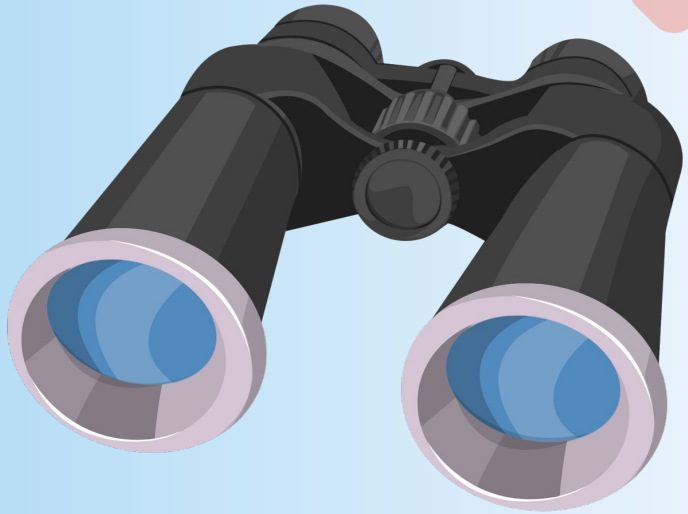
Protein biosynthesis

(Transcription + translation)

By Herman Mageli

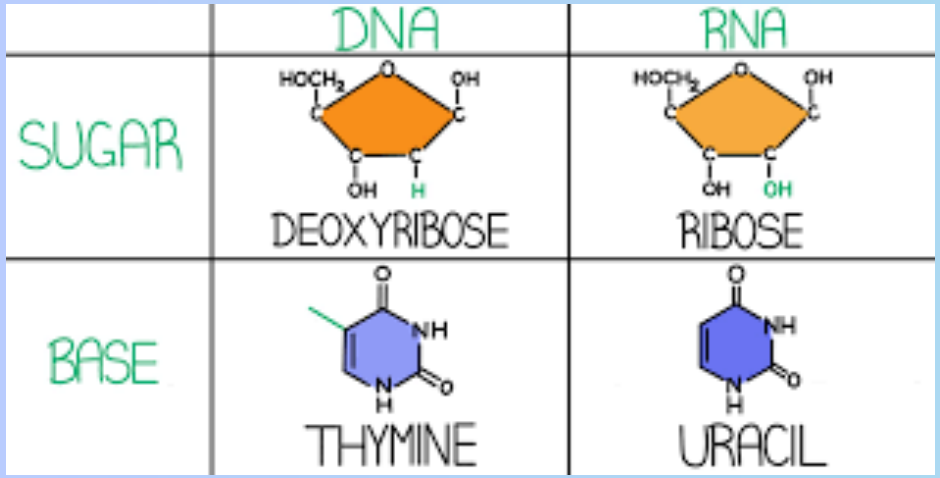
Overview



Transcription
Posttranscriptional modifications
The genetic code
Translation
Posttranslational modifications

DNA vs. RNA

	DNA	RNA
Length	Longer	Shorter
Composition	Deoxyribose	Ribose
Strands	Double stranded	Single stranded
Bases	Thymine	Uracil



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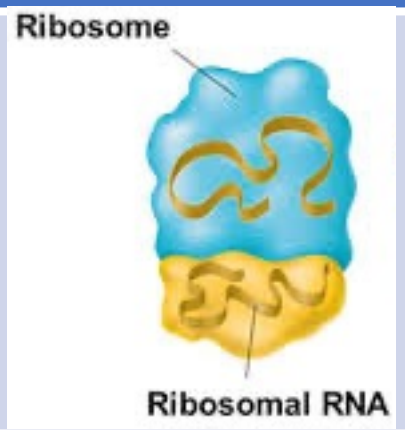
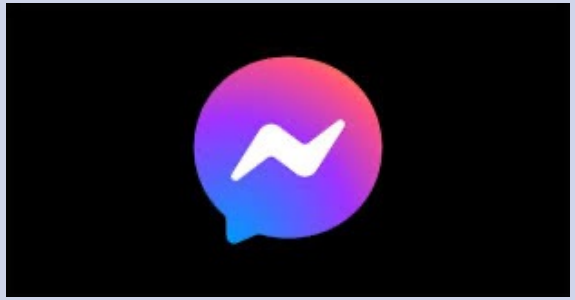
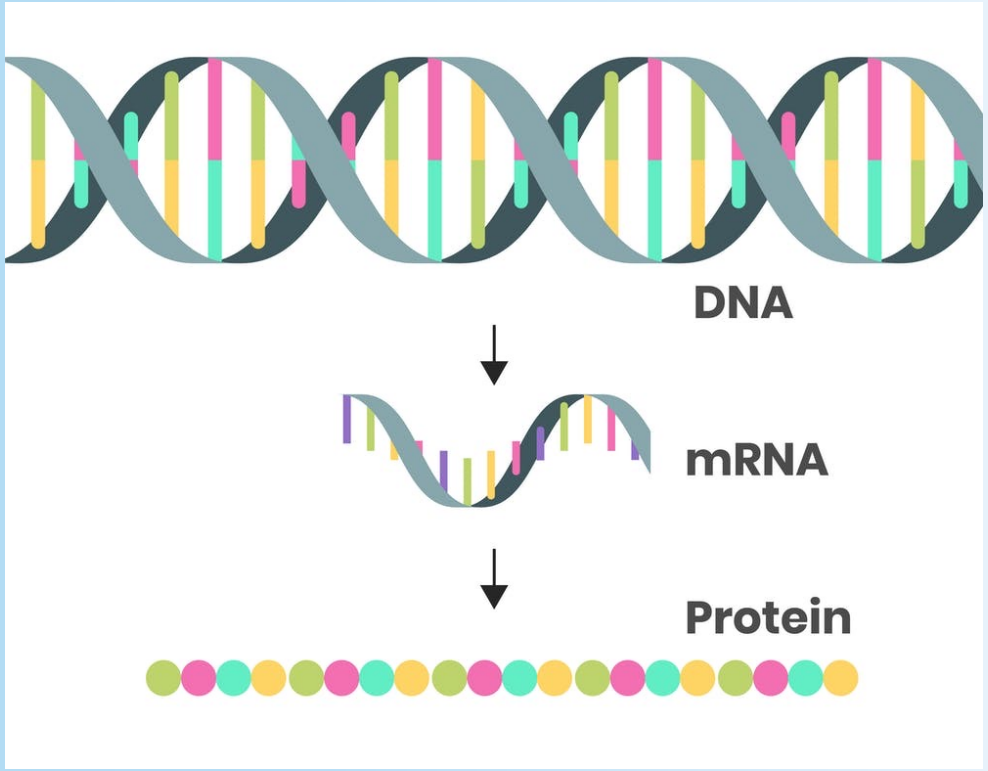
RNA

rRNA	tRNA	mRNA
		
• Most **abundant** • 80% total RNA • **Prokaryotes:** 23S, 16S, 5S • **Eukaryotes:** 18S, 28S, 5.8S, 5S	• 15% of all RNA • **Smallest**	• 5% of all • Carry genetic info from nucleus to cytosol • **Polycistronic vs. Monocistronic**

Transcription

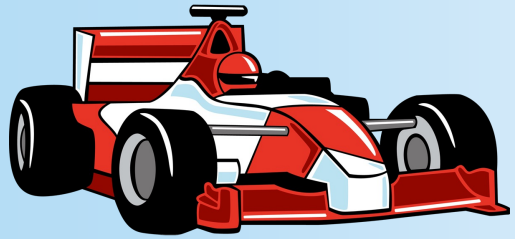
- «The copying process during which a DNA strand serve as a template for the synthesis of RNA»

- Copying a DNA into **mRNA** strands
  - Used to produce **aminoacid chains** in cytoplasm
- Occur in 3 phases
  1. Initiation
  2. Elongation
  3. Termination



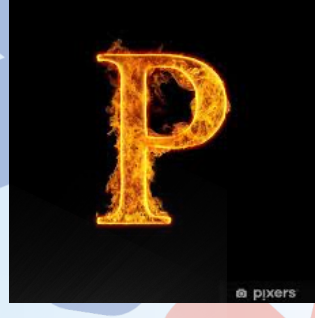
RNA polymerase

- RNA strand → **RNA polymerase**
  - 5´3´ polymerase activity
    - ➢ Attach to 3´-end of DNA and **DRIVE to FIVE**
- **NO primer, NO proofreading activity**
- ONLY 1!!
  - Create all RNA in prokaryotes

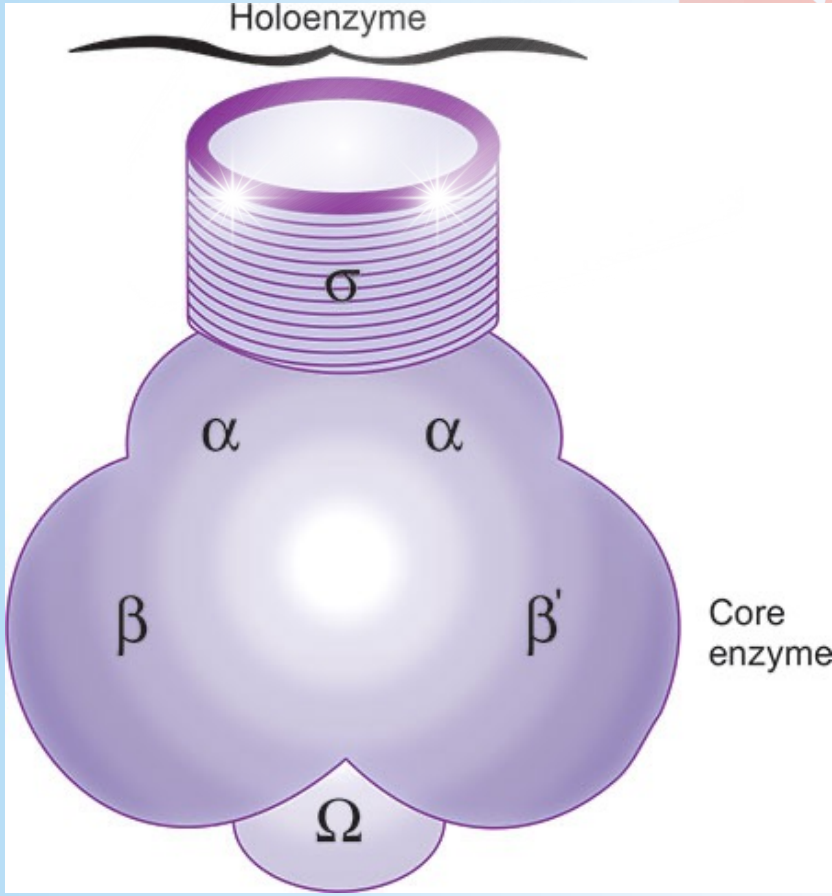


- 3 types
  1. RNA pol. I
  2. RNA pol. II
  3. RNA pol. III

Structure of RNA polymerase



- **Core** enzyme
  - 4 subunits
  - Subunits complete the maschinery
- **Sigma factor**
- **Holoenzyme** : core enzyme + sigma factor



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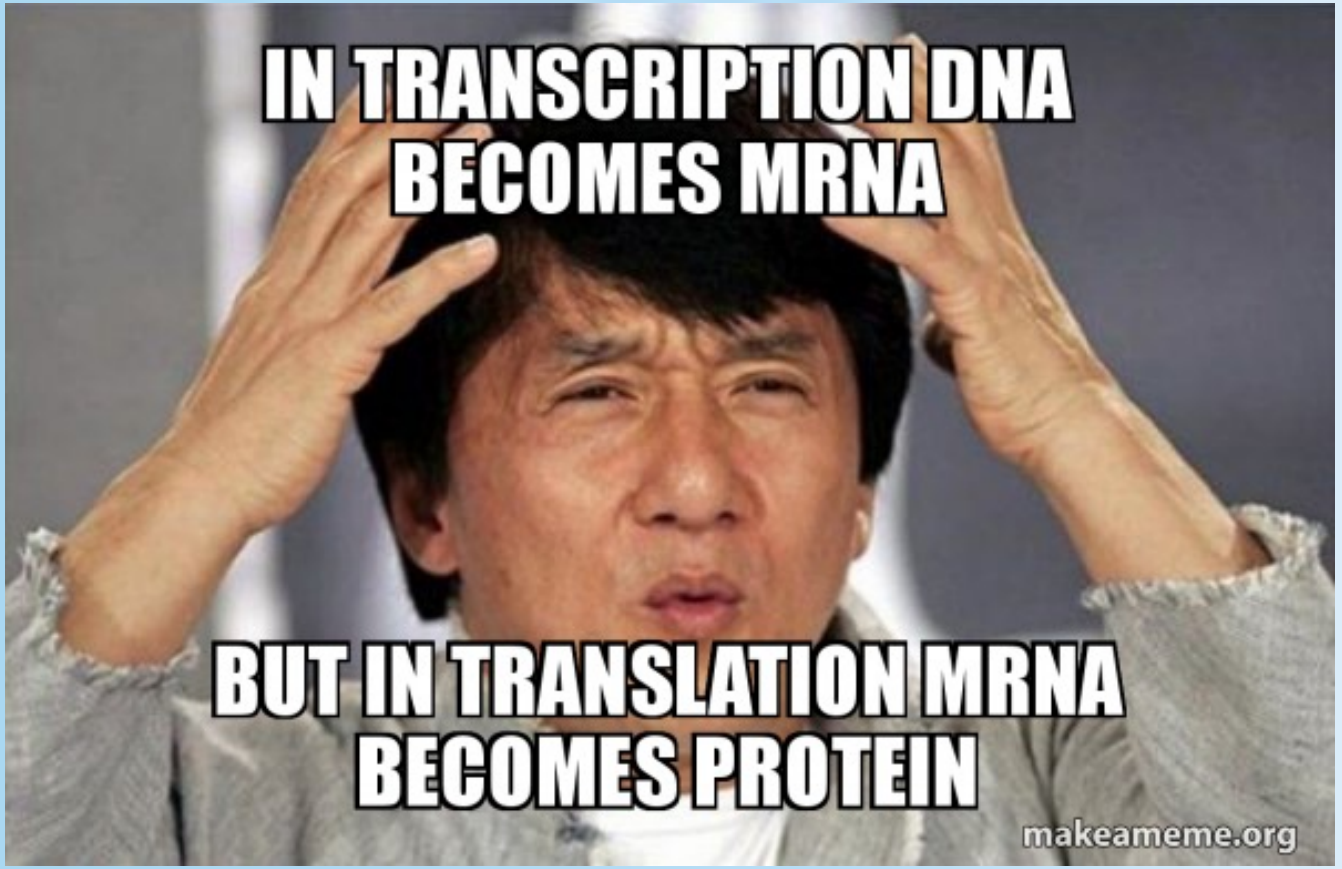
Transcription

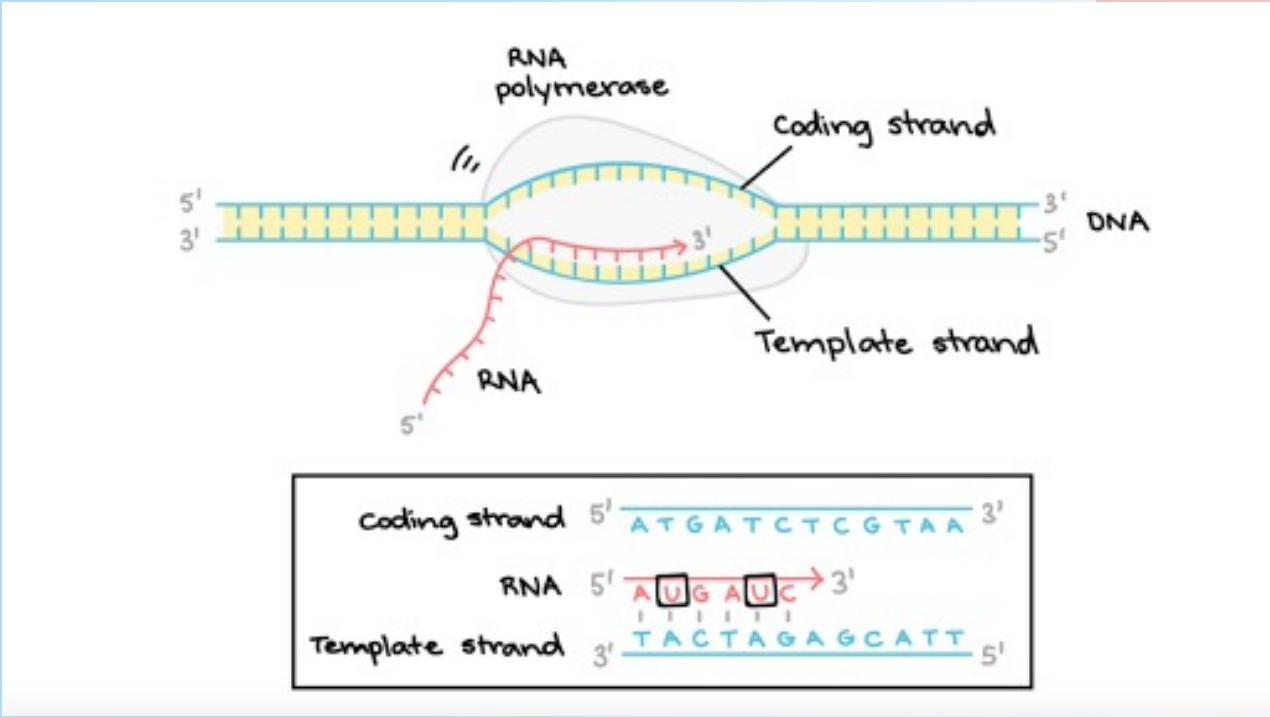
1. Initiation
2. Elongation
3. Termination



1. Initiation

- Holoenzyme recognize promoter region: 2 sequences

1. **-35 sequence**
   - 5´- TTGACA -3´
   - **Closed** complex
2. **Pribnow box**
   - 5´-TATAAT-3´
   - Site of initial DNA melting
     - ➢ **OPEN** complex
     - ➢ Transcription bubble



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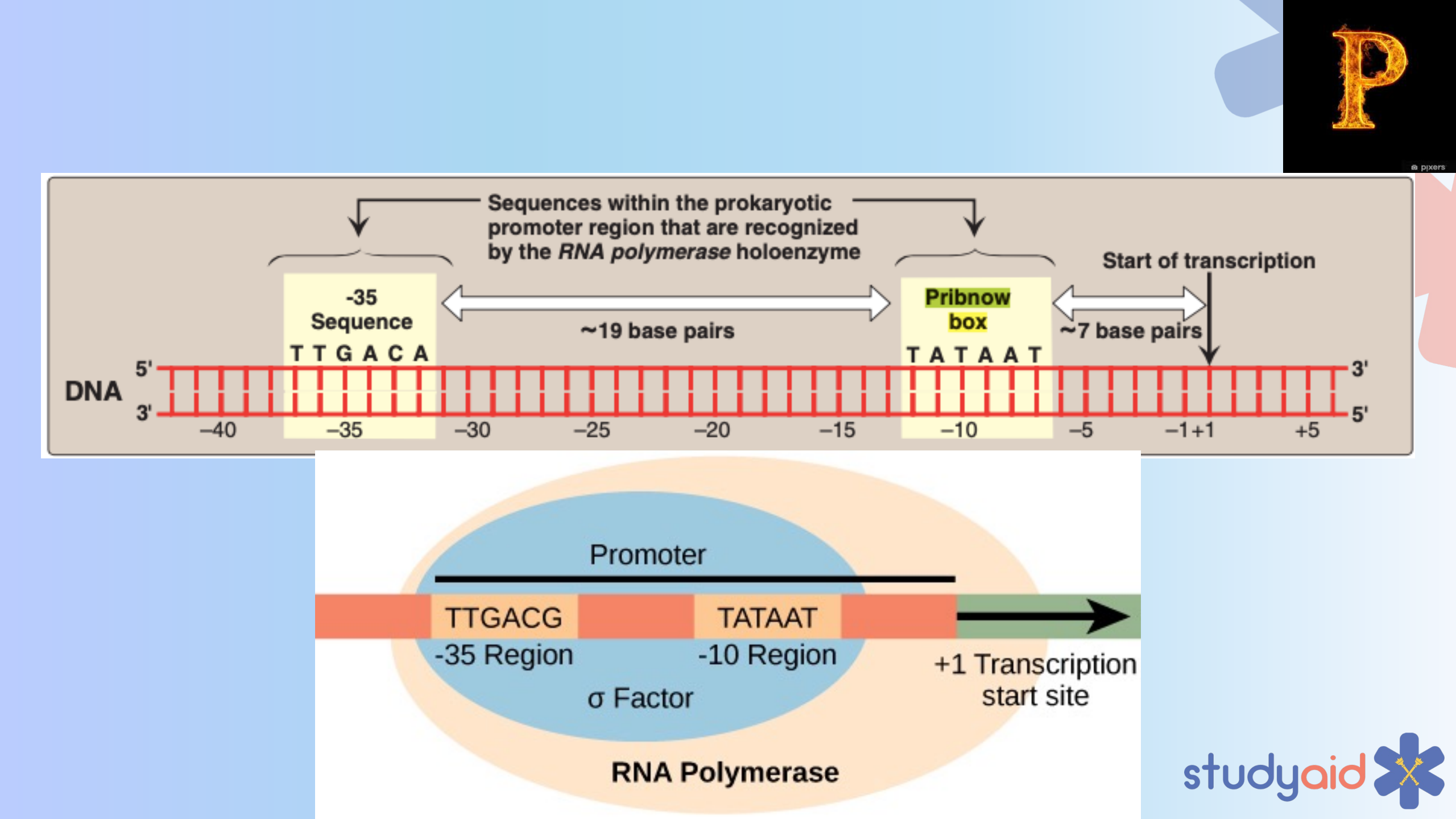
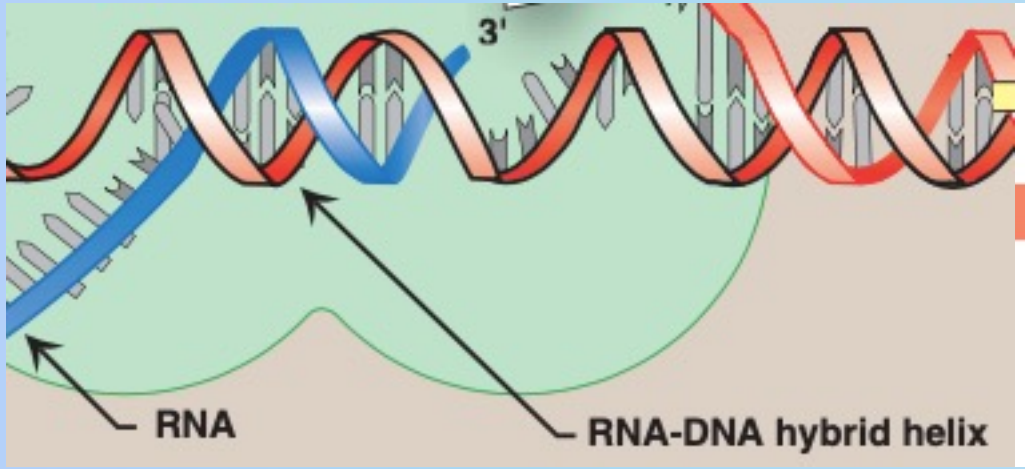
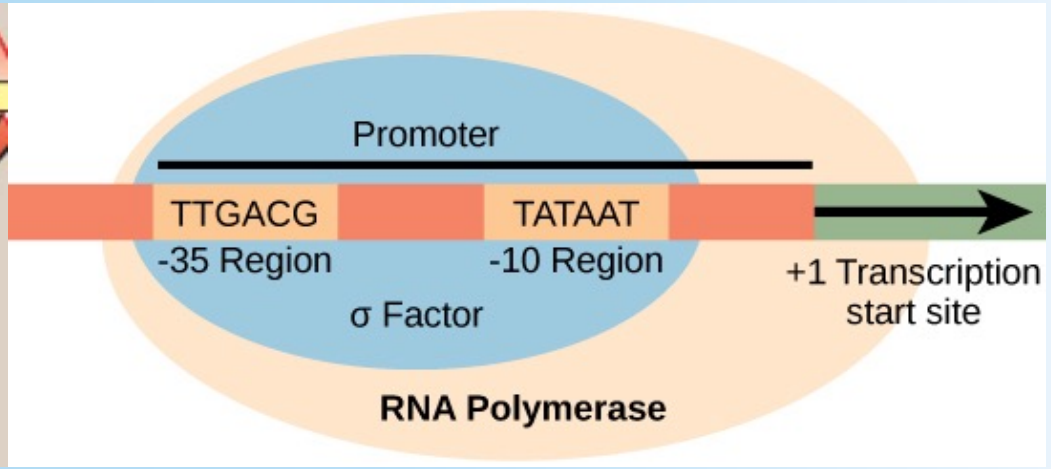

Sequences within the prokaryotic promoter region that are recognized by the RNA polymerase holoenzyme
-35 Sequence
T T G A C A
~19 base pairs
Pribnow box
T A T A A T
Start of transcription
~7 base pairs
DNA
5'
3'
3'
5'
–40
–35
–30
–25
–20
–15
–10
–5
–1 +1
+5
Promoter
TTGACG
TATAAT
-35 Region
-10 Region
σ Factor
+1 Transcription start site
RNA Polymerase

2. Elongation

- As unwinding continues, **supercoils** are formed
  - Removed by **DNA topoisomerases**
- **10 nucleotide** length
  - Sigma factor dissociates
  - DNA-RNA hybrid helix

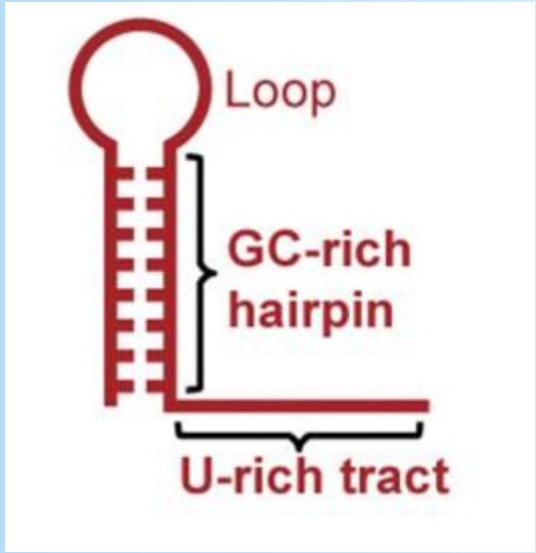




3. Termination

- 2 types
  1. **p-Independent**
     - Most common
     - **Self-complimentary** strand form hairpin-loop
     - GC-rich stem

  2. **p-Dependent**
     - Protein rho(p)
     - **ATPase with helicase activity**
     - Binds C-rich region on RNA 5´-end
       - ➢ Move with ATPase activity to termination site
       - ➢ Helicase activity to cut out.



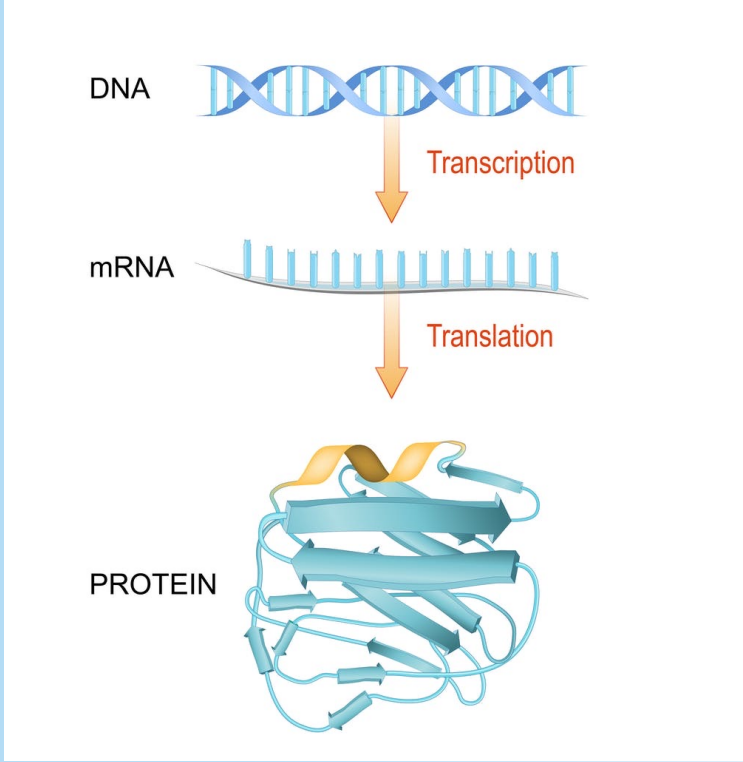
Eukaryotic transcription

- More complicated!
  - 3 instead of 1 RNA polymerase!
    - ➢ One for **each** RNA type
  - **Transcription factors** (TFs)
    - ➢ Assemble transcription comlex
    - ➢ Melt DNA
    - ➢ Bind promoter region
    - ➢ Each RNA polymerase has **its own** transcription factors and promoters

RNA polymerase I	Synthesize precursor to the 28S, 18S, 5.8S **rRNA**
RNA polymerase II	Synthesize precursor of **mRNA** Also small noncodingRNA (snRNA, snoRNA, miRNA)
RNA polymerase III	Synthesize **tRNA** and 5S rRNA Also synthesize some snRNA and snoRNA

RNA polymerase II

- Produce **mRNA**
- Sequences acts as binding sites for TFs
- 2 sequences in promotor region:
  1. **TATA/Hogness box**
     - Almost identical to Pribnow (TATAAT)
     - Full sequence: TATAAA
     - -25
  2. **CAAT box**
     - 70-80 nucleotides upstream

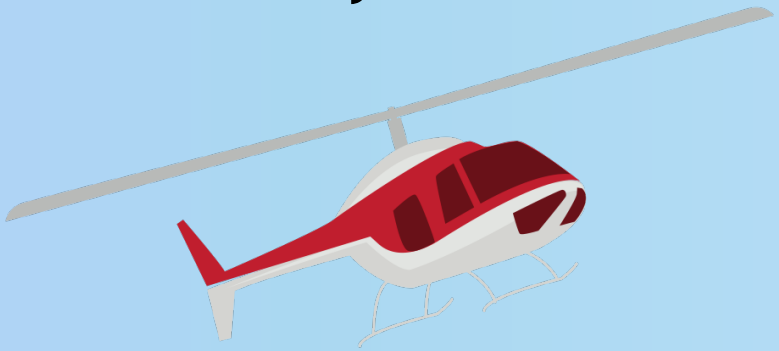


Transcription factors - RNA polymerase II

1. **TFIID:** recognize and binD TATA box

2. **TFIIF**: Fetch the polymerase to the promotor

3. **TFIIH:** Helicase activity that melts the DNA and phosphorylates polymerase



Antibiotics and inhibitors

Rifampin 	• Bind to **b-subunit** of prokaryotic RNA polymerase • **Tuberculosis**
Dactinomycin (actinomycin D) 	• Bind **DNA template** → interfere with movement of RNA polymerase along the DNA • Tumor **chemotherapy**
Alpha-amanitin	• Toxin produced by the mushroom **Amanite phalloides** («death cap») • Form a tight **complex** with **RNA pol.II** → inhibit mRNA synthesis
Streptomycin 	• Bind **30S subunit** → Inhibit translational initiation

Overview

~~Transcription~~
Posttranscriptional modifications
The genetic code
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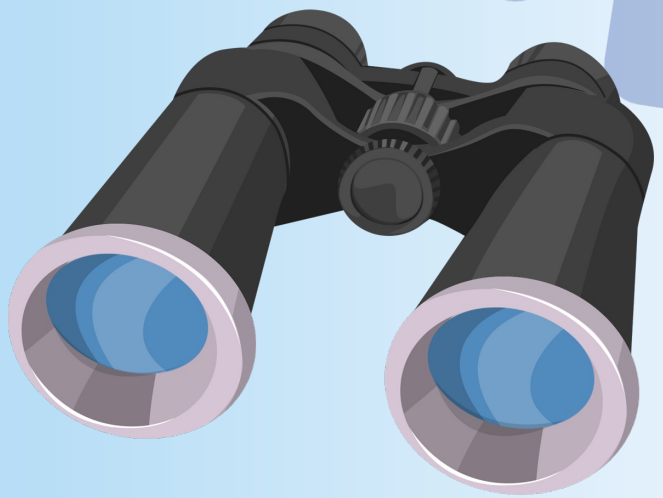


Posttranscriptional modifications

- Initial product = «**primary transcript**»
- Both prokaryotic and eukaryotic rRNA and tRNA are **cleaved** by ribonucleases
- In prokaryotes, mRNA is **almost identical** to primary transcript mRNA
- In eukaryotes, mRNA is **extensively modified.**

	rRNA	tRNA
Origin	• 16S, 28S etc. Are ALL generated from a **long precursor** molecule that is cleaved	• Also made from **longer precursor** molecules that are cleaved
Introns		• Noncoding regions of RNA that may be present. If so, they are removed by **nucleases**.
-CCA ending added		• Added on 3´-end by **nucleotidyltransferase**

Pre-mRNA modifications

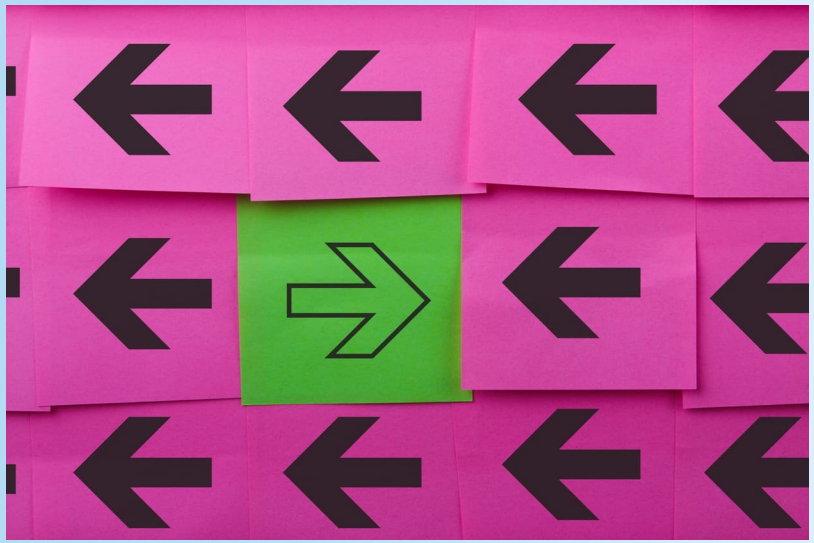
1. 5´-capping
2. Addition of a poly-A tail
3. Removal of introns

5' cap

5´capping

Poly-A tail

- It is a **7-methylguanosine** attached «backwards» to the 5´-end of pre-mRNA»
  - This create a **5´-5´linkage**
- **Goal:**
  - Stabilize mRNA
  - **Allows initiation** phase of translation

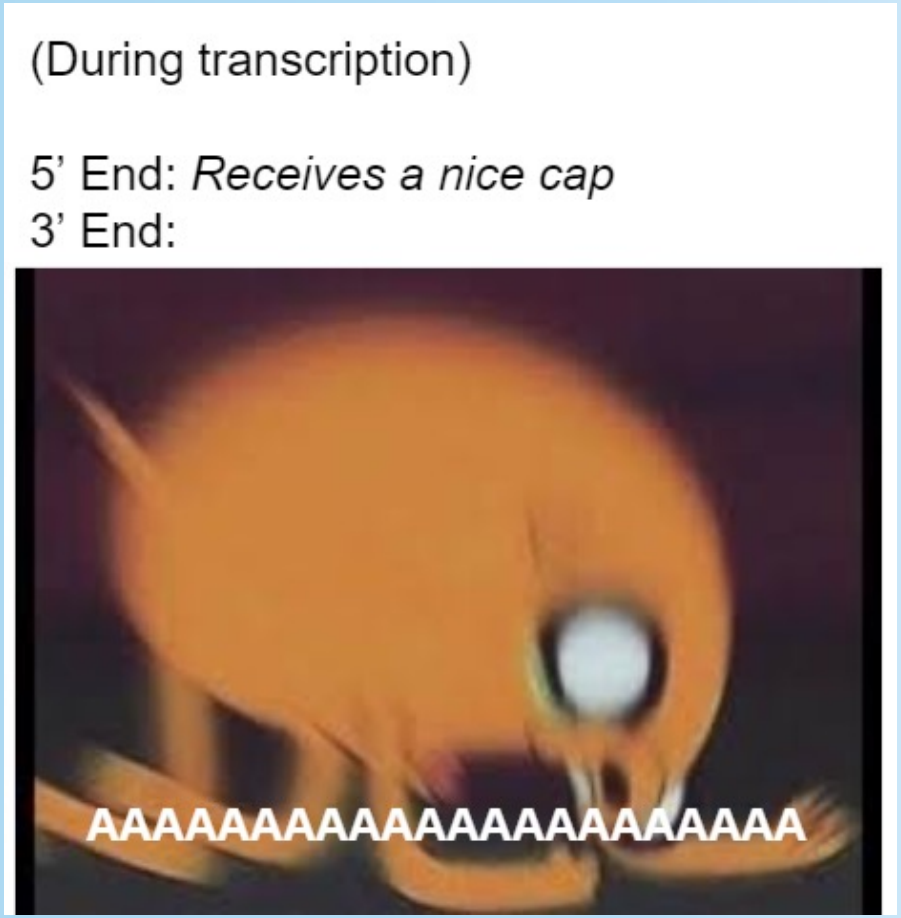


5' cap

Poly-A tail addition

Poly-A tail

- It is a series of 40-200 **adenine** (A) nucleotides added to the end of pre-mRNA
- **Goal:**
  - Stabilize mRNA
  - Facilitate exit from nucleus
  - Aid in translation



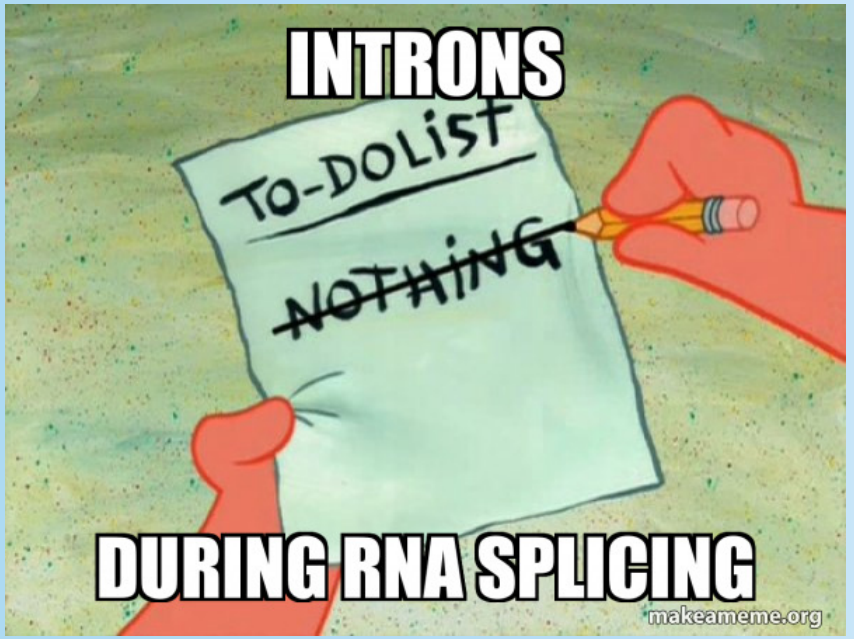
5' cap

Removal of introns

Poly-A tail

- **Introns:** sequences of RNA that do **NOT** code for proteins
- **Exons:** remaining sequences, joined together
- **Splicing:** the process of removing introns and joining exons
  - Occur in **spliceosome**

- **snRNA** = «snurps»
  - Facilitate removal of introns



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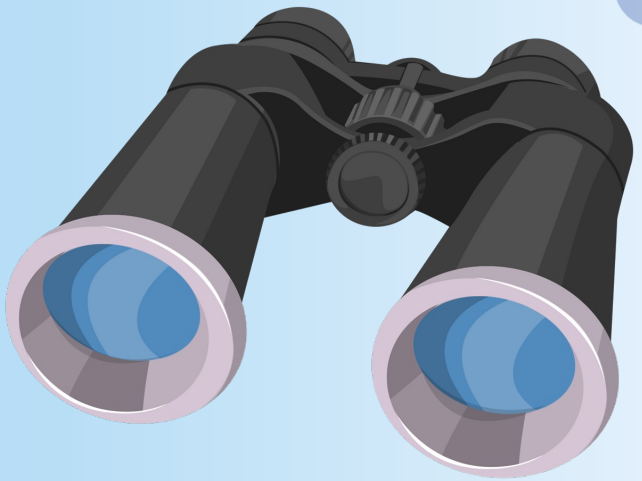


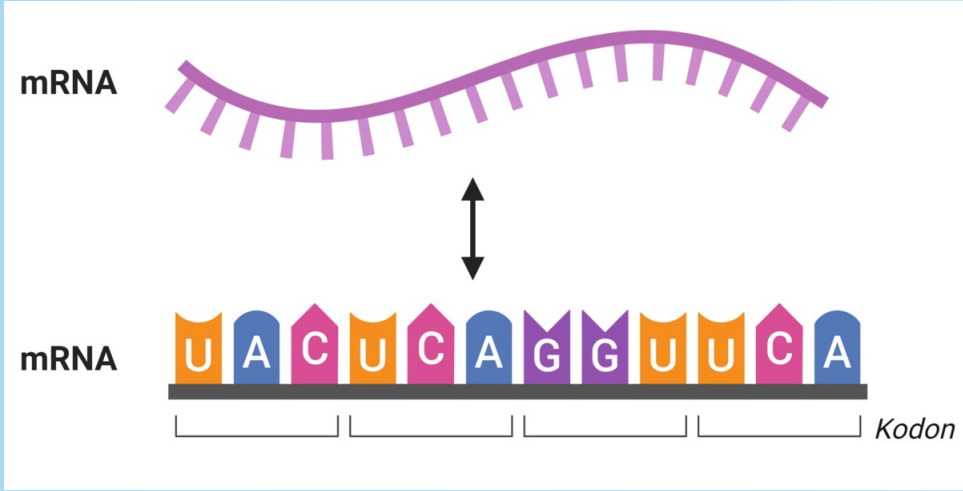
The genetic code

- A «dictionary that identifies correspondence between nucleotide bases and amino acids»
  - 4 letters: A (adenine), G (guanine), C (cytosine), and U (uracil)
- Three letters form **«codons».**
- These codons represents certain **amino acids**

Codons

U Are Gone
U Go Away
U Are Away

- «Codons» are present in mRNA
  - They are 3 base pairs
- **Start codon**: AUG = methionine
- **Stop codons**: at the end, stop the translation = UAG, UGA, UAA (don´t code for amino acids)
- **ALWAYS** read 5´-3´unless specifically written otherwise
  - AUG = 5´-AUG-3´



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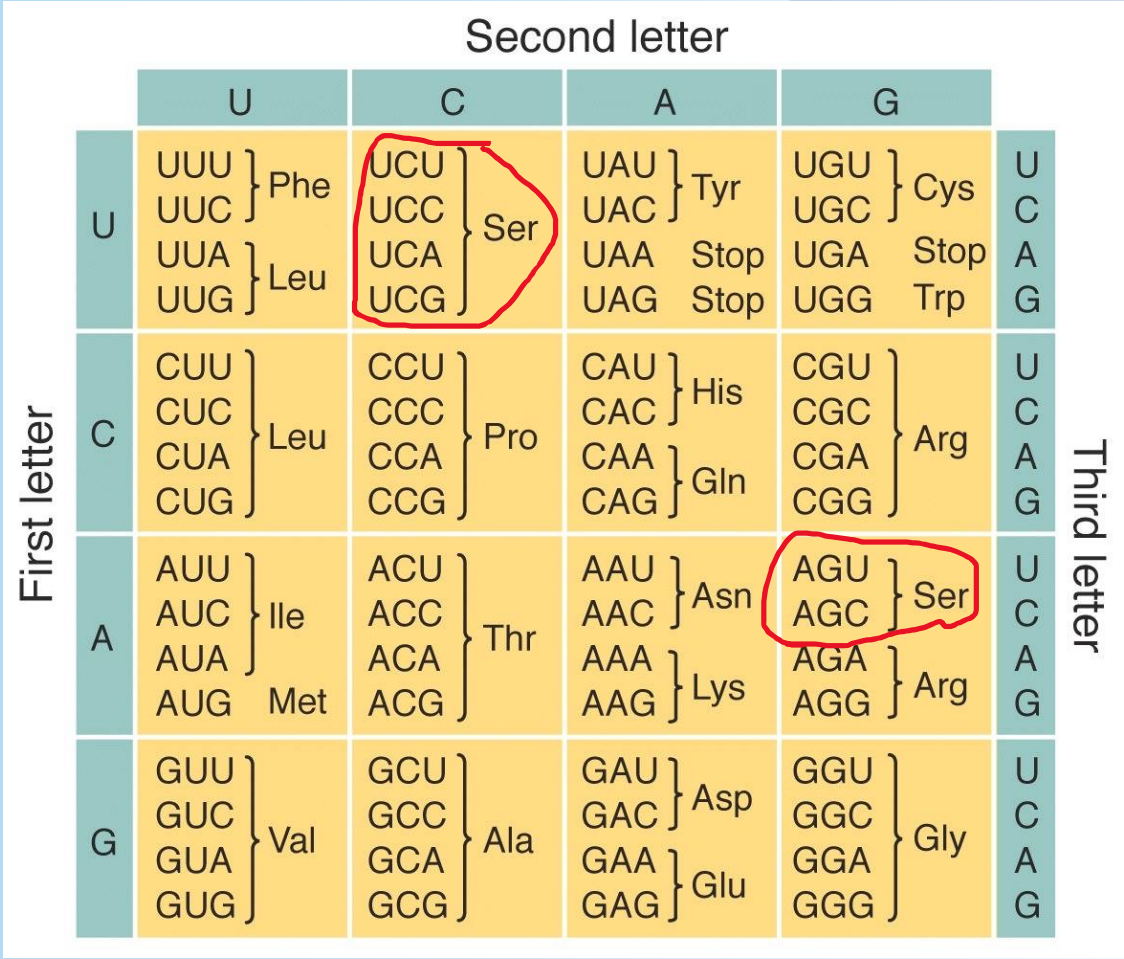
Degenerative genetic code

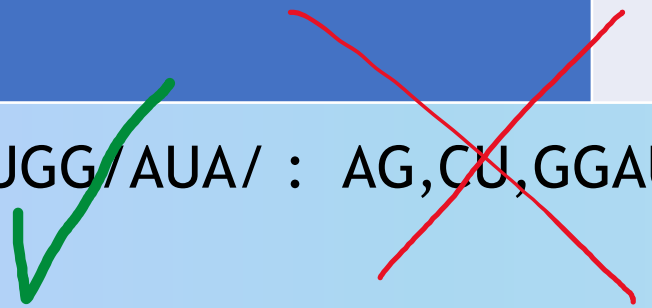
- 4 letters (A,G,C,U)
- We have **4^3 combinations** = 64.
  - 20 amino acids in total
    - ➢ One amino acid can be coded for by several codons



Vocabulary of the genetic code

Specificity (unambigous)	A codon only code for **ONE** amino acid
Degeneracy (redundant)	An amino acid may have **several codons** representing it
Universality	Code is **equal** in all organisms → **AUG** means methionine for me and for you
Non-overlapping	Code is read three bases at the time, **without** any overlap or commas

- AGC/UGG/AUA/ : AG,CU,GGAUA



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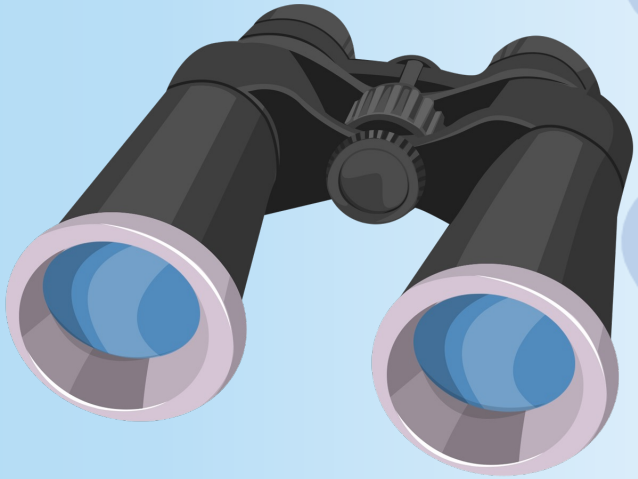
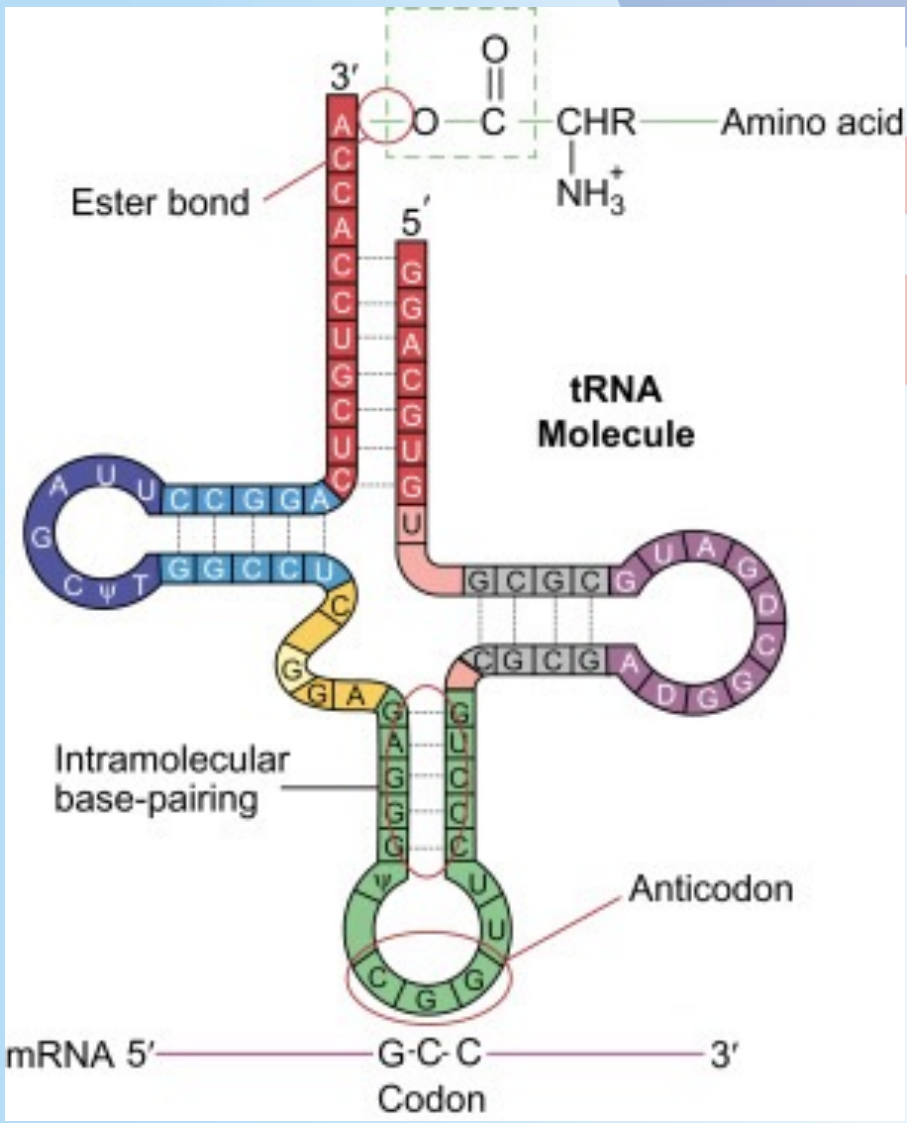


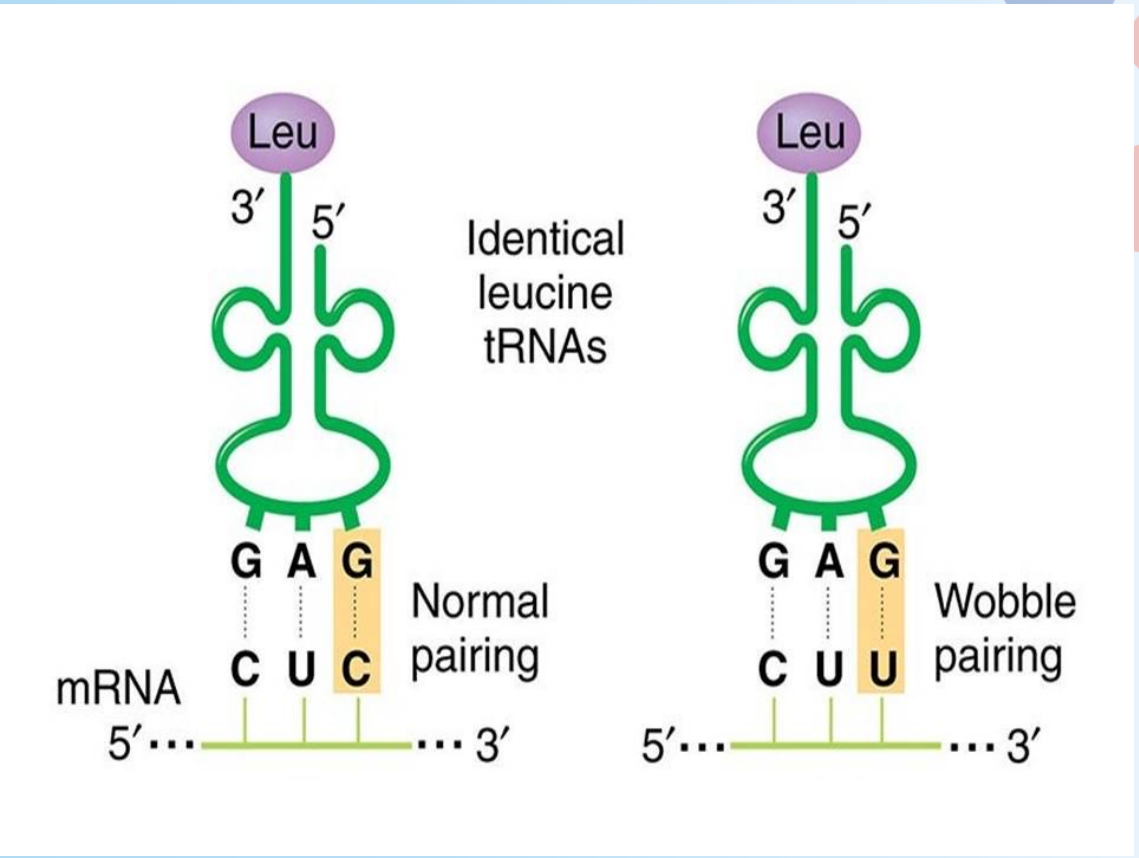
tRNA

- The job of tRNA is to Transport AA!
- **Anticodon vs. Codon**
- tRNAs carry amino acids
  - **Aminoacyl-tRNA synthetase** (ATP→AMP)
  - Attached to the **3´-end (ester bond)**
- tRNA with attached AA = **charged tRNA**
  - No amino acid = uncharged tRNA



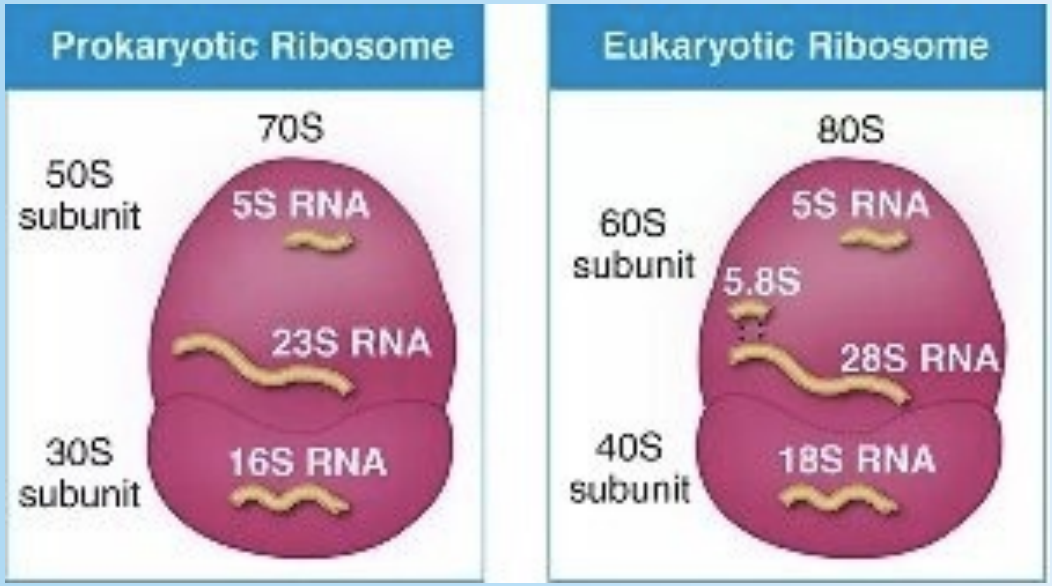
Wobble hypothesis

- 50 tRNAs for **61** codons...
- I base



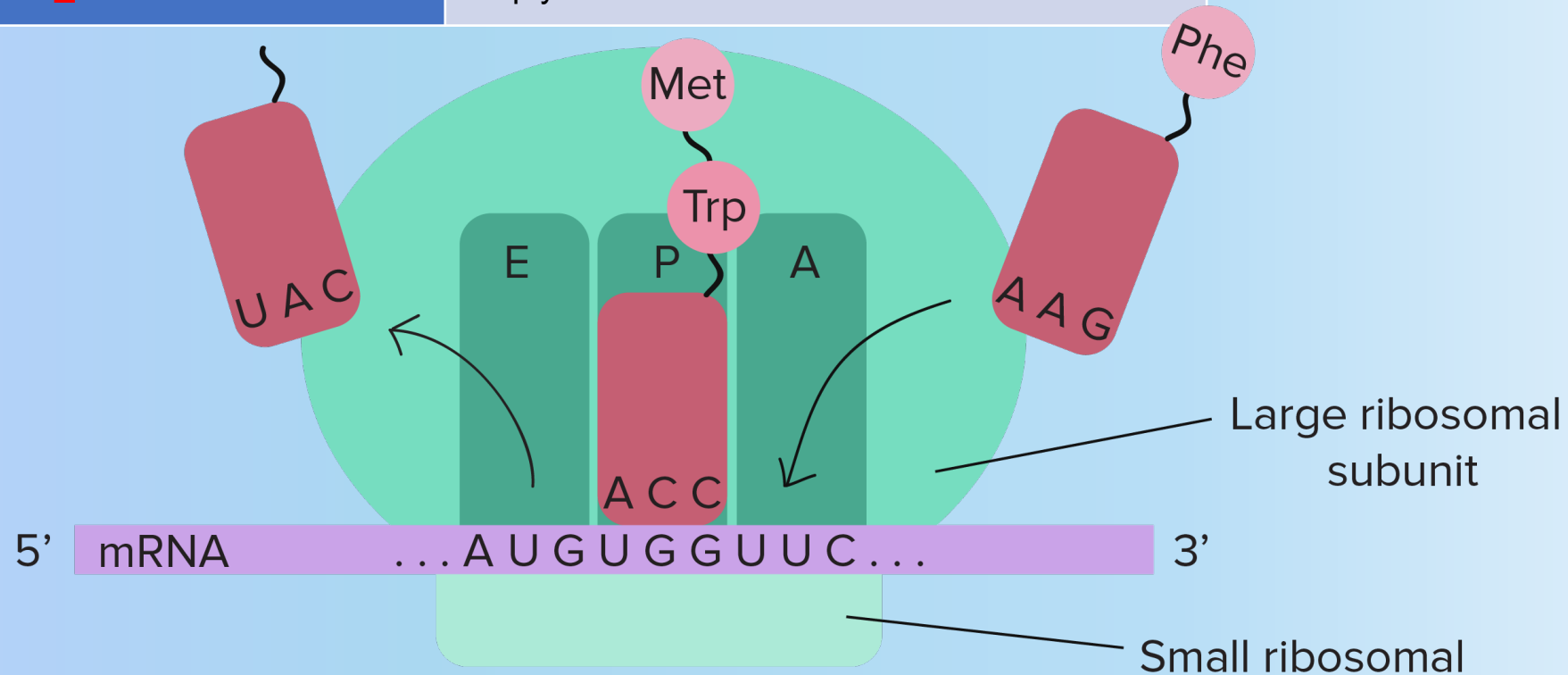
Ribosomes

- **Complexes** of proteins and ribosomal RNA (rRNA)
- A ribosome consist of **2 subunits**: small and large
  - Prokaryotes: **30S + 50S = 70S**
    - prOkaryotes are Odd
  - Eukaryotes: **40S + 60S = 80S**
- Site of **translation!**



Ribosomes - APE

A	Add Aminoacyl-tRNA
P	Peptidyl-tRNA with Polypeptide chain
E	Empty-tRNA → Exit



TRANSLATION - translating mRNA into polypeptide chain

1. Initiation
2. Elongation
3. Termination

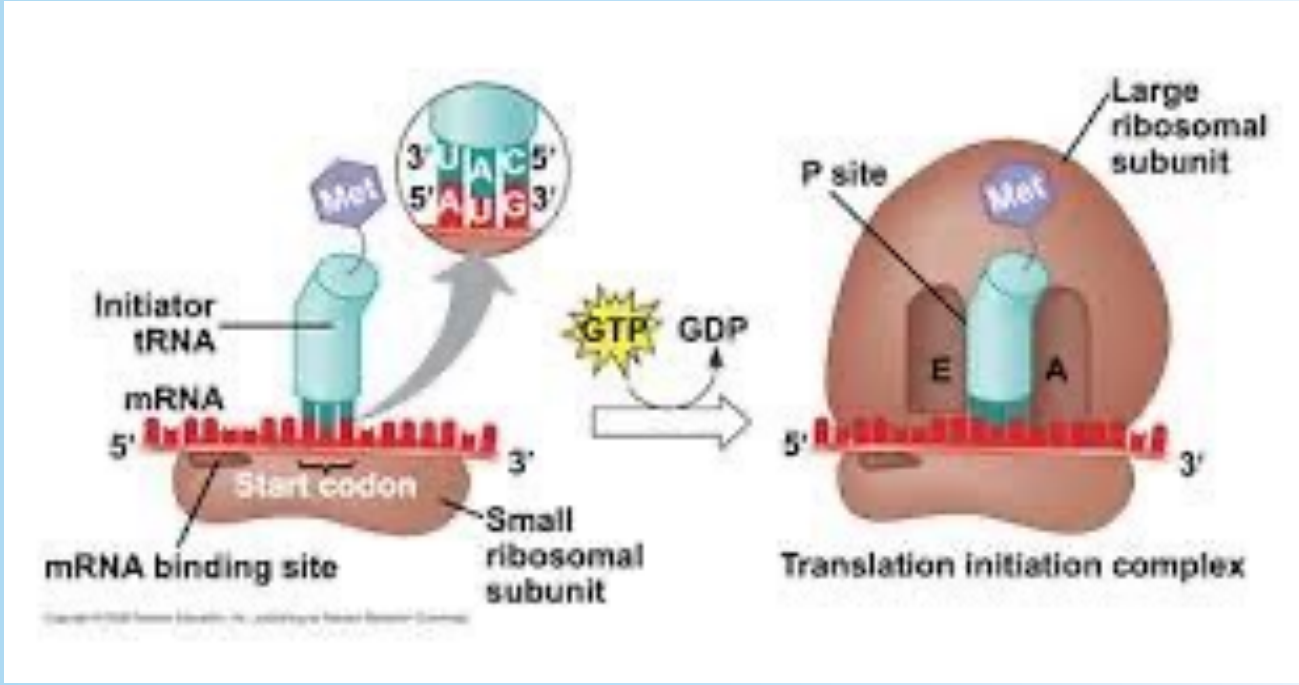
- Aiding factors: (e)IF, (e)EF, (e)RF

1. Initiation

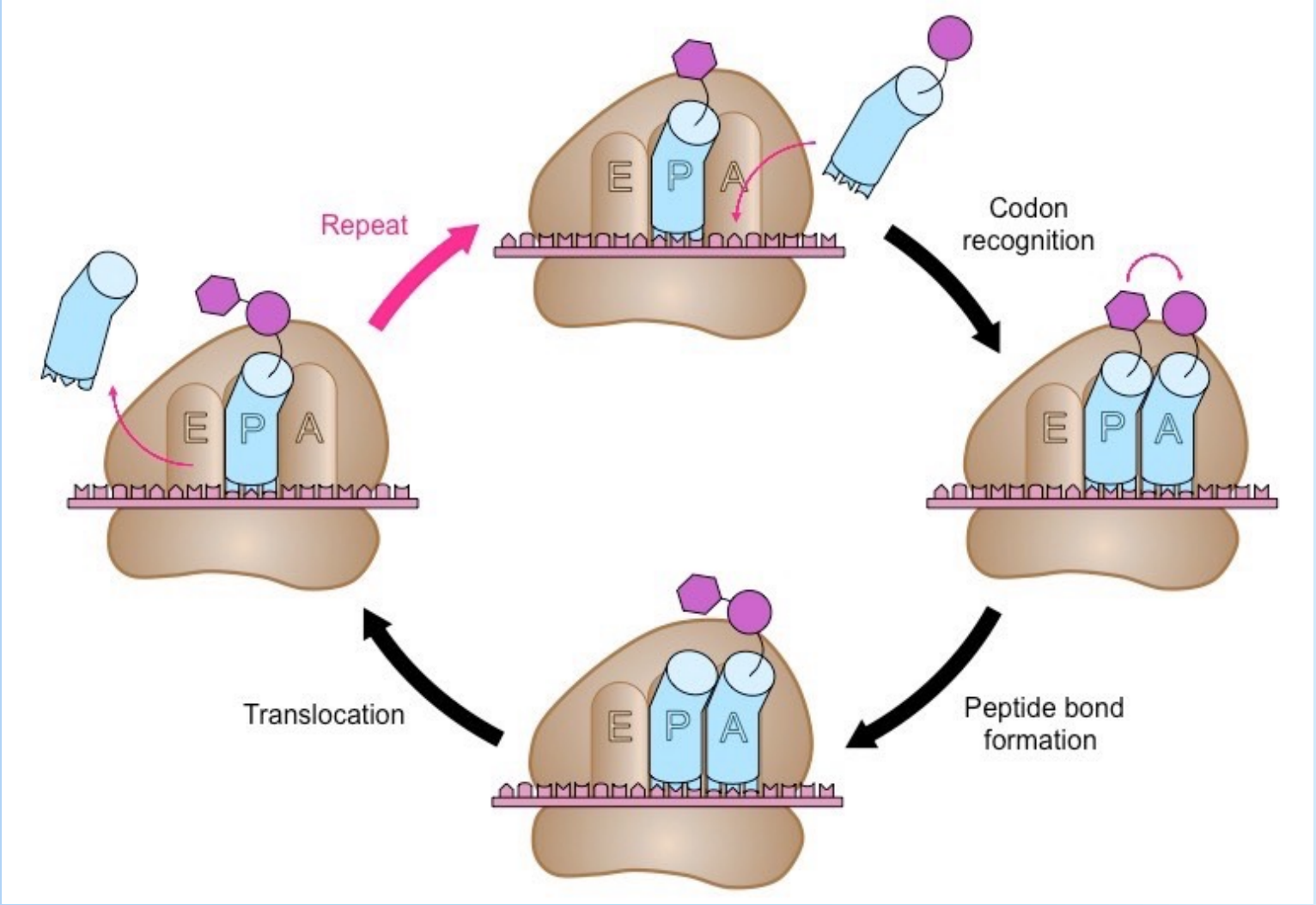
- **Assemble!**
  - Ribosomal subunits
  - mRNA
  - Aminoacyl-tRNA
  - GTP
  - Initiation factors

- Small subunit bind mRNA
  - **Prokaryotes:**
    - **Shine-Dalgarno**
  - **Eukaryotes:**
    - **5´-CAP**
    - «Scanning»: 5´→ 3´
    - **ATP**

- Upon **reaching AUG:**
  - **Unique initator tRNA** recognize
  - Facilitated by eIF-2-GTP
  - Charged tRNA enter P!!



2. Elongation



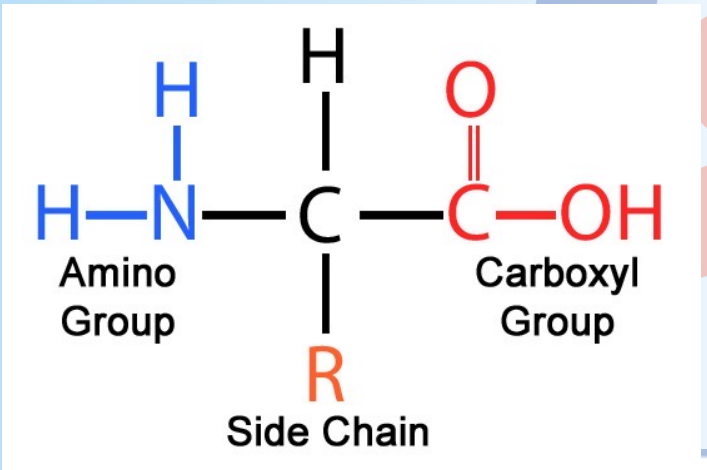
2. Elongation

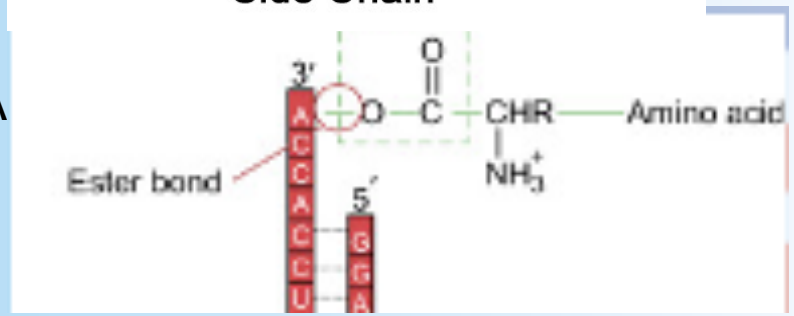
- The ribosome works like a **clamp** reading 5´→ 3´
- Adding AA via **aminoacyl-tRNA** (GTP → GDP)
  - A site
  - Remember that P is already occupied from initiation phase!

- Synthesize from **N-terminal**
  - New strands are attached to the carboxyl group of the previous AA
  - Polypeptide chain in P «jumps» to A

- Peptide bonds linked **by peptidyl transferase**
  - Large subunit
  - Ribozyme

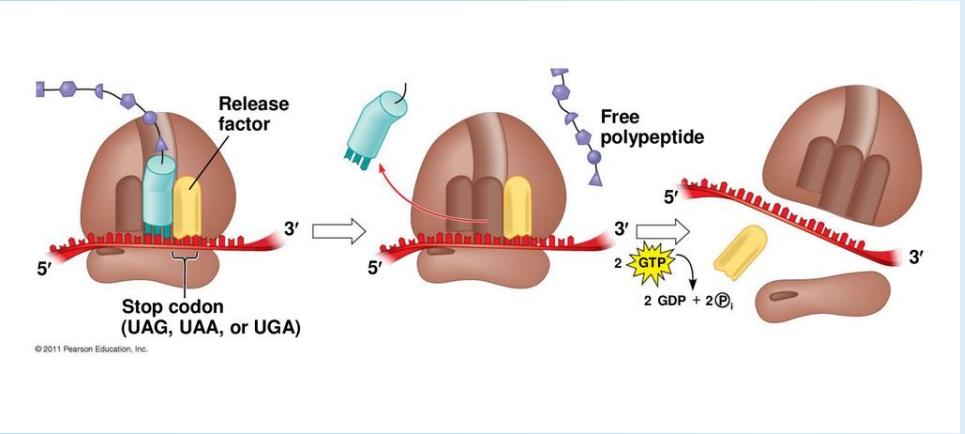
- **Translocation:** GTP → GDP





3. Termination

- STOP codon enters A site
- Release factors
  - **Prokaryotes:**
    - ❖ RF-1: UAA, UAG
    - ❖ RF-2: UAA, UGA
  - **Eukaryotes:**
    - ❖ eRF: all 3 codons
- Binding of **release factors** cause release of polypeptide chain!



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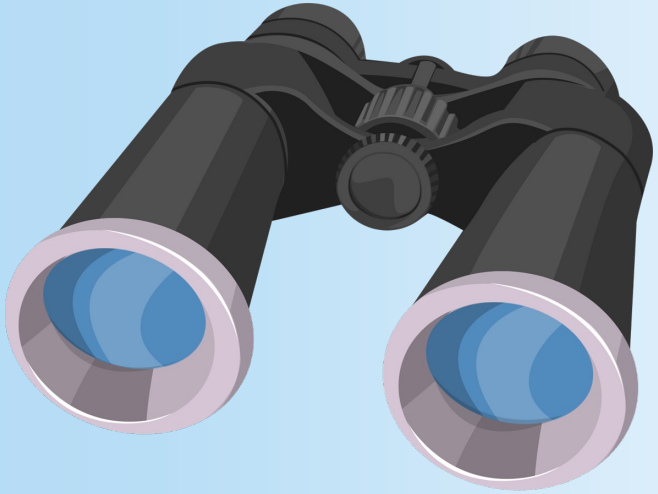


Posttranslational modifications

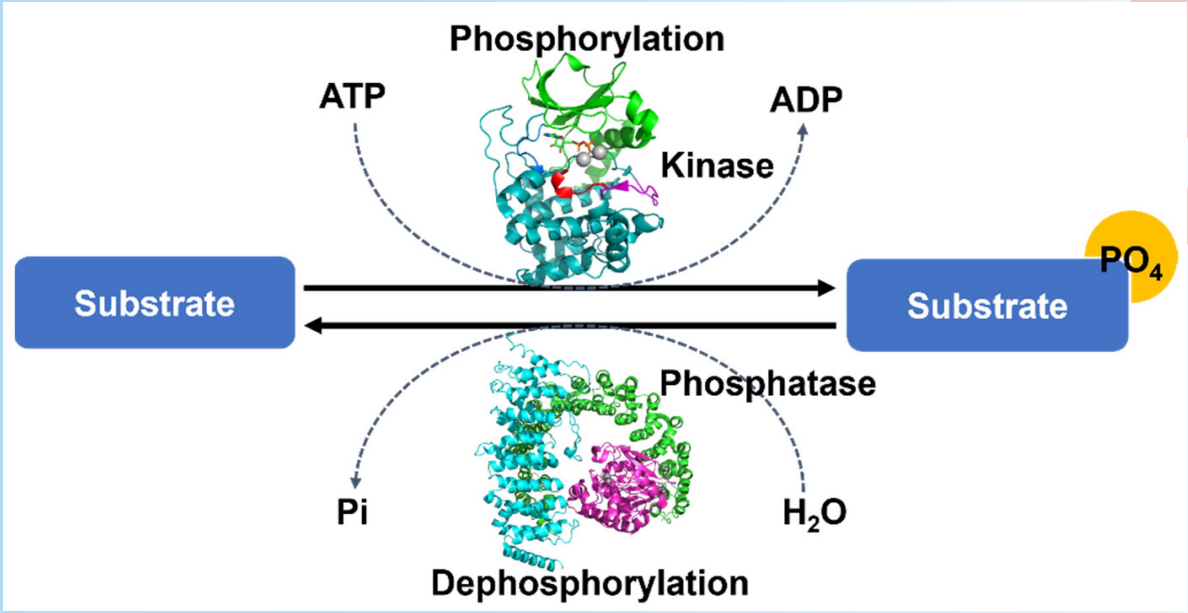
- Polypeptide chain (PP) is **NOT** a protein!!
- May also arise during translation = cotranslational modification
- Some types of posttranslational modifications:

1. **Trimming**
2. **Phosphorylation**
3. **Glycosylation**
4. **Protein folding**

Trimming & phosphorylation

- **Trimming:**
  - Initially large precursor molecules
  - Endoproteases
  - Zymogens

- **Phosphorylation**
  - ATP + protein = phosphoprotein + ADP
  - **Serine, threonine, tyrosine**
  - Kinases/phosphatases



Glycosylation and protein folding

- **Adding carbohydrates**
- Proteins entering plasma membrane or leaving cell
- 2 types:
  1. O-glycosylation: HydrOxyl group of serine and threonine
     - GOlgi
  2. N-glycosylation: amide group of asparagiNe
     - ER

- **Protein folding:**
  - To assume functional state
  - Spontaneous or chaperons

Targeting

Cytoplasmic ribosomes

- **Mitochondria, Nucleus, Cytosol, Peroxisomes**
  - «Targeting sequence»
  - Cleaved upon arrival

RER ribosomes

- **N-Hydrophobic** signal sequence and SRP
- **Plasma, ER, Golgi, lysosomes**
- Travel in vesicles to cis-face of Golgi apparatus
  - Return to RER
  - Stay inside golgi
  - Leave via trans-face