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Review

Argonaute: The executor of small RNA function

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ABSTRACT

The discovery of small non-coding RNAs – microRNA (miRNA), short interfering RNA (siRNA) and PIWI-interacting RNA (piRNA) – represents one of the most exciting frontiers in biology specifically on the mechanism of gene regulation. In order to execute their functions, these small RNAs require physical interactions with their protein partners, the Argonaute (AGO) family proteins. Over the years, numerous studies have made tremendous progress on understanding the roles of AGO in gene silencing in various organisms. In this review, we summarize recent progress of AGO-mediated gene silencing and other cellular processes in which AGO proteins have been implicated with a particular focus on progress made in flies, humans and other model organisms as compliment.

1. Introduction

AGO proteins are the principal players in small non-coding RNA mediated gene silencing in various organisms. These proteins are named after the phenotype of *Arabidopsis thaliana argonaute* mutants which resemble the animal *Argonauta argo* (Bohmert et al., 1998). Although originally discovered in plants, AGO proteins are found to be conserved across eukaryotes. Notable exceptions include the model organism *Saccharomyces cerevisiae*, which does not have any AGO protein and appears not to have small RNA gene-silencing machinery (Hutvagner and Simard, 2008; Drinnenberg et al., 2009). The total number of AGO proteins between organisms varies, in which *Drosophila melanogaster* has five, humans have eight, *A. thaliana* has ten, *Caenorhabditis elegans* has twenty-seven, and *Schizosccharomyces pombe* has only one (Tolia and Joshua-Tor, 2007). Phylogenetic analysis reveals that eukaryotic AGO family can be divided into three major clades: the AGO clade, the PIWI clade and the WAGO clade that can only be found in *C. elegans* (Yigit et al., 2006). The AGO subfamily proteins are ubiquitously expressed and predominantly interact with microRNA (miRNA) and short interfering RNA (siRNA). Meanwhile, PIWI clade proteins are exclusively involved in another class of small RNA, which is PIWI-interacting RNA (piRNA). Biogenesis of all three classes of small RNA are summarized in Figs. 1 and 2.

In the past several years, many novel discoveries have been made on the functions of the AGO proteins in various model organisms. These proteins have been reported to play key roles in germ cell maintenance and division, transcriptional and translational regulation, alternative splicing, and heterochromatin formation (Azzam et al., 2012; Michalik et al., 2012; Wei et al., 2012; Gagnon et al., 2013; Huang and Li, 2014). Our knowledge on the mechanistic details of AGO-mediated gene silencing and AGO post-translational modifications has also been improved. In this review, we will focus on the recent understanding of multiple functional roles of AGO with specific emphasis on the AGO clade across the animal kingdom. The first part of this review discusses recent insights into small RNA biology, after which we will discuss the RNA-induced silencing complex (RISC) assembly and modes of AGO-mediated gene silencing. The later part will address the nuclear functions of AGO and the structural insights with the current understanding on molecular pathways in which AGOs are involved. Although the main focus is on AGO proteins across the metazoa, towards the end of the review, we will briefly introduce AGOs in plants and discuss the differences between the two. As it is well understood that small RNAs cannot catalyze any reactions without first forming a ribonucleoprotein complex called RISC, we shall also

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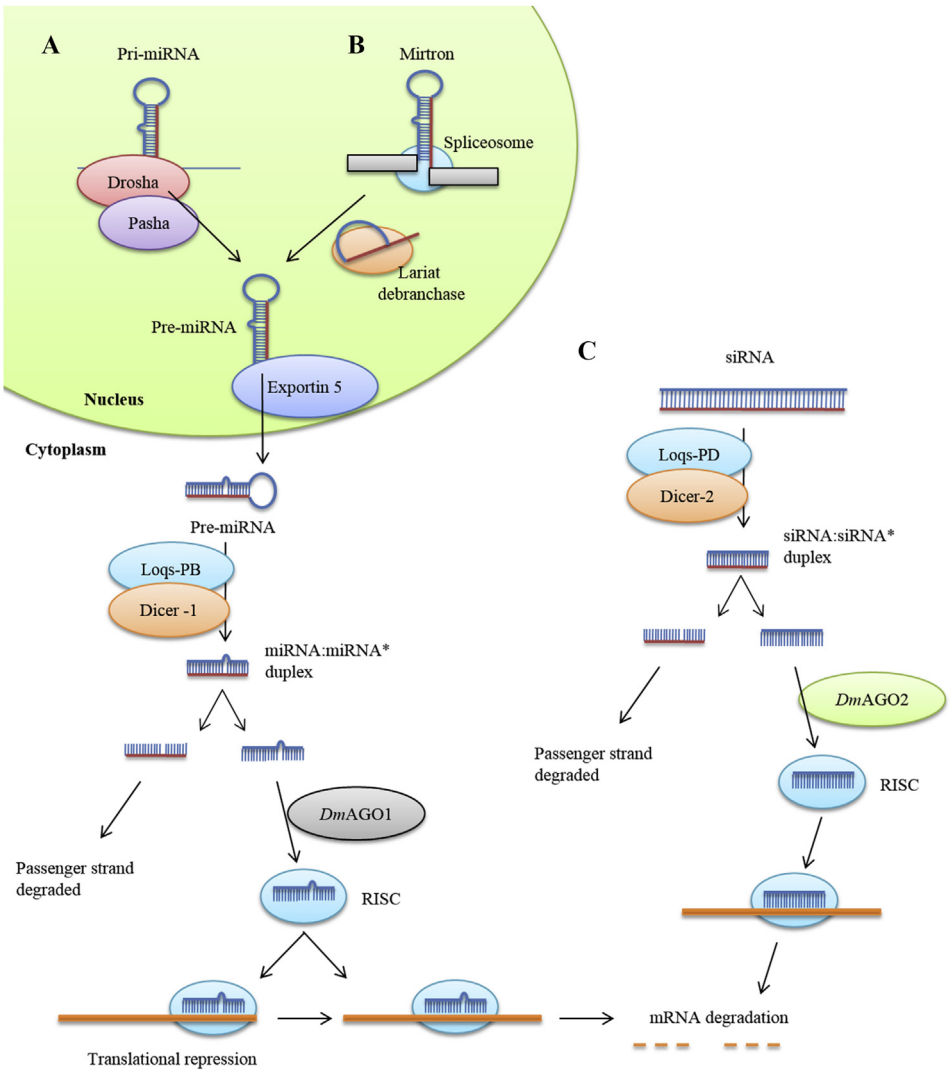

Fig. 1. *Drosophila* miRNA and siRNA biogenesis. **A**: Biogenesis of fly miRNAs starts with RNA polymerase II transcribing the primary transcript (pri-miRNA) that contains hairpin loop domains, a 7-methyl-guanosine cap, and a poly(A) tail. The pri-miRNA is cut by the microprocessor complex, comprising the RNAse III enzyme Drosha and its RNA binding domain-containing partner Pasha (known as DGCR8 in vertebrates), to become a 60–70 nucleotide-long precursor miRNA (pre-miRNA), which is then exported to the cytoplasm by Exportin5, a Ran-GTP-dependent cytoplasmic cargo transporter. In the cytoplasm, Dicer-1, together with its partner, Loquacious (Loq), cleaves the hairpin loop of the pre-miRNA to form a miRNA duplex (miR-5p and miR-3p). This duplex unwinds and one strand is selected as the mature miRNA and preferentially loaded into AGO1 to form the RISC. The strand that is not loaded into AGO1 is presumably degraded most of the time, but in some cases is loaded into the AGO2-containing RISC where it can function as a siRNA. **B**: Mirtron hairpin structure is processed by the spliceosome. Then the lariat is further processed by lariat debranchase enzyme to form the pre-miRNA and loaded into Exportin 5. **C**: siRNAs are made from long dsRNA which can derive endogenously from transposable elements, *cis*-natural antisense transcripts and hairpin RNAs (hpRNA) or dsRNA can be introduced exogenously to generate siRNAs. siRNAs are cleaved from long exogenous RNA into double-stranded siRNAs by Dicer 2 and Loq isoform D (Loqs-PD), then sorted into *Dm*AGO2 by Dicer-2 and R2D2 protein complex. After releasing the passenger strand, *Dm*AGO2 forms RISC (siRISC) with the guide strand, and is capable to cleave its target mRNA. Unlike miRNA, siRNA represses target mRNA by binding to its target sequence with perfect complementarity.

discuss in detail the process of RISC assembly as a key process in small RNA-mediated gene silencing.

2. Small RNAs

Small RNAs are non-coding RNA molecules that encompass different properties and functions. Small non-coding RNAs are characterized by their size (20–30 nucleotides in length), and are highly dependent on their protein partner, the AGO family proteins. In this review, we will cover mostly miRNA, mirtrons, siRNA, and piRNA. For the biogenesis of these small RNAs, we will focus on *Drosophila melanogaster* as it has a distinct miRNA and siRNA pathway for better understanding (Fig. 1). Furthermore, the piRNAs have been extensively studied in flies (Fig. 2).

miRNAs are ~22-nucleotide endogenous short RNAs with a role in the regulation of gene expression in animals and plants by targeting complementary mRNAs for degradation or translational repression (Sempere et al., 2004; Mallory and Vaucheret, 2010). The first characterized miRNA is *lin-4* which was identified from forward genetics in *C. elegans* (Lee et al., 1993; Wightman et al., 1993). Two transcripts of *lin-4* of 61- and 22-nucleotide sequences were identified. These are now recognized to be the precursor and mature sequences, respectively. The mature *lin-4* sequence was later found to regulate *lin-14* mRNA by sequence complementarity. Unlike siRNAs, miRNAs do not bind with perfect complementarity to its mRNA target in animals. miRNA interacts with its target *via* base pairing of its "seed" region (usually nucleotides 2–8) and complementary sequence on the mRNA (Lai, 2002; Lewis et al.,