Replication of viruses

Viral replication: All DNA viruses replicate in the nucleus, except poxviruses, which replicate in the cytoplasm. All RNA viruses replicate in the cytoplasm, except retroviruses, influenza virus, and hepatitis D virus, which require an intranuclear step in their replication. Many viruses encode a replicase, whichis a DNA or RNA polymerase that synthesizes the many copies of the progeny viral genomes.

The steps in viral replication are as follows:

1. Attachment (Adsorption) of the virus to specific receptors on the cell surface.
2. Penetration and uncoating by the virus and intracellular release of nucleic acid.
3. Proliferation of the viral components: virus-coded synthesis of capsid and noncapsid proteins, replication of nucleic acid by viral and cellular enzymes.
4. Assembly of replicated nucleic acid and new capsid protein.
5. Release of virus progeny from the cell.

1. Attachment (Adsorption): The interaction of proteins on the surface of the virus with specific receptor proteins on the surface of the cell is one of the main determinants of both the **species specificity** and the **organ specificity** of the virus.

2. Penetration and uncoating: Viruses adsorbed to the cell surface receptors then penetrate into the cell by means of pinocytosis (a process also known as viropexis). In enveloped viruses, the envelope may also fuse with the cell membrane, releasing the virus into the cytoplasm. Adsorption of such an enveloped virus to two cells at the same time may result in cell fusion. The next step, known as uncoating, involves the release of the nucleic acid from the capsid and is apparently (except in the smallpox virus) activated by cellular enzymes, possibly with a contribution from cell membranes as well. The exact mechanism, which would have to include preservation of the nucleic acid in toto, is not known for all viruses.

3.Replication of the nucleic acid: Different processes are observed corresponding to the types and configurations of the viral genome.

4. Viral maturation (morphogenesis): In this step, the viral capsid proteins and genomes (present in multiple copies after the replication process) are assembled into new, infectious virus particles. In some viral species these particles are also covered by an envelope.

5. Release: The release of viral progeny in some cases correlates closely with viral maturation, whereby envelopes or components of them are acquired when

the particles "bud off" of the cytoplasmic membrane and are expelled from the cell. In nonenveloped viruses, release of viral progeny is realized either by means of lysis of the infected cell or more or less continuous exocytosis of the viral particles.

Viral Growth Curve

• One virion infects a cell and hundreds of progeny virions are produced within hours. This is a remarkable amplification and explains the rapid spread of virus from cell to cell.

• The **eclipse period** is the time when no virus particles are detected within the infected cell. It occurs soon after the cell is infected.

• **Cytopathic effect** (CPE) is the term used to describe the damage, both morphologic and functional, inflicted on the cell by the virus. In the clinical laboratory, the presence of a virus in the patient's specimen is often detected by seeing a CPE in cell culture.

Viral Growth Cycle

• **Infectious nucleic acid**: is viral genome DNA or RNA, purified free of all proteins, that can undergo the entire replicative cycle within a cell and produce infectious progeny viruses. Infectious nucleic acid, because it has no associated protein, can enter and replicate within cells that the intact virion cannot.

• **Polarity of viral genome RNA:** Genome RNA that has the same base sequence as the mRNA is, by definition, positive polarity RNA. Most positive-polarity genomes are translated into viral proteins without the need for a polymerase in the virion. The exception is the retroviruses, which use reverse transcriptase in the virion to transcribe the genome RNA into DNA.Genome RNA that has a base sequence complementary to mRNA has, by definition, negative polarity. A virus with a negative- polarity RNA genome must have an RNA polymerase in the virion to synthesize its mRNA.

• **Viral gene expression:** All viruses require virus-specific messenger RNA to synthesize virus-specific proteins.

• **RNA viruses:** Some RNA viruses, such as poliovirus, have a positive-polarity RNA genome that serves as the mRNA (i.e., the genome is the mRNA). Other viruses, such as influenza virus, have a negative-polarity RNA genome and have an RNA polymerase in the virion that synthesizes the viral mRNA. Rotavirus has a double-stranded RNA genome and has an RNA polymerase in the virion that synthesizes the viral mRNA. Retroviruses, such as HIV, have a positive-polarity RNA genome and have a DNA polymerase in the virion that synthesizes a DNA copy of the RNA genome. This DNA is the template used by the host cell RNA polymerase to synthesize the viral mRNA.

• **DNA viruses:** Most DNA viruses, such as herpesviruses, adenoviruses, and papillomaviruses, have a double-stranded DNA genome and use the host cell RNA polymerase to synthesize the viral mRNA. Poxviruses have a double-stranded DNA genome but have an RNA polymerase in the virion that synthesizes the viral mRNA. Poxviruses have an RNA polymerase in the virion because they replicate in the cytoplasm and do not have access to the host cell RNA polymerase in the nucleus.

• **Lysogeny:** is the process by which viral DNA becomes integrated into host cell DNA, replication stops, and no progeny virus is made. Later, if DNA is damaged by, for example, UV light, viral DNA is excised from the host cell DNA, and progeny viruses are made. The integrated viral DNA is called a **prophage.** Bacterial cells carrying a prophage can acquire new traits, such as the ability to produce exotoxins such as diphtheria toxin. **Transduction** is the process by which viruses carry genes from one cell to another. **Lysogenic conversion** is the term used to indicate that the cell has acquired a new trait as a result of the integrated prophage.

Genetics

The study of viral genetics falls into two general areas: (1) mutations and their effect on replication and pathogenesis; and (2) the interaction of two genetically distinct viruses that infect the same cell. In addition, viruses serve as **vectors** in gene therapy and in recombinant vaccines, two areas that hold great promise for the treatment of genetic diseases and the prevention of infectious diseases.

Mutation

Mutations are changes in the base sequence of a nucleic acid, resulting in a more or less radical alteration of the resulting protein. So-called “silent mutations” (in the second or third nucleotide of a codon) do not influence the amino acid sequence of the protein. Medically important are mutants with weakened virulence that have retained their antigenicity and replication capabilities intact. These are known as “attenuated” viruses. They are the raw material of live vaccines.

INTERACTIONS BETWEEN VIRUSES

When two genetically distinct viruses infect a cell, three different phenomena can ensue.

(1) Recombination: is the exchange of genes between two chromosomes that is based on crossing over within regions of significant base sequence homology. Recombination can be readily demonstrated for viruses with double-stranded DNA as the genetic material and has been used to determine their genetic map. However, recombination by RNA viruses occurs at a very low frequency, if at all.

Reassortment: is the term used when viruses with segmented genomes, such as influenza virus, exchange segments. This usually results in a much higher frequency of gene exchange than does recombination. Reassortment of influenza virus RNA segments is involved in the major antigenic changes in the virus that are the basis for recurrent influenza epidemics.

(2) Complementation: can occur when either one or both of the two viruses that infect the cell have a mutation that results in a nonfunctional protein. The nonmutated virus "complements" the mutated one by making a functional protein that serves for both viruses. Complementation is an important method by which a helper virus permits replication of a defective virus. One clinically important example of complementation is hepatitis B virus providing its surface antigen to hepatitis delta virus, which is defective in its ability to produce its own outer protein. This phenomenon is the basis for the complementation test, which can be used to determine how many genes exist in a viral genome. It is performed by determining whether mutant virus A can complement mutant virus B. If it can, the two mutations are in separate genes because they make different, complementary proteins. If it cannot, the two mutations are in the same gene, and both proteins are nonfunctional. By performing many of these paired tests with different mutants, it is possible to determine functional domains of complementation groups that correspond to genes. Appropriate controls are needed to obviate the effects of recombination.

(3) In phenotypic mixing: the genome of virus type A can be coated with the surface proteins of virus type B. This phenotypically mixed virus can infect cells as determined by its type B protein coat. However, the progeny virus from this infection has a type A coat; it is encoded solely by its type A genetic material. An interesting example of phenotypic mixing is that of **pseudotypes,** which consist of the nucleocapsid of one virus and the envelope of another. Pseudotypes composed of the nucleocapsid of vesicular stomatitis virus (a

rhabdovirus) and the envelope of human immunodeficiency virus (HIV; a retrovirus) are currently being used to study the immune response to HIV.