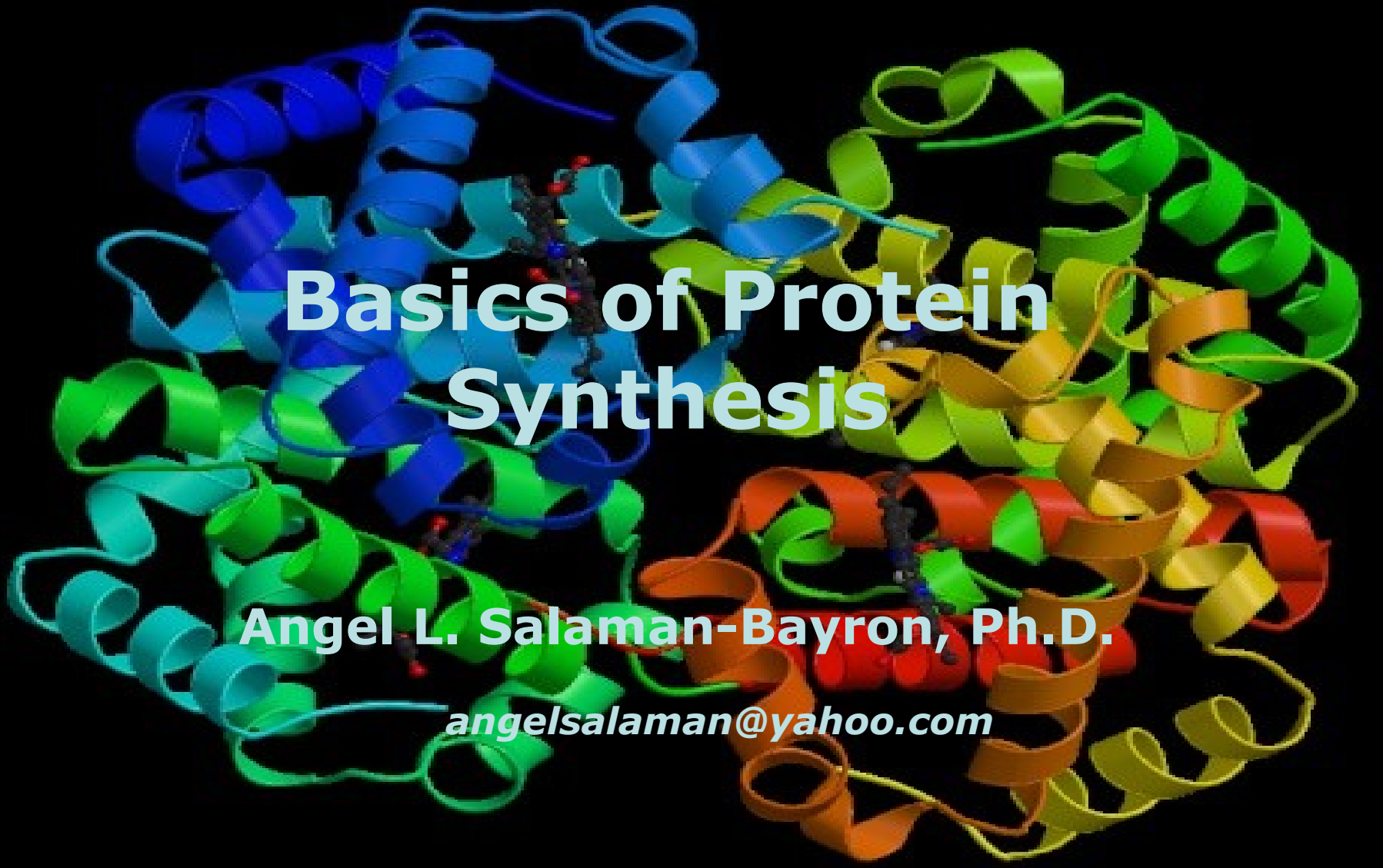


Basics of Protein Synthesis

Angel L. Salaman-Bayron, Ph.D.

angelsalaman@yahoo.com

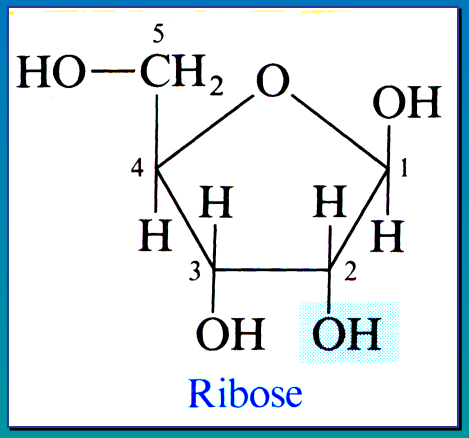
From: Protein Data Bank PDB ID: 1A3N
Tame, J., Vallone, B.: Deoxy Human Hemoglobin. 1998

Presentation Outline

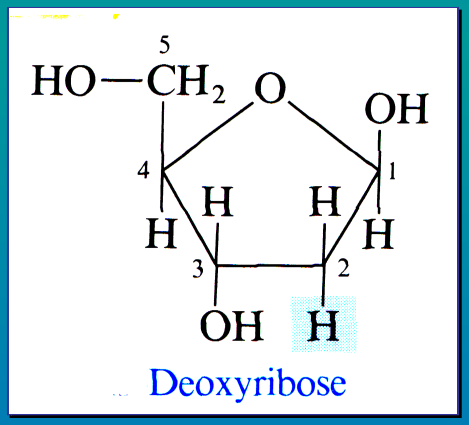
- Nucleic Acids
- DNA is the Genetic Material
- RNA
- RNA Synthesis (Transcription)
- Protein Synthesis (Translation)
- Control of Protein Synthesis
- Protein Degradation

Nucleic Acids

- Nucleic acids made up of chains of nucleotides
- Nucleotides consist of:
  - A base
  - A sugar (ribose)
  - A phosphate
- Two types of nucleic acids in cells:
  - Deoxyribonucleic acid (DNA)
  - Ribonucleic acid (RNA)



Ribose

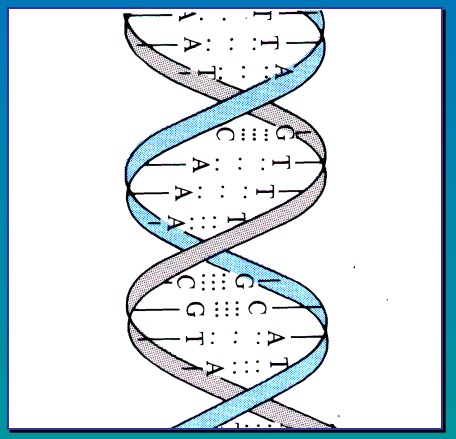


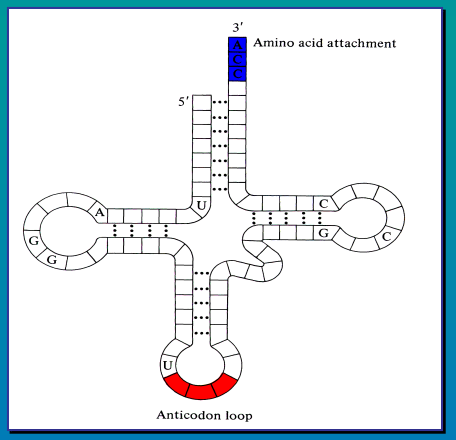
Deoxyribose

Adapted from: Bettelheim FA and March J (1990) Introduction to Organic and Biochemistry (International Edition). Philadelphia: Saunders College Publishing p383.

Nucleic Acids

- Nucleic acids have primary and secondary structures
  - DNA
    - Double-stranded helix
    - H-bonds between strands
  - RNA
    - 3 kinds (mRNA, tRNA, rRNA)
    - All single strands
    - H-bonds within strands





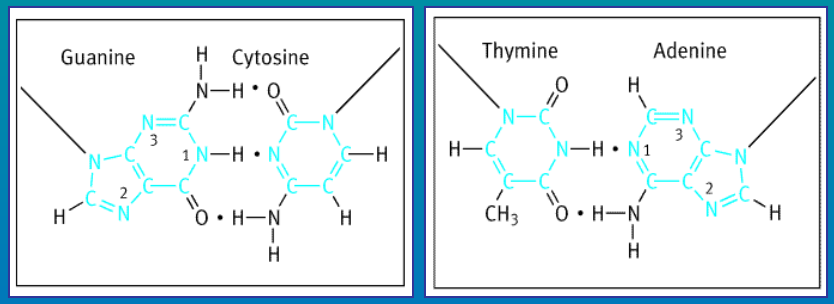
From: Bettelheim FA and March J (1990) Introduction to Organic and Biochemistry (International Edition). Philadelphia: Saunders College Publishing p391 (Left panel) and 393 (Right panel).

DNA base-paring

- **The different bases in the nucleotides which make up DNA and RNA are:**
  - **Adenine**
  - **Guanine**
  - **Cytosine**
  - **Thymine (DNA only)**
  - **Uracil (RNA only)**
- **Chemical structure only allows bases to bind with specific other bases due to chemical structure**

DNA	RNA
Adenine	**Uracil****
Thymine*	Adenine
Guanine	Cytosine
Cytosine	Guanine

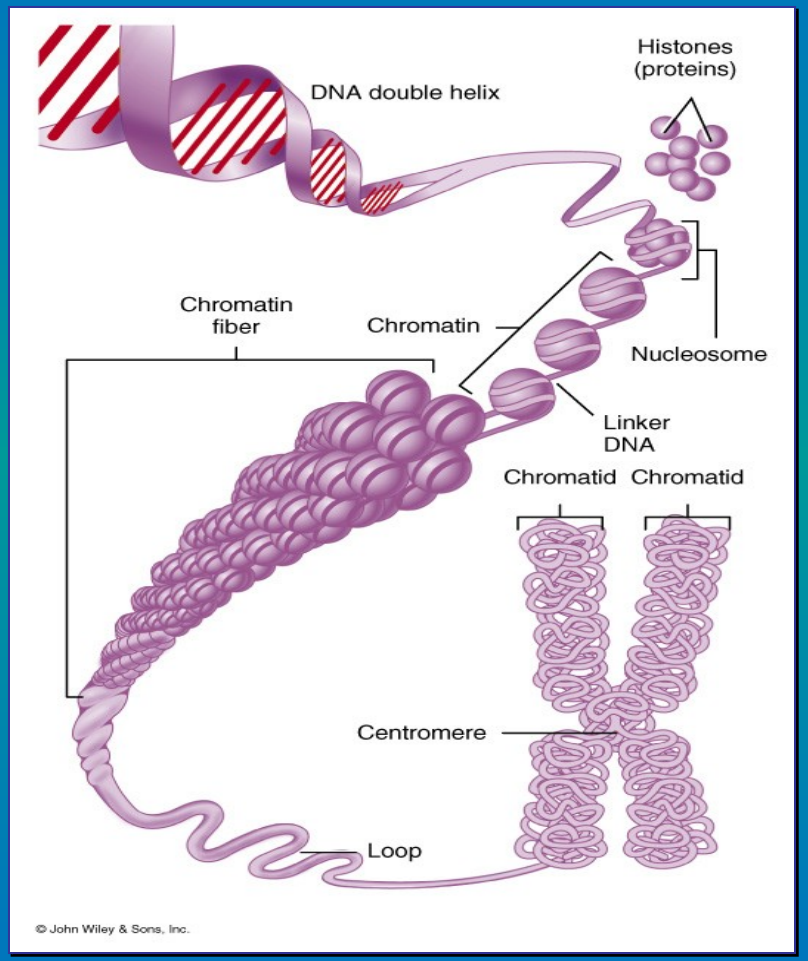
Table showing complementarity of base pairs
* Present only in DNA
**Present only in RNA



From: Elliott WH & Elliott DC. (1997) Biochemistry and Molecular Biology. New York: Oxford University Press. P245.

DNA is the Genetic Material

- **DNA**
  - **Located in 23 pairs of chromosomes in nucleus of cell**
  - **DNA has two functions:**
    - **Replication - reproduces itself when cell divides**
    - **Information transmission**
      - **via protein synthesis**



From: Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P86.

DNA is the Genetic Material

- **DNA contains genetic information**
  - Gene - segment of DNA on a chromosome that codes for a particular protein
  - Coding contained in sequence of bases (on mRNA) which code for a particular amino acid (i.e. genetic code)
  - Genetic code universal in all organisms
    - Mitochondrial DNA slightly different

Table 22.1 The genetic code

5′ base	Middle base				3′ base
	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop*	UGA Stop*	A
	UUG Leu	UCG Ser	UAG Stop*	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA LEU	CCA Pro	CAA Gin	CGA Aeg	A
	CUG Leu	CCG Pro	CAG Gin	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met†	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

*Stop codons have no amino acids assigned to them.
†The AUG codon is the initiation codon as well as that for other methionine residues.

From: Elliott WH & Elliott DC. (1997) Biochemistry and Molecular Biology. New York: Oxford University Press. P294.

RNA has many functions

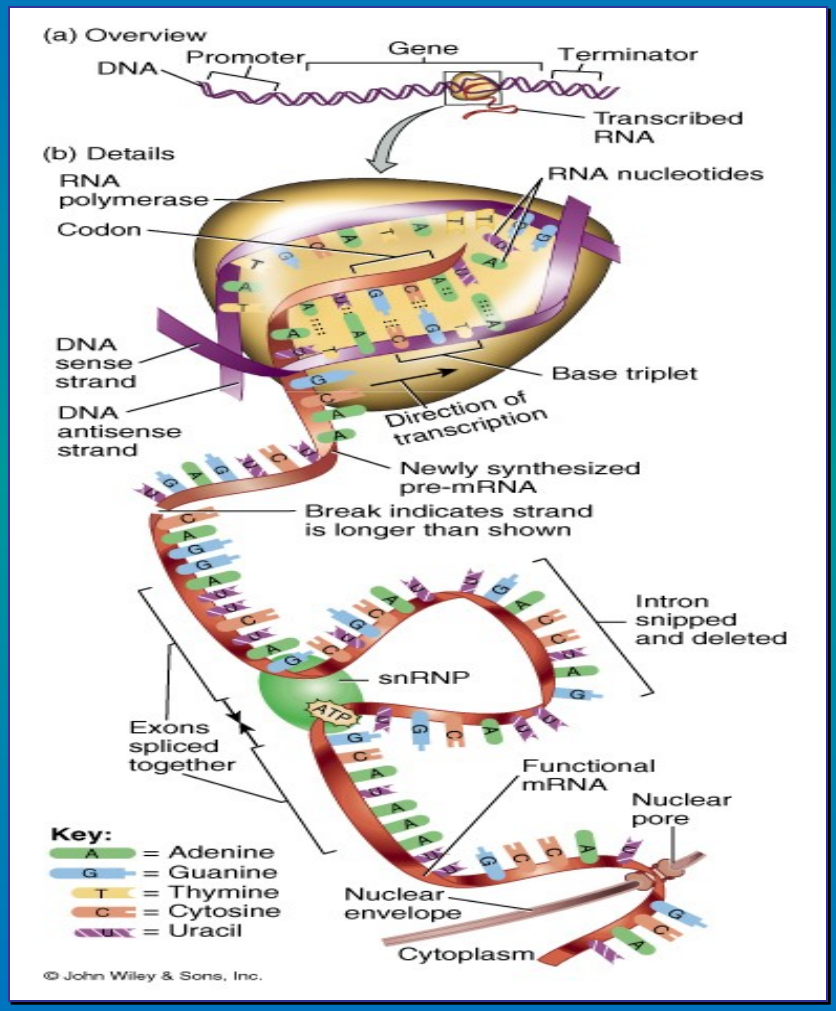
- It have been identified Four principles types of RNA:
  - Messenger RNA (mRNA) - carries genetic information from DNA in nucleus to cytoplasm where proteins synthesized
  - Transfer RNA (tRNA) - carries amino acids from amino acid pool to mRNA
  - Ribosomal RNA (rRNA) - joins with ribosomal proteins in ribosome where amino acids joined to form protein primary structure.
  - Small nuclear RNA (snRNA) - associated with proteins in nucleus to form small nuclear ribonucleoprotein particles (snRNPs) which delete introns from pre-mRNA

Information Flow

- Information stored in DNA is transferred to RNA and then expressed in the structure of proteins
  - Two steps in process:
    - **Transcription** - information transcribed from DNA into mRNA
    - **Translation** - information in mRNA translated into primary sequence of a protein

Transcription

- Information transcribed from DNA into RNA
  - mRNA carries information for protein structure, but other RNA molecules formed in same way
    - RNA polymerase binds to promoter nucleotide sequence at point near gene to be expressed
    - DNA helix unwinds
    - RNA nucleotides assemble along one DNA strand (sense strand) in complementary sequence to order of bases on DNA beginning at start codon (AUG - methionine)
    - Transcription of DNA sense strand ends at terminator nucleotide sequence
    - mRNA moves to ribosome
    - DNA helix rewinds



From: Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P88.

Transcriptional control

- Each cell nucleus contains all genes for that organism but genes only expressed as needed
- Transcription regulated by transcription factors
  - Proteins produced by their own genes
    - If transcription factors promote transcription - activators
    - If transcription factors inhibit transcription - repressors
- General transcription factors interact with RNA polymerase to activate transcription of mRNA
  - Numerous transcription factors required to initiate transcription
  - General transcription factors set base rate of transcription
  - Specific transcription factors interact with general transcription factors to modulate rate of transcription
- Some hormones also cause effects by modulating rate of gene transcription

Protein synthesis occurs in ribosomes

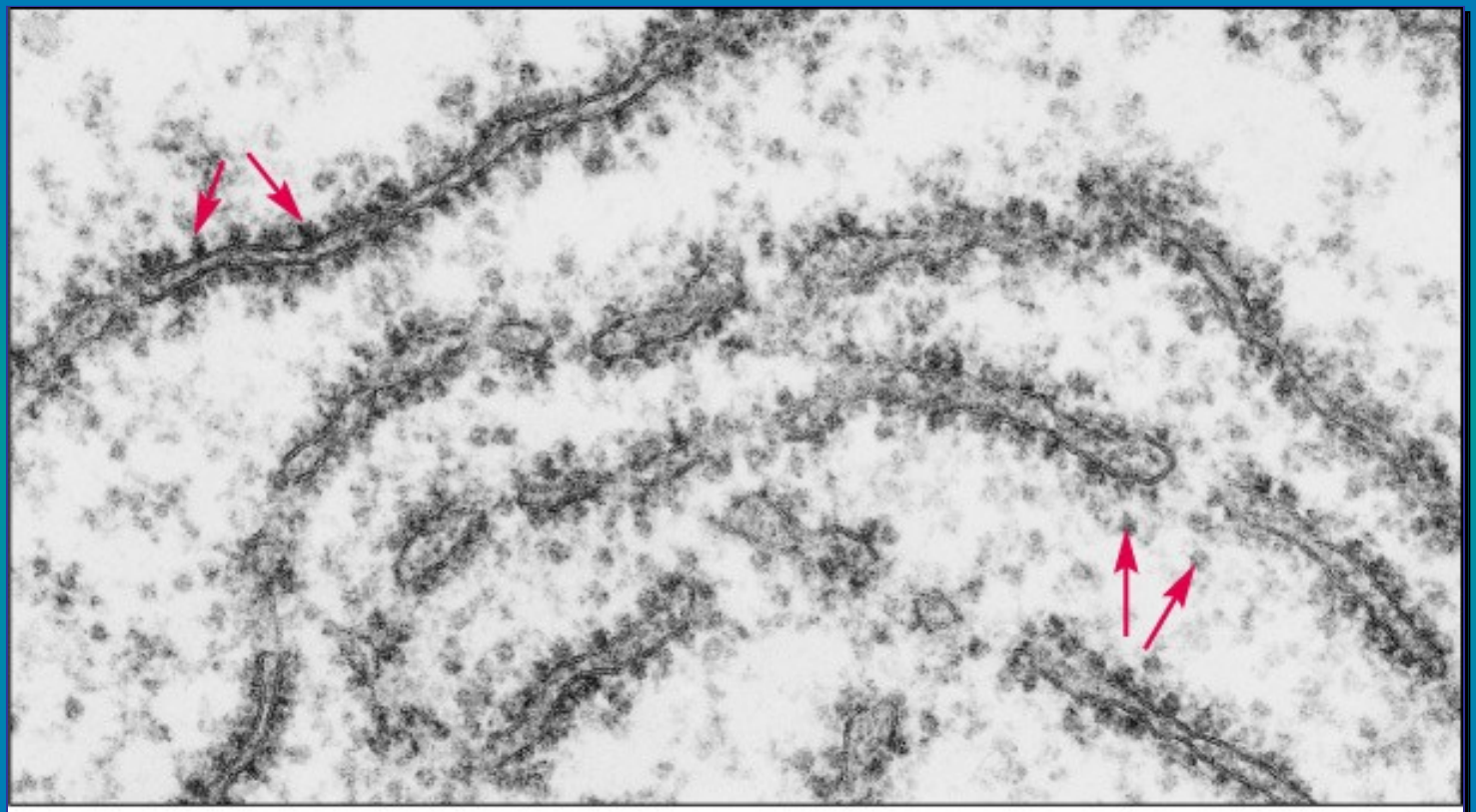
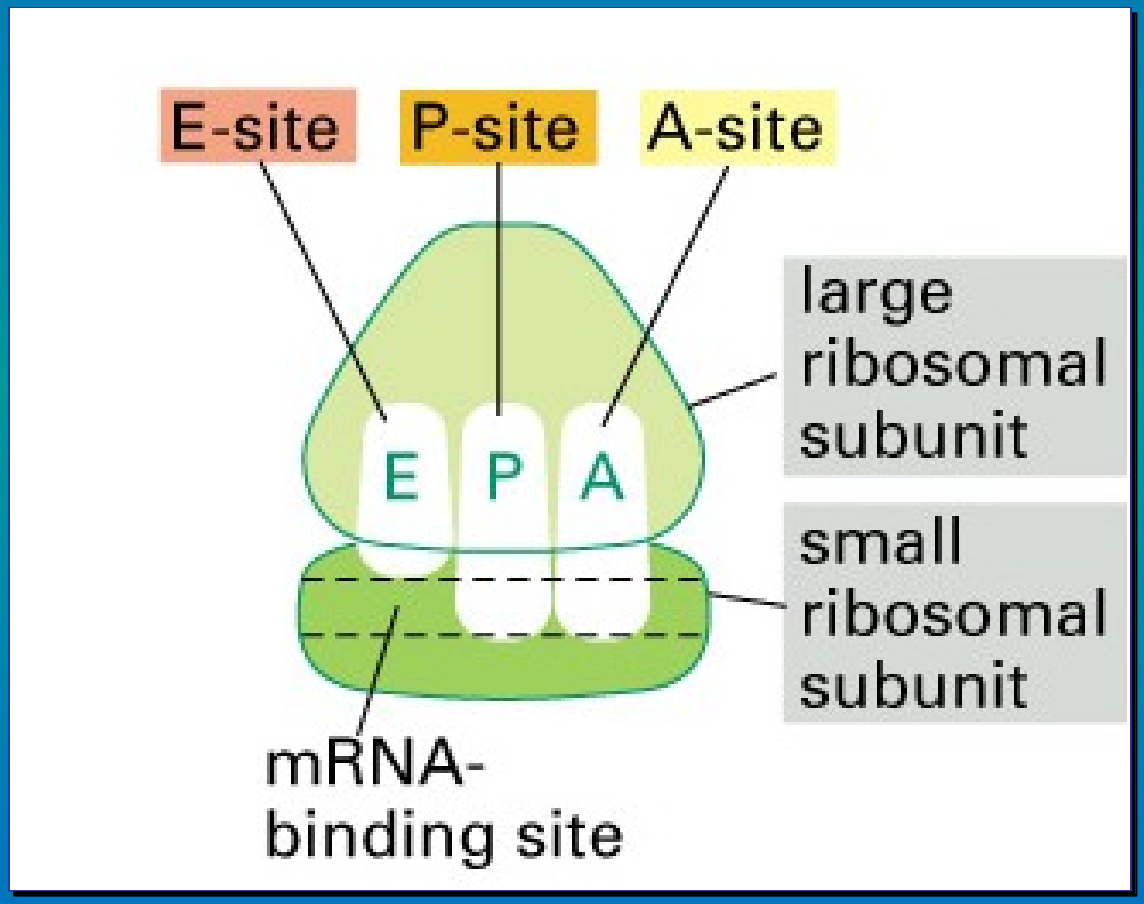


Figure 6–62. Molecular Biology of the Cell, 4th Edition.

Protein synthesis occurs in ribosomes

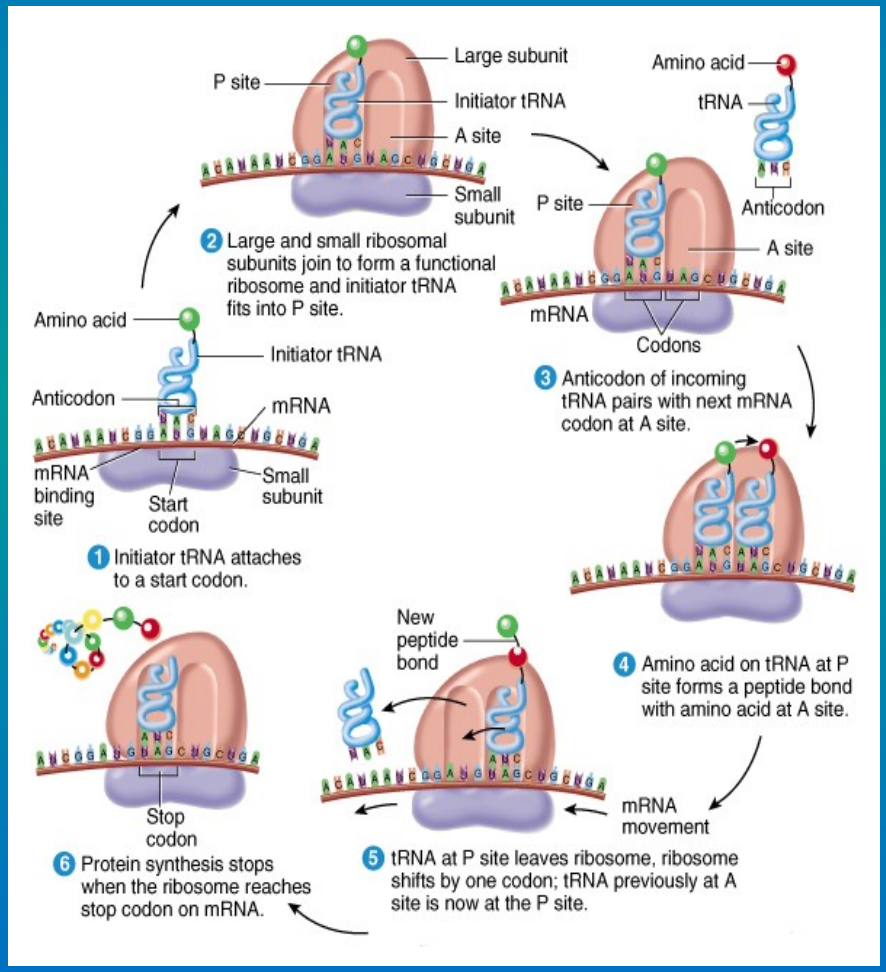


Translation (protein synthesis)

- Information in mRNA translated into primary sequence of a protein in 4 steps:
  - ACTIVATION
  - INITIATION
  - ELONGATION
  - TERMINATION

Translation (protein synthesis)

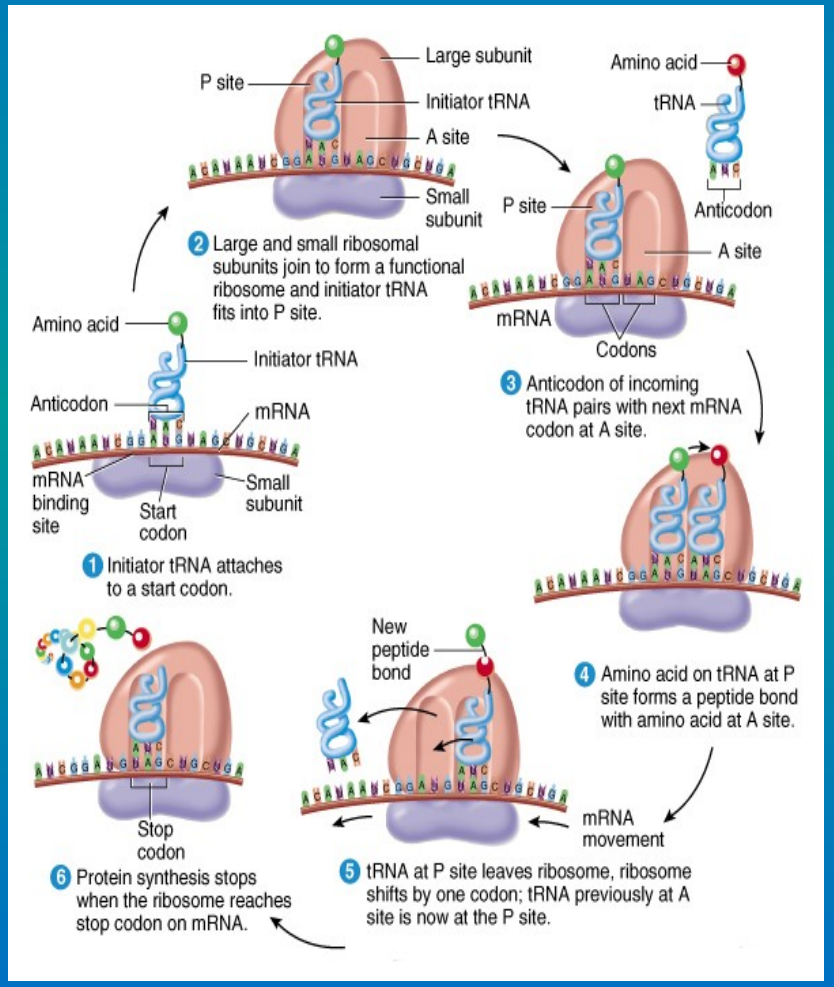
- ELONGATION
  - Anticodon of next tRNA binds to mRNA codon at A site of ribosome
    - Each tRNA specific for one amino acid only, but some amino acids coded for by up to 6 codons
      - Order of bases in mRNA codons determine which tRNA anticodons will align and therefore determines order of amino acids in protein
  - Amino acid at A site linked to previous amino acid
  - Ribosome moves along one codon and next tRNA binds at A site



From: Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P88.

Translation (protein synthesis)

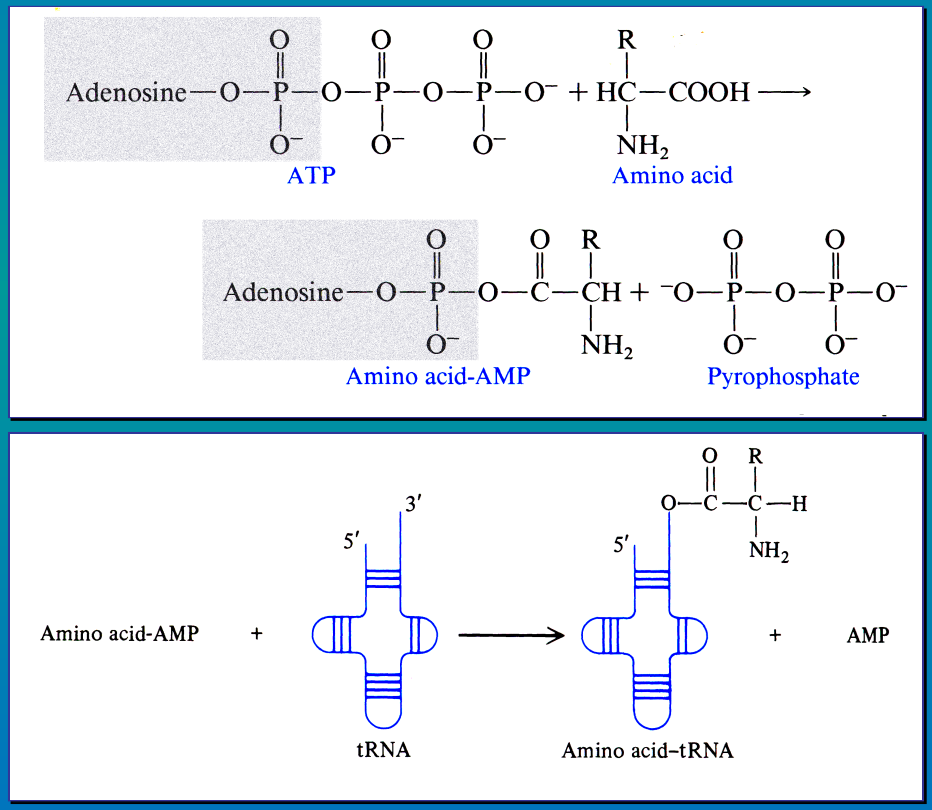
- TERMINATION
  - Final codon on mRNA contains termination signal
  - Releasing factors cleave polypeptide chain from tRNA that carried final amino acid
  - mRNA released from ribosome and broken down into nucleotides



From: Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P88.

Translation Activation

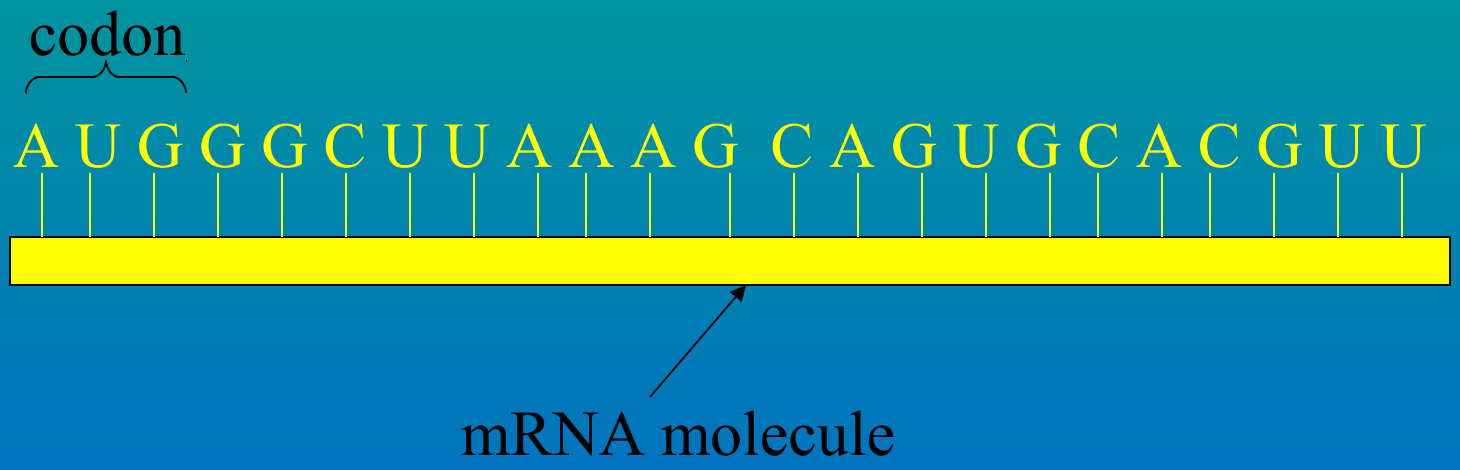
- **ACTIVATION**
  - Each amino acid activated by reacting with ATP
  - tRNA synthetase enzyme attaches activated amino acid to own particular tRNA



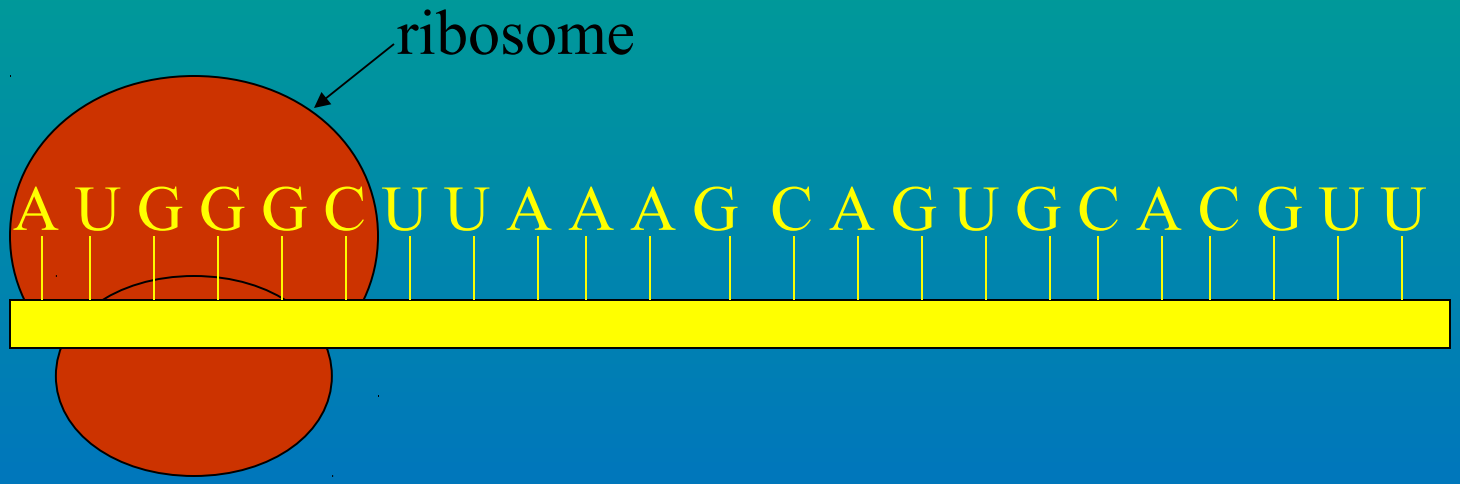
Adapted from: Bettelheim FA and March J (1990) Introduction to Organic and Biochemistry (International Edition). Philadelphia: Saunders College Publishing p398

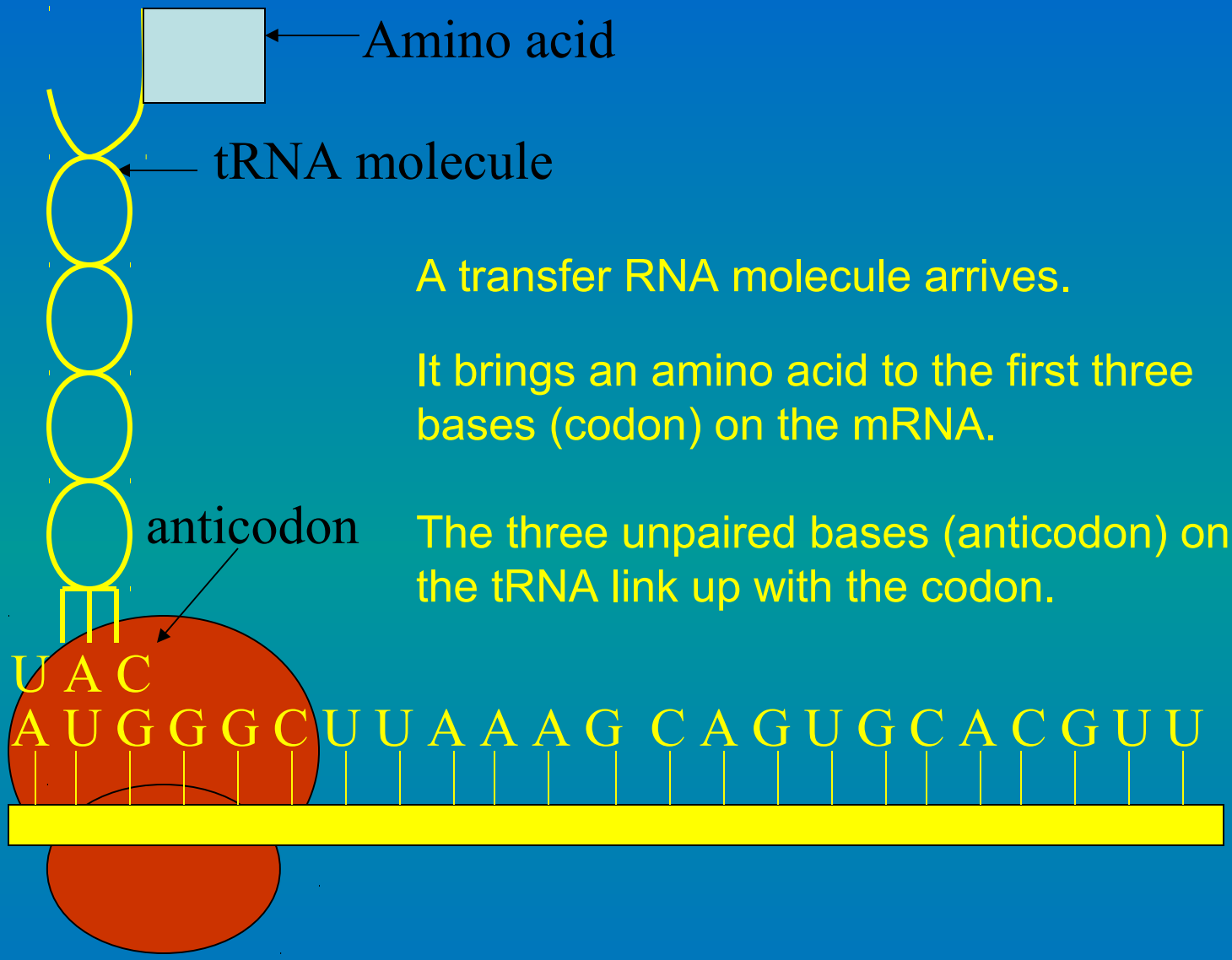
This is a molecule of messenger RNA.

It was made in the nucleus by transcription from a DNA molecule.



A ribosome on the rough endoplasmic reticulum attaches to the mRNA molecule.

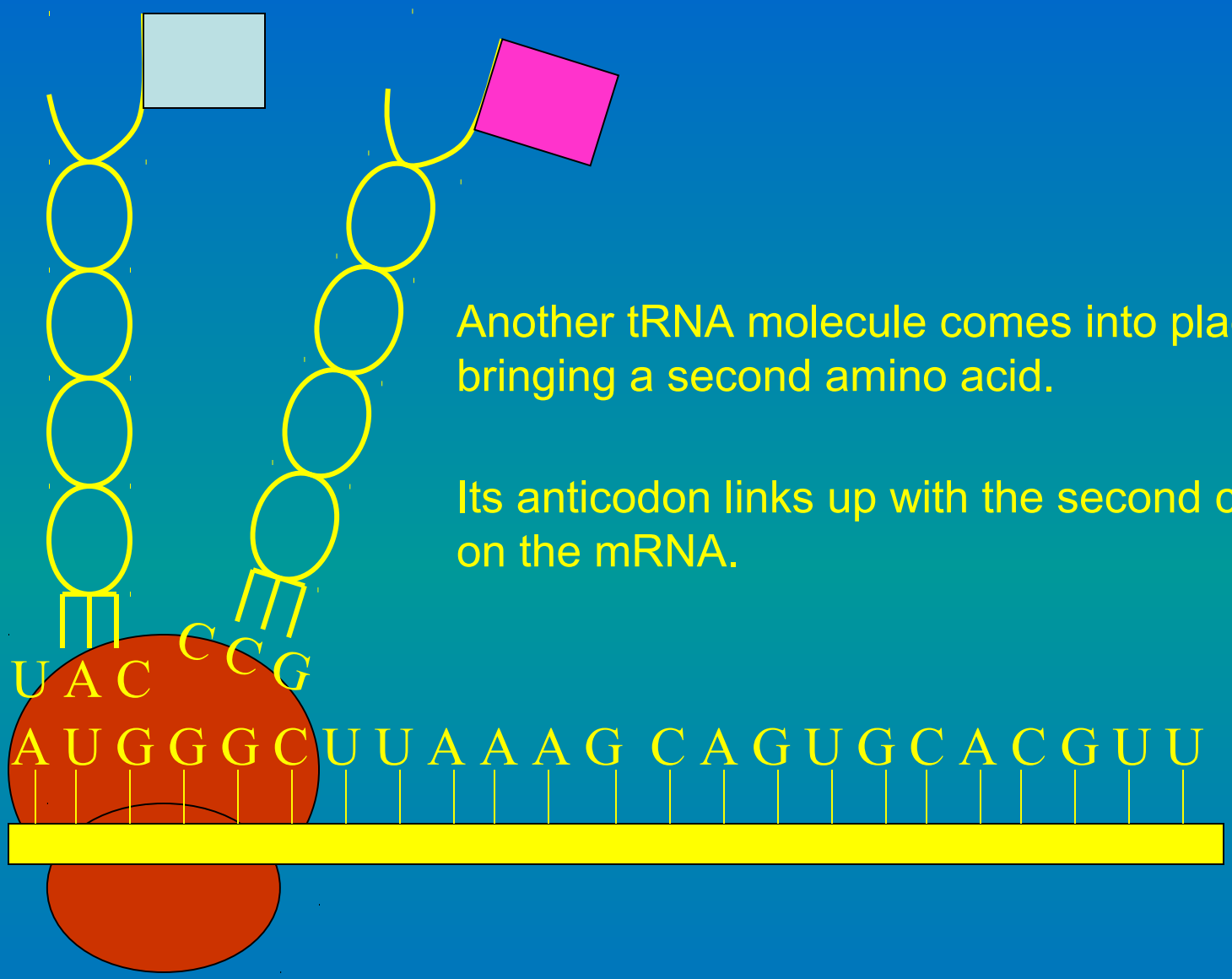




A transfer RNA molecule arrives.

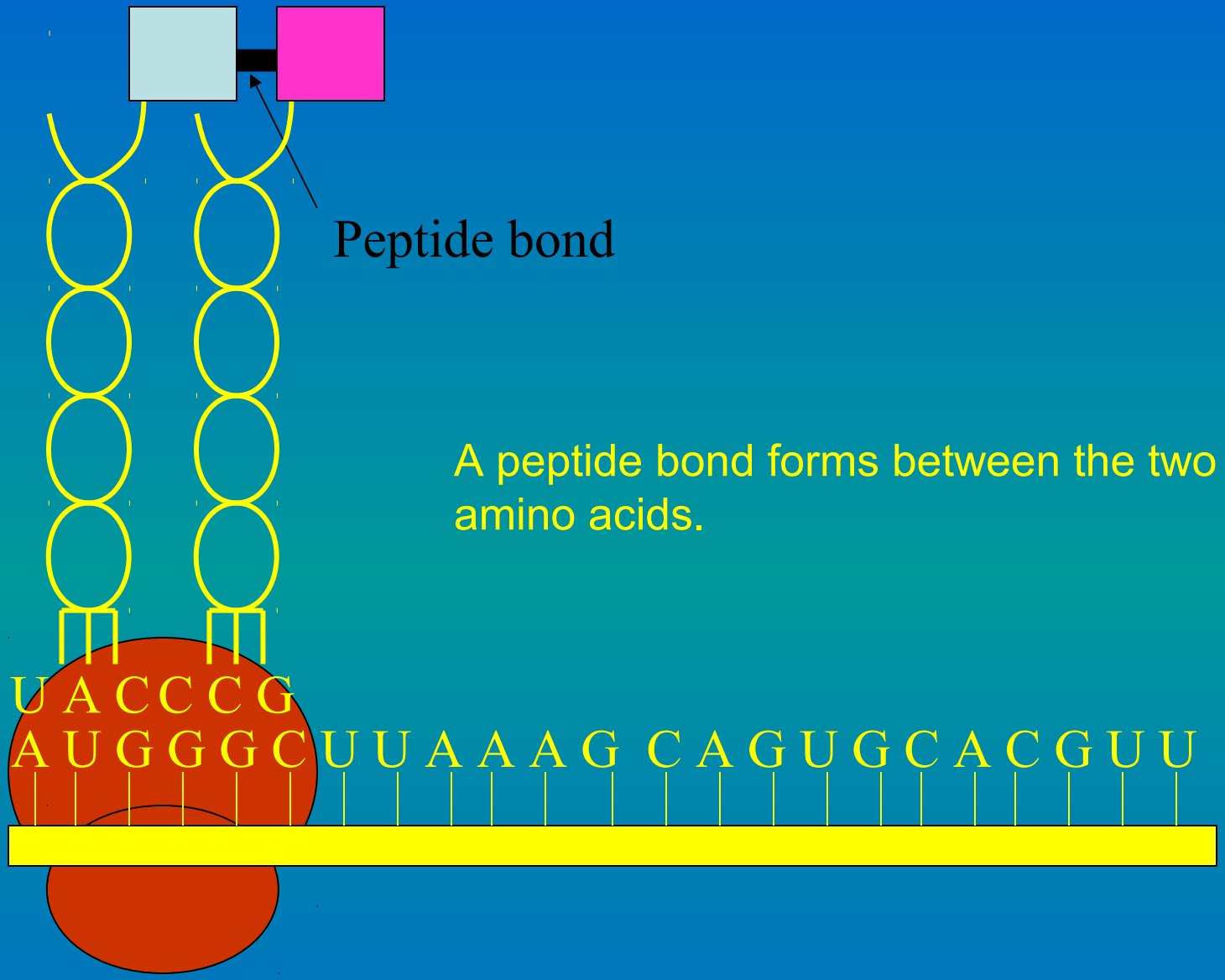
It brings an amino acid to the first three bases (codon) on the mRNA.

The three unpaired bases (anticodon) on the tRNA link up with the codon.



Another tRNA molecule comes into place, bringing a second amino acid.

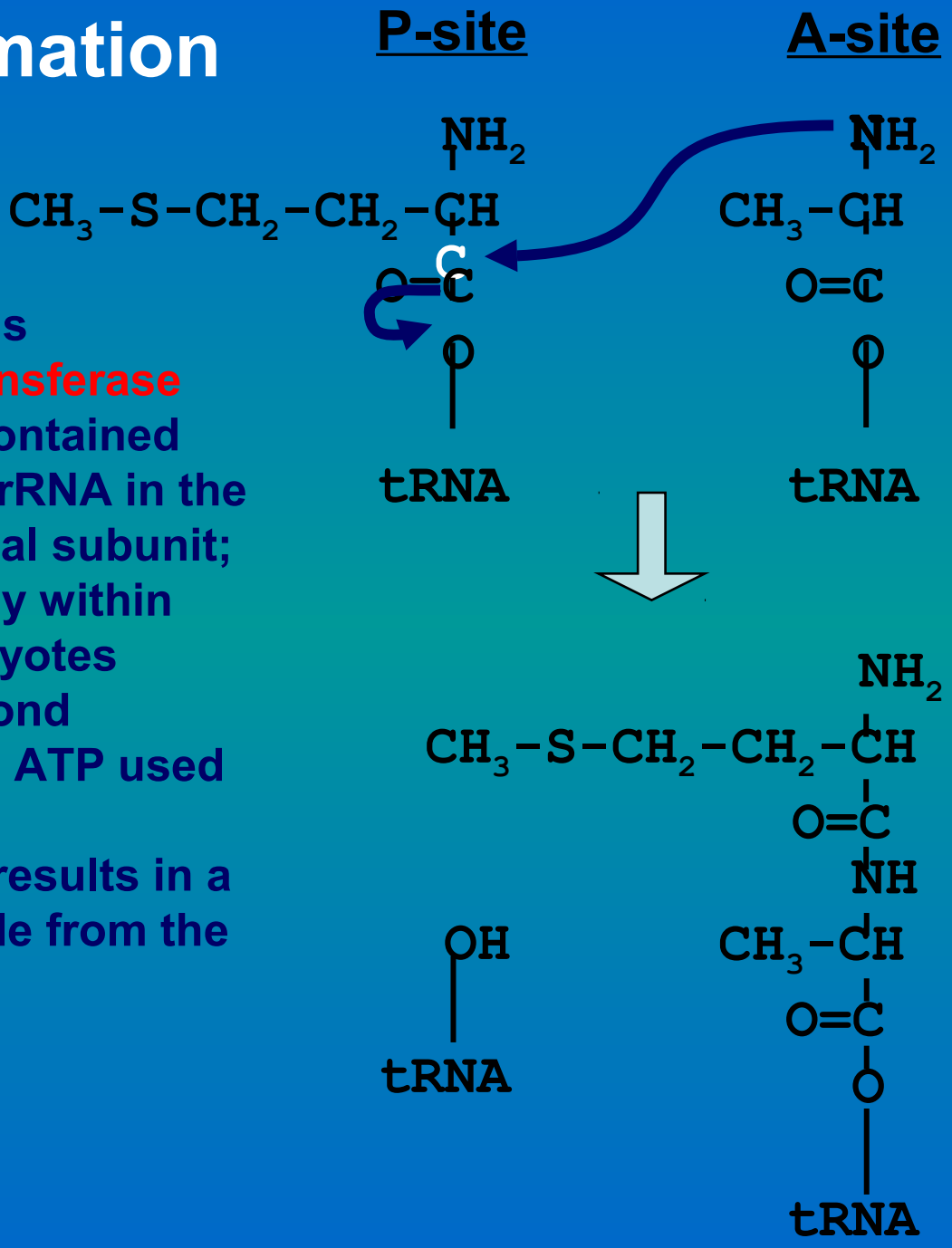
Its anticodon links up with the second codon on the mRNA.

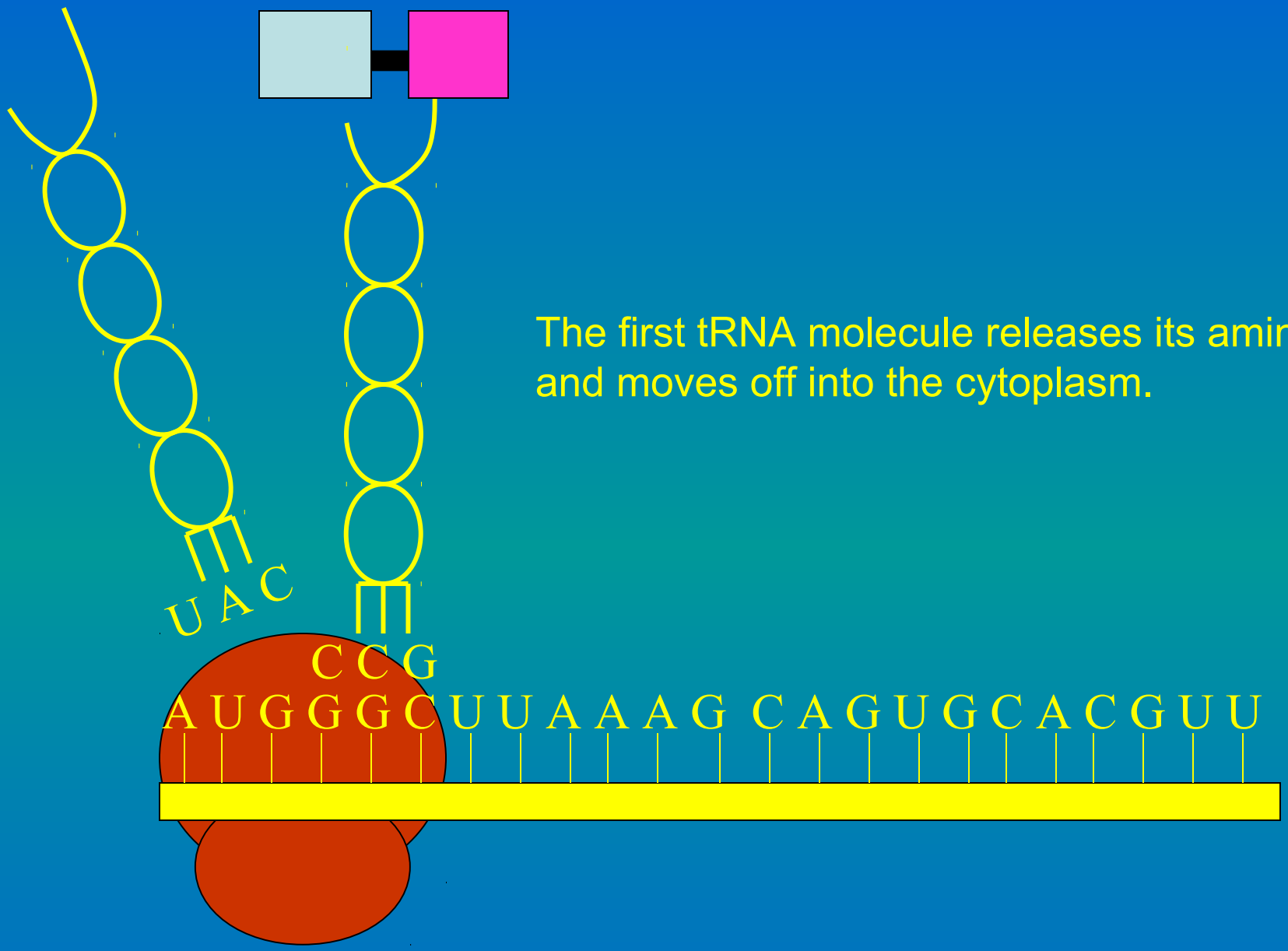


A peptide bond forms between the two amino acids.

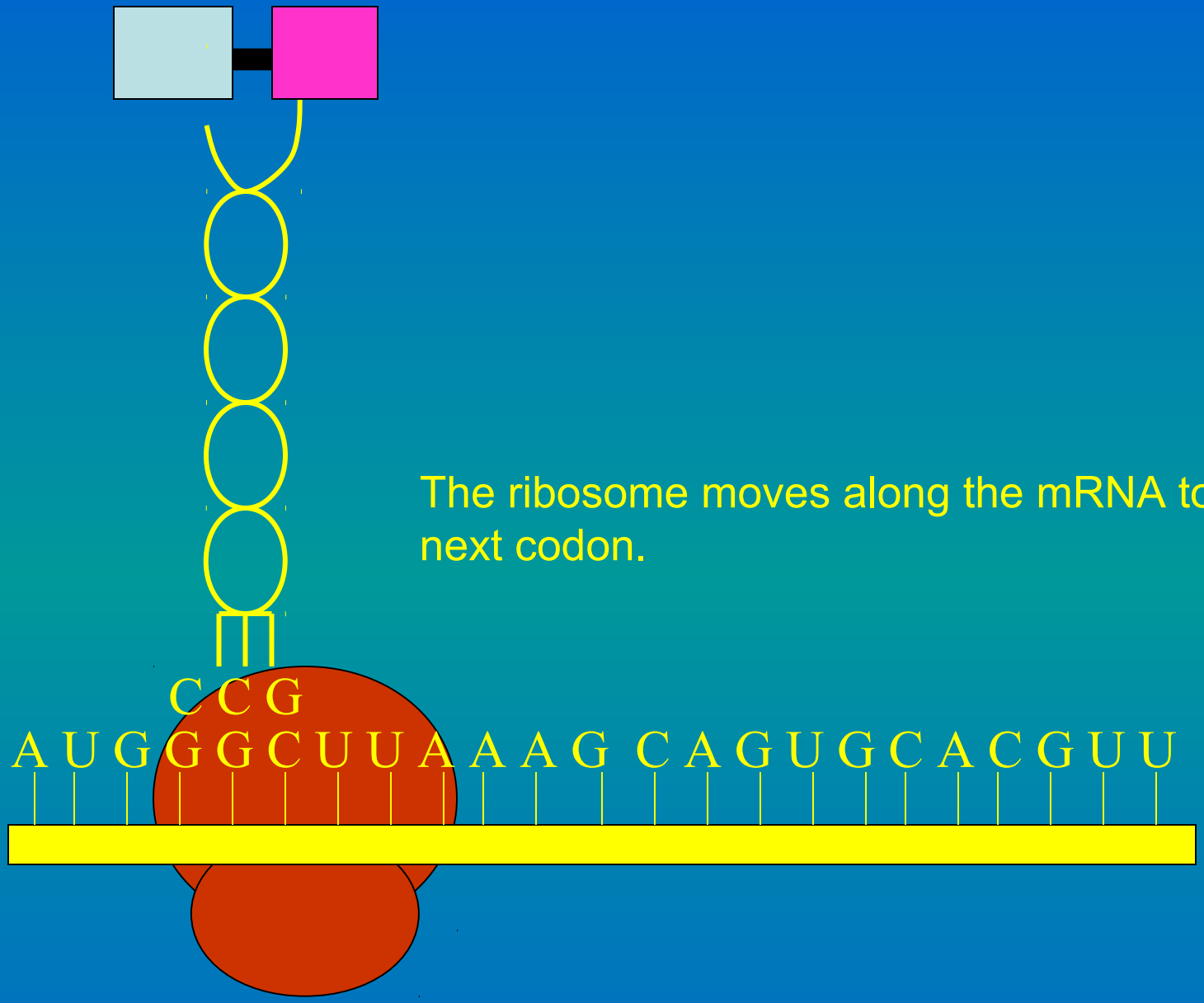
Peptide bond formation

- peptide bond formation is catalyzed by peptidyl transferase
- peptidyl transferase is contained within a sequence of 23S rRNA in the prokaryotic large ribosomal subunit; therefore, it is probably within the 28S rRNA in eukaryotes
- the energy for peptide bond formation comes from the ATP used in tRNA charging
- peptide bond formation results in a shift of the nascent peptide from the P-site to the A-site

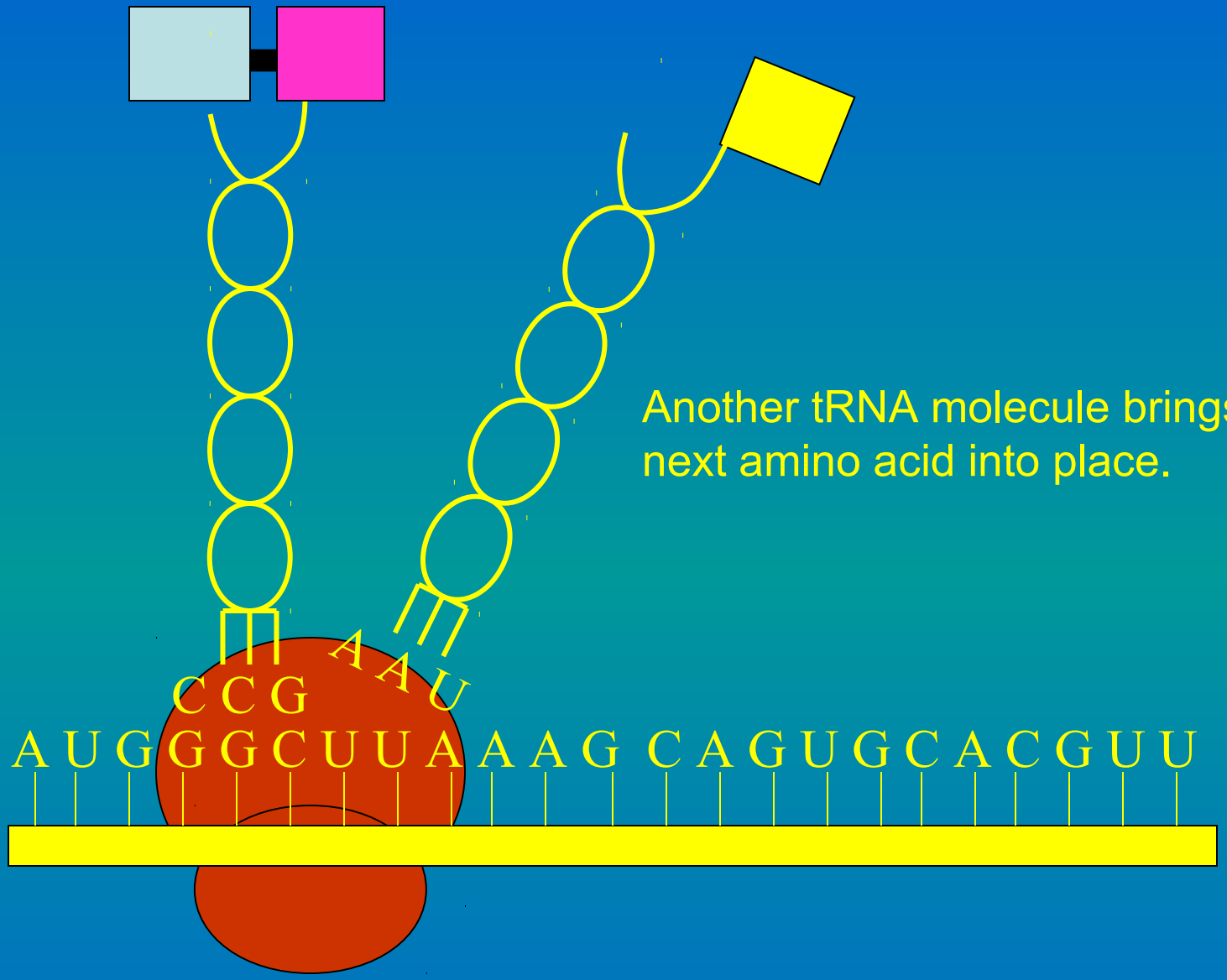




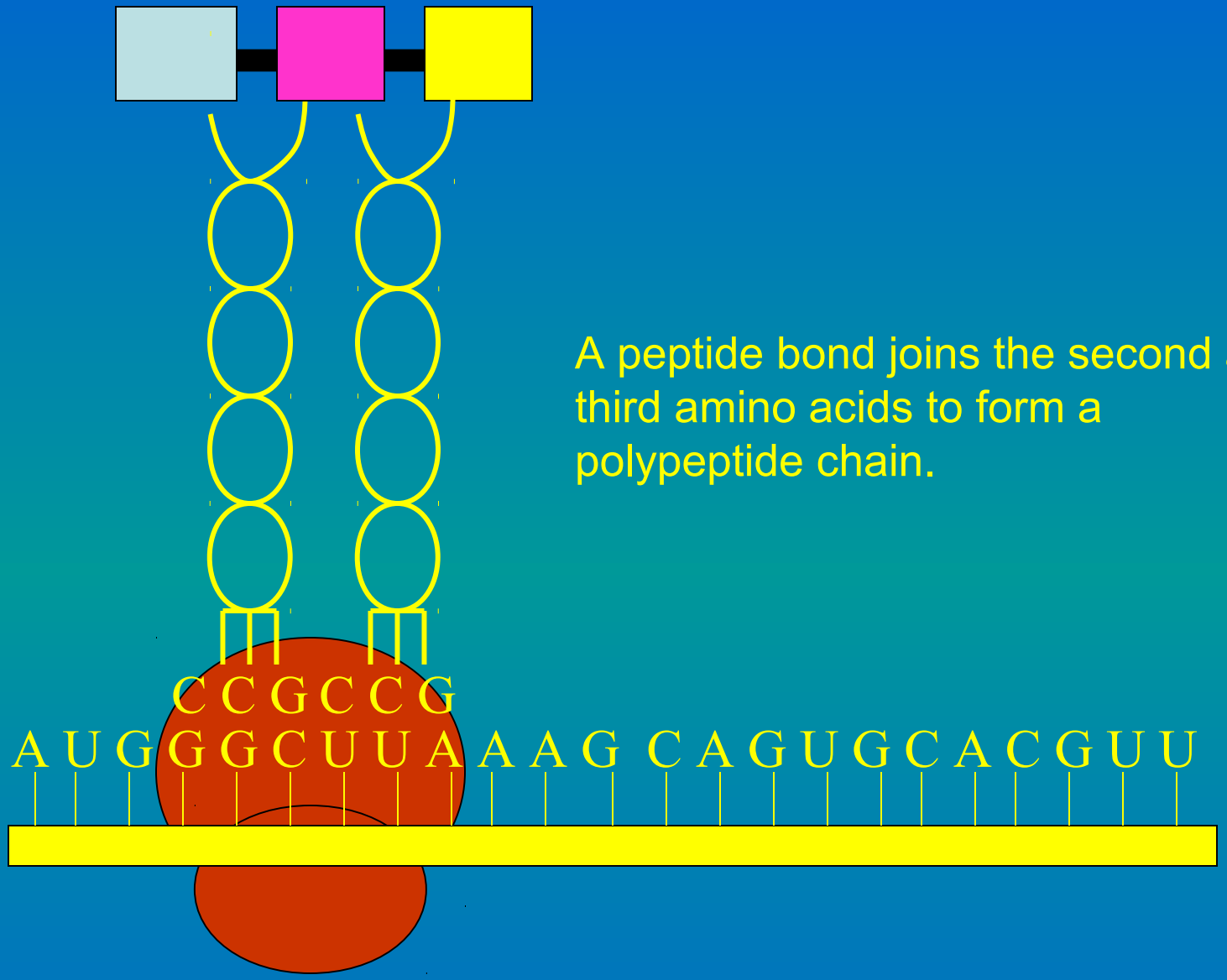
The first tRNA molecule releases its amino acid and moves off into the cytoplasm.



The ribosome moves along the mRNA to the next codon.



Another tRNA molecule brings the next amino acid into place.



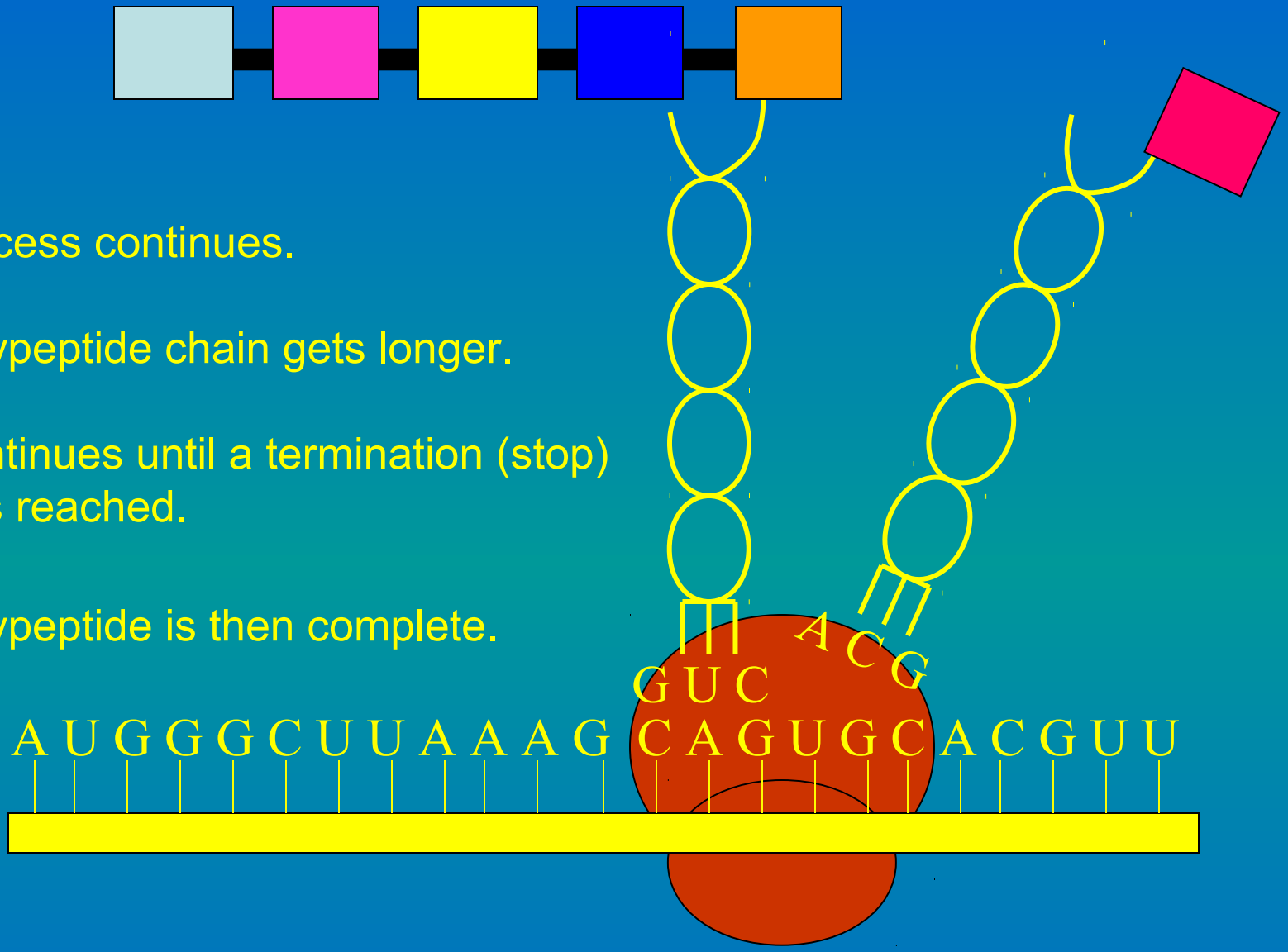
A peptide bond joins the second and third amino acids to form a polypeptide chain.

The process continues.

The polypeptide chain gets longer.

This continues until a termination (stop) codon is reached.

The polypeptide is then complete.



Messenger RNAs are translated on polyribosomes

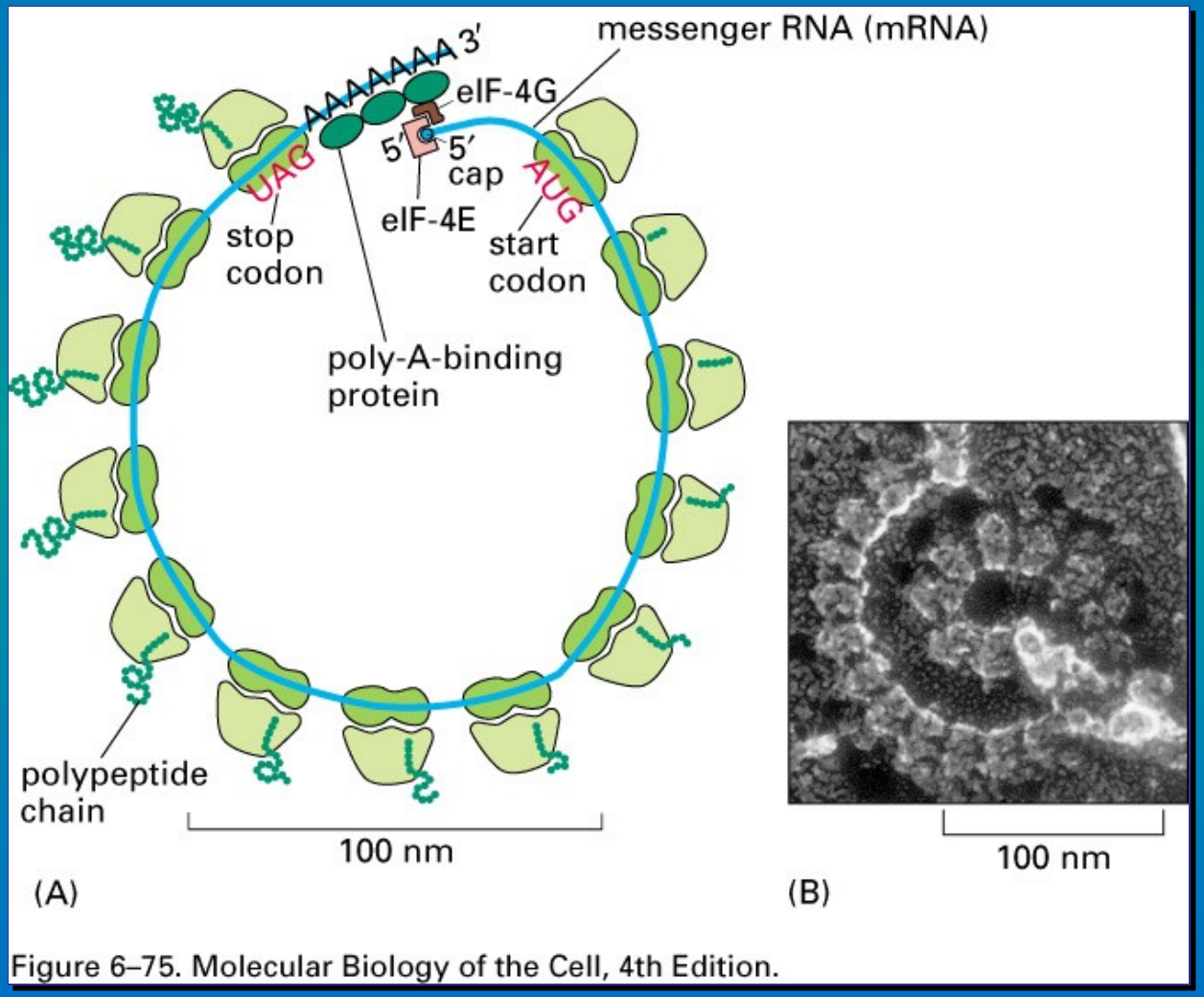


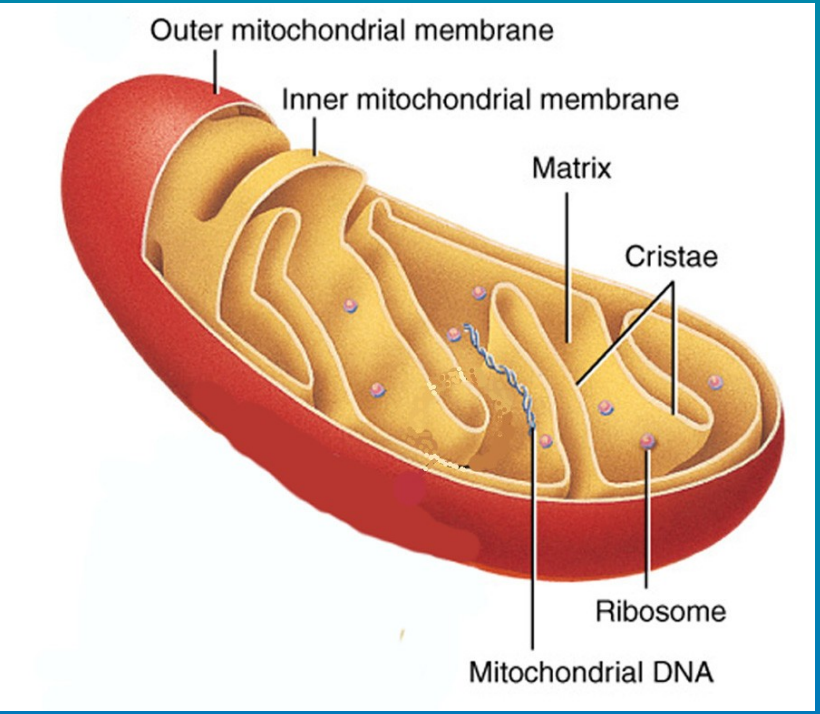
Figure 6–75. Molecular Biology of the Cell, 4th Edition.

Control of protein synthesis

- Rate of protein synthesis:
  - suppressed during exercise
  - increases for up to 48 hours post-exercise
    - Increased protein synthesis during post-exercise period
      - unlikely to be due to increased transcription of RNA
        - » Changes in protein synthesis independent of total RNA
      - more likely due to change in translational control of mRNA
        - » Recent evidence points to involvement of translational initiation factors (eIF4E & eIF4G)
  - Extent of post-exercise protein synthesis also dependent on half-life of mRNA
    - Controlled by ribonucleases (degradative enzymes)
    - Other proteins stabilise and destabilise mRNA against degradation by ribonucleases

Mitochondrial protein synthesis

- Mitochondria contain own DNA and protein synthesizing machinery
- Mitochondrial genetic code slightly different
  - Codon-anticodon interactions simplified
    - Manage with only 22 species of tRNA
- Synthesise only small number of proteins
  - Most mitochondrial proteins coded for in nucleus and transported into mitochondria



Adapted from: Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P84.

Protein degradation

Three main protein degrading systems in muscle:

- Ubiquitin-proteosome
  - Protein marked for degradation by attachment of ubiquitin units
  - Inactive 20S proteosome activated by regulatory protein to become active 26S proteosome
  - 26S proteosome breaks protein into small peptides
    - Small peptides broken down into free amino acids by other processes in cell
- Lysosomal
  - Proteins enter lysosome via endocytosis
    - cathepsins and proteinases degrade bonds
- Calpain
  - Calcium activated proteinase in cytosol of cell
    - Various isomers activated at different calcium concentrations

Protein degradation

- Protein content of a cell depends on balance between protein synthesis and degradation
  - Change in protein = synthesis rate - degradation rate

REFERENCES

1. Elliott WH & Elliott DC. (1997) Biochemistry and Molecular Biology. New York: Oxford University Press. P294.
2. Bettelheim FA and March J (1990) Introduction to Organic and Biochemistry (International Edition). Philadelphia: Saunders College Publishing p398
3. Branden C. and Tooze J. Introduction to Protein Structure,, Garland, New York (1991).
4. Tortora, GJ & Grabowski SR (2000) Principles of Anatomy and Physiology (9th Ed). New York: John Wiley & Sons. P88.