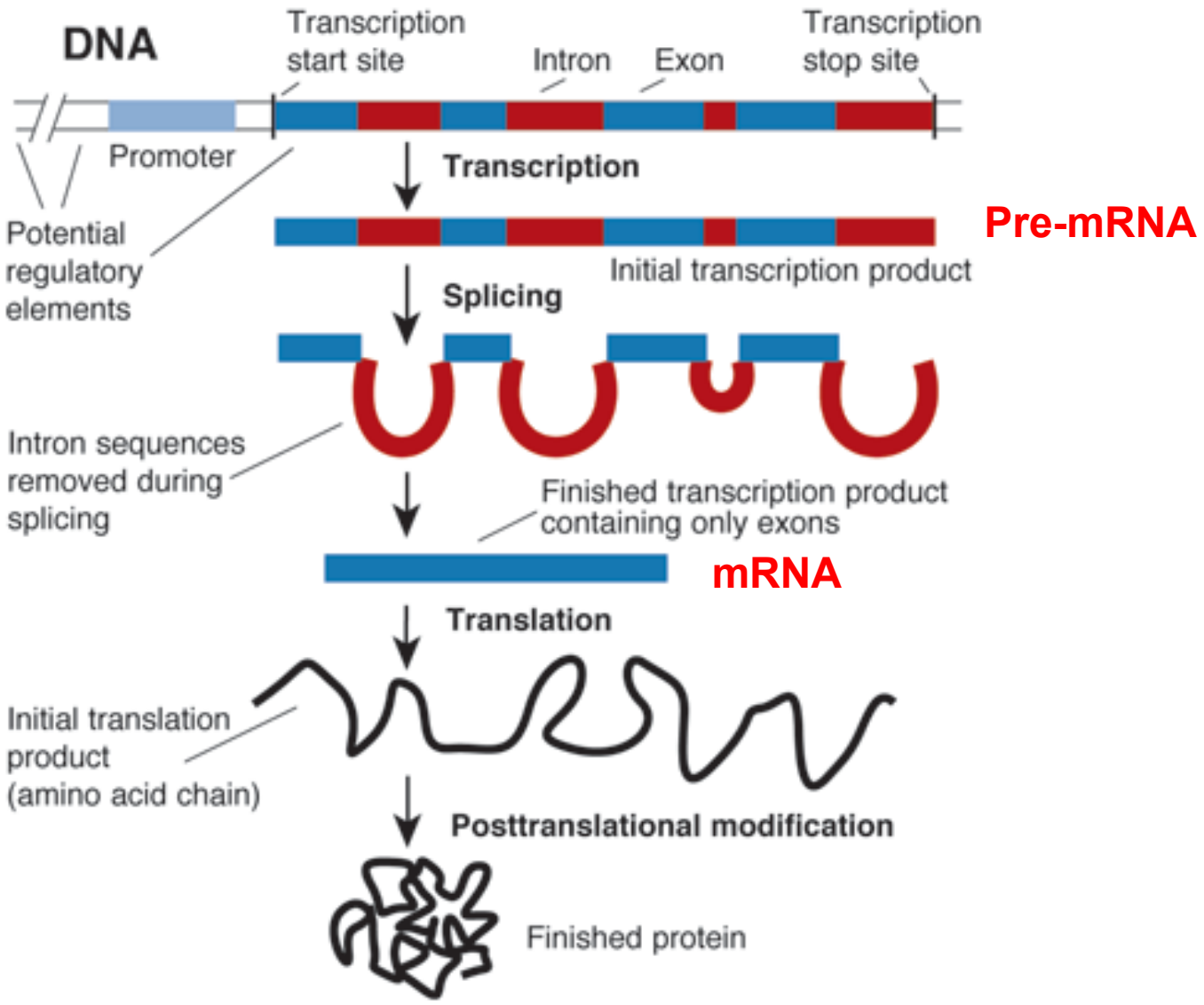
Gene Structure & Protein Synthesis



De-coding a gene sequence

5′-ATGCCTAGGTACCTATGA-3′

3′-TACGGATCCATGGATACT-5′

DNA

↓ Transcription

5′-AUGCCUAGGUACCUAUGA-3′

mRNA

↓ decoded as

5′-AUG CCU AGG UAC CUA UGA-3′

↓ ↓ ↓ ↓ ↓ Translation

N-MET-PRO-ARG-TYR-LEU-C

Protein

The Genetic Code

- **Proteins are made by joining amino acids into long chains called polypeptides. There are 20 different amino acids. The properties of the protein are determined by the order of amino acids.**

- **The genetic code is read three letters at a time so that each "word" is three bases long. Each three letter "word" is a codon.**

- **A codon consists of three consecutive nucleotides that specify a single amino acid that is to be added to the polypeptide.**

The Genetic Code

There are 64 possible codons. Some amino acids can be specified by more than one codon.

There is a start codon, AUG (methionine). There are three stop codons (UAA, UAG, UGA).

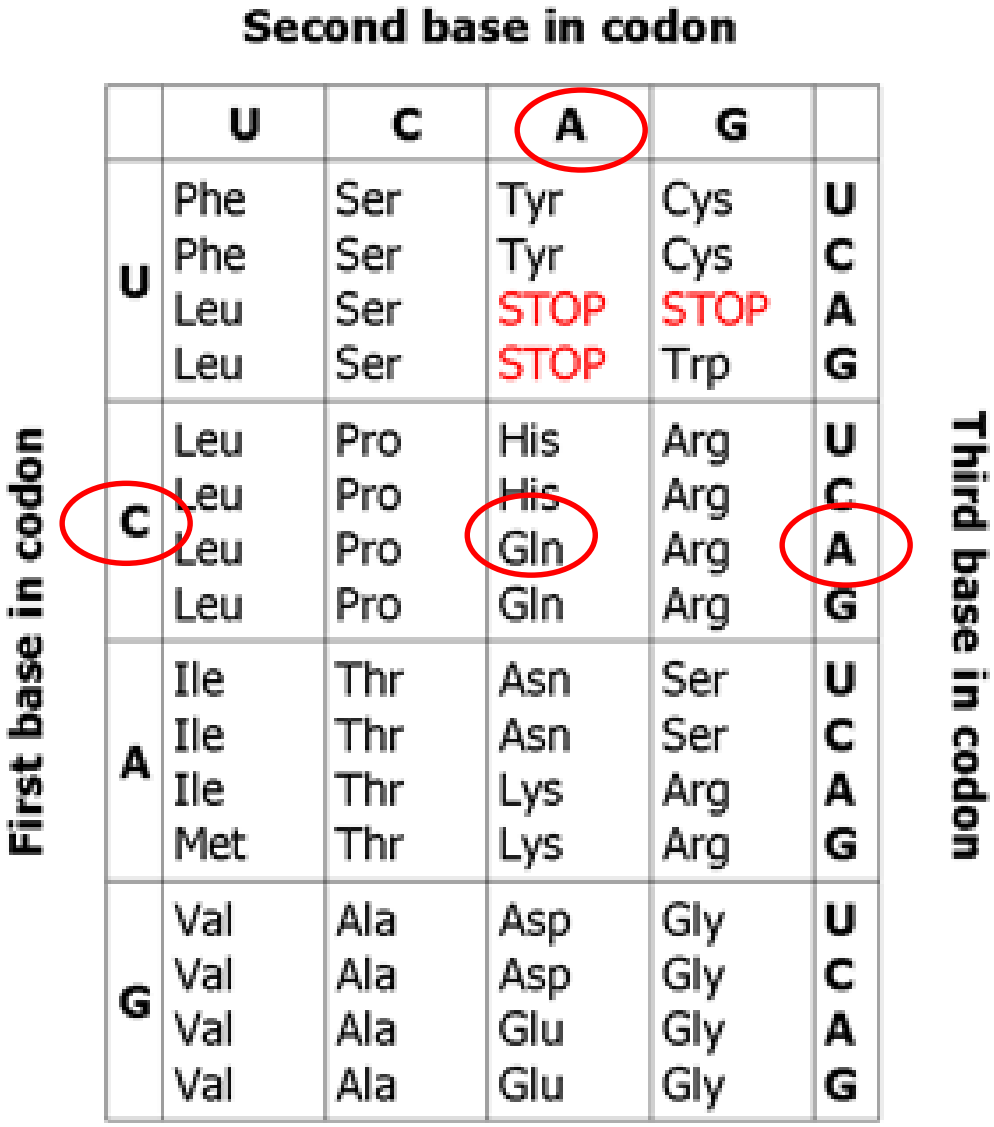
The Genetic Code is Nearly Universal

- The process of making proteins from the information in DNA is used by nearly all cells.
- Nearly all organisms studied to date use the same genetic code.
- Because of this, we are able to use bacteria as factories to make massive amounts of proteins.
  - Insulin, growth factor, etc.

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile
A	R	D	N	C	E	Q	G	H	I

UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

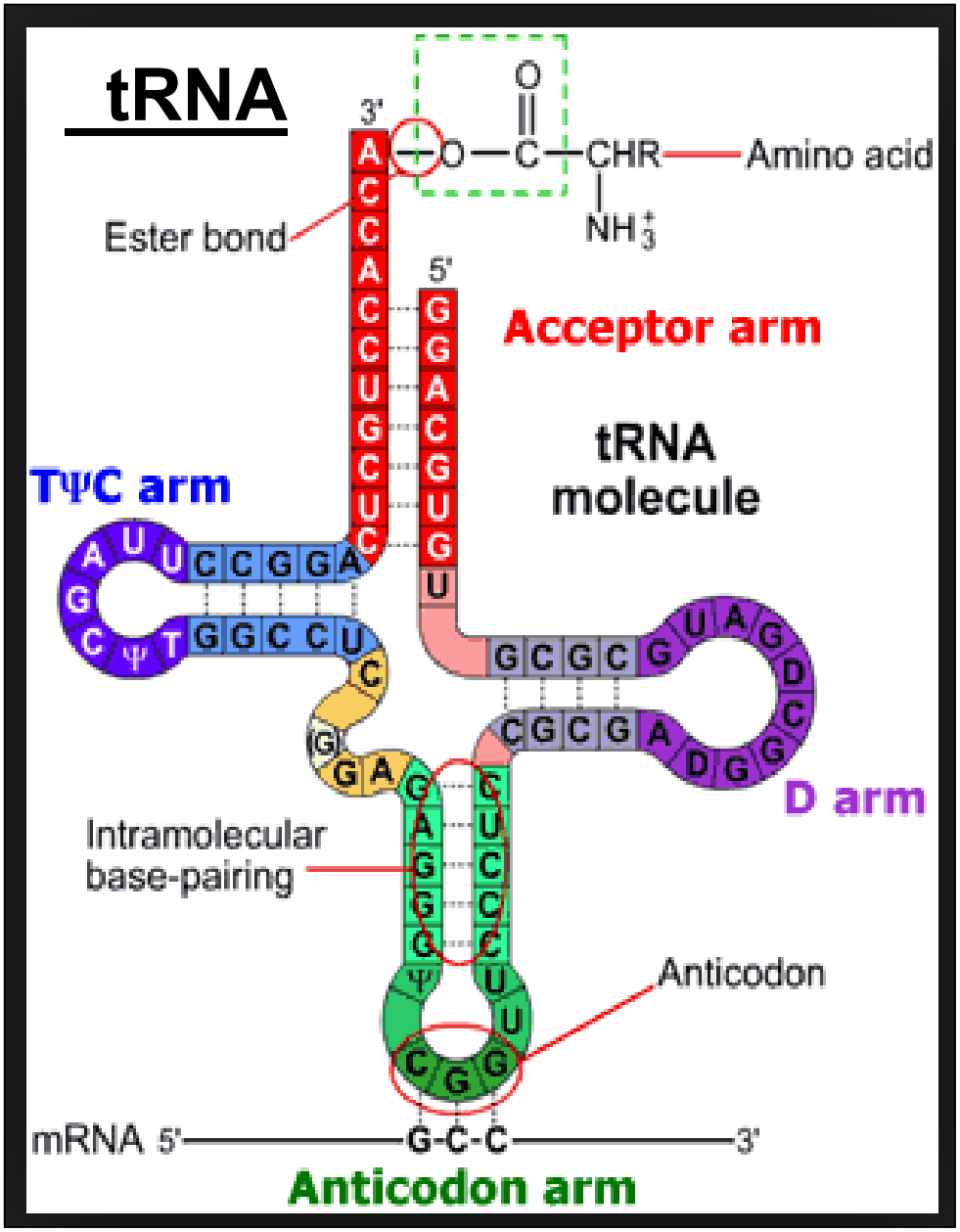
Figure 6–50. Molecular Biology of the Cell, 4th Edition.

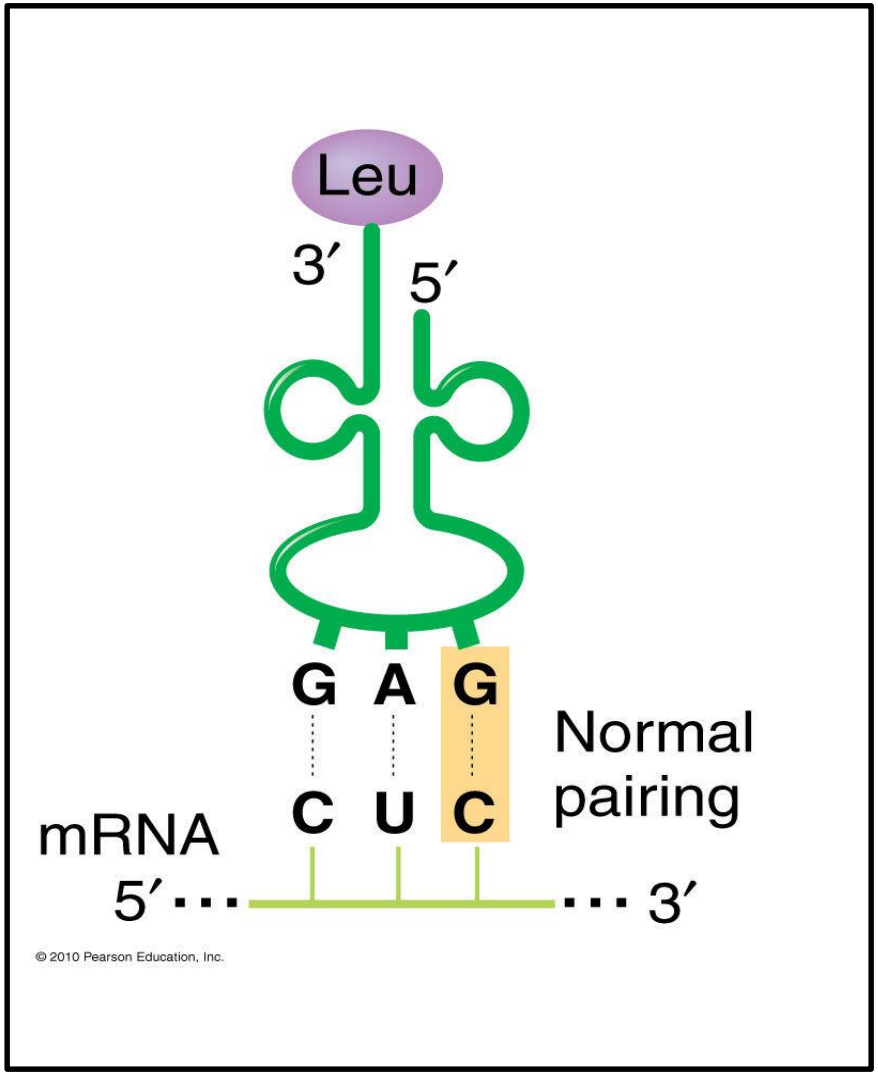

Second base in codon
U C A G
First base in codon
Third base in codon
U Phe Ser Tyr Cys U
Phe Ser Tyr Cys C
Leu Ser STOP STOP A
Leu Ser STOP Trp G
C Leu Pro His Arg U
Leu Pro His Arg C
Leu Pro Gln Arg A
Leu Pro Gln Arg G
A Ile Thr Asn Ser U
Ile Thr Asn Ser C
Ile Thr Lys Arg A
Met Thr Lys Arg G
G Val Ala Asp Gly U
Val Ala Asp Gly C
Val Ala Glu Gly A
Val Ala Glu Gly G

Translation

- Three types of RNA participate in translation.
  - mRNA carries the recipe for making the protein.
  - tRNA and rRNA are used to read the recipe and build the amino acid chain.

- Codons are sets of three nucleotides that code for specific amino acids.
  - tRNA reads the codons and brings the correct amino acids.


tRNA
3'
O
O—C—CHR
Amino acid
NH3+
Ester bond
5'
Acceptor arm
tRNA molecule
TψC arm
D arm
Intramolecular base-pairing
Anticodon
mRNA 5'
G-C-C
3'
Anticodon arm


Leu
3′
5′
G A G
C U C
Normal pairing
mRNA
5′ ··· ··· 3′
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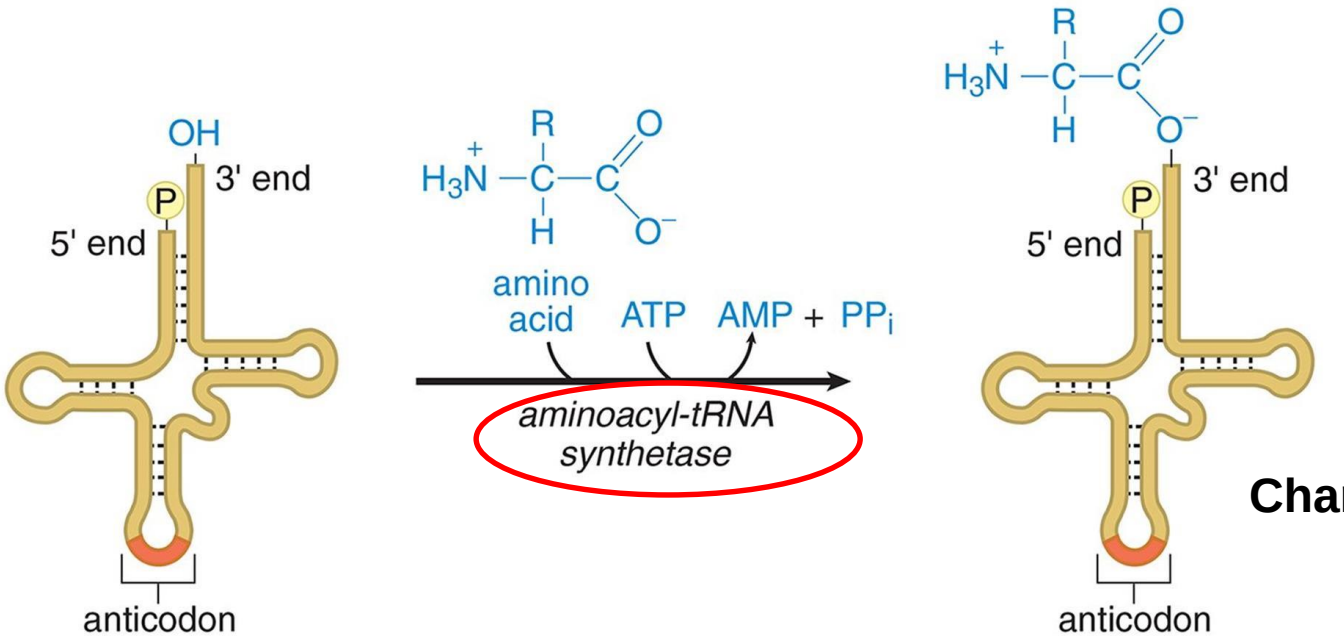
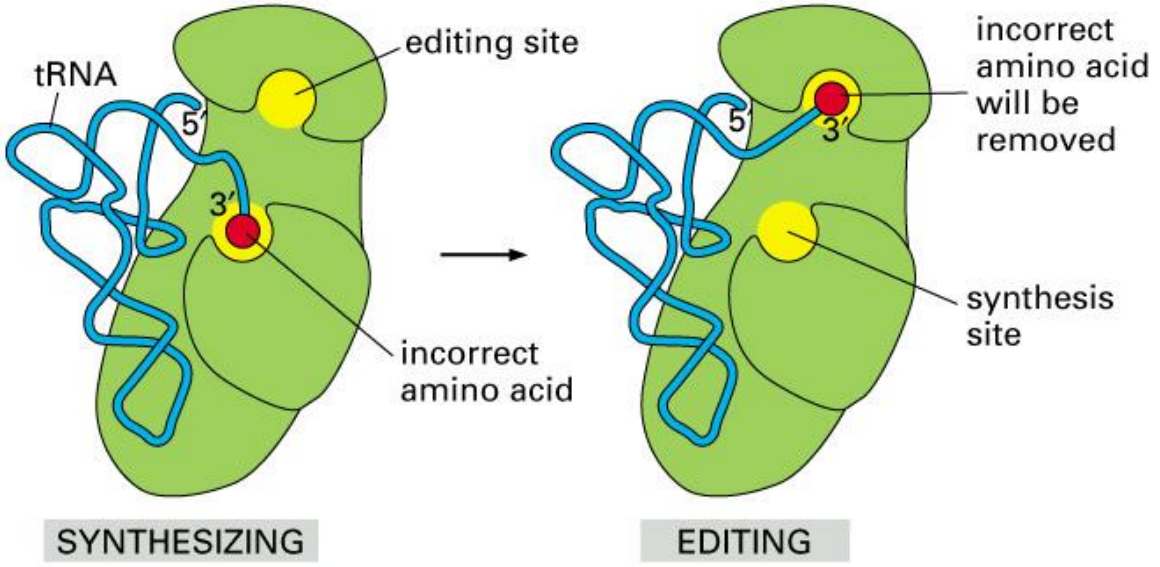
tRNAs are activated by amino-acyl tRNA synthetases



Charged tRNA



High fidelity of amino acyl-tRNA synthetases

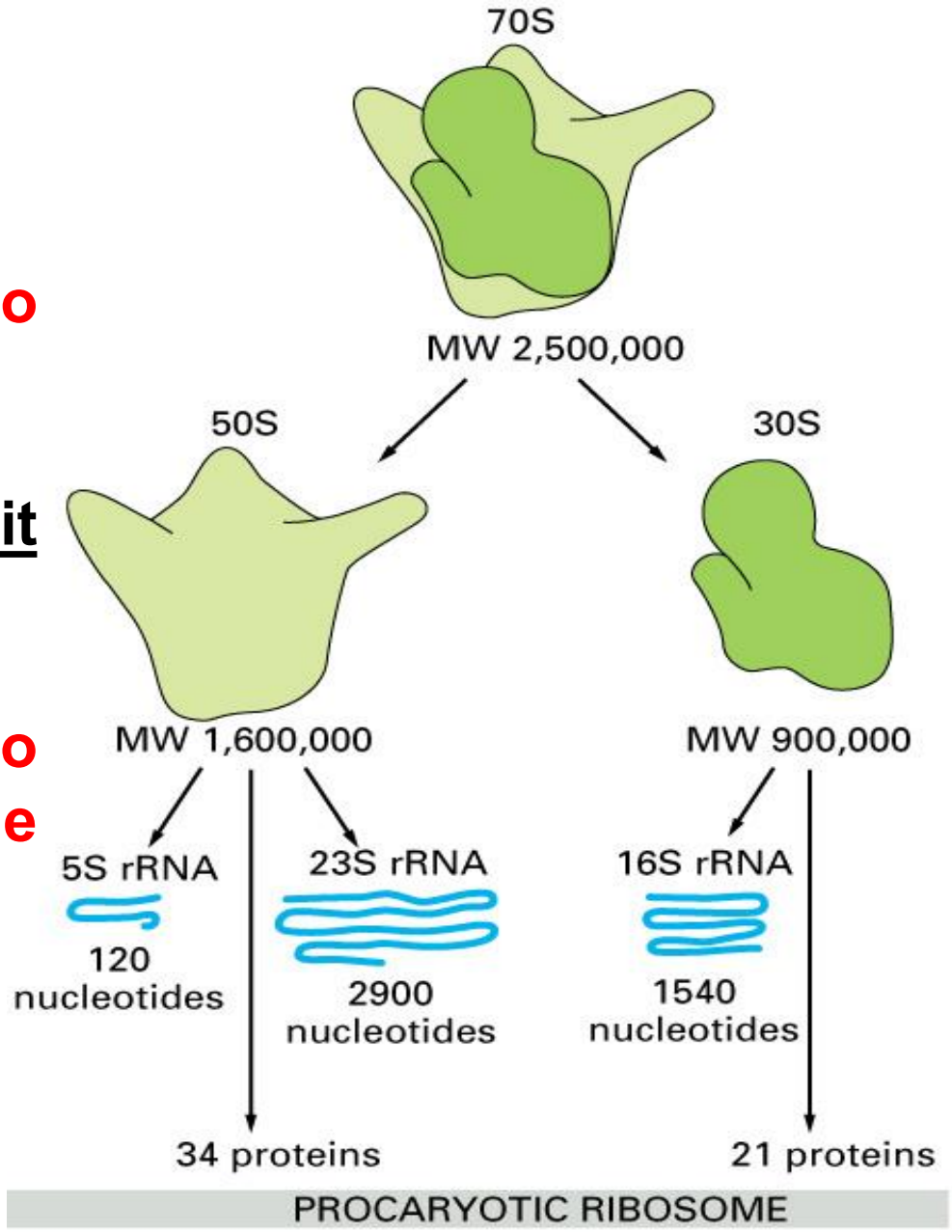
Translation

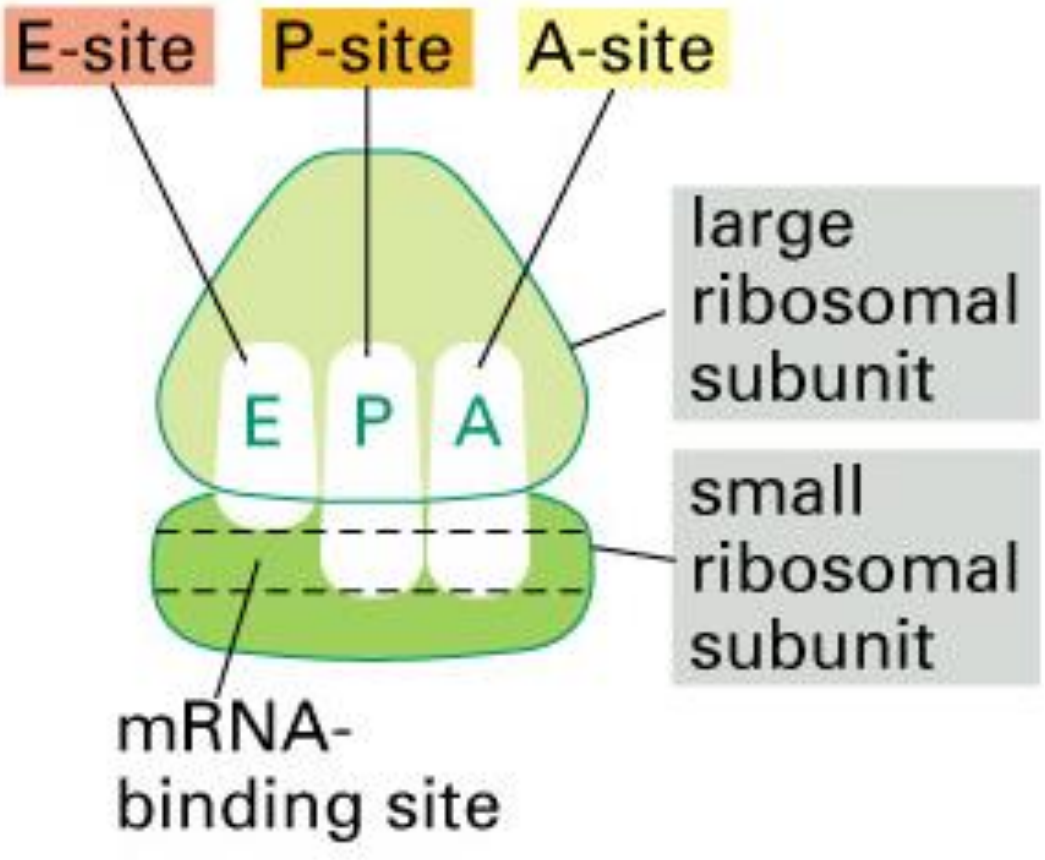
- **Ribosomes are organelles that build proteins.**
  - **rRNA is found in ribosomes.**
  - **mRNA is read on ribosomes.**
  - **Ribosomes are found**
    - **Free-floating in the cytoplasm**
    - **Bound to the endoplasmic reticulum**

Ribosomes

Ribosomes consist of two major components:

- **small ribosomal subunit reads the mRNA**
- **large subunit joins amino acids to form a polypeptide chain**



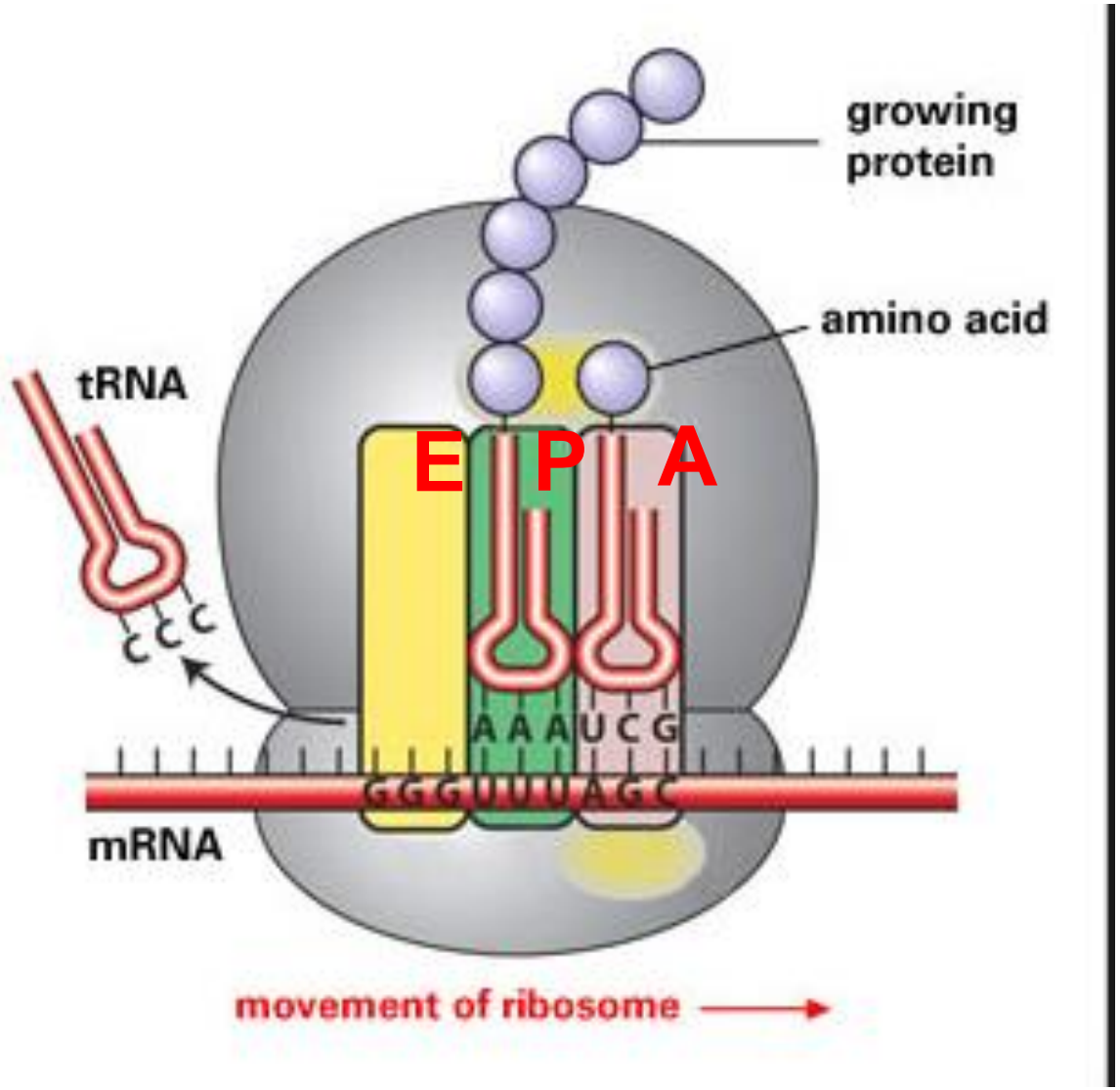


In addition to the E, P, A sites there is an mRNA binding groove that holds onto the message being translated

The *A site* is the arrival site for the aminoacyl tRNA, except for the first aminoacyl tRNA

The *P site* is where the peptidyl tRNA is formed in the ribosome

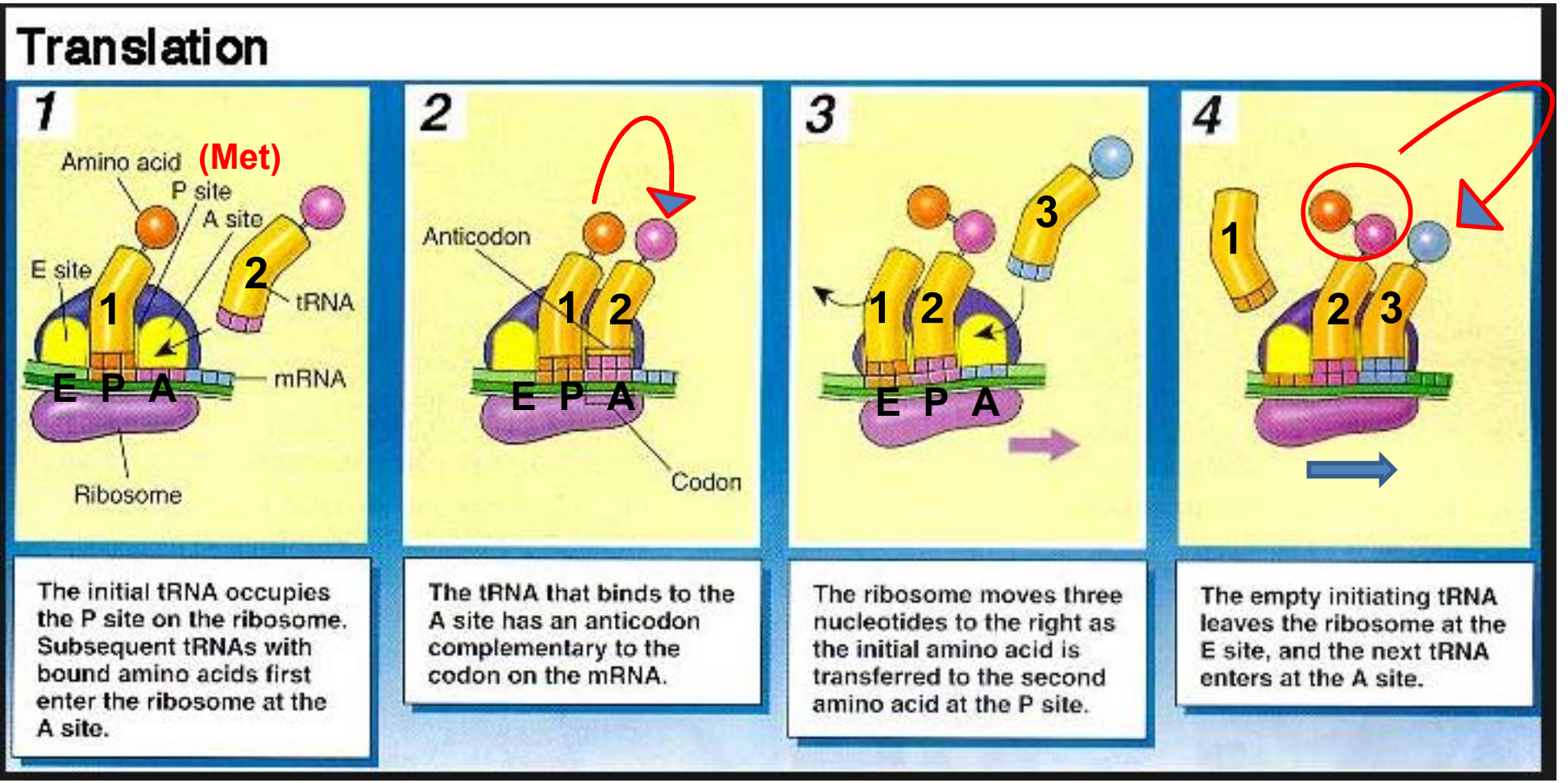
The *E site* is the exit site of the uncharged tRNA after it gives its amino acid to the growing peptide chain.



Each site of E, P and A occupies one codon each at a time, except at the time of initiation.

Translation Initiation

- Translation begins when
  - The small ribosomal subunit binds to the beginning of the mRNA and searches for the AUG start codon.
  - At this point, a tRNA brings the first amino acid.
    - The anticodon in the tRNA matches with a codon on the mRNA.
    - Each tRNA carries a specific amino acid based on its anticodon.
    - The start codon, AUG, binds to a tRNA that carries a methionine.
  - Finally, the large ribosomal subunit joins the complex and the next step, translation elongation, can proceed.



At the time of initiation, P site occupies the start codon, where the first tRNA brings the first amino acid (Met)

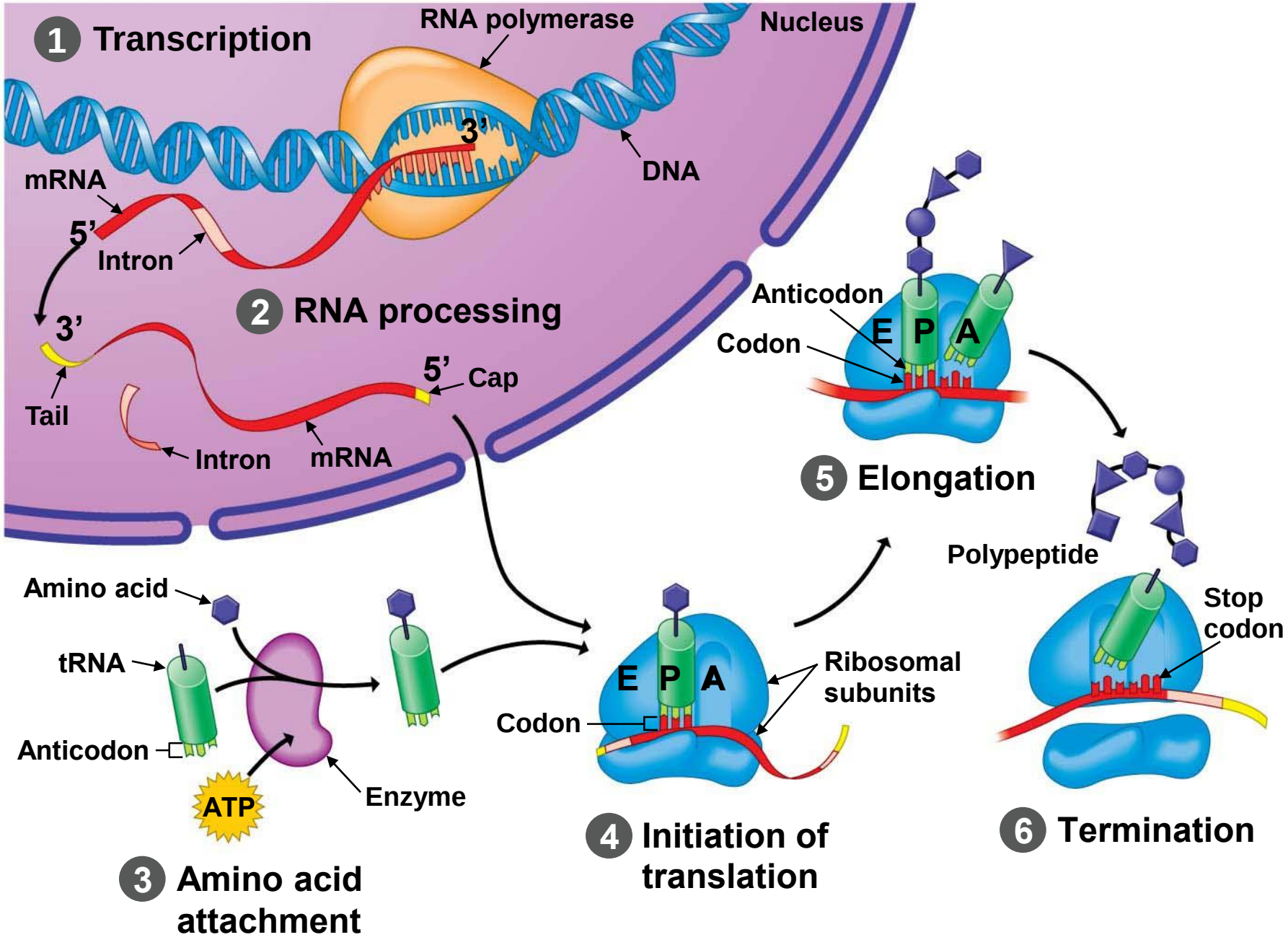
Translation Elongation

- The next tRNA binds with the next codon on the mRNA.
- The ribosome adds this amino acid to the growing polypeptide.
- The ribosome then moves down to the next codon.
- The process repeats itself. For each step, a new amino acid is added to the growing protein.

Translation Termination

- Elongation continues until the ribosome encounters a stop codon.
  - UAA, UAG, UGA are stop codons.
- A release factor binds to the stop codon.
  - This causes the ribosome to release the polypeptide.
  - The ribosomal subunits separate and release the mRNA.
  - The mRNA can be translated again by another ribosome.

Figure 10.19-s6



Control of Protein Synthesis

- **Cell types are different from one another because they express different sets of genes.**
  - **Therefore, have different sets of proteins**
- **Cells control gene expression (the decision to make a protein) in response to different environmental conditions.**
- **Cells can alter gene expression, which controls quantity of a protein**

Control of Protein Quantity

- **Cells can regulate how much of a given protein has to be made by**
  - **Controlling *how much mRNA is available* for translation**
- **Cells do this in a number of ways:**

  1) By regulating how tightly the chromatin is coiled in a certain region

    - **The more tightly the chromatin is coiled, the less likely a gene in that region will be transcribed.**

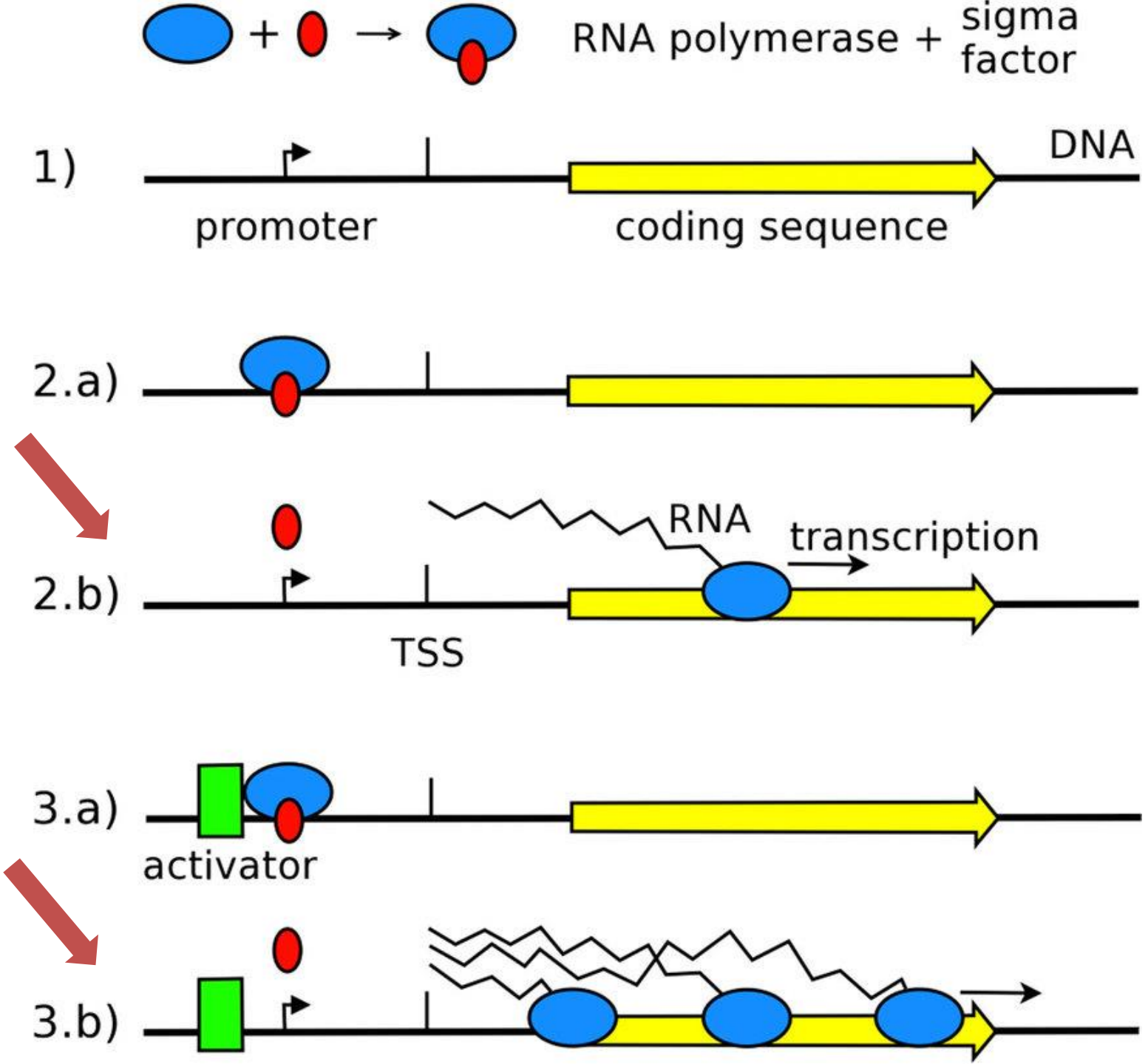
Control of Protein Quantity (Contd…)

2) By increasing or decreasing the rate of transcription of the gene:

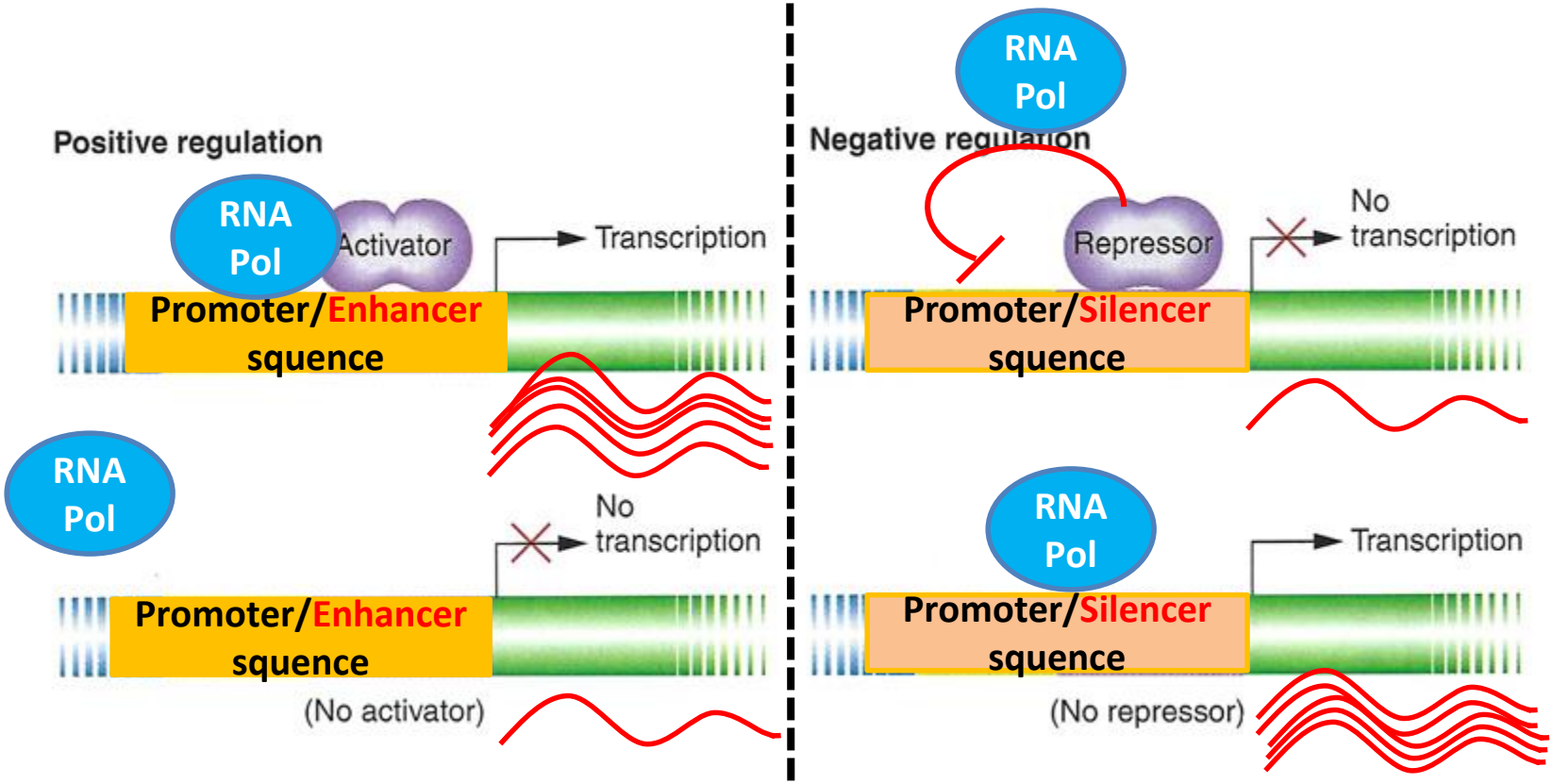
- **Activators bound to *enhancer* regions increases transcription.**
- **Repressors bound to *silencer* regions decreases transcription.**

3) By limiting the *amount of time an mRNA exists* in the cytoplasm:

- **Some mRNA molecules are more stable and will exist longer in the cytoplasm, yielding more protein.**


RNA polymerase + sigma factor
1)
DNA
promoter
coding sequence
2.a)
2.b)
RNA
transcription
TSS
3.a)
activator
3.b)

Activators and repressors of transcription



Without the activator, RNA pol cannot bind to the promoter for some of the genes, genes are turned off or transcribe at a low level

Without the repressor, RNA pol binds to the promoter, genes are turned on. If the Repressor is present, either some of the genes are turned off or transcribe at a low level

Mutations and Protein Synthesis

- A mutation is any change in the DNA sequence of an organism.
- Can be caused by mistakes in DNA replication
- Can be caused by external factors
  - Carcinogens, radiation, drugs, viral infections
- Only mutations in coding regions of gene will change the proteins themselves.

Point Mutations–a Change in a Single Nucleotide of the DNA Sequence

Three types:

A *nonsense mutation* changes a codon to a stop codon.

- This causes the ribosome to stop translation prematurely.
- CAA (Gln) to UAA (stop)

A *missense mutation* causes a change in the type of amino acid added to a polypeptide.

- This may change the way in which a protein functions.
- UUU (Phe) to GUU (Val)

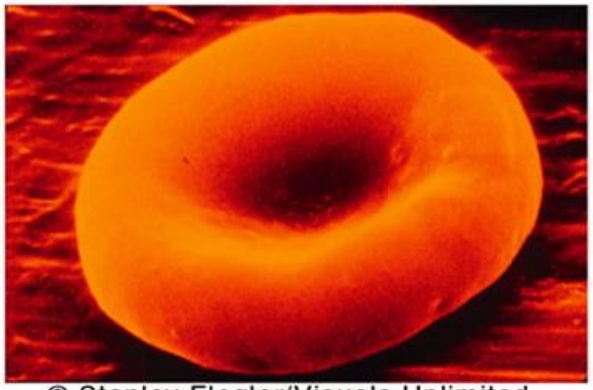
A *silent mutation* does not cause a change in the amino acid sequence.

- UUU to UUC; both code for Phe

Sickle Cell Anemia

- Results from a missense mutation in the gene for hemoglobin
  - GAA to GUA
  - Glutamic acid to valine change
  - Causes the hemoglobin protein to change shape
  - The red blood cells stick together in low oxygen conditions
    - Get stuck in blood vessels, causing the vessels to break-apart easily, leading to anemia
    - Also causes blood vessels to clog, preventing oxygen delivery to tissues, which results in tissue damage
    - Causes weakness, brain damage, painful joints, etc.

Normal and Sickled Red Blood Cells



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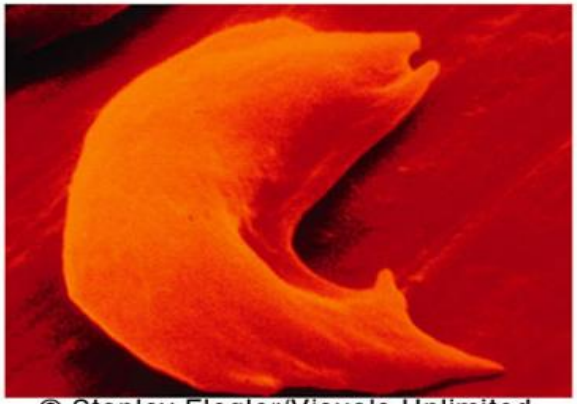

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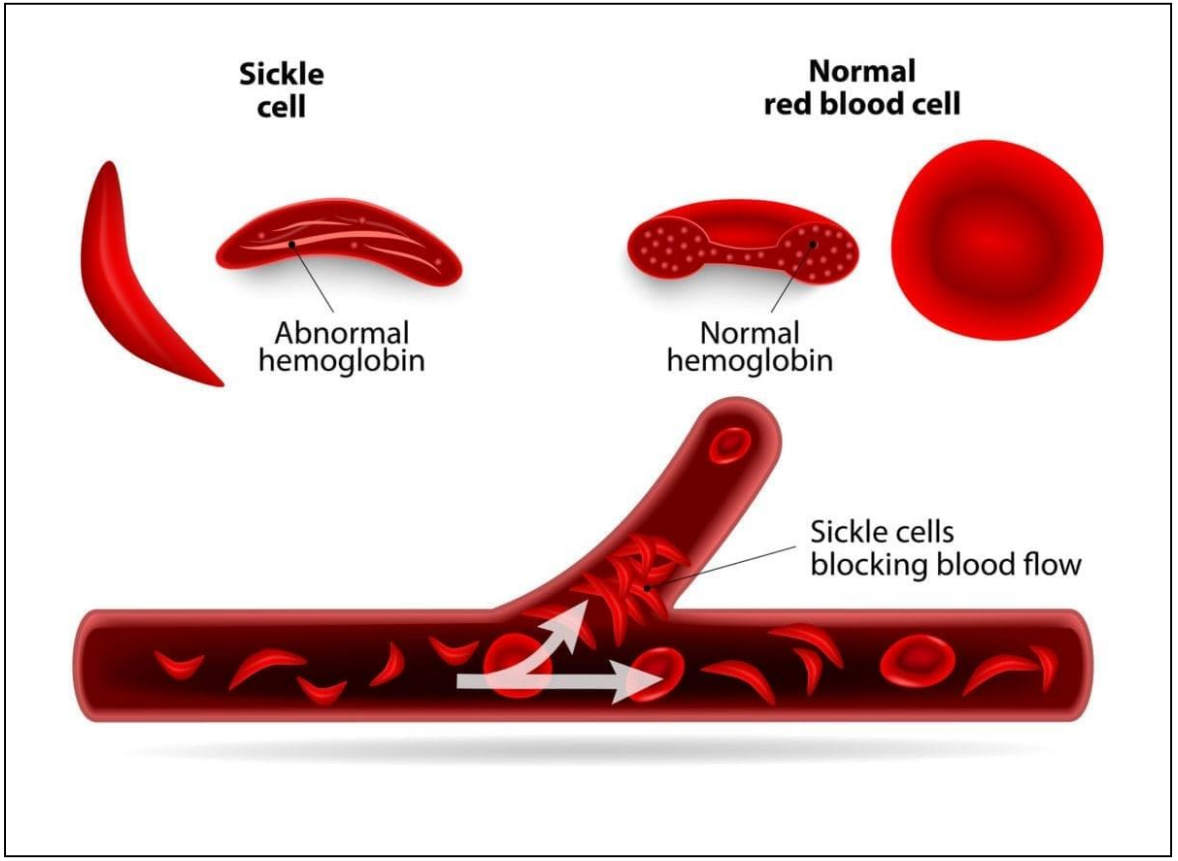
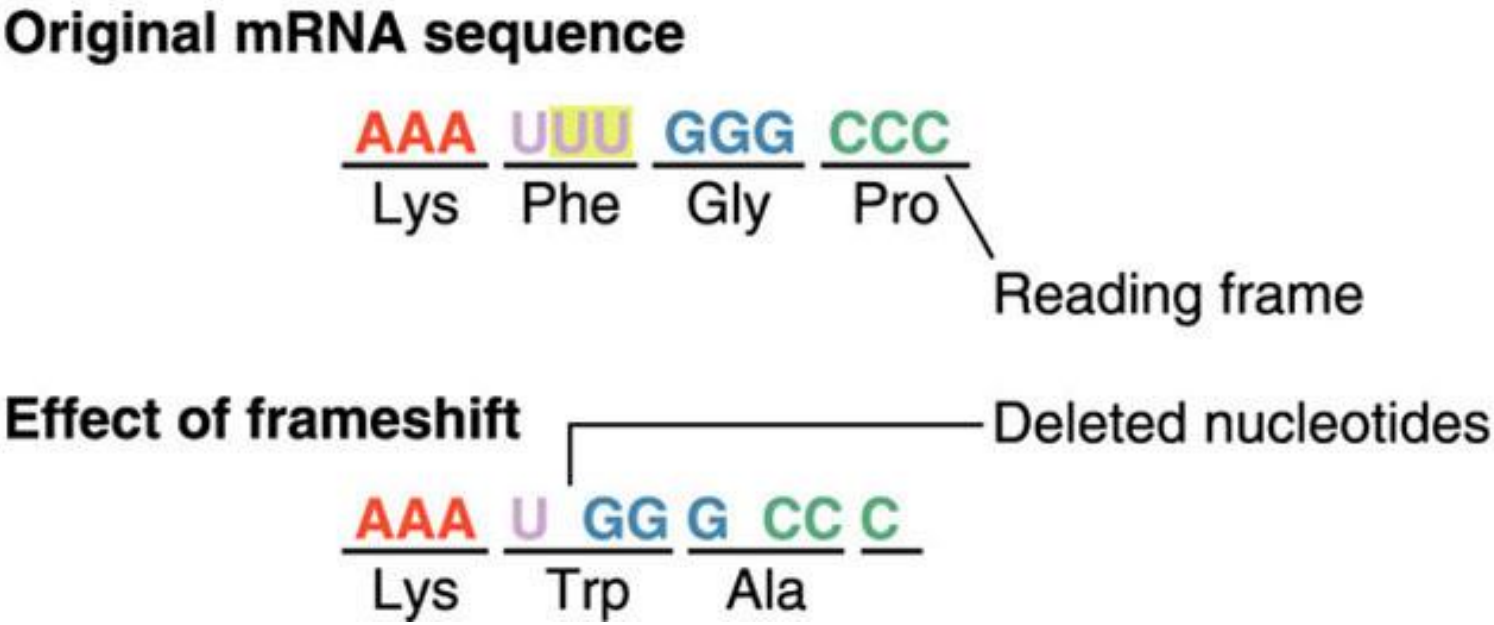


Insertions and Deletions

- An *insertion mutation* occurs when one or more nucleotides is added to the normal DNA sequence.
- A *deletion mutation* occurs when one or more nucleotides is removed from the normal DNA sequence.
- Insertions and deletions cause a frameshift.
  - Ribosomes will read the wrong set of three nucleotides.
  - Changes the amino acid sequence dramatically
  - Changes the function of the protein dramatically

Frameshift



Mutations Caused by Viruses

- **Viruses can insert their genetic material into the DNA of the host cell.**
- **The presence of the viral material may interfere with the host cell's ability to use the genetic material in that area because of this insertion.**
- **Insertion of human papillomavirus (HPV) causes an increased risk of cancer.**

Chromosomal Aberrations

- **Involves a major change in DNA at the level of the chromosome**
  - ***Deletion***
  - ***Duplication***
  - ***Inversion***
  - ***Translocation***

  All of these affect many genes, thus many proteins.
- **In humans, these mutations may cause problems with fetal development.**

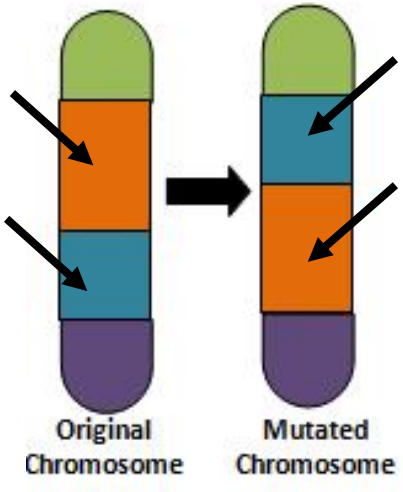
Chromosomal Aberrations

***Deletion*: when a broken piece of chromosome is lost or destroyed**

Deletion

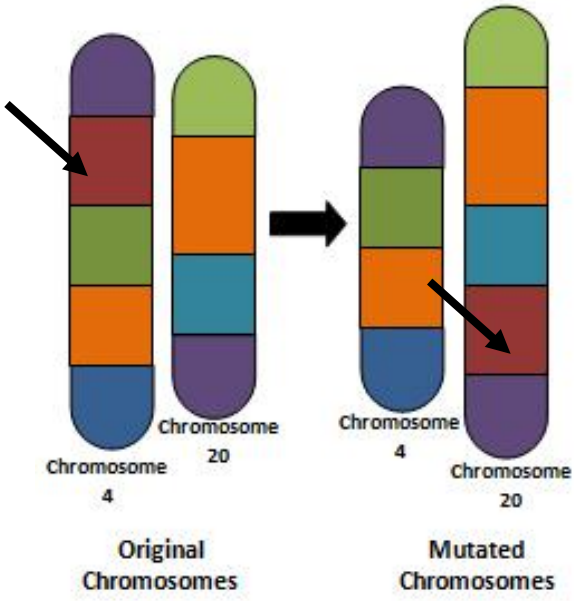
***Duplication*: a chromosomal segment is replicated and reattached in sequence**

Duplication



***Inversions*: a broken piece is reattached in the wrong orientation**

Inversion



***Translocation:* a broken segment is integrated into a different chromosome**

Translocation

5’ AAGGCAAUCCCAUAAGG 3’

Given above an mRNA sequence, write the sequence of the Template strand of DNA from 5’ to 3’

5’ CCTTATGGGATTGCCTT 3’

5’ AAGGCAAUCCCAUAAGG 3’
3’ TTCCG TTAGGGTATT CC 5’

Write the coding strand sequence from the 5’ to 3’

5’ AAGGCAATCCCATAAGG 3’

5’ AAGGCAAUCCCAUAAGG 3’

Write the codons, corresponding anti codons and the amino acids of this mRNA starting from the first base from the 5’ to 3’direction

Codons:	Anti-codons:	Amino acids
5’ AAG 3’	5’ CUU 3’	Lys
5’GCA 3’	5’ UGC 3’	Ala
5’ AUC 3’	5’ GAU 3’	Ile
5’ CCA 3’	5’ UGG 3’	Pro
5’ UAA 3’		

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

5’ AAGGCAAUCCCAUAAGG 3’

In the pre-mRNA, if the nucleotides drawn in the box belong to an intron, write the template strand and the matured mRNA sequence

Template strand:

5’ CCTTATGGGATTGCCTT 3’

Matured mRNA:

5’ AACAUAAGG 3’

Write the codons, anti codons and the amino acids coded by the mRNA:

Codons:
5’ AAC 3’
5’ AUA 3’
5’ AGG 3’

Anti Codons:
5’ GUU 3’
5’ UAU 3’
5’ CCU 3’

Amino acids:
Asn
Ile
Arg

	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Write down the polypeptide sequence of each

5’ AAGGCAAUCCCAUAAGG 3’

5’ AAGGCUAUCCCAUAAGG 3’

Silent Mutation, no change in polypeptide sequence

5’ AAGGCAAUCCCAUAAGG 3’

5’ AAGG UCCCAUAAGG 3’

Lys-Val-Pro

5’ AAGGCAAUCCCAUAAGG 3’

5’ AAGGCCUAAUCCCAUAAGG 3’

Lys-Ala

Original Amino acids:
Lys-Ala-Ile-Pro

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

EXTRA SLIDES

Shine-Dalgarno sequence in prokaryotic translation

-Ribosomal binding site in prokaryotic mRNA

-16S rRNA present in the small subunit of ribosome binds to the mRNA, approximately 8 base pairs upstream to the start codon

-This sequence helps recruiting the ribosome to the mRNA to initiate protein synthesis