# Emerging Roles of RNA

# Recap:

**Watson and Crick**

Proposed **central dogma**

DNA ⟶ RNA ⟶ Protein

**Proteins**

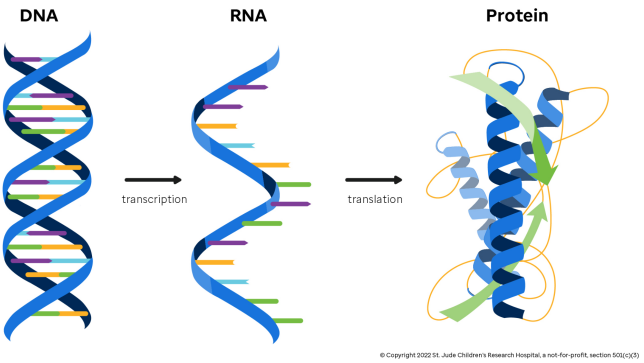
End product, functional unit

**DNA**

Stable molecule, stores genetic information

**RNA**

Considered temporary message between DNA and protein



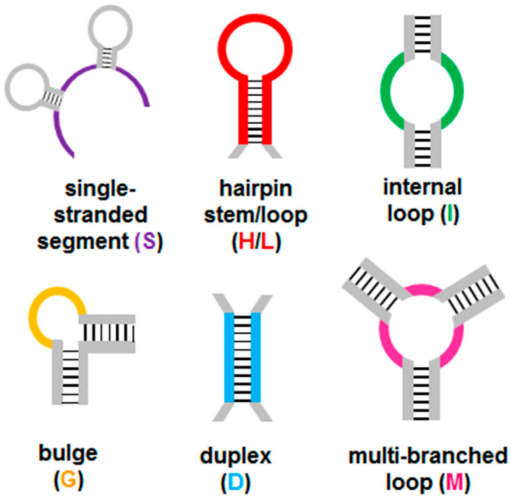

# Emerging Roles of RNA:

**Non Coding RNAs (ncRNAs):**

Watson and Crick model did not consider ncRNAs. Noncoding RNA plays an important role in genetic processes.

**RNA's versatility due to structural diversity:**

- Transcribed as single stranded (mRNA)
- Forms complementary bonds with other nucleic acids
- Creates DNA/RNA duplexes
- Forms hairpin or stem-loop structures
- Associates with proteins and modify their activity

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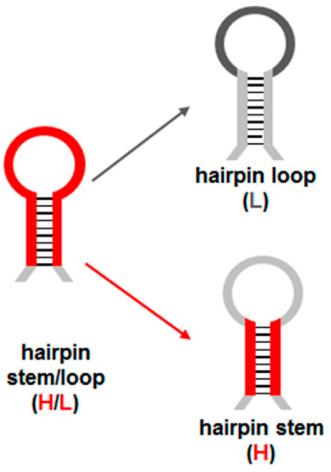



(b)

# Catalytic activity of RNAs:

**Ribozymes:**

Ribozymes are RNAs acting as biological catalysts. They can cut themselves out of larger RNAs (Self-splicing introns). The discovery of ribozymes led to the **RNA world hypothesis**. This hypothesis states that RNA can encode information, catalyze reactions and **self-replicate**. This could allude to RNA being a possible precursor to cellular life. This hypothesis was postulated by Walter Gilbert in 1986.

Ribozyme-catalyzed reactions are limited. They generally can catalyze a reaction **once**.

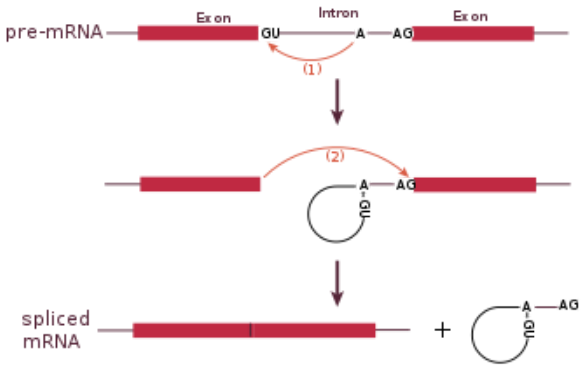
3 Ribozymes are capable of “multiple turnover”:

- Ribosomes
- snRNPs
- RNase P

Naturally occurring ribozymes fall short of **RNA world Hypothesis**. But the possibility remains of self-replicating RNAs existing but being being usurped by DNA during Revolution

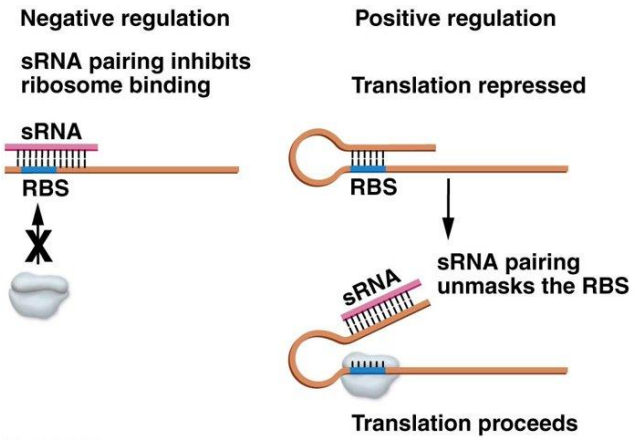
**Self-splicing introns:**

Self-splicing introns demonstrate RNA can break phosphodiester bonds.
They cut themselves out and ligate the remaining exons together. However they can not repeat this reaction nor reverse it.



# sRNAs play regulatory roles in prokaryotes:

**sRNAs (Small noncoding RNAs):**
The regulatory functions of sRNAs are still being discovered. sRNAs have between 50 and 500 nucleotides.
sRNAs are involved in gene regulation. They respond to change in environment and bacterial sRNAs have positive and negative regulation.




sRNAs also play a critical role in bacterial **cell communication.**
sRNAs use **Quorum sensing**: adapting to population density and adjusting behavior accordingly. At low density bacteria are harmless and do not express **virulence factors**. At high density, bacteria launch an attack of **virulence factors**.

sRNAs also modulate protein activity.

# RNA-guided viral defense mechanism:

**Prokaryotes have a RNA-guided viral defense system:**

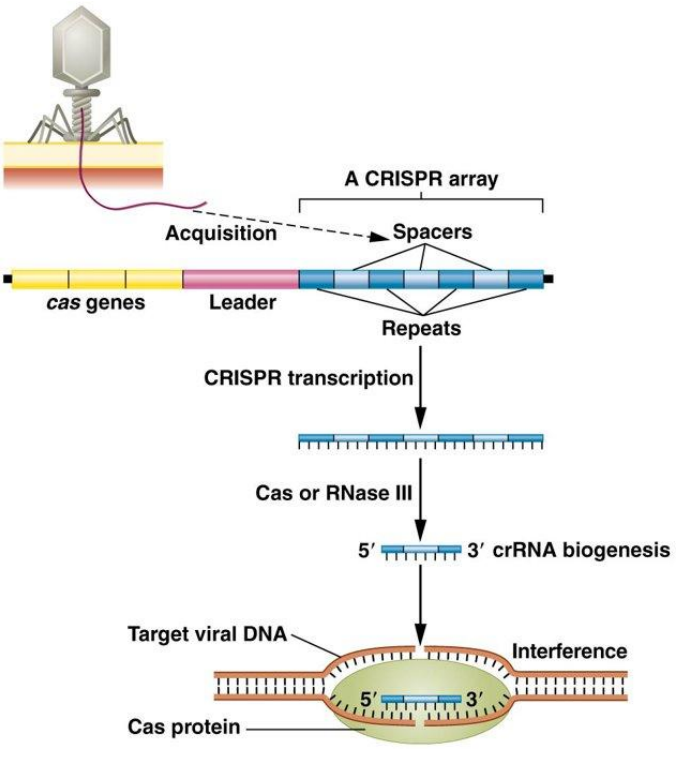
- **Innate response:**
  Their innate response defends against **bacteriophages**, bacteria express restriction enzymes which destroy foreign bacteriophage DNA.
  Other RNAs block bacteriophage adsorption, block DNA insertion and induce suicide in infected cells.

- **Adaptive response:**
  Previous infection by **pathogen** provides immunity to the cell and descendants.
  The Adaptive viral defense mechanism is dependent on **CRISPR**.

**CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats):**

CRISPR's unique spacer sequences are identical to bacteriophage sequences. Phage DNA fragment insertion into CRISPR loci is required for adaptive immunity.

**Cas proteins** have DNase, RNase, helicase domains




## CRISPR/Cas mechanism:

- **Acquisition:** Integration of invading phage DNA into CRISPR loci
- **cRNAbiogenisis:** Transcription of CRISPR loci, processing of crRNA biogenesis; required Cas proteins
- **Interference:** Targeting and cleave of phage DNA sequences complementary to crRNAs

## CRISPR mechanism recognizes "self":

System distinguishes "self" DNA from foreign DNA. Genome Editing with CRISPR-Cas9

"Self" identification is due to crRNA CRISPR repeat sequences being complementary to "self" DNA, which inhibits cleavage. This system is harnessed by scientists as a laboratory tool.

# sncRNAs Mediate Regulation of Eukaryotic Gene Expression:

**sncRNAs(Small noncoding RNAs):**

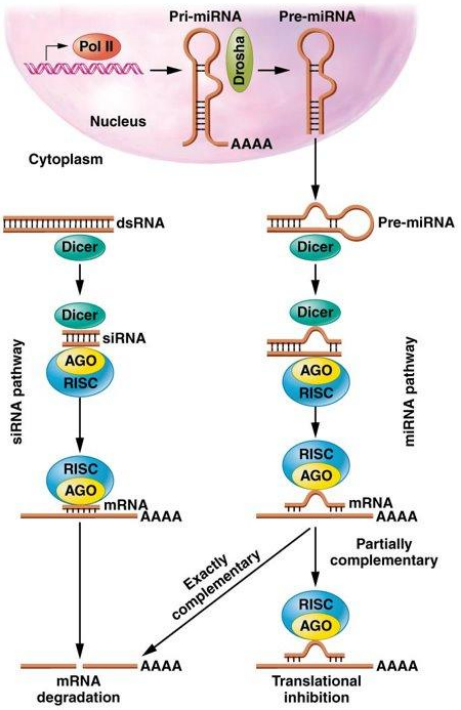
sncRNAs differ from prokaryotic sRNAs in length, biosynthesis, mechanism of action and variety of regulatory activities. sRNA refers to prokaryotes whilst sncRNAs refers to eukaryotes.
sncRNAs are iInvolved in RNA-induced gene silencing at the transcriptional and posttranslational levels. sncRNAs are 20–31 nucleotides long.

**Three types of sncRNAs:**

- **siRNAs** are Small interfering RNAs that protect the cell from exogenous RNAs (retroviral genomes)
- **miRNAs** are MicroRNAs that regulate gene expression
- **piRNAs** are Piwi-interacting RNAs they protect germ cells from mobile DNA elements

# siRNAs and RNA Interference:

**siRNAs and RNA interference:**
RNA interference (RNAi) is the Degradation of complementary mRNAs.



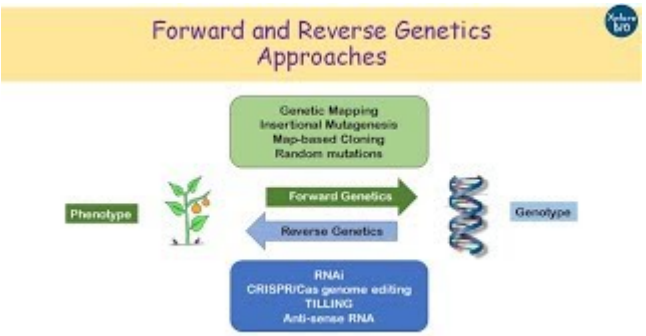

double stranded RNAs cleaved by Dicer (RNAse III protein) into 22 nucleotides with 3′ overhang is **siRNA**.

siRNAs associate with **RISC** (RNA-induced silencing complex).

RISC cleaves/evicts one strand of siRNA but retains the other for recruitment purposes .

**Forward and reverse genetic techniques**:

**Forward genetics:**
Forward genetics is changing the phenotype and finding the difference in the genotype




**Reverse genetics (technique now used):**
Reverse genetics is changing genes and looking at the difference in phenotype

**piRNAs (Piwi-Interacting RNAs):**

piRNAs are important for **transposon silencing** in germ cells and embryonic cells in animals.

**Transposon silencing** is a form of transcriptional gene silencing targeting transposons. Transcriptional gene silencing is a product of histone modifications that prevent the transcription of a particular area of DNA.

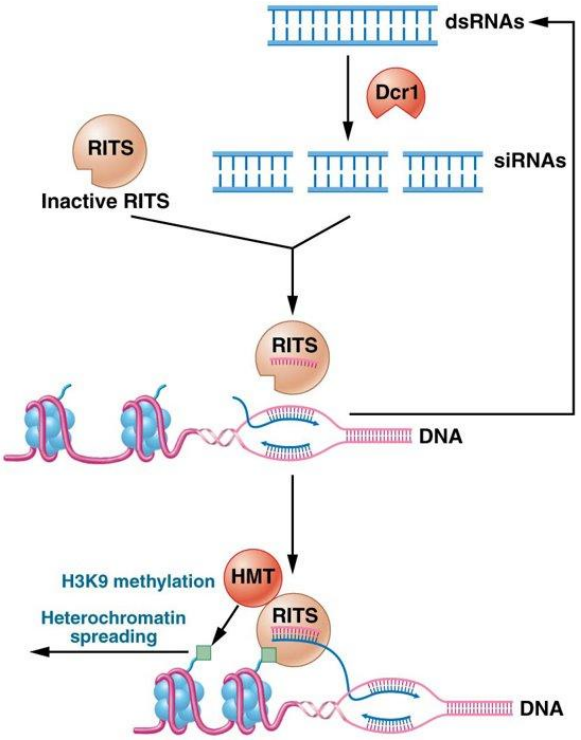
Animals lacking piRNAs have defects in gametogenesis and are sterile.

# RNA-Induced Transcriptional Silencing:

**RITS (RNA-induced transcriptional silencing):**
RITS is a mechanism utilized by sncRNAs involved in **heterochromatin formation**.
siRNAs associated with the **RITS complex** are complementary to centromeric DNA and are required for recruiting complex to centromere and heterochromatin formation

**RNA-directed RNA polymerase (RdRP):**
RdRP Binds to nascent transcripts during centromeric transcription.It then catalyzes formation of dsRNA from single-stranded transcripts.
dsRNA is a substrate for Dicer that leads to siRNA and targets centromeric transcripts .

Recruits DMTs and H3K9 methylation, which triggers heterochromatin formation .




# RISC vs RITS:

RISC (RNA Induced Silencing Complex)
RITS (RNA Induced Transcriptional Silencing)

RISC results in the breakdown of mRNA and RITS inactivates transcription of a gene using histone methylation. With RISC the gene is active but mRNA gets broken down. RITS inactivates the gene and thus doesn't create a mRNA product

RISC uses ssRNA, miRNA and siRNA molecules to break down a target mRNA.

RITS uses siRNA to find the target gene in the genome. The RITS protein complex has enzymes that methylates histones.

RISC is faster at adapting to environmental changes. RITS is a long term effect due to histone methylation having a more lasting effect. Changes given through the RITS complex can be passed on to descendants.

# Long Noncoding RNAs:

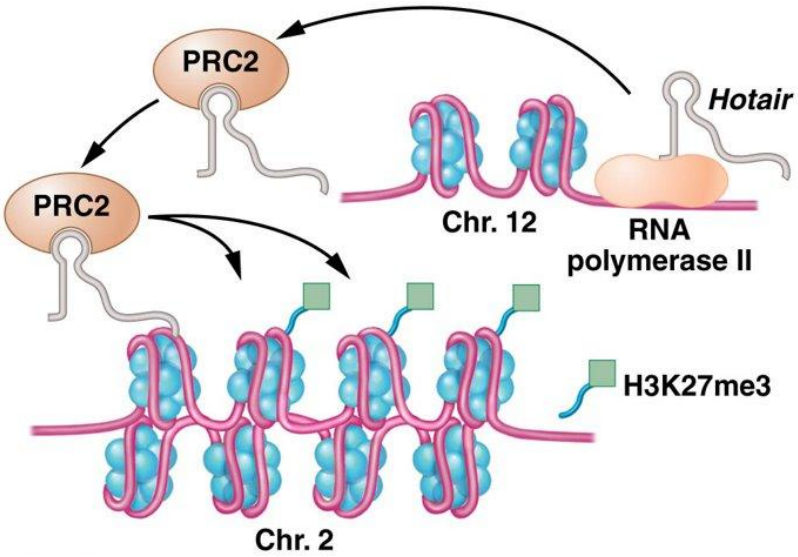
**lncRNAs (Long Noncoding RNAs):**

They are longer than sncRNAs and are produced similar to miRNAs. However they have no start or stop codons so they do not encode proteins. Studies of just a small fraction of lncRNAs have indicated that they have diverse cellular functions.

**lncRNAs functions:**

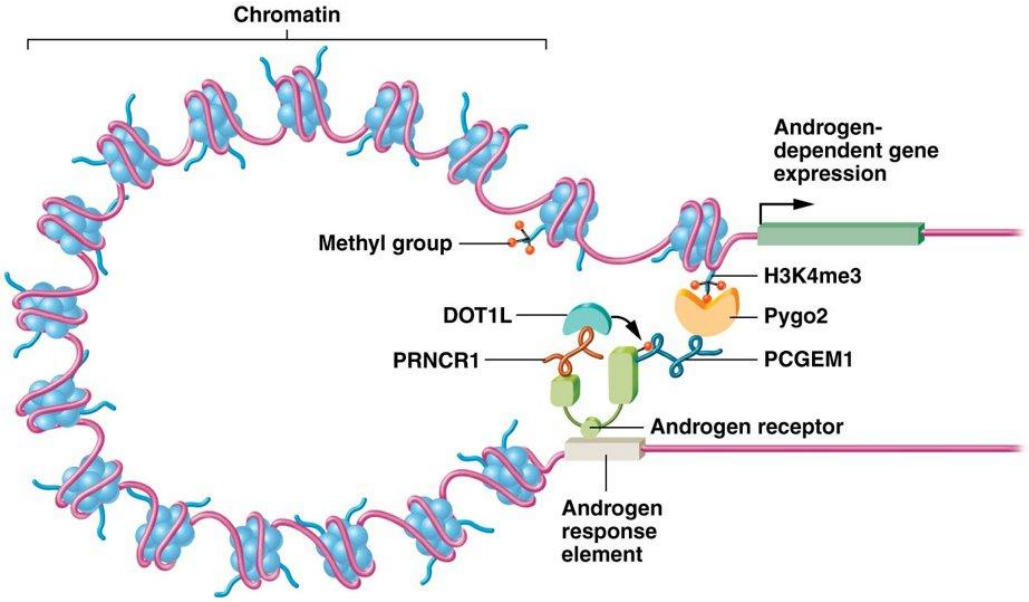
**lncRNA Hotair:**

Hotair is a lncRNA expressed from chromosome 12, it mediates transcriptional repression for target genes on chromosome 2.Overexpression of Hotair in cancer cells has increased chances of metastasis.




**lncRNAs regulate transcription factors:**

The Androgen receptor (AR) binds to testosterone and serves as a transcription factor . The AR function is regulated by two lncRNAs calledP RNCR1 and PCGEM1. They act as liaisons to help DNA loop to bring enhancer and AR closer. Without lncRNA, AR can not activate transcription of target genes

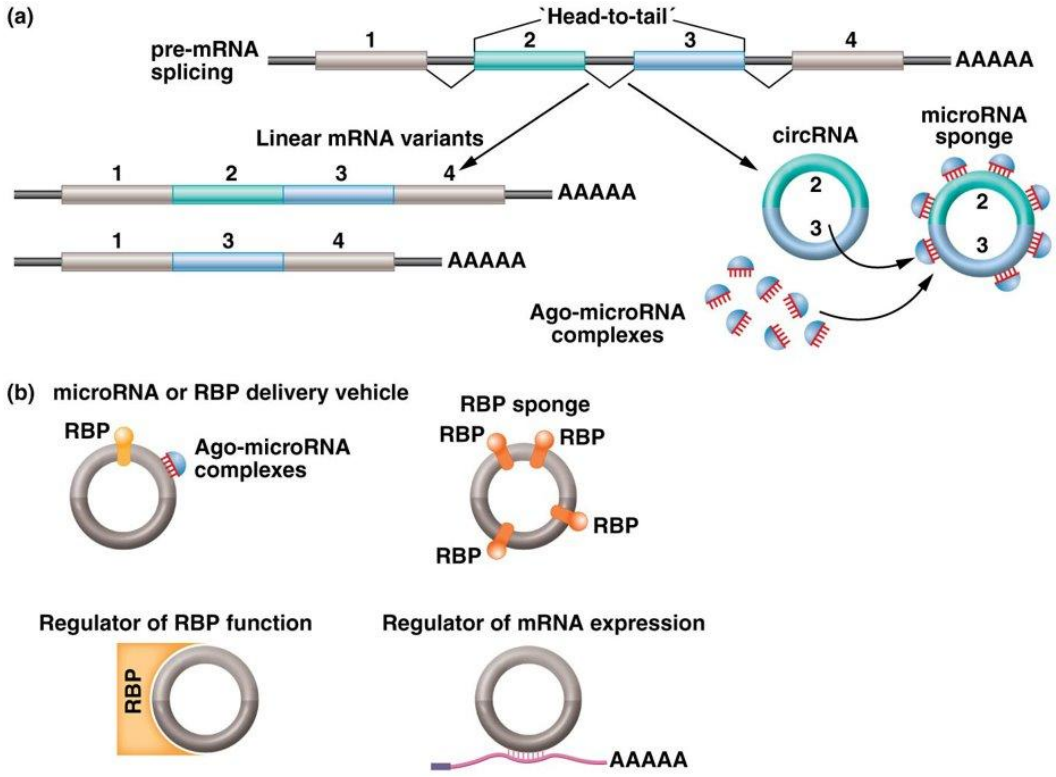


# Circular RNAs:

**Circular RNAs (circRNAs):**

circRNAs have scrambled exon sequences (the fourth exon is followed by the second exon). THey are highly resistant to digestion by RNase and have complementary sites specific to microRNAs. Their purpose is to soak up miRNAs along with their RISC proteins.

circRNA possibly plays an important role in the nervous system.



# mRNA localization and translational regulation in eukaryotes:

### mRNA localized translational control:

mRNA can be locally translated in discrete locations of the cell instead of inside the cytoplasm. mRNA localized and translational control can create asymmetric protein distribution within the cell that define cellular regions with distinct functions. As soon as mRNAs are transcribed they associate with **RBPs** (RNA-binding proteins), which influence splicing, nuclear export, localization, stability, degradation, and translation.

### Actin zip code sequence:

The 54-nucleotide element in actin's 3′ UTR of mRNA serves as a zip code. The binding site for **RBP** is zip code binding protein 1 (**ZPB1**). ZBP1 binds actin mRNAs and prevents translation initiation. Once mRNAs arrive at their final destination, a kinase called Src phosphorylates ZBP1, which disrupts RNA binding and allows translation initiation. Since Src activity is limited to the cell periphery, this mechanism allows cells to transport mRNAs in a translationally repressed state to the cell periphery where they become translated at the site of actin polymerization.