Review

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Post-transcriptional gene silencing in a dynamic RNP world

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Abstract: MicroRNA (miRNA)-guided gene silencing is a key regulatory process in various organisms and linked to many human diseases. MiRNAs are processed from precursor molecules and associate with Argonaute proteins to repress the expression of complementary target mRNAs. Excellent work by numerous labs has contributed to a detailed understanding of the mechanisms of miRNA function. However, miRNA effects have mostly been analyzed and viewed as isolated events and their natural environment as part of complex RNA-protein particles (RNPs) is often neglected. RNA binding proteins (RBPs) regulate key enzymes of the miRNA processing machinery and furthermore RBPs or readers of RNA modifications may modulate miRNA activity on mRNAs. Such proteins may function similarly to miRNAs and add their own contributions to the overall expression level of a particular gene. Therefore, post-transcriptional gene regulation might be more the sum of individual regulatory events and should be viewed as part of a dynamic and complex RNP world.

Keywords: microRNA; RBP; m6A; gene silencing; deadenylation

1 Introduction

When cells change their gene expression program due to external stimuli or during proliferation or differentiation processes, for example, not only transcriptional activity but also post-transcriptional RNA regulation is required to establish robust and homeostatic gene expression patterns. Post-transcriptional gene regulation has been the focus of intense research during the past decades and includes diverse mechanisms directly acting on the transcribed RNA. Its significance is now recognized as a crucial regulatory cellular process and has been associated with numerous diseases. To facilitate these regulatory processes, RNA binding proteins (RBPs) or non-coding RNAs such as microRNAs (miRNAs) contact the RNA and initiate downstream effects on translation efficiency or mRNA stability. More recently, mRNA modifications, commonly referred to as the epi-transcriptome, have also been associated with regulation of gene expression. It is becoming more and more apparent that all these regulatory events can function in parallel on one mRNA and need to be integrated in order to assess the overall level of gene regulation. In addition, such pathways might cross talk and form silenced messenger ribonucleoprotein particles (mRNPs) that often condensate into granules (referred to as biomolecular condensates). This review summarizes findings on miRNA-guided gene silencing and highlights parallels and differences to other gene regulatory pathways.

2 Components and mechanisms of human miRNA-guided gene silencing

MiRNAs are small regulatory RNAs that are present in almost all eukaryotes (Bartel 2018; Treiber et al. 2019). They are encoded in the genome and are transcribed by RNA polymerase II to capped and polyadenylated primary transcripts (Cai et al. 2004; Lee et al. 2004a, b). In the nucleus, the microprocessor complex composed of the RNase III enzyme Drosha and its double stranded (ds) RNA binding partner DGCR8 process such transcripts to hairpins often referred to as miRNA precursors or pre-miRNAs (Denli et al. 2004; Han et al. 2004; Landthaler et al. 2004; Lee et al. 2003). Recent structural and biochemical work shed light on the architecture of the microprocessor. It is composed of two molecules of DGCR8 and one molecule of Drosha (Kwon et al. 2016; Nguyen et al. 2015; Partin et al. 2020). DGCR8 binds the loop of the hairpin and helps positioning Drosha correctly on its

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RNA substrate. Interestingly, DGCR8 binds a heme molecule, which appears to be important for Drosha positioning (Faller et al. 2007; Nguyen et al. 2018; Partin et al. 2017; Quick-Cleveland et al. 2014; Weitz et al. 2014). However, not all hairpin structures are Drosha substrates. Several sequence and structural features have been identified that affect Drosha processing efficiency (Fang and Bartel 2015; Kang et al. 2021; Kim et al. 2021; Rice et al. 2020). The pre-miRNA products are transported to the cytoplasm by the export factor Exportin5 (Bohnsack et al. 2004; Lund et al. 2004; Yi et al. 2003). In the cytoplasm, the RNase III enzyme Dicer cleaves the pre-miRNA and produces a short dsRNA of about 20–24 nucleotides (nts) in length (Grishok et al. 2001; Ketting et al. 2001). Dicer enzymes are widespread and many organisms express several Dicer variants that are specialized on distinct small RNA classes (Henderson et al. 2006; Lee et al. 2004a, b). Besides the two catalytic RNase III domains, a helicase and several other domains, Dicer enzymes possess specific pockets that accommodate the 3′ and the 5′ end of the pre-miRNA substrate. Dicer enzymes can be divided into two classes. Processive Dicer enzymes hydrolyze ATP and actively move along a dsRNA substrate, which is channeled through the helicase domain. They subsequently cleave off 21–23 nt long dsRNAs from the ends of the long dsRNA (Su et al. 2022; Yamaguchi et al. 2022). Non-processive Dicers, such as human Dicer, do not use ATP. The helicase domain is flexibly linked to the core of Dicer and moves up and down to load and accommodate the pre-miRNA during the dicing process (Jouravleva et al. 2022; Lee et al. 2023a,b; Zapletal et al. 2022). Although critical residues are fully conserved, the helicase domain has acquired a different function.

In human, Dicer, with the help of the dsRNA binding protein TRBP, loads the strand with the less stably paired 5′ end directly to a member of the Argonaute protein family to form the RNA-induced silencing complex (RISC) (Khvorova et al. 2003; Noland et al. 2011; Schwarz et al. 2003). MiRNA containing Argonaute proteins are Ago1, 2, 3 and 4 (abbreviated as Ago proteins in the following to distinguish them from Piwi proteins, which also belong to the Argonaute protein family) in human cells and a miRNA-carrying RISC is often referred to as miRISC. During loading, Ago proteins are kept in an open conformation allowing to take over the mature miRNA directly from Dicer (Willkomm et al. 2022). Chaperones such as Hsp90 together with associated co-chaperones stabilize the open conformation and leave the loaded Ago protein when it is transferred to a closed and functional conformation (Iwasaki et al. 2010; Miyoshi et al. 2010). Ago proteins are composed of four distinct domains: the N-domain, the PAZ domain, the MID domain and the PIWI domain. While the N-domain helps loading the small RNA (Kwak and Tomari 2012), the PAZ domain binds the 3′ end and the MID domain the 5′ end of the small RNA (Lingel et al. 2003; Ma et al. 2005; Parker et al. 2005; Schirle and MacRae 2012; Song et al. 2003). The PIWI domain is structurally related to RNase H and some Ago proteins are indeed endonucleases and thus referred to as Slicers. For slicing, Ago proteins contain a conserved catalytic tetrad (Nakanishi 2022; Nakanishi et al. 2012). In human, Ago2 is the only slicer enzyme guided by small RNAs such as miRNAs or siRNAs (Liu et al. 2004; Meister et al. 2004). However, the catalytic tetrad is conserved in Ago3 and it has been shown that an Ago3-specific insertion at the N-terminus inactivates it when loaded with a 21 nt long RNA (Hauptmann et al. 2013; Nakanishi et al. 2013; Schurmann et al. 2013). Interestingly, recent work has shown that Ago3 can accommodate smaller RNA species (referred to as tinyRNAs), which can serve as guides for Ago3-mediated cleavage of complementary RNAs (Park et al. 2020). The physiological relevance and context of this activity, however, is still under investigation. Recently, a role of Mn^{2+}-dependent trimming by the exoribonuclease ISG-20 has been proposed (Sim et al. 2022).

2.1 Cytoplasmic functions of Ago proteins

The best understood aspect of Ago protein function in human cells is miRNA-guided gene silencing in the cytoplasm. MiRNAs guide Ago proteins to specific target sites on mRNAs, which are mainly located in 3′ UTRs. Biophysical and also structural studies revealed that Ago proteins arrange the miRNA in a very distinct conformation. The miRNA itself is characterized by the so-called seed sequence ranging from nts 2–7 or 2–8, a central region that is typically not paired with the mRNA target and a 3′ complementary region that can contribute to miRNA-mRNA pairing but is often not essential (reviewed in (Kilikevicius et al. 2022)). The seed region is pre-ordered and behaves more like an RBP allowing for lateral diffusion on the mRNA for target site identification (Klum et al. 2017). When a target site is recognized, it becomes stably bound by forming an RNA-RNA helix between the seed and the complementary target sequence. For silencing, a member of the TNRC6 protein family is recruited (commonly referred to as GW proteins) (Liu et al. 2005; Meister et al. 2005; Rehwinkel et al. 2005). These remarkable proteins are largely unstructured and bind with two tryptophans (Ws) into specific binding pockets located on the surface of the PIWI domain of Ago proteins (Elkayam et al. 2017; Pfaff et al. 2013; Schirle and MacRae 2012). TNRC6 proteins form a large scaffold and directly bind to the poly(A) binding protein on the poly(A) of the target mRNA and also recruit deadenylases such as the CCR4-NOT complex or PAN2/3 to the mRNA (Braun et al. 2011; Chekulaeva et al. 2011;

Fabian et al. 2009; Huntzinger et al. 2010, 2012; Mathys et al. 2014). Subsequent deadenylation leads to recruitment of the decapping complex to the 5′ cap (Rehwinkel et al. 2005). Decapped mRNA targets are rapidly degraded by the 5′–3′ exoribonuclease Xrn1 (Braun et al. 2012) (Figure 1).

TNRC6 proteins are large scaffold proteins of about 180 kDa in size, which form biomolecular condensates known as cytoplasmic P-bodies (Eystathioy et al. 2002; Liu et al. 2005; Sheu-Gruttadauria and MacRae 2018). Furthermore, TNRC6 proteins can interact with at least three Ago proteins simultaneously and thus form large macroscopically visible condensates (Elkayam et al. 2017; Pfaff et al. 2013; Sheu-Gruttadauria and MacRae 2018). Interestingly, binding of TNRC6 proteins to multiple Ago proteins can enhance the silencing effect suggesting biochemical cooperativity, i.e. binding of an Ago protein to one target site enhances the affinity to a second target site (Briskin et al. 2020). TNRC6 proteins may link Ago proteins on either the same mRNA or different mRNA molecules and thus mediate condensation or recruit such silenced mRNPs to already existing condensates. Contrary, it has been shown that macroscopically visible P-bodies are not essential for miRNA-guided gene silencing and thus the physiological role of P-body condensation in miRNA-guided gene silencing remains still elusive (Eulalio et al. 2007).

MiRNA-guided gene silencing is tightly regulated. Among many other principles, phosphorylation of Ago proteins has been shown to affect gene silencing efficiency. Phospho-proteomics studies identified various residues on Ago proteins that are modified by phosphorylation. For example, Akt-mediated S387 phosphorylation affects localization of Ago proteins in P-bodies and Ago2 cleavage activity (Horman et al. 2013; Zeng et al. 2008). Phosphorylation of a specific residue in the 5′ phosphate binding pocket interferes

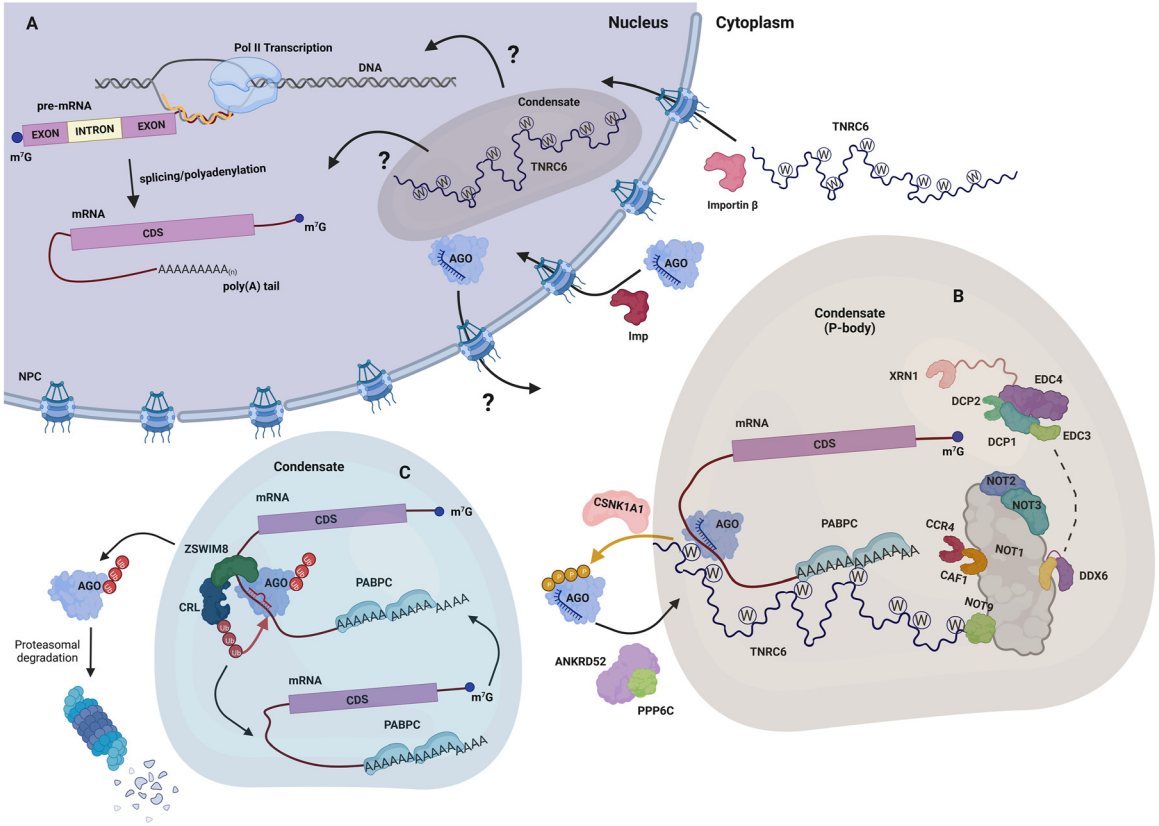

Figure 1: miRNA-guided gene regulation processes and regulation. **(**A) AGO and TNRC6 proteins can shuttle between the nucleus and the cytoplasm. Importin-β and other import receptors are required for nuclear import. Potential export processes are largely unknown. In the nucleus, TNRC6 proteins can form condensates. Ago proteins have been implicated in transcription and nuclear mRNA processing. (B) Regulation of mRNA expression through miRNA-guided deadenylation and subsequent decapping and degradation processes. Both TNRC6 and Ago proteins can form cytoplasmic condensates known as P-bodies. A phosphorylation cycle involving the kinase CSNK1A1 and the phosphates PPP6C and its binding partner ANKRD52 have been shown to be important for Ago dissociation from mRNAs. (C) Extensive pairing of the 3′ end of the miRNA with its target mRNA induces structural changes in Ago that are recognized by the ZSWIM8 ubiquitin ligase, which in turn polyubiquitinates Ago. This tags it for proteasomal degradation. The liberated miRNA is subsequently degraded and therefore, this process is referred to as target-directed miRNA degradation (TDMD). Figure created with BioRender.com.

with miRNA binding (Rudel et al. 2011). Where this modification is physiologically relevant is still unclear. Phosphorylation of Y393 by EGFR modulates binding to Dicer and loading of a specific group of miRNAs (Shen et al. 2013). More recently, we and others have identified a phospho-cluster on human Ago proteins at the very C-terminus (824–834 in human Ago2) (Golden et al. 2017; Quevillon Huberdeau et al. 2017). This cluster appears to be conserved on miRNA-associated Ago proteins in various species. A hierarchical hyper-phosphorylation of this cluster leads to dissociation of Ago proteins from target mRNAs without affecting miRNA binding (Bibel et al. 2022). A genome-wide CRISPR screening approach identified CSNK1A1 as one of the kinases that contribute to hyper-phosphorylation (Golden et al. 2017). It is, however, likely that after a priming phosphorylation event, other kinases might add phosphates to the cluster until a negative charge threshold is reached and the target mRNA dissociates. Such a mechanism would allow for an efficient crosstalk between miRNA-guided gene regulation and various signaling pathways. The same screen also identified two subunits of the PP6 phosphatase complex, the catalytic subunit PP6C together with its regulatory subunit ANKRD52, that can dephosphorylate the cluster (Golden et al. 2017) (Figure 1). The function of the kinase and phosphatase might argue in favor of a phosphorylation cycle allowing Ago proteins to associate and dissociate from target mRNAs upon specific phosphorylation signals. Such a model raises several intriguing questions. First, is there miRNA specificity and if so, how is it achieved? Moreover, how are kinases and phosphatases recruited to Ago proteins? Are there different factors involved that may sense the miRNA load of a specific Ago protein? Future research will help to answer these exciting questions and may also elucidate potential links to disease.

Unloaded Ago proteins are unstable and rapidly degraded by the proteasome (Smibert et al. 2013). In addition, it has been realized that target RNAs with a high degree of complementarity to the miRNA induce miRNA degradation, a process commonly referred to as target-directed miRNA degradation (TDMD). For example, several herpesviruses produce a non-coding RNA that induces the degradation of host miR-27 (Buck et al. 2010; Cazalla et al. 2010; Lee et al. 2013; Libri et al. 2012; Marcinowski et al. 2012). Similarly, introduction of complementary antisense oligonucleotides also induces specific degradation of a miRNA. For degradation, the miRNA is first tailed with non-templated nucleotides, which triggers subsequent recognition by ribonucleases (Ameres et al. 2010). It was further recognized that also endogenous mRNA targets can affect miRNA levels by TDMD in neurons (Bitetti et al. 2018; de la Mata et al. 2015). Studies in mouse brain further identified an extended regulatory circuit composed of the circular RNA cdr1as, the non-coding RNA Cyrano, miR-7 and miR-671. In this complex network, Cyrano directs miR-7 degradation by triggering TDMD thus preventing repression of neuronal miR-7 targets including cdr1as, which is important for neuronal activity. High levels of miR-7 also stimulate cleavage of cdr1as by miR-671 (Hansen et al. 2011; Kleaveland et al. 2018; Piwecka et al. 2017). Cyrano-triggered TDMD is very efficient and was used to screen factors that are involved in this process. Interestingly, a ubiquitin ligase complex centered on ZSWIM8 was identified to ubiquitinate Ago proteins and initiate degradation by the proteasome (Figure 1) (Han et al. 2020; Shi et al. 2020). Furthermore, TDMD is essential in Drosophila since disrupting this pathway leads to embryonic lethality (Kingston et al. 2022). Mechanistically, recent structural as well as single molecule (sm)FRET studies have demonstrated that TDMD targets lock Ago proteins in a specific conformation allowing exoribonucleases or other enzymes to access the miRNA 3′ end (Sheu-Gruttadauria et al. 2019; Willkomm et al. 2022). Taken together, insights into the mechanisms of TDMD are still scarce and it will be interesting to unravel how widespread such target sites in humans are and how important shaping of the miRNAome by highly complementary targets is for pathogenesis.

2.2 Nuclear localization and functions of human Ago and TNRC6 proteins

In many organisms such as plants, fungi and nematodes, specific Argonaute proteins are found in the nucleus and function in transcriptional gene silencing (Gutbrod and Martienssen 2020; Holoch and Moazed 2015; Ketting and Cochella 2021). Human miRNA-specific Ago proteins have been found in the nucleus as well and various functions have been proposed (Gagnon and Corey 2012; Gagnon et al. 2014). For example, early work showed that Ago proteins can be targeted to promoter regions and either silence or enhance gene expression (Chu et al. 2010; Janowski et al. 2006). Whether such effects are directly acting on transcription or through silencing of promoter-associated regulatory transcripts, is only poorly understood and requires further investigation. In addition, Ago proteins have been associated with DNA double strand break repair mechanisms in plants, flies and human (Francia et al. 2012; Michalik et al. 2012; Wei et al. 2012). Finally, alternative splicing could also be affected by nuclear Ago proteins (Allo et al. 2009; Ameyar-Zazoua et al. 2012; Chu et al. 2021; Johnson et al. 2021).

Due to sometimes inconsistent results obtained from immunofluorescence experiments and biochemical fractionations combined with moderate effects in functional assays, nuclear Ago protein functions in human cells remain

a matter of debate. Under steady state conditions, only a minor portion of Ago as well as TNRC6 proteins are detected in nuclei of tissue culture cells using immunofluorescence. However, it was demonstrated that rather specific cell types such as stem cells contain a large Ago-TNRC6 pool in their nuclei (Sarshad et al. 2018). Human TNRC6A contains a nuclear export signal suggesting shuttling between the nucleus and the cytoplasm (Nishi et al. 2013). Interestingly, when the nuclear export signal (NES) is mutated, TNRC6A is trapped and forms condensates in the nucleus clearly demonstrating shuttling activity of this TNRC6 protein (Figure 1). Importin-β is required for this localization and when CRM1-mediated export is inhibited by adding leptomycin b, TNRC6 proteins are retained in the nucleus (Schraivogel et al. 2015). Similarly, Ago proteins tend to accumulate in the nucleus when CRM1 is blocked suggesting that these proteins shuttle as well. Interestingly, TNRC6 or Ago proteins are more abundant in the nucleus when the direct binding partner is reduced in the cytoplasm. We and others thus proposed a model in which nuclear localization of proteins of the Ago and the TNRC6 family are a consequence of balancing equimolar cytoplasmic levels of Ago and TNRC6 proteins (Kalantari et al. 2016; Nishi et al. 2015; Schraivogel and Meister 2014; Schraivogel et al. 2015).

3 Small RNA-guided gene silencing factors in an RNA-protein world

Naked RNA is rarely found in cells and is always associated with proteins. Thus, RNAs should be viewed as RNPs. Moreover, the composition of such RNPs is dynamically changing throughout maturation steps and the lifetime of an RNA molecule (Gehring et al. 2017).

Primary miRNA transcripts (pri-miRNAs) are co-transcriptionally processed (Morlando et al. 2008). Since many miRNA genes are found in introns of human genes, pri-miRNA processing and splicing needs to be coordinated. Nuclear RBPs that associate with the transcription machinery and sequence-specifically interact with RNA transcripts may help to guide RNA maturation processes depending on the cellular demands. Particularly, RBPs can occupy miRNA hairpins and thus inhibit processing. Vice versa, it is also conceivable that RBPs recruit Drosha or Dicer by direct interaction or changing the structure of the miRNA precursor (a recent summary of RBPs involved in miRNA maturation can be found in Treiber et al. 2019). Most prominently, the RBP LIN28a binds to specific sequence motifs present in the loop of members of the let-7 pre-miRNA family and induces decay in stem cells (Heo et al. 2008; Piskounova et al. 2008). To achieve this, LIN28a recruits a TUTase (TUT4 or 7), which adds an oligo-U tail to the 3′ end (Heo et al. 2009; Thornton et al. 2014). Such a tail attracts the 3′–5′ exoribonuclease DIS3L2, which subsequently degrades the pre-miRNA (Chang et al. 2013; Faehnle et al. 2014; Ustianenko et al. 2013). In addition, it has also been shown that pre-miRNA can be mono-uridylated which leads to an optimal Dicer substrate or even miRNA strand switch (Kim et al. 2020).

The lupus autoantigen La is a specialized RBP that interacts with a short poly-U tail of polymerase III transcripts. It can serve as RNA chaperone and support folding of transcripts such as tRNA precursors (pre-tRNAs) (Bayfield and Maraia 2009; Naeeni et al. 2012). Interestingly, in the absence of La, some tRNAs can adopt alternative structures such as hairpins. These pre-tRNA structures are in equilibrium and La shifts it towards correct tRNA folding. When La is not present, these hairpins become Dicer substrates and accidentally end up as miRNAs (Hasler et al. 2016). These examples highlight the complex and dynamic interplay between RBPs and miRNA transcripts during miRNA biogenesis.

In miRNA-guided gene silencing, not only pre-miRNAs during miRNA maturation are bound by RBPs. Ago proteins are also embedded into dynamic mRNPs during gene silencing. In such silenced mRNPs, Ago proteins may crosstalk with other RBPs to enhance or weaken silencing effects. Furthermore, four different Ago proteins exist in humans and it is still not understood what their individual functions are. RIP and miRNA-seq experiments revealed that the four Ago proteins may bind similar subsets of RNAs suggesting redundant functions (Dueck et al. 2012). However, some RNA specificity has also been reported and thus it is possible that individual functions have evolved (Burroughs et al. 2011; Landthaler et al. 2008). Since TNRC6 proteins are large scaffolding proteins with potentially many interactions within a silenced mRNP, it is conceivable that Ago proteins are linked with each other or even with other RBPs in cis on the same RNA target or even in trans on different mRNAs. Consistently, recent work suggests that binding of Ago proteins to 3′ UTRs alone does not well predict gene repression and a more complex interplay between many components on 3′ UTRs might be more realistic (Chu et al. 2020). Therefore, gene silencing in the context of a dynamic mRNP is still not well understood and an exciting aspect for future research.

Mass spectrometry analysis have characterized the interaction landscape of Ago and TNRC6 proteins in the nucleus and the cytoplasm of human cells (Frohn et al. 2012; Hicks et al. 2017; Hock et al. 2007; Kalantari et al. 2016; Meister et al. 2005; Suzawa et al. 2017; Weinmann et al. 2009). A general picture that emerges from all these interactome studies is that Ago proteins are embedded into RNA-dependent and to a lesser extent -independent interaction networks. Many of

these interactions are found both in the nucleus and the cytoplasm of human cells. Thus, these findings underscore a tightly knit regulatory meshwork, in which individual contributions may become important only in the context of the entire RNP interactome.

4 miRNA-guided gene silencing in the context of mRNPs

Human Ago proteins are a very specific class of RBPs since they need a miRNA in order to contact the target mRNAs which they regulate. Yet, similar regulatory processes are conducted by RBPs that do not need a particular guide and directly interact with the substrate RNA. In addition to the approximately 1000 miRNA genes that guide Ago proteins (Kim et al. 2021), over 2000 RBPs are present in the human genome (Corley et al. 2020). RBPs are characterized by RNA binding domains (RBDs), which bind to specific sequence motifs or structural elements (Corley et al. 2020; Katsantoni et al. 2023; Sasse et al. 2018). Many RBDs typically bind rather short sequence motifs and RBPs often possess multiple RBDs, which strongly increase binding specificity due to combined and thus more complex binding motifs (Hennig and Sattler 2015). Similar to miRNAs, the target mRNA repertoire of RBPs is often only partially understood. Different proteins have been found to affect miRNA-mediated gene silencing (Figure 2). Several scenarios can be envisioned how RBPs could influence miRNA-guided silencing as part of an mRNP (Figure 3A).

First, RBPs could directly interact with miRISC and either recruit or inhibit binding to the target mRNA. For example, the AU-rich RBP HuR can oligomerize on specific target mRNAs leading to dissociation of miRISC and loss of P-body localization (Bhattacharyya et al. 2006; Kundu et al. 2012; Tominaga et al. 2011; Young et al. 2012; Zhuang et al. 2013). Vice versa, it has also been reported that HuR can actively recruit miRISC to distinct mRNAs (Kim et al. 2009) suggesting that the complex interplay within an mRNP might be key for the regulatory outcome. Another factor that positively affects miRNA function is Imp8 (Weinmann et al. 2009). Although not an RBP, Imp8 appears to directly interact with Ago proteins and helps recruiting them to mRNA targets by unknown mechanisms. Interestingly, recent work has shown that import receptors can control phase separation of the ALS-associated RBP FUS in addition to their functions in nuclear import (Guo et al. 2018; Hofweber et al. 2018; Yoshizawa et al. 2018). It is tempting to speculate that Imp8 might engage in Ago function by modulating condensates such as P-bodies. However, this hypothesis needs to be evaluated.

Second, and probably more widespread as recognized so far due to technical limitations, is the modulation of local secondary RNA structures nucleated by RBP interactions. Such effects could also be positive or negative depending on whether a miRNA binding site is liberated from or included into a structure initiated by RBP binding. It has been reported that PUM1 binding to the 3′ UTR of the mRNA of the p27 tumor suppressor increases accessibility of miRISC carrying miR-221 and miR-222 (Kedde et al. 2010). Contrary, the RBP Dnd1 blocks target site access (Kedde et al. 2007). Another example for mRNA structure modulating RBPs is FMRP in complex with the RNA helicase MOV10 (Gregersen et al. 2014), which can affect accessibility of miRNA target sites in neurons (Kenny et al. 2014).

Third and perhaps more provocative, RBPs might directly bind to miRNAs and block integration into mRNPs. Despite several reports, it is rather unlikely that protein-free miRNA pools exist in a cell since the ends would not be protected and immediately attacked by ribonucleases. Since miRNAs are generated from dsRNA and very specifically transferred to Ago proteins during RISC loading (likewise involving the PAZ domains of Dicer and Ago proteins), a model in which miRNAs are associated with other RBPs is also not straight-forward. In agreement, depletion of Ago proteins from cellular extracts co-depletes the miRNA pool in such lysates (Hauptmann et al. 2015). It is nevertheless possible that such alternative miRNA-complexes exist in specific cellular compartments, which are not recapitulated by such biochemical studies (Janas et al. 2012). Furthermore, several studies reported that in extracellular vesicles that contain secreted miRNAs, Ago proteins can hardly be found (e.g. (Mukherjee et al. 2016)). In order to explain these observations, a mechanism how miRNAs bypass RISC loading or dissociate from Ago proteins is still awaiting discovery.

Some RBPs are well characterized and biological roles have been unraveled. Several RBPs can recruit deadenylases such as the CCR4-NOT complex to remove the poly(A) tail and silence the mRNA, a process reminiscent to miRNAs (Figure 4).

One example of RBPs that lead to mRNA silencing through deadenylation are members of the highly conserved Pumilio protein family (PUM/Puf proteins, reviewed in Nishanth and Simon 2020). Pumilio proteins contact RNA substrates through a conserved domain, referred to as PUM-homology domain or PUM-HD (Zamore et al. 1997). This domain contains eight rather short repeats each contacting one base of the RNA binding motif (Wang et al. 2002). Pumilio proteins have been implicated in processes as diverse as embryonic development (Lin et al. 2018), innate immunity (Liu et al. 2017) or general cellular pathways such as ribosome biogenesis (Qiu et al. 2014), for example. Among many other mechanisms that have been reported, some Pumilio proteins can recruit the CCR4-NOT

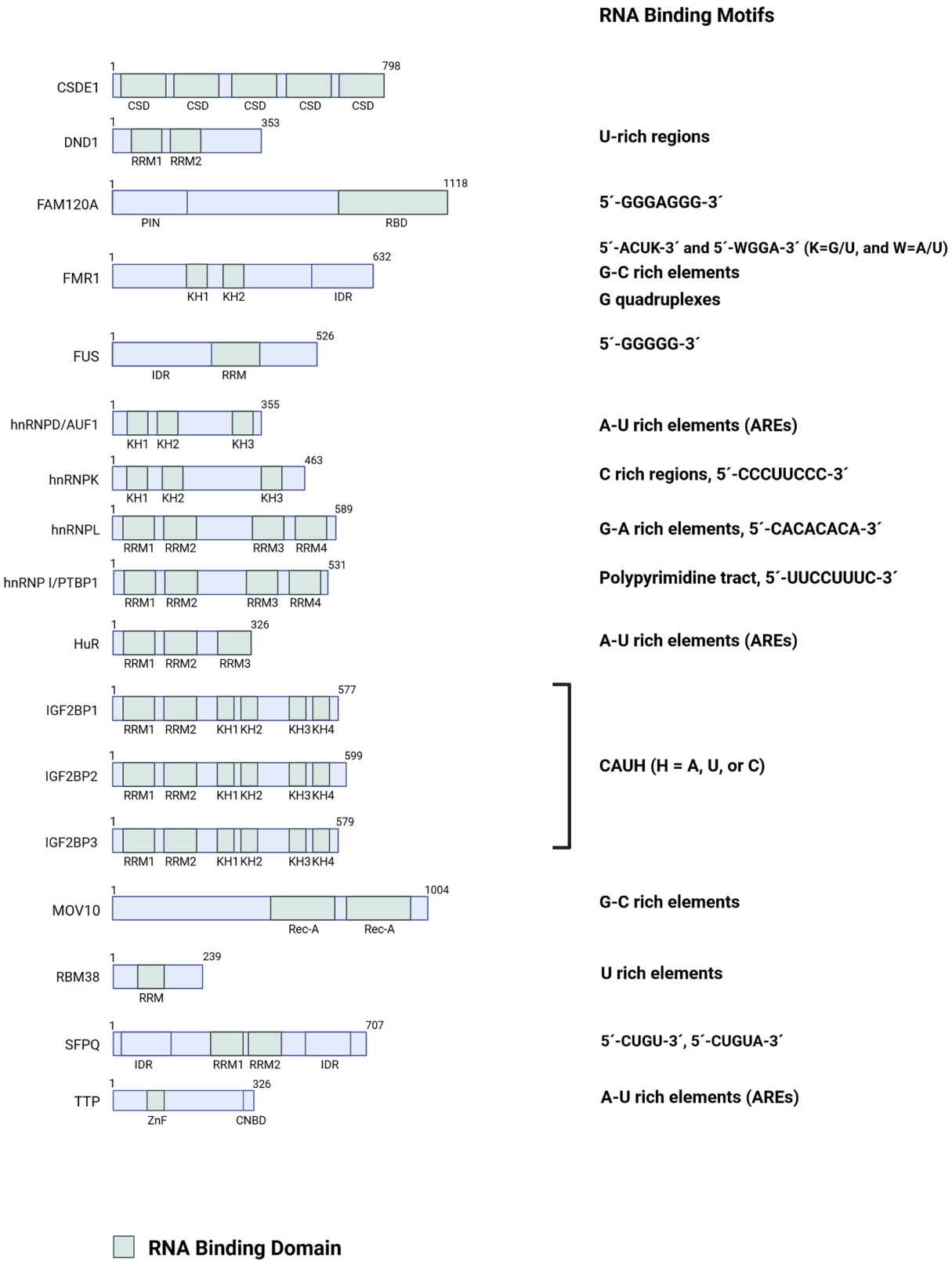

Figure 2: RBPs that have been reported to enhance or suppress miRNA function on specific mRNAs. Domain architecture: Cold Shock Domain [CSD]; RNA Recognition Motif [RRM]; PilT N terminus domain [PIN]; RNA-binding domain [RBD]; K Homology (KH) domain [KH]; Intrinsically Disordered Region [IDR]; Recombination-A domain [Rec-A]; Repression domains [RD]; Pumilio conserved motifs [PCMa and PCMb]; RING-finger E3 ubiquitin ligase domain [RING]; CNOT

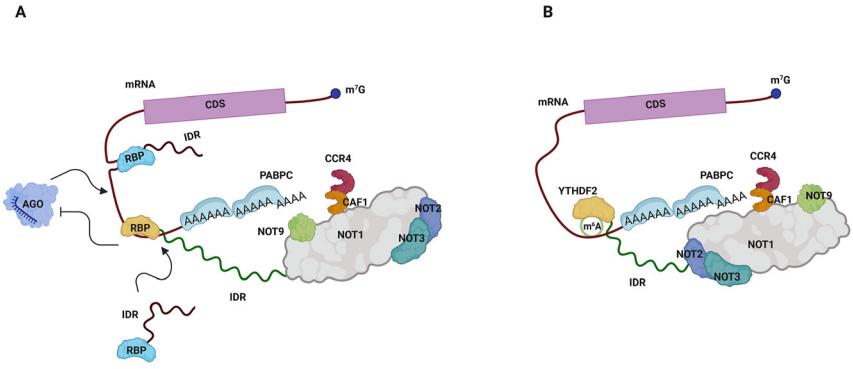

Figure 3: Mechanisms of post-transcriptional gene regulation by RBPs and m6A modification. (A) Synergistic or competitive effects of RNA binding proteins (RBPs) on miRNA-mediated mRNA decay. Such RBPs could either recruit or inhibit binding of miRISC to a specific target mRNA. Some of them can directly contact the CCR4-NOT complex for deadenylation. RBPs typically contain IDRs which engage in RNA or protein interactions. (B) m^6A-mediated mRNA decay. A very specific RBP, YTHDF2, that interacts with m6A can recruit the CCR4-NOT complex for mRNA deadenylation. Intrinsically disordered region [IDR]. Created with BioRender.com.

complex by a direct interaction with CNOT1 and CNOT2 to induce deadenylation-dependent RNA degradation (Arvola et al. 2020; Enwerem et al. 2021).

Another example for a deadenylase recruiting RBP is Nanos (NOS). Similarly to Pumilio proteins, NOS is important for development in various organisms. NOS proteins use a specialized Zn finger motif to contact its substrate RNA (Curtis et al. 1997). NOS can also directly interact with the CCR4-NOT complex through interaction with the NOT module and recruit it to specific target mRNAs (Bhandari et al. 2014; Raisch et al. 2019). Moreover, PUM and NOS have been found together in one repressive complex and since they contact CCR4-NOT at different sites, these concerted interactions could increase the affinity of the CCR4-NOT complex to specific RNAs. In Drosophila, NOS can enhance PUM-mediated repression (Weidmann and Goldstrohm 2012), which would be consistent with such a model. The two examples highlight that recruitment of deadenylase complexes can be facilitated by specific RBPs similarly to miRNAs (through the interaction with TNRC6 proteins). A summary of known RBPs that can recruit the CCR4-NOT complex is presented in Figure 4.

5 M6A-modification: deadenylation-guided by adenine methylation

Besides RBPs and miRNAs, also specific mRNA modifications can regulate gene expression through recruitment of deadenylases such as the CCR4-NOT complex. Methylation of the amino group at the N6 position of the adenine nucleobase (m6A) has emerged as one of the most abundant internal mRNA modifications in eukaryotes (Meyer et al. 2012). This modification is typically clustered around the stop codon and within the 3′ UTR and is found within an GG(m6A)C sequence motif refer to as DRACH motif (Fu et al. 2014). M6A is generated co-transcriptionally by a specific writer complex which is assembled around the catalytic core composed of METTL3/14 (Liu et al. 2014). Recent work reported a model for the enrichment of m6A at 3′ UTRs. The Exon-junction complex (EJC), which is deposited close to a splice junction after splicing, occupies the coding sequence (CDS). Therefore, the DRACH motifs within the CDS are not accessible. Motifs that are not protected by the EJC are accessible and found often close to the 3′ end (He et al. 2023; Uzonyi et al. 2023; Yang et al. 2022). Exceptions are also observed and it is possible that specific sites of distinct mRNAs can escape protection and can still be modified (Slobodin et al. 2017).

Accumulating data suggest that m6A can regulate the stability, processing or translation of the modified mRNA through the recruitment of specific proteins known as “readers” (Wang et al. 2014). The YT521-B homology domain family (YTHDF) proteins are cytoplasmic readers of m6A that selectively recognize m6A through the highly conserved YTH domain (Zhang et al. 2010). The human YTHDF family includes three members, YTHDF1, -2 and -3 and the crystal structure of the isolated YTH domain revealed an aromatic cage that specifically recognizes and stabilizes the m6A through stacking interactions (Zhang et al. 2010). YTHDF proteins are part of dynamic cytoplasmic mRNPs and

Binding Domain [CNBD]. References: CSDE1 (Kakumani et al. 2021); DND1 (Kedde et al. 2007); FAM120A (Katsantoni et al. 2023; Kelly et al. 2019); FMR1 (Ascano et al. 2012; Kenny et al. 2014); FUS (Van Nostrand et al. 2020; Zhang et al. 2018); hnRNPD/AUF1 (Min et al. 2017; Yoon et al. 2015); hnRNPK (Katsantoni et al. 2023; Shin et al. 2017); hnRNPL (Jafarifar et al. 2011; Katsantoni et al. 2023); hnRNPI/PTBP1 (Cui and Placzek 2018; Katsantoni et al. 2023); HuR (Bhattacharyya et al. 2006; Tominaga et al. 2011); IGF2BP1-3 (Busch et al. 2016; Elcheva et al. 2009; Muller et al. 2018; Van Nostrand et al. 2020); MOV10 (Kenny et al. 2014; Meister et al. 2005); RBM38 (Leveille et al. 2011; Yan et al. 2017); SFPQ (Bottini et al. 2017; Van Nostrand et al. 2020); TTP (Jing et al. 2005). Figure created with BioRender.com.

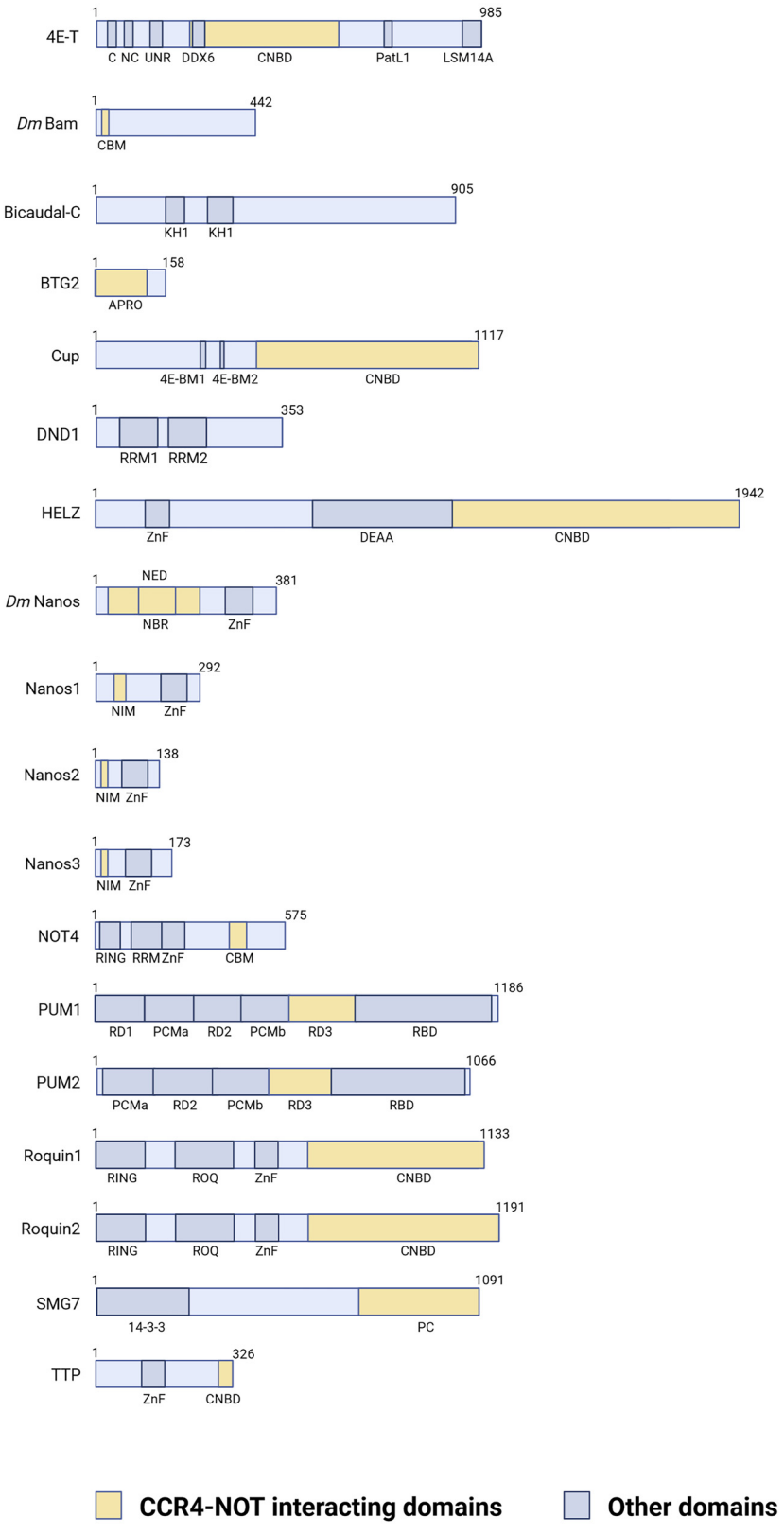

Figure 4: Identity and domain organization of CCR4-NOT recruiting RBPs. Domains interacting with the CCR4-NOT complex are highlighted in yellow. Domain architecture: (Dm) *Drosophila melanogaster*; CNOT Binding Domain [CNBD]; canonical eIF4E-binding motif [C]; noncanonical eIF4E-binding motif [NC]; UNR-interacting domain [UNR]; DDX6-interacting domain [DDX6]; PatL1-interacting domain [PatL1]; Lsm14A-interacting domain [Lsm14A]; CAF40-binding motif [CBM]; K Homology (KH) domain [KH]; AntiPROliferative domain [APRO]; eIF4E-binding motifs [4E-BM1 and 4E-BM2]; RNA Recognition Motif [RRM]; Zinc finger domain [Znf]; putative helicase (Asp, Glu, Ala, Ala) domain [DEAA]; NOT module binding region [NBR]; Nanos effector domain [NED]; NOT1-interacting motif [NIM]; repression domains [RD]; Pumilio conserved motifs [PCMa and PCMb]; RNA-binding domain [RBD]; RING-finger E3 ubiquitin ligase domain [RING]; ROQ RNA-binding domain [ROQ]; 14-3-3-like domain [14-3-3]; Proline-rich C-terminus [PC] region; References: 4E-T (Rasch et al. 2020); *Dm*Bam (Sgromo et al. 2018); Bicaudal-C (Chicoine et al. 2007); BTG2 (Stupfler et al. 2016); CUP (Igreja and Izaurralde 2011); DND1 (Yamaji et al. 2017); HELZ (Hanet et al. 2019); *Dm*Nanos, Nanos1, Nanos2 (Raisch and Valkov 2022; Suzuki et al. 2010); NOT4 (Keskeny et al. 2019); PUM1, PUM2 (Keskeny et al. 2019); Roquin1, Roquin2 (Glasmacher et al. 2010); SMG7 (Loh et al. 2013); TTP (Bulbrook et al. 2018; Fabian et al. 2013). Created with BioRender.com.

particularly YTHDF2 has been shown to regulate RNA decay in a methylation-dependent manner by recruiting the CCR4-NOT complex. This is mediated by directly binding the CNOT1 subunit (Wang et al. 2014). YTHDF2 is able to recruit the CCR4-NOT deadenylase complex by directly interacting with the superfamily homology (SH) domain of CNOT1, the

scaffolding subunit of the complex, through a small region in its N-terminus (residues 101–200) (Figure 3B) (Du et al. 2016). YTHDF1 has been shown to promote translation of modified mRNAs, further highlighting the diverse roles of m6A and its associated proteins in RNA metabolism (Lin et al. 2016; Meyer et al. 2015; Wang et al. 2015). YTHDF3 appears to play a dual role in the regulation of m6A-marked mRNAs by facilitating both mRNA translation and mRNA decay. Specifically, YTHDF3 is involved in mRNA decay through the recruitment of the PAN2/PAN3 deadenylase complex (Liu et al. 2020; Shi et al. 2017). In contrast, it was also reported that YTHDF1, 2, and 3 are rather similar and may play more redundant roles (Lasman et al. 2020; Zaccara and Jaffrey 2020). However, only YTHDF2 seems to interact with the CCR4-NOT complex. Since YTHDF2 is the largest paralog, specific interactions may be contributed by the unstructured N-terminal region. This may explain the many interactions that have been reported for YTHDF2. Besides the CCR4-NOT complex, YTHDF2 recruits UPF1 to induce decapping of m6A-marked mRNAs (Boo et al. 2022) and also the endonuclease complexes RNaseP/MRP to initiate the degradation of mRNAs and circRNAs (Park et al. 2019).

BioID and mass spectrometry analysis of Ago protein interaction networks in miRNA-containing and miRNA--depleted cells also showed that YTHDF2 is in a complex with Ago proteins suggesting a potential crosstalk between the two pathways (Frohn et al. 2012; Youn et al. 2018). However, a clear mechanistic link between miRNA- and m6A-guided cytoplasmic gene silencing still remains to be established. It is conceivable that, similar to RBPs, m6A modification may modulate local RNA structure and thus increase or decrease miRNA target site accessibility. However, it is not yet clear how much m6A modification contributes to structural changes. Future work will shed light on this exciting regulatory concept.

6 Conclusions and outlook

Taken together, miRNAs, RBPs and m6A readers contribute individually to gene regulation through recruiting deadenylases to the poly(A) tail of a mRNA. Individual effects could be weaker or stronger depending on the target site, the identity of the mRNA, cellular stages or developmental stages, for example. As highlighted above, even if there is no physical link between the three processes, the context of an mRNP may alter individual effects and regulation is likewise the sum of all individual contributions. Within silenced mRNPs, large scaffolding proteins such as TNRC6 proteins exist that form many interactions and also induce phase separation and the formation of condensates. It is tempting to speculate that such proteins not only facilitate individual regulatory processes but may also coordinate different pathways by direct physical interactions. Interestingly, it has been reported very recently that TNRC6 proteins can bind to RBPs other than Ago proteins and may connect such pathways (Welte et al. 2023). TNRC6 proteins are largely unstructured and such intrinsically disordered regions (IDRs) are known to establish interactions with RNAs and proteins. Moreover, many RBPs including YTHDF proteins also contain IDRs. Components of the CCR4-NOT complex contain such elements as well and it is likely that a dense meshwork of IDR interactions connects and coordinates gene regulatory pathways and as a consequence form biomolecular condensates such as P-bodies.

Although we only begin to understand the complexity of gene regulation in the context of mRNPs, it is becoming clearer and clearer that such dynamic structures are critical for normal cellular function. Dysregulation may contribute to disease states and developmental defects. Exciting novel insights can be expected in this area of research, which may even impact potential therapeutic developments.

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References

Allo, M., Buggiano, V., Fededa, J.P., Petrillo, E., Schor, I., de la Mata, M., Agirre, E., Plass, M., Eyras, E., Elela, S.A., et al. (2009). Control of alternative splicing through siRNA-mediated transcriptional gene silencing. Nat. Struct. Mol. Biol. 16: 717–724.

Ameres, S.L., Horwich, M.D., Hung, J.H., Xu, J., Ghildiyal, M., Weng, Z., and Zamore, P.D. (2010). Target RNA-directed trimming and tailing of small silencing RNAs. Science 328: 1534–1539.

Ameyar-Zazoua, M., Rachez, C., Souidi, M., Robin, P., Fritsch, L., Young, R., Morozova, N., Fenouil, R., Descostes, N., Andrau, J.C., et al. (2012). Argonaute proteins couple chromatin silencing to alternative splicing. Nat. Struct. Mol. Biol. 19: 998–1004.

Arvola, R.M., Chang, C.T., Buytendorp, J.P., Levdansky, Y., Valkov, E., Freddolino, P.L., and Goldstrohm, A.C. (2020). Unique repression domains of Pumilio

utilize deadenylation and decapping factors to accelerate destruction of target mRNAs. Nucleic Acids Res. 48: 1843–1871.

Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J.B., Nusbaum, J.D., Corcoran, D.L., Langlois, C., Munschauer, M., Dewell, S., Hafner, M., et al. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. Nature 492: 382–386.

Bartel, D.P. (2018). Metazoan MicroRNAs. Cell 173: 20–51.

Bayfield, M.A. and Maraia, R.J. (2009). Precursor-product discrimination by La protein during tRNA metabolism. Nat. Struct. Mol. Biol. 16: 430–437.

Bhandari, D., Raisch, T., Weichenrieder, O., Jonas, S., and Izaurralde, E. (2014). Structural basis for the Nanos-mediated recruitment of the CCR4-NOT complex and translational repression. Genes Dev. 28: 888–901.

Bhattacharyya, S.N., Habermacher, R., Martine, U., Closs, E.I., and Filipowicz, W. (2006). Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 125: 1111–1124.

Bibel, B., Elkayam, E., Silletti, S., Komives, E.A., and Joshua-Tor, L. (2022). Target binding triggers hierarchical phosphorylation of human Argonaute-2 to promote target release. eLife 11:e76908.

Bitetti, A., Mallory, A.C., Golini, E., Carrieri, C., Carreno Gutierrez, H., Perlas, E., Perez-Rico, Y.A., Tocchini-Valentini, G.P., Enright, A.J., Norton, W.H.J., et al. (2018). MicroRNA degradation by a conserved target RNA regulates animal behavior. Nat. Struct. Mol. Biol. 25: 244–251.

Bohnsack, M.T., Czaplinski, K., and Gorlich, D. (2004). Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10: 185–191.

Boo, S.H., Ha, H., Lee, Y., Shin, M.K., Lee, S., and Kim, Y.K. (2022). UPF1 promotes rapid degradation of m(6)A-containing RNAs. Cell Rep 39: 110861.

Bottini, S., Hamouda-Tekaya, N., Mategot, R., Zaragosi, L.E., Audebert, S., Pisano, S., Grandjean, V., Mauduit, C., Benahmed, M., Barbry, P., et al. (2017). Post-transcriptional gene silencing mediated by microRNAs is controlled by nucleoplasmic Sfpq. Nat. Commun. 8: 1189.

Braun, J.E., Huntzinger, E., Fauser, M., and Izaurralde, E. (2011). GW182 proteins directly recruit cytoplasmic deadenylase complexes to miRNA targets. Mol. Cell 44: 120–133.

Braun, J.E., Truffault, V., Boland, A., Huntzinger, E., Chang, C.T., Haas, G., Weichenrieder, O., Coles, M., and Izaurralde, E. (2012). A direct interaction between DCP1 and XRN1 couples mRNA decapping to 5′ exonucleolytic degradation. Nat. Struct. Mol. Biol. 19: 1324–1331.

Briskin, D., Wang, P.Y., and Bartel, D.P. (2020). The biochemical basis for the cooperative action of microRNAs. Proc. Natl. Acad. Sci. U. S. A. 117: 17764–17774.

Buck, A.H., Perot, J., Chisholm, M.A., Kumar, D.S., Tuddenham, L., Cognat, V., Marcinowski, L., Dolken, L., and Pfeffer, S. (2010). Post-transcriptional regulation of miR-27 in murine cytomegalovirus infection. RNA 16: 307–315.

Bulbrook, D., Brazier, H., Mahajan, P., Kliszczak, M., Fedorov, O., Marchese, F.P., Aubareda, A., Chalk, R., Picaud, S., Strain-Damerell, C., et al. (2018). Tryptophan-mediated interactions between tristetraprolin and the CNOT9 subunit are required for CCR4-NOT deadenylase complex recruitment. J. Mol. Biol. 430: 722–736.

Burroughs, A.M., Ando, Y., de Hoon, M.J., Tomaru, Y., Suzuki, H., Hayashizaki, Y., and Daub, C.O. (2011). Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. RNA Biol. 8: 158–177.

Busch, B., Bley, N., Muller, S., Glass, M., Misiak, D., Lederer, M., Vetter, M., Strauss, H.G., Thomssen, C., and Huttelmaier, S. (2016). The oncogenic triangle of HMGA2, LIN28B and IGF2BP1 antagonizes tumor-suppressive actions of the let-7 family. Nucleic Acids Res. 44: 3845–3864.

Cai, X., Hagedorn, C.H., and Cullen, B.R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 10: 1957–1966.

Cazalla, D., Yario, T., and Steitz, J.A. (2010). Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. Science 328: 1563–1566.

Chang, H.M., Triboulet, R., Thornton, J.E., and Gregory, R.I. (2013). A role for the Perlman syndrome exonuclease Dis3l2 in the Lin28-let-7 pathway. Nature 497: 244–248.

Chekulaeva, M., Mathys, H., Zipprich, J.T., Attig, J., Colic, M., Parker, R., and Filipowicz, W. (2011). miRNA repression involves GW182-mediated recruitment of CCR4-NOT through conserved W-containing motifs. Nat. Struct. Mol. Biol. 18: 1218–1226.

Chicoine, J., Benoit, P., Gamberi, C., Paliouras, M., Simonelig, M., and Lasko, P. (2007). Bicaudal-C recruits CCR4-NOT deadenylase to target mRNAs and regulates oogenesis, cytoskeletal organization, and its own expression. Dev. Cell 13: 691–704.

Chu, Y., Kilikevicius, A., Liu, J., Johnson, K.C., Yokota, S., and Corey, D.R. (2020). Argonaute binding within 32032-untranslated regions poorly predicts gene repression. Nucleic Acids Res. 48: 7439–7453.

Chu, Y., Yokota, S., Liu, J., Kilikevicius, A., Johnson, K.C., and Corey, D.R. (2021). Argonaute binding within human nuclear RNA and its impact on alternative splicing. RNA 27: 991–1003.

Chu, Y., Yue, X., Younger, S.T., Janowski, B.A., and Corey, D.R. (2010). Involvement of argonaute proteins in gene silencing and activation by RNAs complementary to a non-coding transcript at the progesterone receptor promoter. Nucleic Acids Res. 38: 7736–7748.

Corley, M., Burns, M.C., and Yeo, G.W. (2020). How RNA-binding proteins interact with RNA: molecules and mechanisms. Mol. Cell 78: 9–29.

Cui, J. and Placzek, W.J. (2018). PTBP1 enhances miR-101-guided AGO2 targeting to MCL1 and promotes miR-101-induced apoptosis. Cell Death Dis. 9: 552.

Curtis, D., Treiber, D.K., Tao, F., Zamore, P.D., Williamson, J.R., and Lehmann, R. (1997). A CCHC metal-binding domain in Nanos is essential for translational regulation. EMBO J. 16: 834–843.

de la Mata, M., Gaidatzis, D., Vitanescu, M., Stadler, M.B., Wentzel, C., Scheiffele, P., Filipowicz, W., and Grosshans, H. (2015). Potent degradation of neuronal miRNAs induced by highly complementary targets. EMBO Rep. 16: 500–511.

Denli, A.M., Tops, B.B., Plasterk, R.H., Ketting, R.F., and Hannon, G.J. (2004). Processing of primary microRNAs by the Microprocessor complex. Nature 432: 231–235.

Du, H., Zhao, Y., He, J., Zhang, Y., Xi, H., Liu, M., Ma, J., and Wu, L. (2016). YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat. Commun. 7: 12626.

Dueck, A., Ziegler, C., Eichner, A., Berezikov, E., and Meister, G. (2012). microRNAs associated with the different human Argonaute proteins. Nucleic Acids Res. 40: 9850–9862.

Elcheva, I., Goswami, S., Noubissi, F.K., and Spiegelman, V.S. (2009). CRD-BP protects the coding region of betaTrCP1 mRNA from miR-183-mediated degradation. Mol. Cell 35: 240–246.

Elkayam, E., Faehnle, C.R., Morales, M., Sun, J., Li, H., and Joshua-Tor, L. (2017). Multivalent recruitment of human argonaute by GW182. Mol. Cell 67: 646–658 e643.

Enwerem, III, Elrod, N.D., Chang, C.T., Lin, A., Ji, P., Bohn, J.A., Levdansky, Y., Wagner, E.J., Valkov, E., and Goldstrohm, A.C. (2021). Human Pumilio

proteins directly bind the CCR4-NOT deadenylase complex to regulate the transcriptome. RNA 27: 445–464.

Eulalio, A., Behm-Ansmant, I., Schweizer, D., and Izaurralde, E. (2007). P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol. Cell. Biol. 27: 3970–3981.

Eystathioy, T., Chan, E.K., Tenenbaum, S.A., Keene, J.D., Griffith, K., and Fritzler, M.J. (2002). A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. Mol. Biol. Cell 13: 1338–1351.

Fabian, M.R., Frank, F., Rouya, C., Siddiqui, N., Lai, W.S., Karetnikov, A., Blackshear, P.J., Nagar, B., and Sonenberg, N. (2013). Structural basis for the recruitment of the human CCR4-NOT deadenylase complex by tristetraprolin. Nat. Struct. Mol. Biol. 20: 735–739.

Fabian, M.R., Mathonnet, G., Sundermeier, T., Mathys, H., Zipprich, J.T., Svitkin, Y.V., Rivas, F., Jinek, M., Wohlschlegel, J., Doudna, J.A., et al. (2009). Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deadenylation. Mol. Cell 35: 868–880.

Faehnle, C.R., Walleshauser, J., and Joshua-Tor, L. (2014). Mechanism of Dis3l2 substrate recognition in the Lin28-let-7 pathway. Nature 514: 252–256.

Faller, M., Matsunaga, M., Yin, S., Loo, J.A., and Guo, F. (2007). Heme is involved in microRNA processing. Nat. Struct. Mol. Biol. 14: 23–29.

Fang, W. and Bartel, D.P. (2015). The menu of features that define primary MicroRNAs and Enable De Novo design of MicroRNA genes. Mol. Cell 60: 131–145.

Francia, S., Michelini, F., Saxena, A., Tang, D., de Hoon, M., Anelli, V., Mione, M., Carninci, P., and d'Adda di Fagagna, F. (2012). Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 488: 231–35.

Frohn, A., Eberl, H.C., Stohr, J., Glasmacher, E., Rudel, S., Heissmeyer, V., Mann, M., and Meister, G. (2012). Dicer-dependent and -independent Argonaute2 protein interaction networks in mammalian cells. Mol. Cell. Proteomics 11: 1442–1456.

Fu, Y., Dominissini, D., Rechavi, G., and He, C. (2014). Gene expression regulation mediated through reversible m(6)A RNA methylation. Nat. Rev. Genet. 15: 293–306.

Gagnon, K.T. and Corey, D.R. (2012). Argonaute and the nuclear RNAs: new pathways for RNA-mediated control of gene expression. Nucleic Acid Ther. 22: 3–16.

Gagnon, K.T., Li, L., Chu, Y., Janowski, B.A., and Corey, D.R. (2014). RNAi factors are present and active in human cell nuclei. Cell Rep. 6: 211–221.

Gehring, N.H., Wahle, E., and Fischer, U. (2017). Deciphering the mRNP code: RNA-bound determinants of post-transcriptional gene regulation. Trends Biochem. Sci. 42: 369–382.

Glasmacher, E., Hoefig, K.P., Vogel, K.U., Rath, N., Du, L., Wolf, C., Kremmer, E., Wang, X., and Heissmeyer, V. (2010). Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression. Nat. Immunol. 11: 725–733.

Golden, R.J., Chen, B., Li, T., Braun, J., Manjunath, H., Chen, X., Wu, J., Schmid, V., Chang, T.C., Kopp, F., et al. (2017). An Argonaute phosphorylation cycle promotes microRNA-mediated silencing. Nature 542: 197–202.

Gregersen, L.H., Schueler, M., Munschauer, M., Mastrobuoni, G., Chen, W., Kempa, S., Dieterich, C., and Landthaler, M. (2014). MOV10 is a 5′ to 3′ RNA helicase contributing to UPF1 mRNA target degradation by translocation along 3′ UTRs. Mol. Cell 54: 573–585.

Grishok, A., Pasquinelli, A.E., Conte, D., Li, N., Parrish, S., Ha, I., Baillie, D.L., Fire, A., Ruvkun, G., and Mello, C.C. (2001). Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell 106: 23–34.

Guo, L., Kim, H.J., Wang, H., Monaghan, J., Freyermuth, F., Sung, J.C., O'Donovan, K., Fare, C.M., Diaz, Z., Singh, N., et al. (2018). Nuclear-import receptors reverse aberrant phase transitions of RNA-binding proteins with prion-like domains. Cell 173: 677–692 e620.

Gutbrod, M.J. and Martienssen, R.A. (2020). Conserved chromosomal functions of RNA interference. Nat. Rev. Genet. 21: 311–331.

Han, J., LaVigne, C.A., Jones, B.T., Zhang, H., Gillett, F., and Mendell, J.T. (2020). A ubiquitin ligase mediates target-directed microRNA decay independently of tailing and trimming. Science 370: eabc9546.

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H., and Kim, V.N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev. 18: 3016–3027.

Hanet, A., Rasch, F., Weber, R., Ruscica, V., Fauser, M., Raisch, T., Kuzuoglu-Ozturk, D., Chang, C.T., Bhandari, D., Igreja, C., et al (2019). HELZ directly interacts with CCR4-NOT and causes decay of bound mRNAs. Life Sci. Alliance 2: e201900405.

Hansen, T.B., Wiklund, E.D., Bramsen, J.B., Villadsen, S.B., Statham, A.L., Clark, S.J., and Kjems, J. (2011). miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. EMBO J. 30: 4414–4422.

Hasler, D., Lehmann, G., Murakawa, Y., Klironomos, F., Jakob, L., Grasser, F.A., Rajewsky, N., Landthaler, M., and Meister, G. (2016). The Lupus Autoantigen La prevents mis-channeling of tRNA fragments into the human MicroRNA pathway. Mol. Cell 63: 110–124.

Hauptmann, J., Dueck, A., Harlander, S., Pfaff, J., Merkl, R., and Meister, G. (2013). Turning catalytically inactive human Argonaute proteins into active slicer enzymes. Nat. Struct. Mol. Biol. 20: 814–817.

Hauptmann, J., Schraivogel, D., Bruckmann, A., Manickavel, S., Jakob, L., Eichner, N., Pfaff, J., Urban, M., Sprunck, S., Hafner, M., et al. (2015). Biochemical isolation of Argonaute protein complexes by Ago-APP. Proc. Natl. Acad. Sci. U. S. A. 112: 11841–11845.

He, P.C., Wei, J., Dou, X., Harada, B.T., Zhang, Z., Ge, R., Liu, C., Zhang, L.S., Yu, X., Wang, S., et al. (2023). Exon architecture controls mRNA m(6)A suppression and gene expression. Science 379: 677–682.

Henderson, I.R., Zhang, X., Lu, C., Johnson, L., Meyers, B.C., Green, P.J., and Jacobsen, S.E. (2006). Dissecting Arabidopsis thaliana DICER function in small RNA processing, gene silencing and DNA methylation patterning. Nat. Genet. 38: 721–725.

Hennig, J. and Sattler, M. (2015). Deciphering the protein-RNA recognition code: combining large-scale quantitative methods with structural biology. Bioessays 37: 899–908.

Heo, I., Joo, C., Cho, J., Ha, M., Han, J., and Kim, V.N. (2008). Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA. Mol. Cell 32: 276–284.

Heo, I., Joo, C., Kim, Y.K., Ha, M., Yoon, M.J., Cho, J., Yeom, K.H., Han, J., and Kim, V.N. (2009). TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. Cell 138: 696–708.

Hicks, J.A., Li, L., Matsui, M., Chu, Y., Volkov, O., Johnson, K.C., and Corey, D.R. (2017). Human GW182 paralogs are the central organizers for RNA-mediated control of transcription. Cell Rep. 20: 1543–1552.

Hock, J., Weinmann, L., Ender, C., Rudel, S., Kremmer, E., Raabe, M., Urlaub, H., and Meister, G. (2007). Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. EMBO Rep. 8: 1052–1060.

Hofweber, M., Hutten, S., Bourgeois, B., Spreitzer, E., Niedner-Boblenz, A., Schifferer, M., Ruepp, M.D., Simons, M., Niessing, D., Madl, T., et al. (2018). Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. Cell 173: 706–719 e713.

Holoch, D. and Moazed, D. (2015). RNA-mediated epigenetic regulation of gene expression. Nat. Rev. Genet. 16: 71–84.

Horman, S.R., Janas, M.M., Litterst, C., Wang, B., MacRae, I.J., Sever, M.J., Morrissey, D.V., Graves, P., Luo, B., Umesalma, S., et al. (2013). Akt-mediated phosphorylation of Argonaute 2 downregulates cleavage and upregulates translational repression of MicroRNA targets. Mol. Cell 50: 356–367.

Huntzinger, E., Braun, J.E., Heimstadt, S., Zekri, L., and Izaurralde, E. (2010). Two PABPC1-binding sites in GW182 proteins promote miRNA-mediated gene silencing. EMBO J. 29: 4146–4160.

Huntzinger, E., Kuzuoglu-Ozturk, D., Braun, J.E., Eulalio, A., Wohlbold, L., and Izaurralde, E. (2012). The interactions of GW182 proteins with PABP and deadenylases are required for both translational repression and degradation of miRNA targets. Nucleic Acids Res 41: 978–994.

Igreja, C. and Izaurralde, E. (2011). CUP promotes deadenylation and inhibits decapping of mRNA targets. Genes Dev. 25: 1955–1967.

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., and Tomari, Y. (2010). Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. Mol. Cell 39: 292–299.

Jafarifar, F., Yao, P., Eswarappa, S.M., and Fox, P.L. (2011). Repression of VEGFA by CA-rich element-binding microRNAs is modulated by hnRNP L. EMBO J. 30: 1324–1334.

Janas, M.M., Wang, B., Harris, A.S., Aguiar, M., Shaffer, J.M., Subrahmanyam, Y.V., Behlke, M.A., Wucherpfennig, K.W., Gygi, S.P., Gagnon, E., et al. (2012). Alternative RISC assembly: binding and repression of microRNA-mRNA duplexes by human Ago proteins. RNA 18: 2041–2055.

Janowski, B.A., Huffman, K.E., Schwartz, J.C., Ram, R., Nordsell, R., Shames, D.S., Minna, J.D., and Corey, D.R. (2006). Involvement of AGO1 and AGO2 in mammalian transcriptional silencing. Nat. Struct. Mol. Biol. 13: 787–792.

Jing, Q., Huang, S., Guth, S., Zarubin, T., Motoyama, A., Chen, J., Di Padova, F., Lin, S.C., Gram, H., and Han, J. (2005). Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 120: 623–634.

Johnson, S.T., Chu, Y., Liu, J., and Corey, D.R. (2021). Impact of scaffolding protein TNRC6 paralogs on gene expression and splicing. RNA 27: 1004–1016.

Jouravleva, K., Golovenko, D., Demo, G., Dutcher, R.C., Hall, T.M.T., Zamore, P.D., and Korostelev, A.A. (2022). Structural basis of microRNA biogenesis by Dicer-1 and its partner protein Loqs-PB. Mol. Cell 82: 4049–4063 e4046.

Kakumani, P.K., Guitart, T., Houle, F., Harvey, L.M., Goyer, B., Germain, L., Gebauer, F., and Simard, M.J. (2021). CSDE1 attenuates microRNA-mediated silencing of PMEPA1 in melanoma. Oncogene 40: 3231–3244.

Kalantari, R., Hicks, J.A., Li, L., Gagnon, K.T., Sridhara, V., Lemoff, A., Mirzaei, H., and Corey, D.R. (2016). Stable association of RNAi machinery is conserved between the cytoplasm and nucleus of human cells. RNA 22: 1085–1098.

Kang, W., Fromm, B., Houben, A.J., Hoye, E., Bezdan, D., Arnan, C., Thrane, K., Asp, M., Johnson, R., Biryukova, I., et al. (2021). MapToCleave: high-throughput profiling of microRNA biogenesis in living cells. Cell Rep 37: 110015.

Katsantoni, M., van Nimwegen, E., and Zavolan, M. (2023). Improved analysis of (e)CLIP data with RCRUNCH yields a compendium of RNA-binding protein binding sites and motifs. Genome Biol. 24: 77.

Kedde, M., Strasser, M.J., Boldajipour, B., Vrielink, J.A., Slanchev, K., le Sage, C., Nagel, R., Voorhoeve, P.M., van Duijse, J., Orom, U.A., et al. (2007). RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. Cell 131: 1273–1286.

Kedde, M., van Kouwenhove, M., Zwart, W., Oude Vrielink, J.A., Elkon, R., and Agami, R. (2010). A Pumilio-induced RNA structure switch in p27-3′ UTR controls miR-221 and miR-222 accessibility. Nat. Cell Biol. 12: 1014–1020.

Kelly, T.J., Suzuki, H.I., Zamudio, J.R., Suzuki, M., and Sharp, P.A. (2019). Sequestration of microRNA-mediated target repression by the Ago2-associated RNA-binding protein FAM120A. RNA 25: 1291–1297.

Kenny, P.J., Zhou, H., Kim, M., Skariah, G., Khetani, R.S., Drnevich, J., Arcila, M.L., Kosik, K.S., and Ceman, S. (2014). MOV10 and FMRP regulate AGO2 association with microRNA recognition elements. Cell Rep. 9: 1729–1741.

Keskeny, C., Raisch, T., Sgromo, A., Igreja, C., Bhandari, D., Weichenrieder, O., and Izaurralde, E. (2019). A conserved CAF40-binding motif in metazoan NOT4 mediates association with the CCR4-NOT complex. Genes Dev. 33: 236–252.

Ketting, R.F. and Cochella, L. (2021). Concepts and functions of small RNA pathways in C. elegans. Curr Top Dev Biol. 144: 45–89.

Ketting, R.F., Fischer, S.E., Bernstein, E., Sijen, T., Hannon, G.J., and Plasterk, R.H. (2001). Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. 15: 2654–2659.

Khvorova, A., Reynolds, A., and Jayasena, S.D. (2003). Functional siRNAs and miRNAs exhibit strand bias. Cell 115: 209–216.

Kilikevicius, A., Meister, G., and Corey, D.R. (2022). Reexamining assumptions about miRNA-guided gene silencing. Nucleic Acids Res. 50: 617–634.

Kim, H., Kim, J., Yu, S., Lee, Y.Y., Park, J., Choi, R.J., Yoon, S.J., Kang, S.G., and Kim, V.N. (2020). A mechanism for microRNA arm switching regulated by uridylation. Mol. Cell 78: 1224–1236 e1225.

Kim, H.H., Kuwano, Y., Srikantan, S., Lee, E.K., Martindale, J.L., and Gorospe, M. (2009). HuR recruits let-7/RISC to repress c-Myc expression. Genes Dev. 23: 1743–1748.

Kim, K., Baek, S.C., Lee, Y.Y., Bastiaanssen, C., Kim, J., Kim, H., and Kim, V.N. (2021). A quantitative map of human primary microRNA processing sites. Mol. Cell 81: 3422–3439 e3411.

Kingston, E.R., Blodgett, L.W., and Bartel, D.P. (2022). Endogenous transcripts direct microRNA degradation in Drosophila, and this targeted degradation is required for proper embryonic development. Mol. Cell 82: 3872–3884 e3879.

Kleaveland, B., Shi, C.Y., Stefano, J., and Bartel, D.P. (2018). A network of noncoding regulatory RNAs acts in the mammalian brain. Cell. 174: 350–362 e317.

Klum, S.M., Chandradoss, S.D., Schirle, N.T., Joo, C., and MacRae, I.J. (2017). Helix-7 in Argonaute2 shapes the microRNA seed region for rapid target recognition. EMBO J 37: 75–88.

Kundu, P., Fabian, M.R., Sonenberg, N., Bhattacharyya, S.N., and Filipowicz, W. (2012). HuR protein attenuates miRNA-mediated repression by promoting miRISC dissociation from the target RNA. Nucleic Acids Res. 40: 5088–5100.

Kwak, P.B. and Tomari, Y. (2012). The N domain of Argonaute drives duplex unwinding during RISC assembly. Nat. Struct. Mol. Biol. 19: 145–151.

Kwon, S.C., Nguyen, T.A., Choi, Y.G., Jo, M.H., Hohng, S., Kim, V.N., and Woo, J.S. (2016). Structure of human DROSHA. Cell 164: 81–90.

Landthaler, M., Gaidatzis, D., Rothballer, A., Chen, P.Y., Soll, S.J., Dinic, L., Ojo, T., Hafner, M., Zavolan, M., and Tuschl, T. (2008). Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. RNA 14: 2580–2596.

Landthaler, M., Yalcin, A., and Tuschl, T. (2004). The human DiGeorge syndrome critical region gene 8 and its D. melanogaster homolog are required for miRNA biogenesis. Curr. Biol. 14: 2162–2167.

Lasman, L., Krupalnik, V., Viukov, S., Mor, N., Aguilera-Castrejon, A., Schneir, D., Bayerl, J., Mizrahi, O., Peles, S., Tawil, S., et al. (2020). Context-dependent functional compensation between Ythdf m(6)A reader proteins. Genes Dev. 34: 1373–1391.

Lee, S., Song, J., Kim, S., Kim, J., Hong, Y., Kim, Y., Kim, D., Baek, D., and Ahn, K. (2013). Selective degradation of host MicroRNAs by an intergenic HCMV noncoding RNA accelerates virus production. Cell Host Microbe 13: 678–690.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., et al. (2003). The nuclear RNase III Drosha initiates microRNA processing. Nature 425: 415–419.

Lee, Y., Kim, M., Han, J., Yeom, K.H., Lee, S., Baek, S.H., and Kim, V.N. (2004a). MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 23: 4051–4060.

Lee, Y.S., Nakahara, K., Pham, J.W., Kim, K., He, Z., Sontheimer, E.J., and Carthew, R.W. (2004b). Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell 117: 69–81.

Lee, Y.Y., Kim, H., and Kim, V.N. (2023a). Sequence determinant of small RNA production by DICER. Nature 615: 323–330.

Lee, Y.Y., Lee, H., Kim, H., Kim, V.N., and Roh, S.H. (2023b). Structure of the human DICER-pre-miRNA complex in a dicing state. Nature 615: 331–338.

Leveille, N., Elkon, R., Davalos, V., Manoharan, V., Hollingworth, D., Oude Vrielink, J., le Sage, C., Melo, C.A., Horlings, H.M., Wesseling, J., et al. (2011). Selective inhibition of microRNA accessibility by RBM38 is required for p53 activity. Nat. Commun. 2: 513.

Libri, V., Helwak, A., Miesen, P., Santhakumar, D., Borger, J.G., Kudla, G., Grey, F., Tollervey, D., and Buck, A.H. (2012). Murine cytomegalovirus encodes a miR-27 inhibitor disguised as a target. Proc. Natl. Acad. Sci. U. S. A. 109: 279–284.

Lin, K., Zhang, S., Shi, Q., Zhu, M., Gao, L., Xia, W., Geng, B., Zheng, Z., and Xu, E.Y. (2018). Essential requirement of mammalian Pumilio family in embryonic development. Mol. Biol. Cell 29: 2922–2932.

Lin, S., Choe, J., Du, P., Triboulet, R., and Gregory, R.I. (2016). The m(6)A methyltransferase METTL3 promotes translation in human cancer cells. Mol. Cell 62: 335–345.

Lingel, A., Simon, B., Izaurralde, E., and Sattler, M. (2003). Structure and nucleic-acid binding of the Drosophila Argonaute 2 PAZ domain. Nature 426: 465–469.

Liu, J., Carmell, M.A., Rivas, F.V., Marsden, C.G., Thomson, J.M., Song, J.J., Hammond, S.M., Joshua-Tor, L., and Hannon, G.J. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. Science 305: 1437–1441.

Liu, J., Gao, M., Xu, S., Chen, Y., Wu, K., Liu, H., Wang, J., Yang, X., Wang, J., Liu, W., et al. (2020). YTHDF2/3 are required for Somatic reprogramming through different RNA deadenylation pathways. Cell Rep 32: 108120.

Liu, J., Rivas, F.V., Wohlschlegel, J., Yates, J.R., 3rd, Parker, R., and Hannon, G.J. (2005). A role for the P-body component GW182 in microRNA function. Nat. Cell Biol. 7: 1161–1166.

Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., Jia, G., Yu, M., Lu, Z., Deng, X., et al. (2014). A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat. Chem. Biol. 10: 93–95.

Liu, Y., Qu, L., Liu, Y., Roizman, B., and Zhou, G.G. (2017). PUM1 is a biphasic negative regulator of innate immunity genes by suppressing LGP2. Proc. Natl. Acad. Sci. U. S. A. 114: E6902–E6911.

Loh, B., Jonas, S., and Izaurralde, E. (2013). The SMG5-SMG7 heterodimer directly recruits the CCR4-NOT deadenylase complex to mRNAs containing nonsense codons via interaction with POP2. Genes Dev. 27: 2125–2138.

Lund, E., Guttinger, S., Calado, A., Dahlberg, J.E., and Kutay, U. (2004). Nuclear export of microRNA precursors. Science 303: 95–98.

Ma, J.B., Yuan, Y.R., Meister, G., Pei, Y., Tuschl, T., and Patel, D.J. (2005). Structural basis for 5′-end-specific recognition of guide RNA by the A. fulgidus Piwi protein. Nature 434: 666–670.

Marcinowski, L., Tanguy, M., Krmpotic, A., Radle, B., Lisnic, V.J., Tuddenham, L., Chane-Woon-Ming, B., Ruzsics, Z., Erhard, F., Benkartek, C., et al. (2012). Degradation of cellular mir-27 by a novel, highly abundant viral transcript is important for efficient virus replication *in vivo*. PLoS Pathog. 8: e1002510.

Mathys, H., Basquin, J., Ozgur, S., Czarnocki-Cieciura, M., Bonneau, F., Aartse, A., Dziembowski, A., Nowotny, M., Conti, E., and Filipowicz, W. (2014). Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in MicroRNA repression. Mol. Cell 54: 751–765.

Meister, G., Landthaler, M., Patkaniowska, A., Dorsett, Y., Teng, G., and Tuschl, T. (2004). Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol. Cell 15: 185–197.

Meister, G., Landthaler, M., Peters, L., Chen, P.Y., Urlaub, H., Luhrmann, R., and Tuschl, T. (2005). Identification of novel argonaute-associated proteins. Curr. Biol. 15: 2149–2155.

Meyer, K.D., Patil, D.P., Zhou, J., Zinoviev, A., Skabkin, M.A., Elemento, O., Pestova, T.V., Qian, S.B., and Jaffrey, S.R. (2015). 5′ UTR m(6)A promotes cap-independent translation. Cell 163: 999–1010.

Meyer, K.D., Saletore, Y., Zumbo, P., Elemento, O., Mason, C.E., and Jaffrey, S.R. (2012). Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell 149: 1635–1646.

Michalik, K.M., Bottcher, R., and Forstemann, K. (2012). A small RNA response at DNA ends in Drosophila. Nucleic Acids Res. 40: 9596–9603.

Min, K.W., Jo, M.H., Shin, S., Davila, S., Zealy, R.W., Kang, S.I., Lloyd, L.T., Hohng, S., and Yoon, J.H. (2017). AUF1 facilitates microRNA-mediated gene silencing. Nucleic Acids Res. 45: 6064–6073.

Miyoshi, T., Takeuchi, A., Siomi, H., and Siomi, M.C. (2010). A direct role for Hsp90 in pre-RISC formation in Drosophila. Nat. Struct. Mol. Biol. 17: 1024–1026.

Morlando, M., Ballarino, M., Gromak, N., Pagano, F., Bozzoni, I., and Proudfoot, N.J. (2008). Primary microRNA transcripts are processed co-transcriptionally. Nat. Struct. Mol. Biol. 15: 902–909.

Mukherjee, K., Ghoshal, B., Ghosh, S., Chakrabarty, Y., Shwetha, S., Das, S., and Bhattacharyya, S.N. (2016). Reversible HuR-microRNA binding controls extracellular export of miR-122 and augments stress response. EMBO Rep. 17: 1184–1203.

Muller, S., Bley, N., Glass, M., Busch, B., Rousseau, V., Misiak, D., Fuchs, T., Lederer, M., and Huttelmaier, S. (2018). IGF2BP1 enhances an aggressive tumor cell phenotype by impairing miRNA-directed downregulation of oncogenic factors. Nucleic Acids Res. 46: 6285–6303.

Naeeni, A.R., Conte, M.R., and Bayfield, M.A. (2012). RNA chaperone activity of human La protein is mediated by variant RNA recognition motif. J. Biol. Chem. 287: 5472–5482.

Nakanishi, K. (2022). Anatomy of four human Argonaute proteins. Nucleic Acids Res. 50: 6618–6638.

Nakanishi, K., Ascano, M., Gogakos, T., Ishibe-Murakami, S., Serganov, A.A., Briskin, D., Morozov, P., Tuschl, T., and Patel, D.J. (2013). Eukaryote-specific insertion elements control human ARGONAUTE slicer activity. Cell Rep 3: 1893–1900.

Nakanishi, K., Weinberg, D.E., Bartel, D.P., and Patel, D.J. (2012). Structure of yeast Argonaute with guide RNA. Nature 486: 368–374.

Nguyen, T.A., Jo, M.H., Choi, Y.G., Park, J., Kwon, S.C., Hohng, S., Kim, V.N., and Woo, J.S. (2015). Functional anatomy of the human microprocessor. Cell 161: 1374–1387.

Nguyen, T.A., Park, J., Dang, T.L., Choi, Y.G., and Kim, V.N. (2018). Microprocessor depends on hemin to recognize the apical loop of primary microRNA. Nucleic Acids Res. 46: 5726–5736.

Nishanth, M.J. and Simon, B. (2020). Functions, mechanisms and regulation of Pumilio/Puf family RNA binding proteins: a comprehensive review. Mol. Biol. Rep. 47: 785–807.

Nishi, K., Nishi, A., Nagasawa, T., and Ui-Tei, K. (2013). Human TNRC6A is an Argonaute-navigator protein for microRNA-mediated gene silencing in the nucleus. RNA 19: 17–35.

Nishi, K., Takahashi, T., Suzawa, M., Miyakawa, T., Nagasawa, T., Ming, Y., Tanokura, M., and Ui-Tei, K. (2015). Control of the localization and function of a miRNA silencing component TNRC6A by Argonaute protein. Nucleic Acids Res. 43: 9856–9873.

Noland, C.L., Ma, E., and Doudna, J.A. (2011). siRNA repositioning for guide strand selection by human Dicer complexes. Mol. Cell 43: 110–121.

Park, M.S., Sim, G., Kehling, A.C., and Nakanishi, K. (2020). Human Argonaute2 and Argonaute3 are catalytically activated by different lengths of guide RNA. Proc. Natl. Acad. Sci. U. S. A. 117: 28576–28578.

Park, O.H., Ha, H., Lee, Y., Boo, S.H., Kwon, D.H., Song, H.K., and Kim, Y.K. (2019). Endoribonucleolytic cleavage of m(6)A-containing RNAs by RNase P/MRP complex. Mol. Cell 74: 494–507 e498.

Parker, J.S., Roe, S.M., and Barford, D. (2005). Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. Nature 434: 663–666.

Partin, A.C., Ngo, T.D., Herrell, E., Jeong, B.C., Hon, G., and Nam, Y. (2017). Heme enables proper positioning of Drosha and DGCR8 on primary microRNAs. Nat. Commun. 8: 1737.

Partin, A.C., Zhang, K., Jeong, B.C., Herrell, E., Li, S., Chiu, W., and Nam, Y. (2020). Cryo-EM structures of human Drosha and DGCR8 in complex with primary MicroRNA. Mol. Cell 78: 411–422 e414.

Pfaff, J., Hennig, J., Herzog, F., Aebersold, R., Sattler, M., Niessing, D., and Meister, G. (2013). Structural features of Argonaute-GW182 protein interactions. Proc. Natl. Acad. Sci. U. S. A. 110: E3770–E3779.

Piskounova, E., Viswanathan, S.R., Janas, M., LaPierre, R.J., Daley, G.Q., Sliz, P., and Gregory, R.I. (2008). Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. J. Biol. Chem. 283: 21310–21314.

Piwecka, M., Glazar, P., Hernandez-Miranda, L.R., Memczak, S., Wolf, S.A., Rybak-Wolf, A., Filipchyk, A., Klironomos, F., Cerda Jara, C.A., Fenske, P., et al (2017). Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. Science 57: eaam8526.

Qiu, C., McCann, K.L., Wine, R.N., Baserga, S.J., and Hall, T.M. (2014). A divergent Pumilio repeat protein family for pre-rRNA processing and mRNA localization. Proc. Natl. Acad. Sci. U. S. A. 111: 18554–18559.

Quevillon Huberdeau, M., Zeitler, D.M., Hauptmann, J., Bruckmann, A., Fressigne, L., Danner, J., Piquet, S., Strieder, N., Engelmann, J.C., Jannot, G., et al. (2017). Phosphorylation of Argonaute proteins affects mRNA binding and is essential for microRNA-guided gene silencing *in vivo*. EMBO J. 36, 2088–2106.

Quick-Cleveland, J., Jacob, J.P., Weitz, S.H., Shoffner, G., Senturia, R., and Guo, F. (2014). The DGCR8 RNA-binding heme domain recognizes primary microRNAs by clamping the hairpin. Cell Rep. 7: 1994–2005.

Raisch, T., Chang, C.T., Levdansky, Y., Muthukumar, S., Raunser, S., and Valkov, E. (2019). Reconstitution of recombinant human CCR4-NOT reveals molecular insights into regulated deadenylation. Nat. Commun. 10: 3173.

Raisch, T. and Valkov, E. (2022). Regulation of the multisubunit CCR4-NOT deadenylase in the initiation of mRNA degradation. Curr. Opin. Struct. Biol. 77: 102460.

Rasch, F., Weber, R., Izaurralde, E., and Igreja, C. (2020). 4E-T-bound mRNAs are stored in a silenced and deadenylated form. Genes Dev. 34: 847–860.

Rehwinkel, J., Behm-Ansmant, I., Gatfield, D., and Izaurralde, E. (2005). A crucial role for GW182 and the DCP1:DCP2 decapping complex in miRNA-mediated gene silencing. RNA 11: 1640–1647.

Rice, G.M., Shivashankar, V., Ma, E.J., Baryza, J.L., and Nutiu, R. (2020). Functional atlas of primary miRNA maturation by the microprocessor. Mol. Cell 80: 892–902 e894.

Rudel, S., Wang, Y., Lenobel, R., Korner, R., Hsiao, H.H., Urlaub, H., Patel, D., and Meister, G. (2011). Phosphorylation of human Argonaute proteins affects small RNA binding. Nucleic Acids Res. 39: 2330–2343.

Sarshad, A.A., Juan, A.H., Muler, A.I.C., Anastasakis, D.G., Wang, X., Genzor, P., Feng, X., Tsai, P.F., Sun, H.W., Haase, A.D., et al. (2018). Argonaute-miRNA complexes silence target mRNAs in the nucleus of mammalian stem cells. Mol. Cell 71: 1040–1050 e1048.

Sasse, A., Laverty, K.U., Hughes, T.R., and Morris, Q.D. (2018). Motif models for RNA-binding proteins. Curr. Opin. Struct. Biol. 53: 115–123.

Schirle, N.T. and MacRae, I.J. (2012). The crystal structure of human Argonaute2. Science 336: 1037–1040.

Schraivogel, D. and Meister, G. (2014). Import routes and nuclear functions of Argonaute and other small RNA-silencing proteins. Trends Biochem. Sci. 39: 420–431.

Schraivogel, D., Schindler, S.G., Danner, J., Kremmer, E., Pfaff, J., Hannus, S., Depping, R., and Meister, G. (2015). Importin-beta facilitates nuclear import of human GW proteins and balances cytoplasmic gene silencing protein levels. Nucleic Acids Res. 43: 7447–7461.

Schurmann, N., Trabuco, L.G., Bender, C., Russell, R.B., and Grimm, D. (2013). Molecular dissection of human Argonaute proteins by DNA shuffling. Nat. Struct. Mol. Biol. 20: 818–826.

Schwarz, D.S., Hutvágner, G., Du, T., Xu, Z., Aronin, N., and Zamore, P.D. (2003). Asymmetry in the assembly of the RNAi enzyme complex. Cell 115: 199–208.

Sgromo, A., Raisch, T., Backhaus, C., Keskeny, C., Alva, V., Weichenrieder, O., and Izaurralde, E. (2018). Drosophila Bag-of-marbles directly interacts with the CAF40 subunit of the CCR4-NOT complex to elicit repression of mRNA targets. RNA 24: 381–395.

Shen, J., Xia, W., Khotskaya, Y.B., Huo, L., Nakanishi, K., Lim, S.O., Du, Y., Wang, Y., Chang, W.C., Chen, C.H., et al. (2013). EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. Nature 497: 383–387.

Sheu-Gruttadauria, J. and MacRae, I.J. (2018). Phase transitions in the assembly and function of human miRISC. Cell 173: 946–957 e916.

Sheu-Gruttadauria, J., Pawlica, P., Klum, S.M., Wang, S., Yario, T.A., Schirle Oakdale, N.T., Steitz, J.A., and MacRae, I.J. (2019). Structural basis for target-directed MicroRNA degradation. Mol. Cell 75: 1243–1255 e1247.

Shi, C.Y., Kingston, E.R., Kleaveland, B., Lin, D.H., Stubna, M.W., and Bartel, D.P. (2020). The ZSWIM8 ubiquitin ligase mediates target-directed microRNA degradation. Science 370: eabc9359.

Shi, H., Wang, X., Lu, Z., Zhao, B.S., Ma, H., Hsu, P.J., Liu, C., and He, C. (2017). YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. Cell Res. 27: 315–328.

Shin, C.H., Lee, H., Kim, H.R., Choi, K.H., Joung, J.G., and Kim, H.H. (2017). Regulation of PLK1 through competition between hnRNPK, miR-149-3p and miR-193b-5p. Cell Death Differ. 24: 1861–1871.

Sim, G., Kehling, A.C., Park, M.S., Secor, J., Divoky, C., Zhang, H., Malhotra, N., Bhagdikar, D., Abd El-Wahab, E.W., and Nakanishi, K. (2022). Manganese-dependent microRNA trimming by 3′–>5′ exonucleases generates 14-nucleotide or shorter tiny RNAs. Proc. Natl. Acad. Sci. U. S. A. 119: e2214335119.

Slobodin, B., Han, R., Calderone, V., Vrielink, J., Loayza-Puch, F., Elkon, R., and Agami, R. (2017). Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation. Cell 169: 326–337 e312.

Smibert, P., Yang, J.S., Azzam, G., Liu, J.L., and Lai, E.C. (2013). Homeostatic control of Argonaute stability by microRNA availability. Nat. Struct. Mol. Biol. 20: 789–795.

Song, J.J., Liu, J., Tolia, N.H., Schneiderman, J., Smith, S.K., Martienssen, R.A., Hannon, G.J., and Joshua-Tor, L. (2003). The crystal structure of the Argonaute2 PAZ domain reveals an RNA binding motif in RNAi effector complexes. Nat Struct Biol. 10: 1026–1032.

Stupfler, B., Birck, C., Seraphin, B., and Mauxion, F. (2016). BTG2 bridges PABPC1 RNA-binding domains and CAF1 deadenylase to control cell proliferation. Nat. Commun. 7: 10811.

Su, S., Wang, J., Deng, T., Yuan, X., He, J., Liu, N., Li, X., Huang, Y., Wang, H.W., and Ma, J. (2022). Structural insights into dsRNA processing by Drosophila Dicer-2-Loqs-PD. Nature 607: 399–406.

Suzawa, M., Noguchi, K., Nishi, K., Kozuka-Hata, H., Oyama, M., and Ui-Tei, K. (2017). Comprehensive identification of nuclear and cytoplasmic TNRC6A-associating proteins. J. Mol. Biol. 429: 3319–3333.

Suzuki, A., Igarashi, K., Aisaki, K., Kanno, J., and Saga, Y. (2010). NANOS2 interacts with the CCR4-NOT deadenylation complex and leads to suppression of specific RNAs. Proc. Natl. Acad. Sci. U. S. A. 107: 3594–3599.

Thornton, J.E., Du, P., Jing, L., Sjekloca, L., Lin, S., Grossi, E., Sliz, P., Zon, L.I., and Gregory, R.I. (2014). Selective microRNA uridylation by Zcchc6 (TUT7) and Zcchc11 (TUT4). Nucleic Acids Res. 42: 11777–11791.

Tominaga, K., Srikantan, S., Lee, E.K., Subaran, S.S., Martindale, J.L., Abdelmohsen, K., and Gorospe, M. (2011). Competitive regulation of nucleolin expression by HuR and miR-494. Mol. Cell. Biol. 31: 4219–4231.

Treiber, T., Treiber, N., and Meister, G. (2019). Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat. Rev. Mol. Cell Biol. 20: 5–20.

Ustianenko, D., Hrossova, D., Potesil, D., Chalupnikova, K., Hrazdilova, K., Pachernik, J., Cetkovska, K., Uldrijan, S., Zdrahal, Z., and Vanacova, S. (2013). Mammalian DIS3L2 exoribonuclease targets the uridylated precursors of let-7 miRNAs. RNA 19: 1632–1638.

Uzonyi, A., Dierks, D., Nir, R., Kwon, O.S., Toth, U., Barbosa, I., Burel, C., Brandis, A., Rossmanith, W., Le Hir, H., et al. (2023). Exclusion of m6A from splice-site proximal regions by the exon junction complex dictates m6A topologies and mRNA stability. Mol. Cell 83: 237–251 e237.

Van Nostrand, E.L., Freese, P., Pratt, G.A., Wang, X., Wei, X., Xiao, R., Blue, S.M., Chen, J.Y., Cody, N.A.L., Dominguez, D., et al. (2020). A large-scale binding and functional map of human RNA-binding proteins. Nature 583: 711–719.

Wang, X., Lu, Z., Gomez, A., Hon, G.C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G., et al. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505: 117–120.

Wang, X., McLachlan, J., Zamore, P.D., and Hall, T.M. (2002). Modular recognition of RNA by a human pumilio-homology domain. Cell 110: 501–512.

Wang, X., Zhao, B.S., Roundtree, I.A., Lu, Z., Han, D., Ma, H., Weng, X., Chen, K., Shi, H., and He, C. (2015). N(6)-methyladenosine modulates messenger RNA translation efficiency. Cell 161: 1388–1399.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew Danielsen, J.M., Yang, Y.G., and Qi, Y. (2012). A role for small RNAs in DNA double-strand break repair. Cell 149: 101–112.

Weidmann, C.A. and Goldstrohm, A.C. (2012). Drosophila Pumilio protein contains multiple autonomous repression domains that regulate mRNAs independently of Nanos and brain tumor. Mol. Cell. Biol. 32: 527–540.

Weinmann, L., Hock, J., Ivacevic, T., Ohrt, T., Mutze, J., Schwille, P., Kremmer, E., Benes, V., Urlaub, H., and Meister, G. (2009). Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. Cell 136: 496–507.

Weitz, S.H., Gong, M., Barr, I., Weiss, S., and Guo, F. (2014). Processing of microRNA primary transcripts requires heme in mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 111: 1861–1866.

Welte, T., Goulois, A., Stadler, M.B., Hess, D., Soneson, C., Neagu, A., Azzi, C., Wisser, M.J., Seebacher, J., Schmidt, I., et al (2023). Convergence of multiple RNA-silencing pathways on GW182/TNRC6. Mol Cell 83: 2478–2492.e8.

Willkomm, S., Jakob, L., Kramm, K., Graus, V., Neumeier, J., Meister, G., and Grohmann, D. (2022). Single-molecule FRET uncovers hidden conformations and dynamics of human Argonaute 2. Nat. Commun. 13: 3825.

Yamaguchi, S., Naganuma, M., Nishizawa, T., Kusakizako, T., Tomari, Y., Nishimasu, H., and Nureki, O. (2022). Structure of the Dicer-2-R2D2 heterodimer bound to a small RNA duplex. Nature 607: 393–398.

Yamaji, M., Jishage, M., Meyer, C., Suryawanshi, H., Der, E., Yamaji, M., Garzia, A., Morozov, P., Manickavel, S., McFarland, H.L., et al. (2017). DND1 maintains germline stem cells via recruitment of the CCR4-NOT complex to target mRNAs. Nature 543: 568–572.

Yan, W., Zhang, Y., and Chen, X. (2017). TAp63gamma and DeltaNp63gamma are regulated by RBM38 via mRNA stability and have an opposing function in growth suppression. Oncotarget 8: 78327–78339.

Yang, X., Triboulet, R., Liu, Q., Sendinc, E., and Gregory, R.I. (2022). Exon junction complex shapes the m(6)A epitranscriptome. Nat. Commun. 13: 7904.

Yi, R., Qin, Y., Macara, I.G., and Cullen, B.R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 17: 3011–3016.

Yoon, J.H., Jo, M.H., White, E.J., De, S., Hafner, M., Zucconi, B.E., Abdelmohsen, K., Martindale, J.L., Yang, X., Wood, W.H., 3rd, et al. (2015). AUF1 promotes let-7b loading on Argonaute 2. Genes Dev. 29: 1599–1604.

Yoshizawa, T., Ali, R., Jiou, J., Fung, H.Y.J., Burke, K.A., Kim, S.J., Lin, Y., Peeples, W.B., Saltzberg, D., Soniat, M., et al. (2018). Nuclear import receptor inhibits phase separation of FUS through binding to multiple sites. Cell 173: 693–705 e622.

Youn, J.Y., Dunham, W.H., Hong, S.J., Knight, J.D.R., Bashkurov, M., Chen, G.I., Bagci, H., Rathod, B., MacLeod, G., Eng, S.W.M., et al. (2018). High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and bodies. Mol. Cell 69: 517–532 e511.

Young, L.E., Moore, A.E., Sokol, L., Meisner-Kober, N., and Dixon, D.A. (2012). The mRNA stability factor HuR inhibits microRNA-16 targeting of COX-2. Mol. Cancer Res. 10: 167–180.

Zaccara, S. and Jaffrey, S.R. (2020). A unified model for the function of YTHDF proteins in regulating m(6)A-modified mRNA. Cell 181: 1582–1595 e1518.

Zamore, P.D., Williamson, J.R., and Lehmann, R. (1997). The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. RNA 3: 1421–1433.

Zapletal, D., Taborska, E., Pasulka, J., Malik, R., Kubicek, K., Zanova, M., Much, C., Sebesta, M., Buccheri, V., Horvat, F., et al. (2022). Structural

and functional basis of mammalian microRNA biogenesis by Dicer. Mol. Cell 82: 4064–4079 e4013.

Zeng, Y., Sankala, H., Zhang, X., and Graves, P.R. (2008). Phosphorylation of Argonaute 2 at serine-387 facilitates its localization to processing bodies. Biochem. J. 413: 429–436.

Zhang, T., Wu, Y.C., Mullane, P., Ji, Y.J., Liu, H., He, L., Arora, A., Hwang, H.Y., Alessi, A.F., Niaki, A.G., et al. (2018). FUS regulates activity of MicroRNA-mediated gene silencing. Mol. Cell 69: 787–801 e788.

Zhang, Z., Theler, D., Kaminska, K.H., Hiller, M., de la Grange, P., Pudimat, R., Rafalska, I., Heinrich, B., Bujnicki, J.M., Allain, F.H., et al. (2010). The YTH domain is a novel RNA binding domain. J. Biol. Chem. 285: 14701–14710.

Zhuang, R., Rao, J.N., Zou, T., Liu, L., Xiao, L., Cao, S., Hansraj, N.Z., Gorospe, M., and Wang, J.Y. (2013). miR-195 competes with HuR to modulate stim1 mRNA stability and regulate cell migration. Nucleic Acids Res. 41: 7905–7919.