Mechanism of RNAi in Genomics and Therapeutics: A Review

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ABSTRACT
RNA interference (RNAi) has the potential to address gene regulatory role in an efficient manner. Short interfering RNA (siRNA), the small RNA deriving from RNAi can control the gene regulation, by a technique called gene silencing. Gene silencing is "switching off" genes which are over expressed, in a sequence specific manner. SiRNA has been experimentally studied in disease therapy and is expected to be developed as a nucleic acid based medicine for incurable diseases such as cancer. This paper reviews the mechanism of RNAi as a gene regulator in genomics and therapeutics.

Keywords
Double stranded RNA (dsRNA), short interfering RNA (siRNA), RNA interference (RNAi), gene regulation, gene silencing.

1. INTRODUCTION
The balance between cell growth and cell death (apoptosis) account for a large number of malignancies [1], [2]. Maintaining this balance plays a crucial gene regulatory role in human health. Although some of the secrets behind apoptosis have been uncovered in the recent past, there is not yet enough clarity about the specific molecular mechanism and regulatory role associated with it. Different studies show that RNA interference (RNAi) has the potential to address regulatory role in an efficient manner. RNAi performs gene regulation mediated by double stranded RNA (dsRNA) and short interfering RNA (siRNA). The siRNA, small RNA deriving from RNAi, can control the gene regulation, by a technique called gene silencing. Gene silencing is "switching off" genes which are over expressed, in a sequence specific manner. SiRNA has been experimentally studied in cancer therapy and is expected to be developed as a nucleic acid based medicine for incurable diseases such as cancer. Here in designing efficient siRNA to control the gene regulation by gene silencing is one among the important research directions in genomics and cancer therapy.

The goal of this article is to review the role of RNAi as a gene regulator in genomics and disease therapy. The paper is structured as follows. Section 2 presents the central dogma of molecular biology and basic principle behind protein synthesis. Section 3 reviews the mechanism of RNAi and describes the formation of siRNA from RNAi. Section 4 describes RNAi in genomics and therapeutics by briefly describing the role of siRNA in gene regulation and cancer therapy. This section also describes the role of oncogenes and tumor suppressors in formation of cancer. Section 5 briefly concludes the scope of siRNA in molecular biology and therapeutics.

2. CENTRAL DOGMA OF MOLECULAR BIOLOGY
The central dogma of molecular biology (Fig 1) was first stated by Francis Crick in 1958 [3] and re-stated in 1970 [4]. The Central Dogma involves three major players: DNA, RNA, and Proteins. The flow of information from DNA to RNA to proteins is the fundamental principles of molecular biology. After replication, DNA is first transcribed into RNA; RNA is then translated into proteins. Through the processes of transcription and translation, information from genes is used to make proteins. Together, transcription and translation are known as gene expression.

2.1 DNA Replication
DNA replication is a biological process which is the basis for biological inheritance. The process starts with one double stranded DNA and produces two identical copies of DNA molecule. Each strand of original double stranded molecule serves as a template for production of complementary strand. Helicase is the enzyme that splits original DNA into two strands.

2.2 Transcription
The process of copying genetic information from one strand of DNA to RNA is called transcription. An entire DNA contains non coding sequence of base pairs called introns and coding sequence of base pairs called exons. In the cell nucleus, all introns and exons of a particular gene is first transcribed into a complementary RNA copy called nuclear RNA or nRNA. In the next step, introns are removed from nRNA, such that the resulting sequence contains only exons. This edited sequence is stored in messenger RNA or mRNA. Thus transcription is the process of synthesis of RNA from DNA and it is taking place in the cell nucleus. The type of RNA which is transcribed from DNA, called messenger RNA (mRNA), contains information for making protein.

2.3 Translation
The mRNA produced by transcription carries information from DNA out of nucleus into the cytoplasm. Translation is the third step of protein synthesis which is taking place in cytoplasm. It is the process of translating the information containing in mRNA to protein. The mRNA interacts with a specialized complex called a ribosome, which reads the sequence of mRNA bases. Each sequence of three bases, called a codon, usually codes for one particular amino acid. Amino acids are the building blocks of proteins. A type of RNA called transfer RNA (tRNA) assembles the protein, one amino acid at a time. Protein assembly continues until the ribosome encounters a "stop" codon.

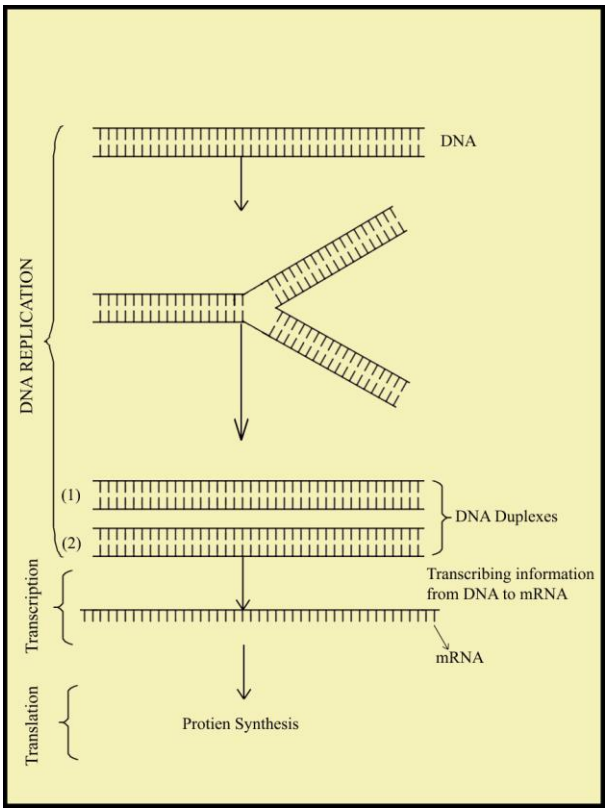

Fig 1: Central Dogma of Molecular Biology

3. RNA INTERFERENCE (RNAI) MECHANISM

3.1 Biogenesis of siRNA

The discovery of RNAi has lead to its wide application as a powerful tool in genomic research. The RNAi pathway was discovered by Fire and Mello in 1998 [5]. RNAi is a biological process capable of controlling the gene regulation by a sequence specific post transcriptional gene silencing mechanism [6] mediated by double stranded RNA (dsRNA). RNAi was first discovered in C.elegans [5] and plants [7], but it can also be used to induce gene silencing in a diverse range of organisms including fungi, protozoan and metazoan animals. It helps in developing various therapeutic applications because of its ability to do specific target silencing. RNAi has been successfully used to target diseases such as AIDS [8], neurodegenerative diseases [9], cholesterol [10] and cancer [11] on mice with the hope of extending these approaches to treat humans.

RNAi mainly targets the protein-producing mRNA and thereby controls disease earlier in the transcription phase by generating a non coding RNA called Short Interfering RNA (siRNA). The biogenesis of RNAi is divided into 4 steps. (Fig 3)

3.1.1 dsRNA cleavage by Dicer generating siRNAs

When long double stranded RNA (siRNA) from an external source is introduced into the cell, it is recognized by Dicer. The Dicer is a Ribonuclease III type protein. It is present in all organisms from unicellular to multi cellular. The dicer cleaves the dsRNA at randomly to generate SiRNAs of ~22 nucleotide (nt) [12], [13]. Each siRNA strand has a 5' phosphate group and a 3' hydroxyl group and has a 2 nt overhang at the ends (Fig 2).

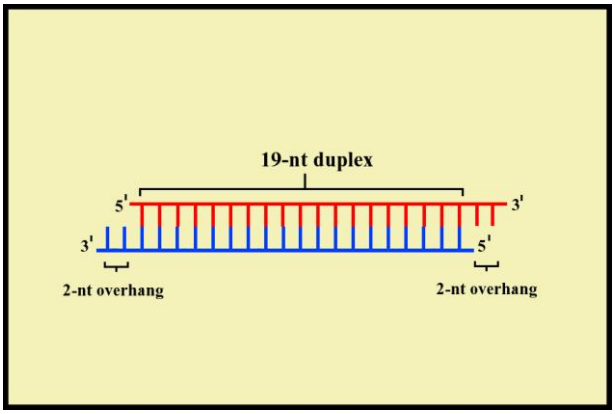

Fig 2: Structure of siRNA

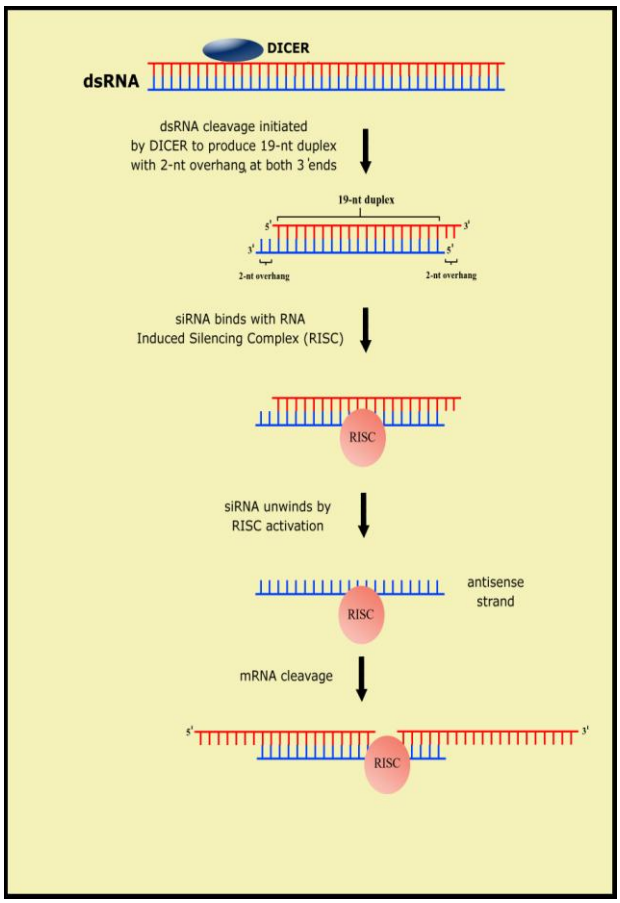

Fig 3: Biogenesis of siRNA

3.1.2 Formation of RISC

The siRNAs created by Dicer initiated cleavage get incorporated into nuclease complex called RISC (RNA Induced Silencing Complex). The complex formed is inactive.

3.1.3 SiRNA unwinding and RISC activation

Due to RISC activation, siRNA duplexes are unwinded and separate into sense and anti sense strands. Both the sense and anti sense strands of the siRNA are capable of directing RNAi but specificity depends on the anti-sense strand. The anti

sense strand is taken up by RISC. Due to the unwinding the siRNA duplex losses one strand that is not bound to the RISC. This single strand RISC complex thus gets activated.

3.1.4 mRNA targeting and degradation

The activated RISC complex along with siRNA joins with the complementary mRNA that are transcribed in the cytoplasm and then matches them. If the match is perfect then the captured mRNA is cleaved into useless pieces. If the match is imperfect then the RISC remains stuck to the mRNA, thus inhibiting its translation.

4. RNAI IN GENOMICS AND THERAPEUTICS

RNAi has become a powerful biological technique for gene function studies and drug discovery [14], [15]. It is also becoming increasingly important in developing therapeutic applications for a number of diseases due to its potential for specific targeted silencing [16], [17].

Widely used applications of RNAi and siRNA in genomics and therapeutics are

- Selection of possible targets for Tumor therapy [19]
- Gene Therapy[20]
- Better understanding of viral infections[21]
- Gene Silencing [22]

4.1 Role of siRNA in Genomics

A gene is the basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. The Human Genome Project has estimated that humans have between 20,000 and 25,000 genes. Every person has two copies of each gene, one inherited from each parent. Most genes are same in all people, but a small number of genes are slightly different between people. These small differences contribute to each person's unique physical features.

Each cell expresses or turns on only a fraction of its genes. The rest of the genes are repressed, or turned off. The process of turning genes on and off is known as gene regulation. Gene regulation is an important part of normal development. Genes are turned on and off in different patterns during development and allows cells to react quickly to changes in their environments.

Regulation process ensures that a dividing cell's DNA is copied properly, any errors in the DNA are repaired, and each daughter cell receives a full set of chromosomes. The cell division cycle has checkpoints which allow certain genes to check for mistakes and halt the cycle for repairs if something goes wrong. If a cell has an error in its DNA that cannot be repaired, it may undergo programmed cell death called apoptosis.

The uncontrolled cell growth or apoptosis affects the gene regulation process which results in mutations. We can apply RNAi with efficient siRNA which can do cleavage or translational inhibition of mRNA, of mutated cells, and there by inhibiting the unwanted cell division and apoptosis. Thus RNAi along with siRNA plays important role in gene regulation.

4.2 Role of siRNA in Therapeutics

Apoptosis is a common process throughout life that helps the body get rid of cells it doesn't need. Apoptosis protects the body by removing genetically damaged cells that could lead to cancer.

During mitosis, a cell duplicates all of its contents and splits to form two identical daughter cells. Mitosis is carefully controlled and regulated by certain genes. When the cycle proceeds without control, cells can divide without order and accumulate genetic defects that can lead to a cancerous tumor.

Generally the abnormalities that lead to cancer is resulted from mutations in protein-encoding genes that regulate cell division. Consequently, mutations begin to increase in the cell, causing further abnormalities in that cell and the daughter cells. Some of these mutated cells die, but other alterations may give the abnormal cell a selective advantage that allows it to multiply much more rapidly than the normal cells. This enhanced growth describes most cancer cells, which have gained functions repressed in the normal, healthy cells.

The uncontrolled cell growth or apoptosis results in mutation. These malfunctioning genes can be broadly classified into three groups. The first group, called proto-oncogenes, produces protein products that normally enhance cell division or inhibit normal cell death. The mutated forms of these genes are called oncogenes[18]. The second group, called tumor suppressors [18], makes proteins that normally prevent cell division or cause cell death. The third group contains DNA repair genes, which help prevent mutations that lead to cancer.

Mutations that produce oncogenes accelerate growth while those that affect tumor suppressors prevent the normal inhibition of growth. In either case, uncontrolled cell growth occurs. So cell growth can be controlled and maintained by regulation of proto-oncogenes, which accelerate growth, and tumor suppressor genes, which slow cell growth.

RNAi with efficient siRNA can be used to do cleavage or translational inhibition at appropriate target positions of the protein producing mRNA, of mutated cells, and there by inhibiting the unwanted cell division and apoptosis, which may reduce the possibility of mutation and there by cancer formation. Thus RNAi along with siRNA plays important role in cancer genetics and cancer therapy.

5. CONCLUSION

RNAi is a sequence specific post transcriptional gene silencing mechanism. Because of this gene knock down efficiency, RNAi has been successfully applied in functional genomics, therapeutics and new drug target identification in mammals and other eukaryotes. Designing RNAi with efficient siRNA which can do translational inhibition is one among the important research directions in genomics and cancer therapy. Hopefully, within a few years, this powerful technique will be used to uncover the secrets behind the regulatory role and molecular biology, to better understand and treat human diseases.

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