Picornaviruses: Pathogenesis and Molecular Biology

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Glossary

***Cre* (*cis*-acting replication element)** An RNA hairpin in picornavirus genomic RNA that acts as a template for uridylylation of VPg (viral protein, genome linked) to VPg-pU-pU by the RNA polymerase 3D.

Enteric virus A virus that preferentially replicates in the intestine or gut of a host. For picornaviruses, these include poliovirus, coxsackievirus, echovirus, and enterovirus 71.

IRES (Internal Ribosome Entry Site) A highly structured RNA element at the 5′ end of some cellular and viral mRNAs that directs translation via a cap-independent mechanism.

Myocarditis Inflammation of the heart muscle, often caused by viruses (e.g., coxsackievirus).

Neurovirulence The ability of a pathogen to invade the nervous system and cause disease (e.g., poliovirus).

Positive-strand RNA An RNA molecule that is functional as mRNA and can be used in translation. Picornavirus genomes exist as positive-sense RNAs.

Quasi-species A collection of variant but related genotypes or individuals that make up a species. In viruses, this refers to the genetic diversity that allows viral populations to adapt to changing environments.

Uridylylation The addition of uridylyl groups to a protein or nucleic acid. In the case of picornaviruses, the viral RNA polymerase 3D uridylylates VPg using an RNA template for use as a protein primer for replication.

VPg (viral protein, genome linked) Also known as 3B, a small, basic viral protein linked to the 5′ end of replicating picornavirus RNAs. VPg is used as a protein primer for the polymerase 3D during viral RNA replication.

Introduction

Picornaviruses are small RNA viruses that cause a wide array of diseases in humans and animals with large economic repercussions. The importance of these small (Latin *pico*) viruses extends beyond their public health implications; as the first mammalian viruses discovered, the study of picornaviruses spearheaded many research programs still alive today. From the study of the molecular virology of picornaviruses, scientists have discovered internal ribosome entry site (IRES)-driven translation, uncovered antiviral responses of mammalian cells, gained insights in the maturation of proteins synthesized as precursors, and accomplished the first chemical synthesis of a virus. In this article, we will highlight the most common diseases of picornaviruses that affect public health, followed by a broad overview of the molecular biology of picornaviruses, including classification, structure, and the basics of the viral replication cycle.

Diseases of Picornaviruses

Poliovirus

Poliovirus (PV), perhaps the most well studied of the *Picornaviridae*, is the causative agent of the crippling and sometimes fatal disease poliomyelitis (Table 1), which is still endemic in parts of Africa and the Middle East despite ongoing eradication efforts. Although poliomyelitis may now seem like a disease of developing nations, in the 1940s and 1950s it was one of the most feared diseases in the United States, crippling above 35 000 people per year. PV is mainly an enteric virus, but approximately 1% of infected individuals experience neurodegeneration caused by the spread of the virus to the central nervous system.

Coxsackievirus

While coxsackievirus is most well known as the cause of the common childhood illness, hand, foot, and mouth disease, these viruses have also been implicated in a wide range of diseases from myocarditis to diabetes. Research suggests that a genomic RNA deletion near the 5′ terminus allows coxsackieviruses to maintain a low level of viral RNA replication, perhaps leading to persistent infection and chronic myocarditis. Additionally, the possible role of coxsackievirus in pancreatitis has been reported in children and in a mouse model. Recently, coxsackievirus has been linked to insulin-dependent type 1 diabetes, an autoimmune disease. Mouse models have indicated that coxsackievirus infection may trigger the onset of diabetes in prediabetic individuals who have not been previously exposed to coxsackievirus (Tracy and Gauntt, 2008).

Enterovirus 71

In the past few decades, large-scale epidemics of enterovirus 71 (EV71), another virus that can cause hand, foot, and mouth disease, have afflicted countries of Southeast Asia. EV71 infection can lead to severe neurological disease, including encephalitis and meningitis, especially in children. Phase I clinical trials have begun for several vaccine candidates; however, it is not yet clear if these are effective (Shang et al.,

Table 1 Picornavirus receptors and diseases

Genus	*Representative species*	*Host*	*Associated disease*	*Cellular receptor*[a]
Aphthovirus	Foot-and-mouth disease virus	Even-toed hoofed animals	Foot-and-mouth disease	Integrin
Cardiovirus	Encephalomyocarditis virus	Mammals, birds, invertebrates	Myocarditis, encephalitis	VCAM-1
	Theiler's murine encephalomyelitis virus	Mice	Neurological disease	Sialic acid
Enterovirus	Coxsackievirus B1-6	Humans	Hand, foot, and mouth disease	CAR
	Enterovirus 71	Humans	Hand, foot, and mouth disease	PSGL-1, SCARB2
	Poliovirus 1-3	Humans	Poliomyelitis	CD155 (PVR)
	Major group rhinovirus	Humans	Respiratory disease	ICAM-1
	Minor group rhinovirus	Humans	Respiratory disease	LDL
Hepatovirus	Hepatitis A virus	Humans, monkeys	Hepatitis	HAVcr-1
Kobuvirus	Aichi virus	Humans	Gastroenteritis	

[a]VCAM, vascular cell adhesion molecule; CAR, coxsackie and adenovirus receptor; PSGL, P-selectin glycoprotein ligand; SCARB, scavenger receptor class B member; PVR, poliovirus receptor; ICAM-1, intercellular adhesion molecule; LDL, low-density lipoprotein; HAVcr-1, hepatitis A virus cell receptor.
Reproduced from Lin, J.Y., Chen, T.C., Weng, K.F., Chang, S.C., Chen, L.L., Shih, S.R., 2009. Viral and host proteins involved in picornavirus life cycle. J. Biomed. Sci. 16, 103; Whitton, J.L., Cornell, C.T., Feuer, R., 2005. Host and virus determinants of picornavirus pathogenesis and tropism. Nat. Rev. Microbiol. 3, 765–776; Rossmann, M.G., He, Y., Kuhn, R.J., 2002. Picornavirus-receptor interactions. Trends Microbiol. 10, 324–331.

2013). The common outbreaks in Asia, easy spread of the virus, and potentially fatal neurological complications make it a significant and dangerous emerging pathogen.

Human Rhinovirus

Human rhinovirus (HRV) infection in humans is the most frequent cause of common cold infections, which is the number one cause of missed work in the United States. Since there is no vaccine and little to no protection across serotypes, each season brings renewed threat of infection. Additionally, recent studies show greater implications for rhinoviruses in the exacerbation of chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease, and cystic fibrosis (Kennedy et al., 2012).

Hepatitis and Gastroenteritis

Viruses are one of the most common causes of gastroenteritis. Given that many picornaviruses enter via the fecal-oral route, it is not surprising that new picornaviruses are being discovered associated with symptoms of gastroenteritis. One such virus, Aichi virus, was discovered in 1989 in Japan (Reuter et al., 2011). Hepatitis A virus (HAV), which causes symptoms similar to gastroenteritis in addition to cirrhosis of the liver, is the only common vaccine-preventable food-borne infection. As such, Hepatitis A is relatively rare in developed countries but still plays a role in food-borne outbreaks across the world.

Nonhuman Diseases of Picornaviruses

While most picornaviruses have a strict host tropism, foot-and-mouth disease virus (FMDV) and encephalomyocarditis virus (EMCV) infect a wide range of species. FMDV causes a devastating disease of the same name that affects many economically important livestock, especially cattle and pigs. Large-scale culling of infected animals and consequent restrictions on international trade during outbreaks have caused huge economic losses for affected countries. Use of the FMDV vaccine to prevent the spread of the disease is controversial, as vaccinated animals can become persistently infected if exposed to FMDV postvaccination, potentially acting as carriers of the virus. EMCV (the prototypic member of the cardiovirus genus; refer to Table 1) has the most diverse host range of picornaviruses, known to infect mammals, birds, invertebrates, and on rare occasions humans, but has the largest economic repercussions for livestock. When sows are infected, EMCV can cause reproductive problems and result in fatal encephalitis or myocarditis in infected piglets. Because of the uniquely wide host range of EMCV, rodents are thought to be major carriers of the virus, making rodent population control a major concern in animal husbandry. Another virus of the rodents, Theiler's murine encephalomyelitis virus (TMEV), may not have much direct impact on public health but is commonly used for the study of demyelination in a mouse model of multiple sclerosis. After establishing a persistent infection of the central nervous system, TMEV induces immune-mediated demyelinating disease and allows for study of the mechanisms of and treatment for this affliction (Roos, 2010). Until recently, cardioviruses were not thought to play major roles in human disease; however, during the past decade, Saffold virus was found in children displaying paralysis, as well as gastroenteritis and respiratory illness. The ongoing discovery of novel picornaviruses that impact global health further emphasizes the ubiquitous and diverse nature of this family of viruses.

Treatment and Control

Vaccines and Antiviral Drugs

Given that the above section is not an extensive review of the diverse diseases that can result from picornavirus infections, the importance of this family of viruses to human health and the economy is clear. Effective vaccines have been developed against PV, HAV, FMDV, and EMCV; however, no antiviral drugs exist to effectively control the infection and spread of picornaviruses. Traditional antivirals include capsid binding proteins, which block receptor binding or uncoating, as well as inhibitors of enzymatic functions of viral proteins which, for picornaviruses, include the RNA-dependent RNA polymerase 3D; the proteinases L, 2A, and 3C; and the ATPase 2C. Drugs

targeting nonstructural proteins have been administered clinically, but, to date, they have failed to produce significant results. These include enviroxime, which is thought to target either viral protein 3A or host proteins interacting with 3A, and rupintrivir, a peptide inhibitor of the 3C proteinase. Neither of these drugs made it past phase II clinical trials. While many other inhibitors to viral nonstructural proteins exist and are used in research settings, capsid inhibitors are the only antipicornavirus drugs currently in clinical trials. Pleconaril, which prevents HRV both from attaching to cells and from uncoating the viral RNA, completed phase II clinical trials in 2007, the results of which have not been released as of 2012. The Australian company Biota successfully completed phase IIb clinical trials in early 2012 with another capsid binding drug, vapendavir. If these compounds are approved for use, they could potentially offer the first treatment for HRV, and they may even act as a broad-spectrum antiviral. Another capsid binding compound, V-073, is in preclinical development for use in the PV eradication campaign (Thibaut et al., 2012). As of late 2013, despite many promising candidates, there are currently no FDA-approved drugs for treatment of any picornavirus. With the rise of high-throughput drug discovery, targeted drug design, and novel antiviral techniques, the development of antipicornaviral compounds is within reach.

Polio Global Eradication Initiative

Since 1988, the World Health Organization, in collaboration with the Center for Disease Control and Prevention, Rotary International, and the United Nations Children's Fund, among others, has been working to eradicate PV infections across the world. Like smallpox, the only human pathogen to be eradicated to date, PV has no natural reservoir and humans are the only natural hosts. That, and the availability of two highly effective vaccines, make PV a potential target for global eradication. As of 2012, the goal of global eradication seems all the more attainable and only three countries continue to have wild PV: Nigeria, Pakistan, and Afghanistan.

Critics of the eradication strategy argue that the current use of live, attenuated oral poliovirus vaccine (OPV) threatens the success of the program. These vaccines, while safe, have the potential to revert to wild-type, neurovirulent virus in vaccinated individuals and pose a threat of disease in unvaccinated individuals. Accordingly, any break in vaccination could pose a threat of a reestablishment of vaccine-derived disease in polio-free areas. One solution to this dilemma could be the prophylactic and therapeutic use of inexpensive antivirals to PV; however, these drugs have yet to be developed. The World Health Organization plans for posteradication maintenance include a complete switch to inactivated poliovirus vaccine (IPV) in countries continuing vaccination and/or a stockpile of monovalent OPV to protect against vaccine-derived PV in countries where vaccination is stopped. This switch will likely require the development of affordable IPV options and mass stockpiling of monovalent OPV, but could eventually lead to the eradication of PV (Heymann et al., 2005).

Replication Cycle Overview

At the most basic level, cell tropism of a virus is determined by the expression of a given receptor; however, in addition to their inability to infect cells lacking the corresponding cellular receptor, picornaviruses show specific tissue tropism and differences in virulence. Tissue tropism has been studied using poliovirus as a model for other picornaviruses and has been linked to the host immune response, genetics of the infecting virus, and to cellular factors including internal ribosome entry site (IRES) trans-acting factors (Whitton et al., 2005). After receptor binding, picornaviruses must use host and viral proteins to complete their replication cycle. Initially, the cellular translation machinery is usurped and modified to translate viral proteins (Figure 1). After sufficient translation,

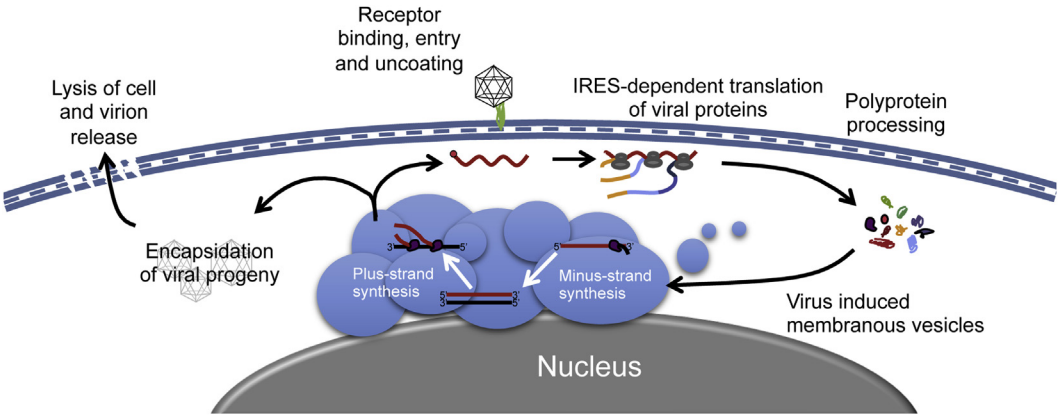

Figure 1 Overview of the picornavirus replication cycle. The virus attaches to the host cell via a cellular receptor. After entry and uncoating, viral RNA enters the cell cytoplasm as positive-sense single-stranded RNA, ready to be translated via cap-independent IRES translation. Once sufficient translation and polyprotein processing have occurred, virus-induced membranous vesicles begin to form in the nuclear periphery. Viral RNA replication takes place on the cytoplasmic face of these vesicles with the original positive-strand serving as a template for the synthesis of a negative-strand intermediate. From the negative-strand RNAs, multiple positive-strand RNAs are synthesized, and then used in further rounds of translation or encapsidation and release from the cell.

the virus co-opts membranes from various cellular compartments to form membranous replication complexes. RNA replication proceeds through multiple steps, with the incoming positive-strand RNA first serving as a template for an intermediate negative strand. Negative-strand RNAs can then be used to synthesize multiple positive-strand RNAs for use in further translation or packaging into progeny virions. An overview of the steps of the picornaviral replication cycle is discussed below, highlighting the interplay between the virus and the cellular environment required for efficient infection.

Capsid Structure and Viral Entry

Picornavirus virions are nonenveloped with an icosahedral capsid ~28 nm in diameter. For nearly all picornaviruses, the capsid is made up of 60 triangular subunits comprised of viral structural proteins VP1, VP2, VP3, and myristoylated VP4 (Figure 2). Prior to RNA encapsidation, empty-capsid precursors form from VP0, VP3, and VP1. VP4 is generated upon virion maturation by cleavage of VP0 into VP2 and VP4, generally believed to be an autocatalytic event upon RNA encapsidation that stabilizes the capsid structure. Triangular subunits of the wedge-shaped VP1, VP2, and VP3 make up the outer surface of the capsid. Each triangular subunit is associated with the smaller viral structural protein VP4, located on the internal side of the capsid and interacting with the viral RNA. The capsid is also the main target for antigenic sites, with VP1 being the most accessible, as five VP1 proteins form star-shaped mesas or peaks along the fivefold axis. Surrounding the mesas along the threefold axis are propeller-shaped protrusions made up of VP2 and VP3. For most enteroviruses, deep canyons form between the mesas and propellers and are the sites of receptor binding; however, for some picornaviruses these canyons are much less pronounced (e.g., some coxsackievirus strains, including CAV9) or absent (e.g., FMDV).

Infection begins with the binding of virus to the host cellular receptor, often a member of the immunoglobulin (Ig) superfamily (Table 1). For enteroviruses, VP1 contains a cavity accessible from the surface of the capsid. A fatty acid, dubbed the pocket factor, is associated with this cavity and may be involved in capsid stability. The amino-terminal domain of the immunoglobulin-like protein binds to the canyon of the viral capsid; the binding of the receptor displaces the pocket factor, likely destabilizing the capsid and initiating uncoating. For picornaviruses like rhinoviruses and aphthoviruses, uncoating is apparently not triggered by receptor binding but instead is initiated by the low pH of endosomes (Tuthill et al., 2010).

After receptor binding, capsids are either endocytosed or possibly rearranged at the plasma membrane, and genomic RNA is subsequently released into the cytoplasm with the loss of VP4, with the rest of the capsid remaining intact. In some models for PV RNA release, the viral RNA exits via the fivefold axis, through a channel that opens during the conformational change induced by receptor binding or pH changes. Recently, cryo-EM studies have revealed a different possible mechanism, where RNA exits from a site at the base of the canyon closer to the twofold axis (Bostina et al., 2011). In both models, channel opening results in the extrusion of myristoylated VP4, followed by the exposure of the *N*-terminus of VP1 and the release of genomic RNA.

Genome Structure and Genetic Diversity

The viral capsid surrounds a single molecule of positive-sense RNA of about 7500 nucleotides in length, which will act as both the messenger RNA for translation and as the template for initial rounds of RNA replication (Figure 3). Besides the structural capsid proteins, the only nonstructural protein to accompany the RNA is the viral protein, genome linked (VPg), which is covalently linked to the 5′ end of the RNA genome. Downstream of the VPg-RNA linkage is the 5′ noncoding region (NCR), which contains multiple secondary structures: (1) stem-loop I, a cloverleaf-like structure located at the 5′ end of the positive-strand, important in the initiation of RNA replication for enteroviruses and rhinoviruses; and (2) the IRES, important for viral translation via a cap-independent

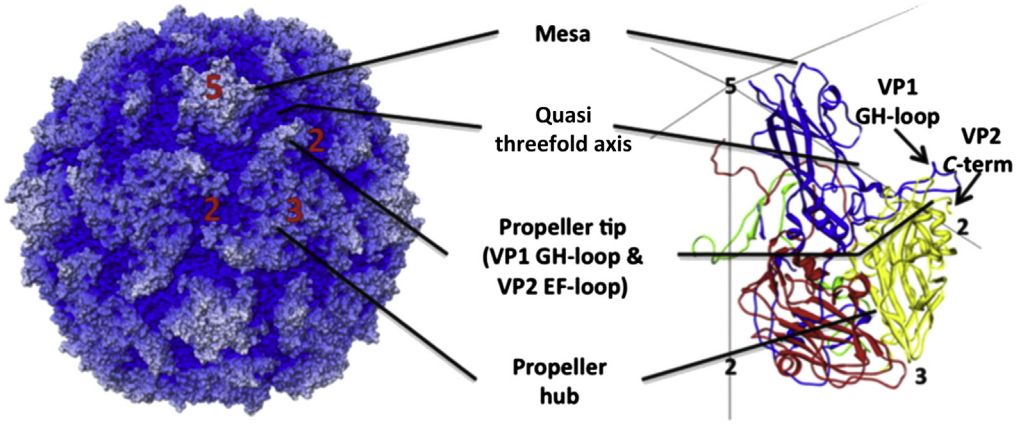

Figure 2 Structure of the poliovirus capsid. A depth-cued representation of the mature poliovirus particle, with light blue representing the star-shaped mesas and dark blue in the canyons. Red numbers indicate axes of symmetry (left panel). A ribbon diagram of a single triangular protomer, with VP1 (blue), VP2 (yellow), VP3 (red), and VP4 (green) overlaid on the axes of symmetry (gray) (right panel). Reproduced from Levy, H.C., Bostina, M., Filman, D.J., Hogle, J.M., 2010. Catching a virus in the act of RNA release: a novel poliovirus uncoating intermediate characterized by cryo-electron microscopy. J. Virol. 84, 4426–4441 with permission from the American Society for Microbiology.

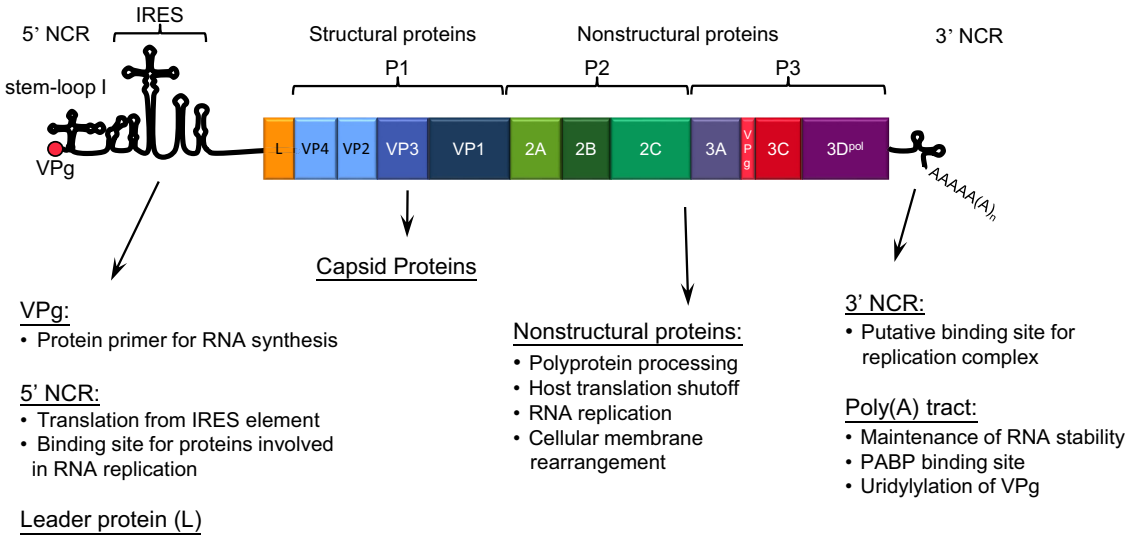

Figure 3 Functional map of a generalized picornavirus genome. The small viral protein VPg is attached at the 5′ end of replicating positive- and negative-sense viral RNA to serve as the protein primer for replication. For enteroviruses, the 5′ NCR includes the stem-loop I RNA element involved in RNA replication initiation. The 5′ NCR of all picornaviruses includes the highly structured IRES translation element, represented here as a type I IRES. Preceding the P1 region in cardio-, aphtho-, and kobuviruses is the L leader protein. The coding region consists of the P1 structural proteins, followed by the P2 and P3 nonstructural proteins. At the 3′ end of the genome is a 3′ NCR and a genetically encoded poly(A) tract. The 3′ NCR is represented here as two stem-loops (X and Y), but can contain one (rhinoviruses) to three (coxsackieviruses) stem-loop structures. Modified from Semler, B.L., Ertel, K.J., 2008. Picornaviridae: molecular biology. In: Mahy, B.W., Van Regenmortel, M. (Eds.), Encyclopedia of Virology, third ed. Elsevier, Boston, MA with permission from Elsevier.

mechanism. Picornaviruses are broadly classified by their 5′ NCRs, including the highly structured RNA of the IRES. As shown in **Figure 3**, following the 5′ NCR is a single open reading frame encoding a large polyprotein that is cleaved during infection by the viral-encoded proteinases. For some genera (cardiovirus, aphthovirus, and kobuvirus), the polyprotein is preceded by a leader protein (L), which is proteolytically active only in aphthoviruses. The P1 region of the polyprotein encodes the viral capsid proteins. Nonstructural proteins of the P2 and P3 regions are responsible for membrane rearrangement of the host cell, proteolytic cleavage of both host and viral proteins, and viral RNA replication. Within the positive-strand of the RNA is a structured hairpin termed the *cis*-acting replication element (*cre*), used during replication to prime uridylylation of the protein primer VPg (see RNA Replication section, below). The location of *cre* varies between genera and can be found in the 2C coding region (PV and coxsackievirus), the VP2 coding region (cardioviruses), or the 5′ NCR (aphthoviruses). Near the 3′ terminus of positive-strand RNA is the 3′ NCR, which in PV is made up of two predicted RNA secondary structures, termed stem-loops X and Y, followed by a genetically encoded poly(A) tract. The precise role of the 3′ NCR in PV replication is not clear, but it is involved in positive-strand RNA synthesis. The 3′ poly(A) tract is required for infectivity and is the putative binding site for the replication complex on positive-strand RNA for negative-strand RNA synthesis (Ogram and Flanegan, 2011).

Like all RNA viruses, picornaviruses exist as a mixture of different genotypes termed quasi-species. The lack of proofreading by the low fidelity RNA-dependent RNA polymerase (3D) contributes to the high error rate of replication (upward of 10^{-4}), which correlates to roughly one substitution per genome. This genetic plasticity allows RNA virus populations to quickly adapt to new environments; however, there is a fine line between tolerable genetic diversity and 'error catastrophe.' Enterovirus mutants with altered polymerase fidelity are attenuated *in vivo*, with high error rates leading to viral extinction and low error rates leaving the virus unable to adapt during environmental bottlenecks (Vignuzzi et al., 2006; Pfeiffer and Kirkegaard, 2005). Recombination between genomes also contributes to the genetic diversity of viral populations, with polymerase (3D) switching between different positive-strand RNA templates during negative-strand synthesis (Lukashev, 2010).

Translation and Polyprotein Processing

Unlike cellular mRNAs, picornavirus genomes lack a 7 methyl guanosine cap at the 5′ terminus, and the highly structured 5′ end of the genome would prevent ribosome scanning. Instead, the viral genome is translated by a cap-independent mechanism driven by an internal ribosome entry site located in the 5′ NCR of the viral mRNA (Pelletier and Sonenberg, 1988; Jang et al., 1988). During the initial stages of infection, modification of host proteins, including the cleavage of eIF4G (enteroviruses and FMDV) and dephosphorylation of 4E-BP1 (EMCV), effectively shuts down cap-dependent translation (**Table 2**); as such, picornavirus translation requires a unique set of IRES transactivating factors (ITAFs) to recruit ribosomes in a cap-independent manner. The RNA structural elements determine the ITAF requirements for successful translation and are grouped as

Table 2 Alteration of host cell proteins and functions

Cellular proteins	*Picornavirus proteins*	*Potential function*
GBF1, BIG1/2	PV 3A, PV 3CD	Recruits Arf-1 to membranes
ACBD3, PI4KIIIβ	Aichi 3A, CVB 3A, HRV 3A, PV 3A	May be involved in formation of membranous vesicles
eIF4G	CVB $2A^{pro}$, FMDV L^{pro}, HRV $2A^{pro}$, PV $2A^{pro}$	Cleavage shuts off cap-dependent translation
PABP	CVB $2A^{pro}$, PV $2A^{pro}$, PV 3C	Cleavage contributes to inhibition of host cell translation
4E-BP1	EMCV, PV (unknown viral protein)	Dephosphorylation inhibits host cell translation
Histone H3	FMDV 3C	Inhibits host cell transcription
TBP, CREB, Oct-1, TFIIIC	PV 3C	Inhibits host cell transcription
RIG-1	EMCV 3C, HRV 3C, PV 3C	Cleavage inhibits cellular immune response
NF-κB	FMDV L^{pro}, PV 3C	Cleavage disrupts IFN signaling
IPS-1	HAV 3ABC, HRV 3C, HRV $2A^{pro}$	Cleavage disrupts IFN signaling pathway
IRF-3	TMEV L	Inhibition of IRF-3 dimerization blocks IFN-β transcription
Gemin3	PV $2A^{pro}$	Cleavage inhibits cellular splicing complex assembly
Nucleoporins	EMCV L, HRV $2A^{pro}$, PV $2A^{pro}$	Cleavage (2A) or phosphorylation (L) disrupts nucleo-cytoplasmic transport
Dystrophin	CVB $2A^{pro}$	Cleavage disrupts cytoskeleton
G3BP	PV 3C	Disrupts cellular stress-granule formation

GBF1, Golgi-specific brefeldin A resistance factor 1; BIG, Brefeldin A-inhibited guanine nucleotide exchange factor; Arf-1, ADP-ribosylation factor 1; ACBD3, Acyl coenzyme A binding domain protein 3; PI4KIIIβ, Phosphatidylinositol 4-kinase-β; CVB, Coxsackievirus B; PABP, Poly(A)-binding protein; TBP, TATA-box binding protein; CREB, Cyclic AMP-responsive element binding protein; Oct-1, Octamer-binding protein-1; RIG-I, Retinoic acid inducible gene I; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; IPS-1, Interferon-β promoter stimulator 1; IFN, Interferon; IRF-3, Interferon regulatory factor 3; G3BP, Ras-GAP SH3 domain-binding protein.
Reproduced from Chase, A.J., Semler, B.L., 2012. Viral subversion of host functions for picornavirus translation and RNA replication. Future Virol. 7, 179–191; Lin, J.Y., Chen, T.C., Weng, K.F., Chang, S.C., Chen, L.L., Shih, S.R., 2009. Viral and host proteins involved in picornavirus life cycle. J. Biomed. Sci. 16, 103; Castello, A., Alvarez, E., Carrasco, L., 2011. The multifaceted poliovirus 2A protease: regulation of gene expression by picornavirus proteases. J. Biomed. Biotechnol. 2011, 369648; Racaniello, V.R., 2010. Innate immune responses. In: Ehrenfeld, E., Domingo, E., Roos, R.P. (Eds.), The Picornaviruses. ASM Press, Washington, D.C.; Greninger, A.L., Knudsen, G.M., Betegon, M., Burlingame, A.L., Derisi, J.L., 2012. The 3A protein from multiple picornaviruses utilizes the golgi adaptor protein ACBD3 to recruit PI4KIIIβ. J. Virol. 86, 3605–3616.

type I IRES (enteroviruses), type II IRES (cardioviruses and aphthoviruses), and the unique hepatitis A IRES, as well as the recently described IRES of kobuviruses (**Figure 4**) (Yu et al., 2011). Specific RNA elements in the IRES are known to interact with both canonical (e.g., eIF2, eIF3) and non-canonical (e.g., La, poly(rC) binding protein 2 (PCBP2), polypyrimidine tract binding protein (PTB)) cellular ITAFs to recruit initiation complexes for translation beginning at an internally located AUG start codon. Each IRES has a unique set of factors necessary for efficient translation. For example, while type II IRES-driven translation is efficient in rabbit reticulocyte lysates (an extract from cells lacking nuclear factors), translation of PV in rabbit reticulocyte lysates must be supplemented by the addition of a HeLa cell extract or an La autoantigen. In uninfected cells, La is involved in transcription and is localized to the nucleus. During enterovirus infection, La is cleaved by the viral proteinase 3C, and the *C*-terminal product of La is relocalized to the cytoplasm where it stimulates IRES translation. Additionally, stem-loop IV of the type I IRES is essential for translation through its interaction with the cellular mRNA stability protein, PCBP2. Normally involved in alternative splicing of pre-mRNAs, PTB also acts to enhance IRES-driven translation and can interact with both type I and type II IRES elements. Ribosome recruitment to the viral RNA may occur directly, with ITAFs perhaps acting to stabilize RNA structures necessary for ribosome binding, or via the interaction between ITAFs bound to the viral RNA and ribosomal components (for proteins that bind the IRES, refer to **Figure 4**) (Fitzgerald and Semler, 2009; Belsham, 2009).

Translation of the viral mRNA occurs through one open reading frame, resulting in a single polyprotein approximately 250 kDa in size. This polyprotein is proteolytically processed by proteinases encoded by the virus, including L (aphthoviruses), 2A (enteroviruses), and 3C(D) (all picornaviruses). Polyprotein P1, the region containing all capsid proteins, is released either by the autocatalytic activity of 2A in enterovirus-infected cells or by 3C for other picornaviruses. Protein 3C, or precursors thereof, is the main proteinase responsible for processing of the polyprotein, including the release of nonstructural proteins encoded by the P2 and P3 regions. The P2 proteins, including 2BC and 2C, are involved in the induction of membranous vesicles, which are the sites of viral RNA replication. Proteins of the P3 region are directly involved in RNA replication, including 3AB which stimulates polymerase activity, and 3D the RNA-dependent RNA polymerase (for known functions of viral proteins, refer to **Figure 5**) (Palmenberg, 1990).

Positive-strand RNAs serve as templates for both translation and negative-strand RNA synthesis; as such, there is thought to be a molecular switch during infection to clear the RNA of translating ribosomes and allow the polymerase to read through the RNA. The cleavage of several host proteins by the 3C(D) proteinase is proposed to mediate this switch. An example of one such event is the cleavage of PCBP2 during PV infection, which occurs with kinetics similar to that of increasing replication. Full-length PCBP2 is active in viral translation via its interaction with stem-loop IV, while cleaved PCBP2 can no longer interact with the IRES but can participate in ternary complex formation with stem-loop I RNA (see RNA Replication section, below). Another player in translation, PTB, is cleaved by the PV 3C proteinase during infection. The cleavage products are thought to effectively inhibit IRES translation by binding to the viral RNA in place of full-length PTB. It is likely that multiple events play a role in mediating the switch from translation to RNA replication, including

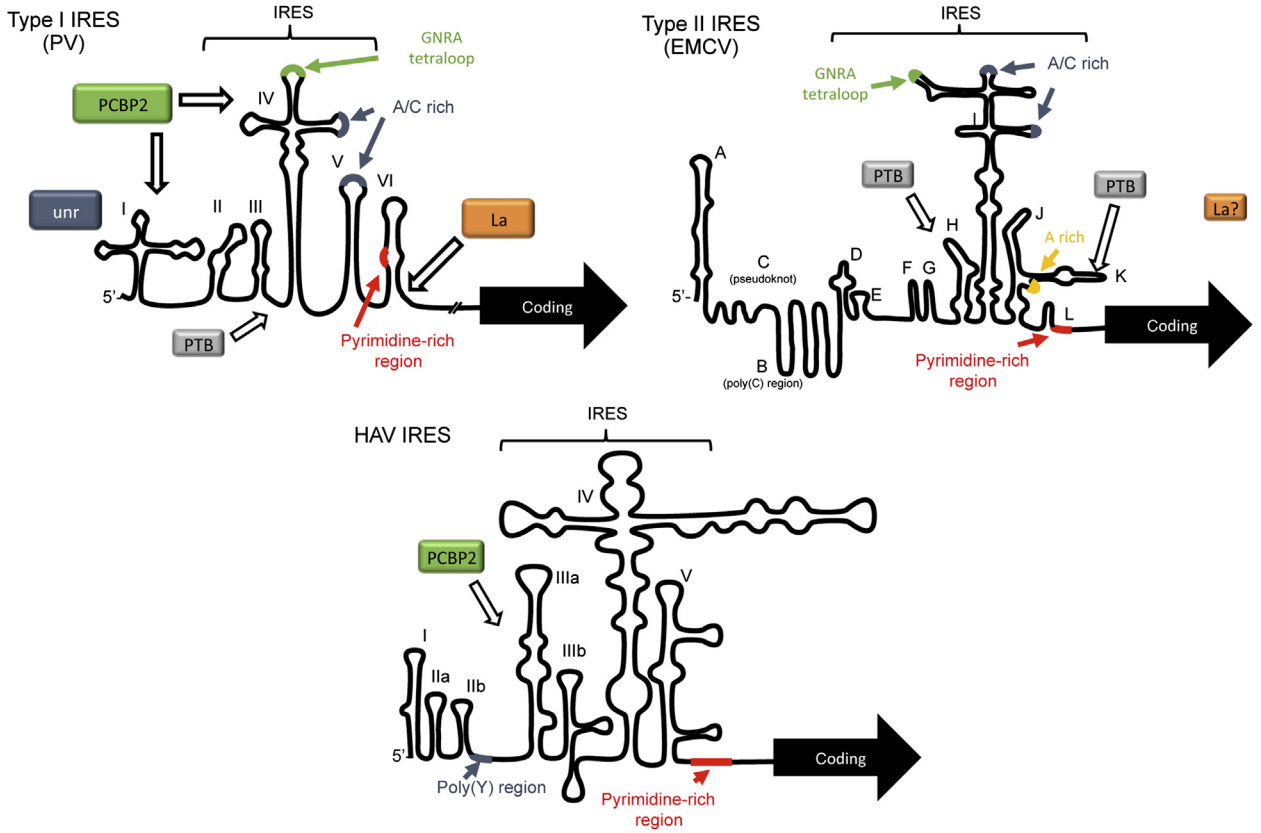

Figure 4 RNA secondary structures of picornavirus IRES elements. Picornavirus genera are broadly classified by their IRES structures, which are grouped into type I (e.g., poliovirus), type II (e.g., EMCV), and hepatitis A virus IRES. Representative structures for type I (top left), type II (top right), and HAV (bottom) IRES elements are depicted with some of the proteins known to bind to the respective IRES. Type I IRES elements are contained within stem-loops II–VI, type II IRES elements are located in stem-loops D-L, and the HAV IRES consists of stem-loops IIIa–V. Both canonical (eIF1A, eIF2-GTP-met, eIF3) and noncanonical (PCBP2, La, unr, PTB) proteins bind to the IRES during ribosome recruitment and assembly, allowing for translation of the viral RNA. Some cellular proteins shown to bind with each IRES are shown with arrows directed at their putative binding sites. Conserved RNA structural elements are depicted, including the GNRA tetraloop (green), A/C-rich regions (gray), and pyrimidine-rich regions (red). Illustration derived from Semler, B.L., Ertel, K.J., 2008. Picornaviridae: molecular biology. In: Mahy, B.W., Van Regenmortel, M. (Eds.), Encyclopedia of Virology, third ed. Elsevier, Boston, MA with permission from Elsevier.

cleavage of ITAFs, accumulation of replication proteins, and spatial segregation of RNA templates via modification of the cellular environment (Daijogo and Semler, 2011).

Membranous Vesicle Formation

Once the initial viral proteins are synthesized and sufficient polyprotein processing has taken place, virus-induced membranous vesicles begin to form from the endoplasmic reticulum (ER) and the Golgi apparatus as early as 2-h post-infection. These vesicles become the sites where RNA replication occurs and are thought to act as scaffolds for RNA synthesis, in the protection of nascent viral RNA from antiviral responses, or in sequestration of proteins required for RNA replication. Much debate exists over the source of replication complex membranes, as they have been reported to contain markers from the ER, the Golgi, the secretory pathway, and autophagic membranes. Recent electron tomography studies during PV or FMDV infection have suggested that initially, during the exponential phase of viral replication, membranes are predominantly single walled (Belov et al., 2012; Limpens et al., 2011). These data also suggest that double membranes are only formed later in infection, by the enveloping of multiple single membranes, and are not formed *de novo* as is seen in autophagy. For most picornaviruses, including PV and FMDV, translation is thought to occur on ER-associated polyribosomes, with replication complexes forming adjacent to the ER, potentially incorporating modified ER membranes, and relocalizing to a perinuclear region by peak times of replication.

It is still unknown exactly how these single and double membranes form or the origin of their components; however, it is clear that viral proteins are responsible for the alteration of the cellular membrane environment. Most of what is known about membrane rearrangement has been studied in cells infected by enteroviruses, namely PV. Ectopic expression of PV 2BC alone can cause membrane rearrangements similar to those seen during infection. While little is known about the function and structure of 2C, this protein binds to ER-associated protein Reticulon 3 and can alter membranes when expressed alone. Enterovirus protein 2B, which contains

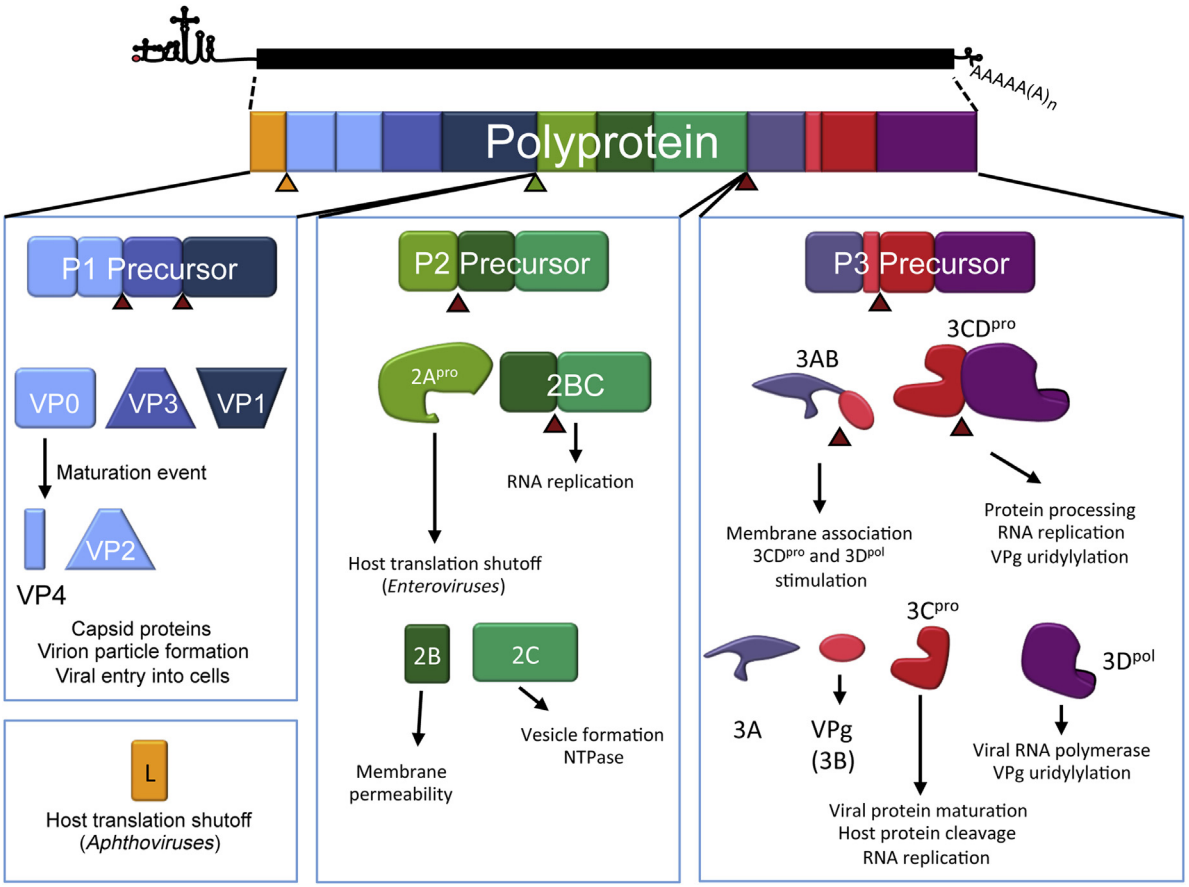

Figure 5 Polyprotein processing cascade and functions of viral proteins. Picornavirus vRNA (in black) is translated into a single polyprotein that is proteolytically processed into the Leader protein (orange), P1 (blue), P2 (green), and P3 (red/violet). Cleavage events by the picornavirus proteinases are indicated as triangles below each cleavage site (orange, L; green, 2A; red, 3C(D)). For cardioviruses, the L protein is not a proteinase and is released from P1 by 3C proteinase activity. Known functions for both precursor and fully processed proteins are described. Modified from Semler, B.L., Ertel, K.J., 2008. Picornaviridae: molecular biology. In: Mahy, B.W., Van Regenmortel, M. (Eds.), Encyclopedia of Virology, third ed. Elsevier, Boston, MA with permission from Elsevier.

ionopore motifs, modifies the permeability of ER membranes and alters calcium ion levels in both the ER and the Golgi. Components of COPII secretory vesicles, responsible for ER to Golgi anterograde transport, colocalize with 2B, suggesting a role for COPII secretory vesicles in viral replication. Additionally, viral proteins 3A and 3CD of PV and coxsackievirus may recruit the guanine–nucleotide exchange factors GBF1, BIG1/2, and consequently the GTPase Arf, all normally responsible for formation of secretory vesicles (Table 2). Enteroviral 3A protein contains a hydrophobic domain and blocks ER to Golgi traffic when expressed alone, potentially contributing to the build up of secretory proteins at the ER (van Kuppeveld et al., 2010). While the complete mechanism of membrane rearrangement for picornaviruses is not yet defined, it is clear that viral proteins containing 2B, 2C, and 3A sequences are largely responsible for transforming the cellular environment to the advantage of the virus.

RNA Replication

Viral replication complexes form on the cytoplasmic face of virus-induced membranous vesicles. Whether this is concurrent with or separate from translation is not clear; however, it is known that positive-strand RNA templates for replication must first be translated during infection. This may contribute to the anchoring of viral RNAs and replication complexes to the membrane, possibly directed through multiple protein–RNA interactions required in *cis*. For example, proteins 2B, 2C, and 3A (or precursors thereof) all have both membrane-binding and RNA-binding activity. Additionally, the RNA polymerase 3D is known to interact with membrane-associated 3AB, which is sufficient to recruit 3D to membranes.

Once replication complexes form, the incoming positive-strand RNA serves as a template for negative-strand RNA synthesis by the viral-encoded RNA-dependent RNA polymerase 3D. A negative-strand RNA intermediate is synthesized to serve as a template for more positive-strand RNAs, which are then used for further rounds of translation, RNA replication, and ultimately packaging into progeny virions (Figure 1). VPg is cleaved from the viral mRNA before translation can occur; however, during RNA replication, two uridylate residues are added to this protein, or a precursor thereof, by the RNA polymerase 3D. The uridylylated VPg then serves as a protein primer for initiation of both negative- and positive-strand RNA

synthesis. For priming of negative-strand RNA synthesis, the 3′ poly(A) tract is thought to be the template for uridylylation, while the *cis*-acting replication element (*cre*) acts as the template for priming positive-strand RNA synthesis. The source of VPg is still unclear, as separate complementation studies have indicated that both 3AB and 3BC, but not 3B (VPg) alone, may rescue mutations to VPg. Polypeptide 3CD is the precursor to the viral-encoded RNA polymerase 3D and the proteinase 3C. Although 3CD lacks polymerase activity, it does play an integral role in viral RNA replication both via the stimulation of VPg uridylylation by 3D and, in enteroviruses, by the formation of a ternary complex at the 5′ end of the viral RNA (Steil and Barton, 2009).

For enteroviruses and rhinoviruses, negative-strand RNA synthesis is thought to initiate following the formation of a ternary complex with the 5′ stem-loop I of viral RNA, the 3CD proteinase, and cellular protein PCBP2. Since negative-strand RNA synthesis is initiated at the 3′ poly(A) tract of the genome, this ternary complex may act to circularize the genome and could contribute to the specificity of 3D-RNA recognition. This long-range interaction may also be facilitated by the cellular protein poly(A) binding protein (PABP) via its interaction with the 3′ poly(A) tract and with the ternary complex; however, functional data for this interaction on full-length RNAs are lacking. Another possibility for circularization is the interaction of viral protein 3AB with both the 3′ NCR and 3CD or 3D, since 3AB is known to interact with and stimulate the enzymatic activities of both of these proteins.

Negative-strand RNA has not been observed in a single stranded form. Instead, the product of negative strand synthesis is a duplex of negative- and positive-sense RNA termed the replicative form. During positive-strand RNA synthesis, the negative strand exists as the replicative intermediate, a partially double-stranded complex with multiple elongating positive strands, creating asymmetric replication with positive-strand RNA exceeding negative-strand RNA as high as 70:1. As noted above, for uridylylation of VPg to prime RNA synthesis, 3CD binds to the *cre* of the viral RNA to then interact with 3D and stimulate the uridylylation of VPg or its precursors. Currently, the only cellular protein known to play a role in positive-strand RNA synthesis is heterogeneous ribonuclear protein C (hnRNP C), which can interact with both the 5′ and 3′ ends of the negative-strand RNA of PV, possibly circularizing the replication template by oligomerization of hnRNP C. The 3′ NCR of enteroviruses can be deleted; however, this results in a defect of positive-strand RNA synthesis. This could be due to the loss of interaction of the 3′ NCR with cellular proteins, including hnRNP C or nucleolin, or viral proteins, such as 3AB and 3CD. Numerous attempts to isolate RNA replication complexes have been made, but unfortunately the precise composition of the complex is unknown, including the cleavage state of viral proteins when entering the complex and the exact cadre or temporal roles of host proteins involved (Paul et al., 2009).

Encapsidation and Release

The process of picornavirus packaging is not well described, including the signal conferring specificity for RNA packaging. It is known that replication is necessary for efficient encapsidation. This requirement may account for the viral RNA specificity observed during packaging of picornaviruses. Virion precursors localize to sites of viral RNA replication, and viral nonstructural proteins participate in the encapsidation process. Replication-competent viral replicons, including those containing sequence deletions or substitutions, can be effectively packaged, suggesting a mechanism for encapsidation specificity other than genome length. Inhibition of PV 2C by the drug hydantoin blocks a step after positive-strand RNA synthesis, implicating 2C in the encapsidation process. Additionally, PV 2C interacts with the capsid protein VP3. It is hypothesized that 2C may act in the release of viral RNA from replication complexes, allowing for the encapsidation of newly replicated progeny RNAs (Liu et al., 2010). Proper VPg linkage has also been hypothesized to serve as an additional signal for encapsidation. Regardless of the mechanism, VPg-linked positive-strand RNA is packaged into provirons containing VP0, VP1, and VP3, followed by cleavage of VP0 to form mature virions (*see* Capsid Structure and Viral Entry section, above). Cell egress is poorly understood but may result, in part, from the cytopathic effects of viral infection. Recently, an alternative model for prelytic release of mature virions via the autophagic pathway has been proposed, although this mechanism is also not well understood (Richards and Jackson, 2012).

Conclusions

The picornaviruses have been a plague on public health for centuries, including the PV epidemics of the 1940s and 1950s, the devastation of livestock by FMDV, and the current threat of EV71 infections in children throughout Asia (**Figure 6**). The discovery of new risks of common picornaviruses, including the exacerbation of asthma by HRV and the potential link between coxsackievirus and type 1 diabetes, demonstrates the broad health implications for this diverse group of viruses. As with many viruses, treatment for picornaviruses is limited. In addition, some existing vaccines are controversial; for example, the FMDV vaccine is serotype-specific, and at best offers only a short-lived protection. The continuing threat to public health posed by picornaviruses emphasizes the need for ongoing research into both the molecular biology and the treatment of these prolific viruses.

Research on picornaviruses has provided key insights into the biology of RNA viruses, host antiviral responses, and noncanonical translation (**Figure 6**); however, gaps in knowledge still exist. Despite decades of research, basic biological questions of these complex pathogens remain. It is still unclear exactly how ribosomes are recruited during viral translation; how the composition of the replication complexes changes during negative- or positive-strand RNA synthesis; how templates are distinguished for translation, replication, and packaging; and if the host factors required for these processes contribute to the differences in tissue tropism among picornaviruses. Further understanding of these complex questions will require an in-depth study of the interplay between viral and cellular factors.

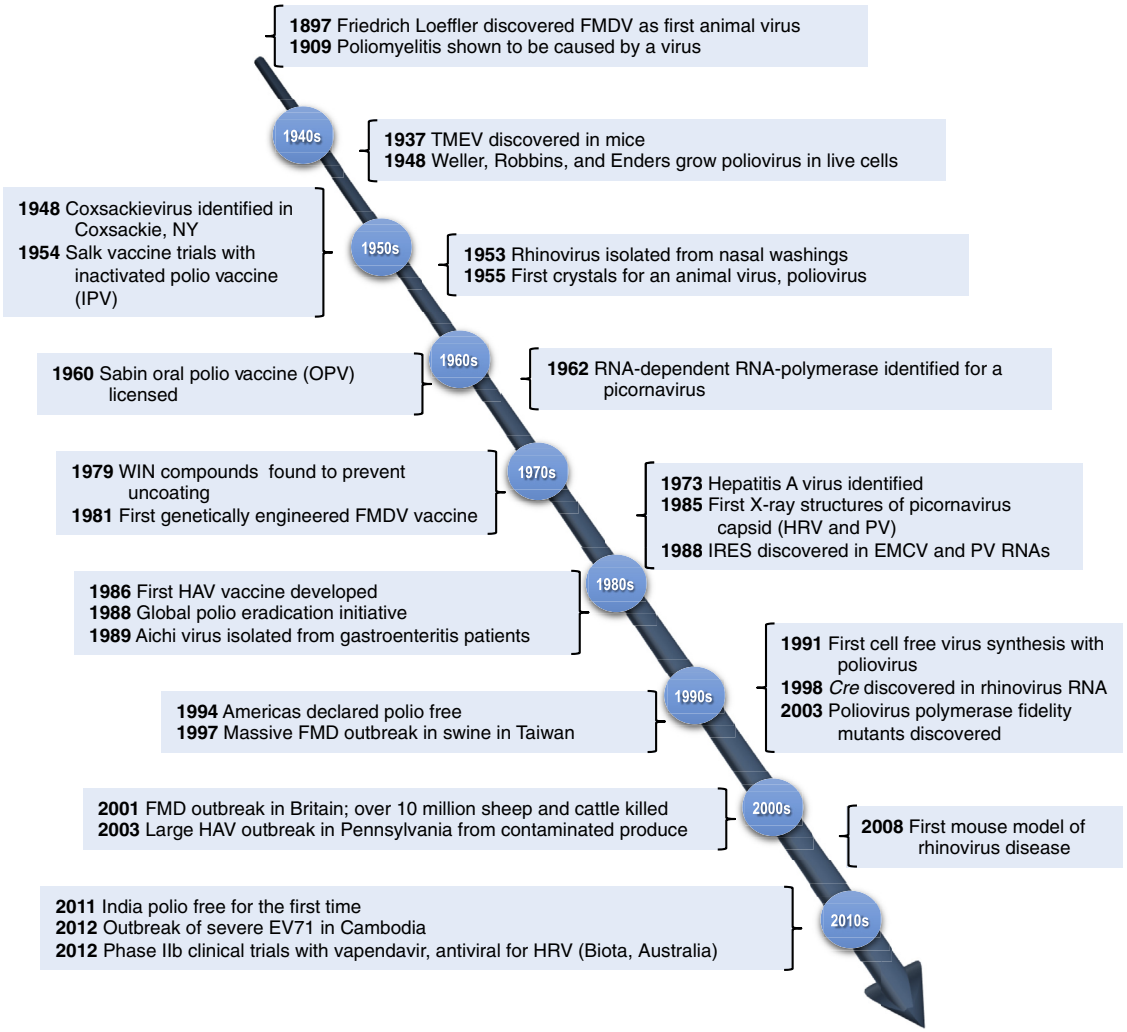

Figure 6 Historical timeline of picornaviruses. Significant events in the history of picornaviruses are shown, including notable scientific findings (right of the timeline) and public health events (left of the timeline). Research discoveries providing insight into noncanonical translation (discovery of the IRES), replication (RNA-dependent RNA polymerase identification), and viral structure are noted. Key events illustrating the role of poliovirus as an important model system for the study of other viruses are noted, including the propagation of poliovirus in live cells, the first atomic structure of an animal virus, and the first infectious cDNA of an animal virus. To the left, public health events are shown, including the identification of novel picornaviruses, events in vaccine and antiviral development, examples of large-scale picornavirus outbreaks, and efforts in the eradication of poliomyelitis. FMDV, food-and-mouth disease virus; TMEV, Theiler's murine encephalomyelitis virus; HRV, human rhinovirus; PV, poliovirus; EMCV, encephalomyocarditis virus; *cre*, *cis*-acting replication element; HAV, hepatitis A virus; EV71, enterovirus 71. Reproduced from Kleid, D.G., Yansura, D., Small, B., Dowbenko, D., Moore, D.M., Grubman, M.J., Mckercher, P.D., Morgan, D.O., Robertson, B.H., Bachrach, H.L., 1981. Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. Science 214, 1125–1129; Pelletier, J., Sonenberg, N., 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334, 320–325; Jang, S.K., Krausslich, H.G., Nicklin, M.J., Duke, G.M., Palmenberg, A.C., Wimmer, E., 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. J. Virol. 62, 2636–2643; Feinstone, S.M., Kapikian, A.Z., Purceli, R.H., 1973. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. Science 182, 1026–1028; Binn, L.N., Bancroft, W.H., Lemon, S.M., Marchwicki, R.H., Leduc, J.W., Trahan, C.J., Staley, E.C., Keenan, C.M., 1986. Preparation of a prototype inactivated hepatitis A virus vaccine from infected cell cultures. J. Infect. Dis. 153, 749–756; Andrewes, C.H., Chaproniere, D.M., Gompels, A.E., Pereira, H.G., Roden, A.T., 1953. Propagation of common-cold virus in tissue cultures. Lancet 265, 546–547; Bartlett, N.W., Walton, R.P., Edwards, M.R., Aniscenko, J., Caramori, G., Zhu, J., Glanville, N., Choy, K.J., Jourdan, P., Burnet, J., Tuthill, T.J., Pedrick, M.S., Hurle, M.J., Plumpton, C., Sharp, N.A., Bussell, J.N., Swallow, D.M., Schwarze, J., Guy, B., Almond, J.W., Jeffery, P.K., Lloyd, C.M., Papi, A., Killington, R.A., Rowlands, D.J., Blair, E.D., Clarke, N.J., Johnston, S.L., 2008. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat. Med. 14, 199–204; Schaffer, F.L., Schwerdt, C.E., 1955. Crystallization of purified MEF-1 poliomyelitis virus particles. Proc. Natl. Acad. Sci. USA 41, 1020–1023; Molla, A., Paul, A.V., Wimmer, E., 1991. Cell-free, de novo synthesis of poliovirus. Science 254, 1647–1651.

See also: Viral Pathogenesis; Viral Vaccines.

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