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Review

RNA Interference – A fine tuner of gene regulation: a Review

S.K. Dash[1]*, Sushil K. Mohapatra[2] and H.N. Malik[2]

[1]Department of Animal Genetics and Breeding, COVS, GADVASU, India.
[2]Animal Biotechnology Centre, ICAR-NDRI, India.

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The discovery of RNA interference by Fire and Mello has opened up a new arena for research in molecular biology. The interfering RNAs (iRNAs) are 21-23 nucleotide RNA duplexes, which have the ability to reduce or abolish gene activity, and this property is being used in reverse genetics. Dicer and RISC (RNA-induced silencing complex) play important role in mechanism of RNA interference. The specificity, efficiency and potency make RNAi an attractive tool for analyzing the function of genes. Researchers are investigating the possibility of using RNA interference in treatment of various important diseases such as HIV, cancer, neurodegenerative diseases, malaria and various other diseases.

Key words: RNA interference, Dicer, RISC, reverse genetics.

INTRODUCTION

Gene expression has fundamental importance in all living organisms. RNA was supposed to be the key genetic material before its role was taken over by DNA in most of the living organisms. The discovery of catalytic RNA (ribozyme) has evolutionary implications and it is now realized that RNA plays a key role in gene expression. The regulation of gene expression is done at two stages, first is the transcriptional level in which transcriptional factors are involved along with other mechanisms such as histone acetylation and DNA methylation and second is the post transcriptional level that is by mRNA processing, translation of mRNA and RNA interference (RNAi). Mechanism of RNA interference (RNAi) was first given by A. Fire and C. Mello and this is a conserved biological response to double stranded RNA, which mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, as well as regulates the expression of protein coding genes (Hannon, 2002). Earlier RNAi was called by other names, including post transcriptional gene silencing and transgene silencing, but it was obvious that they were the same phenomenon only after these were characterized at the molecular level. RNAi can be defined as the ability of exogenous or endogenous double stranded RNA to suppress the expression of the gene which corresponds to the sequence of double stranded RNA. The RNAi is a knockdown and not knockout mechanism of gene silencing, with the advancement of genetic engineering (Volpe and Martienssen, 2011). RNAi is proving to be an alternative approach for stable silencing of genes in a

*Corresponding author. E-mail: shaktikant07@gmail.com.

variety of different animal species. RNAi has been linked to various cellular processes, including the formation of centromeric structure (Djupedal and Ekwall, 2009) and gene regulation, through microRNAs and heterochromatin formation (Dehio, 1994; Wassenegger, 2005). *Science* named RNAi as the 'Technology of the Year' in 2002. During the last few years RNAi has move to the cutting edge of molecular biology.

DISCOVERY OF RNA INTERFERENCE

At first gene silencing in plants drew the interest of researchers. It was reported that introduction of transgenes homologous to endogenous genes resulted to genes suppression of these genes, this process was known as co-suppression, in which there is degradation of the endogenous and the transgene mRNA (Paddison et al., 2002). A similar process of silencing, called Quelling was also reported in fungi (*N. crassa*) (Romano and Macino, 1992). The explanation for the gene silencing studied in plants for several years was provided by A. Fire et al. (1998) through post transcriptional gene silencing (PTGS) called RNA interference (RNAi). They received Nobel Prize in Physiology or Medicine in 2006 for this novel discovery. In their work Fire and Mello studied the phenotypic effect after injection of single stranded and double stranded unc-22 RNA into gonad of three different groups of *C. elegans,* the unc-22 gene encodes a myofilament protein and the decrease in unc-22 activity is known to produce severe twitching movements. They found that the group that was injected with double stranded RNA produced severe twitching movement while the other two groups exhibited normal or wild type movements. Similar process of RNAi has been reported in Drosophila (Filippo et al., 2011) and in mammals (Yang et al., 2001).

RNA INTERFERENCE (RNAi)

Double stranded (dsRNAs) are produced in cell either through transcription of microRNA genes, which consists of inverted repeats or through viral replication (dsRNA Rotavirus). In RNAi long (70-90 nts) dsRNA is digested into short interfering or siRNA (21-23 nts). This siRNA silences homologous genes by destruction of its mRNA or through inhibition of translation of its mRNA or by inducing chromatin modifications in its promoter region (Fire et al., 1998). The interfering RNAs (iRNAs) may be classified into two types, short interfering RNA and micro RNA. The siRNA are exogenous and 21-23 neucleotides in length. They can be designed *in vitro* or the single stranded and double stranded RNA virus infecting the cell are other sources of siRNA. The non protein coding microRNA (miRNA) genes are the other source of iRNAs These miRNA genes produce iRNAs of 21- 22 nucleotides. length (Zamore et al., 2000).

MECHANISM OF RNAi

Endogenous miRNA

The Primary miRNA produced from the transcription of miRNA genes, undergo first stage processing in the nucleus and are acted upon by the complex consisting of enzymes Drosha and Pasha, to produce short hairpin pre miRNAs or sh- pre miRNAs. These pre miRNAs are exported into the cytoplasm by the enzyme Exportin5. In the cytoplasm the pre miRNA undergo second stage processing by the activity of enzyme Dicer (a type of ribonuclease III), which cleaves the pre miRNA to produce siRNA duplex. The Dicer then loads the guide strand into the RISC (RNA induced silencing complex); the guide strand is the strand of siRNA duplex which participates in silencing of its complementary mRNA sequence and the one that does not is the passenger strand. The RISC after loaded with the guide strand which carries it to the complementary mRNA so that the guide strand can bind to the complementary sequence in mRNA. The mRNA is then cleaved or degraded if there is a complete complementarity between the two strands. When there is lesser complementarity, leads to suppression of synthesis of the protein in which mRNA is involved.

Exogenous miRNA

The mechanism of down regulation of exogenous miRNA is similar to the endogenous one, except that in endogenous iRNA processing pathway, the first phase processing does not occur, so the Drosha Pasha enzymes do not take part in the processing of mRNA.

Non-Stoichiometric effect of RNAi

RNA interference mechanism involves only small amounts of dsRNA that can wipe out excess of mRNA. This enzymatic mechanism involves RNA dependent RNA polymerase (RdRp). The sense strand which does not participate in RNAi is acted upon by RdRp as a result of which a double stranded RNA is produced which in turn again participates in RNAi pathway (Zamore et al., 2000).

Dicer

The dicer is a cytoplasmic RNase III enzyme with endo-nuclease activity and it functions as a molecular ruler producing dsRNA fragments 21-23 nucleotides in length. Structurally the dicer is composed of three rigid regions

namely the RNase domain, Platform domain and PAZ domain; these domains are connected by flexible hinges (Filipowicz et al., 2005 and Macrae et al., 2006). Dicer recognizes the end of a dsRNA with PAZ domain and then cuts the dsRNA with its RNase III domains. The distance between PAZ and RNase III domains determines the length of small RNAs generated. Homologs of Dicer exist in many organisms including *C. elegans*, *Drosphila*, yeast and humans. After hydrolysis of substrate RNAs, Dicer aids in loading its small dsRNA product into RISC. The thermodynamic asymmetry along the siRNA or miRNA duplex determines “guide” and “passenger” strands. The dicer presents the strand of the siRNA duplex (whose 5′ end is thermodynamically less stable) as the guide strand to the RISC (Bernstein et al., 2001).

RISC complex

The RNA induced silencing complex otherwise called RISC is approximately 500-kDa RNA-multiprotein complex, which triggers mRNA degradation in response to siRNA. RISC is involved in three major functions a) *Helicase*- unwinding of double-stranded siRNA b) *Nuclease*- ribonuclease component cleaves mRNA c) *RNA-dependent RNA polymerase RdRp*- amplification of silencing signal (Cogoni et al., 1999). Argonaute protein is the signature component of RISC and it has two characteristic domains PAZ (also found in Dicer) and PIWI. The cleft of the PAZ domain binds to the characteristic two-nucleotide 3' overhang of the guide strand; the RISC then carries the guide strand to the complementary mRNA to participate in the process of silencing (Macrae et al., 2006).

APPLICATIONS OF RNAi

Reverse genetics

The function of a gene can be identified by blocking the translation of mRNA and studying the resultant phenotypic change, this branch of genetics is termed as reverse genetics. This is one of the major area of research in which RNA interference is applied. A number of genes whose function has been identified through RNAi are SRP72 (Signal Recognition Particle) which functions in the cytoplasmic protein translocation pathway; PEPCK- a rate-controlling enzyme in gluconeogenesis; BCL2- a potent inhibitor of cell death; CAMK2G- which plays an important role in mammary gland development of goat, β-lactoglobulin- whey protein gene in swine.

Therapeutic applications

Diseases caused by microbes are the major cause of death worldwide. Research using RNAi technique has been found to be effective in the treatment of a number of prominent viral diseases such as HIV and influenza, hepatitis and west nile virus and bacterial infections such as pneumonia and sepsis. RNAi when used in cell culture studies has given promising results in inhibiting the replication or cellular uptake of viruses and other infectious agents. RNAi does not interact with the DNA of an individual rather it interferes with the translation process of gene expression. This would alleviate the patients concern as no changes to ones DNA is made as incase of gene therapy which suggests that this method of treatment would no more be feared than taking any prescription drug. For this reason therapies based on RNAi have attracted much interest of researchers in the pharmaceutical sector.

Transfection of human cells with siRNAs directed against different genes in the poliovirus genome resulted in resistance of the cells to infection with poliovirus (Gitlin et al., 2002). The ability of siRNAs targeting gene encoding the death receptor Fas to protect mice from liver failure and fibrosis in two models of autoimmune hepatitis has been tested. Intravenous injection of Fas siRNA reduced Fas protein levels in the livers of mice during 10-day period. Fas siRNA treatment was found to inhibit hepatocyte necrosis and inflammatory infiltration and markedly reduced serum concentrations of transaminases demonstrating a clear hepatoprotective effect of siRNA therapy (Song et al., 2003).

Potent and specific inhibition of HIV type 1 replication has been reported by RNAi, despite the success of RNAi mediated inhibition of HIV-encoded RNAs in cell culture, targeting the virus directly represents a substantial challenge as the viruses have a high mutation rate. Therefore down regulation (through RNAi) of cellular cofactors required for HIV infection presents an attractive alternative approach; some of the cellular cofactors that have been successfully downregulated by RNAi are NF-B (Surabhi and Gaynor, 2002) and HIV receptor CD4 (Novina et al., 2002).

The cancer cells exhibit two general abnormalities; firstly, dysregulation of cell cycle resulting in uncontrolled growth and secondly loss of apoptosis due to abnormalities in one or more proteins that mediate apoptosis. miRNAs such as let-7 and miR-15/miR-16 block Ras and BCL2 oncogenes and are promising candidates for cancer treatment (Hannon, 2002). Lentiviral vectors were used to produce transgenic porcine cells expressing shRNAs that inhibit FMD viron replication and mutation (Long and Westhusin, 2007). Transgenic *Anopheles* strains carrying miRNA constructs can silence the genes in the immune system of the mosquito that prevent *Plasmodium* recognition by immune system, such strain of Anopheles can be left to the field to replace the wild type and thus malaria can be controlled (Malhotra et al., 2002).

The signal recognition particle (SRP) is known to be

involved in the synthesis of membrane and secretory proteins across or into the endoplasmic reticulum (ER) membrane in eukaryotes (Köch et al., 2003). RNAi silencing of SRP72 and SRP68 lead to the depletion of these particles and induced sudden death in *T. brucei*, due to toxicity leading to accumulation of the pre-SRP in the nucleolus (Lustig et al., 2005). Suppression of prion (PrP) protein in transgenic goats was possible by RNAi and thus a significant (>90%) decrease in PrP expression levels was observed (through Western Blotting) in transgenic goats, carrying miRNA constructs that silence the genes coding for prion particles (Golding et al., 2006). Investigations are going to find out whether RNAi can be used as a remedy for treating various neurodegenerative disorders such as Alzheimer's, Huntington's or Parkinson's disease. One of the strategies for treating neurodegenerative disorders is by blocking disease specific events that initiate these disorders. Pro-apoptotic members of the Bcl-2 family were targeted by RNAi and neuronal death was successfully prevented (Colussi et al., 2000).

CHALLENGES FOR RNAi THERAPY

The RNAi can be used as a major tool in therapeutics however various challenges are there in developing RNAi as a therapy, such as i) efficient targeted delivery to specific cell or tissue types is still not a practical reality for oligonucleotide-based therapeutics ii) Systemic delivery of viral vectors is still a major hurdle iii) Off-target effects (OTE) - arise when an introduced RNA can pair with multiple genes at a time. Today the delivery related research in RNAi is receiving much desired attention.

Conflict of Interests

The author(s) have not declared any conflict of interests.

CONCLUSION

Studies started first in plants, flies, and worms paved way for RNAi for being discovered as an accidental observation. RNAi machinery can handle both endogenous and exogenous dsRNA and the faster identification of gene function is possible through RNAi. It is a powerful experimental tool in reverse genetics and this technique has wide spread future applications in medicine. RNA interference (RNAi) is a relatively new technology that is revolutionizing the way that researchers study mammalian gene expression. RNAi has had significant impact on the ease, speed, and specificity with which the loss of gene function analysis can be done in mammalian cells and animal models. This is becoming the method of choice for researchers doing loss of function studies.

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