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Picornavirus IRES elements: RNA structure and host protein interactions

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ABSTRACT

Internal ribosome entry site (IRES) elements were discovered in picornaviruses. These elements are *cis*-acting RNA sequences that adopt diverse three-dimensional structures and recruit the translation machinery using a 5′ end-independent mechanism assisted by a subset of translation initiation factors and various RNA binding proteins termed IRES transacting factors (ITAFs). Many of these factors suffer important modifications during infection including cleavage by picornavirus proteases, changes in the phosphorylation level and/or redistribution of the protein from the nuclear to the cytoplasm compartment. Picornavirus IRES are amongst the most potent elements described so far. However, given their large diversity and complexity, the mechanistic basis of its mode of action is not yet fully understood. This review is focused to describe recent advances on the studies of RNA structure and RNA–protein interactions modulating picornavirus IRES activity.

1. Picornavirus genome organization

Picornaviruses are non-enveloped positive strand RNA viruses with an icosahedral capsid, which cause important diseases in humans and animals, such as common-cold illnesses, polio, or chronic livestock infections. Picornaviruses are currently classified into 26 genera (Table 1), and unassigned species continue to be described (http://www.picornaviridae.com/). It is widely accepted that the entire life cycle of all picornaviruses occurs in the cytoplasm of the infected cell. Their genome consists of a single-stranded RNA that harbors a single open reading frame (ORF) region flanked by a long 5′UTR and a short poly-(A) tail at the 3′ end. The genome size ranges between about 7100 nts in Tremovirus and 9200 nts in Erbovirus, not taking into consideration the poly(C) tracts (which vary from 50 nts in Cardiovirus to more than 500 nts in some Aphthovirus isolates), and the poly(A) tail (about 50 nts).

Viral proteins are encoded in a long ORF, translated into a single polyprotein (~2300 amino acids), which is co- and post-translational processed. Picornavirus polyproteins share a common general organization (Fig. 1). The P1 region comprises the capsid proteins while the P2 and P3 regions comprise the replication proteins. In a few cases, the leader (L) protein precedes the P1 region. Only in the genome of Dicipivirus (a dicistronic virus isolated from dogs), an intergenic region (IGR) separates two ORFs (Woo et al., 2012).

The polyprotein of all picornaviruses contain embedded proteinases that catalyze cleavages in *cis* and in *trans* in a processing cascade (Hanecak et al., 1982; Parks et al., 1989; Skern et al., 1991; Toyoda et al., 1986). Primary cleavages of the polyprotein are mediated by the 3C proteinase ($3C^{pro}$), a serine-proteinase with a cysteine active site (Seipelt et al., 1999). Enterovirus $3C^{pro}$ cleaves between Q and G pairs while aphthovirus $3C^{pro}$ accepts E or Q at the first position (Sweeney et al., 2007). In all picornaviruses, $3C^{pro}$ is responsible for specific secondary cleavages of the capsid and replication protein precursors.

The 2A protein, located at the junction between the capsid and replication proteins, differs among picornaviruses. In the case of the entero- and sapeloviruses, 2A is a serine proteinase with a HDC catalytic triad (Baxter et al., 2006) that mediates a primary cleavage at the junction of the capsid protein precursor and the replicative domains of the polyprotein. In other genera, 2A is either an oligopeptide sequence mediating a translational recoding event (Table 1, aphtho-, erbo-, tescho-, cosa-, and senecaviruses) or a longer protein in which this activity resides in their C-terminal region (cardio-, parecho- and avihepatovirus). Insertion of the aphthovirus 2A oligopeptide into a polyprotein was sufficient to detect the individual polypeptides, although the upstream 2A product was in molar excess relative to the downstream product

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Table 1
Picornavirus genus, proteases encoded and type of IRES.

Genus	Species	Proteases	IRES type
Enterovirus	Human enterovirus A (HEV), enterovirus 71 (EV71)	2A, 3C	I
	Coxsackievirus B (CVB) (human enterovirus B)	2A, 3C	I
	Poliovirus (PV) (human enterovirus C)	2A, 3C, 3CD	I
	Bovine enterovirus (BEV)	2A, 3C	I
	Human rhinovirus (HRV)	2A, 3C, 3CD	I
Cardiovirus	Encephalomyocarditis virus (EMCV)	3C, (2A CHISEL)	II
	Theilovirus (TMEV)	3C, (2A CHISEL)	II
Aphthovirus	Foot-and-mouth disease virus (FMDV)	L, 3C, (2A CHISEL)	II
	Equine rhinitis A virus (ERAV)	L, 3C, (2A CHISEL)	II
	Bovine rhinitis B virus (BRBV)	L, 3C, (2A CHISEL)	II
Erbovirus	Equine rhinitis B virus (ERBV)	L, 3C, (2A CHISEL)	II
Hepatovirus	Hepatitis A virus (HAV)	3C	III
Parechovirus	Human parechovirus (HPeV)	3C, (2A CHISEL)	II
Kobuvirus	Aichi virus (AiV)	3C	II
Teschovirus	Porcine teschovirus (PTV-1)	3C, (2A CHISEL)	HCV-like B
Sapelovirus	Porcine sapelovirus (PSV, PEV-8), simian sapelovirus (SPV9), simian virus (SV2)	2A, 3C	HCV-like B
Senecavirus	Seneca Valley virus (SVV)	3C, (2A CHISEL)	HCV-like A
Tremovirus	Avian encephalomyelitis virus (AEV)	3C	HCV-like A
Avihepatovirus	Duck hepatitis A virus (DHAV)	3C, (2A CHISEL)	HCV-like
Megrivirus	Duck megrivirus	2A1-2A2-2A3, 3C	HCV-like B
Dicipivirus	Cadicivirus A	3C, 2A	IRES, IGR
Aquamavirus	Aquamavirus A	3C	?
Avisivirus	Avisivirus A	3C	II
Cosavirus	Cosavirus A	3C, (2A CHISEL)	II
Gallivirus	Gallivirus A	3C	II?
Hunnivirus	Hunnivirus A	3C	II
Mischivirus	Mischivirus A	3C	?
Mosavirus	Mosavirus A	3C	?
Oscivirus	Oscivirus A	2A, 3C	HCV-like B
Pasivirus	Pasivirus A	3C	?
Passerivirus	Passerivirus A	3C	?
Rosavirus	Rosavirus A	3C	?
Salivirus	Salivivirus A	2A?, 3C	HCV-like B

? represents unknown.

(Donnelly et al., 2001). These results lead to the proposal that 2A was a translational recoding element (CHYSEL for *cis*-acting *hy*drolytic *e*lement, also referred to as stop/go translation).

Aphtho- and erbovirus polyproteins undergo an additional processing in which the leader protein (L^{pro}) self-cleaves from the polyprotein (Fig. 1). The aphthovirus proteinase exists in two forms (designated Lab and Lb) derived from initiation of translation at either of two in-frame AUG codons (Cao et al., 1995). L^{pro} is a papain-like protease (Guarne et al., 1998) that recognizes substrates rich in basic residues (Pineiro et al., 2012; Steinberger et al., 2014).

2. Features of the picornavirus untranslated region

The genomic RNA of picornaviruses differs from the cellular RNAs in two critical features. First, they do not contain a cap structure at the 5′ end. Instead, a viral protein (VPg) is covalently linked to the 5′UTR. Second, an internal *cis*-acting region within the 5′UTR,

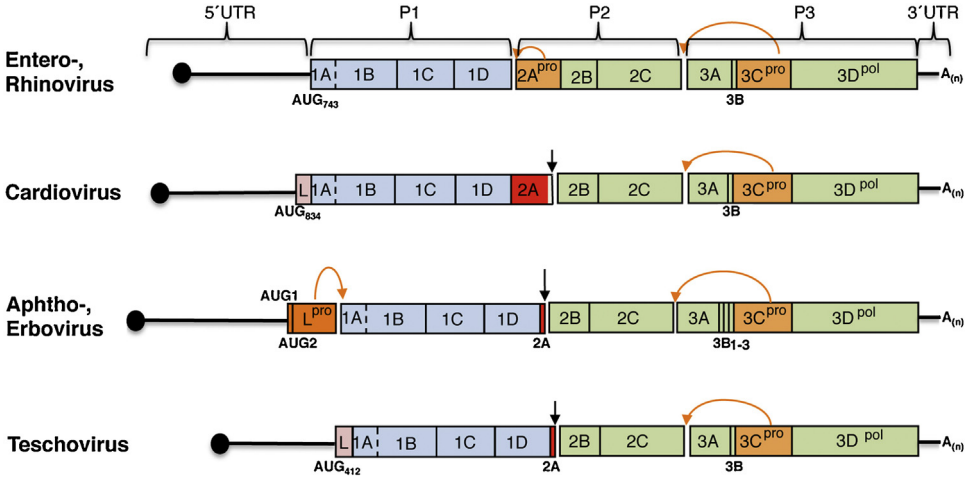

Fig. 1. Schematic of representative picornavirus genomes. A black line depicts 5′ and 3′UTR; a solid black circle depicts the viral VPg protein covalently linked to the 5′ end. The initiator AUG and its nucleotide position are indicated on each genome. Processing of the polyprotein by 3C, 2A and L proteases gives rise to the mature viral proteins (1A to 3Dpol). P1, P2 and P3 correspond to the protein precursors. A red box depicts regions where 2A polypeptide acts as a recoding translational element (marked by an arrow), while pink box depicts cases where the L protein lacks protease activity.