



*RNA interference for post
transcriptional regulation of
gene expression*

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Introduction

- ▶ Eukaryotic gene regulation mechanisms:
 - ❖ Transcriptional
 - ❖ Post-transcriptional – RNAi/PTGS
- ▶ Small non-coding RNAs/micro RNAs – role in some RNAi/PTGS mechanisms.



- ▶ RNAi is a highly conserved mechanism of posttranscriptional gene silencing in which double stranded RNA causes sequence-specific degradation of mRNA sequences.



▶ RNAi is well studied in animal models for genetic studies:

- ❖ *Caenorhabditis elegans*
- ❖ *Drosophila*
- ❖ *Arabidopsis*

Mammals
(including humans) and
many other
eukaryotes

RNAi
mediated
gene
regulation

tool for
gene
analyses

Genes that
can't be
manipulated by
standard
genetic
techniques.

Pathways of RNAi



- ▶ ncRNAs and microRNAs: 21-28 bp long

source: large dsRNA – enzymatic cleavage by endonucleases (specific to dsRNA)

- ▶ “Dicer” enzymes: break large dsRNAs to small pieces

Caenorhabditis elegans: 1 dicer

Drosophila: 2 different dicers



Act in
cytoplasm

Arabidopsis: 3 or more dicers



Probably act in
nucleus



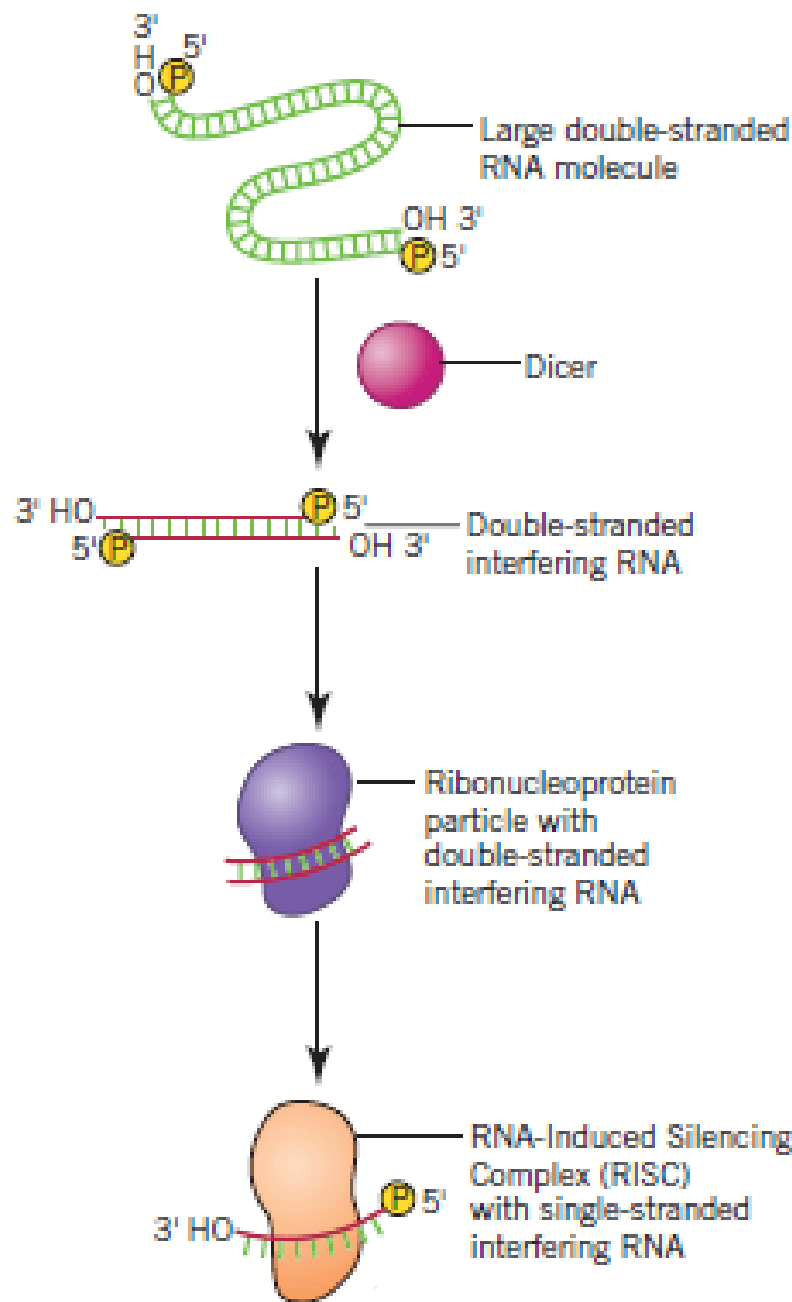
Events in RNAi pathway



- ▶ The dicer's activity results in: siRNAs and miRNAs.
- ▶ The interfering RNAs have 2 unpaired nucleotides at the 3' ends.
- ▶ Both types of small RNAs integrate with ribonucleoprotein particles in the cytoplasm.
- ▶ The dsRNA (siRNA/miRNA) unwinds from the ribonucleoprotein – one of the strands is degraded.



- ▶ Interfering RNA's single remaining strand can interact with the target mRNA.
- ▶ ssRNA of RNA ribonucleoprotein complex base pairs with a complementary sequence in mRNA.
- ▶ The interaction between the single stranded interfering RNA and the target mRNA sequence prevents the gene expression.
- ▶ RNA – protein particle with ssRNA: RISC (RNA Induced Silencing Complex).



STEP
1

A large double-stranded RNA molecule is diced into small, double-stranded interfering RNAs 21-28 base pairs long.

STEP
2

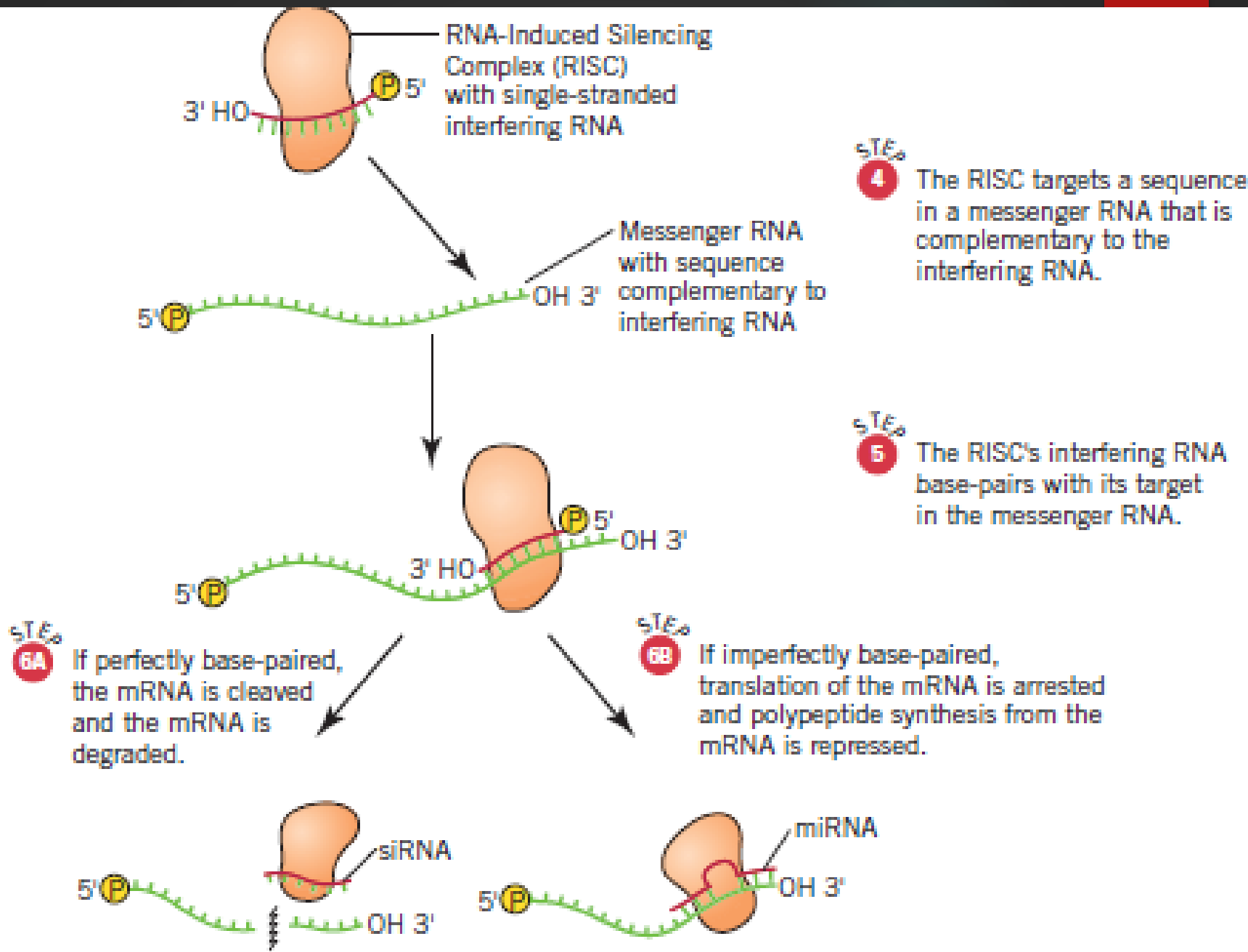
The small interfering RNAs and proteins assemble into ribonucleoprotein particles.

STEP
3

The small interfering RNA in a ribonucleoprotein particle is unwound to produce an RNA-Induced Silencing Complex (RISC).

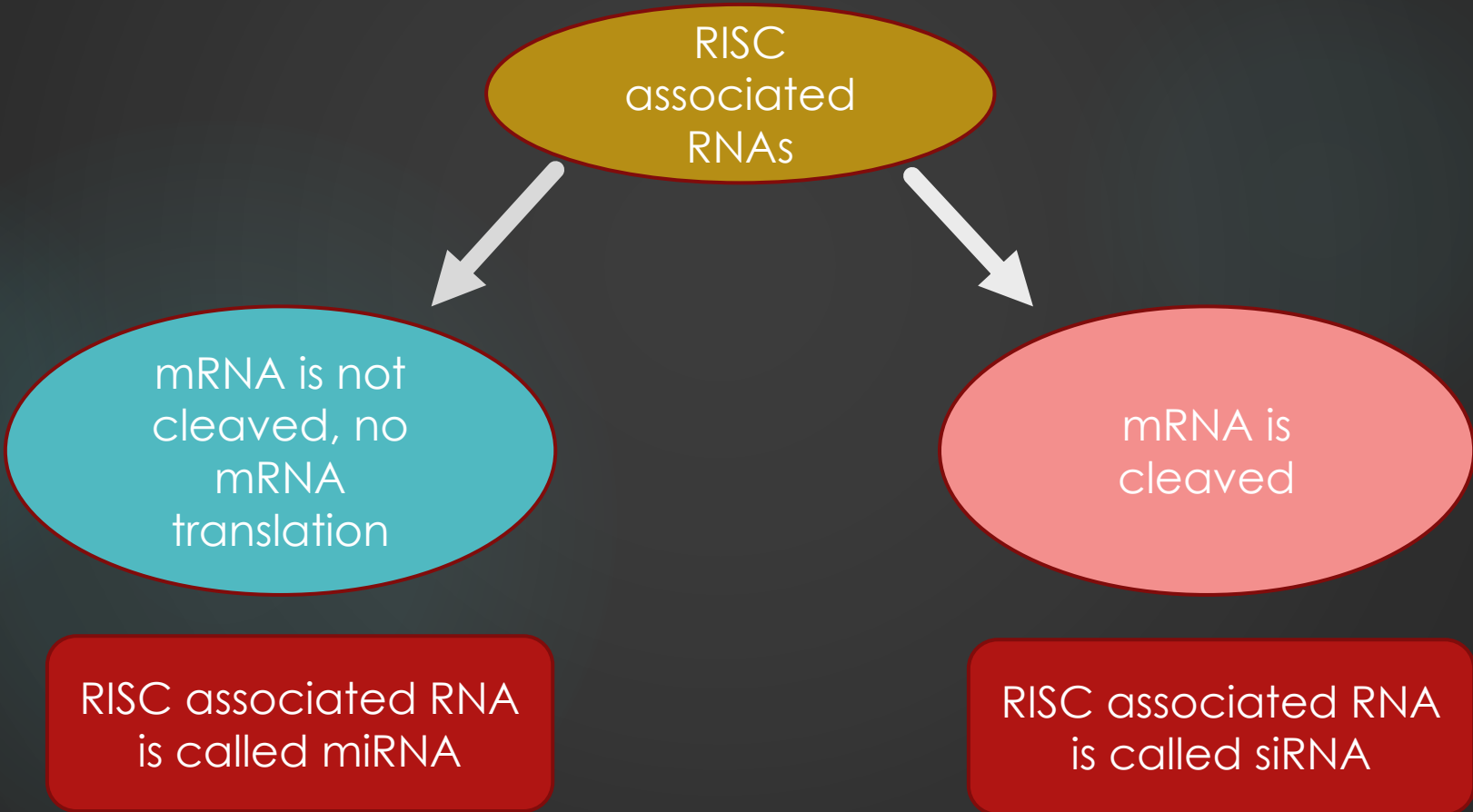


- ▶ “Argonaute” family of proteins: a single molecule or more (with unexplained function) is common in RISCs from different organisms.
- ▶ RISC cleaves at a site of mRNA present at middle of the perfectly or slightly imperfectly paired ssRNA-mRNA region.
- ▶ After cleavage of mRNA, it is degraded and the RISC can be reused in a second run of targeted mRNA cleavage.





► RISC can act as an catalyst as it can be used again and again to cleave the mRNA.



Locations of the mRNA sequences targeted by RISC in plants and animals are different

