Published in final edited form as:

Cell Signal. 2013 August ; 25(8): 1699–1710. doi:10.1016/j.cellsig.2013.03.026.

Signaling Pathways That Control mRNA Turnover

Roopa Thapar^{1,2,3,*} and Andria P. Denmon³

¹Hauptman-Woodward Medical Research Institute, 700 Ellicott Street, Buffalo, NY 14203, USA

²Department of Structural Biology, SUNY at Buffalo, 700 Ellicott Street, Buffalo, NY 14203, USA

³Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251-1892, USA

Abstract

Cells regulate their genomes mainly at the level of transcription and at the level of mRNA decay. While regulation at the level of transcription is clearly important, the regulation of mRNA turnover by signaling networks is essential for a rapid response to external stimuli. Signaling pathways result in posttranslational modification of RNA binding proteins by phosphorylation, ubiquitination, methylation, acetylation etc. These modifications are important for rapid remodeling of dynamic ribonucleoprotein complexes and triggering mRNA decay. Understanding how these posttranslational modifications alter gene expression is therefore a fundamental question in biology. In this review we highlight recent findings on how signaling pathways and cell cycle checkpoints involving phosphorylation, ubiquitination, and arginine methylation affect mRNA turnover.

Keywords

Phosphorylation; Ubiquitination; Arginine Methylation; Signal transduction; mRNA turnover; mRNA decay

1. Introduction

mRNA turnover plays an important role in determining the steady state levels of mRNA transcripts and is a significant control point in gene expression [1–3]. In the early days of molecular biology, most of the attention was focused on transcription and mRNA synthesis. However, it is now well established that the rates of gene synthesis and decay are tightly coordinated [4, 5]. The mRNA abundance in the cell is controlled in large part by mRNA decay pathways that include general decay mechanisms and small regulatory RNAs. mRNA turnover is defined by the mRNA half-life i.e. the length of time an mRNA exists in the cell before it is degraded. In eukaryotes, mRNA transcripts have a range of half-lives. Whereas housekeeping genes such as actin and β -globin have very slow decay rates (on the order of days), several proto-oncogenes such as *c-Myc*, cell-cycle regulated histone mRNAs, and mRNAs that play regulatory roles such as cytokines have half-lives < 1 hr [6, 7]. A change

© 2013 Elsevier Inc. All rights reserved.

*To whom correspondence should be addressed: Tel: 713-201-7875; rthapar@hwi.buffalo.edu or rthapar@rice.edu.

Conflict Of Interest Statement

The authors declare no competing financial interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

in the half-life of these short-lived mRNAs can dramatically alter their mRNA abundance, leading to serious changes in gene regulatory networks, cell growth, and differentiation. Not surprisingly, deregulation of mRNA stability plays an important role in development of several disease states such as cancer, inflammation, cystic fibrosis, and muscular dystrophy [8–10].

mRNA decay rates are influenced by the cellular microenvironment and external stimuli such as hormones, growth factors, stress, cytokines, and viral infections. Several *cis*-acting sequences such as the poly (A) tail, AU-rich elements (AREs), the iron-responsive element (IRE), Jun-kinase response elements, and the histone stem-loop have been identified in the 5' and 3' untranslated regions (UTRs) that are important for the stability of the mRNA transcripts (Figure 1). These *cis*-elements bind *trans*-acting factors such as RNA-binding proteins and non-coding RNAs (Figure 1) that play important roles in either protecting the mRNA from exonucleases, in recruiting decay factors that facilitate rapid decay, or in shuttling the mRNAs into different cellular compartments/organelles such as the nucleus or P-bodies [11]. mRNA decay also requires nucleases and the mRNA transcript is either degraded by the exosome, by specific ribonucleases, or both. The exosome is a large multi-subunit complex of 3'-5' exoribonucleases that scavenges and degrades mRNAs in the cell [12].

To understand how mRNA decay is regulated, it is important to identify the factors and clarify their roles in the RNA decay mechanism. Second, it is not clear how signals transmitted from the extracellular compartment of the cell by exogenous factors or cell-cycle checkpoint signals are relayed to the cytoplasm to regulate mRNA stability. Signaling pathways involving posttranslational modifications (PTMs) that target RNA binding proteins in ribonucleoprotein complexes need to be characterized in detail. PTMs can modulate RNA binding affinity or alter protein-protein interactions with effectors and hence, control gene expression. At least 200 regulatory PTMs such as ubiquitination, SUMOylation and other ubiquitin-like modifications, arginine and lysine methylation, N-acetylation, N-linked glycosylation, lipidation, sulfation, S-nitrosylation, proline hydroxylation, etc are known to exist. Very little is known about how these chemical modifications function to alter the biochemical properties and localization of the RNA-binding protein and hence, affect the stability of the associated mRNA. RNA decay mechanisms have already been summarized in several reviews [1, 2, 13–16]. In this review, we will focus on our current understanding of how signaling pathways and PTMs regulate mRNA turnover.

2. Phosphorylation-dependent pathways

Since its discovery in the 1950s, protein phosphorylation has been considered to be the most prevalent reversible posttranslational modification *in vivo*. Of the ~20,000 genes in the human genome, ~20% have been predicted to be involved in phosphorylation dependent signaling processes. It is estimated that there are ~518 protein kinases [17] and ~150 protein phosphatases in the human genome. Proteomic data predicts that most proteins in the cell are phosphorylated at least at one site [18]. It seems logical to assume that many RNA-binding proteins would also be regulated by protein phosphorylation. Protein kinase mediated signaling networks are used to transmit information from external stimuli such as cytokines, hormones, growth factors, environmental stress, UV irradiation, as well as intracellular cell-cycle dependent pathways to rapidly alter gene expression patterns. How do these pathways affect mRNA decay? Most of our current understanding of the effects of phosphorylation on mRNA turnover comes from the immune system. Several cytokine mRNAs and mRNAs involved in inflammation have ARE sequences in their 3'UTRs and the associated ARE-BPs are regulated by kinase mediated signal transduction pathways. The pathways that have been most well characterized are outlined in this section.

2.1 Mitogen Activated Pathways: p38, JNK, ERK, and PI3K-AKT

2.1.1. The p38 MAPK pathway in ARE-mediated decay (AMD)—The p38 pathway is activated by environmental stresses (such as UV irradiation, heat shock, growth factors, lipopolysaccharides) and inflammatory cytokines [19]. Stimulation of this pathway is strongly correlated with the development of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, cancer, and aging [20, 21]. The kinase cascade begins with activation of a MAPKKK, which in turn activates the MAP kinases MKK-3 and MKK-6 by phosphorylation (Figure 2). These upstream kinases are the two major activators of p38 MAPK, which is the nodal point for activation of several downstream kinases (Figure 2). p38 can also be activated by the general MAPKKs (MKK4) or MAPKK independent signals (TAB1). There are four isoforms of p38 MAPK: α , β , γ , and δ . Whereas the α and β isoforms are ubiquitous, the γ and δ isoforms are neuronal specific.

The p38 pathway is one of the most frequently utilized kinase cascades to regulate the stability of mRNAs that have AU-rich (ARE) elements in their 3' UTRs and undergo ARE-mediated decay (AMD) [22] (Figure 2). mRNAs whose stability increases due to activation of the p38 MAPK pathway include TNF- α [23–25], MIP-1 α [23], GM-CSF [26], COX-2 [27, 28], VEGF [28], MMP-1 [29], and MMP-3 [29]. These mRNAs have Class II AREs in their 3' UTR i.e. have multiple copies of overlapping pentameric AUUUA sequences or at least one copy of the pentameric AUUUA sequence along with U-rich sequences. These ARE sequences are known to bind ARE binding proteins (ARE-BPs) that are phosphorylated by the p38 pathway. They include HuR, AUF1, AUF2, KSRP proteins, TIA-1, TIAR, TIS11b, and the TTP family.

The zinc finger protein Tristetraprolin (TTP) is a prototypical member of a family of ARE-BPs and it is the most well characterized target of the p38 pathway [30, 31]. TTP destabilizes several proinflammatory cytokine mRNAs such as TNF- α , GM-CSF, and IL-2, as well as proangiogenic factors such as VEGF by promoting deadenylation of these mRNAs (Figure 2). In unstimulated cells, TTP binds the ARE sequence in these mRNAs via its two CCCH-type zinc finger motifs causing rapid degradation of these mRNAs. Although the exact mechanism is unclear, TTP has been reported to associate with several protein complexes important for RNA decay such as the PM-Scf75 subunit of the exosome, proteins Dcp2 and Dcp1a of the decapping complex, the CCR4-NOT deadenylase complex, the 5'-3' exonuclease Xrn1, as well as Ago2 and Ago4 subunits of the RNA-induced silencing complex (RISC) [31]. The current view is that TTP recruits the deadenylases Not1 and Caf1 to the mRNA 3' end and also associates with decapping factors to accelerate mRNA decay after deadenylation has occurred. Upon activation of the p38 pathway in response to growth factors and other stimuli, the kinase MK2 phosphorylates TTP at two serines (Ser⁵² and Ser¹⁷⁸ in mouse TTP correspond to Ser⁶⁰/Ser¹⁸⁶ in human TTP) [32]. Phosphorylation of TTP by MK2 has been reported to reduce binding affinity of TTP for the TNF- α ARE and promote its interaction with 14-3-3 proteins thereby altering its sub-cellular distribution and inhibiting its mRNA decay activity [32]. In addition to TTP, the K-homology splicing regulator protein (KSRP) is also phosphorylated by the p38 pathway at Thr692 in its extreme C-terminus [33, 34]. Phosphorylation at Thr692 reduces the affinity of KSRP for the ARE, thereby stabilizing mRNA transcripts. Besides the effect of the p38 pathway on mRNA stability, the p38 pathway has been shown to regulate translation by phosphorylating eIF-4E [35] and heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) [36].

2.1.2. The c-Jun NH (2)-terminal protein kinase (JNK) pathway—The JNK MAPK cascade plays an important role in regulating the stability of IL-2 mRNA in T-cells in response to cytokines and stress stimuli such as UV-irradiation and heat shock [37]. The IL-2 mRNA has a very short half-life of ~40 min in unstimulated Jurkat cells and activation

of the JNK pathway by mitogen stimulation results in stabilization of IL-2 mRNA ($t_{1/2}$ increases to ~90 min). It has been shown that the increased stability is due to a cis-element present in the 5' UTR of the IL-2 mRNA called the JNK-response element (JRE) [38]. The JRE specifically binds nucleolin and YB-1, and this mRNP complex is essential for regulation of mRNA stability via the JNK pathway [38]. However nucleolin and YB-1 are not the phosphorylation targets of the JNK pathway, suggesting another factor exists in the mRNP complex that is regulated by phosphorylation. Besides the JRE, IL-2 mRNA is also regulated by ARE sequences present in its 3' UTR.

2.1.3. The Extracellular signal-regulated (ERK) MAPK pathway—The ERK or MAPK pathway (also known as the Ras-Raf-MEK-ERK) pathway is a major signaling pathway that is implicated in cell growth, cell cycle progression, uncontrolled cell proliferation in cancer, as well as the inflammatory response in eosinophils and macrophages [39, 40]. The binding of extracellular growth factors or mitogens such as epidermal growth factor (EGF) and fibroblast growth factor (FGF) to receptor linked tyrosine kinases such as the EGFR or FGFR receptors, respectively, activates the kinase activity of these receptors (Figure 3). Phosphorylated EGFR binds the Src homology 2 (SH2) domain of the adaptor protein GRB2, which in turn recruits the guanine nucleotide exchange factor SOS. Activated SOS facilitates the exchange of the guanine nucleotide GTP for GDP in H-Ras and K-Ras proteins. Ras-GTP can bind to and stimulate Raf kinase, a key component of the MAPK pathway that activates the tyrosine/threonine kinases MEK (MEK1 and MEK2) by phosphorylation. MEK phosphorylates the Ser/Thr kinase MAPK (or ERK) (Figure 3). The optimal sequence that is phosphorylated in MAPK substrates is a Pro-Xaa-Ser/Thr-Pro motif, although a number of substrates have only a Ser/Thr-Pro dipeptide as the minimal consensus sequence. The substrate specificity of MAP kinase overlaps with other proline-directed protein kinases (e.g. cyclin-dependent protein kinases). Several RNA-binding proteins involved in RNA processing, splicing, translation, and mRNA turnover have phosphorylated Ser/Thr-Pro motifs and are likely regulated by the MAPK pathway.

A well-characterized example is the decay of the GM-CSF mRNA, which is regulated by AU-rich binding factor 1 (AUF1; also called hnRNP D) (Figure 3) [41–43]. In unstimulated eosinophils, unphosphorylated AUF1 binds ARE sequences in the GM-CSF 3' UTR, and recruits the exosome to facilitate mRNA decay via direct interactions with the PM-Scl75 subunit of the exosome. Upon stimulation of eosinophils with hyaluronic acid, Erk-mediated signaling results in phosphorylation of AUF1 at Ser-Pro motifs. Phosphorylated AUF1 becomes a substrate for the prolyl isomerase Pin1 that is specific for Ser/Thr-Pro sequences and catalyzes cis-trans isomerization about the peptide bond, thereby introducing a conformational change in AUF1 (Fig. 3). Pin1 dissociates AUF1 from the GM-CSF mRNA and the released GM-CSF mRNA associates with hnRNP C, thereby stabilizing the mRNA and preventing it from AUF1-mediated degradation.

There is growing evidence that phosphorylation of proteins by proline-directed kinases such as CDKs, MAPKs, and GSK-3 β likely creates binding sites for Pin1 in several RNA-binding proteins, modulating their affinity for RNA, thereby controlling mRNA decay rates. The ARE-BP KSRP, that also binds to ARE sequences, is phosphorylated at Ser181. It is not clear whether Ser181 is phosphorylated by MAPK, but the Ser181-Pro182 sequence is targeted by Pin1 for isomerization and dephosphorylation [44]. KSRP dephosphorylation mediated by Pin1 at Ser181 regulates the interaction of KSRP with *PTH* mRNA and accelerates *PTH* mRNA decay *in vivo*. Pin1 has also been shown to bind Stem-Loop Binding Protein (SLBP), a critical regulator of histone biogenesis. SLBP binds a 16 nt stem-loop sequence in the histone mRNA 3' UTR. SLBP is phosphorylated at Thr171 in the RNA association domain (RBD) [45], and phosphorylation at this site is important for the

assembly of the histone processing complex and the kinetics of RNA binding [46]. Pin1 binds the T171-Pro172 sequence in the SLBP RBD, dissociating SLBP from the histone mRNA, facilitating histone mRNA decay [47].

Recently, MAPK/ERK-mediated phosphorylation has also been implicated in miRNA maturation [48]. ERK phosphorylates HIV TAR RNA-binding protein (TRBP), a component of the core Dicer complex resulting in up regulation of miRNAs. Since miRNAs bind the 3' UTRs of mRNAs to regulate mRNA degradation or facilitate translational repression, the MAPK/ERK signaling pathway is directly implicated in control of mRNA turnover by miRNAs.

2.1.4 The PI3K-AKT pathway—The phosphatidylinositol 3-kinases (PI3Ks) form a unique group of lipid kinases that phosphorylate the 3'-hydroxyl group of phosphatidylinositol and phosphoinositides [49, 50]. They play an important role in signal transduction since a number of proteins bind the phosphoinositide products of PI3Ks and are hence activated by the PI3K pathway. There are three classes of PI3Ks based on their substrate preferences for lipids [50]. Class I PI3Ks primarily generate phosphatidylinositol-3, 4, 5-trisphosphate (PIP₃) from phosphatidylinositol-4, 5-bisphosphate (PI-4, 5-P₂) *in vivo*, class II PI3Ks preferentially generate PI-3-P and phosphatidylinositol-3, 4-bisphosphate (PI-3, 4-P₂) *in vitro*, and may generate PI-3-P, PI-3, 4-P₂ and possibly PIP₃ *in vivo*. Class III PI3Ks generate phosphatidylinositol-3-phosphate (PI-3-P) from phosphatidylinositol (PI). The PI3K-AKT pathway, especially the Class IA PI3Ks, play a key role in cell growth, survival, and cancer progression.

The Class IA PI3Ks are heterodimers consisting of a p85 regulatory subunit and a p110 catalytic subunit [50]. The p85 subunit is transcribed from three different genes such that several isoforms, namely p85 α , p85 β , p55 γ , p55 α , and p50 α exist. The regulatory subunit binds activated receptor tyrosine kinases (RTKs) either directly via its SH2 domains (as in the case of PDGF), or via adaptor-like molecules (as in the case of the insulin receptor substrate, IRS). This binding recruits the p85–p110 heterodimer to its substrate (PI-4, 5-P₂) at the plasma membrane, allowing for conversion of PIP₂ to PIP₃. PIP₃ binds numerous target substrates via their pleckstrin homology (PH) domains (Figure 4). One of the most important targets of PIP₃ is the Ser/Thr kinase AKT (also known as PKB). AKT is recruited to the plasma membrane via its interaction with PIP₃ and upon recruitment; AKT is phosphorylated by mTOR and PDK1 kinases, resulting in the fully activated form of AKT. The substrate consensus sequence usually recognized as the preferred AKT phosphorylation site is RXXXS. There are numerous downstream targets of AKT that are involved in regulation of cell metabolism, cell cycle, cell survival, and regulation of protein synthesis.

Global expression analysis in T38G human glioblastoma cells has shown that there are approximately 50 genes whose mRNA levels are altered in response to PI3K inhibition [51, 52]. Examination of mRNA decay rates showed that 20 out of 50 genes are regulated at the level of mRNA stability and not transcription. siRNA knockdown experiments reveal that BRF1 and KSRP are the primary ARE-BPs responsible for mediating PI3Ks effects on mRNA turnover, whereas AUF1 and HuR are not likely to be targeted. RNA binding proteins that are AKT substrates include KSRP, BRF1 (or TIS11b), NF90, CELF1, and YB1.

KSRP is a well characterized substrate of the PI3K-AKT signaling pathway that has been shown to affect the stability of β -catenin and myogenin mRNAs [53] (Figure 4). In C2C12 myoblasts, phosphorylation of KSRP at Ser193 by AKT dissociates KSRP from myogenin mRNA in the cytoplasm, thereby inhibiting the decay function of KSRP and stabilizing the mRNA. KSRP phosphorylation by AKT also increases KSRP association with 14-3-3 ζ ,

translocating it from the cytoplasm to the nucleus where KSRP regulates myogenic miRNA maturation. In a mouse KSRP knockout as well as cultured cells, KSRP has been shown to affect the processing of miR-206, miR-133b, and miR-1a microRNA precursors (pri-miRs and pre-miRs). KSRP also binds the let-7 pri-miR and affects processing by preventing association of Drosha and Dicer. Therefore PI3K signaling acts as a functional switch, inhibiting decay of myogenin mRNA in the cytoplasm while promoting miRNA processing in the nucleus.

BRF1, a CCCH zinc finger containing protein, has also been shown to be a substrate of AKT [54]. In response to insulin stimulation, BRF1 is phosphorylated by AKT at Ser90, Ser92, and Ser203 *in vivo*. Phosphorylation at these sites promotes interaction of BRF1 with 14-3-3 proteins in the cytoplasm, preventing BRF1 from associating with target mRNAs, thereby stabilizing the mRNA transcripts.

2.2 Cell-cycle regulated pathways

Replication-dependent histone mRNAs encode the bulk of histone proteins to package DNA and form chromatin during S-phase of the cell cycle [55]. Histone mRNA levels are cell cycle regulated [56–58]; their synthesis and degradation being tightly coupled to DNA replication [59, 60]. During the G₁/S-phase transition of the cell cycle there is a 35-fold increase in the steady state histone mRNA level [56], but only a 3–5 fold increase in the rate of transcription [61]. The increase in the abundance of histone mRNAs during G₁/S-phase is attributed to an increased rate of histone pre-mRNA processing, which increases by 8–10 fold [61]. Histone mRNAs have a half-life of ~110 min in early S-phase and ~40 min in late S/G₂-phase [62]. At the G₂/S-phase transition, histone mRNAs need to be rapidly degraded. At this time the rate of processing decreases back to what it was during G₁-phase, and the rate of degradation increases such as the histone mRNA half-life is only ~10 min at the end of S-phase [62, 63]. The half-life also decreases in response to DNA replication inhibitors such as hydroxyurea (HU) [64–66]. The 16 nt stem-loop is the only cis-element at the 3' end of histone mRNAs required for rapid decay of the mRNA transcript [59, 67]. Since this stem-loop binds SLBP, the histone mRNP that assembles at the 3' end, plays an important role in histone mRNA degradation (Figure 6). Several RNA degradation factors such as the Lsm (1-7) complex [68], the exosome [68], the nuclease ERI-1 [69], the decapping factors Dcp1/Dcp2 [68], and the helicase Upf1 [70] have been implicated in histone mRNA decay, although their specific roles and the mechanism of degradation is not clear (Figure 6). Like poly A⁺ transcripts, histone mRNA decay is bidirectional, occurring from both the 5' → 3' and 3' → 5' ends [68, 71]. However, as part of the degradative pathway, histone mRNAs are uridylated by a unique mechanism [68] that requires Tutase4 [71, 72] and possibly a second unidentified Tutase (Figure 6) [71]. Oligouridylation of the histone 3' end appears to be the rate limiting step for degradation [71]. In addition to the requirement of these factors, like poly A⁺ mRNAs, histone mRNAs must actively translate the message in order for efficient decay to occur [73]. The distance between the stem-loop and the stop codon is important suggesting that translation termination by the ribosome and the presence of release factors is a trigger for efficient histone mRNA decay [74].

The signal transduction pathways that couple histone mRNA degradation to inhibition of DNA replication are not well characterized, however, there is emerging evidence that cell cycle checkpoints required for the maintenance of genomic integrity, such as the DNA damage checkpoint, may also play a role in regulating histone mRNA turnover. The phosphoinositide 3-kinase (PI3 kinase)-related kinase (PIKK) family (Figure 5), which includes the sensor kinases ataxia telangiectasia-mutated (ATM), ATM and Rad3-related (ATR), SMG-1, and DNA protein-activated kinase (DNA-PK) play important roles during the DNA damage checkpoint [75, 76]. Although the PIKK family members are Ser/Thr kinases, the kinase domain of PIKK kinases is structurally related to the PI3K lipid kinases

[77]. These kinases are activated in response to double strand breaks and DNA damage (Figure 5). ATM is activated by double-strand breaks due to ionizing radiation (IR) and ATR is activated in response to ssDNA damage and replication stress due to DNA replication inhibitors such as hydroxyurea, aphidicolin as well as UV radiation. DNA-PK is activated by stalled replication forks due to double-strand breaks, and SMG-1 is activated by genotoxic stress and is a component of the nonsense mediated decay (NMD) machinery. A well-known target of SMG-1 is Upf1, which is required NMD, but is also implicated in other cellular processes such as histone mRNA decay, staufen mediated decay (SMD), and telomere maintenance. SMG-1 plays a role in mRNA surveillance and is a component of the SURF complex consisting of SMG-1, Upf-1, eRF-3, and eRF-1 [78]. These kinases act as sensors of the DNA damage response. ATR and DNA-PK have been implicated in linking replication stress with histone mRNA decay [70, 79]. Accessory proteins such as the prolyl isomerase Pin1 that are required for the replication checkpoint [80, 81], are also important for triggering histone mRNA decay (Fig. 5) [47]. Pin1 acts to re-model the histone mRNP by binding directly to the SLBP RNA binding domain and dissociating the SLBP-RNA complex. Pin1 also targets several effectors of the DNA damage checkpoint, such as Cdc25 [82, 83], Cyclin E [84], p53 [85], and p21 [86]. The proteins involved in these signaling pathways, the sites of phosphorylation, and the precise mechanism/s that trigger histone mRNA decay in response to the different DNA damage checkpoints remain to be uncovered. Our current understanding of the signaling pathway and mechanism of histone mRNA degradation is summarized in Figure 6.

2.3 The serine/threonine kinase mammalian target of rapamycin (mTOR) pathway

The target of rapamycin (TOR) signaling pathway controls cell growth, proliferation, survival, protein synthesis, and ribosomal biogenesis [87]. There are two multi-protein mTOR complexes. The components of the mTOR complex 1 (mTORC1) include mTOR, the adaptor protein raptor, the GTPase GβL, deptor, and PRAS40 [87, 88]. Components of mTORC2 include mTOR, GβL, deptor, rictor, mSIN1, and protor [89]. While mTORC1 regulates protein synthesis in response to nutrients, hormones, and growth factors, mTORC2 regulates the cytoskeleton and cell survival. For the discussion on mRNA translation and turnover, we will primarily focus on mTORC1. mTORC1 can be activated by a number of pathways that includes PI3K/AKT, TSC1/TSC2/Rheb, LKB1/AMPK, and Vam6/Rag GTPases [90, 91]. There are two major downstream targets of mTORC1 in the control of translation: 4E-BP proteins that bind the cap binding protein eIF4E and the S6 kinases S6K1 and S6K2. 4E-BPs are translational repressors that compete with eIF4G for binding eIF4E. mTORC1 inhibits the activity of 4E-BPs by phosphorylating them on Ser/Thr residues. S6 kinases phosphorylate downstream targets that include the ribosomal protein S6, the translation initiation factors PDCD4 and eIF4B. mRNAs that are particularly sensitive to the activity of mTORC1 have 5' terminal oligopyrimidine (TOP) motifs [92]. Since mRNA decay is coupled to translation, and mRNAs must be actively translating to trigger mRNA decay, any environmental factor that activates the mTORC1 complex also affects the rate of translation-dependent decay.

2.4 Wnt/β-catenin pathway

The Wnt signaling pathway is implicated in tumor progression and is important for cell-cell communication, cell survival, proliferation, and cell migration [93, 94]. The Wnt family consists of secreted glycoproteins that bind receptors called Frizzled (FRZ) as well as the transmembrane co-receptors LRP5 and LRP6. Wnt signaling activates the Dishevelled (DVL) family of proteins. Activated DSH inhibits the axin/GSK-3/APC complex, which is required for ubiquitination and proteolytic degradation of β-Catenin in the cytoplasm. Inhibition of β-catenin proteolysis results in translocation of β-catenin into the nucleus

where it acts as a co-activator for the TCF/LEF family of transcription factors to promote transcription of TCF/LEF target genes.

An interesting example of a TCF/LEF target gene that is regulated by the Wnt pathway at the level of both transcription and mRNA turnover is Pitx2 [95]. Pitx2 is a homeodomain transcription factor that has U-rich sequences resembling Class III AREs in its 3' UTR. Wnt activation increases the transcription of the Pitx2 gene via TCF/LEF, and also stabilizes Pitx2 mRNAs in mouse pituitary α T3-1 cells, increasing its half-life from 40 min to >120 min. The increase in mRNA half-life is directly correlated with decreased binding of the ARE destabilizing factors TTP and KSRP to the Pitx2 ARE as well as increased association of HuR with the Pitx2 mRNA. The increased protein levels of Pitx2 upon Wnt activation leads to enhanced transcription of Pitx2 targets such as the growth regulatory genes Cyclin D1, Cyclin D2, and c-Jun. This is an example of a signaling pathway that acts to temporally up-regulate a network of genes involved in cell growth by influencing both transcription and the rate of decay of the Pitx2 mRNA.

2.5 Regulation of Nonsense Mediated mRNA Decay (NMD)

To ensure the fidelity and quality of the mRNA transcript that is synthesized, cells have developed mRNA surveillance mechanisms that degrade abnormal transcripts [1, 96]. There are three major decay pathways that are important for quality control: non-stop decay (NSD), which detects and decays transcripts that do not have a stop-codon; no-go decay (NGD), which decays transcripts that are stalled on the ribosome during translation elongation; and nonsense-mediated decay (NMD), a mechanism that detects and degrades transcripts that have a premature termination codon (PTC) [96]. Of these, NMD is the most important and well-characterized mechanism that is essential for cellular fitness and is under the control of phosphorylation [97, 98]. Recent studies show that the NMD pathway plays a role not only in degradation of aberrant transcripts, but is also important for telomere maintenance [99] and cell cycle progression [100].

NMD is triggered when an intron is detected more than 50 nucleotides downstream of a stop codon. After splicing occurs in the nucleus, the cell deposits a protein complex called the exon-exon junction complex (EJC) 20–24 nt upstream of the splice site, thereby marking the position of the removed intron [101]. The position of the terminal EJC is a key determinant for triggering NMD. If an EJC is detected >50 nt downstream of the stop codon, the stop codon is considered to be a PTC and the mRNA is degraded [101]. The complex that catalyzes NMD consists of the suppressors of morphogenetic defects in genitalia (SMG) proteins of which the Upf proteins are the most important [102, 103]. The core Upf complex consists of Upf1 (also known as RENT1 or SMG-2), Upf2 (also called RENT2 or SMG-3), and Upf3 (also called RENT3 or SMG-4). Upf1 is the main effector protein of the complex. It is a large (~130 kDa) ATP-dependent 5' to 3' RNA helicase that has a zinc binding CH domain in the N-terminus that is important for association with Upf2 and two Recombinase-A (RecA) like helicase domains in the remainder of the protein that binds RNA. Upf1 is a phosphoprotein that can be hyperphosphorylated by the PIKK family kinase SMG-1 at 4 S/Q sites *in vivo*, although there are 28 possible S/Q sites in Upf1 that potentially could be phosphorylated by SMG-1 [104–107]. Sequential Upf1 phosphorylation/dephosphorylation cycles are necessary to activate NMD (Figure 7). Other SMG proteins that play regulatory roles include the adaptor proteins SMG-5, SMG-6, SMG-7, SMG-8, and SMG-9. SMG-5 and SMG-7 aid in dephosphorylation of Upf1 and co-purify with the Upf (1-3) complex, SMG-1, and the protein phosphatase PP2A (Figure 7) [102]. SMG-7 has a 14-3-3 like domain that likely serves as an adaptor protein during Upf1 dephosphorylation. SMG-6 is an endonuclease that likely functions to degrade the RNA. SMG-8 and SMG-9 regulate the kinase activity of SMG-1. The interaction between the SMG proteins and the phosphorylation/dephosphorylation cycles are summarized in Figure 7. The actual

mechanism of NMD and the role of the individual proteins in initiating and regulating NMD, in recruitment of decay factors, and in translation termination are being studied and are summarized in some excellent reviews [97, 102, 108]. While it is clear that NMD is under the control of Upf1 phosphorylation, the precise role of the phosphorylation/dephosphorylation cycles in the NMD reaction mechanism is unclear.

3. Ubiquitin-dependent pathways

The ubiquitin-proteasome pathway (UPS) was originally identified as the primary pathway for protein degradation by the proteasome. For its discovery Aaron Ciechanover, Avram Hershko, and Irwin Rose were awarded the Nobel Prize in Chemistry in 2004. Ubiquitin is a small ~8.5 kDa polypeptide that is attached to lysines of target substrates by a multi-enzyme complex [109–111]. When the target protein is polyubiquitinated, it marks the substrate for protein degradation. In recent years, several non-degradative and regulatory functions of mono- and oligo-ubiquitination have been identified [112]. There are several similarities between protein ubiquitination and phosphorylation. Similar to phosphorylation, protein ubiquitination is an ATP-driven reaction that affects almost every cellular process. Both modifications are under the control of several enzymes. More than 600 E3 ligases and 100 deubiquitinating enzymes have been identified in mammalian cells. Finally, ubiquitinated proteins can be specifically recognized by specific ubiquitin binding domains such as UBA, UBZ, CUE, UIM, etc. and, thereby regulate protein-protein interactions.

Ubiquitination occurs via a cascade of events that begins with E1-mediated adenylation of the ubiquitin C-terminal glycine [111]. The ubiquitin is then transferred to a ubiquitin conjugating enzyme, E2, via a thioester linkage which in turn transfers ubiquitin to the ϵ -amino group of a lysine in the target protein with the help of an E3 ubiquitin ligase. Substrate specificity is usually dictated by the E3 ligase. At least fifteen E3 ligases have been identified (Figure 8) in mammalian cells that have RNA binding domains, implicating a role for ubiquitin in RNA metabolism [113]. Several of these E3 ligases have been shown to bind mRNA directly and play a role in mRNA turnover. The mechanism by which ubiquitination of RNA binding proteins affects mRNA turnover is only beginning to be understood.

The first observation that ubiquitination of an RNA binding protein could affect mRNA turnover was reported in 1999 by R. Schneider and co-workers [114]. It was observed that polyubiquitination of AUF1, an ARE-BP that regulates the decay of several cytokine and proto-oncogene mRNAs, was linked to efficient decay of the associated mRNA. In addition, over-expression of deubiquinating enzymes of the UBP family also altered the half-life of a GM-CSF ARE containing mRNA reporter, suggesting that the degradation of RNA and AUF1 protein components of an mRNP may be temporally correlated [115]. Since then several RNA binding proteins such as HuR [116], TTP [117], and SLBP [47] have been shown to be polyubiquitinated.

Ubiquitin regulates both translation and degradation of the TNF- α mRNA by different mechanisms. Similar to AUF1, TTP polyubiquitination has been shown to affect the stability of the TNF- α mRNA [118]. Additionally, TTP has also been reported to directly associate with Cullin4B, a component of the Cullin ring finger ligase family of ubiquitin E3 ligases [117]. RNAi knockdown of Cullin4B results in a decrease in the half-life of TNF- α mRNA from 69 min to 33 min in LPS stimulated cells and an increase in the activity of TTP. However, Cullin4B knockdown has no effect on TTP protein stability. Cullin4B co-localizes with TTP in P-bodies and Cullin4B shRNA treated cells have reduced polysome-associated TNF- α mRNA. The proposed model is that Cullin4B is recruited by TTP to target the

degradation of an unknown protein in the TNF- α mRNP, a key step that licenses the loading of TNF- α mRNP onto polysomes.

Some progress has recently been made in understanding the varied functions of newly discovered RNA associated E3 ligases shown in Figure 8 [113]. An interesting example is MEX-3C, an E3 ligase that binds the 3' UTR of HLA-A2 mRNA and regulates the degradation of the major histocompatibility complex-1 (MHC-1) mRNA [119]. The MEX-3 E3 ligase family consists of four members in humans (Figure 8) of which MEX-3A and MEX-3B co-localize with Ago1 and Dcp1a in P-bodies [120]. MEX-3A and MEX-3B associate with Ago proteins in an mRNA dependent manner, whereas MEX-3C binds Ago1 and Ago2 directly [120]. All MEX-3 proteins interact with poly (A) binding protein (PABP) [120]. MEX-3C binds the HLA-A2 mRNA 3'UTR directly via its two tandemly arranged KH domains and it has a RING domain at the C-terminus that exhibits E3 ligase activity [119]. Overexpression of MEX-3C was found to decrease HLA-A2 mRNA levels in HEK293T cells, whereas expression of the RING deletion mutant had no effect on HLA-A3 mRNA levels. USP7 has been identified as the deubiquitinase for MEX-3C and USP7 counters the effects of MEX-3C *in vivo*. Therefore, the ubiquitin ligase activity of MEX-3C is important for HLA-A2 mRNA degradation. MEX-3C is specific for HLA-A2, but does not bind HLA-B/C, indicating MEX-3C likely recognizes a specific cis-element in the HLA-A 3' UTR that remains to be identified [119].

Mouse Roquin (called MNAB in humans) is another example of a large RNA-binding E3 ligase that has an N-terminal RING finger, a unique ROQ domain, and a CCCH type of zinc finger [121, 122]. Roquin-deficient mice that have elevated levels of IL-2 develop a severe autoimmune lupus type of phenotype and possess higher inducible T-cell co-stimulator mRNA levels. In T-cells, Roquin represses *Icos* mRNA via the miRNA pathway, specifically mediated by miR-101. Roquin and MNAB are both recruited to stress granules, although not much is known about their E3 ligase activity [123].

A fascinating group of E3 ligases that act to repress translation of mRNAs by associating with Argonaute protein containing miRNA complexes (miRNPs) are members of the TRIM-NHL family (Figure 8) [124]. TRIM proteins consist of almost 70 members in mammals and function as ubiquitin E3 ligases, although some also have SUMOylation activity. They are involved in cell cycle progression, apoptosis, transcription, signaling, and regulation of mRNA turnover. TRIM proteins are distinguished by the presence of a tripartite motif (TRIM) consisting of a tandem arrangement of a RING domain, a B-box, and a coiled-coil region. B-box domains are structurally similar to RING domains. The C-terminal domain can be variable and proteins with an NHL domain in the C-terminus are abbreviated TRIM-NHL (Figure 8). There are four TRIM-NHL proteins expressed in mammals: TRIM2, TRIM3, TRIM32, and TRIM71. TRIM-NHL proteins act as cofactors of miRNA-mediated mRNA repression or degradation. They have been characterized in humans, mice, *C. elegans*, and *Drosophila*. In *C. elegans*, NHL-2 associates physically and functionally with the P-body associated DEAD-box helicase CGH-1, and they function together with the core miRISC components ALG-1, ALG-2, and AIN-1 to positively regulate *let-7* and *lys-6* miRNA function [125]. It is not known whether NHL-2 ubiquitinates components of miRISC.

In the mouse neocortex, TRIM32 (also called Brat and Mei-P26 in *Drosophila*) plays a critical role in neuronal differentiation [126]. TRIM32 is an inhibitor of cell proliferation. TRIM32 ubiquitinates and degrades c-Myc. It also binds Argonaute-1 via its NHL domain and increases the activity of miRNAs. However TRIM32 does not ubiquitinate Argonaute-1. TRIM32 regulates 34 miRNAs and one of its targets is *Let-7a*. In contrast to mouse

TRIM32, *Drosophila* Mei-P26 (which also binds Ago-1) has been reported to inhibit miRNAs [127].

Finally, in humans, TRIM71 has been shown to promote translational repression and mRNA decay [128]. TRIM71 associates with Ago2, Hsp90, Hsp70, PABP1, PUM1, PUM2, and Xrn1 proteins in HEK293 cells. Several mRNAs have been identified as TRIM71 targets by RNA-immunoprecipitation and TRIM71 associates with their 3'UTRs. Some of these targets have been validated by qRT-PCR. Exogenous expression of TRIM71 leads to a 3-fold repression of *CCNE2* mRNA and a 1.6–2 fold repression of *HOXA5*, *STAT5B*, *ZNF362*, and *E2F7*. Intriguingly, the RING/B1 domain that exhibits E3 ubiquitin ligase activity is dispensable for translational repression but the NHL domain is required to target TRIM71 to its mRNA substrates.

4. Arginine methylation-dependent pathways

Arginine methylation is a ubiquitous posttranslational modification that controls gene expression via modifying histone proteins, regulating transcription, RNA processing, mRNA turnover, and signal transduction pathways [129, 130]. Arginines can exist in unmethylated, monomethylated (MMA or ω -N^G), asymmetrically dimethylated (aDMA or ω -N^G, N^G), or symmetrically dimethylated (sDMA or ω -N^G, N^G) states (Figure 9). There are at least eleven arginine methyltransferases (PRMTs) in the human genome [131] that are classified as Type I, II, III, or IV based on their substrate specificity (Fig. 9A). Type I, II, and III perform monomethylation but differ in their choice of substrates (Fig. 9A). Type IV methylates only on the δ -nitrogen. RNA binding proteins that play roles in RNA processing and mRNA turnover such as hnRNPs (A1, A2, K, R, and U) [132–134], Sm proteins (D1, D3 B/B', LSm4) [135–138], Piwi [139], Vasa [140], FMRP [141, 142], PABP1 [143, 144], PABPN1 [145, 146], HuR [147, 148], HuD [149], ribosomal proteins [150, 151], the HIV Tat protein [152], among many others are methylated at arginine residues. The addition of a methyl group to the arginine side-chain increases its hydrophobicity and adds steric bulk to the side-chain. Since arginines are preferentially utilized to contact the RNA phosphate backbone, arginine methylation of an RNA binding protein can affect its interaction with RNA by either loss of a hydrogen bond, may facilitate base stacking, or interfere with binding due to steric effects [153]. Similarly, arginine methylation could sterically hinder protein-protein interactions in ribonucleoprotein complexes or promote such interactions via hydrophobic contacts. Methylation is not necessarily an irreversible process and the sites of arginine methylation can be regulated. Arginine deiminases can convert arginine and MMA into citrulline [154, 155] while amide oxidases [156] may demethylate arginine and lysine residues (Figure 9B). Tudor domains have been identified in many proteins as being capable of specifically recognizing lysine-methylated and arginine-methylated motifs (Figure 9C) [157].

Arginine methylation plays a particularly important role in the assembly of “nuage” type of germline granules that regulate the PIWI-piRNA pathway in *Drosophila* and mice [158–160] as well as in the formation of stress granules in mammals [157, 161]. piRNAs are the largest class of small RNAs in eukaryotes being slightly longer (26–31 nt) than miRNAs [162–164]. PIWI (P-element induced wimpy testis) proteins belong to the Argonaute family of regulators that bind and cleave RNA, and are specific for the piRNA (PIWI-interacting RNA) silencing pathway in animal germ (spermatogenic or testes) cells [158, 165]. PIWI proteins protect the genome against retrotransposons [166], are essential for piRNA biogenesis, and regulate mRNA turnover during germ cell differentiation to generate functional haploid gametes. Piwi proteins regulate the decay of transposon mRNAs and, thereby control transposon expression in animal cells. PIWI proteins are essential components of the nuage type of germline granules that act as silencing centers and also

contain the PRMT5 methyosome, Tudor domain containing proteins, and RNA helicases [167]. PIWI proteins undergo symmetric di-methylation on arginine residues by PRMT5 and the methylated arginine is specifically recognized by Tudor domain containing proteins in piRNA containing complexes. Arginine methylation is required for proper assembly and function of PIWI and Tudor proteins in these complexes. Human piRNAs (called Hiwi) are overexpressed in cancers [168–171] and are implicated in stem cell self-renewal [166].

In addition, several proteins that are components of stress granules are known to be arginine methylated and methylation may be required for formation of aggregates observed in these bodies. Stress granules are cytoplasmic foci that form in response to environmental stresses such as heat shock, oxidative stress, viral infection, and UV irradiation [172, 173]. They are short-term storage sites generally composed of translationally repressed, stalled, 48S ribosomal complexes consisting of mRNA bound to initiation factors such as eIF4E, eIF3, eIF4G, and eIF4A. Stress granules are also enriched in RNA decay factors, nucleases, and Argonaute proteins that are involved in miRNA mediated mRNA decay. Stress granule components known to be methylated at arginines include the fragile X mental retardation protein (FMRP) and the spinal motor atrophy gene (SMN1).

Concluding Remarks

We have described several examples of how PTMs play important roles in modulating mRNA turnover. Posttranslational modifications of RNA binding proteins can affect protein-protein and protein-RNA interactions and are important for the remodeling of mRNP complexes for rapidly regulating gene expression. Studies in mRNA turnover have so far been focused on the identification of cis-elements, trans-acting factors, and the mechanism of RNA degradation. While these studies will continue to be important, attention is slowly shifting to understanding the protein-protein interaction networks and signaling pathways that regulate RNA binding proteins. Identifying the signal transduction pathways and cross-talk between these networks remains a challenge. Recent advances in proteomics and high throughput approaches make it possible to characterize these regulatory networks in detail and are likely to provide new insights into the mechanisms that regulate mRNA decay in the future.

Acknowledgments

R.T. was supported by NIH 1RO1-GM076660 and faculty startup funds from HWI. Support for A.P.D. was provided by National Institutes of Health Training Grant 2T32 GM008280.

Abbreviations

PTM	posttranslational modification
ARE	AU-rich elements
UTR	untranslated region
ARE-BP	ARE binding protein
IRE	Iron-responsive element
JRE	Jun-kinase response element
TTP	Tristetraprolin
KSRP	K-homology splicing regulator protein
RISC	RNA-induced silencing complex

AUF1	AU-rich binding factor 1
SLBP	Stem-Loop Binding Protein
NMD	Nonsense Mediated Decay
mTOR	mammalian target of rapamycin

References

1. Garneau NL, Wilusz J, Wilusz CJ. *Nat Rev Mol Cell Biol.* 2007; 8:113–126. [PubMed: 17245413]
2. Chen CY, Shyu AB. *Wiley Interdiscip Rev RNA.* 2011; 2:167–183. [PubMed: 21957004]
3. Schwanhaussner B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M. *Nature.* 2011; 473:337–342. [PubMed: 21593866]
4. Moore MJ, Proudfoot NJ. *Cell.* 2009; 136:688–700. [PubMed: 19239889]
5. Bellofatto V, Wilusz J. *Cell.* 2011; 147:1438–1439. [PubMed: 22196721]
6. Herrick D, Parker R, Jacobson A. *Mol Cell Biol.* 1990; 10:2269–2284. [PubMed: 2183028]
7. Ross J. *Microbiol Rev.* 1995; 59:423–450. [PubMed: 7565413]
8. Chen CY, Shyu AB. *Trends Biochem Sci.* 1995; 20:465–470. [PubMed: 8578590]
9. Farazi TA, Hoell JI, Morozov P, Tuschl T. *Adv Exp Med Biol.* 2013; 774:1–20. [PubMed: 23377965]
10. Cooper TA, Wan L, Dreyfuss G. *Cell.* 2009; 136:777–793. [PubMed: 19239895]
11. Balagopal V, Parker R. *Curr Opin Cell Biol.* 2009; 21:403–408. [PubMed: 19394210]
12. Mitchell P, Tollervey D. *Adv Exp Med Biol.* 2011; 702:1–8. [PubMed: 21713673]
13. Dickson AM, Wilusz J. *Trends Genet.* 2011; 27:286–293. [PubMed: 21640425]
14. Li Y, Kiledjian M. *Wiley Interdiscip Rev RNA.* 2010; 1:253–265. [PubMed: 21935889]
15. Schoenberg DR, Maquat LE. *Nat Rev Genet.* 2012; 13:246–259. [PubMed: 22392217]
16. Wu X, Brewer G. *Gene.* 2012; 500:10–21. [PubMed: 22452843]
17. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. *Science.* 2002; 298:1912–1934. [PubMed: 12471243]
18. Olsen JV, Blagoev B, Gnäd F, Macek B, Kumar C, Mortensen P, Mann M. *Cell.* 2006; 127:635–648. [PubMed: 17081983]
19. Cuadrado A, Nebreda AR. *Biochem J.* 2010; 429:403–417. [PubMed: 20626350]
20. Wagner EF, Nebreda AR. *Nat Rev Cancer.* 2009; 9:537–549. [PubMed: 19629069]
21. Huang G, Shi LZ, Chi H. *Cytokine.* 2009; 48:161–169. [PubMed: 19740675]
22. Dean JL, Sully G, Clark AR, Saklatvala J. *Cell Signal.* 2004; 16:1113–1121. [PubMed: 15240006]
23. Wang SW, Pawlowski J, Wathen ST, Kinney SD, Lichenstein HS, Mantley CL. *Inflamm Res.* 1999; 48:533–538. [PubMed: 10563470]
24. Dean JL, Wait R, Mahtani KR, Sully G, Clark AR, Saklatvala J. *Mol Cell Biol.* 2001; 21:721–730. [PubMed: 11154260]
25. Wilson GM, Lu J, Sutphen K, Sun Y, Huynh Y, Brewer G. *J Biol Chem.* 2003; 278:33029–33038. [PubMed: 12819195]
26. Tebo J, Der S, Frevel M, Khabar KS, Williams BR, Hamilton TA. *J Biol Chem.* 2003; 278:12085–12093. [PubMed: 12556523]
27. Ridley SH, Dean JL, Sarsfield SJ, Brook M, Clark AR, Saklatvala J. *FEBS Lett.* 1998; 439:75–80. [PubMed: 9849881]
28. Cha HJ, Lee HH, Chae SW, Cho WJ, Kim YM, Choi HJ, Choi DH, Jung SW, Min YJ, Lee BJ, Park SE, Park JW. *Hepatogastroenterology.* 2011; 58:790–795. [PubMed: 21830391]
29. Reunanen N, Li SP, Ahonen M, Foschi M, Han J, Kahari VM. *J Biol Chem.* 2002; 277:32360–32368. [PubMed: 12060661]
30. Ross CR, Brennan-Laun SE, Wilson GM. *Ageing Res Rev.* 2012; 11:473–484. [PubMed: 22387927]

31. Brooks SA, Blackshear PJ. *Biochim Biophys Acta*. 2013
32. Chrestensen CA, Schroeder MJ, Shabanowitz J, Hunt DF, Pelo JW, Worthington MT, Sturgill TW. *J Biol Chem*. 2004; 279:10176–10184. [PubMed: 14688255]
33. Briata P, Chen CY, Ramos A, Gherzi R. *Biochim Biophys Acta*. 2012
34. Briata P, Forcales SV, Ponassi M, Corte G, Chen CY, Karin M, Puri PL, Gherzi R. *Mol Cell*. 2005; 20:891–903. [PubMed: 16364914]
35. Shveygert M, Kaiser C, Bradrick SS, Gromeier M. *Mol Cell Biol*. 2010; 30:5160–5167. [PubMed: 20823271]
36. Ziaei S, Shimada N, Kucharavy H, Hubbard K. *Exp Cell Res*. 2012; 318:500–508. [PubMed: 22227431]
37. Chen CY, Del Gatto-Konczak F, Wu Z, Karin M. *Science*. 1998; 280:1945–1949. [PubMed: 9632395]
38. Chen CY, Gherzi R, Andersen JS, Gaietta G, Jurchott K, Royer HD, Mann M, Karin M. *Genes Dev*. 2000; 14:1236–1248. [PubMed: 10817758]
39. Young A, Lou D, McCormick F. *Cancer Discov*. 2013; 3:112–123. [PubMed: 23103856]
40. Young A, Lyons J, Miller AL, Phan VT, Alarcon IR, McCormick F. *Adv Cancer Res*. 2009; 102:1–17. [PubMed: 19595305]
41. Esnault S, Shen ZJ, Whitesel E, Malter JS. *J Immunol*. 2006; 177:6999–7006. [PubMed: 17082615]
42. Esnault S, Shen ZJ, Malter JS. *Crit Rev Immunol*. 2008; 28:45–60. [PubMed: 18298383]
43. Shen ZJ, Esnault S, Malter JS. *Nat Immunol*. 2005; 6:1280–1287. [PubMed: 16273101]
44. Nechama M, Uchida T, Mor Yosef-Levi I, Silver J, Naveh-Many T. *J Clin Invest*. 2009; 119:3102–3114. [PubMed: 19770516]
45. Borchers CH, Thapar R, Petrotchenko EV, Torres MP, Speir JP, Easterling M, Dominski Z, Marzluff WF. *Proc Natl Acad Sci U S A*. 2006; 103:3094–3099. [PubMed: 16492733]
46. Zhang M, Lam TT, Tonelli M, Marzluff WF, Thapar R. *Biochemistry*. 2012; 51:3215–3231. [PubMed: 22439849]
47. Krishnan N, Lam TT, Fritz A, Rempinski D, O'Loughlin K, Minderman H, Berezney R, Marzluff WF, Thapar R. *Mol Cell Biol*. 2012; 32:4306–4322. [PubMed: 22907757]
48. Paroo Z, Ye X, Chen S, Liu Q. *Cell*. 2009; 139:112–122. [PubMed: 19804757]
49. Wymann MP, Schultz C. *Chembiochem*. 2012; 13:2022–2035. [PubMed: 22965647]
50. Engelman JA, Luo J, Cantley LC. *Nat Rev Genet*. 2006; 7:606–619. [PubMed: 16847462]
51. Terragni J, Graham JR, Adams KW, Schaffer ME, Tullai JW, Cooper GM. *BMC Cell Biol*. 2008; 9:6. [PubMed: 18226221]
52. Graham JR, Hendershott MC, Terragni J, Cooper GM. *Mol Cell Biol*. 2010; 30:5295–5305. [PubMed: 20855526]
53. Briata P, Lin WJ, Giovarelli M, Pasero M, Chou CF, Trabucchi M, Rosenfeld MG, Chen CY, Gherzi R. *Cell Death Differ*. 2012; 19:478–487. [PubMed: 21886180]
54. Schmidlin M, Lu M, Leuenberger SA, Stoecklin G, Mallaun M, Gross B, Gherzi R, Hess D, Hemmings BA, Moroni C. *EMBO J*. 2004; 23:4760–4769. [PubMed: 15538381]
55. Marzluff WF, Wagner EJ, Duronio RJ. *Nat Rev Genet*. 2008; 9:843–854. [PubMed: 18927579]
56. Harris ME, Bohni R, Schneiderman MH, Ramamurthy L, Schumperli D, Marzluff WF. *Mol Cell Biol*. 1991; 11:2416–2424. [PubMed: 2017161]
57. Luscher B, Stauber C, Schindler R, Schumperli D. *Proc Natl Acad Sci U S A*. 1985; 82:4389–4393. [PubMed: 3925455]
58. Stauber C, Schumperli D. *Nucleic Acids Res*. 1988; 16:9399–9414. [PubMed: 3141900]
59. Levine BJ, Chodchoy N, Marzluff WF, Skoultchi AI. *Proc Natl Acad Sci U S A*. 1987; 84:6189–6193. [PubMed: 2888112]
60. Robbins E, Borun TW. *Proc Natl Acad Sci U S A*. 1967; 57:409–416. [PubMed: 16591485]
61. Alterman RB, Ganguly S, Schulze DH, Marzluff WF, Schildkraut CL, Skoultchi AI. *Mol Cell Biol*. 1984; 4:123–132. [PubMed: 6583492]

62. Morris TD, Weber LA, Hickey E, Stein GS, Stein JL. *Mol Cell Biol.* 1991; 11:544–553. [PubMed: 1986245]
63. Heintz N, Sive HL, Roeder RG. *Mol Cell Biol.* 1983; 3:539–550. [PubMed: 6406835]
64. Baumbach LL, Marashi F, Plumb M, Stein G, Stein J. *Biochemistry.* 1984; 23:1618–1625. [PubMed: 6426507]
65. Sive HL, Heintz N, Roeder RG. *Mol Cell Biol.* 1984; 4:2723–2734. [PubMed: 6441888]
66. Gallwitz D. *Nature.* 1975; 257:247–248. [PubMed: 1161028]
67. Pandey NB, Marzluff WF. *Mol Cell Biol.* 1987; 7:4557–4559. [PubMed: 3437896]
68. Mullen TE, Marzluff WF. *Genes Dev.* 2008; 22:50–65. [PubMed: 18172165]
69. Hoefig KP, Rath N, Heinz GA, Wolf C, Dameris J, Schepers A, Kremmer E, Ansel KM, Heissmeyer V. *Nat Struct Mol Biol.* 2013; 20:73–81. [PubMed: 23202588]
70. Kaygun H, Marzluff WF. *Nat Struct Mol Biol.* 2005; 12:794–800. [PubMed: 16086026]
71. Su W, Slepnev SV, Slevin MK, Lyons SM, Ziemniak M, Kowalska J, Darzynkiewicz E, Jemielity J, Marzluff WF, Rhoads RE. *RNA.* 2013; 19:1–16. [PubMed: 23188809]
72. Schmidt MJ, West S, Norbury CJ. *RNA.* 2011; 17:39–44. [PubMed: 21051505]
73. Graves RA, Pandey NB, Chodchoy N, Marzluff WF. *Cell.* 1987; 48:615–626. [PubMed: 3028643]
74. Kaygun H, Marzluff WF. *Mol Cell Biol.* 2005; 25:6879–6888. [PubMed: 16055702]
75. Sperka T, Wang J, Rudolph KL. *Nat Rev Mol Cell Biol.* 2012; 13:579–590. [PubMed: 22914294]
76. Lovejoy CA, Cortez D. *DNA Repair (Amst).* 2009; 8:1004–1008. [PubMed: 19464237]
77. Lempiainen H, Halazonetis TD. *EMBO J.* 2009; 28:3067–3073. [PubMed: 19779456]
78. Kashima I, Yamashita A, Izumi N, Kataoka N, Morishita R, Hoshino S, Ohno M, Dreyfuss G, Ohno S. *Genes Dev.* 2006; 20:355–367. [PubMed: 16452507]
79. Muller B, Blackburn J, Feijoo C, Zhao X, Smythe C. *J Cell Biol.* 2007; 179:1385–1398. [PubMed: 18158334]
80. Winkler KE, Swenson KI, Kornbluth S, Means AR. *Science.* 2000; 287:1644–1647. [PubMed: 10698738]
81. Joseph JD, Daigle SN, Means AR. *J Biol Chem.* 2004; 279:32373–32384. [PubMed: 15178679]
82. Crenshaw DG, Yang J, Means AR, Kornbluth S. *EMBO J.* 1998; 17:1315–1327. [PubMed: 9482729]
83. Shen M, Stukenberg PT, Kirschner MW, Lu KP. *Genes Dev.* 1998; 12:706–720. [PubMed: 9499405]
84. van Drogen F, Sangfelt O, Malyukova A, Matskova L, Yeh E, Means AR, Reed SI. *Mol Cell.* 2006; 23:37–48. [PubMed: 16818231]
85. Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP, Xiao ZX. *Nature.* 2002; 419:849–853. [PubMed: 12397361]
86. Wulf GM, Liou YC, Ryo A, Lee SW, Lu KP. *J Biol Chem.* 2002; 277:47976–47979. [PubMed: 12388558]
87. Guertin DA, Sabatini DM. *Cancer Cell.* 2007; 12:9–22. [PubMed: 17613433]
88. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM. *Cell.* 2009; 137:873–886. [PubMed: 19446321]
89. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, Sabatini DM. *Curr Biol.* 2006; 16:1865–1870. [PubMed: 16919458]
90. Efeyan A, Zoncu R, Chang S, Gumper I, Snitkin H, Wolfson RL, Kirak O, Sabatini DD, Sabatini DM. *Nature.* 2013; 493:679–683. [PubMed: 23263183]
91. Zoncu R, Efeyan A, Sabatini DM. *Nat Rev Mol Cell Biol.* 2011; 12:21–35. [PubMed: 21157483]
92. Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM. *Nature.* 2012; 485:109–113. [PubMed: 22552098]
93. Anastas JN, Moon RT. *Nat Rev Cancer.* 2013; 13:11–26. [PubMed: 23258168]
94. Lim X, Nusse R. *Cold Spring Harb Perspect Biol.* 2013; 5
95. Briata P, Ilengo C, Corte G, Moroni C, Rosenfeld MG, Chen CY, Gherzi R. *Mol Cell.* 2003; 12:1201–1211. [PubMed: 14636578]

96. Ghosh S, Jacobson A. *Wiley Interdiscip Rev RNA*. 2010; 1:351–361. [PubMed: 21132108]
97. Isken O, Maquat LE. *Nat Rev Genet*. 2008; 9:699–712. [PubMed: 18679436]
98. Nicholson P, Yepiskoposyan H, Metze S, Zamudio Orozco R, Kleinschmidt N, Muhlemann O. *Cell Mol Life Sci*. 2010; 67:677–700. [PubMed: 19859661]
99. Azzalin CM. *Nucleus*. 2012; 3:16–21. [PubMed: 22156744]
100. Azzalin CM, Lingner J. *Curr Biol*. 2006; 16:433–439. [PubMed: 16488880]
101. Maquat LE, Tarn WY, Isken O. *Cell*. 2010; 142:368–374. [PubMed: 20691898]
102. Kervestin S, Jacobson A. *Nat Rev Mol Cell Biol*. 2012; 13:700–712. [PubMed: 23072888]
103. Conti E, Izaurralde E. *Curr Opin Cell Biol*. 2005; 17:316–325. [PubMed: 15901503]
104. Yamashita A, Ohnishi T, Kashima I, Taya Y, Ohno S. *Genes Dev*. 2001; 15:2215–2228. [PubMed: 11544179]
105. Ohnishi T, Yamashita A, Kashima I, Schell T, Anders KR, Grimson A, Hachiya T, Hentze MW, Anderson P, Ohno S. *Mol Cell*. 2003; 12:1187–1200. [PubMed: 14636577]
106. Pal M, Ishigaki Y, Nagy E, Maquat LE. *RNA*. 2001; 7:5–15. [PubMed: 11214180]
107. Denning G, Jamieson L, Maquat LE, Thompson EA, Fields AP. *J Biol Chem*. 2001; 276:22709–22714. [PubMed: 11331269]
108. Schweingruber C, Rufener SC, Zund D, Yamashita A, Muhlemann O. *Biochim Biophys Acta*. 2013
109. Weissman AM. *Nat Rev Mol Cell Biol*. 2001; 2:169–178. [PubMed: 11265246]
110. Hershko A, Ciechanover A, Varshavsky A. *Nat Med*. 2000; 6:1073–1081. [PubMed: 11017125]
111. Pickart CM, Eddins MJ. *Biochim Biophys Acta*. 2004; 1695:55–72. [PubMed: 15571809]
112. Pickart CM. *Mol Cell*. 2001; 8:499–504. [PubMed: 11583613]
113. Cano F, Miranda-Saavedra D, Lehner PJ. *Biochem Soc Trans*. 2010; 38:1621–1626. [PubMed: 21118137]
114. Laroia G, Cuesta R, Brewer G, Schneider RJ. *Science*. 1999; 284:499–502. [PubMed: 10205060]
115. Laroia G, Sarkar B, Schneider RJ. *Proc Natl Acad Sci U S A*. 2002; 99:1842–1846. [PubMed: 11842200]
116. Chu PC, Chuang HC, Kulp SK, Chen CS. *J Biol Chem*. 2012; 287:43639–43650. [PubMed: 23115237]
117. Pfeiffer JR, Brooks SA. *J Immunol*. 2012; 188:1828–1839. [PubMed: 22262661]
118. Deleault KM, Skinner SJ, Brooks SA. *Mol Immunol*. 2008; 45:13–24. [PubMed: 17606294]
119. Cano F, Bye H, Duncan LM, Buchet-Poyau K, Billaud M, Wills MR, Lehner PJ. *EMBO J*. 2012; 31:3596–3606. [PubMed: 22863774]
120. Buchet-Poyau K, Courchet J, Le Hir H, Seraphin B, Scoazec JY, Duret L, Domon-Dell C, Freund JN, Billaud M. *Nucleic Acids Res*. 2007; 35:1289–1300. [PubMed: 17267406]
121. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, Yu D, Domasch H, Whittle B, Lambe T, Roberts IS, Copley RR, Bell JI, Cornall RJ, Goodnow CC. *Nature*. 2005; 435:452–458. [PubMed: 15917799]
122. Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG, Hutloff A, Giles KM, Leedman PJ, Lam KP, Goodnow CC, Vinuesa CG. *Nature*. 2007; 450:299–303. [PubMed: 18172933]
123. Athanasopoulos V, Barker A, Yu D, Tan AH, Srivastava M, Contreras N, Wang J, Lam KP, Brown SH, Goodnow CC, Dixon NE, Leedman PJ, Saint R, Vinuesa CG. *FEBS J*. 2010; 277:2109–2127. [PubMed: 20412057]
124. Loedige I, Filipowicz W. *Cell*. 2009; 136:818–820. [PubMed: 19269362]
125. Hammell CM, Lubin I, Boag PR, Blackwell TK, Ambros V. *Cell*. 2009; 136:926–938. [PubMed: 19269369]
126. Schwamborn JC, Berezikov E, Knoblich JA. *Cell*. 2009; 136:913–925. [PubMed: 19269368]
127. Neumuller RA, Betschinger J, Fischer A, Bushati N, Poernbacher I, Mechtler K, Cohen SM, Knoblich JA. *Nature*. 2008; 454:241–245. [PubMed: 18528333]
128. Loedige I, Gaidatzis D, Sack R, Meister G, Filipowicz W. *Nucleic Acids Res*. 2013; 41:518–532. [PubMed: 23125361]

129. Yang Y, Bedford MT. *Nat Rev Cancer*. 2013; 13:37–50. [PubMed: 23235912]
130. Bedford MT, Richard S. *Mol Cell*. 2005; 18:263–272. [PubMed: 15866169]
131. Wolf SS. *Cell Mol Life Sci*. 2009; 66:2109–2121. [PubMed: 19300908]
132. Liu Q, Dreyfuss G. *Mol Cell Biol*. 1995; 15:2800–2808. [PubMed: 7739561]
133. Ong SE, Mittler G, Mann M. *Nat Methods*. 2004; 1:119–126. [PubMed: 15782174]
134. Yu MC, Bachand F, McBride AE, Komili S, Casolari JM, Silver PA. *Genes Dev*. 2004; 18:2024–2035. [PubMed: 15314027]
135. Brahms H, Raymackers J, Union A, de Keyser F, Meheus L, Luhrmann R. *J Biol Chem*. 2000; 275:17122–17129. [PubMed: 10747894]
136. Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G. *Mol Cell*. 2001; 7:1111–1117. [PubMed: 11389857]
137. Brahms H, Meheus L, de Brabandere V, Fischer U, Luhrmann R. *RNA*. 2001; 7:1531–1542. [PubMed: 11720283]
138. Boisvert FM, Cote J, Boulanger MC, Cleroux P, Bachand F, Autexier C, Richard S. *J Cell Biol*. 2002; 159:957–969. [PubMed: 12486110]
139. Kirino Y, Kim N, de Planell-Saguer M, Khandros E, Chiorean S, Klein PS, Rigoutsos I, Jongens TA, Mourelatos Z. *Nat Cell Biol*. 2009; 11:652–658. [PubMed: 19377467]
140. Kirino Y, Vourekas A, Kim N, de Lima Alves F, Rappsilber J, Klein PS, Jongens TA, Mourelatos Z. *J Biol Chem*. 2010; 285:8148–8154. [PubMed: 20080973]
141. Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. *Cell*. 2001; 107:489–499. [PubMed: 11719189]
142. Schaeffer C, Bardoni B, Mandel JL, Ehresmann B, Ehresmann C, Moine H. *EMBO J*. 2001; 20:4803–4813. [PubMed: 11532944]
143. Lee J, Bedford MT. *EMBO Rep*. 2002; 3:268–273. [PubMed: 11850402]
144. Smith JJ, Rucknagel KP, Schierhorn A, Tang J, Nemeth A, Linder M, Herschman HR, Wahle E. *J Biol Chem*. 1999; 274:13229–13234. [PubMed: 10224081]
145. Fronz K, Otto S, Kolbel K, Kuhn U, Friedrich H, Schierhorn A, Beck-Sickinger AG, Ostareck-Lederer A, Wahle E. *J Biol Chem*. 2008; 283:20408–20420. [PubMed: 18495660]
146. Fronz K, Guttinger S, Burkert K, Kuhn U, Stohr N, Schierhorn A, Wahle E. *J Biol Chem*. 2011; 286:32986–32994. [PubMed: 21808065]
147. Brennan CM, Steitz JA. *Cell Mol Life Sci*. 2001; 58:266–277. [PubMed: 11289308]
148. Li H, Park S, Kilburn B, Jelinek MA, Henschen-Edman A, Aswad DW, Stallcup MR, Laird-Offringa IA. *J Biol Chem*. 2002; 277:44623–44630. [PubMed: 12237300]
149. Fujiwara T, Mori Y, Chu DL, Koyama Y, Miyata S, Tanaka H, Yachi K, Kubo T, Yoshikawa H, Tohyama M. *Mol Cell Biol*. 2006; 26:2273–2285. [PubMed: 16508003]
150. Staley JP, Woolford JL Jr. *Curr Opin Cell Biol*. 2009; 21:109–118. [PubMed: 19167202]
151. Bachand F, Silver PA. *EMBO J*. 2004; 23:2641–2650. [PubMed: 15175657]
152. Boulanger MC, Liang C, Russell RS, Lin R, Bedford MT, Wainberg MA, Richard S. *J Virol*. 2005; 79:124–131. [PubMed: 15596808]
153. Gary JD, Clarke S. *Prog Nucleic Acid Res Mol Biol*. 1998; 61:65–131. [PubMed: 9752719]
154. Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ, Kouzarides T. *Cell*. 2004; 118:545–553. [PubMed: 15339660]
155. Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y, Roeder RG, Clarke S, Stallcup MR, Allis CD, Coonrod SA. *Science*. 2004; 306:279–283. [PubMed: 15345777]
156. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA. *Cell*. 2004; 119:941–953. [PubMed: 15620353]
157. Chen C, Nott TJ, Jin J, Pawson T. *Nat Rev Mol Cell Biol*. 2011; 12:629–642. [PubMed: 21915143]
158. Arkov AL, Ramos A. *Trends Cell Biol*. 2010; 20:482–490. [PubMed: 20541937]

159. Aravin AA, van der Heijden GW, Castaneda J, Vagin VV, Hannon GJ, Bortvin A. *PLoS Genet.* 2009; 5:e1000764. [PubMed: 20011505]
160. Thomson T, Lasko P. *Cell Res.* 2005; 15:281–291. [PubMed: 15857583]
161. Dolzhanskaya N, Merz G, Aletta JM, Denman RB. *J Cell Sci.* 2006; 119:1933–1946. [PubMed: 16636078]
162. Klattenhoff C, Theurkauf W. *Development.* 2008; 135:3–9. [PubMed: 18032451]
163. Ghildiyal M, Zamore PD. *Nat Rev Genet.* 2009; 10:94–108. [PubMed: 19148191]
164. Kim VN, Han J, Siomi MC. *Nat Rev Mol Cell Biol.* 2009; 10:126–139. [PubMed: 19165215]
165. Siomi MC, Mannen T, Siomi H. *Genes Dev.* 2010; 24:636–646. [PubMed: 20360382]
166. Bamezai S, Rawat VP, Buske C. *Stem Cells.* 2012; 30:2603–2611. [PubMed: 22996918]
167. Juliano C, Wang J, Lin H. *Annu Rev Genet.* 2011; 45:447–469. [PubMed: 21942366]
168. Qiao D, Zeeman AM, Deng W, Looijenga LH, Lin H. *Oncogene.* 2002; 21:3988–3999. [PubMed: 12037681]
169. Sun G, Wang Y, Sun L, Luo H, Liu N, Fu Z, You Y. *Brain Res.* 2011; 1373:183–188. [PubMed: 21138738]
170. He W, Wang Z, Wang Q, Fan Q, Shou C, Wang J, Giercksky KE, Nesland JM, Suo Z. *BMC Cancer.* 2009; 9:426. [PubMed: 19995427]
171. Suzuki R, Honda S, Kirino Y. *Front Genet.* 2012; 3:204. [PubMed: 23087701]
172. Ohn T, Anderson P. *Wiley Interdiscip Rev RNA.* 2010; 1:486–493. [PubMed: 21956944]
173. Anderson P, Kedersha N. *Trends Biochem Sci.* 2008; 33:141–150. [PubMed: 18291657]

Highlights

- RNA-binding proteins (RBDs) undergo posttranslational modifications (PTMs).
- RBDs are regulated by phosphorylation, ubiquitination, and methylation.
- Signaling pathways involving PTMs regulate mRNA turnover by remodeling mRNPs.
- Understanding regulation by PTMs provides insight into the mechanism of mRNA decay.

Figure 1. Commonly found cis-elements in the 3' UTRs of mRNAs that influence the rate of mRNA decay are shown

The trans-acting factors that bind these elements are depicted. (A) The poly (A) tail is present in most eukaryotic mRNA transcripts and binds poly (A) binding protein, PABP. (B) AU-rich elements are present in a number of cytokine, chemokine, and proto-oncogene mRNAs such as c-Myc, c-Jun, and GM-CSF mRNA. Several ARE-BPs such as AUF1, TTP, KSRP are known to exist. (C) The 16 nt stem-loop in the 3' UTRs of histone mRNAs is a regulator of mRNA stability and binds SLBP (D) A stem-loop sequence specific for the iron response element binding protein (IRE-BP) is found in the transferrin receptor mRNA. (E) Several mRNAs are regulated by microRNAs that bind in the 3' UTR of the mRNA target and recruit the RISC complex.

Figure 2. Regulation of TTP mRNA targets by the p38 MAPK pathway

The p38 MAPK is activated by environmental stress and inflammatory cytokines. p38 MAPK activates MK2 which in turn phosphorylates the ARE-binding and mRNA-destabilizing protein tristetraprolin (TTP) at serines 52 and 178 (Mouse TTP numbering). Phosphorylation of TTP by MK2 reduces binding affinity of TTP for the TNF- α , GM-CSF, and IL-2 ARE sequence, and promotes its interaction with 14-3-3 proteins thereby altering its subcellular distribution and inhibiting its mRNA decay activity.

Figure 3. Regulation of the proinflammatory cytokine GM-CSF mRNA stability by the MAPK pathway

When eosinophils are activated by hyaluronic acid, it triggers ERK phosphorylation by the Ras/Raf/MEK pathway. Activated ERK phosphorylates the AU-rich binding protein AUF-1 at Ser⁸³-Pro⁸⁴, a binding site for the prolyl isomerase Pin1. Pin1 regulates cytokine decay by interacting with and modulating the mRNA-binding activity of AUF1.

Figure 4. Regulation of the myogenin mRNA stability by the PI3K-AKT signaling pathway and the ARE-BP KSRP

PI3K/AKT activation in myoblasts C2C12 regulates myomiR maturation via the ARE-BP KSRP. Phosphorylation of KSRP by AKT inhibits its ability to promote the decay of myogenin mRNA by dissociating KSRP from myogenin mRNA in the cytoplasm. The released KSRP binds 14-3-3, translocates to the nucleus where it promotes the maturation of myogenic miRNAs.

Figure 5. A schematic illustration of activation of the replication checkpoint in response to inhibition of DNA synthesis

When DNA replication stalls due to DNA damage or cessation of DNA synthesis, a checkpoint response is activated. Three members of the phosphoinositide 3-kinase (PI3 kinase)-related kinase (PIKK) family, namely ATR, DNA-PK, and SMG-1 have been implicated in histone mRNA decay. Pin1 is also activated in response to the replication checkpoint. Activation of these kinases and Pin1 triggers histone mRNA decay via the RNA binding protein SLBP and the helicase Upf1 by an unknown mechanism.

Figure 6. Steps in histone mRNA degradation in response to cell-cycle regulated phosphorylation events

A schematic of the various steps involved in bidirectional decay of histone mRNA and the factors involved is shown.

Figure 7. Upf1 phosphorylation and dephosphorylation cycles that regulate NMD

Upf1 is phosphorylated by the SMG-1 complex on several S/Q motifs in vivo. SMG-1 activity is regulated by SMG8 and SMG9. Phosphorylation of Upf1 also requires Upf2 and Upf3 and association with the exon junction complex (EJC), triggering NMD. Upf1 is dephosphorylated by PP2A associated with SMG5, SMG6, and SMG7.

Figure 8.
Domain organization of E3 Ubiquitin Ligases that have RNA binding domains.

Figure 9. Arginine methylation reactions regulate assembly of small RNA containing granules (A) The different types of arginine methylation reactions characterized by specific arginine methyltransferases (PRMTs) are shown. Types I, II and III PRMTs generate monomethylarginine (MMA) on one of the terminal (ω) guanidino nitrogen atoms. Type I enzymes can act on MMA substrates to yield asymmetric dimethylarginine (aDMA) whereas Type II enzymes generate the symmetric dimethylarginine product (sDMA). (B) Arginine and MMA can be converted to citrulline by Arginine Deiminases whereas all three modifications can be demethylated back to arginine by Amine Oxidases. (C) PIWI proteins are modified to generate dDMA products by the PRMT5/WDR77 complex. The sDMA is specifically recognized by Tudor domain containing proteins in nuage like granules in animal gonads.