ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 2, pp.085-091, March, 2010

REVIEW ARTICLE

RNA INTERFERENCE AND ITS APPLICATION IN MANAGEMENT OF VIRAL DISEASES IN PLANTS

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ARTICLE INFO

Article History:
Received 2nd, February, 2010
Received in revised form
25th Febryary, 2010
Accepted 5th March, 2010
Published online 25th March, 2010

Key words:
Transgenes
Gene silencing
mRNA
RNA interference

ABSTRACT

The discovery of RNAi was preceded first by observations of transcriptional inhibition by antisense RNA expressed in transgenic plants and more directly by reports of unexpected outcomes in experiments performed by plant scientists in the U.S. and the Netherlands in the early 1990s. In an attempt to alter flower colors in petunias, researchers introduced additional copies of a gene encoding chalcone synthase, a key enzyme for flower pigmentation into petunia plants of normally pink or violet flower color. The over expressed gene was expected to result in darker flowers, but instead produced less pigmented, fully or partially white flowers, indicating that the activity of chalcone synthase had been substantially decreased; in fact, both the endogenous genes and the transgenes were down regulated in the white flowers. Soon after, a related event termed quelling was noted in the fungus Neurospora crassa, although it was not immediately recognized as related. Further investigation of the phenomenon in plants indicated that the down regulation was due to post-transcriptional inhibition of gene expression via an increased rate of mRNA degradation. This phenomenon was called co-suppression of gene expression, but the molecular mechanism remained unknown. Not long after, plant virologists working on improving plant resistance to viral diseases observed a similar unexpected phenomenon. While it was known that plants expressing virus-specific proteins showed enhanced tolerance or resistance to viral infection, it was not expected that plants carrying only short, non-coding regions of viral RNA sequences would show similar levels of protection. Researchers believed that viral RNA produced by transgenes could also inhibit viral replication. The reverse experiment, in which short sequences of plant genes were introduced into viruses, showed that the targeted gene was suppressed in an infected plant.

INTRODUCTION

RNA interference (RNAi) is a system within living cells that helps to control which genes are active and how active they are. Two types of small RNA molecules-microRNA (miRNA) and small interfering RNA (siRNA) are central to RNA interference. RNAs are the direct products of genes, and these small RNAs can bind to specific other RNAs and either increase or decrease their activity, for example by preventing a messenger RNA from producing a protein. RNA interference has an important role in defending cells against parasitic genes – viruses and transposons – but also in directing development as well as gene expression in general. The RNAi pathway is found in many eukaryotes including animals and is initiated by the enzyme Dicer, which cleaves long double-stranded RNA (dsRNA) molecules into short fragments of ~20 nucleotides. One of the two strands of each fragment, known as the *guide strand*, is then incorporated into the RNA-induced silencing complex (RISC). The well-studied outcome is post-transcriptional gene silencing, which occurs when the guide strand base pairs with a complementary sequence of a messenger RNA molecule and induces cleavage by Argonaute, the catalytic component of the RISC complex. This process is known to spread systemically throughout the organism despite initially limited molar concentrations of siRNA. The selective and robust effect of RNAi on gene expression makes it a valuable research tool, both in cell culture and in living organisms because synthetic dsRNA introduced into cells can induce suppression of specific genes of interest. RNAi may also be used for large-scale screens that systematically shut down each gene in the cell, which can help identify the components necessary for a particular cellular process or an event such as cell division. Exploitation of the pathway is also a promising tool in biotechnology and medicine. Historically, RNA interference was known by other names, including post

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transcriptional gene silencing and quelling. Only after these apparently unrelated processes were fully understood did it become clear that they all described the RNAi phenomenon.

Variations in Homology-Dependent Gene Silencing

Homology dependent gene silencing has been recorded in various organisms, ranging from plants to animals to fungi. Because of the different experimental systems used to study this phenomenon, several terms have emerged, often describing potentially similar processes. Silencing is generally defined on the basis of two properties-

a) The inducing agent

b) The mechanism of silencing

(1) Post-Transcriptional Gene Silencing (Ptgs)

This is a general term that applies to RNA interference (RNAi) in animals and to some types of virally and transgene-induced silencing in plants. The transcription of the gene is unaffected; however, gene expression is lost because mRNA molecules become unstable (Ingelbrecht et al., 1994).

(2) Transcriptional Gene Silencing (Tgs)

This is generally observed in plants but has also been seen in animals. Gene expression is reduced by a blockade at the transcriptional repression might be caused by chromatin modification or DNA methylation (Steger et al., 2008).

(3) Transgene Induced Silencing

Silencing is caused by the presence of transgenes in the genome. Repression is usually related to copy number. Tandemly arrayed transgenes are more effective inducers of silencing than dispersed transgenes, with inverted repeats being the most effective. Silencing can occur transcriptionally and post- transcriptionally Fagard and Voucherat (2000).

(4) Virus-Induced Silencing

Silencing is induced by the presence of viral genome RNA. Only replication- competent virus cause silencing, indicating that double stranded RNA molecules might be the inducing agents**.**

(5) Co suppression

Silencing of an endogenous gene due to the presence of a homologous transgene or virus. Cosuppression can occur at the transcriptional or post-transcriptional level (Dernberg et al., 2002).

(6) RNAi

This type of PTGS is induced directly by dsRNA. It was first defined in *Caenorhabditis elegans* and seems to be mechanistically related, if not identical, to PTGS in plants.

(7) Quelling

This term is specific for transgene-induced PTGS in *Neurospora crassa.*

MECHANISM of RNA INTERFERENCE

As the various pieces of the RNAi machinery are being discovered, the mechanism of RNAi is emerging more clearly. In the last few years, important insights have been gained in elucidating the mechanism of RNAi. A combination of results obtained from several in vivo and in vitro experiments have gelled into a two-step mechanistic model for RNAi/PTGS. The first step, referred to as the RNAi initiating step, involves binding of the RNA nucleases to a large dsRNA and its cleavage into discrete ≈21- to ≈25-nucleotide RNA fragments (siRNA). In the second step, these siRNAs join a multinuclease complex RISC, which degrades the homologous single-stranded mRNAs. At present, little is known about the RNAi intermediates, RNA-protein complexes, and mechanisms of formation of different complexes during RNAi. In addition to several missing links in the process of RNAi, the molecular basis of its systemic spread is also largely unknown.

Processing of dsRNA into siRNAs

Studies of PTGS in plants provided the first evidence that small RNA molecules are important intermediates of the RNAi process. Hamilton and Baulcombe (1999), while studying transgene-induced PTGS in five tomato lines transformed with a tomato 1-aminocyclopropane-1-carboxyl oxidase (ACO), found accumulation of *aco* small RNAs of 25 nucleotides. More direct evidence about the generation of siRNAs in RNAi came from an in vitro cell-free system obtained from a *Drosophila* syncytial blastoderm embryo. These authors were able to reproduce many of the features of RNAi in this system. When dsRNAs radiolabeled within either the sense or the antisense strand were incubated with *Drosophila* lysate in a standard RNAi reaction, 21- to 23-nucleotide RNAs were generated with high efficiency (Al-Anouti et al., 2003). Single-stranded ^{32}P-labeled RNA of either the sense or antisense strand was not efficiently converted to 21- to 23-nucleotide products. The formation of the 21- to 23-nucleotide RNAs did not require the presence of corresponding mRNAs (Elbashir et al., 2001).

The role of the small RNAs in RNAi was confirmed independently that synthetic 21- to 23-nucleotide RNAs, when added to cell-free systems, were able to guide efficient degradation of homologous mRNAs. To assess directly if the siRNAs were the true intermediates in an RNAi reaction, (Zamore et al., 2000) fractionated both the unprocessed dsRNAs and processed dsRNAs from the *Renilla luc* dsRNA-treated cell-free *Drosophila* system and showed that only the fractions containing native siRNAs were able to bring about the cognate RNA degradation and their ability to degrade RNA was lost when these fractions were treated at 95°C for 5 min. These in vivo and in vitro studies thus provided the evidence that siRNAs are the true intermediates of the RNAi reaction. Together with the experiments to identify siRNAs as the key molecules for the RNAi effect, several investigators carried out the logical search for polypeptides that could generate such molecules. Based on the binding and cleavage properties of *E. coli* RNase III enzymes, predicted the involvement RNase III-type endonucleases in the degradation of dsRNA to siRNAs Bass (2000). The RNase III enzyme makes staggered cuts in both strands of dsRNA, leaving a 3′ overhang of 2 nucleotides. The first evidence for the involvement of RNase III enzyme in RNAi was provided, chemically analyzed the sequences of the 21- to 23-nucleotide RNAs generated by the processing of dsRNA in the *Drosophila* cell-free system. The presence of 5′-phosphate, 3′-hydroxyl, and a 3′ 2-nucleotide overhangs and no modification of the sugar-phosphate backbone in the processed 21- to 23-nucleotide RNAs Donze and Picard (2002).Experimental studies were carried out with *C. elegans* extract, and an ortholog of Dicer naA number of in vivo and in vitro experimental

studies have shown that the production of 21- to 23-nucleotide RNAs from dsRNA requires ATP. The rate of 21- to 23-nucleotide RNA formation from corresponding dsRNAs has been shown to be six times slower in the *Drosophila* extract depleted for ATP by treatment with hexokinase and glucose (Donech et al., 2003). Dicer simmunoprecipitates from *D. melanogaster* as well as S2 cell extracts and DCR1 immunoprecipitates from *C. elegans* extract required ATP for the production of 22-nucleotide RNAs. These experiments suggest that ATP controls the rate of siRNA formation. However, it is still unclear whether ATP is absolutely rate limiting for the production of siRNAs from dsRNA.

The RNase activity and dsRNA binding of 218-kDa recombinant human Dicer have also been examined in vitro. The enzyme generated siRNA products from dsRNA quite efficiently in the presence of Mg^{2+} and the absence of ATP. The RNase activity was sensitive to ionic interactions, whereas the dsRNA binding was quite effective in presence of high salt and did not require Mg^{2+} at all. The dsRNA binding domain is located at the C terminus of Dicer, which is separable from the helicase and PAZ motifs. Human Dicer expressed in mammalian cells colocalized with calreticulin, a resident protein of the endoplasmic reticulum. In other systems, Dicer has also been found to complex with various other proteins. Hence, it is possible that the Dicer RNase activity functions as a complex of proteins in vivo (Caudy et al., 2002); (Ischizuka et al., 2002).

Amplification of siRNA

One of the many intriguing features of RNA interference is the apparently catalytic nature of the phenomenon. A few molecules of dsRNA are sufficient to degrade a continuously transcribed target mRNA for a long period of time. Although the conversion of long dsRNA into many small siRNAs results in some degree of amplification, it is not sufficient to bring about such continuous mRNA degradation. Since mutations in genes encoding RNA-dependent RNA polymerase (RdRP) affect RNAi, it was proposed that this type of polymerase might replicate siRNAs as epigenetic agents, permitting their spread throughout plants and between generations in *C. elegans*. Recent studies provided convincing biochemical and genetic evidence that RdRP indeed plays a critical role in amplifying RNAi effects (Hammond et al., 2000). In RNAi reaction, they observed the formation of new siRNA species corresponding to target mRNAs but different from trigger dsRNAs. They named these new siRNAs secondary siRNAs. With a primary trigger dsRNA specific for the *lacZ* region of the target mRNA that encoded a GFP-LacZ fusion protein, these authors demonstrated the degradation of a separate GFP mRNA target. This kind of RNAi induced by secondary siRNAs was named transitive RNAi. These authors demonstrated the requirement for the *rrf1* gene, a *C. elegans* gene with sequence homology to RdRP, in the generation of secondary siRNAs and transitive RNAi .Amplification of siRNAs might occur at various stages of the RNAi reaction and has been documented in plants, *C. elegans*, *N. crassa*, and *Dictyostelium discoideum* but not in flies and mammals). Though the RdRP activity is present in *Drosophila* embryo extract, as mentioned earlier, it is surprising that the fly genome does not code for RdRP. Additionally, numerous experiments also suggest that RdRP is not required for RNAi in *D. melanogaster* (Hannon et al., 2002).

In the effector step of RNAi, the double-stranded siRNAs produced in the first step are believed to bind an RNAi-specific protein complex to form a RISC. This complex might undergo activation in the presence of ATP so that the antisense component of the unwound siRNA becomes exposed and allows the RISC to perform the downstream RNAi reaction. This activated complex cleaved the substrate. The size and constitution of the precursor as well as the activated RISC might vary depending on the choice of system. The antisense siRNAs in the activated RISC pair with cognate mRNAs, and the complex cuts this mRNA approximately in the middle of the duplex region Hamilton and Baulcombe (1999).A few independent studies demonstrated the importance of the RISC complex in this part of RNAi reactions. The mRNA-cleaving RNA-protein complexes have also been referred to as siRNP (small interfering ribonucleoprotein particles). It is widely believed that this nuclease is probably different from Dicer, judging from the substrate requirements and the nature of the end products. Since the target cleavage site has been mapped to 11 or 12 nucleotides downstream of 5′ end of the guide siRNA, a conformational rearrangement or a change in the composition of an siRNP ahead of the cleavage of target mRNA is postulated. Finally, the cleaved mRNAs are perhaps degraded by exoribonucleases (Hammond et al., 2000). A part of cleaved fragments of mRNA at the end of step 2 might also be converted to the duplex forms by the RdRP-like activity. These forms might have siRNA-like functions and eventually enter the pool of the amplification reaction. Thus, it is likely that amplification of the RNAi reaction takes place at both step 1 and step 2 of RNAi. In another model, it has been proposed that siRNAs do not act as primers for the RdRP-like enzymes, but instead assemble along the length of the target RNA and are then ligated together by an RNA ligase to generate cRNA. The cRNA and target RNA hybrid would then be diced by the DCR protein. All these models were summarized by Morris (2008).

MECHANISTIC BASIS of RNAi

One of the great mysteries surrounding RNAi is how a cell can respond to virtually any incoming dsRNA by efficiently and specifically silencing genes that are homologous to it. Since, the seminal discovery of small RNA species, progress in both genetic and biochemical dissection of silencing process has begun to produce a basic understanding of the interference mechanism.Shortly after RNAi was discovered in *C. elegans*, geneticists began to search for mutants that were defective in this response. In parallel, genetic screens were being done in Neurospora and Arabidopsis and more recently in Chlamydomonas reinhardtii to identify genes that are required for transgene and virally induced PTGS.Mutants have been identified that affect the formation of silencing activity, the effector step of silencing and the persistence of silencing. These mutants indicate that formation of the silencer and the actual silencing events might be distinct and seperable processes.

In particular, mutant plants and animals have additional phenotypes that at important biological functions for RNAi and related silencing phenomena. Two mutants have been shown to affect the formation of silencing activity. There are C. elegans RNAi deficient mutants rde-4 and rde-1. The rde-1 protein is homologous to the quelling defective (qde-2) gene in Neurospora and ARGONAUTE (AGO-1) in Arabidopsis. Rde-1 and qde-2 have no apparent phenotypes other than defects in RNAi or quelling, the Arabidopsis AGO-1 mutant, which has recently been shown to be defective in PTGS, has several developmental abnormalities - namely – alteredleaf differentiation, a reduced number of secondary meristems and are sterile because of defects in flower development.

Two mutants have also been identified in the second, effector step of silencing activity, these have been defined as such because a heterozygous mutant offspring. Worms with mutants in the RNAi deficient-2 (rde-2) and mutator-7 (mut-7) genes are less deficient in RNAi than the initiator-step mutants. In addition to defects in RNAi, the effector mutants have increased levels of transposons activity and defects in transgene silencing. These other phenotypes imply that part of the RNAi machinery is involved in silencing transposons and cosuppression. This strong genetic relationship between RNAi and transposon silencing supports the idea that RNAi is involved in genome defence and maintenance of genome stability and raises questations concerning the route by which transposons and other multicopy genetic elements provoke an RNAi response (Dernburg et al., 2000).

As genetic routes continue to link proteins to the silencing process, the challenge is to understand precisely how each one fits into the silencing mechanism. Tuschl and colleagues reported that extracts from Drosophila embryos specifically prevented the synthesis of luciferase from synthetic mRNA upon addition of cognate dsRNA. Finally, the authors showed that the inhibition of gene expression was most probably due to degradation of mRNAs that correspond to added dsRNA.

The nuclease, which was designated RISC could b extracted from cells in association with ribosomes. This proposed that RNAi acts as transcriptional surveillance mechanism.Through Partial purification it aws found that 22-nucleotide RNA was homologous to the substrate that fractionated together with the RISC and was required for its activity. These small RNAs direct a multicomponent nuclease towards destruction of homologous mRNA. Therefore, new term was coined “Guide RNAs”.

RNAi mechanism do not involves changes in DNA sequence. One explanation of these epigenetic phenomena invokes an interaction at the DNA or chromatin level and consistent with this idea, it is known that there are RNA-DNA interactions associated with PTGS. These interactions lead to sequence-specific methylation of the corresponding DNA sequences. (Steger et al., 2008). RNAi can be separated into three steps. The study of how PTGS is triggered has revealed the existence of at least three steps:

1. INITIATION

Most data concerning the control of initiation are indirect and result from the analysis of parameters that increase or decrease the efficiency of spontaneous triggering of PTGS.

II. HIGHLY TRANSCRIBED SINGLE TRANSGENE COPIES

Several arguments suggest that the efficiency of triggering could depend on the probability that the transgene produces a particular form of RNA above a threshold level. Indeed PTGS is triggered mostly when plants are homozygous for the transgene locus. PTGS is triggered more efficiently when strong promoters are used. PTGS is inhibited when transgene transcription is blocked.

III. TWO TRANSGENE COPIES ARRANGED IN AN INVERTED REPEAT (IR)

These IRs are usually transcribed at very low levels, IRs produce dsRNA by read through transcription and that dsRNA efficiently triggers PTGS, even when produced at a low level.Introduction of single transgene copies that have a panhandle structure(i.e, carry the same sequence cloned in sense and antisense orientations downstream of the promoter) leads to efficient silencing of homologous (trans) gene, which suggests that such dsRNA are efficient initiators of PTGS. PTGS mechanisms triggered by highly transcribed single transgene loci and transgene IRs in plants are different.

EVIDENCE FOR SYSTEMIC SIGNALLING IN GENE EXPRESSION

There is a systemic signal that can mediate gene silencing. From the gene-specificity of the systemic silencing, it may be inferred that the signal molecule is likely to be nucleic acid.

1. Silencing of nitrate reductase gnes was transmitted to a non-transgenic tobacco mutant scion over expressing the endogenous Nia-2 gene owing to metabolic derepression but not to a wild scion, which indicates that over accumulation of Nia mRNA above the level of that in willd-type plants, rather than the presence of a transgene in the scion, is required for triggering of RNA degradation during PTGS. The transmission of PTGS also occurred when silenced stocks and non-silenced target scion were physically separated by upto 30 cm of stem of a non-target wild type plant indicating long distance propagation.
2. The mobile silencing signal Another strategy used by species to improve their silencing efficiency is based on the fact that RNAi is not cell-autonomous. In both C. elegans and plants, locally initiated silencing can spread to distant sites throughout the organism. This indicates that an as yet unidentified mobile silencing signal exists that cooperates with RNAi. In nematodes, this mobile signal can travel long distances via cell-to-cell movements. Several proteins required for this trafficking have been described, including the multispan transmembrane protein SID-1 or Rsd-3, a homolog of the human enthoprotin, which was previously shown to be involved in vesicle-trafficking). In plants, two types of RNA-silencing spread exist: a cell-to-cell movement, very similar to the nematode one, and a long-range spreading. Indirect evidence indicates that RNA-silencing moves over long distances through the phloem and activates silencing in cells by spreading through plasmodesmata reported that the mobile signal is able

to spread to nearby cells using specific proteins, where it can activate RNAi and, if an amplification system is present, reinitiate a local cell-to-cell movement. While genes required for an active systemy have been partly identified, the mobile signal involved remains to be determined. siRNAs are very good candidates, as they are always associated with RNA silencing and have the ability to reinitiate RNAi. However, mutants in plants and worms defective for siRNA accumulation or production are not defective for systemic silencing. Other candidates could be the long dsRNAs themselves or the targets bearing defects or tagged by proteins. In all these models, the fact that the same species exhibiting systemy also show transitivity suggests that a link exists between these mechanisms

Advantages of RNA interference

One specific advantage of RNAi over other methods previously employed is that dsRNA activates a normal cellular process leading to a highly specific RNA more importantly, a cell-to-cell spreading of this gene silencing effect in several RNAi models (Shuey et al., 2002). It is also a relatively quick method enhancing the evaluate their physiological roles in plants and human (Ge et al., 2003).

There are many examples of experiments in which RNAi is used to assess the functions of particular proteins and to aid in the discovery of what enzymes and proteins are involved in certain metabolic pathways as in the Gibberellins/Abscisic acid signaling pathway (Zentella et al., 2002). Another type of experiment that is currently being done involves attempts to induce RNA interference by bi-directional transcription of a particular gene of interest or by flanking the gene to be silenced by two convergent promoters (Bieri et al., 2002).So far we have seen that RNA interference can be an extremely valuable genetic tool when studying plants, insects and small invertebrates such as nematodes.

APPLICATION

1. Sweet potato (*Ipomoea batatas*) is an important subsistence and famine reserve crop grown in developing countries where *Sweet potato chlorotic stunt virus* (SPCSV; *Closteroviridae*), a single stranded RNA degradation and perhaps (ssRNA) crinivirus, synergizes unrelated viruses in

RNAi in virus resistance in plants

S. No.	Pathogen	Target region	Result
1	*Potao virus- X*	*RDR6*	Meristem exclusion of Virus infection
2	*Potao virus-Y*	*PVXp25*	Block cell to cell movement of virus
3	*Potyvirus*	*HC-pro*	Immunity
4	*Cymbidium ring spot virus*	p^{19}	Blocking of virus spread
5	*Tobacco mosaic virus*	*Phytiene desaturnase (PDA) gene*	Inhibition of TMV replication
6	*Tobacco etch virus*	*CP*	No virus specific symptoms occurs
7	*Cucumber mosaic virus*	*CMV2b*	Block cell to cell movement of virus
8	*Tombusvirus*	*P19*	Sequestration of siRNA and prevent incorporation into RISC complex
9	*Turnip yellow mosaic virus*	*P69*	RDR dependent secondary dsRNA synthesis
10	*Citrus tristeza virus*	*P20,p23, CP*	Inhibition of intracellular silencing
11	*Red clover necrotic mosaic virus*	*Rep gene*	Reduced virus accumulation
12	*Rice dwarf virus*	*Pns10*	Interference with upstream step of dsRNA formation
13	*Tomato golden mosaic virus*	*AC2*	Adenosine kinase inhibition
14	*Tomato bushy stunt virus*	*p19*	Sequestration of siRNA and prevent incorporation into RISC complex
15	*Mungbean yellow mosaic India virus*	*Bidirectional promotor*	Recovery from infection
16	*Alfalfa mosaic virus*	*Arbitrary sequence*	Recovery from infection
17	*Beet necrotic yellow vein virus*	*Coat protein*	tolerance
18	*Pepper mild mottle virus*	*Arbitrary sequence*	Block in viral infectivity
19	*African cassava mosaic virus*	*Rep gene*	Reduced virus accumulation
20	*Tomato yellow leaf curl Sardinia virus*	*Rep gene*	Poor resistance

genetic analysis of traditional model organisms and has provided a means of performing reverse genetics experiments on organisms lacking any established genetic tools. RNAi activity plays a role in host-cell protection from viruses and transposons in plants and insects. From a practical perspective, RNAi can therefore be used to target gene expression and has been proved to be a very powerful technique to knock down specific genes to co-infected sweet potato plants. The most severe disease (and yield losses are caused by co-infection with SPCSV and a potyvirus, *Sweet potato feathery mottle virus* (SPFMV; *Potyviridae*). Potyviruses synergize unrelated viruses by suppression of RNA silencing with the P1/HC-Pro polyprotein; however, the SPCSV-SPFMV synergism is unusual in that the potyvirus is the beneficiary. Transformation of an SPFMV-resistant

sweet potato variety with the double-stranded RNA (dsRNA)-specific class 1 RNA endoribonuclease III (RNase3) of SPCSV broke down resistance to SPFMV, leading to high accumulation of SPFMV antigen and severe disease symptoms similar to the synergism in plants co-infected with SPCSV and SPFMV. RNase3-transgenic sweet potatoes also accumulated higher concentrations of 2 other unrelated viruses and developed more severe symptoms than non-transgenic plants.In leaves, RNase3 suppressed ssRNA-induced gene silencing (RNAi) in an endonuclease activity-dependent manner. It cleaved synthetic double-stranded small interfering RNAs (siRNAs) of 21, 22, and 24 bp in vitro to products of approximately 14 bp that are inactive in RNAi. It also affected total siRNA isolated from SPFMVinfected sweet potato plants, suggesting a viral mechanism for suppression of RNAi by cleavage of siRNA. Results implicate RNase3 in suppression of antiviral defense in sweet potato plants and reveal RNase3 as a protein that mediates viral synergism with several unrelated viruses, a function previously described only for P1/HC-Pro. (Wilmer, et al., 2009).

2. Gene silencing is an important but little understood regulatory mechanism in plants. Here we report that a viral sequence, initially identified as a mediator of synergistic viral disease, acts to suppress the establishment of both transgene-induced and virus-induced posttranscriptional gene silencing. The viral suppressor of silencing comprises the 5*-proximal region of the tobacco etch potyviral genomic RNA encoding P1, helper component-proteinase (HC-Pro) and a small part of P3, and is termed the P1yHCPro sequence. A reversal of silencing assay was used to assess the effect of the P1yHC-Pro sequence on transgenic tobacco plants (line T4) that are posttranscriptionally silenced for the *uidA* reporter gene. Silencing was lifted in offspring of T4 crosses with four independent transgenic lines expressing P1yHC-Pro, but not in offspring of control crosses. Viral vectors were used to assess the effect of P1yHC-Pro expression on virus-induced gene silencing (VIGS). The ability of a potato virus X vector expressing green fluorescent protein to induce silencing of a green fluorescent protein transgene was eliminated or greatly reduced when P1yHC-Pro was expressed from the same vector or from coinfecting potato virus X vectors. Expression of the HC-Pro coding sequence alone was sufficient to suppress virus-induced gene silencing, and the HC-Pro protein product was required for the suppression. This discovery points to the role of gene silencing as a natural antiviral defense system in plants and offers different approaches to elucidate the molecular basis of gene silencing (Anandlakshmi, et al., 1998).RNA interference is a new immunity mechanism that protects fruit flies, mosquitoes, nematodes as well as plants against viral infections' Following are the examples of gene silencing against viral infections in plants

Conclusion

RNA- based silencing systems were independently discovered, named and studied in different systems were discovered, named and studied. Posttranscriptional gene silencing and compression or RNA- mediated virus resistance in pants in animals and plants are all based on this mechanism of short RNAs guiding and edonucleaese to a target RNA sequence for degredation.

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