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REVIEW ARTICLE

Interactions between short and long noncoding RNAs

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It is now evident that noncoding RNAs play key roles in regulatory networks determining cell fate and behavior, in a myriad of different conditions, and across all species. Among these noncoding RNAs are short RNAs, such as MicroRNAs, snoRNAs, and Piwi-interacting RNAs, and the functions of those are relatively well understood. Other noncoding RNAs are longer, and their modes of action and functions are also increasingly explored and deciphered. Short RNAs and long noncoding RNAs (lncRNAs) interact with each other with reciprocal consequences for their fates and functions. LncRNAs serve as precursors for many types of small RNAs and, therefore, the pathways for small RNA biogenesis can impinge upon the fate of lncRNAs. In addition, lncRNA expression can be repressed by small RNAs, and lncRNAs can affect small RNA activity and abundance through competition for binding or by triggering small RNA degradation. Here, I review the known types of interactions between small and long RNAs, discuss their outcomes, and bring representative examples from studies in mammals.

Keywords: ceRNA; long noncoding RNAs microRNA; piRNA; post-transcriptional regulation; TDMD

Studies profiling transcription on a genome-wide level over the past 15 years showed that regions between protein-coding genes are frequently transcribed into RNA molecules of various lengths [1–5]. In addition, protein-coding genes are alternatively spliced and produce a variety of isoforms, some of which are unlikely to encode functional proteins. The majority of stable noncoding RNAs that are > 200 nt are capped, spliced, and polyadenylated, and are collectively called long noncoding RNAs (lncRNAs) [6]. A minority of lncRNAs are processed into smaller RNAs that carry out relatively well-defined functions in cells, such as MicroRNAs (miRNAs), piRNAs, siRNAs, and snoRNAs [7–9]. Other types of small noncoding RNAs, such as snRNAs and tRNAs are typically transcribed independently [10]. Small RNAs are usually recognized on the basis of specific sequences and RNA structures by various proteins and form ribonucleoprotein (RNP) complexes. For example, the Microprocessor complex and Dicer recognize short motifs and structural elements in pri- or pre-miRNAs at various stages of processing and Dicer assists loading of the processed miRNA duplex into the RISC complex [7]. Many of these RNPs are then guided by the small RNA to other RNAs that carry short regions of sequence complementarity. For example, miRNAs guide RISC to RNAs containing seed matches (defined by complementarity to positions 2–8 of the miRNA), mostly found in the 3′ UTRs of protein-coding genes [11]. snoRNAs, on the other hand, guide complexes that deposit RNA modifications, such as 2′*O*-methylation and pseudouridylation, to specific RNA targets, usually in other noncoding RNAs, such as ribosomal RNAs [12].

The functions of the vast majority of lncRNAs remain unknown, but an increasing number is

Abbreviations
ceRNAs, competing endogenous RNAs; lncRNAs, long noncoding RNAs; mCMV, murine cytomegalovirus; piRNA, Piwi-interacting RNA; PWS, Prader-Willi Syndrome; RNP, ribonucleoprotein; SPAs, 5′ snoRNA capped and 3′ polyadenylated.

implicated in a myriad of biological processes [13–16]. Some lncRNAs are differentially expressed or genetically perturbed in a variety of human diseases [17,18], which further increases the interest in understanding lncRNA functions and mechanism of action. It is clear that the presently annotated lncRNAs are composed of a number of families that utilize drastically different mechanisms, and which currently are all bundled together under the 'lncRNA' title due to our limited understanding and hence very poor classification abilities. The common modes of action that were proposed have been reviewed extensively elsewhere [6,19], and include regulation of gene expression in *cis* and in *trans*, scaffolding of subcellular domains and complexes, and regulation of protein activity and abundance. Here I focus on the interface between lncRNAs and small RNAs, and the implications of the interactions between them on their functions. Most of the examples I will present come from mammalian cells, but the principles are likely applicable to other eukaryotic species as well, as while lncRNAs evolve fast, lncRNA features are largely similar in the species that have been profiled [20]. As known interactions between various ncRNAs have been recently quite exhaustively listed elsewhere [21], I will focus here on the general principles and possible outcomes of those interactions (Fig. 1) and will not attempt to cover all reported examples.

Long noncoding RNAs as precursors for small RNAs

Short RNAs, including miRNAs and snoRNAs, are in many cases produced from introns or exons of longer 'hosts'. Some of these hosts are protein-coding genes, but many are lncRNAs. If the small RNA is processed from exonic sequence of those hosts, the processing reaction typically exposes free RNA ends that lead to rapid exonucleolytic degradation of the host. When the small RNA is excised from an intron, the host RNA stability is typically not affected. Recent studies have assigned small RNA-independent functions for hosts of snoRNAs [22–25] and miRNAs [26–28]. In some cases, like *H19*, the lncRNA function was described before a miRNA was discovered to be encoded by the lncRNA locus [29–31]. *H19* is also an intriguing example in which regulated processing of the host results in different relative abundances of the host RNA and the encoded small RNA in different cells [31]. In other cases, specific cellular decay pathways target the hosts and limit their accumulation. For example, nonsense mediated decay [32] was shown to preferentially degrade snoRNA host genes in the cytoplasm [33].

Piwi-interacting RNAs (piRNAs), small RNAs expressed primarily in the germline, are also produced in many cases from lncRNA precursors [34,35]. In some cases these lncRNAs are expressed in tissues where the Piwi pathway is not active without the lncRNAs being processed into piRNAs. It is thus likely that lncRNAs that serve as precursors for small RNAs sometimes function independently, with the processing event mediating their stability, and potentially offering an opportunity for post-transcriptional regulation of lncRNA accumulation.

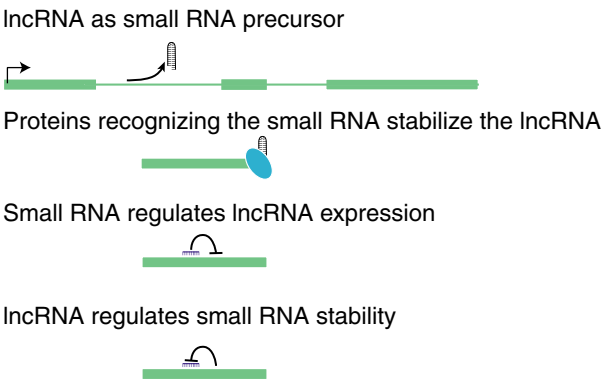

Fig. 1. Modes of possible interactions between small and long RNAs.

Small RNAs related to termini of long noncoding RNAs

The vast majority of lncRNAs are capped at their 5′ end and polyadenylated at their 3′ end, by the same complexes and proteins that process mRNAs. However, there are notable exceptions where the termini of lncRNAs are specified and/or stabilized by pathways that typically produce small RNAs. For example, the 3′ ends of the MALAT1 lncRNA and of the long isoform of the NEAT1 lncRNA are formed by cleavage by RNAse P, that is typically processing the 5′ ends of tRNAs [36]. This cleavage also specifies the 5′ end of tRNA-like small RNAs (called mascRNA in the case of MALAT1), whose functions remain unknown. The 3′ ends of MALAT1 and NEAT1 are then stabilized by triple–helical structures that include a short genomically encoded poly(A) tail [37,38].

As mentioned above, snoRNAs are occasionally encoded in introns of lncRNAs. In most cases, the intron host of the snoRNA is rapidly degraded from both ends, and the snoRNA is stabilized by proteins that form the snoRNP complex. In some cases, however, a single intron can encode two snoRNAs, and

following degradation, lncRNAs with snoRNAs in both ends are formed, denoted sno-lncRNAs [39]. These lncRNAs are stable, accumulate in the nucleus and can regulate alternative splicing globally by binding splicing regulators [39]. Interestingly, most of these cases occur in the region shown to be critical for the Prader-Willi Syndrome (PWS), and may be related to the pathogenesis of this disease. Another sno-lncRNA, called SLERT was recently shown to act in regulation of RNA Polymerase I activity though binding DDX21 [40]. In the PWS region, there are also transcripts that are 5′ snoRNA capped and 3′ polyadenylated (SPAs). These are formed when a snoRNP protects the 5′ end of the transcript, allowing RNA polymerase to continue until a polyadenylation site. SPAs are stable transcripts and they were also shown to bind several splicing regulators and regulate alternative splicing [41].

Regulation of lncRNA expression by small RNAs

As lncRNAs are largely indistinguishable from mRNAs on the molecular level, including a cap, a polyA tail, and introns, it is expected that they would be also regulated by small RNAs in the same way as mRNAs. This indeed appears to be the case, and in some systems, it was shown that such regulation has interesting consequences for the lncRNA.

In *Caenorhabditis elegans*, ALG-1 argonaute protein loaded with the let-7 miRNA binds the pri-let-7 precursor and promotes its processing, resulting in a positive feedback loop [42]. Conceptually similarly, in mammals, miR-709 localizes to the nucleus through an unknown mechanism and binds through an extensively complementary sequence to the polycistronic pri-miRNA of miR-15/16 miRNAs, inhibiting its processing [43].

Extensive complementarity between a miRNA and a lncRNA can also result in lncRNA cleavage, as first exemplified by the cleavage of the CDR1as circular RNA by miR-671 [44]. There are also numerous examples of lncRNAs that are targeted by miRNAs through conventional seed sites (recently listed in [21]), though the functional importance of these interactions remains mostly unclear. Mechanistically, regulation of lncRNA by miRNAs presumably occurs through the same pathway that acts on mRNA targets – recruitment of the cytoplasmic deadenylation complexes, followed by decapping and RNA degradation [45]. Two aspects of lncRNA biology may limit the relevance of regulation by miRNAs: lncRNAs are typically more nuclear than mRNAs [46,47], which makes them less accessible to cytoplasmic RISC complexes, and they are somewhat less stable (the observed difference in stability between lncRNAs and mRNAs is variable, largely due to differences in sets of considered lncRNAs [48–50]). Less stable RNAs are less susceptible to regulation by miRNAs [51], and so miRNAs may have limited impact on expression levels of lncRNAs as a group.

Small RNAs other than miRNAs can also regulate lncRNAs accumulation. Many lncRNAs are expressed specifically in the testis, in particular in late-stage spermatocytes [35,52,53], where the piRNA machinery is also active. The expression of hundreds of these lncRNAs is increased by more than twofold in the testis of *Piwil1*$^{-/-}$ mice which do not express piRNAs [35]. Only a minor fraction of the up-regulated lncRNAs are piRNA precursors, and the sequences of lincRNAs up-regulated in Piwil1 null mice match piRNA antisense sequences, suggesting that piRNAs directly repress some lncRNAs. Similarly, piRNAs were shown to regulate lncRNA expression in flies [54].

Regulation of small RNA activity by long noncoding RNAs

While regulation of long RNAs by small RNAs has so far received relatively limited attention in the scientific literature, the reverse activity – regulation of small RNA activity by lncRNAs, has been the subject of extensive study, and non-negligible controversy, in the last few years. The main reason for this extensive interest is the relative ease with which one can predict possible interactions between lncRNAs and miRNAs, and the considerable understanding of the functions and targets of individual miRNAs. Therefore, when one is faced with the formidable problem of hypothesizing a mode of action or the regulatory targets of a lncRNA, it is often appealing to propose that the lncRNA regulates a particular pathway through binding and affecting the activity of a miRNA. Regulation of miRNA stability by lncRNAs is also an appealing mode of action, since turnover of miRNAs remains quite poorly understood. On the one hand, miRNAs are typically very stable [55], presumably protected from general RNA decay pathways by the Argonaute proteins. On the other hand, developmental transitions and response to stimuli sometimes result in abrupt down-regulation of some miRNAs [56–58], suggesting active and specific turnover, and making target-dependant decay an attractive possibility.

The interest in lncRNAs as potential 'competing endogenous RNAs' (ceRNAs) increased in 2010 following a report from the Pandolfi lab that *PTENP1*, a

transcribed pseudogene of the *PTEN* tumor suppressor, can compete with *PTEN* mRNA for binding of miRNAs [59]. This report also began the skepticism about this phenomenon, as PTENP1 is expressed at much lower levels than PTEN [60], and has to complete for miRNA binding not just with PTEN, but also with tens of thousands of other binding sites each miRNA has throughout the transcriptome (Fig. 2). Since individual miRNA-binding sites confer limited repression, it has been proposed that multiple shared sites result in more efficient crosstalk [61,62], but this does not resolve the stoichiometric concerns about the 'ceRNA hypothesis'. Several recent studies used theoretical and experimental tools to try and address the question of what magnitude of changes in abundance of a single RNA species are required for affecting expression of other genes through competition for binding of short RNAs. Jens and Rajewsky [63] estimated ~ 22 700 binding sites compete for miR-20a binding in unperturbed monocytes. Under these conditions, thousands of new binding sites need to be introduced for meaningfully altering the occupancy of miR-20a on any of its targets. Indeed, artificial 'miRNA sponges' introducing such numbers of sites were shown to lead to increases in levels of individual targets without markedly affecting miRNA expression levels [64]. In stark contrast, changes in expression of endogenous genes, in particular the typically lowly expressed lncRNAs, almost never reach levels that are predicted to have regulatory impact via simple competition for binding.

Consistent with these predictions, an experimental study in mouse liver and hepatocytes [65] found that target overexpression that effectively doubles by the number of available binding sites in the transcriptome is needed for detectable changes in gene expression by competition. Specifically, for miR-122, which is expressed at 1.2×10^5 copies per cell in the liver, addition of at least ~ 200 000 of copies of the *AldoA* target, which contains three potent-binding sites for miR-122, was required for detectable up-regulation of miR-122 targets without affecting miR-122 levels. Similar results were obtained when miR-122 was reduced by ~ 3-fold using antagomiRs, suggesting that miRNA levels are less important for the threshold of expression above which competition becomes observable [65]. In an *in vivo* setting, 20-fold increase in AldoA levels, adding thousands of new binding sites, did not have any detectable effect on miR-122 target expression levels [65]. These findings were recently corroborated in a follow-up study using mouse embryonic stem cells (mESCs) as an additional system and testing other miRNAs with different abundance ranges [66], supporting the concept that the threshold above which a ceRNA can start to influence abundance of other miRNA targets is determined not by miRNA abundance, but rather by the total number of miRNA-binding sites, including low-affinity ones, throughout the transcriptome.

Perhaps the most striking candidate for an endogenous 'miRNA sponge' is the CDR1as circular lncRNA that contains > 60 binding sites for the miR-7 miRNA, and is expected to be resilient to repression by miR-7 due to its circular structure [67,68]. CDR1as indeed acts as a miR-7 sponge in artificial settings [67,68], but loss of CRD1as in mice is surprisingly associated with decrease, rather than increase, in miR-7 levels in the brain, and with increased levels of miR-7 targets [69], suggesting that even the abundant CDR1as with its dozens of high affinity sites for a single miRNA, likely does not act as a miRNA sponge in the endogenous

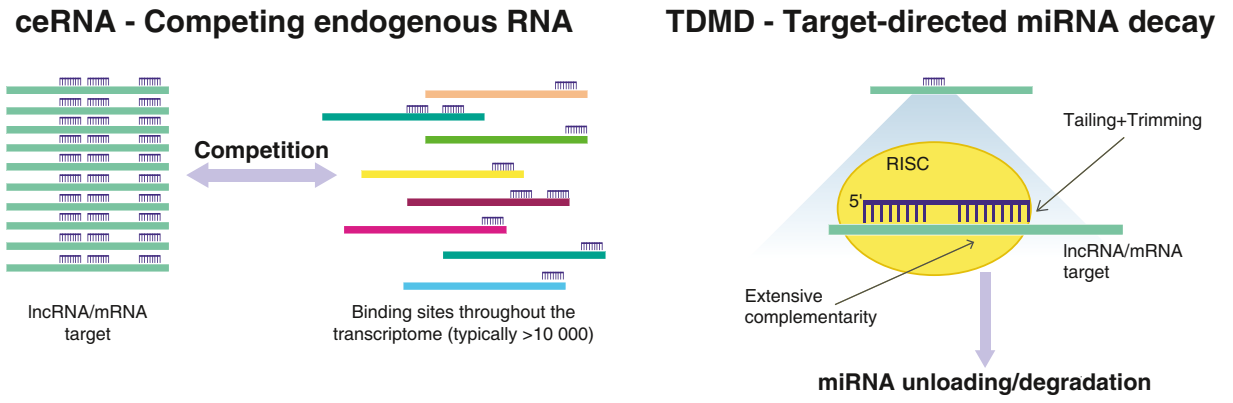

Fig. 2. Left: schematic illustration of the ceRNA activity, in which a single target (potentially with multiple binding sites) needs to be very abundant to effectively compete with many other miRNA-binding sites throughout the transcriptome. Right: schematic illustration of TDMD, in which a target harboring a highly complementary miRNA-binding site triggers miRNA decay.

setting. The reduction in miR-7 expression in CDR1as null mice could be related to the activity of Cyrano lncRNA, as described below.

Despite the doubt cast on the prevalence of ceRNA activity, there is a rapidly growing number of studies reporting ceRNA effect of individual lncRNAs. Most of these studies are performed in cancer cell lines. For example, as of March 2018, there are at least 17 studies reporting ceRNA activity of PVT1 [70–86], a moderately abundant lncRNA, that is almost exclusively nuclear [87,88], and therefore not expected to effectively bind miRNAs. Strikingly, these studies collectively implicate 10 different miRNAs as being 'sponged' by PVT1. The experimental evidence in such studies is typically limited to over-expression of the competitor (which typically pushes its levels way above the physiologically relevant levels), or knockdown followed by qRT-PCR of selected targets, which is typically difficult to interpret, as changes in expression can result from other, miRNA-unrelated effects [63]. The suggested 'gold-standard' for proving ceRNA activity has been editing of endogenous miRNA target sites (e.g., using CRISPR/Cas9) [63,66] and comprehensive evaluation of the effect on other targets (e.g., by RNA-seq followed by Sylamer analysis [89], testing for specific de-repression of the miRNA targets), but to the best of my knowledge, such experiments have not yet been performed for any ceRNA candidate.

LncRNAs that degrade miRNAs through extensively complementary-binding sites

One way through which a relatively non-abundant lncRNA can nevertheless regulate the activity of typically more abundant miRNAs is through binding sites with special sequences or pairing topology, that would trigger miRNA degradation upon binding [90]. Indeed, the first example of lncRNAs acting on a miRNA was the IPS1 lncRNA in plants that binds the phosphate starvation-induced miR-399 through an extensively complementary, yet uncleavable binding site [91]. This activity leads to up-regulation of PHO2, which is an endogenous target of miR-399. The same mechanism was used to design inhibitors for other plant miRNAs [91].

Although animal miRNAs typically do not act through target cleavage and rarely have extensive complementarity with their targets, there is accumulating evidence that such target sites can efficiently affect miRNA accumulation in animal cells. In 2010, Phil Zamore and colleagues have shown through experiments in flies that binding of miRNAs loaded in Ago1 to targets with extensive sequence complementarity triggers tailing of the miRNA with non-templated nucleotides (mostly adenines and uridines), miRNA trimming, and eventual miRNA degradation [92], a phenomenon referred to as target RNA-directed miRNA degradation, or TDMD (Fig. 2). Similar results were shown in HeLa cells *in vitro* [92]. Artificial constructs containing highly complementary miRNA-binding sites were shown to direct efficient miRNA destruction in liver cells and mouse neurons, which was also correlated with tailing and trimming of the miRNA [93,94].

Recent studies have described endogenous targets that cause strong TDMD through extensively complementary sites. The lab of Alena Shkumatava found that a conserved RNA region, part of the *libra* lncRNA in fish and of the 3′ UTR of *Nrep* protein-coding gene in mammals [95,96], binds and degrades miR-29b. This has functional consequences *in vivo*, as animal behavior is altered in zebrafish and mouse mutants where this binding site is lost [96]. A preprint from the Bartel lab [97] describes similar activity by the highly conserved *Cyrano* (*OIP5-AS1*) lncRNA, which harbors an extensively complementary binding site for miR-7 [95] (an additional recent study suggested that Cyrano inhibits miR-7 also in mESCs [98], but changes in miR-7 abundance were not demonstrated in those cells).

There appear to be numerous parallels between the TDMD caused by *Nrep* and *Cyrano*. Both RNAs are quite abundant and predominantly cytoplasmic [96–98] and both contain unusually complementary sites – the highly conserved region in *Cyrano* contains an 8mer pairing to the 5′ of miR-7 and another 13 bases pairing to its 3′, thus pairing with all bases of miR-7 except 9 and 10 (Fig. 3) [95,97]. *Nrep*/*libra* conserved region pairs with 11 bases at the 5′ end of miR-29 and nine bases at the 3′ end, thus binding all bases of

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Cyrano   AAGAACAACAAAAUCACCAAUGUCUUCCAUU 3′
           ||||||||||||||  ||||||||||
miR-7      UUGUUGUUUUAGUG--AUCAGAAGGU   5′

Nrep     UGAGACACUGAU---GAAUGGUGCUAUUUU 3′
           |||||||||   ||||||||||
miR-29b    UUGUGACUAAAGUUUACCACGAU     5′

UL114-145 AATUCCUGCACUAAAAAAAGAAGCACUUUACG 3′
              ||||||||       ||||||||||
miR-17     GAUGGACGUGACA-----UUCGUGAAAAC
miR-20a    GAUGGACGUGAUA-----UUCGUGAAAAC 5′
```

Fig. 3. Base pairing patterns between targets (top) and miRNA (bottom) that result in TDMD. The seed pairing is highlighted in bold.

miR-29b except 12–14 (Fig. 3). These binding sites lead to very efficient mRNA degradation. Scrambling of the miR-29b-binding site in *Nrep* leads to a sharp increase in miR-29b levels in the cerebellar granule cell layer in mice, and ~ 5-fold increase of miR-29b in *in vitro* differentiated neuronal progenitors [96]. Loss of *Cyrano* or small changes in the seed of the miR-7-binding site leads to a > 40-fold increase in mature miR-7 levels in the mouse cerebellum and appreciable increases in other tissues where miR-7 is expressed, as well as in cultured neurons from *Cyrano*-deficient animals [97]. In both cases, pri- or pre-miRNA levels are not affected. The activity of *Cyrano* appears much more efficient than other described examples of TDMD when copy numbers are considered, as a single molecule of *Cyrano* accounts for loss of ~ 17 molecules of miR-7, presumably because of other elements in this lncRNA, or because of the specific neuronal context in which it is active [97]. In any case, consistent with previous results [93], a target with an extensively complementary binding site can cause degradation of multiple miRNA molecules. *Cyrano* activity is associated with tailing and trimming of miR-7, though tailing does not appear to contribute to trimming or miR-7 degradation [97]. *Nrep* is required for miR-29b trimming (no substantial tailing was observed), and it is not clear if this trimming is needed for miR-29b degradation [96].

Interestingly, the main consequence of *Cyrano* loss is reduction in the levels of Cdr1as, a circular RNA, which as mentioned above harbors a large number of miR-7 sites. We observed a similar reduction with transient knockdown of Cyrano using siRNAs in SH-Y5Y cells (H. Hezroni and I. Ulitsky, unpublished results). The mechanism through which this reduction in *Cdr1as* occurs is still largely unclear, but it appears to involve miR-671 that cleaves Cdr1as [97].

TDMD is also used by some viral ncRNAs, including the *Herpesvirus saimiri* HSUR1 ncRNA and the murine cytomegalovirus (MCMV) m169 mRNA both containing binding sites for miR-27 that trigger miRNA degradation [99–101]. These degradation processes also trigger trimming and tailing of miR-27. Similarly, the human CMV UL144–145 transcript causes degradation of miR-17 and miR-20a though an extensively complementary binding site (Fig. 3) [102].

The prevalence of TDMD by endogenous mammalian lncRNAs remains unclear, but its likely rare, as efficient TDMD requires both seed matching and extensive 3′ complementarity [93,97] which is exceedingly rare. The endogenous transcripts shown to cause TDMD indeed both have binding sites conserved throughout vertebrates, with perfect conservation of at least 8 bases complementary to the 3′ end of the mRNA and extensive sequence conservation outside of the miRNA-binding site. Further, TDMD appears to be much more efficient in primary neurons than in other cell types [93,97], though effects are seen in other tissues as well [97], and the reasons underlying the preference for neurons are not known. Therefore, the vast majority of miRNA-binding sites in lncRNAs are not expected to trigger TDMD.

Conclusions and future prospects

As miRNAs and related small RNAs are already known to act in virtually every biological process in mammalian cells, and the spread of lncRNA influence is also increasing, it is likely that we will also see a dramatic increase in the known interactions between members of these two RNA classes. As lncRNAs are in general very similar in their structure and modifications to mRNAs, the modes and outcomes of their interactions with small RNAs also resemble those already seen with mRNAs, and indeed, none of the examples presented here, be it TDMD or cleavage by piRNAs appear to be unique to lncRNAs. As mentioned above, lncRNAs and mRNAs differ in their average abundance, stability, and localization, and these properties may affect the prevalence of their interactions with small RNAs, but it is important to keep in mind that there are thousands of lncRNAs that closely resemble mRNAs in each of those properties. Thus, the small-long RNA network, that is just now beginning to be uncovered, is expected to remain a vibrant and fertile ground for future discoveries, and potentially even therapeutic interventions in a wide array of contexts.

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References

1 Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J,

Hofacker IL *et al*. (2007) RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* **316**, 1484–1488.
2 Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, Nikaido I, Osato N, Saito R, Suzuki H *et al*. (2002) Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* **420**, 563–573.
3 Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP *et al*. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* **458**, 223–227.
4 Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S *et al*. (2015) The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet* **47**, 199–208.
5 Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG *et al*. (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res* **22**, 1775–1789.
6 Ulitsky I and Bartel DP (2013) lincRNAs: genomics, evolution, and mechanisms. *Cell* **154**, 26–46.
7 Bartel DP (2018) Metazoan MicroRNAs. *Cell* **173**, 20–51.
8 Luteijn MJ and Ketting RF (2013) PIWI-interacting RNAs: from generation to transgenerational epigenetics. *Nat Rev Genet* **14**, 523–534.
9 Dupuis-Sandoval F, Poirier M and Scott MS (2015) The emerging landscape of small nucleolar RNAs in cell biology. *Wiley Interdiscip Rev RNA* **6**, 381–397.
10 Dieci G, Fiorino G, Castelnuovo M, Teichmann M and Pagano A (2007) The expanding RNA polymerase III transcriptome. *Trends Genet* **23**, 614–622.
11 Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233.
12 Kiss T (2002) Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions. *Cell* **109**, 145–148.
13 Engreitz JM, Ollikainen N and Guttman M (2016) Long non-coding RNAs: spatial amplifiers that control nuclear structure and gene expression. *Nat Rev Mol Cell Biol* **17**, 756–770.
14 Chen YG, Satpathy AT and Chang HY (2017) Gene regulation in the immune system by long noncoding RNAs. *Nat Immunol* **18**, 962–972.
15 Perry RB-T and Ulitsky I (2016) The functions of long noncoding RNAs in development and stem cells. *Development* **143**, 3882–3894.
16 Ransohoff JD, Wei Y and Khavari PA (2018) The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol* **19**, 143–157.
17 Wapinski O and Chang HY (2011) Long noncoding RNAs and human disease. *Trends Cell Biol* **21**, 354–361.
18 Shi X, Sun M, Liu H, Yao Y and Song Y (2013) Long non-coding RNAs: a new frontier in the study of human diseases. *Cancer Lett* **339**, 159–166.
19 Schmitz SU, Grote P and Herrmann BG (2016) Mechanisms of long noncoding RNA function in development and disease. *Cell Mol Life Sci* **73**, 2491–2509.
20 Ulitsky I (2016) Evolution to the rescue: using comparative genomics to understand long non-coding RNAs. *Nat Rev Genet* **17**, 601–614.
21 Yamamura S, Imai-Sumida M, Tanaka Y and Dahiya R (2018) Interaction and cross-talk between non-coding RNAs. *Cell Mol Life Sci* **75**, 467–484.
22 Damas ND, Marcatti M, Côme C, Christensen LL, Nielsen MM, Baumgartner R, Gylling HM, Maglieri G, Rundsten CF, Seemann SE *et al*. (2016) SNHG5 promotes colorectal cancer cell survival by counteracting STAU1-mediated mRNA destabilization. *Nat Commun* **7**, 13875.
23 Christensen LL, True K, Hamilton MP, Nielsen MM, Damas ND, Damgaard CK, Ongen H, Dermitzakis E, Bramsen JB, Pedersen JS *et al*. (2016) SNHG16 is regulated by the Wnt pathway in colorectal cancer and affects genes involved in lipid metabolism. *Mol Oncol* **10**, 1266–1282.
24 Askarian-Amiri ME, Crawford J, French JD, Smart CE, Smith MA, Clark MB, Ru K, Mercer TR, Thompson ER, Lakhani SR *et al*. (2011) SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. *RNA* **17**, 878–891.
25 Kino T, Hurt DE, Ichijo T, Nader N and Chrousos GP (2010) Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal* **3**, ra8.
26 Montes M, Nielsen MM, Maglieri G, Jacobsen A, Højfeldt J, Agrawal-Singh S, Hansen K, Helin K, van de Werken HJG, Pedersen JS *et al*. (2015) The lncRNA MIR31HG regulates p16INK4A expression to modulate senescence. *Nat Commun* **6**, 6967.
27 Yang H, Liu P, Zhang J, Peng X, Lu Z, Yu S, Meng Y, Tong W-M and Chen J (2016) Long noncoding RNA MIR31HG exhibits oncogenic property in pancreatic ductal adenocarcinoma and is negatively regulated by miR-193b. *Oncogene* **35**, 3647–3657.
28 Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A and Bozzoni I (2011) A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* **147**, 358–369.
29 Dey BK, Pfeifer K and Dutta A (2014) The H19 long noncoding RNA gives rise to microRNAs miR-675-3p

and miR-675-5p to promote skeletal muscle differentiation and regeneration. *Genes Dev* **28**, 491–501.
30 Cai X and Cullen BR (2007) The imprinted H19 noncoding RNA is a primary microRNA precursor. *RNA* **13**, 313–316.
31 Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G and Reik W (2012) The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol* **14**, 659–665.
32 Chang Y-F, Imam JS and Wilkinson MF (2007) The nonsense-mediated decay RNA surveillance pathway. *Annu Rev Biochem* **76**, 51–74.
33 Lykke-Andersen S, Chen Y, Ardal BR, Lilje B, Waage J, Sandelin A and Jensen TH (2014) Human nonsense-mediated RNA decay initiates widely by endonucleolysis and targets snoRNA host genes. *Genes Dev* **28**, 2498–2517.
34 Ha H, Song J, Wang S, Kapusta A, Feschotte C, Chen KC and Xing J (2014) A comprehensive analysis of piRNAs from adult human testis and their relationship with genes and mobile elements. *BMC Genom* **15**, 545.
35 Watanabe T, Cheng EC, Zhong M and Lin H (2015) Retrotransposons and pseudogenes regulate mRNAs and lncRNAs via the piRNA pathway in the germline. *Genome Res* **25**, 368–380.
36 Wilusz JE, Freier SM and Spector DL (2008) 3′ end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. *Cell* **135**, 919–932.
37 Wilusz JE, JnBaptiste CK, Lu LY, Kuhn CD, Joshua-Tor L and Sharp PA (2012) A triple helix stabilizes the 3′ ends of long noncoding RNAs that lack poly(A) tails. *Genes Dev* **26**, 2392–2407.
38 Brown JA, Valenstein ML, Yario TA, Tycowski KT and Steitz JA (2012) Formation of triple-helical structures by the 3′-end sequences of MALAT1 and MENβ noncoding RNAs. *Proc Natl Acad Sci USA* **109**, 19202–19207.
39 Yin QF, Yang L, Zhang Y, Xiang JF, Wu YW, Carmichael GG and Chen LL (2012) Long noncoding RNAs with snoRNA ends. *Mol Cell* **48**, 219–230.
40 Xing Y-H, Yao R-W, Zhang Y, Guo C-J, Jiang S, Xu G, Dong R, Yang L and Chen L-L (2017) SLERT regulates DDX21 rings associated with Pol I transcription. *Cell* **169**, 664–678.e16.
41 Wu H, Yin Q-F, Luo Z, Yao R-W, Zheng C-C, Zhang J, Xiang J-F, Yang L and Chen L-L (2016) Unusual processing generates SPA LncRNAs that sequester multiple RNA binding proteins. *Mol Cell* **64**, 534–548.
42 Zisoulis DG, Kai ZS, Chang RK and Pasquinelli AE (2012) Autoregulation of microRNA biogenesis by let-7 and Argonaute. *Nature* **486**, 541–544.
43 Tang R, Li L, Zhu D, Hou D, Cao T, Gu H, Zhang J, Chen J, Zhang C-Y and Zen K (2012) Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: evidence for a microRNA hierarchy system. *Cell Res* **22**, 504–515.
44 Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ and Kjems J (2011) miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J* **30**, 4414–4422.
45 Jonas S and Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* **16**, 421–433.
46 Lubelsky Y and Ulitsky I (2018) Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells. *Nature* **555**, 107–111.
47 Tilgner H, Knowles DG, Johnson R, Davis CA, Chakrabortty S, Djebali S, Curado J, Snyder M, Gingeras TR and Guigo R (2012) Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs. *Genome Res* **22**, 1616–1625.
48 Mukherjee N, Calviello L, Hirsekorn A, de Pretis S, Pelizzola M and Ohler U (2017) Integrative classification of human coding and noncoding genes through RNA metabolism profiles. *Nat Struct Mol Biol* **24**, 86–96.
49 Schlackow M, Nojima T, Gomes T, Dhir A, Carmo-Fonseca M and Proudfoot NJ (2017) Distinctive patterns of transcription and RNA processing for human lincRNAs. *Mol Cell* **65**, 25–38.
50 Melé M, Mattioli K, Mallard W, Shechner DM, Gerhardinger C and Rinn JL (2017) Chromatin environment, transcriptional regulation, and splicing distinguish lincRNAs and mRNAs. *Genome Res* **27**, 27–37.
51 Larsson E, Sander C and Marks D (2010) mRNA turnover rate limits siRNA and microRNA efficacy. *Mol Syst Biol* **6**, 433.
52 Soumillon M, Necsulea A, Weier M, Brawand D, Zhang X, Gu H, Barthes P, Kokkinaki M, Nef S, Gnirke A *et al*. (2013) Cellular source and mechanisms of high transcriptome complexity in the mammalian testis. *Cell Rep* **3**, 2179–2190.
53 Davis MP, Carrieri C, Saini HK, van Dongen S, Leonardi T, Bussotti G, Monahan JM, Auchynnikava T, Bitetti A, Rappsilber J *et al*. (2017) Transposon-driven transcription is a conserved feature of vertebrate spermatogenesis and transcript evolution. *EMBO Rep* **18**, 1231–1247.
54 Sytnikova YA, Rahman R, Chirn G-W, Clark JP and Lau NC (2014) Transposable element dynamics and PIWI regulation impacts lncRNA and gene expression

diversity in Drosophila ovarian cell cultures. *Genome Res* **24**, 1977–1990.
55 Guo Y, Liu J, Elfenbein SJ, Ma Y, Zhong M, Qiu C, Ding Y and Lu J (2015) Characterization of the mammalian miRNA turnover landscape. *Nucleic Acids Res* **43**, 2326–2341.
56 Rissland OS, Hong S-J and Bartel DP (2011) MicroRNA destabilization enables dynamic regulation of the miR-16 family in response to cell-cycle changes. *Mol Cell* **43**, 993–1004.
57 Avraham R, Sas-Chen A, Manor O, Steinfeld I, Shalgi R, Tarcic G, Bossel N, Zeisel A, Amit I, Zwang Y *et al*. (2010) EGF decreases the abundance of microRNAs that restrain oncogenic transcription factors. *Sci Signal* **3**, ra43.
58 Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Richter J, Duebel J, Bicker S, Fehling HJ, Schübeler D *et al*. (2010) Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. *Cell* **141**, 618–631.
59 Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ and Pandolfi PP (2010) A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* **465**, 1033–1038.
60 Ebert MS and Sharp PA (2010) Emerging roles for natural microRNA sponges. *Curr Biol* **20**, R858–R861.
61 Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP (2011) A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? *Cell* **146**, 353–358.
62 Tay Y, Rinn J and Pandolfi PP (2014) The multilayered complexity of ceRNA crosstalk and competition. *Nature* **505**, 344–352.
63 Jens M and Rajewsky N (2015) Competition between target sites of regulators shapes post-transcriptional gene regulation. *Nat Rev Genet* **16**, 113–126.
64 Ebert MS, Neilson JR and Sharp PA (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* **4**, 721–726.
65 Denzler R, Agarwal V, Stefano J, Bartel DP and Stoffel M (2014) Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol Cell* **54**, 766–776.
66 Denzler R, McGeary SE, Title AC, Agarwal V, Bartel DP and Stoffel M (2016) Impact of MicroRNA levels, target-site complementarity, and cooperativity on competing endogenous RNA-regulated gene expression. *Mol Cell* **64**, 565–579.
67 Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M *et al*. (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* **495**, 333–338.
68 Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK and Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. *Nature* **495**, 384–388.
69 Piwecka M, Glažar P, Hernandez-Miranda LR, Memczak S, Wolf SA, Rybak-Wolf A, Filipchyk A, Klironomos F, Cerda Jara CA, Fenske P *et al*. (2017) Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. *Science* **357**, eaam8526.
70 Chang Z, Cui J and Song Y (2018) Long noncoding RNA PVT1 promotes EMT via mediating microRNA-186 targeting of Twist1 in prostate cancer. *Gene* **654**, 36–42.
71 Li H, Chen S, Liu J, Guo X, Xiang X, Dong T, Ran P, Li Q, Zhu B, Zhang X *et al*. (2018) Long non-coding RNA PVT1-5 promotes cell proliferation by regulating miR-126/SLC7A5 axis in lung cancer. *Biochem Biophys Res Commun* **495**, 2350–2355.
72 Wu D, Li Y, Zhang H and Hu X (2017) Knockdown of Lncrna PVT1 enhances radiosensitivity in non-small cell lung cancer by sponging Mir-195. *Cell Physiol Biochem* **42**, 2453–2466.
73 Chen W, Zhu H, Yin L, Wang T, Wu J, Xu J, Tao H, Liu J and He X (2017) lncRNA-PVT1 facilitates invasion through upregulation of MMP9 in nonsmall cell lung cancer cell. *DNA Cell Biol* **36**, 787–793.
74 Zhao L, Kong H, Sun H, Chen Z, Chen B and Zhou M (2018) LncRNA-PVT1 promotes pancreatic cancer cells proliferation and migration through acting as a molecular sponge to regulate miR-448. *J Cell Physiol* **233**, 4044–4055.
75 Lan T, Yan X, Li Z, Xu X, Mao Q, Ma W, Hong Z, Chen X and Yuan Y (2017) Long non-coding RNA PVT1 serves as a competing endogenous RNA for miR-186-5p to promote the tumorigenesis and metastasis of hepatocellular carcinoma. *Tumour Biol* **39**, 1010428317705338.
76 Song J, Wu X, Liu F, Li M, Sun Y, Wang Y, Wang C, Zhu K, Jia X, Wang B *et al*. (2017) Long non-coding RNA PVT1 promotes glycolysis and tumor progression by regulating miR-497/HK2 axis in osteosarcoma. *Biochem Biophys Res Commun* **490**, 217–224.
77 Li Y, Li S, Luo Y, Liu Y and Yu N (2017) LncRNA PVT1 regulates chondrocyte apoptosis in osteoarthritis by acting as a sponge for miR-488-3p. *DNA Cell Biol* **36**, 571–580.
78 Li P-D, Hu J-L, Ma C, Ma H, Yao J, Chen L-L, Chen J, Cheng T-T, Yang K-Y, Wu G *et al*. (2017) Upregulation of the long non-coding RNA PVT1 promotes esophageal squamous cell carcinoma progression by acting as a molecular sponge of miR-203 and LASP1. *Oncotarget* **8**, 34164–34176.
79 Ma Y, Wang P, Xue Y, Qu C, Zheng J, Liu X, Ma J and Liu Y (2017) PVT1 affects growth of glioma

microvascular endothelial cells by negatively regulating miR-186. *Tumour Biol* **39**, 1010428317694326.
80 Li T, Meng X-L and Yang W-Q (2017) Long noncoding RNA PVT1 acts as a "Sponge" to inhibit microRNA-152 in gastric cancer cells. *Dig Dis Sci* **62**, 3021–3028.
81 Conte F, Fiscon G, Chiara M, Colombo T, Farina L and Paci P (2017) Role of the long non-coding RNA PVT1 in the dysregulation of the ceRNA-ceRNA network in human breast cancer. *PLoS ONE* **12**, e0171661.
82 Huang T, Liu HW, Chen JQ, Wang SH, Hao LQ, Liu M and Wang B (2017) The long noncoding RNA PVT1 functions as a competing endogenous RNA by sponging miR-186 in gastric cancer. *Biomed Pharmacother* **88**, 302–308.
83 Yang S, Ning Q, Zhang G, Sun H, Wang Z and Li Y (2016) Construction of differential mRNA-lncRNA crosstalk networks based on ceRNA hypothesis uncover key roles of lncRNAs implicated in esophageal squamous cell carcinoma. *Oncotarget* **7**, 85728–85740.
84 Panda AC, Grammatikakis I, Kim KM, De S, Martindale JL, Munk R, Yang X, Abdelmohsen K and Gorospe M (2017) Identification of senescence-associated circular RNAs (SAC-RNAs) reveals senescence suppressor CircPVT1. *Nucleic Acids Res* **45**, 4021–4035.
85 Zhou Q, Chen F, Zhao J, Li B, Liang Y, Pan W, Zhang S, Wang X and Zheng D (2016) Long non-coding RNA PVT1 promotes osteosarcoma development by acting as a molecular sponge to regulate miR-195. *Oncotarget* **7**, 82620–82633.
86 Zheng J, Yu F, Dong P, Wu L, Zhang Y, Hu Y and Zheng L (2016) Long non-coding RNA PVT1 activates hepatic stellate cells through competitively binding microRNA-152. *Oncotarget* **7**, 62886–62897.
87 Cabili MN, Dunagin MC, McClanahan PD, Biaesch A, Padovan-Merhar O, Regev A, Rinn JL and Raj A (2015) Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. *Genome Biol* **16**, 20.
88 Chujo T, Yamazaki T, Kawaguchi T, Kurosaka S, Takumi T, Nakagawa S and Hirose T (2017) Unusual semi-extractability as a hallmark of nuclear body-associated architectural noncoding RNAs. *EMBO J* **36**, 1447–1462.
89 Bartonicek N and Enright AJ (2010) SylArray: a web server for automated detection of miRNA effects from expression data. *Bioinformatics* **26**, 2900–2901.
90 Figliuzzi M, Marinari E and De Martino A (2013) MicroRNAs as a selective channel of communication between competing RNAs: a steady-state theory. *Biophys J* **104**, 1203–1213.
91 Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, Rubio-Somoza I, Leyva A, Weigel D, Garcia JA and Paz-Ares J (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat Genet* **39**, 1033–1037.
92 Ameres SL, Horwich MD, Hung J-H, Xu J, Ghildiyal M, Weng Z and Zamore PD (2010) Target RNA-directed trimming and tailing of small silencing RNAs. *Science* **328**, 1534–1539.
93 de la Mata M, Gaidatzis D, Vitanescu M, Stadler MB, Wentzel C, Scheiffele P, Filipowicz W and Großhans H (2015) Potent degradation of neuronal miRNAs induced by highly complementary targets. *EMBO Rep* **16**, 500–511.
94 Xie J, Ameres SL, Friedline R, Hung J-H, Zhang Y, Xie Q, Zhong L, Su Q, He R, Li M *et al*. (2012) Long-term, efficient inhibition of microRNA function in mice using rAAV vectors. *Nat Methods* **9**, 403–409.
95 Ulitsky I, Shkumatava A, Jan CH, Sive H and Bartel DP (2011) Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell* **147**, 1537–1550.
96 Bitetti A, Mallory AC, Golini E, Carrieri C, Carreño Gutiérrez H, Perlas E, Pérez-Rico YA, Tocchini-Valentini GP, Enright AJ, Norton WHJ *et al*. (2018) MicroRNA degradation by a conserved target RNA regulates animal behavior. *Nat Struct Mol Biol* **25**, 244–251.
97 Kleaveland B, Shi CY, Stefano J and Bartel DP (2018) A network of noncoding regulatory RNAs acts in the mammalian brain. *bioRxiv* [PREPRINT].
98 Smith KN, Starmer J, Miller SC, Sethupathy P and Magnuson T (2017) Long noncoding RNA moderates MicroRNA activity to maintain self-renewal in embryonic stem cells. *Stem Cell Reports* **9**, 108–121.
99 Cazalla D, Yario T and Steitz JA (2010) Down-regulation of a host microRNA by a *Herpesvirus saimiri* noncoding RNA. *Science* **328**, 1563–1566.
100 Libri V, Helwak A, Miesen P, Santhakumar D, Borger JG, Kudla G, Grey F, Tollervey D and Buck AH (2012) Murine cytomegalovirus encodes a miR-27 inhibitor disguised as a target. *Proc Natl Acad Sci USA* **109**, 279–284.
101 Marcinowski L, Tanguy M, Krmpotic A, Rädle B, Lisnić VJ, Tuddenham L, Chane-Woon-Ming B, Ruzsics Z, Erhard F, Benkartek C *et al*. (2012) Degradation of cellular mir-27 by a novel, highly abundant viral transcript is important for efficient virus replication *in vivo*. *PLoS Pathog* **8**, e1002510.
102 Lee S, Song J, Kim S, Kim J, Hong Y, Kim Y, Kim D, Baek D and Ahn K (2013) Selective degradation of host MicroRNAs by an intergenic HCMV noncoding RNA accelerates virus production. *Cell Host Microbe* **13**, 678–690.