RNA Interference (RNA i): A novel strategy in health care and crop Improvement

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Abstract

RNA interference (RNAi) is a RNA dependent gene silencing mechanism which includes endogenously induced gene silencing effects of miRNA as well as silencing triggered by foreign dsRNA. The dsRNA binds with a protein complex DICER which cleaves it into short fragments with a few unpaired overhung bases at both ends. The short dsRNA fragments (SiRNA) or miRNA integrate with another active protein complex RISC. Consequently, one of the RNA strands (anti-guide strand) is degraded while the other is selected as a guide strand which remains bound to RISC complex. When a complementary mRNA is located by an RISC bound guide strand, it binds to it and is cleaved and degraded. The RNAi offers a new technology in healthcare and crop improvement.

Key Words: RNAi, gene silencing, applications, medicine, agriculture.

Introduction

RNA interference is a natural cellular mechanism involved in regulation of gene expression. It involves double stranded ribonucleic acid (dsRNA) that can specifically silence the expression of 2 genes with sequences, which are complementary to the dsRNA. The components of double stranded RNA initiate the process of RNAi when an antisense RNA strand is activated which targets a complementary gene transcript such as a messenger RNA for cleavage by a ribonuclease. RNAi has been shown to be an important regulator of gene expression in many eukaryotes including plants, animals and humans.

In 2006, the Nobel prize1 was awarded to Andrew Z. Fire of Stanford University and Craig C. Mello of the University of Massachusetts Medical School, for unraveling the mechanism of gene silencing by double stranded RNA, based on their studies in nematode worm Caenorhabditis elegans. Their initial observations related to this phenomenon were published in Nature (1998)2. Before this mechanism of RNA interference is discovered and fully understood at the molecular level, it was known by other names like 'post transcriptional gene silencing', 'transgene silencing' and 'quelling'3. Around 1990, Scientists working on petunia plants (Napoli *et. Al* 1990)4 reported some unexpected genetic expression. The goal of their experiments was to breed

petunia plants to increase the colour intensity of the flowers. For the target to be achieved, they introduced cloned gene encoding a key enzyme for red pigmentation of flowers. Surprisingly, many of the petunia plants with additional copies of this gene showed petals which appeared fully white or partially white instead of the expected colour. The scientists, based on their evidences, explained the phenomenon as post transcriptional gene inhibition or "co-suppression of gene expression". Similar observations were recorded in few years later in viruses but the molecular mechanism remained unknown until the recent land mark discovery by Fire and Mello.

Cellular Mechanism

The discovery of Fire and Craig revolutionizes our understanding of the cellular role of RNA. The RNAi mechanism goes beyond the fundamental process known as the central dogma of 3 biology. RNA interference is an RNA dependent gene silencing mechanism that includes the endogenously induced gene silencing effects of miRNA as well as silencing triggered by foreign dsRNA. The dsRNA binds with a protein complex DICER which is an endonuclease, which cleaves it into short fragments with 20 to 25 base pairs with a few unpaired overhung bases at both ends. The short dsRNA fragments produced by DICER, is called small interfering RNAs (Si RNAs). Thus, miRNA and SiRNA share same cellular machinery as well as functional analogy.

These fragments (SiRNA or miRNA) integrate with another active protein complex RISC (RNA induced silencing complex). The catalytic active component of the RISC complex is known as argunaute protein which acts as endonuclease and mediates SiRNA induced cleavage of the target mRNA strand. Consequently, one of the RNA strands (anti-guide strand or passenger strand) is degraded while the other is selected as a guide strand which remains bound to RSIC complex. When a complementary mRNA is located by an RSIC bound guide strand it binds to it and is cleaved and degraded. The expression of the gene corresponding to the mRNA is silenced. RNAi has been particularly well studied in certain organisms such as C. elegans, Drosophila (Hammand and Bernstein,2002)5, and in plants where the effect can be spread from cell to cell within the organism. The protein complex like RISC and the enzyme DICER have been found to be conserved throughout eukaryotes. The RNA interference pathway, as well, is conserved across all eukaryotes. It has been established that RNAi mechanism provides genomic stability, especially in plants, keeping the transposon production under control.

Applications in Medicine

The discovery of RNAi makes it a valuable experimental tool for its ability to selectively reduce the expression of an individual gene in a cell. Synthetic dsRNA can be injected to the cell to artificially produce RNAi. RNAi can be used for large scale screens that systematically turn off each gene in the cell in order to identify the necessary components of a particular cellular process. It may be possible to exploit RNA interference in medical therapy. However interferon response in mammalian cells interfere with the introduction of long dsRNA strands; the use of short interfering RNA mimics has been more successful.

There has been, already, a considerable progrees in its application in treatment of macular degeneration and respiratory syncytial virus, which already reached the level of clinical trial developed by Sirna Therapeutics and Alnylam Pharmaceuticals respectively[6]. In another study, RNAi has also been shown to cause the reversal of induced liver failure in mouse system. The applications of RNAi holds promise in antiviral therapies, including the suppression of viral gene expression in cancerous cells[7], knockdown of host receptors and co-receptors for HIV[8], the silencing of hepatitis A[9] and hepatitis B genes[10], silencing of influenza gene expression[11], and inhibition of measles viral replication[12].

It is considered to be effective in treatment of neurodegenerative diseases particularly focussed on polyglutamine diseases such as Huntington's disease[13]. Other applications that are considered promising are to treat cancer by silencing genes- differentially upregulated in tumor cells or genes involved in cell division[14,15]. A major area of concern, however, associated with RNAi application is that it poses a risk because of its potent "off-target" effects in which a gene with a coincidentally similar sequence to the targeted gene is also silenced. About 10% error, due to this off-target mechanism, has been estimated in a computerised genomic study (Qui and Lane, 2005)[16]. In an experimental study, application of RNAi in treatment of liver disease in mice resulted high death rates in the 5 experimental animals which is considered an outcome due to "oversaturation" of the dsRNA pathway (Salazar and Kay, 2006)[17].

Applications in Agriculture

RNAi holds potential too in genetic engineering of crop plants particularly, targetting to reduce level of natural toxin products. The techniques are advantageous in plants for their stable and heritable RNAi phenotype.

The application of RNAi proved successful in a major way, in cotton plant (Sunilkumar *et al,* 2006)[18]. The cotton seeds are rich in dietary protein but unsuitable for human consumption because of its toxic terpenoid product, gossypol. RNAi has been used to produce cotton stocks with seeds containing lower levels of delta-cadinene synthase, a key enzyme in gossypol production, without affecting the enzyme's production in other parts of the plant, where gossypol is important in preventing damage from plant pests.

By using the same technique it has been possible to reduce linamarin content, a cyanogenic substance in cassava plants (Siritunga and Sayre, 2003)[19]. Efforts have been also directed to improve many other useful crop plants, keeping in view a specific target as above, by use of RNAi. (Le *et al,* 2006)[20].

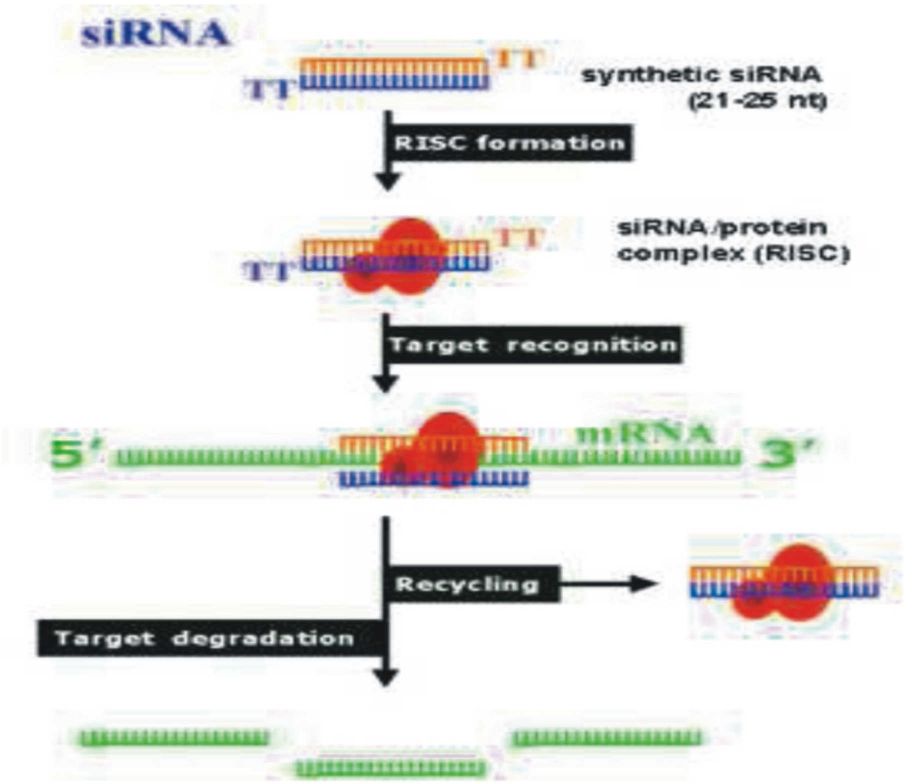

Figure - RNAi mechanism: The mRNA degrading pathway

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