TITLE:

Replication mechanisms of plant RNA viruses: current understanding and perspectives

AUTHOR(S):

Okuno, Tetsuro

CITATION:

Okuno, Tetsuro. Replication mechanisms of plant RNA viruses: current understanding and perspectives. *Journal of General Plant Pathology* 2012, 78(6): 404-408

ISSUE DATE:

2012-11

URL:

<http://hdl.handle.net/2433/162960>

RIGHT:

The final publication is available at www.springerlink.com; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。 ; This is not the published version. Please cite only the published version.

Presidential Address, JGPP (2012)

1 **Replication mechanisms of plant RNA viruses**2 **-Current understanding and perspective-**

3

4 **Tetsuro Okuno**

5

6 T. Okuno

7 Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

8 okuno@kais.kyoto-u.ac.jp

9

10 This article is an abstract of the Presidential Address presented at the 2012 Annual

11 Meeting of the Phytopathological Society of Japan in Fukuoka.

12

13

14

15

16

17

18

19

Presidential Address, JGPP (2012)

20 **Introduction**

21

22 Viruses cause numerous diseases in economically important plants and animals,
23 including humans. However, there are no efficient measures to counter viral infection,
24 with very few exceptions for animal viral diseases. Although virus resistance genes
25 have been used to minimize the loss of crop production in several important plant
26 species, the number of genes that have been used to confer virus resistance is very
27 limited. The delay in the development of efficient measures for the control of viral
28 diseases might be caused in part by the lack of critical information on the mechanisms
29 underlying viral replication, especially regarding the host proteins used by viruses.

30 Positive-strand RNA viruses with a genome composed of messenger-sense
31 single-stranded RNAs, represent the largest group among the seven genetic classes of
32 all viruses. Upon entry into host cells, the genomic RNAs of these viruses serve as
33 mRNAs, and viral replication proteins are translated by exploiting the host translational
34 machinery via diverse strategies, which include cap- and poly(A)-independent
35 translation mechanisms. Subsequently, viral genomes are synthesized on intracellular
36 membranes in infected cells by RNA replicase complexes that consist of viral
37 RNA-dependent RNA polymerase (RdRp), viral auxiliary proteins, host-encoded
38 proteins, and viral RNAs (Ahlquist et al. 2003; den Boon et al. 2010; Nagy and Pogany

Presidential Address, JGPP (2012)

2012). Increasing evidence has demonstrated that viral replication proteins are multifunctional and play critical roles in recruiting the viral genomic RNAs to, and rearranging the specific cellular membranes, which are the sites of replication of positive-strand RNA viruses. This paper presents a brief review of viral RNA translation and replication mechanisms and refers to recent data obtained in the study of the *Red clover necrotic mosaic virus* (RCNMV), which is used by our group as a model virus.

Red clover necrotic mosaic virus

RCNMV is a positive-strand RNA plant virus and a member of the genus *Dianthovirus* in the family *Tombusviridae*. This genus includes the *Carnation ring spot virus* (CRSV) as the type member and the *Sweet clover necrotic mosaic virus* (SCNMV) (Hiruki 1987). The genome of RCNMV consists of two RNAs (RNA1 and RNA2). The bipartite genome is unique among viruses of the family *Tombusviridae*, the genome of which is monopartite. RNA1 encodes putative RNA replicase components, an auxiliary 27 kDa protein (p27), and an 88 kDa protein (p88) with an RdRP motif. RNA1 also encodes a 37 kDa coat protein (CP) that is expressed from a subgenomic RNA (CPsgRNA). Transcription of the CPsgRNA requires an intermolecular interaction

Presidential Address, JGPP (2012)

58 between RNA1 and RNA2 (Sit et al. 1998; Tatsuta et al. 2005). RNA2 is a
59 monocistronic RNA that encodes a movement protein (MP), which is required for viral
60 cell-to-cell movement in plants (Kaido et al. 2011; Xiong et al. 1993).

61

62 **Translation of RCNMV replicase proteins**

63

64 Many plant RNA viruses, including members of the *Tombusviridae* and the
65 *Luteoviridae* families, lack both a 5' cap and a 3' poly(A) tail. Instead, they have
66 cap-independent translation elements (CITEs) in the 3' untranslated region (UTR) of
67 their genomic RNAs (Nicholson and White 2011). In some of these viruses, 3'CITEs
68 bind the eukaryotic translation initiation factor complex eIF4F or eIFiso4F (Iwakawa et
69 al. 2012; Nicholson and White 2011).

70 RCNMV RNA1 and RNA2 possess neither a cap structure at the 5' end nor a
71 poly(A) tail at the 3' end (Mizumoto et al. 2003). Therefore, RCNMV proteins must be
72 translated via cap-independent translation mechanisms. Two RNA elements play
73 essential roles in the cap-independent translation of the replicase proteins encoded in
74 RNA1. One element is the 3'CITE that consists of five stem-loop structures in the 3'
75 UTR of RNA1 (Mizumoto et al. 2003). Another element is an A-rich sequence
76 (ARS) located upstream of the 3'CITE (Iwakawa et al. 2012). A search for cellular

Presidential Address, JGPP (2012)

77 factors that bind the 3' UTR of RNA1 using RNA aptamer-based one-step affinity
78 chromatography and mass spectrometry analysis led to the identification of the ARS as
79 an RNA element that binds to poly(A)-binding protein (PABP) (Iwakawa et al. 2012).
80 Mutagenesis and a tethering assay revealed that the direct interaction between PABP
81 and ARS stimulates the 3'CITE-mediated translation of RCNMV RNA1, and that the
82 PABP-ARS interaction is required for the recruitment of eukaryotic translation
83 initiation factors (eIFs), such as eIF4s or eIF(iso)4Fs, to the 3' UTR and of the 40S
84 ribosomal subunit to the viral mRNA (Iwakawa et al. 2012). Dianthoviruses might have
85 evolved the ARS and 3'CITE as substitutes for the 3' poly(A) tail and the 5' cap of
86 eukaryotic mRNAs for the efficient recruitment of eIFs, PABP, and ribosomes to the
87 uncapped/nonpolyadenylated viral mRNA.

88 p88, which overlaps N-terminally with p27, is produced by programmed -1
89 ribosomal frameshifting (-1 PRF) (Kim and Lommel 1994). In addition, the production
90 of p88 requires the long-distance base pairing between a bulge sequence in the
91 stem-loop structure predicted just downstream of the -1 PRF site and the loop sequence
92 of a small stem-loop structure predicted between the ARS and the 3'CITE (Tajima et al.
93 2011). Because p88, but not p27, is required in *cis* for the replication of RNA1
94 (Okamoto et al. 2008), the existence of RNA elements responsible for -1 PRF and
95 3'CITE-mediated translation in the 3' UTR of RNA1 might be important for switching

Presidential Address, JGPP (2012)

96 translation to replication of RNA1 and for regulating the ratio of p88 to p27.

97

98 **Roles of viral replication proteins in RNA replication**

99

100 Viral RdRP is a key enzyme in the synthesis of viral RNAs. In addition to the RdRP,
101 many positive-strand RNA viruses encode an auxiliary replication protein.
102 Accumulating evidence indicates that the viral auxiliary replication proteins play crucial
103 roles in recognizing viral RNA templates, targeting them to RNA replication sites, and
104 assembling the viral replicase complexes. Thus, the auxiliary replication proteins play
105 multiple roles in the accomplishment of viral RNA replication.

106 For example, the auxiliary replicase protein p33 of *Tombusvirus* binds directly to an
107 internal replication element present in the viral replicon RNA (Pogany et al. 2005),
108 interacts with p92 RdRP (p92^{pol}) via a protein–protein interaction, and recruits the
109 replicon RNA and p92^{pol} to peroxisomal membranes (Panavas et al. 2005). These
110 protein–RNA and protein–protein interactions are required for the assembly of the
111 *Tombusvirus* replicase complex. In the Brome mosaic virus (BMV), which has a
112 tripartite genome, the 1a auxiliary replication protein recognizes RNA elements that are
113 present at the 5' end of RNA2 and in the intergenic region of RNA3 (Chen et al. 2001;
114 Schwartz et al. 2002). The 1a protein, 2a RdRP (2a^{pol}), and viral nucleotide sequences

Presidential Address, JGPP (2012)

115 are required for the assembly of the functional BMV replicase complex. The 1a protein
116 recruits replication templates and 2a^{pol} to the endoplasmic reticulum (ER) membrane,
117 which is the site of BMV RNA replication (Liu et al. 2009; Schwartz et al., 2002).

118 In RCNMV, the p27 auxiliary replication protein binds specifically and directly to a
119 Y-shaped RNA element (YRE) located in the 3' UTR of RNA2. The replicase–YRE
120 interaction is critical for recruiting RNA2 to the membrane fraction (Hyodo et al. 2011;
121 Iwakawa et al. 2011). The domains in p27 that are required for YRE binding were
122 mapped to its central and C-terminal regions, and the critical amino acids of these
123 regions were identified (Hyodo et al. 2011). The C-terminal half of p27 is also involved
124 in the interaction with p88 (Mine et al. 2010b) and localizes at the ER membrane,
125 together with p88 (Turner et al. 2004). The p27–p88 interaction is required for the
126 formation of RCNMV replication complexes, as described below.

127

128 **Viral RNA replication complexes and host proteins involved in RNA replication**

129

130 One of the useful approaches to identify host genes affecting virus RNA replication is a
131 systematic genome-wide screen using yeast single-gene deletion or essential-gene
132 libraries, when the viruses of interest can replicate in yeast (Nagy 2008). Using the
133 yeast systems, about 130 and 100 genes that affect the replication of tomato bushy stunt

Presidential Address, JGPP (2012)

134 virus (TBSV) and BMV, respectively, have been identified (Nagy 2008). Functional
135 analyses in several of these genes demonstrated that the encoded proteins include
136 RNA-binding proteins, cellular chaperons, membrane-shaping proteins, proteins
137 associated with membrane remodeling and lipid synthesis, and others (Nagy and
138 Pogany 2012). Screens of Arabidopsis mutant plants also led to the identification of
139 several genes, such as TOM1 and TOM3, that encode membrane proteins and affect
140 tomato mosaic virus replication (Yamanaka et al. 2000).

141 Host proteins required for plant RNA virus replication have also been identified by
142 purifying membrane-associated viral RNA replication complexes using biochemical and
143 immunological methods, followed by mass spectrometry and other methods. This
144 approach led to the identification of several proteins, including a yeast homologue of
145 mammalian glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for TBSV, the
146 eukaryotic translation elongation factor 1A for TBSV and *Turnip mosaic virus*, heat
147 shock protein 70 (Hsp70) for TBSV (Nagy and Pogany 2012), and Hsp70, Hsp90, and
148 ADP-ribosylation factor 1 (ARF1) for RCNMV, as described below.

149 RCNMV RNA replication complexes were isolated from virus-infected plant tissues
150 using immunoprecipitation and the membrane-associated replication complexes were
151 analyzed using blue native polyacrylamide gel electrophoresis (BN-PAGE) (Mine et al.
152 2010a). The purified fractions contained viral and host proteins and retained two types

Presidential Address, JGPP (2012)

153 of RdRP activities. One was an endogenous template-bound RdRP activity that
154 synthesizes virus-related RNAs without adding RNA templates. The other was an
155 exogenous template-dependent RdRP activity capable of *de novo* initiation of
156 complementary RNA synthesis from selected RNA templates. The RCNMV replication
157 complexes are bound tightly with membranes and their apparent molecular weight is
158 480 kDa. The complex with a template-dependent RdRP synthesizes RNA fragments by
159 specifically recognizing the 3' terminal core promoter sequences of RCNMV RNAs.

160 Using mass spectrometry, we identified possible host proteins present in the
161 affinity-purified RCNMV RNA-dependent RNA polymerase fraction, or in the 480 kDa
162 complex fraction that was purified further via BN-PAGE. The former fraction
163 contained Hsp70, Hsp90, ubiquitin, actin, hin1-like protein, several ribosomal proteins,
164 ARF1, and other proteins in addition to viral replication proteins and CP (Mine et al.
165 2010a; A. Mine and T. Okuno, unpublished data). The latter fraction contained
166 ubiquitin, ATP synthase subunit α , hin1-like protein, and other proteins, in addition to
167 viral replicase proteins (Mine et al. 2010a). Among these candidate proteins, we
168 analyzed Hsp70, Hsp90, and ARF1 for their contribution to RCNMV RNA replication.
169 Hsp70 and Hsp90 are well-known protein chaperones and ARF1 is a highly conserved,
170 ubiquitous, small GTPase that is implicated in the formation of the COPI vesicles on
171 Golgi membranes. Our recent experimental data suggest that these three proteins

Presidential Address, JGPP (2012)

172 interact directly and colocalize with p27 within the virus-induced large punctate
173 structures of ER membranes. Downregulation of these proteins by virus-induced gene
174 silencing decreased RCNMV RNA accumulation in plants. Furthermore, specific
175 inhibitors of Hsp70, Hsp90, and ARF1, such as 2-phenylethynesulfonamide (PES),
176 geldanamycin, and brefeldin A, respectively, inhibited the formation of the 480 kDa
177 replication complex, and the synthesis of RCNMV RNA in an *in vitro* cell-free viral
178 translation/replication system and in protoplast experiments (A. Mine, K. Hyodo and T.
179 Okuno, unpublished data). These data suggest that Hsp70, Hsp90, and ARF1 play
180 important roles in the formation of replication complexes and viral RNA synthesis.
181 Confocal microscopy using GFP-fused Arf1 suggested that p27 induces the
182 redistribution of Arf1 to large aggregate structures from small punctate structures that
183 could be the Golgi apparatus (K. Hyodo and T. Okuno, unpublished data). Interestingly,
184 inhibition of the nucleotide exchange activity of ARF1 by brefeldin A disrupts the
185 ER-localization of p27 in RCNMV-infected cells. Although the precise role of Arf1 in
186 the replication of RCNMV RNA is unclear at present, it is tempting to speculate that
187 p27 functions as the recruiter of Arf1 to the replication site and acts in viral RNA
188 replication via membrane modification and the formation of RNA replication
189 complexes at the membrane. It is noteworthy that enteroviral 3A proteins bind and
190 modulate Arf1 and its guanine nucleotide exchange factor GBF1 to enhance the

Presidential Address, JGPP (2012)

191 preferential recruitment of phosphatidylinositol-4-kinase IIIb (PI4KIIIb) to membranes,
192 yielding a phosphatidylinositol 4-phosphate (PI4P) lipid-enriched microenvironment
193 that differs from that found in uninfected cells (Hsu et al. 2010). Thus, plant and animal
194 viruses might use common host factors to create a favorable environment for viral
195 replication. Conversely, many host proteins used by viruses seem to differ among
196 viruses. TBSV is affected by a set of host factors that is vastly different from that
197 observed for BMV (Li et al. 2009). Down regulation of GAPDH, which inhibits TBSV
198 accumulation, does not affect TMV accumulation in GAPDH-silenced plants (Wang
199 and Nagy 2008).

200

201 **Perspective**

202

203 Over the past 10 years, a large amount of information has been accumulated on
204 *cis*-acting RNA elements and host proteins that play important roles in the replication of
205 positive-strand RNA viruses. The cumulative evidence supports the idea that viral
206 genomic RNAs that encode replication proteins can be conductors that orchestrate
207 multiple processes using viral and host proteins and viral RNA elements. However, our
208 current understanding of viral RNA replication and translation mechanisms is far from
209 complete. Viral RNAs appear to change their structure regarding either local or global

Presidential Address, JGPP (2012)

210 structures in a spatially and temporally regulated manner, probably via the concerted
211 action of viral and host proteins.

212 Screenings of host proteins that bind viral RNAs may represent a way to study the
213 regulatory mechanisms of viral gene expression. One of the approaches is the use of
214 RNA aptamer-based one-step affinity chromatography and mass spectrometry analysis
215 to identify host proteins that bind specifically to viral RNAs. Several host proteins have
216 been identified for RCNMV (Iwakawa et al. 2012). Another approach is a
217 high-throughput method using yeast protein microarrays. This method has led to the
218 identification of as many as 50 viral RNA-binding host proteins in the studies of BMV
219 and TBSV; (Li et al. 2009; Zhu et al. 2007). Further studies using proteomics and
220 genome wide-screens of co-opted host factors will also expand this field of research.
221 Functional analyses of the candidate host proteins using biochemical methods and
222 single-molecule techniques in cell-free systems in combination with analyses in living
223 cells will be needed to test the roles of candidate proteins in virus replication processes.
224 Confocal and electron microscopy experiments will also be useful to study the manner
225 via which the candidate proteins function spatially and temporally in virus replication
226 processes. Our understanding of the roles of viral and host proteins and viral RNA
227 elements in viral replication may lead to the development of novel strategies aimed at
228 controlling viral diseases.

Presidential Address, JGPP (2012)

229

230 **Acknowledgments**

231

232 The author thanks the staff and students of the Laboratory of Plant Pathology, Kyoto

233 University, and all collaborators who supported our research. The author also thanks the

234 researchers who provided materials and experimental protocols used in the research.

235 Research in the author's laboratory was supported in part by a Grant-in-Aid for

236 Scientific Research from the Japan Society for the Promotion of Science.

237

238 **References**

239

240 Ahlquist P, Noueiry AO, Lee WM, Kushner DB, Dye BT (2003) Host factors in

241 positive-strand RNA virus genome replication. J Virol 77: 8181-8186

242 den Boon JA, Diaz A, Ahlquist P (2010) Cytoplasmic Viral Replication Complexes.

243 Cell Host Microbe 8: 77-85

244 Chen J, Noueiry A, Ahlquist P (2001) *Brome mosaic virus* protein 1a recruits viral

245 RNA2 to RNA replication through a 5' proximal RNA2 signal. J Virol

246 75:3207-3219

247 Hiruki C (1987) The dianthoviruses: A distinct group of isometric plant viruses with

Presidential Address, JGPP (2012)

- 248 bipartite genome. *Adv Virus Res* 33:257-300
- 249 Hsu, NY, Ilnytska O, Belov G, Santiana M, Chen YH, Takvorian PM, Pau C, van der
250 Schaar H, Kaushik-Basu N, Balla T, Cameron CE, Ehrenfeld E, van Kuppeveld
251 FJM, Altan-Bonnet N (2010) Viral reorganization of the secretory pathway
252 generates distinct organelles for RNA replication. *Cell* 141:799–811
- 253 Hyodo K, Mine A, Iwakawa H-O, Kaido M, Mise K, Okuno T (2011) Identification of
254 amino acids in auxiliary replicase protein p27 critical for its RNA-binding activity
255 and the assembly of the replicase complex in *Red clover necrotic mosaic virus*.
256 *Virology* 413:300–309
- 257 Iwakawa H-O, Mine A, Hyodo K, An M, Kaido M, Mise K, Okuno T (2011) Template
258 recognition mechanisms by replicase proteins differ between bipartite
259 positive-strand genomic RNAs of a plant virus. *J Virol* 85:497–509
- 260 Iwakawa H-O, Tajima Y, Taniguchi T, Kaido M, Mise K, Taniguchi H, Okuno T
261 (2012) Poly(A)-binding protein facilitates translation of an
262 uncapped/nonpolyadenylated viral RNA by binding to the 3' untranslated region. *J*
263 *Virol* 86 (in press)
- 264 Kaido M, Funatsu N, Tsuno Y, Mise K, Okuno T (2011) Viral cell-to-cell movement
265 requires formation of cortical punctate structures containing Red clover necrotic
266 mosaic virus movement protein. *Virology* 413:205–215

Presidential Address, JGPP (2012)

- 267 Kim KH, Lommel SA (1994) Identification and analysis of the site of -1 ribosomal
268 frameshifting in red clover necrotic mosaic virus. *Virology* 200:574–582
- 269 Li Z, Pogany J, Panavas T, Xu K, Esposito AM, Kinzy TG, Nagy PD (2009)
270 Translation elongation factor 1A is a component of the tombusvirus replicase
271 complex and affects the stability of the p33 replication co-factor.
272 *Virology*:385:245-60
- 273 Liu L, Westler WM, den Boon JA, Wang X, Diaz A, Steinberg HA, Ahlquist P (2009)
274 An amphipathic α -helix controls multiple roles of Brome mosaic virus protein 1a
275 in RNA replication complex assembly and function. *PLoS Pathog* 5(3): e1000351
- 276 Mine A, Takeda A, Taniguchi T, Taniguchi H, Kaido M, Mise K, Okuno T. (2010a)
277 Identification and characterization of the 480-kilodalton template-specific
278 RNA-dependent RNA polymerase complex of *Red clover necrotic mosaic virus*. *J*
279 *Virol* 84:6070-6081
- 280 Mine A, Hyodo K, Takeda A, Kaido M, Mise K, Okuno T (2010b) Interactions between
281 p27 and p88 replicase proteins of *Red clover necrotic mosaic virus* play an
282 essential role in viral RNA replication and suppression of RNA silencing via the
283 480-kDa viral replicase complex assembly. *Virology* 407:213-224
- 284 Mizumoto H, Tatsuta M, Kaido M, Mise K and Okuno T (2003) Cap-independent
285 translational enhancement by the 3' untranslated region of *Red clover necrotic*

Presidential Address, JGPP (2012)

- 286 *mosaic virus* RNA1. J Virol 77:12113–12121
- 287 Nagy PD (2008) Yeast as a model host to explore plant virus-host interactions. Ann
288 Rev Phytopathol 46:217-242.
- 289 Nagy PD, Pogany J (2012) The dependence of viral RNA replication on co-opted host
290 factors. Nat Rev Microbiol 10:137-149
- 291 Nicholson BL, White KA (2011) 3'cap-independent translation enhancers of
292 positive-strand RNA plant viruses. Curr Opin Virol 1:373-380
- 293 Okamoto K, Nagano H, Iwakawa H, Mizumoto H, Takeda A, Kaido M, Mise K, Okuno
294 T (2008) *cis*-Preferential requirement of a-1 frameshift product p88 for the
295 replication of *Red clover necrotic mosaic virus* RNA1. Virology 375:205-212
- 296 Panavas T, Hawkins CM, Panaviene Z, Nagy PD (2005) The role of the p33:p33/p92
297 interaction domain in RNA replication and intracellular localization of p33 and
298 p92 proteins of *Cucumber necrosis* tombusvirus. Virology 338:81-95
- 299 Pogany J, White KA, Nagy PD (2005) Specific binding of tombusvirus replication
300 protein p33 to an internal replication element in the viral RNA is essential for
301 replication. J Virol 79:4859-4869
- 302 Sit TL, Vaewhongs AA, Lommel SA (1998) RNA-mediated trans-activation of
303 transcription from a viral RNA. Science 281:829–832

Presidential Address, JGPP (2012)

- 304 Schwartz M, Chen J, Janda M, Sullivan M, den Boon J, Ahlquist P (2002) A
305 positive-strand RNA virus replication complex parallels form and function of
306 retrovirus capsids. *Mol Cell* 9:505–514
- 307 Tajima Y, Iwakawa H, Kaido M, Mise K, Okuno T (2011) A long-distance RNA-RNA
308 interaction plays an important role in programmed-1 ribosomal frameshifting in
309 the translation of p88 replicase protein of *Red clover necrotic mosaic virus*.
310 *Virology* 417:169-178
- 311 Turner KA, Sit TL, Callaway AS., Allen NS, Lommel SA (2004) Red clover necrotic
312 mosaic virus replication proteins accumulate at the endoplasmic reticulum.
313 *Virology* 320:276-290
- 314 Tatsuta M, Mizumoto H, Kaido M, Mise K, Okuno T (2005) The *Red clover necrotic*
315 *mosaic virus* RNA2 trans-activator is also a cis-acting RNA2 replication element.
316 *J Virol* 79:978–986
- 317 Wang RY-L, Nagy PD (2008) *Tomato bushy stunt virus* co-opts the RNA-binding
318 function of a host metabolic enzyme for viral genomic RNA synthesis. *Cell Host*
319 *Microbes* 3:178–187
- 320 Xiong Z, Kim KH, Giesman-Cookmeyer D, Lommel SA (1993) The roles of the red
321 clover necrotic mosaic virus capsid and cell-to-cell movement proteins in
322 systemic infection. *Virology* 192:27–32

Presidential Address, JGPP (2012)

- 323 Yamanaka T, Ohta T, Takahashi M, Meshi T, Schmidt R, Dean C, Naito S, Ishikawa M
324 (2000) *TOM1*, an *Arabidopsis* gene required for efficient multiplication of a
325 tobamovirus, encodes a putative transmembrane protein. Proc Natl Acad Sci USA
326 97:10107–12
- 327 Zhu J, Gopinath K, Murali A, Yi G, Hayward SD, Zhu H, Kao C (2007) RNA-binding
328 proteins that inhibit RNA virus infection. Proc Natl Acad Sci USA 104:3129–34