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Comparative In Silico Analysis of MicroRNA Binding Mechanisms in Plants and Mammals

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ABSTRACT

MicroRNAs (miRNAs) are essential regulators of gene expression in eukaryotes, playing critical roles in various physiological and pathological processes. While the conserved core functions of miRNAs are well-documented, significant gaps remain in understanding the contrasting mechanisms of miRNA-mediated gene regulation between plants and mammals. Specifically, the differences in target binding and regulatory outcomes are not fully elucidated, hindering a comprehensive view of miRNA functionality across biological systems. This study addresses this gap by investigating these differences using well-characterized examples of miRNAs: miR-156 and miR-172 in plants, and miR-21 and miR-29 in mammals. These miRNAs were selected because they are extensively studied and represent well-established models of miRNA function in their respective systems, enabling robust comparisons. Target prediction tools, such as psRNA Target for plants and TargetScan for mammals, were employed to analyze binding mechanisms. Bioinformatic analysis revealed multiple target genes for both plant and mammalian miRNAs. Comparisons of the best alignments indicate that plant miRNAs exhibit near-perfect complementarity with target mRNAs, leading to direct cleavage, whereas mammalian miRNAs bind with partial complementarity, primarily at the 3' UTR, resulting in translational repression or mRNA destabilization. These findings bridge the knowledge gap by providing valuable insights into the molecular interactions underlying miRNA-target gene regulation, highlighting the unique characteristics and broader implications of miRNA-mediated processes in diverse biological systems.

1. Introduction

MicroRNAs (miRNAs) are small, noncoding RNAs approximately 20 nucleotides long, found across mammals, plants, and viruses. According to miRBase version 22, the human genome is estimated to encode approximately 2,300 mature miRNAs, of which 1,115 are currently annotated [1]. Each miRNA can regulate the expression of tens to hundreds of genes, influencing nearly all cellular and metabolic processes [2,3]. Since their discovery in 1993, miRNAs have been recognized as key molecules in gene regulation [4].

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Plant-derived miRNAs are synthesized in the nucleus and undergo maturation in the cytoplasm. In the nucleus, MIR genes are transcribed by RNA polymerase II (Pol II) to produce primary miRNAs (pri-miRNAs), which are long transcripts containing hairpin structures. The stability of these pri-miRNAs is maintained by the Dawdle (DDL) protein. In plants, the key protein involved in miRNA processing is Dicer-like 1 (DCL1), a type III ribonuclease (RNase) responsible for cleaving pri-miRNAs into shorter fragments. This process is facilitated by two additional proteins: the dsRNA-binding protein Hyponastic leaves 1 (HYL1) and the C2H2 zinc-finger protein Serrate (SE), which enhance the efficiency and precision of cleavage activity. Pri-miRNA processing takes place within the D-body compartment of the nucleus. DCL1 cleaves pri-miRNAs in two sequential steps: first, it trims the imperfectly folded end of the pri-miRNA, forming a hairpin-shaped pre-miRNA. Then, it further processes the pre-miRNA to produce a double-stranded miRNA/miRNA* duplex [5]. In the final stage, one strand of the miRNA/miRNA* duplex is selected as the guide strand and incorporated into the Argonaute (AGO) protein to form the RNA-induced silencing complex (RISC), enabling it to carry out its regulatory functions. The remaining strand is discarded and subsequently degraded [6].

Meanwhile, in mammals, miRNA biogenesis occurs through either canonical or noncanonical pathways. In the canonical pathway, RNA polymerase II transcribes the miRNA gene, producing a primary miRNA that is processed by the microprocessor complex. This complex consists of the nuclear RNase III enzyme DROSHA and two copies of the double-stranded RNA (dsRNA)-binding protein DiGeorge Syndrome Critical Region 8 (DGCR8), ultimately forming the mature miRNA. In contrast, noncanonical pathways generate mature miRNAs from alternative precursors such as introns, DGCR8-independent miRNAs, small nucleolar RNAs (snoRNAs), and tRNAs [7,8].

Understanding the binding mechanisms of miRNAs in plants and mammals is critical for elucidating their regulatory roles and functional differences [9]. The mode of action of plant miRNAs is primarily determined by the degree of complementarity between the miRNAs and their target genes, whereas mammalian miRNAs primarily rely on interactions with untranslated regions (UTRs). Notably, plant miRNAs can also suppress their targets via translational inhibition, highlighting their dual regulatory mechanisms [10]. Despite extensive research, the distinctions in miRNA binding sites and their interactions with target genes across plant and mammalian systems remain poorly characterized, limiting a comprehensive understanding of miRNA functionality in diverse biological contexts. To bridge this knowledge gap, a bioinformatics approach was utilized to predict and analyze miRNA binding sites, uncovering both unique and shared features of miRNA targeting mechanisms in plants and mammals. This study focused on two well-established miRNAs from plants (miR-156 and miR-172) and two from mammals (miR-21 and miR-29), chosen for their extensively studied roles, to serve as robust models for a comparative analysis of miRNA binding mechanisms.

2. Methodology

2.1 MiRNA Sequence Retrieval

For plant miRNAs, miR156 and miR172 from *Arabidopsis thaliana* while for mammals, miR-21 and miR-29 from *Mus musculus* were selected, respectively. The selection of these miRNAs is based on their well-documented biological relevance and roles in critical regulatory pathways. All miRNA sequences were retrieved from miRBase database version 22 (https://www.mirbase.org/), a comprehensive and widely used repository for miRNA sequences and annotations [11].

These miRNAs were selected due to their extensive characterization and their status as well-established models of miRNA function within their respective systems, ensuring a solid foundation for robust comparative analysis. Their well-documented roles in regulating gene expression provide a reliable benchmark for studying miRNA-target interactions [8,16]. Additionally, selecting these

specific miRNAs helps mitigate the risk of false-positive predictions, which can occur due to inaccuracies or incomplete annotation of other miRNAs in databases. Many miRNAs in public repositories may not be accurately annotated or fully validated, leading to potential errors in target prediction and downstream analyses.

2.2 Target Prediction

Two target prediction tools were used in this study. For plant miRNA target prediction, psRNA target version 2 (Release 2007) (https://www.zhaolab.org/psRNATarget/) was used [12]. psRNATarget was designed to identify target transcripts for miRNA by: (i) analyzing the complementary pairing between the miRNA and its target using a predefined scoring system, and (ii) assessing the accessibility of the target site by calculating the unpaired energy (UPE). The tool was set to the "User Submit Small RNA" option, where the sequences for miR156 or miR172 were manually entered. Target prediction was conducted against *Arabidopsis thaliana* transcripts, excluding miRNA genes, using the TAIR database (version 10) with the default parameters of Schema V2 (release 2017).

For mammal target prediction, TargetScan version 8.0 (2021) (https://www.targetscan.org/vert_80/) was deployed [13]. This tool identifies biological targets of miRNAs by detecting conserved 8mer, 7mer, and 6mer sites that align with the seed region of each miRNA [14]. The tool was configured using TargetScan (Mouse), with *Mus musculus* selected as the species and miR-21 or miR-29 chosen as the target miRNAs.

The selection of psRNATarget and TargetScan as the bioinformatic tools for this study was based on their robust features, user-friendly interfaces, and suitability for the specific requirements of plant and mammalian miRNA target prediction, respectively.

3. Results

3.1 MiRNA Sequence Retrieval

The sequence of miR156, miR172, miR-21 and miR-29 were retrieved from miRbase database. The details were recorded as shown in Table 1.

Table 1
The information of miR156, miR172, miR-21 and miR-29 retrieved from miRbase

miRNA	Sequence (5' to 3')	Accession number
ath-miR156a-5p	UGACAGAAGAGAGUGAGCAC	MIMAT0000166
ath-miR172a	AGAAUCUUGAUGAUGCUGCAU	MIMAT0000203
mmu-miR-21a-5p	UAGCUUAUCAGACUGAUGUUGA	MIMAT0000530
mmu-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA	MIMAT0000535

The table presents essential information on four microRNAs (miRNAs): ath-miR156a-5p, ath-miR172a, mmu-miR-21a-5p, and mmu-miR-29a-3p retrieved from the miRBase database. The table includes specific miRNAs identified by species-specific prefixes, such as *ath* for *Arabidopsis thaliana* (a model plant species) and *mmu* for *Mus musculus* (mouse, commonly used in biomedical research). Each miRNA is listed with its nucleotide sequence in the mature, biologically active form, which guides the miRNA to complementary target mRNAs to regulate gene expression. Additionally, unique accession numbers from the miRBase database are provided, enabling researchers to access detailed annotations and associated data for each miRNA [11].

In the context of miRNAs, the designations 5p and 3p indicate the strands derived from the 5' and 3' arms of the precursor miRNA (pre-miRNA) hairpin structure, respectively. These strands are processed by the enzyme Dicer, and typically, only one known as the guide strand is incorporated into the RNA-induced silencing complex (RISC) to mediate gene silencing, while the other strand, referred to as the passenger strand, is usually degraded [5]. The selection of the 5p or 3p strand as the guide strand depends on factors such as the thermodynamic stability of the strand and the specific cellular context. For example, ath-miR156a-5p originates from the 5' arm of the miR156a precursor, while mmu-miR-29a-3p comes from the 3' arm of the miR-29a precursor. The importance of strand selection and its biological implications were emphasized by Gebert and MacRae (2018), who reviewed the structural and functional dynamics of miRNAs in guiding gene regulation [15].

3.2 Target Prediction by PsRNA Target

The targets and binding mechanisms of ath-miR156a-5p and ath-miR172a were identified using the psRNATarget prediction tool, and the target candidates are summarized in Table 2 and Table 3.

Table 2
The potential target candidates of ath-miR156a-5p are listed. Here, only the top five potential target genes are shown

Target Description (Symbol)	Expect value	UPE	Inhibition mode	Target Accession number
Squamosa promoter binding protein-like 3 (SPL3)	0.5	N/A	Cleavage	AT2G33810.1
Squamosa promoter binding protein-like 15 (SPL15)	1.0	N/A	Cleavage	AT3G57920.1
Squamosa promoter-like 11 (SPL11)	1.0	N/A	Cleavage	AT1G27360.2 AT1G27360.3
Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein (SPL13B)	1.0	N/A	Cleavage	AT5G50670.1
Squamosa promoter binding protein-like 9 (SPL9)	1.0	N/A	Cleavage	AT2G42200.1

Table 3
The potential target candidates of ath-miR172a are listed. Here, only the top five potential target genes are shown

Target Description (Symbol)	Expect value	UPE	Inhibition mode	Target Accession number
Target of early activation tagged (EAT) 2 (TOE2)	0.5	N/A	Cleavage	AT5G60120.1 AT5G60120.2
Integrase-type DNA-binding superfamily protein (AP2)	0.5	N/A	Cleavage	AT4G36920.1 AT4G36920.2
Target of early activation tagged (EAT) 3 (TOE3)	0.5	N/A	Cleavage	AT5G67180.1
Related to AP2.7 (RAP2.7)	0.5	N/A	Cleavage	AT2G28550.2 AT2G28550.1 AT2G28550.3
Integrase-type DNA-binding superfamily protein (SMZ)	1.5	N/A	Cleavage	AT3G54990.1

Tables 2 and 3 present predictions for the potential target genes of the miRNAs ath-miR156a-5p and ath-miR172a, as determined by the psRNATarget analysis. Both miRNAs are predicted to target a range of genes involved in key plant processes [16]. For ath-miR156a-5p, potential targets include

several members of the Squamosa promoter-binding protein-like (SPL) family, which play roles in regulating developmental transitions and flowering time, with Expect values as low as 0.5, indicating high confidence in the predicted interactions [12]. In *Arabidopsis*, the SPL gene family is a well-established target of miR156. Among the 17 SPL genes, 11 have been reported to be downregulated by miR156 via mRNA cleavage and translational inhibition. As plants age, the gradual decline in miR156 levels leads to an increased expression of SPL transcription factors, which promote flowering by activating FT, LFY, and MADS-box genes. Conversely, overexpression of miR156 in transgenic plants delays flowering and prolongs the juvenile phase [16].

Similarly, ath-miR172a targets genes such as the Target of early activation tagged (EAT) proteins (TOE2), and the Integrase-type DNA-binding superfamily protein (AP2). The inhibition mode for all these interactions is cleavage, and the identified targets are associated with genes related to plant growth and development, transcriptional regulation, and DNA binding. The Expect values, ranging from 0.5 to 1.5, further highlight the likelihood of these interactions [12]. This comprehensive analysis underscores the pivotal role of miRNAs in regulating gene expression, particularly genes involved in developmental timing and stress responses in plants. In *Arabidopsis*, miRNA172 targets the mRNA of the floral homeotic gene *AP2*, which plays a crucial role in floral development. Elevated accumulation of miRNA172 leads to defects in floral organ identity, resembling the phenotype of *ap2* loss-of-function mutants. Conversely, high levels of mutant *AP2* mRNA with disrupted miR172 binding sites result in abnormalities in floral patterning [16].

3.3 Target Prediction by TargetScan

The targets and binding mechanisms of mmu-miR-21a-5p and mmu-miR-29a-3p were identified by TargetScan, and the target candidates are summarized in Table 4 and Table 5, respectively.

Table 4
The potential target candidates of mmu-miR-21a-5p are listed. Here, only the top five potential target genes are shown

Target Description (Symbol)	Conserve site	Poorly conserve site	Target Accession number
G protein-coupled receptor 64 (Gpr64)	2 (8mer)	N/A	0112408.3
Cytoplasmic polyadenylation element binding protein 3 (Cpeb3)	1 (8mer)	1 (7mer-A1)	0079754.5
Kelch-like 15 (Klhl15)	1 (8mer)	1 (7mer-A1)	0113915.1
Family with sequence similarity 46, member A (Fam46a)	1 (7mer-m8)	1 (7mer-m8)	0034802.9
PR domain containing 11 (Prdm11)	1 (8mer) 2 (7mer-m8)	N/A	0111274.2

Table 5
The potential target candidates of mmu-miR-29a-3p are listed. Here, only the top five potential target genes are shown

Target Description (Symbol)	Conserve site	Poorly conserve site	Target Accession number
Tet methylcytosine dioxygenase 3 (Tet3)	2 (8mer) 4 (7mer-m8) 1 (7mer-A1)	2 (7mer-m8) 1 (7mer-A1)	0089622.5
A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 17 (Adamts17)	1 (8mer) 1 (7mer-m8)	 1 (7mer-A1)	0107478.2
Collagen, type IV, alpha 5 (Col4a5)	2 (8mer)	1 (8mer)	0112931.2
Tet methylcytosine dioxygenase 2 (Tet2)	2 (8mer) 2 (7mer-m8)	 1 (7mer-m8)	0098603.3
Collagen, type I, alpha 1 (Col1a1)	1 (8mer)	2 (7mer-A1)	0001547.7

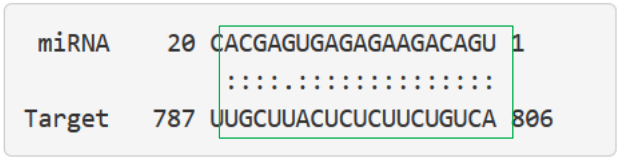
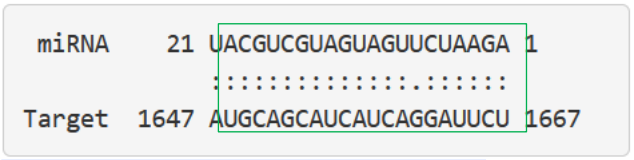
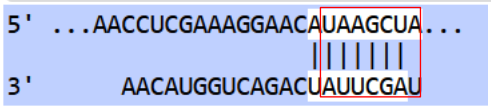
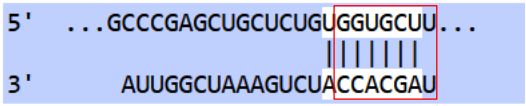
Based on Table 4 and 5, the analysis of the potential targets for mmu-miR-21a-5p and mmu-miR-29a-3p highlights distinct regulatory roles aligned with their biological functions, respectively. TargetScan predicts target genes by analyzing the binding complementarity between the seed region (nucleotides 2–8) of the miRNA and the 3' UTR of the mRNA target [17]. The strongest canonical target sites are 8mer sites, characterized by Watson–Crick pairing with miRNA positions 2–8 and an adenosine (t1A) opposite position 1. These are followed in strength by sites with complementary nucleotides 2–8 lacking a t1A and sites with complementary nucleotides 2–7 paired with a t1A [8, 15, 18]. For mmu-miR-21a-5p, the top targets, including Gpr64 (with two conserved 8mer sites) and Prdm11 (with a mix of conserved and poorly conserved sites), suggest its involvement in oncogenic pathways, focusing on cellular signaling, protein binding, and epigenetic regulation. miR-21 is a key contributor to fibrosis in various organs, including the kidneys. Lademirsen, a miR-21 inhibitor, targets pathways involved in ATP generation, ROS production, and inflammatory signaling. Early studies in Alport mice demonstrated that miR-21 silencing improved survival and mitigated glomerulosclerosis, interstitial fibrosis, tubular damage, and inflammation [8].

In contrast, mmu-miR-29a-3p targets such as Tet3 (comprehensive conserved and poorly conserved binding) and collagen-related genes Col4a5 and Col1a1 (important in extracellular matrix dynamics) point to its antifibrotic and epigenetic regulatory roles, particularly in fibrotic diseases. While mmu-miR-21a-5p exhibits primarily conserved site interactions reflecting its strong oncogenic impact, mmu-miR-29a-3p shows a combination of conserved and poorly conserved sites, consistent with its therapeutic potential in fibrosis and related disorders [19]. A prior study suggested that remlarsen, a synthetic mimic of miR-29, could serve as an effective therapy for preventing fibrotic scars, including hypertrophic and keloid scars, as well as cutaneous fibrosis conditions like scleroderma [8].

3.4 Comparison of the Binding Mechanisms of Plant and Mammalian MiRNAs

Table 6 below presents the alignment of plant and mammalian miRNAs with their targets. For simplicity, only the best predictions are included in the table.

Table 6
A comparison of the alignment between plant and mammalian miRNAs with their respective targets

miRNA and target	Alignment (s)
ath-miR156a-5p and SPL13	
ath-miR172a and TOE2	
mmu-miR-21a-5p and Gpr64	
mmu-miR-29a-3p and Tet3	

In plants, miRNAs exhibit perfect or near-perfect complementarity to their target genes, ensuring precise binding. For instance, ath-miR156a-5p and ath-miR172a (Table 6) bind perfectly to their respective targets (as indicated in the green boxes). This binding can occur within the 5' UTR, 3' UTR, or coding region, with the coding region being the predominant site. Such interactions primarily lead to the cleavage of the target gene, although translational inhibition may also occur as an alternative mechanism [17,20,21].

In contrast, mammalian miRNAs predominantly rely on seed region complementarity and conservation to recognize target sites, typically within the 3' UTR of mRNAs. The seed sequence refers to a highly conserved 6–8 nucleotide region at the 5' end of the miRNA, crucial for target recognition and binding. Binding can also occur in the 5' UTR or coding region [17,22,23]. For example, mmu-miR-21a-5p and mmu-miR-29a-3p (Table 6) bind to their targets through their seed sequence alone, rather than the entire sequence (as indicated in the green boxes). Consequently, while plant miRNAs serve as precise regulators, mammalian miRNAs act as versatile modulators, reflecting distinct evolutionary strategies in adapting to different biological complexities.

4. Conclusions

In conclusion, this study marks the distinct mechanisms by which miRNAs regulate gene expression in plants and mammals. While plant miRNAs generally exhibit perfect complementarity leading to target gene cleavage, mammalian miRNAs function through partial complementarity, predominantly in the 3' UTR, resulting in translational repression or mRNA destabilization. These differences highlight the diverse strategies evolved by miRNAs to control gene expression across species. Understanding these unique regulatory mechanisms provides deeper insights into miRNA function and their potential applications in therapeutic strategies targeting gene regulation. Additionally, to further validate miRNA-target interactions, experimental approaches such as luciferase reporter assays, real-time qPCR or degradome sequencing are recommended to confirm the biological relevance of predicted target genes.

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