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MINIREVIEW

Exploring the world of RNA interference in plant functional genomics: a research tool for many biology phenomena

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Abstract

The discoveries of RNA interference (RNAi), known as remarkable process where specific genes are silenced by small noncoding RNAs, have revolutionized our understanding of gene regulation in plants and other organisms. Recently, RNAi is used as a tool for reduction of expression of several genes or to control de expression of specific genes in eukaryotic genome. The aim of this review is to underline the most contribution in the "world of RNAi" from biological mechanism to several applications in plant system.

Key words: siRNA, plant gene silencing, functional genomics

Introduction

Initially discovered in *Petunia* plants, RNA interference has been described in almost all eukaryotic organisms, including protozoa, flies, nematodes, insects, parasites, and mouse and human cell lines (6, 5, 1, 11 26). Harnessed as an experimental tool, RNAi has revolutionized approaches to decoding gene function. It also has the potential to be exploited therapeutically, and clinical trials to test this possibility are already being planned (7, 18 **)**.

RNA interference represents the biological mechanism by which double-stranded RNA (dsRNA) induces gene silencing by targeting complementary mRNA for degradation. RNA silencing is a form of gene suppression that occurs at the level of RNA and includes posttranscriptional gene silencing in plants and fungi and RNA interference (RNAi) in *Caenorhabditis elegans*, *Drosophila*, and animals (1, 8, 13, 14, 22, 24, 23). Two types of RNA, double-stranded RNA (dsRNA) and short interfering RNA (siRNA), play key roles in this phenomenon (17, 21).

Extensive genetic and biochemical studies in various species have yielded a model of RNA silencing in which trigger dsRNA, either introduced into the cell or transcribed from transgenes, is cleaved into small siRNAs of 21 to 26 nucleotides (nt) by an enzyme, RNAse, named Dicer (10, 12). The siRNAs are incorporated into an RNA-induced silencing complex (RISC) to be associated with the target mRNAs (11), and the activated RISC functions to degrade the target mRNAs and suppress gene expression at various levels (18, 27).

The natural function of RNAi is referring to the mechanism involved in cellular defense against viruses, genomic containment of retrotransposons, and post-transcriptional regulation of gene expression. RNAi can specifically silence individual genes, creating knock-out phenotypes, either in transformants that can produce the required hairpin RNAs, or upon infection with recombinant RNA viruses that carry the target gene (VIGS, viral-induced gene silencing). RNAi can also reduce activity of related genes in addition to its target, particularly

in mammals, or affect adjacent but unrelated genes through local heterochromatization. In plant system the RNAi remains an important technique to reduce gene expression and it has been extensively used in functional genomics studies.

Pathways for RNA silencing in plants

Over the past, several pathways for silencing genes have been described. For instance, **cytoplasmic siRNA silencing**, is a mechanism by which the dsRNA could be a replication intermediate or a secondary-structure feature of single-stranded viral RNA and maybe important for virus-infected plant cells. The dsRNA may be form by annealing of overlapping complementary transcripts (1).

Silencing of endogenous messenger RNAs by miRNAs (microRNA)

miRNAs negatively regulate gene expression by base pairing to specific mRNAs, resulting in either RNA cleavage or arrest of protein translation. Like siRNAs, the miRNAs are short 21-24-nucleotide RNAs derived by Dicer cleavage of a precursor (9).

In plants, the prototype miRNAs were identified as a subset of the short RNA population, and are derived from an inverted repeat precursor RNA with partially double-stranded regions, and they target a complementary single-stranded mRNA. However, there are differences between the miRNAs of plants and animals (2, 3, 4).

Silencing associated with DNA methylation and suppression of transcription

The first evidence for this type of silencing was the discovery in plants that transgene and viral RNAs guide DNA methylation to specific nucleotide sequences. More recently, these findings have been extended by the observations that siRNA-directed DNA methylation in plants is linked to histone modification (1, 2). An important role of RNA silencing at the chromatin level is probably protecting the genome against damage caused by transposons.

Application of using RNAi in plant system

Because plants represent the principal source of human foods and livestock feeds, many efforts to improve the nutritional content of plants have focused on plant breeding. This can be done either by means of classical breeding based on selection of the natural or induced genetic variations, or by means of genetic engineering of transgenic plants. Genetic engineering technologies have advantages over classical breeding not only because they increase the scope of genes and the types of mutation that can be manipulated, but also because they have the ability to control the spatial and temporal expression patterns of the genes of interest.

The principle of using RNAi in functional genomics is quite simple and its starts with introduction of a dsDNA in cell, followed of activation of DICER gene and RISC complex which eventually leads to loss of gene expression. Of course, RNAi have many advantages and limitations when is used in functional genomics studies. An advantage of using RNAi is that a specific gene can be silenced if the target sequence is better chosen. This is also one of the limitation of using RNAi because, unlike insertional mutagenesis the exactly sequence of the gene is required. Secondly, the methods to delivery RNAi is very important, some species are easily transformable and some not.

Today, several groups are using RNAi methodology in plant functional genomics studies and numerous projects are employed today to determine gene function. For example, AGRIKOLA consortium (*Arabidopsis* genomic RNAi knock-out line analysis) is using the PCR products to generate gene-specific RNAi constructs for each *Arabidopsis* gene used in large scale gene silencing studies (16, 25); CATMA consortium (Complete *Arabidopsis* Transcriptome MicroArray), is generating gene sequence tags (GSTs) representing each *Arabidopsis* gene, designed so that they will hybridize on *Arabidopsis* cDNA microarrays in a

gene-specific manner; the *Medicago truncatula* RNAi database (www.medicago.org/rnai/) is a NSF-funded project planning to silence 1500 genes involved in symbiosis in this model legume; amiRNAi Central (http://2010.cshl.edu/scripts/main2.pl) is a new NSF-funded project to provide a comprehensive resource for knockdown of *Arabidopsis* genes.

However, by using RNAi, scientists can quickly and easily reduce the expression of a particular gene in mammalian and plant cell systems, often by 90% or greater, to analyze the effect that gene has on cellular function (19, 20).

Currently, several vectors are used for performing RNAi in plant system. For example a set of binary vectors, called ChromDB's RNAi vectors are described for producing dominant negative RNAi mutants using a target sequence cloning strategy that is based on the inclusion of two restriction enzyme cleavage sites in each of two primers used to amplify gene-specific fragments from cDNA. This design minimizes the number of PCR primers and results in the placement of unique restriction enzyme recognition sites to allow for flexibility in future manipulations of the plasmid, *e.g.*, moving the inverted repeat target sequence to a different vector (Chrom database). These vectors are based on pCAMBIA binary vectors, a set of plasmids developed by the Center for Application of Molecular Biology to International Agriculture (CAMBIA).

The pHELLSGATE, high-throughput gene silencing vector and a high throughput tobacco rattle virus (TRV) based Virus-induced gene silencing (VIGS) vector are binary vectors developed by Invitrogen are used for expression of GUS and GFP proteins. These vectors are base on Gateway recombination-based technology, which replaced the conventional cloning strategy. It is based on the phage lambda system of recombination. It enables segments of DNA to be transferred between different vectors while orientation and reading frame are maintained. It can also be used for transfer of PCR products.

It saves valuable time, because once the DNA has been cloned into a Gateway vector, it can be used as many genome function analysis systems as is required. In this way, the use of vectors in the process of plant functional genomics has been made much easier, while the process has also been made faster. This allows for higher throughput analysis to occur (www.invitrogen.com) (15).

Hairpin RNAi has been extensively used for research purposes and is increasingly being employed with the aim of developing plants for commercial use: alter oil content in oilseeds to improve their nutritional value; produce alternative potential pharmaceutical ingredients in poppies; develop immunity to Barley yellow Dwarf virus in Barley; develop high amylose wheat varieties with health benefits (CSIRO plant industry).

Hairpin RNAi have been used to understand complex metabolic pathways in non-model plants. Alkaloid synthesis in poppies is one example. The enzyme codeinone reductase (COR) converts codeinone to codeine, which is demethylated to morphine. An hairpin RNAi construct designed to target all seven members of the COR gene family yielded transgenic plants displaying varying degrees of diminished morphine production, from 25 to 100 per cent, along with the compensatory accumulation of the morphine precursor reticuline. This result was unexpected because reticuline lies eight steps upstream from morphine.

Another approach is to down-regulate the activity if a target enzyme to accumulate the intermediate product (27). For example, transgenic California poppy cells transformed with BBE (berberine bridge enzyme) RNAi vector showed a reduction in BBE expression and accumulation of the pathway intermediate reticuline. This was the first success in targeting genes involved in secondary metabolism.

Conclusions

The study of RNAi has led to a revolution in the understanding of gene expression. Several groups of scientists have used the RNAi strategies as a new tool for cheap screen of gene function in transformable plants. For example, REGIA, an EU project on functional genomics of transcription factors from *Arabidopsis thaliana*, is a promising project for characterization of all *Arabidopsis* genes by the year 2010. Taken together, RNAi represent a significant tool for the accomplishment of these goals, and will undoubtedly be used to address many other challenges in plant functional genomics.

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