The highways and byways of mRNA decay

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Abstract | When considering the control of gene expression, the focus has traditionally been on transcriptional regulation. Recently, however, the large contribution made by mRNA decay has become difficult to ignore. Large-scale analyses indicate that as many as half of all changes in the amounts of mRNA in some responses can be attributed to altered rates of decay. In this article, we discuss some of the mechanisms that are used by the cell to mediate and regulate this intriguing process.

mRNA-surveillance pathway
Mechanism used by the cell to identify and destroy aberrant mRNAs.

Poly(A) tail
A homopolymeric stretch of ~25–200 adenine nucleotides that is present at the 3′ end of most eukaryotic mRNAs.

Exonuclease
Also known as an exoribonuclease. An enzyme that catalyses the removal of mononucleotides from either the 5′ or 3′ end of an RNA molecule.

Polysome
Two or more ribosomes that are bound to different sites on the same mRNA.

5′ cap
The 7-methylguanosine structure found at the 5′ end of a eukaryotic mRNA.

Decapping
The removal of the mRNA 5′-cap structure.

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Transcription — the 'birth' of an mRNA — is just one of many stages in the process of gene expression that can be regulated. From splicing and polyadenylation, through mRNA export to translation, every aspect of a transcript's life is subject to elaborate control. It is therefore no surprise that many cellular factors and mechanisms are devoted entirely to modulating the rate of mRNA degradation.

Over the past five years, most of the enzymes involved in mRNA decay have been identified and we are beginning to unravel the complex regulation that determines the path and rate of mRNA degradation. Furthermore, the recent discovery that there are specific cytoplasmic sites, known as mRNA-processing (P) bodies, where mRNA turnover occurs, was quite unexpected.

Various facets of mRNA decay have been reviewed in several excellent articles[1–6]. Here, our main goal is to summarize the wealth of current literature and to touch on major advances in all aspects of the field. We first review the factors and enzymes that are required for mRNA turnover and describe the pathways that are used to degrade normal transcripts. We then discuss P bodies as sites of mRNA decay and storage. We describe alternative mRNA-decay pathways, including surveillance pathways that target aberrant mRNAs. Several mRNA-stability elements, particularly AU-rich elements (AREs), are discussed in detail along with their binding factors, and we finish by focusing on how the mRNA-decay machinery interfaces with other aspects of mRNA metabolism to facilitate coordinated gene expression. We hope that the reader will come away with an increased appreciation of the power and complexity of a process that might otherwise be misconstrued as a biological waste-disposal system.

The superhighway to destruction

Deadenylation-dependent mRNA decay. Eukaryotic mRNAs are created with two integral stability determinants — the 5′ 7-methylguanosine cap and the 3′ poly(A) tail — that are incorporated co-transcriptionally. These two structures interact with the cytoplasmic proteins eIF4E and the poly(A)-binding protein (PABP), respectively, to protect the transcript from exonucleases and to enhance translation initiation. To initiate decay, either one of these two structures must be compromised or the mRNA must be cleaved internally by endonucleolytic attack.

In eukaryotes, the bulk of mRNAs undergo decay by a pathway that is initiated by poly(A)-tail shortening. This first step in the turnover pathway is unique in that it is reversible — transcripts that bear the correct signals can be readenylated and return to polysomes. Nevertheless, once the cell determines that an mRNA must be destroyed, one of two irreversible routes is taken (FIG. 1a). Either the 5′ cap is removed by a process known as decapping, which allows the mRNA body to be degraded in the 5′→3′ direction by the XRN1 exoribonuclease, or the unprotected 3′ end is attacked by a large complex of 3′→5′ exonucleases known as the exosome. These two pathways are not mutually exclusive and the relative contribution of each mechanism remains a subject of debate. In *Saccharomyces cerevisiae*, knocking out components of either the 3′→5′ or the 5′→3′ pathway had minimal effects on the transcriptome, which implies redundancy[7,8]. Results obtained by using a sensitive assay to detect decay intermediates indicate that both 5′→3′ and 3′→5′ pathways are involved in the decay of unstable ARE-containing mRNAs in mammalian cells (E. L. Murray and D. R. Schoenberg, personal communication). It therefore seems that the precise pathway of mRNA decay might be flexible.

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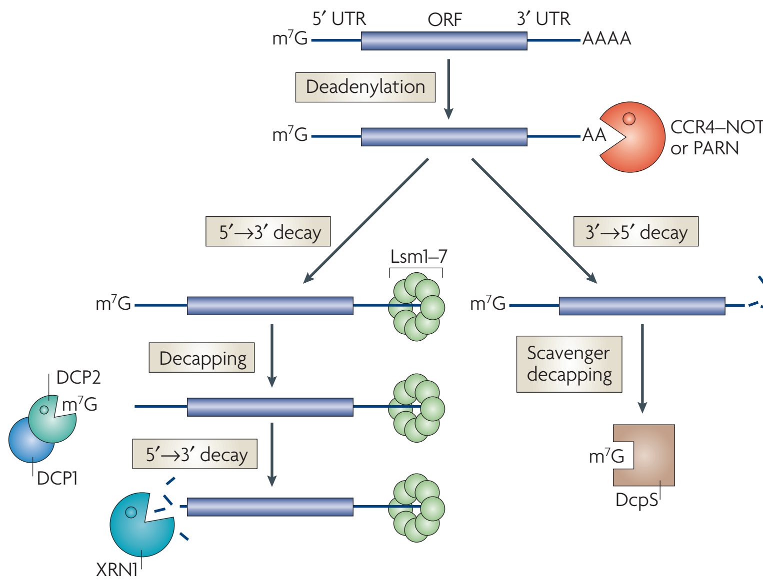

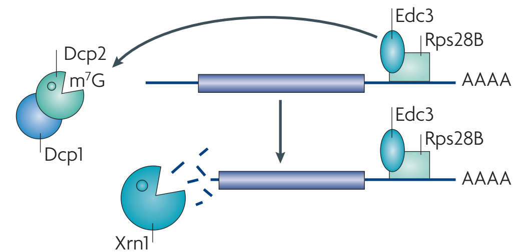

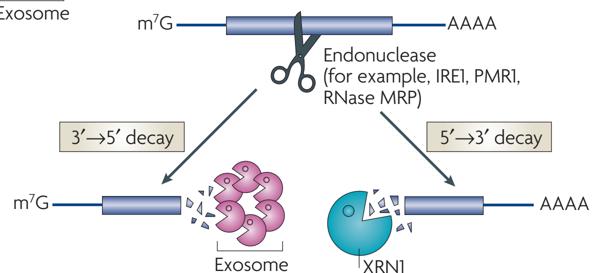

Figure 1 | **Mechanisms of normal mRNA degradation. a** | Most mRNAs undergo decay by the deadenylation-dependent pathway. The poly(A) tail is removed by a deadenylase activity, shown here as either CCR4–NOT or PARN. Following deadenylation, two mechanisms can degrade the mRNA: either decapping followed by 5′→3′ decay or 3′→5′ decay. In the decapping pathway, the Lsm1–7 complex associates with the 3′ end of the mRNA transcript and induces decapping by the DCP1–DCP2 complex. This leaves the mRNA susceptible to decay by the 5′→3′ exoribonuclease XRN1. Alternatively, the deadenylated mRNA can be degraded in the 3′→5′ direction by the exosome, with the remaining cap structure being hydrolysed by the scavenger-decapping enzyme DcpS. **b** | In *Saccharomyces cerevisiae*, deadenylation-independent pathways require recruitment of the decapping machinery. Here, Rps28B interacts with enhancer of decapping-3 (Edc3) to engage the decapping enzyme. Following decapping, the mRNA is degraded by Xrn1. **c** | Endonuclease-mediated mRNA decay initiates with internal cleavage of the mRNA, which generates two fragments each with one unprotected end. The fragments are degraded by XRN1 and the exosome.

The enzymes that perform each step in decay are outlined below and summarized in TABLE 1.

Deadenylation. As discussed above, deadenylation is the first step in bulk mRNA decay, although so far it remains unclear exactly how and when deadenylation is triggered. There are several characterized eukaryotic deadenylases, including PAN2–PAN3, CCR4–NOT and PARN (poly(A)-specific ribonuclease), each with unique properties.

PAN2–PAN3 is a PABP-dependent poly(A) nuclease that is involved in trimming the poly(A) tails of nascent mRNAs to the standard length of 60–80 nucleotides in *S. cerevisiae*[9]. Mammalian PAN2–PAN3 carries out the initial shortening of the tail of a β-globin reporter transcript from the usual 200 nucleotides to a length of ~80 nucleotides[10]. At this point, the deadenylation is handed over to another deadenylase, CCR4–NOT (see below). Whether PAN2–PAN3 has a role in deadenylating all mRNAs in higher eukaryotes is unclear — it seems likely that this function can be carried out by other deadenylases.

CCR4–NOT is the main deadenylase in *S. cerevisiae*[11] and is a large complex of nine proteins, two of which, Ccr4 and Caf1 (also known as Pop2), have exonuclease domains. In contrast to PAN2–PAN3, CCR4–NOT activity is inhibited by PABP[12]. In mammalian cells, CCR4–NOT has been shown to deadenylate reporter mRNAs[10]. CCR4 is a member of a large family of proteins in higher eukaryotes, at least some of which are also deadenylases[13].

PARN is unique in that it has cap-dependent deadenylase activity — that is, its processivity is enhanced by the presence of a 5′ cap on the mRNA[14–16]. Moreover, this deadenylase is inhibited by cap-binding proteins[15,17]. PARN has been implicated in the mass deadenylation of maternal mRNAs that occurs in *Xenopus laevis* oocytes during maturation[18] and is also the main deadenylase activity in cytoplasmic extracts derived from various cell lines[15,19,20]. PARN is essential for embryogenesis in plants[21,22], and is found in many higher eukaryotes including mammals and several insect species[14,20], although there is no obvious counterpart in *S. cerevisiae* or *Drosophila melanogaster*[23].

Interestingly, PAN2, CCR4, CAF1 and PARN all have ribonuclease D-type (RNAse D-type), Mg^{2+}-dependent, 3′→5′-exonuclease domains, as does the nuclear exosome subunit RRP6 (also known as PM-Scl100)[18,24], which is indicative of divergent evolution. Given that there are over a dozen candidate deadenylases in

Exosome
A large complex of 3′→5′ exonucleases that functions in the nucleus and the cytoplasm in several different RNA-processing and RNA-degradation pathways.

Transcriptome
The pool of mRNAs that is present in the cell under a given condition.

Deadenylase
An enzyme that removes the 3′-poly(A) tail from RNA in a 3′→5′ direction.

RNase D
Escherichia coli RNase D is a 3′→5′ exoribonuclease that is required for processing of tRNA precursors. Many eukaryotic proteins with similar exoribonuclease domains have been identified.

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Table 1 | **mRNA-decay factors**

Decay factor	Protein components	Protein domains	Functions and characteristics	Localization	Other functions
Deadenylation					
CCR4–NOT	Ccr4	3′→5′ exonuclease	Main deadenylase in *Saccharomyces cerevisiae*; inhibited by PABP	Nucleus; cytoplasm; P bodies	Transcription; protein degradation
	Caf1 (Pop2)	3′→5′ exonuclease			
	Caf40 (Rcd1)	Rcd1-like			
	Caf130	Not known			
	Not1	Not known			
	Not2	Not2, Not3 and Not5 share a domain of unknown function			
	Not3	Not2, Not3 and Not5 share a domain of unknown function			
	Not4	Ubiquitin ligase			
	Not5	Not2, Not3 and Not5 share a domain of unknown function			
PAN2–PAN3	PAN2	WD40 repeat	Involved in first phase of poly(A) shortening	Nucleus; cytoplasm	Not known
	PAN3	3′→5′ exonuclease			
PARN	PARN	3′→5′ exonuclease	Cap-dependent deadenylase activity; inhibited by PABP and nuclear cap-binding complex	Nucleus; cytoplasm	Translation inhibition
5′→3′ decay					
Decapping enzyme	DCP1A, DCP1B	EVH1	Produces ^{7me}GDP; Hedls not found in yeast	Cytoplasm; P bodies	mRNA localization
	DCP2	Nudix Mut T			
	Hedls (Ge-1)	WD40 repeat			
Dhh1 (RCK/p54, Me31B)	Dhh1	DExD/H-box RNA helicase	Required for decapping	Cytoplasm; P bodies	Translational repression
Edc proteins	Edc1, Edc2 (*S. cerevisiae*)	Not known	Enhance decapping	P bodies (EDC3)	Not known
	EDC3 (Lsm16)	Sm-like; YjeF_N			
Lsm complex	Lsm1–7	Sm-like	Required for decapping; heptameric	Cytoplasm; P bodies	Nuclear Lsm2–8 involved in splicing
Pat1	Pat1	Not known	Yeast specific	Cytoplasm	Translational repression
XRN1, (Sep1, Kem1)	XRN1	5′→3′exonuclease	Degrades decapped 5′-monophosphate RNA	Cytoplasm; P bodies	Microtubule polymerization
3′→5′ decay					
Cytoplasmic exosome	RRP41 (Ski6), RRP42, RRP45 (PM-Scl75), RRP46, Mtr3, OIP2	RNase PH	RNase PH domain subunits form a six-membered ring structure; RNAse PH domain has affinity for AU-rich sequences; RRP44 is not present in human exosome preparations	Nucleus; cytoplasm; evidence for cytoplasmic foci; Ski7 is exclusively cytoplasmic	Processing of small RNAs; nuclear mRNA surveillance
	CSL4 (Ski4), RRP4 , RRP40	S1 RNA binding; KH RNA binding			
	RRP44 (Dis3)	*PIN* RNA binding; RNase II			
	Ski2	DEVH RNA helicase			
	Ski3	Tetratricopeptide repeat			
	Ski7	EF1α-like GTPase			
	Ski8	WD40 repeat			
Scavenger-decapping enzyme	DcpS	HIT pyrophosphatase	Produces ^{7me}GMP from ^{7me}GpppG	Predominantly nucleus	Facilitates 5′→3′ decay

Entries in brackets indicate alternative protein names. EVH1, Ena/VASP homology-1; Hedls, human enhancer of decapping large subunit; HIT, histidine triad; PABP, poly(A)-binding protein; *PIN*, Pilt N terminal; RNase, ribonuclease.

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Box 1 | **microRNAs, P bodies and mRNA decay**

As small regulatory RNAs, including small interfering RNAs (siRNAs), microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs) and repeat-associated siRNAs (rasiRNAs), have recently been the focus of several reviews[123–125], we will not cover them in detail here. However, there are several aspects of their function that link them closely to the mRNA-turnover machinery. Perhaps most significantly, components of the miRNA machinery, including Argonaute (Ago) proteins and miRNAs themselves, localize to P bodies. Moreover, recent experiments indicate that, in the absence of mature miRNAs, P bodies dissolve, which indicates that a major role of P bodies is to facilitate translational repression and/or mRNA decay that is mediated through the miRNA pathway. In support of this, P-body components CCR4–NOT, DCP1 and DCP2 are required for miRNA-mediated mRNA decay in *Drosophila melanogaster* cells[126]. Indeed, miRNA-associated transcripts seem to undergo decay by a deadenylation-dependent pathway that is similar to the pathway used to degrade mRNAs[127,128].

Two examples of intimate interactions between the miRNA pathway and AU-rich elements (AREs) have now come to light. The first example involves the translational regulation of *CAT1* mRNA[52]. In some cell types, the 3′ UTR of *CAT1* is a target for translational repression by an miRNA (*miR-122*). Association with *miR-122* results in the localization of *CAT1* to P bodies and consequent translational silencing. However, AU-rich sequences that are also found in the 3′ UTR of the *CAT1* mRNA interact with HuR, a well-characterized ARE-binding protein. During stress, HuR is transported from the nucleus to the cytoplasm where it can bind *CAT1* mRNA and induce its release from P bodies, thereby relieving translational repression. This experiment is doubly significant, because it shows for the first time that mammalian P bodies are sites of mRNA storage, and not only decay[52].

The second example of the connection between ARE function and miRNAs involves decay of the ARE-containing *TNFα* mRNA[129]. It now seems that at least some of the extensive post-transcriptional control of the amounts of *TNFα* mRNA might be a result of interaction between the *TNFα* 3′ UTR and an miRNA, *miR-16*. The function of *miR-16* seems to require tristetraprolin (TTP), which is thought to interact with Ago-family members[129].

These recent findings might signify additional intricate modulation of mRNA decay and portend countless interactions between miRNAs and RNA-binding proteins.

mammalian cells, it has proved difficult to decipher their respective roles. It seems likely that there is some redundancy, but it is also possible that they are recruited to specific targets or show restricted spatial and temporal expression patterns.

3′→5′ decay. The 3′→5′ turnover of mRNAs is carried out by the exosome, a 10–12-subunit complex that consists of 6 proteins with significant homology to 3′→5′-phosphorolytic exoribonucleases and several accessory proteins, including factors with homology to hydrolytic 3′→5′ exonucleases and RNA helicases[4]. Each of the core exosome subunits has an RNase PH domain, which might contribute to the catalytic activity. However, it remains unclear whether all subunits are catalytically active or if some have roles in substrate recognition and placement. The exosome has several roles in addition to cytoplasmic mRNA decay, including 3′ processing of noncoding RNAs in the nucleus. Following 3′→5′ decay, the 5′ cap on the remaining oligomer is metabolized by the scavenger decapping enzyme DcpS[25].

Decapping and 5′→3′ decay. The 5′→3′ mRNA-decay pathway initiates with decapping. In *S. cerevisiae*, the decapping enzyme is a dimer that consists of Dcp1 and Dcp2 proteins. Dcp2, which contains a MutT domain, supplies the catalytic activity[26]. In higher eukaryotes, there is a third bridging component known as Hedls or Ge-1, which stimulates decapping activity[27,28]. Following decapping, the 5′→3′ exoribonuclease Xrn1 degrades the RNA.

Several accessory factors are required for efficient decapping. First, the Sm-like (Lsm) proteins seem to be involved in the 5′→3′ mRNA-decay process. The heptameric Lsm1–7 complex associates with the 3′ end of deadenylated mRNAs and promotes decapping[29,30]. Other Lsm proteins, namely EDC3 (enhancer of decapping-3; also known as Lsm16)[31], the *S. cerevisiae* PABP-binding protein-1 (Pbp1)[32] and mammalian RAP55 ((RNA-associated protein of 55 kDa; also known as Lsm14)[33], have been implicated in mRNA turnover. Even the bacterial Sm-like protein Hfq is an important modulator of mRNA decay[34]. Additional decapping accessory proteins include the Edc proteins[35], the DExD/H-box RNA helicase Dhh1 (also known as RCK/p54 or Me31B)[36] and Pat1 (REF. 37). Dhh1 and Pat1 have a role in mediating translational repression as well as decapping[38].

RNA helicase
An enzyme that catalyses the unwinding of the RNA secondary structure.

RNase PH
E. coli RNase PH is a phosphorolytic 3′→5′ exoribonuclease that is involved in the maturation of tRNA precursors.

Sm-like domain
A protein domain that is similar to that found in the Sm proteins associated with the small nuclear RNAs of the splicing machinery.

Many roads lead to P bodies

Components of the 5′→3′ mRNA-decay pathway are enriched in granular cytoplasmic foci known as P bodies or GW bodies[3,5]. Factors that are involved in translation initiation[39,40], deadenylation[41], decapping[42], 5′→3′-exonucleolytic decay[43], nonsense-mediated decay[44,45] and microRNA (miRNA)-mediated RNA decay[46] (BOX 1) have been shown to colocalize in these granular structures. Recent evidence indicates that at least some components of the exosome are localized in cytoplasmic foci, although these foci have not been definitively identified as P bodies[47]. It is important to emphasize, however, that the factors found in P bodies are not exclusively localized to these bodies, and their relative distribution between P bodies and the cytoplasm is unclear. Moreover, not all cells display P bodies unless exposed to stress[48] (FIG. 2). Current models propose that P bodies are cellular sites of decay[42], although it is unknown what percentage of mRNA decay actually occurs in these structures.

P bodies assemble when the 5′→3′ decay system is overloaded with RNA substrates or when mRNA decay is impaired, for example, by mutation of decapping factors

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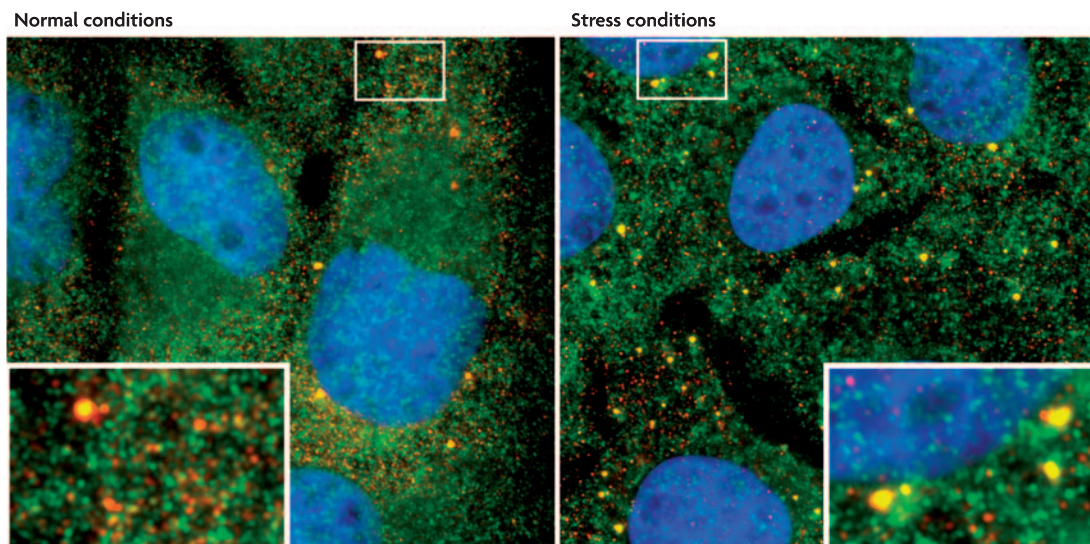

Figure 2 | **Mammalian P bodies and their induction by stress.** Human U2OS cells grown under normal conditions (left) or exposed to 0.5 mM sodium arsenite for 45 minutes (right) were fixed and stained for Lsm1 (green), XRN1 (red) and DNA (blue) and examined by confocal microscopy. P bodies are stained yellow where Lsm1 and XRN1 colocalize. Few or no P bodies are seen in the absence of stress, but conditions that promote mRNA release from polysomes cause the rapid assembly of the 5′→3′ mRNA-decay machinery in P bodies. Note that only a small fraction of total Lsm1 or XRN1 staining is present in P bodies under normal conditions. Insets show an enlarged view of the boxed regions.

or the XRN1 exonuclease[41,42]. On the other hand, P bodies substantially decrease in size and number or disappear when the amount of mRNA to be decayed is reduced. Blocking transcription or translation elongation[41,49], or inhibiting communication between the translation and mRNA-turnover machineries by mutating or depleting Dhh1, Pat1 (REF. 38), the nucleocytoplasmic-shuttling protein eIF4E transporter (eIF4E-T)[39,40] or the single-stranded-RNA-binding protein Sbp1 (REF. 50), results in a marked reduction in the size and number of P bodies, as does the mutation of factors involved in the early steps of mRNA decay (such as the CCR4–NOT deadenylase[40,42] or Lsm proteins[42]). These studies imply that mRNA is required for the formation of visible P bodies. Therefore, there is little evidence that P bodies are preformed structures that 'lie in wait' to degrade targeted mRNAs. Rather, P bodies are dynamic structures that are likely to form when required to regulate the interplay between translation and mRNA decay.

The identification of mRNA-decay intermediates in P bodies[42], together with the observations above, indicates that P bodies are indeed sites of mRNA turnover. However, P bodies have additional roles in the cell. For example, they might represent structures that are formed only when the 5′→3′ mRNA-decay pathway is disrupted or overloaded, or they might sequester mRNAs that are targeted for decay but cannot be immediately degraded. In support of this idea, glucose deprivation in *S. cerevisiae* causes a marked inhibition of mRNA turnover and concomitant accumulation of P bodies[51]. That normal mRNAs can be released from P bodies[52,53] supports a broader, regulatory role for P bodies in RNA sorting and sequestration rather than simply functioning as sites of mRNA decay. Sequestration of mRNA in P bodies might be a way of compartmentalizing mRNAs that are targeted for destruction to ensure that they cannot reassociate with ribosomes and generate unwanted or aberrant proteins.

Unusual routes to decay

Although most transcripts undergo deadenylation-dependent decay as described above, there are several exceptions. Specific mRNAs seem to bypass the standard pathways to allow unique regulation of decay.

Deadenylation-independent decapping. Two unrelated transcripts, *RPS28B* and *EDC1* mRNAs, bypass the deadenylation step to be directly decapped. The *S. cerevisiae RPS28B* mRNA undergoes decay through an intriguing autoregulatory mechanism (FIG. 1b). The Rps28B protein binds directly to a stem-loop structure in the 3′ UTR of its own mRNA and recruits Edc3 — an enhancer of decapping. This presumably leads to association of other decapping factors and allows deadenylation-independent decapping of the transcript[54].

The *S. cerevisiae EDC1* mRNA, which encodes the decapping-regulatory protein Edc1, also decays by a deadenylation-independent decapping pathway[55]. In this instance, deadenylation seems to be prevented by a novel interaction between the poly(A) tail and a poly(U) stretch in the 3′ UTR. This intramolecular pairing blocks access to the deadenylase. That Edc1 encodes a decapping regulator and is decayed by a pathway that is highly sensitive to decapping indicates the possible existence of feedback regulation. Also, decapping of *EDC1* mRNA requires some subunits of the CCR4–NOT complex, which signifies an interesting link between the deadenylation complex and decapping[55].

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Box 2 | **Nuclear mRNA surveillance**

Although most transcripts are destined for decay in the cytoplasm after a productive life encoding proteins, errors in transcription, mRNA export or mRNA processing frequently result in nuclear mRNA decay. This process has been studied almost exclusively in *Saccharomyces cerevisiae*. As in the cytoplasm, both 3′→5′ and 5′→3′ mRNA-decay pathways have a role in nuclear mRNA turnover, although the favoured pathway seems to be determined by the substrate. Transcripts that are restricted to the nucleus because of a defect in export undergo decay through both mechanisms[130]. By contrast, unspliced pre-mRNAs and mRNAs with defective polyadenylation are subject to more rapid decay mediated mainly by the nuclear exosome[131,132]. Similar to cytoplasmic 5′→3′ mRNA decay, nuclear 5′→3′ decay requires an Lsm complex (nuclear Lsm2–8) for decapping and a 5′→3′ exonuclease, Rat1, which is homologous to Xrn1 (REF. 133). Although nuclear mRNAs undergo deadenylation, nuclear deadenylation differs from that usually observed in the cytoplasm in that it is more processive[131]. It remains unclear what enzyme removes the poly(A) tail in the nucleus.

In addition to its function as a surveillance pathway, nuclear mRNA decay is used to regulate gene expression. The amounts of several mRNAs change in cells with mutations in nuclear exosome subunits[8,134]. One characterized example is the *S. cerevisiae NAB2* mRNA, which is regulated through a poly(A) tract within its 3′ UTR[135]. The poly(A) tract interferes with 3′-end formation and sensitizes the transcript to decay by the nuclear exosome subunit Rrp6 and the nuclear exosome. This effect is mediated by a heterogeneous nuclear ribonucleoprotein, known as Nab2, which interacts with the poly (A) tract and enhances exosomal decay.

Recent studies in *S. cerevisiae* have uncovered a new enzyme complex, TRAMP, which is involved in the decay of several types of nuclear RNA[136,137]. The TRAMP complex contains a poly(A)-polymerase activity, Trf4, and catalyses the polyadenylation of mRNAs. Intriguingly, instead of stabilizing the mRNA, in this case, polyadenylation results in the recruitment and activation of the exosome, leading to rapid decay. This mechanism is reminiscent of bacterial mRNA decay.

Endonuclease
Also known as an endoribonuclease. An enzyme that catalyses the hydrolysis of ester linkages within an RNA molecule by creating internal breaks.

Stress granule
A cytoplasmic structure that develops in mammalian cells during certain types of cellular stress. It is believed to be a site where translationally repressed mRNAs are stored or sorted for decay. P bodies often assemble close to stress granules.

Cartilage hair hypoplasia
A recessively inherited developmental disorder characterized by short stature, fine and sparse hair, immune deficiency and a predisposition to malignancy. The defect is caused by mutations in RNase MRP.

Messenger ribonucleoprotein
(mRNP). A complex that comprises an mRNA and associated RNA-binding proteins.

Endoribonucleolytic decay. Perhaps the most efficient means of destroying an mRNA is through endonucleolytic cleavage, which produces two fragments that are susceptible to exonucleases (FIG. 1c). Several cellular endonucleases that target mRNA have been characterized in recent years, including PMR1, IRE1 and, somewhat unexpectedly, the predominantly nucleolar ribosomal RNA (rRNA)-processing enzyme RNase MRP. Furthermore, unidentified endonucleases have been implicated in the decay of aberrant mRNAs (see below)[56,57], and short interfering RNAs (siRNAs) initiate mRNA decay through endonucleolytic cleavage mediated by Argonaute protein-2 (AGO2)[58,59].

PMR1 was first identified as a polysome-associated endonuclease involved in the oestrogen-induced destabilization of albumin mRNA in *X. laevis*[60]. Unlike most mRNA decay, endonucleolytic cleavage by PMR1 occurs on polysomes, targeting actively translating mRNAs. Recent studies have identified two polysome-targeting domains[61] in PMR1 as well as a domain that binds the translational regulator T-cell-restricted intracellular antigen-1 (TIA-1). This interaction diverts TIA-1 to stress granules during arsenite stress[62].

IRE1 is another endonuclease that targets actively translating mRNAs. In this case, transcripts that are usually targeted to the endoplasmic reticulum (ER) are rapidly cleaved as part of the unfolded-protein response that is induced as a result of ER stress in *D. melanogaster* S2 cells[63]. Although IRE1 has not yet been shown to be directly responsible for this cleavage, it does have endonuclease activity that catalyses splicing of the *XBP1* mRNA[64].

RNase MRP is a multi-component complex with an RNA core that is known to be involved in processing rRNAs and mitochondrial RNAs[65]. However, recent studies in *S. cerevisiae* have identified a novel role for RNase MRP in degrading the *CLB2* mRNA, which encodes a B-type cyclin, at the end of mitosis[66]. RNase MRP was found to cleave *CLB2* mRNA within its 5′ UTR leading to degradation of the 3′ product by Xrn1. Intriguingly, decay of *CLB2* seems to occur in a previously undescribed type of P body, known as a temporal asymmetric MRP (TAM) body, which contains RNase MRP and Xrn1, but not other P-body components such as Dcp1 or Lsm1 (REF. 67). Whether RNAse MRP has a comparable role in mammalian cells is not known, although some of the defects seen in the syndrome cartilage hair hypoplasia, which is caused by mutations in RNase MRP, are consistent with defects in cell-cycle regulation, and *cyclin B* mRNA is upregulated in these patients[68].

Owing to their potency, cellular endoribonucleases are highly specific and/or regulated. IRE1 and PMR1 are activated only when needed[63,69], whereas RNase MRP is restricted to the nucleolus and mitochondria except at the end of mitosis when it is transported to TAM bodies[67]. RNase MRP also has restricted specificity — only three substrates have so far been identified[66].

Re-routing faulty transcripts: mRNA surveillance

Each step in the production of a mature mRNA transcript provides an opportunity for introducing errors. To preserve translational fidelity, the cell has evolved means for detecting and degrading aberrant transcripts, thereby protecting it from potentially toxic protein products. Surveillance for inappropriate mRNA processing occurs in the nucleus (BOX 2), whereas the three pathways discussed below are translation dependent and detect aberrant messenger ribonucleoprotein (mRNP) structures in the cytoplasm.

Nonsense-mediated decay. The most well-studied surveillance mechanism is nonsense-mediated decay (NMD)[2] (FIG. 3a). NMD detects and degrades transcripts that contain premature termination codons (PTCs). PTCs can arise from mutations, frame-shifts, inefficient processing, leaky translation initiation and extended 3′ UTRs. These transcripts, if translated, could produce truncated proteins with aberrant functions. The NMD pathway has been found in all eukaryotes and the core proteins of the NMD complex, UPF1, UPF2 and UPF3, are highly conserved[2]. Beyond this conservation, however, the detection of the PTC and the method of decay of the transcript seem to have diverged. Nevertheless, a common thread is the recognition of an aberrant mRNP conformation.

In mammalian cells, one well-defined feature of an aberrant transcript is retention of the exon junction complex (EJC). The EJC is the residual 'mark' of splicing, and this protein complex is deposited 20–24 nucleotides upstream of every exon junction[70]. As most introns are located in coding regions, EJCs are usually displaced by translating ribosomes. But in mRNAs that contain a PTC, the EJC remains inappropriately associated with

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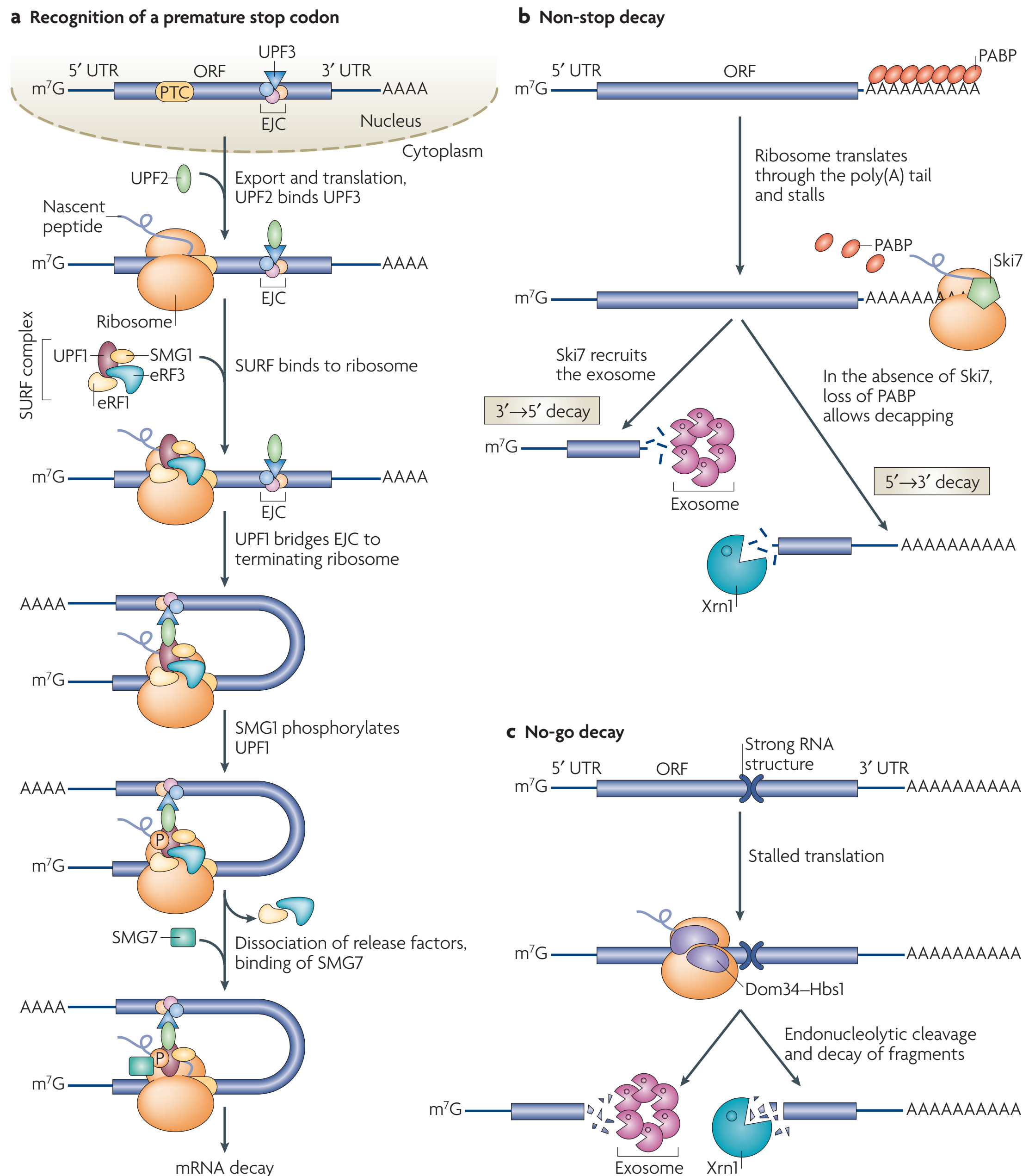

Figure 3 | **mRNA-surveillance mechanisms. a** | Nonsense-mediated decay (NMD). Following splicing in the nucleus, the exon junction complex (EJC), which contains UPF3 (a core protein of the NMD pathway), is associated with the transcript, and the resulting messenger ribonucleoprotein is exported to the cytoplasm. In the cytoplasm, a second NMD core protein, UPF2, binds to UPF3. Ribosomes associate and translate the mRNA, but are stalled on encountering a premature termination codon (PTC). This results in binding of the SURF complex (comprising SMG1, UPF1 and the peptide-release factors eRF1 and eRF3) to the ribosome. UPF1 also binds UPF2, thereby linking the EJC to the PTC. Phosphorylation of UPF1 by SMG1 leads to dissociation of eRF1 and eRF3 and binding of the SMG7 adaptor protein. Subsequent steps that are still being elucidated lead to mRNA decay by various pathways. **b** | Non-stop decay. Translation of an mRNA that lacks a stop codon results in ribosomes traversing the poly(A) tail, displacing poly(A)-binding protein (PABP) and stalling at the 3′ end of the mRNA. One model proposes that, in yeast and mammalian cells, Ski7, an adaptor protein that functions as a molecular mimic of tRNA, binds to the A site on the stalled ribosome to release the transcript, and then recruits the exosome. The exosome degrades the poly(A) tail and mRNA body. In another pathway described in *Saccharomyces cerevisiae*, in the absence of Ski7, the displacement of PABP by the translating ribosome renders the mRNA susceptible to decapping and 5′→3′ decay by the 5′→3′ exoribonuclease Xrn1. **c** | No-go decay. Ribosomes can stall within the open reading frame (ORF), for example, on encountering a strong secondary RNA structure. The Dom34 and Hbs1 proteins bind the transcript near the stalled ribosome and initiate an endonucleolytic cleavage event near the stall site. This releases the ribosome and generates two mRNA fragments, each with a free end exposed for exonucleolytic decay by the exosome and Xrn1, respectively.

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the mRNA downstream of the PTC, where it is detected by the surveillance machinery (FIG. 3a). However, an inappropriately positioned EJC is not a universal trigger for mRNA decay. In *D. melanogaster* and *S. cerevisiae*[71], as well as in mammalian immunoglobulin μ transcripts[72], the EJC has no role and, instead, the distance between the stop codon and the poly(A) tail is crucial for triggering mRNA decay. By extending the 3′ UTR, the PTC increases the distance from the terminating ribosome to the poly(A) tail, and the resultant mRNP conformation is then recognized as abnormal[71–73].

Despite these alternative mechanisms for identifying NMD substrates, all characterized pathways have in common the requirement for the NMD-complex component UPF1. In EJC-dependent NMD, the role of UPF1 and other surveillance-complex components is becoming clear. During a normal translation-termination event, the stop codon is recognized by the peptide-release factors eRF1 and eRF3 leading to peptidyl-transfer RNA (tRNA) hydrolysis. When termination occurs at a PTC, peptide release is delayed and the ribosome remains stalled at the stop codon. This allows the binding of UPF1 and the SMG1 kinase to the release factors, forming a complex, known as SURF, on the stalled ribosome[74]. At this point, through binding to UPF2, UPF1 interacts with the EJC, which lies at an exon–exon junction downstream of the termination site. Formation of this bridge between SURF at the ribosome and the EJC leads to phosphorylation of UPF1 by SMG1 and dissociation of the release factors[74]. Finally, SMG7, a 14-3-3-related protein, associates with the complex and triggers decay[44]. The precise mechanism of decay is not known, but dephosphorylation of UPF1, which involves the adaptor protein SMG5 and the protein phosphatase PP2A[75,76], seems to be important.

Recent studies indicate that the decay pathways used by PTC-containing mRNAs are redundant. In *S. cerevisiae*, the predominant pathway involves deadenylation-independent decapping and occurs in P bodies[45], although there is also evidence for a deadenylation-dependent mechanism that culminates in 3′→5′ decay[77]. In at least one instance, deadenylation can occur prior to decay in mammalian NMD, in which case both 5′→3′ and 3′→5′ mRNA-decay pathways are subsequently used[78]. However, it remains possible that there are also deadenylation-independent pathways in mammals. Interestingly, NMD in *D. melanogaster* is mediated by endonucleolytic cleavage near the PTC, followed by exonucleolytic decay of the resultant fragments[56].

Finally, we should emphasize that the NMD pathway is not solely reserved for the decay of aberrant transcripts, but has an important role in the regulation of normal gene expression. Microarray analysis of the transcriptome in UPF1-depleted mammalian cells identified numerous upregulated normal transcripts, some of which contained upstream ORFs, 3′-UTR introns, frameshifts or other features that mimic a PTC[79]. Furthermore, mRNAs that apparently lack any feature resembling a PTC, namely histone mRNAs[80] and the *ARF1* mRNA[81], degrade in a UPF1-dependent manner. Intriguingly, in these cases, UPF1 is recruited to the transcript through interaction with a factor bound to the 3′ UTR — the stem-loop-binding protein (SLBP) in the case of histone mRNAs[80] and Staufen in the case of *ARF1* (REF. 81). Neither of these mechanisms requires UPF2 or UPF3, which indicates that these pathways are distinct from classic NMD.

Non-stop decay. Non-stop decay (NSD) targets mRNAs that lack a stop codon (FIG. 3b). Such transcripts can be generated by breakage, or by the absence of an in-frame stop codon, causing translation to proceed along the poly(A) tail. Premature polyadenylation might be an important contributor to the production of substrates for this decay pathway. In addition to protecting cells from aberrant proteins, NSD facilitates the release of the ribosome.

Currently, there is evidence for two distinct pathways of NSD, which probably function in concert. The original model for NSD, which is conserved in yeast and mammalian cells, proposes the requirement for the cytoplasmic exosome, the SKI complex (Ski2, Ski3 and Ski8), and the adaptor protein Ski7 (REFS 82,83). The stalled ribosome at the 3′ end of a transcript initiates NSD as follows: the C terminus of Ski7, which is similar in structure to the GTPase domain of elongation factor-1A (EF1A) and eRF3, binds to the empty A site of the ribosome, thereby releasing the ribosome. Ski7 then recruits the exosome and the associated SKI complex to deadenylate and then rapidly decay the transcript in the 3′→5′ direction[82,83]. In the absence of Ski7, a second pathway, the 5′→3′ mRNA-decay pathway can mediate NSD in *S. cerevisiae*[84]. This seems to be due to the removal of PABP by the translating ribosome, which is known to render transcripts susceptible to decapping as well as reducing translational efficiency.

No-go decay. The final mRNA-surveillance mechanism, no-go decay[85] (NGD) (FIG. 3c), was only recently discovered in yeast and consequently is the least understood pathway. NGD prevents the sequestration of translation factors to faulty transcripts by detecting stalled ribosomes on an mRNA and endonucleolytically cleaving the mRNA near the stall site. This releases the stalled ribosome and mRNA fragments, which are decayed by the exosome and Xrn1 (REF. 85). Curiously, this is reminiscent of NMD in *D. melanogaster*[56]. The full mechanism of NGD is not understood and the endonuclease has not been identified; however, it has been shown that the initial cleavage event requires Dom34 and Hbs1. These proteins are related to eRF1 and eRF3, respectively, and might therefore interact directly with the stalled ribosome. Also, Hbs1 is related to the NSD factor Ski7. As Ski7 mediates the release of stalled ribosomes at the 3′ end of transcripts without a stop codon, perhaps Hbs1 functions in a similar manner in NGD[85]. It is not as yet known how important this mechanism is for viability. However, ribosome stalling has been implicated in transcript decay in *Arabidopsis thaliana*, which indicates that it could be a conserved process[86].

14-3-3 proteins
A large family of small, acidic proteins that interact predominantly with proteins that are phosphorylated on serine or threonine residues. 14-3-3 proteins have been implicated in many intracellular-signalling events through their ability to regulate catalytic activity, subcellular localization and protein–protein interactions.

A site
The site at which the ribosome binds an aminoacylated tRNA for addition to the elongating peptide chain.

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Table 2 | **ARE-binding proteins**

RNA-binding protein	Function	RNA-binding domain	Mode of action	Modifications	Other functions
AUF1 (hnRNP D) and its four splice isoforms (p37, p40, p42, p45)	Usually destabilizing	RRM	Recruit the exosome; remodel mRNA to allow other proteins to bind	Phosphorylation allows isomerization by PIN1 leading to dissociation from RNA; interacts with 14-3-3 proteins	DNA binding
CUG-BP	Destabilizing	RRM	Recruits PARN; modulates ARE function	Phosphorylated by myotonic dystrophy protein kinase	Splicing; translation
ELAV proteins, for example, HuR and HuD	Stabilizing	RRM	Compete with destabilizing proteins for ARE-binding; might relocalize mRNAs away from decay machinery	CARM1-mediated methylation reduces stabilizing function	Translation; RNA localization
KSRP	Destabilizing	KH domain	Recruits decay enzymes: PARN and the exosome	Phosphorylation by p38-MAPK pathway leads to reduced RNA-binding affinity	Splicing
RHAU	Destabilizing	RNA helicase	Recruits decay enzymes: PARN and the exosome	Not known	Not known
TIA-1, TIAR	Translational silencing	RRM	Induce aggregation into stress granules	Phosphorylated by FAST	Alternative splicing
Tristetraprolin (TTP, TIS11, ZFP36), BRF1 (TIS11B, ZFP36L1), BRF2 (TIS11D, ZFP36L2)	Destabilizing	CCCH-type zinc finger	Recruit decay enzymes: CCR4, DCP1, PM-Scl75, RRP4	Phosphorylation by p38-MAPK pathway leads to association with 14-3-3 proteins	Transcription

Entries in brackets indicate alternative protein names. AUF1, AU-rich binding factor-1; CUG-BP, CUG-binding protein; ELAV, embryonic lethal abnormal vision; FAST, Fas-activated serine/threonine kinase; hnRNP, heterogeneous nuclear ribonucleoprotein; KSRP, KH splicing regulatory protein; MAPK, mitogen-activated protein kinase; PARN, poly(A)-specific ribonuclease; RHAU, RNA helicase associated with AU-rich element; RRM, RNA-recognition motif; TIA-1, T-cell-restricted intracellular antigen-1; TIAR, TIA-1-related; TIS, TPA-induced sequence; ZFP, zinc finger protein.

Signals that control mRNA decay

Microarray analyses have revealed that 40–50% of changes in gene expression in response to cellular signals occurs at the level of mRNA stability[87,88]. These changes are usually induced by alterations in the composition of mRNPs that either inhibit or facilitate decay. Not surprisingly, mRNA-stability determinants are found predominantly in the 3′ UTR where protein complexes are sheltered from traversing ribosomes, but they are also found in the 5′ UTR and coding region.

AU-rich elements and their binding proteins. By far the most well-studied class of mRNA-stability element is the AU-rich element (ARE). This varied sequence element is found in the 3′ UTR of many transcripts that encode cytokines, proto-oncogenes and transcription factors, among others[6]. AREs are classified into several classes on the basis of the number and context of the AUUUA pentamer. However, it seems that no two AREs are identical. Even when the ARE sequences are similar, the flanking sequence can influence the overall effect on mRNA stability. For example, the function of the ARE in the 3′ UTR of *TNFα* mRNA is kept in check by a downstream constitutive decay element[89], and UG-rich sequences adjacent to AREs modulate ARE function through interaction with the CUG-binding protein (CUG-BP) and related proteins[90,91].

Many ARE-binding proteins have been identified, including AU-rich binding factor-1 (AUF1), tristetraprolin (TTP), KH splicing regulatory protein (KSRP), RHAU, embryonic lethal abnormal vision (ELAV) proteins, and TIA-1 and TIA-1-related protein (TIAR) (TABLE 2). However, only recently have we started to understand how these factors modulate mRNA turnover. The key to enhancing decay lies in recruiting the mRNA-decay machinery. In some cases, the ARE can perform this function alone — for example, the exosome shows affinity for AREs[92]. Alternatively, AUF1, KSRP, RHAU and TTP can all interact directly or indirectly with mRNA-decay factors. The 37-kDa isoform of AUF1 interacts with the exosome[93]. KSRP and RHAU both bind to the PARN deadenylase and to the exosome, and this results in enhanced decay of the target mRNA[94,95]. Indeed, tethering of KSRP to HIV RNA was recently shown to be sufficient to inhibit viral replication, presumably by destabilization of the tethered transcript[96]. TTP interacts with the exosome, the decapping enzyme and CCR4 deadenylase[97]. Also, binding of TTP modulates PARN activity *in vitro*, although a direct interaction has not been detected[98]. Finally, CUG-BP, which has affinity for AREs and for UG-rich sequences, interacts directly with PARN to recruit the enzyme to the bound mRNA and promote deadenylation[90].

It is not clear how stabilizing mRNA-binding proteins perform their function. They might function simply by removing the mRNAs from the sites of decay or by competing for the binding site of destabilizing factors. Alternatively, they could directly interact with and inhibit the mRNA-decay machinery or somehow strengthen the PABP–poly(A) interaction, which prevents

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deadenylation. There is evidence to support two of these models. The well-characterized stability factor, HuR, has been shown to compete for binding sites with the destabilizing proteins AUF1, KSRP and TTP[99,100]. Therefore, under conditions in which HuR binding is favoured, target mRNAs are stabilized. Binding of HuR has also been shown to coincide with the relocation of transcripts from P bodies to polysomes[52].

Modulation of RNA-binding proteins. Although many studies have been devoted to identifying the ARE-binding proteins, perhaps the most interesting aspect of current research is how this binding is modulated in response to cellular signals. One reason for keeping an mRNA inherently unstable is to facilitate rapid changes in its abundance when required. Therefore, remodelling of the mRNP can allow conversion from an unstable to a stable form of the transcript and result in increased mRNA abundance and protein production over a short time. When these changes are coordinated with elevated transcription or translation, massive changes in gene expression can be observed.

Several signalling pathways have been found to impinge on ARE function, for example, p38 mitogen-activated protein kinase (p38 MAPK), extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and Wnt/β-catenin. These signalling pathways can modulate mRNP structure and mRNA stability by mediating phosphorylation of ARE-binding proteins. Phosphorylation can either alter the affinity of the protein for its substrate or result in the binding of other factors that change its function. The p38-MAPK pathway modulates the stability of many labile mRNAs. Both TTP and KSRP are putative targets of the p38-MAPK pathway. Several ARE-containing mRNAs, including *p21* and myogenin mRNA, are induced during differentiation of myoblasts into myocytes. These transcripts are usually unstable and associate with KSRP[101]. However, on activation of the p38-MAPK pathway during differentiation, KSRP is directly phosphorylated by p38 and dissociates from the ARE[101]. The ARE is then free to associate with stabilizing factors such as HuR[102]. This regulation facilitates the controlled expression of muscle-specific transcripts at appropriate times.

By contrast, phosphorylation of TTP through the p38-MAPK pathway seems to have little effect on its RNA-binding properties[103]. However, there are data indicating that phosphorylation allows TTP to interact with 14-3-3 proteins and this could modulate its function or subcellular localization[104]. At present, there are conflicting data as to whether 14-3-3 interaction alters the ability of TTP to mediate rapid mRNA decay[103,105].

The decay of *GM-CSF* mRNA is regulated through the ERK pathway in eosinophils[106]. In resting cells, *GM-CSF* transcripts are bound to AUF1 and are inherently unstable. Interestingly, activation of the ERK pathway leads to phosphorylation of AUF1 isoforms and simultaneous dephosphorylation of the AUF1-associated peptidylprolyl isomerase PIN1. This change in the phosphorylation state activates PIN1, allowing it to isomerize AUF1 and induce its dissociation from the *GM-CSF* mRNA. The mRNA is now free to associate with the heterogeneous nuclear ribonucleoprotein hnRNP C and it is consequently stabilized to increase GM-CSF expression[106].

Puf proteins and post-transcriptional operons. AREs are by no means the only mRNA-stability elements. In *S. cerevisiae*, the Puf proteins, which are related to the *D. melanogaster* translational regulator Pumilio, recognize UG-rich sequences and direct the accelerated decay of their substrate mRNAs by recruiting the CCR4–NOT deadenylase through direct interaction with Caf1 (REF. 107). Global analyses have revealed that each Puf protein has its own set of functionally related target transcripts. For example, Puf3 preferentially interacts with mRNAs that encode mitochondrial proteins, whereas Puf4 associates with mRNAs that encode nucleolar rRNA-processing factors[108]. This implies that each Puf protein can coordinately regulate certain cellular processes. In support of this, Puf3 function is dependent on the cellular carbon source — Puf3-target mRNAs are unstable in *S. cerevisiae* grown in glucose but are stable in *S. cerevisiae* grown in ethanol media[109].

Stabilizing elements. Specific RNA-sequence elements can also confer stability on transcripts. Unusually stable transcripts often encode proteins with housekeeping roles. Three such transcripts, α-globin, β-globin[110,111] and α-collagen[112], share pyrimidine-rich elements in their 3′ UTRs that are required for stability and function through binding of the KH-domain RNA-binding proteins αCP1 and αCP2 (also known as hnRNP E1 and E2). These proteins were first found as components of the α-complex associated with the C-rich stability determinant of α-globin. The αCPs have also been found to be associated with a pyrimidine-rich element, the differentiation-control element (DICE) in the lipoxygenase-15 mRNA, which is another erythroid-specific transcript[113]. DICE has been implicated as a translational and an mRNA-stability regulator[113,114]. The mode of action of αCPs remains unclear, although their interaction with PABP indicates that they might stabilize mRNA by protecting the poly(A) tail against deadenylation[115].

Interfacing with other cellular mechanisms

Several mRNA-decay factors function in other processes to facilitate coordinated changes in gene expression. One aspect of gene expression that has been linked with mRNA decay is translation. Furthermore, other steps in mRNA metabolism, such as transcription and mRNA localization, have also been connected with specific mRNA-turnover factors. Finally, less obvious processes, such as protein turnover and even genome stability, might also be coupled with mRNA decay.

Translation. General inhibition of translation elongation results in the stabilization of mRNAs on polysomes, whereas inhibition of translation initiation diverts transcripts to P bodies for decay. Observations like these indicate a strong link between translation and mRNA turnover. They also signify the complexity of the interaction:

Heterogeneous nuclear ribonucleoprotein
(hnRNP). A historic term used to describe protein complexes that associate with mRNAs and pre-mRNAs in the nucleus.

KH domain
An RNA-binding domain that was first identified in the hnRNP K protein.

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translation is necessary for, but also competes with, mRNA decay. Many mRNA-binding proteins (for example, ELAV proteins, CUG-BP and αCP) that influence mRNA turnover also regulate translation. The poly(A) tail itself is both a determinant of mRNA stability and of translation efficiency. The first step in mRNA decay, deadenylation, is therefore also a means to inhibit the translation of mRNAs.

The PARN deadenylase is an integral part of the machinery that is associated with the cytoplasmic polyadenylation element (CPE) in *X. laevis* oocytes[116]. CPE-containing transcripts are silenced at the level of translation by maintaining a short poly(A) tail. The CPE is involved in activating translation of specific mRNAs on oocyte maturation through binding to the CPE-binding protein CPEB, which, in turn, recruits the GLD2 poly(A) polymerase. This leads to extension of the poly(A) tail and concomitant enhancement of translation. PARN is essential for this regulation[116]. PARN interacts directly with CPEB and constantly removes the poly(A) tail, counteracting the GLD2 poly(A)-polymerase activity. When maturation is induced, CPEB is phosphorylated, expelling PARN from the mRNP. GLD2 is then free to polyadenylate the mRNA. Interestingly, CPEB is a component of P bodies, which indicates that localization of mRNA to P bodies might also have a role in this phenomenon[117].

Transcription. The first step in gene expression, transcription, can be connected with the last, mRNA decay, through at least two factors: the CCR4–NOT deadenylase and the Rpb4 transcription factor. The cell might conserve energy by coordinating these two processes so that they work together to modulate gene expression.

The CCR4–NOT complex was first identified in *S. cerevisiae* as a glucose-regulated transcription complex that represses RNA polymerase II. The entire complex is evolutionarily conserved, and is required for both transcription and deadenylation. Furthermore, CCR4–NOT has a role in protein turnover through the Not4 subunit, which is an E3 ubiquitin ligase[118]. Although it remains unclear how all these functions are related, it seems likely that this complex detects and responds to signals to control gene expression at several levels.

The *S. cerevisiae* Rpb4 protein is an integral subunit of RNA polymerase II, but is also required for deadenylation and decay of a subset of transcripts that encode proteins involved in protein synthesis[119]. Rpb4 interacts with the Lsm–Pat1 complex and localizes to P bodies, which indicates a bona fide role in mRNA turnover. It is thought that Rpb4 has an essential role in modulating gene expression in response to stresses such as glucose deprivation and heat shock.

mRNA localization. The correct localization of mRNAs is important during development. Both DCP1 and CCR4 have been implicated in the localization of *D. melanogaster* transcripts. During oogenesis, DCP1 has a similar localization pattern to the *oskar* mRNA and is required for correct localization to the posterior pole of the oocyte[120]. Another protein with a role in mRNA decay, Dhh1, which is known as Me31B in *D. melanogaster*, is also found in the *oskar* mRNP[121]. However, DCP2, the catalytic subunit of the decapping enzyme, is absent from the localized mRNP, which indicates that decapping activity is separable from this localization function[120]. Another component of the *oskar* mRNP, Exuperantia, which is also required for *oskar* mRNA localization, contains an RNase D-type exonuclease domain that is similar to those found in deadenylases[24].

In the early *D. melanogaster* embryo, maternal *hsp83* mRNA is localized by a degradation/protection mechanism that removes mRNAs from the bulk cytoplasm while maintaining mRNAs at the posterior pole. *Hsp83* mRNA is bound directly by the smaug protein, which recruits the CCR4–NOT deadenylase, thereby destabilizing the transcript and contributing to localization[122].

Conclusions and future perspectives

Over the past five years, the study of mRNA turnover has matured significantly and mRNA decay is now considered a key player in regulated gene expression. However, there are still many gaps in our understanding of the enzymes, pathways and regulation of mRNA turnover. The roles of several factors that are integral to the turnover pathways remain unclear. The Lsm1–7 complex and the other Lsm-related proteins are clearly implicated in mRNA decay, but their specific functions are only vaguely defined. Similarly, there are numerous deadenylases, but the precise role of each remains controversial. Understanding these factors better might allow us to tease out the mechanisms by which deadenylation is initiated and how it triggers the subsequent steps in decay.

P bodies are clearly destined to be the focus of many studies in the next few years. The relationship between P bodies and other cytoplasmic RNA granules, such as stress granules, TAM bodies and germ-cell granules, needs to be defined. Also, the factors and steps in the various pathways that target and deliver mRNA to P bodies should be dissected. Specifically, the switch that diverts a transcript from translation to turnover warrants investigation.

Although we now have an idea of how PTCs are recognized in the EJC-dependent pathway, the alternative pathway that is induced by extended 3′ UTRs remains somewhat ambiguous. In addition, it is not known how the surveillance machinery induces mRNA decay, or even how the precise pathway of NMD is specified. Several studies now indicate the existence of unidentified endonucleases that might have important roles in mRNA surveillance. Undoubtedly, these factors will soon be characterized.

The regulation of mRNA stability in response to cellular signals is clearly as complex as that occurring at the transcriptional level. Phosphorylation, isomerization, relocalization and methylation are just some of the ways that RNA-binding proteins can be modulated, leading to marked changes in mRNP structure. Furthermore, each mRNA has its own complement of RNA-binding proteins that define stability, localization and translation efficiency. By permitting RNA-binding proteins to be

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shared between transcripts with similar functions, the cell has developed a powerful means to coordinate gene expression. It is easy to predict that there will be numerous studies uncovering various aspects of regulation at this level.

Note added in proof

Two groups recently reconstituted and showed catalytic activity of the *S. cerevisiae*[138,139] and human[138] exosomes *in vitro*. A crystal structure for the nine-subunit human exosome was also reported[138]. Interestingly, the yeast exosome activity seems to be principally contributed by Dis3 (also known as Rrp44), an RNase R-like protein with processive hydrolytic activity that is not associated with the human exosome[138,139]. By contrast, the human exosome has processive phosphorolytic activity, which is thought to be contained within the RRP41–RRP45 active site[138]. These two studies show that many of the exosome subunits lack catalytic activity and instead have structural roles, and also uncover intriguing differences among archaeal, yeast and human exosome activities.

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