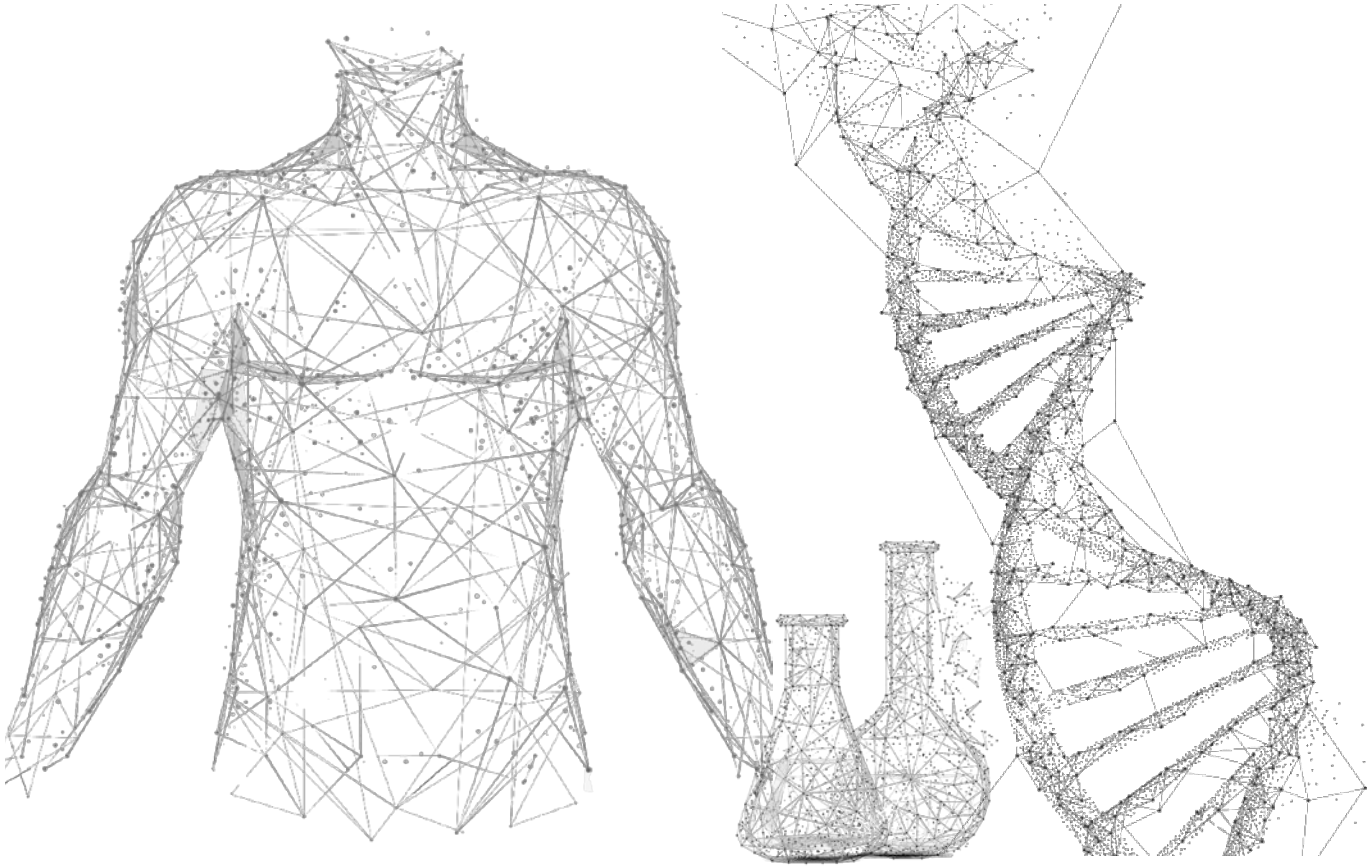


mRNA, tRNA, rRNA and ncRNAs

San Luis Potosi State University (UASLP) Mexico
Molecular Biology Course, Faculty of Medicine graduate program

Dr. Christian A. García-Sepúlveda

Viral & Human Genomics BSL-3 Laboratory

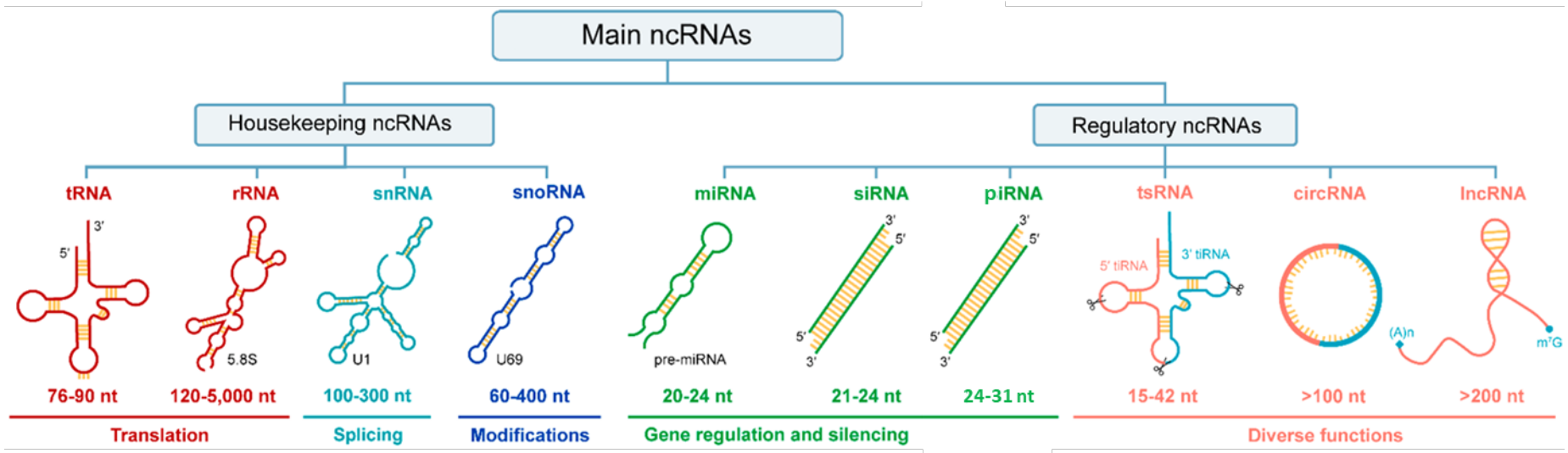
Last updated February 10, 2025 v3

Cellular RNAs

RNA chemically unstable due to the 2'-OH group in ribose, making it prone to hydrolysis.

Major types of RNA in eukaryotic cells:

- Messenger RNA (mRNA) – Protein coding RNA
- Ribosomal RNA (rRNA) – Structural & functional component of ribosomes
- Transfer RNA (tRNA) – Adapter for translation
- Non-Coding RNAs (ncRNAs) and their Regulatory Roles



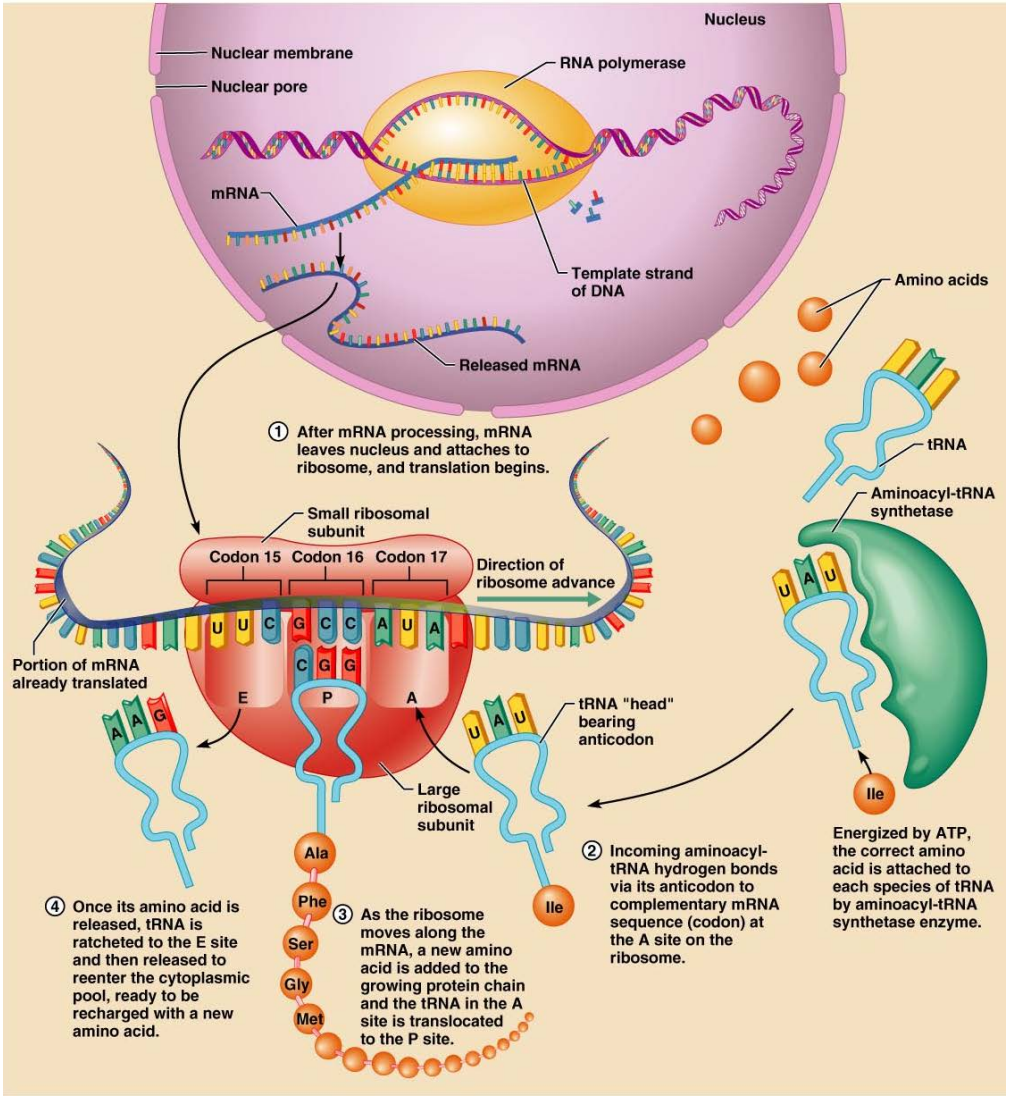
Messenger RNA (mRNA)

Carries genetic information from DNA to ribosomes for translation into proteins.

Synthesized as pre-mRNA, undergoing 5' capping, splicing, and 3' polyadenylation in the nucleous before export to the cytoplasm.

Contains codons (triplet sequences) that specify amino acids.

Processed by RNA polymerase II.



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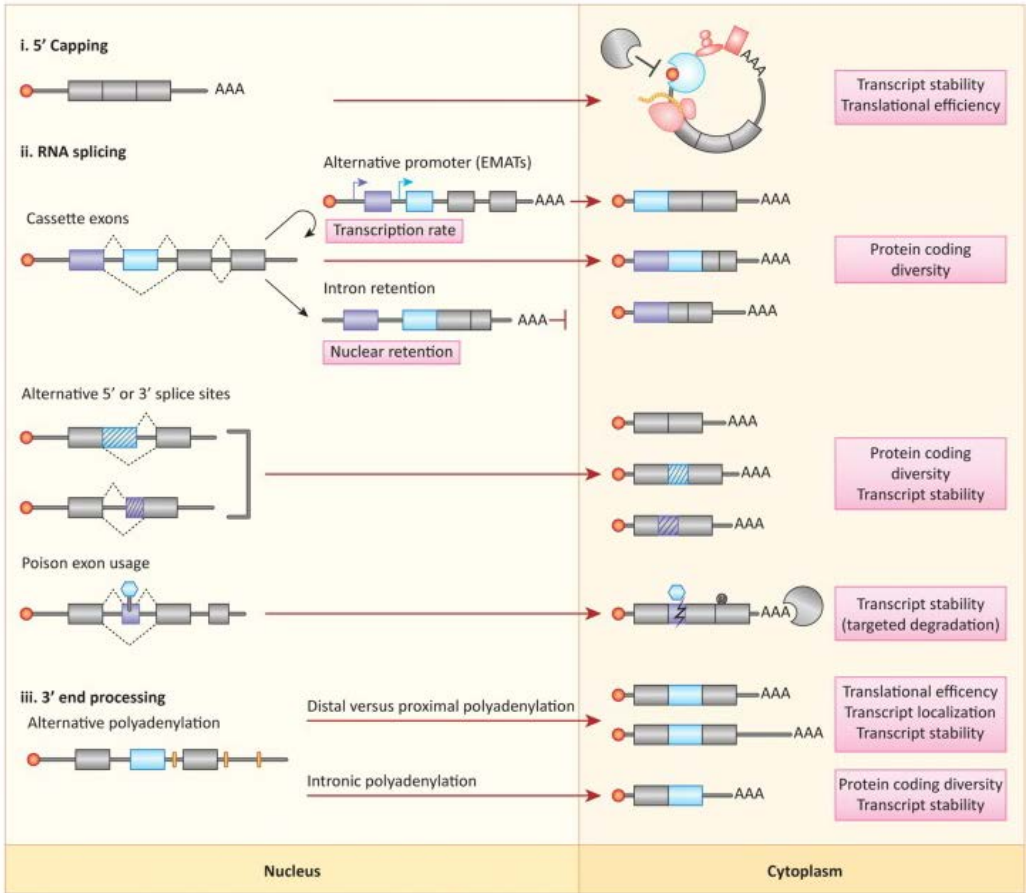

Reprogramming RNA processing. Neil CR, et al. Trends Pharmacol Sci. 2022 May;43(5):437-454.

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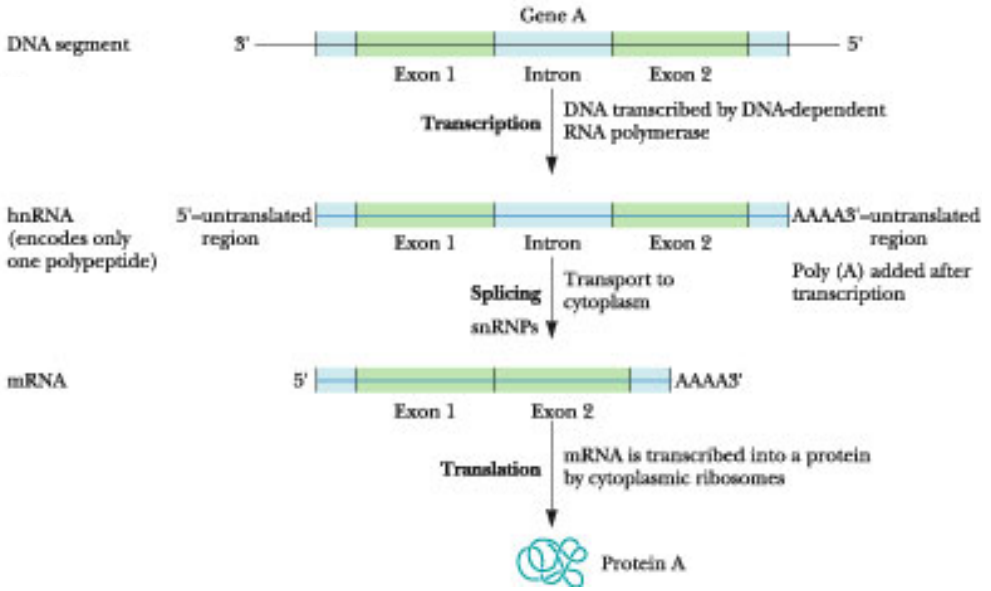


Ribosomal RNA (rRNA)

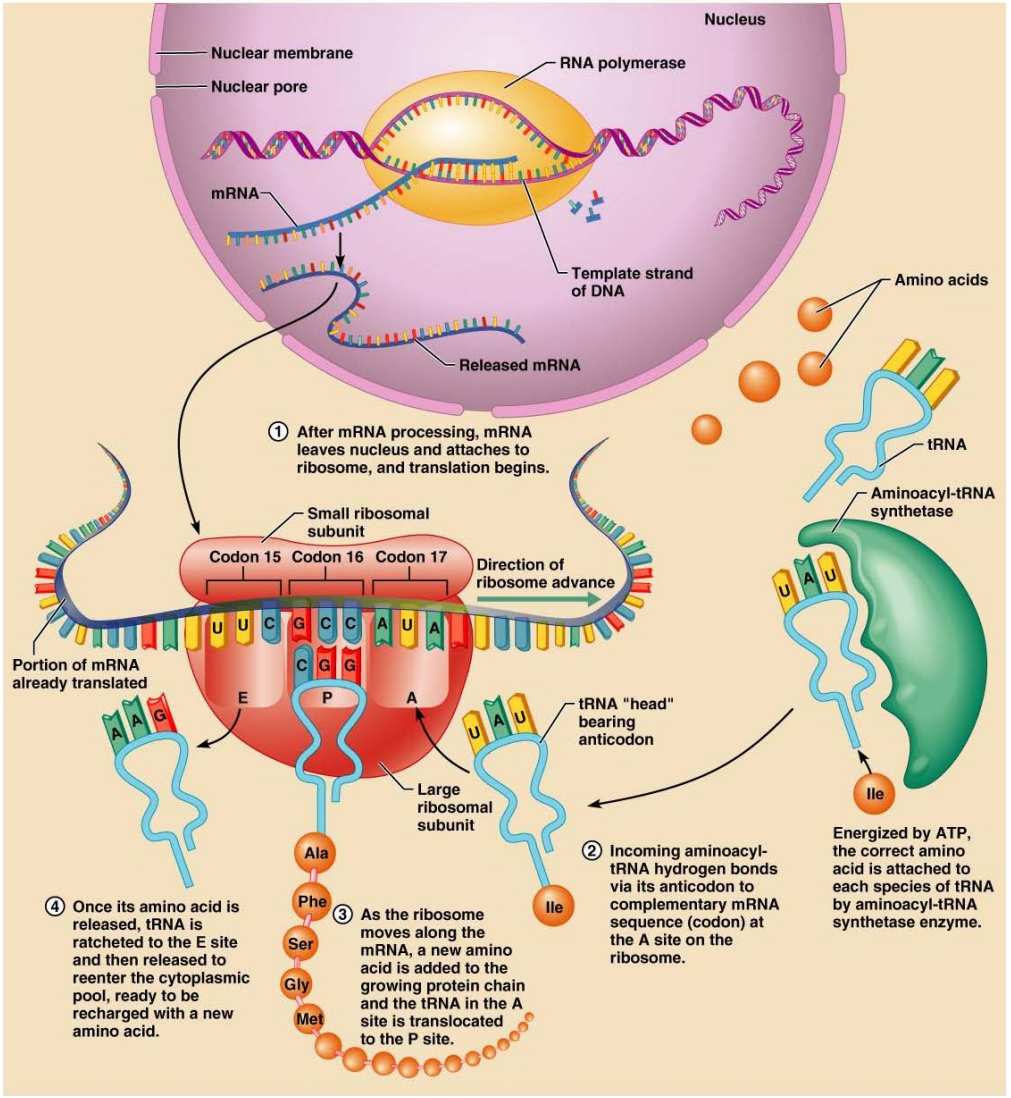
Actually a non-coding RNA.

Most abundant RNA, constituting 80-90% of total cellular RNA.

Major rRNA components
28S, 18S, 5.8S (processed from 45S precursor by RNA Pol I).

5S rRNA transcribed separately by RNA Pol III.

Forms the catalytic peptidyl transferase center (ribozyme function) of ribosomes.



Transfer RNA (tRNA)

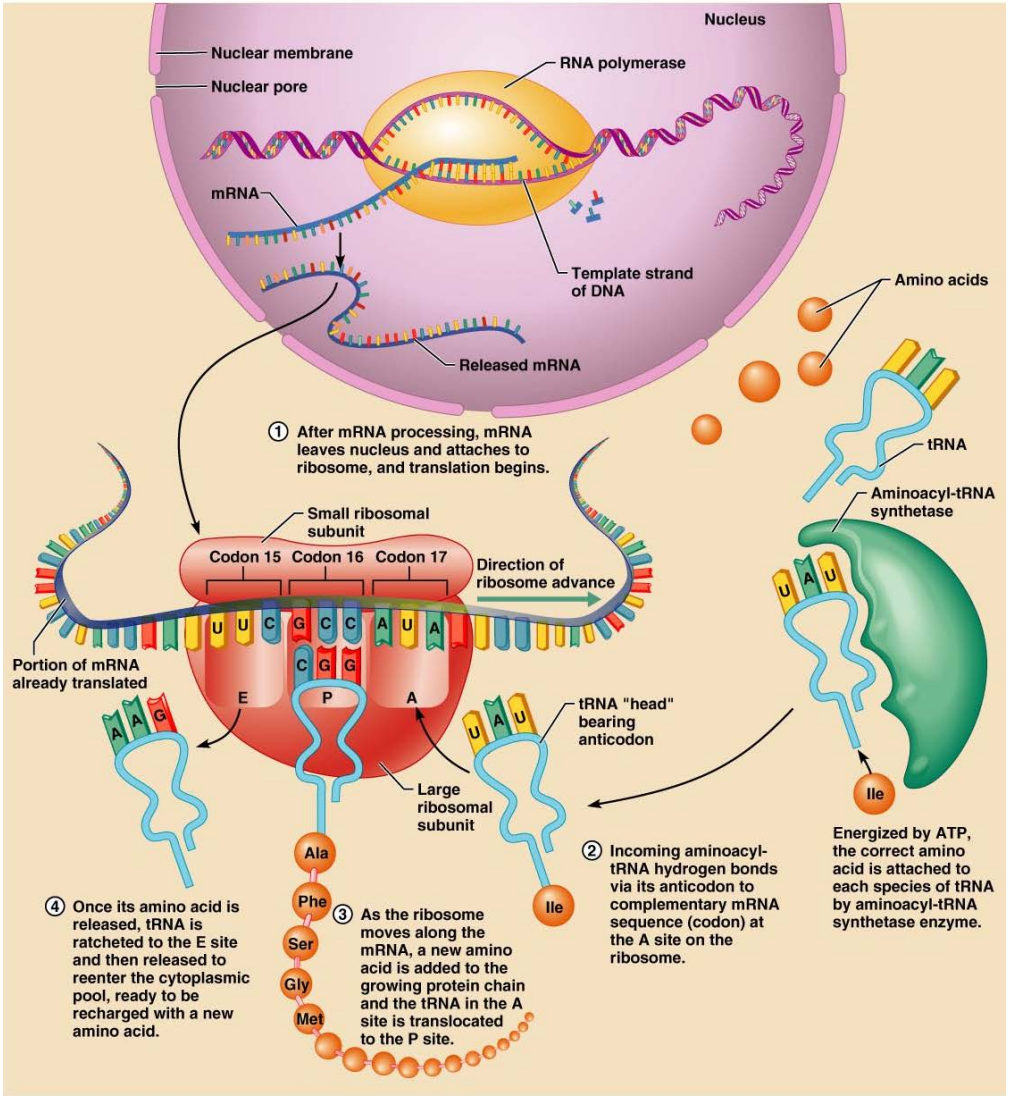
Actually another non-coding RNA.

Smallest RNA (~76–90 nucleotides) that transports amino acids to ribosomes.

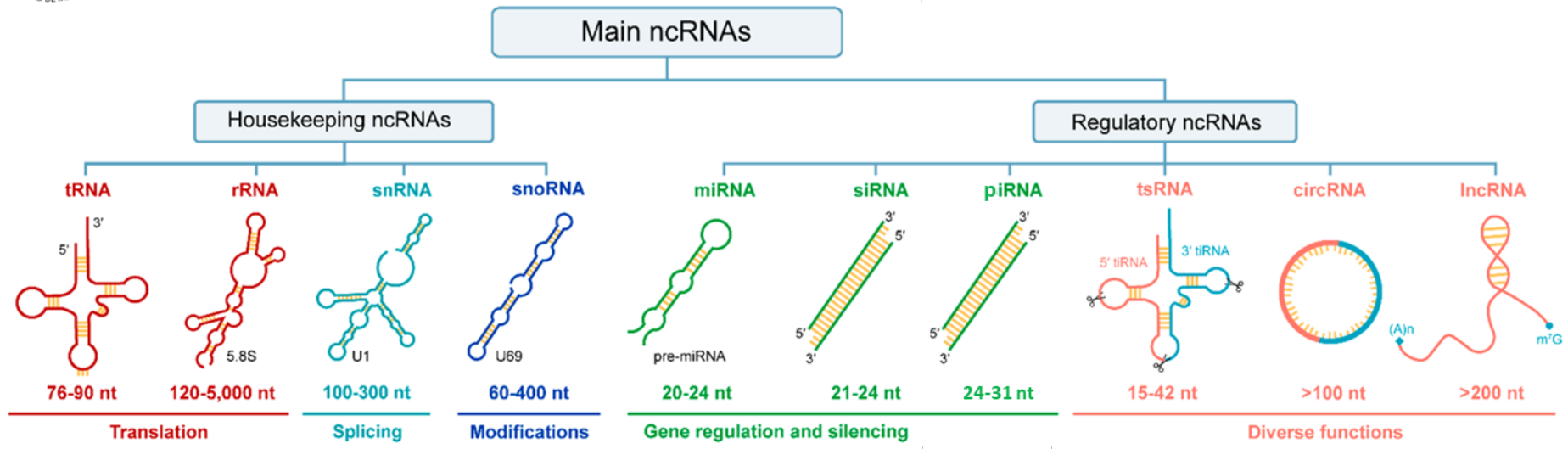
Includes:
Anticodon loop (recognizes codons in mRNA).

Acceptor stem (3'-CCA terminus), where amino acids are attached.

Transcribed by RNA polymerase III.



Non-Coding RNAs (ncRNAs) and their regulatory roles



MicroRNAs (miRNAs) – Post-Transcriptional Gene Silencing

Small Interfering RNAs (siRNAs) – RNA Interference (RNAi)

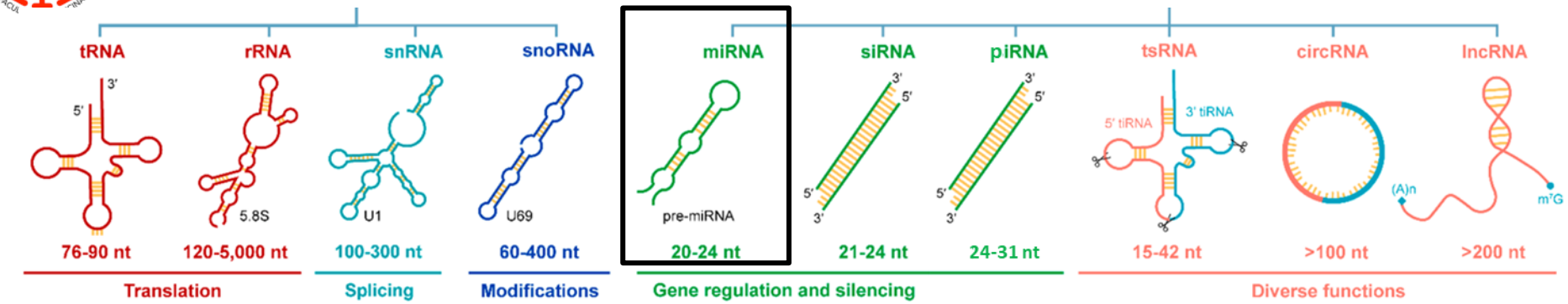
Piwi-Interacting RNAs (piRNAs) – Germline-Specific RNA Regulation

tRNA-derived small RNAs (tsRNAs) – Gene regulation, stress response, & disease processes.

Circular RNAs (circRNAs) – Regulatory RNA with Stability

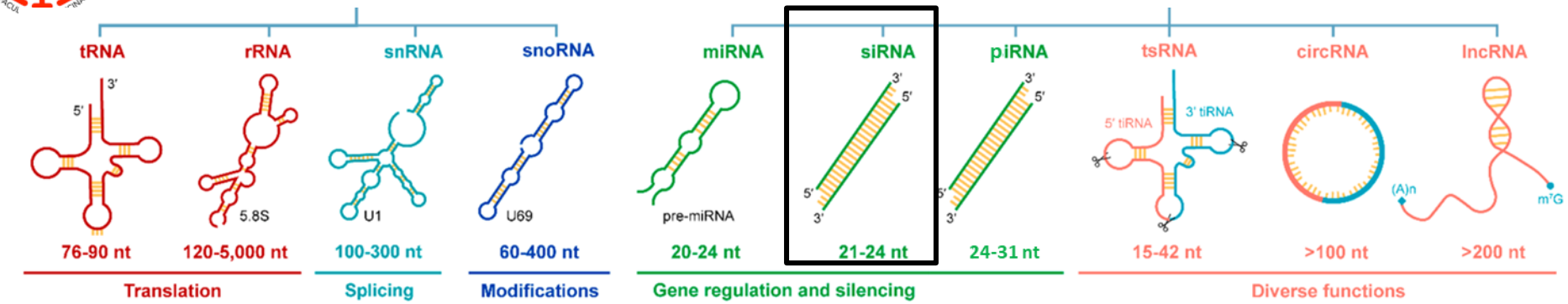
Long Non-Coding RNAs (lncRNAs) – Chromatin and Transcription Regulation

MicroRNAs (miRNAs)



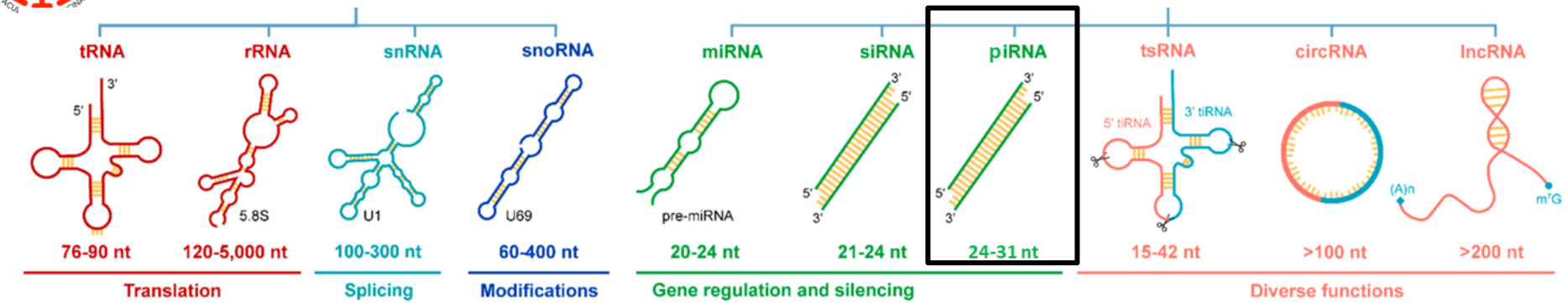
- Post-Transcriptional Gene Silencing
- Short (~22 bp) RNA molecules that regulate gene expression by binding to the 3'-UTR of target mRNAs.
- Formed from pri-miRNA → pre-miRNA → mature miRNA (via Drosha & Dicer enzymes).
- Suppress protein synthesis by mRNA degradation or translation inhibition.
- Important in cancer, immune response, and metabolic disorders.

Small Interfering RNAs (siRNAs)



- RNA Interference (RNAi)
- Exogenous or endogenous double-stranded RNAs (~21-23 nucleotides) that silence specific mRNAs.
- Cleaved by Dicer, loaded onto RISC (RNA-induced silencing complex) for gene silencing.
- Used in therapeutic gene silencing (e.g., siRNA therapy for genetic diseases, viral infections, cancer).

Piwi-Interacting RNAs (piRNAs)



- Germline-Specific RNA Regulation
- ~24-31 nucleotides, primarily expressed in germ cells, involved in transposon silencing and genome integrity maintenance.
- Interact with PIWI proteins to suppress transposable elements in the genome.

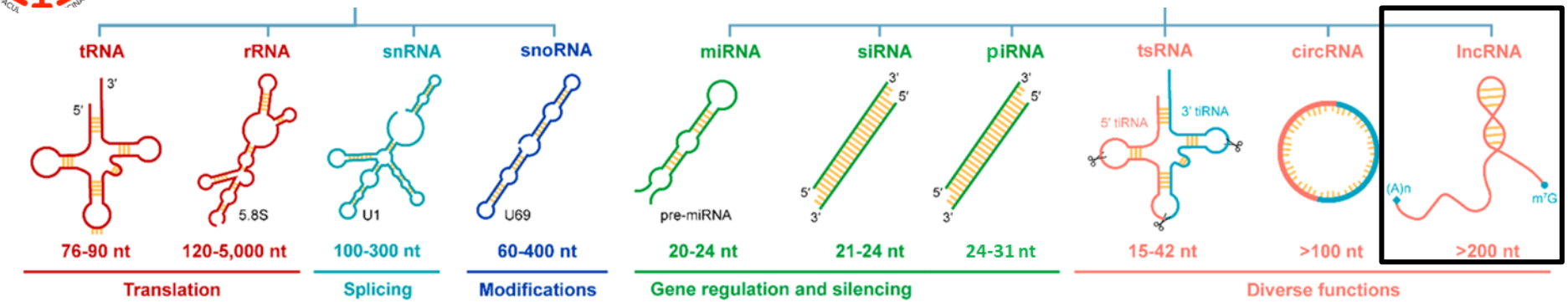
tRNA-derived small RNAs (tsRNAs)

- Small RNA fragments derived from transfer RNAs (tRNAs).
- Roles in gene regulation, stress response, and disease (cancer, neurodegeneration, & viral infections).
- Act similarly to microRNAs (miRNAs) by binding Argonaute proteins and regulating mRNA expression.
- Some tsRNAs inhibit translation by interacting with ribosomal subunits or translation initiation factors.
- Classified based on their size and the part of the tRNA they originate from:
  - tRF-5 (5' tRNA-derived fragments)
  - tRF-3 (3' tRNA-derived fragments)
  - tRF-1 (3' trailer-derived tRFs)
  - tRF-2 (Internal tRFs)
  - tiRNAs (tRNA-derived stress-induced RNAs) – Larger (~30–40 nt) arise under stress conditions.

Circular RNAs (circRNAs)

- Regulatory RNA with Stability
- Covalently closed-loop RNA molecules derived from alternative splicing.
- Act as miRNA sponges, sequestering miRNAs to regulate gene expression.
- Implicated in cancer biology and neurodegenerative diseases.

Long Non-Coding RNAs (lncRNAs)



- Chromatin and Transcription Regulation
- >200 nucleotides, non-coding RNAs involved in epigenetic regulation, chromatin remodeling, and transcriptional activation/repression.
- Example: Xist RNA mediates X-chromosome inactivation in females.
- Dysregulation associated with cancer, neurological diseases, and metabolic disorders.

Prokaryote mRNAs

5’ and 3’ ends not modified

Ribosomes recruited to Shine-Dalgarno site (do not require a free 5’ end)

Can contain many open reading frames (ORFs)

Translated 5’ → 3

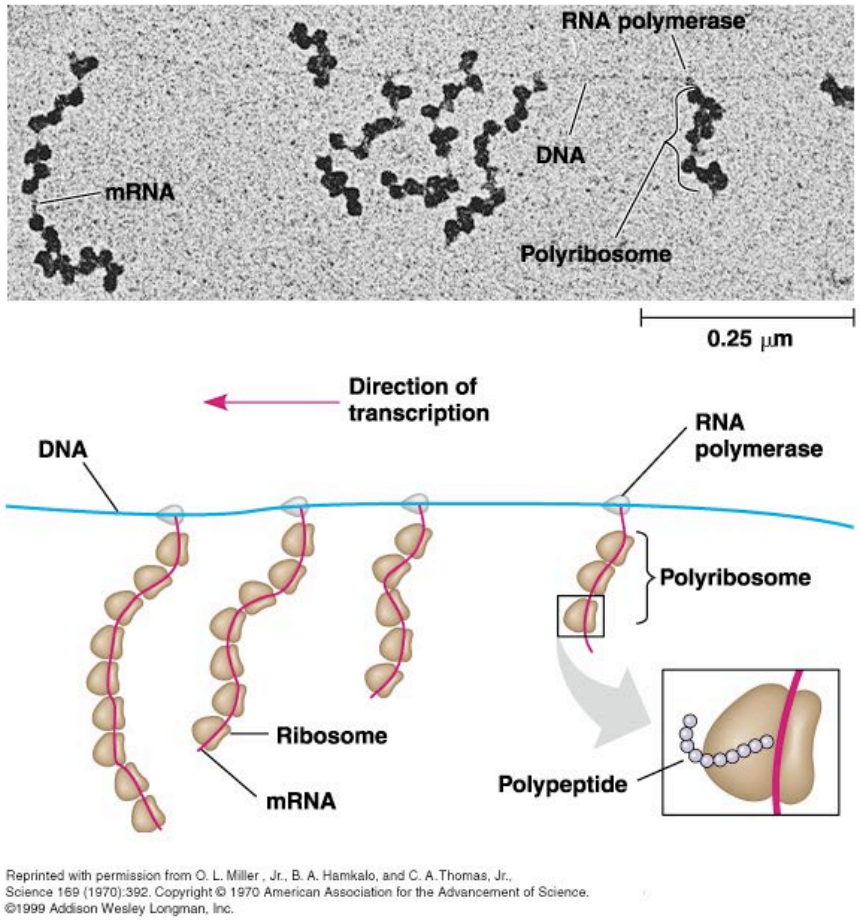
Transcripto-translational coupling

Translated (once or many times)

Degraded by RNAses

Steady state level depends on the rates of both synthesis and degradation

Transcription of protein coding RNA



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Eukaryote mRNAs

Encode a single peptide (monocistronic).

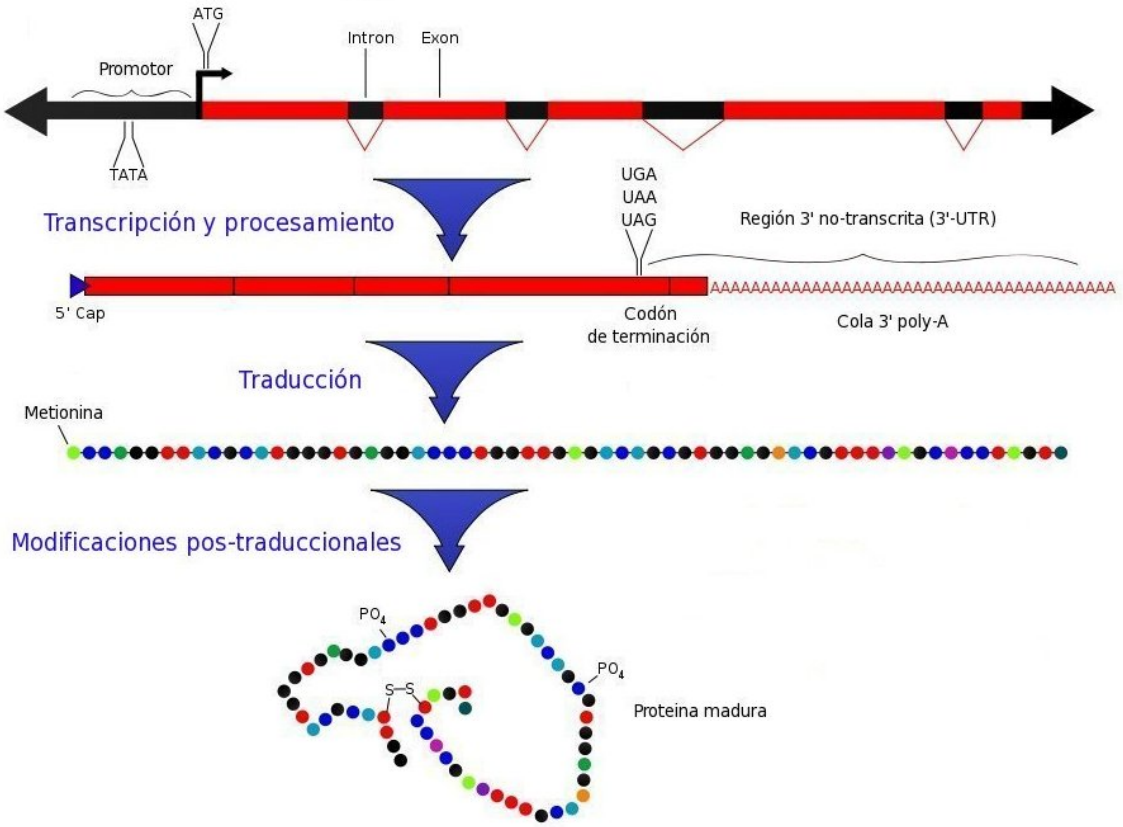
Polyproteins are observed in eukaryoti viruses, but these are a single translation product, cleaved into separate proteins after translation.

Catalysed by RNA Polymerase II

Initiation at the Promoter sequence

Regulation of gene expression is at the initiation stage

Transcription factors binding to promoter and regulate rate of initiation of RNA Polymerase



Eukaryote mRNA 5’-end capping

Addition of a 7-methylguanosine cap for stability and translation initiation.

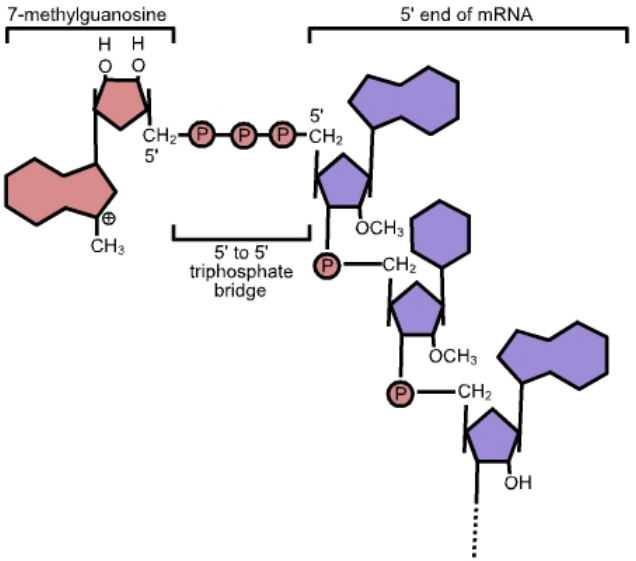
Guanine nucleotide connected to the mRNA via an unusual 5' to 5' triphosphate linkage.

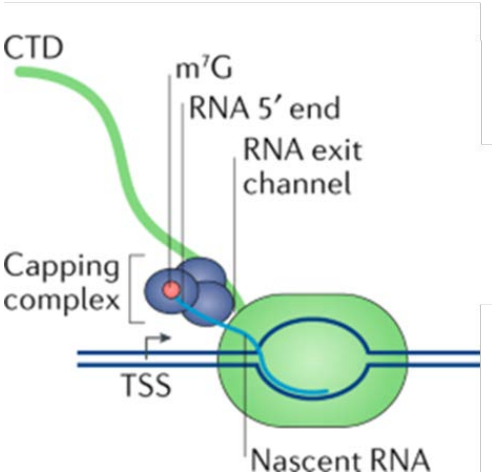
This guanosine is methylated on C7 (7-methylguanosine) or m7G.

Methylation of the 2’-OH of the first 3 ribose sugars.

Functionally the 5’-cap looks like the 3' end of an RNA molecule.

Offers resistance to 5' exonucleases.





Viral 5’-end cap snatching

First step of transcription for some ssRNA(-) viruses.

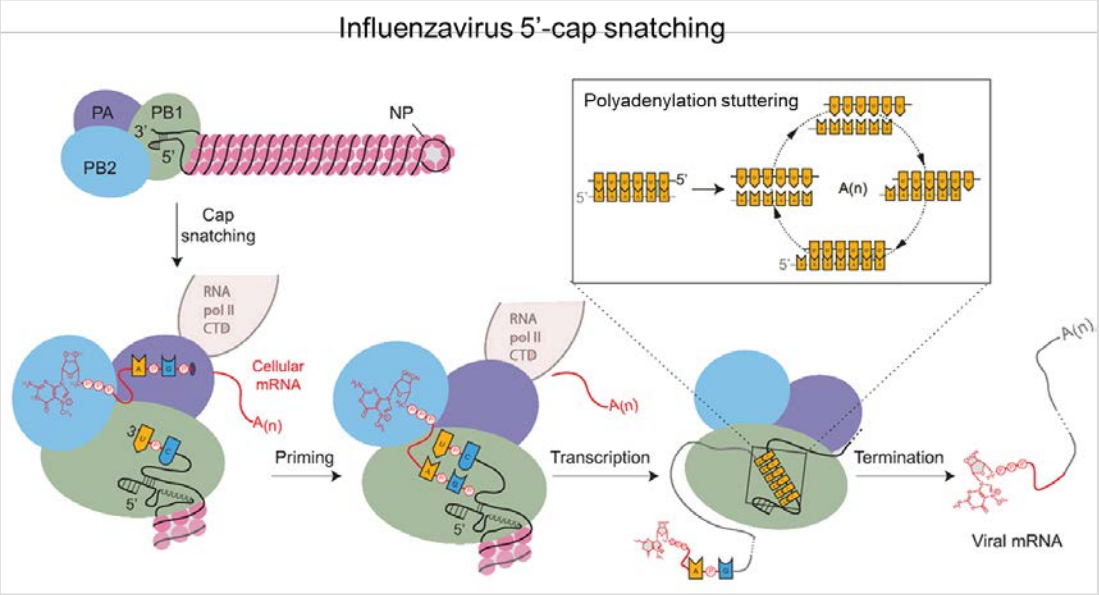
- Influenza viruses (Orthomyxoviridae)
- Lassa virus (Arenaviridae)
- Hantaan virus (Hantaviridae) and
- Rift valley fever virus (Phenuiviridae).

First 10 - 20 bp of host RNA removed (snatched) and used as 5’ cap for vRNA.

The viral RNA-dependent RNA polymerase (RdRp) transcribes viral mRNA (+) from ssRNA(-) template.

Cap snatching best described in influenza A viruses.

Orthomyxoviridae RdRp has three subunits: **PA**, **PB1** & **PB2**.



1. PB1 binds the 5’ end of vRNA.
2. PB2 binds host m7G.
3. PA cleaves host mRNA 10 to 13 bp after cap.
4. PB1 adds joins host primer to vRNA
5. PB1 stutters to produce 3’-en poly-A tail.

Eukaryote mRNA 3'-end polyadenylation

Covalent linkage of a poly(A) tail to RNA.

Poly-A protects mRNA from exonucleases.

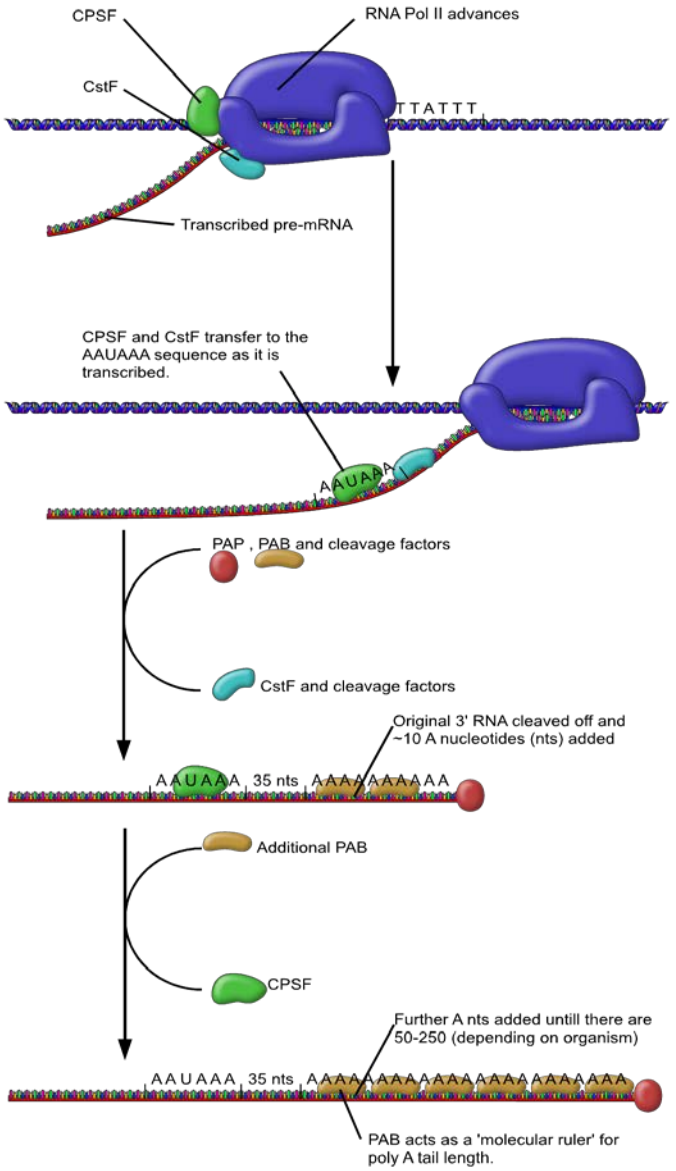
Required for:

- Transcription termination
- mRNA nuclear export
- Translation.

Some prokaryotic mRNAs also polyadenylated.

Poly-A signal is transcribed, mRNA is cleaved (endonuclease) in a special site (AAUAAA) then 50 to 250 adenine residues are added.

- CPSF recognizes poly-A sequence
- CstF cuts mRNA after poly-A sequence after 35 bp.
- PAP polyadenylates mRNA
- PABP indicates poly-A length



mRNA 3’ end polydenylation

Addition of 50 to 250 Adenosine residues to the 3’ end.

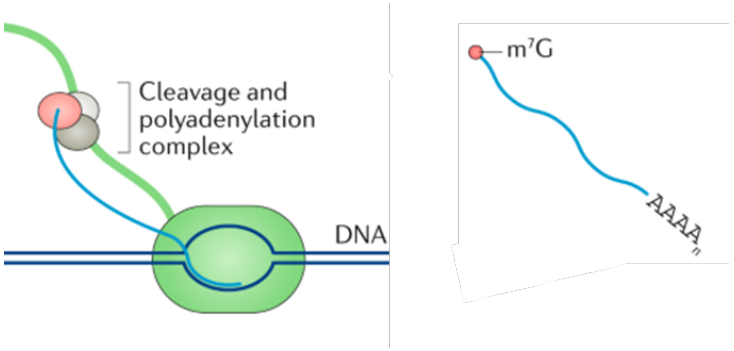
The mRNA needs to be modified to stabilize it (by labeling it or altering its conformation).

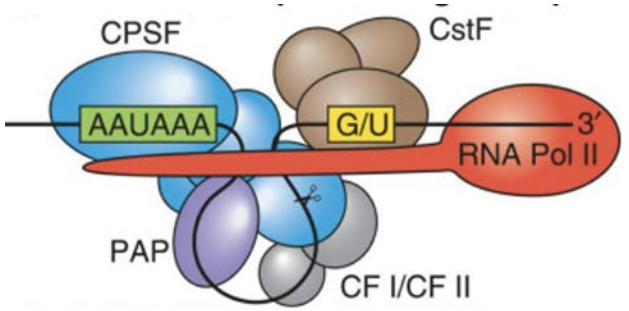
Places between 50–250 adenosine residues.

Enhances mRNA stability (prevents rapid degradation).

Facilitates nuclear export.

Regulates gene expression (affects mRNA half-life).





Protein Complex	Function
Cleavage & Polyadenylation Specificity Factor (CPSF)	Recognizes AAUAAA signal and cleaves pre-mRNA.
Cleavage Stimulation Factor (CstF)	Binds GU-rich region, promotes cleavage.
Cleavage Factors (CFI & CFII)	Aid in RNA cleavage.
Poly(A) Polymerase (PAP)	Adds adenosine residues after cleavage.
Poly(A) Binding Proteins (PABP)	Regulates poly(A) length and mRNA stability.
RNA Polymerase II CTD	Recruits CPSF and CstF

Splicing related diseases

Disease	Splicing Defect	Consequence
Spinal Muscular Atrophy	SMN2 exon 7 skipping	Motor neuron loss
Myotonic Dystrophy	Sequestration of MBNL1	Muscle wasting, cardiac defects
Frontotemporal Dementia & Amyotrophic Lateral Sclerosis	Mutations in TDP-43, FUS, and C9orf72 genes.	Accumulation of RNA/protein aggregates, neuronal death.
Bcl-x Splicing in Cancer	Bcl-xL ↑ (anti-apoptotic)	Chemotherapy resistance
p53 Splicing in Tumors	Loss of functional p53	Uncontrolled cell growth
Cystic Fibrosis	CFTR exon skipping	Thick mucus, lung infections
Beta-Thalassemia	Incorrect HBB splicing	Severe anemia
Duchenne Muscular Dystrophy	Dystrophin exon skipping	Muscle degeneration
Diabetes	Altered INSR splicing	Insulin resistance
Lupus (SLE)	Autoantibodies target snRNPs (U1 snRNA).	Autoimmune inflammation
Cancer and Tumorigenesis	Increased Bcl-xL via splicing factor SRSF1)	Resistance to apoptosis
	Mutations in MDM2 and p53 mRNA splicing	Inhibit tumor suppression
	Aberrant tumor suppressors (BRD9, BAP1)	Incr. tumor growth, metastasis.
	Bcl-xL increase (anti-apoptotic)	Chemotherapy resistance

Transfer RNA (tRNA)

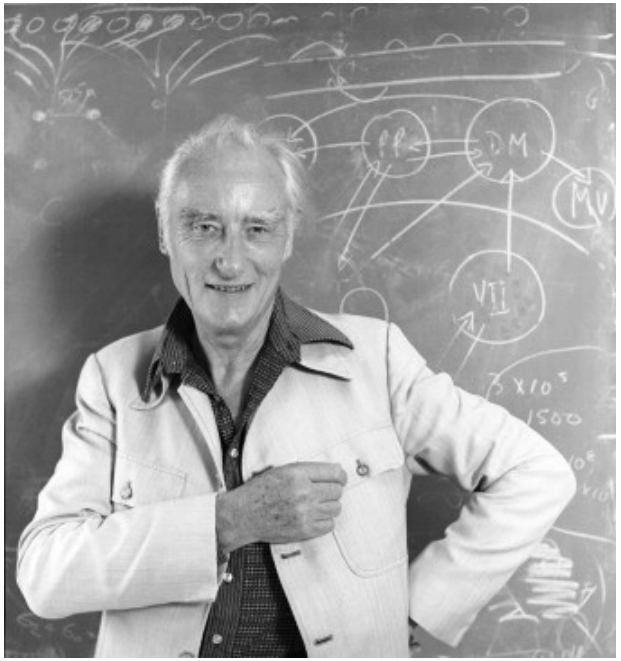
tRNA first hypothesized by Francis Crick.

Small RNA that transfers a specific amino acid to a growing polypeptide chain in the ribosome.

3' terminal site charged with amino acid.

Anticodon loop complements mRNA's codon.

tRNA molecules with different anticodons may carry the same amino acid (degenerecy).



"He also suggested that there would have to be a series of intermediate adaptor molecules specific for each amino acid—a remarkable prediction of the existence of tRNA molecules with their three-base anticodons."

Robin Holliday 2004

Francis Crick (1916–2004). Robin Holliday, Cell, Vol. 119, 1–2, October 1, 2004

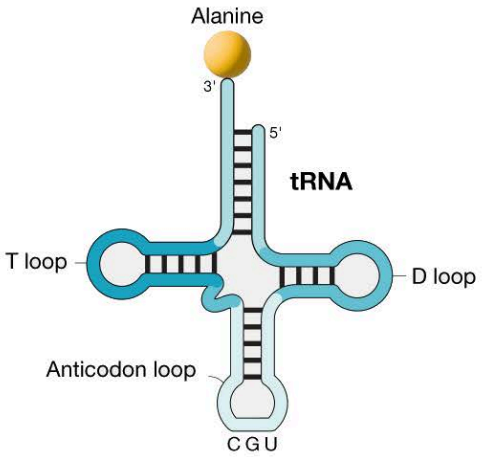
Transfer RNA (tRNA)

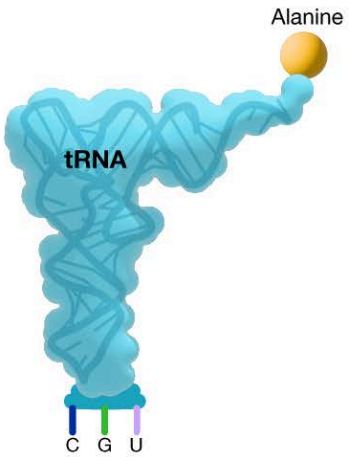
tRNA has primary (sequence), secondary (cloverleaf), and tertiary structure (L-shape).

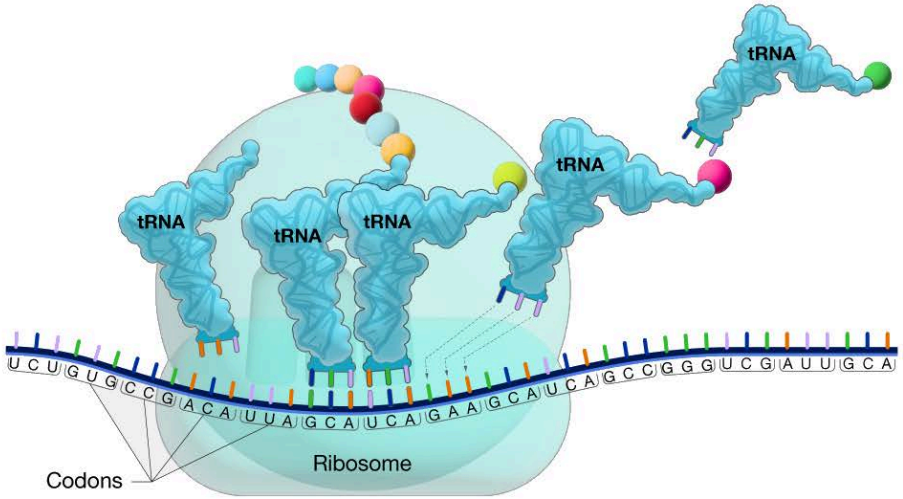
Small RNAs 75 - 85 bases in length

Highly conserved secondary and tertiary structures in prokaryotes and eukaryotes.

- Acceptor stem (7 bp) is duplex of 5’- and 3'-termi (non-Watson-Crick base pairing).
- D arm (18 bp) contains dihydrouridine.
- Anticodon arm (17 bp).
- The T arm (17 bp) contains pseudouridine TψC sequence







Transfer RNA (tRNA)

Organisms have varying amounts of tRNA genes.

C. elegans has 29,647 genes of which 620 code for tRNA (2%).

Saccharomyces cerevisiae has 275 tRNA genes in its genome.

In the human genome there are:

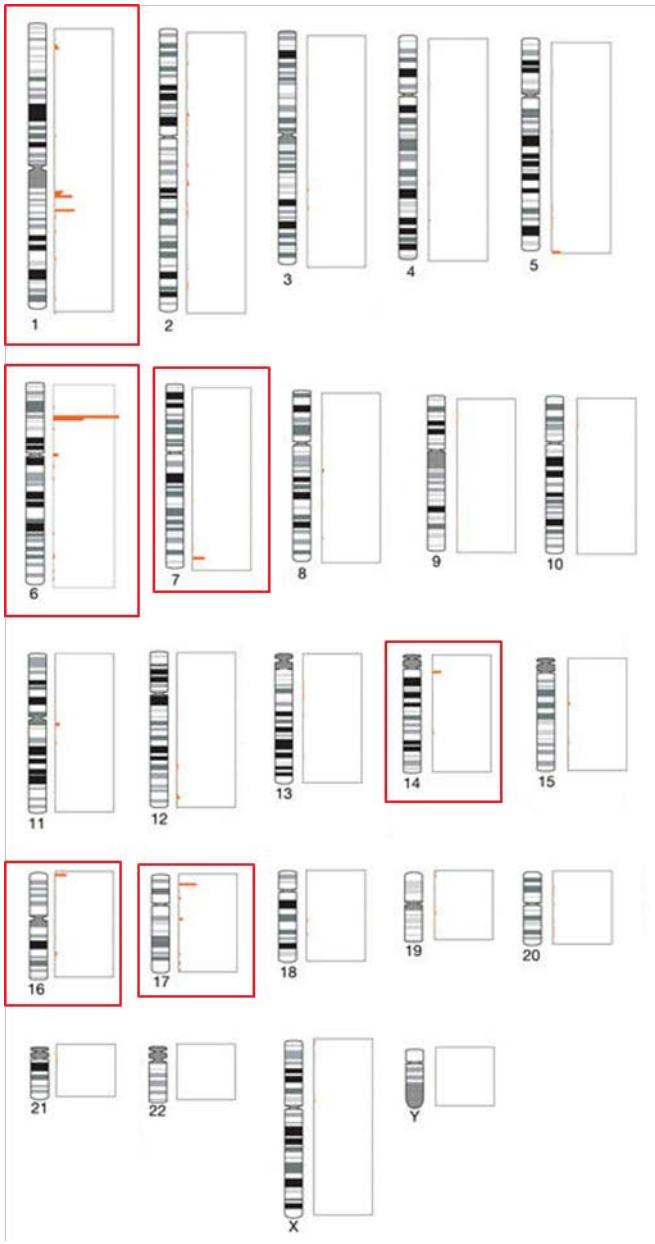
- 4,421 non-coding RNA genes (which include tRNA genes).
- 22 mitochondrial tRNA genes
- 497 nuclear encoding cytoplasmic tRNA genes and
- 324 tRNA-derived putative pseudogenes.

Cytoplasmic tRNA genes are grouped into 49 families

tRNA genes are found on all chromosomes, except 22 and Y.

High clustering on 6p and 1.

tRNA molecules are transcribed (in eukaryotic cells) by RNA polymerase III



Ribosomal RNA (rRNA)

Most abundant RNA in cells (~80% of total cellular RNA).

Prokaryotic ribosomes smaller than eukaryotes.

The ribosomes in eukaryote mitochondria resemble those in bacteria.

Sponsor translation: Catalyze amino acids polymerization into polypeptide chains.

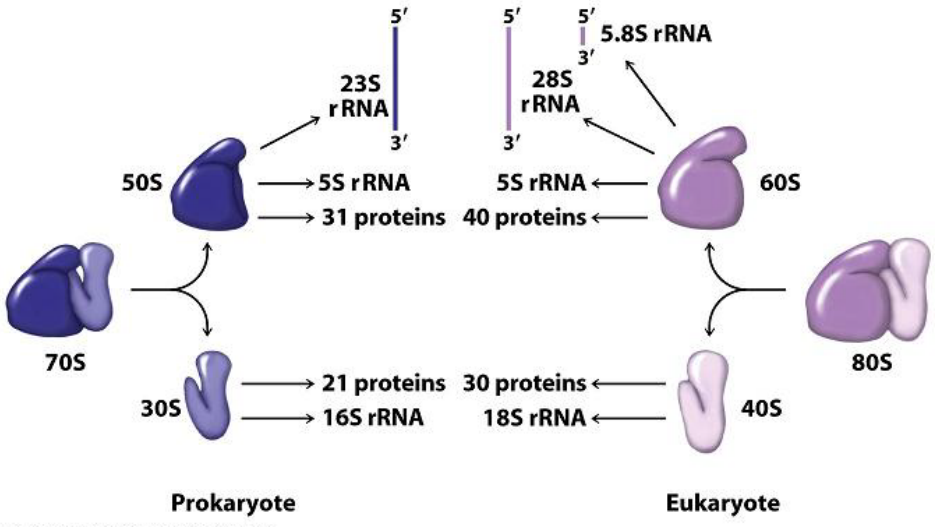
Rely on tRNA adapters.

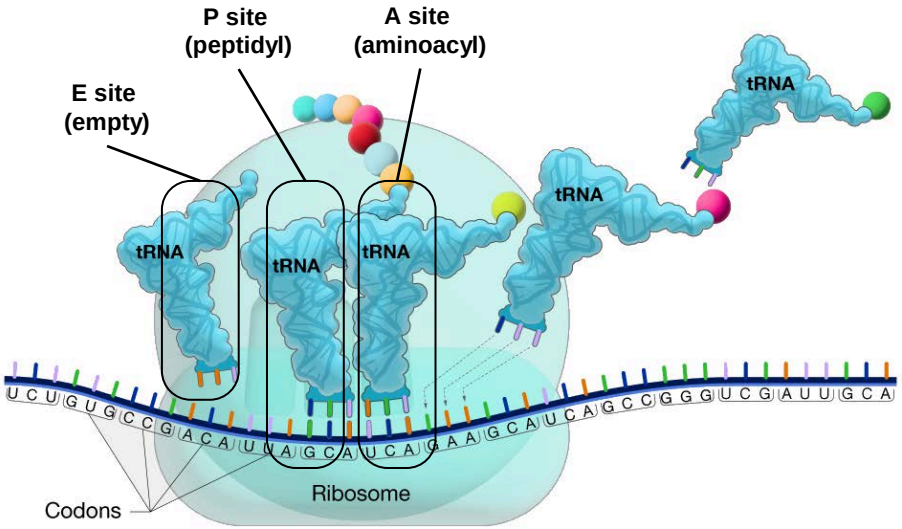
Synthesized in nucleolus rRNA gene clusters.

Mutations in mitochondrial rRNA genes lead to mitochondrial diseases.

Target of antibiotics (macrolides & aminoglycosides).

rRNA sequencing (16S/18S rRNA) and tree of life.





Ribosomal RNA (rRNA) A site

Binds an incoming aminoacyl-tRNA as directed by the codon currently occupying this site.

This codon specifies the next amino acid to be added to the growing peptide chain.

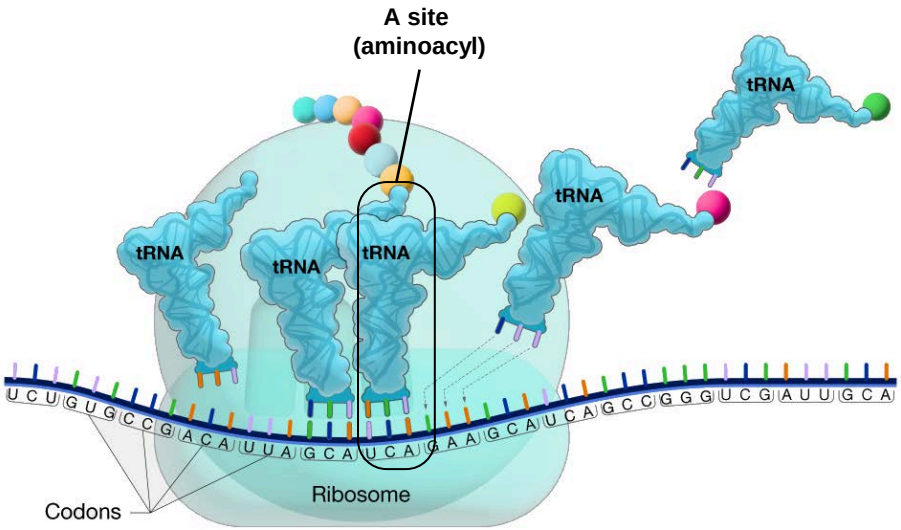
The A site is only working after the first aminoacyl-tRNA has attached to the P site.

First site to bind tRNA during elongation.

Found in both prokaryotic (70S) and eukaryotic (80S) ribosomes.

A site dome: Transpeptidation reaction

A site floor: Codon – anticodon interaction



Ribosomal RNA (rRNA) P site

The P-site codon is occupied by peptdyl-tRNA (a tRNA with multiple amino acids attached).

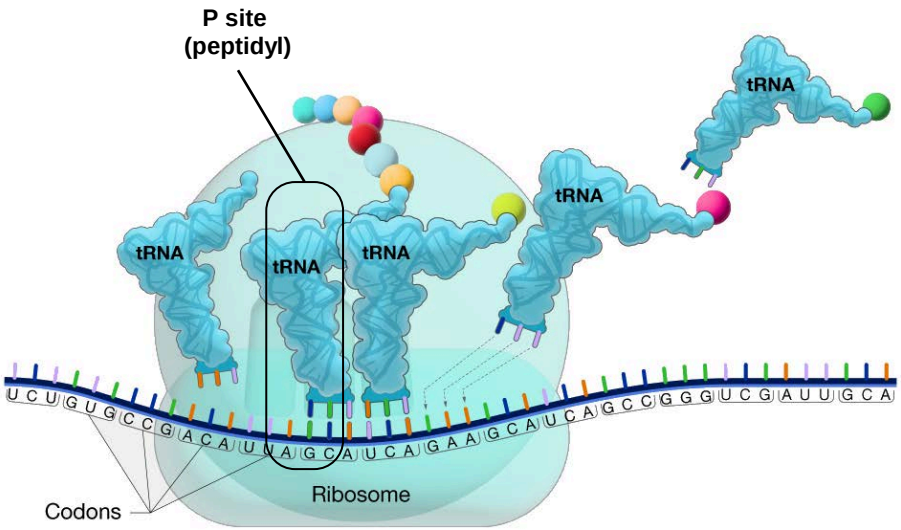
The P site is actually the first to bind to aminoacyl tRNA during initiation.

This tRNA in the P site carries the chain of amino acids that has already been synthesized.

Found in both prokaryotic (70S) and eukaryotic (80S) ribosomes.

P site dome: Transpeptidation reaction

P site floor: Codon – anticodon interaction



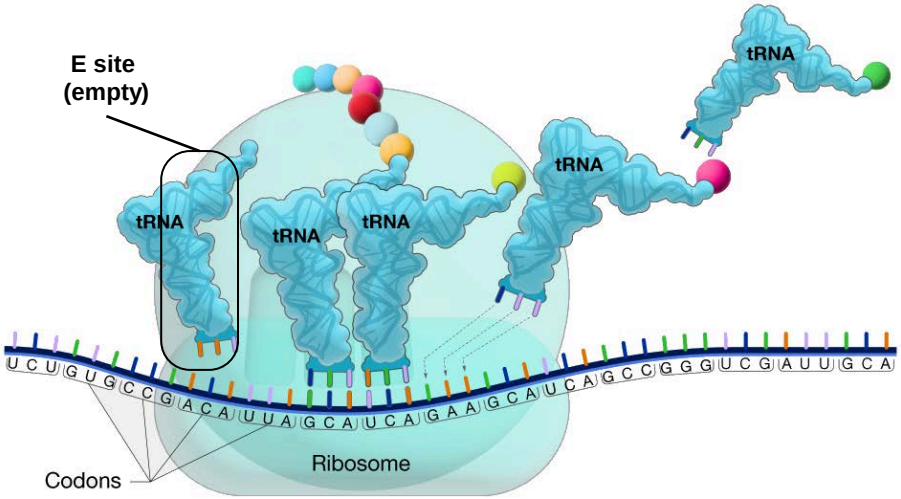
Ribosomal RNA (rRNA) E site

The E site is occupied by the empty tRNA as it is about to exit the ribosome.

Found only in prokaryotic (70S) ribosomes.

E site dome: Harbours empoty tRNA

P site floor: Inexistent



Prokaryote rRNA and antibiotics

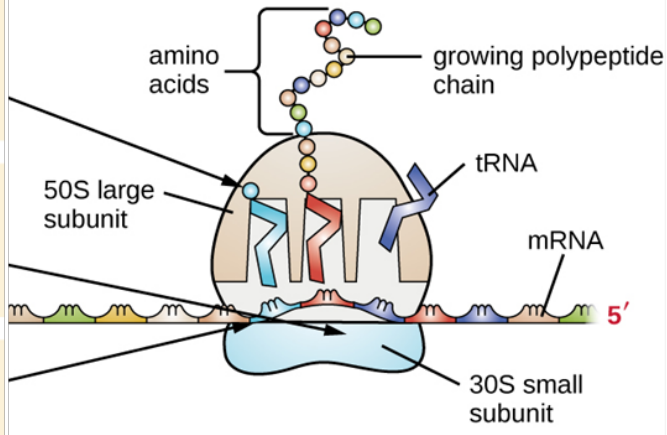
Prokaryote and eukaryote ribsome differences lie outside of functional parts.

Differences exploited by antibiotics that harm bacteria but not cells of the infected person.

Mitochondrial membrane prevents rRNA intoxication in the infected person.

Antibiotics such as macrolides, aminoglycosides and others:

	Major classes of protein synthesis–inhibiting antibacterials
Erythromycin, Clarithromycin, Azithromycin, Roxithromycin, Telithromycin ⇦	**Chloramphenicol, macrolides, and lincosamides** • Bind to the 50S ribosomal subunit • Prevent peptide bond formation • Stop protein synthesis
Gentamicin, , neomycin, tobramycin, amikacin, plazomicin, streptomycin and paromomycin ⇦	**Aminoglycosides** • Bind to the 30S ribosomal subunit • Impair proofreading, resulting in production of faulty proteins
Tetracycline, Doxycycline, Minocycline, Lymecycline, Oxytetracycline, Tigecycline, Chlortetracycline, Demeclocycline and Rolitetracyclin. ⇦	**Tetracyclines** • Bind to the 30S ribosomal subunit • Block the binding of tRNAs, thereby inhibiting protein synthesis



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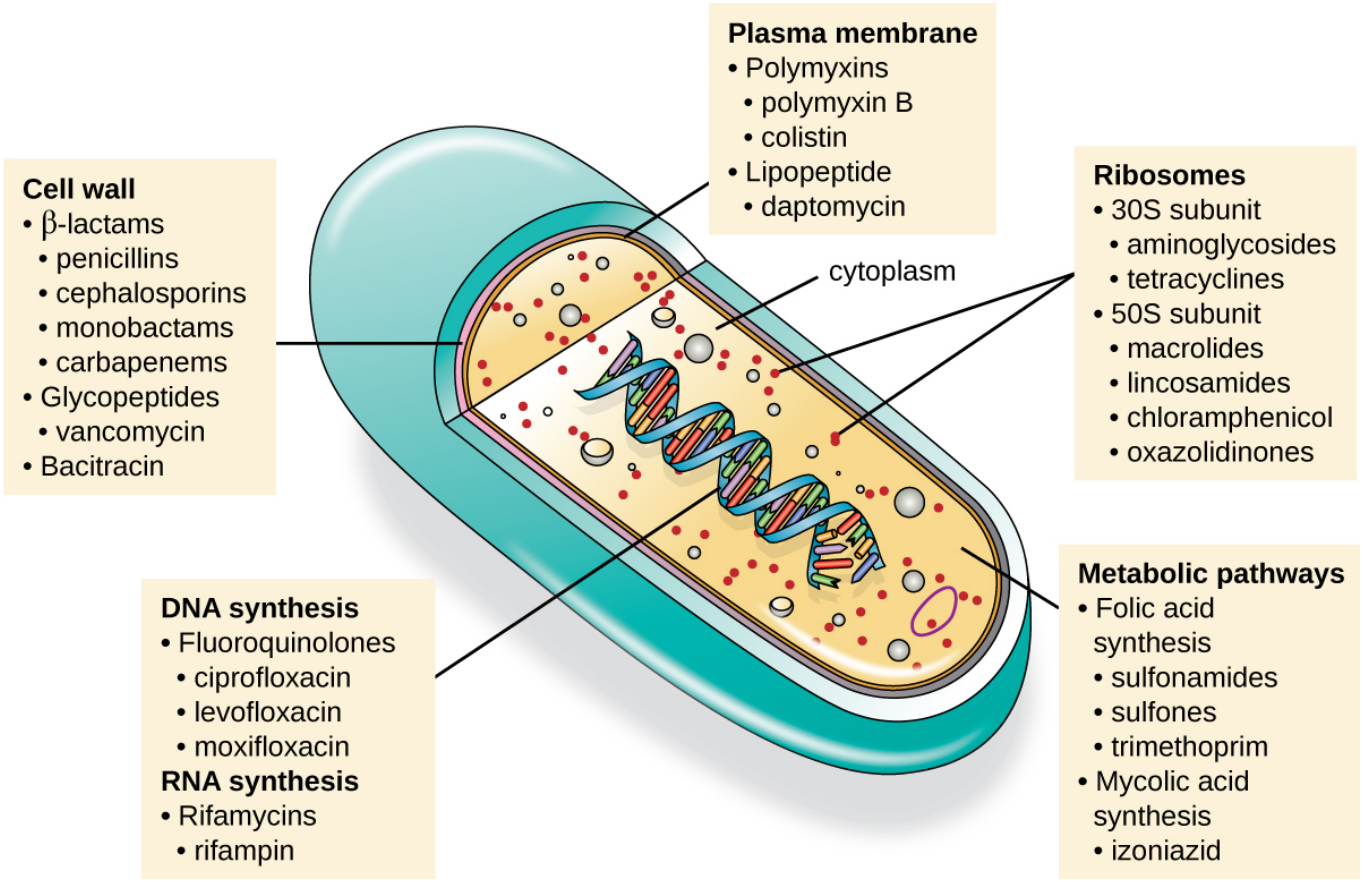
Other antibiotic exploits

Other antibiotic exploits



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Instalaciones de Alta Contención Biológica Nivel de Bioseguridad 3 (BSL-3) CDC-certificadas

Facultad de Medicina UASLP
San Luis Potosí, México

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