Stepping into small RNA biology

Introduction

Until now, we have discussed genetics on the level of the DNA, where mutations are introduced into DNA sequences and the phenotypic effects of these mutations are assessed. However, the function of a gene can also be affected by changes that are not associated with a change in the DNA sequence, in which case the gene is said to undergo epigenetic regulations, which will be discussed in the high-level lecture 1. In the introduction lecture, we have already discussed the phenomenon of RNA silencing, and in particular the process of RNA interference, where the down regulation of gene expression is mediated by short noncoding RNAs (small RNAs) derived from long, perfectly complementary dsRNA molecules. RNA silencing via small RNAs suppress gene expression post-transcriptionally by base pairing to mRNAs, but we will see that in some organisms small RNAs can also anneal to nascent transcripts of target DNA and promote epigenetic modifications of the DNA via cytosine methylation.

The concept of sRNA-mediated gene regulation is now widely accepted and has been shown to affect a large variety of cellular processes, but the identification of small regulatory RNAs and their importance for biology around 20 years ago was, at the time, revolutionary. Until then, scientists focused on protein-coding genes in their research, following the central dogma of DNA being transcribed into RNA, which is then translated into protein. Thus, RNA was mostly considered as a mere mediator, but not a direct actor, in the genetic information flux. Studies on non-protein-coding sequences were largely dismissed as was the non-coding part of the genome, considered by most as "junk DNA".

In this lesson, we will start with a short introduction into the world of RNA biology and discuss the relevant research that led to the identification of small RNAs. Finally, we will discuss the process of small RNA biogenesis and the mechanisms they employ to silence gene expression.

The RNA world

That RNA occupies a central position in biology is undisputed: it is the main result of transcription and the mediator of translation. RNA was the first nucleic acid to appear in evolution, long before DNA evolved. The biochemical properties of RNA make it a unique macromolecule that can fulfill several cellular functions: First, the linear sequence of RNA makes it a simple source of genetic information. Further, the single-stranded nature of RNA allows the formation of secondary structures to facilitate interactions with other molecules. RNAs can even assume tertiary structures that present surfaces for interactions and contain internal environments that create binding sites for metal ions, such that some RNAs can promote catalytic reactions. In 1982, the discovery of ribozymes demonstrated that RNA is unique in being both a genetic material and a biological catalyst. Ribozymes are catalytic RNAs that function within the ribosome to link amino acids during translation. They also participate in other processes, like RNA processing (e.g., RNA splicing). So-called hammerhead ribozymes were also discovered in viroids, plant sub-viral pathogens with a circular, highly complementary RNA genome that does not encode any protein. The viroid hammerhead ribozyme cleaves and linearizes the genomic RNA to allow subsequent replication in a rolling-circle mechanism. We will see later how studies of viroids in plants became also instrumental in the discovery of how small RNAs can mediate epigenetic silencing of target genes (high-level lecture 1).

The central role of RNA in these critical life processes led to the proposition of an RNA world, where RNA was the genetic material and RNA was used to synthesize proteins. Although RNA was now accepted to be able to catalyze certain cellular functions, it was still believed that proteins would be the main regulators of cellular processes.

In the case of gene regulation, the common model was that gene expression was controlled on the level of transcription. Extensive studies identified regulatory sequences on the DNA as well as proteins

binding to these sequences, categorized as transcription enhancers or suppressors. It was generally accepted that transcriptional control mediated by proteins was the gene-expression regulatory mechanism. However, about 20 years ago, scientists discovered that small, non-coding RNAs can control gene expression by recognizing the expressed mRNAs of genes, either directing their degradation or inhibiting their utilization in translation. In this process, double-stranded RNA of various cellular origins is processed to small RNAs that are subsequently incorporated into protein complexes mediating direct translational silencing and/or mRNA degradation. This novel mode of gene regulation was termed post-transcriptional gene silencing or PTGS. PTGS is conserved throughout evolution and was shown to affect many biological processes.

Classes of small non-coding RNAs: common aspects and differences

The small non-coding RNAs that mediate post-transcriptional silencing (PTGS) can be grouped into three main categories: Short interfering RNAs (siRNAs), microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs). These RNAs are only known to be present in eukaryotes. The borders between the three classes become more and more blurred with increasing research on these species; however, there are some characteristics that can be used to distinguish these classes. Both siRNAs and miRNAs are double-stranded sequences produced by Dicer RNase III enzymes (as discussed in the introduction lecture on RNA interference) and both are present in many cell types of eukaryotic organisms. In contrast, piRNAs are Dicer-independent, single stranded and found exclusively in metazaons where they exert their function in the germline. In this course, we will mainly focus on the biogenesis, modes of action and biological roles of miRNAs and siRNAs, but will discuss the role of piRNAs in the second high-level lecture at the end of the course.

We will first shortly review the similarities and differences between miRNAs and siRNAs before looking at how these RNAs were discovered. siRNAs act in the biological gene-silencing process called RNA interference (RNAi). The stimulus that triggers RNAi is the presence of a long dsRNA in the cell, and this molecule was originally introduced artificially into organisms such as plants, worms or flies using injection or transgenic expression. Of course, RNAi does not exist in cells for the sake of silencing transgenes or experimental dsRNA! In fact, it was found that RNAi is an evolutionarily conserved response to exogenous dsRNA, which reflects an ancient endogenous defense against foreign or invasive nucleic acids, such as viruses or transposons (see figure 1-1). Recently, it has become clear that there are also endogenous sources of siRNAs, a phenomenon we will discuss later in this course.

miRNAs, on the other hand, are encoded by the cell's own genome and serve to regulate the organism's gene expression. Both miRNAs and siRNAs start out as precursor molecules that are processed within the cell into short, double-stranded RNA fragments of 20-30 nucleotides. While miRNAs are processed from stem-loop or hairpin-like precursors (they arise due to mismatches between the two not completely complementary strands), siRNA precursors are usually longer and fully complementary dsRNAs. Processing of the precursor is, in both cases, mediated by the enzyme Dicer. One of the two strands of the small dsRNAs is loaded onto so-called RNA-induced silencing complexes (RISCs) and the target mRNA is recognized through Watson-Crick base pairing. Once the target is recognized, its expression is silenced (see figure 1-1).

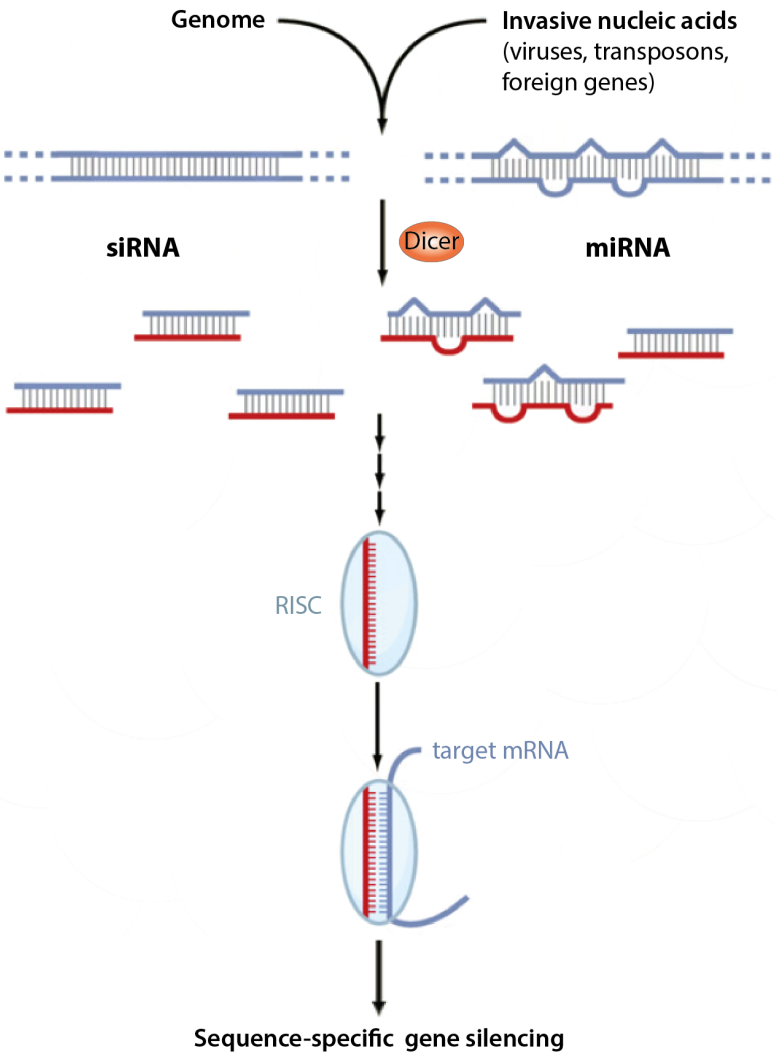

Figure 1-1 Common features of miRNA and siRNA silencing. Double-stranded RNA precursors of various kinds are processed by a Dicer protein into short fragments of 20–30 nucleotides in length. One strand of the processed RNA is incorporated into RNA-induced silencing complexes (RISCs) to mediate gene silencing of the target mRNA. (Adapted from R.W. Carthew and E.J. Sontheimer, *Cell*, 2009)

So, superficially, both siRNAs and miRNAs seem to be very similar in both their biogenesis and activity in the cell. However, the main criteria to differentiate these molecules are the following:

- miRNAs are excised by Dicer from their short (roughly 70 nucleotide long in mammals) and imperfect stem loop precursor as a single small RNA species, which may accumulate to tremendous levels in the cell (up to 50'000 molecules/cell).
- siRNAs, on the other hand are produced by consecutive cuts by Dicer along a perfect, long dsRNA molecule (remember the movie in the introduction lecture). Therefore, siRNAs, unlike miRNAs, are always part of a population that is distributed along their long dsRNA precursor.

The discovery of small regulatory RNAs: The first chapter of a *C. elegans* story

The era of the small non-coding RNAs began in 1993 with *lin-4* being the first microRNA to be discovered. *lin-4* was discovered already in 1981 in a forward genetic screen in *C. elegans* as a mutant with defects in developmental timing (*lin* mutants are lineage-defective; that show defects in the pattern of cell divisions that occur during development). Worms with *lin-4* loss-of-function mutations recapitulate cell-division patterns that are specific to the first larval stage (L1) at inappropriate later stages of development, and *lin-4* activity was shown to be required for the transition from the L1 to L2 stage. When researchers around Victor Ambros cloned the corresponding DNA fragment containing the *lin-4* gene, it was found to be only 700 bp long and to contain no conventional start nor stop codons as it would be expected for a mutation that maps to a protein-coding gene. Introducing mutations into what the researchers thought was the reading frame of the gene did not affect the function of *lin-4* - a situation very unusual for a protein-coding sequence. They also found two very small *lin-4* transcripts of only 61 and 22 nucleotides in length. Thus, Ambros and his colleagues reasoned that *lin-4* did not encode a protein and was perhaps exerting its regulatory role as RNA.

Shortly after *lin-4* was found in the screen, the group around Gary Ruvkun found a mutation in a protein-coding gene, *lin-14*, that was able to revert the *lin-4* mutant phenotype (thus, *lin-14* is a suppressor of the *lin-4* phenotype). Interestingly, the *lin-14* mutation caused exactly the opposite phenotype: in *lin-14* mutants, the L1 stage-specific cell fates were skipped, and the larvae developed prematurely into L2 stage larvae. Indeed, epistasis analysis detected interaction between *lin-4* and *lin-14*, and placed them in a hierarchy within the same regulatory pathway.

While the two mutants were analyzed separately in different laboratories, more and more evidence accumulated how these two genes might work together to control *C. elegans* development. The researchers around *lin-14* found that it was down-regulated at a post-transcriptional level, and that this down-regulation was dependent on a 3' untranslated region (UTR) of *lin-14*. After finally sharing their results, the two groups came to the same conclusion: the short *lin-4* RNAs were partially complementary to a repeated sequence in the 3' UTR of the *lin-14* gene. Thus, the non-coding transcript of *lin-4* regulates *lin-14* mRNA through binding to its 3' UTR (see figure 1-2A). This direct base pairing was essential for the ability of *lin-4* to control *lin-14* expression by disrupting its translation. Interestingly, *lin-4* is partially complementary to seven different sites in the *lin-14* 3' UTR (see figure 1-2B). This feature is common; many miRNA target transcripts carry multiple miRNA target sites.

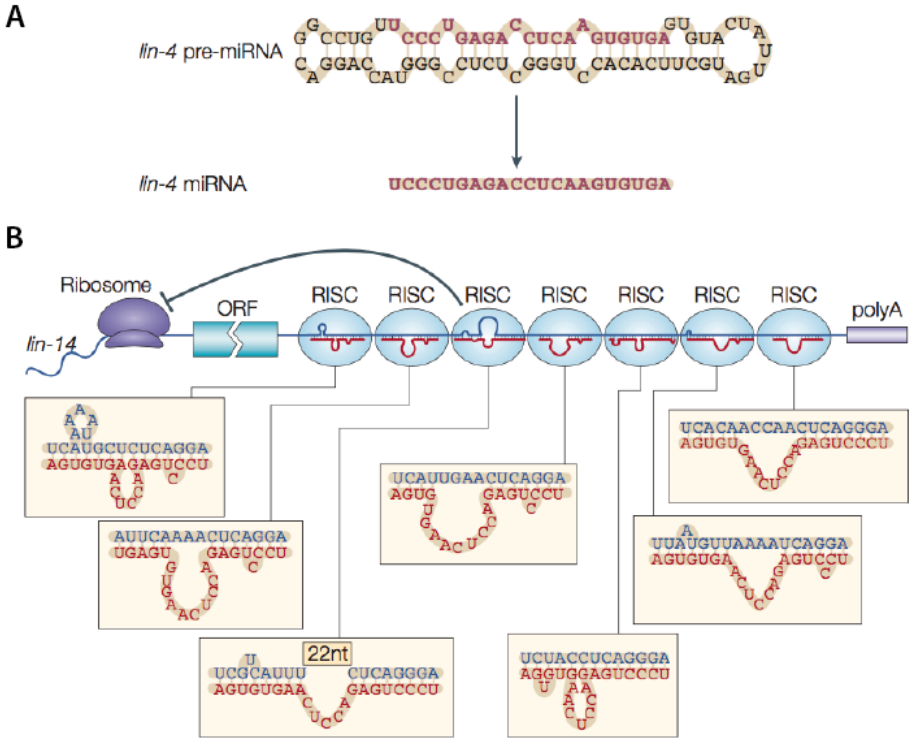

Figure 1-2 Molecular characteristics of *lin-4* and the binding to its target mRNA *lin-14*. (A) The precursor structure and mature microRNA (miRNA) sequence of *lin-4*. **(B)** Sequence complementary between *lin-4* (red) and the 3′ UTR of its target, the *lin-14* mRNA (blue). *lin-4* is partially complementary to seven different sites in the *lin-14* 3′ UTR. The binding of *lin-4* to these target sites leads to repression of LIN-14 protein synthesis. (Adapted from L. He and G.J. Hannon, *Nat. Rev. Genet.*, 2004)

The second chapter: The identification of *let-7* opens the door for miRNA research

In 2001, seven years after the identification of *lin-4*, another forward genetic screen in *C. elegans* identified the second miRNA, *let-7*. The researchers around Gary Ruvkun identified a mutation in *let-7* that causes the reappearance of larval cell fates during adult development. *let-7* encodes a small, 21-nucleotide long RNA. Interestingly, the researchers found that the *let-7* mutant phenotype could be partially suppressed by a loss-of-function mutation in the protein-coding gene *lin-41*. Analogous to *lin-4*, *let-7* is complementary to sequences in the 3' UTR of *lin-41*, and exerts its function by binding to this region to inhibit the translation of *lin-41* (see figure 1-3).

Figure 1-3 Potential duplexes between the miRNA *let-7* and its target mRNA *lin-41*. (Adapted from wormbook.org)

In summary, during *C. elegans* development, the accumulation of the two miRNAs *lin-4* and *let-7* is inversely correlated to the expression of their targets, *lin-14* and *lin-41*, respectively (see figure 1-4). This allows to perfectly control the timing of their expression and therefore an adequate larval-to-adult transition. Unlike *lin-4*, *let-7* is highly conserved across species from flies to humans, a finding that raised the idea that miRNAs could also be present in organisms other than *C. elegans*. By now, thanks to the advent of ultra-deep sRNA sequencing technologies (see lesson 2), we know that there are thousands of miRNA genes encoded by the genomes of most eukaryotic organisms, including animals and plants.

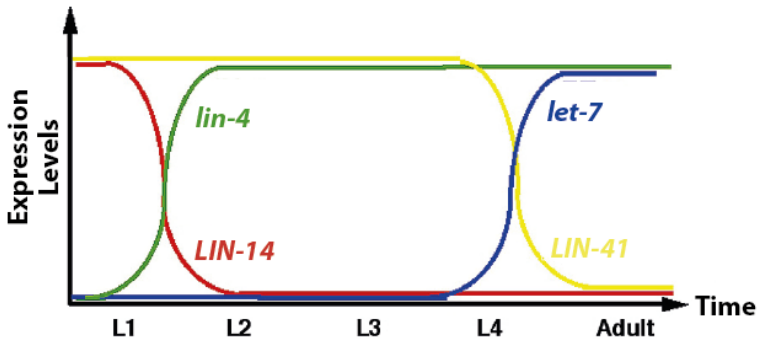

Figure 1-4 Temporal expression of the miRNAs *lin-4* and *let-7* and their targets. *lin-4* acts to down-regulate LIN-14 by antisense base pairing with sequences in the *lin-14* 3′ UTR. *lin-4* is required for a normal L1-to-L2 transition during *C. elegans* post-embryonic development. *lin-14* encodes a putative transcription factor present at high levels in newly hatched L1 animals, which decreases by the L2 stage. *lin-4* loss-of-function mutants fail to turn off LIN-14 expression after the L2 stage. This leads to retarded development by repeating the L1 stage patterns. In a similar manner, *let-7* controls the L4-to-adult transition by interacting with the 3′ UTR of multiple target genes, including *lin-41*. *let-7* RNA is expressed at the late L3 and early L4 stages and controls larval-to-adult transition by down-regulating LIN-41 and thereby allowing the animals to advance to the adult cell-fate programs. (Adapted from wormbook.org)

The connection to the siRNA pathway revealed the mode of biogenesis of miRNAs

At the time when miRNAs were discovered, RNA interference mediated by siRNAs had been already already studied in quite some detail in other organisms, as we have discussed in the introduction lecture. It was known that RNA silencing occurs in some plants and fungi transformed with foreign or endogenous DNA. In 1999, plant scientists discovered that in all cases of transgene silencing studied, RNA complementary to the transgene mRNA was detected. Interestingly, these RNA molecules were unusually small and of a uniform length estimated at 20 nucleotides; they had sense and antisense strands, suggesting they were short double-stranded RNAs most likely derived form a longer dsRNA precursor (see figure 1-5A). Indeed, it later turned out that most of the plant transgenes that triggered RNA silencing were organized into inverted repeats as a result of aberrant transgene insertion into the genome, a feature that allows the production of long dsRNA molecules. In parallel, direct injection of dsRNA into model organisms, such as *C. elegans* or *Drosophila*, was found to induce RNA silencing via RNA interference, and, interestingly, labelled dsRNA molecules incubated with *Drosophila* embryo extracts were converted into small RNAs of a uniform length, estimated at 20 nucleotides (see figure 1-5B). These synchronous observations between species therefore forged a link between transgene suppression in plants and RNAi in animals.

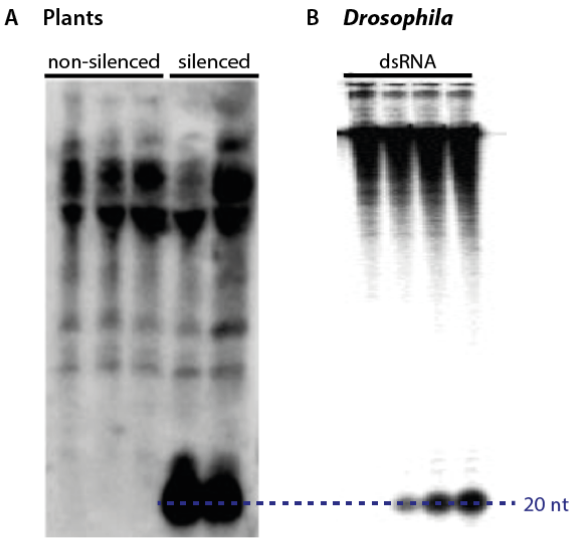

Figure 1-5 In both plants and animals, RNAi is characterized by the presence of RNAs of about 20 nucleotides in length that are homologous to the gene that is being suppressed. Lysates from **(A)** plants that initiated a gene-silencing response against transgenes, and from **(B)** *Drosophila* embryos treated with dsRNA for 0, 15, 30, or 60 min (left to right). In both cases, small RNAs with similar sizes of approximately 20 nucleotides appear that are the mediators of the RNAi response. (A: Adapted from A.J. Hamilton and D.C. Baulcombe, *Science*, 1999, B: Adapted from E. Bernstein *et al.*, *Nature*, 2001)

Further biochemical work conducted in *Drosophila* lysates showed that the dsRNA is converted into siRNAs by one of three RNAse III enzymes encoded in the fly genome. This enzyme was coined "Dicer" and was sufficient, when purified, to perform the conversion of long dsRNA into sRNA of about 20-25 nucleotide in length. Strikingly, the size of these sRNA was the same as the ones of the miRNAs *lin-4* and *let-7*. This led to the idea that the siRNA and miRNA pathways could be connected via Dicer, producing both types of molecules. Indeed, worms with a partial loss-of-function mutation in the *C. elegans* homolog of Dicer showed similar phenotypes as *lin-4* or *let-7* mutants, suggesting that a common dsRNA processing machinery is used by in the siRNA and miRNA pathways

The connection to the siRNA pathway revealed the mode of biogenesis of miRNAs

Thus, we have now seen that siRNAs and miRNAs are similar regarding their molecular characteristics, their biogenesis, and their AGO effector proteins. The fundamental function they exert is also the same: both regulate gene expression at the post-transcriptional level. Both siRNAs and miRNAs depend on the enzyme Dicer for their maturation from precursor molecules into the 20-nucleotide long, small fragments, and both small RNAs need to be incorporated into the RISC to mediate their gene-silencing function. Initially, siRNAs were found to bind to their mRNA targets with perfect complementarity and direct the cleavage of the mRNA at the site of complementarity (see figure 6). Meanwhile, animal miRNAs were found to act as translational repressors (see figure 1-6). In many cases, miRNAs bind to the 3' UTR of the target mRNA through multiple imperfect matches and therefore negatively regulate expression by repressing translation of the target mRNA, as originally found for *lin-4* and *lin-14* (see figure 1-6). However, evidence is now emerging that these rules are not always true, or at least not necessarily mutually exclusive: in plants, for example, most miR-

NAs anneal to their target with nearly complete complementary, either in the 3' UTR or the coding region, and therefore mediate cleavage of their target mRNAs just like siRNAs, but the remaining non-cleaved mRNA fraction may also undergo translational repression. Likewise, some (albeit only a few) miRNAs in animals promote target cleavage via extended miRNA-target complementarity while, conversely, some mammalian siRNAs imperfectly paired with their target can trigger translational repression rather than mRNA degradation. Thus, it is important to remember that both mechanisms of silencing genes exist, and that both mechanisms can be used by either siRNAs and miRNAs. The flexibility of the system makes sense as it allows cells to switch from one mode of silencing to the other, depending on the need of the cell. In cases where gene expression has to be adapted rapidly, e.g., as a response to an external stress, a regulation that is based on translational inhibition is more flexible, because it is quickly reversible, enabling translation to resume as soon as the stress is over. By contrast, mRNA degradation is irreversible, and therefore some delay will occur before the cell has produced new mRNA that can be used for translation of the protein.

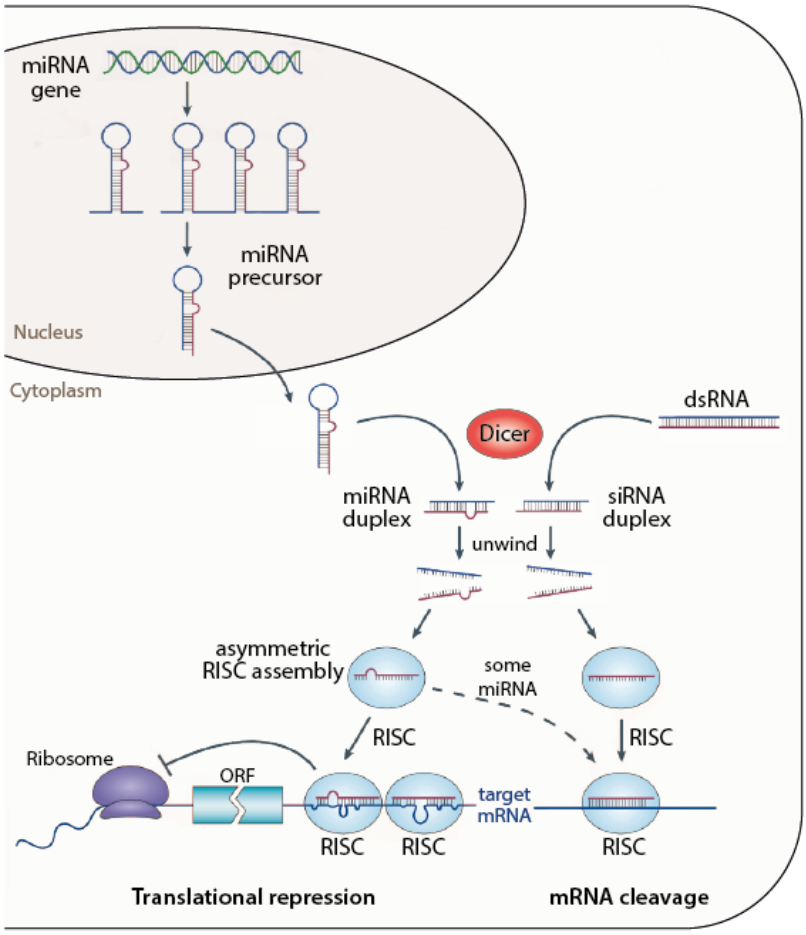

Figure 1-6 Mode of action of microRNAs (miRNAs) and small interfering RNAs (siRNAs). Note that, although not represented on the scheme, some imperfectly matched siRNAs may also trigger translational repression. (Adapted from L. He and G.J. Hannon, *Nature*, 2004)

Biogenesis of miRNA and RISC assembly

We will now discuss the biogenesis and processing of small RNAs in more detail, concentrating on the miRNA pathway. As discussed above, miRNAs have to be processed from small precursor molecules to be able to perform their action. There are two processing events that lead to mature miRNA. In the first step, the miRNA transcripts (called pri-miRNA) are processed into precursors of 70 nucleotides (called pre-miRNA) that contain the typical stem-loop (see figure 1-7). In animals, this first process is mediated by a nuclear ds-RNA-specific nuclease called Drosha. The second processing step cuts off the loop from the pre-miRNA to create a mature miRNA of approximately 22 nucleotides. In plants, the two steps take place in the nucleus, while in animals the pre-miRNA is exported into the cytoplasm where the enzyme Dicer carries out the final cleavage reaction to produce a single, discrete species of miRNA. Plants use the Dicer homolog Dicer-like 1 (DCL1) to carry out both cleavage processes in the nucleus (see figure 1-7).

Another specificity of plants is that miRNAs, like all plant sRNAs, undergo 3'-O-methylation on both strands via the action of the RNA methylase HEN1. This modification protects the end of the miRNAs from undergoing oligouridylation and subsequent degradation. Animal miRNAs are not methylated, in contrast to endogenous siRNAs and piRNAs found in these organisms.

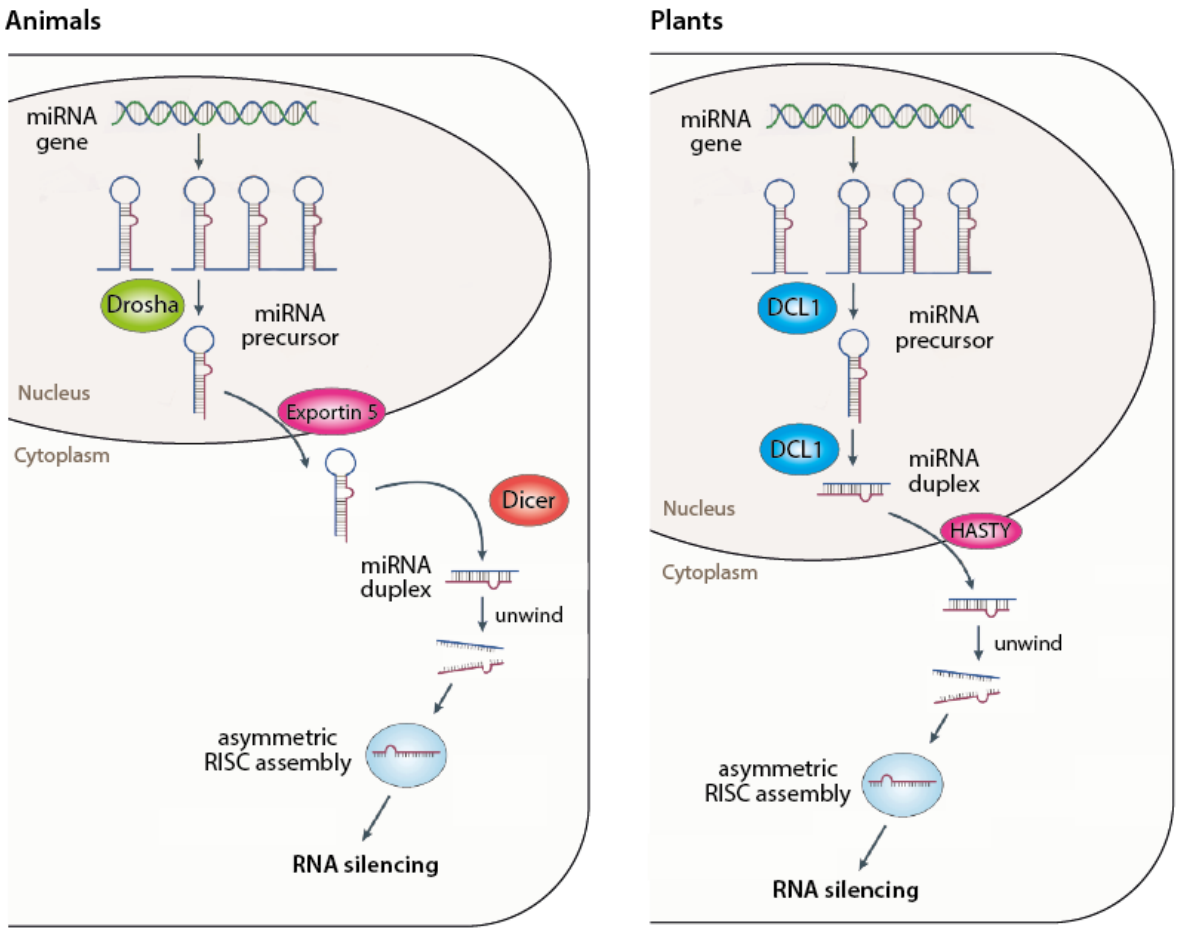

Figure 1-7 Biogenesis and RISC assembly in animals (left) and plants (right). (Adapted from L. He and G.J. Hannon, *Nature*, 2004 and E.J. Chapman and J.C. Carrington, *Nat. Rev. Genet.*, 2007)

The pre-miRNA (in animals) as well as the miRNAs (in plants) are exported into the cytoplasm by Exportin 5 or HASTY, respectively, a Ran-GTP dependent transporter. In the cytoplasm, the miRNAs associate with the RISC. To be able to specifically bind the target mRNA, it is important that only one strand (the so called guide strand) is incorporated into the RISC, while the other one (so called passenger strand or miRNA* strand) is discarded. The separation of the two miRNA strands and the selection of one specific strand to be incorporated into RISC is based on thermodynamic properties of the duplex as the unwinding begins at the extremity displaying the highest free energy (i.e., the weakest base-pairing). The process does not require energy due to the relaxed nature of miRNA:miRNA* strand interactions (because their precursors are themselves imperfectly matched). On the contrary, strand separation from perfect siRNA duplexes requires energy and is catalyzed by AGO-mediated "slicing" (see later in the text). The selected miRNA guide strand then becomes incorporated into AGO, where it acts as a guide as part of AGO (Figure 1-8). Structurally, AGO exposes the most 5'-end nucleotides of the miRNAs used for initial target scanning. The nucleotides 2-8 in this region, called the seed region, are essential for scanning and initial nucleation of the base-paring between the miRNA and its target. If the strength of pairing is not sufficient, then the AGO-miRNA complex might release the target and move on to sample other, unrelated mRNAs.

There are multiple AGO homologs in each animal species (e.g., 24 in *C. elegans* and 4 in mammals), suggesting that the composition of RISCs might be different to act in a tissue-specific or developmentally regulated fashion. Some of the AGO proteins have endonuclease activity and can catalyze the cleavage of target base pairs. This initial cut, called "slicing" represents the critical first step in some of the RNA-silencing events that proceed through RNA destabilization (see figure 1-8). In addition to AGOs, there are several other proteins associated with various RISCs, and the association of these proteins may also vary extensively for different AGO proteins, depending on the small RNA pathway involved.

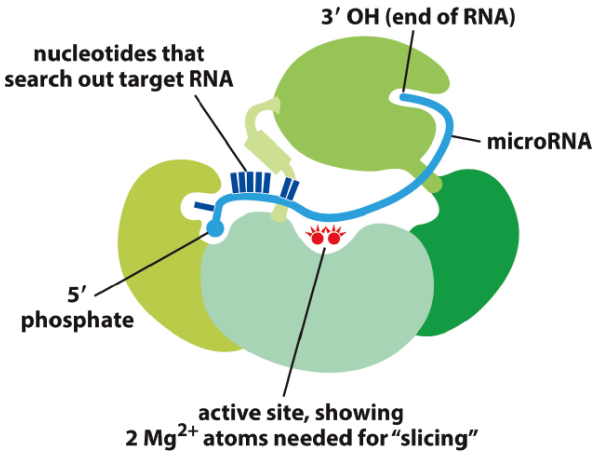

Figure 1-8 A schematic drawing of the human AGO protein carrying a miRNA. The protein is folded into four domains (each indicated by a different color). The miRNA is held in an extended form that is optimal for forming RNA-RNA base pairs between target mRNA. Initially, nucleotides 2-8 from the 5′-end of the miRNA define the seed region (in blue), which serves as a probe for sequence complementarity. Note that nucleotide 1 is excluded from the seed. The active site of AGO that slices a target RNA when it is base-paired with full complementary with the miRNA is indicated in red. Several AGO proteins lack this catalytic site and bind mRNAs without slicing them. (Figure 7-76, *Molecular Biology of the Cell*, Alberts, 6th edition, Garland Science)

Post-transcriptional gene silencing by miRNAs

Almost all animal miRNAs bind to the 3' UTRs of their target genes with mismatches. In contrast, most plant miRNAs bind with near-perfect complementary to their target site within the coding region of their targets. In animals, the degree of miRNA-mRNA complementarity determines the regulatory mechanism: perfect complementary allows AGO-catalyzed endonucleolytic cleavage (slicing) of the complementary mRNA strand. On the other hand, mismatches at the active catalytic site of AGO - the situation that prevails in the vast majority (99%) of animal miRNAs - preclude cleavage and promote repression of mRNA translation (see figure 1-9). It is believed that translational repression might be the default mechanism to repress gene expression in animals. Perfectly or near-perfectly matching miRNAs, as seen in plants, may induce slicing to some extent, and the remaining mRNA pool may undergo translational repression. The prevalence for each mechanism is different for each miRNA:target pair.

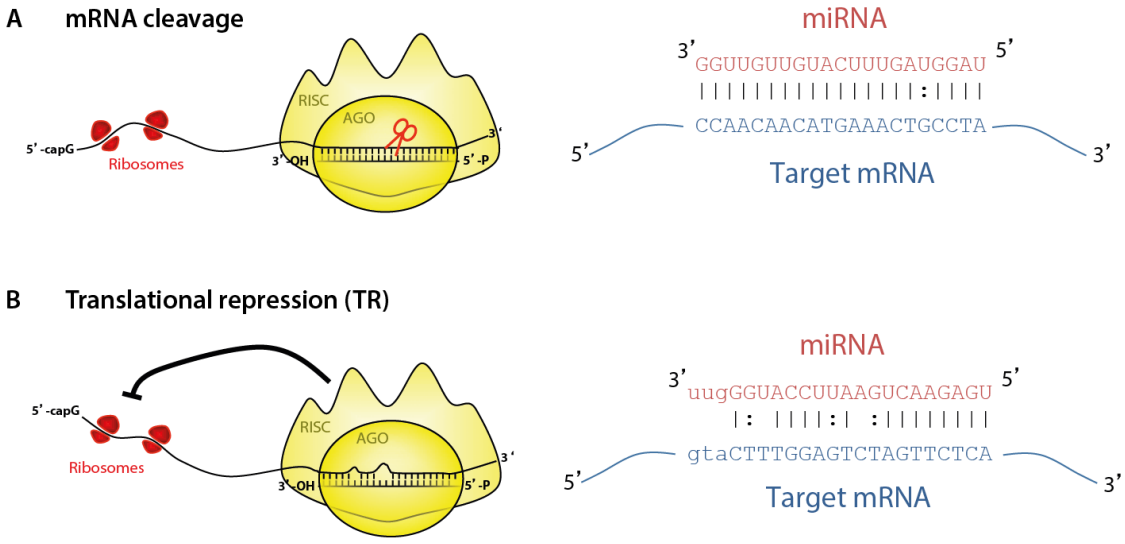

Figure 1-9 **(A)** In rare cases, animal miRNAs display perfect or near-perfect complementarity to their target transcripts (like most miRNA in plants), which they therefore regulate via slicing operated by the catalytic residues of the AGO nuclease. **(B)** Most animal miRNAs (99%) display imperfect base-pairing to their target sequence. A highly conserved central mismatch prevents slicing, leading to a mode of regulation known as translational repression.

Mechanisms of translational repression by miRNAs

How and at which step do miRNAs repress translation? Theoretically, translation could be repressed at the initiation step or after initiation. Translation of an mRNA molecule is initiated by the protein complex eIF4F (figure 1-10, green open circle) that recognizes the 5'-cap structure of the mRNA. eIF4F is the protein complex that regulates translation initiation. After binding to the 5' cap of the mRNA, proteins of the initiation complex recruit the 40S subunit of the ribosome (figure 1-10, dark purple) together with another initiation factor, eIF6 (see figure 1-10, olive circle). At the AUG start codon, the 40S subunit joins the 60S subunit (light purple in figure 1-10) and elongation begins. The initiation complex also interacts with the 3' end of the RNA through interaction with proteins that specifically bind to the poly(A) tail of the mRNA (blue circles in figure 1-10). This simultaneous interaction of the initiation complex with the 5' and the 3' ends of the mRNA leads to a circularization of the molecule, which greatly enhances translation efficiency. There are several ways how post-translational repression is mediated by miRNAs (see figure 1-10, B-F). Examples for all these mechanisms have been documented and there is currently no clear consensus as to which

mechanism is prevalent in animals. In all cases, however, translational repression is coupled to a form of accelerated mRNA decay that is induced by deadenylation and decapping of the target mRNA. This is mediated by a deadenylation and decapping (yellow and green in figure 1-10, respectively) machinery, containing 3'-to-5' (for deadenylation) and 5'-to-3' (for decapping) nucleases. Thus, in animals, the action of miRNA ultimately results in decreased mRNA levels, although this effect is usually very modest (1.5 to 3 fold reduction) for any single miRNA. It is very important to understand that mRNA decay coupled to translational repression is completely distinct from mRNA endonucleolytic cleavage (slicing) mediated by AGOs in the context of perfect miRNA:target base pairing.

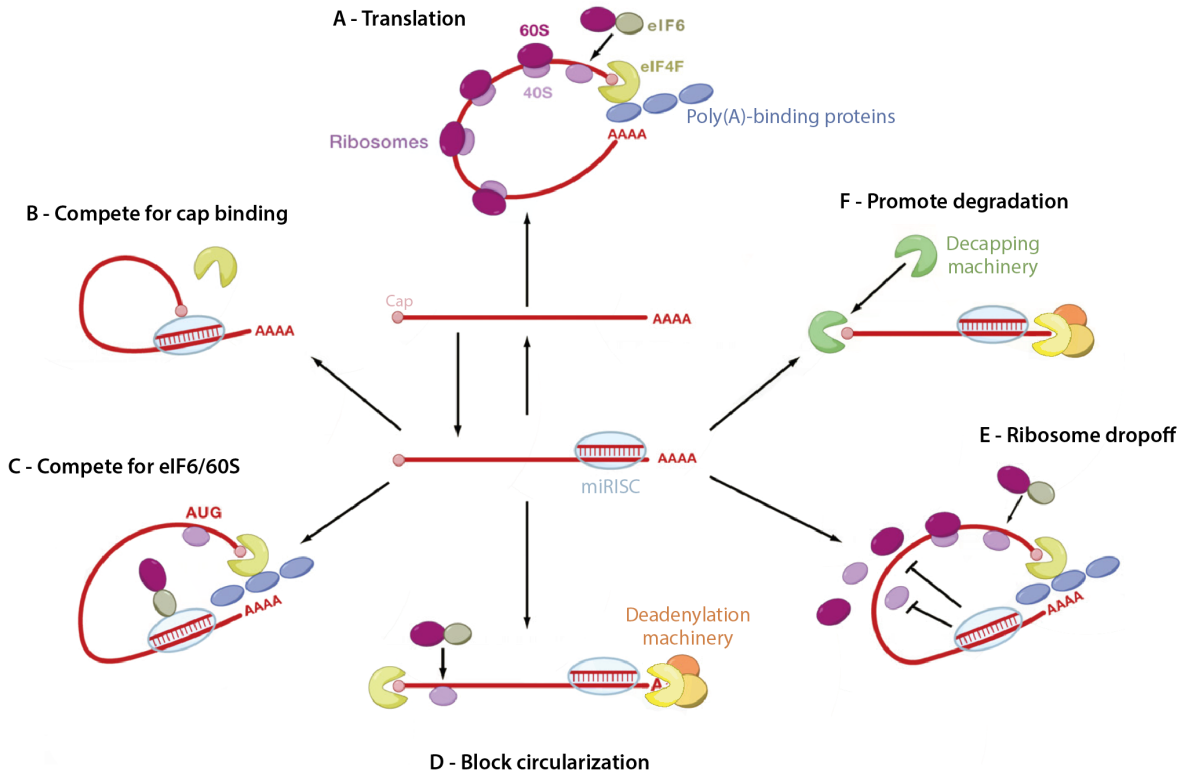

Figure 1-10 Possible mechanisms of translational suppression by miRNA-containing RISCs (miRISCs). (A) Non-repressed mRNAs recruit initiation factors and ribosomal subunits and form circularized structures that enhance translation. **(B)** When miRISCs bind to mRNAs, they can repress initiation at the cap-recognition stage or **(C)** the 60S-recruitment stage. **(D)** miRISCs can induce deadenylation of the mRNA and thereby inhibit circularization of the mRNA. **(E)** miRISCs can also repress a post-initiation stage of translation by inducing ribosomes to drop off prematurely. **(F)** Finally, miRISCs can promote mRNA degradation by inducing deadenylation followed by decapping. (Adapted from R.W. Carthew and E.J. Sontheimer, *Cell*, 2009)