

Interaction Between Argonaute2 and RNA Molecules: AGO2 Molecular Structure and Different Regions in Nucleotide Chain

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Abstract. Argonaute2 (AGO2) is an important protein connecting the construction of RNA-induced silencing complex (RISC) and micro-RNA (miRNA) biogenesis. This paper explores the mechanism during the function through previous studies which reveal the crystal structure and affinity comparisons. It is concluded that the AGO2 have compartmentalized domains for certain functions, which have both individual and cooperative role in the entire process. The characters of AGO2 and miRNA suggest a model of region-different nucleotide chains in miRNA, which means its epigenetic information is based on base sequence and its special information. According to these findings, further studies are advised to monitor the reactions in a dynamical method, which would be a new potential entry point for clinical utilization.

1. Introduction

Epigenetic regulation is a systematic regulation of genetic expression levels without alteration to genetic codes. The currently most well-known and widely studied epigenetic modifications involve changes to DNA and histone proteins, which alter the structure of chromatin and influence the accessibility of DNA to transcription factors and other regulatory proteins, ultimately affecting gene expression. Compared to the more heritability and stability of this modification, RNA-involved mechanism and their effects on gene expression are generally considered more dynamic and reversible. (VAISSIERE, SAWAN and HERCEG, 2008) There are many epigenetic regulations with RNA participating: RNA-directed DNA methylation (RdDM), Long noncoding RNAs (lncRNAs), RNA editing and RNA interference (RNAi). RNAi, as one of the essential methods, is based on guidance by miRNA pairing to mRNA, which furtherly suppresses translation and degradation of the mRNA before translation. Therefore, a study to reveal this mechanism strikes a new direction for adjustment of cellular physiology, which has a foreseeable way to be applied clinically[1]. Because of their versatility and specificity, these non-protein-coding RNAs are more likely to serve as drug targets for multifactorial diseases, especially those for which a miracle drug is currently lacking.

This paper focuses on one detailed step in miRNA biogenesis. Firstly, a briefing on the biogenesis of miRNA indicates the detailed part, AGO2 interacts with double-strand miRNA for further formation of RNA-induced silencing complex (RISC), which would be discussed in this paper. Then, according to the AGO2 crystal structure,

it is able to conclude that the AGO2 has a specific molecular structure comprised by several functional domains, which work together to interact with miRNA based on its sequence and conformational information. In this process, miRNA shows high flexibility due to the compartmentalization of the entire chain. It reveals the function of different parts of the miRNA and molecular interaction during RISC formation.

These experimental data are from comparisons of the activities between the wild type and certain mutant alleles. Certain residue changes alter the conformational properties of interesting proteins and their interaction results with target molecules. To observe certain molecular structures in 3D view, an electron microscope is applied for some atom special positions. Electron microscopy (EM) is a powerful technique to study the molecular interactions between proteins and nucleic acids. For example, cryo-electron microscopy (cryo-EM) is used to study the transcription initiation complex and the ribosome. This technique helps analysis of the specificity and affinity between the miRNA and AGO2. The sequencing of the residues in the peptide chain and nucleotide chain gives information about proteins and RNA molecules.

2. Overall mechanism of miRNA biogenesis is for RISC

The processing of the miRNA begins with the transcription of DNA in the nucleus by RNA polymerase II or III, which depends on the types of target gene transcribed.[2] Transcribed products are called primary miRNA. Before it leaves the nucleus to the cytoplasm,

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several proteins would cut partial primary-miRNA segments and generate precursor-miRNA, which are called microprocessor often including RNase III enzyme Droscha and the dsRNA-binding protein DGCR8. After the cleavage, the pre-miRNAs are hairpin-shaped. Then precursor-miRNA is transported by exportin-5 (XPO5), which recognizes the pre-miRNA hairpins and binds through its interaction with the double-stranded stem region of the hairpin. This complex furtherly is furtherly transported through nuclear pore complex (NPC) to the cytoplasm. The NPC is a large protein complex that spans the nuclear envelope and acts as a gatekeeper to regulate the transport of molecules between the nucleus and cytoplasm.

In the cytoplasm, miRNA is generated and capable to guide the RISC for certain silencing in genetic expression. After entering cytoplasm, the precursor miRNA (pre-miRNA) is first recognized and bound by the double-stranded RNA-binding protein TRBP (transactivation response RNA-binding protein), which recruits the RNase III enzyme Dicer to the pre-miRNA. This step allows precursor miRNA to get further processing by Dicer because the TRBP functions for the recruitment of Dicer to pre-mRNA. Dicer is a type of large RNA protein which cleaves the precursor-miRNA into a short double-strand RNA by cutting off the precursor-miRNA loop region. However, there are some alternative pathways for miRNA biogenesis that do not require Dicer. Once the cleavage generates the double-strand miRNA, miRNA is capably bound by the AGO2. AGO2 unwinds the double-strand miRNA and organizes it into a certain orientation. The unwound miRNA has two strands, guide strands for RISC functioning and a passenger strand gets degradation during the RISC assembly[3]. Finally, the RISC is capable to silence specific mRNA and prevent further translation in cells.

3. Molecular structure of AGO2 promises the correct function of each component

In a general view of precursor-miRNA processing, AGO2 is one of the important roles in epigenetic regulation involving RISC. From the overall processes to detailed steps, AGO2 is an essential participant element in precursor-miRNA processing and miRNA loading tools for further RISC. Dicer is canonically considered a necessary component in miRNA production. However, the AGO2-dependent pathway has also been observed as no requirement for Dicer to participate. One example is the miRNA-451 is produced in an AGO2 mediating pathway, which not only directly represses the physiological metabolism of tumour cells, but also uses exosomes for secretion to alter the local microenvironment to repress the invasion and metastasis of tumour cells[4]. Highly conserved Argonaute protein family members have several important domains, which promise the proper function in the regulation of gene expression.

AGO2 protein is the first and most comprehensively studied protein in this family. AGO2 and AGO families

of proteins have four highly conserved domains from the amino acid sequence. The first is the N-terminal domain (N). Then there is the PIWI/Argonaute/Zwille (PAZ) domain. It is followed by the MID domain and p-element-induced wimpy tested (PIWI) domain[5]. The entire peptide chain would be folded into certain 3D structure for functional conformation. Each domain has its specific function and also works together with other domains even if they are not closed in simply adjacent view but in functional conformation.

Insight into the molecular structure reveals the principle of AGO2 interacting with RNA and other proteins. The 2.3-angstrom resolution crystal structure of human Ago2 was observed as a bilobed molecule. The bilobed structure consists of four globular domains (N, PAZ, MID and PIWI). This bilobed molecular structure has a cleft in the centre that binds guide and target RNA. The second to sixth Nucleotides in A heterogeneous mixture of guide RNA is an A-form conformation. This conformation allows the base pairing of the guide RNA with the target messenger RNA. In the region between No. 6 and 7 nucleotides, it is possible to combine miRNA target recognition and play a role in slicing RNA product release. There are tandem tryptophan binding pockets within the PIWI domain. This shapes an interaction surface for recruiting glycine-tryptophan-182 or other cofactors[6]. In the AGO2, the main function of the N-terminal is the catalysis of cleavage of the mRNA and initiating RISC activation. Compared to other members in the family, AGO2 has two motifs comprising to promise its catalytic activity, which is due to the requirement of the correct position of certain residues[7]. PAZ and PIWI domain (PPD) proteins form a ribonucleoprotein complex, RISC, with the RNA cleavage product of Dicer. RISC mediates gene silencing by targeting messenger RNA cleavage and translational repression. The PAZ domain is not essential for PPD protein-Dicer interactions. In contrast, the PIWI-box, a subregion of the PIWI domain in the PPD protein, binds directly to the Dicer RNase III domain through the activity of Hsp90. The binding of the PPD protein to Dicer inhibited the RNase activity of this enzyme in vitro. At the same time, PPD proteins and Dicer are present in soluble and membrane-associated fractions, suggesting that interactions between these two proteins may occur in multiple compartments. This suggests that the interaction between Dicer and PPD proteins is direct. Meanwhile, the Dicer RNase III domain is required for binding to PPD proteins[8]. According to studies of these domains, different parts of AGO2 ensure the RNA is loaded into a certain orientation and interacts with other proteins for the construction of the RISC.

4. AGO2 Interacts with miRNA for RISC to Function Properly

AGO2 separates the passenger RNA from spliced double-strand miRNA to mature RISC for further target mRNA silence with the remained guide RNA. This step produces RISC with ability of RNAi from the pre-RISC which contains AGO2 elements carrying the double-strand miRNA[9]. In vivo, a mutant allele of endogenous AGO2

was isolated with lost ability of RNAi as the defective splicing activities compared to wild type. Focus on a molecular level, guide RNA is removed from a stable complex containing AGO2 and guide RNA through complementary target RNA, which destabilizes the entire complex. This propensity for segregation would be enhanced by a mismatch between the 5' end of the target and guide RNA. It is also weakened by the mismatch with the guide RNA 3' end [10]. This means that the entire miRNA chain has a different functional region for interaction with AGO2 proteins. Common mRNA is in a chain shape, which means the AGO2 has different compartmental conditions for contacting with miRNA during the formation of RISC.

5. The miRNA is divided into several regions by AGO2

In animal cells, it has been found that siRNA and miRNA have a different mechanism for binding to target mRNA. Several domains are established by AGO2. From the 5' to 3' ends, there are the anchor, seed, central, 3' supplementary, and tail regions. Each of them has a specific biochemical role during binding to the RNA target, which leads to mRNA silencing and or viral RNA against. In most cases, the anchoring and tail-strand regions are located at 1 and 18 to 21 nucleotides, respectively. Their molecular structural properties aid the loading of miRNA onto AGO2 proteins. This part of the region helps ensure miRNA guidance to AGO2 after the passenger chain is removed. However, these terminal domains usually do not form base pairs with the target RNA, even if their sequence-predicted loyalties are paired [11]. Central base pairing, the ninth to twelfth bases, is required for target cleavage. If mismatch in the central region, it prevents RISC from acquiring a catalytically active conformation. The nucleotides in the seed region are located in the 2-7 bases of the miRNA, the most core binding target mRNA fragment. Studying the molecular structure, it is known that the AGO-bound miRNA seed sequence can be used for initial pairing to the target site. The pairing of partial miRNA seed regions to targets is not perfect. Thus, the extended pairing of the 3' part of the miRNA interacts to compensate, thereby producing pairs whose sequences are not exactly coincident. This is because sequences immediately adjacent to the seed can affect the targeted region. In the seed-pairing architecture, targets with miRNAs alone were generally less inhibited than targets paired with eighth position [12]. Also, the pairing between the 3' supplementary regions of the miRNA does not require wrapping the target RNA around the lead strand. This avoids topological problems when RISC cuts the target [11]. These results indicate the conformational and thermodynamical factors matter in the mechanism of AGO2 and miRNA binding for a certain function, which is based on and extends from simple miRNA sequence information.

6. Conclusion

Overall process of biogenesis of miRNA begins with the transcription in nucleus and is generated in cytoplasm through processing by Dicer and AGO. During this production in cytoplasm, AGO2 is considered as an essential role in loading into certain orientations and base-pairing to target mRNA for epigenetic regulation. In the molecular level, AGO2 has a special crystal structure comprised of 4 main domains for function in the formation of the RISC: N-terminal, PAZ domain, PIWI domain and MID domain. Its bilobed molecular structure created by PDD ensures the RNA molecules are embedded into its cleft. During its functioning, miRNA was recognized mainly by its seed region with the assistance of a 3' supplementary region and anchored through its affinity affected by its 3' and 5' terminal. It was found that the complementary sequence is not only conditioned during the mRNA target binding to miRNA but also affected by another region, the 3' supplementary region. Explanation of how miRNA has both high specificity and also flexibility in silence is supported by this finding.

According to this review, many details are shown during AGO2 processing double-strand miRNA. In future study, researchers are capable to explore the thermodynamics of the reactions in the molecular level when docking. It is the fundamental theory for development in pharmacology and clinical treatment. However, most studies in this field are still not under dynamic observation. Therefore, potential differences between the real physiological conditions and experimental phenomenon still remain unknown.

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