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Viral Replication and Genetics

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Viral Replication

Viral replication is a very complex and varied process. The mechanics of replication depends largely upon the type of nucleic acid and genomic organization of each particular virus. Despite the variation in replication strategies, there is commonality in several steps of viral replication. All viral replication schemes contain the following basic steps: attachment, penetration, uncoating (if necessary), protein synthesis (or gene expression), genome replication, assembly (morphogenesis), and release.

- Attachment depends on the physical interaction between the virion and the surface of the host/target cell. Typically, the interaction is a receptor-ligand interaction. As a result, species specificity or specific cell type specificity is determined. Without attachment, viral infection cannot occur. On the other hand, not all attachments result in productive infection. In other words, attachment is required yet does not assure that replication will follow.
- Penetration refers to the introduction of viral nucleic acid into the cell, the internalization of the nucleocapsid via receptor-mediated endocytosis or the fusion of the viral envelope with the plasma membrane. As an immediate result, the nucleic acid is either located in the cytosol or within an endocytic vesicle.
- Uncoating of the nucleic acid from the nucleocapsid may require the participation of host proteins or other factors. Uncoating is a prerequisite for the genome be expressed. Following uncoating, the viral nucleic acid either continues in the replication cycle or a copy of it becomes integrated within the host genome and is quiescent until triggered to become active (retroviruses).
- Protein synthesis (gene expression)- mRNA is produced and translated into viral proteins. Regardless of whether the virus has a DNA or RNA genome, ss, ds, segmented or monopartite, it has to produce mRNAs that are recognized and translated by the host cell machinery.
- As will be described for each of group of viruses, there is a unique mechanism whereby the host cell machinery becomes largely dedicated to synthesis of viral products and away from the synthesis of host cell products.
- Genome replication: The mechanism of genome replication varies with the type of nucleic acid, its structure and topology. For the simplest viruses, it is a task of host cell enzymes; most viruses encode their own replication enzymes.

- Maturation is the assembly of complete virus particles. Maturation of nonenveloped viruses is primarily the assembly of genome + capsid proteins forming the nucleocapsid. This occurs spontaneously through protein-protein and protein-nucleic acid interactions. In the maturation of enveloped virions, the nucleocapsid acquires an external envelope consisting of the host cell membranes (nuclear, Golgi, endoplasmic reticulum, or plasma membranes), which contains a lipid bilayer of cellular origin and viral encoded proteins. The envelope is acquired by a process known as budding.
- Release of virions. For nonenveloped viruses, thousands of progeny virions are released by host cell death and lysis. For enveloped viruses, the progeny virions are released by budding out from the cell. Budding does not necessarily result in cell death, yet some enveloped viruses may be also released by cell lysis.

Replication of DNA Viruses

- In general, DNA viruses replicate within the nucleus. Exceptions are poxviruses and iridoviruses (viruses of insects and fish), which use cytoplasmic "factories".
- Those DNA viruses that multiply within the nucleus use host DNA-dependent RNA polymerase for transcription. The majority of poxviruses and iridoviruses have virion-encoded transcriptases that allow them to replicate in the cytoplasm.
- Replication of viral DNA is semiconservative and symmetrical with both strands being replicated. In dsDNA viruses, such as adenoviruses, the replication of both daughter strands does not necessarily follow the same mechanism.
- Host DNA polymerases may be involved in replicating small- to moderate-sized genome viruses (papillomaviruses, polyomaviruses), whereas larger sized genome viruses usually code for their own polymerases (adenoviruses, herpesviruses, poxviruses).
- Maturation of DNA viruses, with the exception of poxviruses and iridoviruses, occurs in the nucleus.
- Structural proteins are transported from the cytoplasm to the nucleus, where they interact with each other and with the genome and are assembled into capsids that surround the nucleic acid.
- Enveloped viruses complete maturation by budding through the nuclear membrane (iridoviruses) or the plasma membrane.

<u>Double-Stranded DNA Virus Replication</u>

These include the following animal-associated virus families: Asfarviridae, Poxviridae, Iridoviridae, Herpesviridae, Polyomaviridae, Papillomaviridae, and the Adenoviridae (Figure 3.1).

- The genomes range in size from 5 - 8 kb (Polyomaviridae) to over 300 kb (Poxviridae, and Iridoviridae).
- In general, replication takes place in the nucleus by host enzymes (for small viruses such as polyoma and papillomaviruses) or by virus-encoded replicases (adenovirus, herpesvirus). Replication of poxviruses and some iridoviruses takes place in the cytoplasm resulting in the formation of inclusion bodies, which contain necessary enzymes of viral origin associated with replication, such as viral DNA-dependent DNA polymerases.
- The dsDNA may be in the form of circular, linear, circularly permuted, or linear with covalently closed ends.
- The small circular genomes replicate bidirectionally in a manner similar to plasmids. The replication of polyomavirus DNA (closed, circular and double-stranded) is postulated to be mediated by a "swivel mechanism" consisting of endonuclease and ligase. The endonuclease "nicks" one strand, allowing a short region to be replicated. The nick is then repaired by the ligase.

Figure 3-1. General replication scheme of dsDNA viruses. - To view this image in full size go to the IVIS website at www.ivis.org . -

<u>Single-Stranded DNA Virus Replication</u>

Includes the following animal-associated virus families: Circoviridae and Parvoviridae.

- The genomes range in size from 3 kb to 6 kb.
- The single-stranded circular DNA of circoviruses is thought to be replicated by a rolling circle mechanism.
- Replication occurs in the nucleus and involves the generation of a - sense DNA strand to serve as a template for the + sense DNA genome for the progeny virions. This involves the production of a dsDNA intermediate, known as the replicative form.
- Entry of the viral ssDNA into the nucleus stimulates its "repair" by host enzymes into the replicative form. In the case of the circular forms, the replicative form is associated with host histones and other nuclear proteins and thus "treated" as a host chromosome. Linear forms have derived mechanisms that allow the genome to be replicated without a loss of DNA following each replication.
- The ssDNA may be in the forms of linear single-component (parvoviridae), circular single-component (circoviruses).

Figure 3-2. General replication scheme of ssDNA viruses. - To view this image in full size go to the IVIS website at www.ivis.org . -

Double-Stranded DNA Viruses with Reverse Transcriptase

- Includes the Hepadnaviridae.
- Genome arrangement is a partially double-stranded non-covalently closed circular DNA, 3.2 kb in size.
- Following attachment, penetration, and partial uncoating of the virion, the partially dsDNA enters the nucleus and is completed by viral polymerase/ and/or cellular enzymes. Once completed, the backbone is sealed by the action of host ligase.
- In the nucleus, the viral DNA acts like a "mini-chromosome" following its association with host histones, etc. However, host DNA polymerase cannot replicate it.
- In the replication cycle a large mRNA called the pg-RNA (pre-genomic RNA), which is longer than the DNA template from which it was transcribed due to the addition of a poly A tail is produced. It is this RNA intermediate that serves as the template for the virion DNA.
  Smaller mRNAs are also produced, leave the nucleus, and serve as template for translation, giving origin for viral polymerase and capsid protein. Partial assembly of capsids ensues.
- Some of the pgRNA is encapsidated into these recently assembled, immature virions. Within the capsid, a cDNA copy of the pgRNA is made by encapsidated reverse transcriptase (viral polymerase). Following synthesis of the first complementary cDNA strand, the viral polymerase degrades the pgRNA template and begins synthesizing the second DNA strand. The virions are then released from the cells by budding, containing a DNA genome that is only partially double stranded.

Replication of RNA Viruses

- Replication of most RNA viruses occurs strictly within the cytoplasm of cells and is independent of nuclear machinery. Exceptions are orthomyxoviruses (bunyaviruses) that require factors from host DNA transcription and retroviruses that replicate via a DNA intermediate.
- Attachment is an electrostatic interaction between the virions and specific cell receptors.
- Viruses then enter the cell by receptor-mediated endocytosis or by fusing with the cell membrane or with the endocytic vesicle (enveloped viruses).
- Uncoating occurs in the cytoplasm, or during passage (translocation) through the cell membrane, as appears to be the case for picornaviruses. The RNA of reoviruses, however, is never completely uncoated, but remains in viral cores during genome expression and replication.
- The genome of some RNA viruses is a single molecule of RNA (monopartite); in others, the genome is segmented (multipartite).
- The RNA of some animal viruses has mRNA function (+ sense) and can be directly translated, whereas the genome in others is antisense (- sense), and must first be transcribed into + RNAs by a viral-encoded RNA-dependent RNA polymerase (transcriptase).
- Retroviruses have the enzyme reverse transcriptase (RNA-dependent DNA polymerase) permitting the formation of a dsDNA intermediate (provirus DNA), which becomes incorporated into the host genome, and is subsequently transcribed into mRNA by the host DNA-dependent RNA polymerase.
- In general, replication of viral RNA is semiconservative and proceeds via a replicative intermediate (R1). The R1 consists of parental viral RNA that serves as a template for the transcription of several RNA strands, which eventually "peel off" and serve as templates for the synthesis of viral RNA.
- Replication of double-stranded RNA of reoviruses is conservative and asymmetrical; only one strand is replicated, unlike double-stranded DNA. The replication processes requires RNA-dependent RNA polymerases (replicases) that are virus encoded.
- Maturation occurs in the cytoplasm with the viral RNA becoming associated with the capsid proteins forming the nucleocapsid. Enveloped viruses complete maturation by budding through the endoplasmic reticulum, Golgi apparatus, or the plasma membrane.

Double-Stranded RNA Viruses

- Includes the following animal-associated virus families: Reoviridae and Birnaviridae.
- The genomes of these viruses range in size from 4 kb to 20 - 27 kb in length.
- Attachment is via receptor-mediated endocytosis. The virion is partially uncoated and the core particle remains in the endocytic vesicle.

- Replication is by a conservative mechanism, the dsRNA serves as a template for the production of mRNA by a viral RNA-dependent RNA polymerase. Much of the remainder of the replication mechanism is poorly understood at this time.
- Replication does not involve the formation of R1 intermediates. No free dsRNA is formed in the cytoplasm of the host cell.
- All have segmented, linear genomes. Each segment corresponds to a monocistronic mRNA.
- All of the genomes are linear, but may be two-component (Birnaviridae), or multi-component (reoviruses have 10 - 12 components).

Figure 3-3. General replication scheme of dsRNA viruses. - To view this image in full size go to the IVIS website at www.ivis.org . -

Single-Stranded Positive Sense RNA Viruses

- Includes the following animal-associated virus families: Caliciviridae, Picornaviridae, Astroviridae, HEV-like viruses, Nodaviridae, Flaviviruses, Coronaviridae, Togaviridae, and Arteriviridae.
- Genome sizes range from less than 5 kb to 20 - 30 kb.
- Attachment is via receptor-mediated endocytosis. There, the virion is uncoated and the ssRNA released to the cytoplasm.
  The viral genomes that are messenger-sense are totally or partially translated into proteins as the first step of virus replication.
- Picornaviruses and Flaviviruses possess a positive sense RNA genome as genome, which behaves as a polycistronic mRNA. The genome is directly translated into one large polypeptide, which is co-translationally cleaved into a number of proteins by viral encoded or host cell proteases.
- Coronaviruses have a complex transcription pattern, involving several rounds of translation in order to complete the replication cycle.
- Linear forms of the following are possible: single-component with single open reading frames (ORFs) (picornaviruses), single-component with multiple ORFs (togaviruses and caliciviruses), and two-component with single ORF (nodaviruses).

Figure 3-4. Replication scheme of positive-sense ssRNA viruses. - To view this image in full size go to the IVIS website at www.ivis.org . -

Single-Stranded Negative Sense RNA Viruses

- Includes the following animal-associated virus families: Orthomyxoviridae, Rhabdoviridae, Paramyxoviridae, Bornaviridae, Filoviridae, Deltavirus, Arenaviridae, and Bunyaviridae.
- Genomes range from 10 - 14 kb to 11 - 20 kb in size.
  As the genomes are negative-sense, they are not translated. Therefore, these viruses need to bring their replicases in the virion to proceed with transcription and replication of the genome.
- Orthomyxoviruses have segmented genomes. The first step in the replication process is the transcription of - sense RNA by a virus encoded RNA-dependent-RNA polymerase.
- Rhabdoviruses have nonsegmented genomes. Replication still requires the transcription via a viral RNA-dependent RNA polymerase.
- In the case of the ambisense viruses, the transcriptase is encoded within the positive-sense portion that will eventually mediate the transcription of the negative-sense regions.
- The following linear genome arrangements include single-component with multiple ORFs (filoviruses, paramyxoviruses, and rhabidoviruses), two-component ambisense (arenaviruses), three-component negative sense or ambisense (bunyaviruses), and six- to eight-component (orthomyxoviruses).

Figure 3-5. Replication scheme of negative-sense ssRNA viruses. - To view this image in full size go to the IVIS website at www.ivis.org . -

Single-Stranded Positive Sense RNA Viruses with Reverse Transcriptase

- Includes the vertebrate-associated viruses in the family Retroviridae.
- This viral genome arrangement is comprised of diploid linear ssRNA held together by protein. It is 5'-capped and has a 3' poly A tail, and has four characteristic coding regions (*gag-pro-pol-env*). These regions are: *gag* (group specific antigen: matrix protein, nucleoprotein, capsid) genes; *pro* (protease) gene; *pol* (reverse transcriptase and RNase-H); and *env* (envelope, receptor binding) genes.
- The conversion of RNA to ssDNA and then to dsDNA is mediated by the viral enzyme reverse transcriptase. The resulting dsDNA, called provirus DNA, is ultimately integrated into the host chromosome by the viral enzyme integrase.
- Once integrated into the host genome, the viral dsDNA (or provirus) remains latent until "triggered" into active virion production. The provirus is then transcribed into mRNA by cellular RNA polymerase II.

Viral Genetics

Natural selection acting on viral genomes over the years has resulted in great genetic diversity for some viruses. Viral genomes are the key to understanding how viruses interact with the host cells they infect. The rapidly growing knowledge of viral genetics has led to many important applications and new fruitful techniques. Some of the important areas of interest are discussed below.

Mutation

A mutation is a change in the nucleic acid sequence of an organism. The organism possessing a mutation is referred to a mutant. This change is based on comparison with the wild type (reference) virus. From this information, strains (wild types of the same virus), types (serological or biological), and variants (phenotypically different from wild type where genetic reason is unknown) can be identified.

Mutations are neutral events that can be acted upon by natural selection. If the mutation enhances the survival (transmission and replication) of the organism, it has a selective advantage. If the mutation is detrimental to the growth and survival, the organism is eventually eliminated from the population. If the mutation does not alter the survivability or its phenotype, then the mutation may not be easily detected. Mutations occur by two different mechanisms, spontaneous mutation and induced mutation.

- Spontaneous mutations are endogenous, being the result of DNA or RNA polymerase errors or the result of incorporation of naturally occurring tautomeric forms of nucleotides.
  DNA viruses are typically more genetically stable than RNA viruses; the spontaneous mutation rate is 10^{-8} to 10^{-11} per incorporated nucleotide. This is due to the fact that DNA polymerases often have some error correction ability.
  RNA viruses are considerably less genetically stable, with a spontaneous mutation rate of 10^{-3} to 10^{-4} per incorporated nucleotide. RNA polymerases typically lack error correction ability. In spite of this, some RNA viruses (e.g., poliovirus) are relatively genetically stable. It is thought that these viruses have the same high mutation rate as other RNA viruses, but are so precisely adapted for transmission and replication that minor changes result in their elimination.
- Induced mutations are exogenous, the result of exposure to mutagens (either chemical or radiation) that significantly increase the mutation rate for a given organism. Chemical mutagens act either directly on the bases or indirectly by enhancing mispairing. Ultra violet radiation can induce the formation of pyrimidine dimers, ionizing radiation can damage DNA directly by breaking chemical bonds or indirectly by forming free radicals that in turn damage DNA.

There are a variety of phenotypes that are generated as the result of mutation. Some of the more common ones include:

Host Range Mutation

Mutation allows for a change in the host range of a particular virus from the original one associated with the wild type virus. This type of change is believed to have occurred with feline parvovirus, which extended its host range and became capable of infecting dogs.

Conditional Lethal Mutations

This includes a series of mutations that replicate under a specific range of conditions, beyond this range wild type viruses are capable of replication but the conditional is not. Examples of conditional lethal mutants include temperature-sensitive mutants and cold-sensitive mutants. Temperature-sensitive mutants have been used in vaccine development and cold-sensitive mutants have been used in the analysis of viral replication cycles.

Plaque Size Mutation

As a result of mutation, these viruses produce plaques that deviate from those of the wild type. This information sometimes

correlates with the infectivity of a particular virus strain.

Nonsense (amber) Mutations
Refers to point mutations that result in the formation of a translational stop codon at a position where an amino acid is incorporated in the wild type protein. As a result, the protein is truncated and frequently nonfunctional. The most common nonsense mutation is to the UAG codon, called amber.

Deletion Mutations
These are the result of a loss of nucleotides at some point in the genome, varying from a single nucleotide to whole sections of the genome. These can either occur in nature or be voluntarily produced in the lab and used in the development of viral vectors or to attenuate a virus for vaccine development.

Antigenic Shift and Drift
Antigenic shift refers to the change an antigen associated with a viral pathogen due to the acquisition of a novel entire gene or a change in an existing one. Typically antigenic shift is observed readily with those viruses that possess multipartite genomes, such orthomyxoviruses, arenaviruses and bunyaviruses. Coinfection of different strains in the same cell may result in packaging mixed genomes, containing some segments from one virus and other from the other.
Antigenic drift is a result of accumulation of point mutations (single bases substitutions) has been identified as the mechanism associated with the antigenic variation observed with influenza viruses and may be the mechanism associated with the variability observed with rhinoviruses.

Interactions Between Two Viruses

Viral infections with two or more different viruses are known to occur in nature as well as in culture. These are referred to as mixed infections and can result in new viral combinations, and thus new variants of the virus. The following are some of the interactions that can occur during mixed infections.

Complementation
Complementation can occur during a mixed infection when one of the two viruses is deficient in a particular gene product. Without this protein, the virus is incapable of transmission and replication and is therefore a defective particle. In a mixed infection, if the second virus involved does make the product (thus complementing of the defect), the defective particle is capable of completing the transmission and replication processes. In nature, complementation occurs with the human Hepatitis D virusoid. The virusoid is defective in a surface antigen that is provided by Hepatitis B in mixed infections, allowing the replication of the Hepatitis D life cycle to be completed.

Recombination
Genetic recombination is the exchange of genetic material between two viral chromosomes in regions where a high level of genetic homology exists. As a result, the progeny are genetically distinct from the two "parental" viruses. Recombination is common in DNA viruses and those RNA viruses having a DNA phase (e.g., retroviruses). Currently, three mechanisms of recombination have been identified:

Intramolecular Recombination
Recombination that is mediated by cellular enzymes between two regions on a single dsDNA molecule, resulting in a looping-out of the intermediate region, yielding a shorter dsDNA molecule and a separate circular dsDNA molecule. The reverse of this reaction can also occur, resulting in the integration of a circular dsDNA molecule into another dsDNA molecule. This type of recombination is typically associated with monopartite DNA viruses.

Copy-choice Recombination
A genetic recombination in which the new nucleic acid molecule comes about by replicating selected parts of each parental molecule and by alternating between the two (maternal and paternal). This mechanism is poorly understood and occurs in monopartite RNA viruses.

Reassortment
This occurs in mixed infections with variant viruses having segmented genomes infecting a single cell. The progeny virions can contain some segments from one parent, some from the other. This is an efficient process observed with orthomyxoviruses, reoviruses, arenaviruses, and bunyaviruses. The mechanism is not well understood. Reassortment has been implicated in the appearance of new, highly virulent influenza virus strains throughout the 20th century.

Genetic Reactivation
Genetic reactivation is a special case recombination/reassortment that occurs in mixed infections when one or both of the viruses is noninfectious. The progeny, resulting from either recombination or reassortment, are now infectious and carry markers of both parents. If only one parent was noninfectious, the process is called cross-reactivation or marker rescue. If

both parents were noninfectious, the process is called multiplicity reactivation.

Phenotypic Mixing
Phenotypic mixing is an example of non-genetic interactions between two viruses. As a result of mixed infection, the individual progeny possess structural proteins (envelope, capsid) from either or both parents. The genome of either parent virus can be encapsidated within any of the three types of capsids (envelopes), yielding six different types of progeny. Therefore, the genotype and phenotype of many of the progeny virions do not match.

Virus Applications: Gene Therapy and Recombinant Vaccines

Perhaps two of the most intriguing applications utilizing the knowledge of viral replication and genetics are gene therapy and development of recombinant vaccines. These techniques hold great promise for the development of novel ways to treat genetic diseases or afford protection against diseases in humans and animals.

Gene Therapy
Gene therapy is based on the premise of using viruses with no pathogenic properties, but retains their ability to selectively interact with and transmit their genes (plus any genetically-engineered genes) into specific host cells and tissues. Retroviruses are an excellent means for gene delivery into target host cells. The dsDNA of their genome is stable and readily integrated into the host genome. The viruses are engineered in such a manner that once the genome has been integrated into the genome they cannot replicate. Often this means using helper viruses to aid in the initial uptake of the engineered virions by the host cells by complementation. The limitation of this method of gene therapy is that in some cases the gene in question needs to be present in all cells of the host and not just a select group of cells or tissues. Retroviruses have been used in gene therapy for incorporation of the adenine deaminase (ADA) gene into the immune cells of patients with an ADA immunodeficiency. In addition to retroviruses, some other viruses currently being studied as potential vectors for gene therapy include the adenoviruses, adeno-associated viruses (parvoviruses) and herpesviruses.

Recombinant Vaccines
The three kinds of vaccines prepared with recombinant nucleic acid techniques are discussed in Chapter 6. Several of these recombinant vaccines are in current use to prevent animal and human viral diseases.

Glossary
Ambisense: Refers to an RNA genome containing sequence information that is both-positive sense (can be used directly as mRNA) and negative-sense (must be transcribed to form mRNA).
Conservative Replication: Replication of dsDNA or dsRNA in such a manner that the original strands do not become a part of the newly formed progeny dsDNA or dsRNA.
Endocytic Vesicle: A vesicle formed in the process of endocytosis, the "engulfment" of the virus, which can be mediated by surface receptors or cell membrane interactions.
Golgi Membrane: A membrane associated with the Golgi apparatus of a eukaryotic cell. The Golgi apparatus receives newly synthesized lipids and proteins from the endoplasmic reticulum and chemically modifies and traffics them to the appropriate locations in the cell.
Inclusion Bodies: These represent virus "factories" in which viral nucleic acid or protein is being synthesized.
Ligase: A host enzyme that creates covalent bonds in nucleic acids associated with breaks in the sugar-phosphate backbone of the molecule.
Monocistronic: Contains information for a single gene or gene product.
Monopartite: A viral genome having a single segment.
Multi-component Genomes: Genomes having more than one nucleic acid molecule making up its total genome.
Mutagens: Chemical or physical agents that increase the mutation rate of the DNA of an organism.
Negative-sense DNA: DNA whose transcription does not produce an RNA molecule that can be used directly as mRNA. It is the template for creation of negative-sense RNA genomes.
Polycistronic: Contains information for several genes or gene products.
Positive-sense DNA: DNA whose transcription produces the genome of positive-sense RNA genomes or can be used directly as mRNA.
Reverse Transcriptase: A viral enzyme that uses a RNA template to synthesize DNA.
Semiconservative Replication: Replication of dsDNA or dsRNA in such a manner that the original strands (one original, one newly synthesized) become a part of the newly formed progeny dsDNA or dsRNA.
Single-component Genomes: Genomes having a single nucleic acid molecule making up its total genome.
Tautomers: These are isomeric forms of organic compounds and when two of them exist in equilibrium it is referred to as tautomerism.
Transcriptase: A viral enzyme capable of using an RNA molecule as a template for transcription.
Wild Type: The natural virus; such viruses are used as reference strains for the comparisons of mutants and variants of a particular virus.