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Principles and Properties of Eukaryotic mRNPs

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The proper processing, export, localization, translation, and degradation of mRNAs are necessary for regulation of gene expression. These processes are controlled by mRNA-specific regulatory proteins, noncoding RNAs, and core machineries common to most mRNAs. These factors bind the mRNA in large complexes known as messenger ribonucleoprotein particles (mRNPs). Herein, we review the components of mRNPs, how they assemble and rearrange, and how mRNP composition differentially affects mRNA biogenesis, function, and degradation. We also describe how properties of the mRNP "interactome" lead to emergent principles affecting the control of gene expression.

Introduction

mRNA is the intermediate between information and action and is consequently a highly regulated molecule subject to a diversity of RNA-processing reactions in eukaryotic cells. Moreover, recent studies have indicated that mRNA concentrations have little correlation with protein concentrations and that much of the regulation of gene expression occurs at the level of protein synthesis (Schwanhäusser et al., 2011; Sonenberg and Hinnebusch, 2009). These observations highlight the importance of understanding the mechanisms and regulation of mRNA biogenesis and function.

The control of mRNA function is modulated through interactions between individual mRNAs and proteins or noncoding RNAs (ncRNAs). These assemblies form complex structures referred to as messenger ribonucleoprotein particles (mRNPs). Formation of each mRNP follows the same general steps (Figure 1). mRNPs are first assembled during transcription and remodeled by cotranscriptional RNA-processing reactions. After export from the nucleus, some mRNPs are transported to specific regions of subcellular localization. Cytoplasmic mRNPs undergo structural rearrangements in order for translation to occur. Ultimately, all mRNPs are disassembled by mRNA degradation. The components of each mRNP play a significant role in controlling each step of biogenesis and function. Thus, understanding the composition, assembly, remodeling events, and function of mRNPs is fundamental to an understanding of the control of eukaryotic mRNAs. Herein, we review the components and principles that determine mRNP composition, the general manners by which mRNPs regulate gene expression, and emergent mRNP "interactome" properties that affect gene expression (Castello et al., 2012).

Components of Eukaryotic mRNPs

Three types of cellular molecules bind to mRNAs to make up the components of mRNPs. These include proteins, small ncRNAs (such as miRNA and PIWI-interacting RNAs), and large ncRNAs (Fabian et al., 2010; Juliano et al., 2011; Yoon et al., 2013).

mRNA-Binding Proteins

Historically, mRNA-binding proteins were identified from the study of RNA processing, translation, or degradation. A number of mRNA-binding proteins were also identified by the presence of known RNA-binding domains. One useful approach has been to purify mRNPs under denaturing conditions after crosslinking in vivo and then identify the crosslinked proteins. This method was used for identifying the hnRNP proteins (Dreyfuss et al., 1984). Recently, such approaches combined with mass spectroscopy techniques have allowed for the identification of hundreds of new mRNA-binding proteins in both yeast and mammals (Baltz et al., 2012; Castello et al., 2012; Kwon et al., 2013; Mitchell et al., 2013).

mRNA-binding proteins commonly contain a set of previously identified RNA-binding domains. For example, 40%–50% of yeast and mammalian mRNA-binding proteins contain a known RNA-binding domain (Castello et al., 2012; Mitchell et al., 2013). Interestingly, as determined by sequence analysis, many mRNP proteins contain multiple RNA-binding domains. To illustrate this point, we analyzed 120 known yeast RNA-binding proteins with one or more canonical RNA-binding domains (RRM, KH, S1, zinc-finger, Pumilio, or DEAD-box domain; SMART domain website, http://smart.embl-heidelberg.de/) for their domain architecture. These 120 proteins have an average of 1.9 RNA-binding domains, and one-third (41) have more than one. This is a lower limit of RNA-binding domains per protein, since over 50% of the proteins that crosslink to mRNAs do not contain canonical RNA-binding domains (Castello et al., 2012; Mitchell et al., 2013).

RNA-binding proteins contain multiple RNA-binding domains for several reasons. First, RNA-binding domains are frequently combined to create a larger binding site for a single RNA ligand, leading to increased affinity and specificity. For example, the *Drosophila* protein Sex-lethal, involved in gender determination, extends its recognition site to nine bases by aligning two RRM domains (Handa et al., 1999). Consistent with this usage, the number of RNA-binding domains per protein is generally inversely correlated with the length of the RNA binding site per domain (RNA-binding proteins with multiple domain types were included in this analysis; Figure 2). For example, the Pumilio domain, recognizing eight bases, is often the sole RNA-binding domain. In contrast, zinc-finger domains contact as few as two bases, and proteins with this domain have multiple RNA-binding

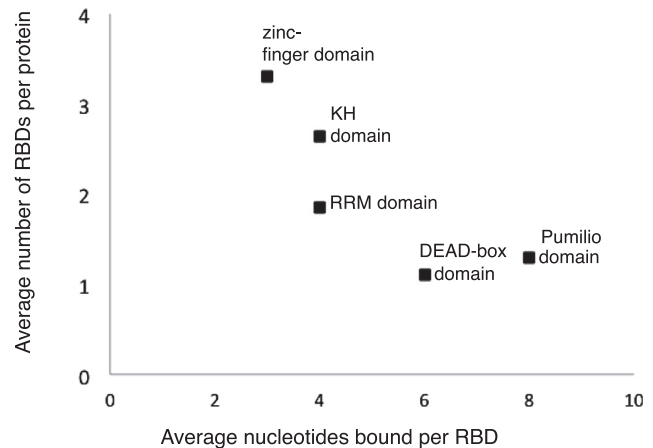CrossMark

mRNA Life Event
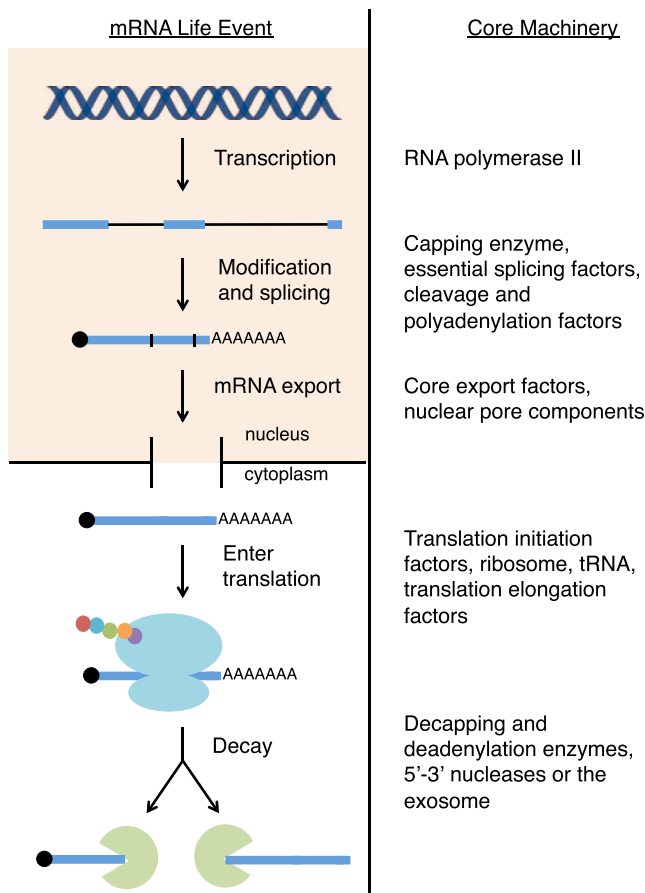
Figure 1. The Majority of mRNAs Pass through the Same Central Steps of mRNA Metabolism

These central steps, shown on the left, involve mRNPs, which are referred to as "core machineries" containing components listed in the right panel. mRNAs are synthesized in the process of transcription. Cotranscriptionally (shown as a second step for clarity), the mRNA is modified, and introns are removed by splicing. After the mature mRNA has been created, it is exported from the nucleus through the nuclear pore. In the cytoplasm, the mRNP is remodeled before and during translation. Translation involves multiple steps (initiation, elongation, and termination), each of which brings a number of factors to the mRNA in addition to the ribosome. At the end of the mRNA lifetime, it is degraded. This process occurs through two main pathways: (1) deadenylation-dependent decapping and 5′-to-3′ decay or (2) deadenylation followed by 3′-to-5′ degradation by the cytoplasmic exosome.
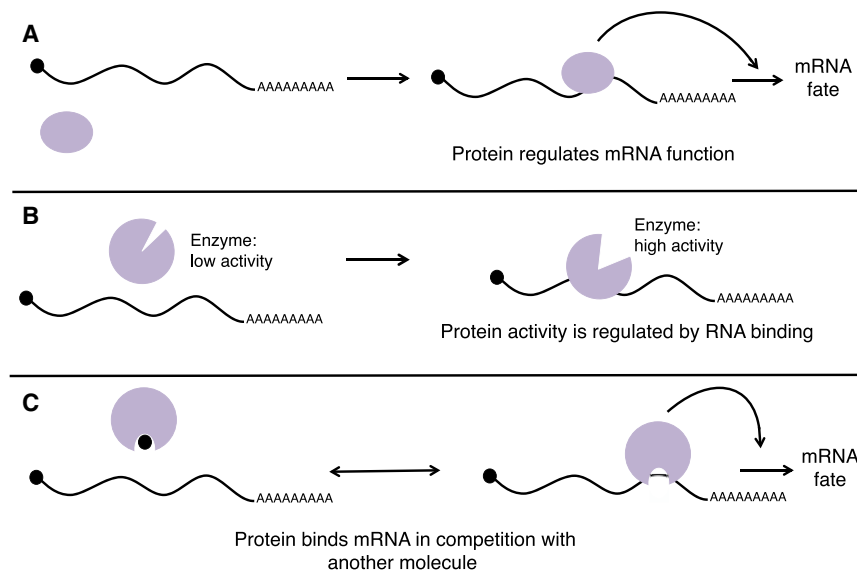

Figure 2. The Number of RBDs in a Protein Is Inversely Correlated with the Number of Nucleotides Commonly Bound by that Type of RBD

RNA-binding domains (RBDs) shown are the zinc-finger domain (binding two to four nucleotides), KH and RRM domains (binding four nucleotides), the DEAD-box domain (binding six nucleotides), and the Pumilio domain (binding eight to ten nucleotides) (Daubner et al., 2013; Del Campo and Lambowitz, 2009; Hall, 2005; Valverde et al., 2008; Wang et al., 2002). The average number of RBDs per protein was calculated for 120 *S. cerevisiae* RNA-binding proteins (Mitchell et al., 2013). Values for the average number of nucleotides bound per RBD were taken from structural studies cited above.

domains in 80% of cases for yeast RNA-binding proteins (Hall, 2005). For instance, seven zinc fingers are found in the *S. cerevisiae* polysome-associated protein Gis2.

Multiple RNA-binding domains in a single protein can also be used in more complex manners. For example, PARN, (poly(A)-specific ribonuclease) is a processive, tight-binding, and poly(A)-specific exonuclease. Its R3H RNA-binding domain is responsible for the high affinity of PARN for RNA but does not have specificity for poly(A) or contribute to its processivity (He et al., 2013). In contrast, the RRM and catalytic domains have lower affinity for RNA but bind to poly(A) preferentially. In principle, proteins with multiple RNA-binding domains could also be used to link together multiple mRNAs into a higher-order struc-

ture and thereby potentially affect the subcellular organization of mRNPs (see below).

The large repertoire of mRNA-binding proteins raises the question of the functional significance of these protein-mRNA interactions. In principle, new mRNA-binding proteins could function akin to many of the well-understood mRNA-binding proteins and control key steps in mRNA biogenesis and function (Figure 3A). However, there are precedents for additional roles of protein-mRNA interaction. For instance, mRNA binding may regulate the activity of the bound protein, modulating either enzymatic or nonenzymatic activities such as protein or ncRNA binding (Figure 3B). This possibility is suggested by the observation that many new RNA-binding proteins are enzymes, including kinases, ubiquitin proteases, and ligases, as well as metabolic enzymes (Baltz et al., 2012; Castello et al., 2012; Kwon et al., 2013; Mitchell et al., 2013). In addition, proof of principle for RNA modulating enzymatic activity comes from the fact that kinases PKR and GCN2 are activated by binding RNA (Dabo and Meurs, 2012; Wek et al., 1995). Such RNA-dependent activation of kinases or other protein-modification enzymes could lead to local modification of mRNP components to regulate only specific mRNAs containing the sequence recognized by the modification enzyme. The concentration of a given protein is typically much higher than that of an individual mRNA, preventing mRNA binding from having a large effect on the bulk of the protein. Consequently, effects on enzymes are likely to be limited to an increase in enzyme activity or to *cis* effects within an mRNP. Consistent with a positive role, RNA-protein interactions have also been shown to play a stimulatory role in the assembly of a signaling complex in yeast cells during the unfolded-protein response (Aragón et al., 2009).

Figure 3. Consequences of Protein-mRNA Interactions
(A) Proteins bind to mRNA to regulate mRNA function.
(B) Protein function is regulated by binding to RNA substrates.
(C) Proteins bind to mRNA in competition with other binding partners, thus controlling a particular set of mRNAs in response to the level of other molecules.
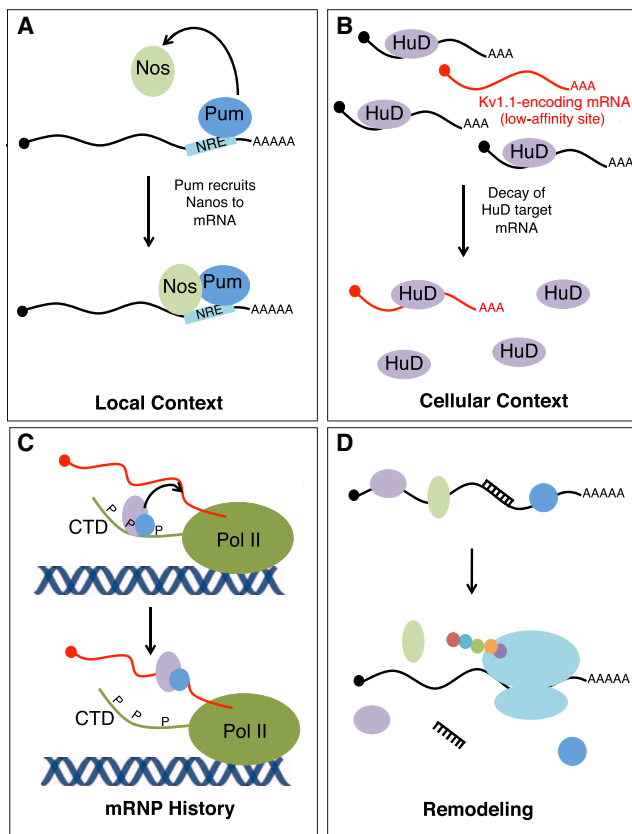
the central tasks of mRNA metabolism. Many stages in the biology of mRNAs involve large, canonical machineries that bind to mRNAs in a coordinated manner (Figure 1). These core mRNPs regulate splicing, export, translation, and decay of the mRNA. Each involves the binding of many proteins that are deposited during earlier biosynthetic events (see below) or bound in a manner that is primarily determined by mRNA landmarks, such as the cap structure, splice sites, and poly(A)-tail. For instance, during translation initiation, a closed-loop structure is thought to form when PABP, which binds to the poly(A) tail, interacts with the eIF4G subunit of the cap-binding complex (Sonenberg and Hinnebusch, 2009).

To a certain extent, these common machineries create a degree of homogeneity across mRNPs. For example, all mRNAs with introns interact with small nuclear RNAs and other essential splicing factors. Thus, one key to understanding the assembly and control of mRNAs is to understand the manner in which these large central machineries interact with specific mRNAs, how this interaction can vary, and how that variation can affect mRNA regulation (see below).

A second key step of mRNP formation is the binding of components that interact with sequence-specific features of individual mRNAs. These mRNA-specific interactors often bind the mRNA concurrently with core machineries, regulate specific steps in mRNA biogenesis, and function on subclasses of mRNAs. For example, many proteins regulate splicing on particular mRNAs, leading to alternative splicing events. These include the SR proteins, a family of proteins with both an RNA-binding domain and a serine/arginine-rich domain that can function in protein-protein interactions to recruit the splicing machinery to the mRNP. Some of these proteins can bind to exon sequences, called splicing enhancers, that promote splice-site selection (Zhou and Fu, 2013). Consistent with their role in alternative splicing on select messages, consensus sequences have been identified for a number of SR proteins (Zhou and Fu, 2013).

Not all mRNA-binding proteins fall into the categories of high sequence specificity or general machineries. Rather, mRNA-binding proteins exist on a continuum of specificity, ranging from extremely selective to quite promiscuous. Many proteins operate in the middle ground and bind to a range of sequences, but not all mRNAs. Pumilio-domain-containing proteins are an example of RNA-binding proteins with high specificity. These domains typically bind to specific sequences of eight to ten bases. In the middle of the spectrum, hnRNP A1 and other

The interaction between mRNAs and enzymes has also suggested a complex interconnection between metabolic pathways and the control of mRNAs, wherein metabolites regulate mRNA-binding proteins (Figure 3C). Such a model, called the "REM (RNA, enzyme, and metabolite) model" has been proposed for enzymes of intermediary metabolism (Hentze and Preiss, 2010). Cytosolic aconitase is known to act in this manner; it binds mRNAs in competition with its iron-sulfur cluster to regulate expression of the genes encoded by these mRNAs, which include those involved in iron homeostasis (Hentze and Preiss, 2010). This type of competitive binding between mRNA and a small molecule does not require enzymatic activity. In this way, a metabolic enzyme can "moonlight" as a regulator of mRNA translation and stability.

Finally, one has to consider the possibility that some identified RNA-protein interactions are the results of recent genetic changes and have little function. This might be particularly common for proteins that bind other RNAs, and therefore a simple mutational event could make a new binding site in a given mRNA. Such new interactions would then be fixed if they conferred some advantage.

ncRNAs Are Also mRNP Components
Both small ncRNA and long ncRNA (lncRNA) can interact with and regulate mRNAs to control gene expression. miRNAs, and other small RNAs, essentially serve as guides to dictate the specificity of Argonaute or PIWI proteins interacting with mRNAs. Argonautes, either directly or through the recruitment of GW182 proteins, then repress translation and promote mRNA degradation (reviewed in Fabian et al., 2010). The roles played by lncRNAs in gene expression are quite diverse. lncRNAs regulate both mRNA stability and translation through direct interactions with mRNAs (Yoon et al., 2013). Like miRNAs, lncRNAs may recruit proteins to the mRNP in order to carry out this regulation.

mRNP Components Range from General to Highly Specific
Components of mRNPs can act either as mRNA-specific regulators or as components of core mRNP machineries that perform

Figure 4. Four Types of Input Regulate mRNP Composition
(A) Local context of an mRNP: shown here, Pum binds to NREs (Nanos [Nos] response elements) and recruits Nos to a ternary complex with the mRNA. In the absence of Pum, Nos does not bind.
(B) Cellular context: shown here, HuD does not bind to the Kv1.1-encoding mRNA when other mRNAs with higher-affinity HuD binding sites are available. When those mRNAs decay, HuD is released and binds the Kv1.1-encoding mRNA.
(C) Deposition of mRNP factors during biogenesis: shown here, the poly-adenylation factors CPSF and CstF are transferred from the C-terminal domain of RNA polymerase II to the nascent RNA.
(D) mRNPs are remodeled during transitions between different states: shown here, during the first round of translation, the force of the translocating ribosome removes proteins and ncRNA from the open reading frame.

hnRNP family proteins bind a wide variety of targets but have a discernable sequence preference (Burd and Dreyfuss, 1994; Jean-Philippe et al., 2013). At the opposite end, having very little sequence specificity are some of the DEAD-box helicases, which rearrange a variety of mRNA substrates during central processes of gene expression. For instance, eIF4A is thought to rearrange the 5′ UTR of most translating mRNAs to facilitate ribosome binding and scanning. In vivo studies of model mRNAs have shown little dependence on RNA sequence with a low affinity varying between 1 and 2 μM (Lorsch and Herschlag, 1998; Rajagopal et al., 2012).

What Determines the Composition of an mRNP?
Hundreds of proteins and RNAs bind to and regulate mRNAs. How do these components build a complex mRNP structure? We have found that all together, four inputs into each mRNA-pro-

tein or mRNA-RNA interaction dictate the dynamic composition of each mRNP (Figure 4). Each interaction is dictated by the local context of the binding site (including mRNA sequence and other mRNP components), the cellular context (including local and active concentrations), the deposition of mRNP components during biogenesis, and transitions in mRNP composition and structure as an mRNA matures.

Local Context
Local context is defined by the primary sequence and structure of the mRNA, as well as the proteins and RNA molecules bound to that mRNA. The local context is what determines the basic affinity of an mRNA-binding factor for a region of an mRNA. Since many mRNA-binding proteins and miRNAs recognize sequence, the primary sequence of an mRNA is often a key factor in this interaction. Other mRNA features, such as the cap structure and poly(A) tail, can also contribute.

Some RNA-binding proteins recognize structural features, either predominantly or in addition to sequence. Yeast Rps28b binds to a hairpin in the 3′ UTR of its own mRNA, stimulating mRNA decay by recruiting decapping factors (Badis et al., 2004). Proteins with dsRNA-binding domains, such as Staufen, are thought to bind to dsRNA regions primarily by recognizing RNA shape, although dsRNA-binding proteins can be sequence specific (Masliah et al., 2013).

Local context also includes nearby mRNP components, which can increase the affinity of a binding site through protein-protein interactions or inhibit binding via overlapping binding sites on the RNA. For example, limiting Pum binding to some *Drosophila* mRNAs reduces the binding of the interacting protein Nanos (Figure 4A; Sonoda and Wharton, 1999). Several examples of proteins competing for miRNA binding sites have been observed (Jafarifar et al., 2011, and references therein). One well-characterized example of such competition is the ability of the AU-rich-element (ARE)-binding protein HuR to compete for miRNA binding sites, located both directly at its recognition site and nearby, through its polymerization activity (Kundu et al., 2012; Mukherjee et al., 2011).

Cellular Context
Several observations argue that the binding of any protein or ncRNA to an mRNA is dictated by both the concentration of the binding component and the number of competing binding sites. For example, increasing the cellular concentration of Mpt5, a yeast Pumilio protein that regulates mRNA translation and stability, leads to additional translational repression of its target mRNA, *HO*. This suggests that all binding sites in the *HO* mRNA are not saturated under normal cellular conditions and that the level of saturation can be increased by increasing levels of Mpt5 (Tadauchi et al., 2001). Similarly, the amount of functional eIF4E is regulated by Tor signaling through the phosphorylation of eIF4E-binding proteins, thereby regulating the function of eIF4E on mRNA (Sonenberg and Hinnebusch, 2009).

The ability of a factor to bind any site is influenced by its competing sites in the transcriptome. This has been most clearly demonstrated for miRNA binding sites. ncRNA "decoys" or "sponges" compete for miRNAs, reducing the number of miRNAs bound to their target mRNAs and upregulating the expression of those genes (Hansen et al., 2013; Memczak et al., 2013). Moreover, binding sites for a factor can become

occupied once a competing site is degraded, as appears to be the case with the CamKII-encoding mRNA in neurons, where degradation of this and several other mRNAs releases the translational activator HuD to bind and regulate the Kv1.1-encoding mRNA (Figure 4B) (Sosanya et al., 2013).

Posttranslational modifications can change mRNP structure by altering the binding or function of RNA-binding proteins. mRNA-binding proteins are known to undergo a variety of modifications, including phosphorylation, methylation, and ubiquitination, which can alter their affinity for mRNA or other proteins (Thandapani et al., 2013; Zhou and Fu, 2013). Consequently, the pool of free protein is not always equivalent to the pool of available protein if a modification is needed to facilitate binding. This type of change can be used to regulate binding globally or in specific regions of the cell. One example of such regulation is the use of "phosphogradients" across a cell to modulate mRNP structure by changing local concentrations of active mRNA-binding proteins. This can be achieved by differential localization of kinases and phosphatases. For example, in the *C. elegans* embryo, a phosphogradient of the RNA-binding protein Mex5 is created by localization of the kinase Par-1 to the posterior cytoplasm. Phosphorylation of Mex5 reduces mRNA-binding activity, leading to its release from mRNP complexes. Because phosphorylated Mex5 is released from large complexes, it diffuses faster, thus creating an asymmetric accumulation of Mex5 in the anterior cytoplasm (Griffin et al., 2011).

Recruitment of Factors during mRNA Biogenesis

mRNP components can be deposited on mRNAs during the process of biogenesis. Some RNA-binding proteins are placed on the mRNA through an interaction with the C-terminal domain (CTD) of RNA polymerase II; the CTD consists of highly phosphorylated repeat sequences. Two such examples are polyadenylation factors CPSF and CstF, which interact with the CTD of RNA polymerase II (Figure 4C). This interaction is required for efficient mRNA 3′ processing in vitro (Hsin and Manley, 2012). Interactions with RNA polymerase II can also lead to the delivery of sequence-specific mRNA-binding proteins. For example, She2 in yeast interacts with RNA polymerase II through Spt4 and Spt5 and can then be transferred to mRNAs that have a She2 binding site (Shen et al., 2010). Because the components of the polymerase complex are involved in mRNP assembly, mRNP structure may be influenced by the identity of the DNA promoter sequence, which can have downstream consequences for the fate of the mRNA.

Other aspects of mRNA processing can also deposit specific proteins on the mRNA. The process of polyadenylation is thought to lead to the deposition of nucleophosmin upstream of the polyadenylation site (Palaniswamy et al., 2006). Similarly, the process of splicing delivers the exon junction complex (EJC) to splice sites (Kataoka et al., 2000; Le Hir et al., 2000). In mammalian cells, the EJC is known to play an important role in quality control, as it remains bound to the mRNA during export from the nucleus into the cytoplasm. The presence of the EJC 3′ to a stop codon indicates to the quality-control machinery that it is a premature stop codon and that the mRNA should undergo nonsense-mediated mRNA decay (NMD) (Popp and Maquat, 2013).

The ability of a deposited protein or complex, such as the EJC, to function at a downstream step relies on a tight interaction with a slow off-rate; otherwise, it is unlikely to remain bound at the downstream step. The EJC is clearly tightly bound in this manner and does not freely dissociate from its bound mRNA (Le Hir et al., 2000). Instead, it requires the force of translation, or the activity of PYM, a cytoplasmic protein that interacts with the EJC, to be removed from the mRNA (Dostie and Dreyfuss, 2002; Gehring et al., 2009).

The coupling of biogenesis and mRNP assembly can be important for imprinting features onto an mRNP before it enters the cytosol. For example, many mRNAs that are localized to specific subcellular regions are loaded in the nucleus with mRNP components that both dictate their subcellular destination and repress translation until the mRNA is properly localized (Shen et al., 2010; Trcek and Singer, 2010).
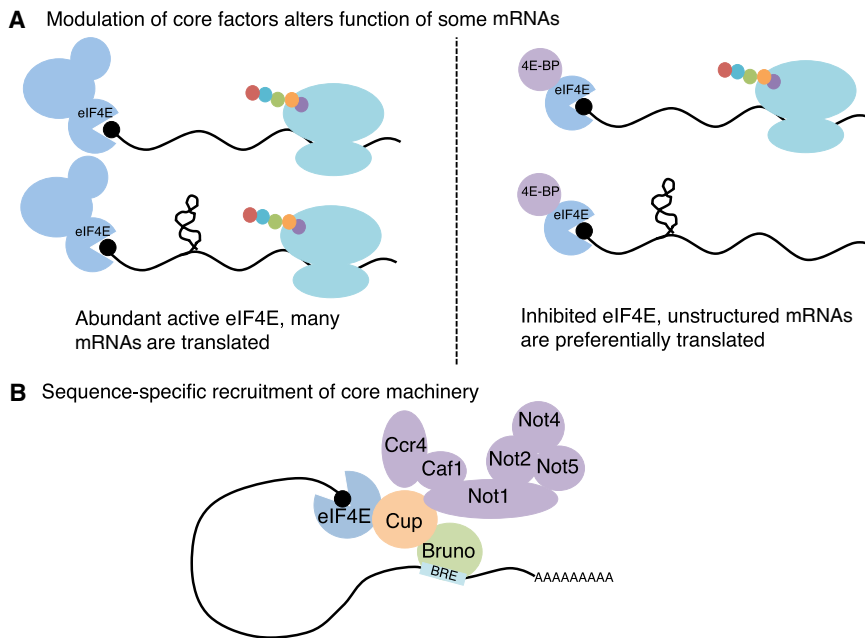
The coupling of biogenesis and assembly may affect the specificity of mRNP-component binding in two manners. First, if only specific mRNA-binding proteins are recruited to a particular genomic locus, then the apparent specificity of binding to the resulting mRNA will be enhanced. Alternatively, if an mRNA-binding factor is recruited to a genomic locus, it might bind to lower-affinity sites than it would in bulk solution as a result of its higher local concentration. An understanding of how assembly is coupled to biogenesis will be important in ultimately determining how mRNPs are structured.

Orchestrated Remodeling Events

As an mRNA matures, it passes through stages common to most transcripts. Most mRNAs are transcribed and modified, spliced, exported from the nucleus, possibly localized to a specific cellular region, translated, and degraded (Figure 1). Each of these transitions requires alterations in the mRNP. In addition, mRNA-specific transitions can occur. For instance, upon localization to a site of active actin polymerization, β-actin-encoding mRNA is released from the translation repressor ZBP1 when this protein is phosphorylated by a localized kinase, initiating β-actin translation (Hüttelmaier et al., 2005).

mRNPs are remodeled by several different mechanisms:

1. Proteins are released from mRNA through the activity of localized modification complexes. For example, after the yeast SR protein Npl3 is exported from the nucleus as part of an mRNP, phosphorylation by the cytoplasmic kinase Sky1 is required for its release from the mRNA and reimport into the nucleus (Gilbert et al., 2001). Npl3 interacts with the translation initiation factor eIF4G to repress translation (Rajyaguru et al., 2012). Cytosolic removal of this factor from an mRNA may facilitate translation initiation.

2. Proteins are released from mRNA through the activity of DEAD-box ATPases. In one example, the DEAD-box ATPase Dbp5 is associated with the cytoplasmic side of the nuclear pore. Dbp5 releases the export factors Mex67 and Nab2 from mRNA, ending the process of export and releasing the mRNA into the cytoplasm (Lund and Guthrie, 2005; Tran et al., 2007).

3. Proteins are bound to and removed from mRNPs on the basis of different local availability. Once in the cytoplasm,

A Modulation of core factors alters function of some mRNAs

Abundant active eIF4E, many mRNAs are translated

Inhibited eIF4E, unstructured mRNAs are preferentially translated

B Sequence-specific recruitment of core machinery

Figure 5. mRNP Components Regulate mRNA Function in Two Primary Ways
(A) Changes in components of core machineries can alter the functions of specific mRNAs. Here, due to the inhibitor eIF4E-binding protein, change in the activity of eIF4E has a more significant effect on some mRNAs than on others.
(B) Sequence-specific binding proteins can regulate target mRNAs, often by recruiting components of core machineries. Here, Cup and Bruno are recruited to *oskar* mRNA by Bruno-recognition elements (BREs) in the 3′ UTR.

the mRNP is exposed to cytosol-localized factors that can bind to the mRNA. These factors include the cap-binding protein eIF4E and other 5′ proximal factors that promote binding of the small ribosomal subunit, as well as the ribosome itself. The cytoplasmic process of translation also causes mRNP rearrangements, including removal of proteins and ncRNAs from the open reading frame (Figure 4D).

4. Some proteins are removed from cytoplasmic mRNPs by the RAN-GTPase gradient, which imports proteins with a nuclear localization signal and thus allows for exchange with cytoplasmic proteins. The nuclear cap-binding complex is removed from the mRNA by binding to importins, which release the cap-binding complex from the mRNA and promote reimport of the cap-binding complex to the nucleus by interacting with the nuclear pore complex and releasing the cap-binding complex in the nucleus via the activity of nuclear-localized RAN-GTP (Görlich et al., 1996). The cap of the newly exported mRNA is now available to bind eIF4E (Sato and Maquat, 2009).

5. An mRNP can also be remodeled through direct modification of mRNA by deadenylation, readenylation, uridinylation, or base-specific modifications. These modifications can change the bound proteins and ultimately the function of the mRNP. For example, the addition of a poly(U) tail can stimulate 3′-to-5′ degradation by the exonuclease Dis3L2, or in the case of histone mRNA, decapping and decay (Malecki et al., 2013; Mullen and Marzluff, 2008).

Modulation of mRNA Function by mRNP Components

A critical issue in mRNA regulation is how the proteins and ncRNAs present in mRNPs influence mRNA biogenesis, function, and degradation. We have identified two general mechanisms by which the properties of individual mRNAs are regulated.

Differential Interactions with Core Machineries

Individual mRNAs interact in different manners with the core mRNA machineries, which we define as those required for essential steps in mRNA biogenesis, function, and decay (Figure 1). Differential interactions between mRNAs and core mRNA machineries occur because the assembly of each core machinery involves a number of different biochemical interactions and activities. Intrinsic mRNA qualities, such as differences in sequence and structure, cause individual mRNAs to interact with these machineries in unique ways, although in each case the mRNA has to interact with the machinery in a manner sufficient to allow function. A classic example of such "nonconforming conformity" from a different area of cell biology is that although all charged tRNAs bind eF-Tu with similar affinity, the individual contributions of amino acids and the tRNA backbone vary significantly (LaRiviere et al., 2001). Similarly, different substeps in assembly or function can be rate limiting for different mRNAs without creating significant variation in the overall rate of the process (Koromilas et al., 1992; Nissan and Parker, 2008). Binding to eIF4E (discussed in detail below) is one such interaction. However, in some cases, these differences dictate different overall output from individual mRNAs. For example, mammalian mRNAs differ in the context of the AUG codon, and this influences the efficiency with which ribosomes enter translation and produce proteins (Kozak, 1989).

Given the differential interaction between mRNAs and the core machineries, changes in the active concentration of core components have both general and specific effects on gene expression. In this manner, modulation of general pathways can lead to mRNA-specific regulation (Figure 5A). For instance, eIF4E is a translation initiation factor that binds to the cap structure to help recruit the ribosome to the 5′ end of mRNAs. Overexpression of eIF4E has a particularly positive effect on translation of those mRNAs with highly structured 5′ UTRs (Koromilas et al., 1992). Consistent with its ability to modulate the expression of key genes, eIF4E is one of the most highly regulated mRNA-binding proteins in the cell and is controlled by a variety of signaling pathways, including that of the eIF4E-binding protein family of inhibitors (Sonenberg and Hinnebusch, 2009). Whereas these proteins cause a general reduction in translation, some

genes, including several associated with autism, are more sensitive to this mechanism than others (Figure 5A; Gkogkas et al., 2013).

Sequence-Specific Regulation
A second mechanism of differential mRNA regulation is by the sequence-specific binding of accessory proteins and/or ncRNAs that either promote or inhibit the function of core machineries (Figure 5B). An example of a sequence-specific mRNP component that alters mRNA fate via core factors is miRNA. The RISC machinery recruited to mRNAs by miRNA includes the protein GW182, which interacts directly with the Ccr4-Caf-Not and Pan2-Pan3 deadenylation complexes (Braun et al., 2013). In this way, miRNA binding recruits general deadenylases, stimulating decay. ARE-binding proteins associate with core decapping, exonuclease, and deadenylation factors to stimulate decay of mRNAs with these sequences (Lykke-Andersen and Wagner, 2005). mRNA-specific binding proteins can also recruit core machineries to stimulate translation. For example, HuD binding to specific mRNAs can enhance their recruitment of the translation initiation factor eIF4A to mRNAs and thereby promote translation (Fukao et al., 2009).

Sequence-specific regulators can affect multiple steps in the life of an mRNA, and such coupling of regulation can be required for proper mRNA control. For example, during *Drosophila* development, the protein Cup is recruited to *oskar* mRNA in a sequence-dependent manner (via an interaction with Bruno); there, it binds to eIF4E to inhibit translation (Figure 5B) (Nakamura et al., 2004). Cup also stimulates deadenylation of *oskar* mRNA by recruiting the Ccr4-Caf-Not complex, further inhibiting translation (Igreja and Izaurralde, 2011). Cup then stabilizes the deadenylated mRNA via a noncanonical interaction with eIF4E, presumably by strengthening the interaction of this protein with the cap structure to inhibit decapping (Igreja and Izaurralde, 2011). In this way, Cup is able to inhibit translation without triggering mRNA decapping. Similarly, many proteins can affect RNA-processing events in the nucleus and then modulate translation, localization, and/or mRNA decay in the cytosol. For example, HuR influences mRNA processing in the nucleus and stability and translation in the cytoplasm (Mukherjee et al., 2011).

We hypothesize that sequence-specific binding proteins also regulate mRNA function through direct modification of the core machineries once recruited to the same mRNP (in *cis*). This is analogous to the local chromatin modification due to the recruitment of modification enzymes and is suggested by the observation that a number of mRNA-binding proteins, including kinases (yeast protein Ste20) and ubiquitin proteases (yeast protein Ubp3), have posttranslational modification activity (Baltz et al., 2012; Castello et al., 2012; Mitchell et al., 2013; Tsvetanova et al., 2010). In order to increase the specificity for modifying proteins in the mRNP as opposed to unbound substrates, mRNA binding might increase enzymatic activity of the modifier (Figure 3B). As mentioned above, it has been shown that RNA binding can activate enzymatic activity, as in the case of the kinase PKR (Dabo and Meurs, 2012).

Sequence-specific binding proteins can also regulate a group of mRNAs by directly altering the mRNA structure, thereby changing the way in which these mRNAs interact with core machineries. For example, cytoplasmic polyadenylation can stimulate translation and increase mRNA stability and is an important point of regulation in many biological processes, including development, mitosis, and synaptic plasticity (Charlesworth et al., 2013). It is generally thought that the addition of a poly(A) tail stimulates translation through the binding of PABP to the newly added tail and the formation of a closed-loop structure via the interaction between PABP and the cap-associated factor eIF4G. Thus, the addition of a poly(A) tail most likely changes which step is the rate-limiting step of translation initiation, increasing the rate of translation. In *Xenopus* oocytes, cytoplasmic polyadenylation element binding protein (CPEB) recognizes a sequence located in the 3′ UTR of dormant mRNAs with shortened poly(A)-tails. CPEB recruits a cytoplasmic poly(A) polymerase to these mRNAs, activating translation and facilitating maturation of *Xenopus* oocytes (Charlesworth et al., 2013).

For two reasons, sequence-specific regulators should not be expected to affect all bound mRNAs. First, because individual mRNAs have different rate-limiting interactions with core machineries, a sequence-specific binding protein might alter a step that is not rate limiting for an mRNA. Second, given that binding sites for many factors are quite simple, it should be expected that new interactions are constantly forming and being lost as a result of genetic mutations and that at any snapshot in time, there are a number of nonfunctional interactions in the mRNP interaction map.

Emergent Properties of the mRNP Interactome
The features of mRNPs and their interactions give rise to some emergent properties that affect the biogenesis, function, and degradation of eukaryotic mRNAs.
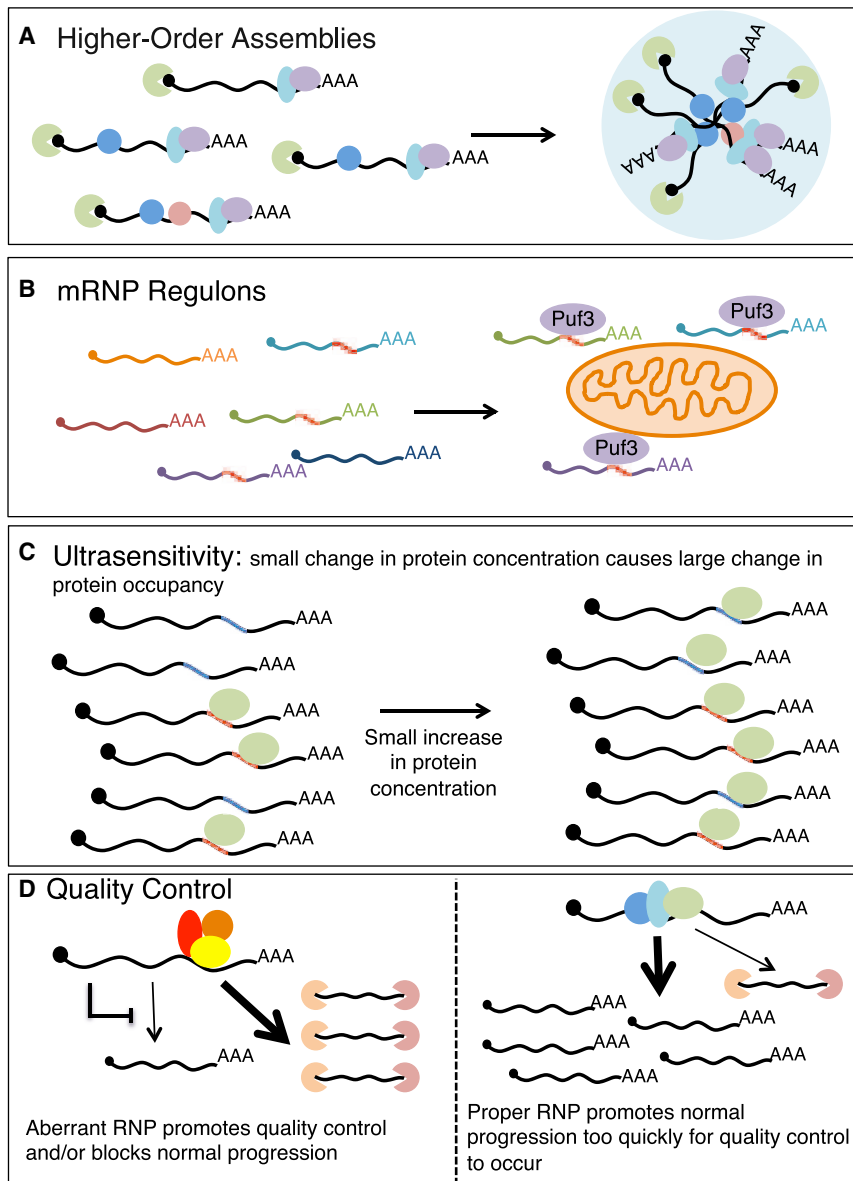
Higher-Order Assemblies: mRNP Granules
A conserved feature of mRNPs is that they can assemble into higher-order structures as a result of the interaction and aggregation domains of mRNA-binding proteins (Figure 6A). When such assemblies are large enough to be visible in the light microscope, they are referred to as mRNP granules, but similar structures are likely to form on smaller scales. These assemblies are generally observed for mRNPs that are not engaged in translation. Examples include (1) RNA-transport granules, which are prevalent in neurons and oocytes and are thought to play a role in mRNA localization; (2) P-bodies, which contain translation repressors and the mRNA decay machinery and are thought to play a role in mRNA storage and degradation; and (3) stress granules, which contain some translation initiation factors and are thought to represent zones where mRNAs assemble a translation initiation complex (Buchan and Parker, 2009).

mRNP granules create a high local concentration of mRNAs and mRNA-binding components. As such, the assembly of mRNAs into these structures is likely to affect the rates and specificity of transitions in mRNP composition, as well as the accessibility of mRNAs to the degradation machinery. Sequestration of mRNAs into granules can also limit their interactions with other nongranule components.

Structure of Higher-Order mRNP Assemblies
Some features of the assembly and structure of mRNP aggregates are known. First, granules are highly dynamic structures, as measured by FRAP, suggesting that the interactions within them are weak enough to allow for rapid exchange (Kedersha

Figure 6. Emerging Themes in the mRNP "Interactome"
(A) Higher-order assemblies of mRNPs form.
(B) Regulons of mRNAs encoding proteins of related function are coordinately regulated by the same mRNA-binding proteins. Shown here, mRNAs that have mitochondrial-related function are bound by Puf3.
(C) Ultrasensitivity is created when multiple mRNA sequences are bound by the same protein or ncRNA.
(D) Many different forms of quality control for mRNAs exist. In these pathways, quality-control factors either inhibit the rate of the normal forward pathways or increase the rate at which the quality-control reaction occurs. Simultaneously, binding of mRNP factors for a competing metabolic step increases the rate of that step, preventing sufficient time for quality-control steps to occur.

tions between different mRNP components that crosslink individual mRNAs together.

Higher-order mRNP assemblies can also be formed by RNA-binding proteins that interact with two or more mRNAs at the same time. For example, both Bruno and PTB bind the 3′ UTR of *oskar* mRNA and can simultaneously interact with multiple molecules of *oskar* (Besse et al., 2009). This multimerization may contribute to granule formation, given that a mutant reducing the expression of PTB has been shown to reduce the size of *oskar*-silencing granules (Besse et al., 2009).

mRNAs may play an active role in higher-order assembly formation and/or stabilization through direct intermolecular base pairing. For example, the *bicoid* mRNA forms an inter-mRNA loop-loop interaction to create a Staufen binding site (Ferrandon et al., 1997). Injection of the 3′ UTR of *bicoid* can cause the formation of mRNA-protein granules dependent upon this inter-mRNA interaction, supporting the hypothesis that mRNA-mRNA contacts can stimulate higher-order assembly formation (Ferrandon et al., 1997; Wagner et al., 2001). Base-pairing interactions have the ability to alter the contents of mRNP granules in two ways. First, some of these base-pairing interactions are known to recruit proteins to the mRNP, as for Staufen in the example above. Second, because mRNA-mRNA base pairing is sequence specific, it could lead to biases in the mRNA content of individual granules. mRNAs that base pair may be likely to segregate into granules with a subset of mRNAs that have complementary sequences.

mRNP Regulons
Another property of the mRNP interactome map is the emergence of mRNP regulons, which are groups of mRNAs that are coordinately regulated by one or more mRNA-binding proteins

et al., 2005). Second, protein-protein interactions play an important role in granule formation. Such interactions can include protein dimerization domains, as for Edc3 in the formation of yeast P-bodies (Decker et al., 2007; Ling et al., 2008), as well as aggregation domains, which are predicted to have a high probability of beta-zipper formation by computational methods and are concentrated in mRNA-binding proteins (Decker et al., 2007; Kato et al., 2012; Li et al., 2013; Reijns et al., 2008). Such aggregation domains have been shown to be important for stress granule assembly in mammalian cells and for P-bodies in yeast (Decker et al., 2007; Gilks et al., 2004; Reijns et al., 2008). Moreover, many RNA-binding proteins can self-aggregate when expressed either in vivo or in vitro (Alberti et al., 2009; Kato et al., 2012; Kim et al., 2013). This suggests a working model in which mRNPs assemble into larger structures in part through interac-

or miRNAs (Figure 6B). These functionally related and coregulated sets of mRNAs are referred to as "regulons" in reference to the bacterial operon, in which functionally related genes are coordinately regulated by their location on the same transcript (Keene, 2007). Such regulons are a consequence of mRNA regulators controlling groups of mRNAs and of mRNP proteins or miRNAs being influenced by various signaling pathways. Thus, changes in the abundance or modification status of mRNA-binding proteins or miRNAs can control a group of mRNAs with a related biological role. One example of such a regulon is the control of mRNA stability and localization of mRNAs targeted to the mitochondria by Puf3 (Figure 6B; Gerber et al., 2004; Olivas and Parker, 2000; Saint-Georges et al., 2008).

Ultrasensitivity

An interesting feature of the mRNP interactome map is ultrasensitivity to changes in the available pool of an mRNA-binding component (ultrasensitivity is defined as a nonlinear occupancy increase relative to a change in intracellular concentration of the binding partner). Ultrasensitivity in this case is due to the fact that there are a number of different binding sites for mRNP components. These various binding sites have different levels of occupancy depending on their particular affinities and cellular concentrations. Once a tight binding site has been saturated by a protein, increases in the levels of this protein cause greater than linear changes in the occupancy of a lower-affinity site that was previously unoccupied (Figure 6C; Zhang et al., 2013). The high-affinity site no longer competes with the lower-affinity site for binding, allowing for binding to increase to a greater amount than the increase in concentration of the binding protein. Thus, a single protein can bind to a variety of binding sites under various conditions, and this variety can lead to tight regulation of function and a regulatory output that is determined by the overall transcriptome.

Two examples of ultrasensitivity due to this type of "molecular titration" have been suggested to occur at the level of mRNA regulation (Zhang et al., 2013). Modeling suggests that most mRNAs are ultrasensitive to changes in the concentration of ribosomes (De Vos et al., 2011). Single-cell studies of miRNA regulation found that when miRNA binding sites are not saturated, they are highly sensitive to changes in concentration of available miRNA and miRNA binding sites (Mukherji et al., 2011).

Quality Control

Quality control exists at many of the central steps of mRNA function. For example, pre-mRNAs that fail to complete the first or second step of pre-mRNA splicing are degraded by both nuclear and cytoplasmic decay mechanisms (Harigaya and Parker, 2012; Hilleren and Parker, 2003; Volanakis et al., 2013). Similarly, mRNAs that are defective in various aspects of translation are preferentially degraded (Mitchell and Tollervey, 2001). The earliest identified translation-dependent quality-control pathway is NMD, which disposes of mRNAs with premature stop codons (Popp and Maquat, 2013). Moreover, quality-control systems exist for the destruction of mRNAs without stop codons (nonstop decay), with inefficient signal sequences, and with paused translation (no-go decay) (Doma and Parker, 2007).

Two properties of the mRNP interactome create inherent quality-control systems that preferentially degrade nonfunctional mRNAs. First, quality-control systems are an inherent property of the competing activities of mRNA-binding proteins that lead to different outcomes for the mRNA (Figure 6D). For example, competition between the NMD quality-control factor Upf1 and translation termination factors at translation stop codons is thought to play a role in triggering NMD (Popp and Maquat, 2013). Similarly, competition between Ago2 and the signal recognition particle binding to signal sequences has been proposed to play a role in triggering the decay of mRNAs with defective signal sequences during the translation of secreted proteins (Karamyshev et al., 2014). Second, quality-control systems are reinforced by the coupling of steps in the mRNA biogenesis pathway. Specifically, whenever an upstream event in mRNA biogenesis loads a positive factor for a downstream event, the presence of this factor enhances the downstream event at the expense of any competing quality-control pathway. For example, proper poly(A) tail addition facilitates mRNA export, whereas hypo- or hyperadenylation slows export, allowing for the nuclear exosome to dispose of the aberrant message (Hilleren et al., 2001). Similarly, when an upstream event removes a factor that promotes a downstream quality-control step, failure to complete an upstream event, as signaled by the remaining factor, triggers quality control. For example, in mammalian cells the EJC is loaded on the mRNA during splicing and must be removed by translation elongation so that the NMD quality-control pathway does not trigger mRNA degradation (Popp and Maquat, 2013).

Four Unresolved Issues

Eukaryotic mRNPs are a complex assembly of proteins and RNAs whose composition is affected by the cellular context of the cell and the dynamic history of an mRNA's life. We have identified four major issues that remain to be resolved to allow a fuller understanding of the mechanisms and regulation of eukaryotic mRNPs. For example, it will be of interest to understand how the higher-order assembly of mRNPs into mRNP granules such as P-bodies, RNA-transport granules, and stress granules affects the specificity and control of gene expression.

A second key challenge is to understand the dynamics of mRNP assembly and exchanges. Currently, essentially nothing is known about the dynamics of mRNPs in cells. How often do proteins vacate mRNA binding sites? Are transient, unstable interactions a major component of mRNP structure, and if so, can they influence function? Are off-rates slow enough that proteins deposited on mRNA during biogenesis remain bound until the time of mRNA decay? If off-rates are this slow, does that indicate that many mRNPs do not have the opportunity to reach thermodynamic equilibrium but are instead composed of low-stability interactions that are kinetically trapped? Both in vitro and in vivo characterization of mRNP dynamics will be required for understanding what role kinetics plays in establishing the structure of mRNPs.

A third issue, related to the dynamics of mRNPs, is to understand the diversity of mRNPs, both from an individual gene and between different genes. Since a given transcript can produce different alternatively spliced forms, which are the output of different assembled mRNPs, it is self-evident that an individual gene can produce multiple different mRNPs. If mRNP composition is often kinetically determined, we should anticipate that

variation in initial assembly reactions can yield a diversity of different mRNPs from a single transcription unit; this might have downstream consequences for multiple regulatory fates of individual mRNAs from a single gene. Conversely, if mRNP composition is thermodynamically controlled and mRNPs are in equilibrium with free proteins, then any initial variation in assembly should be lost over time as the mRNP approaches equilibrium. A related issue is how much mRNP composition varies between different genes. Given the diversity of components, it could be that every mRNP is essentially unique. Alternatively, there could be subtypes, and many mRNPs from different genes could have related and highly similar composition, which would eventually be revealed as more data on each mRNP component and its mRNA targets accumulate.

A final challenge is to understand how the fate of an mRNA is dictated by a particular assembly of associated proteins and ncRNAs. On one extreme, each mRNP component could act independently, and the informational output of the mRNP would be a simple summation of the effects of each individual component. Alternatively, individual mRNP components are likely to synergize, or antagonize, the functions of each other, thereby giving more complex outputs dependent on the specific composition. Thus, gaining insight into the functional interactions between different mRNP components when in *cis* on an mRNA will be needed for reliably predicting the output from mRNP compositions.

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