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RNA Interference (RNAi) and Its Application in Plant Metabolic Engineering

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RNA Interference (RNAi) is double stranded (dsRNA) mediated gene silencing phenomenon in which dsRNA triggers a marked reduction in either transcription of the corresponding gene or direct degradation of the corresponding mRNA. With the thorough going research in RNAi mechanisms and the complete development of RNAi technology, researchers will create a new biological science and see enormous economic and social spinoffs.

Introduction

In 1988, it was first reported that when a double stranded RNA having sequence of specific mRNA transcript is introduced into the cell; the corresponding mRNA is degraded, resulted in selective inhibition of gene expression, this phenomenon was termed as RNA interference or RNAi. In other words, RNA Interference (RNAi) is double stranded (dsRNA) mediated gene silencing phenomenon in which dsRNA triggers a marked reduction in either transcription of the corresponding gene or direct degradation of the corresponding mRNA.

Andrew Fire and Craig C. Mello shared the Nobel Prize in Physiology or Medicine for their work on RNAi in 2006. This interference appears to be conserved among various eukaryotes such as protozoa, fungi, plants and animals. RNAi has been used to silence gene expression in diverse organisms such as plants, fungi and animals like *Drosophila melanogaster, Caenorhabditis elegans* and mice.

RNAi is more of an endogenous mechanism of post-transcriptional gene silencing (such as control of viral infection and prevention of transposome movement) and a reverse genetics tool, strongly down-regulates the expression of the target gene in a sequence-specific manner. Exploitation of this pathway is a promising tool in biotechnology, biomedicine and agriculture.

RNA interference is initiated by the presence of double stranded RNA, which may arise in several ways: by the transcription of inverted repeats in DNA into a single RNA molecule that base pairs with itself; by the simultaneous transcription of two different RNA molecules that are complementary to one another and pair; or by the replication of double-stranded RNA virus. RNA silencing leads to the degradation of mRNA and methylation of DNA.

Salient Features of RNAi

- dsRNA rather than single stranded antisense RNA is the interfering agent.
- High degree of specific gene silencing with less effort.
- Highly potent and effective gene silencing mechanism.
- Silencing can be introduced in different development stages.
- Systemic (sequencing specific) silencing and this mechanism effectively passed through generations.

Components Involved in RNAi Mechanism

- **The RNA Components:** These are central to RNA interference.

1. siRNA- small interfering RNA & consist of 21-25 nucleotides.
   a. Encoded by endogenous genes.
   b. Hairpin precursors.
   c. Recognize multiple targets.
2. miRNA- microRNA & also consist of 21-25 nucleotides.
   a. Mostly exogenous origin.
   b. dsRNA precursors.
   c. Target specific.

- **The Protein Components:** Drosha, Dicer, RNA Dependent RNA polymerase (RDRP), RNA Induced Silencing Complex (RISC) and Argonaute (Ago).

Molecular Mechanism of RNAi

In the mechanism of this event, firstly the dicer protein, RNase like enzyme attaches to long double stranded RNA which enters the cytoplasm of the cell. The long dsRNA is processed into 20-25 nucleotide small interfering RNAs (siRNAs) by dicer enzyme. These siRNA then pair with complementary sequences in mRNA and attract an RNA-protein complex that cleaves the mRNA approximately in the middle of the bound siRNA. The siRNAs assemble into endoribonulease containing complexes known as RNA induced silencing complexes (RISC) that either cleave or unwind the sense strand. The siRNA antisense strand guides the RISC to complementary RNA molecule. The siRNA binds to complementary RNA molecule, once bound, RISC cleaves the target RNA. Cellular nucleases degrade the target RNA. This halts translation of the protein, thereby suppressing protein expression.

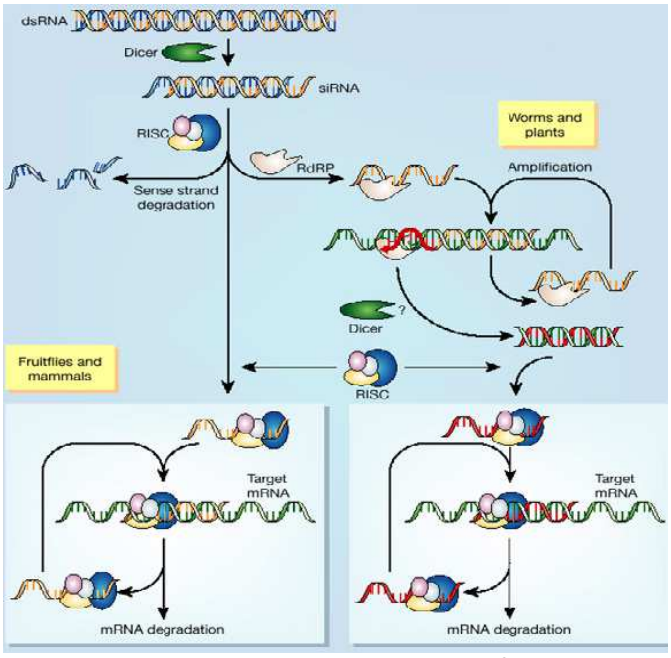

Fig. 1: Molecular Mechanism of RNAi

Metabolic Engineering of Plants Using RNAi

Metabolic engineering using RNAi provides a promising means of overcoming some of the limitations of plants by engineering of food plants that produce lower levels of natural plant toxins. RNAi has been used to engineer metabolic pathways to overproduce secondary products with health, yield or environmental benefits. For crop improvement, exogenous dsRNAs complimentary to known mRNA's, are introduced into a cell to specifically destroy that particular mRNA, thereby diminishing or abolishing gene expression. The plant metabolic engineering using RNAi has an increasing role in the creating and improving novel traits of plants. Specific dsRNA molecules should be designed to silence specific genes in plants. Future dissection of miRNA gene structures will greatly facilitate the development of RNAi vectors with high silencing efficiency and fewer side-effects.

Genetic engineering of highly nutritional food crops requires both gene silencing and counter-silencing technologies. Developing vectors that can suppress the RNAi pathway, while

over expressing transgenes will revolutionize this field. Such vectors might be based on viral suppressors of RNA silencing. With the thorough going research in RNAi mechanisms and the complete development of RNAi technology, researchers will create a new biological science and see enormous economic and social spinoffs. Some of the well known examples of RNAi to engineer novel traits in plants: High-oleic cottonseed oil**,** Low glutelin Rice**,** Transgenic opium poppy (Non-narcotic), transgenic tomato, transgenic cotton having reduced gossypol level.

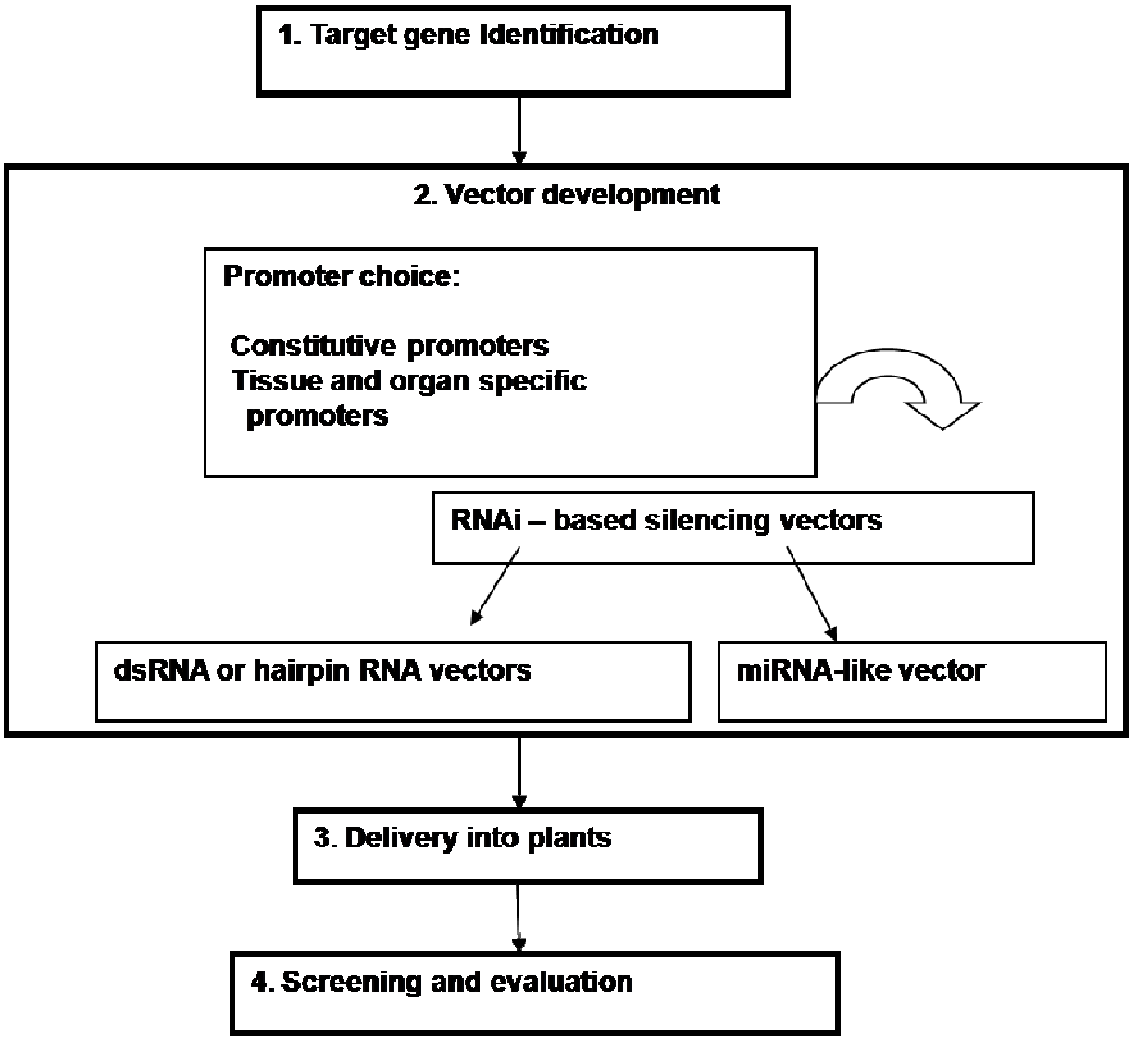

Fig. 2: The important steps in designing experiments based on RNAi for targeting traits of interest in plants.

Conclusion

Recent evidence indicates that expression of some genes may be suppressed through RNA interference also known as RNA Silencing and post-transcriptional gene silencing. Although many of the details of this mechanism are still poorly understood, it appears to be widespread, existing in fungi, plants and animals. It may also prove to be a powerful tool for artificially regulating gene expression in genetically engineered organisms. RNA interference is thought to have evolved as a defense against RNA viruses and transposable elements that move through an RNA intermediate. The extent to which it contributes to normal gene regulation is uncertain, but dramatic phenotypes effects result from some mutations that occur in the enzymes that carry out RNA interference.