MicroRNA Processing by Dicer

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MicroRNAs (miRNAs) are small non-coding RNAs that are about 22 nucleotides in length. They regulate gene expression post-transcriptionally with the effector protein complex, containing Argonaute or trinucleotide repeat containing 6 (TNRC6) proteins, and target mRNAs in a sequence-dependent manner, causing the translational repression and destabilization of the target mRNAs. Both Drosha and Dicer, members of the RNase III family proteins, are essential components in the canonical miRNA biogenesis pathway. miRNA is transcribed into primary-miRNA (pri-miRNA) from genomic DNA. Drosha then cleaves the flanking regions of pri-miRNA into precursor-miRNA (pre-miRNA), while Dicer cleaves the loop region of the pre-miRNA to form a miRNA duplex. In this report, we summarized and discussed the current reports in which double-stranded RNA binding proteins (dsRBPs), such as TAR RNA binding protein (TRBP) or the adenosine deaminase acting on RNA (ADAR), modulate the processing of miRNA by Dicer in various manners.

microRNA biogenesis | Dicer-associated proteins | dsRBP | TRBP | ADAR | PACT | LGP2 | miRNA–mRNA network

1. Introduction

MicroRNAs (miRNAs) are single-stranded RNAs of approximately 22 nucleotides in length and are classified as small non-coding RNAs. The miRNAs regulate gene expression post-transcriptionally by a mechanism known as RNA silencing, where miRNA is loaded onto Argonaute (AGO), a core component of the miRNA-induced silencing complex (miRISC) [1]. While on AGO, the miRNA recognizes target mRNAs that have sequences that are complementary to the “seed region” (positions 2–8 from the 5′ end) of the miRNA in their 3′ untranslated region [2]. As the seed region only consists of seven nucleotides, each miRNA is capable of recognizing and regulating many types of mRNAs, indicating that miRNA–mRNA gene expression networks are highly complicated.

Some of the miRNAs that have been discovered in diverse eukaryotes are evolutionally conserved, while others are species specific [3][4][5][6]. The first two miRNAs, lin-4 and let-7, were discovered in Caenorhabditis elegans (C. elegans) through the analysis of the heterochronic gene mutants that undergo development/differentiation at an abnormal time within the organism [7][8][9]. Let-7 is evolutionally conserved across various species, including in humans. In human lung cancer cells, let-7 regulates cell proliferation and also suppresses the expression levels of NRAS and KRAS, two genes that induce oncogenic transformation when mutated [10][11][12]. Thus, although let-7 is conserved in both C. elegans and humans, its function in the two species is different.

At the initial stage of miRNA biogenesis, miRNA is transcribed by RNA polymerase II (Pol II) as primary-miRNA (pri-miRNA) , which has stem loop structures [13][14] (**Figure 1**). In the canonical miRNA biogenesis pathway, the flanking regions of the pri-miRNA are cleaved to generate precursor-miRNA (pre-miRNA) in the nucleus by a microprocessor complex consisting of Drosha, a member of the RNase III family proteins, and its cofactor DiGeorge syndrome critical region 8 (DGCR8), a double-stranded RNA (dsRNA) binding protein (dsRBP) [15][16][17]. The pre-miRNA is transported from the nucleus to the cytoplasm by Exportin-5 (EXP5), which then couples with GTP-bound Ran [18]. In the cytoplasm, Dicer, an RNase III family protein, cleaves off the loop region of the pre-miRNA to generate a miRNA duplex in collaboration with the trans-activation response (TAR) RNA binding protein (TRBP) in the canonical miRNA biogenesis pathway [19][20]. The interaction of the Dicer–TRBP complex with Argonaute (AGO) facilitates the loading of the miRNA duplex onto AGO to form the RISC-loading complex (RLC) [21][22][23]. The miRNA duplex is then unwound into single stranded miRNAs; the RNA strand that remains on the AGO protein acts as the miRNA, while the other strand is discarded [24]. The former is called the guide strand, and the latter is called the passenger strand. The mature miRNA on the AGO protein guides the RISC to target mRNAs that have sequences that are complementary to the seed region of the miRNA. After binding to the mRNA, AGO recruits the trinucleotide repeat containing 6 (TNRC6) protein, a scaffold protein tethering effector proteins to destabilize and translationally repress target mRNAs by inducing their decapping and deadenylation [25].

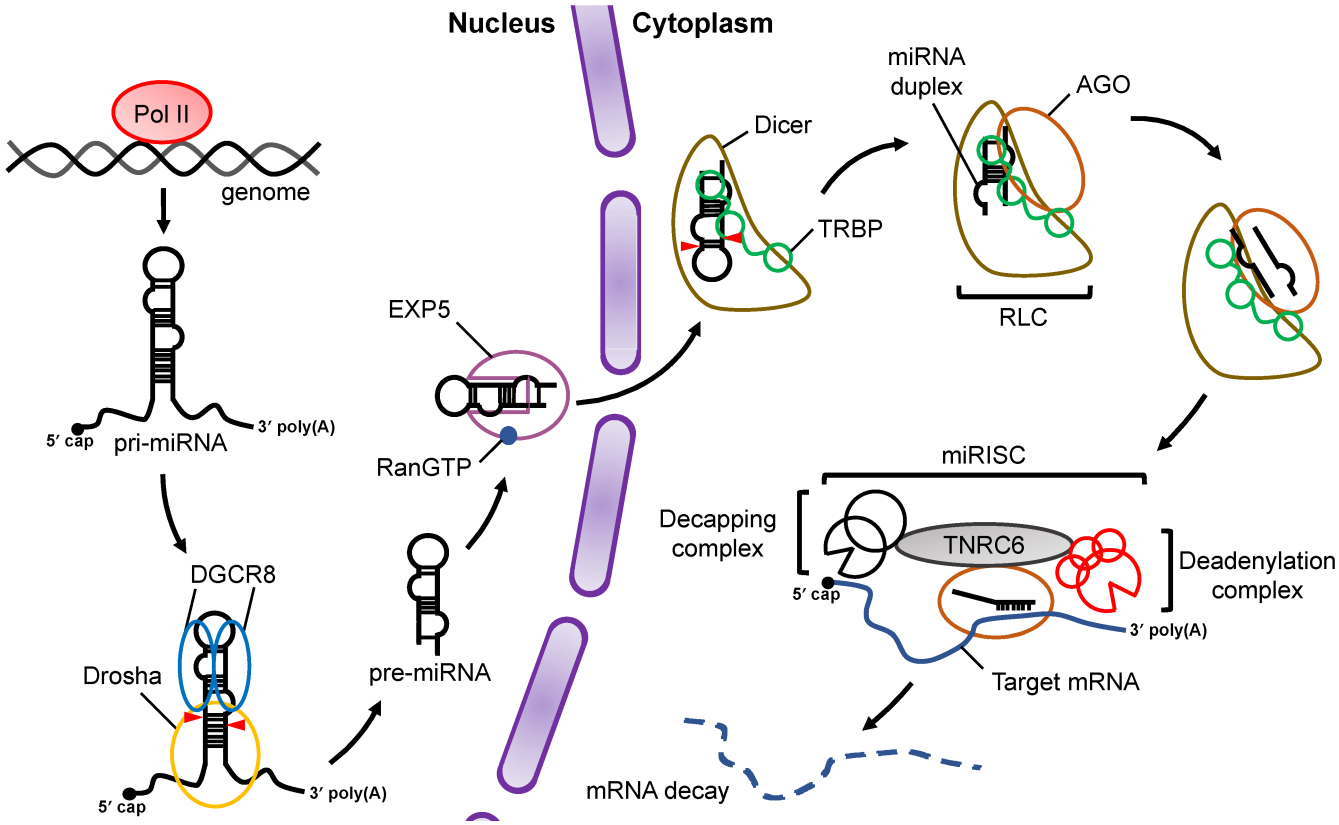

Figure 1. Overview of miRNA biogenesis and the RNA silencing pathway. Pri-miRNAs are transcribed from the genome by Pol II. In the nucleus, pri-miRNA is cleaved by a microprocessor complex consisting of Drosha and DGCR8 to produce pre-miRNA, which is then transported from the nucleus to the cytoplasm by EXP5 coupled with GTP-bound Ran (RanGTP). Cleavage of pre-miRNA is performed by Dicer and its cofactor, TRBP, in a canonical miRNA biogenesis pathway. After pre-miRNA cleavage, the miRNA duplex is loaded onto AGO proteins through

formation of the RLC complex. The mature miRNA guides the RISC complex to target mRNAs that are complementary to the seed region of miRNA. Recruitment of the TNRC6 protein induces the destabilization and translational repression of the target mRNA.

2. Processing of Pre-miRNA by Dicer

In eukaryotes, the domain structures of Dicer proteins are widely conserved; however, depending on the species, there are different mechanisms of substrate recognition. *Drosophila* has two Dicer paralogs that have different functions: Dicer-1 (Dcr-1) acts on the miRNA maturation pathway, and Dcr-2 acts on the small interfering RNA (siRNA) pathway, the purpose of which is for dicing the long dsRNA [26]. Plants also have at least four distinct classes of Dicer-like (DCL) proteins (DCL1–4) [27]. The DCL1 functions for miRNA processing and the DCL2-4 redundantly function for siRNA production. Humans, on the other hand, have only one *Dicer* gene, which functions in both pathways. It has been reported that Dicer cleaves pre-miRNAs more efficiently than dsRNAs for siRNA production in vitro [28][29][30][31]. Human Dicer is a multiple-domain protein (**Figure 2**a). An electron microscopy study demonstrated that Dicer forms an L-shaped structure [32][33] and recognizes the 3′-overhang of pre-miRNA by the Piwi, Argonaute, and Zwille (PAZ) domain, while the phosphorylated 5′-end of the pre-miRNA is captured by the platform domain [34][35] (**Figure 2**c). These two domains are arranged to be able to recognize the structure of pre-miRNA [36][37]. In the 5′ and 3′ counting rules, Dicer measures the nucleotide lengths from both ends of the pre-miRNA and cleaves the terminal loop of the pre-miRNA by means of the intramolecular dimerization of two RNase III domains, RIIIDa and RIIIDb, generating a miRNA duplex [38][39]. Unlike human Dicer, *Drosophila* Dcr-1 recognizes the terminal loop of pre-miRNA by its DExD/H-box helicase domain and specifically cleaves pre-miRNAs in a loop size-dependent manner [40]. On the other hand, *Drosophila* Dcr-2 recognizes the blunt end of dsRNA via its helicase domain and processes the dsRNA for siRNA production [41]. The DExD/H-box helicase domain of human Dicer does not have such substrate selectivity. The dsRBD of human Dicer also cannot distinguish the stem region of the pre-miRNA from that of the dsRNA of the siRNA in vitro [42], indicating that Dicer, on its own, has low substrate selectivity.

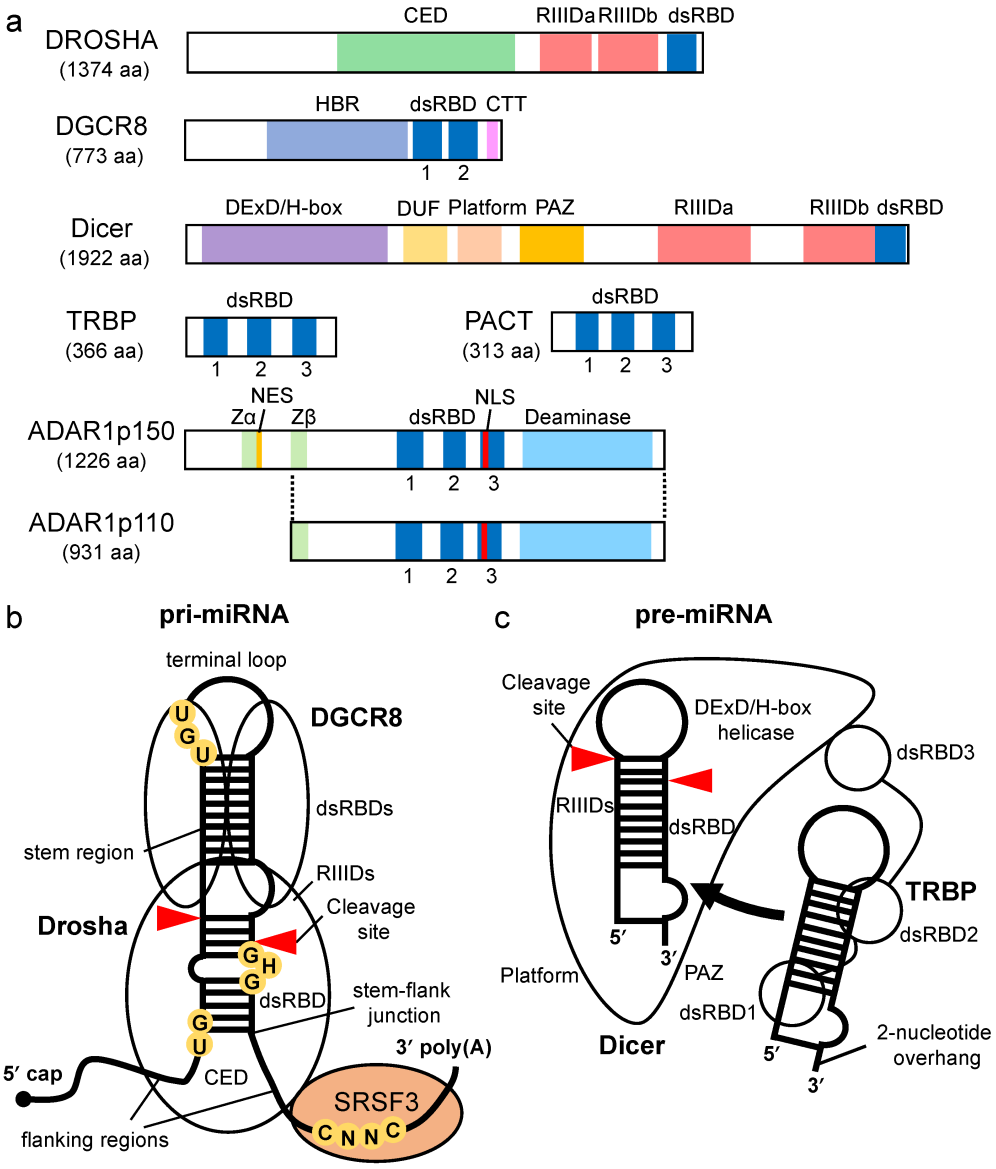

Figure 2. Domain structure of proteins involved in miRNA biogenesis and structural features of processing complexes of pri-miRNA and pre-miRNA. (**a**) The domain structures of Drosha, DGCR8, Dicer, TRBP, PACT, ADAR1p150, and ADAR1p110. CED indicates central domain; RIIIDa, RNase III domain a; RIIIDb, RNase III domain b; dsRBD, double-stranded RNA binding domain; HBR, heme-binding region; DExD/H-box, DExD/H-box helicase; CTT, C-terminal tail; DUF283, domain of unknown function; PAZ, Piwi–Argonaute–Zwille; Zα and Zβ, Z-DNA binding domain α and β, respectively; NES, nuclear export signal; NLS, nuclear localization signal. (**b**) Pri-miRNA has a terminal loop, stem region, and flanking region as common structural features, and some pri-miRNAs have conserved sequence motifs. UG is recognized by Drosha, UGU is recognized by DGCR8, GHG is recognized by dsRBD of Drosha, and CNNC is recognized by Ser/Arg-rich splicing factor 3 (SRSF3). (**c**) Pre-miRNA is structurally characterized by a terminal loop, a stem region, and a 3′ end overhang.

3. Enhancement of Pre-miRNA Processing by Dicer via TRBP/PACT

Dicer-associated proteins regulate the substrate recruitment and the cleavage activity of Dicer [43][44]. The processing of pre-miRNA is not only promoted by TRBP but also by other Dicer-associated proteins, such as PACT, a protein activator of protein kinase R (PKR) [43][45][46]. TRBP is a protein that binds to TAR RNA, a hairpin-structured RNA that is encoded by human immunodeficiency virus type I [47]. PACT is a protein that was initially identified as an activator of PKR [48]. TRBP and PACT have highly conserved domain structures with three dsRBDs [49] (**Figure 2**a). The dsRBDs are divided into two subclasses, type-A and type-B. Type-A has a conserved αββββα motif with a high affinity to dsRNA [50][51]. Type-B, also termed half dsRBD, has poorly conserved N-terminal sequences in the αββββα motif and is associated with protein–protein interactions [52][53]. The first and second dsRBDs of TRBP and PACT are type-As, and the third dsRBD is a type-B that binds to Dicer through the DExD/H-box helicase domain [43][45][46].

Both TRBP and PACT interact with Dicer to promote the cleavage of the pre-miRNA in the RLC containing AGO protein and the facilitate loading of the miRNA duplex onto AGO [20][23][43][54][55]. It was reported that deletion or mutation of the DExD/H-box helicase domain of Dicer activated the cleavage of its substrates, which suggested that this domain inhibits catalytic activity rather than affecting RNA-substrate binding [56]. TRBP binds to the DExD/H-box helicase domain of Dicer and stimulates the cleavage activity of Dicer. Therefore, the DExD/H-box helicase domain functions as an intramolecular structural switch that maintains Dicer in a low-activity state until the partner proteins interact with its DExD/H-box helicase domain. In addition, it was reported that TRBP facilitates the processing activity of pre-miRNA by Dicer in RNA-crowded molecular environments [57] and that it also facilitates the recruitment of pre-miRNAs to the PAZ domain of Dicer. Furthermore, the sliding motion of TRBP on dsRNA with Dicer has been reported [58]. This was associated with the higher substrate cleavage activity of Dicer compared to Dicer alone, which suggests that TRBP facilitates the cleavage activity of Dicer by guiding Dicer to the substrates. To date, no studies on the mechanism by which PACT promotes Dicer-mediated cleavage of pre-miRNAs have been reported. However, the amino acid sequence of the Dicer-interacting dsRBD of PACT was found to be similar to that of TRBP. It has yet to be determined if PACT interacts with Dicer by a mechanism similar to that of TRBP and if it enhances the processing of similar types of pre-miRNAs.

TRBP and PACT have different functions. Although TRBP preferentially binds to simple duplex RNA, PACT inhibits Dicer-mediated dsRNA cleavage for siRNA production [59]. Unlike PACT, the cleavage site for Dicer-TRBP shifts when compared to cleavage by Dicer alone [60][61]. PACT and TRBP have no redundant effects on the production of isomiRs , different-sized miRNAs that alter the downstream target-binding specificities. Such differences in dsRNA recognition and processing behavior are attributed to two N-terminal RNA-binding domains in each protein.

Several studies have addressed the TRBP-mediated maturation of specific miRNAs and its effect on downstream pathways. It was reported that the TRBP-mediated maturation of miR-208a decreased the expression level of SRY-Box Transcription Factor 6 (Sox6), which is required for normal heart function [62]. It was also reported that disruptions of TRBP-dependent maturations of tumor suppressor certain miRNAs (TS-miRs), miR-143 and miR-145, were related to the self-renewal and tumor maintenance of cancer stem cells [63]. These results suggest that TRBP regulates biogenesis and the downstream gene regulatory pathways of specific miRNAs.

4. Enhancement of Pre-miRNA Processing by Dicer via ADAR1

ADAR1 is classified as an adenosine deaminase acting on the RNA (ADAR) family protein that edits adenosine into inosine on dsRNA (A-to-I RNA editing) [64][65][66]. In addition to having zinc finger domains, ADAR1 has three dsRBDs and a deaminase domain (**Figure 2**a). The ADAR1-mediated regulation of miRNA biogenesis is classified into two types: one is the regulation of Drosha and Dicer cleavage by the A-to-I RNA editing of their substrate miRNAs/siRNAs [67][68][69], and the other is the promotion of miRNA maturation, which is achieved by forming a complex with Dicer via protein–protein interaction [54]. ADAR1 interacts directly with the DExD/H-box helicase domain of Dicer via its second dsRBD in the absence of dsRNA [54], while all ADAR dsRBDs that are type-A are involved in binding to dsRNA. Thus, the interaction mechanism between ADAR1 and Dicer is different from that of TRBP–Dicer or PACT–Dicer interaction. It has been reported that the DExD/H-box helicase domain of Dicer is essential for interaction with dsRBP during viral infection [70]. ADAR1 facilitates Dicer-mediated pre-miRNA cleavage and loading onto RISC for miRNA maturation and siRNA production [54]. However, the detailed mechanism for promoting Dicer-mediated processing by ADAR1 is not clear. Unlike TRBP and PACT, ADAR1 may regulate pre-miRNA processing by Dicer in combination with additional deaminase domain activity.

ADAR1 regulates the maturation of specific miRNAs in a spatiotemporal manner by interacting with Dicer. It was reported that *Adar*-knockout mice underwent systemic apoptosis at embryonic day 12 (E12) followed by death [71]. Analysis of these *Adar1*-knockout mice showed that the expression of ADAR1 and Dicer increased gradually from E9 to E12, and the expression of specific miRNAs, including miR-1 and miR-181a, increased at E12, suggesting the importance of miRNA maturation via Dicer–ADAR1 interaction during embryogenesis [54]. Several research groups have reported that miRNA maturation mediated by Dicer-ADAR1 affects global gene expression profiles in various diseases. It was reported that ADAR1p150 promotes the maturation of specific miRNAs by interacting with Dicer during viral infection [72]. In mice models, the overexpression of ADAR1p150 induced the expression of miR-222, which, in turn, repressed the expression of phosphatase and the tensin homolog (PTEN), an apoptosis-related gene, leading to increased cell survival. A recent report indicated that the upregulation of the ADAR1 expression level facilitated the Dicer-mediated processing of specific miRNA in oral cancer patients [73]. The expression levels of six oncogenic miRNAs (onco-miRs) were increased by Dicer–ADAR1 interaction. These results suggested that ADAR1-mediated miRNA maturation regulates the downstream gene pathways.

5. TRBP-LGP2 Interaction Inhibits Pre-miRNA Processing by Dicer

In virus infected mammalian cells, virus-derived RNAs are captured by viral sensor proteins such as retinoic acid-inducible gene I-like receptors (RLRs), inducing the production of type I interferon [74][75][76]. Recently, we reported that the TRBP-mediated maturations of pre-miRNAs were inhibited through the competitive binding of the laboratory of genetics and physiology 2 (LGP2) to Dicer–TRBP interaction during Sendai virus infection [77]. Interferons enhanced the expression of LGP2, which interacted with TRBP to inhibit the Dicer–TRBP interaction [78]. Following LGP2-dependent inhibition of Dicer–TRBP interaction, the maturations of TRBP-bound pre-miRNAs,

including miR-106b, were suppressed. The inhibition of the maturation of such miRNAs increased the expression of apoptosis-related genes downstream of miRNA processing. This finding suggested that the crosstalk between antiviral response and miRNA biogenesis is regulated by TRBP binding to the specific pre-miRNAs.

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