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## Chapter 10

### Introduction to RNA Viruses

| Outline | Page |
|---------|------|
| Definition and Basic Properties of RNA Viruses | 97 |
| Subgroups of RNA Viruses | 98 |
| **Positive Strand RNA Viruses** | 98 |
| **Negative Sense and Ambisense RNA Viruses** | 98 |
| dsRNA Viruses | 100 |
| Structural Features of RNA Virus Genomes | 100 |
| Priming Viral RNA Synthesis | 102 |
| Mechanisms to Generate Capped mRNAs | 102 |
| Mechanisms to Generate Polyadenylated mRNAs | 103 |
| Mechanisms to Regulate Synthesis of Genomes and Transcripts | 103 |
| RNA Viruses and Quasispecies | 103 |
| References | 105 |

After studying this chapter, you should be able to:

- Define “positive-strand RNA virus.”
- Describe “negative-strand RNA virus” and “ambisense RNA virus.”
- Understand the activities of RNA-dependent RNA polymerase (RdRp).
- Describe three general methods for priming RNA synthesis.
- Describe some functions of the noncoding regions (NCRs) of an RNA virus genome.

### Definition and Basic Properties of RNA Viruses

RNA viruses replicate their genomes using virally encoded RNA-dependent RNA polymerase (RdRp). The RNA genome is the template for synthesis of additional RNA strands (a molecule of RNA is the template and molecules of RNA are produced). During replication of RNA viruses, there are at least three types of RNA that must be synthesized: the genome, a copy of the genome (copy genome), and mRNAs. Some RNA viruses also synthesize copies of subgenomic mRNAs. RdRp is the key player for all of these processes (Fig. 10.1).

RdRps of RNA viruses probably arose from a common ancestor. The RdRp, in association with other proteins required for viral genome synthesis is often called the replicase complex. The replicase complex consists of the set of proteins required to produce infectious genomes. In addition to the RdRp, the replicase complex may contain RNA-helicases (to unwind base-paired regions of the RNA genome) and ATPases (to supply energy for the polymerization process). The number of proteins in the replicase complex differs among virus families. There may also be a requirement for host cell proteins.

The biochemical requirements for genome synthesis may or may not be identical to those required for synthesis of mRNAs. If the two processes differ, the term transcription complex is sometimes used to describe the particular set of proteins required for viral mRNA synthesis. Some RdRps have methylase activities that synthesize mRNA “caps” and RdRp may “stutter” at poly U tracts to generate polyadenylated mRNAs.
SUBGROUPS OF RNA VIRUSES

Positive Strand RNA Viruses

RNA viruses can be subdivided into groups based on type of RNA that serves as the genome. Positive or plus (+)-strand RNA viruses have genomes that are functional mRNAs (Table 10.1). Upon penetration into the host cell, ribosomes assemble on the genome to synthesize viral proteins. Genomes of positive-strand RNA viruses are single-stranded molecules of RNA and may be capped and polyadenylated. During the replication cycle of positive-strand RNA viruses, among the first proteins to be synthesized are those needed to synthesize additional genomes and mRNAs. Thus the infecting genome has two functions: It is an mRNA and also serves as the template for synthesis of additional viral RNAs. A functional definition of a positive-strand virus is that purified or chemically synthesized genomes are infectious (Fig. 10.2 and Box 10.1).

Positive-strand RNA viruses often use large complexes of cellular membranes for genome replication. They actively modify host cell membranes to construct viral replication scaffolds. Positive-strand RNA viruses of animals also use a common strategy to express RdRp. RdRp is a nonstructural protein, meaning that it is not found within the assembled virion. Instead it is translated directly from the infecting genome shortly after penetration. RdRp and other viral proteins needed for viral RNA synthesis are encoded as a polyprotein that is cleaved by virally encoded proteases. In the case of the picornaviruses and the flaviviruses, all viral proteins (structural and nonstructural) are synthesized as part of a single long polyprotein. Other positive-strand RNA viruses (i.e., togaviruses, coronaviruses, arteriviruses) synthesize an RdRp-containing polyprotein from genome-length mRNA, but use subgenomic mRNAs to encode structural and other proteins.

Negative Sense and Ambisense RNA Viruses

There are three additional groups of RNA viruses whose genomes are not mRNAs. They are the negative or minus (−)-strand RNA viruses, the closely related ambisense RNA viruses, and double-stranded RNA viruses (Table 10.2 and Box 10.2). For each of these groups of viruses, the first synthetic event after genome penetration is transcription (Fig. 10.3). This is accomplished by viral proteins (including viral RdRp) that enter cell with the genome. Transcription/replication complexes usually contain between two and four proteins. They associate with the genome through interactions with RNA-binding nucleocapsid (N) or capsid proteins. Therefore, naked (purified away from protein) genomic RNA is not infectious, cannot be translated, and will eventually be degraded if transcription is blocked. Before genome replication can proceed, viral mRNAs must be transcribed and translated. Because the virion of a negative-strand

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TABLE 10.1 Positive-Strand RNA Viruses of Animalsa

| Virus family     | Virion morphology         | Genome type   |
|------------------|---------------------------|---------------|
| Arteriviridae    | Enveloped, helical nucleocapsid | Unsegmented  |
| Astroviridae     | Nonenveloped, icosahedral  | Unsegmented   |
| Caliciviridae    | Nonenveloped, icosahedral  | Unsegmented   |
| Coronavirusidae  | Enveloped, helical nucleocapsid | Unsegmented  |
| Flaviviridae     | Enveloped, icosahedral nucleocapsid | Unsegmented  |
| Hepaviridae      | Nonenveloped, icosahedral  | Unsegmented   |
| Nodaviridae      | Nonenveloped, icosahedral  | Bisegmented   |
| Picornaviridae   | Nonenveloped, icosahedral  | Unsegmented   |
| Togaviridae      | Enveloped, icosahedral nucleocapsid | Unsegmented  |

*aOnly those families with members that infect vertebrates are included in this list.
RNA virus contains RdRp, it is possible to synthesize viral mRNAs in a test tube (Fig. 10.4). If purified virions are gently lysed under appropriate buffer conditions, with the addition of NTPs, mRNAs will be transcribed in the test tube. However, genome RNA will not be synthesized under these conditions (Table 10.1). Finally, the genomes of negative-strand RNA viruses are often shown in diagrams with the 3´ end to the left, opposite to the usual convention of illustrating a strand of nucleic acid.

### Box 10.1
**Positive-strand RNA viruses**

- Purified genomes (or chemically synthesized genomes) are infectious if introduced into a permissive cell.
- The genome serves as an mRNA.
- The first synthetic event in the replication cycle is protein synthesis.
- Genome replication is cytoplasmic.

- Genomes of positive-strand RNA viruses fold into complex structures. These RNA structural elements have key roles in genome replication, transcription, and translation.
- Families include: Picornaviridae, Flaviviridae, Togaviridae, Hepeviridae, Coronaviridae, Arteriviridae, Toroviridae, among many others.

Negative-strand RNA viruses use the genome sense strand as the template for synthesis of all mRNAs. In contrast, viruses that use an ambisense coding strategy transcribe some mRNAs from the copy genome. There are virus families in which some members are considered negative-strand RNA viruses while others use an ambisense strategy. Thus these two strategies are closely related. Some ambisense viruses *package copy genomes* that can be used as templates for transcription, such that the full complement of viral genes...
can be transcribed soon after infection. It should be noted that packaged copy genomes are not RNAs and are not translated.

**dsRNA Viruses**

Family *Reoviridae* is a large family of dsRNA viruses (Table 10.3). Reoviruses also have segmented genomes, packaging 11–12 segments of dsRNA. Reoviruses are nonenveloped and particles consist of two or three concentric icosahedral capsid layers. The genome segments are found within the innermost, $T = 1$ icosahedral shell. A unique feature of the reovirus replication cycle is that the genome segments are transcribed from within the capsid. The mRNA products leave the capsid through pores at the vertices of the capsid (see Chapter 26: Family *Reoviridae*).

**TABLE 10.2** Negative-Strand RNA Viruses of Animals

| Virus family         | Virion morphology | Genome type                                |
|----------------------|-------------------|--------------------------------------------|
| Arenaviridae         | Enveloped, helical nucleocapsid | Bisegmented (some use ambisense coding strategy) |
| Bornaviridae         | Enveloped, helical nucleocapsid | Unsegmented                                |
| Bunyaviridae         | Enveloped, helical nucleocapsid | Trisegmented (some use ambisense coding strategy) |
| Filoviridae          | Enveloped, helical nucleocapsid | Unsegmented                                |
| Nymaviridae          | Enveloped, helical nucleocapsid | Unsegmented                                |
| Orthomyxoviridae     | Enveloped, helical nucleocapsid | Segmented                                  |
| Paramyxoviridae      | Enveloped, helical nucleocapsid | Unsegmented                                |
| Pneumoviridae        | Enveloped, helical nucleocapsid | Unsegmented                                |
| Rhabdoviridae        | Enveloped, helical nucleocapsid | Unsegmented                                |

*Only those families with members that infect vertebrates are included in this list.

**STRUCTURAL FEATURES OF RNA VIRUS GENOMES**

The genomes of RNA viruses have some common general features. Obviously there are one or more open reading frames that encode the viral proteins. But there are also regions of RNA that do not code for protein. These non-coding regions (NCRs) or untranslated regions (UTRs) are often highly conserved within a virus family, indicating that they have important functions. The preferred usage can vary by virus family but the terms NCR and UTR are largely interchangeable. NCRs may have specific, critical nucleotide sequences but in some cases they are regions of the genome that fold into conserved structures, and structure may be more critical than a specific sequence.

All RNA genomes have NCRs at their 5' and 3' ends. NCRs vary in size, from very long (several hundred nt) in the case of picornaviral 5' NCRs to just a short base-paired hairpin in the case of flavivirus 3' NCRs. Complementary RNA sequences are sometimes found at the 5' and 3' ends of RNA genomes, allowing them to circularize.

The 5' and 3' NCRs are required, and are often sufficient, to direct genome replication. Virally encoded proteins, such as the RdRp recognize specific sequences and/or structures at the ends of the genome. To simplify studies of the genome replication process, researchers often generate “minigenomes” that lack some virally encoded proteins. Of course a source of RdRp must be supplied. RdRp may be encoded in the minigenome or may be supplied *in trans* (by using a

**BOX 10.2**

**NEGATIVE AND AMBISENSE-STRAND RNA VIRUSES**

- Purified genomes (or chemically synthesized genomes) are NOT infectious.
- RdRp is packaged within the virion.
- Genomes serve as templates for transcription and the first synthetic event in the replication cycle is mRNA synthesis.
- Many replicate in the cytoplasm, a few replicate in the nucleus.
- Viral genomes are often tightly associated with a nucleocapsid (N) protein.
- Families of negative-strand RNA viruses include Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Bornaviridae, and Filoviridae. The Bunyaviridae includes some members that use a negative sense coding strategy and other that use an ambisense coding strategy.
The genomes of viruses in the order Mononegavirales are unsegmented, negative-strand RNA. If RNA is extracted from purified virions and analyzed by gel electrophoresis, a single RNA is seen (top panel). However, virions contain RdRp and if they are gently lysed, in a buffer solution containing NTPs, mRNAs are synthesized. The lower panel shows the products of mRNA synthesis as a set of subgenomic mRNAs of different sizes. Note that genome synthesis does not occur under these conditions.
cell line stably expressing the viral RdRp, for example). The sequences required to direct RNA replication are often fairly simple and can be linked to virtually any RNA sequence to drive its replication.

Many RNA genomes also have promoters to direct synthesis of subgenomic mRNAs. These promoter sequences can be rather short but provide a means to direct the RdRp to internal sites on the genome. There may also be specific RNA sequences that signal polyadenylation. There are a variety of different strategies that RNA viruses use to regulate transcription and genome replication, but all involve RNA sequences found in the genome.

The RNA genomes of some viruses are highly structured and extensively base paired. An example is the internal ribosome entry sites (IRES) of picornaviruses (Chapter 11: Family Picornaviridae). The IRES serves as a platform for ribosome assembly. Picornaviruses are among the RNA viruses that encode RNA-helicases to unwind highly structured regions of the genome, such as the IRES. Without the help of a helicase, the RdRp would not be able to disrupt the base-paired RNA in the IRES and genome replication would stall.

**PRIMING VIRAL RNA SYNTHESIS**

While all RNA viruses use an RdRp for replication and transcription, there are a variety of strategies used for priming RNA synthesis, regulating RNA synthesis, and capping and polyadenylating mRNAs.

In the eucaryotic cell, RNA synthesis (from a DNA template) is primer independent. DNA sequences (promoters) direct RNA polymerases to the “correct” position on the DNA genome. Promoters can be quite long and complex and promoter regions themselves are not transcribed. What mechanisms do viral RdRps use to prime viral RNA synthesis? It is particularly important, in the case of genome synthesis, that genetic information not be lost or modified; however, mRNAs are often capped and polyadenylated. Are the methods for priming viral mRNA synthesis the same or different from the methods of priming genome replication? The RNA viruses seem to have experimented widely. Various mechanisms by which RdRps initiate RNA synthesis are described below.

- De novo or primer-independent transcription and replication employs the RdRp, the RNA template, an initiation NTP, and a second NTP. The initial NTP can be considered the primer. The initial complex is not highly stable.
- De novo initiation is sometimes used to generate short RNA oligonucleotides that are subsequently used as primers in a mechanism known as prime and realign. The short internally initiated oligos slip back to extend from 5’ end of the template. Bunyaviruses and arenaviruses use this process. As a result the 5’ ends of the genome and copy genome contain nontemplated nucleotides.
- RNA structures such as hairpins can be used for priming in a process where the 3’ end of the template RNA loops back upon itself to serve as the primer. This process is “template priming” as part of the template (the genome) functions as the primer.
- Some RNA viruses (i.e., picornaviruses) use a protein to prime RNA replication. A tyrosine hydroxyl group on the primer protein (VPg) is linked to GTP to serve as the first nucleotide in the new strand. The protein interactions between VPg, RdRp, and the RNA template form a stable initiation complex (Chapter 11: Family Picornaviridae).
- Some RNA viruses (for example, influenza viruses) “steal” short (10–15 nt) capped oligonucleotides from cellular mRNAs to prime viral mRNA synthesis (a process called cap-snatching) (Chapter 23: Family Orthomyxoviridae). This priming process cannot be used for genome synthesis and indeed, the genome segments of influenza viruses are primed by a de novo process.

**MECHANISMS TO GENERATE CAPPED mRNAs**

RNA viruses use different mechanisms to cap their mRNAs. In the case of the influenza viruses the fragment of cellular mRNA used for priming viral mRNA synthesis also provides the methyl-G cap (Chapter 23: Family Orthomyxoviridae). The RdRp proteins of many RNA viruses have methylase activities, thus the RdRp also synthesizes the cap. Some RNA viruses (for example, piconaviruses) do not use capped mRNAs.

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**TABLE 10.3 Double-Stranded RNA Viruses of Animals**

| Virus family   | Virion morphology                                      | Genome type          |
|---------------|--------------------------------------------------------|----------------------|
| Birnaviridae  | Nonenveloped, icosahedral                               | Bisegmented          |
| Picobirnaviridae | Nonenveloped, icosahedral                               | Bisegmented          |
| Reoviridae    | Nonenveloped, icosahedral with multilayered capsids    | Segmented (10–12)    |

*Only those families with members that infect vertebrates are included in this list.*

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VIRUSES
RNA viruses also use a few different mechanisms to polyadenylate their mRNAs. For example, the picornaviruses use poly A tracts encoded in the genome. Among the negative-strand RNA viruses, those in the order Mononegavirales use a stuttering mechanism to synthesize long poly A tracts from short poly U tracts (Fig. 10.5). Other RNA viruses do not polyadenylated their mRNAs. The flaviviruses have a short hairpin at the 3′ end of their genome-length mRNA.

**Mechanisms to Generate Polyadenylated mRNAs**

Even with fairly simple genomes, RNA viruses must, and do, regulate the amounts of genome, copy genome, and mRNAs that are synthesized during an infection. It would be “wasteful” if a positive-strand RNA virus had to make a new copy genome for synthesis of every genome. It is much more efficient to synthesize many genomes from each copy genome. Having different structures at the 5′ and 3′ ends of the genome and copy RNA would be one way to accomplish this. We will see in a later chapter that togaviruses (Chapter 16: Family Togaviridae) use slightly different versions of the replicase complex to synthesize genomes versus copy genomes and that the replicase proteins are “regulated” by proteolytic cleavage. Internal promoters for mRNA synthesis can vary in sequence, controlling the relative affinity of the transcription complex for each mRNA.

**Mechanisms to Regulate Synthesis of Genomes and Transcripts**

A large group of unsegmented negative-strand RNA viruses (Order Mononegavirales) synthesize mRNAs sequentially, from the 3′ end to the 5′ end of the infecting genome (Chapters 19–22: Family Rhabdoviridae, Families Paramyxoviridae and Pneumoviridae, Family Filoviridae, and Family Bornaviridae) (Fig. 10.5). Those genes closest to the 3′ end of the genome are more abundantly expressed, as the transcription complex always initiates synthesis at the 3′ end of the genome.

**RNA Viruses and Quasispecies**

An important feature of RNA viruses is that many exist in nature as quasispecies. The term quasispecies is
used to describe a group of closely related, but nonidentical genomes (Fig. 10.6). (Quasispecies are not unique to RNA viruses, some retroviruses, HIV for example, exist as quasispecies).

Poliovirus (PV) is a good example of a virus that forms a quasispecies. If one examines genome sequences from a mouse experimentally infected with PV serotype 1, we find that the genomes are not...
identical, although they are all clearly related to one another. So, which one of these genomes is the “best” or the “fittest”? To the surprise of many virologists, it turns out that the population (quasispecies) may be more fit than any individual genome. Or put another way, we cannot find any single genome in the population that replicates better than the group as a whole and in fact, most individual genomes replicate more poorly than the group. Why this occurs is not always clear, but an animal is a very complex ecosystem. Different members of the quasispecies may be better adapted to different niches in the animal.

How does a quasispecies form? PV genomes can be chemically synthesized and/or cloned to generate a single genome sequence. But as the cloned virus replicates, mutations accumulate (generating a quasispecies). Measurable levels of mutation occur because the fidelity of PV RdRp is low. RdRps do not have proof-reading activities (as do many DNA polymerases). If a mistake occurs, there are only two possibilities: RNA synthesis can stop, or RNA synthesis can continue beyond the mistake to generate a point mutation. The fidelity of RdRps has been studied and they generate transition mutations at a frequency of \(10^{-5} \text{ to } 10^{-7}\) (one transition mutation for every \(10^3 \text{ to } 10^5\) nt synthesized) and transversion mutations at a frequency of \(10^{-6} \text{ to } 10^{-7}\) (one transversion mutation for every \(10^6 \text{ to } 10^7\) nt synthesized). A rate of one mutation per \(10^5\) nucleotides synthesized ensures that during an infection, many progeny will contain a mutation. The significance of the quasispecies model for RNA viruses is that there is no single “fittest” or “best” genome sequence. The population is fitter than the individual (Box 10.3).

In this chapter we have learned:

- The definition of an RNA virus.
- The subgroups within the RNA virus group (positive strand, negative strand, ambisense, and double strand) and the differences in their replication strategies.
- The types of noncoding regions commonly found in the genomes of RNA viruses.
- The different mechanisms used to prime RNA synthesis.
- That some RNA viruses exist as quasispecies, mixed populations that are more fit than any individual member.

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