

Intro II – Viral Replication

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.

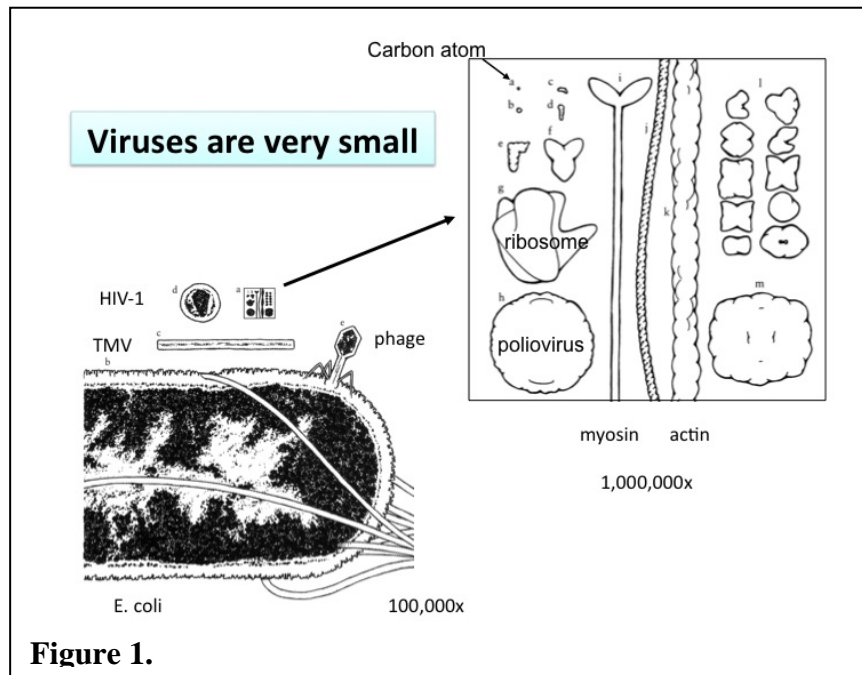
Viruses Defined

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.

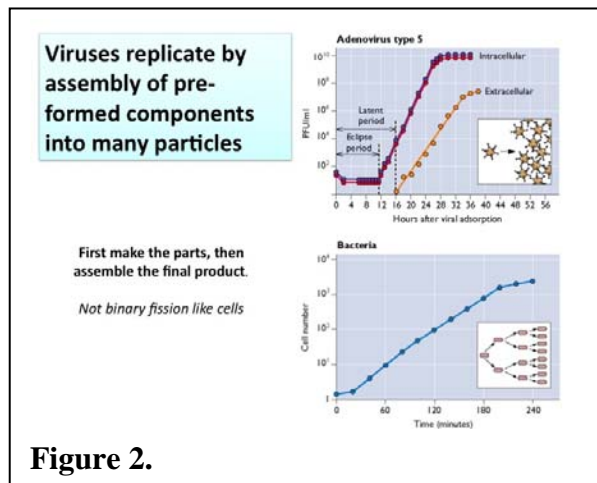
Other defining viral attributes include:

- 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.

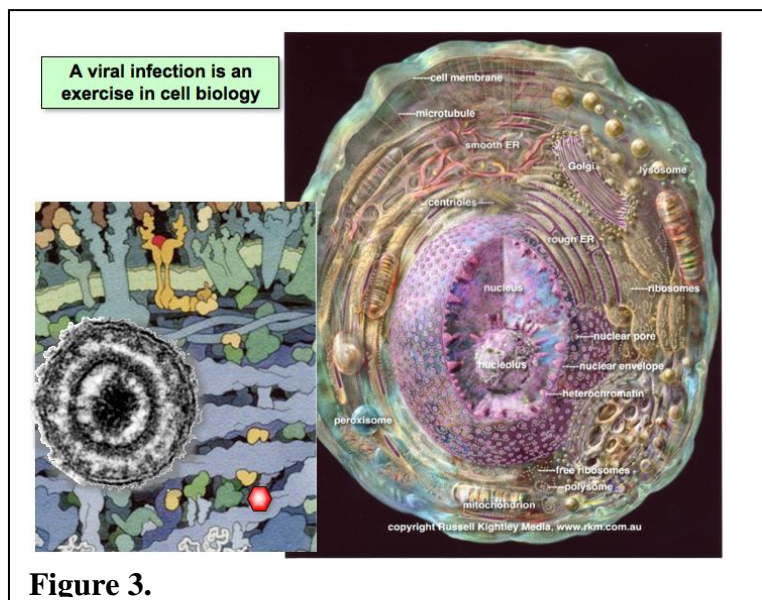
2. The viral genome contains the information to initiate and complete an infectious cycle within a susceptible and permissive cell. The infectious cycle allows attachment and entry of the particle, decoding of genome information, translation of viral mRNA by host ribosomes, genome replication, assembly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

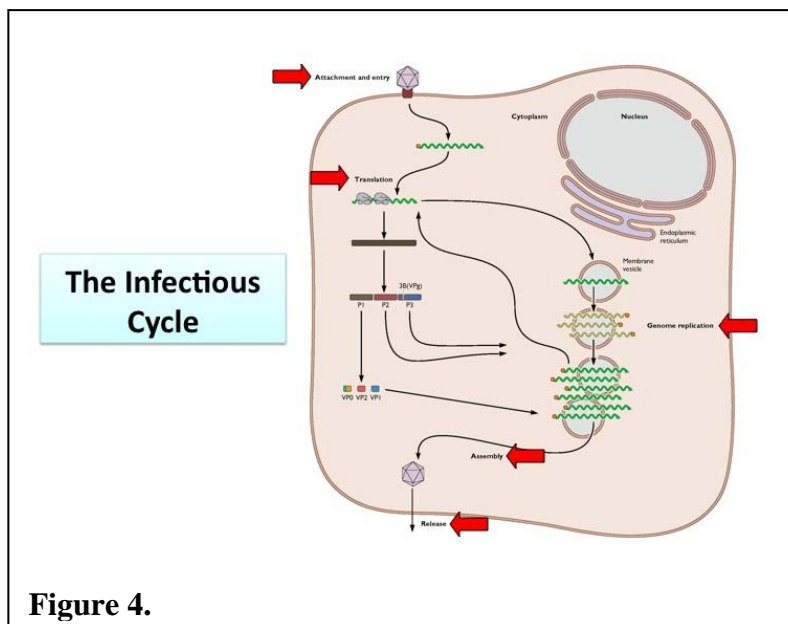
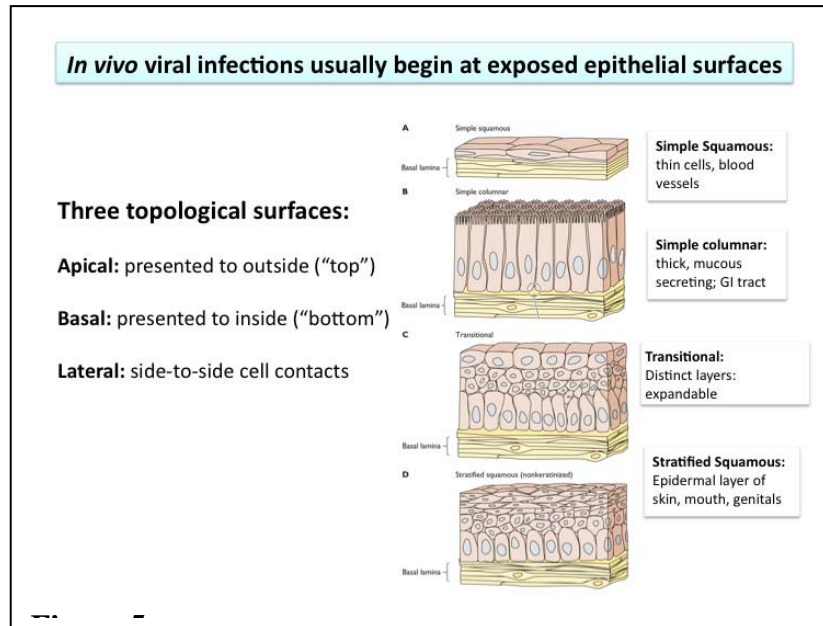


Figure 4.

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 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.

The Cell Surface

In animals, viral infections usually begin at the epithelial surfaces of the body that are exposed to the environment (Fig. 5). Cells cover these surfaces, and the part of these cells exposed to the environment is called the apical surface. Conversely, the basolateral surfaces of such cells are in contact with adjacent or underlying cells or tissues. These cells exhibit a differential (polar) distribution of proteins and lipids in the plasma membranes that creates the two distinct surface domains. As illustrated in Fig. 5, these cell layers differ in thickness and organization. Movement of macromolecules between the cells in the epithelium is prevented by tight junctions, which circumscribe the cells at the apical edges of their lateral membranes. Many viral infections are initiated upon entry of epithelial or endothelial cells at their exposed apical surfaces, often by attaching to cell surface molecules specific for this domain. Viruses that both enter and are released at apical membranes can be transmitted laterally from cell to cell without ever transversing the epithelial or endothelial layers; they generally cause localized infections. In other cases, progeny virions are transported to the basolateral surface and released into the underlying cells and tissues, a process that facilitates viral spread to other sites of replication.



The plasma membrane of every cell type is composed of a similar phospholipid/glycolipid bilayer, but different sets of membrane proteins and lipids allow the cells of different tissues to carry out their specialized functions. The lipid bilayer consists of molecules that possess both hydrophilic and hydrophobic portions; they are known as amphipathic molecules (Fig. 6). They form a sheet-like structure in which polar head groups face the aqueous environment of the cell’s cytoplasm (inner surface) or the surrounding environment (outer surface). The polar head groups of the inner and outer leaflets bear side chains with different lipid compositions. The fatty acyl side chains form a continuous hydrophobic interior about 3 nm thick.

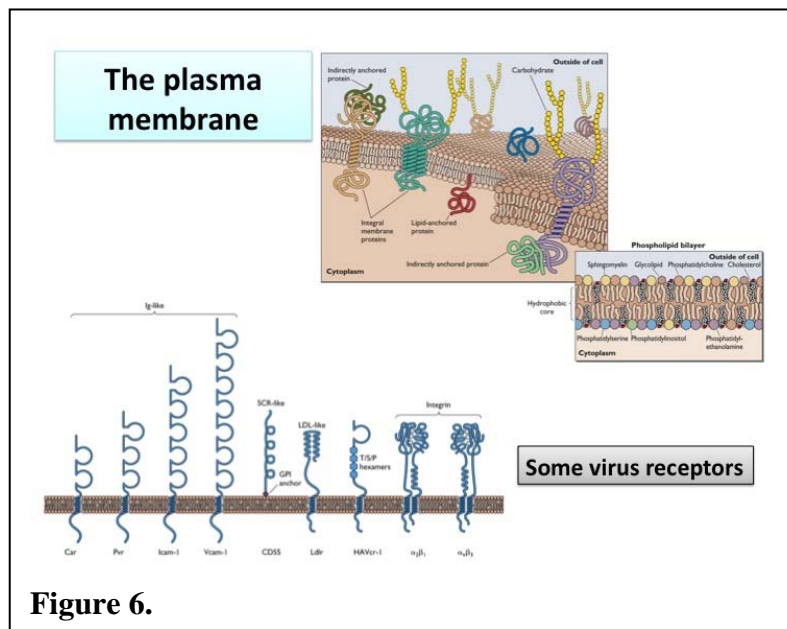


Figure 6.

Membrane proteins are classified into two broad categories, integral membrane proteins and indirectly anchored proteins (Fig. 6). Integral proteins are embedded in the lipid bilayer, because they contain one or more membrane-spanning domains, as well as portions that protrude out into

the exterior and interior of the cell (Fig. 6). Many membrane-spanning domains consist of an α -helix typically 3.7 nm long. Some proteins with multiple membrane-spanning domains form critical components of molecular pores or pumps, which mediate the internalization of required nutrients, or expulsion of undesirable material from the cell, or maintain homeostasis with respect to cell volume, pH, and ion concentration.

The external portions of membrane proteins may be decorated by complex or branched carbohydrate chains linked to the peptide backbone. Such membrane glycoproteins quite frequently serve as viral receptors.

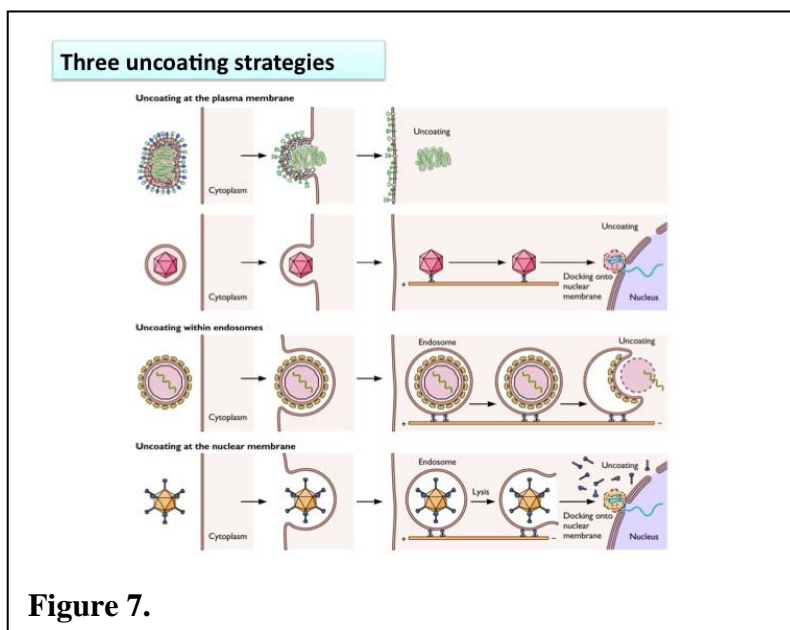
Some membrane proteins do not span the lipid bilayer, but are anchored in the inner or outer leaflet by covalently attached hydrocarbon chains. Indirectly anchored proteins are bound to the plasma membrane lipid bilayer by interacting either with integral membrane proteins or with the charged sugars of the glycolipids within the membrane lipid.

Entering Cells

Viral infection is initiated by a collision between the virus particle and the cell, a process that is governed by chance. Therefore, a higher concentration of virus particles increases the probability of infection. However, a virion may not infect every cell it encounters. It must come in contact with the cells and tissues in which it can replicate. Such cells are normally recognized by means of a specific interaction of a virion with a cell surface receptor. This process can be either promiscuous or highly selective, depending on the virus and the distribution of the cell receptor. The presence of such receptors determines whether the cell will be **susceptible** to the virus. However, whether a cell is **permissive** for the replication of a particular virus depends on other, intracellular components found only in certain cell types. Cells must be both susceptible **and** permissive if an infection is to be successful.

In general, viruses have no means of locomotion, but their small size facilitates diffusion driven by Brownian movement. Propagation of viruses is dependent on essentially random encounters with potential hosts and host cells. Viral propagation is critically dependent on the production of large numbers of progeny virions with surfaces composed of many copies of structures that enable the virions to attach to susceptible cells.

Successful entry of a virus into a host cell requires that the virus cross the plasma membrane, and often the nuclear membrane (Fig. 7). The virus particle must also disassemble to make the viral genome accessible in the cytoplasm, and the nucleic acid must be targeted to the correct cellular compartment. These are not simple processes. Cell membranes are not permeable to virus particles. Virions or critical subassemblies are brought across such barriers by specific transport pathways. To survive in the extracellular environment, the viral genome must be encapsidated in a protective coat that will shield viral nucleic acid from the variety of potentially harsh conditions that a virion may meet in the environment. For example, mechanical shearing, ultraviolet (UV) irradiation (from sunlight), extremes of pH (in the gastrointestinal tract), dehydration (in the air), and enzymatic attack (in body fluids) are all capable of damaging viral nucleic acids. However, once in the host cell, the protective structures must become sufficiently unstable to release the genome. Virus particles cannot be viewed only as passive vehicles: they must be able to undergo structural transformations that are important for attachment and entry into a new host cell and for the subsequent disassembly required for viral replication.



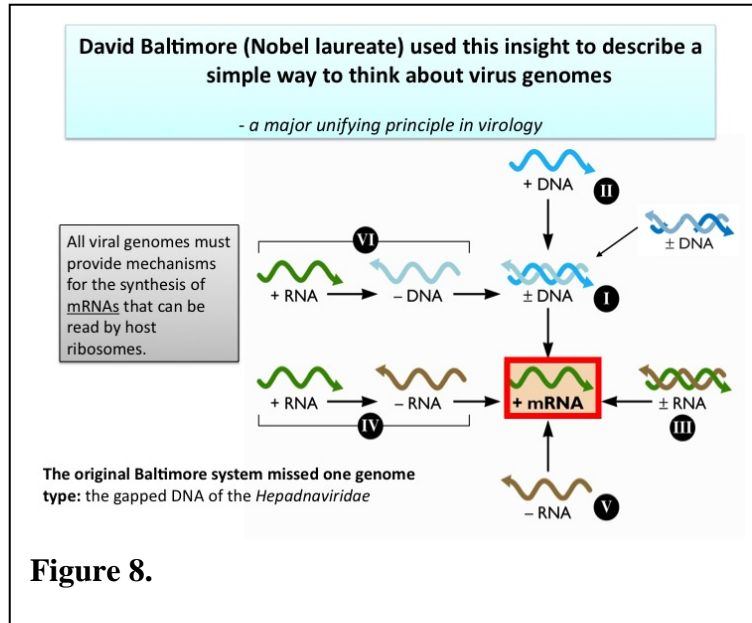
Viral Genomes

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. 8). 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.

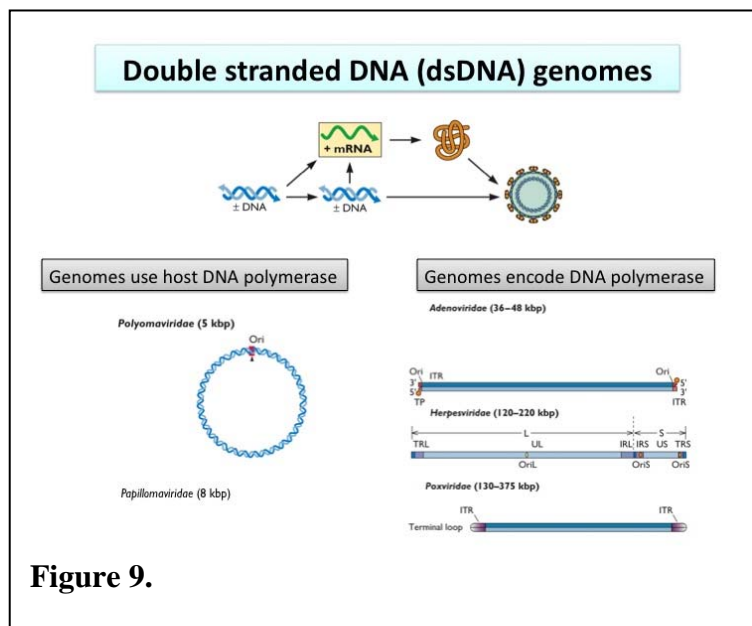
DNA Genomes

The strategy of having DNA as a viral genome appears at first glance to be simplicity itself: the host genetic system is based on DNA, so the viral genome replication and expression could simply emulate the host's system. Many surprises await those who believe that this is all such a strategy entails.



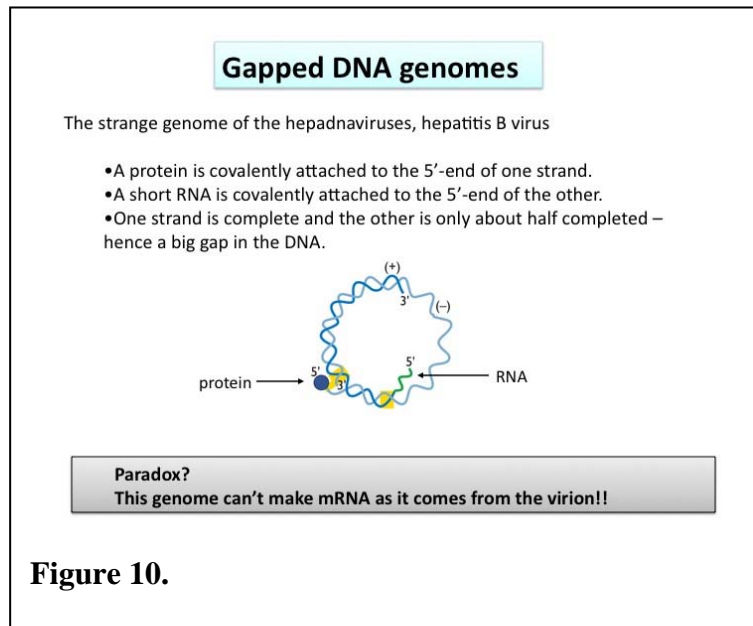
Double-Stranded DNA (dsDNA) (Fig. 9)

Viral genomes may consist of double-stranded or partially double-stranded nucleic acids. There are 22 families of viruses with dsDNA genomes; those that include mammalian viruses are the *Adenoviridae*, *Herpesviridae*, *Papillomaviridae*, *Polyomaviridae*, and *Poxviridae*. These dsDNA genomes may be linear or circular. mRNA is produced by copying of the genome by host or viral DNA-dependent RNA polymerase.

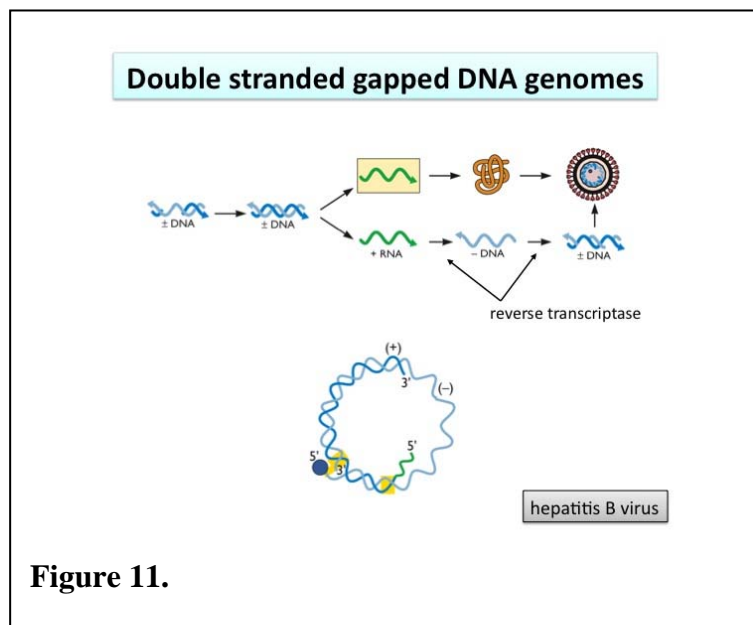


Gapped DNA (Fig. 10, 11)

For partially double-stranded DNA, such as the gapped DNA of *Hepadnaviridae*, the gaps must be filled to produce perfect duplexes. This repair process must precede mRNA synthesis. The

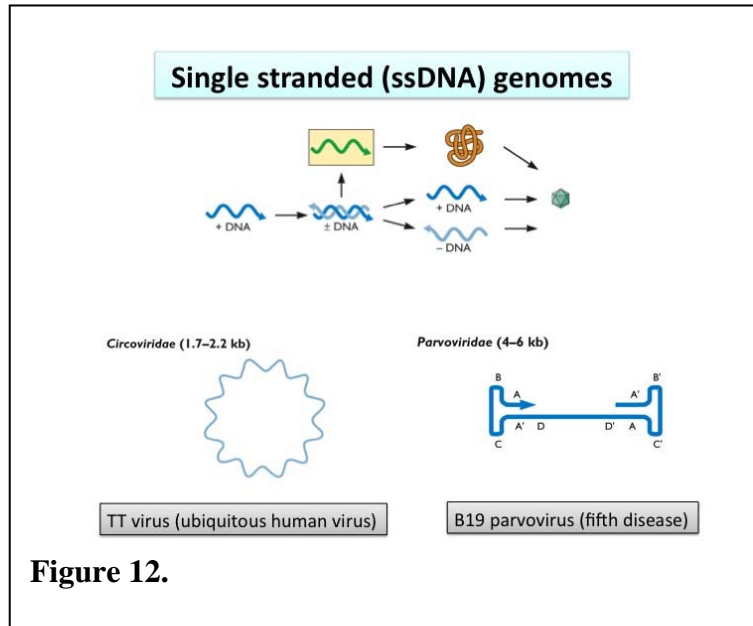


unusual gapped DNA genome is produced from an RNA template by a virus-encoded reverse transcriptase homologous to that of the retroviruses.



Single-Stranded DNA (ssDNA) (Fig. 12)

Five families and one genus of viruses containing ssDNA genomes have been recognized; the *Circoviridae* and *Parvoviridae* include viruses that infect mammals. ssDNA must be copied into mRNA before proteins can be produced. However, as RNA can only be made from a double-stranded DNA template, no matter what sense the single-stranded DNA is, DNA synthesis **must** precede mRNA production in the replication cycles of these viruses. The single-stranded genome is produced by cellular DNA polymerases.

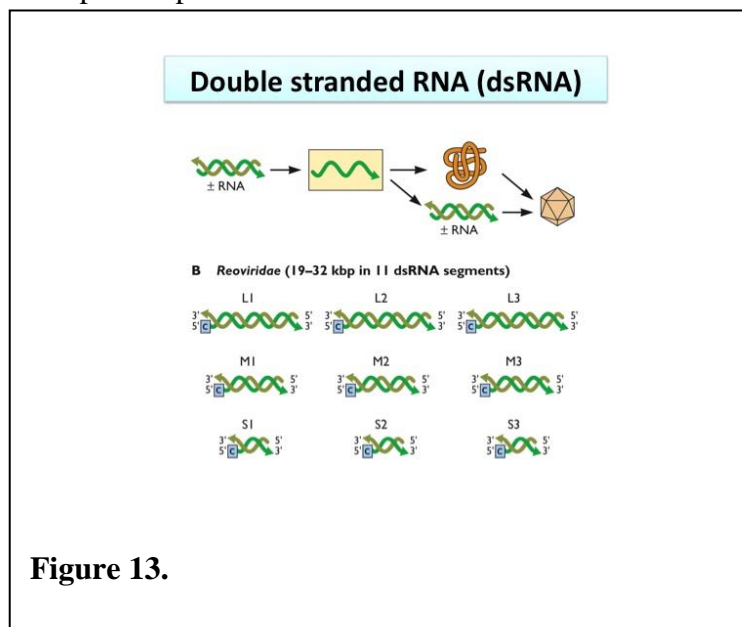


RNA Genomes

Cells have no RNA-dependent RNA polymerase that can replicate the genomes of RNA viruses or make mRNA from RNA. The simple solution that has evolved is that RNA virus genomes encode novel RNA-dependent RNA polymerases that produce RNA (both genomes and mRNA) from RNA templates. The mRNA produced is readable by host ribosomes. One notable exception is retrovirus mRNAs, which are produced from DNA templates by host RNA polymerase II.

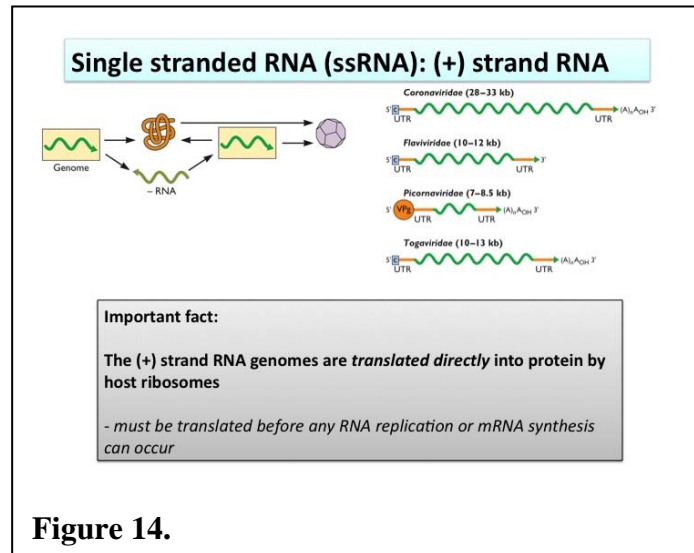
dsRNA (Fig. 13)

There are seven families of viruses with dsRNA genomes. The number of dsRNA segments ranges from 1 (*Totiviridae* and *Hypoviridae*, viruses of fungi, protozoans, and plants) to 10 to 12 (*Reoviridae*, viruses of mammals, fish, and plants). While dsRNA contains a (+) strand, it cannot be translated as part of a duplex. The (–) strand RNAs are first copied into mRNAs by a viral RNA-dependent RNA polymerase to produce viral proteins. Newly synthesized mRNAs are encapsidated and then copied to produce dsRNAs.



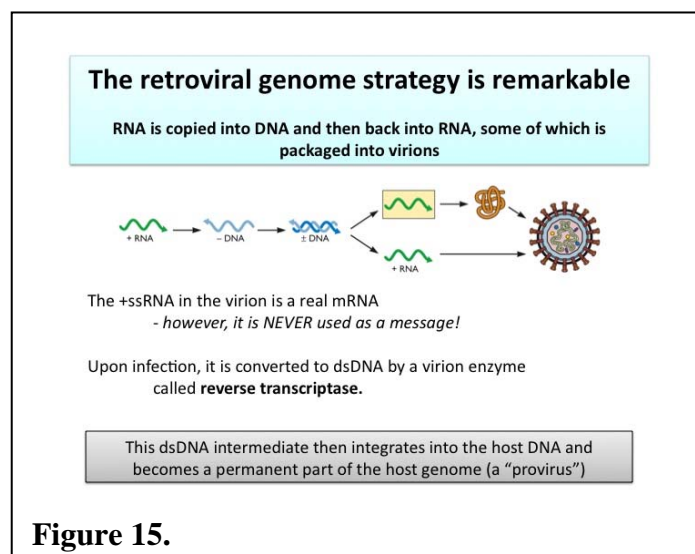
(+) Strand RNA (Fig. 14)

The (+) strand RNA viruses are the most plentiful on this planet; 22 families and 22 genera have been recognized. The *Arteriviridae*, *Astroviridae*, *Caliciviridae*, *Coronaviridae*, *Flaviviridae*, *Picornaviridae*, *Retroviridae*, and *Togaviridae* include viruses that infect mammals. (+) strand RNA genomes usually can be translated directly into protein by host ribosomes. The genome is replicated in two steps. First, the (+) strand genome is copied into a full-length (-) strand. The (-) strand is then copied into full-length (+) strand genomes. A subgenomic mRNA is produced in cells infected with some viruses of this class.



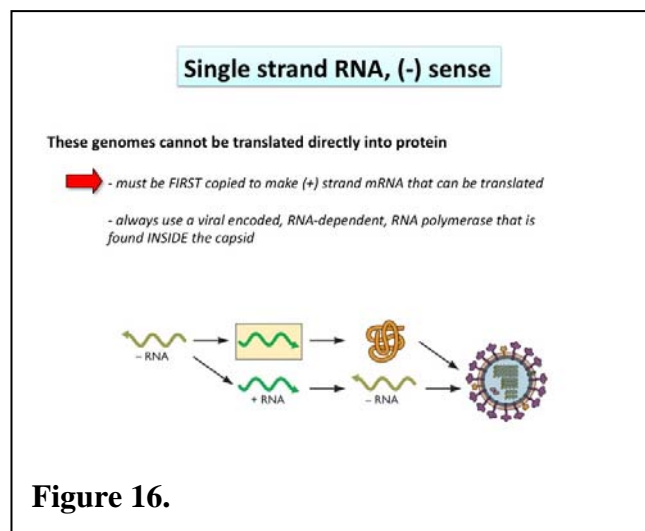
(+) Strand RNA with DNA Intermediate (Fig. 15)

The retroviruses are unique (+) strand RNA viruses because a dsDNA intermediate is produced from genomic RNA by an unusual viral RNA-dependent DNA polymerase called reverse transcriptase. This DNA then serves as the template for viral mRNA and genome RNA synthesis by cellular enzymes.

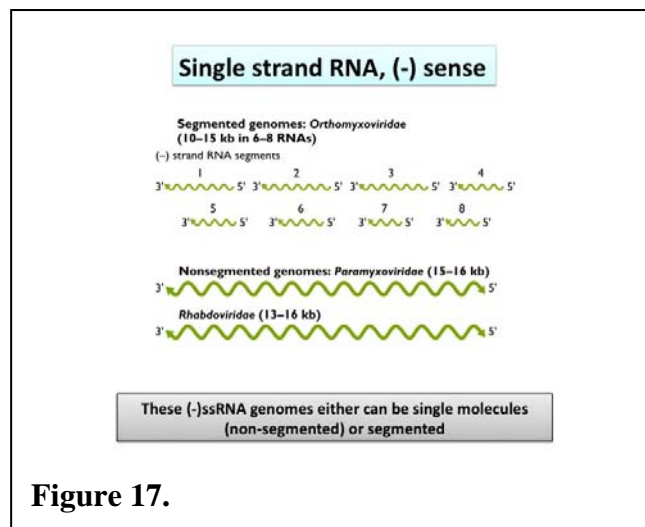


(-) Strand RNA (Fig. 16, 17)

Viruses with (-) strand RNA genomes are found in seven families and three genera. Viruses of this class that can infect mammals are found in the *Bornaviridae*, *Filoviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, and *Rhabdoviridae*. By definition, (-) strand RNA genomes cannot be translated directly into protein but first must be copied to make (+) strand mRNA by a viral RNA-dependent RNA polymerase. There are no enzymes in the cell that can produce mRNAs from the RNA genomes of (-) strand RNA viruses. These virus particles therefore contain virus-encoded, RNA-dependent RNA polymerases that produce mRNAs from the (-) strand genome. This strand is also the template for the synthesis of full-length (+) strands, which in turn are copied to produce (-) strand genomes. (-) strand RNA viral genomes can be either single molecules (nonsegmented) or segmented.



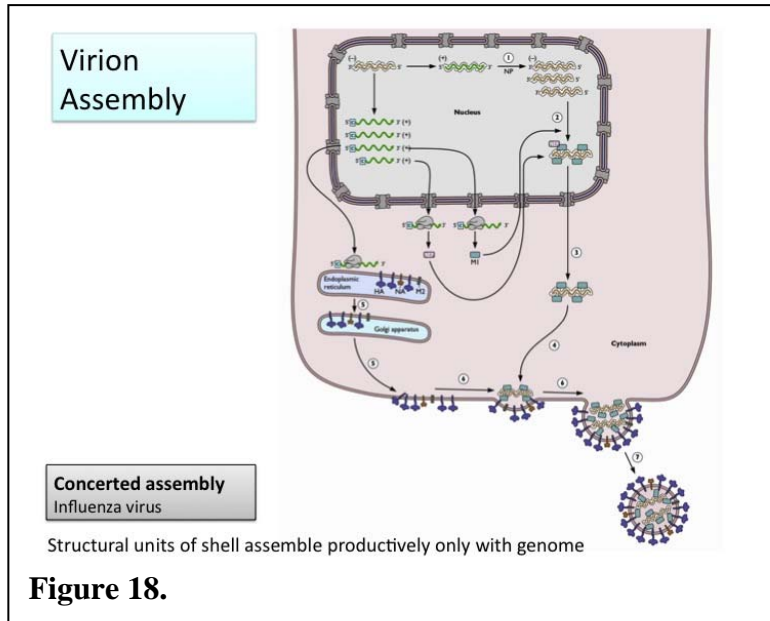
Forming Progeny Virions



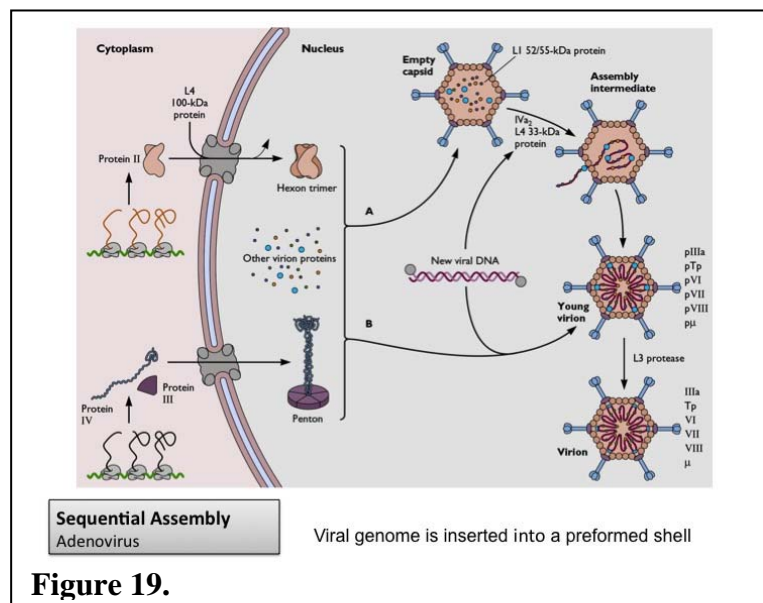
The various components of a virion, the nucleic acid genome, capsid protein(s), and in some cases envelope proteins, are often synthesized in different cellular compartments. Their trafficking through and among the cell's compartments and organelles requires that they be equipped with the proper homing signals. Virion components must be assembled at some central

location, and the information for assembly must be preprogrammed in the component molecules (Chapter 13). The primary sequences of virion proteins contain sufficient information to specify assembly.

In concerted assembly, the structural units of the protective protein shell assemble productively



only in association with the genomic nucleic acid. The nucleocapsids of (-) strand RNA viruses form by a concerted mechanism (Fig. 18), as do retrovirus particles. In sequential assembly, the genome is inserted into a preformed protein shell. The formation of herpesviral capsid is an example of this type of assembly (Fig. 19).



Release of Virus Particles

The majority of viruses leave an infected cell by one of two general mechanisms. Some viruses are released into the external environment upon budding from, or lysis of the cell. Others move directly into a new host cell without physical release of the particle, so-called cell-to-cell spread.

Many enveloped viruses assemble at, and bud from, the plasma membrane. Consequently, the final assembly reaction releases the newly formed virus particle into the extracellular milieu. The assembly of enveloped viruses is therefore mechanistically coupled with and coincident with their exit from the host cell. The egress of viruses without envelopes is frequently by destruction (lysis) of the host cell. Large quantities of assembled virions may accumulate in the infected cell, prior to release upon lysis.

Many viruses spread from one host cell to another as extracellular virions released from an infected cell (Fig. 20). Such extracellular dissemination is necessary to infect another host. Some viruses, such as alphaherpesviruses, can also spread from cell to cell without passage through the extracellular environment. Such cell-to-cell spread is dependent on the viral fusion machinery. Virus spread by this mechanism avoids exposure to host defense mechanisms that target extracellular virus.

