Ten years ago, Andrew Z. Fire and Craig C. Mello described a potent and sequence-specific interference with gene expression mediated by double-stranded RNA [1]. Their landmark discovery of “RNA interference” (RNAi) was honoured with the Nobel Prize for Physiology or Medicine in 2006 and has spurred intense research of this novel mechanism of gene regulation. Within a few years, we have learned a lot about the biochemistry of RNAi and related silencing mechanisms, including the molecular steps during the process and the composition of involved protein machineries. In parallel, our appreciation of the exceptional importance of RNAi has grown rapidly as its fundamental role for the orchestration of gene expression and the defence against invading foreign nucleic acids is beginning to unfold.

It has emerged that short ca. 22 nt long RNA duplexes are the key effectors of RNAi and that a common core mechanism exists that is responsible for processing double-stranded RNA into these guide structures directing sequence-specific gene silencing (Fig. 1A). In addition, we are now aware of a large family of endogenous RNA molecules, consisting of over 400 different members in humans, called micro RNAs (miRNAs) which together form an intricate meshwork of gene regulation. During miRNA biogenesis initiated by RNA polymerase II transcription of a so called “pri-miRNA”, two processing steps carried out by the nuclear RNase III enzyme Drosha and the cytoplasmic Dicer yield an imperfect RNA duplex carrying 2 nt 3’ overhangs on each strand. One of the strands is subsequently incorporated into an RNA-induced silencing complex (RISC). In this context, the miRNA guides the assembly to its cognate mRNA resulting in cleavage of the mRNA in case of perfect or nearly perfect complementarity or translational repression for imperfectly base paired miRNAs.

While it has been observed for some time that miRNAs play a key role in regulating gene expression of cellular mRNAs, evidence is now mounting that they also profoundly influence virus replication and pathogenesis [2]. Given the intimate relation between viruses and its host, the marked dependence of viruses on cellular factors and the key role of miRNAs in shaping the host cell proteome, this may not be so surprising. Yet the skill by which viruses have evolved to usurp the miRNA system is remarkable: while various DNA viruses like for instance human cytomegalovirus (hCMV) encode miRNAs modulating the host cell environment conducive for virus propagation thus taking advantage of the host’s miRNA machinery [3,4], the hepatitis C virus (HCV) was recently recognized to usurp an endogenous miRNA highly expressed in liver cells to promote virus replication [5,6]. HCV is an RNA virus of the family Flaviviridae that replicates in hepatocytes and is a major cause of chronic hepatitis, liver cirrhosis...
and hepatocellular carcinoma worldwide. Unlike the canonical outcome of miRNA-dependent gene regulation where binding of the miRNA to its cognate target sequence in the 3' untranslated region of an mRNA results in translational repression, occupation of a tandem binding site within the 5' non-translated region of the HCV genome by miRNA122 results in elevated HCV RNA levels [5,6]. Although the molecular mechanism of this surprising positive regulation has not been completely elucidated, this represents an intriguing example where a miRNA upregulates the abundance of a replicating viral RNA.

In a study presented in this issue of the Journal [7], Murakami and colleagues employed an algorithm-based search to identify endogenous miRNAs that may control HCV RNA replication. Using this approach, miR-199a* was recognized to have partial complementarity to a conserved region within the HCV 5' untranslated region (UTR). MiR-199a* is expressed in liver cells, albeit with much lower abundance when compared to miR-122 [7–9]. Based on a comprehensive set of experiments the authors conclusively show that this miRNA down-regulates RNA replication of full length HCV replicons of the Con1 (genotype 1b) and the JFH1 (genotype 2a) strain. This inhibition seems to be directly mediated by miR-199a* since administration of antisense oligonucleotides (ASOs) sequestering the miRNA increased HCV RNA replication to an extent that was statistically significant. Further confirming the sequence-specificity of the miRNA-based negative regulation, Murakami et al. mutated the HCV sequences complementary to miR-199a* within the 5'NTR of a genotype 1b replicon, or alternatively used miRNAs with 3 or 5 mismatch mutations. In both cases, responsiveness of HCV RNA replication was lost, indicating that a direct sequence specific interaction between the HCV RNA and the miRNA was responsible for the observed modulation. As expected for miRNA-medi-
ated gene regulation, the observed anti-viral effect of the miRNA appeared to be independent of the interferon (IFN) pathway, since – at least at the transcriptional level – typical IFN-induced anti-viral effectors like 2'-5' oligoadenylate synthase (2'-5'OAS), protein kinase R (PKR) or MxA were not upregulated by administration of miR-199a*.

Finally, when using infection assays either with patient serum-derived HCV particles (genotype 1b or 2a) or cell culture-derived JFH1 viruses, pre-treatment of target cells with miR199a* reduced HCV copy numbers in the infected cells, while depletion of endogenous levels of the miRNAs by ASO slightly raised HCV RNA abundance. Together, these data confirm a regulatory role of miR-199a* on HCV replication also upon infection.

The novel findings by Murakami and colleagues extend our knowledge of interactions between pathogens and endogenous miRNAs and suggest that HCV replication is limited by miR-199a*. Although not explicitly analyzed in their study, according to the normal mode of gene regulation by miRNAs, it is reasonable to assume that this miRNA down-regulates HCV RNA replication by repressing translation of the HCV polyprotein or by mediating cleavage of the viral genome. Irrespective of the precise molecular mechanism, the aforementioned miRNA clearly operates in a different mode compared to miR-122 which is known to increase HCV RNA abundance [5,6]. Why the interaction of HCV with different endogenous miRNAs has discrepant outcome and how interaction with miR122 causes increased HCV RNA replication rather than translational repression or genome degradation is presently unclear. Of note, Jopling et al. recently observed that transfer of the miR-122 binding site to the 3'UTR of a reporter mRNA mediates translational repression of this mRNA, indicating that miR-122 exerts dual functions in a context dependent manner [5]. Since insertion of the miR-122 site into the 5'UTR of a reporter RNA had no effect on translation or mRNA stability and due to the finding of Murakami that miR-199a* which also targets a stretch within the 5'UTR of HCV decreases HCV replication it is unlikely that miR-122 exerts its positive effect on HCV replication simply due to the positioning of its binding site within the 5'UTR of HCV. Nevertheless, given the high degree of secondary RNA structures contained within the 5'UTR of HCV which are mediated by complementary basepairing and play a pivotal role for HCV translation and replication, it is possible that interaction with an endogenous miRNA may fine tune the composition or conformation of these structures in a highly position dependent manner thus affecting replication efficiency.

To which extent these miRNAs modulate HCV replication or influence HCV tropism in vivo remains to be determined. It is conceivable that expression of a set of miRNAs with target sequences in conserved regions of the HCV genome could significantly limit virus replication. Alternatively, absence of a miRNA essential for efficient HCV replication may similarly restrict HCV propagation. In agreement with the latter hypothesis, Chang et al. recently described substantially increased HCV replication in a human kidney-derived cell line (293 cells) upon ectopic expression of miR-122 [10]. In accordance with these data, limited availability of miR-122 which is expressed in a liver-specific manner may contribute to the restricted tropism of HCV by preventing spread to tissues with low levels of miR-122. In turn, while miR-199a* is expressed in liver cells only at low levels [7-9], high quantities of these miRNAs in extrahepatic tissues may preclude efficient HCV replication. Therefore, depending on the host cell and the tissue-specific (or species-specific) profile of expressed miRNAs, HCV may encounter more or less favourable conditions for infection and replication. Recent data by Randall et al. suggest that within human liver cells, the machinery essential for processing of dsRNA into mature miRNAs is important for efficient HCV replication and infection [8]. Using Huh-7 hepatoma cells as a model and cell culture-derived infectious HCV particles, this group silenced expression of various host proteins required for miRNA biogenesis which caused clearly reduced HCV infection and replication. In combination with the findings of Murakami et al. these data therefore imply that in these cells the positive effect of miR-122 on HCV replication and possibly its effect on additional host genes as well as regulatory effects of other miRNAs are dominant over the miR199a*-dependent restriction. Considering the aptitude of HCV to establish life long persistent infection of the liver, the virus apparently has well adapted not only to the specific set of host proteins expressed in this tissue, but also to the hepatocyte-specific miRNA environment.

The excellent potential of RNAi for therapeutic applications has been well recognized. However, when considering miRNAs as possible effectors the possibly broad specificity of these regulators of gene expression needs to be carefully evaluated. Since recognition of target mRNAs does not rely on perfect complementarity, a set of miRNAs rather than a single transcript can be affected by a single miRNA usually not expressed in a given tissue. Accordingly, Murakami et al. observed a large number of differentially regulated host genes upon introduction of miR-199a* into human hepatoma cells [7]. Nevertheless, studies like the one by Murakami and Jopling are beginning to unravel novel “Achilles heels” in the intricate network of interactions between a viral pathogen and its host. Considering the highly efficient endogenous RNAi machinery and hoping that the remarkable findings about the mechanisms of RNAi will be paralleled by rapid progress optimizing in vivo stability, delivery and specificity, therapeutic application may become within reach in the near future.
Acknowledgement

Work in the author’s laboratory is supported by an Emmy Noether fellowship of the Deutsche Forschungsgemeinschaft (PI 734/1-1), by grants from the Helmholtz Association SO-024, by the Initiative and Networking Fund of the Helmholtz Association within the Helmholtz Alliance on Immunotherapy of Cancer, by the International Research Training Group IRTG1273, and by the Indo-German Science Center. The author is grateful to Sandra Ciesek and Eike Steinmann for critical reading of the manuscript.

References


