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Overview of RNA Interference Technology

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RNA interference is gene knockdown. A cellular mechanism for degrading undesirable RNAs from the cytoplasm without effect in the nucleus. It is way for cell to defend itself.

- **Who discovered RNA interference?**

American scientists Craig Mello and Andrew Fire discovered RNA interference (RNAi) in 1998 and were awarded the 2006 Nobel Prize in Physiology or Medicine for their discovery.

- **Basics of RNAi**

✓ Gene silencing is a technique that aims to reduce or eliminate the production of a protein from it's corresponding gene.
✓ It generally describes the "switching off" of a gene by a mechanism other than genetic modification.
✓ That is, a gene which would be expressed ("turned on") under normal circumstances is switched off by machinery in the cell.
✓ It occurs when RNA is unable to make a protein during translation.
✓ Gene silencing is same as gene knock down but is totally different from gene knock out.
✓ When genes are knocking down, there expression is reduced, where in contrast when genes are knocked out; they are completely erased from organism's genome and thus have no expression.

- **How does it work?**

✓ This is accomplished by binding a specific strand of RNA to an existing m-RNA strand.
✓ The m-RNA creates a copy of DNA strand.
✓ By binding the RNA to the m-RNA, m-RNA is prevented from replicating that portion of the DNA.
✓ Specific genes can be targeted and prevented from replicating in to new DNA strands.

- **Types of Gene silencing**

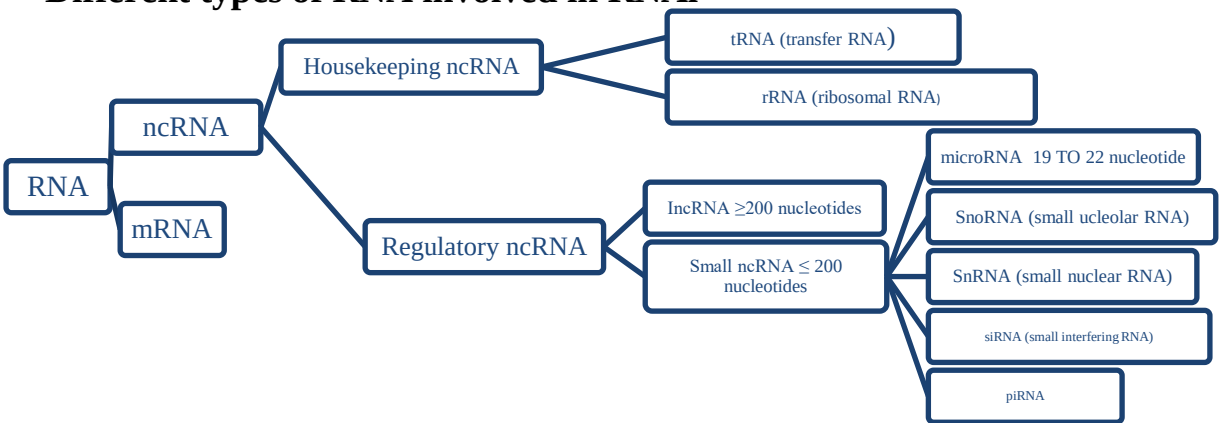
Genes are regulated either from the transcriptional or post-transcriptional level, so silencing can occur at transcriptional level or post-transcriptional level.
There are mainly two types of gene silencing
1. Transcriptional gene silencing-1.
2. Post transcriptional gene silencing or RNA interference (RNAi)

- **Why is gene silencing treatment needed?**

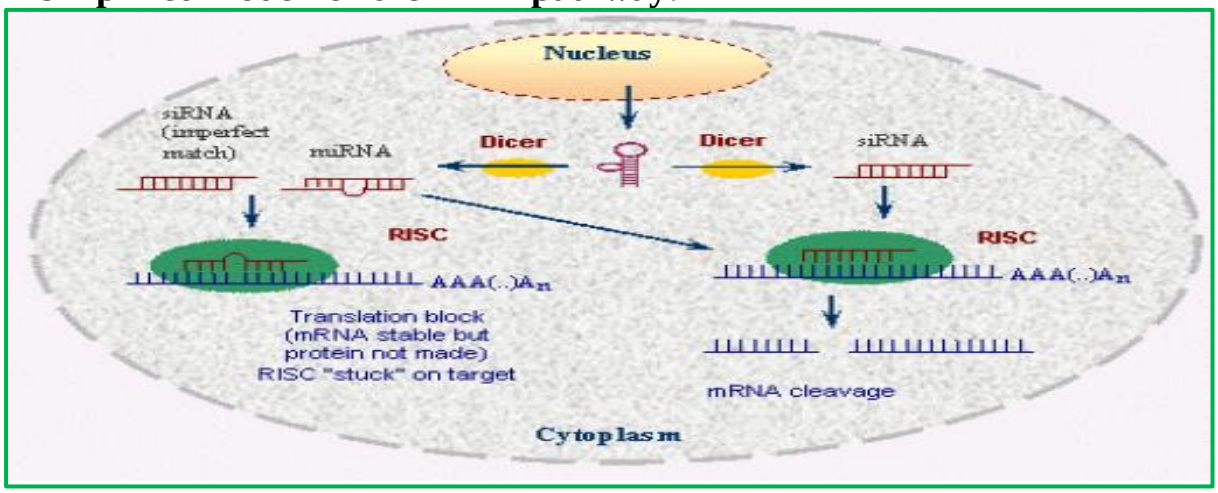
✓ Sometimes the cell production process can become faulty and mistakes are made. For example, specific elements of DNA may be removed out of the genetic instructions, causing cells to mutate (change).

- ✓ Cells may produce too much of a particular protein or the protein it produces does not work correctly. This can lead to a variety of genetic diseases, including cystic fibrosis, Huntington's disease, thalassemia, and some types of cancer. Some of these diseases are difficult to treat using conventional approaches.
- ✓ Gene silencing treatment offers a potential solution for conditions with a genetic cause.

- **Different types of RNA involved in RNAi**



A simplified model for the RNAi pathway:



1. A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short, interfering RNA (siRNA) by the RNase II enzymes Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC).
2. The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. Gene silencing is a result of nucleolytic degradation of the targeted mRNA by the RNase H enzyme Argonaute (Slicer).
3. If the siRNA/mRNA duplex contains mismatches the mRNA is not cleaved. Rather, gene silencing is a result of translational inhibition.
4. The nature has evolved an efficient mechanism for gene silencing through the use of small 21-25 nucleotide long, non-coding RNAs that are double stranded and bind to the target RNAs in a sequence-specific manner.

Pathways involved in RNA Induced Gene Silencing

There are three main classes of small RNA namely

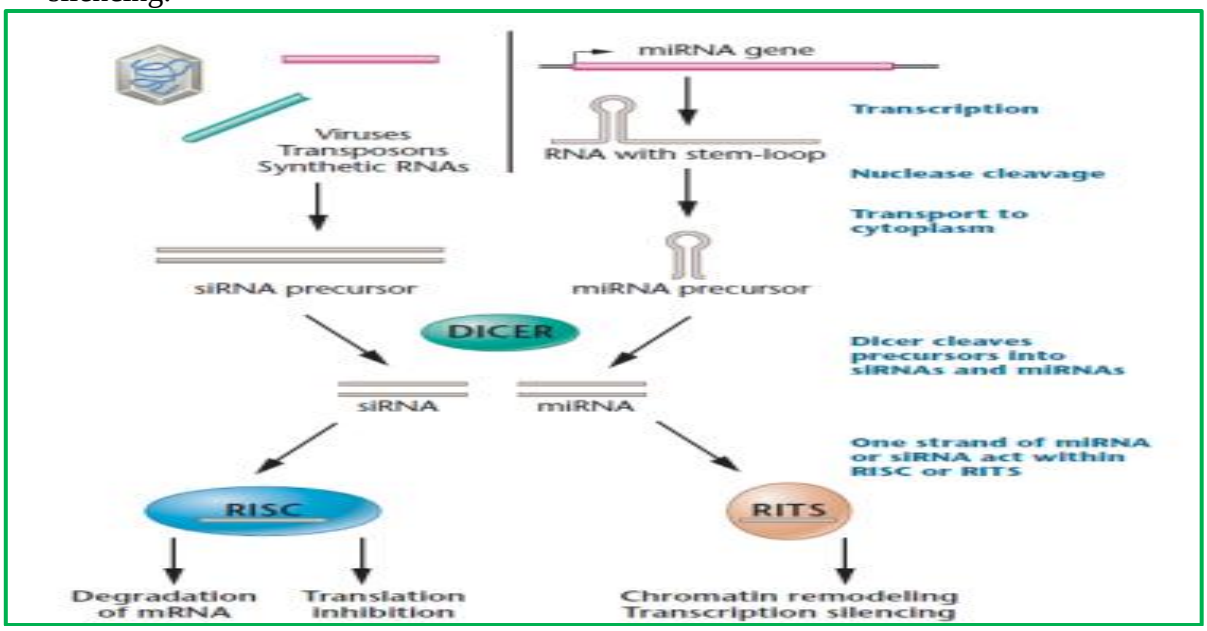
1. Short interfering RNAs (siRNAs)
2. MicroRNAs (miRNAs)

3. PIWI-interacting RNAs (piRNAs)

Although these three RNA's arise from different sources, their mechanisms of action are similar.

The RNA interference pathway takes several steps.

1. First of all either the siRNA or them iRNA molecules associate with the RNA-induced silencing complex (RISC).
2. Secondly within RISC, the short double-stranded RNA is denatured followed by the degradation of the sense strand.
3. Thirdly the RNA/RISC complex becomes fully functional and highly specific by finding mRNA molecules that have a sequence complementary to the antisense RNA present in the RISC.
4. At this juncture RNAi pathway proceeds in two different directions depending upon the complementarity of target mRNA to the antisense mRNA present in the RISC.
5. If the sequence of the target mRNA is perfectly complementary to antisense RNA in the RISC then the RISC will cleave the mRNA which is subsequently degraded by ribonucleases. But if sequence of the target mRNA is not exactly complementary to that of the antisense RNA within the RISC then the RISC complex binds to the mRNA, which represses its translation.
6. RNAi is a powerful biological mechanism for silencing the gene expression by either affecting the stability or the translation of mRNA; heterochromatinization of the genome leading to repression of gene transcription. These functions are performed by associating with the RNA-induced initiation of transcription silencing complex (RITS).
7. The antisense RNA strand within the RITS directs the RITS complex to definite gene promoters or to the larger regions of chromatin. The function of RITS is to recruit the chromatin remodeling enzymes to these genomic regions which in turn methylate histones and DNA, causing heterochromatin formation and subsequent transcriptional silencing.



Gene regulatory mechanisms by RNA induced gene silencing Source: Klug, W. S., and Cummings, M. R. 2010. Concepts of Genetics. 10th Edition. Pearson Education Pte. Ltd.

Advantages and Disadvantages of RNAi

Advantages of RNAi :

1. Downregulation of gene expression simplifies "knockout" analysis.
2. Powerful tool for analyzing unknown genes in sequenced genomes.

3. Inducting viral resistance.
4. Oligonucleotide can be manufactured quickly, some within one week; the sequence of the mRNA is all that needed.
5. Useful approach in future gene therapy.
6. Blocking expression of unwanted genes and undesirable substance.
7. This type of screening has the promise to be more efficient and have greater potential. Redundancies can be discovered by targeting a group of genes that are related in sequence.

Disadvantages of RNAi :

1."High pressure injection" electroporation can cause significant injection damage to the integrity of the normal tissue, organs and thus preclude the utilization in a clinical set-up.
2. Liposomes/cationic encapsulated SiRNA may also be toxic to the host and may cause severe host severe host immune responses.
3. Others emerging strategies includes chemical modification of SiRNA molecules and encapsulated with different molecules are still ln their infancy and need to be thoroughly investigated before used in therapeutic applications.
4. Lipid base nanoparticles are toxicity at high dose, preparation is difficult and low transformation efficiency.
5. Hybrid nanoparticles toxicity have very high doses.

Application of RNAi

- Plant architecture alteration
- Alteration of biotic and abiotic susceptible genes
- Transgenic plants
- Nutritional improvement, removal of toxic compound, prolongation of shelf life
- Modulation of flower colour and scent
- Seedless fruit development
- Development of male sterile plant
- Delection of allergens
- Insecticide
- Antiviral RNAi therapy
- Cancer RNAi technology

Conclusion

RNA interference (RNAi) is a cellular process that silences specific genes by degrading their mRNA or inhibiting its translation. Discovered by Craig Mello and Andrew Fire in 1998, RNAi has applications in plant biotechnology (e.g., enhancing traits, pest resistance) and medicine (e.g., treating genetic disorders and cancers). While it offers advantages like rapid design and potential for gene therapy, challenges include delivery system limitations and potential toxicity.

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