Mammalian Genome (2022) 33:412–420
https://doi.org/10.1007/s00335-021-09915-y

Learning noncoding RNA biology from viruses

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Received: 1 July 2021 / Accepted: 2 September 2021 / Published online: 7 September 2021

Abstract
Insights into interactions between viral factors and the cellular machinery usually lead to discoveries concerning host cell biology. Thus, the gene expression field has historically relied on viral model systems to discover mechanisms underlying different cellular processes. In recent years, the functional characterization of the small nuclear noncoding RNAs expressed by the oncogenic *Herpesvirus saimiri,* called HSURs, resulted in the discovery of two mechanisms for the regulation of gene expression. HSUR1 and HSUR2 associate with host microRNAs, which are small noncoding RNAs that broadly regulate gene expression by binding to messenger RNAs. HSUR1 provided the first example of a process known as target-directed miRNA degradation that operates in cells to regulate miRNA populations. HSUR2 functions as a miRNA adaptor, uncovering an entirely new, indirect mechanism by which miRNAs can inhibit mRNA function. Here, I review the path that led to these discoveries and their implications and postulate new exciting questions about the functions of these fascinating viral noncoding RNAs.

Introduction

Virology has historically nourished many of the current fields comprised by the biomedical sciences, and RNA biology is no exception. Research on virus biology led to many of the early discoveries that established the field of RNA biology as such. The capping of messenger RNAs (mRNAs), mRNA splicing and alternative splicing, alternative polyadenylation, internal ribosomal entry sites, translational frameshifting, the discovery of nuclear mRNA export factors: these are just a few examples of important discoveries in RNA biology that happened by studying viruses. The analysis of viral models continues to shed light into mechanisms underlying RNA function.

Herpesvirus saimiri (HVS) is a herpesvirus that naturally persists in T cells of squirrel monkeys (*Saimiri sciureus*) and displays oncogenic potential when transmitted to other New World primates like the common marmoset (*Callithrix jacchus*) (Ensser and Fleckenstein 2005). In transformed T cells, HVS expresses seven small nuclear RNAs (snRNAs) called *Herpesvirus saimiri* U-rich RNAs (HSURs). HSURs were serendipitously discovered in experiments aimed at identifying an RNA transcript responsible for viral transformation (Murthy et al. 1986). HSURs are expressed during latency and are not expressed during the lytic phase of infection (Murthy et al. 1986). Soon after their discovery, HSURs were shown to bind to Sm proteins and to carry a 5′ trimethylguanosine cap structure (Lee et al. 1988), relating them to the cellular small nuclear ribonucleoproteins (snRNPs) involved in pre-mRNA splicing and 3′ end formation of histone pre-mRNAs. Despite continued efforts to characterize them, their functions remain unknown for decades until the discovery that two of these viral snRNAs, HSUR1 and HSUR2 (Fig. 1), interact with host-encoded microRNAs (miRNAs) (Cazalla et al. 2010). miRNAs are small noncoding RNAs (ncRNAs) that regulate gene expression by promoting decay via deadenylation and decapping, and/or translational repression of target mRNAs (Bartel 2018). miRNAs associate with Argonaute (Ago) proteins to form RNA-induced silencing complexes (RISC) and they exert their canonical function by interacting with target mRNAs via base-pairing through their seed sequence (nucleotides 2 through 7 from the miRNA 5′ end) with additional base-pairing involving nucleotides 13–16 of miRNA in some cases (Bartel 2018). If a miRNA associated with Ago2 (one of the four Ago proteins present in mammals) and its target RNA exhibit extensive and perfect complementarity including the central region (nucleotides 9–12) of the miRNA, then Ago2 cleaves the target RNA. This cleavage results in the

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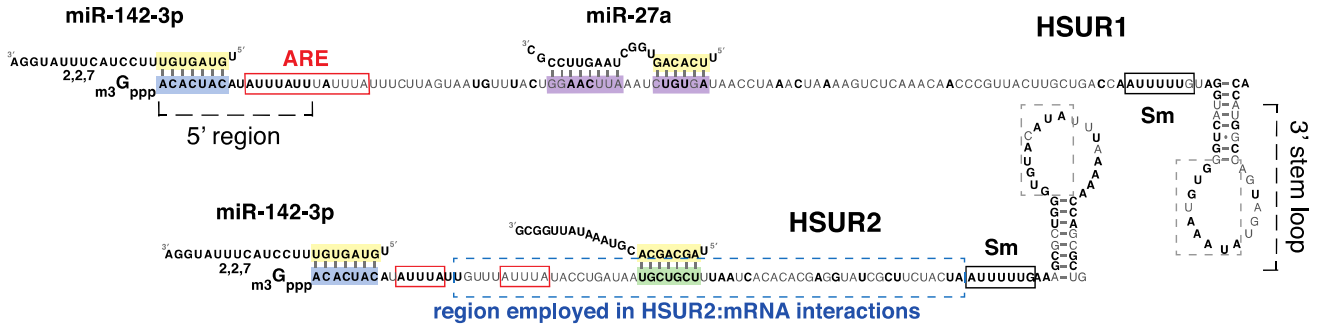

Fig. 1 HSUR1 and HSUR2. Nucleotide sequences of HSUR1, HSUR2 and miR-142-3p, miR-16 and miR-27a. Black, bold nucleotides are perfectly conserved in all available genome sequences of HVS A, B, and C strains and also in *H. ateles* (Cazalla et al. 2010). Binding sites for miR-142-3p (blue), miR-16 (green), and miR-27a (purple) are highlighted. Seed regions of miRNAs are highlighted in yellow. AU-rich element (ARE)-like sequences are shown in red boxes, whereas the Sm-binding site is shown in a black box. The HSUR2 region involved in interactions with target mRNAs (Gorbea et al. 2019) is shown in a dashed blue box

degradation of the target RNA by exonucleases (Liu et al. 2004; Meister et al. 2004). HSUR1 and HSUR2 interact with the hematopoietic lineage-specific miRNA miR-142-3p (Chen et al. 2004). HSUR1 also interacts with miR-27, whereas HSUR2 interacts with miR-16 (Fig. 1). In all cases, interactions occur via base-pairing with the seed region of these miRNAs. HSUR1 also shows extensive complementarity to the 3′ region of miR-27, but not the central region, of the miRNA (Fig. 1). In addition to miRNA binding sites, HSUR1 and HSUR2 contain sequences that resemble AU-rich elements or AREs (Cook et al. 2004; Fan et al. 1997; Myer et al. 1992). The AREs present in these viral snRNAs bind ARE-binding proteins (ARE-BPs) and, in the case of HSUR1, modulate its stability (Fan et al. 1997).

The discovery of interactions between these two classes of ncRNAs was unexpected and initially puzzling. Until then, all the functional interactions described for miRNAs were with protein-coding mRNAs. Since HSURs are not translated and are not polyadenylated, it seemed unlikely that interactions with miR-142-3p and miR-16 would affect HSURs' functions. Also, since the interaction between HSUR1 and miR-27 does not involve the central region of the miRNA, HSUR1 cannot be cleaved by Ago2 and its stability is not affected. Subsequent research into the functions of HSUR:miRNA interactions provided novel insights into ncRNA function and led to the discovery of novel mechanisms for gene regulation.

HSUR1 and target-directed miRNA degradation (TDMD)

Direct comparison of miRNA levels in T cells transformed with either wild type or a mutant version of HVS deleted for HSUR1 and HSUR2 clearly indicated that the overall abundance of miR-27 was diminished in the presence of HSURs. In contrast, the abundance of miR-16 and miR-142-3p was not affected (Cazalla et al. 2010). HSUR1 did not affect the transcription or processing of miR-27, but rather promoted the degradation of the mature miRNA. Mutational analysis showed that the targeting of miR-27 for degradation by HSUR1 occurs in a binding-dependent and sequence-specific manner. By destabilizing miR-27, HVS promotes an increase in the abundance of miR-27 targets, such as FOXO1, a transcription factor that regulates multiple cellular processes that are relevant for viral infection (Burgering and Kops 2002; Cabrera-Ortega et al. 2017; Kitamura 2013; Zhang et al. 2011). Further examination of miR-27 targets in HVS-transformed marmoset T cells revealed that downregulation of miR-27 could help promote the activation of infected T cells and viral latency (Guo et al. 2014). The discovery of miR-27 degradation by HSUR1 provided the first example of a virus using this strategy to manipulate host gene expression. Beyond the significance to herpesvirus biology, the discovery of HSUR1-mediated miR-27 degradation constitutes the first example of a mechanism that, we now know, is used by host cells to regulate miRNA populations. Moreover, it also demonstrated that snRNAs can have functions outside pre-mRNA processing.

Concurrently with the discovery of HSUR:miRNA interactions, it was reported that murine cytomegalovirus (MCMV), another herpesvirus, also promotes degradation of miR-27 during the lytic phase of infection by an unknown mechanism (Buck et al. 2010). This was followed by two reports describing mRNA m169, which contains a miR-27 binding site in its 3′ untranslated region (3′UTR), as the transcript responsible for miR-27 degradation in MCMV infections (Libri et al. 2012; Marcinowski et al. 2012). Mutation of the miR-27 binding site in m169 results in virus attenuation in an *in* vivo infection model (Marcinowski et al. 2012). A third example of a herpesvirus using a similar strategy to downregulate the levels of specific miRNAs was provided

by human cytomegalovirus (HCMV). This virus expresses a long ncRNA (lncRNA) from the UL144-145 region that binds and promotes degradation of miR-17 and miR-20a, two miRNAs that belong to the oncogenic miR-17-92 cluster (He et al. 2005). Degradation of these miRNAs results in accelerated HCMV production during lytic infection (Lee et al. 2013).

Although the three examples described above occur in herpesviruses, they do not seem to share a common evolutionary origin. Three different classes of RNA transcripts (an snRNA, a lncRNA, and an mRNA) are employed to target the degradation of the miRNA in each case; although HVS and MCMV target the same miRNA, miR-27, HVS does so during latency in T cells, while MCMV does so in lytic infections of diverse cell types (Buck et al. 2010); MCMV and HCMV are both β-herpesviruses but they target different miRNAs for degradation with unrelated transcripts. These examples are diverse not only in class and origin of the RNA transcripts employed but also show flexibility in terms of base-pairing with miRNAs to promote their degradation despite the fact that in all cases, the interaction involves the seed region as well as extensive complementarity with the 3′ region of the miRNA (Pawlica et al. 2019). This diversity suggested that selective miRNA decay induced by extensive target complementarity could be a widespread phenomenon.

Accumulating evidence suggested that this is indeed the case. Using artificial target RNAs, it was shown that extensive target complementarity induces the addition of non-templated nucleotides to the miRNA ("tailing"), trimming and degradation of the miRNA in *Drosophila* and HeLa cells (Ameres et al. 2010). Another study utilized artificial constructs carrying extensive complementary sites to miR-122 and let-7 to efficiently downregulate these miRNAs in mouse liver cells (Xie et al. 2012). Through the use of lentiviruses expressing artificial mRNAs with highly complementary sites to miR-124 and miR-132, the same phenomenon was described to operate with remarkable potency in mouse primary neurons; this phenomenon was termed target RNA-directed miRNA degradation, or TDMD (de la Mata et al. 2015). More recent studies described endogenous RNA transcripts with extensive miRNA binding sites that elicit TDMD. The lncRNA libra is expressed by zebrafish and shows extensive sequence similarity with the 3′UTR of a mammalian protein-coding gene, neuronal regeneration-related protein (*NREP*), which regulates animal behavior. These two transcripts carry a highly conserved, extensive binding site for miR-29b that promotes the degradation of the miRNA in the brain. Mutant zebrafish and mice, in which the biding sites for miR-29b were impaired in *libra* or *Nrep*, showed altered behavior, highlighting the importance of regulating miR-29b by TDMD in these two animal models (Bitetti et al. 2018). Cyrano, a lncRNA broadly conserved in vertebrates that carries a highly extensive binding site for miR-7 (Ulitsky et al. 2011), provides another interesting example of TDMD in the brain (Kleaveland et al. 2018).

HSUR1 has also proven instrumental in the dissection of mechanistic details of TDMD. Mutational analysis and structure mapping experiments showed that the miR-27 binding region in HSUR1 must be available in a conformationally flexible segment for TDMD to occur (Pawlica et al. 2016). Crystal structures of miR-27 loaded into Ago2 and bound to HSUR1 showed that the complex adopts a previously unobserved conformation. The extensive complementarity of HSUR1 to the 3′ portion of miR-27 promotes the widening of Ago2's central RNA binding cleft and the release of the miRNA 3′ end from its binding pocket (the PAZ domain) in Ago2. This conformational change was initially postulated to make the miRNA available for enzymatic attack; however, recent evidence suggests that this may not be the case (see below). Similar results were obtained for other pairs of miRNAs and TDMD targets, suggesting this is a general principle in TDMD (Sheu-Gruttadauria et al. 2019).

Tailing and 3′ to 5′ exonucleolytic trimming of the miRNA has been described to occur in most examples of TDMD. This correlation led to the assumption that these two processes were essential for TDMD (Ameres et al. 2010; Sheu-Gruttadauria et al. 2019). However, the search for evidence of tailing as a necessary step in TDMD has been so far unsuccessful, and the cellular enzymes responsible for miRNA decay in TDMD have remained unknown until very recently. Surprisingly, two recent reports have implicated a ubiquitin E3 ligase-substrate adaptor associated with proteolysis, not RNA degradation, in TDMD (Han et al. 2020; Shi et al. 2020). These studies utilized cells naturally expressing Cyrano and a miR-7-sensitive mRNA encoding a florescent protein as reporter to perform genome-wide CRISPR-based screens. This approach led to the identification of the Cullin-RING E3 ubiquitin ligase (CRL)-substrate adaptor ZSWIM8 as a factor required for TDMD. In addition to Cyrano/miR-7, both reports showed that ZSWIM8 mediates TDMD in many of the previously described examples, including the degradation of miR-27 mediated by HSUR1 (Shi et al. 2020). Both reports suggest a model in which TDMD downregulates miRNAs by removal of Ago. According to this model, RNAs that trigger TDMD change Ago's conformation (Sheu-Gruttadauria et al. 2019). This conformational change allows binding of ZSWIM8, addition of polyubiquitin chains, and degradation of Ago by the proteasome, leaving the miRNA unprotected and accessible to nucleases (Fig. 2). Therefore, TDMD destroys the miRNA indirectly. Interestingly, knockout of ZSWIM8 orthologs in mouse and *Drosophila* results in upregulation of several miRNAs, indicating that TDMD is a mechanism that is conserved in diverse species, and that many more TDMD-triggering RNAs remain to be identified.

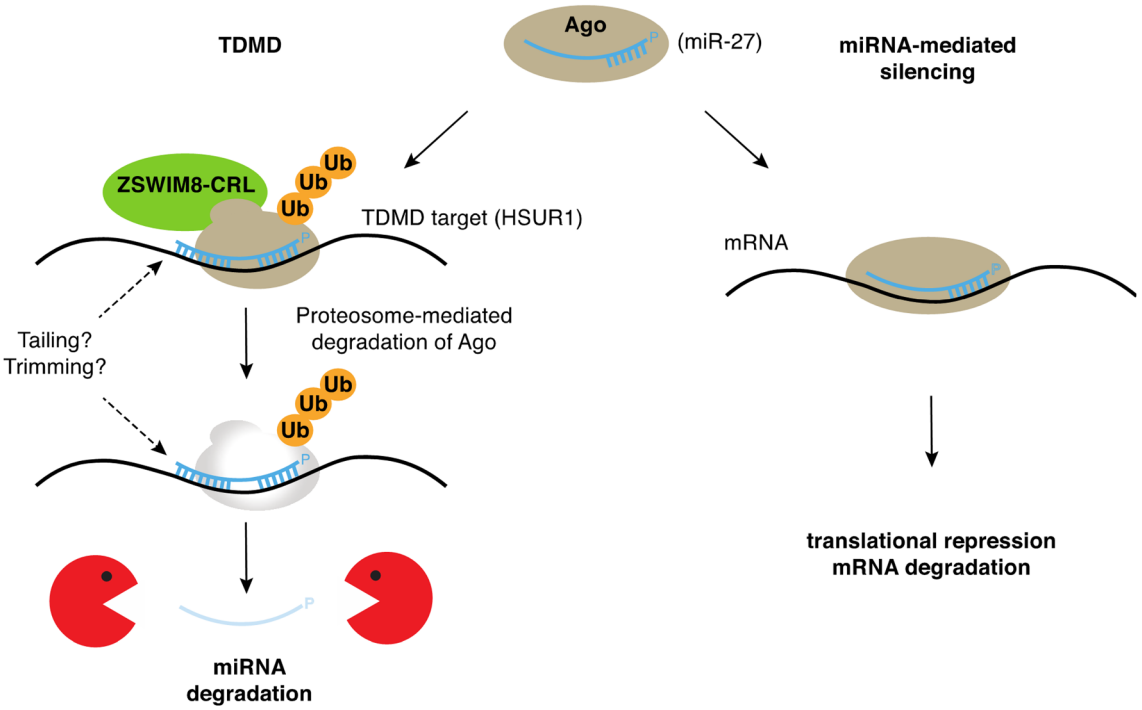

Fig. 2 Target-directed miRNA degradation. In canonical miRNA-mediated silencing of target mRNAs, the interaction involves primarily the seed region of the miRNA and results in mRNA repression. In TDMD, the target RNA (HSUR1) interacts extensively with the seed and 3′ regions of the miRNA (miR-27). This type of interaction results in a conformational change in Ago that exposes the 3′ end of the miRNA to enzymes responsible for tailing and trimming and allows binding of ZSWIM8-CRL, addition of polyubiquitin chains, and degradation of Ago by the proteasome, with the subsequent degradation of the miRNA

As it happened many times in the history of molecular biology, a virus served as a "window to the cell." The study of HSUR1 led to the discovery of a widespread cellular mechanism to regulate miRNA populations. Likewise, the search for functions for HSUR2:miRNA interactions led to the discovery of a new mechanism to regulate gene expression.

HSUR2: the miRNA adaptor

miR-142-3p and miR-16 interact with HSURs exclusively through their seed region (Fig. 1) and their abundance is not affected in HVS-transformed T cells (Cazalla et al. 2010). Other classes of ncRNAs, for example circular RNAs (circRNAs), can bind miRNAs to function as miRNA decoys or sponges (Hansen et al. 2013; Memczak et al. 2013). Efficient miRNA sponges are expressed at higher levels than, and in the same subcellular compartment as, the target miRNA. miRNAs localize mostly to the cytoplasm and HSUR2 is found mainly in the nucleus; also, HSUR2 is less abundant than miR-16 and miR-142-3p (Gorbea et al. 2017). These observations argued against the possibility that HSUR2 functions as a sponge for these two host miRNAs. So, why does HSUR2 bind host miRNAs? The puzzle was solved with the discovery that HSUR2 functions as a miRNA adaptor that utilizes these two host miRNAs to regulate host gene expression. HSUR2 directly interacts with host mRNAs and tethers miR-16 and miR-142-3p to downregulate the expression of HSUR2-bound mRNAs (Gorbea et al. 2017). This is the first example of a ncRNA functioning as a miRNA adaptor that uncovers an entirely new, indirect mechanism by which miRNAs can inhibit mRNA function. Also, HSUR2 provides the second example of an snRNA functioning outside pre-mRNA processing.

Using this mechanism, HVS decreases the abundance of multiple host mRNAs encoding proteins that play important roles in processes that viruses modulate during infection. HSUR2 targets include genes involved in apoptosis (e.g., *FAS*), cell cycle progression (e.g., *RB1*), and immune response (e.g., *IFNγ*). HSUR2 associated with actively translated polyribosomes, indicating that HSUR2 remains associated with mRNAs after the first round of translation as it has been described for miRNAs (Maroney et al. 2006; Nottrott et al. 2006). This observation suggested that HSUR2 binds primarily 3′UTRs of target mRNAs. Since HSUR2 is mostly nuclear (Chou et al. 1995), it is likely that HSUR2 binds to target mRNAs in the nucleus and travels to the cytoplasm bound to target mRNAs. Once in the cytoplasm, it can bind host miRNAs miR-142-3p and miR-16, tethering them

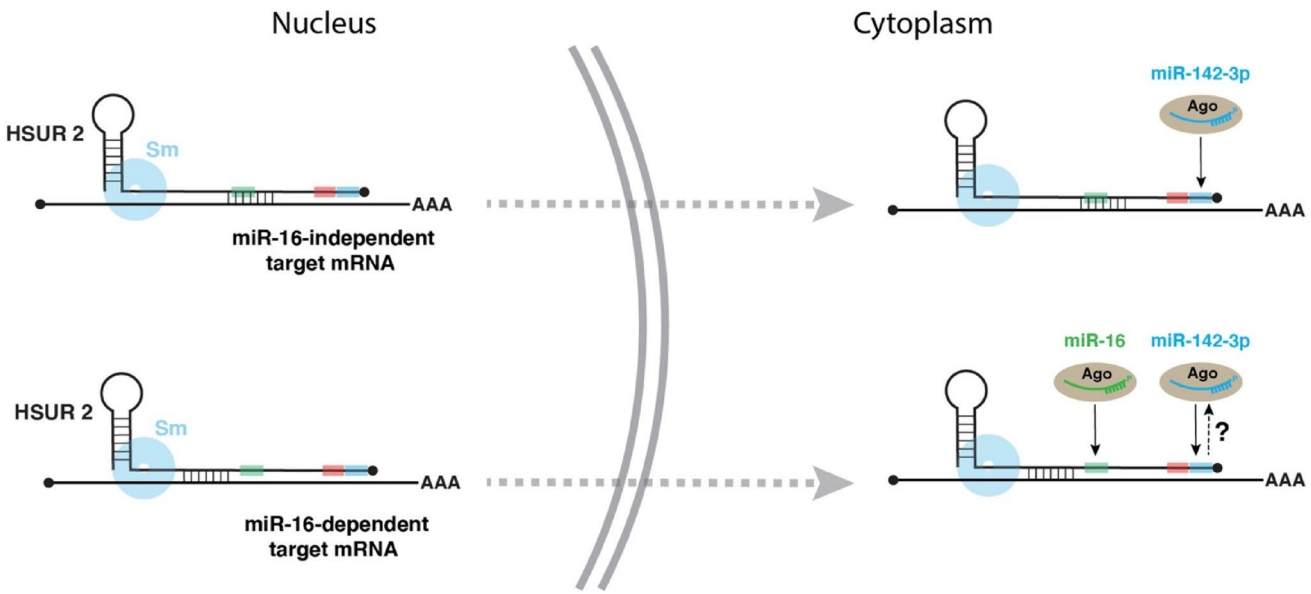

Fig. 3 HSUR2-mediated mRNA silencing. HSUR2 basepairs with target mRNAs in the nucleus and travels to cytoplasm bound to the target mRNA. miR-16-independent targets basepair with the miR-16 binding site (green box) in HSUR2, allowing the tethering of miR-142-3p only. miR-16-dependent target basepair with HSUR2 with sequences that exclude the miR-16 binding sites, allowing the recruitment of miR-16 for mRNA repression. In these cases, interaction with miR-142-3p is also required for HSUR2 function; however, it is not known if miR-142-3p remains bound to HSUR2 and is used for mRNA repression

to the bound target mRNA (Fig. 3). In addition to HSUR2 association with polyribosomes, this working hypothesis is supported by the observation that only small fractions of HSUR2, miR-16, and miR-142-3p are associated in marmoset T cell extracts (Cazalla et al. 2010). Further experimentation will be required to test this model.

Marmoset T cells transformed with a mutant version of HVS deleted of HSUR2 grow slower than cells transformed with wild-type virus (Murthy et al. 1989). This phenotype could be explained at least in part by the observation that these cells undergo apoptosis more often than cells transformed with wild-type HVS (Gorbea et al. 2017). Ectopic expression of HSUR2 renders cells resistant to apoptosis, a phenotype that requires binding of HSUR2 to both miR-142-3 and miR-16 (Gorbea et al. 2017). This report also provided the first example of a virus using such a mechanism to regulate apoptosis during infection.

The discovery of HSUR2's function posed a mechanistic conundrum: how can HSUR2 simultaneously target multiple seemingly unrelated mRNAs? To answer this question, it was required to determine the sequences mediating interactions between HSUR2 and target mRNAs. The task of accurately predicting RNA–RNA interactions is hard even when the binding properties of the RNA of interest are known. For example, despite the large amount of information accumulated over the years regarding miRNA-target interactions, bioinformatic searches for miRNA targets usually yield a high number of false-positive predictions that are not supported by experimental data (Oliveira et al. 2017). Without any knowledge of the binding properties of HSUR2, it was practically impossible to make predictions about how this viral snRNA could interact with its target mRNAs. Thus, an unbiased, biochemical approach to determine RNA–RNA interactions was required to determine how HSUR2 binds to target mRNAs.

Most available methods (Aw et al. 2016; Cai et al. 2020; Lu et al. 2016; Nguyen et al. 2016; Sharma et al. 2016) that determine RNA–RNA interactions rely on *in* vivo psoralen crosslinking, a proximity ligation approach (Kudla et al. 2011) and use total or ribosomal RNA-depleted RNA samples to generate chimeric sequences used to infer RNA–RNA interactions. These approaches presented two caveats that made them unsuitable for determining HSUR2:mRNA interactions: (1) the number of chimeric sequences indicating possible RNA–RNA interactions strongly correlates with the abundance of the RNA species, dramatically lowering the chances of obtaining a comprehensive list of RNA–RNA interactions for low-abundance RNAs like HSUR2 and its target mRNAs; and (2) to sequence chimeric reads, crosslinks need to be reversed before preparation of libraries, thus losing the only piece of information that distinguishes RNA–RNA interactions that occur in vivo from interactions that occur in the test tube and are present during the RNA ligation step required to generate chimeric RNAs. To circumvent these caveats, a general method for individual-nucleotide resolution RNA–RNA interaction identification

by crosslinking and capture (iRICC) was developed (Gorbea et al. 2019). iRICC relies on psoralen-mediated in vivo crosslinking of base-paired RNAs and reverse-transcriptase stalling for detection of residues involved in psoralen-mediated crosslinking. Furthermore, iRICC is designed to obtain a comprehensive list of RNA binding partners of a single RNA of interest, allowing the study of low-abundance RNA species. This approach is conceptually similar to the CLIP-seq family of methods that transformed the way the field studies RNA–protein interactions during the last decade (Lee and Ule 2018).

iRICC identified binding sites for HSUR2 and confidently determined the sequences mediating HSUR2:mRNA interactions. HSUR2 binding sites reside mostly in the 3′UTRs of target mRNAs. This was expected since HSUR2's main function is to deliver miRNAs to mRNAs, and most miRNA binding sites also occur at 3′UTRs (Chi et al. 2009; Hafner et al. 2010). A quarter of HSUR2 binding sites were in the coding sequence of target mRNAs and less than 2% of interactions with the 5′UTR of target mRNAs. It is unclear why HSUR2 binds these two regions of mRNAs. One possibility is that HSUR2 further downregulates mRNA expression by recruiting miRNAs to coding sequences (Brummer and Hausser 2014; Fang and Rajewsky 2011; Schnall-Levin et al. 2010). Alternatively, HSUR2 could regulate target mRNAs through miRNA-independent mechanisms when binding to coding sequences and 5′UTRs.

A luciferase-based reporter system was used to validate the novel, uncharacterized interactions found by iRICC. Mutational analyses confirmed all interactions tested, emphasizing the power of iRICC to identify novel, uncharacterized, biologically-relevant RNA–RNA interactions in vivo. Perhaps the most surprising finding is that HSUR2 does not present a "seed" or specialized region to interact with most targets, but rather, acts as a flexible adaptor that interacts through different base-pairing arrangements with different mRNAs, explaining how HSUR2 can target multiple unrelated transcripts (Gorbea et al. 2019). One possible advantage of this flexible mode of binding is that it makes it hard for the host to escape regulation by HSUR2. Seed-based RNA regulatory systems are not permissive to mismatches in the seed regions (Bartel 2018; Chipman and Pasquinelli 2019; Gorski et al. 2017; Kunne et al. 2014) and single mismatches in the seed region of miRNAs can dramatically reduce repression (Chandradoss et al. 2015; Salomon et al. 2015; Schirle et al. 2014). Thus, a single point mutation can allow the host to escape viral miRNA-based regulation, but it would not allow it to escape the regulation exerted by ncRNAs like HSUR2.

The data obtained by iRICC could also partially account for a previously unexplained observation. The interaction between HSUR2 and miR-142-3p is required for repression of all targets of HSUR2 tested, but the interaction between HSUR2 and miR-16 is required for repression of a subset of targets only (Gorbea et al. 2017, 2019). iRICC revealed that some HSUR2:mRNA interactions involve the region of HSUR2 that is complementary to miR-16 (highlighted in green, Fig. 1) and, in those cases, miR-16 is not required for inhibition. Mutagenesis of HSUR2 binding sites demonstrated that HSUR2 can indeed recognize multiple base-pair arrangements within the same target mRNA, and that the arrangement of base pairs determines if HSUR2 utilizes miR-16 for target repression. Interestingly, miR-16-dependent and miR-16-independent targets are repressed by HSUR2 to the same extent, indicating that miR-142-3p and miR-16 do not work in an additive manner in HSUR2-mediated mRNA repression (Gorbea et al. 2019).

As it happened with HSUR1, the study of HSUR2 led to the discovery of a new, indirect way in which miRNAs can be used to regulate gene expression. Interactions between different classes of ncRNAs and miRNAs are usually interpreted within a paradigm in which miRNA function is affected (Yamamura et al. 2018). The discovery that ncRNAs can work as miRNA adaptors provides an alternative to this paradigm. The study of HSUR2 has also led to the development of a powerful technique to determine RNA–RNA interactions in vivo that provided insights into the mechanism of action of this surprising viral snRNA.

Outstanding questions

Several mechanistic aspects of HSUR2 function remain unknown. For example, it is unclear how HSUR2 can specifically recognize binding sites in target mRNAs without using a seed region. Although base-pairing is required for repression (Gorbea et al. 2019), HSUR2's flexibility in base-pair arrangements indicate that potential sequence complementarity is not enough to specifically select target mRNAs. One possibility is that, like cellular snRNPs that rely on *cis*-acting signals like splicing enhancers and *trans*-acting factors like SR proteins for recruitment to the correct binding sites during splicing (Kohtz et al. 1994; Lavigueur et al. 1993; Roscigno and Garcia-Blanco 1995; Sun et al. 1993; Tarn and Steitz 1995; Tian and Maniatis 1993), additional *cis*- and *trans*-acting factors may assist HSUR2 in recognition of binding sites in target mRNAs.

The molecular mechanisms underlying the use of miR-142-3p and miR-16 in HSUR2-mediated mRNA repression are not fully understood either. While miR-16 is required for repression of only a subset of HSUR2 target mRNAs, miR-142-3p is required for repression of all targets tested so far. The use of luciferase-based reporter allowed for quantification of HSUR2-mediated repression and clearly showed that these two miRNAs are not used in a mutually exclusive or additive manner (Gorbea et al. 2019). These

observations could be explained by an allosteric activation model in which binding of miR-142-3p to HSUR2 promotes an "active" state of HSUR2, and, after allosteric activation, miR-142-3p could then also be used for repression (i.e., destabilization) of target mRNAs depending on the specific arrangement of base pairs between HSUR2 and the target mRNA. If HSUR2 binds to the target mRNA in a way that does not engage the miR-16 binding site (Fig. 3), then miR-16 is preferred over miR-142-3p and used for repression. Thus, in certain cases, binding of miR-142-3p is required for allosteric activation of HSUR2, but miR-16 is required for the actual repression. If HSUR2 cannot recruit miR-16 because its binding site is engaged in the interaction with the target mRNA, then miR-142-3p is used for both allosteric activation and repression of the target mRNA (Fig. 3). Further experimentation will be required to test the fascinating possibility that a miRNA functions as an allosteric regulator of HSUR2 function.

Another outstanding question is why HSUR2 evolved to specifically bind miR-142-3p and miR-16 to repress mRNAs. miR-142-3p is highly expressed in hematopoietic tissues and is important for normal hematopoietic lineage development and differentiation (Kramer et al. 2015; Lu et al. 2013; Nimmo et al. 2013) but is largely absent in non-hematopoietic tissues (Chen et al. 2004). HVS establishes latency in T cells (Johnson and Jondal 1981a, b; Cazalla et al. 2011; Kiyotaki et al. 1986) where miR-142-3p is abundantly expressed (Cazalla et al. 2011). Since miR-142-3p activity is required for repression of all HSUR2 targets, it is conceivable that HSUR2 would not be able to modulate host gene expression in tissues where this miRNA is absent. This mechanism could at least partially explain why HVS establishes latent infections in lymphoid cells. It would be interesting to test if mutant versions of HSUR2 engineered to bind other miRNAs can repress mRNAs in cell types where miR-142-3p is missing.

The miR-16 family of miRNAs regulates the cell cycle by modulating the expression of genes with important roles in the G1-S transition including those encoding cyclins D1, D2, D3, E1, and CDK6 (Bonci et al. 2008; Cimmino et al. 2005; Liu et al. 2008). Accordingly, overexpression of miR-16 induces G1 arrest (Liu et al. 2008). In addition, the abundance of miR-16 family members is dynamically regulated throughout the cell cycle, with highest levels of expression when cells arrest in G0 (Rissland et al. 2011). HSUR2 directly binds and regulates the mRNA encoding pRb, the master regulator of the G1-S transition, in a miR-16-dependent manner to modulate pRb levels in infected cells (Gorbea et al. 2017). One intriguing possibility is that HSUR2 binds a cycling miRNA like miR-16 to more effectively downregulate pRb levels when the abundance of miR-16 family members increases, i.e., when the infected cell increases the levels of miR-16 family members to arrest in G0. By lowering pRb levels when miR-16 family members are more abundant, HSUR2 could force the cell to go through the G1-S checkpoint even if levels of G1-S cyclins and CDKs are low.

HSUR1 binds miR-142-3p without affecting its abundance or activity (Cazalla et al. 2010) and carries an active ARE that regulates HSUR1 levels (Fan et al. 1997), but that is not required for miR-27 degradation (Cazalla et al. 2010). Why does HSUR1 bind miR-142-3p and ARE-BPs? One exciting possibility is that, in addition to its function as a TDMD-triggering RNA that regulates miR-27 levels, HSUR1 also functions as a miRNA and ARE-BPs adaptor that binds target mRNAs to tether miRNAs and ARE-BPs to regulate their expression. Finally, it would be interesting to know if other viruses or host cells also express ncRNAs like HSUR2 that can function as miRNA adaptors. What is certain is that these fascinating viral snRNAs promise to continue teaching us exciting new RNA biology in the future.

Acknowledgements I would like to thank Carlos Gorbea for critical commentary and discussions.

Declarations

Conflict of interest The author declares that no competing interests exist.

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