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Structural analysis provides insights into the modular organization of picornavirus IRES

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ABSTRACT

Picornavirus RNA translation is driven by the internal ribosome entry site (IRES) element. The impact of RNA structure on the foot-and-mouth disease virus (FMDV) IRES activity has been analyzed using Selective 2'Hydroxyl Acylation analyzed by Primer Extension (SHAPE) and high throughput analysis of RNA conformation by antisense oligonucleotides printed on microarrays. SHAPE reactivity revealed the self-folding capacity of domain 3 and evidenced a change of RNA structure in a defective GNRA mutant. A modified RNA conformation of this mutant was also evidenced by RNA accessibility to oligonucleotides. Interestingly, comparison of nucleotide reactivity with RNA accessibility revealed that SHAPE reactive nucleotides corresponding to the GNRA motif were not accessible to their respective target oligonucleotides. The differential response was observed both in domain 3 and the entire IRES. Our results demonstrate distant effects of the GNRA motif in the domain 3 RNA conformation, and highlight the modular organization of a picornavirus IRES.

Introduction

Initiation of translation of picornavirus RNAs is driven by the internal ribosome entry site (IRES) element, a region within the 5′ untranslated region (5′UTR) that recruits the ribosomal subunits internally using a 5′ end-independent mechanism. IRES elements are present in a large variety of RNA viruses (Balvay et al., 2007; Filbin and Kieft, 2009; Lukavsky, 2009; Martinez-Salas et al., 2008). Nevertheless, and despite performing the same function, IRES elements differ in nucleotide sequence, RNA structure and trans-acting factors requirement. Even within the group of picornavirus RNAs, IRES elements are divergent in sequence and structure having been classified in four groups in terms of RNA structural organization (Belsham, 2009; Fernandez-Miragall et al., 2009). The large heterogeneity of RNA sequence and factor requirement poses many questions concerning our understanding of a general mechanism behind IRES function. Moreover, the diversity of the currently known IRES elements imposes serious problems to accurately predict the presence of IRES elements in eukaryotic mRNAs.

Despite the lack of conserved primary sequence, a typical attribute of picornavirus IRES structure is the presence of stable stem-loops, a property shared with other viral IRES elements (Martinez-Salas, 2008; Belsham, 2009; Lukavsky, 2009). In addition, a distinctive feature of picornavirus IRES is their long length, ranging from about 300 to 460 nt, presumably needed to generate a plethora of recognition motifs involved in the interaction with a large number of host factors regulating IRES-mediated translation initiation (Fitzgerald and Semler, 2009; Pacheco and Martinez-Salas, 2010). RNA structure plays a fundamental role in picornavirus IRES translation initiation. As it occurs in many other RNAs, compensatory substitutions in base-paired regions allowed secondary structure conservation during viral evolution in the field. Besides, functional analysis of nucleotide substitutions in conserved regions has proven extremely valuable for the identification of essential motifs required either at the primary sequence level, or as secondary structural elements (Martinez-Salas, 2008).

Foot-and-mouth disease virus (FMDV) is a picornavirus that causes a devastating disease worldwide (Grubman et al., 2008). Regulatory elements on the 5′ and 3′ untranslated regions of the FMDV genome control translation and replication of the viral RNA (Belsham and Brangwyn, 1990; Kuhn et al., 1990; Saiz et al., 2001; Lopez de Quinto et al., 2002; Serrano et al., 2006; Lawrence and Rieder, 2009). The IRES element of FMDV encompasses about 460 nts organized in five domains (Fig. 1A) with the peculiarity that domain 1 forms part of the *cis*-acting replication element (*cre*) (Mason et al., 2002). While the 5′ and 3′ end sequences of the IRES element determine the interaction with various translation initiation factors (eIFs) and other RNA-binding proteins (Kolupaeva et al., 1996; Lopez

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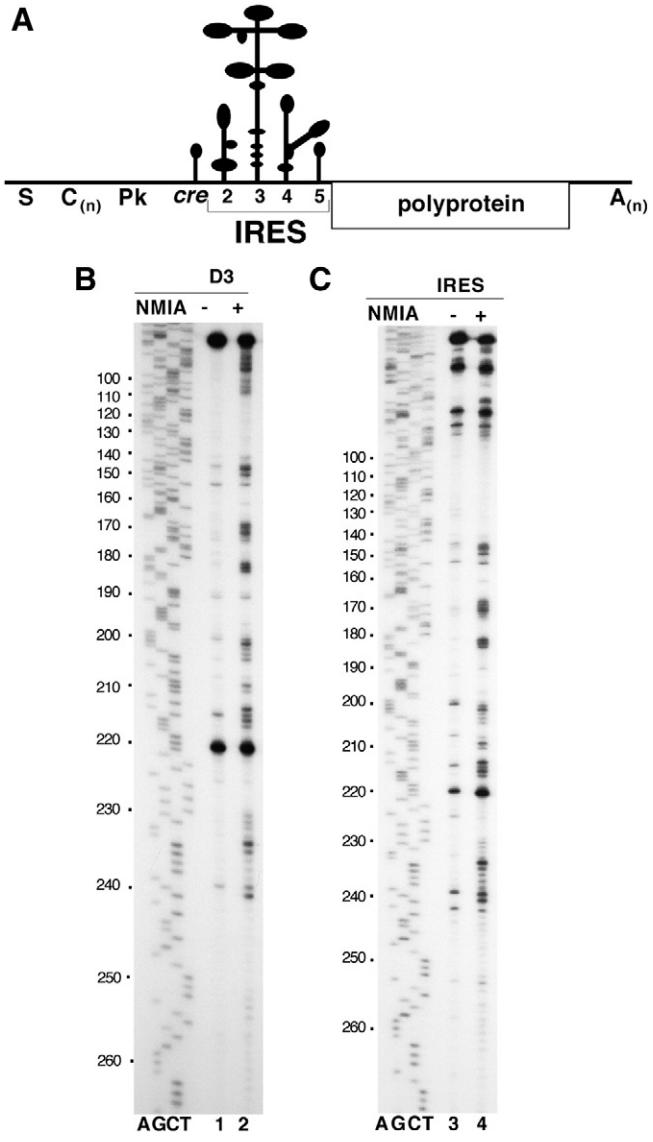

Fig. 1. SHAPE analysis of the FMDV IRES. (A) Schematic representation of the viral genome. Relevant features within the 5′ UTR are indicated; S stands for the S region, C (n) for poly C tract, Pk for pseudoknots, *cre* for cis-replicative element, and IRES for the internal ribosome entry site. IRES domains (2–5) referred to in the text are indicated; domain 1 overlaps with the *cre* element. The single open reading frame encodes a long polyprotein, flanked by a 3′UTR region with a poly(A) tail. (B) SHAPE analysis of the transcript domain 3 (D3). Primer extension analysis of RNA incubated with DMSO (−) or NMIA (+) conducted with a 5′-end labeled primer. Nucleotide positions are indicated on the left according to the sequencing lanes (AGCT) obtained with the same labeled primer; cDNA full-length products are shown at the top of each lane. (C) SHAPE analysis of the transcript encompassing the entire IRES, performed with the same labeled primer as in (B).

de Quinto and Martinez-Salas, 2000; Pilipenko et al., 2000; Lopez de Quinto et al., 2001; Stassinopoulos and Belsham, 2001; Andreev et al., 2007; Pacheco et al., 2008, 2009), the central region (termed domain 3, hereafter D3) contains RNA structural elements absolutely required for IRES activity (Lopez de Quinto and Martinez-Salas, 1997). In support of the essential role played by D3 during internal initiation, a transcript encompassing domains 4 and 5 does not possess IRES activity (Fernandez-Miragall et al., 2009), despite that it is endowed with the capacity to recruit translation initiation factors eIF4G, eIF4B, eIF3 and other IRES-binding factors.

The apical region of D3 in the IRES elements of encephalomyocarditis virus (EMCV) and FMDV contains two conserved purine-rich motifs, GNRA and RAAA (N stands for any nucleotide and R for purine). RNA structure studies have shown that the GNRA motif of FMDV, EMCV and poliovirus IRES adopts a tetraloop conformation at the tip of a stem-loop (Fernandez-Miragall and Martinez-Salas, 2003; Phelan et al., 2004; Du et al., 2004). Furthermore, as demonstrated by functional analysis, this motif is essential for IRES function (Lopez de Quinto and Martinez-Salas, 1997; Robertson et al., 1999).

The role played by D3 during IRES-dependent translation remains elusive. On the basis of its capacity to mediate intra-molecular and inter-molecular RNA-RNA interactions (Ramos and Martinez-Salas, 1999; Fernandez-Miragall et al., 2006), it has been proposed that this region instructs the functional conformation of the whole IRES element. In support of this idea, RNA probing analysis showed that mutations in the GNRA motif modified the RNA structure of this domain (Fernandez-Miragall and Martinez-Salas, 2003). Although RNA probing has provided important clues about the secondary structure of this region (Fernandez-Miragall et al., 2009) little is known about its three-dimensional structure.

RNA structure is tightly linked to biological function. Thus, as found in other RNA regulatory signals, the IRES secondary structure is phylogenetically conserved (Martinez-Salas, 2008). However, to understand the mechanism of IRES-dependent translation it is crucial to determine the spatial arrangement of the stem-loops shaping up its three-dimensional structural organization. We have previously used different chemical and enzymatic probes to gather information about the structure of FMDV IRES element in solution (Fernandez-Miragall et al., 2009). In order to deepen into the structure-function relationship of picornavirus IRES elements, we have undertaken the study of the FMDV IRES RNA structural organization using the wild type RNA sequence as well as a defective mutant carrying a single nucleotide substitution in the conserved GNRA motif. To this end, we have taken advantage of Selective 2′Hydroxyl Acylation analyzed by Primer Extension (SHAPE) reactivity. This method reveals flexible regions or nucleotides constrained in a conformation where the ribose 2′-OH is particularly susceptible to modification (Merino et al., 2005; Wilkinson et al., 2006; Vicens et al., 2007). In contrast, nucleotides involved in canonical base pairing or certain non-canonical but stable U:G, A:A, and A:G pairs have values of reactivity close to the background (Mortimer and Weeks, 2007). SHAPE analysis performed with the wild type IRES of FMDV verified our previous results regarding the RNA structure of D3 at the nucleotide level. Additionally, SHAPE reactivity studies demonstrated a change in RNA structure induced by the GUAG single nucleotide substitution at the GNRA motif that also affects the adjacent stem-loops within the apical region of D3. These results have been confirmed using an independent and complementary approach, with different level of resolution. High throughput analysis of RNA conformation by antisense oligonucleotide microarrays has been shown to be a sensitive method to detect structural differences in related RNA molecules (Duan et al., 2006; Kierzek et al., 2009; Mandir et al., 2009), including the 5′ end genomic regions of human immunodeficiency virus type 1 (HIV-1) (Ooms et al., 2004) and hepatitis C (HCV) (Martell et al., 2004). The study of the defective GUAG IRES mutant demonstrated discrete differences in RNA accessibility of distant stem-loops, also identified by SHAPE analysis. Together, the results obtained by SHAPE probing and RNA accessibility revealed a profound implication of the GNRA tetraloop in the FMDV IRES conformation and in its capacity to drive internal initiation.

Results

SHAPE analysis of IRES RNAs

To gain information about the RNA structure of the FMDV IRES we have performed a detailed RNA SHAPE analysis using wild type IRES transcripts and N-methylisatoic anhydride (NMIA) as the modifying agent (Wilkinson et al., 2006). SHAPE reactivity correlates inversely