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Targeting of viral RNAs by Upf1-mediated RNA decay pathways

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Abstract

Viral RNAs are susceptible to co-translational RNA decay pathways mediated by the RNA helicase Upstream frameshift 1 (Upf1). Upf1 is a key component in nonsense-mediated decay (NMD), Staufen1-mediated mRNA decay (SMD), and structure-mediated RNA decay (SRD) pathways, among others. Diverse families of viruses have features that predispose them to Upf1 targeting, but have evolved means to escape decay through the action of *cis*- or *trans*-acting viral factors. Studies aimed at understanding how viruses are subjected to and circumvent NMD have increased our understanding of NMD target selection of host mRNAs. This review focuses on the knowledge gained from studying NMD in viral systems as well as related Upf1-dependent pathways and how these pathways restrict virus replication.

INTRODUCTION

Upf1 is an ATP-dependent RNA helicase that is required for several RNA quality control pathways and is highly conserved across eukaryotic systems [1–4]. Nonsense-mediated decay (NMD), the most extensively studied Upf1-dependent pathway, targets aberrant transcripts containing a premature termination codon (PTC) to prevent the production of truncated proteins with potentially detrimental effects [5, 6]. Phosphorylation of Upf1 is necessary for NMD [7–9] and inhibiting NMD results in increased steady-state levels of ~10% of cellular transcripts in multiple eukaryotic systems, demonstrating that NMD effectively regulates a considerable amount of gene expression post-transcriptionally [10–14]. mRNAs that do not contain PTCs make up a portion of cellular transcripts targeted by Upf1 and these transcripts often have upstream open reading frames (uORFs) [15] or introns in their 3’ untranslated region (3’ UTR) [16]. Upf1 predominantly binds 3’ UTRs and translocates over long distances using its RNA helicase activity, efficiently displacing bound proteins [17]. Transcriptome-wide mapping experiments revealed that Upf1 binding is enhanced in GC-rich regions, possibly as a result of reduced translocation [18–20]. The

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elevated levels of Upf1 binding to highly-structured, GC-rich 3' UTRs regions [19] increases the likelihood that phosphorylated Upf1 will interact with a terminating ribosome and initiate mRNA decay [21]. The multi-cistronic organization of viral genomes predisposes viruses to NMD targeting since internal stop codons are perceived as PTCs by the host. Furthermore, viral 3' UTRs are often GC-rich and highly structured for recruiting host factors [22] and/or components of the translation machinery [23], which also predisposes viral RNAs to NMD. As such, diverse families of viruses are targeted by NMD [24, 25], which the virus can evade through the presence of either specific *cis*-acting RNA sequences or viral proteins [26, 27]. In addition to NMD, Upf1 is essential for additional RNA degradation pathways that are initiated by various host factors (Fig. 1A) [21]. However, the roles of additional Upf1-dependent pathways in restricting viruses are poorly understood.

Diverse viruses are Upf1 targets for NMD

Both animal and plant viruses have been used for studying NMD. Studies using the retrovirus *Rous sarcoma virus* (RSV) were the first to demonstrate that Upf1 can target unspliced viral RNAs for NMD in a co-translational manner [28]. Interestingly, Upf1 is a component of the HIV-1 ribonucleoprotein (RNP) and positively regulates retrovirus replication (Table 1) [29–31]. The first evidence that NMD broadly inhibits RNA viruses came from studies demonstrating that NMD restricts *Semliki forest virus* (SFV) accumulation in mammalian cells [32] as well as *Potato virus X* (PVX) and *Turnip crinkle virus* (TCV) in plants [33]. Interestingly, the potyvirus *Turnip mosaic virus* was not targeted by NMD, which was attributed to its polyprotein expression strategy that prevents Upf1 accumulation along the viral mRNA [33]. These findings are in agreement with studies that demonstrate Upf1 is displaced by the translating ribosome and therefore only accumulates in 3' UTRs [34]. NMD also targets several flaviviruses including *Hepatitis C virus* (HCV) [35] and *Zika virus* (ZIKV) [36], and the coronavirus *Murine hepatitis virus* (MHV) [37]. The DNA virus *Kaposi's sarcoma-associated herpesvirus* (KSHV) was the first DNA virus determined to be targeted by Upf1 and NMD [38]. pre-mRNA splicing in the KSHV transcriptome results in retention of several introns downstream of termination codons that in turn predispose KSHV transcripts to NMD [39].

Virus evasion of NMD

Both *cis*-acting RNA sequences and *trans*-acting viral proteins can disrupt NMD and are summarized in Figure 2. Many viruses require ribosome recoding to produce C-terminally extended proteins and maximize coding potential [40, 41]. Viral ribosome recoding sequences (i.e., readthrough or frameshifting) just downstream from termination codons confer NMD-resistance in several plant viruses [42] as well as mammalian viruses like *Moloney murine leukemia virus* (MoMLV)[43, 44], likely as a result of ribosome displacement of Upf1 when the termination codon is by-passed [45].

Ribosomes terminating at the *gag* stop codon in retroviral mRNAs can leave a 3' UTR that is greater than 7 kb in length, predisposing retroviruses to Upf1 targeting. However, NMD-resistance is conferred by RNA stability elements (RSEs; about 400 nt) located downstream of the *gag* stop codon, which are strongly conserved among retroviruses [46–49]. *Human T-cell leukemia virus type 1* (HTLV-1) expresses Tax, a viral protein that directly binds

Upf1 and INT6, a factor associated with eukaryotic initiation factor 3 (eIF3) and NMD [50]. Intricate studies have demonstrated that Tax binding to Upf1 inhibits Upf1 helicase activity and reduces RNA binding, thus effectively inhibiting NMD [51]. Upf1 activity is also antagonized by the SFV helicase and nsP3 in viruses in the *Togaviridae* [32]. Additionally, the HTLV-1 Rex protein has been proposed to protect both viral and host transcripts against NMD [52], however the mechanism remains unclear.

The NMD pathway is disrupted during HCV infection as a result of the viral core protein interfering with the interaction between Within bgcn homolog (WIBG) and the exon-junction complex (EJC) components Y14 and Magoh [35]. Y14, Magoh, and PYM1, an additional EJC component, are antiviral towards ZIKV, *West Nile virus* (WNV), and *Dengue virus* (DENV) as a result of Y14 directly binding viral RNA in a Magoh- and PYM1-dependent manner [53]. The WNV capsid inhibits interactions between PYM1, Magoh, and Y14, and WNV infection causes mis-localization of EJC components [53]. The ZIKV capsid interacts with nuclear Upf1 which leads to Upf1 degradation by the proteasome [36]. The targeting of cytoplasmic RNA viruses by the EJC is somewhat surprising since EJC proteins are largely associated with spliced mRNAs that form in the nucleus [54, 55]. However, studies in *Drosophila* have revealed that Y14 and Magoh can bind intronless transcripts [56] making it possible that EJC components could associate with viral RNAs promiscuously.

Studies using plant viruses have uncovered additional *cis- and trans*-acting viral elements that can thwart NMD. The long-distance p26 movement protein from *Pea enation mosaic virus 2* (PEMV2) confers resistance to both viral and non-viral 3' UTRs [57]. RNA-seq analyses revealed that both an NMD inhibitor and PEMV2 infection resulted in a high degree of overlap between down- and up-regulated transcripts, suggesting that PEMV2 severely impairs the NMD pathway [57]. The transactivator protein (TAV) from *Cauliflower mosaic virus* (CaMV), a DNA virus, disrupts the VARICOSE decapping complex and confers NMD resistance to host transcripts, but it remains unknown if CaMV transcripts are themselves subjected to NMD [58]. A short unstructured region (USR) immediately downstream of the TCV subgenomic RNA termination codon confers NMD resistance to both viral and non-viral NMD targets [42]. Introducing a 2-nt mutation downstream of the USR that forces the USR to form a stable hairpin abolishes NMD protection, demonstrating that the unstructured RNA is inherently NMD-resistant.

Viral RNA sequences provide insight into NMD target selection

The discovery of *cis*-acting viral RNA sequences that confer NMD-resistance has shed light on how cellular transcripts with NMD-inducing features evade NMD surveillance. The RSV RSE has long been studied for its role in conferring stability and NMD-resistance [49, 59]. Polypyrimidine tract binding protein 1 (PTBP1) binds polypyrmidine tracts within the RSV RSE [60], promoting Upf1 dissociation from NMD targets [61]. Nearly 200 human NMD targets are protected by PTBP1 and these transcripts are enriched for polypyrimidine hexamers downstream of their termination codons that bind PTBP1 and confer NMD-resistance [60]. Similar findings have revealed that hnRNP L protects cellular transcripts with long 3' UTRs against NMD by binding to 3' UTRs with CA repeats [62].

Interestingly, highly unstructured RNA sequences immediately downstream of the termination codon, as is found in TCV, are over 4-fold enriched in NMD-resistant human transcripts when compared to target transcripts [19, 42]. Another study identified several NMD-inhibiting termination-proximal *cis* elements in human mRNAs that were enriched for A/U nucleotides (~70%) in the first 200-nt downstream of the stop codon and lack stable secondary structures [63].

In *Arabidopsis thaliana*, alternative splicing generates three eukaryotic release factor 1 (eRF1) transcripts, where eRF1–1 contains an NMD-inducing intron in the 3' UTR [64]. Interestingly, the eRF1–1 termination codon is followed by a 6-nt sequence (CAAUCA) that is similar to the *Tobacco mosaic virus* (TMV) ribosome readthrough signal [64, 65] and readthrough of this codon confers NMD-resistance and contributes to autoregulation of eRF1 protein levels [66]. A human genome-wide study found that the TMV readthrough signal was heavily favored among transcripts that undergo ribosome readthrough when treated with aminoglycosides and become NMD-resistant [67]. Collectively, these examples demonstrate how *cis*-acting viral sequences that confer NMD-resistance can provide insight into cellular NMD target selection, a process that is not fully understood [6].

Timing and efficiency of virus inhibition by NMD

Suppressing Upf1 expression results in increased virus accumulation across eukaryotic systems. However, the ability of Upf1 and NMD to limit virus replication in a biologically significant way at the organismal level remains unknown. NMD has been proposed to mainly occur during the pioneer round of mRNA translation [68], however multiple studies have shown that NMD can occur by chance during all rounds of translation [69–71]. Using single-molecule imaging and tracking individual ribosomes on an NMD target, Hoek *et. al.* (2019) demonstrated that approximately 10% of terminating ribosomes induce NMD [71]. This inefficiency of NMD activation presents a challenge for host cells to use NMD to eliminate viral RNAs. NMD may be most effective at restricting virus replication during early stages of infection when few copies of the viral RNAs are present. After an infection is established, the 10% overall efficiency of NMD is unlikely to appreciably limit virus replication since the cell is overcome with viral RNAs. In support of this conjecture, MHV is most efficiently inhibited by NMD in the first five hours of infection before expression of the viral N protein that inhibits NMD [37].

Additional Upf1-mediated decay pathways

Staufen1-mediated mRNA decay (SMD) requires that the double-stranded RNA binding protein Stau1 bind the 3' UTRs of target mRNAs [72, 73]. Stau1 then recruits Upf1 to the 3' UTRs of target mRNAs for subsequent target degradation [74]. Stable 3' UTR stem-loop structures or intermolecular base-pairing between an mRNA 3' UTR and long non-coding RNA (lncRNA) are sufficient to recruit Stau1 [75]. Knockdown of Stau1 results in the upregulation of 1% of human transcripts demonstrating that SMD, like NMD, can also regulate gene expression post-transcriptionally [76]. The efficiencies of NMD and SMD are inversely correlated with one another since Upf1 is required for both pathways but both Stau1 and the NMD factor Upf2 share binding sites within Upf1 [77, 78]. Whereas Stau1 plays a pro-viral role in multiple virus lifecycles (see Table 1) [79–91], there is currently

limited evidence for Stau1 and SMD targeting viral RNAs. Importantly, Cho *et. al.* found that *Influenza A virus* (IAV) NS1 binding to Stau1 prevents Stau1 from interacting with Upf1 and initiating SMD decay of IAV RNAs [92]. Transcriptome-wide identification of Stau1 binding sites has revealed striking similarities to Upf1 where Stau1 preferentially binds long 3' UTRs containing GC-rich secondary structures absent of specific sequence motifs [93–95]. Although most studies to date have focused on NMD-targeting of viruses, the similarities and intertwined functions of Stau1 and Upf1 suggest that SMD could also restrict accumulation in diverse families of viruses.

Recently, structure-mediated RNA decay (SRD) was demonstrated to selectively degrade transcripts with highly-structured 3' UTRs in a Upf1- and Ras GTPase-activating protein-binding protein 1 (G3BP1)-dependent manner [96]. G3BP1 and Upf1 interact via base-paired RNA sequences that do not overlap with Stau1 binding sites as determined by CLIP-seq [95, 96]. G3BP1 forms stress granule cores during stress leading to sequestration and translational repression of cellular transcripts [97–100]. G3BP1 has several pro-viral (Table 1) and anti-viral roles in virus lifecycles. G3BP1 inhibits *Sendai virus* and *Vesicular stomatitis virus* by facilitating the RIG-I antiviral response [101, 102], G3BP1 sequesters HIV-1 RNA transcripts [103], and G3BP1 is specifically targeted and cleaved by the viral proteases from several picornaviruses [104–106]. Interestingly, SRD targets non-coding circular RNAs (circRNAs) in addition to mRNAs [96] opening the possibility that non-coding viral RNAs could be targeted by Upf1 and SRD.

CONCLUSIONS

The necessity of all viruses to either translate their genomes or antigenomes or produce mRNAs predisposes all viruses to Upf1-targeting (Fig. 1B) and future work will undoubtedly identify additional families of viruses subjected to Upf1-mediated decay. Most research to date has focused on the role of NMD in virus lifecycles, and the identification of *cis*- and *trans*-acting viral factors that interfere with NMD has broadened our understanding of NMD itself. Global inhibition of the NMD pathway has been observed during plant and animal virus infections and may contribute to pathogenesis. However, future work is needed to determine if viruses manipulate host gene expression through NMD-inhibition as a means to shape the transcriptome in a way that favors virus replication. Upf1's involvement in additional pathways like SMD or SRD suggest that viruses are likely affected by additional Upf1-dependent pathways and additional studies dissecting the involvement of individual pathways in virus replication is needed.

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HIGHLIGHTS

- Upf1 targets viral RNAs for degradation through multiple pathways
- Viruses disrupt Upf1-mediated decay in different way through *cis*- and *trans*-acting factors
- Viral systems have yielded insight into Upf1 target selection within host cells

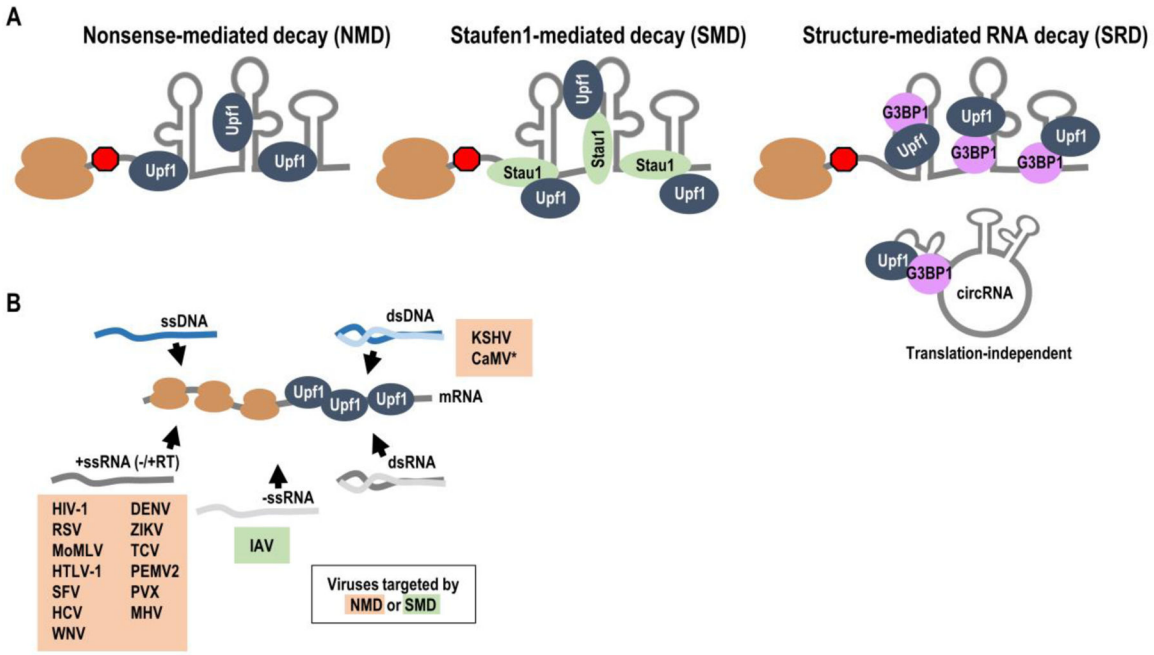

Figure 1: Upf1-dependent decay and targeting of viral mRNAs.

(**A**) Upf1 is essential for nonsense-mediated decay (NMD), Staufen1-mediated decay (SMD) and structure-mediated RNA decay (SRD). Upf1 associates with the 3' UTRs of viral and host 3' UTRs and can initiate NMD. Stau1 binds dsRNA structures in the 3' UTRs of mRNAs and recruits Upf1 for subsequent SMD. G3BP1 binds highly structured regions in conjunction with Upf1 to target mRNAs or non-coding circRNAs for SRD. (**B**) All viruses produce mRNAs that are potential targets of Upf1. Most viruses that are known to be targeted by Upf1 are +ssRNA viruses. IAV (-ssRNA) is targeted by Upf1 and SMD. dsDNA viruses produce mRNAs that are targeted by NMD. *CaMV interferes with NMD, but direct evidence of susceptibility of viral RNAs towards NMD is lacking.

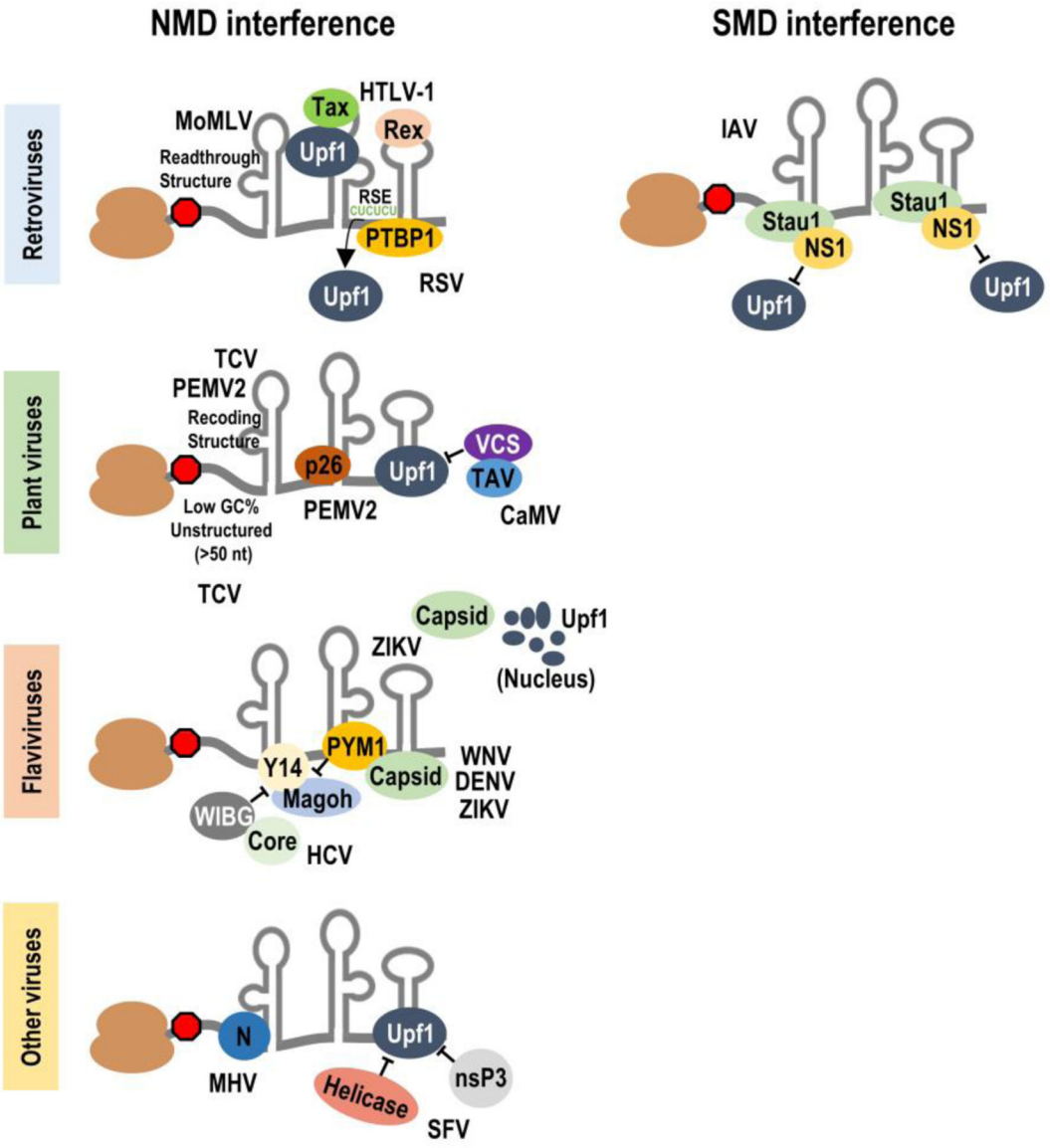

Figure 2: Viruses disrupt NMD and SMD.

Both *cis*-acting RNA sequences and *trans*-acting viral proteins interfere with NMD. Flavivirus-mediated inhibition of NMD generally involves interfering with EJC components. SMD is disrupted by IAV NS1 blocking Upf1 association with Stau1.

Table 1:

Pro-viral roles of Upf1 and associated decay factors

Protein	Virus	Function	Refs.
Upf1	HIV-1	Required for efficient translation of pr55(Gag)	[29]
Upf1	HIV-1	Upf1 incorporates into viral RNPs to promote nuclear export of HIV-1 genomic RNA	[30]
Upf1	HIV-1	Upf1 helicase activity is required for HIV-1 reverse transcription and infectivity	[31]
Stau1	Ebola	Stau1 facilitates interactions between viral RNA and replication machinery	[79]
Stau1	EV-A71	Promotes efficient viral protein translation by binding to 5’ UTR of viral RNAs	[80]
Stau1	HIV-1	Disassembles stress granules to promote efficient translation and trafficking of viral RNAs	[81]
Stau1	IBDV, HCV	Stau1 binds viral dsRNA and interferes with type I interferon response and protein kinase R (PKR)	[82, 88]
Stau1	HIV-1	Component of HIV-1 RNP and regulates HIV-1 assembly and infectivity	[84–87]
Stau1	IAV	Interacts with NS1 and viral ribonucleoprotein and is required for efficient replication	[89–91]
G3BP1	SINV	Limits polyprotein expression, possibly by sequestering viral RNAs in nsP4 replication complexes	[107]
G3BP1	SFV, CHIKV	nsP3 interacts with G3BP1 to sequester viral replication complexes and recruit translation factors	[108]
G3BP1	MNV, HuNoV	Facilitates interactions between VPg, viral RNA, and translation machinery	[109]