Regulation of mRNA Translation and Stability by microRNAs

Marc Robert Fabian,[1] Nahum Sonenberg,[1] and Witold Filipowicz[2]

[1]Department of Biochemistry and Goodman Cancer Research Center, McGill University, Montreal, Quebec, H3G 1Y6, Canada; email: marc.fabian@mail.mcgill.ca, nahum.sonenberg@mcgill.ca

[2]Friedrich Miescher Institute for Biomedical Research, 4002 Basel, Switzerland; email: Witold.Filipowicz@fmi.ch

Annu. Rev. Biochem. 2010. 79:351–79

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

This article's doi: 10.1146/annurev-biochem-060308-103103

0066-4154/10/0707-0351$20.00

Key Words

Argonaute, deadenylation, GW182, microRNA, repression

Abstract

MicroRNAs (miRNAs) are small noncoding RNAs that extensively regulate gene expression in animals, plants, and protozoa. miRNAs function posttranscriptionally by usually base-pairing to the mRNA 3′-untranslated regions to repress protein synthesis by mechanisms that are not fully understood. In this review, we describe principles of miRNA-mRNA interactions and proteins that interact with miRNAs and function in miRNA-mediated repression. We discuss the multiple, often contradictory, mechanisms that miRNAs have been reported to use, which cause translational repression and mRNA decay. We also address the issue of cellular localization of miRNA-mediated events and a role for RNA-binding proteins in activation or relief of miRNA repression.

Contents

INTRODUCTION

MicroRNAs (miRNAs) comprise a large family of small ~21-nucleotide-long noncoding RNAs that have emerged as key posttranscriptional regulators of gene expression in metazoan animals, plants, and protozoa. In mammals, miRNAs are predicted to control the activity of more than 60% of all protein-coding genes (1) and participate in the regulation of almost every cellular process investigated to date (reviewed in References 2–4). miRNAs regulate protein synthesis by base-pairing to target mRNAs. In animals, most studied miRNAs form imperfect hybrids with sequences in the mRNA 3′-untranslated region (3′ UTR), with the miRNA 5′-proximal "seed" region (positions 2–8) providing most of the pairing specificity (reviewed in References 2 and 5). Until very recently, it appeared that plant miRNAs generally base-pair to mRNAs with nearly perfect complementarity and trigger endonucleolytic mRNA cleavage by the RNA interference (RNAi) mechanism. However, new findings indicate that animal-like mechanisms also broadly operate in plants and that plant miRNAs can repress mRNA translation without a pronounced effect on mRNA stability (6, 7).

Generally, miRNAs inhibit protein synthesis either by repressing translation and/or by bringing about deadenylation and subsequent degradation of mRNA targets (reviewed in References 5, 8, and 9). More recently, however, some miRNAs were reported to activate mRNA translation (10–14). miRNAs function in the form of ribonucleoprotein complexes, miRISCs (miRNA-induced silencing complexes). Argonaute (AGO) and GW182 [glycine-tryptophan (GW) repeat-containing protein of 182 kDa] family proteins represent the best-characterized protein components (reviewed in References 8 and 9). Components of miRISC (including miRNAs as well as AGO and GW182 proteins) and repressed mRNAs are enriched in processing bodies (P bodies, also known as GW bodies), which are

cytoplasmic structures thought to be involved in the storage or degradation of translationally repressed mRNAs (15, 16). Some P-body components are important for effective repression of protein synthesis by miRNAs (17–20). Recently, multivesicular bodies (MVBs) and endosomes were also identified as cellular organelles contributing to miRNA function or miRISC turnover (21, 22).

The mechanistic details of miRNA's function in repressing protein synthesis are not well understood. In addition, the results from studies conducted in different systems have often been contradictory. It is difficult to conclude whether the reported discrepancies are artifacts of different experimental approaches or whether miRNAs are indeed able to exert their repressive effects by disparate mechanisms (5, 8, 9, 23). This article reviews the current knowledge on mechanistic aspects of miRNA-induced repression of protein synthesis in animal and insect cells and discusses the disparities regarding different modes of miRNA function. We also highlight new findings indicating that miRNA-mediated repression is a regulated process. For example, under specific cellular conditions, miRNA-mediated repression can be prevented or reversed (24, 25). Moreover, factors have been identified that control repression by distinct subsets of miRNAs (26, 27). For recent reviews addressing mechanistic aspects of miRNA repression, see References 5, 8, 9, 23, 28, and 29. Other reviews discuss biogenesis (30, 31) and biological functions (2–4, 32) of miRNAs.

PRINCIPLES OF TARGET RECOGNITION BY miRNAS

miRNAs interact with their mRNA targets via base-pairing. With few exceptions, metazoan miRNAs base-pair with their targets imperfectly, following a set of rules which have been formulated based on experimental and bioinformatics analyses (2). The most stringent requirement is a contiguous and perfect Watson-Crick base-pairing of the miRNA 5′ nucleotides 2–8, representing the seed region nucleating the interaction. In addition, an A residue across position 1 of the miRNA and A or U across position 9 improve miRNA activity, although they do not need to base-pair with mRNA nucleotides. However, functional miRNA sites containing mismatches or even bulged nucleotides in the seed have also been identified as exemplified by the *Lin-41* mRNA targeted by *let*-7 miRNA in *Caenorhabditis elegans* (33). Complementarity of the miRNA 3′ half is quite relaxed, though it stabilizes the interaction, particularly when the seed matching is suboptimal. Generally, miRNA-mRNA duplexes contain mismatches and bulges in the central region (miRNA positions 10–12) that prevent endonucleolytic cleavage of mRNA by an RNAi mechanism. AU-rich sequence context and structural accessibility of the sites may improve their efficacy (2). Usually, multiple sites, either for the same or different miRNAs, are required for effective repression, and when the sites are close to each other, they tend to act cooperatively (34, 35).

Most of the predicted and experimentally characterized miRNA sites are positioned in the mRNA 3′ UTR. However, animal miRNAs may also target 5′ UTR and coding regions of mRNAs, as documented by experiments involving both artificial and natural mRNAs and also by bioinformatic predictions (12, 36–39). Sites located in coding regions appear to be less robust than those in the 3′ UTR (36, 37), but inclusion of rare codons to slow down the ribosome transit through the miRNA site region can increase their potency, likely owing to the facilitated occupancy of the site by miRISC (37). Interestingly, in some instances, association of miRNAs with 5′-UTR target sites appears to activate rather than repress translation [(12, 13); and see below].

PROTEIN COMPONENTS OF miRNA RIBONUCLEOPROTEINS

The key components of miRISCs are proteins of the Argonaute family. These proteins contain three evolutionarily conserved domains, PAZ, MID, and PIWI, which interact with the

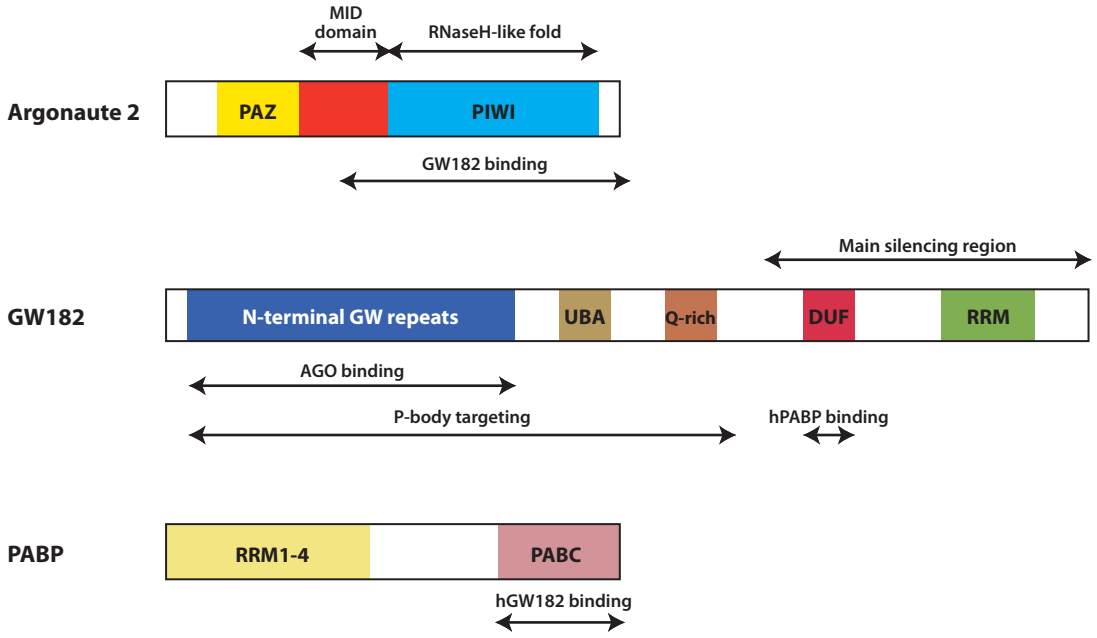

Figure 1

Schematic diagram of human Argonaute 2 (AGO2), GW182, and poly(A)-binding protein (PABP). Of the four human AGO proteins, only AGO2 functions in both miRNA repression and RNAi. It contains an enzymatically competent RNaseH-like PIWI domain, which endonucleolytically cleaves perfectly complementary RNA targets. There are three human GW182 paralogs (TNRC6A, -B, and -C), whereas *Drosophila* contains only one GW182 protein (dGW182, also known as Gawky), with a similar domain organization. *C. elegans* contains two proteins, AIN-1 and -2, which differ substantially from GW182s but perform analogous functions. The N-terminal region of GW182, containing glycine-tryptophan (GW) repeats, interacts with AGO proteins. The region, including GW-rich, ubiquitin-associated (UBA), and glutamine-rich (Q-rich) domains, is responsible for targeting GW182 proteins to P bodies. The C-terminal part of mammalian and *Drosophila* proteins (the main silencing region), containing DUF (domain of unknown function) motifs and RNA recognition motifs (RRMs), is a major effector domain, mediating translational repression and deadenylation of mRNA. Domains of PABP include four RRMs and a conserved C-terminal domain, PABC. Mammalian PABP binds directly to the silencing region of human GW182 proteins via PABC. Abbreviation: PAZ, Piwi-Argonaute-Zwilli domain.

3′ and 5′ ends of the miRNA, respectively (40, 41). The details of miRISC assembly are not well understood. The process may be coupled to miRNA processing by Dicer and to the selection of the mature miRNA strand from the complementary passenger strand (referred to as miRNA*).

Many Argonaute paralogs are encoded in metazoan and plant genomes but only some, known as AGO proteins, function in miRNA or both miRNA and siRNA pathways; others are dedicated to the function of piRNAs in germ cells or to other classes of small RNAs (40). In mammals, four AGO proteins, AGO1 through AGO4, function in miRNA repression, but only AGO2 (**Figure 1**), having an enzymatically competent RNaseH-like PIWI domain, which cleaves mRNA at the center of the siRNA-mRNA duplex, also functions in RNAi (42). Involvement of AGO1 through AGO4 in miRNA repression is demonstrated by their association with similar sets of miRNAs and proteins identified in immunoprecipitation experiments (42–45) and also by their ability to repress protein synthesis when artificially tethered to the mRNA 3′ UTR (46–48). Although these and some other data argue against paralog-specific functions of mammalian AGO proteins (49), there are indications that some AGO proteins are more potent repressors than others when tethered to reporter mRNAs (48). The cell- or tissue-specific differences in the

relative abundance of individual AGOs suggest that the robustness of miRNA-mediated repression may differ between different types of cells (48). There are also indications that AGO2 in mammals may have some specific functions that cannot be complemented by the other Argonaute proteins. For example, knockdown of AGO2 in human HEK293 cells engenders a much more profound effect on miRNA-mediated repression than knockdowns of the other AGO proteins (50), and knockout of AGO2 but not the other Argonuates is embryonically lethal in mice (42). In addition, AGO2 is essential for hematopoiesis in mice (51). Because this requirement is independent of the endonucleolytic activity of AGO2, the specific role of AGO2 in hematopoiesis involves miRNA regulation rather than its potential role as an RNAi factor. In *Drosophila*, it was thought that AGO1 is exclusively dedicated to the miRNA pathway, whereas AGO2 functions in RNAi. However, recent data indicate that AGO2 also gets loaded with a subclass of miRNAs and represses protein synthesis via a mechanism that differs from the one induced by AGO1 [(52–54); and see below]. In *C. elegans*, which expresses 27 Argonaute proteins, only ALG (Argonaute-Like Gene)-1 and -2 function in the miRNA pathway (40).

Biochemical studies (55, 56) and X-ray structures of prokaryotic AGO-like proteins in complex with small RNAs (or their mimics), or in a ternary complex also including a target RNA (41, 57, 58), offer a molecular basis for some of the rules for mRNA recognition by miRNAs. The 5′-terminal miRNA nucleotide, in a monophosphorylated form, is anchored in a deep pocket at the junction of the MID and PIWI domains, with the terminal phosphate and the base interacting, either directly or via a magnesium ion, with conserved AGO amino acids. Nucleotides at miRNA positions 2–6 contact AGO through the phosphate-ribose RNA backbone and are displayed on the protein surface in a semihelical conformation, with the bases available for hydrogen bonding to the target mRNA. These properties explain why the nucleotide at the miRNA position 1 does not need to base-pair to the target and why perfect complementarity in the seed sequence is crucial for nucleating the miRNA-mRNA interaction (41, 57, 58). Association of the miRNA 3′ end with PAZ may be transiently disrupted to relieve the topological constraints during propagation of the miRNA-mRNA duplex over two helical turns (59).

GW182 proteins are another group of factors, which is crucial for the miRNA-induced repression (**Figure 1**) (60). They interact directly with and act downstream of AGOs. There are three mammalian GW182 proteins (known as TNRC6A, -B, and -C) (**Figure 1**) and a single *Drosophila* homolog (dGW182, also known as Gawky). GW182 proteins contain GW repeats in the N-terminal portion, followed by a glutamine (Q)-rich region, a domain of unknown function (DUF), and an RRM (RNA recognition motif) domain. Some GW182s contain in addition a putative ubiquitin-associated (UBA) domain. The *C. elegans* counterparts of GW182 proteins, AIN-1 and AIN-2, contain GW repeats but lack the DUF and RRM domains (61, 62). The GW repeats are responsible for the interaction of GW182 with the AGO proteins (63, 64). Disruption of the GW182-AGO interaction, by point mutations or a peptide competing with GW182 for AGO binding, abrogates miRNA-mediated repression (64, 65). The region extending from the N terminus to the Q-rich domain is responsible for targeting dGW182 to P bodies (17).

Direct tethering of GW182 to an mRNA in *Drosophila* cells leads to repression of protein synthesis even in the absence of AGO1, consistent with a mechanism whereby GW182 is the effector of AGO function (17). Recent mutagenesis analyses of mammalian GW182 proteins identified their C-terminal segment, encompassing the DUF and RRM domains as well as sequences C proximal to RRM as a minimal protein fragment, which effectively causes both translational repression and mRNA destabilization when tethered to the mRNA (66, 67). An equivalent fragment was also found to act as a repressive domain in dGW182

(63, 68), although one of these studies identified the Q-rich and N-terminal GW-rich regions of dGW182 as additional autonomous domains active in inducing repression (68). Structural and mutagenic analyses of the C-terminal inhibitory fragment of GW182 proteins indicate that regions flanking the RRM, rather than the DUF and RRM themselves, are important for repressing protein synthesis (63, 67, 69). In addition, the RRM appears not to exhibit RNA-binding activity (70; H. Mathys & W. Filipowicz, unpublished results).

miRISCs interact with several additional proteins that may function as regulatory factors that modulate miRNA function (reviewed in Reference 40). One example is the fragile X mental retardation protein, FMRP, and its *Drosophila* ortholog, dFXR, which are RNA-binding proteins known to act as modulators of translation (71, 72). Other examples include the RNA helicase RCK/p54, a P-body component that is essential for inducing repression (17, 20), and Importin 8 (Imp8), which, in addition to its role in transporting AGO2 to the nucleus, functions in miRNA repression in mammalian cells by enhancing the association of AGO2 complexes with target mRNAs (73). The TRIM-NHL family proteins, TRIM32 in mammalian cells and NHL-2 in *C. elegans*, were recently reported to enhance the activity of selected miRNAs by binding to core miRISC components (26, 27). Very little is known about how these proteins function in miRNA-mediated repression.

INTRODUCTION TO EUKARYOTIC TRANSLATION

The process of translation is divided into three steps: initiation, elongation, and termination. Initiation involves the assembly of an 80S ribosome complex positioned at the translation start site of the mRNA. This is followed by the elongation of the peptide chain. Termination entails the release of the newly synthesized protein and dissociation of ribosomal subunits from the mRNA. In eukaryotes, the rate-limiting step under most circumstances is initiation. Consequently, initiation is the most common target for translational control. All nuclear transcribed eukaryotic mRNAs contain at their 5′ end an m^7GpppN group (where N is any nucleotide) termed the 5′ cap, which facilitates ribosome recruitment to the mRNA. Some cellular and viral mRNAs are translated via alternative cap-independent mechanisms.

Cap-Dependent Translation

Cap-dependent translation requires the participation of at least 13 different eukaryotic initiation factors (eIFs). It is accomplished through a mechanism whereby the small (40S) ribosomal subunit, in a complex with a number of eIFs, binds the mRNA near the 5′ cap and scans the mRNA in a 5′→3′ direction until it encounters an AUG (or a near-cognate) codon in an optimal context (reviewed in Reference 74). Recruitment of ribosomes to the mRNA is facilitated by the 5′ cap and the 3′ poly(A) tail, via protein factors bound to these structures, the eIF4F complex, and the poly(A)-binding protein (PABP), respectively. The eIF4F contains three subunits (75, 76): (*a*) the eIF4A, an ATP-dependent RNA helicase that is thought to unwind the mRNA 5′-UTR secondary structure; (*b*) the eIF4E, a 24-kDa polypeptide that specifically interacts with the cap (77); and (*c*) the eIF4G, a large scaffolding protein that binds to both eIF4E and eIF4A and other proteins. The poly(A) tail functions as a translational enhancer whereby the PABP directly interacts with the eIF4G to effectively circularize the mRNA (78). The PABP-eIF4G interaction promotes mRNA circularization to stabilize the interaction of the eIF4E with the cap, thus enhancing the rate of translation initiation (79).

Cap-Independent Translation

The discovery of the internal ribosome entry sites (IRESs) in picornaviruses two decades ago (80, 81) has provided an alternative mechanism of translation initiation. IRESs provide an internal ribosome-binding site, thus bypassing the requirement for the cap. Subsequently, IRESs have been documented in a multitude

of cellular mRNAs (82). Always (with the exception of hepatitis A virus), IRESs function independently of eIF4E. Certain IRESs [such as those of poliovirus and encephalomyocarditis virus (EMCV)] function via direct binding of the eIF4G subunit of the eIF4F complex to the IRES (83, 84). The hepatitis C virus (HCV) IRES bypasses the need for the entire eIF4 family of proteins and binds directly to eIF3 and the 40S ribosomal subunit (85). The CrPV (cricket paralysis virus) contains an intergenic IRES, which recruits the ribosome via a mechanism completely independent of initiation factors, whereby the IRES mimics an aminoacylated tRNA and positions itself within the P site of the ribosome (86, 87). This allows the CrPV IRES to initiate translation from a non-AUG codon.

miRNA-MEDIATED REPRESSION OF TRANSLATION

A large number of in vivo and in vitro studies addressed the mechanisms by which miRNAs suppress protein synthesis. These studies showed that miRNAs either inhibit translation of target mRNAs (**Figure 2**) or facilitate their deadenylation and subsequent degradation (**Figure 3**). In the following sections, we summarize these findings and analyze the molecular mechanisms involved. We discuss a complex relationship between mRNA translation and its deadenylation and decay, which could shed light on the source of diversity in the outcome of the miRNA mechanistic studies.

miRNA-Mediated Repression of Translation Initiation

Lin-4, the original miRNA, which was discovered in *C. elegans*, was initially shown to cause inhibition of translation of lin-14 without a reduction in mRNA levels or a shift in polysomes, leading to the conclusion that miRNAs inhibit mRNA translation at the elongation step (88–90). Although additional reports supported such a conclusion in other experimental systems, many other results pointed to defects in the control of translation initiation and mRNA stability.

miRNA-mediated repression of translation initiation was first observed in HeLa cells using both mono- and bicistronic reporter mRNAs whose 3′ UTRs were targeted by either endogenous (let-7) (46) or artificial (CXCR4) (91) miRNAs. Analysis of mRNA levels failed to detect pronounced degradation of miRNA-targeted mRNAs, demonstrating that translation was indeed inhibited (46, 91). Importantly, let-7 targeted mRNAs shifted to lighter fractions of polysomal density gradients, an event that is indicative of repressed translation at the initiation step caused by a defect in ribosome recruitment to the mRNA. This was not an isolated observation, as similar shifts were observed in Huh7 cells for the miR-122-targeted CAT-1 mRNA (24), in HEK293T cells for a miR-16-targeted reporter mRNA (92), and in *C. elegans* for multiple miRNA-targeted mRNAs, including the daf-12 and lin-41 mRNAs, which are regulated by the let-7 miRNA (93).

Targeting of cap-dependent translation. Several groups reported that mRNAs that lack a functional 5′-cap structure, or whose translation is cap-independent, are refractory to miRNA-mediated translational repression (46, 91, 94–97). mRNAs with a nonfunctional ApppG cap structure, targeted by the CXCR4 miRNA mimic, were not repressed as well (~twofold repression) as mRNAs bearing the m7G cap (~fivefold repression) in HeLa cells (91). mRNAs containing HCV (46), EMCV (46, 91) or CrPV (91) IRESs were refractory to miRNA-mediated repression in transfected HeLa cells. Moreover, tethering of either the eIF4E or the eIF4G to the intercistronic region of bicistronic mRNAs promoted translation of the second cistron regardless of whether let-7 target sites were present in its 3′ UTR (46). Collectively, these investigations in cultured cells pointed to the possibility that miRNAs interfere with either eIF4E function or eIF4E recruitment to the 5′-cap structure of miRNA-targeted mRNAs (**Figure 2*a***).

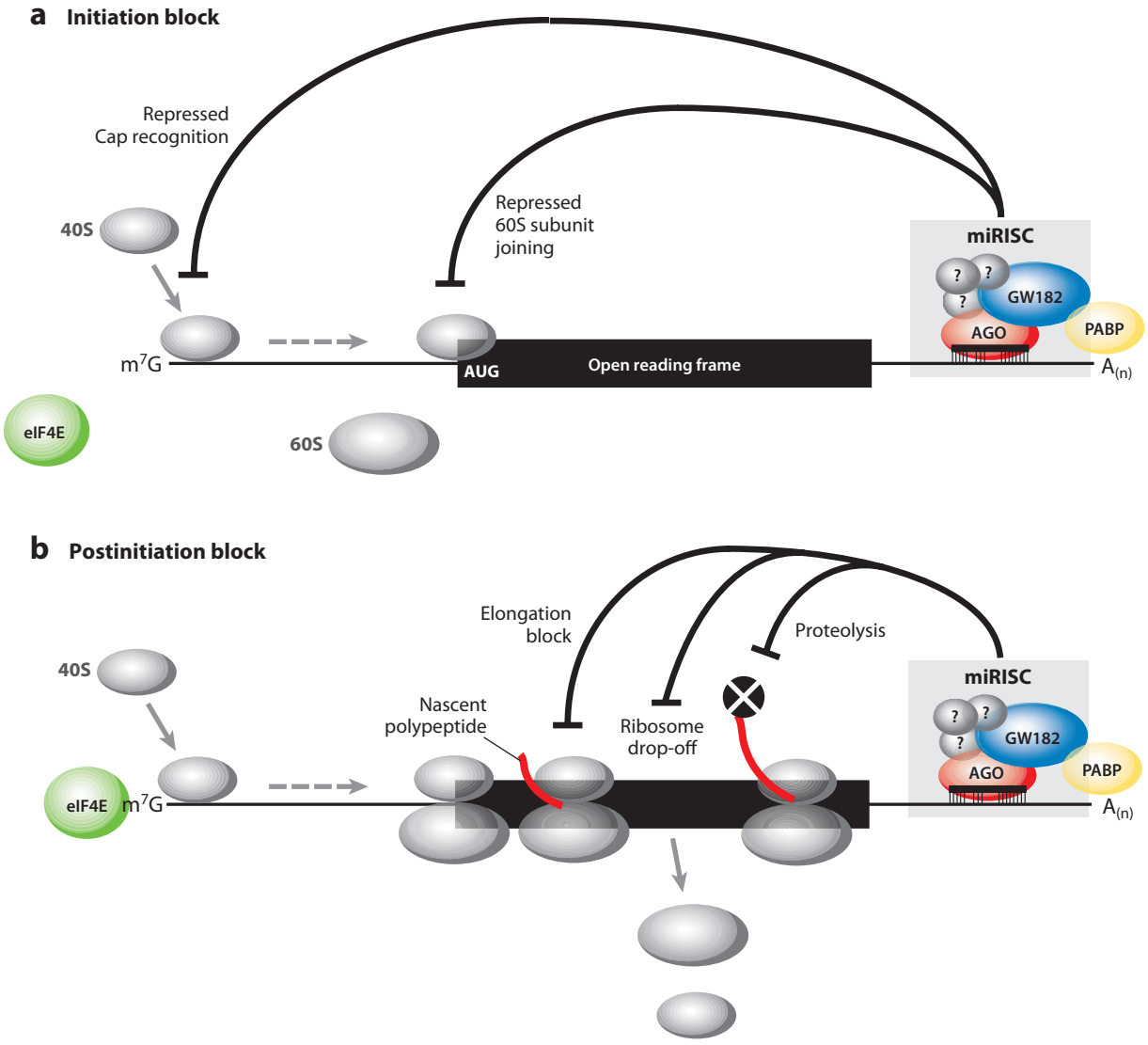

Figure 2

Schematic diagram of miRNA-mediated translational repression. (*a*) Initiation block: The miRISC inhibits translation initiation by interfering with eIF4F-cap recognition and 40S small ribosomal subunit recruitment or by antagonizing 60S subunit joining and preventing 80S ribosomal complex formation. The reported interaction of the GW182 protein with the poly(A)-binding protein (PABP) (106, 156) might interfere with the closed-loop formation mediated by the eIF4G-PABP interaction and thus contribute to the repression of translation initiation. (*b*) Postinitiation block: The miRISC might inhibit translation at postinitiation steps by inhibiting ribosome elongation, inducing ribosome drop-off, or facilitating proteolysis of nascent polypeptides. There is no mechanistic insight to any of these proposed "postinitiation" models. The 40S and 60S ribosomal subunits are represented by small and large gray spheres, respectively. Ovals with question marks represent potential additional uncharacterized miRISC proteins that might facilitate translational inhibition. Abbreviations: AGO, Argonaute; $A_{(n)}$, poly(A) tail; m^7G, the 5′-terminal cap.

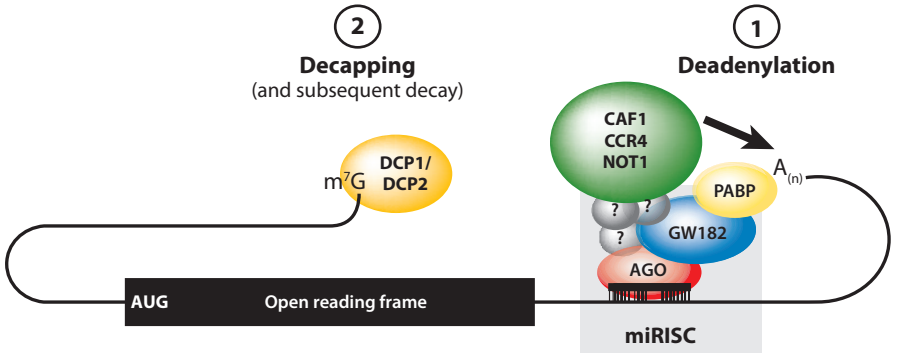

Figure 3

Schematic diagram of miRNA-mediated mRNA decay. The miRISC interacts with the CCR4-NOT1 deadenylase complex to facilitate deadenylation of the poly(A) tail [denoted by $A_{(n)}$]. Deadenylation requires the direct interaction of the GW182 protein with the poly(A)-binding protein (PABP) (see previous figures). Following deadenylation, the 5′-terminal cap (m^7G) is removed by the decapping DCP1-DCP2 complex. The open reading frame is denoted by a black rectangle. Abbreviations: AGO, Argonaute; CAF1, CCR4-associated factor; CCR4, carbon catabolite repression 4 protein; NOT1, negative on TATA-less.

The conclusions from the cell culture studies are strongly supported by in vitro experiments using cell-free systems that faithfully recapitulate the action of miRNAs in cells. In cell-free extracts from mouse Krebs II ascites cells (referred to as Krebs extracts) (97), *Drosophila* embryos (96), and HEK293 cells (95), inhibition of mRNA translation or deadenylation was dependent on the ability of the "seed sequence" of the miRNA to base-pair to the target sequence in the mRNA. Addition of oligonucleotides, which are complementary to miRNAs (antimiRs), to the extract prevented miRNA function. The three systems mentioned above made use of an endogenous miRNA targeting for in vitro synthesized mRNAs. All these aforementioned studies concluded that the miRNA-mediated translation inhibition occurs at the initiation step and is due to the interference with the cap recognition process. This is further supported by the findings that miRNAs failed to inhibit IRES-dependent translation or translation from ApppG-capped mRNAs (95–97). More detailed analyses revealed that miRNAs inhibited ribosome initiation complex formation; miR-2 inhibited both 40S ribosomal subunit recruitment and 80S initiation complex formation in fly embryo extract (96), and 80S initiation complex formation was impaired in mouse Krebs extract (97). A study by Zdanowicz et al. (98) points to the 5′-cap structure itself as being a direct target of miRNA-mediated translational repression. miR-2-targeted mRNAs bearing modifications to the triphosphate bridge of the 5′ cap demonstrated increased translational repression in both *Drosophila* embryonic extracts and S2 cells (98). Strong evidence for the notion that the cap recognition machinery is indeed the target for miRNA-mediated translational repression was the demonstration that adding a purified eIF4F complex to the Krebs extract alleviated translational repression of let-7-targeted mRNAs (97). In contrast to these results, Wang et al. (99) showed that in a rabbit reticulocyte lysate the CXCR4 artificial miRNA impairs translation by inhibiting the joining of the 60S subunit, even though the 5′ cap was required for the inhibition. It is possible that this inconsistence with former results stems from nuclease-treated rabbit reticulocyte lysate not displaying cap-poly(A) tail translational synergy (100, 101), which may, in turn, alter the outcome of miRNA-mediated repression.

Efforts to elucidate the mechanism by which miRNAs impede the cap recognition step of translation initiation have not been successful. In a promising study, Kiriakidou et al. (102) observed that AGO2 binds directly, albeit weakly, to the cap structure and suggested that this binding competes with eIF4E and results in inhibition of translation initiation. The authors reported that the AGO MID domain exhibits limited sequence homology to the cap-binding protein eIF4E and contains two aromatic residues that could function in a similar manner to those in eIF4E in sandwiching the cap structure. Mutating the two aromatics to valines abolished AGO2 interaction with m^7GTP-Sepharose and impaired its ability to repress translation when tethered to an mRNA 3′ UTR. Although this model is appealing, it was brought into question by Eulalio et al. (65), who demonstrated that mutation of the two aromatic residues interfered with the binding of AGO proteins to GW182, interacations that are required for miRNA-dependent repression. Moreover, structural modeling by Kinch & Grishin (103) indicated that AGO2 shares extremely limited, if any, structural similarity to eIF4E. Thus, it is questionable whether AGO proteins bind directly to the cap structure, and even if this is the case, the binding might not occur via the reported aromatic residues.

Another mechanism that the miRISC might utilize to inhibit cap-dependent translation is by interacting with a component of the cap-binding complex, eIF4F. Using a *Drosophila* embryo extract, Iwasaki et al. (52) demonstrated that both dAGO1, which associates with most miRNAs in *Drosophila*, and dAGO2, which is loaded only with a subclass of miRNAs (53, 54, 104, 105), can induce miRNA-mediated repression, albeit through different mechanisms of action. dAGO1 inhibits protein synthesis by repressing translation and inducing mRNA deadenylation and subsequent decay through its interaction with dGW182 (17, 65). In contrast, dAGO2 repression appears not to involve dGW182. Instead, dAGO2 was found to bind to eIF4E on targeted mRNAs, and the authors propose that dAGO2 represses cap-dependent translation by competing with eIF4G for binding to eIF4E. Notably, the dAGO2-eIF4E interaction mechanism is most likely not evolutionarily conserved as eIF4E has not been found to interact with mammalian AGO2 (106). Moreover, translation driven by tethered eIF4E was found to be refractory to let-7 repression in HeLa cells (46).

Repression by inhibiting the 80S complex assembly. As mentioned above, Wang et al. (99) reported the enrichment of 40S but not 60S ribosomal subunits in complexes formed by mRNA undergoing miRNA-mediated repression in reticulocyte lysates. The authors postulated that miRNAs may repress initiation by inhibiting 60S subunit joining (**Figure 2*a***), but the mechanism of the inhibition was not investigated. Another study also concluded that miRNAs might affect 60S joining (107). The 60S ribosomal subunit and its associated protein, eIF6, which prevents the 60S joining to 40S (108) and regulates translation (109), coimmunoprecipitated with the AGO2-Dicer-TRBP (TAR RNA-binding protein) complex (107). Depletion of eIF6 from either human cells or *C. elegans* partially alleviated the inhibition of let-7 or lin-4 miRNA targets, leading the authors to propose that miRISC association with eIF6 disrupts polysome formation by inhibiting 80S complex assembly. However, the validity of these results was brought into question by experiments showing that depletion of eIF6 from *Drosophila* S2 cells has had no noticeable effect on miRNA-mediated repression (65). Of note, knocking down eIF6 in *C. elegans* strongly interferes with the production of mature lin-4 miRNA (107), raising the possibility that it may have impacted the maturation and/or loading of miRNAs into the active miRISC. Also, Ding et al. (110) reported that knockdown of eIF6 in *C. elegans* enhanced rather than diminished let-7-mediated repression.

Translational repression of initiation and the poly(A) tail. It is well established that the PABP enhances cap-dependent translation of

mRNAs, most probably by interacting with the eIF4G of the eIF4F complex (79, 111). Thus, miRNA-mediated mRNA deadenylation is expected to cause a decrease in translation initiation. Some discrepancies exist regarding the role of the poly(A) tail in miRNA-mediated translational repression. Both the 5′ cap and poly(A) tail were required for optimal translational repression of mRNA by a miRNA mimic in HeLa cells in one study (91), but no substantial difference in the repression between capped poly(A)$^+$ and poly(A)$^-$ mRNAs was noted by others (46). More recently, Beilharz et al. (112) reported that deadenylation of the miRNA-targeted mRNA promotes translational repression. Using a mammalian cell extract derived from HEK293 cells overexpressing AGO2 and GW182 proteins and let-7 miRNA, Wakiyama et al. (95) observed no miRNA repression of a nonadenylated mRNA reporter. However, as miRNA induced rapid mRNA deadenylation in the HEK293 cell extract, this result could reflect a potential mechanistic bias of the system favoring (because of the overexpression of miRISC components) the miRNA-mediated deadenylation rather than translational repression. Using an alternative strategy, several groups addressed the role of the poly(A) tail by replacing it with a stem loop, which acts in the 3′-end maturation of nonpolyadenylated histone mRNAs (65, 113), or by removing the poly(A) tail by the action of a ribozyme inserted in the reporter 3′ UTR (114). These experiments have revealed that in both mammalian and *Drosophila* cells, nonpolyadenylated mRNAs undergo miRNA-mediated repression, although the repression was not always as strong as for polyadenylated mRNAs (112–114). Taken together, these data demonstrate that miRNAs repress protein synthesis via both poly(A) tail–dependent and –independent mechanisms.

miRNA Repression at Postinitiation Steps

A number of studies concluded that miRNAs inhibit translation at postinitiation steps (**Figure 2**) (37, 88, 115–117). The most persuasive observations that have led to this conclusion originate from polysomal sedimentation analyses. Early investigations in *C. elegans* (88, 118) indicated that the lin-14 and lin-28 mRNAs, which are targets of lin-4 miRNA, remain associated with translating polysomes during larval development in spite of reduced protein levels. However, Ding & Grosshans (93) recently reexamined the polysome profiles of lin-14 and lin-28 mRNAs during *C. elegans* development and reported their shifting into lighter polysome fractions in response to lin-4 miRNA repression. Olsen & Ambros (88) analyzed lin-14 and lin-28 mRNAs at different developmental stages of *C. elegans* when lin-4 miRNA either is or is not expressed, but Ding & Grosshans compared wild-type and lin-4 mutant worms at the same stage of development. Thus, the experimental differences may account for the reported contradictory findings. Association of repressed mRNAs with functional polysomes was also observed in mammalian cells using reporter mRNAs targeted by both endogenous (37, 115, 116) and synthetic (117) miRNAs.

Several miRNAs, as well as AGO proteins, have also been reported to be associated with polysomal fractions in both mammalian (11, 115, 119, 120) and plant cells (7). This served as an argument in favor of the hypothesis that miRNAs inhibit translation elongation (**Figure 2*b***) (116). However, the degree of translational repression is dependent on the number and possibly also positioning of miRNA target sites in the 3′ UTR (34, 35, 46). Thus, identification of miRNAs or other miRISC components in a polysome fraction of a sucrose gradient is not a definitive proof of the repression acting at the postinitiation translational steps, as cosedimenting miRNA-AGO complexes may not always be repressing their associated mRNAs.

Evidence supporting the postinitiation mechanism is not limited to association of miRNP components and repressed mRNA with translating polysomes. Several groups observed IRES-driven translation being repressed

by the miRNA machinery (117, 121), in marked contrast to other studies (46, 91, 95, 97). In particular, Petersen et al. (117) observed cap-independent translation, driven by the HCV IRES and CrPV IRES, being repressed by the CXCR4 miRNA mimic. As IRES elements require fewer (e.g., EMCV and HCV) or any (e.g., CrPV) translation factors to initiate translation, these results are consistent with miRNAs inhibiting translation at a step other than initiation.

What could be the mechanism by which miRNAs inhibit translation at postinitiation steps? Unfortunately, there are no known molecular mechanisms to explain such inhibition. Conclusions drawn from metabolic labeling and ribosome run-off experiments led Petersen et al. (117) to propose that miRNAs may antagonize translation elongation by causing premature termination and subsequent ribosome drop-off (**Figure 2*b***). Interestingly, similar observations and conclusions were made for translationally repressed mammalian nonSTOP mRNAs that lack in-frame termination codons (122).

On the basis of the demonstrated association of miRNA targets with polysomes, Nottrott et al. (115) proposed that the miRNA machinery recruits proteolytic enzymes to polysomes, which leads to the degradation of nascent polypeptides (**Figure 2*b***); a similar model had been previously put forward by Olsen & Ambros (88). Although not completely excluded, this model is highly improbable, as targeting polypeptides to the endoplasmic reticulum (ER) (using an ER signal recognition sequence), which should have made nascent proteins inaccessible to proteolysis, had no effect on the degree of the miRNA-mediated translational repression in HeLa cells (46). Moreover, high-throughput profiling of mammalian miRNA targets demonstrated an overrepresentation of mRNAs coding for membrane and ER proteins among translationally repressed mRNAs (123).

MODULATION OF miRNA-MEDIATED REPRESSION

miRNAs act preferentially by binding to the mRNA 3′ UTR. Hence, it comes as no surprise that 3′ UTR–binding proteins, such as HuR (24) or Dead-end 1 (Dnd1) (25), modulate miRNA-mediated repression. Regulation of miRNA repression by RNA-binding proteins is probably a widespread phenomenon. A comparative study of mRNAs interacting with Pumilio (PUF) proteins, which have been linked to let-7 repression of hbl-1 mRNA in *C. elegans* (124), showed a considerable enrichment of PUF-binding sites in the vicinity of predicted miRNA recognition sequences in human mRNAs (125). APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G) also appears to interfere with miRNA repression (92).

HuR is an AU-rich element (ARE)-binding protein, which counteracts the action of ARE-associating proteins known to destabilize mRNAs. It does so by competing with destabilizing proteins for binding to the mRNA 3′ UTR (reviewed in Reference 126). Bhattacharyya et al. (24) demonstrated that HuR relieves the miR-122-mediated repression of CAT-1 mRNA. In human hepatoma cells, CAT-1 mRNA is translationally repressed and localizes to P bodies in an miR-122-dependent manner. In response to cellular stress, such as amino acid starvation or ER stress, HuR translocates from the nucleus to the cytoplasm and, by binding to the CAT-1 mRNA 3′ UTR, causes the release of the mRNA from P bodies and into actively translating polysomes. In contrast to the situation with CAT-1 mRNA, repression of c-Myc mRNA by the let-7 miRNA is enhanced by the HuR binding to adjacent AREs (127). Consistently, depleting cells of HuR abrogates let-7-mediated inhibition of c-Myc. It is possible that HuR binding to the 3′ UTR modifies mRNA folding or accessibility of RISC to miRNA-binding sites. This scenario may apply to c-Myc, but is unlikely to operate during CAT-1 mRNA regulation. In the latter case,

HuR functions even when its binding site is positioned far away from the miR-122 sites. In addition, HuR derepresses mRNAs targeted not only by miR-122 but also by let-7 miRNA (24).

Dnd1 is an RNA-binding protein that is essential for primordial germ cell (PGC) survival in zebrafish (128) and mouse (129). Dnd1 prevents miR-221-mediated repression of the p27 mRNA in mammalian cells and miR-430-mediated repression of nanos1 and TDRD7 mRNAs in the PGCs of zebrafish (25). In zebrafish embryos, the nanos1 and TDRD7 mRNAs are deadenylated by miR-430 in somatic cells, but not in the PGCs regardless of the fact that miR-430 is present in both cell types (130). Depleting Dnd1, using an antisense strategy, led to a marked miR-430-dependent decrease in both nanos1 and TDRD7 mRNAs. It appears that Dnd1 prevents miRNA-mediated repression of nanos1 and TDRD7 mRNAs by binding to U-rich sequences adjacent to miRNA-binding sites. In this way, it interferes with the miRNA binding to the 3′ UTR of target mRNAs (25).

Two TRIM-NHL family proteins, NHL-2 in *C. elegans* (26) and TRIM32 in mice (27), were identified as positive regulators of miRNA activity. The *C. elegans* NHL-2 is required for full potency of let-7 and lsy-6 miRNAs. It interacts genetically and physically with the worm equivalents of AGO, GW182, and RCK/p54 proteins; the latter protein is implicated in miRNA repression in flies and mammals (20). The mouse TRIM32 enhances miRISC activity by interacting (via the NHL domain) with AGO1. Intriguingly, both TRIM-NHL proteins appear to enhance the activity of only some miRNAs. How these proteins enhance the repression of selected miRNAs and what specific features of miRISC or miRNA-mRNA complexes they recognize remain unknown.

miRNA-MEDIATED TRANSLATIONAL ACTIVATION

It was reported that miRNAs, in specific situations, activate rather than repress translation (11–13, 131). Vasudevan and coworkers (11, 131) found that miRNAs repress translation in proliferating cells but upregulate it in quiescent cells arrested in G0/G1. For example, under serum starvation conditions, the AGO2-miR369-3 complex bound to the 3′ UTR of TNFα mRNA was found to recruit the fragile X–related protein 1 (FXR1) and stimulate mRNA translation. Also, tethering of AGO2 or FXR1 to a reporter mRNA 3′ UTR activated translation in growth arrested cells. Broad translational activation by miRNAs or AGO2 in quiescent cells is rather unexpected. Of note, in G1-arrested cells in the *Drosophila* eye, miRNAs were found to repress translation of mRNAs (132). Thus, miRNA-mediated activation of translation is probably not a general mechanism in nonproliferating cells.

Examples of stimulatory effects of miRNAs interacting with the mRNA 5′ UTR were also reported. Orom et al. (12) found that miR-10a interacts with the 5′ UTR of many mRNAs encoding ribosomal proteins and is responsible for increased translation of these mRNAs in response to stress or nutrient shortage. The miRNA interaction was mapped to a region immediately downstream of the 5′ TOP (5′-terminal oligopyrimidine tract) motif characteristic of mRNAs encoding ribosomal proteins and some translation factors (133). Surprisingly, miR-10a binding to the 5′ UTR of ribosomal protein mRNAs does not seem to follow the classical miRNA-mRNA interaction rules for seed region base-pairing. Previously, a similar nonorthodox base-pairing was proposed for the interaction of miR-16 with the ARE-like element in mRNA 3′ UTR (134). It is important to establish what rules apply to these noncanonical miRNA-mRNA interactions.

miR-122, a liver-specific miRNA, stimulates replication of the HCV RNA in hepatoma cells by binding to the 5′ UTR upstream of the HCV RNA IRES (135). More recently, Henke et al. (13) found that miR-122 may also stimulate HCV RNA translation, possibly by increasing ribosome loading on the HCV IRES. As the HCV IRES can be translationally repressed by artificial tethering of multiple AGO2 molecules upstream of the IRES (121), it is

possible that, in the experiments of Henke et al., miR-122 does not function by recruiting AGO and GW182 proteins, but rather acts as a chaperone modifying RNA structure and facilitating ribosome access to the HCV mRNA. Additional experimentation is required to understand why miR-122 enhances translation when binding to the HCV 5′ UTR but inhibits translation of cellular and reporter mRNAs bearing miR-122 sites in the 3′ UTR (24, 127, 136).

miRNA-MEDIATED mRNA DEADENYLATION AND DECAY

mRNA decay most often starts with the removal of the poly(A) tail by 3′–5′ exoribonucleases, which include (*a*) the CCR4 (carbon catabolite repression 4)-NOT1 (negative on TATA-less) complex, which contains, in addition to other proteins, the deadenylases CCR4/CNOT6 and CAF1/CNOT7 (CCR4-associated factor, an RNase D family deadenylase); (*b*) poly(A)-specific ribobonuclease (PARN); and (*c*) poly(A) nuclease (PAN) (137, 138). Either the mRNA is degraded in a 3′–5′ direction, or the 5′-terminal cap is first removed by the decapping enzyme (i.e., DCP1-DCP2 complex), and the body of the RNA is then degraded by Xrn1, a 5′–3′ exonuclease (139). mRNA stability is often under the control of *cis*-acting elements within the 3′ UTR, which recruit protein factors that, in turn, recruit deadenylation enzymes. Examples of these *cis*-acting elements include AU-rich elements, the *c-fos* RNA coding determinant, and miRNA target sites (28, 140, 141). miRNAs cause mRNA target degradation in human cells, *C. elegans*, *Drosophila* S2 cells, and zebrafish (17, 50, 142, 143). Many studies showed that perturbing the levels of specific miRNAs, or the activity of the miRNA machinery, has dramatic effects on the level of hundreds of miRNA targets. Several of these studies demonstrated that miRNA-mediated downregulation of target levels has important biological consequences (143–145).

Much evidence supports the idea that miRNAs destabilize target mRNAs through deadenylation and subsequent decapping and 5′–3′ exonucleolytic digestion. First, poly(A) length determination assays have demonstrated that miRNAs mediate deadenylation of a wide array of targets in a variety of systems. In zebrafish, miR-430 mediates the deadenylation of hundreds of maternal transcripts at the early stage of embryo development (143). Using mouse P19 embryonal carcinoma cells, Wu & Belasco (146) demonstrated that *lin-28* mRNA, whose levels decrease during retinoic acid-induced neuronal differentiation, is deadenylated through the activity of miR-125, a miRNA whose levels increase during differentiation (113). miRNA-mediated deadenylation has also been recapitulated in both mammalian and *Drosophila* cell-free extracts (52, 95, 106) (see below).

GW182 and miRNA-Mediated Deadenylation

Deadenylation and the subsequent decapping and decay of mRNAs targeted by miRNAs require the AGO and GW182 components of the miRISC (17). Knocking down or immunodepleting human AGO2 (106) or *Drosophila* AGO1 (17) abrogates miRNA-mediated deadenylation and stabilizes miRNA-targeted mRNAs. GW182 proteins, which interact with all mammalian AGO proteins (reviewed in References 43, 60, and 147), and *Drosophila* AGO1 (17), are also required for miRNA-mediated deadenylation and decapping (17, 18, 52, 65, 95, 106). The GW182-AGO interaction is mediated via GW repeats in the GW182 N terminus through binding to the AGO MID/PIWI domain (17, 64, 148–150). The relevance of this interaction for miRNA-mediated repression was tested using a GW-rich fragment of GW182 termed the AGO hook. A GW182 fragment encompassing the AGO hook region when expressed in *Drosophila* cells competes with GW182 for binding to AGO and interferes with miRNA-mediated repression (65). Moreover, adding the hook peptide blocked miRNA-mediated translational repression or deadenylation in vitro (64, 106, 148).

As mentioned above, tethering GW182 proteins to the mRNA represses translation and causes mRNA decay even in the absence of AGO proteins (17, 65, 68, 151), demonstrating that AGO proteins act as scaffolds to recruit GW182 to the mRNA. Thus, although AGO recruitment to mRNA can be circumvented, GW182 is indispensible. Knocking down GW182 in *Drosophila* S2 cells abrogates both translational repression and decay of miRNA-targeted mRNAs (17, 52, 68), demonstrating that GW182 is integral to both miRNA mechanisms of action. Similar results were observed in mammalian cells (67) and in *C. elegans* (62, 93). Thus, even though GW182 proteins interact with and recruit the mRNA decay machinery to miRNA-targeted mRNAs (see below), they likely also interact with translation factors and/or ribosomal subunits on target mRNAs to antagonize translation.

CCR4-NOT1 Complex and miRNA-Mediated Deadenylation

GW182 recruits the CCR4-NOT1 deadenylase complex to promote deadenylation of miRNA-targeted mRNAs (**Figure 3**). The complex can also be recruited to the mRNA by tethering GW182 to the 3′ UTR (17). CAF1 was identified as an AGO-interacting protein by MudPIT (multidimensional protein identification technology) analysis of human AGO1 and AGO2 immunoprecipitates from HEK293 cells (106). CAF1 was also pulled down from micrococcal nuclease-treated mouse Krebs ascites extracts using a biotin-labeled antilet-7 2′-*O*-methylated oligonucleotide, indicating that it interacts with let-7-loaded AGO proteins in an RNA-independent manner (106). Knocking down CAF1 or NOT1 blocked the majority of miRNA-mediated deadenylation and mRNA destabilization (114). In addition, immunodepleting CAF1 from a mammalian cell-free extract dramatically blocked let-7-mediated deadenylation (106). Thus, the requirement of the CCR4-NOT1 deadenylase complex for miRNA-mediated deadenylation is evolutionarily conserved.

Poly(A)-Binding Protein and miRNA-Mediated Deadenylation

The PABP is also required for miRNA-mediated deadenylation in vitro. Depleting the PABP from Krebs extracts blocked let-7-mediated deadenylation, which could be rescued by adding a recombinant PABP (106). How might a PABP function in miRNA-dependent deadenylation? Glutathione *S*-transferase pull-down experiments, in parallel with in vivo coimmunoprecipitations, revealed that the C terminus of GW182 directly interacts with the C terminus of the PABP in an RNA-independent manner. Moreover, blocking this interaction in Krebs extracts antagonized miRNA-mediated deadenylation in vitro (106). C-terminal fragments of human (67, 69) and *Drosophila* (63, 68) GW182 proteins, which contain both the DUF and RRM regions, mediate deadenylation and decay of mRNAs with an efficiency comparable to that of the full-length proteins when tethered to the reporter 3′ UTR. Of note, a sequence within the DUF region of both mammalian and *Drosophila* GW182 proteins shares similarity to a motif [termed a PAM2 motif (152–154)] in PABP-interacting proteins that binds the PABP C terminus (106). Moreover, the recently solved crystal structure of the mammalian DUF oligopeptide in a complex with the PABP C-terminal domain demonstrates that DUF exhibits a fold similar to the PAM2 motif (155). Thus, the DUF most likely functions as a PAM2-like motif to bind directly to the PABP C terminus.

Taken together, these data demonstrate that miRNA-mediated deadenylation requires the GW182 C terminus to interact with the PABP (**Figure 3**). Immunoprecipitated *Drosophila* GW182 protein interacts with the PABP (156), demonstrating that this interaction is evolutionarily conserved. A GW182-PABP interaction may have multiple roles in miRNA-mediated repression. It is conceivable that this interaction juxtaposes the PABP-associated poly(A) tail with the miRISC-associated deadenylase complex to facilitate initiation of the deadenylation reaction (106). The PABP is

also a bona fide translation initiation factor that stimulates 40S ribosomal subunit recruitment and 80S complex formation (79). The PABP interacts with eIF4G (79, 157), Paip1 (a PABP-interacting protein that stimulates translation) (152, 158, 159), and the termination factor eRF3 (160). Thus, GW182 binding to the PABP may interfere with both translation initiation (by interfering with the mRNA "closed loop" conformation) and termination by blocking PABP binding to the factors listed above, through either competitive binding or steric hindrance. Consistent with this idea, adding a fragment of eIF4G that binds the PABP blocked miRNA-mediated deadenylation in vitro (106). In addition, overexpression of a fragment of GW182 in *Drosophila* S2 cells that binds PABP competed with PABP-eIF4G complexes (156). Using a dsRNA-mediated knockdown strategy to deplete various proteins in *Drosophila* S2 cells, Izaurralde and coworkers (17–19, 65) screened for factors involved in miRNA-mediated mRNA deadenylation, decapping, and decay. In addition to GW182, they identified the decapping DCP1-DCP2 complex and the decapping enhancer proteins Ge-1, EDC3, HPat, and Me31B. Knocking down key decapping factors led to the stabilization of the deadenylated miRNA targets. Thus, although miRNA-mediated deadenylation (**Figure 3**, step 1) is not sufficient for destabilization of target mRNAs, it mechanistically precedes the miRNA-mediated decapping (**Figure 3**, step 2) (18).

miRNA-mediated deadenylation is translation independent. It can proceed even when translation is blocked by translation inhibitors, such as cycloheximide (95, 106) or hippuristanol (106). It is also observed when the start codon of the mRNA is blocked by an antisense oligonucleotide (143) or by insertion of stable stem-loop structures in 5′ UTRs that block ribosome scanning (114). Likewise, it can occur on a model ApppN-capped mini-mRNA devoid of the coding region (106). These results demonstrate that a miRNA-targeted mRNA does not need to be translationally competent for deadenylation to proceed. Whether an actively translating mRNA first needs to be translationally repressed by the miRNA machinery to undergo deadenylation is an important question. Addition of a recombinant fragment of eIF4G that binds the PABP to Krebs extract blocks miRNA-mediated deadenylation (106), suggesting that the eIF4G-PABP interaction must be disrupted before deadenylation can commence. As the eIF4G-PABP interaction enhances cap-dependent translation, these data suggest that miRNA-mediated inhibition of translation may precede deadenylation. Indeed, kinetic analysis has revealed that let-7-mediated translational repression occurs prior to deadenylation in a mammalian cell-free extract (106).

miRNA-mediated deadenylation might also proceed on actively translating mRNAs. Using Xrn1 deletion yeast strains, Hu et al. (161) demonstrated that an actively translating mRNA can be cotranslationally deadenylated and decapped. In keeping with this result, Beilharz et al. (112) observed that the miRNA-mediated deadenylation precedes translational repression of let-7-target mRNAs in mammalian cells. Thus, miRNA-mediated deadenylation may be a cotranslational event as well. This might explain why miRNA-targeted mRNAs are frequently found in association with polysomes (115, 116, 119, 120). It would be interesting to investigate, using high-resolution poly(A) tail length determination assays, whether the polysome-associated miRNA targets are deadenylated.

Although miRNA-mediated deadenylation and subsequent mRNA decay appear to be widespread events (114, 123, 162), not all miRNA-targeted mRNAs are destabilized (for a review, see Reference 5). In addition, the Dicer mRNA in mammalian cells is translationally repressed by the let-7 miRNA, but its mRNA level remains for the most part unaffected (123). Moreover, even though deadenylation can contribute to miRNA-mediated repression, it is not absolutely required. Depletion of the CAF1 deadenylase, which abrogates let-7-mediated deadenylation, alleviates some but not all repression in Krebs extracts (106). Likewise,

inhibiting deadenylation in mammalian cells, using an antisense blocking strategy, reduces but does not abolish miRNA-mediated repression (112). In addition, efficient miRNA-mediated deadenylation in vitro may require the PABP to contact the GW182 DUF motif (106), but the tethered C-terminal GW182 fragments lacking the DUF or containing mutations in its sequence are still able to efficiently repress protein synthesis in both *Drosophila* (63, 68) and mammalian (66, 67) cells. Thus, miRNA-mediated mRNA deadenylation and decay cannot account for the entirety of miRNA action.

A MULTITUDE OF INHIBITORY MECHANISMS?

On the basis of the data summarized above, it is clear that the molecular mechanisms by which miRNAs inhibit cap-dependent initiation of translation and mediate mRNA deadenylation and decay have begun to emerge. Nonetheless, there is considerable documentation to support alternative mechanisms in addition to translation initiation. One obvious and logical possibility is that miRNAs effect repression via several disparate or potentially overlapping mechanisms in a cell- or development-dependent manner. It is also possible that differences in the experimental design favor one mode of repression over another. For example, Lytle et al. (121) reported that the method of cell transfection (for example, cationic lipid versus electroporation) strongly influences the degree of miRNA-mediated repression. Differences in the transcriptional promoters used for driving expression of mRNA reporters might also account for some contradictory data (163). Although SV40 promotor-driven mRNAs shift into subpolysomal fractions upon repression by miRNA, the TK promotor-driven mRNAs do not. Therefore, promoter-dependent loading of specific RNA-binding proteins and/or differences in mRNA nuclear processing might dictate whether initiation or postinitiation repression dominates. Another attempt to explain the observed discrepancies is based on modeling of rate-limiting steps during translation. Nissan & Parker (164) postulated that some of the discrepancies could result from differences in rate-limiting steps in translation systems or in the mRNA reporters used by different investigators. For example, they argue that mRNAs containing IRESs may be refractory to miRNA inhibition because initiation is not the rate-limiting step during translation of these mRNAs.

CELLULAR COMPARTMENTALIZATION OF miRNA REPRESSION

Much evidence exists indicating that many components of the miRNA machinery and the repression process itself may not be localized in the cytosol but that they occur in association with different cellular organelles or structures.

Roles of P Bodies

Translationally repressed mRNAs can accumulate in discrete cytoplasmic foci known as P or GW bodies (15, 16) or in another class of cytoplasmic aggregates, stress granules (SGs), which form in response to various stress conditions (165). P bodies also seem to act as sites of the final steps of mRNA degradation (15, 16), although a recent report indicates that decapping and 5′–3′ exonucleolytic degradation of mRNA in yeast already occur on polysomes when mRNA still continues to be translated (161). P bodies are enriched in proteins involved in mRNA deadenylation, decapping, and degradation. For example, they contain the CCR4-NOT1 deadenylase complex, the decapping enzyme complex DCP1-DCP2, and the 5′–3′ exonuclease XRN1 (15, 16), key factors responsible for mRNA decay. P bodies are also enriched in a group of proteins referred to as decapping activators, including the helicase RCK/p54 (Me31B in *Drosophila*), HPat1, RAP55, EDC3, Ge-1/Hedls, and the heptameric LSm1-7 complex. As described above, some of the latter proteins (e.g., RCK/p54 and HPat1), or their homologs, act as translational

repressors (15, 16, 165). P bodies lack ribosomes and most of the translation initiation factors.

Consistent with their role in translational repression and mRNA decay, P bodies in metazoans are also enriched in proteins participating in miRNA repression, such as AGOs and GW182s [the GW182 proteins are actually among the "founding" components of P bodies and this is why these structures are also known as GW bodies (166)], and in miRNAs themselves. Moreover, there are several reports describing a correlation between miRNA-mediated translational repression and accumulation of target mRNAs in P bodies and an inverse relationship between P-body localization and polysome association of target mRNAs (24, 46, 92, 167). For example, CAT-1 mRNA, a target of miR-122 in human hepatoma Huh7 cells, localizes to P bodies when translation is repressed but is redistributed outside of P bodies by stress. Moreover, transfecting miR-122 into cells that do not express miR-122 results in accumulation of CAT-1 mRNA in P bodies (24). Recently, Nathans et al. (168) demonstrated that miR-29a, by interacting with the 3′ UTR of the human immunodeficiency virus-1 (HIV-1), targets HIV-1 RNA to P bodies in human T lymphocytes and that disruption of P bodies by depletion of their components enhances HIV-1 viral production and infectivity. Consistent with their enrichment in P bodies, AGO and GW182 proteins and miRNAs interact with different P-body components (17, 20, 45, 46, 62, 167, 169), and the knockdown of some P-body components, e.g., RCK/p54 in mammalian cells (20) or Ge-1 and combinations of other decapping activators in *Drosophila* S2 cells (18), compromises miRNA-induced repression. Notably, a functional miRNA pathway is essential for formation of P-body aggregates. Global inhibition of miRNA biogenesis or depletion of proteins involved in miRNA repression results in dispersal of P bodies in mammalian and *Drosophila* S2 cells (169, 170).

Despite the aforementioned observations, which implicate P bodies in the miRNA-mediated silencing, important issues regarding the miRNA-P-body relationship remain unresolved. P bodies are highly dynamic structures, altering in both size and number during the cell cycle and in response to changes in the translational status of the cell (15, 16, 169–171). Depletion of certain P-body components has a strong effect on their integrity, as visualized by light microscopy. Interestingly, knockdowns of certain P-body components result in the dispersion of P bodies but do not prevent miRNA-mediated repression. These findings indicate that microscopically visible P bodies are not essential for the repression and that the presence of large P-body aggregates is a consequence rather than the cause of miRNA-induced silencing (170). However, these findings do not exclude the possibility that submicroscopic structures, possibly escaping elimination in knockdown experiments, contribute to the persisting repression. Indeed, studies of the interactions between core protein components during P-body formation in yeast indicate that assembly of submicroscopic complexes, consisting of P-body components, with individual mRNAs is sufficient to engender translational repression and/or decay (172).

The relative distribution of miRISC components between P bodies and the cytosol represents another issue of contention. Only ~1.3% of enhanced green fluorescent protein (EGFP)-tagged AGO2 localizes to P bodies in HeLa cells (173). Moreover, in FRAP (fluorescence recovery after photobleaching) experiments, the P-body-associated EGFP-AGO2 and also GFP-GW182 exchanged with the cytoplasm at a much slower rate than some P-body components involved in mRNA decay (173–175). In another study, it was found that only ~20% of ectopically expressed let-7 miRNA and repressed reporter mRNA localized to visible P bodies (46). Collectively, these data suggest that the repression either involves submicroscopic P bodies or occurs outside of them. Because many P-body components, including AGO proteins, are also found throughout the cytosol (16), a possible scenario is that repression by miRISCs is initiated in the cytosol and that the

repressed mRNAs form P-body aggregates, either small or large, upon run off of ribosomes. P-body proteins having established inhibitory activity on translation (see above) might assist miRISCs in initiating the repression. The fact that miRNA repression can be recapitulated in cell-free extracts also argues against a primary role of P bodies in this process, inasmuch as these aggregates are unlikely to exist in cell-free extracts. However, pseudopolysomes that are formed in extracts from *Drosophila* embryos (96) might represent P-body-like aggregates, and it will be interesting to analyze them in some detail.

Role of Stress Granules

SGs form upon global repression of translation initiation (165). SGs share some protein components with P bodies, and SGs and P bodies are frequently located adjacent to each other, possibly exchanging their cargo material (175, 176). AGO proteins, artificial miRNA mimics, and repressed reporter mRNAs accumulate in SGs (173). Because SGs are known to form not only in response to stress, but also upon general inhibition of translation initiation (177, 178), SGs, like P bodies, may play a role in miRNA-mediated repression (173, 178). However, it is also possible that localization of miRISC components to SGs is solely due to pulling the mRNA-associated, but not necessarily inhibitory, miRISCs to SGs formed in response to stress. The latter possibility could explain why localization of AGO proteins to SGs, but not P bodies, is miRNA dependent (173). AGO proteins directly interact with core P-body components (17, 20, 64), but their localization to SGs might depend on association with miRNA to allow interaction with mRNA by base-pairing.

Role of Multivesicular Bodies and Endosomes

Association of a large fraction of AGO proteins with cellular membranes, such as the Golgi and ER, was noted some time ago, but its possible significance remained unexplored (179–181). Recent work carried out in *Drosophila* and in mammalian cells identified MVBs, specialized late endosomal compartments with a characteristic multivesicular morphology, as cellular organelles contributing to miRNA function or miRISC turnover (21, 22). MVBs sort endocytosed proteins into different compartments, including lysosomes (for proteolysis) and exosomes (for secretion). In both *Drosophila* and mammalian cells, blocking of MVB formation inhibits silencing by siRNAs and miRNAs, whereas blocking their turnover stimulates silencing. Dissection of the miRNA pathway, in vivo and in vitro, identified the loading of AGO proteins with small RNAs as a step that is enhanced when MVB turnover is impaired by inactivation of *HPS4* (Hermansky-Pudlak syndrome 4), a gene originally identified in a *Drosophila* screen, whose mutation enhances small RNA silencing (22). Gibbings et al. (21) reached similar conclusions by silencing different mammalian genes involved in MVB metabolism. Both studies investigating the role of MVBs/endosomes concluded that a large fraction of GW bodies [we use this nomenclature because GW182 proteins were mainly used to follow the localization of P/GW-body aggregates; in addition, Gibbings et al. (21) found that a considerable fraction of GW182-containing structures does not contain classical P-body markers] colocalizes with MVBs. Also, GW182 and some miRNAs, but not AGO proteins, were enriched in purified exosome-like vesicles secreted by MVBs. Whether the latter phenomenon represents a specific way of elimination of miRISC components from the cell or is indicative of the miRNA-mediated intercellular communication (182) remains to be established.

In summary, much still needs to be learned about the cellular localization of different steps in the assembly, function, and recycling of miRISCs. It will be interesting to establish how specific miRNAs are transported in neurons to get to dendritic spines, where they are implicated in regulation of local translation in response to synaptic stimulation (183, 184).

PERSPECTIVES

It is astonishing that miRNAs have evaded the radar of molecular biologists for so long, considering their paramount involvement and impact on organism and organ development, cellular differentiation, viral infection, and oncogenesis. What might we expect in the coming decade? One can anticipate solving the three-dimensional structures of the individual miRISC components and the complex itself. The knowledge generated from these structures, and possibly intermediates in miRISC assembly, should provide a comprehensive view of the molecular mechanism of miRISC formation and function.

An important challenge is to elucidate the interactions of miRISC with components of the translation and deadenylation machinery and to obtain three-dimensional structures of these supercomplexes. *Drosophila* has only one GW182 protein; however, there are several mammalian GW182 paralogs and isoforms. It is important for future studies to determine their tissue expression profiles during development and to establish whether they have redundant or unique functions. Another important challenge is to determine what dictates whether an mRNA-bound miRISC inhibits translation or initiates mRNA decay, or both. Indeed, 3′-UTR architecture, in combination with RNA-binding proteins, such as HuR and Dnd1, has already been shown to regulate miRNA accessibility and/or repressive function. Possibly, similar types of RNA-protein interactions may determine which mechanism, translational repression or deadenylation, is favored for miRNA-mediated repression.

Another important new field of miRNA research is identifying new posttranslational modifications to miRISC proteins and determining how these modifications impact miRISC function (reviewed in References 185 and 186). The Dicer-interacting protein TRBP was recently shown to be positively regulated through phosphorylation by the mitogen-activated protein kinase (MAPK) pathway (187). Moreover, human AGO2 undergoes several forms of posttranslational modification. AGO2 can be hydroxylated at proline 700 by the type I collagen prolyl-4-hydrolylase, a modification that stabilizes AGO2 and localizes it to P bodies (188). AGO and GW182 proteins are also known to be phosphorylated (166, 189). AGO2 phosphorylation at serine 387 facilitates its localization to P bodies (189). AGO2 may be negatively regulated by posttranslational modification, as phosphorylation of tyrosine 529 in the small RNA 5′ end-binding pocket interferes with small RNA loading (G. Meister, personal communication). Uncovering new posttranslational modifications and the signaling cascades that lead to these modifications is of paramount importance. As our understanding of the molecular biology of miRNA action increases, it will be possible to gain important insights into the role of miRNAs in health and disease.

SUMMARY POINTS

1. miRNAs inhibit protein synthesis by repressing translation and/or by bringing about deadenylation and subsequent degradation of mRNA targets. Generally, miRNAs function as part of ribonucleoprotein complexes, miRISCs (miRNA-induced silencing complexes), with miRNAs base-pairing to partially complementary sequences in the 3′ UTRs of target mRNAs. In certain instances, miRNAs have been also reported to activate translation of targeted mRNAs.
2. Core components of miRISCs include the AGO family of proteins, which directly anchor miRNAs in a deep pocket, and the GW182 family of proteins, which directly interact with AGO proteins via their GW repeats. GW182 proteins act downstream of AGO proteins to effect miRNA-mediated repression. AGO proteins function to bridge the miRNA to the silencing effectors, the GW182 proteins.

3. miRNAs have been found to repress translation at initiation, either by targeting the cap recognition step or by inhibiting ribosome 80S complex assembly, but repression at postinitiation steps has also been reported.
4. miRNA-mediated repression can be modulated by 3′ UTR-binding proteins such as HuR and Dnd1, and two AGO-interacting proteins of the TRIM-NHL protein family, the *C. elegans* NHL-2 protein and the mouse TRIM32 protein.
5. miRISC was shown to recruit the CCR4-NOT1 deadenylase complex to promote deadenylation of miRNA-targeted mRNAs. The PABP enhances miRNA-mediated deadenylation via its direct interaction with GW182.
6. miRNA-targeted translationally repressed mRNAs can accumulate in discrete cytoplasmic foci, such as P or GW bodies, or stress granules. A fraction of GW bodies colocalizes with multivesicular bodies (MVBs), membrane structures that play a role in miRNA-mediated repression.

FUTURE ISSUES

1. The detailed molecular mechanisms of how the miRISC represses translation are not known. Key issues include understanding how the miRISC directly contacts the deadenylation and translation machinery. In vitro reconstituted systems are likely to prove essential in addressing these issues.
2. AGO and GW182 proteins can undergo posttranslational modification. Future studies will eludicate the dynamics of these modifications and their importance for activity of the proteins. These studies will uncover the signaling pathways that posttranslationally regulate the activity of miRISC components.
3. While several RNA-binding proteins that modulate miRISC activity on specific target mRNAs have been discovered, many more are likely to be involved.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by a grant from the Canadian Institutes of Health Research (N.S.); the European Commission Framework Program 6 Project "Sirocco" and the Friedrich Miescher Institute, which is supported by the Novartis Research Foundation (W.F.); and by a postdoctoral fellowship from the Canadian Cancer Society (M.R.F.).

LITERATURE CITED

1. Friedman RC, Farh KK, Burge CB, Bartel DP. 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 19:92–105
2. Bartel DP. 2009. MicroRNAs: target recognition and regulatory functions. *Cell* 136:215–33

3. Bushati N, Cohen SM. 2007. microRNA functions. *Annu. Rev. Cell Dev. Biol.* 23:175–205
4. Ghildiyal M, Zamore PD. 2009. Small silencing RNAs: an expanding universe. *Nat. Rev. Genet.* 10:94–108
5. Filipowicz W, Bhattacharyya SN, Sonenberg N. 2008. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nat. Rev. Genet.* 9:102–14
6. Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M, Dunoyer P, Yamamoto YY, et al. 2008. Widespread translational inhibition by plant miRNAs and siRNAs. *Science* 320:1185–90
7. Lanet E, Delannoy E, Sormani R, Floris M, Brodersen P, et al. 2009. Biochemical evidence for translational repression by *Arabidopsis* microRNAs. *Plant Cell* 21:1762–68
8. Eulalio A, Huntzinger E, Izaurralde E. 2008. Getting to the root of miRNA-mediated gene silencing. *Cell* 132:9–14
9. Chekulaeva M, Filipowicz W. 2009. Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. *Curr. Opin. Cell Biol.* 21:452–60
10. Vasudevan S, Tong Y, Steitz JA. 2008. Cell-cycle control of microRNA-mediated translation regulation. *Cell Cycle* 7:1545–49
11. Vasudevan S, Tong Y, Steitz JA. 2007. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 318:1931–34
12. Orom UA, Nielsen FC, Lund AH. 2008. MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. *Mol. Cell* 30:460–71
13. Henke JI, Goergen D, Zheng J, Song Y, Schuttler CG, et al. 2008. microRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J.* 27:3300–10
14. Niepmann M. 2009. Activation of hepatitis C virus translation by a liver-specific microRNA. *Cell Cycle* 8:1473–77
15. Parker R, Sheth U. 2007. P bodies and the control of mRNA translation and degradation. *Mol. Cell* 25:635–46
16. Eulalio A, Behm-Ansmant I, Izaurralde E. 2007. P bodies: at the crossroads of post-transcriptional pathways. *Nat. Rev. Mol. Cell Biol.* 8:9–22
17. Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E. 2006. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev.* 20:1885–98
18. Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, et al. 2007. Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes Dev.* 21:2558–70
19. Rehwinkel J, Behm-Ansmant I, Gatfield D, Izaurralde E. 2005. A crucial role for GW182 and the DCP1:DCP2 decapping complex in miRNA-mediated gene silencing. *RNA* 11:1640–47
20. Chu CY, Rana TM. 2006. Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. *PLoS Biol.* 4:e210
21. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. 2009. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat. Cell Biol.* 11:1143–49
22. Lee YS, Pressman S, Andress AP, Kim K, White JL, et al. 2009. Silencing by small RNAs is linked to endosomal trafficking. *Nat. Cell Biol.* 11:1150–56
23. Wu L, Belasco JG. 2008. Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs. *Mol. Cell* 29:1–7
24. Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. 2006. Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* 125:1111–24
25. Kedde M, Strasser MJ, Boldajipour B, Oude Vrielink JA, Slanchev K, et al. 2007. RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell* 131:1273–86
26. Hammell CM, Lubin I, Boag PR, Blackwell TK, Ambros V. 2009. *nhl-2* modulates microRNA activity in *Caenorhabditis elegans*. *Cell* 136:926–38
27. Schwamborn JC, Berezikov E, Knoblich JA. 2009. The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. *Cell* 136:913–25
28. Standart N, Jackson RJ. 2007. MicroRNAs repress translation of m7Gppp-capped target mRNAs in vitro by inhibiting initiation and promoting deadenylation. *Genes Dev.* 21:1975–82
29. Nilsen TW. 2007. Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet.* 23:243–49

30. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. 2009. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* 11:228–34
31. Kim VN, Han J, Siomi MC. 2009. Biogenesis of small RNAs in animals. *Nat. Rev. Mol. Cell Biol.* 10:126–39
32. Flynt AS, Lai EC. 2008. Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nat. Rev. Genet.* 9:831–42
33. Vella MC, Choi EY, Lin SY, Reinert K, Slack FJ. 2004. The *C. elegans* microRNA *let-7* binds to imperfect let-7 complementary sites from the *lin-41* 3′UTR. *Genes Dev.* 18:132–37
34. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. 2007. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol. Cell* 27:91–105
35. Doench JG, Sharp PA. 2004. Specificity of microRNA target selection in translational repression. *Genes Dev.* 18:504–11
36. Easow G, Teleman AA, Cohen SM. 2007. Isolation of microRNA targets by miRNP immunopurification. *RNA* 13:1198–204
37. Gu S, Jin L, Zhang F, Sarnow P, Kay MA. 2009. Biological basis for restriction of microRNA targets to the 3′ untranslated region in mammalian mRNAs. *Nat. Struct. Mol. Biol.* 16:144–50
38. Rigoutsos I. 2009. New tricks for animal microRNAS: targeting of amino acid coding regions at conserved and nonconserved sites. *Cancer Res.* 69:3245–48
39. Kloosterman WP, Wienholds E, Ketting RF, Plasterk RH. 2004. Substrate requirements for let-7 function in the developing zebrafish embryo. *Nucleic Acids Res.* 32:6284–91
40. Peters L, Meister G. 2007. Argonaute proteins: mediators of RNA silencing. *Mol. Cell* 26:611–23
41. Jinek M, Doudna JA. 2009. A three-dimensional view of the molecular machinery of RNA interference. *Nature* 457:405–12
42. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, et al. 2004. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305:1437–41
43. Landthaler M, Gaidatzis D, Rothballer A, Chen PY, Soll SJ, et al. 2008. Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. *RNA* 14:2580–96
44. Azuma-Mukai A, Oguri H, Mituyama T, Qian ZR, Asai K, et al. 2008. Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. *Proc. Natl. Acad. Sci. USA* 105:7964–69
45. Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, et al. 2005. Identification of novel Argonaute-associated proteins. *Curr. Biol.* 15:2149–55
46. Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, et al. 2005. Inhibition of translational initiation by let-7 microRNA in human cells. *Science* 309:1573–76
47. Pillai RS, Artus CG, Filipowicz W. 2004. Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. *RNA* 10:1518–25
48. Wu L, Fan J, Belasco JG. 2008. Importance of translation and nonnucleolytic Ago proteins for on-target RNA interference. *Curr. Biol.* 18:1327–32
49. Su H, Trombly MI, Chen J, Wang X. 2009. Essential and overlapping functions for mammalian Argonautes in microRNA silencing. *Genes Dev.* 23:304–17
50. Schmitter D, Filkowski J, Sewer A, Pillai RS, Oakeley EJ, et al. 2006. Effects of Dicer and Argonaute down-regulation on mRNA levels in human HEK293 cells. *Nucleic Acids Res.* 34:4801–15
51. O'Carroll D, Mecklenbrauker I, Das PP, Santana A, Koenig U, et al. 2007. A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. *Genes Dev.* 21:1999–2004
52. Iwasaki S, Kawamata T, Tomari Y. 2009. *Drosophila* Argonaute1 and Argonaute2 employ distinct mechanisms for translational repression. *Mol. Cell* 34:58–67
53. Förstemann K, Horwich MD, Wee L, Tomari Y, Zamore PD. 2007. *Drosophila* microRNAs are sorted into functionally distinct Argonaute complexes after production by Dicer-1. *Cell* 130:287–97
54. Tomari Y, Du T, Zamore PD. 2007. Sorting of *Drosophila* small silencing RNAs. *Cell* 130:299–308
55. Parker JS, Parizotto EA, Wang M, Roe SM, Barford D. 2009. Enhancement of the seed-target recognition step in RNA silencing by a PIWI/MID domain protein. *Mol. Cell* 33:204–14
56. Ameres SL, Martinez J, Schroeder R. 2007. Molecular basis for target RNA recognition and cleavage by human RISC. *Cell* 130:101–12

57. Parker JS, Roe SM, Barford D. 2005. Structural insights into mRNA recognition from a PIWI domain-siRNA guide complex. *Nature* 434:663–66
58. Wang Y, Juranek S, Li H, Sheng G, Tuschl T, Patel DJ. 2008. Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. *Nature* 456:921–26
59. Wang Y, Juranek S, Li H, Sheng G, Wardle GS, et al. 2009. Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes. *Nature* 461:754–61
60. Eulalio A, Tritschler F, Izaurralde E. 2009. The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. *RNA* 15:1433–42
61. Zhang L, Ding L, Cheung TH, Dong MQ, Chen J, et al. 2007. Systematic identification of *C. elegans* miRISC proteins, miRNAs, and mRNA targets by their interactions with GW182 proteins AIN-1 and AIN-2. *Mol. Cell* 28:598–613
62. Ding L, Spencer A, Morita K, Han M. 2005. The developmental timing regulator AIN-1 interacts with miRISCs and may target the Argonaute protein ALG-1 to cytoplasmic P bodies in *C. elegans*. *Mol. Cell* 19:437–47
63. Eulalio A, Helms S, Fritzsch C, Fauser M, Izaurralde E. 2009. A C-terminal silencing domain in GW182 is essential for miRNA function. *RNA* 15:1067–77
64. Till S, Lejeune E, Thermann R, Bortfeld M, Hothorn M, et al. 2007. A conserved motif in Argonaute-interacting proteins mediates functional interactions through the Argonaute PIWI domain. *Nat. Struct. Mol. Biol.* 14:897–903
65. Eulalio A, Huntzinger E, Izaurralde E. 2008. GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. *Nat. Struct. Mol. Biol.* 15:346–53
66. Baillat D, Shiekhattar R. 2009. Functional dissection of the human TNRC6 (GW182-related) family of proteins. *Mol. Cell. Biol.* 29:4144–55
67. Zipprich JT, Bhattacharyya S, Mathys H, Filipowicz W. 2009. Importance of the C-terminal domain of the human GW182 protein TNRC6C for translational repression. *RNA* 15:781–93
68. Chekulaeva M, Filipowicz W, Parker R. 2009. Multiple independent domains of dGW182 function in miRNA-mediated repression in *Drosophila*. *RNA* 15:794–803
69. Lazzaretti D, Tournier I, Izaurralde E. 2009. The C-terminal domains of human TNRC6A, TNRC6B, and TNRC6C silence bound transcripts independently of Argonaute proteins. *RNA* 15:1059–66
70. Eulalio A, Tritschler F, Buttner R, Weichenrieder O, Izaurralde E, Truffault V. 2009. The RRM domain in GW182 proteins contributes to miRNA-mediated gene silencing. *Nucleic Acids Res.* 37:2974–83
71. Jin P, Alisch RS, Warren ST. 2004. RNA and microRNAs in fragile X mental retardation. *Nat. Cell Biol.* 6:1048–53
72. Ishizuka A, Siomi MC, Siomi H. 2002. A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev.* 16:2497–508
73. Weinmann L, Hock J, Ivacevic T, Ohrt T, Mutze J, et al. 2009. Importin 8 is a gene silencing factor that targets Argonaute proteins to distinct mRNAs. *Cell* 136:496–507
74. Pestova TV, Hellen CU. 2001. Functions of eukaryotic factors in initiation of translation. *Cold Spring Harb. Symp. Quant. Biol.* 66:389–96
75. Edery I, Humbelin M, Darveau A, Lee KA, Milburn S, et al. 1983. Involvement of eukaryotic initiation factor 4A in the cap recognition process. *J. Biol. Chem.* 258:11398–403
76. Grifo JA, Tahara SM, Morgan MA, Shatkin AJ, Merrick WC. 1983. New initiation factor activity required for globin mRNA translation. *J. Biol. Chem.* 258:5804–10
77. Sonenberg N, Rupprecht KM, Hecht SM, Shatkin AJ. 1979. Eukaryotic mRNA cap binding protein: purification by affinity chromatography on sepharose-coupled m7GDP. *Proc. Natl. Acad. Sci. USA* 76:4345–49
78. Sachs A. 2000. Physical and functional interactions between the mRNA cap structure and the poly(A) tail. In *Translational Control of Gene Expression*, ed. N Sonenberg, JWB Hershey, MB Mathews, pp. 447–66. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
79. Kahvejian A, Svitkin YV, Sukarieh R, M'Boutchou MN, Sonenberg N. 2005. Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes Dev.* 19:104–13

80. Pelletier J, Sonenberg N. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* 334:320–25
81. Jang SK, Krausslich HG, Nicklin MJ, Duke GM, Palmenberg AC, Wimmer E. 1988. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* 62:2636–43
82. Hellen CU, Sarnow P. 2001. Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev.* 15:1593–612
83. Hellen CU, Wimmer E. 1995. Translation of encephalomyocarditis virus RNA by internal ribosomal entry. *Curr. Top. Microbiol. Immunol.* 203:31–63
84. Kolupaeva VG, Pestova TV, Hellen CU, Shatsky IN. 1998. Translation eukaryotic initiation factor 4G recognizes a specific structural element within the internal ribosome entry site of encephalomyocarditis virus RNA. *J. Biol. Chem.* 273:18599–604
85. Pisarev AV, Shirokikh NE, Hellen CU. 2005. Translation initiation by factor-independent binding of eukaryotic ribosomes to internal ribosomal entry sites. *C. R. Biol.* 328:589–605
86. Spahn CM, Jan E, Mulder A, Grassucci RA, Sarnow P, Frank J. 2004. Cryo-EM visualization of a viral internal ribosome entry site bound to human ribosomes: The IRES functions as an RNA-based translation factor. *Cell* 118:465–75
87. Jan E, Sarnow P. 2002. Factorless ribosome assembly on the internal ribosome entry site of cricket paralysis virus. *J. Mol. Biol.* 324:889–902
88. Olsen PH, Ambros V. 1999. The *lin-4* regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev. Biol.* 216:671–80
89. Wightman B, Ha I, Ruvkun G. 1993. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75:855–62
90. Lee RC, Feinbaum RL, Ambros V. 1993. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75:843–54
91. Humphreys DT, Westman BJ, Martin DI, Preiss T. 2005. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc. Natl. Acad. Sci. USA* 102:16961–66
92. Huang J, Liang Z, Yang B, Tian H, Ma J, Zhang H. 2007. Derepression of microRNA-mediated protein translation inhibition by apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G) and its family members. *J. Biol. Chem.* 282:33632–40
93. Ding XC, Grosshans H. 2009. Repression of *C. elegans* microRNA targets at the initiation level of translation requires GW182 proteins. *EMBO J.* 28:213–22
94. Wang B, Love TM, Call ME, Doench JG, Novina CD. 2006. Recapitulation of short RNA-directed translational gene silencing in vitro. *Mol. Cell* 22:553–60
95. Wakiyama M, Takimoto K, Ohara O, Yokoyama S. 2007. Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. *Genes Dev.* 21:1857–62
96. Thermann R, Hentze MW. 2007. *Drosophila* miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature* 447:875–78
97. Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, et al. 2007. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. *Science* 317:1764–67
98. Zdanowicz A, Thermann R, Kowalska J, Jemielity J, Duncan K, et al. 2009. *Drosophila* miR2 primarily targets the m^7GpppN cap structure for translational repression. *Mol. Cell* 35:881–88
99. Wang B, Yanez A, Novina CD. 2008. MicroRNA-repressed mRNAs contain 40S but not 60S components. *Proc. Natl. Acad. Sci. USA* 105:5343–48
100. Munroe D, Jacobson A. 1990. mRNA poly(A) tail, a 3′ enhancer of translational initiation. *Mol. Cell. Biol.* 10:3441–55
101. Soto Rifo R, Ricci EP, Décimo D, Moncorgé O, Ohlmann T. 2007. Back to basics: the untreated rabbit reticulocyte lysate as a competitive system to recapitulate cap/poly(A) synergy and the selective advantage of IRES-driven translation. *Nucleic Acids Res.* 35:e121
102. Kiriakidou M, Tan GS, Lamprinaki S, De Planell-Saguer M, Nelson PT, Mourelatos Z. 2007. An mRNA m7G cap binding-like motif within human Ago2 represses translation. *Cell* 129:1141–51

103. Kinch LN, Grishin NV. 2009. The human Ago2 MC region does not contain an eIF4E-like mRNA cap binding motif. *Biol. Direct* 4:2
104. Kawamura Y, Saito K, Kin T, Ono Y, Asai K, et al. 2008. *Drosophila* endogenous small RNAs bind to Argonaute 2 in somatic cells. *Nature* 453:793–97
105. Seitz H, Ghildiyal M, Zamore PD. 2008. Argonaute loading improves the 5′ precision of both microRNAs and their miRNA strands in flies. *Curr. Biol.* 18:147–51
106. Fabian MR, Mathonnet G, Sundermeier T, Mathys H, Zipprich JT, et al. 2009. Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deadenylation. *Mol. Cell* 35:868–80
107. Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, et al. 2007. MicroRNA silencing through RISC recruitment of eIF6. *Nature* 447:823–28
108. Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N, et al. 2003. Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. *Nature* 426:579–84
109. Gandin V, Miluzio A, Barbieri AM, Beugnet A, Kiyokawa H, et al. 2008. Eukaryotic initiation factor 6 is rate-limiting in translation, growth and transformation. *Nature* 455:684–88
110. Ding XC, Slack FJ, Grosshans H. 2008. The let-7 microRNA interfaces extensively with the translation machinery to regulate cell differentiation. *Cell Cycle* 7:3083–90
111. Tarun SZ Jr, Wells SE, Deardorff JA, Sachs AB. 1997. Translation initiation factor eIF4G mediates in vitro poly(A) tail-dependent translation. *Proc. Natl. Acad. Sci. USA* 94:9046–51
112. Beilharz TH, Humphreys DT, Clancy JL, Thermann R, Martin DI, et al. 2009. MicroRNA-mediated messenger RNA deadenylation contributes to translational repression in mammalian cells. *PLoS One* 4:e6783
113. Wu L, Fan J, Belasco JG. 2006. MicroRNAs direct rapid deadenylation of mRNA. *Proc. Natl. Acad. Sci. USA* 103:4034–39
114. Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M, Izaurralde E. 2009. Deadenylation is a widespread effect of miRNA regulation. *RNA* 15:21–32
115. Nottrott S, Simard MJ, Richter JD. 2006. Human let-7a miRNA blocks protein production on actively translating polyribosomes. *Nat. Struct. Mol. Biol.* 13:1108–14
116. Maroney PA, Yu Y, Fisher J, Nilsen TW. 2006. Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nat. Struct. Mol. Biol.* 13:1102–7
117. Petersen CP, Bordeleau ME, Pelletier J, Sharp PA. 2006. Short RNAs repress translation after initiation in mammalian cells. *Mol. Cell* 21:533–42
118. Seggerson K, Tang L, Moss EG. 2002. Two genetic circuits repress the *Caenorhabditis elegans* heterochronic gene *lin-28* after translation initiation. *Dev. Biol.* 243:215–25
119. Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, et al. 2004. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc. Natl. Acad. Sci. USA* 101:360–65
120. Nelson PT, Hatzigeorgiou AG, Mourelatos Z. 2004. miRNP:mRNA association in polyribosomes in a human neuronal cell line. *RNA* 10:387–94
121. Lytle JR, Yario TA, Steitz JA. 2007. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. *Proc. Natl. Acad. Sci. USA* 104:9667–72
122. Akimitsu N, Tanaka J, Pelletier J. 2007. Translation of nonSTOP mRNA is repressed post-initiation in mammalian cells. *EMBO J.* 26:2327–38
123. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. 2008. Widespread changes in protein synthesis induced by microRNAs. *Nature* 455:58–63
124. Nolde MJ, Saka N, Reinert KL, Slack FJ. 2007. The *Caenorhabditis elegans* pumilio homolog, *puf-9*, is required for the 3′UTR-mediated repression of the *let-7* microRNA target gene, *hbl-1*. *Dev. Biol.* 305:551–63
125. Galgano A, Forrer M, Jaskiewicz L, Kanitz A, Zavolan M, Gerber AP. 2008. Comparative analysis of mRNA targets for human PUF-family proteins suggests extensive interaction with the miRNA regulatory system. *PLoS One* 3:e3164
126. Hinman MN, Lou H. 2008. Diverse molecular functions of Hu proteins. *Cell Mol. Life Sci.* 65:3168–81
127. Kim HH, Kuwano Y, Srikantan S, Lee EK, Martindale JL, Gorospe M. 2009. HuR recruits let-7/RISC to repress c-Myc expression. *Genes Dev.* 23:1743–48

128. Weidinger G, Stebler J, Slanchev K, Dumstrei K, Wise C, et al. 2003. dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. *Curr. Biol.* 13:1429–34
129. Youngren KK, Coveney D, Peng X, Bhattacharya C, Schmidt LS, et al. 2005. The *Ter* mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature* 435:360–64
130. Mishima Y, Giraldez AJ, Takeda Y, Fujiwara T, Sakamoto H, et al. 2006. Differential regulation of germline mRNAs in soma and germ cells by zebrafish miR-430. *Curr. Biol.* 16:2135–42
131. Vasudevan S, Steitz JA. 2007. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* 128:1105–18
132. Lee YS, Nakahara K, Pham JW, Kim K, He Z, et al. 2004. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 117:69–81
133. Hornstein E, Tang H, Meyuhas O. 2001. Mitogenic and nutritional signals are transduced into translational efficiency of TOP mRNAs. *Cold Spring Harb. Symp. Quant. Biol.* 66:477–84
134. Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, et al. 2005. Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* 120:623–34
135. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. 2005. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* 309:1577–81
136. Jopling CL, Schutz S, Sarnow P. 2008. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 4:77–85
137. Meyer S, Temme C, Wahle E. 2004. Messenger RNA turnover in eukaryotes: pathways and enzymes. *Crit. Rev. Biochem. Mol. Biol.* 39:197–216
138. Yamashita A, Chang TC, Yamashita Y, Zhu W, Zhong Z, et al. 2005. Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. *Nat. Struct. Mol. Biol.* 12:1054–63
139. Coller J, Parker R. 2004. Eukaryotic mRNA decapping. *Annu. Rev. Biochem.* 73:861–90
140. Grosset C, Chen CY, Xu N, Sonenberg N, Jacquemin-Sablon H, Shyu AB. 2000. A mechanism for translationally coupled mRNA turnover: interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. *Cell* 103:29–40
141. Barreau C, Paillard L, Osborne HB. 2005. AU-rich elements and associated factors: Are there unifying principles? *Nucleic Acids Res.* 33:7138–50
142. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, et al. 2005. Regulation by *let-7* and *lin-4* miRNAs results in target mRNA degradation. *Cell* 122:553–63
143. Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, et al. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 312:75–79
144. Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, et al. 2005. The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science* 310:1817–21
145. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, et al. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433:769–73
146. Wu L, Belasco JG. 2005. Micro-RNA regulation of the mammalian *lin-28* gene during neuronal differentiation of embryonal carcinoma cells. *Mol. Cell. Biol.* 25:9198–208
147. Hock J, Weinmann L, Ender C, Rudel S, Kremmer E, et al. 2007. Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. *EMBO Rep.* 8:1052–60
148. Takimoto K, Wakiyama M, Yokoyama S. 2009. Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression. *RNA* 15:1078–89
149. Lian SL, Li S, Abadal GX, Pauley BA, Fritzler MJ, Chan EK. 2009. The C-terminal half of human Ago2 binds to multiple GW-rich regions of GW182 and requires GW182 to mediate silencing. *RNA* 15:804–13
150. El-Shami M, Pontier D, Lahmy S, Braun L, Picart C, et al. 2007. Reiterated WG/GW motifs form functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. *Genes Dev.* 21:2539–44
151. Li S, Lian SL, Moser JJ, Fritzler ML, Fritzler MJ, et al. 2008. Identification of GW182 and its novel isoform TNGW1 as translational repressors in Ago2-mediated silencing. *J. Cell Sci.* 121:4134–44

152. Roy G, De Crescenzo G, Khaleghpour K, Kahvejian A, O'Connor-McCourt M, Sonenberg N. 2002. Paip1 interacts with poly(A) binding protein through two independent binding motifs. *Mol. Cell. Biol.* 22:3769–82
153. Kozlov G, De Crescenzo G, Lim NS, Siddiqui N, Fantus D, et al. 2004. Structural basis of ligand recognition by PABC, a highly specific peptide-binding domain found in poly(A)-binding protein and a HECT ubiquitin ligase. *EMBO J.* 23:272–81
154. Khaleghpour K, Svitkin YV, Craig AW, DeMaria CT, Deo RC, et al. 2001. Translational repression by a novel partner of human poly(A) binding protein, Paip2. *Mol. Cell* 7:205–16
155. Jinek M, Fabian MR, Coyle S, Sonenberg N, Doudna JA. 2010. Structural insights into the human GW182-PABC interaction in microRNA-mediated deadenylation. *Nat. Struct. Mol. Biol.* 17:238–40
156. Zekri L, Huntzinger E, Heimstädt S, Izaurralde E. 2009. The silencing domain of GW182 interacts with PABPC1 to promote translational repression and degradation of microRNA targets and is required for target release. *Mol. Cell. Biol.* 29:6220–31
157. Tarun SZ Jr, Sachs AB. 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *EMBO J.* 15:7168–77
158. Martineau Y, Derry MC, Wang X, Yanagiya A, Berlanga JJ, et al. 2008. Poly(A)-binding protein-interacting protein 1 binds to eukaryotic translation initiation factor 3 to stimulate translation. *Mol. Cell. Biol.* 28:6658–67
159. Craig AW, Haghighat A, Yu AT, Sonenberg N. 1998. Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. *Nature* 392:520–23
160. Uchida N, Hoshino S, Imataka H, Sonenberg N, Katada T. 2002. A novel role of the mammalian GSPT/eRF3 associating with poly(A)-binding protein in cap/poly(A)-dependent translation. *J. Biol. Chem.* 277:50286–92
161. Hu W, Sweet TJ, Chamnongpol S, Baker KE, Coller J. 2009. Co-translational mRNA decay in *Saccharomyces cerevisiae*. *Nature* 461:225–29
162. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. 2008. The impact of microRNAs on protein output. *Nature* 455:64–71
163. Kong YW, Cannell IG, de Moor CH, Hill K, Garside PG, et al. 2008. The mechanism of micro-RNA-mediated translation repression is determined by the promoter of the target gene. *Proc. Natl. Acad. Sci. USA* 105:8866–71
164. Nissan T, Parker R. 2008. Computational analysis of miRNA-mediated repression of translation: implications for models of translation initiation inhibition. *RNA* 14:1480–91
165. Kedersha N, Anderson P. 2007. Mammalian stress granules and processing bodies. *Methods Enzymol.* 431:61–81
166. Eystathioy T, Chan EK, Tenenbaum SA, Keene JD, Griffith K, Fritzler MJ. 2002. A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. *Mol. Biol. Cell* 13:1338–51
167. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. 2005. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* 7:719–23
168. Nathans R, Chu CY, Serquina AK, Lu CC, Cao H, Rana TM. 2009. Cellular microRNA and P bodies modulate host-HIV-1 interactions. *Mol. Cell* 34:696–709
169. Pauley KM, Eystathioy T, Jakymiw A, Hamel JC, Fritzler MJ, Chan EK. 2006. Formation of GW bodies is a consequence of microRNA genesis. *EMBO Rep.* 7:904–10
170. Eulalio A, Behm-Ansmant I, Schweizer D, Izaurralde E. 2007. P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Mol. Cell. Biol.* 27:3970–81
171. Lian S, Jakymiw A, Eystathioy T, Hamel JC, Fritzler MJ, Chan EK. 2006. GW bodies, microRNAs and the cell cycle. *Cell Cycle* 5:242–45
172. Decker CJ, Teixeira D, Parker R. 2007. Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in *Saccharomyces cerevisiae*. *J. Cell Biol.* 179:437–49
173. Leung AK, Calabrese JM, Sharp PA. 2006. Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. *Proc. Natl. Acad. Sci. USA* 103:18125–30
174. Andrei MA, Ingelfinger D, Heintzmann R, Achsel T, Rivera-Pomar R, Luhrmann R. 2005. A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA* 11:717–27

175. Kedersha N, Stoecklin G, Ayodele M, Yacono P, Lykke-Andersen J, et al. 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J. Cell Biol.* 169:871–84
176. Wilczynska A, Aigueperse C, Kress M, Dautry F, Weil D. 2005. The translational regulator CPEB1 provides a link between dcp1 bodies and stress granules. *J. Cell Sci.* 118:981–92
177. Mazroui R, Sukarieh R, Bordeleau ME, Kaufman RJ, Northcote P, et al. 2006. Inhibition of ribosome recruitment induces stress granule formation independently of eukaryotic initiation factor 2alpha phosphorylation. *Mol. Biol. Cell* 17:4212–19
178. Leung AK, Sharp PA. 2006. Function and localization of microRNAs in mammalian cells. *Cold Spring Harb. Symp. Quant. Biol.* 71:29–38
179. Cikaluk DE, Tahbaz N, Hendricks LC, DiMattia GE, Hansen D, et al. 1999. GERp95, a membrane-associated protein that belongs to a family of proteins involved in stem cell differentiation. *Mol. Biol. Cell* 10:3357–72
180. Tahbaz N, Carmichael JB, Hobman TC. 2001. GERp95 belongs to a family of signal-transducing proteins and requires Hsp90 activity for stability and Golgi localization. *J. Biol. Chem.* 276:43294–99
181. Tahbaz N, Kolb FA, Zhang H, Jaronczyk K, Filipowicz W, Hobman TC. 2004. Characterization of the interactions between mammalian PAZ PIWI domain proteins and Dicer. *EMBO Rep.* 5:189–94
182. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 9:654–59
183. Kosik KS. 2009. MicroRNAs tell an evo-devo story. *Nat. Rev. Neurosci.* 10:754–59
184. Fiore R, Siegel G, Schratt G. 2008. MicroRNA function in neuronal development, plasticity and disease. *Biochim. Biophys. Acta* 1779:471–78
185. Rudel S, Meister G. 2008. Phosphorylation of Argonaute proteins: regulating gene regulators. *Biochem. J.* 413:e7–9
186. Heo I, Kim VN. 2009. Regulating the regulators: posttranslational modifications of RNA silencing factors. *Cell* 139:28–31
187. Paroo Z, Ye X, Chen S, Liu Q. 2009. Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling. *Cell* 139:112–22
188. Qi HH, Ongusaha PP, Myllyharju J, Cheng D, Pakkanen O, et al. 2008. Prolyl 4-hydroxylation regulates Argonaute 2 stability. *Nature* 455:421–24
189. Zeng Y, Sankala H, Zhang X, Graves PR. 2008. Phosphorylation of Argonaute 2 at serine-387 facilitates its localization to processing bodies. *Biochem. J.* 413:429–36

Annual Review of Biochemistry

Volume 79, 2010

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Biochemistry* articles may be found at http://biochem.annualreviews.org