PÉCSI TUDOMÁNYEGYETEM
UNIVERSITY OF PÉCS

TRANSCRIPTION

Renáta Schipp

Gene expression

Gene expression:

- is the process by which information from a gene is used for the synthesis of gene products. These products are proteins, but in the case of non-protein coding genes the products are functional RNAs.

- has several steps

- during gen expression the genotype gives rise to the phenotype (observable trait)

Levels of gene expression

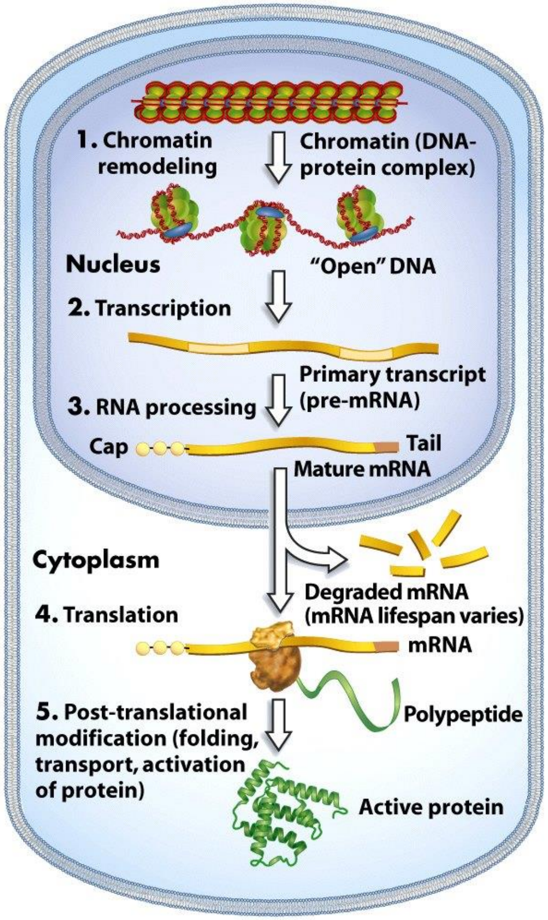
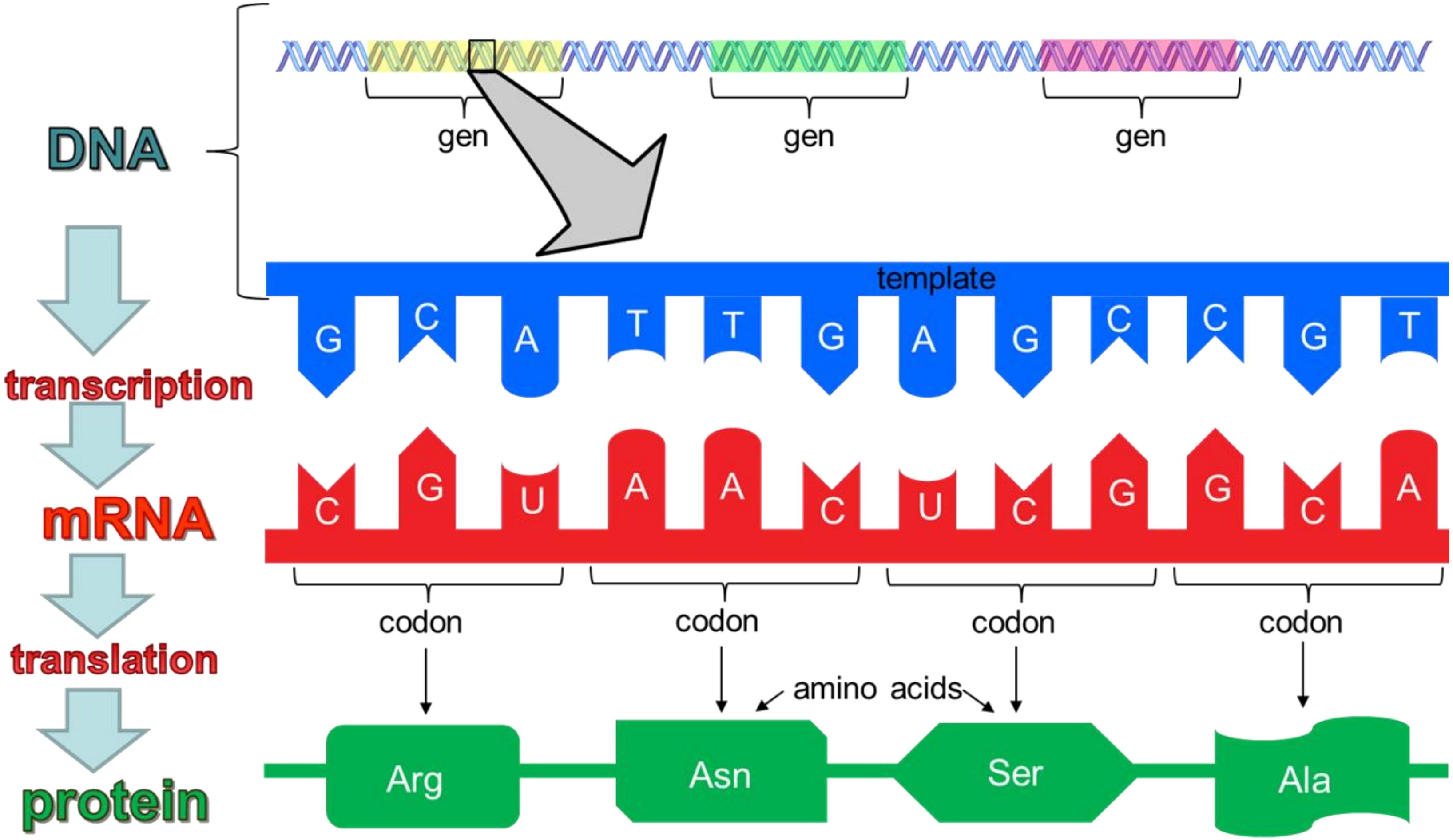


Figure 18-1 Biological Science, 2/e © 2005 Pearson Prentice Hall, Inc.

The central dogma of molecular biology

the flow of information in the cells



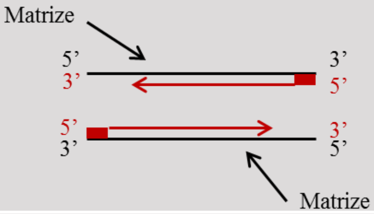
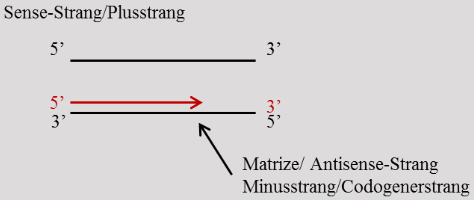
Transcription

General features: Replication-Transcription

Replication	Transcription	
		Symmetry
		Enzym
		Proofreading
		Frequency
		Direction
		Starting point
		Primer

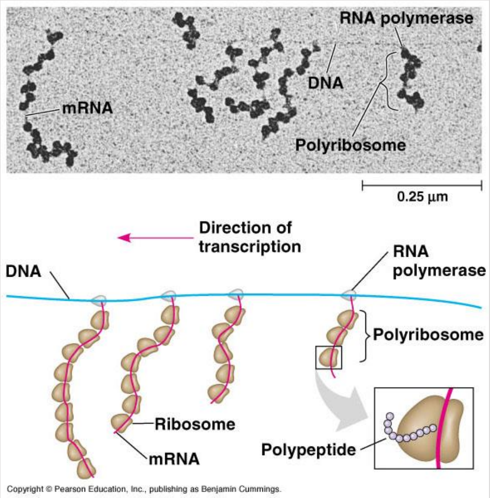
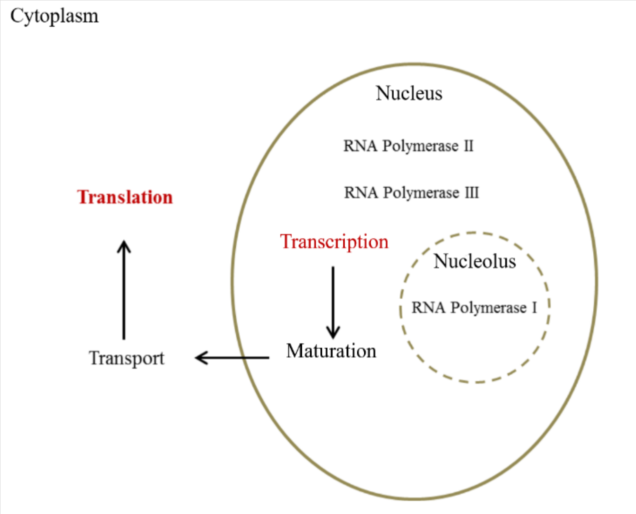
Transcription

General features: Replication-Transcription

Replication	Transcription	
symmetrical 	asymmetrical 	**Symmetry**
DNA-polymerase	DNA-dependent RNA-polymerase	**Enzym**
✓	—	**Proofreading**
1/ cell cycle	1.000-10.000/cell cycle	**Frequency**
5’-3’	5’-3’	**Direction**
origo	promoter	**Starting point**
✓	—	**Primer**

Transcription

General features: Prok. vs. Euk.

Prokaryotes	**Eukaryotes**
 Copyright © Pearson Education, Inc., publishing as Benjamin Cummings. **chromosome-polysome complex**	Cytoplasm Nucleus RNA Polymerase II RNA Polymerase III Translation Transcription Nucleolus RNA Polymerase I Transport Maturation
coupled transcription-translation	transcription and translation are chronologically and areal separated

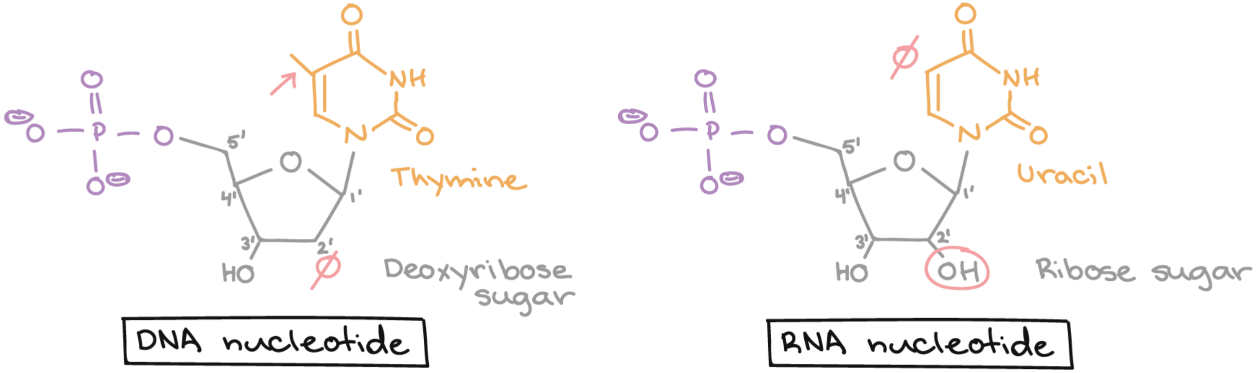
Transcription

General features: Prok. vs. Euk.

Prokaryotes	**Eukaryotes**
coupled transcription-translation no nucleus no maturation same directions: transcription: 5’- 3’ translation: 5’- 3’	transcription and translation are separated
from 1 promoter more genes	from 1promotor 1gen
1 RNA polymerase	3 RNA polymerases

General features of transcription

- DNA serves as a template
- enzyme: RNA polymerase (forms 3’-5’ phosphodiester bonds)
- the direction of the synthesis is 5’-3’ from the point of view from the new RNA molecule!!! (but from the point of view of DNA it is 3’ to 5’)
- substrate: ribonucleoside-triphosphate

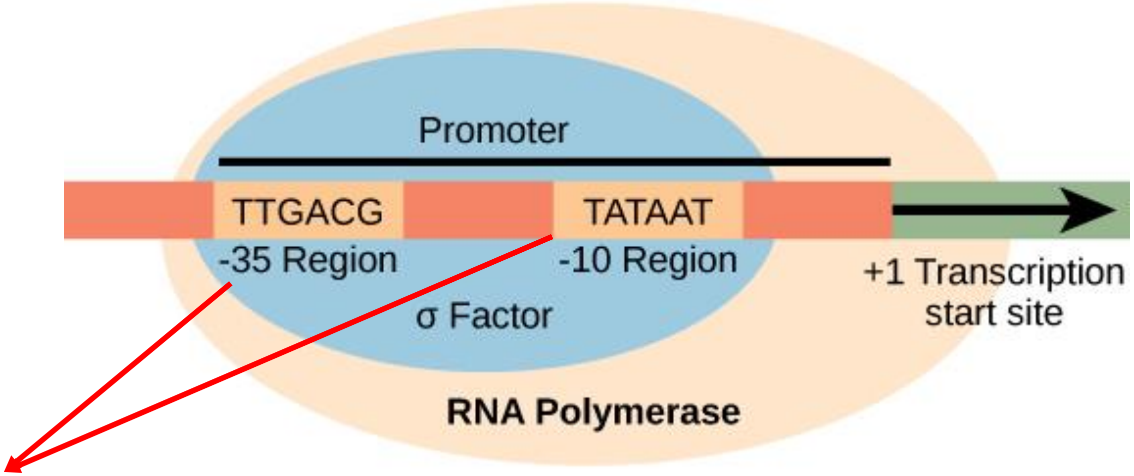


- β (beta) and γ (gamma) phosphate groups are released during the synthesis
- bases in RNA: A, U, G, C (no T)
- 3 steps: initiation, elongation, termination

https://www.khanacademy.org/science/biology/gene-expression-central-dogma/transcription-of-dna-into-rna/a/stages-of-transcription

Prokaryotic transcription: promoter

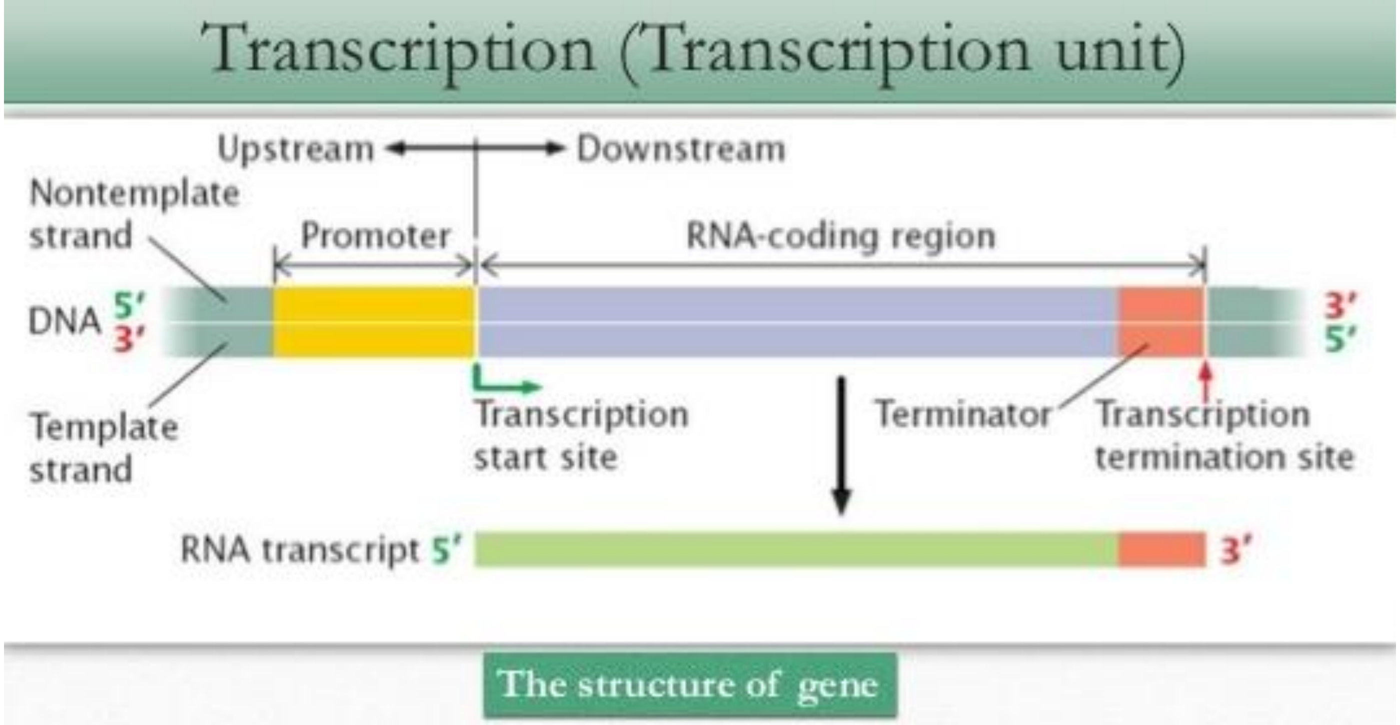
A promoter is a region of DNA that initiates transcription of a particular gene.
Promoters are located upsteram of the genes they regulated.



consensus sequences: are conserved, are located at the -10 and -35 regions upstream of the initiation site, are similar across all promoters

https://courses.lumenlearning.com/wm-biology1/chapter/prokaryotic-transcription

Prokaryotic transcription: promoter



https://www.slideshare.net/Mona_Albureikan/microbial-genetics-lectures-7-8-and-9

Prokaryotic transcription: RNA polymerase

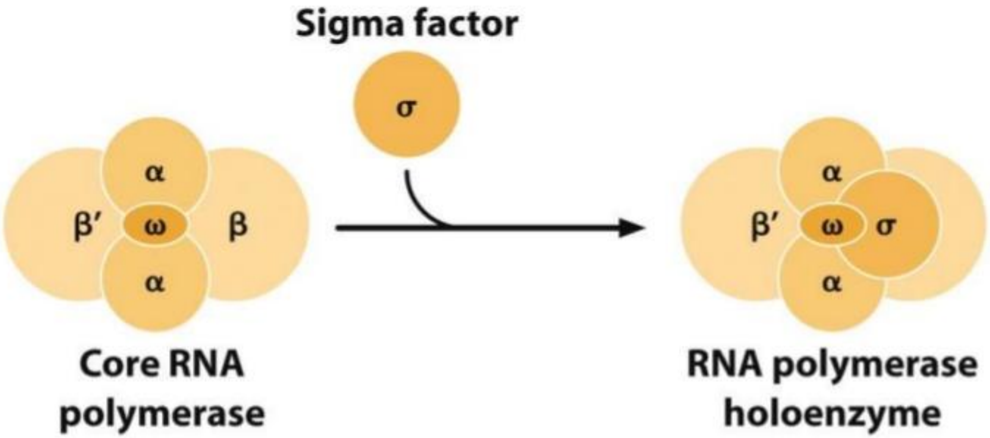
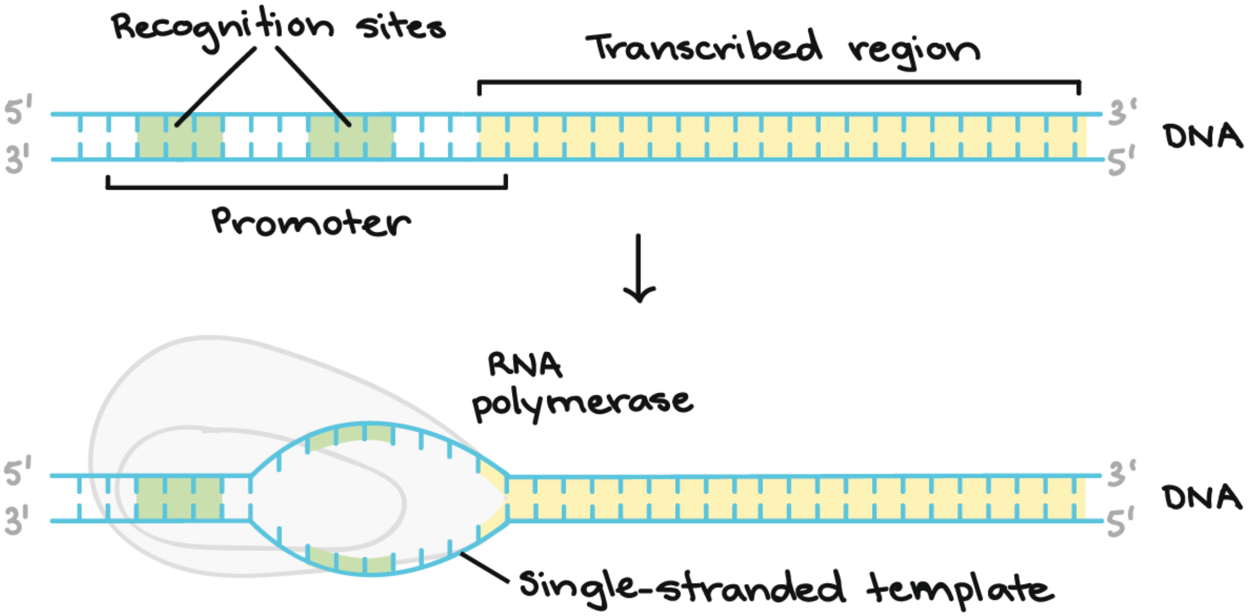


Figure 13.9a
Genetics: A Conceptual Approach, Fourth Edition
© 2012 W. H. Freeman and Company

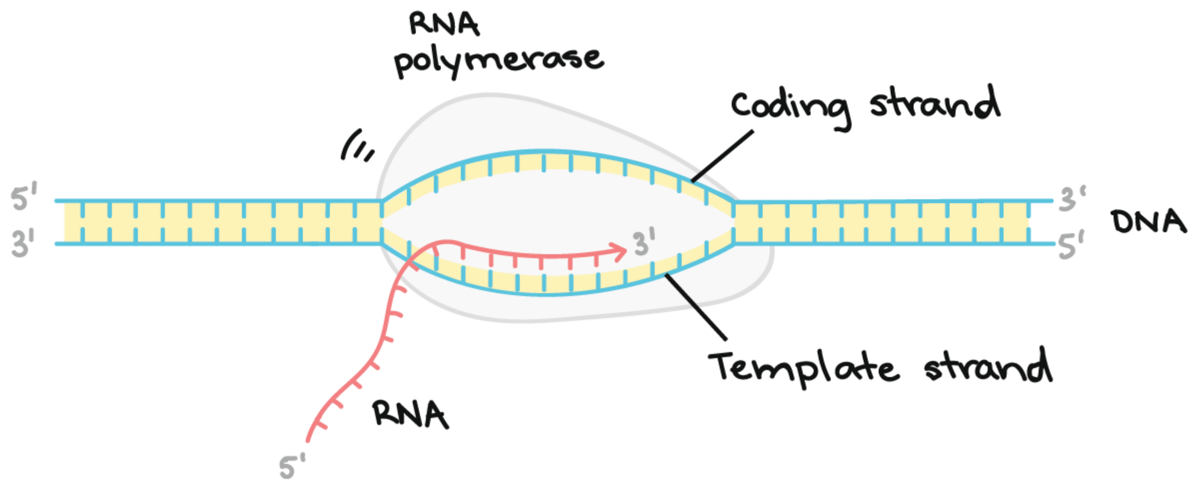
α: interaction with regulatory proteins
β: initiation , elongation
β’: DNA binding
σ: promoter recognation

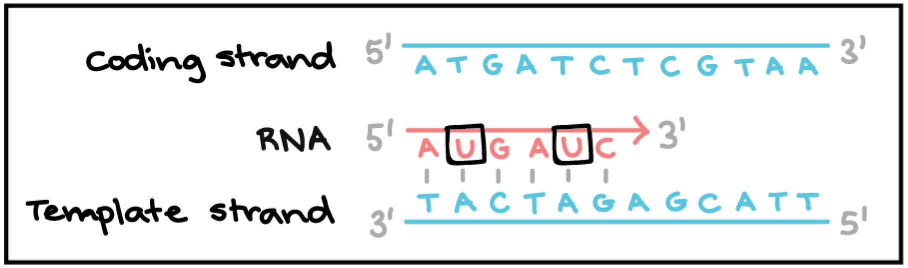
Prokaryotic transcription: initiation



https://www.khanacademy.org/science/biology/gene-expression-central-dogma/transcription-of-dna-into-rna/a/stages-of-transcription

Prokaryotic transcription: elongation

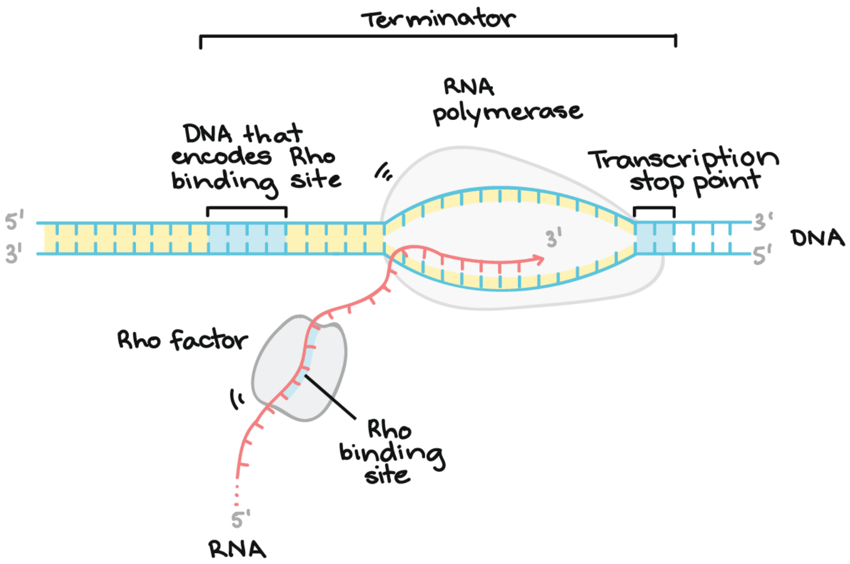


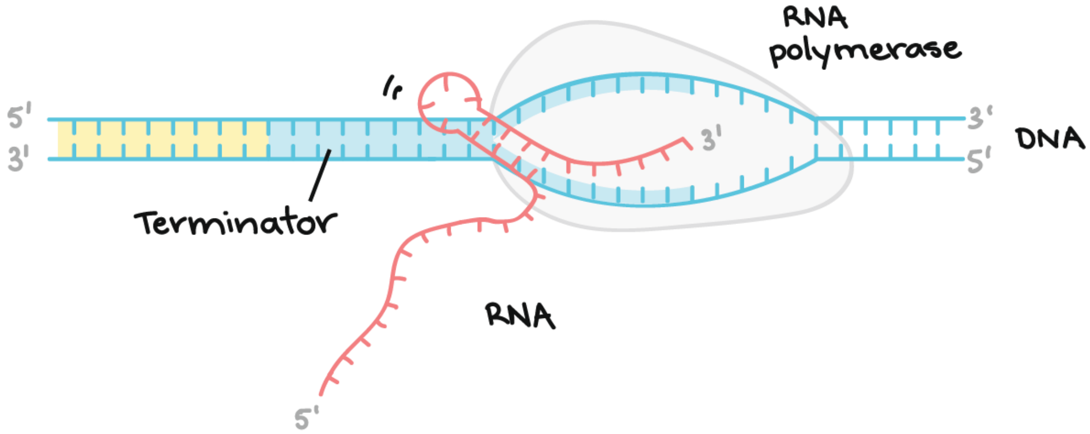


https://www.khanacademy.org/science/biology/gene-expression-central-dogma/transcription-of-dna-into-rna/a/stages-of-transcription

Prokaryotic transcriptin: termination

Rho-factor dependent





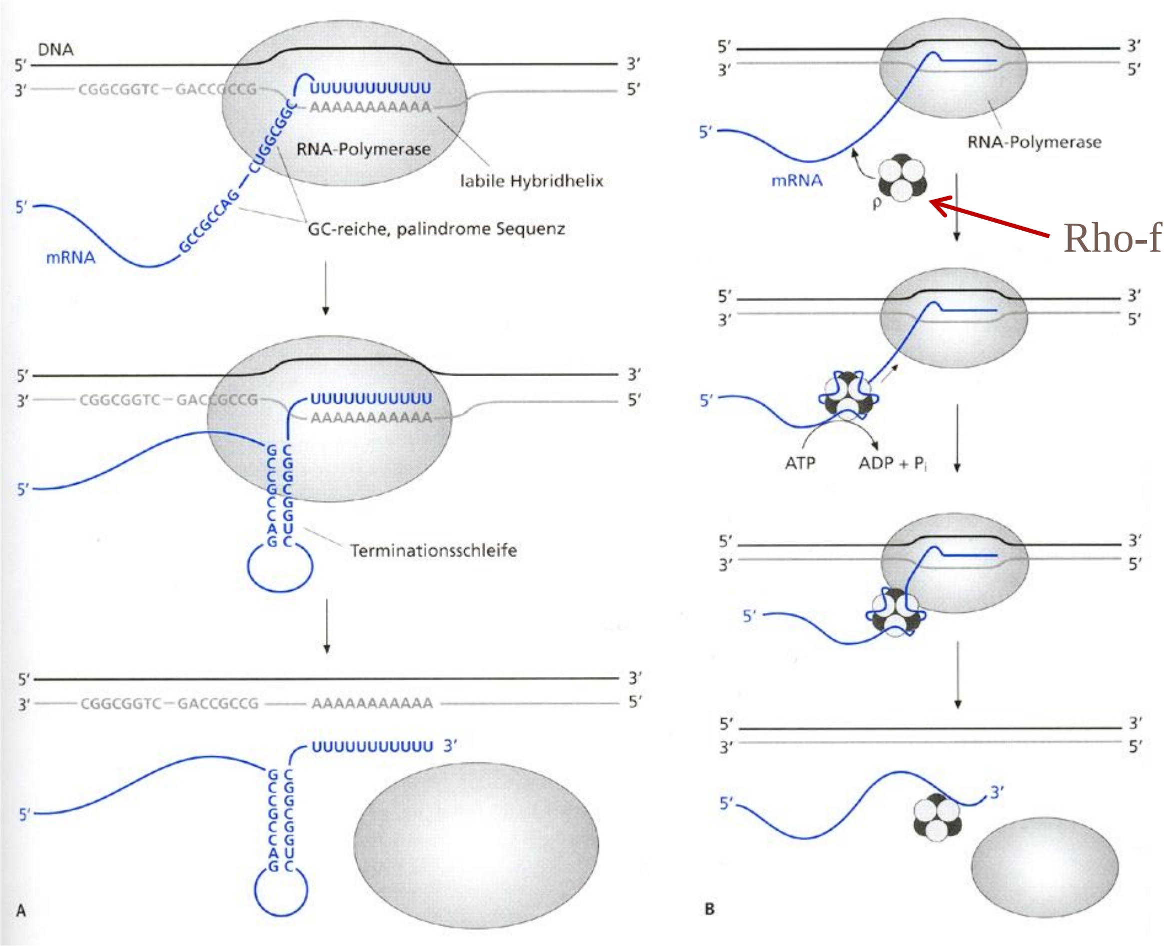
Rho-factor independent

https://www.khanacademy.org/science/biology/gene-expression-central-dogma/transcription-of-dna-into-rna/a/stages-of-transcription

prokaryotische Transkription: Termination

Rho-factor dependent

Rho-Factor independent



Rho-factor

hankeln@uni-mainz.de

Prokaryotic transcription: RNA processing

RNA processing

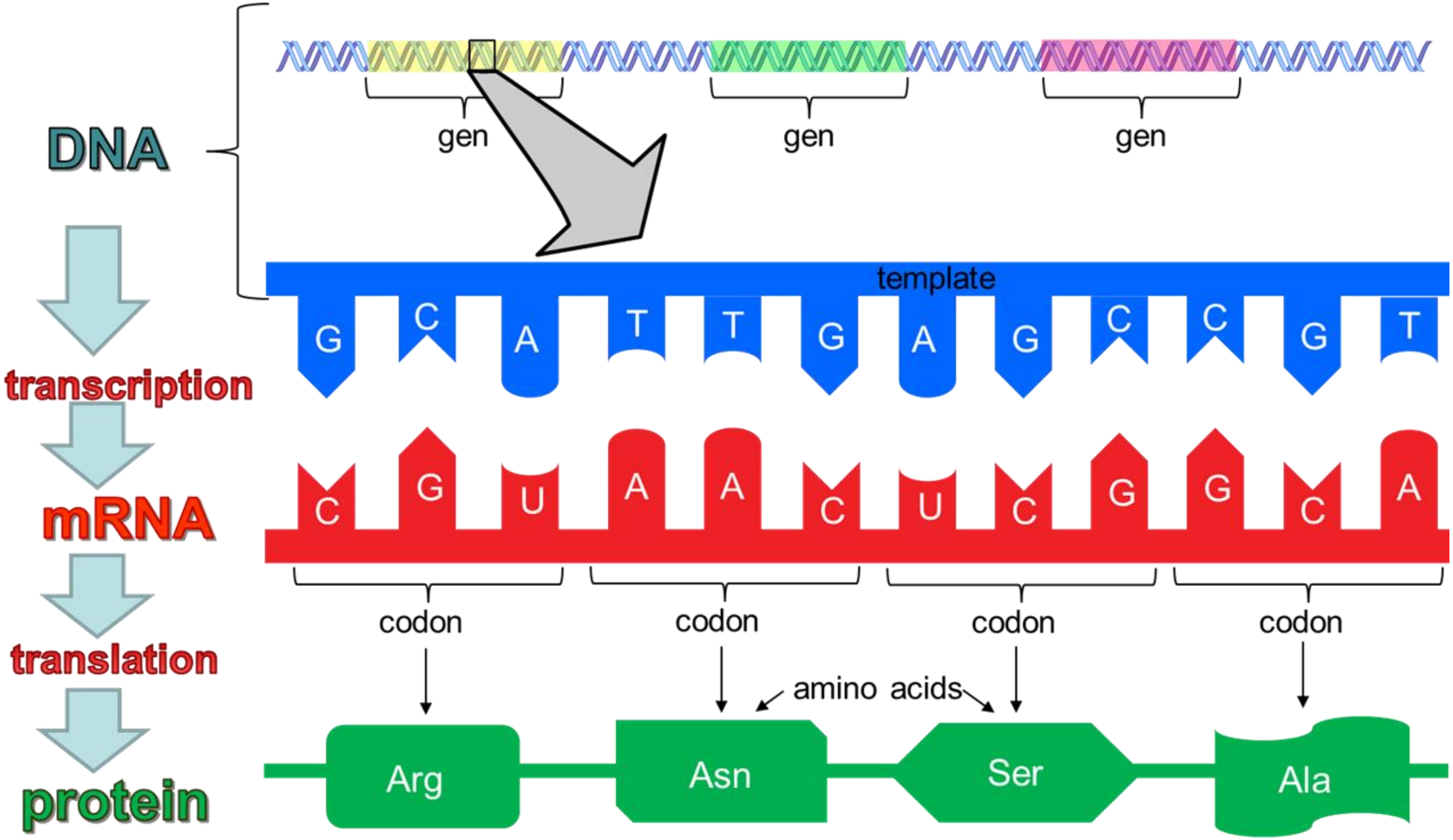
- characteristic of tRNA and rRNA
- - *nucleolytic cleavages* (endonucleases, exonucleases)
- - *nucleotide addition* (e.g. tRNA)
- - *nucleoside modification* (e.g. methylation)

GENETIC CODE

Renáta Schipp

The central dogma of molecular biology

the flow of information in the cells



Definition of the genetic code

- genetic code is the nucleotide sequence on DNA (and subsequently on mRNA by transcription) which determines the sequence of the amino acids in proteins
- the code is composed of codons
- codons are on mRNA, anticodons on tRNA
- each codon consists of three nucleotides

20 amino acid

4^2=16 combinations

4^3=64 combinations

Features of the genetic code

61 sense codons → amino acids

3 nonsense codons → stopcodons

- ***the code is redundant (degenerate)*** *”more than needed”*

  = one amino acid can be coded by more than one codons

  (Leucin: 6, Glycin: 4, Tryptophan: 1)

- ***the code is unambiguous***

  = a codon codes for a single amino acid

- ***the code is universal***

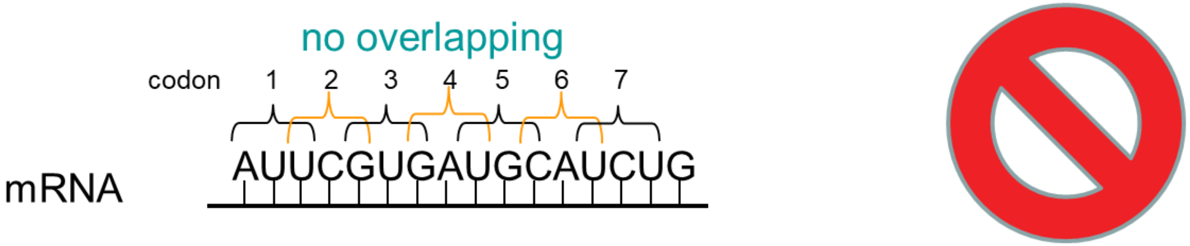
  = highly conserved in the living world

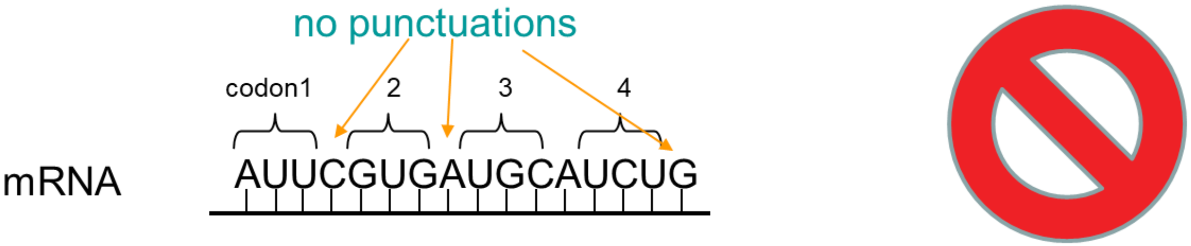
  exceptions (e.g. mitochondrial genetic apparatus)

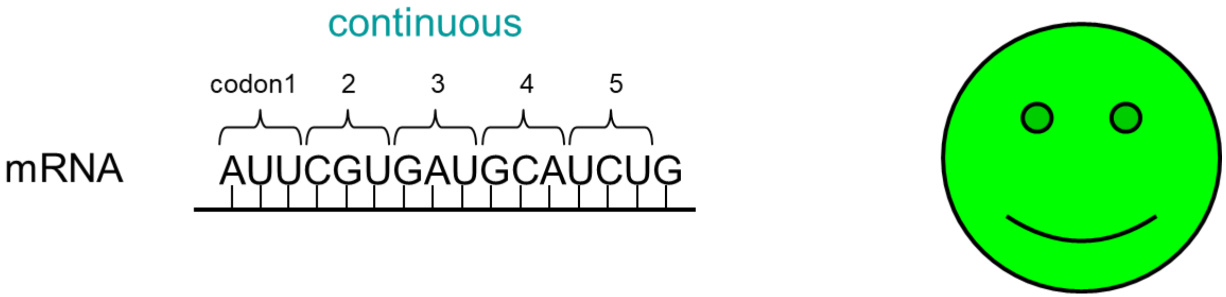
Features of the genetic code

- ***the code is continuous***

  = no punctuations, no overlapping



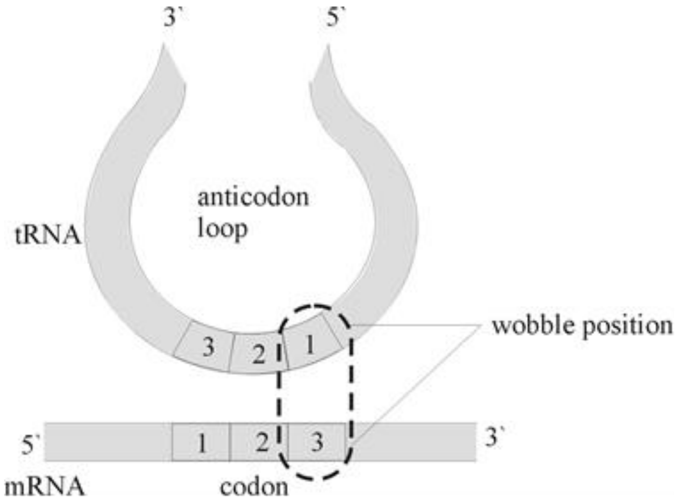




- ***wobble***

  = uncertainty at the third codon position

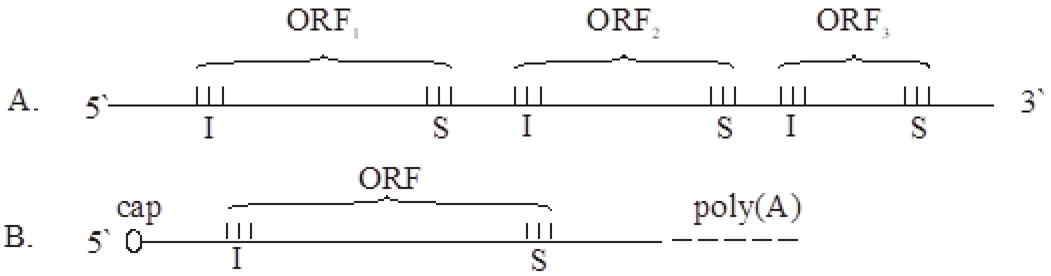
- usually, the first two nucleotides are enough for the translation of the amino acid
- in the first two positions the pairing of the codon-anticodon is specific
- in the 3. position the pairing is less specific



Reading frame

- ***open reading frame*** **(ORF)**

monocystronic and *polycystronic* mRNAs

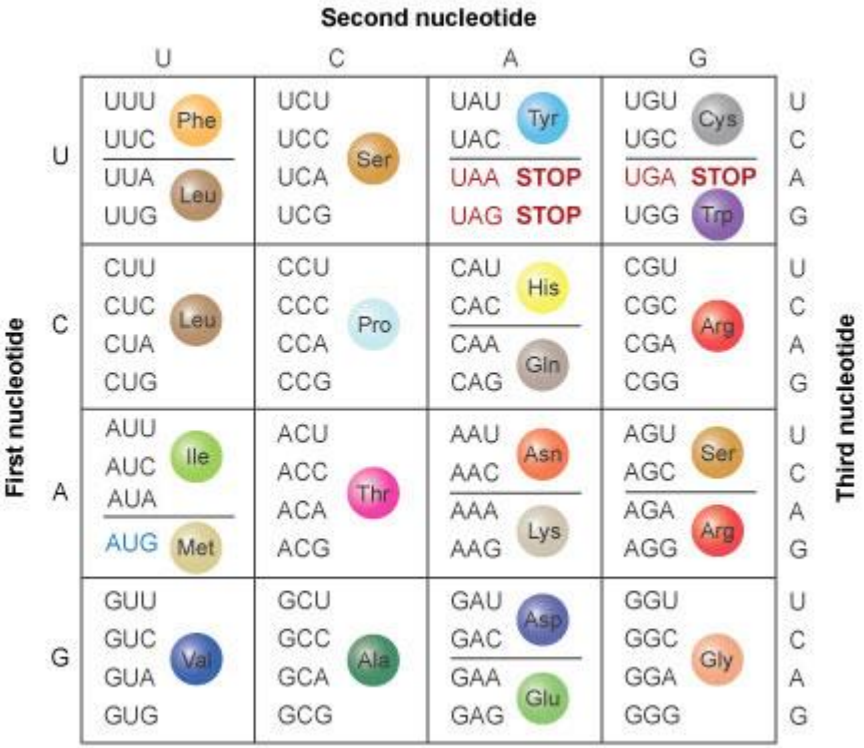


polycistronic mRNA from prokaryotes (A.); **monocistronic** mRNA from eukaryotes (B.). (I = initiation codon, S = stop codon, ORF = open reading frame)

How to translate a codon

A table (or dictionary) can be used to translate any codon sequence.

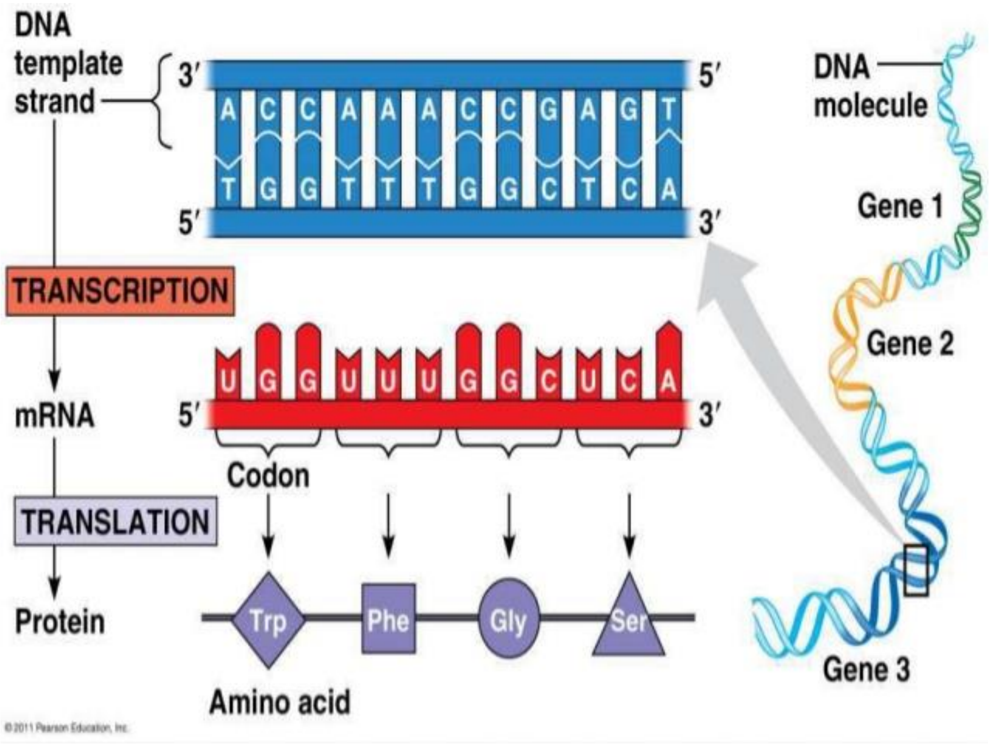
- Each triplet is read from 5′ → 3′ direction on the mRNA

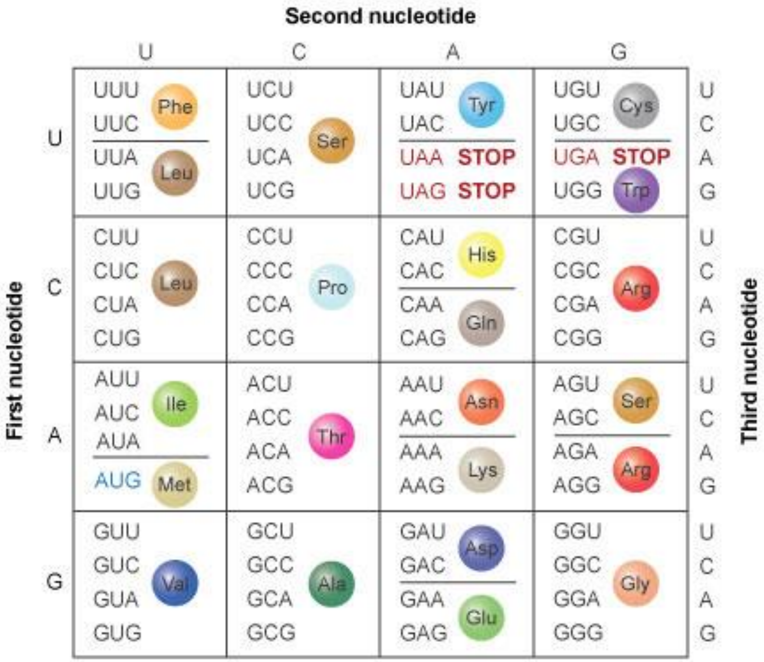


	Second nucleotide				
First nucleotide	U	C	A	G	Third nucleotide
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC	UCC	UAC	UGC	C
	UUA Leu	UCA	UAA STOP	UGA STOP	A
	UUG	UCG	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Ile	ACU Thr	AAU Asn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

https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393

How to translate a codon





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

Third nucleotide

https://www.slideshare.net/GleeCillo/transcriptiontranslation-and-genetic-codecell-biologyby-welfredo-yu

Genetic code excersise

Given the following DNA sequence, transcribe it into mRNA, then translate this sequence into protein.

5’-ATGTTTTATGCACGTACGGAGCTTCGGTAG-3’ DNA

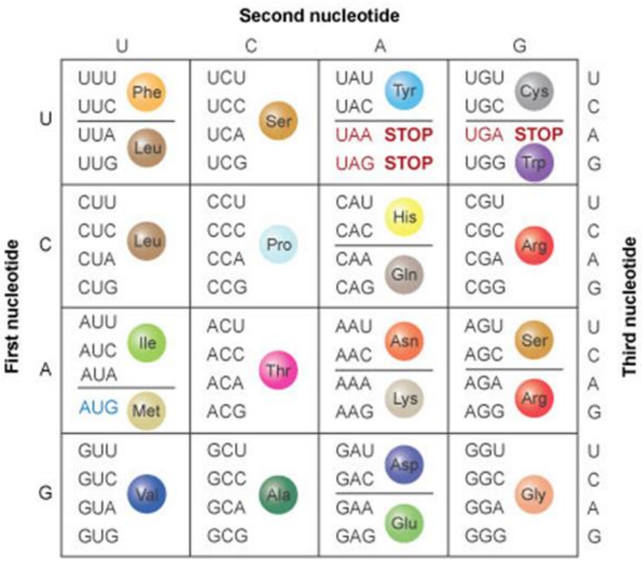
Results

5’-ATGTTTTATGCACGTACGGAGCTTCGGTAG-3’ DNA (coding strand)

3’-TACAAAATACGTGCATGCCTCGAAGCCATC-5’ DNA (template strand)

5’-AUGUUUUAUGCACGUACGGAGCUUCGGUAG-3’mRNA

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop



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

Modification

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAG ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand)

5’-AUG UUC UAU GCA CGU ACG GAG CUU CGG UAG-3’mutated mRNA

Met ? Tyr Ala Arg Thr Glu Leu Arg stop

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)
3’-TAC AAA ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (template strand)

Silent mutation

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAG ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand)

5’-AUG UUC UAU GCA CGU ACG GAG CUU CGG UAG-3’mutated mRNA

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop original amino acid sequence

no change in amino acid sequence !

Modification

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAA ATA CCT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand)

5’-AUG UUU UAU GGA CGU ACG GAG CUU CGG UAG-3’mutated mRNA

Met Phe Tyr ? Arg Thr Glu Leu Arg stop

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)
3’-TAC AAA ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (template strand)

↑

Missense mutation

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAA ATA CCT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand)

5’-AUG UUU UAU GGA CGU ACG GAG CUU CGG UAG-3’mutated mRNA

Met Phe Tyr Gly Arg Thr Glu Leu Arg stop

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop original amino acid sequence

different amino acid sequence !

Modification

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAA ATT CGT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand)

5’-AUG UUU UAA GCA CGU ACG GAG CUU CGG UAG-3’mutated mRNA

Met Phe ? Ala Arg Thr Glu Leu Arg stop

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)
3’-TAC AAA ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (template strand)

↑

Nonsense mutation

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

3’-TAC AAA ATT CGT GCA TGC CTC GAA GCC ATC-5’ DNA (mutated template strand

5’-AUG UUU UAA GCA CGU ACG GAG CUU CGG UAG-3’ mutated mRNA

Met Phe stop

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop original amino acid sequence

polypeptide synthesis stop !

Modification(insertion)

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

Insertion

3’-TAC AAA TAT ACG TGC ATG CCT CGA AGC CAT C-5’ DNA (template strand)

5’-AUG UUU AUA UGC ACG UAC GGA GCU UCG GUA G-3’mRNA

Met Phe ??????????????????????????????????????

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)
3’-TAC AAA ATA CGT GCA TGC CTC GAA GCC ATC-5’ DNA (template strand)

Frameshift mutation

5’-ATGTTTTATGCACGTACGGAGCTTCGGTAG-3’ DNA (coding strand)

Insertion

3’-TACAAATATACGTGCATGCCTCGAAGCCATC-5’ DNA (template strand)

5’-AUGUUUAUAUGCACGUACGGAGCUUCGGUAG-3’mRNA

Met Phe Ile Cys Thr Tyr Gly Ala Ser Val

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop original amino acid sequence

major difference in amino acid sequence !!!!!!!!!!!!!!!!!!!

Modification(deletion)

5’-ATG TTT TAT GCA CGT ACG GAG CTT CGG TAG-3’ DNA (coding strand)

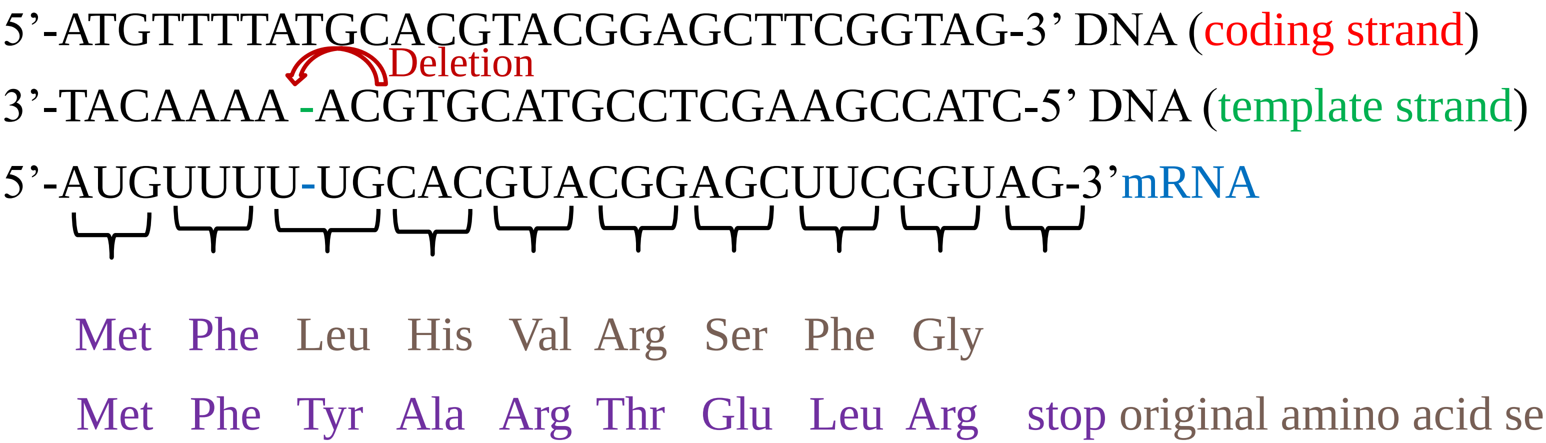
Deletion

3’-TAC AAA A –AC GTG CAT GCC TCG AAG CCA TC-5’ DNA (template strand)

5’-AUG UUU U-UG CAC GUA CGG AGC UUC GGU AG-3’mRNA

Met Phe ?????????????????????????????????????

Frameshift mutation



5’-ATGTTTTATGCACGTACGGAGCTTCGGTAG-3’ DNA (coding strand)

Deletion

3’-TACAAAA -ACGTGCATGCCTCGAAGCCATC-5’ DNA (template strand)

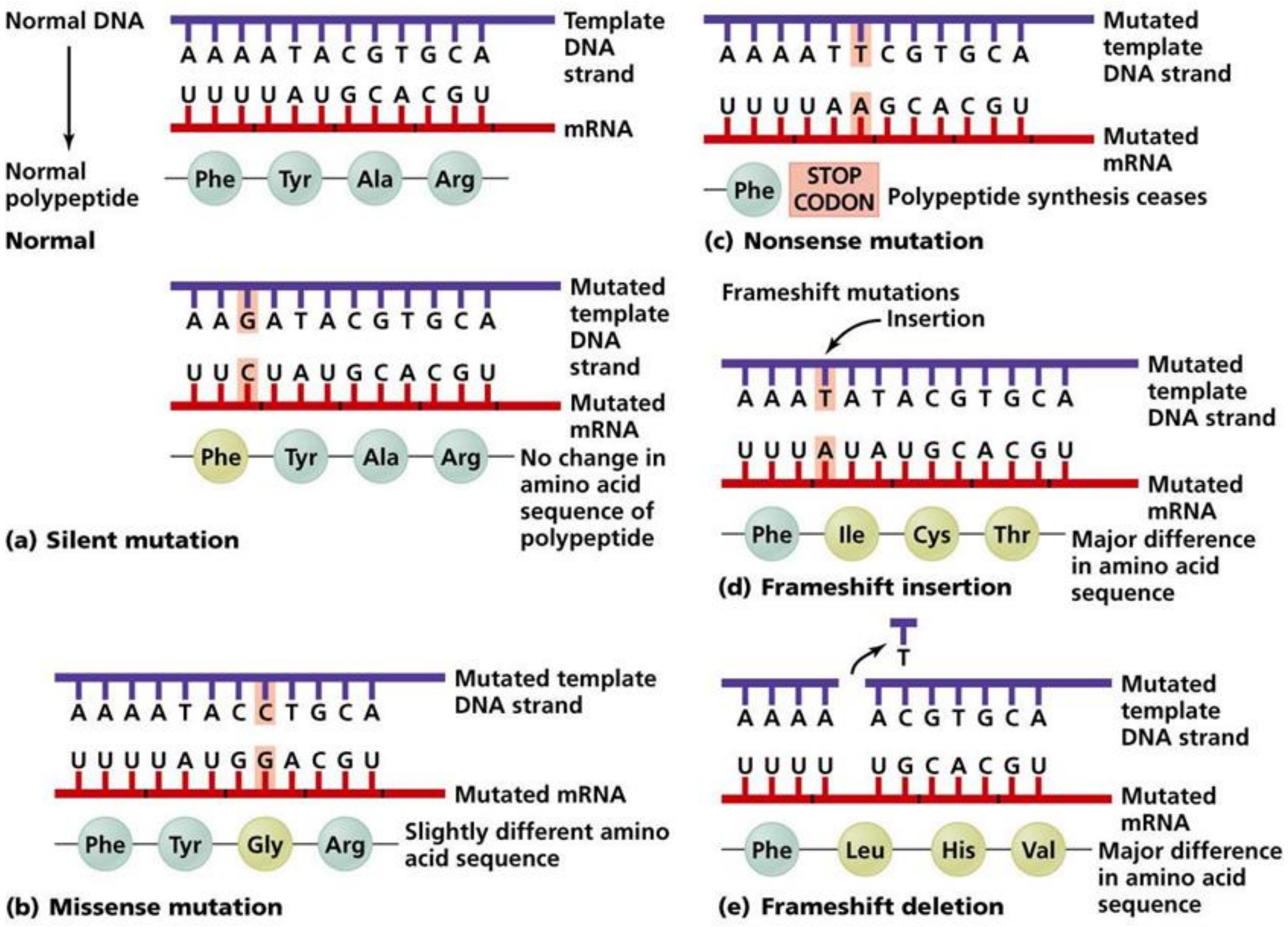
5’-AUGUUUU-UGCACGUACGGAGCUUCGGUAG-3’mRNA

Met Phe Leu His Val Arg Ser Phe Gly

Met Phe Tyr Ala Arg Thr Glu Leu Arg stop original amino acid sequence

major difference in amino acid sequence !!!!!!!!!!!!!!!!!!!!

Summary of mutations



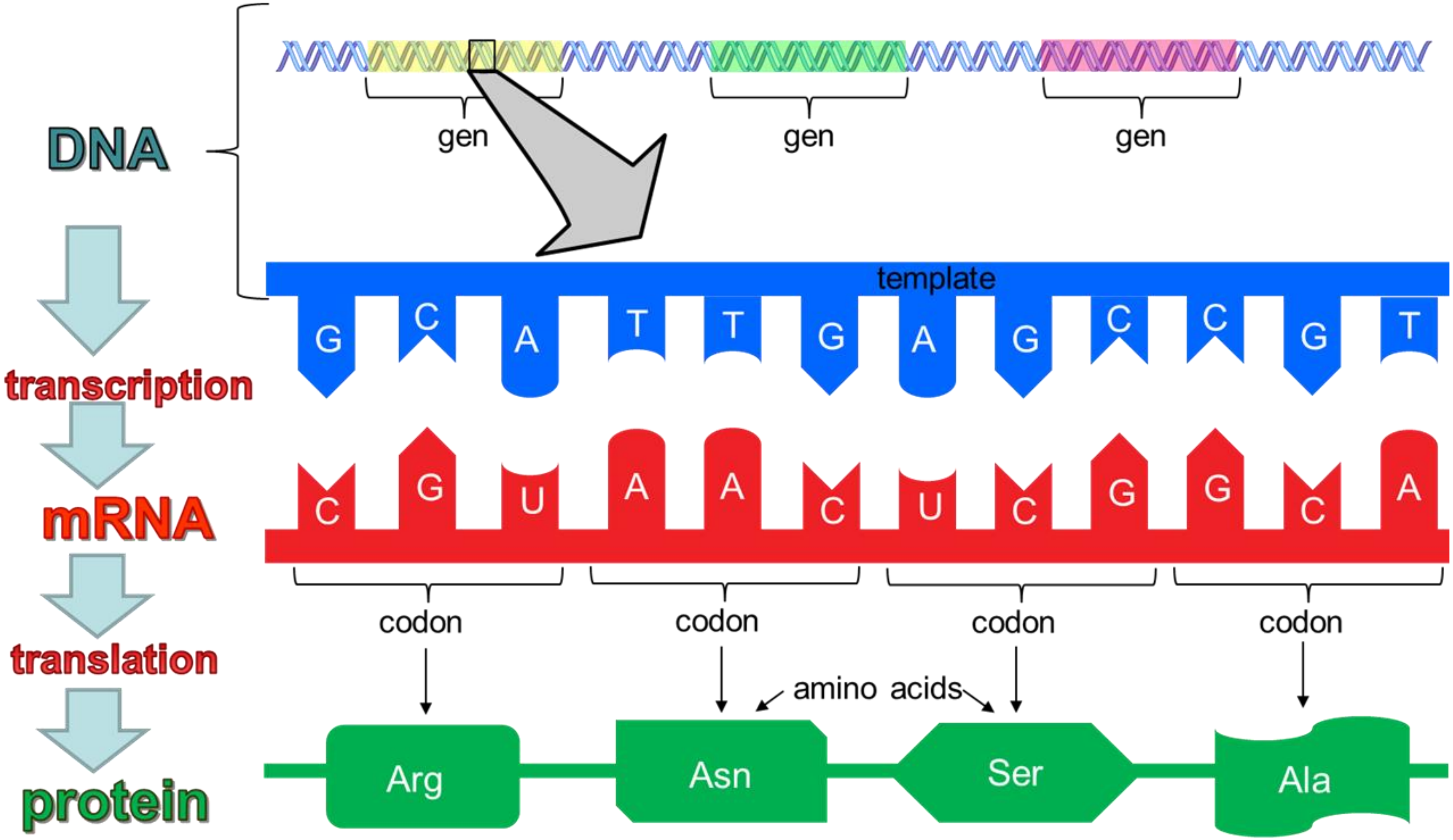
Thank you for your attentions!

TRANSLATION

Renáta Schipp

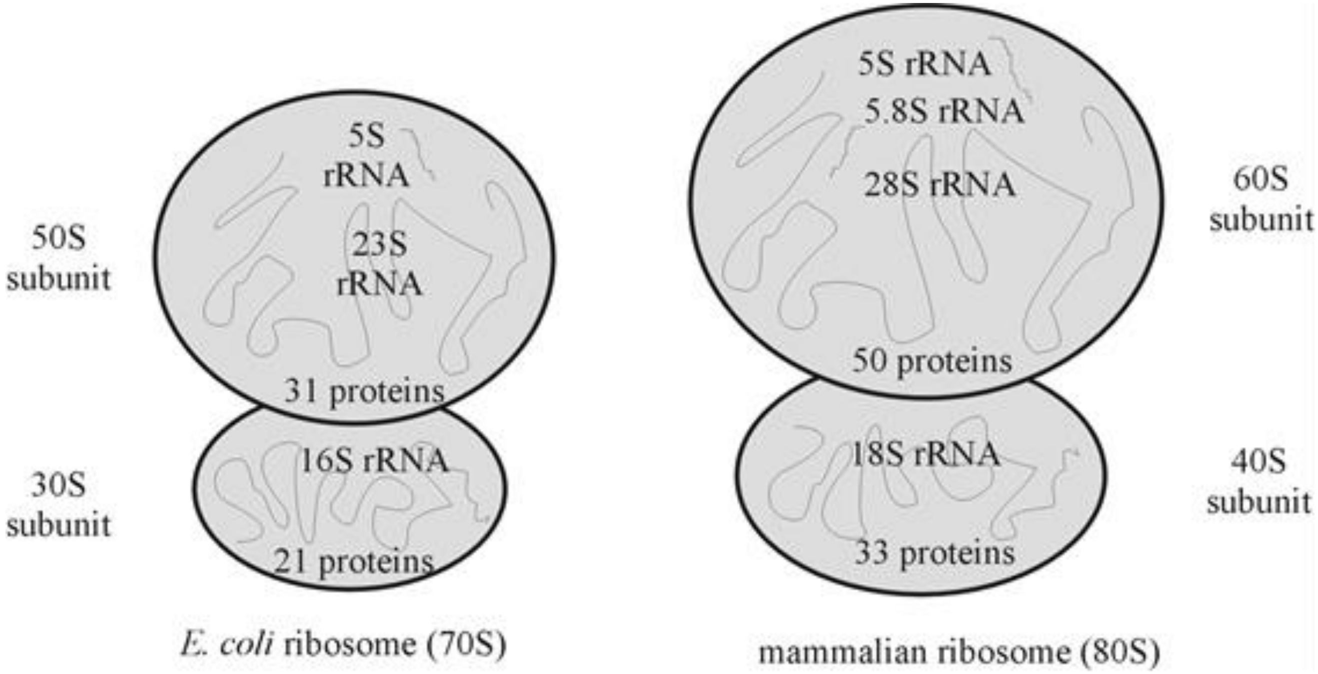
The central dogma of molecular biology

the flow of information in the cells



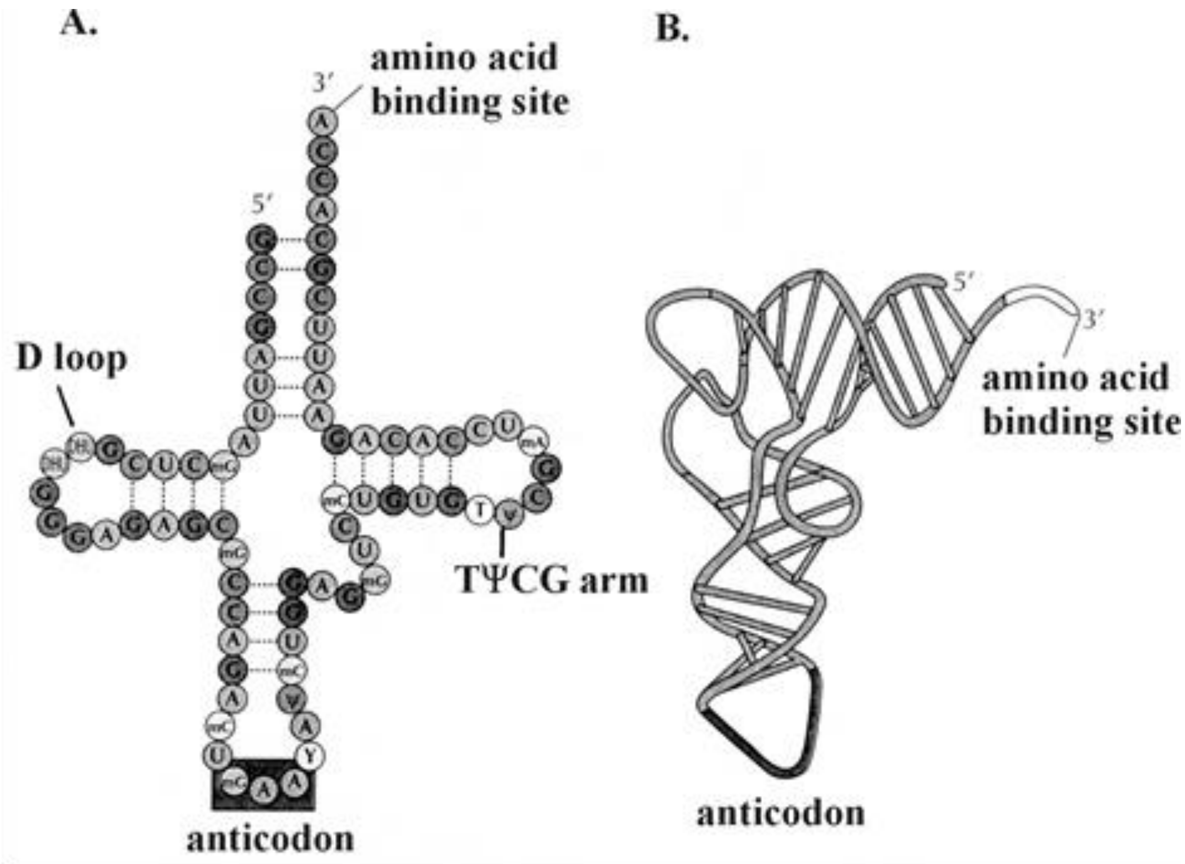
Ingredients of translation

ribosomes



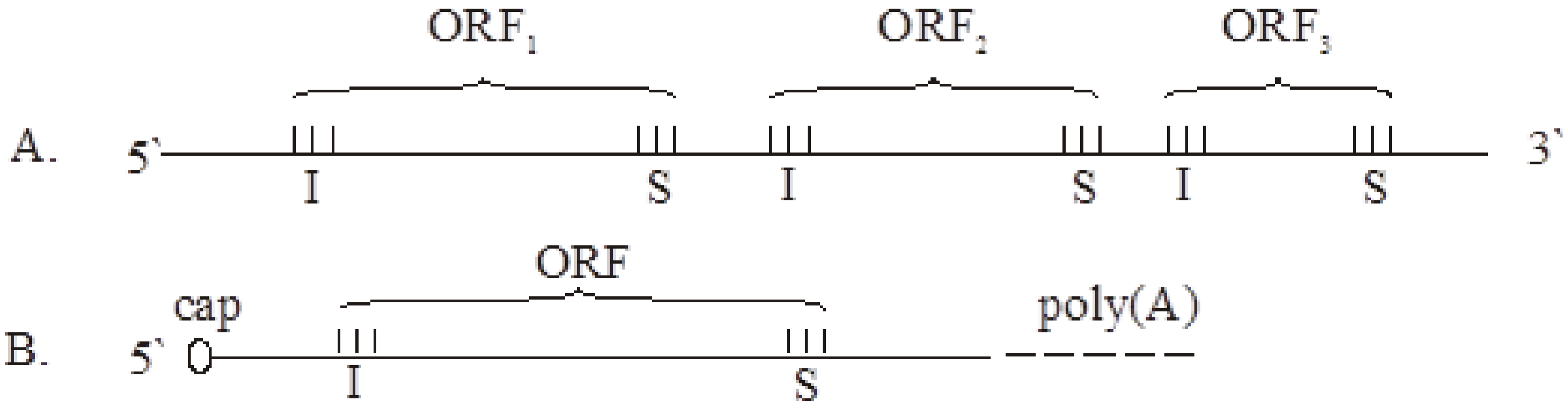
Ingredients of translations

transfer RNA: tRNA



Ingredients of translation

messenger RNA: mRNA



Polycistronic mRNA from prokaryotes (A.); monocistronic mRNA from eukaryotes (B.). (I = initiation codon, S = stop codon, ORF = open reading frame)

Ingredients of translation

amino acid: 20 essential amino acid

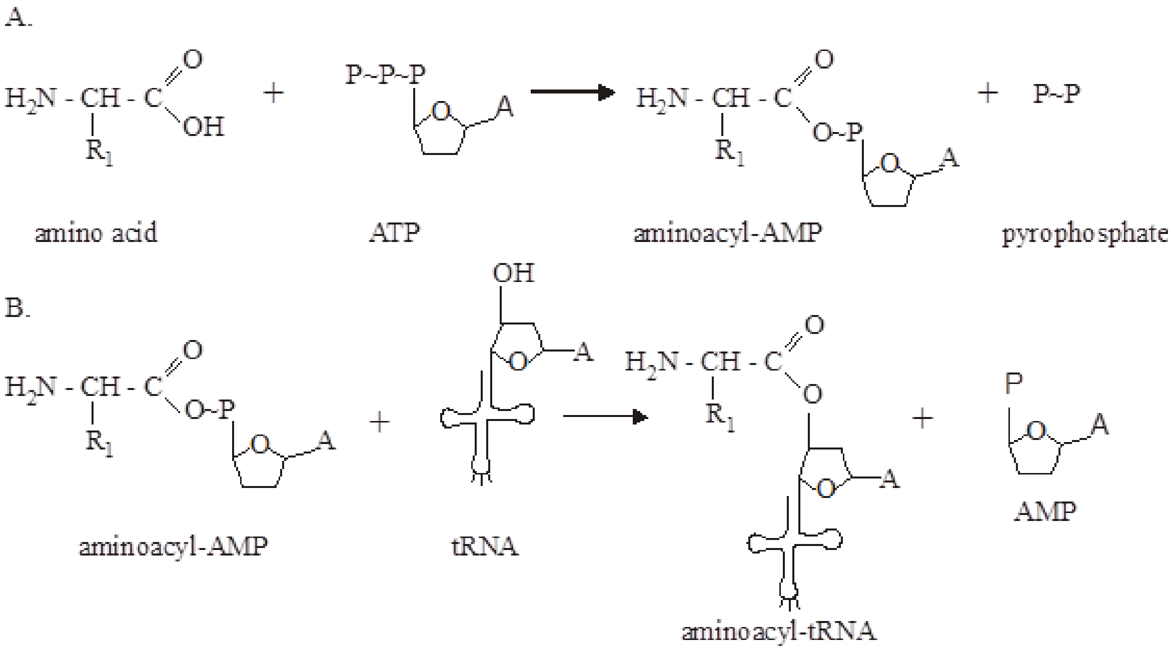
synthesis of **aminoacyl-tRNA by aminoacyl-tRNA synthases**

step 1 - activation of amino acid

amino acid + ATP → aminoacyl-AMP + pyrophosphate

step 2 - formation of aminoacyl-tRNA

aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP



Steps of translation

Initiation

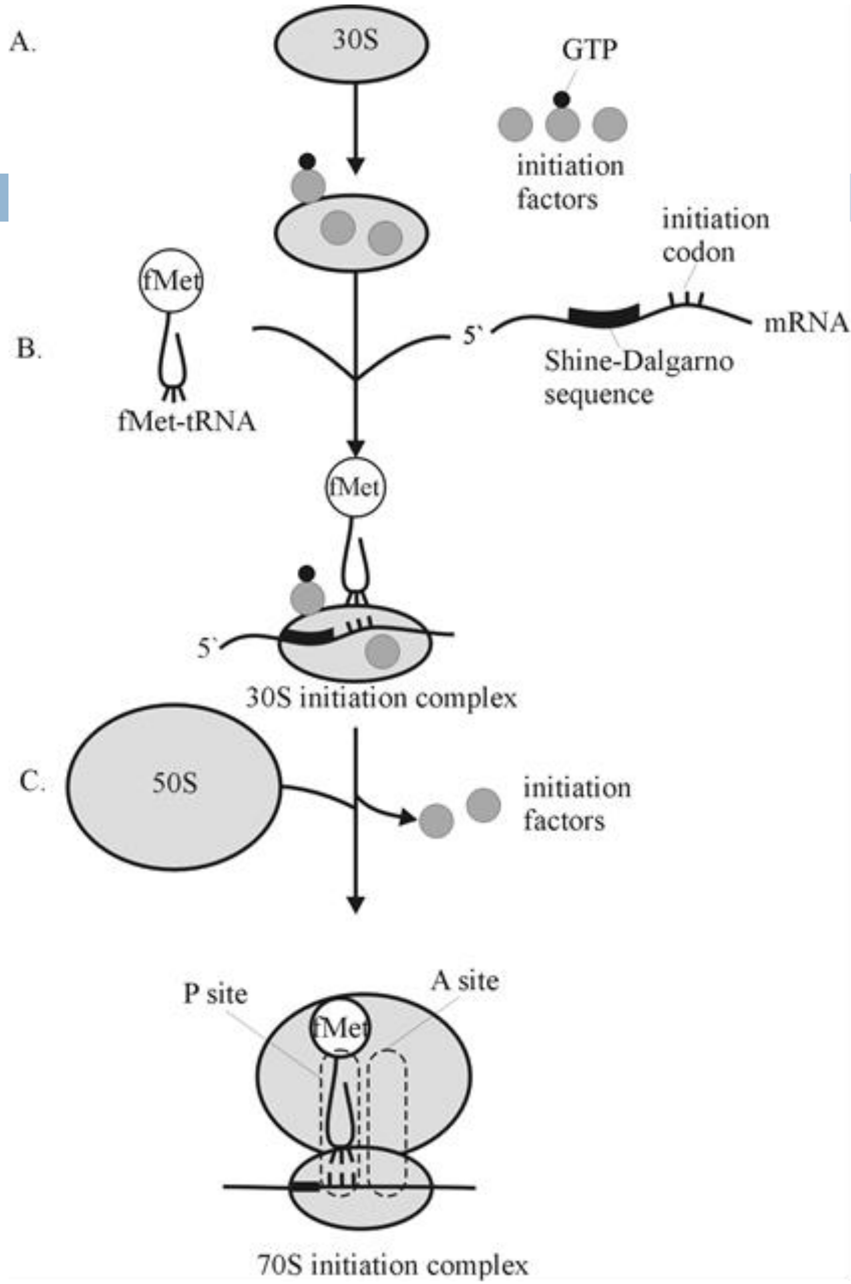
A: binding of initiation factors to the small subunit

B: assembly of 30S initiation complex

C: assembly of 70S initiation complex.

30S initiation complex
= mRNA + 30S subunit + fMet-tRNA + initiation factors

70S initiation complex
= 30S initiation complex + 50S subunit
P site: fMet-tRNA
A site: empty



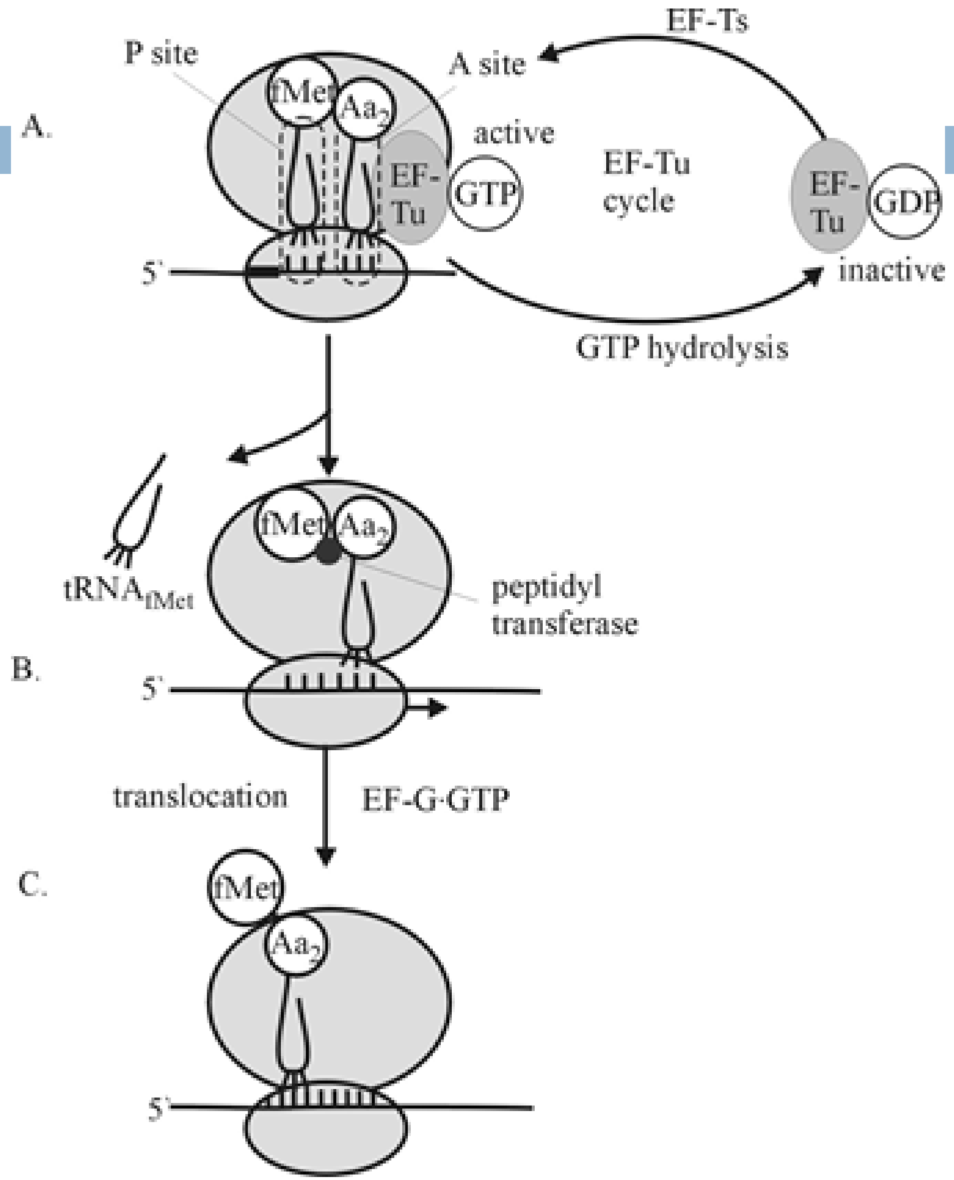
Steps of translation

Elongation

A. ***Aa-tRNA binding*** with the help of *EF-Tu* • *GTP* to A site

B. ***peptide bond formation***
peptidyl transferase (ribozyme)

C. ***translocation***
with the help of *EF-G* • *GTP*

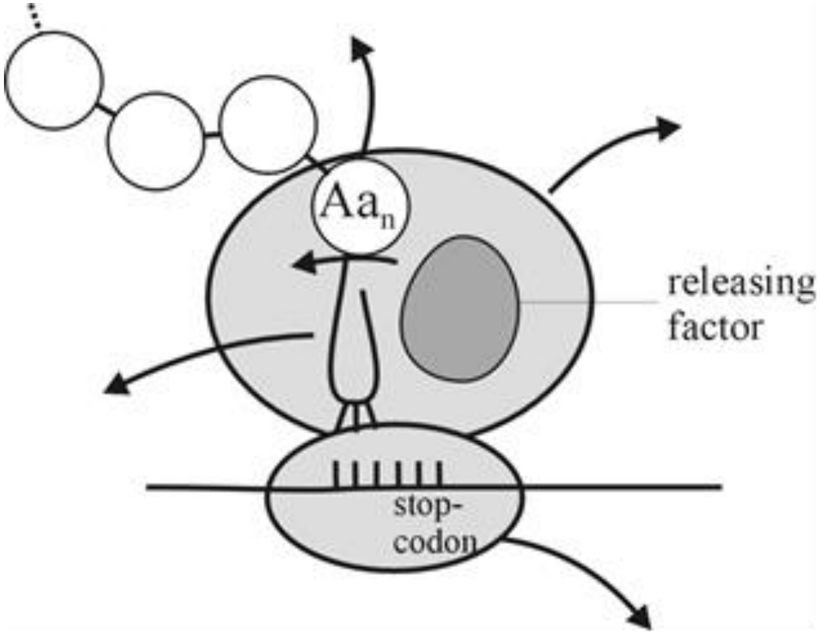


Steps of translation

Termination

stop codon:
dose not code for any amino acid

releasing factors:
release of ribosomal subunites, mRNA, polypeptide chain



Unique features of eukaryotic translation

5`-cap → ribosome binding

ribosomes

- *free ribosomes* → proteins of cytosol, nucleus, mitochondria

- *bound ribosomes* → secretory proteins, proteins of endoplasmic reticulum, Golgi complex, lysosomes, cell membrane

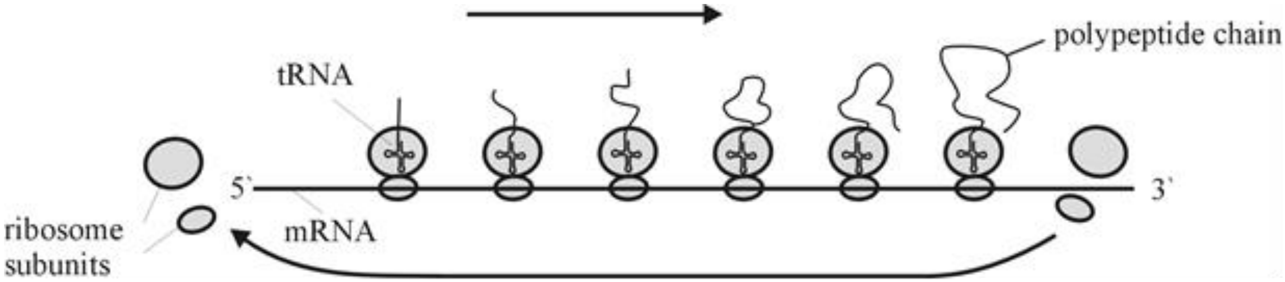
General features of translation

- ***direction*:**

5`end → 3`end on mRNA

N terminus → C terminus on polypeptide chain

- ***polysomes*** are formed



- **one polypeptide chain/ribosome**

- requires **4 high energy bonds**/peptide bonds (ATP → AMP, 2GTP → 2GDP)

for amino acid activation: ATP→AMP=2 high energy bonds

for Aa-tRNA binding (EF-Tu) GTP→GDP= 1 high energy bond

for translocation (EF-G) GTP→GDP= 1 high energy bond

Inhibitors of protein synthesis

- **chloramphenicol** ⊣ peptidyl transferase
- **erythromycin** ⊣ translocation
- **tetracyclin** ⊣ aa-tRNA binding
- **streptomycin** ⊣ 30S subunit
- **puromycin** → early termination

Antibiotics are a group of medicines that are used to treat infections caused by some prokaryotes.

Thank you for your attentions!