Mechanisms Involved in Gene Silencing

GENE SILENCING

Gene silencing is the process a cell uses to reduce or block the expression of a specific gene. This can happen during transcription or translation. The idea is simple: the gene is still there, but the cell chooses not to use it.

Gene-silencing strategies are now central to therapeutics. They are being explored for cancer, viral infections, metabolic disorders, and neurological diseases, where turning down a harmful gene can change a disease's course.

Gene knockdown

Gene silencing and gene knockdown are frequently equated.

The two terms are often used interchangeably, but there is a difference.

- **Gene silencing:** reduces gene expression, usually by 70 percent or more.
- **Gene knockout:** removes the gene entirely from the genome.

Silencing is therefore a **knockdown**, not a deletion. The gene remains present but largely inactive.

History

- The modern field began in 1998, when Andrew Fire and Craig Mello showed that introducing double-stranded RNA (dsRNA) into a cell triggers a powerful gene-silencing pathway.

- Their discovery of RNA interference (RNAi) earned them the 2006 Nobel Prize.

Types of Gene Silencing

Silencing can be triggered either at the transcriptional level or the post-transcriptional level since genes can be controlled at any of these levels. Gene silencing generally comes in two flavors.

1. Transcriptional gene silencing

- Promoters are being silenced
- Hypermethylated genes residing the specific region i.e., promotor.
- Goal: to boost viral immunity

2. Post transcriptional gene silencing

- Promoter are activated
- Hypermethylation of the coding region associated genes
- Viral immunity as a goal

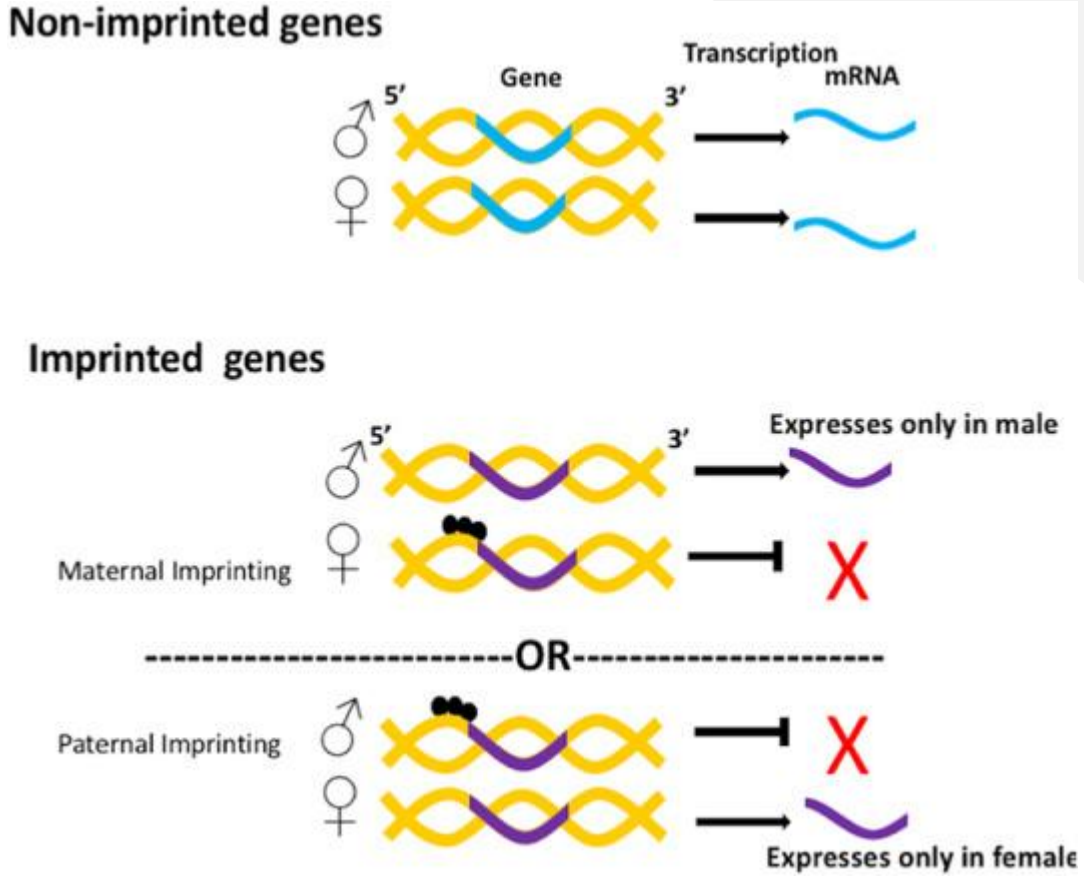
Transcriptional Gene Silencing

It is brought on by histone changes, which result in a heterochromatin environment surrounding a gene and prevent the transcriptional machinery (RNA polymerase, transcription factors, etc.) from accessing it.

1. Genomic Imprinting
2. Paramutation
3. Transposon silencing (or Histone Modifications)
4. Transgene silencing
5. Position effect

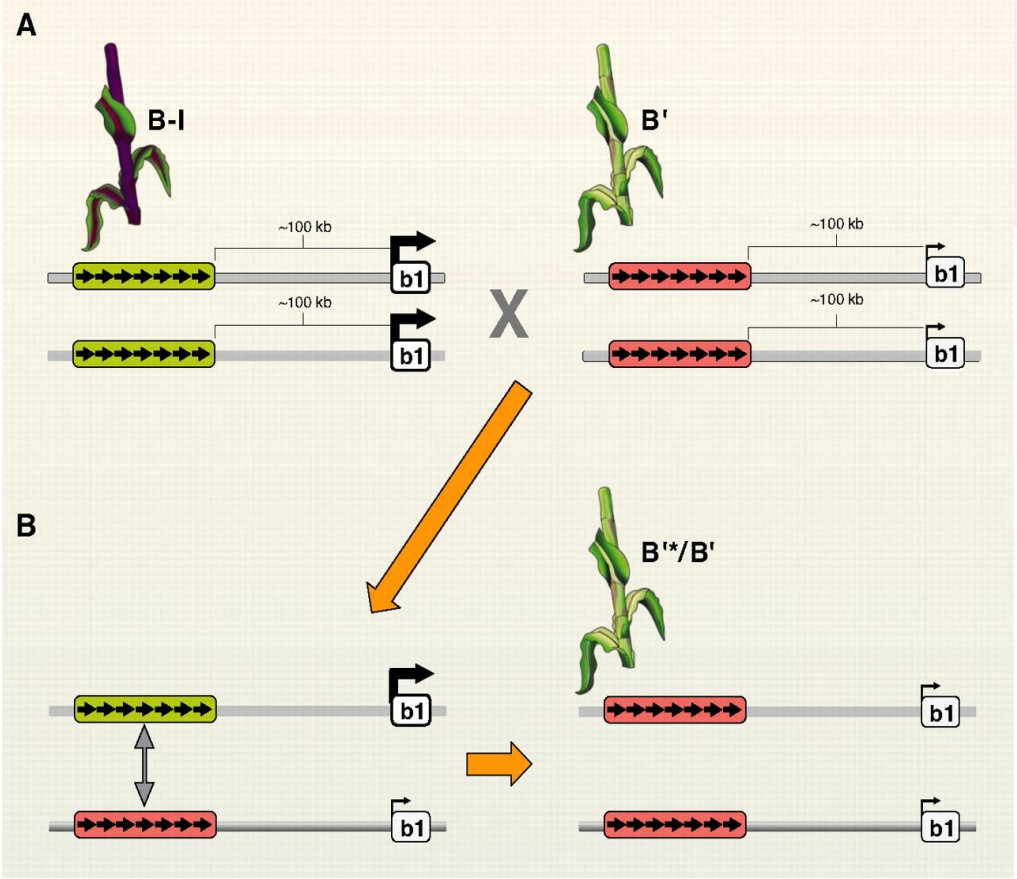
1.Genomic Imprinting

- Some genes are expressed only from the maternal or paternal allele. The other allele is epigenetically silenced.
- Imprinting occurs in plants, insects, and mammals and represents a specialized form of non-Mendelian inheritance.



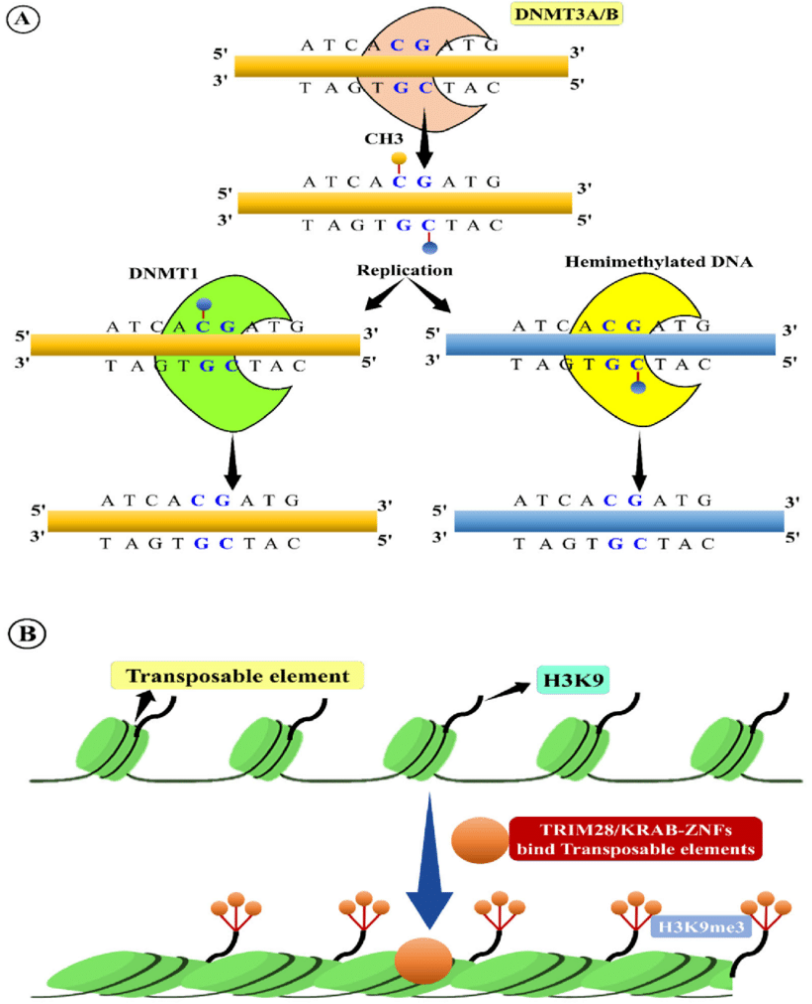
2. Paramutation

- One allele at a locus can alter the expression pattern of the other allele in a heritable way.
- The classic example comes from maize, where kernel pigmentation changes due to this interaction.



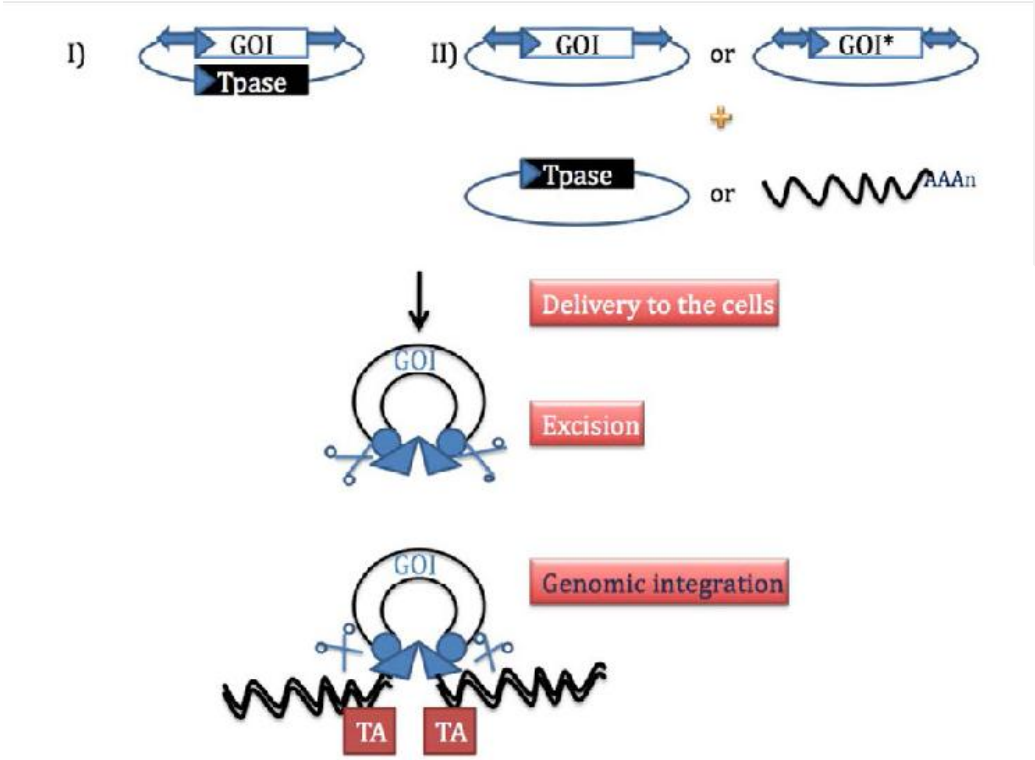
3. Transposon silencing (or Histone Modifications)

- Transposable elements can destabilize the genome by inserting into genes.
- Cells silence them via DNA methylation and histone modifications.
- Uncontrolled transposons are linked to disorders such as SCID, hemophilia, and certain cancers.



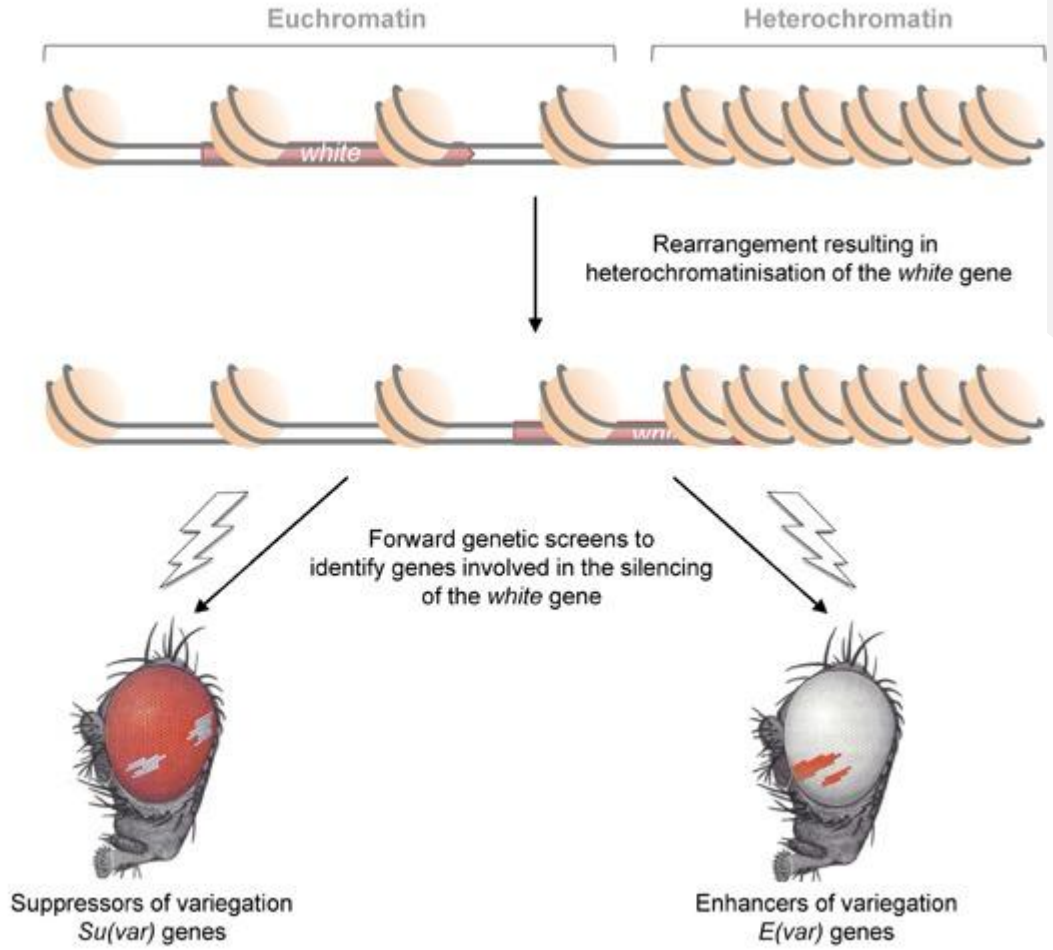
4. Transgene silencing

- Inserted transgenes sometimes land in inactive chromatin or become unstable, causing their expression to fade over time.
- This is a major challenge in plant and animal biotechnology.
- For instance, tomato softening is slowed by lowering polygalactouronase expression.



5. Position effect

- A gene's expression can change when its chromosomal location changes.
- In Drosophila, for example, the white-eye gene shows variegation when moved near heterochromatin.



Post-Transcriptional Gene Silencing

The capacity of exogenous or occasionally endogenous RNA to inhibit the expression of the gene whose sequence matches an m-RNA.

1. RNA interference
2. Nonsense mediated decay
3. Anti sense RNA technology

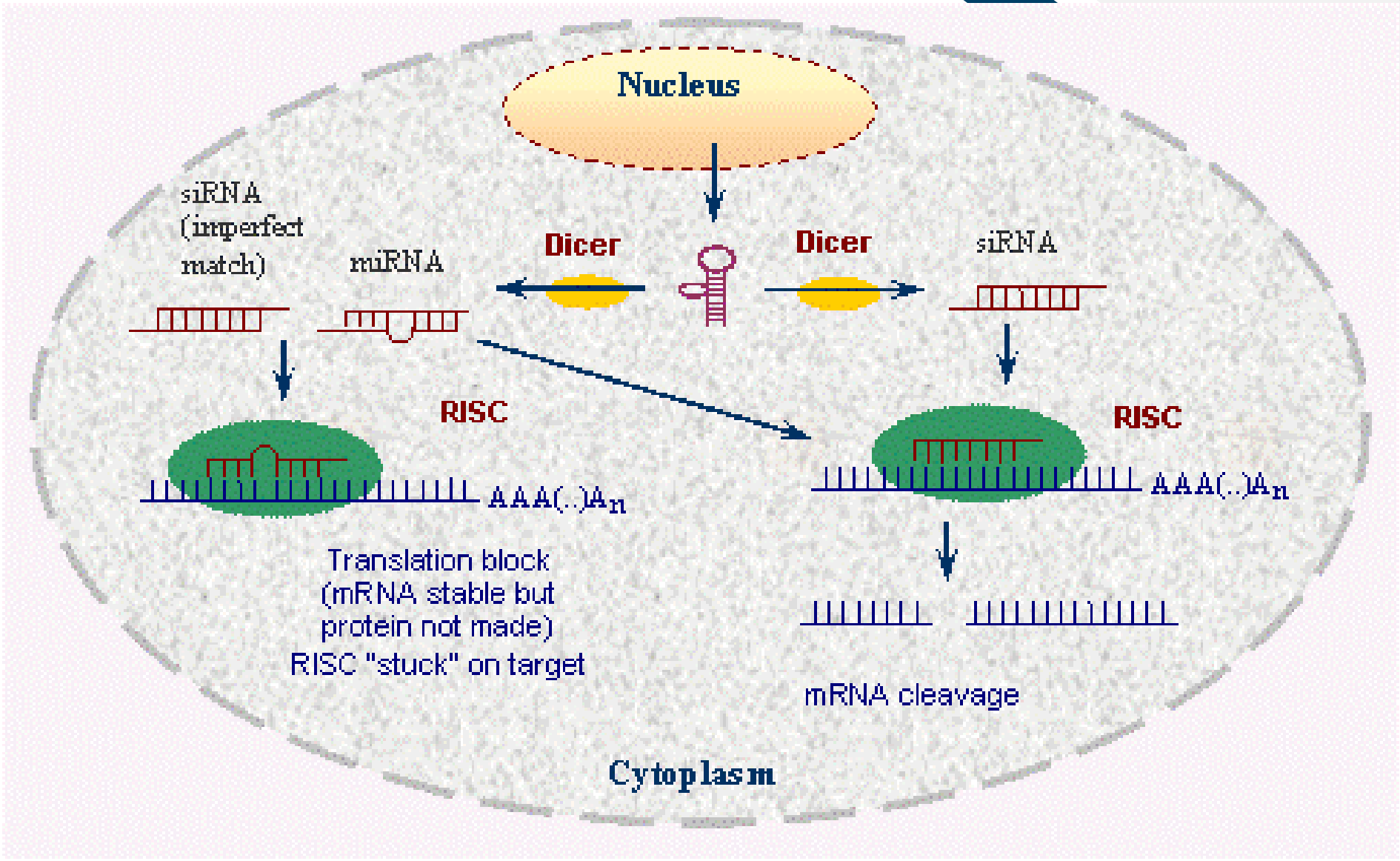
1. RNA interference

- RNAi begins when dsRNA enters a cell. The enzyme **Dicer** cuts this dsRNA into small interfering RNAs (siRNAs).

- These siRNAs guide the **RISC complex** to complementary mRNA, leading to either mRNA cleavage or translation block.

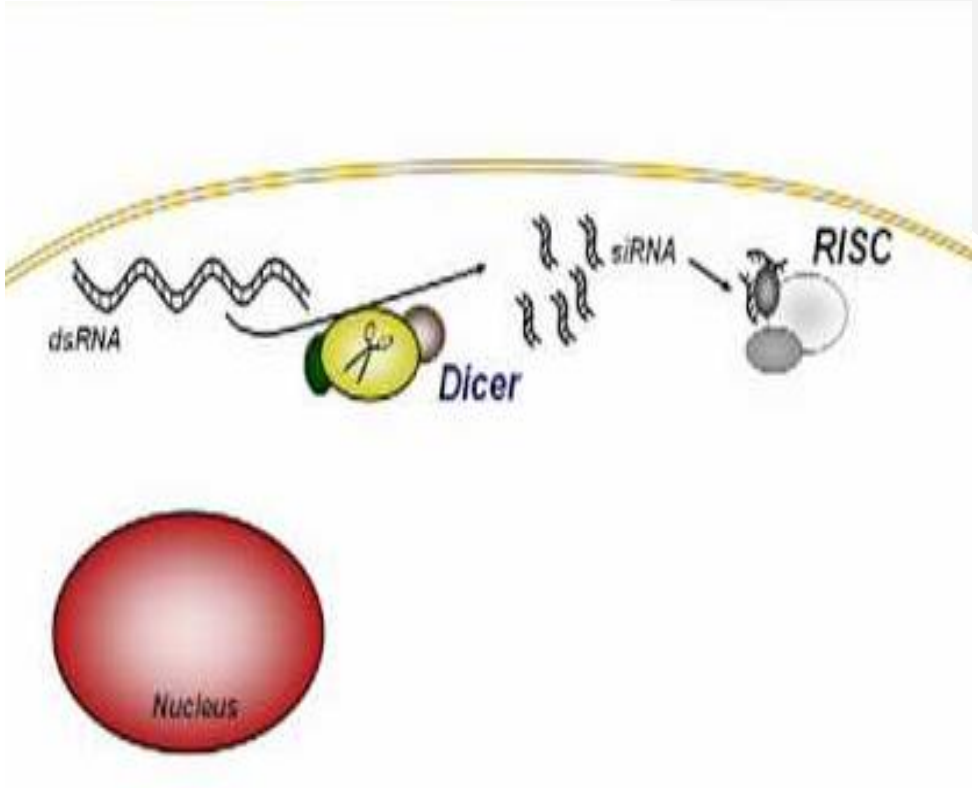
Key points:

•RNAi is highly specific.
•It functions across eukaryotes.
•Roughly 30 percent of human genes are estimated to be regulated by small RNAs.


Nucleus
siRNA
(imperfect
match)
miRNA
Dicer
Dicer
siRNA
RISC
RISC
AAA(..)An
AAA(..)An
Translation block
(mRNA stable but
protein not made)
RISC "stuck" on target
mRNA cleavage
Cytoplasm

Dicer

- A dsRNA-processing enzyme that produces uniform siRNAs and initiates RNAi.
- It is ATP-dependent and structurally related to RNase III enzymes.



Si RNA (Small interfering RNA)

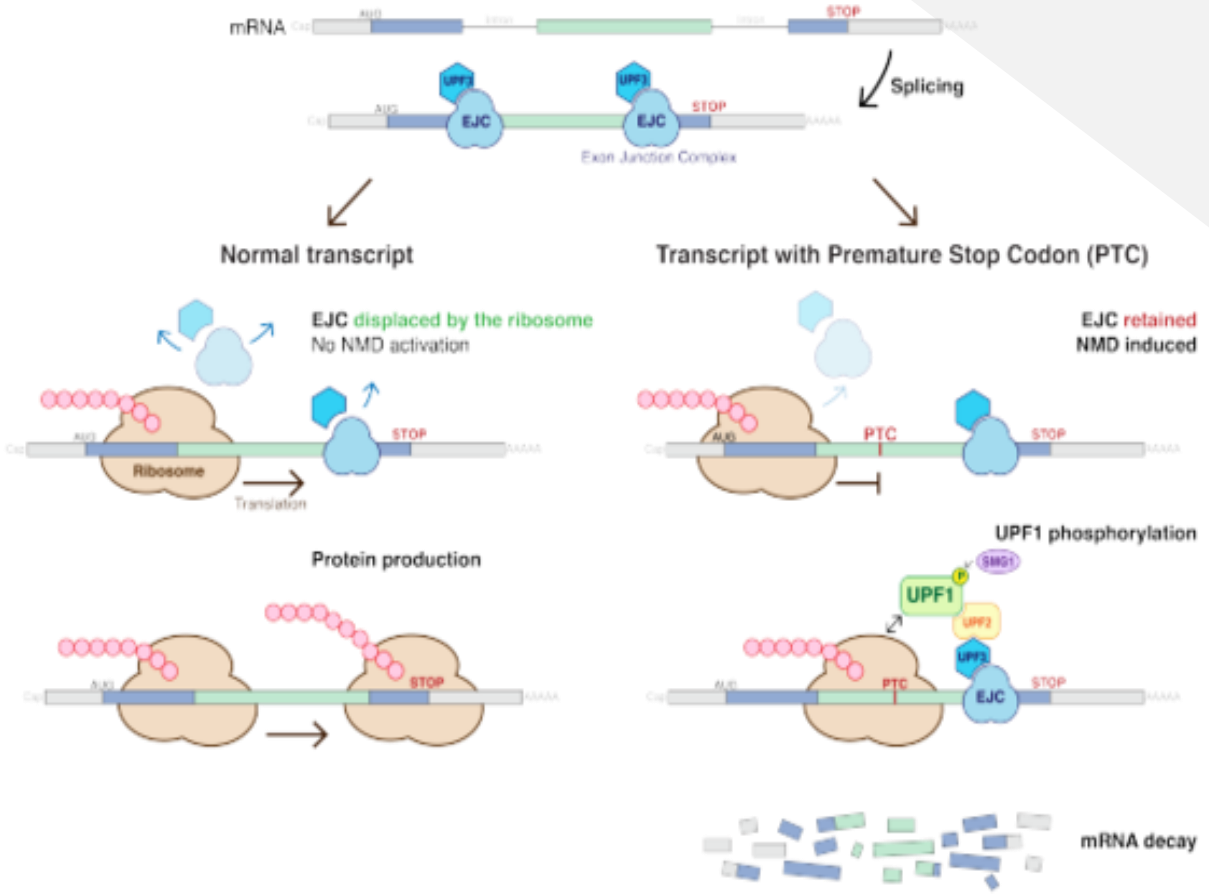
- Short (≈21 nt) duplex RNAs that direct RISC to perfectly matching mRNA targets.
- The end result is targeted mRNA degradation.

Mi RNA (Micro RNA)

- MicroRNAs originate from endogenous hairpin precursors.
- 70 nt hairpin precursor (pre-mi RNA)
- They often bind imperfectly to their targets, leading to translational repression rather than direct cleavage.

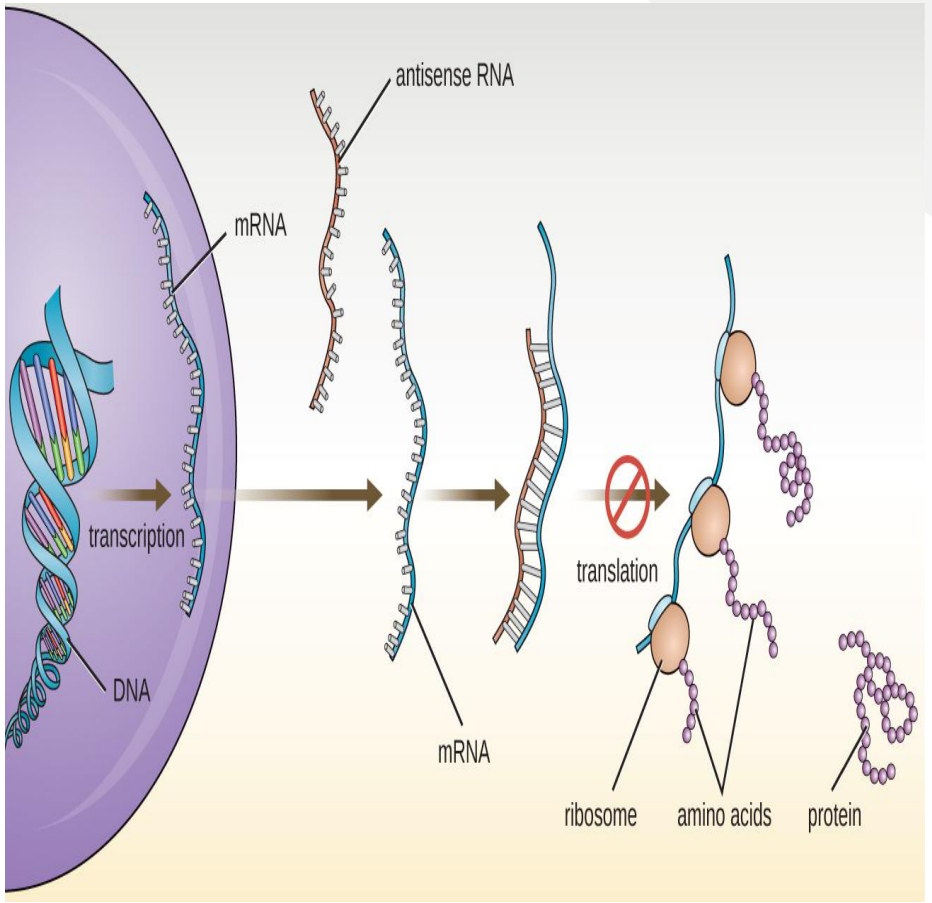
2. Nonsense Mediated Decay

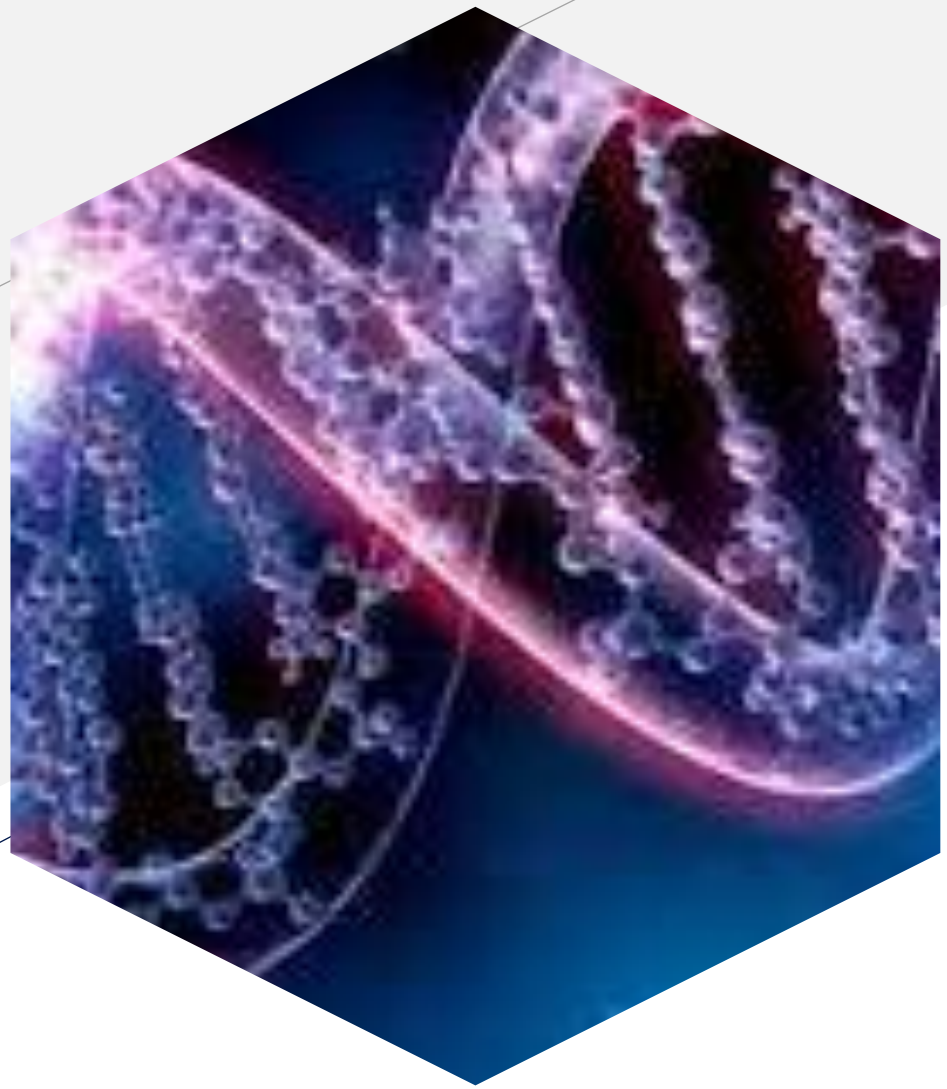
- NMD detects mRNAs containing premature stop codons and prevents the production of incomplete or incorrect proteins.
- Exon-junction complexes (EJCs) left behind during mRNA splicing help the cell recognize aberrant transcripts.



3. Anti sense RNA technology

- Antisense RNA is complementary to a target mRNA.
- When both are present in the same cell, they form a duplex that blocks mRNA processing or translation.
- This approach is widely used in plant biotechnology and is the basis for several antisense drugs.





Thank You.