Cellular and Molecular Life Sciences (2025) 82:197
https://doi.org/10.1007/s00018-025-05663-3

REVIEW

Decoding microRNA arm switching: a key to evolutionary innovation and gene regulation

Danillo Pinhal[1] · Leandro de B. Gonçalves[1] · Vinícius F. Campos[2] · James G. Patton[3]

Received: 4 December 2024 / Revised: 20 February 2025 / Accepted: 17 March 2025

Abstract

miRNA arm switching is a pivotal regulatory mechanism that allows organisms to fine-tune gene expression by selectively utilizing either the 5p or 3p strand of a miRNA duplex. This process, conserved across species, facilitates adaptive responses to developmental cues, environmental changes, and disease states. By dynamically altering strand selection, arm switching reshapes gene regulatory networks, contributing to phenotypic diversity and evolutionary innovation. Despite its growing recognition, the mechanisms driving arm switching—such as thermodynamic properties and enzyme-mediated processing—remain incompletely understood. This review synthesizes current findings, highlighting arm switching as a highly conserved mechanism with profound implications for the evolution of regulatory networks. We explore how this phenomenon expands miRNA functionality, drives phenotypic plasticity, and co-evolves with miRNA gene duplications to fuel the diversification of biological functions across taxa.

Keywords miRNA strand selection mechanisms · Post-transcriptional gene regulation · Evolution of gene regulatory networks · Phenotypic plasticity in development

Introduction

The world of microRNAs

Since its discovery in 1993 during studies on nematode development, microRNAs (miRNAs) have been identified in several eukaryotic groups, including humans [1]. These small, abundant molecules introduce an additional layer of complexity in genetic and physiological regulatory systems, through which evolutionary adaptive mechanisms operate. Over time, numerous discoveries have illuminated the regulatory functions of RNA, including aspects of its structure, biogenesis, interactions with biological pathways, and the role of natural selection in shaping these processes.

MiRNAs, like other non-coding RNAs, are recognized as fundamental regulators of various biological processes, including ontogenesis, cellular growth, differentiation, regeneration, and disease progression. For this reason, an expanding research field has been established over the last two decades, focusing on miRNA function, regulation, and evolution, and it continues to grow.

MiRNAs are small non-coding transcripts (~22 nucleotides) that regulate gene expression by binding to target mRNAs. A defining feature of miRNAs is the seed region, a short and highly conserved sequence spanning nucleotides 2–7 at the 5′ end. This region is crucial for target recognition, as it typically forms complementary base pairs with miRNA recognition elements (MREs) located in the 3′ untranslated region (3′UTR) of target mRNAs, thereby determining specificity. In addition to the seed region, downstream nucleotides—particularly nucleotide 8 and, to a lesser extent, nucleotides 13–16—also contribute to target binding, further refining miRNA–mRNA interactions [1]. Perfect base pairing between miRNA and its mRNA target leads to

Danillo Pinhal and Leandro de B. Gonçalves equally contributed to this work.

✉ Danillo Pinhal
danillo.pinhal@unesp.br

[1] Genomics and Molecular Evolution Laboratory, Department of Chemical and Biological Sciences, Institute of Biosciences, DCQB, IBB, UNESP, Botucatu, SP CEP 18618-689, Brazil

[2] Structural Genomics Laboratory, Graduate Program in Biotechnology, Technological Development Center, Federal University of Pelotas, Pelotas, RS, Brazil

[3] Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA

Published online: 10 May 2025

cleavage of the target transcript, similar to the mechanism of small interfering RNAs (siRNAs). However, the vast majority of miRNAs bind imperfectly to their targets, even within the seed region, leading to translation inhibition and eventual target degradation. Because of this mechanism, each miRNA can regulate multiple mRNA targets, exerting broad effects on gene expression networks.

Generally, miRNA genes are transcribed and processed by the enzymes Drosha and Dicer, resulting in a short RNA duplex. Each strand of this RNA duplex is referred to as a miRNA "arm", designated as either 5p or 3p. Both arms can be loaded into the RNA-induced silencing complex (RISC), a protein complex whose core component is an Argonaut (AGO) enzyme. Once loaded into RISC, one strand is preferentially selected while the complementary "passenger strand" is rapidly degraded. Different miRNA arms of the same precursor (pre-miRNA) can be preferentially selected by distinct RISCs [2]. This process, known as strand selection, determines which miRNA arm will actively regulate gene expression. However, miRNA arms of the same precursor (pre-miRNA) are not always selected in a fixed manner. Instead, arm selection can vary depending on tissue type, developmental stage, or species [1, 3, 4].

One or both arms may become enriched or silenced under specific conditions, leading to shifts in the relative proportion of miRNA strands. For example, *miR-21-5p*, a well-known oncomiR (a miRNA associated with cancer development), is upregulated in response to TGF-β (Transforming Growth Factor Beta) and BMP4 (Bone Morphogenetic Protein 4), despite *pri-miR-21* levels remaining unchanged [5]. While the precise molecular mechanisms driving strand selection shifts remain unclear, target mRNA abundance appears to be a key influencing factor [6]. These shifts, referred to as differential strand selection or alternative strand selection, involve variations in the relative usage of the 5p and 3p arms but do not necessarily lead to a complete dominance switch.

In contrast, arm switching occurs when the previously minor arm becomes the predominant strand loaded into RISC, reversing the usual selection pattern (Fig. 1). Since each arm has a distinct seed sequence and regulates different

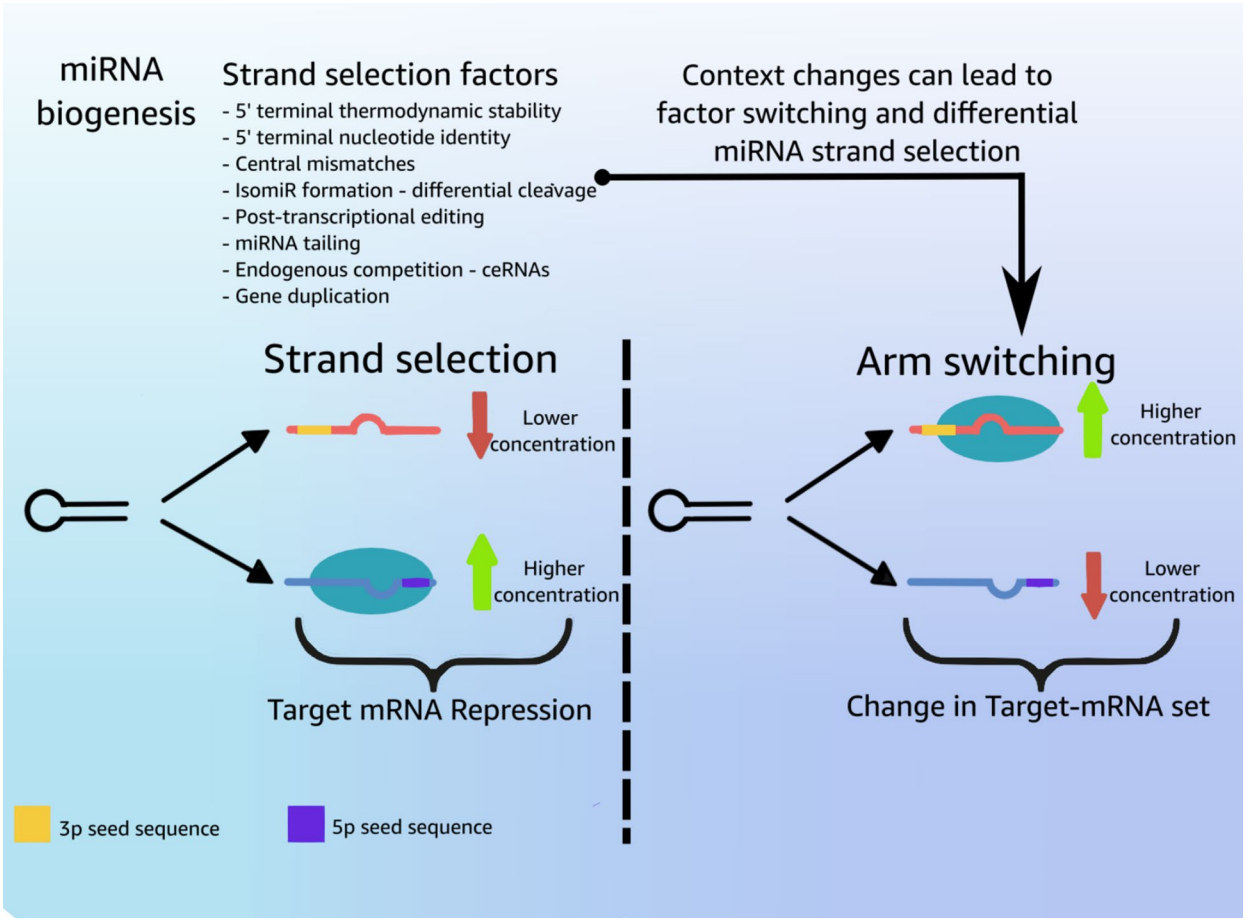

Fig. 1 *Arm switching* refers to events involving changes in the preferentially selected arm of a precursor miRNA, which presumably leads to alterations in the repertoire of target mRNAs. Various factors reviewed in this study may determine miRNA arm selection and potentially result in *arm switching*

target mRNAs, this process fundamentally alters the miRNA's regulatory network [7–9].

Moreover, arm switching is considered an active regulatory process, allowing for rapid and localized adjustments in miRNA function. This mechanism is believed to play a role in adaptive responses across different eukaryotic taxa. In humans, arm switching has been linked to various diseases, including cancer, positioning it as a potential biomarker for tumor development [10]. Characterizing arm switching events could provide valuable insights for biomedical and biotechnological applications.

Despite the growing recognition of miRNA arm switching and its functional and evolutionary significance, many questions remain unanswered, particularly regarding the molecular determinants of strand selection. This study aims to systematically review current knowledge on arm selection mechanisms and arm switching events, particularly in animal groups, while discussing their causal factors, underlying biological processes, and broader evolutionary implications.

Canonical miRNA biogenesis in metazoan

The miRNA biogenesis pathway is well described (Fig. 2) [1, 11, 12]. During biogenesis, miRNA genes are transcribed by RNA polymerase ll (Fig. 2a) with nuclear (Drosha) and cytoplasmic (Dicer) processing steps. Nascent initial transcripts are known as pri-miRNAs (primary miRNAs) that are usually capped at their 5′ ends and contain a poly (A) tail at their 3′ ends and include multiple stem-loops or hairpin secondary structures (Fig. 2b) [11, 13, 14]. Within the nucleus, the first processing step occurs, mediated by the Microprocessor Complex, composed of the metazoan conserved enzyme Drosha and coenzyme DGCR8 (DiGeorge syndrome critical region 8; homologous to Pasha in *Drosophila melanogaster* and PASH-1 in *Caenorhabditis elegans*) (Fig. 2b). The Drosha-DGCR8 complex exhibits endoribonucleolytic activity, typical of RNase III enzymes and recognizes hairpins and cleaves the pre-miRNA 11 pb from the base of the double-stranded region, leaving a two-nucleotide 3′ overhang and exposing a 5′ phosphate. This overhang has a crucial role in miRNA recognition due to its resulting structural thermodynamic asymmetry [15–17]. Of note, approximately 15% of miRNAs are classified as miRtrons that are encoded in introns and are processed independent of Drosha.

After nuclear cleavage, the resulting 65–70 nucleotide precursor miRNA (pre-mRNA) containing a single stem-loop structure (Fig. 2c) [14] is exported to the cytoplasm via Exportin 5 (XPO5) and the RAN-GTP/GDP (RAs-related Nuclear protein-GTPase) concentration gradient (Fig. 2c) [18, 19]. Once in the cytoplasm, the pre-miRNA is recognized by Dicer, another conserved RNAse lll family enzyme. Dicer is often referred to as a "molecular ruler" because it can "measure" a set of nucleotides from the terminal end of pre-miRNA and cleaves at this precise distance due to precise association of its catalytic domains with the RNA. Pre-miRNA terminal overhangs are generated by the PAZ (PIWI-Argonaute-ZWILLE) domain from the Dicer enzyme (Fig. 3a). In vertebrates, Dicer works alongside two auxiliary paralogous proteins TRBP (Transactivation Response element RNA-Binding Protein) and PACT (Protein ACTivator of PKR), which recognize double-stranded RNAs (dsRNAs) through dsRBP domains. Dicer cleaves pre-miRNA adjacent to the loop region according to its molecular ruler property (Fig. 2d).

Two methods exist for determining the precise cleavage point within pre-miRNAs. The first is if the 5′ terminal

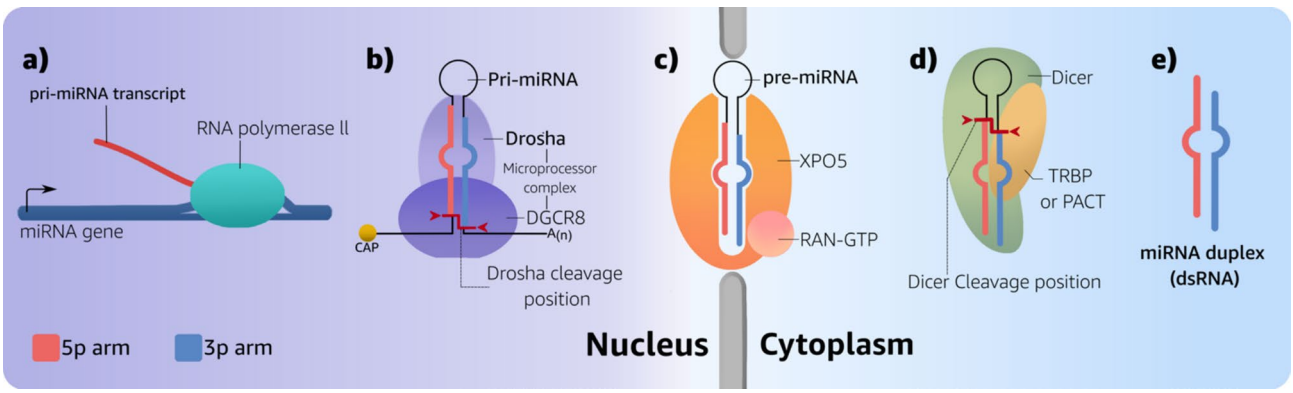

Fig. 2 miRNA biogenesis. **a** The transcription of the miRNA gene is carried out by RNA polymerase II. **b** The primary transcript, *pri-miRNA*, is incorporated into the microprocessor complex composed of Drosha and DGCR8, which cleaves the *pri-miRNA* 11 nucleotides from its basal junction, represented by the red line and arrows. **c** The resulting molecule is the *pre-miRNA*, which is exported to the cytoplasm by XPO5 in association with RAN-GTP. **d** In the cytoplasm, the complex formed by Dicer, TRBP, and/or PACT (in humans) cleaves the loop region of the *pre-miRNA*. Dicer is recognized as a "molecular ruler" for its ability to measure 22 nucleotides for cleavage. **e** A duplex of miRNA arms is formed, one of which will be incorporated into the RISC

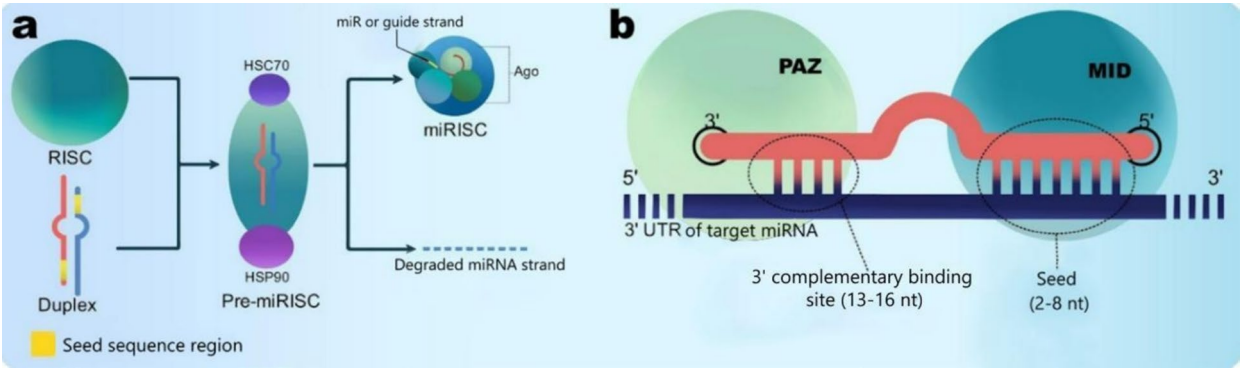

Fig. 3 Incorporation of the duplex into the RISC and miRNA-mRNA target interaction. **a** The RISC incorporates the miRNA duplex (pre-miRISC) with the assistance of the chaperones HSP90 and HSC70, which induce conformational changes in the Ago protein. After the passenger strand is ejected, it is rapidly degraded, leading to the formation of the active miRISC. **b** Target recognition by the miRISC occurs through the identification of the 3′ UTR regions of mRNAs via MREs (miRNA recognition elements), particularly involving the complementary region 3 (13–16 nucleotides), but predominantly through the seed region (2–8 nucleotides)

portion is relatively more stable, the pre-miRNA binds into the 3′ PAZ domain of Dicer and counting initiates from the 3′ terminus with cleavage between 21–25 nt from this terminus. Alternatively, in certain instances where the 5′ pre-miRNA terminus is relatively unstable due to mispairing, the miRNA is adjusted to the platform domain of Dicer, shifting the nucleotide counting and resulting in a cleavage point 22 nt from the 5′ terminus (5′-counting rule) (Fig. 2a) [20]. This distinct cleavage system may lead to the generation of miRNA isoform variants, also known as isomiRs [21, 22].

The product of Dicer cleavage typically results in a dsRNA molecule with two complementary stands (arms) and a two nucleotide-long 3′ overhang (Fig. 2e). The arm typically found in biological samples is referred to as the "guide strand" or the mature miRNA, while the less prevalent complementary arm is called the "passenger strand" or star strand (miR*) [1, 23]. However, the current trend is moving away from this nomenclature. Instead, it is now recommended to use suffixes 5p or 3p [24] to denote miRNA sequences, indicating their position within the pre-miRNA structure. For example, a miRNA arm generated from the 5′ position of a pre-miRNA is labeled a 5p arm (Fig. 2e).

The next step of miRNA processing involves the duplex loading into an RNA-induced silencing complex (RISC) (Fig. 3), resulting in a temporary complex miRISC (miRNA + RISC) with one or more members of the Argonaute protein family (AGO). AGO proteins play the effector function for mRNA silencing.

In animals, different orthologs encode AGO proteins, which are classified into three groups: AGO proteins, similar to *Arabidopsis thaliana* AGO1 which are capable of interacting with miRNAs and other siRNAs, PIWI proteins which are mainly expressed in the germline to control transposable elements and maintain genomic integrity through interaction with piRNAs. (PIWI-interacting RNAs; another class of small RNA), and WAGO proteins which are ubiquitous in nematodes [25]. There are two copies of genes encoding AGO proteins (AGO1 and AGO2) in flies, and there are 4 copies (AGO1-4) in mammals. AGO proteins have four domains: N, PAZ, MID, and PIWI (Fig. 4). The N-terminal domain performs duplex unwinding during the RISC loading. The PAZ (Piwi/Argonaute/Zwille) domain recognizes and binds to the 3′ terminal end. Residues located between the MID (middle) and PIWI (P-Element induced wimpy testis) domains form a region for recognition of 5′ nucleotides. In humans, the AGO2 PIWI domain exhibits endoribonuclease activity known as "slicer" function [26–28].

Within the RISC, the loaded miRNA serves as a template sequence that imperfectly matches to target mRNA 3′UTR through Watson–Crick pairing. Nucleotides 2–8 located at 5′ termini correspond to the seed region and generally pairs with more complementarity than the rest of the miRNA [1] (Fig. 3b). Different miRNA genes can generate mature miRNAs with the same or very similar seed sequences (miRNA families) and it is assumed these miRNAs recognize and regulate overlapping sets of target mRNAs [29]. There are many examples of miRNAs that recognize MREs with imperfect seed pairing [30]. These pairing arrangements often extend to regions beyond the seed sequence to compensate for mismatches in the seed region or even shifting the binding motif to nucleotides 11–20 located on the nt 3′ portion of miRNA, despite this pairing structure showing insufficient [31]. Once the target mRNAs are identified, AGO and other proteins within the miRISC are responsible for carrying out effector function leading to translation repression, destabilization, and eventual degradation of the mRNA targets thereby altering the RNome. Evolutionarily, target mRNAs recognized by imperfect pairing can drift allowing non-canonical MREs to mediate relevant biological regulation [32–34].

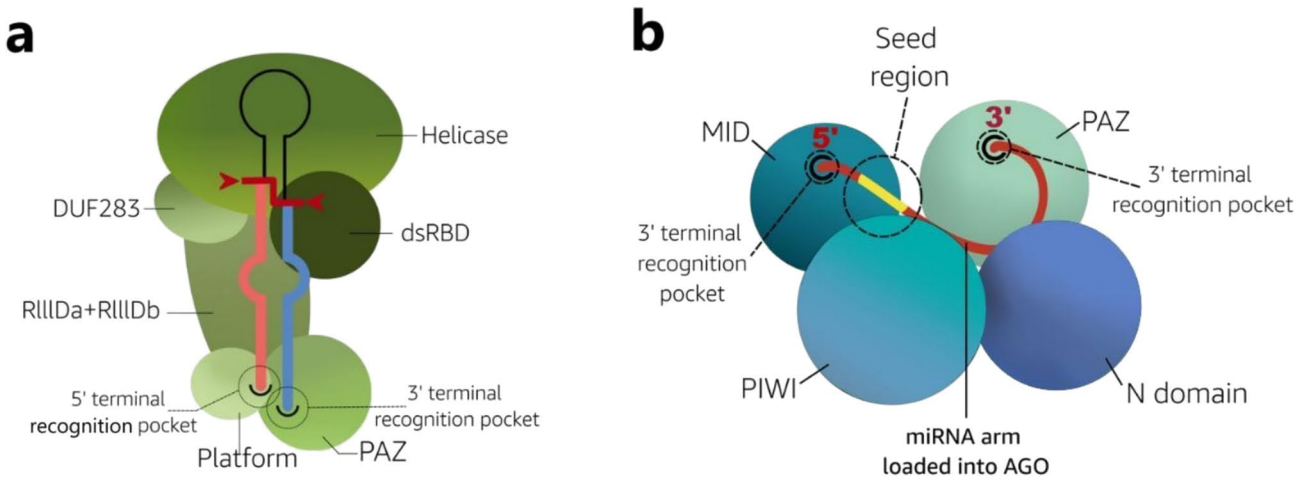

Fig. 4 Structures and domains of the Dicer and Argonaute 2 (Ago2) proteins. **a** The Dicer structure consists of the following domains: Helicase, responsible for recognizing and cleaving the loop; DUF238; Platform, responsible for recognizing the 5′ end of the pre-miRNA; PAZ, responsible for recognizing the 3′ end of the pre-miRNA; and the RNase III catalytic domains (RIIIa/RIIIb), which recognize and cleave the regions indicated by red arrows. **b** The structure of Ago2, the key component of the RNA-induced silencing complex (RISC), includes the MID and PIWI domains, whose interface recognizes the 5′ end of the guide strand, the N domain, which is responsible for duplex unwinding, and the PAZ region, which recognizes the 3′ end of the miRNA. The seed region exposure is highlighted in yellow

MiRNA strand selection mechanisms

Strand selection or arm selection is a nonrandom step of miRNA biogenesis through which one strand of duplex is selected for loading into RISC and, in this way, driving a distinct 5p/3p stoichiometric ratio. The prevalence and abundance of each arm in a given cellular context is referred to as arm usage and it has a direct influence on cell phenotype expression, as changes in the loaded miRNA sequence lead to alteration in the set of targets. This highlights strand selection as a context-dependent, biased process that fine-tunes gene regulation.

As mentioned, only one of the duplex strands is loaded into RISC. From small RNAseq data, one arm is usually favored over its complementary strand, generating a 5p/3p relative abundance ratio. In *D. melanogaster*, miRNA loading readily occurs with Ago1 due to alterations in positions 9–10 of the duplex. However, incorporation of the duplex into Ago2 is possible through the RNA-Induced Silencing Complex (RISC)-Loading Complex (RLC) composed of Dicer (Dcr-2) and an accompanying enzyme R2D2 [35–37]. In *C. elegans,* loading occurs through the action of the ALG family (Argonaute-like Genes) [38]. In mammals, all four members of the Argonaute family can associate with miRNAs with a preference for duplexes containing internal mismatches at nucleotide positions 8–11 [39, 40]. Although different Argonaute family members exhibit distinct characteristics, there appears to be no significant difference in miRNA loading activity among these proteins in mammals [41].

The mechanisms that determine strand selection are not completely elucidated, but evidence suggests that the determination of which strand is preferentially selected is driven by the thermodynamics properties of miRNA duplex and the biogenesis enzymes involved. The consensus is that the two main properties are: (I) 5′ terminal thermodynamic stability and (II) 5′ nucleotide identity. These thermodynamic properties contribute simultaneously and, following this framework, there are two methods of passenger strand ejection, either slicer-independent or slicer-dependent [41].

Recent observations have shown cases where the prevalence of a miRNA arm does not consistently follow the established rules, suggesting that additional factors may influence strand selection. In many cases, mature miRNAs previously established as the guide strand, either due to expression prevalence or thermodynamic properties, were found to not be the predominant miRNA in different tissues. For example, Ro et al. [3] analyzed human miRNA sequences accessible in the miRBase database and discovered that 75% of miRNAs classified as guide strand (57 out of 76 pairs) did not match with the expected arm selection dictated by the thermodynamic rule. They also identified 32 pairs expressed in other species that were not deposited in miRBase which did not follow this rule. Medley et al. [24] performed a separate analysis, considering the 5′ nucleotide identity, and concluded that these two rules alone are insufficient to determine strand selection. By analyzing miRBase data of *C. elegans*, *D. melanogaster*, and *H. sapiens*, Kozomara et al. [42] found that more than half of miRNA strand selection is not explained by any of these collective properties with between 17 and 25% not explained by any of these

properties individually. Moreover, there are cases, such as human *miR-34*, for which annotations indicate distinct functions for each arm, yet they are expressed in significantly equivalent 5p/3p ratios in certain tissues [43, 44] making it difficult to determine which is the guide strand. This shows that the 5p/3p ratio is not constant and can fluctuate depending on cellular context [3, 45]. Considering these findings, the stoichiometric relationship between simultaneously produced arms, or the context-dependent pattern of miRNA expression, is often referred to as arm usage. This demonstrates that the bias in arm selection is not fixed and varies with the organism's context [7, 8]. Arm usage can vary not only among different tissues but also the 5p/3p ratio can fluctuate based on cell context, tissue specificity, developmental stage, sex, environment, and pathophysiological states [3, 4, 46–69]. Additionally, arm usage can be interspecifically inconsistent, meaning that the 5p/3p ratio pattern can be different when comparing the miRNA expression profile in homologous tissues in different species. This is thought to be a factor involved in the variability of eukaryotic biodiversity of phenotypes [7, 8, 45, 70–75] These findings indicate that there is an active mechanism for modulating miRNA expression profiles depending on context. Thus, identifying the factors and properties that influence strand selection and arm usage across various contexts is crucial for understanding this mechanism.

Differential strand selection and arm switching

The prevailing view for the past ~ 10 years has been that the so-called "passenger strand" is discarded and immediately degraded by cell machinery. However, advancements in high-throughput sequencing have enabled the detection of passenger strands in tissues, even at low but physiologically relevant concentrations [3, 23, 76]. Like guide strands, passenger strands can generate functional mature miRNA sequences that interact with mRNA targets and promote silencing [3]. The conservation of passenger strands within the genome suggests that these sequences play an important biological role, even if not directly involved in target repression. If passenger strands were inert, conservation of bases beyond the terminal sequences would be unlikely [76, 77]. Specific modifications to the wild-type ALG that lead to differential strand selection have a more prominent impact on the biology of the model than the total deletion of its gene, which underscores the importance of proper arm selection [78, 79]. This contrasts with initial conceptions that attached limited importance to passenger strands as these were rarely detected in biological samples. It is now understood that the regulatory capacity of miRNAs occurs not only through the action of the preferentially selected strand, but also through the set of complementary strands, the relative abundance of 5p/3p arms and its concentration towards the targets in the tissue in a given context [76, 80].

The variation in patterns of arm usage switching in response to cellular contexts indicates that strand selection is driven by processes within the cells that actively regulate the abundance of miRNAs. Situations have been observed where this process leads to a total inversion of selective strand preference, resulting in opposite 5p/3p ratios. Although they remain understudied, arm switching events are of profound importance in the evolution and diversification of new miRNAs and functions in eukaryotes, contributing to the fine-tuning of phenotype regulation and playing a significant role in the adaptive processes [7, 81]. Additionally, arm switching is particularly relevant in early development, where it refines gene regulatory networks during tissue differentiation and morphogenesis. Arm switching events have been observed across a variety of biological processes, organisms, and tissues (Table 1).

A notable subset of these documented arm switching events occurs during early development, where shifts in arm preference refine gene networks in response to differentiation cues. For example, in *Gallus gallus*, deep sequencing of embryonic tissues revealed distinct arm switching events at different developmental stages, particularly during the formation of neural and muscular structures [53]. Similarly, in *Bombyx mori*, a detailed temporal analysis uncovered sequential shifts in strand selection during metamorphosis, indicating that arm usage is adjusted in response to major physiological transformations [51].

At the tissue-specific level, differential arm selection has been documented across various vertebrate organs. In *Danio rerio*, arm switching events in miRNAs such as *miR-202* influence sex differentiation, with gonad-specific shifts in 5p/3p expression regulating key reproductive pathways [55]. In mammals, *miR-34* exemplifies how strand selection can influence tumorigenesis, as its 5p and 3p isoforms regulate distinct oncogenic and tumor-suppressive pathways [43, 44]. Likewise, in immune regulation, *miR-146a* exhibits differential arm usage depending on inflammatory conditions, with shifts between 5 and 3p strands altering key immune-related pathways [68].

While these developmental shifts in arm usage shape early differentiation pathways, post-embryonic arm switching events further modulate phenotypic expression in response to environmental and physiological changes. Beyond these examples, the biological consequences of arm switching extend to structural interactions within the RISC complex, affecting how miRNAs recognize and regulate target genes. This mechanistic interplay is crucial to understanding the trade-off between complementary strands and their associated functions.

Arm switching has an important impact on the biology of organisms because the arms or strands are antisense paired

Table 1 Identified arm switching events in literature review

Arm switching category	Compared organisms	Compared tissues/organs	Annotated biological contexts	Source of analyzed sequences	References
Development	*G.gal*	Embryo	Embryos(5, 7 e 9 days)	in vivo	[53]
Development	*B. mor*	–	Larval, pre-pupal, pupal and imago	In vivo	[51]
Development; Interspecific; Intertissue	*T.tri, C.rot, I.sca, D.pul, T.cas, D.mel, H.mel* e *A.aeg* (Arthropoda)	Leg muscle (*T.tri* e *C.rot*)	Development and intertissue (*T.tri vs. C.rot*); Interspecific (all organisms)	in vivo *(T.tri e C.rot);* other organisms: miRGeneDB	[75] (supplementary material)
Development; Intersexual	*O.nil*	Heart (female), brain (male and female), gonads (male and female),white and red skeletal muscle (male and female), eye and liver	Development: embryos vs. adults;	In vivo	[54]
Development; intertissue; intersexual	*D.rer*	Ovary, testicle, eye, heart, brain, intestine, liver and embryos	Dev.: (2.5 horas post-fertilization- hpf), spherical (4hpf), shield (6hpf) and 24hpf	Database (NCBI SRA)	[62]
Health condition	*H.sap*	Diverse cancer cell lineages and adjacent (normal) tissue	Cancer	Database (TCGA). in vivo	[10]
Health states; Intertissue	*H.sap; M.mus e R.nov* (Mammalia; Euarchontoglires)	256 sRNA library of 26 organs from diverse cell lineages	Tissues; adult and embryos; normal, e Tumor	in vivo	[4]
Health state	*H.sap*	Cell L-02 (normals hepatocytes HepG2 (hepatoma)	Cancer	Obtained from other studies (miRBase)	[109]
Interspecific	*C.car; D.rer; O.lat; A.bur; M.zeb; N.bri; O. nil; P.nye* (Teleostei)	blood, brain, eye, embryo, heart, kidney, liver, muscle, ovary, skin and testis	health; *O.nil, M.zeb, A.bur, N,bir* = female. *P.nye* = male. *M.zeb* caught in the wild	in vivo (cichlids: *A.bur, M.zeb. N.bri, O.nil, P.nye*). miRBase (others)	[65]
Interspecific	*D.mel, T.cas, C.ele, C.tel, H.sap, C. int, B.flo* e *N.vec* (Eumetazoa)	–	Adult; normal	In vitro *(T.cas, D. mel);* other organisms obtained from miRBase	[7]
Interspecific	*C.ele, C.bri* e *C.rem* (Nematoda)	–	Normal; Diverse developmental stages	In vivo	[70] (supplementary material)
Interspecific	*T.cas, D.mel, B.mor* (Insecta)	–	Embryos with 0–5 days (*T. cas*)	In vivo (*T.cas, D,mel*); Obtained from Cai et al., 2010 (*B.mori*)	[8]
Interspecific	*T.cas, D.mel, B.mor* e *S.Fru* (Insecta)	cellular lineages (Sf9 (*S. fru*)	Cell culture (*S.fru*)	In vitro (*S.fru*); Database (miRBase; *D.mel* e *T.cas*)	[71]
Interspecific	*P.tep, T.cas* e *D.mel* (Arthropoda)	Embryos	Normal; stages 1–10 (*P.tep*)	In vivo (*P.tep*) *e* Databases (miRBase; *D.mel* and *T.cas)*	[73]
Interspecific	*H.hol, T.cor, D.mel* e *T.cas* (Arthropoda)	–	Imago; normal (*H.hos* e *T.cor*)	in vivo (*H.hol, T.cor);* Obtained from other works	[74] (supplementary text)
Interspecific	*B.ter* e *A.mel* (Insecta; Hymenoptera)	–	Larvae; female; normal	In vivo *(B. ter);* Database *(*miRBase*; A. mel)*	[72]

Table 1 (continued)

Arm switching category	Compared organisms	Compared tissues/organs	Annotated biological contexts	Source of analyzed sequences	References
Intersexual	*H.hip*	Brain and gonads	Sexual development	In vivo	[56]
Intersexual	*H. sap*	354 *datasets* from human tissues	Gender: male and female; normal and tumor tissues	*Database* (NCI-TCGA)	[57]
Intertissue	*S.Mal*	Rhopalium vs. tentacles	Adult (medusa); normal	In vivo	[50]
Intertissue	*M.Mus*	Stomach vs. spleen	Normal	In vivo	[3]
Intertissue	*H.sap*	61 human tissues	–	in vivo	[49]
Intertissue	*H.sap e M.mus* (Mammalia; Euarchontoglires)	15 tissues from *M.mus* and 9 cellular lineages from *H.sap*		in vivo	[20]
Intertissue	*H. sap*	Brain, heart, kidney, liver, lung, ovary, placenta, spleen, testicle and thymus	Normal;	Database (NCBI SRA)	[46]
Intertissue	*H.sap*	410 sRNA *datasets* divided in 15 tissues and cellular lineages	Normal and diseased	Database (NCBI SRA)	[47]
Intertissue	*H.sap*	Blood; brain; breasts; cervix; epithelium; embryo stem cells; liver; lung; skin	Diverse cellular lineages	Database (NCBI GEO)	[48]

Studies that identified arm switching events of miRNAs were found in this review. A.aeg = *Aedes Aegypti*; A.bur = *Astatotilapia burtoni*; A.gam = *Anopheles gambiae*; A.mel = *Apis mellifera*; A.mal = *Sanderia malayensis*; B.flo = *Branchiostoma floridae*; B.mor = *Bombyx mori*; B.ter = *Bombus terrestris*; C.bri = *Caenorhabditis briggsae*; C.car = *Cyprinus carpio*; C.ele = *Caenorhabditis elegans*; C.int = *Ciona intestinalis*; C.ram = *Caenorhabditis remanei*; C.rot = *Carcinoscorpius rotundicauda*; C.tel = *Capitella teleta*; D.mel = *Drosophila melanogaster*; D.pul = *Daphnia pulex*; D.rer = *Danio rerio*; G.gal = *Gallus gallus*; H.hip = *Hippoglossus hippoglossus*; H.hol = *Helicorthomorpha holstii*; H.mel = *Heliconius melpomene*; H.sap = *Homo sapiens*; I.sca = *Ixodes scapularis*; M. zeb = *Metriaclima zebra*; M.mus = *Mus musculus*; N.bri = *Neolamprologus brichardi*; N.vec = *Nematostella vectensis*; O.lat = *Oryzias latipes*; O.nil = *Oreochromis niloticus*; P.nye = *Pundamilia nyererei*; P.tep = *Parasteatoda tepidariorum*; R.nov = *Rattus norvegicus*; S.fru = *Spodoptera frugiperda*; T.cas = *Tribolium castaneum*; T.cor = *Trigoniulus corallinus*; T.tri = *Tachypleus tridentatus*

in a duplex. For this reason, the seed region, other MREs and molecular sites do not directly pair (Fig. 4a). During arm switching, alternative RISC loading can alter the repertoire of affected mRNA targets and repression or regulation of a divergent set of genes. This, in turn, can lead to variations in phenotypic expression [20, 82]. Given the dichotomy of strand selection (i.e., selection of 5p or 3p by RISC), arm switching events imply the suppression of certain regulatory pathways and biological functions in favor of amplifying others. This suggests a regulatory trade-off between complementary miRNA strands, where their associated functions compete in a context-dependent manner. For example, ratio alterations of *miR-155* in humans occur according to the plasmacytoid dendritic cell (pDCs) activation period. *miR-155* arms have antagonistic effects on cytokine production. The *miR-155-3p* strand, previously classified as a passenger strand, shows increased expression upon stimulation of Toll-like receptor 7 (TLR7) in pDCs, whereas *miR-155-5p* is reduced. At 4 h after stimulation, *miR-155-3p* is highly expressed leading to the promotion of cytokine expression. About 8 h later, the 5p/3p ratio inverts, with *miR-155-5p* becoming more prevalent and suppressing cytokine expression [83–85]. The increment of one biological function at the cost of suppression of another by changes in the relative expression of a given miRNA arm may confer adaptive advantages [7, 70]. Therefore, arm switching has evolved as an endogenous biologic mechanism that allows miRNA expression profiles to fluctuate dynamically regulating distinct sets of target mRNAs and biological pathways. This ensures that organisms maintain fitness across a range of environmental conditions. However, the molecular processes underlying the context-dependent regulation of miRNAs arms remain only partially understood.

Arm switching is considered a comparative phenomenon, meaning its detection requires an analysis of miRNA expression profiles in different biological contexts. As a result, arm switching can be subclassified as (I) Intraspecific, when differential miRNA arm expression occurs within the same species, across different tissues or conditions, and (II) Interspecific, when the differential expression of a miRNA arm occurs in homologous tissues of different species.

Phylogenetically close species tend to show similarity in terms of miRNA gene composition but less frequently in terms of miRNA expression profiles, with this similarity diminishing as phylogenetic distance increases. Griffiths-Jones et al. [7] suggested that miRNA expression provides a mechanism for the emergence of novel phenotypes, which are then shaped by natural selection. Despite the growing number of reported cases, there is no accurate estimate of the overall frequency of arm switching events. However, some studies have attempted to quantify this process. Kim et al. [20] used accurate quantification by sequencing (aq-seq) on 15 mouse tissues and 9 human cell lineages, identifying that approximately 13% of miRNAs (with a standard deviation of $\sigma < 3\%$) display fluctuations in miRNA arm ratios. Among *D. melanogaster* and *Tribolium castaneum*, the rate of arm switching is ~ 11% [8]. These data suggest that not all complementary miRNAs undergo arm switching, indicating that this process is highly regulated rather than a stochastic occurrence. As a result, arm switching adds another dimension of complexity to miRNA expression and function, influencing evolutionary dynamics by creating an additional layer of regulatory plasticity. Since the two miRNA strands compete for RISC loading, each has the potential to regulate distinct biological functions and undergo independent selective pressures.

Thus, understanding the mechanisms governing miRNA arm selection is essential for elucidating how these processes influence cellular function, development, and evolutionary adaptation. The following section describes the molecular and environmental factors that regulate arm selection and arm switching.

Factors regulating arm selection and arm switching

Intrinsic to dsRNA

Relative thermodynamic stability of pre-miRNA 5′ termini

Early studies on bias in arm selection sought to understand this phenomenon on RNAi (RNA interference) molecules. Intrinsic properties of miRNAs, specifically those of their precursors pri-miRNA and pre-miRNA, play a crucial role in the duplex unwinding and its loading into RISC. In 2003, two studies conducted by Khvorova et al [86] and Schwarz et al. [87] arrived at similar conclusions when investigating the physicochemical properties that lead one strand (i.e. guide strand) to be incorporated into mRNA silencing complexes while the complementary strand (i.e. passenger strand) is discarded. Both studies found evidence that the miRNA strand with less relative thermodynamic stability at its 5′ terminus (i.e. with less stable hydrogen bounds) is more common in the guide strand. The proposed explanation for this finding is that the strand with less internal stability at the 5′ terminus is more likely to be selected by AGO. A single mismatch within the first four 5′ nucleotides determines guide strand selection [88]. Initial studies indicated that RISC loading in humans does not require ATP and ATP would be consumed by a hypothetical helicase [89, 90]. However, experiments performed by Kawamata and Tomari research group found that fly and human AGOs indeed require ATP for miRNA duplex loading, but not for unwinding nor passenger ejection [89–93].

From this, the question arises: where does the energy for unwinding could come from? The Rubber Band model states that chaperones Hsc70/Hsp90 forcefully adjust the AGO conformation, with energy expenditure that is transferred and stored to an enzyme like rubber bands, thus allowing duplex loading into RISC despite it generating a structural tension inside the complex [93, 94]. In this instant, the complex is known as pre-miRISC as it is a temporary structure that contains two miRNA strands. The tension within the molecules provides the action of wedging the duplex by the AGO N domain and consequently ejecting the less interacting strand, the passenger strand. Hence, the tension release returns AGO to its more stable conformation ATP independently. This postulate is according to the idea that the guide strand has more binding stability with the MID and PIWI nucleotide-binding pockets of AGO. This thermodynamic affinity contrast between the strands, combined with AGO conformational changes from less stable to more stable results in ATP-independent passenger strand ejection. A critique of this model is that AGO should not be compared to a real rubber band as it is not capable of storing potential energy due to its structural properties; this competence is associated with chaperones. Given these criticisms, a new and revised model has been called the Energy Slope model. This model proposes that RISC duplex loading and passenger ejection "rolls down" an energy slope from the RNA-free state of AGO (unstable) caused by chaperones, to the double-stranded RNA-bound state to the single strand RNA-bound state (stable) (Fig. 4a [93]. The role of chaperones in this model is to modify the structure of AGO in an ATP dependent manner to an active and flexible state. This lowers the activation energy required for miRNA duplex loading. This activation energy is relatively low for the yeast *Kluyveromyces polysporus* KpAgo and Ciliate *Tetrahymena* Twi1p, which can adopt the active conformation without the help of chaperones [95, 96]. In this situation, passenger strand ejection would be explained by wedging the duplex hydrogen bonds starting by the 3′ guide strand. At this point, duplex unwinding is slowly started by mismatches within seed region or the 3′ supplementary bond region. Single amino acid substitutions on MID, PIWI of ALG-1 *C. elegans* models promote differential selection for some miRNAs [97].

Central mismatches within duplex

Mismatches in miRNA duplexes are common [89] and are directly related to thermodynamic stability due to differences in hydrogen bonding between paired strands. Central mismatches are a prerequisite to duplex loading into *D. melanogaster* AGO1, however, this may be restricted to a species or group due to RNAi sorting preferences between dme-Ago1 and dme-Ago-2. The same applies between ALG-1 and ALG-2 of *C. elegans*. Inserting mismatches at positions 12–15 of the guide strand facilitates unwinding and leads to "inverted asymmetry selection" by fly Ago1 [89]. Okamura et al. [40] suggested that dme-Ago1 selects so-called miR while dme-Ago2 selects miR*. Both enzymes act in parallel in miRNA arm generation. However, as far as is known, these findings have not been reproduced.

The RNAi sorting mechanism is apparently less rigorous in humans and other animals. Mismatches are important for strand ejection in AGO with reduced or absent slicer activity. Inserting mismatches in the central or 3′ complementary positions of guide strands promotes strand selection and unwinding (i.e. increased guide strand production) [35, 40, 89, 91]. These results suggest that, at least in part, overall duplex stability is important for strand selection, but it is not the only property assessed by AGO for strand selection but also includes stability inside the center of the duplex and 5′ terminal identity [45, 79]. However, besides the apparent lack of a sorting system of siRNAs for the four mammals AGO family members, central mismatches do not seem to have an apparent effect on arm selection in this group. Nevertheless, mismatches in the seed region (2-8nt) and the 3′ complementary region (12-15nt) facilitates unwinding for AGO 1, 3, and 4 which have a lack of catalytic activity (N domain).

Exposure and identity of terminal 5′ nucleotide

The RNase III domain in AGO proteins typically cleaves double-stranded RNAs (dsRNA) leaving 2 nt 3′ overhangs that expose the 3′ terminal hydroxyl and 5′ phosphate groups [17]. The AGO MID domain recognizes the phosphorylated 5′ terminus of the guide strand, which is considered a prerequisite for miRNA-RISC assembly. The MID domain of the human, mouse, fly, nematode and *Arabidopsis* Ago2 exhibited a preference for U or A 5′ terminal nucleotides. Additionally, it is believed that AGO would have a sorting system that correlates 5′ nucleotide identity with affinity to AGO, where affinity to U nucleotides is usually higher, A or C nucleotides that sometimes appear with greater affinity than the other and G nucleotides has the lowest affinity [20, 98–100]. Consistent with this, guide strands display an uracil bias at their 5′ termini, whereas passenger strands exhibit a 5′ terminal cytosine bias. Despite that, the 5′ terminal region of more expressed strands often shows an excess of pyrimidines (U and C) indicating that various factors may be associated with the selection of distinct miRNA strands [45, 99, 100]. Suzuki et al. [41] identified which nucleotides contributed the most to strand selection. The outcomes reaffirm what was established: U, A, G, C from most to least favorable, with greater relative intensity for the first two.

Experiments conducted by Okamura et al. [40] successfully reversed the arm selection by Ago1 in flies, i.e., arm switching, by changing the terminal 5′ nucleotide to 'U' in the passenger strand. Interestingly, Young et al. [101] published an article demonstrating the inversion of strand selection preference from 3 to 5p of *miR-140* exclusive to chondrocytes, termed stoichiometric reversion. This result was achieved by introducing C → U mutations on the last 5′ nucleotide of the 5p strand (passenger strand) and UA → CG mutations on the same site on the 3p strand (guide strand). This experiment is consistent with the aforementioned sorting pattern, and these findings confirm the premise of the duplex sorting model, at least partially.

Determining how decisional the MID domain affinity sorting system is difficult without isolating this variable from others, including thermodynamic stability and the variety of AGO paralogs. In vitro experiments demonstrate that blocking 5′ phosphorylation of the guide strand of artificial dsRNAs leads to passenger strand loading into RISC, despite stable thermodynamic asymmetry caused by artificially designed mismatches [102].

Overall, these findings indicate that a sorting system for 5′ terminal nucleotide selection points to an arm selection mechanism and can compensate for undue inversion of arm selection (Fig. 4) [35, 79, 100]. However, the identity of the 5′ terminal nucleotide alone cannot serve as a definitive determinant for the guide strand. Furthermore, this model has limited capacity to elucidate the mechanisms underlying arm switching. Given the significance of this sorting system, it is evident that it functions concurrently with other factors, the most prominent of which is the aforementioned thermodynamic stability rule.

Twin-drive model

As we advance in understanding miRNA arm selection, it becomes increasingly evident that this process is likely the result of multiple coexisting mechanisms that, together, may account for the entirety of the phenomenon. Hu et al. [45], aiming to evaluate the determinant factors regulating strand selection in humans, analyzed 895 miRNAs with differential arm expression from 1:10 fold upregulation. They found that 103 miRNAs (11.5%) did not show any thermodynamic or nucleotide identity properties to explain strand selection. Nevertheless, as the abundance ratio increases (max. 1:1000), other properties related to 5′ nucleotide identity gradually become more prominent.

Suzuki et al. [41] proposed the "Twin-drive model" to explain how the terminal thermodynamic asymmetry rule and 5′ nucleotide identity rule can coexist and how they superposition. In this model, the authors stated that the MID domain of AGO is composed of two superimposed sensors or recognition sites: a G drive (referring to Gibbs free energy) for identification of the phosphate on the RNA backbone and an N drive (referring to Nucleobase) for identification of nucleotides (Fig. 5). The G drive, composed by phosphate-binding pocket, is proposed to be capable of sensing thermodynamic stability within the duplex through interaction with terminal phosphate and it is responsible for the thermodynamic asymmetry rule. According to the authors, the phosphate-binding pocket serves as a tract that steadily guides the RNA duplex backbone toward the MID domain, enabling the detection of collateral internucleotide interactions and negatively selects stable ends. The N drive would be responsible for the 5′ nucleotide identity rule through the nucleotide-selection loop of the MID domain. To evaluate this hypothesis, a set of human Ago2 mutants was analyzed together with *pri-miR-154*, whose arms are equivalently selected, but, the 5p arm is favorable for selection by nucleotide identity and the 3p arm has properties to be selected by the thermodynamic rule. They found that the 5p/3p ratio increased or decreased corresponding to the position of the mutation—mutations in the phosphate-binding pocket attenuates the thermodynamic sensor and mutation increases the ratio in the nucleotide-selection loop. Additional miRNAs with asymmetric expression profiles were

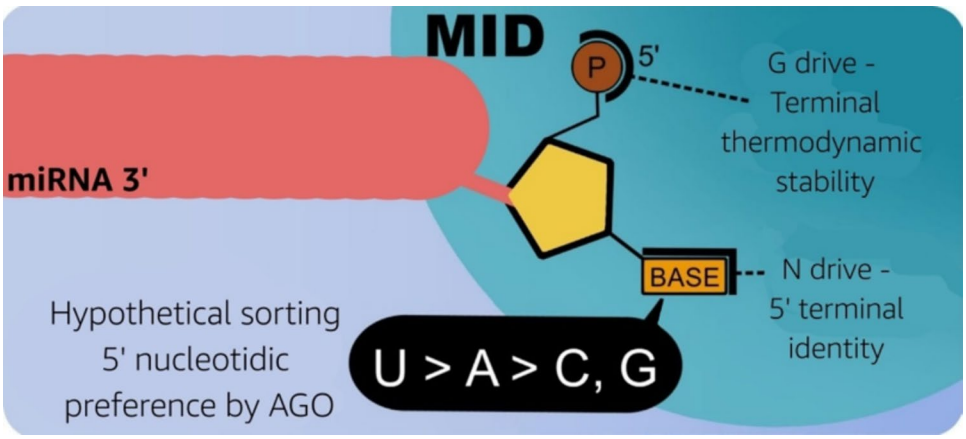

Fig. 5 Twin drive model: This model was developed to explain the coexistence of thermodynamic stability rules and 5′ terminal identity in strand selection. The MID domain of Ago is proposed to contain sensors capable of recognizing duplex stability through the phosphate backbone, represented by the "P" circle, and sensors that identify the nucleotide identity at the 5′ end. Additionally, the base preference hierarchy for Ago is suggested to be U > A > C > G

analyzed in this analysis too. Also, observation that essential amino acids for sensoring are conserved across four mammalian Ago, suggesting similar properties of selection. The authors further pointed out that this model describes how relative thermodynamic asymmetry alone does not explain the strand selection mechanisms and that non-passive processes contribute to strand selection.

The twin-drive model not only provides a mechanistic framework for understanding how thermodynamic stability and 5′ nucleotide identity influence miRNA strand selection, but it also has significant evolutionary implications. While this model explains key aspects of miRNA processing, its evolutionary relevance remains an area of ongoing investigation. Comparative genomic studies suggest that variations in AGO proteins across species may influence strand selection preferences, potentially leading to lineage-specific miRNA regulatory adaptations. For example, research in *C. elegans* has demonstrated that miRNA strand selection and sorting are linked processes, shaped by the differential loading preferences of AGO proteins [78, 79]. These findings indicate that natural selection may have acted on AGO's ability to discriminate between miRNA arms, refining strand selection mechanisms over evolutionary time. However, further studies are needed to determine whether these differences reflect adaptive changes or neutral divergence in AGO function.

The model is consistent with observed patterns of miRNA arm switching across taxa, where certain miRNAs undergo arm switching in some species but not in others [8, 10]. These differences may be associated with evolutionary modifications in AGO affinity for specific 5′ nucleotides or the thermodynamic stability of miRNA duplexes. Such changes could enable species to modulate miRNA regulatory activity in response to environmental pressures, though additional experimental validation is required. Additionally, the co-evolution of miRNA gene duplication and arm switching may be influenced by mechanisms predicted by the twin-drive model. Newly duplicated miRNAs can undergo modifications in their 5′ stability or nucleotide identity, leading to shifts in strand selection [60, 103]. This process may facilitate functional diversification between paralogous miRNAs, as observed in vertebrates and arthropods, where duplicated miRNAs exhibit distinct strand preferences [8]. However, direct evidence linking these evolutionary shifts to selection pressures on twin-drive sorting mechanisms remains limited.

By integrating these findings, the RISC twin drive model serves as a useful conceptual tool for exploring miRNA evolution, particularly in relation to strand selection and regulatory diversification. Nonetheless, additional functional and evolutionary studies are necessary to determine the extent to which selection pressures have directly shaped miRNA sorting mechanisms across different taxa. Furthermore, there is still much to investigate regarding how the cellular machinery can respond to context, inducing arm switching. We have evidence that other cellular processors, in addition to RISC, act upon miRNA precursors, and these processing can affect arm switching and RNA interference. As research advances, the twin-drive model is likely to continue evolving, incorporating new insights into the intricate balance of miRNA regulatory mechanisms and their evolutionary consequences.

Extrinsic to dsRNA

Differential terminal cleavage

Considering that proper strand selection depends on the properties of the terminal nucleotides, precise cleavage of the strands becomes important. Occasionally, the enzymes responsible for cleavage, Drosha and Dicer, do not always generate consistent cleavage patterns, which occasionally leads to changes in the final product and global interaction with mRNA repression machinery. These alterations have been observed to be relevant in biological processes. The identity of a mature miRNA can vary due to a mutation (indel and substitution) of a single base in its sequence. In this scenario, miRNA variants can be produced from the same gene leading to what are called miRNA isoforms,

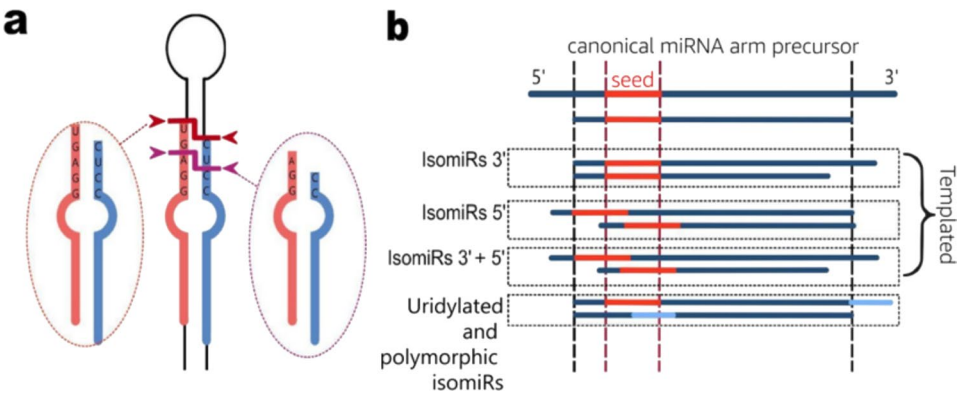

Fig. 6 IsomiRs. **a** Differential cleavage, represented by red and pink arrows, can cause changes in the size and nucleotide sequence of miRNAs. These variants are called isomiRs. **b** The diagram shows the formation of different types of isomiRs, categorized as templated and non-templated. This image also highlights the displacement of the seed region (seed shifting) due to differential cleavage

better known as isomiRs [21, 22] (Fig. 6). IsomiRs are canonically annotated miRNA isoforms that exhibit heterogeneous lengths and/or sequence discrepancies (Fig. 6b). They exhibit diverse expression patterns in various biological contexts. IsomiR diversity has been cataloged, not only between cellular lineages and tissues but also between species, with evidence of the functional and evolutionary significance of miRNAs in adaptation [20, 57, 81, 104]. The diversity of isomiRs is fundamentally linked to miRNA regulation processes, such as target identification, modulation or dysfunction of expression of the cellular targets.

In the case of isomiRs generated during biogenesis by Dicer or Drosha imprecise cleavage, the shortening or lengthening of pri-miRNA or pre-miRNA is the main consequence of differential cleavage. Additionally, the function of exoribonucleases (such as Nbr and exosomes) can also result in the formation of miRNAs through shortening of the ends. Conversely, through the process of RNA tailing, polymerases can add adenine (adenylation) or uracil (uridylation) monomers to increase 3′ ends, thus elongating strand lengths. Depending on these processes, isomiRS are known as 5′ or 3′ based on the portion modified, and as 5′ + 3′ when the added bases occur at both terminals. These isomiRs correspond to a genomic sequence and are thus referred to as templated isomiRs. Non-templated isomiRS or polymorphic isomiRs are rarer (< 1%) and depend on post-transcriptional nucleotide modifications, commonly mediated by ADAR enzymes, which will be mentioned later [21, 105, 106].

5′ isomiRs exhibit a shift in the start of the sequence, altering the start of the seed sequence (Fig. 6b) [105, 107, 108]. This phenomenon is known as seed shifting [81, 107]. When this occurs, cellular function can be impacted due to the altered target repertoire. However, 5′ isomiRs are rarer than 3′ isomiRs, likely due to a negative pressure on these variants to favor the maintenance and stability of cells [109]. Nevertheless, natural selection may favor sets of isomiRs based on seed shifting, gradually making one arm dominant over its counterpart, as identified in different species with varying degrees of relatedness [8, 81, 107].

3′ isomiR variants are more commonly detected in sequencing and also exhibit greater variability. These also play an important role in biological modulation, as altering the length of the sequence or the overhangs at the miRNA termini, or even the identity of the terminal nucleotide, can affect both the relative thermodynamic stability and the identity of the miRNA termini. To elucidate this issue, Lee and Doudna [110] conducted experiments using two isoforms of *miR-200* derived from Dicer cleavage or Dicer associated with its TRBP enzyme, which were 1 nt longer at the 3′ terminal for the 5p arm and 1 nt longer at the 5′ terminal for the 3p arm. They found an increased 5p/3p ratio in the isoform generated solely by Dicer. This indicates that isomiR formation can alter RISC selectivity of each arm and induce arm switching. The same research group replicated these findings in 2015 and found that 13% of miRNA duplexes analyzed display alternate arm selection in the absence of TRBP and PACT cofactors [111].

There are reports that Dicer is dispensable for asymmetric RISC loading, as previously evidenced [112]. For miRNAs with unfavorable terminal nucleotide identity (G) and mismatches in terminal and central regions, the presence of the Dicer/PACT or TRBP complex may be necessary, depending on the secondary structure of the duplex. Human AGO2, in these experiments, was able to select the guide strand alone, but the actions of Dicer, TRBP and PACT were also necessary to direct strand selection, termed the RISC-loading checkpoint by the authors.

As with mature miRNA nomenclature, isomiRs usually annotated as canonical are not necessarily the dominant form [113, 114]. The processes of isomiR formation share similarities with the contextual change in miRNA expression profiles, integrating mechanisms of epigenetic flexibilization in phenotype expression. In the context of strand selection, isomiRs demonstrate an important role in the loading and selection of the strand by RISC. Consistent with this, Guo et al. [109] used expression profile data to compare expression levels of different isomiRs and identified expression patterns between arms and constant isoforms in different samples. The authors noted that functional pressures, especially in pathological conditions, have a relation to changes in expression profiles. This indicates, once again, isomiR expression diversification is not random and corresponds to context. The authors attribute the arm switching phenomenon to modulation of expression profiles between different isomiRs. Thus, the ability of an organism to produce different isomiRs and the alternation of dominance between these variants or between sets of variants of the complementary arm are additional resources that influence arm switching. Therefore, it can be stated that differential cleavage, isomiR production, and strand selection intertwine as mechanisms that can promote the plasticity of phenotype expression. However, better elucidation is needed to gain a deeper understanding of how these phenomena interact, the form and degree of this interaction, and the underlying elements that dictate their processes.

MiRNA tailing

RNAs usually require posttranscriptional processes that regulate and ensure proper expression of its products. The terminal structure of an RNA is crucial for determining its purpose and viability. Various classes of RNAs, including miRNAs, have extra nucleotides added post-transcriptionally at its termini by nucleotidyltransferase enzymes (TENTs),

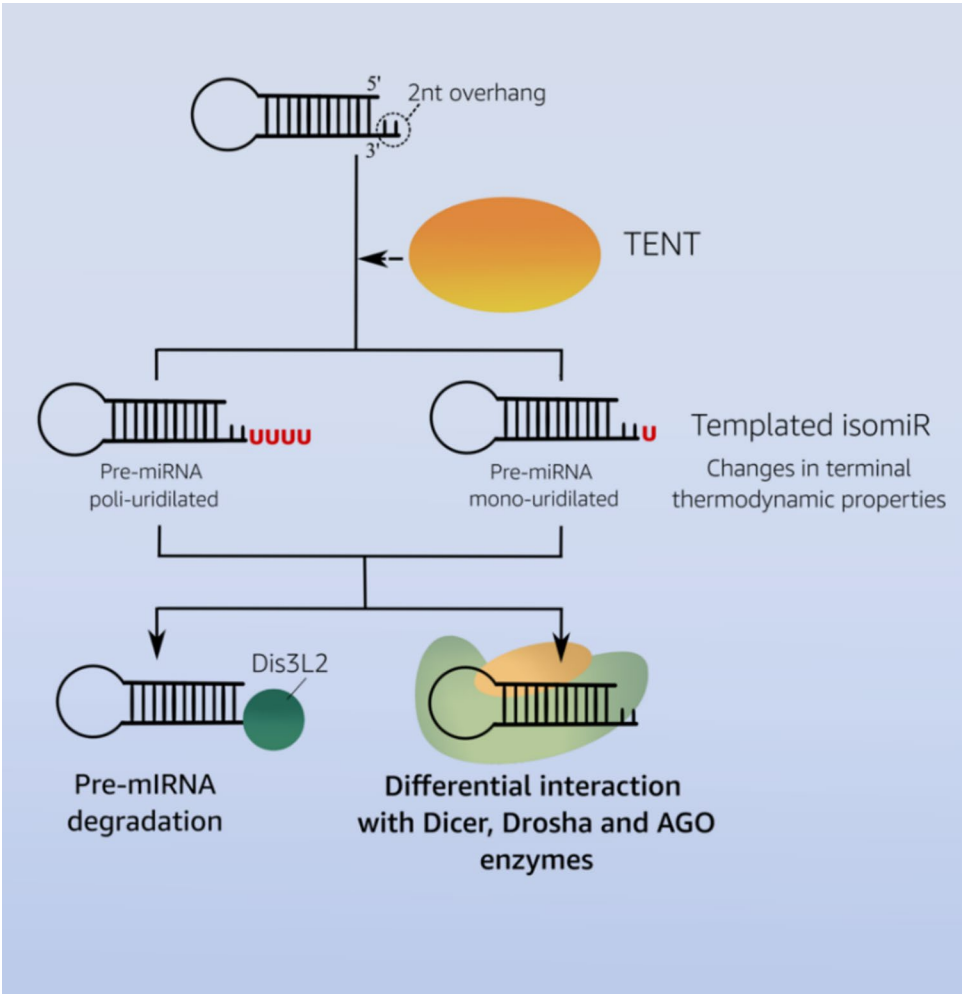

Fig. 7 miRNA tailing process. **a** The miRNA precursor undergoes the addition of uridine nucleotides at the 3′ ends (uridylation) via TENT (nucleotidyltransferase enzymes). **b** Depending on the number of nucleotides added, templated isomiRs are formed. There are two main outcomes: **c** tagging of the precursor and miRNA for subsequent degradation, or **d** differential interaction with the enzymes Drosha, Dicer, and Ago due to terminal thermodynamic modifications

primarily at the 3′ ends [115] (Fig. 7). In most known cases, the added nucleotides are adenines, through the process of adenylation catalyzed by Poly(A) polymerases (PAPs), or uridines, added via uridylation, catalyzed by Terminal Uridyltransferase (TUTases) or poly(U) polymerase enzymes. This feature, known as RNA tailing, is common and widely conserved in eukaryotes [60, 116]. RNA tailing has already been described as involved in different biological pathways, ranging from protecting extremities to marking RNAs for subsequent degradation by ribonucleolytic enzymes (Fig. 7). Mature miRNA tailing can affect target repression since adenylation or uridylation expands target recognition or shifts seed position. Nevertheless, RNA Tailing may participate in the process of arm selection and arm switching.

Adenylation during pri-miRNA transcription occurs as usual in polymerase 2 products. Nonetheless, uridylation comes into focus, as posterior adenylation is less common in miRNAs precursors. Uridylation primarily influences its fate and strand selection at the pre-miRNA stage, following Drosha cleavage. Cleavage of pri-miRNA by Drosha typically results in pre-miRNAs with a 2 nt overhang at the 3′ position (group 1 miRNAs). The subsequent recognition by Dicer in the cytoplasm requires the presence of this overhang. If Drosha cleavage results in an unusual miRNA with 1 nt 3′ overhang (group 2 miRNAs), it may prevent recognition or reduce Dicer efficiency due to being a suboptimal substrate. Nevertheless, Posttranscriptional uridylation can "correct" the miRNAs terminal length. TUTases, specifically TUT4 (also named Zcchc11), TUT7 (also known named Zcchc6) and in vitro cases TUT2 (also named Gld2 or PAPD4. It remains uncertain whether its effect is due to adenylation or uridylation) intervene in group ll miRNAs an it outcomes in templated isomiRs with 2nt overhang [60, 105, 116, 117] (Fig. 7). For example, using pre-let-7 isomiRs as a model, it has been shown that TUT4/7/2 promotes uridylation on group ll let-7 members in absence of pluripotency factor Lin28. Uridylation can fine-tuning of let-7 miRNA family expression depending on cellular context: mono-uridylation can enhance Dicer processing while poly-uridylation mediated by Lin28 inhibits [118–120].

Dicer "molecular ruler" function can be affected by alteration in the size of precursors due to uridylation at

pre-miRNA stage. Additionally, insertion of 3′ uridine can impact the duplex terminal thermodynamic stability and exchange the terminal nucleotide identity. (Fig. 7). This raises the question of whether uridylation could be a process that leads to arm switching. To investigate this, Kim et al. [20] conducted experiments using the conserved mammalian *miR-324*, which exhibits intertissue arm switching. The canonical arms of miR-324 have distinct and possibly antagonistic biological functions in mammals: *miR-324-3p* becoming overexpressed in carcinoma progression. Furthermore, it was observed that the levels of TUT4/7 is relatively low in tissues, but increased in glioblastomas. This suggests that expression of these TUTases promotes *miR-324* uridylation and, consequently, induces the arm switching. Experiments revealed that mono- or oligo-uridylation (approximately + 2 nt) at the 3′ end of *pre-miR-324* elongates the strand, leading to its repositioning within Dicer. This repositioning alters the interaction with the PAZ and platform domains, which recognize the 5′ end, reducing their efficiency. As a consequence, the dsRNA binding domain (dsRBD) of Dicer can more effectively position the pre-miRNA, causing a shift in the 'molecular ruler' and altering the nucleotide counting starting point, ultimately changing the pre-miRNA cleavage site. (Figs. 3, 7). In other words, uridylation by TUT7/4 alters the miRNA-Dicer interaction, potentially inducing arm switching, which in the case of *miR-324*, may contribute to glioblastoma formation. TUTases are differentially expressed in tissues or during development and are more expressed in development and some cancers and other disorders, as described [6, 20, 60, 121, 122].

With RNA tailing mechanism, TENTs have the potential to act as the 5p/3p ratio or arm usage controller. With evidence that expression of TUTases, especially TUT7, TUT4 and TUT2, add nucleotides to miRNAs, Dicer differential interaction and cleavage form different isomiRs that eventually induces context-specific arm switching or isomiR expression. In both cases, uridylation can influence the RNome expression profile.

This suggests that arm switching is strongly influenced by extrinsic factors beyond the enzymes or miRNAs themselves. Similar to how lin28 indirectly regulates certain members of the let-7 family, other unknown factors may modulate arm usage and induce switching in various miRNAs upon expression. However, further studies are needed to clarify the extent and mechanisms through which specific factors or contexts drive arm switching. This hypothesis is plausible, as miRNA strand selection likely integrates within a complex cellular framework that synergistically regulates miRNA and RNA expression. Given their roles as post-transcriptional length and terminal modifiers, TENTs (TUTases and PAPases) warrant further investigation in this process.

Edition of miRNAs by nucleotide substitution

Non-templated isomiRs are those with single nucleotide variations compared to the parental transcript. This is due to post-transcriptional RNA editing that replaces nucleotides in transcript sequences. RNA editing is an epigenetic regulatory mechanism that has gained increasing attention, although their biological significance is under investigation. The main enzymes responsible for editing dsRNAs are the ADARs (Adenosine Deaminases Acting on RNA), a highly conserved enzyme family in animals [123]. They catalyze deamination reactions, converting adenosine to inosine (A → I editing). This process is considered sequence editing because the translation machinery interprets inosine derived from adenosine as guanine paired with cytosine, which may lead to codon change [123]. Additionally, the AID/APOBEC enzyme family (Activation Induced Cytidine Deaminases/ Apolipoprotein B mRNA Editing Enzyme Cytidine deaminases) promotes C → U editing. However, APOBEC family enzymes edit preferentially single stranded RNAs (ssRNAs) and are mainly expressed in immune cells. Despite miRNA editing through APOBEC was suggested by bioinformatics prediction, there is a significant lack of studies on the prevalence and implications of this enzyme family upon miRNAs [124].

MiRNA editing can encompass a range of biological processes and profoundly influence their biogenesis. These enzymes can bind to dsRNAs during any step of miRNA biogenesis step (i.e., acts upon pri-, pre-miRNA, duplex and mature miRNA.) ADAR-mediated miRNA editing mostly results in impaired miRNA biogenesis [123]. However, it is uncertain whether this represents the core functional role of ADAR in miRNA regulation. Notably, the capacity of ADAR to bind dsRNA enable a diverse array of biological functions, for example, ADAR can form complexes with Dicer that quadruple its miRNA processing speed to promote miRNA loading into RISC [123, 125] ADAR-mediated miRNA editing can alter the repertoire of miRNA targets, particularly when editing occurs in the seed region, as seen in *miR-455-5p*, which is linked to human melanoma progression [126]. The same occurs in the case of *miR-376a-5p* [102] or *miR-411*, where seed region diversity was described to interact with the differential cleavage mechanisms already described. Additionally, editing affects the weakening of MRE recognition, as reported for human *miR-376*, *miR-22*, and *miR-191*, reducing their silencing efficiency [128].

Data obtained by Kawahara et al. show that about 16% of pri-miRNAs in the adult human brain undergo ADAR miRNA editing [129]. 40% of miRNAs in vivo have their level altered in C. elegans ADAR1 and ADAR2 mutants compared with wild-type [130]. Warnefors et al., in their 2014 study [106], found evidence that site-specific miRNA

editing (i.e., occurring at the same nucleotide positions) is evolutionarily conserved, identifying numerous conserved editing events in mammals and different vertebrates, suggesting that miRNA editing is not random free from selective pressure, but represents a controlled mechanism that favors increased miRNA functional diversity. Additionally, less than 5% of sites are significantly editing levels, indicating that it is a rare and possibly inconsistent event. Furthermore, the biological significance of this is uncertain [131]. Versely et al. found that 64% of miRNA edits in the mouse brain occur in the seed region. Similarly, Warnefors et al. [106] found that 73% of edits in their study were in the seed region analyzing 5 mammals and chicken. Furthermore, Nigita et al. [132] identified 83% of edits in the seed region under hypoxic conditions in a breast adenocarcinoma cell line, indicating that miRNA editing is again a non-random mechanism of cell regulation [106, 124, 132, 133]. This suggests that miRNA editing events are under stabilizing selection, as uncontrolled it would lead to miRNA dysfunction.

These processes can create a mismatch effect due to thermodynamic changes in the precursor molecule. Consequently, miRNA editing can directly contribute to a fluctuation and balance of thermodynamic asymmetry between the termini and, which may also impact RISC selectivity [125, 134]. Thus, miRNA editing can influence the thermodynamic stability of the precursor. For example, *pri-miR-367a-1* editing at position + 4, conserved across various primate tissues, increases RNA stability, presumably disfavoring the selection of the 5p arm in this context [135]. Considering the factors discussed here that influence strand selection, increased stability at one terminus favors the selectivity of the other terminus, thereby increasing or decreasing the 5p/3p ratio or arm usage. Finally, it is worth noting that no studies or cases were found in the examined literature indicating that miRNA editing occurs at the terminal nucleotide position (neither at the 5′ nor the 3′ end) or that it influences strand selection or arm switching based on terminal nucleotide identity.

Deletion of the ADAR1 gene leads to variation in concentration or arm usage in various miRNAs [124, 136]. Despite that, no direct expression comparison between arms from the same precursor has been conducted to infer arm switching. Li et al. [137] established a correlation between editing values in an index they called PMD (pri- versus mature divergence), finding a correspondence between the level of editing and mature miRNA abundance. Using this approach, they found that edits on one arm promote the production of the complementary arm, depending on the miRNA and the editing site. To test this, they analyzed adult brain samples from wild-type and ADAR1−/− mutant mice and detected significant 5p/3p ratio variation. This suggests that editing may influence arm switching in vivo.

MiRNA editing is a post-transcriptional regulatory mechanism that works in conjunction with miRNA target regulation. It is worth pointing out that epigenetic regulatory processes can overlap, as exemple, *miR-1* in mouse embryos can act upon ADAR mRNA silencing expression [138]. Or, in the particularly interesting case of the miRNA encoded by the Epstein-Barr virus genome (EBV) *miR-BART6-5p*, which specifically targets human Dicer mRNA (acting as RNAi), presumably reducing the immune response and maintaining the infection latency state. Additionally, *miR-BART6-5p* undergoes A-to-I editing resulting in the inhibition of loading onto RISC and activating lytic state. Nishikura [123] have suggested that this editing may have evolved with an immune-regulatory function on EBV.

Despite the influence of miRNA editing on the thermodynamic properties and architecture of miRNA precursors and, at least in arm usage, much remains unknown about the underlying influencing factors and contribution of miRNA editing factors. Further research is necessary to better understand how these modifications integrate into miRNA biogenesis and expression, as well their potential role in driving arm switching.

miRNA competitive inhibition through transcriptome

It is known that the processes underlying miRNA-induced target repression involve recognition through base-pairing of miRNAs with their targets. Conversely, it is reasonable to state that RNAs containing MREs might interfere with miRNA target acquisition through the RNAi principle and thus impact the 5p/3p ratio of these molecules. The competing endogenous RNA (ceRNA) hypothesis [139–141] proposes that lncRNAs (long non-coding RNAs), circular RNAs (circRNAs), pseudogene-transcribed RNAs, and cognate mRNAs can, in contrast to miRNAs, positively regulate target genes through competitive inhibition of miRNAs. The introduction of this concept were called "miRNA sponges" that are artificial RNAs (introduced or transgenic) acting as competitors to endogenous miRNA/siRNAs that attenuates, even inhibit their activity by sequestering them [142, 143]. Through this sequestration capacity, miRNA sponges can limit the activity of specific miRNAs. Subsequently, this process, in which miRNA targets can trigger the degradation of these miRNAs, was termed Target RNA-directed microRNA degradation (TDMD) [144, 145]. The possibility that various transcriptome molecules can affect miRNA availability by acting as sequestrators has generated considerable interest. Over the past decade, studies have suggested that certain endogenous, artificial, or viral lncRNAs within cells might exert this miRNA inhibition effect [144, 146–149]. These competing RNAs, mostly lncRNAs, are antisense oligonucleotides complementary to endogenous miRNAs

and are referred as antagomiRs or anti-miRs. The ceRNA hypothesis postulates a stoichiometric competition among MREs in the transcriptome for miRNA binding. Thus, miRNAs can pair with a network of target lncRNAs through MREs (the ceRNAs) and vice versa. In this way, the transcriptome can establish a network of recognition and synergistic regulation with one or more target miRNAs. AntagomiRs can establish a differential binding architecture between ceRNAs and miRNAs, competing with Ago for binding this competition reduces the stoichiometric availability of miRNAs, thereby diminishing their regulatory activity. As a consequence, in scenarios of extensive ceRNA competition, mRNA targets would have a higher chance of translation.

However, the real effect of ceRNAs in inducing TDMD is viewed with skepticism, as the expression of a ceRNA constitutes only a small fraction of the target repertoire. High expression of a single ceRNA may have a minor effect on miRNA repression and is possibly physiologically rare. One highlighted reason is the lack of highly complementary material to miRNAs in metazoan genomes, and the induction of ceRNA-involving mechanisms does not lead to miRNA elongation and degradation, which is required for better TDMD efficiency [145]. Studies controlling ceRNA levels in vivo indicate that to assert some level of influence on miRNA activity, the target miRNA concentration needed to be equimolar and far beyond physiologically plausible concentrations, at least for highly expressed miRNAs [150, 151]. This is because ceRNAs can establish TDMD only through miRNA sequestration, with their concentration being the only effective influencing factor. The controversy surrounding the ceRNA hypothesis involves the idea that if ceRNA regulatory effects were a driving force in miRNA expression regulation, a single target would represent only a small fraction of the miRNA target repertoire and thus be indistinguishable in its level of influence. The mechanism of miRNA repression by targets emerges from the cytoplasmic transcript pool and the conformation of the miRNA-target binding architecture. Moreover, experiments in viral models with mutations or the removal of MREs from artificial targets showed that miRNA regulation could still be observed [150, 152].

This dynamic of TDMD regulation and degradation affects miRNA levels. However, it is suggested that this is not an emergent effect of the total cytoplasmic transcriptome but is triggered by specific RNA molecules that recruit the cellular machinery for the tagging and degradation of specific miRNAs [145, 149]. Greater complementarity with the miRNA and encompassing the target's 3′ region, appears to be a requirement for TDMD. Ameres et al. observed that drosophila miRNAs sometimes show extensive complementarity to target mRNAs [144]. When exposed to artificial miRNAs with extensive complementarity, their efficiency is reduced, and the miRNAs are decoupled from RISC or degraded, in the case of AGO2 [145, 153]. This extended complementarity is generally the cause of the loss of regulatory capacity of miRNAs, as it is believed to initiate signaling and degradation mechanisms (by terminal nucleotidyltransferases—TENTs and by ribonucleases, respectively, as mentioned above) or, at least, promote the unloading of miRNAs from Ago, leading to their independent degradation [144, 154–156]. Therefore, TDMD is not about repressing miRNA activity by interfering with their biogenesis or AGO affinity but rather by the availability of strands in the cytoplasmic transcriptome pool. Thus, it is assumed that this can interfere with the 5p/3p ratio adjustment, not by their concentration per se, but by the functionally available 5p/3p strand ratio, meaning strands not sequestered and thus free to perform their regulatory roles. Hence, TDMD indicates that, without necessarily altering transcript templates, shifting miRNA precursors, interfering with cleavage patterns or AGO strand preference, it is theoretically possible to induce arm switching—or analogous effect in the practice—solely through miRNA sequestration.

If TDMD occurs endogenously, it likely operates through multiple pathways, with thermodynamic properties playing a crucial role in guide strand selection. Additionally, homologous long non-coding RNAs (lncRNAs) have been shown to perform conserved functions [157–159]. If lncRNAs consistently mediate TDMD, miRNA regulation through this mechanism may represent an evolutionarily conserved trait. However, many aspects of TDMD remain uncertain, requiring further investigation into its biological significance and regulatory complexity.

MiRNA gene duplication

Each miRNA arm is typically associated with distinct and often independent biological functions compared to its complementary arm. Marco et al. [2] demonstrated that mature miRNAs from humans and flies generated from the same precursor have significantly different target sets and are linked to distinct biological processes. This trade-off does not necessarily imply that the functions associated with different arm expression profiles cannot coexist within the cellular infrastructure. However, this coexistence arises despite the mRNA silencing machinery responding to the selection factors previously mentioned, which tend to be similar in paralogous genes [81].

The evolutionary process that would allow the concomitant expression of phenotypes associated with both complementary arms is gene duplication. Indeed, paralogous miRNAs tend to produce functional miRNAs from the same arm [8]. Despite this, arm switching between paralogous miRNAs, where each copy expresses a different arm, has been observed. For instance, in Griffiths-Jones et al. [7],

among the *mir-100/10* family genes in protostomes one copy in particular, the *miR-933* gene, underwent arm switching from 5 to 3p, but the dominance of the 5p arm was conserved in other paralogs in the model organisms studied, except in *D. melanogaster*.

However, the evolutionary implications of gene duplication in miRNAs extend beyond simple redundancy. Duplicated miRNA copies may undergo functional specialization through subfunctionalization, where each paralog retains only a subset of the ancestral functions, or neofunctionalization, where novel functions emerge due to sequence divergence. One key regulatory advantage of arm switching in duplicated miRNAs is that it allows for differential target selection without requiring sequence modifications in the mature miRNA strand. For example, in teleost fish, different *miR-181* paralogs exhibit distinct 5p/3p expression profiles across species, suggesting that miRNA duplication coupled with arm switching can drive lineage-specific regulatory diversification [54, 55]. Similarly, in insects, shifts in *miR-100* arm selection have been associated with variations in developmental timing, underscoring the role of arm switching in fine-tuning gene expression without the need for additional genomic rearrangements [7, 8].

In the context of miRNA, gene duplication followed by the processes of neofunctionalization or subfunctionalization, where these events could reduce the pressure of arm selection, would allow for flexible arm usage patterns. Neofunctionalization would occur in situations where mutations in one of the copies cause fluctuations in Ago preference. Gene duplication followed by subfunctionalization could influence the stoichiometric imbalance of the pre-miRNA/target ratio in favor of the pre-miR, allowing the expression of the passenger strand without compromising the regulatory effects of the guide strand. This points to miRNA gene duplication as a potential source of attenuation of AGO's selective dichotomy or, at least, could contribute to the expression of different arm expression profiles, possibly leading to arm switching [7, 8, 70, 73, 81]

However, it is important to emphasize that the stoichiometric factor appears to be crucial for this phenomenon. In studies conducted by [73, 75] genome analysis was performed to search for WGS (Whole Genome Duplication) events [160]. Despite the duplication of miRNA genes in these events, no significant variation in miRNA expression profiles was found. One possible explanation for this is that whole genome duplication events simultaneously duplicate their targets. This may indicate that the duplication of a miRNA gene can be a source or possibly a prerequisite for arm switching, but for this to occur, the miRNA/target abundance ratio must be unbalanced (Supplementary Material of [161]).

Additionally, regulatory flexibility arising from arm switching may provide an adaptive advantage by enabling species to adjust gene expression in response to environmental pressures. For instance, in mammals, hypoxia-responsive miRNAs have been observed to shift arm preference under oxygen deprivation, illustrating how dynamic strand selection can serve as a mechanism for rapid physiological adaptation [66, 132]. This suggests that, in addition to lineage-specific regulatory divergence, miRNA duplication combined with arm switching can enhance an organism's ability to respond to fluctuating environmental conditions.

In summary, current evidence suggests that miRNA duplication does not necessarily induce arm switching or directly influence the expression profile, but it allows each miRNA arm associated with distinct biological functions to be expressed without competing for AGO selection. However, it is evident that, despite the effects of miRNA gene duplication being reasonably well studied, especially in explaining the emergence of new miRNAs, further investigation is needed to understand how miRNA gene duplication directly affects the occurrence of arm switching.

Conclusions and final remarks

Understanding the mechanisms of miRNA arm selection and arm switching is essential for elucidating the foundations of gene and physiological regulation in eukaryotic organisms. These processes provide an additional layer of complexity to the biological regulatory system, enabling cells to modulate their responses to various biological contexts and situations. In this work, we conducted a comprehensive and up-to-date review of the current state of knowledge regarding miRNA arm selection and arm switching.

Research on arm switching events across diverse intra- and interspecific contexts remains limited, which hinders our capability to better understand the evolution of miRNA regulatory mechanisms and arm switching, as well as how these may have favored the biological evolution of organisms. Investigating miRNA components in different biological groups can elucidate part of the evolutionary process of organisms and explain, at least partially, how organisms genetically adapt to different environments and contexts. To achieve this, it is crucial to understand arm selection and the processes controlling it. Characterizing the frequency and functional impact of these events in various contexts and biological groups presents a significant challenge. The integration of experimental and computational approaches, along with the use of new sequencing technologies and data analysis, can contribute to important advances in this field.

Although much has been discovered about miRNA arm selection in the past decade, significant challenges remain

in identifying the factors and variables regulating differential strand selection processes, understanding the evolution of these mechanisms, and characterizing them in different organisms and contexts. While it is known that thermodynamic stability and the identity of the 5′ nucleotides play important roles, current literature indicates that much remains to be elucidated about other determinants involved in arm selection and arm switching events, such as the regulation of biogenesis enzymes, environmental factors triggering arm switching, or determinants in precursor structures. Another challenge is characterizing the occurrence and diversity of functional impact of these events in different organisms. To date, insufficient research has been conducted on arm switching events from an evolutionary perspective. It is known that this is not a process free from natural selection, as some miRNAs and miRNA families have already been investigated phylogenetically [7]. Therefore, it is presumed to be fixed by natural selection. It should be noted that RNAi regulatory mechanisms may have importance for multicellularity, as these evolved independently in animals, plants, and fungi 162]. Intertissue arm switching events have been reported in cnidarians [50], indicating that the presence of this mechanism in metazoans predates the emergence of bilaterians. Furthermore, the presence of miRNA genes increased during the diversification of this group, potentially conferring an adaptive advantage. Moreover, as mentioned, gene duplication appears to favor miRNA regulatory control and be a factor in the occurrence of arm switching. There are a range of challenges regarding the identification of arm switching in different organisms and evaluating its biological impact. This requires assessing the expression of miRNA arm pairs in various tissues, species, and contexts and then comparing, in a normalized manner, expression profiles. Additionally, a functional step to identify the targets of the involved miRNAs and the affected biological pathways is necessary to measure the biological impact these miRNAs have on phenotypic expression.

While the mechanisms governing miRNA arm switching—such as thermodynamic stability and enzyme-mediated processing—have been extensively studied, their direct impact on species fitness remains untested. Most studies addressing these mechanisms focus on their biochemical properties rather than their role in evolutionary adaptation. However, miRNAs have been widely recognized as drivers of regulatory evolution, influencing gene expression networks across taxa.

For instance, rapidly evolving miRNA clusters have been shown to rewire their target networks, contributing to lineage-specific regulatory adaptations in *Drosophila* [163]. Similarly, de novo miRNAs have been proposed as key players in adaptive evolution, with studies testing their emergence under the Red Queen Hypothesis framework [103]. These findings underscore the broader evolutionary relevance of miRNAs, even though the specific mechanisms of arm switching remain to be investigated in this context.

This review represents a significant effort in advancing the understanding of these phenomena and highlights the importance of studying arm selection and arm switching in gene regulation and the phenotypic plasticity of organisms. This review identified gaps in the current knowledge about miRNA selection and underscored the importance of future research to fill these gaps.

The future prospects for studying miRNAs and the factors that modulate functional arm selection are promising. Understanding arm selection and arm switching will open doors to the development of biotechnological techniques as well as potential therapeutic applications and significant advances in understanding gene regulation and its impact on the evolution of eukaryotic organisms. Continued research focused on elucidating the factors that impact arm selection and arm switching is essential for uncovering the mechanisms that govern gene regulation and, ultimately, modulate the biological pathways and processes inherent to life.

Acknowledgements We are grateful to the members of the LGEM lab for their invaluable support throughout this research study.

Author contributions Conceptualization, L.G. and D.P.; methodology, data curation, writing—review and editing, L.G., V.C., J.P., and D.P. All authors read and approved the final manuscript.

Funding This work was supported by grants from Sao Paulo Research Foundation (FAPESP; #2019/15494-5 and 2020/13577-8), Rio Grande do Sul Research Foundation (FAPERGS; #19/2551-0002283-2) and National Council for Scientific and Technological Development (#310693/2023-3).

Data availability Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

References

1. Bartel DP (2018) Metazoan micrornas. Cell 173(1):20–51
2. Marco A et al (2012) MicroRNAs from the same precursor have different targeting properties. Silence 3(1):1–7
3. Seungil RO et al (2007) Tissue-dependent paired expression of miRNAs. Nucleic acids Res 35(17):5944–5953
4. Landgraf P et al (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129(7):1401–1414
5. Kumarswamy R, Volkmann I, Thum T (2011) Regulation and function of miRNA-21 in health and disease. RNA Biol 8(5):706–713
6. Tsai K-W et al (2016) Strand selection preference of microRNA-193a varies in breast cancer. Sci Rep 6(1):1–13
7. Griffiths-Jones S et al (2011) MicroRNA evolution by arm switching. EMBO Rep 12(2):172–177
8. Marco A et al (2010) Functional shifts in insect microRNA evolution. Genome Biol Evol 2:686–696
9. Kern F et al (2020) miRSwitch: detecting microRNA arm shift and switch events. Nucleic Acids Res 48(W1):W268–W274
10. Chen L et al (2018) miRNA arm switching identifies novel tumour biomarkers. EBioMedicine 38:37–46
11. Ha M, Kim VN (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15(8):509–524
12. Gebert LFR, Macrae IJ (2019) Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 20(1):21–37
13. Han J et al (2006) Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 125(5):887–901
14. Kwon SC et al (2016) Structure of human DROSHA. Cell 164(1–2):81–90
15. Denli AM et al (2004) Processing of primary microRNAs by the microprocessor complex. Nature 432(7014):231–235
16. Gregory RI et al (2004) The microprocessor complex mediates the genesis of microRNAs. Nature 432(7014):235–240
17. Nicholson AW (2014) Ribonuclease III mechanisms of double-stranded RNA cleavage. Wiley Interdiscip Rev RNA 5(1):31–48
18. Kim Y-K, Kim B, Kim VN (2016) Re-evaluation of the roles of DROSHA, Exportin 5, and DICER in microRNA biogenesis. Proc Natl Acad Sci 113(13):E1881–E1889
19. Clancy JW et al (2019) An ARF6–Exportin-5 axis delivers pre-miRNA cargo to tumour microvesicles. Nat Cell Bioly 21(7):856–866
20. Kim H et al (2020) A mechanism for microRNA arm switching regulated by uridylation. Mol Cell 78(6):1224.e5-1236.e5
21. Dhanoa JK et al (2019) Biogenesis and biological implications of isomiRs in mammals-a review. ExRNA 1(1):1–8
22. Neilsen CT, Goodall GJ, Bracken CP (2012) IsomiRs—the overlooked repertoire in the dynamic microRNAome. Trends Geneti 28(11):544–549
23. Guo L, Lu Z (2010) The fate of miRNA* strand through evolutionary analysis: implication for degradation as merely carrier strand or potential regulatory molecule? PLoS ONE 5(6):e11387
24. Medley JC, Panzade G, Zinovyeva AY (2021) MicroRNA strand selection: unwinding the rules. Wiley Interdiscip Rev RNA 12(3):e1627
25. Meister G (2013) Argonaute proteins: functional insights and emerging roles. Nat Rev Genet 14(7):447–459
26. Liu J et al (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305(5689):1437–1441
27. Willkomm S et al (2022) Single-molecule FRET uncovers hidden conformations and dynamics of human Argonaute 2. Nat Commun 13(1):3825
28. Mallick B et al (2019) Understanding the molecular interaction of human argonaute-2 and miR-20a complex: a molecular dynamics approach. J Cell Biochem 120(12):19915–19924
29. Pasquinelli AE (2012) MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat Rev Genet 13(4):271–282
30. Marco A (2018) SeedVicious: analysis of microRNA target and near-target sites. PLoS ONE 13(4):e0195532
31. Broughton JP et al (2016) Pairing beyond the seed supports microRNA targeting specificity. Mol Cell 64(2):320–333
32. Seok H et al (2016) MicroRNA target recognition: insights from transcriptome-wide non-canonical interactions. Mol Cells 39(5):375
33. Zhang K et al (2018) A novel class of microRNA-recognition elements that function only within open reading frames. Nat Struct Mol Biol 25(11):1019–1027
34. Chipman LB, Pasquinelli AE (2019) miRNA targeting: growing beyond the seed. Trends Genet 35(3):215–222
35. Czech B et al (2009) Hierarchical rules for argonaute loading in Drosophila. Mol Cell 36(3):445–456
36. Förstemann K et al (2007) Drosophila microRNAs are sorted into functionally distinct argonaute complexes after production by dicer-1. Cell 130(2):287–297
37. Tomari Y, Du T, Zamore PD (2007) Sorting of Drosophila small silencing RNAs. Cell 130(2):299–308
38. Aalto AP et al (2018) Opposing roles of microRNA Argonautes during *Caenorhabditis elegans* aging. PLoS Genet 14(6):e1007379
39. Su H et al (2009) Essential and overlapping functions for mammalian Argonautes in microRNA silencing. Genes Dev 23(3):304–317
40. Okamura K, Liu N, Lai EC (2009) Distinct mechanisms for microRNA strand selection by Drosophila Argonautes. Mol Cell 36(3):431–444
41. Suzuki HI et al (2015) Small-RNA asymmetry is directly driven by mammalian Argonautes. Nat Struct Mol Biol 22(7):512–521
42. Kozomara A, Birgaoanu M, Griffiths-Jones S (2019) miRBase: from microRNA sequences to function. Nucleic Acids Res 47(D1):D155–D162
43. Córdova-Rivas S et al (2019) 5p and 3p strands of miR-34 family members have differential effects in cell proliferation, migration, and invasion in cervical cancer cells. Int J Mol Sci 20(3):545
44. Feng H et al (2019) MiR-34b-3p represses cell proliferation, cell cycle progression and cell apoptosis in non-small-cell lung cancer (NSCLC) by targeting CDK4. J Cell Mol Med 23(8):5282–5291
45. Hu HY et al (2009) Sequence features associated with microRNA strand selection in humans and flies. BMC Genom 10(1):1–11
46. Cloonan N et al (2011) MicroRNAs and their isomiRs function cooperatively to target common biological pathways. Genome Biol 12(12):1–20
47. Gong J et al (2014) Comprehensive analysis of human small RNA sequencing data provides insights into expression profiles and miRNA editing. RNA Biol 11(11):1375–1385
48. Pundhir S, Gorodkin J (2015) Differential and coherent processing patterns from small RNAs. Sci Rep 5(1):1–16
49. Ludwig N et al (2016) Distribution of miRNA expression across human tissues. Nucleic Acids Res 44(8):3865–3877
50. Nong W et al (2020) Jellyfish genomes reveal distinct homeobox gene clusters and conservation of small RNA processing. Nat Commun 11(1):1–11
51. Jagadeeswaran G et al (2010) Deep sequencing of small RNA libraries reveals dynamic regulation of conserved and novel microRNAs and microRNA-stars during silkworm development. BMC Genom 11:1–18

52. Sayed D, Abdellatif M (2011) MicroRNAs in development and disease. Physiol Rev 91(3):827–887
53. Glazov EA et al (2008) A microRNA catalog of the developing chicken embryo identified by a deep sequencing approach. Genome Res 18(6):957–964
54. Pinhal D et al (2018) Genome-wide microRNA screening in Nile tilapia reveals pervasive isomiRs' transcription, sex-biased arm switching and increasing complexity of expression throughout development. Sci Rep 8(1):1–18
55. de Oliveira AC et al (2024) MicroRNA transcriptomes reveal prevalence of rare and species-specific arm switching events during zebrafish ontogenesis. Evolut Bioinform 20:11769343241263230
56. Bizuayehu TT et al (2012) Sex-biased miRNA expression in Atlantic halibut (Hippoglossus hippoglossus) brain and gonads. Sex Dev 6(5):257–266
57. Guo L et al (2016) A comprehensive analysis of miRNA/isomiR expression with gender difference. PLoS ONE 11(5):e0154955
58. Brites D, Fernandes A (2015) Neuroinflammation and depression: microglia activation, extracellular microvesicles and microRNA dysregulation. Front Cell Neurosci 9:476
59. Quintanilha BJ et al (2017) Nutrimiromics: role of microRNAs and nutrition in modulating inflammation and chronic diseases. Nutrients 9(11):1168
60. Menezes MR, Balzeau J, Hagan JP (2018) 3′ RNA uridylation in epitranscriptomics, gene regulation, and disease. Front Mol Biosci 5:61
61. Çakmak HA, Demir M (2020) MicroRNA and cardiovascular diseases. Balkan Med J 37(2):60
62. Ribeiro AO et al (2022) MicroRNA roles in regeneration: multiple lessons from zebrafish. De Dyn 251(4):556–576
63. Simone NL et al (2009) Ionizing radiation-induced oxidative stress alters miRNA expression. PLoS ONE 4(7):e6377
64. Nallamshetty S, Chan SY, Loscalzo J (2013) Hypoxia: a master regulator of microRNA biogenesis and activity. Free Radic Biol Med 64:20–30
65. Brawand D et al (2014) The genomic substrate for adaptive radiation in African cichlid fish. Nature 513(7518):375–381
66. Yan Y et al (2015) Influence of a high-altitude hypoxic environment on human plasma microRNA profiles. Sci Rep 5(1):1–10
67. Zhang J et al (2021) Molecular mechanism of Chinese alligator (Alligator sinensis) adapting to hibernation. J Exp Zool Part B Mol Dev Evol 336(1):32–49
68. Croston TL et al (2018) MicroRNA regulation of host immune responses following fungal exposure. Front Immunol 9:170
69. Weidner J et al (2021) Spotlight on microRNAs in allergy and asthma. Allergy 76(6):1661–1678
70. De Wit E et al (2009) Repertoire and evolution of miRNA genes in four divergent nematode species. Genome Res 19(11):2064–2074
71. Mehrabadi M, Hussain M, Asgari S (2013) MicroRNAome of Spodoptera frugiperda cells (Sf9) and its alteration following baculovirus infection. J Gen Virol 94(6):1385–1397
72. Sadd BM et al (2015) The genomes of two key bumblebee species with primitive eusocial organization. Genome Biol 16(1):1–32
73. Leite DJ et al (2016) Pervasive microRNA duplication in chelicerates: insights from the embryonic microRNA repertoire of the spider Parasteatoda tepidariorum. Genome Biol Evol 8(7):2133–2144
74. Qu Z et al (2020) Millipede genomes reveal unique adaptations during myriapod evolution. PLoS Biol 18(9):e3000636
75. Nong W et al (2021) Horseshoe crab genomes reveal the evolution of genes and microRNAs after three rounds of whole genome duplication. Commun Biol 4(1):1–11
76. Okamura K et al (2008) The regulatory activity of microRNA* species has substantial influence on microRNA and 3′ UTR evolution. Nat Struct Mol Biol 15(4):354–363
77. Yang J-S et al (2011) Widespread regulatory activity of vertebrate microRNA* species. RNA 17(2):312–326
78. Zinovyeva AY et al (2014) Mutations in conserved residues of the *C. elegans* microRNA Argonaute ALG-1 identify separable functions in ALG-1 miRISC loading and target repression. PLoS Genet 10(4):e1004286
79. Zinovyeva AY et al (2015) Caenorhabditis elegans ALG-1 antimorphic mutations uncover functions for Argonaute in microRNA guide strand selection and passenger strand disposal. Proc Natl Acad Sci 112(38):E5271–E5280
80. Huang C-J et al (2014) Frequent co-expression of miRNA-5p and-3p species and cross-targeting in induced pluripotent stem cells. Int J Med Sci 11(8):824
81. Berezikov E (2011) Evolution of microRNA diversity and regulation in animals. Nat Rev Genet 12(12):846–860
82. Li S-C et al (2012) MicroRNA 3′end nucleotide modification patterns and Strand selection preference in liver tissues. BMC Syst Biol 6:1–12
83. Zhou H et al (2010) miR-155 and its star-form partner miR-155* cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. Blood J Am Soc Hematol 116(26):5885–5894
84. Alivernini S et al (2018) MicroRNA-155—at the critical interface of innate and adaptive immunity in arthritis. Front Immunol 8:1932
85. Dawson O, Piccinini AM (2022) miR-155-3p: processing by-product or rising star in immunity and cancer? Open Biol 12(5):220070
86. Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. Cell 115(2):209–216
87. Schwarz DS, Hutvágner G, Tingting Du, Zuoshang Xu, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. Cell 115(2):199–208
88. Sun G et al (2009) SNPs in human miRNA genes affect biogenesis and function. RNA 15(9):1640–1651
89. Kawamata T, Seitz H, Tomari Y (2009) Structural determinants of miRNAs for RISC loading and slicer-independent unwinding. Nat Struct Mol Biol 16(9):953–960
90. Maniataki E, Mourelatos Z (2005) A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. Genes Dev 19(24):2979–2990
91. Yoda M et al (2010) ATP-dependent human RISC assembly pathways. Nat Struct Mol Biol 17(1):17–23
92. Kwak PB, Tomari Y (2012) The N domain of Argonaute drives duplex unwinding during RISC assembly. Nat Struct Mol Biol 19(2):145–151
93. Kobayashi H, Tomari Y (2016) RISC assembly: coordination between small RNAs and Argonaute proteins. Biochim Biophys Acta BBA Gene Regul Mech 1859(1):71–81
94. Kawamata T, Tomari Y (2010) Making risc. Trends Biochem Sci 35(7):368–376
95. Sasaki HM, Tomari Y (2012) The true core of RNA silencing revealed. Nat Struct Mol Biol 19(7):657–660
96. Woehrer SL et al (2015) A tetrahymena Hsp90 co-chaperone promotes si RNA loading by ATP-dependent and ATP-independent mechanisms. EMBO J 34(4):559–577
97. Chatterjee S et al (2011) Target-mediated protection of endogenous microRNAs in *C. elegans*. Dev Cell 20(3):388–396
98. Shijun MI et al (2008) Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5′ terminal nucleotide. Cell 133(1):116–127

99. Frank F, Sonenberg N, Nagar B (2010) Structural basis for 5′-nucleotide base-specific recognition of guide RNA by human AGO2. Nature 465(7299):818–822
100. Seitz H, Tushir JS, Zamore PD (2011) A 5′-uridine amplifies miRNA/miRNA* asymmetry in Drosophila by promoting RNA-induced silencing complex formation. Silence 2:1–10
101. Young C et al (2022) Reversing the miRNA-5p/-3p stoichiometry reveals physiological roles and targets of miR-140 miRNAs. RNA 28(6):854–864
102. Kawamata T, Yoda M, Tomari Y (2011) Multilayer checkpoints for microRNA authenticity during RISC assembly. EMBO Rep 12(9):944–949
103. Zhao Y, Lu G-A, Yang H, Lin P, Liufu Z, Tang T, Xu J (2020) Run or die in the evolution of new microRNAs—testing the red queen hypothesis on de novo new genes. Mol Biol Evol 38(4):1544–1553
104. Fernandez-Valverde SL, Taft RJ, Mattick JS (2010) Dynamic isomiR regulation in Drosophila development. RNA 16(10):1881–1888
105. Bofill-DeRos X, Yang A, Gu S (2020) IsomiRs: expanding the miRNA repression toolbox beyond the seed. Biochim Biophys Acta (BBA) Gene Regul Mech 1863(4):194373
106. Warnefors M et al (2014) Conserved microRNA editing in mammalian evolution, development and disease. Genome Biol 15(6):1–14
107. Tan GC et al (2014) 5′ isomiR variation is of functional and evolutionary importance. Nucleic Acids Res 42(14):9424–9435
108. Tan GC, Dibb N (2015) IsomiRs have functional importance. Malays J Pathol 37(2):73–81
109. Guo L et al (2014) Selected isomiR expression profiles via arm switching? Gene 533(1):149–155
110. Lee HY, Doudna JA (2012) TRBP alters human precursor microRNA processing in vitro. RNA 18(11):2012–2019
111. Wilson RC et al (2015) Dicer-TRBP complex formation ensures accurate mammalian microRNA biogenesis. Mol Cell 57(3):397–407
112. Noland CL, Doudna JA (2013) Multiple sensors ensure guide strand selection in human RNAi pathways. RNA 19(5):639–648
113. Guo L et al (2013) Close association between paralogous multiple isomiRs and paralogous/orthologues miRNA sequences implicates dominant sequence selection across various animal species. Gene 527(2):624–629
114. Guo L, Chen F (2014) A challenge for miRNA: multiple isomiRs in miRNAomics. Gene 544(1):1–7
115. Warkocki Z et al (2018) Terminal nucleotidyl transferases (TENTs) in mammalian RNA metabolism. Philos Trans R Soc B Biol Sci 373(1762):20180162
116. Liudkovska V, Dziembowski A (2021) Functions and mechanisms of RNA tailing by metazoan terminal nucleotidyltransferases. Wiley Interdiscip Rev RNA 12(2):e1622
117. De Almeida C et al (2018) RNA uridylation: a key posttranscriptional modification shaping the coding and noncoding transcriptome. Wiley Interdiscip Rev RNA 9(1):e1440
118. Thornton JE et al (2012) Lin28-mediated control of let-7 microRNA expression by alternative TUTases Zcchc11 (TUT4) and Zcchc6 (TUT7). RNA 18(10):1875–1885
119. Heo I et al (2012) Mono-uridylation of pre-microRNA as a key step in the biogenesis of group II let-7 microRNAs. Cell 151(3):521–532
120. Chang H-M et al (2013) A role for the Perlman syndrome exonuclease Dis3l2 in the Lin28–let-7 pathway. Nature 497(7448):244–248
121. Zhi T et al (2017) EZH2 alteration driven by microRNA-524-5p and microRNA-324-5p promotes cell proliferation and temozolomide resistance in glioma. Oncotarget 8(56):96239
122. Li S-C et al (2012) miRNA strand selection and isomiR distribution in gastric cancer. BMC genomics 13(1):1–10
123. Nishikura K (2016) A-to-I editing of coding and non-coding RNAs by ADARs. Nat Rev Mol Cell Biol 17(2):83–96
124. Correia De Sousa M et al (2019) Deciphering miRNAs' action through miRNA editing. Int J Sci 20(24):6249
125. Mingardi J et al (2018) miRNA editing: new insights into the fast control of gene expression in health and disease. Mol Neurobiol 55:7717–7727
126. Shoshan E et al (2015) Reduced adenosine-to-inosine miR-455–5p editing promotes melanoma growth and metastasis. Nat Cell Biol 17(3):311–321
127. Kawahara Y et al (2007) Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. Science 315(5815):1137–1140
128. Kume H et al (2014) A-to-I editing in the miRNA seed region regulates target mRNA selection and silencing efficiency. Nucleic Acids Res 42(15):10050–10060
129. Kawahara Y et al (2008) Frequency and fate of microRNA editing in human brain. Nucleic Acids Res 36(16):5270–5280
130. Warf MB et al (2012) Effects of ADARs on small RNA processing pathways in *C. elegans*. Genome Res 22(8):1488–1498
131. Alon S et al (2012) Systematic identification of edited microRNAs in the human brain. Genome Resh 22(8):1533–1540
132. Nigita G et al (2016) MicroRNA editing in seed region aligns with cellular changes in hypoxic conditions. Nucleic Acids Res 44(13):6298–6308
133. Vesely C et al (2014) ADAR2 induces reproducible changes in sequence and abundance of mature microRNAs in the mouse brain. Nucleic Acids Res 42(19):12155–12168
134. Iizasa H et al (2010) Editing of Epstein–Barr virus-encoded BART6 microRNAs controls their Dicer targeting and consequently affects viral latency. J Biol Chem 285(43):33358–33370
135. Gallego A et al (2017) RNA editing independently occurs at three mir-376a-1 sites and may compromise the stability of the microRNA hairpin. Gene 628:109–116
136. Cho CJ et al (2018) Combinatory RNA-sequencing analyses reveal a dual mode of gene regulation by ADAR1 in gastric cancer. Dig Dis Sci 63:1835–1850
137. Li L et al (2018) The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting. Genome Res 28(1):132–143
138. García-López J, de Hourcade JD, Del Mazo J (2013) Reprogramming of microRNAs by adenosine-to-inosine editing and the selective elimination of edited microRNA precursors in mouse oocytes and preimplantation embryos. Nucleic Acids Res 41(10):5483–5493
139. Salmena L et al (2011) A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146(3):353–358
140. Thomson DW, Dinger ME (2016) Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 17(5):272–283
141. Lin W et al (2021) The development and controversy of competitive endogenous RNA hypothesis in non-coding genes. Mol Cell Biochem 476:109–123
142. Ebert MS, Neilson JR, Sharp PA (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods 4(9):721–726
143. Panda AC (2018) Circular RNAs act as miRNA sponges. Circ RNAs 1087:67–79
144. Ameres SL et al (2010) Target RNA-directed trimming and tailing of small silencing RNAs. Science 328(5985):1534–1539
145. Fuchs Wightman F et al (2018) Target RNAs strike back on microRNAs. Front Genet 9:435

146. Cazalla D, Yario T, Steitz JA (2010) Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. Science 328(5985):1563–1566
147. Memczak S et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495(7441):333–338
148. Hansen TB et al (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495(7441):384–388
149. Kingston ER, Blodgett LW, Bartel DP (2022) Endogenous transcripts direct microRNA degradation in Drosophila, and this targeted degradation is required for proper embryonic development. Mol Cell 82(20):3872.e9-3884.e9
150. Denzler R et al (2014) Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. Mol Cell 54(5):766–776
151. Bosson AD, Zamudio JR, Sharp PA (2014) Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. Mol Cell 56(3):347–359
152. Park JK et al (2007) The miRNA pathway intrinsically controls self-renewal of Drosophila germline stem cells. Curr Biol 17(6):533–538
153. Rüegger S, Großhans H (2012) MicroRNA turnover: when, how, and why. Trends Biochem Sci 37(10):436–446
154. Baccarini A et al (2011) Kinetic analysis reveals the fate of a microRNA following target regulation in mammalian cells. Curr Biol 21(5):369–376
155. De La Mata M et al (2015) Potent degradation of neuronal mi RNA s induced by highly complementary targets. EMBO Rep 16(4):500–511
156. Han J, Mendell JT (2022) MicroRNA turnover: a tale of tailing, trimming, and targets. Trends Biochem Sci 48(1):26–39
157. Bitetti A et al (2018) MicroRNA degradation by a conserved target RNA regulates animal behavior. Nat Struct Mol Biol 25(3):244–251
158. Kleaveland B et al (2018) A network of noncoding regulatory RNAs acts in the mammalian brain. Cell 174(2):350.e17-362.e17
159. Ghini F et al (2018) Endogenous transcripts control miRNA levels and activity in mammalian cells by target-directed miRNA degradation. Nat Commun 9(1):1–15
160. Pasquier J et al (2017) Evolution of gene expression after whole-genome duplication: New insights from the spotted gar genome. J Exp Zool Part B Mol Dev Evol 328(7):709–721
161. Peterson KJ et al (2022) MicroRNAs as indicators into the causes and consequences of whole-genome duplication events. Mol Biol Evol 39(1):msab344
162. Shabalina SA, Koonin EV (2008) Origins and evolution of eukaryotic RNA interference. Trends Ecol Evol 23(10):578–587
163. Lyu Y, Liufu Z, Xiao J, Tang T (2021) A rapid evolving microRNA cluster rewires its target regulatory networks in *Drosophila*. Front Genet 12:760530