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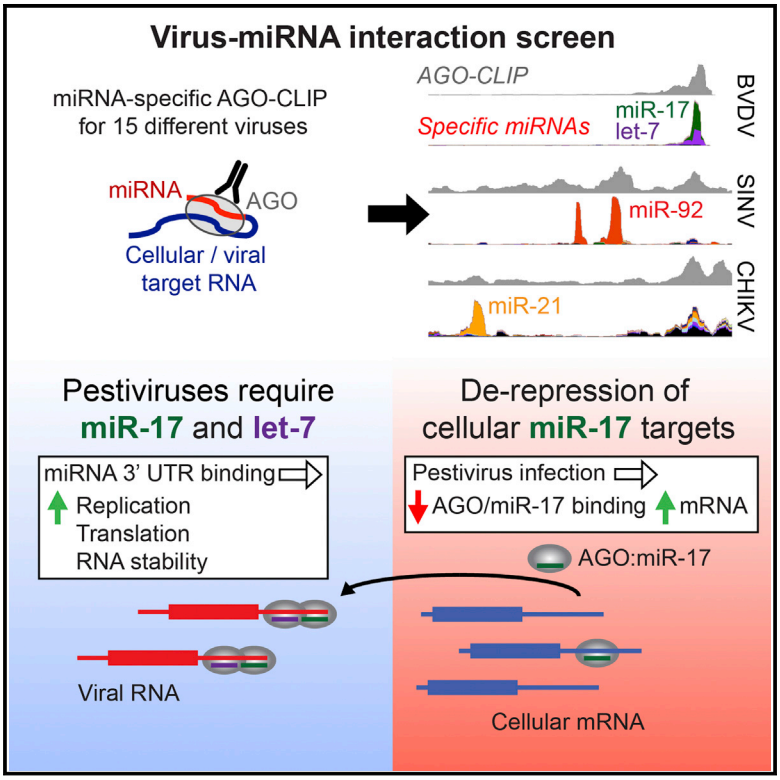
A Broad RNA Virus Survey Reveals Both miRNA Dependence and Functional Sequestration

Graphical Abstract



Highlights

- miRNA binding profiles for 15 different viruses elucidated by Argonaute (AGO)-CLIP
- Groups of viruses sequester specific miRNAs or AGO in general
- Pestiviruses critically depend on cellular miR-17 and let-7
- Pestiviral RNA functionally reduces miR-17 binding on endogenous mRNA targets

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In Brief

To assess the landscape of miRNA interactions on viral RNA, Scheel et al. screened 15 different RNA virus infections using Argonaute crosslinking immunoprecipitation (AGO-CLIP). Unlike canonical miRNA functions, miR-17 and let-7 interact with the pestivirus 3′ UTR to promote virus replication. Pestivirus RNA functionally sequesters miR-17 leading to de-repression of its cellular targets.

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A Broad RNA Virus Survey Reveals Both miRNA Dependence and Functional Sequestration

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SUMMARY

Small non-coding RNAs have emerged as key modulators of viral infection. However, with the exception of hepatitis C virus, which requires the liver-specific microRNA (miRNA)-122, the interactions of RNA viruses with host miRNAs remain poorly characterized. Here, we used crosslinking immunoprecipitation (CLIP) of the Argonaute (AGO) proteins to characterize strengths and specificities of miRNA interactions in the context of 15 different RNA virus infections, including several clinically relevant pathogens. Notably, replication of pestiviruses, a major threat to milk and meat industries, critically depended on the interaction of cellular miR-17 and let-7 with the viral 3′ UTR. Unlike canonical miRNA interactions, miR-17 and let-7 binding enhanced pestivirus translation and RNA stability. miR-17 sequestration by pestiviruses conferred reduced AGO binding and functional de-repression of cellular miR-17 targets, thereby altering the host transcriptome. These findings generalize the concept of RNA virus dependence on cellular miRNAs and connect virus-induced miRNA sequestration to host transcriptome regulation.

INTRODUCTION

Direct and indirect interactions with host miRNAs play important roles for DNA viruses, many of which encode their own miRNAs (Kincaid and Sullivan, 2012). For RNA viruses, interactions with host miRNAs remain poorly characterized with the exception of HCV, which requires the liver-specific miR-122 (Wilson and Sagan, 2014). The discovery of interactions between miR-122 and the HCV 5′ UTR (Jopling et al., 2005) at two binding sites stimulating viral replication was unexpected, as miRNAs typically interact with the 3′ UTRs of mRNAs to destabilize transcripts or repress translation (Bartel, 2009). The HCV/miR-122 interaction has proven promising as therapeutic target (Janssen et al., 2013). In contrast, Eastern equine encephalitis virus (EEEV) is an example of canonical miRNA action, in which conserved miR-142-3p sites limit viral replication and thereby activation of innate immunity specifically in hematopoietic cells, a mechanism that enhances neuropathogenesis (Trobaugh et al., 2014).

Studies of miRNA action were enhanced by AGO-CLIP methods (Chi et al., 2009; Hafner et al., 2010), which were used to characterize miRNA expression and regulation for several DNA viruses (Haecker et al., 2012; Riley et al., 2012; Skalsky et al., 2012). More recently, we generated small RNA binding landscapes for HCV RNA, confirming the 5′ UTR miR-122 interaction and unexpectedly demonstrating that sequestration of miR-122 by HCV RNA leads to transcriptome-wide de-repression of cellular miR-122 targets (Luna et al., 2015). A major drawback of standard AGO-CLIP methods is the inability to link miRNAs directly to their targets. miRNA-target chimeras produced through direct ligation allow unambiguous identification of such interactions (Grosswendt et al., 2014; Helwak et al., 2013). Through covalent ligation of endogenous argonaute-bound RNAs (CLEAR)-CLIP on endogenous AGO complexes, we recently improved target identification efficiency (Moore et al., 2015).

In the current study, we used AGO-CLIP and analysis of miRNA-target chimeras to examine a broad panel of 15 different RNA viruses for miRNA interactions in mammalian cells. Of particular interest were uncovered interactions between miR-17 and let-7 and the 3′ UTR of bovine viral diarrhea virus (BVDV), which were critical for viral replication. BVDV and classical swine fever virus (CSFV) are members of the *Pestivirus* genus, an important group of animal pathogens distantly related to HCV within the *Flaviviridae* family. By examining host transcriptomes in vitro and ex vivo, we observed reduced AGO binding and functional mRNA de-repression of cellular miR-17