MicroRNA (miRNA)

miRNAs are small non-coding RNAs and have an average length of 22 nucleotides. Their function is to serve as gene regulatory molecule.

First miRNA was discovered by Victor Ambros and colleagues in 1983. They reported that lin-4, a gene known to control the timing of C. elegans larval development does not code for a protein but a pair of small RNAs. Later on labs of Ambros and Ruvkun discovered that lin-4 miRNA has anti-sense complementarity to many sites on the 3`-UTR of the lin-14 gene.

Ruvkun lab then demonstrated that lin-4 binds to the 3`-UTR in lin-14 mRNA and by this it can regulate the amount of LIN-14 protein without affecting the levels of lin-14 mRNA.

These discoveries supported the model that lin-4 RNAs pair with lin-14 3`-UTR and cause translational repression of lin-14. This repression is part of the regulatory mechanisms that trigger the transition from the cell divisions of the first larval stage to those of the second.

The second miRNA to be discovered was let-7 in C. elegans and it was also a 22 nucleotide RNA. The let-7 RNA acts to promote transition from late-larval to adult cell fates. Due to their roles in controlling the timing of the developmental transitions, the lin-4 and let-7 RNAs were called small temporal RNAs (stRNAs).

Biogenesis of miRNAs:

It starts with processing of the transcripts generated by RNA pol II/III.

- The processing can be co-transcriptional or post-transcriptional.
- About half of the known miRNAs are coded by introns and processed out of them. Such miRNAs are intragenic.
- Some miRNAs are also coded by the exons and are processed out of them. Such miRNAs are also intragenic.
- Remaining miRNAs are transcribed using their own promoters and are independent of any other gene. Such miRNAs are called as intergenic.
- Sometimes miRNAs are transcribed as a long transcript and it is called as cluster.
- miRNAs are first transcribed into primary miRNAs (pri-miRNAs) and then processed into precursor-mRNA (pre-mRNA) and mature miRNAs respectively.

1. **Canonical pathway of miRNA biogenesis:** Canonical means most common pathway or usual mode of synthesis.

- Pathway starts at formation of pri-miRNA transcript.
- The microprocessor complex cleaves pri-mRNA into precursor-mRNA (pre-mRNA).
- Microprocessor complex is made of Drosha and DiGeorge syndrome critical region 8 (DGCR8) proteins.
- The pre-miRNA is exported to cytoplasm in an exportin5/RanGTP-dependent manner.
- In cytoplasm, RNase III endonuclease called Dicer cleaves the pre-miRNA into mature miRNA.
- Dicer removes the terminal loop leading to the formation of mature miRNA duplex.
- The mature miRNA consists of two strands called as 5p and 3p. They are derived respectively from the 5` end and 3` end of pre-miRNAs.
- When either or both the strands of the mature miRNA are loaded on the Argonaute(AGO) family of proteins in an ATP-dependent manner.
- This leads to formation of miRNA-induced silencing complex (miRISC).

Note: Proportion of the 5p or 3p strand loaded on the AGO varies greatly among cell types and environments and ranges from nearly equal proportions to the predominance of one form over other. The selection of 5p or 3p strand is partially based on the thermodynamic stability at 5` end of the miRNA duplex or a 5` U at nucleotide position 1.

Generally, the strand with lower 5` stability or 5` uracil is loaded preferentially into AGO and considered as guide strand. The unloaded strand is called a passenger strand and is unwound from the guide strand and degraded.

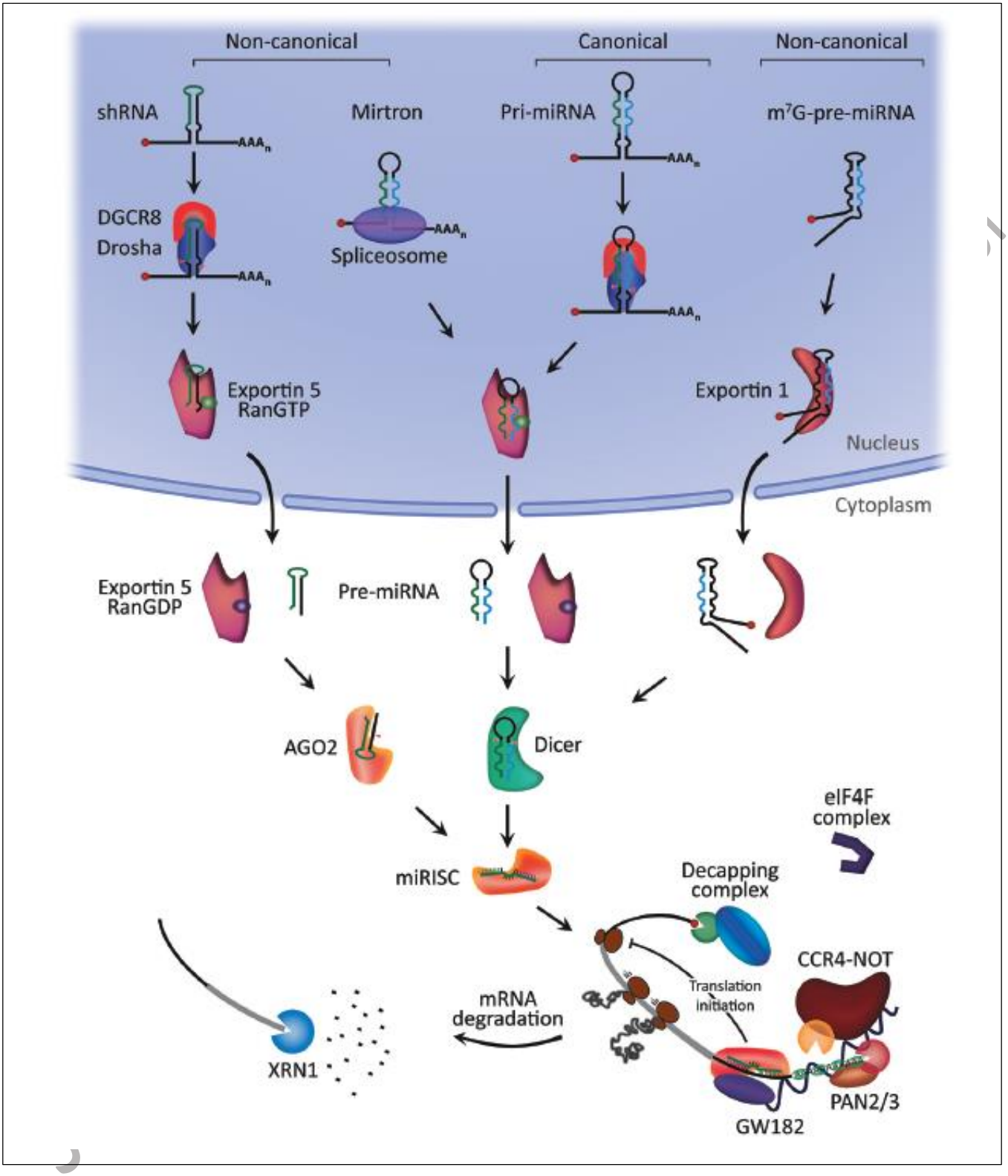

Figure: Pathways of miRNA synthesis and their mechanism of action

2. **Non-canonical pathway of miRNA biogenesis:** These pathways are less common therefore they are called as non-canonical. They use different combinations of the proteins involved in canonical pathway.

- **Drosha-DGCR8-independent pathway:**
    - The pre-miRNA resembles Dicer substrates.
    - It is exported directly to the cytoplasm through Exportin-1.
    - Thus, Drosha cleavage is not required in this pathway.

- After this miRISC is formed.
- E.g. pre-miRNA produced from introns (Mirtrons) and 7-methylguanosine capped pre-miRNA.

- **Dicer-independent pathway:**
  - Endogenous short hairpin RNA (shRNA) transcripts are produced.
  - They are processed by Drosha into pre-miRNA.
  - Their length is small to be a Dicer substrate so for maturation they need Argonaute-2 in the cytoplasm.
  - Thus, entire pre-miRNA is loaded onto the Argonaute-2 and undergoes processing to become mature miRNA.
  - After this miRISC is formed.

Functions of miRNA: Major role of miRNAs is in gene regulation by repression of translation and degradation of the mRNA. However, in some cases miRNA binding activates the expression than repression.

General mechanism of translational repression mediated by miRNA is outlined below:

- miRNA in miRISC binds to the 3`UTR of their target mRNA.
- miRISC initiates translational repression most likely by interfering with eIF4F complex.
- GW182 family proteins are bound to the Argonaute protein and they recruit poly(A)-deadenylases called PAN2/3 and CCR4-NOT.
- PAN2/3 initiates the deadenylation and CCR4-NOT completes the process.
- After this, decapping complex acts to remove m^7G cap on target mRNA.
- Decapped mRNA may then be degraded in 5`-3` manner by an exoribonuclease XRN1.

Long non-coding RNAs (lncRNAs)

1. Introduction

Long non-coding RNAs (lncRNAs) are a class of RNA molecules longer than 200 nucleotides that do not code for proteins. They are involved in various biological processes, including gene regulation, chromatin remodeling, and cellular differentiation.

2. Characteristics of lncRNAs

- Length: More than 200 nucleotides.
- Lack of protein-coding potential.
- Often transcribed by RNA polymerase II.
- May undergo splicing, polyadenylation, and other post-transcriptional modifications.
- Low expression levels but highly specific to tissues and developmental stages.

3. Biogenesis of lncRNAs

lncRNAs are transcribed from different regions of the genome:

- **Intergenic lncRNAs**: Located between protein-coding genes.
- **Intronic lncRNAs**: Derived from introns of protein-coding genes.
- **Antisense lncRNAs**: Overlap with a protein-coding gene on the opposite strand.
- **Bidirectional lncRNAs**: Transcribed in the opposite direction from a promoter region.
- **Enhancer-associated lncRNAs (e-lncRNAs)**: Transcribed from enhancer regions and regulate gene expression.

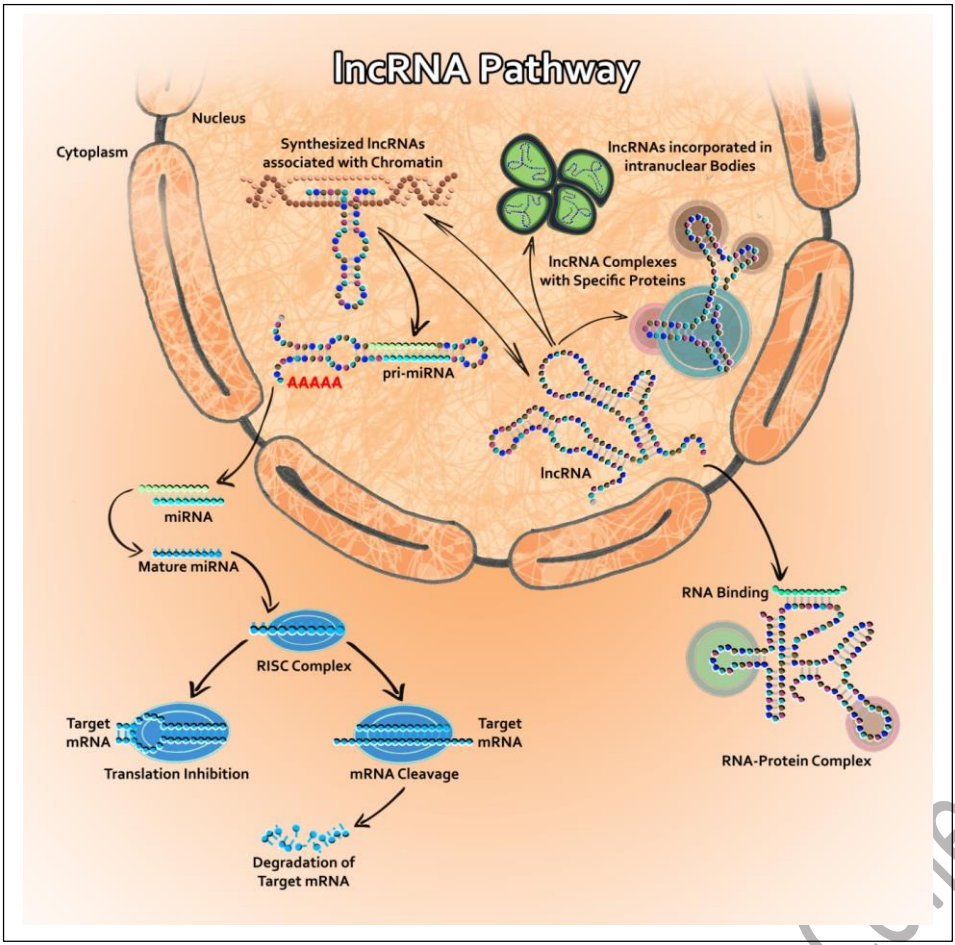

Figure: Comparative account of miRNA and lncRNAs biosynthesis.

4. Functions of lncRNAs

lncRNAs regulate gene expression at multiple levels:

A. Transcriptional Regulation

- **Chromatin remodeling**: lncRNAs interact with chromatin-modifying complexes like PRC2 (Polycomb Repressive Complex 2) to alter chromatin structure (e.g., Xist in X-chromosome inactivation).
- **Enhancer activation**: lncRNAs act as enhancers to promote gene transcription (e.g., HOTTIP regulates HOXA genes).

B. Post-Transcriptional Regulation

- **RNA stability**: lncRNAs can stabilize or degrade target mRNAs.
- **miRNA sponging**: lncRNAs act as "sponges" by sequestering microRNAs, preventing them from binding to their target mRNAs (e.g., MALAT1).

C. Translational and Post-Translational Regulation

- **Regulation of translation**: lncRNAs can bind to ribosomes or translation initiation factors.
- **Protein scaffolding**: lncRNAs facilitate interactions between proteins involved in signaling pathways.

5. Mechanisms of Action

lncRNAs exert their functions via different molecular mechanisms:

A. Signal lncRNAs

- Act as molecular indicators of transcriptional activity.
- Example: Xist lncRNA silences the X chromosome in female mammals.

B. Decoy lncRNAs

- Bind and sequester proteins or RNAs to prevent their activity.

- Example: GAS5 lncRNA inhibits glucocorticoid receptor signaling.

C. Guide lncRNAs

- Recruit chromatin modifiers to specific genomic locations.
- Example: HOTAIR recruits PRC2 to repress target genes.

D. Scaffold lncRNAs

- Serve as platforms for assembling multiple proteins into complexes.
- Example: TERC (telomerase RNA component) is essential for telomerase function.

6. Role of lncRNAs in Diseases

A. Cancer

- Dysregulated lncRNAs contribute to tumorigenesis.
- Example: HOTAIR promotes metastasis in breast cancer.

B. Neurological Disorders

- lncRNAs influence neuronal differentiation and synaptic function.
- Example: BACE1-AS regulates β-amyloid production in Alzheimer's disease.

C. Cardiovascular Diseases

- Involved in heart development and disease.
- Example: ANRIL is linked to atherosclerosis.

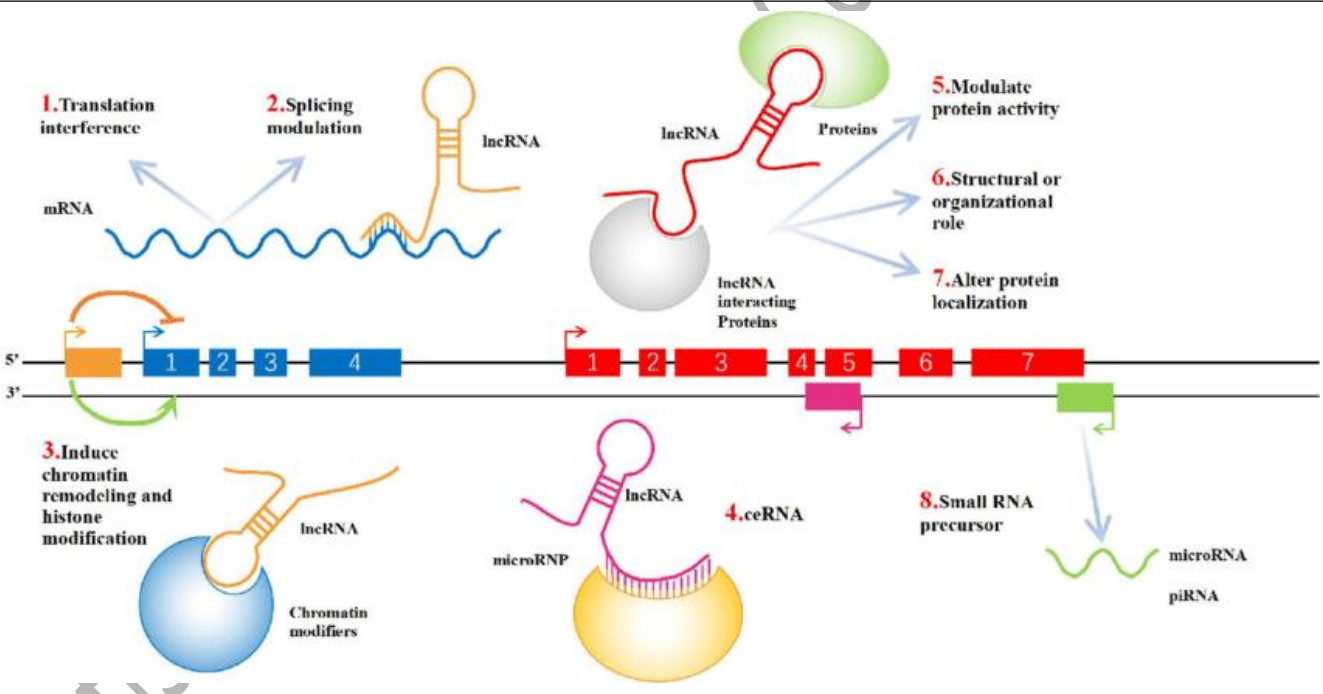

Figure: Different effects of lncRNAs.