

## Introduction to Virology I

All living things survive in a sea of viruses. We take up billions of them regularly: we breathe 6 liters of air per minute, eat thousands of grams of food and its allied contaminants per day, touch heaven knows what and put our fingers in our eyes and mouths. Every milliliter of seawater contains more than a million virus particles. We carry viral genomes as part of our own genetic material. Viruses infect our pets, domestic food animals, wildlife, plants, insects; even viruses have "viruses". Viral infections can cross species barriers, and do so constantly (zoonotic infections).

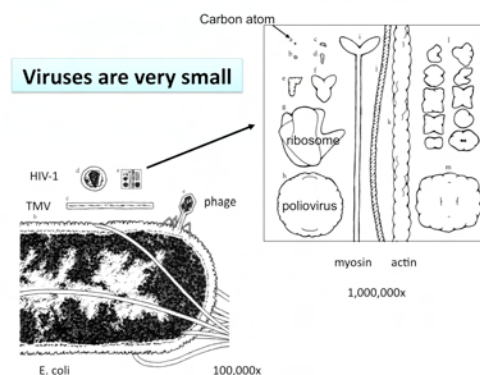
The number of viruses impinging upon us is staggering. There are more than  $10^{30}$  bacteriophage particles (viruses that infect bacteria) in the world's water supply. If lined up head to tail,  $10^{30}$  bacteriophages would form a line more than 200 million light years in length. The biomass of this number of particles exceeds that of elephants by more than 1000-fold. Whales are commonly infected with a member of the *Caliciviridae* family, viruses that can also infect humans and cause gastroenteritis. Infected whales secrete more than  $10^{13}$  caliciviruses daily, a huge amount. Keep this fact in mind the next time you swim in the ocean. There are about  $10^{16}$  HIV genomes on the planet today. With this number of genomes, it is highly probable that HIV genomes already exist that are resistant to every one of the antiviral drugs that we have now, or ever will have.

Amazingly, the vast majority of viruses that infect us have little or no impact on our health or well-being. We exist because we have a defense system that evolved to fight infections. If our immune system is down (e.g. due to AIDS, malignancy, or immunosuppression needed during organ transplants) even the most common viral infection can be lethal.

This lecture will define and discuss the basic principles of **viral replication**, the sum total of all the events whereby a single virus particle attaches to a cell and subsequently produces many new viruses.

### Viruses Defined

A virus is a very small (Fig. 1), infectious, obligate intracellular parasite. Virus particles are chemicals: they are not alive. They are complex chemicals to be sure, but by themselves virus particles cannot do much at all. It is the infected cell that does 'something'. Infected cells, infected tissues, infected organisms, and infected populations of hosts are the primary units of selection. These infected entities are the living manifestation of what is encoded in a viral genome.



**Figure 1**

Other defining viral attributes include:

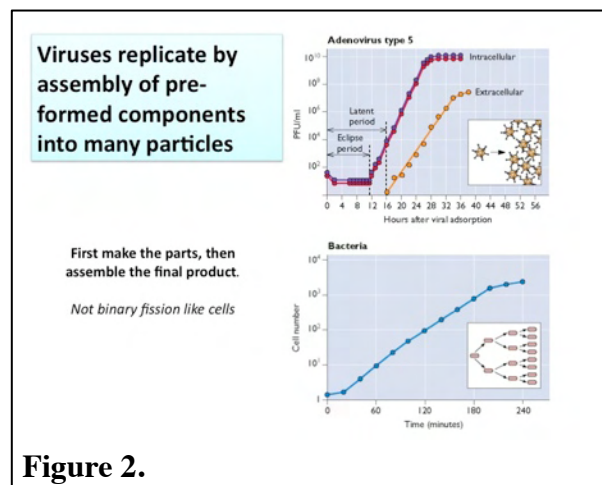
- The genome is comprised of either DNA or RNA.
- Within an appropriate host cell, the viral genome directs the synthesis, by cellular systems, of the components needed for replication of the viral genome and its transmission within virus particles.
- New virus particles are formed by *de novo* assembly from newly-synthesized components within the host cell.
- The progeny particles are the vehicles for transmission of the viral genome to the next host cell or organism.
- The particles are then disassembled inside the new cell, initiating the next infectious cycle.

Viruses replicate by assembly of pre-formed components into many particles (Fig. 2). First the individual parts are synthesized, then they are assembled into the final product. They do not replicate by binary fission, as do bacterial and eukaryotic cells.

### The Three-Part Strategy

All viruses follow this three-part strategy:

1. All viruses have a nucleic acid genome packaged in a proteinaceous particle. This particle is the vehicle for transmission of the viral genome from host to host. The particle is a delivery device, but it is not alive.



2. The viral genome contains the information to initiate and complete an infectious cycle within a susceptible and permissive cell. The infectious cycle allows attachment and entry of the particle, decoding of genome information, translation of viral mRNA by host ribosomes, genome replication, assembly and release of particles containing the genome.

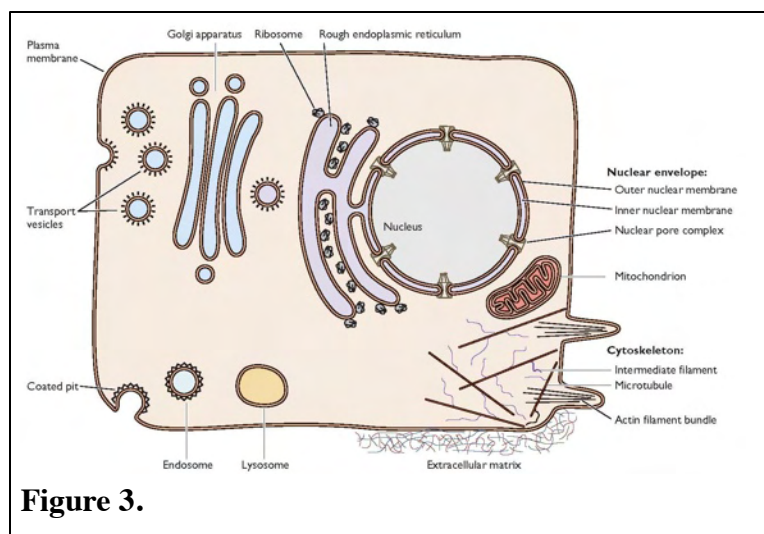
3. All viral genomes are able to establish themselves in a host population so that virus survival is ensured.

This three-part strategy achieves one goal: survival.

Despite this simple three-part strategy, the tactical solutions encoded in genomes of viruses from individual families are incredibly diverse. There are countless virus particles out there with amazing diversity with respect to size, nature and topology of genomes, coding strategies, tissue and cell tropism, and degrees of pathogenesis from benign to lethal. Nevertheless, there is an underlying simplicity and order to all this diversity because of two simple facts: All viral genomes are obligate molecular parasites that can only function after they replicate in a cell; and all viruses must make mRNA that can be translated by host ribosomes. They are parasites of the host protein synthesis machinery.

Viruses require many different functions of the host cell (Fig. 3) for propagation. Cells provide the machinery for translation of viral mRNAs, sources of energy, enzymes for genome replication, and sites of nucleic acid replication and viral assembly. The cellular transport apparatus brings viral genomes to the correct cellular compartment, and ensures that viral subunits reach locations where they may be assembled into virus particles.

Viruses cannot reproduce extracellularly; the production of new infectious viruses takes place within a cell. Virologists divide the viral infectious cycle into discrete steps to facilitate their study, although in virus-infected cells no such artificial boundaries occur. The **infectious cycle** (Fig. 4) comprises attachment and entry of the particle, translation of viral mRNA by host ribosomes, genome replication, and assembly and release of particles containing the genome. New virus particles produced during the infectious cycle may then infect new cells. The term



**virus replication** is another name for the sum total of all events that occur during the infectious cycle.

Because all viral infections begin in a single cell, the virologist can use cultured cells to study stages of the infectious cycle. There are events common to virus replication in animals and in cultured cells, but there are also many important differences. While viruses readily attach to cells in culture, in nature, a virus particle must encounter a host, no mean feat for nano-particles

without any means of locomotion. After encountering a host, the virus particle must pass through physical host defenses, such as dead skin, mucous layers, and the extracellular matrix. Host

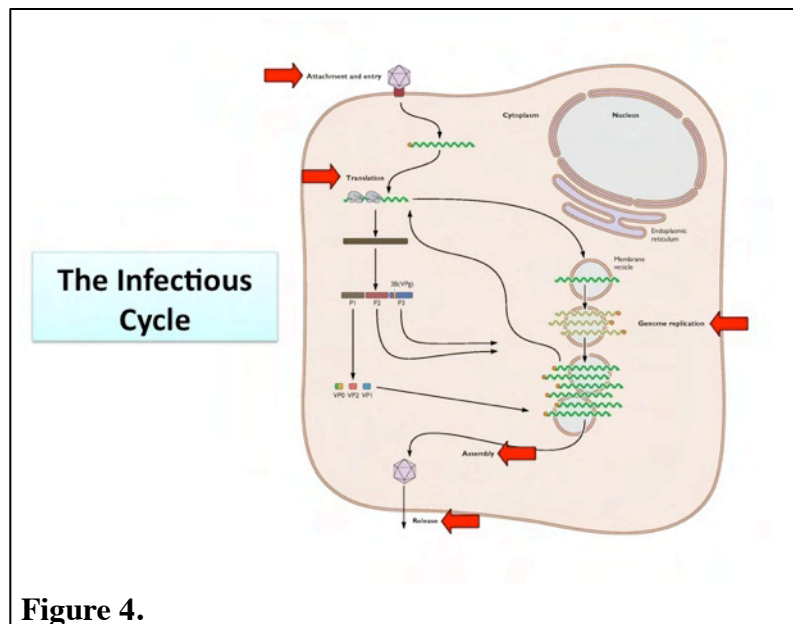


Figure 4.

defenses such as antibodies and immune cells, which exist to combat virus infections, are not found in cultured cells. Virus infection of cultured cells has been a valuable tool for understanding viral life cycles, but the differences compared with infection of a living animal must always be considered.

## Virus Cultivation

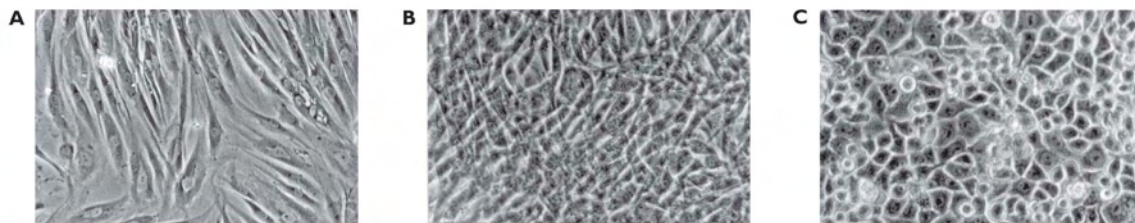


Figure 5.

Cell culture is the most common method for the propagation of animal viruses. There are three main kinds of cell cultures (Fig. 5). **Primary cell cultures** are prepared from animal tissues and have a limited life span. Some viral vaccines are now prepared in **diploid cell strains**, a homogeneous population of a single type of cell that can divide up to 100 times before dying. **Continuous cell lines** consist of a single cell type that can be propagated indefinitely in culture. They are usually derived from tumor tissue or from transformed cells. Some viruses kill the cells in which they replicate, and the infected cells may eventually detach from the cell culture plate. These changes are called **cytopathic effects**, and can be seen with a simple light microscope. These changes include rounding up and detachment of cells from the culture dish (Fig. 6) and sometimes the formation of a group of fused cells called a syncytium (Fig. 7).

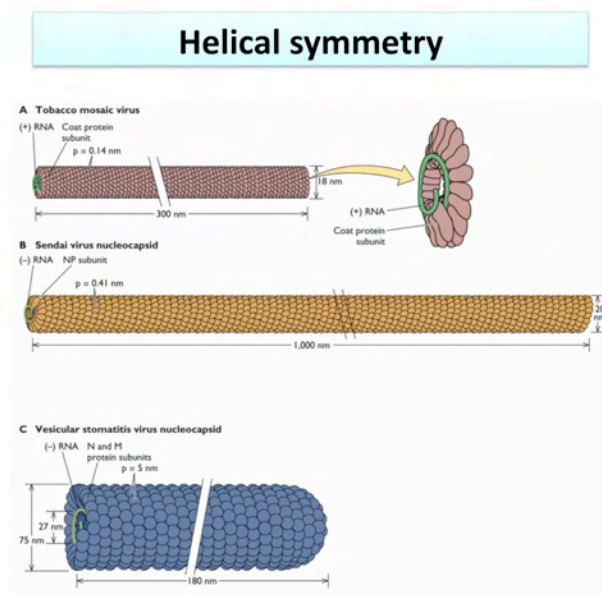


interaction with cell receptors, induction of fusion with host cell membranes, and interaction with cell components to transport the genome to the correct compartment.

To protect the nucleic acid genome, virus particles must be stable structures. However, virions must also attach to an appropriate host cell and deliver the genome to the interior of the cell. Virus particles are therefore metastable structures that have not yet attained the minimum free energy conformation. This state can be attained only when an unfavorable energy barrier is surmounted, following induction of the irreversible conformational transitions associated with attachment and entry. Virions are not simply inert structures. Rather, they are molecular machines that play an active role in delivery of the nucleic acid genome to the appropriate host cell.

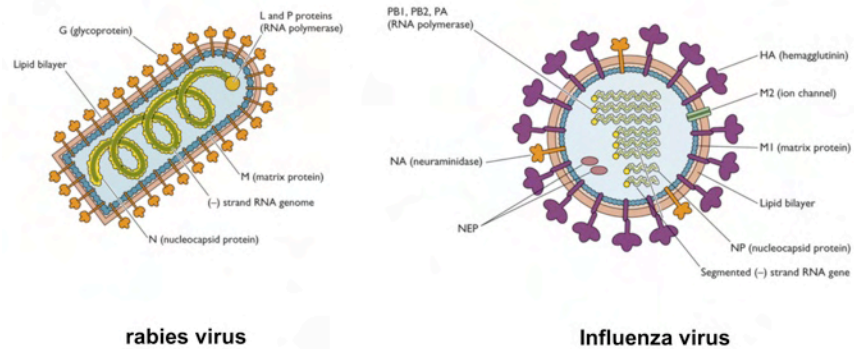
All virions contain at least one protein coat, the capsid or nucleocapsid, that protects the nucleic acid genome. Genetic economy dictates that capsids and nucleocapsids be built from identical copies of a small number of viral proteins with structural properties that permit regular and repetitive interactions among them. These protein molecules are arranged to provide maximal contact and noncovalent bonding among subunits and structural units. The repetition of such interactions among a limited number of proteins results in a regular structure, with symmetry that is determined by the spatial patterns of the interactions. The protein coats of all but a few viruses display **helical** or **icosahedral** symmetry.

The nucleocapsids of some enveloped animal viruses, plant viruses, and bacteriophages are rodlike or filamentous structures with helical symmetry (Fig. 9). In such structures, the viral RNA is coated with multiple protein subunits that assemble as a long-rodlike helix. The virions of paramyxoviruses, rhabdoviruses, and orthomyxoviruses contain internal structures with helical symmetry encased within an envelope (Fig. 10).



**Figure 9**

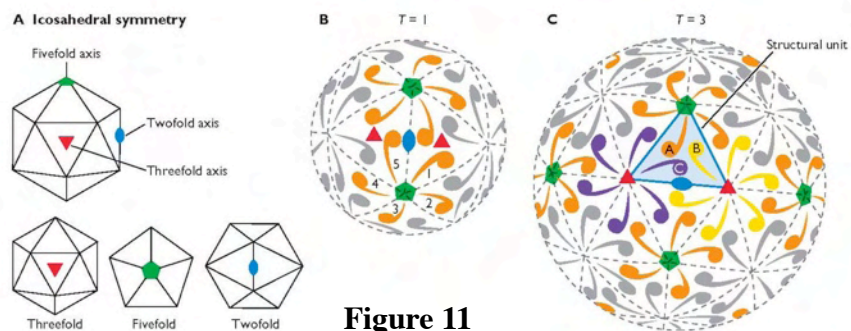
## Helical symmetry



**Figure 10**

Many virus particles have icosahedral symmetry (Fig. 11). An icosahedron is a solid with 20 triangular faces and 12 vertices. Each of the 20 faces of an icosahedron is an equilateral triangle. In the simplest protein shells, a trimer of a single viral protein corresponds to each triangular face of the icosahedron. As an icosahedron has 20 faces, 60 identical subunits is the minimum number needed to build a capsid or nucleocapsid with icosahedral symmetry. An example is adeno-associated virus type 2, a member of the parvovirus family. These small animals viruses, with particles 250 Å in diameter, encase a single stranded DNA genome of less than 5 kb. The particles are built from 60 copies of a single protein subunit.

## Icosahedral symmetry

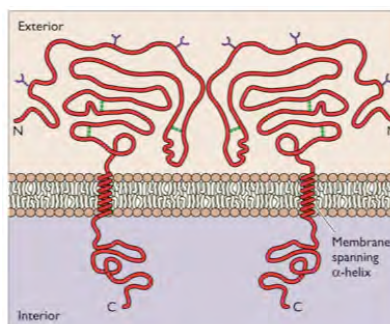


**Figure 11**

Some naked viruses are considerably larger and composed of many more viral proteins than adeno-associated virus type 2. A feature of such viruses is the presence of proteins devoted to specialized structural or functional roles. An example of such complex viruses is adenovirus. A striking feature of the adenovirus particle (diameter, 1,500 Å) is the presence of long fibers at the 12 vertices. Each fiber is attached to 1 of the 12 penton bases. The remainder of the shell is built from 240 additional subunits, the hexons, each of which is a trimer of viral protein II.

Often icosahedral virus particles are enveloped, such as is the case for the herpesviruses. These large (2,000 Å), DNA-containing viruses comprise an icosahedral capsid that encases a double-stranded DNA genome. The capsid is surrounded by a lipid membrane that is acquired from the host cell during assembly. The herpesvirion is remarkable because its protein components are encoded by over half of the 80 genes present in viral DNA.

Embedded in the membrane of enveloped viruses are viral proteins, most of which are glycoproteins that have one or more covalently linked sugar chains. Such **viral glycoproteins** are integral membrane proteins firmly embedded in the lipid bilayer by a short membrane-spanning domain (Fig. 12).



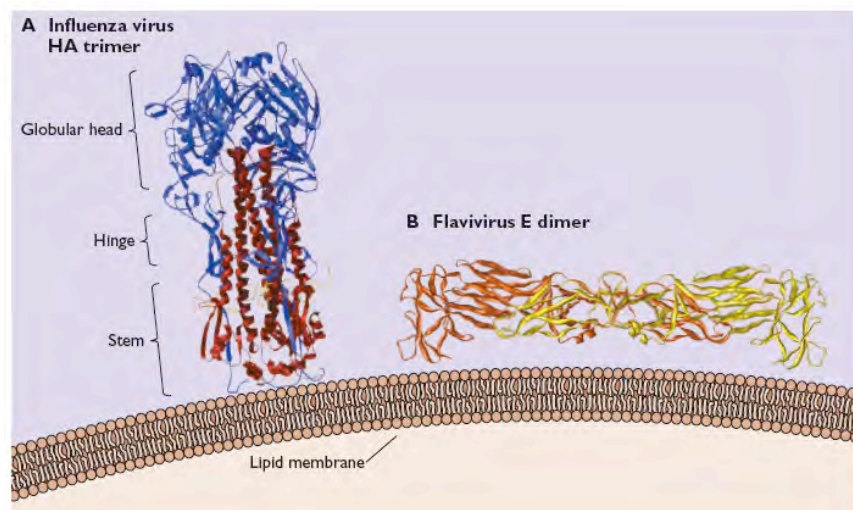
**Figure 12**

The external domains contain binding sites for cell surface virus receptors, major antigenic determinants, and sequences that mediate fusion of viral with cellular membranes during entry. Internal domains, which make contact with other components of the virion, are often essential for virus assembly.

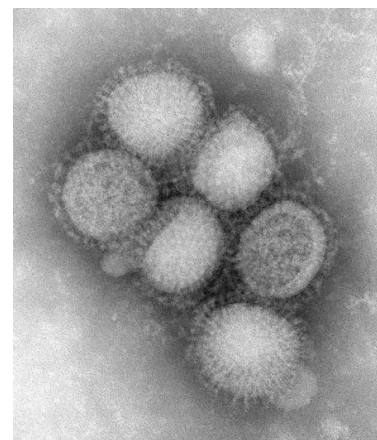
An example of a viral membrane glycoprotein is the hemagglutinin (HA) protein of human influenza A virus, which is a trimer. This protein contains a globular head with a top surface that is projected about 135 Å from the viral membrane by a long stem. The membrane-distal globular domain contains the binding site for the virus receptor. Another viral glycoprotein that mediates cell attachment and entry is the E protein of the flavivirus tick-borne encephalitis virus. The external domain of E protein is quite different: it is a flat, elongated dimer that would lie on the surface of the viral membrane rather than projecting from it as does the HA (Fig. 13).

With few exceptions, viral membrane glycoproteins are oligomeric. On the exterior of the virion, these oligomers form surface projections, often called spikes, that are readily visible in electron micrographs (Fig. 14 - 2009 pandemic H1N1 influenza virus). Because of their critical roles in initiating infection, the structures of many viral glycoproteins have been determined.





**Figure 13**

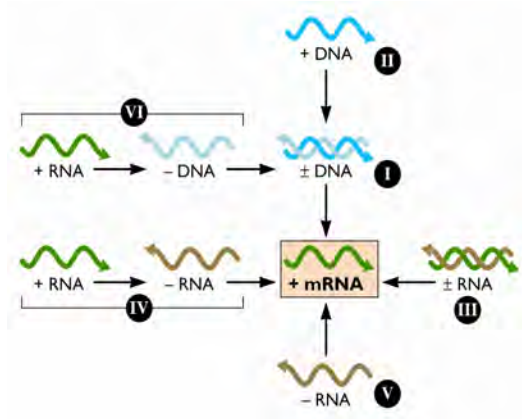


**Figure 14**

## Viral Genomes

The results of studies in the 1950s demonstrated that the **viral genome** is the nucleic acid-based repository of the information needed to build, replicate, and transmit a virus. Although this conclusion seems obvious today, this discovery constitutes one of the building blocks of modern molecular biology. The structures of viral genomes are far less complex than is implied by the existence of thousands of distinct entities defined by classical taxonomic methods. In fact, it is possible to organize the known viruses into seven groups, based on the structures of their genomes.

One universal function of viral genomes once inside a cell is to specify proteins. However, viral genomes do not encode the machinery needed to carry out protein synthesis. An important principle is that all viral genomes must be copied to produce messenger RNAs (mRNAs) that can be read by host ribosomes. Literally, all viruses are parasites of their host cells' mRNA translation system. The Baltimore classification system integrates this principle to construct an elegant molecular algorithm for virologists (Fig. 15). When the bewildering array of viruses is classified by this system, we find 7 pathways to mRNA. The 7 classes of viral genomes include dsDNA, gapped dsDNA, ssDNA, dsRNA, ss(+)-RNA, ss(-)-RNA, and ss(+)-RNA with DNA intermediate. The elegance of the Baltimore system is that by knowing only the nature of the viral genome, one can deduce the basic steps that must take place to produce mRNA. Perhaps more pragmatically, the system simplifies comprehension of the extraordinary life cycles of viruses.



**Figure 15**