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Standing your ground to exoribonucleases: Function of *Flavivirus* long non-coding RNAs

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ABSTRACT

Members of the *Flaviviridae* (e.g., Dengue virus, West Nile virus, and Hepatitis C virus) contain a positive-sense RNA genome that encodes a large polyprotein. It is now also clear most if not all of these viruses also produce an abundant subgenomic long non-coding RNA. These non-coding RNAs, which are called subgenomic flavivirus RNAs (sfRNAs) or Xrn1-resistant RNAs (xrRNAs), are stable decay intermediates generated from the viral genomic RNA through the stalling of the cellular exoribonuclease Xrn1 at highly structured regions. Several functions of these flavivirus long non-coding RNAs have been revealed in recent years. The generation of these sfRNAs/xrRNAs from viral transcripts results in the repression of Xrn1 and the dysregulation of cellular mRNA stability. The abundant sfRNAs also serve directly as a decoy for important cellular protein regulators of the interferon and RNA interference antiviral pathways. Thus the generation of long non-coding RNAs from flaviviruses, hepaciviruses and pestiviruses likely disrupts aspects of innate immunity and may directly contribute to viral replication, cytopathology and pathogenesis.

1. Introduction

The transcripts of RNA viruses serve numerous functions in addition to directing protein synthesis and serving as genomes/genomic templates. An examination of the various roles played by untranslated regions present at the 3′ ends of viral transcripts illustrates the interesting breadth of non-coding activities of these RNAs. First, the 3′ untranslated region (UTR) of viral transcripts often contains identifiable domains that play key roles in facilitating both local and long range structural interactions of the RNA. Local structures in the 3' UTR can serve a variety of functions, including influencing the formation of binding sites for regulatory proteins (Paingankar and Arankalle, 2015), ribosome/translation factor recruitment (Bai et al., 2013; Sharma et al., 2015) and RNA stabilization (Weil et al., 2009). Long-range 3′ UTR structural interactions, particularly with the 5' UTR (Fricke et al., 2015), can play important roles in viral replication and translation (Nicholson and White, 2014; de Borba et al., 2015). The primary sequence of the viral 3′ UTR often contains target elements for cellular RNA binding proteins (Oakland et al., 2013; Dong et al., 2015). These protein-RNA interactions can play key roles in virus replication, gene expression and host-virus interactions. Given the huge amount (up to ~10^5 copies or more) of viral RNA present in a cell during infection, it is also possible that viral UTRs can serve as sponges for cellular RNA binding proteins and micro-RNAs (miRNA) s that can disrupt cellular post-transcriptional regulation of gene expression (Barnhart et al., 2013). The 3′ UTR also has the capacity to contain rather novel regulatory features that are either naturally present or engineered. MiRNA binding sites present in the 3′ UTR, for example, can have a major effect on virus biology in terms of affecting tissue-specificity of infection and pathogenesis (Bogerd et al., 2014; Trobaugh et al., 2014). MiRNA sites can also be engineered into 3′ UTRs to regulate virus gene expression on a cell-specific basis and perhaps increase the safety/efficacy of vaccine vectors (Langlois et al., 2013; Tsetsarkin et al., 2015). Riboswitches, RNA structures that can signal through an effector module to alter transcript function upon the binding of small molecules (Mellin and Cossart, 2015), can also be effectively engineered into a viral 3′ UTR (Bell et al., 2015). Finally, it should not be overlooked that the 3′ UTR also contains important and often highly conserved promoter elements that aid in viral polymerase binding and replication (Gebhard et al., 2011). Thus it should not be surprising that viral 3′ UTRs often contain conserved evolutionary signatures within a virus family (Gritsun et al., 2014) and have a demonstrable association with viral virulence (Chen et al., 2013; Sakai et al., 2015; Manokaran et al., 2015). It is now coming to light that the non-coding regions of a viral RNA may have additional unforeseen functions beyond conventional UTR-related activities.

Cellular long non-coding RNAs (lncRNAs), which are arbitrarily defined as non-coding transcripts >200 nucleotides in length,

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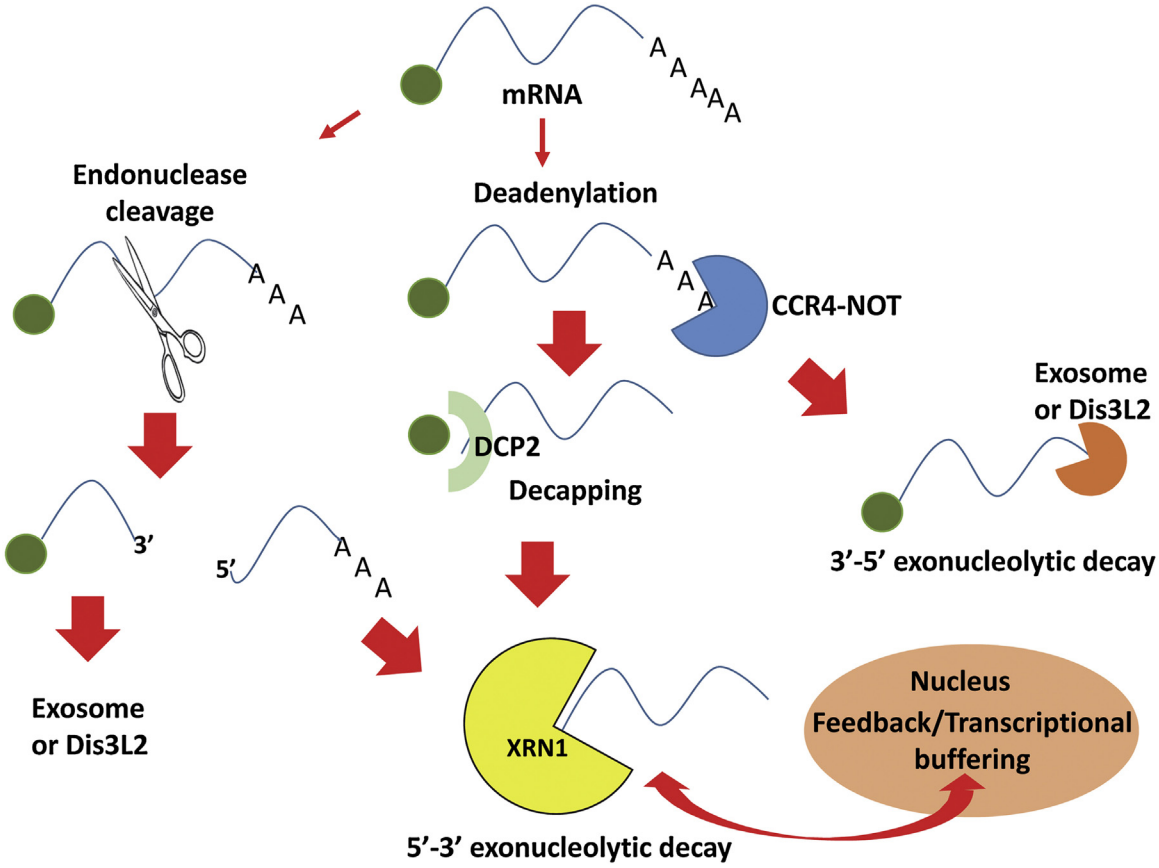

Fig. 1. An overview of the general pathways of cytoplasmic mRNA decay in eukaryotic cells.
The degradation of mRNAs is generally initiated by deadenylation via the CCR4-NOT deadenylase complex (as well as some contribution from other deadenylases). As seen in the center portion of the diagram, deadenylated mRNAs then generally undergo decapping by DCP2 to generate a 5′ monophosphate containing RNA. This decapped mRNA is then rapidly and processively degraded by the 5′-3′ exonuclease Xrn1. Xrn1-mediated mRNA decay can feedback in some fashion to the nucleus where it helps to buffer mRNA synthesis at the level of transcription to maintain cellular homeostasis. As seen on the right side of the diagram, deadenylated mRNAs can also be degraded by an alternative 3′-5′ exonucleolytic pathway mediated by the Dis3 enzyme associated with the RNA exosome or an independent exonuclease called Dis3L2. Finally (left side of the diagram), if an mRNA is subjected to endonucleolytic cleavage (e.g., by RNAi-mediated decay, RNAse L digestion, etc.), the newly formed and fully accessible 5′ monophosphate and 3' OH containing RNA fragments are rapidly degraded by the two exonucleolytic pathways as indicated.

are extensively generated from the cellular genome and have been implicated in the regulation of fundamental aspects of cell biology such as proliferation, differentiation and apoptosis (Bassett et al., 2014; Wilusz, 2015). While the study of lncRNA-mediated mechanisms is still in its infancy, the transcripts have been implicated in fundamental cellular processes such as chromatin remodeling (Kawaguchi et al., 2015), transcriptional enhancers (Pefanis et al., 2015), splicing (Gonzalez et al., 2015), mRNA stability (Gong and Maquat, 2011), translation (Ruiz-Orera et al., 2014; Essers et al., 2015), miRNA function (Dhir et al., 2015), and subcellular organization (Quinodoz and Guttman, 2014) through interactions with DNA, proteins, and other RNAs. Taking advantage of the key role played by these transcripts in the cell, viruses have developed ways to usurp cellular lncRNAs – or make some of their own (Tycowski et al., 2015) – to promote dysregulation of cellular functions to enhance viral infections.

Interestingly, it is now clear that in addition to conventional protein-encoding transcripts, all members of the *Flaviviridae* likely produce a long non-coding RNA (lncRNA) that impacts viral biology and host-virus interactions (Roby et al., 2014). These RNAs have been referred to as sfRNAs (subgenomic flavivirus RNAs) or xrRNAs (Xrn1–resistant RNAs) (Pijlman et al., 2008; Chapman et al., 2014a). Arthropod-borne flaviviruses produce large amounts of a ~300-500 base sfRNA which corresponds to the 3′ UTR region of the genomic RNA (Wengler et al., 1978; Lin et al., 2004; Pijlman et al., 2008). Hepaciviruses (e.g., Hepatitis C virus (HCV)) and pestiviruses (e.g. bovine viral diarrhea virus (BVDV)) produce a long subgenomic RNA with a 5' end that maps 30–120 bases from the original 5′ end of the genomic RNA (Moon et al., 2015). The goal of this review is to provide an overview of our understanding of the generation and functions of these flaviviral lncRNAs as well as speculate upon additional roles for these long non-coding transcripts in virus biology and pathogenesis.

2. Flaviviral lncRNAs are generated via stalling of a cellular RNA decay enzyme

Transcription is not the sole way to regulate gene expression in eukaryotic cells. The degradation of cellular RNAs plays a significant role in regulating both the level of mRNAs (Braun and Young, 2014) as well as quality-controlling gene expression to remove unwanted or deleterious transcripts (Miller and Pearce, 2014). Since viral mRNAs in the cytoplasm clearly fall into the 'unwanted' category of transcripts, the cellular RNA decay machinery also actively acts upon virus RNAs during infection (Moon and Wilusz, 2013). We envision this attack on viral RNAs early in infection by the cellular RNA decay machinery as an aspect of innate immunity that attempts to control the infection prior to activation of classical innate pathways such as interferon. Cellular mRNAs are generally degraded in a two-step process (Schoenberg and Maquat, 2012) (Fig. 1). First, the poly(A) tail is shortened/removed by regulated deadenylase enzymes followed by decapping mediated by