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Gene silencing by double stranded Ribonucleic acid (RNA)

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ABSTRACT

Ribonucleic acid (RNA) silencing, RNA interference (RNAi) or post-transcriptional gene silencing takes place in a variety of eukaryotes and it was discovered firstly in the plants. The RNA silencing process is activated by a trigger from dsRNA predecessor. A very important step in the silencing pathways the conversion of dsRNA into small duplexes of RNA of the representative length and arrangement. Then these small dsRNA monitor RNA silencing by different mechanisms. Post transcriptional gene silencing mechanisms were initially identified as an anti-viral process that give protection to the organisms from the viruses or which inhibit the unsystematic incorporation of transposable components. The basic aim of this review article is to study the mechanism of gene silencing by dsRNA and the roles of certain proteins in cellular post transcriptional RNA silencing machinery and finally we also discuss the RNA silencing as an anti-viral defense mechanism in the plants.

Keywords: RNA, gene, silencing, pathway and plant

INTRODUCTION: The appliance of gene silencing was initially learned in plants and also termed as silencing of post-transcriptional genes. RNA silencing is prompted by the originators of double stranded ribo-nucleic acids. These double stranded or two pleated ribo-nucleic acids are commonly processed into the small ribo-nucleic acid duplexes and its length vary from 21-28 nucleotides nearly and which then direct recognition". It is worth notice that silencing ribo-nucleic acid is double stranded or two crumpled strands. First one is sense strand and the other one is anti-sense strand. The sense strand is of sequenced as a base sequence of messenger ribo-nucleic acid. And the further one, anti-sense RNA have the opposite base sequences (Bartel, 2004). Silencing of double stranded or two folded strand RNA (ribo-nucleic acid) have the two phases/stages. First one is the initial or starting or beginning phase/stage and the second one is the effective phase also called as a successful and viable stage or phase. In the preparatory stage, the long stranded ribo-nucleic acids are divided into the less interfering ribo-nucleic acid with the help of an enzyme that we called DICOR (Mello and Conte, 2004). The additional one is the effective stage, in which the parting of two-folded silencing RNAs strands and the conveyance of anti-sense strand occurs into a proteins group." (Hori *et al.*, 2014). Until that time, the mechanism of silencing ribo-nucleic acid can be conducted as the mechanism of anti-viral that gave protection to the living organisms from RNA viruses. Application of the transgene is the most increasing technology in plant growth and now days it becomes the most important point. The presence of duplicated homologous sequences causes the inactivation of transgenes (Mok *et al.*, 2010). Apparently, homology can trigger gene inactivation and serves as single. In transcriptional process it also triggers gene inactivation. RNA silencing is the term which is generally use to describe post-transcriptional gene silencing in plant (Tinoco *et al.*, 2010). Basic procedure of RNA silencing is simple and straight forward. In this process double stranded RNA or hairpin RNA is cleaved by Dicer RNase enzyme interfere RNA, which consist of 21-26 nucleotides, these nucleotides guides silencing complex of induced RNA to destroy single stranded RNA (Fire, 2015). RNA-RNA, RNA-DNA, RNA-protein and protein-protein interactions, all these processes involve RNA silencing which is a complex process (Aregger *et al.*, 2012). Eukaryotes conserved ancient RNA surveillance system. This mechanism act against invasive nucleic acids, including viruses and other highly repetitive genomic sequences (Bora *et al.*, 2012). In various organisms double stranded RNA shows gene expression in a specific sequence manner. This biological process termed as RNA interference or (RNAi) (Fire, 2007). RNAi is a potent method which only few molecules of dsRNA per cell to silence expression. Silencing is not only spread from digestive tract of worms but it can also be transmitted through the germ line for several generations (Christiaens *et al.*, 2014).

RNA silencing system: RNA silencing mechanisms have formerly been boosted as antiviral mechanisms that secure organisms from RNA-viruses, or that preclude random integration of transient elements but the general role of silence in the regulation of gene expression only becomes seeming when it is manipulated that definite genes in plants and animals encode the short form of fold-back dsRNA (Liu *et al.*, 2012). Many of these miRNA genes are evolutionarily conserved, in plants miRNA acts primarily as siRNAs that guide the cleavage of sequence complementary mRNAs (Garbutt *et al.*, 2013). In animals such as nematodes, miRNAs appear to inhibit translation by targeting partially complimentary sequences located primarily within the 3 'untranslated region (UTR) of mRNAs (Wynant *et al.*, 2014).

Gene silencing in c-RNA gene: Small interfering RNA is the finest approach to effectively influence gene expression to study protein function in a wide range of cell types (Garbutt *et al.*, 2013). The si-RNA in the prevention of cancerous cells,

including the corporation and enquiry of cancer-related siRNA archives and their submissions in anticancer drug target detection and cancer therapy The detection of the RNA interface is an operative mode to circumvent the expression of separable genes by double-stranded RNA (Luo *et al.*, 2013). Sooner or later, it will be thinkable to custom gene-specific remedy to act towards human's syndromes as well as cancer (Bolognesi *et al.*, 2012).

Small interfering RNAs [siRNAs], **ressed siRNAs** have not been existed in the category of mammals, but they can be turn out from dsRNA as well as a minute hairpin (shRNA) by dicer cleavage or RNase-3 nuclease activities or chemical synthesis (Woppmann *et al.*, 2010). RNA (ADAR), an adenosine deaminaminase effecting on an RNA-editing enzyme, has been revealed to contend for dsRNA so that it is uncomplimentary as a substrate for dicer and thus hinders cRNA formation (Hori *et al.*, 2014). correspondingly acknowledged as short inhibiting RNAs or else suppressing RNAs, to be precise as actually passionate technology that can be effortlessly permits the silencing of the genes of living creatures such as mammals or animals by means of extraordinary level of potential capacity in addition to it also be made up of twenty to twenty five base pairs (Mok *et al.*, 2010).

Endogenously (RISC): Dicer distributes sRNA to a group of proteins called RNA-inducing silencing complex (RISC), where the catalytic component argonaute (Ago) is capable to fix siRNA to the equivalent strand to drag the consequential mRNA and additional, harm the mRNA, resulting in gene silencing (Ota *et al.*, 2013). ATP is mandatory throughout the unwinding of the siRNA duplex (Allen and Walker III, 2012).

The Molecular mechanism of RNA that triggered Gene Silencing: RNA triggers of gene silencing is precise ancient as well as developmentally trained and far accomplishment phenomenon (Thakur *et al.*, 2014). This performance is furthermore acknowledged as RNA interference. It takes place at what time dual stranded RNA helices prompt cleavage of their correlative mRNAs (Kreutzer and Limmer, 2012; Thakur *et al.*, 2014). Since these RNA subdivisions can be presented exogenously as little intrusive RNAs for instance siRNAs. RNA interference has become an ordinary investigative scheme in laboratory look (Rossi *et al.*, 2011). Also, the quantity, of RNA-based therapeutics that are as of now in clinical preliminaries for an assortment of human toxicities spectacle the remedial capability of RNA interference (Masliah *et al.*, 2013). Like so, we center all over the place our present appreciative of the conformation and magnitudes of not the same programmes of RNA interference triggers and how this evidence has been added to our conception of the biogenesis and synergist essentials of siRNA and micro-RNA in mammalian cells (Komor *et al.*, 2016). Nevertheless of their reputation in science and drugs, the atomic and cell-constituents of micro RNA biogenesis and capability are not absolutely comprehended. Captivating inquiries stay both for accepting the impressions of alterations and altering on micro RNAs and the suggestions among micro RNAs and the other cell RNAs, as we give an instance such as, long non-coding RNAs (Komor *et al.*, 2016).

Pathways of RNA silencing: It can be classified into four functional based pathways in plants. These are as follows: (Liu and Paroo, 2010; Bologna and Voinnet, 2014; Borges and Martienssen, 2015; D'Ario *et al.*, 2017).

(1)mi-RNA pathway ___ microRNA pathway**:** MicroRNA can be generate by the transcription of an enzyme RNA-Polymerase-ll

(2)Trans-RNA pathway___trans-acting-small-interfering RNA: The Trans-acting small interfering RNA (tasiRNA) can be initiated by the specific types of miRNA (micro RNA).

(3)RNA directed DNA methylation Pathway.

(4)Exogenic RNA silencing pathway: The RNA-silencing induced by the viruses and trans-genes, is termed as Exogenic RNA

RNA silencing and its role in plant with virus interactions: During the plant transgenic studies the result of the RNA silencing was unexpected. This was observed in 1990 into a diverse of molecular biology to now recent research is also observed (Ghoshal and Sanfaçon, 2015). In biological processes and the other scientific mechanisms of RNA silencing, viruses have been a critical tool in unveiling. In fact, the study in 1990s on the viruses' resistance and the effect of the pathogens provided the first evidence of introducing the gene silencing (Wang *et al.*, 2012). The phenomenon of RNA- directing DNA methylation was firstly introduced in the tobacco plants. The basic processes of the RNA silencing process in plant is now well understood (Bivalkar-Mehla *et al.*, 2011; Burgyán and Havelda, 2011; Jaubert *et al.*, 2011; Incarbone and Dunoyer, 2013). Double stranded RNA is promoted by the Dicer-like (DCL) proteins into 21-24 nucleotides small RNAs which are loaded to member of Argonatue (AGO) family to from an RNA silencing complex (RISC). These RISC along with sRNAs which are guide to direct RNA degradation, translational repression or DNA methylation of homologous target genes (Redfern *et al.*, 2013). Mostly there are three basic RNA-silencing pathway in the plants, the first one is micro (mi) RNA pathway, the second is small interfering (si) RNA-directed RNA degradation pathway and the last but not the least is RdDM (RNA directed DNA Methylation) pathway (Yoo *et al.*, 2011). In miRNA pathway, imperfect short hpRNA (hairpin RNA) are formed between the complementary regions of a primary miRNA transcript is processed in the mucleus by the DCL1, one of these four DCL proteins in Arabidopsis, into a single 21 -24 nt miRNA. miRNA play critical role in the control of the development of the plant by repressing the expression of regulatory genes such as the transcription factors, although recent research suggested that plants miRNA can also perform translational repression (Kim and Kim, 2012). The RdDM pathway is very unique in plants and play important role in silencing transposons and the repetitive DNA elements to maintain genome stability and integrity (Sand *et al.*, 2012). The RdDM is directed by 24-nt siRNAs that are processed by DCL3 from dsRNA synthesized by the plant-specific DNA dependent RNA polymerase IV and RDR2. The molecular details of the RdDM is not fully described yet (Yoo *et al.*, 2011).

RNA silencing as an antiviral defense mechanism in plants: Positive-sense RNA viruses are mostly include in the majority of the plants viruses. Which has either ssRNA or dsRNA genome which mostly depends on the viral RNA dependent DNA polymerase (replicase) for the process of multiplication (Agius *et al.*, 2012). Like fungal and the bacterial pathogens, the viruses can also be subject of innate immune responses conferred by host encoded disease resistance gene. Resistance gene of natural virus are continuing to be sought for use in breeding of durable virus resistance in crop plant (Nuss, 2011).

The tobacco *N* gene against *Tobacco mosaic virus* (TMV) was firstly identified *R* gene conferring the resistance to a virus (Scholthof *et al.*, 2011). *Turnip mosaic virus* (TuMV) induced lethal necrosis in some crucifer plants such as *Arabidopsis* (Ying *et al.*, 2010; Wang *et al.*, 2012) and *Brassica napus* in response to the corresponding *R* genes.

Applications of RNA-silencing in plants: The applications of RNA-Silencing in Plants Biotechnology are as follows; (Simón-Mateo and García, 2011; Ma *et al.*, 2015; Guo *et al.*, 2016). (1) The RNA silencing can be used for the development of seedless fruits. (2) It plays a vital role in alteration of plants architecture and also involved in flowering time. (3) It can enhance the resistance to biotic stresses of plants (Ahmed *et al.*, 2017). (4)RNA silencing functioning as up or down in the regulation of genes in plants. (5)RNA silencing is the method that provide immunity against viruses or transposons.

CONCLUSION: This review assertively supports the fact that the RNA silencing play an actual significant part in the development of plants and animals by giving an efficient system of gene controlling. RNA silencing may also prove beneficial for human disease controlling because many linkages between the inherited or acquired genetic disorders and RNA silencing factors have identified. There is much more to study about the biological roles and the molecular process of the gene silencing in plants. Most likely, there may be more than one mechanism of gene silencing to exist. The understanding of gene silencing and the molecular basis of the gene silencing would enhance the application of transgene technology, and will also reveal new mechanisms which are involved in regulating the development of plants.

CONFLICT OF INTEREST: Author has no conflict of interest

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