

Family *Coronaviridae*

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After reading this chapter, you should be able to answer the following questions:

- What are the major structural and replicative features of coronaviruses?
- Which coronavirus protein is responsible for attachment and fusion?
- By what mechanism are coronavirus mRNAs synthesized?
- Why do coronaviruses undergo high rates of RNA recombination?
- What human diseases are caused by coronaviruses?
- How does the virus that causes feline infectious peritonitis (FIP) virus differ from the virus that causes feline enteritis?

Members of the family *Coronaviridae* are large, enveloped, single-stranded RNA viruses. They are the largest known RNA viruses, with genomes ranging from 25 to 32 kb and a virion of 118–136 nm in diameter. Virions are roughly spherical and are notable for the large spike (S) glycoprotein that extends 16–21 nm from the virus envelope (Fig. 17.1 and Box 17.1). The family contains two subfamilies, the *Coronavirinae* and the *Torovirinae* (Box 17.2). The toroviruses are notable for having a helical, doughnut-shaped nucleocapsid (Fig. 17.2).

Members of the subfamily *Coronavirinae* are widespread among mammals, often causing only mild respiratory or enteric infections. Over 60 coronaviruses (CoVs) have been isolated from bats (BtCoV) and most of these are in the genus betacoronavirus. Bats serve as large (and highly mobile) CoV reservoirs; many bat species have their own unique BtCoV, suggesting a very long history of coevolution. Until 2002 CoVs were considered only minor pathogens of humans. However an outbreak of severe acute respiratory syndrome (SARS) that began in 2002 was linked to infection with a new CoV (SARS-CoV). The outbreak increased interest in CoV replication, distribution, evolution, transmission, and pathogenesis. In 2014 another coronavirus (distinct from SARS-CoV) was isolated in connection with an outbreak of severe respiratory disease in the Middle East. This virus, called Middle East respiratory syndrome coronavirus (MERS-CoV), continues to cause sporadic cases of severe respiratory illness.

Several important animal diseases are caused by CoVs. Infectious bronchitis virus (IBV) of chickens was the first CoV identified (in the 1930s). A pig coronavirus caused the deaths of millions of piglets in the United States in 2014. Later in this chapter, we consider the unique pathogenesis of the feline CoV

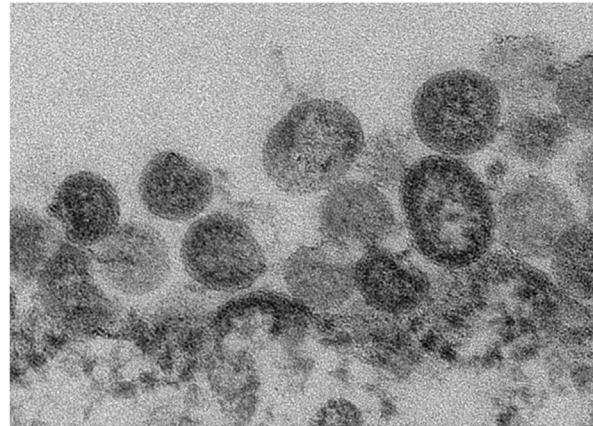
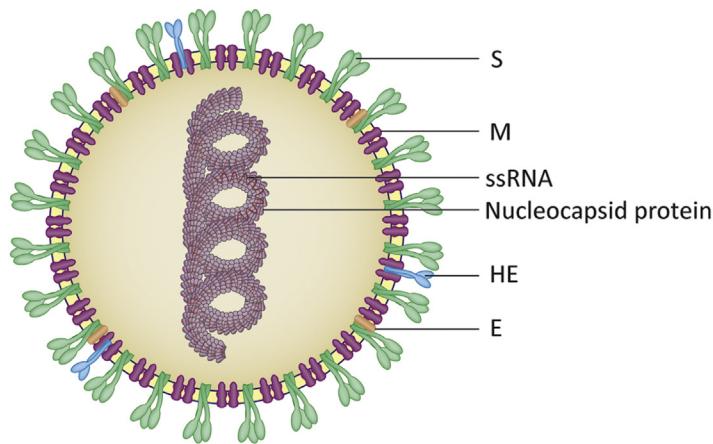


FIGURE 17.1 Virion structure (subfamily *Coronavirinae*) (right). TEM image of MERS-CoV virions in culture (left). From CDC/Cynthia Goldsmith, Azaibi Tamin, Ph.D. Public Health Image Library Image #17280.

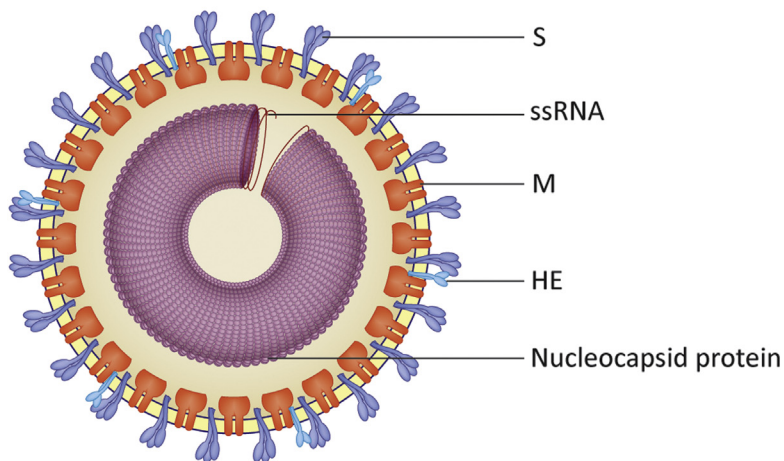


FIGURE 17.2 Torovirus structure (left) and transmission electron micrograph of torovirus particles (right). From Graham Beards.

BOX 17.1

GENERAL CHARACTERISTICS

Genomes are monopartite, single-strand RNA, positive sense, capped, and polyadenylated, 25–32 kb.

Virions are enveloped with prominent spikes. Virion size is 118–140 nm. Within the envelope is a flexible (subfamily *Coronavirinae*) or a doughnut-shaped (subfamily *Torovirinae*) nucleocapsid.

Transcription and genome replication are cytoplasmic. Genome-length RNA serves as mRNA for a long polyprotein precursor [encoding several non-structural proteins (nsps) including RdRp]. A set of 3' coterminal mRNAs encode the structural proteins and some nsps. Transcription is discontinuous, leading to high rates of template switching.

(FeCoV) causative agent of FIP, a deadly disease of domestic cats. A rodent coronavirus, mouse hepatitis virus (MHV), has served for many years as a useful model system for investigating CoV replication and pathogenesis.

CORONAVIRUS GENOME ORGANIZATION

The 25–32 kb positive-strand RNA genome contains 7–10 open reading frames (ORFs). Almost two-thirds

BOX 17.2

TAXONOMY

Family *Coronaviridae*
 Subfamily *Coronavirinae*
 Genus *Alphacoronavirus*
 Genus *Betacoronavirus*
 Genus *Deltacoronavirus*
 Genus *Gammacoronavirus*

Subfamily *Torovirinae*

Genus *Torovirus*

Toroviruses have been isolated from mammals with gastroenteritis, but rarely from humans. Virions are enveloped, about 120–140 nm in diameter. They have surface spikes and a doughnut-shaped nucleocapsid.

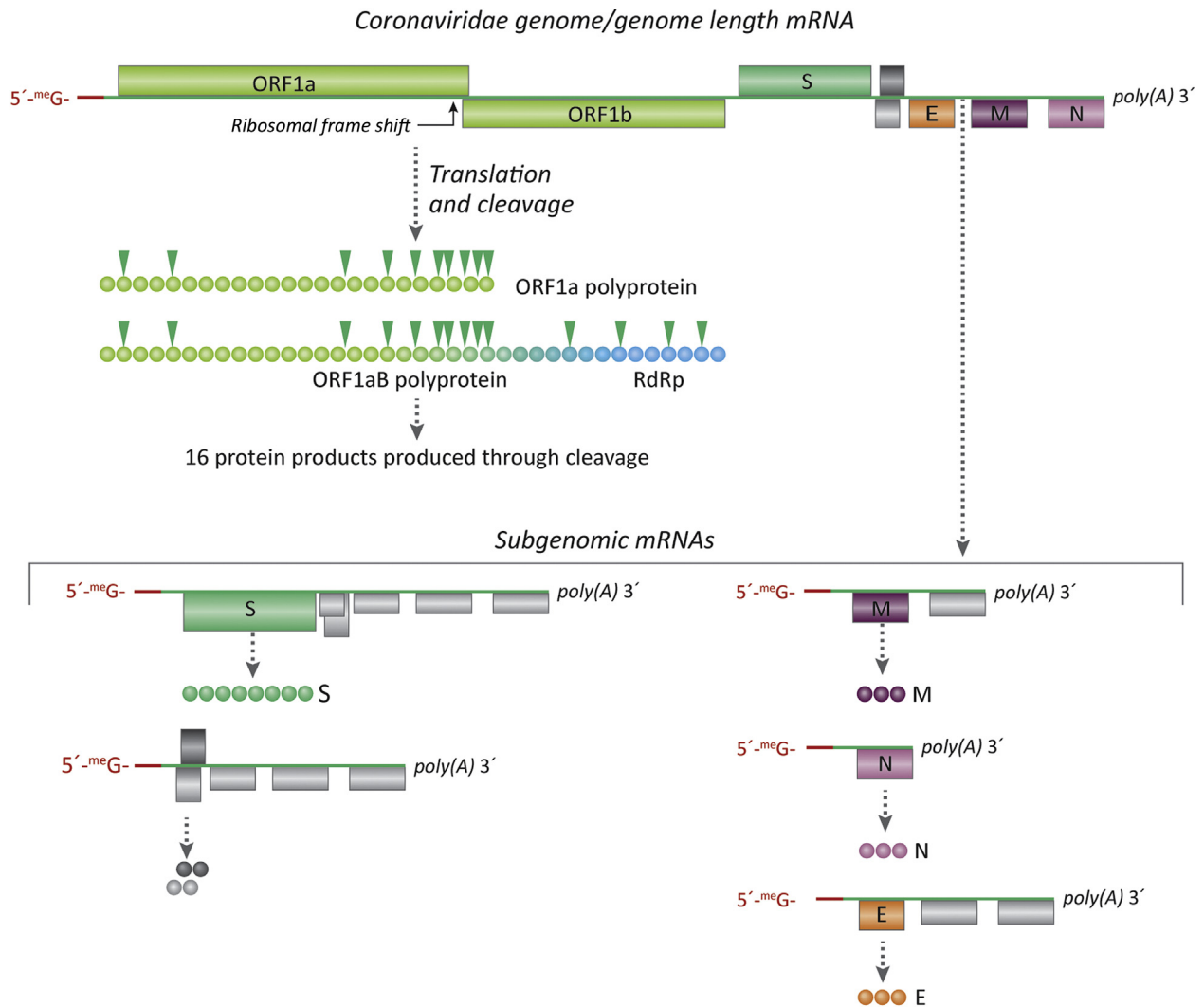


FIGURE 17.3 Coronavirus genome organization and gene expression strategy.

of the genome encodes nonstructural proteins (nsps) that are required for transcription and genome replication. Among these is nsp12, the large 930 amino acid RNA-dependent RNA polymerase (RdRp). Nsp12

forms a multiprotein complex with other CoV nsps. CoV nsps are synthesized as long precursor polypeptides, cleaved by virally encoded proteases (Fig. 17.3). The REP1a polyprotein is cleaved to produce 11

TABLE 17.1 Coronavirus nsps That form the Replicase/Transcriptase Complex*

Nonstructural protein	Function
nsp1	Interferon antagonist (not present in all coronaviruses)
nsp2	Not known
nsp3	Papain-like protease domains and several other protein interaction domains. May tether the RNA genome to the replicase/transcriptase complex.
nsp4	Transmembrane scaffold. Involved in membrane remodeling.
nsp5	Main protease (M ^{Pro}) (also called 3C-like protease)
nsp6	Transmembrane scaffold. Involved in membrane remodeling.
nsp7	Forms a large complex with nsp8.
nsp8	Forms a large complex with nsp7. The complex may function as a processivity clamp for the RdRp.
nsp9	Single-stranded RNA-binding protein
nsp10	Zinc-binding cofactor for 2'-O-methyltransferase (nsp16)
nsp12	RdRp
nsp13	RNA 5' triphosphatase (cap synthesis), RNA helicase
nsp14	N7-methyltransferase, Exo N 3'-5' exonuclease (provides a proofreading function for the coronavirus RdRp)
nsp15	Nendo U endonuclease (cleaves single- and double-stranded RNA downstream of uridylate residues, producing 2-3 cyclic phosphates).
nsp16	2'-O-methyltransferase (cap synthesis)

*Products of polyproteins 1a and 1b.

smaller products (nsp1–nsp11; listed in Table 17.1). *Rep1b* follows *Rep1a* in the genome, separated from it by a frame-shifting site that is slightly upstream from the *Rep1a* stop codon. About 20%–25% of the time, ribosomes reaching the frame-shift site will slip into the minus-1 reading frame and a longer polyprotein, called REP1b is synthesized. REP1b codes for five additional proteins, nsps 12–16, among which are the RdRp (nsp12), a protein with helicase and phosphatase activities (nsp13), a protein with exonuclease and methyl transferase activities (nsp14), an endoclease (nsp15) and a second methyltransferase (nsp16). The guanine-N7-methyltransferase, nsp13, and the 2' O-methyltransferase, nsp16, synthesize the CoV 5'-cap. In addition to the final cleavage products, long-lived intermediates may have activities different from those of the fully cleaved products.

Seven to 10 additional ORFs lie downstream of the replicase-associated genes. The largest of these encodes the spike (S) protein. The order (S, E, M, N) of the structural proteins on the coronavirus genome is well conserved (their specific structural roles will be discussed later). Dispersed among the structural protein genes are a variable number of additional small ORFs. These lie in-between, or overlap, the structural protein genes and they are not well conserved among CoVs. These are called *accessory proteins* (by convention they are named using the number of the shortest mRNA on which they are encoded, Box 17.3). Mutation studies show that at least some of the accessory proteins are not required for coronavirus replication in cell cultures. However, mutating the accessory proteins does have a profound effect on the ability of the CoVs to replicate in their hosts and impacts viral pathogenesis.

As expected for an RNA virus, the CoV genome contains regulatory elements. Cis-acting regulatory elements are required for replication, transcription, and genome packaging. At the 5' end of the genome, elements that participate in viral RNA synthesis extend past the 5' untranslated region (UTR) and into the coding region of *rep1a*. This region folds up into a set of seven stem-loops. Folding of the CoV 5' RNA is dynamic, allowing for changing conformations that control critical aspects of RNA and protein synthesis. Functional analyses of cis-acting regulatory regions have shown that stability of RNA stem-loops can be critical for viral fitness.

At the 3' end of the CoV genome, additional cis-acting elements are found in the 3' UTR. The 3' cis-acting elements are conserved (and are interchangeable) among betacoronaviruses. As with the 5' UTR, this region is probably dynamic, with different conformations controlling different steps in RNA replication/synthesis.

Other important cis-acting sequences are those that direct ribosomal frame shifting near the end of the *rep1a* gene. Finally, genome packaging signals have been localized to internal regions of the HCoV-SARS genome.

VIRION STRUCTURE

Coronavirus particles are enveloped with prominent spikes. Virions are spherical and range in size from 118 to 140 nm. Within the envelope is a flexible (subfamily *Coronavirinae*) or a doughnut-shaped (subfamily *Torovirinae*, see Box 17.4) nucleocapsid that consists of genomic RNA associated with the nucleoprotein (N). The spike (S) protein is the major glycoprotein that extends from the surface of the virion. Other

BOX 17.3

CORONAVIRUSES ACCESSORIZER

Coronaviruses encode a heterogeneous group of proteins, many of which are not absolutely necessary for replication in cultured cells, collectively called accessory proteins. The numbers of accessory proteins encoded by CoVs differ among even closely related coronaviruses. The genes encoding accessory proteins are interspersed among the structural genes. They are numbered according to the mRNA from which they are produced. SARS-CoV expresses eight accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b). They are all small peptides, ranging in size from about 45 to 180 amino acids. A few are found in purified virions and several are membrane-associated. Several have been identified in infected cells from

autopsy patients, good evidence that they are expressed during a natural infection. For the most part, their functions are not well characterized but a few activities have been determined: The SARS-CoV 3a protein forms an ion channel in the plasma membrane and may modulate virus releases. SARS-CoV 3b interferes with the interferon pathway, and protein 6 may be an antagonist of type I IFN. Both SARS and MERS-CoV infections are characterized by a delay in type I IFN production and poor development of adaptive immune responses. It is likely that accessory proteins are involved in their pathogenesis.

BOX 17.4

TOROVIRUSES

The family *Coronaviridae* contains two subfamilies, the *Coronavirinae* and the *Torovirinae*. As expected, toroviruses share many characteristics with the other members of the family *Coronaviridae*, including basic virion architecture, genome organization, and mechanism for synthesizing a 3'-nested set of mRNAs. Torovirus particles are round, pleomorphic, enveloped viruses about 120–140 nm in diameter with obvious surface spike proteins. What are the unique features of toroviruses that have resulted in their being assigned to a distinct subfamily? Their most distinctive morphologic feature is a doughnut-shaped nucleocapsid (Fig. 17.2).

All toroviruses identified to date have a glycoprotein with HE activities (Fig. 17.4). HE mediates binding to sialic acid residues but is also a so-called receptor-destroying enzyme because it cleaves (thus destroys) sialic acid. One theory is that the activities of HE allow toroviruses to penetrate the mucus layer in the respiratory and digestive tracts. Toroviruses infect vertebrates and many have been isolated from mammals with gastroenteritis. Toroviruses have been isolated from humans, cattle, pigs, sheep, goats, and horses. Toroviruses have not been extensively studied as most are difficult to grow in cultured cells.

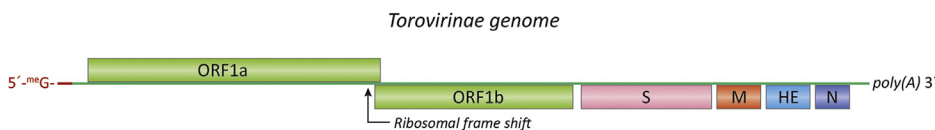


FIGURE 17.4 Torovirus genome organization.

membrane-associated proteins include membrane (M) and envelope (E).

CORONAVIRUS STRUCTURAL PROTEINS

All CoVs encode four structural proteins: Three membrane-associated proteins (S, M, and E) and a single nucleocapsid (N) protein. However some betacoronaviruses have an additional membrane protein with hemagglutinating and esterase activities, hence it is

called HE. The order of the structural genes on the CoV genome is (HE) S, M, E, N (Fig. 17.3). The order of structural genes on the torovirus genome is S, M, HE, and N (Fig. 17.4).

The spike protein (S) forms a prominent projection from the virus envelope and gives CoVs their characteristic appearance. S is glycosylated and is the attachment and fusion protein. Among the CoVs there are some differences in the manner in which S is processed (cleaved into S1 and S2 fragments). S is always ER associated and modified by N-linked glycosylation.

In many beta- and gammacoronaviruses S is partially or completely cleaved by host furin-like proteases in the ER (prior to assembly of new virions). The extent of proteolysis correlates with the number of highly basic residues at the S1/S2 cleavage site. The S1 (N-terminal) and S2 (C-terminal) products remain noncovalently associated. In contrast, HCoV-SARS S is *not* cleaved during assembly or release. Instead it is cleaved in an acidified endosome during entry/penetration. It appears that there are two critical cleavage events that act in concert to mediate fusion: A cleavage at the S1/S2 boundary and a second cleavage within S2 (called the S2' cleavage site). Some CoV S proteins are not cleaved, but the terms S1 and S2 are still used to refer to the corresponding N- and C-terminal domains of the protein. It is also likely that, in some cases, processing of CoV S in cultured cells is not identical to the process in the infected host.

Comparisons of S1 sequences show that they diverge extensively and are not highly conserved, even within multiple isolates of a single type of coronavirus. We can reasonably speculate that sequence divergence is, at least in part, a result of host immune responses. In contrast to S1, the S2 product is highly conserved across the subfamily *Coronavirinae*.

The membrane protein (M) is the most abundant protein in the virion. M contains three hydrophobic domains, thus is tightly associated with the virus envelope. M plays a major role in promoting membrane curvature. It has a short ectodomain (extracellular domain) that is modified by glycosylation. M also interacts with the N and E proteins. Expression of HCoV-SARS M (in the absence of other viral proteins) results in self-assembly and release of membrane-enveloped vesicles. Virus-like particles are released when M is coexpressed with either N or E.

The envelope (E) protein is found in very small amounts in the virion (about 20 molecules per virion), although larger amounts of E are present in infected cells. Different CoVs have variable requirements for E during particle formation, ranging from required for particle formation to not essential. In fact, virus titers close to 1×10^6 pfu per mL have been reported for HCoV-SARS lacking the E protein. Studies show that E assembles in membranes to form ion channels, thus E is a viroporin. Viroporins influence the electrochemical balance in subcellular compartments.

The nucleocapsid (N) protein is the only protein found in the ribonucleoprotein particle. N forms homodimers and homooligomers and binds genomic RNA, packaging it into a long flexible nucleocapsid. In the infected cell, N localizes to the cytoplasm, and for some CoVs N is also found in the nucleolus. N interacts with other CoV structural proteins, thus has a role in assembly and budding. N also colocalizes with

replicase–transcriptase components and is required for RNA synthesis. Other roles for N include modulating cell-cycle (promoting cell-cycle arrest) and inhibiting host cell translation.

An additional structural protein is found in most betacoronaviruses, including the human coronavirus HCoV-HKU1. The hemagglutinin-esterase (HE) is 48 kDa glycoprotein that projects outwards 5–10 nm from the virion. HE binds sialic acid units on glycoproteins and glycolipids. The esterase activity removes acetyl groups from O-acetylated sialic acid, thus may be a receptor-destroying enzyme.

CORONAVIRUS REPLICATION CYCLE

The overall scheme of CoV replication is similar to that of other positive-strand RNA viruses but the process for synthesis of subgenomic mRNAs is unique.

Attachment

CoV S is the receptor-binding protein. The receptors for some CoVs have been identified. Two human CoVs, HCoV-SARS, and HCoV-NL63 bind different regions of angiotensin converting enzyme 2 (ACE2). ACE2 is a cell-surface, zinc-binding carboxypeptidase important for regulation of cardiac function and blood pressure. ACE2 is expressed in epithelial cells of the lung and small intestine, as well as many other organs. HCoV-MERS binds to dipeptidyl peptidase 4 (DPP4). Several other CoVs (FeCoV, FIPV, CCoV, and TGEV) bind to aminopeptidase N. (We might well ask if it is significant that many CoV receptors are proteases.) However, not all CoVs bind to protein receptors. Bovine CV and the HCoV-OC43 bind to sialic acid units found on glycoproteins and glycolipids.

Penetration

CoVs are enveloped, so penetration occurs as a consequence of a membrane fusion event. Just as cleavage of the S protein varies among CoVs, so do the sites and requirements of the fusion process. CoV S proteins contain a hydrophobic “fusion peptide” that becomes exposed upon a large-scale rearrangement of S. The location and details of S rearrangement are variable, but can be triggered by factors such as proteolytic cleavage of S and/or acid pH. Processing of HCoV-SARS has been well studied and will serve as our example. HCoV-SARS S is found as an uncleaved product on extracellular virions. It is cleaved only after attachment and endocytosis. There are two critical cleavage events that precede (and are required for)

fusion. One cleavage is at the S1/S2 boundary and the other is a cleavage within S2 (S2'). Evidence supports cleavage at the S1/S2 boundary by cathepsin L; however, if one exposes HCoV-SARS to extracellular proteases (such as trypsin or elastase) in cell cultures, infection is *greatly enhanced*, suggesting that the endosomal proteases are not highly efficient. This may be relevant to HCoV-SARS-infection and pathogenesis: In the human respiratory tract, a transmembrane serine protease (TMPRSS2) is expressed in pneumocytes and binds to ACE2 (the receptor for HCoV-SARS). The interaction between ACE2 and TMPRSS2 would conveniently put uncleaved S in close proximity to a host cell protease upon initial binding.

Amplification

The first synthetic event in the CoV replication cycle is translation of the viral genome by host cell ribosomes. REP1a and REP1b are translated from genomic RNA and these polyproteins are necessary for virus replication to move forward. Some of the REP1a products (nsp3, nsp4, nsp6) have transmembrane domains and these serve to anchor the replication–transcription complex to cell membranes, a prerequisite for synthesis of additional viral RNAs. The interaction also causes remodeling of host cell membranes to form structures dedicated to virus RNA synthesis.

RNA Synthesis

In vitro, the CoV RdRp requires a primer, specifically a short RNA oligonucleotide. It so happens that the CoVs encode two different proteins with RdRp

activity. The nsp8 gene product is thought to be a primase capable of synthesizing short oligonucleotides. Nsp12 is the elongating polymerase. Other viral nsps in the replication–transcription complex are involved in cap synthesis (Table 17.1). CoVs also encode two ribonucleases. NendoU (nsp15) is a Nidovirales endonuclease that cleaves both single- and double-stranded RNA, cutting downstream of uridylylate residues. ExoN (nsp14) is a 3'–5' exonuclease. CoVs with mutations in ExoN have an *enhanced mutation rate* suggesting a role for ExoN in proofreading during RNA synthesis. Quite an unexpected finding for an RNA virus!

In addition to genome-length RNA, a set of subgenomic (sg) mRNAs is found in the infected cell. The sg mRNAs are used for the expression of the structural and accessory proteins. All are capped and polyadenylated and they share a common 3'-end forming a so-called “nested set” of mRNAs. A closer look at the sg mRNAs reveals that each contains an identical leader sequence of 70–100 nt at the 5'-end. The leader sequences found on all sg mRNAs are identical; however, this sequence is found only *once* in the genome, near the 5'-end. Leader sequences are fused to downstream sequences (sometimes called the body RNAs) at short, 8–9 nt motifs called the transcription regulating sequence (TRS). A TRS is found upstream of the ORFs-encoding structural proteins (these are called TRS body or TRS-B). A TRS is also present just downstream of the leader sequence in the 5' UTR. These findings provide clues to the unique strategy used for CoV mRNA synthesis: CoV sg mRNAs are generated by a process of discontinuous transcription illustrated (simplified) in Fig. 17.5.

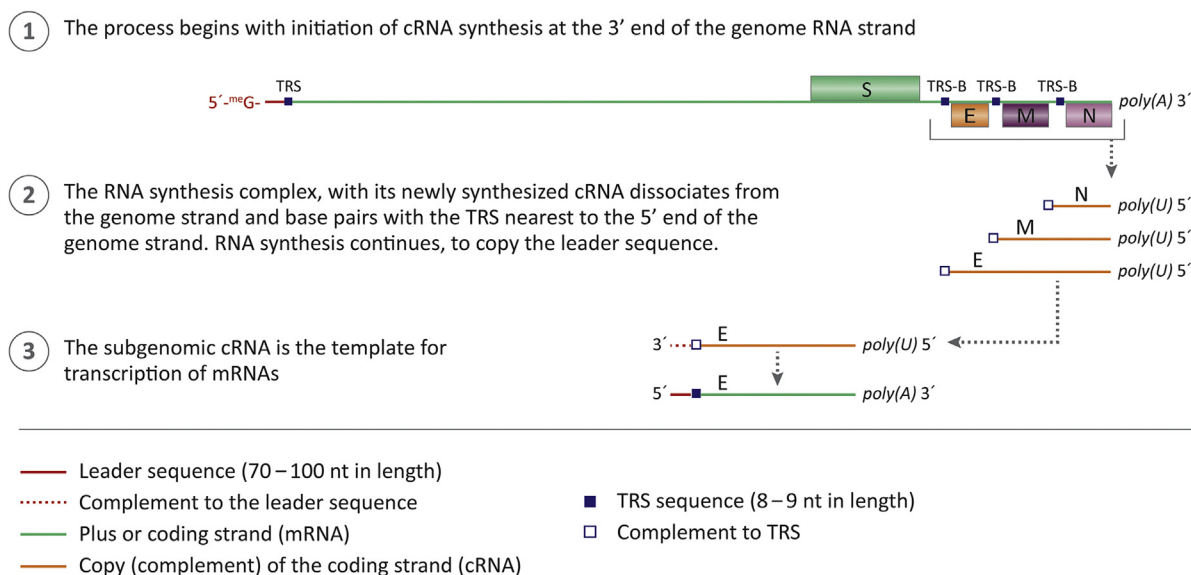


FIGURE 17.5 Coronavirus discontinuous transcription.

Discontinuous transcription very likely contributes to the high level of CoV genome recombination (Box 17.5). This type of RNA virus genome recombination is called a *copy-choice* mechanism (Fig. 17.6). Quantification of CoV RNAs presents in infected cells

shows that cRNAs (negative-sense RNAs with 5' oligo (U)) are present at levels 0.1–0.01 times lower than “positive-sense” genomes and mRNAs. This finding suggests that recombinants are more likely to occur during synthesis of negative strands.

BOX 17.5

CORONAVIRUS RECOMBINATION

Discontinuous transcription likely contributes to the readily observed examples of CoV genome recombination. If the replicase–transcriptase complex *must* dissociate/reassociate with template to generate subgenomic cRNAs, it follows that the same process sometimes occurs during synthesis of genome-length cRNA. Studies in the MHV experimental system show that genome recombination is a relatively common event. Examination of feline and canine CoVs (CCoVs) provide real world examples of this phenomenon. FeCoVs are common in cats and usually cause mild enteric illness. On occasion enteric FeCoVs mutate within an infected cat and gain the ability to infect macrophages, an event which is

accompanied by production of severe systemic disease called FIP. However, among cats with FIP, some researchers found a few “novel” viruses. Sequencing these novel viruses revealed that they contained genes from both feline and CCoV. As CCoV is a relatively common dog virus (and it is common to have both cats and dogs in a household), it was hypothesized that the novel FIP-associated virus arose in cats that became infected with *both* FeCoV and CCoV; in the coinfecting cats, macrophage-infecting recombinants emerged, causing the clinical disease, FIP (Terada et al., 2014). The FIP-associated recombinant viruses do not appear to be transmissible from one cat to another.

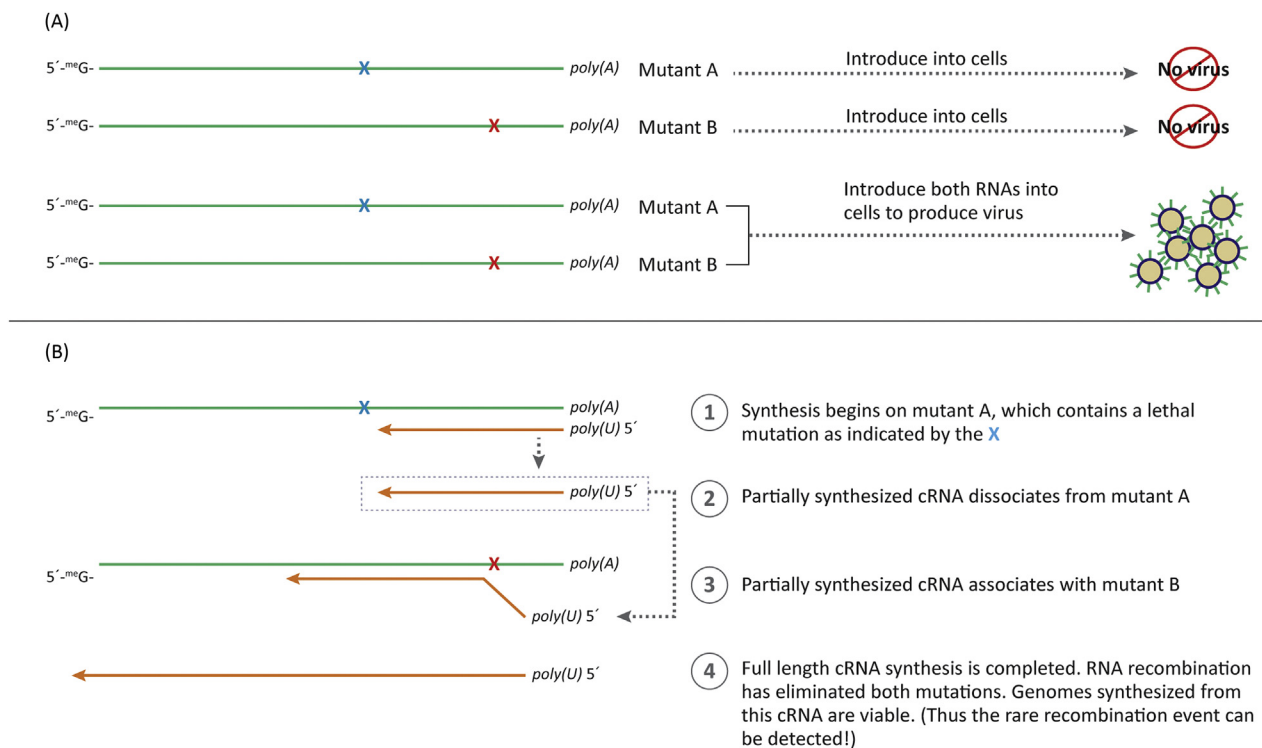


FIGURE 17.6 Coronavirus RNA recombination. (A). If individual mutant coronaviruses are used to infect cells there is no virus growth. However, if mutants are used together to infect cells, virus growth is restored suggesting that genome recombination has occurred. (B). This panel illustrates the mechanism of RNA copy choice ‘recombination’.

Assembly and Release

Virion assembly takes place on membranes. Genomic RNA is bound by N protein, associates with M protein and buds into ER/Golgi membranes. M packs tightly into membranes and is thought to cause the membrane curvature that drives budding. S and E are also membrane proteins and are acquired during the budding process.

The ion channel activity of E is that of a *viroporin*; it alters cell secretory pathways to promote virus release. A function of E may be to increase the pH of the transport vesicles. Virus particles contained within membrane bound vesicles are released from cells by exocytosis.

DISEASES CAUSED BY CORONAVIRUSES

The first coronaviruses isolated were from poultry with respiratory disease (infectious bronchitis) in the 1930s. IBV remains a worldwide problem, particularly in high-density commercial production facilities. Until 2002 human coronaviruses (HCoV) were associated only with mild respiratory tract disease, with estimates that they caused 15%–25% of all “common colds.” That changed in 2002 when a human coronavirus was identified as the cause of an apparently new disease called SARS. The SARS outbreak was controlled, but in 2014 another novel CoV was isolated from patients hospitalized with severe respiratory disease in Saudi Arabia. As most infected patients lived in or had traveled to Middle East countries, the new disease was named MERS and the coronavirus responsible is called HCoV-MERS. Most animal and human CoVs are transmitted by the fecal-oral route and their initial replication site is in epithelial cells where virus production causes local respiratory symptoms or diarrhea. However, on occasion, CoVs cause severe to fatal disease. Some examples are presented here.

Severe Acute Respiratory Syndrome

SARS facts:

- SARS-CoV emerged in the human population in China in 2002.
- Epidemiologic studies and genetic analysis indicated the virus most likely jumped from bats into farm-raised Himalayan palm civets (*Paguma larvata*) and then into humans.
- Human to human transmission was by respiratory and fecal routes.
- Approximately 8000 cases were reported worldwide.

- Twenty-six countries were affected.
- Seven hundred and seventy-four deaths occurred (~10% case mortality rate).
- Economic losses in Hong Kong were ~5.9 billion (\$US).
- In July 2003, WHO reported that the last known human chain of transmission was broken.
- Bats and birds are natural reservoirs of SARS-like viruses.
- Laboratory-associated infections occurred in China in 2004.

SARS-infection causes a triphasic pattern of disease. The first phase is nonspecific with fever, cough, sore throat, and myalgia. Breathing difficulties (dyspnea) show up 7–14 days after appearance of the first symptoms. The second phase of the disease includes shortness of breath, fever, onset of hypoxia, and often diarrhea. In the most serious cases, patients progress to a third phase with development of acute respiratory distress requiring hospitalization and mechanical respiration. Three viral proteins have been implicated in HCoV-SARS pathogenesis. An accessory protein (encoded from orf3a) interferes with cell signaling pathways, another accessory protein (encoded from orf6) interferes with interferon signaling and the E glycoprotein is a strong inducer of proinflammatory cytokines. All of these proteins are dispensable for virus replication in cell cultures, but in mouse models of disease their deletion reduces disease.

Middle East Respiratory Syndrome

MERS facts:

- MERS begins with coughing, fever, and breathing problems but may progress to pneumonia and kidney failure.
- Over 1600 human cases and the outbreak is ongoing.
- Case fatality rate >30%.
- Cases are sporadic and cannot be linked to a single source (based on genome sequencing).
- Countries most affected include those in the Arabian Peninsula (Bahrain, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, United Arab Emirates, and Yemen).
- Casual transmission from person to person very rare.
- Most person to person transmission occurs in a hospital setting.
- Many healthy camels in the Arabian Peninsula have antibodies specific to CoV-MERS (indicating past infection) but infections often occur among people with no known contact with camels.

- A virus very similar to CoV-MERS has been found in some bats (sequence analysis suggests that the virus moved from bats to camels).
- Many questions about the epidemiology of CoV-MERS remain unanswered.

Sporadic cases of MERS continue to be reported. Strict infection control procedures in hospitalized patients limit person to person spread in that setting and transmission among casual or household contacts is rare.

While MERS-CoV and SARS-CoV are both betacoronaviruses, they derive from different sources and have unique characteristics. SARS-CoV has a tropism for ciliated respiratory epithelial cells and its receptor is ACE2. In contrast MERS-CoV has a tropism for nonciliated respiratory epithelial cells and its receptor is DPP4. Continued comparison of these two viruses will provide new insights into CoV transmission and pathogenesis. The propensity for CoV recombination, and their ubiquitous presence in bats, makes a case for close surveillance and study of these potential human pathogens.

Feline Coronavirus and Feline Infectious Peritonitis

FeCoV is the most common virus found in cat fecal samples and is spread through the fecal-oral route. Infection is usually subclinical or associated with a transient, mild diarrhea. Immunity to FeCoV is neither solid nor long-lasting. As antibody levels decrease, cats may be reinfected and once again experience mild diarrhea. Most kittens infected with FeCoV clear the virus, but about 15% become chronic shedders. Chronic shedders are at highest risk for developing FIP, a systemic (multiorgan), lethal disease. FIP develops when enteric FeCoV mutates to become capable of infecting monocytes and macrophages. This results in systemic viral infection, as the virus is no longer confined to the digestive tract. The virus associated with monocytes/macrophages is called the FIP *biotype*.

What kinds of mutations change the cell tropism of enteric FeCoV and result in development of FIP? Mutation occurs independently within each cat, with every FIP biotype virus having unique genetic features. Specific viral genes (for example, the orf3C protein) are important for the change in cell tropism. After changing cell tropism, the virus continues to mutate,

becoming better adapted to peritoneal macrophages. This occurs despite preexisting host immune responses. Early signs of FIP are nonspecific and include anorexia, weight loss, inactivity, and dehydration. FIP can occur in any age cat, but cats less than 1 year of age or greater than 10 years of age are more susceptible. Constant virus replication in macrophages leads to B-cell activation and production of nonprotective (nonneutralizing) antibodies. In fact, immune complexes are damaging as they activate the complement system and lead to immune-mediated vasculitis. The classic lesions associated with the severest form of FIP are aggregates of macrophages, neutrophils, and lymphocytes that form in very small veins. These aggregates are called pyogranulomas and they are associated with development of edema and accumulation of large volumes of protein-rich fluids.

In this chapter we have learned that:

- Members of the family *Coronaviridae* are large, positive-strand RNA viruses.
- They are enveloped, with a helical nucleocapsids.
- A long spike (S) protein forms extends 16–21 nm from the surface of virions. S is the attachment and fusion protein.
- mRNAs are synthesized by a process of discontinuous transcription, a process that leads to high rates of RNA recombination.
- There are two subfamilies, *Coronavirinae* and *Torovirinae*. Most HCoVs cause mild respiratory or enteric disease. Notable exceptions are CoV-SARS and HCoV-MERS that can cause severe respiratory disease. The toroviruses are less well studied but have been isolated from a variety of mammals (intestinal and respiratory tracts).
- Members of the family *Coronaviridae* are notable for the mechanism of transcription, which is discontinuous (the RdRp moves from one location on the template RNA to a distant location). This process leads to high rates of RNA recombination during genome replication.

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