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Review Article

Stable Intronic Sequence RNAs (sisRNAs): a Newer Insight to Cellular Regulatory Network

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ABSTRACT

With the advances in life science sector and its successful collaboration with cutting-edge technologies, newer aspects of cell regulatory pathways are discovered in a progressive manner. Intronic sequences, previously proclaimed as 'junks', were subsequently studied in depth to demonstrate their several biological functions and resulted in the discovery of small nucleolar RNA, micro RNA, small cajal body associated RNA, long non coding RNAs etc. In the year 2012, one more milestone was built in this aspect by the innovation of stable intronic sequence RNA (sisRNA) that was isolated from the oocyte cytoplasm of Xenopus tropicalis and confirmed by high-throughput sequencing. Surprisingly, this intronic RNA was found to be stable for a minimum of period of two days and this feature urged the scientists to explore its functional activities. Within half a decade sisRNA was found to be involved in several cellular metabolic activities like embryo development, immunoglobulin evolution, homeostasis maintenance etc. Therefore, this complex molecule can be explored in detail for its exploitation in the field of therapeutics, diagnostics and cancer biomarkers in the future course of work.

Keywords: *sisRNA, biomarkers, Xenopus tropicalis, Sequence RNAs*

INTRODUCTION

Introns are the non-coding RNAs interspersed between the exonic sequences, generated from their host mRNAs by the mechanism of post-transcriptional splicing. Introns were discovered independently by Phillip Sharp and Richard Roberts in adenovirus (Berget et al., 1977, Chow et al., 1977) while the nomenclature was done by Walter Gilbert in 1978 (Gilbert, 1978). Sometimes introns are also regarded as intervening sequences (Tilghman et al., 1978) but the latter also includes other sequences like inteins, untranslated sequences (UTR) etc. Introns are ubiquitously found in all eukaryotes, but rarely present in prokaryotes. Few eukaryotic genes also lack introns like histones (Nelson, 2008).

During post-transcriptional processing, splicing of mRNA is done to remove and degrade the intronic sequences. Nevertheless, the discovery of various non-coding RNAs viz. snoRNA (small nucleolar RNA), miRNA (micro RNA), scaRNA (small cajal body associated RNA) uncovered the fact that introns are not merely junk rather they play many important biological functions (Cech & Steitz, 2014). In 2012, Joseph Gall's laboratory reported the presence of some strangely stable introns in the oocyte nucleus of *Xenopus tropicalis*, which remained stable up to 48hrs (Gardner et al., 2012). Later, a high-throughput RNA sequencing by the team revealed the fact that these are maternally deposited non-coding sequences derived from their cognate coding strand, hence they termed these as stable intronic sequence RNAs (sisRNAs) (Gardner et al., 2012). Two years later, again the same group reported its presence in the cytoplasm of germinal oocyte in *X. tropicalis* (Talhouarne & Gall, 2014). Further research in this field uncovered the fact that sisRNAs are also found in *Drosophila*, *Arabidopsis*, viruses, yeast and mouse (Osman et al., 2016). Chan and Pek (2018) have recently defined sisRNAs as "the intronic sequences (i) are derived from the coding strands of their cognate host genes, (ii) are not rapidly degraded and (iii) may contain exonic sequences, 5' caps, and/or polyA tails". In this review, we intend to outline the research applications of sisRNAs in various aspects of biological science. Here we have summarized the recent discoveries, mechanism of biogenesis, functional roles, methods for laboratory isolation and various research applications of sisRNA.

1. Recent discoveries of sisRNAs:

As mentioned earlier, the first announcement about the presence of a new class of non-coding RNA i.e. sisRNA was published by Gardner in 2012 (Gardner et al., 2012). In addition to this, the following discovery was also reported in *Xenopus tropicalis*, while the former was found in the nucleus and the latter in the cytoplasm (Gardner et al., 2012, Talhouarne et al., 2014). Soon after, there are many researchers reported its presence in

various organisms like human, viruses, yeast, mouse etc. and are explained in detail in a review by Chan and Pek, 2019.

2. Features conferring stability to sisRNA:

Physiological generation of sisRNAs usually follows two different mechanisms, either splicing-dependent or splicing-independent pathway. Based on their biogenesis, they either can be linear, circular or branched lariat (Moss and Steitz, 2013). Usually, introns are degraded immediately after splicing as a turnover mechanism but sisRNAs avoid this canonical degradation pathway and hence becomes stable. In the case of circular sisRNAs, it is evident that they resist degradation by debranching mechanism (Chan & Pek, 2018). But very few is known about the mechanism behind the stability of linear sisRNAs. In 2018, Ng et al., reported that sisR-3 in Drosophila is transcribed directly from its cognate template and undergoes polyadenylation by a protein named 'Wispy' to maintain its stability in the cell. SisRNAs can be found in the nucleus and/or cytoplasm based on the type of tissue, organism and the developmental stage. In a recent review, Chan & Pek, 2018 has mentioned that most of the sisRNAs share a conserved secondary structure at their 3'-terminal (but the sequences are not conserved) that guides in targeting and binding to the complementary sequences.

3. Generation of sisRNAs:

There are two mechanisms for biogenesis of sisRNAs: splicing-dependent (Fig:1) and splicing-independent. In the first case, the intron is spliced out in the form of a lariat (circular intronic RNA with 3′ tail) during pre-mRNA splicing and hence gets processed by the lariat debranching enzyme or undergoes 3′-end trimming to form a linear or circular sisRNA, respectively. Recircularization of linear sisRNAs can also result in the formation of circular sisRNAs after debranching (Talhouarne and Gall, 2018, Li et al., 2015). Alternatively, exon back-splicing mechanism can also generate sisRNAs those may get circularised further and this latter process is highly promoted by the complementary elements (such as Alu repeats) flanking the

exons. This process competes with the production of linear mRNA during regular pre-mRNA splicing mechanism. A class of circular sisRNAs is found in the cells of mammals that was shown to contain both exons and retained intronic sequences (termed exon–intron circular RNAs, EIciRNAs) (Li et al., 2015).

The ebv-sisRNA-1 and ebv-sisRNA-2 from the Epstein–Barr virus (EBV) are reported to be independently transcribed to form unspliced transcripts of various lengths with or without polyA tails (Moss, 2014, Moss & Seitz, 2013). In few cases, sisRNAs were found to be transcribed from the promoter sites of the 5'-untranslated regions (5'-UTR) of genes (Tompkins et al., 2018). Intronic sequences can be fully retained in intron-retained (IR) transcripts or partially retained via intronic cleavage and polyadenylation (Chan and Pek, 2018).

4. SisRNAs- biological functions:

SisRNAs can regulate gene expression either by activation or repression which is mostly mediated by their binding to the target nucleotide sequence (DNA or RNA), besides they can also bind to proteins to regulate the function of the later.

In recent years many scientists reported the role of sisRNAs in activation of genes in *Drosophila* and human. In H9 cells of human, ci-ankrd52 (a circular intronic RNA) was found to enhance the transcription of its host gene *ANKRD52* by colocalizing with the RNA polymerase II complex at the transcription start site (Zhang et al., 2013). Another sisRNA named *sisR-4* in case of *Drosophila* binds to the intronic enhancer DNA of *deadpan* (*dpn*) gene and activates expression of the latter, which has an important role in *Drosophila* embryo development (Tay & Pek, 2017). Williamson et al. (2017) found that in normal cells, complete transcription recovery of *ASCC3* resumes 48hrs after UV irradiation which is mediated by the short *ASCC3* isoform (intronic sequence) that is synthesized due to slower transcription rate in early hours (Williamson et al., 2017). Further, intronic switch RNA was reported to be regulating the class switch

recombination for the generation of various immunoglobulin heavy chains (IgH). It was found that intronic switch RNAs (S), spliced from their parental gene i.e. immunoglobulin heavy chain gene, forms G-quadruplexes and bind to the RNA-binding site of activated cytosine deaminase (AID) thus guiding the latter to the parental gene locus (Zheng et al., 2015). AID helps in occurrence of class switch recombination to allow synthesis of heavy chains for IgG, IgA etc. (Stavnezer et al., 2008).

Besides activating gene, sisRNA also represses the activity of some genes and this phenomenon has been found in many organisms. The host gene *regene* (*rga*) in *Drosophila* is upregulated by its cis- natural antisense transcript (NAT) '*ASTR*' but the latter is downregulated by *sisR-1* via anti-sense mediated degradation (Pek et al., 2015). However, Wong et al., (2017) reported that the cytosolic sisR-1 in Drosophila can be regulated by a protein named DIP-1, which is synthesized from the fourth chromosome, binds to sisR-1 and forms satellite bodies. So, complete homeostasis in germinal stem cell development is maintained via DIP-1-*sisR-1-ASTR-rga* pathway (Wong et al. 2017). *SisR-2* and *sisR-3* repress the DFAR-1 gene and lncRNA *CR44148* respectively (Osman & Pek, 2018, Ng et al., 2018).

SisRNA can also interact with proteins and regulate their function. lnc-Lsm3 competes with viral RNAs for binding to RIG-1 during late stage of interferon (IFN) production to maintain the homeostasis of IFN production (Jiang et al., 2018). Further, it was reported that few lariat RNAs (escaped normal debranching) can compete with microRNAs to bind with miRNA-processing enzyme DCL1/HYL1 complex, thus, prevents maturation of miRNA from primary miRNA.

5. Method of sisRNA isolation and analysis:

Enrichment step is one of the essential procedures in the study of sisRNAs. The first high-throughput discovery of sisRNAs was reported in 2012 from Joseph Gall's laboratory where this was made possible by using clean and well-separated nuclear and cytoplasmic

fractions from the *Xenopus tropicalis* oocytes (Gardner et al, 2012). It is crucial to have a clean separation as even a small amount of cytoplasmic cross-contamination will completely mask the detection of sisRNAs in the nuclear fraction. The mature *X. tropicalis* oocyte is a good model for studying sisRNAs and nuclear-cytoplasmic fractionation as it contains a giant nucleus that can be manually dissected in an optimal isolation medium. For cell culture, nuclear-cytoplasmic fractionation can be conducted with centrifugation following homogenization in cold cell disruption buffer. Alongside nuclear-cytoplasmic fractionation, Other enrichment strategies can be employed as well to further improve the purity of sisRNAs (Moss et al., 2014). The $3' \rightarrow 5'$ exoribonuclease RNase-R has the ability to degrade all linear and branched RNAs leaving behind intact circular RNAs, have been used in labs for enrichment of circular sisRNAs and intronic lariats (Zhang et al., 2013). sisRNAs with or without polyA tails can be obtained by the method of polyA tail selections coupled with subsequent depletion of ribosomal RNA (Ng et al., 2018). Additional strategies like transcription inhibition by utilizing chemicals such as actinomycin D or α-amanitin would be able to differentiate sisRNAs from pre-mRNAs alongside conferring stability to the former (Talhouarne and Gall, 2018). All these mentioned procedures inclusive of handling techniques are going to significantly affect the quality of the isolated sisRNAs. Preferably, natural cells lacking nuclear compartments such as mammalian red blood cells, transcriptionally quiescent cells such as mature oocytes and early embryos should be significantly opted by researchers for the isolation of sisRNAs.

6. Future perspectives:

Study of sisRNAs is still in its infancy, so a lot must be explored for more of its applications to the field of medicine, diagnostics and research. Being relatively stable, sisRNAs can serve as ideal diagnostic targets for various diseases. Although, it is little known about the role of sisRNA in cancer, its function as cancer biomarkers may get unmasked in recent future. Its closeness to the site of transcription can be explored to use this for regulating its host gene & as a therapeutic target. Since introns are located at upstream sites, its targeting may result in a better outcome than targeting the downstream mRNA or proteins. Future studies in this regard are hence highly recommended.
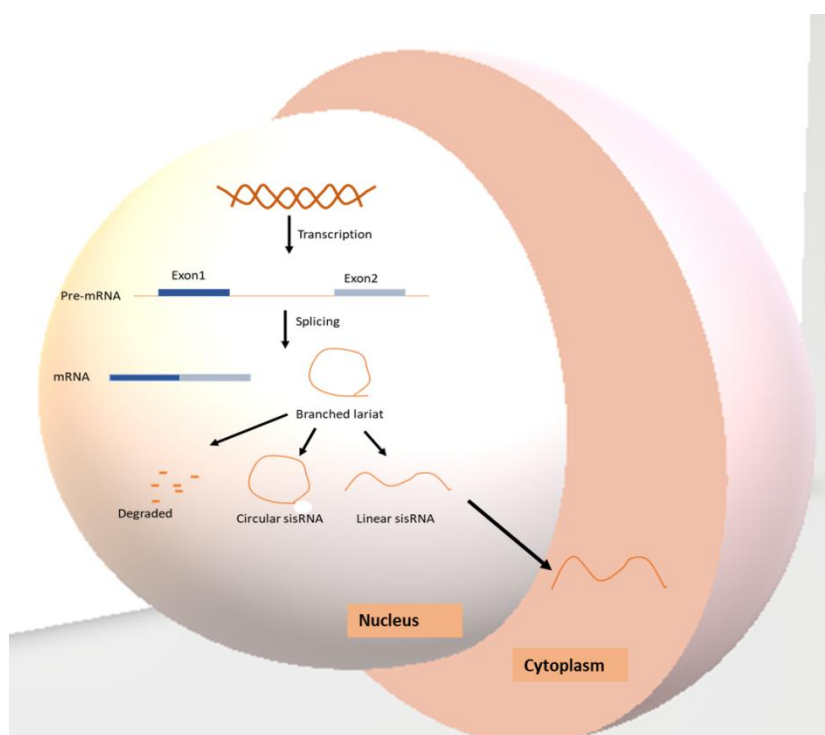

Fig. 1: Mechanism of sisRNA biogenesis by RNA splicing

CONCLUSION

SisRNA is the very recent addition to the class of non-coding RNAs. Since its discovery, a number of experiments were performed to provide the evidence of its presence in many organisms and in addition, some defined activities like its role during the course embryogenesis and development, class switch recombination in synthesis of multiple heavy chains, homeostasis of interferon production etc. have been already revealed. Therefore, this can be helpful in unfolding multiple circuits in synthetic biology as well as a regulatory molecule in the field of research. Its stability can be exploited for preparing diagnostics and therapeutics. But many facts are yet to be explored regarding sisRNAs such as more possible pathways for its degradation and stability, its role in various diseases and the pathway involved in its evolution etc.

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