# RNAi: Gene Silencing

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*Abstract*: **Gene Editing is a major challenge existing in the era to cope up the genetic diseases prevailing. With the advancement in Technology in Molecular Biology. It's a gene regulatory approach which is highly stimulated by small size molecules of interfering RNA to suppress the gene of interest effectively. This method involves silencing of a single stranded RNA by binding of another RNA molecule. Interference occurs naturally in Plants and animals too. The phenomenon has evolved so as to provide a barrier and defence mechanism against Viruses. Within time it has been found that RNAi, play a crucial role in regulation and alteration of different pathways in animals and Plants too. It has been found, with more intensive studies and researches, it could be possible to implement this process to enhance different pathways in living systems.**

*Keywords*: **RNAi, Gene Silencing**

## 1. Introduction

RNAi is a mechanism for silencing gene expression. In other words, it prevents translation of RNA. RNAi takes place in all eukaryotes organisms and act as a method of cellular defence (found by Craig Mello and Andrew Fire in 1998) against foreign DNA or RNA sequence or when a particular mRNA is over expressing itself which can cause problems in the homeostasis of cell or organisms. This method provides new platform through which ecofriendly molecular tools can be developed for improvement in crops by inhibiting the gene responsible for various stresses and by improving the inherent property of disease resistance. Transgenic plants that can produce RNAi inducers throughout its life would be cost effective.

This method involves silencing of a specific mRNA using a complementary RNA to make it a double stranded RNA. Translation of double stranded RNA is not possible so this process neutralize the problematic RNA.

This type of silencing provides protection to genetic material by suppressing the action of transposons and retro elements.

## 2. Mechanism of RNAi

RNA interference is initiated by the enzyme endoribonuclease also called dicer which cleaves long double stranded RNA (dsRNA) into short fragments of 20-25 nucleotides. These fragments are double stranded and called small interfering RNA (siRNA). These siRNA are then unwound into two single strands. one of these strands is degraded and other is integrated into Argonaut protein, part of RNA induced silencing complex(RISC). siRNA can also silence the gene by methylation of DNA (George Sen and Helen Blau 2006).

*DICER:* The dicer is a cytoplasmic RNAse III enzyme having endonuclease activity. The function of dicer is to cut long strand of dsDNA into small fragments of 20-25 nucleotides. Dicer have three regions called RNase domain, platform domain and PAZ. The dsDNA recognizes the end of dsDNA with the help of PAZ domain and cuts it with RNAse III domains.

*RISC Complex:* RNA inducing silencing complex also called RISC in a multiprotein complex which activates the degradation of mRNA by responding to siRNA(Zofia et al 2003) .RISC include three enzymes helicase ,nuclease-ribonuclease, RNA-dependent RNA polymerase .Helicase enzyme unwinds the double stranded siRNA ,Nuclease-ribonuclease cleaves mRNA and RNA-dependent RNA polymerase (RdRp) amplifies the silencing signal .the main component of RISC is Argonaute protein that cleaves target mRNA strand complementary to their bound siRNA. Dicer produces short double stranded fragments so there are two functional single stranded siRNA produced. But only one of the single-stranded RNA here will be utilized to base pair with target mRNA .it is known as guide strand, incorporated into the Argonaute protein and leads gene silencing. The other single stranded named passenger strand is degraded during the RNA – induced silencing complex process.

*RNA helicase:* RNA helicase cause unwinding of double stranded RNA. Helicase works downstream of the RISC complex. Two major families of RNA helicase are involved in RNAi (Devi et al. 2016).

*RNA-dependent RNA polymerase (RdRp):* RdRp catalyzes amplification of RNAi, which is in small amounts. RdRp catalyzes the siRNA-plasmid amplification by polymerase chain reaction to convert mRNA into dsRNA, a long form that is cleaved to produce new siRNAs .

## 3. RNAi for plant disease resistance

Pathogens causes huge reduction in crop yield that have significant negative impact on economy. These pathogens can

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become threat to eliminate the entire plant species. Traditionally pesticides have been used to prevent crop damage done by the pathogens but when it was discovered that these pesticides pollute the environment and are harmful to human by bio magnifications scientists began to find safer inexpensive alternative that can be used to protect crops.

In early 1990s Napoli and colleagues tried to deepen the colour of petunia by introducing a strong promoter gene called chalcone synthase but unexpectedly the flower become de-pigmented as transcription of endogenous gene was reduced. Due to the fact that both endogenous gene and transgene were suppressed, this phenomenon was termed as "co-suppression".

The biggest challenge in RNAi research field is the transportation of molecules that will activate the RNAi pathway in the plants. There are several method through which these molecules (dsRNA or siRNA) can be transported into the plant cells such as agroinfilteration, virus induced gene silencing (VIGS), micro-bombardment, etc.

### 4. Methods to induce RNAi in plants and animal cell

#### A. Gene gun

Gene gun or biolistic particle delivery system is device used to transport exogenous DNA (transgenes) to cells .in this method a particle of heavy metal like tungsten, gold, or titanium are coated with DNA or RNA. Transfer of gene causes minimal damage to cell but it is efficient. The injected particle coated with gene activates the RNAi mechanism in cell. the result of RNAi silencing can be observed in no time.

#### B. Virus Induced Gene Silencing (VIGS)

Viruses that have modified RNA are used to activate the RNAi mechanism of cell. These viruses act as vectors for gene expression, such as tobacco mosaic virus, potato virus etc.

#### C. Agrobacterium tumifaciens

Agrobacterium is a plant pathogen that causes crown gall disease in many species of plants. This bacterium contain tumor inducing gene in its plasmid (non-chromosomal DNA) along with some additional gene that help this T-DNA to integrate into the host genome. For genetic engineering purposes, this bacterium must be disarmed which can be done by removing T-DNA leaving those additional genes that help in integrating the plasmid into host genome.

#### D. Electroporation

Electroporation is not a common method to transport foreign DNA in host cell. This method uses electrical field which increases the permeability of cell membrane.

### 5. Medical Applications

RNAi has several applications in biomedical research, immune system and health care for example in treatment of HIV, hepatitis, cardiovascular disease metabolic disease, cancer, neurodegenerative diseases etc. The ability of SiRNA

and longer dsRNA to induce innate immune responses is still being debated Mammalian cells are severely sensitive to the introduction of dsRNA , it is believed that molecules less than 30 bp in length are generally avoid induction of interference pathways. Gene therapy attempts to treat genetic diseases using normal copies of defected gene by transferring the nucleic acid as drug. RNAi can be used for gene therapy and it have several advantages over normal gene therapy such as high efficiency, sequence specificity (Mengyu et al.). RNAI not only suppress transcription but also initiate the degradation of mRNA by post-transcriptional gene silencing (PTGS), these two process reduce the transcription of coding gene. RNAi based gene therapy is becoming an alternative which can permanently cure deadly disease such as Haemophilia, leukaemia, multiple myeloma etc.

#### A. Haemophilia

Haemophilia is an X-linked blood disorder that weakens the body's ability to make blood clots, process that stops bleeding. Rnai based gene therapy using both viral and non-viral vectors have been attempted to treat haemophilia. Treatment of haemophilia is still in primary stage of development.

#### B. Cancer

Cancer is a genetic disease that is caused due to mutation in genome which leads to abnormal cell growth and cell division that have the potential to invade the other organs of the body. Mutation in proto oncogene, which is responsible for normal cell division can cause uncontrolled cell growth which causes cancer. Mutation in tumour suppressor gene is also a cause of cancer. Due to its high efficiency and potential, RNAi is very effective in cancer treatment. oncogene and tumor suppressing gene are the main target to be silenced by RNAi. Inhibition of multiple gene that are responsible for cancer at the same time is an effective approach to treat cancer and it also reduces the possibility of multiple drugs resistance caused by overdose of chemical drugs. Personalized drugs are more effective than other drugs to control tumor growth. These personalized drugs can be developed by this method (Behzad et. al. 2014).

#### C. HIV

Human deficiency virus have 9 viral gene that are required for all process including receptor binding, viral entry, replicative cycle etc. HIV infects CD4+ T lymphocytes leading to reduction in cell number which leads to acquired immune deficiency syndrome (AIDS).RNAi is designed to block crucial steps in the replication cycle of virus .despite the success of RNAi mediated inhibition of HIV-encoded RNAs in cell culture ,targeting the virus the is present in humans is difficult because of high mutation rate in the genome of virus (John Rossi 2018).This problem can be avoided by targeting cellular transcripts that encode functions that required by the virus for entry and replication.

#### D. Hepatitis B

Hepatitis is an infectious disease caused by HBV and causes

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liver cirrhosis and hepatocellular carcinoma. HBV infection is currently treated by using interferons but treatment is partially successful. RNAi has the potential to treat HBV infection. RNAi efficiency was tested in laboratory involving co-delivery of HBV replicon and an expression unit encoding an anti-HBV shRNA. This study demonstrated significant result in destroying HBV core antigen in liver hepatocytes.

### E. Metabolic disease

RNAi can be used to silence endogenous gene that are involved in the pathway of metabolic disease. Through RNAi those genes can be silenced that code for non-drugable proteins. Phosphoenol pyruvate carboxykinase (PEPCK) is rate controlling enzyme in gluconeogenesis and different rate of gluconeogenesis is responsible for change in hepatic glucose output and sustained hyperglycaemia.

## 6. Applications in plants

The RNAi technology can be considered as eco-friendly, biosafe as it eliminates risks that are associated with the development of transgenic plants. Through RNAi genetically modified plants are being developed that are resistant to nematodes, insect, fungi, Viruses, parasitic weeds etc.

Some examples of genetically modified plants,
1. Transgenic Arabidopsis thaliana is resistant Heterodera schachti (a nematode).
2. Transgenic tobacco and soybean.
3. Transgenic rice resistant to phloem feeding insects.
4. Transgenic potato, banana, barley resistant to fungi.
5. Cassava resistant to virus ACMV.
6. Tomato resistant to tomato yellow leaf curl virus (TYLC).

Metabolism manipulation by RNAi can improve fibre quality, early flowering, early maturity, increased oil content in seeds, increased carotenoid and flavinoid in fruits without affecting any other components, and productivity in plants. These qualities are beneficial from industrial point of view.

RNAi can be used to find the function of gene by blocking them and studying the result of change. The main cause of most deaths worldwide is the disease caused by microbes.

With well-organized research in the field of RNAi mechanism and understanding its entire process could create a new branch of biological science that can offers genetically modified plants that high nutrition and economic value.

### References

[1] George L. Sen, and Helen M. Blau, "A brief history of RNAi: the silence of the genes," in *The FASEB Journal,* vol. 20, pp. 1293-1299, July 2006.
[2] Szweykowska-Kulinska, Zofia, Artur Jarmołowski and Marek Figlerowicz, "RNA interference and its role in the regulation of eucaryotic gene expression," in *Acta biochimica Polonica*, vol. 50, no. 1, pp. 217-229, 2003.
[3] Devi Singh, Sarika Chaudhary, Rajendra Kumar, Preeti Sirohi, Kamiya Mehla, Anil Sirohi, Shashi Kumar, Pooran Chand and Pankaj Kumar Singh, "RNA Interference Technology — Applications and Limitations," in Intechopen.com, April 2016.
[4] Mansoori, Behzad, Siamak Sandoghchian Shotorbani and Behzad Baradaran, "RNA interference and its role in cancer therapy," in *Advanced pharmaceutical bulletin,* vol. 4, no. 4, pp. 313-321, 2014.