Current Biology, Vol. 12, 735–739, April 30, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00809-6

Identification of Tissue-Specific MicroRNAs from Mouse

Mariana Lagos-Quintana, Reinhard Rauhut, Abdullah Yalcin, Jutta Meyer, Winfried Lendeckel, and Thomas Tuschl[1]
Department of Cellular Biochemistry
Max-Planck-Institute for Biophysical Chemistry
Am Fassberg 11
D-37077 Göttingen
Germany

Summary

MicroRNAs (miRNAs) are a new class of noncoding RNAs, which are encoded as short inverted repeats in the genomes of invertebrates and vertebrates [1, 2]. It is believed that miRNAs are modulators of target mRNA translation and stability, although most target mRNAs remain to be identified. Here we describe the identification of 34 novel miRNAs by tissue-specific cloning of approximately 21-nucleotide RNAs from mouse. Almost all identified miRNAs are conserved in the human genome and are also frequently found in nonmammalian vertebrate genomes, such as pufferfish. In heart, liver, or brain, it is found that a single, tissue-specifically expressed miRNA dominates the population of expressed miRNAs and suggests a role for these miRNAs in tissue specification or cell lineage decisions. Finally, a miRNA was identified that appears to be the fruitfly and mammalian ortholog of *C. elegans* lin-4 stRNA.

Results and Discussion

MicroRNAs (miRNAs) represent a new class of gene products, which are believed to sequence-specifically control translation of target mRNAs by binding to sites of antisense complementarity in 3′ untranslated regions (UTRs) [1–5]. Several miRNAs, such as let-7 RNA, miR-1, miR-34, miR-60, and miR-87, are highly conserved between invertebrates and vertebrates, implicating that they may recognize multiple sites and/or multiple targets of presumably conserved function [3–6]. The small temporal RNAs (stRNAs) lin-4 and let-7 represent a subclass of miRNAs identified by genetic analysis in *Caenorhabditis elegans*, which are developmentally regulated and themselves control developmental programs, such as timing of neuronal rewiring, Dauer larva formation, vulva formation, and the terminal differentiation of hypodermal cells [7–11].

miRNAs are typically excised from 60- to 70-nucleotide foldback RNA precursor structures, which are sometimes detected at the onset of miRNA precursor expression [12–14] or during expression of very abundant miRNAs [3–5]. Generally, only one of the strands of the hairpin precursor molecule is excised and accumulates, presumably because it is protected by associated proteins from RNA degradation. These putative proteins may as well mediate the translational suppression. The miRNA precursor processing reaction requires Dicer RNase III and Argonaute family members [12–14]; Dicer and Argonaute proteins are also involved in RNAi [15–18].

To gain insights into the distribution and function of miRNAs in mammals, we investigated the tissue-specific distribution of miRNAs in adult mouse. Cloning of miRNAs from specific tissues was preferred over whole organism-based cloning because low-abundance miRNAs that normally go undetected by Northern blot analysis are identified clonally. Also, in situ hybridization techniques for detecting 21-nt RNAs have not yet been developed. Therefore, 19- to 25-nucleotide RNAs were cloned and sequenced from total RNA, which was isolated from 18.5-week-old BL6 mice (see the Supplementary Material available with this article online). Because RNA was prepared from combining tissues of several mice, minor sequence variations that were detected multiple times in multiple clones may reflect polymorphisms rather than RT/PCR mutations. Public database searching was used to identify the genomic sequences encoding the ~21-nt RNAs. The occurrence of a 20 to 30 basepair foldback structure involving the immediate upstream or downstream flanking sequences was used to assign miRNAs [1, 3–5].

We examined nine different mouse tissues and identified 34 novel miRNAs, some of which are highly tissue-specifically expressed (Table 1 and Figure 1). miR-1 was previously shown by Northern analysis to be strongly expressed in human adult heart but not in human brain, liver, kidney, lung, or colon [5]. Here we show that miR-1 accounts for 45% of all mouse miRNAs found in heart, yet miR-1 was still expressed at a low level in liver and midbrain, even though it remained undetectable by Northern analysis. Three copies or polymorphic alleles of miR-1 were found in mice. The conservation of tissue-specific miR-1 expression between mouse and human provides additional evidence for a conserved regulatory role of this miRNA. In liver, variants of miR-122 account for 72% of all cloned miRNAs, and miR-122 was undetected in all other tissues analyzed. In spleen, miR-143 appeared to be most abundant, at a frequency of ~30%. In colon, miR-142-as was cloned several times and also appeared at a frequency of 30%. In small intestine, too few miRNA sequences were obtained to permit statistical analysis. This was due to strong RNase activity in this tissue, which caused significant breakdown of abundant noncoding RNAs, e.g., rRNA, so that the fraction of miRNA in the cloned sequences was very low. For the same reason, no miRNA sequences were obtained from pancreas.

To gain insights in neural tissue miRNA distribution, we analyzed cortex, cerebellum, and midbrain. Similar to heart, liver, and small intestine, variants of a particular miRNA, miR-124, dominated and accounted for 25% to 48% of all brain miRNAs. miR-101, -127, -128, -131, and -132, also cloned from brain tissues, were further

[1]Correspondence: ttuschl@mpibpc.gwdg.de

Table 1. Mouse miRNA Sequences Identified by Cloning from Distinct Mouse Tissues

		Number of Clones[a]							
miRNA	Sequence (5′ to 3′)	ht	lv	sp	si	co	cx	cb	mb
let-7a	UGAGGUAGUAGGUUGUAUAGUU		3			1	1		7
let-7b	UGAGGUAGUAGGUUGUGUGGUU		1	1				2	5
let-7c	UGAGGUAGUAGGUUGUAUGGUU		2				2	5	19
let-7d	AGAGGUAGUAGGUUGCAUAGU	2				2	2		2
let-7e	UGAGGUAGGAGGUUGUAUAGU			1					2
let-7f	UGAGGUAGUAGAUUGUAUAGUU			2				3	3
let-7g	UGAGGUAGUAGUUUGUACAGUA						1	1	2
let-7h	UGAGGUAGUAGUGUGUACAGUU						1	1	
let-7i	UGAGGUAGUAGUUUGUGCU						1	1	
miR-1b	UGGAAUGUAAAGAAGUAUGUAA	4	2						1
miR-1c	UGGAAUGUAAAGAAGUAUGUAC	7							
miR-1d	UGGAAUGUAAAGAAGUAUGUAUU	16							1
miR-9	UCUUUGGUUAUCUAGCUGUAUGA						3	4	4
miR-15a	UAGCAGCACAUAAUGGUUUGUG	1							2
miR-15b	UAGCAGCACAUCAUGGUUUACA	1							
miR-16	UAGCAGCACGUAAAUAUUGGCG	1			1	2	1	2	3
miR-18	UAAGGUGCAUCUAGUGCAGAUA			1					
miR-19b	UGUGCAAAUCCAUGCAAAACUGA			1					
miR-20	UAAAGUGCUUAUAGUGCAGGUAG					1			
miR-21	UAGCUUAUCAGACUGAUGUUGA	1		1	2	1			
miR-22	AAGCUGCCAGUUGAAGAACUGU	2	1		1			1	2
miR-23a	AUCACAUUGCCAGGGAUUUCC	1							
miR-23b	AUCACAUUGCCAGGGAUUACCAC						1		
miR-24	UGGCUCAGUUCAGCAGGAACAG	1				1	1		1
miR-26a	UUCAAGUAAUCCAGGAUAGGCU							3	2
miR-26b	UUCAAGUAAUUCAGGAUAGGUU		2				4	1	
miR-27a	UUCACAGUGGCUAAGUUCCGCU	1		2		1	1	2	1
miR-27b	UUCACAGUGGCUAAGUUCUG								1
miR-29a	CUAGCACCAUCUGAAAUCGGUU	1				1		1	
miR-29b/miR-102	UAGCACCAUUUGAAAUCAGUGUU	1				1	5		3
miR-29c/	UAGCACCAUUUGAAAUCGGUUA	1					3		1
miR-30a-s/miR-97	UGUAAACAUCCUCGACUGGAAGC			1			1		1
miR-30a-as[b]	CUUUCAGUCGGAUGUUUGCAGC							1	
miR-30b	UGUAAACAUCCUACACUCAGC			1				2	
miR-30c	UGUAAACAUCCUACACUCUCAGC	2					1	1	
miR-30d	UGUAAACAUCCCCGACUGGAAG		1						
miR-99a/miR-99	ACCCGUAGAUCCGAUCUUGU						1		
miR-99b	CACCCGUAGAACCGACCUUGCG							1	
miR-101	UACAGUACUGUGAUAACUGA						2	1	1
miR-122a	UGGAGUGUGACAAUGGUGUUUGU		3						
miR-122b	UGGAGUGUGACAAUGGUGUUUGA		11						
miR-122a,b	UGGAGUGUGACAAUGGUGUUUG		23						
miR-123	CAUUAUUACUUUUGGUACGCG	1	2						
miR-124a[c]	UUAAGGCACGCGG-UGAAUGCCA				1		37	41	24
miR-124b	UUAAGGCACGCGGGUGAAUGC						1	3	
miR-125a	UCCCUGAGACCCUUUAACCUGUG						1	1	
miR-125b	UCCCUGAGACCCU--AACUUGUGA						1		
miR-126	UCGUACCGUGAGUAAUAAUGC	4						1	
miR-127	UCGGAUCCGUCUGAGCUUGGCU							1	
miR-128	UCACAGUGAACCGGUCUCUUUU						2	2	2
miR-129	CUUUUUUCGGUCUGGGCUUGC							1	
miR-130	CAGUGCAAUGUUAAAAGGGC							1	
miR-131	UAAAGCUAGAUAACCGAAAGU						1	1	1
miR-132	UAACAGUCUACAGCCAUGGUCGU							1	
miR-133	UUGGUCCCCUUCAACCAGCUGU	4					1		
miR-134	UGUGACUGGUUGACCAGAGGGA						1		
miR-135	UAUGGCUUUUUAUUCCUAUGUGAA						1		
miR-136	ACUCCAUUUGUUUUGAUGAUGGA						1		
miR-137	UAUUGCUUAAGAAUACGCGUAG						1		1
miR-138	AGCUGGUGUUGUGAAUC						1		
miR-139	UCUACAGUGCACGUGUCU					1	1		
miR-140	AGUGGUUUUACCCUAUGGUAG				1				
miR-141	AACACUGUCUGGUAAAGAUGG			1	1		1		
miR-142-s	CAUAAAGUAGAAAGCACUAC				1	1			
miR-142-as[c]	UGUAGUGUUUCCUACUUUAUGG			1	1	6			
miR-143	UGAGAUGAAGCACUGUAGCUCA	3		7			2		1

(*continued*)

Table 1. Continued

miRNA	Sequence (5′ to 3′)	Number of Clones[a]							
		ht	lv	sp	si	co	cx	cb	mb
miR-144	UACAGUAUAGAUGAUGUACUAG	2				1			
miR-145	GUCCAGUUUUCCCAGGAAUCCCUU	1							
miR-146	UGAGAACUGAAUUCCAUGGGUUU	1							
miR-147	GUGUGUGGAAAUGCUUCUGCC			1					
miR-148	UCAGUGCACUACAGAACUUUGU			1					
miR-149	UCUGGCUCCGUGUCUUCACUCC	1							
miR-150	UCUCCCAACCCUUGUACCAGUGU					1			
miR-151	CUAGACUGAGGCUCCUUGAGGU					1			
miR-152	UCAGUGCAUGACAGAACUUGG					1			
miR-153	UUGCAUAGUCACAAAAGUGA								1
miR-154	UAGGUUAUCCGUGUUGCCUUCG								1
miR-155	UUAAUGCUAAUUGUGAUAGGGG					1			

The sequences indicated represent the longest miRNA sequences identified by cloning. The 3′ terminus of miRNAs is often truncated by one or two nucleotides. miRNAs that are more than 85% identical in sequence (i.e., share 18 out of 21 nucleotides) or contain 1- or 2-nucleotide internal deletions are referred to by the same gene number followed by a lowercase letter. Minor sequence variations between related miRNAs are generally found near the ends of the miRNA sequence and are thought to not compromise target RNA recognition. Minor sequence variations may also represent A to G and C to U changes, which are accommodated as G-U wobble base pairs during target recognition. miRNAs with the suffix -s or -as indicate RNAs derived from either the 5′ half or the 3′ half of a miRNA precursor. Mouse brains were dissected into midbrain (mb), cortex (cx), and cerebellum (cb). The tissues analyzed were heart (ht), liver (lv), small intestine (si), colon (co), cortex (ct), cerebellum (cb), and midbrain (mb).

[a] The total number of clones, including breakdown products of noncoding RNAs and yet to be identified sequences, is listed in the Supplementary Material.

[b] The originally described miR-30 [3] was renamed to miR-30a-as in order to distinguish it from the miRNA derived from the opposite strand of the precursor encoded by the *mir-30a* gene. miR-30a-s is equivalent to miR-97 [22].

[c] A 1-nt length heterogeneity is found on both the 5′ and 3′ end. The 22-nt miR sequence is shown, but only 21-nt miRNAs were cloned.

analyzed by Northern blotting and shown to be predominantly brain specific (see Supplementary Material). Members of another class of noncoding RNAs, C/D-box small nucleolar RNAs (snoRNAs) and H/ACA-box snoRNA, in mouse and human have also shown brain-specific expression patterns [23].

miR-125a and miR-125b are very similar to the sequence of *C. elegans* lin-4 stRNA and may represent its orthologs (Figure 2A). This is of great interest because, unlike let-7 that was readily detected in other species, lin-4 has acquired a few mutations in the central region and thus escaped bioinformatic database searches. Using the mouse sequence miR-125b, we could readily identify its ortholog in the *D. melanogaster* genome (see Supplementary Material). miR-125a and miR-125b differ only by a central diuridine insertion and a U to C change. miR-125b is very similar to lin-4 stRNA with the differences located only in the central region, which is presumed to be bulged out during target mRNA recognition [11]. miR-125a and miR-125b were cloned from brain tissue, but expression was also detected by Northern analysis in other tissues, consistent with the role for lin-4 in regulating neuronal remodeling by controlling *lin-14* expression [19]. Unfortunately, orthologs to *C. elegans lin-14* have not been described, and miR-125 targets remain to be identified in *D. melanogaster* or mammals. Finally, miR-125b expression is also developmentally regulated and only detectable in pupae and adult but not in embryo or larvae of *D. melanogaster* (Figure 2B).

Sequence comparison of mouse miRNAs with previously described miRNA reveals that miR-99 variants are similar to *D. melanogaster*, mouse, and human miR-10 [3] as well as *C. elegans* miR-51 [4]; miR-141 is similar to *D. melanogaster* miR-8 [3]; miR-29 variants are similar to *C. elegans* miR-83 [4]; and miR-131 and miR-142-s are similar to *D. melanogaster* miR-4 [3] and *C. elegans* miR-79 [4]. miR-124a is conserved between invertebrates and vertebrates. In this respect, it should be noted that almost every miRNA cloned from mouse was also encoded in the human genome and frequently detected in other vertebrates, such as the pufferfish, *Fugu rubripes*, and the zebrafish, *Danio rerio*. Sequence conservation may point to conservation in function of these miRNAs. Comprehensive information about orthologous sequences is listed in Table S2 in the Supplementary Material).

In two cases, both strands of miRNA precursors were cloned (Table 1), which was previously observed once for a *C. elegans* miRNA [4]. It is thought that the most frequently cloned strand of a miRNA precursor represents the functional miRNA, which is miR-30c-s and miR-142-as, "s" and "as" indicating the 5′ or 3′ side of the foldback structure, respectively.

The *mir-142* gene is located on chromosome 17 but was also found at the breakpoint junction of a t(8;17) translocation, which causes an aggressive B cell leukemia due to strong upregulation of a translocated *MYC* gene [20]. The translocated *MYC* gene, which was also truncated at the first exon, was located only 4-nt downstream of the 3′ end of the miR-142 precursor. This suggests that translocated *MYC* was under the control of the upstream *mir-142* promoter. Alignment of mouse and human miR-142 containing EST sequences indicate an ~20 nt conserved sequence element downstream of the *mir-142* hairpin. This element was lost in the translocation. It is conceivable that the absence of the conserved downstream sequence element in the putative miR-142/mRNA fusion prevented the recognition of the

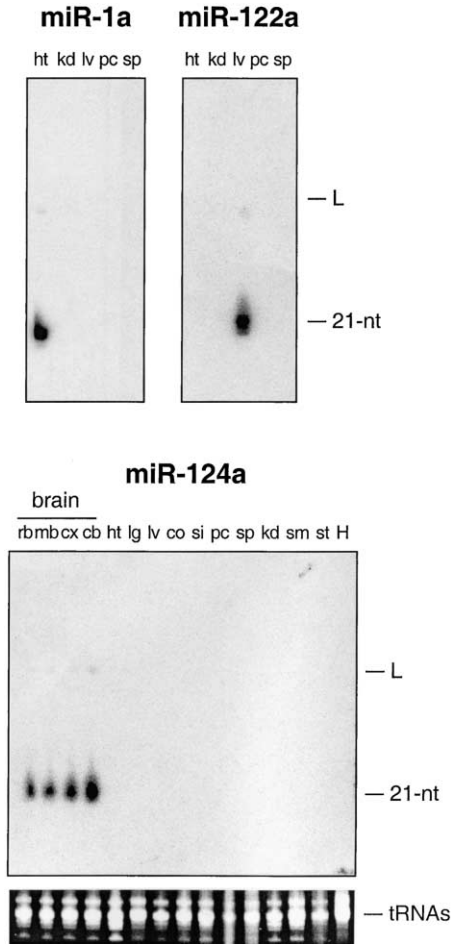

Figure 1. Northern Blot Analysis of Tissue-Specific miRNAs

Total RNA from different mouse tissues was blotted and probed with a 5′-radiolabeled oligodeoxynucleotide complementary to the indicated miRNA. Equal loading of total RNA on the gel was verified by ethidium bromide staining prior to transfer; the band representing tRNAs is shown. The foldback precursors are indicated with capital "L." Mouse brains were dissected into midbrain (mb), cortex (cx), and cerebellum (cb). The rest of the brain (rb) was also used. Other tissues were heart (ht), lung (lg), liver (lv), colon (co), small intestine (si), pancreas (pc), spleen (sp), kidney (kd), skeletal muscle (sm), stomach (st); H, human HeLa SS3 cells.

transcript as a miRNA precursor and therefore may have caused accumulation of fusion transcripts and overexpression of *MYC*.

miR-155, which was cloned from colon, is excised from the known noncoding BIC RNA [21]. *BIC* was originally identified as a gene transcriptionally activated by promoter insertion at a common retroviral integration site in B cell lymphomas induced by avian leukosis virus. Comparison of *BIC* cDNAs from human, mouse, and chicken revealed 78% identity over 138 nucleotides [21]. The identity region covers the miR-155 foldback precursor and a few conserved boxes downstream of the foldback sequence. The relatively high level of expression of *BIC* in lymphoid organs and cells in human, mouse, and chicken implies an evolutionary conserved function, but BIC RNA has also been detected at low levels in nonhematopoietic tissues [21].

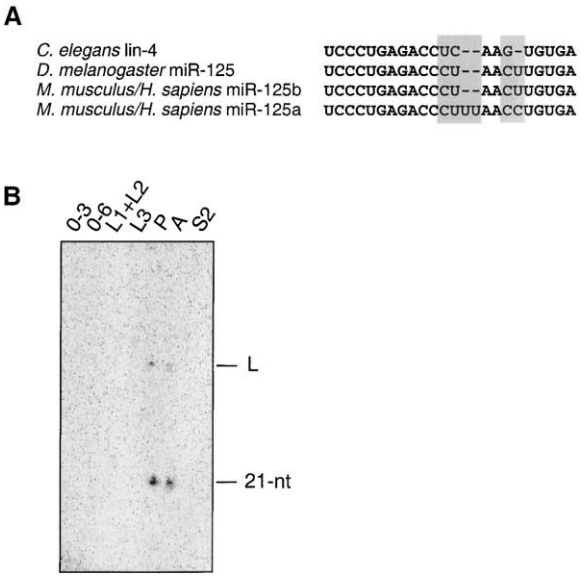

Figure 2. Potential Orthologs of lin-4 stRNA

(A) Sequence alignment of *C. elegans* lin-4 stRNA with mouse miR-125a and miR-125b and the *D. melanogaster* miR-125. Differences are highlighted by gray boxes.

(B) Northern blot of total RNA isolated from staged populations of *D. melanogaster*, probed for miR-125. E, embryo; L, larval stage; P, pupae; A, adult; S2, Schneider-2 cells.

Another interesting observation was that segments of perfect complementarity to miRNAs are not observed in mRNA sequences or in genomic sequences outside the miRNA inverted repeat. Although this could be fortuitous, based on the link between RNAi and miRNA processing [12–14], it may be speculated that miRNAs retain the potential to cleave perfectly complementary target RNAs. Because translational control without target degradation could provide more flexibility, it may be preferred over mRNA degradation.

In summary, 34 novel miRNAs were identified from mouse, which are conserved in human and often also in other nonmammalian vertebrates. A few of these miRNAs appear to be extremely tissue specific, suggesting a critical role for some miRNAs in tissue specification and cell lineage decisions. We may have also identified the fruitfly and mammalian ortholog of *C. elegans* lin-4 stRNA. The establishment of a comprehensive list of miRNA sequences will be instrumental for bioinformatic approaches that make use of completed genomes and the power of phylogenetic comparison in order to identify miRNA-regulated target mRNAs.

Supplementary Material

Supplementary Material including additional methodological details, figures, and tables can be found online at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgments

We are very grateful to S.M. Elbashir and C. Karschin for preparation of mouse tissues and sectioning of mouse brains; G. Dowe, G. Heyne, and M. Killian for sequencing; J. Aach for bioinformatic assistance; and R. Lührmann for support. This work was funded by a

Bundesministerium für Bildung und Forschung (BMBF) Biofuture grant, number 0311856.

Received: February 19, 2002
Revised: March 6, 2002
Accepted: March 6, 2002
Published: April 30, 2002

References

1. Ambros, V. (2001). microRNAs: tiny regulators with great potential. Cell *107*, 823–826.
2. Moss, E.G. (2002). MicroRNAs: hidden in the genome. Curr. Biol. *12*, R138–R140.
3. Lagos-Quintana, M., Rauhut, R., Lendeckel, W., and Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. Science *294*, 853–858.
4. Lau, N.C., Lim, L.P., Weinstein, E.G., and Bartel, D.P. (2001). An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science *294*, 858–862.
5. Lee, R.C., and Ambros, V. (2001). An extensive class of small RNAs in Caenorhabditis elegans. Science *294*, 862–864.
6. Pasquinelli, A.E., Reinhart, B.J., Slack, F., Martindale, M.Q., Kuroda, M.I., Maller, B., Hayward, D.C., Ball, E.D., Degnan, B., Müller, P., et al. (2000). Conservation of the sequence of *let-7* heterochronic regulatory RNA. Nature *408*, 86–89.
7. Ambros, V., and Horvitz, H.R. (1984). Heterochronic mutants of the nematode Caenorhabditis elegans. Science *226*, 409–416.
8. Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). The C. elegans heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell *75*, 843–854.
9. Wightman, B., Ha, I., and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in C. elegans. Cell *75*, 855–862.
10. Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettinger, J.C., Rougvie, A.E., Horvitz, H.R., and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature *403*, 901–906.
11. Rougvie, A.E. (2001). Control of developmental timing in animals. Nat. Rev. Genet. *2*, 690–701.
12. Grishok, A., Pasquinelli, A.E., Conte, D., Li, N., Parrish, S., Ha, I., Baillie, D.L., Fire, A., Ruvkun, G., and Mello, C.C. (2001). Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell *106*, 23–34.
13. Hutvágner, G., McLachlan, J., Bálint, É., Tuschl, T., and Zamore, P.D. (2001). A cellular function for the RNA interference enzyme dicer in small temporal RNA maturation. Science *93*, 834–838.
14. Ketting, R.F., Fischer, S.E., Bernstein, E., Sijen, T., Hannon, G.J., and Plasterk, R.H. (2001). Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. *15*, 2654–2659.
15. Bernstein, E., Caudy, A.A., Hammond, S.M., and Hannon, G.J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature *409*, 363–366.
16. Hammond, S.M., Boettcher, S., Caudy, A.A., Kobayashi, R., and Hannon, G.J. (2001). Argonaute2, a link between genetic and biochemical analyses of RNAi. Science *293*, 1146–1150.
17. Billy, E., Brondani, V., Zhang, H., Muller, U., and Filipowicz, W. (2001). Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. Proc. Natl. Acad. Sci. USA *98*, 14428–14433.
18. Knight, S.W., and Bass, B.L. (2001). A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in C. elegans. Science *2*, 2.
19. Hallam, S.J., and Jin, Y. (1998). *lin-14* regulates the timing of synaptic remodelling in Caenorhabditis elegans. Nature *395*, 78–82.
20. Gauwerky, C.E., Huebner, K., Isobe, M., Nowell, P.C., and Croce, C.M. (1989). Activation of *MYC* in a masked t(8;17) translocation results in an aggressive B-cell leukemia. Proc. Natl. Acad. Sci. USA *86*, 8867–8871.
21. Tam, W. (2001). Identification and characterization of human BIC, a gene on chromosome 21 that encodes a noncoding RNA. Gene *274*, 157–167.
22. Mourelatos, Z., Dostie, J., Paushkin, S., Sharma, A., Charroux, B., Abel, L., Rappsilber, J., Mann, M., and Dreyfuss, G. (2002). miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev. *16*, 720–728.
23. Cavaille, J., Buiting, K., Kiefmann, M., Lalande, M., Brannan, C.I., Horsthemke, B., Bachellerie, J.P., Brosius, J., and Hüttenhofer, A. (2000). Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. Proc. Natl. Acad. Sci. USA *97*, 14311–14316.
24. Lai, E.C. (2002). MicroRNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. Nat. Genet. *30*, 363–364.

Note Added in Proof

It was recently noted that the 5′ ends of a subset of *Drosophila* microRNAs are perfectly complementary to 3′ UTR sequence motifs that mediate negative posttranscriptional regulation [24].