siRNA and miRNA

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Topic - siRNA and miRNA

Dr. Punam Ranjan

Assistant Professor

Department of Botany

Patna Science College, Patna.

e-mail gayatripunam@gmail.com

Trio of DNA, RNA and Protein

- As proteins took over a greater share of the workload in the primitive cell, the RNA world was gradually transformed into an "RNA–protein world." At a later point in time, RNA was presumably replaced by DNA as the genetic material, which propelled life forms into the present "DNA–RNA– protein world."
- The evolution of DNA may have required only two types of enzymes: a ribonucleotide reductase to convert ribonucleotides into deoxyribonucleotides and a reverse transcriptase to transcribe RNA into DNA.
- The fact that RNA catalysts do not appear to be involved in either DNA synthesis or transcription supports the idea that DNA was the last member of the **DNA–RNA–protein** triad to appear on the scene.

Small Regulatory RNAs and RNA Silencing Pathways

- The idea that RNA molecules are directly involved in the regulation of gene expression began with a puzzling observation.
- The petals of a petunia plant are normally light purple. In 1990, two groups of investigators reported on an attempt to deepen the color of the flowers by introducing extra copies of a gene encoding a pigment-producing enzyme.
- To the surprise of the researchers, the presence of the extra genes caused the petals to lose their pigmentation rather than become more darkly pigmented as expected (Figure in next page).
- Subsequent studies indicated that, under these experimental conditions, both the added genes and their normal counterparts within the genome were being transcribed, but the resulting mRNAs were somehow degraded.

- The phenomenon became known as ***posttranscriptional gene silencing*** **(*PTGS*).**

RNA interference has been studied largely in plants and nematode worms.

Source- Karp, Cell and Molecular Biology, 7th edition, 2013

- **RNA interference (RNAi)**
- Petunia plants normally have purple flowers. The flowers of this plant appear white because the cells contain an extra gene (a transgene) that encodes an enzyme required for pigment production.
- The added gene has triggered RNA interference leading to the specific destruction of mRNAs transcribed from both the transgene and the plant's own genes, causing the flowers to be largely unpigmented.

Molecular basis of RNAi mediated Gene silencing; Experiment on *C. elegans*

- Andrew Fire and Craig Mello, injected these worms with several different preparations of RNA hoping to stop production of a particular muscle protein.
- One of the preparations contained "sense" RNA, that is, an RNA having the sequence of the mRNA that encoded the protein being targeted; another preparation contained "antisense" RNA, that is, an RNA having the complementary sequence of the mRNA in question; and a third preparation consisted of a double-stranded RNA containing both the sense and antisense sequences bound to one another.
- Neither of the single-stranded RNAs had much of an effect, but the double-stranded RNA was very effective in stopping production of the encoded protein.
- Fire and Mello described the phenomenon as **RNA interference** (**RNAi**). They demonstrated that double-stranded RNAs (dsRNAs) were taken up by cells where they induced a response leading to the selective destruction of mRNAs having the same sequence as the added dsRNA.

- The phenomenon of dsRNA-mediated RNA interference is an example of the broader phenomenon of **RNA silencing**, in which small RNAs, typically working in conjunction with protein machinery, act to inhibit gene expression in various ways.
- RNAi is thought to have evolved as a type of **"genetic immune system"** to protect organisms from the presence of foreign or unwanted genetic material.
- **To be more specific, RNAi probably evolved as a mechanism to block the replication of viruses and/or to suppress the movements of transposons within the genome because both of these potentially dangerous processes can involve the formation of double-stranded RNAs. Cells can recognize dsRNAs as "undesirable" because such molecules are not produced by the cell's normal genetic activities.**

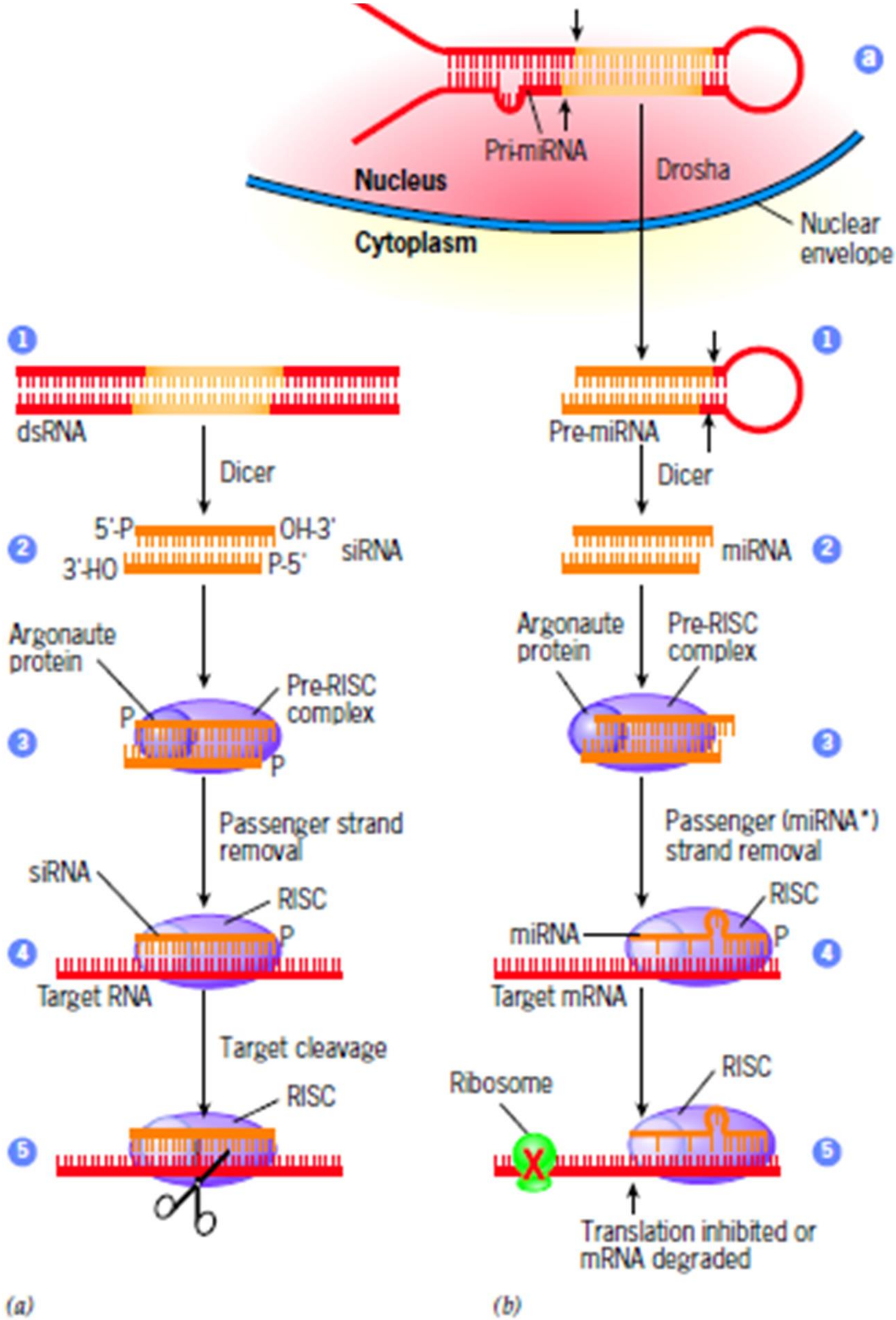
➢ The formation and mechanism of action of siRNAs and miRNAs.

- See Figure - (*a*) In step 1, both strands of a double-stranded RNA re cleaved by the endonuclease Dicer to form a small (21–23 nucleotide) siRNA, which has overhanging ends (step 2).
- In step 3, the siRNA becomes associated with a protein complex, a pre-RISC, that contains an Argonaute protein (typically Ago2) capable of cleaving and removing the passenger strand of the siRNA duplex.
- In step 4, the single-stranded guide siRNA, in association with proteins of the RISC complex, binds to a target RNA that has a complementary sequence.
- The target RNA might be a viral RNA, a transcript from a transposon, or an mRNA, depending on circumstances. In step 5, the target RNA is cleaved at a specific site by the Argonaute protein and subsequently degraded.

- See Figure – (b) MicroRNAs are derived from single-stranded precursor RNAs that contain complementary sequences that allow them to fold back on themselves to form a double-stranded RNA with a stem-loop at one end.

- This pseudo-dsRNA (or pri-miRNA) is cleaved at a specific site near its terminal loop by a protein complex containing an endonuclease named Drosha to generate a pre-miRNA that has a 3’ overhang at one end.
- The pre-miRNA is exported to the cytoplasm (step 1) where it is cleaved by Dicer into a small duplex miRNA (step 2) that has a 3 overhang at both ends. In step 3, the double-stranded RNA becomes associated with a protein complex containing an Argonaute protein (typically Ago1), leading to the separation of the strands and removal of the passenger strand (called miRNA*).
- The single-stranded guide miRNA then binds to a complementary region on an mRNA (step 4) and inhibits translation of the message as shown in step 5 (or alternatively leads to deadenylation and degradation of the mRNA.
- Unlike siRNAs, miRNAs that inhibit translation are only partially complementary to the target mRNA, hence the bulge.
- Most plant miRNAs and a handful of animal miRNAs are precisely complementary to the mRNA, or nearly so; in these cases, the outcome of the interaction tends to be cleavage of the mRNA by Ago2 in the same manner shown in *a*. (A certain class of miRNAs derived from introns do not form long hairpin pri-miRNAs and do not require Drosha for processing.)

Mechanism of action of siRNA and miRNA



Reference ----Karp, Cell and Molecular Biology, 7th edition, 2013