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RNA interference: A review

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Abstract
RNA interference (RNAi), as commonly defined, is a phenomenon leading to post-transcriptional gene silencing (PTGS) after endogenous production or artificial introduction into a cell of small interfering double strand RNA (siRNA or miRNA) with sequences complementary to the targeted gene (Bosher and Labouesse, 2000). Whereas the transcription of the gene is normal, the translation of the protein is prevented by selective degradation of its encoded mRNA. However, PTGS is not restricted to RNAi and has emerged as a more complex mechanism that involves several different proteins and small RNAs.

Keywords: RNA, phenomenon, siRNA or miRNA

Introduction

Napoli and Jorgensen were the first to report an RNAi type of phenomenon in 1990. The goal of their studies was to determine whether chalcone synthase (CHS), a key enzyme in flavonoid biosynthesis, was the rate-limiting enzyme in anthocyanin biosynthesis. The anthocyanin biosynthesis pathway is responsible for the deep violet coloration in petunias. In an attempt to generate violet petunias, Napoli and Jorgensen overexpressed chalcone synthase in petunias, which unexpectedly resulted in white petunias. The levels of endogenous as well as introduced CHS were 50-fold lower than in wild-type petunias, which led them to hypothesize that the introduced transgene was "cosuppressing" the endogenous CHS gene.

Petunia

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Enzyme chalcone synthase (CHS) for synthesis of anthocyanin(flavonoid)

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Results in BLUE color
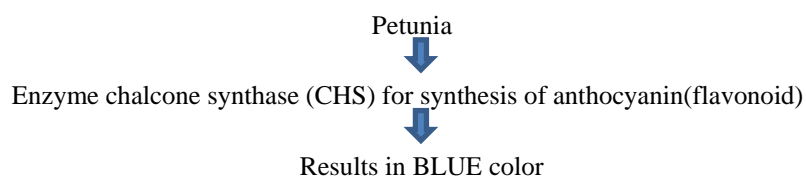
While reports of PTGS in plants were piling up, Romano and Macino reported a similar phenomenon in *Neurospora crassa*, noting that introduction of homologous RNA sequences caused "quelling" of the endogenous gene. During attempts to boost the production of an orange pigment made by the gene *al1* of the fungus *Neurospora crassa*. An *N.crassa* strain containing a wild-type *al1 gene* (orange phenotype) was transformed with a plasmid containing a 1,500-bp fragment of the coding sequence of the *al1 gene*. A few transformants were stably quelled and showed albino phenotypes.
RNA silencing was first documented in animals by Guo and Kemphues, who observed that the introduction of sense or antisense RNA to par-1 mRNA resulted in degradation of the par-1 message in *Caenorhabditis elegans.*
These findings "cosuppression, quelling and sense mRNA" caused investigators to rethink the current dogma.

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Antisense Technology: When a gene is known to be causative of a particular disease, it is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the mRNA produced by that gene and effectively turning that gene "off". This is because mRNA has to be single stranded for it to be translated. Results were highly variable, and in light of the discovery of RNAi, dsRNA probably inhibited its target by inducing RNAi rather than inhibiting translation.

Elucidation of the silencing trigger

In 1998, Fire and Mello Working with *C.elegans* published paper that provided an explanation for the previously reported silencing of endogenous genes by "cosuppression, quelling and sense mRNA". They tested the hypothesis that the trigger for gene silencing was not single-stranded RNA (ssRNA) but double-stranded RNA (dsRNA). Reasoned that the seemingly finding of Guo and Kemphues showing that introduction of sense RNA leads to gene silencing could have been due to the contamination of preparations of ssRNA by dsRNA resulting from the activity of bacteriophage RNA polymerases.

They extensively purified the sense and antisense ssRNA preparations, then directly compared their effects to dsRNA on the unc-22 gene. The purified ssRNAs (sense or antisense) were consistently found to be 10- to 100-fold less effective than dsRNA targeting the same mRNA. ssRNA was found to be effective only if the sense strand was injected into the animals, followed by the antisense strand or vice versa, suggesting that hybridization of the ssRNA to form dsRNA occurred *in vivo*. This work established an entirely new conceptual framework for the effects of RNA on gene silencing by highlighting a role for dsRNA.
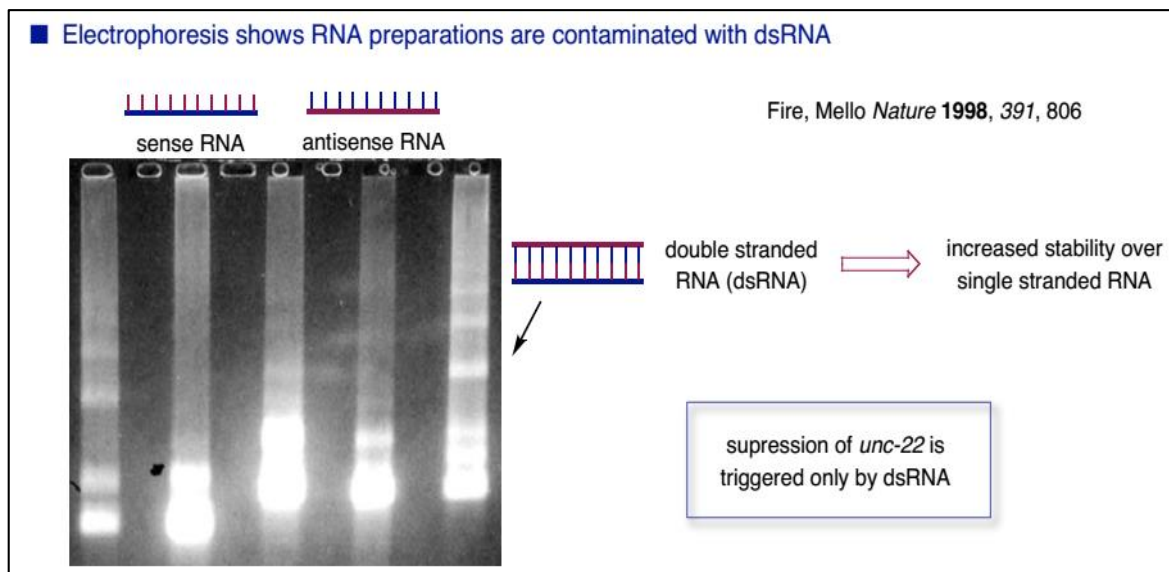

Mechanism of RNAi
Components
- **Dicer**

It is endonuclease enzyme dimer cleaves RNA belongs to RNAse III family which show specificity for dsRNAs. This enzyme have ability to digest dsRNA into uniformly sized small RNAs (siRNA) having overhangs of 2 to 3 nucleotides at 3' end. Dicer family proteins are ATP-dependent nucleases. These nucleases are evolutionarily conserved in worms, flies, fungi, plants, and mammals.
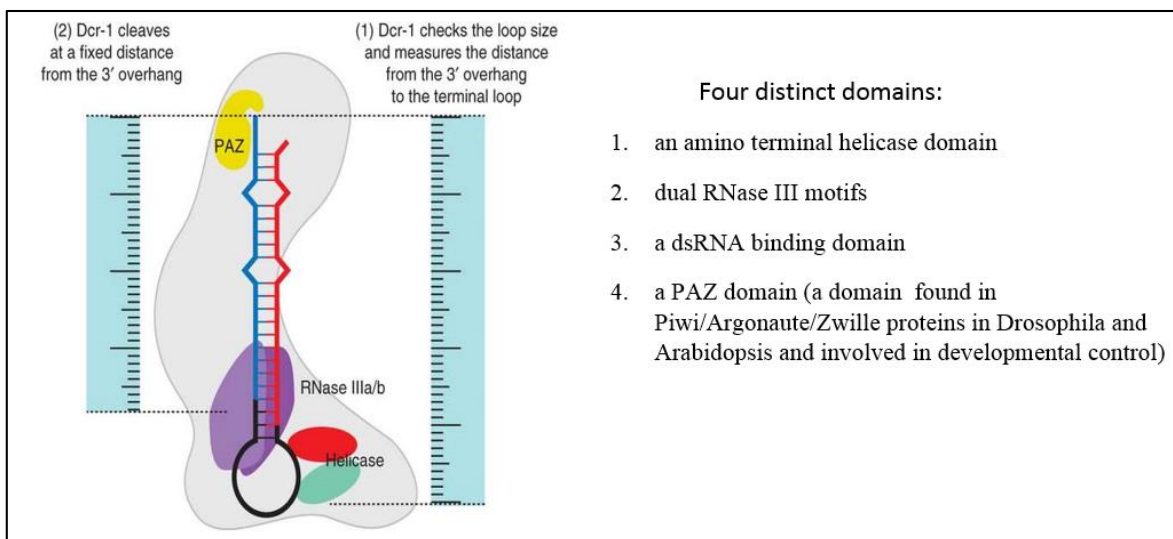
- **Guide RNAs**
1. miRNA:
A small non-coding RNA molecule (22 nucleotides) found in plants, animals, and some viruses, which functions in post-transcriptional regulation of gene expression. It base-pairs with complementary sequences within mRNA molecules,

resulting in gene silencing via translational repression or target degradation. Well conserved in eukaryotic organisms and are thought to be a vital and evolutionarily ancient component of genetic regulation. The human genome may encode over 1000 miRNAs.

The *lin-4* was the first microRNA discovered in 1993 by Victor Ambros, Rosalind Lee and Rhonda Fein Baum during a study of the gene *lin-14* in *C. elegans* development. Found that LIN-14 protein abundance was regulated by product encoded by the *lin-4* gene. From the *lin-4* gene a 22-nucleotide miRNA that have partially complementary to the *lin-14* mRNA. In 2000 second miRNA characterized: *let-7*, which repressed *lin-41*, *lin-14*, *lin-28*, *lin-42*, and *daf-12* expression during developmental stage transitions in *C. elegans*. *let-7* was soon found to be conserved in many species. Since then, miRNA research has revealed multiple roles in negative regulation and possible involvement in positive regulation. By affecting gene regulation, miRNAs are likely to be involved in most biological processes
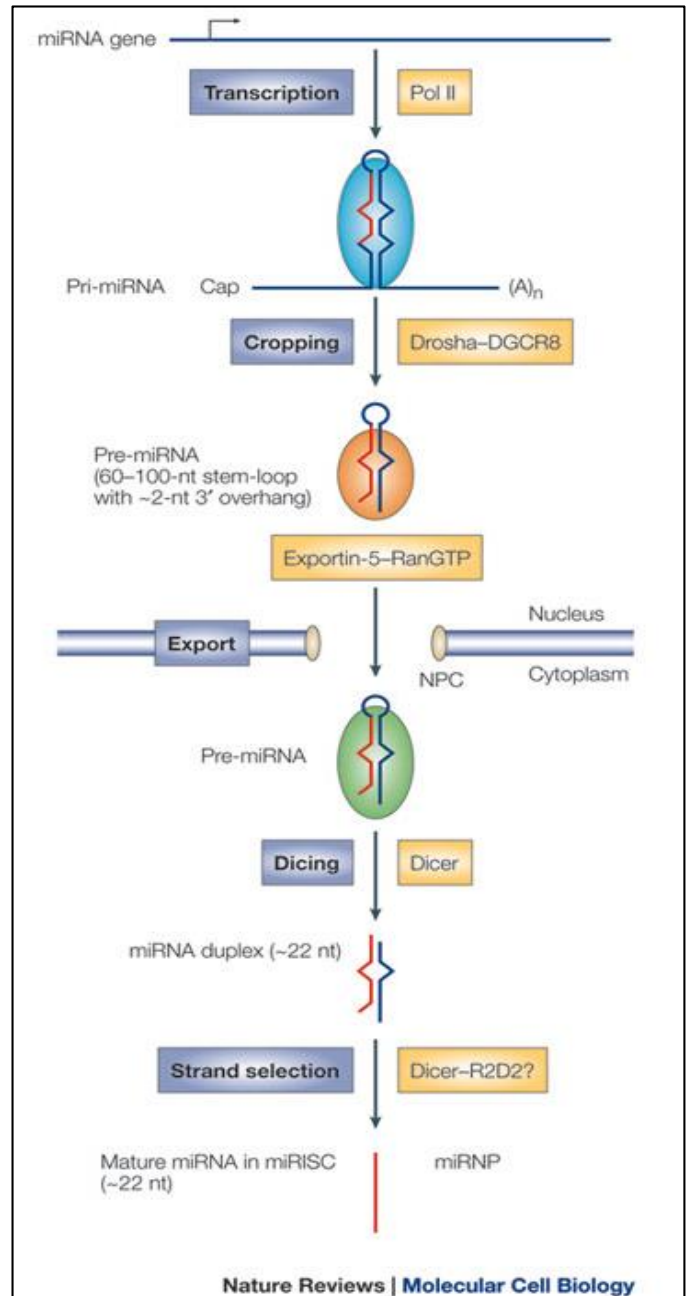
2. siRNA
Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 base pairs in length.

RNA-Induced Silencing Complex (RISC)
RISC is a large (~500-kDa) RNA-multiprotein complex, which triggers mRNA degradation in response to siRNA. The active components of an RNA-induced silencing complex (RISC) are endonucleases called argonaute proteins, which cleave the target mRNA strand complementary to their bound siRNA. RISC contains siRNA, endo- and exonuclease, argonaute(ARO):PAZ domain (assembly).

miRNA Mechanism

Primary microRNAs (pri-miRNAs) are transcribed by RNA polymerases from DNA

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Trimmed by the microprocessor complex (comprising Drosha and microprocessor complex subunit DCGR8) into ~70 nucleotide precursors, called pre- miRNAs

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Pre- miRNAs contain a loop and usually have interspersed mismatches along the duplex.

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Dicer processes the pre- miRNAs into miRNA– miRNA* duplexes

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The duplex associates with an Argonaute (AGO) protein within the precursor RNAi-induced silencing complex (pre-RISC).

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One strand of the duplex (the passenger strand) is removed.

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The mature RISC contains the guide strand, which directs the complex to the target mRNA for post-transcriptional gene silencing.

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siRNA mechanism

Long dsRNAs are processed by Dicer into small interfering RNAs (siRNAs).

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siRNAs are 20–24-mer RNAs and harbour 3′OH and 5′phosphate (PO4) groups, with 3′dinucleotide overhangs.
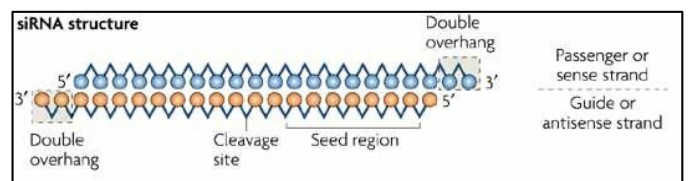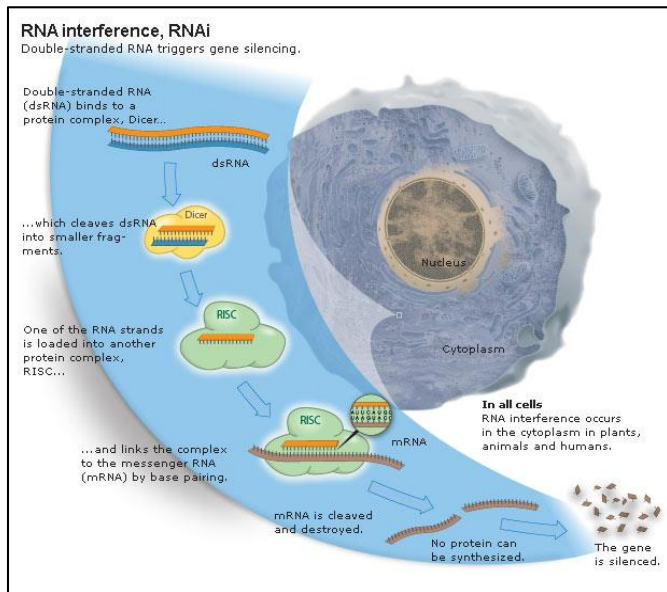

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The duplex associates with an Argonaute (AGO) protein within the precursor RNAi-induced silencing complex (pre-RISC).

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One strand of the duplex (the passenger strand) is removed.

Then, the mature RISC, containing an AGO protein and the guide strand, associates with the target mRNA for cleavage.

RNA interference, RNAi
Double-stranded RNA triggers gene silencing.

Applications
Functional Genomics: RNAi technology can be used to identify and functionally assess the thousands of genes within the genome that potentially participate in disease phenotypes. In addition, RNAi technology provides an efficient means for blocking expression of a specific gene and evaluating its response to chemical compounds or changes in signaling pathways. RNAi changed the way of scientists to attack genetic problems and understand cellular mechanics. Previous methods of studying gene function is by manipulation of DNA by generation of random mutants by exposing to mutagens or through the use of DNA inserts. RNAi is a form of reverse genetics, researchers can systematically pick genes with predetermined genetic location rather than beginning with mutants and then searching for the genes affected which removes a large portion of previous labor.

Macular Degeneration: One of the first diseases to test RNAi as a treatment, the reason being that the RNA can be directly injected into the diseased tissue. Occurs when the protein, vascular endothelial growth factor (VEGF), is overproduced in the eye causes a buildup of blood vessels behind the retina leading to blurred vision and possible blindness. To destroy the mRNA that codes for VEFG, dsRNA is injected into the whites of the eyes which leads to reduced blood vessel formation and the shrinkage of present blood vessels. The first RNAi treatment trial for macular degeneration started in 2004, where a quarter of the participants saw a significant improvement in their vision after two months. Presently, Acuity Pharmaceuticals, Inc. is sponsoring a study with siRNA called Cand5 for the treatment of macular degeneration.

Cancer: A lot of research is currently being conducted investigating the use of RNAi as a future cancer therapeutic. Results from *in vitro* and *in vivo* animal studies look promising. As more genes involved in causing cancer are being discovered and sequenced the efficiency of RNAi increases. Research is being conducted to design specific siRNA that targets telomerase. Telomerase is an enzyme that produces telomeres which are tandem repeats of DNA (TTAGGG) located at the ends of chromosomes. Telomerase increase telomere length, thereby enabling cancerous cells to evade senescence and allows enhanced replicative potential resulting in virtual immortality. Through RNAi Inhibition of telomerase activity prevents telomere extension, potentially causing replicative senescence and apoptosis of cancerous cells. Altering the telomerase will hinder cancer development and essentially make the cancerous cells susceptible to "old age". The future use of siRNA is appealing since there are very few or no effects on normal diploid cells. However, only *in vitro* and animal studies have been conducted so far.

HIV: Future application of RNAi includes inhibition of HIV, where proteins critical to HIV's survival are targeted. The siRNA's are constructed to destroy complementary HIV mRNA's, ultimately resulting in inhibition of HIV replication and its ability to attach to immune cells. Due to HIV's ability to mutate, multiple genes can be targeted at once to ensure inhibition of HIV's ability to make critical proteins. Namely, HIV-1 cellular receptor CD4's (controlled by the nef gene), Envelope associated proteins (controlled by the env gene). Capsid proteins (controlled by the gag protein).

Cardiovascular and Cerebrovascular Diseases: Progressive occlusion of arteries in a process called atherosclerosis. Atherosclerosis - damage to vascular endothelial cells, local production of inflammatory cytokines, and the recruitment of macrophages to the site forming foam cells; in addition, apoptosis of foam cells and vascular smooth muscle cells occurs (Geng and Libby, 2002). The severe ischemia that occurs in heart or brain cells during a myocardial infarction or stroke results in the death of cardiac muscle cells or neurons. It may be possible to use RNAi technology to intervene in the process of atherosclerosis. A key step in the process of atherosclerosis is the up-regulation of cell adhesion molecules in vascular endothelial cells, which play an essential role in the recruitment of macrophages to the site of endothelial damage. The production of cell adhesion molecules can be selectively suppressed in cultured cells by RNAi.

Neurodegenerative Disorders: Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis are examples of neurodegenerative disorders, characterized by the dysfunction and death of specific populations of neurons. Specific genetic mutations are responsible for a small percentage of cases of Alzheimer's and Parkinson's disease and amyotrophic lateral sclerosis. Huntington's disease result from mutations (polyglutamine expansions) in the huntington protein (Rubinsztein, 2002) [5]. Studies of patients, and of animal and cell culture models of each disease, have revealed shared biochemical cascades that result in neuronal death. Those cascades include increased oxidative stress, dysregulation of cellular calcium homeostasis and apoptosis (Mattson, 2000) [7]. For example, an abnormality in the proteolytic processing of the amyloid precursor protein is believed to be a key early event in Alzheimer's disease pathogenesis, and two enzymes calledβ- and ɣ- secretases that are responsible for cleaving of amyloid precursor protein to generate the neurotoxic amyloid β-peptide. Recent studies have shown that cultured neurons can be efficiently transfected with siRNAs and that the targeted genes are effectively silenced.

Pitfalls: Over recent years various complex barriers to achieving efficient RNAi have become evident. These hurdles include:

- Specificity for the target gene;
- Delivery to the correct cell or tissues;
- RNAi may activate the interferon response," a nonspecific viral defense mechanism," makes RNAi ineffective (Tuschl 2001) [6].
- Durability of RNAi activity and the ability to redose (if needed); and
- Considerations of the stability of the target mRNA and encoded protein.

Conclusion

Although induced RNAi is able to trigger profound and specific inhibition of virus replication, it is becoming clear that RNAi therapeutics are not as straightforward as we had initially hoped. Difficulties concerning toxicity and delivery to the right cells that earlier hampered the development of antisense-based therapeutics may also apply to RNAi. In addition, there are indications that viruses have evolved ways to escape from RNAi. Proper consideration of all of these issues will be necessary in the design of RNAi-based therapeutics for successful clinical intervention.

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