Trends in Biochemical Sciences

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Review

Deciphering the mRNP Code: RNA-Bound Determinants of Post-Transcriptional Gene Regulation

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Eukaryotic cells determine the final protein output of their genetic program not only by controlling transcription but also by regulating the localization, translation and turnover rates of their mRNAs. Ultimately, the fate of any given mRNA is determined by the ensemble of all associated RNA-binding proteins (RBPs), non-coding RNAs and metabolites collectively known as the messenger ribonucleoprotein particle (mRNP). Although many mRNA-associated factors have been identified over the past years, little is known about the composition of individual mRNPs and the cooperation of their constituents. In this review we discuss recent progress that has been made on how this 'mRNP code' is established on individual transcripts and how it is interpreted during gene expression in eukaryotic cells.

The mRNP Is a Central Unit of Eukaryotic Gene Expression

mRNA is the key intermediate in the expression of protein-coding genes, connecting information (contained within the genome) and function (carried out by encoded proteins). Although transcriptional regulation allows genomic information to be retrieved selectively in a quantitatively controlled manner, regulation does not stop here: processing can generate many different mRNA versions from one precursor and also affect the amount of mRNA produced. Protein output of any particular mRNA can be adjusted by regulation of its rate of translation and also by vastly different half-lives of different mRNAs. In addition, localized protein production can be achieved by localization of mRNA to specific parts of the cell. The cellular fates of mRNAs depend on their primary structures which in turn determine the formation of the **messenger ribonucleoprotein particle** (mRNP; see Glossary), in other words mRNA association with RBPs, *trans*-acting RNAs, and even small molecules [1]. This molecular ensemble is read by 'effectors' – the factors that participate in the various steps of mRNA biogenesis and function [2]. The sequence-dependent and -independent endowment of mRNAs with possibly unique sets of regulatory molecules and their interpretation by the machineries of RNA processing, translation, and mRNA decay is referred to as the 'mRNP code'. A detailed insight into the composition of mRNPs, their assembly, and remodeling is pivotal for understanding how they control gene expression. Substantial advances have been made towards this goal through recent technological inventions in the analysis of RNAs and RNPs (Box 1), and we highlight some recent findings in this review.

A Diverse Spectrum of Molecules Interact with Eukaryotic mRNA

mRNA as the backbone of mRNPs interacts directly with two major types of biological macromolecules, namely proteins and non-coding RNAs (Figure 1, Key Figure). In turn, both

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The mRNA-bound proteome is more complex than previously anticipated and comprises up to 1000 RBPs.

Because there are many mRNA-interacting factors, and each mRNA is the blueprint of a particular protein, the resulting ribonucleoprotein particles (mRNPs) are likely to be unique in their composition.

The 'mRNP code' concept implies that specific sets of proteins, non-coding RNAs, and other molecules bind to individual mRNAs and control their fate and function in every cell.

The mRNP code is highly dynamic and reflects the functional status of each mRNA.

Previously unknown RNA-binding domains show unconventional modes of RNA–protein interactions.

Recent technologies such as high-throughput analyses of RNA–protein interactions and ribosome profiling have revealed binding sites and sequences of multiple RBPs *in vivo* and *in vitro*.

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Box 1. Approaches Used to Study RNA–Protein Interactions

One of the most basic methods is RNA immunoprecipitation (RIP) which utilizes immunoprecipitation of a (putative) RNA-binding protein (RBP). This method allows the isolation and subsequent analysis of RNAs bound to the RBP by medium- or high-throughput techniques such as microarray analysis and RNA-seq [99]. Similarly to RIP, crosslinking and immunoprecipitation (CLIP) uses immunoprecipitation of an RBP of interest in combination with UV-crosslinking to identify the binding sites of RBPs by high-throughput sequencing [100]. Complementary to methods using crosslinking are approaches in which RBPs are coupled to a catalytically active enzyme that modifies mRNAs to which the RBPs bind. In two recent reports the RNA-editing enzyme ADAR or poly(U) polymerase (PUP) were fused to different RBPs, leading to the successful identification of target mRNAs [101,102].

Traditional biochemical approaches allow the analysis of only a limited number of RNA–protein interactions at a time. An alternative to the above-mentioned *in vivo* methods are *in vitro* techniques for high-throughput identification of RBP-bound RNA sequences [103,104]. SEQRS [*in vitro* selection, high-throughput sequencing of RNA, and SSLs (sequence specificity landscapes)], for example, uses the same principle as systematic evolution of ligands by exponential enrichment (SELEX). Immobilized RBPs are incubated with complex RNA libraries and, after a few rounds of selection, the protein-bound RNAs are identified by deep sequencing, yielding a wide range of RNA affinities [105].

Interactome capture is a technique to identify components of mRNPs. This method uses UV-crosslinking of proteins to their bound mRNAs and subsequent oligo(dT) capture of mRNA–protein complexes from cell lysates under denaturing conditions [8,9]. The obvious limitation of this method is that it enriches for RBPs bound to polyadenylated mRNAs, but not to premature and non-polyadenylated RNAs. It also fails to identify components of mRNPs that do not bind directly to mRNAs or cannot be efficiently crosslinked. These limitations may be overcome using integrated approaches that combine purification of RBPs with the computational analysis of RNA-dependent protein–protein interaction networks (e.g., SONAR) [106]. Because UV-crosslinking can capture even transient interactions, an independent validation of the RNA-binding function of proteins identified in proteome-wide screens is desirable.

Ribosome profiling (RP) is a recent technique to globally monitor the translation status of cellular mRNAs [107]. RP is based on ribosome footprinting and uses nuclease treatment to eliminate RNA sequences that are not protected by ribosomes. The pool of *in vivo* ribosome footprints is analyzed by high-throughput sequencing, enabling the positions of translating ribosomes to be mapped. RP has been used not only to quantify protein synthesis but also to annotate coding regions and upstream open reading frames.

Glossary

Crosslinking and immunoprecipitation (CLIP): method to systematically map RNA target sequences of RNPs *in vivo*.
Electron cryo-microscopy (cryo-EM): a variant of transmission electron microscopy (TEM) where samples are studied at cryogenic temperatures. Recent technological progress allows the use of cryo-EM to analyze macromolecular complexes such as mRNPs at high resolution.
Messenger ribonucleoprotein particle (mRNP): mRNA bound to its interactors (proteins, ncRNAs, ions and metabolites). The mRNP represents the functional unit of mRNA in cells.
mRNA interactome capture: a recently invented systematic method to purify and identify proteins that interact with cellular mRNAs *in vivo*.
Ribosome profiling (RP): a recently developed technique to globally monitor the translation status of cellular mRNAs. RP generates a snapshot of actively translating ribosomes in a cell at a particular moment.

can also function as interaction platforms for additional factors that are not directly bound to the mRNA [3,4].

The diverse group of mRNA-binding proteins (mRBPs) is characterized by the presence of one or more RNA-binding domains or modules. Most common are the RNA recognition motif (RRM), the K-homology (KH) domain, the Larp module, zinc fingers, double-stranded RNA-binding motifs, and DEAD-box domains [5]. However, prediction of (m)RBPs solely from their amino acid sequence or the presence of known RNA-binding domains has proven difficult or misleading. On the one hand, not all designated RNA-binding domains indeed mediate the interaction with RNA; some contact other proteins instead [6,7]. On the other, recently established catalogs of RBPs include many factors that have not been previously linked to RNA [8–10]. Furthermore, up to one third of candidate RBPs do not contain 'classical' RNA-binding domains. In fact, domains previously implicated in scaffolding functions now turn out to confer specific RNA binding. For example, the NHL domain of the *Drosophila* protein BRAT was recently shown to form an unconventional RNA-binding module that binds to single-stranded RNA in a sequence-specific manner via a positively charged platform [11]. Likewise, a WD40 repeat, which typically forms a propeller-like protein interaction scaffold in signaling molecules, has recently been shown to bind to RNA in a specific manner in the context of the protein Gemin5 [12–15]. Even unstructured protein regions such as arginine-glycine-glycine (RGG) stretches can mediate RNA binding [16–18]. It is therefore very difficult to predict whether a protein is indeed an RNA binder without experimental validation.

Many RBPs such as members of the heterogeneous nuclear (hn)RNP protein family contain more than one RNA-binding domain or even combinations of several different types thereof [19]. This enables binding to longer stretches of RNA and typically leads to an increased affinity

Key Figure

Components, Determinants and Readers of the Messenger Ribonucleoprotein Particle (mRNP) Code

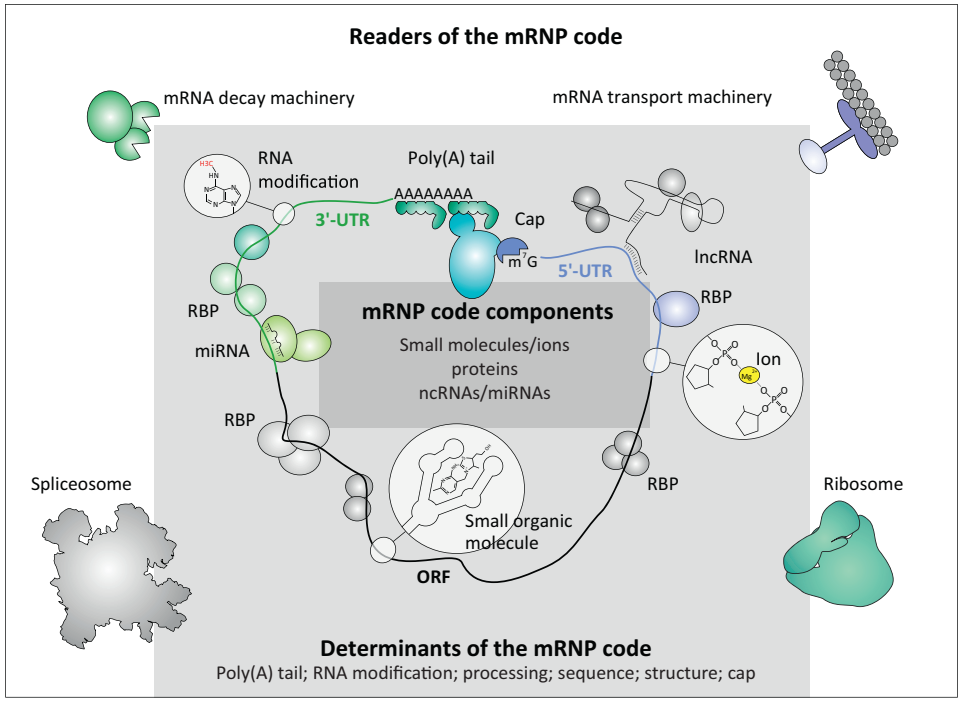


Figure 1. The figure shows a canonical eukaryotic mRNA with different types of interacting factors and their mode of interaction (mRNP components). The determinants of the mRNP code are known to influence the composition of the mRNP, whereas the readers of the mRNP code are regulated by components of the mRNP. Abbreviations: m^7G, 7-methylguanosine; miRNA, microRNA; ORF, open reading frame; RBP, RNA-binding protein; 3′/5′-UTR, 3′/5′ untranslated region.

and specificity of the RBP. Alternatively, multiple RNA-binding domains may also be combined to recognize non-contiguous binding sites on mRNA, thereby assisting topological organization of mRNAs and/or properly positioning other components of the mRNP [20]. Indeed, recent studies suggest that the combinatorial use of RNA binding domains in proteins may be even more common than was previously assumed [8,21].

Small and long non-coding RNAs (sncRNAs and lncRNAs) comprise the second major class of macromolecules interacting with mRNA (Figure 1). Although their interaction with mRNA may require cofactors, specificity is often solely determined by sequence complementarity. sncRNAs, such as microRNAs (miRNAs) or PIWI-interacting RNAs (piRNAs) primarily serve as guides to direct common effectors (i.e., Argonaute or PIWI proteins) to specific mRNA targets [22]. The function of lncRNAs on mRNA may be more diverse, ranging from directly affecting mRNA metabolism to the recruitment of proteins, as in the case of sncRNAs [23]. Whereas nuclear lncRNAs may regulate pre-mRNA processing, cytoplasmic lncRNAs have been implicated in translation regulation. For example, the so-called SINEUPs are natural

antisense lncRNAs that stimulate the translation of their target mRNAs [24]. SINEUPs contain a SINEB2 element which acts as a translation enhancer [25]. By contrast, lincRNA-p21 has been shown to inhibit the translation of its target mRNAs, JUNB and CTNNB1, through a mechanism that involves reduced polysome sizes and possibly ribosome 'drop-off' [26]. Given the increasing number and complexity of known lncRNAs, a significant number of them are likely to contribute to gene regulation.

Lastly, the mRNP may also interact with small organic molecules or ions. The enzymatic cofactor thiamin pyrophosphate (TPP), for example, binds to structured RNA elements (riboswitches) within introns of specific mRNAs and regulates their processing [27] (Figure 1). Ions, by contrast, often contribute to the stabilization of RNA secondary and tertiary structure, or regulate the binding of proteins to their mRNA target, as described for zinc-finger proteins [28].

Division of Labor: Common and Specific mRNP Components

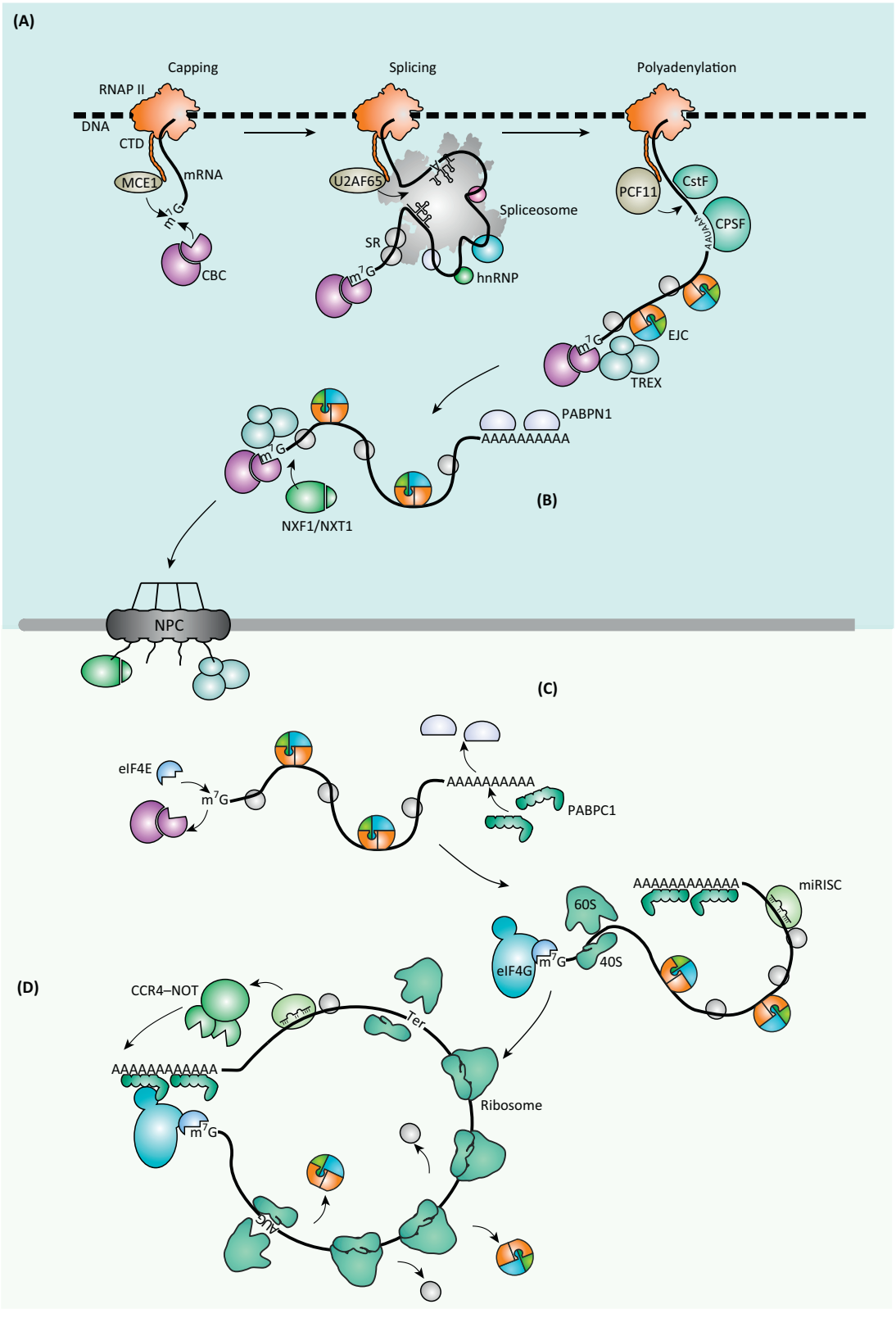
Despite the large variety of molecules interacting with mRNAs, one can classify mRNP components into three major groups: (i) common 'core' proteins that bind to all mRNAs in defined phases and that may possess or promote enzymatic (i.e., processing) activities, (ii) mRNA-specific factors that target only a specific subgroup of the mRNA pool, and (iii) non-specific binders [2]. The core factors comprise mRNP components performing important general functions during mRNA metabolism. For example, all intron-containing pre-mRNAs bind to essential splicing factors to form spliceosomes [29]. Likewise, machineries carrying out other post-transcriptional processes such as capping, polyadenylation, or mRNA export are also common to most if not all mRNPs (Figure 1).

By contrast, mRNA-specific proteins recognize defined sequences or structural features of particular mRNAs with high specificity and affinity. Thus, such proteins function only on subclasses of mRNAs and regulate their fate by affecting the function of common factors in processing, stability, or translation. For example, proteins of the SR family target specific RNA sequences in *cis*, thereby regulating alternative splicing events [30]. The third group comprises proteins that have little or no sequence specificity. Prototypes of such proteins are some DEAD-box helicases, which to execute their functions, interact with the phosphate backbone of the RNA [31–33]. However, many of these 'nonspecific' proteins acquire specificity towards particular mRNAs by interacting with other factors that have defined targets in *cis*. In the following section we give examples of how common and specific mRNP components are recruited to mRNAs and how the resulting mRNPs exert their function.

Gene Expression and the Formation of mRNPs

The life cycle of mRNA and the formation of mRNPs in eukaryotes comprise several general steps (summarized in Figure 2). The starting point in mRNP production is the generation of mRNA precursors (pre-mRNA) by RNA polymerase II (RNAP II). Pre-mRNAs are then processed to reach maturity and are transported to the cytoplasm for translation. The lifespan of mature mRNA in the cytoplasm is, however, typically short, thus enabling cells to adjust to changing growth conditions. To ensure mRNP turnover, cells employ elaborate enzymatic machineries that disassemble mRNPs and degrade the mRNA [34]. In the course of this pathway the mRNA attracts the *trans*-acting factors that make up the mRNP and are crucial for each respective phase. After having served their functions, factors can be actively ejected or released by simple dissociation.

The initial association of proteins with mRNAs occurs as soon as nascent mRNA precursors of sufficient length emerge from RNAP II. Naturally, many factors associating with the mRNA in this initial phase are connected to its processing and subsequent nuclear export. These include



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the capping and polyadenylation machinery as well as the spliceosome, all of which associate with the pre-mRNA cotranscriptionally [35]. Nuclear processing considerably alters the RNA backbone of the mRNP and hence generates a binding platform for a new set of proteins [36]. This process, which is referred to as mRNP remodeling, licenses the mRNP for nuclear export [37]. Important marks of this licensing process are the splicing-dependent deposition of exon junction complexes (EJCs) and the recruitment of mRNA export factors such as the metazoan TREX complex. After their translocation into the cytoplasm, some mRNPs require localization to specific cellular regions for their functionality [38]. Eventually, mRNPs are decoded (i.e., translated) by ribosomes and are ultimately targeted for degradation. The events leading to mRNP formation and remodeling, which are described in more detail in the next section, are subject to intense communication and regulation between mRNP components and the respective effector machineries.

Molecular Principles Determining the Formation of an mRNP

Even though all mRNAs pass through a common choreography of synthesis, processing, transport, translation, and finally decay, it is believed that each individual mRNA is packaged into a unique mRNP [39]. Theoretically, mRNPs could be formed simply by the reversible association of RBPs, regulatory RNAs, and associated factors with *cis* elements in the mRNA (Box 2). However, there are more sophisticated processes which are integrated into the process of mRNA biogenesis. For example, some RBPs are deposited on the mRNA by the interaction with additional factors, such as the C-terminal domain (CTD) of RNAP II. Depending on its post-translational modification status, the CTD can serve as a binding platform for proteins that are destined to associate with the nascent transcript [40–42] and mediate essential or regulatory steps of mRNA metabolism [43–45]. Association of proteins with the nascent RNA via the CTD is not as straightforward as it may seem: according to thermodynamic principles, the equilibrium between an RNA–protein complex and its free constituents is independent of the pathway of association – that is, the 'handover' from the CTD cannot 'push' the binding equilibrium. There are three ways in which the CTD could be helpful. First, there is no handover, but the factor binds to the CTD and the nascent transcript simultaneously, perhaps cooperatively. This possibility is dramatically illustrated by the capping enzyme, which is normally recruited by the Ser5-phosphorylated CTD [46]. Lethality caused by the absence of Ser5 phosphorylation can be complemented by covalent attachment of the enzyme to the CTD [47]. Similarly, the CTD appears to participate directly in 3′ cleavage/polyadenylation [48]. Second, the system is not at equilibrium. Instead, handover of the hypothetical factor from the CTD to the nascent RNA is 'pulled' by an ensuing irreversible reaction such as splicing or cleavage/polyadenylation. In this case, transient binding to the CTD would serve to increase the on-rate for RNA binding. Third, handover from the CTD to the nascent transcript could be driven by NTP hydrolysis. This would be analogous to the handover of the signal peptide of secreted proteins from the signal recognition particle to the translocon in the ER membrane, which is promoted by GTP hydrolysis.

mRNA processing also has a major impact on mRNP formation, as illustrated by the process of splicing-dependent deposition of the EJC on spliced mRNA. This event determines the fate of

Figure 2. Establishing, Remodeling, Reading, and Disassembly of the Messenger Ribonucleoprotein Particle (mRNP). (A) mRNAs are transcribed by polymerase II (RNAP II) and are co-transcriptionally processed. The C-terminal domain (CTD) recruits, among potentially other proteins, the capping enzyme MCE1, the splicing factor U2AF65, and the 3′-processing factor PCF11 to couple transcription with mRNA maturation. The spliceosome deposits the exon junction complex (EJC) as a marker on exon–exon junctions. (B) Nuclear remodeling on the processed mRNA ensures the formation of export-competent mRNPs and the binding of crucial export factors such as NXF1/NXT1. (C) After passage through the nuclear pore complex (NPC) the mRNP undergoes cytoplasmic remodeling: the cap-binding complex (CBC) is replaced by eIF4E, and the nuclear poly(A)-binding protein (PABPN1) is replaced by its cytosolic counterpart PABPC1. (D) The mRNP is then ready for translation, which involves the recruitment of the ribosome on the cap-binding complex eIF4F and the translation-dependent replacement of the EJC. Eventually, all mRNPs are disassembled and degraded by specialized enzymes. As an example, the miRNA-dependent degradation of mRNA via the CCR–NOT complex is shown (text for more details). Abbreviations: hnRNP, heterogeneous nuclear ribonucleoprotein; m^7G, 7-methylguanosine; Ter, termination codon.

Box 2. Biophysical Considerations of RNA–Protein Interactions

The affinity of an RBP for a given sequence can be described by the equilibrium dissociation constant, K_d. An interaction with a K_d in the low nanomolar range is usually considered to have high affinity. It is important to remember that the K_d is defined not only by the law of mass action but also by the ratio of rate constants of dissociation ('off') and association ('on') ($K_d = k_{off}/k_{on}$). Association rate constants are typically in the range of 10^7 to 10^8 M^{-1} s^{-1}, close to the diffusion limit. If, for example, a protein binds with a K_d of 10 nM and an association rate constant of 10^7 M^{-1} s^{-1}, the dissociation rate constant will be 0.1 s^{-1}, which corresponds to a half-life of ~7 s. In other words, even a high-affinity association can be the result of a rapid on–off equilibrium and the complex may possess a relatively short half-life. This has important consequences for the experimental analysis of mRNPs, with typical procedures taking on the order of hours. During this time, complexes may go through several rounds of dissociation and reassociation with *trans*-acting factors or even form new mRNPs from scratch (see Mili and Steitz for an experimental demonstration [108]). Thus, a complex isolated from a cell extract by some type of pull-down procedure need not be the same that existed in the cell. Despite macromolecular crowding, kinetic stabilities of complexes may not be very different *in vivo* compared to what is measured in dilute solutions *in vitro* [109]. Thus, postulating that a protein associates with an RNA molecule at some point in time and space, and then remains bound to the RNA for extended periods of time until the RNA has arrived at its destination, assumes a kinetic stability of the complex that cannot be taken for granted.

spliced mRNAs by influencing several different post-transcriptional steps of gene expression [49]. The EJC consists of four core components: EIF4A3, MAGOH, RBM8A, and MLN51 (also referred to as Barentsz or CASC3), and includes several additional peripheral factors [50]. Of the four core components, only the DEAD-box protein EIF4A3 binds directly to the mRNA. Recruitment of the EJC does not involve a specific RNA-sequence element in *cis*. Instead, the EJC is assembled by the spliceosome in a stepwise manner from its individual components. EJC deposition requires the interaction of EIF4A3 with the MIF4G domain of the spliceosomal protein CWC22 [51–53]. Within the spliceosome CWC22 is located close to the first exon of the pre-mRNA, and the orientation of its MIF4G domain directs EIF4A3 to a position 20 nt upstream of the splice junction [54,55]. How and when MAGOH and RBM8A join EIF4A3 remains an open question, but the formation of an intermediate pre-EJC is completed within the spliceosomal C-complex. EJCs remain bound to their mRNA during and after transport to the cytoplasm [56]. The particular ability of the EJC (or a similar complex) to regulate later steps of mRNA metabolism requires its tight and stable interaction with the mRNA. In fact, the MAGOH–RBM8A heterodimer inhibits dissociation of the EJC by keeping EIF4A3 in the closed conformation and preventing the release of bound ADP. Eventually, the EJC is actively removed from the mRNA by translating ribosomes or by the EJC disassembly factor PYM [57,58]. Hence, in contrast to interactions occurring via an association–dissociation equilibrium, some components of the mRNP are deposited and/or dissociated via catalyzed processes rather than spontaneously.

The coupling of mRNA biogenesis and mRNP assembly may have several advantages for the gene expression machinery. On the one hand, the integration of both processes may serve as quality control mechanism to ensure that only properly processed mRNAs are loaded with important functional components. On the other, the increased local concentration of an RBP effected by a third component (i.e., EJC, CTD, or other modules) may promote binding of the RBP and ultimately influence its function in the context of the mRNP.

Adjusting Function: Remodeling of mRNPs

The mRNPs initially formed in the nucleus during transcription and processing (often termed 'early' mRNPs) are by no means static. Instead, mature mRNPs undergo extensive remodeling as they enter the cytoplasm and progress in their life cycle [59]. Remodeling events may occur in an active manner (i.e., linked to NTP hydrolysis) or occur by diffusion-driven replacement reactions (see also Box 2). Major remodeling events occur in the cytosol at the 5′ and 3′ ends of mRNAs to prepare the mRNP for translation. The 5′-terminal 7-methylguanosine (m^7G) cap is bound in the nucleus by the cap-binding complex (CBC) which ensures stability and participates in the processing of mRNAs [60]. The CBC stays associated with mRNAs during

transport to the cytosol but is then replaced by eIF4E (a subunit of the translation initiation factor complex eIF4F), which promotes the recruitment of the small ribosomal subunit and eventually translation initiation [61,62]. Likewise, the nuclear poly(A) binding protein PABPN1, which is required for efficient polyadenylation of mRNA, is replaced by its cytosolic cousin (PABPC1) after nuclear export, although PABPN1 and PABPC1 do not have functions only in the nucleus and cytoplasm, respectively [63]. This enables circularization of the mRNA via a contact of PABPC1 with the eIF4G subunit of eIF4F and, as a consequence, efficient translation. The initial encounter with the translational machinery is thought to strip many RBPs off the mRNA, leaving only the 3′ untranslated regions (3′-UTRs) of the mRNA as sites for long-term RBP interactions.

Remodeling can also occur on mRNP transit through the nuclear pore complex or upon mRNA localization at specific sites within the cell. In yeast, the DEAD-box helicase Dbp5 localizes at the cytoplasmic side of the nuclear pore. Once mRNAs emerge from the nucleus at this site, the helicase strips off the export factors Mex67 and Nab2 (and potentially other factors) in an ATP-dependent fashion and allows their release into the cytoplasm [64,65]. Site-specific post-translational modifications in RBPs can also lead to reorganization of mRNPs. The zipcode-binding protein 1 (ZBP1), for example, inhibits the translation of bound β-actin mRNPs during their transport in neurons. Once the mRNP reaches its destination in axons, phosphorylation causes ZBP1 dissociation and the local translation of β-actin [66].

Remodeling of mRNPs may also be initiated by covalent alteration of the mRNA itself. This can occur by deadenylation, readenylation, uridylation, editing, and/or base modifications [67–69]. Obviously, such covalent modifications can change the binding of mRNP components and ultimately the function of the mRNP. For example, deadenylation causes dissociation of poly(A) binding proteins and consequently affects the translatability of mRNPs [70]. Important types of editing include adenosine to inosine changes, which are mediated by ADARs [71], as well as cytidine to uridine conversions mediated by apobec-1 [72]. Among the RNA modifications that affect mRNP composition, stability, and remodeling, the reversible methylation of adenosines to *N*6-methyladenosine (m^6A) is the most abundant and currently most intensely studied. This modification is very frequent in mammals (several thousand sites have been mapped in humans and mice) and is enriched in 5′-UTRs, around stop codons, and in 3′-UTRs adjacent to stop codons. m^6A modifications reportedly perform their function in mRNP biology via at least two different mechanisms: by altering the structure of the methylated transcripts to enable or inhibit mRNA interactions with *trans*-acting factors, or by serving as a platform for m^6A-binding proteins that elicit downstream events [59,73].

Hence, the emerging picture is that the mRNP can remain stable during particular periods of gene expression but will be remodeled when the mRNA enters the next functional stage of its life cycle. Similar to the initial establishment of the mRNP, its remodeling is an important process during the life of an mRNA, which may involve *trans*-acting factors and complex molecular mechanisms. This remodeling can occur in an active, ATP-driven manner, as exemplified by the action of helicases, or in a passive manner by simple association and dissociation events.

Reading and Regulating the mRNP Code

How is the information carried by components of the mRNP decoded? We will highlight here factors and mechanisms acting on mature mRNPs in the cytoplasm, but similar principles apply to mRNPs in the nucleus. The primary function of mRNPs is their translation into protein. The mRNP therefore carries factors and sequence information that not only allow the translation machinery to recognize and translate the mRNA but also permit translation to be regulated (reviewed in [74,75]). Regulators controlling the translation of specific mRNAs, such as miRNAs or RBPs, typically affect the rate of initiation, often by interfering with the cap-dependent assembly of the pre-initiation complex [74,76]. However, regulation at later steps has also been

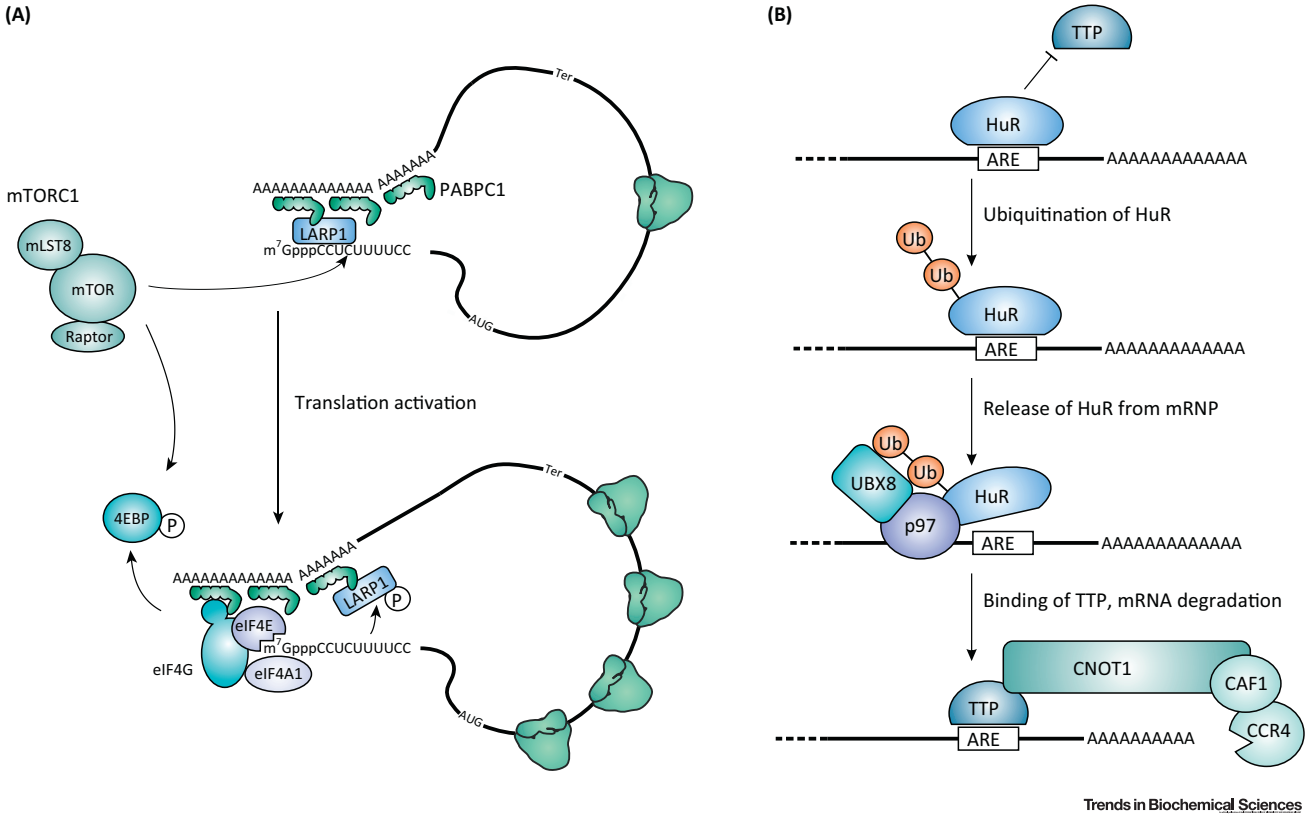

Figure 3. Regulation of Translation and Stability through RNA-Binding Proteins (RBPs). (A) Translation is often regulated by factors binding to the 5′ end of the messenger ribonucleoprotein particle (mRNP). Shown is a 5′-terminal oligopyrimidine tract (5′-TOP) mRNA whose 5′ end can bind to LARP1 or eIF4E (text for details). mTORC1 phosphorylation of LARP1 stimulates translation by dissociating the protein from 5′-TOP. Conversely, phosphorylation (P) leads to 4EBP dissociation from eIF4E and enables translation (text for details). (B) Regulation of mRNP stability by the HuR–thiamine pyrophosphate (TTP) axis. HuR binding to an AU-rich element (ARE) prevents association of the mRNA destabilizer TTP. Ubiquitinylation (Ub) of HuR allows its displacement from the mRNA through the segregase activity of p97/UBX8 and enables degradation. Abbreviations: m^7G, 7-methylguanosine; Ter, termination codon.

observed [77]. Two examples of RBP-mediated regulation of mRNP translation and stability are given in Figure 3.

mRNP translation is often controlled by specific signals arriving from inside or outside the cell. An interesting group of transcripts that shows tight regulation in response to mitogenic and nutritional signals are the mRNAs encoding ribosomal proteins and translation factors. These transcripts undergo normal processing in the nucleus but, owing to a defined 5′-terminal oligopyrimidine tract (5′-TOP), their translation can be selectively inhibited upon shortage of cellular resources [78]. A factor termed La-related protein 1 (Larp1) as a crucial component of the TOP mRNA-specific mRNP code has recently been linked to this regulation [79,80] (Figure 5A). Larp1 is phosphorylated by the kinase mTOR, a major nutrient sensor and signaling module in eukaryotes. In its phosphorylated state, Larp1 does not bind to the TOP motif, allowing translation of TOP mRNAs under normal growth conditions. Dephosphorylation of Larp1, however, increases its affinity for the TOP motif, which leads to the displacement of eIF4E and the sequestration of TOP mRNAs in translationally inactive mRNPs. Translation of other transcript classes can be regulated in a related manner by eIF4E-binding proteins (eIF4EBPs) [60]. Members of this protein family repress translation upon binding to eIF4E. Phosphorylation in response to various signals (for example UV irradiation or insulin signaling) results in their dissociation and the activation of translation.

The protein output from mRNA depends not only on the rate of translation but also on the stability of the mRNA, which again is regulated by both miRNAs and RBPs binding to mRNA-specific sequences, typically in the 3′-UTR. Prototypes of such regulatory sequences are the AU-rich elements (AREs). Probably the best-understood protein acting via binding to AREs is tristetraprolin, which destabilizes RNAs primarily by recruiting the CCR4–NOT complex, the major enzyme responsible for shortening the poly(A) tail [81]. miRNAs destabilize mRNAs by a similar mechanism [76]. Although most factors known to regulate mRNA half-life do so by promoting mRNA decay, proteins belonging to the HuR/ELAV family bind to AREs and lead to increased stability of bound mRNAs [82,83]. Interestingly, the ATPase p97 has been shown to release ubiquitinated HuR from the mRNP formed on p21 mRNA, thereby destabilizing the p21 transcript [84] (Figure 3B).

mRNPs that are translationally repressed or undergoing 5′ decay tend to aggregate in P bodies, stress granules, or related RNA–protein granules that are found in the early stages of development [85]. By contrast, mRNAs can be transported to specific parts of the cell to serve in localized translation. For example, this principle is used extensively to structure the early syncytial embryo of *Drosophila* [86]. A particularly well-studied case in simpler organisms is the SHE complex of *S. cerevisiae*, which transports specific mRNAs into the daughter cell during cell division [87].

Whereas the factors discussed in this section belong to the group of proteins targeting only a limited set of mRNAs in a specific context, more multifunctional proteins have also been described. Heterogeneous nuclear ribonucleoprotein (hnRNP) proteins of the A class or SR proteins are well known as splicing factors but have more recently also been implicated in mRNA transport, translation, and stability issues. These examples illustrate that many mRNA-associated proteins can act individually on their bound targets. Their individual modes of action are very diverse but in many cases are well understood (reviewed in [88,89]). However, mRNPs always represent an ensemble of many different factors that may act in a combinatorial manner and whose functions need to be coordinated. How this is achieved is currently only beginning to be understood and is certainly a major challenge for future studies.

The mRNP Code and Human Disease

Because the mRNP composition determines the fate of any given mRNA in virtually all phases of its life cycle, it is perhaps not surprising that disturbances in mRNP composition or function are increasingly recognized as causes of human disease ([90] and references therein). One can distinguish two major groups of ‘mRNP-linked’ diseases. The first group comprises genetic disorders that affect the mRNA itself. Thus, mutations in splice sites, polyadenylation signals, or regulatory sequences affecting mRNA processing represent most likely >10% of all known human genetic diseases. Because those mutations typically affect only one (or a small number of) mRNA(s), the molecular defect and the disease phenotype are often predictable. The blood disease β-thalassemia, for example, can result from mutations in splice sites or the poly-adenylation signal of the β-globin mRNA, causing aberrant processing and, as a consequence, insufficient hemoglobin formation [91].

Diseases of the second group arise from mutations in components that act in *trans* on mRNAs. These diseases often display very complex etiologies because typically large sets of mRNPs are simultaneously affected *in vivo*. General pre-mRNA splicing factors are mutated in the eye disease retinitis pigmentosa, leading to blindness [92–94]. Overexpression of the alternative splice factor SRSF1, by contrast, leads to malignant transformation by affecting splice patterns [95]. Whereas the above-mentioned diseases arise from aberrant mRNA processing, many neuropsychiatric diseases such as fragile X syndrome and schizophrenia are caused by the lack of, or defects in, regulatory mRNA binding proteins [45]. In these cases, the mRNPs that are

bound and/or influenced by these proteins appear to be altered. In most cases, however, the disease-causing consequences for mRNA metabolism are unclear. Systematic investigation of the mRNP composition and function under normal and disease conditions will be a central aspect of future studies to achieve a deeper understanding of the above-mentioned and many other human disorders [96].

Concluding Remarks

As we have outlined in this review, mRNPs undergo an elaborate pathway of assembly and maturation before being decoded by the translation machinery. These post-transcriptional events are enabled and regulated by factors interacting in *trans* with the mRNA backbone. Although today we have a good knowledge of the biochemical composition of mRNPs as a bulk entity, and many RBPs have been identified, our knowledge about the assembly, composition, and adjustment of individual mRNP species at different functional stages has remained limited. Therefore, an improved methodology as well as interdisciplinary approaches will be necessary to draw a comprehensive picture of mRNPs in eukaryotes.

While current techniques often use UV irradiation to detect proteins that directly interact with mRNA, these methods fail to identify components of mRNPs that do not bind directly to mRNAs or that cannot be efficiently crosslinked. Further improvements in the specificity and sensitivity of **mRNA interactome capture** methods will enable the detection of transient or rare mRNP components. Finally, there is an urgent need for new methods that allow the purification of other components of mRNPs, particularly non-coding RNAs and metabolites (Box 1).

The development of **crosslinking and immunoprecipitation** (CLIP)-based techniques (iCLIP, PAR-CLIP, CLAP, CRAC) represented a major breakthrough in mapping protein–RNA interactions *in vivo* [97]. In recent years CLIP has been successfully utilized to identify the binding sites of many individual RBPs. We envisage that the construction of transcriptome-wide occupancy profiles for larger sets of RBPs will allow maps to be generated of virtually all potential RNA binding sites and their frequency of use by interacting proteins. Furthermore, it will be important to determine the affinities of RBPs within their normal cellular environment, and this will require the establishment of quantitative CLIP methods (Box 1).

In theory, each component of an mRNP could act independently of other components of the same particle. Accordingly, the fate of the mRNP as a whole would be determined by the sum of the effects of its individual components. However, individual mRNP components are likely to influence each other, and, in extreme cases, some components may have opposing effects depending on the specific context of the mRNP. Hence, to interpret individual mRNP compositions and to reliably predict the readout of the mRNP code, we need to gain insight into the functional interactions between different mRNP components. Structural biology has greatly contributed to our understanding of interactions between RBPs and their substrate RNAs. Although structures of RBPs bound to RNA are less broadly available than genome-wide RNA-binding data, they are often an indispensable element for functional analyses. Structural insight into complexes of non-canonical RNA-binding domains with RNA and of larger mRNP units will be an essential goal for future studies. Non-canonical or higher-order interactions may involve unstructured or flexible protein regions, and may therefore not be amenable to X-ray crystallography. However, recent exciting technological advances in **electron cryo-microscopy** or nuclear magnetic resonance (NMR) may soon allow these challenges to be tackled [98].

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Outstanding Questions

What is the composition (proteins, ncRNAs) of a given mRNP? How does the composition change during the life cycle of an mRNP?

How are mRNPs assembled *in vivo*, and what are the mechanisms of assembly? How are proteins specifically loaded or unloaded?

What proteins, albeit not directly interacting with the mRNA, are functional components of the mRNP?

How do multifunctional RBPs execute the correct function at the right time and right place?

How do the components of an mRNP act together in a combinatorial manner? How is the effect of RBPs with opposing functions calculated?

Is the binding of an RBP regulated by post-translational modifications or by the interaction with other proteins?

What are the functions of the newly identified RBPs? What are their binding sites in the transcriptome?

What is the structure of an mRNP? Is an mRNP compact or are there extended regions? How are long-distance interactions within an mRNP established?

What is the affinity of a given RBP to its binding site *in vivo*? How do RBPs chose between different possible binding sites?

Will identical mRNA molecules always form the same mRNP?

What is the minimum set of RBPs to form a functional mRNP?

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