

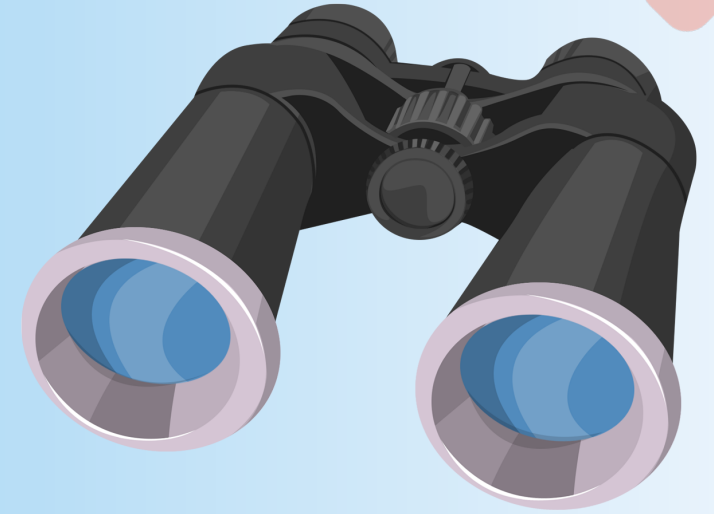
Protein biosynthesis

(Transcription + translation)

By Herman Mageli

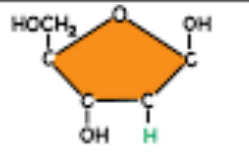
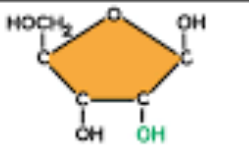
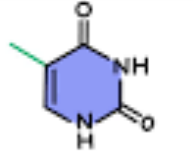
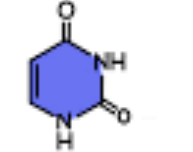
Overview

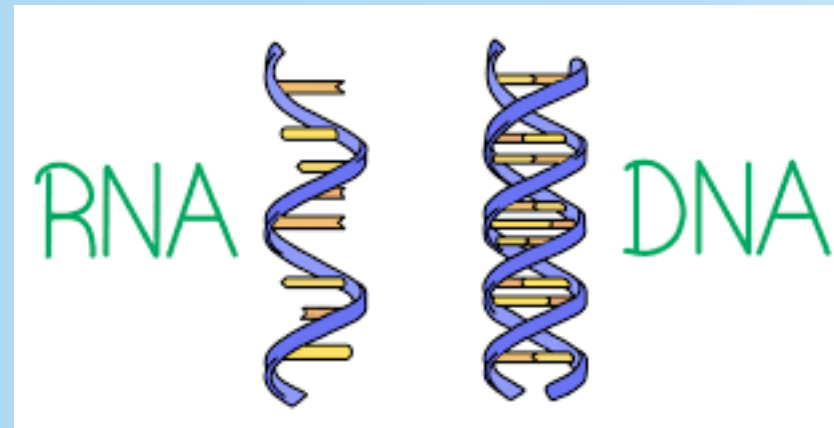
Transcription
Posttranscriptional modifications
The genetic code
Translation
Posttranslational modifications



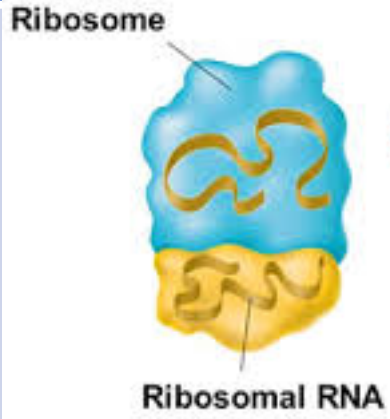
DNA vs. RNA

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

	DNA	RNA
SUGAR	 DEOXYRIBOSE	 RIBOSE
BASE	 THYMINE	 URACIL

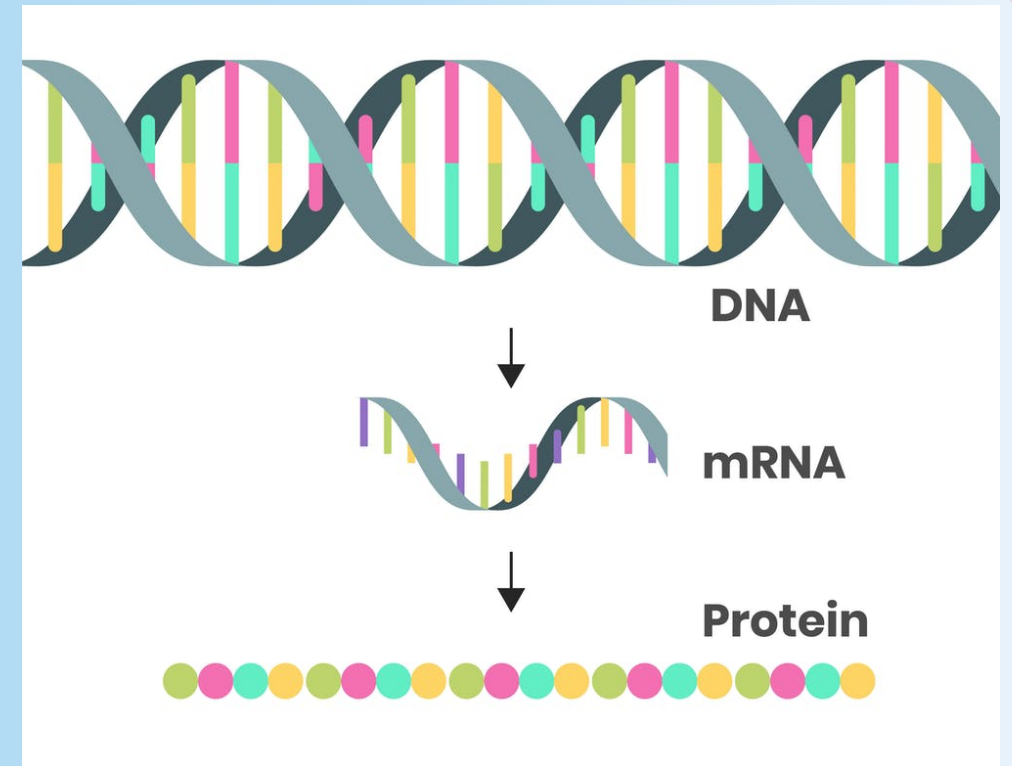


RNA

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

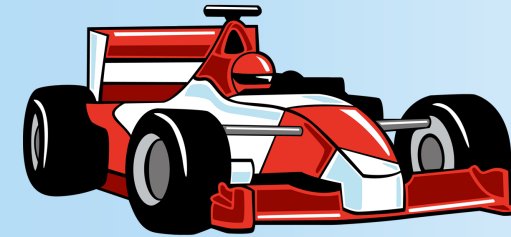
Transcription

- «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



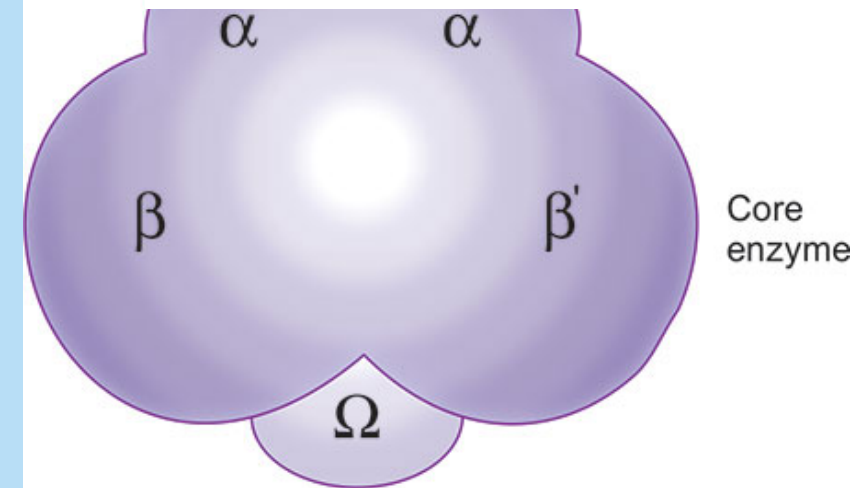
RNA polymerase

- 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



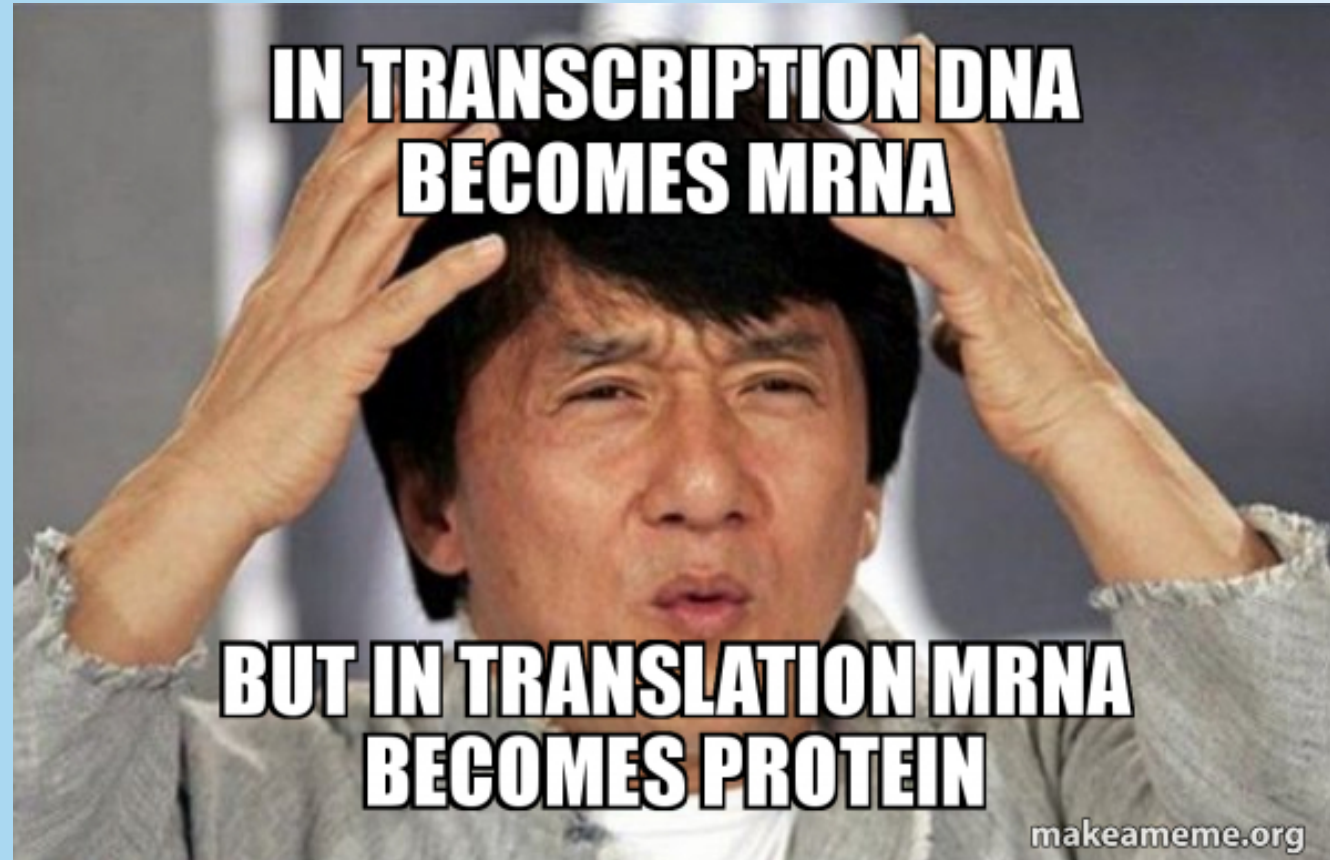
Structure of RNA polymerase

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



Transcription

1. Initiation
2. Elongation
3. Termination



1. Initiation

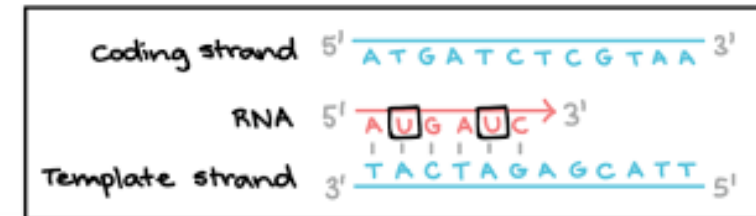
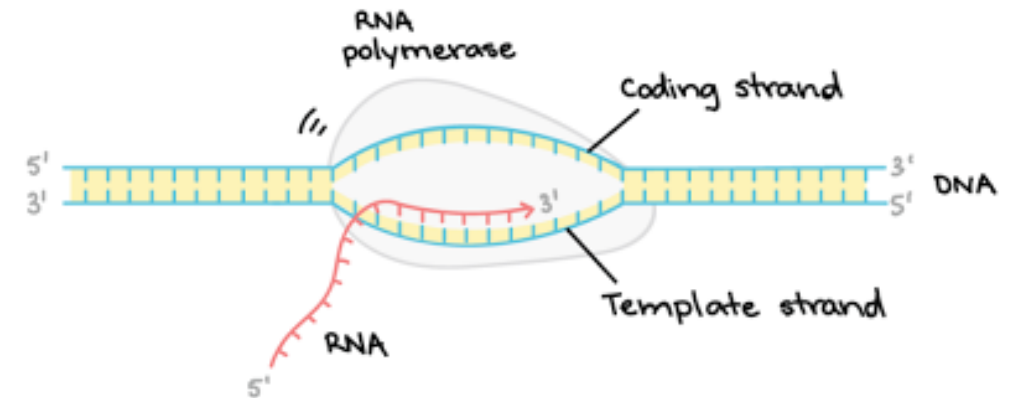
- Holoenzyme recognize promoter region: 2 sequences

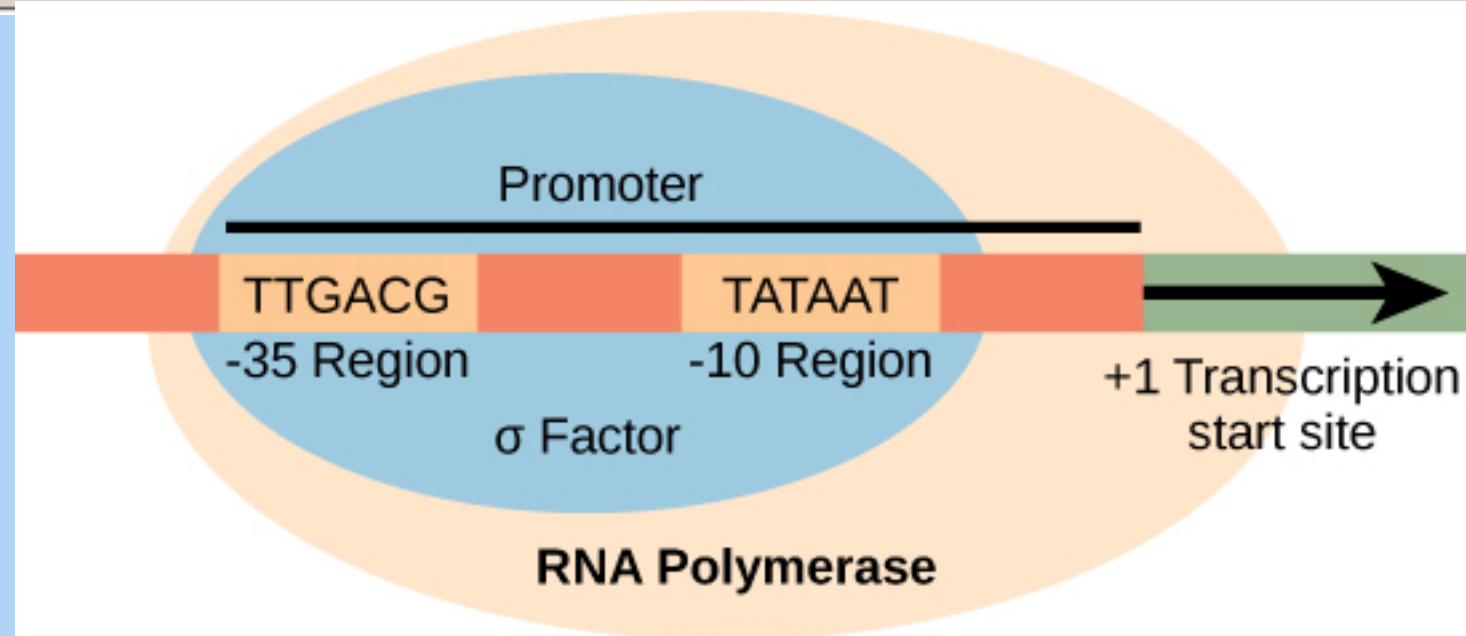
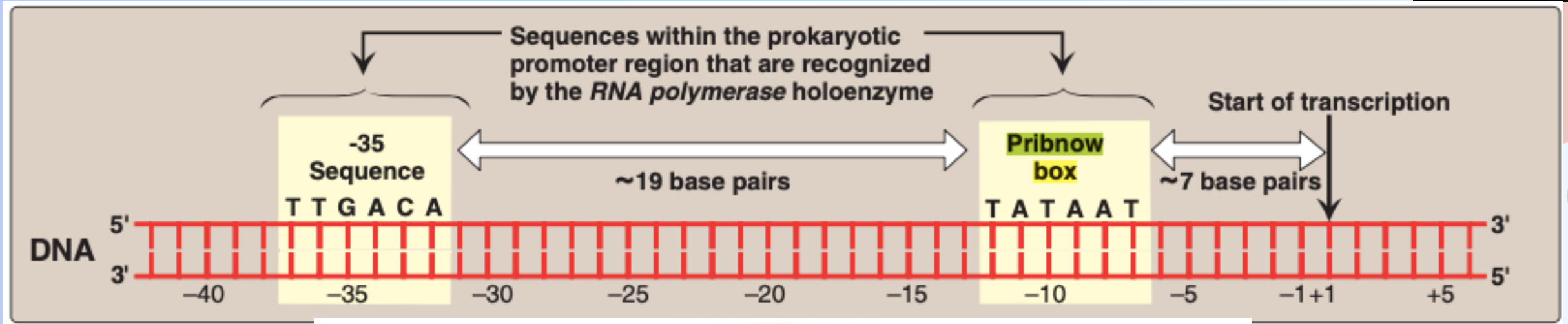
1. -35 sequence

- 5' - TTGACA - 3'
- Closed complex

2. Pribnow box

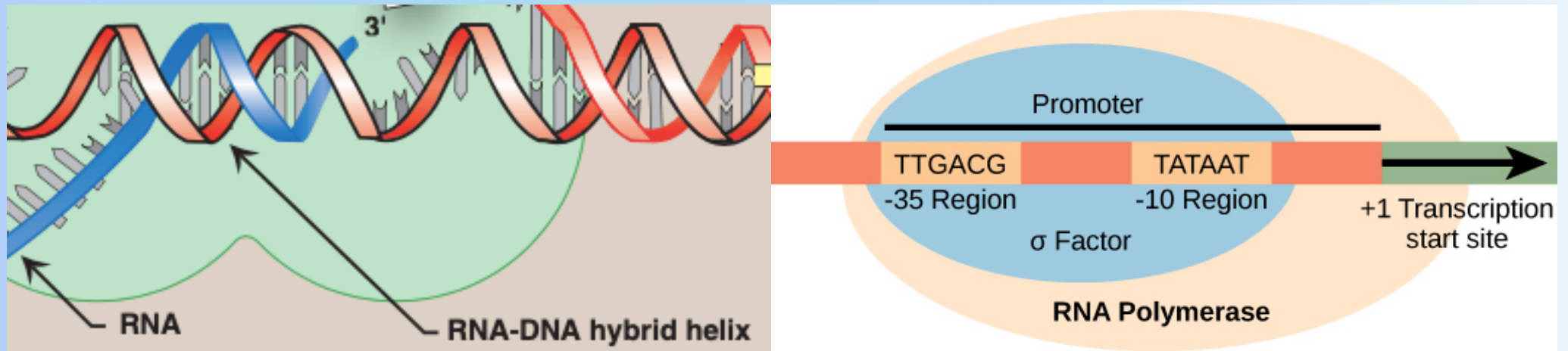
- 5' - TATAAT - 3'
- Site of initial DNA melting
 - OPEN complex
 - Transcription bubble





2. Elongation

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

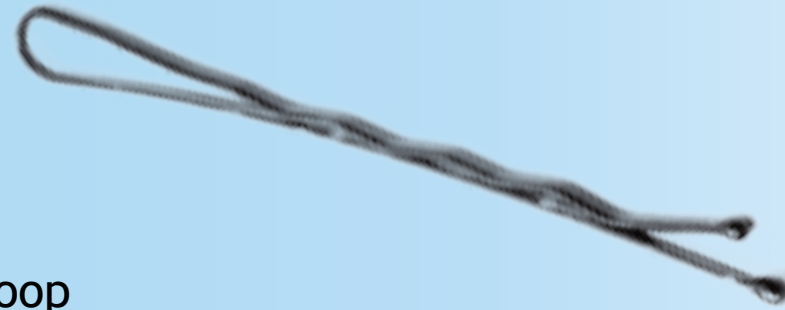


3. Termination

- 2 types

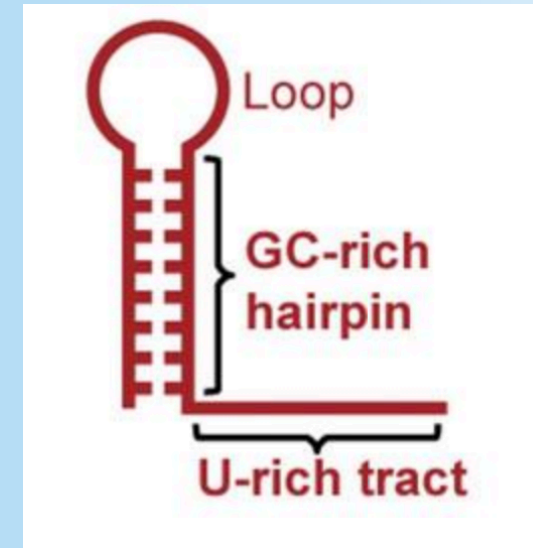
1. **p-Independent**

- 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

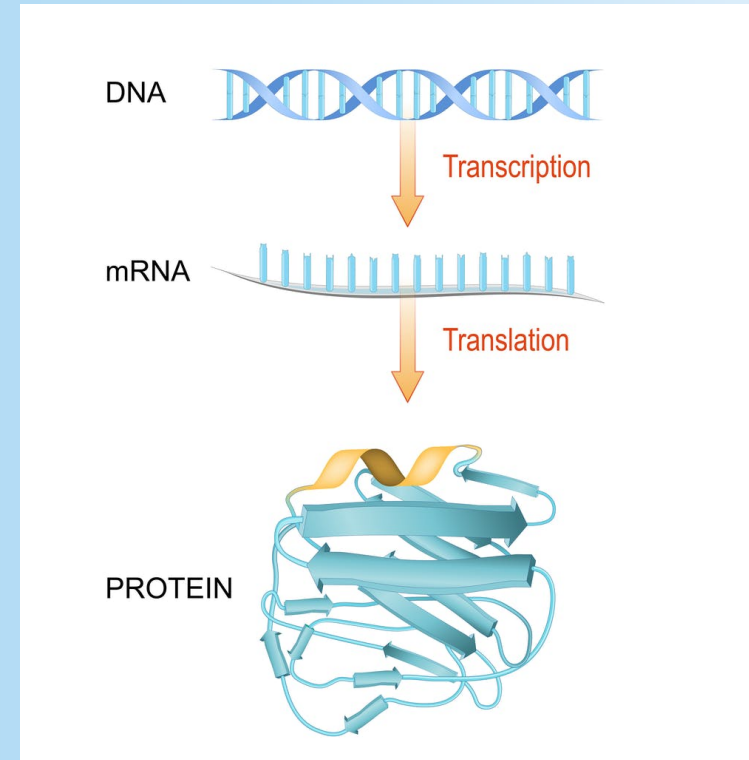
- More complicated!
 - 3 instead of 1 RNA polymerase!
 - One for each RNA type
 - **Transcription factors (TFs)**
 - Assemble transcription complex
 - Melt DNA
 - Bind promoter region
 - Each RNA polymerase has **its own** transcription factors and promoters



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



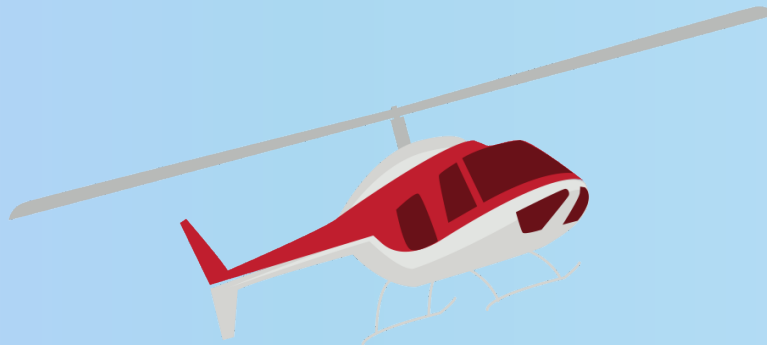
1. **TFIID**: recognize and bind TATA box



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



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



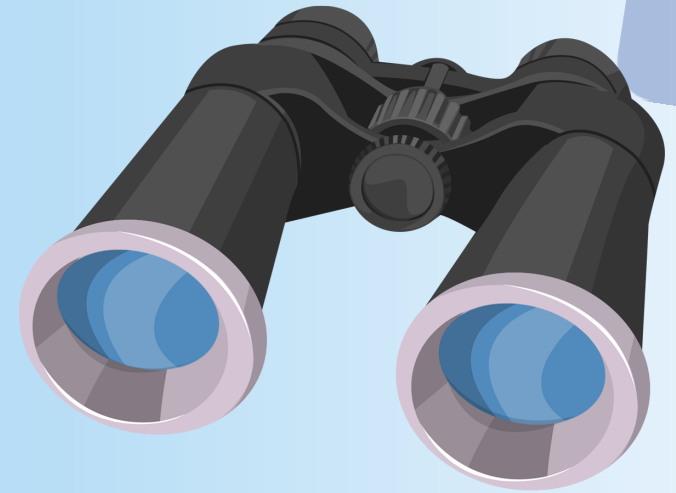
Antibiotics and inhibitors



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

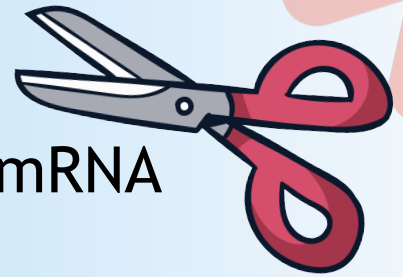
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Posttranscriptional modifications

- 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**.

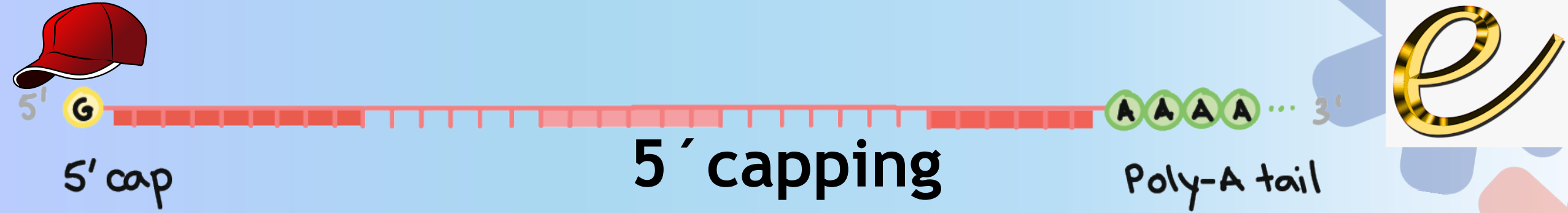


	rRNA	tRNA
Origin	• 16S, 28S etc. Are ALL generated from a long precursor molecule that is cleaved	• Also made from longer precursor molecules that are cleaved
Introns		• Noncoding regions of RNA that may be present. If so, they are removed by nucleases.
-CCA ending added		• Added on 3'-end by nucleotidyltransferase

Pre-mRNA modifications

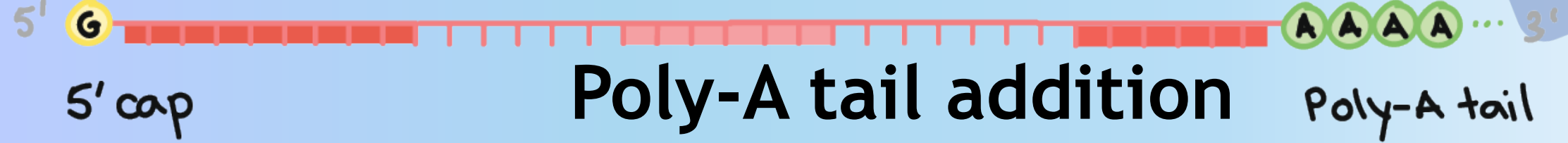
1. 5' -capping
2. Addition of a poly-A tail
3. Removal of introns





- 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





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

(During transcription)

5' End: *Receives a nice cap*

3' End:



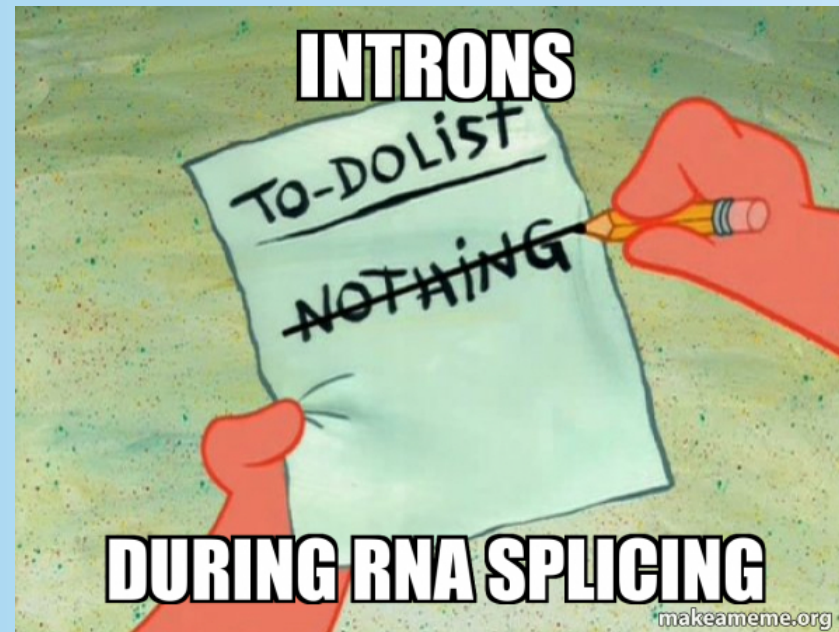


5' cap

Removal of introns

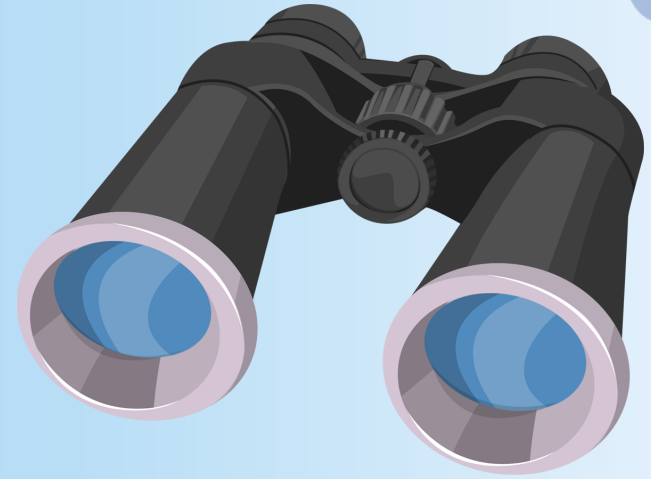
Poly-A tail

- **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

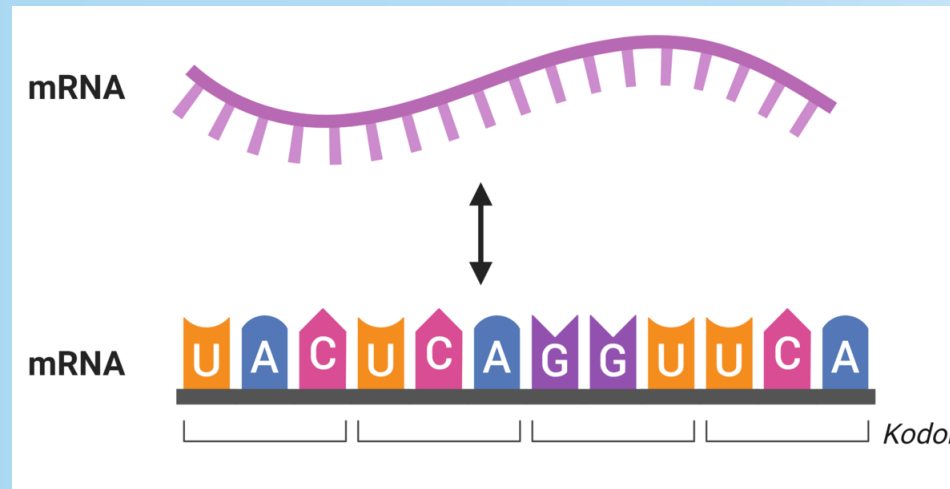
- A «dictionary that identifies correspondence between nucleotide bases and amino acids»
 - 4 letters: A (adenine), G (guanine), C (cytosine), and U (uracil)
- Three letters form «**codons**».
- These codons represents certain amino acids



Codons

- «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'

U Are Gone
U Go Away
U Are Away



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

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

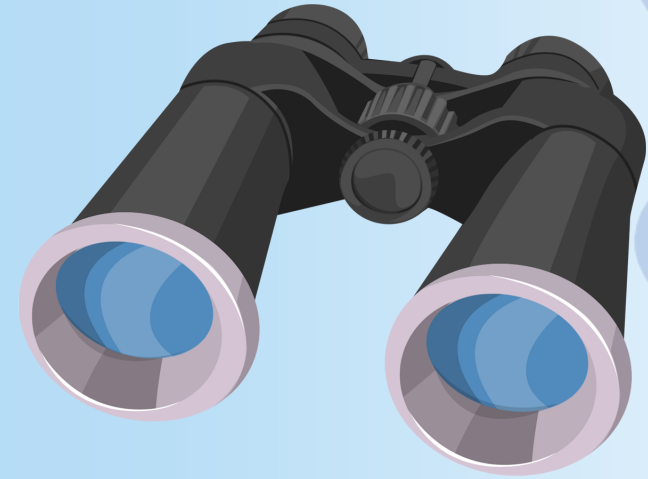
Vocabulary of the genetic code

Specificity (unambiguous)	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

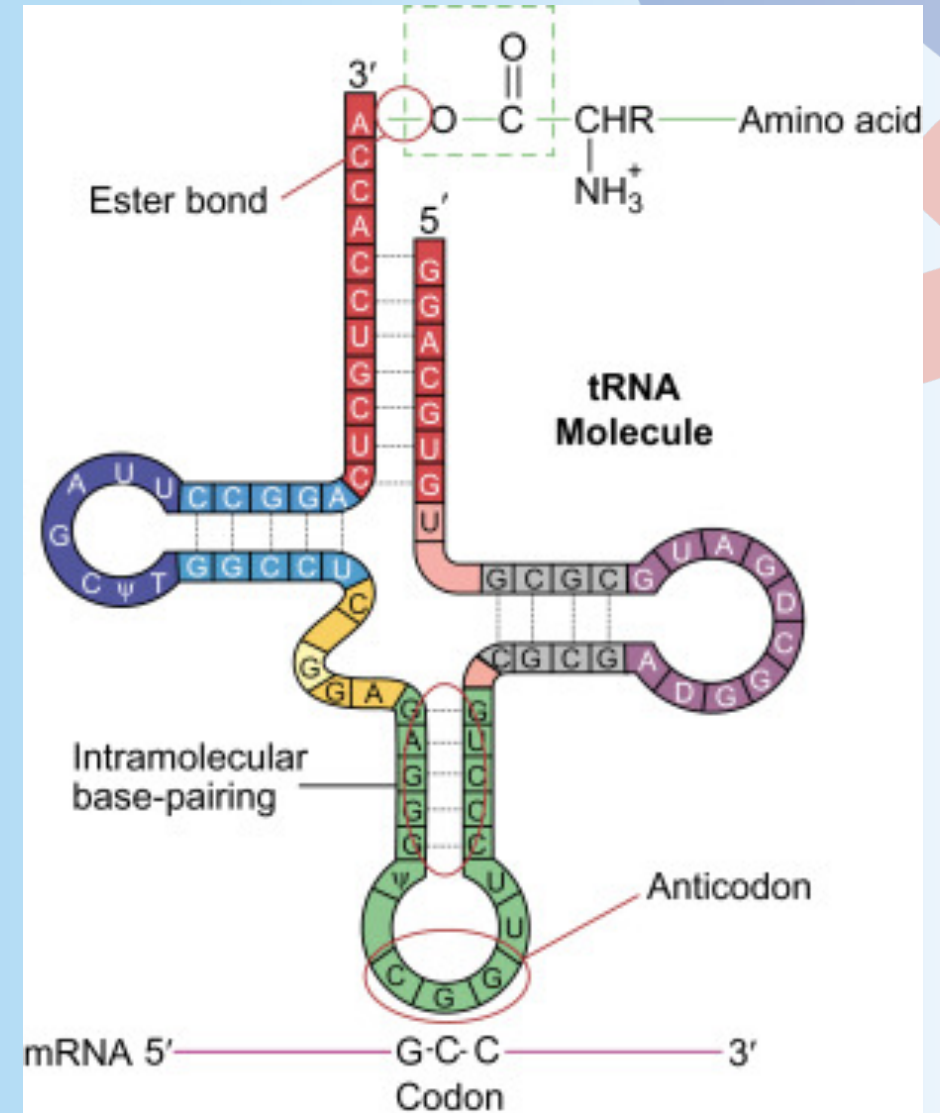
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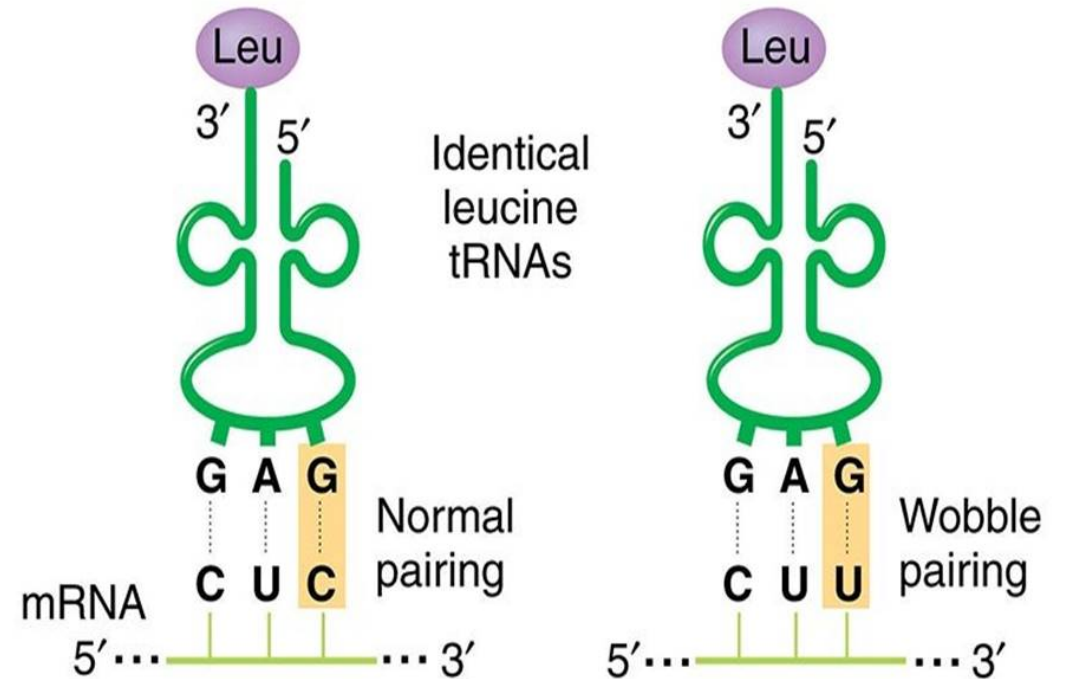
tRNA

- The job of **t**RNA is to **T**ransport 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



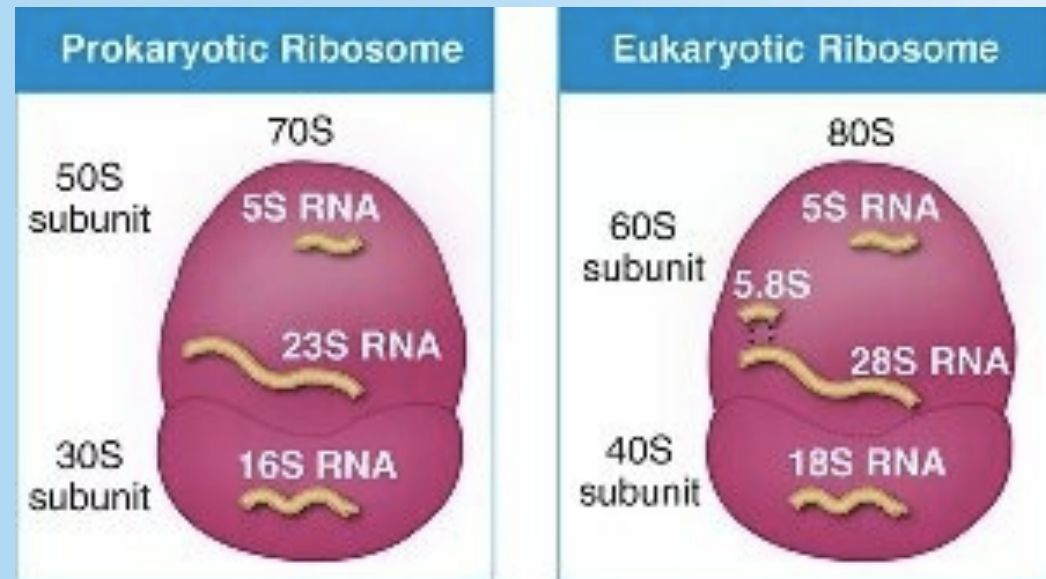
Wobble hypothesis

- 50 tRNAs for 61 codons...
- 1 base



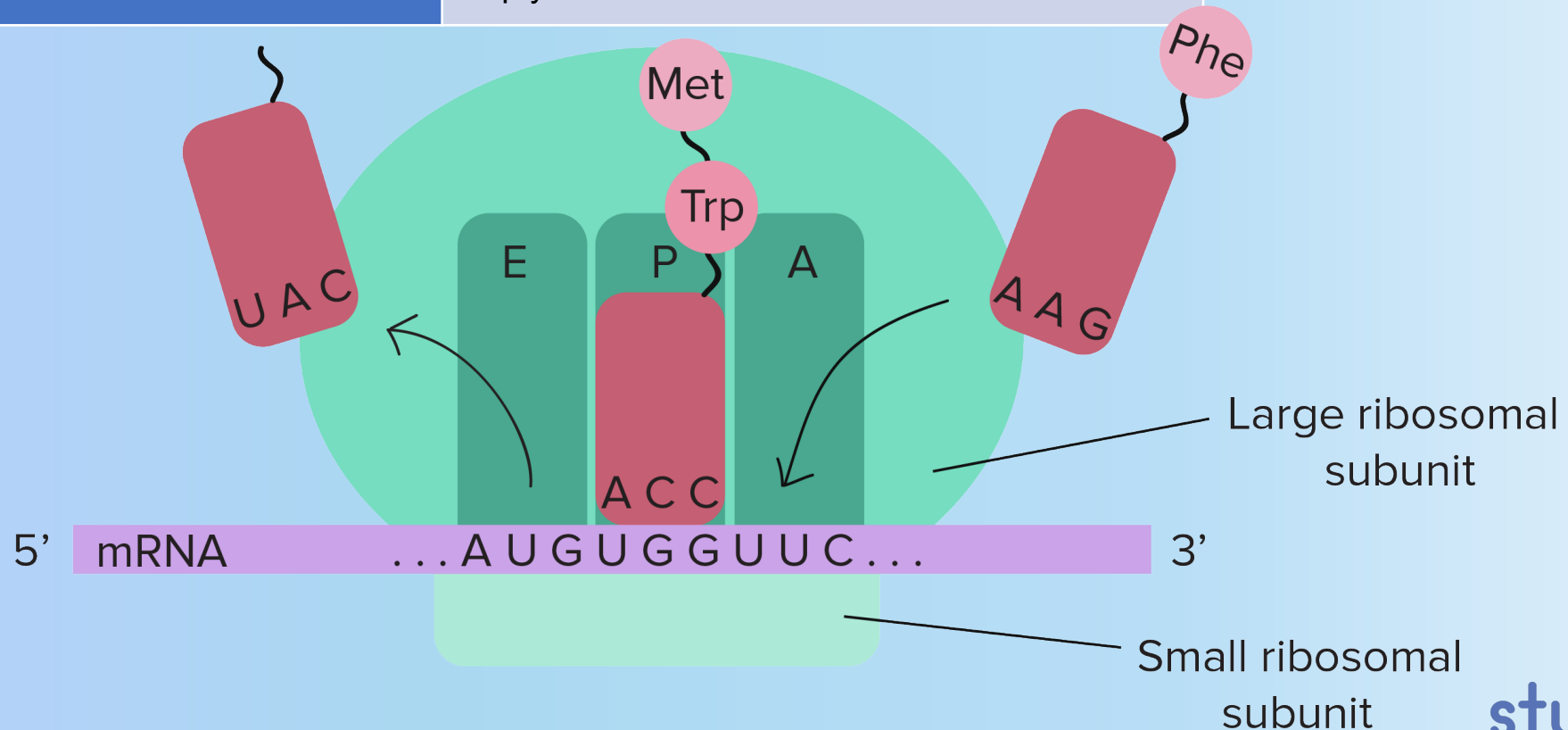
Ribosomes

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



Ribosomes - APE

A	Add A minoacyl-tRNA
P	P eptidyl-tRNA with P olypeptide chain
E	E mpy-tRNA → E xit



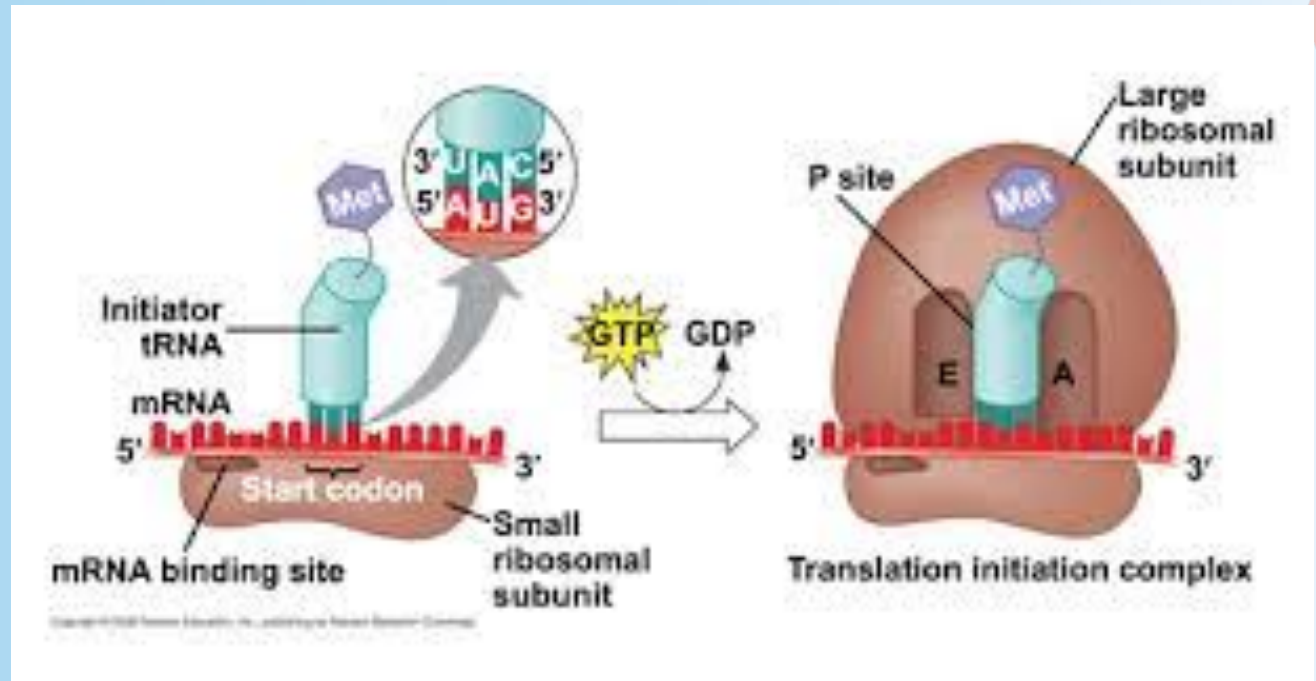
TRANSLATION - translating mRNA into polypeptide chain

1. Initiation
 2. Elongation
 3. Termination
- Aiding factors: (e)IF, (e)EF, (e)RF

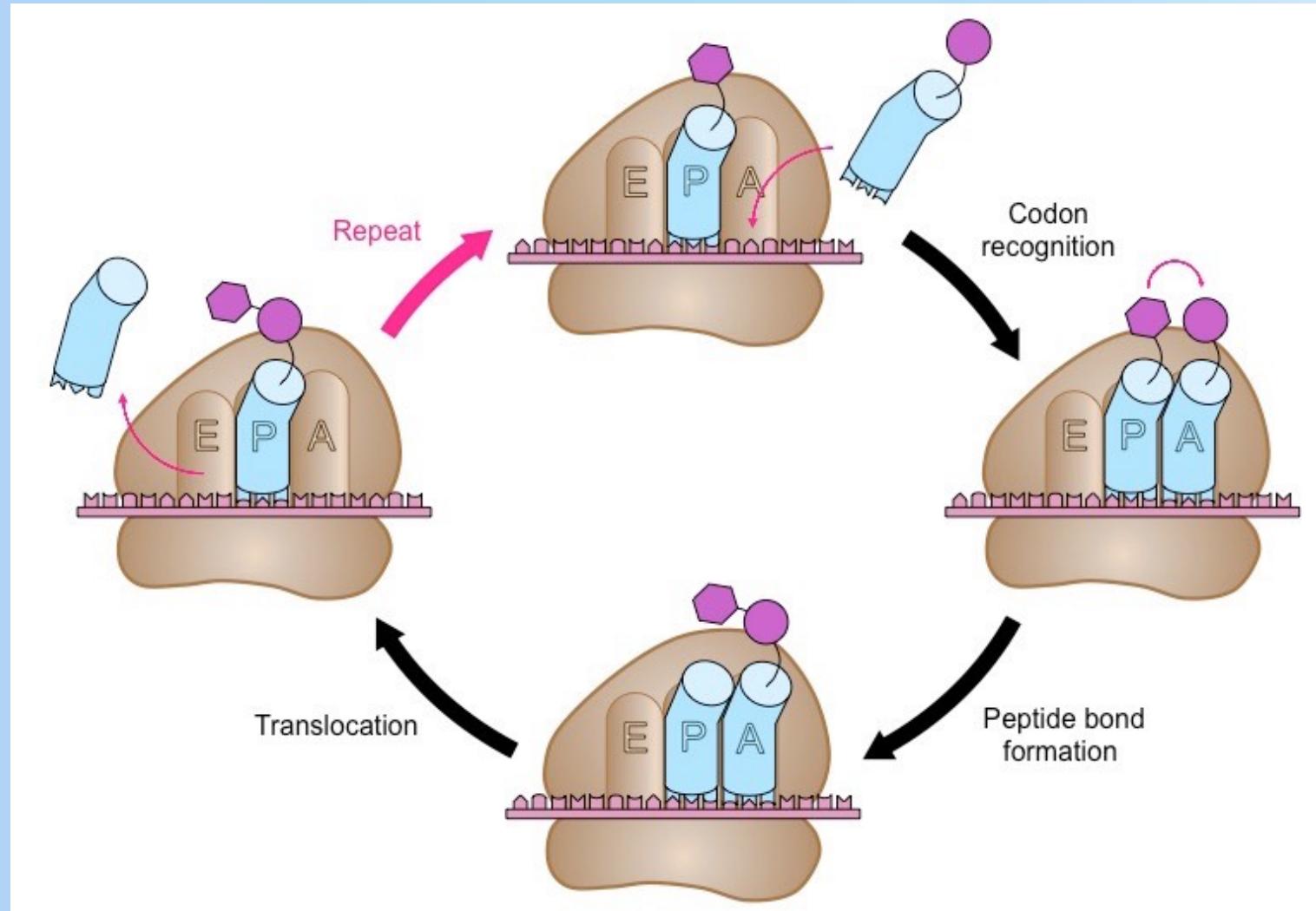


1. Initiation

- **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 initiator tRNA recognize
 - Facilitated by eIF-2-GTP
 - Charged tRNA enter P!!

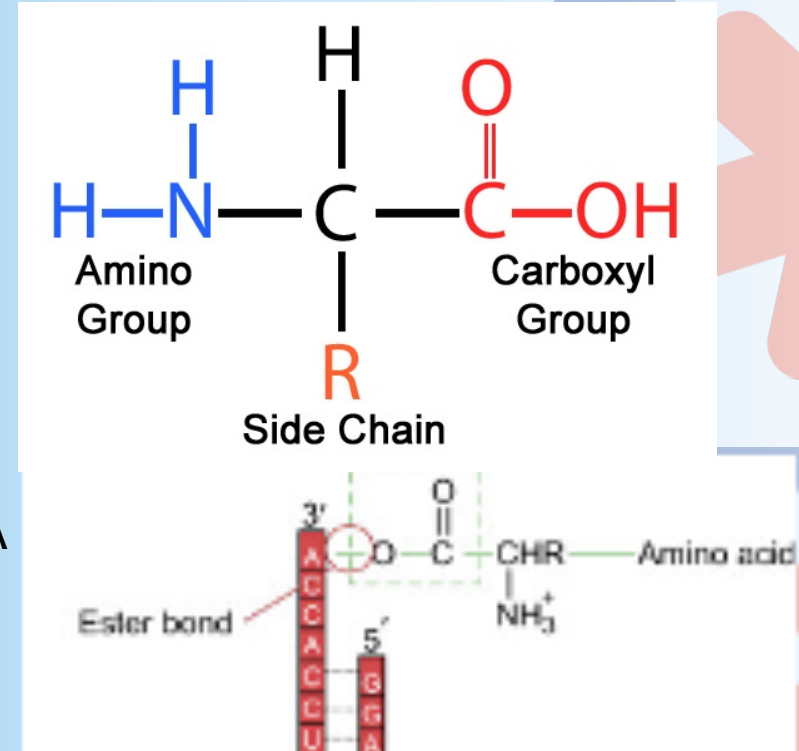


2. Elongation



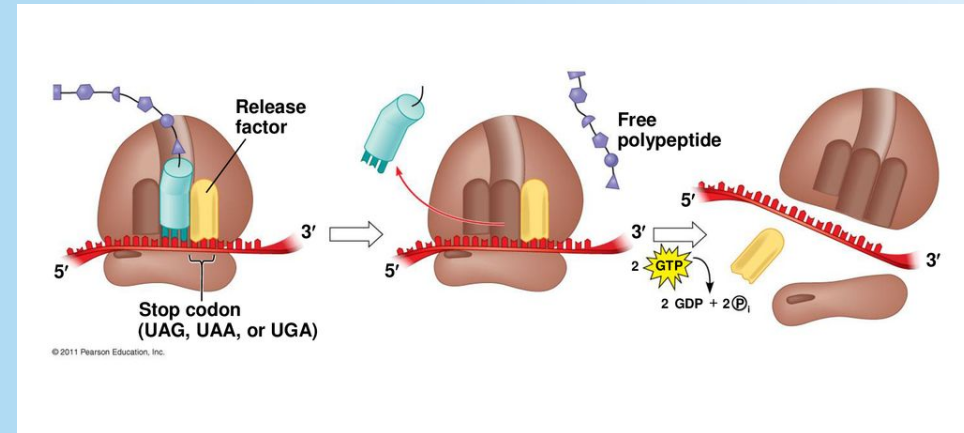
2. Elongation

- 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



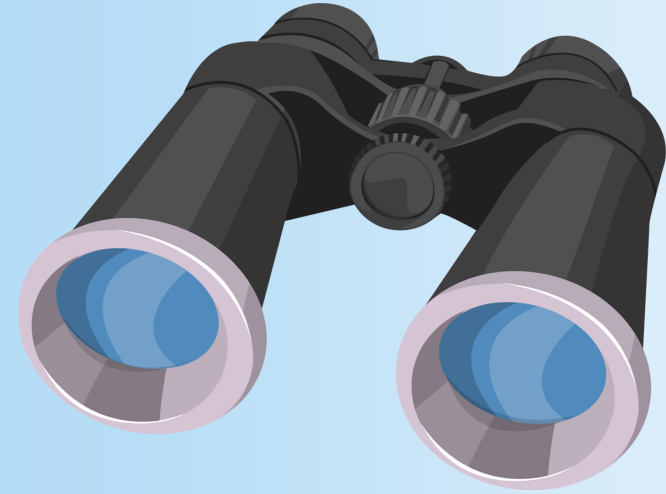
3. Termination

- 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:

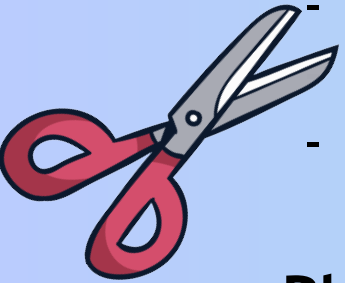
- 
1. Trimming
 2. Phosphorylation
 3. Glycosylation
 4. Protein folding



Trimming & phosphorylation

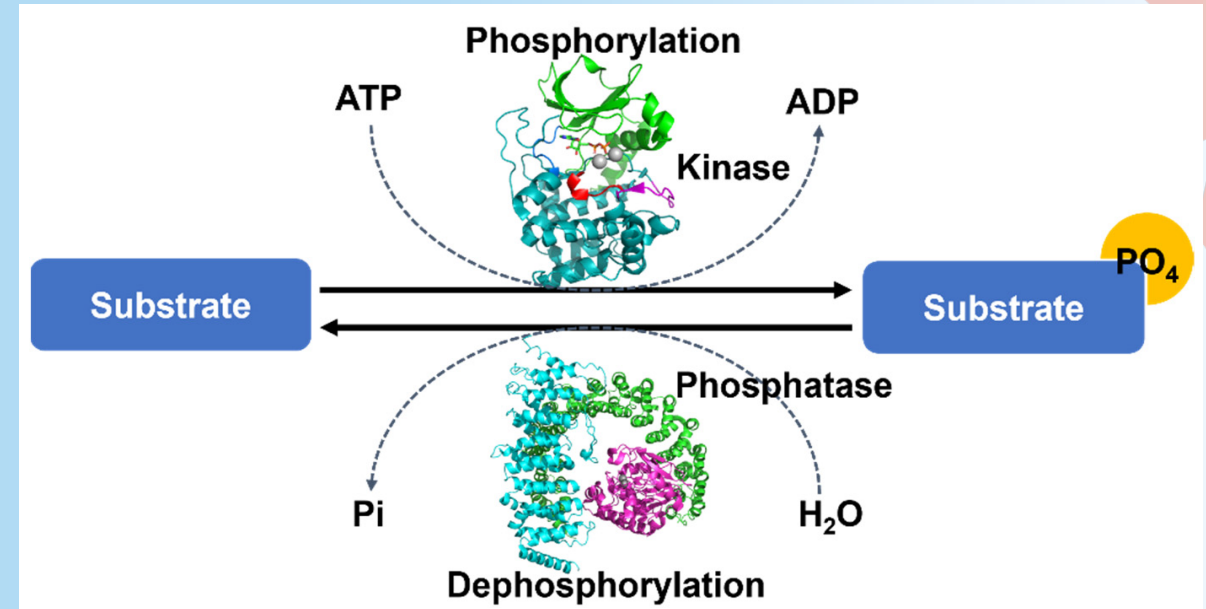
- **Trimming:**

- Initially large precursor molecules
- Endoproteases
- Zymogens



- **Phosphorylation**

- $\text{ATP} + \text{protein} = \text{phosphoprotein} + \text{ADP}$
- **Serine, threonine, tyrosine**
- Kinases/phosphatases



Glycosylation and protein folding

- Adding carbohydrates
- Proteins entering plasma membrane or leaving cell
- 2 types:
 1. **O**-glycosylation: Hydr**O**xyl group of serine and threonine
 - **G****O**lgi
 2. **N**-glycosylation: amide group of asparagi**N**e
 - 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

