CC-I: Microbial World and Principles of Microbiology

Physiochemical and Biological Characteristics of Microorganisms (including viruses)

There are more microbes on Earth than stars in the universe. This vast diversity reflects the roughly 3.5 billion years in which microbes have evolved to adapt to seemingly every environment on (and in) the planet, regardless of how inhospitable it may seem to a plant or animal.

MAJOR GROUPS OF THE MICROBIAL COMMUNITY

BACTERIA

Bacteria are microbes with a cell structure simpler than that of many other organisms.

VIRUSES

Viruses are the smallest of all the microbes.

FUNGI

Fungi can be single celled or very complex multicellular organisms.

PROTOZOA

Protozoa are single celled organisms.

ALGAE

Single celled to multicellular organisms

ARCHAEA

Archaea can be spherical, rod, spiral, lobed, rectangular or irregular in shape.

PRIONS

A prion is a type of protein that can cause disease in animals and humans by triggering normally healthy proteins in the brain to fold abnormally.

Characteristics of Microorganisms

Characteristics of microorganisms can be divided into two groups:

1. Classical
2. Molecular

- The most durable identifications are those that are based on a combination of approaches.

Classical Characteristics:

Classical approaches to taxonomy make use of morphological, physiological, biochemical, and ecological characteristics. These characteristics have been employed in microbial taxonomy for many years and form the basis for phenetic classification.

Molecular Characteristics

The study of DNA, RNA, and proteins has advanced our understanding of microbial evolution and taxonomy. Evolutionary biologists studying plants and animals draw from a rich fossil record to assemble a history of morphological changes; in these cases, molecular approaches supplement such data. In contrast, microorganisms have left almost no fossil record, so molecular analysis is the only feasible means of collecting a large and accurate data set that explores microbial evolution. When scientists are careful to make only valid comparisons, phylogenetic inferences based on molecular approaches provide the most robust analysis of microbial evolution.

Morphology, Chemical and Molecular Characteristics

Morphology

1. Cell shape
2. Cell size (diameter, length)
3. Motility
4. Flagellation
5. Type of cell division
6. Cell differentiation
7. Internal or external structures (endospores, gas vesicles, etc.)
8. Gram stain
9. Ultrastructure.

Chemical composition and molecular analyses

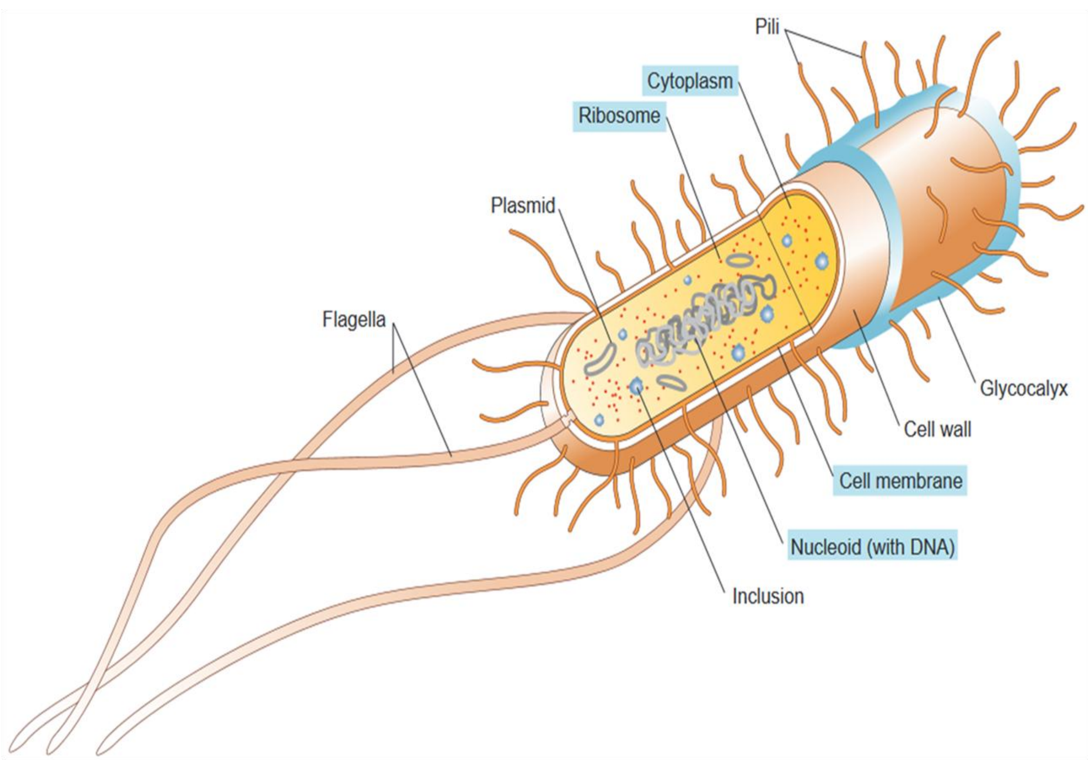
1. Color of cell suspension
2. Pigments
3. Reserve materials
4. DNA base composition
5. 16S rRNA sequence
6. DNA/DNA reassociation for species determination
7. Whole cell fatty acid composition

Physiological Characteristics

Physiology

1. Growth medium
2. Temperature range and optimum
3. pH range and optimum
4. Phototrophic or lithotrophic growth
5. Vitamin requirements
6. List of carbon sources used for growth
7. List of nitrogen sources used for growth
8. Relation to oxygen
9. Modes of energy generation
   I. electron donors: either organic or inorganic
   II. electron acceptors: oxygen, nitrate, sulfate, carbon dioxide, iron oxides, etc
10. Extracellular enzymes
    I. amylase
    II. lipase
    III. gelatinase
    IV. cellulase
    V. xylanase
11. Other enzymes
    I. catalase
    II. oxidase
12. Glucose fermentation
13. Denitrification

Bacteria



Distribution and Occurrence Bacteria are almost omnipresent.

Morphology

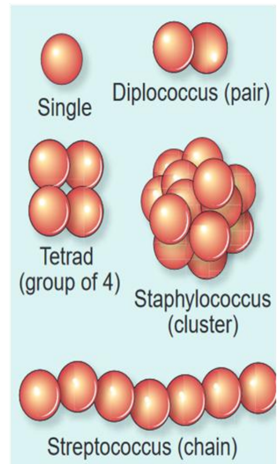
Shape and size:

Many bacterial cells have a rod, spherical, or spiral shape and are organized into a specific cellular arrangement.

We can divided bacterial shape mainly into three types-

1. Spherical or Coccus bacteria
2. Rod-shaped or Bacilli bacteria
3. Spiral bacteria
4. Other forms (unique shape)

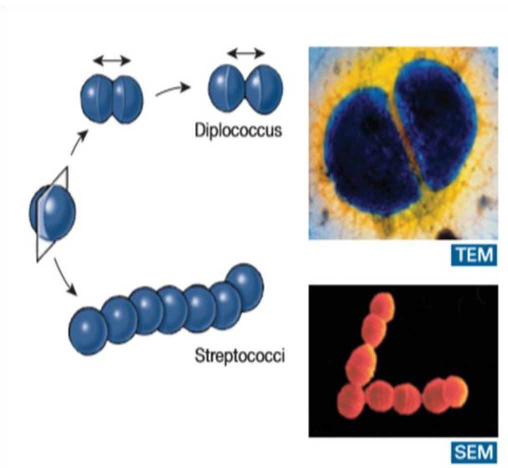
Spherical or Coccus bacteria

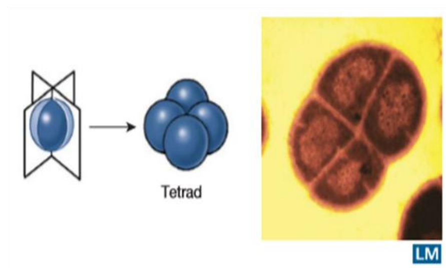


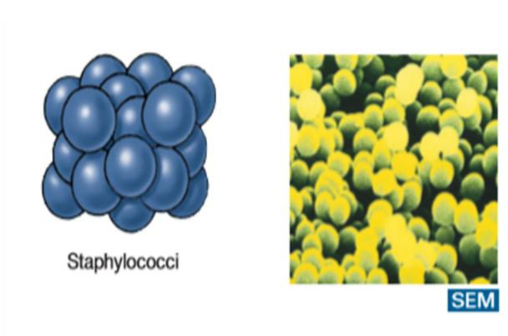
- The ellipsoidal or spherical bacteria are called cocci (singular coccus)
- The cocci measure 0.5-1.25µm in diameter.
- They are atrichous, thereby non-motile.
- They may occur singly or in group in different orientation.

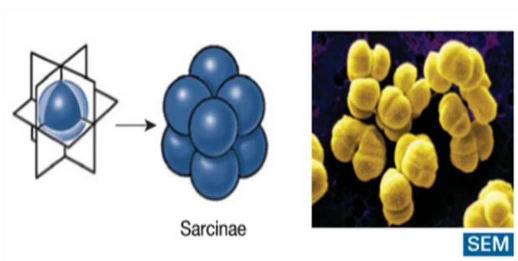
Cocci can be divided based on the of cells and its arrangement in an aggregation, these are-

I. Micrococci
II. Diplococci
III. Tetracocci
IV. Staphylococci
V. Streptococci
VI. Sarcinae





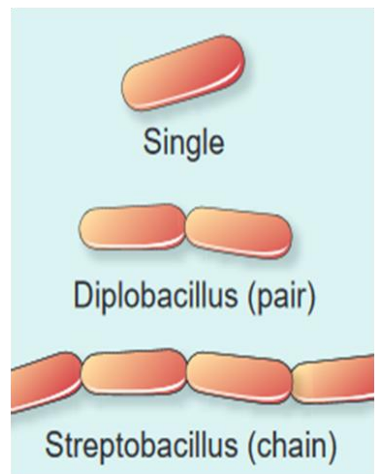




Rod-shaped or Bacilli bacteria

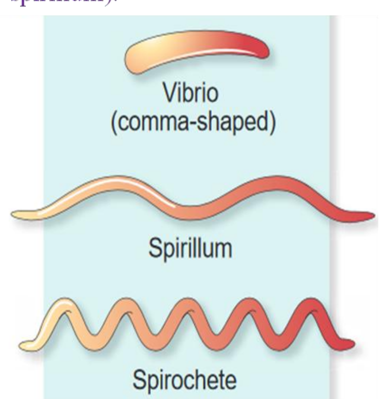
- The bacterial cells are rod-shaped or cylindrical shaped called bacilli (singular, bacillus).
- The bacilli measure 0.5-1.0 μm x 2-3 μm.
- They may be motile or non-motile.
- They may be very short or long, narrow and have blunt or round ends.
- They may occur either singly or in groups.

- Based on the number of cells and its arrangement in an aggregation bacilli are further divided into following categories,
  I. Bacillus
  II. Diplobacillus
  III. Streptobacillus



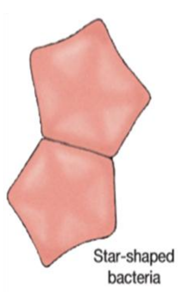
Spiral bacteria

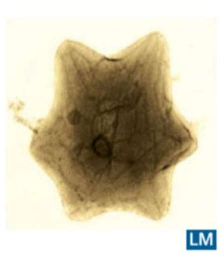
- The cells are slightly larger and spirally coiled rods, called spirilli (singular spirillum).
- Each bacterium has more than one turn of a helix.
- Has one or more flagella at each pole.
- Occur either singly or in chain.
- Their cell measure 1μm - 100μm.

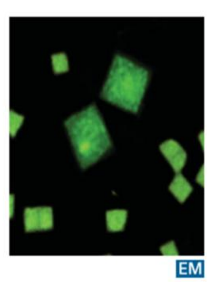


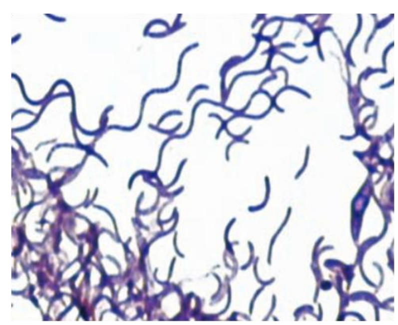
Other forms of bacteria

i. Vibrios
ii. Filamentous
iii. Pleomorphic
iv. Star shaped
v. Square shaped









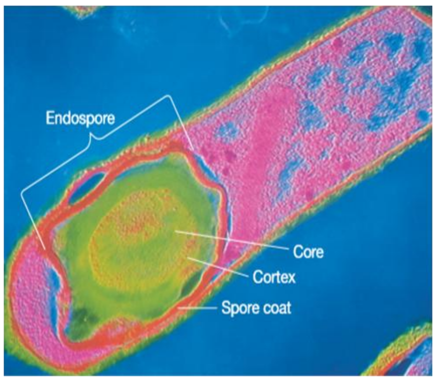
Habitat	Examples
Soil	*Arthrobacter, Streptomyces, Pseudomonas, Bacillus* etc.
Rhizosphere	*Rhizobium, Frankia, Pseudomonas* etc.
Air	*Mycobacterium tuberculosis, Streptococcus pneumoniae* etc.
Water	*Chlorobium, Rhodopseudomonas, Escherichia coli, Campylobacter, Salmonella* etc.
Human gut	*Helicobacter pylori, Escherichia coli, Klebsiella, Firmicutes, Bacteroidetes, Actionobacteria* etc.
Ruminant gut	*Streptococcus bovis, Methanomicrobium mobile, Ruminococcus albus* etc.

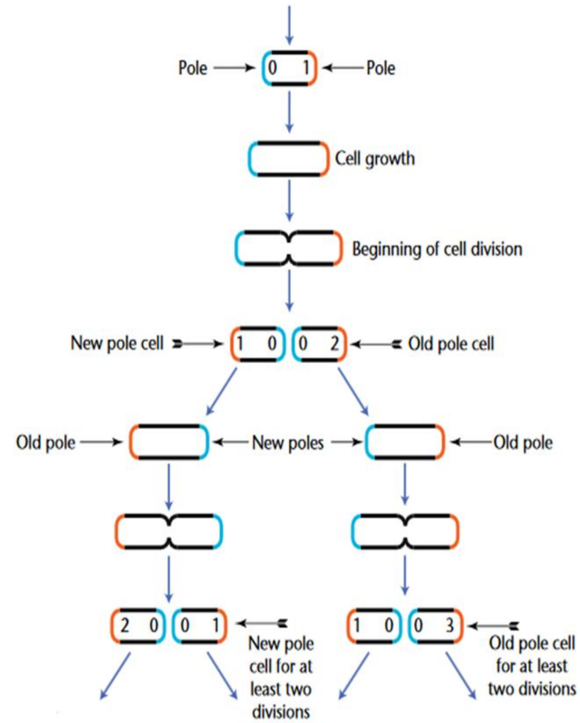
Unique shape	Examples
Vibrios	*Vibrio cholerae, V. coli.*
Filamentous	*Beggiatoa, Thiothrix* etc.
Pleomorphic	*Acetobactor* may occur as singly rod (bacillus) or chain of small rods (Streptobacillus), in response to environmental variation.
Star shaped	*Stella* sp.
Square shaped	*Haloarcula* sp.

Shapes	Examples
Spherical shape	
Micrococci	*Micrococcus nigra, M. lutens*
Diplococci	*Diplococcus pneumoniae*
Tetracocci	*Pedicoccus cerevisiae*
Staphylococci	*Staphylococcus aureus*
Streptococci	*Streptococcus lactis*
Sarcinae	*Sarcina verticuli*
Rod shaped	
Bacillus	*Bacillus polymaxa, Lactobacillus*
Diplobacillus	*Corynebacterium diphtheriae*
Streptobacillus	*Bacillus cereus, B. tuberculosis*

Mode of reproduction

- Bacteria usually reproduce by asexual methods.
- The asexual reproduction takes place by the following methods-
  - I. Binary fission
  - II. Conidia formation
  - III. By Budding
  - IV. Cyst formation
  - V. Endospore formation





VIRUS

Properties of viruses: General nature and important features

It's just a piece of bad news wrapped up in protein.
—Nobel laureate **Peter Medawar** (1915–1987) describing a virus

The study of viruses is called virology.

Virus, infectious agent of small size and simple composition that can multiply only in living cells of animals, plants, or bacteria.

A complete virus particles have following attributes-

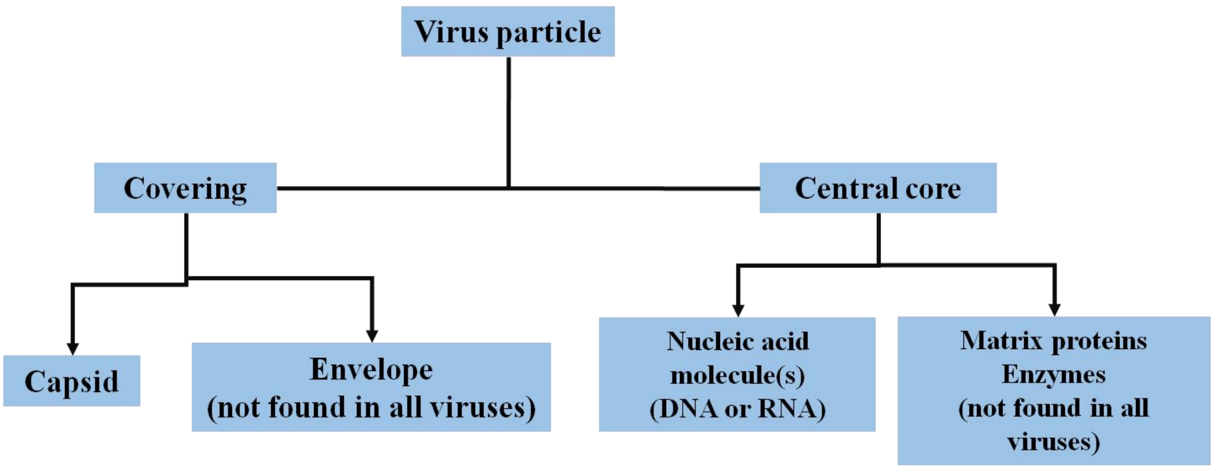
- Contain a single type of nucleic acid, either DNA or RNA.
- Contain a protein coat (sometimes itself enclosed by an envelope of lipids, proteins, and carbohydrates) that surrounds the nucleic acid.
- Multiply inside living cells by using the synthesizing machinery of the cell.
- Cause the synthesis of specialized structures that can transfer the viral nucleic acid to other cells.

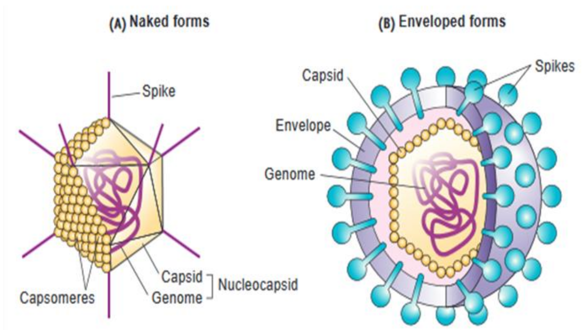
Virion: A complete virus particles, which have the ability to infect living cells are known as virion.

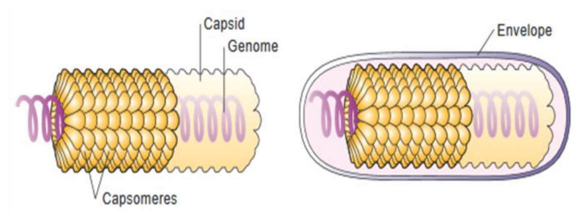
Virions are generally acts as transport mediators, they can infect new healthy cells.

Virus can be intra or extracellular but virions are always extracellular in nature.

Viral Components: Capsids, Nucleic Acids, and Envelopes



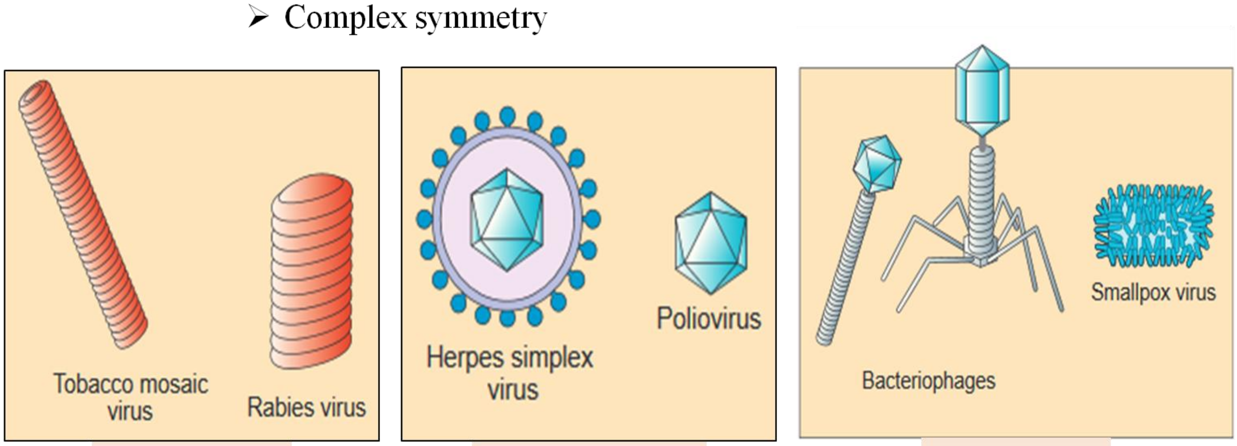




Viruses Are Grouped by Their Shape

Viruses can be separated into groups based on their nucleocapsid symmetry; that is, their three-dimensional shapes.

- Helical symmetry
- Icosahedral (icos = "twenty,"edros = "side") symmetry.
- Complex symmetry



Helical viruses

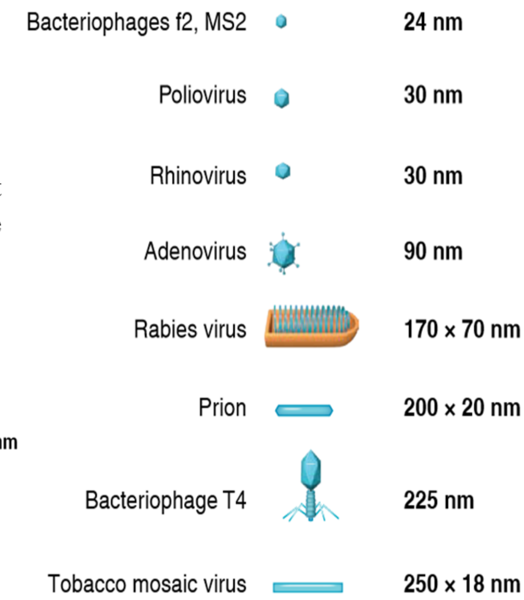
Icosahedral viruses

Complex viruses

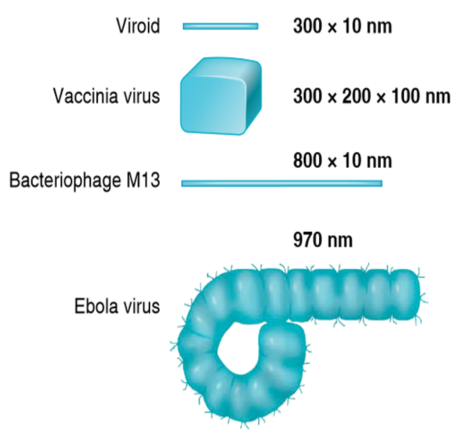
General nature of viruses

1. Viruses are small particles.

- ✓ Most viruses are too small to be seen with a light microscope, viral sizes are determined with the aid of electron microscopy.
- ✓ Although some viruses are variable in shape, most viruses have a specific shape that is determined by the capsomeres or the envelope.
- ✓ They can easily be passed through bacterial filters.



❖ The units in which virions are normally measured are nano meters (1 nm=10^{-9}m). Although virions are very small, their dimensions cover a large range.

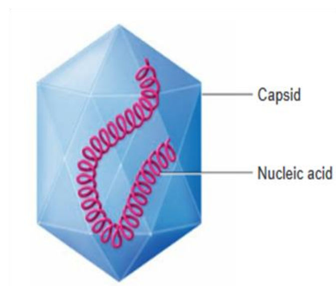


General nature of viruses

2. Viruses have genes.

The virion contains the genome of the virus. Whereas the genomes of cells are composed of double-stranded DNA, there are four possibilities for a virus genome:

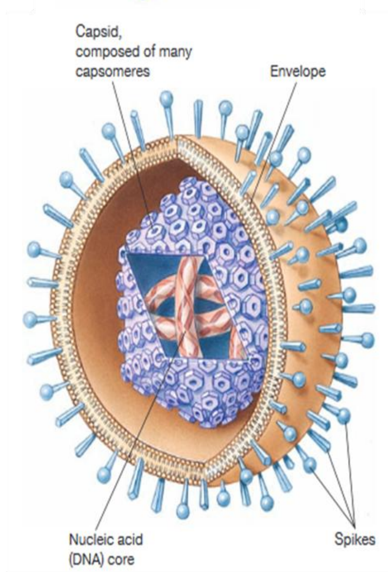
- double-stranded DNA
- single-stranded DNA
- double-stranded RNA
- single-stranded RNA.



The viral genome of almost all viruses contains either DNA or RNA, but not both, and the nucleic acid occurs in either a double-stranded or a single-stranded form. Usually the nucleic acid is a linear or circular molecule, although in some instances (as in influenza viruses) it exists as separate, nonidentical segments. The viral genome is folded or coiled, which allows the viruses to maintain their extremely small size.

They don't have any synthetic or metabolic machinery, they use host's machinery to synthesize required components. they followed several mechanisms to acquire it, like-

- ✓ *Viruses use host cell proteins.*
- ✓ *Viruses code efficiently.*
- ✓ *Many virus proteins are multifunctional.*



General nature of viruses

3. Viruses are parasites.

- **They are obligate intracellular parasites:** A new cell is always formed directly from a pre-existing cell, but a new virion is never formed directly from a pre-existing virion. New virions are formed by a process of replication, which takes place inside a host cell and involves the synthesis of components followed by their assembly into virions. Viruses are therefore parasites of cells, and are dependent on their hosts for most of their requirements, including-
  - Building-blocks such as amino acids and nucleosides;
  - Protein-synthesizing machinery (ribosomes);
  - Energy, in the form of adenosine triphosphate.
- A virus modifies the intracellular environment of its host in order to enhance the efficiency of the replication process.
- **Viruses Have a Host Range and Tissue Specificity:**

  Host range: The virus can infect and it is based on a virus' capsid structure. Most viruses have a very narrow host range.

  For example, a specific bacteriophage, only infects specific bacterial species.

  Tissue tropism: Within its host range, many viruses only infect certain cell types or tissues within a multicellular plant or animal. This limitation is called tissue tropism (tissue attraction).

  For example, the host range for the human immunodeficiency virus (HIV) is a human. In humans, HIV primarily infects a specific group of white blood cells called T helper cells.

General nature of viruses

4. Are viruses living or non-living?

The living and non-living matter of a virus is a skeptical issue.

Viruses are on the edge of life. But on which side of the edge are they? Are they living or, being able to be crystallized, are they nonliving? If nonliving, how can they cause disease?

Most biologist use certain emergent properties of life to define something as a living organism. These properties include an ability to:

- ✓ Grow and develop.
- ✓ Reproduce.
- ✓ Establish a complex organization.
- ✓ Regulate its internal environment.
- ✓ Transform energy.
- ✓ Respond to the environment.
- ✓ Evolve by adapting to a changing environment.

- Many scientists consider them living because they can reproduce when they infect a host cell, and they do contain genetic information like all living organisms.
- Other scientists would say they are not living because they do not satisfy all seven emergent properties of life.

5. Viruses are ubiquitous on Earth

Viruses are found everywhere on the earth and have the capacity to infect all living cell of that particular habitats.

Properties of Viruses

- Obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants, and animals.
- Ultramicroscopic size, ranging from 20 nm up to 450 nm (diameter).
- Not cellular in nature; structure is very compact and economical.
- Do not independently fulfill the characteristics of life.
- Inactive macromolecules outside the host cell and active only inside host cells.
- Basic structure consists of protein shell (capsid) surrounding nucleic acid core.
- Nucleic acid can be either DNA or RNA but not both.
- Nucleic acid can be double-stranded DNA, single-stranded DNA, single-stranded RNA, or double-stranded RNA.
- Molecules on virus surface impart high specificity for attachment to host cell.
- Multiply by taking control of host cell's genetic material and regulating the synthesis and assembly of new viruses.
- Lack enzymes for most metabolic processes.
- Lack machinery for synthesizing proteins.

Subviral particles; viroids, prions, and their importance.

Subviral particles

Subviral particles

- ✓ Small in size.
- ✓ An infectious agent without any genome at all.
- ✓ Their genome consists of nucleotide sequences only.
- ✓ This infectious agent without a genome, cause disease in animals (including humans) and plants.

e.g., Satellites, viroids and prions

Satellites And Viroids

- ✓ **Satellites** are small RNA molecules that are absolutely dependent on the presence of another virus (Helper virus) for multiplication. Even viruses have their own parasites!
- ✓ Most satellites are associated with plant viruses, but a few are associated with bacteriophages or animal viruses (e.g., the Dependovirus genus) that are satellites of adenoviruses.

Helper virus:
Viruses which enable defective viruses to replicate or to form a protein coat by complementing the missing gene function of the defective (satellite) virus. Helper and satellite may be of the same or different genus.

Two classes of satellites can be distinguished:

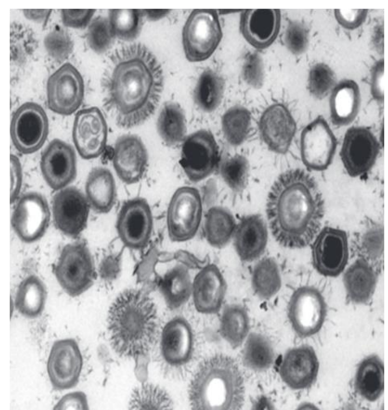
I. **Satellite Viruses,** which encode their own coat proteins.

II. **Satellite RNAs Or Satellite Nucleic Acids** (or"virusoids"),which use the coat protein of the helper virus.

- Satellites replicate in the cytoplasm using an *RNA-dependent RNA polymerase*, an enzymatic activity found in plant but not animal cells.

Typical properties of satellites

- ✓ Their genomes have approximately 500-2,000 nt of single-stranded RNA.
- ✓ There is little or no nucleotide sequence similarity between the satellite and the helper virus genome.
- ✓ They cause distinct disease symptoms in plants that are not seen with the helper virus alone.
- ✓ Replication of satellites usually interferes with the replication of the helper virus.
- ✓ Most satellites are associated with plant viruses.
- ✓ Transmission from animal host to host, or cell to cell within a host, does not seem able to pass on complete sets of multiple particles.
- ✓ One exception appears to be the delta hepatitis virus, which does infect only humans, and appears to be a kind of hybrid between a satellite and a viroid



Virophages —"eaters of viruses."

- o Reduce helper virus production.
- o It is not actually a phage but acts similarly in that it impairs its host virus's replication, by 70% in fact.
- o "Sputnik"virophage, reduce the number of its helper virus, *Mimivirus*. The Mimivirus infects amoeba (*Acanthamoeba polyphaga*).

Delta hepatitis

Hepatitis delta virus (HDV) is a unique chimeric molecule with some of the properties of a satellite virus and some of a viroid which causes disease in humans.

Hepatitis delta virus (HDV) needed helper virus hepatitis B virus (HBV) for infection. HDV initially was thought to be part of the hepatitis B virus (HBV) because it was never found without the presence of hepatitis B infection.

Later, it was found to be a separate, defective pathogen that required coinfection with the hepatitis B virus in order to replicate.

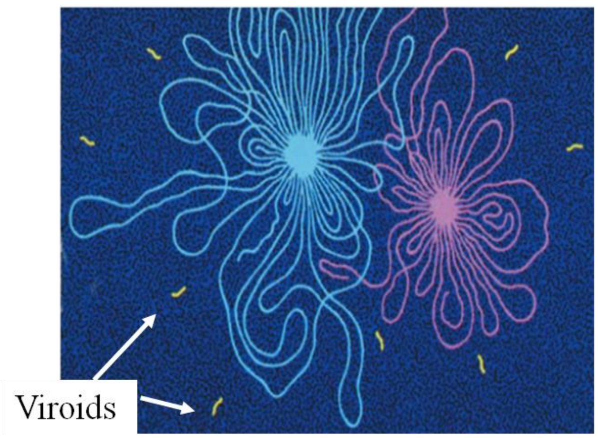
- o It cannot replicate its own capsid material as do satellite viruses, but uses the HBV capsid.
- o It can be prevented by vaccinating against HBV, because it cannot infect without its helper virus.

Viroids →

- ✓ "virus-like" agent.
- ✓ An infectious RNA particle smaller than a virus.
- ✓ Generally cause several plant disease.
- ✓ e.g., Potato spindle tuber disease, Chrysanthemum stunt disease, Cucumber pale fruit disease, and Tomato apical stunt disease.

Discovery of Viroids:

- ✓ In 1971 the plant pathologist T. O. Diener (Theodor Otto Diener) described a new type of infectious agent. He was studying potato tuber spindle disease, which was thought to be caused by a virus. However, no virions could be detected. Rather, Diener discovered molecules of RNA in the nuclei of diseased plant cells .
- ✓ He proposed the concept of a viroid.



Tomato apical stunt disease

How viroids cause disease?

It is not clear how viroids and their RNA cause disease.

Hypothesis

- ➢ Viroids must disrupt host cell metabolism in some way.
- ➢ They may interfere with the cell's ability to process mRNA molecules.
- ➢ Diener speculates the viroids originated as introns, the sections of RNA spliced out of messenger RNA molecules before the messengers are able to function. Because the viroid RNA encodes no proteins.
- ➢ The viroid RNA interacts with host cell RNA, inactivating proteins that bring about disease through loss of cell function.
- ➢ The viroid RNA "silences" host cell "target RNA" again bringing about disease through loss of cell regulation.

Two hypotheses have been proposed to account for the origin of viroids.

I. One suggests that they originated early in precellular evolution when the primary genetic material probably consisted of RNA.

II. A second suggests that they are relatively new infectious agents that represent the most extreme example of parasitism.

Satellites Vs Viroids

Characteristic	Satellites	Viroids
Helper virus required for replication	Yes	No
Protein(s) encoded	Yes	No
Genome replicated by	Helper virus enzymes	Host-cell RNA polymerase II
Site of replication	Same as helper virus(nucleus or cytoplasm)	Nucleus

PRIONS

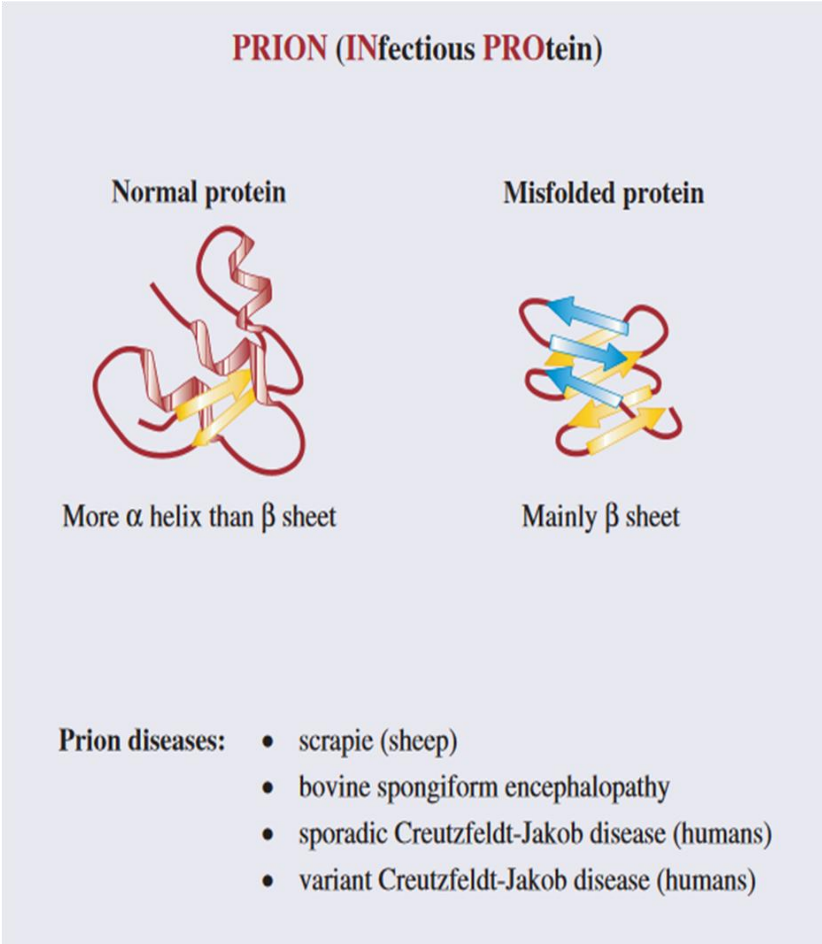
A particular group of transmissible, chronic, progressive infections of the nervous system and are invariably fatal.

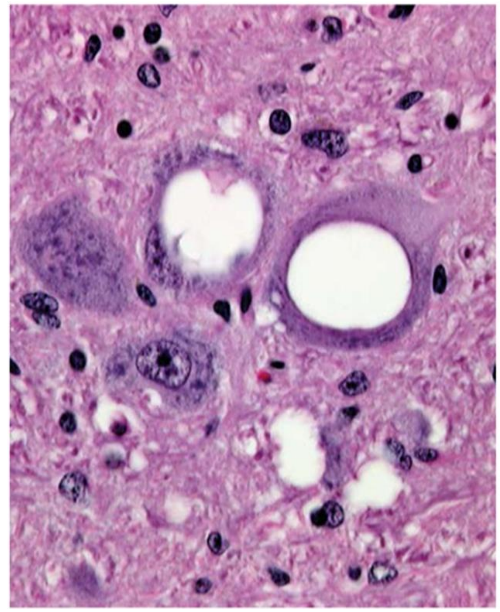
The evidence suggests that the causative agents are protein molecules from within the cells of the host; no nucleic acid has been found associated with them.

- ✓ The prion diseases are characterized by very **long incubation periods**, measured in years.
- ✓ **Signs of prion disease** include dementia and loss of co-ordination; the patient gradually deteriorates, and death is inevitable.

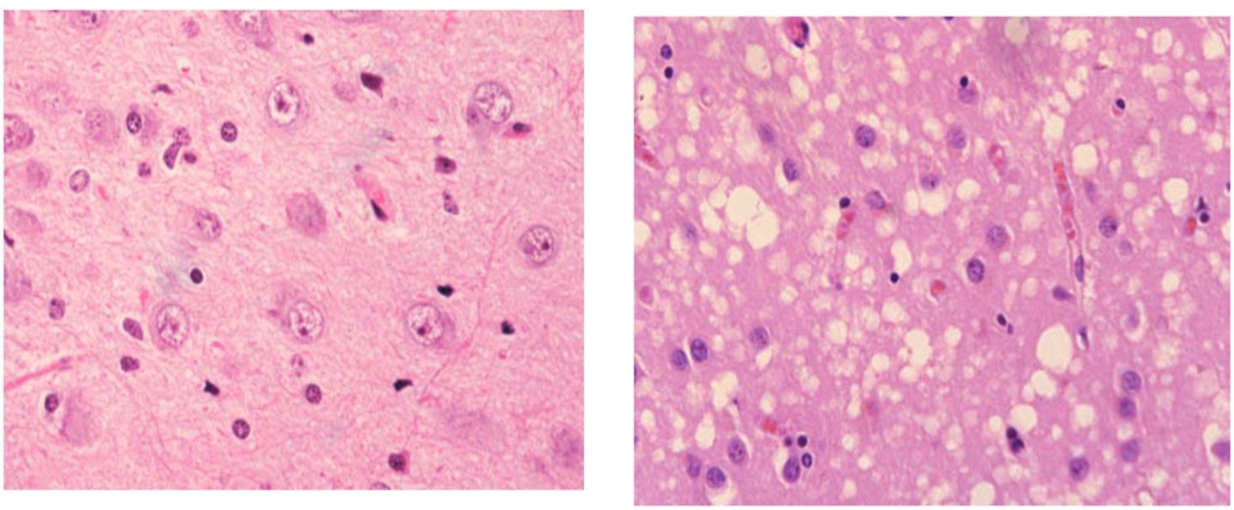
Transmissible spongiform encephalopathies

- ❑ **Encephalopathy** -means disease of the brain;
- ❑ **Spongiform** -refers to the development of holes in the brain, making it appear like a sponge.
- ❑ **Transmissible** -refers to the fact that the causative agent is infectious.





Brain section from a sheep with scrapie. The spongiform appearance (holes in the tissue)is evident.



A prion-caused disease, Creutzfeldt-Jakob disease.
(a) A section through the cerebral cortex of a normal human brain reveals a solid structure whereas
(b) A section through the brain of a patient with Creutzfeldt-Jakob disease shows many holes. It is clear why CJD is referred to as a subacute spongiform encephalopathy.

THE NATURE OF PRIONS

- ✓ There is no evidence that the infectious agents that cause TSEs contain any nucleic acid; the agents appear to be misfolded forms of normal cell proteins.
- ✓ This ***'protein-only'*** hypothesis was proposed by Stanley Prusiner, who also suggested the term prion, derived from infectious protein.
- ✓ Versions of the normal protein have been found in mammals, birds and reptiles; in humans it is encoded by the *Prnp* gene.
- ✓ It is found on many cell types, but especially on cells of the central nervous system.

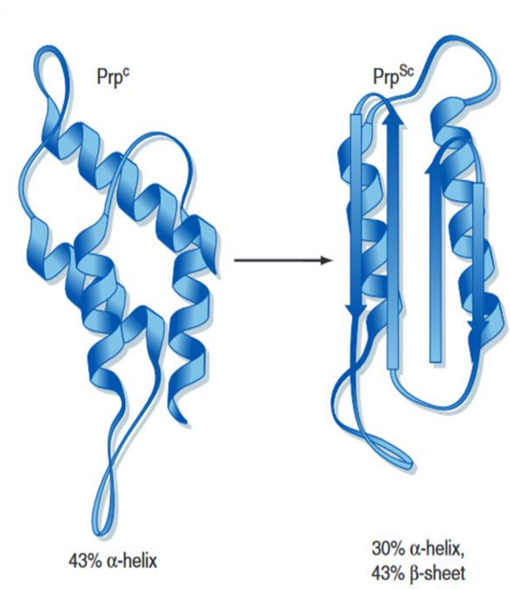
Molecular biology of the Prion

The conformation of much of the normal protein is α-helix. When the protein misfolds there is a decrease in ***α helix*** structure and an increase in ***βsheet*** . This change in conformation is accompanied by changes in properties of the protein.

Various terminologies are used for the normal and the misfolded forms of the protein.

- ❖ The normal protein is commonly designated as **PrP^c^** (**PrP**=prion protein; **c**=cell),
- ❖ While the misfolded form is designated as **PrP^Sc^** (**Sc**=scrapie) or **PrP^res^** (**res**=resistant to proteinase).

The fundamental difference between the infectious, pathogenic form (**PrP^Sc^**) and the endogenous form (**PrP^c^**) results from a change in the conformation of the folded protein, which adopts a conformation rich in β-sheet.

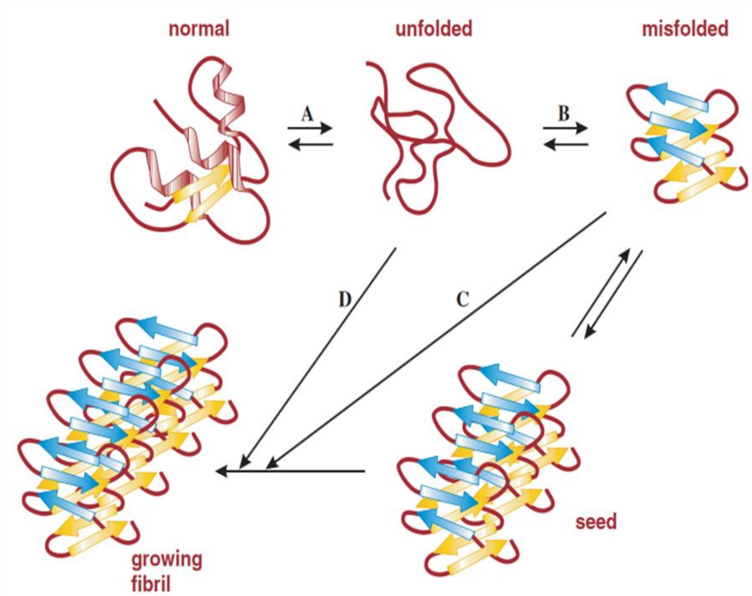


Conformational changes in PrP.

Molecular biology of the Prion

Model for prion replication.

- Copies of the normal protein unfold (A) and refold (B) into a form comprised mainly of βsheet (shown in yellow and blue).
- Replication may require a ***critical 'seed' size.***
- Further recruitment of misfolded molecules (C) or unfolded molecules (D) then occurs as an irreversible process.



Molecular biology of the Prion

- ✓ *The misfolded protein accumulates* in endosomes and lysosomes, and quantities build up especially in neurones.
- ✓ In some prion diseases the protein can also be found in a number of other organs and tissues, *including the spleen and lymph nodes*.
- ✓ ***Prion infectivity*** is remarkably heat resistant and some infectivity can survive autoclaving for prolonged periods .
- ✓ ***Prion infectivity*** is also very resistant to inactivation by irradiation and by some chemicals that inactivate virus infectivity.
    - *Treatments that are used to inactivate prion infectivity include exposure to 2.5 – 5 per cent sodium hypochlorite solution or 1 M NaOH for 1 – 2 hours.*

TSE IN ANIMALS

Bovine Spongiform Encephalopathy

Affected cattle showed altered behaviour and a staggering gait, giving the disease its name in the press of "mad cow disease." On microscopic examination, the brains of affected cattle showed extensive spongiform degeneration. It was concluded that BSE resulted from the use of contaminated foodstuffs.

Scrapie

Scrapie is a naturally occurring disease of sheep found in many parts of the world, although it is not universally distributed.

Scrapie is primarily a disease of sheep although it can also affect goats. The scrapie agent has been intensively studied and has been experimentally transmitted to laboratory animals many times.

Symptoms

Infected sheep show severe and progressive neurological symptoms such as abnormal gait; they often repeatedly scrape against fences or posts.

Transmissible Mink Encephalopathy (TME)

TME is a rare disease of farmed mink caused by exposure to a scrapie-like agent in feed.

Feline Spongiform Encephalopathy (FSE)

A scrapie-like syndrome in domestic cats resulting in ataxia (irregular and jerky movements) and other symptoms typical of spongiform encephalopathies.

Chronic Wasting Disease (CWD)

CWD is a disease similar to scrapie which affects deer and captive exotic ungulates (e.g., nyala, oryx, kudu).

HUMAN TSEs

Disease	Description	Comments
CJD	Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical gray matter, or encephalopathy with prion protein (PrP) immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types).	Three forms: sporadic, iatrogenic (recognized risk, e.g., neurosurgery), familial (same disease in first degree relative).
Familial fatal insomnia (FFI)	Thalamic degeneration, variable spongiform change in cerebrum.	Occurs in families with PrP_{178} asp-asn mutation.
Gerstmann–Straussler–Scheinker disease (GSS)	Encephalo(myelo)pathy with multicentric PrP plaques.	Occurs in families with dominantly inherited progressive ataxia and/or dementia.
Kuru	Characterized by large amyloid plaques.	Occurs in the Fore population of New Guinea due to ritual cannibalism, now eliminated.

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Search

Advanced Search

Prion Diseases

Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce inflammatory response.

The causative agents of TSEs are believed to be prions. The term "prions" refers to abnormal, pathogenic agents that are transmissible and are able to induce abnormal folding of specific normal cellular proteins called prion proteins that are found most abundantly in the brain. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. Prion diseases are usually rapidly progressive and always fatal.

Source: https://www.cdc.gov/prions/index.html

Comparison of Viruses, Viroids, and Prions

	Virus	Viroid	Prion
Nucleic acid	+ (ssDNA, dsDNA, ssRNA, or dsRNA)	+ (ssRNA)	–
Presence of capsid or envelope	+	–	–
Presence of protein	+	–	+
Need for helper viruses	+/– (Needed by some of the smaller viruses such as the parvoviruses)		
Viewed by	Electron microscopy	Nucleotide sequence identification	Host cell damage
Affected by heat and protein denaturing agents	+	–	–
Affected by radiation of enzymes that digest DNA or RNA	+	+	–
Host	Bacteria, animals, or plants	Plants	Mammals