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Review

RNAi: An emerging field of molecular research

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RNA silencing, named as co-suppression or post transcriptional gene silencing (PTGS) was found in transgenic plants which was the result of cellular mRNA degradation and silencing of gene expression. RNA interference (RNAi) is a specific technique using only a few double stranded RNA (dsRNA) molecules to stop the expression which has made it one of the important areas in molecular biology. By introducing a gene into the host genome which is highly homologous to an endogenous gene, the RNA silencing is initiated. Double-stranded RNA (dsRNA) is cut by the enzyme "Dicer" producing small interfering RNAs (siRNAs) which combine with RNA-induced silencing complex (RISC). RISC, a protein complex, binds one strand of siRNA with mRNA of native target gene for destruction, resulting in gene silencing. The mechanism of RNAi offers a quick and easy way to determine the function of a gene. In this review, we discuss the history, components, mechanism and the application of RNA interference.

Key words: Dicer, dsRNA, gene silencing, RISC, RNAi.

INTRODUCTION

RNA interference is an exciting new field of research over the world in the field of biotechnology and molecular biology. In this process, double stranded RNA (dsRNA) is incorporated into cells which causes the specific degradation of mRNA of the same sequence (Hayafune et al., 2006). It was found on the nematode *Caenorhabditis elegans* that the double stranded RNA (dsRNA) was more effective to produce interference than either strand individually (Fire et al., 1998). When this dsRNA species was purified away from the single-stranded RNA, the antisense ssRNA lost most of its interfering activity, and the sense ssRNA was totally inactive. On the other hand, the dsRNA was a very potent inducer of the so-called interference effect. Thus, a new phenomenon was discovered, it was named RNA interference (RNAi). RNAi silence mRNA expression, has revoulationalized biological sciences, can be used to develop novel drugs against any disease target (Antonin and de Fougerolles, 2008). The RNAi can be an excellent mechanism to control viral diseases in plants, animals and humans (Rahman et al., 2008). RNAi techniques have been working with mRNA degradation, gene silencing, gene expression regulation, resistance to virus infection, regulation of chromatin structure and genome integrity (Hannon, 2002; Grewal and Moazed, 2003). RNAi provided a great opportunity in the basic biological research and the development of RNAi tools for therapeutic applications (Fabani, 2007). In this report, we have tried to explain the various components of RNAi, its process known to date, mode of action and application. This phenomenon can result in highly specific suppression of gene expression and have the capability to revolutionize key areas of biological sciences such as molecular biology, biotechnology, clinical research and therapeutics.

DISCOVERY OF RNAi

An observation of RNAi was made in Petunia flower that introduction of a pigment- producing gene (*CHS*) suppressed expression of both the introduced gene and the homologous endogenous gene, known as co-suppression (Napoli et al., 1990). The RNAi was discovered in *Caenorhabditis elegans* where the worm responded biologically due to the exogenous induction of

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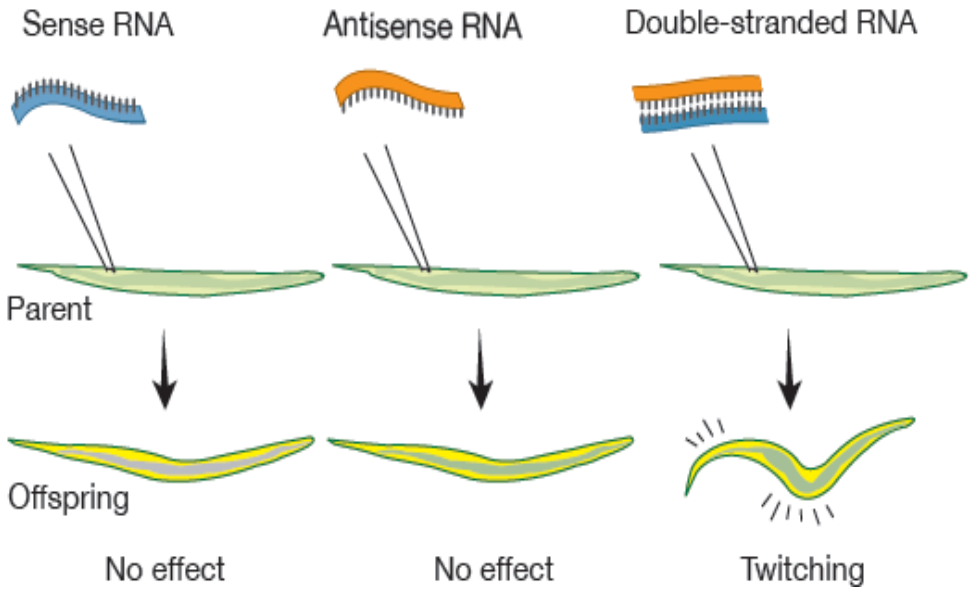

Figure 1. Expression of genes encoding a muscle protein in the worm *Caenorhabditis elegans*. Unique application of sense and antisense RNA have no effect but combined application shows twitching movements (Daneholt, 2006).

dsRNA (Grishok, 2007) (Figure 1). dsRNA was injected into *C. elegans* observing specific and effective gene silencing effect which was named at RNAi (Fire et al., 1998). Andrew Fire and Craig Mello won the Nobel Prize for medicine for their discoveries on gene silencing (Daneholt, 2006). Their achievements showed a natural mechanism regulating the stream of genetic database. RNAi has thus emerged as a basic mechanism for the regulation of gene expression and to study the gene function.

COMPONENTS OF RNAi

Among the components of gene silencing process, some serve as initiators and others serve as effectors, amplifiers and transmitters. Many other components and their interrelations are expected to be available in future. Here we have outlined what is known so far.

Dicer

RNAi mechanism involves dsRNA processing, mRNA degradation where the Dicer acts as an essential component in the process. Dicer is a ribonuclease in RNase III family enzyme that's functions is the processing of dsRNA to short double-stranded RNA fragments called small interfering RNA (siRNA) (Bernstein et al., 2001). dsRNA continuously cleaves by dicer at 21-25 bp distance and produce siRNA with 2-nt 3′ overhangs and 5' phosphorylated ends.

Dicer contains helicase domain, dual RNase III motifs and a region homologous to the protein of RDE1 or QDE2 or ARGONAUTE family (Bernstein et al., 2001). Dicer works on the first step of RNAi pathway as a catalyst starting production of RNA-induced silencing complex (RISC). Argonaute, a catalytic component of dicer, have the capability to degrade mRNA complementary to that of the siRNA guide strand (Jaronczyk et al., 2005). Dicer digests dsRNA to siRNA of uniformed size.

RNA induced silencing complex (RISC)

RISC or RNA-induced silencing complex is a siRNA directed endonuclease contains proteins and siRNA. It targets and destroys mRNAs in the cell complementary to the siRNA strand. When RISC finds the mRNA complementary to siRNA, it activates RNAse enzyme resulting the cleaves of targeted RNA. About 20-23 bp siRNA are able to associate with RISC and guide the complex to the target mRNA and combined together and degrades them, resulting in decreased levels of protein translation and knockdown the gene function (Hammond et. al., 2000; Hammond et. al., 2001). RISC acts as catalyst to cleave single phosphodiester bond of mRNA (Schwarz et al., 2004).

RNA dependent RNA polymerase

RNA-dependent RNA polymerases (RdRPs) play role in the silencing effect in RNAi and Post Transcriptional Gene Silencing (PTGS) mechanism. Due to the activity of

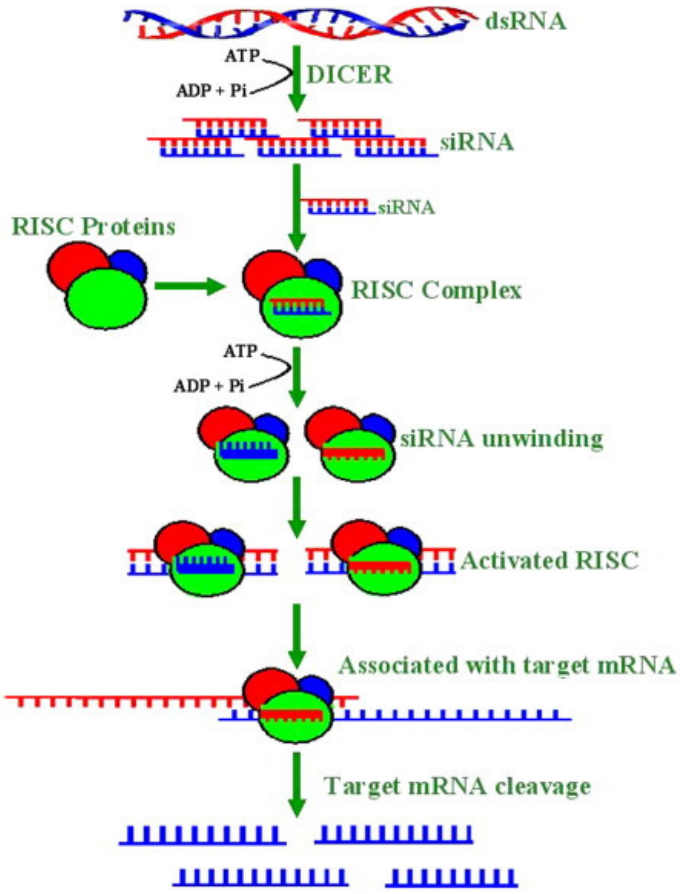

Figure 2. RNAi mechanism. siRNAs associated with RISC. Active RISC binds as well as cleaves mRNA to stop protein synthesis (Rahman et al., 2008).

RdRps, RNAi is more powerful technique than the antisense approach of gene silencing in plants (Dalmay et al., 2000) and *C. elegans* (Smardon et al., 2000). This RdRPs activity was first observed in RNA viruses (Blumenthal and Carmichael, 1979). Transcription and replication of viral genome is the result of the activity of viral RdRp. In some plants, RdRP activity can also be found in healthy tissue. In 1993, RdRP was isolated and purified from tomato leaves (Schiebel et al., 1993a) and characterized its catalytic properties (Schiebel et al., 1993b). RdRP activity was normally demonstrated by performing RNA transcription reactions in the presence of DNA-dependent RNA polymerase inhibitors.

Translation initiation factors

Initiation and effector steps are involved in RNAi mechanism (Hutvagner and Zamore, 2002). In the initiation step, dsRNA is digested into 21-23 nucleotides siRNAs known as "guide RNAs" (Hammond et al., 2001; Hutvagner and Zamore, 2002). It was evident that siRNAs are produced by the activity of dicer on dsRNA strand. Initiation step of RNAi involves the activity of some genes. The function of *rde1* and *rde4* (rde is for RNAi deficient) was observed in *C. elegans* (Sharp, 2001). In the effector step, double stranded siRNA associate with RISC. The RISC activation is linked with ATP dependent siRNA unwinding. The activated RISC then break down the mRNA ~12 nucleotides from the 3' terminus of the siRNA (Naykanen et al., 2001; Hutvagner and Zamore, 2002). There are some genes involved in the effector step of PTGS. In *C. elegans*, *rde2* and *mut7* mutated genes showed defective RNAi. Rapid aging, a disease known as Werner syndrome was the result of mut-7 activity (Grishok et al., 2000).

MECHANISM OF GENE SILENCING

The basic mechanism of RNAi is a multi-step process. When the dsRNA entered in to the cell, it is targeted by the enzyme Dicer. The Dicer cut the dsRNA in to smaller segments of 21-25 nucleotides. The siRNA associate with RISC in the cell cytoplasm, interact with the catalytic RISC component which contains several proteins surrounding siRNAs (Caudy et al., 2003; Zeng et al., 2003). siRNA duplex then loose its double strand and bind to the targeted mRNA, cleave it in the region covered by siRNA (Parrish et al., 2000). The siRNA fragments were first observed in plants undergoing post transcriptional gene silencing (Hamilton and Baulcombe, 1999). Since then siRNAs have been detected in all species having RNAi like phenomena. However, the mechanism of RNAi is outlined in Figure 2 (Rahman et al., 2008).

HOMOLOGY DEPENDENT GENE SILENCING

Researchers have transformed plants with various plant and non-plant genes to study the role of genes for producing plants with special quality and characters (Sijen and Kooter, 2000). It was observed that expression of transgene is variable but the findings of co-suppression were interesting and surprising. The features of co-suppression were associated with simultaneous transgene silencing and homologous endogenous gene expression. This co-suppression was first observed with genes in Petunia flower pigmentation where the flower color became white in the transformants (Napoli et al., 1990). Later on, homology dependent gene-silencing (HDGS) was observed and identified with different genes. It indicated that HDGS is a common and widespread phenomenon in plants all over the world. HDGS are two types, transcriptional gene-silencing (TGS) and post-transcriptional gene-silencing (PTGS).

Transcriptional gene silencing (TGS)

TGS is a form of silencing in which transcription initiation

is blocked in the nucleus. Transpoon regulation through DNA mythylation in nucleus was associated with TGS initially (Vaucheret and Fagard, 2001). It is the result of histone protein modifications which create the environment of heterochromatin around a gene. It serves to down regulate genes pre-transcriptionally that makes it inaccessible to transcriptional pathway (Holmquist and Ashley, 2006). TGS and DNA methylation in plants can be stimulated by either dsRNA or viral infection (Vaucheret and Fagard, 2001). TGS has affected the sequences that are integrated in the genome. Methylation of the coding sequence of gene is sufficient to block expression (Hohn et al., 1996). Since it has not been determined whether these methylated sequences are transcribed or not, it is not possible to classify this type of silencing event as TGS or PTGS. TGS and PTGS, therefore, appear to form two alternative pathways to control incoming, redundant and/or mobile nucleic acids.

Post transcriptional gene silencing (PTGS)

In Post transcriptional gene silencing, target gene is destroyed through mRNA degradation (Vaucheret and Fagard, 2001). Incorporation of homologous dsRNA transgene or virus is the cause of silencing endogenous gene expression where silence gene transcription is synthesized but does not accumulate due to rapid degradation. PTGS is frequently referred to as co-suppression in plants (Hamilton and Baulcombe, 1999) and considered as the hottest topic of molecular biology. Sense transgenes, antisense transgenes and sense/ antisense transgenes or viruses may affect PTGS expression and classification (Fagard and Vaucheret, 2000). The destruction of the mRNA prevents translation to form a protein. It has been shown that PTGS same as TGS can occur in *cis*, simultaneously in *cis* and *trans*, or in *trans* position (Fagard and Vaucheret, 2000). The first reports of dsRNA mediating PTGS were made simultaneously in plants (Waterhouse et al., 1998) and *C. elegans* (Fire et al., 1998). Offspring generated from crossing transgenic plants containing a gene in an antisense orientation silenced endogenous gene from five to ten times more than control line (Waterhouse et al., 1998). PTGS technology has many advantages: It is a targeted approach to determine gene function. Determination of gene function can be done using a partial gene sequence in a silencing vector which avoids the need for laborious identification and isolation of transgenic knockout plants from large quantities of mutants. A selective gene from a gene family or silencing of many members of a family simultaneously can be achieved through careful selection of the sequence for targeting silencing. A highly conserved sequence between members of a family can silence multiple genes of the family (Fire et al., 1998; Schwarz et al., 2002; Rahman et al., 2008; Judge and Maclachlan, 2008).

ACHIEVEMENTS AND POSSIBILITIES

RNAi is an important area of molecular research all over the world. Clear knowledge and understanding of RNAi mechanisms is necessary to know the functions of gene and establishment of this technology. It is now in an advanced state but still did not come out from its infancy. Scientists are thinking that it is a revolution in the field of molecular genetics that has engineered the control of gene expression and manipulation. The application of this field is ranging from molecular biology to gene therapy. The RNAi field is linked with RNA interference, transgene silencing and transgene mobilization (Agami, 2002; Couzin, 2003). dsRNA activates in RNAi as a normal cellular process leading to specific RNA degradation of high specificity and a cell-to-cell dissemination and transfer of this gene silencing effect in several RNAi mechanisms (Shuey et al., 2002). Practically, RNAi is a very effective and powerful technique to block particular gene expression which can be used to know the function of gene in plants or humans or organisms (Schwarz et al., 2002; Ge et al., 2003; Kamath et al., 2003).

Viral attack is a major problem existing worldwide for plants and vertebrates. RNAi technology can be potential tool in this regard that can silence viral genes (Rahman et al., 2008). The reality of intracellular immunization is very near by using small RNA (Li et al., 2006). The potentiality of RNAi and its activity to treat disease and cancer has created interest to the scientists (Li et al., 2005; Ying et al., 2006). The activity of RNAi in cultured cell infected with HIV, human papilloma virus, polio or containing a variety of cancer genes is being used in many laboratories. This technology is used in the production of therapeutic drugs against Hepatitis or immune-deficiency virus in humans. Clinical application of RNAi is near to the door (Rahman et al., 2008). RNAi is established by its application as a therapeutic principle to treat human diseases. The technique of gene turning off created by siRNA has the potentiality to develop siRNA based drugs (Judge and Maclachlan, 2008). It is evident that the RNAi gradually become an impressive field of molecular research which could be the major outcome in the near future.

REFERENCES

Agami R (2002). RNAi and related mechanisms and their potential use for therapy. Curr.Opin. Chem. Biol. 6: 829-834.

Antoni R, de Fougerolles (2008). Delivery Vehicles for Small Interfering RNA In Vivo. Hum. Gene. Ther. 19: 125-132.

Bernstein E, Caudy A, Hammond SM, Hannon GJ (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409(6818): 295-296.

Bluementhal T, Carmichael GG (1979). RNA replication: Function and structure of Qbeta-replicase. Ann. Rev. Biochem. 48: 525-548.

Caudy AA, Ketting RF, Hammond SM, Denli AM, Bathoorn AM, Tops BB (2003). A micrococcal nuclease homologue in RNAi effector complexes. Nature 425: 411-414.

Couzin J (2003). RNA interference. Mini RNA molecules shield mouse liver from hepatitis. Science 299 (5609): 995.

Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe DC (2000). An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell 101: 543-553.

Daneholt B (2006). Advanced Information: RNA interference. The Nobel Prize in Physiology or Medicine 2006. Retrieved on 2007-01-25.

Fabani MM (2007). RNAi at Oxford. J. RNAi and Gene Silencing 3(1): 220-222.

Fagard M, Vaucheret H (2000). (TRANS) gene silencing in plants: How many mechanisms? Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 167-94

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998). Potent and specific genetic interference by double-stranded RNA in *Caenoehabditis elegans*. Nature 391(6669): 806-811.

Ge Q, McManus MT, Nguyen T, Shen CH, Sharp PA, Eisen HN (2003). RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. Proc Natl. Acad. Sci. USA 100: 2718-2723.

Grewal SIS, Moazed D (2003). Heterochromatin and epigenetic control of gene expression. Science 301: 798-802.

Grishok A, Tabara H, Mello CC (2000). Genetic requirements for inheritance of RNAi in *C.elegans*. Science 287: 2494-2497.

Grishok A (2007). The nuclear aspect of RNAi in *C. elegans*. J. RNAi Gene Silencing 3(1): 254-263.

Hamilton AJ, Baulcombe DC (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286: 950-952.

Hammond SM, Caudy AA, Hannon GJ (2001). Post-transcriptional Gene Silencing by Double-stranded RNA. Nat. Rev. Gen. 2: 110-119.

Hammond SM, Bernestein E, Beach D, Hannon GJ (2000). An RNA directed nuclease mediates posttranscriptional gene silencing in Drosophila cells. Nature 404: 293-296.

Hannon GJ (2002). RNA interferance. Nature 418: 244-251.

Hayafune M, Miyano-Kurosaki N, Park WS, Moori Y, Takaku H (2006). Silencing of HIV-1 gene expression by two types of siRNAs expression systems. Anti viral Chem. Chemother. 17: 241-249.

Hohn T, Corsten S, Rieke S, Muller M, Rothnie H (1996). Methylation of coding region alone inhibits gene expression in plant protoplasts. Biochemistry, 93: 8334-8339.

Holmquist G, Ashley T (2006). Chromosome organization and chromatin modification: influence on genome function and evolution. Cytogenet Genome Res. 114(2): 96-125.

Hutvagner G, Zamore PD (2002). A micro RNA in a multiple turn –over RNAi enzyme complex. Science 297:2056-2060.

Jaronczyk K, Carmichael J, Hobman T (2005). Exploring the functions of RNA interference pathway proteins:some functions are more RISCy than others? Biochem. J. 387(3): 561-571.

Judge A, Maclachlan (2008). Overcoming the Innate Immune Response to Small Interferring RNA. Hum. Gene Ther. 19: 111-124.

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M (2003). Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421: 231–237.

Li L, Lin X, Shoemaker AR, Albert DH, Fesik SW, Shen Y (2006). Hypoxia-inducible factor-1 inhibition in combination with temozolomide treatment exhibits robust antitumor efficacy in vivo. Clin. Cancer Res. 12(15): 4747-4754.

Li L, Lin X, Staver M, Shoemaker A, Semizarov D, Fesik SW (2005). Evaluating hypoxia-inducible factor-1alpha as a cancer therapeutic target via inducible RNA interference in vivo. Cancer Res. 16: 7249-7258.

Napoli C, Lemieux C, Joregnsen R (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous gens in trans. Plant Cell 2: 279-289.

Nykanen A, Haley B, Zamore PD (2001). ATP requirements and small interfering RNA structure in the RNA interference pathway. Cell 107: 309-321.

Parrish S, Fleenor J, Xu S, Mello C, Fire A (2000). Functional anatomy of a dsRNA trigger; Differential requirement for the two trigger strands in RNA interference. Mol. Cell 6: 1077-1087.

Rahman M, Ali TH, Riazuddin S (2008). RNA interference: The story of gene silencing in plants and humans. Biotechnol. Adv. 26(3): 202-209.

Schiebel W, Haer B, Marinkovic S, Kianner A, Sanger HL (1993a). RNA-directed RNA polymerase from tomato leaves, purification and physical properties. J. Biol. Chem. 268: 11851-11857.

Schiebel W, Haer B, Marinkovic S, Kianner A, Sanger HL (1993b). RNA directed RNA polymerase from tomato leaves II catalytic in vitro properties. J. Biol. Chem. 268: 11858-11867.

Schwarz D, Tomari Y, Zamore P (2004). The RNA-Induced Silencing Complex Is a Mg-Dependent Endonuclease. Curr. Biol. 14(9): 787-791.

Schwarz DS, Hutvagner G, Haley B, Zamore PD (2002). Evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways. Mol. Cell 10: 537-548.

Sharp PA (2001). RNA interference. Genes Dev. 15: 485-490.

Shuey D, McCallus D, Tony G (2002). RNAi: gene-silencing in therapeutic intervention. Drug Discov. Today 7(20): 1040-1046.

Sijen T, Kooter JM (2000). Post-transcriptional gene-silencing: RNAs on the attack or on the defense? Bio Essays 22: 520-531.

Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM (2000). EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. Curr. Biol. 10: 169-178.

Vaucheret H, Fagard M (2001). Transcriptional gene silencing in plants: targets, inducers and regulators. Trends Genet. 17(1): 29-35.

Waterhouse PM, Graham MW, Wang MB (1998). Virus resistance and gene silencing in plants is induced by double stranded RNA. Proc. Natl. Acad. Sci. 95: 13959-13964.

Ying RS, Fan XG, Zhu C, Li N, Zhang BX (2006). Inhibition of hepatitis B virus replication and expression by RNA interference in vivo. Zhonghua Ganzangbing Zazhi 14(1): 15-18.

Zeng Y, Yi R, Cullen BR (2003). Micro RNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc. Natl. Acad. Sci. USA 100: 9779-9784.