Competing endogenous RNA (ceRNA): a new class of RNA working as miRNA sponges

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MicroRNAs (miRNAs) are widely known as a class of non-coding RNA (ncRNA), which regulate gene expression, largely at the post-transcriptional level, but rarely also at the transcription level. During the last few years, a new class of ncRNA has been discovered, which counteracts the repressive activity of miRNA, by sequestering miRNA within a cell. These novel ncRNA molecules have been variously described as 'miRNA sponges/decoys', 'target mimics' or 'competing endogenous RNA'. Several reports of the occurrence of these ceRNAs and their role in regulating gene expression were published recently, which are briefly described in this article. These discoveries will keep scientists busy for many years to generate new information.

Keywords: Competing endogenous RNA, eukaryotic genome, gene expression, miRNA sponges.

DURING the last half a century, studies on plant and animal genomes have witnessed many surprises. One of the early surprises in late 1960s was the discovery of high proportion of repetitive and noncoding DNA (ncDNA) in majority of eukaryotic genomes. Consequently, it became known that in most eukaryotes, only a small fraction (then called 'unique DNA') of the genome consists of protein-coding genes[1–3]. In parallel with this discovery and thereafter, it was also discovered that a major part of the abundant repetitive DNA is composed of transposable elements (TEs). These TEs were initially described as mobile genetic elements[4], a concept that was put forward as early as 1950 by Barbara McClintock[5]. Another surprise during late 1970s was the Nobel Prize-winning discovery of split genes in 1977, suggesting that a protein-coding gene carried coding sequences (exons) interrupted by non-coding sequences (introns)[6,7]. (Richard J. Roberts and Phillip A. Sharp were awarded the 1993 Nobel Prize in Physiology or Medicine for this discovery.)

During the last two decades, another major surprise was the discovery that a sizable fraction of the eukaryotic genome is transcribed into non-coding RNA (ncRNA, other than tRNA and rRNA) which is never translated, but is involved in post-transcriptional regulation of gene expression. A part of this ncRNA is also involved in RNA interference (RNAi), which was first discovered when introduction of a gene for chalcone synthase resulted in co-suppression of homologous genes and therefore led to the disappearance of purple colour in petunia flowers[8]. It was later shown that RNAi actually involved the production of a double-stranded RNA as an intermediate step[9] (A. Fire and C. C. Mello won the 2006 Nobel Prize in Physiology or Medicine for this discovery). RNAi thus became a popular area of research not only for understanding the mechanism of gene expression, but also for manipulating gene expression for a variety of purposes (healthcare in the field of medicine and crop improvement in the field of agriculture). As a consequence, small interfering RNAs (siRNAs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are now known to constitute an important component of the repertoire of ncRNAs, which are involved in regulation of gene expression.

Another big surprise recently brought to light is the fact that specific miRNAs, each blocking translation of a specific mRNA (may rarely also control transcription), do not function independently in exercising post-transcriptional control on gene expression. A new class of RNAs was discovered, which competes with specific mRNAs for providing binding sites to the corresponding miRNAs. These RNAs are described as 'competing endogenous RNAs' (ceRNAs) and include both linear and circular RNAs. A particular ceRNA controls the suppressive effect of a specific miRNA on mRNA translation through sequestering this miRNA, thus facilitating translation of the target mRNA (Figure 1). It also became known that coding mRNA (which was once looked upon as a mere message for translation) may also be involved in a variety of 'non-coding' functions[10]. It is obvious thus that a network of RNA molecules (involving mRNA, miRNA and ceRNA) is actually involved in regulating gene expression, mainly at the post-transcriptional level, although it may also involve regulation at the transcription level (see later in the text). In this article, a brief summary of the functions of ncRNA in degrading mRNA will be presented first as background information. This

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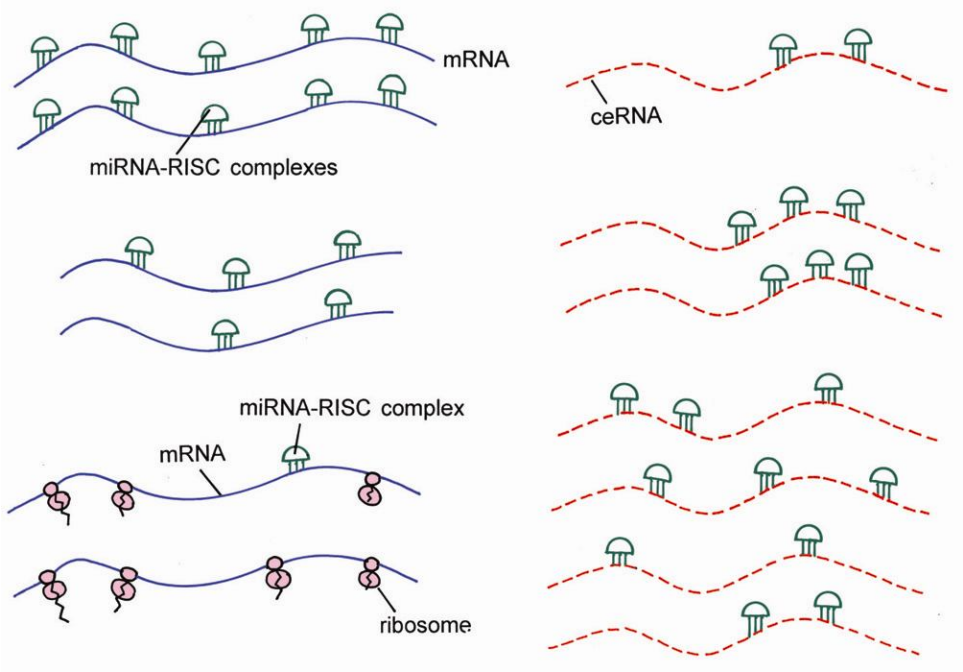

Figure 1. Diagrammatic representation of the role of miRNA in binding to and suppressing translation of mRNA (top left panel) in the absence of adequate level of ceRNA to sequester miRNA (top right panel). The miRNA is unable to suppress mRNA translation (bottom left panel), when the ceRNA level increases and sequesters majority of miRNA (bottom right panel), thus leaving very little miRNA for binding to its target mRNA.

will be followed by an account of ceRNAs (with suitable examples), which work as miRNA sponges, and are involved in regulatory ceRNA networks (ceRNETs).

The transcriptome: discovery of ncRNAs

Transcriptome studies have now been conducted in a number of animal and plant species. In humans, the ENCODE (Encyclopaedia of DNA Elements) project was completed in 2012 (ref. 11) and the modENCODE project (started in 2007), also published its first set of results in 2010 (mod implies model organisms)[12]. The analysis of transcriptome from humans and these model organisms has shown that a transcriptome consists of a mixture of RNA molecules derived from a variety of genes, including protein coding genes, pseudogenes and ncRNA genes (including miRNA, lncRNA and circular RNA)[13]. These are briefly described here (details of ceRNA are presented later in this article).

Protein-coding genes

Approximately 20,000 protein-coding genes have been identified in the human genome, many of which carry a number of miRNA response elements (MREs)[11–13] each for miRNA binding. In a transcriptome study, one can identify these MREs on coding gene transcripts and predict the extent of miRNA-dependent regulation, which may be exercised in a temporal and spatial manner.

Pseudogenes

The pseudogenes that are often found within a genome resemble known genes and were initially believed to represent 'nonfunctional', 'junk' or 'evolutionary relics'. This is because, except for a few cases, they do not encode functional proteins, and the translation of their transcripts (if any) is interrupted by premature stop codons, frameshift mutations, insertions or deletions. Human genome sequencing revealed that there might be as many as ~19,000 pseudogenes in the human genome, many of which are transcribed and are often well conserved[14]. There is at least one popular example, where a pseudogene (*PNETpg1*) has been shown to be transcribed into lncRNA molecules, which control the activity of a miRNA (see later in the text for details).

ncRNA and its role in regulation

As mentioned above, during 1970s and 1980s, it was largely believed that only a tiny fraction of the genome is transcribed, since the major fraction was known to be repetitive or the so called 'junk DNA'. However, contrary to this earlier belief, it is now widely known that a large proportion of the genome (including unique and repetitive fraction) is actually transcribed, and also that a large proportion of this transcriptome is never translated into proteins; this is described as ncRNA[13]. In humans, ENCODE project discovered that ~75% of the genome is actually

Table 1. A timeline of major milestones in the discovery of ncRNA, microRNA and ceRNA

1990	Multiple copies of a pigment transgene result in colourless petunias[8]
1993	First microRNA (*lin-4*) discovered in *Caenorhabditis elegans*[39,40]
1995	Discovery of RNA interference[41]
1998	Discovery of the involvement of double-stranded RNA in RNAi (2006 Nobel Prize in Physiology or Medicine)[9]
2000	First conserved microRNA (*let-7*) in *C. elegans*[42]
2001	RNAi shown to be mediated by small ncRNA (21–22 nu), named microRNA for the first time[43]
2002	microRNA discovered in plants for the first time[44]
2002	microRNAs (*miR15* and *miR16*) were implicated in cancer[45]
2002	miRNAs and siRNAs share components of the effector pathway[46]
2007	miRNA could activate mRNA translation[47]
2007	Target mimicry in *Arabidopsis thaliana*[32]
2008	MiRNA-mediated transcriptional gene activation/silencing[48]
2009	miRNA binding can titrate miRNA[29]
2011	ceRNA hypothesis[26]
2012	Plant miRNA could regulate mouse gene[49]
2010–13	Exonic circular ceRNA (ecircRNA)[22,23,31,33,34,50,51]

transcribed, although only 1–2% of the genome consists of protein-coding genes[11]. A part of this ncRNA is available in the form of small ncRNAs (22 nt) known to be involved in regulation of gene expression. Another class of ncRNA includes the more abundant long ncRNAs (lncRNAs, 100–1000 nt long), whose function and mode of action are being currently studied. The so-called piRNA (PIWI-interacting RNA), involved in germline transposon silencing and gametogenesis, is another important class of ncRNAs now receiving increased attention[15]. The major milestones for the discovery of different types of ncRNA are listed in Table 1.

Of the above ncRNAs, the most important are microRNAs (22 nt long), which are known to be implicated in the suppression of translation. When deregulated, these miRNAs sometimes also cause diseases in humans[16] (http://cmbi.bjmu.edu.cn/hmdd). The miRNAs and lncRNAs are also known to be involved in responses to abiotic stresses in crop plants[17,18].

miRNAs bind to complementary sequences available on the target mRNA transcripts in the form of MREs[19,20]. There may be many different MREs in an individual mRNA, which may, therefore, be degraded by a group of miRNA. As a corollary, in some other cases, the same miRNA may disintegrate hundreds of different types of transcripts, which carry the same MREs. Thus microRNAs suppress translation of a large proportion of the transcriptome[21].

In addition to miRNAs, the long non-coding RNAs or lncRNAs (100s to 1000s nt long) mentioned above, were also found to be important. Human ENCODE project revealed that the human transcriptome may carry as many as 10,000–200,000 lnc-RNAs, which can activate gene expression and/or silence a large number of genes[11].

Circular RNAs

Circular RNAs (circRNAs) were only sporadically reported in the past, and their discovery was largely serendipitous. Only recently, transcriptome analysis in some organisms revealed that circRNAs are ubiquitous and are much more abundant than was previously believed. Since the circRNAs result through circularization due to joining of exons following intron splicing, they have also been described as exonic circular RNAs (ecircRNA)[22]. Human transcriptome has been shown to carry thousands of these cricRNAs[23]. In an archaeon (*Sulfolobus solfataricus* P2) also, multiple circular transcripts were detected and shown to include a large number of non-coding RNAs (ncRNAs)[24]. These results suggested that circular ncRNAs might have important biological roles, which have only been partially understood[25].

The above discussion suggests that coding RNA and ncRNA are generally involved in a network influencing each other's levels in an intricate manner. The miRNAs mainly function through binding to the target mRNA, but often their own activity is also controlled by another set of RNAs, called competing endogenous RNAs or ceRNAs[26] (see next section).

Synthetic transcripts containing MREs: a competition with target mRNA

The miRNAs were initially shown to control expression of specific target mRNA using MREs that are specific and common for the corresponding mRNAs; this repression activity of miRNA was then believed to be universal (there being no adaptive response). However, majority of mRNAs that are the targets of miRNA were later found to be actively translated and expressed in a temporal and spatial manner, despite the occurrence of their corresponding miRNA. This observation suggested that there must be mechanisms, which counteract/restrict the translational repression activity of miRNA. The belief received support in 2007, when Phil Sharp and co-workers observed in cultured cells a competition between synthetic plasmids carrying MREs and the target mRNA for

the miRNAs involved in repression of the target mRNA. These competing artificial transcripts were described as 'miRNA-sponges'[27,28] (due to their role in sequestering miRNA), thus providing for a mechanism for modulating miRNA action. Evidence in favour of this conclusion also came from a number of recent studies that are briefly described later in this article.

Competing endogenous RNA hypothesis

Following the above experiments of Sharp, in 2009 Hervé Seitz (then at University of Massachusetts Medical School in USA) proposed that the phenomenon observed through the use of plasmids carrying MSE by Sharp may have a biological function. He believed that the role of a substantial fraction of computationally identified miRNA targets (later celled ceRNA) may be to sequester miRNAs, preventing the latter from binding to their authentic target mRNA[29]. In 2011, Pandolfi and co-workers (Harvard Medical School, Boston, USA) proposed the 'competitive endogenous hypothesis'[26], according to which, the concentration of a specific miRNA can be temporally reduced within a specific cell type due to ceRNA, which would work as miRNA sponge, thus facilitating translation of the target mRNA, despite the presence of corresponding miRNA. Further, according to this hypothesis, all types of RNA transcripts communicate through 'MREs' and are thus involved in a crosstalk (involving both coding and noncoding; linear and circular RNAs). This leads to the formation of large-scale transcriptome regulatory networks that are described as ceRNA networks (ceRNETs)[26]. The ceRNA hypothesis was later confirmed by four studies published in *Cell* in 2011 (see ref. 25 for a brief review of these studies), providing evidence for the presence of a new species of RNA (ceRNA), which competes with target mRNA for binding of miRNA. Through sharing common MREs in their 3′ untranslated regions (UTRs), the ceRNAs upregulate expression of genes that were the target of a miRNA (Figure 1). Some of the recent studies that elucidated the role of these ceRNAs are briefly discussed here.

Nuclear miRNA and antisense ncRNA

It is widely known that miRNA generally occurs in the cytoplasm and suppresses translation. However, miRNAs were detected in the nucleus also, suggesting that they may also regulate gene expression by a mechanism other than translation inhibition[30]. In a recent study, a circular transcript that was antisense to the gene encoding Cerebellar Degeneration-Related protein 1 (CDR1) was located in the nucleus[31] as well as in the cytoplasm and was shown to take part in regulation of the expression of CDR1 sense mRNA (see later in the text for details).

Cytoplasmic miRNA, lncRNA and circRNA

A more common phenomenon involving suppression of translation by miRNA, and the regulation of miRNA activity by ceRNA occurs in the cytoplasm and not in the nucleus (described above). This phenomenon will be discussed with the help of results from a study in the model plant species, *Arabidopsis thaliana*[32], and three recent studies in animals (including humans)[31,33,34]. These four studies that are highlighted in a recent communication[35] are classified here in three categories, depending upon the organism used and the type of ceRNA involved.

IPS1 inhibits the effect of miR-399 on PHO2 in A. thaliana (target mimicry): In plants, *PHO2* encodes a protein, which causes reduction in shoot phosphorus (P_i) content, but miR-399 (a P_i starvation–responsive miRNA) causes cleavage of *PHO2* mRNA, thus neutralizing the negative effect of *PHO2*. In *A. thaliana*, the gene *IPS1* (INDUCED BY PHOSPHATE STARVATION1) carries a 23 nt long conserved motif, which shows extensive sequence complementarity with miR-399, except at positions 10 and 11 (it is this mismatch region, which causes miRNA-guided cleavage of mRNA targets).

Levels of *PHO2* mRNA and miR-399 were studied in transgenics that over-expressed *IPS1* and miR-399, either alone or in combination[32]. The following results were obtained: (i) over-expression of miR-399 (alone) reduced *PHO2* mRNA accumulation (due to cleavage by miR-399 based on complimentarity at positions 10 and 11), but did not degrade *IPS1* RNA (due to mismatch at positions 10–11); (ii) over-expression of *IPS1* (alone) gave an increased *PHO2* mRNA accumulation and almost no miR-399 in these plants (apparently, IPS1 RNA sequestered miR-399); (iii) simultaneous over-expression of *IPS1* and miR-399 gave high level of *PHO2* mRNA and low level of shoot P_i content (since miR-399 is sequestered by *IPS1* RNA and is not available for cleavage of PHO2 mRNA). This phenomenon was described as 'target mimicry', since *IPS1* ncRNA mimicked the target *PHO2* mRNA for binding of miR-399 and behaved as a miRNA sponge to sequester miR-399 (Figure 2).

lncRNAs of PTENpg1 pseudogene regulate tumour suppression: Phosphate and tensin gene, *PTEN* is a tumour suppressor gene. So the presence of its product blocks cancerous growth, and its absence causes faster cancer growth. In a recent study, it was shown that the expression of *PTEN* gene is regulated both at the transcription and translation levels by its pseudogene (*PTENpg1*). Three different transcripts (including two antisense RNAs, asRNAα and asRNAβ and one sense RNA) take part in the regulation of *PTEN* expression[33], and the regulation is achieved in the following manner (Figure 3): (i) *PTENpg1* asRNAα binds to the *PTEN* promoter and inhibits *PTEN* transcription by recruiting epigenetic repressor

complexes; this leads to the onset of cancer (due to the absence of *PTEN* protein); (ii) *PTENpg1* asRNAβ is partially complementary to *PTENpg1* sense RNA (a lncRNA), and promotes its stabilization by binding to its 5′ end. Since *PTENpg1* lncRNA functions as ceRNA, its stabilized form will sequester miRNA and facilitate translation of *PTEN* mRNA (its activity is generally suppressed by a miRNA), thus suppressing cancer growth. Therefore, any intervention involving suppression of PTENpg1 asRNAα and promoting PTENpg1 lncRNA as well as *PTENpg1* asRNAβ will help in the control of cancerous growth. This study was led by scientists at the Scripps Research Institute (TSRI), La Jolla, USA, who believe that this research may provide a way to control cancer through manipulation of the activity of *PTENpg1*.

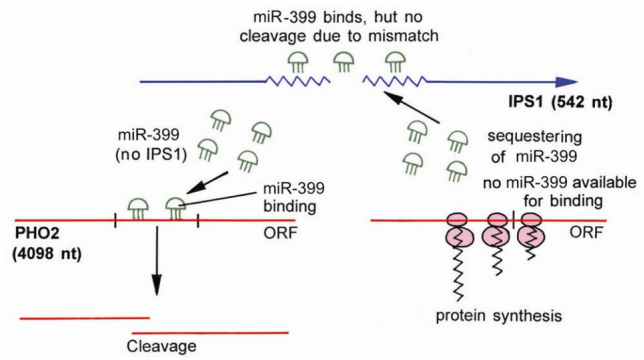

Figure 2. Diagrammatic representation of the results of a study involving effects of miR-399 and ceRNA (IPS1 mRNA) on *PHO2* gene expression in *Arabidopsis thaliana*. Note binding of miR-399 to *PHO2* mRNA (in the absence of *IPS1* mRNA) causing its cleavage (left panel), and sequestering of miR-399 by *IPS1* mRNA (top panel) leading to translation of *PHO2* mRNA (right panel).

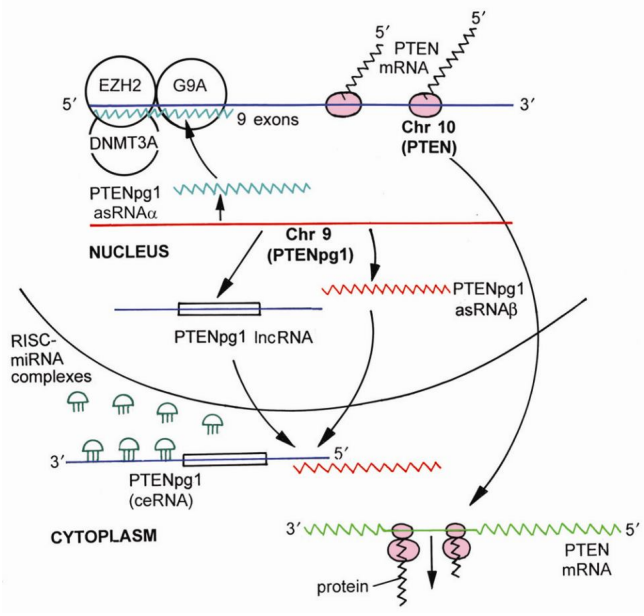

Figure 3. Diagrammatic representation of the roles of a tumour suppressor gene *PTEN* and its pseudogene, *PTENpg1* in development of cancer. Note PTEN translation, which is facilitated due to sequestering of miRNA by PTENpg1 lincRNA, which functions as ceRNA (see text for details).

Exonic circular RNAs work as miRNA sponges: As mentioned above, circRNAs are a new class of RNAs which result due to joining of exons following intron splicing, and are involved in controlling the activity of miRNA (Figure 4). Recently, two studies demonstrated that in a cell, circRNAs are found in thousands and that they function as miRNA sponges[31,34]. The results of these two studies will be briefly described.

(i) CDR1as/ciRS-7 sponges miR-7: In a recent study[31], RNA-seq data were collected from ribosomal RNA-depleted RNA samples from human, mouse and a nematode (*Caenorhabditis elegans*). The RNA-seq data were then examined using a novel computational device leading to the identification of as many as ~2500 circRNAs. Of these, 85% circRNAs were found to align to known protein-coding genes; ~50 circRNAs were also validated using suitable tests. Among these circRNAs, one CDR1as was antisense to the CDR1 locus, which was already known to be involved in a number of cerebellar degeneration disorders. The CDR1as is known to carry >70 MREs for miR-7 (a microRNA involved in downregulation of many genes)[31]. Another study independently identified the same circRNA species in human and mouse brains[34]. So this circRNA was examined in both these

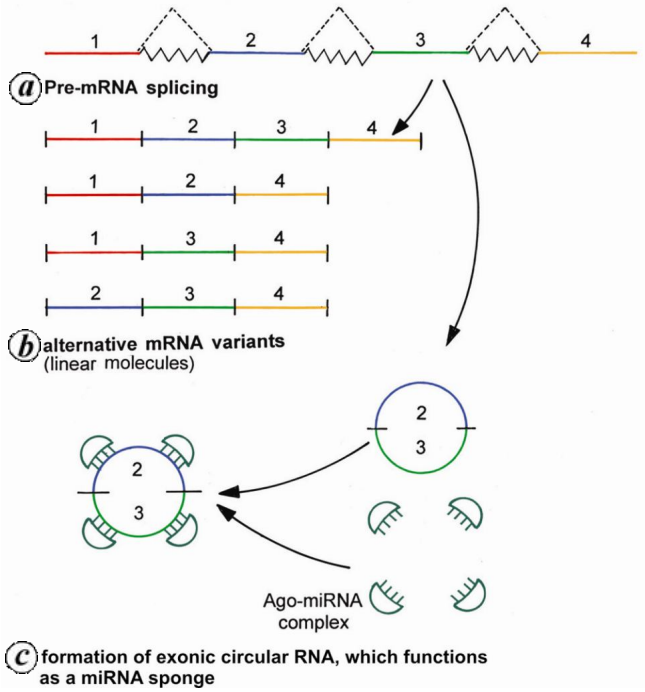

Figure 4. Diagrammatic representation of development of exonic circular RNA (along with alternative linear mRNA variants), which functions as ceRNA and sequesters miRNA.

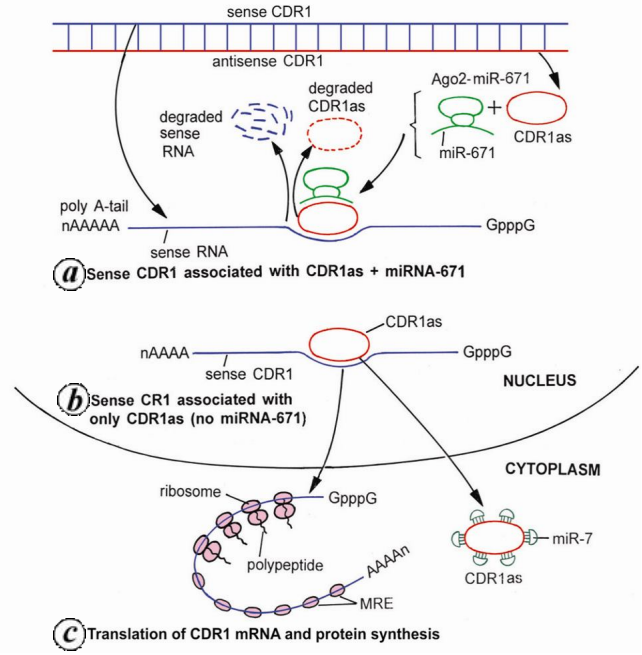

Figure 5. Diagrammatic representation of the two alternative fates of linear sense CDR1 and circular antisense RNA (CDR1as) in the presence and absence of miRNA-671. Circular CDR1as pairs with sense CDR1 mRNA in the nucleus and stabilizes it for export to the cytoplasm, where the mRNA can be translated (due to sequestering of miR-7 by CDR1as, which would otherwise degrade CDR1 mRNA). In the presence of miR-671-Ago2-RISC in the nucleus, the CDR1as RNA is degraded, destabilizing the sense mRNA, which is also degraded (based on ref. 49).

studies, although it was described as CDR1as (antisense RNA) in one study[31] and ciRS-7 (circular RNA sponge for miR-7) in the other study[34]. In these two studies, it was shown that CDR1as/ciRS-7 is involved in regulation of the expression of *CDR1* gene, which is downregulated (at the translation level) by a miR-7. However, the activity of miR-7 in turn is regulated by circular CDR1as/crRS-7, which can bind to as many as 20,000 miR-7 molecules per cell. CDR1as is not cleaved or spliced by miR-7 due to mismatch at nucleotide positions 11 and 12, since matching at these two bases is an essential requirement for mRNA degradation. Obviously, CDR1as works as a miR-7 sponge, so that when knocked down, the target genes for miR-7 were all downregulated, suggesting the role of CDR1as as a ceRNA molecule, which functions as a miRNA sponge (Figure 5).

Further evidence for the role of ciRS-7 as miR-7 sponge became available from the following observations[31,34]: (a) no linear form of ciRS-7 was detectable in human cells; (b) knockdown or over-expression of ciRS-7 in human cells led to changes in the levels of known miR-7 targets; (c) the presence of ciRS-7 reduced the effect of miR-7 on both reporter constructs and endogenous miR-7 targets; (d) miR-7 depletion and ciRS-7 over-expression gave similar phenotype, as verified using zebrafish as a model.

(ii) Sry circRNA sponges miR-138: In addition to the work on ciRS-7 and miR-7 involving the *CDR1* gene, the role of *Sry*-derived testis-specific circRNA was also examined[31]. It was found to serve as a miR-138 sponge in the same manner as ciRS-7 serves as a sponge for miR-7. The study of these two circular RNAs (ciRS-7/CDR1as and Sry) suggested that the effects of circRNAs as miRNA sponges is a general phenomenon and should be widespread in both animal and plant systems.

Kinetics of RNA network: ceRNA dosage and ceRNETs

The interactions among different components of a complex RNA-based regulatory network (including ceRNA, mRNA and miRNA) were recently explained with the help of a mathematical model[10]. It was shown that 50% of miRNA families had 1–400 target mRNA molecules, and that none of the miRNA targeted all or majority of mRNAs. It was also hypothesized that expression levels of different components of ceRNET (ceRNA and miRNA) influence cross-regulation and that optimum regulation is achieved at equimolar concentrations of ceRNA and miRNA within the network. It has also been shown that the perturbation of any component of ceRNET affects the entire network, which exhibits a titration mechanism with rates of transcription, degradation, association and dissociation of different RNAs representing the key parameters of the kinetic model.

Another key element highlighted in the above study is the effect of the dose of individual RNAs. It is argued that a basal expression level for each of the different RNAs of the network is needed for a specific output. This was explained using the example of phosphatase and tensin homologue (PTEN; described earlier in the article), the vesicle-associated membrane protein-associated protein A (VAPA) functioning as ceRNA, and eight miRNAs shown to target both PTEN and VAPA. A series of cell lines with variable VAPA : PTEN ratio but similar levels of miRNAs were used, and then the effect of perturbation due to introduction of siRNA against VAPA was examined. It was observed that when ceRNA : PTEN ratio is more than unity, silencing of ceRNA (VAPA) had a strong effect on the level of PTEN. But if VAPA : PTEN ratio is unity, then increased miRNA expression decreased PTEN expression proportionally to the amount of miRNA expression. These *in vitro* studies confirmed the predictions of the theoretical model, according to which ceRNA dosage is critical for ceRNA activity.

Conclusion, summary and perspectives

The discovery of miRNA during 1993 was a big surprise at that time, but later it provided a system to repress expression of certain genes. More recently, the system of

ceRNAs has been discovered, which in the form of a variety of non-coding and coding RNAs (both linear and circular) provides a mechanism to facilitate translation of mRNA that would otherwise be degraded by miRNA. These ceRNAs together make a network of RNAs involved in regulation of gene expression, not merely at the translation level, but sometimes also at the transcription level. A series of reports that appeared during 2011–13 suggest wide occurrence of these ceRNAs in all systems of living organisms, including archaea and higher plants/animals. It is also known that ceRNA molecules generally carry MREs (described as 'miRNA sensors')[37], which exhibit partial/extensive complementarity with specific miRNA species, and occur in thousands within the same cell to function as 'miRNA sponges'. The high conservation of lncRNA and circRNAs working as ceRNA makes it possible to study their function *in vivo* during development as well as onset of diseases such as cancer. In the case of plants, these molecules may be involved in responses to abiotic stresses like drought, salinity, heat and also in nitrogen and phosphorus use-efficiency. However, it is intriguing to find that the level of complementarity between miRNA and the target mRNA is partial in animals, but is extensive in plant systems[37].

The recent discovery of ceRNAs in the form of circular RNAs in thousands within a cell is a big surprise and offers advantages over linear molecules due to their stability, which allows these ceRNAs to spread in different tissues[36]. CircRNAs can also be used as biomarkers in diagnosis and for the study of response to specific therapies in the case of animals and for the study of response to abiotic stresses in the case of crop species. Ectopic injection of circRNAs to counteract oncogenic miRNAs is also possible. While in some cases we need to counteract the effect of miRNA through the action of ceRNA, in other cases we may have to promote the action of miRNA to counteract the negative effect of certain genes, as in case of *Arabidopsis PHO2* gene.

Another important discovery is the role of pseudogenes, which may be transcribed in some cases to form ceRNA molecules. The recent study involving kinetics of the different components of the RNA network involved in regulation of gene expression suggests that the mechanism of operation of this regulatory system may not be simple and may be much more complicated than originally envisaged, thus warranting further studies. It is certain that many more potential ceRNA molecules with unforeseen biological impacts remain to be discovered. Next-generation sequencing technologies and sophisticated algorithms will certainly help in future to elucidate further the functional significance of these ceRNAs and to decipher in detail the complexity of the relationship between the coding and the non-coding genome.

It has also been shown that there are multiple mechanisms for regulating the turnover of miRNAs in living cells. In addition to ceRNA discussed in this article, the turnover of miRNA (in the form of 'miRNA turnover complexes', described as miRNasomes) is also controlled by several other factors, which include the following[37,38]: (i) presence/absence and the level of a target mRNA for one or more specific miRNAs; (ii) the extent of complementarity between miRNA and the target mRNA; (iii) miRNases, which degrade mature miRNAs, and (iv) targeted–mediated mature miRNA protection. These factors provide another layer of regulation to miRNA activity, thus outlining the complexity of the mechanism involved in determining the turnover of miRNA, which in turn affects gene expression. These aspects will be studied in greater detail in future, and their application in dealing with human diseases will be sought, since both under-expression and over-expression of miRNA have been shown to cause human diseases, including cancer.

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