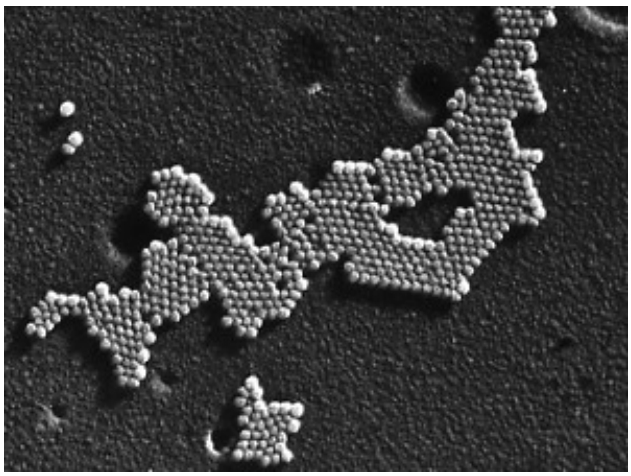
Viruses

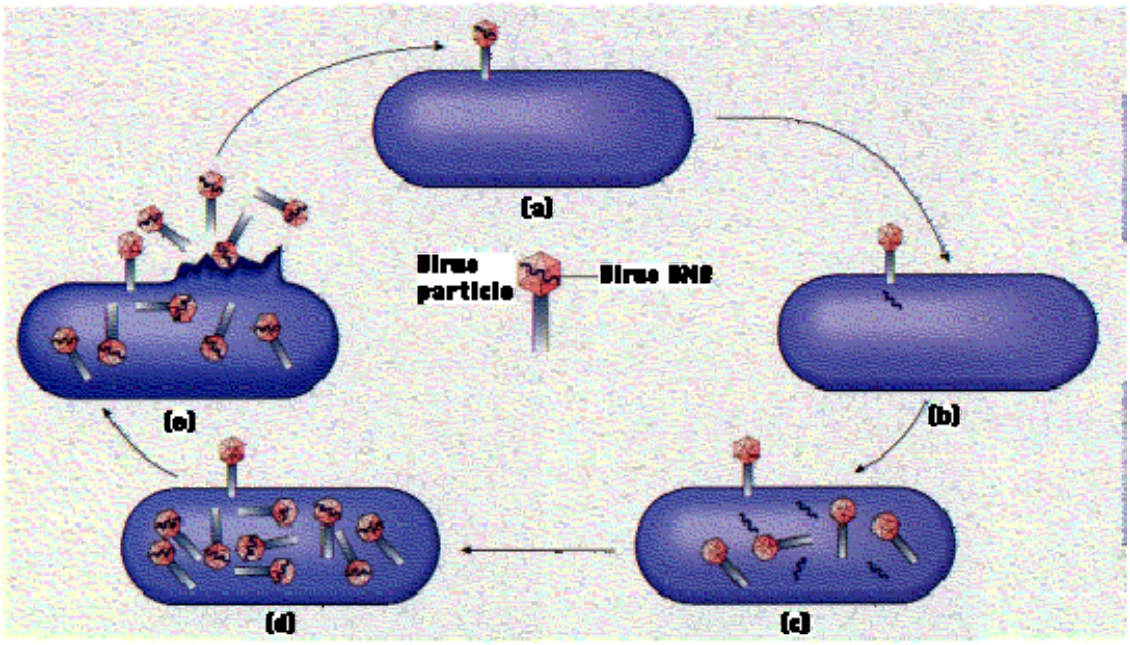


Properties

- They are obligate intracellular parasites.
- Probably there are no cells in nature that escape infection by one or more kinds of viruses. (Viruses that infect bacteria are called **bacteriophages**.)
- Outside the cell, they consist of particles called **virions**.
- Virions range in size from as small as the **poliovirus** shown above magnified some 450,000 times (courtesy of A. R. Taylor), which is 30 nm in diameter (about the size of a ribosome) to as large as the vaccinia virus which, at 230 nm, is larger than some bacteria.
- The virion consists of
    - An outer shell, the **capsid**, made of protein. The capsid is responsible for
        - protecting the contents of the core
        - establishing what kind of cell the virion can attach to
        - infecting that cell

      Some viruses contain other ingredients (e.g., lipids, carbohydrates), but these are derived from their host cells.

    - an interior core containing
        - the **genome**; either **DNA** or **RNA** The genes are few in number (3 - 100 depending on the species). They encode those proteins needed for viral reproduction that the **host cell will not supply**.
        - Often, one or more **proteins** (enzymes) needed to start the process of reproduction within the host cell.



Life Cycle

- The virion attaches to the surface of the host cell (usually binding to a specific cell surface molecule that accounts for the specificity of the infection). Example: **HIV-1**, the cause of **AIDS**, binds to the chemokine receptor **CCR5** found on human lymphocytes and macrophages.
- Once inside the cell, the virions are uncoated.
- Viral genes begin to expressed leading to the synthesis of proteins needed for
    - replication of the genome
    - synthesis of new proteins to make new capsids and cores.
- The details of these processes differ for different types of viruses and are described below for each type.

Viral Genomes

Either DNA or RNA, never both.

DNA viruses can be further divided into

- those that have their genes on a **double-stranded DNA** molecule (**dsDNA**). Example: smallpox
- those that have their genes on a molecule of **single-stranded DNA** (**ssDNA**). Example: Adeno-Associated Virus (AAV).

RNA viruses occur in four distinct groups:

1. Those with a genome that consists of single-stranded **antisense RNA**; that is, RNA that is the complement of the message sense. This is also called **negative-stranded RNA**. Examples: measles, Ebola
2. Those with a genome that consists of single-stranded **sense RNA**; that is, the RNA has message sense (can act as a messenger RNA - mRNA). This is also called **positive-stranded RNA**. Examples: poliovirus
3. Those with a genome made of several pieces of **double-stranded RNA**. Example: reovirus.
4. **Retroviruses**. Their RNA (also single-stranded) is copied by **reverse transcriptase** into a DNA genome within the host cell. Example: HIV-1

DNA Viruses

1. Genome is a molecule of double-stranded DNA

Examples:

- **smallpox** (variola)
- **vaccinia** (used to immunize against smallpox until the disease was eliminated from the planet)
- **varicella-zoster** (causes chicken pox the first time; shingles the second)
- **herpesviruses**
    - **herpes simplex viruses**
        - **HSV-1** — usually infects the trigeminal nerves periodically causing "cold sores" on the lips and face
        - **HSV-2** — usually infects the genitals
    - **KSHV**; causes **Kaposi's sarcoma** in AIDS patients and other people with suppressed immune systems. Also called human herpesvirus 8 (HHV-8).
    - **human cytomegalovirus** (**HCMV**); most of us have it; can cause blindness — even death — in people with suppressed immune systems.
    - **Epstein-Barr virus** (**EBV**); causes mononucleosis and has been implicated in the development of Burkitt's lymphoma (a cancer) and Hodgkin's disease. Its genome has been completely sequenced: 172,282 base pairs of DNA encoding 80 genes.
- **adenoviruses**; some 50 different strains infect humans; responsible for some cases of the common "cold". Two strains have been modified to serve as vectors in gene therapy trials [Link].

Hepatitis B

The genome of hepatitis B ("serum hepatitis") is also **dsDNA**, but its mode of replication is different from the other dsDNA viruses.

- Once inside its host cell (a liver cell), the virion core enters the nucleus.
- The viral DNA is transcribed (by the host's RNAP II) into molecules of **mRNA**.
- These enter the cytoplasm where they are translated (again by host ribosomes, etc.) into the various proteins of the virus, including a viral **reverse transcriptase**.
- These components are assembled into new viral cores, and in each
- one molecule of mRNA is reverse transcribed into a single strand of DNA, which then serves as the template for the synthesis of the second strand.

2. Genome is single-stranded DNA

Examples:

- **phiX-174**, another famous bacteriophage (infects **E. coli**) that helped usher in the modern era of molecular genetics. Its single strand of DNA has 5,386 nucleotides and encodes 10 genes.
-
- **Adeno-associated virus** (**AAV**). This virus, which can only grow in cells infected with adenovirus, shows great promise as a safe and effective vector for introducing therapeutic genes into human patients.

RNA Viruses

1. Negative-stranded RNA viruses: genome consists of one or more molecules of single-stranded "antisense" RNA

Examples:

- **measles**
- **mumps**
- **respiratory syncytial virus** (**RSV**), **parainfluenza viruses** (**PIV**), and human **metapneumovirus**. (In the U.S., these close relatives account for hundreds of thousands of hospital visits each year, mostly by children.)
- **rabies**
- **Ebola**
- **influenza**

Method of replication

- In addition to its antisense RNA genome, the core of the virion contains an **RNA replicase**, which is an RNA-dependent RNA polymerase.
- Once released in the host cell, this polymerase makes many **complementary** copies of the genome, which are "sense" and serve as **messenger RNAs**.
- These are translated into the proteins needed to assemble fresh virions, e.g., capsid proteins and RNA polymerase.

Note that this strategy

- provides many copies of mRNA
- depends on the virion having its own RNA replicase (because the host cell does not) (So, naked RNA molecules of these viruses are not infectious - in contrast to the next group: the positive-stranded RNA viruses)

2. Positive-stranded RNA: genome is a molecule of single-stranded "sense" RNA

Examples:

- **polioviruses**
- **rhinoviruses** (frequent cause of the common "cold"; 99 different strains are known)

- **noroviruses** (frequent cause of outbreaks of gastrointestinal illness — especially in "closed" settings like cruise ships and nursing homes)
- **coronaviruses** (includes the agent of **S**evere **A**cute **R**espiratory **S**yndrome (**SARS**)
- **rubella** (causes "German" measles)
- **yellow fever** virus
- **West Nile** virus
- **dengue fever** viruses
- **equine encephalitis** viruses
- **hepatitis A** ("infectious hepatitis") and **hepatitis C** viruses
- **tobacco mosaic virus** (TMV)

Method of replication

- The "sense" RNA encodes an **RNA replicase** (an RNA-dependent RNA polymerase) that
- is translated by the host machinery (ribosomes, etc.) into the enzyme, which
- catalyzes the synthesis of large numbers of "antisense" **replicative intermediates**.
- These serve as templates for the synthesis of large numbers of **mRNA** molecules that
    - are translated by the host cell machinery into the proteins needed to make fresh virions
    - are incorporated into the new virions.

3. Genome consists of several molecules of double-stranded RNA

Examples:

- reovirus
- several plant viruses

Method of replication

The virus particle contains enzymatic machinery that transcribes each of the dsRNA molecules into a **mRNA** (complete with cap) and exports these into the cytosol of the infected cell.

4. Retroviruses

These viruses contain a **reverse transcriptase** that copies their RNA genome into DNA. Examples:

- The Rous sarcoma virus (RSV)
- **HIV-1 and HIV-2**, that cause **AIDS**

- **HTLV-1** and **HTLV-2**; about 3% of the people infected with HTLV-1 develop leukemia.

Latent Viruses

Most of the infective cycles described for the various viruses end in the death of the host cell. Bacterial cells literally burst, a process called lysis, and similar infective cycles are called **lytic cycles**.

Lysogeny

In some cases, though, the events of the lytic cycle are not completed. E. coli infected by a DNA bacteriophage may resume its normal existence, including reproducing itself.

Where has the virus gone?

It is still there and, in fact, is present in the descendants of the bacterium. That these cells still harbor the virus can be demonstrated by irradiating the cells with ultraviolet rays or treating them with certain chemicals. Such treatment restores the normal lytic cycle. The phage is said to have been "rescued" - hardly the case for its host!

The stable relationship between a bacteriophage and its host is called **lysogeny**. The **viral DNA** actually becomes replicated when the host's DNA is replicated prior to each cell division. During lysogeny, the phage is called a **prophage**.

In some cases, the prophage DNA becomes inserted into the chromosome of its host. In fact, when the phage is "rescued", the released virions may contain some host genes as well as their own. When these virions infect new hosts, they insert these bacterial genes into them. This process of genetic transfer, a virus-mediated transformation, is called **transduction**.

What does the prophage do while it is a part of its host genome? It can express certain of its genes. For example, the gene that encodes diphtheria toxin is the property of a prophage in the diphtheria bacillus, not of the bacillus itself.

Some animal viruses can also establish latent infections. **Simian virus 40** (SV40) is a DNA virus that produces

- a **lytic infection** in the kidney cells of the African green monkey (these cells are used to cultivate viruses in the lab)

- but a **latent infection** in the cells of humans, mice, rats, and hamsters. Like lysogeny in bacteria, the SV40 genome becomes incorporated in the DNA of its host (in chromosome 7 in human cells).

Although a human cell with harboring SV40 shows no outward sign of the virus, its presence can be detected by:

- the appearance of viral-encoded antigens in the host cell
- the ability of these cells to cause a lytic infection in African green monkey cells when fused with them.

Latent infections may also cause the cell to become cancerous. The cell has become **transformed**. (In these cases, the word fulfills both of its biological meanings:

- "transformed" by the incorporation of new DNA
- "transformed" as it becomes cancerous.

In humans,

- **lytic** infections of plasma cells by the **Epstein-Barr virus** (EBV) occur in **mononucleosis**;
- **latent** infections of B cells by EBV predispose the person to lymphoma.

while

- **lytic** infections by **human papilloma virus** (**HPV**) cause genital warts;
- **latent** infections by some strains of HPV lead to cervical cancer.

Evading Cell-Mediated Immunity

Many viruses (all of which are **intracellular** parasites) exploit receptor-mediated endocytosis to sneak their way into their host cell.

They have evolved surface molecules that serve as decoy ligands for receptors on the target cell surface. Binding to these receptors tricks the cell into engulfing the virus.

Some examples:

- **Epstein-Barr Virus** (**EBV**). This virus causes mononucleosis and is a contributing factor in the development of Burkitt's lymphoma, a cancer of B lymphocytes. It binds to receptors present on the surface of B cells.

- **HIV**, the human immunodeficiency virus. It binds to the **CD4** molecule on the surface of the $CD4^+$ subset of T cells.
- **Influenza virus**. The **hemagglutinin** on the surface of the virus binds to carbohydrate on the target cell surface tricking the cell into engulfing it [More].

The process can be remarkably fast. A team in Munich (Seisenberger *et al.*, **Science**, 30 November 2001) succeeded in attaching single fluorescent molecules to single virions of adeno-associated virus (AAV) and watched them infect a HeLa cell:

- After bumping against the host cell's plasma membrane an average of 5 times,
- the virus was engulfed in an average of 64 milliseconds;
- took 15 minutes to pass (in the endosome) through the cytosol and
- enter the nucleus.

Once within a cell, a virus is safe from attack by antibodies. But it is still subject to being destroyed by an attack by $CD8^+$ cytotoxic T lymphocytes (CTL). Fragments of proteins synthesized by the virus will be deposited in the groove of class I histocompatibility molecules and displayed at the cell surface. These will be recognized as "foreign" and elicit an attack which will destroy the host cell along with its content of viruses.

- **SV40**; a virus that infects primate cells and causes tumors in rodent cells.
- Some **bacteriophages**
    - **T2** and **T4**; from which much early information about gene structure and expression was learned. [Links]
    - **lambda**; a popular vector [example]

The essential elements of the infective cycle of DNA bacteriophages consist of:

- The virions attach to the surface of their host cell **(a)**.
- The proteins of the capsid inject the DNA core into the cell **(b)**.
- Once within the cell, some of the bacteriophage genes (the "early" genes) are transcribed (by the host's RNA polymerase) and translated (by the host's ribosomes, tRNA, etc.) to produce enzymes that will make many copies of the phage DNA and will turn off (even destroy) the host's DNA.
- As fresh copies of phage DNA accumulate, other genes (the "late" genes) are transcribed and translated to form the proteins of the capsid **(c)**.
- The stockpile of DNA cores and capsid proteins are assembled into complete virions **(d)**.
- Another "late" gene is transcribed and translated into molecules of **lysozyme**. The lysozyme attacks the peptidoglycan wall (from the inside, of course).
- Eventually the cell ruptures and releases its content of virions ready to spread the infection to new host cells **(e)**.