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RNA interference (RNAi)- A promising technology for sustainable agriculture

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Key words :

RNA silencing is a novel gene regulatory mechanism which regulates the transcript level by either suppressing transcription (transcriptional gene silencing [TGS]) or by activating a sequence-specific RNA degradation process (post- transcriptional gene silencing [PTGS] or RNA interference).

RNA interference (RNAi) is a conserved biological response to double-stranded RNA that medicates 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.

RNAi related events were described first in plants and later on in higher eukaryotes including protozoa, nematodes, insects, flies, parasites, mouse and human cell line. Three phenotypically different but mechanically similar forms of RNAi:

- Cosuppresion or PTGS in plants
- Quellinlg in fungi
- RNAi in animal kingdom.

Mechanism:

A class of small RNA that mediates the silencing of particular gene functions by interacting with mRNA often in 3'UTR, resulting in either mRNA degradation or translational inhibition mRNA and thus gene that produces it, is silenced. Small RNA are sometimes called micro-RNA (miRNA) and many small RNA which present only transiently during development are referred as small temporal RNA (stRNA). RNAs mainly act in 2 ways;

First, miRNA transcribed as precursor RNAs about 70 nucleotides long, with internally complementary sequences that form hairpin like structures. The precursor are cleaved by endonucleases in Dicer family (RNAse Ø) to form short duplexes about 20-25 nucleotides long. One strand of the processed miRNA is transferred to target mRNA, leading to inhibition of translation or degradation of RNA.

Second, double strand RNA can be constructed and introduce into a cell. Dicer processes the duplex RNAs into short segments called small interfering RNA (siRNA).these binds with target mRNA and silence it. This process is called as 'RNA interference'. In plants, virtually any gene can be effectively shut down in this way. In nematodes, simply introducing duplex RNA into worm's diet produces very effective suppression of the target gene.

PTGS in plants:

In plants, the RNA silencing story unfolded during a search for transgenic petunia flowers that were expected to be purple. In 1990 R. Jorgensen's laboratory wanted to up regulate the activity of gene for chalcone synthase (chsA), an enzyme involved in the production of anthocyanin pigments. Surprisingly, some of the transgenic petunia plants harboring the chsA coding region under the control of 35S promoter lost both endogene and transgene chalcone synthase activity, and thus many of the flowers were developed white sectors. The loss of cytosolic chsA mRNA was not associated with reduced transcription, as demonstrated by run-on transcription taste in isolated nuclei. Jorgensen coined the term cosuppression to describe the loss of mRNAse of both the endo and transgene.

Quelling and RNAi:

Homology dependent-dependent phenomenon was observed in fungal systems. These events were called 'quelling'. Quelling came to light during attempts to boost the production of an orange pigment made by the gene of fungus *Neurospora crassa*. An *Neurospora crassa* strain containing a wild-type all +gene (orange phenotype) was transformed with a plasmid containing a 1500bp fragment of the coding sequence of the wild type gene. A few transformants were stably quelled and showed albino phenotypes. In the all +quelled strains, the level of unspliced mRNA was similar to that of the wild-type strain, whereas the native all+ mRNA was highly reduced, indicating that quelling and not the rate of transcription affected the level of mature mRNA in a homology-dependant manner.

Virus-induced gene silencing:

Besides the processes mentioned above, homology-driven RNA degradation also occurs during the growth of viral genomes in infected plants. Viruses can be the source, the target, or both the source and the target of silencing. When *Brrasica napus* was inoculated with cauliflower mosaic virus (a DNA virus), lesions at the site of virus entry were visible 5 to7 days post-inoculation. Symptoms of systemic infections were apparent by 10 to 14 days post-inoculation. A symptom was most prominent at 30 to 40 days post-inoculation and declined thereafter (*i.e.* the plants recovered), with the newly emergent leaves remaining asymptomatie at 50 days post-inoculation.

After these initial observations in plants many laboratories around the world searched for the occurrence of this phenomenon in other organisms. Craig C. Mello and Andrew Fire's 1998 Nature paper based on research conducted with their colleagues at the Carnegie Institute of Washington and the University of Massachusetts reported a potent gene silencing effect after injecting doubled stranded RNA into *C. elegans*. In investigating the regulation of muscle protein production, they observed that neither mRNA and antiscence RNA injections had an effect on protein production, but double-stranded RNA successfully silenced the targeted gene. As a result of this work, they coined the term RNAi. The discovery of RNAi in *C. elegans* is particularly notable, as it represented the first identification of the causative agent (double-stranded RNA) of this inexplicable phenomenon. Fire and Mellow were awarded the Nobel Prize in Physiology and Medicine in 2006 for this work.

Applications:

– Antiviral defense mechanism
– Control of the activity of transposons
– Analysis of gene function as it can disrupt gene function without creating a mutant organism
– DNA vector based-strategy allows suppression of endogenous genes and produce transgenic lines with suitably modified traits.
– Production of low caffeine coffee.
– RNAi as a treatment for HIV
– RNAi as a novel therapeutic agent
– RNAi as a research tool
– RNAi for genetic diseases.

Though RNA interference is a novel phenomenon, it has few limitations as follow:-

– RNAi based on exogenous siRNAs is rather short-lived. E.g. for 4-6 days.
– Longer double-stranded RNA molecules (longer than 30 bp) trigger antiviral response and a general suppression of gene expression.
– Exogenous siRNA must be delivered into cells, which is not highly efficient.

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