Viral RNAs versus the Cellular RNA Decay Machinery

Some RNA viruses disarm the cellular machinery that inactivates RNA, others hijack host factors to protect viral RNA against their effects

Stephanie L. Moon and Jeffrey Wilusz

Understanding the interactions between RNA viruses and the cellular mRNA decay machinery can yield important insights into the biology of many families of viruses and their host cells and may provide novel pathways for drug design. Viral suppression of host cellular exonucleases and the sponging of host stability factors also seem to be crucial for pathogenesis.

The success of a viral infection generally depends on the virus replicating to high levels—a process that requires RNA viral genomes and transcripts to be protected from the cellular RNA decay machinery. These RNA degradation factors constantly police cells to remove any and all unwanted transcripts. In the face of viral infections, the RNA decay machinery cannot be simply overwhelmed by the high replication rate of RNA viruses, nor can such viruses hide in membranous compartments, because they need access to the cytoplasmic milieu to generate proteins.

Research is uncovering how viruses can disarm the cellular RNA decay machinery or hijack cellular factors to protect viral RNAs from degradation. Aside from simply allowing viruses to proliferate, their usurping of cellular RNA stability factors or suppression of RNA decay enzymes has dramatic consequences for cellular gene expression and the ability of cells to respond to viral infections. Indeed, viruses have several means for evading the cellular RNA decay machinery and disrupting it or other host processes in ways that can lead to pathology.

Host RNA Decay Machinery that Viruses Must Evade

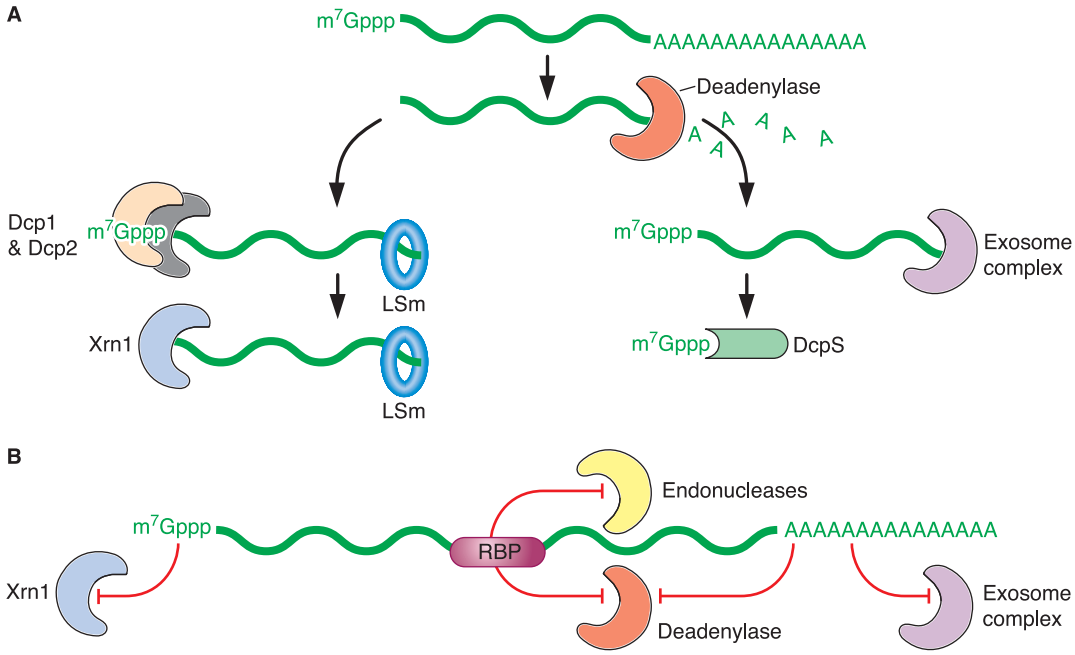
Viruses are intracellular parasites that depend on host cells to proliferate. To any viral RNA, the cell's mechanisms for degrading RNA are likely to be a major impediment to be avoided, manipulated, or suppressed at all costs. The cellular RNA decay machinery normally ensures proper gene expression by rapidly removing aberrant or unneeded cellular mRNAs from the pool of transcripts available for translation. In addition to being a quality control mechanism, the regulation of when and how quickly mRNA molecules are degraded allows for tightly controlled, rapid changes in cellular gene expression that cannot be achieved by changes in transcription alone. In fact, recent evidence indicates the rates of mRNA synthesis and decay are closely coordinated as a way of buffering gene expression.

What are the key components of the RNA decay machinery that dictate RNA stability in a cell? During its journey from nucleus to cytoplasm where it may be translated, an mRNA molecule is subject to both stability and RNA decay factors. Enzymes along the exonucleolytic pathway target capped and polyadenylated mRNAs, typically first removing adenyl groups from such transcripts (Fig. 1A). Next, the mRNA is decapped and degraded by Xrn1 in the 5′-3′ direction as the 3′ end is protected by the LSm1–7

SUMMARY

- **Some RNA viruses enhance their own infectivity by subverting the RNA decay machinery of host cells.**
- **RNA viruses stabilize their own mRNA molecules at the expense of those in host cells by mimicking particular cellular RNA stability factors or directly repressing aspects of the cellular RNA decay machinery.**
- **The mechanisms used by viruses to stabilize their transcripts have a significant impact on the post-transcriptional control of cellular gene expression.**
- **Collectively, the means by which RNA viruses stabilize their own RNA molecules within host cells may greatly affect cytopathology and viral pathogenesis.**

FIGURE 1



Messenger RNAs are generally degraded in a series of regulatable steps. (A) The major cellular mRNA decay pathways. Most mRNA molecules targeted for decay are deadenylated [removal of the poly(A) tail] followed by processive degradation by exoribonucleases in either the 5′-3′ or 3′-5′ direction. (B) Three of the common ways by which a cellular mRNA avoids decay.

complex. Alternatively, when a transcript does not interact with the LSm1–7 complex, the Dis3 component of the exosome may instead degrade it in the 3′-5′ direction. In this case the scavenger enzyme DcpS decaps the resulting short 5′ terminal fragment.

Several mechanisms protect cellular mRNAs from decay, including stabilizing factors that modify the transcript (Fig. 1B). For example, the 5′ cap structure protects mRNA molecules from the Xrn1 exoribonuclease, which degrades it in the 5′-3′ direction, while the 3′ poly(A) tail and other sequence or structural elements within a transcript draw stability factors to the mRNA to protect it from other types of nucleases.

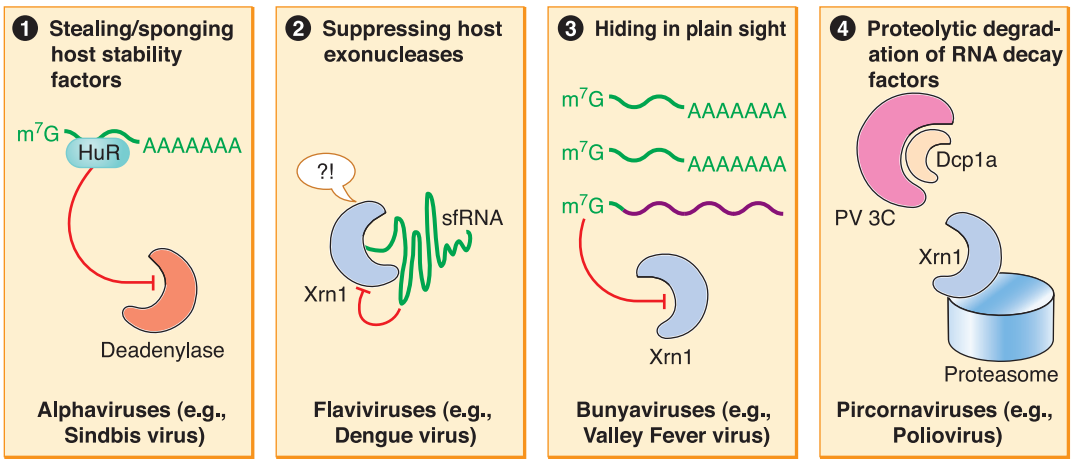
One Viral Means for Withstanding the Cellular RNA Decay Machinery Is Direct

RNA viruses withstand the host RNA decay machinery by means of two broad mechanisms: (1) host nucleases are removed either through virus-induced proteolytic degradation or by competitive inhibitor RNAs that suppress cellular nuclease activity, and (2) viral RNAs escape the cell's decay machinery by disguising themselves as cellular mRNAs, cloaking themselves in proteins and lipids, or relying on cellular stability factors (Fig. 2).

Picornaviruses deal with host mRNA decay factors by generating proteases that target cellular decay factors, such as Xrn1, Dcp2, and AUF1. For example, the cellular RNA decay-promoting protein AUF1 relocalizes from the nucleus to the cytoplasm in infected cells, binds to the 5′ untranslated regions (UTRs) of picornavirus RNAs, and degrades viral RNA molecules. However, picornaviruses counter this cellular response to infection by means of a viral protease that cleaves AUF1, inducing its degradation. AUF1-deficient cells in vitro yield higher poliovirus and rhinovirus titers.

Arthropod-borne members of the *Flavivirus* genus use the cell's decay machinery to generate a product from viral transcripts that then feeds back and inhibits an entire major pathway of RNA decay. Specifically, the short subgenomic flavivirus RNA (sfRNA) represses the cellular

FIGURE 2



Four mechanisms by which RNA viruses suppress or manipulate mRNA decay machinery. Examples of viruses that use each mechanism are depicted.

Xrn1 5′-3′ exoribonuclease, which stalls on and has trouble getting released from the highly structured 3′ untranslated region (UTR) of these flaviviral RNAs that it is trying to degrade. The cell senses this repression of Xrn1 activity, shutting down this entire pathway of RNA decay in infected cells. In addition, sfRNA also represses RNA interference activity in cells, preventing this arm of cellular RNA decay from endonucleolytically attacking viral transcripts. Moreover, sfRNA enhances viral RNA replication in certain cell types, further contributing to pathogenesis.

A More Subtle Viral Means for Withstanding the Cellular RNA Decay Machinery

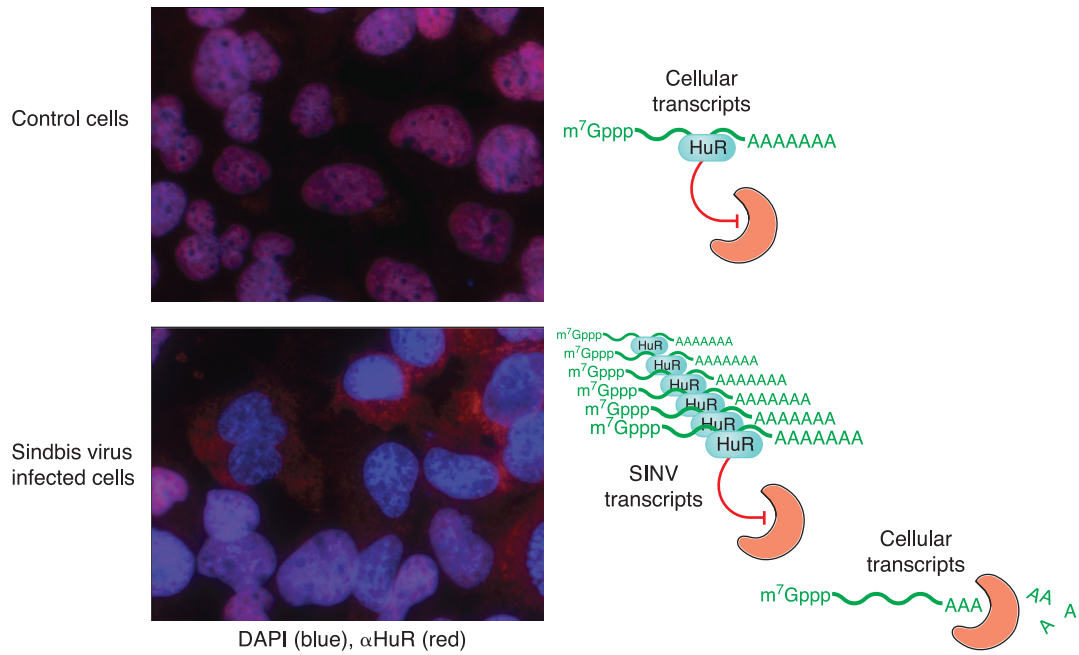
Other RNA viruses follow a more subtle approach to combating the cellular RNA decay machinery by evading host nucleases instead of suppressing or destroying them. This "cloaking" of viral RNAs can take several forms. First, many RNA viruses make transcripts that look a lot like cellular mRNAs. For instance, bunyaviruses, orthomyxoviruses, and arenaviruses all steal the 5′ caps of cellular mRNAs and incorporate them into their own nascent transcripts. In this manner, the 5′ end of the transcripts derived from these negative- and ambi-sense segmented RNA viruses look like they are of cellular origin and can undergo cap-dependent translation and be protected from Xrn1-mediated decay. Similarly, the transcripts of these and other RNA viruses have poly(A) tails that are generated by stuttering of the viral RNA-dependent RNA polymerase that can attract translation-promoting factors and prevent 3′-5′ decay by the exosome complex.

Further, some RNA viruses generate perinuclear membranous compartments for replication and maintain their RNAs within viral ribonucleoprotein complexes. This packaging likely creates a nuclease-free zone in which viral RNAs destined to be part of viral progeny rather than serve as mRNAs are protected from decay before they are packaged.

Still other viral RNAs contain sequence or structural elements that attract host RNA-binding proteins, further cloaking the viral transcripts so they can remain undetected. For instance, alphavirus RNAs contain high-affinity U-rich binding sites for the host mRNA stability protein HuR in their 3′ untranslated regions. This interaction is so favorable that viral transcripts bind and sequester/sponge the HuR protein in the cytoplasm, relocalizing this factor from the nucleus into the cytoplasm (Fig. 3). This binding to the HuR protein makes viral transcripts so stable that they generate measurably fewer infectious particles when they infect HuR-deficient mosquito and human cells.

HuR is not the only cellular mRNA stability factor usurped by RNA viruses. Several other positive-sense RNA viruses use unique mechanisms to steal the host LSm1–7 protein to protect their transcripts from exonucleolytic decay.

FIGURE 3



Alphaviruses can change posttranscriptional control of cellular gene expression by RNA-mediated sponging of the cellular HuR protein, a factor for stabilizing mRNA molecules. Top: The vast majority of HuR protein is found in the nucleus of mock-infected HuH7 human cells (left side). HuR loads onto cellular mRNAs in the nucleus and stabilizes cellular transcripts against the cytoplasmic RNA decay machinery (right side). Bottom: In HuH7 cells infected with Sindbis virus, the HuR protein relocalizes to the cytoplasm when it binds to high-affinity site in the 3′ UTR of Sindbis virus transcripts. These complexes stabilize the viral transcripts, which accumulate while cellular mRNAs that ordinarily rely on HuR protein for their stability are degraded (right side).

Hepatitis C virus (HCV) genomic RNA contains a structured RNA element in its 5′ UTR and a U-rich region in its 3′ UTR that are both capable of binding to LSm1–7, likely stabilizing the viral genome. The LSm-HCV RNA interaction is important for viral proliferation; depletion of LSm1–7 decreases HCV replication and translation in cell culture models of infection. This strategy may not be limited to positive-sense RNA viruses. The glycoprotein mRNA of the negative-sense RNA rabies virus appears to be differentially stabilized in infected cells by interaction with the host cell protein PCBP2. Thus rabies virus appears to depend on differential RNA stability to augment expression of select transcripts.

By mimicking cellular transcripts or RNA-protein complexes, RNA viruses effectively hijack these host cell factors to generate new virions at high efficiencies. It is likely particularly important early during an infection, protecting key viral RNAs against decay while permitting viral proteins to be produced and speeding viral RNA replication before cells can generate an effective antiviral response. Adding insult to injury, viral sponging of cellular RNA stability proteins has detrimental consequences for cellular gene expression.

Dire Consequences for RNA Virus-Infected Cells

By suppressing or destroying host RNA decay factors or stealing RNA stability factors from host cells, RNA viruses render the host cell environment more conducive for their persistence and replication. These changes likely contribute to pathogenesis indirectly by enhancing viral replication. That raises the question whether viral-mediated repression of cellular RNA decay also has direct effects on pathogenesis. It seems likely that something happens to cellular mRNA metabolism when highly abundant viral RNA mole-

cules suppress, destroy, or take over cellular RNA decay and stability factors. Indeed, the mechanisms that viruses use to protect their transcripts may have significant impact on the stability and expression of very important types of cellular mRNAs.

For example, picornaviruses and flaviviruses suppress or destroy host decay factors, which ordinarily maintain proper host gene expression. In particular, Xrn1 and cellular RNA decay play key roles in determining the levels of short-lived transcripts that encode proteins such as cytokines, chemokines, or cell cycle factors.

Throwing a viral-generated wrench into the host-cell RNA decay machinery is therefore likely to dysregulate host gene expression and stabilize these normally unstable cellular mRNAs. Specifically, subgenomic flavivirus RNA (sfRNA)-mediated suppression of Xrn1 stabilizes short-lived cellular mRNAs that encode factors important for cell growth and proliferation, significantly upsetting mRNA abundances. This dysregulation of cellular mRNA stability is important for pathogenesis, as an sfRNA-deficient West Nile (Kunjin) virus has greatly decreased pathogenicity in mice compared to wild-type virus that can produce sfRNA.

Stealing the Cell's RNA Stability Factors

Bunyaviruses steal the 5′ caps from transcripts that include those encoding cell cycle factors. Once the 5′ cap of an mRNA is removed through viral cap-snatching, the transcript is susceptible to the 5′-3′ exonuclease Xrn1. As those transcripts are depleted, they are no longer translated and their functions are lost to the cell. However, those losses can enhance viral replication. For instance, arresting the cell cycle in S/G2 enhances bunyavirus activity in *Drosophila* cells.

Many RNA viruses bind and may sequester cellular RNA-binding proteins, which could alter cellular gene expression. HuR protein binding to cellular mRNAs protects them from deadenylation; HuR also influences alternative splicing and polyadenylation as part of its normal role in the nucleus of mammalian cells. High-affinity binding sites in the 3′ UTR of abundant Sindbis virus RNAs sponges the HuR protein, dramatically changing HuR subcellular localization and dysregulating splicing, alternative polyadenylation, and mRNA stability of transcripts normally post-transcriptionally regulated by HuR (Fig. 3).

Perhaps the binding of the LSm1–7 complex by Hepatitis C virus similarly sequesters this important stability factor away from short-lived transcripts and makes them susceptible to inappropriate decay by 3′-5′ exonucleases. Sponging of cellular RNA stability factors by viral RNAs may contribute to viral pathogenesis by causing a wide variety of specific and/or general defects in cellular gene expression.

Understanding RNA Viruses May Lead to Rational Drug Design

These studies into how RNA viruses interfere with the RNA decay machinery of cells that they infect are identifying viral and host cell targets for rational drug design. For example, because so many arthropod-borne flaviviruses generate subgenomic flavivirus RNA through similar RNA structural elements, it may be possible to identify a compound to treat infections caused by many different flaviviruses. Targeting the poliovirus protease responsible for degrading host decay factors could block viral polyprotein cleavage while also reducing viral damage to host RNA decay factors. Similarly, because several alphaviruses target the HuR protein of host cells, identifying small molecules that block this interaction might serve as a means for treating such infections.

Stephanie L. Moon is a graduate student and Jeffrey Wilusz is a Professor in the Department of Microbiology, Immunology and Pathology at Colorado State University, Fort Collins (jeffrey.wilusz@colostate.edu).

Suggested Reading

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FAQ

HUMAN MICROBIOME

Learn more about your microbiome

American Academy of Microbiology:
http://bit.ly/HumanMicrobiome

AMERICAN SOCIETY FOR MICROBIOLOGY

YOUR BODY: HUMAN AND MICROBES

WHAT IS THE MICROBIOME?

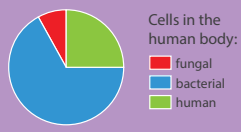
The human body is home to trillions of microbes. The community of microbes living in intimate association with our bodies, and the genes they contain, make up the **human microbiome**.

WAIT ... WHAT'S A MICROBE?

A **microbe** is a microscopic organism - this includes viruses, bacteria, and fungi.

Not all microbes make us sick - the microbes in and on our bodies play many essential roles.

WHO'S THERE?



A human body is actually only about 25% human cells. The rest is many thousands of species of bacteria and other microbes.

WHERE ARE THEY?

Wherever the human body is exposed to the outside world, there is a microbial community.

mouth lungs GI tract skin urogenital tract

Our microbiome helps us extract energy and nutrients from the food we eat, and crowds out or inhibits pathogens.

HOW DO WE GET OUR MICROBIOME?

BIRTH:
A newborn gets its microbes from:
- its mother's birth canal
- skin of its mother and other caregivers

BREAST MILK:
Breast milk has been fine-tuned to provide:
- nutrients, vitamins, and antibodies
- diverse microbes to populate the baby's gut

ENVIRONMENT:
For the rest of the baby's life, it will continuously encounter microbes:
- soil and water
- people, pets, plants
- new, diverse foods