

Research Article

Base Composition Characteristics of Mammalian miRNAs

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MicroRNAs (miRNAs) are short RNA sequences that repress protein synthesis by either inhibiting the translation of messenger RNA (mRNA) or increasing mRNA degradation. Endogenous miRNAs have been found in various organisms, including animals, plants, and viruses. Mammalian miRNAs are evolutionarily conserved, are scattered throughout chromosomes, and play an important role in the immune response and the onset of cancer. For this study, the author explored the base composition characteristics of miRNA genes from the six mammalian species that contain the largest number of known miRNAs. It was found that mammalian miRNAs are evolutionarily conserved and GU-rich. Interestingly, in the miRNA sequences investigated, A residues are clearly the most frequent occupants of positions 2 and 3 of the 5' end of miRNAs. Unlike G and U residues that may pair with C/U and A/G, respectively, A residues can only pair with U residues of target mRNAs, which may augment the recognition specificity of the 5' seed region.

1. Introduction

MicroRNAs (miRNAs) are single-stranded, short (15–27 nucleotides) RNA sequences that repress protein synthesis via base pairing to some portion of a messenger RNA (mRNA), such as the 3' untranslated region (3'UTR), the 5' untranslated region (5'UTR), or the coding region [1, 2]. Endogenous miRNAs have been found in various organisms, including animals, plants, and viruses. In mammals, precursor miRNAs (pre-miRNAs) 60–100 nucleotides in length form a hairpin stem-loop structure and are processed by Dicer, a highly conserved RNase III family endonuclease that is found in almost all eukaryotic organisms [3, 4]. This processing yields a miRNA : miRNA duplex that is approximately 21 nucleotides long. One or both of the duplex's short RNA strands is incorporated into RNA-induced silencing complexes (RISCs, which are composed of Dicer, Argonaute, and other nonspecified proteins) and functions as a mature miRNA that can base pair with mRNA targets, inducing either the degradation or translational repression of the mRNA [3, 4].

The regulation of miRNA is essential for organisms because miRNA plays critical roles in numerous biological processes, including the proliferation and differentiation of

cells, as well as apoptosis. It is predicted that mammalian miRNAs can regulate approximately 30% of protein-coding genes [4]. The dysfunctional posttranscriptional modulation of gene expression caused by miRNAs has been related to diseases such as cancer and neurodegenerative disorders [5–8]. The investigation of miRNAs and their gene targets has thus attracted much attention in recent years and has become a major focus of research in medicine and molecular biology [7, 9–13].

For this study, the author explored the base composition of mature miRNAs from the six mammalian species that contain the largest number of known miRNAs. Position-specific base dominance in certain miRNAs has been investigated by other researchers; for example, Gkirtzou et al. discussed the sequence composition on and around mature miRNAs for humans and mice [14], and Wang et al. described position-specific base dominance in human miRNAs [15]. However, this paper provides more complete, detailed, and updated information regarding the base composition characteristics of mature miRNAs in four primates and two rodents, thus providing additional knowledge regarding mammalian miRNAs. We found that the mammalian miRNAs are evolutionarily conserved and GU-rich, where U residues are more frequent than other bases. Adenosine residues are generally

TABLE 1: Total base composition and base composition at position 1 of both ends of mature miRNAs in six mammalian species. Bases shown in red represent the most frequent residues in position 1 of both ends of the miRNAs, whereas bases in green indicate the least frequent residues in position 1 at the 5' end of these miRNAs.

Species	No. of miRNA sequences investigated	No. and % of A, G, U, and C in miRNAs investigated	Base composition at position 1 of the 5' end of miRNAs	Base composition at position 1 of the 3' end of miRNAs
<i>Homo sapiens</i>	1897	A 9510 (23%)	A 578 (30%)	A 409 (22%)
		G 11383 (28%)	G 263 (14%)	G 463 (24%)
		U 10755 (26%)	U 673 (36%)	U 630 (33%)
		C 9236 (23%)	C 383 (20%)	C 395 (21%)
<i>Pongo pygmaeus</i>	600	A 2945 (23%)	A 178 (30%)	A 110 (18%)
		G 3438 (26%)	G 69 (11%)	G 141 (24%)
		U 3731 (29%)	U 264 (44%)	U 236 (39%)
		C 2928 (22%)	C 89 (15%)	C 113 (19%)
<i>Pan troglodytes</i>	525	A 2653 (23%)	A 166 (32%)	A 103 (20%)
		G 2961 (26%)	G 64 (12%)	G 118 (22%)
		U 3265 (29%)	U 229 (44%)	U 198 (38%)
		C 2527 (22%)	C 66 (12%)	C 106 (20%)
<i>Macaca mulatta</i>	488	A 2479 (23%)	A 144 (29%)	A 89 (18%)
		G 2741 (26%)	G 59 (12%)	G 117 (24%)
		U 3042 (29%)	U 213 (44%)	U 179 (37%)
		C 2392 (22%)	C 72 (15%)	C 103 (21%)
<i>Mus musculus</i>	1157	A 5687 (23%)	A 336 (29%)	A 262 (23%)
		G 6634 (26%)	G 161 (14%)	G 266 (23%)
		U 6901 (27%)	U 417 (36%)	U 379 (33%)
		C 5926 (24%)	C 243 (21%)	C 250 (21%)
<i>Rattus norvegicus</i>	680	A 3496 (24%)	A 203 (30%)	A 162 (24%)
		G 3639 (25%)	G 86 (12%)	G 150 (22%)
		U 4149 (28%)	U 243 (36%)	U 233 (34%)
		C 3472 (23%)	C 148 (22%)	C 135 (20%)

a less frequent occupant, except at positions 2 and 3 in the 5' seed region of these miRNAs. This characteristic may explain the specificity of pairing and target recognition by the 5' seed region in miRNAs.

2. Materials and Methods

The sequences of mature miRNAs were obtained from miRBase (<http://www.mirbase.org/>), a database that contains experimentally supported base sequences for various miRNAs [16–19]. The miRNA data for *Homo sapiens*, *Pongo pygmaeus*, *Pan troglodytes*, *Macaca mulatta*, *Mus musculus*, and *Rattus norvegicus* were exported from the miRBase database and then transferred into Microsoft Excel. In Excel, the miRNA sequences were arranged such that each Excel column contained only the nucleotides at a specific position of the miRNAs studied (e.g., column B contained nucleotides at position 1 of the 5' end of all human miRNAs; column C contained nucleotides at position 2 of the 5' end of all human miRNAs studied). The nucleotides in each column were copied and pasted into an online RNA base composition calculator

(<http://www.currentprotocols.com/WileyCDA/CurrentProtocols/curpro3/tool/7.html>) in order to determine the number and percentage of A, G, U, and C at every position of each miRNA of each species studied.

3. Results and Discussion

The six largest miRNA pools were exported from the miRBase database, including *Homo sapiens* (1921 miRNAs), *Mus musculus* (1157 miRNAs), *Rattus norvegicus* (680 miRNAs), *Pongo pygmaeus* (600 miRNAs), *Pan troglodytes* (525 miRNAs), and *Macaca mulatta* (488 miRNAs). A small number of human miRNAs were found to have slightly different names but identical sequences. The names of miRNAs in miRBase are first assigned to their hairpin precursors and then inherited by the mature miRNAs. The naming problem is caused by the occasional existence of multiple miRNA members in the same family, where the mature sequence from one stem of the hairpin precursor is slightly different from the same sequence in a miRNA family member, while the sequences from the other stem of the hairpin precursor are identical. After deleting these redundant sequences, 1897 out of 1921 of

TABLE 2: Percentages (%) of A, G, U, and C in every mature miRNA of each species studied. This table contains base composition information for the second-through-eighth nucleotides from both ends of the miRNAs. Bases shown in red are the most favored residues at positions 2 and 3 of the 5' end; bases in magenta are the most favored residues at positions 4–6 of the 5' end; bases in orange are the most favored residues at position 2 of the 3' end; bases in green are the most favored residues at position 3 of the 3' end; bases in blue are the most favored residues at position 4 of the 3' end; and bases in purple are the most favored residues at positions 5 and 7 of the 3' end of the miRNAs examined.

Species	Location of base residue, as indicated by end of miRNA (5' or 3') and position number													
	5', 2	5', 3	5', 4	5', 5	5', 6	5', 7	5', 8	3', 2	3', 3	3', 4	3', 5	3', 6	3', 7	3', 8
<i>Homo sapiens</i>	A29	A29	A22	A23	A24	A26	A24	A20	A19	A19	A21	A20	A20	A24
	G28	G28	G36	G33	G27	G28	G26	G32	G29	G30	G26	G28	G29	G27
	U22	U23	U19	U22	U24	U23	U26	U24	U30	U29	U30	U26	U27	U27
	C21	C20	C23	C22	C25	C23	C24	C24	C22	C22	C23	C26	C24	C22
<i>Pongo pygmaeus</i>	A32	A28	A26	A22	A21	A26	A20	A16	A17	A15	A22	A23	A21	A27
	G26	G27	G30	G32	G29	G26	G24	G36	G28	G32	G24	G26	G26	G23
	U24	U23	U21	U25	U24	U22	U28	U27	U34	U31	U33	U25	U28	U28
	C18	C22	C23	C21	C26	C26	C28	C21	C21	C22	C21	C26	C25	C22
<i>Pan troglodytes</i>	A35	A32	A28	A24	A23	A24	A23	A17	A17	A15	A22	A21	A23	A24
	G26	G25	G30	G31	G26	G26	G23	G36	G29	G32	G24	G26	G24	G23
	U20	U23	U20	U25	U25	U23	U28	U25	U33	U31	U32	U27	U29	U31
	C19	C20	C22	C20	C26	C27	C26	C22	C21	C22	C22	C26	C24	C22
<i>Macaca mulatta</i>	A34	A29	A29	A24	A21	A27	A21	A18	A18	A14	A22	A23	A22	A29
	G27	G25	G27	G30	G28	G23	G24	G38	G25	G33	G24	G25	G25	G24
	U23	U23	U22	U24	U25	U24	U28	U24	U36	U31	U32	U27	U28	U25
	C16	C23	C22	C22	C26	C26	C27	C20	C21	C22	C22	C25	C25	C22
<i>Mus musculus</i>	A30	A25	A21	A24	A23	A25	A24	A21	A22	A20	A20	A24	A21	A23
	G29	G27	G31	G27	G26	G28	G25	G32	G23	G26	G24	G27	G26	G28
	U21	U28	U24	U27	U26	U23	U24	U22	U35	U31	U30	U24	U27	U25
	C20	C20	C24	C22	C25	C24	C27	C25	C20	C23	C26	C25	C26	C24
<i>Rattus norvegicus</i>	A31	A28	A25	A25	A23	A30	A24	A21	A19	A20	A21	A22	A20	A25
	G28	G26	G29	G26	G22	G24	G21	G32	G23	G25	G21	G29	G22	G25
	U20	U25	U22	U26	U28	U25	U27	U20	U36	U31	U34	U25	U34	U26
	C21	C21	C24	C23	C27	C21	C28	C27	C22	C24	C24	C24	C24	C24

human miRNAs were included for investigation in this study. No identical sequences were observed within any of the other five species.

Column 3 in Table 1 provides the total base composition, including total numbers and percentages of A, G, U, and C in all mature miRNAs of each species studied. Overall, G and U residues are more abundant than A and C residues in the miRNA sequences investigated. More Us than Gs are present in five of the six species, with human miRNAs being the exception. Based on canonical Watson-Crick base pairing, this bias in miRNA composition indicates the abundance of A residues in the target mRNA regions.

David Bartel's research team discovered the high frequency of U and low occurrence of G at the 5' end of *Caenorhabditis elegans* miRNAs [20]. Data shown in Table 1, column 4, confirmed this tendency in mammalian species. At position 1 of all experimentally confirmed miRNAs in each species studied, the frequency of U residues ranges from 36% to 44% (shown in red in Table 1), whereas G residues range from 11% to 14% (shown in green in Table 1). In addition, C residues are less frequent at the 5' end of mammalian

miRNAs, indicating a preference for base pairs with lower thermodynamic stability (i.e., favoring AU rather than GC base pairs) at position 1.

Data presented in Table 1, column 5, provide the base composition at the 3' end of the miRNAs studied. Interestingly, the 3' end demonstrates the same abundance of U residues as found in the 5' end of miRNAs, indicating an enrichment of A residues within the 5' end of mRNA targets. G and C residues are less frequent in the 3' end of miRNAs as well, however, to a lesser degree than in the 5' end.

It has been reported that the presence of an A residue in the mRNA target, across from position 1 of the 5' end of a miRNA, improves binding between the miRNA and its target. This occurs even though actual base pairing between the A residue and the nucleotide at position 1 of the 5' end of the miRNA is not necessary [3, 4, 21–24]. Fewer studies have focused on the 3' end of miRNAs [14]. The strong bias toward U residues at position 1 of both ends of miRNAs in the six mammalian species studied in this paper implies that both ends, rather than just the 5' end, may participate in target recognition. One study demonstrated that miRNA can

TABLE 3: Percentages (%) of A, G, U, and C in every mature miRNA of each species studied. This table contains base composition information for the ninth-through-sixteenth nucleotides from the 5' end of the miRNAs. Bases shown in red are the most favored residues at positions 9, 13, and 16 of the 5' end; bases in magenta are the most favored residues at position 15 of the 5' end of the miRNAs examined.

Species	Location of base residue, as indicated by end of miRNA (5') and position number							
	5', 9	5', 10	5', 11	5', 12	5', 13	5', 14	5', 15	5', 16
<i>Homo sapiens</i>	A22	A22	A22	A24	A24	A23	A23	A21
	G26	G28	G32	G27	G28	G29	G30	G25
	U29	U25	U24	U26	U28	U28	U25	U28
	C23	C25	C22	C23	C20	C20	C22	C26
<i>Pongo pygmaeus</i>	A21	A22	A22	A22	A21	A24	A24	A25
	G26	G27	G26	G25	G26	G23	G30	G21
	U31	U25	U27	U32	U31	U30	U25	U28
	C22	C26	C25	C21	C22	C23	C21	C26
<i>Pan troglodytes</i>	A20	A22	A20	A24	A22	A24	A22	A25
	G24	G27	G29	G23	G25	G25	G30	G20
	U35	U25	U26	U31	U31	U30	U26	U30
	C21	C26	C25	C22	C22	C21	C22	C25
<i>Macaca mulatta</i>	A20	A23	A23	A23	A21	A23	A26	A26
	G25	G26	G26	G25	G26	G26	G27	G20
	U34	U24	U26	U28	U34	U28	U26	U27
	C21	C27	C25	C24	C19	C23	C21	C27
<i>Mus musculus</i>	A21	A23	A20	A25	A23	A25	A21	A21
	G23	G27	G29	G26	G26	G28	G29	G25
	U31	U24	U26	U26	U28	U27	U24	U29
	C25	C26	C25	C23	C23	C20	C26	C25
<i>Rattus norvegicus</i>	A19	A21	A23	A26	A23	A25	A25	A20
	G23	G26	G25	G26	G26	G28	G28	G22
	U34	U23	U26	U27	U30	U30	U24	U30
	C24	C30	C26	C21	C21	C17	C23	C28

simultaneously interact with both the 3'UTR and 5'UTR of a mRNA target via complementary base pairing using its 5' and 3' regions, respectively [25], thus providing evidence for the possibility that both ends of miRNAs are involved in target recognition.

In metazoans, the second-through-seventh nucleotides at 5' end of a miRNA are known as the seed region, which is the most important region for miRNA complementarity and target recognition [23, 26]. In this study, we investigated the base composition (i.e., the percentages of A, G, U, and C) for two areas of every mature miRNA of each species in our study. We examined the sequences both the 5' seed region and the nucleotide immediately following it (i.e., the eighth nucleotide at the 5' end); our results are presented in Table 2. The corresponding regions at the 3' end were also studied, and those results are also presented in Table 2. In the 5' seed region, there is a preference for A at positions 2 and 3 (shown in red in Table 2), followed by a preference for G at positions 4–6 (shown in magenta in Table 2). The base preference at positions 7 and 8 is not consistent across the six species. In the corresponding 3' regions, there is a strong preference for G at position 2 (shown in orange in Table 2), followed by a preference for U at position 3 (shown in green in Table 2).

Position 4 at the 3' end of these miRNAs is more frequently G in primates and U in rodents (shown in blue in Table 2). Position 5 at the 3' end of these miRNAs is most frequently U (shown in purple in Table 2); U is also the most frequent occupant of position 7 (shown in purple in Table 2), except in human miRNAs. The base preferences at positions 6 and 8 of the 3' end of miRNAs are not consistent across the six species studied.

The specificity of miRNA targeting in mammals is not necessarily restricted to the 5' and 3' ends of the seed pairing regions, and previous reports suggest that seed matches are not always sufficient for the repression of targets in mammals [3]. Therefore, base composition beyond the eighth nucleotide from the 5' end of miRNAs was also investigated in this study. Base composition information for the ninth-through-sixteenth nucleotides from the 5' end of the miRNAs is presented in Table 3. Positions 9, 13, and 16 demonstrate a strong preference for U; whereas position 15 shows a strong preference for G in all six species investigated. The base preferences at positions 10, 11, 12, and 14 from the 5' end of the miRNAs are not consistent in these six species.

Previous studies suggest that the presence of an A or U residue in the mRNA target, across from position 9 of the 5'

end of a miRNA, improves binding between the miRNA and its target. This occurs even though the A or U does not need to base pair with the nucleotide at position 9 of the 5' end of the miRNA [3, 4, 22]. Our study demonstrates that at position 9 of the miRNAs, there is a strong preference for U (ranging from 29% to 35% in the six species investigated), which is able to base pair with the A residue in the mRNA target. This finding implies that the nucleotide at position 9 of the 5' end of a miRNA plays an important role in improving both the recognition of specific targets and the functional efficiency of the miRNA.

It has been reported that residues at positions 13–16 of the 5' end of the miRNAs are also important for stabilizing miRNA-mRNA interactions, especially when base pairing in the 5' seed region is suboptimal [3, 4, 22]. The lengths of the mature miRNAs investigated in this study range from 15 to 27 nucleotides. For some short miRNAs, residues at positions 13–16 are located in the 3' half of the miRNAs; however, for some long miRNAs, these residues are situated in the central region of the miRNAs. Therefore, whether nucleotide pairing at positions 13–16 of the 5' end of a miRNA is important may, at least partially, depend on their exact locations in the miRNA investigated.

Overall, mammalian miRNAs are evolutionarily conserved and GU-rich, where U residues are more frequent than other bases. This finding implies that their target mRNA sites should be A-rich. Another interesting finding is that, in the miRNA sequences investigated, A residues are clearly the most frequent occupants of positions 2 and 3 of the 5' end of miRNAs. Unlike G and U residues that may pair with C/U and A/G, respectively, A residues can only pair with U residues of target mRNAs, which may augment the recognition specificity of the 5' seed region.

Conflict of Interests

The author declares that she has no competing financial interests.

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