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Fated for decay: RNA elements targeted by viral endonucleases

William Rodriguez, Daniel Macveigh-Fierro, Jacob Miles, Mandy Muller *

Microbiology Department, University of Massachusetts, Amherst, MA, United States

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ABSTRACT

For over a decade, studies of messenger RNA regulation have revealed an unprecedented level of connectivity between the RNA pool and global gene expression. These connections are underpinned by a vast array of RNA elements that coordinate RNA-protein and RNA-RNA interactions, each directing mRNA fate from transcription to translation. Consequently, viruses have evolved an arsenal of strategies to target these RNA features and ultimately take control of the pathways they influence, and these strategies contribute to the global shutdown of the host gene expression machinery known as "Host Shutoff". This takeover of the host cell is mechanistically orchestrated by a number of non-homologous virally encoded endoribonucleases. Recent large-scale screens estimate that over 70 % of the host transcriptome is decimated by the expression of these viral nucleases. While this takeover strategy seems extraordinarily well conserved, each viral endonuclease has evolved to target distinct mRNA elements. Herein, we will explore each of these RNA structures/sequence features that render messenger RNA susceptible or resistant to viral endonuclease cleavage. By further understanding these targeting and escape mechanisms we will continue to unravel untold depths of cellular RNA regulation that further underscores the integral relationship between RNA fate and the fate of the cell.

1. Introduction

From synthesis to decay, the regulation of RNA steady state and turnover emerges as an important contributor of the gene expression cascade and is critical to the survival of the cell. A key component of this pathway is the role of RNA structural elements in directing the many facets of RNA life. These RNA elements serve as signals utilized by the cell to monitor the stability of the RNA pool and buffer gene expression accordingly. In particular, the steady-state levels of messenger RNA (mRNA) are tightly regulated through a number of features including transcriptional splice patterns, the 5′ cap, the 3′ poly-A tail, and internal RNA secondary structures. Collectively, these features define the identity of these RNA as mRNAs and direct their fate.

Any perturbation in this carefully regulated expression cascade can have widespread consequences on the overall cell fate. Thus, it is not surprising that viruses extensively remodel these pathways for their own benefit. During infection, virus and host clash for control over gene expression pathways. For the host, holding control of gene expression equates to mounting a robust anti-viral response, while for the virus wresting control away from the host allows it to fulfill its life cycle and dampen host defenses. Viruses have evolved to take over existing pathways governing mRNA fate in order to get an edge over the host, and rapidly hijack the host gene expression environment [1–5]. In fact, viruses are exceptionally powerful manipulators of RNA decay and expertly exploit host RNA surveillance pathways. Emerging evidence has revealed that widespread RNA decay triggers a massive down regulation of transcription and translation rates throughout the cell [6–11]. Interestingly, to reach this global level of gene expression remodeling, multiple viruses seem to have converged onto a strategy of accelerating RNA decay using their own encoded endoribonucleases. Viruses that express these mRNA-specific endonucleases include alpha- and gammaherpesviruses, influenza A virus, SARS and MERS coronaviruses, vaccinia virus, and African swine fever virus [12–19]. The effect of these viral RNAses is extensive, as it is estimated that over two-thirds of the host transcriptome is impacted by viral-mediated decay [17, 20–22]. However, how the host reacts to this massive takeover, or how these viral endonucleases can target mRNA in such a widespread manner remains elusive and is still an active area of research.

In the past five years, studies of viral endonucleases and accelerated RNA decay have greatly enhanced our understanding of RNA regulation. In particular, recent studies have unveiled several RNA elements that confer susceptibility or resistance to endonuclease cleavage [20–26]. Many of these elements consist of RNA structural motifs and/or internal sequences that recruit RNA binding proteins. To date, it is still unclear

* Corresponding author.
E-mail address: mandymuller@umass.edu (M. Muller).

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how prevalent these RNA elements are or their precise nature and characteristics. Further study into their evolutionary implications in the virus-host battle for control of gene expression is also needed. Below, we review our current understanding of RNA elements that facilitate targeting or escape of cellular mRNAs from viral endonuclease cleavage.

2. Viral endonuclease targeting

RNA fate is tightly coupled to the presence or absence of regulatory elements that direct RNA-protein interactions, localization and turnover rates. It is well established that from the moment an RNA emerges from the transcription machinery, it will undergo a number of modifications in both sequence and structure that will direct splicing, translocation, translation, and ultimately decay of mature transcripts [27–29]. As the cell has evolved to utilize RNA elements to regulate gene expression, so have viruses evolved to take advantage of them. Viral endoribonucleases, an exemplary product of this co-evolution, utilize these defining elements as a means of selectively targeting mRNA substrates. Throughout this section we will discuss how each of these RNA features render mRNA susceptible to – and in many cases direct – viral endonuclease cleavage or similar strategies aimed at degradation of the host transcriptome during viral infection.

2.1. Targeting the 5′ cap

5′ Caps are a common and conserved feature of mRNA, so it is perhaps not surprising that viruses have converged onto a mechanism of accelerated RNA decay relying on 5′ cap targeting. Several viruses encode their own “decappers” which are viral enzymes that are surprisingly similar to the prototypical human, plant, and yeast decapping enzyme Dcp2 [30]. 5′ Caps are RNA modifications commonly found in the form of m7GpppNm. Once an mRNA is decapped, what remains is the cap end with an m7GDP modification and the uncapped monophosphoryltated RNA products. This leftover RNA product thus becomes the perfect substrate for 5′-3′ exonucleoytic degradation, in particular by the cellular housekeeping exonuclease Xrn1 [31]. Vaccinia virus (VacV) encodes two proteins (Fig. 1A) that exhibit decapping activity known as D9 and D10 [32–34]. Both of these proteins belong to a superfamily of hydrolases that contain a consensus Nudix sequence, an essential motif for the decapping activity of cellular decapping enzymes such as Dcp2 [35,36]. Although both D9 and D10 are decapping enzymes, there are some differences in their expression kinetics and their target preferences. First, D9 is synthesized early while D10 is synthesized late during infections thus maintaining a prolonged stage of viral-controlled decapping throughout infection. Intriguingly, D9 and D10 also widely differ in their affinity for capped mRNA with D9 showing greater specificity for the biologically relevant mRNA cap structure [33,34]. Furthermore, mRNA length seems to be an important criteria for D9 and D10 targeting, with D9 more efficiently cleaving small RNA substrates whereas D10 was shown to cleave RNA substrates of 24 nucleotides or more with similar efficiency [33,34]. This suggests that both of these decapping proteins can target not only methylated caps but RNA itself. Both D9 and D10 were shown to be inhibited by uncapped mRNA, however D10 needed a 100-fold molar excess of competitive uncapped mRNA to reduce its decapping activity by 50 %, suggesting that D9 has a greater affinity for RNA [33,34]. Since D9 and D10 have different expression kinetics, their affinity and activity for specific types of mRNA may reflect a global attempt by the virus to control its cellular visibility. How viral mRNA escape D9 and D10 activity remains unknown.

Another virus that employs a similar method of mRNA degradation is the African Swine Fever Virus (ASFV), a large, double-stranded DNA virus in the Asfarviridae family. ASFV carries a gene (D250R in strain Ba71V and g5R in strain Malawi) that encodes a decapping protein (ASFV-DP – Fig. 1A) that, similarly to D9, D10 and the host Dcp2, has a Nudix hydrolase motif [37] that can hydrolyze a wide range of substrates [38]. In particular, ASFV-DP activity – much like D9 – was shown to depend on an RNA body and that adding even 1-fold molar excess of uncapped mRNA reduced decapping by more than 81 %. ASFV-DP N-terminus was hypothesized to be sufficient to bind RNA whereas its C-terminal region encompassing the Nudix motif is not required. The N-terminal region fold into a basic channel that may serve as an RNA binding platform and it was proposed that the N-terminal domain interacts with the highly acidic C-terminus to increase the disassociation (off) rate of the RNA-ASFV-DP complex [15]. Furthermore, ASFV-DP activity seems to be independent of methylated nucleotides suggesting that this endonuclease does not actually need a cap structure to locate its substrate [37]. However, the cap structure seems to have a stabilizing influence on the binding of ASFV-DP to its target mRNA. More recently, ASFV-DP was found to preferentially target the elongation-initiation factor elF4E mRNA compared to beta-actin mRNA [15], raising the intriguing question of potential transcript specificity in ASFV-DP targeting mechanism. Given the high level of similarities between

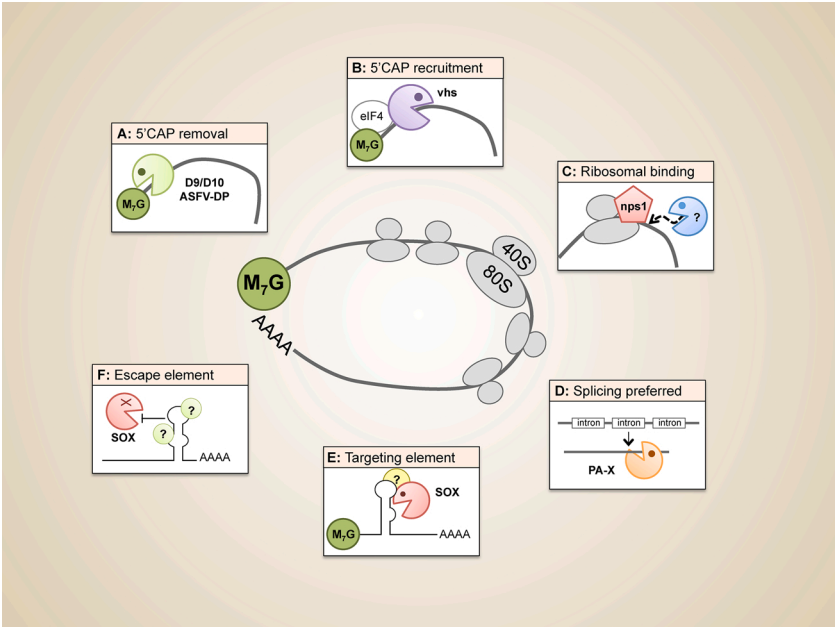

Fig. 1. Messenger RNA features that direct viral endonuclease and decapping enzymes targeting or escape from cleavage. (A) Vaccinia virus (VacV) D9/D10 and African swine flu virus (ASFV) ASFV-DP preferentially cleave mRNA at the site of the 5′ M_7G cap. (B) Herpes simplex virus-1 (HSV-1) vhs recognizes the cap binding complex to facilitate upstream internal cleavage. (C) Coronavirus (CoV) nsp1 indirectly triggers mRNA decay by inactivation of the 40S ribosomal subunit. (D) Influenza A virus (IAV) PA-X preferentially targets and internally cleaves intron-bearing pre-mRNA in the nucleus, coordinating with the host spliceosome and 3′ end processing factors. (E) Kaposi’s sarcoma-associated Herpesvirus (KSHV) SOX internally cleaves mRNA at specific sites. A combination of specific RNA secondary structure and nucleotide sequence directly contribute to SOX target selectivity and cleavage efficiency. (F) RNA elements in the 3′ UTRs of select mRNA have been implicated in facilitating escape from viral endonuclease cleavage.

ASFV-DP, and VacV D9, it would be interesting to investigate whether D9 similarly has sequence-specific capabilities.

Influenza A virus (IAV) has also evolved to target mRNA 5′ caps in a mechanism referred to as "Cap Snatching". The influenza RNA genome is replicated in the host nucleus using a viral-encoded RNA-dependent RNA polymerase (RdRp). While host 5′ caps are added to pre-messenger RNA co-transcriptionally, the RdRp does not allow for the recruitment of this machinery. Instead, it possesses a nuclease activity that will cleave fully formed and assembled 5′ caps from host genes and use it as a primer for viral transcription [39]. Once a host transcript is used as a cap "donor", it is removed from the pool of viable mRNAs and will be quickly decayed by the host machinery. There are limited studies on how this affects the host cell homeostasis but it would be interesting to investigate whether this could play a role in the viral-host interplay.

2.2. Splice-pattern targeting

Influenza A virus (IAV) also uses the power of RNA decay to suppress host gene expression. PA-X is a recently identified endonuclease produced by a ribosomal frameshift during translation of the polymerase subunit PA and PA-X was shown to be responsible for host shutoff in Influenza [19,22,40,41]. PA-X consists of the PA RNase domain fused to a unique carboxy-terminal domain known as the X-open reading frame (X-ORF) [42]. PA-X has been shown to selectively degrade RNAs that are transcribed by the host RNA polymerase II (Pol II), but not by any other polymerase [23]. This specificity allows the virus to protect its own viral RNAs that are expressed from the viral RNA dependent RNA polymerase. PA-X accumulates in the nucleus, and previous research has shown that not all mRNAs are degraded equally by PA-X [22,23,41,43]. This suggests that there is an additional layer or alternate molecular mechanism to the targeting of PA-X. Interestingly, the X-ORF is required for PA-X activity, specifically for nuclear localization and binding to other proteins [22]. In particular, PA-X was shown to interact with host proteins involved in 3′ RNA processing as well as splicing regulators, and thus has been proposed to link splicing to polyadenylation during RNA processing. Furthermore, it was recently showed that even single splicing event can promote degradation by PA-X (Fig. 1D) and that prototypical splice sites AG/GT had higher PA-X susceptibly compared to imperfect matches like TA/GT [22]. However, intronless reporters are still degraded by PA-X to some extent. This was attributed to their robust expression, which may promote association with cellular factors involved in PA-X targeting. It therefore emerges that the important signals that render mRNA susceptible to PA-X are core features of host mRNA, such as Pol II transcription and splicing leftover marks. This is a remarkable example of how viruses rapidly evolve to efficiently hinder host cell immunity while promoting expression of their own viral RNAs.

2.3. Targeting the translation machinery

While the methods described above involved viral enzymes targeting the RNA processing machinery, some viruses trigger RNA decay later in the mRNA life cycle. In particular, Coronaviruses have a unique method of inducing host gene suppression by hijacking the host RNA quality control pathways in a way reminiscent of non-sense and no-go decay pathways. The coronavirus nsp1 protein has been shown to be associated with widespread RNA decay during infection (Fig. 1C). However, it has been documented that nsp1 itself is not directly responsible for endonucleolytic cleavage of host mRNA and does not possess nuclease activity [9,44–46]. Instead, nsp1 was shown to bind and stall the 40 s ribosome leading to inhibition of mRNA translation, rendering the transcript inactive and translationally incompetent [9,47]. The host cell machinery is prepared to deal with stalled ribosomes and once nsp1 triggers the inhibition of ribosome scanning a cascade of events is initiated: recruitment of an unknown cellular endonuclease and 5′ cap removal followed by 5′-3′ decay mediated by XRN1 [44]. Recently, it was discovered that the cellular endonuclease Cue2 is responsible for RNA decay during no-go decay [48], making Cue2 a potential candidate for nsp1-mediated decay during coronavirus infection.

While the binding of nsp1 to the 40 s ribosomal subunit is a major factor in the targeting mechanism, targeting can also be driven in a template dependent manner [47]. Earlier research has suggested that nsp1-mediated RNA cleavage can target internal ribosome entry sites as well as the 5′ cap of non-viral mRNA [49]. It was shown that in transcripts containing internal ribosome entry sites, cleavage occurred upstream of the initiation codon, approximately 100–200 nucleotides away. Nsp1 thus emerges as a thorough RNA decay trigger that uses diverse and non-overlapping strategies to widely target host mRNAs. How the viral transcripts escape nsp-1 mediated is still under investigation. Clues explaining this resistance to decay came from structural studies of the SARS-CoV genome. Located within the 5′ UTR of SARS genome is a structure that has been dubbed stem-loop 1 (SL1) that provides protection from nsp1-mediated gene suppression [47,50]. While the mechanism driving the stabilization of SL1 containing-RNA and escape from nsp1 remains unknown, it has been shown that direct binding by nsp1 to this structured region is important [47,50]. This highlights the dual role of nsp1 when recruited to host vs. viral mRNA.

Overall, SARS coronavirus nsp1 is an interesting regulator of RNA stability: currently, nsp1 does not appear to have any endonucleolytic activity of its own, and instead binds to the 40 s subunit exploiting the host's RNA quality control pathways to trigger mRNA degradation. At the same time, nsp1 is also directly responsible for the protection of viral transcripts. This intrinsic duality of nsp1 makes it a potent regulator of RNA fate during infection. Of note, the newly emerged SARS-CoV2 coronavirus encodes an nsp1 protein with high sequence similarity to the other known coronaviruses [51] reinforcing how pivotal nsp1 is in the regulation of coronavirus infection.

Another master regulator of RNA fate that uses its host translation machinery as a platform to trigger decay is the herpesvirus protein vhs. Early exploration of the herpes simplex virus virion host shutoff protein (vhs) identified that this protein was responsible for destruction of almost all host and viral mRNA [52]. It became quickly evident that vhs preferentially targets mRNA while rRNA and tRNAs levels remain unaffected by vhs activity [53,54]. For these reasons, it was suggested that a particular mRNA features, such as the 5′ cap or the 3′ polyadenylated tail may play an important role in vhs targeting. vhs was reported to interact with eIF4H, eIF4AII and eIF4F [55]. In particular, it was shown that mutations in vhs blocking its interaction with eIF4H render vhs incapable of degrading mRNA and that knock out of eIF4H expression is sufficient to abrogate vhs function [56]. Taken together, these results strongly suggest that eIF4H binding is necessary for vhs targeting (Fig. 1B). Furthermore, vhs interaction with the other eIF4 factors that form the cap-binding complex was demonstrated to contribute to the recruitment of vhs to the 5′ end of its target transcripts [1,55,57,58]. vhs preferentially selects cleavage sites near translation initiation sites within the 5′ UTR and at the start codon which was suggested to be driven by secondary structures of mRNA, blocking the movement of the 40S ribosomal subunit, or factors that may cause stalling [56]. Once initial cuts have been made by vhs, host factors such as XRN1 quickly detect vhs-mediated internal cuts and finish the decay process of the mRNA [59].

Furthermore, it has been shown that *in vitro*-translated vhs is able to cleave viral mRNAs containing IRES slightly downstream of ribosome entry sites [52,56,60]. There seems to be a clear distinction between the mechanisms of recruitment of vhs to cap complexes versus selective structural-dependent recognition of IRES and this difference could play a role in the particular pool of transcript targeted by vhs [58].

2.4. Site-specific targeting

Similar to vhs and nsp1, the gammaherpesviral SOX family of proteins also targets the vast majority of host mRNA for degradation. These gammaherpesviral nucleases include the Kaposi's sarcoma-associated

herpesvirus (KSHV) SOX, the murine herpesvirus 68 (MHV68) muSOX and Epstein–Barr virus (EBV) BGLF5 [12]. While SOX and its homologs selectively target translationally competent mRNAs, the presence of ribosomes or other translation-associated structures is not a strict requirement for target recognition [12]. Rather, targeting by these nucleases has been demonstrated to be directed by recognition of a degenerate RNA motif that facilitates substrate binding and cleavage. This targeting motif was first mapped within three SOX target transcripts to a UGAAG motif [21,61]. Since this discovery, significant progress has been made in understanding how this element directs SOX mediated cleavage. In this section of the review, we discuss the most recent advances made in further defining the SOX-targeting element, its prevalence in the host transcriptome, and the contributions of RNA structure to SOX-target recognition and cleavage.

In 2015, Gaglia *et al.* further defined the SOX-targeting motif and proceeded to search the host transcriptome for the prevalence of this element amongst *in vivo* SOX targets [21]. They uncovered that the SOX targeting mechanism can be simultaneously sequence-specific and promiscuous. They also further defined the targeting element itself as a degenerate pyrimidine-rich sequence pattern adjacent to an unpaired stretch of adenine residues. Consistent with previous observations, these SOX cut sites are present on endogenous mature mRNAs and are not restricted to a particular region of these transcripts. Astoundingly, despite the relatively large size of the targeting element, most well-annotated human and viral transcripts contained at least one sequence fitting the SOX-targeting motif. By defining SOX targeting element and its prevalence in the transcriptome this work set the foundation allowing *in vitro* analyses to further explore the contribution of secondary structures in SOX targeting. Given the existence of multiple cut-sites on target transcripts beyond this consensus sequence previously described, Lee *et al.* argued that the presence/absence of this sequence alone was insufficient to capture the breadth of SOX targeting [24]. Through structural exploration of known cleavage targets they identified several general stem loops and bulges flanking *in silico* predicted targeting sites and showed that SOX substrate processing is entirely dependent on the recognition of loop or bulge RNA folds within the target RNA duplex (Fig. 1E). Crystal structures of SOX bound with RNA further demonstrated that SOX cleavage is restricted to regions within or flanked by an unpaired nucleotide tract, often within a loop fold. Collectively, these results illustrated the critical involvement of RNA secondary structures in SOX targeting and further reinforced a targeting mechanism consistent with dynamic KSHV-induced mRNA decay.

Despite the further mapping of the SOX targeting element and assessment of secondary structure participation one outstanding question remained: what is the direct impact of these RNA features in target recognition? More specifically, how do these features influence SOX affinity and/or the efficiency by which targets are degraded? To answer these questions, Mendez *et al.* developed the first *in vitro* system that accurately mimicked SOX *in vivo* cleavage activity [26] and demonstrated the requirement of an exposed loop structure and specific upstream sequences for SOX cleavage, both serving as a "binding platform" for SOX. Thus, it is the combination of features within the cut site that determines the binding affinity of SOX for its substrate and modulates SOX cleavage efficiency and target recognition. SOX targeting therefore seems to depend on the cooperation of these various RNA features, which can explain why although SOX-mediated turnover is widespread, SOX targeting is still selective.

By further elucidating the prevalence and requirements for SOX cleavage, each of these studies has expanded our understanding of the contributions of RNA elements for endonuclease targeting. However, a broader challenge remains in determining how SOX and related endonucleases preferentially target Pol II transcribed mRNAs, a feature that is not preserved by purified SOX *in vitro* [24,26]. One hypothesis is the involvement of cellular co-factors capable of directing SOX to its targets. The aforementioned IAV PA-X endonuclease actively interacts factors involved in mRNA maturation and processing, thus allowing for efficient PA-X cleavage [22,39]. It is therefore possible that SOX and its homologs are also recruited to Pol II-transcribed mRNA in the cytoplasm via an unknown RNA-binding proteins or related factors. Characterizing the interactions of SOX with these potential protein co-factors will also be instrumental in deciphering SOX target specificity and may reveal the hidden mechanism behind transcripts escaping SOX decay.

3. Escape from endonuclease cleavage

It is commonly accepted that *cis*-acting elements play vital roles in determining mRNA half-life either by extending the life of a transcript, or destabilizing the transcript and labeling it for decay [62,63]. Oftentimes this stabilization is mediated by interactions with *trans*-acting factors that interact with the RNA. These elements can be either sequence-based, such as AU-Rich Elements (ARE), or they can be structurally based such as the Iron-responsive element (IRE) or like the subgeomic RNA produced by Flaviviridae (sfRNA).

As discussed above, RNA elements play an integral part in viral nuclease targeting, but recently, studies have shown that these *cis*-acting elements may also participate in regulating escape from viral-induced degradation [25,64–66]. The most studied example of this is in the context of KSHV infection (Fig. 1F), where it was shown early on that while the vast majority of host transcripts are targeted for degradation, a portion of transcripts are only mildly affected by SOX-mediated degradation [64,65,67,68]. While most of these "escapees" likely simply lack the targeting features required for SOX cleavage, some select transcripts were found to resist SOX decay even in the presence of a robust SOX targeting element [20,25,64,65]. This type of dominant negative effect was shown to stem from a sequence that is confided to the escaping transcript 3′ UTR and was dubbed the SOX-resistant element (SRE) [25,64,65]. Strikingly, despite the limited sequence similarity between the known escaping mRNA, there seems to be a conserved structurally important stem-loop located within the SOX resistant elements [20,25]. This would suggest that from an evolutionary standpoint the structure of the SRE is conserved over sequence and could suggest that it serves as a platform to recruitment of *trans*-acting factors.

While AU-Rich elements have been shown to stabilize RNAs, it was shown that there is a correlation between AU-Rich elements in a given mRNA and its susceptibility to endonucleolytic cleavage. A study by Escalatine *et al.* showed that the herpes simplex virus's endonuclease vhs discrimination between target and non-target mRNA could be based on the presence of AU-Rich elements [69]. The authors suggested that many AU-Rich elements containing mRNA encode immune sensors that could disrupt viral replication, and vhs-mediated decay could thus alleviate some of this stress. Furthermore, one of the vhs-resistant transcripts is tristetraprolin (TTP), a protein that binds AU-rich elements and promotes the decay of the corresponding mRNA. Therefore, by sparing TTP, the effect of vhs on AU-rich element-containing transcript is further enhanced.

Furthermore, while this review focuses on host RNA elements that escape viral endonucleases, there also exist several examples of RNA structures that escape from host nucleases to benefit viral infection. This type of escape relies on complex structural elements and have been identified in a diverse range of viruses including members of flaviviridae, phleboviruses, and arenaviruses [70]. These structural elements hinder the host most prevalent exonuclease Xrn1 [71], and have been dubbed XRN1-resistant RNA or xrRNA. This highly structured RNA is necessary for the production of sub-genomic flavivirus RNA (sfRNA), and is located within the 3′ UTR of the viral genome [72]. These xrRNA have been shown to adopt a pseudoknot conformation at which point Xrn1 is blocked from proceeding [73]. One structure of note is that of an xrRNA element found in the phlebovirus Rift Valley Fever virus, where the element that stalls Xrn1 is found within a G-rich region. This region has similar characteristics to a G-quadruplex, a secondary structure that forms between four stacked guanine that interact in Hoogsteen hydrogen bonding to form stabilizing structures [74] and suggest that

the presence of G-quadruplexes may play an additional role in transcript stability and Xrn1 resistance.

Overall, escape from viral nuclease cleavage may have widespread effects during infection and regulation of this escape mechanism may be viral or host driven. By understanding how RNA elements can trigger targeting versus escape, it could be turned into powerful anti-viral drugs.

4. Conclusions and future perspectives

With the advent of deep sequencing technology, it has emerged that regulation of RNA decay relies heavily on "RNA elements" – both sequence and structural elements. As we discussed in this review, the characterization of these elements have helped us better understand of how viruses seize control of gene expression during infection. The never-ending battle to control the gene expression environment of the host cell has given rise to viral nucleases able to bypass several rate-limiting steps of the cellular RNA decay pathways to accelerate mRNA decay. Insights gleamed from these mechanistic parallels between cellular and viral-mediated decay allow us to draw connections between seemingly distal stages of gene expression. These insights also draw attention to the critical role of RNA structural/sequence elements in governing the fate of mRNA and the widespread consequences of disturbing the equilibrium these RNA elements govern.

Many challenges remain in the study of RNA features that direct cleavage or protection from viral and host endonucleases. To date, very few large-scale screens of the targets/escapees across the eukaryotic transcriptome have been reported. This is due to a number of technical challenges, in particular the fact that most current RNA-seq techniques require significant transcript expression levels to accurately assess shifts in gene expression [75]. This is therefore inherently complicated by the expression of a viral nuclease with widespread RNA decay activity. Looking at RNA stability in pools of infected cells has also historically been a problem during interpretation of sequencing results as infection may progress at different rates in cells. However, with the development single-cell sequencing technology, the next few years will be very essential to provide us with a more accurate overview of RNA decay upon infection.

Identification of RNA elements regulating RNA stability is important to better understand gene expression and the past couple decades have seen a lot of effort in trying to identify and annotate novel regulatory sequences. Using viral nucleases to uncover such elements has been very prolific and we anticipate it will continue to bring to light a vast network of RNA regulatory elements that may be contributing to the regulation of RNA decay.

Furthermore, while this review does not address this aspect of RNA regulation, one of the main "RNA elements" that has taken center stage the past couple of years is that of RNA modifications. There are over 100 of these chemical additions, and they are emerging as potent regulators of mRNAs, miRNAs, and lncRNAs in all aspects of structure, function, and decay. RNA post-transcriptional modifications can control *when* an mRNA is degraded in a complex and nuanced manner [76,77]. Even the localization along the mRNA and stoichiometry of these modifications can influence the fate of the transcript. In the case of N6-methyladenosine (m^6A), where a methyl group is added to an adenine, these modifications serve as platform to recruit m^6A reader proteins. The composition of these m^6A reader complexes will impact multiple aspect of RNA fate, from accelerated degradation by targeting to P-bodies to regulation of splicing and export of mRNAs [78]. Presence of an m^6A modification can also affect the structure of the mRNA, acting like a switch, and creating new platforms for binding. With how deeply ingrained RNA modifications are in the fate of mRNA it only makes sense that an obligate parasite like a virus seeking control over host processes would attempt to subvert or utilize these as well. Future work will reveal how viruses interface with the host post-transcriptional machinery and should yield important insights into the regulation of RNA fate.

Finally, while viral endonucleases can trigger widespread mRNA turnover, they only represent one of many strategies deployed by viruses to control RNA turnover and reshape the host gene expression landscape. Viruses can also encode their own non-coding RNA [79,80] that can have widespread effect on dampening the host anti-viral response. Furthermore, host non-coding RNAs are known to be critical for numerous anti-viral and apoptotic processes, and therefore subverting this arm of the host gene expression machinery is also essential [81]. For example, VacV – in addition to encoding the deccaping enzymes D9 and D10-encodes a poly(A)-polymerase (VP55) that polymerizes a poly-adenosine tract tail onto the vast majority of host microRNA (miRNA), triggering their degradation by the 3′-end uridylation-dependent and the 3′–5′ exonuclease-mediated decay pathways [82]. Some viruses have a more specific strategy to selectively target anti-viral miRNAs. HCMV, for example, encodes a long bicistronic non-coding RNA, UL144–UL145 known as miRDE, that triggers the decay of the cellular miR-17/miR-20a family miRNAs through a non-canonical miRNA–miRNA interaction [83]. These studies emphasize the importance of accounting for the degradation of non-coding RNAs during viral infection and the broader implications of this decay in regard to global RNA interference pathways.

The features that comprise a messenger RNA, from cap-to-tail, define nearly all aspects of RNA life. As such, it is unsurprising that viruses have evolved to take advantage of these RNA elements and the regulatory pathways that are deeply intertwined with these elements. Thus, by studying the mechanisms governing this takeover during infection, we can continue to reveal the integral relationship between the fate of RNA and the fate of the cell.

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