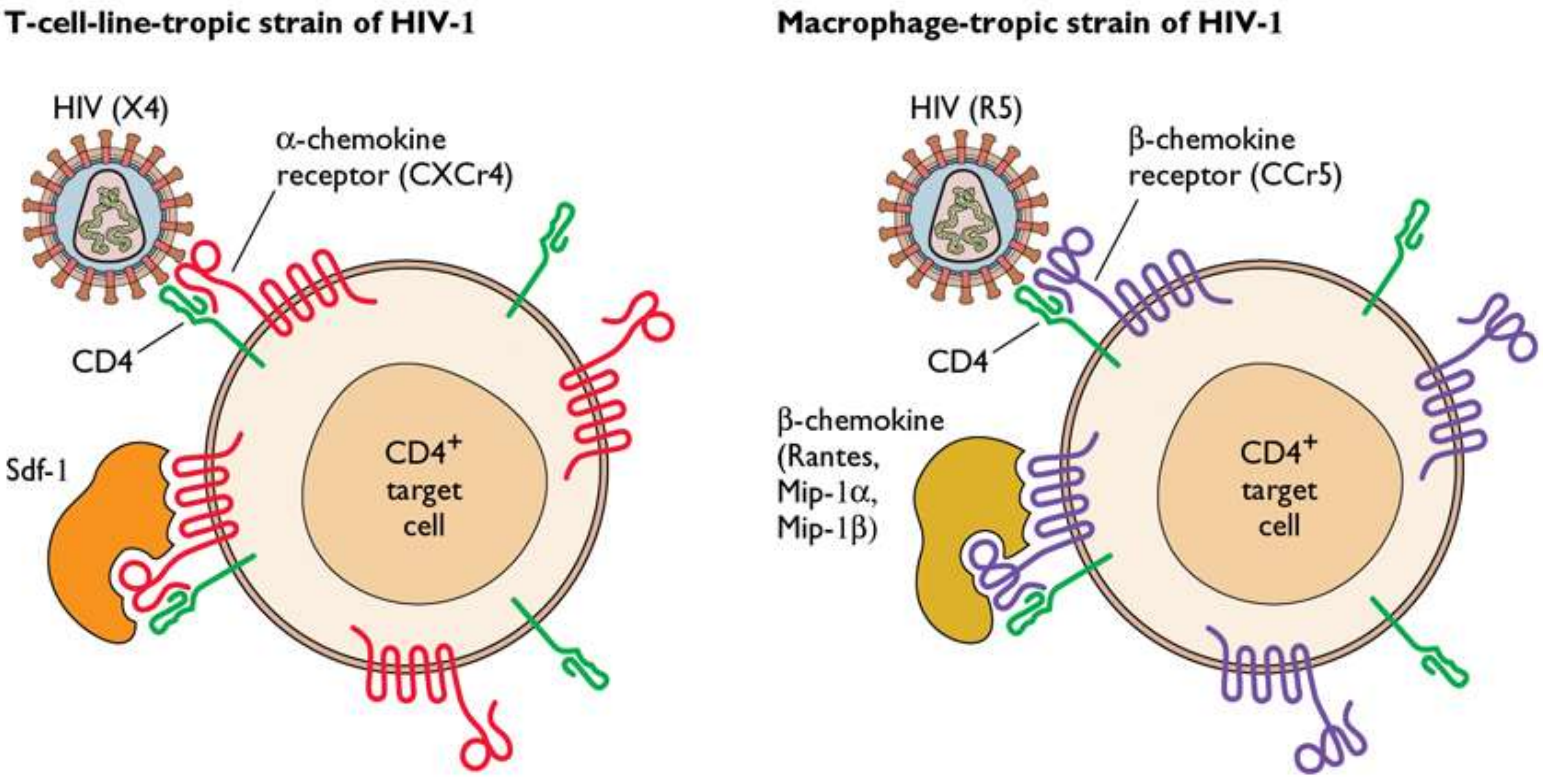
Attachment

- To infect cells viruses need to bind to receptors on cells
- Ligands on viruses aid this process



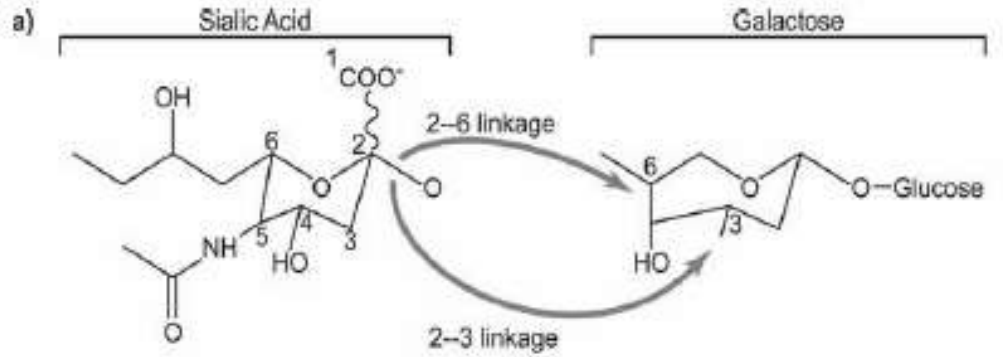
- Examples
  - Orthomyxoviruses use hemaglutinin glycoprotein to bind to sialic acid on cells
  - Other viruses (eg Rhinoviruses use intracellular adhesion molecules (ICAM-1)
  - HIV uses gp120 glycoprotein to bind to CD4 and other chemokine co-receptors (CCR5 and CXCR4) on leukocytes
  - Others use hormone receptors and permeases
- Viral tropism is dependent on receptor binding
  - Viral evolution has given rise to viruses that can adapt and use other receptors
  - Attachment sites are targets for therapeutics

HIV-1 attaches via gp120/gp41 to the CD4 surface receptor and chemokine co-receptors CXCR4 and/or CCR5

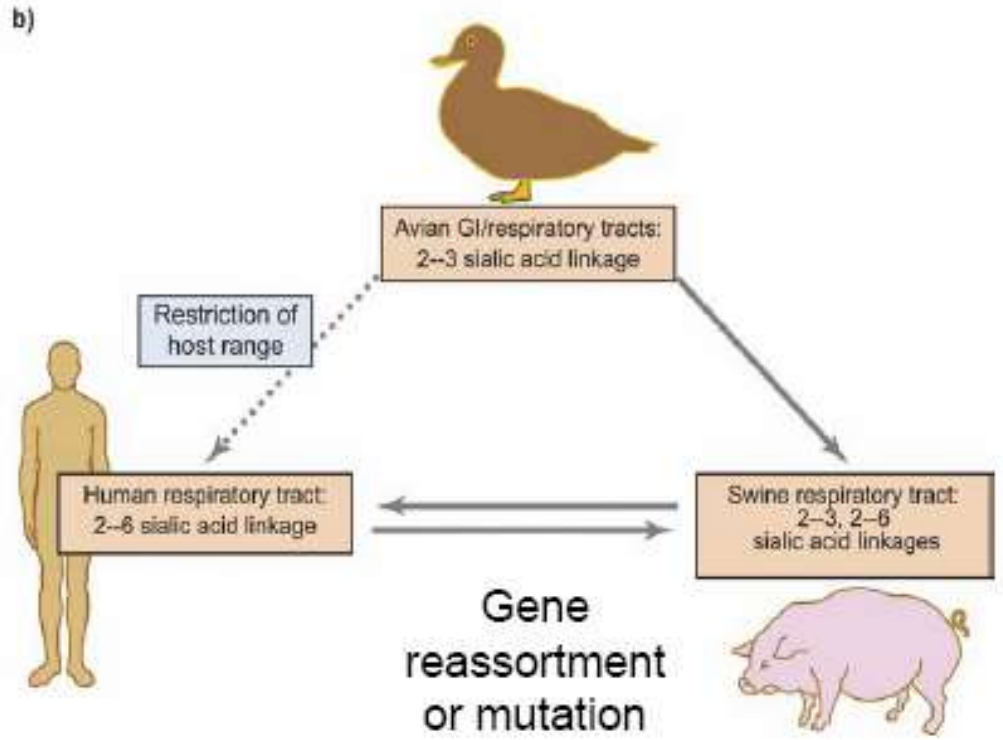


Adapted from Fig. 3 of A. S. Fauci, *Nature* **384:**529–533, 1996, with permission.

Sialic acid residues can be covalently attached to galactose residues of integral glycoproteins and glycolipids via either 2–3 or 2–6 α linkages.



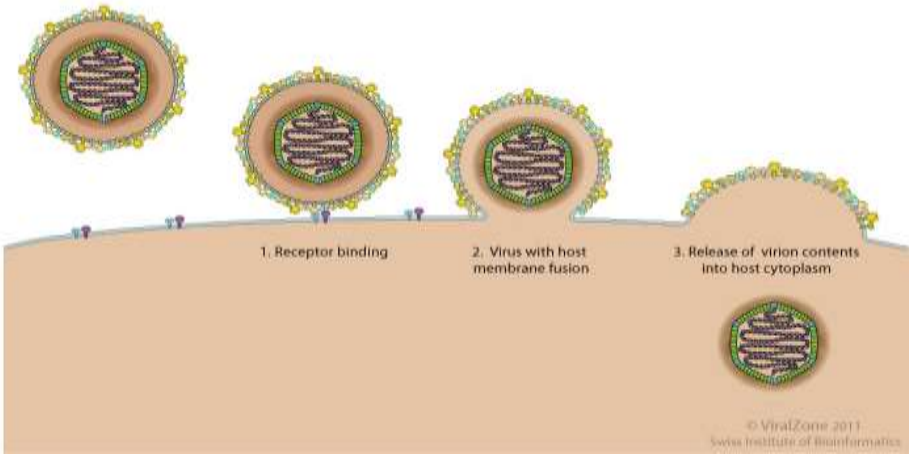
The avian, human, and swine upper respiratory tract epitheliae preferentially express 2–3 linkages, 2–6 linkages, and both 2–3 and 2–6 linkages respectively

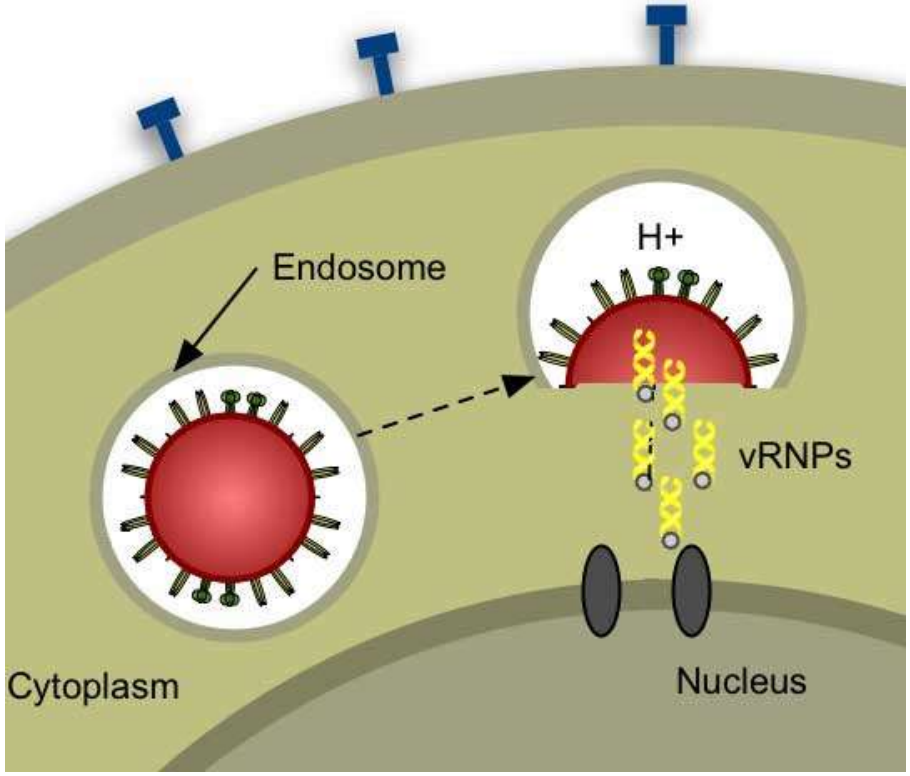


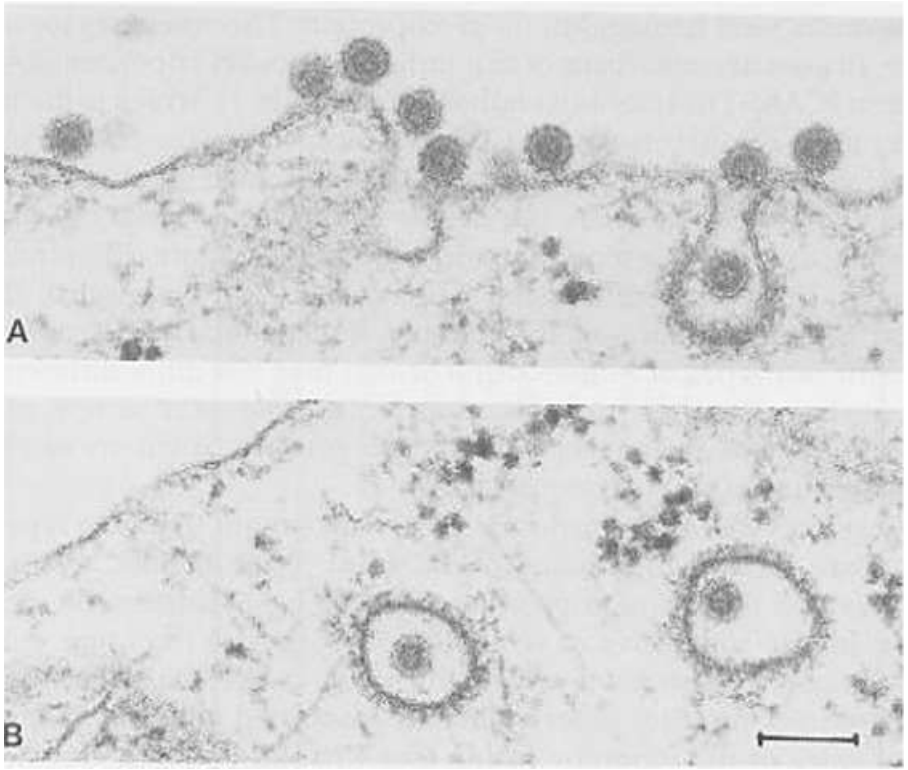
Penetration (Uptake)

- Endocytosis
  - Receptor mediated endocytosis forming clathrin-coated pits
  - Formation of clathrin-coated vessicles that enter the cytoplasm and later fuse with endosomes
  - Acidification of endosomes triggers changes in capsid proteins and release of RNA e.g. polioviruses
  - In influenza viruses acidification causes conformational changes to hemagglutinin enabling fusion of viral envelop and endosome membrane and release of viral nucleocapsid into the cytoplasm.

- Fusion with plasma membrane
  - Fusion glycoprotein of paramyxoviruses causes the envelop to fuse directly with the host cell membrane even at neutral pH
  - Nuncleocapsid is then released into the cytoplasm.
  - Fusion proteins are involved e.g. gp41 in HIV
  - Also target for therapies e.g. CCR5 fusion inhibitor Miraviroc


1. Receptor binding
2. Virus with host membrane fusion
3. Release of virion contents into host cytoplasm
© ViralZone 2011
Swiss Institute of Bioinformatics


Endosome
H+
vRNPs
Cytoplasm
Nucleus


A
B

Uncoating

- For enveloped RNA viruses that enter via membrane fusion or endocytosis transcription commences immediately the nucleocapsid is in the cytoplasm
- With non-enveloped viruses eg Reoviruses, certain capsid proteins are removed and the genome is expressed without being fully removed from the core (nucleocapsid)
- Most viruses, however, the core is completely uncoated
- For viruses that replicate in the nucleus uncoating is completed in the nucleus

Transcription of the viral genome

- DNA viruses
  - Transcription of mRNA from dsDNA and replication of DNA
  - Similar to mammalian cells
    - papoviruses, adenoviruses, herpesviruses
      - Transcription by cellular DNA-dependent RNA polymerase II
      - Cleavage and splicing to produce monocistronic mRNAs
    - Poxviruses
      - Replicate in the cytoplasm
      - Carry their own transcriptase (DNA-dependent RNA polymerase)
      - Produce other proteins that make them replicate outside the nucleus
    - Parvoviruses
      - Use host cellular DNA polymerase to synthesize dsDNA from viral ssRNA genome.
      - dsDNA is then transcribed by cellular DNA-dependent RNA polymerase II
    - Hepadnaviruses
      - Reverse transcription of RNA intermediate for DNA replication
      - Retroviruses use a similar mechanism
      - Uses host cellular DNA-dependent RNA polymerase
      - Also uses viral DNA polymerase to synthesize dsDNA

Transcription of the viral genome

- RNA viruses
  - + sense ssRNA viruses (Picronaviruses, Togaviruses, Flaviviruses and Caliciviruses) require no trancriptase since genomic RNA serves as mRNA
  - The genome is translated directly into a polyprotein which is cleaved to give individual viral proteins including an RNA-depended RNA polymerase which replicates the viral RNA
    - Synthesizes – sense RNA copies which are used as templates to form + sense genomic RNA
  - - sense ssRNA viruses (paramyxoviruses, filoviruses and Rhabdiviruses carry an RNA-depended RNA polymerase (trancriptase) which transcribes + sense RNAs that serve as mRNA. For segmented viruses (Orthomyxoviruses each segment is transcribed by a trancriptase carried in the virion.

Transcription of the viral genome

- RNA viruses
  - **dsRNA viruses** (Reoviruses) the minus strand is transcribed by a virion associated transcriptase in the cytoplasm to yield mRNA
  - The plus strand serves as a template for replication

  - In retroviruses, the **plus sense RNA** is transcribed by the viral associated RNA-dependent DNA polymerase (reverse transcriptase) to produce an RNA-DNA hybrid which is converted into a dsDNA (proviral DNA or *provirus*) which is integrated into host genomic DNA .
  - Proviral DNA can remain latent for a long time
  - Proviral DNA is transcribed by cellular RNA polymerase II.

Transcription Summary

- Viral RNA of most + sense ssRNA viruses bind directly to ribosomes and is translated in full or in part without requiring transcription
- For other RNA viruses, mRNA must be transcribed
- For DNA viruses that replicate in the nucleus, cellular DNA-dependent RNA polymerase II performs transcription
- Other viruses require an in-house transcriptase
- Cytoplasmic RNA viruses carry a DNA-dependent RNA polymerase (ds or ss RNA-dependent RNA polymerase.

Translation

- Capped, polyadenylated and processed (Methylated) monocistronic or polycistronic viral mRNA bind to ribosomes and are translated just like cellular mRNAs from the 5' to the 3' end

- Produced proteins undergo post translational modifications
  - Phosphorylation for nucleotide binding
  - Fatty acid acylation form membrane insertion
  - Glycosylation (membrane proteins) or proteolytic cleavage (polyproteins)

- Proteins are also transported to various parts of the cells

Replication of Viral Nucleic acid

- Replication of viral DNA
  - Requires a helicase (ATPase) to unwind the dsDNA
  - A helix destabilizing protein to keep the duplex apart
  - A DNA polymerase to replicate the strands from 5" to 3" ends
  - An RNAse to degrade the RNA primers
  - A DNA ligase to join the Okazaki fragments together

    - Eg Papovirus genomes have histones and resemble cellular genome, utilizes cellular DNA polymerase α for replication
    - Adenoviruses have linear DNA which is replicated by a virus encoded DNA polymerase from both ends, no Okazaki fragments are generated.
    - Herpesviruses came with all the proteins/enzymes required for replication

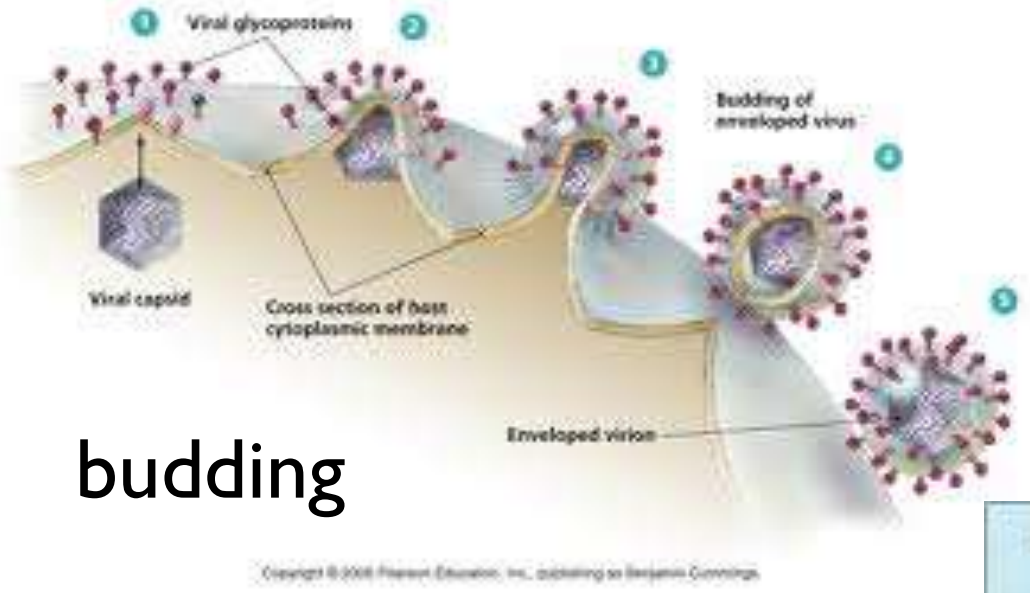
Replication of Viral Nucleic acid

- Replication of viral RNA
  - A phenomenon unique to viruses
  - Requires an RNA-depended RNA polymerase (not found in mammalian cells)
  - Requires synthesis of a complementary strand which serves as a template for replication
  - For retroviruses, replication proceeds via a DNA intermediate which is integrated into host cellular DNA.
    - Replication and transcription of viral RNA occurs from integrated proviral DNA.

Assembly and Release

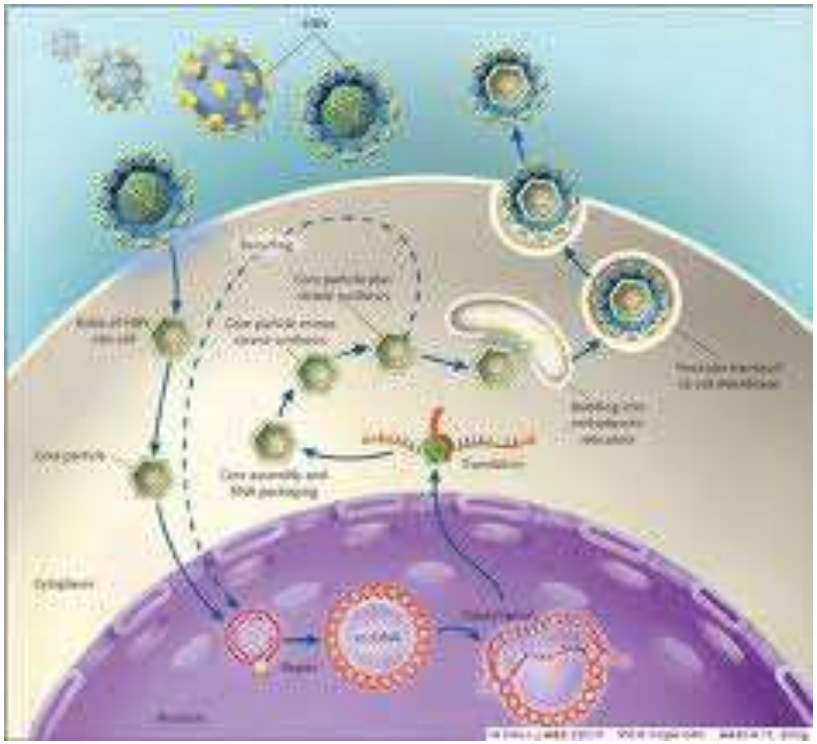
- Non-enveloped (naked) viruses
  - All have icosahedral structures
  - Structural proteins form capsomeres which self-assembles into capsid where viral nucleic acid is packaged
  - Most naked viruses accumulate in the cytoplasm or nucleus until the cell lyses

- Enveloped viruses
  - Mature by acquiring an envelop by budding through cellular membranes
    - Budding from cell membranes
      - Insertion of viral glycoproteins into cell membrane by displacement
      - Eg herpesviruses, togaviruses, retroviruses
    - Exocytosis
      - Bud through Golgi complex or ER into vesicles that migrate to the cell membrane where they fuse and expel viruses by exocytosis
        - Eg Flaviviruses, coronaviruses, bunyaviruses



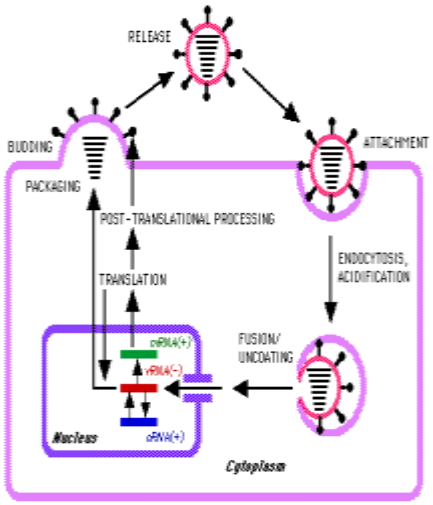
budding

exocytosis



Summary

- General features of viral life cycle
  - Attachment
  - Penetration
  - Uncoating
  - Transcription of early mRNA
  - Translation of early proteins (non-structural proteins)
  - Viral DNA/RNA replication
  - Transcription of late mRNA
  - Translation of late proteins (structural proteins)
  - Assembly of virions
  - Release



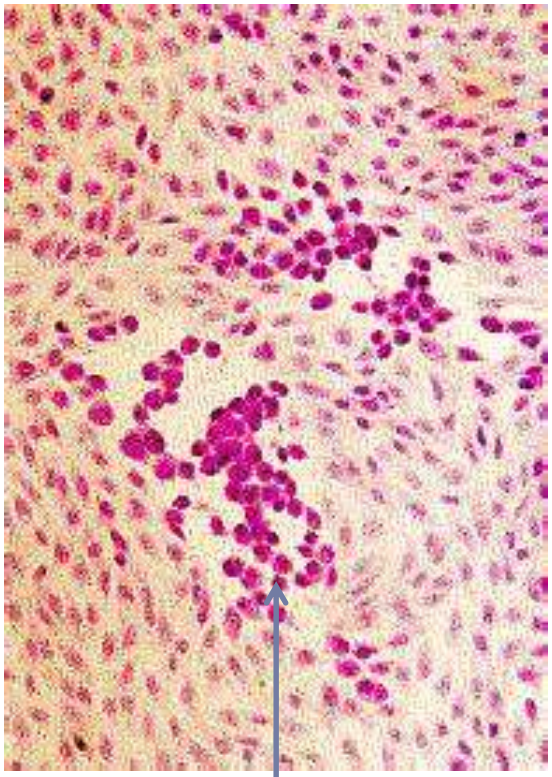
Viral pathogenesis

Types of virus-induced changes in cells

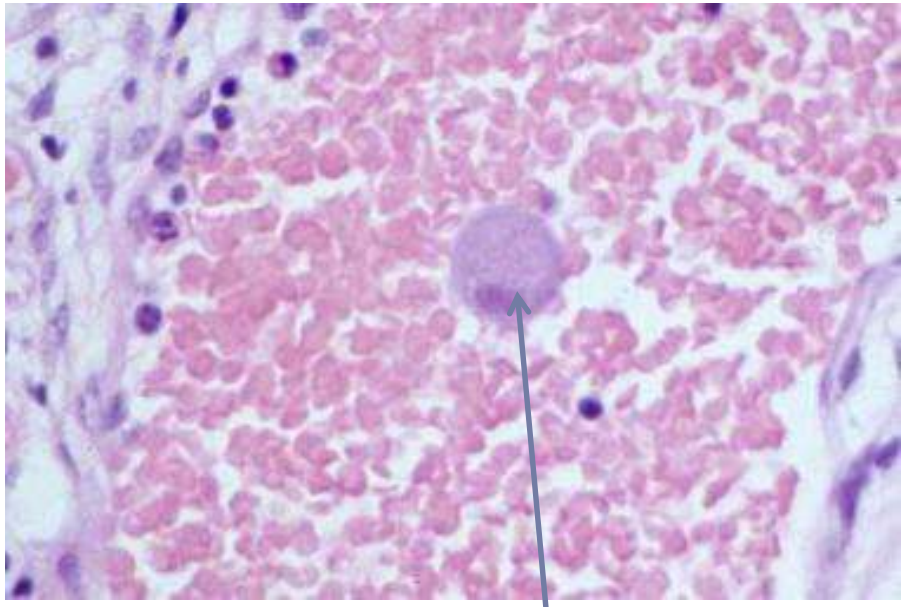
Type of infection	Effects on cells	Production of infectious virions	Examples
Lytic (cytocidal)	Morphological changes (CPE), inhibition of protein, RNA, DNA synthesis and cell death	Yes	Alphaherpesviruses, enteroviruses, reoviruses
Persistent productive	No CPE, little metabolic disturbance, cells continue to divide, some loss of function	Yes	Arenaviruses, rabies virus, most retroviruses
Persistent, nonproductive transformation	Usually nil, Alteration of morphology, cells can be passaged indefinitely, produce tumors when transplanted	No, No, oncogenic DNA viruses Yes, oncogenic retroviruses	Measles in the brain Polyomaviruses, adenoviruses Sarcomaviruses

Cytopathic effects (CPE) of viral infections

- 1. Inclusion bodies
  - Recognized after staining and fixation
  - Single or multiple
  - E.g. poxviruses, paramyxoviruses, reoviruses



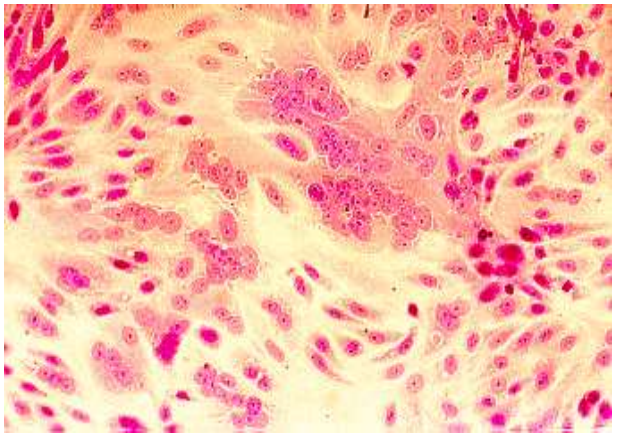
Inclusion bodies in brain: rabies virus



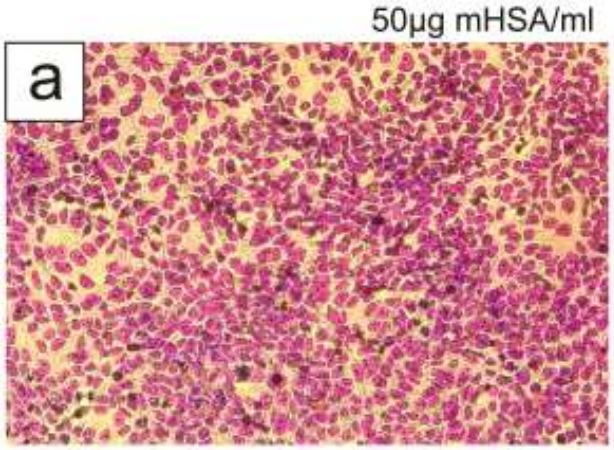
Giant cell inclusion bodies: Cytomegalovirus

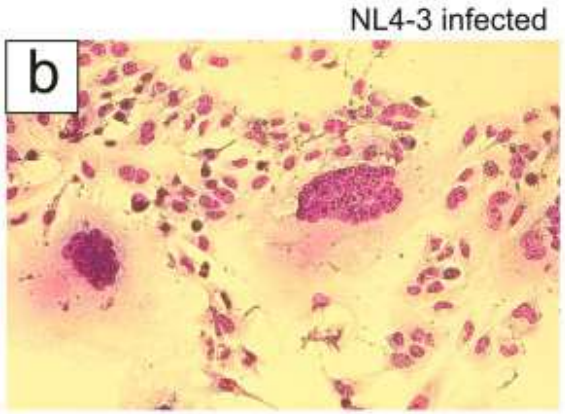
Cytopathic effects (CPE) of viral infections

- 2. Cell fusion (Syncytia formation)
  - Fusion of cells
    - Mechanism of spread and immune evasion (antibody responses)
  - Lentiviruses, paramyxoviruses and some herpesviruses



Syncytia formation in RSV culture





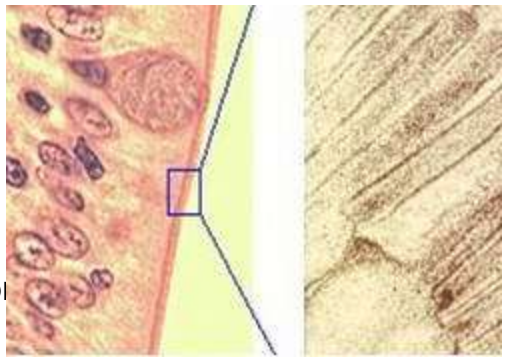
Syncytia formation in HIV culture

Relationship between CPE and disease

- Not direct
  - Lytic viruses like enterovirus may cause mild disease where as non-lytic virus like rabies may cause lethal disease
  - In some organs, a great deal of cellular damage may occur without causing apparent illness (e.g. Liver)
  - Edema may not be important in some organs but can be serious in the brain

Viral damage to tissues and organs

- Direct damage by lytic viruses
  - Paralysis in a polio patient is a direct consequence of death of motor neurons in the anterior horn of the spinal code leaving the muscles nonfunctional
- Damage to epithelium of the respiratory tract
  - Influenza viruses
    - Inflammation and necrosis of epithelial debris
    - Accumulation of fluid and necrotic debris causing obstruction/blockage (hypoxia)
- Damage to epithelium of the intestinal tract
  - Rotaviruses
    - Shortening and fusion of microvilli
      - Fluid accumulation in the gut lumen and diarrhoea
      - Impaired absorption, osmotic loss, electrolyte loss and develop



Viral damage to tissues and organs

- Bacterial superinfection
  - Epithelial damage predisposes to secondary bacterial infection
  - Pneumococcal infection during influenza infection
  - RSV infection predisposes patients to rhinitis, pharyngitis, sinusitis, and otitis media.
  - Rotavirus infection can increase susceptibility to *E coli* diarrhoea

- Physiological changes without causing cell death
  - Viral infection of islets of the pancreas
  - Alter expression of MHC class I
  - Over expression of MHC class II

Viral damage to tissues and organs

- Immunopathology
  - Type I (Anaphylactic hypersensitivity)
    - IgE on mast cells and basophils
    - Release of histamines, leukotrienes and heparin
    - Rushes, acute respiratory syndrome, anaphylaxis
    - Not very important in viral infections but important in helminth infections and allergy
  - Type II (Antibody dependent cototoxic hypersensitivity)
    - ADCC
    - Herpesviruses, unclear
  - Type III (Immune complex mediated hypersensitivity)
    - Common cause in mild inflammation
    - Filoviruses, flaviviruses
  - Type IV (delayed cell-mediated hypersensitivity)
    - E.g. lymphocytic choriomeningitis (LCM)
    - Severe meningitis, cerebral edema, and death

Viral damage to tissues and organs

- Autoimmunity
  - Molecular mimicry

Molecular mimicry between proteins of infectious organisms and human host proteins

Protein*	Residue†	Sequence‡
Human cytomegalovirus IE2	79	P D P L G R P D E D
HLA-DR molecule	60	V T E L G R P D A E
Poliovirus VP2	70	S T T K E S R G T T
Acetylcholine receptor	176	T V I K E S R G T K
Papilloma virus E2	76	S L H L E S L K D S
Insulin receptor	66	V Y G L E S L K D L
Rabies virus glycoprotein	147	T K E S L V I I S
Insulin receptor	764	N K E S L V I S E
Adenovirus 12 E1B	384	L R R G M F R P S Q C N
α-Gliadin	206	L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160	G V E T T T P S
Human IgG constant region	466	G V E T T T P S
Measles virus P3	13	L E C I R A L K
Corticotropin	18	L E C I R A C K
Measles virus P3	31	E I S D N L G Q E
Myelin basic protein	61	E I S F K L G Q E

Viral damage to tissues and organs

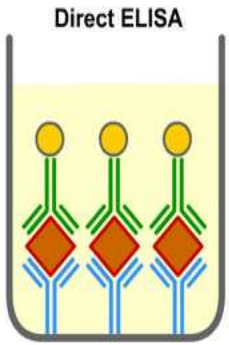
- Autoimmunity
  - Molecular mimicry
  - Polyclonal B cell activation
    - E.g. EBV induced polyclonal B cell activation and antibody production to various tissues/organs
  - Cytokine production of MHC antigens
    - Induction of interferon gamma and tumor necrosis alpha which induce MHC class II on brain cells which start to present antigens (egg myelin) to T cells
      - Multiple sclerosis demyelization
  - Exposure of sequestered cellular proteins
    - Incorporation of cellular proteins into viral envelop
  - T cell dysfunction
    - Down regulation of T cell function

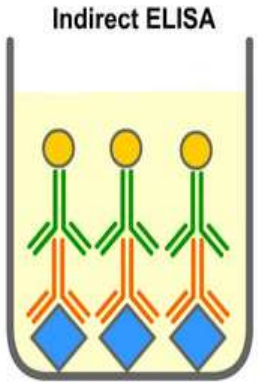
Immunosuppression

- Destruction of T cells
  - CD4+T cell destruction by HIV
    - Impaired antigen processing and presentation, and cytokine production
    - Death by apoptosis, fusion (syncytia formation), lysis by CD8+ T cells
- Abortive infection of monocytes/macrophages and T/B cells
  - CMV, EBV, measles virus

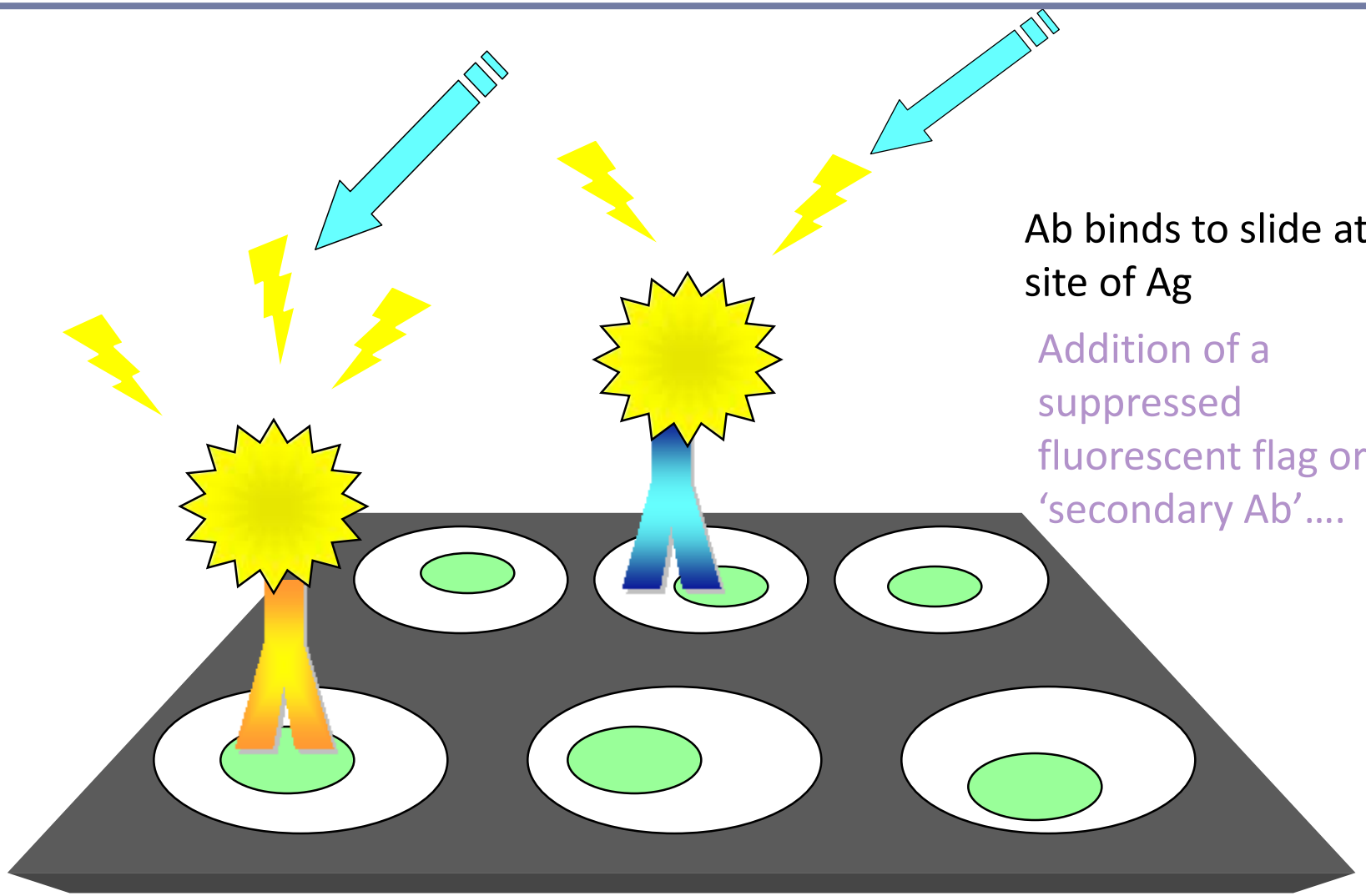
Laboratory diagnosis of Viral Diseases

- Culture
  - Gold standard in many viral diseases
- Serology (Antigen detection)
  - Indirect Immunoflourescence to detect viral proteins
  - Direct ELISA
  - Western blotting
- Serology (antibody detection)
  - Detection of serum antibodies
  - Not very important in clinical decision making
- Nucleic acid detection
  - RT-PCR to detect viral RNA, PCR to detect viral DNA
  - Not available in all settings
  - Nucleic acid sequencing is important in typing and epidemiological investigations



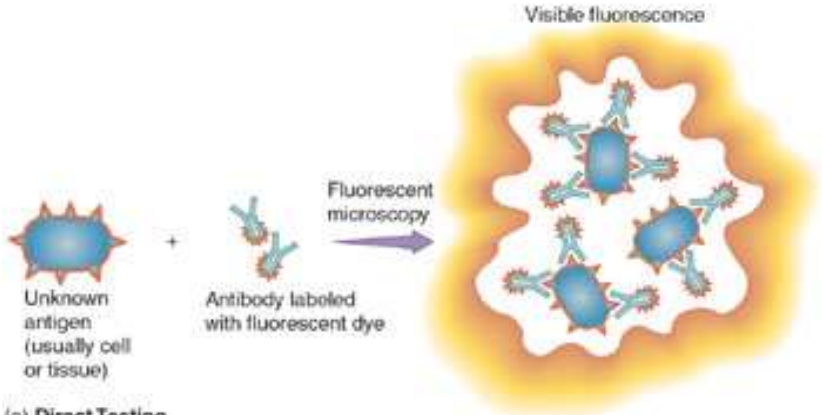


Immunohistochemistry

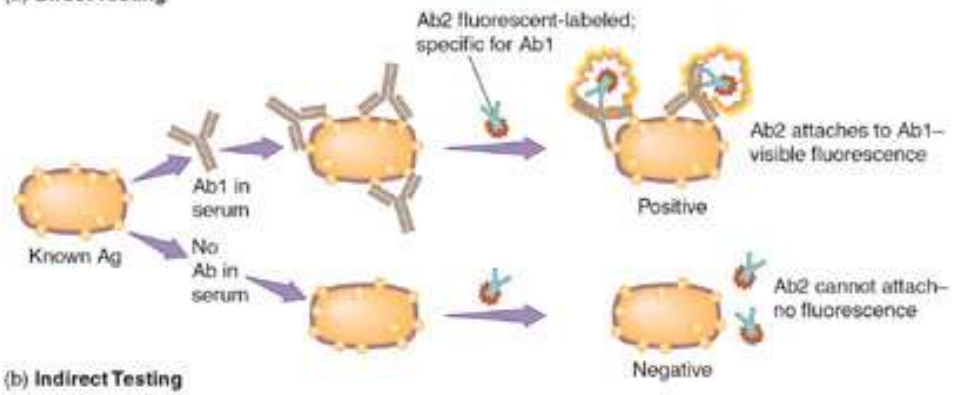


Ab binds to slide at site of Ag

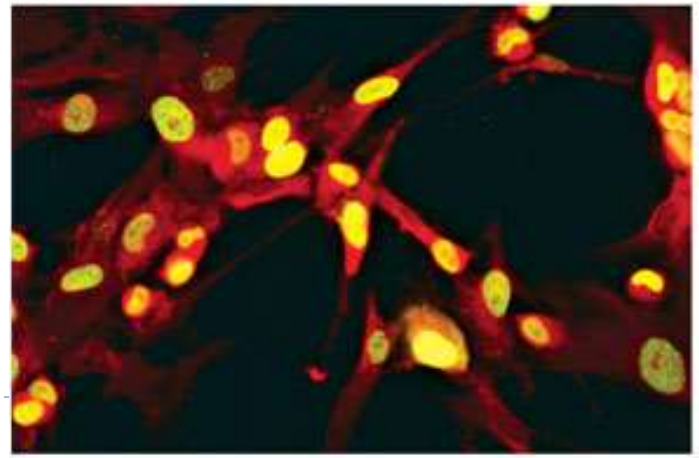
Addition of a suppressed fluorescent flag or ‘secondary Ab’….



(a) **Direct Testing**



(b) **Indirect Testing**



(c) **Indirect Immunofluorescence Testing**

Viral Genetics and Evolution

- Viruses have the greatest genetic diversity
  - Natural selection
    - Mutation (antigenic drift)
    - Recombination
    - Reassortment (antigenic shift)
      - Segmented genomes

- Mutations
  - Caused by errors in replicating (copying) genomes
  - Many are lethal (however, non-lethal mutations result change of phenotype that may confer selective advantage)
    - Non-functional gene product
    - Stop codon that terminates translations
  - Some mutations are neutral

Mutations

- Classified according to kind of nucleic acid change (genotypic) or phenotype change (phenotypic)

- Genotypic mutations
  - **Point mutations- single nucleotide substitutions**
  - Deletions
  - Insertions
  - Duplications
  - Inversions
  - **Incorporation of foreign nucleic acid (Reassortment)**

Mutations

- Classified according to kind of nucleic acid change (genotypic) or phenotype change (phenotypic)

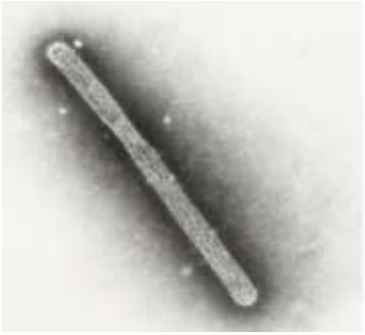
- Phenotypic
  - Type of plaque (plaque mutations)
  - **Resistance mutation (escape mutations)**
  - Conditional lethal mutations
  - **Host range mutations**
  - Temperature sensitive mutations

Mutation Rates

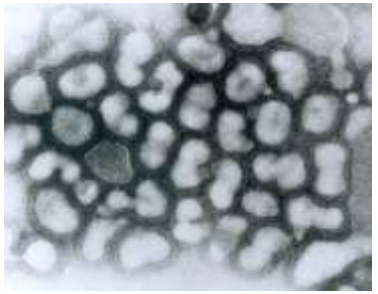
- DNA viruses
  - Rates similar to eukaryotic DNA
    - Viral DNA is subject to same cellular 'proofreading' exonuclease correction
    - Rates occur at 10^{-8} to 10^{-11} per incorporated nucleotide
    - Point mutations in the third nucleotide of a codon are often silent (wobble hypothesis/redundancy of the genetic code)
  - Rates are higher for RNA viruses
    - No cellular proofreading for RNA
    - E.g. 10^{-3} to 10^{-4} nearly every progeny virion is different from parent and from each other
    - Most mutations are lethal but most non-lethal mutations accumulate quickly

Evolution of Influenza Viruses

- Orthomyxovirus group
  - Enveloped
  - Pleiomorphic
  - Single stranded segmented – sense RNA genome
  - Three major groups based on ribonuclear Ags.
    - A: Most studied, Human, swine, avian, equine, marine
    - B: Humans only
    - C: Humans, swine
  - Subtype A
    - Greatest virulence and epidemic spread
  - Virus-specified hemagglutinin and neuraminidase spikes

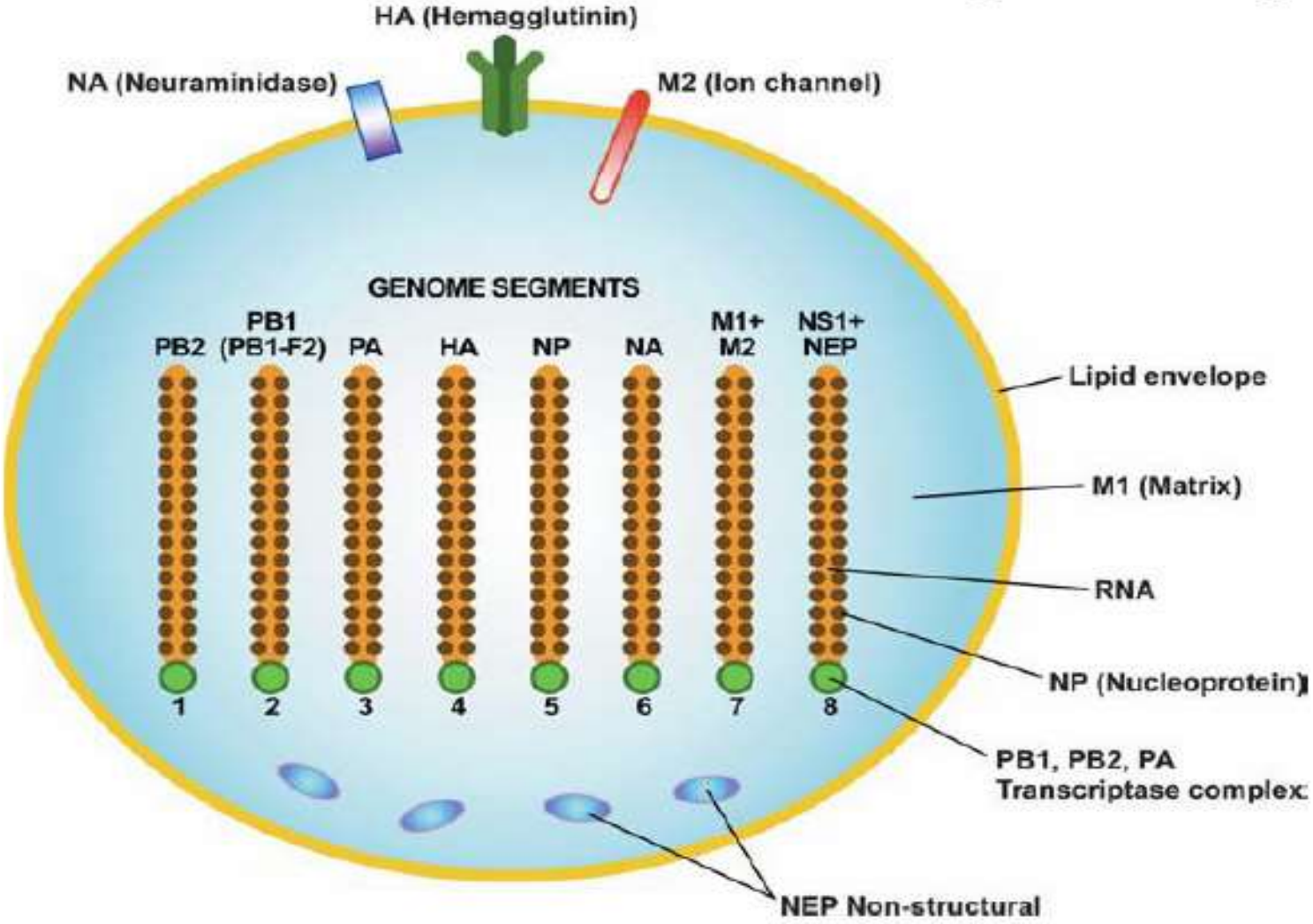


- transmission electron micrograph (TEM) 150,000x
- roughened surface of the proteinaceous coat



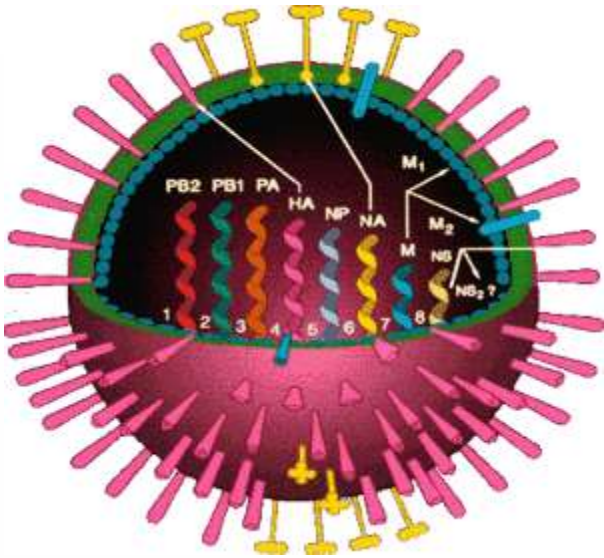
Influenza Viruses

Structure of Influenza Viruses



Membrane proteins

- Hemagglutinin (HA)
  - Attach to cell surface sialic acid receptors
  - Facilitate entry if the virus into the cell
  - Crucial component of current vaccine
- Neuraminidase (NA)
  - Catalyze the cleavage if glycosidic linkages to sialic acid on host cell and the virion surfaces
  - Inhibition of NA— the most effective antiviral treatment
- M2 protein — small amount in influenza A
  - Ion channel
  - Regulate the internal PH of the virus
  - Blocked by antiviral drug



Influenza Virus

Genome

- Consists of s/s (-)sense RNA in 8 segments (7 in Influenza C).
- The structure of the influenza virus genome is known in great detail because of the tremendous amount of genetic investigation (conventional and molecular) which has been done.
- The 5' and 3' terminal sequences of all the genome segments are highly conserved

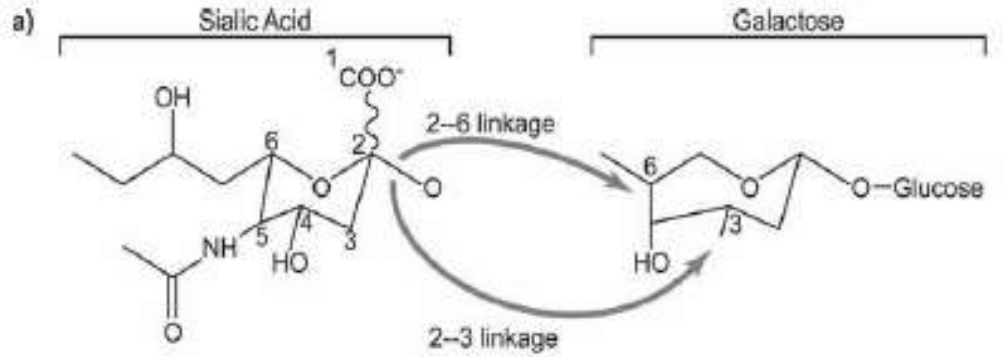
Genome

Segment:	Size (nt)	Polypeptide (s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity (?)
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation. Anti-interferon protein.
		NS2	Non-structural: nucleus+cytoplasm, function unknown

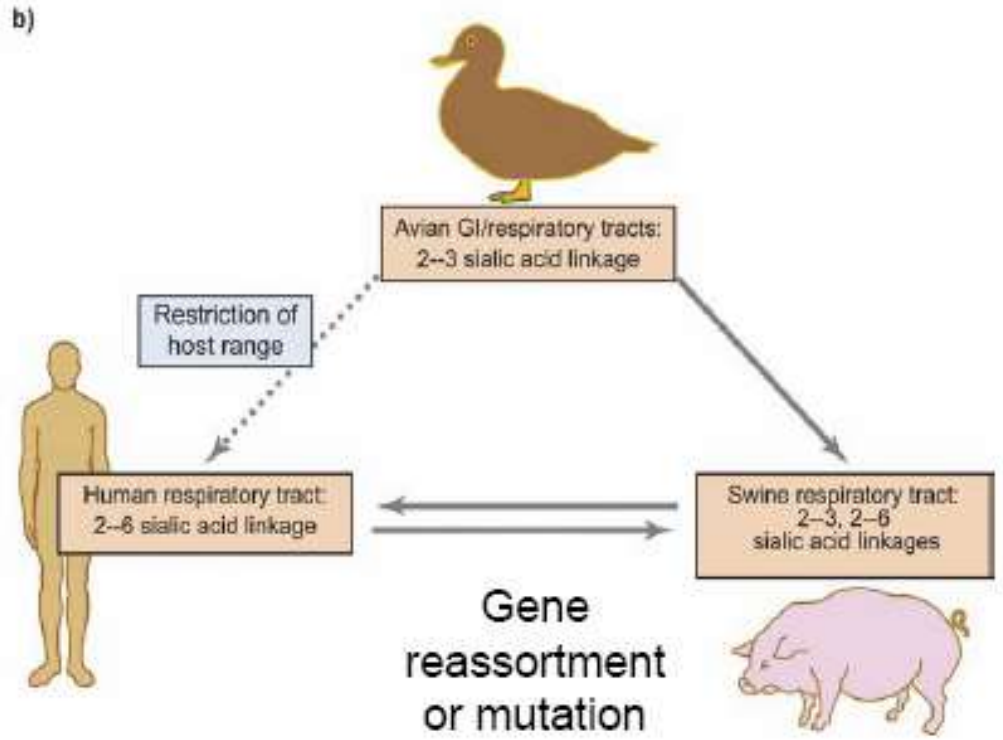
Influenza A

- Clinical and epidemiological importance
- Mutability of virus produces antigenic changes
  - Mutation and whole gene 'swapping' (reassortment) between different strains
  - Recombination
    - Results: antigenic drifts and antigenic shifts
- Subtypes based on H and N antigens
  - H antigens: 15
  - N antigens: 9
- Avian subtypes :
  - 2 H subtypes (H5 and H7)
  - 7 N subtypes
- Only H1, H2 and H3 H subtypes and N1 and N2 N subtypes are associated with stable human infection

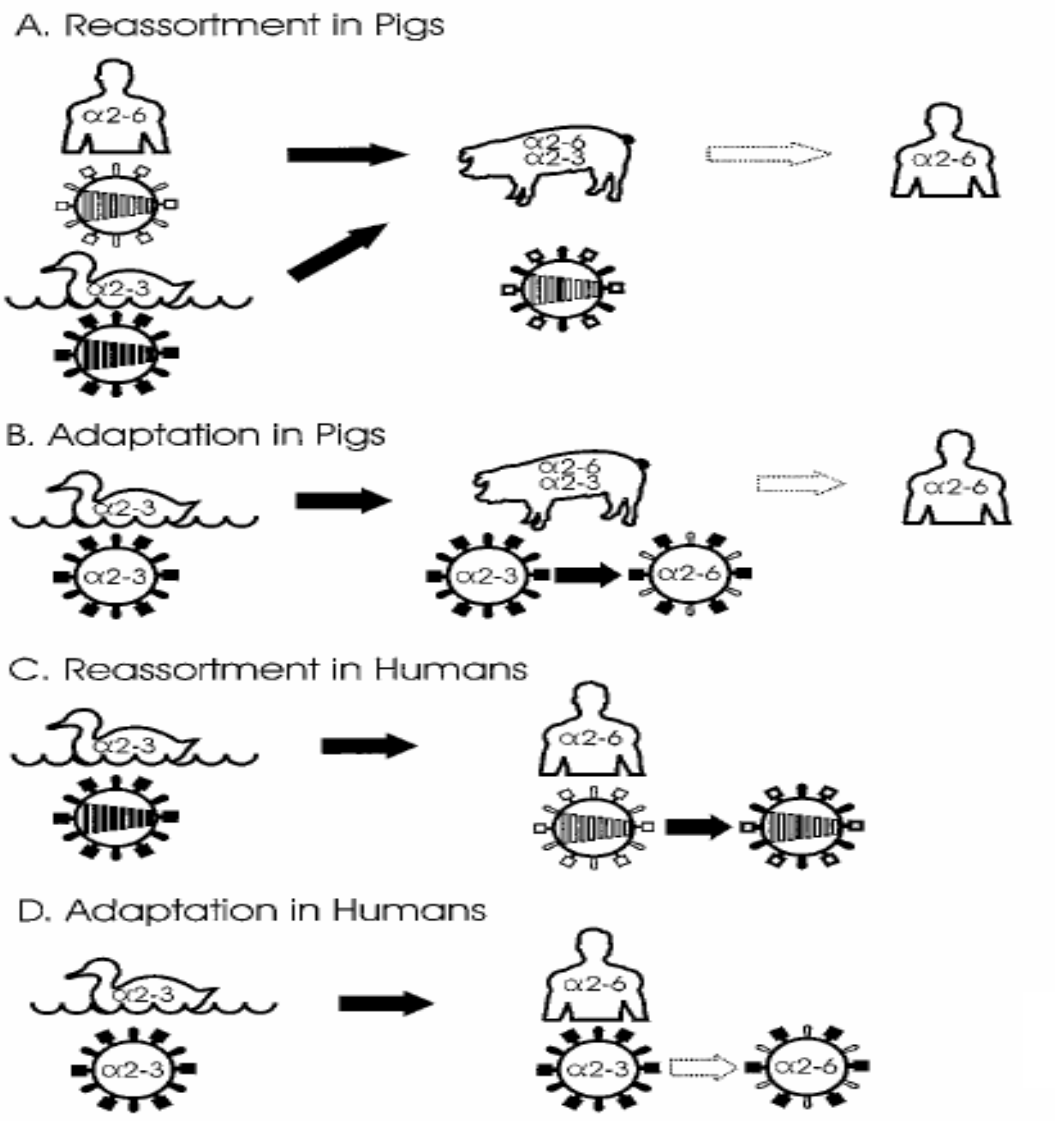
Sialic acid residues can be covalently attached to galactose residues of integral glycoproteins and glycolipids via either 2–3 or 2–6 α linkages.



The avian, human, and swine upper respiratory tract epitheliae preferentially express 2–3 linkages, 2–6 linkages, and both 2–3 and 2–6 linkages respectively



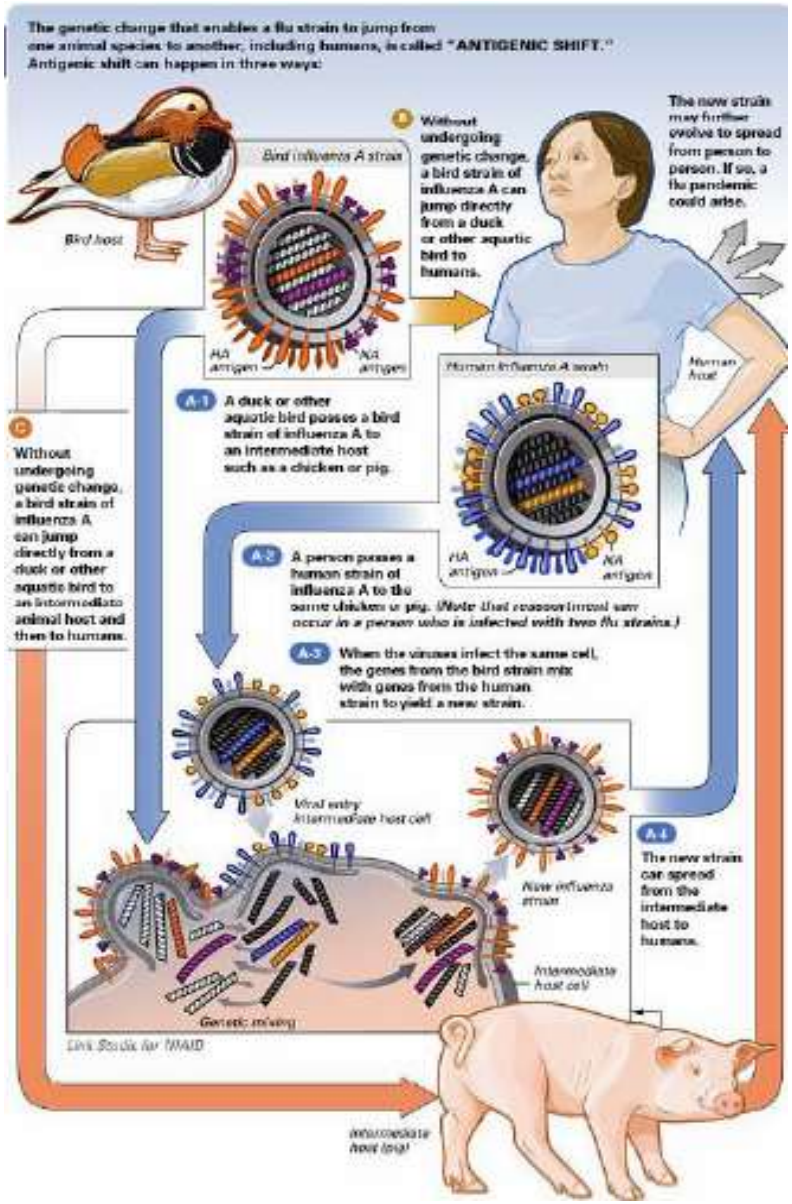
Molecular basis for generation in pigs of Influenza A with pandemic potential

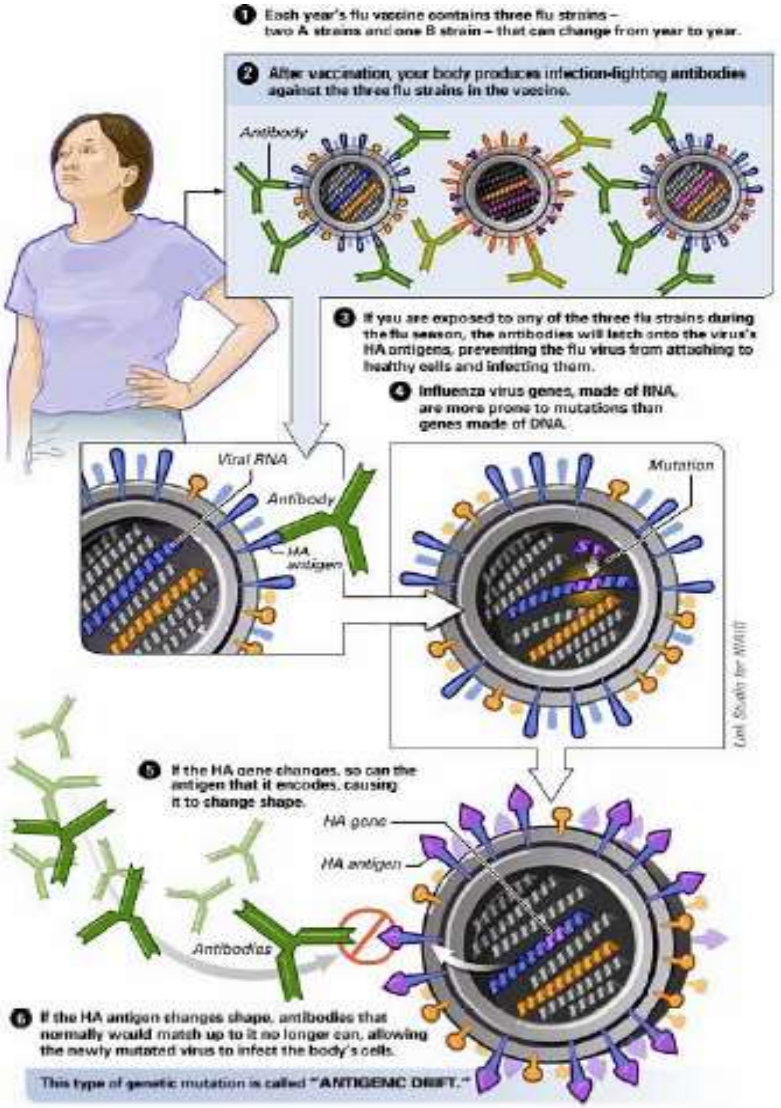


Influenza A

- Antigenic drift
  - Subtle changes (point mutations) in H, N or non structural genes
  - Occurs every few years
  - Allows for maintenance in a population
  - Is associated with seasonal outbreaks

- Antigenic shift
  - Major antigenic changes
    - Due to reassortment or whole gene 'swapping'
    - New subtype may develop mutations too
  - Correlates with epidemics and pandemics
    - Little or no immunity





Antigenic Shift

Antigenic Drift