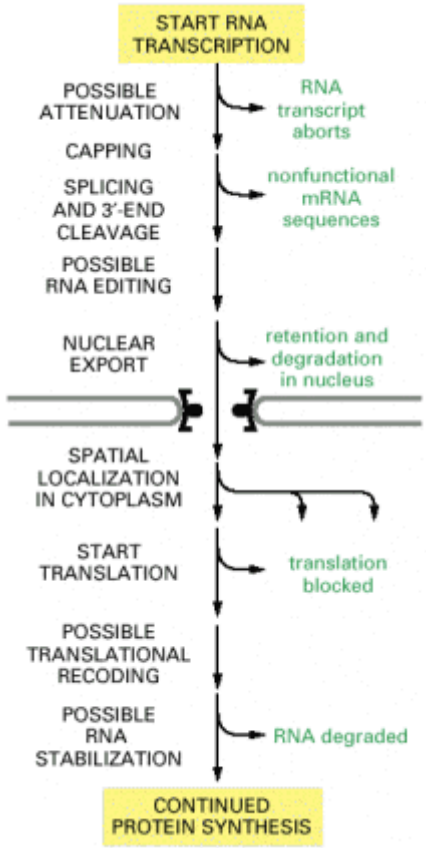
# Post-transcriptional control of gene expression

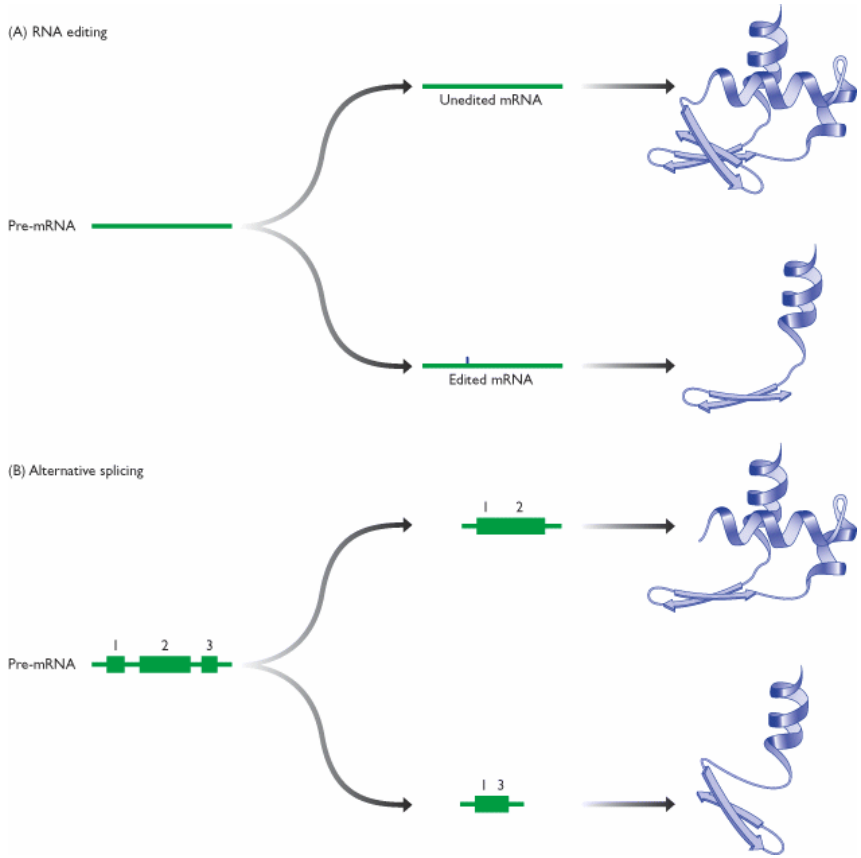
# Possible post-transcriptional controls on gene expression



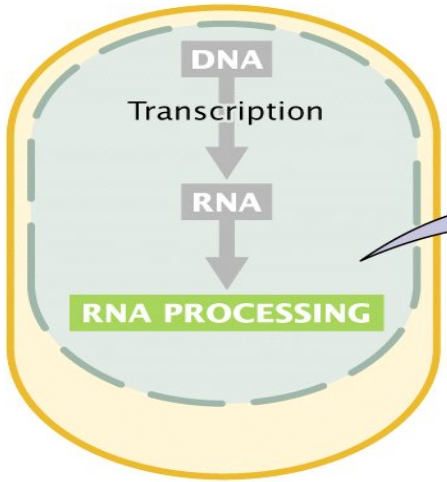

**Only a few of these controls are likely to be important for any one gene**

# *RNA editing*

- Mecanismo de processamento do RNA que altera a sequências dos mRNAs codificantes de proteínas (é diferente do *splicing* alternativo)
- As reacções de *editing* do RNA incluem: inserções, deleções, modificações de base antes da tradução
- É um processo controlado necessitando de um *guide RNA* (gRNA)
- Muito comum em mRNAs mitocondriais, cloroplastidiais. Menos frequente em mRNAs nucleares.
- Alterações específicas. Ex: desaminação de citosinas e adenosinas.

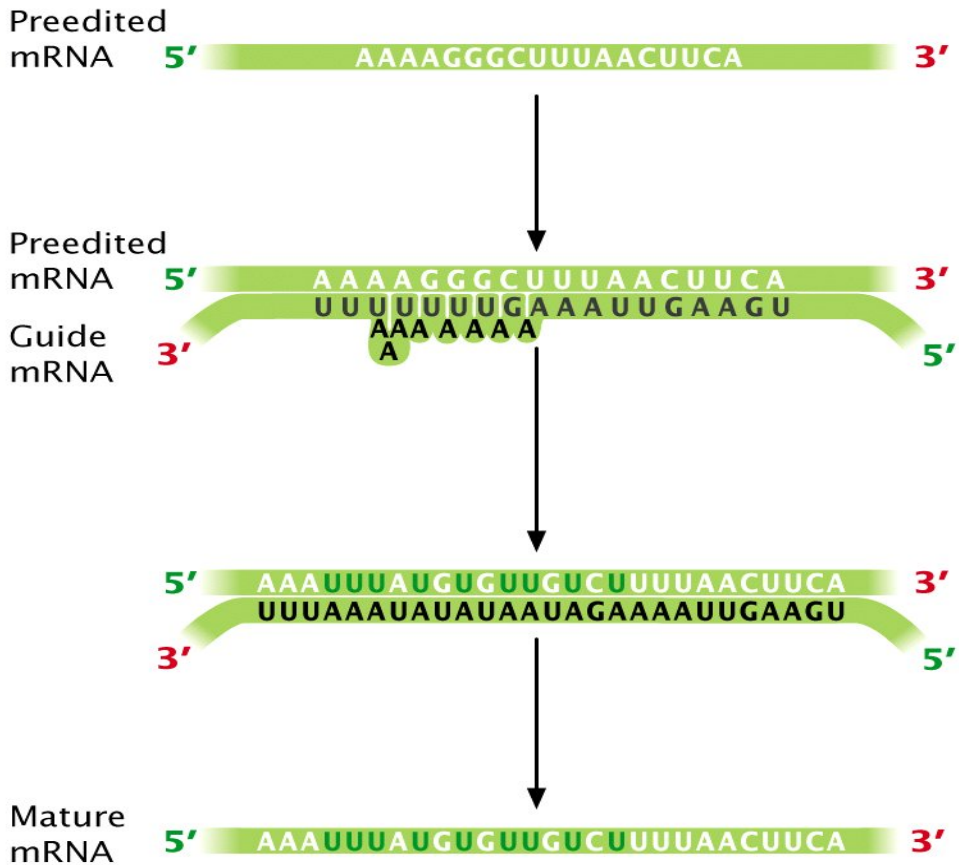


# RNA editing is carried out by guide RNAs

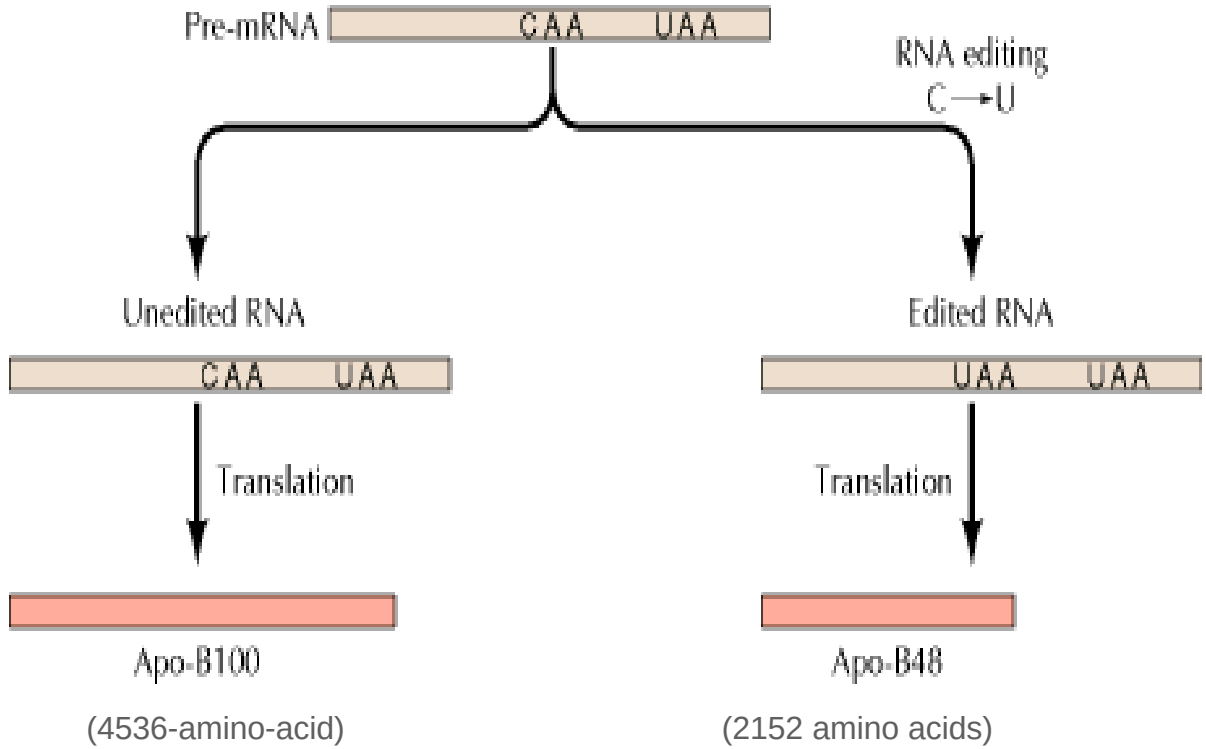



~~All information about a the aa sequence of a protein resides in the DNA~~

**Guide RNAs serves as a template for the addition, deletion or alteration of bases**



# Editing of apolipoprotein B mRNA



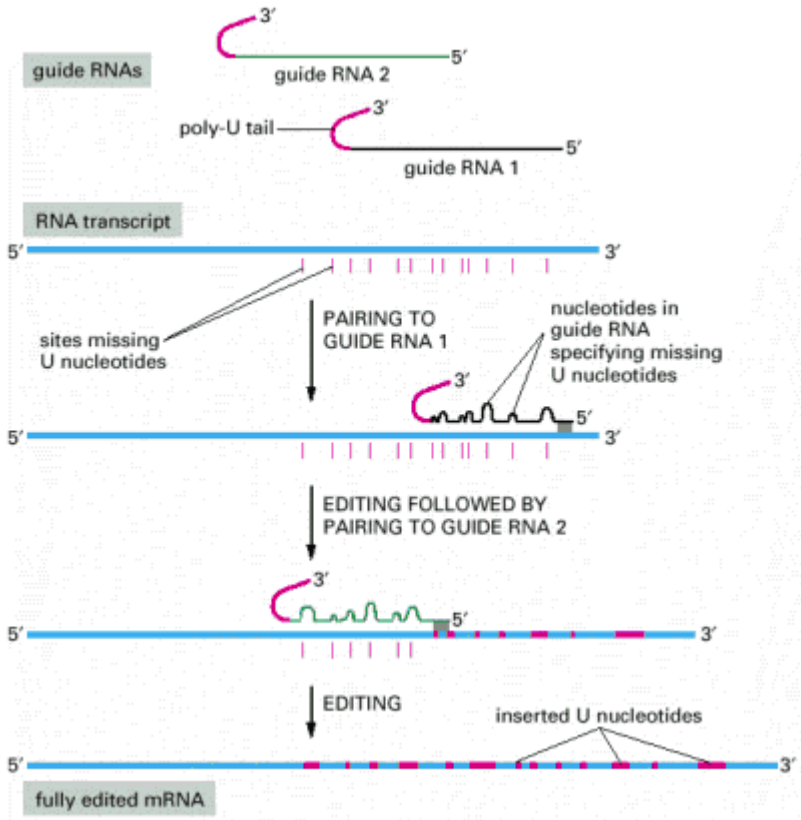

In **human liver**, unedited mRNA is translated to yield a full-length protein (Apo-B100)

Apo-100 is secreted into the blood carrying lipids all over the body

In **human intestine**, the mRNA is edited by a base modification that changes a specific C to a U.

Apo-B48 functions in the absorption of dietary lipids by the intestine

# RNA editing in the mitochondria of trypanosomes

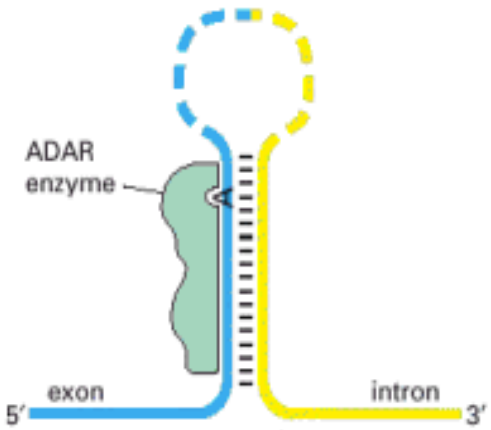



Editing generally starts near the 3’ end and progresses toward the 5’ end of the RNA transcript, as shown, because the "anchor sequence" at the 5’ end of most guide RNAs can pair only with edited sequences.

# Mechanism of A-to-I RNA editing in mammals

**The position of an edit is signaled by RNA sequences carried on the same RNA molecule**

Typically, a sequence complementary to the position of the edit is present in an intron,
and the resulting double-stranded RNA attracts the A-to-I editing enzyme ADAR.



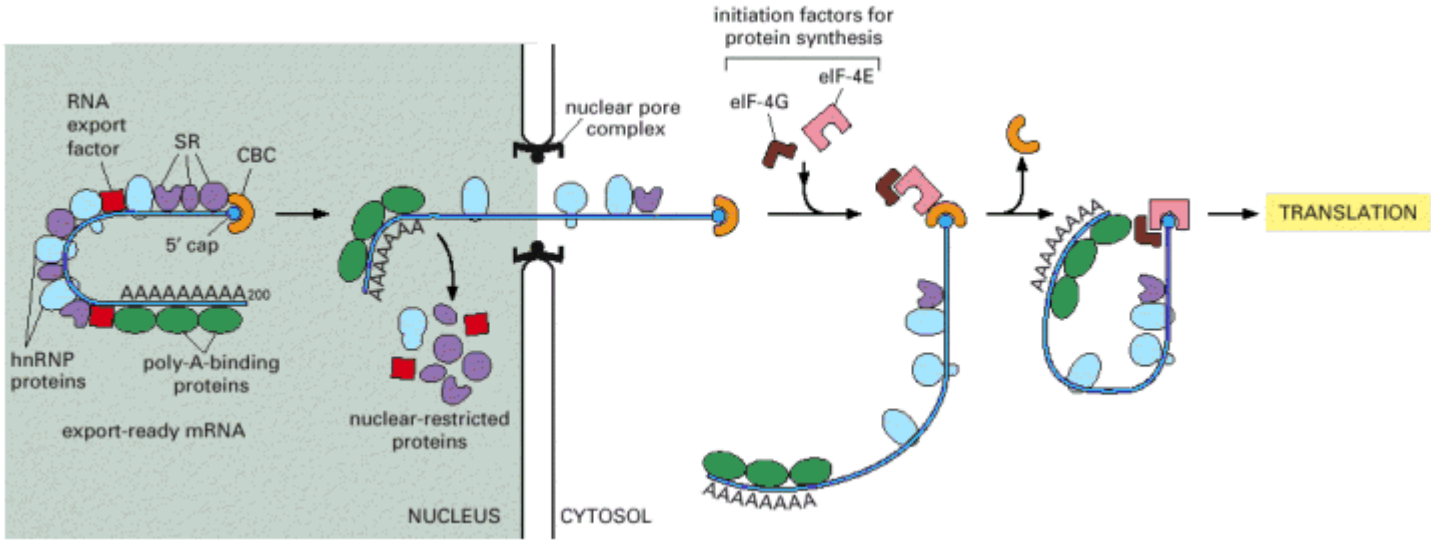

Mice and humans have three ADAR enzymes:
- ADR1 is required in the liver for proper red blood cell development,
- ADR2 is required for proper brain development and
- ADR3 the role of which is not yet known.

A- to –I, deamination of adenosine, converts it into inosine, that base pairs to cytidine

# Examples of RNA editing in mammals

| Tissue | Target RNA | Change | Comments |
|---|---|---|---|
| Intestine | Apolipoprotein B mRNA | C→U | Converts a glutamine codon to a stop codon |
| Muscle | α-galactosidase mRNA | U→A | Converts a phenylalanine codon into a tyrosine codon |
| Testis, tumors | Wilms tumor-1 mRNA | U→C | Converts a leucine codon into a proline codon |
| Tumors | Neurofibromatosis type-1 mRNA | C→U | Converts an arginine codon into a stop codon |
| B lymphocytes | Immunoglobulin mRNA | Various | Contributes to the generation of antibody diversity |
| HIV-infected cells | HIV-1 transcript | G→A,<br>C→U | Involved in regulation of the HIV-1 infection cycle |
| Brain | Glutamate receptor mRNA | A→inosine | Multiple positions leading to various codon changes |

# Schematic illustration of an "export-ready" mRNA molecule and its transport through the nuclear pore



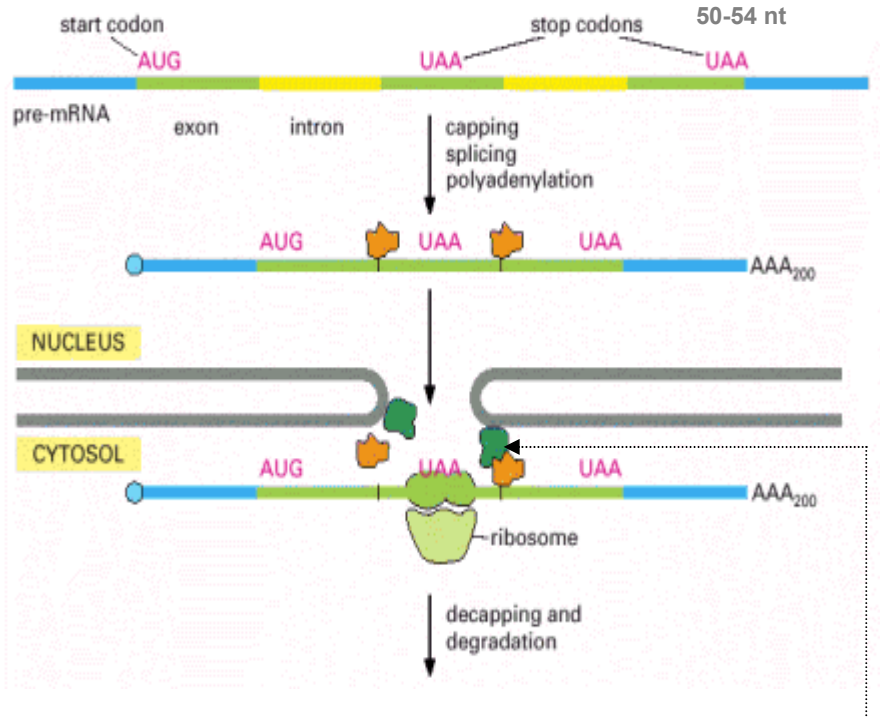

Some proteins travel with the mRNA as it moves through the pore, whereas others remain in the nucleus. In the cytoplasm, the mRNA continues to shed previously bound proteins and acquire new ones; these substitutions affect the subsequent translation of the message.

**Proteins that become bound to an mRNA, when transported, influence its stability and translation in the cytosol**.

RNA export factors, play an active role in transporting the mRNA to the cytosol. Some are deposited at exon-exon boundaries as splicing is completed, signifying those regions of the RNA that have been properly spliced

# A model for nonsense-mediated mRNA decay

**Nuclear proteins** mark the **exon-exon boundaries** on a spliced mRNA molecule. These proteins are thought to assemble in concert with the splicing reaction and may also be involved in the transport of mature mRNAs from the nucleus

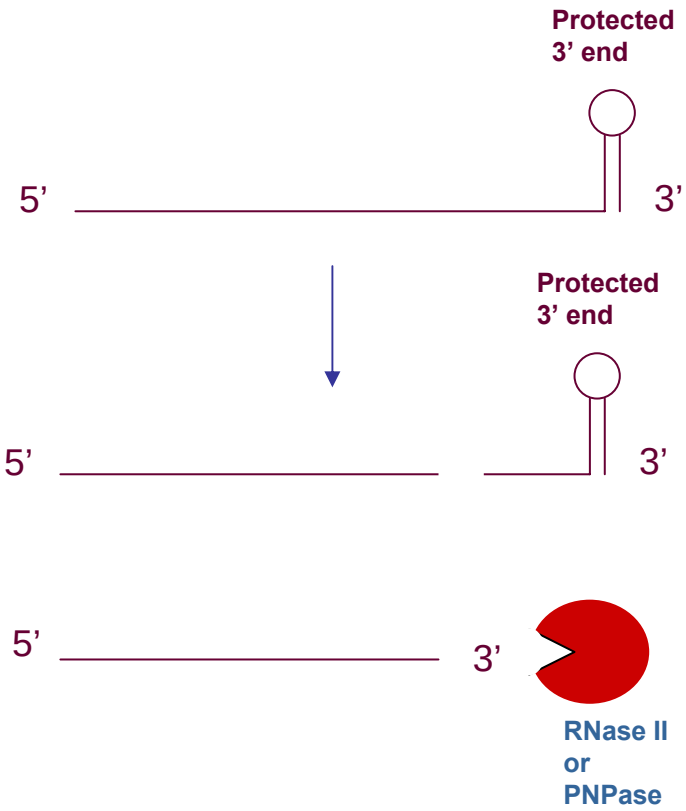



**"test" round of translation is performed by the ribosome**

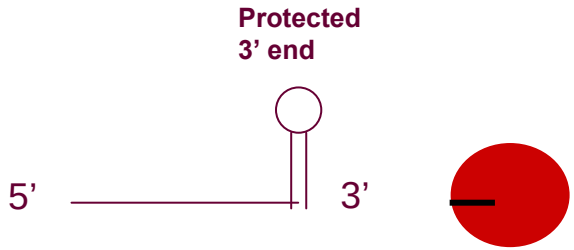
**surveillance proteins**

**If an in-frame stop codon is encountered before the final exon-exon boundary is reached, the mRNA is subject to nonsense-mediated decay.**

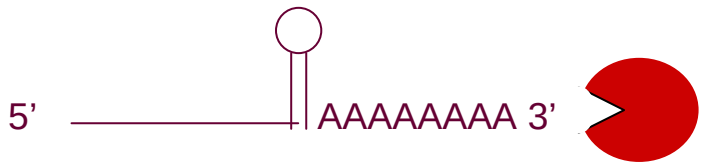
# mRNA degradation in eubacteria



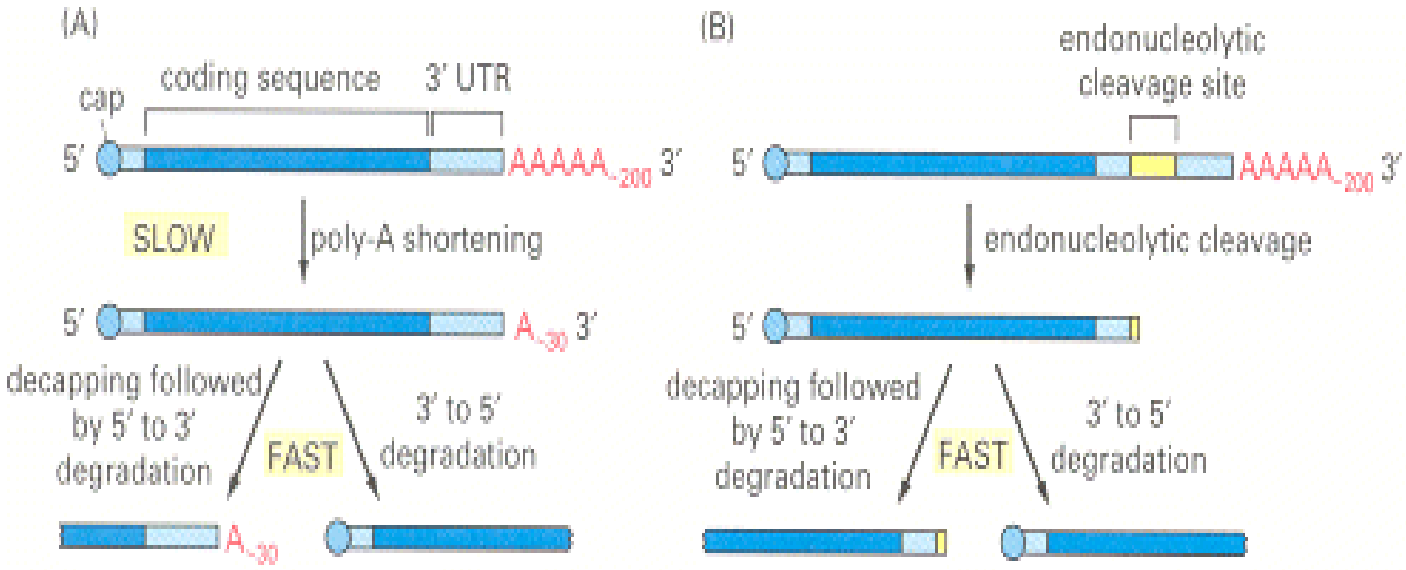

Poly-A promoted degradation




The ***E. coli*** **degradosome**
-RNA helicase
-Exonuclease (PNPase, RNase II)
-Endonuclease (ex. RNase E, RNase III)



# Two mechanisms of eucaryotic mRNA decay




Decapping prevents translation

**Deadenylation-dependent decay**

Most eucaryotic mRNAs are degraded by this pathway. The critical threshold of poly-A tail length that induces decay may correspond to the loss of the poly-A binding proteins

**Deadenylation-independent decay (or decapping pathway)**

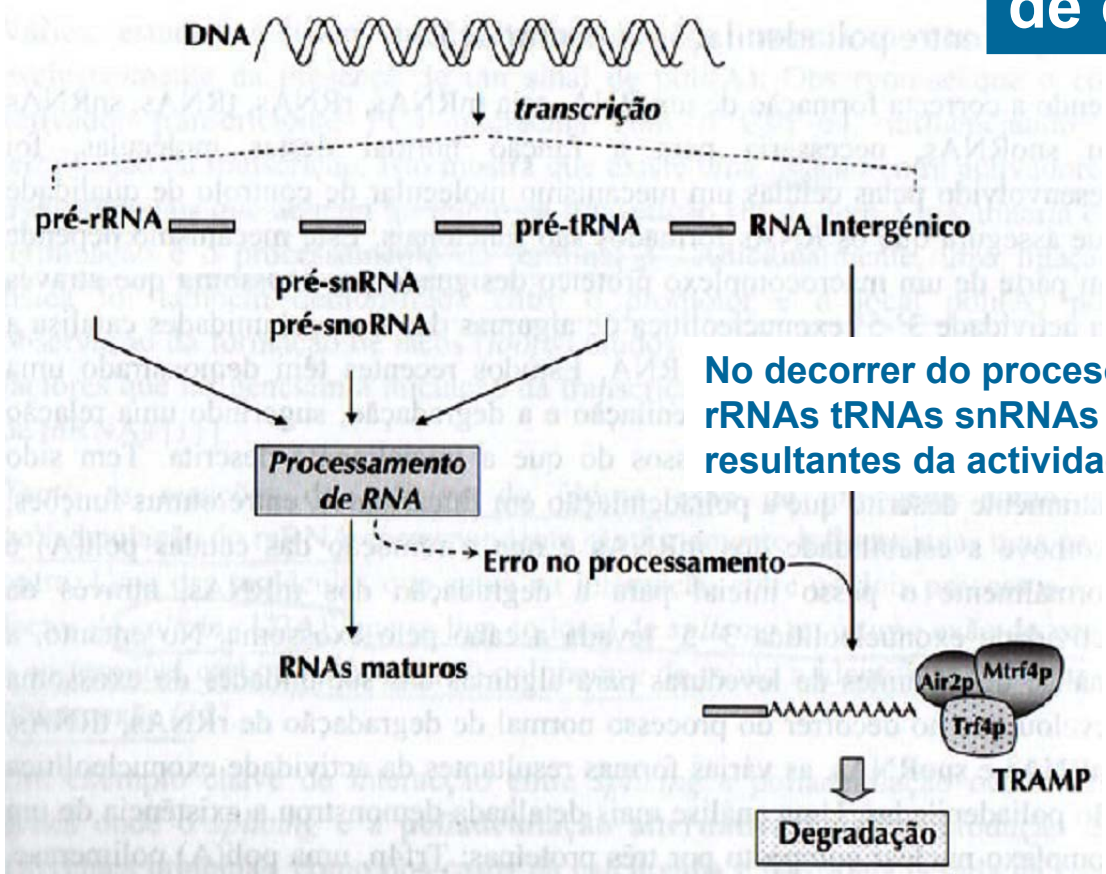
It is not yet known with certainty whether decapping follows endonucleolytic cleavage of the mRNA

The **deadenylation enzyme** associates with both the 3 poly-A tail and the 5' cap, and this arrangement may coordinate decapping with poly-A shortening. Although 5 to 3 and 3 to 5 degradation are shown on separate RNA molecules, these two processes can occur together on the same molecule

**Instability elements**

# Poliadenilação vs degradação

## Mecanismo molecular de controlo de qualidade




No decorrer do proceso normal de degradação dos rRNAs tRNAs snRNAs e snoRNAs, as varias formas resultantes da actividade exonucleolítica são poliadeniladas

O complexo TRAMP juntamente com o exossoma, encontra-se envolvido
no normal processamento de RNAs no núcleo

# RNAi
# (RNA interference)

## PTGS (Post-Transcriptional Gene Silencing)

## General mechanism of RNAi

## siRNAs and miRNAs

# Discovery

- The term **“RNA interference”** (RNAi), was first described for the worm *Caenorhabditis elegans* in **1993** by R. C. Lee of Harvard University to describe targeted destruction of endogenous mRNA upon injection of short dsRNAs into *C. elegans*. It is a PTGS pathway that results in mRNA degradation

- RNAi has been also observed in plants and *Drosophila* for a number of years. Recently RNAi was discovered to work in mammalian system

# RNA interference or RNA silencing

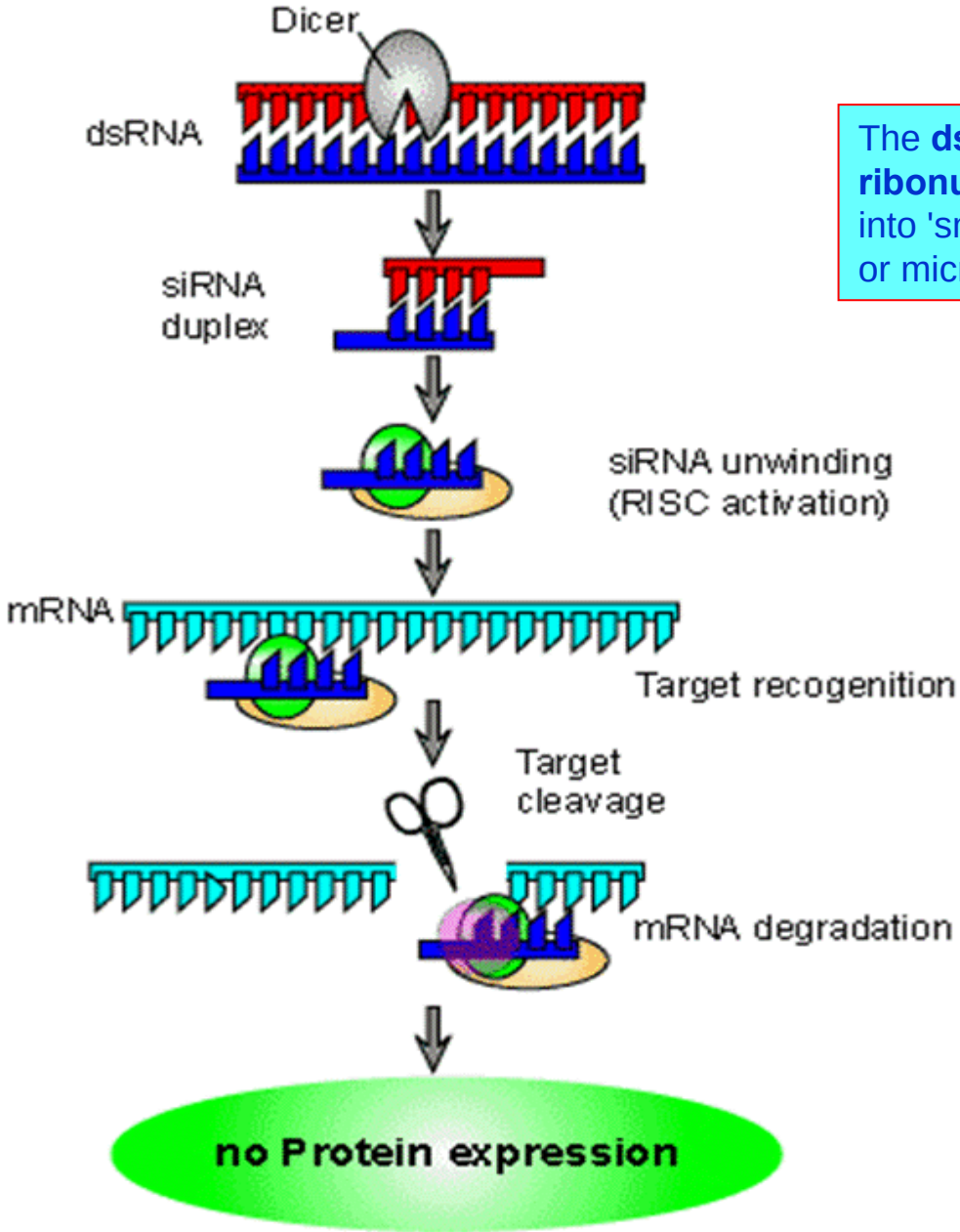
- Post-transcriptional gene silencing
- Co-supression in plants
- RNA-mediated virus resistance in plants
- RNA interference in animals
- Silencing in fungi
- RNA silencing

# PTGS

## (Post-Transcriptional Gene Silencing)

- Naturally occurring cellular process
- Uses double-stranded RNA (dsRNA) to direct homology-dependent suppression of gene expression
- Originally identified as a defense mechanism against foreign genetic material, but it is evolutionarily ancient, an important biological system with roles in transposon silencing and endogenous gene regulation
- Described in: fungi, plants, worms, flies, birds and mammals
- PTGS is not a single, independent cellular response
- Consists of multiple overlapping pathways responsible for sequence-specific gene interference via several mechanisms, like:
  - mRNA degradation, translational inhibition, and even transcriptional silencing through chromatin remodeling.

# General mechanism of action of RNAi




The **dsRNA** molecule is **broken down** by the **Dicer ribonuclease (dsRNA-specific endonuclease)** into 'small interfering RNAs' **(siRNAs)** or microRNAs **(miRNAs)** of **21 -25 bp dsRNA.**

**siRNA** assembles into **RNA-induced silencing complexes (RISCs),** and it then activates the complex by **unwinding its RNA strands**

**The unwound RNA strands subsequently guide the complex to the complementary RNA molecules, where the complex cleaves and destroys the cognate RNA, which results in RNAi phenomenon**

# siRNA and miRNA

- **Two classes of short dsRNA** molecules:
  - **small interfering RNA (siRNA),** *Exogenous origin (ex. viral)*
  - **microRNA (miRNA),** *Endogenous origin*

  have been identified as sequence-specific posttranscriptional regulators of gene expression.

- siRNA and miRNA are incorporated into related

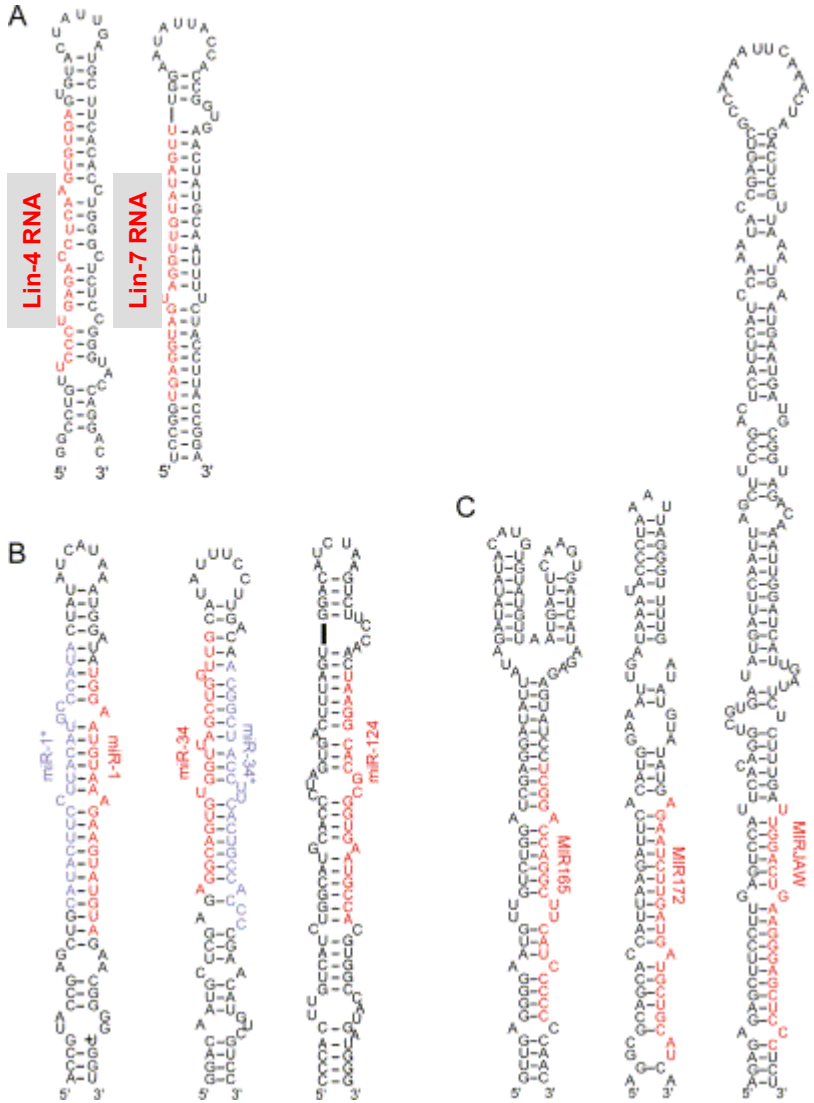
**RNA-induced silencing complexes (RISCs)**
(siRISC and miRISC)

# miRNA: definition and processing

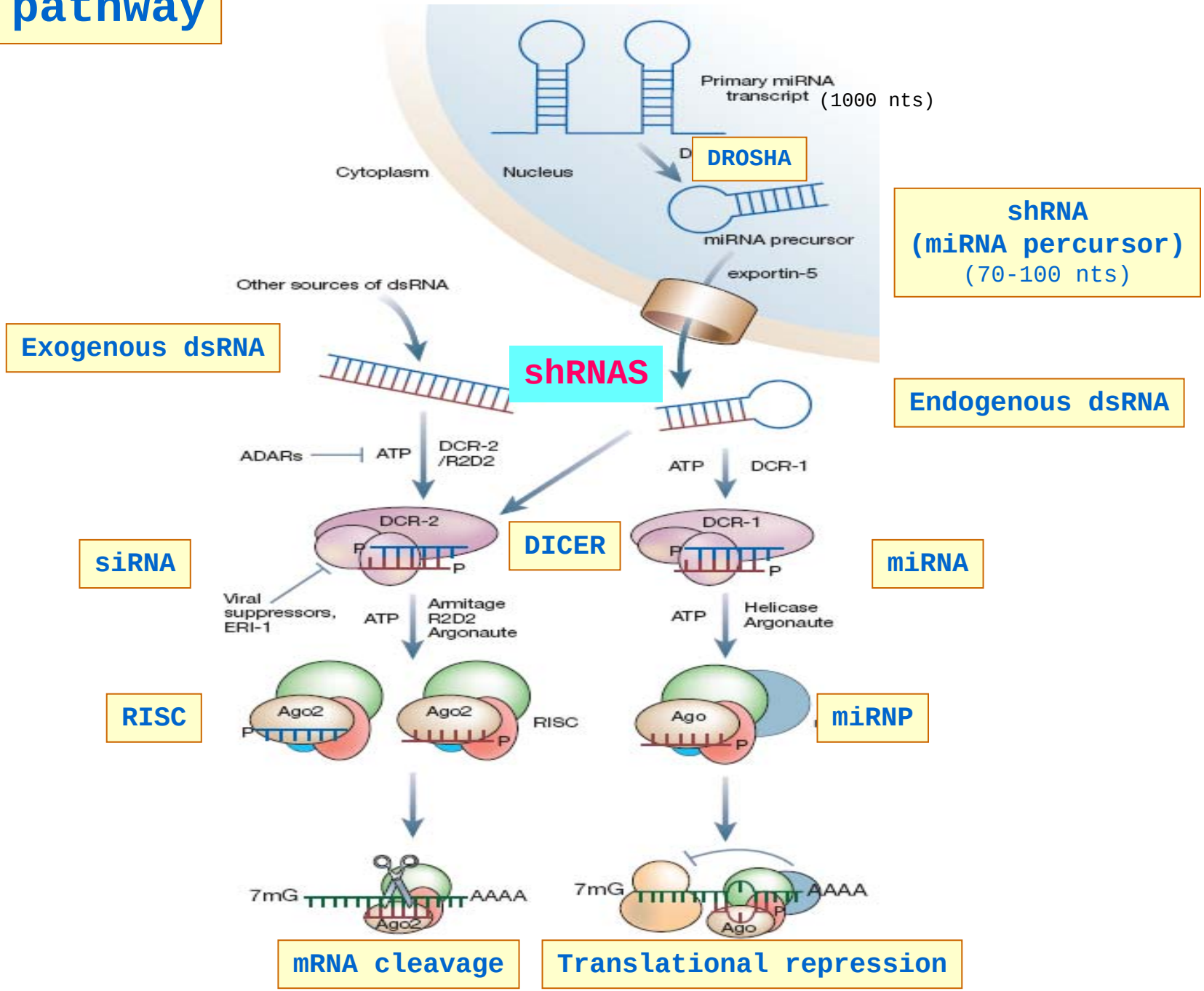
- miRNA are short **endogenous noncoding** RNA molecules. Function as guide molecules in diverse silencing pathways
- miRNAs genes are transcribed from DNA, but are **not translated**
- The DNA sequence that codes for an miRNA gene is **longer** than the miRNA. This forms a primary miRNA structure (**pri-miRNA**) which is a **double stranded RNA hairpin loop**
- In **ANIMALS**, the nuclear enzyme **Drosha** cleaves the base of the hairpin to form **pre-miRNA**. The pre-miRNA molecule is then actively transported out of the nucleus into the cytoplasm by **Exportin 5**, a carrier protein.
- The **Dicer** enzyme cuts **20-25 nucleotides** from the base of the hairpin to release the mature **miRNA**.
- In **PLANTS**, which lack Drosha homologues, pri- and pre-miRNA processing by **Dicer** probably, takes place in the nucleus, and mature miRNA duplexes are exported to the cytosol by Exportin 5.

# Examples of Metazoan pri-miRNAs

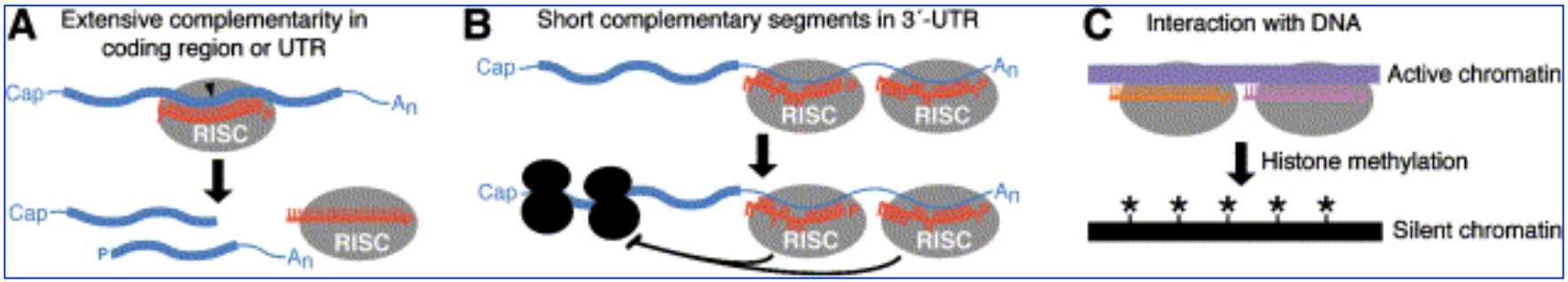
The initial **stem−loop configuration** of the primary transcript provides structural clues that have been used to guide **searches of genomic sequence** for candidate miRNA genes



# RNAi pathway



# The actions of Small Silencing RNAs




Messenger RNA cleavage specified by a miRNA or siRNA.

↓ site of cleavage

Translational repression specified by miRNAs or siRNAs

Transcriptional silencing, thought to be specified by heterochromatic siRNAs

*RNA directs DNA methylation?*
*RNA induces chromatin modifications?*
*RNA blocks promoter sequences?*

RISC- RNA-induced silencing complex (mRNA degradation)
RITS- RNA-induced translational silencing (chromatin modification)
miRNP (translational repression)

**IMPORTANT**

Genes

have been found in

**BACTERIA**

that are similar in the sense that they

control mRNA abundance or translation by binding an mRNA by base pairing,

**however they**

**are not generally considered to be miRNAs**

**because the**

**Dicer enzyme is not involved**

# Summary

**dsRNA is an important regulator of gene expression in many eukaryotes; it triggers different types of gene silencing that are collectively referred to as RNA silencing or RNA interference**

**Key step** → **processing of dsRNA (microRNAs percursors)**
(variable lenght and origin)
**into short RNA duplexes (siRNAs or miRNAs )**

**dsRNA guide RNA silencing by specific and distinct mechanisms**

**RNA cleavage**

**Translational repression of complementary ssRNAs (mRNAs, or viral genomic/antigenomic RNAs)**

**Guiding chromatin modification**

**miRNAs could be as important as transcription factors in regulating gene expression in higher eukaryotes**