



CORRESPONDENCE

REVISED

Does the linear *Sry* transcript function as a ceRNA for miR-138? The sense of antisense [v2; ref status: indexed, <http://f1000r.es/4pd>]

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v2 First published: 11 Apr 2014, 3:90 (doi: [10.12688/f1000research.3872.1](https://doi.org/10.12688/f1000research.3872.1))
 Latest published: 13 Nov 2014, 3:90 (doi: [10.12688/f1000research.3872.2](https://doi.org/10.12688/f1000research.3872.2))

Abstract

Recently, the sex determining region Y (*Sry*) and the cerebellar degeneration-related protein 1 (*CDR1as*) RNA transcripts have been described to function as a new class of post-transcriptional regulatory RNAs that behave as circular endogenous RNA sponges for the micro RNAs (miRNAs) miR-138 and miR-7, respectively. A special feature of the *Sry* gene is its ability to generate linear and circular transcripts, both transcribed in the sense orientation. Here we remark that both sense (e.g. *Sry* RNA) and antisense (e.g. *CDR1as*) transcripts could circularize and behave as miRNAs sponges, and importantly, that also protein-coding segments of mRNAs could also assume this role. Thus, it is reasonable to think that the linear *Sry* sense transcript could additionally act as a miRNA sponge, or as an endogenous competing RNA for miR-138.

Open Peer Review

Referee Status:

	Invited Referees	
	1	2
REVISED		
version 2 published 13 Nov 2014	report	report
	↑	↑
version 1 published 11 Apr 2014	report	report

- 1 **Gordon Carmichael**, University of Connecticut Health Center USA
- 2 **Amy Pasquinelli**, University of California San Diego USA

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How to cite this article: Granados-Riveron JT and Aquino-Jarquin G. **Does the linear Sry transcript function as a ceRNA for miR-138? The sense of antisense [v2; ref status: indexed, <http://f1000r.es/4pd>]** *F1000Research* 2014, 3:90 (doi: [10.12688/f1000research.3872.2](https://doi.org/10.12688/f1000research.3872.2))

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Grant information: Work in the group's lab is supported by grants CB-168661 from the Mexican Council of Sciences and Technology (CONACyT) and Mexican Federal Funds (HIM/2012/010-SSA 1017).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

First published: 11 Apr 2014, 3:90 (doi: [10.12688/f1000research.3872.1](https://doi.org/10.12688/f1000research.3872.1))

First indexed: 19 Dec 2014, 3:90 (doi: [10.12688/f1000research.3872.2](https://doi.org/10.12688/f1000research.3872.2))

REVISED Amendments from Version 1

In the current version we have included additional information in response to the comments and observations made by the reviewers.

We have discussed aspects indicated by both referees about the ceRNA field, genes transcribed bi-directionally, biogenesis of circRNA molecules, requirements for ceRNA functionality, as well as the impact of ceRNA copy number on the miRNA function.

See referee reports

Crosstalk involving RNA–RNA interactions adds a new dimension to our understanding of complex regulatory networks and offers profound implications for the elucidation of gene function¹.

MicroRNAs (miRNAs) are a type of endogenously expressed small regulatory non-protein-coding RNAs that negatively regulate gene expression by base-pairing (with imperfect complementarity) to miRNA response elements (MREs), which are usually located within the 3'-untranslated region (3'-UTR) of target RNA transcripts². According to their number and location, it has become evident that a biological process may involve multiple miRNAs, and that a given gene may be regulated by more than one miRNA. A recently discovered molecular mechanism, named Competing Endogenous RNA (ceRNA) effect, has highlighted the importance of indirect interactions among transcript RNAs competing for the same pool of miRNAs³. In the case of two ceRNAs having MREs in common, both are regulated by the same set of miRNAs. Multiple classes of non-coding RNAs (lncRNAs) including circular endogenous RNA sponges (circRNAs), pseudogenes, and protein-coding mRNAs function as key ceRNAs and “super-sponges” to regulate the expression of mRNAs in plants and mammalian cells⁴. The effectiveness of a ceRNA would depend on the number of miRNAs that it can “absorb”. This, in turn, would depend on the ceRNA’s accessibility to miRNA molecules, which is influenced by its subcellular localization and its interaction with RNA-binding proteins. Furthermore, the specific cellular context in which the ceRNA is expressed would also impact its overall influence because not all microRNAs are present ubiquitously and at all times⁵. Aberrant expression of central nodes of such ceRNA networks may cause a disturbance that could contribute to disease pathogenesis⁶.

Recent bioinformatic and experimental analyses have identified thousands of circRNAs in the mammalian transcriptome, suggesting that circRNAs may in fact represent a new class of ceRNA regulators⁷. These circRNAs are produced mainly through a type of alternative RNA splicing named ‘back-splicing’, in which a splice donor splices to an upstream acceptor rather than a downstream acceptor⁸. Recently Guo *et al.* suggested that this would be the way in which most, if not all, cellular circRNAs are generated⁸.

Recently, Hansen *et al.* and Memczak *et al.* described a new class of post-transcriptional regulatory RNAs that behave as circRNAs in two back-to-back papers published in *Nature*^{9,10}. In both reports, the authors demonstrated that a ~1.5-kb single-stranded antisense circRNA molecule (human *CDRIas* or *ciRS-7*) containing multiple miR-7 binding sites densely arranged, acts as a natural miRNA

sponge, by capturing complexes formed by miR-7/Ago2. Memczak *et al.* observed that human *CDRIas* expression in zebrafish impaired midbrain development, similar to knocking down miR-7⁹.

Hansen *et al.* also showed that another circular RNA molecule, transcribed from the mouse *Sry* gene, could also act as an endogenous sponge. They noted that this transcript contains 16 binding sites for miR-138 and demonstrated *in vitro* that the *Sry* circRNA selectively “absorbs” this specific miRNA. Recently, Kartha and Subramanian asserted, based on the report by Hansen *et al.*, that this *Sry* RNA is an antisense circular transcript that functions as a miRNAs sponge¹¹. Although this apparently is a typographical error (antisense instead of sense), it was also referred as such in the original report by Memczak *et al.* in *Nature*. This suggests that the circular *Sry* transcript is, as occurs with the *CDRIas* sponge, an antisense circular RNA. Although it seems obvious that sponges are antisense to the miRNA they bind to, it should not be assumed that all circRNAs are transcripts in an antisense orientation to a protein coding gene, as occurs with *CDRIas*.

Natural antisense transcripts (NATs) are ncRNAs transcribed from the opposite strand of a coding gene and are capable of regulating the expression of their sense gene pair or of several related genes¹². Genomic loci that express NATs are highly abundant and sense/antisense (SAS) transcript pairs tend to be co-expressed. The most comprehensive studies predict that in human and mice 40–72% of all transcriptional units show evidence of bi-directional transcription¹³. To our knowledge, there are no reports of a circRNA whose sequence in sense and antisense orientation, possesses the ability to function as miRNA sponge. A special feature of the *Sry* gene is that it can generate linear as well as circular transcripts depending on the use of alternative promoters (proximal vs distal)¹⁴. Capel *et al.* reported for the first time that the circular *Sry* RNA is derived from a sense sequence that consists of a single exon. This molecule is formed by the processing of a longer precursor transcript that contains one inverted repeat at each end. This unusual configuration promotes the formation of a stem-loop structure that facilitates the nucleophilic attack of a donor splicing site at the 3' end to an acceptor site at the 5' end, which results in its circularization¹⁴ (Figure 1). Thus, it can be asserted that this is, in fact, a circular sense *Sry* mRNA. Although the notion that the *Sry* circRNA is derived from an antisense transcript does not alter the interpretation of the results obtained by Hansen *et al.*, we consider that this distinction is important, because it implies that both sense (e.g. *Sry* RNA) and antisense (e.g. *CDRIas*) transcripts could be circularized and act as RNA sponges, an observation which is not acknowledged by the authors of either of the original papers. To the best of our knowledge, no antisense transcript of the murine *Sry* gene has been reported. Nevertheless, if the circular version of the *Sry* transcript can soak up miRNAs, can the *Sry* linear transcripts also do the same?

In this respect, there is evidence that certain miRNAs may function by targeting sites in the 5'-UTR¹⁵ and open reading frame (ORF) regions of mRNAs¹⁶, suggesting that miRNAs may modulate gene expression by mechanisms different from canonical 3'-UTR target mRNA suppression. Binding of a miRNA to a ceRNA not only prevents that miRNA from binding to other MREs, but can

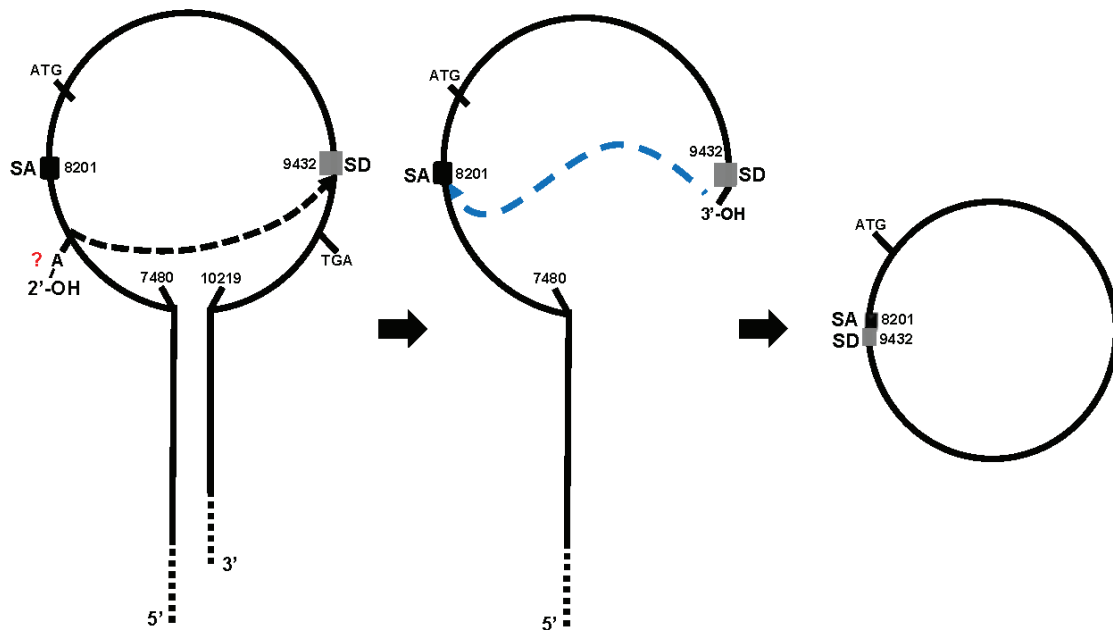


Figure 1. Formation of circular *Sry* RNA. After the *Sry* pre-RNA is transcribed, a stem-loop structure is created due to the presence of inverted repeats at the 5' and 3' ends. A normal splicing reaction takes place when the splice donor (SD) is attacked by a 2'-OH, presumably from a branch site adenosine residue (A) located in the intron, causing the first cleavage of the phosphodiester backbone. The newly formed 3'-OH at the SD, attacks the 5'-P at the splice acceptor (SA) site, resulting in excision of the intron and ligation of the circular exon of 1231 nucleotides. Modified from Capel *et al.*⁸.

also repress translation from the coding segment of the ceRNA¹⁷. A study of the pseudogene of the phosphatase and tensin homolog PTEN, *PTENP1*, provided the first experimental evidence for the cross-talk between coding and non-coding RNAs¹⁸. Tay *et al.* found that several endogenous protein-coding transcripts, such as serine incorporator 1 (*SERINC1*), vesicle-associated membrane protein associated protein A (*VAPA*), *CCR4-NOT* transcription complex and subunit 6-like (*CNOT6L*), act as *PTEN* ceRNAs, which regulate *PTEN* tumor suppressor levels in a miRNA-dependent manner¹⁸. This clearly suggests that mRNAs can function as ceRNAs and we propose that the mouse linear *Sry* sense transcript could also behave as a miRNA sponge, or as a ceRNA for miR-138.

Recently, Denzler *et al.* questioned the biological relevance of ceRNAs in terms of the abundance of these molecules which would be required to induce derepression of the targets of specific miRNAs¹⁹. However, Memczak *et al.* and Hansen *et al.* shown that circRNA behaving as miRNA sponges selectively bind miRNAs forming complexes with Ago proteins, which raises the possibility that ceRNAs modulate gene expression not only by capturing miRNAs but also through the depletion of the pool of available effector molecules of the miRNA pathway. Additionally, Denzler *et al.* based their calculations for target abundance on sites present in transcriptome 3' UTRs, however, they were unable to rule out that unidentified highly abundant and regulated non coding RNAs (including circRNAs) might substantially contribute to the pool of available binding sites, a limitation acknowledged in their paper¹⁹. This may be of particular importance in the adult testis, which express the circular *Sry* transcript and also has been shown to provide a

permissive environment for transcription initiation, a phenomenon that has been called “transcriptional promiscuity”²⁰. Denzler *et al.* also state that their findings in liver tissue can be generalized to other tissue and disease states, given that target abundance did not show large changes in the presence of insulin signaling or liver disease, conditions know to modify gene expression in such tissue. However, the authors also discuss that during cellular processes such as differentiation (like the spermatogenesis in the adult testis), expression of coding and noncoding RNAs changes dramatically, potentially making these systems more amenable to ceRNA-mediated gene regulation¹⁹.

Shortly after the emergence of circRNAs, the first public circRNA database ([circBase version 0.1](#)) was developed by the Rajewsky laboratory as a compendium of thousands of circRNAs sequences that are expressed in eukaryotic cells²¹. Access to this resource allows us to use the information in order to validate those circRNAs that are probably involved in many important cellular processes. Nevertheless, the precise molecular mechanisms that underlie post-transcriptional repression by circRNAs remain still largely unknown, but their discovery demonstrates the importance of this distinct type of non-protein-coding regulatory RNAs for the elucidation of gene function. The extent to which other animal or human antisense or sense circRNAs also behave as miRNA sponges will doubtlessly be a subject of intense research. Moreover, due to their longer half lives *in vivo*, circRNAs may possess a great potential for therapeutic intervention. Thus, manipulating miRNA function, either by mimicking or inhibiting ceRNAs implicated in several disorders such as cancer, could provide a novel strategy to interfere

with disease initiation and/or progression. The antisense modulation of circRNAs/ceRNA→miRNAs→mRNAs→protein regulatory networks could offer ingenious decoy combinations (antisense technology) as well as delivery platforms for concurrently target multiple miRNAs in abnormal or undesired conditions²².

Author contributions

JTGR and GAJ contributed extensively to this work and were involved in the critical revision of the manuscript. Both authors have agreed to the final version of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

GA-J is supported by grants CB-168661 from the Mexican Council of Sciences and Technology (CONACyT) and Mexican Federal Funds (HIM/2014/047-SSA 1123). JTGR is supported by Mexican Federal Funds (HIM/2014/040-SSA 1146).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Vidal M, Cusick ME, Barabasi AL: **Interactome networks and human disease.** *Cell.* 2011; **144**(6): 986–98.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bartel DP: **MicroRNAs: target recognition and regulatory functions.** *Cell.* 2009; **136**(2): 215–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bosia C, Pagnani A, Zecchina R: **Modelling Competing Endogenous RNA Networks.** *PLoS One.* 2013; **8**(6): e66609.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Li JH, Liu S, Zhou H, *et al.*: **starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data.** *Nucleic Acids Res.* 2014; **42**(Database issue): D92–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Salmena L, Poliseno L, Tay Y, *et al.*: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell.* 2011; **146**(3): 353–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Karreth FA, Ala U, Provero P, *et al.*: **Pseudogenes as competitive endogenous RNAs: target prediction and validation.** *Methods Mol Biol.* 2014; **1167**: 199–212.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tay Y, Rinn J, Pandolfi PP: **The multilayered complexity of ceRNA crosstalk and competition.** *Nature.* 2014; **505**(7483): 344–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Guo JU, Agarwal V, Guo H, *et al.*: **Expanded identification and characterization of mammalian circular RNAs.** *Genome Biol.* 2014; **15**(7): 409.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Memczak S, Jens M, Elefsinioti A, *et al.*: **Circular RNAs are a large class of animal RNAs with regulatory potency.** *Nature.* 2013; **495**(7441): 333–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hansen TB, Jensen TI, Clausen BH, *et al.*: **Natural RNA circles function as efficient microRNA sponges.** *Nature.* 2013; **495**(7441): 384–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kartha RV, Subramanian S: **Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation.** *Front Genet.* 2014; **5**: 8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Khorkova O, Myers AJ, Hsiao J, *et al.*: **Natural antisense transcripts.** *Hum Mol Genet.* 2014; **23**(R1): R54–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Werner A, Cockell S, Falconer J, *et al.*: **Contribution of natural antisense transcription to an endogenous siRNA signature in human cells.** *BMC Genomics.* 2014; **15**: 19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Capel B, Swain A, Nicolis S, *et al.*: **Circular transcripts of the testis-determining gene Sry in adult mouse testis.** *Cell.* 1993; **73**(5): 1019–30.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Orom UA, Nielsen FC, Lund AH: **MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation.** *Mol Cell.* 2008; **30**(4): 460–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tay Y, Zhang J, Thomson AM, *et al.*: **MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation.** *Nature.* 2008; **455**(7216): 1124–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Poliseno L, Salmena L, Zhang J, *et al.*: **A coding-independent function of gene and pseudogene mRNAs regulates tumour biology.** *Nature.* 2010; **465**(7301): 1033–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tay Y, Kats L, Salmena L, *et al.*: **Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs.** *Cell.* 2011; **147**(2): 344–57.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Denzler R, Agarwal V, Stefano J, *et al.*: **Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance.** *Mol Cell.* 2014; **54**(5): 766–76.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schmidt EE: **Transcriptional promiscuity in testes.** *Curr Biol.* 1996; **6**(7): 768–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Glazar P, Papavasileiou P, Rajewsky N: **circBase: a database for circular RNAs.** *RNA.* 2014; **20**(11): 1666–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wang Z: **The concept of multiple-target anti-miRNA antisense oligonucleotide technology.** *Methods Mol Biol.* 2011; **676**: 51–7.
[PubMed Abstract](#) | [Publisher Full Text](#)

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Current Referee Status:



Version 2

Referee Report 19 December 2014

doi:10.5256/f1000research.6097.r7098



Amy Pasquinelli

Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA

The revisions are sufficient.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 21 November 2014

doi:10.5256/f1000research.6097.r6689



Gordon Carmichael

Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA

This revised version offers substantial improvements and is generally acceptable. Although the fields of circular RNA and miRNA sponges are popular and active at the moment and papers are appearing frequently, it would help if the authors could include in their citations several recent papers that will be of value to readers of this article.

References

1. Lasda E, Parker R: Circular RNAs: diversity of form and function. *RNA*. 2014; **20** (12): 1829-1842 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
2. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L: Complementary sequence-mediated exon circularization. *Cell*. 2014; **159** (1): 134-147 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
3. Liang D, Wilusz JE: Short intronic repeat sequences facilitate circular RNA production. *Genes Dev*. 2014; **28** (20): 2233-2247 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
4. Bosson AD, Zamudio JR, Sharp PA: Endogenous miRNA and Target Concentrations Determine Susceptibility to Potential ceRNA Competition. *Mol Cell*. 2014; **56** (3): 347-359 [Publisher Full Text](#) | [Reference Source](#)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 24 October 2014

doi:10.5256/f1000research.4147.r4478



Amy Pasquinelli

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This short commentary points out that both sense and antisense transcripts with miRNA target sites could serve to titrate the miRNAs and, thus, release other target RNAs from repression. Recently, there has been much interest in these so called competing endogenous RNAs (ceRNAs) as a form of regulating miRNA activity. While ceRNA activity has been attributed to pseudogenes and transcripts that circularize (circRNAs), theoretically any RNA, including protein coding mRNAs, could serve this function. The correspondence proposes that the linear Sry sense, as well as the previously reported antisense, transcript could function as sponges for miR-138. A major criticism of the proposal that ceRNA activity plays a function in regulating miRNA availability for target regulation is the consideration of cellular RNA concentrations. In many cases, the ceRNAs, even the circRNAs which can be more stable, are expressed at levels far below the miRNA or even its target mRNA. In fact, after this correspondence by Granados-Riveron & Aquino-Jarquin was originally published in April 2014, the hypothesis that ceRNAs can alter miRNA function *in vivo* was rigorously tested and the conclusion was published in May that most ceRNAs simply are not abundant enough to act as competitive inhibitors of miRNA binding to target mRNAs (Denzler *et al.*, 2014). Thus, the authors need to include discussion of this concern over how generally miRNA activity might actually be affected by the presence of any kind of ceRNA, sense, antisense or circular.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 06 Nov 2014

Guillermo Aquino-Jarquin, Hospital Infantil de México, Federico Gómez, Mexico

This short commentary points out that both sense and antisense transcripts with miRNA target sites could serve to titrate the miRNAs and, thus, release other target RNAs from repression. Recently, there has been much interest in these so called competing endogenous RNAs (ceRNAs) as a form of regulating miRNA activity. While ceRNA activity has been attributed to pseudogenes and transcripts that circularize (circRNAs), theoretically any RNA, including protein coding mRNAs, could serve this function. The correspondence proposes that the linear Sry sense, as well as the previously reported

antisense, transcript could function as sponges for miR-138. A major criticism of the proposal that ceRNA activity plays a function in regulating miRNA availability for target regulation is the consideration of cellular RNA concentrations. In many cases, the ceRNAs, even the circRNAs which can be more stable, are expressed at levels far below the miRNA or even its target mRNA. In fact, after this correspondence by Granados-Riveron & Aquino-Jarquín was originally published in April 2014, the hypothesis that ceRNAs can alter miRNA function in vivo was rigorously tested and the conclusion was published in May that most ceRNAs simply are not abundant enough to act as competitive inhibitors of miRNA binding to target mRNAs (Denzler et al., 2014). Thus, the authors need to include discussion of this concern over how generally miRNA activity might actually be affected by the presence of any kind of ceRNA, sense, antisense or circular.

Firstly, we thank Dr Pasquinelli for her insightful comments. Secondly, we would like to clarify that the previously reported circular mouse *Sry* transcript reported by Capel *et al.*¹, now known to behave a sponge for mir-138^{2,3}, is, in fact, not derived from an antisense transcript but from a sense transcript, whose expression is directed by a promoter which is different to the promoter of the canonical linear *Sry* transcript involved in mammalian sex- determination. To the best of our knowledge, no antisense transcript of the murine *Sry* gene has been reported and a recent search of our own in publicly available EST data supports this assertion. Actually, one of our motivations for publish this piece of correspondence was to clarify this misconception (that the circular *Sry* RNA is an antisense transcript), which was first asserted in the report by Memczak *et al.* in Nature³ and later reproduced in a review of the subject by Kartha and Subramanian.⁴

We agree that recent findings by Denzler *et al.* question the biological relevance of ceRNAs in terms of the abundance of these molecules which would be required to induce derepression of the targets of specific miRNAs⁵. However, Memczak *et al.* and Hansen *et al.* shown that miRNA sponges selectively bind miRNAs forming complexes with Ago proteins, which raises the possibility that ceRNAs modulate gene expression not only by capturing miRNAs but also through the depletion of the pool of available effector molecules of the miRNA pathway. Additionally, Denzel *et al.* based their calculations for target abundance on sites present in transcriptome 3'UTRs, however, they were unable to rule out that unidentified highly abundant and regulated non coding RNAs (including circRNAs) might substantially contribute to the pool of available binding sites, a limitation acknowledged in their paper⁵. This may be of particular importance in the adult testis, which express the circular *Sry* transcript and also has been shown to provide a permissive environment for transcription initiation, a phenomenon that has been called "transcriptional promiscuity"⁶. Denzel *et al.* also state that their findings in liver can be generalized to other tissues and disease states, given that target abundance did not show large changes in the presence of insulin signaling or liver disease, conditions know to modify gene expression in hepatocytes. However, the authors also discuss that during cellular processes such as differentiation (like the spermatogenesis in the adult testis), expression of coding and non-coding RNAs changes dramatically, potentially making these systems more amenable to ceRNA-mediated gene regulation⁵.

We agree with Dr Pasquinelli in respect to the need of a discussion on the proposed mechanisms for ceRNAs action and their caveats in light of the findings by Denzel et al. and therefore, the new version of our correspondence includes such discussion.

References

1. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R: Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell*. 1993; **73** (5): 1019-1030 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
2. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J: Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013; **495** (7441): 384-388 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
3. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gergersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N: Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013; **495** (7441): 333-338 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
4. Kartha RV, Subramanian S: Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation. *Front Genet*. 2014; **5**: 8 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
5. Denzler R, Agarwal V, Stefano J, Bartel DP, Stoffel M: Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol Cell*. 2014; **54** (5): 766-776 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
6. Schmidt EE: Transcriptional promiscuity in testes. *Curr Biol*. 1996; **6** (7): 768-769 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)

Competing Interests: No competing interests were disclosed.

Referee Report 19 June 2014

doi:10.5256/f1000research.4147.r5178



Gordon Carmichael

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This article presents a short summary and review of recent work on circular RNAs and on competing endogenous RNAs, which can sequester and “sponge” microRNAs. A point made by the authors is that both sense and antisense RNAs from the same genomic region can act as miRNA sponges and that linear transcripts from the same regions that produce circular RNAs can themselves sequester miRNAs. Overall, I found the article to be provocative, though not novel. During the past several years there have appeared a number of papers describing circular RNAs of various sorts (for example, stable introns and circles resulting from splicing events commonly promoted by strong secondary structures flanking exons). There has also been a wealth of published work suggesting or showing that abundant RNAs can bind miRNAs and thus reduce their availability for the regulation of some mRNAs. Thus, this paper breaks little new ground, though it does point out that many genomic regions express both sense and antisense transcripts, and each of these can affect miRNA availability. To me, the area covered by this paper is a very interesting and important one, and it is of value to present and discuss new concepts in gene regulation. Thus, I think this manuscript would be improved by:

1. More clearly summarizing the ceRNA field.

2. Discussing a bit more the well known fact that sense and antisense transcripts are commonly expressed in cells, though not necessarily from the same locus at the same time.
3. Mentioning the several ways that stable circular RNAs can be produced.
4. Finally, discussing the issue lacking from the current version that, in order to effectively act as a miRNA sponge, an RNA must not only be stable, but also in the proper cellular compartment and containing a molar concentration of miRNA binding sites that is high enough to sequester a biologically significant fraction of the endogenous miRNA of interest.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 06 Nov 2014

Guillermo Aquino-Jarquin, Hospital Infantil de México, Federico Gómez, Mexico

1. More clearly summarizing the ceRNA field.

We are grateful to Dr. Gordon Carmichael for all the comments made to our manuscript.

A recently discovered molecular mechanism, named Competing Endogenous RNA (ceRNA) effect, has highlighted the importance of indirect interactions among transcript RNAs competing for the same pool of miRNAs ¹. ceRNAs share one or more miRNA response elements (MREs) and compete for a restricted pool of common miRNAs. Thus, ceRNAs form complex regulatory networks of miRNAs and MRE-containing transcripts, both protein-coding and noncoding, which ensure a tight control of many biological processes. Aberrant expression of central nodes of such ceRNA networks may cause a disturbance that could contribute to disease pathogenesis ². A discussion along these lines has been included in our new submission.

2. Discussing a bit more the well known fact that sense and antisense transcripts are commonly expressed in cells, though not necessarily from the same locus at the same time.

CDR1as is a circRNA that functions as a sponge for miR-7, deriving from an antisense transcript of the *CDR1* protein-coding gene. On the other hand, the transcript of the male sex-determining gene *Sry*, transcribed in sense orientation ³, is a second circRNA proposed to act as a sponge, in this case, for miR-138. However, Memczak *et al.* stated that "Perhaps the best known circRNA is **antisense** to the mRNA transcribed from the *SRY* (sex-determining region Y) locus and is highly expressed in testes" ⁴. Recently, Kartha and Subramanian asserted, based on the report by Hansen *et al.*, that this *Sry* RNA is an antisense circular transcript that functions as a miRNAs sponge ⁵. Although this apparently is a typographical error (antisense instead of sense), it was also referred as such in the original report by Memczak *et al.* in Nature⁴. This would suggest that the circular *Sry* transcript is, as occurs with the *CDR1as* sponge, an antisense circular RNA. Although it seems obvious that sponges are antisense to the miRNA they bind to, it should not be assumed that all circRNAs are transcripts in an antisense orientation to a protein coding gene, as occurs with

CDR1as. We conducted an *in silico* analysis taking the antisense sequence of the *Sry* mRNA to make a search for potential binding sites for miR-138, using the fuzzy bioinformatics tool (EMBOSS Suite) and we found none. When we used the *Sry* ORF (GenBank NM_011564) for the miR-138 binding site (CACCAGCA), we found 14 potential sites which is consistent with the number of sites found by Hansen and colleagues. For this reason, we remark in our current submission that both sense and antisense *Sry* transcripts could circularize and behave as miRNAs sponges, and importantly, that protein-coding segments of *Sry* mRNA could also assume this role.

To our knowledge, there are no reports of a circRNA whose sequence in sense and antisense orientation possesses the ability to function as miRNA sponge. On the other hand, a particularly interesting family of non-protein coding RNAs consists of natural antisense transcripts (NATs). NATs are ncRNAs transcribed from the opposite strand of a coding gene and are capable of regulating the expression of their sense gene pair or of several related genes ⁶. Genomic loci that express NATs are highly abundant and sense/antisense (SAS) transcript pairs tend to be co-expressed. The most comprehensive studies predict that in human and mice 40-72% of all transcriptional units show evidence of bi-directional transcription ⁷. The regulatory activity of SAS pairs in human tissues has been postulated on protein expression at different levels, such as alternative splicing, post-transcriptional regulation, transport and epigenetic imprinting as well as transcriptional and translational interference through annealing to complementary sequences ⁸. **As requested, we have included this information in the version of our submission.**

3. Mentioning the several ways that stable circular RNAs can be produced.

Recent bioinformatic and experimental analyses have identified thousands of circRNAs in the mammalian transcriptome, suggesting that circRNAs may in fact represent a new class of ceRNA regulators ⁹. These circRNAs are produced mainly through a type of alternative RNA splicing named 'back-splicing', in which a splice donor splices to an upstream acceptor rather than a downstream acceptor ¹⁰. Recently Guo *et al.* suggested that this would be the way in which generates most, if not all, cellular circRNAs. **Please find a relevant discussion in the new version.**

4. Finally, discussing the issue lacking from the current version that, in order to effectively act as a miRNA sponge, an RNA must not only be stable, but also in the proper cellular compartment and containing a molar concentration of miRNA binding sites that is high enough to sequester a biologically significant fraction of the endogenous miRNA of interest.

Increasing experimental evidence supports the hypothesis that multiple non-coding RNA species, including small non-coding RNAs, pseudogenes, lncRNAs and circular RNAs (circRNAs) may possess ceRNA activity ⁹. The effectiveness of a ceRNA would depend on the number of miRNAs that it can "absorb" This, in turn, would depend on the ceRNA's accessibility to miRNA molecules, which is influenced by its subcellular localization and its interaction with RNA-binding proteins. Furthermore, the specific cellular context in which the ceRNA is expressed would also impact its overall influence because not all microRNAs are present ubiquitously and at all times ¹¹. **We have included a discussion of the subject in the current version.**

References

1. Bosia C, Pagnani A, Zecchina R: Modelling Competing Endogenous RNA Networks. *PLoS One*.

- 2013; **8** (6). [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
2. Karreth FA, Ala U, Provero P, Pandolfi PP: Pseudogenes as competitive endogenous RNAs: target prediction and validation. *Methods Mol Biol.* 2014; **1167**: 199-212 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
3. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R: Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell.* 1993; **73** (5): 1019-1030 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
4. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N: Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature.* 2013; **495** (7441): 333-338 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
5. Kartha RV, Subramanian S: Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation. *Front Genet.* 2014; **5** (8). [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
6. Khorkova O, Myers AJ, Hsiao J, Wahlestedt C: Natural antisense transcripts. *Hum Mol Genet.* 2014; **23** (R1): R54-R63 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
7. Werner A, Cockell S, Falconer J, Carlile M, Alnumeir S, Robinson J: Contribution of natural antisense transcription to an endogenous siRNA signature in human cells. *BMC Genomics.* 2014; **15** (19). [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
8. Lapidot M, Pilpel Y: Genome-wide natural antisense transcription: coupling its regulation to its different regulatory mechanisms. *EMBO Rep.* 2006; **7** (12): 1216-1222 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
9. Tay Y, Rinn J, Pandolfi PP: The multilayered complexity of ceRNA crosstalk and competition. *Nature.* 2014; **505** (7483): 344-352 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
10. Guo JU, Agarwal V, Guo H, Vartel DP: Expanded identification and characterization of mammalian circular RNAs. *Genome Biol.* 2014; **15** (7): 409 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)
11. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?. *Cell.* 2011; **146** (3): 353-358 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.