Lecture 16-17

BT 632
Stem Cells, Cancer and Therapy
(3-0-0-6)

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RNAi and miRNA

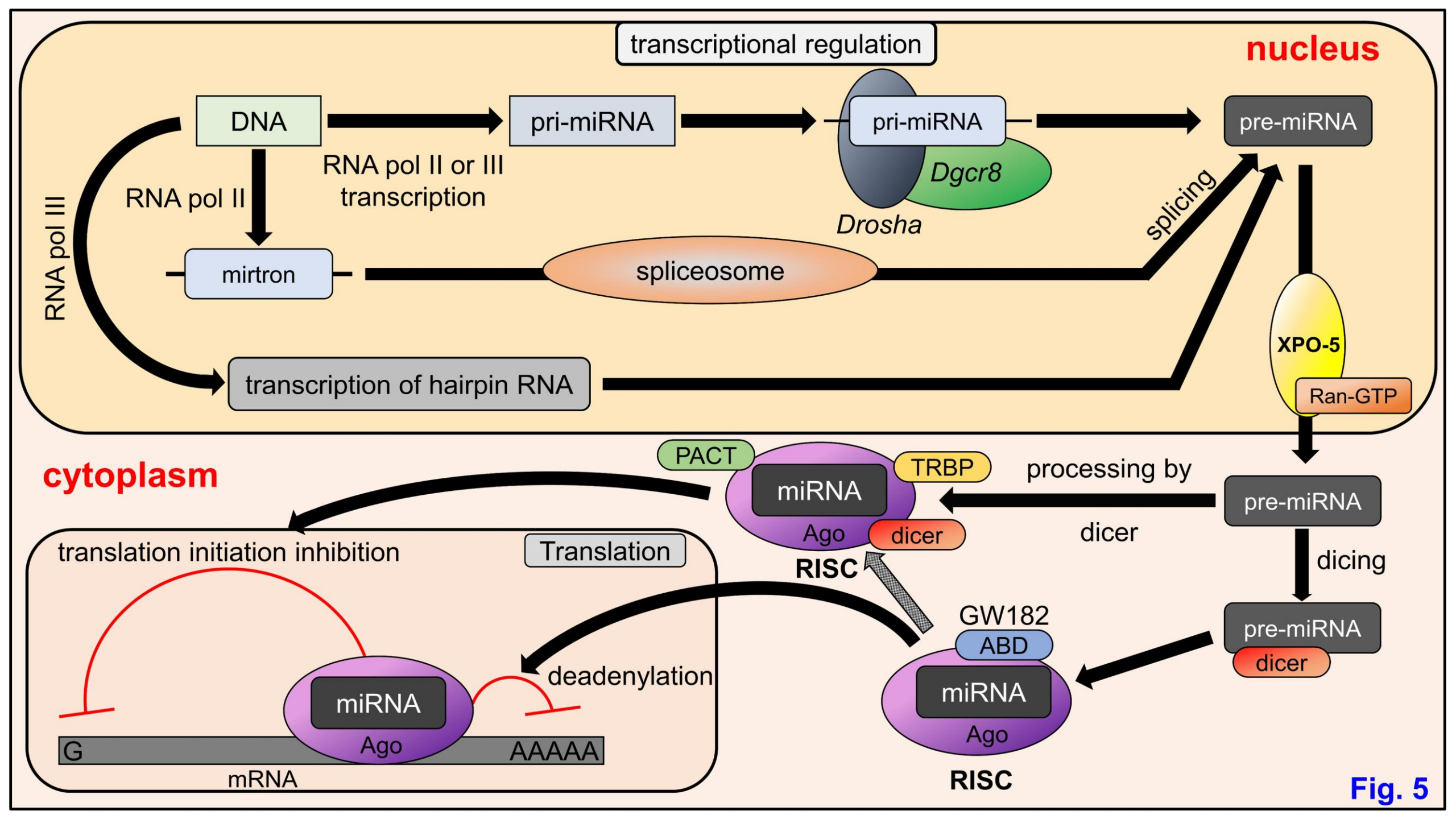
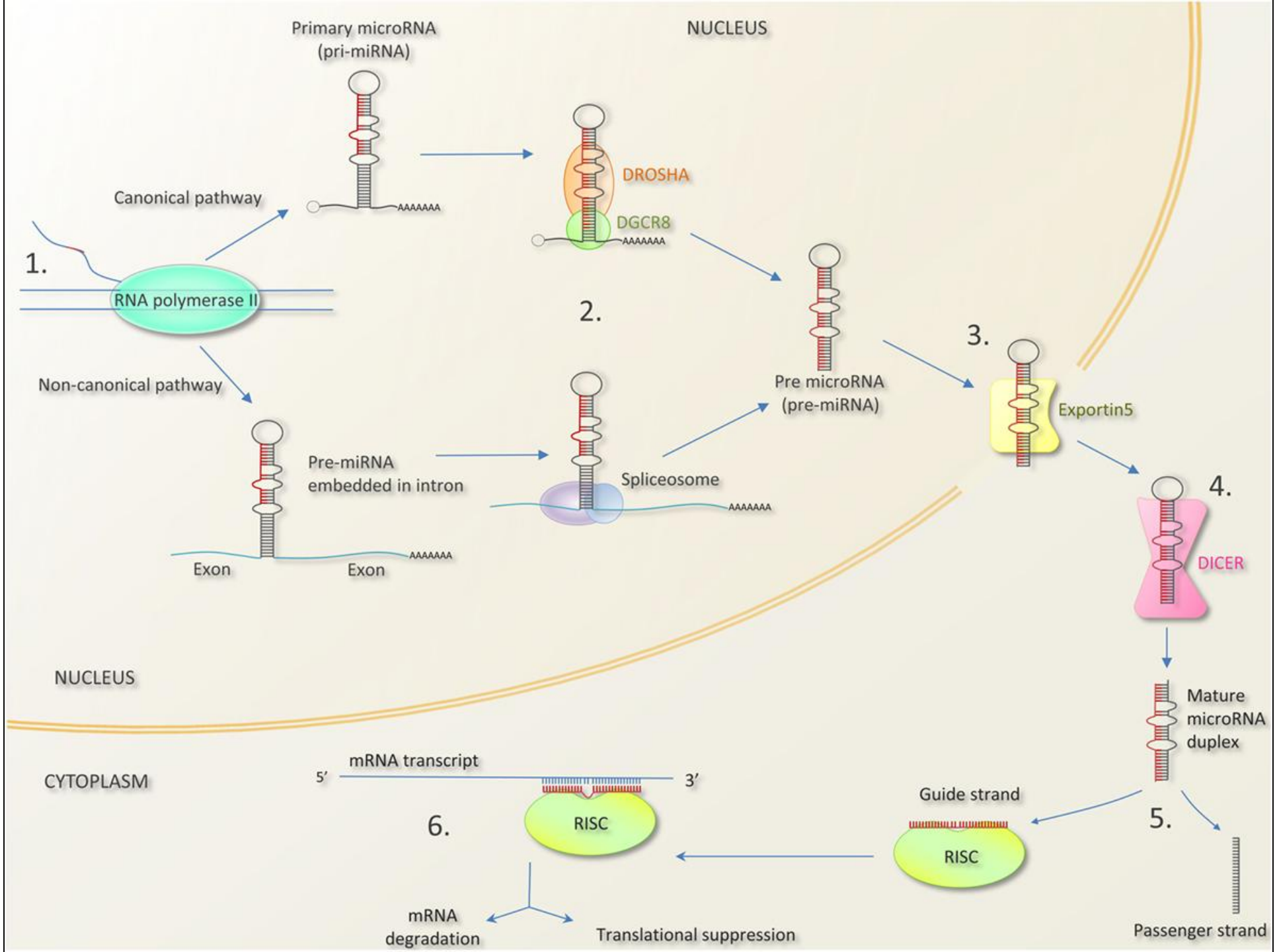

transcriptional regulation
nucleus
DNA
pri-miRNA
pri-miRNA
pre-miRNA
RNA pol II or III
transcription
RNA pol II
RNA pol III
Dgcr8
Drosha
splicing
mirtron
spliceosome
XPO-5
Ran-GTP
transcription of hairpin RNA
cytoplasm
PACT
TRBP
miRNA
Ago
dicer
RISC
processing by
dicer
pre-miRNA
dicing
pre-miRNA
dicer
translation initiation inhibition
Translation
deadenylation
GW182
ABD
miRNA
Ago
RISC
G
AAAAA
mRNA

Fig. 5


Primary microRNA
(pri-miRNA)
NUCLEUS
Canonical pathway
DROSHA
DGCR8
1.
RNA polymerase II
2.
Pre microRNA
(pre-miRNA)
3.
Exportin5
Non-canonical pathway
Pre-miRNA
embedded in intron
Spliceosome
4.
DICER
Exon
Exon
NUCLEUS
Mature
microRNA
duplex
CYTOPLASM
mRNA transcript
5′
3′
Guide strand
6.
RISC
RISC
5.
mRNA
degradation
Translational suppression
Passenger strand

RNA interference (RNAi)

- **Definition: “RNA interference is the process in which the gene expression is inhibited by RNA molecules by neutralizing the targeted mRNA molecules.”**

- RNA interference (RNAi) is a naturally occurring post-transcriptional mechanism found in nearly all cells. It was discovered by by two American scientists Andrew Fire and Craig Mello in 2006 in the cells of C.elegans. They introduced short segments of double-stranded RNA into the cells of C.elegans and inhibited the expression of certain genes.

- RNAi is a gene silencing or gene knockdown technology working at the RNA level. It targets the organism's own mRNA sequence and interferes with protein synthesis. RNA interference acts as a mode of gene regulation or works as a defense mechanism against any exogenous pathogenic RNA.

- It is a gene regulatory mechanism that limits the level of transcript in two ways:
  - Suppressing transcription (Transcriptional gene silencing)
  - Degrading the RNA produced (post-transcriptional gene silencing)

RNA interference (RNAi)

❑ RNA Interference Applications

RNA interference has the following applications:

Gene Knockdown:
RNA interference is often used to study the functions of genes in cell culture and in model organisms. This mechanism is used to reduce the expression of the targeted gene.

Functional Genomics:
It helps in quickly understanding the functions of different genes in various organisms.
It is useful in studying genes that are newly discovered and not well-understood.

RNAi Therapeutics:
With the invention of short hairpin RNA or small interfering RNA, it became possible to silence the specific gene sequences instead of silencing the entire gene. Since then, RNAi has been used to target specific gene sequences that can cause cancer. It can also be used to treat bacterial diseases, viruses, and parasites, relieve pain and even modulate sleep.

Drug Development:
It is used to identify genes that could make cells resistant to certain drugs or genes that are affected by specific drugs, providing insights into their mechanisms of action.

RNA interference (RNAi)

❑ Limitations of RNA Interference (RNAi):

The use of RNA interference (RNAi) technology comes with some challenges, which are as follows:

❑ Difficulty in Designing Effective siRNA Sequences:

- To figure out the right sequence for the siRNA can be difficult. There are specific rules and constraints to follow, and sometimes, even with careful design, it may not work properly.

❑ Cell Receptiveness:

- It may be possible that some cells might not easily take in the siRNA, which can limit its effectiveness.

❑ Efficiency of siRNA in Cell:

- siRNA are relatively less stable and efficient in cells than that in in-vitro conditions. It is important to find the right type of chemical modification that makes the siRNA stable in the body without affecting its ability to silence the target gene.

RNA interference (RNAi)

Gene Knockdown	Gene Knockout
Gene Knockdown is a temporary and often incomplete reduction in the expression of a specific gene.	Gene Knockout is a permanent and complete elimination of a gene's function.
It is achieved by interfering with messenger RNAs (mRNAs) or non-coding RNAs, produced by cells.	It involves the deletion or inactivation of the gene (DNA), rendering it non-functional.
It does not involve alterations to the host genome.	It involves the alteration of genomic DNA sequences.

miRNAs (same machinery used for RNAi)

Please read the below review article to know about microRNAs:

WJBC World Journal of Biological Chemistry

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Biol Chem 2017 February 26; 8(1): 45-56

DOI: 10.4331/wjbc.v8.i1.45

ISSN 1949-8454 (online)

REVIEW

Role of microRNAs in translation regulation and cancer

Stefania Oliveto, Marilena Mancino, Nicola Manfrini, Stefano Biffo

miRNAs

Review 1

A miRNA that binds the 3'UTR of a target mRNA, how can it inhibit its translation?

To date it is very clear that miRNAs contribute to the regulation of protein synthesis in two ways, mRNA destabilization or translational repression. Unfortunately, to date, a general mechanism for the translational inhibition by miRNAs has not been widely accepted; we rely on several different models that will be critically presented.

Review 2

In most cases, miRNAs interact with the 3′ untranslated region (3′ UTR) of target mRNAs to induce mRNA degradation and translational repression. Most studies to date have shown that miRNAs bind to a specific sequence at the 3′ UTR of their target mRNAs to induce translational repression and mRNA deadenylation and decapping.