Principles and Effects of miRNA-mediated Posttranscriptional Gene Regulation

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Abstract

miRNAs are abundant regulatory RNAs involved in the regulation of many key biological processes. Recent advances in understanding the mechanism of RNAi and miRNA-mediated mechanisms shed light on major principals of the formation of the regulatory complex and provide models to explain how these small regulatory RNA species interfere with gene expression and how they influence the translational status of the transcriptome.

Introduction

Pathways that function on the basis of the principles of RNA interference (RNAi) are highly conserved mechanisms of gene regulation. Archean and eukaryotes harbour several key components of the RNAi machinery and the function of RNAi related mechanisms has been studied in diverse eukaryotic model systems (Cerutti & Casas-Mollano, 2006). RNAi, apart from providing an indispensable reverse genetic tool (Elbashir et al., 2001) and promising therapeutic approach (Zimmermann et al., 2006), is key regulator of gene expression on both transcriptional and post-transcriptional level and it is involved in the defence against viruses and bacteria. RNAi provides switch points in many developmental processes and it is a key determinant in regulating mechanisms leading to diseases including cancer (Wienholds & Plasterk, 2005).

The hallmark of all RNA silencing mechanisms is the transformation of the sequence information of some species of long RNAs with double stranded (dsRNA) characteristic into small 20-27 nucleotide (nt) long regulatory RNAs, which serve as the specific determinants of a protein complex, the RNA Induced Silencing Complexes (RISC), that represses gene expression. Among the small regulatory RNAs associated with diverse RNAi based mechanisms microRNAs (miRNA) are probably one of the most abundant ones in plants and metazoan. For instance, miRNAs are predicted to constitute ~ 3% of human genes and they may contribute to the post-transcriptional regulation of at least one third of the human mRNAs.

Here, we review the principles and mechanisms of miRNA-mediated gene regulation taking the assembly of a functional RISC as our starting point.

RISC formation

The machinery of RNAi achieves specificity in transcriptional silencing, targeted RNA cleavage and translational repression through its RNA-programmed protein complexes. RISC was first defined as a large RNA-protein complex with sequence-specific RNA cleavage activity that could be purified by chromatographic fractionation from cells programmed *in vivo* or *in vitro* with longer dsRNA or siRNA (Hammond et al., 2000; Zamore et al., 2000). Biochemical approaches and genetic screens in protists, plants, fungi and *C. elegans* have unambiguously identified the members of the Argonaute protein family as essential protein components of both the cleavage-competent RISCs and the miRNA-containing RNPs (miRNP) (Baumberger & Baulcombe, 2005; Hammond et al., 2001; Liu et al., 2004; Meister et al., 2004; Mourelatos et al., 2002; Qi et al., 2005). Argonaute orthologues have been identified in bacteria, archea and most but not all eukaryotes (Cerutti & Casas-Mollano, 2006).

Argonautes have the four following characteristic conserved protein domains: N terminal, Mid, PAZ and PIWI domain. The three-dimensional structures of bacterial and archaeal Argonaute proteins and the PAZ domains of eukaryotic Argonautes revealed that the PAZ domain binds the short single-stranded 3´ ”tails” characteristic of an siRNA. It has been postulated that the PAZ domain initiates incorporation of a small RNA into RISC by binding the single-stranded 2nt 3´overhang of an siRNA or miRNA duplexes (Lingel et al., 2003; Ma et al., 2005; Song et al., 2003; Song et al., 2004; Yan et al., 2003). More remarkably, these structural studies revealed that the key to understanding the sequence-specific activity of RISC resides in the structure of the PIWI domain, whose three-dimensional fold clearly places in the RNase H enzyme family of nucleic-acid directed RNA-endonucleases (Ma et al., 2005; Parker et al., 2005; Song et al., 2004; Yuan et al., 2005). This finding is consistent with earlier biochemical studies showing that chemistry of cleavage by human and fly RISC has many similarities to RNAse H-mediated catalysis: a requirement for the divalent cation Mg^{2+} and cleavage products with 5´terminal phosphates and 3´terminal hydroxyl groups (Martinez & Tuschl, 2004; Schwarz et al., 2004). A minimal and active human RISC reconstituted *in vitro* from single-stranded siRNA and recombinant human Ago2 (hAgo2) shows similar chemical and kinetic properties to the larger fly and human cleavage-competent RISCs formed in unpurified cell extract (Haley & Zamore, 2004; Martinez & Tuschl,

2004; Rivas et al., 2005). Moreover, tethering hAgo1-hAgo4 to the 3´UTR of an mRNA through a sequence-specific protein RNA-binding domain rather than by a small RNA showed that Argonaute proteins themselves can initiate the repression of mRNA translation (Pillai et al., 2004).

The complexes that form around the minimal RISCs appear to be very diverse. Their sizes, from ~ 160 kD (minimal RISC) to 80S ("holo-RISC"), and protein components differ from report to report, depending on the model system and purification approaches (Hammond et al., 2001; Martinez et al., 2002; Pham et al., 2004; Zamore et al., 2000). For instance, *D.melanogaster* RISC complexes have been shown to contain, in addition to an Argonaute, Fragile X mental retardation 1 protein (dFMR), Vasa intronic gene (VIG) and Tudor staphylococcal nuclease (TSN) (Caudy et al., 2002; Ishizuka et al., 2002). The functions of these co-purified factors are largely unknown yet. In human cells Gemin 3 and 4 have been identified in complex together with hAgo2 (Meister et al., 2005a; Mourelatos et al., 2002). Both hAgo1 and hAgo2 co-purify with the helicase Mov10, the fly orthologue of which is required for RISC assembly, Tncr6 Bisoform1 and Prmt5 proteins with unknown function (Meister et al., 2005b; Tomari et al., 2004a).

There is an increasing number of reports that Argonautes associates with complexes that contain Dicer, the key enzyme in siRNA and miRNA maturation (Duchaine et al., 2006; Gregory et al., 2005; Maniataki & Mourelatos, 2005; Meister et al., 2005b; Okamura et al., 2004; Sasaki et al., 2003; Tabara et al., 2002). The co-association of RISC and the miRNA processing is not surprising. Dicer not only generates short-lived double-stranded siRNA and miRNA intermediates but also participates in the downstream process that lead to the selection of one of the strands, the guide strand of an siRNA, and participates in its incorporation into an Argonaute. The combination of genetic and biochemical approaches in fly identified the RISC loading complex (RLC) that activates and transfers one strand of an siRNA onto the fly Ago2 (Lee et al., 2004; Liu et al., 2003; Pham et al., 2004; Tomari et al., 2004b). RLC contains at least Dicer-2, one of the two fly Dicers that predominantly process long dsRNAs and bind to siRNAs, and its dsRNA-binding interactor R2D2 (Tomari et al., 2004b). The key determinant in strand selection and unwinding is the thermodynamic difference between the two 5´ends of an siRNA (Khvorova et al., 2003; Schwarz et al., 2003). R2D2 recognizes and binds to the relatively more stable 5´end of the siRNA positioning Dicer-2 onto the 5´end of the guide strand, whose

5´end is less tightly paired than the corresponding 5´end of the passenger strand, and facilitates its incorporation into RISC. Both R2D2 and Dicer-2 are necessary but not sufficient to complete the unwinding of the duplex suggesting that other factors are required for this process. Indeed, one such factor is very likely to be the Argonautes themselves. In spite of the fact, that the recombinant hAgo2 are not capable of unwinding short RNA duplexes, genetic data in flies suggest that Ago2 is indispensable for this process (Okamura et al., 2004). In fact, it has been recently demonstrated that the cleavage competent fly and the human Ago2 actively participate in the unwinding mechanism by cleaving the passenger strand of the perfectly double-stranded siRNA (Leuschner et al., 2006; Matranga et al., 2005; Miyoshi et al., 2005).

The pathway has been most thoroughly studied for RISC assembly initiated by long dsRNA or siRNAs, but it is likely that similar RISC loading mechanisms operate when RISC is charged with miRNAs. The majority of miRNAs are represented with one strand of the stem of the pre-miRNA, predominantly with the strand that has thermodynamically weaker 5´ end, at steady state level suggesting that miRNA maturation follows the rule of the asymmetric RISC formation. In addition, orthologues of R2D2 are important for RNAi and miRNA processing in worms flies and mammals. The miRNA specific fly Dicer-1 requires Loquacious, for proper miRNA processing, Dicer in worms co-immunoprecipitates with RDE-4, a dsRNA-binding protein essential for RNAi initiated with long dsRNA, and human Dicer requires two R2D2 othologues, TRBP and PACT, for miRNA-mediated gene silencing (Chendrimada et al., 2005; Forstemann et al., 2005; Haase et al., 2005; Jiang et al., 2005; Lee et al., 2006; Saito et al., 2005; Tabara et al., 2002). Furthermore, Argonautes seem to interact physically with the miRNA maturation and loading machinery since immunoprecipitated hAgo2 complexes contain functional Dicer activity that can asymmetrically process exogenous pre-miRNAs, generating mature RISC that can cleave a perfect complementary target RNA (Gregory et al., 2005; Maniataki & Mourelatos, 2005; Meister et al., 2005b). Together, the data suggest that RISC formation is a highly dynamic process that requires the coordinated interaction of complexes involved in distinct steps of siRNA and miRNA maturation (Fig.1.).

Mechanism(s) of miRNA-mediated gene regulation

According to our current understanding, miRNA-guided posttranscriptional gene-regulation follows at least two distinct mechanisms based on the characteristics of the target recognition by miRNAs. First, the target can be cleaved at a fixed position relative to the miRNA complementary sequence (Hutvagner & Zamore, 2002) . Second, the translation of the target RNA may be inhibited, without initiating sequence-specific mRNA degradation.

There are two basic requirements for miRNA-mediated sequence-specific cleavage. The Argonaute in RISC has to bear catalytic activity, and the complementary between the miRNA and target has to have sufficient to initiate sequence-specific cleavage. Although all Argonaute proteins contain a PIWI domain with its characteristic RNAse H fold not all have retain catalytically active aspartate-aspartates-histidine (DDH motif) (Liu et al., 2004; Meister et al., 2004). However, the loss of DDH motif is not sufficient to explain the loss of catalytic activity for some Argonaute proteins. For example, human Ago3 contains an intact DDH motif but the protein cannot initiate sequence-specific cleavage. Alternatively, hAgo3 catalized target cleavage may require additional cofactors not yet identified or be restricted to a subset of targets or small RNA guides not yet explored (Liu et al., 2004; Song et al., 2004). In flies, both Ago1, which prefers to bind miRNAs and Ago2 that binds siRNAs, can show catalytic activity if a sufficiently complementary target is presented (Okamura et al., 2004). In spite of the fact, that all exogenously introduced human Argonautes equally bind to siRNAs and miRNAs only hAgo2 is capable of targeted RNA cleavage (Liu et al., 2004; Meister et al., 2004).

The outcomes of *in vivo* approaches, structural studies of PIWI and Argonaute proteins, and kinetic analysis of purified or "holo-RISCs" revisited the early view about the degree of specificity of the sequence-specific RNA cleavage. Now, it is clear that target cleavage requires a continuous A-form helix between the small regulatory and the targeted RNAs (Chiu & Rana, 2003; Haley & Zamore, 2004). The helix spans from the second to the 12^{th} nucleotide of an siRNA or miRNA, counted from its 5´end, that result in a cleavage between the 10^{th} and 11^{th} nucleotides as a consequence of placing the scissile phosphate near the active site of the Argonautes (Haley & Zamore, 2004; Ma et al., 2005; Parker et al., 2005; Song et al., 2004). The miRNA mediated cleavage of transcripts results in the production of 5' and 3' fragments characteristic to RNAse H activity (Martinez & Tuschl, 2004; Schwarz et al., 2004), which are subsequently processed by different degradation pathways. In

Drosophila cells, the elimination of the 3' fragments is mediated by Xrn1, and it is not dependent on the exosome components Rrp4 or Ski2. On the other hand, the 5 fragment is likely to be degraded by the exosome. Although both deadenylation and decapping of transcripts seems to facilitate mRNA degradation, neither of the two steps appears to take place before processing of the 3' or 5' fragments by Xrn1 or Ski2 (Orban & Izaurralde, 2005).

In contrast to plants in humans only a few examples are known when a miRNA-target pair exhibits sufficient complementary to initiate sequence-specific target degradation. The interaction between miR-196, and its target RNA, HOXB8, can be traced in mouse embryos by following the characteristic RNA fragments deriving from the sequence-specifically cleaved HOXB8 transcript (Hornstein et al., 2005; Yekta et al., 2004). The imprinted PEG-11/rtl1 gene gives rise to an intronless transcript from its paternal copy, which is maternally repressed. From this locus however, a maternal antisense transcript arises that contains a number of hairpin-loop structures that are processed into miRNAs that have been shown to repress the paternal sense transcript. These miRNAs are necessarily perfectly complementary to their target, which is subsequently cleaved in a sequence specific manner (Davis et al., 2005; Seitz et al., 2003). The estimated number of human genes that are possibly targets of miRNA guided sequence-specific cleavage rather than translational repression, is about 40. Among these are MCL-1, LIMK-1 and MUSASHI-1, likely targeted by miR-298, miR-328 and miR-129 respectively, which encode proteins with anti-apoptotic functions (John et al., 2004).

The majority of animal miRNAs have only limited complementary to their experimentally verified or predicted targets and the level of base pairing between most of the miRNAs and their targeted sequences prevent miRNAs from inducing sequence-specific cleavage. The miRNA-target RNA interaction appears restricted to the 5´end of the miRNA sequence that was shown to contribute the most to the target binding (Doench & Sharp, 2004; Haley & Zamore, 2004; Lewis et al., 2003; Rajewsky & Socci, 2004). In fact, it is well established that the perfect complementary between the miRNA 2-8 nucleotides, so called "seed sequence", and the targeted RNA is absolutely essential for target recognition. In this region, a single mismatch blocks binding, although a shorter seed pairing may be compensated by extensive matching between the target and the 3´ regions of the miRNAs (Brennecke et al., 2005; Kloosterman et al., 2004; Lai et al., 2005; Lewis et al., 2003). Another

function of the 3´region of a miRNA is to give specificity to the members of miRNA families that share identical seeds but they diverge in their 3´sequences (Brennecke et al., 2005). The recognition of the principles of miRNA targeting accelerated the development and improvement of a series of bio-computational approaches to predict miRNA targets in animals that produced a remarkably large number of putative miRNA regulated transcripts further emphasizing the significance of miRNAs (Enright et al., 2003; Kiriakidou et al., 2004; Lall et al., 2006; Lewis et al., 2005; Stark et al., 2003).

In spite of the fact that miRNAs bind their targets by following an almost universal mechanism, the only exception so far is miR-16 that was suggested to base-pair with its target by initiating a non-canonical conformation (Jing et al., 2005), it seems that at the level of gene regulation the outcome of these interactions can be diverse. The worm *lin-4*, the founding member of miRNAs, together with its target RNAs associate with polyribosomes but no protein is made suggesting that the *lin-4* miRNA inhibits translation after the initiation step, most likely slowing down the rate of polypeptide elongation or stability (Olsen & Ambros, 1999; Seggerson et al., 2002). In fact, many miRNAs have been associated with polysomes, in cultured human cells (Nelson et al., 2004) and primary cortical neurons (Kim et al., 2004). Recently, the most detailed analysis of the miRNA-mediated inhibition of translational elongation was carried out in human cells. siRNAs, mimicking miRNA action by imperfectly matching them to the 3´UTR of a reporter, silenced the translation of the target after initiation, concluded from the data that showed efficient regulation of a construct with IRES, by increasing translation termination that eventually resulted in ribosome drop off (Petersen et al., 2006). However, miRNAs also interfere with translation at the initiation step. Analysing of the effects of both exogenously provided miRNA-like siRNAs or endogenous miRNAs on the translation of their reporter and endogenous targets in human cells showed that the 5´cap is required for miRNA-mediated translational repression, because IRES initiated translation or tethering eIF4E and eIF4G to the reporter was immune to miRNAs (Humphreys et al., 2005; Pillai et al., 2005). The two reports disagree with the prerequisite of the poly(A) tail for the inhibition, but in cell free translation system both the 5´ cap and the poly(A) tail has been shown to be required for recapitulating miRNA-mediated translational repression (Wang et al., 2006). These results argue

that miRNA programmed RISCs can prevent the recruitment of translation factors to the 5´cap of the mRNA.

Regulation by miRNAs also appears to be connected to the Processing body (P-bodies, GW-bodies, PBs), a cytoplasmatic structure involved in storage and 5´ to 3´destruction of mRNA. In cultured human cell miRNA-binding caused the targeted mRNA to localize to P-bodies, which is argued to occurs in response to the primary regulatory event, repression of translational initiation (Bhattacharyya et al., 2006; Pillai et al., 2005). Indeed, eIF4e and eIF4e-transporter proteins participate in the transport of mRNAs into the PBs (Andrei et al., 2005) and in yeast inhibition of translation initiation increases localization of an mRNA to PBs (Sheth & Parker, 2003).

Human Argonaute proteins and *C. elegans* ALG-1, a worm Argonaute protein required for *let-7* directed gene regulation, co-localize, co-purify and interact with proteins also located in PBs (Ding et al., 2005; Jakymiw et al., 2005; Liu et al., 2005a; Liu et al., 2005b; Meister et al., 2005b; Sen & Blau, 2005). The localization of Argonaute proteins to PBs requires that the protein bind small RNAs; the target mRNA is transported to the PB only if it contains miRNA binding sites, suggesting that miRNAs bound to Argonaute proteins direct the mRNA to the (Agbottah et al., 2005; Liu et al., 2005a; Liu et al., 2005b; Pillai et al., 2005). Three lines of evidence support the idea that the existence of complexes associated with Argonaute proteins in the PBs is not only a consequence of inhibiting the translation of miRNA-regulated transcripts but PB localized RISCs might have an autonomous role in miRNA mediated repression. First, exogenously transfected siRNAs with no known endogenous mRNA targets localized to PBs (Jakymiw et al., 2005). Second, depletion by RNAi of PB components, such as GW182 protein, decapping enzymes (Dcp1a, Dcp2), RCK/p54 and the 5´-to- 3´exonuclease Xrn1, impair both RNAi and miRNA-mediated repression in flies worm and cultured human cells (Chu, 2006; Ding et al., 2005; Jakymiw et al., 2005; Liu et al., 2005a; Rehwinkel et al., 2005). Finally, Mov10, the human homolog of Armitage, a helicase required in flies for RISC assembly in the germ line, interacts with human Ago1 and Ago2 and is localized in the PBs. Moreover, AIN-1, the worm GW182 ortholog, interacts with DCR-1 suggesting that events before RISC assembly may take place in these structures (Ding et al., 2005; Meister et al., 2005b). The RNA and miRNA independent interaction between hAgo2 and Dcp1a strengthens this hypothesis (Liu et al., 2005b).

What is the fate of the miRNA-targeted mRNAs after their transfer to PBs? PBs contain the RNA decay machinery, suggesting they degrade the miRNA-targeted mRNAs. In fact, miRNA-binding can clearly destabilize target mRNA by a mechanism distinct from the Argonaute-catalyzed sequence specific cleavage that characterize RNAi (Bagga et al., 2005; Farh et al., 2005; Giraldez et al., 2006; Lim et al., 2005). Detailed analyses of miRNA-directed mRNA decay revealed that miRNAs induce translation-independent deadenylation of the targeted mRNA, which is the hallmark of deadenylation-dependent decapping followed by 5´-to-3´ decay (Giraldez et al., 2006; Wu et al., 2006). Remarkably, AU-rich elements (ARE) in the 3´UTRs of unstable mRNAs can also accelerate the shortening of the poly(A) tail triggering decapping and RNA destruction, and there are hints of connections between the miRNA and ARE pathways. For instance, miR-16 may recruit TTP to canonical AREs through the members of the Argonaute family. Interestingly, TTP forms a complex with hDcp1a, hDcp2 and hXrn1, and overexpressed TTP co-localizes with PBs (Jing et al., 2005; Kedersha et al., 2005; Lykke-Andersen & Wagner, 2005). Moreover, the ARE binding protein HuR involved in reversing the miR-122 mediated repression of the CAT-1 mRNA (Bhattacharyya et al., 2006). However, mRNAs do not always respond with significantly decreased stability when targeted by miRNAs. (Bhattacharyya et al., 2006; Humphreys et al., 2005; Petersen et al., 2006; Pillai et al., 2005; Wang et al., 2006). And miRNA targeted, translationally repressed transcripts can be transported to PBs without destruction suggesting that PB may function as a reservoir where quiescent mRNAs stored, possibly allowing their reuse for translation (Bhattacharyya et al., 2006; Pillai et al., 2005).

A new model proposes to integrate the disparate models for miRNA-mediated mechanisms. The model postulates that the inherent stability and turnover rate of a mRNA determines if miRNA-binding manifests in repression of translation (without mRNA destabilization) or in accelerated degradation of the mRNA (causing a decrease in its steady-state abundance) (Valencia-Sanchez et al., 2006). However, it remains possible that miRNAs sequester and/or degrade their mRNA targets by divergent but interrelated mechanisms. For example, different neuronal miRNAs show distinct polysomal distributions patterns in the same cells (Kim et al., 2004). The choice among pathways may be regulated by developmental and environmental cues or may reflect the different stoichiometries between different miRNAs and their targets.

Global effects of miRNAs on gene expression

The first miRNAs were identified as key regulators of developmental timing in *C. elegans* suggesting that miRNAs, in general, may play decisive regulatory roles in transitions between different developmental programmes by switching off a few targets (Lee et al., 1993; Reinhart et al., 2000). Indeed, the results of genetic screens in worm and fly supported this view by presenting additional miRNAs with bold phenotype in developmental processes (Brennecke et al., 2003; Johnston & Hobert, 2003). The targeted mRNAs that are involved in this "switch target" (Bartel & Chen, 2004) type regulation bear multiple miRNA-binding sites in their 3´UTRs that facilitate the radical inhibition of translation of abundant messages via the cooperative actions of miRNAs (Doench & Sharp, 2004). However, current experimental and bioinformatics data argue that the developmental switch function of miRNAs may be exceptional and the role of miRNAs in gene regulation is much more complex. For example, one miRNA can regulate hundreds of targets simultaneously (Giraldez et al., 2006; Lim et al., 2005) and target predictions unambiguously suggest that mRNAs with multiple miRNA binding sites are very rare, about 2% of the predicted targets have more than one predicted miRNA-binding sites. The majority of the putative targets have only a single miRNA-binding site for individual miRNAs which probably is non sufficient for governing switch type regulation. Furthermore, inhibitions of all miRNAs by depleting Dicer do not severely impair differentiation and patterning in vertebrates (Giraldez et al., 2005; Harfe et al., 2005). Indeed, miRNAs have recently been hypothesized to be part of genetic networks wired by feed-forward loops (FFL) that maintain the fidelity of developmental programs, rather than initiating developmental transitions, by conferring robustness with both preventing leaky transcriptions and buffering genetic noise (Hornstein & Shomron, 2006). The robustness provided by miRNAs was experimentally confirmed in flies by examining the expression patterns of abundant, developmentally regulated miRNAs and their conserved targets. In these cases miRNAs and targets with conserved bonding sites have "mutually exclusive" expression patterns meaning that miRNAs and their targets are highly expressing in spatially and temporarily neighbouring tissues but they are generally excluded from the same tissues suggesting that miRNAs prevent misexpression of non-wanted, leaky, transcripts (Stark et al., 2005). In

addition, abundant messages that co-express with miRNAs evolved to avoid miRNA-mediated repression by shortening their 3´UTR and selecting against sequences complementary to seed sequences of miRNAs (Farh et al., 2005; Stark et al., 2005).

In summary, miRNAs are not only involved in directly regulating up to one third of the metazoan transcriptomes but they are a driving force behind the evolution of 3´UTR which may easily affect virtually all existing transcripts.

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Figure legend

RISC formation (1-4) is a dynamic process. The pre-miRNA is cleaved (1) and one strand of an siRNA or a miRNA is selected and loaded by the RLC (2-3) into RISC (4).

miRNAs can be engaged in diverse but interrelated regulatory mechanisms (5-11). Should miRNAs share sufficient complementary with their targets and are incorporated into a cleavage competent Argonaute then miRNAs may initiate endonucleolytic cleave of the mRNA (5). The cleaved mRNA is further degraded by the exosome and by Xrn1 (11). miRNAs can inhibit translation after translation initiation resulting in ribosome drop off (6) or they impede translation initiation (7). mRNAs targeted by RISC and excluded from translating ribosomes are transported to P-bodies (8) where they are stored or degraded (9). RISC can also be localized in P-bodies on a target independent manner and P-body components are required for RISC activity or RISC assembly (10).

 : Dicer

 : Argonautes

 : Dicer associated dsRNA binding protein (RDE-4, R2D2, loquacious, TRBP, PACT)

 : RNA helicase (MOV10, Armitage)

 : GW182

 : Decapping enzyme (Dcp)

 : Xrn1

 : Exosome

 : Poly(A) binding protein

 : sequence-specific cleavage