Published in final edited form as:

Future Virol. 2012 February ; 7(2): 179–191.

Viral subversion of host functions for picornavirus translation and RNA replication

Amanda J Chase and Bert L Semler*

Department of Microbiology & Molecular Genetics, School of Medicine, University of California, Irvine, CA 92697, USA

Abstract

Picornavirus infections lead to symptoms that can have serious health and economic implications. The viruses in this family (*Picornaviridae*) have a small genomic RNA and must rely on host proteins for efficient viral gene expression and RNA replication. To ensure their effectiveness as pathogens, picornaviruses have evolved to utilize and/or alter host proteins for the benefit of the virus life cycle. This review discusses the host proteins that are subverted during infection to aid in virus replication. It will also describe proteins and functions that are altered during infection for the benefit of the virus.

Keywords

coxsackievirus; FMDV; host factor; human rhinovirus; IRES-mediated translation; picornavirus; poliovirus; proteolytic cleavage; RNA replication

The *Picornaviridae* family is made up of single-stranded, positive-sense RNA viruses. These viruses cause illnesses that have important health and economic consequences. Coxsackievirus is known to cause hand, foot and mouth disease, as well as serious heart infections such as myocarditis and pericarditis. The emerging picornavirus enterovirus 71 also causes hand, foot and mouth disease, and in more serious cases, causes neurological symptoms. An enterovirus 71 outbreak in Asia, primarily in China, led to the deaths of over 20 children from severe brain infections or heart and lung complications [1]. Human rhinovirus is a widespread picornavirus that causes the common cold. Although the symptoms of the common cold are not particularly serious for most people, human rhinovirus has economic implications through billions of dollars lost due to many hours of missed work. Additionally, the common cold can be much more serious for people with respiratory dysfunction, such as asthmatics [2]. The most well-studied picornavirus is poliovirus, which can cause paralytic poliomyelitis. Picornaviruses are also known to infect nonhuman animals. For example, foot-and-mouth disease virus (FMDV) infects cattle, sheep, goats and pigs, among others, and encephalomyocarditis virus (EMCV) infects rats, mice, cattle and, most severely, pigs, as well as humans. Viruses that affect livestock can lead to significant economic losses.

© 2012 Future Medicine Ltd

*Author for correspondence: Tel.: +1 949 824 7573, Fax: +1 949 824 2694, blsemmler@uci.edu.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

All picornavirus have a similar genomic RNA structure that lacks a cap at the 5' end and has a highly structured 5' noncoding region (NCR) with several stem-loop structures. Due to the absence of a 7-methyl guanosine cap, picornaviruses initiate translation in a cap-independent manner. Cap-dependent translation involves recognition of the cap structure by the eIF4F cap-binding complex that recruits the ribosome to the mRNA for translation initiation [3]. Translation initiation by picornaviruses is mediated by an internal ribosome entry site (IRES) that is composed of RNA stem-loop structures in the 5' NCR. The 5' NCR of poliovirus, human rhinovirus and coxsackievirus is made up of six stem-loop structures; for these viruses, stem-loops II–VI make up the IRES. Other picornaviruses, such as EMCV and FMDV, have even more RNA stem-loop structures in their 5' NCRs. These highly structured 5' NCR sequences preclude ribosome scanning through the NCR to the authentic initiation site [3]. Thus, ribosomes must be recruited to the RNA by a mechanism that is distinct from ribosome scanning. Once translation is initiated, the coding region is translated into a single polyprotein that is subsequently processed to produce precursor and mature viral proteins that are involved in viral RNA replication and, ultimately, packaging of progeny virus. The viral proteins produced include viral proteinases 3C and 3CD (all picornaviruses), 2A (poliovirus, human rhinovirus and coxsackievirus), and L (FMDV), which cleave the polyprotein to produce mature viral proteins; this review will focus primarily on the viral proteinases. 2A, 3C and FMDV L proteinases also cleave host cell proteins to shut down cellular cap-dependent translation, while allowing viral cap-independent translation. Eventually, there is a switch from translation to RNA replication, which occurs on the same positive-strand RNA template, but not at the same time [4,5]. Viral RNA synthesis occurs in replication complexes, which form at the site of altered host cell membranes [6]. Initiation of negative-strand RNA synthesis produces templates that are used to generate positive-strand RNAs used for further rounds of translation, as templates for additional negative-strand RNA synthesis, or that are packaged and released as virion RNA (Figure 1).

Aside from their role in disrupting cap-dependent translation, viral proteinases are also involved in cleavage of host proteins to disrupt host cell transcription and nucleocytoplasmic trafficking. Disruption of nucleocytoplasmic shuttling is advantageous for viral replication because picornaviruses are strictly cytoplasmic viruses, meaning that all translation and viral RNA replication must occur in the cytoplasm. Several of the host proteins involved in these processes usually shuttle from the nucleus to the cytoplasm or they are primarily nuclear proteins. Therefore, viral infection must require relocalization of specific host cell proteins to the cytoplasm by disrupting nucleocytoplasmic trafficking.

During the intracellular replication cycle of picornaviruses, there are several modifications to host cell proteins that allow the virus to subvert host cell functions in order to favor viral translation and RNA replication. Some of the alterations of host cell proteins may be fortuitous and not necessarily essential for viral translation or RNA synthesis. However, it is clear that cleavage of several host proteins by viral proteinases disrupts host cell functions, resulting in efficient viral translation and RNA replication. This review will focus on the functional modifications and cleavage of cellular proteins that allow viral subversion of host cell functions during picornavirus replication.

Viral subversion of host cell protein functions

As noted above, the functions of several host cell proteins are usurped to benefit picornavirus replication. Examples include PCBP2, PTB and La. PCBP2 is an RNA-binding protein that binds to poly(rC)-rich regions and is expressed in both the nucleus and the cytoplasm of the uninfected host cell [7,8]. PCBP2 and related family members are involved in host cell mRNA stabilization and translation silencing, as well as translation stimulation

[9]. During poliovirus infection, PCBP2 acts as an RNA-binding protein, which is similar to its role in the host cell, and is important for both translation and RNA replication. PCBP2 binds to stem-loop IV of the poliovirus 5' NCR (a major structure of the viral IRES) and forms a ribonucleoprotein (RNP) complex that is required for translation of the viral polyprotein [10–13]. Additionally, PCBP2 interacts outside of the IRES with stem-loop I of the poliovirus 5' NCR to form a ternary complex, along with the viral proteinase 3CD. This complex is required for initiation of negative-strand RNA synthesis, and possibly positive-strand RNA synthesis as well [12,14–17]. PTB is another host cell protein used by poliovirus to mediate efficient cap-independent translation. PTB is a member of the heterogeneous nuclear RNP (hnRNP) family and normally shuttles between the nucleus and the cytoplasm [18]. In the uninfected host cell, PTB is involved in mediating alternative splicing of pre-mRNAs, but during infection, it stimulates viral IRES-dependent translation [19–21]. An additional RNA-binding protein that is utilized by poliovirus and coxsackievirus B3 to enhance IRES-mediated translation is the La autoantigen [22–25]. In uninfected mammalian cells, La is involved in the initiation and termination of RNA polymerase III transcription. It is localized to the nucleus in the host cell, but upon infection, La is relocalized to the cytoplasm, where it binds to the 5' NCR of enteroviruses to stimulate translation [23,25]. During the course of infection, La is cleaved by poliovirus 3C proteinase, and the truncated, C-terminal portion of La is redistributed to the cytoplasm, where it retains the ability to enhance translation [26]. La has been shown to enhance poliovirus translation *in vitro* in rabbit reticulocyte lysate by inhibiting the aberrant initiation of translation at nonauthentic internal sites in the genome [24]. Together, host proteins PTB, PCBP2 and La enhance cap-independent translation of enterovirus RNA, increasing the level of available viral proteins for RNA replication.

After IRES-dependent translation, the newly synthesized polyprotein is then proteolytically processed to produce the viral proteins involved in viral RNA replication, host shut-off and virion assembly. For RNA replication to occur, an alteration of host cell membranes to form replication complexes that are capable of viral RNA synthesis must first occur [6]. Formation of replication complexes is thought to involve membranes derived from the endoplasmic reticulum (ER) and Golgi, ultimately resulting in the formation of single- and double-walled vesicles [27]. In the uninfected host cell, transport from the ER to the Golgi involves COPII coat proteins. COPII are vesicle coat proteins that transport proteins from the ER to the Golgi. Production of transport vesicles occurs at the ER, mediated by the COPII complex, and COPII-coated vesicles subsequently leave the ER [28–30]. After budding, COPII vesicles lose the COPII coat proteins and fuse together to form the ER–Golgi intermediate complex. The nonstructural protein 2B colocalizes with COPII proteins, suggesting that the COPII complex is involved in replication complex formation [28]. High-resolution confocal microscopy shows that the vesicles are ER-derived and accumulate in the cytoplasm during the course of poliovirus infection, which is consistent with COPII involvement [28,31].

Involvement of the secretory pathway in replication complex formation is suggested based on poliovirus RNA synthesis sensitivity to Brefeldin A (BFA) during infection [32–34]. BFA is a fungal antibiotic that blocks steps in the secretory pathway after COPII vesicle formation from the ER and specifically inhibits the function of a small GTPase, Arf, which is required in the secretory pathway [35]. Arf cycles between an active GTP-bound state and an inactive GDP-bound form. Active Arf–GTP can initiate the formation of vesicles by deforming membrane curvature and recruiting the coat and adaptor proteins that form secretory vesicles [35]. Generating the active form of Arf from Arf–GDP requires guanine nucleotide exchange factors (GEFs). BFA inactivates three GEFs (GBF1, BIG1 and BIG2) and traps Arf in the inactive, GDP-bound state [36]. This would also suggest that the GEFs are necessary for poliovirus RNA replication. The viral protein 3A recruits GBF1, and the

viral proteinase 3CD recruits BIG1 and BIG2 to membranes, inducing formation of Arf-GTP and increasing the level of activated Arf by fourfold during poliovirus infection [37]. Recruitment of GBF1 and Arf1 promotes production of a phosphatidylinositol-4-phosphate lipid microenvironment that facilitates membrane binding of the 3D RNA-dependent RNA polymerase [38]. Together, these data suggest that formation of picornavirus replication complexes involves Arf-dependent membrane trafficking and COPII-dependent budding of vesicles from the ER, although the exact mechanism is not known.

Subsequent work looking at replication complexes using electron microscopy has shown that the complexes are bound by a double membrane [27]. The formation of double membrane complexes has been suggested to occur in one of two ways. A single membrane complex could be formed in the COPII-dependent manner, followed by membrane wrapping to form the double membrane structures observed [39]. Alternatively, it has been proposed that membranes derived from the ER or Golgi wrap around small pools of cytoplasm and form replication complexes that are similar to immature autophagic vacuoles [27,40–42]. Cellular autophagosomes can form from the ER, Golgi and lysosome, and comprise cytoplasm surrounded by two lipid bilayers [43,44]. The gene *LC3* is used as a marker for cellular autophagosomes. It has been shown that *LC3* localizes to membrane compartments in infected cells [45]. Picornavirus proteins 2BC and 3A induce the formation of vacuoles that resemble replication complexes, and it has been shown that *LC3* localizes to these complexes [41]. Based on the ER and Golgi markers found in the replication complex bounded by a double membrane and colocalizing with *LC3*, it has been proposed that the poliovirus, human rhinovirus 2 and coxsackievirus replication complexes are formed in a manner that is similar to cellular autophagy [40,46,47]. It is hypothesized that the formation of the replication complexes allows local concentrations of viral and host proteins to favor the initiation of viral RNA synthesis.

Cleavage of host cell proteins by viral proteinases

The picornaviruses encode three viral proteinases, 2A, 3C and L. The 3C precursor protein, 3CD, also has proteolytic activity. The L protein harboring proteolytic activity is only encoded in aphthovirus genomes (e.g., FMDV). The 2A proteins with proteinase activity are only encoded in a subset of picornavirus genomic RNAs. These proteins are responsible for processing of the polyprotein into mature viral proteins, as well as cleavage of host cell proteins to disrupt host transcription and cap-dependent translation (Table 1). Through the use of mutated 2A cleavage sites, it has been shown that cleavage occurs preferentially at phenylalanine–glycine sites, although the consensus cleavage site is not as stringent as that of 3C proteinase (Table 2) [48]. 2A proteinase cleaves primarily at one site in the viral polyprotein, and is also known to cleave the eIF4G. eIF4G is important for cap-dependent translation and is the scaffolding protein of the eIF4F cap-binding complex, which also includes eIF4E (cap-binding protein) and eIF4A (helicase) (Figure 2). eIF4G also interacts with the cellular protein PABP, which binds to the 3′ poly(A) tract of cellular mRNAs, allowing for the functional circularization of mRNAs to provide an efficient mechanism for multiple rounds of translation initiation [3]. eIF4G is cleaved early during poliovirus, human rhinovirus or coxsackievirus infection, and the fragments produced cannot support cap-dependent translation [49,50]. There are two isoforms of eIF4G – I and II – which have similar biochemical activities and are thought to have redundant functions [51]. Cleavage of eIF4GI by itself is not sufficient to completely inhibit cap-dependent translation [52,53]. Cleavage of eIF4GII is slightly delayed during poliovirus infection, and the disappearance of intact eIF4GII corresponds with inhibition of cap-dependent translation, suggesting that both isoforms are cleaved by 2A proteinase [54–56]. However, the C-terminal eIF4G fragment can still interact with eIF3 to recruit the 40S ribosomal subunit to initiate viral IRES-driven translation [57–59]. Interestingly, 2A cleavage of eIF4G is more efficient when

eIF4G is associated with eIF4E, suggesting that eIF4G is preferentially cleaved when it is actively involved in cap-dependent translation [60].

eIF4G is also cleaved during infection by another picornavirus, FMDV. However, FMDV 2A lacks authentic proteolytic activity, and eIF4G is instead cleaved by the L proteinase [61–63]. The L proteinase is a member of the papain-like family of cysteine proteases, whereas the 2A proteinase is a member of the chymotrypsin-like protease family [64]. Cleavage of eIF4G by poliovirus, coxsackievirus and human rhinovirus 2A protein and by FMDV L protein is one way the virus shuts down cap-dependent translation. EMCV shuts down cap-dependent translation in an alternative way, as eIF4G is not cleaved during EMCV infection. Instead, 4E-BP1 is dephosphorylated during EMCV infection, which represses eIF4E activity and disrupts formation of the eIF4F cap-binding complex, thereby inhibiting cap-dependent translation [65–67]. Picornaviruses have developed these mechanisms to shut down cap-dependent translation in order to allow more efficient IRES-dependent translation by usurping the canonical translation factors and machinery to benefit virus translation.

Shut-down of host functions, such as cap-dependent translation, is important for a successful virus infection, and therefore, the picornaviruses have evolved to utilize multiple means of disrupting host cell functions. The viral proteinase 3C and precursor 3CD are known to cleave several host cell proteins, leading to the inhibition of cap-dependent translation, inactivation of host transcription and mediating a switch from viral translation to viral RNA replication. The poliovirus 3C proteinase cleaves primarily at glutamine–glycine sites, with an aliphatic residue in the P4 position (Table 2). 3C cleavage sites for other picornaviruses are somewhat more variable than those of poliovirus (Table 2). 3C cleaves PABP, a host cell protein that binds to the poly(A) tract, has three RNA recognition motifs (RRMs) and a flexible linker region in the C-terminal protein interaction domain [68,69]. PABP is cleaved at three sites, one located in the linker region and the other two between the RRM domains [70]. In the uninfected host cell, PABP is involved in mRNA circularization for initiation of multiple rounds of translation [71]. Therefore, cleavage of PABP is proposed to indirectly interfere with cap-dependent translation via the disruption of template circularization by inhibiting the ability of the protein to bind the poly(A) tract or other host cell proteins.

For poliovirus, cleavage of host proteins by 3C/3CD viral proteinases may contribute to disruption of viral cap-independent translation and allow for a switch from translation to viral RNA replication. Positive-sense picornavirus genomic RNAs are used as templates for both viral translation and RNA replication. Translation proceeds along the template in a 5′ to 3′ direction, while RNA replication is initiated at the 3′ end and proceeds to the 5′ end, creating the potential for a collision between translating ribosomes and replicating polymerase. It has been shown that templates being actively translated cannot function in RNA replication [4,5]. Therefore, there must be a mechanism to mediate a switch in template usage from translation to RNA replication. It has been proposed that cleavage of several host cell proteins leads to template switching (Figure 3) [72,73]. Host cell protein PTB is used by the virus to stimulate translation directed by poliovirus and EMCV, but it is also cleaved by 3C proteinase [74,75]. The PTB fragments produced are redistributed to the cytoplasm, where they appear to inhibit cap-independent translation in poliovirus-infected cells [72]. While the mechanism by which the PTB fragments inhibit translation is unknown, it is postulated that the C-terminal fragment, which can still bind viral RNA, prevents the binding of intact PTB to the IRES [72]. Alternatively, the N-terminal fragments could sequester RNA-binding proteins involved in cap-independent translation, or the cleaved PTB may not be able to recruit the translation machinery to the IRES. By disrupting cap-independent translation through the cleavage of PTB, the template can be cleared of ribosomes to allow the RNA to be used for viral RNA replication (Figure 3). Cleavage of

another host cell protein, PCBP2, is also suggested to potentiate the switch from viral translation to RNA replication. As noted previously, full-length PCBP2 interacts with stem-loop IV of the poliovirus 5' NCR and forms an RNP complex necessary for IRES-mediated translation [10–13]. PCBP2 also binds to stem-loop I of poliovirus 5' NCR and forms a ternary complex with 3CD proteinase that is required for initiation of negative-strand RNA synthesis [5,14–16]. PCBP2 is composed of three hnRNP K-homologous (KH) domains, with a linker region located between domains KH2 and KH3 (for a review, see [9]). During the course of poliovirus, coxsackievirus or human rhinovirus infection, PCBP2 is cleaved in the linker region by the 3C/3CD viral proteinase [73] [Daijogo S, Chase AJ, Semler BL, Unpublished Data]. The cleavage event disrupts the ability of PCBP2 to bind to stem-loop IV, thus inhibiting cap-independent translation. However, the truncated form of PCBP2 is still able to form a functional ternary complex with poliovirus stem-loop I RNA and remains active in viral RNA synthesis [73]. Cleavage of PCBP2 has been shown to occur during poliovirus infection at, and later than, peak times of replication [73]. This would suggest that, although PCBP2 cleavage may lead to more efficient viral RNA replication, other host or viral proteins could also be involved in mediating the switch from translation to RNA replication (Figure 3). Because it may be necessary for shut-down of cap-independent translation to occur before RNA replication, it would benefit the virus to have multiple means of mediating the switch of template usage. For example, cleavage of PTB in conjunction with PCBP2 cleavage by the viral proteinase 3CD may work together to mediate template use switching. The switch could also involve other, as yet undiscovered, host proteins. Alternatively, the cleavage of PCBP2, PTB and PABP may be a redundant function adapted by the virus to ensure that the switch to RNA replication can occur under different metabolic conditions in infected cells.

Cleavage of host proteins in virus-infected cells also inhibits host transcription. Picornavirus proteinases 2A and 3C have a role in disrupting RNA pol I, II and III transcription. RNA pol I transcribes rRNA and makes up 70–90% of all transcription occurring in the cell, and therefore directly influences the rate of cell growth [76]. Virus-induced shut-down of pol I transcription occurs by 90–180 min postinfection, although the means by which pol I transcription is inhibited is not known [77]. More is known about the inhibition of RNA pol III transcription, which transcribes housekeeping genes and is therefore tied to regulation of cell growth and the cell cycle. Pol III transcription initiation occurs when TFIIC binds to a promoter element – the B box – downstream of the transcription start site. Bound TFIIC recruits TFIIB and positions it at the transcription start site. RNA pol III recognizes TFIIB and is recruited to the start site, at which point TFIIC dissociates and transcription is initiated [78–81]. During the course of poliovirus infection, pol III transcription is inhibited by 3C proteinase cleavage of TFIIC. The cleavage inactivates the TFIIC–DNA complex, prohibiting the recruitment of TFIIB and RNA pol III to the start site [77,82].

RNA pol II transcribes host cell mRNAs, and its mode of action is more complex than other previously described means of transcription [77]. Poliovirus has evolved to inhibit pol II transcription in multiple ways. Both 2A and 3C viral proteinases cleave proteins involved in pol II transcription, although only cleavage by 3C appears to mediate inhibition of transcription. TFIID is one of several transcription factors that make up the preinitiation complex, along with TBP. This complex binds to the TATA box in the core promoter of the gene upstream of the start of transcription. Levels of TFIID complex are decreased fivefold during poliovirus infection [83]. Subsequent *in vitro* experiments showed that adding TBP restored levels of pol II transcription. These data suggested that pol II transcription is inhibited by the TFIID complex [84]. Although both 2A and 3C proteinases cleave TBP, only cleavage by 3C inhibits transcription [85,86]. Expression of poliovirus 3C via a CMV promoter following transient cotransfection of HeLa cells with reporter plasmids that could direct TATA or initiator-mediated pol II transcription showed that 3C is necessary and

sufficient for the inhibition of pol II transcription [84]. In addition, pol II transcription is inhibited by the cleavage of CREB, mediated by 3C viral proteinase. CREB is an upstream transcription factor that interacts with cellular and viral promoters for high-level transcription. Phosphorylated CREB is transcriptionally active and is preferentially cleaved by 3C proteinase to inhibit pol II transcription [87].

FMDV also inhibits transcription, albeit by a mechanism that is distinct from that of poliovirus. Early during the course of FMDV infection, the N-terminus of histone H3 is cleaved by the 3C proteinase [88,89]. Histone H3 is a protein involved in the chromatin nucleosome structure and is highly post-translationally modified. The protein is made up of a main globular domain and a long N-terminal tail. After cleavage by FMDV 3C, the larger fragment remains chromatin-associated, and the cleaved fragment contains the domain implicated in regulating transcriptionally active chromatin [90]. Therefore, cleavage of histone H3 by 3C proteinase to remove the regulatory domain may, in part, be responsible for the inhibition of host cell transcription in FMDV-infected cells. Picornaviruses have evolved these different mechanisms of shutting down host cell transcription to the clear benefit of the virus, since it has been shown that inefficient shut-off of host transcription leads to inefficient viral RNA replication [91].

The cleavage of most host proteins leads, directly or indirectly, to improved viral RNA synthesis. By disrupting cap-dependent translation, cap-independent translation proceeds more efficiently, and more viral proteins are produced. Viral proteins are required for the formation of replication complexes and the initiation of viral RNA synthesis. Similarly, cleavage of host cell proteins, such as PCBP2 and PTB, by the viral proteinase 3C disrupts cap-independent translation to allow a switch from translation to RNA replication. Additionally, poliovirus, human rhinovirus and EMCV 3C degrade RIG-1, proposed to be a mechanism for attenuating the innate immune response [92]. Together, these mechanisms alter cellular mechanisms and allow a replicative advantage to be gained over the host cell by the virus.

Disruption of nucleocytoplasmic trafficking

Translation and replication of all picornavirus genomes occur in the cytoplasm of the infected host cell. During viral infection, some of the host cell proteins involved in viral RNA replication or translation accumulate in the cytoplasm, although they are found in both the nucleus and the cytoplasm in uninfected cells. This suggests that nucleocytoplasmic trafficking is altered during picornavirus infection. It has been proposed that picornaviruses inhibit nuclear import of cellular factors such as La, PTB and PCBP2, resulting in cytoplasmic accumulation [93]. Proteins that relocate from the cytoplasm to the nucleus have a sequence motif with a nuclear localization signal (NLS) that is recognized by a specific receptor, and this unique pairing is an essential component of the transport pathway. To determine which transport pathways might be inhibited by picornavirus infection, an assay was used in which green fluorescent protein (GFP) was fused to a canonical NLS (GFP-NLS), and this reporter construct was transiently transfected into cells. The GFP-NLS was found to be predominantly nuclear in uninfected cells, but localized to the cytoplasm in virus-infected cells [94,95]. The same assay was carried out to show that the transportin import pathway and K nuclear shuttling (KNS) import pathway are also inhibited during picornavirus infection [93,94]. The transportin pathway is utilized by different hnRNPs, while the KNS pathway mediates the shuttling of hnRNP K [96,97]. Not all trafficking is inhibited during infection; two examples of pathways remaining intact include the glucocorticoid receptor import pathway and the Crm1 export pathway [93,98,99].

All transport pathways traverse the nuclear pore complex (NPC) that is embedded in the nuclear envelope. The NPC is made up of 50–100 proteins called nucleoporins (Nups) that contain repeating phenylalanine-glycine (FG) repeats [100]. The FG repeats are the essential docking domain for transport receptors to cross the membrane. During poliovirus infection, docking of receptor–cargo complexes at the NPC is inhibited, suggesting that the structure of the NPC is altered [94]. During the course of poliovirus or human rhinovirus infections, the 2A proteinase cleaves at least three Nups: Nup62, Nup98 and Nup153 [101–103]. Cleavage of these Nups corresponds with the observed relocalization of nuclear proteins to the cytoplasm and the inhibition of import [94]. When these Nups are cleaved during infection, the FG-repeat domain is lost, preventing transport vesicles from docking at the NPC and inhibiting the import pathway [104,105]. Cardioviruses, including EMCV and Theiler's murine encephalomyelitis virus, encode 2A and L proteins that lack enzymatic functions. The L protein has been shown to activate kinases that hyperphosphorylate FG-repeat domains in nuclear pore proteins, thereby disrupting nuclear transport [106,107]. The disruption of nuclear import during poliovirus infection leads to the cytoplasmic accumulation of specific nuclear factors that can be utilized by the virus during translation or RNA replication. For example, the cellular RNA-binding protein SRp20 is found predominantly in the nuclei of uninfected cells, but is relocalized to the cytoplasm during poliovirus infection [108]. Based on the interaction of SRp20 with PCBP2, it has been proposed that SRp20 is important for recruitment of the ribosomes to the viral RNA [108,109].

Conclusion

The small genome size of all picornaviruses requires that the viruses utilize host proteins for efficient translation and RNA replication. The viruses have evolved to effectively alter host proteins in order to disrupt host cell functions and alter the cellular environment to favor virus replication. The efficiency of picornavirus infections depends on the ability of viral proteins to subvert host proteins, and therefore it is most advantageous for the virus to evolve redundant mechanisms in order to ensure a favorable environment. Despite the potential for redundancy, it would seem that most changes to the host cell by the virus have evolved for a function, rather than as fortuitous cleavage events. There are undoubtedly numerous host proteins (that are as-yet undiscovered) that are altered or utilized by the virus during infection, and a complete appreciation of how viruses subvert host functions will involve understanding how host proteins act together, synergistically or redundantly, to ensure efficient virus RNA replication.

Future perspective

Host proteins play an integral role in picornavirus replication. During infection, host proteins are altered to allow viral, IRES-mediated translation over that of host cell cap-dependent translation. While it is known that ribosomes are recruited to an IRES and directed to the authentic start codon without scanning, the mechanism by which the ribosome is recruited to viral RNA during picornavirus infection is unknown. To understand the fundamental mechanisms of cap-independent translation, it will be critical to determine the specific process by which ribosomes are recruited to the IRES in order to initiate translation, and also the nature of all host proteins involved. Translation of the polyprotein allows the accumulation of viral proteins, including proteinases 2A, 3C and L. This increases the levels of cleaved host proteins, inhibiting both cap-dependent translation and, eventually, cap-independent translation, as well as altering host membranes and nucleocytoplasmic trafficking. For RNA replication to occur, the template must be cleared of translating ribosomes. While two host proteins, PTB and PCBP2, are hypothesized to help mediate template switching, the exact mechanism is unknown. Future understanding of

viral RNA replication will include a better characterization of how template switching is set in motion, including other host proteins that are modified, as well as whether these proteins work in a synergistic manner or are redundant. Additionally, a complete picture of RNA replication is incomplete without a greater description of the replication complexes that are formed during infection. The method by which host membranes are altered to form vesicles for RNA replication is beginning to be understood; however, it is not known what host proteins localize to the replication complex, the local concentrations of those proteins or whether the proteins are in a cleaved form, are intact or both. Most of the work that has been performed looking at the role of host proteins in picornavirus infection has been carried out with poliovirus. Ongoing studies will provide a closer look at other picornaviruses in order to obtain a more complete understanding of the individual requirements for each virus.

Executive summary

Viral subversion of host cell protein functions

- Host proteins PCBP2, PTB and La enhance viral translation.
- Replication complexes, the site of viral RNA synthesis, are formed from the endoplasmic reticulum in a COPII-dependent manner.
- Arf-dependent membrane trafficking is involved in the formation of replication complexes.
- The formation of double-membrane replication complexes occurs utilizing the autophagocytic pathway.

Cleavage of host cell proteins by picornavirus proteinases

- Viral proteinases 2A and L cleave eIF4G to inhibit cap-dependent translation.
- Cleavage of host cell protein PABP indirectly disrupts cap-dependent translation.
- Viral proteins PCBP2 and PTB are cleaved during poliovirus infection, and the cleavage is proposed to mediate a switch in template usage from viral translation to RNA replication.
- Cleavage of factors involved in RNA pol I, II and III transcription inhibits host transcription.
- Transcription is inhibited during foot-and-mouth disease virus infection, in part by 3C viral proteinase cleavage of histone H3.

Disruption of nucleocytoplasmic trafficking

- Disruption of nuclear import/export pathways leads to cytoplasmic accumulation of some host proteins that are used by the virus.
- The nuclear pore complex is altered during picornavirus infection by cleavage of nucleoporins that make up the nuclear pore complex, leading to inhibition of nuclear trafficking.

Future perspective

- The process by which the ribosome is recruited to a picornavirus internal ribosome entry site is largely unknown. Identification of the host proteins involved in this process will be crucial to obtain a better understanding of viral gene expression.

- Cleavage of host proteins is proposed to mediate template switching from viral translation to replication, and how these mechanisms work in conjunction with each other or act redundantly is an important area of research.
- It is known that viral RNA synthesis occurs in membrane-bound replication complexes, but it is not known which host proteins enter the replication complex or if the proteins are in a cleaved or intact state.
- Poliovirus is the most well-studied picornavirus, and much is known about how poliovirus subverts host cell proteins for the benefit of the virus. Ongoing work is focusing on analyzing the translation and RNA replication mechanisms of other picornaviruses and how they use or alter cellular proteins in a manner that is similar to or distinct from that of poliovirus.

Acknowledgments

The authors are grateful to A Cathcart and J Rozovics for their critical reading of the review.

Research described from the authors' laboratory was supported by Public Health Service Grant AI 26765 from the NIH. BL Semler is a Senior Fellow of the American Asthma Foundation.

References

Papers of special note have been highlighted as:

■ of interest

■ ■ of considerable interest

1. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N. Engl. J. Med.* 1999; 341(13):929–935. [PubMed: 10498487]
2. Gavalá ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol. Rev.* 2011; 242(1):69–90. [PubMed: 21682739]
3. Fitzgerald KD, Semler BL. Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. *Biochim. Biophys. Acta.* 2009; 1789(9–10):518–528. [PubMed: 19631772] ■ ■ Review of cellular cap-dependent translation and viral cap-independent translation.
4. Barton DJ, Morasco BJ, Flanagan JB. Translating ribosomes inhibit poliovirus negative-strand RNA synthesis. *J. Virol.* 1999; 73(12):10104–10112. [PubMed: 10559325] ■ Corroborates that the poliovirus genome cannot serve as a template for viral RNA replication when it is actively being translated, suggesting a switch in template usage must occur.
5. Gamarnik AV, Andino R. Switch from translation to RNA replication in a positive-stranded RNA virus. *Genes Dev.* 1998; 12(15):2293–2304. [PubMed: 9694795] ■ Demonstrates that the positive-sense RNA genome cannot function as a template for translation and replication simultaneously, suggesting a switch from viral translation to RNA replication must occur.
6. Dales S, Eggers HJ, Tamm I, Palade GE. Electron microscopic study of the formation of poliovirus. *Virology.* 1965; 26:379–389. [PubMed: 14319710]
7. Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell.* 1994; 77(1):33–39. [PubMed: 8156595]
8. Leffers H, Dejgaard K, Celis JE. Characterisation of two major cellular poly(rC)-binding human proteins, each containing three K-homologous (KH) domains. *Eur. J. Biochem.* 1995; 230(2):447–453. [PubMed: 7607214]
9. Makeyev AV, Liebhaber SA. The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. *RNA.* 2002; 8(3):265–278. [PubMed: 12003487]

10. Blyn LB, Swiderek KM, Richards O, Stahl DC, Semler BL, Ehrenfeld E. 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. USA.* 1996; 93(20): 11115–11120. [PubMed: 8855318]
11. Blyn LB, Towner JS, Semler BL, Ehrenfeld E. Requirement of poly(rC) binding protein 2 for translation of poliovirus RNA. *J. Virol.* 1997; 71(8):6243–6246. [PubMed: 9223526]
12. Gamarnik AV, Andino R. Two functional complexes formed by KH domain containing proteins with the 5' noncoding region of poliovirus RNA. *RNA.* 1997; 3(8):882–892. [PubMed: 9257647]
13. Walter BL, Parsley TB, Ehrenfeld E, Semler BL. Distinct poly(rC) binding protein KH domain determinants for poliovirus translation initiation and viral RNA replication. *J. Virol.* 2002; 76(23): 12008–12022. [PubMed: 12414943]
14. Andino R, Rieckhof GE, Baltimore D. A functional ribonucleoprotein complex forms around the 5' end of poliovirus RNA. *Cell.* 1990; 63(2):369–380. [PubMed: 2170027]
15. Andino R, Rieckhof GE, Achacoso PL, Baltimore D. Poliovirus RNA synthesis utilizes an RNP complex formed around the 5'-end of viral RNA. *EMBO J.* 1993; 12(9):3587–3598. [PubMed: 8253083]
16. Parsley TB, Towner JS, Blyn LB, Ehrenfeld E, Semler BL. Poly (rC) binding protein 2 forms a ternary complex with the 5'-terminal sequences of poliovirus RNA and the viral 3CD proteinase. *RNA.* 1997; 3(10):1124–1134. [PubMed: 9326487]
17. Vogt DA, Andino R. An RNA element at the 5'-end of the poliovirus genome functions as a general promoter for RNA synthesis. *PLoS Pathog.* 2010; 6(6) E1000936.
18. Michael WM, Choi M, Dreyfuss G. A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. *Cell.* 1995; 83(3):415–422. [PubMed: 8521471]
19. Sawicka K, Bushell M, Spriggs KA, Willis AE. Polypyrimidine-tract-binding protein: a multifunctional RNA-binding protein. *Biochem. Soc. Trans.* 2008; 36(Pt 4):641–647. [PubMed: 18631133]
20. Hellen CU, Witherell GW, Schmid M, et al. A cytoplasmic 57-kDa protein that is required for translation of picornavirus RNA by internal ribosomal entry is identical to the nuclear pyrimidine tract-binding protein. *Proc. Natl Acad. Sci. USA.* 1993; 90(16):7642–7646. [PubMed: 8395052]
21. Gosert R, Chang KH, Rijnbrand R, Yi M, Sangar DV, Lemon SM. Transient expression of cellular polypyrimidine-tract binding protein stimulates cap-independent translation directed by both picornaviral and flaviviral internal ribosome entry sites *in vivo*. *Mol. Cell. Biol.* 2000; 20(5):1583–1595. [PubMed: 10669736]
22. Craig AW, Svitkin YV, Lee HS, Belsham GJ, Sonenberg N. The La autoantigen contains a dimerization domain that is essential for enhancing translation. *Mol. Cell. Biol.* 1997; 17(1):163–169. [PubMed: 8972196]
23. Meerovitch K, Svitkin YV, Lee HS, et al. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J. Virol.* 1993; 67(7):3798–3807. [PubMed: 8389906]
24. Svitkin YV, Meerovitch K, Lee HS, et al. Internal translation initiation on poliovirus RNA: further characterization of La function in poliovirus translation *in vitro*. *J. Virol.* 1994; 68(3):1544–1550. [PubMed: 8107217]
25. Ray PS, Das S. La autoantigen is required for the internal ribosome entry site-mediated translation of coxsackievirus B3 RNA. *Nucleic Acids Res.* 2002; 30(20):4500–4508. [PubMed: 12384597]
26. Shiroki K, Isoyama T, Kuge S, et al. Intracellular redistribution of truncated La protein produced by poliovirus 3Cpro-mediated cleavage. *J. Virol.* 1999; 73(3):2193–2200. [PubMed: 9971802]
27. Schlegel A, Giddings TH Jr, Ladinsky MS, Kirkegaard K. Cellular origin and ultrastructure of membranes induced during poliovirus infection. *J. Virol.* 1996; 70(10):6576–6588. [PubMed: 8794292]
28. Rust RC, Landmann L, Gosert R, et al. Cellular COPII proteins are involved in production of the vesicles that form the poliovirus replication complex. *J. Virol.* 2001; 75(20):9808–9818. [PubMed: 11559814]

29. Klumperman J. Transport between ER and Golgi. *Curr. Opin. Cell. Biol.* 2000; 12(4):445–449. [PubMed: 10873822]
30. Springer S, Spang A, Schekman R. A primer on vesicle budding. *Cell.* 1999; 97(2):145–148. [PubMed: 10219233]
31. Bienz K, Egger D, Pasamontes L. 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.* 1987; 160(1):220–226. [PubMed: 2820130]
32. Cuconati A, Molla A, Wimmer E, Brefeldin A inhibits cell-free *de novo* synthesis of poliovirus. *J. Virol.* 1998; 72(8):6456–6464. [PubMed: 9658088]
33. Irurzun A, Perez L, Carrasco L. Involvement of membrane traffic in the replication of poliovirus genomes: effects of Brefeldin A. *Virology.* 1992; 191(1):166–175. [PubMed: 1329315]
34. Maynell LA, Kirkegaard K, Klymkowsky MW. Inhibition of poliovirus RNA synthesis by brefeldin A. *J. Virol.* 1992; 66(4):1985–1994. [PubMed: 1312615]
35. Belov GA, Ehrenfeld E. Involvement of cellular membrane traffic proteins in poliovirus replication. *Cell Cycle.* 2007; 6(1):36–38. [PubMed: 17245115]
36. Mossessova E, Corpina RA, Goldberg J. Crystal structure of *ARF1***Sec7* complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. *Mol. Cell.* 2003; 12(6):1403–1411. [PubMed: 14690595]
37. Belov GA, Habbersett C, Franco D, Ehrenfeld E. Activation of cellular Arf GTPases by poliovirus protein 3CD correlates with virus replication. *J. Virol.* 2007; 81(17):9259–9267. [PubMed: 17567696] ■ Demonstrates that the Arf pathway is involved in viral replication complex formation.
38. Hsu NY, Illytska O, Belov G, et al. Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. *Cell.* 2010; 141(5):799–811. [PubMed: 20510927]
39. Belov GA, Nair V, Hansen BT, Hoyt FH, Fischer ER, Ehrenfeld E. Complex dynamic development of poliovirus membranous replication complexes. *J. Virol.* 2012; 86(1):302–312. [PubMed: 22072780]
40. Suhy DA, Giddings TH Jr, Kirkegaard K. Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. *J. Virol.* 2000; 74(19):8953–8965. [PubMed: 10982339]
41. Jackson WT, Giddings TH Jr, Taylor MP, et al. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol.* 2005; 3(5) E156.
42. Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. *Nat. Rev. Microbiol.* 2008; 6(5):363–374. [PubMed: 18414501]
43. Dunn WA Jr. Studies on the mechanisms of autophagy: maturation of the autophagic vacuole. *J. Cell Biol.* 1990; 110(6):1935–1945. [PubMed: 2161853]
44. Dunn WA Jr. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. *J. Cell Biol.* 1990; 110(6):1923–1933. [PubMed: 2351689]
45. Taylor MP, Kirkegaard K. Modification of cellular autophagy protein LC3 by poliovirus. *J. Virol.* 2007; 81(22):12543–12553. [PubMed: 17804493]
46. Kemball CC, Alirezai M, Flynn CT, et al. Coxsackievirus infection induces autophagy-like vesicles and megaphagosomes in pancreatic acinar cells *in vivo*. *J. Virol.* 2010; 84(23):12110–12124. [PubMed: 20861268]
47. Klein KA, Jackson WT. Human rhinovirus 2 induces the autophagic pathway and replicates more efficiently in autophagic cells. *J. Virol.* 2011; 85(18):9651–9654. [PubMed: 21752910]
48. Toyoda H, Nicklin MJ, Murray MG, et al. A second virus-encoded proteinase involved in proteolytic processing of poliovirus polypeptide. *Cell.* 1986; 45(5):761–770. [PubMed: 3011278] ■ First demonstration of poliovirus 2A as a viral proteinase, including a description of the cleavage recognition sequence.
49. Etchison D, Milburn SC, Edery I, Sonenberg N, Hershey JW. 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.* 1982; 257(24):14806–14810. [PubMed: 6294080]

50. Lloyd RE. Translational control by viral proteinases. *Virus Res.* 2006; 119(1):76–88. [PubMed: 16303201] ■ Review of picornavirus proteinases and how they can control both cellular and viral translation.
51. Prevot D, Darlix JL, Ohlmann T. Conducting the initiation of protein synthesis: the role of eIF4G. *Biol. Cell.* 2003; 95(3–4):141–156. [PubMed: 12867079]
52. Bonneau AM, Sonenberg N. Proteolysis of the p220 component of the cap-binding protein complex is not sufficient for complete inhibition of host cell protein synthesis after poliovirus infection. *J. Virol.* 1987; 61(4):986–991. [PubMed: 3029432]
53. Keiper BD, Rhoads RE. Cap-independent translation initiation in *Xenopus* oocytes. *Nucleic Acids Res.* 1997; 25(2):395–402. [PubMed: 9016570]
54. Gradi A, Svitkin YV, Imataka H, Sonenberg N. Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. *Proc. Natl Acad. Sci. USA.* 1998; 95(19):11089–11094. [PubMed: 9736694]
55. Marissen WE, Gradi A, Sonenberg N, Lloyd RE. Cleavage of eukaryotic translation initiation factor 4GII correlates with translation inhibition during apoptosis. *Cell Death Differ.* 2000; 7(12):1234–1243. [PubMed: 11175261]
56. Svitkin YV, Gradi A, Imataka H, Morino S, Sonenberg N. Eukaryotic initiation factor 4GII (eIF4GII), but not eIF4GI, cleavage correlates with inhibition of host cell protein synthesis after human rhinovirus infection. *J. Virol.* 1999; 73(4):3467–3472. [PubMed: 10074204]
57. Borman AM, Kirchweiger R, Ziegler E, Rhoads RE, Skern T, Kean KM. eIF4G and its proteolytic cleavage products: effect on initiation of protein synthesis from capped, uncapped, and IRES-containing mRNAs. *RNA.* 1997; 3(2):186–196. [PubMed: 9042945]
58. Hambidge SJ, Sarnow P. Translational enhancement of the poliovirus 5′ noncoding region mediated by virus-encoded polypeptide 2A. *Proc. Natl Acad. Sci. USA.* 1992; 89(21):10272–10276. [PubMed: 1332040]
59. Liebig HD, Ziegler E, Yan R, et al. Purification of two picornaviral 2A proteinases: interaction with eIF-4 gamma and influence on *in vitro* translation. *Biochemistry.* 1993; 32(29):7581–7588. [PubMed: 8338854]
60. Bovee ML, Lamphear BJ, Rhoads RE, Lloyd RE. Direct cleavage of eIF4G by poliovirus 2A protease is inefficient *in vitro*. *Virology.* 1998; 245(2):241–249. [PubMed: 9636363]
61. Devaney MA, Vakharia VN, Lloyd RE, Ehrenfeld E, Grubman MJ. Leader protein of foot-and-mouth disease virus is required for cleavage of the p220 component of the cap-binding protein complex. *J. Virol.* 1988; 62(11):4407–4409. [PubMed: 2845152]
62. Donnelly ML, Hughes LE, Luke G, et al. The ‘cleavage’ activities of foot-and-mouth disease virus 2A site-directed mutants and naturally occurring ‘2A-like’ sequences. *J. Gen. Virol.* 2001; 82(Pt 5):1027–1041. [PubMed: 11297677]
63. Doronina VA, Wu C, de Felipe P, Sachs MS, Ryan MD, Brown JD. Site-specific release of nascent chains from ribosomes at a sense codon. *Mol. Cell. Biol.* 2008; 28(13):4227–4239. [PubMed: 18458056]
64. Roberts PJ, Belsham GJ. Identification of critical amino acids within the foot-and-mouth disease virus leader protein, a cysteine protease. *Virology.* 1995; 213(1):140–146. [PubMed: 7483257]
65. Pause A, Methot N, Svitkin Y, Merrick WC, Sonenberg N. Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation. *EMBO J.* 1994; 13(5):1205–1215. [PubMed: 8131750]
66. Gingras AC, Svitkin Y, Belsham GJ, Pause A, Sonenberg N. Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus. *Proc. Natl Acad. Sci. USA.* 1996; 93(11):5578–5583. [PubMed: 8643618]
67. Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J.* 1996; 15(3):658–664. [PubMed: 8599949]
68. Kozlov G, Trempe JF, Khaleghpour K, Kahvejian A, Ekiel I, Gehring K. Structure and function of the C-terminal PABC domain of human poly(A)-binding protein. *Proc. Natl Acad. Sci. USA.* 2001; 98(8):4409–4413. [PubMed: 11287632]

69. Kozlov G, De Crescenzo G, Lim NS, et al. 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.* 2004; 23(2):272–281. [PubMed: 14685257]
70. Joachims M, Van Breugel PC, Lloyd RE. Cleavage of poly(A)-binding protein by enterovirus proteases concurrent with inhibition of translation *in vitro*. *J. Virol.* 1999; 73(1):718–727. [PubMed: 9847378]
71. Jacobson A, Favreau M. Possible involvement of poly(A) in protein synthesis. *Nucleic Acids Res.* 1983; 11(18):6353–6368. [PubMed: 6137807]
72. Back SH, Kim YK, Kim WJ, et al. Translation of polioviral mRNA is inhibited by cleavage of polypyrimidine tract-binding proteins executed by polioviral 3C(pro). *J. Virol.* 2002; 76(5):2529–2542. [PubMed: 11836431]
73. Perera R, Daijogo S, Walter BL, Nguyen JH, Semler BL. Cellular protein modification by poliovirus: the two faces of poly(rC)-binding protein. *J. Virol.* 2007; 81(17):8919–8932. [PubMed: 17581994]
74. Kafasla P, Morgner N, Poyry TA, Curry S, Robinson CV, Jackson RJ. Polypyrimidine tract binding protein stabilizes the encephalomyocarditis virus IRES structure via binding multiple sites in a unique orientation. *Mol. Cell.* 2009; 34(5):556–568. [PubMed: 19524536]
75. Kafasla P, Morgner N, Robinson CV, Jackson RJ. Polypyrimidine tract-binding protein stimulates the poliovirus IRES by modulating eIF4G binding. *EMBO J.* 2010; 29(21):3710–3722. [PubMed: 20859255]
76. Tower J, Culotta VC, Sollner-Webb B. Factors and nucleotide sequences that direct ribosomal DNA transcription and their relationship to the stable transcription complex. *Mol. Cell. Biol.* 1986; 6(10):3451–3462. [PubMed: 3796588]
77. Weidman MK, Sharma R, Raychaudhuri S, Kundu P, Tsai W, Dasgupta A. The interaction of cytoplasmic RNA viruses with the nucleus. *Virus Res.* 2003; 95(1–2):75–85. [PubMed: 12921997]
- Review focusing on the interaction of cytoplasmic viruses with the host cell nucleus and how these viruses can inhibit host transcription and nuclear trafficking.
78. Kassavetis GA, Braun BR, Nguyen LH, Geiduschek EP. *S. cerevisiae* TFIIB is the transcription initiation factor proper of RNA polymerase. III. while TFIIA and TFIIC are assembly factors. *Cell.* 1990; 60(2):235–245. [PubMed: 2404611]
79. Kassavetis GA, Blanco JA, Johnson TE, Geiduschek EP. Formation of open and elongating transcription complexes by RNA polymerase III. *J. Mol. Biol.* 1992; 226(1):47–58. [PubMed: 1619662]
80. Kovelman R, Roeder RG. Purification and characterization of two forms of human transcription factor IIIC. *J. Biol. Chem.* 1992; 267(34):24446–24456. [PubMed: 1447193]
81. Yoshinaga SK, L'Etoile ND, Berk AJ. Purification and characterization of transcription factor IIIC2. *J. Biol. Chem.* 1989; 264(18):10726–10731. [PubMed: 2732244]
82. Clark ME, Hammerle T, Wimmer E, Dasgupta A. 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.* 1991; 10(10):2941–2947. [PubMed: 1915271]
83. Kliewer S, Dasgupta A. An RNA polymerase II transcription factor inactivated in poliovirus-infected cells copurifies with transcription factor TFIID. *Mol. Cell. Biol.* 1988; 8(8):3175–3182. [PubMed: 2850483]
84. Yalamanchili P, Harris K, Wimmer E, Dasgupta A. Inhibition of basal transcription by poliovirus: a virus-encoded protease (3Cpro) inhibits formation of TBP–TATA box complex *in vitro*. *J. Virol.* 1996; 70(5):2922–2929. [PubMed: 8627767]
85. Das S, Dasgupta A. Identification of the cleavage site and determinants required for poliovirus 3Cpro-catalyzed cleavage of human TATA-binding transcription factor TBP. *J. Virol.* 1993; 67(6):3326–3331. [PubMed: 8388502]
86. Yalamanchili P, Banerjee R, Dasgupta A. Poliovirus-encoded protease 2Apro cleaves the TATA-binding protein but does not inhibit host cell RNA polymerase II transcription *in vitro*. *J. Virol.* 1997; 71(9):6881–6886. [PubMed: 9261414]

87. Yalamanchili P, Datta U, Dasgupta A. Inhibition of host cell transcription by poliovirus: cleavage of transcription factor CREB by poliovirus-encoded protease 3Cpro. *J. Virol.* 1997; 71(2):1220–1226. [PubMed: 8995645]
88. Falk MM, Grigera PR, Bergmann IE, Zibert A, Multhaup G, Beck E. Foot-and-mouth disease virus protease 3C induces specific proteolytic cleavage of host cell histone H3. *J. Virol.* 1990; 64(2): 748–756. [PubMed: 2153239]
89. Grigera PR, Tisminetzky SG. Histone H3 modification in BHK cells infected with foot-and-mouth disease virus. *Virology.* 1984; 136(1):10–19. [PubMed: 6330987]
90. Grubman MJ, Moraes MP, Diaz-San Segundo F, Pena L, de los Santos T. Evading the host immune response: how foot-and-mouth disease virus has become an effective pathogen. *FEMS Immunol. Med. Microbiol.* 2008; 53(1):8–17. [PubMed: 18400012]
91. Kundu P, Raychaudhuri S, Tsai W, Dasgupta A. Shutoff of RNA polymerase II transcription by poliovirus involves 3C protease-mediated cleavage of the TATA-binding protein at an alternative site: incomplete shutoff of transcription interferes with efficient viral replication. *J. Virol.* 2005; 79(15):9702–9713. [PubMed: 16014932]
92. Barral PM, Sarkar D, Fisher PB, Racaniello VR. RIG-I is cleaved during picornavirus infection. *Virology.* 2009; 391(2):171–176. [PubMed: 19628239]
93. Gustin KE. Inhibition of nucleo-cytoplasmic trafficking by RNA viruses: targeting the nuclear pore complex. *Virus Res.* 2003; 95(1–2):35–44. [PubMed: 12921994] ■ Overview of how nucleocytoplasmic trafficking is disrupted during the course of picornavirus infection.
94. Gustin KE, Sarnow P. Effects of poliovirus infection on nucleo-cytoplasmic trafficking and nuclear pore complex composition. *EMBO J.* 2001; 20(1–2):240–249. [PubMed: 11226174]
95. Belov GA, Evstafieva AG, Rubtsov YP, Mikitas OV, Vartapetian AB, Agol VI. Early alteration of nucleocytoplasmic traffic induced by some RNA viruses. *Virology.* 2000; 275(2):244–248. [PubMed: 10998323]
96. Pollard VW, Michael WM, Nakielnny S, Siomi MC, Wang F, Dreyfuss G. A novel receptor-mediated nuclear protein import pathway. *Cell.* 1996; 86(6):985–994. [PubMed: 8808633]
97. Michael WM, Eder PS, Dreyfuss G. The K nuclear shuttling domain: a novel signal for nuclear import and nuclear export in the hnRNP K protein. *EMBO J.* 1997; 16(12):3587–3598. [PubMed: 9218800]
98. Love DC, Sweitzer TD, Hanover JA. Reconstitution of HIV-1 Rev nuclear export: independent requirements for nuclear import and export. *Proc. Natl Acad. Sci. USA.* 1998; 95(18):10608–10613. [PubMed: 9724751]
99. Wolff B, Sanglier JJ, Wang Y. Leptomycin B is an inhibitor of nuclear export: inhibition of nucleo-cytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. *Chem. Biol.* 1997; 4(2):139–147. [PubMed: 9190288]
100. Ryan KJ, Wenthe SR. The nuclear pore complex: a protein machine bridging the nucleus and cytoplasm. *Curr. Opin. Cell. Biol.* 2000; 12(3):361–371. [PubMed: 10801463]
101. Castello A, Izquierdo JM, Welnowska E, Carrasco L. RNA nuclear export is blocked by poliovirus 2A protease and is concomitant with nucleoporin cleavage. *J. Cell Sci.* 2009; 122(Pt 20):3799–3809. [PubMed: 19789179]
102. Park N, Skern T, Gustin KE. Specific cleavage of the nuclear pore complex protein Nup62 by a viral protease. *J. Biol. Chem.* 2010; 285(37):28796–28805. [PubMed: 20622012]
103. Watters K, Palmenberg AC. Differential processing of nuclear pore complex proteins by rhinovirus 2A proteases from different species and serotypes. *J. Virol.* 2011; 85(20):10874–10883. [PubMed: 21835805]
104. Bayliss R, Littlewood T, Stewart M. Structural basis for the interaction between FxFG nucleoporin repeats and importin-beta in nuclear trafficking. *Cell.* 2000; 102(1):99–108. [PubMed: 10929717]
105. Stewart M, Baker RP, Bayliss R, et al. Molecular mechanism of translocation through nuclear pore complexes during nuclear protein import. *FEBS Lett.* 2001; 498(2–3):145–149. [PubMed: 11412846]

106. Porter FW, Brown B, Palmenberg AC. Nucleoporin phosphorylation triggered by the encephalomyocarditis virus leader protein is mediated by mitogen-activated protein kinases. *J. Virol.* 2010; 84(24):12538–12548. [PubMed: 20881039]
107. Porter FW, Palmenberg AC. Leader-induced phosphorylation of nucleoporins correlates with nuclear trafficking inhibition by cardioviruses. *J. Virol.* 2009; 83(4):1941–1951. [PubMed: 19073724]
108. Fitzgerald KD, Semler BL. Re-localization of cellular protein SRp20 during poliovirus infection: bridging a viral IRES to the host cell translation apparatus. *PLoS Pathog.* 2011; 7(7) E1002127.
109. Bedard KM, Daijogo S, Semler BL. A nucleo-cytoplasmic SR protein functions in viral IRES-mediated translation initiation. *EMBO J.* 2007; 26(2):459–467. [PubMed: 17183366]
110. Brunner, JE.; Semler, BL. Positive-strand RNA virus replication in vertebrate hosts. In: Mahy, BWJ.; ter Meulen, V., editors. *Topley and Wilson's Microbiology and Microbial Infections*. UK: Hodder Arnold; 2005. p. 135-146.
111. Daijogo S, Semler BL. Mechanistic intersections between picornavirus translation and RNA replication. *Adv. Virus Res.* 2011; 80:1–24. [PubMed: 21762819]
112. Palmenberg AC. Proteolytic processing of picornaviral polyprotein. *Annu. Rev. Microbiol.* 1990; 44:603–623. [PubMed: 2252396]

Figure 1. Effect of picornavirus infection on the host cell

Picornavirus positive-strand genomes are used as templates for translation of a single polyprotein that is subsequently processed to produce viral proteins used in RNA replication, shown around the viral RdRp, which is depicted in red. Viral proteins contribute to cleavage of host cell proteins. Host protein cleavage downregulates cellular translation while still allowing viral, cap-independent translation. Viral proteins direct the modification of host cell membranes to form replication complexes (shown as green orbs), the sites of viral RNA replication. Negative-strand RNA synthesis generates templates used for synthesis of positive-strand RNA templates, which are used for further rounds of translation of viral proteins, RNA synthesis or are packaged and released as virion RNA. Modification of host proteins also alters the nucleus (shown in blue) to disrupt nucleocytoplasmic trafficking.

RdRp: RNA-dependent RNA polymerase; RI: Replicative intermediate.

Adapted with permission from [110].

Figure 2. Poliovirus as a model for picornavirus cap-independent translation

Viral proteinase 2A cleaves eIF4G, disrupting the eIF4F cap-binding complex. The highly structured 5' noncoding region and lack of a cap at the 5' end prevent cap binding and ribosome scanning. Instead, translation is mediated by the internal ribosome entry site, which recruits the 40S ribosome subunit by an unknown mechanism that could involve other, as yet unknown, ITAFs. The ribosome recognizes the authentic start codon to initiate translation.

SL: Stem-loop.

Adapted with permission from [3].

Figure 3. Switch from translation to RNA synthesis

(A) Picornavirus genome RNA is a template for cap-independent translation under specific conditions until (B) the conditions are altered to allow the genome RNA to be used as a template for RNA synthesis. When the template is being translated, PCBP2 binds to SL-IV, along with SRp20, and PTB interacts at the base of SL-IV to enhance translation. As viral proteins are translated, the concentration of viral proteinases increases, leading to the cleavage of host proteins, including PCBP2 and PTB. Cleavage of PCBP2 prevents the interaction with SL-IV, thereby inhibiting translation. Cleaved PCBP2 remains active in RNA replication and forms a functional ternary complex with SL-I. PTB cleavage is

proposed to prevent binding of full-length PTB, thereby preventing the stimulation of cap-independent translation. The cleavage events are proposed to mediate a switch in template usage from translation to RNA replication.

SL: Stem-loop.

Adapted with permission from [73].

\$watermark-text

\$watermark-text

\$watermark-text

Table 1

Examples of cleavage of cellular proteins by picornavirus proteinases.

Host cell protein	Viral proteinase(s)	Effects of protein cleavage
PTB	PV 3C	Inhibits viral cap-independent translation and helps mediate a switch from viral translation to RNA replication
PCBP2	PV 3C, CVB 3C, HRV 3C	Inhibits viral cap-independent translation and helps mediate a switch from viral translation to RNA replication
La	PV 3C, CVB 3C	Enhances viral IRES-dependent translation
PABP	PV 3C, HRV14 3C, CVB 2A	Indirectly causes inhibition of viral IRES-dependent translation
eIF4G	PV 2A, CVB 2A, FMDV L	Inhibits cap-dependent translation
Histone H3	FMDV 3C	Inhibits host cell transcription
TFIIIC	PV 3C	Inhibits recruitment of TFIIB and RNA polymerase III to the transcription start site
TBP	PV 3C, PV 2A	Inhibits RNA polymerase II transcription
CREB	PV 3C, PV 2A	Inhibits RNA polymerase II transcription
Nucleoporins (Nup62, Nup98 and Nup153)	PV 2A, HRV 2A	Disrupts nuclear import

CVB: Coxsackievirus B; FMDV: Foot-and-mouth disease virus; HRV: Human rhinovirus; IRES: Internal ribosome entry site; PV: Poliovirus. Adapted with permission from [111].

Table 2

Viral proteinase recognition sites.

Viral proteinase	P1	P1'	Additional substrate determinants
Poliovirus 3C/3CD	Q Q Q Q	G A N S	Amino acid with an aliphatic side chain (usually an alanine) in the P4 position
Human rhinovirus 3C/3CD	Q Q Q E	G S A N G	Amino acid with an aliphatic side chain (usually an alanine) in the P4 position
Cardiovirus 3C/3CD	Q Q Q E E	G S A N S	Proline preferred in the P2 or P2' position
Enterovirus 2A	F Y	G G	–

A proteinase recognizes a site that is between the P1 and P1' amino acids, one amino acid upstream or downstream of the cleavage site, respectively. P2 and P2' represent the second amino acid upstream or downstream of the cleavage site, respectively. P4 indicates the amino acid four positions upstream of the cleavage site. The preferred amino acids at the P1 and P1' positions of a cleavage site for each proteinase are indicated in bold.

For a review on the specific cleavage recognition sites for viral proteinases, see [112].