

# An RNA Primer

## Outline

### Part 1).

- A). The central dogma and basic RNA structure
- B). Types of RNA
- C). RNA Control
  - a). Transcriptional Gene Silencing (TGA)
  - b). Post-Transcriptional – RNA Processing (Splicing)
  - c). Post-Transcriptional – Alternative Splicing
  - d). Post-Transcriptional – RNA interference (RNAi)
  - e). Regulation of Translation
  - f). Post-Translational Modifications of Proteins
  - g). Post-Transcriptional vs Post-Translational Modification
- D). Antisense RNA Inhibits Gene Translation
- E). AONs: Antisense oligonucleotides - Antisense oligodeoxynucleotides and Antisense oligoribonucleotides Used to Control Translation
- F). Transgenes and Transgenesis
- G). Transposons (“Jumping Genes”) and Retrotransposons

Major Sources:

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/W/Welcome.html>

<http://en.wikipedia.org/>

<http://www.answers.com/>

### Part 2).

## **Paper: RNA Interference and Gene Silencing — History and Overview.**

**From:** <http://www.ambion.com/hottopics/rnai>

### **Part 1).**

#### **A). The central dogma and basic RNA structure**

The majority of genes are expressed as the proteins they encode. It is now understood that the process occurs in two steps:

- Transcription = DNA  $\rightarrow$  RNA
- Translation = RNA  $\rightarrow$  protein

Taken together, they make up the "central dogma" of biology, often written as:

**DNA  $\rightarrow$  RNA  $\rightarrow$  protein.**

This dogma had also been stated: "one gene = one protein" We now know that this cannot be true because the number of genes (the most up-to-date human genome version contains only 20,000 to 25,000 genes) is far less than the number of proteins. The concept of alternative splicing answers the problem, as we will see below.

Watson and Crick started out with:

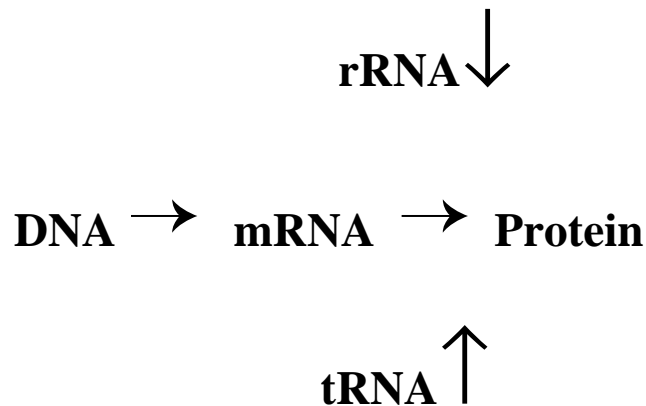
**DNA  $\rightarrow$  ?  $\rightarrow$  protein.**

**This was revised to:**



Crick modified this to the **adaptor hypothesis**: There must also be substances that carry amino acids to the RNA. This led to the discovery of tRNA.

Eventually, rRNA was discovered and mRNA was also differentiated giving us a more complete picture:



Basic Structure:

Both DNA and RNA are composed of repeating units of nucleotides. Each nucleotide consists of a sugar, a phosphate and a nucleic acid base. The sugar in DNA is deoxyribose. The sugar in RNA is ribose, the same as deoxyribose but with one more OH (oxygen-hydrogen atom combination - called a hydroxyl).

The covalent structure of RNA differs from that of DNA in two basic ways (a) RNA is a ribonucleic acid, the sugar unit in the nucleotides is ribose rather than deoxyribose (b) in RNA, thymine (T) is replaced by uracil U. Uracil base pairs with adenine (A).

## B). Types of RNA

There are many types of RNA and it seems more are discovered all the time. It is not clear what some of the types do.

In cells there are three **major** types of RNA found: messenger RNA (mRNA),

transfer RNA (tRNA) and ribosomal RNA (rRNA).

Summary:

- 1) messenger RNA (**mRNA**). This will later be translated into a polypeptide.
- 2) ribosomal RNA (**rRNA**). This will be used in the building of ribosomes: machinery for synthesizing proteins by translating mRNA.
- 3) transfer RNA (**tRNA**). RNA molecules that carry amino acids to the growing polypeptide.
- 4) small nuclear RNA (**snRNA**). DNA transcription of the genes for mRNA, rRNA, and tRNA produces large precursor molecules ("**primary transcripts**") that must be processed within the nucleus to produce the functional molecules for export to the cytosol. Some of these processing steps are mediated by snRNAs.
- 5) small nucleolar RNA (**snoRNA**). These RNAs within the nucleolus have several functions
- 6) microRNA (**miRNA**). These are tiny RNA molecules that appear to regulate the expression of messenger RNA (mRNA) molecules.
- 7) Others.

## 1). Messenger RNA (mRNA)

Messenger RNA (mRNA) copies the DNA base pair sequence and carries this message into the cell – this sequence determines the sequence of amino acids used to make proteins.

Messenger RNA comes in a wide range of sizes reflecting the size of the polypeptide it encodes. Most cells produce small amounts of thousands of different mRNA molecules, each to be translated into a peptide needed by the cell.

Many mRNAs are common to most cells, encoding "housekeeping" proteins needed by all cells (e.g. the enzymes of **glycolysis**).

Other mRNAs are specific for only certain types of cells. These encode proteins needed for the function of that particular cell (e.g., the mRNA for **hemoglobin** in

the precursors of red blood cells).

## **2). Ribosomal RNA (rRNA)**

Ribosomal RNA (rRNA) essentially helps to create ribosomes, the factories of protein production in the cell. Ribosomal RNA is found within the ribosome and ribosomal RNA is the primary constituent of ribosomes.

Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm and attached to the membrane of the endoplasmic reticulum. rRNA is transcribed from DNA, like all RNA, and in eukaryotes it is processed in the nucleolus before being transported through the nuclear membrane as it moves to the ribosome. This type of RNA makes up the vast majority of RNA found in a typical cell (~95%). While other proteins are also present in the ribosomes, the rRNA forms the active site for peptide bond formation, making that molecule a ribozyme.

[Eukaryotes are organisms with complex cells, in which the genetic material is organized into membrane-bound nuclei. They include the animals, plants, and fungi, which are mostly multicellular, as well as the kingdom of the protists, many of which are unicellular.]

## **3). Transfer RNA (tRNA)**

Transfer RNA (tRNA) picks up and carries amino acids, the building blocks of proteins, in the cell's protein manufacturing process.

Transfer RNA is a small RNA chain (74-93 nucleotides) that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. It has sites for amino-acid attachment and codon (a particular sequence of 3 bases) recognition. The codon recognition is different for each tRNA and is determined by the anticodon region, which contains the complementary bases to the ones encountered on the mRNA. Each tRNA molecule binds only one type of amino acid, but because the genetic code is degenerate – redundant – two or more codons may code for the same amino acid.

Transfer RNA is the "adaptor" molecule hypothesized by Francis Crick, which mediates recognition of the codon sequence in mRNA and allows its translation into the appropriate amino acid.

#### **4). Small Nuclear RNA (snRNA)**

Small nuclear RNA (snRNA) is a class of small RNA molecules that are found within the nucleus of eukaryotic cells. They are involved in a variety of important processes such as RNA splicing (removal of introns from hnRNA [see below] ) and maintaining the telomeres. snRNAs are always associated with specific proteins, and these complexes are referred to as small nuclear ribonucleoproteins (snRNP) or sometimes as snurps.

Approximately a dozen different genes for snRNAs, each present in multiple copies, have been identified.

Also: The snRNAs have various roles in the processing of the other classes of RNA. For example, several snRNAs are part of the spliceosome that participates in converting pre-mRNA into mRNA by excising the introns and splicing the exons.

#### **5). Small Nucleolar RNA (snoRNA)**

Small nucleolar RNA (snoRNA) is a class of small RNA molecules that are involved in chemical modifications of ribosomal RNAs (rRNAs) and other RNA genes, for example by the process of methylation.

snoRNAs are a component in the small nucleolar ribonucleoprotein (snoRNP), which contains snoRNA and proteins. The snoRNA guides the snoRNP complex to the modification site of the target RNA gene via sequences in the snoRNA that hybridize to the target site. The proteins then catalyze modification of the RNA gene.

[hybridize: to bind complementary pairs of DNA molecules. A DNA molecule has a very strong preference for its sequence complement, so just mixing

complementary sequences is enough to induce them to hybridize.]

As the name suggests, these RNAs (there are probably over 100 of them) are found in the **nucleolus** where they are responsible for several functions.

In vertebrates, the snoRNAs are made from **introns** removed during **RNA processing**.

## 6). MicroRNAs (miRNAs)

microRNA (miRNA) are RNA genes that are the reverse complement of another gene's mRNA transcript and inhibit the expression of the target gene.

A miRNA is a form of single-stranded RNA which is typically 20-25 nucleotides long, and is thought to regulate the expression of other genes. miRNAs are RNA genes which are transcribed from DNA, but are not translated into protein. The DNA sequence that codes for an miRNA gene is longer than the miRNA. This DNA sequence includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a double stranded RNA hairpin loop; this forms a primary miRNA structure (pri-miRNA). Drosha, a nuclear enzyme, cleaves the base of the hairpin to form pre-miRNA. The pre-miRNA molecule is then actively transported into the nucleus by Exportin 5, a carrier protein. The Dicer enzyme then cuts 20-25 nucleotides from the base of the hairpin to release the mature miRNA.

The function of miRNAs appears to be in gene regulation. For that purpose, a miRNA is complementary to a part of one or more messenger RNAs. The annealing (bonding) of the miRNA to the mRNA inhibits protein translation. In some cases, the formation of the double-stranded RNA through the binding of the miRNA triggers the degradation of the mRNA transcript through a process similar to RNA interference (RNAi), though in other cases it is believed that the miRNA complex blocks the protein translation machinery or otherwise prevents protein translation without causing the mRNA to be degraded.

In the worm *C. elegans*, successful development through its larval stages and on to the adult requires the presence of at least two miRNAs. These small transcripts are generated by the cleavage of larger precursors using the *C. elegans* version of Dicer. They act by **inhibiting translation** of several messenger RNAs in the worm (by binding to a region of complementary sequence in the 3' untranslated region [**3'-UTR**] of the mRNA).

The microRNAs (miRNAs) are a large class of RNAs that:

- are found in humans (we have more than 200 miRNA genes), *Drosophila*, mice, frogs, fish, and plants as well as in *C. elegans*;
- all contain 19–25 nucleotides;
- are cleaved from larger RNA precursors (by **Dicer** in animals);
- may be expressed in
  - only certain cell types and
  - at only certain times in the differentiation of a particular cell type.

While direct evidence of the function of many of these newly-discovered gene products remains to be discovered, they probably regulate gene expression by regulating messenger RNA (mRNA), either by:

- destroying the mRNA when the base-pairing is exact or
- repressing its **translation** when the base-pairing is simply a close match.

So, miRNAs may play as important role as **transcription factors** in **coordinating the expression of multiple genes** in a particular type of cell.

## 7). Some Others:

### **Pre-mRNA**

(preliminary mRNA) is a single strand of ribonucleic acid (RNA), synthesized from the DNA in the nucleus of a cell by the transcription process. It has also been called heterogenous nuclear RNA (hnRNA).

In eukaryotes, the pre-mRNA includes two different types of segments: exons and introns. Exons are those parts that code for polypeptides, while introns are not to be expressed and must be excised before translation may occur. This process is called splicing. Spliceosomes, small organelles found in the nucleus and made up of protein and RNA, perform the excision. They also attach new non-coding segments to the leading and lagging ends of the mRNA. These non-coding segments include a 5' cap of 7-methylguanosine and a poly-A tail, attached through the process of polyadenylation. When the mRNA has been properly processed, it is exported out of the nucleus and to a ribosome for translation.

## **Small interfering RNA**

Small interfering RNA (siRNA) are a class of 20-25 nucleotide-long RNA molecules that interfere with the expression of genes. These are produced as part of the RNA interference (RNAi) process by the enzyme Dicer.

## **Double Stranded RNA**

Double-stranded RNA (or dsRNA) is RNA with two complementary strands, similar to the DNA found in all "higher" cells. dsRNA forms the genetic material of some viruses. In eukaryotes, it may play a role in the process of RNA interference and in microRNAs.

## **gRNAs**

gRNAs (guideRNA) are RNA genes that function in RNA editing. Thus far, RNA editing has been found only in the mitochondria of **kinetoplastids**, in which mRNAs are edited by inserting or deleting stretches of **uridyates** (Us). The gRNA forms part of the **editosome** and contains sequences that hybridize to matching sequences in the mRNA, to guide the mRNA modifications.

The term "guide RNA" is also sometimes used generically to mean any RNA gene that guides an RNA/protein complex via hybridization of matching sequences.

[The kinetoplastids are a group of flagellate protozoa, including a number of

parasites responsible for serious diseases in humans and other animals, as well as various forms found in soil and aquatic environments. They are included in the Euglenozoa, and are distinguished from other such forms mainly by the presence of a kinetoplast, a DNA-containing granule located within the single mitochondrion and associated with the flagellar bases.]

## **Efference RNA**

Efference RNA (eRNA) is derived from **intron** sequences of genes or from non-coding DNA. The function is assumed to be regulation of translational activity by interference with the transcription apparatus or target proteins of the translated peptide in question, or by providing a concentration-based measure of protein expression, basically introducing a fine-tuned **analog** element in gene regulation as opposed to the digital on-or-off regulation by **promoters**. Research into the role of eRNAs is only beginning, but they could theoretically be able to explain much of the molecular fundament of **biodiversity**, which has so far eluded genetics.

[Promoter: A DNA molecule to which RNA polymerase binds, initiating the transcription of mRNA.]

## **Signal recognition particle RNA**

The **signal recognition particle** (SRP) is an RNA-protein complex present in the cytoplasm of cells that binds to the mRNA of proteins that are destined for secretion from the cell. The RNA component of the SRP in eukaryotes is called 4.5S RNA.

The signal recognition particle (SRP) recognizes and transports specific proteins to the endoplasmic reticulum in eukaryotes and the plasma membrane in prokaryotes. The core of the particle is universal, being conserved in all three kingdoms.

[three kingdoms: Bacteria, Archaea, and Eucarya]

The SRP is found in the cytosol. In eukaryotes, it binds to the ER [endoplasmic reticulum] signal sequence found in an emerging secretory protein and

subsequently delivers the protein along with the ribosomes creating it to the ER membrane.

## **pRNA**

At least one species of DNA-containing **phages**, phi-29, uses a complex of six identical short RNA sequences as mechanical components (utilizing ATP for energy) of its DNA packaging machinery. How common this phenomenon is has yet to be determined.

[Phage (also- a bacteriophage): virus parasitic in bacteria; it uses the bacterium's machinery and energy to produce more phage until the bacterium is destroyed and phage is released to invade surrounding bacteria.]

## **XIST RNA.**

The gene *Xist* initiates the chromosomal silencing process of X chromosome inactivation in mammals. Its product, a noncoding RNA, is expressed from and specifically associates with the inactive X chromosome in female cells.

## **C). RNA Control**

### **Summary of RNA control:**

There are four basic opportunities (that we know about) for control as shown below:

= **Transcriptional control:** See C, a). below. Control of the sequence of DNA that gets transcribed into mRNA.

= **Post-Transcriptional** – See C, b), c) and d). below. These mechanisms control or regulate mRNA after it has been produced but before it is translated into protein.

= **Translational Control** – See C, e). below. Control of how the mRNA is translated into protein.

= **Post-Translational control** – see C, f). below. Modifications after the protein

chain has been assembled but before it is the finished protein.

## **a). Transcriptional Gene Silencing (TGA)**

*Gene expression* is the combined process of the transcription of a gene into mRNA, the processing of that mRNA, and its translation into protein (for protein-encoding genes).

Gene activity is controlled first and foremost at the level of transcription. Much of this control is achieved through the interplay between proteins that bind to specific DNA sequences and their DNA-binding sites. This type of control hinges on the idea that if a gene is not transcribed it's not able to be expressed. These mechanisms may slow down or speed up transcription.

### Regulatory Proteins and Transcription

Proteins called transcription factors function by binding to the promoter and to another region called the enhancer. The enhancer region may be located at a distance from the gene. These transcription factors are necessary for RNA polymerase to attach. Transcription begins when the factors at the promoter region bind with the factors at the enhancer region creating a loop in the DNA. Hundreds of different transcription factors have been discovered; each recognizes and binds with a specific nucleotide sequence in the DNA. A specific combination of transcription factors is necessary to activate a gene.

Transcription factors is regulated by signals produced from other molecules. For example, hormones activate transcription factors and thus enable transcription. Hormones therefore activate certain genes.

From: [http://health.ucsd.edu/news/2004/08\\_05\\_Looney.html](http://health.ucsd.edu/news/2004/08_05_Looney.html)

Kevin V. Morris, Simon W. L. Chan, Steven E. Jacobsen, David J. Looney  
*Science*, Vol 305, Issue 5688, 1289-1292, 27 August 2004

An understanding of siRNA begins with a look at the way genes work. First, a

“promoter” region within the gene must be active in order to allow the genetic information encoded in the DNA to be copied (transcribed) into a single strand of RNA called messenger RNA (mRNA). During normal transcription, the mRNA leaves the nucleus and travels to the cytoplasm of the cell, where it works with another cellular component called the ribosome to make proteins.

Researchers use either lentiviral vectors (“chemical ferries”) to open up the nuclear membrane, or special transfection reagents that direct the transfected synthetic siRNA to the nucleus. This allows siRNA access to the promoter, where it stops the first part of the gene-making process called transcription, before it begins – hence its name – Transcriptional Gene Silencing.

Previous research with siRNA used in the nucleus of plants has indicated that this effect can be long lasting, giving rise to the hope that it will be similarly long lasting in humans. Until recently, however, scientists have been unable to detect activity of siRNA directed against gene promoters in the nucleus of human cells.

Kevin V. Morris, Ph.D., the study’s first author and a post-doctoral fellow in Looney’s lab, noted that “theoretically, one could envision targeting virtually any gene at the level of the promoter and silencing that gene. This has implications in most biological processes in which one would want to down regulate the expression of a gene, such as those genes involved in virus infections such as HIV, as well as human cancers and certain genetic disorders.”

## **b). Post-Transcriptional – RNA Processing (Splicing)**

In the case of a protein-coding gene, the amount of protein expressed also can be regulated by controlling the stability of the corresponding mRNA in the cytoplasm and the rate of its translation. In addition, the cellular locations of some mRNAs are regulated, so that newly synthesized protein is concentrated where it is needed. All of the regulatory mechanisms that control gene expression following transcription initiation are referred to as *Post-Transcriptional control*. Post-Transcriptional control involves differential processing of preliminary mRNA before it leaves the nucleus and regulation of transport of mature mRNA.

Differential excision of introns and splicing of mRNA can vary type of mRNA that leaves nucleus.

Eukaryotic DNA transcription takes place in a cell's nucleus and produces what is called a primary RNA transcript or pre-messenger RNA. Before eukaryotic products of transcription can be moved into the cytoplasm, they must undergo modifications that allow them to become mature messenger RNA. Splicing is the name given to the reaction that removes unnecessary segments of the primary RNA transcript, called introns. The removal of the introns produces mRNA. Messenger RNA contains only exons, those portions of the primary RNA transcript that will be translated into a protein.

In eukaryotes, mRNA undergoes several processing steps before it is ready to be translated into protein:

- 1). capping** - The addition of a 5' cap. A modified guanine nucleotide is added to the "front" of the message. This is critical for recognition and proper attachment of the ribosome.
- 2). polyadenylation** - A sequence (often several hundred) of adenine nucleotides is added to the 3' end of the pre-mRNA through the action of an enzyme, polyA polymerase (this modification does not occur in prokaryotic mRNA). The polyadenylated tail is added on to the transcripts that contain a specific sequence, the AAUAAA signal. The importance of the AAUAAA signal is demonstrated by a mutation in the coding DNA sequence (AATAAA) which can lead to some hemoglobin deficiencies.

Polyadenylation helps increase the half-life of the transcript, so that the transcript lasts longer in the cell and consequently is translated more and produces more protein.

- 3). RNA splicing** - The pre-mRNA (unprocessed or partially-processed messenger RNA is called "pre-mRNA" or "hnRNA" for heterogeneous nuclear RNA) is modified to remove certain stretches of non-coding sequences called introns; the stretches that remain include protein-coding sequences and are called exons.

Sometimes one pre-mRNA message may be spliced in several different ways, allowing a single gene to encode multiple proteins. This process is called **alternative splicing**. Most RNA splicing is performed by enzymes, but some RNA molecules are also capable of catalyzing their own splicing (see ribozymes).

**Also: RNA splicing** is an essential, precisely regulated Post-Transcriptional process that occurs prior to mRNA translation. In eukaryotes, protein-coding genes are first transcribed by RNA polymerase II into precursor messenger RNA (pre-mRNA) molecules which are copies of the genomic DNA. These pre-mRNA molecules contain both intronic regions destined to be removed during pre-mRNA processing (RNA splicing), as well as exonic sequences that are retained within the mature mRNA.

RNA Splicing is necessary to remove non-coding sequences called introns from the pre-mRNA. Many pre-mRNAs can generate several different types of mature mRNAs via alternative splicing.

**Also:** With the help of **spliceosomes**, a multi-component protein, the splicing reaction occurs in two steps. The spliceosome contains five small nuclear ribonucleoproteins (snRNPs, pronounced "snurps"). They are called U1, U2, U4, U5, and U6. Each **snRNP** contains protein components that are critical for the splicing reaction. U1 binds directly to the 5' splice site via complementary base pairing. U1 then recruits U2, which forms a complex with branch point A. U4 and U6 work in concert to form a "pre-splicing complex" and U5 helps to hold the exons in place between the first and second steps in the splicing reaction. Once the splicing reactions have occurred and the exons have been joined, the resulting mRNA is freed from the spliceosome machinery and the different snRNP components are recycled for further use.

**4). RNA editing.** A fourth type of modification can be made: RNA editing. RNA editing is a modification that changes the mRNA sequence and as a result alters the protein produced by that mRNA. Editing can occur in two ways. First, by changing one nucleotide to another, and second by inserting or deleting a nucleotide or nucleotides.

Kimball: **RNA editing**: the alteration of the sequence of nucleotides in the RNA

- after it has been **transcribed** from DNA but
- before it is **translated** into protein

RNA editing occurs by two distinct mechanisms:

- **Substitution Editing**: chemical alteration of individual nucleotides (the equivalent of point mutations).

These alterations are catalyzed by enzymes that recognize a specific target sequence of nucleotides (much like restriction enzymes):

- cytidine deaminases that convert a C in the RNA to uracil (U);
- adenosine deaminases that convert an A to inosine (I), which the ribosome translates as a G. Thus a CAG codon (for Gln) can be converted to a CGG codon (for Arg).

○

- **Insertion/Deletion Editing**: insertion or deletion of nucleotides in the RNA.

These alterations are mediated by guide RNA molecules that:

- base-pair as best they can with the RNA to be edited and
- serve as a **template** for the addition (or removal) of nucleotides in the target

### c). **Post-Transcriptional – Alternative Splicing**

The human genome sequencing project initially estimated the number of human genes to be between 30,000-40,000, [now, from 20K to 25K] which is much less than the previous estimates. This increased diversity at the mRNA level can be accounted for, in part, by alternative RNA splicing.

During RNA splicing, exons can either be retained in the mature message or targeted for removal in different combinations to create a diverse array of mRNAs

from a single pre-mRNA, a process referred to as alternative RNA splicing

Alternative splicing provides a mechanism for producing a wide variety of proteins from a small number of genes.

While we humans may turn out to have only 20 to 25 thousand genes, we probably make at least 10 times that number of different proteins. Recent genome-wide analyses of alternative splicing indicate that 40-60% of human genes have alternative splice forms, suggesting that alternative splicing is one of the most significant components of the functional complexity of the human genome.

The processing of pre-mRNA for many proteins proceeds along various paths in different cells or under different conditions. For example, early in the differentiation of a **B cell** (a lymphocyte that synthesizes an antibody) the cell first uses an exon that encodes a transmembrane domain that causes the molecule to be retained at the cell surface. Later, the B cell switches to using a different exon whose domain enables the protein to be secreted from the cell as a circulating antibody molecule.

One of the most dramatic examples of alternative splicing is the DSCAM gene in the fruit fly, **Drosophila**. This single gene contains some 108 exons of which 17 are retained in the final mRNA. Some exons are always included; others are selected from an array. Theoretically this system is able to produce 38,016 different proteins. And, in fact, of 50 **cDNAs** [DNA produced **in vitro** by the **reverse transcription** of a **messenger RNA** ] synthesized at random from mRNAs, 49 of them turned out to be unique.

These DSCAM proteins are involved in guiding neurons to their proper destination. Perhaps the incredible diversity of synaptic junctions in the mammalian central nervous system ( $\sim 10^{14}$ ) is mediated by alternative splicing of a limited number of

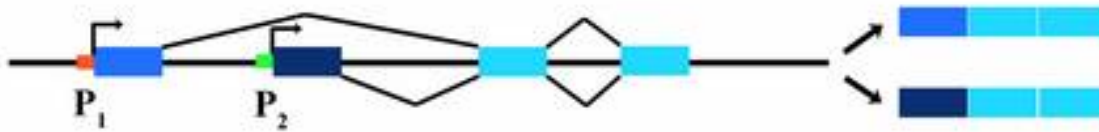
gene transcripts.

So, whether a particular segment of RNA will be retained as an exon or excised as an intron can vary under different circumstances. Clearly the switching to an alternate splicing pathway must be closely regulated.

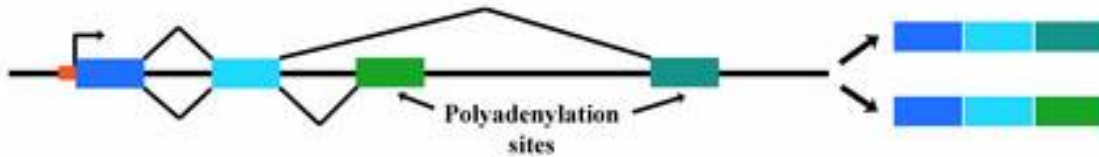
There are four known modes of alternative splicing:

- a. **Alternative selection of promoters:** this is the only method of splicing which can produce an alternative N-terminus domain in proteins. In this case, different sets of promoters can be spliced with certain sets of other exons.
- b. **Alternative selection of cleavage/polyadenylation sites:** this is the only method of splicing which can produce an alternative C-terminus domain in proteins. In this case, different sets of **polyadenylation** sites can be spliced with the other exons.
- c. **Intron retaining mode:** in this case, instead of splicing out an intron, the intron is retained in the mRNA transcript. However, the intron must be properly encoding for **amino acids**. The intron's code must be properly expressible, otherwise a stop codon or a shift in the **reading frame** will cause the protein to be non-functional.
- d. **Exon cassette mode:** in this case, certain exons are spliced out to alter the sequence of amino acids in the expressed protein.

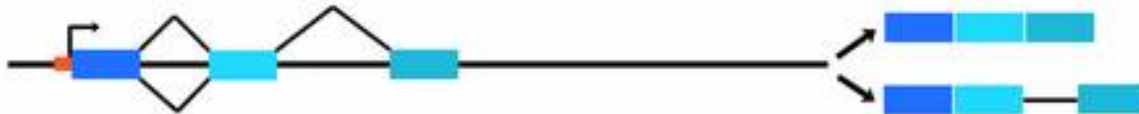
(a) Alternative selection of promoters (e.g., *myosin* primary transcript)



(b) Alternative selection of cleavage/polyadenylation sites (e.g., *tropomyosin* transcript)



(c) Intron retaining mode (e.g., *transposase* primary transcript)



(d) Exon cassette mode (e.g., *troponin* primary transcript)



**d). Post-Transcriptional – RNA interference (RNAi)**

**[RNA interference (RNAi) / Post-Transcriptional gene silencing (PTGS)]**

**1). RNA interference (RNAi), also called Post-Transcriptional Gene Silencing or PTGS.**

[http://en.wikipedia.org/wiki/RNA\\_interference](http://en.wikipedia.org/wiki/RNA_interference)

As the name implies, this process takes place **AFTER** transcription has taken place – **Post-Transcriptional gene silencing.**

RNA interference (RNAi) is a mechanism in which the presence of small fragments of double-stranded RNA (dsRNA) whose sequence matches a given gene interferes with the expression of that gene.

## Overview

RNAi appears to be a highly potent and specific process which is actively carried out by special mechanisms in the cell, known as the RNA interference machinery. While the complete details of how it works are still unknown, it appears that the machinery, once it finds a double-stranded RNA molecule, cuts it up, separates the two strands, and then proceeds to destroy other single-stranded RNA molecules that are complementary to one of those segments. dsRNAs direct the creation of small interfering RNAs (siRNAs) which target RNA-degrading enzymes (RNAses) to destroy transcripts complementary to the siRNAs.

In plants, the use of double stranded RNA to reduce expression has been a common procedure for many years. Antisense mRNA, the reverse complement of a gene, was cloned into a plant. The two complementary RNAs then formed double strands and were degraded. Only after the much more recent discovery of the RNAi machinery (in the plant *Petunia* and later also in *C. elegans*) the use of antisense RNA became more widespread.

The genetic information of many viruses is held in the form of double-stranded RNA, so it is likely that the RNA interference machinery evolved as a defense against these viruses. The machinery is however also used by the cell itself to regulate gene activity: certain parts of the genome are transcribed into microRNA, short RNA molecules that fold back on themselves in a hairpin shape to create a double strand. When the RNA interference machinery detects these double strands, it will also destroy all mRNAs that match the microRNA, thus preventing their translation and lowering the activity of many other genes. This mechanism was first shown in the JAW microRNA of *Arabidopsis*; it is involved in the regulation of several genes that control the plant's shape. The mechanism has also been shown in

many other eukaryotes; by now, some 150 microRNAs have been detected in humans.

RNAi has also been linked to various cellular processes, including the formation of centromeric structure.

Before RNAi was understood, it was called by several names including Post-Transcriptional Gene Silencing (PTGS) and transgene silencing. Only after these phenomena were characterized at the molecular level was it obvious that they were the same phenomenon.

## **Gene Knockout and knockdown**

RNAi has recently been applied as an experimental technique to "knockout" genes in model organisms for experimental analysis in determining the function of a gene. Repressing a gene from being expressed allows for testing of the protein and its role in the life of a cell or larger organism. (Because RNAi may not totally abolish expression of a gene, using it against a gene is sometimes referred as a "knockdown", to distinguish it from procedures in which the DNA sequence encoding a gene is removed, "knockout".) Most functional genomics applications of RNAi were made on *C. elegans*, a nematode worm that is frequently used as a model organism in genetics research.

## **Role in medicine**

Researchers are developing synthetic (man made), short pieces of RNA called short interfering RNA (siRNA), to shut down genes. The synthetic versions are patterned after naturally occurring siRNA in the body that may act as a defense against gene sequences that come from viruses or other genetic parasites. These techniques have given rise in recent years to a multi-million dollar pharmaceutical industry.

The dsRNAs that trigger RNAi may be usable as drugs. For example, dsRNA could repress essential genes in eukaryotic human pathogens or viruses that are dissimilar

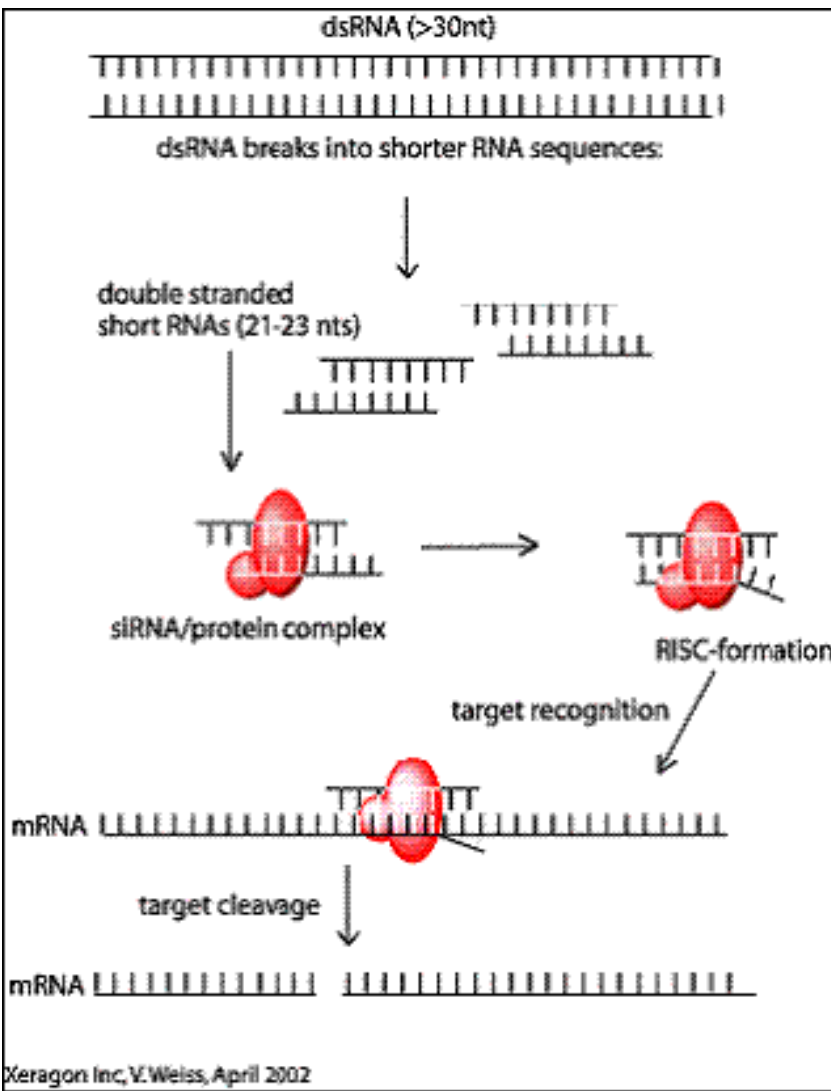
from any human genes; this would be analogous to how existing drugs work. Such applications of RNAi are currently only speculative.

RNAi interferes with the translation process of gene expression and appears not to interact with the DNA itself. Proponents of therapies based on RNAi suggest that the lack of interaction with DNA may alleviate some patients' concerns about alteration of their DNA and suggest that this method of treatment would likely be no more feared than taking any prescription drug. For this reason RNAi and therapies based on RNAi have attracted much interest in the pharmaceutical and biotech industries.

**From:**  
[http://www1.qiagen.com/about/InvestorRelation/corppres/04\\_18\\_02/PTGS.pdf](http://www1.qiagen.com/about/InvestorRelation/corppres/04_18_02/PTGS.pdf)

## **How does RNAi work?**

Genetic and biochemical data indicate a possible two-step mechanism for RNA interference: an initiation step and an effector step. In the first step, input doublestranded RNA, e.g. virus RNA, is processed into 21-25 nucleotide “guidance sequences”. Up until today, it is not known whether they are single or doublestranded. The guide RNAs are incorporated into a nuclease complex, called the RNA-induced silencing complex (RISC), which is part of the natural viral defense and transposon silencing mechanism and acts in the second effector step to destroy (virus) mRNAs that are recognized by the guide RNAs through base-pairing interactions.



Xeragon Inc, V. Weiss, April 2002

This mechanism can also be used in molecular biology and modern drug development by transfecting cells with small interfering RNA (siRNA). Small interfering RNA (siRNAs) are double stranded RNAs of ~21-25 nucleotides in length that have been shown to function as key molecules in triggering sequence specific RNA degradation during post-Transcriptional gene silencing. Synthetic siRNAs can induce gene specific inhibition of expression in a variety of cell lines including humans and mice. The interference by siRNA is always superior to inhibition of gene expression mediated by single stranded antisense oligonucleotides. The siRNAs seem to avoid the nonspecific effects triggered by longer dsRNAs in mammalian cells. These facts may open a path toward the eventual use of siRNAs as reverse genetic and therapeutic tools in mammalian cells.

**From Kimball:**

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/AntisenseRNA.html>

## **Mechanism of RNAi**

The only RNA molecules normally found in the cytoplasm of a cell are molecules of **single-stranded** RNA. If the cell finds molecules of double-stranded RNA (**dsRNA**), it uses an enzyme called **Dicer** to cut them into fragments containing 19 base pairs (~2 turns of a double helix) with two additional nucleotides at the opposite end of each strand.

The two strands of each fragment then separate — releasing the **antisense strand**. With the aid of a protein, it binds to a **complementary sense sequence** on a molecule of **mRNA**. If the base pairing is exact, the mRNA is destroyed.

Because of their action, these fragments of RNA have been named "short (or small) interfering RNA" (**siRNA**).

The complex of siRNA and protein is called the "**RNA-induced silencing complex**" (**RISC**).

### **e). Regulation of Translation**

In some cases, once the mRNA is available for translation, translation can be encouraged or suppressed. For example, iron ( $\text{Fe}^{++}$ ) in cells is stored in a special protein called ferritin. If iron levels are low, a repressor protein attaches to the mRNA for ferritin and prevents its translation into new protein. If iron levels in the cell rise, then some iron binds to the ferritin mRNA repressor protein, altering its shape and causing it to separate from the mRNA. Thus the mRNA can now be translated into ferritin needed to store the increased iron. Similar types of control operate for the synthesis of proteins made from different types of subunits such as hemoglobin.

Here are some ways gene expression is controlled at the level of translation:

## 1). By RNA Interference

Here small RNA molecules bind to the complementary portion of a **mRNA** and

- prevent it from being translated by ribosomes or
- trigger its destruction.

## 2). By Riboswitches

In many bacteria (and some eukaryotes), it turns out that the regulation of the level of certain metabolites is controlled by riboswitches. A riboswitch is a part of a molecule of messenger RNA (mRNA) with a specific binding site for the **metabolite** (or a close relative).

Examples:

- If thiamine pyrophosphate (the active form of **thiamine** [vitamin B<sub>1</sub>]) is available in the culture medium of **E. coli**,
  - it binds to a messenger RNA whose protein product is an enzyme needed to synthesize thiamine from the ingredients in **minimal medium**.
  - Binding induces an **allosteric** shift in the structure of the mRNA so that it can no longer bind to a ribosome and thus cannot be translated into the enzyme.
  - E. coli no longer wastes resources on synthesizing a vitamin that is available pre-formed.
- If **vitamin B<sub>12</sub>** is present in the cell,
  - it binds to the mRNA which encodes a protein needed to import the vitamin from the culture medium.
  - This, too, induces an allosteric shift in the mRNA that prevents it from

binding a ribosome.

- E. coli no longer wastes resources on synthesizing a transporter for a vitamin that it already has enough of.
- Some **gram-positive bacteria** (E. coli is gram-negative) control the level of a sugar needed to synthesize their **cell wall** with a riboswitch. In this case, as the concentration of the sugar builds up, it binds to the messenger RNA (mRNA) whose product is the enzyme that makes the sugar. This causes the mRNA to self-destruct so production of the enzyme — and thus the sugar — ceases.

Other riboswitches act on **transcription** rather than translation.

It has been suggested that these regulatory mechanisms, which do not involve any protein, are a left over relict from an "**RNA world**".

Riboswitchs

<http://www.biologydaily.com/biology/Riboswitch>

In **molecular biology**, a riboswitch is a part of an **mRNA** molecule that can directly bind a small target molecule, and whose binding of the target affects the **gene's** activity. Thus, an mRNA that contains a riboswitch is directly involved in regulating its own activity, depending on the presence or absence of its target molecule.

Some riboswitches activate their gene when binding to the target, while others repress it. Riboswitches also differ as to their mechanism of control. Known riboswitches operate through **transcription** termination, **translation** initiation or self-cleavage (i.e. the riboswitch is a **ribozyme** that cleaves itself in a metabolite-dependent manner).

Most known riboswitches occur in **eubacteria**, but functional riboswitches of one

type (the THI element) have been discovered in **eukaryotes**. Sequences similar to known riboswitches have also been found in **archaea**.

Riboswitches are a demonstration that naturally occurring **RNA** can bind small molecules, a capability that many previously believed was the domain of **proteins** or artificially constructed RNAs called aptamers. The existence of riboswitches in all domains of life therefore adds some support to the **RNA world hypothesis**, which holds that life originally existed using only RNA, and proteins came later; this hypothesis requires that all critical functions performed by proteins could be performed by RNA.

Although the genetic pathways in which riboswitches are involved have been studied for decades, the existence of riboswitches has only recently been found. This oversight may relate to an assumption that genes are regulated by proteins, not by the mRNA transcript itself. Now that riboswitches are a known mechanism of genetic control, it is reasonable to speculate that more riboswitches will be found.

Also - From: <http://www.yale.edu/breaker/riboswitch.htm>

Each cell must regulate the expression of hundreds of different genes in response to changing environmental or cellular conditions. The majority of these sophisticated genetic control factors are proteins, which monitor metabolites and other chemical cues by selectively binding to targets. We have confirmed that RNA also can form precision genetic switches and that these elements can control fundamental biochemical processes.

Riboswitches are a type of natural genetic control element that use untranslated sequence in an mRNA to form a binding pocket for a metabolite that regulates expression of that gene. Seven riboswitches are under investigation and it appears as though this mechanism of genetic regulation is widespread in bacteria. These natural RNA switches are the latest illustration of how functional RNAs are engaged in fundamental cellular processes.

Riboswitches are dual function molecules that undergo conformational changes and that communicate metabolite binding typically as either increased transcription termination or reduced translation efficiency via an expression platform. Some riboswitches have been detected in eukaryotes, supporting the view that riboswitches might be an ancient form of genetic control that has persisted since the "RNA World." The evolutionary consequences of this finding are being explored.

Many antibiotics bind to ribosomal RNAs and selectively inhibit bacterial growth. We are beginning to investigate whether riboswitches might also be targeted by new classes of antibiotics. Given the significant role that riboswitches play in bacterial genetic control and the fact that they have evolved to bind metabolites, we suspect that drug compounds could be created that disrupt bacterial genetic control. Furthermore, engineered riboswitches might function as designer genetic control elements.

### 3). By Proteins

Translation of at least one mRNA in humans is repressed by a protein — **aminoacyl tRNA synthetase**. In response to the inflammatory cytokine **interferon-gamma** [IFN- $\gamma$ ], the synthetase abandons its normal function (adding Glu and Pro to their respective tRNAs) and instead binds to the mRNA blocking its translation.

### f). Post-Translational Modifications of Proteins

Once a peptide (amino acid) chain has been assembled, it usually requires additional processing before the finished protein is ready to perform the task it was created to do. This final stage of processing is known as Post-translational modification.

Post-translational modifications modulate the activity of most eukaryote proteins.

The most common forms of modification include the following:

- a) Breaking the protein into smaller proteins
- b) Combining multiple protein products together, forming a larger protein
- c) Adding sugars to the protein product

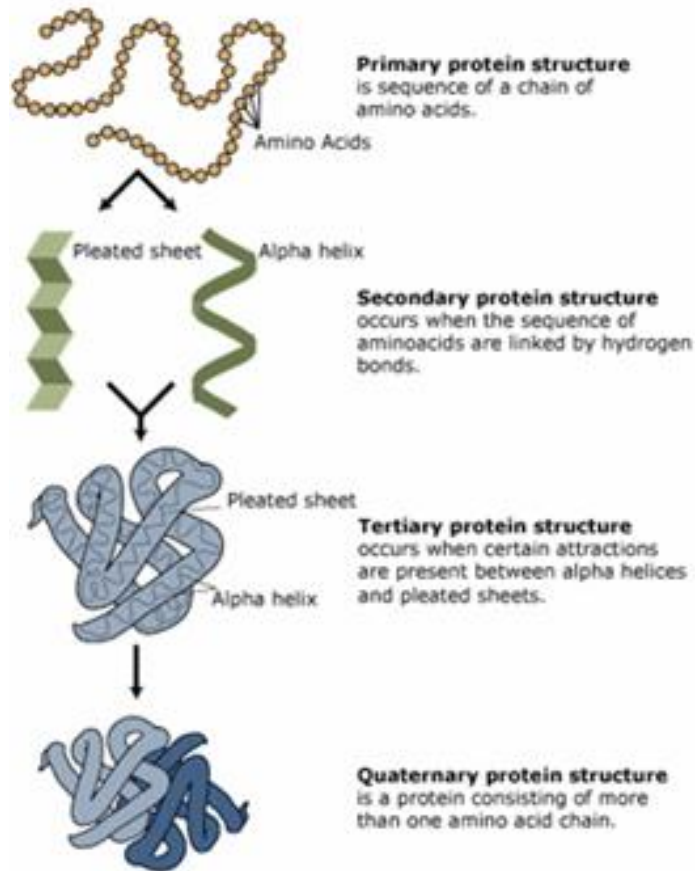


Image adapted from: National Human Genome Research Institute.

Also - from: [http://www.explore-biology.com/genetics/P/Posttranslational\\_modification.html](http://www.explore-biology.com/genetics/P/Posttranslational_modification.html)

**Posttranslational modification** means the **chemical** modification of a **protein** after its translation. It is one of the later steps in **protein biosynthesis** for many proteins.

Posttranslational modification may involve the formation of disulfide bridges and

attachment of any of a number of biochemical **functional groups**, such as **acetate**, **phosphate**, various **lipids** and **carbohydrates**. **Enzymes** may also remove one or more amino acids from the amino end of the polypeptide chain, or cut the polypeptide in the middle of the chain. For instance, the peptide hormone insulin is cut twice after disulfide bond formation to remove a propeptide from the middle of the chain, leaving a protein consisting of two polypeptide chains connected by disulfide bonds. In other cases, two or more polypeptide chains that are synthesized separately may associate to become **subunits** of a protein with **quaternary structure**.

A protein is a chain composed of a long sequence of 20 possible **amino acids**, also called a polypeptide. Some posttranslational modification extends the range of possible functions a protein can have by introducing other chemical groups into the makeup of a protein (e.g., **carbohydrate** chains). Such chemical changes may alter the hydrophobicity of a protein and thus determine if the modified protein is **cytosolic** [Pertaining to or contained in the cytosol – the semi-fluid component of a cell's cytoplasm] or membrane-bound. Other modifications like **phosphorylation** are part of common mechanisms for controlling the behavior of a protein, for instance, activating or inactivating an **enzyme**.

Types of Post-translational modifications include:

- **phosphorylation**, the addition of a **phosphate** group, usually to **serine**, **tyrosine**, **threonine** or **histidine**
- **acetylation**, the addition of an **acetyl** group, usually at the N-terminus of the protein
- **alkylation**, the addition of an **alkyl** group (e.g. methyl, ethyl)
  - **methylation** the addition of a methyl group, usually at **lysine** or **arginine** residues (this is a subtype of alkylation)
- isoprenylation, the addition of an isoprenoid group (e.g. farnesol and geranylgeraniol)
- **glycosylation**, the addition of a **glycosyl** group to either **asparagine**,

**hydroxylysine, serine, or threonine**, resulting in a **glycoprotein**

- **ubiquitination**, the covalent linkage of the protein ubiquitin to a target protein, typically interpreted by the cellular machinery as a "destroy this protein" tag.
- SUMOylation, the covalent linkage of the SUMO protein (Small Ubiquitin-related MOdifier) to a target protein (1).
- ISGylation, the covalent linkage of the ISG15 protein (Interferon-Stimulated Gene 15) to a target protein (2). ISG15 bears significant sequence similarity to **ubiquitin**.

## **g). Post-Transcriptional vs Post-Translational Modifications**

It is important not to confuse Post-Transcriptional and Post-Translational processes:

### **Post-Transcriptional Processing of RNAs**

As described above, eukaryotic RNAs undergo significant Post-Transcriptional processing. All 3 classes of RNA (tRNA, mRNA and rRNA) are transcribed from genes that contain **introns**. The sequences encoded by the intronic DNA must be removed from the primary transcript prior to the RNAs being biologically active.

**Posttranslational modification** means the **chemical** modification of a **protein** after its translation. It is one of the later steps in **protein biosynthesis** for many proteins.

## **D). Antisense RNA Inhibits Gene Translation**

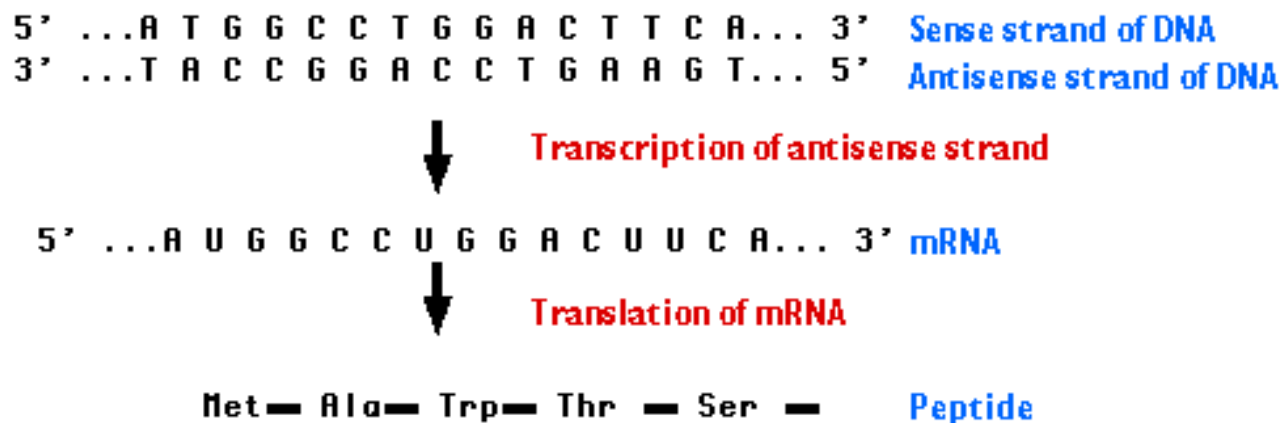
**Antisense mRNA:** An mRNA (messenger RNA) transcript that is complementary to endogenous mRNA. Antisense mRNA is the noncoding strand complementary to the coding sequence of mRNA. Introducing a transgene coding mRNA or antisense mRNA is a strategy used to block expression (translation) of a gene of

interest.

Anti-sense mRNA can inhibit gene translation in many eukaryotes, when the anti-sense RNA's sequence is complementary to that of the mRNA of the gene. [The antisense sequence attaches to the corresponding sense sequence, forming a short segment of double stranded mRNA (dsRNA)]. This means a gene is not expressed as protein if a matching anti-sense mRNA is present in the cell. This may be a defense mechanism against retrotransposons (transposons that use dsRNA as an intermediate state) or viruses, because both can use double-stranded mRNA as an intermediate.

In biochemical research, this effect has been used to study gene function, simply shutting down the studied gene by adding its anti-sense mRNA transcript. Such studies have been done on the worm *C. elegans*.

Also: Messenger RNA (mRNA) is single-stranded. Its sequence of nucleotides is called "sense" because it results in a gene product (protein). Normally, its unpaired nucleotides are "read" by transfer RNA anticodons as the ribosome proceeds to **translate** the message.



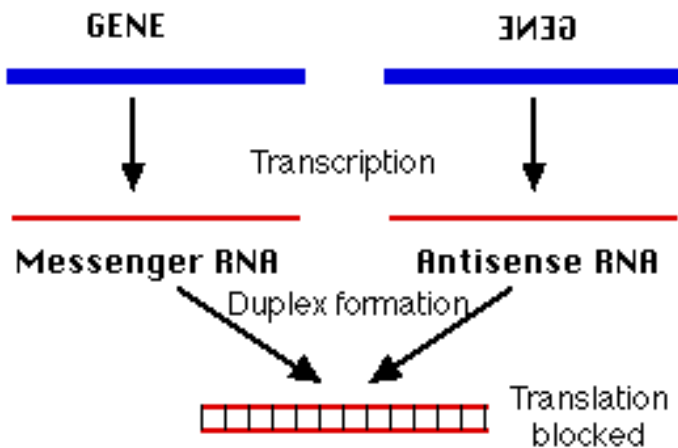
However, RNA **can** form duplexes (double strands) just as DNA does. All that is needed is a second strand of RNA whose sequence of bases is complementary to the first strand; e.g.,

5' C A U G 3' mRNA

3' G U A C 5' Antisense RNA

The second strand is called the **antisense strand** because its sequence of nucleotides is the complement of message sense. When mRNA forms a duplex with a complementary antisense RNA sequence, translation is blocked. This may occur because:

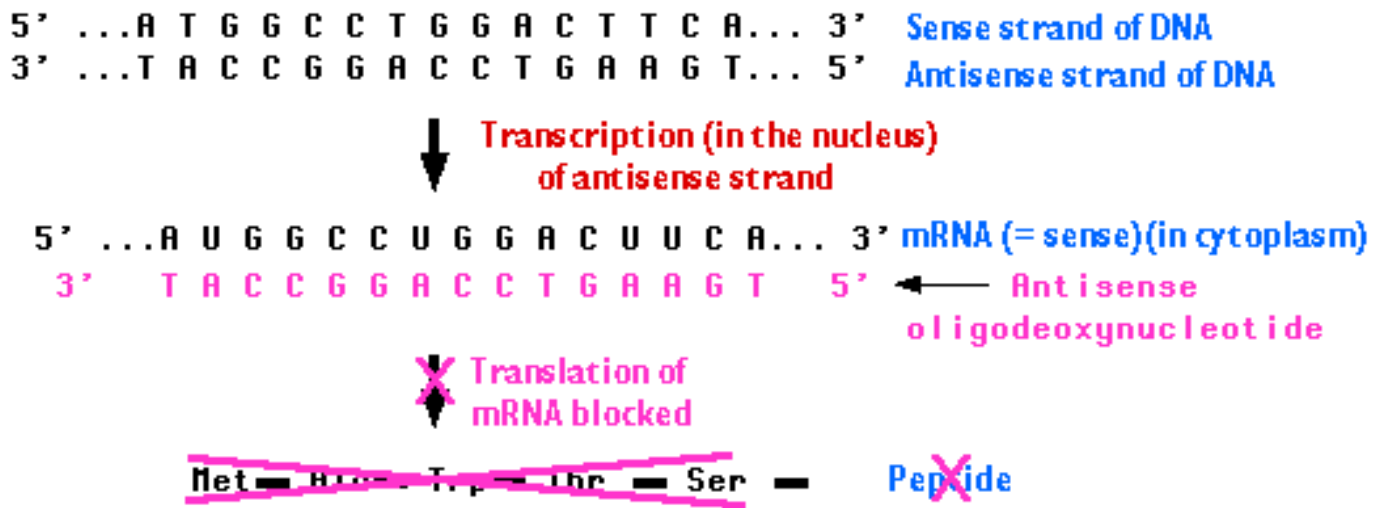
- the ribosome cannot gain access to the nucleotides in the mRNA or
- duplex RNA is quickly degraded by ribonucleases in the cell



## E). AONs: Antisense oligonucleotides - Antisense oligodeoxynucleotides and Antisense oligoribonucleotides Used to Control Translation

AONs are synthetic (man made):

- the monomers can be deoxynucleotides like those in DNA
- the monomers can be ribonucleotides like those in RNA
- there are usually only a few (15–20) of them, hence the name "oligo"
- their sequence (3' → 5') is antisense; that is, complementary to the sense sequence of a molecule of **mRNA**.



Antisense AONs are synthesized in the hope that they can be used as therapeutic agents — blocking disease processes by blocking the synthesis of a particular protein. This would be achieved by the binding of the AON to the mRNA from which that protein is normally synthesized. Binding of the two may:

- physically block the ability of ribosomes to move along the messenger RNA or
- simply hasten the rate at which the mRNA is degraded within the cytosol.

In order to be useful in human therapy, AONs must:

- be able to enter the target cells;
- avoid digestion by DNase;
- not cause unpleasant side-effects.

### F). Transgenes and Transgenesis

Transgenesis consists of introducing an exogenous [derived or originating externally] gene - called a **transgene** - into a living organism so that the organism will exhibit a new property and transmit that property to its offspring. In animals, for the transgene to be passed to descendants, it must be integrated into the **gametes** (germ cells – sperm or eggs). The new property of the animal comes from

the **protein** coded for by the transgene.

This is contrasted with efforts to introduce changes into an individual to control some genetic problem. For example, using an AON approach to therapy may inhibit translation of a mutated and harmful protein and alleviate symptoms. These interventions do not alter the DNA of the patient (or their germ cells) and therefore are not passed on to any subsequent children.

## **G). Transposons (“Jumping Genes”) and Retrotransposons**

Transposons are sequences of DNA that can move around to different positions within the genome of a single cell, a process called Transposition. In the process, they can cause mutations and change the amount of DNA in the genome.

Transposons are also called "jumping genes" or "mobile genetic elements". There are a variety of mobile genetic elements, they can be grouped based on their mechanism of transposition. Class I mobile genetic elements, or retrotransposons, move in the genome by being transcribed to RNA and then back to DNA by reverse transcriptase, while class II mobile genetic elements move directly from one position to another within the genome using a transposase to "cut and paste" them within the genome.

### **Retrotransposons**

Retrotransposons (sometimes Retroposons) work by copying themselves and pasting copies back into the genome in multiple places. Initially retrotransposons copy themselves to RNA (transcription) but, instead of being translated, the RNA is copied into DNA by a reverse transcriptase (often coded by the transposon itself) and inserted back into the genome.

Retrotransposons behave very similarly to retroviruses, such as HIV, giving a clue to their evolutionary origins.

## END part -1

<http://www.ambion.com/hottopics/rnai>

# RNA Interference and Gene Silencing — History and Overview

May 20, 2002

Post-transcriptional gene silencing (PTGS), which was initially considered a bizarre phenomenon limited to petunias and a few other plant species, is now one of the hottest topics in molecular biology (1). In the last few years, it has become clear that PTGS occurs in both plants and animals and has roles in viral defense and transposon silencing mechanisms. Perhaps most exciting, however, is the emerging use of PTGS and, in particular, RNA interference (RNAi) — PTGS initiated by the introduction of double-stranded RNA (dsRNA) — as a tool to knock out expression of specific genes in a variety of organisms (reviewed in 1-3).

How was RNAi discovered? How does it work? Perhaps more importantly, how can it be harnessed for functional genomics experiments? This article will briefly answer these questions and provide you with resources to find in depth information on PTGS and RNAi research.

## A Bizarre Phenomenon is Discovered:

### Cosuppression and PTGS in Plants

More than a decade ago, a surprising observation was made in petunias. While trying to deepen the purple color of these flowers, Rich Jorgensen and colleagues introduced a pigment-producing gene under the control of a powerful promoter. Instead of the expected deep purple color, many of the flowers appeared variegated or even white. Jorgensen named the observed phenomenon "cosuppression", since the expression of both the introduced gene and the homologous endogenous gene was suppressed (1-5).

First thought to be a quirk of petunias, cosuppression has since been found to occur in many species of plants. It has also been observed in fungi, and has been particularly well characterized in *Neurospora crassa*, where it is known as "quelling" (1-3).

But what causes this gene silencing effect? Although transgene-induced silencing in some plants appears to involve gene-specific methylation (transcriptional gene silencing, or TGS), in others silencing occurs at the post-transcriptional level (post-transcriptional gene silencing, or PTGS). Nuclear run-on experiments in the latter case show that the homologous transcript is made, but that it is rapidly degraded in the cytoplasm and does not accumulate (1, 3, 6).

Introduction of transgenes can trigger PTGS, however silencing can also be induced by the introduction of certain viruses (2, 3). Once triggered, PTGS is mediated by a diffusible, trans-acting molecule. This was first demonstrated in *Neurospora*, when Cogoni and colleagues showed that gene silencing could be transferred between nuclei in heterokaryotic strains (1, 7). It was later confirmed in plants when Palauqui and colleagues induced PTGS in a host plant by grafting a silenced, transgene-containing source plant to an unsilenced host (8). From work done in

nematodes and flies, we now know that the trans-acting factor responsible for PTGS in plants is dsRNA (1-3).

## Gene Silencing by dsRNA:

### RNA Interference

#### RNAi Is Discovered in Nematodes

The first evidence that dsRNA could lead to gene silencing came from work in the nematode *Caenorhabditis elegans*. Seven years ago, researchers Guo and Kemphues were attempting to use antisense RNA to shut down expression of the *par-1* gene in order to assess its function. As expected, injection of the antisense RNA disrupted expression of *par-1*, but quizzically, injection of the sense-strand control did too (9).

This result was a puzzle until three years later. It was then that Fire and Mello first injected dsRNA — a mixture of both sense and antisense strands — into *C. elegans* (10). This injection resulted in much more efficient silencing than injection of either the sense or the antisense strands alone. Indeed, injection of just a few molecules of dsRNA per cell was sufficient to completely silence the homologous gene's expression. Furthermore, injection of dsRNA into the gut of the worm caused gene silencing not only throughout the worm, but also in its first generation offspring (10).

The potency of RNAi inspired Fire and Timmons to try feeding nematodes bacteria that had been engineered to express dsRNA homologous to the *C. elegans unc-22* gene. Surprisingly, these worms developed an *unc-22* null-like phenotype (11-13). Further work showed that soaking worms in dsRNA was also able to induce silencing (14). These strategies, whereby large numbers of nematodes are exposed to dsRNA, have enabled large-scale screens to select for RNAi-defective *C. elegans* mutants and have led to large numbers of gene knockout studies within this organism (15-18).

#### RNAi in *Drosophila*

RNAi has also been observed in *Drosophila*. Although a strategy in which yeast were engineered to produce dsRNA and then fed to fruit flies failed to work, microinjecting *Drosophila* embryos with dsRNA does effect silencing (2). Silencing can also be induced by "shooting" dsRNA into *Drosophila* embryos with a "gene gun" or by engineering flies to carry DNA containing an inverted repeat of the gene to be silenced. Over the last few years, these RNAi strategies have been used as reverse genetics tools in *Drosophila* organisms, embryo lysates, and cells to characterize various loss-of-function phenotypes (2, 19-23).

### The Biochemical Mechanism of RNAi

So how does injection of dsRNA lead to gene silencing? Many research groups have diligently worked over the last few years to answer this important question. A key finding by Baulcombe and Hamilton provided the first clue. They identified RNAs of ~25 nucleotides in plants undergoing cosuppression that were absent in non-silenced plants. These RNAs were complementary to both the sense and antisense strands of the gene being silenced (24).

Further work in *Drosophila* — using embryo lysates and an in vitro system derived from S2 cells — shed more light on the subject (3, 25, 26). In one notable series of experiments, Zamore and colleagues found that dsRNA added to *Drosophila* embryo lysates was processed to 21-23 nucleotide species. They also found that the homologous endogenous mRNA was cleaved only in the region corresponding to the introduced dsRNA and that cleavage occurred at 21-23 nucleotide intervals (26). Rapidly, the mechanism of RNAi was becoming clear.

## Current Models of the RNAi Mechanism

Both biochemical and genetic approaches (see "The Genes and Enzymes Involved in PTGS and RNAi" below for a discussion of genetic approaches used to understand RNAi) have led to the current models of the RNAi mechanism. In these models, RNAi includes both initiation and effector steps ([27](#), see also a Flash animation of "[How Does RNAi Work?](#)", from reference [3](#)).

In the initiation step, input dsRNA is digested into 21-23 nucleotide small interfering RNAs (siRNAs), which have also been called "guide RNAs" (reviewed in [3](#), [18](#), [27](#)). Evidence indicates that siRNAs are produced when the enzyme Dicer, a member of the RNase III family of dsRNA-specific ribonucleases, processively cleaves dsRNA (introduced directly or via a transgene or virus) in an ATP-dependent, processive manner. Successive cleavage events degrade the RNA to 19-21 bp duplexes (siRNAs), each with 2-nucleotide 3' overhangs ([27](#), [28](#)).

In the effector step, the siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. An ATP-dependent unwinding of the siRNA duplex is required for activation of the RISC. The active RISC then targets the homologous transcript by base pairing interactions and cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA ([3](#), [18](#), [27](#), [29](#)). Although the mechanism of cleavage is at this date unclear, research indicates that each RISC contains a single siRNA and an RNase that appears to be distinct from Dicer ([27](#)).

Because of the remarkable potency of RNAi in some organisms, an amplification step within the RNAi pathway has also been proposed. Amplification could occur by copying of the input dsRNAs, which would generate more siRNAs, or by replication of the siRNAs themselves (see "Possible Role for RNA-dependent RNA Polymerase" below). Alternatively or in addition, amplification could be effected by multiple turnover events of the RISC ([3](#), [18](#), [27](#)).

## The Genes and Enzymes Involved in PTGS and RNAi

### Possible Role for RNA-dependent RNA Polymerase

Genetic screens in *Neurospora*, *C. elegans*, and *Arabidopsis* have identified several genes that appear to be crucial for PTGS and RNAi. Several of these, including *Neurospora qde-1*, *Arabidopsis SDE-1/SGS-2* and *C. elegans ego-1*, appear to encode RNA-dependent RNA polymerases (RdRPs). At first glance, it might be assumed that this is proof that an RdRP activity is required for RNAi. Certainly the existence of an RdRP might explain the remarkable efficiency of dsRNA-induced silencing if it amplified either the dsRNA prior to cleavage or the siRNAs directly. But mutants of these genes have varying phenotypes, which makes the role of RdRP in RNAi difficult to discern ([1](#), [3](#), [17](#), [18](#)).

In *C. elegans ego-1* mutants ("ego" stands for "enhancer of *glp-1*"), RNAi functions normally in somatic cells, but is defective in germline cells where *ego-1* is primarily expressed. In *Arabidopsis SDE-1/SGS-2* mutants ("SGS" stands for suppressor of gene silencing), siRNAs are produced when dsRNA is introduced via an endogenously replicating RNA virus, but not when introduced by a transgene. It has been proposed that perhaps the viral RdRP is substituting for the *Arabidopsis* enzyme in these mutants ([1](#), [3](#), [17](#), [18](#)). Although no homolog of an RdRP has been found in flies or humans, an RdRP activity has recently been reported in *Drosophila* embryo lysates ([30](#)). One model of amplification, termed the "random degradative PCR" model, suggests that an RdRP uses the guide strand of an

siRNA as a primer for the target mRNA, generating a dsRNA substrate for Dicer and thus more siRNAs ([27](#), [30](#)). Evidence supporting this model has been found in worms, whereas experimental results refuting the model have been obtained from *Drosophila* embryo lysates ([26](#), [27](#)).

### RNAi Initiators

Two *C. elegans* genes, *rde-1* and *rde-4* ("rde" stands for "RNAi deficient"), are believed to be involved in the initiation step of RNAi. Mutants of these genes produce animals that are resistant to silencing by injection of dsRNA, but silencing can be effected in these animals by the transmission of siRNA from heterozygous parents that are not silencing deficient. The *C. elegans rde-1* gene is a member of a large family of genes and is homologous to the *Neurospora qde-2* ("qde" stands for "quelling deficient") and the *Arabidopsis AGO1* genes ("AGO" stands for "argonaute"; *AGO1* was previously identified to be involved in *Arabidopsis* development). Although the function of these genes in PTGS is unclear, a mammalian member of the RDE-1 family has been identified as a translation initiation factor. Interestingly, *Arabidopsis* mutants of *AGO1*, which are defective for cosuppression, also exhibit defects in leaf development. Thus some processes or enzymes involved in PTGS may also be involved in development ([1](#), [3](#), [17](#), [18](#)).

### RNAi Effectors

Important genes for the effector step of PTGS include the *C. elegans rde-2* and *mut-7* genes. These genes were initially identified from heterozygous mutant worms that were unable to transmit RNAi to their homozygous offspring ([16](#)). Worms with mutated *rde-2* or *mut-7* genes exhibit defective RNAi, but interestingly, they also demonstrate increased levels of transposon activity. Thus, silencing of transposons appears to occur by a mechanism related to RNAi and PTGS. Although the *rde-2* gene product has not yet been identified, the *mut-7* gene encodes a protein with homology to the nuclease domains of RNase D and a protein implicated in Werner syndrome (a rapid aging disease) in humans ([1](#), [3](#), [17](#), [18](#), [31](#)). Perhaps this protein is a candidate for the nuclease activity required for target RNA degradation.

### PTGS Has Ancient Roots

Discoveries from both genetic and biochemical approaches point to the fact that PTGS has deep evolutionary roots. Proposals have been put forth that PTGS evolved as a defense mechanism against transposons or RNA viruses, perhaps before plants and animals diverged ([1](#), [3](#), [17](#), [18](#)).

Interestingly, it was noted by many researchers that disruption of genes required for RNAi often causes severe developmental defects. This observation suggested a link between RNAi and at least one developmental pathway. A group of small RNA molecules, known as small temporal RNAs (stRNAs), regulates *C. elegans* developmental timing through translational repression of target transcripts. Research indicates that the *C. elegans lin-4* and *let-7* stRNAs are generated from 70-nt transcripts following the folding of these longer transcripts into a stem-loop structure. The folded RNA molecules are cleaved to produce 22-nt stRNAs by the enzyme Dicer (called DCR-1 in *C. elegans*). Thus Dicer generates both siRNAs and stRNAs, and represents an intersection point for the RNAi and stRNA pathways ([32-34](#)).

Recently, nearly 100 additional ~22 nt RNA molecules, termed microRNAs (miRNAs), were identified in

*Drosophila*, *C. elegans*, and HeLa cells (35-38). Much like *lin-4* and *let-7*, these miRNAs are formed from precursor RNA molecules that fold into a stem-loop secondary structure. The newly discovered ~22 nt miRNAs are believed to play a role in regulation of gene expression, and at least two of them are known to require Dicer for their production (37). It appears that the use of small RNAs for both gene regulation and RNAi is a common theme throughout evolution.

## Inducing RNAi in Mammalian Cells — From Mechanism to Application

### Non-specific Gene Silencing by Long dsRNAs

While the natural presence of RNAi had been observed in a variety of organisms (plants, protozoa, insects, and nematodes), evidence for the existence of RNAi in mammalian cells took longer to establish. Transfection of long dsRNA molecules (>30 nt) into most mammalian cells causes nonspecific suppression of gene expression, as opposed to the gene-specific suppression seen in other organisms. This suppression has been attributed to an antiviral response, which takes place through one of two pathways.

In one pathway, long dsRNAs activate a protein kinase, PKR. Activated PKR, in turn phosphorylates and inactivates the translation initiation factor, eIF2a, leading to repression of translation. (39) In the other pathway, long dsRNAs activate RNase L, which leads to nonspecific RNA degradation (40).

A number of groups have shown that the dsRNA-induced antiviral response is absent from mouse embryonic stem (ES) cells and at least one cell line of embryonic origin. (41, 42) It is therefore possible to use long dsRNAs to silence specific genes in these specific mammalian cells. However, the antiviral response precludes the use of long dsRNAs to induce RNAi in most other mammalian cell types.

### siRNAs Bypass the Antiviral Response

Interestingly, dsRNAs less than 30 nt in length do not activate the PKR kinase pathway. This observation, as well as knowledge that long dsRNAs are cleaved to form siRNAs in worms and flies and that siRNAs can induce RNAi in *Drosophila* embryo lysates, prompted researchers to test whether introduction of siRNAs could induce gene-specific silencing in mammalian cells (43). Indeed, siRNAs introduced by transient transfection were found to effectively induce RNAi in mammalian cultured cells in a sequence-specific manner. The effectiveness of siRNAs varies — the most potent siRNAs result in >90% reduction in target RNA and protein levels (44-46). The most effective siRNAs turn out to be 21 nt dsRNAs with 2 nt 3' overhangs. Sequence specificity of siRNA is very stringent, as single base pair mismatches between the siRNA and its target mRNA dramatically reduce silencing (44, 47). Unfortunately, not all siRNAs with these characteristics are effective. The reasons for this are unclear but may be a result of positional effects (46, 48, 49). For current recommendations on designing siRNAs, see "[siRNA Design](#)".

## RNAi as a Tool for Functional Genomics

Although the history and mechanism of RNAi and PTGS are fascinating, many researchers are most excited about RNAi's potential use as a functional genomics tool. Already RNAi has been used to ascertain the function of many genes in *Drosophila*, *C. elegans*, and several species of plants. With the knowledge that RNAi can be induced in mammalian cells by the transfection of siRNAs, many more researchers are beginning to use RNAi as a tool in human, mouse and other mammalian cell culture systems.

In early experiments with mammalian cells, the siRNAs were synthesized chemically (Ambion is one of several companies that offer [custom siRNA synthesis](#)). Recently, Ambion introduced a kit (the [Silencer™ siRNA Construction Kit](#)) to produce siRNAs by in vitro transcription, which is a less expensive alternative to chemical synthesis, particularly when multiple different siRNAs need to be synthesized. Once made, the siRNAs are introduced into cells via transient transfection. Due to differences in efficacy, most researchers will synthesize 3–4 siRNAs to a target gene and perform pilot experiments to determine the most effective one. Transient silencing of more than 90% has been observed with this type of approach ([44-46](#), [48](#), [49](#)).

So far, injection and transfection of dsRNA into cells and organisms have been the main method of delivery of siRNA. And while the silencing effect lasts for several days and does appear to be transferred to daughter cells, it does eventually diminish. Recently, however, a number of groups have developed expression vectors to continually express siRNAs in transiently and stably transfected mammalian cells ([50-56](#)). Some of these vectors have been engineered to express small hairpin RNAs (shRNAs), which get processed in vivo into siRNAs-like molecules capable of carrying out gene-specific silencing ([50](#), [53](#), [54](#), [56](#)). The vectors contain the shRNA sequence between a polymerase III (pol III) promoter and a 4-5 thymidine transcription termination site. The transcript is terminated at position 2 of the termination site (pol III transcripts naturally lack poly(A) tails) and then folds into a stem-loop structure with 3' UU-overhangs. The ends of the shRNAs are processed in vivo, converting the shRNAs into ~21 nt siRNA-like molecules, which in turn initiate RNAi ([50](#)). This latter finding correlates with recent experiments in *C. elegans*, *Drosophila*, plants and Trypanosomes, where RNAi has been induced by an RNA molecule that folds into a stem-loop structure (reviewed in [3](#)).

Another siRNA expression vector developed by a different research group encodes the sense and antisense siRNA strands under control of separate pol III promoters ([52](#)). The siRNA strands from this vector, like the shRNAs of the other vectors, have 5 thymidine termination signals. Silencing efficacy by both types of expression vectors was comparable to that induced by transiently transfecting siRNA.

The recent studies on RNAi have taken the research world by storm. The ability to quickly and easily create loss-of-function phenotypes has researchers rushing to learn as much as they can about RNAi and the characteristics of effective siRNAs. In the future, RNAi may even hold promise for development of gene-specific therapeutics. Much has been learned about this powerful technique, but additional information becomes available on an almost daily basis (see [The RNA Interference Resource](#) to learn about the very latest RNAi research and tools). It is not an understatement to say that the field of functional genomics is being revolutionized by RNAi.

## References

1. Cogoni C, and Macino G. (2000) Post-transcriptional gene silencing across kingdoms. *Genes Dev* **10**: 638-643. ([Abstract](#))
2. Guru T. (2000). A silence that speaks volumes. *Nature* **404**, 804-808. ([Article](#))
3. Hammond SM, Caudy AA, Hannon GJ. (2001) Post-transcriptional Gene Silencing by Double-stranded RNA. *Nature Rev Gen* **2**: 110-119. ([Abstract](#))

4. Napoli C, Lemieux C, and Jorgensen R. (1990) Introduction of a chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans. *Plant Cell* **2**: 279-289.
5. Jorgensen RA, Cluster PD, English J, Que Q, and Napoli CA. (1996) Chalcone synthase cosuppression phenotypes in petunia flowers: comparison of sense vs. antisense constructs and single-copy vs. complex T-DNA sequences. *Plant Mol Biol* **31**: 957-973. ([Abstract](#))
6. Ingelbrecht I, Van Houdt H, Van Montagu M, and Depicker A. (1994) Posttranscriptional silencing of reporter transgenes in tobacco correlates with DNA methylation. *Proc Natl Acad Sci USA* **91**: 10502-10506. ([Abstract](#), [PDF of Article](#))
7. Cogoni C, Irelan JT, Schumacher M, Schmidhauser T, Selker EU, and Macino G. (1996) Transgene silencing of the *al-I* gene in vegetative cells of *Neurospora* is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation. *EMBO J* **15**: 3153-3163. ([Abstract](#))
8. Palauqui JC, Elmayan T, Pollien JM, and Vaucheret H. (1998) Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J* **16**: 4738-4745. ([Article](#))
9. Guo S, and Kempheus KJ. (1995). *Par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**: 611-620. ([Abstract](#))
10. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, and Mello CC. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**: 806-811. ([Article](#))
11. Timmons, L., and Fire, A. (1998) Specific interference by ingested dsRNA. *Nature* **395**: 854.
12. Timmons L, Court D, and Fire A. (2001) Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**:103-112. ([Abstract](#))
13. Hunter CP. (2000) Shrinking the Black Box of RNAi. *Current Biology* **10**: R137-R140. ([Article](#))
14. Tabara H, Grishok A, and Mello CC. (1998) RNAi in *C. elegans*: soaking in the genome sequence. *Science* **282**: 430-431.
15. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, and Ahringer J. (2000) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biology* **2**: 2.1-2.10. ([Article](#))
16. Grishok A, Tabar H, and Mello CC. (2000) Genetic requirements for inheritance of RNAi in *C. elegans*. *Science* **287**: 2494-2497. ([Abstract](#))
17. Sharp PA, and Zamore PD. (2000) RNA Interference. *Science* **287**: 2431-2433.
18. Sharp PA. RNA Interference-2001. (2001) *Genes Dev* **15**: 485-490.

19. Kennerdell JR, and Carthew RW. (1998) Use of dsRNA-mediated genetic interference to demonstrate that *frizzled* and *frizzled 2* act in the *wingless* pathway. *Cell* **95**: 1017-1026. ([Abstract](#))
20. Kennerdell JR, and Carthew RW. (2000) Heritable gene silencing in *Drosophila* using double-stranded RNA. *Nature Biotech* **18**: 896-898. ([Abstract](#))
21. Dzitoyeva S, Dimitrijevic N, Manev H. (2001) Intra-abdominal injection of double-stranded RNA into anesthetized adult *Drosophila* triggers RNA interference in the central nervous system. *Mol Psychiatry* **6(6)**:665-670.
22. Worby CA, Simonson-Leff N, Dixon JE. (2001) RNA interference of gene expression (RNAi) in cultured *Drosophila* cells. *Sci STKE* **Aug 14, 2001(95)**:PL1.
23. Schmid A, Schindelholz B, Zinn K. (2002) Combinatorial RNAi: a method for evaluating the functions of gene families in *Drosophila*. *Trends Neurosci* **25(2)**:71-74.)
24. Hamilton AJ, Baulcombe DC. (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**: 950-952. ([Abstract](#))
25. Hammond S, Bernstein E, Beach D, and Hannon G. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature*, **404**: 293-298. ([Abstract](#))
26. Zamore PD, Tuschl T, Sharp PA, and Bartel DP. (2000). RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**: 25-33. ([Abstract](#))
27. Hutvagner G, and Zamore PD. (2002) RNAi: nature abhors a double-strand. *Curr Opin Genetics & Development* **12**:225-232.
28. Bernstein E, Caudy AA, Hammond SA, and Hannon GJ. (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**:363-366.
29. Nykanen A, Haley B, and Zamore PD. (2001) ATP requirements and small interfering RNA structure in the RNA interference pathway. *Cell* **107**:309-321.
30. Lipardi C, Wei Q, and Paterson BM. (2001) RNAi as random degradative PCR. siRNA primers convert mRNA into dsRNA that are degraded to generate new siRNAs. *Cell* **107**:297-307.
31. Ketting RF, Haverkamp TH, van Luenen HG, and Plasterk RH. (1999). *Mut-7* of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNase D. *Cell* **99**: 133-141. ([Abstract](#))
32. Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, and Mello CC. (2001) Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* **106**:23-34.
33. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, and Zamore PD. (2001) A cellular function for the

RNA-interference enzyme Dicer in the maturation of the *let-7* small temporal RNA. *Science* **293**(5531):834-838.

34. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, and Plasterk RH. (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* **15**(20):2654-2659.
35. Lagos-Quintana M, Rauhut R, Lendeckel W, and Tuschl T. (2001) Identification of novel genes coding for small expressed RNAs. *Science* **294**:853-858.
36. Lau NC, Lim LP, Weinstein EG, and Bartel DP. (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* **294**:858-862.
37. Lee RC, and Ambrose V. (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* **294**:862-864.
38. Ruvkun G. (2001) Glimpses of a tiny RNA world. *Science* **294**:797-799.
39. Manche L, Green SR, Schmedt C, and Mathews MB. (1992). Interactions between double-stranded RNA regulators and the protein kinase DAI. *Mol. Cell. Biol.* **12**:5238-5248.
40. Minks MA, West DK, Benveniste S, and Baglioni C. (1979). Structural requirements of double-stranded RNA for the activation of 2'-5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. *J. Biol. Chem.* **254**:10180-10183.
41. Yang S, Tutton S, Pierce E, and Yoon K. (2001) Specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells. *Mol. Cell. Biol.* **21**(22):7807-7816.
42. Paddison PJ, Caudy A, and Hannon GJ. (2002). Stable suppression of gene expression by RNAi in mammalian cells. *Proc. Natl. Acad. Sci. USA* **99**(3):1443-1448.
43. Elbashir SM, Lendeckel W, and Tuschl T. (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev* **15**(2):188-200.
44. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, and Tuschl T. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**: 494-498. ([Abstract](#))
45. Caplen NJ, Parrish S, Imani F, Fire A, and Morgan RA. (2001) Specific inhibition of gene expression by small double-stranded RNAs in invertebrates and vertebrate systems. *Proc. Natl. Acad. Sci. USA* **98**: 9746-9747.
46. Holen T, Amarzguioui M, Wiiger M, Babaie E, and Prydz H. (2002) Positional effects of short interfering RNAs targeting the human coagulation trigger Tissue Factor. *Nucleic Acids Research* **30**(8):1757-1766.
47. Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T. (2001) Functional anatomy of siRNA for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J* **20**: 6877-6888.
48. Jarvis RA, and Ford LP. (2001) The siRNA Target Site Is an Important Parameter for Inducing RNAi in Human Cells. *TechNotes* **8**(5): 3-5. ([Article](#))

49. Brown D, Jarvis R, Pallotta V, Byrom M, and Ford L. (2002) RNA Interference in Mammalian Cell Culture: Design, Execution and Analysis of the siRNA Effect. *TechNotes* **9(1)**: 3-5. ([Article](#))
50. Brummelkamp TR, Bernards R, and Agami R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. *Science* **296**:550-553.
51. Lee NS, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P, and Rossi J. (2002). Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nature Biotechnol.* **20**:500-505.
52. Miyagishi M, and Taira K. (2002). U6-promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells. *Nature Biotechnol.* **20**:497-500.
53. Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, and Conklin DS. (2002). Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. *Genes & Dev.* **16**:948-958.
54. Paul CP, Good PD, Winer I, and Engelke DR. (2002). Effective expression of small interfering RNA in human cells. *Nature Biotechnol.* **20**:505-508.
55. Sui G, Soohoo C, Affar E-B, Gay F, Shi Y, Forrester WC, and Shi Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. *Proc. Natl. Acad. Sci. USA* **99(6)**:5515-5520.
56. Yu J-Y, DeRuiter SL, and Turner DL. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. *Proc. Natl. Acad. Sci. USA* **99(9)**:6047-6052.

## Additional Resources

### RNA interference

[www.nature.com/nature/fow/000316.html](http://www.nature.com/nature/fow/000316.html)

### Flash Animation: How Does RNAi Work?

Hammond, S.M., Caudy, A.A., Hannon, G.J. (2001) Post-transcriptional Gene Silencing by Double-stranded RNA. *Nature Rev Gen* **2**: 110-119.

[www.nature.com/nrg/journal/v2/n2/animation/nrg0201\\_110a\\_swf\\_MEDIA1.html](http://www.nature.com/nrg/journal/v2/n2/animation/nrg0201_110a_swf_MEDIA1.html)

**Cosuppression** - Silencing of an endogenous gene caused by the introduction of a transgene or infection by a virus. This term, which can refer to silencing at the post-transcriptional (PTGS) or transcriptional (TGS) level, has been primarily adopted by researchers working with plants.

**Post-transcriptional Gene Silencing (PTGS)** - Silencing of an endogenous gene caused by the introduction of a homologous dsRNA, transgene or virus. In PTGS, the transcript of the silenced gene is synthesized but does not accumulate because it is rapidly degraded. This is a more general term than RNAi, since it can be triggered by several different means.

**Quelling** - PTGS in *Neurospora crassa* induced by the introduction of a transgene.

**RISC** - RNA-induced silencing complex. A nuclease complex, composed of proteins and siRNA (see below), that targets and destroys endogenous mRNAs complementary to the siRNA within the complex.

**RNA interference (RNAi)** - Post-transcriptional gene silencing (PTGS) induced by the direct introduction of dsRNA. The term "RNA interference" was first used by researchers studying *C. elegans*.

**siRNAs** - Small interfering RNAs. Current models of PTGS indicate that these 21-23 nucleotide dsRNAs mediate PTGS. Introduction of siRNAs can induce PTGS in mammalian cells. siRNAs are apparently produced in vivo by cleavage of dsRNA introduced directly or via a transgene or virus. Amplification by an RNA-dependent RNA polymerase (RdRP) may occur in some organisms. siRNAs are incorporated into the RNA-induced silencing complex (RISC), guiding the complex to the homologous endogenous mRNA where the complex cleaves the transcript.

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/>