Chapter 11
Core-Associated Genome Replication Mechanisms of dsRNA Viruses

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Introduction

The double-stranded RNA (dsRNA) viruses are a diverse group, which infect a wide assortment of prokaryotic and eukaryotic hosts. Although not all dsRNA viruses are considered pathogens, many cause devastating disease in their hosts and have widespread medical, veterinary, and agricultural impacts. Currently, the International Committee for the Taxonomy of Viruses (ICTV) recognizes seven distinct families of dsRNA viruses (Hypoviridae, Totiviridae, Birnaviridae, Partitiviridae, Cystoviridae, Chrysoviridae, and Reoviridae) (Table 11.1) (http://phene.cpmc.columbia.edu). Of these families, Reoviridae is composed of the largest number of individual species, which are categorized into 12 separate genera, and includes some of the most severe dsRNA viral pathogens of humans and domestic animals (Mellor and Boorman, 1995; Parashar et al., 2003). In particular, rotaviruses are members of the Reoviridae family and a leading cause of lethal gastroenteritis in young children and infants (Parashar et al., 2003). As such, the Reoviridae family has been studied in detail, providing insights into the general strategies dsRNA viruses use to propagate. Members of the Totiviridae and Cystoviridae families, which infect fungi and bacteria, respectively, have replication strategies similar to Reoviridae and are often viewed as models for understanding dsRNA virus biology (Mindich, 2004; Poranen and Tuma, 2004; Wickner, 1996). Together, studies of these three virus families have elucidated several common themes in dsRNA virus replication: (i) RNA synthesis occurs within a protected core via an anchored RNA-dependent RNA polymerase (RdRp); (ii) genome replication and capsid assembly occur simultaneously; and (iii) *cis*-acting elements in the viral RNA determine template specificity. This chapter will explore these themes regarding the core-associated genome replication of dsRNA viruses by reviewing structural and biochemical studies of individual members of Totiviridae, Cystoviridae, and Reoviridae families.

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C.E. Cameron et al. (eds.), *Viral Genome Replication*,
DOI 10.1007/b135974_11,

Table 11.1 dsRNA virus families, genome segments, and particle types

Family	# Genome segments	Particle type
Hypoviridae	1 (unpackaged)	Enveloped; no protein shell
Totiviridae*	1 (packaged singly)	Single-shelled; pseudo $T = 1$
Partitiviridae	2 (packaged separately)	Single-shelled; unknown structure
Birnaviridae	2 (co-packaged)	Single-shelled; $T = 13$
Cystoviridae*	3 (co-packaged)	Triple-shelled; pseudo $T = 1$ core, Middle $T = 13$ layer, and outer membrane
Chrysoviridae	4 (packaged separately)	Single-shelled; classic $T = -1$ core
Reoviridae*	10, 11, or 12 (co-packaged)	Single, double, or triple-shelled; pseudo $T = 1$ core; Middle and outer $T = 13$ layers

*dsRNA virus families that exhibit a pseudo $T = 1$ core.

RNA Synthesis Occurs Inside a Protected Core

To replicate successfully in the cytoplasm of a eukaryotic host cell, a dsRNA virus must overcome elaborate intracellular defense mechanisms (Garcia-Sastre and Biron, 2006). Specifically, the detection of viral dsRNA by the cell triggers an antiviral response that drastically impedes viral replication (Garcia-Sastre and Biron, 2006; Levy and Garcia-Sastre, 2001). Consequently, dsRNA viruses have evolved to escape this antiviral response by confining their genomes, throughout the entire course of infection, within one to three concentric protein shells (Lawton et al., 2000; Mertens, 2004). The innermost protein shell not only houses the segments of viral genomic dsRNA but also encases the viral RdRp and other enzymes necessary for mediating RNA synthesis (Ahlquist, 2006). Together, the proteins and RNA of the innermost shell make up a proteinaceous structure referred to as the viral core (Fig. 11.1). During the entry of a dsRNA virus into a cell, the outer layers of the virion are sequentially lost, triggering the enzymes within the core to begin viral transcription ((+)RNA synthesis) using the endogenous dsRNA genome as template (Fig. 11.1A) (Mertens and Diprose, 2004; Mindich, 2004; Patton, 2001; Patton and Spencer, 2000; Patton et al., 2004, 2007). Following transcription, the (+)RNA molecules are extruded from the virion core and into the host cell cytoplasm where they are translated into viral proteins (Mertens and Diprose, 2004; Mindich, 2004; Patton, 2001; Patton and Spencer, 2000; Patton et al., 2004, 2007). For Reoviridae, newly synthesized viral proteins accumulate in large cytoplasmic inclusions where the initial stages of virion particle assembly occur simultaneously with genome replication (dsRNA synthesis) (Patton et al., 2006, 2007; Roy and Noad, 2006). Particularly, viral core proteins assemble into intermediate structures, which package (+)RNA molecules at the same time as the core-associated viral enzymes convert them into dsRNA (Fig. 11.1B) (Patton et al., 2007; Roy and Noad, 2006). Because viral cores contain all the components necessary for transcription and genome replication, these processes occur within a protected environment that sequesters the precious viral dsRNA genome away from host cell antiviral sentries (Lawton et al., 2000; Mertens, 2004).

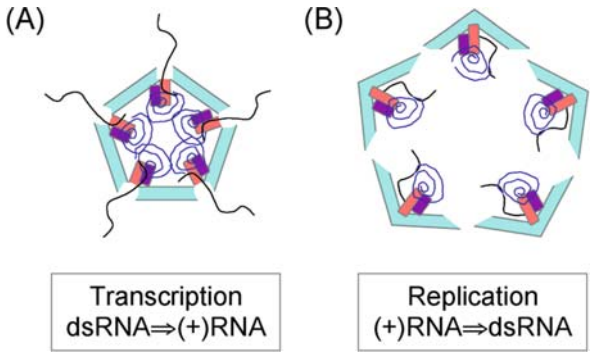

Fig. 11.1 Core-associated transcription and genome replication of dsRNA viruses. The schematics shown above diagram the two stages of RNA synthesis for dsRNA virus. Core components are not drawn to scale. (**A**) Transcription. Entry of a dsRNA virus into a cell triggers the enzymes (*pink* and *purple*) within the core shell (*light blue*) to begin (+)RNA synthesis using the endogenous dsRNA genome (*blue spirals*) as template. Following transcription, the (+)RNA molecules (*black lines*) are extruded from the virion core through channels at the fivefold axes. (**B**) Replication. Viral core proteins assemble into intermediate structures, which package (+)RNA molecules at the same time as the core-associated viral enzymes convert them into dsRNA.

Core Components of Model dsRNA Viruses

Viruses belonging to the Totiviridae, Cystoviridae, and Reoviridae families have been studied in great detail, yielding significant information about the structure and function of dsRNA viral cores. The prototypical member of Totiviridae is L-A virus, a pathogen of the yeast *Saccharomyces cerevisiae*. L-A is one of the simplest dsRNA viruses, having only one genome segment, which is encased by a single shell made up of the viral coat protein (Gag) (Fig. 11.2A) (Wickner, 1996). The viral RdRp (Pol) is expressed from the genome as a Gag–Pol fusion protein due to a –1 ribosomal frameshift and is incorporated into L-A particles (Dinman et al., 1991). Pol is required for mediating the concerted replication and packaging of the viral genome segment while anchored inside the core; however, Gag alone is sufficient for particle formation (Ribas and Wickner, 1992). The observation that Totiviridae members do not have outer layers is likely a reflection of their obligate intracellular life cycle. These viruses do not exit their host fungal cell, but rather spread via cytoplasmic mixing (Wickner, 1996). Despite their simplicity, L-A particles are strikingly similar in structure to cores of the Reoviridae and Cystoviridae families (Naitow et al., 2002). Furthermore, because L-A particles can synthesize dsRNA in vitro using exogenous templates, they remain a straightforward and elegant system for studying dsRNA viral genome replication (Fujimura and Wickner, 1989).

The bacteriophage phi 6 (Φ6) is the best-characterized member of the Cystoviridae family (Mindich, 2004; Poranen and Tuma, 2004). The Φ6 virion is a double-layered nucleocapsid (NC) surrounded by a host cell-derived lipid envelope, which is embedded with several viral proteins (Poranen and Tuma, 2004). The outer protein layer of the NC shows $T = 13$ icosahedral symmetry and is composed entirely

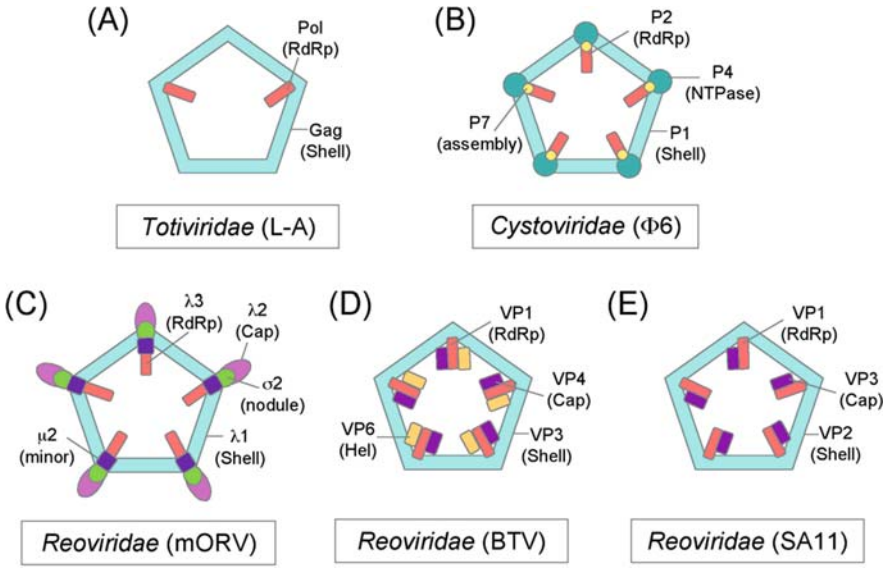

Fig. 11.2 Core components of model dsRNA viruses. The schematics above show the approximate locations of viral core components and are not drawn to scale. (**A**) L-A virus is the prototypical Totiviridae member. (**B**) The bacteriophage phi 6 (Φ6) is the best-characterized member of the Cystoviridae family. (**C**) Mammalian orthoreovirus (mORV) is the type species for turreted members of Reoviridae. (**D**) The well-studied non-turreted Reoviridae members are the orbivirus, blue tongue virus (BTV), and (**E**) the rotavirus simian agent 11 (SA11).

of the viral P8 protein (Butcher et al., 1997; Huiskonen et al., 2006; Jaalinoja et al., 2007; Kainov et al., 2003). Also referred to as the polymerase complex (PC), the Φ6 core consists of four viral proteins: a shell protein (P1), a nodule-like hexameric NTPase (P4), an assembly cofactor (P7), and an internally anchored viral RdRp (P2) (Fig. 11.2B) (Makeyev and Grimes, 2004). Inside the Φ6 core are one copy each of three dsRNA genome segments: small (S), medium (M), and large (L) (Mindich, 2004). Because the Φ6 core fully reproduces RNA packaging in vitro, in addition to template-dependent in vitro RNA synthesis, it has become an important model system (Makeyev and Grimes, 2004; Mindich, 2004). Even more, a high-resolution structure of the catalytically active Φ6 RdRp has suggested mechanisms for semi-conservative transcription and the initiation of viral RNA synthesis (Butcher et al., 2001).

The virion architecture of Reoviridae family members is similar to Totiviridae and Cystoviridae. Yet, this family is more complex due to the increased number of genome segments and capsid proteins. Despite a few rare exceptions, the cores of Reoviridae family members encapsidate 10, 11, or 12 equimolar dsRNA genome segments and are surrounded by two $T = 13$ icosahedral proteins shells (Mertens, 2004). Some Reoviridae genera have turrets composed of the viral capping enzyme(s) (5′-triphosphatase, guanylytransferase, methyltransferase, etc.) that

protrude outward from their core shells at each fivefold axis (Mertens, 2004). The type species for turreted members of Reoviridae is the mammalian orthoreovirus (mORV), a ubiquitous non-pathogenic animal virus. The mORV core is composed of five proteins, many with multiple functions (Fig. 11.2C). The core shell protein (λ1) also possesses NTPase, 5′-triphosphatase, and helicase activities. The nodule-forming clamp protein (σ2) helps stabilize the capsid, whereas the turret-forming (λ2) mediates most of the roles related to capping the (+)RNA following transcription. Inside the core resides the anchored RdRp (λ3) and another protein (μ2) that acts as a cofactor during RNA synthesis (Mertens, 2004). In contrast, other genera of Reoviridae are described as being non-turreted because they lack protruding core structures and retain their capping enzymes within their cores. Two well-characterized non-turreted Reoviridae members are the orbivirus, blue tongue virus (BTV), and the rotavirus, simian agent 11 (SA11). BTV is a deadly pathogen of ruminants and has a viral core that consists of a shell protein (VP3) with internal, anchored enzyme complexes composed of an RdRp (VP1), an RNA capping enzyme (VP4), and a helicase (VP6) (Fig. 11.2D) (Mertens and Diprose, 2004; Roy and Noad, 2006). The core of the prototypic rotavirus SA11 is similar to BTV, but lacks the helicase protein. The SA11 core has a shell protein (VP2) that surrounds anchored enzyme complexes made of an RdRp (VP1) and an RNA capping enzyme (VP3) (Fig. 11.2E) (Jayaram et al., 2004). In vitro RdRp activities have been described for mORV λ3 and BTV VP1, and a high-resolution structure of λ3 has been determined (Boyce et al., 2004; Tao et al., 2002). Undoubtedly, these discoveries have greatly enhanced our understanding of Reoviridae genome replication. Nonetheless, SA11 VP1 is the only Reoviridae RdRp with in vitro activities that recapitulate those needed to support virus replication in vivo, making it the most well-studied RdRp of the family (Patton, 2001; Patton and Spencer, 2000; Patton et al., 2004, 2007).

Core Shells Exhibit Pseudo $T = 1$ Icosahedral Symmetry

The structures of viral cores from individual members of the Totiviridae, Cystoviridae, and Reoviridae families have been analyzed to various degrees using cryo-electron microscopy (cryo-EM), X-ray crystallography, and three-dimensional (3D) image reconstruction (Butcher et al., 1997; Caston et al., 2006, 1997; Cheng et al., 1994; Fang et al., 2005; Grimes et al., 1998, 1997; Hewat et al., 1992; Hill et al., 1999; Huiskonen et al., 2006; Jaalinoja et al., 2007; Kainov et al., 2003; Lawton et al., 1997b; Lu et al., 1998; Metcalf et al., 1991; Naitow et al., 2002; Nakagawa et al., 2003; Prasad et al., 1996; Reinisch et al., 2000; Xia et al., 2003; Yeager et al., 1990; Zhang et al., 2005, 2003; Zhou et al., 2003). Although these core proteins show minimal primary amino acid sequence similarities, their architectural organizations are markedly conserved, even among diverse virus families. Specifically, the viral core shells are simple, relatively smooth, icosahedrons composed of 120 subunits of a thin protein (Fig. 11.3) (Jayaram et al., 2004; Kim et al., 2004; Mertens and

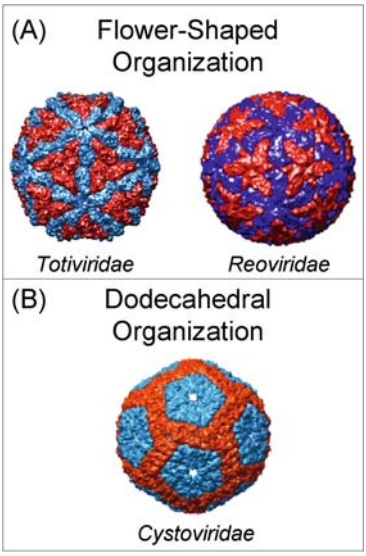

Fig. 11.3 Organization of pseudo $T = 1$ core shell proteins as visualized using cryo-EM. The core shells of Totiviridae, Cystoviridae, and Reoviridae are simple icosahedrons composed of 120 subunits of a thin protein organized as 60 asymmetric dimers. (**A**) Flower-like organization. The core proteins of Totiviridae and Reoviridae family members are arranged as a decamer around each icosahedral fivefold axis like the petals of a flower. Five copies of the A-form of the shell proteins (*blue*) cluster around the fivefold axis, while five copies of the structurally distinct B-form (*red*) are situated further away from the vertex, interdigitated between the A-forms. Cryo-EM images of L-A virus and the mORV core were generated using VIPERdb (Shepherd et al., 2006). (**B**) Dodecahedral organization. For the Cystoviridae family, five copies of the A-form (*blue*) tightly encircle the fivefold axis, whereas the B-form (*red*) makes a dodecahedral skeleton bordering the A-forms. The cryo-EM image of the Φ6 core was adapted with permission from S.J. Butcher (Huiskonen et al., 2006).

Diprose, 2004). The stoichiometry of these cores indicates that they have a forbidden triangulation number of $T = 2$ (Caspar and Klug, 1962). However, the 120 subunits are organized in the core as 60 asymmetric dimers, allowing these structures to be more accurately described as having pseudo $T = 1$ symmetry (Caspar and Klug, 1962). The arrangement of the core shell proteins in such a manner requires that the individual monomers within a dimer unit adopt slightly different conformations (Caspar and Klug, 1962; Steven et al., 1997). Thus, even though the monomers are chemically identical, they are structurally quasi-equivalent molecules. Large aqueous channels traverse the core through each fivefold axis, providing portals for entry of nucleotides and divalent cations and conduits for the exit of viral (+)RNA following transcription (Jayaram et al., 2004; Kim et al., 2004; Mertens and Diprose, 2004). This arrangement of quasi-equivalent core shell proteins to yield pseudo $T = 1$ icosahedrons is a general feature of many dsRNA viruses, but is unique in that it is not seen elsewhere in nature (Steven et al., 1997).

Structural analyses demonstrate that the overall architectures of the pseudo $T = 1$ viral cores of dsRNA viruses are the same; yet, the organization of quasi-equivalent protein dimers is slightly different among some families. For example, the core proteins of Totiviridae and Reoviridae family members, such as L-A Gag, reovirus λ1, BTV VP3, and SA11 VP2, are arranged as a decamer around each icosahedral fivefold axis like the petals of a cupped, inverted flower (Fig. 11.3A) (Grimes et al., 1998; Naitow et al., 2002; Prasad et al., 1996; Reinisch et al., 2000). Precisely, five copies of one type of shell protein (A-form) cluster around the fivefold axis, while five copies of the second, structurally distinct type of the same protein (B-form) are situated further away from the vertex, interdigitated between the A-forms (Fig. 11.3A). In contrast to this flower-like organization seen in Totiviridae and Reoviridae, the core shell proteins of the Cystoviridae Φ6 show a dodecahedral organization (Fig. 11.3B) (Huiskonen et al., 2006; Jaalinoja et al., 2007; Kainov et al., 2003). Specifically, five copies of the A-form of Φ6 P1 tightly encircle the fivefold axis, whereas the B-form makes a dodecahedral skeleton bordering the A-forms (Fig. 11.3B). While the functional significance of these two types of pseudo $T = 1$ arrangements is not known, it has been proposed that the distinctive manner in which Φ6 P1 is organized allows for a generous expansion of the core upon RNA packaging (Huiskonen et al., 2006).

Locations of Viral Enzymes and dsRNA Inside the Core

One of the major functions of the pseudo $T = 1$ core shell of a dsRNA virus is to serve as a platform to which the viral RdRp and associated enzymes are attached. For the Totiviridae member L-A, the viral RdRp Pol is covalently linked to the pseudo $T = 1$ core shell protein Gag as a result of a translational fusion event (Wickner, 1996). The efficiency of this event suggests that two copies of Gag–Pol are incorporated into assembled L-A particles (Wickner, 1996). Although the Pol domains are not visualized in the L-A core structure, the orientation of Gag termini dictates that these RdRps be situated within the viral particle, essentially fixed to the inner wall and proximal to the icosahedral fivefold axis (Naitow et al., 2002). For the more complex Cystoviridae and Reoviridae families, there is an emerging view that the locations of the viral enzymes mirror what is seen for L-A, with the RdRp-containing complexes positioned underneath the vertices of the core shell. In support of this idea, the enzyme complexes of the SA11 have been visualized in cryo-EM image reconstructions as densities beneath the core shell layer at each of the 12 fivefold axes (Fig. 11.4) (Grimes et al., 1998; Prasad et al., 1996). Moreover, the high-resolution crystal structure of the mORV RdRp (λ3) has been modeled into the cryo-EM density map of the core (Zhang et al., 2003). These results are consistent with the other viruses, showing that mORV λ3 is anchored to the inner surface of the λ1 shell, slightly off-center from each fivefold axis (Zhang et al., 2003). Furthermore, the structural organization of dsRNA has been determined for several viruses and, in all cases, is seen as dodecahedral tubules packed in a larger radius around the viral enzymes (Fig. 11.4) (Grimes et al., 1998; Huiskonen et al.,

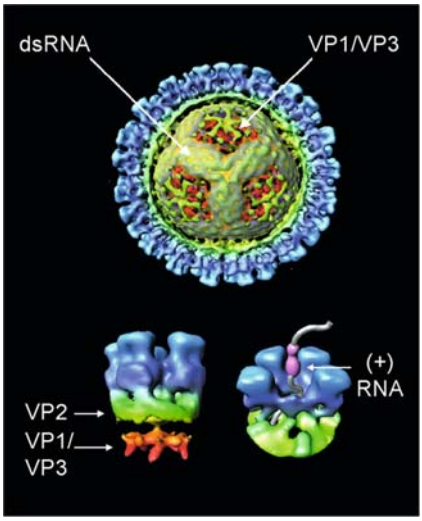

Fig. 11.4 Locations of viral enzymes and dsRNA inside the core. The enzyme complexes (*red*) of the rotavirus SA11 have been visualized in cryo-EM image reconstructions as densities beneath the VP2 core shell layer (*green*) at each of the 12 fivefold axes, and dsRNA (*yellow–gray*) is seen as dodecahedral tubules packed in a larger radius around the viral enzymes. A newly transcribed (+)RNA (*gray*) would acquire a 5′-cap (*purple*) prior to extrusion from the core via a fivefold channel. Images were modified with permission from B.V.V. Prasad (Prasad et al., 1996).

2006; Naitow et al., 2002; Prasad et al., 1996). This observation suggests that, for Cystoviridae and Reoviridae, each of the 12 enzyme complexes is dedicated to transcribing and replicating a single genome segment. Thus, for dsRNA viruses with less than 12 genome segments, some of the vertices will have orphaned enzyme complexes. Alternatively, it is possible that such viruses package only one enzyme complex per dsRNA segment, meaning that they would have some empty vertices. Either way, viral cores may be viewed as a collection of RdRp units, which operate independently and simultaneously during viral replication.

The structural location of the viral enzymes suggests a system for RNA synthesis in which a freely moving template RNA is pulled through a tethered RdRp. During genome replication, the RdRp would be bound to an assembling core shell intermediate, such as a decamer or pro-core (see later section). The (+)RNA would either be pulled into the core during the assembly process or be inserted into an already assembled core-like structure. While anchored near the vertex of the core shell intermediate, the RdRp catalyzes (–)RNA strand synthesis, converting the (+)RNA template into a complete dsRNA genome segment. During transcription, the RdRp uses the (–)RNA strand of the endogenous dsRNA segment, which encircles the enzyme, as template for (+)RNA synthesis. The position of the RdRp near the fivefold axis would allow the nascent (+)RNA to pass directly into the channel along or near the fivefold axis en route to virion exit. Although Totiviridae and Cystoviridae synthesize uncapped (+)RNAs during transcription, the transcripts of Reoviridae family members must acquire a

5′-cap in order to be efficient templates for translation. For mORV, nascent (+)RNAs would be capped as they navigate the hollow chamber of each projecting fivefold λ2 turret (Zhang et al., 2003). Because non-turreted BTV and SA11 have their RNA capping enzymes juxtaposed to their RdRps, a newly synthesized (+)RNA molecule would obtain a 5′-cap prior to being extruded from the core (Fig. 11.4) (Grimes et al., 1998; Prasad et al., 1996). A reconstruction of SA11 and BTV particles in the act of transcription revealed that they have the capacity to synthesize high levels of (+)RNA for several hours, indicating that the RdRp efficiently reengages the dsRNA template numerous times (Diprose et al., 2001; Lawton et al., 1997a). Indeed, the core shell protein might serve as a scaffold on which the dsRNA duplex is melted and repeatedly transcribed by the anchored RdRp. Nonetheless, the precise protein–protein and protein–RNA interactions that govern the placement of enzymes and RNA within the core shell are not fully understood.

RdRp Structures Highlight Mechanisms of RNA Synthesis

The recently solved high-resolution crystal structures of two dsRNA viral RdRps, Cystoviridae Φ6 P2 and Reoviridae mORV λ3, have greatly enhanced our understanding of how these viruses mediate RNA synthesis (Butcher et al., 2001; Tao et al., 2002). The overall fold of these proteins is analogous to that of all known RdRps and can be described as resembling a hollow, cupped right hand with fingers, palm, and thumb sub-domains and an internally located active site (Fig. 11.5) (O'Reilly and Kao, 1998). However, the structures of Φ6 P2 and mORV λ3 have embellishments on the basic RdRp architecture. Specifically, Φ6 P2 has a small amino-terminal extension that straps together the finger and thumb sub-domains, essentially closing the enzyme (Fig. 11.5A) (Butcher et al., 2001). The Φ6 P2 structure also has a carboxy-terminal loop that protrudes into the central cavity of the enzyme (Fig. 11.5A) (Butcher et al., 2001). This loop is referred to as the initiation platform and is important for the de novo initiation of RNA synthesis (see below). The mORV λ3 structure shows large amino- and carboxy-terminal elaborations that form a cage around the catalytic right hand (Fig. 11.5B–E). Like that of Φ6 P2, the amino-terminal domain of mORV λ3 reinforces the bridge between the fingers and thumb, also supporting the closure of the polymerase (Fig. 11.5D) (Tao et al., 2002). Yet, the carboxy terminus of mORV λ3 forms a ring-shaped bracelet that is entirely absent in all other RdRps whose structures are known (Fig. 11.5E) (Tao et al., 2002). This bracelet domain is reminiscent of the clamps of DNA polymerases, which open and close upon templates (Mossi and Hubscher, 1998). Even so, unlike DNA polymerase clamps, the bracelet domain of λ3 likely remains closed and slides along the template during polymerization. The structures of Φ6 P2 and mORV λ3 also show several hollow tunnels that allow the RNA template, nucleotides, and divalent cations to access the catalytic site and to permit the exit of nascent RNA (Fig. 11.6). Both enzymes have a single nucleotide entry tunnel on one side as well as a single template entry tunnel approximately 90° away near the top of the protein (Fig. 11.6). Nonetheless, Φ6 P2 and mORV λ3 have different

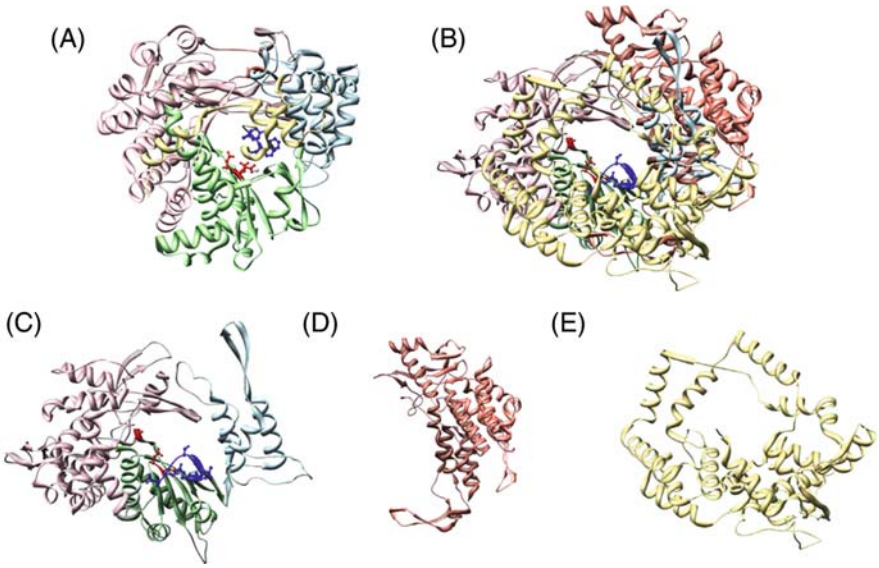

Fig. 11.5 Structures of the Φ6 and mORV RdRps. The overall fold of the enzymes resembles a hollow, cupped right hand with fingers (*light pink*), palm (*green*), and thumb (*light blue*) sub-domains and an internally located active site (catalytic aspartic acids; *red*). Flexible regions of the enzymes involved in initiation complex formation are shown in *blue*. (**A**) Φ6 P2 structure. The small amino-terminal extension that straps together the finger and thumb sub-domains, closing the enzyme is shown at the top of the molecule in *dark pink*. The carboxy-terminal loop that protrudes into the central cavity of the enzyme is shown in *yellow*. (**B**) mORV λ3 structure. The large amino- and carboxy-terminal elaborations that form a cage around the catalytic right hand are shown in *dark pink* and *yellow*, respectively. (**C**) mORV λ3 right-hand pol domain structure with fingers, palm, and thumb sub-domains. (**D**) mORV λ3 amino-terminal elaboration. (**E**) mORV λ3 carboxy-terminal bracelet. The PDB numbers for Φ6 P2 and mORV λ3 are 1MUK and 1HI8, respectively (Butcher et al., 2001; Tao et al., 2002). Images were generated using Chimera computer program (Petterson et al., 2004).

numbers of RNA exit tunnels, reflecting the divergent transcription mechanisms of these viruses. Because Cystoviridae transcription occurs using a semi-conservative mechanism, Φ6 P2 has a single tunnel for the exit of a dsRNA product, making it a three-tunneled RdRp (Fig. 11.6A). In contrast, members of the Reoviridae family use a fully conservative mechanism of transcription, meaning that the RdRp separates the dsRNA product into the nascent (+)RNA and parental (–)RNA strands prior to their exit. This separation requires mORV λ3 to have two RNA exit tunnels, making it a four-tunneled RdRp (Fig. 11.6B).

The semi-conservative and conservative transcription mechanisms of Cystoviridae and Reoviridae, respectively, both use the endogenous dsRNA genome as template for (+)RNA synthesis (Fig. 11.7A and B). For mORV and Φ6, a dsRNA genome segment is separated into a (–)RNA strand, which accesses the RdRp via the template entry tunnel, and a (+)RNA strand that is "peeled-off" away from the enzyme (Fig. 11.7A and B). During Φ6 transcription, the parental (+)RNA strand is shuttled out of the core, while the parental (–)RNA strand is used as a template

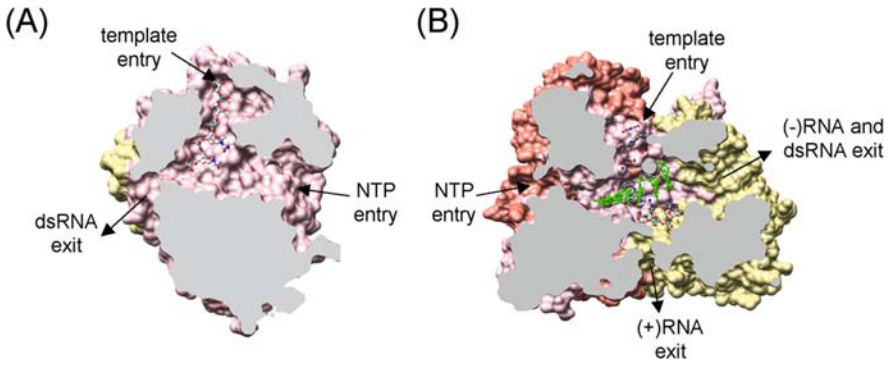

Fig. 11.6 Locations of tunnels within the Φ6 P2 and mORV λ3 structures. The structures of Φ6 P2 and mORV λ3 show several hollow tunnels that allow the RNA template, nucleotides, and divalent cations to access the catalytic site and to permit the exit of nascent RNA. Protein domains are colored as described in Fig. 11.5. An RNA template is shown in the entry tunnels of both structures and is element colored. (**A**) Φ6 P2 has a single tunnel for the exit of a dsRNA product, making it a three-tunneled RdRp. (**B**) mORV λ3 has two RNA exit tunnels, one for (+)RNA and one for (–)RNA and dsRNA, making it a four-tunneled RdRp. A nascent RNA strand is shown in *green*. The PDB numbers for Φ6 P2 and mORV λ3 are 1HHT and 1N35, respectively (Butcher et al., 2001; Tao et al., 2002). Images were generated using the Chimera computer program (Petterson et al., 2004).

for nascent (+)RNA strand synthesis (Fig. 11.7A). The product of semi-conservative Φ6 transcription is a dsRNA duplex composed of nascent the (+)RNA strand paired with the parental (–)RNA strand, which is released from P2 via the single RNA exit tunnel (Fig. 11.7A). This dsRNA molecule is separated again, the (+)RNA transcript is shuttled out of the core, and the (–)RNA strand is used as a template for another round of transcription. On the contrary, during fully conservative mORV transcription, the parental (+)RNA strand that is "peeled-off" the dsRNA segment stays inside the core and waits to reanneal with its complementary (–)RNA strand (Fig. 11.7B). Meanwhile, the parental (–)RNA strand enters λ3 and is used as a template for nascent (+)RNA strand synthesis, made initially as a dsRNA duplex (Fig. 11.7B). Unlike what is described for Φ6 P2, however, mORV λ3 quickly separates the strands of the newly made dsRNA duplex, allowing the parental (–)RNA strand and the nascent (+)RNA strand to exit the enzyme via individual tunnels (Fig. 11.7B). Following release of the two strands from the enzyme, the parental (–)RNA strand base pairs with its initial (+)RNA partner to reform the original dsRNA segment, while the nascent (+)RNA transcript acquires a 5′-cap as it is extruded from the core (Fig. 11.7B). By allowing the parental (+)RNA strand to remain in the core and reanneal with its (–)RNA partner, mORV ensures multiple rounds of transcription from the same dsRNA genome segment. Although dsRNA viral RdRps catalyze several cycles of transcription from a single dsRNA segment, packaged (+)RNA strand is used just once as template during genome replication. This (+)RNA template accesses the active site via the template entry channel and serves to catalyze (–)RNA strand synthesis, forming a dsRNA duplex. For Cystoviridae family members, the dsRNA duplex exits the RdRp in the same manner as

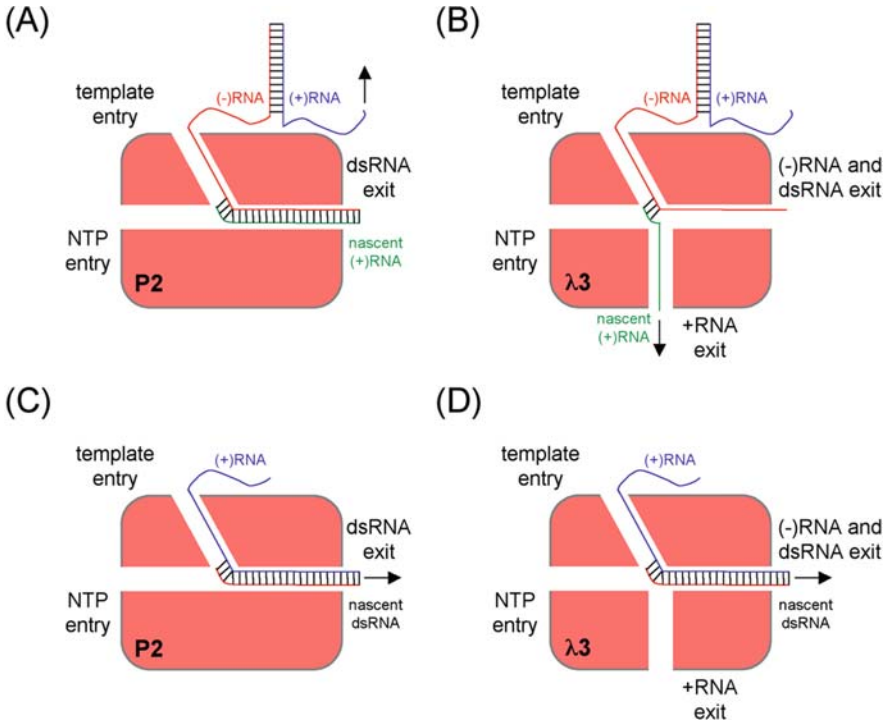

Fig. 11.7 Semi-conservative and conservative mechanisms of RNA synthesis. The schematic shown above illustrates the RNA synthesis mechanisms of Φ6 P2 and mORV λ3. (**A**) Φ6 P2 semi-conservative transcription. A dsRNA genome segment is separated into a (–)RNA strand (*red*), which accesses the RdRp via the template entry tunnel, and a (+)RNA strand (*blue*) that is "peeled-off" away from the enzyme. The parental (+)RNA strand is shuttled out of the core (*arrow*), while the parental (–)RNA strand (*red*) is used as a template for nascent (+)RNA strand synthesis (*green*). The product of semi-conservative Φ6 transcription is a dsRNA duplex, which is released from P2 via the single RNA exit tunnel. (**B**) mORV λ3 conservative transcription. The parental (+)RNA strand (*blue*) that is "peeled-off" the dsRNA segment stays inside the core; meanwhile, the parental (–)RNA strand (*red*) enters λ3 and is used as a template for nascent (+)RNA strand synthesis (*green*), made initially as a dsRNA duplex that is quickly separated. The parental (–)RNA strand (*red*) and the nascent (+)RNA strand (*green*) exit the enzyme via individual tunnels. The nascent (+)RNA transcript (*green*) acquires a 5′-cap as it is extruded from the core (*arrow*). (**C**) Φ6 P2 genome replication. The (+)RNA template (*blue*) accesses the active site via the template entry channel and serves to catalyze (–)RNA strand synthesis (*red*), forming a dsRNA duplex that exits the RdRp in the same manner as during transcription. (**D**) mORV λ3 genome replication. The RdRp uses the (+)RNA template (*blue*) to catalyze (–)RNA strand synthesis (*red*), forming a dsRNA duplex that leaves the enzyme using the (–)RNA exit tunnel.

during transcription (Fig. 11.7C). The Reoviridae members allow the dsRNA duplex to leave the enzyme using the tunnel that, during transcription, is designated for (–)RNA exit (Fig. 11.7D). Cystoviridae and Reoviridae RdRps differentiate among transcription and replication templates by recognizing *cis*-acting RNA elements with different affinities (see later section).

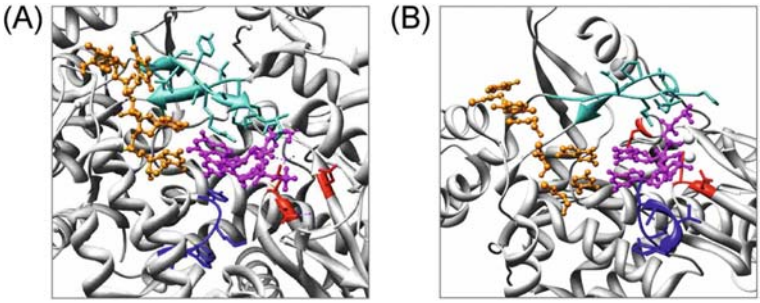

Fig. 11.8 Initiation complex formation for Φ6 P2 and mORV λ3. The Φ6 and mORV RdRps each have a region of the protein that functions as a "stage" on which an initiation complex is constructed. (**A**) The Φ6 P2 initiation platform. During Φ6 RNA synthesis, incoming initiatory nucleotides (*pink*) are stabilized by the P2 carboxy-terminal plug (*blue*) and a motif-F-like structure (*teal*), allowing them to base pair with the RNA template (*gold*) near the catalytic aspartic acids (*red*). (**B**) The mORV λ3 priming loop. For mORV RNA synthesis, the RNA template (*gold*) forms tight stacking interactions against the priming loop (*blue*), which is formed by the residues in the tip of the fingers and the palm sub-domains and stabilized by motif-F (*teal*). The priming loop allows the template (*gold*) to base pair with incoming nucleotides (*pink*). The PDB numbers for Φ6 P2 and mORV λ3 are 1HIO and 1NIH, respectively (Butcher et al., 2001; Tao et al., 2002). Images were generated using the Chimera computer program (Petterson et al., 2004).

The structures of Φ6 P2 and mORV λ3, complexed with nucleotides and RNA, also suggest a mechanism for the de novo initiation of RNA polymerization (Fig. 11.8) (Butcher et al., 2001; Tao et al., 2002). The defining features of de novo RNA synthesis are that no information in the viral genome is lost, and that additional proteins for priming are unnecessary (Makeyev and Grimes, 2004). However, initiation without a primer requires specific molecular interactions to occur between the template and incoming nucleotides in order to keep them correctly positioned at the RdRp active site (Makeyev and Grimes, 2004). To aid in forming these stable interactions, many viral RdRps have a region of the protein that functions as a "stage" on which an initiation complex is constructed. For the Cystoviridae member Φ6, the carboxy-terminal plug provides such a "stage" and is referred to as the initiation platform (Butcher et al., 2001). During Φ6 RNA synthesis, the RNA template enters P2 and is stabilized by the plug, allowing it to base pair with incoming initiatory nucleotides near the active site (Fig. 11.8A). In the course of elongation, the carboxy-terminal plug presumably moves to allow the dsRNA product to egress from the RdRp active site. For mORV, the incoming nucleotides enter λ3 and are stabilized against the priming loop, which is formed by the residues in the tip of the fingers and the palm sub-domains (Fig. 11.8B) (Tao et al., 2002). Thus, the priming loop functions as a "stage" for λ3, allowing the incoming nucleotides to base pair with the RNA template. Like the Φ6 P2 plug, the λ3 priming loop shifts its location following initiation of phosphodiester bond formation so as not to block the elongating dsRNA duplex. Thus, the structures of Φ6 P2 and mORV λ3 demonstrate that these viruses have evolved different strategies for forming a stable initiation complex that allows effective RNA synthesis in the absence of a primer.

Biochemical Studies of dsRNA Viral Genome Replication

In addition to structural studies, elegant biochemical experiments have elucidated many important details regarding Totiviridae, Cystoviridae, and Reoviridae genome replication. The catalytic process of viral dsRNA synthesis has predominantly been studied using core particles that have been disrupted by incubation in hypotonic conditions. For the Totiviridae and Reoviridae members, L-A and SA11, such disrupted cores possess RdRp activity when incubated in the presence of nucleotides, divalent cations, and exogenous (+)RNA (Chen et al., 1994; Fujimura and Wickner, 1989). These assays were instrumental in locating the *cis*-acting replication signals in (+)RNA templates for these viruses (see later section). However, the open core systems of L-A and SA11 fail to package the dsRNA products of replication, suggesting that they do not fully recapitulate in vivo processes. Moreover, these systems do not allow for directed mutagenesis of individual core protein components to study their roles during dsRNA synthesis. The discovery that recombinant SA11 VP1 is capable of catalyzing dsRNA synthesis was a breakthrough for clarifying the functions of proteins during each stage of genome replication (Patton et al., 1997). An interesting feature of this enzyme is that it requires the core shell protein VP2 for biochemical activity (Patton et al., 1997). This phenomenon suggests that in vitro VP1 catalyzes dsRNA synthesis in a manner connected to core assembly. Using these recombinant proteins, the molar ratio of VP1:VP2 required for maximum dsRNA synthesis was determined to be 1:10 (Patton et al., 1997; Tortorici et al., 2003). This ratio mimics that of each decamer of the rotavirus core, indicating that activation of the RdRp might require the formation of an assembly intermediate. Deletion mutagenesis studies have shown that the amino terminus of VP2 contains a domain critical for interactions with VP1, VP3, and RNA. Specifically, an amino-terminally truncated VP2 fails to induce dsRNA synthesis and does not encapsidate the enzyme complex or RNA, but does assemble into core-like particles (Labbe et al., 1994; Lawton et al., 1997b; Patton et al., 1997; Zeng et al., 1998). Although the precise mechanism by which VP2 triggers the function of VP1 is unknown, it is possible that the amino terminus of VP2 forms an internal platform inside the core at the fivefold axis and on which the RdRp operates.

Biochemical studies of SA11 dsRNA synthesis using open cores or recombinant proteins have characterized the requirements for the initiation of genome replication. Particularly, the formation of a (–)RNA strand initiation complex was shown to be a salt-sensitive process that requires the RdRp VP1, the core shell protein VP2, rGTP, Mg^{2+}, and template (+)RNA (Chen and Patton, 2000; Tortorici et al., 2003). The need for rGTP is likely a reflection of the 3′-terminal nucleotides (CC) of all rotavirus template (+)RNAs (see later section). The divalent cation Mg^{2+} is a common cofactor for RdRps, but in these reactions, the addition of Mn^{2+} stimulates the activity of recombinant VP1 (O'Reilly and Kao, 1998; Patton et al., 2004; Tortorici et al., 2003). Importantly, the observation that VP2 must be preincubated with VP1 prior to the elongation step suggests that this protein functions at the initiation step of dsRNA synthesis. VP1 has been shown to interact with (+)RNA and rNTPs in

the absence of VP2 and in the presence of salt, demonstrating that (i) VP2 does not merely function to bring together the template, nucleotides, and enzyme and (ii) the salt-sensitivity of complex formation is not due to the lack of template or nucleotide recognition by VP1. Still, it remains unclear exactly how VP2 activates VP1 to initiate dsRNA synthesis (Chen and Patton, 2000; Patton, 1996; Tortorici et al., 2003). Currently, there is no atomic structure for VP1, but sequence comparisons would lead to the prediction that this enzyme is very similar to mORV λ3 (Patton et al., 2007; Tao et al., 2002). The RdRp activity of recombinant mORV λ3, while very minimal in comparison to both mORV open cores and SA11 VP1, does not require the presence of the core shell protein λ1. It will be interesting to determine whether the structures of SA11 VP1 and mORV λ3 show differences in the locations of residues required for initiating RNA synthesis.

The Cystoviridae Φ6 RdRp P2 is capable of catalyzing both RNA replication and transcription activities in vitro as a single viral protein (Makeyev and Grimes, 2004). The minimal requirements for in vitro initiation and elongation using purified P2 are nucleotides, Mg^{2+}, and (+)RNA template (Ojala and Bamford, 1995). The recombinant enzyme shows a high specific activity, but exhibits decreased template specificity in comparison to the Φ6 open core system. Still, purified P2 self-assembles together with P1, P4, and P7 into viral cores that are fully functional for both RNA packaging and genome replication (Mindich, 2004; Poranen and Tuma, 2004). No such packaging system exists for the Totiviridae and Reoviridae families, making studies of Φ6 important to our understanding of how dsRNA synthesis is connected to capsid assembly.

Genome Replication and Capsid Assembly Occur Simultaneously

To protect newly made dsRNA from the host cell antiviral response, (+)RNA packaging into a core-like intermediate is thought to precede genome replication. Core assembly intermediates of the Cystoviridae member Φ6, called pro-cores, can be made using recombinant proteins P1, P2, P4, and P7 (Mindich, 2004; Poranen and Tuma, 2004). These pro-cores are stimulated to package and replicate the (+)RNA templates (S+, M+, and L+) by incubation in polyethylene glycol, ADP, Mg^{2+}, and rNTPs (Mindich, 2004; Poranen and Tuma, 2004). The reaction is consecutive in that S+ is packaged first, followed by M+ and then L+. A hexamer of the NTPase protein (P4) mediates the bulk of (+)RNA packaging at a single fivefold axis, but a cofactor protein (P7) enhances the efficiency of this process (Mindich, 2004; Poranen and Tuma, 2004). Studies indicate that P4 functions like a molecular motor, powering the entry of the (+)RNA molecules into the pro-core. Only after all three templates are packaged inside the pro-core does (–)RNA strand synthesis begin, converting the (+)RNAs into the full-length genome segments (S, M, and L). Forceful expansion of the core shell as a result of RNA packaging is thought to trig-

ger dsRNA synthesis. The prerequisite packaging of (+)RNA into pre-formed core intermediates undoubtedly links this process with genome replication.

Much less is known about how Totiviridae and Reoviridae members package their (+)RNA templates, but limited studies support the hypothesis that this process is linked to genome replication. The fact the L-A viral RdRp (Pol) is fused to the core shell protein (Gag) makes is difficult to argue against the model in which the single (+)RNA template is bound by Gag–Pol during Totiviridae particle formation (Wickner, 1996). For the Reoviridae, however, the presence of 10–12 different (+)RNAs and numerous separate proteins makes understanding the precise pathway of assembly and replication more difficult. Attempts have been made to define this pathway for the rotavirus SA11 by isolating replication intermediates (RIs) from infected cells (Gallegos and Patton, 1989; Patton and Gallegos, 1990). These studies suggest that an initial interaction occurs between the RdRp VP1, the capping enzyme VP3, and a single (+)RNA template, forming a pre-core RI that lacks polymerase activity. Thereafter, a VP2 decamer interacts with a pre-core RI to form a core RI, which is capable of initiating dsRNA synthesis. It is unclear whether genome replication occurs prior to, at the same time as, or after the core RIs close into a complete pseudo $T = 1$ icosahedron. Treatment of core RIs with RNase causes the degradation of (+)RNA templates and abolishes genome replication. In contrast, replicated dsRNAs are protected from RNase degradation, suggesting that the products of replication are protected, possibly in a closed core. In further support of the idea that packaging precedes dsRNA synthesis, free dsRNA has never been detected in rotavirus-infected cells and the *cis*-acting packaging signals are located in the (+)RNAs, but are masked in the dsRNA products (Patton et al., 2007). Importantly, the requirement of SA11 VP2 for binding VP1, VP3, and RNA, and for triggering genome replication ensures that dsRNAs are not produced until cores are available for their protection. Nonetheless, it remains a complete mystery how Reoviridae family members incorporate one of each genome segment in equimolar amounts into viral cores. Also, the functions of SA11 nonstructural proteins during packaging and replication are unclear.

The role of the BTV core shell protein (VP3) during the early stages of particle assembly has been analyzed using deletion mutagenesis (Kar et al., 2004). These studies showed that deletion of residues at either the amino or carboxy terminus of VP3 did not affect its interactions with the enzyme complex proteins (VP1, VP4, and VP6), but that these mutant cores were extremely unstable. Still, deletion of the VP3 carboxy terminus abolished VP3–RNA binding and the formation of complete icosahedrons. These results suggest that the amino terminus of BTV VP3 is dispensable for encapsidating the RdRp-containing enzyme complex and RNA during assembly. The results further suggest that the RNA-binding domain of BTV lies in the carboxy terminus of the protein, overlapping with an oligomerization domain. These results with BTV are in contrast to what has been determined for SA11 VP2, which forms stable cores in the absence of its amino terminus, but does not bind VP1, VP3, or RNA (Labbe et al., 1994; Lawton et al., 1997b; Patton et al., 1997; Zeng et al., 1998). Because the BTV RdRp VP1 has in vitro activity in the absence of its core shell protein, it remains unknown how these assembly

mutations alter genome replication. Yet, the reported differences in the core protein–protein interactions between BTV and SA11 suggest that individual Reoviridae family members might have distinct pathways of forming core replication intermediates.

Cis-Acting RNA Signals Determine Template Specificity

In the course of packaging and replication, a dsRNA virus must pick the correct viral (+)RNA molecules from a sea of cellular ones. This specificity is attributed to the presence of *cis*-acting signals that selectively channel the viral RNAs into the assembly and replication complexes. For L-A, a stem-loop structure (internal site) in the (+)RNA forms the packaging signal that is recognized by the Pol domain of the Gag–Pol fusion protein during assembly (Esteban et al., 1989). Conversion of this packaged (+)RNA to dsRNA requires this internal site, as well as sequences in the 3′-end of the template (Fujimura et al., 1992; Wickner et al., 1986). Because the internal site is 400 nucleotides from the 3′-end, it is thought that secondary and tertiary folding of the molecule brings these two sites together during L-A replication. The Cystoviridae member Φ6 specifically recognizes its RNA template based on a conserved 18-nt sequence at the 5′-end, as well as an upstream *pac* sequence that is unique in each segment. The *pac* sequence of each segment folds into a distinctive stem-loop structure required for organized packaging (Gottlieb et al., 1994; Mindich, 2004). Efficient Φ6 (–)RNA strand synthesis follows packaging and requires that the templates have the 3′-sequence 5′-CUCUCUCUCU-3′ (Mindich, 2004; Onodera et al., 1993). Template RNAs lacking this 3′-sequence are packaged, but not replicated, demonstrating that an additional level of specificity occurs during dsRNA synthesis (Onodera et al., 1993).

Little is known regarding the precise *cis*-acting signals underlying gene-specific packaging of (+)RNA for the Reoviridae family. Recent studies using an in vitro rescue system have provided evidence that the packaging signals of mORV reside at the 5′-end of (+)RNAs (Roner and Steele, 2007). Specifically, chimeric (+)RNA molecules that contain the 5′-end of mORV m1 or s2 genes fused to the open reading frame (ORF) of reporter genes are specifically packaged and replicated by helper virus cores (Roner and Steele, 2007). The 5′-ends of the m1 and s2 genes show little sequence similarities, but each is predicted to form a stem-loop structure that might serve as a recognition signal for core proteins. For rotavirus, sequences in the 5′- and 3′-untranslated regions (UTRs) of homologous genes from distantly related strains maintain a high level of conservation (Patton et al., 2007). This sequence conservation is observed for the same gene of different strains, even when the ORF is extremely variable, suggesting that UTRs might contain important *cis*-acting signals for packaging. Moreover, the observation that UTRs from heterologous genes of the same virus are different suggests that these regions form gene-specific signals. Though it is not well understood, the mechanism of (+)RNA packaging for Reoviridae is undoubtedly a strictly regulated process. The result of this meticulous

process is the formation of a viral core that contains exactly one copy each of the 10–12 dsRNA genome segments.

The SA11 open core system has allowed considerable progress in defining the *cis*-acting signals in (+)RNAs that support genome replication for the Reoviridae family. These studies have identified two elements within all SA11 template RNAs that promote efficient dsRNA synthesis: (i) a 3′-terminal consensus sequence (3′CS) 5′-UGUGACC-3′ and (ii) a panhandle structure formed by sequences in the 5′- and 3′-UTRs (Chen et al., 2001; Patton et al., 1999, 1996; Tortorici et al., 2003, 2006; Wentz et al., 1996). Of these two elements, the highly conserved 3′CS is the most important, as a deletion of this region in the context of a viral (+)RNA template completely abolishes replication (Patton et al., 1996). The 3′CS is composed of two partially overlapping determinants that mediate specific template recognition by VP1 and productive initiation complex formation. As defined by electromobility shift assays, the UGUG(A) portion of the 3′CS drives high-affinity interactions with VP1 (Chen et al., 2001; Tortorici et al., 2003). In contrast, the terminal ACC nucleotides of the 3′CS are dispensable for VP1 binding, but are important for the formation of a (–)RNA strand initiation complex in vitro (Chen et al., 2001; Tortorici et al., 2003). In addition to the 3′CS, the formation of a panhandle structure, as a result of base pairing between the 5′-UTR and 3′-UTR, is important for genome replication. Specifically, mutations that prevent the panhandle structure from forming a free single-stranded 3′-end completely inhibit replication, even in the presence of an intact 3′CS (Chen and Patton, 1998; Tortorici et al., 2006). This result suggests that the 3′CS must be presented to the RdRp as a single-stranded template, allowing it to be sterically accessible for initiation complex formation. It is also possible that these panhandle structures reveal the putative gene-specific packaging signals in UTRs. Thus, besides determining specificity during dsRNA synthesis, the panhandle structure might also promote the proper assortment of the (+)RNAs during packaging.

Indeed, these *cis*-acting signals in (+)RNA templates are important to efficient (–)RNA strand synthesis during SA11 genome replication. However, it is important to note that SA11 VP1 is capable of using (–)RNA templates, which lack both a 3′CS and a panhandle structure, for multiple rounds of transcription. The 3′-ends of SA11 (–)RNA strands show a less conserved sequence of 5′-$(A/U)_6$AGCC-3′ that is thought to be recognized by VP1 during transcription, but with a lower affinity than the 3′CS of (+)RNAs (Patton et al., 2007). From a biological standpoint, this possibility is realistic because the (–)RNA strand of a genomic dsRNA segment would already be paired with the RdRp inside the core. Thus, there would be no need for VP1 to be selective about which template to use during transcription; essentially, the enzyme has only one choice. Quite the opposite occurs during genome replication when the RdRp must identify the correct (+)RNA among a cadre of cellular RNAs. This requires that the RdRp be very particular, as the wrong choice would cause the generation of a defective particle. The idea that the affinity of template binding determines specificity is supported experimentally for RdRps of the Reoviridae family, yet these principles are likely to apply to the Totiviridae and Cystoviridae RdRps as well.

Summary and Conclusions

Structural and biochemical studies of prototypical members of the Totiviridae, Cystoviridae, and Reoviridae families have identified common themes in the replication strategies of dsRNA viruses. From these studies, it is clear that viral RNA synthesis occurs within a protected core via an anchored RdRp. The evidence to date is also consistent with the ideas that genome replication and capsid assembly occur simultaneously and *cis*-acting elements in the viral RNA determine template specificity. Such precise regulation of core-associated RNA synthesis ensures that the precious dsRNA genome is protected during the entire replication cycle of these viruses. However, several unanswered questions remain about the exact mechanisms Totiviridae, Cystoviridae, and Reoviridae members use to mediate RNA packaging, core assembly, and genome replication. What region(s) within the core shell proteins are important for interactions with the viral enzymes and RNA? Which domain(s) of the RdRp directly engage the core shell and/or other viral enzymes? Do the viral enzymes remain tethered to the inside of the core shell during all stages of viral RNA synthesis? What changes occur inside the core following viral entry/uncoating that trigger transcription? What changes occur inside the core during RNA packaging that trigger genome replication? How do segmented dsRNA viruses package equimolar ratios of genome segments? Is RNA packaging coordinated by protein–RNA interactions only or are RNA–RNA interactions among segments important too? What are the roles of viral nonstructural proteins during packaging and replication? Certainly, future studies addressing these important questions related to dsRNA replication are warranted.

This chapter focuses on the replication strategies of dsRNA viruses that retain their genomes within a pseudo $T = 1$ core, as they are the most numerous in terms of individual species. Yet, it is important to mention that not all dsRNA virus families have this pseudo $T = 1$ core-associated mechanism of genome replication; instead, there are some intriguing exceptions to the described themes (Table 11.1). For example, the core shells of Chrysoviridae family members are similar to pseudo $T = 1$ cores, but are composed of 60 protein subunits instead of dimers, making them classic $T = 1$ structures (Mertens, 2004). Interestingly, viruses within this family package their four dsRNA segments in separate core shells, rather than together, circumventing the need for gene-specific packaging signals (Mertens, 2004). In addition, viruses belonging to the Birnaviridae family have single-shelled particles that show $T = 13$ icosahedral symmetry and are nearly identical to the structure of Reoviridae outer virion layers (Coulibaly et al., 2005). The Birnaviridae members also have a VPg-like protein linked to the 5′-ends of their bisegmented genome, a feature that is seen in several positive-strand RNA viruses. Furthermore, the Hypoviridae family members have a replication strategy that is more similar to that of positive-strand RNA viruses than to other dsRNA viruses. Specifically, members of this family completely lack a core shell and replicate their single dsRNA genome segment in association with cellular membranes (Jacob-Wilk et al., 2006). It is thought that positive-strand RNA viruses mediate RNA synthesis in association with vesicular or invaginated membranes to protect their dsRNA replication

intermediates from detection by the host cell antiviral system (Ahlquist, 2006). Therefore, membranous positive-strand viral replication complexes and dsRNA viral cores can be thought of as functionally analogous structures. Although these shared features cannot distinguish divergent from convergent evolution, these parallels suggest that positive-strand RNA and dsRNA viruses might have an ancestral linkage. Ongoing and future studies of viral genome replication are sure to reveal more unifying themes that link seemingly diverse families to each other. Such studies will not only enhance our understanding of how viruses spread but will also help identify important targets for limiting the impact of viral diseases.

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