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Hijacking host functions for translation and RNA replication by enteroviruses

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Running title: Use and abuse of host functions for enterovirus replication

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Abstract

The enterovirus genus includes the species poliovirus, coxsackievirus, rhinovirus, and enterovirus. These viruses can cause severe diseases in certain individuals, including poliomyelitis, myocarditis, and meningitis. Rhinovirus is responsible for one of the most prevalent human diseases in the world, the common cold. Although diseases caused by these infections can be severe, no antiviral against enteroviruses is currently available. To develop broad-spectrum antivirals, the molecular components and mechanistic steps of the viral replication cycle must be identified. Due to the small genomic RNA (~7.5 kb) of enteroviruses, host proteins are utilized to mediate viral replication. Although some of these cellular proteins have been identified and their roles in picornavirus replication have been characterized, it is necessary to identify and elucidate the replication functions of additional cellular proteins to develop new potential targets for antiviral therapeutics. Enteroviruses are known to modify cellular proteins to stimulate their levels of gene expression and RNA synthesis, but there are some cases where unaltered host proteins can aid in viral replication. Enteroviruses can also evade the antiviral response by altering host proteins involved in the immune and stress response to ensure efficient viral replication. How enteroviruses modify and utilize these host proteins will be discussed in this chapter.

I. Introduction

The diverse enterovirus genus of the *Picornaviridae* family encompasses 12 species, including poliovirus, coxsackievirus, rhinovirus, enterovirus, and echovirus serotypes (ictvonline.org/virusTaxonomy.asp). These viruses are responsible for the most prevalent human diseases worldwide (Khetsuriani et al., 2006), such as the common cold. Other illnesses include poliomyelitis, pericarditis, myocarditis, hand, foot, and mouth disease, and meningitis, which can severely affect infants, the elderly, and immunocompromised individuals. Although the symptoms of respiratory illnesses caused by picornaviruses are almost never fatal, these viral infections have a negative economic impact due to lost work time and can severely affect individuals with respiratory dysfunction, such as asthma (Gavala et al., 2011). Such respiratory infections are commonly caused by human rhinovirus, coxsackievirus, and enterovirus D68. Enterovirus D68 was first identified in California in 1962, but has recently had recurring outbreaks in North America, Europe, and Asia (Tokarz et al., 2012). Enterovirus D68 outbreaks have been associated with severe respiratory illnesses and are quickly spreading throughout the United States (Midgley et al., 2014). Another enterovirus with recurring outbreaks is enterovirus 71. Enterovirus 71 is a neurotropic enterovirus with symptoms similar to hand, foot, and mouth disease and remains endemic in the Asia-Pacific region. Neurological diseases caused by enterovirus 71 infection can cause aseptic meningitis and brainstem encephalitis, which can lead to mortality [reviewed in (Shih et al., 2011)]. Although a vaccine against poliovirus is available, no effective antivirals for treating enterovirus infections currently exist. Since symptoms caused by such infections can lead to severe complications in certain individuals, it is necessary to develop antiviral

therapeutics against enteroviruses. Antivirals can target a host protein required for enterovirus replication, a viral protein, or the viral RNAs. Antivirals targeting the host can lead to cell toxicity, while antivirals against a viral protein can lead to antiviral-resistant mutants. To develop an effective broad-spectrum antiviral, the steps of the enterovirus replication mechanism and the roles of key molecular players, both host and viral, must be elucidated.

While infection may result in diverse diseases, all enteroviruses have a small (~7.5 kb) positive-sense, single stranded RNA genome that is replicated in the cytoplasm of infected cells. The genome contains a highly structured 5' noncoding region (NCR) that is necessary for viral translation and RNA replication. Following the 5' NCR is the coding region, which encodes both the structural and nonstructural proteins, a 3' NCR, and a short genetically encoded poly(A) tract at the 3' terminus (Kitamura et al., 1981; Wimmer et al., 1993; Yogo and Wimmer, 1972). Enteroviruses lack a 7-methylguanosine (7mG) cap at the 5' end of their RNA and instead contain a small viral protein known as VPg covalently linked to the 5' end by a tyrosyl-RNA phosphodiester bond (Ambros and Baltimore, 1978; Flanegan et al., 1977; Lee et al., 1977; Rothberg et al., 1978). Enteroviruses have evolved to use VPg as a protein-primer for RNA synthesis, since their RNA-dependent RNA polymerase (RdRP) 3D ($3D^{pol}$) cannot initiate viral RNA replication *de novo* (Flanegan and Baltimore, 1977; Paul et al., 1998). The position of VPg on the genomic RNA is outlined in Figure 1, which depicts an overview of the enterovirus genome and the structural and nonstructural viral proteins it encodes.

Following virion uncoating after the onset of infection, enterovirus genomic RNA is localized in the cell cytoplasm where it is then translated into a single viral polyprotein.

Since enterovirus genomic RNAs lack a 7mG cap at the 5' end, the viral polyprotein is translated in a cap-independent manner via an internal ribosome entry site (IRES). The IRES is located in the 5' NCR of viral RNA and is composed of a number of stem-loop secondary structures. Due to their limited coding capacity (~7.5 kb), enteroviruses have evolved to hijack host cellular functions to carry out the translation and replication of their genomes. Viral translation is mediated by cellular IRES trans-acting factors (ITAFs). After translation of the open reading frame of genomic RNA, the viral polyprotein is proteolytically processed by viral proteinases. These viral proteinases can also cleave host proteins, resulting in the shut down of cellular translation and transcription and the alteration of nucleo-cytoplasmic trafficking. These cleavage events are advantageous to the virus since the predominantly nuclear host proteins involved in viral replication become more concentrated in the cell cytoplasm. Following viral protein synthesis, specific viral proteins alter cytoplasmic membranes to form replication complexes, where viral RNA is synthesized. These newly synthesized RNAs can either undergo further rounds of translation and replication or become encapsidated into virions that go on to infect neighboring cells. In addition to the modifications of host proteins for viral replication, viral proteinases can also alter cellular proteins to suppress antiviral response pathways, such as the type I interferon (IFN) response, generation of stress granules (SGs), and processing body (P body) formation. This review will focus on how enteroviruses subvert host cellular proteins to enhance viral replication while evading antiviral and stress response pathways.

II. Viral proteinase disruption of host machinery

Enteroviruses can disrupt host cell translation and transcription machinery to benefit viral replication by using viral-encoded proteinases to cleave host cell proteins. To carry out these modifications, enteroviruses utilize the virus-encoded proteinases 2A and 3C (and the precursor protein, 3CD). In addition to recognizing multiple cleavage sites in host cellular proteins, these proteins are responsible for proteolytically processing the enterovirus polyprotein. Poliovirus proteinase 2A cleaves between phenylalanine-glycine or tyrosine-glycine residues in the viral polyprotein (Toyoda et al., 1986), while 3C/3CD cleaves primarily at glutamine-glycine sites but can cleave at additional sites as well. 3C/3CD cleavage activity is dependent on surrounding sequences, specifically an amino acid with an aliphatic side chain in the amino acid located four positions (P4) proximal to the cleavage site (Blair and Semler, 1991; Nicklin et al., 1986). The somewhat divergent recognition sites for these proteinases allow for cleavage of host proteins, which disrupts cellular functions and alters protein activities. This section will focus on how enterovirus proteinases cleave host proteins to shut down the cellular translation and transcription machinery, subverting host functions to augment viral translation and RNA synthesis. These cleavage events and cellular function alterations are outlined in Table 3.1.

During cap-dependent translation of cellular mRNAs, eukaryotic initiation factors are recruited to the 7mG cap structure at the 5’ ends of mRNA. These factors form a complex that interacts with the 43S pre-initiation complex (PIC) to recruit ribosomes for translation initiation. Eukaryotic initiation factor 4G (eIF4G) serves as a scaffold protein that aids in the recruitment of eIF4E and eIF4A, to form a ribonucleoprotein (RNP)

complex termed eIF4F, as well as additional proteins such as poly(A) binding protein (PABP), for initiation of cap-dependent translation (Jackson et al., 2005; Wells et al., 1998). Poliovirus and coxsackievirus 2A proteinases have been shown to cleave both eIF4G isoforms, eIF4GI and eIF4GII, early in viral infection, resulting in the loss of the N-terminal domain required for both eIF4E and PABP interaction [reviewed in (Daijogo and Semler, 2011)] (Devaney et al., 1988; Etchison et al., 1982; Krausslich et al., 1987). 2A proteinase preferentially cleaves eIF4G when the cellular protein is bound to cap-binding protein eIF4E, thus leading to rapid shut down of cap-dependent cellular translation (Bovee et al., 1998). This host machinery shut down allows for resource allocation to efficient cap-independent translation benefiting viral protein synthesis. Additionally, evidence suggests that the cleaved form of eIF4G is required to stimulate IRES-dependent translation (Lamphear et al., 1995; Liebig et al., 1993). eIF4G interacts with stem-loop V of the poliovirus and coxsackievirus IRES (de Breyne et al., 2009). The central domain of eIF4G interacts with eIF3, a component of the 43S PIC, *in vitro* (Sweeney et al., 2014). This direct interaction between eIF4G and eIF3 at stem-loop V suggests that this interaction may be required in recruiting the 43S PIC to the proximal stem-loop IV, an essential step in 48S complex formation for viral translation initiation to occur.

In addition to viral-mediated cleavage of the cap-binding complex scaffold protein eIF4G, enterovirus infection also results in the cleavage of host protein PABP to disrupt cap-dependent translation. PABP is a cellular protein that binds to the 3' poly(A) tract of mRNAs and interacts with eIF4G to functionally circularize the mRNA for efficient translation and mRNA stability in the uninfected cell [reviewed in (Fitzgerald

and Semler, 2009; Smith et al., 2014)]. During infection, PABP is cleaved by poliovirus and coxsackievirus 2A proteinases (Joachims et al., 1999; Kerekatte et al., 1999). In addition, poliovirus and human rhinovirus 3C proteinases preferentially cleave ribosome-associated PABP (Kuyumcu-Martinez et al., 2002). PABP has three conserved putative cleavage sites in the flexible linker domain between its RNA recognition motifs (RRMs) and C-terminal domain and two additional putative cleavage sites in the RRMs (Kozlov et al., 2004; Kozlov et al., 2001; Lloyd, 2006). Cleavage of PABP at these different sites by either 2A or 3C results in cellular translation inhibition by disrupting mRNA circularization.

Enterovirus proteinases 2A and 3C play roles in shutting down host cellular transcription during infection by disrupting RNA polymerases (pol) I, II, and III. Poliovirus proteinase 3C is responsible for inhibiting RNA pol I transcription activity approximately 90 to 180 minutes post-infection by targeting the pol I transcription factor upstream binding factor (UBF), which is a sequence-specific DNA-binding protein that stabilizes the selectivity factor (SL-1) protein complex on the rRNA promoter for pol I transcription, and the SL-1 protein complex subunit, TATA-binding protein (TBP)-associated factor (TAF), TAF_{110} (Banerjee et al., 2005). RNA pol II is responsible for transcribing host cellular mRNAs and is targeted by both poliovirus proteinases 2A and 3C. These enzymes cleave TBP, which is involved in forming a PIC containing transcription factor II D (TFIID) for pol II binding to transcription start sites (Das and Dasgupta, 1993; Yalamanchili et al., 1997a). However, it is 3C-mediated cleavage of TBP and phosphorylated CREB, both upstream cellular transcription factors, that is required for pol II transcription inhibition (Das and Dasgupta, 1993; Yalamanchili et al.,

1997b; Yalamanchili et al., 1996). Poliovirus 3C activity can also lead to the degradation of transcription activator p53, in a non-ubiquitin mediated pathway, but in the presence of an unknown cellular protein (Weidman et al., 2001). RNA pol III is responsible for transcribing ribosomal RNA genes, tRNA genes, and genes encoding other small RNAs. Pol III activity is inhibited during poliovirus infection by 3C-mediated cleavage of TFIIIC (Clark et al., 1991; Shen et al., 2004). TFIIIC binds to the promoter element, B box, downstream of the transcription start site to recruit TFIIIB, which recruits pol III to the transcription start site. Once TFIIIB recruits pol III, TFIIIC dissociates, allowing pol III-mediated transcription to occur. 3C cleavage of TFIIIC inhibits recruitment of TFIIIB, thus indirectly inhibiting pol III transcription (Clark et al., 1991; Kassavetis et al., 1990). These proteinase-mediated cleavages of host proteins all work together to inhibit cellular transcription.

The cleavage of host proteins can directly or indirectly lead to the disruption of cellular translation and transcription. Such cleavage events are summarized in Table 3.1. Viral modifications of cellular proteins are not restricted to down regulation of host cell machinery but can also extend to the enhancement of viral IRES-mediated translation, viral RNA synthesis, and evasion of the host antiviral and stress response mechanisms. These topics will be further discussed in the following sections of this chapter.

III. Use and abuse of host cell functions for viral translation and RNA replication

Use of host factors for IRES-dependent translation and viral RNA synthesis

Since enteroviruses replicate in the host cytoplasm, and a number of cellular proteins involved in viral replication are predominantly nuclear, enteroviruses modify the cellular nucleo-cytoplasmic trafficking mechanism to accumulate proteins used for viral

replication in the cell cytoplasm. Some of the cellular proteins modified during viral infection that will be discussed in this section include La autoantigen, polypyrimidine tract-binding protein (PTB), poly(rC)-binding protein 2 (PCBP2), and serine/arginine (SR)-rich protein (SRp20) (Table 3.2). These shuttling proteins contain an amino acid sequence known as a nuclear localization signal (NLS) that is recognized by a specific import receptor complex (Gorlich and Kutay, 1999). Protein-receptor complexes then relocalize from the cytoplasm to the nucleus through nuclear pore complexes (NPCs) embedded in the nuclear envelope of the host cell. The NPC is made up of nucleoporins (Nups) that contain phenylalanine-glycine repeats necessary for shuttling the protein-receptor complex through the nuclear membrane. During enterovirus infection, the NPC becomes modified when poliovirus or human rhinovirus proteinase 2A cleaves Nup62, Nup98, and Nup153. These cleavage events correlate with proteins accumulating in the cytoplasm and inhibition of import pathways (Belov et al., 2000; Castello et al., 2009; Fitzgerald et al., 2013; Gustin and Sarnow, 2001; Park et al., 2008; Park et al., 2010; Watters and Palmenberg, 2011). Cleavage of Nups results in loss of the phenylalanine-glycine repeats necessary for the protein-receptor complex docking in the NPC domain during shuttling through the nuclear membrane (Bayliss et al., 2000; Stewart et al., 2001). Pathways that are inhibited during enterovirus infection include the transportin import pathway and K nuclear shuttling (KNS) import pathway. The KNS import pathway mediates the transport of RNA-binding proteins required for enterovirus replication known as heterogeneous nuclear ribonucleoproteins (hnRNPs) (Gustin, 2003; Gustin and Sarnow, 2001; Michael et al., 1997; Pollard et al., 1996). This viral-mediated disruption

of nucleo-cytoplasmic trafficking results in accumulation of nuclear proteins in the cytoplasm necessary to enhance viral translation and RNA synthesis.

Host protein La was initially described as an autoantigen found in sera from patients with systemic lupus erythematosus and Sjögren syndrome (Tan, 1989). La is predominantly a nuclear protein in the uninfected cell and plays a role in the maturation of RNA pol III transcripts, due to its ability to bind various RNA structures via its RNA binding domain (Gottlieb and Steitz, 1989; Kenan et al., 1991). During poliovirus or coxsackievirus B3 (CVB3) infection, La becomes relocalized to the cell cytoplasm and interacts with the 5' NCR of the viral RNA to enhance IRES-mediated viral translation (Meerovitch et al., 1993; Ray and Das, 2002). During the course of poliovirus infection, La is cleaved by viral proteinase 3C but is still able to bind the viral IRES and mediate translation of the viral genome (Shiroki et al., 1999). Previous studies showed that the addition of purified La protein to rabbit reticulocyte lysate enhances translation while inhibiting accumulation of aberrant translation products (Meerovitch et al., 1993; Svitkin et al., 1994). La is only one of the several known IRES trans-acting factors (ITAFs) that interact with the viral IRES to enhance translation of the enterovirus genome. Another nuclear RNA binding protein, nucleolin, has also been shown to interact with both the 5' and 3' NCR of poliovirus RNA to stimulate viral translation and replication, although the exact mechanism remains unclear (Izumi et al., 2001; Waggoner and Sarnow, 1998).

Host cell shuttling protein PTB is a member of the hnRNP complex and also functions as a cellular ITAF. In the uninfected cell, PTB functions as a repressive regulator of alternative splicing (Mulligan et al., 1992). During poliovirus infection, PTB, like La, relocalizes to the cell cytoplasm and becomes redistributed (Back et al., 2002).

Full length PTB has been implicated in enhancing viral translation during infection (Florez et al., 2005) by binding to stem-loop V of the poliovirus IRES and modulating adjacent eIF4G binding (Kafasla et al., 2010). Full-length PTB also interacts with PCBP2 when bound to stem-loop IV of the poliovirus 5' NCR to stimulate translation (Kim et al., 2000). However, the multiple isoforms of PTB are cleaved between the RRM domains by poliovirus 3C/3CD proteinase late during viral infection (Back et al., 2002). Cleavage of the N-terminal domain of PTB could result in loss of interaction with PCBP2 and the hnRNP complex. Alternatively, cleavage of the C-terminal domain could result in loss of interaction with the IRES element (Back et al., 2002). The accumulation of cleaved PTB corresponded with a decrease in viral translation levels *in vitro*, suggesting a role in mediating the switch from viral translation to negative-strand RNA synthesis during the replication cycle (Back et al., 2002). A switch in viral translation to RNA synthesis is required during viral replication since the positive-strand viral RNA is translated in a 5' to 3' direction by the translation machinery, while the negative-strand viral RNA is synthesized using the same template but in the opposite direction by the viral-encoded RdRP $3D^{pol}$ [reviewed in (Daijogo and Semler, 2011)]. Interestingly, cleavage of PTB seems to be specific to poliovirus- or human rhinovirus-infected HeLa cells. A recent study shows that PTB is not efficiently cleaved in human rhinovirus-infected WisL cells, a human lung fibroblast cell line, suggesting that host proteins may be differentially cleaved by enteroviruses in different cell lines (Chase and Semler, 2014). RNA-binding host protein unr has been shown to act synergistically with PTB to enhance human rhinovirus IRES-mediated translation but has minimum enhancement of poliovirus IRES translation (Hunt et al., 1999). This difference in host protein usage among enteroviruses

suggests that these viruses may utilize different cellular proteins to mediate the same viral functions, including the switch in viral translation to RNA replication.

PCBP2 is a host cell RNA-binding protein that functions as an ITAF for enterovirus translation and has been shown to be involved in the switch from viral translation to RNA synthesis. PCBP2 binds to poly(rC) regions of RNA and is expressed in both the nucleus and cytoplasm of the uninfected cell. During poliovirus infection, PCBP2 binds to RNA secondary structure stem-loop IV of the viral IRES, along with host splicing factor SRp20, to help form the hnRNP complex necessary for viral translation (Bedard et al., 2007; Blyn et al., 1996; Blyn et al., 1997). PCBP2 can also bind stem-loop I in the 5' NCR of the poliovirus genome to form a ternary complex with viral precursor proteinase 3CD (Parsley et al., 1997). This complex is required for initiation of negative-strand RNA synthesis and has been suggested to also be involved in positive-strand RNA synthesis (Gamarnik and Andino, 1997; Parsley et al., 1997; Vogt and Andino, 2010). During poliovirus, coxsackievirus, or human rhinovirus infection of HeLa cells, PCBP2 is cleaved in the linker region between its K-homologous (KH) domains, KH2 and KH3, by viral proteinase 3C/3CD (Chase et al., 2014; Perera et al., 2007). Cleaved PCBP2 can no longer bind stem-loop IV or interact with SRp20, resulting in inhibition of IRES-mediated translation, but it can still bind stem-loop I for viral RNA synthesis (Bedard et al., 2007; Chase et al., 2014; Perera et al., 2007). It has also been suggested that poliovirus 3CD binds to stem-loop I to increase the binding affinity of PCBP2 to stem-loop I, thus decreasing its availability for binding to stem-loop IV for translation (Gamarnik and Andino, 1998). Cleavage of PCBP2, along with the cleavage of other proteins such as PTB, can help mediate the switch from viral translation to RNA

synthesis. Interestingly, cleavage of PCBP2 does not occur in human rhinovirus-infected human lung fibroblasts, WisL cells, while it does when they are infected with poliovirus, as determined by Western blot analysis (Chase and Semler, 2014). Such a differential cleavage pattern suggests that cleavage of specific host proteins may be required to mediate the switch to RNA synthesis only in certain cell types. It is also possible that the concentration of PCBP2 in WisL cells is low and below the level of detection of the Western blot analysis used in this study (Chase and Semler, 2014). The mechanism that brings about the switch from viral translation to RNA replication remains incompletely understood and will require future studies.

Host protein SRp20, a shuttling protein involved in mRNA splicing and translation, contains an RRM domain at its N-terminus for RNA binding and a serine/arginine (RS)-rich domain at its C-terminus for nucleo-cytoplasmic shuttling and protein-protein interactions (Caceres et al., 1997; Caceres et al., 1998). During poliovirus or CVB3 infection, and to a lesser extent during human rhinovirus 16 infection, SRp20 relocalizes to the infected cell cytoplasm (Fitzgerald et al., 2013; Fitzgerald and Semler, 2011). Poliovirus proteinase 2A activity is required for this redistribution pattern in the cell cytoplasm (Fitzgerald et al., 2013). In the cytoplasm, SRp20 enhances poliovirus translation by binding the KH3 domain of PCBP2 with its RS domain and recruiting ribosomes to the IRES for translation (Bedard et al., 2007). Whether SRp20 recruits the ribosomes directly or indirectly to stem-loop IV for IRES-mediated translation remains to be determined. It is possible that additional undiscovered ITAFs are required to recruit ribosomes to the IRES for translation or that SRp20 may recruit the ribosomes via direct interactions.

Following the initial rounds of translation, viral polyproteins are processed by the viral-encoded proteinases. The nonstructural viral proteins can go on to function in viral RNA synthesis. To allow for efficient viral RNA synthesis to occur, there is a switch from viral translation to RNA synthesis. As discussed above, this switch is currently thought to occur when host factors, such as PTB and PCBP2, are cleaved by enterovirus proteinase 3C/3CD. Cleavage of ITAFs inhibits IRES-mediated translation but still allows viral RNA replication to proceed, since the presence of these cleaved proteins favors the clearing of ribosomes from the RNA template. Overall, viral translation and RNA replication are dependent on the modifications of host proteins by the viral-encoded proteinases. In addition to host protein modifications, the cellular environment becomes altered in enterovirus-infected cells so efficient viral RNA replication can occur.

Alteration of host cell membranes for viral RNA synthesis

For viral RNA replication to occur, cellular organelles must be modified to form virus-induced membranous vesicles that serve as sites of replication complexes for viral RNA synthesis (Caliguiri and Tamm, 1969; Dales et al., 1965). The specific localization of these membranous vesicles may physically separate RNA synthesis from IRES-mediated translation in the cytoplasm and increase the local concentrations of viral proteins required for viral replication. The virus-induced vesicles are derived from the endoplasmic reticulum (ER), Golgi, and from components of autophagic vesicles to form single- and double-walled vesicles (Bienz et al., 1987; Jackson et al., 2005; Schlegel et al., 1996).

The COPII complex components, Sec13 and Sec31, have been shown to colocalize with viral protein 2B, suggesting that COPII may be involved in the formation

of replication complexes (Rust et al., 2001). COPII is a vesicle coat protein complex that transports proteins from the ER to the Golgi in the uninfected cell (Barlowe et al., 1994). COPII vesicle proteins are made in the ER with the help of COPII complexes, which include coat proteins. Once the COPII-coated vesicles are formed, they bud from the ER, lose their coat proteins, and become fused to the Golgi (Klumperman, 2000; Rust et al., 2001; Springer et al., 1999). During poliovirus infection, it has been shown that these ER-derived vesicles accumulate in the cytoplasm, and there is a transient increase in COPII vesicle budding from the ER (Bienz et al., 1987; Rust et al., 2001; Trahey et al., 2012). Alterations in the secretory pathway also mediate the formation of viral replication complexes. During poliovirus infection, nonstructural viral protein 3A recruits guanine nucleotide exchange factor (GEF), GBF1, and viral proteinase 3CD recruits GEFs, BIG1, and BIG2, to membranes to activate the secretory pathway by converting the small GTPase Arf1 into its GTP-active form (Belov et al., 2007). Arf1-GTP can alter membrane curvature and recruit coat proteins to form secretory transport vesicles (Belov and Ehrenfeld, 2007). The activation of Arf1 leads to the production of phosphatidylinositol-4-phosphate (PI4P), a lipid with an important role in vesicle transport. Expression of CVB3 3A can lead to an accumulation of PI4P and PI4-kinase III β (PI4KIIIβ) (Hsu et al., 2010). During CVB3 infection, PI4KIIIβ has also been shown to colocalize with sites of viral RNA replication and to be required for both poliovirus and CVB3 replication (Hsu et al., 2010). 3A has been shown to associate with acyl coenzyme A [acyl-CoA]-binding protein domain 3 (ACBD3), a protein that binds to an integral Golgi protein known as giantin (Greninger et al., 2012). However, it was recently shown that although ACBD3 does interact with CVB3 3A and PI4KIIIβ directly,

this interaction is not required for the recruitment of PI4KIIIβ to replication complexes (Dorobantu et al., 2014). Additionally, depletion of GBF1 and Arf1 by pharmalogical inhibition or small interfering RNA (siRNA) treatment in CVB3-infected cells did not inhibit PI4KIIIβ recruitment (Dorobantu et al., 2014). These contradictory findings suggests that the mechanism for virus-induced host membrane reorganization remains poorly understood and additional studies are required to dissect the involvement of the secretory pathway during enterovirus replication.

Host cell membrane organization throughout poliovirus infection has been observed by electron microscopy (Belov et al., 2012; Caliguiri and Tamm, 1970). At 3 hours post-infection (h.p.i.), replication complexes appear to be single-membraned, while at 4 h.p.i. the complexes appear to be convoluted. At later times of infection, replication complexes appear to be double-membraned, illustrating the dynamic nature of viral-induced, membranous vesicles throughout the replication cycle. The convoluted membranes observed at peak times of infection resemble the crescent-shaped precursor membranes seen during autophagy. During poliovirus infection, LC3, a marker for autophagy, localizes to these membranous vesicles. This localization is induced by viral proteins 2BC and 3A (Jackson et al., 2005; Taylor and Kirkegaard, 2007). One hypothesis to explain these observations is that enteroviruses induce formation of replication complexes via a mechanism similar to autophagy (Kemball et al., 2010; Klein and Jackson, 2011; Suhy et al., 2000). However, a recent study using an antibody specific for double-stranded RNA (dsRNA), which is an RNA intermediate formed during viral RNA synthesis, to identify replication complexes found that dsRNA does not significantly colocalize with LC3 early during infection but does at late times of infection

(Richards et al., 2014). Although this contradicts previous studies suggesting that LC3 plays a role in replication complex formation, the authors of this report alternatively suggest that LC3 may have a role in viral replication, but not in complex formation (Richards et al., 2014). This apparent discrepancy in findings may be due to previous studies using antibodies against viral proteins to analyze the role of the autophagy pathway during viral RNA synthesis instead of antibodies specific for viral RNA replication intermediates. In summary, although previous studies have attempted to elucidate the mechanisms utilized in enterovirus replication complex formation, there are many features of this process that remain to be determined.

Additional host proteins usurped for viral translation and replication

Enteroviruses require multiple host factors to carry out their viral replication cycles. It is apparent that the host factors described above are not sufficient to carry out translation, replication, and encapsidation of the viral RNA. In a comprehensive attempt to identify host factors binding to the viral RNA during infection, numerous experimental approaches have been employed. A recent study using thiouracil cross-linking mass spectrometry (TUX-MS) has identified host factors binding to poliovirus RNA during replication in HeLa cells (Lenarcic et al., 2013). In addition to previously identified host factors known to interact with the viral RNA, 66 putative host proteins have been identified using this methodology. From these 66, eight proteins were selected for validation. Knockdown of two of these proteins, NONO (non-POU-domain-containing octamer-binding protein) and CNBP (cellular nucleic acid-binding protein), decreased poliovirus replication similar to levels when PCBP2, La, PTB, or hnRNP C was knocked down. Further analysis of these two host proteins revealed that CNBP was required for

efficient viral translation and NONO was required for efficient positive-strand RNA synthesis (Lenarcic et al., 2013). This methodology proved to be an effective way to identify proteins associated with the viral RNA during enterovirus replication. One of these proteins is AU-rich binding factor 1 (AUF1) (Lenarcic et al., 2013), which had been previously identified via an RNA affinity screen for proteins interacting with the 5' NCR (Rozovics et al., 2012).

AUF1, also known as hnRNP D, is a cellular protein that binds to AU-rich elements in the 3' NCR of mRNAs in the uninfected cell (Zhang et al., 1993). It is involved in RNA stability and can target RNAs for degradation via an mRNA-decay pathway (Kiledjian et al., 1997). AUF1 has four isoforms due to alternative splicing that contain tandem RRMs to bind RNA (Kajita et al., 1995). During poliovirus and CVB3 infection, AUF1 relocalizes from the nucleus to the cytoplasm in a proteinase 2A-driven manner and colocalizes with the nonstructural viral protein 2B (Cathcart et al., 2013). AUF1 is cleaved by poliovirus or human rhinovirus 3CD and CVB3 3C (Rozovics et al., 2012; Wong et al., 2013). AUF1 has also been shown to directly interact with the poliovirus 5' NCR, specifically full length 5' NCR and stem-loop IV (Cathcart et al., 2013; Rozovics et al., 2012). This interaction is inhibited by the cleavage of AUF1 by 3CD (Cathcart et al., 2013). AUF1 has been shown to interact with the 3' NCR of CVB3 RNA as well, due to the AU-rich sequence at the 3' end (Wong et al., 2013). When AUF1 is genetically ablated or knocked down, poliovirus, human rhinovirus 16, or CVB3 viral titers increase, suggesting an inhibitory role for AUF1 during enterovirus infection (Cathcart et al., 2013; Wong et al., 2013). AUF1 has been shown to decrease poliovirus translation *in vitro*, suggesting that AUF1 functions as an antiviral factor during

enterovirus infection (Cathcart et al., 2013). It is possible that enteroviruses cleave AUF1 to disrupt the interaction of this protein with viral RNA as a mechanism to evade the cellular RNA decay pathway. AUF1 has also been shown to interact with other host factors involved in viral replication, including PCBP2, nucleolin, and PABP (Dempsey et al., 1998; Kiledjian et al., 1997; Lu et al., 2006). AUF1 cleavage may disrupt these host protein-protein interactions so that these host factors can bind to the viral RNA and stimulate viral translation and replication. Another possible role for AUF1 during enterovirus replication stems from the fact that AUF1 has been shown to bind to both the 5' and 3' NCRs of the viral RNA. Such interactions could aid in the functional circularization of viral RNA through homo-multimerization of AUF1 via its dimerization domain present at the N-terminus. Further studies are necessary to define the role of AUF1 during enterovirus infection and how the virus might evade the RNA degradation pathway initiated by this protein.

Another recently identified host protein that is utilized during enterovirus replication is 5' tyrosyl-DNA phosphodiesterase 2 (TDP2) (Virgen-Slane et al., 2012). In the uninfected cell, TDP2, also known as TRAF and TNF receptor-associated protein (TTRAP) and ETS-1 associated protein II (EAPII), functions as a DNA repair enzyme that hydrolyzes 5' phosphotyrosyl DNA linkages in topoisomerase II-mediated double strand breaks (Cortes Ledesma et al., 2009). TDP2 was shown to harbor the activity, discovered decades ago, that cleaves the phosphotyrosyl bond between VPg and the 5' end of poliovirus virion RNA (Ambros and Baltimore, 1978; Ambros et al., 1978; Virgen-Slane et al., 2012). This activity was initially referred to as VPg unlinkase and was shown to be present in both the nucleus and the cytoplasm of uninfected and

poliovirus infected cells (Ambros et al., 1978). TDP2 is a predominantly nuclear protein but is also found in the uninfected cell cytoplasm. During poliovirus infection, TDP2 relocalizes from the nucleus to the cell cytoplasm, and during peak times of infection, is sequestered to the cell periphery in sites distinct from putative replication complexes (Virgen-Slane et al., 2012). Earlier studies had shown that VPg is absent from actively translating viral RNAs but present on newly synthesized viral RNAs or encapsidated virion RNAs (Fernandez-Munoz and Darnell, 1976; Hewlett et al., 1976; Nomoto et al., 1977; Nomoto et al., 1976). However, another early study reported that poliovirus RNA with a VPg linked to the 5' end was capable of forming a translation initiation complex in rabbit reticulocyte lysate (RRL), suggesting that the cleavage of VPg may not be required for viral translation *in vitro* (Golini et al., 1980). Limitations to the experiments in this latter study include that they were done in a cell-free system using RRL, a lysate deficient in TDP2 (Rozovics et al., 2011), and that the sucrose gradients could not determine if the VPg linked RNA represented a small or larger population of the viral RNA associated with the ribosomes.

In accordance with the early findings described above, a study using a replicon with an uncleavable bond between VPg and the viral RNA and a reporter gene shows that following transfection of this mutated CVB3 or poliovirus RNA, viral translation and replication are unaltered (Langereis et al., 2014). Although these results suggest that cleavage of the VPg-RNA linkage is not required for viral translation and replication, the caveats of the study must also be considered. The viral RNA harboring this uncleavable bond and reporter gene was transfected into the cells, thus bypassing the normal receptor-mediated entry pathway and the uncoating step. These steps that occur during normal

infection may be essential in determining the orientation of viral RNA during uncoating and its initial exposure to the cell cytoplasm following uncoating. It is possible that since the viral RNA has been transfected into the cell and thus only undergoes primary rounds of translation and replication, the cleavage of VPg by VPg unlinkase/TDP2 may be necessary to determine the fate of nascent viral RNAs to either be encapsidated in progeny virus particles or undergo an additional round of translation and replication. Another study showed that TDP2 is necessary for efficient enterovirus replication in murine cells (Maciejewski et al., 2016). Although viral yields were significantly reduced in the absence of TDP2, this study shows that TDP2 is differentially required among enteroviruses. The greatest dependency on TDP2 was seen during CVB3 infection, with CVB3 yields remaining unchanged in the absence of TDP2. However, in agreement with Langereis *et al.* (2014), CVB3 replicated at reduced levels in the absence of TDP2 following transfection using a CVB3 replicon encoding a reporter gene (Maciejewski et al., 2016). As stated above, it is important to note the experimental caveats of performing a transfection versus infection. Together these results show that TDP2 plays a critical role during the course of enterovirus infections in murine cells and could serve as a putative target for antiviral development.

Most studies characterizing host proteins required for the enterovirus replication cycle have been carried out using the prototypic enterovirus, poliovirus. As discussed in this section, not all enteroviruses require the same host proteins for their viral replication cycles. This variation leaves room for other host proteins to be utilized during replication. Since there have been only a few reports of ITAFs required for EV71, a study was undertaken to identify cellular proteins bound to a biotinylated EV71 5'NCR. From this

study, 12 cellular proteins were identified to interact with the 5' NCR (Lin et al., 2008). Of these 12 proteins, previously identified proteins were purified, including PTB, poly(C)-binding protein 1 (PCBP1, also known as hnRNP E1), PCBP2, La, and Unr. In addition, proteins previously unidentified as EV71 5' NCR-binding proteins were reported, including hnRNP K, hnRNP A1, far-upstream element-binding protein 1 (FBP1), and FBP2 (Lin et al., 2008). More recently, these latter proteins have been shown to redistribute from the cell nucleus to the cytoplasm and stimulate EV71 infection (Huang et al., 2011; Lin et al., 2008; Lin et al., 2009). Although the roles of hnRNP K, hnRNP A1, FBP1, and FBP2 during enterovirus 71 infection remain to be determined, this finding suggests that although enteroviruses have a highly conserved genome, they utilize several different host factors to enhance their viral replication.

IV. Evasion of host antiviral and stress response pathways and mRNA surveillance

Enteroviruses, like any invading pathogen, elicit immune and stress responses in the host. This leads to the activation of pathways that induce the interferon response (IFN) and stress granule (SG) formation. Similar to the enterovirus-mediated modifications of host proteins involved in cellular translation and transcription, enteroviruses can also modify host proteins involved in antiviral and stress response pathways. Additionally, enteroviruses can evade cellular mRNA surveillance by disrupting processing bodies (P bodies). This section will discuss how enteroviruses modify host proteins involved in cellular defense pathways to suppress their activation and, thereby, stimulate viral replication.

RIG-I-like receptor detection of enteroviruses

The host must be able to recognize invading pathogens to avoid eliciting a response against itself. To accomplish this, cells have specialized pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs). PRRs fall into several different families and include the Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-Type lectin receptors (CLRs), and RIG-like receptors (RLRs). Two essential immune receptors in the RLR family that detect double stranded viral RNA in the cytoplasm are retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5). Ligand binding causes these receptors to oligomerize and serve as signaling platforms to recruit and activate signaling adaptors, including the mitochondrial adaptor, MAVS. This activates a cascading signaling pathway that leads to the transcription of NF-κB factors and interferon regulatory factors (IRFs). These transcription factors then go on to induce expression of type I IFN. Such cellular response pathways induced by the virus serve as the front line of host defense against invading pathogens [reviewed in (Reikine et al., 2014)].

RLRs belong to the DExD/H-box helicase family and use ATP binding and hydrolysis to drive formation of an oligomer filament composed of repeating dimers, which extends from their target protein to downstream signals to activate IFN signaling (Peisley et al., 2013). The DExD/H-box helicase family includes three sensors for detecting viruses: RIG-I, MDA5, and LGP2. RIG-I discriminates between host and viral RNA by specifically binding to short double-stranded RNAs (dsRNAs) and single-stranded RNAs (ssRNAs) (less than 300 bp) with a 5’ triphosphate (5’ppp) moiety (Hornung et al., 2006). The C-terminal domain of RIG-I recognizes and binds 5’ppp,

while its helicase domains bind the RNA to form oligomer filaments (Hornung et al., 2006; Peisley et al., 2013). In contrast to RIG-I, MDA5 binds specifically to long dsRNA regardless of its 5' end moiety. MDA5 also forms a filament around dsRNA. Both RIG-I and MDA5 contain an N-terminal caspase recruitment domain (CARD), which aids in MAVS recruitment and activation (Kato et al., 2006). However, LGP2, the third member of the family, lacks a CARD and therefore cannot recruit or activate MAVS. Instead, LGP2 responds to viral stimuli by modulating RIG-I and MDA5 signaling (Childs et al., 2013). Since enterovirus RNAs lack the 5'ppp moiety, the interaction between RIG-I and enterovirus RNAs is not well understood but will be discussed later in this section. To date, LGP2 has not been shown to interact with enterovirus RNAs but has been recently shown to interact with the RNA of encephalomyocarditis virus, another member of the picornavirus family belonging to the cardiovirus genus (Deddouche et al., 2014). However, MDA5 along with its adaptor molecule MAVS, has been shown to induce the antiviral response pathway during enterovirus infection and thus will be the focus of this section.

MDA5 serves as a cytoplasmic sensor for enterovirus infections (Abe et al., 2012; Wang et al., 2010). MDA5 was shown to specifically bind to two dsRNA enterovirus species, the replicative form (RF) and the replicative intermediate (RI) (Feng et al., 2012; Triantafilou et al., 2012). The RF is formed when the negative-strand is being synthesized from the positive-strand template, and the RI is formed when there are multiple positive-strands being synthesized from the negative-strand [reviewed in (Daijogo and Semler, 2011)] (Baltimore et al., 1964). These two viral species could induce type I IFN response following transfection of enterovirus RNA into the cell (Feng et al., 2012; Triantafilou et

al., 2012). Gel-purified CVB3 RF alone was also shown to activate the ATPase activity of recombinant MDA5 (Feng et al., 2012). Interestingly, transfecting CVB3 genomic RNA into mouse embryonic fibroblasts knocked out for RIG-I did not significantly alter its ability to produce an IFN response (Feng et al., 2012). These results are not surprising since RIG-I requires a 5'ppp moiety in short dsRNA to interact with the target RNA. When mice lacking MDA5 and its adaptor molecule MAVS were infected with CVB3, there was an increase in mortality and a reduced IFN response (Wang et al., 2010). However, poliovirus-infected mice expressing the poliovirus receptor but lacking MDA5 and MAVS did not display increased mortality nor a decrease in IFN response even though it was shown that MDA5 was required for the production of IFNs *in vitro* (Abe et al., 2012; Oshiumi et al., 2011). It is important to note that the apparent discrepancy seen between the *in vitro* versus *in vivo* data may be due to differences in the viral RNA delivery method, host protein association with the viral RNA, or overall physiological environment of the cell.

A recent study was carried out to measure the type I IFN response, specifically IFN-α/β, at the different steps of the CVB3 replication cycle. When negative-strand RNA synthesis was inhibited, thereby inhibiting the production of RF, the IFN-α/β response was abolished; when positive-strand RNA synthesis was inhibited, resulting in inhibition of the production of RI, the IFN-α/β response was not impaired (Feng et al., 2012). These results suggest that RF is required to activate the type I IFN response during an enterovirus infection. Also, MDA5 and MAVS are required to sense the dsRNA intermediates produced during enterovirus infection. To evade the IFN response pathway,

enteroviruses modify the RLRs directly as well as modify the host proteins downstream of the RLRs signaling pathway.

Enterovirus evasion of the RIG-I-like receptor-mediated antiviral response

Enteroviruses employ different mechanisms to disrupt the type I IFN pathway. Typically, they modify host proteins in a proteinase-dependent manner. However, there are differing reports for virus-mediated modifications of the antiviral response among enteroviruses. Poliovirus has been shown to degrade MDA5 and MAVS in a proteasome- and caspase-dependent manner (Barral et al., 2007; Rebsamen et al., 2008). CVB3 proteinase 3C cleaves MAVS, resulting in a functionally inactive fragment that can no longer participate in both NF-κB and type I IFN signaling (Mukherjee et al., 2011). CVB3 has also been shown to target other components of the RLR pathway, including 3C-mediated cleavage of focal adhesion kinase (FAK), which is responsible for MAVS induction (Bozym et al., 2012). For EV71, proteinase 2A cleaves MAVS (Wang et al., 2013). EV71 3C can either directly cleave a downstream signaling protein, interferon regulatory factor 7 (IRF7), or block the recruitment of MAVS to RIG-I, thus interrupting the relocalization of downstream signaling protein IRF3 to the nucleus and reducing the IFN response (Lei et al., 2010; Lei et al., 2013). Similar to poliovirus, EV71 also induces capsase-mediated degradation of MDA5 (Kuo et al., 2013). Human rhinovirus type 1a proteinases 2A and 3C and cellular caspase 3 have all been suggested to cleave MAVS (Drahos and Racaniello, 2009).

Recently a comprehensive study was carried out to analyze the viral-mediated cleavage of key players involved in the RLR-mediated IFN response. In this study, enterovirus modulation of the major components of the RLR signaling pathway was

studied. CVB3 proteinase 2A was shown to cleave both MDA5 and MAVS (Feng et al., 2014). MDA5 and MAVS cleavage was also observed when using recombinant EV71, CVB3, and poliovirus 2A proteinases, suggesting that enteroviruses uniformly utilize proteinase 2A to cleave MDA5 and MAVS and thus inhibit the type I IFN response (Feng et al., 2014) (Figure 2). The apparent differences in these findings compared to previous reports may be due to the fact that in previous studies, cells were pre-treated before infection with cell stress inducer, polyI:C, which may have activated caspase-mediated apoptosis. The previous study (Mukherjee et al., 2011) showing 3C-mediated cleavage of MAVS was carried out using exogenous 3C versus the recent study, which used endogenous 3C (Feng et al., 2014). Overexpression of 3C may have preferentially cleaved MAVS. Although it is possible that both 2A and 3C can cleave MAVS, it is likely that during a normal infection 2A preferentially cleaves MAVS (Feng et al., 2014). Additionally, RIG-I is cleaved by recombinant CVB3 3C (Feng et al., 2014). It was unknown if RIG-I recognized enteroviruses, since previous studies have shown that the type I IFN response is unaltered when RIG-I is knocked out; however, the newly identified CVB3 3C-mediated cleavage event may help elucidate the mechanisms of enterovirus evasion of the RIG-I pathway (Feng et al., 2012). This function may be redundant, since enteroviruses cleave MDA5 and MAVS to inhibit the IFN response pathway. Perhaps enteroviruses modify multiple pathways to ensure efficient replication of their genomic RNAs. Cleavage events in the RLR pathway allow the virus to shut down the host antiviral response and efficiently replicate in the cell cytoplasm. Given that enteroviruses use similar replication mechanisms due to their conserved genomic RNA

sequences, it is likely that they mediate similar cleavage events to evade the type I IFN response.

Enterovirus evasion of the stress response

Cells form stress granules (SGs) in the presence of environmental stress stimuli. In the uninfected cell, SGs form cytosolic aggregates that contain cap-dependent stalled pre-initiation complexes, translationally silenced mRNAs, and canonical translation eIFs. These aggregates form following the phosphorylation of eukaryotic initiation factor 2α (eIF2α), which is a required component of the eIF2 complex responsible for loading the tRNA onto the 40S ribosome (Ernst et al., 1978). Although the exact mechanism of SG generation is not yet understood, SGs typically form as a result of hypoxia, nutrient deprivation, or oxidative stress. SG formation is canonically used to halt cap-dependent translation. Host proteins proposed to be important during SG formation include the RNA binding proteins, T-cell restricted intracellular antigen 1 (TIA-1), TIA-1-related protein (TIAR), and RasGAP-SH3 domain binding protein (G3BP) (Kedersha et al., 1999; Tourriere et al., 2003). Previous studies have shown that SG formation can reduce the levels of enterovirus replication. It has been suggested that viral RNAs are sequestered by SGs or that the host proteins required for viral replication can become trapped in SGs and are thus unavailable for viral replication (White et al., 2007). However, the exact role SGs play during enterovirus infection remains poorly understood.

SGs can form independently of eIF2α phosphorylation. Poliovirus infection can lead to stress granule formation as a stress response from the host (Mazroui et al., 2006). This stress granule formation occurs at the same time eIF4G is cleaved, suggesting that poliovirus-mediated cleavage of eIF4G, leading to shut down of cap-dependent

translation, may induce SG formation (Mazroui et al., 2006). SG formation induced by poliovirus is then disrupted by viral proteinase 3C as infection proceeds (White et al., 2007). 3C can cleave G3BP, leading to SGs dispersing at a later time in infection. When a recombinant, non-cleavable G3BP was introduced into the cell, SG formation was restored and viral replication was reduced (White et al., 2007). This viral-mediated modification of G3BP, a major component in SG formation, may be one mechanism that enteroviruses use to evade the stress response. However, another study showed that TIA-1-dependent SGs, in the presence of low levels of G3BP and eIF4G, may still form at a later time in poliovirus infection (Piotrowska et al., 2010).

As mentioned above, SGs can be formed due to different stress stimuli. The presence of an RNA-binding protein previously shown to interact with poliovirus RNA-dependent RNA polymerase $3D^{pol}$, Sam68, was used as a marker for SGs unique to poliovirus infection (McBride et al., 1996; Piotrowska et al., 2010). Sam68 localized with SGs induced during poliovirus infection but not with SGs formed due to heat shock or arsenite stress (Piotrowska et al., 2010). This finding suggests that SGs observed during poliovirus infection are unique to stress induced directly by the virus (Piotrowska et al., 2010). Another study showed that TIA-1-positive SGs did form early during poliovirus infection, but as infection proceeded, TIA-1-positive SGs lacking translation factors, RNA-binding proteins, and mRNA were observed (White and Lloyd, 2011). This type of SG observed later in infection suggests that poliovirus most likely disrupts SGs to release any sequestered translation factors to aid in its replication.

During CVB3 infection, SGs are formed early during infection but are disassembled at 5 h.p.i.. CVB3 3C can also cleave G3BP, suggesting a similar

mechanism of stress response evasion to poliovirus (Fung et al., 2013). These findings suggest that the viral-mediated modification of the stress response may be conserved among enteroviruses. Although the exact role of SG formation during enterovirus infection remains unclear, another study reported that host factor, SRp20, which was previously summarized in Table 3.2, colocalizes with SG marker TIA-1 at 3 h.p.i. during a poliovirus infection of HeLa cells. Although not all SRp20 was present in SGs in these experiments, colocalization was unique to poliovirus-induced SG formation, as this colocalization was not observed during oxidative stress (Fitzgerald and Semler, 2013). These results further confirm that translation factors involved during viral replication may be sequestered into SGs and suggest that perhaps viral-mediated disruption of SGs must be achieved to release factors necessary for efficient viral replication.

Evasion of cellular mRNA surveillance

P bodies are another type of cytoplasmic granules that contain translationally silenced, deadenylated mRNAs and may serve in mRNA surveillance of the uninfected cell. P bodies have been suggested to be involved in mRNA decay pathways and include proteins involved in the formation of mRNA decapping complexes, such as Dcp1a/2 and Edc3, major 5' exonuclease Xrn1, and proteins involved in mRNA deadenylation, Ccr4 and Pan 2/3 [reviewed in (Adjibade and Mazroui, 2014)]. P bodies form aggregates in a similar fashion to SGs, although the mechanism of their formation remains unknown. Since enteroviruses have positive-strand RNAs lacking a cap at the 5' end, this makes them susceptible to the mRNA decay pathways. One study demonstrated that P bodies are disrupted at 4 h.p.i. during poliovirus or CVB3 infection (Dougherty et al., 2011). This dispersion of P bodies correlated with an increase of poliovirus 3C/3CD expression.

During poliovirus infection, the number of P bodies was shown to decrease in the presence of the viral RNA replication inhibitor, guanidine HCl; however, the P bodies that did not disperse appeared to increase in size (Dougherty et al., 2011). P body protein components Xrn1, Dcp1a, and Pan 3 were also shown to undergo accelerated proteasome-mediated degradation during poliovirus infection. Only Dcp1a appeared to be cleaved by poliovirus proteinase 3C. An increase in turnover of Dcp1a during infection was observed, suggesting that poliovirus targets the mRNA decay pathway to disrupt P body formation (Dougherty et al., 2011). Although the mechanism of enterovirus disruption of P body formation remains to be discovered, it is likely that enteroviruses disrupt this host function to avoid the mRNA decay pathway.

V. Summary

Due to their limited coding capacity, enteroviruses hijack host cell functions to stimulate viral translation and replication. They typically do this by cleaving cellular proteins to modify their canonical functions. Enteroviruses modify proteins involved in cellular nucleo-cytoplasmic trafficking, translation, and transcription to make these proteins available for viral translation and RNA replication. The viral proteinases can also further modify specific host proteins to mediate the switch from viral translation to RNA synthesis. Nonstructural viral proteins without proteolytic activity also alter cellular functions, such as membrane reorganization for viral replication complex formation. Additionally, enteroviruses can evade the host antiviral or stress response to ensure efficient replication. Although it is known that enteroviruses can modify the host functions in multiple ways, many of the mechanisms remain unclear. It is necessary to elucidate these mechanisms and identify viral specific protein-protein interactions so that

antivirals may be generated targeting either the host proteins or viral proteins involved. Although antivirals against cellular proteins can be potentially toxic to the cell, targeting a non-canonical function of a host protein or a novel protein-protein interface may circumvent such an issue. Importantly, identifying cellular targets required for enterovirus replication avoids the issues of generating resistant viral variants due to the high mutation rates of viral RNA-dependent RNA polymerases. Such a prospect holds considerable promise for development of broad-spectrum antiviral therapies to treat enterovirus infections.

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References

1. Abe, Y., Fujii, K., Nagata, N., Takeuchi, O., Akira, S., Oshiumi, H., Matsumoto, M., Seya, T., and Koike, S. (2012). The toll-like receptor 3-mediated antiviral response is important for protection against poliovirus infection in poliovirus receptor transgenic mice. J Virol *86*, 185-194.
2. Adjibade, P., and Mazroui, R. (2014). Control of mRNA turnover: Implication of cytoplasmic RNA granules. Semin Cell Dev Biol *34*, 15-23.
3. Ambros, V., and Baltimore, D. (1978). Protein is linked to the 5' end of poliovirus RNA by a phosphodiester linkage to tyrosine. J Biol Chem *253*, 5263-5266.
4. Ambros, V., Pettersson, R.F., and Baltimore, D. (1978). An enzymatic activity in uninfected cells that cleaves the linkage between poliovirion RNA and the 5' terminal protein. Cell *15*, 1439-1446.
5. Back, S.H., Kim, Y.K., Kim, W.J., Cho, S., Oh, H.R., Kim, J.E., and Jang, S.K. (2002). Translation of polioviral mRNA Is inhibited by cleavage of polypyrimidine tract-binding proteins executed by polioviral 3C(pro). J Virol *76*, 2529-2542.
6. Baltimore, D., Becker, Y., and Darnell, J.E. (1964). Virus-Specific Double-Stranded Rna in Poliovirus-Infected Cells. Science *143*, 1034-1036.
7. Banerjee, R., Weidman, M.K., Navarro, S., Comai, L., and Dasgupta, A. (2005). Modifications of both selectivity factor and upstream binding factor contribute to poliovirus-mediated inhibition of RNA polymerase I transcription. J Gen Virol *86*, 2315-2322.

8. Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M.F., Ravazzola, M., Amherdt, M., and Schekman, R. (1994). COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell *77*, 895-907.
9. Barral, P.M., Morrison, J.M., Drahos, J., Gupta, P., Sarkar, D., Fisher, P.B., and Racaniello, V.R. (2007). MDA-5 is cleaved in poliovirus-infected cells. J Virol *81*, 3677-3684.
10. Bayliss, R., Littlewood, T., and Stewart, M. (2000). Structural basis for the interaction between FxFG nucleoporin repeats and importin-beta in nuclear trafficking. Cell *102*, 99-108.
11. Bedard, K.M., Daijogo, S., and Semler, B.L. (2007). A nucleo-cytoplasmic SR protein functions in viral IRES-mediated translation initiation. EMBO J *26*, 459-467.
12. Bedard, K.M., and Semler, B.L. (2004). Regulation of picornavirus gene expression. Microbes Infect *6*, 702-713.
13. Belov, G.A., and Ehrenfeld, E. (2007). Involvement of cellular membrane traffic proteins in poliovirus replication. Cell Cycle *6*, 36-38.
14. Belov, G.A., Evstafieva, A.G., Rubtsov, Y.P., Mikitas, O.V., Vartapetian, A.B., and Agol, V.I. (2000). Early alteration of nucleocytoplasmic traffic induced by some RNA viruses. Virology *275*, 244-248.
15. Belov, G.A., Habbersett, C., Franco, D., and Ehrenfeld, E. (2007). Activation of cellular Arf GTPases by poliovirus protein 3CD correlates with virus replication. J Virol *81*, 9259-9267.

16. Belov, G.A., Nair, V., Hansen, B.T., Hoyt, F.H., Fischer, E.R., and Ehrenfeld, E. (2012). Complex dynamic development of poliovirus membranous replication complexes. Journal of virology *86*, 302-312.
17. Bienz, K., Egger, D., and Pasamontes, L. (1987). Association of polioviral proteins of the P2 genomic region with the viral replication complex and virus-induced membrane synthesis as visualized by electron microscopic immunocytochemistry and autoradiography. Virology *160*, 220-226.
18. Blair, W.S., and Semler, B.L. (1991). Role for the P4 amino acid residue in substrate utilization by the poliovirus 3CD proteinase. J Virol *65*, 6111-6123.
19. Blyn, L.B., Swiderek, K.M., Richards, O., Stahl, D.C., Semler, B.L., and Ehrenfeld, E. (1996). Poly(rC) binding protein 2 binds to stem-loop IV of the poliovirus RNA 5' noncoding region: identification by automated liquid chromatography-tandem mass spectrometry. Proc Natl Acad Sci of the U S A *93*, 11115-11120.
20. Blyn, L.B., Towner, J.S., Semler, B.L., and Ehrenfeld, E. (1997). Requirement of poly(rC) binding protein 2 for translation of poliovirus RNA. J Virol *71*, 6243-6246.
21. Bovee, M.L., Lamphear, B.J., Rhoads, R.E., and Lloyd, R.E. (1998). Direct cleavage of elF4G by poliovirus 2A protease is inefficient in vitro. Virology *245*, 241-249.
22. Bozym, R.A., Delorme-Axford, E., Harris, K., Morosky, S., Ikizler, M., Dermody, T.S., Sarkar, S.N., and Coyne, C.B. (2012). Focal adhesion kinase is a

component of antiviral RIG-I-like receptor signaling. Cell Host Microbe *11*, 153-166.

23. Caceres, J.F., Misteli, T., Screaton, G.R., Spector, D.L., and Krainer, A.R. (1997). Role of the modular domains of SR proteins in subnuclear localization and alternative splicing specificity. J Cell Biol *138*, 225-238.
24. Caceres, J.F., Screaton, G.R., and Krainer, A.R. (1998). A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. Genes Dev *12*, 55-66.
25. Caliguiri, L.A., and Tamm, I. (1969). Membranous structures associated with translation and transcription of poliovirus RNA. Science *166*, 885-886.
26. Caliguiri, L.A., and Tamm, I. (1970). The role of cytoplasmic membranes in poliovirus biosynthesis. Virology *42*, 100-111.
27. Castello, A., Izquierdo, J.M., Welnowska, E., and Carrasco, L. (2009). RNA nuclear export is blocked by poliovirus 2A protease and is concomitant with nucleoporin cleavage. J Cell Sci *122*, 3799-3809.
28. Cathcart, A.L., Rozovics, J.M., and Semler, B.L. (2013). Cellular mRNA decay protein AUF1 negatively regulates enterovirus and human rhinovirus infections. J Virol *87*, 10423-10434.
29. Chase, A.J., Daijogo, S., and Semler, B.L. (2014). Inhibition of poliovirus-induced cleavage of cellular protein PCBP2 reduces the levels of viral RNA replication. J Virol *88*, 3192-3201.
30. Chase, A.J., and Semler, B.L. (2014). Differential cleavage of IRES trans-acting factors (ITAFs) in cells infected by human rhinovirus. Virology *449*, 35-44.

31. Childs, K.S., Randall, R.E., and Goodbourn, S. (2013). LGP2 plays a critical role in sensitizing mda-5 to activation by double-stranded RNA. PLoS One *8*, e64202.
32. Clark, M.E., Hammerle, T., Wimmer, E., and Dasgupta, A. (1991). Poliovirus proteinase 3C converts an active form of transcription factor IIIC to an inactive form: a mechanism for inhibition of host cell polymerase III transcription by poliovirus. EMBO J *10*, 2941-2947.
33. Cortes Ledesma, F., El Khamisy, S.F., Zuma, M.C., Osborn, K., and Caldecott, K.W. (2009). A human 5'-tyrosyl DNA phosphodiesterase that repairs topoisomerase-mediated DNA damage. Nature *461*, 674-678.
34. Daijogo, S., and Semler, B.L. (2011). Mechanistic intersections between picornavirus translation and RNA replication. Adv Virus Res *80*, 1-24.
35. Dales, S., Eggers, H.J., Tamm, I., and Palade, G.E. (1965). Electron microscopic study of the formation of poliovirus. Virology *26*, 379-389.
36. Das, S., and Dasgupta, A. (1993). Identification of the cleavage site and determinants required for poliovirus 3CPro-catalyzed cleavage of human TATA-binding transcription factor TBP. J Virol *67*, 3326-3331.
37. de Breyne, S., Yu, Y., Unbehaun, A., Pestova, T.V., and Hellen, C.U. (2009). Direct functional interaction of initiation factor eIF4G with type 1 internal ribosomal entry sites. Proc Natl Acad Sci of the U S A *106*, 9197-9202.
38. Deddouche, S., Goubau, D., Rehwinkel, J., Chakravarty, P., Begum, S., Maillard, P.V., Borg, A., Matthews, N., Feng, Q., van Kuppeveld, F.J., *et al.* (2014).

Identification of an LGP2-associated MDA5 agonist in picornavirus-infected cells. eLife *3*, e01535.

39. Dempsey, L.A., Hanakahi, L.A., and Maizels, N. (1998). A specific isoform of hnRNP D interacts with DNA in the LR1 heterodimer: canonical RNA binding motifs in a sequence-specific duplex DNA binding protein. J Biol Chem *273*, 29224-29229.
40. Devaney, M.A., Vakharia, V.N., Lloyd, R.E., Ehrenfeld, E., and Grubman, M.J. (1988). Leader protein of foot-and-mouth disease virus is required for cleavage of the p220 component of the cap-binding protein complex. J Virol *62*, 4407-4409.
41. Dorobantu, C.M., van der Schaar, H.M., Ford, L.A., Strating, J.R., Ulferts, R., Fang, Y., Belov, G., and van Kuppeveld, F.J. (2014). Recruitment of PI4KIIIbeta to coxsackievirus B3 replication organelles is independent of ACBD3, GBF1, and Arf1. J Virol *88*, 2725-2736.
42. Dougherty, J.D., White, J.P., and Lloyd, R.E. (2011). Poliovirus-mediated disruption of cytoplasmic processing bodies. J Virol *85*, 64-75.
43. Drahos, J., and Racaniello, V.R. (2009). Cleavage of IPS-1 in cells infected with human rhinovirus. J Virol *83*, 11581-11587.
44. Ernst, V., Levin, D.H., and London, I.M. (1978). Inhibition of protein synthesis initiation by oxidized glutathione: activation of a protein kinase that phosphorylates the alpha subunit of eukaryotic initiation factor 2. Proc Natl Acad Sci U S A *75*, 4110-4114.

45. Etchison, D., Milburn, S.C., Edery, I., Sonenberg, N., and Hershey, J.W. (1982). Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000-dalton polypeptide associated with eucaryotic initiation factor 3 and a cap binding protein complex. J Biol Chem *257*, 14806-14810.

46. Feng, Q., Hato, S.V., Langereis, M.A., Zoll, J., Virgen-Slane, R., Peisley, A., Hur, S., Semler, B.L., van Rij, R.P., and van Kuppeveld, F.J. (2012). MDA5 detects the double-stranded RNA replicative form in picornavirus-infected cells. Cell Reports *2*, 1187-1196.

47. Feng, Q., Langereis, M.A., Lork, M., Nguyen, M., Hato, S.V., Lanke, K., Emdad, L., Bhoopathi, P., Fisher, P.B., Lloyd, R.E.*, et al.* (2014). Enterovirus 2Apro targets MDA5 and MAVS in infected cells. J Virol *88*, 3369-3378.

48. Fernandez-Munoz, R., and Darnell, J.E. (1976). Structural difference between the 5' termini of viral and cellular mRNA in poliovirus-infected cells: possible basis for the inhibition of host protein synthesis. J Virol *18*, 719-726.

49. Fitzgerald, K.D., Chase, A.J., Cathcart, A.L., Tran, G.P., and Semler, B.L. (2013). Viral proteinase requirements for the nucleocytoplasmic relocalization of cellular splicing factor SRp20 during picornavirus infections. J Virol *87*, 2390-2400.

50. Fitzgerald, K.D., and Semler, B.L. (2009). Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. Biochim Biophys Acta *1789*, 518-528.

51. Fitzgerald, K.D., and Semler, B.L. (2011). Re-localization of cellular protein SRp20 during poliovirus infection: bridging a viral IRES to the host cell translation apparatus. PLoS Pathog *7*, e1002127.

52. Fitzgerald, K.D., and Semler, B.L. (2013). Poliovirus infection induces the co-localization of cellular protein SRp20 with TIA-1, a cytoplasmic stress granule protein. Virus Res *176*, 223-231.

53. Flanegan, J.B., and Baltimore, D. (1977). Poliovirus-specific primer-dependent RNA polymerase able to copy poly(A). Proc Natl Acad Sci of the U S A *74*, 3677-3680.

54. Flanegan, J.B., Pettersson, R.F., Ambros, V., Hewlett, N.J., and Baltimore, D. (1977). Covalent linkage of a protein to a defined nucleotide sequence at the 5'-terminus of virion and replicative intermediate RNAs of poliovirus. Proc Natl Acad Sci U S A *74*, 961-965.

55. Florez, P.M., Sessions, O.M., Wagner, E.J., Gromeier, M., and Garcia-Blanco, M.A. (2005). The polypyrimidine tract binding protein is required for efficient picornavirus gene expression and propagation. J Virol *79*, 6172-6179.

56. Fung, G., Ng, C.S., Zhang, J., Shi, J., Wong, J., Piesik, P., Han, L., Chu, F., Jagdeo, J., Jan, E., *et al.* (2013). Production of a dominant-negative fragment due to G3BP1 cleavage contributes to the disruption of mitochondria-associated protective stress granules during CVB3 infection. PLoS One *8*, e79546.

57. Gamarnik, A.V., and Andino, R. (1997). Two functional complexes formed by KH domain containing proteins with the 5' noncoding region of poliovirus RNA. RNA *3*, 882-892.

58. Gamarnik, A.V., and Andino, R. (1998). Switch from translation to RNA replication in a positive-stranded RNA virus. Genes Dev *12*, 2293-2304.

59. Gavala, M.L., Bertics, P.J., and Gern, J.E. (2011). Rhinoviruses, allergic inflammation, and asthma. Immunol Rev *242*, 69-90.

60. Golini, F., Semler, B.L., Dorner, A.J., and Wimmer, E. (1980). Protein-linked RNA of poliovirus is competent to form an initiation complex of translation in vitro. Nature *287*, 600-603.

61. Gorlich, D., and Kutay, U. (1999). Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol *15*, 607-660.

62. Gottlieb, E., and Steitz, J.A. (1989). The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription in vitro. EMBO J *8*, 841-850.

63. Greninger, A.L., Knudsen, G.M., Betegon, M., Burlingame, A.L., and Derisi, J.L. (2012). The 3A protein from multiple picornaviruses utilizes the golgi adaptor protein ACBD3 to recruit PI4KIIIbeta. J Virol *86*, 3605-3616.

64. Gustin, K.E. (2003). Inhibition of nucleo-cytoplasmic trafficking by RNA viruses: targeting the nuclear pore complex. Virus Res *95*, 35-44.

65. Gustin, K.E., and Sarnow, P. (2001). Effects of poliovirus infection on nucleo-cytoplasmic trafficking and nuclear pore complex composition. EMBO J *20*, 240-249.

66. Hewlett, M.J., Rose, J.K., and Baltimore, D. (1976). 5'-terminal structure of poliovirus polyribosomal RNA is pUp. Proc Natl Acad Sci U S A *73*, 327-330.

67. Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.K., Schlee, M.*, et al.* (2006). 5'-Triphosphate RNA is the ligand for RIG-I. Science *314*, 994-997.

68. Hsu, N.Y., Ilnytska, O., Belov, G., Santiana, M., Chen, Y.H., Takvorian, P.M., Pau, C., van der Schaar, H., Kaushik-Basu, N., Balla, T.*, et al.* (2010). Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell *141*, 799-811.

69. Huang, P.N., Lin, J.Y., Locker, N., Kung, Y.A., Hung, C.T., Lin, J.Y., Huang, H.I., Li, M.L., and Shih, S.R. (2011). Far upstream element binding protein 1 binds the internal ribosomal entry site of enterovirus 71 and enhances viral translation and viral growth. Nucleic Acids Res *39*, 9633-9648.

70. Hunt, S.L., Hsuan, J.J., Totty, N., and Jackson, R.J. (1999). unr, a cellular cytoplasmic RNA-binding protein with five cold-shock domains, is required for internal initiation of translation of human rhinovirus RNA. Genes Dev *13*, 437-448.

71. Izumi, R.E., Valdez, B., Banerjee, R., Srivastava, M., and Dasgupta, A. (2001). Nucleolin stimulates viral internal ribosome entry site-mediated translation. Virus Res *76*, 17-29.

72. Jackson, W.T., Giddings, T.H., Jr., Taylor, M.P., Mulinyawe, S., Rabinovitch, M., Kopito, R.R., and Kirkegaard, K. (2005). Subversion of cellular autophagosomal machinery by RNA viruses. PLoS Biol *3*, e156.

73. Joachims, M., Van Breugel, P.C., and Lloyd, R.E. (1999). Cleavage of poly(A)-binding protein by enterovirus proteases concurrent with inhibition of translation in vitro. J Virol *73*, 718-727.
74. Kafasla, P., Morgner, N., Robinson, C.V., and Jackson, R.J. (2010). Polypyrimidine tract-binding protein stimulates the poliovirus IRES by modulating eIF4G binding. EMBO J *29*, 3710-3722.
75. Kajita, Y., Nakayama, J., Aizawa, M., and Ishikawa, F. (1995). The UUAG-specific RNA binding protein, heterogeneous nuclear ribonucleoprotein D0. Common modular structure and binding properties of the 2xRBD-Gly family. J Biol Chem *270*, 22167-22175.
76. Kassavetis, G.A., Braun, B.R., Nguyen, L.H., and Geiduschek, E.P. (1990). S. cerevisiae TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. Cell *60*, 235-245.
77. Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K.J.*, et al.* (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature *441*, 101-105.
78. Kedersha, N.L., Gupta, M., Li, W., Miller, I., and Anderson, P. (1999). RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. J Cell Biol *147*, 1431-1442.
79. Kemball, C.C., Alirezaei, M., Flynn, C.T., Wood, M.R., Harkins, S., Kiosses, W.B., and Whitton, J.L. (2010). Coxsackievirus infection induces autophagy-like

vesicles and megaphagosomes in pancreatic acinar cells in vivo. J Virol *84*, 12110-12124.

80. Kenan, D.J., Query, C.C., and Keene, J.D. (1991). RNA recognition: towards identifying determinants of specificity. Trends Biochem Sci *16*, 214-220.
81. Kerekatte, V., Keiper, B.D., Badorff, C., Cai, A., Knowlton, K.U., and Rhoads, R.E. (1999). Cleavage of Poly(A)-binding protein by coxsackievirus 2A protease in vitro and in vivo: another mechanism for host protein synthesis shutoff? J Virol *73*, 709-717.
82. Khetsuriani, N., Lamonte-Fowlkes, A., Oberst, S., Pallansch, M.A., Centers for Disease, C., and Prevention (2006). Enterovirus surveillance--United States, 1970-2005. MMWR Surveill Summ *55*, 1-20.
83. Kiledjian, M., DeMaria, C.T., Brewer, G., and Novick, K. (1997). Identification of AUF1 (heterogeneous nuclear ribonucleoprotein D) as a component of the alpha-globin mRNA stability complex. Mol Cell Biol *17*, 4870-4876.
84. Kim, J.H., Hahm, B., Kim, Y.K., Choi, M., and Jang, S.K. (2000). Protein-protein interaction among hnRNPs shuttling between nucleus and cytoplasm. J Mol Biol *298*, 395-405.
85. Kitamura, N., Semler, B.L., Rothberg, P.G., Larsen, G.R., Adler, C.J., Dorner, A.J., Emini, E.A., Hanecak, R., Lee, J.J., van der Werf, S.*, et al.* (1981). Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature *291*, 547-553.

86. Klein, K.A., and Jackson, W.T. (2011). Human rhinovirus 2 induces the autophagic pathway and replicates more efficiently in autophagic cells. J Virol *85*, 9651-9654.

87. Klumperman, J. (2000). Transport between ER and Golgi. Cur Opin Cell Biol *12*, 445-449.

88. Kozlov, G., De Crescenzo, G., Lim, N.S., Siddiqui, N., Fantus, D., Kahvejian, A., Trempe, J.F., Elias, D., Ekiel, I., Sonenberg, N.*, et al.* (2004). Structural basis of ligand recognition by PABC, a highly specific peptide-binding domain found in poly(A)-binding protein and a HECT ubiquitin ligase. EMBO J *23*, 272-281.

89. Kozlov, G., Trempe, J.F., Khaleghpour, K., Kahvejian, A., Ekiel, I., and Gehring, K. (2001). Structure and function of the C-terminal PABC domain of human poly(A)-binding protein. Proc Natl Acad Sci of the U S A *98*, 4409-4413.

90. Krausslich, H.G., Nicklin, M.J., Toyoda, H., Etchison, D., and Wimmer, E. (1987). Poliovirus proteinase 2A induces cleavage of eucaryotic initiation factor 4F polypeptide p220. J Virol *61*, 2711-2718.

91. Kuo, R.L., Kao, L.T., Lin, S.J., Wang, R.Y., and Shih, S.R. (2013). MDA5 plays a crucial role in enterovirus 71 RNA-mediated IRF3 activation. PLoS One *8*, e63431.

92. Kuyumcu-Martinez, N.M., Joachims, M., and Lloyd, R.E. (2002). Efficient cleavage of ribosome-associated poly(A)-binding protein by enterovirus 3C protease. J Virol *76*, 2062-2074.

93. Lamphear, B.J., Kirchweger, R., Skern, T., and Rhoads, R.E. (1995). Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G

(eIF4G) with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation. J Biol Chem *270*, 21975-21983.

94. Langereis, M.A., Feng, Q., Nelissen, F.H., Virgen-Slane, R., van der Heden van Noort, G.J., Maciejewski, S., Filippov, D.V., Semler, B.L., van Delft, F.L., and van Kuppeveld, F.J. (2014). Modification of picornavirus genomic RNA using 'click' chemistry shows that unlinking of the VPg peptide is dispensable for translation and replication of the incoming viral RNA. Nucleic Acids Res *42*, 2473-2482.
95. Lee, Y.F., Nomoto, A., Detjen, B.M., and Wimmer, E. (1977). A protein covalently linked to poliovirus genome RNA. Proc Natl Acad Sci U S A *74*, 59-63.
96. Lei, X., Liu, X., Ma, Y., Sun, Z., Yang, Y., Jin, Q., He, B., and Wang, J. (2010). The 3C protein of enterovirus 71 inhibits retinoid acid-inducible gene I-mediated interferon regulatory factor 3 activation and type I interferon responses. J Virol *84*, 8051-8061.
97. Lei, X., Xiao, X., Xue, Q., Jin, Q., He, B., and Wang, J. (2013). Cleavage of interferon regulatory factor 7 by enterovirus 71 3C suppresses cellular responses. J Virol *87*, 1690-1698.
98. Lenarcic, E.M., Landry, D.M., Greco, T.M., Cristea, I.M., and Thompson, S.R. (2013). Thiouracil cross-linking mass spectrometry: a cell-based method to identify host factors involved in viral amplification. J Virol *87*, 8697-8712.
99. Liebig, H.D., Ziegler, E., Yan, R., Hartmuth, K., Klump, H., Kowalski, H., Blaas, D., Sommergruber, W., Frasel, L., Lamphear, B., *et al.* (1993). Purification of

two picornaviral 2A proteinases: interaction with eIF-4 gamma and influence on in vitro translation. Biochemistry *32*, 7581-7588.

100. Lin, J.Y., Li, M.L., Huang, P.N., Chien, K.Y., Horng, J.T., and Shih, S.R. (2008). Heterogeneous nuclear ribonuclear protein K interacts with the enterovirus 71 5' untranslated region and participates in virus replication. J Gen Virol *89*, 2540-2549.

101. Lin, J.Y., Li, M.L., and Shih, S.R. (2009). Far upstream element binding protein 2 interacts with enterovirus 71 internal ribosomal entry site and negatively regulates viral translation. Nucleic Acids Res *37*, 47-59.

102. Lloyd, R.E. (2006). Translational control by viral proteinases. Virus Res *119*, 76-88.

103. Lu, J.Y., Bergman, N., Sadri, N., and Schneider, R.J. (2006). Assembly of AUF1 with eIF4G-poly(A) binding protein complex suggests a translation function in AU-rich mRNA decay. RNA *12*, 883-893.

104. Maciejewski, S., Nguyen, J.H., Gomez-Herreros, F., Cortes-Ledesma, F., Caldecott, K.W., and Semler, B.L. (2016). Divergent Requirement for a DNA Repair Enzyme during Enterovirus Infections. mBio *7*, e01931-01915.

105. Mazroui, R., Sukarieh, R., Bordeleau, M.E., Kaufman, R.J., Northcote, P., Tanaka, J., Gallouzi, I., and Pelletier, J. (2006). Inhibition of ribosome recruitment induces stress granule formation independently of eukaryotic initiation factor 2alpha phosphorylation. Mol Biol Cell *17*, 4212-4219.

106. McBride, A.E., Schlegel, A., and Kirkegaard, K. (1996). Human protein Sam68 relocalization and interaction with poliovirus RNA polymerase in infected cells. Proc Natl Acad Sci U S A *93*, 2296-2301.

107. Meerovitch, K., Svitkin, Y.V., Lee, H.S., Lejbkowicz, F., Kenan, D.J., Chan, E.K., Agol, V.I., Keene, J.D., and Sonenberg, N. (1993). La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. J Virol *67*, 3798-3807.

108. Michael, W.M., Eder, P.S., and Dreyfuss, G. (1997). The K nuclear shuttling domain: a novel signal for nuclear import and nuclear export in the hnRNP K protein. EMBO J *16*, 3587-3598.

109. Midgley, C.M., Jackson, M.A., Selvarangan, R., Turabelidze, G., Obringer, E., Johnson, D., Giles, B.L., Patel, A., Echols, F., Oberste, M.S.*, et al.* (2014). Severe respiratory illness associated with enterovirus d68 - missouri and illinois, 2014. MMWR Morb Mortal Wkly Rep *63*, 798-799.

110. Mukherjee, A., Morosky, S.A., Delorme-Axford, E., Dybdahl-Sissoko, N., Oberste, M.S., Wang, T., and Coyne, C.B. (2011). The coxsackievirus B 3C protease cleaves MAVS and TRIF to attenuate host type I interferon and apoptotic signaling. PLoS Pathog *7*, e1001311.

111. Mulligan, G.J., Guo, W., Wormsley, S., and Helfman, D.M. (1992). Polypyrimidine tract binding protein interacts with sequences involved in alternative splicing of beta-tropomyosin pre-mRNA. J Biol Chem *267*, 25480-25487.

112. Nicklin, M.J.H., Toyoda, H., Murray, M.G., and Wimmer, E. (1986). Proteolytic processing in the replication of polio and related viruses. Biotechnology *4*, 36-42.

113. Nomoto, A., Kitamura, N., Golini, F., and Wimmer, E. (1977). The 5'-terminal structures of poliovirion RNA and poliovirus mRNA differ only in the genome-linked protein VPg. Proc Natl Acad Sci of the U S A *74*, 5345-5349.

114. Nomoto, A., Lee, Y.F., and Wimmer, E. (1976). The 5' end of poliovirus mRNA is not capped with m7G(5')ppp(5')Np. Proc Natl Acad Sci U S A *73*, 375-380.

115. Oshiumi, H., Okamoto, M., Fujii, K., Kawanishi, T., Matsumoto, M., Koike, S., and Seya, T. (2011). The TLR3/TICAM-1 pathway is mandatory for innate immune responses to poliovirus infection. J Immunol *187*, 5320-5327.

116. Park, N., Katikaneni, P., Skern, T., and Gustin, K.E. (2008). Differential targeting of nuclear pore complex proteins in poliovirus-infected cells. J Virol *82*, 1647-1655.

117. Park, N., Skern, T., and Gustin, K.E. (2010). Specific cleavage of the nuclear pore complex protein Nup62 by a viral protease. J Biol Chem *285*, 28796-28805.

118. Parsley, T.B., Towner, J.S., Blyn, L.B., Ehrenfeld, E., and Semler, B.L. (1997). Poly (rC) binding protein 2 forms a ternary complex with the 5'-terminal sequences of poliovirus RNA and the viral 3CD proteinase. RNA *3*, 1124-1134.

119. Paul, A.V., van Boom, J.H., Filippov, D., and Wimmer, E. (1998). Protein-primed RNA synthesis by purified poliovirus RNA polymerase. Nature *393*, 280-284.

120. Peisley, A., Wu, B., Yao, H., Walz, T., and Hur, S. (2013). RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner. Mol Cell *51*, 573-583.

121. Perera, R., Daijogo, S., Walter, B.L., Nguyen, J.H., and Semler, B.L. (2007). Cellular protein modification by poliovirus: the two faces of poly(rC)-binding protein. J Virol *81*, 8919-8932.

122. Piotrowska, J., Hansen, S.J., Park, N., Jamka, K., Sarnow, P., and Gustin, K.E. (2010). Stable formation of compositionally unique stress granules in virus-infected cells. J Virol *84*, 3654-3665.

123. Pollard, V.W., Michael, W.M., Nakielny, S., Siomi, M.C., Wang, F., and Dreyfuss, G. (1996). A novel receptor-mediated nuclear protein import pathway. Cell *86*, 985-994.

124. Ray, P.S., and Das, S. (2002). La autoantigen is required for the internal ribosome entry site-mediated translation of Coxsackievirus B3 RNA. Nucleic Acids Res *30*, 4500-4508.

125. Rebsamen, M., Meylan, E., Curran, J., and Tschopp, J. (2008). The antiviral adaptor proteins Cardif and Trif are processed and inactivated by caspases. Cell Death Differ *15*, 1804-1811.

126. Reikine, S., Nguyen, J.B., and Modis, Y. (2014). Pattern Recognition and Signaling Mechanisms of RIG-I and MDA5. Front Immunol *5*, 342.

127. Richards, A.L., Soares-Martins, J.A., Riddell, G.T., and Jackson, W.T. (2014). Generation of unique poliovirus RNA replication organelles. mBio *5*, e00833-00813.

128. Rothberg, P.G., Harris, T.J., Nomoto, A., and Wimmer, E. (1978). O4-(5'-uridylyl)tyrosine is the bond between the genome-linked protein and the RNA of poliovirus. Proc Natl Acad Sci of the U S A *75*, 4868-4872.

129. Rozovics, J.M., Chase, A.J., Cathcart, A.L., Chou, W., Gershon, P.D., Palusa, S., Wilusz, J., and Semler, B.L. (2012). Picornavirus modification of a host mRNA decay protein. mBio *3*, e00431-00412.

130. Rozovics, J.M., Virgen-Slane, R., and Semler, B.L. (2011). Engineered picornavirus VPg-RNA substrates: analysis of a tyrosyl-RNA phosphodiesterase activity. PLoS One *6*, e16559.

131. Rust, R.C., Landmann, L., Gosert, R., Tang, B.L., Hong, W., Hauri, H.P., Egger, D., and Bienz, K. (2001). Cellular COPII proteins are involved in production of the vesicles that form the poliovirus replication complex. J Virol *75*, 9808-9818.

132. Schlegel, A., Giddings, T.H., Jr., Ladinsky, M.S., and Kirkegaard, K. (1996). Cellular origin and ultrastructure of membranes induced during poliovirus infection. J Virol *70*, 6576-6588.

133. Shen, H., Kan, J.L., and Green, M.R. (2004). Arginine-serine-rich domains bound at splicing enhancers contact the branchpoint to promote prespliceosome assembly. Mol Cell *13*, 367-376.

134. Shih, S.R., Stollar, V., and Li, M.L. (2011). Host factors in enterovirus 71 replication. J Virol *85*, 9658-9666.

135. Shiroki, K., Isoyama, T., Kuge, S., Ishii, T., Ohmi, S., Hata, S., Suzuki, K., Takasaki, Y., and Nomoto, A. (1999). Intracellular redistribution of truncated La protein produced by poliovirus 3Cpro-mediated cleavage. J Virol *73*, 2193-2200.

136. Smith, R.W., Blee, T.K., and Gray, N.K. (2014). Poly(A)-binding proteins are required for diverse biological processes in metazoans. Biochem Soc Trans *42*, 1229-1237.

137. Springer, S., Spang, A., and Schekman, R. (1999). A primer on vesicle budding. Cell *97*, 145-148.

138. Stewart, M., Baker, R.P., Bayliss, R., Clayton, L., Grant, R.P., Littlewood, T., and Matsuura, Y. (2001). Molecular mechanism of translocation through nuclear pore complexes during nuclear protein import. FEBS Letters *498*, 145-149.

139. Suhy, D.A., Giddings, T.H., Jr., and Kirkegaard, K. (2000). Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. J Virol *74*, 8953-8965.

140. Svitkin, Y.V., Meerovitch, K., Lee, H.S., Dholakia, J.N., Kenan, D.J., Agol, V.I., and Sonenberg, N. (1994). Internal translation initiation on poliovirus RNA: further characterization of La function in poliovirus translation in vitro. J Virol *68*, 1544-1550.

141. Sweeney, T.R., Abaeva, I.S., Pestova, T.V., and Hellen, C.U. (2014). The mechanism of translation initiation on Type 1 picornavirus IRESs. EMBO J *33*, 76-92.

142. Tan, E.M. (1989). Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol *44*, 93-151.

143. Taylor, M.P., and Kirkegaard, K. (2007). Modification of cellular autophagy protein LC3 by poliovirus. J Virol *81*, 12543-12553.

144. Tokarz, R., Firth, C., Madhi, S.A., Howie, S.R., Wu, W., Sall, A.A., Haq, S., Briese, T., and Lipkin, W.I. (2012). Worldwide emergence of multiple clades of enterovirus 68. J Gen Virol *93*, 1952-1958.

145. Tourriere, H., Chebli, K., Zekri, L., Courselaud, B., Blanchard, J.M., Bertrand, E., and Tazi, J. (2003). The RasGAP-associated endoribonuclease G3BP assembles stress granules. J Cell Biol *160*, 823-831.

146. Toyoda, H., Nicklin, M.J., Murray, M.G., Anderson, C.W., Dunn, J.J., Studier, F.W., and Wimmer, E. (1986). A second virus-encoded proteinase involved in proteolytic processing of poliovirus polyprotein. Cell *45*, 761-770.

147. Trahey, M., Oh, H.S., Cameron, C.E., and Hay, J.C. (2012). Poliovirus infection transiently increases COPII vesicle budding. J Virol *86*, 9675-9682.

148. Triantafilou, K., Vakakis, E., Kar, S., Richer, E., Evans, G.L., and Triantafilou, M. (2012). Visualisation of direct interaction of MDA5 and the dsRNA replicative intermediate form of positive strand RNA viruses. J Cell Sci *125*, 4761-4769.

149. Virgen-Slane, R., Rozovics, J.M., Fitzgerald, K.D., Ngo, T., Chou, W., van der Heden van Noort, G.J., Filippov, D.V., Gershon, P.D., and Semler, B.L. (2012). An RNA virus hijacks an incognito function of a DNA repair enzyme. Proc Natl Acad Sci of the U S A *109*, 14634-14639.

150. Vogt, D.A., and Andino, R. (2010). An RNA element at the 5'-end of the poliovirus genome functions as a general promoter for RNA synthesis. PLoS Pathog *6*, e1000936.

151. Waggoner, S., and Sarnow, P. (1998). Viral ribonucleoprotein complex formation and nucleolar-cytoplasmic relocalization of nucleolin in poliovirus-infected cells. J Virol *72*, 6699-6709.

152. Wang, B., Xi, X., Lei, X., Zhang, X., Cui, S., Wang, J., Jin, Q., and Zhao, Z. (2013). Enterovirus 71 protease 2Apro targets MAVS to inhibit anti-viral type I interferon responses. PLoS Pathog *9*, e1003231.

153. Wang, J.P., Cerny, A., Asher, D.R., Kurt-Jones, E.A., Bronson, R.T., and Finberg, R.W. (2010). MDA5 and MAVS mediate type I interferon responses to coxsackie B virus. J Virol *84*, 254-260.

154. Watters, K., and Palmenberg, A.C. (2011). Differential processing of nuclear pore complex proteins by rhinovirus 2A proteases from different species and serotypes. J Virol *85*, 10874-10883.

155. Weidman, M.K., Yalamanchili, P., Ng, B., Tsai, W., and Dasgupta, A. (2001). Poliovirus 3C protease-mediated degradation of transcriptional activator p53 requires a cellular activity. Virology *291*, 260-271.

156. Wells, S.E., Hillner, P.E., Vale, R.D., and Sachs, A.B. (1998). Circularization of mRNA by eukaryotic translation initiation factors. Mol Cell *2*, 135-140.

157. White, J.P., Cardenas, A.M., Marissen, W.E., and Lloyd, R.E. (2007). Inhibition of cytoplasmic mRNA stress granule formation by a viral proteinase. Cell Host Microbe *2*, 295-305.

158. White, J.P., and Lloyd, R.E. (2011). Poliovirus unlinks TIA1 aggregation and mRNA stress granule formation. J Virol *85*, 12442-12454.

159. Wimmer, E., Hellen, C.U., and Cao, X. (1993). Genetics of poliovirus. Annu Rev Genet *27*, 353-436.

160. Wong, J., Si, X., Angeles, A., Zhang, J., Shi, J., Fung, G., Jagdeo, J., Wang, T., Zhong, Z., Jan, E.*, et al.* (2013). Cytoplasmic redistribution and cleavage of AUF1 during coxsackievirus infection enhance the stability of its viral genome. FASEB J *27*, 2777-2787.

161. Yalamanchili, P., Banerjee, R., and Dasgupta, A. (1997a). Poliovirus-encoded protease 2APro cleaves the TATA-binding protein but does not inhibit host cell RNA polymerase II transcription in vitro. J Virol *71*, 6881-6886.

162. Yalamanchili, P., Datta, U., and Dasgupta, A. (1997b). Inhibition of host cell transcription by poliovirus: cleavage of transcription factor CREB by poliovirus-encoded protease 3Cpro. J Virol *71*, 1220-1226.

163. Yalamanchili, P., Harris, K., Wimmer, E., and Dasgupta, A. (1996). Inhibition of basal transcription by poliovirus: a virus- encoded protease

(3Cpro) inhibits formation of TBP-TATA box complex in vitro. J Virol *70*, 2922-2929.

164. Yogo, Y., and Wimmer, E. (1972). Polyadenylic acid at the 3'-terminus of poliovirus RNA. Proc Natl Acad Sci U S A *69*, 1877-1882.

165. Zhang, W., Wagner, B.J., Ehrenman, K., Schaefer, A.W., DeMaria, C.T., Crater, D., DeHaven, K., Long, L., and Brewer, G. (1993). Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. Mol Cell Biol *13*, 7652-7665.

Host protein	Viral proteinase	Host function disrupted	References
eIF4GI/II	PV and CVB3 2A	Cap-dependent translation	Devaney et al. 1988; Etchison et al., 1982; Krausslich et al., 1987; Bovee et al., 1998; Lamphear et al., 1995
PABP	PV and CVB3 2A; PV 3C	Cap-dependent translation	Joachims et al., 1999; Kuyumcu et al., 2002; Kerekatte et al., 1999
UBF	PV 3C	RNA pol I transcription	Banerjee et al., 2005
TAF_{110}	PV 3C	RNA pol I transcription	Banerjee et al., 2005
TBP	PV 2A; PV 3C	PIC formation for RNA pol II transcription	Yalamanchili et al., 1997a; Das and Dasgupta, 1993; Yalamanchili et al., 1996
CREB-P	PV 3C	RNA pol II transcription	Yalamanchili et al., 1997b
p53	PV 3C	Transcription	Weidman et al., 2001
TFIIIC	PV 3C	RNA pol III transcription	Clark et al., 1991; Shen et al., 1996

Table 3.1. Enterovirus cleavage targets to disrupt the host cell translation and transcription machinery. Viral-encoded proteinases mediate cleavage of cellular proteins to shut down host cell functions, including cap-dependent translation and cellular transcription. Viral disruption allows host functions to become available for viral translation and RNA synthesis activities. The cellular proteins involved in cellular translation and transcription targeted by viral proteinases are outlined in Table 3.1.

Host protein	Viral Protein	Function of host protein	Role in viral replication	References
Nup62, Nup98, Nup153	PV and HRV 2A	Nucleo-cytoplasmic trafficking	Concentrates host proteins in the cytoplasm	Park et al., 2010; Park et al., 2008; Watters and Palmenberg, 2011; Castello et al., 2009 Gustin and Sarnow, 2001
La	PV 3C	Maturation of RNA pol III transcripts	Binds IRES for translation	Shiroki et al., 1999
PTB	PV and HRV14 3C/3CD	Alternative splicing	Full length PTB enhances viral translation; cleaved PTB mediates switch to RNA synthesis	Back et al., 2002
PCBP2	PV, CVB3, and HRV 3C/3CD	RNA binding; mRNA stability	Full length PCBP2 binds SLIV to enhance translation; full length PCBP2 binds SLI to initiate RNA synthesis; cleaved PCBP2 binds SLI for RNA synthesis	Perera et al., 2007; Chase et al., 2014
SRp20	PV 2A	Shuttling RNA binding protein; mRNA splicing	Interacts with PCBP2 to enhances translation	Fitzgerald et al., 2013
COPII	PV 2B	Protein transport from the ER to Golgi	Forms replication complexes	Rust et al., 2001
GBF1, BIG1/2	PV 3A and 3CD	Guanine nucleotide exchange factors	Activates Arf1 to produce PI4KIIIβ for replication complex formation	Belov et al., 2007
LC3	PV 2BC and 3A	Autophagy	Forms replication complexes	Jackson et al., 2005; Taylor and Kirkegaard, 2007
AUF1	PV and HRV 3CD; CVB3 3C	mRNA decay; RNA stability	Negative regulator of translation	Rozovics et al., 2012; Wong et al., 2013; Cathcart et al., 2013
TDP2	none	DNA repair	Cleaves VPg from 5' end	Virgen-Slane et al., 2012

Table 3.2. Use and abuse of host cell functions for enterovirus translation and RNA replication. Enterovirus proteins alter host proteins to stimulate viral translation and replication. Viral proteinases 2A, 3C, and 3CD can mediate cleavage of host proteins to change their canonical functions to non-canonical activities to aid in viral replication. However, some nonstructural proteins, including 2B, 2BC, and 3A, can modify the microenvironment of the cytoplasm to generate replication complexes so that viral RNA synthesis can be carried out.

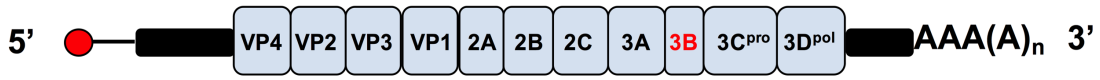

Figure 3.1. Overview of the enterovirus genome. Enteroviruses have a small (~7.5 kb), positive-sense RNA genome. At the 5’ end is the viral protein genome-linked (VPg) in red. VPg is covalently linked to the 5’ end of the RNA via an O4-(5'-uridylyl)tyrosine phosphodiester bond. VPg serves as a protein-primer for RNA synthesis since the RNA dependent RNA polymerase $3D^{pol}$ cannot initiate RNA synthesis *de novo*. The highly structured 5’ noncoding region (NCR) consists of stem-loops I-VI. Stem-loop I, also known as the cloverleaf, is required for viral RNA synthesis. Stem-loops II-VI encode the internal ribosome entry site (IRES), which initiates translation of the viral genome via a cap-independent mechanism. Following the 5’ NCR is the coding region. The coding region is translated into a single polyprotein, containing the structural (VP4-VP1) and nonstructural (2A, 2B, 2C, 3A, 3B, 3C, and 3D) proteins. The polyprotein is then proteolytically processed by viral-encoded proteinases. The nonstructural proteins are involved in multiple steps throughout the replication cycle, including modifying the host cell environment to aid in replication. Following the coding region is the 3’ NCR and genetically-encoded poly(A) tract, which are necessary for efficient viral replication [reviewed in (Bedard and Semler, 2004)].

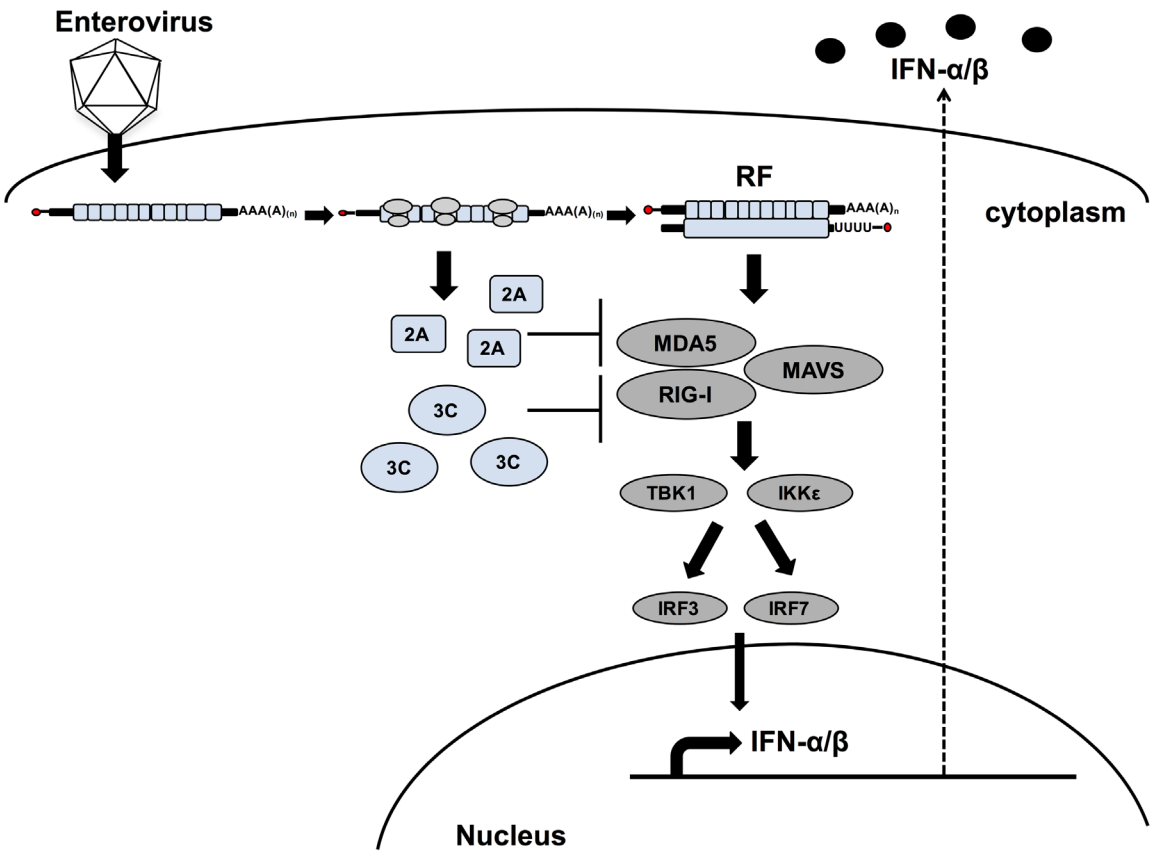

Figure 3.2. Enterovirus evasion of the type I IFN response. The host elicits an immune response when it detects invading pathogens. The immune receptors of the RLR pathway (RIG-I and MDA5) can detect double stranded RNAs. Although enteroviruses have single stranded RNAs, a double stranded replicative form (RF) is generated during viral replication. The RLR pathway is induced by detection of this RF. In order to bring about efficient replication of their viral genomes, enteroviruses have evolved mechanisms to evade the host antiviral response. A recent comprehensive study has shown that enterovirus proteinase 2A can cleave both MDA5 and MAVs and proteinase 3C can cleave RIG-I to inhibit the type I IFN response (Feng et al., 2014).

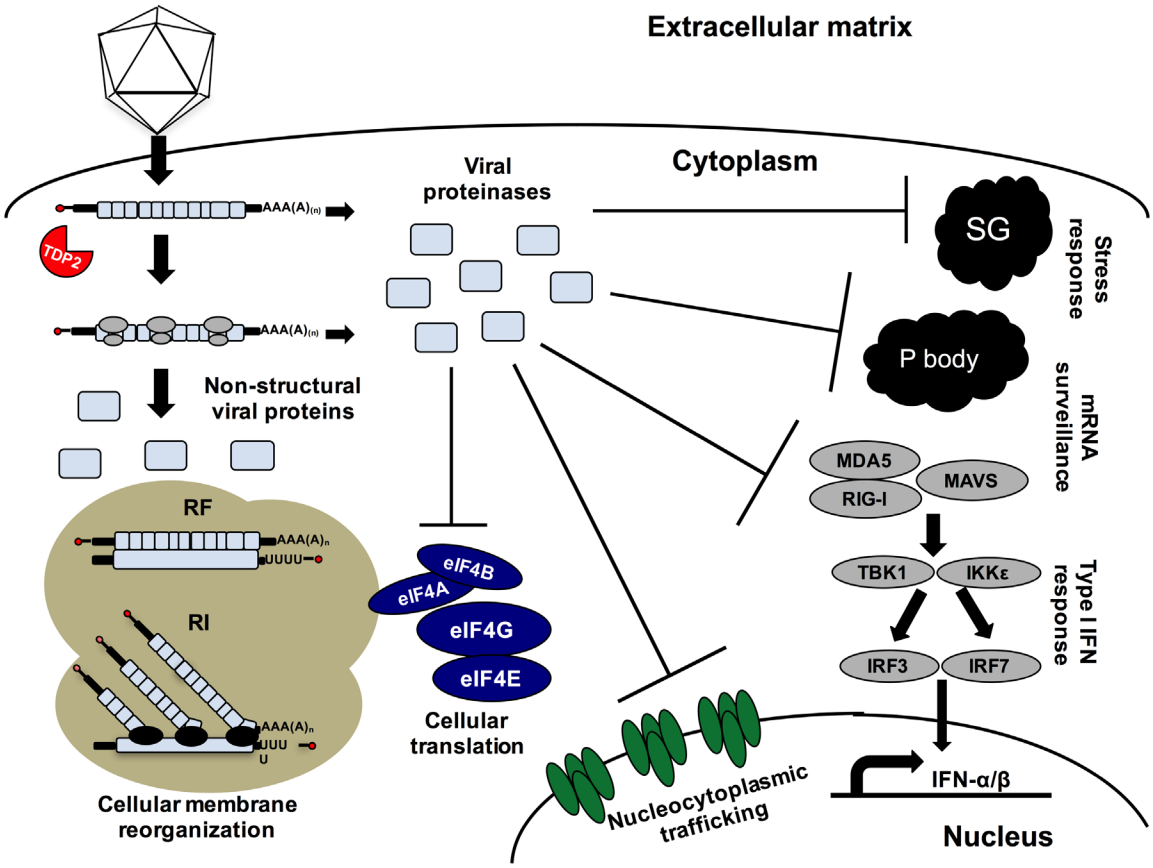

Figure 3.3. Summary of enterovirus-mediated host modifications to enhance viral translation and RNA replication. Enteroviruses modify cellular functions to stimulate viral replication. Upon release into the cytoplasm, the positive-strand viral RNA is translated. It has been suggested that cellular enzyme TDP2 cleaves the covalently linked viral protein, VPg, from the 5' end of the RNA to allow for polysome association. Following translation of the viral genome, the viral polyprotein is proteolytically processed. The viral proteins can then alter a number of cellular proteins, resulting in the hijacking of host functions for viral replication. Viral proteinases 2A, 3C, and 3CD are responsible for cleaving host proteins involved in cellular nucleo-cytoplasmic trafficking, translation, transcription, and the antiviral response. Nonstructural viral proteins, such as 2B, 2BC, and 3A, are also responsible for inducing conformational changes in the host

cytoplasmic membranes to serve as replication sites for viral RNA synthesis. Together these virus-induced modifications of cellular proteins result in an altered microenvironment that allows the virus to replicate efficiently.