Perspective:

Recent transcriptome-wide mapping of UPF1 binding sites reveals evidence for its recruitment to mRNA before translation

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Summary

The ATP-dependent RNA helicase UPF1, a key factor in nonsense-mediated mRNA decay (NMD), was so far thought to be recruited specifically to NMD-targeted mRNAs by aberrantly terminating ribosomes. However, two recent publications reporting independently transcriptome-wide mapping of UPF1 occupancy on RNA challenge this model and instead provide evidence that UPF1 binds to mRNA already before translation. According to the new data, UPF1 appears to initially bind all mRNAs along their entire length and gets subsequently stripped off the coding sequence by translating ribosomes. This re-poses the question of where and how UPF1 engages with mRNA and how the NMD-targeted transcripts are selected among the UPF1-bound mRNAs.

Keywords: posttranscriptional gene regulation; mRNA turnover; mRNA quality control; nonsense-mediated mRNA decay; ribosome; translation termination; UPF1; RENT1; SMG-2

Nonsense-mediated mRNA decay

During their entire lifespan, mRNAs are accompanied by a changing set of proteins and small non-coding (nc)RNAs forming complex messenger ribonucleoprotein particles (mRNPs) [1]. The components of an mRNP and their positioning on the mRNA to a large extent determine the fate of the mRNA molecule and hence play important roles in the regulation of gene expression. Cells possess multiple surveillance mechanisms that detect aberrant mRNPs and promote the degradation of the concerned mRNA. Arguably the best-studied surveillance mechanism is nonsense-mediated mRNA decay (NMD), which was discovered more than 30 years ago and initially described as a translation-dependent process that selectively degrades mRNAs with truncated open reading frames (ORFs) due to premature translation-termination codons (PTC) [2, 3], thereby preventing the expression of potentially deleterious C-terminally truncated proteins. In humans, one out of three disease-associated mutations leads to the production of PTC-containing mRNA and NMD therefore influences the severity of the clinical manifestations caused by these mutations [4-6]. Besides its quality control function, genome-wide mRNA profiling experiments revealed that NMD also regulates the abundance of many physiological mRNAs coding for functional proteins (reviewed in [7]).

Unraveling UPF1 functions – the key to understand NMD

In spite of intensive research over the past decades, our understanding of the molecular mechanism of NMD is still fragmentary. Among the so far identified proteins involved in NMD, UPF1 plays a – if not the – key role: with 48.5% amino acid identity between human and baker's yeast it is the most conserved NMD factor [8], it is essential in vertebrates and flies [9-11], and binds RNA unlike most other NMD factors. With an estimated $3*10^6$ molecules per cell, UPF1 is a quite abundant protein [12]. In relation to the approx. $3*10^5$ mRNA molecules per cell [13], there are on average about 10 UPF1 proteins available per mRNA, which is comparable to the approx. 20 molecules of poly(A) binding protein (PABP) per mRNA [14].

UPF1 is an ATP-dependent 5'-3' RNA helicase belonging to the superfamily 1 (SF1) [15]. Two RecA-like domains form the helicase domain with the ATP-binding pocket located in between [16]. The helicase and the ATPase activities of UPF1 are repressed by an N-terminal cysteine/histidine-rich zinc finger (CH) domain and a C-terminal

domain rich in serine-glutamine motifs (SQ domain) [17-19]. Since UPF1's ATPase activity is required for NMD, the inhibitory configurations of the flanking CH and SQ domains must be relieved at some point during the NMD process. While the inhibitory effect of the CH domain is suspended by its interaction with UPF2, which pulls the CH domain from the Rec2A subdomain to the Rec1A subdomain on the opposite side of the helicase core [17], it is currently unknown how the SQ domain-mediated inhibition is overcome [19]. There is evidence that the ATPase activity of UPF1 is required for disassembling the NMD complex after the endonucleolytic cleavage of the target mRNA, since UPF1 mutants unable to bind or hydrolyze ATP led to the accumulation of 3' RNA decay intermediates [20]. As expected for a helicase, UPF1 has the capacity to unwind RNA duplexes *in vitro*, an activity that is promoted by the other two conserved NMD factors UPF2 and UPF3B [18], but it remains to be seen if UPF1 also translocates on RNA *in vivo*.

In metazoans, UPF1 is a phosphoprotein with more than a dozen serine or threonine followed by glutamine (S/TQ) motifs, and half of them can be phosphorylated *in vitro* by the phosphatidylinositol 3-kinase-related protein kinase SMG1. *In vivo*, UPF1 undergoes a cycle of phosphorylation and dephosphorylation, which is essential for NMD in metazoans (reviewed in [21]). The exact function of UPF1 phosphorylation during NMD and its relation to ATP binding and hydrolysis is not known, but the endonuclease SMG6 and the decay factor-adaptors SMG5/SMG7 and PNRC2 preferentially interact with phosphorylated UPF1 and ATPase-deficient UPF1 mutants accumulate in a hyper-phosphorylated state [22, 23, 24]. Recently, Yamashita and colleagues identified phospho-T28 and phospho-S1096 to be required for the interaction of UPF1 with SMG6 and SMG7, respectively [25].

Current model: selective and translation-dependent recruitment of UPF1 to NMD-targeted mRNAs

A central unsolved question is how the NMD pathway selects its target mRNAs. Based on a wealth of biochemical and genetic data, including the above-mentioned characteristics of UPF1, the following working model has emerged during the last years: in a nutshell, NMD ensues on mRNAs with termination codons in an environment unfavorable for efficient translation termination [7, 26]. The underlying assumption is that the translation termination is a highly regulated process involving the orchestrated function of many factors, comparable to translation initiation, and

that in the absence of a termination promoting factor, ribosomes stall at the termination codon [27, 28] and lead to the assembly of NMD factors that ultimately degrade the mRNA. Supporting this model, UPF1 was found to interact with the release factors eRF1 and eRF3 [23, 29, 30] and this interaction is antagonized by the interaction between eRF3 and poly(A) binding protein C1 (PABPC1) [31]. PABPC1 and its yeast homologue Pab1p have been shown to stimulate termination [32, 33, 34] and tethering of PABPC1/Pab1p near NMD-triggering termination codons efficiently suppresses NMD [27, 31, 34-36]. This model provides an explanation why not only PTCs but also long 3' UTRs can elicit NMD [36-38]. The detection of an immunoprecipitable complex consisting of SMG1, UPF1, eRF1 and eRF3 (called SURF complex [23]) suggested that SURF might form at ribosomes that fail to terminate properly. According to this model, UPF1 would be recruited in a translation-dependent manner and only to NMD-targeted transcripts. This view was corroborated by studies reporting preferential association of UPF1 with PTC-containing transcripts in C. elegans [39] and in human cells [40].

In the SURF complex, SMG1 kinase is kept in an inactive state by two regulatory proteins, SMG8 and SMG9, until UPF2 interacts with UPF1 [41]. The conformational change induced by UPF2 leads to the dissociation of SMG8 and SMG9 and UPF1 phosphorylation by the activated SMG1 kinase [41, 25]. As already mentioned above, UPF1 phosphorylation is then thought to commit the mRNA to degradation by the recruitment of the decay promoting factors SMG6, SMG5/SMG7 and PNRC2 (reviewed in [7]).

In the case of exon junction complex (EJC)-independent NMD, free UPF2 would have to join the SURF complex by diffusion, which is expected to be a rate-limiting step and results in rather inefficient NMD [37, 42]. In contrast, classical NMD-targeted mRNAs harbor an NMD-enhancing EJC downstream of the termination codon. The tetrameric EJC core consisting of eIF4AIII, MAGO, MLN51 and Y14 binds to UPF3, which in turn recruits UPF2 [43, 44, 45, 46]. EJCs assemble at exon-exon junctions during splicing [47, 48, 49, 50] and are displaced from the coding sequence during translation by the scanning ribosomes [51, 52], so that after onset of translation most mRNAs will be devoid of EJCs. However, if the termination codon is located more than 30 nucleotides upstream of the 3'-most EJC, which often is the case for PTCs, these EJCs remain bound to the mRNA and enhance NMD by providing pre-bound UPF2 ready to interact with the SURF complex (reviewed in [7]).

Evidence for translation-independent mRNA interaction of UPF1

The recent development of CLIP-seq (crosslinking and immunoprecipitation followed by high-throughput sequencing) techniques allows the transcriptome-wide detection of the binding sites for RNA-binding proteins [53, 54]. To explore how NMD shapes the embryonic transcriptome, Hurt and colleagues performed integrated genome-wide analyses of UPF1 binding locations, NMD-regulated gene expression, and translation in murine embryonic stem cells (mESCs) [55], and we used CLIP-seq to map the RNA-binding sites of UPF1 in human cells [56]. In both cell types, UPF1 was detected predominantly in 3' UTR sequences, where it was quite evenly distributed, whereas coding sequence (CDS) was much less covered with UPF1. This high enrichment of UPF1 in 3' UTRs is consistent with a study from Hogg and Goff, who investigated UPF1 association with PP7-tagged reporter transcripts and found that the amount of UPF1 interacting with the transcripts correlated with their 3' UTR length [57]. Intriguingly and contradicting the above-described NMD model, Hogg and Goff further reported that the UPF1 interaction with the tested 3' UTRs occurred even in the absence of translation. To explore this discrepancy, both CLIP-seq studies also analyzed RNA binding of UPF1 in translation-suppressed HeLa cells [56] and mESCs [55], respectively. Translation inhibition unveiled a striking redistribution of UPF1 towards CDS [55, 56], indicating that before translation UPF1 interacts with mRNAs along its entire length and that during translation elongating ribosomes displace it from the CDS (Figure 1). Displacement of UPF1 from RNA by elongating ribosomes is consistent with results from Hogg and Goff, who showed that even a low frequency of translational read-through induced by retroviral RNA elements preceding the stop codon reduced the UPF1 association with long 3' UTRs and stabilized the otherwise NMD-targeted reporter transcripts [57]. Since CLIP yields relative data, i.e. enrichment of sequence x in the IP relative to its abundance in the transcriptome, the shift of UPF1 occupancy from 3' UTR to CDS upon translation inhibition could result from an increased binding to CDS, a decreased binding to 3' UTRs or a combination of both. To distinguish between these possibilities, we performed RNA-immunoprecipitations (RIP) in which CDS and 3' UTR of selected endogenous mRNAs were separated by an oligo-mediated RNase H cleavage during the IP [56]. This assay confirmed the marked increase of UPF1 association with CDS upon translation inhibition,

corroborating the conclusion that UPF1 binds to CDS in the absence of translation and gets displaced from CDS during translation.

Furthermore, association of UPF1 with long ncRNAs was also detected, providing additional evidence for translation-independent binding [56]. As expected for untranslated RNA, the UPF1 binding on these transcripts was not altered by translation inhibition.

Specificity of UPF1 for mRNA

What do the two CLIP papers tell us about the specificity of the UPF1-RNA interaction? As one might have expected for a helicase, motif searches did not reveal any preferred RNA binding sequence for UPF1. Nevertheless, UPF1 was clearly distributed along RNA in a non-random fashion. Hurt and colleagues found an enriched UPF1 density in G-rich areas with an increased propensity to form secondary structures [55]. Our analysis did not reveal any sequence preference except for a bias towards U at the position of the crosslink [56], which most likely just reflects the better UV crosslinking efficiency of U compared to the other bases [58].

Interestingly with regards to the question of when and how UPF1 is recruited to RNA, UPF1 density was 10 – 30-fold higher in exons than introns in both studies, and also very little UPF1 association was detected with rRNA and tRNA. This suggests that UPF1 associates with mRNA during or after splicing. UPF1 recruitment to mRNA during splicing together with the EJC seemed to be an attractive hypothesis at first glance, because UPF1 interacts with the EJC through UPF2 and UPF3 [46], but both CLIP studies did find no correlation between UPF1 binding and EJC deposition. Hurt and colleagues showed that genes encoding for transcripts with high UPF1 binding density in their 3' UTR were not enriched for the expression of downstream EJC-containing isoforms [55], and we did not find any correlation between the UPF1 and the recently reported EJC binding sites [56, 59]. Moreover, knockdown of the EJC core factor eIF4AIII did not affect UPF1 association with mRNA [60]. Thus, the available data indicates that UPF1 engages with mRNA independently of EJCs and the specificity of UPF1 for mRNA remains to be explored in the future. It is conceivable that UPF1 binds RNA in the cytoplasm and that mRNA specificity results from the fact that the highly structured tRNAs and the rRNAs, which assemble through a highly orchestrated process into ribosomal subunits, are simply not accessible for UPF1 binding.

If not UPF1 binding, what is the distinctive step in NMD target selection?

According to the above-described NMD model, UPF1 recruitment to an mRNA marks this mRNA for NMD and UPF1 should therefore preferentially – if not exclusively – be associated with NMD targeted mRNAs. Consistent with this model, a recent study conducted with a set of reporter transcripts reported the accumulation of more UPF1 molecules per 3' UTR length unit if the mRNA was an NMD target [40]. However, the transcriptome-wide analyses gave a more complex picture: the results of Hurt and colleagues indicate a correlation between UPF1 binding and NMD only for transcripts with short 3' UTRs, most of which harbor an EJC as the NMD-inducing signal [55]. However, no significant difference in UPF1 density was detected when we compared two sets of experimentally identified endogenous NMD targets [38, 61] with corresponding control groups of transcripts that are not affected by NMD [56].

The lack of a strict correlation between UPF1 binding and NMD together with the evidence for translation-independent binding of UPF1 to mRNA challenges the idea that UPF1 binding would be the step that commits an mRNA to the NMD pathway. At the same time, it re-opens the question about what the NMD-triggering step then would be. At this moment, one can only speculate and more research is clearly needed. An obvious possibility is that the SMG1-mediated phosphorylation of UPF1, which leads to recruitment of RNA degradation factors (see above), represents the activation step of NMD that specifically occurs when ribosomes fail to properly terminate translation (Figure 1). A variation of the same theme is that UPF1 indiscriminately binds to mRNA and acts there like a ticking bomb: if not stripped off by ribosomes within a certain time window, it will become phosphorylated and induce the decay of the mRNA. Both scenarios predict that hyper-phosphorylated UPF1 would be found associated specifically with NMD targeted transcripts, a prediction that can be tested by purification of affinity-tagged reporter transcripts. It could also be that ATP binding or hydrolysis by UPF1 are the crucial steps to activate NMD.

Crosslink sites do not necessarily represent the locations of initial binding

Another open question is whether UPF1 initially engages with mRNA at specific sites. The crosslinking sites detected in the two CLIP studies do not necessarily represent the positions of initial UPF1 binding to mRNA, because UPF1 can probably translocate on RNA by virtue of its 5'-to-3' helicase activity. An interesting follow-up

study would therefore be to CLIP exogenously expressed tagged UPF1 mutants defective in ATP binding, ATP hydrolysis and/or helicase activity.

UPF1 reveals its secrets only slowly

While it is obvious to all researchers in the field that understanding UPF1 function is key to understand NMD, this protein does not divulge its secrets easily. Our current understanding can be summarized by the following statement by J.R. Hogg: "*…UPF1 accumulation in mRNPs is a prerequisite for decay but is not sufficient to consign the mRNP to destruction. Instead the initiation of decay requires the completion of one or more additional rate-limiting steps…"* [62]. Additional investigations of the activities, regulation and interactions of UPF1 and other NMD proteins in the context of endogenous mRNPs are needed to reveal the order of events leading from UPF1 accumulation in mRNPs to the decay of the mRNAs.

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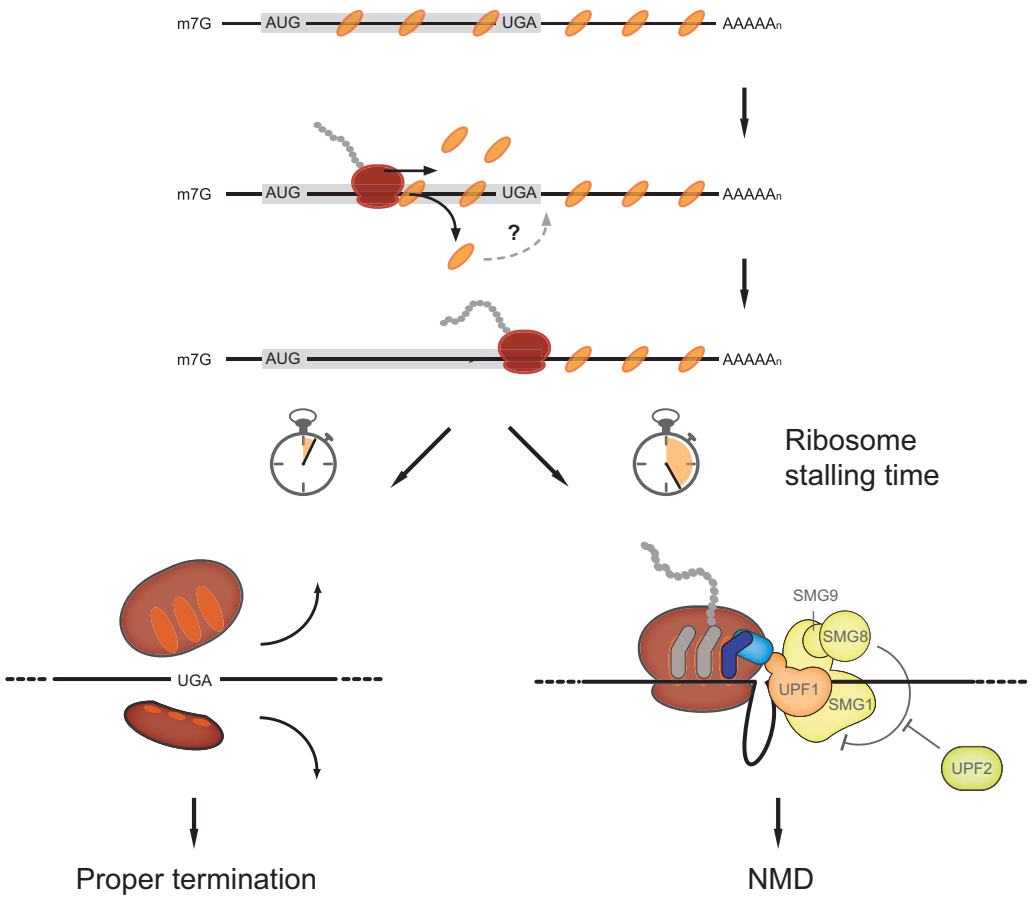
Figure legend

Figure 1. Modified working model for NMD based on the new evidence for UPF1 interaction with mRNA before translation. The coding sequence (CDS), starting with AUG and ending with a stop codon (UGA in this Figure), is depicted by a light grey box. UPF1 (orange ovals) associates along the entire mRNA during or after splicing, but before translation starts. The ribosome (red) then displaces UPF1 from the CDS. It is not known whether displaced UPF1 can rebind in the UTR regions (indicated by the question mark). According to the model, the kinetics of translation termination determines if NMD ensues. Proper termination is typified by a short residence time of the ribosme at the stop codon (bottom left), while the absence of termination promoting signals (e.g. PABPC1) stalls the ribosome at the stop codon, allowing remaining UPF1 to interact with the release factors (eRFs, light blue) and the SMG1 complex (yellow; bottom right). Interaction of UPF2 (light green) with the UPF1:SMG1:eRFs (SURF) complex induces a conformational change that leads to dissociation of SMG8 and SMG9 and to the activation of SMG1's kinase activity. Phosphorylated UPF1 subsequently recruits directly (SMG6) and/or indirectly (SMG5/SMG7) RNA decay factors, leading to the degradation of the mRNA.


m7G
AUG
UGA
AAAAAn
?
Ribosome
stalling time
UGA
SMG9
SMG8
UPF1
SMG1
UPF2
Proper termination
NMD