Regulation of miRNA strand selection: follow the leader?

Hedda A. Meijer*[1], Ewan M. Smith* and Martin Bushell*[1]
*Medical Research Council (MRC) Toxicology Unit, Hodgkin Building, Lancaster Road, Leicester LE1 9HN, U.K.

Abstract

miRNA strand selection is the process that determines which of the two strands in a miRNA duplex becomes the active strand that is incorporated into the RISC (RNA-induced silencing complex) (named the guide strand, leading strand or miR) and which one gets degraded (the passenger strand or miR*). Thermodynamic features of the duplex appear to play an important role in this decision; the strand with the weakest binding at its 5′-end is more likely to become the guide strand. Other key characteristics of human miRNA guide strands are a U-bias at the 5′-end and an excess of purines, whereas the passenger strands have a C-bias at the 5′-end and an excess of pyrimidines. Several proteins are known to play a role in strand selection [Ago (Argonaute), DICER, TRBP (trans-activation response RNA-binding protein), PACT (protein activator of dsRNA-dependent protein kinase) and Xrn-1/2]; however, the mechanisms by which these proteins act are largely unknown. For several miRNAs the miR/miR* ratio varies dependent on cell type, developmental stage and in different disease states, suggesting that strand selection is a tightly controlled process. The present review discusses our current knowledge regarding the factors and processes involved in strand selection and the many questions that still remain.

Introduction

The human genome contains ∼1900 miRNA hairpins (miRBase) [1]; this has been traditionally interpreted to reflect that in humans there are ∼1900 functional miRNAs. However, it is coming to light that the processing of miRNAs is a dynamic process by which each strand of the precursor can be selected to become a functional mature miRNA, potentially doubling the total of encoded miRNAs. Moreover, miRNAs can be modified in several different ways resulting in isomiRs, therefore increasing the total number of functional miRNAs even more. Each of these miRNAs has the potential to exert distinct functional consequences via subtle or dramatic changes to their target repertoire. The ratio of the two mature strands of many miRNA duplexes can vary depending on cell type, developmental stage and in several diseases. This implies that both transcription and processing of miRNAs is strictly regulated.

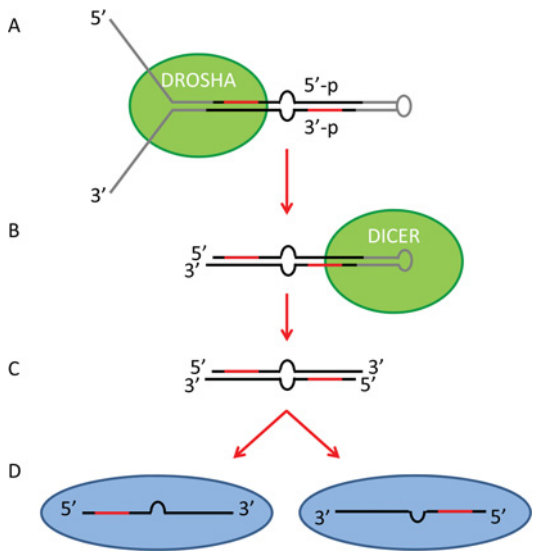
Most miRNAs are transcribed as a long precursor by RNA polymerase II and undergo extensive processing before they are integrated into the active RISC (RNA-induced silencing complex) (Figure 1). Initially, these pri-miRNAs (primary miRNAs) get trimmed by Drosha to a hairpin duplex of approximately 70 nt, called pre-miRNAs (precursor miRNAs), subsequently, these pre-miRNAs get transported to the cytoplasm by Exportin5 [2]. In the present review, another round of trimming takes place, this time by Dicer in combination with TRBP [TAR (trans-activation response) RNA-binding protein], which results in an approximately 22 nt long duplex with short 3′ overhangs [2]. Dicer then transfers the duplex to one of the four human Ago (Argonaute) proteins; this requires Ago to undergo conformational changes to allow binding of the duplex [3]. One of the strands of the duplex is discarded (called the passenger strand or miR*) in a process called strand selection, leaving an activated RISC containing Ago and one of the ssRNA molecules (called the guide strand, leading strand or miR). Incorporation of the miRNA into the RISC allows the presentation of miRNA nt 2–8 (the seed sequence) at an interface where it can interact with the target mRNA, typically with a region within its 3′-UTR. The target recognition is enabled by a strong complementarity between the seed sequence of the miRNA and the target mRNA. Each miRNA can have hundreds of different targets resulting in a complex system of post-transcriptional regulation [4,5]. Interaction of miRNAs with their targets generally results in translational repression followed by degradation of the target mRNA [6–9].

The cellular repertoire of miRNAs is controlled at many levels: transcription of the precursor, processing of the duplex, formation and activation of RISC and lastly stability of the miRNA [10]. Deep sequencing has demonstrated how varied the miRNA expression profiles are across different cell types, developmental stages and in disease states [11–13]. Both alternative transcription patterns and alternative strand selection play important roles in determining the miRNA expression pattern [11].

Key words: guide strand, isomiR, leading strand, miR*, miRNA biogenesis, passenger strand.
Abbreviations: Ago, Argonaute; Hsp90, heat-shock protein 90; PACT, protein activator of dsRNA-dependent protein kinase; PAZ, PIWI/Argonaute/Zwille; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; RISC, RNA-induced silencing complex; TRBP, TAR (trans-activation response) RNA-binding protein.
[1]**Correspondence may be addressed to either of these authors (email hm16@le.ac.uk or mb446@le.ac.uk).**

Figure 1 | miRNA processing and strand selection

(**A**) Drosha cleaves the 5′-end of the 5p arm and the 3′-end of the 3p arm of the pri-miRNA, whereas (**B**) Dicer cleaves the 3′-end of the 5′ arm and the 5′-end of the 3′ arm of the pre-miRNA, resulting in (**C**), a miRNA duplex where the seed sequence of the 5p arm is determined by Drosha and the seed sequence of the 3p arm by Dicer. (**D**) Both strands can be incorporated into mature RISC. pri-miRNA is shown in grey, mature miRNA is shown in black, seed sequence is shown in red, Drosha and Dicer are shown in green, and Ago is shown in blue.



RISC assembly

The Ago proteins form the heart of the RISC. It has been shown that just Ago2 and an siRNA can form a functional RISC *in vitro* that is capable of cleaving a target mRNA [14]. However, in the cell, Ago is associated with several other proteins [Dicer, Hsp90 (heat-shock protein 90), TRBP and/or PACT (protein activator of dsRNA-dependent protein kinase)] to help it function efficiently [15–17]. These proteins assist with the two steps of RISC assembly: (i) RISC loading (a conformational change to Ago and loading of the RNA duplex resulting in the formation of pre-RISC) and (ii) RISC maturation (the duplex is unwound and one of the strands is removed, resulting in the mature RISC) [18].

A protein critical for RISC loading is Hsp90, which binds directly to the N-terminus of Ago2 [15,16] and is predicted to mediate the conformational change to Ago allowing the duplex to enter, a process strongly dependent on ATP hydrolysis [19]. The RNA duplex is a bulky rigid structure and Ago is required to go through a conformational change in order to accommodate the duplex [3]. The 5′-end of the guide strand then binds to the Ago2 MID (middle) domain, assisted by the PIWI (P-element-induced wimpy testes) domain, whereas the 3′-end interacts with the PAZ (PIWI/Argonaute/Zwille) domain [20]. This process is stimulated by central mismatches in the duplex, which might give the duplex a degree of flexibility to enhance efficient duplex loading [21].

The first stage of the unwinding is a change in the position of the N-domain of Ago2 which pries open the end of the duplex therefore presenting the 5′-end of the passenger strand to the Ago PIWI domain [22]. The subsequent step is dependent on the nature of the duplex and which Ago is in the complex. Unlike siRNAs, which are normally processed by Ago2 cleaving the passenger strand, miRNAs when loaded on to Ago2 are processed through a cleavage-independent bypass mechanism, which involves the unwinding of the duplex before discarding the passenger strand. miRNA/miRNA* duplexes typically contain multiple mismatches which are presumed to be responsible for the preference of Ago2 to use the bypass mechanism [23]. Ago1, Ago3 and Ago4 are not able to process siRNAs so this task is exclusive for Ago2; however, all Ago proteins are able to use the bypass mechanism for the processing of miRNAs [18]. The four Ago proteins might have similar preferences for RNA duplexes [21], but the different processes for RISC maturation do not have the same efficiency. The bypass mechanism is much slower than the cleavage-dependent pathway, making the activation of RISC the rate-limiting step for non-cleaving RISC [18].

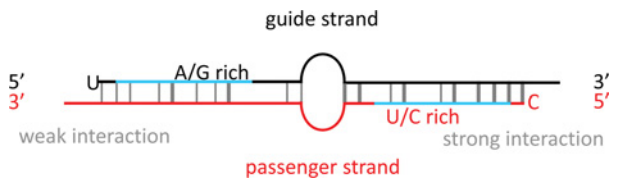
Features of active strands

The activation of RISC is strongly stimulated by the thermodynamic instability of the miRNA duplex [24]. The thermodynamic stability is only important for miRNA duplexes processed via the bypass mechanism, it does not apply to duplexes with a (nearly) completely base-paired stem (such as siRNAs); in these cases, Ago2 can cleave one of the strands and it does not have to pry the two strands apart starting from the end of the duplex [18]. The strand of the miRNA duplex with the weakest binding at the 5′-end of the duplex is more frequently incorporated into RISC, whereas the strand with the stronger interaction at their 5′-end is generally degraded [24] (Figure 2). Presumably, the weaker 5′ interaction will make it easier for the N-domain of Ago to pry open the end of the duplex and start the unwinding process. The first four nucleotides of the duplex determine the thermodynamic asymmetry, with a single extra hydrogen bond being sufficient for preferential loading [24,25]. Most miRNA duplexes have a double nucleotide overhang at the 3′-end. These nucleotides can affect the stability of the duplex and are therefore predicted to have an impact on strand selection [26,27].

For human miRNAs, the guide strand has a uracil-bias at the 5′-end, whereas the passenger strand has a cytosine-bias at the 5′-end [28] (Figure 2). Presumably, these nucleotides contribute to the thermostability of the duplex. Another feature of the guide strand is an excess of purines (A/G), whereas the passenger strand consequently has an excess of pyrimidines (U/C) [28]. The purine residues have been suggested to form hydrophobic interactions with the aromatic residues in the Ago PAZ domain, resulting in preferential loading of the duplex in an orientation that results in the purine-rich strand becoming the guide strand. This

Figure 2 | Features of guide and passenger strands

The guide strand (black) usually starts with a 5′ uracil base and is generally A/G rich, whereas the passenger strand (red) frequently has a 5′ cytosine base and is generally U/C rich. The interaction at the 5′-end of the guide strand is typically weaker than at the 5′-end of the passenger strand. Weak interactions are shown as thin grey lines, strong interactions are shown as thick grey lines and seed sequence is show in blue.



implies that the orientation of the duplex when it is loaded on to RISC determines which strand will be incorporated into the mature RISC. miRNAs where both strands of the duplex are present at similar levels do not show either of the biases described above [28]. A single point mutation in the duplex can change the preferred guide strand as demonstrated by an SNP (single nucleotide polymorphism) discovered in an individual with atrial fibrillation [29]. This mutation is located within the duplex at the 3′-end of the mature strand of *miR-133a-3p*, which results in alternative processing and accumulation of the *miR-133a-5p* strand. Normally, the 3p strand is the only one detected; however, this point mutation appears to reduce the thermostability of *miR-133a-3p* resulting in a change of strand selection [29].

Post-transcriptional modifications to the duplex can also have a major impact on strand selection. The resulting isomiRs can be very abundant in certain cell types [30,31]. They are more likely to have modified 3′-ends than 5′-ends [28,32]. This is probably the result of the more stringent requirement of the seed sequence, which would be changed when the 5′-end processing is altered and would have a major impact on the selection of genes to be silenced. However, the terminal modifications of the 3′-end of miRNAs, mostly non-templated mono- or di-nucleotide additions, can also have a major effect since they appear to affect the thermostability of the duplex and therefore strand selection [33]. It has been shown that for the vast majority of miRNAs in brain samples, isomiRs are in abundance [30]. Most of these modifications are 3′-end trimmings or additions. For some of these miRNAs, the expression levels of the corresponding isomiR are strongly up-regulated in Huntington's disease and these modifications to the miRNA duplex can potentially affect strand selection [30].

Some pre-miRNAs are transcribed by PolII (without any trimming by Drosha) and therefore have an m7G cap structure at the 5′-end, which strongly biases the guide strand selection by Ago in favour of the 3p miRNA [34]. In summary, any changes to the 5′- or 3′-end of each strand of the duplex can affect strand selection, therefore the processes that modify miRNA ends are expected to be tightly regulated.

Protein factors involved in strand selection

Ago2 has intrinsic strand selection capability which can be enhanced by DICER, TRBP and PACT binding [17]. The extent of the stimulating effect of TRBP and PACT is dependent on the duplex parameters: thermodynamics, 5′ nucleotide identity and structure. When DICER and TRBP/PACT are in complex with Ago2, the thermodynamic characteristics of the duplex are more important than 5′-end nucleotides. The effect of TRBP and PACT on strand selection is dependent on the nature of the duplex, certain miRNAs are much more sensitive than others to the presence of TRBP and/or PACT, implying that there is an interplay between the protein factors and the duplex involved [35]. The choice of Ago can also affect strand selection. The PAZ and MID domains of Ago3 can change the guide/passenger strand ratio of the tumour suppressor miRNA let-7a in comparison with let-7a processed by Ago1, Ago2 or Ago4 [36].

Guide strands and passenger strands

A testament to the importance of miRNAs is the strong conservation of the sequence of guide strands (especially the seed region) during vertebrate evolution. However, a substantial fraction of passenger strands is also conserved [37], whereas others show high levels of nucleotide divergence even when the guide strand is highly conserved [38]. The lack of divergence in some cases clearly suggests that these passenger strands play an important role, at least in some tissues/under certain circumstances. The name 'passenger strand or miR*' is most likely not very accurate in these cases and therefore using a nomenclature which is based on the 5p–3p system would be preferable since that does not suggest that one strand is more important than the other (Figure 1). Highly abundant miRNAs originate more frequently from the 5p strand than the 3p strand [28]. This might be a result of trying to reduce the error rate of highly expressed miRNAs. Drosha is a more accurate enzyme than Dicer; consequently, the seed sequence will be less error prone if it is located on the 5p arm of the duplex because Drosha is responsible for the cutting of the 5′-end of the 5p arm and Dicer cuts the 5′-end of the 3p arm [28] (Figure 1). The location where each arm of the duplex gets trimmed at the 5′-end determines the seed sequence and therefore the target repertoire.

For many miRNAs, both strands are readily detected. Expression profiling shows that in some tissues, both strands can be equally abundant, whereas in other tissues there is a strong preference for one of the strands [11,33,39–41]. In general, both strands can be equally functional when selected [4,5]. For miRNA genes that have more than one copy in the genome, each sister miRNA can be regulated independently, during both transcription and processing; the resulting ratio of 5p to 3p strand incorporation into Ago can be different for each copy, implying that each copy originating from a different genomic location has the potential to regulate its own set of mRNAs [39,42]. The variable abundance of both strands of miRNA duplexes is clear evidence of the critical

role strand selection plays in miRNA-mediated regulation, as shown in the examples discussed below.

Consequences of strand selection

The fact that both strands of a particular miRNA are present does not necessarily mean that they are counteracting each other even though they will have near complementary sequences. Guide strands can act synergistically with their passenger strands by interacting with different targets reinforcing the same phenotype [43–47]. For example, *miR30e* and *miR30e** are up-regulated in primary human glioma cells and are markers for malignant progression and predict poor survival. Both strands have different targets and co-operate to support the progression of glioma [44].

Alternatively, guide and passenger strands can each have their own targets, which can directly counteract each other [29]. This is demonstrated by *miR-155* and *miR-155**, which both play a role in the activation of plasmacytoid dendritic cells. The ratio between the two strands changes throughout the different activation stages and have opposite effects on type I interferon expression [48]. In contrast, *miR-155* and *miR-155** are both induced in cytokine-treated astrocytes and co-operatively play a role in the inflammatory response [49].

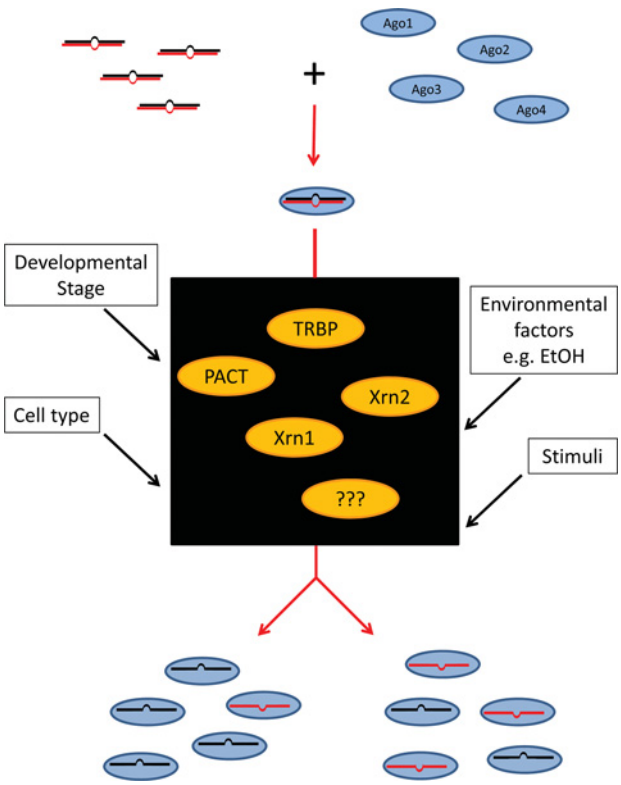
These examples show that the regulation of strand selection is a tightly controlled process which has critical biological functions. The general rules regarding the duplex characteristics and their general consequences for strand selection might be well established, however, our knowledge regarding how these rules change depending on cell type, development and in health and disease are still fairly limited and deserve more attention. It is clear that strand selection plays a critical role under many circumstances and understanding how this is regulated will give us a better grasp of its full potential to control gene expression pathways.

Conclusion and perspective

Currently, there is not much known about how the thermodynamic stability rules can be over-ruled under certain circumstances. However, since cell type, developmental stage and disease state can all affect the ratio of the miRNA strands that can be detected, there are clearly certain circumstances that affect strand selection. A number of factors have been shown to play a role in strand selection, which is discussed below. Generally, miRNA levels and their target mRNAs levels demonstrate a strong anti-correlation [50]. Interestingly, target mRNAs have been shown to have the ability to protect their cognate miRNAs from degradation [51]. It has been suggested that miRNAs which are bound to Ago, but are not interacting with targets are susceptible to degradation, whereas miRNAs that are interacting with mRNAs are protected. Most miRNAs are relatively stable, with an average half-life of approximately 5 days [52]. However, it has been shown that certain miRNAs can be very unstable in neuronal cells (half-life as little as 1 h) [53]. The

Figure 3 | Possible factors/circumstances affecting strand selection

Strand selection changes under different circumstances and several factors are known to play a role; how the circumstances affect these factors and how these factors convey this preference is currently unknown. The guide strand is shown in black and the passenger strand is shown in red. EtOH, ethanol.



degradation of miRNAs is mediated by Xrn-1 and Xrn-2. This is, at least for certain miRNAs, a regulated process since the expression of Xrn-1/Xrn-2 can affect the ratios of certain miR/miR* combinations [51,54].

TRBP and PACT stimulate the inherent strand selection capability of Ago2 [17]. However, a complex containing Ago and TRBP can have a different preferred guide strand than a complex formed of Ago and PACT. This is a potential level of regulation since any factors that can affect expression levels of TRBP and/or PACT can affect strand selection of at least certain miRNAs [35]. Therefore different expression levels of Xrn-1/Xrn-2, TRBP and PACT in different cell types/under different circumstances have the potential to regulate strand selection. Whether these proteins change the strand selection ability of Ago or act directly on the miRNA duplexes themselves facilitating post-transcriptional modifications is currently unclear.

Even lifestyle could have an impact on strand selection. It has been shown that alcohol can alter guide/passenger strand ratios [55]. There are most likely several more factors that determine strand selection. The main questions are now what are these factors and how are they regulated, what is their

impact on strand selection and subsequently how do they affect miRNA targeting (Figure 3).

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