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Review

MicroRNAs: Synthesis, mechanism, function, and recent clinical trials

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ABSTRACT

MicroRNAs (miRNAs) are a class of small, endogenous RNAs of 21–25 nucleotides (nts) in length. They play an important regulatory role in animals and plants by targeting specific mRNAs for degradation or translation repression. Recent scientific advances have revealed the synthesis pathways and the regulatory mechanisms of miRNAs in animals and plants. miRNA-based regulation is implicated in disease etiology and has been studied for treatment. Furthermore, several preclinical and clinical trials have been initiated for miRNA-based therapeutics. In this review, the existing knowledge about miRNAs synthesis, mechanisms for regulation of the genome, and their widespread functions in animals and plants is summarized. The current status of preclinical and clinical trials regarding miRNA therapeutics is also reviewed. The recent findings in miRNA studies, summarized in this review, may add new dimensions to small RNA biology and miRNA therapeutics.

1. Introduction

The first small RNA, *lin-4*, was discovered in 1993 through a genetic screening in nematodes. Later in the same year, the regulation of *lin-14* by *lin-4* was discovered, which demonstrated the regulatory function of small RNAs [1,2]. The shorter *lin-4* RNA is now recognized as the origin of an abundant class of small regulatory RNAs, known as microRNAs (miRNAs). Currently, miRNA-directed gene regulation is an active area of study. Hundreds of miRNAs have been discovered by cloning and size-fractionated RNA techniques [3–5]. The recent development of high-throughput sequencing technologies [6,7] and computational and bioinformatics prediction methods has greatly enhanced research on miRNAs including regulatory targets and possible functions [8–11]. A number of miRNAs are known for functions in diverse processes including cell proliferation, cell death, fat metabolism, neuronal patterning, hematopoietic differentiation, immunity, and control of leaf and flower development [12]. Computational techniques and bioinformatics algorithms for finding genes regulated by miRNAs have suggested that these examples represent very few of the total miRNA system.

In animals, miRNAs are synthesized from primary miRNAs (pri-miRNAs) in two stages by the action of two RNase III-type proteins: Drosha in the nucleus and Dicer in the cytoplasm [13]. In plants, the two-step processing of pri-miRNA into mature miRNA occurs entirely in the nucleus and is carried out by a single RNase III enzyme, DCL1 (Dicer-like 1) [14]. The mature miRNAs are then bound by Argonaute (Ago) subfamily proteins. These miRNAs target mRNAs and thereby function as posttranscriptional regulators [13].

Developments in the miRNA field are increasing steadily. This is clearly evident in the studies of miRNAs in various diseases, ranging from Alzheimer's to diabetes. Recently, miRNA research has been accelerated by technological advancements in RNA-based therapies. miRNAs are now being studied for their potential as a new generation of drugs.

This review highlights our understanding of miRNAs following the report of *lin-4* RNA and its regulation of *lin-14*. The major topics discussed include miRNA synthesis and regulatory mechanisms. The functions of miRNAs in gene regulatory pathways and several recent preclinical and clinical trials are also summarized.

2. miRNA synthesis in animals

miRNAs are defined as 21–25 nucleotide single-stranded RNAs (ssRNAs), which are produced from hairpin shaped precursors [15]. miRNAs transcripts are then processed after their synthesis. In recent years, there has been significant effort to investigate the processing of miRNAs in animals and plants. In animals, genes for miRNAs are transcribed to a primary miRNA (pri-miRNA). The pri-miRNA is processed within the nucleus to a precursor miRNA (pre-miRNA) by Drosha, a class 2 RNase III enzyme. Next, the transport of pre-miRNAs to the cytoplasm is mediated by exportin-5 (EXP-5). In the cytoplasm, they are further processed to become mature miRNAs by Dicer an RNase III type protein and loaded onto the Argonaute (ago) protein to produce the effector RNA-induced silencing complex (RISC).

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2.1. Genome, genes, and transcriptions

The identification of the *lin-4* RNA in 1993 opened windows for a new era in the field of miRNA genomics; this era truly, began in 2000 with the discovery of the *let-7* RNA in *Caenorhabditis elegans* [16,17]. In the same year, the *let-7* gene and *let-7* RNA were detected in humans, *Drosophila*, and other bilateral animals [18]. Since then, thousands of miRNAs and miRNA genes have been reported by cloning and other molecular biology techniques. Moreover, other miRNAs and miRNA genes have been predicted with the help of bioinformatics and computational technology tools. A recent study reported 154 *C. elegans*, 152 *Drosophila melanogaster*, 337 *Danio rerios* (zebrafish), 475 *Gallus gallus* (chicken), 695 human, and 187 *Arabidopsis thaliana* miRNAs [13]. It is worth noting that the miRNA database "miRBase" reports an indeed larger number of human miRNA than the reported figures. miRNAs have even been reported in simple multicellular organisms [19]. Evolutionary studies show that some miRNAs are phylogenetically conserved in bilaterian animals. More than half of the *C. elegans* miRNA genes have been found to have homologs in humans [13].

Early researchers discovered that the majority of miRNAs are located in intergenic regions, whereas a few were annotated in intronic regions [3,5]. Approximately half of all known miRNAs are found in close proximity to other miRNAs. These clustered miRNAs are expressed as poly-cistronic primary transcripts. A few cases showed that some miRNAs can be transcribed from their own promoter as mono-cistronic primary transcripts [20,21]. Based on their genomic locations, miRNA genes can be classified as intronic miRNAs in coding transcription units (TUs), intronic miRNAs in noncoding TU, exonic miRNAs in coding TU, and exonic miRNAs in noncoding TU (Fig. 1).

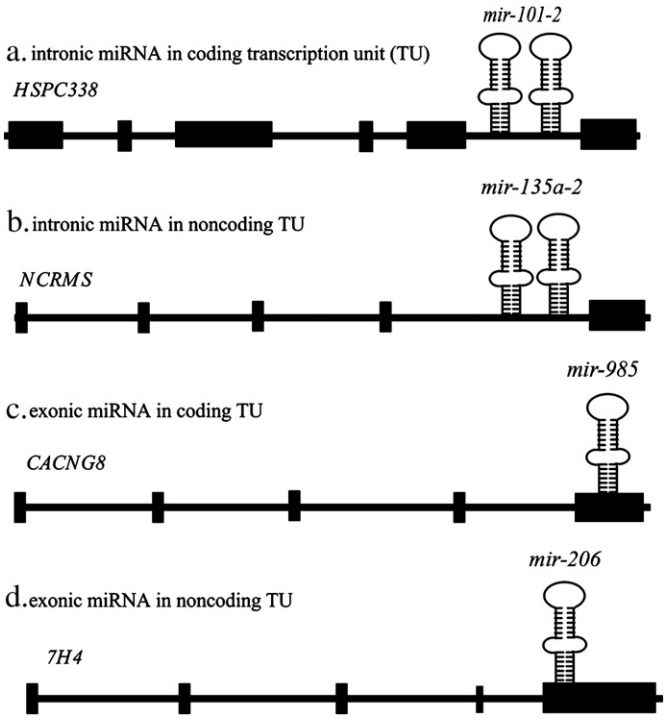

Fig. 1. Schematic illustration of the genomic organization and structure of miRNA genes. The miRNAs can be divided into four distinct groups on the basis of their genomic location (a) intronic miRNAs in coding transcription units (TUs), for example, the *mir-101-2* cluster. The *mir-101-2* cluster is found in the intron of a non-coding RNA gene, HSPC338. (b) Intronic miRNAs in noncoding TU, such as the *mir-135a-2* cluster. (c) Exonic miRNAs in coding TU, of which the *mir-985* is a well known example that is found in the CACNG8 gene. (d) Exonic miRNAs in noncoding TU, such as *mir-206*. The hairpin illustrates miRNA stem loops, and boxes show the protein coding regions (exon). The figure shows a rough schematic.

RNA polymerase II (Pol II) is mainly responsible for the transcription of miRNA genes [21,22], but a small group associated with Alu repeats can be transcribed by RNA polymerase III (Pol III) [23]. Pol II-dependent miRNA gene expression enables temporal control, so that a specific set of miRNAs can be synthesized according to specific conditions and cell types. The product of Pol II- or Pol III-mediated expression is known as the primary miRNA (pri-miRNA), which are usually several kilobases long and contain local stem loop structures.

2.2. Nuclear processing

A number of different proteins are involved in miRNA processing (Fig. 2). All animal miRNAs are first processed in the nucleus. The pri-miRNA produced by Pol II is cleaved at the stem of the hairpin structure, which releases an approximately 60–70 nt hairpin structure, known as the precursor miRNA (pre-miRNA) [24,25]. This processing step is performed by Drosha, which requires the DiGeorge syndrome critical region in gene 8 (DGCR8) in humans and Pasha in *D. melanogaster* or *C. elegans* as a cofactor [20,26–29]. Drosha, in conjunction with either DGCR8 or Pasha, forms a large complex known as the microprocessor complex [26,28]. Mouse models showed that DGCR8 genes are important for developmental processes. DGCR8 and Drosha are largely conserved in animals [30–33]. Typically, metazoan pri-miRNAs are comprised of about 33 base pairs (bp) of the stem loop and a terminal loop and single-strand RNA (ssRNA) flanking segments. DGCR8 interacts with the ssRNA segment and guides Drosha to slice pri-miRNA. Drosha cleaves RNA duplexes about 11 bp away from the ssRNA-stem loop junction and thus processes the pri-miRNA to the pre-miRNA with a 5′-phosphate group and an approximately 2 nt 3′ overhang [20,34,35].

2.3. Transportation by exportin-5

Pre-miRNAs are transported into the cytoplasm for further processing to become mature miRNAs. The transport of the pre-miRNA occurs through nuclear pore complexes, which are large proteinaceous channels embedded in the nuclear membrane [36]. The transport of the pre-miRNA is mediated by the RanGTP-dependent nuclear transport receptor exportin-5 (EXP5) [37–39]. One proposed model of miRNA transport posits that the export of the pre-miRNA is initiated when the EXP5 recognizes the >14-bp double-stranded RNA (ds-RNA) stem loop with a 3′ overhang followed by cooperative binding to both the pre-miRNA and GTP-bound cofactor Ran in the nucleus. The pre-miRNA bound EXP5 exports out of the nucleus, where hydrolysis of the GTP results in the release of the pre-miRNA [37,40–42].

2.4. Cytoplasmic processing and Argonaute loading

The nuclear cleavage process by Drosha defines one end of the mature miRNA. The pre-miRNA is released in the cytoplasm by means of EXP5 and is subsequently processed by an endonuclease cytoplasmic RNase III enzyme Dicer to create a mature miRNA [43–46]. Dicer is a highly specific enzyme that measures about 22 nt from the preexisting terminus of the pre-miRNA and cleaves the miRNA strand. Dicer is a highly conserved protein that exists in almost all eukaryotic organisms. Some organisms have multiple types of Dicers; for example, *D. melanogaster* contains Dicer-1 and Dicer-2, each having different roles. Dicer-1 is required for miRNA maturation, whereas Dicer-2 is required for the maturation of siRNA [47].

Dicer works in close proximity with other proteins including RNAi deficient-4 (RDE-4) in *C. elegans*, R2D2, fragile X mental retardation 1 (FMR1) in *D. melanogaster*, and the Argonaute family proteins (Ago family protein) in several other organisms [48–51]. Recently, it was shown that *D. melanogaster* Dicer-1 requires Loquacious (LOQS; also

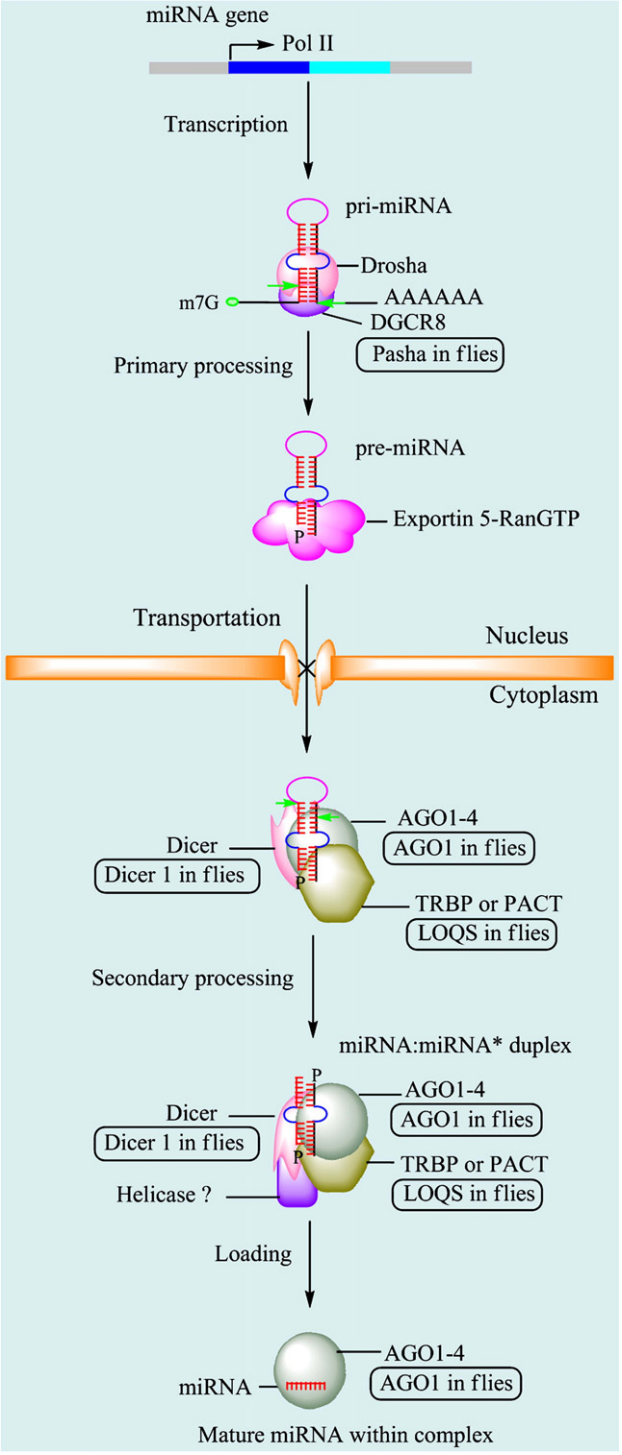

Fig. 2. The animal miRNA synthesis pathway. The microRNA (miRNA) genes are transcribed by RNA polymerase II (Pol II), which results in the production of a pri-miRNA. Drosha, along with DiGeorge syndrome critical region gene-8 (DGCR-8; Pasha in flies), mediates the initial processing step (primary processing) that produces a ~65 nucleotide (nt) pre-miRNA. The pre-miRNA has a short stem of 2–3 nt 3′ overhangs, which is recognized by exportin 5 (EXP5) that mediates transport to cytoplasm. In the cytoplasm, RNase III Dicer is thought to catalyze the second processing step (secondary processing), which generates the miRNA/miRNA* duplex. Dicer, TRBP or PACT (LOQS in flies), and Argonaute1–4 (Ago 1–4) (Argonaute 1 in flies) are responsible for pre-miRNA processing and RISC (RNA-induced silencing complex) assembly. An unknown helicase is thought to mediate unwinding of the duplex. One strand of the duplex remains the mature miRNA (miRNA) on Ago, whereas the miRNA* or passenger strand is degraded. The figure shows the mammalian miRNA synthesis pathway and fly factors are in the squares.

known as R3D1), which contains three dsRNA binding motifs for pre-miRNA processing [52–54]. The human Dicer is associated with two closely related proteins, trans-activation response RNA-binding protein (TRBP) and protein kinase, interferon-inducible double-stranded RNA-dependent activator (PRKRA, also known as PACT) [55–57]. The Dicer-associated proteins do not seem to be required for any processing activity themselves, but rather they contribute in the formation of the RNA-induced silencing complex [55–57]. However, the specific roles of these proteins have yet to be determined.

According to the current model, after the generation of an approximately 22 nt miRNA duplex by Dicer cleavage, the miRNA duplex is incorporated into an Ago family protein complex. This generates an effector complex. Mostly one strand of the miRNA (passenger strand or miRNA*) is degraded, whereas the other strand remains bound to Ago as mature miRNA (guide strand or miRNA). Yet, in a few cases, miRNA* are loaded into RISC and therefore remains functional. Recent evidence has shown that the thermodynamic stability of the two ends of the duplex may determine which strand is to be selected [58]. Dicer, in conjunction with other interacting proteins (TRBP and/or PACT in human and LOQS in fly) and Ago family proteins, contributes to RISC assembly by forming a RISC loading complex (RLC) [55,59–62]. The exact mechanism regarding to the role of RLC in RNA loading to Ago is not known. However, evidence suggests that after the processed miRNA duplexes are released from Dicer, the stable end of the miRNA duplex binds to interacting proteins in the RLC, and the unstable end associates with the Ago proteins [49,62]. It has been demonstrated that the endo-nucleolytic enzyme activity of the Ago protein is responsible for the removal of the miRNA passenger strands [63]. Most of the miRNAs contain mismatches in the middle, and some Ago proteins lack “slicer” activity, making the passenger strand of the miRNA resistant to cleavage. Evidence suggests that an RNA Helicase (yet to be identified) mediates the unwinding and removal of the unselected strand of the miRNA duplex. After loading, the miRNA guides the RISC to its target mRNA, which is silenced through degradation or translation repression [13,14].

3. miRNA synthesis in plants

Homologs of Drosha and its cofactors (DGCR8/Pasha) have not been confirmed in plants, which suggests that Drosha-dependent stepwise processing is absent in plants. Genetic studies showed that Dicer like-1 (DCL-1) is solely responsible for plant miRNA processing. The HASTY (HST) homologue of exportin-5 mediates the export of miRNAs from the nucleus to the cytoplasm. The loading of the miRNA to the Argonaute family proteins (Ago) is carried out in the nucleus or in the cytoplasm (Fig. 3).

3.1. Genes and their transcription in plants

In 2002, the first small RNA in plants was discovered through cloning of a small RNA in rice and *Arabidopsis*. This suggested that a small portion of cloned small RNAs correspond to miRNAs [64,65]. Recently, advanced genetics, direct cloning and sequencing, and bioinformatics and computational prediction methods have revealed many new miRNAs and their functions in *Arabidopsis* and other plant species [66]. A recent study reported 959 miRNAs genes from 10 plant species including mosses, dicots, and monocots [66]. Some plant miRNA genes have multiple isoforms (paralogs) that probably arose by the process of gene duplication and diversification. Plant miRNAs are generally conserved in evolutionary processes ranging from mosses to flowering plants [67–69]. Most of the miRNA genes are annotated to intergenic regions, and unlike animal miRNAs, plants miRNAs are not arranged in clusters [66]. The majority of plant miRNAs analyzed have been found to have their own transcriptional units that are transcribed into a primary transcript (pri-miRNA) by

polymerase II [66]. Plant miRNA precursors are quite diverse in structure, and the stem loops are usually longer than those of animal pri-miRNAs. Studies have shown that the 5′ cap is present in most plant miRNAs [70]. Most of the plant miRNAs have poly-adenylated tails; however, the exact role of polyadenylation is still unknown [71].

3.2. Dicer processing and methylation

Plant miRNA processing is entirely dependent on Dicer-like proteins. Various studies in *A. thaliana* and other plants have revealed that DCL1 is important for miRNA processing [72]. DCL1 is a nuclear protein which indicates that mature miRNAs in plants might be synthesized in the nucleus [73]. The functional loss of DCL1 greatly reduces the accumulation of miRNAs and causes pleiotropic developmental defects, revealing the role of DCL1 in miRNA maturation [72,74–76]. Recent studies have shown that the processing of pri-miRNAs to pre-miRNAs by DCL1 also requires two other proteins, HYPONASTIC LEAVES1 (HYL1) and SERRATE (SE). HYL1 is a member of the ds-RNA-binding protein family in *Arabidopsis*, and SE encodes a C_2H_2 zinc finger motif, which plays a general role in the biogenesis of miRNAs [77–80].

Plant miRNA methylation occurs after Dicer processing, which distinguishes it from animal miRNAs. Hua Enhancer (HEN1), a methyltransferase, may be responsible for methylation and has a general role in miRNA processing in plants [66]. Recently, it was demonstrated that HEN1 adds a methyl group onto the 2′ OH of the 3′ terminal nucleotide [81]. The molecular mass of an endogenous miRNA is approximately 14 Da larger than that of an *in vitro* synthesized unmodified miRNA, indicating the presence of a methyl group in plants [82].

3.3. Argonaute loading and transportation

The resulting methylated miRNA/miRNA* duplex is loaded onto the Ago protein to generate RISC. The Ago family proteins are composed of three distinctive domains: the PAZ, MID, and PIWI domains [83]. The Ago protein PAZ domains bind to RNA and PIWI domains in a folded structure similar to RNase H [84]. The miRNA* strand is degraded, which results in the formation of RISC with one mature miRNA. Like in animals, the strand selection is made through thermodynamic stability [58,85]. Different types of Ago proteins have been reported in *Arabidopsis*, and most of these contain the catalytic site for slicer activity [86].

HST is a plant homolog of exportin-5 and plays a role in plant miRNA export from the nucleus to cytoplasm [87,88]. An HST mutant showed pleiotropic phenotypes and a reduced accumulation of miRNAs, indicating that this protein functions as a nuclear export receptor [88–90]. Evidence showed that mature miRNA abundance is higher than that of miRNA* in both cellular compartments. These facts suggest that either RISC loading occurs in the nucleus followed by transportation of miRISC to the cytoplasm or RISC loading occurs in the cytoplasm after the transportation of the miRNA/miRNA* duplex.

4. Mechanism

miRNAs guide miRISC to specifically recognize messenger RNA (mRNA) and downregulate gene expression by one of the two posttranscriptional mechanisms: (i) translational repression and (ii)

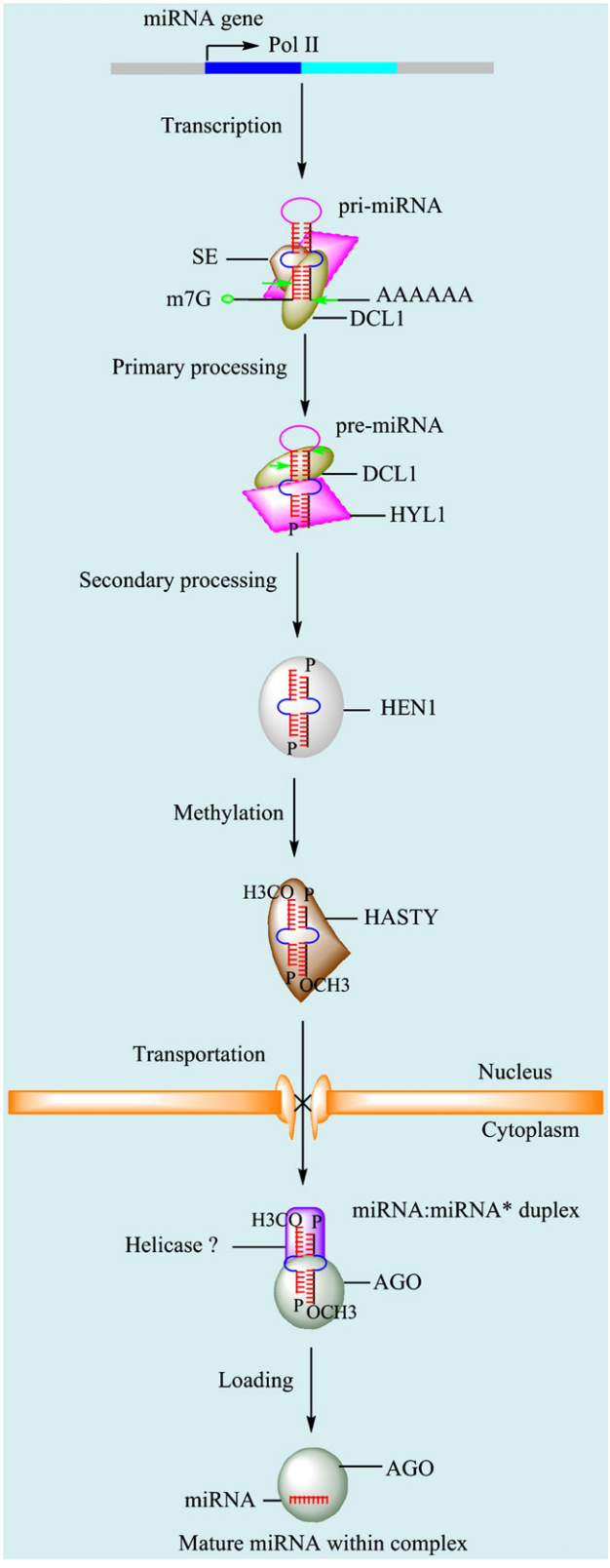

Fig. 3. The plant miRNA synthesis pathway. The miRNA genes are transcribed by RNA polymerase II (Pol II), which results in the production of the pri-miRNA. DCL1 in association with SE and HYL1 performs the first processing step (primary processing), which produces the pre-miRNA. DCL1 and HYL1 are also responsible for the second processing step (secondary processing) to produce the miRNA/miRNA* duplex. HEN1 mediates methylation in plant miRNA synthesis, which adds methyl groups to both strand of the miRNA/miRNA* duplex. Hasty (HST) is thought to be responsible for nuclear export of miRNA in plants. Argonaute loading occurs in the nucleus or cytoplasm (figure shows cytoplasmic Argonaute loading). Some unknown helicase is thought to mediate unwinding of the duplex. The passenger strand (miRNA*) is degraded, and the other strand remains the mature strand with the Ago proteins.

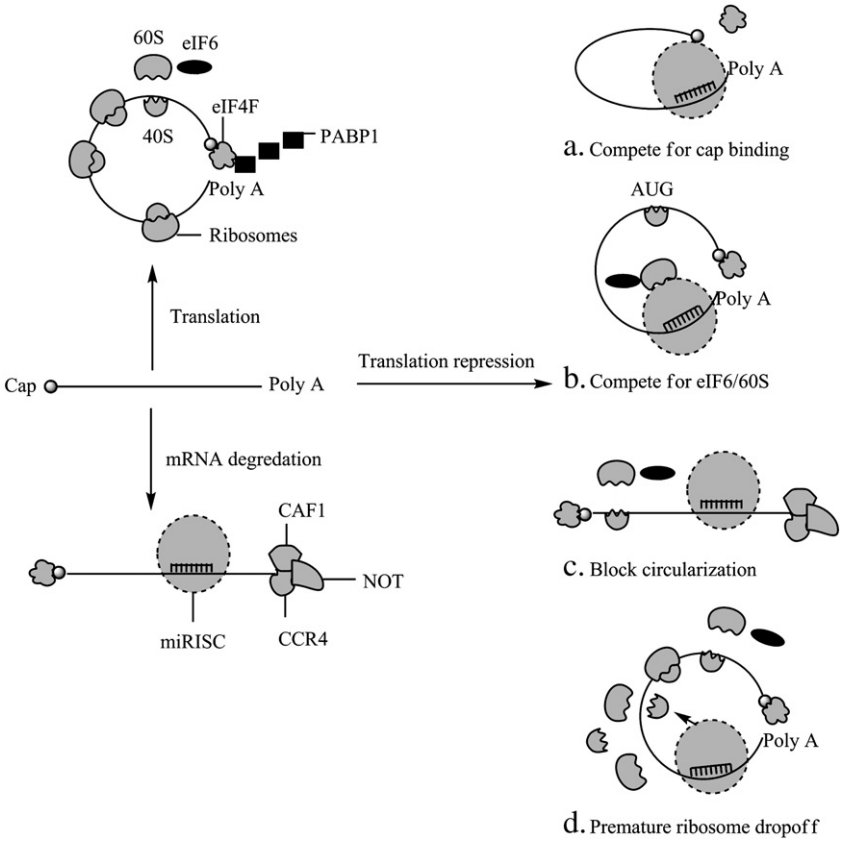

Fig. 4. Possible mechanisms for miRNA gene regulation. Unregulated mRNAs engage with the initiation factor eIF4F complex, which is composed of eIF4A, eIF4E and eIF4G subunits and recruits ribosomal subunits, which form circularized structures that enhance translation (upper left). When miRISC binds to target mRNAs, a high degree of miRNA–mRNA complementarity facilitates Ago-catalyzed degradation of target mRNA sequences through mRNA cleavage mechanisms (lower left). Alternatively, central mismatches prevent degradation and facilitate translational repression by any of four (a–d) possible mechanisms (right): (a) miRISCs bind to target mRNAs and represses initiation at the cap recognition stage, or at (b) the 60S ribosomal recruitment stage, (c) miRISC can prevent mRNA to circularize (d) miRISC attachment to target mRNAs also facilitates premature separation from ribosomes, which represses translation at the postinitiation stage.

mRNA cleavage (Fig. 4). Initially, it was proposed that *lin-4* RNA represses translation of *C. elegans lin-14* mRNA [2]. Current studies suggest that if miRISC contains a heterologous RNA recognition factor, then it facilitates miRISC to recognize and specifically represses mRNA in spite of lacking miRNA binding sites [90]. Studies indicate that most miRNA binding sites in animal mRNAs lie in the 3′ UTR as multiple copies. Animal miRNAs bind with mismatches and bulges through Watson–Crick base pairing [91]. In contrast, the miRNA binding sites in plant mRNAs lie in the centre of the complementary regions, and most plant miRNAs contain a high degree of sequence complementarity to their target mRNA sequence [92–94].

The degree of miRNA–mRNA complementarity is a major determinant of the regulatory mechanism process. The high degree of complementarity enables the Ago-catalyzed degradation of target mRNA sequences through the mRNA cleavage mechanism process. In contrast, a central mismatch omits degradation and facilitates the translational repression mechanism.

4.1. Translation repression

The exact mechanism for the repression of target mRNA translation by miRISC is still unknown. Whether repression occurs at the translational initiation or posttranslational level still needs to be determined. However, the current model suggests that the eIF4F complex is involved in translational initiation. The subunits of the eIF4F complex include eIF4A, eIF4E, and eIF4G. The mRNA 5′ terminal cap is recognized by eIF4E and thus starts the initiation process. eIF3, another initiation factor, interacts with eIF4G and contributes to the 40S ribosomal subunit assembly at the 5′ end of the mRNA to enable the preinitiation complex. The elongation process is initiated by joining of the 60S ribosomal subunit at the AUG codon of the mRNA and the 40S preinitiation complex. eIF4G and eIF3 also interact with the polyA-binding protein PABP1. The mRNA molecule becomes circular as a result of this process, and the translation efficiency is thereby improved. In some viral mRNAs, the translation initiation process is facilitated without any initiation factors through internal ribosome sites (IRES), which require only a subset of the initiation factors [92].

Whether a miRNA inhibits translation through inhibition of initiation or elongation is typically determined by two sets of criterion. For the first option, the density gradient centrifugation technique is used to determine whether mRNAs are present in the complex mRNA–protein (mRNP) system (initiation inhibition), or in the form of large polysomes (elongation inhibition). The second criterion is tested by determining whether inhibited mRNAs containing IRES sequences are resistant to repression [92,95]. In testing this, some studies reported data supporting repressed initiation [95–98], whereas others provide evidence for inhibition of the post-initiation processes [99–101]. However, none of the above criteria alone is sufficient to explain repressed initiation or inhibition of postinitiation processes. The existing discrepancies show that repression may occur either at the initiation step or at a later stage in the translation process.

In 2006, Petersen et al. proposed a possible mechanism through which miRISC may exert its action by repressing the elongation process. An inhibited mRNA can be associated with polysomes, but when the initiation process is rapidly blocked with hippuristanol, the ribosomes quickly become detached in a miRNA-dependent manner. Based on these results, it was suggested that miRISC promotes early ribosome dissociation from mRNAs. Recently, three different models

have been proposed to explain the mechanism by which miRISC represses the initiation mechanism (Fig. 4). First, miRISCs were shown to compete with eIF4E for binding to the mRNA 5′ cap structure, which results in the failure of the translation initiation process [97,102]. However, some studies contradict this model and suggest that either GW182 or a downstream factor could be the eIF4E competitor [103]. The second model suggests that miRISC prevents the mRNA from circularizing, resulting in translation inhibition [104–107]. The C-C chemokine receptor 4-negative on TATA (CCR4–NOT) complex is composed of multiple proteins, namely chemokine (C-C motif) receptor 4 (CCR4), chromatin assembly factor 1 subunit (CAF1), and NOT1–NOT5. These regulate gene expression and may be involved in miRISC translation inhibition [107–110]. The third model proposes that miRISC may inhibit the assembly of the 60S ribosomal subunit with the 40S preinitiation complex. In this process, the 40S ribosomes are attached to the targeted mRNA, but the 60S ribosomal subunit fails to join the 40S subunit, resulting in translation repression [111,112].

Another possible mechanism of miRNA mediated translational repression is that miRNA/RISC may mediate translation repression through accumulation of target mRNAs in processing bodies (P-bodies) [113]. P-bodies lack any translation machinery, and thus, it is suggested that P-bodies containing mRNAs are not involved in the translation process [113]. The accumulation of mRNA in a miRNAs-dependent manner suggests that miRNAs are increasing the ribosome-free mRNA and cause translation repression.

4.2. mRNA degradation

Previously, it has been shown that when miRNAs have a high degree of sequence complementarity, then target mRNA degradation processes are facilitated through Ago protein slicer activity. The fact that mRNAs are reduced with an abundance of miRNAs suggests that miRNAs are responsible for mRNA degradation processes [104–106,114,115]. Recent studies have suggested that not only the Ago-catalyzed mRNA degradation process is responsible for the mRNA degradation, but other mechanisms such as deadenylation, decapping, and exonucleolytic digestion of mRNA are also involved [104–106]. mRNA degradation by miRNA requires Ago, GW182, and the cellular decapping and deadenylation machinery [103]. The exact process of target selection has yet to be determined. However, it has been shown that the number, type, and position of mismatches in the miRNA/mRNA duplex play a critical role in the selection of the degradation or translational repression mechanisms [116].

5. Functions of miRNAs in animals

miRNAs have key roles in the regulation of distinct processes in mammals. They provide a key and powerful tool in gene regulation and thus a potential novel class of therapeutic targets. miRNAs play an evolutionarily conserved developmental role and diverse physiological functions in animal. miRNAs largely exhibit limited complementarity with their target mRNAs in animals, but this is still sufficient to regulate several physiological processes. It has been suggested that they repress the initiation step of the translation process, which may be followed by mRNA degradation [117]. Loss-of-function mutations of the first two identified miRNAs in *C. elegans*, *lin-4* (abnormal cell lineage-4) and *let-7* (lethal-7), caused defects in larvae developmental processes [1,118]. It has been suggested that *lin-4* regulates the early developmental stages, whereas *let-7* plays an important role in the late developmental processes in *C. elegans* and possibly some other animals [119,120]. The *lsy-6* (laterally symmetric-6) miRNA induces cell fate of two morphologically distinct neurons, ASE left (ASEL) and ASE right (ASER). *lsy-6* is expressed in the ASEL neurons and inhibits the expression of its target gene, cog-1 (connection of gonad defective-1), which results in the loss of asymmetry. *mir-273* in the ASER neurons, activated by the *lsy-6* target cog-1, inhibits the translation of die-1 (dorsal intercalation and elongation defect-1). This leads to the down-regulation of *lsy-6* and the subsequent expression of the GCY-5 (guanylyl cyclase-5) receptor in the ASER [121,122].

Two miRNAs, bantam and *lin-14*, were identified in *D. melanogaster*, and studies suggest that overexpression of bantam induces growth and inhibits apoptosis [123]. It is known that miR-14 suppresses cell death and is involved in fat metabolism by acting on *D. melanogaster* IL1-beta convertase (DRICE), which is upregulated in the absence of miR-14 [124]. Furthermore, two groups of Notch target genes contain conserved motifs in their 3′ UTR, which are complementary to the sequences of a related group of miRNAs [125,126]. miR-7 regulates the GY-box motif, and reduction in mir-7 expression leads to a reduced expression of downstream Notch targets, such as Cut, resulting in reduced vein spacing and thickening of the veins [125,126].

A knockout gene strategy has been used in different mammals to study the role of miRNAs in mammalian developmental processes. A Dicer knockout was made in zebrafish [127], and this revealed a role of the *mir-430* family members, which are highly expressed in zebrafish zygotic development, in neurogenesis. *mir-430* expression was also observed in the early developmental processes in frogs [128,129]. Recent studies have suggested that late-stage mouse development is under the control of miRNAs, which is supported by the regulation of Hox genes by miR-196. *mir-196* is expressed in the hind limb, it cleaves its target Hoxa B8, and it inhibits the translation of Hoxc8, Hoxd8, and Hoxa7 [130,131]. miR-196 acts upstream of Hox B8 and Sonic hedgehog (SHH) in limb development [131]. The muscle-specific miRNA, miR-1, targets heart and neural crest derivatives-expressed protein 2 (HAND2), which results in muscle degeneration and premature differentiation of cardiomyocytes [132]. *mir-181*, which is expressed in the B lymphocytes of bone marrow and the thymus of mice, causes an increase in B lymphocytes and regulates mouse hematopoietic lineage differentiation [133]. Similarly, the expression of *mir-143* has been reported in human fat tissues and has been shown to regulate fat differentiation by increasing the extracellular signal-regulated kinase-5 (ERK5) level [134]; indeed, ERK5 is the predicted potential target gene for miR-143 [135]. This shows the involvement of miR-143 in adipocyte differentiation by the regulation of ERK5 protein levels. Some miRNAs regulate diverse physiological processes, including miR-375 and miR-16. *mir-375* is expressed in the pancreatic islet and inhibits glucose-induced insulin secretion through regulation of its target gene Myotrophin, indicating that miR-375 is an inhibitor of glucose-stimulated insulin secretion [136]. It has also been shown that *mir-375* is highly expressed in the pituitary gland of zebrafish embryos, indicating a role for miR-375 in the secretion of hormones [137]. Similarly, miR-16 causes AU-rich element-mediated mRNA instability and degradation [138]. miR-16 in humans has a limited complementarity to AREs but is sufficient to destabilize ARE-containing mRNAs. In addition, *mir-155* lies in the noncoding BIC RNA transcript and is involved in innate immunity, as evidenced by the rapid induction in B lymphocytes and T lymphocytes either after antigen exposure or due to some inflammatory mediators [139]. miR-155 targets PU.1 and c-Maf transcription factors, which result in the negative regulation of IgG1 and T-cell lineage by differentiation of T helper type 1 and type 2 cells [140,141]. Recently, it has been found that some endogenous miRNAs participate in antiviral defense mechanisms. miR-32 exhibits inhibitory effects against the retrovirus type 1 (PFV-1) and protects human cells from PFV-1 [142]. The role of miRNAs in different types of cancer was shown in a study on chronic lymphocyte leukemia; *mir-15a* and *mir-16-1* are located at chromosome 13q14 and have been found to be deleted in the majority of cases of chronic lymphocytic leukemia [143]. *mir-15a* and *mir-16-1* are upregulated in B-cell lymphomas and exert tumor suppressor activities by inhibiting B-cell lymphoma 2

(Bcl2) functions [144]. Similarly, *mir-17-92* clusters are located on human chromosome 13q31, which is augmented in some tumors and is frequently amplified in B-cell lymphomas. This overexpression of *mir-17-92* induces c-Myc-mediated tumorigenesis and suppresses apoptosis in mouse models of human B-cell lymphoma [145]. In addition, *mir-372* and *mir-373*, which are expressed in primary human fibroblasts, induced tumorigenesis through targeting the tumor suppressor gene LATS2 [146]. Specifically, *mir-372* and *mir-373* are expressed in testicular tumors of the germ cell [146].

Based on the above evidence, it can be concluded that miRNAs control various physiological processes in humans and other animals through diverse targets. Several of the reported animal miRNAs and their biological functions are summarized in Table 1.

6. Functions of miRNAs in plants

Like in animals, miRNAs also play crucial roles in plants at various developmental stages and facilitate organ identity maintenance [14]. Plant development is a highly regulated process that is controlled at many levels. Plant miRNAs are highly complementary to conserved target mRNAs, which allows fast and confident bioinformatics identification of plant miRNA targets [156]. The major class of miRNA-targeted genes is comprised of transcription factors and F-box (a motif that was first identified in cyclin F) proteins, which constitute major plant developmental regulatory networks [157]. In plants, miRNA regulatory functions can be divided into three major categories. First, miRNAs are capable of defining distinct expression patterns of their targets, in which miRNAs and their targets are expressed on adjoining nonoverlapping domains. Second, miRNAs prevent variations in the pattern and expression levels of their targets by sharing overlapping expression domains. Third, miRNAs are involved in the temporal regulation of target gene accumulation [158], which regulates developmental transitions.

The first evidence for the importance of miRNAs in plant development came from mutants impaired in small RNA biogenesis or function, which exhibited altered growth patterns. Many developmental defects result from this type of impaired miRNA activity. The role of miRNAs in target accumulation was demonstrated by target gene expression pattern expansion in the absence of miRNA regulation. This restricting action was proposed based on *mir-165/166* regulation of prohibitin (PHB) in *Arabidopsis* and maize rolled leaf 1 (RLD1) in maize [159]. The *mir-165/166* genes are important for establishing and maintaining abaxial polarity. In the same way, mutations within the *mir-165/166* complementary site of the maize homeodomain leucine zipper (HD-ZIP) gene RLD1 adaxialize leaf primordia causes an overaccumulation of RLD1-mRNA [160]. When *mir-165/166* was identified and the mutations were mapped to the miRNA complementary site, it was hypothesized that the altered phenotypes resulted from the loss of miRNA-directed regulation [161]. The prediction tools for plant miRNA targets and other methodologies have been used to study the regulatory impact of miR-167 and its target genes, ADP ribosylation factors 6 and 8 (ARF6 and 8). Two recent reports revealed the regulatory role of miR-167 in plant reproductive development [162]. The ARF6 and ARF8 genes regulate stamen development in the immature flowers. It was shown that miR-167 causes the degradation of ARF6- and ARF8-encoded mRNAs [163]. miR-167 may also repress ARF6 expression at the translational level. The *mir-167*-overexpressing *Arabidopsis* recapitulates ARF6/ARF8 double-mutant phenotypes, in which the plants produce flowers with short stamens and anthers lose the ability to release pollen. Mutations of the *mir-167* target sites for ARF6 or ARF8 result in abnormal expression of these genes in both ovules and anthers, where *mir-167* is normally present. The promoter activity of *mir-167* was studied with respect to four members of the *mir-167* family, which illustrated the essential roles of these members. The plant hormones auxin, gibberellic acid (GA), and abscisic acid (ABA) play critical roles in the regulation of developmental processes such as embryogenesis, cell division, elongation, differentiation, and organogenesis [164].

One of the important mediators in the GA-dependent pathway is GAMYB, which controls GA-activated genes. *mir-159* is regulated by GA and targets the GAMYB genes MYB33 and MYB65 [165]. Overexpression of *mir-159* leads to a late flowering phenotype [165,166]. Developmental defects such as hyponastic leaves were observed in transgenic plants expressing the miRNA-resistant version of MYB33 and the double-mutant *mir-159ab* [166,167]. These defects were diminished in the quadruple mutant of *mir-159ab*, MYB33 and MYB65, conclusively demonstrating the role of miRNA-based regulation of the MYB genes in these phenotypes [168].

Furthermore, miR-164 prevents the alteration and facilitates the precise control of the expression level of target genes in the Auxin signal transduction pathways and leaf patterning [169]. miR-164

Table 1
Animal miRNAs and their biological functions.

miRNAs	Target gene	Biological functions	Species	Reference
bantam	HID	Cell death and proliferation	*D. melanogaster*	[149]
let-7	*lin-41*, HBL-1	Regulation of developmental timing	*C. elegans*	[17,148]
lin-4	*lin-14*, lin-28	Physiological condition and developmental timing	*C. elegans*	[1,147]
lsy-6	COG-1	Neuronal cell fate and developmental timing	*C. elegans*	[121]
miR-1	HAND 2	Cardiomyocyte differentiation and proliferation	*Mus musculus*	[132]
miR-7	Notch targets	Notch signaling	*D. melanogaster*	[125,126]
miR-14	Caspase?	Cell death and proliferation	*D. melanogaster*	[124]
miR-15a, miR-16-1	Bcl_2	Down-regulated in B cell chronic lymphocyte leukemia		[143,144]
miR-16	Several	AU-rich element mediated mRNA instability	*Homo sapiens*	[138]
miR-17-92	c-Myc, E2F1	Upregulated in B-cell lymphoma	*H. sapiens*	[145,155]
miR-32	Retrovirus PFV1	Antiviral defense	*H. sapiens*	[142]
miR-143	ERK5	Adipocyte differentiation		[134]
miR-143, miR-145	Unknown	Downregulated in colonic adenocarcinoma	*H. sapiens*	[154]
miR-146	c-Myc, ROCK1	Development and function of immune system	*H. sapiens*	[151,152]
miR-155	PU-1, c-Maf	T-cell development and in innate immunity	Mouse	[139–141]
miR-181	unknown	Regulation of hematopoietic cell fate	*M. musculus*	[137]
miR-196	HOXA7, HOXB8, HOXC8, HOXD8	Development?	*M. musculus*	[130]
miR-223	NFI-A, Mef2c	Regulation of granulocytic maturation	*H. sapiens*	[150]
miR-273	DIE-1	Neuronal cell fate and developmental timing	*C. elegans*	[122]
miR-372, miR-373	LATS2			[146]
miR-375	Myotrophin	Insulin secretions	*M. musculus*	[136]
miR-430	?	Brain morphogenesis	*D. rerio*	[128]
SVmiRNAs	SV40 viral mRNAs	Susceptibility to cytotoxic T cells		[153]

controls the activity of the NAM, ATAF, and CUC (NAC) transcription factors, which regulate signaling processes. A balance exists between these, but overexpression of *mir-164* causes the down-regulation of CUP SHAPED COTYLEDON (CUC1), (CUC2), and the NAC family genes, which results in the induction of lateral leafing and rooting [170]. These observations suggest that closely interrelated miRNA family members that target the same set of genes can have different functions in plant development, which expands the role of miRNAs in the Auxin signaling pathways. A similar role has been observed in the process of leaf initiation by miR-156, which regulates 10 members of the SQUAMOSA promoter-binding-like (SPL) gene family and is expressed in an opposite pattern than these factors. miR-156 regulates SPL9 and has the same temporal expression pattern. Reduced activity of miR-156 results in an increased expression of SPL9 [171].

In *Arabidopsis*, the miR-172 targeted gene APETALA2 (AP2) controls the developmental timing of flowers. The overexpression of *mir-172* leads to loss-of-function mutants, which exhibit developmental floral defects such as absence of petals and sepal transformation into carpels [172]. Many miRNA families target a single class of gene products including miR-319, which targets the TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP) transcription factors. Furthermore, the overexpression of *mir-319* leads to patchy leaf shapes and delayed flowering times [173]. The miR-319-resistant TCP4 gene causes deviant seedlings with no apical meristems. Several miRNAs are closely connected to signaling mediators that respond to plant hormones, such as miR-393, which targets TRANSPORT INHIBITOR RESPONSE 1 (TIR1) and three F-box proteins [156]. The F-box proteins act as auxin receptors and mediate degradation in response to auxin [174]. In addition, miRNAs target transcription factors, such as in the case of miR-162 that targets DCL1 or miR-168 that targets Ago1 [175,176]. miRNA targeting of DCL1 and Ago1 suggests a feedback mechanism, whereby miRNAs negatively regulate their activity. Some of the known plant miRNAs and their biological functions are summarized in Table 2. Based on the above discussion, it is clear that miRNAs play a vital role in the regulation of many developmental and other processes in plants.

7. Current clinical trials

Recent understanding of the importance of miRNAs has attracted the interest of the biomedical research community. Researchers believe that miRNAs are the next important class of therapeutic molecules after siRNA. These will have significant advantages over siRNAs due to many therapeutic applications.

The misregulation of several miRNAs is linked to the development of certain diseases in humans and other organisms [184]. It has been demonstrated that the restoration of misregulated miRNAs to their normal levels can reduce or even eliminate diseases including tumors in animal models [184]. Because miRNAs are naturally occurring molecules, there are certain advantages in their application as therapeutic agents. Worldwide researchers have validated the theory of "miRNA replacement therapy," which involves introducing synthetic miRNAs or miRNA mimetics into diseased tissues in an attempt to restore normal proliferation, apoptosis, cell cycle, and other cellular functions that have been affected by the misregulation of one or more miRNAs [185,186]. In contrast, some researchers have utilized miRNA inhibitors in an effort to increase the endogenous levels of therapeutic proteins [187]. Thus, in theory, inhibition of a specific miRNA linked to a given disease can remove the block of expression of a therapeutic protein. On the other hand, the administration of a miRNA mimetic can increase the endogenous miRNA population, therefore suppressing a harmful gene. In many cases, the reactivation or inhibition of these miRNA-regulated pathways leads to a significant therapeutic responses [188]. The pioneering groups of specialized pharmaceutical companies have initiated studies on creating viable therapeutic candidates with miRNA inhibitors and miRNA mimetics in diverse fields such as cancer, cardiovascular diseases, neurological disorders, and viral infections [185]. miRNAs are making their way in the pharmaceutical industry as therapeutic and diagnostic targets.

A miRNA-dependent posttranscriptional gene silencing process has been proven effective in organisms ranging from plants to nematodes and from fruit flies to humans at cell culture level. In 2008, a leading pharmaceutical company called Santaris announced the initiation of clinical trials with SPC3649, an LNA-based (locked nucleic acid) antisense molecule against miR-122, for the treatment of hepatitis C [185]. miR-122 has been found to affect hepatitis C virus (HCV) replication, which also has a role in cholesterol synthesis [189]. Due to these potential applications and its expression in the liver, miR-122 has become a favorite target for first-generation miRNA-based therapeutic development programs. Trials are already in progress and include 48 healthy volunteers to evaluate the safety of the drug and other factors. So far, the company has reported that results are encouraging, and phase 2 clinical trials in HCV patients are planned [190].

According to the National Cancer Institute (USA), liver cancer is the third most common cause of cancer deaths in men and the tenth most

Table 2
Plant miRNAs and their biological functions in different plant species.

miRNAs	Target gene	Biological functions	Species	Reference
miR156	SPL	Development transition time	*A. thaliana*	[171]
miR157	SPL	Developmental timing	*Gossypium hirsutum*	[177]
miR158	PP2	Unknown	*A. thaliana*	[72]
miR159	MYBTFS:GAMYB, MYB33	Floral identity and flower development	*A. thaliana*	[165]
miR160	ARF	Leaf and root development, auxin response, floral organ identity	*Glycine max*	[178]
miR162	DCL1		*A. thaliana*	[175]
miR164	NAC-TF: CUC1, CUC2	Shoot and root development	*A. thaliana*	[182]
miR164a	NAC-TF: CUC1, CUC2	Leaf development, patterning, and polarity	*A. thaliana*	[169]
miR164c	NAC-TF: CUC1, CUC2	Floral identity and flower development	*A. thaliana*	[183]
miR165/miR166	HD-ZIP, PHB	Meristem maintenance, vascular development and organ polarity	*A. thaliana*	[159]
miR167	ARF6 and 8	Auxin response	*A. thaliana*	[163]
miR168	AGO1		*A. thaliana*	[176]
miR170/171	SCL	Root development	*Populus trichocarpa*	[179]
miR172	AP2	Developmental timing and floral organ identity	*Oryza sativa*, *A. thaliana*	[172,180]
miR319	TCP	Leaf development	*A. thaliana*	[173]
miR319/JAW	BHLH TFS: TCPS	Leaf development, patterning and polarity		[173]
miR390	TAS3	Auxin response, developmental timing, lateral organ polarity	*Zea mays*	[181]
miR393	F-box protein: TIR1	Hormone signaling for plant development	*A. thaliana*	[157]
miR395	Sulfate transporter	Stress response	*O. sativa*	[156]
miR408	Plantacyanin, laccases	Stress response	*A. thaliana*	[157]

Table 3
Recent preclinical and clinical trials based on miRNA therapeutics.

Disease or condition	Trial title	Targeted status	Location	Government identifier[a] or reference
Asthma	miRNA analysis in premenstrual asthma	Unspecified	The Ohio State University, Columbus, United States	NCT00837395
Barrett's esophagus, esophageal adenocarcinoma	miRNA expression in upper gastroin-testinal mucosal tissue	Unspecified	Mayo Clinic, Florida, United States	NCT00909350
Cancer and liver infection	miR-34a mimetics	miRNA 34a and tumor p53 protein	Rosetta Genomics	[186]
Cancer, acute leukemia myelogenous	AML miRNA therapy	Unspecified	Mirna Therapeutic	185
Epstein–Barr virus and herpes simplex virus infection	Herpes virus therapy	Unspecified	Rosetta Genomics	[185]
HCV infection	Hepatitis C therapy	Unspecified	Rosetta Genomics	[185]
HCV infection	Anti-mir-122 oligo	miRNA122	Alnylam	[189]
HCV infection, hypercholesterolemia	SPC-3649	miRNA122	Santaris Pharma	[190]
Hepatitis C	miRNA-122 clinical course of patients with chronic HCV infection	Unspecified	National Taiwan University Hospital	NCT00980161
Healthy	Safety study of SPC3649 in healthy men	Unspecified	Hvidovre University Hospital, Denmark, Santaris Pharma	NCT00688012
Heart failure	miRNA inhibitors	miRNA 208a	Miragen Therapeutics	[185]
Heart failure	miRNA mimetics	Unspecified	Miragen Therapeutics	[185]
HIV/AIDS infection	HIV therapy	Unspecified	Rosetta Genomics	[185]
Inflammatory bowel disease	miRNA in inflammatory bowel disease	Unspecified	Tel Aviv Sourasky Medical Center, Israel	NCT00734331
Leukemia	Studying biomarkers in cell samples from patients with acute myeloid leukemia	Unspecified	National Cancer Institute (NCI)	NCT01057199
Lungs and non-small cell cancer	Osolo miRNA therapy	miRNA let-7a-1	Mirna Therapeutic	[185]
Melanoma	miRNA expression and function in cutaneous malignant melanoma	Unspecified	Rigshospitalet, Denmark	NCT00536029
Naevi malignant melanoma	Expression patterns of miRNA processing enzyme Dicer	Unspecified	Ruhr-University Bochum, Germany	NCT00862914
Pregnant women	miRNA profile in umbilical cord blood NK cells	Unspecified	National Taiwan University Hospital, Taiwan	NCT00751569
Prostate cancer	Prostate cancer miRNA	Unspecified	Mirna Therapeutics	[185]
Pulmonary arterial hypertension	Expression and significance of miRNA	Unspecified	The Ohio State University, Columbus, U.S.	NCT00806312
Renal cell carcinoma	miRNA expression in renal cell carcinoma	Unspecified	The 1st Affiliated Hospital, Sun Yet-sen University, China	NCT00743054
Sepsis	Circulating miRNAs as biomarkers of sepsis	Unspecified	Changhai Hospital, Shanghai, China	NCT00862290
Skin Cancer	Expression levels of miRNA processing enzymes Dicer and Drosha in epithelial skin cancer	Unspecified	Ruhr-University Bochum, Germany	NCT00849914
Transplant	CMV miRNA expression *in vivo* and Immune Evasion	Unspecified	University of Alberta Hospital, Canada	NCT00677482
Unspecified	Antagomirs	Unspecified	Alnylam	[185]
Unspecified	Anti-inflammatory miRNA	Unspecified	Alnylam	[185]
Unspecified	Anticancer miRNA	Unspecified	Alnylam	[185]

[a] Source: www.clinicaltrials.gov.

common in women. Rosetta Genomics has commenced in *in vivo* studies with its miRNA-based liver cancer therapeutic program in collaboration with Isis Pharmaceuticals. The project, joining Isis's widespread understanding of antisense chemistry and Rosetta Genomics' knowledge in miRNA technologies, is the companies' first attempt at exploring the role of miRNAs as master switches of the human body to treat cancer. In September 2008, Rosetta Genomics moved its miRNA-based liver cancer therapeutics project with Isis Pharmaceuticals to Regulus Therapeutics, a joint venture between Alnylam Pharmaceuticals and Isis Pharmaceuticals that is focused on the development of miRNA-based therapeutics [191,192].

Rosetta Genomics together with Columbia University Medical Center (CUMC) has proposed its first diagnostic test for regulatory approval. The test distinguishes between squamous and nonsquamous lung cancer [193]. GlaxoSmithKline and Regulus Therapeutics formed a strategic alliance for the development of novel miRNA-targeted drugs for inflammatory diseases [194]. Asuragen initiated the first ever miRNA-based diagnostic test. The test is designed to differentiate between pancreatic cancer and pancreatitis, which often have similar symptoms. Asuragen performed a trial in which 60 samples from patients were evaluated by the newly developed assay. The assay demonstrated remarkable results in distinguishing between the two conditions, in which 95% of the samples were accurately identified [195]. Miragen has announced plans to focus on identifying targets which are related to cardiovascular diseases, primarily relating to heart failure [196]. The recent preclinical and clinical trials based on miRNA therapeutics are summarized in Table 3.

The process of developing any drug is very expensive and is characterized by numerous hurdles with a very high chance of failure. The development of miRNA-targeted drugs is very challenging due to the lack of experience, and studies are still in their early stages. Although miRNA clinical trials are still in their infancy; nevertheless, the available data indicate the great potential of miRNAs in diagnosis and therapy.

8. Future prospectives

A large number of miRNAs and their functions have been discovered, and more are expected to be explored in the near future due to rapidly expanding sequencing power. Although the miRNA synthesis pathway in animals and plants has been well researched over the past decade, many questions have yet to be answered. Specifically, the precise structures of the complexes including the

Microprocessor, EXP5, HST, and Dicer-RISC in association with the targeted mRNA remain to be determined. The exact biochemical role of many factors associated with the miRNA biogenesis such as PACT, LOQS, HEN1, SE, and HYL1 have yet to be revealed. In addition, more protein factors associated with miRNA synthesis and mechanisms are expected to be determined in future. Moreover, the significance and enzymology of the modifications such as uridylation, adenylation, and methylation of miRNAs are still a mystery.

The majority of evolutionarily conserved miRNAs belong to multiple gene families, and one of the challenges is to understand the functional relationship among the members of the miRNA families. A large number of miRNAs have multiple target genes; therefore, researchers will have to determine the regulatory relationship between multiple members of a miRNA gene family and multiple target genes.

The scaling up of miRNAs from the laboratory to the pharmaceutical industry is ongoing. Whereas the swift technological developments to date are encouraging, there are still a number of risks associated with this research. For example, it is unclear whether successfully inhibiting miRNA in chronic diseases will have meaningful results. Typically, tissue culture cell lines express less miRNAs than tissues and thus may not be as rate limiting for disease treatment *in vivo*. Furthermore, there are additional general hurdles such as drug delivery to the right organs or tissues and choosing the appropriate technology to modulate the miRNA expression. These hurdles will certainly make the road towards miRNA therapeutics a very rough one; however, a number of therapeutic programs with similar initial problems have been proven to be successful.

References

[1] R.C. Lee, R.L. Feinbaum, V. Ambros, The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*, Cell 75 (1993) 843–854.

[2] B. Wightman, I. Ha, G. Ruvkun, Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*, Cell 75 (1993) 855–862.

[3] N.C. Lau, P.L. Lim, E.G. Weinstein, D.P. Bartel, An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*, Science 294 (2001) 858–862.

[4] R.C. Lee, V. Ambros, An extensive class of small RNAs in *Caenorhabditis elegans*, Science 294 (2001) 862–864.

[5] M. Lagos-Quintana, R. Rauhut, W. Lendeckel, T. Tuschl, Identification of novel genes coding for small expressed RNAs, Science 294 (2001) 853–858.

[6] C. Lu, S.S. Tej, S. Luo, C.D. Haudenschild, B.C. Meyers, P.J. Green, Elucidation of the small RNA component of the transcriptome, Science 309 (2005) 1567–1569.

[7] M. Margulies, M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J. Berka, M.S. Braverman, Y.J. Chen, Z. Chen, S.B. Dewell, L. Du, J.M. Fierro, X.V. Gomes, B.C. Godwin, W. He, S. Helgesen, C.H. Ho, G.P. Irzyk, S.C. Jando, M.L. Alenquer, T.P. Jarvie, K.B. Jirage, J.B. Kim, J.R. Knight, J.R. Lanza, J.H. Leamon, S.M. Lefkowitz, M. Lei, J. Li, K.L. Lohman, H. Lu, V.B. Makhijani, K.E. McDade, M.P. McKenna, E.W. Myers, E. Nickerson, J.R. Nobile, R. Plant, B.P. Puc, M.T. Ronan, G.T. Roth, G.J. Sarkis, J.F. Simons, J.W. Simpson, M. Srinivasan, K.R. Tartaro, A. Tomasz, K.A. Vogt, G.A. Volkmer, S.H. Wang, Y. Wang, M.P. Weiner, P. Yu, R.F. Begley, J.M. Rothberg, Genome sequencing in microfabricated high-density picolitre reactors, Nature 437 (2005) 376–380.

[8] E.C. Lai, P. Tomancak, R.W. Williams, G.M. Rubin, Computational identification of *Drosophila* MicroRNA genes, Genome Biol. 4 (2003) R42.

[9] J.W. Nam, K.R. Shin, J. Han, Y. Lee, V.N. Kim, B.T. Zhang, Human microRNA prediction through a probabilistic co-learning model of sequence and structure, Nucleic Acids Res. 33 (2005) 3570–3581.

[10] S.C. Li, C.Y. Pan, W.C. Lin, Bioinformatics discovery of microRNA precursor from human ESTs and introns, BMC Genomics 7 (2006) 164.

[11] T.H. Huang, B. Fan, F.M. Rothschild, Z.L. Hu, K. Li, S.H. Zhao, MiR Finder: an improved approach and software implementation for genome-wide fast microRNA precursor scans, BMC Bioinform. 8 (2007) 341.

[12] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (2004) 522–531.

[13] V.N. Kim, J. Han, M.C. Siomi, Biogenesis of small RNAs in animals, Nat. Rev. Mol. Biol. 10 (2009) 126–139.

[14] W.M. Jones-Rhoades, D.P. Bartel, B. Bartel, MicroRNAs and their regulatory roles in plants, Annu. Rev. Plant Biol. 57 (2006) 19–53.

[15] V. Ambros, B. Bartel, D.P. Bartel, C.B. Burge, J.C. Carrington, X. Chen, G. Dreyfuss, S.R. Eddy, S. Griffiths-Jones, M. Marshall, M. Matzke, G. Ruvkun, T. Tuschl, A uniform system for microRNA annotation, RNA-Publ. RNA Soc. 9 (2003) 277–279.

[16] B.J. Reinhart, F.J. Slack, M. Basson, J.C. Bettinger, A.E. Pasquinelli, A.E. Rougvie, H.R. Horvitz, G. Ruvkun, The 21 nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*, Nature 403 (2000) 901–906.

[17] F.J. Slack, M. Basson, Z. Liu, V. Ambros, H.R. Horvitz, G. Ruvkun, The *lin-41* RBCC gene acts in the *C. elegans* heterochronic pathway between the *let-7* regulatory RNA and the LIN-29 transcription factor, Mol. Cell. Biol. 5 (2000) 659–669.

[18] A.E. Pasquinelli, B.J. Reinhart, F. Slack, M.Q. Martindale, M.I. Kuroda, B. Maller, D.C. Hayward, E.E. Ball, B. Degnan, P. Müller, J. Spring, A. Srinivasan, M. Fishman, J. Finnerty, J. Corbo, M. Levine, P. Leahy, E. Davidson, G. Ruvkun, Conservation across animal phylogeny of the sequence and temporal regulation of the 21 nucleotide let-7 heterochronic regulatory RNA, Nature 408 (2000) 86–89.

[19] A. Grimso, M. Srivastava, B. Fahey, B.J. Woodcroft, R.H. Chiang, N. King, B.M. Degnan, D.S. Rokhsar, D.P. Bartel, Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals, Nature 455 (2008) 1193–1197.

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

[21] X. Cai, C.H. Hagedorn, B.R. Cullen, Human MicroRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs, RNA-Publ. RNA Soc. 10 (2004) 1957–1966.

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

[23] G.M. Borchert, W. Lanier, B.L. Davidson, RNA polymerase III transcribes human microRNAs, Nat. Struct. Mol. Cell Biol. 13 (2006) 1097–1101.

[24] Y. Lee, K. Jeon, J.T. Lee, S. Kim, V.N. Kim, MicroRNA maturation: stepwise processing and subcellular localization, EMBO J. 21 (2002) 4663–4670.

[25] Y. Zeng, B.R. Cullen, Sequence requirements for micro RNA processing and function in human cells, RNA-Publ. RNA Soc. 9 (2003) 112–123.

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

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

[28] R.I. Gregory, K.P. Yan, G. Amuthan, T. Chendrimada, B. Doratotaj, N. Cooch, R. Shiekhattar, The Microprocessor complex mediates the genesis of microRNAs, Nature 432 (2004) 235–240.

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

[30] Y. Wang, R. Medvid, C. Melton, R. Jaenisch, R. Blelloch, DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal, Nat. Genet. 39 (2007) 380–385.

[31] V. Filippov, V. Solovyev, M. Filippova, S.S. Gill, A novel type of RNase III family proteins in eukaryotes, Gene 245 (2000) 213–221.

[32] H. Wu, H. Xu, L.J. Miraglia, S.T. Crooke, Human RNase III is a 160-kDa protein involved in preribosomal RNA processing, J. Biol. Chem. 275 (2000) 36957–36965.

[33] K.R. Fortin, R.H. Nicholson, A.W. Nicholson, Mouse ribonuclease III cDNA structure, expression analysis, and chromosomal location, BMC Genomics 3 (2002) 26.

[34] J. Han, Y. Lee, K.H. Yeom, J.W. Nam, I. Heo, J.K. Rhee, S.Y. Sohn, Y. Cho, B.T. Zhang, V.N. Kim, Molecular basis for the recognition of primary microRNAs by the Drosha–DGCR8 complex, Cell 125 (2006) 887–901.

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

[36] S. Nakielny, G. Dreyfuss, Transport of proteins and RNAs in and out of the nucleus, Cell 99 (1999) 677–690.

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

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

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

[40] C. Gwizdek, B.O. Nazari, A.M. Brownawell, A. Doglio, E. Bertrand, L.G. Macara, C. Dargemont, Exportin-5 mediates nuclear export of minihelix-containing RNAs, J. Biol. Chem. 278 (2003) 5505–5508.

[41] E. Basyuk, F. Suavet, A. Doglio, R. Bordonne, E. Bertrand, Human let stem-loop precursors harbor features of RNase III cleavage products, Nucleic Acids Res. 31 (2003) 6593–6597.

[42] Y. Zeng, B.R. Cullen, Structural requirements for pre-microRNA binding and nuclear export by Exportin 5, Nucleic Acids Res. 32 (2004) 4776–4785.

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

[44] G. Hutvagner, J. McLachlan, A.E. Pasquinelli, E. Bálint, T. Tuschl, P.D. Zamore, A cellular function for the RNAinterference enzyme Dicer in the maturation of the let 7 small temporal RNA, Science 293 (2001) 834–838.

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

[46] S.W. Knight, B.L. Bass, A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*, Science 293 (2001) 2269–2271.

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

[48] H. Tabara, E. Yigit, H. Siomi, C. Mello, The dsRNA binding protein RDE-4 interacts with RDE-1, DCR-1, and a DExH-box helicase to direct RNAi in *C. elegans*, Cell 109 (2002) 861–871.
[49] Q. Liu, T.A. Rand, S. Kalidas, F. Du, H.E. Kim, D.P. Smith, X. Wang, R2D2, a bridge between the initiation and effector steps of the *Drosophila* RNAi pathway, Science 301 (2003) 1921–1925.
[50] S.M. Hammond, S. Boettcher, A.A. Caudy, R. Kobayashi, G.J. Hannon, Argonaute 2, a link between genetic and biochemical analyses of RNAi, Science 293 (2001) 1146–1150.
[51] M.A. Carmell, Z. Xuan, M.Q. Zhang, G.J. Hannon, The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis, Genes Dev. 16 (2002) 2733–2742.
[52] K. Forstemann, Y. Tomari, T. Du, V.V. Vagin, A.M. Denli, D.P. Bratu, C. Klattenhoff, W.E. Theurkauf, P.D. Zamore, Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein, PLoS Biol. 3 (2005) e236.
[53] F. Jiang, X. Ye1, X. Liu, L. Fincher, D. McKearin, Q. Liu, Dicer-1 and R3D1-L catalyze microRNA maturation in *Drosophila*, Genes Dev. 19 (2005) 1674–1679.
[54] K. Saito, A. Ishizuka, H. Siomi, M.C. Siomi, Processing of pre-microRNAs by the Dicer-1–Loquacious complex in *Drosophila* cells, PLoS Biol. 3 (2005) e235.
[55] T.P. Chendrimada, R.I. Gregory, E. Kumaraswamy, J. Norman, N. Cooch, K. Nishikura, R. Shiekhattar, TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing, Nature 436 (2005) 740–744.
[56] A.D. Haase, L. Jaskiewicz, H. Zhang, S. Laine, R. Sack, A. Gatignol, W. Filipowicz, TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing, EMBO Rep. 6 (2005) 961–967.
[57] Y. Lee, I. Hur, S.Y. Park, Y.K. Kim, M.R. Suh, V.N. Kim, The role of PACT in the RNA silencing pathway, EMBO J. 25 (2006) 522–532.
[58] A. Khvorova, A. Reynolds, S.D. Jayasena, Functional siRNAs and miRNAs exhibit strand bias, Cell 115 (2003) 209–216.
[59] E. Maniataki, Z. Mourelatos, A human, ATP-independent, RISC assembly machine fueled by pre-miRNA, Genes Dev. 19 (2005) 2979–2990.
[60] I.J. MacRae, E. Ma, M. Zhou, C.V. Robinson, J.A. Doudna, *In vitro* reconstitution of the human RISC-loading complex, Proc. Natl Acad. Sci. USA 105 (2008) 512–517.
[61] R.I. Gregory, T.P. Chendrimada, N. Cooch, R. Shiekhattar, Human RISC couples microRNA biogenesis and posttranscriptional gene silencing, Cell 123 (2005) 631–640.
[62] Y. Tomari, C. Matranga, B. Haley, N. Martinez, P.D. Zamore, A protein sensor for siRNA asymmetry, Science 306 (2004) 1377–1380.
[63] S. Diederichs, D.A. Haber, Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression, Cell 131 (2007) 1097–1108.
[64] C. Llave, K.D. Kasschau, M.A. Rector, J.C. Carrington, Endogenous and silencing-associated small RNAs in plants, Plant Cell 14 (2002) 1605–1619.
[65] M.F. Mette, J.V.D. Winden, M. Matzke, A.J. Matzke, Short RNAs can identify new candidate transposable element families in *Arabidopsis*, Plant Physiol. 130 (2002) 6–9.
[66] X. Chen, MicroRNA metabolism in plants, Curr. Top. Microbiol. Immunol. 20 (2008) 117–136.
[67] C. Maher, L. Stein, D. Ware, Evolution of *Arabidopsis* microRNA families through duplication events, Genome Res. 16 (2006) 510–519.
[68] T. Arazi, M. Talmor-Neiman, R. Stav, M. Riese, P. Huijser, D.C. Baulcombe, Cloning and characterization of micro-RNAs from moss, Plant J. 43 (2005) 837–848.
[69] M.J. Axtell, D.P. Bartel, Antiquity of microRNAs and their targets in land plants, Plant Cell 17 (2005) 1658–1673.
[70] Z. Xie, E. Allen, N. Fahlgren, A. Calamar, S.A. Givan, J.C. Carrington, Expression of *Arabidopsis* MIRNA genes, Plant Physiol. 138 (2005) 2145–2154.
[71] M. Megraw, V. Baev, V. Rusinov, S.T. Jensen, K. Kalantidis, A.G. Hatzigeorgiou, MicroRNA promoter element discovery in *Arabidopsis*, RNA-Publ. RNA Soc. 12 (2006) 1612–1619.
[72] B.J. Reinhart, E.G. Weinstein, M.W. Rhoades, B. Bartel, D.P. Bartel, MicroRNAs in plants, Genes Dev. 16 (2002) 1616–1626.
[73] I. Papp, M.F. Mette, W. Aufsatz, L. Daxinger, S.E. Schauer, A. Ray, J.V.D. Winden, M. Matzke, A.J. Matzke, Evidence for nuclear processing of plant micro RNA and short interfering RNA precursors, Plant Physiol. 132 (2003) 1382–1390.
[74] A. Ray, J.D. Lang, T. Golden, S. Ray, SHORT INTEGUMENT (SIN1), a gene required for ovule development in *Arabidopsis*, also controls flowering time, Development 122 (1996) 2631–2638.
[75] S.E. Jacobsen, M. Running, E.M. Meyerowitz, Disruption of an RNA helicase/RNAse III gene in *Arabidopsis* causes unregulated cell division in floral meristems, Development 126 (1999) 5231–5243.
[76] W. Park, J. Li, R. Song, J. Messing, X. Chen, CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*, Curr. Biol. 12 (2002) 1484–1495.
[77] C. Lu, N. Fedoroff, A mutation in the *Arabidopsis* HYL1 gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin, Plant Cell 12 (2000) 2351–2366.
[78] A. Hiraguri, R. Itoh, N. Kondo, Y. Nomura, D. Aizawa, Y. Murai, H. Koiwa, M. Seki, K. Shinozaki, T. Fukuhara, Specific interactions between Dicer-like proteins and HYL1/DRB-family dsRNA-binding proteins in *Arabidopsis thaliana*, Plant Mol. Biol. 57 (2005) 173–188.
[79] D. Lobbes, G. Rallapalli, D.D. Schmidt, C. Martin, J. Clarke, SERRATE: a new player on the plant microRNA scene, EMBO Rep. 7 (2006) 1052–1058.
[80] L. Yang, Z. Liu, F. Lu, A. Dong, H. Huang, SERRATE is a novel nuclear regulator in primary microRNA processing in *Arabidopsis*, Plant J. 47 (2006) 841–850.
[81] Z. Yang, Y.W. Ebright, B. Yu, X. Chen, HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group onto the 2′ OH of the 3′ terminal nucleotide, Nucleic Acids Res. 34 (2006) 667–675.
[82] B. Yu, Z. Yang, J. Li, S. Minakhina, M. Yang, R.W. Padgett, R. Steward, X. Chen, Methylation as a crucial step in plant microRNA biogenesis, Science 307 (2005) 932–935.
[83] F. Wahid, T. Khan, K.H. Hwang, Y.Y. Kim, Piwi-interacting RNAs (piRNAs) in animals: the story so far, Afr. J. Biotechnol. 8 (2009) 4002–4006.
[84] J.J. Song, S.K. Smith, G.J. Hannon, L. Joshua-Tor, Crystal structure of Argonaute and its implications for RISC slicer activity, Science 305 (2004) 1434–1437.
[85] D.S. Schwarz, G. Hutvagner, T. Du, Z. Xu, N. Aronin, P.D. Zamore, Asymmetry in the assembly of the RNAi enzyme complex, Cell 115 (2003) 199–208.
[86] A. Herr, Pathways through the small RNA world of plants, FEBS Lett. 579 (2005) 5879–5888.
[87] K.M. Bollman, M.J. Aukerman, M.Y. Park, C. Hunter, T.Z. Berardini, R.S. Poethig, HASTY the *Arabidopsis* ortholog of exportin 5/MSN5, regulates phase change and morphogenesis, Development 130 (2003) 1493–1504.
[88] M.Y. Park, G. Wu, A. Gonzalez-Sulser, H. Vaucheret, R.S. Poethig, Nuclear processing and export of microRNAs in *Arabidopsis*, Proc. Natl Acad. Sci. USA 102 (2005) 3691–3696.
[89] G. Meister, M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng, T. Tuschl, Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs, Mol. Cell 15 (2004) 185–197.
[90] A. Telfer, R.S. Poethig, HASTY: a gene that regulates the timing of shoot maturation in *Arabidopsis thaliana*, Development 125 (1998) 1889–1898.
[91] R.S. Pillai, C.G. Artus, W. Filipowicz, Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis, RNA-Publ. RNA Soc. 10 (2004) 1518–1525.
[92] W.C. Richard, J.S. Erik, Origins and mechanisms of miRNAs and siRNAs, Cell 136 (2009) 642–655.
[93] C. Llave, Z. Xie, K.D. Kasschau, J.C. Carrington, Cleavage of scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA, Science 297 (2002) 2053–2056.
[94] G. Tang, B.J. Reinhart, D.P. Bartel, P.D. Zamore, A biochemical framework for RNA silencing in plants, Genes Dev. 17 (2003) 49–63.
[95] D.T. Humphreys, B.J. Westman, D.I. Martin, T. Preiss, Micro-RNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly (A) tail function, Proc. Natl Acad. Sci. USA 102 (2005) 16961–16966.
[96] M. Kiriakidou, G.S. Tan, S. Lamprinaki, M.D. Planell-Saguer, P.T. Nelson, Z. Mourelatos, An mRNA m7G cap binding-like motif within human Ago2 represses translation, Cell 129 (2007) 1141–1151.
[97] G. Mathonnet, M.R. Fabian, Y.V. Svitkin, A. Parsyan, L. Huck, T. Murata, S. Biffo, W.C. Merrick, E. Darzynkiewicz, R.S. Pillai, MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F, Science 317 (2007) 1764–1767.
[98] X.C. Ding, H. Grosshans, Repression of *C. elegans* microRNA targets at the initiation level of translation requires GW182 proteins, EMBO J. 28 (2009) 213–222.
[99] K. Seggerson, L. Tang, E.G. Moss, Two genetic circuits repress the *Caenorhabditis elegans* heterochronic gene *lin-28* after translation initiation, Dev. Biol. 243 (2002) 215–225.
[100] P.A. Maroney, Y. Yu, J. Fisher, T.W. Nilsen, Evidence that micro-RNAs are associated with translating messenger RNAs in human cells, Nat. Struct. Mol. Biol. 13 (2006) 1102–1107 1.
[101] C.P. Petersen, M.E. Bordeleau, J. Pelletier, P.A. Sharp, Short RNAs repress translation after initiation in mammalian cells, Mol. Cell 21 (2006) 533–542.
[102] R. Thermann, M.W. Hentze, *Drosophila* miR2 induces pseudo-polysomes and inhibits translation initiation, Nature 447 (2007) 875–878.
[103] A. Eulalio, E. Huntzinger, E. Izaurralde, GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay, Nat. Struct. Mol. Biol. 15 (2008) 346–353.
[104] I. Behm-Ansmant, J. Rehwinkel, T. Doerks, A. Stark, P. Bork, E. Izaurralde, mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes, Genes Dev. 20 (2006) 1885–1898.
[105] A.J. Giraldez, Y. Mishima, J. Rihel, R.J. Grocock, S.V. Dongen, K. Inoue, A.J. Enright, A.F. Schier, Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs, Science 312 (2006) 75–79.
[106] L. Wu, J. Fan, J.G. Belasco, MicroRNAs direct rapid deadenylation of mRNA, Proc. Natl Acad. Sci. USA 103 (2006) 4034–4039.
[107] M. Wakiyama, K. Takimoto, O. Ohara, S. Yokoyama, Let-7microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system, Genes Dev. 21 (2007) 1857–1862.
[108] B. Yongli, S. Christopher, C. Yueh-Chin, A.C. Martine, L. Hai-Yan, L.D. Clyde, The CCR4 and CAF1 proteins of the CCR4–NOT complex are physically and functionally separated from NOT2, NOT4, and NOT5, Mol. Cell. Biol. 10 (1999) 6642–6651.
[109] L. Hai-Yan, B. Vasudeo, C.A. Deborah, R. Juri, M. Matthias, L.D. Clyde, The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively, EMBO J. 17 (1998) 1096–1106.
[110] R.S. Pillai, S.N. Bhattacharyya, C.G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand, W. Filipowicz, Inhibition of translational initiation by Let-7 microRNA in human cells, Science 309 (2005) 1573–1576.
[111] T.P. Chendrimada, K.J. Finn, X. Ji, D. Baillat, R.I. Gregory, S.A. Liebhaber, A.E. Pasquinelli, R. Shiekhattar, MicroRNA silencing through RISC recruitment of eIF6, Nature 447 (2007) 823–828.
[112] Y. Wang, S. Juranek, H. Li, G. Sheng, T. Tuschl, D.J. Patel, Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex, Nature 456 (2008) 921–926.
[113] M.A. Valencia-Sanchez, J. Liu, G.J. Hannon, R. Parker, Control of translation and mRNA degradation by miRNAs and siRNAs, Genes Dev. 20 (2006) 515–524.

[114] S. Bagga, J. Bracht, S. Hunter, K. Massirer, J. Holtz, R. Eachus, A.E. Pasquinelli, Regulation by *let-7* and *lin-4* miRNAs results in target mRNA degradation, Cell 122 (2005) 553–563.
[115] L.P. Lim, N.C. Lau, P. Garrett-Engele, A. Grimson, J.M. Schelter, J. Castle, D.P. Bartel, P.S. Linsley, J.M. Johnson, Microarray analysis shows that some microRNAs down-regulate large numbers of target mRNAs, Nature 433 (2005) 769–773.
[116] L.M. Aleman, J. Doench, P.A. Sharp, Comparison of siRNA induced off-target RNA and protein effects, RNA-Publ. RNA Soc. 13 (2007) 385–395.
[117] M. Kato, F.J. Dlacj, MicroRNAs: small molecules with big roles—*C. elegans* to human cancer, Biol. Cell 100 (2008) 71–81.
[118] B.J. Reinhart, F.J. Slack, M. Basson, A.E. Pasquinelli, J.C. Bettinger, The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*, Nature 403 (2000) 901–906.
[119] M. Boehm, F. Slack, A developmental timing microRNA and its target regulate life span in *C. elegans*, Science 310 (2005) 1954–1957.
[120] S.Y. Lin, S.M. Johnson, M. Abraham, M.C. Vella, A. Pasquinelli, The *C. elegans* hunchback homolog, *hbl-1*, controls temporal patterning and is a probable microRNA target, Dev. Cell 4 (2003) 639–650.
[121] R.J. Johnston, O. Hobert, A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*, Nature 426 (2003) 845–849.
[122] S. Chang, R.J. Johnston Jr, C. Frøkjær-Jensen, S. Lockery, O. Hobert, MicroRNAs actsequentially and asymmetrically to control chemosensory laterality in the nematode, Nature 430 (2004) 785–798.
[123] R. Nolo, C.M. Morrison, C. Tao, X. Zhang, G. Halder, The *bantam* microRNA is a target of the hippo tumor-suppressor pathway, Curr. Biol. 16 (2006) 1895–1904.
[124] P. Xu, S.Y. Vernooy, M. Guo, B.A. Hay, The *Drosophila* microRNA *mir-14* suppresses cell death and is required for normal fat metabolism, Curr. Biol. 13 (2003) 790–795.
[125] A. Stark, J. Brennecke, R.B. Russell, S.M. Cohen, Identification of *Drosophila* microRNA targets, PLoS Biol. 1 (2003) 1–13.
[126] E.C. Lai, B. Tam, G.M. Rubin, Pervasive regulation of *Drosophila* Notch target genes by GY-box, Brd-box, and Kbox- class microRNAs, Genes Dev. 19 (2005) 1067–1080.
[127] E. Wienholds, M.J. Koudijs, E.F.J. Van, E. Cuppen, R.H. Plasterk, The microRNA-producing enzyme Dicer1 is essential for zebrafish development, Nat. Genet. 35 (2003) 217–218.
[128] A.J. Giraldez, R.M. Cinalli, M.E. Glasner, A.J. Enright, J.M. Thomson, S. Baskerville, S.M. Hammond, D.P. Bartel, A.F. Schier, MicroRNAs regulate brain morphogenesis in zebrafish, Science 308 (2005) 833–838.
[129] T. Watanabe, A. Takeda, K. Mise, T. Okuno, T. Suzuki, N. Minami, H. Imai, Stage-specifi expression of microRNAs during *Xenopus* development, FEBS Lett. 579 (2005) 318–324.
[130] S. Yekta, I.H. Shih, D.P. Bartel, MicroRNA-directed cleavage of *HOXB8* mRNA, Science 304 (2004) 594–596.
[131] E. Hornstein, J.H. Mansfield, S. Yekta, J.K. Hu, B.D. Harfe, The microRNA miR-196 acts upstream of Hoxb8 and Shh in limb development, Nature 438 (2005) 671–674.
[132] Y. Zhao, E. Samal, D. Srivastava, Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis, Nature 436 (2005) 214–220.
[133] C.Z. Chen, L. Li, H.F. Lodish, D.P. Bartel, MicroRNAs modulate hematopoietic lineage differentiation, Science 303 (2004) 83–86.
[134] C. Esau, X. Kang, E. Peralta, E. Hanson, E.G. Marcusson, L.V. Ravichandran, Y. Sun, S. Koo, R.J. Perera, R. Jain, N.M. Dean, S.M. Freier, C.F. Bennett, B. Lollo, R. Griffey, MicroRNA-143 regulates adipocyte differentiation, J. Biol. Chem. 279 (2004) 52361–52365.
[135] B.P. Lewis, I.H. Shih, M.W. Jones-Rhoades, D.P. Bartel, C.B. Burge, Prediction of mammalian microRNA targets, Cell 115 (2003) 787–798.
[136] M.N. Poy, L. Eliasson, J. Krutzfeldt, S. Kuwajima, X. Ma, P.E. Macdonald, S. Pfeffer, T. Tuschl, N. Rajewsky, P. Rorsman, M. Stoffel, A pancreatic islet-specific microRNA regulates insulin secretion, Nature 432 (2004) 226–230.
[137] E. Wienholds, W.P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, D.E. Bruijn, H.R. Horvitz, S. Kauppinen, R.H. Plasterk, MicroRNA expression in zebrafish embryonic development, Science 309 (2005) 310–311.
[138] Q. Jing, S. Huang, S. Guth, T. Zarubin, A. Motoyama, J. Chen, D.F. Padova, S.C. Lin, H. Gram, J. Han, Involvement of microRNA in AU-rich element-mediated mRNA instability, Cell 120 (2005) 623–634.
[139] T.H. Thai, D.P. Calado, S. Casola, K.M. Ansel, C. Xiao, Y. Xue, A. Murphy, D. Frendewey, D. Valenzuela, J.L. Kutok, M. Schmidt-Supprian, N. Rajewsky, G. Yancopoulos, A. Rao, K. Rajewsky, Regulation of the germinal center response by microRNA-155, Science 316 (2007) 604–608.
[140] E. Vigorito, K.L. Perks, C. Abreu-Goodger, S. Bunting, Z. Xiang, S. Kohlhaas, P.P. Das, E.A. Miska, A. Rodriguez, A. Bradley, K.G. Smith, C. Rada, A.J. Enright, K.M. Toellner, I.C. Maclennan, M. Turner, MicroRNA-155 regulates the generation of immunoglobulin classswitched plasma cells, Immunity 27 (2007) 847–859.
[141] A. Rodriguez, E. Vigorito, S. Clare, V.M. Warren, P. Couttet, R.D. Soond, V.S. Dongen, J.S. Grocock, P.P. Das, A.E. Miska, D. Vetrie, K. Okkenhaug, J.A. Enright, G. Dougan, M. Turner, A. Bradley, Requirement of bic/microRNA-155 for normal immune function, Science 316 (2007) 608–611.
[142] C.H. Lecellier, P. Dunoyer, K. Arar, J. Lehmann-Che, S. Eyquem, C. Himber, A. Saib, O. Voinnet, A cellular microRNA mediates antiviral defense in human cells, Science 308 (2005) 557–560.
[143] G.A. Calin, C.D. Dumitru, M. Shimizu, R. Bichi, S. Zupo, Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia, Proc. Natl Acad. Sci. USA 99 (2002) 15524–15529.
[144] A. Cimmino, G.A. Calin, M. Fabbri, M.V. Iorio, M. Ferracin, miR-15 and miR-16 induce apoptosis by targeting BCL2, Proc. Natl Acad. Sci. USA 102 (2005) 13944–13949.
[145] L. He, J.M. Thomson, M.T. Hemann, E. Hernando-Monge, D. Mu, A microRNA polycistron as a potential human oncogene, Nature 435 (2005) 828–833.
[146] P.M. Voorhoeve, C. le Sage, M. Schrier, A.J. Gillis, H. Stoop, A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors, Cell 124 (2006) 1169–1181.
[147] E.G. Moss, R.C. Lee, V. Ambros, The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the *lin-4* RNA, Cell 88 (1997) 637–646.
[148] J.E. Abrahante, A.L. Daul, M. Li, M.L. Volk, J.M. Tennessen, E.A. Miller, A.E. Rougvie, The *Caenorhabditis elegans* hunchback-like gene lin-57/hbl-1 controls developmental time and is regulated by microRNAs, Dev. Cell 4 (2003) 625–637.
[149] J. Brennecke, D.R. Hipfner, A. Stark, R.B. Russell, S.M. Cohen, *bantam* encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene *hid* in *Drosophila*, Cell 113 (2003) 25–36.
[150] F. Fazi, A. Rosa, A. Fatica, V. Gelmetti, M.L. De Marchis, C. Nervi, I. Bozzoni, A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPα regulates human granulopoiesis, Cell 123 (2005) 819–831.
[151] T.C. Chang, D. Yu, S.Y. Lee, A.E. Wentzel, E.D. Arking, M.K. West, V.C. Dang, T.A. Tikhonenko, T.J. Mendell, Widespread microRNA repression by Myc contributes to tumorigenesis, Nat. Genet. 40 (2008) 43–50.
[152] S.L. Lin, A. Chiang, D. Chang, S.Y. Ying, Loss of mir-146a function in hormone refractory prostate cancer, RNA-Publ. RNA Soc. 14 (2008) 417–424.
[153] C.S. Sullivan, A.T. Grundhoff, S. Tevethia, J.M. Pipas, D. Ganem, SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells, Nature 435 (2005) 682–686.
[154] M.Z. Michael, S.M. O, Connor, H.P.N.G. Van, G.P. Young, R.J. James, Reduced accumulation of specific microRNAs in colorectal neoplasia, Mol. Cancer Res. 1 (2003) 882–891.
[155] K.A. O'Donnell, E.A. Wentzel, K.I. Zeller, C.V. Dang, J.T. Mendell, c-Myc-regulated microRNAs modulate E2F1 expression, Nature 435 (2005) 839–843.
[156] M.W. Rhoades, D.P. Bartel, Computational identification of plant micro-RNAs and their targets, including a stress-induced miRNA, Mol. Cell 14 (2004) 787–799.
[157] R. Sunkar, J.K. Zhu, Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*, Plant Cell 16 (2004) 2001–2019.
[158] G. Damien, miRacle in plant development: role of microRNAs in cell differentiation and patterning, Semin. Cell Dev. Biol. 19 (2008) 586–595.
[159] J. Kim, J.H. Jung, J.L. Reyes, Y.S. Kim, S.Y. Kim, K.S. Chung, MicroRNA-directed cleavage of ATHB15mRNA regulates vascular development in *Arabidopsis* inflorescence stems, Plant J. 42 (2005) 84–94.
[160] M.T. Juarez, J.S. Kui, J. Thomas, B.A. Heller, M.C. Timmermans, MicroRNA-mediated repression of rolled leaf1 specifies maize leaf polarity, Nature 428 (2004) 84–88.
[161] M.W. Rhoades, B.J. Reinhart, L.P. Lim, C.B. Burge, B. Bartel, D.P. Bartel, Prediction of plant microRNA targets, Cell 110 (2002) 513–520.
[162] P. Ru, L. Xu, H. Ma, H. Huang, Plant fertility defects induced by the enhanced expression of microRNA167, Cell Res. 16 (2006) 457–465.
[163] M.F. Wu, Q. Tian, J.W. Reed, *Arabidopsis* microRNA167 controls patterns of ARF6 and ARF8 expression, and regulates both female and male reproduction, Development 133 (2006) 4211–4421.
[164] D.E. Richards, K.E. King, T. Ait-Ali, N.P. Harberd, How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 67–88.
[165] P. Achard, A. Herr, D.C. Baulcombe, N.P. Harberd, Modulation of floral development by a gibberellin-regulated microRNA, Development 131 (2004) 3357–3365.
[166] R. Schwab, J.F. Palatnik, M. Riester, C. Schommer, M. Schmid, D. Weigel, Specific effects of microRNAs on the plant transcriptome, Dev. Cell 8 (2005) 517–527.
[167] A.A. Millar, F. Gubler, The *Arabidopsis* GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development, Plant Cell 17 (2005) 705–721.
[168] R.S. Allen, J. Li, M.I. Stahle, A. Dubroue, F. Gubler, A.A. Millar, Genetic analysis reveals functional redundancy and the major target genes of the *Arabidopsis* miR159 family, Proc. Natl Acad. Sci. USA 104 (2007) 16371–16376.
[169] K. Nikovics, T. Blein, A. Peaucelle, T. Ishida, H. Morin, M. Aida, P. Laufs, The balance between the MIR164A and CUC2 genes controls leaf margin serration in *Arabidopsis*, Plant Cell 18 (2006) 2929–2945.
[170] H.S. Guo, Q. Xie, J.F. Fei, N.H. Chua, MicroRNA directs mRNA cleavage of the transcription factor NAC1 to down-regulate auxin signals for *Arabidopsis* lateral root development, Plant Cell 17 (2005) 1376–1386.
[171] J.W. Wang, R. Schwab, B. Czech, E. Mica, D. Weigel, Dual effects of miR156-targeted SPL genes and CYP78A5/KLUH on plastochron length and organ size in *Arabidopsis thaliana*, Plant Cell 20 (2008) 1231–1243.
[172] M.J. Aukerman, H. Sakai, Regulation of flowering time and floral organ identity by a microRNA and its *APETALA2-like* target genes, Plant Cell 15 (2003) 2730–2741.
[173] J.F. Palatnik, E. Allen, X. Wu, C. Schommer, R. Schwab, J.C. Carrington, D. Weigel, Control of leaf morphogenesis by microRNAs, Nature 425 (2003) 257–263.
[174] S. Kepinski, O. Leyser, The *Arabidopsis* F-box protein TIR1 is an auxin receptor, Nature 435 (2005) 446–451.
[175] Z. Xie, K.D. Kasschau, J.C. Carrington, Negative feedback regulation of *Dicer-Like1* in *Arabidopsis* by microRNA-guided mRNA degradation, Curr. Biol. 13 (2003) 784–789.
[176] H. Vaucheret, F. Vazquez, P. Crete, D.P. Bartel, The action of *ARGONAUTE1* in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development, Genes Dev. 18 (2004) 1187–1197.
[177] C.X. Qiu, F.L. Xie, Y.Y. Zhu, K. Guo, S.Q. Huang, L. Nie, Z.M. Yang, Computational identification of microRNAs and their targets in *Gossypium hirsutum* expressed sequence tags, Gene 395 (2007) 49–61.

[178] T. Dezulian, J.F. Palatnik, D. Huson, D. Weigel, Conservation and divergence of microRNA families in plants, Genome Biol. 6 (2005) P13.
[179] S. Lu, Y.H. Sun, R. Shi, C. Clark, L. Li, V.L. Chiang, Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*, Plant Cell 17 (2005) 2186–2203.
[180] X. Chen, A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development, Science 303 (2004) 2022–2025.
[181] B. Zhang, X. Pan, T.A. Anderson, Identification of 188 conserved maize microRNAs and their targets, FEBS Lett. 580 (2006) 3753–3762.
[182] H.S. Guo, Q. Xie, J.F. Fei, N.H. Chua, MicroRNA directs mRNA cleavage of the transcription factor NAC1 to down-regulate auxin signals for *Arabidopsis* lateral root development, Plant Cell 17 (2005) 1376–1386.
[183] C.C. Baker, P. Sieber, F. Wellmer, E.M. Meyerowitz, The early extra petals1 mutant uncovers a role for microRNA miR164c in regulating petal number in *Arabidopsis*, Curr. Biol. 15 (2005) 303–315.
[184] G. Vicki, Tapping miRNA-regulated pathways, Genet. Eng. Biotechnol. News 28 (2008) No.5.
[185] B. Janet, H. Amir, Therapy analysis—microRNA; update analysis, Pharmaprojects 29 (2008).
[186] R.S. Nina, E. Marciano, E. Meiri, Y. Spector, N. Rosenfeld, N. Moskovits, Z. Bentwich, M. Oren, Transcriptional activation of miR-34a contributes to p53-mediated apoptosis, Mol. Cell 26 (2007) 731–743.
[187] S.M. George, MicroRNA gets down to business, Nat. Biotechnol. 25 (2007) 631–638.
[188] E.C. Maria, O. Teague, H. Kylie, D.H. Van, B. Mark, D.H. Van, N. Perry, P. Wagaarachch, A.R. Sarah, C.G. Print, L.M. Hull, MicroRNA-regulated pathways associated with endometriosis, Mol. Endocrinol. 23 (2009) 265–275.
[189] J. Elmen, M. Lindow, S. Schutz, M. Lawrence, A. Petri, S. Obad, M. Lindholm, M. Hedtnarn, H. Frydenlund, U. Berger, S. Gullans, P. Kearney, P. Sarnow, E.M. Straarup, S. Kauppinen, LNA-mediated microRNA silencing in non-human primates, Nature 452 (2008) 896–899.
[190] Santaris Pharma, News Release, Santaris Pharma begins human clinical testing of the world's first medicine targeted at a human microRNA, May 28 2008, pp. 1–4.
[191] Rosetta Genomics, Initiates in vivo studies, Drug Clinical Trials (2007) (http://www.emaxhealth.com/95/18136.html).
[192] Company focus: Diagnostics, Rosetta Genomics (2009) (http://www.rosetta-genomics.com).
[193] H. Ohad, Rosetta Genomics: Mining genes from junk (Part II), (2008) (http://seekingalpha.com/article/72184-rosetta-genomics-mining-genes-from-junk-part-ii).
[194] L.J. O Neill, A renaissance of interest innate immunity: will new treatments for rheumatoid arthritis emerge? Future medicine–future, Rheumatol 3 (2008) 203–205.
[195] Asuragen, press release, Austin, Texas, Asuragen Launches the First Validated microRNA Diagnostic Assay (2008) (http://www.asuragen.com).
[196] Portfolio Company News, Boulder, CO, Miragen Therapeutics, Inc Receives $8M in Venture Capital Financing for Development of MicroRNA-based Therapies (2008) (http://www.atlasventure.com).