**RNA Interference**

* In 1998, Andrew Fire, Craig Mello, and their colleagues observed a strange phenomenon.
* They were inhibiting the expression of genes in the nematode *Caenorohabditis elegans* by inserting single- stranded RNA molecules that were complementary to a gene’s DNA sequence. Called antisense RNA, such molecules are known to inhibit gene expression by binding to the mRNA sequences and inhibiting translation.
* Fire, Mello, and colleagues found that more potent gene silencing was triggered when double stranded RNA was injected into the animal.
* **For their discovery of RNA interference, Fire and Mello were awarded with the Nobel Prize in physiology or medicine in 2006.**
* It is an Important Mechanism of Gene Regulation. The expression of a number of eukaryotic genes is controlled through RNA interference, also known as RNA silencing and post transcriptional gene silencing.
* Research suggests that as much as 30% of human genes are regulated by RNA interference. RNA interference is widespread in eukaryotes, existing in fungi, plants, and animals.
* This mechanism is also used as a powerful technique for artificially regulating gene expression in genetically engineered organisms.

**Small Interfering RNAs and MicroRNAs**

* RNA interference is triggered by microRNAs (miRNAs) and small interfering RNAs (siRNAs), depending on their origin and mode of action.
* An enzyme called Dicer cleaves and processes double stranded RNA to produce single stranded siRNAs or miRNAs that are from 21 to 25 nucleotides in length and pair with proteins to form RNA induced silencing complex (RISC).
* The RNA component of RISC then pairs with complementary base sequences of specific mRNA molecules, most often with sequences in the 31 UTR of the mRNA.
* Small interfering RNAs tend to base pair perfectly with the mRNAs, whereas miRNAs often form less than perfect pairings.

**Mechanisms of Gene Regulation by RNA Interference**

Small interfering RNAs and microRNAs regulate gene expression through at least four distinct mechanisms:

(1) Cleavage of mRNA

(2) Inhibition of translation

 (3) Transcriptional silencing

(4) Degradation of mRNA

**(1) mRNA CLEAVAGE**

* RISCs that contain an siRNA (and some that contain an miRNA) pair with mRNA molecules and cleave the mRNA near the middle of the bound siRNA.
* This cleavage is carried out by a protein that is sometimes referred to as ''Slicer’. After cleavage, the mRNA is further degraded.
* Thus, the presence of siRNAs and miRNAs increase the rate at which mRNAs are broken down and decrease the amount of protein produced.

**(2) INHIBITION OF TRANSLATION**

* Some miRNAs regulate genes by inhibiting the translation of their complementary mRNAs.
* For example, an important gene of flower development in *Arabidopsis thaliaua* is *APETALA2.* The expression of this gene is regulated by a miRNA that base pairs with nucleotides in the coding region of *APETALA2* mRNA and inhibits its translation.
* The exact mechanism by which miRNAs repress translation is not known, but some research suggests that it can inhibit both the initiation step of translation and steps after translation initiation such as those causing premature termination.
* Many miRNAs have multiple miRNA binding sites, and translation is most efficiently inhibited when mlultiple miRNAs are bound to the mRNA.

**(3) TRANSCRIPTIONAL SILENCING**

* Other siRNAs silence transcription by altering chromatin structure.
* These siRNAs combine with proteins to form a complex called RITS, which is analogous to RISC.
* The siRNA component of RITS then binds to its complementary sequence in DNA or an RNA molecule in the process of being transcribed and represses transcription by attracting enzymes that methylate the tails of histone proteins.
* The addition of methyl groups to the histones causes them to bind DNA more tightly, restricting the access of proteins and enzyn1es necessary to carry out transcription.
* Some miRNAs bind to complementary sequences in DNA and attract enzymes that methylate the DNA directly, which also leads to the suppression of transcription.

**(4) SLICER·INDEPENDENT DEGRADATION OF mRNA**

* A final mechanism by which miRNAs regulate gene expression is by triggering the decay of mRNA in a process that does not require Slicer activity.
* For example, a short lived mRNA with an AU-rich element in its *3’* UTR is degraded by an RNA-silencing mechanism.
* Researchers have identified an miRNA with a sequence that is complementary to the consensus sequence in the AU-rich element.
* This miRNA binds to the AU-rich element and, in a way that is not yet fully understood, brings about the degradation of the mRNA in a process that requires Dicer and RISC.

