

## REVIEW ARTICLE

# The role of microRNAs (miRNA) in circadian rhythmicity

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### Abstract

MicroRNA (miRNA) is a recently discovered new class of small RNA molecules that have a significant role in regulating gene and protein expression. These small RNAs (~22 nt) bind to 3' untranslated regions (3'UTRs) and induce degradation or repression of translation of their mRNA targets. Hundreds of miRNAs have been identified in various organisms and have been shown to play a significant role in development and normal cell functioning. Recently, a few studies have suggested that miRNAs may be an important regulators of circadian rhythmicity, providing a new dimension (posttranscriptional) of our understanding of biological clocks. Here, we describe the mechanisms of miRNA regulation, and recent studies attempting to identify clock miRNAs and their function in the circadian system.

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### Introduction

MicroRNA (miRNA) is a recently discovered new class of small RNA molecules that have a significant role in regulating gene and protein expression. A brief survey of the World Wide Web reveals that two of the most common phrases associated with miRNA are 'small RNA revolution' and 'tip of the iceberg'. This reflects well the astonishing fact that the importance of these molecules was obscured for so many years (Lee *et al.* 1993; Wightman *et al.* 1993), and long after the principles of gene regulation were thought to be generally understood. It also conveys the consensus among researchers about the future promise of studying the functional role of miRNAs in diverse fields related to regulation of gene expression. It is, therefore, not surprising that in addition to their role in the development, and normal cell function (Ambros 2004; Bushati and Cohen 2007), evidence began to emerge that miRNAs may play an important role in circadian rhythmicity. Here we describe the mechanism of miRNA regulation, and recent studies attempting to identify clock miRNAs and their function in circadian systems.

#### *How are miRNAs produced?*

miRNAs are short, single stranded RNA molecules, 22–24 nucleotides long, transcribed from noncoding

genomic regions (Ambros *et al.* 2003; Bushati and Cohen 2007). This process is carried out by RNA polymerase II through long pri-miRNA precursors that encode one or more miRNAs, each of which is organized in a 60–70 nucleotide hairpin structure separated by a single-stranded RNA (figure 1) (Ambros *et al.* 2003; Rana 2007).

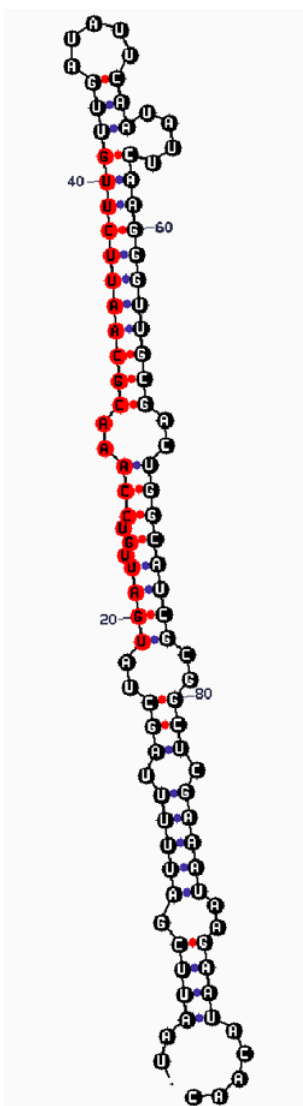
In the cell nucleus, the pri-miRNA hairpin is processed by a multi-protein complex that includes the RNase III Droscha, and the double-stranded RNA binding protein Pasha (Denli *et al.* 2004; Landthaler *et al.* 2004), producing stem loop pre-miRNA sequences (figure 2). Exportin-5 (a Ran-GTP dependent transporter) exports these pre-miRNAs to the cytoplasm, where the RNase III Dicer cuts out the single-stranded loop producing a miRNA duplex (Bernstein *et al.* 2001; Bohnsack *et al.* 2004; Lee *et al.* 2004). Dicer also participates in the assembly of mi-RISC (miRNA RNA-induced silencing complex) where one of the two strands of miRNA duplex is degraded (Matranga *et al.* 2005; Miyoshi *et al.* 2005).

#### *How do miRNAs work?*

Accumulating evidence suggests that miRNAs regulate expression in various context-dependent ways by different miRNA processing complexes. The interaction of miRNA

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**Figure 1.** Secondary structure of pre-miRNA. The predicted stem-loop structure of pre-mir-219-1, a representative circadian miRNA in the mouse (see text) is depicted. The mature sequence is shown in red. The free energy  $\Delta G$  predicted for this structure is  $-30.2$  kcal/mole. Pre-miRNA sequence was obtained from miRNAMap (Hsu *et al.* 2006) (<http://mirnamap.mbc.nctu.edu.tw>) and the structure was derived using the mfold algorithm (Zuker 2003) available at (<http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi>).

with target transcripts can be broadly grouped into two different pathways (figure 2). Perfect base pairing between a miRNA and mRNA-target induces mRNA cleavage through the ‘silencing’ mechanism. In contrast, imperfect pairing of miRNA with 3’UTR of target mRNA drives binding of miRISC to the target, leading to repression of translation.

The mechanism of action of miRNAs is not completely understood but it involves base pairing between the 5’ end of the miRNA (7–8 nucleotides, named ‘seed’), and the 3’UTR of the target mRNA (Lewis *et al.* 2003; Farh *et al.* 2005;

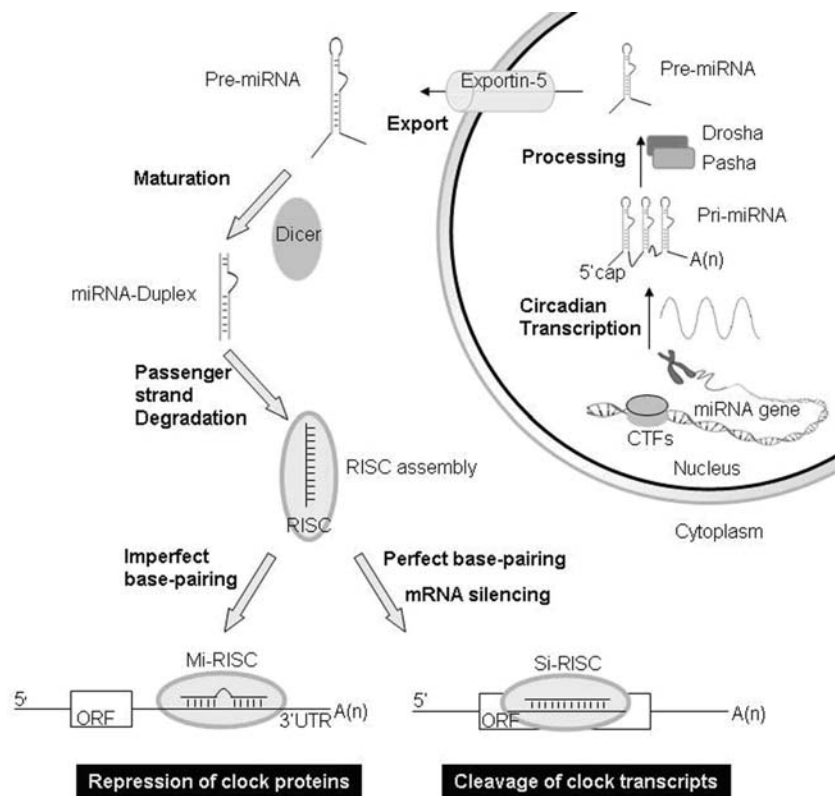
Stark *et al.* 2005). This pairing may either lead to mRNA degradation, or can affect the efficiency of translation by either promoting the ribosomal drop-off from the mRNA, or inhibiting the formation of 80S ribosome at the beginning of the translation (Humphreys *et al.* 2005; Pillai *et al.* 2005; Nottrott *et al.* 2006; Petersen *et al.* 2006). It was shown that miRNAs can also affect the stability of mRNA by de-adenylation and de-capping of the target mRNA (Behm-Ansmant *et al.* 2006; Giraldez *et al.* 2006; Mishima *et al.* 2006; Wu *et al.* 2006).

Identifying targets of miRNA is tricky. Different algorithms have been developed to recognize the ‘seed’ region to identify putative target genes, and their predictions may not always overlap. Currently, four different depositories of miRNA exist, offering different methods for target searching: the EMBL server (Brennecke *et al.* 2005), mirBase at the Wellcome Trust Sanger Institute (Griffiths-Jones *et al.* 2008), TargetScan (Grimson *et al.* 2007), and PicTar (Krek *et al.* 2005). Recently, efforts were made to integrate information of sequences, secondary structure and multiple polyA sites from 3’UTR regions (Vella *et al.* 2004a,b; Brennecke *et al.* 2005; Didiano and Hobert 2006; Rajewsky 2006; Long *et al.* 2007). To add to the complexity, recent studies indicate that base pairing of sequence outside the miRNA’s ‘seed’ region can also contribute to the binding to the target (Brennecke *et al.* 2005; Grun *et al.* 2005; Stark *et al.* 2005; Long *et al.* 2007).

Finally, a recent study showed that in contrast to their traditional role in downregulation, miRNA can also increase protein translation (Vasudevan *et al.* 2007). HeLa cell quiescence induced by serum starvation is associated with translational upregulation mediated by tumour necrosis factor- $\alpha$  (TNF $\alpha$ ). Vasudevan *et al.* (2007) found that the human miRNA miR369-3 that targets TNF $\alpha$  3’UTR is required for the interaction of this protein with two other proteins AGO and FXR1, leading to upregulation of the protein translation. Two other miRNAs, Let-7 and miRxcrc4, that usually repress translation in proliferating cells, were also found to cause upregulation of translation of their targets during cell quiescence (Vasudevan *et al.* 2007).

#### **Circadian clocks: posttranscriptional and translational regulation**

The daily cycling of light and temperature, generated by the earth’s rotation, is one of the most important driving forces in the evolution of the circadian clock, allowing organisms to anticipate and adapt to their daily (and seasonally) changing environment (Zheng and Sehgal 2008). Although the molecular details of the circadian clock may vary somewhat in different taxa, the principle of a system of self-sustained transcriptional–translational feedback loops is well conserved (Dunlap 1999; Gallego and Virshup 2007; Zheng and Sehgal 2008): Transcription factors, such as the



**Figure 2.** Synthesis and function of miRNA. Circadian transcription factors (CTFs) induce the transcription of miRNA genes (pri-miRNA). Drosha–Pasha multi-protein complex processes the pri-miRNA and produces 60–70 nt pre-miRNA. Exportin-5 translocates the pre-miRNA from the nucleus to the cytoplasm where Dicer cuts the pre-miRNA single stranded loop and generates miRNA duplex. At the RISC, the passenger strands of the miRNA duplex are degraded. The mature single strand miRNA is loaded at the RISC. Perfect base pairing between miRNA and target mRNA induces mRNA cleavage (si-RISC dependent silencing), while imperfect base-pairing between 3'UTR of target mRNA and miRNA induces mi-RISC-dependent inhibition of translation.

mammalian CLOCK and BMAL1, bind to clock-specific motifs (e.g. E-box) and drive the transcription of negative regulators, which are exported to the cytoplasm and then translated. These negative factors, which physically interact with each other, producing heterodimers (e.g. mouse PER-CRY, or fly PER-TIM), are later shuttled back to the nucleus where they repress the positive circadian transcription factors, thereby downregulating their own transcription (Darlington *et al.* 1998; Kume *et al.* 1999; Lee *et al.* 2000; Alabadi *et al.* 2001). The decrease in abundance in the negative factors later in the circadian cycle gradually leads to their own derepression, allowing the positive transcription factor to drive a new circadian cycle.

The daily oscillation of the core clock proteins drives a transcription rhythm of downstream clock-controlled genes (ccgs). Genomewide expression analysis from several microarray studies in different organisms indicates that the expression of as much as 5%–10% of transcripts in a specific tissue oscillates in a circadian manner (Claridge-Chang *et al.*

2001; McDonald and Rosbash 2001; Akhtar *et al.* 2002; Ceriali *et al.* 2002; Lin *et al.* 2002; Panda *et al.* 2002; Storch *et al.* 2002; Ueda *et al.* 2002; Duffield 2003; Keegan *et al.* 2007).

Yet, analysis of the mammalian cycling proteome revealed that the proportion of cycling cytosolic proteins is rather higher than that found in microarray studies, and that many of the cycling proteins show a constant abundance at the level of the mature transcript (Reddy *et al.* 2006). This discrepancy indicates that posttranscriptional and posttranslational mechanisms (largely unknown) are important components of circadian rhythmicity. Additional evidence (reviewed in Zheng and Sehgal 2008) shows that cycling of clock transcripts and proteins is not entirely essential for clock function, indicating that the 'central dogma' of the transcriptional-negative-feedback loop is probably sufficient but not necessary, and posttranscriptional/translational mechanisms are an important part of the circadian clock.

Before we embark on reviewing miRNA as a possible circadian posttranscriptional mechanism, we should point out that regulation of the circadian clock by endogenous antisense RNA (which conceptually includes miRNA) has already been reported in the past (reviewed by Crosthwaite 2004). For example, in *Neurospora*, several (5–5.5 kb) antisense *frequency (frq)* RNA are present (Kramer *et al.* 2003) which completely overlap with the sense transcript. The levels of the *frq* antisense RNAs cycle in antiphase with the sense *frq* RNA under free-running conditions, suggesting that the *frq*-antisense transcript has a clock function. This was elegantly demonstrated using mutant strains where the expression of the antisense, which is normally light-induced, is abolished. In these strains, circadian rhythmicity was delayed and phase response to light pulses was dramatically enhanced compared with the response of the wild-type (Kramer *et al.* 2003).

#### Role of miRNA in the circadian clock

A recent microarray study of *Drosophila* heads tested the expression of 78 miRNAs from flies entrained to light–dark cycles and compared to the corresponding expression in the clock mutant *cyc<sup>01</sup>* (Yang *et al.* 2008). Two miRNAs *dme-miR-263a* and *263b*, showed significant cycling that was abolished in the mutant. The expression of these miRNAs was validated by qPCR, which also revealed that both these products cycled in continuous darkness. The fold-change of *mir-263a* and *263b* was rather small (1.7-fold and 2-fold oscillations, respectively). Compared with the fold-change of circadian clock transcripts (e.g. 4–5: McDonald and Rosbash (2001)), the modest change of miRNA may suggest that they are not biologically relevant. However, Yang *et al.* (2008) profiled the expression in whole heads, and it is rather likely that expression of these specific miRNAs change in individual neurons at a higher level (which could be tested by *in situ* hybridization). Also, it is possible that moderate-fold change is inherent property of miRNAs, reflecting the role of these regulators in fine tuning of expression. For example, miRNA levels after sleep deprivation in the rat's brain also show modest magnitude of fold-change (1.5–2.5: Davis *et al.* (2007)).

Clearly, some clock miRNA may not show any daily oscillation and yet play an important role in circadian regulation. The constant level of miRNA may serve as a threshold that 'gates' circadian oscillations. These miRNA may respond to various cues (e.g. temperature) by changing the gating of oscillation of other clock proteins. Indeed, Yang *et al.* (2008) identified six miRNAs that did not cycle, but had a significantly different profile compared with that of the *cyc<sup>0</sup>* mutant.

Using the different prediction algorithms (Yang *et al.* 2008), a number of clock genes were identified that might provide a target for these circadian miRNAs, including *per*, *Clock*, *tim*, *dbt*, *cwo* and *tws*. However, how the cycling of *dme-miR-263a* and *263b* is generated is not yet clear. In-

terestingly, expression levels of both miRNAs, which have similar profiles, are high in the mutant, suggesting that their expression is normally repressed by CYC in the wild-type, indirectly by driving an as-yet-unknown transcriptional inhibitor.

A different approach was made in a survey of circadian miRNA in the mouse (Cheng *et al.* 2007). Here, previously published data on genomewide targets of the transcription factor CREB in the SCN in response to light were used (Impey *et al.* 2004). One of the targets of CREB was found to be a region upstream of *miR-132*. This prediction was confirmed using chromatin immunoprecipitation (ChIP) and RT-PCR. Interestingly, in addition to *miR-132*, *miR-219-1* (figure 1) was also enriched in the CREB immunoprecipitate. To identify miRNA whose transcription is driven by *Clock*, another ChIP experiment was carried using CLK antibodies; here, only *miR-219-1* was identified (Cheng *et al.* 2007).

When the promoter region of *miR-219-1* was analysed, E-box and CRE motifs (CRE-1 and CRE-2) were identified. In addition, the coexpression of BMAL1 and CLK in mammalian PC12 cells induced increased expression of *miR-219-1* (Cheng *et al.* 2007). However, *miR-132* was not responding to BMAL1 and CLK expression. In continuous darkness, both miRNAs show daily oscillation in the SCN (but not in other regions), peaking during the subjective day, which was abolished in *mCry1/mCry2* double mutant, strongly indicating a clock control of the expression of these miRNAs *in vivo* (Cheng *et al.* 2007).

The role of *miR-219* was further tested using antagomirs, oligoribonucleotides complementary to the miRNAs that block miRNA activity. Antagomir against *miR-219* reduced the level of this miRNA in the SCN and induced a lengthening of the circadian period. The *miR-132* antagomir induced two-fold increase in phase shift in light-pulse experiments, suggesting that *miR-132* is a negative regulator of the light-dependent resetting of the clock.

To identify the targets of *miR-132* and *miR-219-1*, the prediction algorithms TargetScan, miRanda and miRBase were used, revealing an overall enrichment of ion-channel proteins, suggesting that these two miRNAs modulate cellular excitability. Indeed, when cortical neuronal cultures were transfected with each of the miRNAs, a significant change in internal Ca<sup>2+</sup> level was observed in response to depolarization or activation of the glutamate receptor: introduction of *miR-132* increased Ca<sup>2+</sup> responsiveness, while *miR-219* slightly reduced it (Cheng *et al.* 2007). Transfecting HEK293T cells with either of the two miRNAs in combination with CLK and BMAL1 increased *Per1::LUC* reporter activity. Increase in *Per1::LUC* reporter was also shown in primary neuronal cultures, indicating that these miRNAs are involved in the core clock timing mechanism.

In another recent study on the mouse, the daily cycling of expression of a number of miRNAs (*miR-96*, *miR-124a*, *miR-103*, *miR-182*, *miR-106b*, *miR-422a*, and *miR-422b*) was found in the retina (Xu *et al.* 2007). It was found that the

mRNA encoding adenylyl cyclase VI (*Adcy6*), which was shown earlier to be expressed rhythmically (Han *et al.* 2005), is a predicted target (using PicTar and TargetScan) of two putative circadian miRNAs: *miR-182* and *miR-96*. The expression of these miRNAs is in anti-phase to the *Adcy6* transcript. In a luciferase assay, *miR-182* and *miR-96* are able to repress *Adcy6* expression.

As in the circadian clock in mammals, posttranscriptional regulation also appears to play an important role in the avian circadian system (Karaganis *et al.* 2008). For example, although global cycling of the transcriptome is reduced in the chick pineal gland, a robust cycling of melatonin still persists. This cycling may be driven as a posttranscriptional process by four recently identified miRNAs that show circadian oscillation in their expression levels (Shende *et al.* 2008). Among the predicted targets of these miRNAs are the chicken orthologues of mammalian clock genes *NPAS2* and *Period3*.

Some miRNA may not show diurnal cycling but still have a significant impact in regulation of clock, or clock-controlled proteins. Nocturnin, for example is a cycling deadenylase downstream of the circadian clockwork, serving as the clock output in metabolic regulation (Green *et al.* 2007). Nocturnin has recently been shown to be targeted by miR-122 (Kojima *et al.* 2008), which is consistent with an earlier study suggesting that this miRNA is involved in lipid metabolism (Esau *et al.* 2006). As large proportions of the transcriptome and proteome are under circadian control, one may assume that many more of these circadian-controlled genes are targeted by miRNAs. The miRNA system may represent an additional module that integrates various stimuli and modulates circadian rhythmicity either directly by targeting clock genes, or indirectly by acting on clock-controlled proteins.

Finally, miRNA also seems to be important for seasonal timing. In *Arabidopsis thaliana*, the circadian clock is involved in photoperiodic timing of flowering, with many flowering genes exhibiting circadian rhythmicity (Samach and Coupland 2000; Hayama and Coupland 2003). GIGANTEA (GI), is an *Arabidopsis* clock protein that links the circadian pacemaker and the photoperiodic flowering response through interaction with COSTANT (CO) and FLOWERING LOCUS T (FT) (Yanovsky and Kay 2003; Mizoguchi *et al.* 2005). Interestingly, a plant miRNA (*miR-172*) was identified that responds to day length (Schmid *et al.* 2003). A recent study showed that GI regulates the response of *miR-172* to change in day length (Jung *et al.* 2007). The *miR-172* and its targets, mediate flowering by regulating FT and constitute a separate pathway for seasonal timing.

## Conclusion

For almost two decades, the transcription–translation feedback model provided a framework for understanding how the

molecular clock works (Hardin *et al.* 1990). Yet, accumulating evidence indicates a gap in our understanding that was often assumed to represent an elusive posttranscriptional mechanism. The recent discovery of miRNAs and their function will probably contribute to narrowing this gap. MicroRNAs play an important role in various biological functions and it was only a matter of time before their role in the circadian system would be revealed. Although research on circadian miRNAs is still in its infancy, we are confident that future research will show that this type of regulation has a major impact on the circadian clock. The ‘small revolution’ is here.

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## References

- Akhtar R. A., Reddy A. B., Maywood E. S., Clayton J. D., King V. M., Smith A. G. *et al.* 2002 Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr. Biol.* **12**, 540–550.
- Alabadi D., Oyama T., Yanovsky M. J., Harmon F. G., Mas P. and Kay S. A. 2001 Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science* **293**, 880–883.
- Ambros V. 2004 The functions of animal microRNAs. *Nature* **431**, 350–355.
- Ambros V., Bartel B., Bartel D. P., Burge C. B., Carrington J. C., Chen X. *et al.* 2003 A uniform system for microRNA annotation. *RNA* **9**, 277–279.
- Behm-Ansmant I., Rehwinkel J., Doerks T., Stark A., Bork P. and Izaurralde E. 2006 mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev.* **20**, 1885–1898.
- 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.
- Bohsack M. T., Czaplinski K. and Gorlich D. 2004 Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* **10**, 185–191.
- Brennecke J., Stark A., Russell R. B. and Cohen S. M. 2005 Principles of microRNA-target recognition. *PLoS Biol.* **3**, e85.
- Bushati N. and Cohen S. M. 2007 microRNA functions. *Annu. Rev. Cell Dev. Biol.* **23**, 175–205.
- Ceriani M. F., Hogenesch J. B., Yanovsky M., Panda S., Straume M. and Kay S. A. 2002 Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *J. Neurosci.* **22**, 9305–9319.
- Cheng H. Y., Papp J. W., Varlamova O., Dziema H., Russell B., Curfman J. P. *et al.* 2007 microRNA modulation of circadian-clock period and entrainment. *Neuron* **54**, 813–829.
- Claridge-Chang A., Wijnen H., Naef F., Boothroyd C., Rajewsky N. and Young M. W. 2001 Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* **32**, 657–671.
- Crosthwaite S. K. 2004 Circadian clocks and natural antisense RNA. *FEBS Lett.* **567**, 49–54.
- Darlington T. K., Wager-Smith K., Ceriani M. F., Staknis D., Gekakis N., Steeves T. D. *et al.* 1998 Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. *Science* **280**, 1599–1603.

- Davis C. J., Bohnet S. G., Meyerson J. M. and Krueger J. M. 2007 Sleep loss changes microRNA levels in the brain: a possible mechanism for state-dependent translational regulation. *Neurosci. Lett.* **422**, 68–73.
- Denli A. M., Tops B. B., Plasterk R. H., Ketting R. F. and Hannon G. J. 2004 Processing of primary microRNAs by the microprocessor complex. *Nature* **432**, 231–235.
- Didiano D. and Hobert O. 2006 Perfect seed pairing is not a generally reliable predictor for miRNA-target interactions. *Nat. Struct. Mol. Biol.* **13**, 849–851.
- Duffield G. E. 2003 DNA microarray analyses of circadian timing: the genomic basis of biological time. *J. Neuroendocrinol.* **15**, 991–1002.
- Dunlap J. C. 1999 Molecular bases for circadian clocks. *Cell* **96**, 271–290.
- Esau C., Davis S., Murray S. F., Yu X. X., Pandey S. K., Pear M. et al. 2006 miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab.* **3**, 87–98.
- Farh K. K., Grimson A., Jan C., Lewis B. P., Johnston W. K., Lim L. P. et al. 2005 The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science* **310**, 1817–1821.
- Gallego M. and Virshup D. M. 2007 Post-translational modifications regulate the ticking of the circadian clock. *Nat. Rev. Mol. Cell Biol.* **8**, 139–148.
- Giraldez A. J., Mishima Y., Rihel J., Grocock R. J., Van Dongen S., Inoue K. et al. 2006 Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* **312**, 75–79.
- Green C. B., Nicholas D., Shihoko K., Carl A. S., Joseph F., David L. et al. 2007 Loss of nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity. *Proc. Natl. Acad. Sci. USA* **104**, 9888–9893.
- Griffiths-Jones S., Saini H. K., van Dongen S. and Enright A. J. 2008 miRBase: tools for microRNA genomics. *Nucleic Acids Res.* **36**, 154–158.
- Grimson A., Farh K. K., Johnston W. K., Garrett-Engele P., Lim L. P. and Bartel D. P. 2007 MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol. Cell* **27**, 91–105.
- Grun D., Wang Y. L., Langenberger D., Gunsalus K. C. and Rajewsky N. 2005 microRNA target predictions across seven *Drosophila* species and comparison to mammalian targets. *PLoS Comput. Biol.* **1**, e13.
- Han S., Kim T., Ha D. and Kim K. 2005 Rhythmic expression of adenylyl cyclase VI contributes to the differential regulation of serotonin N-acetyltransferase by bradykinin in rat pineal glands. *J. Biol. Chem.* **280**, 38228–38234.
- Hardin P. E., Hall J. C. and Rosbash M. 1990 Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature* **343**, 536–540.
- Hayama R. and Coupland G. 2003 Shedding light on the circadian clock and the photoperiodic control of flowering. *Curr. Opin. Plant Biol.* **6**, 13–19.
- Hsu P. W. C., Huang H., Hsu S., Lin L., Tsou A., Tseng C. et al. 2006 miRNAMap: genomic maps of microRNA genes and their target genes in mammalian genomes. *Nucleic Acids Res.* **34**, D135–139.
- Humphreys D. T., Westman B. J., Martin D. I. and Preiss T. 2005 MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc. Natl. Acad. Sci. USA* **102**, 16961–16966.
- Impey S., McCorkle S. R., Cha-Molstad H., Dwyer J. M., Yochum G. S., Boss J. M. et al. 2004 Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell* **119**, 1041–1054.
- Jung J. H., Seo Y. H., Seo P. J., Reyes J. L., Yun J., Chua N. H. et al. 2007 The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in *Arabidopsis*. *Plant Cell* **19**, 2736–2748.
- Karaganis S., Kumar V., Beremand P., Bailey M., Thomas T. and Cassone V. 2008 Circadian genomics of the chick pineal gland *in vitro*. *BMC Genomics* **9**, 206.
- Keegan K. P., Pradhan S., Wang J. P. and Allada R. 2007 Meta-analysis of *Drosophila* circadian microarray studies identifies a novel set of rhythmically expressed genes. *PLoS Comput. Biol.* **3**, e208.
- Kojima S., Gatfield D. and Green C. 2008 Nocturnin expression is regulated post-transcriptionally by miR-122. 20th meeting, Society for research on biological rhythms, Destin, Florida.
- Kramer C., Loros J. J., Dunlap J. C. and Crosthwaite S. K. 2003 Role for antisense RNA in regulating circadian clock function in *Neurospora crassa*. *Nature* **421**, 948–952.
- Krek A., Grun D., Poy M. N., Wolf R., Rosenberg L., Epstein E. J. et al. 2005 Combinatorial microRNA target predictions. *Nat. Genet.* **37**, 495–500.
- Kume K., Zylka M. J., Sriram S., Shearman L. P., Weaver D. R., Jin X. et al. 1999 mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. *Cell* **98**, 193–205.
- Landthaler M., Yalcin A. and Tuschl T. 2004 The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. *Curr. Biol.* **14**, 2162–2167.
- Lee K., Loros J. J. and Dunlap J. C. 2000 Interconnected feedback loops in the *Neurospora* circadian system. *Science* **289**, 107–110.
- 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.
- Lee Y. S., Nakahara K., Pham J. W., Kim K., He Z., Sontheimer E. J. et al. 2004 Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* **117**, 69–81.
- Lewis B. P., Shih I. H., Jones-Rhoades M. W., Bartel D. P. and Burge C. B. 2003 Prediction of mammalian microRNA targets. *Cell* **115**, 787–798.
- Lin Y., Han M., Shimada B., Wang L., Gibler T. M., Amarakone A. et al. 2002 Influence of the period-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **99**, 9562–9567.
- Long D., Lee R., Williams P., Chan C. Y., Ambros V. and Ding Y. 2007 Potent effect of target structure on microRNA function. *Nat. Struct. Mol. Biol.* **14**, 287–294.
- Matranga C., Tomari Y., Shin C., Bartel D. P. and Zamore P. D., 2005 Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* **123**, 607–620.
- McDonald M. J. and Rosbash M. 2001 Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* **107**, 567–578.
- Mishima Y., Giraldez A. J., Takeda Y., Fujiwara T., Sakamoto H., Schier A. F. et al. 2006 Differential regulation of germline mRNAs in soma and germ cells by zebrafish miR-430. *Curr. Biol.* **16**, 2135–2142.
- Miyoshi K., Tsukumo H., Nagami T., Siomi H. and Siomi M. C. 2005 Slicer function of *Drosophila* argonautes and its involvement in RISC formation. *Genes Dev.* **19**, 2837–2848.
- Mizoguchi T., Wright L., Fujiwara S., Cremer F., Lee K., Onouchi H. et al. 2005 Distinct roles of GIGANTEA in promoting flowering and regulating circadian rhythms in *Arabidopsis*. *Plant Cell* **17**, 2255–2270.
- Nottrott S., Simard M. J. and Richter J. D. 2006 Human let-7a miRNA blocks protein production on actively translating polyribosomes. *Nat. Struct. Mol. Biol.* **13**, 1108–1114.
- Panda S., Antoch M. P., Miller B. H., Su A. I., Schook A. B.,

- Straume M. *et al.* 2002 Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109**, 307–320.
- Petersen C. P., Bordeleau M. E., Pelletier J. and Sharp P. A. 2006 Short RNAs repress translation after initiation in mammalian cells. *Mol. Cell* **21**, 533–542.
- Pillai R. S., Bhattacharyya S. N., Artus C. G., Zoller T., Cougot N., Basyuk E. *et al.* 2005 Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* **309**, 1573–1576.
- Rajewsky N. 2006 microRNA target predictions in animals. *Nat. Genet.* **38**, 8–13.
- Rana T. M. 2007 Illuminating the silence: understanding the structure and function of small RNAs. *Nat. Rev. Mol. Cell Biol.* **8**, 23–36.
- Reddy A. B., Karp N. A., Maywood E. S., Sage E. A., Deery M., O'Neill J. S. *et al.* 2006 Circadian orchestration of the hepatic proteome. *Curr. Biol.* **16**, 1107–1115.
- Samach A. and Coupland G. 2000 Time measurement and the control of flowering in plants. *Bioessays* **22**, 38–47.
- Schmid M., Uhlenhaut N. H., Godard F., Demar M., Bressan R., Weigel D. *et al.* 2003 Dissection of floral induction pathways using global expression analysis. *Development* **130**, 6001–6012.
- Shende V. R., Beremand P. D. and Cassone V. M. 2008 MicroRNA rhythms in the chick pineal gland. 20th meeting, Society for research on biological rhythms, Destin, Florida.
- Stark A., Brennecke J., Bushati N., Russell R. B. and Cohen S. M. 2005 Animal microRNAs confer robustness to gene expression and have a significant impact on 3'UTR evolution. *Cell* **123**, 1133–1146.
- Storch K., Lipan O., Leykin I., Viswanathan N., Davis F. C., Wong W. H. *et al.* 2002 Extensive and divergent circadian gene expression in liver and heart. *Nature* **417**, 78–83.
- Ueda H. R., Matsumoto A., Kawamura M., Iino M., Tanimura T. and Hashimoto S. 2002 Genome-wide transcriptional orchestration of circadian rhythms in *Drosophila*. *J. Biol. Chem.* **277**, 14048–14052.
- Vasudevan S., Tong Y. and Steitz J. A. 2007 Switching from repression to activation: microRNAs can up-regulate translation. *Science* **318**, 1931–1934.
- Vella M. C., Choi E. Y., Lin S. Y., Reinert K. and Slack F. J. 2004a The *C. elegans* microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev.* **18**, 132–137.
- Vella M. C., Reinert K. and Slack F. J. 2004b Architecture of a validated microRNA::target interaction. *Chem. Biol.* **11**, 1619–1623.
- 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.
- Wu L., Fan J. and Belasco J. G. 2006 MicroRNAs direct rapid deadenylation of mRNA. *Proc. Natl. Acad. Sci. USA* **103**, 4034–4039.
- Xu S., Witmer P. D., Lumayag S., Kovacs B. and Valle D. 2007 MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. *J. Biol. Chem.* **282**, 25053–25066.
- Yang M., Lee J. E., Padgett R. W. and Edery I. 2008 Circadian regulation of a limited set of conserved microRNAs in *Drosophila*. *BMC Genomics* **9**, 83.
- Yanovsky M. J. and Kay S. A. 2003 Living by the calendar: how plants know when to flower. *Nat. Rev. Mol. Cell Biol.* **4**, 265–275.
- Zheng X. and Sehgal A. 2008 Probing the relative importance of molecular oscillations in the circadian clock. *Genetics* **178**, 1147–1155.
- Zuker M. 2003 Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31**, 3406–3415.

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