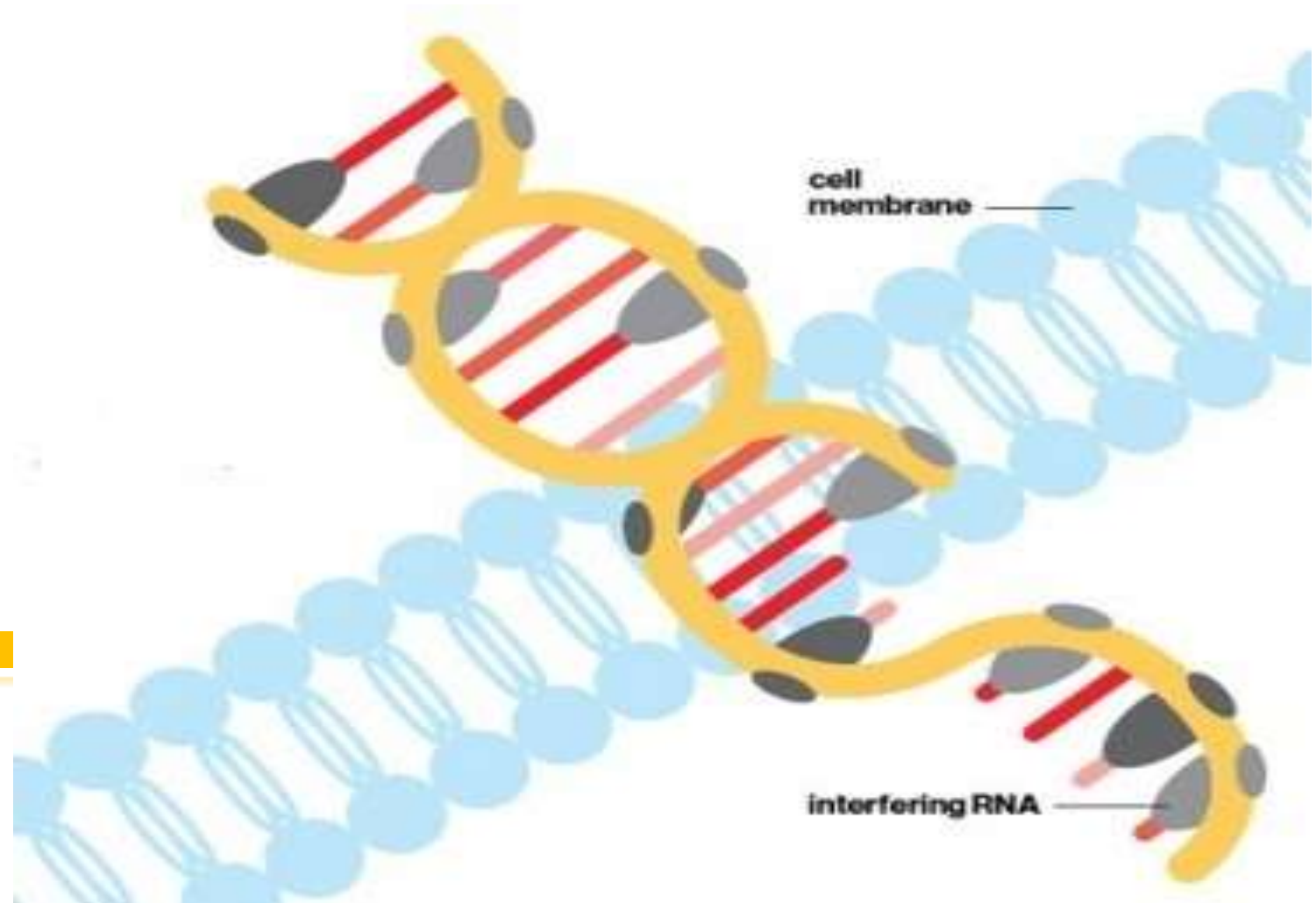


GENE SILENCING



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Introduction

- Gene silencing is a technique that aims to reduce or eliminate the production of a protein from its 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 knock down, their expression is reduced, whereas in contrast when genes are knocked out, they are completely erased from organism’s genome and thus have no expression.

Short history of gene silencing

- 1990 Jorgensen:
- To deepen the pigmentation in petunias Introduction of transgenes homologous to endogenous genes often resulted in plants with both gene suppressed called **co suppression**.
- Resulted in degradation of the endogenous and transgene mRNA.
- 1995 Guo and Kemphues:
- injection of either antisense or sense RNAs in the germline of *C.elegans* was equally effective at silencing at homologous target genes.
- 1998 Mello and Fire:
- Extension of above experiments, combination of sense and antisense RNA(=dsRNA) was 10 times more effective than single strand RNA.

- The discovery of the mechanism of RNA interference by ds RNA by prof. Andrew Fire and prof. Craig Mello in 1998, gave them the Nobel prize in 2006.

- **HOW does it works?**
 1. This is accomplished by binding a specific strand of RNA to an existing m-RNA strand.
 2. The m-RNA creates a copy of DNA strand.
 3. By binding the RNA to the m-RNA, m-RNA is prevented from replicating that portion of the DNA.
 4. Specific genes can be targeted and prevented from replicating in to new DNA strands.

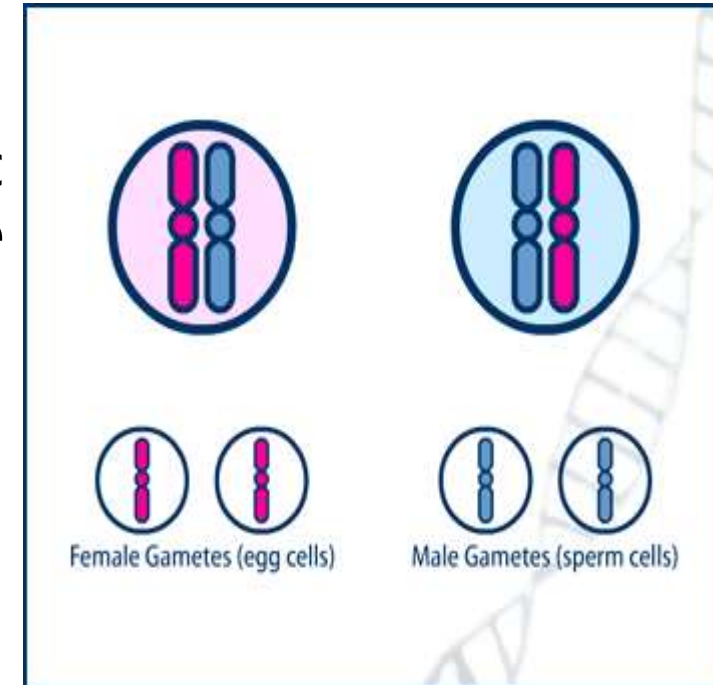
Types of Gene silencing

- genes are regulated at either the transcriptional level or post-transcriptional level, therefore silencing can be induced either at transcriptional level or post-transcriptional level.
- There are mainly two types of gene silencing
 1. Transcriptional gene silencing
 2. Post transcriptional gene silencing

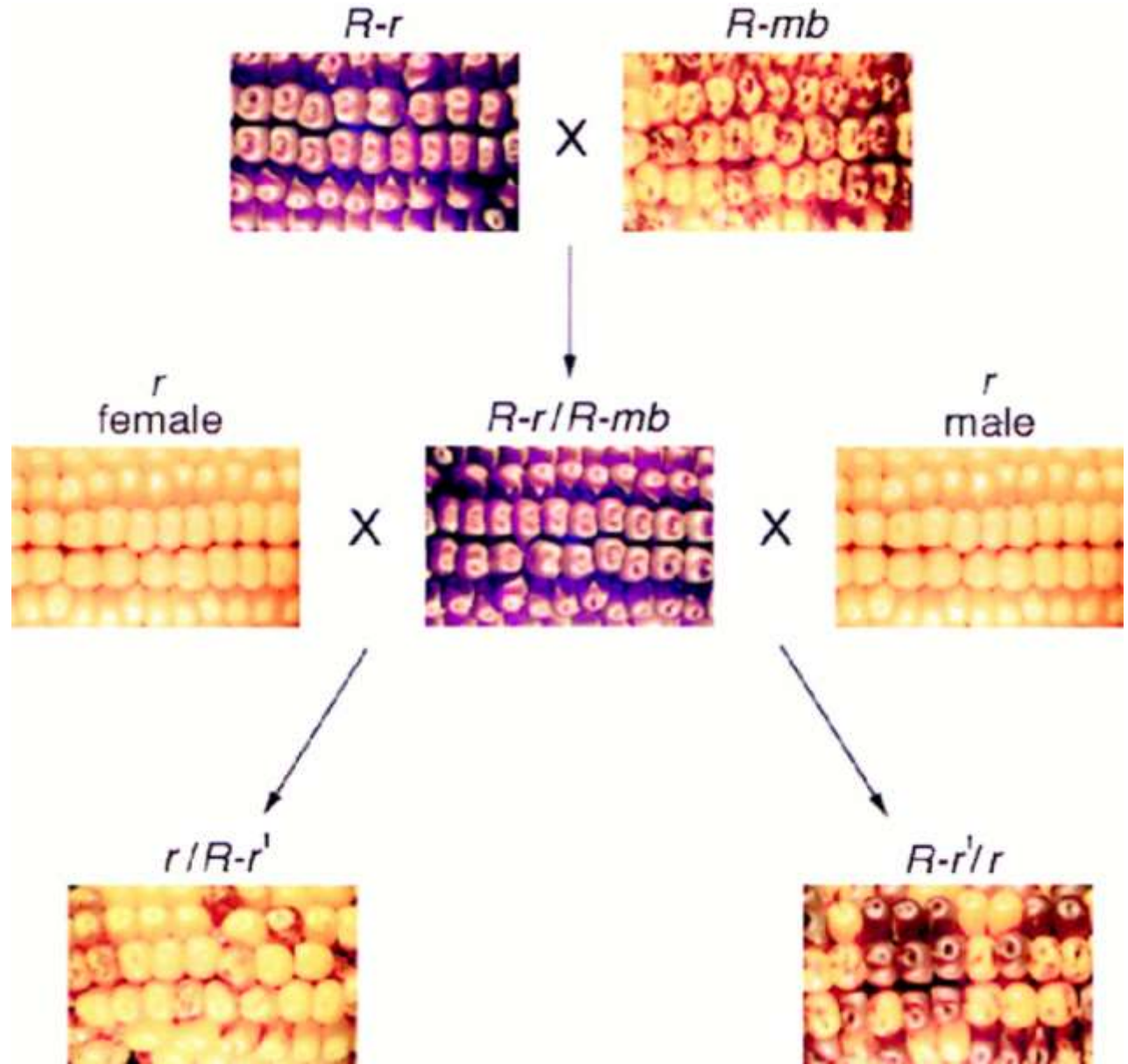
Transcriptional gene silencing	Post transcriptional gene silencing
Promoter silenced	Promoter active
Genes hypermethylated in promoter region	Genes hyper methylated in coding region
Purpose – viral immunity	Purpose - viral immunity

Transcriptional gene silencing

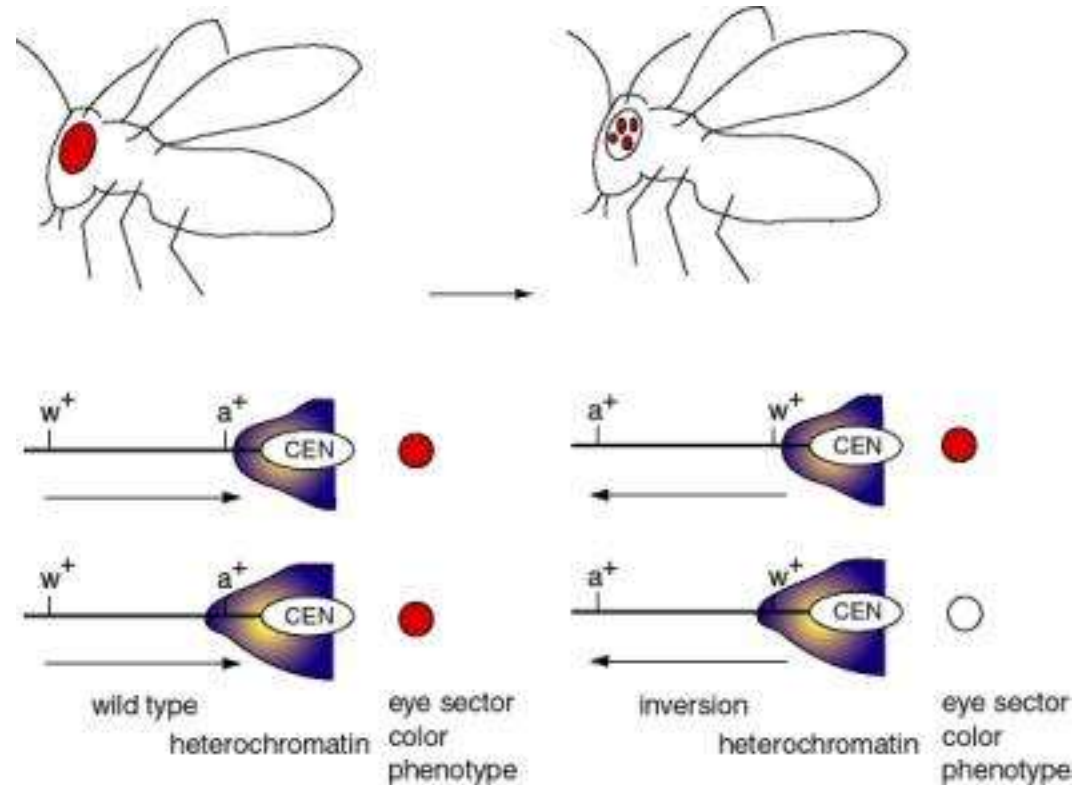
- is the result of histone modifications, creating an environment of heterochromatin around a gene that makes it inaccessible to transcriptional machinery (RNA polymerase, transcription factors, etc.).
- **Genomic imprinting:**
- genomic imprinting is a genetic phenomenon by which certain genes are expressed in a parent-of-origin-specific manner. It is an inheritance process independent of the classical Mendelian inheritance.
- genomic imprinting have been demonstrated in insects, mammals and flowering plants.



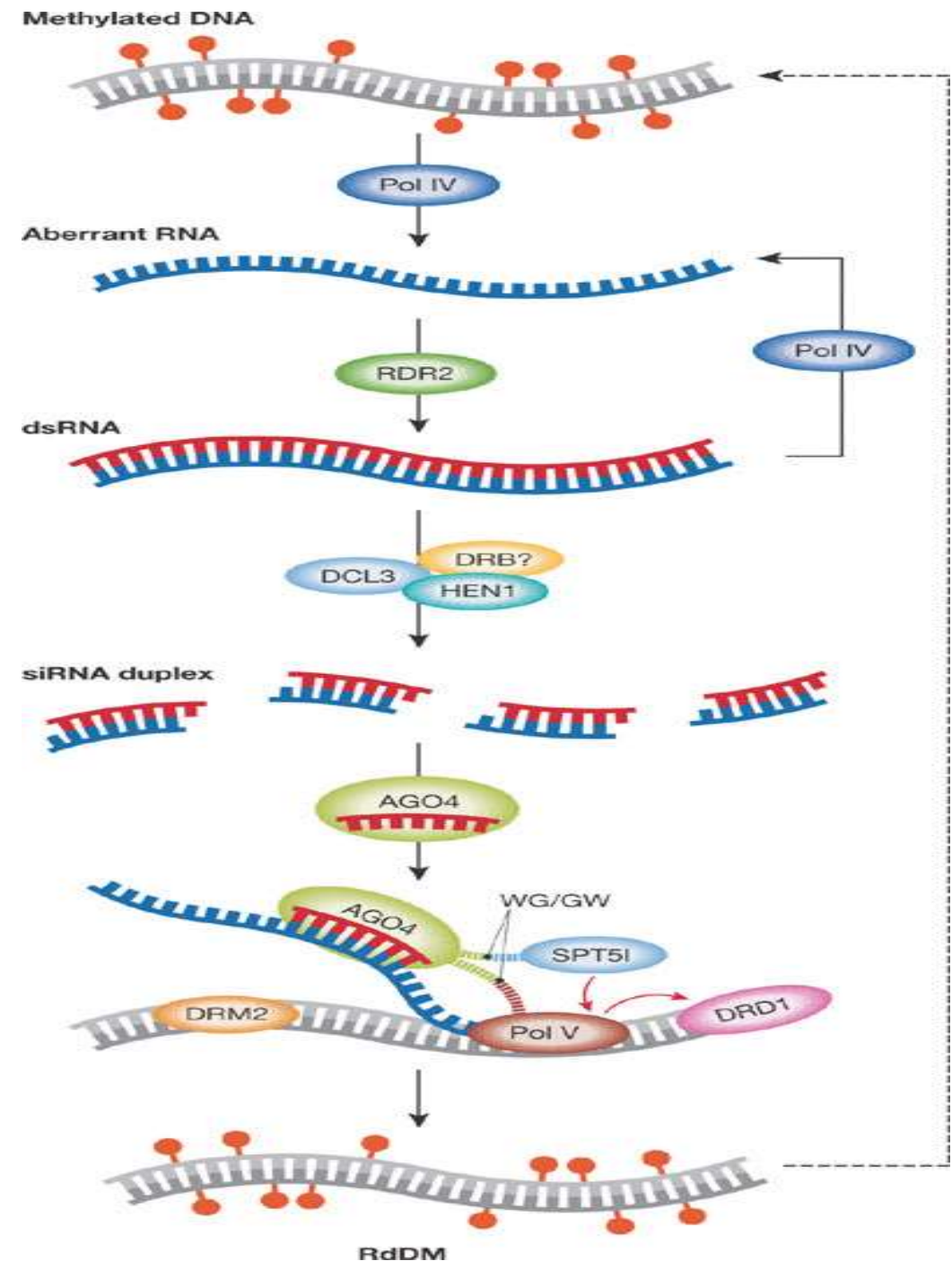
- **Paramutation:**
- paramutation is an interaction between two alleles of a single locus, resulting in a heritable change of one allele that is induced by the other allele.
- Paramutation was first observed by the effect it had on the colour of corn kernels in maize plants.



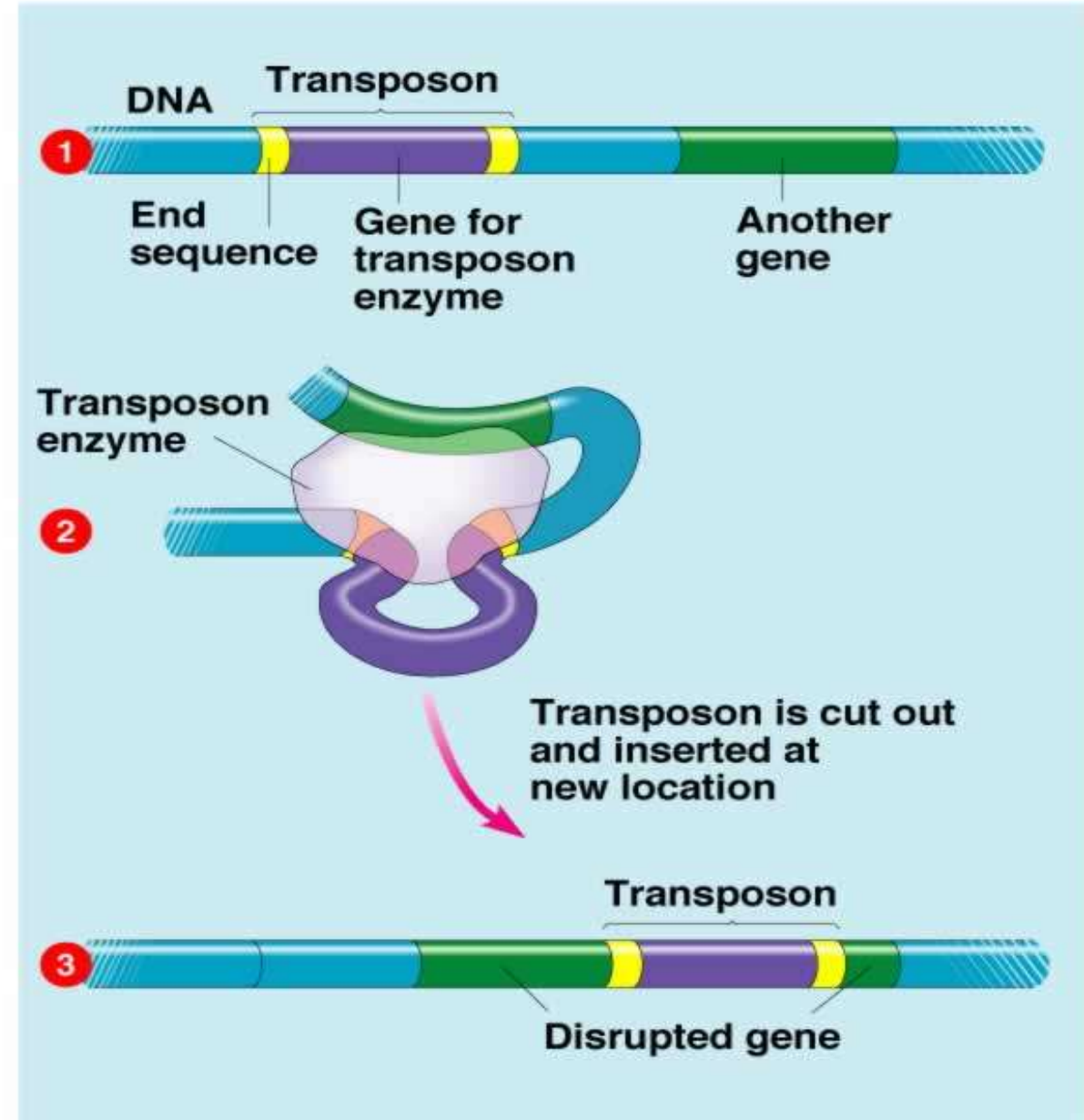
- **Position effect:**
- Position effect is the effect on the expression of a gene when its location in a chromosome is changed, often by translocation. This has been well described in *Drosophila* with respect to eye colour and is known as position effect variegation (PEV).



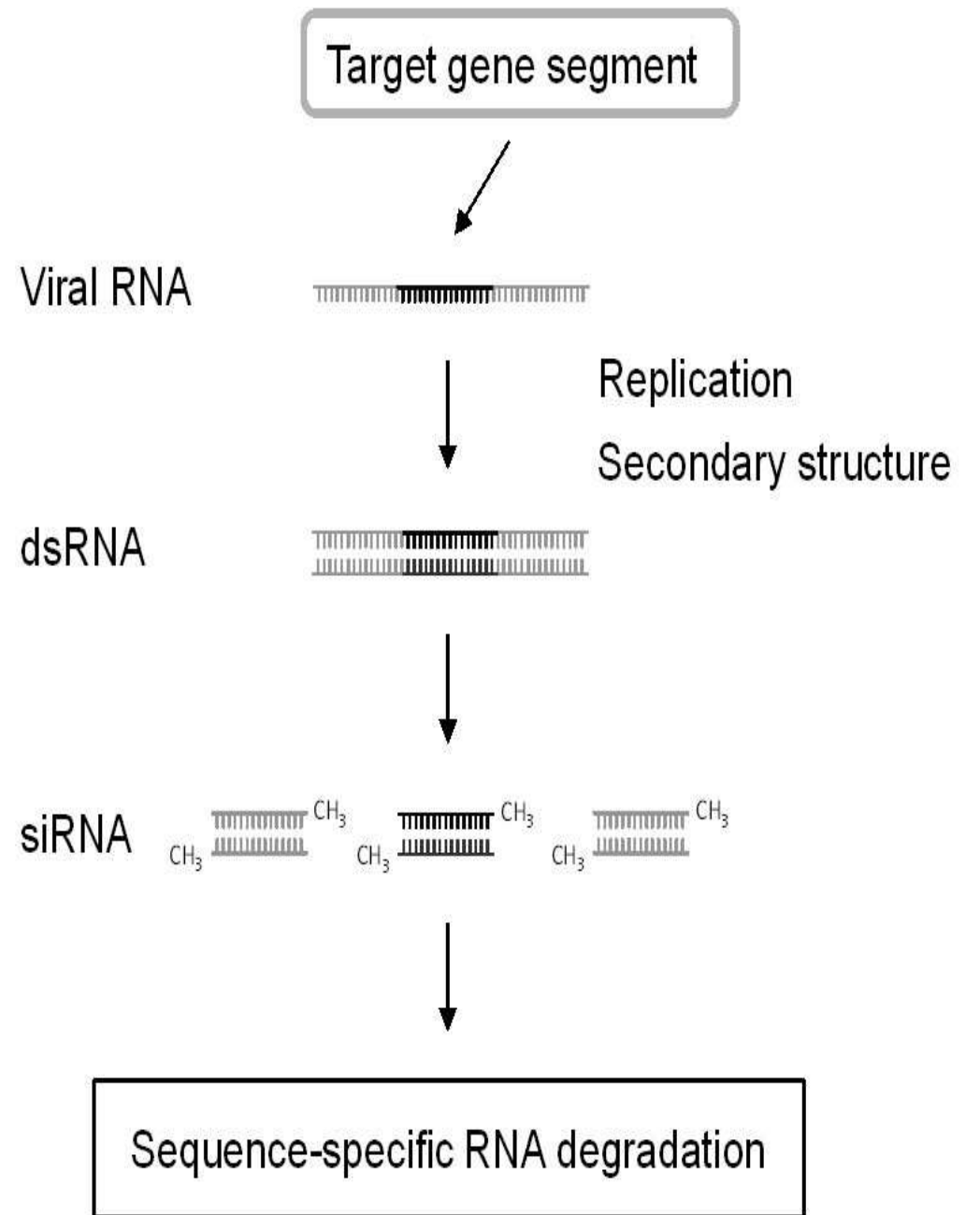
- RNA Directed DNA Methylation:
- RNA-directed DNA methylation is an epigenetic process first elucidated in plants whereby small double-stranded RNAs (dsRNA's) are processed to guide methylation to complementary DNA loci. In the model plant organism *Arabidopsis thaliana*



- **Transposon silencing:**
- Transposon silencing is a form of transcriptional gene silencing targeting transposons. Transcriptional gene silencing is a product of histone modification that prevent the transcription of that area of DNA.
- The “jumping” of transposon generates the genomic instability and cause the extremely deleterious mutations.
- Transposable element insertion have been linked to many disease including haemophilia ,SCID and predisposition to cancer.



- **Transgene silencing:**
- Unfortunate insertion of transgene into a transcriptionally inactive part of genome. When an insertion of any transgene it does not show activity as per desire and this is because of its instability.
- The loss of transgene stability is because of gene silencing.
- E.g. slow fruit softening tomato, by reducing expression of polygalactouronase enzyme.

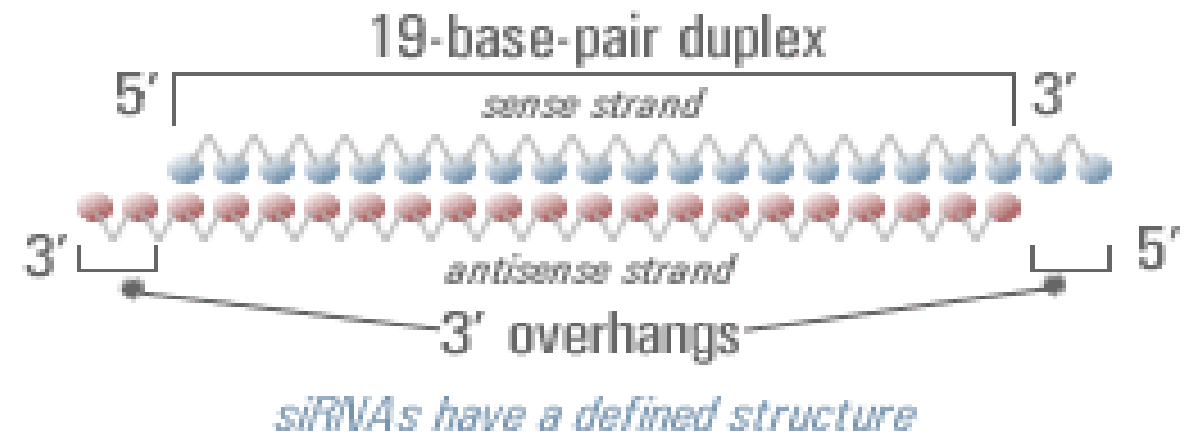


Post transcriptional gene silencing

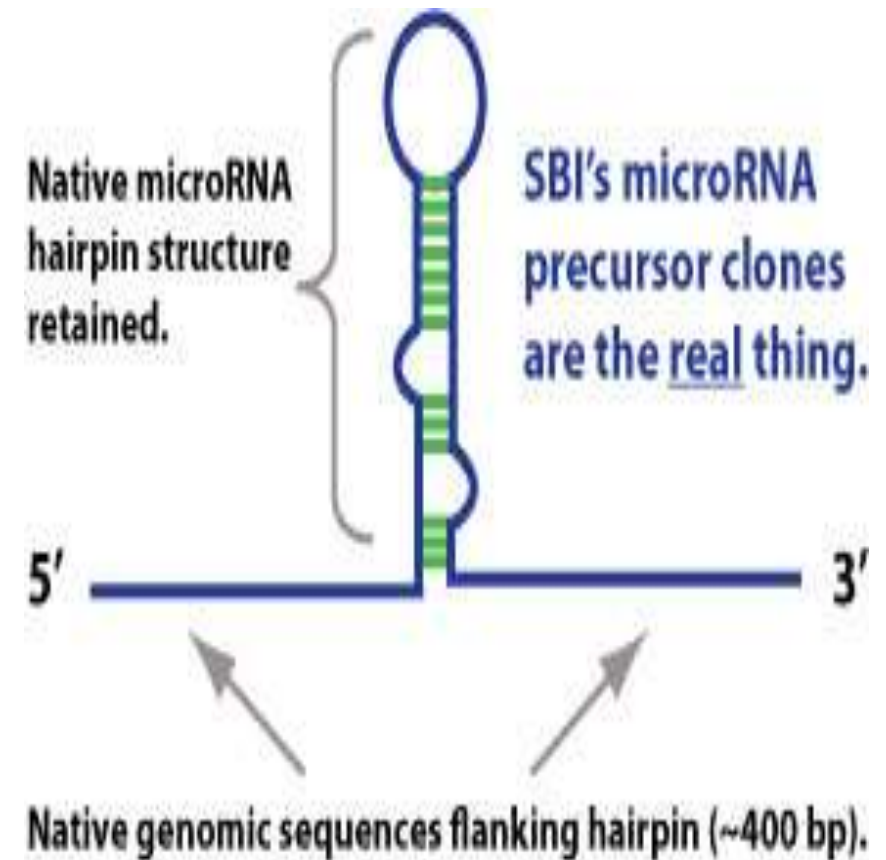
- The ability of exogenous or sometimes endogenous RNA to suppress the expression of the gene which corresponds to the m-RNA sequence.
- **RNA i (RNA interference):**
- it is a post transcriptional process triggered by the introduction of double stranded RNA (ds RNA) which leads to the gene silencing in a sequence specific manner.
- First evidence came from studies on nematode *caenorhabditis elegans*. further analysis in fruit fly *drosophila melanogaster*.
- It is also known as post transcriptional gene silencing / co suppression and quelling.

- **over view of RNA i:**
- During RNA i Double-stranded RNAs cut into short double-stranded RNAs, s(small) i(interfering) RNAs, by an enzyme called Dicer. These then base pair to an mRNA through a dsRNA-enzyme complex. This will either lead to degradation of the mRNA strand
- Highly specific process
- Very potent activity
- So far only been seen in eukaryotes
- Evidence 30% of genome is regulated by RNA I
- RNA i pathway guided by,
- **Si RNA (small interfering RNA):**
- Small interfering RNAs that have an integral role in the phenomenon of RNA interference (RNA i), a form of post- transcriptional gene silencing
- RNA i: 21-25 nt fragments, which bind to the complementary portion of the target mRNA and tag it for degradation

- A single base pair difference between the Si RNA template and the target mRNA is enough to block the process.
- Each strand of Si RNA has:
 - a. 5'-phosphate termini
 - b. 3'-hydroxyl termini
 - c. 2/3-nucleotide 3' overhangs

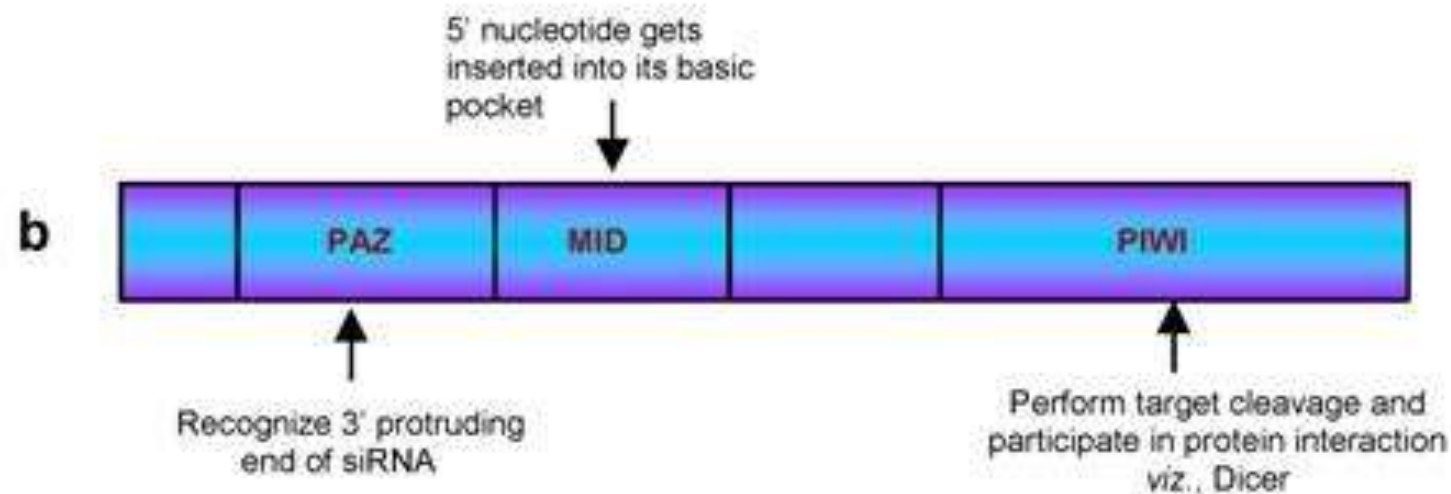
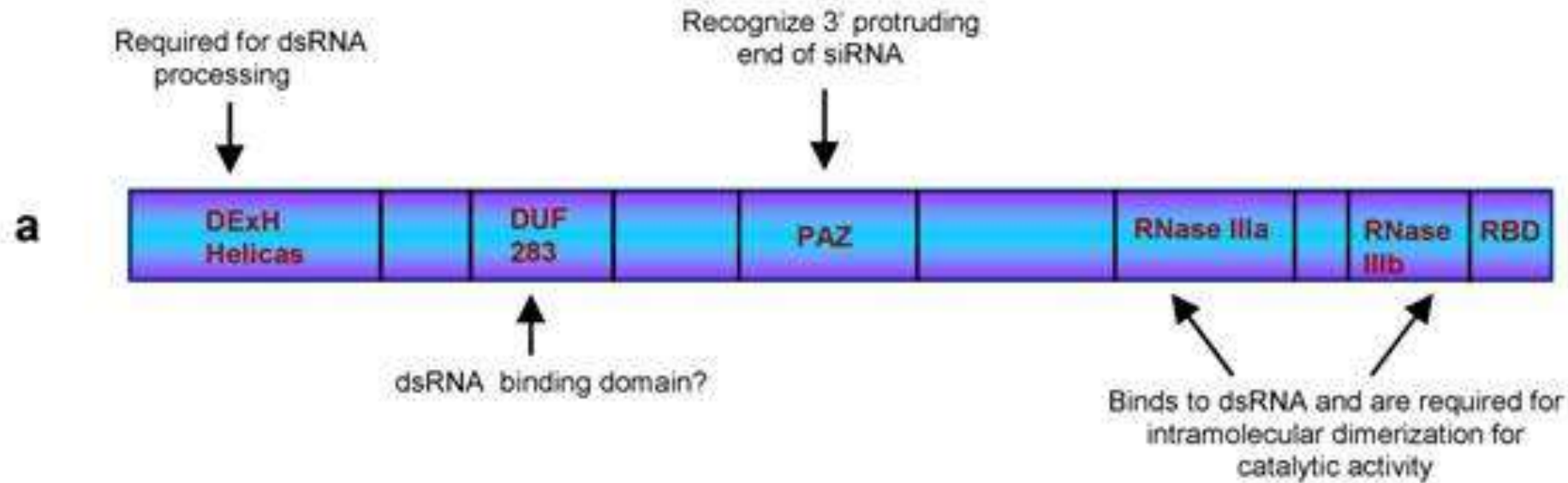


- **Mi RNA (micro RNA):**
- mi RNA Originate from capped & polyadenylated full length precursors (pri-miRNA)
- Hairpin precursor ~70 nt (pre-mi RNA) Mature mi RNA ~22 nt (mi RNA)
- mi RNA originates with SS RNA that forms a hairpin secondary structure.
- Mi RNA regulates post-transcriptional gene expression and is often not 100% complementary to the target.
- And also mi RNA help to regulate gene expression, particularly during induction of heterochromatin formation serves to down regulate genes pre- transcriptionally (RNA induced transcriptional silencing or RITS) RITS.



- **Dicer:**
- RNase III-like dsRNA-specific ribonuclease
 - Enzyme involved in the initiation of RNA i.
 - It is able to digest dsRNA into uniformly sized small RNAs (si RNA)
- Dicer family proteins are ATP- dependent nucleases.
- RNase III enzyme acts as a dimer
- Loss of dicer→loss of silencing processing in vitro
- Dicer homologs exist in many organisms including *C.elegans*, *Drosophila*, yeast and humans (Dicer is a conserved protein)
- DICER's domain:
- Dicer is a ribonuclease (RNase III family) with 4 distinct domains:
 - 1) Amino-terminal helicase domain
 - 2) Dual RNase III motifs in the carboxyl terminal segment
 - 3) dsRNA binding domain
 - 4) PAZ domain (110-130 amino-acid domain present in protein like Argo, Piwi.);it is thought to be important for protein-protein interaction

- DICER' s domain:



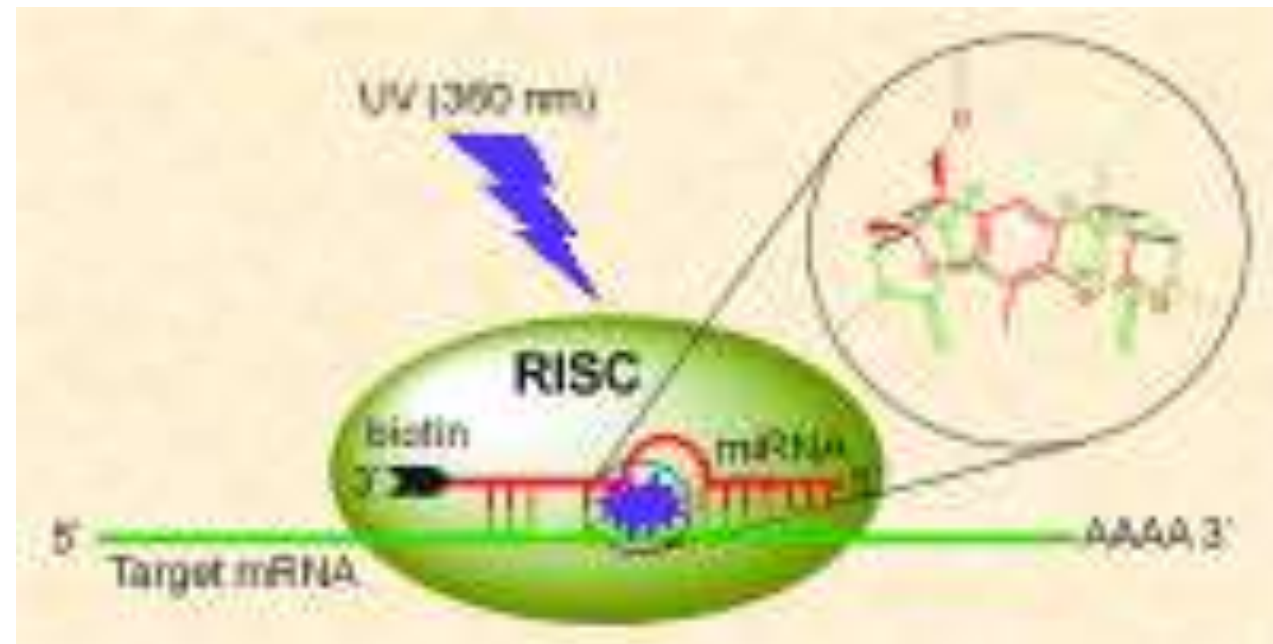
DUF: Domain of Unknown Function

RBD: RNA Binding Domain

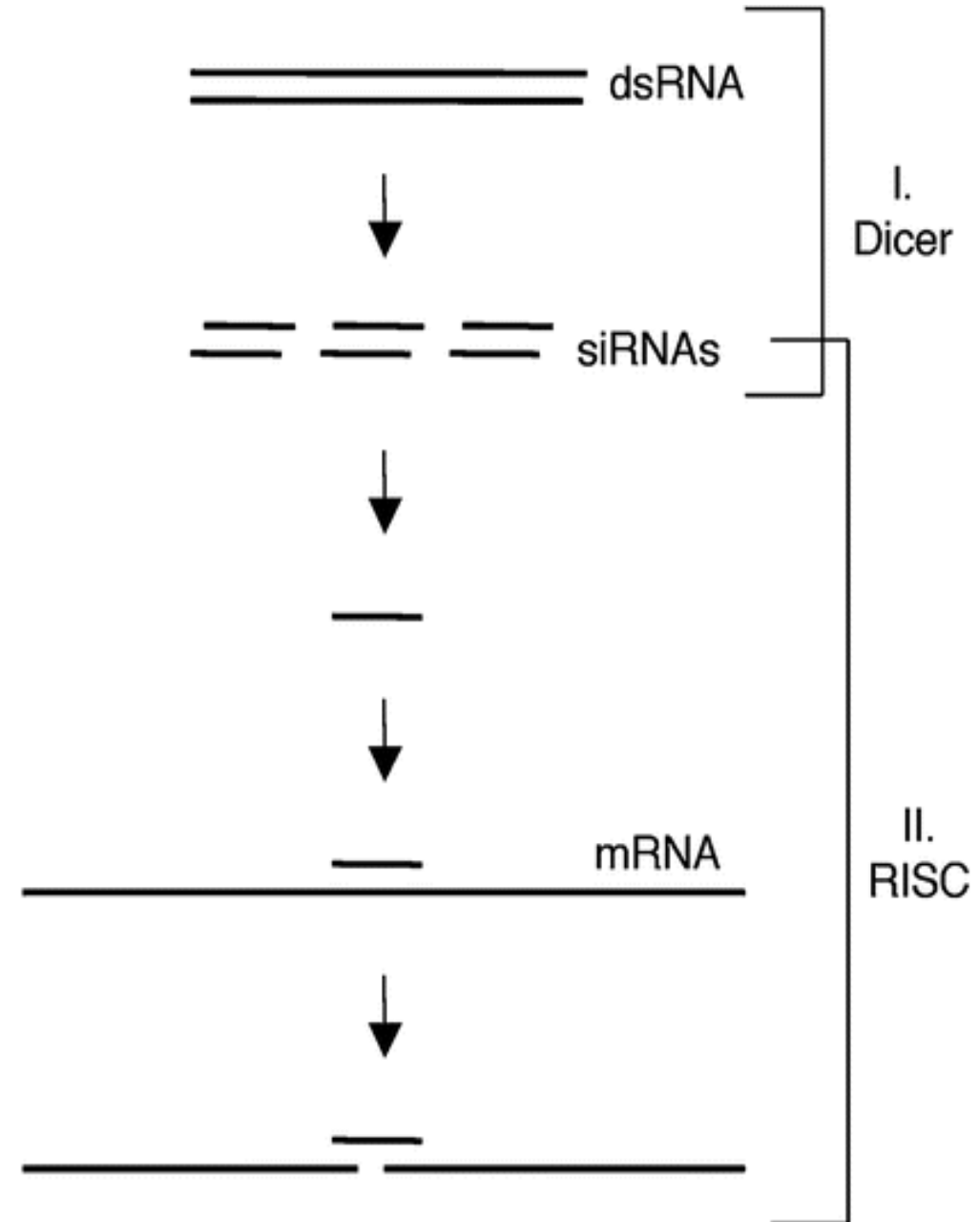
PAZ : Piwi, Argonaute and Zwille

PIWI: P element-induced wimpy testes

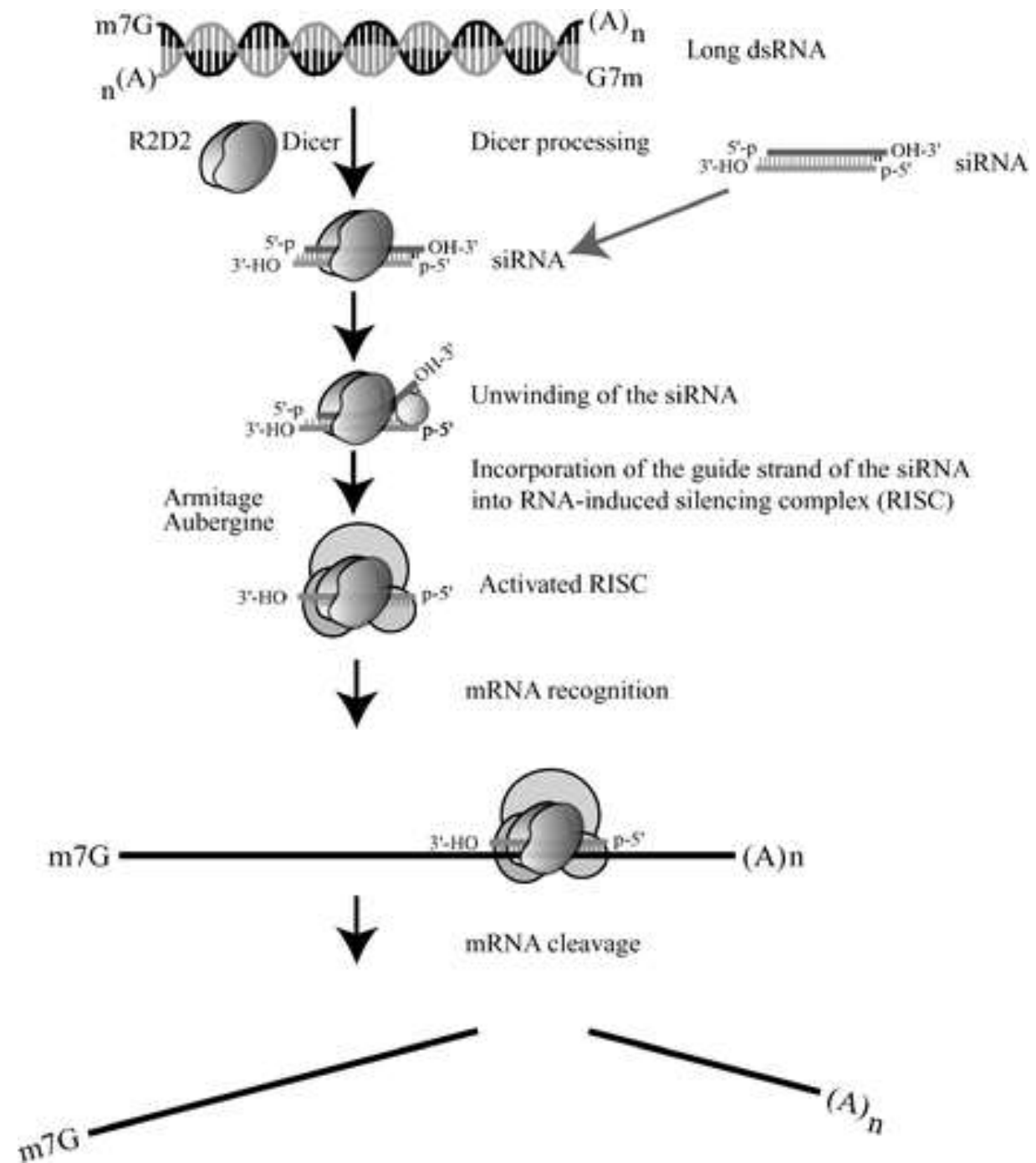
- **RISC (RNA Inducing Silencing Complex):**
- RISC is a large (~500-kDa) RNA-multi protein complex, which triggers mRNA degradation in response to Si RNA
- Unwinding of double- stranded Si RNA by ATP independent helicase.
- The active components of an RISC are endonucleases called argonaute proteins which cleave the target mRNA strand.



- **Steps of the RNA i pathway :**
- I. ds RNA is sliced by an ATP-dependent ribonuclease (Dicer) into short interfering RNAs (si RNAs).
- duplexes of 21-23 nucleotides bearing two-nucleotide 3' overhanging ends.
- II. Si RNAs are transferred to a second enzyme complex, designated RISC for RNA i -induced silencing complex. The si RNA guides RISC to the target mRNA, leading to its destruction.
- the antisense strand of the Si RNA is perfectly complementary

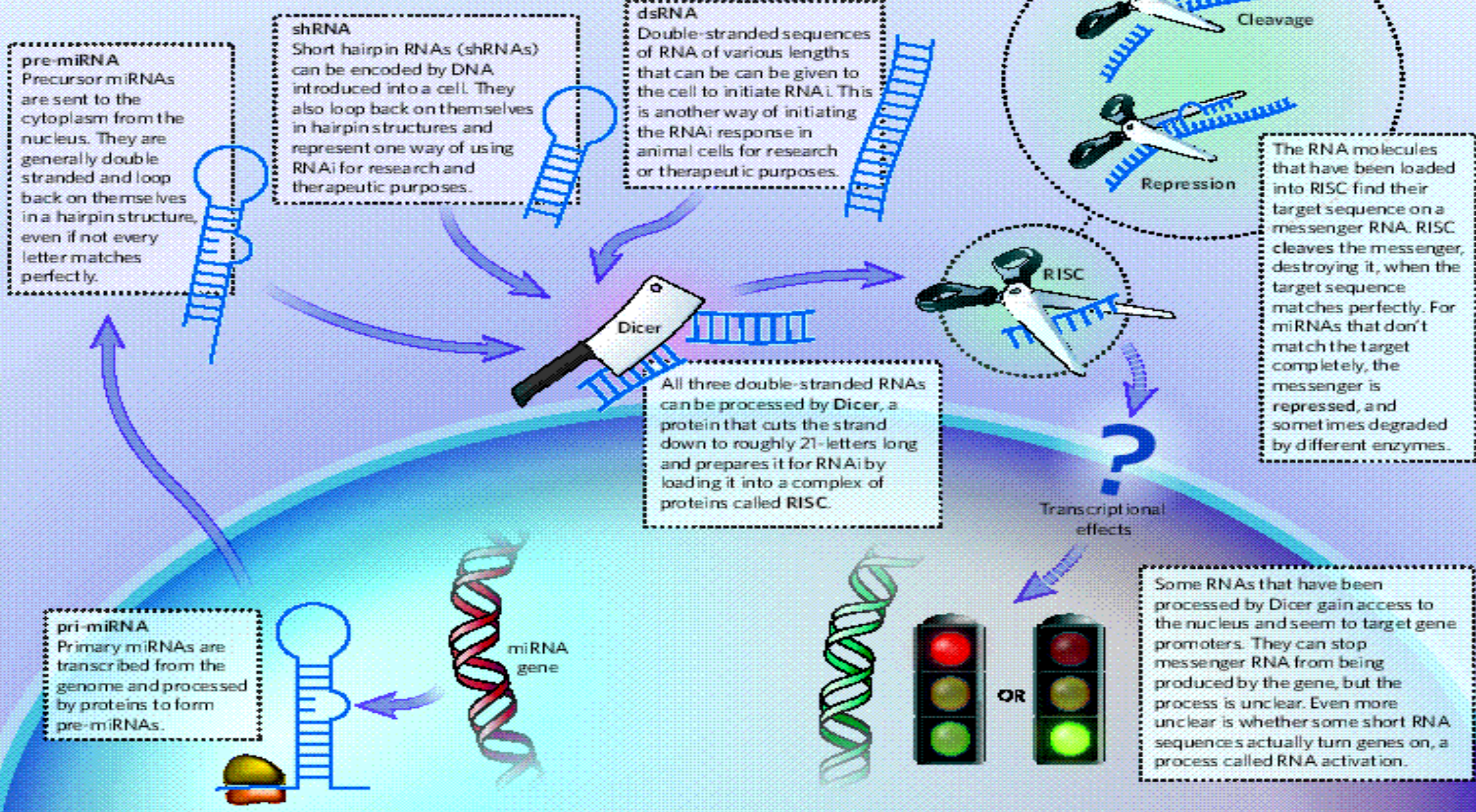


- **RNA i PATHWAY:**
- dsRNA are chopped into short interfering RNAs (siRNA) by Dicer.
- The si RNA-Dicer complex recruits additional components to form an RNA-Induced Silencing Complex (RISC). The siRNA unwinds.
- The unwound siRNA base pairs with complementary mRNA, thus guiding the RNAi machinery to the target mRNA.
- The target mRNA is effectively cleaved and subsequently degraded – resulting in gene silencing.

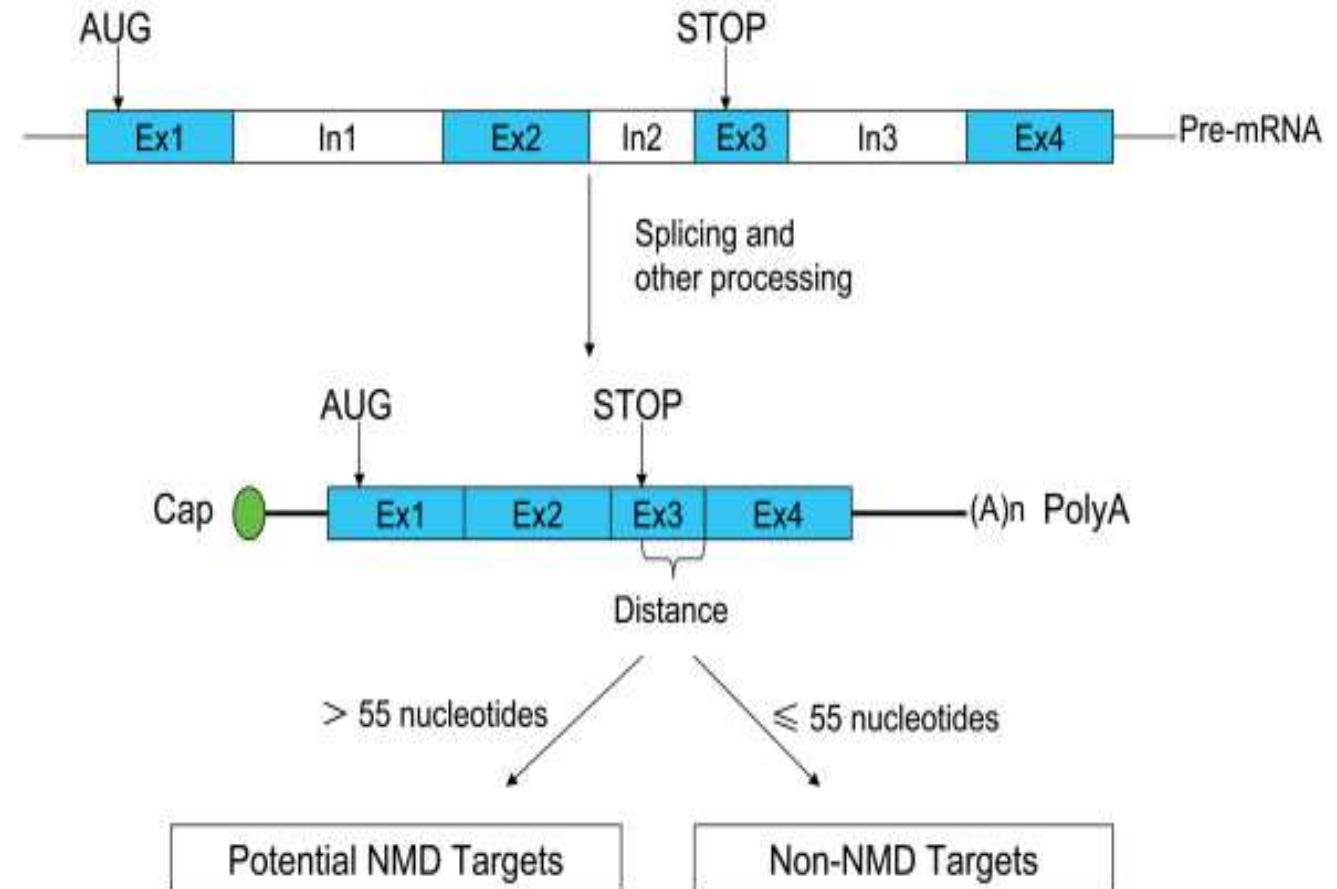


BEYOND INTERFERENCE

RNA interference (RNAi) has been one of the hottest fields of research in the past decade. As more details are clarified about the various triggers — the short RNAs that do the dirty work — more questions arise such as how RNA interference works in the nucleus to stop transcription (or possibly amplify transcription).



- **Non sense Mediated Decay:**
- Nonsense mediated decay (NMD) is a cellular mechanism of mRNA surveillance that functions to detect nonsense mutations and prevent the expression of truncated or erroneous proteins.
- NMD is triggered by exon junction complexes (EJCs) (components of the assembled RNP) that are deposited during pre-mRNA processing.

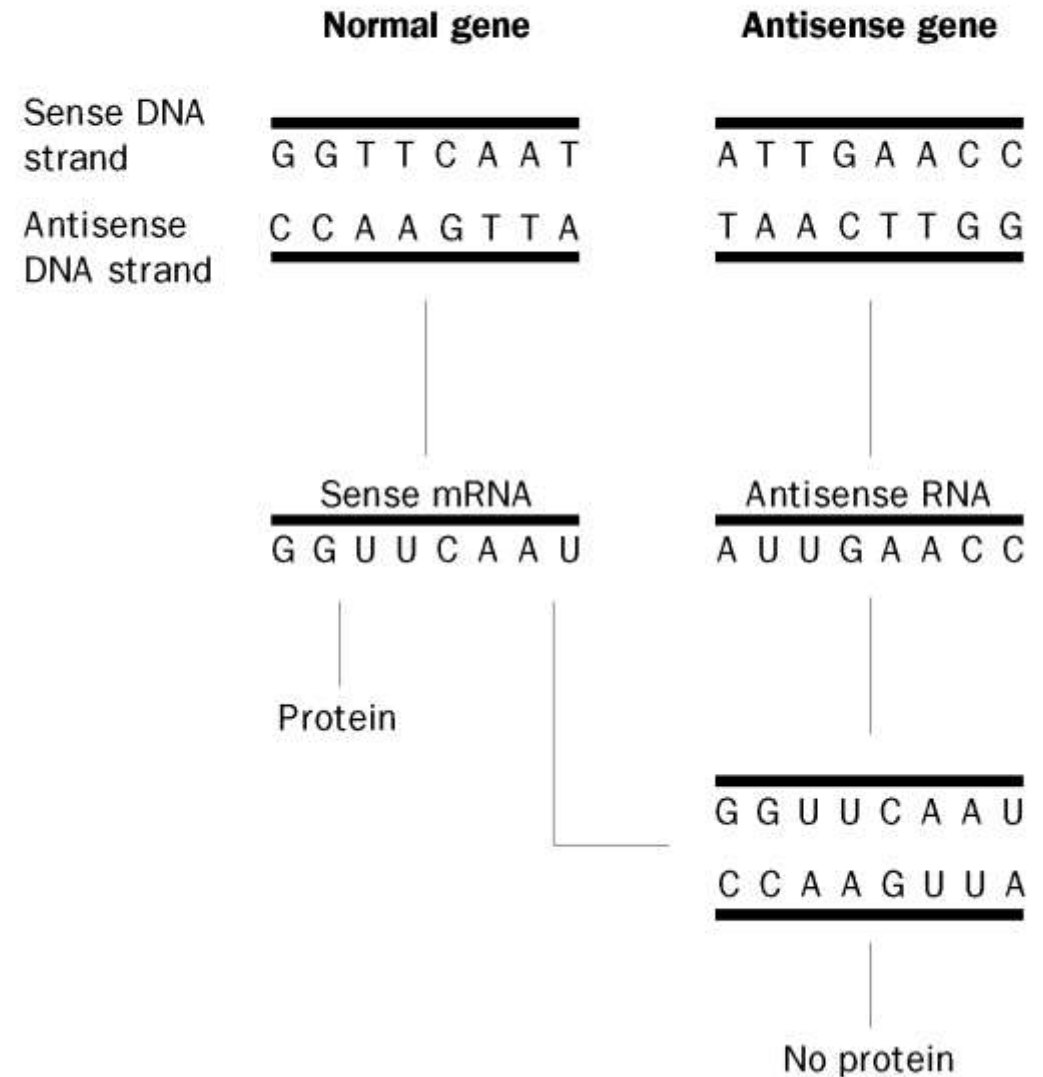


Ex*: Exons **In*:** Introns **AUG:** start codon **STOP:** termination codon

- **Anti sense RNA technology:**
- It blocks the activity of the m RNA in a stoichiometric manner.
- Antisense RNA has the opposite sense to m RNA.
- The presence of complimentary sense and antisense RNA in the in the same cell can lead to the formation of a stable duplex which interferes with gene expression at the level of RNA processing or possible translation.
- This technology widely used in plants for gene inhibition.

Figure 1

Outline model for the down regulation of a gene by the use of antisense RNA technology



- **Advantages of gene silencing:**
- Downregulation of gene expression simplifies "knockout" analysis.
- Easier than use of antisense oligonucleotides. Si RNA more effective and sensitive at lower concentration.
- Cost effective
- High Specificity middle region 9-14 are most sensitive With Si RNA, the researcher can simultaneously perform experiments in any cell type of interest
Can be labelled Ease of transfection by use of vector
- blocking expression of unwanted genes and undesirable substances.
- Inducing viral resistance
- Powerful tool for analysing unknown genes in sequenced genomes.
- Useful approach in future gene therapy.
- Oligonucleotides can be manufactured quickly, some within one week; the sequence of the mRNA is all that is needed

- **Disadvantages of gene silencing:**
- “High pressure injection” and electroporation can cause significant injection damage to the integrity of the normal tissues and organs and thus preclude the utilisation in a clinical set-up.
- Liposomes/cationic encapsulated Si RNA may also be toxic to the host and may cause severe host immune responses.
- Other emerging strategies includes chemical modification of Si RNA molecules and encapsulated with different molecules are still in their infancy and need to be thoroughly investigated before used in therapeutic applications.

Application of Gene silencing

- Specific gene silencing using RNA i in cell culture.
- Cancer treatments
- RNA interference has been used for applications in biotechnology, particularly in the engineering of food plants that produce lower levels of natural plant toxins.
- Such techniques take advantage of the stable and heritable RNA i phenotype in plant stocks. For example, cotton seeds.
- Modulation of HIV-I replication by RNA i.
- Small RNA and it's application in andrology and urology.
- Developing technologies for epigenomic analysis and clinical application of molecular diagnosis.
- Currently there are at least six oligonucleotide drugs inducing RNA i for illness including cancer.

conclusion

- it is the epigenetic regulation of gene expression and widely used in agriculture and in biotechnology.
- Besides the all types of gene silencing the RNA i is the important post transcriptional gene silencing.

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