Chapter 1
Understanding How miRNAs Post-Transcriptionally Regulate Gene Expression

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Contents

Abstract The discovery of microRNA (miRNA)-mediated gene silencing has added a new level of complexity to our understanding of post-transcriptional control of gene expression. Considering the ubiquity of miRNA-mediated repression throughout basic cellular processes, understanding its mechanism of action is paramount to obtain a clear picture of the regulation of gene expression in biological systems. Although many miRNAs and their targets have been identified, a detailed understanding of miRNA action remains elusive. miRNAs regulate gene expression at the post-transcriptional level, through both translational inhibition and mRNA destabilization. Recent reports suggest that many miRNA effects are mediated through proteins of the GW182 family. This chapter focuses on the multiple and potentially overlapping mechanisms that miRNAs utilize to regulate gene expression in eukaryotes.

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1.1 Introduction

MicroRNAs (miRNAs) are small RNA molecules, approximately 22 nucleotides in length, encoded within the genomes of more eukaryotes. miRNAs direct an intricate mechanism that regulates eukaryotic gene expression at the post-transcriptional level. miRNA functions in mammals include modulating hematopoietic lineage differentiation, insulin secretion, apoptosis, heart muscle development, neuron development, and many other processes (Chen and Lodish 2005; Poy et al. 2004; Welch et al. 2007). Furthermore, control of gene expression through miRNA activity has been shown to play a significant role in numerous human pathologies, including cancer (Calin and Croce 2006a, 2006b; Chang and Mendell 2007; Croce and Calin 2005; Cummins and Velculescu 2006; Dalmay and Edwards 2006; Garzon et al. 2006; Giannakakis et al. 2007; Hammond 2006; He et al. 2007; Mattes et al. 2008; Stefani 2007). Recent research has shed some light on the mechanisms by which miRNAs regulate gene expression; however, many studies have yielded contradictory conclusions. Overall, miRNAs regulate gene expression by inhibiting mRNA translation and/or facilitating mRNA degradation.

1.1.1 Eukaryotic Translation

Translation may be divided into three steps: initiation, elongation, and termination. Initiation involves the assembly of an 80S ribosome complex positioned at the appropriate start site on the mRNA to be translated. Elongation is the polypeptide synthesis step, where the nucleotide sequence carried on the mRNA molecule is translated into the amino acid sequence of the growing peptide chain. Termination involves the release of the newly synthesized protein. 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 the structure m^7GpppN (where N is any nucleotide) termed the "cap," which facilitates ribosome recruitment to the mRNA. This canonical mechanism of translation initiation is termed as cap-dependent translation initiation. In contrast, many eukaryotic and viral mRNAs are translated via alternative, cap-independent, mechanisms.

1.1.1.1 Cap-Dependent Translation Initiation

Cap-dependent translation depends on the activities of a variety of eukaryotic initiation factors (eIFs). It is accomplished through mRNA scanning mechanism, whereby the small (40S) ribosomal subunit, in complex with a number of eIFs, binds the mRNA near the 5′ cap structure and scans the mRNA in a 5′–3′ direction until it encounters an AUG start codon in an optimal context (Kozak 1978; Kozak and Shatkin 1979) (Fig. 1.1a). Recruitment of ribosomes to a given mRNA is

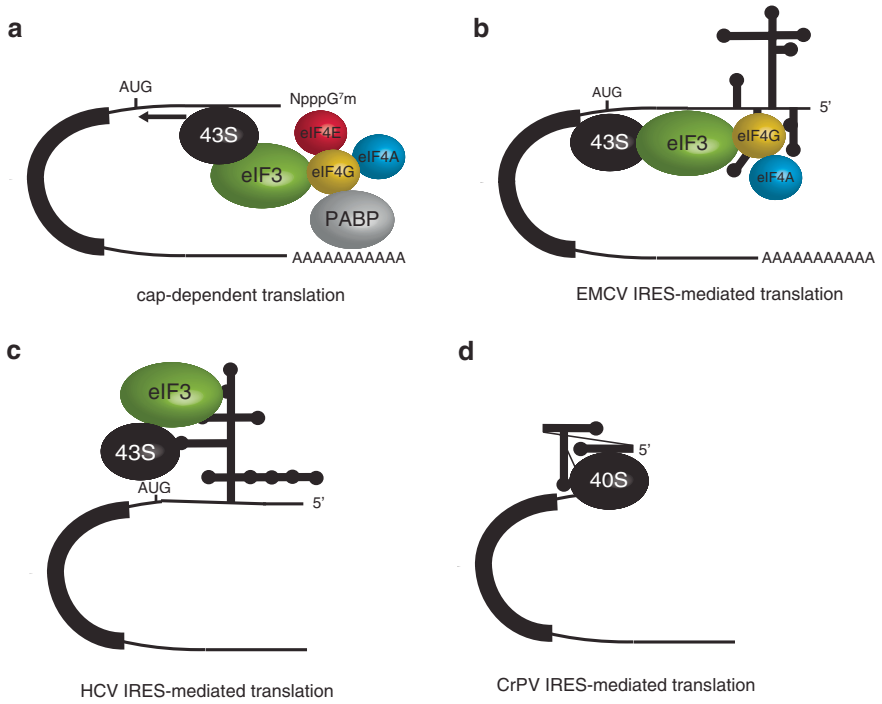

Fig. 1.1 Mechanisms of translation initiation. (**a**) cap-dependent translation. (**b**) EMCV IRES-mediated cap-independent translation. (**c**) HCV IRES-mediated cap-independent translation. (**d**) CrPV intergenic IRES-mediated cap-independent translation. Open reading frames are denoted as *thick curved black lines*. IRES secondary structures are presented as *thick black lines* bound by translation factors and/or ribosomal subunits

facilitated by the 5′ cap, the 3′ poly(A) tail, the poly(A) binding protein (PABP), and the eIF4F complex. eIF4F is a three subunit complex (Edery et al. 1983; Grifo et al. 1983) composed of (1) eIF4A, an ATP-dependent RNA helicase that unwinds secondary structures, (2) eIF4E, a 24 kDa polypeptide that specifically interacts with the cap structure (Sonenberg et al. 1979), and (3) eIF4G, a large scaffolding protein that binds to both eIF4E and eIF4A. The poly(A) tail functions as a translational enhancer (Sachs 2000), as the 3′ poly(A) and 5′ cap structure act synergistically to enhance translation initiation (Gallie 1991; Sachs and Varani 2000). This synergy can be explained by the physical interaction between PABP and eIF4G that brings about the circularization of the mRNA. mRNA circularization is thought to increase the affinity of eIF4E for the cap, thus enhancing the rate of translation initiation. A given mRNA is activated when the eIF4F complex binds to the 5′ cap (through eIF4E) and interacts with the 3′ poly(A) tail (through eIF4G–PABP–poly(A) interaction). The activated mRNA is then bound by the 43S preinitiation complex (PIC), which contains the 40S ribosomal subunit, an initiator tRNA (Met-tRNAi),

as well as eIFs 1, 1A, 2, 3, and 5. eIF3 is a large multisubunit scaffolding protein that bridges the mRNA complex to the ribosome through interaction with eIF4G (Imataka et al. 1997).

Once the PIC associates with the mRNA, it proceeds to scan the 5′ untranslated region (UTR) until an appropriate initiation codon is encountered. The RNA helicase activity of eIF4A is thought to promote scanning. eIF4B functions, at least in part, to enhance the RNA helicase activity of eIF4A, likely by increasing the affinity of eIF4A for ATP (Bi et al. 2000; Rogers et al. 1999). eIF1 and eIF1A are thought to promote scanning and enhance the fidelity of start codon selection (Pestova and Kolupaeva 2002), while eIF5 is the GTPase-activating protein for eIF2 (Mitchell and Lorsch 2008). Once the start codon is recognized, the partially hydrolyzed phosphate from the eIF2-bound GTP is released; this is then followed by release of eIF1 (Algire et al. 2005; Maag et al. 2005). The 60S ribosomal subunit then joins the 43S initiation complex, with the assistance of eIF5B, and translation elongation commences (Pestova et al. 2000). eIF6 is the only initiation factor currently known to regulate the availability of the 60S subunit (Ceci et al. 2003). Free 60S ribosomal subunits bound by eIF6 are unable to bind to the 40S subunit to form 80S ribosome complexes. Only when an eIF6-bound 60S is phosphorylated by RACK1/PKC, it can dissociate from the 60S and allow it to join the 40S subunit upon start codon recognition.

1.1.1.2 Poly(A) Tail-Independent Translation Initiation

The poly(A) tail plays a critical role in the control of translation initiation under many physiological conditions (Wickens et al. 2000). Histone mRNAs are the only mammalian mRNAs that lack poly(A) tails; nevertheless, they are efficiently translated. A terminal stem-loop on histone mRNAs binds the histone stem-loop binding protein, which functionally substitutes for PABP by interacting with eIF4G (Ling et al. 2002).

1.1.1.3 Cap-Independent Translation Initiation

The discovery, in picornaviruses two decades ago (Jang et al. 1988; Pelletier and Sonenberg 1988), of internal ribosome entry sites (IRESes) has added a new degree of complexity to our understanding of translation initiation. IRESes are generally (but not always (Gilbert et al. 2007)) highly structured *cis*-acting RNA elements that function to enhance translation initiation in a cap-independent manner. Although originally discovered in viral genomes, IRESes have since been found in several mRNAs (i.e., myc, XIAP, and DAP5 (Henis-Korenblit et al. 2000; Holcik et al. 1999; Stoneley et al. 1998)). Often, but not always (as in the case of the Cricket paralysis virus (CrPV) intergenic IRES (Wilson et al. 2000)) located in the 5′UTR, IRESes enhance translation in the absence of eIF4E by recruiting the 40S subunit to the mRNA through unconventional means. Certain IRESes (such as

those of poliovirus and encephalomyocarditis virus (EMCV)) (Fig. 1.1b) can directly bind the eIF4G subunit of the eIF4F complex, thus bypassing the requirement for eIF4E and the 5′ cap (Hellen and Wimmer 1995; Kolupaeva et al. 1998). The Hepatitis C virus IRES bypasses the need for the entire eIF4F complex and binds directly to eIF3 and the 40S ribosomal subunit (Pisarev et al. 2005) (Fig. 1.1c). The CrPV intergenic IRES enhances translation via a factorless mechanism, whereby the IRES mimics an aminioacylated tRNA and positions itself within the P-site of the ribosome (Jan and Sarnow 2002; Spahn et al. 2004). This allows the CrPV intergenic IRES to initiate translation from a non-AUG codon (Fig. 1.1d).

1.2 miRNA Biogenesis

miRNAs are small (~22 nucleotides) noncoding RNAs that were first discovered in *C. elegans* but have since been found to exist in almost all eukaryotes ranging from plants to insects to mammals (Lee and Ambros 2001) (*S. cerevisiae* is an exception). More recently, miRNAs have also been found within the genomes of several viruses including Epstein–Barr virus and several herpesviruses (Cullen 2009). miRNAs are processed from primary transcripts via a two-step mechanism involving two RNase III-type enzymes known as Drosha and Dicer (Fig. 1.2). miRNAs are transcribed, either from discreet miRNA genes or as parts of introns of protein coding genes. These initial miRNA precursors, known as pri-miRNAs, are processed into ~70 nt hairpin structures known as pre-miRNAs by a nuclear enzyme complex known as the microprocessor. The microprocessor contains an endoribonuclease known as Drosha as well as a double-stranded RNA binding protein known as DiGeorge syndrome critical region 8 (DGCR8) in mammals and Partner of Drosha (Pasha) in *D. melanogaster* and *C. elegans*. DGCR8/Pasha is required for proper pri-miRNA processing (Han et al. 2006; Zeng and Cullen 2003, 2005). Drosha contains two RNase domains that cleave the 5′ and 3′ ends, releasing the pre-miRNA (Han et al. 2004). By binding specifically to the pri-miRNA dsRNA hairpin, DGCR8/Pasha determines the cleavage sites on the pri-miRNA, and hence the length of the pre-miRNA (Han et al. 2006). In some instances, the sequence of the mature pre-miRNA corresponds precisely to the sequence of a spliced intron. These spliced-out pre-miRNAs, known as mirtrons, no longer require microprocessor activity in order to generate mature miRNAs (Berezikov et al. 2007; Okamura et al. 2007; Ruby et al. 2007). In the case that pre-miRNAs are present in introns, recent results question the temporal order of pre-mRNA splicing and miRNA processing. Drosha-dependent pre-miRNA processing can still occur on pre-mRNAs that are splicing-deficient, suggesting that Drosha can process intronic pre-miRNAs directly from pre-mRNAs (Kim and Kim 2007). A more recent study, using an in vitro system displaying both splicing and pre-miRNA processing, demonstrated that microprocessor-dependent pre-miRNA cropping can occur kinetically faster than splicing (Kataoka et al. 2009). This study concluded that the microprocessor and spliceosome may be functionally linked such that Drosha-mediated miRNA processing and pre-mRNA

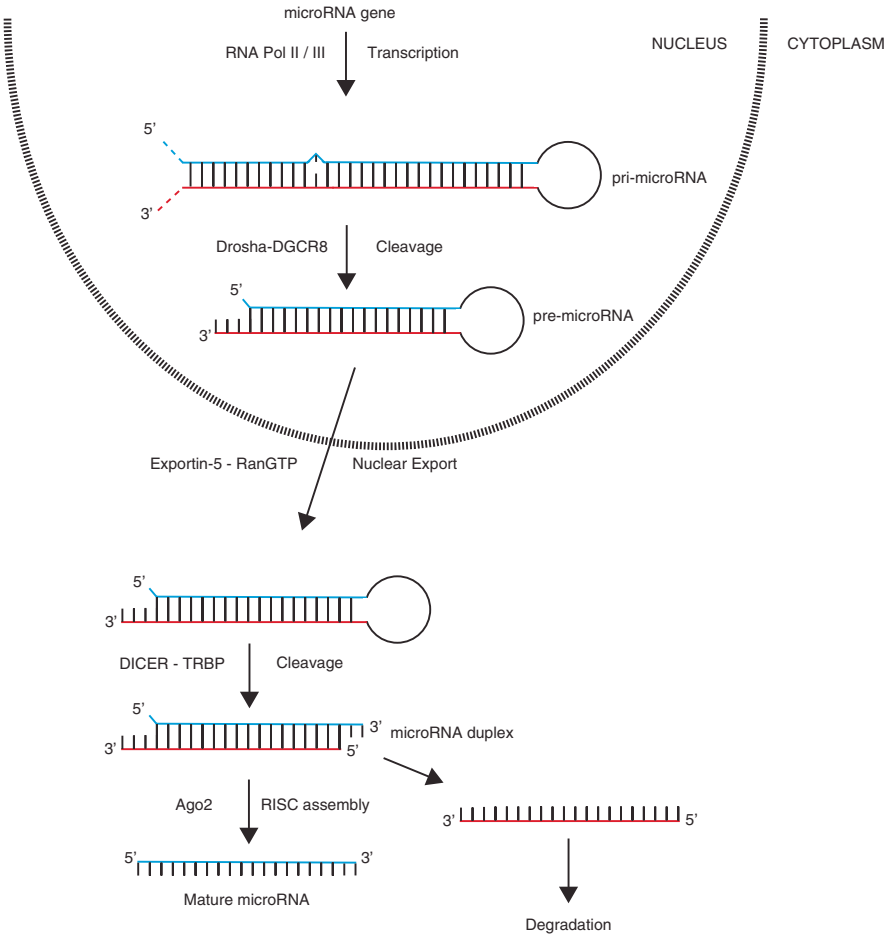

Fig. 1.2 Mechanism of microRNA biogenesis. The nuclear membrane is presented as a *broken curved black line*

splicing may occur simultaneously. Pre-miRNAs are transported from the nucleus to the cytoplasm (by a complex of Exportin5 and Ran-GTP (Yi et al. 2003)), where they are processed into ~22 bp double stranded RNAs by the RISC loading complex. The RISC loading complex consists of the RNase Dicer, the dsRNA binding protein TRBP (product of the *loquacious* gene in flies), PACT (protein activator of PKR), and Argonaute proteins. While the number of Dicer paralogues varies evolutionarily, vertebrates have only one gene coding for a Dicer-like protein. Once processed, one of two strands of the miRNA is loaded into a ribonucleoprotein complex, referred to as a miRNA-induced silencing complex (miRISC). The most widely studied protein components of miRISCs are proteins of the Argonaute family.

Pre-miRNA processing by Dicer and miRISC complex assembly (loading of the mature miRNA onto Argonaute proteins) are thought to occur simultaneously at the RISC loading complex.

1.3 miRNA-Mediated Regulation of Eukaryotic Gene Expression

In most cases, miRNA-targeted sites are located in mRNA 3′UTRs. miRNAs can also regulate gene expression of mRNAs that contain miRNA target sites in their 5′UTR (Lytle et al. 2007); however, there is currently only one known example of a miRNA targeting the 5′UTR of naturally occurring mRNA (Orom et al. 2008). A recent report has shed some light on the nature of the evolutionary preference for miRNA target sites to reside in the mRNA 3′UTR. Gu et al. reported that, mutating the stop codon of reporter mRNAs such that the coding sequence extends past miRNA target sites, thus positioning the target sites within the mRNA coding sequence, significantly impairs miRNA-dependent repression of reporter mRNA translation (Gu et al. 2009). However, placing rare codons upstream of target sites within the coding sequence partially restored miRNA-mediated repression. These results suggest that actively translating ribosomes may displace the miRISC complex from target sites positioned within the coding sequence. Nevertheless, experimentally validated miRNA target sites have been reported in the coding sequences of several genes (Forman et al. 2008; Rigoutsos 2009). One interesting example is the presence of three let-7 target sites within the coding sequence of *dicer*, which could represent a negative feedback loop for production of the mature form of this miRNA.

Specificity of miRNA function is controlled through the direct base pairing of a miRNA-loaded RISC to miRNA-complementary target sites on targeted mRNAs (Doench and Sharp 2004). miRNA-regulated mRNAs often harbor multiple miRNA target sites within their 3′UTRs, sites that in many cases are phylogenetically conserved between species (Stark et al. 2005). miRNAs are roughly the same size as small-interfering RNAs (siRNAs) but are not generated and do not act for the most part in the same manner. Although both miRNAs and siRNAs interact with Argonaute (Ago) proteins, miRNAs are distinct from siRNAs in that, unlike siRNAs, miRNAs imperfectly base pair to target sites and do not lead to endonucleolytic cleavage of targeted mRNAs, but rather regulate their expression by other means (Bartel 2004). Interestingly, siRNAs can act as miRNAs if made to base pair imperfectly to target sites (Zeng et al. 2003), and miRNAs can act as siRNAs if made to base pair perfectly (Doench et al. 2003).

Considering the short length of time that has past since their discovery, a wealth of effort and resources have been expended in an attempt to elucidate exactly how miRNAs mediate their effects. However, the mechanism by which miRNAs exert post-transcriptional control of gene expression remains highly controversial. Early reports generally suggested that miRNAs inhibit gene expression at the

post-transcriptional level, at some stage post-translation initiation. These reports also suggested that miRNA action had little or no effect on the abundance or stability of target mRNAs. More recent results challenge these data as results from both in vitro and in vivo studies have shown that miRNAs can inhibit translation initiation as well as promote decay of target mRNAs. As such, the literature now contains reports favoring three different potential modes of miRNA-mediated repression: miRNAs may (1) destabilize target mRNAs, (2) inhibit translation initiation, or (3) block translation at some stage after initiation (Fig. 1.3). These three possible inhibitory mechanisms are by no means mutually exclusive. It is possible that the primary mode of miRNA mediated gene regulation may vary by cell type or developmental stage, possibly controlled by miRNA levels or miRISC complex components. Perhaps the most compelling evidence for cellular regulation of the nature of the miRNA response comes from recent reports suggesting that serum starvation can switch the miRNA response from inhibition of target gene expression to enhancement (Vasudevan et al. 2007, 2008). Indeed, the nature of miRNA control of gene expression is much more complex than initially thought.

1.3.1 miRNA-Mediated Translational Control

miRNAs are studied in a variety of in vivo and in vitro systems derived from mammals, flies, and worms. miRNAs first made their grand entrance in the study of developmental timing in *C. elegans*. Genetic analyses carried out by Victor Ambros'

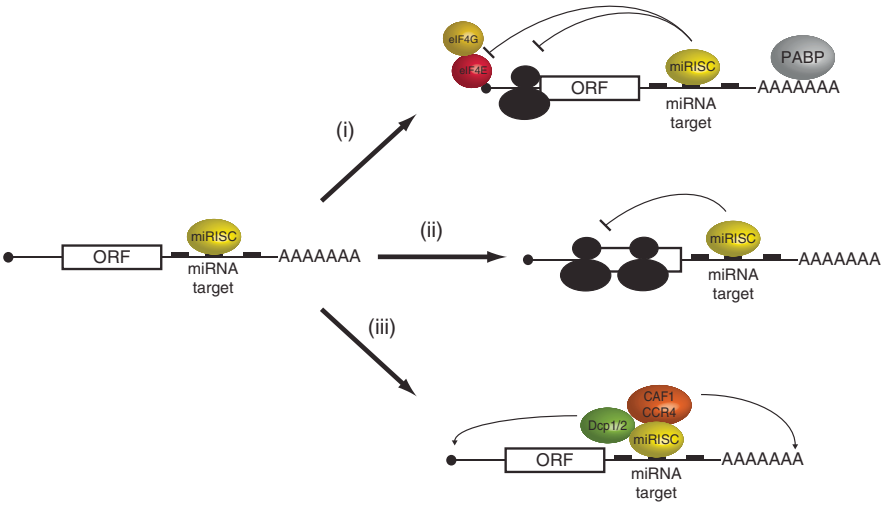

Fig. 1.3 Mechanisms of miRNA-mediated repression. (1) inhibition of translation initiation. (2) inhibition of polysome elongation. (3) miRNA-mediated deadenylation and decapping via the CAF1/CCR4 deadenylase complex and Dcp1/2 decapping complex

and Gary Ruvkun's laboratories determined that the *lin-4* gene functioned to repress the production of *lin-14* protein. The key discoveries were that the *lin-4* gene did not encode a protein but rather a short noncoding RNA (what is now called a miRNA), and that the *lin-4* miRNA exhibited partial complementarity to conserved sequences within the 3′UTR of *lin-14* mRNA, sites that were important for developmental regulation (Lee et al. 1993; Wightman et al. 1991, 1993). It was later demonstrated that lin-4 repressed lin-14 protein production at the translational level with no observed effect on lin-14 mRNA transcription or stability (Olsen and Ambros 1999). The possibility that miRNAs represented a general phenomenon, rather than a species-specific one, came with the discovery of a second miRNA in *C. elegans*, let-7 (Pasquinelli et al. 2000; Reinhart et al. 2000). Just like the lin-14 miRNA, the let-7 miRNA also regulated the expression of a target mRNA (*lin-41*); however, unlike the lin-14 miRNA, the let-7 miRNA was phylogenetically conserved in both flies and animals. Data for both the lin-4 and let-7 miRNAs suggested that they did not influence mRNA biogenesis or stability (although mRNA degradation of let-7 and lin-4 targeted mRNAs has since been reported in *C. elegans* (Bagga et al. 2005; Ding and Grosshans 2009)), but rather inhibited translation. Polyribosome sedimentation experiments conducted by the Ambros' laboratory determined that both lin-4 miRNA and its targeted *lin-14* mRNA were still associated with polyribosomes, suggesting that the lin-4 miRNA inhibits translation at a post-initiation step (Olsen and Ambros 1999). An identical distribution of polyribosomes was described for the lin-4 miRNA-repressed *lin-28* mRNA as well (Seggerson et al. 2002). Subsequently, several other miRNAs (let-7b, and miR-128, -129-2, 326 and -344) were also found associated with polyribosomal fractions in mammalian neurons (Kim et al. 2004; Nelson et al. 2004). However, a recent study concluded that both the lin-4 and let-7 miRNAs in *C. elegans* facilitate inhibition of translation initiation rather than at post-initiation step (Ding and Grosshans 2009). In addition, this report demonstrated that miRNA-mediated mRNA decay often occurs alongside translational inhibition; however, the level of decay varies between miRNA-targeted transcripts (Ding and Grosshans 2009). It is possible that some of the inconsistencies in observations for miRNA-mediated effects could be partially due to the larval stage at which miRNA effects in *C. elegans* were studied, or differences in assays used to measure mRNA decay (i.e., oligo d(T) versus gene-specific oligonucleotides used for qPCR assays) (Ding and Grosshans 2009).

Although several other groups also arrived at the same conclusion as the Ambros lab (i.e., that miRNAs inhibit translation post-initiation (Maroney et al. 2006; Petersen et al. 2006) and do not lead to mRNA degradation (Brennecke et al. 2003; Doench et al. 2003; Zeng et al. 2003)), miRNAs have also demonstrated that they can inhibit translation initiation. Using both the tethering approach and reporters targeted by endogenous let-7 miRNAs they demonstrated that the let-7 miRNA could inhibit translation initiation in HeLa cells (Pillai et al. 2005). In contrast to earlier work, polyribosomal profiling of let-7-targeted mRNAs demonstrated a shift of the targeted mRNA into the upper gradient fractions when the reporter mRNA

contained let-7 target sites, consistent with inhibition of translation initiation. Furthermore, cap-independent translation was refractory to miRNA action. This was determined using cap analogs as well as bicistronic constructs containing an IRES or tethered eIF4E or eIF4G initiation factors. Work from the Preiss lab was published soon thereafter, and came to similar conclusions using an artificial miRNA (CXCR4) that targeted a transfected reporter mRNA (Humphreys et al. 2005). In addition, their work also concluded that an mRNA requires both a 5′-cap and 3′ poly(A) tail in order for translation to be efficiently inhibited by the miRNA RISC.

Interestingly, miRNA-mediated translational repression could be derepressed in human cells subject to stress conditions (Bhattacharyya et al. 2006a, 2006b). miR-122 translational repression of endogenous CAT-1 mRNA in Huh7 cells can be reversed upon amino acid deprivation. The translational derepression of CAT-1 mRNA involves a redistribution of the mRNA out of processing bodies and into actively translating polysomes and requires binding of the AU-rich element binding protein HuR to the CAT-1 mRNA 3′UTR. Soon thereafter, the RNA-binding protein Dead end 1 (Dnd1), which binds to U-rich sequences, demonstrated that it too could derepress specific miRNA-targeted mRNAs in both zebrafish and human germ cells (Kedde et al. 2007). Dnd1 binds to U-rich sequences adjacent to miRNA target sites, and interferes with miRNA–RISC access thereby derepressing specific miRNA-targeted mRNAs.

Although the majority of miRNA research has been conducted in vivo, several groups have developed cell-free extracts that recapitulate miRNA repression in vitro. The first in vitro system to be published came from the Novina lab, and was established using a rabbit reticulocyte lysate (Wang et al. 2006, 2008). Using the artificial CXCR4 miRNA and in vitro transcribed reporter mRNAs, they demonstrated that miRNA silencing was cap- and poly(A)-dependent; however, increasing the length of the poly(A) tail seemed to offset the cap-dependence of the system. In addition, using a biotin pulldown approach to capture factors associated with the miRNA-targeted mRNA, they found that targeted mRNAs associated with the 40S ribosomal subunit and eIF2 and eIF3 translation factors, but not with the 60S. Toe printing assays suggested that miRNA-targeted mRNAs exhibited a characteristic 40S subunit toe print, suggesting a miRNA-mediated initiation block at the 60S subunit joining step. A compelling argument in support of a miRNA-mediated block of 60S subunit joining came from the work conducted by the Shiekhattar and Pasquinelli laboratories (Chendrimada et al. 2007). They demonstrated that the 60S ribosomal subunit antiassociation factor eIF6 associates with the human miRISC. They further demonstrated that depletion of eIF6 in either human cells or worms abrogates miRNA-mediated repression (Basu et al. 2001; Sanvito et al. 1999). However, eIF6 has been shown to play a prominent role in 60S subunit biogenesis, complicating interpretation of data regarding a role of eIF6 in the miRNA response.

Several other groups have described additional in vitro systems that recapitulated miRNA-mediated repression. Extracts were made from *D. melanogaster*

embryos (Thermann and Hentze 2007), mouse Krebs-2 ascites (Mathonnet et al. 2007), and transfected HEK-293 cells (Wakiyama et al. 2007). Overall, all the three results concluded that miRNAs inhibit translation at the initiation step and that this inhibition is a cap-dependent event. In addition, each group reported unique observations. Experiments with *D. melanogaster* embryonic extracts demonstrated that miR-2 inhibited 40S ribosomal subunit recruitment to the miRNA-targeted mRNA, suggesting a miRNA-mediated block of early events in translation initiation (Thermann and Hentze 2007). In this study, miRNA-targeted mRNAs were associated with "pseudopolysomes" that sedimented faster than 80S ribosomes in a density gradient. Experiments in HEK-293-derived extracts demonstrated that miRNAs induced deadenylation of target mRNAs in vitro (Wakiyama et al. 2007). Work in mouse Krebs-2 extracts demonstrated that addition of recombinant eIF4F antagonized miRNA-mediated translational repression (Mathonnet et al. 2007). This result strongly suggested that early events in translation initiation are targeted by miRNAs (i.e., eIF4F/cap interactions). Kiriakidou et al. presented a compelling hypothesis about how the miRISC might inhibit eIF4F–cap interactions when they demonstrated that the central domains of AGO proteins possess sequence homology to the cap binding region of eIF4E (Kiriakidou et al. 2007). They found that AGO2 binds a cap column and that mutations to two aromatic residues in the central domain of AGO2 blocked its interaction with the cap column. These mutations also inhibited mutant AGO2 translational repression activity when the AGO2 mutant was tethered to the 3′UTR of a reporter mRNA (Kiriakidou et al. 2007). This led to a model whereby AGO2–cap interaction competes with eIF4E for cap binding, thus decreasing the rate of translation initiation. However, a more recent report calls these results into question. Izaurralde and colleagues showed, using *Drosophila* AGO homologues, that the AGO mutant that led to a loss of cap column interaction in the previous study abrogated association of Argonaute with miRNA and with GW182 (a P-body component with affinity for AGO proteins) (Eulalio et al. 2008b). Further, they observed no difference in binding of the mutant *Drosophila* Ago homologue to a cap affinity column. These studies directly contradict one another with regard to cap affinity and miRNA binding capability of mutant Ago. Clearly, additional experiments will be required to determine whether Ago proteins directly interact with the 5′-cap. It is possible that AGO proteins or some other component of the miRISC complex can compete with eIF4E for cap binding. It is also possible that miRNAs exert their effect by preventing proper circularization of the mRNA. It is thought that interaction of PABP with eIF4G, a component of the eIF4F cap binding complex, leads to circularization of mRNAs. This circularization is thought to increase the affinity of eIF4E (the cap binding component of eIF4F) for the cap. Hence, if miRNAs inhibit circularization, this would lead to a loss of affinity of eIF4F for the cap, and hence a decrease in efficiency of translation initiation. This model is consistent with the requirement of both a 5′ cap and a 3′ poly(A) tail to elicit miRNA-mediated repression observed in many systems.

1.3.2 Enhancing Eukaryotic Translation

miRNAs repress gene expression by inhibiting mRNA translation and/or initiating mRNA decay. However recent studies in the Steitz lab suggest that miRNAs may in fact enhance translation, rather than inhibit, under certain cellular conditions (Vasudevan et al. 2007, 2008). Specifically, when mammalian cells are starved of serum, miR369-3 interacts with the 3′UTR of tumor necrosis factor-alpha mRNA and enhances its translation. miRNA-mediated enhancement of translation requires the interaction of FXR1 protein with Ago2. Translational enhancement is not limited to miR369-3, as let-7 and CXCR4 miRNAs also enhanced translation of target mRNAs under serum-starvation conditions. Overall, they present a model whereby miRNAs repress translation in proliferating cells, but enhance translation when cells quiesce. These provocative results introduce a new level of complexity with regard to the mechanism of the miRNA response. It will be interesting to see what cellular events and signaling cascades elicit the switch from miRNA-mediated repression of gene expression to enhancement.

1.3.3 miRNA-Mediated Regulation of mRNA Stability

The original discovery of the lin-4 and let-7 miRNAs in *C. elegans* was accompanied by the demonstration that they inhibit translation without affecting mRNA stability (Lee et al. 1993; Reinhart et al. 2000; Wightman et al. 1993). In contrast to the early reports in *C. elegans*, many groups now observe some level of miRNA-mediated mRNA destabilization. This observation suggests that miRNA-mediated translational repression and mRNA decay act in tandem to facilitate repression of gene expression. This assertion is supported by a report that miRNAs elicit a 95% reduction in reporter expression, as well as a 50% decrease in target mRNA levels (Petersen et al. 2006). In addition, Wu et al. reported that miR-125b expression reduced target protein production by 90%, while mRNA levels were reduced by around 70% (Wu et al. 2006). Hence, translational repression and mRNA destabilization appear to have an additive effect on miRNA-mediated repression of gene expression.

In contrast to siRNA-mediated mRNA endonucleolytic cleavage, miRNA mediated enhancement in the rate of mRNA decay appears to be enacted via more traditional deadenylation-dependent degradation pathways. The first evidence that miRNAs mediate deadenylation of target mRNAs came from work conducted in zebrafish in the Schier laboratory. Studies on clearance of maternal mRNAs following activation of zygotic transcription demonstrated that miR-430 targets a few hundred maternal transcripts and mediates their deadenylation and decay (Giraldez et al. 2006). Studies were also published at the same time demonstrating that miRNAs can induce deadenylation in both *Drosophila melanogaster* S2 cells and as well as in

HeLa and NIH-3T3 cells (Behm-Ansmant et al. 2006; Wu et al. 2006). miRNA mediated deadenylation appears to be mediated by the Caf1–CCR4–Not1 deadenylation complex. Work published by the Izaurralde group (Behm-Ansmant et al. 2006; Eulalio et al. 2008a) demonstrated, in *D. melanogaster* cells, that miRNA-dependent mRNA decay is inhibited by siRNA knockdown of deadenylation factors Not1 and Ccr4 as well as the decapping enzyme Dcp1/2 (Behm-Ansmant et al. 2006). miRNA-mediated deadenylation, in this system, also required the GW182 homolog Gawky, as artificially tethering of Gawky to a reporter 3′UTR stimulated deadenylation in the absence of Ago1 protein, the core Argonaute required for miRNA-mediated deadenylation in *Drosophila*.

Interestingly, miRNA-mediated mRNA degradation and translational repression are suggested to function as independent mechanisms of action. Several groups have demonstrated that mRNAs that are not actively translating can still undergo miRNA-mediated deadenylation and/or decay (Eulalio et al. 2007; Wakiyama et al. 2007). In mammalian cells, miRNA-dependent deadenylation and subsequent complete decay of target mRNA was accelerated by miR-125b expression (Wu et al. 2006). This effect required only a single miR-125b target site and was not affected by inhibition inserting a stable hairpin structure into the reporter mRNA's 5′UTR that prevents translation. miRNA-dependent mRNA deadenylation has also been observed in vitro. Wakiyama et al. reported target mRNA deadenylation in extracts derived from HEK293 cells (Wakiyama et al. 2007). Consistent with in vivo results, deadenylation was not dependent on translation, as mRNAs containing a nonfunctional ApppN cap or IRES-containing mRNAs were subject to deadenylation, despite the fact that these constructs exhibited no translational repression (Wakiyama et al. 2007). mRNA decapping often occurs subsequent to mRNA deadenylation and precedes mRNA decay. Several groups have suggested that miRNAs also function, at least for specific mRNAs, to mediate removal of the 5′-cap structure. Specifically, knocking down decapping factors Dcp1 and/or Dcp2 leads to a stabilization of miRNA-targeted reporter mRNAs (Eulalio et al. 2007; Rehwinkel et al. 2006).

Two recent reports have provided a large-scale picture of miRNA mediated control of both target protein and mRNA levels using mass spectrometric proteomic approaches in parallel with microarray-based analysis of mRNA levels. Selbach et al. introduced five different miRNAs (miR-1, miR-155, miR-16, miR30a, and let7b) into HeLa cells by transfection and also used a locked nucleic acid to knockdown let7b and looked at changes in protein and mRNA levels on a genome-wide scale (Selbach et al. 2008). They report that most targets are repressed at both the mRNA and protein level, with the relative contributions of mRNA destabilization and translation inhibition varying from miRNA to miRNA and from target to target. Interestingly, they found that proteins translated at the endoplasmic reticulum were overrepresented in the class of targets that were repressed mainly at the protein level. Baek et al. used a similar, large-scale approach to look at the effect of transfection of miR-1, miR-124, and miR-181 into HeLa cells as well as the effect of deleting miR-223 from mouse neutrophils (Baek et al. 2008). They found a similar correlation between effects at the mRNA and protein level, reporting that

targets exhibiting more than 33% repression at the protein level were also repressed at the mRNA level. Both studies demonstrated the ubiquity of the miRNA response, showing that transfection of single miRNAs generally repressed hundreds of genes at the post-transcriptional level, although few targets were repressed by more than three or fourfold. These results lend credence to the notion that miRNA repression serves to fine-tune gene expression. The Izaurralde lab depleted *D. melanogaster* S2 cells of either AGO1 (the only Argonaute protein involved in the miRNA response in flies), CAF1, or NOT1 and monitored changes in cellular mRNA levels by microarray (Eulalio et al. 2009b). They found that 60% of genes regulated by AGO1 were also regulated by CAF1 and/or NOT1. These results also suggest that mRNA deadenylation plays a significant role in the miRNA response in vivo.

It is clear, then, that miRNA-mediated translational repression and mRNA destabilization act synergistically to inhibit gene expression. mRNA deadenylation removes the binding site for PABP at the mRNA's 3′ end, efficiently disrupting mRNA circularization. As such, deadenylation may be seen as a component of miRNA-mediated translational repression, in addition to its role in initiating decay of the entire mRNA.

1.3.4 The Role of GW182 Proteins and P-Bodies in the miRNA Response

GW182 proteins have recently become a popular topic for studies directed at elucidating mechanistic details of the miRNA response. GW182 is part of a conserved group of proteins characterized by multiple glycine–tryptophan repeat regions that has been found localized to cellular processing bodies (P-bodies, dynamic subcellular structures involved in mRNA storage and decay). GW182 proteins also interact with Argonaute proteins (through GW repeat regions), representing a link between the miRISC and the P-body (Jakymiw et al. 2005; Lian et al. 2009; Liu et al. 2005a, 2005b; Meister et al. 2005; Sen and Blau 2005; Takimoto et al. 2009). It was initially demonstrated in *Drosophila* S2 cells that knockdown of the GW182 homolog *gawky* disrupted miRNA-mediated repression of reporter protein production (Rehwinkel et al. 2005), a result that was reproduced in human cells (Liu et al. 2005a) and in *C. elegans* (Ding and Grosshans 2009). Results from *D. melanogaster* later suggested that miRNA-mediated repression is enacted through GW182 proteins as depletion of Ago1 or Gawky resulted in strikingly similar changes in gene expression by microarray (Behm-Ansmant et al. 2006). Also, tethering of Gawky to the 3′UTR of a reporter gene in the absence of Ago1 resulted in mRNA destabilization as well as a decrease in reporter protein production (Behm-Ansmant et al. 2006), while disruption of Gawky–Ago1 interactions through overexpression of the Ago1 binding domain of Gawky blocked miRNA-mediated silencing of reporters (Eulalio et al. 2008a). A similar result was later reported in human cells (Takimoto et al. 2009). Further evidence for the idea that miRNA effects are mediated through GW182 proteins came from work in human cells. Lian et al. showed that the C-terminal half of all four human Argonaute proteins bind

GW182 and that tethering of this C-terminal half of hAgo2 to the 3′UTR of reporters results in similar levels of repression as tethering full length Ago2 (Lian et al. 2009). Three recent studies have implicated the C-terminus of GW182 proteins as the region responsible for mediating gene silencing. GW182 tethering assays, in concert with deletion analyses, have demonstrated that the C-terminal domain of human GW182 proteins, that cannot bind Argonaute, is sufficient to drive repression of reporter gene expression (Lazzaretti et al. 2009; Zipprich et al. 2009). Importantly, tethering of C-terminal fragments results in repression at both the protein and mRNA levels (Lazzaretti et al. 2009; Zipprich et al. 2009). Genetic analysis in *D. melanogaster* has demonstrated that both the N-terminal Argonaute binding domain and the C-terminal effector domain of Gawky are necessary for miRNA-mediated repression (Eulalio et al. 2009a). Interestingly, a Gawky mutant that fails to localize to P-bodies, but contains the N-terminal Arogonaute binding domain and C-terminal silencing domain, is able to support the miRNA response, while this mutant fails to rescue association of Ago1 to P-bodies (Eulalio et al. 2009a).

Taken together, these results suggest that miRNA mediated translational repression, as well as mRNA destabilization is mediated through GW182 proteins. In effect, the role of the miRNA and Argonaute appears to be to recruit target mRNAs to GW182 proteins, which facilitate translational repression and decay of these transcripts.

1.4 Summary

As data continue to emerge regarding the mechanism of the miRNA response, it has become increasingly apparent that a single concise mechanism cannot account for all examples of miRNA-mediated repression. miRNAs have been reported to repress translation at the level of initiation as well as post-initiation, and to facilitate decay of target mRNAs. It is likely that different cell types, different developmental stages, or different miRNAs may exhibit repression via different mechanisms or combination of mechanisms. One remaining challenge will be to determine what molecular cues determine which mode of repression (or activation) is enacted. Recent reports strongly suggest that miRNA effects are mediated through GW182 proteins and that GW182 effects are not limited to bringing miRNA targeted transcripts to P-bodies. The next step, then, is to dissect the molecular events, downstream of recruitment of GW182 proteins to targeted mRNAs, involved in the various modes of miRNA-mediated control of gene expression.

References

Algire MA, Maag D, Lorsch JR (2005) Pi release from eIF2, not GTP hydrolysis, is the step controlled by start-site selection during eukaryotic translation initiation. Mol Cell 20:251–262

Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP (2008) The impact of microRNAs on protein output. Nature 455:64–71

Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122:553–563

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Basu U, Si K, Warner JR, Maitra U (2001) The Saccharomyces cerevisiae TIF6 gene encoding translation initiation factor 6 is required for 60S ribosomal subunit biogenesis. Mol Cell Biol 21:1453–1462

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 20:1885–1898

Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC (2007) Mammalian mirtron genes. Mol Cell 28:328–336

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

Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006b) Stress-induced reversal of microRNA repression and mRNA P-body localization in human cells. Cold Spring Harb Symp Quant Biol 71:513–521

Bi X, Ren J, Goss DJ (2000) Wheat germ translation initiation factor eIF4B affects eIF4A and eIFiso4F helicase activity by increasing the ATP binding affinity of eIF4A. Biochemistry 39:5758–5765

Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM (2003) Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 113:25–36

Calin GA, Croce CM (2006a) MicroRNA-cancer connection: the beginning of a new tale. Cancer Res 66:7390–7394

Calin GA, Croce CM (2006b) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866

Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N, Marchisio PC, Biffo S (2003) Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. Nature 426:579–584

Chang TC, Mendell JT (2007) microRNAs in vertebrate physiology and human disease. Annu Rev Genom Hum Genet 8:215–239

Chen CZ, Lodish HF (2005) MicroRNAs as regulators of mammalian hematopoiesis. Semin Immunol 17:155–165

Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI, Liebhaber SA, Pasquinelli AE, Shiekhattar R (2007) MicroRNA silencing through RISC recruitment of eIF6. Nature 447:823–828

Croce CM, Calin GA (2005) miRNAs, cancer, and stem cell division. Cell 122:6–7

Cullen BR (2009) Viral and cellular messenger RNA targets of viral microRNAs. Nature 457:421–425

Cummins JM, Velculescu VE (2006) Implications of micro-RNA profiling for cancer diagnosis. Oncogene 25:6220–6227

Dalmay T, Edwards DR (2006) MicroRNAs and the hallmarks of cancer. Oncogene 25:6170–6175

Ding XC, Grosshans H (2009) Repression of C. elegans microRNA targets at the initiation level of translation requires GW182 proteins. EMBO J 28:213–222

Doench JG, Sharp PA (2004) Specificity of microRNA target selection in translational repression. Genes Dev 18:504–511

Doench JG, Petersen CP, Sharp PA (2003) siRNAs can function as miRNAs. Genes Dev 17:438–442

Edery I, Humbelin M, Darveau A, Lee KA, Milburn S, Hershey JW, Trachsel H, Sonenberg N (1983) Involvement of eukaryotic initiation factor 4A in the cap recognition process. J Biol Chem 258:11398–11403

Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dorner S, Bork P, Boutros M, Izaurralde E (2007) Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. Genes Dev 21:2558–2570

Eulalio A, Huntzinger E, Izaurralde E (2008a) GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol 15 :346–353

Eulalio A, Huntzinger E, Izaurralde E (2008b) GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol

Eulalio A, Helms S, Fritzsch C, Fauser M, Izaurralde E (2009a) A C-terminal silencing domain in GW182 is essential for miRNA function. RNA

Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M, Izaurralde E (2009b) Deadenylation is a widespread effect of miRNA regulation. RNA 15:21–32

Forman JJ, Legesse-Miller A, Coller HA (2008) A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. Proc Natl Acad Sci U S A 105:14879–14884

Gallie DR (1991) The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. Genes Dev 5:2108–2116

Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM (2006) MicroRNA expression and function in cancer. Trends Mol Med 12:580–587

Giannakakis A, Coukos G, Hatzigeorgiou A, Sandaltzopoulos R, Zhang L (2007) miRNA genetic alterations in human cancers. Expert Opin Biol Ther 7:1375–1386

Gilbert WV, Zhou K, Butler TK, Doudna JA (2007) Cap-independent translation is required for starvation-induced differentiation in yeast. Science 317:1224–1227

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF (2006) Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science 312:75–79

Grifo JA, Tahara SM, Morgan MA, Shatkin AJ, Merrick WC (1983) New initiation factor activity required for globin mRNA translation. J Biol Chem 258:5804–5810

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 16:144–150

Hammond SM (2006) MicroRNAs as oncogenes. Curr Opin Genet Dev 16:4–9

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

Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho Y, Zhang BT, Kim VN (2006) Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 125:887–901

He L, He X, Lowe SW, Hannon GJ (2007) microRNAs join the p53 network–another piece in the tumour-suppression puzzle. Nat Rev Cancer 7:819–822

Hellen CU, Wimmer E (1995) Translation of encephalomyocarditis virus RNA by internal ribosomal entry. Curr Top Microbiol Immunol 203:31–63

Henis-Korenblit S, Strumpf NL, Goldstaub D, Kimchi A (2000) A novel form of DAP5 protein accumulates in apoptotic cells as a result of caspase cleavage and internal ribosome entry site-mediated translation. Mol Cell Biol 20:496–506

Holcik M, Lefebvre C, Yeh C, Chow T, Korneluk RG (1999) A new internal-ribosome-entry-site motif potentiates XIAP-mediated cytoprotection. Nat Cell Biol 1:190–192

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 U S A 102:16961–16966

Imataka H, Olsen HS, Sonenberg N (1997) A new translational regulator with homology to eukaryotic translation initiation factor 4G. EMBO J 16:817–825

Jakymiw A, Lian S, Eystathioy T, Li S, Satoh M, Hamel JC, Fritzler MJ, Chan EK (2005) Disruption of GW bodies impairs mammalian RNA interference. Nat Cell Biol 7:1267–1274

Jan E, Sarnow P (2002) Factorless ribosome assembly on the internal ribosome entry site of cricket paralysis virus. J Mol Biol 324:889–902

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 62:2636–2643

Kataoka N, Fujita M, Ohno M (2009) Functional association of the Microprocessor complex with spliceosome. Mol Cell Biol

Kedde M, Strasser MJ, Boldajipour B, Oude Vrielink JA, Slanchev K, le Sage C, Nagel R, Voorhoeve PM, van Duijse J, Orom UA, Lund AH, Perrakis A, Raz E, Agami R (2007) RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. Cell 131:1273–1286

Kim YK, Kim VN (2007) Processing of intronic microRNAs. EMBO J 26:775–783
Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G (2004) Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proc Natl Acad Sci U S A 101:360–365
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: 1141–1151
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 273:18599–18604
Kozak M (1978) How do eucaryotic ribosomes select initiation regions in messenger RNA? Cell 15:1109–1123
Kozak M, Shatkin AJ (1979) Characterization of translational initiation regions from eukaryotic messenger RNAs. Methods Enzymol 60:360–375
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
Lee RC, Ambros V (2001) An extensive class of small RNAs in Caenorhabditis elegans. Science 294:862–864
Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854
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 15:804–813
Ling J, Morley SJ, Pain VM, Marzluff WF, Gallie DR (2002) The histone 3'-terminal stem-loop-binding protein enhances translation through a functional and physical interaction with eukaryotic initiation factor 4G (eIF4G) and eIF3. Mol Cell Biol 22:7853–7867
Liu J, Rivas FV, Wohlschlegel J, Yates JR 3rd, Parker R, Hannon GJ (2005a) A role for the P-body component GW182 in microRNA function. Nat Cell Biol 7:1261–1266
Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R (2005b) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol 7:719–723
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 U S A 104:9667–9672
Maag D, Fekete CA, Gryczynski Z, Lorsch JR (2005) A conformational change in the eukaryotic translation preinitiation complex and release of eIF1 signal recognition of the start codon. Mol Cell 17:265–275
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 13:1102–1107
Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, Murata T, Biffo S, Merrick WC, Darzynkiewicz E, Pillai RS, Filipowicz W, Duchaine TF, Sonenberg N (2007) MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 317:1764–1767
Mattes J, Collison A, Foster PS (2008) Emerging role of microRNAs in disease pathogenesis and strategies for therapeutic modulation. Curr Opin Mol Ther 10:150–157
Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, Luhrmann R, Tuschl T (2005) Identification of novel argonaute-associated proteins. Curr Biol 15:2149–2155
Mitchell SF, Lorsch JR (2008) Should I stay or should I go? Eukaryotic translation initiation factors 1 and 1A control start codon recognition. J Biol Chem 283:27345–27349
Nelson PT, Hatzigeorgiou AG, Mourelatos Z (2004) miRNP:mRNA association in polyribosomes in a human neuronal cell line. RNA 10:387–394
Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC (2007) The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 130:89–100
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 216:671–680

Orom UA, Nielsen FC, Lund AH (2008) MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30:460–471

Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 408:86–89

Pelletier J, Sonenberg N (1988) Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334:320–325

Pestova TV, Kolupaeva VG (2002) The roles of individual eukaryotic translation initiation factors in ribosomal scanning and initiation codon selection. Genes Dev 16:2906–2922

Pestova TV, Lomakin IB, Lee JH, Choi SK, Dever TE, Hellen CU (2000) The joining of ribosomal subunits in eukaryotes requires eIF5B. Nature 403:332–335

Petersen CP, Bordeleau ME, Pelletier J, Sharp PA (2006) Short RNAs repress translation after initiation in mammalian cells. Mol Cell 21:533–542

Pillai RS, Artus CG, Filipowicz W (2004) Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. RNA 10:1518–1525

Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W (2005) Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science 309:1573–1576

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

Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M (2004) A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432:226–230

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 11:1640–1647

Rehwinkel J, Natalin P, Stark A, Brennecke J, Cohen SM, Izaurralde E (2006) Genome-wide analysis of mRNAs regulated by Drosha and Argonaute proteins in Drosophila melanogaster. Mol Cell Biol 26:2965–2975

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 403:901–906

Rigoutsos I (2009) New tricks for animal microRNAS: targeting of amino acid coding regions at conserved and nonconserved sites. Cancer Res 69:3245–3248

Rogers GW Jr, Richter NJ, Merrick WC (1999) Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A. J Biol Chem 274:12236–12244

Ruby JG, Jan CH, Bartel DP (2007) Intronic microRNA precursors that bypass Drosha processing. Nature 448:83–86

Sachs A (2000) Physical and functional interactions between the mRNA cap structure and the poly(A) tail. In Sonenberg N, Hershey JWB, Mathews MB (eds) Translational control of gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 447–466

Sachs AB, Varani G (2000) Eukaryotic translation initiation: there are (at least) two sides to every story. Nat Struct Biol 7:356–361

Sanvito F, Piatti S, Villa A, Bossi M, Lucchini G, Marchisio PC, Biffo S (1999) The beta4 integrin interactor p27(BBP/eIF6) is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly. J Cell Biol 144:823–837

Seggerson K, Tang L, Moss EG (2002) Two genetic circuits repress the Caenorhabditis elegans heterochronic gene lin-28 after translation initiation. Dev Biol 243:215–225

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

Sen GL, Blau HM (2005) Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nat Cell Biol 7:633–636

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 U S A 76:4345–4349

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 118:465–475

Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM (2005) Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3'UTR evolution. Cell 123:1133–1146

Stefani G (2007) Roles of microRNAs and their targets in cancer. Expert Opin Biol Ther 7:1833–1840

Stoneley M, Paulin FE, Le Quesne JP, Chappell SA, Willis AE (1998) C-Myc 5' untranslated region contains an internal ribosome entry segment. Oncogene 16:423–428

Takimoto K, Wakiyama M, Yokoyama S (2009) Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression. RNA

Thermann R, Hentze MW (2007) Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. Nature 447:875–878

Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science 318:1931–1934

Vasudevan S, Tong Y, Steitz JA (2008) Cell-cycle control of microRNA-mediated translation regulation. Cell Cyc 7:1545–1549

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 21:1857–1862

Wang B, Love TM, Call ME, Doench JG, Novina CD (2006) Recapitulation of short RNA-directed translational gene silencing in vitro. Mol Cell 22:553–560

Wang B, Yanez A, Novina CD (2008) MicroRNA-repressed mRNAs contain 40S but not 60S components. Proc Natl Acad Sci U S A 105:5343–5348

Welch C, Chen Y, Stallings RL (2007) MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. Oncogene 26:5017–5022

Wickens M, Goodwin EB, Kimble J, Strickland S, Hentze MW (2000) Translational control of developmental decisions. In Sonenberg N, Hershey JWB, Mathews MB (eds) Translational control of gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 295–370

Wightman B, Burglin TR, Gatto J, Arasu P, Ruvkun G (1991) Negative regulatory sequences in the lin-14 3'-untranslated region are necessary to generate a temporal switch during Caenorhabditis elegans development. Genes Dev 5:1813–1824

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 75:855–862

Wilson JE, Powell MJ, Hoover SE, Sarnow P (2000) Naturally occurring dicistronic cricket paralysis virus RNA is regulated by two internal ribosome entry sites. Mol Cell Biol 20:4990–4999

Wu L, Fan J, Belasco JG (2006) MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci U S A 103:4034–4039

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

Zeng Y, Cullen BR (2003) Sequence requirements for micro RNA processing and function in human cells. RNA 9:112–123

Zeng Y, Cullen BR (2005) Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. J Biol Chem 280:27595–27603

Zeng Y, Yi R, Cullen BR (2003) MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci U S A 100:9779–9784

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, Epub March 20, 2009