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Review article

# Cytoplasmic mRNA decay and quality control machineries in eukaryotes

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## Abstract

mRNA degradation pathways have key regulatory roles in gene expression. The intrinsic stability of mRNAs in the cytoplasm of eukaryotic cells varies widely in a gene- and isoform-dependent manner and can be regulated by cellular cues, such as kinase signalling, to control mRNA levels and spatiotemporal dynamics of gene expression. Moreover, specialized quality control pathways exist to rid cells of non-functional mRNAs produced by errors in mRNA processing or mRNA damage that negatively impact translation. Recent advances in structural, single-molecule and genome-wide methods have provided new insights into the central machineries that carry out mRNA turnover, the mechanisms by which mRNAs are targeted for degradation and the general principles that govern mRNA stability at a global level. This improved understanding of mRNA degradation in the cytoplasm of eukaryotic cells is finding practical applications in the design of therapeutic mRNAs.


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## Introduction

Gene expression is regulated at multiple levels, including mRNA turnover. The intrinsic stability of different mRNAs varies by orders of magnitude, and individual mRNAs can be stabilized or destabilized in response to cellular cues[1–3]. Moreover, mRNAs that are produced with errors or that undergo damage that compromises the translation process need to be rapidly turned over[4,5]. mRNA degradation can also be activated globally during developmental transitions, by cell conditions that require dramatic changes in gene expression[6,7], or when cells are infected by viruses[8]. Proper degradation of mRNAs has a key role in processes such as inflammation, in which coordinated degradation of mRNAs encoding inflammatory factors prevents chronic inflammation[9]; embryogenesis, in which large numbers of maternal mRNAs need to be turned over during the maternal-to-zygotic transition[10]; and the warfare between mRNA degradation machineries activated by viruses and their hosts, which compete to deplete cells of viral or antiviral mRNAs[11]. These events require carefully choreographed activation of the enzymes that carry out mRNA degradation to ensure that targeted mRNAs are degraded while others remain untouched.

Studies over the past few decades have identified the central enzyme complexes responsible for degradation of mRNAs in the cytoplasm of eukaryotic cells and the main pathways that control the stability of mRNAs. However, these early studies were generally limited to mRNA reporters or the mRNAs of a few select genes, which limited the scope of conclusions, and most mRNA decay complexes lacked structural information to guide detailed mechanistic insights. Recent advances in structural, single-molecule and genome-wide studies have now provided unprecedented new insights into the mechanisms and regulation of mRNA degradation.

In this Review, we discuss the ensemble of pathways that orchestrate mRNA degradation in the cytoplasm of eukaryotes and their intimate link to the translation process. We describe the enzymes that carry out cytoplasmic mRNA degradation and what determines whether and when they act on mRNAs. We discuss what dictates the intrinsic and regulated stability of mRNAs, how mRNA surveillance pathways identify and degrade aberrant mRNAs that are compromised for translation, and how these pathways may impact therapeutic mRNAs. An equally complex set of pathways that coordinate RNA degradation in the nucleus has been the subject of recent reviews and will not be discussed here[12,13]. We focus on human cytoplasmic mRNA decay pathways, but the proteins and mechanisms are highly conserved among eukaryotes, and organism-specific differences will be pointed out where they are known.

## The cytoplasmic mRNA degradation complexes

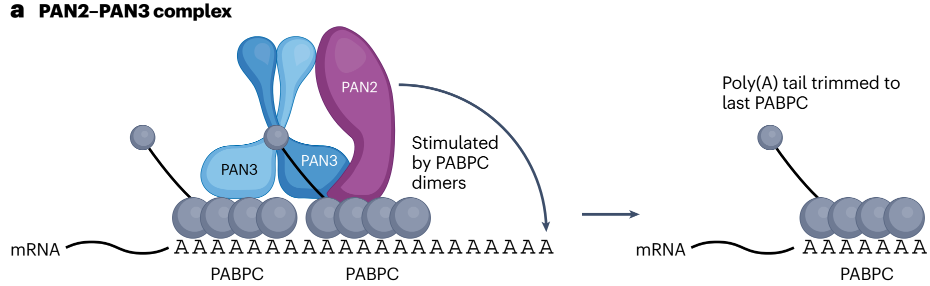
The central enzymes responsible for mRNA degradation in eukaryotes have been identified over the past few decades. All eukaryotic mRNAs are born with 5′ 7-methyl-guanosine ($m^7G$) caps and, with the exception of metazoan replication-dependent histone mRNAs, 3′ end poly(A) tails. These two modifications distinguish mRNAs from other RNAs of the cell and, via the association in the cytoplasm of the $m^7G$ cap with the eIF4F complex and the poly(A) tail with the cytoplasmic poly(A)-binding protein (PABPC), promote the engagement of mRNAs with translation machinery[14,15]. All mRNAs are transient molecules with lifetimes that vary widely between mRNAs and between tissues and cell types. For example, in human cells some mRNAs needed for transient bursts of protein production degrade within 15–30 min of their initial synthesis, whereas others that produce proteins in continuous demand can be stable for many hours. Moreover, some mRNAs are kept stable for longer periods of time when stored for future use, such as mRNAs that undergo long journeys to neurite outgrowths and maternal mRNAs that are stored in oocytes for days to weeks, if not longer, awaiting fertilization. When mRNAs reach the end of their lifetime they are subject to degradation, which, in most cases, is initiated by removal of the poly(A) tail by the process of deadenylation[16–18]. This is generally followed by excision of the $m^7G$ cap by decapping, which exposes the mRNA to exonucleolytic degradation from the 5′ end. In specialized cases, mRNA degradation can also be initiated by endonucleolytic cleavage, which exposes the mRNA body to exonucleolytic degradation from the 5′ and 3′ ends produced at the site of cleavage.

### The complexes that carry out mRNA deadenylation

There are two major complexes responsible for mRNA deadenylation in the eukaryotic cytoplasm: the PAN2–PAN3 complex[19,20] and the CCR4–NOT complex[21] (Fig. 1). These complexes differ greatly in their specificity for poly(A) tails and their effects on mRNA turnover. Whereas numerous studies have implicated the CCR4–NOT complex in deadenylation leading to degradation of mRNAs, the PAN2–PAN3 complex may instead function primarily to stochastically trim poly(A) tails in the absence of mRNA turnover.

The PAN2–PAN3 complex consists of a catalytic subunit — the DEDD exonuclease PAN2 — in complex with a PAN3 protein dimer[22,23] (Table 1 and Fig. 1a). PAN2 (and CNOT7 and CNOT8, CNOT7/8, its homologues in the CCR4–NOT complex) achieves specificity towards poly(A) tails not through direct interaction with adenine bases but instead by recognizing the ability of poly(A) tails to form stacked helical structures[24]. Biochemical and genetic studies have demonstrated that the PAN2–PAN3 complex is activated by PABPC, which interacts directly with PAN3, that the preferred substrates of the PAN2–PAN3 complex are mRNAs with long poly(A) tails and that PAN2–PAN3 does not degrade the poly(A) tail beyond the last PABPC molecule[19,22,23,25–27]. Recent structural studies have revealed that the PAN2–PAN3 complex interacts specifically with dimerization interfaces of PABPC[28], which binds cooperatively to poly(A) tails of mRNAs with each monomer covering approximately 30 adenosines[29,30]. This provides a structural rationale for how PAN2–PAN3 specifically acts on long poly(A) tails: only poly(A) tails longer than approximately 60 adenosines can accommodate the multiple PABPC molecules required for PAN2–PAN3 interaction. It has long been known that even stable mRNAs slowly deadenylate over time[16], and genome-wide studies have revealed the accumulation of long-lived mRNAs with short poly(A) tails in somatic cells[31,32]. Thus, PAN2–PAN3 may act as a molecular clock that trims long poly(A) tails over time, perhaps to prevent stable mRNAs from titrating out cytoplasmic pools of PABPC or to facilitate eventual degradation of ageing mRNAs.

The CCR4–NOT complex contains two exonucleases: one each of the EEP exonucleases CNOT6/6L (Ccr4 in yeast) and the DEDD exonucleases CNOT7/8 (Caf1, also known as Pop2, in yeast), a homologue of PAN2 (ref. 21). These exonucleases assemble into the large multisubunit CCR4–NOT complex via interaction with the scaffold protein CNOT1 (Not1 in yeast), which in turn interacts with other subunits of the CCR4–NOT complex[33,34] (Table 1 and Fig. 1b). Although both exonucleases of the CCR4–NOT complex show specificity towards adenosines, biochemical studies have demonstrated differences in their activities towards PABPC-associated poly(A) tails. CNOT7/8 activity is inhibited by PABPC, whereas CNOT6/6L can displace PABPC from the poly(A) tail and is indeed stimulated by PABPC in vitro[26,35]. Although the specific workload between these two deadenylases is not fully understood, their



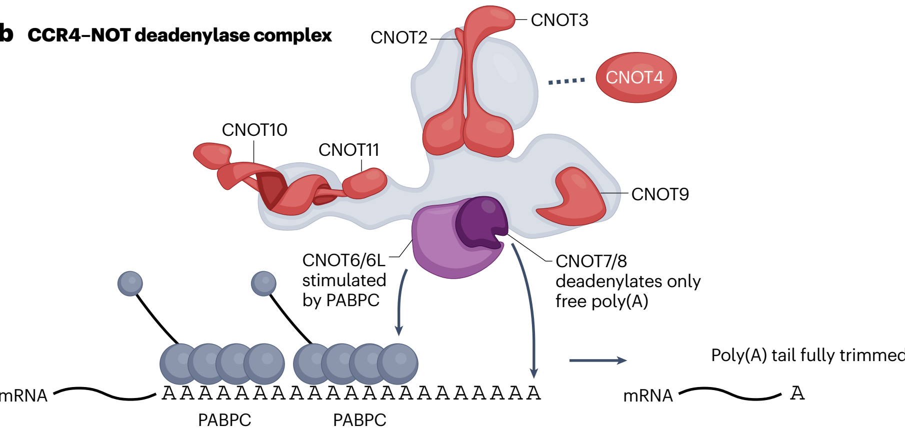




**Fig. 1 | Complexes responsible for deadenylation of cytoplasmic mRNAs. a**, The cytoplasmic poly(A) binding protein (PABPC) binds cooperatively to the poly(A) tail of mRNAs. The PAN2–PAN3 deadenylase complex is recruited to PABPC dimerization interfaces and can deadenylate poly(A) tails until the last PABPC. **b**, The CCR4–NOT deadenylase complex promotes deadenylation through the combined action of deadenylases CNOT6/6L, which are stimulated by PABPC, and CNOT7/8, which deadenylate only free poly(A). CNOT co-factors of the CCR4–NOT complex are shown on top of the CNOT1 scaffold protein, which is illustrated in grey.

combined activities allow the CCR4–NOT complex to promote deadenylation beyond the last PABPC and initiate degradation of the mRNA body. This ability of the CCR4–NOT complex to degrade the poly(A) tail beyond the last PABPC molecule is coupled to subsequent decapping and degradation of the mRNA from the 5′ end.

## mRNA degradation from the 5′ end

The coupling of deadenylation by the CCR4–NOT complex with mRNA decapping occurs via both direct and indirect mechanisms. The primary machinery responsible for mRNA decapping in the eukaryotic cytoplasm is highly dynamic and consists of multiple alternative complexes (Table 1 and Fig. 2A). At the core of these complexes is the NUDIX-domain-containing DCP2 decapping enzyme and its binding partner DCP1, which is required for DCP2 activity[36–39]. The DCP2–DCP1 complex is stimulated by various enhancers of decapping. In metazoans, the DCP2–DCP1 interaction is stabilized by EDC4 (no yeast homologue), which interacts with both DCP1 and DCP2 (refs. 40,41). In some organisms, DCP1 also interacts with small proline-rich proteins PNRC1/2 (Edc1/2 in yeast), which stimulate decapping activity[42–45]. Two other enhancers of decapping, EDC3 and LSM14A (Scd6 in yeast), associate with the DCP1–DCP2 complex in a mutually exclusive manner, and these complexes in turn interact with decapping activators PATL1 (Pat1 in yeast) and the RNA helicase DDX6 (Dhh1 in yeast)[46]. The many interactions between the proteins of the decapping complex network are carried out by a mixture of folded protein domains and intrinsically disordered regions (IDRs), many of which are rapidly evolving[46].

Decapping is coupled to CCR4–NOT-mediated deadenylation by at least two mechanisms (Fig. 2B). First, deadenylation beyond the last PABPC molecule promotes decapping by an indirect mechanism that relies on the creation of a binding site for the LSM1–7–PATL1 complex, which specifically associates with deadenylated mRNA 3′ ends. PATL1-mediated interactions with multiple decapping components subsequently activate decapping[47–50]. In some organisms, LSM1–7–PATL1 binding to deadenylated mRNA 3′ ends is stimulated by uridylation by terminal uridylyl transferases (TUT4/7 in human, Cid1 in *Schizosaccharomyces pombe*), which can also activate decapping independently of deadenylation[51,52]. A second link between deadenylation and decapping is mediated by the decapping enhancer DDX6, which interacts directly with the CNOT1 scaffold of the CCR4–NOT complex[53–56] and engages with the decapping network via mutually exclusive interactions with PATL1, EDC3 and LSM14A[55,57,58]. Although these direct and indirect interactions promote decapping as a consequence of deadenylation, decapping rates have been reported to vary widely between mRNAs, suggesting that the decapping step can also be regulated independently of deadenylation[18,59].

It is still not fully understood how the decapping machinery gains access to the m$^7$G cap, which is normally occupied by the eIF4F translation initiation complex[60]. The budding yeast homologues of decapping activators DDX6 (Dhh1), LSM14A (Scd6) and PATL1 (Pat1) are known to inhibit translation initiation[61,62], which may be related to remodelling of the eIF4F complex at the m$^7$G cap. The act of deadenylation itself may also help to destabilize the eIF4F complex by the release of PABPC, which interacts with eIF4F via its eIF4G subunit[63]. Moreover, some mRNA decay activators interact with translation repressors that may help to destabilize the eIF4F–cap complex, for instance, proteins that compete with eIF4F for cap-binding (for example, 4EHP in human[64]) or CUP proteins, which disrupt the eIF4E–eIF4G interaction within

**Table 1 | Complexes responsible for degradation of mRNA in the cytoplasm**

| Complex | Subunit name | | Function |
|---|---|---|---|
| | *Homo sapiens* | *Saccharomyces cerevisiae* | |
| PAN2–PAN3 | PAN2 | Pan2 | DEDD exonuclease (deadenylase) |
| | PAN3 | Pan3 | Binds PABPC dimers |
| CCR4–NOT | CNOT1 | Not1 | Scaffold |
| | CNOT2 | Not2 | NOT box scaffold |
| | CNOT3 | Not3/5 | NOT box scaffold/binds ribosome E-site |
| | CNOT4 | Not4 | RING E4 ligase |
| | CNOT6/6L | Ccr4 | EEP exonuclease (deadenylase) |
| | CNOT7/8 | Caf1 | DEDD exonuclease (deadenylase) |
| | CNOT9 | Caf40 | Protein–protein interaction |
| | CNOT10 | NA | RNA binding |
| | CNOT11 | NA | RNA binding |
| Decapping–5′-to-3′ decay | DCP1A/1B | Dcp1 | Decapping subunit |
| | DCP2 | Dcp2 | Decapping catalytic subunit (NUDIX domain) |
| | EDC4 | NA | Decapping subunit |
| | PNRC1/2 | Edc1/2 | Decapping enhancer |
| | EDC3 | Edc3 | Decapping enhancer |
| | LSM14A | Scd6 | Decapping enhancer/translation repressor |
| | DDX6 | Dhh1 | RNA helicase/translation repressor |
| | PATL1 | Pat1 | mRNA binding, scaffold |
| | LSM1–7 | Lsm1–7 | Deadenylated mRNA 3′ end binding |
| | XRN1 | Xrn1 | 5′-to-3′ exonuclease |
| SKI–exosome | SKIV2L | Ski2 | RNA helicase |
| | TTC37 | Ski3 | TPR protein |
| | WDR61 | Ski8 | WD-repeat protein |
| | HBS1L3 | Ski7 | GTP-binding protein |
| | EXOSC1 | Csl4 | Exosome cap component |
| | EXOSC2 | Rrp4 | Exosome cap component |
| | EXOSC3 | Rrp40 | Exosome cap component |
| | EXOSC4 | Rrp41 | Exosome core component |
| | EXOSC5 | Rrp46 | Exosome core component |
| | EXOSC6 | Mtr3 | Exosome core component |
| | EXOSC7 | Rrp42 | Exosome core component |
| | EXOSC8 | Rrp43 | Exosome core component |
| | EXOSC9 | Rrp45 | Exosome core component |
| | DIS3L | Rrp44 | Exosome processive exonuclease |

NA, not applicable; PABPC, cytoplasmic poly(A) binding protein.

the eIF4F complex[65,66]. Although the processes of deadenylation and decapping inhibit recruitment of new ribosomes to mRNAs via the release of PABPC and eIF4F, respectively, several lines of evidence suggest that elongating ribosomes can complete translation even while the mRNA is undergoing degradation, which may explain why most mRNA degradation occurs in the same 5′-to-3′ direction as translation[67].

Decapping is further coupled to subsequent degradation of the mRNA body from the 5′ end, which is mediated by association of the cytoplasmic 5′-to-3′ exonuclease XRN1 with the mRNA decapping network via interactions that seemingly differ between organisms[41,46,62,68,69] (Fig. 2A). These interaction networks, which connect the CCR4–NOT deadenylase with the decapping machinery and the 5′-to-3′ exonuclease XRN1, ensure efficient decapping and exonucleolytic degradation of the mRNA body following mRNA deadenylation.

The dynamic network of folded domain and IDR interactions within the decapping factor network, and their assembly with RNA targets, underlie their capacity to form RNA granules, or biomolecular condensates, known as P-bodies[70–72] (Fig. 2C). The extent of P-body formation in cells is dictated by the concentration of decapping factor-associated, translationally repressed mRNAs. Therefore, conditions that promote the targeting of mRNAs for decapping or slow down the downstream degradation steps cause increased P-body formation in cells. The capacity to form P-bodies is conserved among eukaryotes and thus must be an important property of cells. However, although there has been much progress in understanding the mechanisms that underlie P-body formation and dissolution, the importance of P-body formation in mRNA repression and degradation remains poorly defined and under active investigation[72–76].

## mRNA degradation from the 3′ end

In addition to the coupled deadenylation–decapping–5′-to-3′ exonucleolytic decay pathway described above, cytoplasmic mRNAs can also be exonucleolytically degraded from exposed 3′ ends produced by deadenylation or endonucleolytic cleavage. A major factor in 3′ end degradation is the cytoplasmic exosome complex[77] (Table 1 and Fig. 3a). The exosome consists of a nine-subunit barrel-like core structure, which forms a channel that can accommodate a single-stranded RNA[78]. Located at the bottom of the channel is a processive DIS3 3′-to-5′ exonuclease (DIS3L, also known as DIS3L1, in the human cytoplasmic exosome)[79,80]. The top of the cytoplasmic exosome core is associated with a complex centred around the RNA helicase SKI2 (also known as SKIV2L), which unwinds and feeds RNA substrates into the exosome core channel for degradation by the DIS3 exonuclease[81–84]. The SKI2 helicase associates with co-factors SKI3 (also known as TTC37) and SKI8 (also known as WDR61) to form the cytoplasmic exosome-associated SKI complex, which is linked to the exosome core via another SKI protein, SKI7 (also known as HBS1L3).

Recent genome-wide studies in mouse embryonic stem cells identified mRNAs, as opposed to non-coding RNAs, as the primary targets of the SKI–exosome complex and showed enriched association of SKI2 within mRNA coding regions[85]. Meanwhile, structural studies have demonstrated a direct interaction of the SKI complex with the ribosome near the mRNA entry tunnel[86–88], and biochemical studies have demonstrated the ability of the SKI complex to extract RNA from stalled ribosomes[89] (Fig. 3b). These findings indicate that a primary function of the cytoplasmic SKI–exosome complex lies in the degradation of translating mRNAs that have undergone internal cleavage that causes, or results from, an aberrant translation event (see mRNA decay governed by translation section).

Many eukaryotes encode a DIS3 exonuclease, DIS3L2, that exists in the cytoplasm independently of the exosome. DIS3L2 has been identified as a 3′-to-5′ exonuclease that is activated by 3′ uridylation of RNAs by terminal uridylyl transferases (such as TUT4/7 in human) and targets non-coding RNAs as well as a subset of mRNAs, including replication-dependent histone mRNAs[90,91], the only mRNAs known to be produced without poly(A) tails. Recent cryo-electron microscopy (cryo-EM) studies of DIS3L2 have determined how it recognizes and degrades its targets and have shown that by undergoing dramatic structural rearrangements it can degrade both single- and double-stranded RNA regions[92]. Another 3′-to-5′ exonuclease, the DEDD-family enzyme ERI1, has also been implicated in degradation of replication-dependent histone mRNAs[93,94]. There are multiple additional predicted 3′-to-5′ exonucleases encoded in eukaryotic genomes[95], but whether these enzymes function in cytoplasmic mRNA degradation remains unclear.

## How mRNA decay activators promote decay

The stability of mRNAs can be regulated according to cellular or developmental cues. For example, processes that require transient bursts in gene expression, such as the transient production of cytokines that occurs during inflammation, require rapid mRNA degradation to allow the completion of the gene expression burst. Similarly, processes that require permanent changes in gene expression, such as developmental transitions, require degradation of mRNAs that are no longer needed in the new cellular state. Central to these processes are mRNA decay activators that promote the recruitment of mRNA decay complexes to mRNAs destined for degradation. Some mRNA decay activators are sequence-specific RNA binding proteins, whereas others are proteins that recognize mRNA structures or nucleotide modifications[96,97]. There are also mRNA decay activators that globally activate mRNA degradation by recognizing features common to all mRNAs. mRNA decay activators interface with mRNA decay machineries through various means. A common entry point is the CCR4–NOT deadenylase complex (Fig. 4), but some factors activate decapping without prior deadenylation and others initiate degradation through endonucleolytic cleavage.

### Sequence-specific mRNA decay activators

A well-studied sequence-specific mRNA decay activator, which has a central role in developmental transitions as well as many other biological processes, is the microRNA (miRNA)-induced silencing complex (miRISC) (Fig. 4a). miRISC consists of an Argonaute (AGO) protein and an associated ~21 nucleotide miRNA that provides specificity by base-pairing with a complementary sequence on target mRNAs[98–100]. More than 1,000 miRNAs encoded in the human genome are expressed during specific biological processes and developmental transitions[101–104]. miRISC promotes the repression of translation initiation and initiates mRNA degradation via deadenylation, which is mediated by GW182/TNRC6 adapter proteins[105]. These adapter proteins interact directly, via tryptophan-containing motifs, with the CNOT9 subunit of the CCR4–NOT complex as well as through additional interactions with CNOT1 that have yet to be fully mapped[54,55]. miRISC also promotes decapping through decapping factors PATL1, DCP1 and DDX6 (ref. 106), although this may occur indirectly through the recruitment of CCR4–NOT.

Another well-studied mRNA decay activator is the protein TTP, which activates degradation of mRNAs with 3′ untranslated region (UTR) AU-rich elements during the inflammatory response and is regulated by kinase pathways including the p38-MAPK pathway[107,108]. TTP interacts with the CCR4–NOT complex via at least two direct interactions[109] (Fig. 4b). One is mediated by a highly conserved TTP C-terminal motif known as the CNOT1-interaction motif (CIM), which interacts with a HEAT domain of CNOT1 (ref. 110). A second interaction forms between conserved tryptophan residues of TTP and the CNOT9 component of the CCR4–NOT complex[111], similar to the miRISC GW182/TNRC6–CNOT9 interaction. In addition, TTP directly interacts with DCP2 of the decapping complex[112] and can promote decapping independently of deadenylation in vitro[40]. TTP also interacts directly with a GIGYF–4EHP complex, which promotes translation repression and may stimulate degradation by displacing the eIF4F complex from the mRNA cap[113,114].

Several other sequence-specific mRNA decay activators similarly promote mRNA degradation via direct interaction with the CCR4–NOT complex such as Pumilio/FBF (PUF)[35,115], Roquin[116,117], DND1 (ref. 118) and m$^6$A-binding YTHDF proteins[119] to name a few[120,121]. There are also mRNA decay activators known to recruit decapping machinery to mRNA in the absence of deadenylation such as Rps28b[122], Edc3 and Not proteins[123] in yeast. Moreover, some mRNA decay activators initiate degradation through endonucleolytic cleavage, including Regnase[124] and AGO proteins associated with small interfering RNAs. Thus, sequence- or modification-specific mRNA degradation can be initiated by various mechanisms, each of which involves activation of mRNA decay machinery that exposes the mRNA body to exonucleolytic decay.

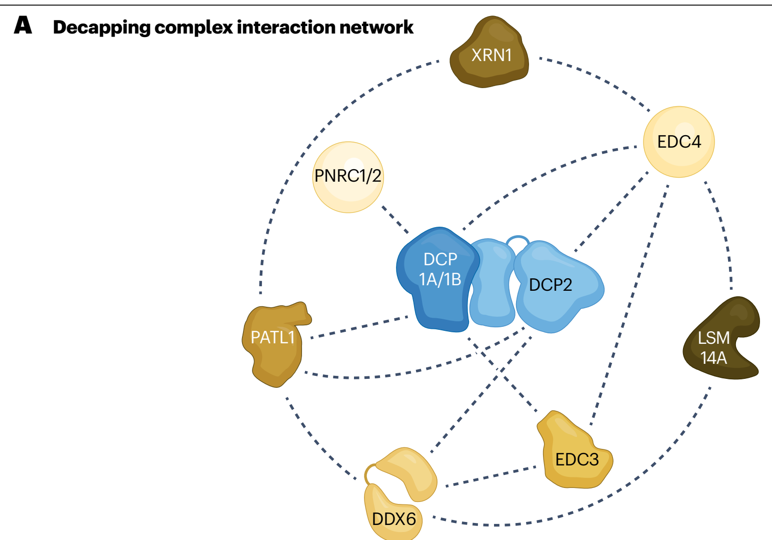
### Global mRNA decay regulation

In addition to sequence-specific mRNA degradation, there are also factors that more globally affect mRNA turnover. A well-studied example is the BTG/TOB family of anti-proliferative proteins. These proteins promote bulk mRNA degradation by directly linking PABPC with the CCR4–NOT complex (Fig. 4c). Specific members of this protein family have been shown to be important in T cell quiescence (BTG1 and BTG2)[125] and mammalian embryogenesis (BTG4)[126], both of which are conditions that involve large-scale mRNA degradation. Moreover, mutations in the genes encoding BTG and TOB have been linked to various cancers[127,128]. BTG/TOB proteins activate mRNA deadenylation via an interaction between a conserved BTG domain in these proteins and the catalytic CNOT7/8 subunit of CCR4–NOT[129–132]. In addition, four of six vertebrate BTG/TOB proteins are known to directly bind to PABPC: TOB1/2 proteins via a PAM2 motif that interacts with PABPC C-terminal MLLE motifs[133] and BTG1/2 proteins via a conserved motif that interacts with the PABPC RRM1 domain[134].

Additional mechanisms that globally regulate CCR4–NOT activity have been identified. These include regulation of CCR4–NOT via apparent acetylation of the CNOT7/8 subunit[135] and repression of CCR4–NOT via interaction of the CNOT9 subunit with the protein RNF219 (refs. 136–138). The exact biological roles of these CCR4–NOT-modulating activities are not fully understood.

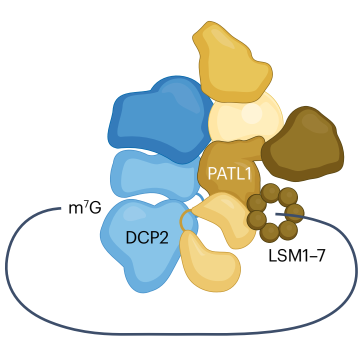
Global mRNA degradation also has an important role in the response to infection by RNA viruses. Double-stranded RNAs (dsRNAs) produced during RNA virus infection are detected by multiple innate immunity factors, which, in vertebrates, include enzymes that produce 2′,5′-phosphodiester-linked oligoadenylates (2-5A)[139]. 2-5A in turn activates the endonuclease RNase L. Subsequent widespread mRNA degradation by RNase L globally downregulates protein production[140,141]. Meanwhile, interferon-stimulated mRNAs activated by additional dsRNA sensors are, by a poorly understood mechanism, partially resistant to RNase L, causing reprogramming of infected cells to mount an antiviral response[140,141]. Conversely, some viruses encode their own global-acting RNases, which negatively impact the host

**A** Decapping complex interaction network

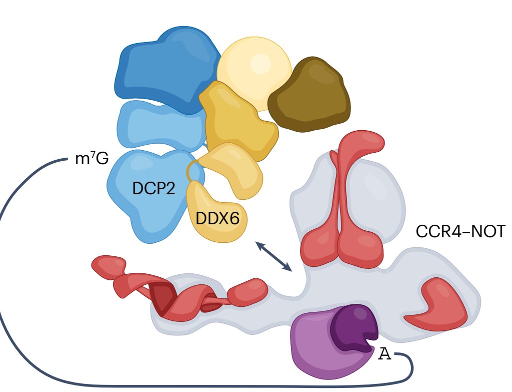



**B** Decapping complex recruitment

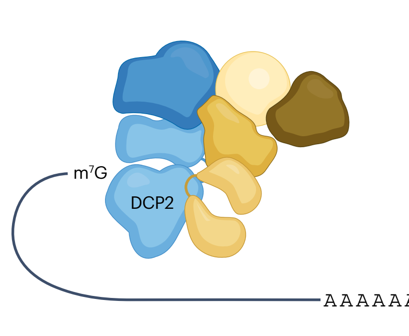
**a** Recruitment by LSM1–7–PATL1



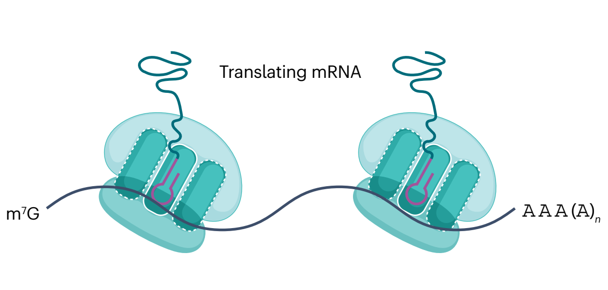

**b** Recruitment by CCR4–NOT–DDX6




**c** Deadenylation-independent recruitment




**C** P-body formation



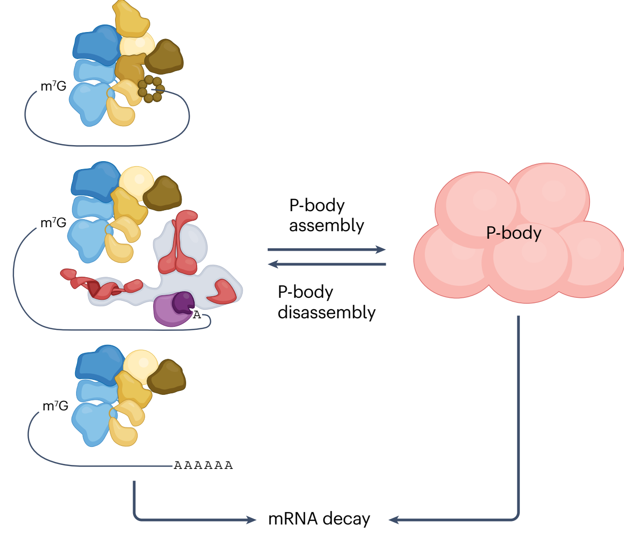



**Fig. 2 | The cytoplasmic decapping machinery. A**, The human decapping machinery interaction network. Lines indicate known direct interactions between the human DCP1A/1B–DCP2 decapping complex, shown enhancers of decapping and the 5′-to-3′ exonuclease XRN1. **B**, Mechanisms of recruitment of the decapping complex to target mRNAs. Recruitment of the decapping complex can occur through the LSM1–7–PATL1 complex associating with deadenylated mRNA 3′ ends (part **Ba**), direct interaction between decapping enhancer DDX6 and the CCR4–NOT deadenylase complex (part **Bb**) or deadenylation-independent mechanisms (part **Bc**). **C**, P-body formation is determined by the concentration of mRNAs that are translationally repressed and associated with the decapping complex. Decapping complex-associated mRNAs may be degraded or become derepressed and re-enter the translation pool.

antiviral response without shutting down virus-produced mRNAs[11]. The specificity in these systems that allows certain mRNAs to escape degradation remains poorly defined.

## mRNA decay governed by translation

It has long been appreciated that the process of translation plays a central part in mRNA stability[142–145]. Not only is the initiation of mRNA decay associated with loss of recruitment of new ribosomes, but the process of translation itself is monitored by several distinct mRNA decay machineries (Fig. 5). These machineries inspect the state of the translating ribosome, triggering degradation of the mRNA and, in some cases, the nascent polypeptide and other components of the associated translation machinery when translation slows down or goes awry[4,5].

### mRNA decay and codon optimality

One such pathway monitors codon usage. mRNAs enriched for non-optimal codons are substantially less stable than those with optimal codons[146–148]. This destabilizing effect of non-optimal codons was first observed for specific mRNAs in budding yeast[149,150] and has more recently been generalized at the global level, first in budding yeast[146] and subsequently in a range of other organisms from bacteria to humans[148,151].

In relation to mRNA decay, the optimality of a codon is dictated by the efficiency with which it recruits its cognate aminoacylated tRNA into the A-site of the ribosome during translation elongation. Although it is difficult to measure levels of charged and uncharged tRNAs in natural tissues, codon optimality seems to be affected not only by the cellular abundance of the cognate tRNA, but also by the modification status of the tRNA, how well the tRNA recognizes its codons, and how efficiently the tRNA is charged with its cognate amino acid[146,148,151–153]. Thus, an optimal codon in one organism, tissue or condition can be a non-optimal codon in another depending on the availability and identity of functional aminoacylated tRNAs.

Evidence suggests that codon optimality can contribute substantially to the overall intrinsic stability of individual mRNAs[146,154,155]. Indeed, mRNAs encoding proteins in similar pathways have been observed in budding yeast to be co-regulated at the level of mRNA stability based on similar codon usage[146]. Moreover, codon optimality has been reported to have a key role in dictating changes in mRNA abundance over important developmental transitions such as the maternal-to-zygotic transition[152,156].

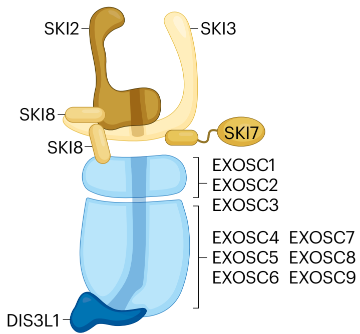
Recent structural studies have provided some clarity on the mechanism by which codon optimality impacts mRNA degradation. Both yeast and human ribosomes were observed by cryo-EM to interact directly with the CNOT3 subunit (Not5 in yeast) of the CCR4–NOT complex[157,158]. This subunit was observed to interact with the E-site of ribosomes in which both the A- and E-sites are empty. That observation has led to the idea that codon-dependent mRNA stability is dictated by continuous competition between A-site recruitment of charged tRNAs and E-site recruitment of the CCR4–NOT deadenylase complex during translation elongation (Fig. 5). Interestingly, ribosomes associated with the CCR4–NOT complex are also mono-ubiquitylated at the small ribosomal subunit protein eS7 by the CCR4–NOT-associated ubiquitin ligase CNOT4, but the importance of this ubiquitylation remains unclear[157–159].

This model is consistent with findings from genetic and biochemical experiments in which mRNAs with non-optimal codons have been observed to undergo enhanced deadenylation[146,152,156], although a poly(A) tail is not required for degradation[153]. Moreover, depletion in budding yeast of the CCR4–NOT complex catalytic subunit Caf1, or of the CCR4–NOT-associated decapping activator Dhh1, which is also known to associate with the ribosome, specifically stabilizes mRNAs with non-optimal codons[35,160]. Thus, recruitment of the CCR4–NOT complex probably activates not only deadenylation but also decapping and 5′-to-3′ exonucleolytic decay consistent with the interactions between these machineries (Fig. 2). Genetic studies have also implicated the CCR4–NOT complex and DDX6 in this decay pathway in human cells[161,162], although some studies did not observe a role for DDX6 (refs. 161,163). In addition to undergoing deadenylation and decay, mRNAs with non-optimal codons have also been observed to undergo repression of translation initiation[161]. The specific mechanism for this repression remains unknown, but it seems to occur independently of DDX6 and CCR4–NOT[161].

### mRNA decay by translation elongation stalls

Another pathway that monitors translation detects stalls in translation elongation. This pathway, which is distinct from the codon optimality pathway[164,165], was initially discovered to degrade mRNAs that lack stop codons (referred to as non-stop decay; NSD)[166,167] or contain an RNA structure that stalls elongating ribosomes (referred to as no-go decay; NGD)[168] (Table 2 and Fig. 5). It was later discovered that these types of stall also trigger degradation of the nascent polypeptide by a process termed ribosome-associated quality control (RQC)[169,170]. Most studies of this pathway have been performed on reporter mRNAs that stall translation elongation with difficult-to-translate codon repeats, RNA structures or the absence of stop codons. Although a few endogenous mRNA targets have been identified in various organisms[171,172], the primary targets of this pathway are thought to be mRNAs that have been rendered non-functional owing to chemical- or irradiation-mediated nucleotide damage[173,174] or aberrant processing, such as premature polyadenylation within the mRNA coding region[167,175].

The mechanism by which the elongating ribosome stalls has been structurally and biochemically dissected in some detail at some stall sites. For example, a ribosome undergoing elongation into an oligo(A) sequence (such as a poly(A) tail) stalls owing to unfavourable interactions between the encoded oligo-lysine peptide and the exit tunnel of the ribosome, which provides the oligo(A) tail positioned in the A-site of the ribosome with time to form a stacked helical structure that impairs access for the next incoming tRNA[176,177]. CGA–CGA codon pairs, which stall the ribosome in budding yeast, similarly form a structure that prevents access for the incoming tRNA. Formation of this structure occurs because translation elongation is slowed down by suboptimal I–A base-pairing with an inosine-containing cognate tRNA[177].



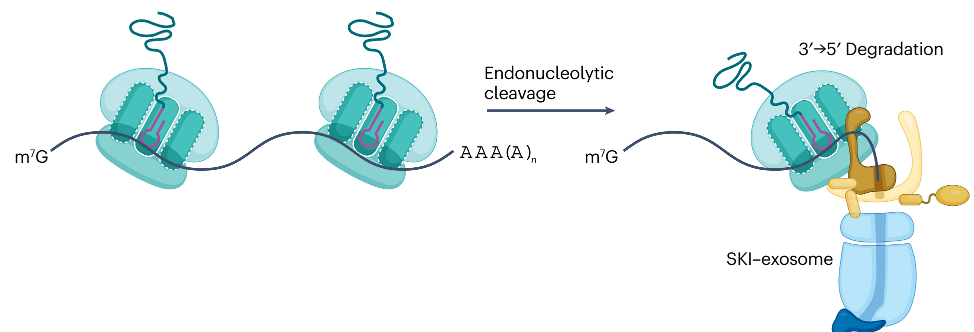




**Fig. 3 | The cytoplasmic 3′-to-5′ exonuclease SKI–exosome complex.** **a**, Diagram of the human SKI–exosome complex. Processive 3′-to-5′ exonuclease DIS3L interacts with the EXOSC4–9 exosome core structure, which is capped by EXOSC1–3 proteins. SKI2 helicase, with SKI3 and SKI8 co-factors, associates with the exosome cap via SKI7 adapter protein. A tunnel through the exosome, from SKI2 to exonuclease DIS3L, can accommodate a single-stranded RNA. **b**, The SKI–exosome complex extracts and degrades endonucleolytically cleaved ribosome-associated mRNAs via threading of the RNA from its 3′ end through the SKI2–3–8 helicase complex into the exosome core to reach exonuclease DIS3L. The structure of the exosome complex in parts **a** and **b** is adapted with permission from ref. 84, Elsevier.

Multiple lines of evidence support that ribosome stalling is detected when a trailing ribosome collides with the stalled ribosome[178,179] (Fig. 5). This creates a unique small subunit dimer surface that is recognized by downstream sensor proteins. One such sensor is the E3 ubiquitin ligase ZNF598 (Hel2 in yeast) which ubiquitylates the eS10 and uS10 proteins of the small ribosomal subunit[178,180]. This recognition begins a signalling cascade for decay of the mRNA, splitting of the stalled ribosome and degradation of the nascent polypeptide. The specific mechanism by which the mRNA undergoing ribosome stalling is degraded seems to depend on the type of stall. Both NSD and NGD substrates undergo endonucleolytic cleavage by an endonuclease first identified in budding yeast (Cue2) and the worm *Caenorhabditis elegans* (NONU-1) (N4BP2 in human)[181,182], which seems to recognize the collided ribosome via its ubiquitylation by ZNF598 (ref. 183). This cleavage takes place either in the A-site of the collided ribosome, or, if splitting of the stalled ribosome is inhibited, upstream of the trailing ribosome[181,183]. In addition to endonucleolytic cleavage, degradation can be initiated from the 5′ or 3′ end of the mRNA dependent on the type of stall. Whereas degradation of an NSD substrate was observed to depend on the activity of the 3′-to-5′ exonucleolytic SKI–exosome complex[166], degradation of an NGD substrate was instead dependent on the 5′-to-3′ exonuclease XRN1 (ref. 181).

Another factor that recognizes the collided ribosomes is EDF1 (Mbf1 in yeast)[184]. Biochemical evidence in human cells suggests that EDF1 promotes repression of further translation of the stall-inducing mRNA via recruitment of the translation initiation repressor GIGYF2–4EHP complex[184,185]. In budding yeast, GIGYF2 homologues Syh1 and Smy2 also associate with the collided ribosome, although this seems to occur independently of the EDF1 homologue Mbf1, and stimulate mRNA degradation instead of translation repression[164,184,185]. When the number of stalled ribosomes overwhelms the degradation machinery, translation initiation is repressed globally via activation of the eIF2α kinase GCN2 and the integrated stress response[186]. When further overwhelmed, a MAPKKK, ZAKα, becomes activated by the collided ribosomes to trigger a stress response, ultimately resolving the stress or leading to apoptosis[186].

In addition to promoting translation repression and mRNA degradation, the stalled and collided ribosomes need to be actively split. This is followed by degradation of the nascent polypeptide associated with the released large ribosomal subunit by RQC. The mechanisms of ribosome splitting and RQC have been extensively discussed in recent reviews[187–190].

### mRNA surveillance of translation termination

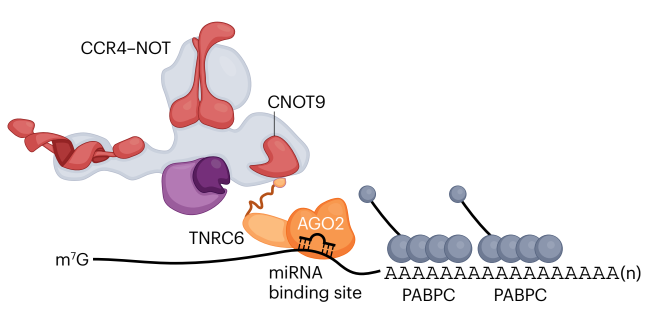
The final step of translation, translation termination, is also subject to surveillance[5,191,192] (Table 2 and Fig. 5). This is carried out by the nonsense-mediated mRNA decay (NMD) pathway, which targets mRNAs that contain premature termination codons (PTCs) through genetic mutation, defects in pre-mRNA splicing, or alternative splicing that causes inclusion of exons with stop codons. NMD also targets a subset of seemingly normal mRNAs[193–195] presumably because they have evolved to be repressed by the NMD pathway via PTC-like translation termination. However, despite having been discovered more than 40 years ago[196,197], NMD is, paradoxically, the translation-dependent mRNA surveillance pathway that is least understood with respect to how the ribosome differs between its normal and aberrant states[198].

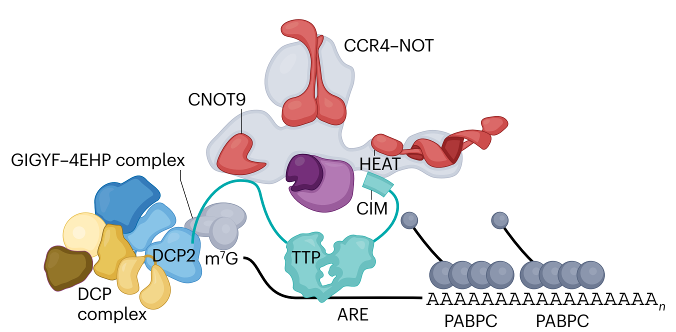
A great amount of research has been conducted on the mRNA–protein (mRNP) components that help the NMD pathway to distinguish between a normal termination codon and a PTC. An abundance of evidence suggests that the mRNP composition that surrounds the termination event has a central role in NMD by a combination of effects on the efficiency or mechanics of the translation termination process and/or on recruitment of NMD factors. For example, PABPC inhibits NMD when positioned in the proximity of a stop codon, probably by positively impacting the translation termination process[199–202]. Other RNA binding proteins, such as hnRNP L and PTBP1, inhibit NMD of human mRNAs when PABPC is located distal to the termination codon owing to a long 3′ UTR[203,204]. By contrast, the exon junction complex (EJC), a protein complex that in metazoans is deposited at mRNA splice junctions and directly interacts with NMD factors, accelerates NMD when positioned on an mRNA downstream of a termination event[205–207]. Other mRNP components that affect NMD have also been identified[5].

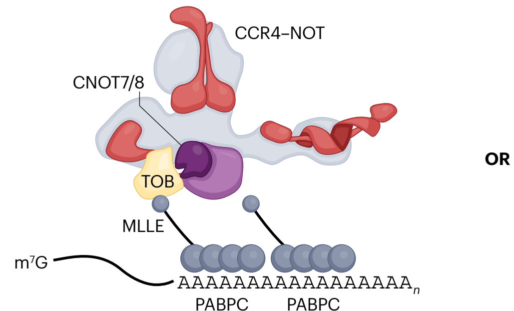
The central trans-factor in NMD is the RNA helicase UPF1 (refs. 208–210). Although UPF1 has been extensively studied, the specific features of ribosomes terminating at PTCs recognized by UPF1 remain poorly understood[201,211]. Biochemical studies have identified interactions between UPF1 and the ribosome close to the E-site[212] as well as with the translation termination factor eRF3 (refs. 213–216). Cross-linking followed by immunoprecipitation (CLIP) studies have shown UPF1 to broadly associate with mRNA 3′ UTRs in mammals[217–219] and ribo-seq studies in budding yeast have identified association with elongating ribosomes[220]. These interactions are seen on both substrate and non-substrate mRNAs of the pathway, consistent with a hypothesis in which UPF1 continuously samples all mRNAs of the cell[192,219,221]. The ATPase activity of UPF1 has an essential role in NMD and has been implicated in substrate recognition[219,222] as well as mRNP remodelling, including removal of the terminating ribosome, to allow completion of mRNA degradation[223,224]. An important unresolved question is exactly how these interactions contribute to the discrimination between regular and prematurely terminating ribosomes.

The activity of UPF1 is regulated by its evolutionarily conserved co-factors UPF2 and UPF3, which stimulate UPF1 substrate recognition and UPF1 ATPase activity[225–230]. Once the NMD substrate is identified, UPF1 recruits downstream mRNA decay machinery. This is mediated by SMG protein co-factors, which in metazoans include the endonuclease SMG6 (refs. 231,232) and the proteins SMG5 and SMG7, which in turn activate general mRNA decay factors including the CCR4–NOT complex and decapping machinery[233–237]. In metazoans, UPF1 phosphorylation by the SMG1–SMG8–SMG9 kinase complex promotes SMG protein recruitment by generating phospho-dependent SMG5–7 binding sites[238–241]. A slew of additional factors have been identified that affect the recognition and degradation steps of NMD (reviewed in ref. 5). In a parallel with the RQC system, there is evidence that the nascent polypeptides associated with ribosomes terminating at PTCs are also targeted for degradation[242,243]. UPF1 had been proposed to function as a ubiquitin ligase acting to promote nascent polypeptide degradation[242,244], but a more recent study has put this idea in doubt[243].

A controversial question in the field was whether NMD is restricted to early, pioneer, rounds of translation[245], a hypothesis that had been challenged by studies showing that NMD can occur on mRNAs after they had already been exposed to multiple rounds of translation[246–248]. This controversy seems to have been fully settled by a recent study that provides evidence through single-molecule assays that NMD is equally likely to be induced by every translating ribosome encountering a PTC[249]. Thus, efficiently targeted NMD substrates may be stochastically targeted for degradation primarily during early rounds of translation but there is no difference between early or late ribosomes in their ability to trigger NMD.



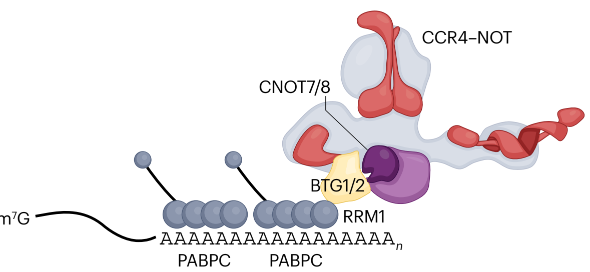




**Fig. 4 | Recruitment of mRNA degradation machinery by mRNA decay activators. a**, The microRNA (miRNA)-induced silencing complex (miRISC), which consists of an Argonaute (AGO) protein in complex with a miRNA, recruits TNRC6/GW182 proteins, which in turn recruit the CCR4–NOT deadenylase complex via direct interaction with CNOT9. **b**, TTP binds mRNA 3′ untranslated region (UTR) AU-rich elements (AREs) and recruits the CCR4–NOT complex by direct interactions with CNOT9 and a HEAT domain of CNOT1. TTP also interacts, via tetraproline motifs, with cap-binding repressor complex GIGYF–4EHP and, via the TTP unstructured N terminus, with the decapping (DCP) complex. **c**, BTG/TOB proteins promote global mRNA degradation via direct interaction with the cytoplasmic poly(A)-binding protein (PABPC) and deadenylases CNOT7/8 of the CCR4–NOT complex. TOB proteins interact with the MLLE domain of PABPC, whereas BTG1 and BTG2 interact with RRM1. CIM, CNOT1-interaction motif.

### mRNA surveillance during localized translation

Although most studies of translation-dependent mRNA surveillance pathways have focused on translation occurring in the general cytoplasm of cells, there is also evidence for surveillance of mRNAs undergoing localized translation[250], including at the endoplasmic reticulum[251–253], mitochondria[254–256] and at axons and dendrites of neurons[257,258]. Some of these pathways seem to require specialized factors.

For example, in the case of NMD, evidence suggests that the protein NBAS, which is involved in Golgi-to-endoplasmic reticulum retrograde transport, serves an additional role in NMD at the endoplasmic reticulum[259]. NBAS is thought to recruit the central NMD factor UPF1 to targets at the endoplasmic reticulum, which then activates a localized NMD response. Moreover, a splice variant of UPF1 ($UPF1_{LL}$) was found to favour endoplasmic reticulum-localized mRNAs[260]. NMD has been previously implicated in modulating the endoplasmic reticulum stress response[261–263], but whether this activity relates to localized NMD remains to be addressed. Another example of specialized machinery required for localized translation-dependent quality control is RQC at the endoplasmic reticulum. Here, recent studies have identified UFMylation, a protein modification similar to ubiquitylation, as essential for degradation of nascent peptides produced from RQC targets at the endoplasmic reticulum[264–266]. It remains to be determined whether specialized factors are required also for degradation of the mRNAs producing such RQC-targeted polypeptides at the endoplasmic reticulum.

## Cytoplasmic degradation and therapeutic mRNAs

The development of therapeutic mRNAs has seen rapid progress in recent years as dramatically highlighted by the success of the mRNA-based coronavirus virus disease 2019 (COVID-19) vaccines. Therapeutic mRNAs, which take advantage of host cell translation machinery to produce therapeutic proteins, are being explored not only for production of antigens for vaccination purposes, but also for cancer-targeting therapeutic vaccinations and for protein-replacement therapies[267]. One of several important considerations in the generation of effective therapeutic mRNAs is the efficiency and duration of protein production from the mRNAs after they enter the cytoplasm of target cells. This, in turn, is dictated by the translation efficiency and stability of the therapeutic mRNAs.

Given the central roles of the mRNA $m^7G$ cap and poly(A) tail in both translation and mRNA stability, these are obvious targets of modification to enhance protein expression of therapeutic mRNAs. Indeed, much effort has gone into the development of $m^7G$ cap analogues, for example, through modifications to the $m^7G$ base and the cap triphosphate, that inhibit decapping and/or increase the association of the therapeutic mRNA with the translation initiation eIF4F complex[268]. Efforts to modify the poly(A) tail are in earlier stages of exploration, but such modifications may have even greater potential for modulation of the duration of protein production given the key role of the poly(A) tail in mRNA stability. It would be predicted that poly(A) tail modifications that inhibit deadenylation by the CCR4–NOT complex, while maintaining or enhancing PABPC association, could dramatically increase the duration of protein expression by therapeutic mRNAs. Modifications of poly(A) tail phosphates and of poly(A) tail 3′ ends have been attempted but so far with varied effects on translation and stability[268,269].

Translation and stability of mRNAs are additionally affected by 5′ and 3′ UTR sequences. Indeed, recent studies have demonstrated large effects of UTR sequences on the translation efficiencies of synthetic mRNAs[270]. What seems to have received less attention are

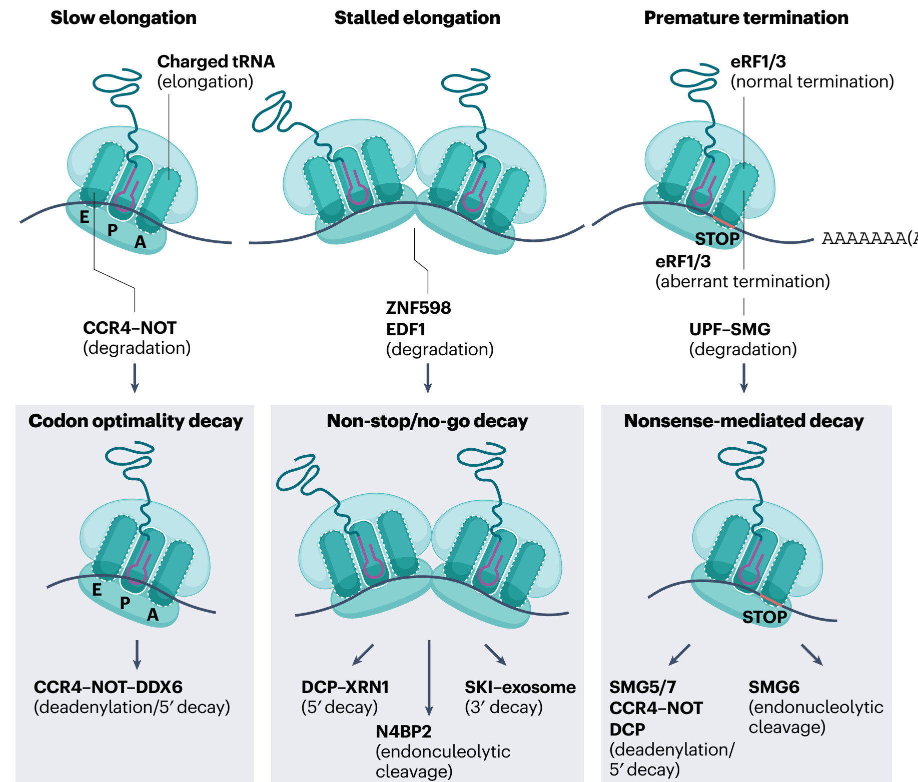



**Fig. 5 | mRNA decay pathways that monitor the translation process.** mRNA decay pathways activated by suboptimal or abnormal translation, triggered by competition between normal translation factors and factors that initiate mRNA decay. When translation elongation is slowed owing to non-optimal codons (left), the CCR4–NOT deadenylase complex and DDX6 are recruited to the translating ribosome, promoting deadenylation and degradation from the mRNA 5′ end. Stalled elongation (middle) promotes ribosome collisions, which trigger mRNA degradation via non-stop decay or no-go decay by the recruitment of ribosome collision sensors ZNF598 and EDF1, leading to mRNA endonucleolytic cleavage by N4BP2 and subsequent degradation of mRNA fragments by DCP–XRN1 and the SKI–exosome complex. When a ribosome encounters a premature termination codon (right), nonsense-mediated decay is triggered via recruitment of UPF and SMG proteins. This leads to endonucleolytic cleavage by SMG6 as well as degradation via deadenylation and decay from the 5′ end.

**Table 2 | Proteins involved in mRNA surveillance by the ribosome**

| Pathway | Subunit name | | Function |
|---|---|---|---|
| | *Homo sapiens* | *Saccharomyces cerevisiae* | |
| NSD/NGD | ZNF598 | Hel2 | Recognizes collided ribosomes, E3 ubiquitin ligase |
| | N4BP2 | Cue2 | RNA endonuclease |
| | EDF1 | Mbf1 | Recognizes collided ribosomes, represses translation |
| | GIGYF1/2 | Syh1/Smy2 | Represses translation initiation |
| | 4EHP | NA | Binds $m^7G$ cap/represses translation initiation |
| NMD | UPF1 | Upf1 | RNA helicase |
| | UPF2 | Upf2 | Stimulates UPF1 |
| | UPF3 | Upf3 | Stimulates UPF1 |
| | SMG1–SMG8–SMG9 | NA | Serine/threonine kinase complex |
| | SMG6 | NA | Endonuclease |
| | SMG5/7 | Ebs1/Nmd4 | Recruits deadenylase/decapping factors |

$m^7G$, 7-methyl-guanosine; NA, not applicable; NGD, no-go decay; NMD, nonsense-mediated mRNA decay; NSD, non-stop decay.

the translation-dependent mRNA decay pathways. Because some of these pathways initiate mRNA degradation via endonucleolytic cleavage – from which modified $m^7G$ caps and poly(A) tails will not offer protection – and because they are all associated with translation repression, this may be an area in which therapeutic mRNAs could be further optimized. Although efforts are being made to use optimal codons for efficient translation[271], efforts may also be needed to prevent translation elongation stalls that could activate NGD, and to promote efficient termination to prevent NMD. Although EJCs should not be deposited on therapeutic mRNAs, which lack introns and a nuclear history, there are several additional factors that affect whether translation termination is interpreted as normal or premature by NMD machinery[5]. Thus, generating therapeutic mRNAs that allow efficient translation elongation and termination may help to improve the effectiveness of mRNA therapies.

## Conclusions and future perspectives

The past decade, with its rapid developments in structural, single-molecule and genome-wide molecular techniques, has seen much progress in our understanding of how gene expression is controlled at the level of mRNA turnover in the cytoplasm of eukaryotes. There are, however, many important questions that remain to be addressed. Although the central players in mRNA deadenylation have been identified, the precise division of labour between CCR4–NOT and PAN2–PAN3 deadenylation complexes, and between the CNOT7/8 and CNOT6/6L catalytic subunits of the CCR4–NOT complex, remains to be fully understood. Also, the extent to which other deadenylases of the cell, such as PARN and TOE1, may take part in cytoplasmic mRNA deadenylation in addition to their roles in small non-coding RNA processing[95] remains poorly defined. There are also multiple additional predicted exonucleases and endonucleases in eukaryotes that have yet to be fully characterized and could have roles in cytoplasmic mRNA degradation. The extent to which mRNP remodelling is required for mRNA degradation also remains poorly understood, including the case of the eIF4F complex and how it is dislodged from the $m^7G$ cap to allow decapping. Moreover, one of the most controversial unresolved questions is the precise role of P-bodies in mRNA metabolism. Recent advances in cellular single-molecule techniques[272,273] and methods of P-body in vitro assembly[274,275] are promising avenues for future studies.

There are also several outstanding questions regarding mRNA surveillance pathways. In the case of the NMD pathway, a key unresolved question is exactly how a ribosome terminating at a PTC differs from a normal terminating ribosome and how this is read by the central NMD factor UPF1. For the NGD pathway, it is not yet known how pervasive mRNA damage is in cells nor how important clearing out such damaged mRNAs by the NGD pathway is for cell function.

Finally, a key question is what dictates the intrinsic and regulated stability of mRNAs in various tissues and conditions. How much does the translation process, for example, via codon optimality, contribute to the intrinsic stability of mRNAs compared with the composition of 5′ and 3′ UTRs? What are the key principles by which mRNA decay activators are controlled by cellular cues? How does intrinsic and regulated stability of mRNAs differ between different cell types and tissues? New developments in approaches to monitor mRNA turnover genome-wide at the single-cell level[276,277] promise to pave the way to decipher how mRNA turnover is choreographed within and between individual cell types and tissues. With an increasing understanding of the general principles that govern mRNA turnover, it may one day be possible to decipher the mRNA code that controls the stability of mRNAs in different cell types, tissues and developmental transitions.

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## Author contributions

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## Competing interests

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