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The Regulation of mRNA Stability in Mammalian Cells: 2.0

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Abstract

Messenger RNA decay is an essential step in gene expression to set mRNA abundance in the cytoplasm. The binding of proteins and/or noncoding RNAs to specific recognition sequences or secondary structures within mRNAs dictates mRNA decay rates by recruiting specific enzyme complexes that perform the destruction processes. Often, the cell coordinates the degradation or stabilization of functional subsets of mRNAs encoding proteins collectively required for a biological process. As well, extrinsic or intrinsic stimuli activate signal transduction pathways that modify the mRNA decay machinery with consequent effects on decay rates and mRNA abundance. This review is an update to our 2001 *Gene* review on mRNA stability in mammalian cells, and we survey the enormous progress made over the past decade.

Keywords

mRNA degradation pathways; RNA surveillance; AU-rich elements; RNA-binding proteins; noncoding RNAs

1. Introduction

We now thoroughly appreciate that the abundance of an mRNA is a function not only of its synthesis, processing, and nuclear export rates, but also of its degradation rate in the cytoplasm. Messenger RNA degradation rates often change in response to a stimulus, thereby quickly increasing or decreasing mRNA abundance to meet a cell's needs for individual proteins. RNA-binding proteins and/or noncoding RNAs decorate mRNAs via their binding to *cis*-acting elements within mRNAs. The constellations of bound proteins and noncoding RNAs dictate mRNA degradation rates via their ability to recruit (or exclude) the mRNA degradation machinery. In a 2001 issue of *Gene*, we reviewed the regulation of mRNA stability in mammalian cells. At the end of the article, we posed a few key questions for the future, including the nature of the mRNA degradation machinery and how RNA-binding proteins recruit this machinery to mRNAs; the role that RNA structure might have for protein recognition of *cis*-acting elements; and how signal transduction pathways might affect mRNA degradation. In this updated review article, we address these questions together with those we did not even know to ask 10 or so years ago. We first describe the various complexes of mRNA degradation enzymes, and then detail the amazing progress

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related to mRNA surveillance pathways, including nonsense-mediated mRNA decay. We follow this with last decade's unveiling of coordinated regulation of mRNA subsets, i.e., posttranscriptional operons. In this context, we describe progress towards understanding how AU-rich and G-rich elements in select mRNAs promote their degradation. Finally, we describe what has become a major frontier in the field of posttranscriptional gene expression: the roles of noncoding RNAs in mRNA degradation. Progress in the field of mRNA degradation has been enormous during the past decade. We thus aim to entice, and implore the interested reader to seek the array of excellent, detailed reviews available on virtually every topic we discuss here.

2. Messenger RNA degradation pathways and enzymes

Work during the last decade has revealed that, with a few exceptions, the mRNA decay enzymes in *S. cerevisiae* have homologous counterparts in mammalian cells. Deadenylation-dependent mRNA decay is the major pathway in mammalian cells (Fig. 1). One or a combination of three deadenylases performs poly(A) tail removal, and the particular enzymes utilized appear to be mRNA specific (Garneau et al., 2007; Goldstrohm and Wickens, 2008). Pan2-Pan3 is a PABP-dependent poly(A) ribonuclease, absent in *S. cerevisiae*, involved in the initial trimming of poly(A) tails. The Ccr4-Not complex (also known as Ccr4-Caf complex) performs subsequent deadenylation. Ccr4-Not is the major deadenylase in eukaryotes and consists of nine protein subunits. A third deadenylase is the poly(A)-specific exoribonuclease PARN, also absent in *S. cerevisiae*.

Each of the deadenylases has a distinct mode of action. The current model for PARN action is that once the 5'-cap structure on an mRNA becomes accessible by movement of the translation initiation complex, PARN binds the cap. This interaction stimulates the poly(A) exoribonuclease activity of PARN, resulting in the removal of the poly(A) tail. For mRNAs not subject to deadenylation by PARN, the process is a bit more complicated. The translation termination factor eRF3 acts through both Pan2-Pan3 and Ccr4-Not (Funakoshi et al., 2007). eRF1-eRF3, Pan2-Pan3, and Ccr4-Not complexes competitively interact with PABPC1. eRF3, Pan3, and Tob (an antiproliferative transcription factor) mediate the respective interactions of each complex with PABPC1. A translation-dependent exchange of eRF1-eRF3 for deadenylases occurs on PABPC1, leading to activation of Pan2-Pan3 and Ccr4-Not. Pan2-Pan3 initiates deadenylation via poly(A) trimming and Ccr4-Not then completes it (Yamashita et al., 2005).

Following deadenylation, degradation of an mRNA continues in a 5'-3' or 3'-5' direction. 3'-5' decay of the mRNA body requires the 10-12-subunit exosome, which contains RNase PH-domain enzymes, S1- and KH-family RNA-binding proteins, RNase D-like enzymes, and RNA helicases (Mukherjee et al., 2002; Houseley et al., 2006). Once a few 5' nucleotides of the mRNA remain, the scavenger decapping enzyme DcpS removes the cap structure (Liu et al., 2002). 5'-3' mRNA decay begins with decapping by the Dcp1-Dcp2 proteins. Decapping requires numerous other proteins including Lsm1-7 (bound at the 3'-end of the deadenylated product), hEdc3, Rck/p54, and Hedls. Hedls enhances the decapping activity of Dcp2 and promotes complex formation between Dcp1-Dcp2 (Fenger-Gron et al., 2005). Xrn1-mediated 5'-3' exoribonucleolytic destruction of the mRNA follows decapping.

Histone mRNAs decay by 5'-3' and 3'-5' decay pathways, though with some interesting modifications. Mammalian histone mRNAs lack poly(A) tails and instead have a 3'-terminal stem-loop structure (6-bp stem, 4-nt loop). The 3' stem-loop binds several proteins, including SLBP, Upf1 (an NMD factor), ATR (a regulator of the DNA damage checkpoint), Lsm1, and the 3' exoribonuclease 3'hExo (Kaygun and Marzluff, 2005; Yang et al., 2006b).

3' hExo is a member of the DEHHh 3'-exoribonuclease family and is homologous to *C. elegans* ERI-1, which degrades siRNAs. At the end of S phase, 3' addition of short oligo(U) tracts by TUTase-1 and -3 permits binding by the Lsm1–8 complex of proteins (Mullen and Marzluff, 2008). The oligo(U) serves as the substrate for 3' exoribonucleolytic degradation for a short distance into the body of the mRNA; TUTases then add short oligo(U) to the 3' ends of partially degraded mRNAs, permitting additional 3'-exoribonucleolytic degradation. Decapping and 5'-3' decay occur simultaneously with 3'-5' degradation; this ensures quick removal of all remnants of histone mRNA upon completion of DNA synthesis. The elaborate destruction of histone mRNAs is apparently essential, as improper synthesis of histones outside S phase results in genome instability (Osley and Hereford, 1981).

The endoribonucleolytic pathway occurs perhaps less frequently than deadenylation, but is nonetheless still prevalent (Fig. 1) (Bracken et al., 2011). During the past five years, a number of labs identified endoribonucleases, including PMR1, ERN1/IRE1, and Zc3h12a. These enzymes often target actively translating mRNAs (Yang et al., 2004; Isken and Maquat, 2007; Houseley and Tollervey, 2009). PMR1 likely responds to one or more signaling pathways, since its phosphorylation by a tyrosine kinase is critical for its association with polyribosomes (Yang et al., 2004). Additionally, arsenite stress drives PMR1 into stress granules (defined below), which affect mRNA degradation (Yang et al., 2006a). Endotoxin-dependent TLR activation induces expression of the endoribonuclease, Zc3h12a, which targets cytokine mRNAs (Matsushita et al., 2009). Zc3h12a has a PIN-like domain and a domain required for its degradation activity *in vitro* and *in vivo*. There is also an endoribonuclease activity that cleaves *MYC* and *MDR-1* mRNAs upon unmasking of the cleavage sites; the *MYC* coding region determinant-binding protein, CRD-BP (also known as IMP1) is the masking protein (Sparanese and Lee, 2007). In at least some cases, endoribonucleolytic cleavage of *MYC* mRNA may be miRNA-mediated (Elcheva et al., 2009); however, we will defer discussion of miRNA- and small interfering RNA-mediated endoribonucleolytic activities to section 6.1.

Many proteins involved in mRNA degradation concentrate in cytoplasmic foci referred to as processing bodies (P-bodies) (Parker and Sheth, 2007). Though originally described as sites for translational repression, P-bodies contain Dcp1-Dcp2, Lsm proteins, Pan2-Pan3, Ccr4-Not complex, Rck/p54, Xrn1, and others (Cougot et al., 2004; Parker and Sheth, 2007; Zheng et al., 2008). Mutation of mRNA decay factors decreases the size and number of P-bodies suggesting they are indeed cytoplasmic foci for mRNA degradation (Kedersha et al., 2005). Transcripts entering P-bodies can exit them and return to polyribosomes for translation (Brenques et al., 2005). Thus, P-bodies may be sites for mRNA sorting, sequestration, and storage (Kedersha et al., 2005; Parker and Sheth, 2007).

Stress granules (SGs) are cytoplasmic structures that form during certain types of cellular stress. There, translationally repressed mRNAs aggregate, then are sorted and targeted for storage, degradation, or translation. (Kedersha et al., 2005; Anderson and Kedersha, 2006). While SGs and P-bodies are distinct cytoplasmic structures for mRNA processing, the two are linked spatially, compositionally, and functionally. Cellular stress induces both of them, but SGs require eIF2 α phosphorylation while P-bodies do not. Both share Fas-activated S/T phosphoprotein, Xrn1, eIF4E, and the RNA-binding protein TTP. However, eIF3, G3BP, eIF4G, and PABPC1 are confined to P-bodies. The model is that SGs contain sorted and remodeled mRNAs that are released from disassembled polyribosomes; SGs then deliver these mRNAs to P-bodies for degradation (Kedersha et al., 2005).

3. Messenger RNA surveillance pathways

Work during the past 10 years has contributed significantly to our understanding of the interworkings of mRNA surveillance (Doma and Parker, 2007; Isken and Maquat, 2007). In general, nonsense-mediated mRNA decay, nonstop mRNA decay, and no-go mRNA decay ensure the destruction of mRNAs that would otherwise produce potentially defective proteins.

3.1 Nonsense-mediated mRNA decay (NMD)

NMD is a translation-coupled, mRNA-surveillance mechanism that minimizes synthesis of truncated proteins due to a premature-termination codon, or PTC, (i.e., nonsense codon) in an mRNA (Mendell et al., 2004; Chang et al., 2007; Isken and Maquat, 2007; Frischmeyer-Guerrero et al., 2011; Hwang and Maquat, 2011). These can be due to mutations in genes, errors in pre-mRNA splicing that retain introns, or nonproductive chromosomal rearrangements (e.g., T cell receptor- β recombination). However, there is evidence that NMD also regulates 10–20% of normal mRNAs, such as those with alternatively spliced exons in the 3'-UTR (Hwang and Maquat, 2011). Additionally, bioinformatic analyses indicate that 10% of protein-coding genes in eukaryotic genomes harbor -1 programmed ribosomal frameshifting (-1 PRF) sites; this often introduces a nonsense codon in the -1 frame, leading to mRNA destruction (Belew et al., 2011).

Numerous proteins are essential for the NMD machinery, including up-frameshift proteins (UPF) and proteins from the family called suppressor with morphogenetic effects on genitalia (SMG) (Chiu et al., 2003). The key issue for NMD is distinguishing PTC-bearing mRNAs from normal mRNAs. As such, there is a second signal located downstream of a PTC that determines whether or not an mRNA undergoes NMD (Tange et al., 2005; Brogna and Wen, 2009). The exon-exon junction complex (EJC) is the second signal in mammalian mRNAs. The EJC is an ~350-kDa protein complex that affects translation, surveillance, and localization of spliced mRNAs (Tange et al., 2004). The EJC contains several protein subunits including Y14, MAGOH, eIF4AIII, PYM, and SKAR (Maquat et al., 2010). The EJC assembles approximately 20–24 nt upstream of an exon-exon junction during pre-mRNA splicing and remains bound to mRNAs after entry into the cytoplasm. It thus acts as an imprint providing position-specific memory of splicing events (Le Hir et al., 2001; Ballut et al., 2005). During pre-mRNA synthesis, the cap-binding complex (CBC), a heterodimer of CBP20 and CBP80 subunits, binds the 5' cap and promotes pre-mRNA splicing (Lejeune et al., 2004). The karyopherin importin α (IMP α) binds CBP80, and PABPC1/PABPN1 bind the poly(A) tail (Sato and Maquat, 2009). CBP80 promotes the interaction of UPF1 with UPF2 (Hosoda et al., 2005). UPF2 and UPF3 link UPF1 to the EJC via interaction of UPF3b with MAGOH-Y14 and eIF4AIII (Chamieh et al., 2008). As the mRNP emerges from the nucleus, a pioneer round of translation displaces EJCs and exchanges PABPN1 with PABPC1. If the ribosome encounters a PTC, defined usually as a stop codon >50–55 nt upstream of an EJC, the SURF complex, comprised of protein kinase SMG1, UPF1, and eRFs (hence the name), assembles at the stalled ribosome. Upon binding of UPF1 and SMG1 to the downstream EJC, SMG1 phosphorylates UPF1, thereby recruiting endoribonuclease SMG6 and/or deadenylation, decapping, and exoribonucleolytic factors for rapid decay of the mRNA (Fig. 2) (Lejeune et al., 2003; Yamashita et al., 2005; Kashima et al., 2006; Isken et al., 2008; Ivanov et al., 2008; Brogna and Wen, 2009; Eberle et al., 2009). P-bodies may serve as cytoplasmic foci for NMD due to their locally high concentrations of mRNA decay factors and SMG proteins (Kedersha et al., 2005; Parker and Sheth, 2007). For mRNAs that pass muster and are not destroyed, importin β (IMP β) binds CBP80-bound IMP α to promote exchange of CBC for eIF4E; this permits normal translation.

Several studies suggest that a sufficiently long distance between a termination codon and the 3'-end of an mRNA can also trigger NMD. In this case, failure of PABPC1 or other proteins to interact with the terminating ribosome or EJC may signal distance-dependent NMD (Silva et al., 2008). This is known as the faux 3'-UTR model of NMD (Amrani et al., 2004). During normal translation, the proper distance between the stop codon and 3'-end allows interaction of PABPC1 with eRF3-eRF1 for termination. However, a sufficiently long distance between a termination codon and 3'-end disrupts PABPC1-eRF interactions. As a result, PABPC1 interacts with UPF1 to trigger NMD (Amrani et al., 2004; Amrani et al., 2006; Ivanov et al., 2008).

In the ribosome release model of NMD, eIF3 bound to the ribosome terminating at the PTC promotes ribosome dissociation; UPF1 may participate in ribosome dissociation as well. The resulting 60S and 40S subunits dissociate from the mRNA and unmask the region downstream of the PTC. This unprotected region causes rapid mRNA decay (Lejeune et al., 2004; Amrani et al., 2006; Morris et al., 2007; Brogna and Wen, 2009).

PTCs do not always trigger mRNA degradation. Most notable are T-cell receptor β (*TCR β*) mRNAs, which are the products of chromosomal rearrangements in T-cells (Bhalla et al., 2009). PTCs promote an increase in the nuclear:cytoplasmic ratio of these mRNAs due to their retention in the nucleus, not cytoplasmic degradation. There are sequences in the VDJ region of the mRNAs responsible for nuclear retention, which requires UPF1 and EJC subunit eIF4AIII, but not UPF3b.

3.2 Nonstop mRNA decay (NSD)

Translation of mRNAs that lack a termination codon altogether permits ribosome transit to the very 3'-end of the mRNA, leading to its degradation (Fig. 1) (van Hoof et al., 2002). In contrast to NMD, polypeptides do not release from the ribosome, and thus, NSD seems to involve mRNA decay factors distinct from NMD. The C-terminal domain of Ski7 recognizes the unoccupied A-site of the ribosome once it reaches the 3'-end. This is possible since Ski7 is a molecular mimic of the GTPase domains of EF1A and eRF3. Ski7 recruits Ski2, Ski3, and Ski8 through its N-terminal domain. This brings exosomes to the 3'-end of the mRNA for its destruction beginning at the poly(A) tail (Frischmeyer et al., 2002; Maquat, 2002; van Hoof et al., 2002; Isken and Maquat, 2007). Sequential decapping and Xrn1-mediated degradation can also act on NSD targets, though less efficiently (Fig. 3) (Frischmeyer et al., 2002). In addition to degrading translationally dead-end templates, NSD facilitates ribosome release; the resulting ribosome subunits are now available for translation of normal mRNAs (Frischmeyer et al., 2002; Pisarev et al., 2007).

A variation of NSD, referred to as ribosome extension-mediated mRNA decay, or REMD, occurs in the α -thalassemia known as α -Constant Spring, α^{CS} (Kong and Liebhaber, 2007). These patients have a mutation that eliminates the stop codon in *α -globin* mRNA. As such, the ribosome continues into the 3'-UTR and through a CU-rich element that normally binds an mRNA-stabilizing, protein complex known as the α -complex. Ribosome extension triggers deadenylation and mRNA degradation independent of UPF1.

3.3 No-go mRNA decay (NGD)

NGD is a pathway observed in *S. cerevisiae* (but not yet in mammalian cells, to our knowledge). Messenger RNAs that harbor ribosome barriers, e.g., strong secondary structures, or ribosomes that cannot properly elongate, elicit NGD (Doma and Parker, 2006; Doma and Parker, 2007; Houseley and Tollervy, 2009). The stall promotes ribosome release and endoribonucleolytic decay that require GTPase family member Hbs1 and endoribonuclease Dom34 (Pisarev et al., 2007). The empty A-site of the stalled ribosome

and the ensuing interaction of the Hbs1/Dom34 complex with the ribosome promotes peptide-tRNA hydrolysis, peptide release and endoribonucleolytic cleavage of the mRNA (Doma and Parker, 2006; Lee et al., 2007; Graille et al., 2008). Exosomes and Xrn1 degraded the resulting mRNA cleavage products (Fig. 4). There is also evidence that, in addition to NMD, NGD may participate in destruction of mRNAs with -1 PRF sites, as noted in section 3.1 (Belew et al., 2011). It is not yet clear why -1 PRF sites that promote mRNA degradation might require two decay pathways.

4. *Cis*-acting elements and *trans*-acting factors involved in mRNA decay

The interactions between *cis*-acting elements within mRNAs and their RNA-binding proteins (RBPs) control posttranscriptional gene expression (Keene and Tenenbaum, 2002; Kishore et al., 2010). During the past decade, microarray analyses of mRNPs from mammalian cells revealed that mRNA-binding proteins associate with unique subsets of mRNAs to coordinately regulate their localization, translation, and/or degradation (Tenenbaum et al., 2000; Keene and Tenenbaum, 2002; Tenenbaum et al., 2003; Keene, 2007; Hogan et al., 2008). These mRNA subsets represent posttranscriptional operons, or regulons, that integrate synthesis of functionally related proteins needed collectively for a biological process. Coordinate expression of a given mRNA subset is facilitated by related untranslated sequence elements for regulation (USERS) (Keene and Tenenbaum, 2002). Below, we review two USERS – AU-rich elements and GU-rich elements – and the proteins that bind them.

4.1 AU-rich elements and their binding proteins

One of the best characterized USERS is the family of AU-rich elements (AREs) found in 3'-UTRs of many labile transcripts. AREs can modulate mRNA stability and/or translation depending on the particular ARE-binding proteins (AUBPs) associated. The mRNA decay function of AREs was first discovered in 1986 in a classic experiment fusing an AT-rich segment of the 3'-UTR from the granulocyte-macrophage colony stimulating factor (*GM-CSF*) gene to the rabbit β -globin gene, which encodes a normally stable mRNA. The *GM-CSF* 3'-UTR segment induced rapid degradation of the chimeric mRNA (Shaw and Kamen, 1986). The Shaw and Kamen paper also noted similar sequences in cDNAs encoding numerous cytokines, oncoproteins, and growth factors. Similar experiments identified a number of AREs (Chen and Shyu, 1995). Subsequent bioinformatic analyses estimated that 5–8% of the transcriptome contains AREs and these transcripts additionally encode proteins required for apoptosis, immune responses, and intracellular signaling, to name just a few processes (Halees et al., 2008).

AREs vary widely in sequence but were originally classified into three broad types (Chen and Shyu, 1995; Wilusz et al., 2001). Class I AREs, found in mRNAs such as *FOS* and *MYC*, contain 1–3 interspersed copies of the AUUUA pentamer surrounded by U-rich regions. Class II AREs, found in cytokine mRNAs such as *GM-CSF* and tumor necrosis factor α (*TNF α*), contain multiple overlapping copies (typically 5–8) of the AUUUA motif. Class III AREs, such as the one in *JUN* mRNA, lack AUUUA pentamers but contain predominantly U-rich sequence. These unique features of AREs suggest that different AUBPs, or more likely, combinations of AUBPs, may differentially regulate the classes of AREs (Chen and Shyu, 1995; Halees et al., 2008).

There are 20 AUBPs so far, including those that promote mRNA degradation, such as AUF1/hnRNP D, tristetraprolin (TTP), butyrate-regulated factor-1 (BRF1), KH domain-splicing regulatory protein (KSRP), and exosome subunit PM-Scl75; mRNA stabilizing proteins such as HuR; and translational control proteins such as T-cell intracellular antigen 1 (TIA-1) and TIA-1-related protein (TIAR) (Barreau et al., 2005). As we will describe

below, most AUBPs act to recruit the degradation machinery to mRNAs for their destruction.

AUF1 consists of four isoforms p37^{AUF1}, p40^{AUF1}, p42^{AUF1}, and p45^{AUF1} generated by alternative pre-mRNA splicing (Gratacos and Brewer, 2010). Experiments with RNAi-induced knockdown of AUF1 or *auf1*^{-/-} mice revealed it to promote mRNA degradation (Lu et al., 2006b). AUF1 forms complexes with translation initiation factor eIF4G, PABP, lactate dehydrogenase, heat-shock proteins Hsp/Hsc70 and Hsp27, and other unidentified proteins (Lu et al., 2006a; Sinsimer et al., 2008; Gratacos and Brewer, 2010; Knapinska et al., 2011). Hsp/Hsc70, Hsp27 and lactate dehydrogenase are AUBPs as well, though their roles in mRNA degradation are unclear.

AUF1 binding to at least some AREs alters local RNA structure, which may provide requisite surfaces to nucleate assembly of additional protein-protein and protein-RNA interactions required for translation and mRNA degradation (Wilson et al., 2003; Zucconi et al., 2010). For example, AUF1 binds directly to both PABP and eIF4G; however, its binding to an ARE or to Hsp70 opposes the AUF1-PABP interaction whereas it does not affect the AUF1-eIF4G interaction (Lu et al., 2006a). During ongoing translation, the ribosome might remodel AUF1-mRNA interactions, thereby displacing it from the ARE in a complex with PABP to expose the poly(A) tail to deadenylase. Alternatively, AUF1 may preclude poly(A) binding by PABP, allowing deadenylases access to the mRNA (Sagliocco et al., 2006). AUF1 may also facilitate degradation of the mRNA body following deadenylation by recruiting exosomes to ARE-mRNAs (Chen et al., 2001; Lal et al., 2004; Torrisani et al., 2007). The exosome subunit PM-Scf175 is also an AUBP, thus providing an additional means of recruiting exosomes to ARE-mRNAs (Mukherjee et al., 2002; Anderson et al., 2006).

AUF1 proteins shuttle between the nucleus and cytoplasm (Sarkar et al., 2003; Chen et al., 2004; Suzuki et al., 2005). Changes in shuttling and/or AUF1 protein degradation rates can alter its cytoplasmic abundance, thereby affecting ARE-mRNA degradation rates. For example, cell cycle-dependent fluctuations in proteasome-dependent AUF1 degradation lead to changes in its cytoplasmic abundance. Thus, during S phase, a low AUF1 level stabilizes the ARE-mRNA *DNMT1* (DNA methyltransferase-1); DNMT1 is responsible for maintenance of DNA methylation during cell division (Torrisani et al., 2007). By contrast, the circadian rhythm oscillates cytoplasmic abundance of AUF1 by somehow controlling its shuttling from the nucleus (Woo et al., 2010). When AUF1 abundance is high, levels of its target mRNA cryptochrome 1, *CRY1*, are low. Thus, AUF1 controls the circadian amplitude of *CRY1* mRNA. Perhaps this involves 14-3-3 σ , which facilitates nucleocytoplasmic transport of the p37 and p40 AUF1 isoforms to accelerate ARE-mRNA decay (He and Schneider, 2006).

Likewise, KSRP and the zinc-finger proteins TTP and BRF1 nucleate assembly of numerous mRNA decay factors on ARE-mRNPs. The central KH domains of KSRP are essential for degradation activity via their interaction with deadenylase PARN, decapping factor Dcp2, and exosome subunit Rps4 (Gherzi et al., 2004; Chou et al., 2006). TTP and BRF1 possess two activation domains that interact with exosomes, deadenylase Ccr4, decapping enzymes, and the 5'-3' exoribonuclease Xrn1 to promote mRNA degradation in P-bodies. The interaction map of all these enzyme subunits with TTP suggests highly orchestrated degradation processes (Fenger-Gron et al., 2005; Lykke-Andersen and Wagner, 2005; Franks and Lykke-Andersen, 2007).

The Hu family of RNA-binding proteins, which are related to *Drosophila melanogaster* embryonic lethal, abnormal vision-like (ELAV-like) proteins, consists of four members – HuA/HuR, HuB, HuC and HuD. Binding by these proteins generally stabilizes mRNAs

bearing AU- and U-rich sequences (Park-Lee et al., 2003; Lopez de Silanes et al., 2004; Barreau et al., 2005; Lebedeva et al., 2011). While HuR is predominantly nuclear, it shuttles between the nucleus and cytoplasm. This suggests that HuR binds its mRNA targets initially in the nucleus and cotransports with them to the cytoplasm; this could provide a mechanism to shift mRNA-binding equilibrium to HuR at the expense of AUBPs that promote mRNA degradation. HuC and HuD are neural-specific, while both neurons and reproductive organs express HuB.

Numerous signaling pathways, particularly p38 MAP kinase signaling, affect the abundance, localization, and/or activity of AUBPs to control ARE-mRNA degradation. For example, exposure of macrophages to endotoxin activates the p38 MAP kinase pathway and stabilizes *IL1 β* and *TNF α* mRNAs to initiate an innate immune response; *auf1*^{-/-} mice are hypersensitive to endotoxin-induced sepsis due to a failure to degrade those mRNAs during the anti-inflammatory phase of innate immunity (Lu et al., 2006b). The p38 MAP kinase pathway phosphorylates KSRP during differentiation of myoblasts to myocytes; this lowers its ARE-binding affinity and stabilizes mRNAs required for conversion to myocytes (Briata et al., 2005). p38 also phosphorylates TTP and BRF1 to stabilize ARE-mRNAs (Stoecklin et al., 2004; Benjamin et al., 2006; Brook et al., 2006; Sun et al., 2007; Maitra et al., 2008). Phosphorylation of TTP or BRF1 promotes their interaction with 14-3-3 proteins to either sequester them in the nucleus or exclude them from stress granules. The net effect is ARE-mRNA stabilization. Finally, gamma irradiation induces p38 MAP kinase signaling and phosphorylation of HuR (Lafarga et al., 2009). This releases HuR from the nucleus to accumulate in the cytoplasm, bind and stabilize *p21*^{Cip1} mRNA, and cause G₁/S checkpoint arrest.

Numerous other signaling pathways regulate mRNA degradation as well. For example, AUF1 is subject to tyrosine phosphorylation by the kinase NPM-ALK, a fusion protein expressed in a subset of anaplastic large cell lymphomas (Fawal et al., 2006). Hyperphosphorylation of AUF1 correlated with increased stability of mRNAs encoding numerous cyclins and the MYC oncoprotein. Likewise, phosphorylation of AUF1 during eosinophil activation stabilizes *GM-CSF* mRNA (Shen et al., 2005). In this case, phosphorylation leads to *cis-trans* isomerization of serine-proline and serine-threonine peptide bonds by the peptidyl-prolyl isomerase Pin1, thereby lowering association of AUF1 with the ARE and stabilizing the mRNA. Heat shock alters mRNA decay rates, in part by promoting degradation of HuR by proteasomes (Abdelmohsen et al., 2009). However, phosphorylation of HuR at three residues (two serines, one threonine) by the Chk2 kinase protects HuR from degradation. Similarly, phosphorylation of BRF1 by protein kinase B, PKB, protects it from proteasomal degradation (Benjamin et al., 2006). Phosphorylation carries out this function by permitting binding of BRF1 to a 14-3-3 protein; this protein-protein interaction sequesters BRF1 from both proteasomes and the mRNA decay machinery. Functionally, this serves to maintain high levels of BRF1 in an unavailable form that can be released to resume its decay function once the mRNA stabilizing signal dissipates. Undoubtedly, there are many more interactions between signaling pathways and the mRNA decay machinery that await discovery.

4.2 GU-rich elements and binding proteins

While AREs and their associated proteins have garnered much attention, additional elements and proteins regulate mRNA stability. For example, CUG-binding protein 1 (CUGBP1), a member of the CELF (CUGBP and embryonic lethal abnormal vision-like factor) family, was identified by its binding to CUG-repeat sequences within the mRNA encoding the myotonin protein kinase (Vlasova and Bohjanen, 2008). However, CUGBP1 promotes rapid decay of GU-rich element-bearing mRNAs. CUGBP1 is multifunctional as well, and

regulates posttranscriptional processes including alternative pre-mRNA splicing, mRNA degradation, and translation (Vlasova et al., 2008; Rattenbacher et al., 2010).

5. Combined effects of RNA-binding proteins on mRNA degradation

An important question is whether binding of one RBP affects the binding of another RBP to its target mRNAs. The number and complexity of RNA motifs recognized by RBPs make possible the highly orchestrated, posttranscriptional processing events that control abundance and translation of their mRNA targets (Lopez de Silanes et al., 2004; Raineri et al., 2004; Mazan-Mamczarz et al., 2009). As well, multiple RBPs can bind a common mRNA target in a competitive, cooperative, or independent fashion. For example, Hu proteins often stabilize mRNAs via competitive binding with destabilizing proteins to AREs: polyamines shift the ARE-binding equilibrium toward AUF1 at the expense of HuR to promote degradation of *JunD* mRNA (Zou et al., 2010). By contrast, for *p16^{INK4}* mRNA, HuR and AUF1 act as cofactors to promote mRNA degradation (Chang et al., 2010). Regulation of inducible nitric oxide synthase (*iNOS*) expression involves five RBPs: AUF1, HuR, KSRP, polypyrimidine tract-binding protein (PTB), and TTP (Fechir et al., 2005; Linker et al., 2005; Pautz et al., 2006; Pautz et al., 2009). TTP does not bind the ARE directly, but rather, it binds KSRP, which does bind the ARE and competes with HuR for binding the same site within the ARE. Thus, binding of TTP-KSRP and HuR are reciprocal. KSRP also binds PTB; cytokine induction of a human colon carcinoma cell line, DLD-1, increases association of PTB with *iNOS* mRNA and stabilizes it. While cytokine induction of DLD-1 cells increases *iNOS* mRNA levels; AUF1 serves to limit the magnitude of induction. Clearly, association of multiple RBPs with *iNOS* mRNA permits its highly tuned regulation in response to cytokine signaling. Finally, AUBPs themselves comprise a complex circuitry of self- and cross-regulatory RNP interactions (Pullmann et al., 2007). Examination of AUF1, HuR, KSRP, NF90, TIA-1, and TIAR revealed they each associate with their own mRNA and mRNAs encoding all the other proteins. RNAi-mediated knockdown of one protein affected expression of all the others. This provides a mechanism to control abundance of each protein and this will have obvious effects on their competitive or cooperative ARE-binding equilibria.

6. Noncoding RNAs in mRNA degradation

During the past decade, the *cis*-acting elements and *trans*-acting factors that have revolutionized the field of posttranscriptional regulation involve noncoding (nc)RNAs. In the following sections we will describe some of these ncRNAs, how they interact with the mRNA decay machinery to promote degradation, and how they interface with RBPs to co-regulate mRNAs.

6.1 MicroRNAs and small interfering RNAs

MicroRNAs (miRNAs) were discovered in *C. elegans* in the early 1990s. These 21–25-nt RNAs may comprise about 3% of all human genes and regulate gene expression by controlling translation and/or mRNA degradation (Bartel, 2009; Fabian et al., 2010; Huntzinger and Izaurralde, 2011). They are transcribed as long primary (pri)-miRNAs from noncoding RNA genes or as embedded passengers within protein-coding genes. RNase III-like enzyme Drosha processes a pri-miRNA into a 70-nt precursor (pre)-miRNA, which possesses stem-loop structures containing multiple bulges and mismatches. Ran-GTP-dependent Exportin-5 transports a pre-miRNA to the cytoplasm and Dicer then cleaves it to yield a duplex RNA. Usually, only one strand (the guide strand) incorporates into miRNPs called RNA-induced silencing complexes (miRISC). Thermodynamic instability and weaker base-pairing of the guide strand relative to the other (passenger) strand dictate preferential incorporation. The passenger strand is often degraded (Hutvagner and Zamore, 2002; Bartel,

2004; Kim, 2005; Pham and Sontheimer, 2005). In some cases, however, both strands incorporate into their respective miRISC (Okamura et al., 2008). We also note that alternative pathways for miRNA biogenesis have emerged as well (Yang and Lai, 2011).

miRISC is similar to small interfering RNA (siRNA)-loaded RISC, which is responsible for RNAi. siRNAs derive from long dsRNA precursors endogenously produced (e.g., viral infection) or exogenously provided (Zeng et al., 2003; Wu and Belasco, 2008). Dicer processes long dsRNA into siRNAs for assembly of one strand into RISC. Like miRISC, the siRNA guides siRISC to its target mRNA through base-pairing with it (Meister et al., 2004; Grosshans and Filipowicz, 2008).

An essential mode of miRNA action is the specificity of their interaction with their target 3'-UTRs. For mammalian miRNAs, complementarity to nucleotides 2–8 at the 5'-end of miRNAs, referred to as the “seed” sequence, plays a primary role in establishing miRISC-mRNA interactions (Behm-Ansmant et al., 2006b). There are five additional criteria important for binding site context (Grimson et al., 2007): (i) AU content near the site; (ii) proximity to additional miRNA-binding sites; (iii) mRNA base pairing with miRNA nucleotides 13–16; (iv) binding greater than 15 nt downstream of the termination codon; and (v) for long 3'-UTRs, binding away from its center. However, there are instances of miRNA binding sites in 5'-UTRs and coding regions. Multiple, partially complementary sites direct inhibition of target mRNA translation (Bartel, 2004; Bartel, 2009), though subsequent findings indicated that interaction between miRNAs and target mRNAs often promotes mRNA degradation (Guo et al., 2010).

Argonaute proteins (Ago1–4) are RISC subunits central to its function. Ago proteins contain PAZ and PIWI domains. MicroRNA binding requires the PAZ domain; PIWI domains, which are related to endoribonuclease RNase H, facilitate mRNA “slicer” activity (Hock et al., 2007). In mammalian cells, only Ago2 catalyzes endoribonucleolytic cleavage (Liu et al., 2004; Meister et al., 2004; Chen et al., 2009). The “slicer” activity of Ago2 generates 5'- and 3'-cleavage products when there is perfect or near-perfect complementarity between the miRNA and target mRNA. Exoribonucleases such as exosomes and Xrn1 degrade these 5'- and 3'-products, respectively (Behm-Ansmant et al., 2006b). For example, mammalian miR-196 promotes endoribonucleolytic cleavage and degradation of *HoxB8* mRNA through perfect complementarity (though there is one G:U base pair) (Yekta et al., 2004). Additional RISC subunits include GW182/TNRC6, TRBP, PACT, SMN complex, FMRP, and Tudor-SN (Hutvagner and Zamore, 2002; Chendrimada et al., 2005; Meister et al., 2005; Zhang et al., 2007; Eulalio et al., 2008). There is also evidence that NMD factor UPF1 interacts with Ago1 and Ago2 to assist recruiting RISC to mRNAs for degradation (Jin et al., 2009).

An alternate mode of miRNA action that degrades mRNAs is deadenylation/decapping-dependent and slicer-independent (Wu et al., 2006; Filipowicz et al., 2008; Fabian et al., 2009). Reporter assays revealed that RISC subunit GW182 promotes biphasic deadenylation. Pan2-Pan3 provide trimming of the poly(A) tail and Ccr4-Not completes its removal, followed by Dcp1-Dcp2-dependent decapping (Behm-Ansmant et al., 2006a; Chen et al., 2009; Piao et al., 2010). The N-terminal end of GW182 contains numerous repeats of glycine and tryptophan (GW) to form the Ago-binding domain; the C-terminal end of GW182 is necessary for mRNA degradation and is referred to as the silencing domain (SD) (Fabian et al., 2009; Zekri et al., 2009). The SD contains a PABP-interacting motif (PAM2), which also binds Pan2-Pan3 by contacting Pan3. Gly/Ser/Thr-Trp (G/S/TW) and Trp-Gly/Ser/Thr (WG/S/T) motifs within the GW182-SD also form protein-protein contacts with the Cnot1 subunit of Ccr4-Not (Fig. 5) (Braun et al., 2011; Chekulaeva et al., 2011; Fabian et al., 2011). Those two motifs are thus referred to as CIM-1 and CIM-2, respectively (Ccr4-Not-interacting motifs-1 and -2). These studies at long last elucidate how binding of

miRISC to mRNAs promotes deadenylation for translational repression and/or mRNA degradation. Undoubtedly, there will soon be reports detailing miRISC interactions with additional mRNA degradation enzymes and translation factors. Finally, artificially tethering any Ago protein or GW182 to the 3'-UTR of a reporter mRNA is generally sufficient to promote deadenylation and decapping of the mRNA without need for a miRNA at all. Thus, it is clear that miRNAs serve as the targeting vehicle to latch miRISC onto mRNAs via base-pairing.

Also perhaps not surprising is the emerging theme of co-regulation of mRNAs by RBPs and miRNAs. This was predicted several years ago by Tenenbaum and colleagues, who envisioned a number of mechanisms by which RBPs and miRNAs might act in concert or opposition to control translation and/or mRNA degradation (George and Tenenbaum, 2006). A few examples will suffice to provide an illustration of two fields intersecting: (i) while miR-16 is partially complementary to the *TNFA* ARE, miR-16 requires the AUBP TTP to trigger mRNA degradation. miR-16-RISC recruits TTP to *TNFA* mRNA via TTP-Ago interactions, thereby promoting mRNA degradation (Jing et al., 2005). (ii) AUBPs AUF1 and HuR act as cofactors to recruit RISC to *p16^{INK4}* mRNA for its degradation. Knockdown of either AUBP reduces RISC binding to the mRNA (Chang et al., 2010); and (iii) one example that nicely illustrates nature's sublime beauty – RNA structural changes that unmask miRNA binding sites. Quiescent cells synthesize cell cycle inhibitor protein p27^{Kip1}. Under these conditions, local RNA structure in the p27^{Kip1} 3'-UTR masks binding sites for miR-221/222, permitting p27^{Kip1} synthesis. Upon exposure of cells to EGF, phosphorylation of RNA-binding protein Pumilio-1 (PUM1) induces its binding to the p27^{Kip1} 3'-UTR. This in turn restructures RNA and exposes the miR-221/222 target sites to permit their binding and a block to p27^{Kip1} synthesis. Cells then proliferate (Kedde et al., 2010).

While examples of RBPs and miRNAs as co-regulators are bountiful, a recent bioinformatic analysis provides an estimate as to how widespread protein binding sites are coupled to miRNA target sites (Jacobsen et al., 2010). The authors concluded that upon overexpression of si/miRNAs, down-regulated mRNAs have U-rich motifs (i.e., class III AREs); up-regulated mRNAs have AUUUA motifs (i.e., class I and II AREs). They also looked for 7-nt “words” in mRNAs from Ago1–4 PAR-CLIP data (Hafner et al., 2010) and found that 84% of miRNA targets have U-rich motifs, and on average they are 50 nt upstream of miRNA target sites. Thus, it would appear that AUBPs are often coupled to miRNA target sites, and binding may act cooperatively or competitively to control mRNA degradation/translation.

6.2 Small vault RNAs

Vaults are barrel-shaped, RNP structures associated with multidrug resistance (Kedersha and Rome, 1986). They consist of three proteins and noncoding, vault RNAs (vRNAs) from which several small vault RNAs (svRNAs) are produced. Processing is Drosha-independent and Dicer-dependent. svRNAs assemble with Ago proteins and guide sequence-specific cleavage of target mRNAs in a manner similar to miRNAs (Persson et al., 2009; Gopinath et al., 2010). Based on the similarities between svRNA- and miRNA-based degradation mechanisms, Rovira and colleagues identified several hundred mRNAs as predicted svRNA targets. Indeed, they validated one of the top candidates, *CYP3A4*, which encodes a cytochrome P450 isoform, as a target of svRNAb (Persson et al., 2009). Thus, svRNAs add additional complexity to small noncoding RNA-mediated gene silencing in eukaryotes

6.3 Long noncoding RNAs

Transcription of eukaryotic genomes yields abundant long noncoding RNAs (lncRNAs). It is becoming increasingly clear that lncRNAs can function to regulate expression of protein-

coding mRNAs. Like small noncoding RNAs such as miRNAs, lncRNAs partially base-pair with their target mRNAs (Hannon et al., 2006; Ponting et al., 2009; Wilusz et al., 2009). Formation of RNA duplexes between lncRNAs and mRNA can mask regulatory elements or provide binding sites for *trans*-acting factors. For example, a cytoplasmic, polyadenylated lncRNA containing antisense Alu sequence can base pair with selected mRNAs bearing Alu elements in their 3'-UTRs. STAU1 is an RNA-binding protein that recognizes the resulting dsRNA (i.e., STAU1-binding site, SBS). This protein-RNA interaction recruits UPF1 and the mRNA degradation machinery to elicit STAU1-mediated mRNA decay (SMD) (Kim et al., 2005; Gong and Maquat, 2011b). lncRNAs can act at another level by generating endogenous siRNAs that trigger degradation of target mRNAs (Wilusz et al., 2009; Gong and Maquat, 2011a). Like miRNAs, multiple lncRNAs can control one or more mRNAs. Clearly, lncRNAs offer yet another mechanism for coordinated, posttranscriptional regulation of gene networks.

7. Conclusion and perspectives

As we noted on our 2001 *Gene* review, the importance of mRNA stability lies in the need for the cell to quickly adjust mRNA levels in response to intrinsic or extrinsic stimuli, with or without altering transcription. Some of the questions posed at the end of our last review have been answered. These include identification of degradation enzyme complexes and how they assemble; how binding of RBPs to *cis*-elements promotes mRNA degradation; how RNA structure within *cis*-elements affects recognition and binding of RBPs (and miRNAs); and identification of numerous signaling pathways that affect degradation and how they do so. The past decade has seen exciting, unexpected discoveries as well. These include the discoveries of localized centers, or foci, in the form of P-bodies and stress granules for mRNA degradation/translation repression; and ncRNAs and their roles in mRNA degradation in concert with RBPs. But, there are many important issues and questions for the future as well. For example, there has been considerable effort to elucidate the roles for individual ncRNAs in biological processes and defining ncRNA expression profiles specific to normal *versus* disease tissue; pluripotency *versus* differentiation of embryonic stem cells, and nonactivated *versus* activated cells during an immune response. This approach will likely continue long into the future. How do RBPs and ncRNAs act to coordinate large subsets of posttranscriptional operons in a time-dependent fashion, for example, during embryonic development or in response to signaling pathways? Transgenic knockout models exist for many AUBPs, and these will continue to reveal insights into their roles in biology, particularly the immune system. Finally, RNAi was a surprising mechanism – what other currently inconceivable mechanisms lie out there unbeknownst to us? The next decade should provide exciting answers.

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Abbreviations

Ago	Argonaute
ALY/REF	Ally of AML-1 and LEF-1
ARE	AU-rich element
ATR	ataxia telangiectasia and Rad3 related
AUBP	AU-rich element-binding protein

BRF	butyrate-regulated factor
Caf	Ccr4-associated factor(s)
CBC	cap binding complex
CBP20/80	20-kDa/80-kDa cap-binding protein
Ccr4	carbon catabolite repressor 4
Cdk1	cyclin-dependent kinase 1
CELF	CUGBP and embryonic lethal abnormal vision-like factor
Chk2	checkpoint kinase 2
CIM	Ccr4-Not-interacting motif
CRD-BP	coding region determinant (CRD)-binding protein
CUGBP1	CUG-binding protein 1
DNMT1	DNA methyltransferase 1
dsRNA	double-stranded RNA
Edc3	enhancer of decapping 3
EGF	epidermal growth factor
EJC	exon junction complex
ERI-1	exoribonuclease-1
FMRP	fragile X mental retardation protein
G3BP	Ras GTPase-activating protein binding protein
GM-CSF	granulocyte/macrophage-colony stimulating factor
GRE	G-rich element
Hedls	human enhancer of decapping/large subunit
GW182	glycine-tryptophan repeat protein 182
Hsp/Hsc	heat shock protein/heat shock cognate protein
IMP1	insulin-like growth factor-II mRNA-binding protein 1
IMPα/β	importin α/β
IRE1	inositol-requiring protein 1
KH	hnRNP K homology
KSRP	KH-domain splicing regulatory protein
lncRNA	long noncoding RNA
Lsm proteins	like Sm proteins
MAGO	mago nashi (grandchildless)
MAP	mitogen-activated protein
miRNA	microRNA
ncRNA	noncoding RNA
NGD	no-go mRNA decay

NIMA	G2-specific protein kinase nimA
NMD	nonsense-mediated mRNA decay
Not proteins	negative-on-transcription proteins
NSD	nonstop mRNA decay
nt	nucleotide
PABP	poly(A)-binding protein
PACT	protein activator of the interferon-induced protein kinase
PAM2	PABP-interacting motif 2
Pan2/3	PABP-dependent poly(A)-specific ribonuclease 2/3
PAR-CLIP	photoactivatable ribonucleoside-enhanced-crosslinking and immunoprecipitation
PARN	poly(A)-specific ribonuclease
PAZ	PIWI-Argonaute-Zwille
PIN	PilT N-terminal
Pin1	peptidyl-prolyl <i>cis-trans</i> isomerase NIMA-interacting 1
PIWI	P element-induced wimpy testis
PMR1	polysomal ribonuclease 1
PM-Scl75	75-kDa polymyositis-systemic sclerosis protein
PRF	programmed ribosomal frameshifting
PTB	polypyrimidine tract-binding protein
PTC	premature termination codon
PYM	partner of Y14-MAGOH
RF	release factor
RBP	RNA-binding protein
Rck/p54	54-kDa RNA helicase cloned from B-cell lymphoma cell line RC-K8
RISC	RNA-induced silencing complex
RNAi	RNA interference
Rrp4	ribosomal RNA-processing protein 4
SBS	STAU1-binding site
SKAR	S6 kinase 1 ALY/REF-like target
SLBP	stem-loop binding protein
SMG	suppressor with morphogenetic effects on genitalia
SMN	spinal muscular atrophy
STAU1	Staufen 1
SMD	STAU1-mediated mRNA decay
SURF	SMG1-UPF1-eRF1-eRF3

svRNA	small vault RNA
TIA-1	T cell intracellular antigen 1
TIAR	TIA-1-related protein
TLR	Toll-like receptor
TNF	tumor necrosis factor
TNRC6	trinucleotide repeat containing 6A/B/C
TRBP	HIV <i>trans</i> -activating response RNA-binding protein
TTP	tristetraprolin
TUTase	terminal uridylyl transferase
Tudor-SN	Tudor staphylococcal nuclease domain-containing protein
UPF	up-frameshift
USERS	untranslated sequence elements for regulation
UTR	untranslated region
vRNA	vault RNA
Xrn1	exoribonuclease 1

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Highlights

- Ribonucleases orchestrate mRNA degradation via defined pathways
- Messenger RNA surveillance pathways ensure protein coding fidelity
- *Cis*-acting elements and *trans*-acting factors dictate mRNA decay rates
- RNA-binding proteins act in concert to control mRNA degradation rates
- Noncoding RNAs are revolutionizing mRNA degradation paradigms

Fig. 1. Messenger RNA decay pathways in mammalian cells. The deadenylation-dependent and deadenylation-independent (i.e., endonucleolytic) pathways are depicted. See text for detailed descriptions of the relevant enzymes that catalyze each decay step.

Fig. 2. Nonsense-mediated mRNA decay (NMD). During pre-mRNA splicing, EJCs deposit 20–24 nt upstream of exon-exon junctions. The mRNA exports to the cytoplasm where a pioneer round of translation begins. UPF1 is loosely bound to the CBC. If a ribosome encounters a stop codon, CBC-UPF1 promotes SMG1-UPF1 formation and binding to eRF1-eRF3 to form the SURF complex. SMG1-UPF1 binds UPF2-UPF3 within the downstream EJC, signifying the stop codon as a PTC. SMG1 phosphorylates UPF1 to recruit endoribonucleolytic and/or deadenylation/decapping enzymes, resulting in rapid mRNA degradation.

Fig. 3.

Nonstop mRNA decay (NSD). Translation of mRNAs lacking a stop codon allows the ribosome to traverse the 3'-UTR and poly(A) tail and stall at the 3'-end. This leads to dissociation of PABP and mRNA degradation. The Ski7 protein binds the stalled ribosome to release the transcript and facilitate exosome-mediated 3'-5' degradation. Decapping by Dcp1-Dcp2 and 5'-3' degradation by Xrn1 is a minor pathway in NSD (dashed arrow), e.g., in the absence of Ski7.

Fig. 4.

No-go mRNA decay (NGD). Translation stalls upon encountering a barrier to ribosome progression. The endoribonucleases Dom34 and Hbs1 bind the transcript near the stall site and initiate endonucleolytic cleavage. Exosomes and Xrn1 degrade the resulting 5'- and 3'-mRNA fragments, respectively. It is not yet known if NGD occurs in mammalian cells.

Fig. 5. Model of the interactions between Ccr4-Not subunits and GW182 for miRNA-mediated deadenylation. GW repeats, the PAM2 domain, and CIM-1/2 in GW182 are shown. Ccr4 is also known as Cnot6, and Cnot7 is also known as Caf1. Ccr4/Cnot6 and Caf1/Cnot7 are the subunits with deadenylase activity. Pan2-Pan3 are not shown. See text for additional details.