www.journalworlds.com
AGRI JOURNAL WORLD
VOLUME 2 ISSUE 1 JANUARY, 2022

GENE SILENCING: AN IMPORTANT TOOL IN THE BIOLOGICAL SCIENCES

S.S. Pawar[1], D. Malakar[2], N.P. Kurade[1], A.V. Nirmale[1]

[1] ICAR-National Institute of Abiotic Stress Management, Baramati- 413115
[2] ICAR-National Dairy Research Institute, Karnal-132001

**Corresponding author email:sachinndri@gmail.com*

ABSTRACT

Gene silencing refers to the phenomenon of inhibiting gene function. It can be achieved by gene knockouts or sequence-specific knockdown of the messenger R.N.A. (mRNA) or by inhibiting protein function. Gene silencing is used to study genes with unknown function, target validation in drug development and therapeutic purpose. Gene silencing is done at the gene level (gene knockouts), protein level (antibodies and aptamers) or mRNA level (RNAi, Ribozymes and DNAzymes).

INTRODUCTION

Gene silencing has confirmed to be an influential instrument in the hands of genetic engineers over the years. Gene silencing denotes to the phenomenon of inhibiting gene function either by gene knockouts or sequence-specific knockdown of the messenger R.N.A. (mRNA) or by inhibiting protein function. After injection into adult animals, purified single strands had a modest effect, whereas double-stranded mixtures caused potent and specific interference. Gene silencing is the popular method to study genes with unknown function, target validation in drug development and therapeutic purpose for treating several diseases.

The history of gene silencing can be traced back to the 1970s. In the late 1970s, synthetic antisense oligonucleotides were used for inhibiting R.S.V. replication. In the early 1980s, there was the discovery of Ribozymes. The late 1990s saw the development of DNAzymes. Inhibition of complementary mRNA translation by exogenous single-stranded D.N.A. (ssDNA) in the cell free system triggered the gene silencing era. Jorgensen et al. (1996) was reported a surprising observation in petunias. They introduced a pigment-producing gene with a powerful promoter to increase the purple colour in petunia flowers. This leads to change in colour pattern from purple to variegated even white which was contrast to the normal expectation of getting deep purple flowers. Since the expression of the imported gene and the corresponding endogenous gene was inhibited, Jorgensen coined the term "cosuppression". Guo and Kemphues (1995) attempted to examine the function of the Par-1 gene by using antisense R.N.A. to silence it. The most recent development in the antisense world is the discovery of Ribonucleic acid interference (RNAi), as reported by Fire and Mello (1998). They started by injecting *C. elegans* with dsRNA, a combination of sense and antisense strands. Silencing was far more effective with this injection than with the sense or antisense strands alone. They had experimentally proved that the capability of the exogenous dsRNA to mask the expression of the gene that corresponds to the dsRNA sequence and Single-strand R.N.A. was ten times more efficient than a combination of sense and antisense R.N.A. (dsRNA).

APPROACHES FOR INDUCING GENE FUNCTION LOSS

Gene silencing or loss of function of a gene can be brought about at three levels (Fig 1), namely, at the gene level (gene knockouts) or mRNA level (RNAi, AS ONs, Ribozymes and DNAzymes) or protein level (antibodies and aptamers).

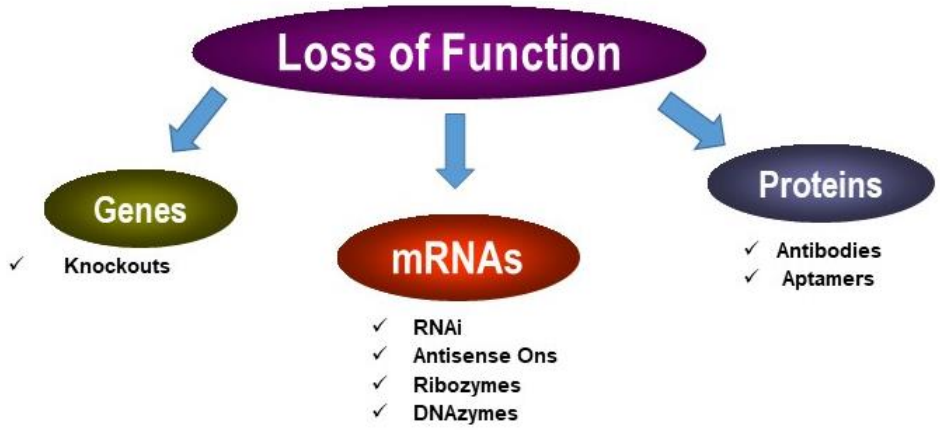

Fig 1: Various approaches for inducing loss of gene function

GENE KNOCKOUT

A gene knockout, abbreviated as K.O. Gen knockout, can be brought about by using a homologous recombination method that involves a series of steps in which recombinant vector is designed first and then introduced in the cell. The final construct is designed to bring about recombination in the gene of interest, which results in the insertion of a foreign sequence of the specific target gene in the genome of the organism, which causes loss of function by disruption of the gene of interest. Site-specific nucleases such as Zinc finger nucleases (Z.F.N.s), Transcription activator-like effector (TALE)-nucleases (T.A.L.E.N.s), Meganucleases or CRISPR/Cas9 system are also used to bring about gene knockout. They cause site-specific double stranded breaks in the gene leading to gene knockout.

GENE SILENCING AT mRNA LEVEL

RNA interference

RNAi takes advantage of an old aspect of the immune system that protects plants and animals from invaders by depleting viral genomic R.N.A. targets in a sequence-specific manner using tiny interfering R.N.A.s (siRNAs). RNAi is an evolutionally highly conserved process of P.T.G.S. (Post-transcriptional gene silencing) by which dsRNA causes sequence-specific mRNA-degradation was first discovered by Fire and Mello (1998) in the nematode, *Caenorhabditis elegans.* RNAi has provided a theoretically exciting and practically within reach methodology to manipulate gene expression. The outstanding achievement of R.N.A. interference (RNAi) in life sciences is centered on its high potency to silence genes in a sequence-specific manner. In various animals and cell types, R.N.A.s (dsRNAs; typically >200 nt) can be employed to silence the expression of target genes. The RNAi pathway is mediated via the initiation step (siRNA generation) and effector step (degradation of mRNA). The initiation step involves processing long dsRNA

by the RNase III family nucleases (Dicer) enzyme into small dsRNA molecules called siRNA. Effector step is where siRNAs are bound by multi-protein complex "RISC – R.N.A. induced silencing complex" (with RNase activity) guiding targeted mRNA to degradation.

Antisense oligonucleotides (AS-ONs)

Antisense oligonucleotides are the 13-15 nucleotides long D.N.A. or R.N.A. complementary to the region of target mRNA, which can suppress its expression selectively. The AS-ONs were discovered in the late 1970s, where it was demonstrated to inhibit R.S.V. replication in cell culture. Automation of ONs synthesis in the early 1980s facilitated the use of AS-ONs as antisense agent. Based on the mechanism of action, the AS-ONs can be grouped as RNase H dependent and steric hindrance AS-ONs. RNase H dependent AS-ONs cleave the R.N.A. moiety of a DNA-RNA heteroduplex resulting in degradation of the target mRNA. The steric hindrance AS-ONs exhibit antisense action by inhibiting translation by inhibiting splicing by intron removal or blocking the translation initiation or elongation.

Ribozymes

These are the catalytic R.N.A. molecules with the ability to cleave phosphodiester linkages in R.N.A. without proteins. They bind to substrate R.N.A. through Watson-Crick base pairing, which offers sequence-specific cleavage of transcripts. Ribozymes can be produced using a vector which can cause the continued production & prolonged intracellular effect, although dissociation of cleavage product may be the rate-limiting step. For successful substrate cleavage, several ribozymes require high divalent metal ion concentrations, limiting their intracellular utilization.

Based on sequence and secondary structure, the ribozymes can be grouped as hammerhead and hairpin ribozymes. The hammerhead ribozymes were first isolated from viroid R.N.A. and dissected into enzyme and substrate strands. It is less than 40 nucleotides long & consists of two substrate-binding arms and a catalytic domain. The hairpin ribozymes were derived from the satellite R.N.A.'s negative strand of the tobacco ringspot virus.

GENE SILENCING AT PROTEIN LEVEL

Antibodies

Antibodies are the proteins that are secreted by the body in response to antigenic stimuli. At the protein level, the effect can be blocked by specifically targeting the protein molecules by use of antibodies. However, selection requires a biological system; therefore, raising antibodies to toxins or non-immunogenic targets is difficult. Moreover, antibodies have a limited shelf-life and are temperature sensitive. These factors limit the use of antibodies as gene silencing agents.

Aptamers

These are the small single-stranded nucleic acid molecules or peptides that assume specific three-dimensional folded conformations that bind to target molecules. Aptamer" is deduced from Latin aptus, 'to fit' and Greek meros implies 'part of region'. They can be divided into R.N.A. aptamers, D.N.A. aptamers, and Peptide aptamers. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs and can be targeted to various molecules for causing desired effects (Fig 1).

CONCLUSION

The Gene slicing technology is new advanced technique which can benefit the human kinds in various way. This will help to understand and unnerve the complex phenomenon in life science. It also can be successfully utilized in the crop production and resistance development in both plants and animals.

REFERENCES

Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. (2003) R.N.A. interference: biology, mechanism, and applications. Microbiology and Molecular Biology Reviews. 67:657-685.

Bunka DH, Stockley PG. (2006) Aptamers come of age - at last. Nature Reviews Microbiology. 4:588-596.

Dias N, Stein CA. (2002) Antisense oligonucleotides: basic concepts and mechanisms. Molecular Cancer Therapeutics. 1:347-355.

El-Sappah AH, Yan K, Huang Q, Islam MM, Li Q, Wang Y, Khan MS, Zhao X, Mir R.R., Li J, El-Tarabily KA and Abbas M (2021) Comprehensive Mechanism of Gene Silencing and Its Role in Plant Growth and Development. Front. Plant Sci. 12:705249.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. (1998) Potent and specific genetic interference by double-stranded R.N.A. in Caenorhabditis elegans. Nature. 391:806-811.

Guo S, Kemphues KJ. (1995) par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell. 81:611-620.

Han H. R.N.A. Interference to Knock Down Gene Expression. Methods Mol Biol. 2018;1706:293-302.

__