

GENE SILENCING IS ONE OF THE MOST EFFICIENT AND PROMISING
FUNCTIONAL GENOMICS TOOLS

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ABSTRACT

Gene silencing is defined as epigenetic of gene expression leading to inactivation of previously active individual genes or larger chromosome regions. Silencing of a target gene can be achieved at two levels-transcriptional and post-transcriptional stages. The transcriptional gene silencing occurs through the repression of the process of transcription & the post transcriptional gene silencing occurs by the degradation of mRNA. There is also another type called Meiotic. Mechanisms responsible for repression of genes involve changes in level of DNA methylation & alterations in covalent modifications of histone proteins which leads to chromatin compaction. These are the effects of small RNA regulators such as small interfering RNAs (siRNAs), microRNAs (miRNA), Piwi-associated RNAs (piRNAs) etc. Virus can also conduct gene silencing which is known as Virus Induced Gene Silencing by cloning and inserting plant endogenous gene sequence in recombinant viral vectors. Even Gene Silencing helps in buildup resistance against biotic & abiotic stress & helps in knock down any kind of undesirable genes.

KEYWORDS: Gene silencing, miRNA, dsRNA, RNAi.

INTRODUCTION

The world population has been increasing day by day at an exploding rate and as per UN report, 2017 & FAO Revised Report 2012 there will be 9.8 billion people by 2050. To feed this population a huge amount of food is needed. But there are various factors like climate change, pollution leads to rise in temperature, irregular rain

distribution & intensity, hail-storm, earthquake etc. which directly or indirectly effecting the growth & development of crops & other food sources & decreases the productivity of crops. Even there is also a significant loss of crops are due to attack of pathogens in all most all the horticultural & agricultural species.^[1-5]

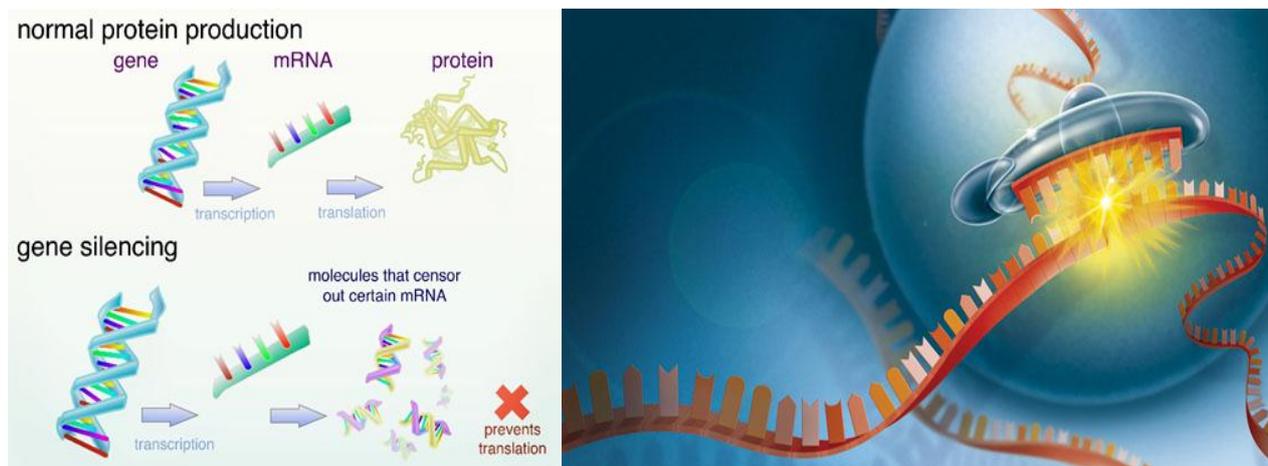


Figure-1: Gene silencing.

To fix all these problems the advanced biotechnology & genetic engineering come up with various tools like Gene Silencing, Genetic Transformation, rDNA technology etc. The discovery of mechanisms that suppress gene activity in plants has extended the horizontal for research on control of gene expression. Gene mapping, haploid mapping etc. helps in identifying different genes. To silence or down regulate the targeted gene, gene silencing mechanisms offers an explicit opportunity. Gene Silencing is also used in food quality modification such as the reduction of caffeine levels in coffee beans & to increase the nutrition value of corn protein & tomatoes. Research on forest tree yield & quality has included the study of gene silencing related to lignin synthesis. Even research on fruit crops has targeted application of gene silencing on viral &

bacterial resistance and physiological aspects such as self-fertility. Plant gene function by effecting gene expression through silencing technique (PTGS/RNAi and VIGS) is also under recent investigation.^[6]

Short history of gene silencing

✓ **1990 JORGENSEN:** Richard A. Jorgensen (born 1951) is an American molecular geneticist and an early pioneer in the study of post transcriptional gene silencing. To deepen the pigmentation in petunias, introduction of endogenous genes often resulted in plants with both gene suppressed called **Co-suppression**, which resulted in degradation of endogenous & transgene mRNA.^[7]



Figure-2: Scientists of gene silencing.

✓ **1995 GUO & KEMPHUES:** Injection of either antisense & sense RNAs in the germline of *Celegans* was equally effective at silencing at homologous target gene.

✓ **1998 MELLO & FIRE:** Extension of above experiments, combination of sense and antisense RNA (=dsRNA) was 10 times more effective than single strand RNA.

The discovery of the mechanism of RNA interference by dsRNA by **Prof. Andrew Fire and Prof. Craig Mello** in 1998, gave them the Nobel prize in 2006.^[8]

How does it work?

- This is accomplished by binding a specific standard of RNA to an existing m-RNA standard.
- The m-RNA creates a copy of DNA standard.
- By binding the RNA to the m-RNA, m-RNA is prevented from replicating that portion of DNA.
- Specific genes can be targeted & prevented from replicating in to a DNA strands.

Mechanisms of gene silencing: Some early findings regarding GS occurred when a team of researchers tried

to obtain transgenic petunias with greater amounts of anthocyanin pigments, by amplifying the gene activity of chalcone synthase. Instead of obtaining deeper purples in the petals, white or chimeric flowers were produced. Apparently, the transgene was not expressed, and ended up silencing a homologue endogenous gene. The phenomenon, named "co-suppression", was unstably transmitted within generations leading to the hypothesis that it was mediated by a nucleic acid, presumably RNA.^[9]

Similar phenomena were named "quelling" in fungi and "RNA interference" (RNAi) in *Caenorhabditis elegans*. The research indicated that the presence of double stranded RNA (dsRNA), a non-occurring form in normal cells, was related to the silencing of sequence homologue genes. The fact that the phenomenon seemed to be triggered by the presence of doubled stranded RNA (dsRNA) suggested that this could be originally a defense mechanism against viruses and transposable elements, since these originate dsRNA.^[10]

Most of the GS phenomena are related to RNA activity within the cell. Therefore, the term RNA silencing is often used to describe GS and comprise all mechanisms

by which RNA sequences regulate gene expression, except those sequences characterized as mRNAs, tRNAs, or ribosomal RNAs. Genetic and biochemical studies have confirmed that the mechanisms of RNAi, co-suppression, and virus-induced gene silencing are similar. Moreover, the biological pathways underlying dsRNA-induced GS exist in many, if not most,

eukaryotic organisms. The study of similar phenomena in different organisms (*Caenorhabditis elegans*, *Neurospora crassa*, *Drosophila melanogaster*, *Arabidopsis thaliana* and *Petunia x hybrida*) allowed the proposal of models for different but interacting forms of silencing.^[11]

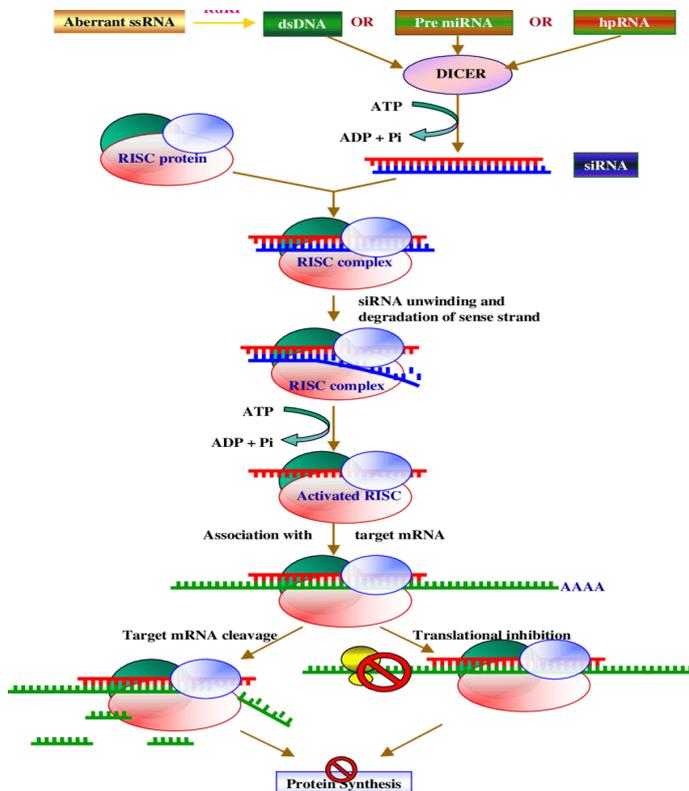


Figure-3: The molecular mechanism of post-transcriptional gene silencing.

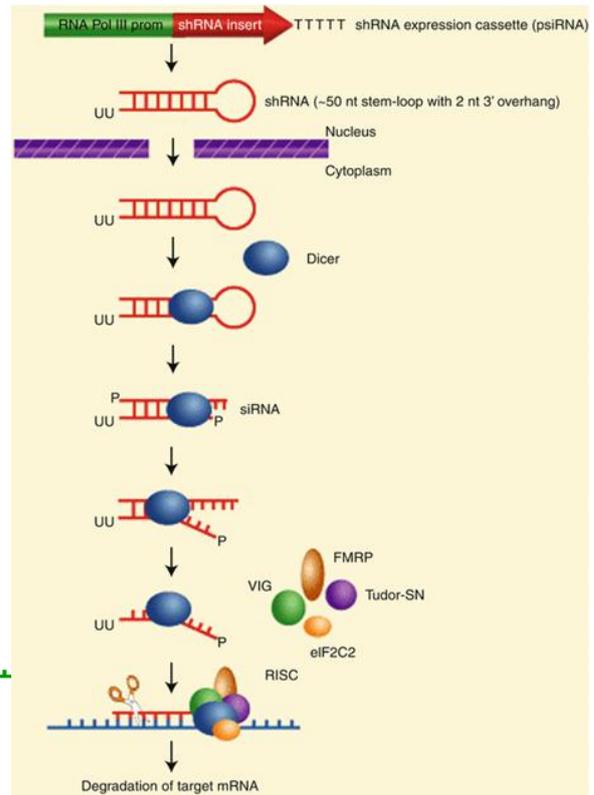
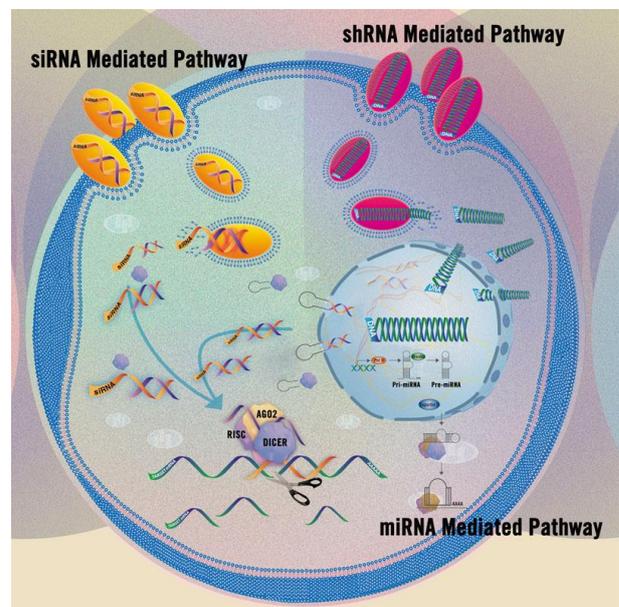


Figure-4: Transcriptional Gene Silencing.

Characteristics: Gene silencing is a general term describing epigenetic processes of gene regulation. This process is important for the differentiation of many different types of cells. Genes are regulated at either the transcriptional or post-transcriptional level. Transcriptional gene silencing is the result of histone modifications, creating an environment of heterochromatin around a gene that makes it inaccessible to transcriptional machinery (RNA polymerase, transcription factors, etc.).^[12]

Types of gene silencing: There are various gene silencing methods currently employed in research and being developed as potential disease therapeutics. Nearly all of them involve disabling the function of mRNA by preventing it from being translated into a protein. However, they differ in the design of the molecule used to disrupt mRNA and the manner of mRNA breakdown. As a result, different silencing methods have specific advantages and drawbacks. Two of the leading and most understood methods of gene silencing are -

1. Transcriptional Gene Silencing (TGS)
2. Post Transcriptional Gene Silencing (PTGS)



Transcriptional Gene Silencing: The result of histone modifications, creating an environment of hetero-

chromatin around a gene that makes it inaccessible to transcriptional machinery (RNA polymerase, transcription factors etc).

Genomic Imprinting

- Genomic imprinting is an inheritance process independent of the classical Mendelian inheritance.

It is an epigenetic process that involves DNA methylation and histone methylation without altering the genetic sequence.

- It has been demonstrated in insects, mammals & flowering plants.^[13]

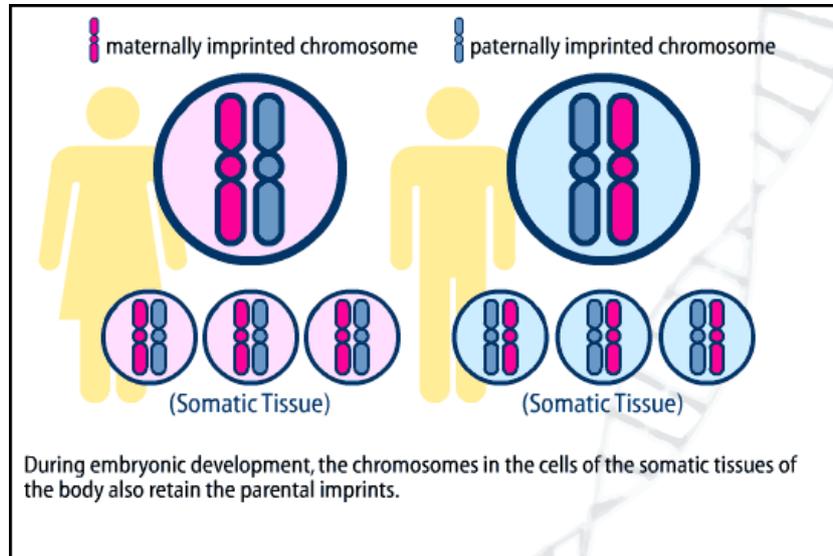


Figure-5: Genomic Imprinting.

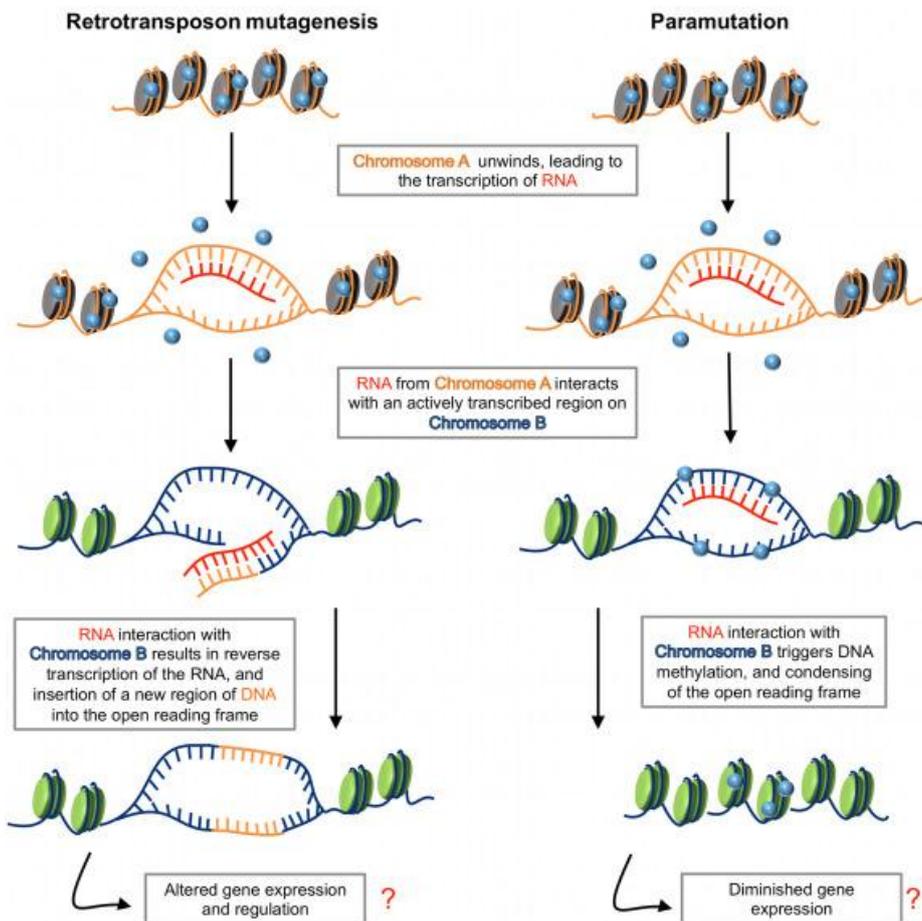


Figure-6: Paramutation.

Paramutation: In epigenetics, a paramutation is an interaction between two alleles at a single locus, whereby one allele induces a heritable change in the other allele. The change may be in the pattern of DNA methylation or histone modifications.

Position Effect: Position effect is the effect on the expression of a gene when its location in a chromosome is changed, often by translocation. This has been well described in *Drosophila* with respect to eye color and is known as position effect variegation (PEV).^[14]

- It was first observed by the effect it had on the colour of corn kernels in maize plants.

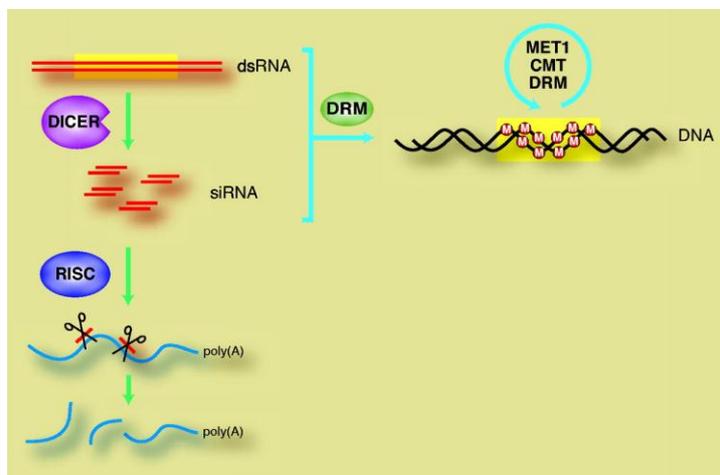


Figure 7: RNA Directed DNA Methylation.

RNA Directed DNA Methylation: RNA-directed DNA methylation (RdDM) is prevalent in flowering plants and induces transcriptional silencing at repetitive DNA, including all types of transposons.

particular area of DNA. Transcriptional silencing of transposons is crucial to the maintenance of a genome.

In the model plant organism *Arabidopsis thaliana* Transposon Silencing: Silencing is a form of transcriptional gene silencing targeting transposons. Transcriptional gene silencing is a product of histone modifications that prevent the transcription of a

- "Jumping" of transposon generates the genomic instability & cause the extremely deleterious mutations.
- Transposable element insertion has been linked to many diseases including haemophilia, SCID & predisposition to Cancer.^[15]

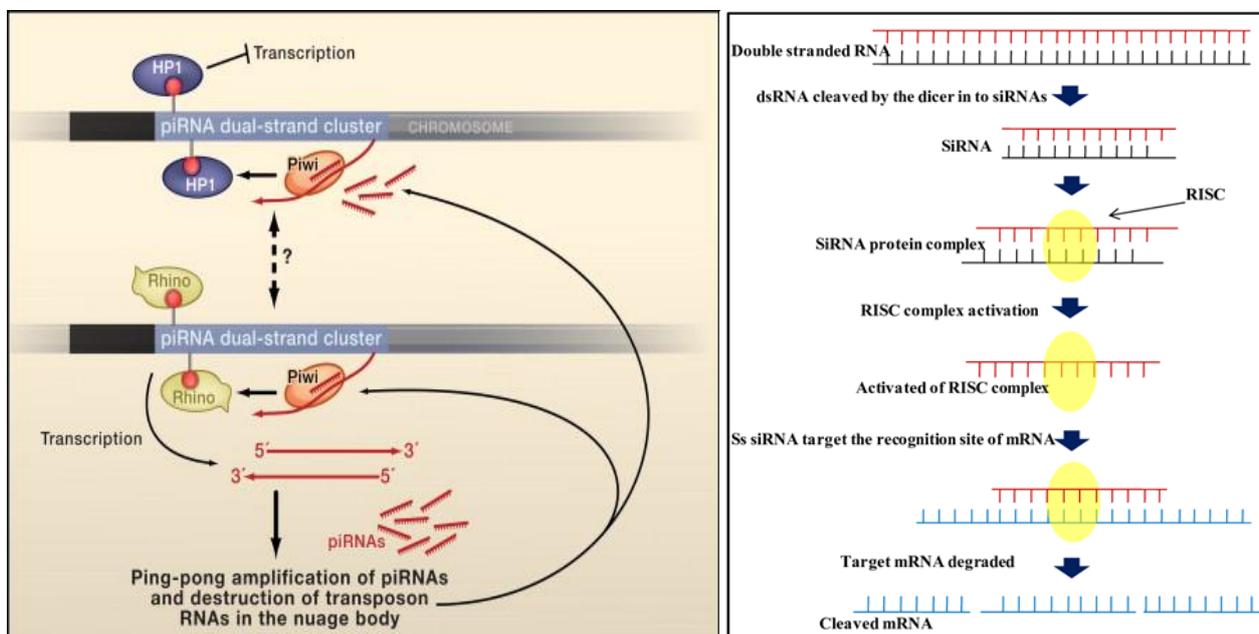


Figure-8: Transgene Silencing.

Transgene Silencing

- Unfortunate insertion of transgene in to a transcriptionally inactive part of a genome. When an insertion of any transgene it doesn't show activity as per desire & this is because of its instability.
- The loss of transgene stability is because of gene silencing.
- E.g. slow fruit softening tomato, by reducing expression of polygalactouronase enzyme.

Post Transcriptional Gene Silencing: RNA silencing, also known as post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) is a mechanism regulating gene expression in a wide range of eukaryotes. RNA silencing is a mechanism in which small RNAs block gene expression by targeting homologous mRNAs without impacting nuclear DNA.^[16]

RNA Interference (RNAi): RNA interference (RNAi) or Post-Transcriptional Gene Silencing (PTGS) is a conserved biological response to double-stranded RNA that mediates resistance to both endogenous parasitic and

exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes. This natural mechanism for sequence-specific gene silencing promises to revolutionize experimental biology and may have important practical applications in functional genomics, therapeutic intervention, agriculture and other areas. Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference. RNAs are the direct products of genes, and these small RNAs can direct enzyme complexes to degrade messenger RNA (mRNA) molecules and thus decrease their activity by preventing translation, via post-transcriptional gene silencing. Moreover, transcription can be inhibited via the pre-transcriptional silencing mechanism of RNA interference, through which an enzyme complex catalyzes DNA methylation at genomic positions complementary to complexed siRNA or miRNA. RNA interference has an important role in defending cells against parasitic nucleotide sequences – viruses and transposons. It also influences development.

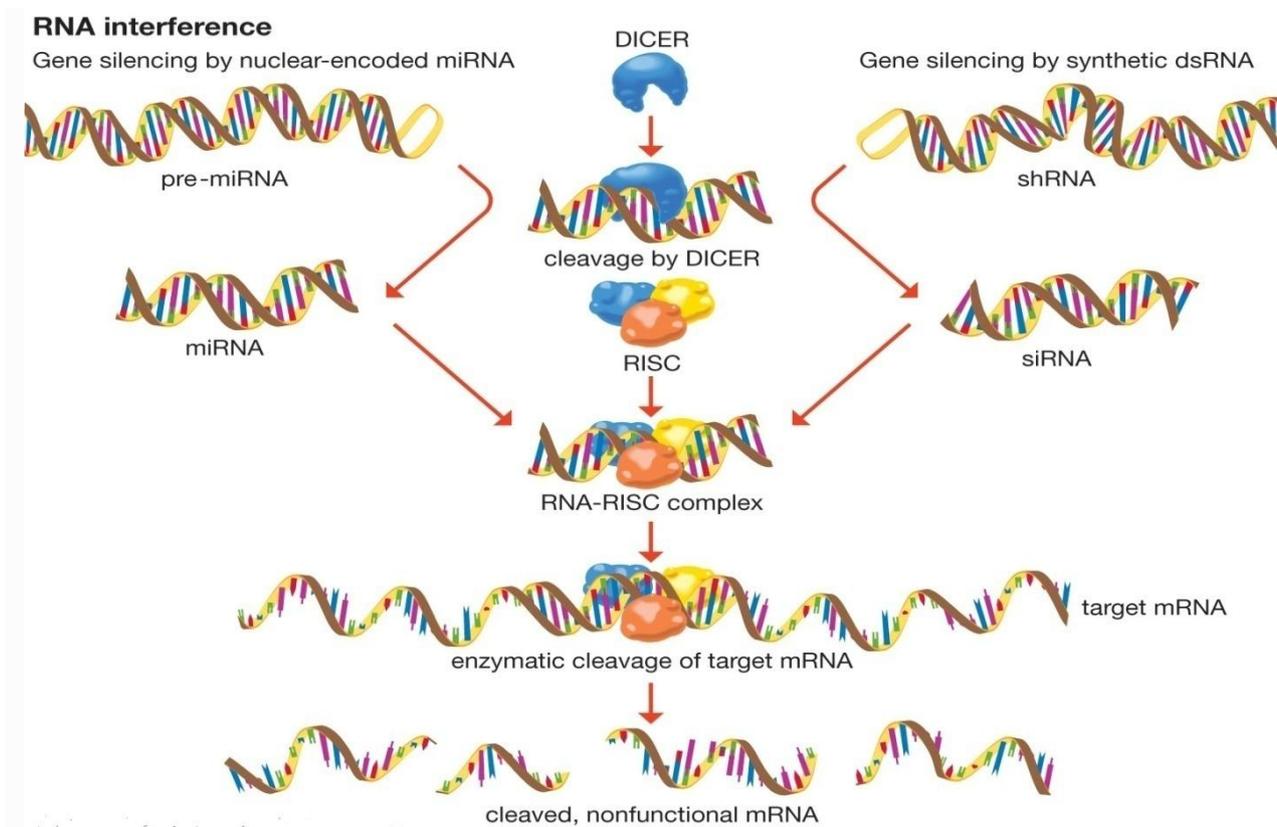


Figure-9: RNA Interference (RNAi).

miRNAs are small non-coding RNAs, with an average 22 nucleotides in length. Most miRNAs are transcribed from DNA sequences into primary miRNAs (pri-miRNAs) and processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. In most cases, miRNAs interact with the 3' UTR of target mRNAs to suppress expression. However, interaction of miRNAs with other

regions, including the 5' UTR, coding sequence, and gene promoters, have also been reported. Furthermore, miRNAs have been shown to activate gene expression under certain conditions. Recent studies have suggested that miRNAs are shuttled between different subcellular compartments to control the rate of translation, and even transcription.^[17]

Endogenous triggers of RNAi pathway

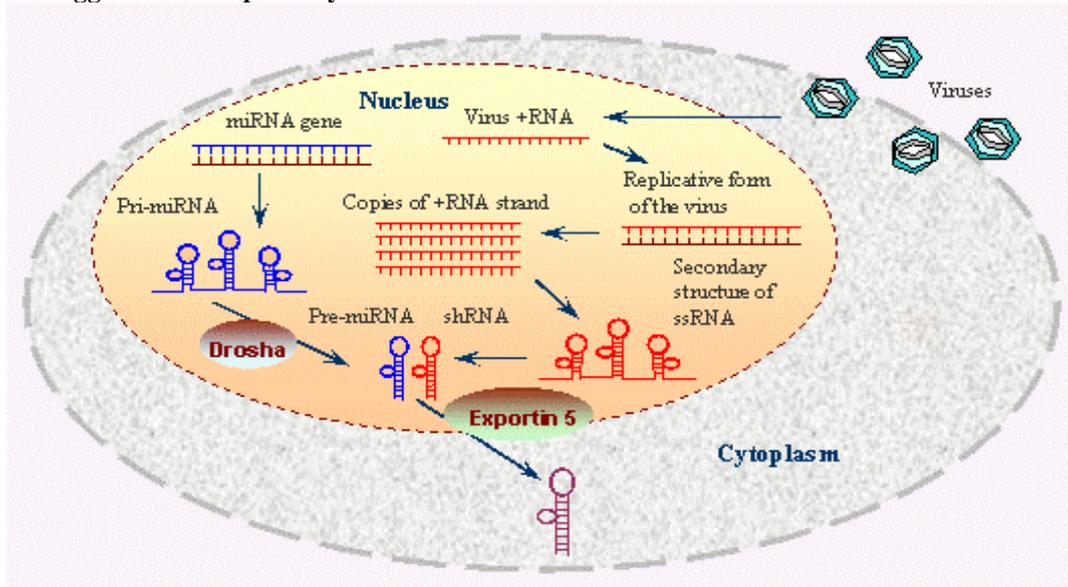


Figure-10: Endogenous triggers of RNAi pathway.

Endogenous triggers of RNAi pathway include foreign DNA or double-stranded RNA (dsRNA) of viral origin, aberrant transcripts from repetitive sequences in the genome such as transposons, and pre-microRNA (miRNA). In plants, RNAi forms the basis of virus-induced gene silencing (VIGS), suggesting an important

role in pathogen resistance. A possible mechanism underlying the regulation of endogenous genes by the RNAi machinery was suggested from studies of *C. elegans*. In mammalian cells long (>30nt) double-stranded RNAs usually cause Interferon response.^[18]

A simplified model for the RNAi pathway

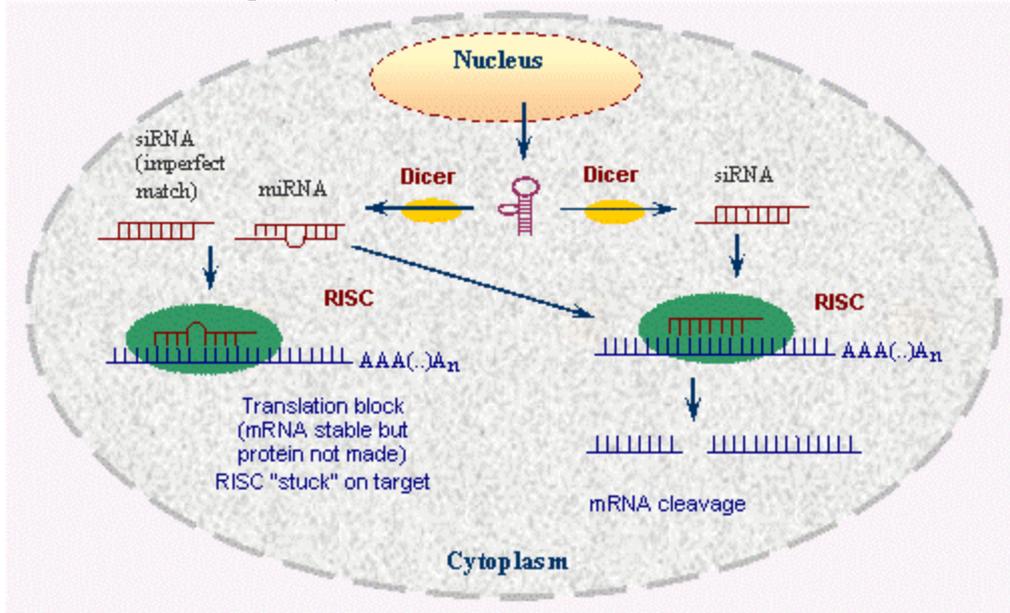


Figure-11: RNAi pathway.

A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into an short, interfering RNA (siRNA) by the RNase II enzymes Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC

assembly and the single-stranded RNA hybridizes with mRNA target. Gene silencing is a result of nucleolytic degradation of the targeted mRNA by the RNase H enzyme Argonaute (Slicer). If the siRNA/mRNA duplex contains mismatches the mRNA is not cleaved. Rather, gene silencing is a result of translational inhibition.^[19]

RNAi in experiments and therapeutics: how it works

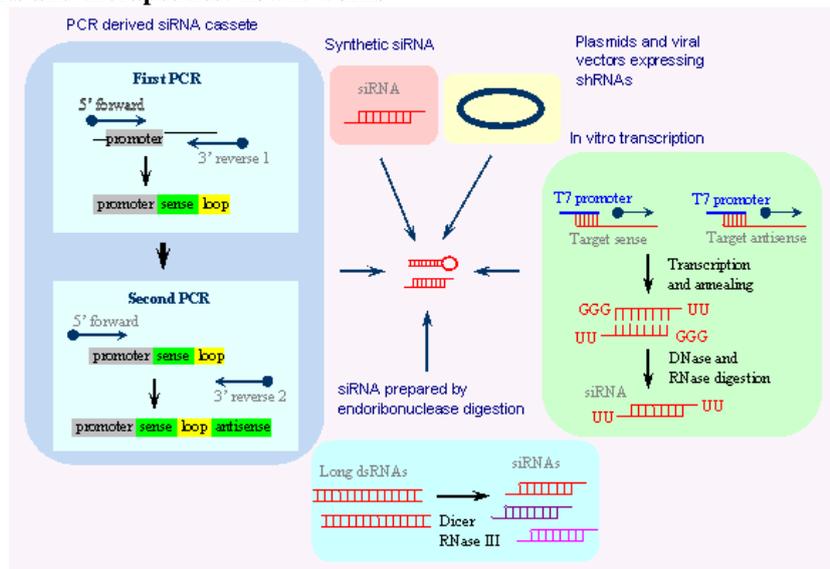


Figure-12: RNAi implementation.

RNAi can be triggered experimentally by exogenous introduction of dsRNA or constructs which express shRNAs. The high degrees of efficiency and specificity are the main advantages of RNAi. Consequently, RNAi is used in functional genomics (systematic analysis of loss-of-function phenotypes induced by RNAi triggers) and developing therapies for the treatment of viral infection, dominant disorders, neurological disorders, and many types of cancers (*in-vivo* inactivation of gene products linked to human disease progression and pathology).

The RNA interference pathway is often exploited in experimental biology to study the function of genes in cell culture and *in vivo* in model organisms. Double-stranded RNA is synthesized with a sequence complementary to a gene of interest and introduced into a cell or organism, where it is recognized as exogenous genetic material and activates the RNAi pathway. Using this mechanism, researchers can cause a drastic decrease in the expression of a targeted gene. Studying the effects of this decrease can show the physiological role of the gene product. Since RNAi may not totally abolish expression of the gene, this technique is sometimes referred as a "knockdown", to distinguish it from "knockout" procedures in which expression of a gene is entirely eliminated. In a recent study validation of RNAi silencing efficiency using gene array data showed 18.5% failure rate across 429 independent experiments. Depending on the organism and experimental system, the exogenous RNA may be a long strand designed to be cleaved by dicer, or short RNAs designed to serve as siRNA substrates. In most mammalian cells, shorter RNAs are used because long double-stranded RNA molecules induce the mammalian interferon response, a form of innate immunity that reacts nonspecifically to foreign genetic material. Mouse oocytes and cells from early mouse embryos lack this reaction to exogenous

dsRNA and are therefore a common model system for studying mammalian gene-knockdown effects. Specialized laboratory techniques have also been developed to improve the utility of RNAi in mammalian systems by avoiding the direct introduction of siRNA, for example, by stable transfection with a plasmid encoding the appropriate sequence from which siRNAs can be transcribed, or by more elaborate lentiviral vector systems allowing the inducible activation or deactivation of transcription, known as conditional RNAi.^[20]

Application of Gene Silencing: Considering the successful results from different applications of the various mechanism of the gene silencing in wide range of agricultural and horticultural crop plants, it has been proved that the techniques of gene silencing are very effective in solving of production constraints like increasing the quality of produce, building resistance against different pathogens and pests and creating the new useful variation by suppressing the endogenous genes as well. Hence, this technology can be seen as to lower the production gap. Considering the successful results from different applications of the various mechanism of the gene silencing in wide range of agricultural and horticultural crop plants, it has been proved that the techniques of gene silencing are very effective in solving of production constraints like increasing the quality of produce, building resistance

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- Specific gene silencing using RNA I in cell culture.
- Cancer treatments.
- RNA interference has been used for applications in biotechnology, particularly in the engineering of food plants that produce lower levels of natural plant toxins.
- Such techniques take advantage of the stable and heritable RNA i phenotype in plant stocks. For example, cotton seeds.
- Modulation of HIV-I replication by RNA i.
- Small RNA and its application in andrology and urology.
- Developing technologies for epigenomic analysis and clinical application of molecular diagnosis.
- Currently there are at least six oligonucleotide drugs inducing RNA i for illness including cancer.

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Advantages of gene silencing: The advantages of RNA include the high efficiency of the gene knockdown, the ability to easily Target the gene of interest, as well as stable and long-term silencing by expressing shRNAs. This makes for a powerful tool that has been successfully applied to answer many questions in cell biology.

1. Downregulation of gene expression simplifies "knockout" analysis.
2. Powerful tool for analyzing unknown genes in sequenced genomes.
3. Inducing viral resistance.
4. Oligonucleotide can be manufactured quickly, some within one week; the sequence of the mRNA is all that needed.
5. Useful approach in future gene therapy.
6. Blocking expression of unwanted genes and undesirable substance.
7. This type of screening has the promise to be more efficient and have greater potential.

Redundancies can be found by targeting a number of genes that are connected in sequence.

Disadvantages of gene silencing

1. "High pressure injection" electroporation can cause significant injection damage to the integrity of the normal tissue, organs and thus preclude the utilization in a clinical set-up.
2. Liposomes/cationic encapsulated SiRNA may also be toxic to the host and may cause severe host severe host immune responses.
3. Others emerging strategies includes chemical modification of SiRNA molecules and encapsulated with different molecules are still In their infancy and need to be thoroughly investigated before used in therapeutic applications.
4. Lipid base nanoparticles are toxicity at high dose, preparation is difficult and low transformation efficiency.
5. Hybrid nanoparticles toxicity have very high doses.

getting inspiration to frame article on **Gene Silencing** supported by Director of School of Pharmacy [**Dr. Beduin Mahanti**] Techno India University, Kolkata.

This article helps us to know the full details of Gene Silencing followed by molecular genetics. This article has been reviewed and appreciated by one of the author **Dr. Dhananjay Saha** who belongs from the field of biomedical science as well as biotechnology.

CONCLUSION

Gene silencing as a therapeutic strategy is a highly active area of research and may one day yield an effective treatment for HD, since it acts by directly reducing the production of the mutant huntingtin protein. Considering the successful results from different application of the various mechanism of the gene silencing in wide range of agricultural and horticultural crop plants, it has been proved that the techniques of gene silencing are very effective in solving of production constraints like increasing the quality of produce, building resistance against different pathogens and pests and creating the new useful variation by suppressing the endogenous genes as well. Hence, this technology can be seen as to lower the production gap. RNA I technology can be considered an eco-friendly, biosafe & ever technology as it elements even certain risk associated with development of transgenics.

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