



REVIEW

RNA chaperones encoded by RNA viruses

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RNAs are functionally diverse macromolecules whose proper functions rely strictly upon their correct tertiary structures. However, because of their high structural flexibility, correct folding of RNAs is challenging and slow. Therefore, cells and viruses encode a variety of RNA remodeling proteins, including helicases and RNA chaperones. In RNA viruses, these proteins are believed to play pivotal roles in all the processes involving viral RNAs during the life cycle. RNA helicases have been studied extensively for decades, whereas RNA chaperones, particularly virus-encoded RNA chaperones, are often overlooked. This review describes the activities of RNA chaperones encoded by RNA viruses, particularly the ones identified and characterized in recent years, and the functions of these proteins in different steps of viral life cycles, and presents an overview of this unique group of proteins.

KEYWORDS RNA viruses; RNA chaperone; ATP-independent helix-destabilizing activity; kinetic trap; viral life cycle

INTRODUCTION

Most RNA molecules rely on correct tertiary structures for their proper functions. However, owing to their extreme structural flexibility, correct folding of RNAs appears to be a difficult process. RNAs can easily become kinetically trapped in alternative secondary and tertiary structures that are misfolded and inactive. However, because these alternative misfolded structures of RNAs contain a multitude of base-pairings and duplexes that are locally stable, they are very hard to refold. Such a state of RNA misfolding is named a “kinetic trap” (Chauhan and Woodson, 2008).

To deal with this problem, hosts and viruses encode “helper” or RNA remodeling proteins, such as ATP-independent RNA chaperones and ATP-dependent RNA helicases. These proteins can destabilize RNA-RNA or RNA-DNA base-pairings, lowering the thermodynamic barrier between RNA conformations to help RNA es-

cape from kinetic traps and refold to form proper RNA structures (Chauhan and Woodson, 2008). RNA remodeling proteins play critical roles in almost all processes of RNA metabolism.

RNA remodeling proteins are comprised of two large, distinct groups of proteins: helicases and chaperones. RNA helicases are highly similar to DNA helicases and use the energy provided by ATP hydrolysis to unwind RNA duplexes. These proteins are believed to participate in most ATP-dependent rearrangements of structured RNAs and are classified into six superfamilies (SFs) based on their conserved helicase motifs (Kadare and Haenni, 1997). Among these helicase superfamilies, SF1 and SF2 are the two largest and most closely related ones, and these SF1 and SF21 enzymes are monomeric and contain at least seven conserved motifs that mediate nucleic acid-binding, ATP-binding, ATP hydrolysis and conformational change of the helicases (Kadare and Haenni, 1997). Various RNA viruses have been found to encode their own RNA helicases, including brome mosaic virus (BMV) (Ahola et al., 2000; Wang et al., 2005), enterovirus (Mirzayan and Wimmer, 1992; Teterina et al., 1992; Xia et al., 2015), flavivirus (Gu et al., 2000), alphavirus (Rikkonen, 1996), alphatetravirus (Wang et al., 2012), and nidovirus (Oleksiewicz et al., 2000).

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Unlike RNA helicases, RNA chaperones are heterogeneous proteins that have no conserved motifs but are still able to destabilize or unwind RNA duplexes and promote the formation of correctly folded RNA structures by helping misfolded RNAs escape kinetic traps. RNA chaperones, however, do not require ATP binding or hydrolysis for RNA remodeling (Musier-Forsyth, 2010). The definition of RNA chaperones is somewhat uncertain, and some authors define RNA helicases as an ATP-dependent subgroup of RNA chaperones (Oleksiewicz et al., 2000). In this review, RNA helicases and RNA chaperones are defined as two separate classes of RNA remodelers.

Both RNA helicases and RNA chaperones function to destabilize or unwind RNA duplexes by binding to single-stranded RNA (ssRNA) strands. Their ssRNA binding and helicase/chaperone activities are not usually sequence-specific. Interestingly, another group of proteins, named ssRNA-binding proteins (sRBPs), are also able to destabilize RNA duplexes in an ATP-independent manner. The main difference between RNA chaperones and sRBPs, which stabilize a specific RNA conformation, is that the ssRNA binding by RNA chaperones is normally weak and transient, while sRBPs remain bound to ssRNAs to stabilize one strand and displace the other strand (Rajkowitsch et al., 2007; Zúñiga et al., 2009; Musier-Forsyth, 2010).

In viruses, particularly RNA viruses, the RNA molecules (vRNAs) can adopt various structures. They need proper structures to form diverse cis-acting elements in their 5'-untranslated region (UTR), 3'-UTR, or protein coding region; these elements are important for vRNA functions including translation, replication, and encapsidation (Rajkowitsch et al., 2007; Zúñiga et al., 2009; Musier-Forsyth, 2010). Formation of these highly structured RNA elements requires RNA chaperones to facilitate. The growing list of virus-encoded RNA chaperones currently includes the nucleocapsid (NC) protein of retroviruses, coronavirus nucleocapsid (N) protein, hantavirus nucleocapsid (N) protein, flavivirus core protein, enterovirus nonstructural proteins 3AB and 2C, iflavivirus 2C, hepatitis D virus small delta antigen (SdAg), HIV-1 Tat and Vif, and cypovirus VP5. In addition, rotavirus nonstructural protein NSP2 is a putative RNA chaperone (Taraporewala and Patton, 2001). This review focuses on the RNA chaperones encoded by RNA viruses, including positive-stranded (+), negative-stranded (-), and double-stranded RNA viruses (Table 1).

RNA CHAPERONES ENCODED BY (+)-RNA VIRUSES

Core proteins of *Flaviviridae*

The viruses belonging to the family *Flaviviridae* have

a single-stranded positive-sense RNA genome with a single open reading frame (ORF) which encodes a single polyprotein that is subsequently cleaved to produce individual structural and nonstructural proteins (Shepard et al., 2005). The family *Flaviviridae* comprises three genera, *Flavivirus*, *Pestivirus* and *Hepacivirus* (Shepard et al., 2005). The genus *Hepacivirus* includes hepatitis C virus (HCV), an important human pathogen causing chronic hepatitis and liver cirrhosis. The genus *Flavivirus* includes a variety of arthropod-borne viruses such as Dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and others. The genus *Pestivirus* includes a number of important pathogens infecting livestock, such as classical swine fever virus (CSFV) and bovine viral diarrhea virus (BVDV).

The genomic RNA 5'UTR and 3'UTR of HCV contain a number of structured RNA elements that have been reported to be involved in viral replication (Ito and Lai, 1997; Friebe et al., 2005; Song et al., 2006). The HCV 5'-UTR contains 341 nucleotides (nt) and is folded into an internal ribosome entry site (IRES) structure that mediates cap-independent translation, while its 3'-UTR is 230-nt long and contains three structured RNA elements, including a variable sequence with VSL1 and VSL2 stem-loops, a poly U/UC tract, and a 98-nt 3'X region that forms three stem-loops (Kolykhalov et al., 1996; Blight and Rice, 1997; Friebe and Bartenschlager, 2002). On cleavage, the HCV polyprotein yields three structural proteins, named core protein, E1 and E2. The core protein is located in the N-terminal region of the polyprotein, contains many basic residues, and has RNA-binding activity. The HCV core protein may oligomerize upon packaging of the genomic RNA to form the viral nucleocapsid. The mature core protein has two domains, a hydrophobic C-terminal domain and an N-terminal domain. The three very basic clusters in the N-terminal domain of HCV core protein have potent RNA chaperone activity that functions on the conserved 3'X region of the 3'-UTR of HCV genomic RNA and is able to direct the dimerization of HCV RNA 3'-UTRs (Kolykhalov et al., 2000; Boulant et al., 2006). Although the RNA chaperone activity of HCV core protein has been well characterized (Sharma et al., 2010), its functions in the life-cycle of HCV remain largely unknown.

Although RNA chaperones have been studied for many years, their ATP independent helix destabilizing and annealing mechanism has not been well understood. To explain the RNA chaperone activity, an "entropy transfer" model has been proposed in which the intrinsic disordered of chaperone proteins can transfer their entropy or disorder to RNA molecules. Such transfer of disorder destabilizes misfolded RNAs, helps them escape the trap of kinetic energy, and is conducive to the proper RNA structure reform. (Rajkowitsch et al., 2007;

Table 1. The RNA chaperones encoded by RNA viruses.

RNA chaperone encoded by	Family	Genus	Species	Protein Name	Reference
(+)RNA viruses	<i>Flaviviridae</i>	<i>Hepacivirus</i>	Hepatitis C virus (HCV), GB virus B (GBV-B)	core protein	Cristofari et al., 2004; Ivanyi-Nagy et al., 2006
		<i>Flavivirus</i>	West nils virus	core protein	Ivanyi-Nagy et al., 2008
		<i>Pestivirus</i>	Bovine viral diarrhoea virus (BVDV)	core protein	Ivanyi-Nagy et al., 2008
	<i>Coronaviridae</i>	<i>Alphacoronavirus</i>	Transmissible Gastroenteritis virus (TGEV)	N protein	Zúñiga et al., 2007
		<i>Betacoronavirus</i>	SARS-CoV	N protein	Cui et al., 2015
	<i>Picornaviridae</i>	<i>Enterovirus</i>	Poliovirus	3AB	DeStefano and Titilope, 2006
		<i>Enterovirus</i>	Enterovirus 71 (EV71)	3AB, 2C	Tang et al., 2014; Xia et al., 2015
<i>Iflaviridae</i>	<i>Iflavirus</i>	Ectropis obliqua picorna-like virus (EoV)	2C	Cheng et al., 2013	
(-)RNA virus	<i>Bunyaviridae</i>	<i>Hantavirus</i>	Sin Nombre hantavirus	N nucleocapsid protein	Mir and Panganiban, 2006
dsRNA viruses	<i>Reoviridae</i>	<i>Cypovirus</i>	Cypovirus 5	VP5	Yang et al., 2014
		<i>Rotavirus</i>	Rotavirus	NSP2	Taraporewala and Patton, 2001

Zúñiga et al., 2009).

Interestingly, HCV core protein has been predicted to contain a disordered region, providing support to the “disorder or entropy transfer” model of RNA chaperones. Intrinsically disordered regions have also been predicted in the core proteins of other genera of *Flaviviridae*, including flavivirus and pestivirus. Biochemical analyses confirmed that these core proteins are also potent RNA chaperones (Ivanyi-Nagy et al., 2008).

Coronavirus N protein

Coronaviruses (CoVs) are enveloped viruses, belonging to the family *Coronaviridae*, including a large number of pathogens with global distribution that cause significant diseases in humans, such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) (Gorbalenya et al., 2006). The coronavirus genome is a single-stranded, positive-sense RNA, the largest viral RNA genome known (around 30 kb), which is 5'-capped and 3'-polyadenylated (Sawicki and Sawicki, 2005). Six conserved genes are encoded, in the order 5'-ORF1a-ORF1b-S-E-M-N-3'. The 5' two-thirds of the genomic RNA encode ORFs1a/b, the viral replicase. The 3' third of the genome encodes four structural proteins, membrane (M), envelope (E), spike (S) and nucleocapsid (N), and a number of nonstructural proteins scattered among them (Sawicki and Sawicki, 2005; Zúñiga et al., 2007).

Coronavirus N protein has a structural role in assembly of the virus, and is probably also involved in vir-

al RNA synthesis (Zúñiga et al., 2007). Although different coronaviruses encode N proteins that vary in size and nucleotide sequence, the three-domain structure and some functional motifs are conserved, including a RNA binding domain, oligomerization domain, and M protein binding domain (Xia et al., 2015). Based on protein disorder analysis of coronavirus N proteins, RNA chaperone activity was first demonstrated for transmissible gastroenteritis virus (TGEV) N protein (Brian and Baric, 2005). RNA chaperone activity was also reported for SARS-CoV N protein, and it was suggested that RNA chaperone activity is a property of all coronavirus N proteins (Cui et al., 2015). Like other RNA chaperones, coronavirus N proteins also have activities such as facilitating nucleic acid annealing, RNA duplex destabilization, and enhancement of ribozyme self-cleavage (Cui et al., 2015).

TGEV N protein has RNA chaperone activity and is able to facilitate template switching *in vitro* (Zúñiga et al., 2007). Template switching during discontinuous RNA synthesis is a complicated and indispensable process that includes the stoppage of nucleic acid synthesis, the template switch, nascent nucleic acid strand re-association, and elongation of the nascent strand (Zúñiga et al., 2007). It is supposed that the N protein could decrease the thermodynamic barrier between the nascent (-)RNA strand and the genomic RNA template, thereby promoting the nascent RNA strand template switch to hybridize with the transcription-regulating sequences of the leader sequence (Zúñiga et al., 2004).

Picornavirus 3AB protein

Picornaviruses are a large group of single, plus-stranded RNA viruses, including many important human and animal pathogens, such as poliovirus (PV), foot-and-mouth disease virus (FMDV), enterovirus 71 (EV71), and encephalomyocarditis virus (EMCV). Human picornaviruses cause over 3 billion human infections per year, and relating diseases ranging from mild (common cold, upper respiratory illness, and hand-foot-and-mouth disease), to severe (encephalitis, aseptic meningitis, neonatal sepsis-like disease, myocarditis, and poliomyelitis) conditions (Brian and Baric, 2005). The RNA genome of picornavirus is a single positive strand, containing a long 5'-UTR, a single ORF and a 3'-UTR. This RNA genome encodes a long polyprotein that is processed via cleavage at specific sites to produce mature viral proteins.

Protein 3AB is the precursor of nonstructural proteins 3A and 3B. Protein 3A is a membrane anchoring protein that anchors the replication complex to endoplasmic reticulum (ER) membrane (DeStefano and Titilope, 2006). Protein 3B, also called VPg, is a small peptide (21–24 amino acids (a.a.)) that is covalently linked to the 5' end of vRNAs and is believed function as a primer for initiating vRNA synthesis by the RNA-dependent RNA polymerase (RdRP), $3D^{Pol}$. DeStefano and colleagues have showed that poliovirus protein 3AB possesses properties consistent with classical RNA chaperone proteins, and was able to greatly accelerate the rate of annealing between complementary nucleic acids and destabilize short double-stranded RNA substrates (DeStefano and Titilope, 2006). Moreover, our group has also demonstrated that protein 3AB from EV71 has RNA chaperone activity (Tang et al., 2014). These findings suggest that RNA chaperone activity is a general function of non-structural protein 3AB in picornaviruses.

Poliovirus 3AB was the first RNA chaperone described in picornaviruses (DeStefano and Titilope, 2006). Domain organization and disorder prediction analyses showed that poliovirus or EV71 protein 3AB does not contain any intrinsically disordered regions, implying that the entropy transfer mechanism does not apply to enteroviral 3AB (Tang et al., 2014). However, the RNA chaperone activity of 3AB was moderately inhibited by various divalent metallic ions, implying that 3AB remodels RNA molecules through transient ionic interactions with the negatively charged RNA backbone (Woodson, 2010). The studies by Gangaramani *et al.* and Tang *et al.* have reported that the 3B region plus the C-terminal 7 a.a. of protein 3A is the key region for the RNA chaperone activity of 3AB (Gangaramani et al., 2010; Tang et al., 2014). Moreover, residues Lys-81, Phe-83, His-86, Tyr-90 and Arg-104 of 3AB have been reported to be critical for the RNA chaperone activity of poliovirus or EV71 3AB (Gangaramani et al., 2010; Tang et al., 2014).

During the life cycle of picornaviruses, the RNA chaperone activity of 3AB may play critical roles in coating and protecting viral genomic RNA, facilitating the formation and/or deformation of vRNA cis-acting elements, vRNA synthesis, and translation. 3AB could strongly stimulate the RNA synthesis activity of $3D^{Pol}$, especially when low concentrations of $3D^{Pol}$ were used (Byströff and Shao, 2002; James et al., 2003). However, for technical reasons, it is hard to determine the precise role of an RNA chaperone in a particular process in cells. Based on studies of protein 3AB from both poliovirus and EV71, several possibilities have been proposed, including: enhancement of primer binding to the template, facilitation and stabilization of picornaviral $3D^{Pol}$ binding to the primer-template, and lowering of the dissociation rate constant (James et al., 2003; James et al., 2004).

$2C^{ATPase}$ proteins of picorna-like virus and picornavirus

The picorna-like virus superfamily is a loosely defined group of positive-strand RNA viruses including 14 families of viruses and several unclassified genera and species. Among this superfamily, five families of viruses (*Dicistroviridae*, *Iflaviridae*, *Marnaviridae*, *Picornaviridae* and *Secoviridae*) make up the order *Picornavirales*. The genomes of picorna-like viruses are characterized by highly conserved genes, including an RdRP (a $3D^{Pol}$ for viral RNA replication), a chymotrypsin-like $3C^{Pro}$ or $3C^{Pro}$ -like (3CL) protease, and a putative helicase (non-structural protein $2C^{ATPase}$). Based on conserved superfamily 3 (SF3) signature A, B, and C motifs, $2C^{ATPase}$ has been predicted to be a SF3 helicase (Rodriguez and Carrasco, 1993; Pfister and Wimmer, 1999). Motifs A and B are “Walker” NTP binding motifs that appear in ATP- and/or GTP-binding proteins (Rodriguez and Carrasco, 1993). Motif C has been recognized as characteristic of SF3 helicases (Pfister and Wimmer, 1999). Although $2C^{ATPase}$ is one of the most conserved nonstructural proteins in the order *Picornavirales*, and multiple functional domains have been identified in genetic and drug inhibition studies, the exact roles of this protein in the viral life cycle remain largely unclear. Previous studies reported that $2C^{ATPase}$ participates in diverse processes, including RNA binding, RNA replication, inhibition of autophagy, suppression of nuclear factor-kappaB (NFκB) activation, membrane anchoring and rearrangement, and encapsidation and viral morphogenesis (Echeverri and Dasgupta, 1995; Teterina et al., 1997; Krogerus et al., 2003; Zheng et al., 2011; Gladue et al., 2012). Moreover, this protein appears to associate with vRNA in vRNA replication complexes in infected cells, implying a direct role in viral RNA replication (Rodriguez and Carrasco, 1995).

$2C^{ATPase}$ proteins of an insect picorna-like virus. RNA chaperone activity of a picorna-like virus $2C^{ATPase}$ protein was first reported for the $2C^{ATPase}$ from *Ectropis obliqua* picorna-like virus (EoV; genus *Iflavirus*, family *Iflaviridae*). The study by our group showed that EoV $2C^{ATPase}$ does have RNA remodeling activity that is able to destabilize RNA duplex. Surprisingly, the RNA remodeling activity associated with EoV $2C^{ATPase}$ is more like the activity of an RNA chaperone than the RNA helicase it was predicted to be (Cheng et al., 2013). EoV $2C^{ATPase}$ can nonspecifically destabilize nucleic acid helices and accelerate strand annealing in an ATP-independent manner; and divalent metal ions (e.g. Mg^{2+} and Zn^{2+}) inhibit $2C^{ATPase}$ -mediated helix destabilization. EoV $2C^{ATPase}$ does have ATPase activity similar to that of other picornaviral $2C^{ATPase}$ proteins, but the RNA chaperone and ATPase activities of EoV $2C^{ATPase}$ are independent and can be functionally separated (Cheng et al., 2013).

$2C^{ATPase}$ proteins of picornaviruses. Enteroviruses are a large group of positive-stranded RNA viruses in the genus *Enterovirus* of the *Picornaviridae* family, and include many closely related human pathogens such as poliovirus, EV71, and coxsackie viruses. After the global eradication of poliovirus, EV71 is regarded as the most important neurotropic enterovirus. Recently, the highly conserved and multifunctional protein $2C^{ATPase}$ from EV71 has been demonstrated that functions as both an RNA helicase and an ATP-independent RNA chaperone, and that it is involved in viral RNA replication.

$2C^{ATPase}$ contains conserved motifs typical of SF3 helicases, but previous attempts to discover helicase activity associated with $2C^{ATPase}$ were unsuccessful (Rodriguez and Carrasco, 1993). A computation-modeled three-dimensional structure of enteroviral $2C^{ATPase}$ indicated that this protein is similar to other viral SF3 helicases in terms of both conserved motifs and structure, implying that the protein should have RNA remodeling activity. Like other RNA remodeling proteins, EV71 $2C^{ATPase}$ has helix unwinding activity for both RNA and DNA duplexes, and the unwinding activity is bidirectional. Moreover, destabilization of RNA duplexes by EV71 $2C^{ATPase}$ requires ATP or other NTPs. Interestingly, EV71 $2C^{ATPase}$ also has an ATP-independent helix unwinding activity. Furthermore, EV71 $2C^{ATPase}$ unwinds 3'-protruded helices in an ATP-dependent manner, consistent with all known SF3 helicases, but the enzyme unwinds 5'-protruded helices exactly like an RNA chaperone, i.e., in the absence of ATP (and such that increasing ATP concentrations did not further enhance the helix unwinding). Thus, one may speculate that EV71 $2C^{ATPase}$ has both RNA helicase and chaperone activities. The latter includes the ability to destabilize structured RNA strands, to stimulate RNA strand annealing, and to en-

hance hammerhead ribozyme cleavage activity. Functional domain analysis showed that the C-terminal domain of $2C^{ATPase}$ plays an indispensable role in the RNA chaperone activity, probably due to the predicted coiled-coil structure that links the C-terminal domain with the helicase core domain of $2C^{ATPase}$ and the coiled-coil structure is intrinsically disordered. This finding also provides support for the "entropy or disorder transfer" model of RNA chaperones.

We proposed that during enteroviral RNA replication, particularly (+)-vRNA synthesis, $2C^{ATPase}$ initially associates with the 3'-end of the (-)-vRNA template, and its RNA chaperone activity functions to remodel and make the 3'-end accessible to enteroviral RdRP ($3D^{Pol}$) and primers (VPg), resulting in the initiation of (+)-vRNA replication (Figure 1, A–B). During the process of (+)-vRNA synthesis, the helicase activity of $2C^{ATPase}$ functions to unwind the replicative dsRNA, and even facilitates the release of $3D^{Pol}$ from the (-)-vRNA template, thereby releasing the nascently synthesized progeny (+)-vRNAs from the template (Figure 1, C–E), and recycling the (-)-stranded template and RdRP for another cycle of vRNA replication (Figure 1, E–A).

RNA CHAPERONE ENCODED BY (-)-RNA VIRUS (BUNYAVIRUS N PROTEIN)

Hantavirus, belonging to the family *Bunyaviridae*, is an enveloped, segmented, negative-stranded RNA virus (Khaiboullina et al., 2005). The virus genome includes three fragments, L, M and S, respectively encoding polymerase protein, G1 and G2 glycoproteins, and nuclear protein (Plyusnin et al., 1996; Barr and Wertz, 2005). There are two types of hantavirus, one causing hantavirus pulmonary syndrome (HPS), and the other causing hantavirus hemorrhagic fever with renal syndrome (HFRS). The former is mainly epidemic in the United States, and is also found in Argentina, Brazil, Paraguay, Bolivia and Germany. The latter is the common hemorrhagic fever with renal syndrome in China. It has been proven that the virus is the direct pathogen (Mei and Cosgriff, 1989). The kidney is the primary organ suffering injury in the early stage of infection, and the virus is the factor that directly causes renal damage.

Hantavirus N nucleocapsid protein plays an important role in the virus replication cycle. N protein has different activities and biological functions when interacting with, respectively, viral (-)RNA, (+)cRNA, or mRNA. No mRNA was detected in encapsidation complex; the virus encapsidation process needs viral (-)RNA or (+)cRNA, but not mRNA.

The 5' and 3' ends of full-length the viral RNA of Bunyaviridae may base paired with each other and formed the intramolecular "panhandle" structures which

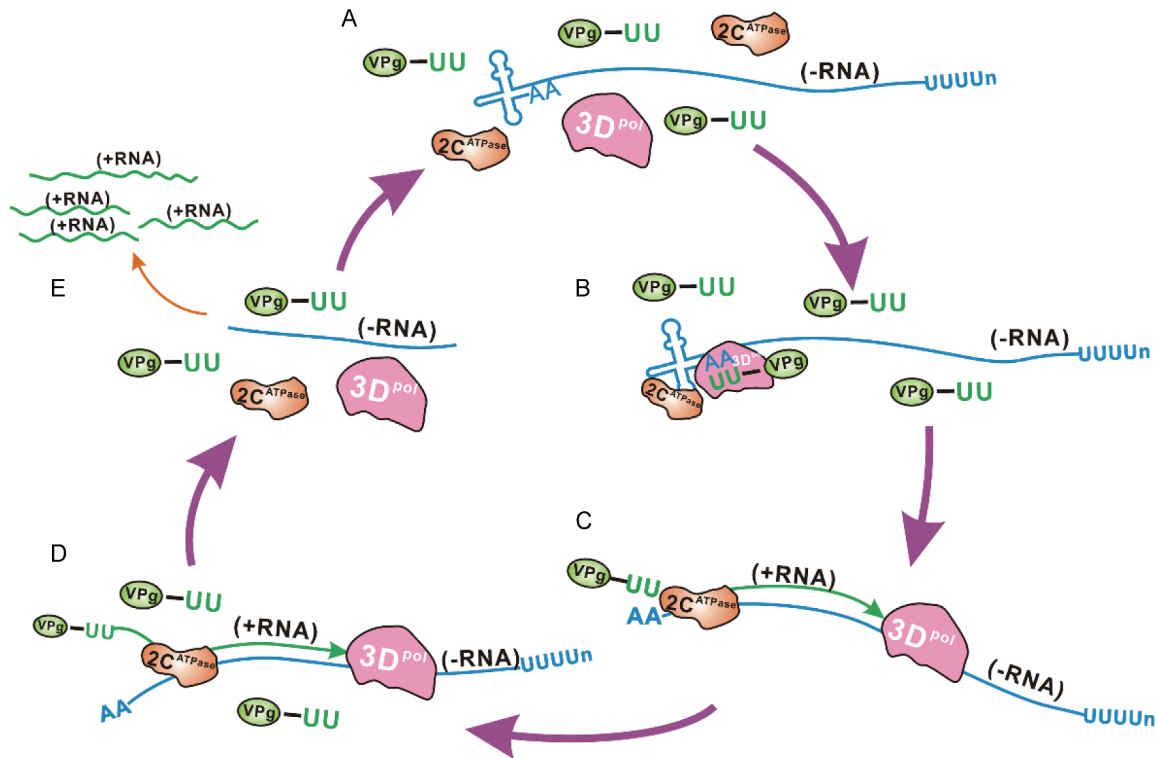


Figure 1. Model of 2C^{ATPase} involvement in enteroviral replication. (A–B) Once enteroviral (-)vRNA synthesis is complete, 3D^{pol}, the primer VPgUpU, and other replication complex proteins are assembled at the 3'-end of (-)vRNA template, and then 3D^{pol} initiates the synthesis of (+)vRNA. In this step, the RNA chaperone activity of 2C^{ATPase} functions to remodel and make the (-)vRNA 3'-end accessible for 3D^{pol} and the primers. (C–E) The process of (+)vRNA synthesis on the (-)vRNA template generates a replicative intermediate dsRNA region. 2C^{ATPase} binds to the dsRNA (C) and its helicase activity functions to unwind the replicative dsRNA (D), resulting in the separation and release of nascently synthesized progeny (+)vRNAs, and probably 3D^{pol} and VPgUpU primers, from the (-)vRNA template (E). (E–A) The (-)vRNA template, 3D^{pol}, and VPgUpU primers are then recycled for new rounds of vRNA replication.

is important for the recognition and encapsidation of vRNA during particle assembly (Mir and Panganiban, 2006). The RNA chaperone activity of N protein can facilitate panhandle formation and is likely to be involved in initiation of translation (Mir and Panganiban, 2006). The N-terminal 100 amino acids of N protein form a domain that is both necessary and sufficient for the RNA chaperone activity. This region may be in one of two states: either highly disordered and functional in N-mediated RNA chaperone activity, or, in a trimeric form, ordered and functional in high-affinity vRNA panhandle recognition (Brown and Panganiban, 2010).

RNA CHAPERONES ENCODED BY DSRNA VIRUSES

Cypovirus capsid protein VP5

Reoviruses, belonging to the family *Reoviridae*, contain a segmented double-stranded RNA (dsRNA) gen-

ome, and the genome was packaged in a single- or double-layered inner capsid. These viruses include important pathogens responsible for diseases in plants, insects, humans and livestock animals (Mertens, 2004). Unlike positive-stranded and negative-stranded RNA viruses, dsRNA viruses, including reoviruses, utilize their inner capsid not only as the shield for escaping antiviral defenses, but also as the machinery for vRNA synthesis, because the inner capsids of dsRNA viruses contain several copies of RdRPs and mRNA-capping enzyme (Mertens, 2004; Coulibaly et al., 2007).

Cypovirus (cytoplasmic polyhedrosis virus, CPV) is one of 15 genera within the family *Reoviridae*. Cypovirus contains 10 genomic dsRNA segments and is further classified into 16 types based on the electrophoretic migration profiles of the genome segments (King et al., 2011). CPV particles are packaged in polyhedra to enable survival in unfavorable external environments (Coulibaly et al., 2007). Cypovirus is used as a relatively simple model for studying dsRNA virus gene rep-

lication because of its single layered capsid. The CPV capsid is comprised of three major capsid proteins, VP1, VP3, and VP5. One-hundred and twenty copies of VP1 form the capsid shell, 60 copies of VP3 are located on top of the five-fold axis and function as an mRNA capping enzyme, while 60 copies of VP5 are clamp proteins that stabilize the CPV capsid shell. It has recently been reported that VP5 from the type 5 *Helicoverpa armigera* cypovirus (HaCPV-5) possesses RNA chaperone activity and can destabilize RNA helices and accelerate strand annealing process ATP-independently (Yang et al., 2014). Further exploration of VP5 revealed that its helix-destabilizing activity is RNA-specific, lacks directional properties, and could be inhibited by divalent metal ions (e.g. Mg^{2+} , Mn^{2+} , Ca^{2+} and Zn^{2+}) to varying degrees. The 5' and 3' ends of the (+)-vRNA strand contact and form a panhandle structure that has to be unlocked during genome replication. Moreover, it has been demonstrated that the VP5 protein of HaCPV-5 can facilitate the initiation of transcription through a CPV panhandle-structured RNA template by an alternative polymerase (i.e. a reverse transcriptase), which strongly suggests a direct function of the VP5 RNA chaperone activity in the initiation of cypoviral dsRNA replication (Yang et al., 2014).

Rotavirus nonstructural protein 2 (NSP2)

Rotavirus, another genus of the family *Reoviridae*, is the major cause of gastroenteritis in young children. It has been reported that the multifunctional enzyme NSP2 of rotavirus, involved in rotaviral dsRNA replication, has ATP-independent nucleic acid helix-destabilizing activity (Taraporewala and Patton, 2001). This activity lacks unwinding directionality and has no sequence specificity. The helix-destabilizing activity of RNA chaperones normally needs a large excess of protein over RNA helix substrate (DeStefano and Titilope, 2006; Cheng et al., 2013), and the optimal activity of rotavirus NSP2 has been detected at molar ratios of 1000:1 to 2000:1 (protein : RNA) (Taraporewala and Patton, 2001). These data suggest that CPV VP5 may not be the sole RNA chaperone protein in the family *Reoviridae*. However, rotavirus NSP2 and CPV VP5 have some differences. For example, CPV VP5 has RNA specific helix-destabilizing activity while NSP2 can unwind both DNA-RNA and RNA-RNA helices (Taraporewala et al., 1999; Taraporewala and Patton, 2001). This difference implies that these two reoviral proteins may use different mechanism to destabilize RNA helices. Since no study has yet determined whether rotavirus NSP2 possesses any strand annealing acceleration activity which is an important characteristic of RNA chaperones, further characterization of NSP2 is required to determine whether this protein should be recognized as an nucleic acid chaperone (Qiu et al., 2014).

DISCUSSION

Although the molecular mechanisms and structural bases governing ATP-dependent translocation and unwinding/destabilization by RNA helicases have been extensively studied (Kadare and Haenni, 1997; Rajkowitsch et al., 2007; Musier-Forsyth, 2010), the mechanism(s) by which RNA chaperones destabilize RNA duplexes and stimulate RNA strand annealing in an ATP-independent manner remain largely unknown. This discrepancy is partially due to the lack of consensus sequences or motifs among various RNA chaperones. Thus far, two hypotheses have been proposed. The most popular mechanistic hypothesis for RNA chaperone activity is the “disorder or entropy transfer” model, in which intrinsically disordered or unstructured regions of RNA chaperone proteins transfer their entropy (disorder) to the RNA molecules that they bind. This transfer of disorder destabilizes misfolded RNA base-pairings, helping them to escape kinetic traps and facilitating refolding to the correct RNA structure (Rajkowitsch et al., 2007; Zúñiga et al., 2009). Many virus-encoded RNA chaperone proteins, including HIV-1 Vif, NC, and Tat, flavivirus core, hantavirus N and coronavirus N, are predicted to contain intrinsically disordered regions (Ivanyi-Nagy et al., 2008; Zúñiga et al., 2009; Brown and Panganiban, 2010), a prediction often made using the software DisProt Predictor VL3 (<http://www.dabi.temple.edu/disprot/predictor.php>) (Obradovic et al., 2003; Peng et al., 2005; Zúñiga et al., 2009). The disorder of hantavirus N was predicted using another algorithm, VL-XT (Brown and Panganiban, 2010); we reassessed it using VL3, and predict similar disordered regions. However, disorder prediction for poliovirus or EV71 3AB using VL3 suggests that these proteins do not contain disordered regions, and therefore that the “disorder or entropy transfer” model may not apply to all RNA chaperones.

Another model is that some RNA chaperones can remodel the RNA structures through transient electrostatic or ionic interactions with RNA molecules. For nucleic acids have negative charges on the phosphodiester backbone, ionic interactions may seriously affect the dynamics and stability of RNA (Woodson, 2010). In this model, RNA chaperone proteins having a large, positively charged region could destabilize the structure of RNA and promote rearrangements of stable structure, and the cationic may inhibit strand annealing and RNA destabilization by stabilizing the RNA conformation and competing with RNA chaperones for non-specific RNA binding. (Vo et al., 2006; Wu et al., 2007; Woodson, 2010). Because RNA chaperone proteins lack sequence similarity, it is highly likely that RNA chaperones employ diverse mechanisms for their functions. Future studies may elucidate these mechanisms.

RNA chaperones and RNA helicases have been identified and studied extensively. However, the RNA remodeling activities of these proteins have normally been studied *in vitro* biochemically and biophysically; it is very hard to determine the cellular or *in vivo* roles of RNA helicase or chaperone activities on the structures and functionalities of RNAs. These technical limitations hinder our understanding of these important RNA-binding proteins and their roles in viral life cycles. Future studies may overcome these technical difficulties, providing a mechanistic view of how host or viral RNA remodeling proteins function *in vivo*.

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COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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