REPORT

Numerous microRNPs in neuronal cells containing novel microRNAs

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ABSTRACT

Spinal muscular atrophy (SMA) is a common neurodegenerative disease that is caused by deletions or loss-of-function mutations in the Survival of Motor Neuron (SMN) protein. SMN is part of a large complex that functions in the assembly/restructuring of ribonucleoprotein (RNP) complexes. We recently showed in HeLa cells that two components of the SMN complex, Gemin3 and Gemin4, together with the argonaute protein eIF2C2, also associate with microRNAs (miRNAs) as part of a novel class of RNPs termed miRNPs. Here we report on miRNPs isolated from neuronal cell lines of mouse and human, and describe 53 novel miRNAs. Several of these miRNAs are conserved in divergent organisms, including rat, zebrafish, pufferfish, and the nematode *Caenorhabditis elegans*. The chromosomal locations of most of the novel miRNAs were identified and indicate some phylogenetic conservation of the likely precursor structures. Interestingly the gene locus of one miRNA, miR-175, is a candidate region for two neurologic diseases: early-onset parkinsonism (Waisman syndrome) and X-linked mental retardation (MRX3). Also, several miRNAs identified as part of miRNPs in these cells appear to constitute two distinct subfamilies. These subfamilies comprise multiple copies of miRNAs on different chromosomes, suggesting an important function in the regulation of gene expression.

Keywords: Gemin3; miRNAs; SMA; motor neurons

INTRODUCTION

MicroRNAs (miRNAs) are a large family of short (20–24-nt) single-stranded noncoding regulatory RNAs that includes the small temporal RNAs (stRNAs) *lin-4* and *let-7* (Ambros and Horvitz 1984; Reinhart et al. 2000). Based on knowledge gained from studies on stRNAs, miRNAs are thought to modulate gene expression by partially base-pairing with target mRNA sequences. Members of this new class of RNAs have been found in several metazoans including *Caenorhabditis elegans* (Lau et al. 2001; Lee and Ambros 2001), mouse (Lagos-Quintana et al. 2002), and humans (Lagos-Quintana et al. 2001; Mourelatos et al. 2002). miRNAs appear to be derived from the processing of larger precursors of approximately 70 nt or longer. The precursors are predicted to form imperfect stem-loop structures from

which the mature miRNAs are excised from one half of the stem by a process that involves the RNase III enzyme Dicer (Hutvagner et al. 2001).

miRNAs were recently shown to be components of a novel class of ribonucleoprotein (RNP) complexes termed miRNPs (Mourelatos et al. 2002). These complexes sediment as 15S particles in sucrose gradients and were found to contain the proteins Gemin3 (a DEAD-box putative RNA helicase; Charroux et al. 1999), Gemin4 (Charroux et al. 2000), and eIF2C2, a member of the argonaute family of proteins (Mourelatos et al. 2002). Gemin3 and Gemin4 are also components of the SMN complex, a large multiprotein complex containing the Survival of Motor Neuron (SMN) protein, Gemin2 (formerly SIP 1; Liu et al. 1997), Gemin5 (Meister et al. 2001; Gubitz et al. 2002), Gemin6 (Paushkin et al. 2002; Pellizzoni et al. 2002), and Gemin7 (Baccon et al. 2002). The SMN complex functions in the assembly/restructuring of RNPs, including spliceosomal small nuclear RNPs (snRNPs; Fischer et al. 1997; Pellizzoni et al. 1998; Meister et al. 2001), and possibly small nucleolar RNPs (snoRNPs; Jones et al. 2001; Pellizzoni et al. 2001a), heterogeneous nuclear RNPs (hnRNPs; Mourelatos et al. 2001), and transcriptosomes (Pellizzoni et al. 2001b).

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Deletion or loss-of-function mutations in SMN cause spinal muscular atrophy (SMA), a common genetic disease characterized by progressive degeneration of motor neurons (Melki 1997). Because Gemin3 and Gemin4 are shared components of the SMN and miRNP complexes, deletion or loss-of-function mutations of SMN in SMA may also affect the activity of miRNPs due to possible redistribution or changes in the levels of Gemin3 and Gemin4. Thus, it is possible that specific or general changes in the activity of the miRNPs play a role in the development of SMA. However, it was not known whether Gemin3 also associates with miRNAs in motor neuron cells, which are specifically affected in SMA. Furthermore, miRNAs associated with miRNPs in this cell type have not been previously identified. Here we report on the characterization of miRNAs associated with miRNPs isolated from neuronal cells. We identified 53 novel miRNAs from mouse and human neuronal cell lines, several of which are phylogenetically conserved in divergent organisms. The predicted precursor structure of most of these miRNAs was determined from known genomic sequences. Several miRNAs were found to constitute distinct subfamilies comprising multiple copies on different chromosomes, suggesting that these miRNAs play an important role in the regulation of gene expression.

RESULTS AND DISCUSSION

Gemin3 associates with miRNAs in neuronal cell lines

Because SMA specifically affects motor neurons, we wished to study miRNPs in well characterized neuronal cell lines. To do so, total extracts from mouse motor neuron MN-1 cells (Salazar-Grueso et al. 1991; Brooks et al. 1997) and human retinoblastoma Weri cells (McFall et al. 1977) were incubated with either the anti-Gemin3 antibody (11G9) or with the nonimmune mouse serum (NMS) as control. The immunoprecipitates were digested with proteinase K, and the associated RNAs were isolated. The extracted RNAs were 3'-end-labeled with [5'-³²P]-pCp, resolved by gel electrophoresis, and visualized by autoradiography. As shown in Figure 1, short (~24-nt) RNAs were specifically coimmunoprecipitated with the anti-Gemin3 antibody but not with the control antibody in both MN-1 and Weri cells. This indicates that Gemin3 is associated with small RNAs not only in HeLa cells (Mourelatos et al. 2002), and suggests that Gemin3 interacts with miRNAs in various cell types including motor neuron cells, as part of miRNPs.

Identification and characterization of miRNAs

To identify the miRNAs in these cells, the RNAs were cloned as previously described (Elbashir et al. 2001; Mourelatos et al. 2002). A total of 107 clones from Weri and MN-1

FIGURE 1. miRNPs from mouse and human neuronal cells. Total extracts from MN-1 (mouse) and Weri (human) cells were incubated with either nonimmune mouse serum (NMS) as a control or the anti-Gemin3 antibody (11G9). Immunoprecipitates were treated with proteinase K, and associated RNAs were purified by phenol-chloroform extraction and precipitated. Isolated RNAs were 3'-end-labeled with [5'-³²P]-pCp and resolved by electrophoresis on 15% denaturing polyacrylamide gels. *Left:* molecular weight marker.

cells were sequenced. We found 41 different miRNAs associated with miRNPs in Weri cells, six of which were also found with miRNPs in HeLa cells (Table 1, miRNAs in bold letters; Mourelatos et al. 2002). Several miRNAs previously identified from total RNA, including let-7, miR-15, miR-20, miR-21, miR-29a, and the more recently described miR-15b, miR-27b, miR-29c, and neuronal-specific miR-124a (Lagos-Quintana et al. 2002) were also found, indicating that miRNPs also play a role in the metabolism of these miRNAs. Interestingly, 25 novel miRNAs were found in the miRNP from human neuronal cells, and three additional miRNAs were identified by sequence homology to the new miRNAs identified in the present study. Most miRNAs can be localized in the human genome, and are highly conserved in other organisms with little sequence variation. The precursor sequence and secondary structure of miRNAs that are found on several chromosomes in one species and/

TABLE 1. miRNA sequences associated with miRNPs in the Weri human neuronal cell line

miRNA	Number of clones	miRNA sequence	Size (nt)	Source	Predicted Precursors							
					Hs (Chr.)	Dr	Fr	Dm	Ce	Mm	Rn	
let-7a	1	UGAGGUAGUAGGUUGUAUAGUU	22	Hs	+	(9, 11, 22)	-	+	+	+	+	+
miR-15a	1	UAGCAGCACAUAAUGGUUUGUGA	23	His	+	(13)	+	+	-	-	-	-
miR-15b	1	UAGCAGCACAUCAUGGUUUAC	21	His, Mm	+	(3)	-	-	-	-	+	-
miR-19a	4	UGUGCAAUUCUUAUGCAAACUGA	23	His	+	(13)	-	+	-	+	-	+
miR-19b	4	UGUGCAAUCCAUUGCAAACUGA	23	His	+	(13, X)	-	+	-	-	-	-
miR-20	8	UAAAGUGCUUUAUGUGCAGGUAG	23	His	+	(13)	-	+	-	-	-	+
miR-21	1	AGUUAUCUUAUCAGACUGAUGUUGA	25	His	+	(13)	-	+	-	-	-	+
miR-27b	1	GUUCACAGUGGCUAAGUUCUG	21	His, Mm	+	(9)	-	+	-	-	+	+
miR-29a	1	UAGCACCACUGGAAUUCGGUUA	22	His	+	(7.1, 7.2)	-	-	-	-	+	+
miR-29c	1	UAGCACCACUUGGAAUUCGGUUA	22	His	+	(1)	-	+	-	-	-	+
miR-91	5	CAAAGUGCUUACAGUGCAGGUAG	23	His	+	(13)	-	+	-	-	-	+
miR-92	3	UAUUGCACUUGUCCCGGCCUGU	22	His	+	(13, X)	-	+	+	-	-	+
miR-93	1	AAAGUGCUGUUCGUGCAGGUAG	22	His	+	(7.1, 7.2)	-	+	-	-	+	-
miR-94	1	UAAAGUGCUGACAGUGCAGUAU	22	His	+	(7.1, 7.2)	-	-	-	-	+	-
miR-106	1	AAAAGUGCUUACAGUGCAGGUAGC	24	His	+	(X)	-	+	-	-	-	-
miR-124a	3	UAGGACACGCGGUGAAUGCCAA	22	His	+	(8.1, 8.2, 20)	-	+	+	+	-	-
miR-172	2	UGGCAGUGUCUUAAGCUGGUUGUU	23	His	+	(1)	-	+	-	-	+	-
miR-173	1	CACGGGAGGUAGUGACGAAA	21	His	+	(2.1, 2.2)	-	-	+	-	-	+
miR-174	1	UAUGGCACUGGUAGAAUUCACU	22	His	+	(7)	+	+	+	-	-	-
miR-175	1	CAAGUCACUAGUGGUUCCGUUUA	23	His	+	(X)	-	-	+	-	+	-
miR-176	1	GGACUGCCUCAGCUGUGC	18	His	+	(2)	-	-	-	-	-	-
miR-177	1	GUGUCCUAAGGUGAGCUCAG	20	His	+	(8)	-	-	-	+	+	+
miR-178	1	AACAUAUCAAACGUGUGCGUGAGUU	25	His, Mm	+	(1, 9)	-	+	-	-	+	+
miR-179	1	GGUUGAUAGGUGCGGGGUGUAA	22	His, Mm	-	-	-	-	-	-	-	-
miR-180	1	AUUUCAGGUGAAGUUAUUAAGAGUC	24	His, Mm	-	-	-	-	-	-	-	-
miR-181	1	GUGCUAGGGAUUGGGGCUUG	20	His, Mm	-	-	-	-	-	-	+	+
miR-182a	1	CAAAGUGCUGUUAAGUGCAGGUAG	23	His	-	-	-	+	-	-	-	-
miR-182b	-	CAAAGUGCUGUUAUUGCAGG	20	-	+	(11)	-	-	-	-	-	+
miR-183	2	ACUACGAUAUGAUAAAUCCGUGG	23	His	-	-	-	-	-	-	-	-
miR-184	1	AUGCAAGUCGAGCUUGAAGUUC	23	His	-	-	-	-	-	-	-	-
miR-185	1	AUCGUUAUUAUUCGAUGGCGUGA	22	His	-	-	-	-	-	-	-	-
miR-186	1	CGGCCUUAUGUCUGCGGGGUGAUU	23	His	-	-	-	-	-	-	-	-
miR-187	1	AGGAGCACAGCUGGGUAUCUAAU	24	His	-	-	-	-	-	-	-	-
miR-188	1	CAACCUUGGGAUACCAACCCUGUA	23	His	-	-	-	-	-	-	-	-
miR-189	1	CGUAGACCCGAAACCGGGUGAC	22	His	-	-	-	-	-	-	-	-
miR-190	1	UACCGCAUUAUUGUAAAGAUUG	23	His	-	-	-	-	-	-	-	-
miR-191	1	CGGUUCAUACCCGAAGGGUCCGAA	24	His	-	-	-	-	-	-	-	-
miR-192	1	AUAACGUUGAAGAAGAUUGCAUC	21	His	-	-	-	-	-	-	-	-
miR-193	1	CGAGCCGUCGUAGACACGACGUU	24	His	-	-	-	-	-	-	-	-
miR-194	1	GCCGUCGUCGACGAGUGCACUU	22	His	-	-	-	-	-	-	-	-
miR-195	1	CAAAGUGCUGACAGUUCAGGUAG	23	His	-	-	-	-	-	-	-	-
miR-196a	1	UAAGGGCUGGGCCGGUCGGGCU	22	His, Mm	-	-	-	-	-	-	-	-
miR-196b	-	UAAGGGCUGGGCUGGGCUGGGCU	22	-	+	(13)	-	-	-	-	-	+
miR-196c	-	GGGCGGGCCGGUCGGGC	18	-	+	(10)	-	-	-	-	-	-

The short (22–25 nt) RNAs associated with miRNPs in Weri cells were cloned as previously described (Elbashir et al. 2001), and a total of 64 clones were sequenced. **miRNAs** in bold letters were previously shown to associate with miRNPs in HeLa cells. The miRNA sequences are shown 5' to 3'. The source (Hs; *Homo sapiens*, Mm; *Mus musculus*) indicates the species of the cell line from which the miRNAs were identified. In this column, - indicates miRNAs identified by sequence homology in the human genome. (Chr.); chromosomal localization of the miRNA in the human genome. Dr; *Danio rerio*, Fr; *Fugu rubripes*, Dm; *Drosophila melanogaster*, Ce; *Caenorhabditis elegans*, Mm; *Mus musculus*, Rn; *Rattus norvegicus*. + indicates fully conserved miRNAs, and + in red color represents conserved miRNAs containing one or two mismatches.

or in several species are often moderately conserved (data not shown). Seven miRNAs were identified in both Weri and MN-1 miRNPs (Table 1). Of these RNAs, three could not be mapped in either the human or mouse genome, and one, miRNA-181, is found in the mouse and rat genomes but not in the available sequences of the human genome. The miRNAs that have no database entry do not correspond to any known structural or messenger RNAs, and thus are most likely present in repetitive genomic regions or in genomic domains that are not yet fully sequenced.

Including the miRNAs found in both cell lines (Table 1), 40 different miRNAs were found associated with miRNPs in MN-1 cells (Table 2; Mourelatos et al. 2002). These include

10 miRNAs previously shown to be in miRNPs in HeLa cells (miRNAs in bold letters), and miR-23b and miR-26b that were recently described from total mouse RNA (Lagos-Quintana et al. 2002). We found 23 new miRNAs in the miRNPs from mouse neuronal cells, and identified two additional miRNAs by sequence homology in the human genome. Similarly to human miRNAs, most mouse sequences are conserved and can be localized by database search in the genome of at least one organism. The miRNAs that have not been localized in the human genome are either not present, not sufficiently conserved in human to be identified, or present in genomic regions that are not yet fully sequenced. Although several of the miRNAs were analyzed

TABLE 2. miRNA sequences associated with miRNPs in the MN-1 mouse neuronal cell line

miRNA	Number of clones	miRNA sequence	Size (nt)	Source	Predicted Precursors							
					Hs (Chr.)	Dr	Fr	Dm	Ce	Mm	Rn	
miR-16	1	UAGCAGCAGUAAAUATUGGCG	22	Mm	+ (3, 13)	-	+	-	-	+	-	
miR-19a	1	UGUGCAAAUCCUAGCAAAACUGA	23	Mm	+ (13)	-	+	-	+	-	+	
miR-19b	2	UGUGCAAAUCCUAGCAAAACUGA	23	Mm	+ (13, X)	-	+	-	-	-	+	
miR-21	1	UAGCUUUAUCAGACUGAUGUUGAC	22	Mm	+ (13)	-	+	-	-	-	+	
miR-22	1	AAGCUGCCAGUUGAAGAACUG	21	Mm	+ (17)	-	-	-	-	+	+	
miR-23b	2	AUCACAUUGCCAGGGAUJACCAC	23	Mm	+ (9)	-	+	-	-	-	-	
miR-24	2	UGGCUAGUUCAGCAGGAACAGIA	23	Mm	+ (9, 19)	-	+	-	-	+	+	
miR-26b	1	UUCAGUAATUICAGGATJAGGUTU	23	Mm	+ (2)	-	+	-	-	+	+	
miR-27	2	UUCACAGUGGCUAAGUCCGC	21	Mm	+ (19)	-	+	-	-	+	-	
miR-103	1	AGCAGCAUUGUACAGGGCUAUGA	23	Mm	+ (5, 10, 20)	-	+	-	-	+	+	
miR-197	1	CAAAGAAUUCUCCUUUUGGCUU	23	Mm	+ (1)	-	-	-	-	-	-	
miR-198	1	ACUGGACUUGGAGUCAAAAGG	21	Mm	+ (4)	-	-	-	-	-	-	
miR-199a	1	UGAUUCGGUGGGUGGUGUGGC	21	Mm	-	-	-	-	-	-	-	
miR-199b	-	UGAUUCGGUGGGUGGUGUGGC	21	-	-	-	-	-	-	-	+	
miR-199c	-	UGAUUCGGUGGGUGGUGUGGC	21	-	+ (11, 17)	-	-	-	-	+	+	
miR-200	1	UGUAAACAUCCCGACUGGAA	22	Mm	+ (8)	-	+	-	-	+	-	
miR-201	1	CCUGGUGGCCAAGUJUGAC	20	Mm	+ (4)	-	-	-	-	-	-	
miR-202	1	CAGUGCAAUGUUAAGGGCAU	22	Mm	+ (11)	-	+	-	-	-	+	
miR-203	1	GUGCUAGGAUUGGGGCU	17	Mm	+ (4)	-	-	-	-	-	+	
miR-204	1	ACAGUAGUCGACAUUGGUUA	22	Mm	+ (1, 9, 19)	-	-	-	-	+	+	
miR-205	1	AGCCAAUGGUGCGAAGCUA	19	Mm	+ (8, 11, 17)	-	-	-	-	+	-	
miR-206	1	UGUUGAAAAAGCAUGGGAUG	20	Mm	+ (13)	-	-	-	-	+	+	
miR-207	1	UAAAGUGCTUGACAGCUCAGUA	22	Mm	-	-	-	-	-	-	-	
miR-208	1	CCGUAGGCCGUUGAAGCGAUC	21	Mm	-	-	-	-	-	-	-	
miR-209	1	UUAGUAGUGGUGCCUUCCAA	20	Mm	-	-	-	-	-	-	+	
miR-210	1	UCGUCGCGAGACCGAUUATUU	21	Mm	-	-	-	-	-	-	-	
miR-211	1	GACCUGAGAGGGUGAUCGGCCAC	23	Mm	-	-	-	-	-	-	-	
miR-212	1	AGGCAUGGCCAGGUUGAAGCGAGG	24	Mm	-	-	-	-	-	-	-	
miR-213	1	GUACUJAGUAGAGCAGCCAC	20	Mm	-	-	-	-	-	-	-	
miR-214	1	GGCGUAAAGGGAGCGUACGGGAU	24	Mm	-	-	-	-	-	-	-	
miR-215	1	AGACCCACCAGGCGUUCGGCC	21	Mm	-	-	-	-	-	-	-	
miR-216	1	UUAGAUAGAGUAACAGGUUCU	22	Mm	-	-	-	-	-	-	-	
miR-217	1	CAAAGCAGCAGUAUCGCCU	19	Mm	-	-	-	-	-	-	-	
miR-218	1	AUGCCUGUCGUUACUGCCUGCU	23	Mm	-	-	-	-	-	-	+	
miR-219	2	CAGACAGGGUGUACAUGACCUU	22	Mm	-	-	-	-	-	+	-	

The short (22–25 nt) RNAs associated with miRNPs in MN-1 cells were cloned as previously described (Elbashir et al. 2001), and a total of 43 clones were sequenced. **miRNAs** in bold letters were previously shown to associate with miRNPs in HeLa cells. The miRNA sequences are shown 5' to 3'. The source (Hs; *Homo sapiens*, Mm; *Mus musculus*) indicates the species of the cell line from which the miRNAs were identified. In this column, - indicates miRNAs identified by sequence homology in the human genome. (Chr.); chromosomal localization of the miRNA in the human genome. Dr; *Danio rerio*, Fr; *Fugu rubripes*, Dm; *Drosophila melanogaster*, Ce; *Caenorhabditis elegans*, Mm; *Mus musculus*, Rn; *Rattus norvegicus*. + indicates fully conserved miRNAs, and + in red color represents conserved miRNAs containing one or two mismatches.

by northern blotting, it is not certain whether these are novel neuronal-specific or ubiquitously miRNAs. The miRNAs with an entry in the human genome database are predicted to fold into stem-loop structures with surrounding genomic sequences (Fig. 2). The predicted secondary structure of the miRNA precursors localized on more than one chromosome or that can be mapped in the genome of other species is often partially conserved (data not shown). Two of the miRNAs, miR-23b and miR-27b, were found as part of a novel cluster in the human genome on chromosome 9 (Fig. 3A; solid boxes). In addition, two computer-predicted putative miRNA precursors were found surrounding miR-27b (dashed boxes in Fig. 3).

Interestingly, miR-175 from Weri cells, which is located on the X chromosome in humans and is conserved in *D. melanogaster* and *M. musculus*, was also found as part of a longer human Expressed Sequence Tag (EST). This EST contains the miR-175 miRNA precursor and possibly another miRNA precursor that was computer-predicted, and

encodes a putative isoform of the epsilon subunit of the gamma-aminobutyric acid (GABA) A receptor (Fig. 3A; Wilke et al. 1997). This receptor is a multisubunit chloride channel that inhibits synaptic transmission in the central nervous system. The gene locus of the epsilon subunit is a candidate region for two neurologic diseases: early-onset parkinsonism (Waisman syndrome; Gregg et al. 1991), and X-linked mental retardation (MRX3; Gedeon et al. 1991). Thus, it will be of interest to determine whether miR-175 synthesis or activity are affected and whether deregulation of this miRNA plays a role in the development of these diseases.

Two distinct subfamilies of miRNAs associate with miRNPs in neuronal cells

Analysis of the miRNPs indicates that they contain a large family of highly conserved miRNAs in this cell type (Fig. 3B). This miRNA family encompasses at least nine mem-

FIGURE 2. Predicted secondary structures of the novel human miRNA putative precursors. Human genomic sequences upstream and downstream of the novel miRNAs were folded with the computer program mfold. Predicted secondary structures are shown 5' to 3'. Red areas represent the mature miRNA. Chromosome number represents the localization of the miRNA in the human genome.

bers, including miR-182a, miR-182b, miR-188, and miR-207, which are described herein. This result suggests that these miRNPs may play an important role in the maturation and/or delivery of their miRNAs to a common class of putative RNA targets in neuronal cells. In addition, several miRNAs with homologous 5' ends were found in miRNPs (Fig. 3C). Most members of this miRNA class were associ-

ated with miRNPs identified from the neuronal cells, including the novel miR-29c, miR-187, and miR-217. The 5' end of several miRNAs was recently found to be complementary to the K box, Brd box, and GY box motifs found in the 3' UTR of basic helix-loop-helix (bHLH) repressors and the Bearded (Brd) family members (Lai 2002). The K and Brd box sequences mediate negative posttranscriptional

mRNA regulation by affecting the polyadenylation status of transcripts, resulting in decreased stability and translational efficiency of the mRNA (Lai and Posakony 1997; Lai et al.

1998). Thus, the identification of these miRNA families should help in the identification of putative mRNA targets and in the elucidation of their functions.

FIGURE 3. Novel miRNA clusters and families of miRNA paralogs associated with miRNPs in neuronal cells. (A) Novel miRNA clusters. The putative precursors of identified miRNAs are presented as solid boxes, and the mature miRNAs identified in the study are shown in red. Computer-predicted putative miRNA precursors are presented as dashed boxes, and the predicted mature miRNAs are shown in light blue. The chromosomal localization in the human genome is indicated on the right. The size of the region containing the miRNAs is indicated at the top of each cluster. (B) miRNPs contain a conserved miRNA family in neuronal cells. miRNA family members associated with miRNPs in neuronal cells are aligned. The names of miRNAs are indicated on the left. miRNA length is shown at the end of each miRNA. Conserved residues are in bold letters and gray shaded areas. Consensus is shown at the bottom: Nucleotides shown in red were invariable. (C) miRNPs contain a class of miRNAs with homologous 5' ends in neuronal cells. miRNA class members with homologous 5' ends are aligned. The names of miRNAs are indicated on the left. miRNA length is shown at the end of each miRNA. Conserved residues are in bold letters and gray shaded areas. Consensus is shown at the bottom: Nucleotides shown in red were invariable.

MATERIALS AND METHODS

Cell culture and cell extract preparation

MN-1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; complete DMEM). Weri cells were grown in DMEM supplemented with 10% fetal bovine serum. At 80% confluence, cells (1×10^7) were collected by centrifugation at 1000 g for 15 min at 4°C. The cell pellet was washed with cold phosphate-buffered saline (PBS), and with RSB 200 (20 mM Tris-HCl pH 7.4, 2.5 mM magnesium chloride, 200 mM sodium chloride). Cells were resuspended in one volume of lysis buffer (RSB 200 containing 0.05% NP-40, RNasin (1 U/ μ L) and protease inhibitors (CompleteTM; Roche), and disrupted by brief sonication. Extract was clarified by centrifugation at 11,000 g for 15 min at 4°C.

RNA immunoprecipitation, 3'-end labeling, and miRNA cloning

For immunoprecipitation, cell extract was incubated for 1 h at 4°C with either normal mouse serum (NMS) as control, or with anti-Gemin3 (11G9) antibodies immobilized on GammaBindTM G SepharoseTM (Amersham Pharmacia Biotech). Immunoprecipitates were washed five times with lysis buffer, treated with DNase I (0.5 U/ μ L; Roche) for 15 min at 30°C, followed by proteinase K digestion (0.2 μ g/ μ L; Roche) for 30 min at 37°C. RNA was extracted with phenol followed by two phenol/chloroform extractions and ethanol precipitation. The RNA was 3'-end labeled with [5'-³²P]-pCp and analyzed on a denaturing 15% polyacrylamide gel. The ~22-nt miRNAs were cloned by using the protocol developed by Elbashir and coworkers (2001).

RNA analysis

miRNA sequences were determined by automated DNA-cycle sequencing. Chromosomal locations and genomic se-

quences of the novel miRNAs were identified by searching various genomes with the Basic Local Alignment Sequence Tool (BLAST®) algorithm, available at the National Center for Biotechnology Information (NCBI). Secondary structures of the likely precursors of the novel miRNAs were predicted by folding upstream and downstream genomic miRNA sequences with the computer program mfold (Zuker et al. 1999). miRNA alignments were established with the macvector program.

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Numerous microRNPs in neuronal cells containing novel microRNAs

JOSÉE DOSTIE, ZISSIMOS MOURELATOS, MICHAEL YANG, ANUP SHARMA, and GIDEON DREYFUSS

We wish to revise the designation of the microRNAs (miRNAs) reported in Tables 1 and 2 of the original manuscript. The simultaneous publication of numerous new miRNA sequences in the same issue of *RNA* (Lagos-Quintanas, M., Rauhut, R., Meyer, J., Borkhardt, A., and Tuschl, T. 2003. New microRNAs from mouse and human. *RNA* 9: 175–179), and the report of several miRNA sequences which were not available at the time of submission of our manuscript resulted in an extensive overlap in the designation of many of the miRNAs we reported. To rectify this, we changed the designations of many of the miRNAs included in Tables 1 and 2 and adopted the designation used by others. The novel miRNAs—those not included in publications by others—are numbered sequentially beginning with miRNA 224. In addition, many of the miRNAs we

Table 1. miRNA and miRNA-like sequences associated with miRNPs in the Weri human neuronal cell line

					Predicted Precursors							
miRNA	Number of	miRNA sequence clones	Size	Source (nt)	Hs (Chr.)	Dr	Fr	Dm	Ce	Mm	Rn	
let-7a	1	UGAGGUAGUAGGUUGUUAUAGUU	22	Hs	+	(9, 11, 22)	-	+	+	+	+	
miR-15a	1	UAGCAGCACAUAAUGGUUUGUGA	23	Hs	+	(13)	+	+	-	-	-	
miR-15b	1	UAGCAGCACAUCAUGGUUUAC	21	Hs, Mm	+	(3)	-	-	-	+	-	
miR-19a	4	UGUGCAAUUCUAGCAAACUGA	23	Hs	+	(13)	-	+	-	+	+	
miR-19b	4	UGUGCAAUUCUAGCAAACUGA	23	Hs	+	(13, X)	-	+	-	-	+	
miR-20	8	UAAAGUGCUUAUAGUGCAGGUAG	23	Hs	+	(13)	-	+	-	-	+	
miR-21	1	AGUUAGCUUAUCAGACUGAUGUUGA	25	Hs	+	(13)	-	+	-	-	+	
miR-27b	1	GUUCACAGUGGCUAAGUUCUG	21	Hs, Mm	+	(9)	-	+	-	+	+	
miR-29a	1	UAGCACCAUCUGAAAUCGGUUA	22	Hs	+	(7.1, 7.2)	-	-	-	+	+	
miR-29c	1	UAGCACCAUUAUGAAAUCGGUUA	22	Hs	+	(1)	-	+	-	-	+	
miR-34	2	UGGCAGUGUCUUAUCGUGGUUU	23	Hs	+	(1)	-	+	-	+	-	
miR-91	5	CAAAGUGCUUACAGUGCAGGUAG	23	Hs	+	(13)	-	+	-	-	+	
miR-92	3	UAUUGCACUUGUCCCGGCCUGU	22	Hs	+	(13, X)	-	+	+	-	+	
miR-93	1	AAAGUGCUGUUCGUGCAGGUAG	22	Hs	+	(7.1, 7.2)	-	+	-	+	-	
miR-94	1	UAAAGUGCUGACAGUGCAGAU	22	Hs	+	(7.1, 7.2)	-	-	-	+	-	
miR-106	1	AAAAGUGCUUACAGUGCAGGUAGC	24	Hs	+	(X)	-	+	-	-	-	
miR-124a	3	UAAGGCACGCGUGAAUGCCAA	22	Hs	+	(8.1, 8.2, 20)	-	+	+	-	-	
miR-181	1	AACAUUAACGCUGUCGGUGAGUU	25	Hs, Mm	+	(1, 9)	-	+	-	+	+	
miR-183	1	UAUGGCACUGGUAGAAUUCACU	22	Hs	+	(7)	+	+	+	-	-	
miR-224	1	CAAGUCACUAGUGGUUCCGUUUA	23	Hs	+	(X)	-	-	+	+	-	
miR-Lk-1	1	GGACUGCCUCAGCUGUGC	18	Hs	+	(2)	-	-	-	-	-	
miR-Lk-2	1	GUGUCCUAAAGGUGAGCUCAG	20	Hs	+	(8)	-	-	-	+	+	
miR-Lk-3	1	GGUUGAUAGGUCGGGGUGUAA	22	Hs, Mm	-	-	-	-	-	-	-	
miR-Lk-4	1	AUUUCAGGUGAAGUUUCAAGAGUC	24	Hs, Mm	-	-	-	-	-	-	-	
miR-Lk-5	1	GUGCAGGGAUUGGGGCUUG	20	Hs, Mm	-	-	-	-	-	+	+	
miR-Lk-6a	1	CAAAGUGCUGUUAUGCAGGUAG	23	Hs	-	-	+	-	-	-	+	
miR-Lk-6b	-	CAAAGUGCUGUUAUUGCAGG	20	-	+	(11)	-	-	-	-	+	
miR-Lk-7	2	ACUACGAAUGAUACAUCGUGG	23	Hs	-	-	-	-	-	-	-	
miR-Lk-8	1	AUGCAAGUCGAGCUUGAAGUUUC	23	Hs	-	-	-	-	-	-	-	
miR-Lk-9	1	AUCGUUAUUUAUCGAGGCGUGA	22	Hs	-	-	-	-	-	-	-	
miR-Lk-10	1	CGGCCUUAGUCGUCGGGGUGAUU	23	Hs	-	-	-	-	-	-	-	
miR-Lk-11	1	AGGAGCACAGCUGGGUUAUCUAGU	24	Hs	-	-	-	-	-	-	-	
miR-Lk-12	1	CAACCUUGGGAUACACCCUGUA	23	Hs	-	-	-	-	-	-	-	
miR-Lk-13	1	CGUAGACCCGAAACCGGGUGAC	22	Hs	-	-	-	-	-	-	-	
miR-Lk-14	1	UACCGCAUAAUGUUGAAAGAUGG	23	Hs	-	-	-	-	-	-	-	
miR-Lk-15	1	CGGUUCAUACCCGAAGGGUGCCAA	24	Hs	-	-	-	-	-	-	-	
miR-Lk-16	1	AUAACGUUGAAAGAUGGCAUC	21	Hs	-	-	-	-	-	-	-	
miR-Lk-17	1	CGAGCCGUCGUAGACCACGACGUU	24	Hs	-	-	-	-	-	-	-	
miR-Lk-18	1	GCCGUCGUCGACGAGUGCAUU	22	Hs	-	-	-	-	-	-	-	
miR-Lk-19	1	CAAAGUGCUUACAGUUCAGGUAG	23	Hs	-	-	-	-	-	-	-	

The short (22–25 nt) RNAs associated with miRNPs in Weri cells were cloned as previously described (Elbashir et al., 2001), and a total of 64 clones were sequenced. **miRNAs** in bold letters were previously shown to associate with miRNPs in HeLa cells. miR-Lk-number represents small RNA sequences, identified in the miRNP complexes, that cannot be classified as miRNAs under the novel classification code for miRNAs. The sequences are shown 5' to 3'. The source (Hs; *Homo sapiens*, Mm; *Mus musculus*) indicates the species of the cell line from which the RNAs were identified. In this column, - indicates RNAs identified by sequence homology in the human genome. (Chr.); chromosomal localization of the miRNA in the human genome. Dr; *Danio rerio*, Fr; *Fugu rubripes*, Dm; *Drosophila melanogaster*, Ce; *Caenorhabditis elegans*, Mm; *Mus musculus*, Rn; *Rattus norvegicus*. + indicates fully conserved RNAs, and + represents conserved RNAs containing one or two mismatches.

reported do not, in the absence of additional data, fulfill the stringent criteria suggested as guidelines for classification of newly discovered RNAs as miRNAs as was recently published (Ambros, V., Bartel, B., Bartel, D.P., Burge, C.B., Carrington, J.C., Chen, X., Dreyfuss, G., Eddy, S.R., Griffiths-Jones, S., Marshall, M., et al. 2003. A uniform system for microRNA annotation. *RNA* 9: 277–279). However, we are confident in the authenticity of these RNAs as assessed by co-immunoprecipitation with anti-Gemin3 antibodies and sequence determination often from multiple clones. In light of this, we refer to these RNAs as miRNA-like (miRNA-Lk-#) in the revised Tables. Four RNAs—miR-173, miR-196a, miR-196b, and miR-196c—were deleted from the Tables because their relevance to the corresponding genome could not be confirmed by additional criteria. The new designations of the novel miRNAs will be corrected in the NCBI database. We regret any potential confusion in nomenclature that our original publication may have caused. All the conclusions of the paper remain.

Table 2. miRNA and miRNA-like sequences associated with miRNPs in the MN-1 mouse neuronal cell line

miRNA	Number of clones	miRNA sequence	Size (nt)	Source	Predicted Precursors							
					Hs (Chr.)	Dr	Fr	Dm	Ce	Mm	Rn	
miR-16	1	UAGCAGCAGCUAAAUUUGGCG	22	Mm	+	(3, 13)	-	+	-	-	+	-
miR-19a	1	UGUGCAAUUCUAUGCAAACUGA	23	Mm	+	(13)	-	+	-	+	-	+
miR-19b	2	UGUGCAAUCCAUUGCAAACUGA	23	Mm	+	(13, X)	-	+	-	-	-	+
miR-21	1	UAGCUUAUCAGACUGAUGUUGAC	22	Mm	+	(13)	-	+	-	-	-	+
miR-22	1	AAGCUGCCAGUUGAAGAACUG	21	Mm	+	(17)	-	-	-	-	+	+
miR-23b	2	AUCACAUUGCCAGGGAUUAACCAC	23	Mm	+	(9)	-	+	-	-	-	-
miR-24	2	UGGCUAGUUCAGCAGGAACAGA	23	Mm	+	(9, 19)	-	+	-	-	+	+
miR-26b	1	UUCAAGUAAUUCAGGAUAGGUUU	23	Mm	+	(2)	-	+	-	-	+	+
miR-27	2	UUCACAGUGGCUAAGUUCGCG	21	Mm	+	(19)	-	+	-	-	+	+
miR-30d	1	UGUAAACAUGCCCGACUGGAA	22	Mm	+	(8)	-	+	-	-	+	-
miR-103	1	AGCAGCAUUGUACAGGGCUAUGA	23	Mm	+	(5, 10, 20)	-	+	-	-	+	+
miR-130	1	CAGUGCAAUGUUAAAAGGGCAU	22	Mm	+	(11)	-	+	-	-	-	+
miR-186	1	CAAAGAAUUCUCCUUUUGGGCUU	23	Mm	+	(1)	-	-	-	-	-	-
miR-199-as	1	ACAGUAGUCUGCACAUUGGUUA	22	Mm	+	(1, 9, 19)	-	+	-	-	+	+
miR-20	1	ACUGGACUUGGAGUCAAAAGG	21	Mm	+	(4)	-	-	-	-	-	-
miR-21	1	CCUGGGUGCCAAGUUUUGAC	20	Mm	+	(4)	-	-	-	-	-	-
miR-Lk-22a	1	UGAUUCGGUGGGUGGUGGUGC	21	Mm	-	-	-	-	-	-	-	-
miR-Lk-22b	-	UGAUUCCGUGGGUGGUGGUGC	21	-	+	(19)	-	-	-	-	-	+
miR-Lk-22c	-	UGAUUCUGUGGGUGGUGGUGC	21	-	+	(11, 17)	-	-	-	-	+	+
miR-Lk-23	1	GUGCUAGGAUUGGGGCU	17	Mm	+	(4)	-	-	-	-	-	+
miR-Lk-24	1	AGCCAAUGGUGCGAAGCUA	19	Mm	+	(8, 11, 17)	-	-	-	-	+	-
miR-Lk-25	1	UGUUGAAAAAGCAUGGGAUG	20	Mm	+	(13)	-	-	-	-	+	+
miR-Lk-26	1	UAAAGUGCUGACAGCUCAGAU	22	Mm	-	-	-	-	-	-	-	-
miR-Lk-27	1	CCGUAGGCCGUGAAGCGAUC	21	Mm	-	-	-	-	-	-	-	-
miR-Lk-28	1	UUAGUAUGGUUGCCUCCAA	20	Mm	-	-	-	-	-	-	-	+
miR-Lk-29	1	UCGUCCGAGACCGAUUAUUU	21	Mm	-	-	-	-	-	-	-	-
miR-Lk-30	1	GACCUGAGAGGGUGAUCGGCCAC	23	Mm	-	-	-	-	-	-	-	-
miR-Lk-31	1	AGGCAUUGGCCAGGUUGAAGCGAGG	24	Mm	-	-	-	-	-	-	-	-
miR-Lk-32	1	GUACUUAGUAGAGCAGCCAC	20	Mm	-	-	-	-	-	-	-	-
miR-Lk-33	1	GGCGUAAAGGGAGCGUACGCGGAU	24	Mm	-	-	-	-	-	-	-	-
miR-Lk-34	1	AGACCCACCAGGCGUUCGGCC	21	Mm	-	-	-	-	-	-	-	-
miR-Lk-35	1	UUAGAUGAGAUACAGGUUUUCU	22	Mm	-	-	-	-	-	-	-	-
miR-Lk-36	1	CAAAGCAGCAGUAUCGCCU	19	Mm	-	-	-	-	-	-	-	-
miR-Lk-37	1	AUGCCUGUCGGUUAACUGCCUGCU	23	Mm	-	-	-	-	-	-	-	+
miR-Lk-38	2	CAGACAGGGUGUACAUGACCUU	22	Mm	-	-	-	-	-	-	+	-

The short (22–25 nt) RNAs associated with miRNPs in MN-1 cells were cloned as previously described (Elbashir et al., 2001), and a total of 43 clones were sequenced. **miRNAs** in bold letters were previously shown to associate with miRNPs in HeLa cells. miR-Lk-number represents small RNA sequences, identified in the miRNP complexes, that cannot be classified as miRNAs under the novel classification code for miRNAs. The sequences are shown 5' to 3'. The source (Hs; *Homo sapiens*, Mm; *Mus musculus*) indicates the species of the cell line from which the RNAs were identified. In this column, - indicates RNAs identified by sequence homology in the human genome. (Chr.); chromosomal localization of the miRNA in the human genome. Dr; *Danio rerio*, Fr; *Fugu rubripes*, Dm; *Drosophila melanogaster*, Ce; *Caenorhabditis elegans*, Mm; *Mus musculus*, Rn; *Rattus norvegicus*. + indicates fully conserved RNAs, and + represents conserved RNAs containing one or two mismatches.

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