Published in final edited form as:

Wiley Interdiscip Rev RNA. 2014 ; 5(5): 713–722. doi:10.1002/wrna.1241.

Emerging mechanisms of mRNP remodeling regulation

Chyi-Ying A. Chen and

Department of Biochemistry and Molecular Biology, The University of Texas, Medical School at Houston, Houston, Texas 77030, USA

Ann-Bin Shyu

Department of Biochemistry and Molecular Biology, The University of Texas, Medical School at Houston, Houston, Texas 77030, USA

Ann-Bin Shyu: Ann-Bin.Shyu@uth.tmc.edu

Abstract

The assembly and remodeling of the components of messenger ribonucleoprotein particles (mRNPs) are important in determining the fate of an mRNA. A combination of biochemical and cell biology research, recently complemented by genome-wide high-throughput approaches, has led to significant progress on understanding the formation, dynamics and function of mRNPs. These studies also advanced the challenging process of identifying the evolving constituents of individual mRNPs at various stages during an mRNA's lifetime. While research on mRNP remodeling in general has been gaining momentum, there has been relatively little attention paid to the regulatory aspect of mRNP remodeling. Here, we discuss the results of some new studies and potential mechanisms for regulation of mRNP remodeling.

Early biochemical studies in 1970's^{1–3} showed that mammalian nuclear pre-mRNAs are packaged into heterogeneous nuclear ribonucleoprotein particles (hnRNPs) by a group of approximately twenty conserved RNA-binding proteins (RNA-BP), collectively termed hnRNP proteins^{4,5}. The list of RNA-associated proteins has subsequently grown. Some of the proteins bind mRNAs in a sequence-specific manner, while others associate with RNAs non-specifically or are non-RNA-binding proteins (non-RNA-BPs) that associate with RNAs through binding to RNA-BPs. Recent results from transcriptome-scale mRNA-interactome capture experiments revealed that the pool of poly(A)⁺ mRNAs associate with ~800 RNA-BPs and a plethora of non-RNA-BPs at the steady-state^{6–8}. It has become increasingly clear that many different proteins are part of mRNP complexes during the lifetime of eukaryotic mRNAs, from their biogenesis in the nucleus and export through the nuclear pore complex to their metabolism in the cytoplasm^{9–11}.

One prominent feature of mRNP complexes is that they are not static. Instead, there are highly dynamic exchanges of mRNP protein constituents, which dictate their functions and fate at each step during their metabolism^{9–11}. These alterations in mRNP protein composition are collectively termed mRNP remodeling. Any failure to appropriately

assemble or disassemble an mRNP complex potentially disrupts downstream events that determine its fate (such as mRNA export, translation, localization, and decay) and function^{12–14}. Thus, mRNPs represent highly dynamic, functional units of mRNAs^{15, 16}, and precise regulation of mRNP remodeling process is vital to proper gene expression.

Results from recent studies point to emerging roles for mRNP remodeling in controlling the fate of mRNA, but understanding the regulation and physiological implications of mRNP remodeling remains in its infancy. In this review, we focus on some new studies to highlight potential mechanisms for regulation of mRNP remodeling. Readers are referred to some excellent recent reviews on general mRNP remodeling and its influence on mRNA fate^{9–11}. Also, complementing the topics discussed here are several interesting reviews on mRNP surveillance¹⁵ and nuclear export dynamics¹⁷.

PROTEIN PHOSPHORYLATION, INTRINSICALLY DISORDERED REGIONS, AND mRNP REMODELING

Reversible protein phosphorylation is a key regulatory mechanism for many signal transduction processes in eukaryotic cells^{18–20}. Most protein phosphorylation occurs at serine (Ser) or threonine (Thr) residues, altering the protein's function or its interaction with binding partners^{21, 22}. The reversible phosphorylation of certain key RNA decay factors illustrates how mRNP remodeling can be controlled in a signal-dependent manner to modulate the stability of a specific group of mRNAs. For instance, Upf1, a key effector of the nonsense-mediated decay (NMD) pathway, becomes phosphorylated during premature translation termination in aberrant mRNPs containing a nonsense codon^{23, 24}.

Phosphorylated Upf1 triggers a specific mRNP remodeling to allow binding of the endonuclease Smg6 and other proteins that recruit some decay factors (such as decapping complex and 5' to 3' exonuclease) to the mRNP. As a result, the aberrant mRNP undergoes rapid degradation. Another example is TTP, a potent RNA-destabilizing factor that binds AU-rich elements (AREs) in mRNAs to recruit deadenylase for rapid deadenylation and decay of the transcript. Phosphorylation of TTP during an inflammatory response prevents TTP from recruiting deadenylase, thus leading to transient stabilization of ARE-containing mRNAs¹⁶.

The mechanisms underlying the actions of Upf1 and TTP have been well covered previously^{16, 23}. Here, we will discuss another way of regulating mRNP remodeling during mRNA metabolism, namely through reversible Ser/Thr phosphorylation within intrinsically disordered segments of RNA-BPs.

Intricate interactions between Poly(A)-binding protein and its PAM2-containing partners

Cytoplasmic poly(A)-binding protein (PABP) C1 is a highly conserved and abundant RNA-BP that binds to the 3' poly(A) tails of mRNAs and recruits several interacting partners to regulate the mRNA fates^{25, 26}. In these critical cytoplasmic processes, PABPC1 serves as a binding scaffold for the other proteins. Among mammalian PABPC1-interacting proteins, at least sixteen contain a motif of ~12 amino acids which constitute the PABP-interacting Motif 2 (PAM2) that binds the MLLE domain near the C-terminus of PABPC1^{27, 28}.

Several PAM2-containing proteins are involved in translation and mRNA decay. These include GW182²⁹, translation termination factor 3 (eRF3)³⁰, PABP-dependent poly(A) nuclease subunit 3 (Pan3)³¹, and transducer of erbB-2 (Tob2) proteins³².

The number of PAM2-containing proteins and their varied roles in mRNA metabolism suggest several important questions. For example, how does the cell remodel the mRNP to alter interactions between PABPC1 and its binding partners to accommodate different biological processes? What molecular mechanisms control association and dissociation between PABPC1 and individual binding partners during mRNP remodeling? The observations that PAM2 motifs are located outside of globular or structured protein domains³³ and are generally within intrinsically disordered regions (IDRs)³⁴ hint at possible answers to these questions.

Phosphorylation in intrinsically disordered regions of PAM2-containing proteins

IDRs can retain unstructured segments under physiological conditions, even when the IDR is flanked by one or more structured domains^{35–37}. One prominent feature of IDRs is that they are rich in polar, uncharged amino acids, such as serine (Ser) and threonine (Thr)³⁸. As the amino acid composition, sequence complexity, hydrophobicity, and charge adjacent to many phosphorylation sites resemble those of IDRs^{38, 39}, IDRs are predicted to be “hotspots” for protein phosphorylation. Recently, an *in silico* analysis revealed that PAM2 motifs are generally located near cluster(s) of potential Ser- or Thr- phosphorylation sites within an IDR³⁴, further suggesting that reversible phosphorylation of Ser/Thr clusters near the PAM2 motifs modulates interactions between PABPC1 and its PAM2-containing partners³⁴. Several lines of evidence support this notion. For example, introducing phospho-resistant mutations in the IDRs near PAM2 motifs in PAM2-containing proteins such as Pan3, Tob2, and GW182 enhances interactions between these proteins and PABPC1; conversely, introduction of phospho-mimetic mutations near the PAM2 motifs decreases interactions with PABPC1. Importantly, phospho-mimetic mutations that compromise the interaction of the three PAM2-containing proteins with PABPC1 also result in appreciable loss of the proteins' functions in mRNA turnover and gene silencing³⁴.

The interaction between PABPC1 and a PAM2 motif-containing partner may involve a two-step mechanism³⁴. In this mechanism, the PAM2 motif first contacts the MLLE domain in PABPC1, which induces folding in the IDRs that promote secondary contacts with the MLLE domain and thus strengthen the interaction. In this concept, reversible phosphorylation in IDRs near PAM2 motifs modulates the interactions with PABPC1 (Fig. 1). It will be interesting to see whether this mechanism represents a general way to regulate the functions of PAM2-containing proteins in eukaryotes. In this regard, it is worth noting that another recent study of the mRNA interactome found that most proteins associated with mRNAs are highly enriched in IDRs⁷. It will be interesting to learn how many of them are phospho-proteins. The dynamic nature of IDRs combines structural flexibility with a high functional density and multiple interaction interfaces^{38, 40, 41}, so the two-step mechanism described above may represent a new paradigm in which reversible phosphorylation of IDRs in RNA-associated proteins (or mRNP protein constituents) has a key role in the dynamic and signal-dependent control of mRNP remodeling.

UBIQUITIN MAKES ITS MARK ON mRNP REMODELING

Ubiquitin is a polypeptide that is conjugated to many proteins to regulate a wide range of different cellular processes^{42, 43}. Generally, ubiquitin is conjugated to lysine residues on substrate proteins in a cascade of enzymatic reactions catalyzed by E1, E2, and E3 ligases. Downstream factors then recognize particular ubiquitin modifications and trigger subsequent molecular events. Ubiquitin modification can involve a single ubiquitin (monoubiquitin), two ubiquitins (diubiquitin), or a polyubiquitin chain with distinct lysine linkages; each type of modification has distinct biological roles. In addition to their well-known role in targeting proteins for proteasomal degradation, ubiquitin modifications have many roles in regulating non-degradative cellular processes^{44–46}. Here, we discuss two recent investigations of how non-degradative ubiquitin modifications modulate mRNP remodeling.

Ubiquitination of HuR and regulation of ARE-mediated mRNA decay

Although several mRNP remodeling steps regulated by non-degradative ubiquitination have been well described⁴⁷, little is known about the molecular events leading to ejection of ubiquitin-conjugated proteins from mRNPs. A recent study focusing on remodeling of mRNPs containing mRNA with AREs bound by HuR, a ubiquitous RNA-BP, provides new insights into the underlying mechanism of mRNP regulation by ubiquitination⁴⁸.

HuR and the stability of ARE-containing mRNAs—AREs are a class of loosely defined RNA-regulatory elements that represent the most common destabilizing element in eukaryotic mRNAs^{49, 50}. A varying array of ARE-binding proteins (ARE-BPs) associate with ARE-containing transcripts during cellular stress responses, leading to alterations in the stability of the bound transcripts^{51, 52}. Exchanges of a destabilizing ARE-BP (such as TTP or KSRP) in favor of a stabilizing ARE-BP (predominantly HuR) are known to account for stabilization of many ARE-containing mammalian mRNAs in response to environmental or physiological stimuli^{53–55}.

HuR probably represents the best studied ARE-BP that recognizes AREs present in the 3' UTRs of transcripts coding for cytokines, proto-oncogenes, and transcription factors^{49, 50, 56}. The affinity of HuR for ARE targets appears to be one of the highest among ARE-BPs^{57–59}. Thus, HuR may simply outcompete destabilizing ARE-BPs at AREs on target mRNAs, thereby leading to stabilization of otherwise unstable messages⁶⁰. An intriguing and important question then arises as to how HuR may be displaced from an mRNP by a destabilizing ARE-BP when a subsequent change in cellular conditions calls for destabilization of the transcript involved.

HuR and the p97- protein-complex remodeling machine—It has recently been shown that non-degradative, ubiquitin signaling-dependent disassembly of HuR-containing mRNPs can be promoted by the p97-cofactor complex, a protein-complex remodeling machine⁴⁸. p97, also known as valosin-containing protein (VCP), is a member of the AAA (ATPases associated with diverse cellular activities) family⁶¹. In its segregase activity^{62, 63}, p97 recognizes a ubiquitinated substrate through one of the p97 cofactors and then uses energy from ATP hydrolysis to “extract” the ubiquitinated protein from the protein

complex⁶¹. An important feature of this segregase action is that the ejected protein can be deubiquitinated and recycled for subsequent use.

During the remodeling of mRNPs containing mRNA with AREs bound by HuR (Fig. 2), HuR was found to be di-ubiquitinated at K313 and K326 in its third RNA-binding domain (RRM3). This creates a strong binding site for UBXD8, a p97 cofactor that helps recruit p97 to the target substrate. The ubiquitin lysine residue involved is K29, which does not constitute a proteolytic signal, in clear distinction with the effect of ubiquitination using the K48 residue of ubiquitin⁶⁴. The p97-UBXD8 complex utilizes energy from ATP hydrolysis to release HuR from the target ARE-containing mRNA^{48, 65}.

Regulation of the interaction between p97-UBXD8 complex and RNA-binding proteins

—It remains unclear how the above-mentioned remodeling activity is regulated, but the process appears to occur during the resolution of a cellular stress response. Many stressors are known to induce HuR phosphorylation^{54, 60, 66, 67}. Depending on the phosphorylation sites used, HuR either relocates from the nucleus to the cytoplasm or exhibits stronger binding affinity for AREs^{54, 60, 66, 67}. As a result, HuR's target mRNAs become stabilized in the cytoplasm during a stress response. One possibility is that stress-related phosphorylation of HuR prevents it from being ubiquitinated for subsequent remodeling by p97-UBXD8. Indeed, two kinds of stress, UV-irradiation and heat-shock, are found to diminish ubiquitination of HuR⁴⁸. This implies a lower chance that HuR is recognized and released from the mRNP by p97-UBXD8, thus stabilizing HuR-ARE-containing mRNPs.

The p97-UBXD8 complex also interacts with several other RNA-BPs besides HuR⁴⁸; thus, it will be interesting to find out whether compromising the mRNP remodeling function of p97/UBXD8 has more general effects on mRNA metabolism. A recent report showing that clearance of mammalian stress granules containing aberrant mRNPs is diminished when p97 function is compromised¹⁴ lent support for this notion. Moreover, as p97 can associate with an array of distinct cofactors, one could imagine that different p97-cofactor complexes might interact with additional RNA-BPs and thus participate in many different mRNP remodeling steps. The non-degradative effects of protein ubiquitination involving p97 segregase may emerge as an exciting and novel regulatory mechanism of mRNP remodeling.

mRNP remodeling and mRNA export

Following synthesis in the nucleus, an eukaryotic mRNA becomes associated with various proteins that aid the pre-mRNA processing steps during maturation^{10, 68}. While some of the proteins eventually accompany the mRNA into the cytoplasm, others are removed from the mRNA and recycled for re-use in the nucleus. Thus, mRNP remodeling has a critical role in the highly intricate and dynamic upstream events in mRNA biogenesis, such as transcription, splicing, and/or 3' end processing⁶⁹. In other words, export of mature mRNAs from the nucleus to the cytoplasm entails major and dramatic mRNP remodeling. Here, we discuss an example in which ubiquitination of Yra1 by the E3 ligase Tom1 mediates a key mRNP remodeling step during mRNA export in budding yeast.

Ubiquitination of Yra1 and mRNP remodeling

From yeasts to humans, properly processed and mature mRNPs are known to associate with Mex67/TAP^{47, 69}. In budding yeast, Mex67 plays a major role in mRNA export through direct interaction with nucleoporins lining the nuclear pore. It does this by bridging the nuclear pore complex (NPC) with mature mRNPs ready to leave the nucleus^{47, 69}.

Mex67 also directly interacts with Nab2, a nuclear poly(A) tail-binding protein essential for mRNA export⁷⁰. However, Mex67 binds mRNAs weakly and requires an adaptor protein to stabilize its association with mRNPs⁴⁷. The best characterized adaptor protein for Mex67 in yeast is Yra1 (Aly/REF is the homolog in higher eukaryotes)⁷¹. In the nucleus, Yra1 forms a trimeric complex through interactions with both Mex67 and Nab2, enhancing the interaction between Mex67 and Nab2 as well as their association with mRNPs (Fig. 3, step 1)⁷². Consequently, this trimeric complex helps define an mRNP as being matured properly and thus ready for export. While Nab2 and Mex67 go through the NPC with the mRNP, Yra1 remains in the nucleus^{71, 73}. The remodeling of the mRNP to dissociate Yra1 from the trimeric complex is accomplished through mono- or di-ubiquitination of Yra1 by Tom1 and another E3 ligase (Fig. 3, step 2). It appears that Tom1-mediated ubiquitination largely accounts for dissociation of Yra1 from the Mex67-Nab2-mRNP⁷².

Several important questions about the Mex67-Nab2-Yra1 processes remain to be answered, particularly concerning regulatory aspects. For instance, what signals the ubiquitination of Yra1 by Tom1 E3 ligase? Why does ubiquitin conjugation to Yra1 elicit its dissociation from the Mex67-Nab2-mRNP complex? Does the dissociation involve additional factors? Interestingly, at least two additional factors that are required for mRNA export, Hpr1 and Npl3, are subject to ubiquitination by a different E3 ligase, Rsp5, to facilitate mRNA export^{74, 75}. It is plausible that ubiquitination regulates mRNA export by controlling the dynamics of mRNP packaging and remodeling at multiple points along the export pathway. As both Nab2 and Tom1 are conserved in higher eukaryotes⁷⁶, such a mechanism of ubiquitin-mediated mRNP remodeling may be evolutionarily conserved.

RNA HELICASE-MEDIATED ATP-DEPENDENT mRNP REMODELING

Remodeling of mRNP involving RNA helicases of the DEAD-box family is arguably the most commonly found and studied mechanism⁷⁷. Different RNA helicases play important yet distinct roles in almost every step of RNA metabolism⁷⁷. For example, the RNA helicase Upf1 can disassemble the NMD complex, a remodeling that is required to complete the NMD process and recycle the factors involved⁷⁸. RNA helicases are ATPases, and the use of energy from ATP hydrolysis to unwind RNA duplexes is a general feature of remodeling RNP complexes^{77, 79}. One exception to this general rule is the case of yeast Dbp5, which utilizes a different strategy, as discussed below.

The curious case of ATP-dependent mRNP remodeling mediated by Dbp5

Dbp5 is a DEAD-box RNA-dependent ATPase in budding yeast that plays a key role in mRNP remodeling just before a mature mRNP leaves the nucleus through the NPC^{80, 81}. Dbp5 is required to remove at least two key proteins from an export-competent mRNP, i.e., Mex67 and Nab2^{47, 82}. As discussed above, Mex67 and its other binding partners facilitate

mRNP export by triggering specific mRNP remodeling at the NPC (Fig. 3, step 2)^{47, 69}. Recent evidence suggests that Mex67 is first recruited to RNA pol II co-transcriptionally and probably becomes associated with pre-mRNA upon 3' end formation^{83, 84}. On the other hand, Nab2, which is a yeast nuclear poly(A)-binding protein involved in poly(A) tail length control and mRNA export, later joins Mex67-containing mRNPs to constitute an export competent mRNP^{47, 69}.

Although the sub-cellular compartment where Dbp5 becomes associated with mRNPs containing both Mex67 and Nab2 has not been identified, it may be the nucleus (Fig. 3)^{85, 86}. Once the mature mRNP exits the NPC aided by Mex67, Dbp5 interacts in the cytoplasm with Gle1 loaded with IP₆. This interaction stimulates Dbp5 to bind ATP (Fig. 3, step 3). Subsequently, hydrolysis of ATP by Dbp5 and release of Pi from Dbp5-ADP lead to a conformational change in Dbp5 and a significant decrease in its affinity for RNA^{87, 88}. This conformational change in Dbp5 not only releases Dbp5-ADP from the mRNP but also triggers loss of both Mex67 and Nab2 from the mRNP in a yet-to-be determined manner (Fig. 3, steps 4 and 5). Released Mex67 and Nab2 return to the nucleus, while the mRNP is recognized by ribosomes for translation. The rapid depletion of these export factors from the cytoplasm ensures that transport of the mRNP is directional. Thus, Dbp5 plays a vital role in remodeling nuclear mRNP at the NPC so that only properly matured mRNPs proceed through the NPC to the cytoplasm. One distinct feature of Dbp5-mediated mRNP remodeling is that it involves a conformational change in Dbp5 upon ATP hydrolysis and Pi release. This is in contrast with several known DEAD-box proteins that use the energy from ATP hydrolysis to trigger a conformational change (unwinding) in the RNA duplexes during mRNP remodeling, rather than in the proteins themselves^{77, 79}.

It is unclear whether the Dbp5's mode of action also occurs in other members of the DEAD-box RNA-dependent ATPase family. Another key aspect of Dbp5 function that begs for further study concerns its regulation. Dbp5 needs to physically interact with two other proteins, Gle1 and Nup159, for recycling its ATPase activity during mRNP remodeling^{87, 88}. Are these protein-protein interactions modulated as a way to control mRNP export? If yes, what are the signals and post-translational modifications involved? As the mRNP remodeling function of Dbp5 is not mRNA-specific, one can imagine that mechanisms modulating Dbp5 activity and thus nuclear mRNP export would have a significant impact on the transcriptome profile of cytoplasmic mRNA.

Conclusion

The molecular constituents comprising individual mRNPs and their dynamic changes during the lifetime of mRNAs are emerging as exciting yet underexplored areas of research in RNA biology. With over 800 putative human RNA-BPs and the myriad proteins that the RNA-BPs interact with, identifying the makeup of individual mRNPs and defining specific roles for individual RNA-BPs is a challenging but important task. The post-translational modification mechanisms discussed here illustrate how mRNP remodeling and its dynamics may be modulated to alter the fate of mRNA in a transcript-specific or transcriptome-wide manner. At this point, few signaling pathways associated with mRNP remodeling have been identified. To address this open issue, one might employ a comprehensive approach of

RNA-affinity chromatography and quantitative proteomics to characterize modifications of the RNA-binding proteome. This could identify many RNA-BPs that are modified by ubiquitin or phosphorylation in response to proteasome or phosphatase inhibition.

The physiological importance of RNA-BPs and RNP complexes is underscored by their involvement in many human diseases. One recent example is the malfunction of hnRNPA1 that results in mRNP aggregation and is linked to neuronal degenerative diseases⁸⁹. Further, mutations in p97 have been genetically linked with inclusion body myopathy, Paget's disease, frontotemporal dementia, and amyotrophic lateral sclerosis^{90–92}. Nevertheless, the molecular pathogenesis of these diseases are not well understood. It is tempting to propose that some of these severe neurodegenerative diseases associated with p97 mutations involve a defect in mRNP remodeling that result in mRNP aggregation and aberrant mRNA translation. A recent study showing that p97 is critical for mammalian cells to perform autophagic clearance of stress granule-related and pathogenic RNP granules that arise in p97-linked degenerative diseases¹⁴ sheds new light on this important issue. Studies of the regulation of mRNP remodeling could provide a basis for development of approaches to manipulate a subset of mRNPs under particular physiological or pathological conditions, an extension of the "RNP infrastructure" concept put forward over a decade ago⁹³. In the near future, we should have a more comprehensive grasp of the constituents of individual mRNPs, of mRNP dynamics, and of the underlying regulatory mechanisms.

References

1. Beyer A, Christensen M, Walker B, LeSturgeon W. Identification and characterization of the packaging proteins of core 40S hnRNP particles. *Cell*. 1977; 11:127–138. [PubMed: 872217]
2. Kumar A, Pederson T. Comparison of proteins bound to heterogeneous nuclear RNA and messenger RNA in HeLa cells. *J Mol Biol*. 1975; 96:353–365. [PubMed: 1165583]
3. Stevenin J, Gallinaro-Matringe H, Gattoni R, Jacob M. Complexity of the structure of particles containing heterogeneous nuclear RNA as demonstrated by ribonuclease treatment. *Eur J Biochem*. 1977; 74:589–602. [PubMed: 856587]
4. Dreyfuss G. Structure and Function of Nuclear and Cytoplasmic Ribonucleoprotein Particles. *Annual Review of Cell Biology*. 1986; 2:459–498.
5. Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG. hnRNP proteins and the biogenesis of mRNA. *Annu Rev Biochem*. 1993; 62:289–321. [PubMed: 8352591]
6. Baltz Alexander G, Munschauer M, Schwanhäusser B, Vasile A, Murakawa Y, Schueler M, Youngs N, Penfold-Brown D, Drew K, Milek M, et al. The mRNA-Bound Proteome and Its Global Occupancy Profile on Protein-Coding Transcripts. *Molecular Cell*. 2012; 46:674–690. [PubMed: 22681889]
7. Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, Strein C, Davey NE, Humphreys DT, Preiss T, Steinmetz LM, et al. Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins. *Cell*. 2012; 149:1393–1406. [PubMed: 22658674]
8. Mitchell SF, Jain S, She M, Parker R. Global analysis of yeast mRNPs. *Nat Struct Mol Biol*. 2013; 20:127–133. [PubMed: 23222640]
9. Muller-McNicoll M, Neugebauer KM. How cells get the message: dynamic assembly and function of mRNA-protein complexes. *Nat Rev Genet*. 2013; 14:275–287. [PubMed: 23478349]
10. Moore MJ. From birth to death: the complex lives of eukaryotic mRNAs. *Science*. 2005; 309:1514–1518. [PubMed: 16141059]
11. Keene JD, Tenenbaum SA. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol cell*. 2002; 9:1161–1167. [PubMed: 12086614]

12. Hieronymus H, Silver PA. A systems view of mRNP biology. *Genes & Development*. 2004; 18:2845–2860. [PubMed: 15574591]
13. Wilusz CJ, Wilusz J. Consequences of mRNA Wardrobe Malfunctions. *Cell*. 2010; 143:863–865. [PubMed: 21145451]
14. Buchan JR, Kolaitis R-M, Taylor JP, Parker R. Eukaryotic Stress Granules Are Cleared by Autophagy and Cdc48/VCP Function. *Cell*. 2013; 153:1461–1474. [PubMed: 23791177]
15. Tutucci E, Stutz F. Keeping mRNPs in check during assembly and nuclear export. *Nat Rev Mol Cell Biol*. 2011; 12:377–384. [PubMed: 21602906]
16. Lee SR, Lykke-Andersen J. Emerging roles for ribonucleoprotein modification and remodeling in controlling RNA fate. *Trends in Cell Biology*. 2013; 23:504–510. [PubMed: 23756094]
17. Grünwald D, Singer RH. Multiscale dynamics in nucleocytoplasmic transport. *Current Opinion in Cell Biology*. 2012; 24:100–106. [PubMed: 22196930]
18. Hunter T. The Age of Crosstalk: Phosphorylation, Ubiquitination, and Beyond. *Molecular Cell*. 2007; 28:730–738. [PubMed: 18082598]
19. Temporini C, Calleri E, Massolini G, Caccialanza G. Integrated analytical strategies for the study of phosphorylation and glycosylation in proteins. *Mass Spectrometry Reviews*. 2008; 27:207–236. [PubMed: 18335498]
20. Gnad F, Gunawardena J, Mann M. PHOSIDA 2011: the posttranslational modification database. *Nucleic Acids Research*. 2011; 39:D253–D260. [PubMed: 21081558]
21. Sugiyama N, Nakagami H, Mochida K, Daudi A, Tomita M, Shirasu K, Ishihama Y. Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in Arabidopsis. *Molecular Systems Biology*. 2008;4.
22. Reiland S, Messerli G, Baerenfaller K, Gerrits B, Endler A, Grossmann J, Gruissem W, Baginsky S. Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks. *Plant Physiol*. 2009; 150:889–903. [PubMed: 19376835]
23. Kervestin S, Jacobson A. NMD: a multifaceted response to premature translational termination. *Nat Rev Mol Cell Biol*. 2012; 13:700–712. [PubMed: 23072888]
24. Durand S, Lykke-Andersen J. SnapShot: Nonsense-Mediated mRNA Decay. *Cell*. 2011; 145:324–324. e322. [PubMed: 21496649]
25. Kuhn U, Wahle E. Structure and function of poly(A) binding proteins. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*. 2004; 1678:67–84.
26. Mangus DA, Evans MC, Jacobson A. Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol*. 2003; 4:233. <http://genomebiology.com/2003/2004/2007/2223>. [PubMed: 14611650]
27. Tritschler F, Huntzinger E, Izaurralde E. Role of GW182 proteins and PABPC1 in the miRNA pathway: a sense of déjà vu. *Nat Rev Mol Cell Biol*. 2010; 11:379–384. [PubMed: 20379206]
28. Albrecht M, Lengauer T. Survey on the PABC recognition motif PAM2. *Biochemical and Biophysical Research Communications*. 2004; 316:129–138. [PubMed: 15003521]
29. Eystathiou T, Jakymiw A, Chan EKL, Seraphin B, Cougot N, Fritzler MJ. The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. *RNA*. 2003; 9:1171–1173. [PubMed: 13130130]
30. Hoshino S, Imai M, Kobayashi T, Uchida N, Katada T. The eukaryotic polypeptide chain releasing factor (eRF3/GSPT) carrying the translation termination signal to the 3'-Poly(A) tail of mRNA. Direct association of erf3/GSPT with polyadenylate-binding protein. *J Biol Chem*. 1999; 274:16677–16680. [PubMed: 10358005]
31. Uchida N, Hoshino S-i, Katada T. Identification of a Human Cytoplasmic Poly(A) Nuclease Complex Stimulated by Poly(A)-binding Protein. *J Biol Chem*. 2004; 279:1383–1391. [PubMed: 14583602]
32. Ikematsu N, Yoshida Y, Kawamura-Tsuzuku J, Ohsugi M, Onda M, Hirai M, Fujimoto JTY. Tob2, a novel anti-proliferative Tob/BTG1 family member, associates with a component of the CCR4 transcriptional regulatory complex capable of binding cyclin-dependent kinases. *Oncogene*. 1999; 18:7432–7441. [PubMed: 10602502]
33. Albrecht M, Lengauer T. Survey on the PABC recognition motif PAM2. *biochem Biophys Res Commun*. 2004; 316:129–138. [PubMed: 15003521]

34. Huang K-L, Chadee AB, Chen C-YA, Zhang Y, Shyu A-B. Phosphorylation at intrinsically disordered regions of PAM2 motif-containing proteins modulates their interactions with PABPC1 and influences mRNA fate. *RNA*. 2013; 19:295–305. [PubMed: 23340509]
35. Brown CJ, Johnson AK, Dunker AK, Daughdrill GW. Evolution and disorder. *Current Opinion in Structural Biology*. 2011; 21:441–446. [PubMed: 21482101]
36. Radivojac P, Iakoucheva LM, Oldfield CJ, Obradovic Z, Uversky VN, Dunker AK. Intrinsic Disorder and Functional Proteomics. *Biophysical Journal*. 2007; 92:1439–1456. [PubMed: 17158572]
37. Uversky VN. Intrinsically disordered proteins from A to Z. *The International Journal of Biochemistry & Cell Biology*. 2011; 43:1090–1103. [PubMed: 21501695]
38. Iakoucheva LM, Radivojac P, Brown CJ, O'Connor TR, Sikes JG, Obradovic Z, Dunker AK. The importance of intrinsic disorder for protein phosphorylation. *Nucleic Acids Research*. 2004; 32:1037–1049. [PubMed: 14960716]
39. Johnson LN, Lewis RJ. Structural Basis for Control by Phosphorylation. *Chemical Reviews*. 2001; 101:2209–2242. [PubMed: 11749371]
40. Tompa P, Csermely P. The role of structural disorder in the function of RNA and protein chaperones. *The FASEB Journal*. 2004; 18:1169–1175.
41. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol*. 2005; 6:197–208. [PubMed: 15738986]
42. Pickart CM, Cohen RE. Proteasomes and their kin: proteases in the machine age. *Nat Rev Mol Cell Biol*. 2004; 5:177–187. [PubMed: 14990998]
43. Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2004; 1695:55–72.
44. O'Neill LAJ. Regulation of Signaling by Non-degradative Ubiquitination. *Journal of Biological Chemistry*. 2009; 284:8209. [PubMed: 19008216]
45. Clark K, Nanda S, Cohen P. Molecular control of the NEMO family of ubiquitin-binding proteins. *Nat Rev Mol Cell Biol*. 2013; 14:673–685. [PubMed: 23989959]
46. Sadowski M, Suryadinata R, Tan AR, Roesley SNA, Sarcevic B. Protein monoubiquitination and polyubiquitination generate structural diversity to control distinct biological processes. *IUBMB Life*. 2012; 64:136–142. [PubMed: 22131221]
47. Iglesias N, Stutz F. Regulation of mRNP dynamics along the export pathway. *FEBS Letters*. 2008; 582:1987–1996. [PubMed: 18394429]
48. Zhou H-L, Geng C, Luo G, Lou H. The p97-UBXD8 complex destabilizes mRNA by promoting release of ubiquitinated HuR from mRNP. *Genes & Development*. 2013; 27:1046–1058. [PubMed: 23618873]
49. Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: are there unifying principles? *Nucl Acids Res*. 2006; 33:7138–7150. [PubMed: 16391004]
50. Chen AC-Y, Shyu A-B. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem Sci*. 1995; 20:465–470. [PubMed: 8578590]
51. Clement SL, Scheckel C, Stoecklin G, Lykke-Andersen J. Phosphorylation of Tristetraprolin by MK2 Impairs AU-Rich Element mRNA Decay by Preventing Deadenylase Recruitment. *Molecular and Cellular Biology*. 2011; 31:256–266. [PubMed: 21078877]
52. Stoecklin G, Anderson P. Posttranscriptional mechanisms regulating the inflammatory response. *Adv Immunol*. 2006; 89:1–37. [PubMed: 16682271]
53. Brennan CM, Gallouzi IE, Steitz JA. Protein ligands to HuR modulate its interaction with target mRNAs in vivo. *Journal of Cell Biology*. 2000; 151:1–14. [PubMed: 11018049]
54. Hinman M, Lou H. Diverse molecular functions of Hu proteins. *Cellular and Molecular Life Sciences*. 2008; 65:3168–3181. [PubMed: 18581050]
55. Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL, Gorospe M. Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J*. 2004; 23:3092–3102. [PubMed: 15257295]

56. von Roretz C, Marco SD, Mazroui R, Gallouzi I-E. Turnover of AU-rich-containing mRNAs during stress: a matter of survival. *Wiley Interdisciplinary Reviews: RNA*. 2011; 2:336–347. [PubMed: 21957021]
57. Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL, Gorospe M. Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J*. 2004; 23:3092–3102. [PubMed: 15257295]
58. Ma WJ, Cheng S, Campbell C, Wright A, Furneaux H. Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. *J Biol Chem*. 1996; 271:8144–8151. [PubMed: 8626503]
59. Chen C-YA, Xu N, Shyu A-B. Highly Selective Actions of HuR in Antagonizing AU-Rich Element-Mediated mRNA Destabilization. *Mol Cell Biol*. 2002; 22:7268–7278. [PubMed: 12242302]
60. Srikantan S, Gorospe M. HuR function in disease. *Front Biosci*. 2012; 17:189–205.
61. Meyer H, Bug M, Bremer S. Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat Cell Biol*. 2012; 14:117–123. [PubMed: 22298039]
62. Ramadan K, Bruderer R, Spiga FM, Popp O, Baur T, Gotta M, Meyer HH. Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. *Nature*. 2007; 450:1258–1262. [PubMed: 18097415]
63. Acs K, Luijsterburg MS, Ackermann L, Salomons FA, Hoppe T, Dantuma NP. The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat Struct Mol Biol*. 2011; 18:1345–1350. [PubMed: 22120668]
64. Pickart CM. Targeting of substrates to the 26S proteasome. *The FASEB Journal*. 1997; 11:1055–1066.
65. Chen C-YA, Shyu A-B. Protein segregase meddles in remodeling of mRNA–protein complexes. *Genes & Development*. 2013; 27:980–984. [PubMed: 23651853]
66. Gallouzi IE, Brennan CM, Stenberg MG, Swanson MS, Eversole A, Maizels N, Steitz JA. HuR binding to cytoplasmic mRNA is perturbed by heat shock. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:3073–3078. [PubMed: 10737787]
67. Wang W, Furneaux H, Cheng H, Caldwell MC, Hutter D, Liu Y, Holbrook N, Gorospe M. HuR Regulates p21 mRNA Stabilization by UV Light. *Molecular and Cellular Biology*. 2000; 20:760–769. [PubMed: 10629032]
68. Keene JD. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet*. 2007; 8.
69. Natalizio BJ, Wenthe SR. Postage for the messenger: designating routes for nuclear mRNA export. *Trends in Cell Biology*. 2013; 23:365–373. [PubMed: 23583578]
70. Batisse J, Batisse C, Budd A, Böttcher B, Hurt E. Purification of Nuclear Poly(A)-binding Protein Nab2 Reveals Association with the Yeast Transcriptome and a Messenger Ribonucleoprotein Core Structure. *Journal of Biological Chemistry*. 2009; 284:34911–34917. [PubMed: 19840948]
71. Strasser K, Hurt E. Yra1p, a conserved nuclear RNA-binding protein, interacts directly with Mex67p and is required for mRNA export. *EMBO J*. 2000; 19:410–420. [PubMed: 10722314]
72. Iglesias N, Tutucci E, Gwizdek C, Vinciguerra P, Von Dach E, Corbett AH, Dargemont C, Stutz F. Ubiquitin-mediated mRNP dynamics and surveillance prior to budding yeast mRNA export. *Genes & Development*. 2010; 24:1927–1938. [PubMed: 20810649]
73. Lund MK, Guthrie C. The DEAD-Box Protein Dbp5p Is Required to Dissociate Mex67p from Exported mRNPs at the Nuclear Rim. *Molecular cell*. 2005; 20:645–651. [PubMed: 16307927]
74. Gwizdek C, Hobeika M, Kus B, Ossareh-Nazari B, Dargemont C, Rodriguez MS. The mRNA Nuclear Export Factor Hpr1 Is Regulated by Rsp5-mediated Ubiquitylation. *Journal of Biological Chemistry*. 2005; 280:13401–13405. [PubMed: 15713680]
75. Gupta R, Kus B, Fladd C, Wasmuth J, Tonikian R, Sidhu S, Krogan NJ, Parkinson J, Rotin D. Ubiquitination screen using protein microarrays for comprehensive identification of Rsp5 substrates in yeast. *Molecular Systems Biology*. 2007; 3.
76. Hall J, Kow E, Nevis K, Lu C, Luce K, Zhong Q, Cook J. Cdc6 stability is regulated by the Huw1 ubiquitin ligase after DNA damage. *Mol Biol Cell*. 2007; 18.
77. Linder P, Jankowsky E. From unwinding to clamping — the DEAD box RNA helicase family. *Nat Rev Mol Cell Biol*. 2011; 12:505–516. [PubMed: 21779027]

78. Franks TM, Singh G, Lykke-Andersen J. Upf1 ATPase-Dependent mRNP Disassembly Is Required for Completion of Nonsense-Mediated mRNA Decay. *Cell*. 2010; 143:938–950. [PubMed: 21145460]
79. Pyle AM. RNA helicases and remodeling proteins. *Curr Opin Chem Biol*. 2011; 15:636–642. [PubMed: 21862383]
80. Alcazar-Roman AR, Tran EJ, Guo S, Wentz SR. Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nat Cell Biol*. 2006; 8:711–716. [PubMed: 16783363]
81. Weirich CS, Erzberger JP, Flick JS, Berger JM, Thorner J, Weis K. Activation of the DEXD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator InsP6 is required for mRNA export. *Nat Cell Biol*. 2006; 8:668–676. [PubMed: 16783364]
82. Kohler A, Hurt E. Exporting RNA from the nucleus to the cytoplasm. *Nat Rev Mol Cell Biol*. 2007; 8:761–773. [PubMed: 17786152]
83. Gwizdek C, Iglesias N, Rodriguez MS, Ossareh-Nazari B, Hobeika M, Divita G, Stutz F, Dargemont C. Ubiquitin-associated domain of Mex67 synchronizes recruitment of the mRNA export machinery with transcription. *Proceedings of the National Academy of Sciences*. 2006; 103:16376–16381.
84. Diepkins G, Iglesias N, Stutz F. Cotranscriptional Recruitment to the mRNA Export Receptor Mex67p Contributes to Nuclear Pore Anchoring of Activated Genes. *Molecular and Cellular Biology*. 2006; 26:7858–7870. [PubMed: 16954382]
85. Zhao J, Jin S-B, Bjorkroth B, Wieslander L, Daneholt B. The mRNA export factor Dbp5 is associated with Balbiani ring mRNP from gene to cytoplasm. *EMBO J*. 2002; 21:1177–1187. [PubMed: 11867546]
86. Estruch F, Cole CN. An Early Function during Transcription for the Yeast mRNA Export Factor Dbp5p/Rat8p Suggested by Its Genetic and Physical Interactions with Transcription Factor IIH Components. *Molecular Biology of the Cell*. 2003; 14:1664–1676. [PubMed: 12686617]
87. Noble KN, Tran EJ, Alcázar-Román AR, Hodge CA, Cole CN, Wentz SR. The Dbp5 cycle at the nuclear pore complex during mRNA export II: nucleotide cycling and mRNP remodeling by Dbp5 are controlled by Nup159 and Gle1. *Genes & Development*. 2011; 25:1065–1077. [PubMed: 21576266]
88. Hodge CA, Tran EJ, Noble KN, Alcazar-Roman AR, Ben-Yishay R, Scarcelli JJ, Folkmann AW, Shav-Tal Y, Wentz SR, Cole CN. The Dbp5 cycle at the nuclear pore complex during mRNA export I: dbp5 mutants with defects in RNA binding and ATP hydrolysis define key steps for Nup159 and Gle1. *Genes & Development*. 2011; 25:1052–1064. [PubMed: 21576265]
89. Bekenstein U, Soreq H. Heterogeneous nuclear ribonucleoprotein A1 in health and neurodegenerative disease: From structural insights to post-transcriptional regulatory roles. *Molecular and Cellular Neuroscience*. 2013; 56:436–446. [PubMed: 23247072]
90. Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, Gibbs JR, Brunetti M, Gronka S, Wu J, et al. Exome Sequencing Reveals VCP Mutations as a Cause of Familial ALS. *Neuron*. 2010; 68:857–864. [PubMed: 21145000]
91. Kimonis VE, Fulchiero E, Vesa J, Watts G. VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: Review of a unique disorder. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2008; 1782:744–748.
92. Tresse E, Salomons FA, Vesa J, Bott LC, Kimonis V, Yao T-P, Dantuma NP, Taylor JP. VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. *Autophagy*. 2010; 6:217–227. [PubMed: 20104022]
93. Keene JD. Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome. *Proceedings of the National Academy of Sciences*. 2001; 98:7018–7024.

Figure 1.

A model illustrating how reversible phosphorylation (designated by “P⁻”) in the intrinsically disordered regions (IDRs; represented by curvy orange lines) near the PAM2 motif may regulate interaction between the C-terminal MLLE domain of eukaryotic PABPC1 and a PAM2-containing protein. RRM, RNA Recognition Motif. Details are described in the text.

Figure 2.

A hypothetical scheme for remodeling of HuR-associated mRNPs by p97 segregase activity through non-degradative ubiquitin signaling. Note that HuR has three RNA recognition motifs (RRMs). The first two RRM bind HuR to the ARE in the initial mRNP. Subsequent ubiquitination on RRM3 leads to recruitment of UBXD8 and p97. ATP hydrolysis by p97 changes its conformation and “extracts” HuR from the mRNA and its mRNP. Details are described in the text.

Figure 3.

A schematic diagram showing key mRNP remodeling steps during export of a mature mRNP from the nucleus to the cytoplasm. NPC, nuclear pore complex. Details are described in the text.