
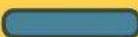





## **RNA** **RIBONUCLEIC ACID**



-  **ADEINE**
-  **URACIL**
-  **GUANINE**
-  **CYTOSINE**
-  **PHOSPHATE  
BACKBONE**

# **Regulatory RNAs**

**殷昊**

**2023年5月19日**



# What is regulatory RNAs ?



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- ◆ Regulatory RNAs are a class of **non-coding RNA molecules** that play important roles in gene expression regulation.
- ◆ Regulatory RNAs interact **with DNA, RNA, and/or proteins** to modulate gene expression at transcriptional, post-transcriptional, and/or epigenetic levels.
- ◆ Regulatory RNAs play important roles in cellular differentiation, development, immune response, and disease processes.



# Types of regulatory RNAs



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- **MicroRNAs (miRNAs)**
- **Small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs)**
- **Long non-coding RNAs (lncRNAs)**
- **Piwi-interacting RNAs (piRNAs)**
- **Circular RNAs (circRNAs)**
- **Small nucleolar RNAs (snoRNAs)**
- **Enhancer RNAs (eRNAs)**
- **tRNA-Derived Small RNAs**



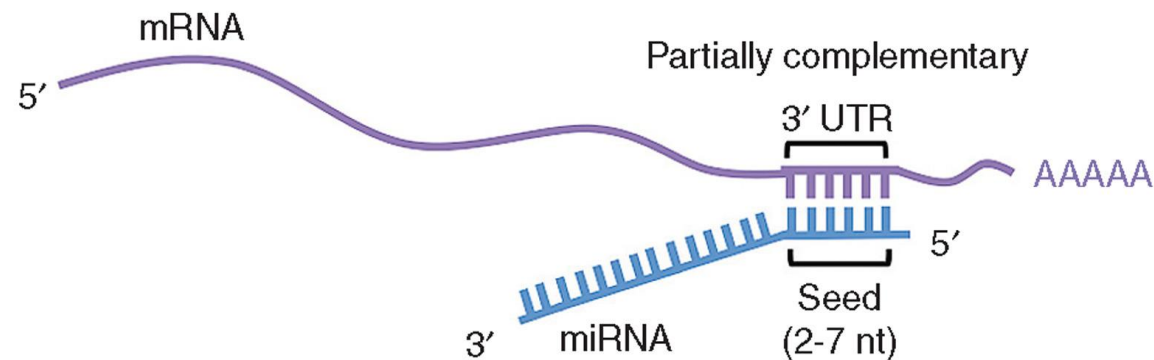
# MicroRNAs (miRNAs)

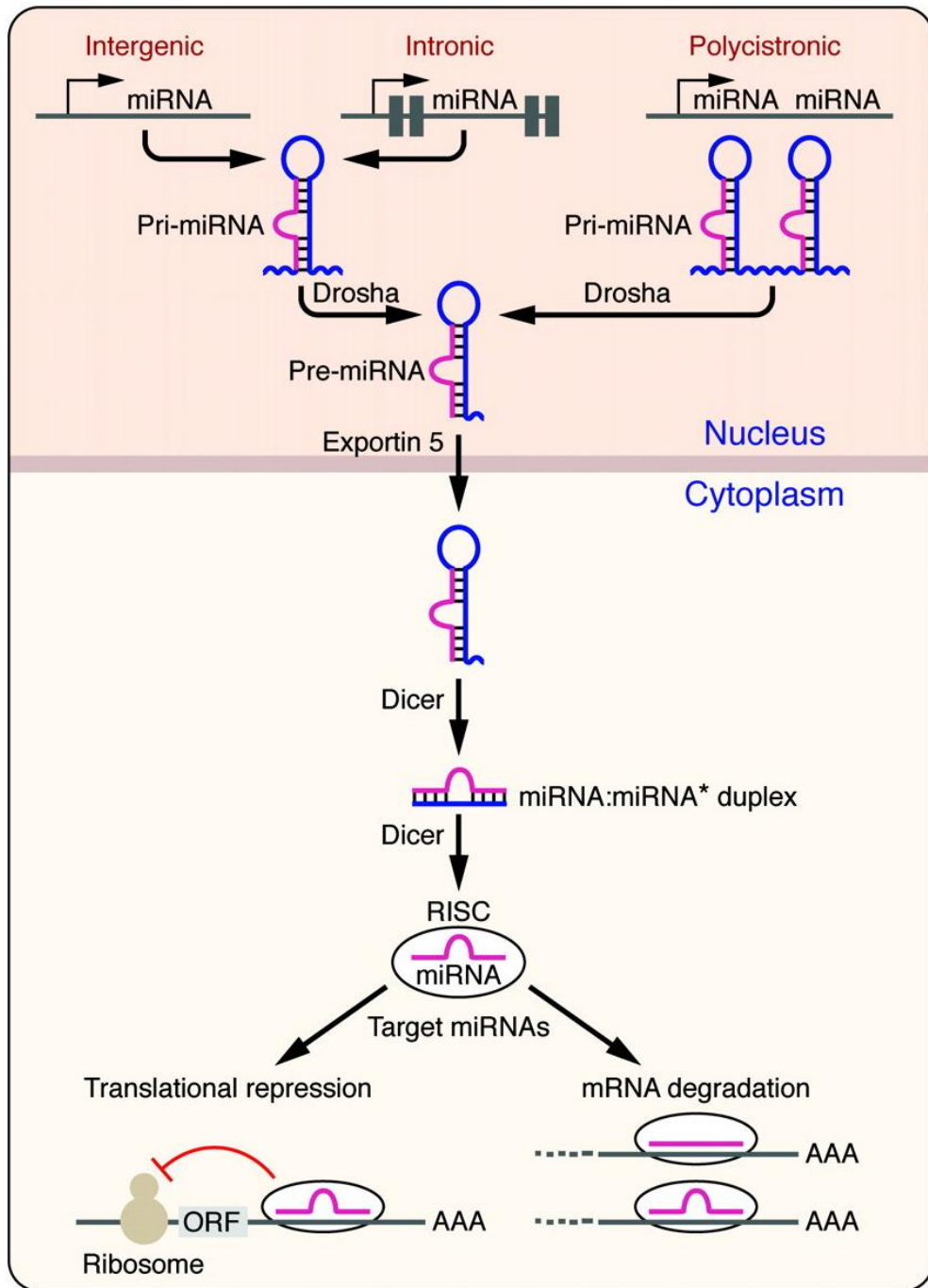


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- miRNAs are **small endogenous RNAs**, ~21-25 nt in length.
- Typically miRNAs interact with specific mRNAs through complementary base-pairing to **influence the translation or stability** of the target mRNA molecule.

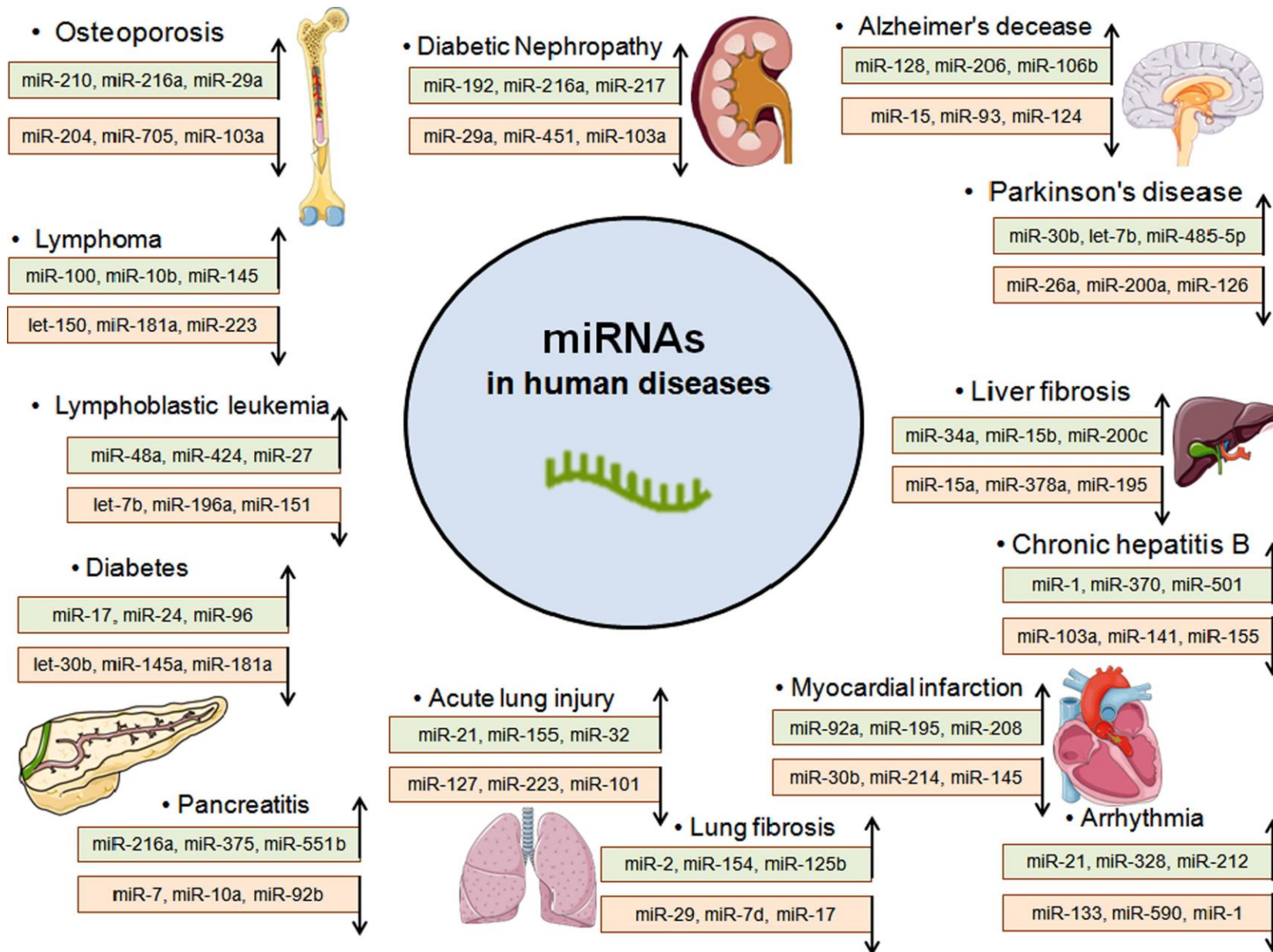




**Drosha**—An endonuclease that processes double-stranded primary RNAs into short, ~70 base pair precursors for Dicer processing.

**Dicer**—An endonuclease that processes double-stranded precursor RNA to 21 to 23 nucleotide RNAi molecules.

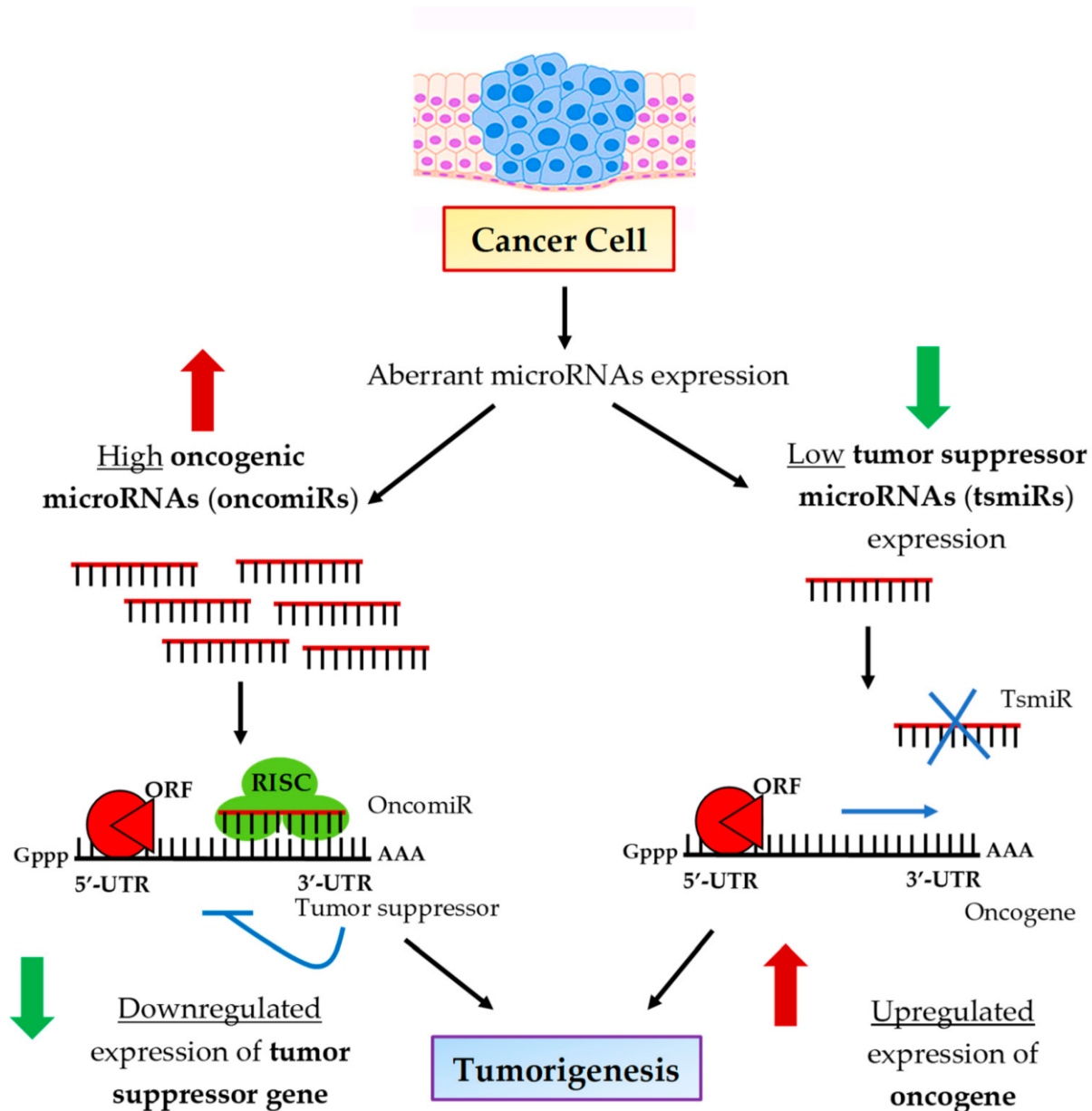
**RISC**—RISC—RNA-induced silencing complex is a complex composed of RNA molecules and proteins. Its main function is to mediate **RNA interference (RNAi)** and **RNA target regulation** by binding to **miRNA or siRNA**, thereby inhibiting or reducing the expression of specific target genes.



- Early disease diagnosis
- Disease prognosis evaluation
- New drug target screening



# miRNA and Cancer



**Dysregulation of miRNA expression** has been observed in various types of cancer, and abnormal miRNA expression can contribute to the initiation and progression of cancer by regulating the expression of oncogenes or tumor suppressor genes.



# Types of regulatory RNAs



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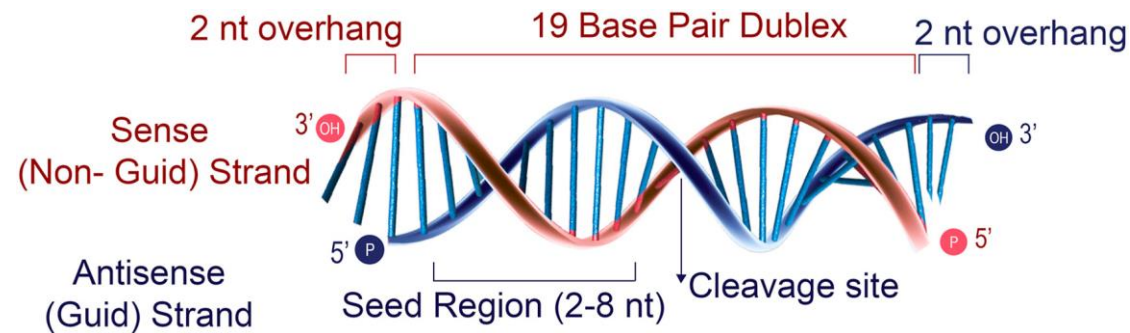




# Small interfering RNAs (siRNAs)

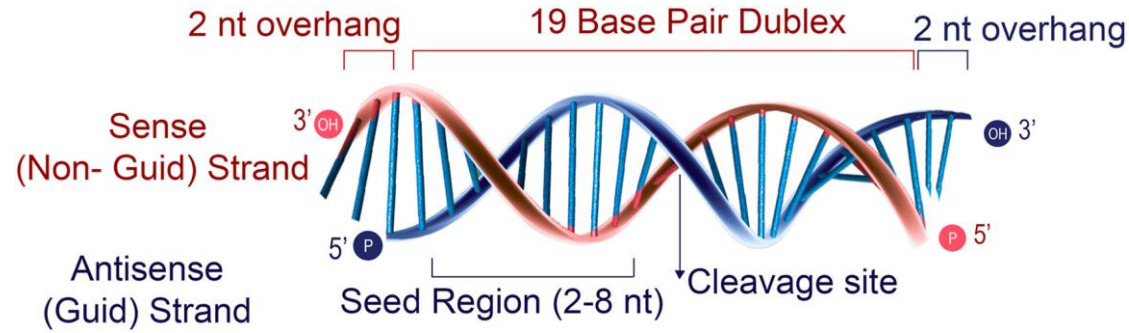
- ◆ Small interfering RNA (siRNA): 1998 by Andrew Fire and Craig Mello, who were awarded the Nobel Prize in Physiology or Medicine in 2006 for their discovery.
- ◆ They found that RNA interference (RNAi), a process in which RNA molecules can silence or "interfere" with gene expression, could be induced in cells by introducing double-stranded RNA (dsRNA).

## The siRNA structure:





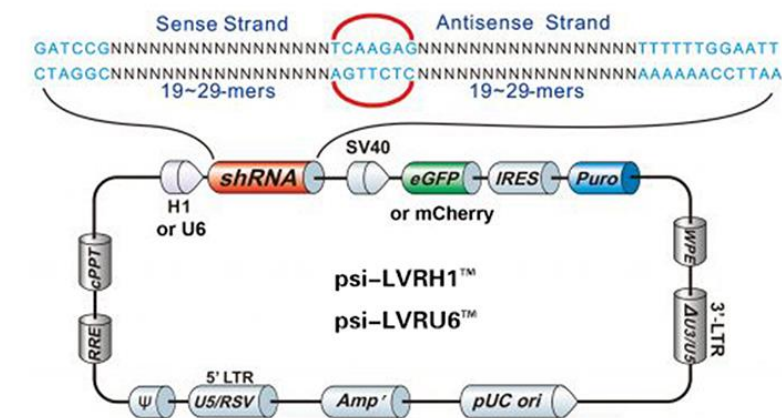
# siRNA structure



- siRNA is a small, double-stranded RNA molecule that typically contains 21-23 nucleotides in each strand.
- One of the strands, called the **guide strand**, is loaded into a multi-protein complex called the RNA-induced silencing complex (RISC), while the other strand, known as the passenger strand (Non-Guide strand), is degraded.



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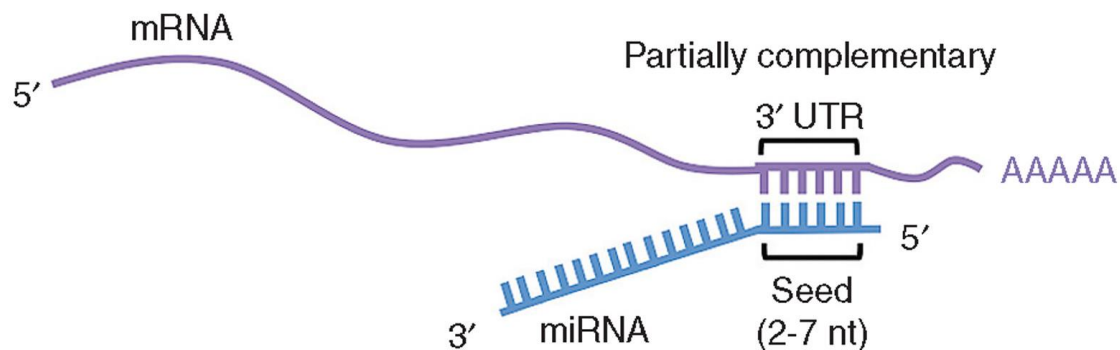




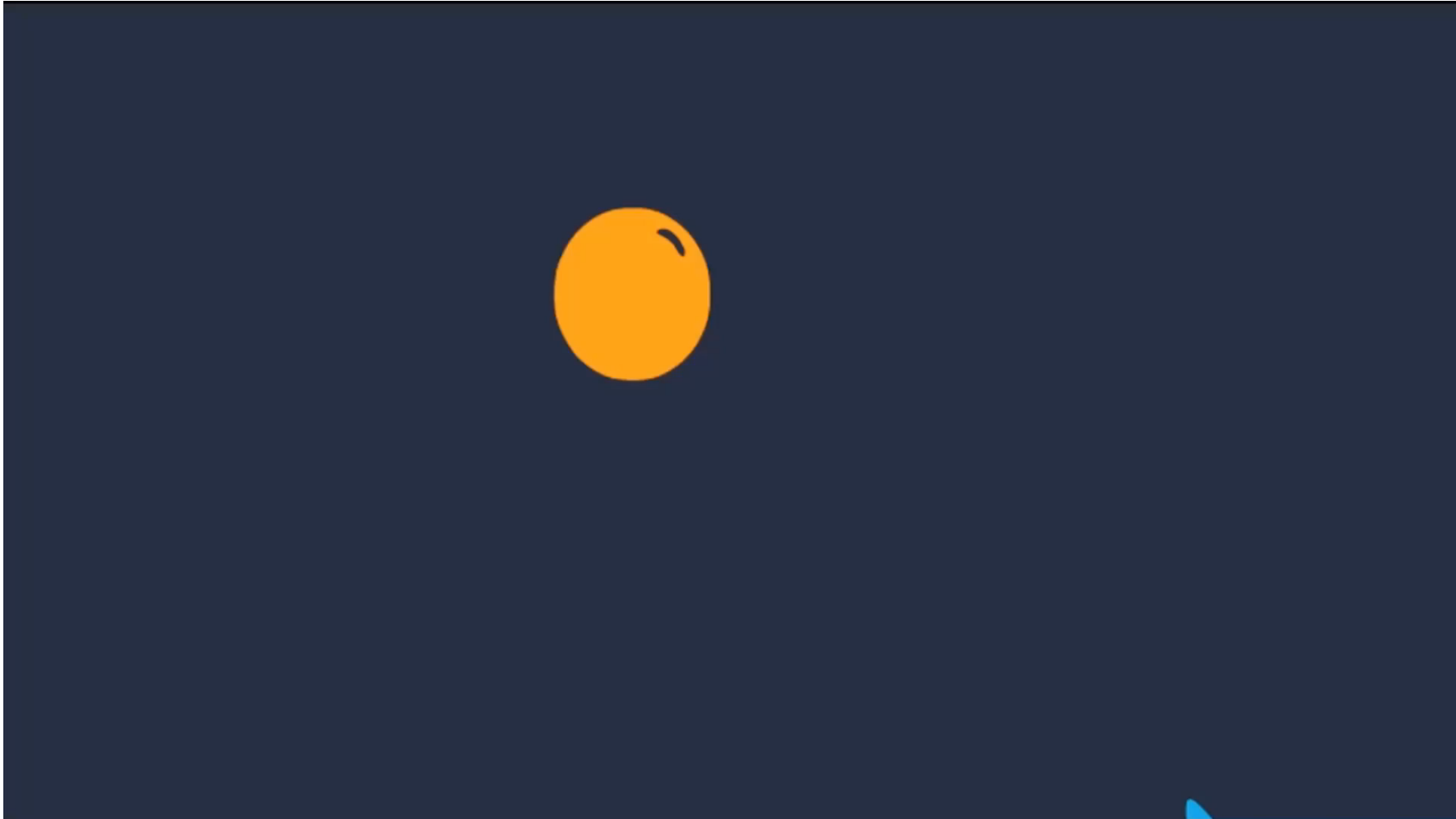
# Comparison between miRNA and siRNA

	miRNA	siRNA
序列长度	<ul style="list-style-type: none"><li>• ~22 nt dsRNA</li></ul>	<ul style="list-style-type: none"><li>• ~22 nt dsRNA</li></ul>
功能状态	<ul style="list-style-type: none"><li>• Dicer酶切割产物</li><li>• 与AGO2结合后获得功能</li></ul>	<ul style="list-style-type: none"><li>• Dicer酶切割产物</li><li>• 与AGO2结合后获得功能</li></ul>
序列来源	<ul style="list-style-type: none"><li>• 内源性：细胞自身合成的天然分子；来源于具“茎-环”结构的miRNA前体</li></ul>	<ul style="list-style-type: none"><li>• 外源性：人工合成和病毒感染</li><li>• 内源性：asRNA与mRNA形成的dsRNA</li></ul>
表达特性	<ul style="list-style-type: none"><li>• 表达具有时序性、保守性、组织特异性和疾病特异性</li></ul>	<ul style="list-style-type: none"><li>• 由人工导入或病毒感染</li></ul>
主要作用	<ul style="list-style-type: none"><li>• 转录后基因微调，mRNA功能下调</li></ul>	<ul style="list-style-type: none"><li>• 转录后基因沉默，mRNA功能丧失</li></ul>
作用机制	<ul style="list-style-type: none"><li>• 抑制蛋白翻译</li><li>• 降解靶mRNA</li><li>• 种子序列（5'端2-8位7个碱基）与靶基因3'UTR互补配对结合</li></ul>	<ul style="list-style-type: none"><li>• 降解靶 mRNA</li><li>• 以其全序列与靶mRNA编码区结合，位点完全互补结合</li></ul>

	miRNA	siRNA
作用方式	<ul style="list-style-type: none"> <li>种子序列特异性</li> <li>多靶基因作用</li> <li>基因抑制强度效率较低</li> </ul>	<ul style="list-style-type: none"> <li>基因特异性</li> <li>单靶基因作用</li> <li>基因抑制强度效率极高</li> </ul>
应用潜力	<ul style="list-style-type: none"> <li>可接受miRNAi的干扰而改变功能状态（增强或抑制）</li> <li>可在体液中检测和定量</li> <li>可能作为人类疾病诊断和预后的生物标志物</li> <li>可作为人类疾病治疗靶点而发展 miRNA—based 新药</li> </ul>	<ul style="list-style-type: none"> <li>本身就是RNAi的“执行者”</li> <li>治疗“药物”</li> </ul>



# RNA interference (RNAi)





# Types of regulatory RNAs



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- Enhancer RNAs (eRNAs)
- tRNA-Derived Small RNAs
- Antisense RNAs





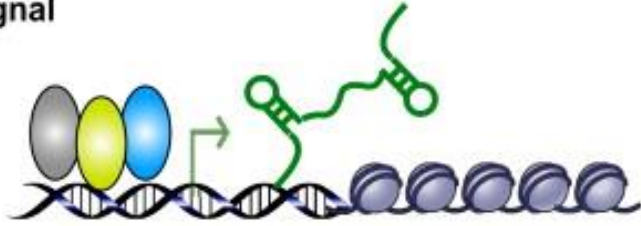
# Long non-coding RNAs (lncRNAs)

- Long non-coding RNAs (lncRNAs) are RNA molecules that are longer than 200 nucleotides and do not encode proteins.
- The majority of lncRNAs are transcribed by RNA polymerase II.
- They are widely present in cells and are closely related to various biological processes, including gene expression regulation, cell cycle regulation, cell differentiation, and tumor formation.

**Video:**

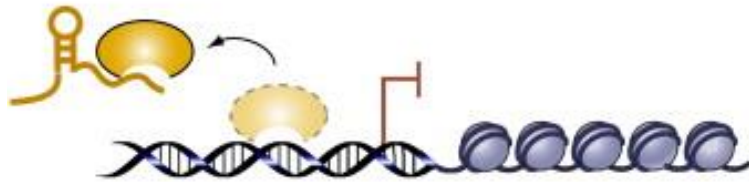


#### I. Signal



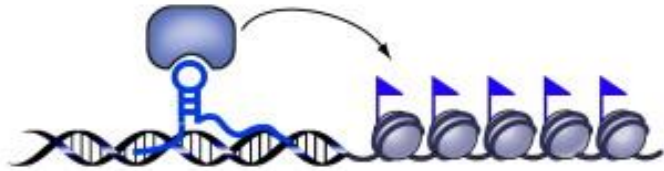
- lncRNAs as signals marking space, time, developmental stage, and expression for gene regulation.

#### II. Decoy



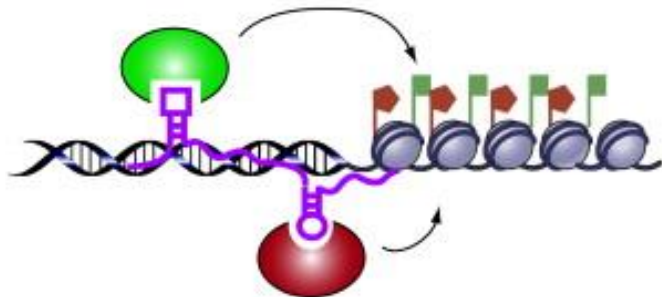
- lncRNAs bind to specific protein targets and occupy their active sites, thereby inhibiting their function (transcription factors, chromatin modifiers, or other regulatory factors).

#### III. Guide



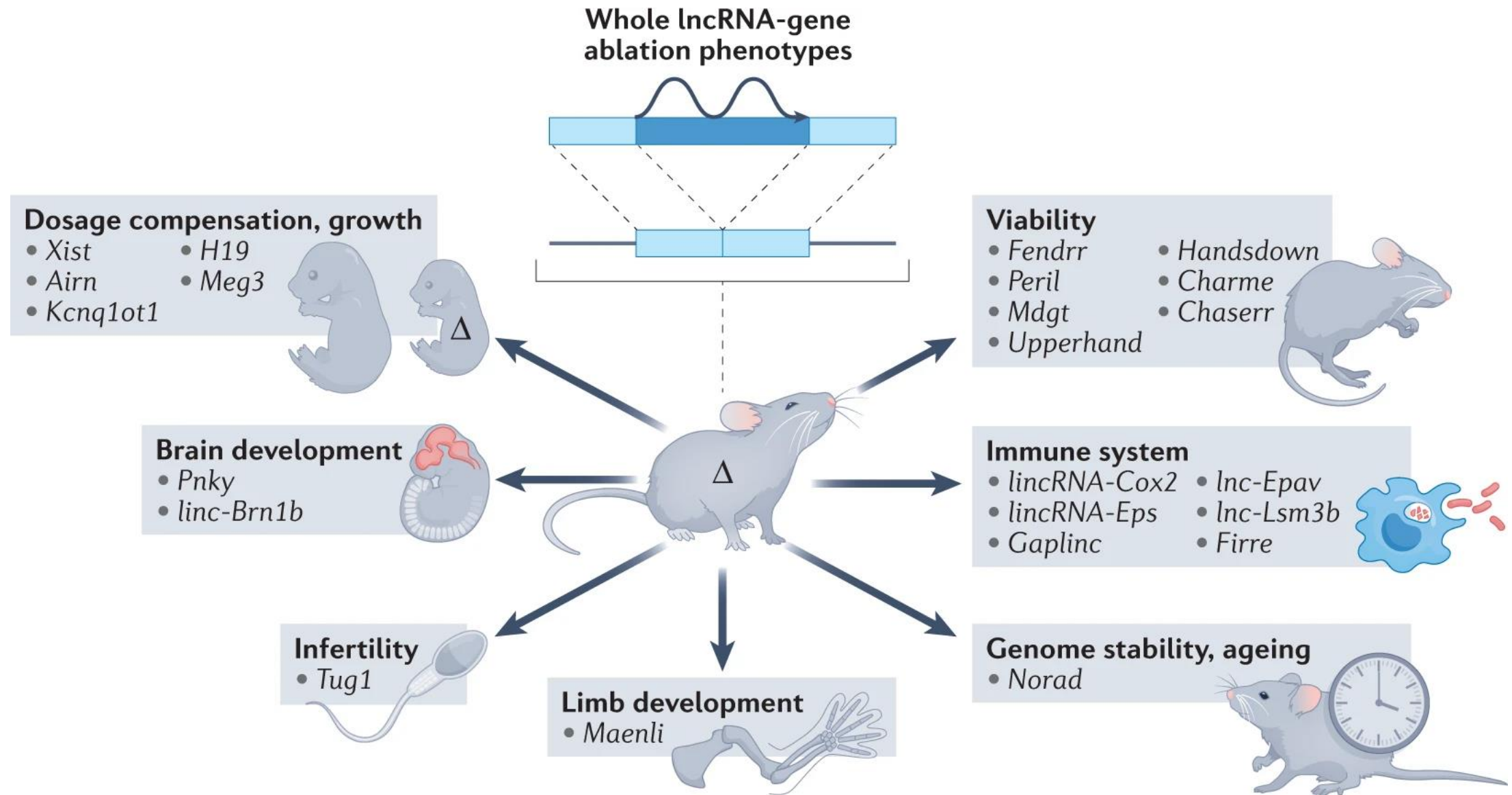
- lncRNAs act as guides and direct ribonucleoprotein complexes to specific targets. lncRNAs can guide changes in gene expression either in *cis* or in *trans*.

#### IV. Scaffold



- lncRNAs can function as scaffolds, serving as central platforms for the assembly of multiple effector molecules.

# Visible phenotypes of mutations in long non-coding RNA genes in mice





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# Piwi-interacting RNAs (piRNAs)



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- piRNAs were first discovered in *Drosophila* and were later found to be present in many other eukaryotes including mammals.
- piRNA (piwi-interacting RNA) is typically 24-35 nucleotides in length and interacts with proteins of the **PIWI** clade of the Argonaute family.
- piRNAs are mainly expressed in **germ cells** and function in transposon silencing.
- The main function of piRNA is to regulate genome stability and protect germ cells from invasion by exogenous genetic materials.

Video:

## Non-coding RNAs

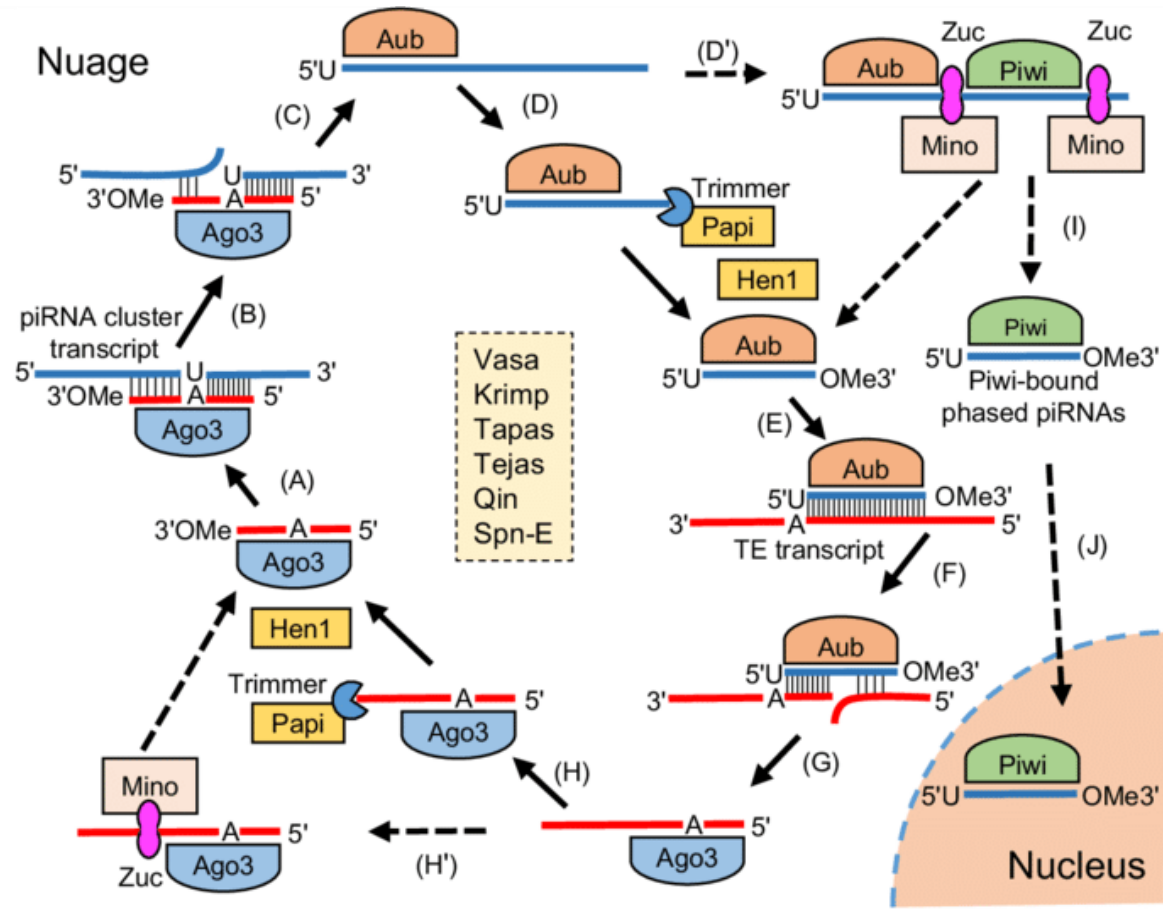
- Many classes of small, mid-sized and long noncoding RNAs, only some of which we will cover
- MicroRNAs (miRNAs) – post-transcriptional gene silencing
- **Piwi-interacting RNAs** (piRNAs) – control transposable elements and direct DNA methylation at transposable elements
- Long non-coding RNAs (lncRNAs) – appear to direct epigenetic machinery and establish different epigenetic states



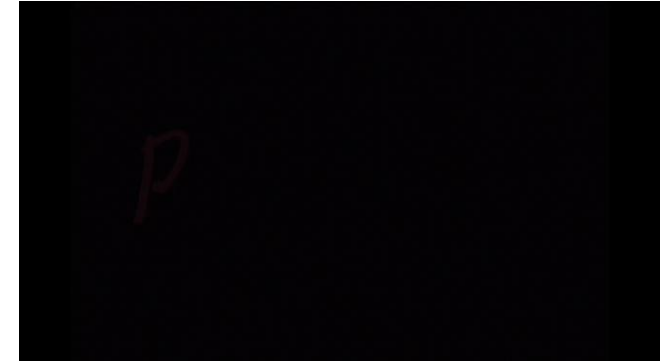




# The Ping-Pong Cycle



Video:



- **Sense piRNA** - Ago3 complex
- **Antisense piRNA** - Aub complex





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# Circular RNAs (circRNAs)

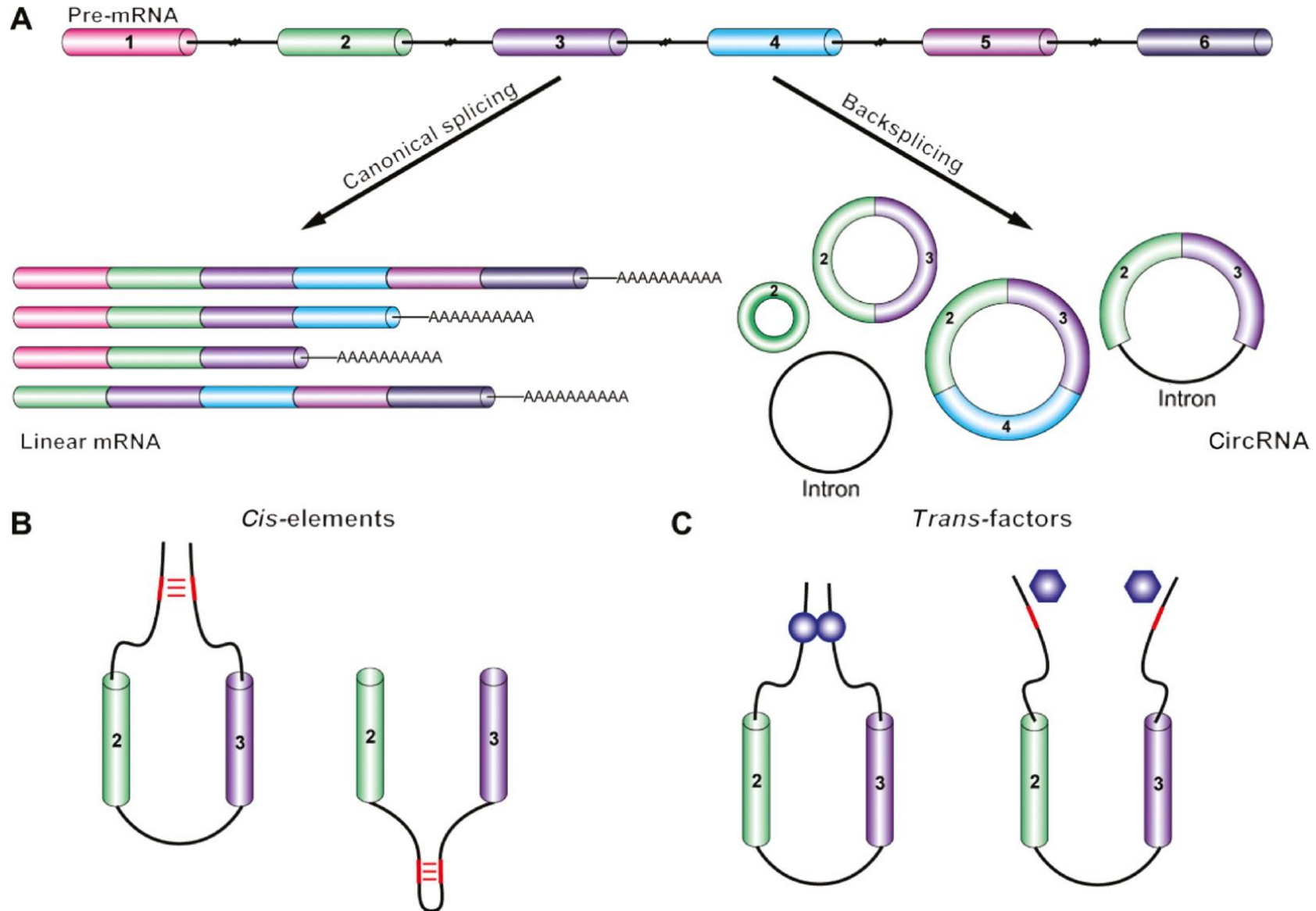


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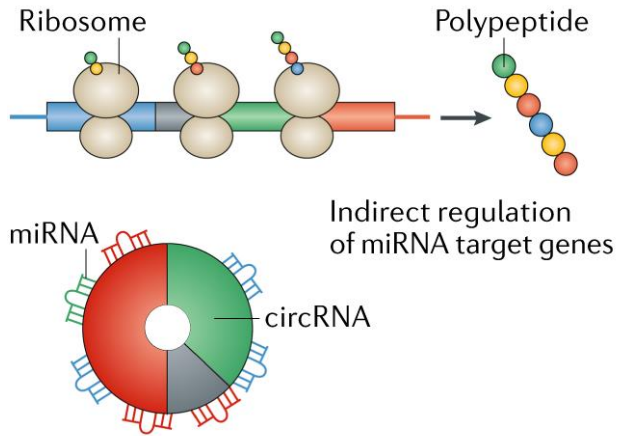
- ◆ Circular RNAs (circRNAs) comprise a large class of non-coding RNAs that are produced by a non-canonical splicing event called **backsplicing**.
- ◆ Most circRNAs are expressed from known protein-coding genes and consist of a single exon or of multiple exons.
- ◆ Despite a lack of polyadenylation (poly(A)) and capping, circRNAs generally localize to the cytoplasm.
- ◆ CircRNAs are emerging as important regulators of gene expression and are thought to play important roles in various biological processes, including development, differentiation, and disease.

# Back-splicing for circRNA biogenesis

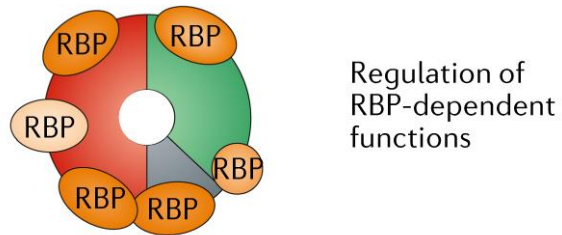


# General mechanisms of circRNA functions

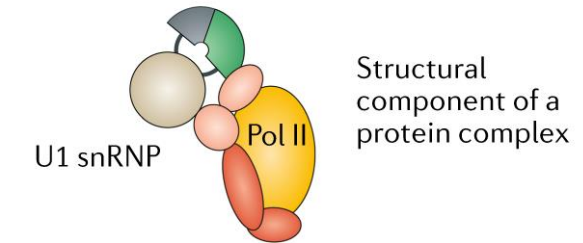
## a miRNA sponges or decoys



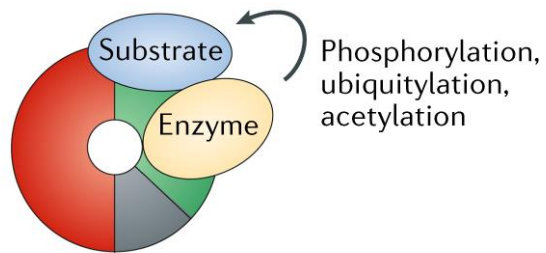
## b Protein sponges or decoys



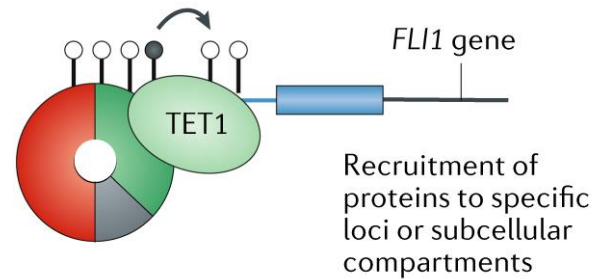
## c Enhancer of protein function



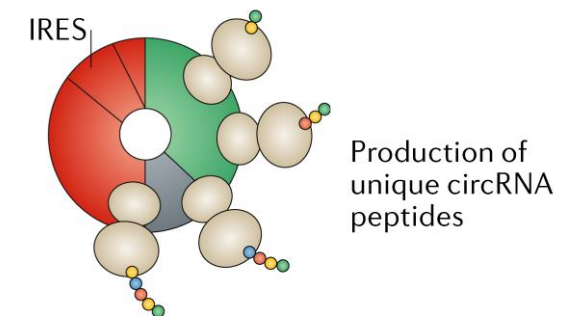
## d Protein scaffolding



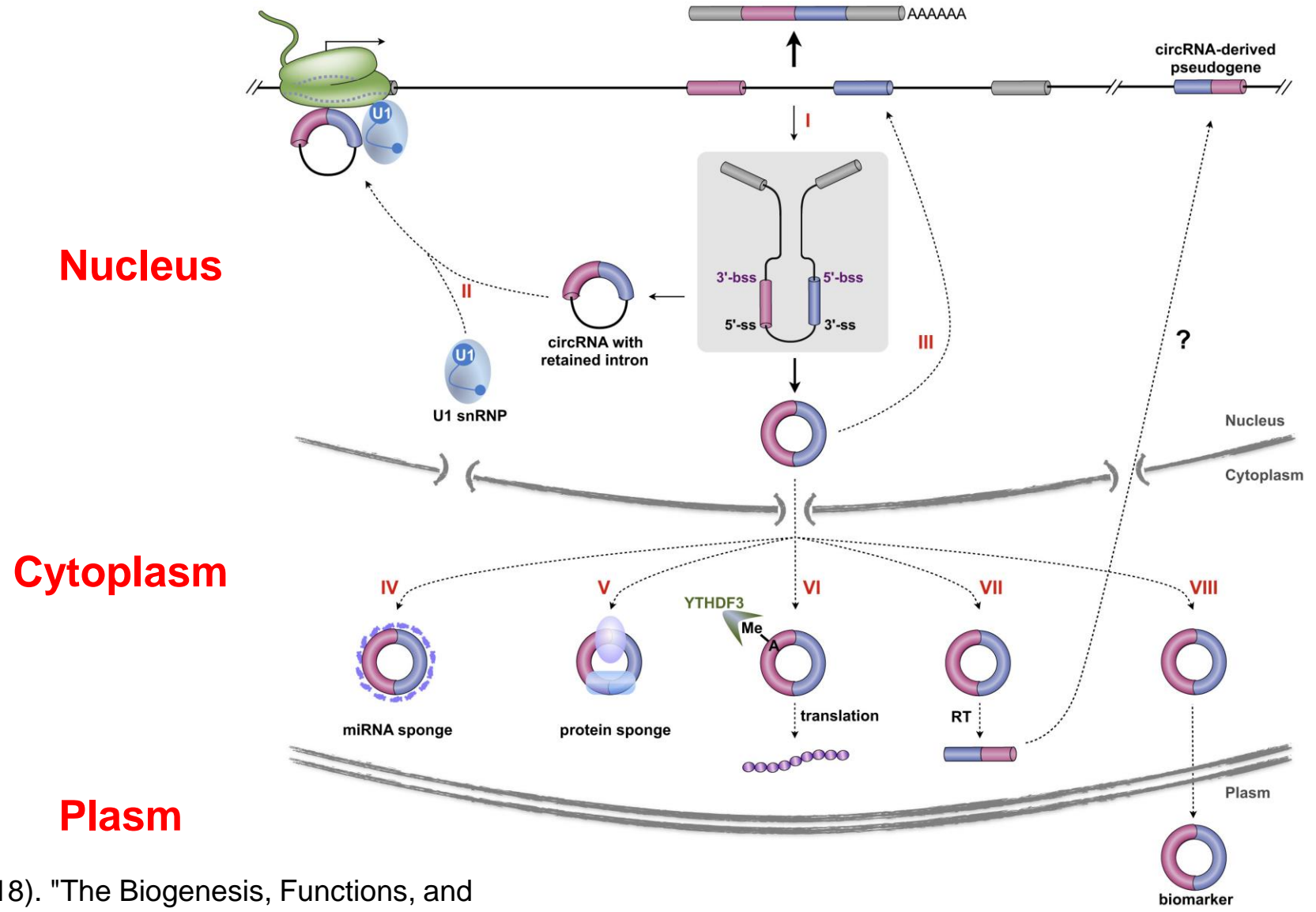
## e Protein recruitment



## f Templates for translation



# General mechanisms of circRNA functions





# Types of regulatory RNAs



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# Small nucleolar RNAs (snoRNAs)



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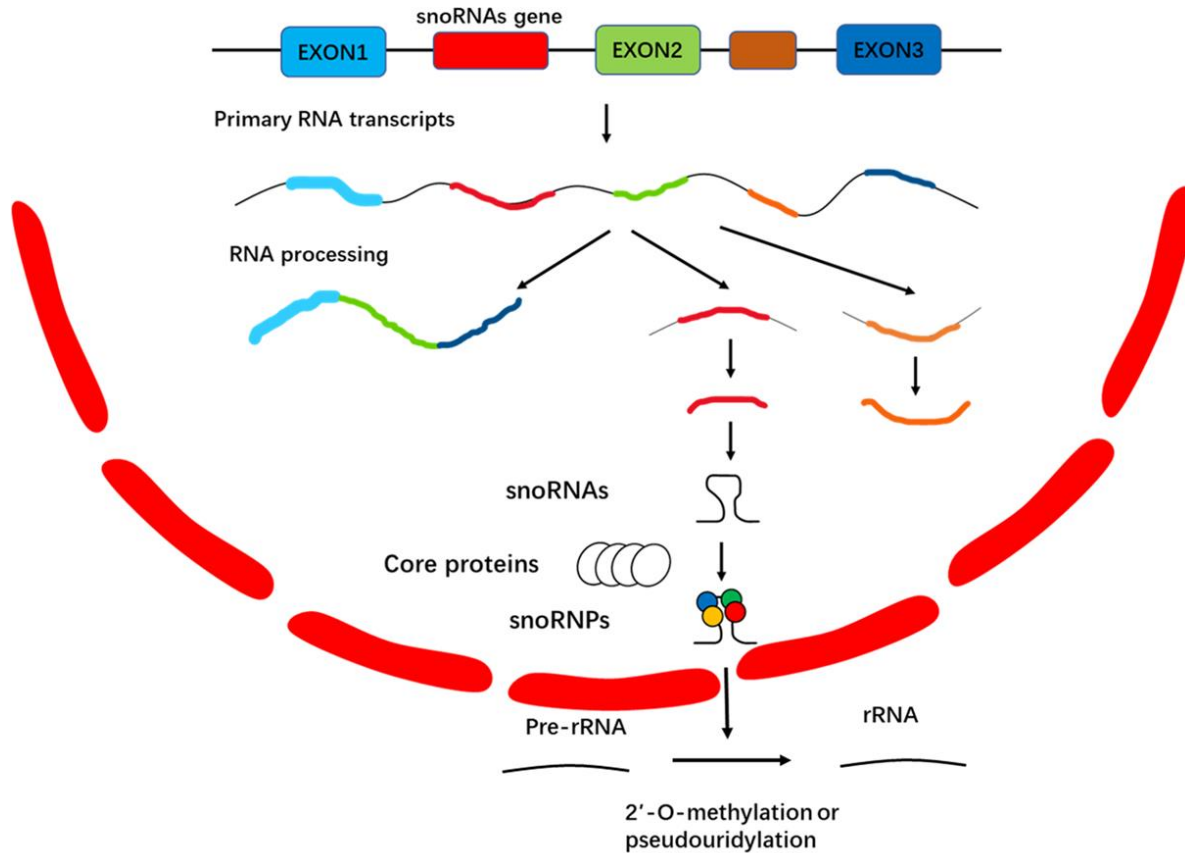
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- ◆ snoRNAs are a class of small non-coding RNAs that guide chemical modifications of other RNA molecules, particularly ribosomal RNAs and small nuclear RNAs, which are essential components of the protein synthesis machinery.
- ◆ They typically range in size from **60 to 300 nucleotides** and are found in both the nucleus and cytoplasm of eukaryotic cells.
- ◆ In addition to their role in RNA modification, snoRNAs have been implicated in other cellular processes, such as regulating alternative splicing, mRNA stability, and DNA methylation.

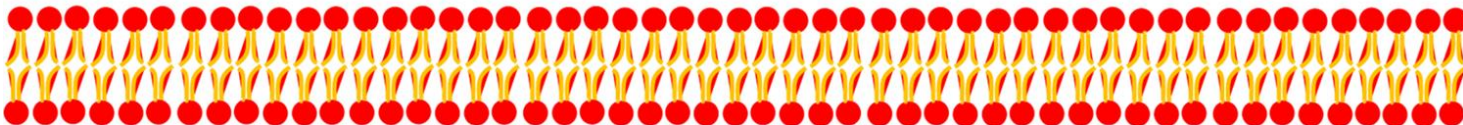




# The biosynthesis of snoRNAs and snoRNPs

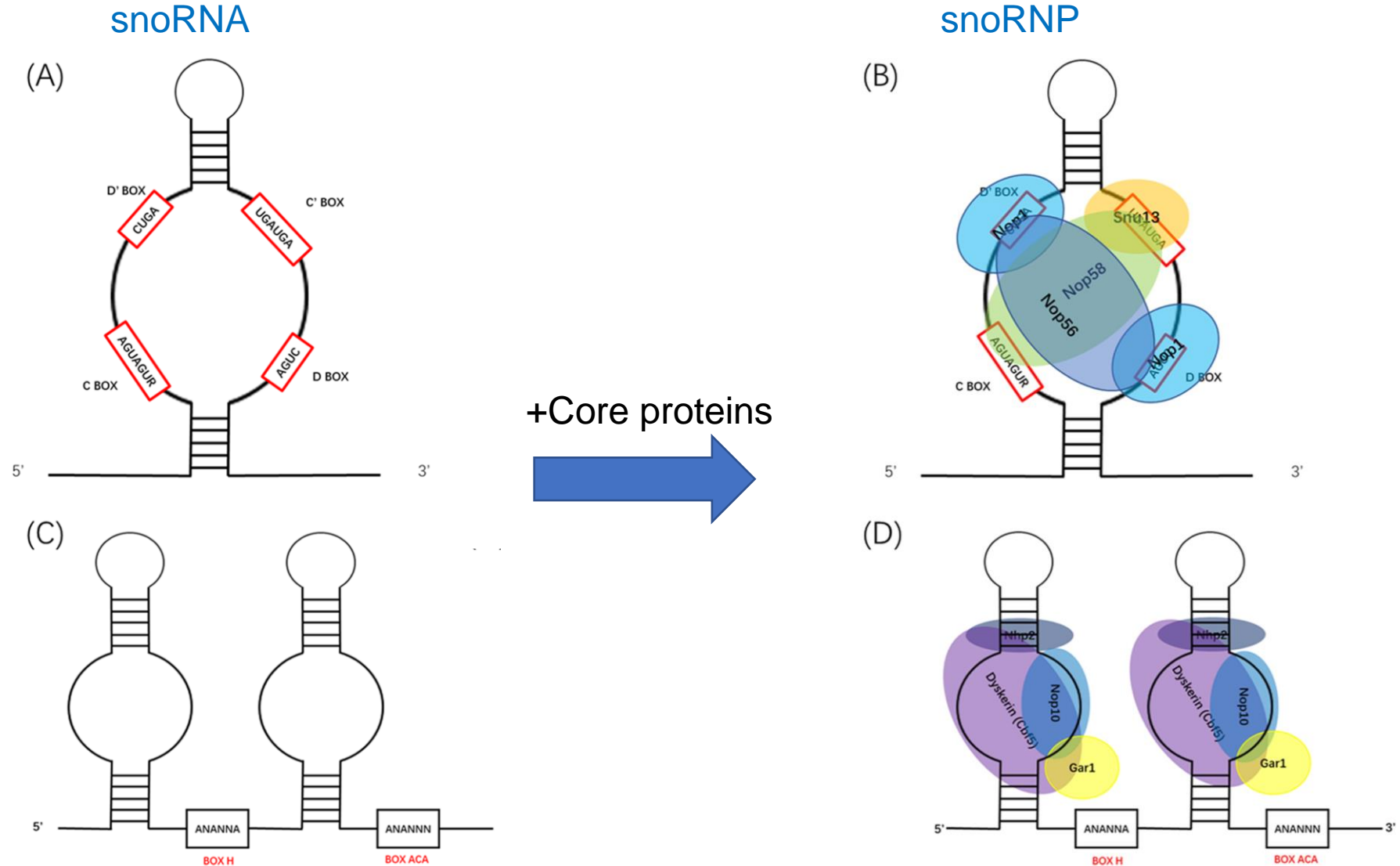


- Originate from the nucleolus, snoRNAs are mainly encoded in the intron region of the gene transcribed by RNA polymerase II.
- SnoRNAs form functional snoRNPs through binding to core proteins.
- SnoRNAs stabilize the structure of rRNA through modifying rRNA with 2' O-methylation and pseudouridylation.

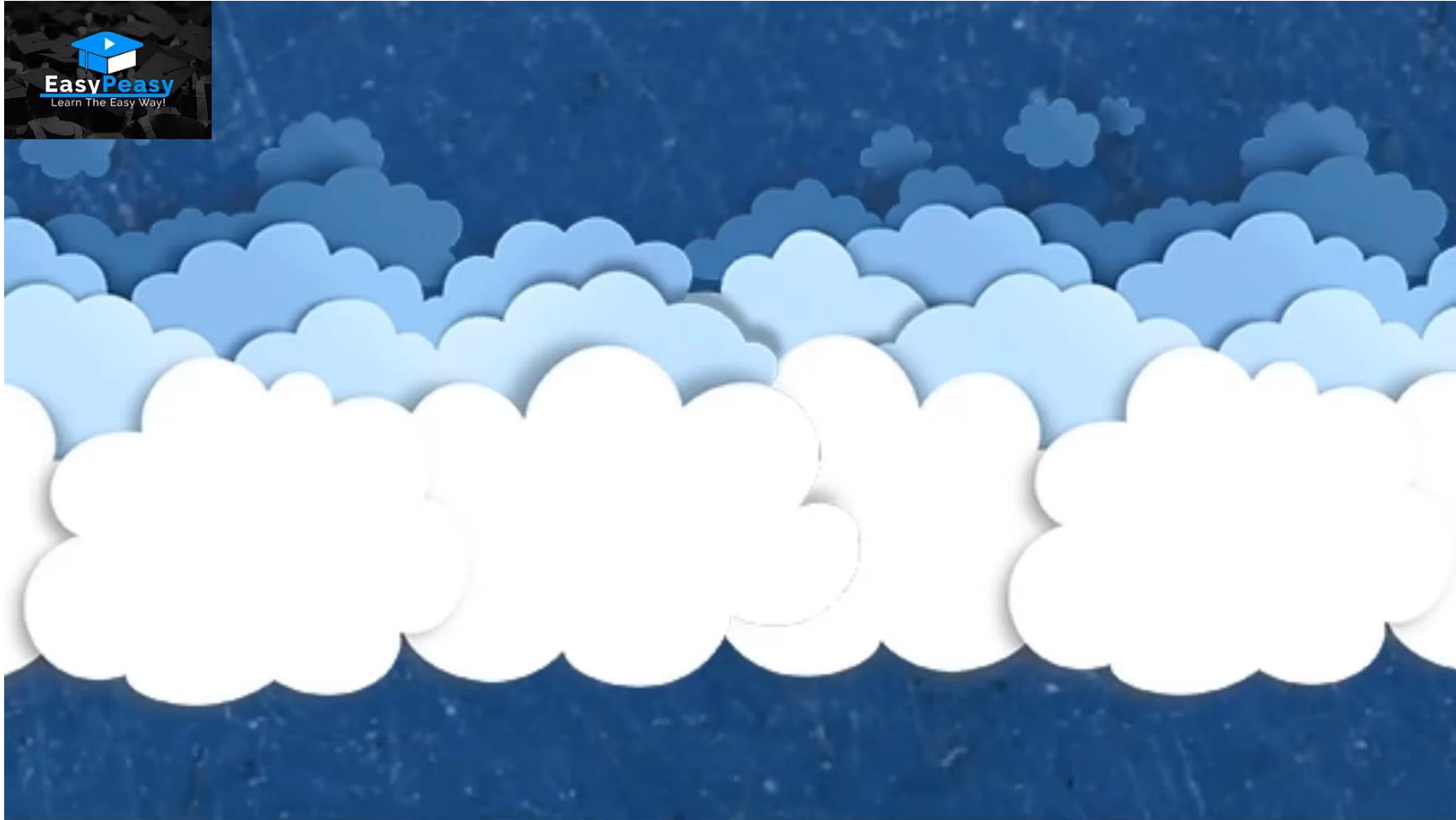




# Structures of snoRNAs and snoRNPs



# How do snoRNAs work?





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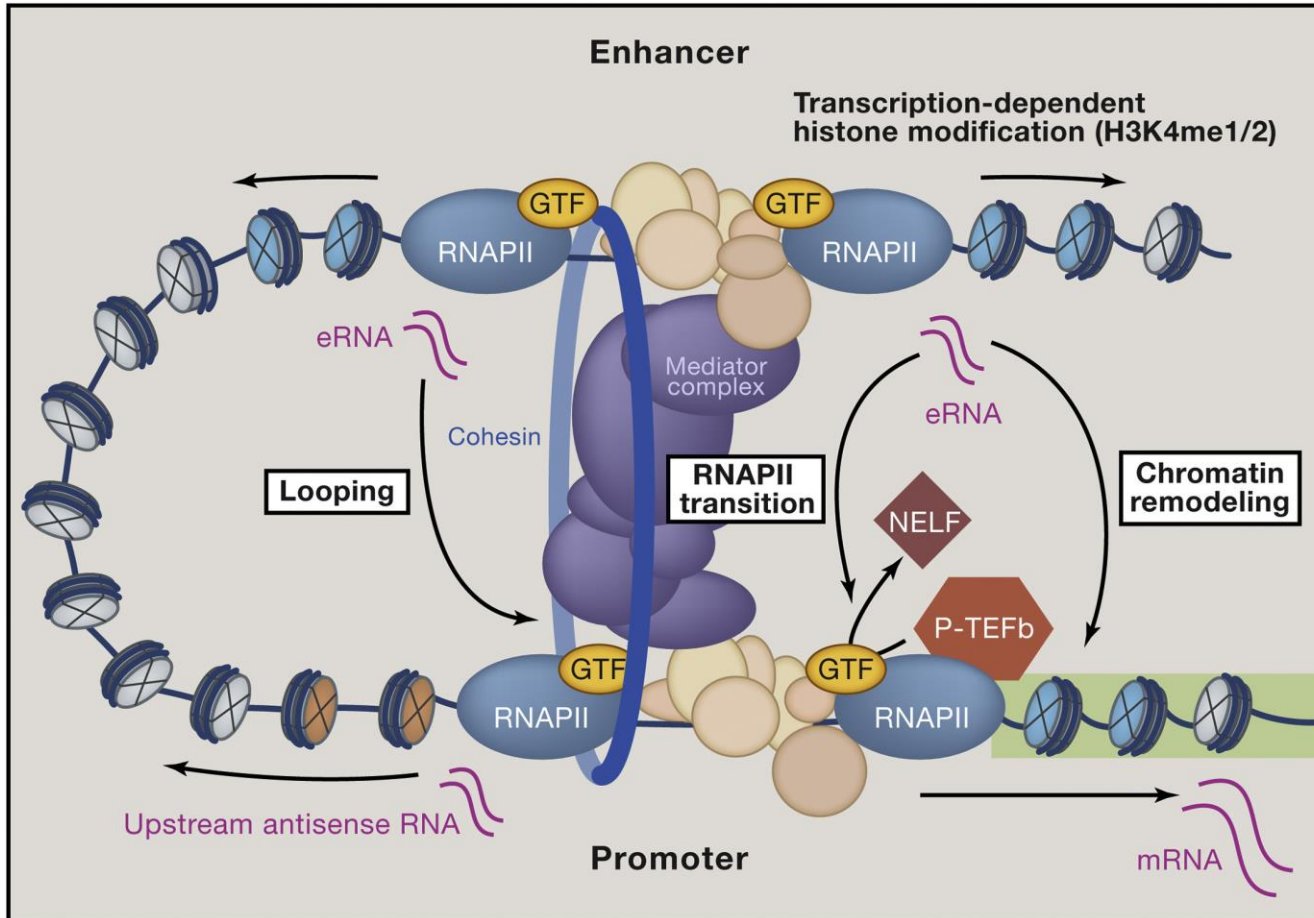
# Enhancer RNAs (eRNAs)



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- Enhancer RNAs (eRNAs) were first discovered by Professor Howard Chang's team at Stanford University in 2010.
- Global profiling showed that eRNAs are transcribed bi-directionally from the center of enhancers, where CBP and RNAPII are bound.
- eRNAs are typically 200-2,000 nucleotides in length and are transcribed by RNA polymerase II.
- They are often expressed in a cell type-specific manner and play important roles in gene regulation.



- Facilitating interactions between enhancers and gene promoters
- Regulating chromatin state
- Influencing transcription factor binding

The mechanism by which eRNAs regulate gene expression is complex and not fully understood. However, it is thought that they function by interacting with transcription factors and chromatin-modifying enzymes to control the accessibility of DNA to the transcriptional machinery. This can either promote or inhibit gene expression, depending on the context.



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# tRNA-Derived Small RNAs (tsRNA)

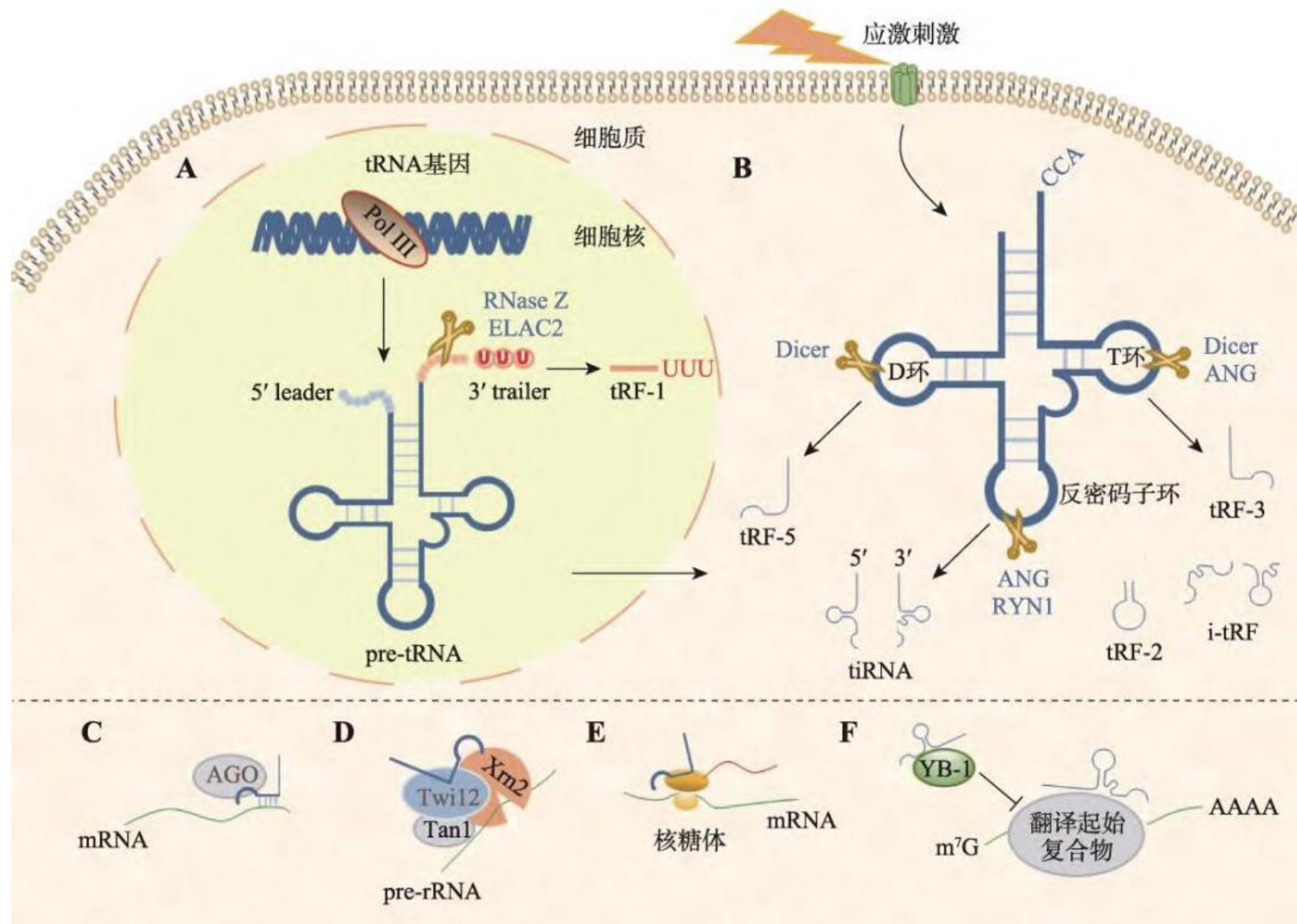


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- ◆ tsRNA (tRNA-derived small RNA) refers to small RNA molecules generated from processing of mature tRNAs.
- ◆ tsRNAs typically range in length from 15-35 nt and can be classified into two types: tRFs (tRNA-related fragments) and tiRNAs (tRNA halves).
- ◆ tsRNAs play various biological functions in cells, such as regulation of gene expression, cell differentiation, apoptosis, and tumorigenesis.

# tsRNA classification and mechanism



C、调节mRNA 稳定性

D、通过影响核糖体的生物发生  
增强翻译

E、通过与核糖体结合抑制翻译

F、调控翻译起始过程