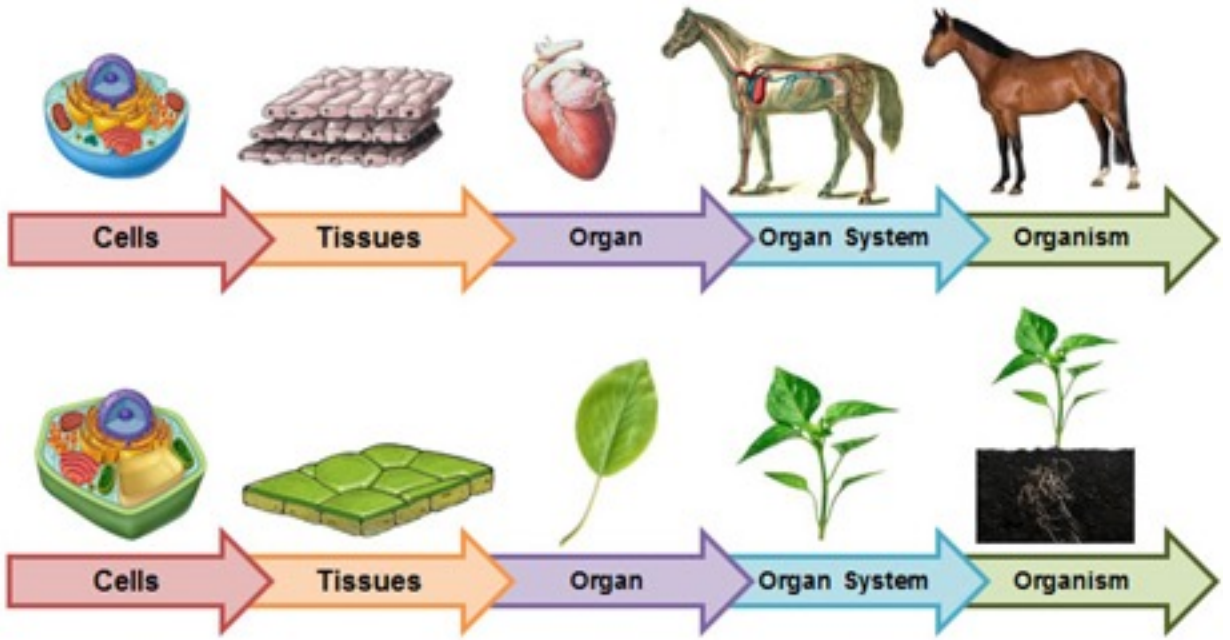
LSM1301



Gene Expression

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Announcements/Reminders

- Midterm review by Dr. Nalini (after lecture today)
- Laboratory assignment 5 is due tomorrow (30th March, 23.59 hrs)
  - Make sure to check the Similarity Report
- Practical 6 is this Thursday (31th March)
  - Please wear long pants and shoes
  - Bring labcoat or purchase one
  - Read the handout before coming to lab

Lecture Topics and Assignments

This is a temporary plan, and is subject to change.

- Introduction (1 Lecture Session)

- Dr. NP Lectures/labs/Museum (5 Lects, 2 Labs, 1 Museum, 3 Tutorials)
  - Total Assignments (25%)

- Chemistry of Life (1 Lect session)
- Cell Structure and Function (1 Lect)
- Energy and Life (1 Lect)
- DNA and Gene Expression (2 lect, DNA and Heredity; Gene Expression)
- Biotechnology (1 Lect)
- Summary & Tutorial (2 Sessions)
  - Total Assignments 25%= 4 lab assignments (25%)

Today

Learning Plan (Gene Expression)

Topic	Learning outcomes for the WK	Activities for online session	Activities for face-to-face session	Assignments/ Assessments
Gene Expression	• *Describe the enzymes required for DNA replication, transcription and translation* • *Explain the information flow from genes to proteins* • *Connect DNA replication with cell division, and explain how DNA integrity is maintained* • *Analyse possible consequences of DNA mutation and forms of mutation* • *Understand how gene expression is regulated* • *Explain how gene expression is regulated in prokaryotic cells using Lac Operon as an example* • *Explain how gene expression is regulated in eukaryotic cells using RNA splicing as an example*	• *Read lecture notes to gain an overall picture about this topic* • *Watch video: Transcription and Translation http://www.youtube.com/watch?v=h3b9ArupXZg (12 min)* • *Participate discussion on LumiNUS forum via posting questions and answering questions (peer learning)* • *Practice more questions on DNA Replication, Gene Expression, and Gene Regulation via online resources https://highered.mheducation.com/sites/0073031208/student_view0/chapter14/multiple_choice.html (optional)*	• *Practice questions on DNA replication* • *Explain template strand and non-template strand; sense strand and antisense strand* • *Discuss various mutations and consequences* • *Practice questions on gene expression*	• *Enhance understanding with Track-learning MCQs* • *Complete assignments related to lab sessions* • *Watch webcast lecture if needed*

Intended Learning Outcomes

- At the end of this class, the student should be able
  - To describe the central dogma of molecular biology, and to relate the dogma to the roles of the three types of RNA molecules involved, and the genetic code
  - To describe the process of transcription, and to relate the differences in transcription between prokaryotic and eukaryotic cells to concepts acquired in the topic of ‘Cell Structure and Function’
  - To describe the process of translation, and to relate the process to the effects of mutations
  - To describe the regulation of gene expression in prokaryotic and eukaryotic cells, and to apply the concept of gene regulation to real-life examples, such as the androgen insensitivity syndrome

Outline

- DNA to Proteins
  - RNA
  - Genetic code
- Transcription
  - Prokaryotic cells
  - Eukaryotic cells
- Transfer RNA
- Ribosomes
- Translation
  - Initiation
  - Elongation
  - Termination
- Regulation of Gene Expression
  - Prokaryotic regulation
  - Eukaryotic regulation
  - Androgen insensitivity syndrome

Outline

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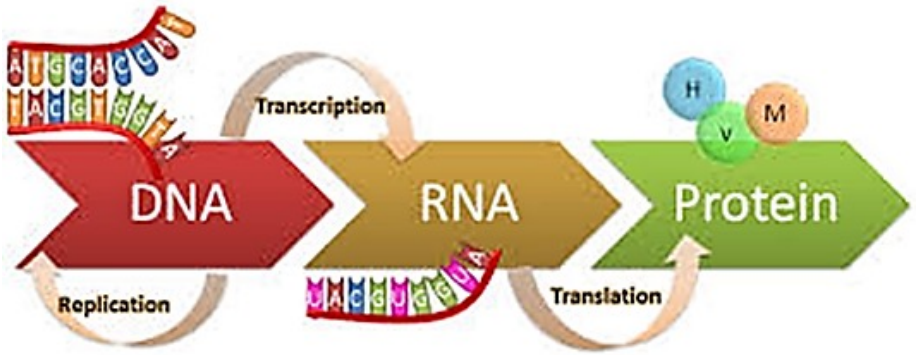
DNA to Proteins

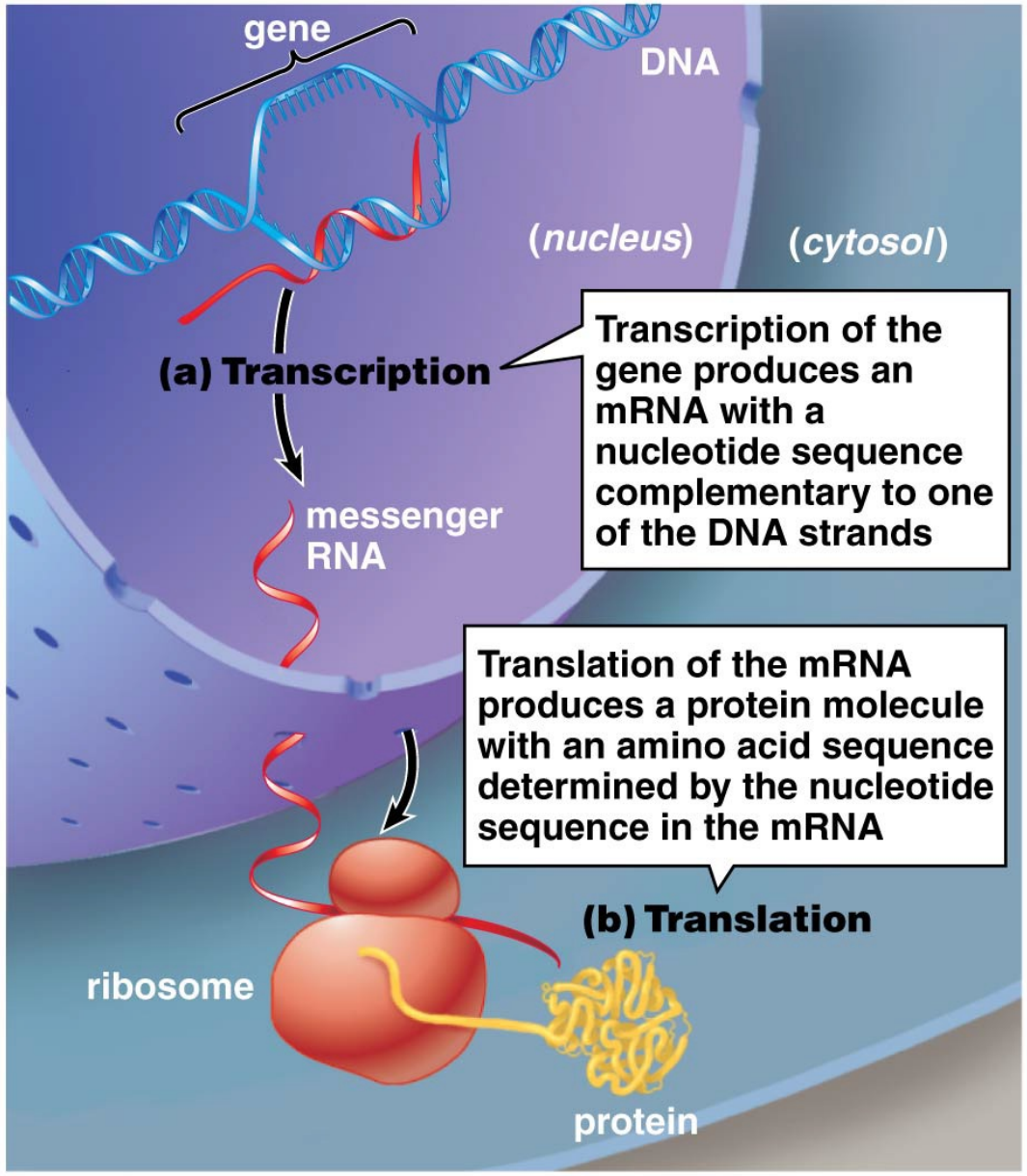


DNA → RNA → protein

1. Transcription
   - Information in DNA copied into mRNA
   - In eukaryotes, occurs in nucleus, then mRNA moves into cytoplasm
2. Translation
   - mRNA translated to form polypeptide chains, which fold to form proteins

DNA to Proteins

- Genetic information flows through intermediary – RNA
  - Central dogma of molecular biology
- Transcription – information in gene converted to RNA
  - In eukaryotes, occurs in nucleus
- Translation – information in mRNA converted to polypeptide chain that folds into protein
  - In eukaryotes, occurs in cytoplasm



From DNA to Proteins

- The information content of DNA is in the form of specific sequences of nucleotides
- The DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins
- Proteins are the links between genotype and phenotype (involved in cell shape, function, reproduction, and synthesis of biomolecules)
- **Gene expression**, the process by which DNA directs protein synthesis, includes two stages: transcription and translation

RNA

- Differences between RNA and DNA

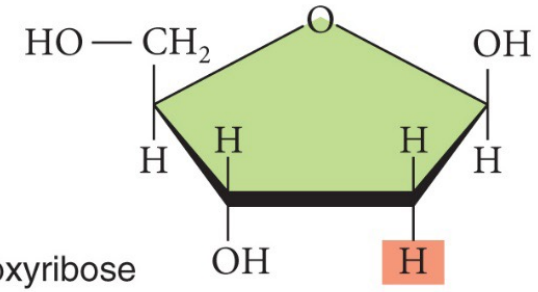
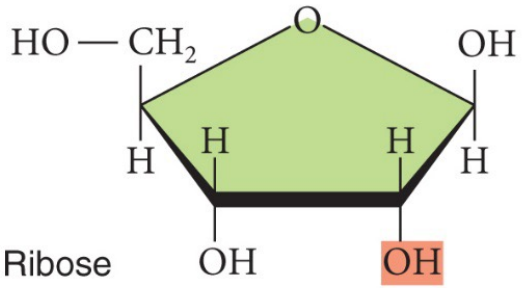
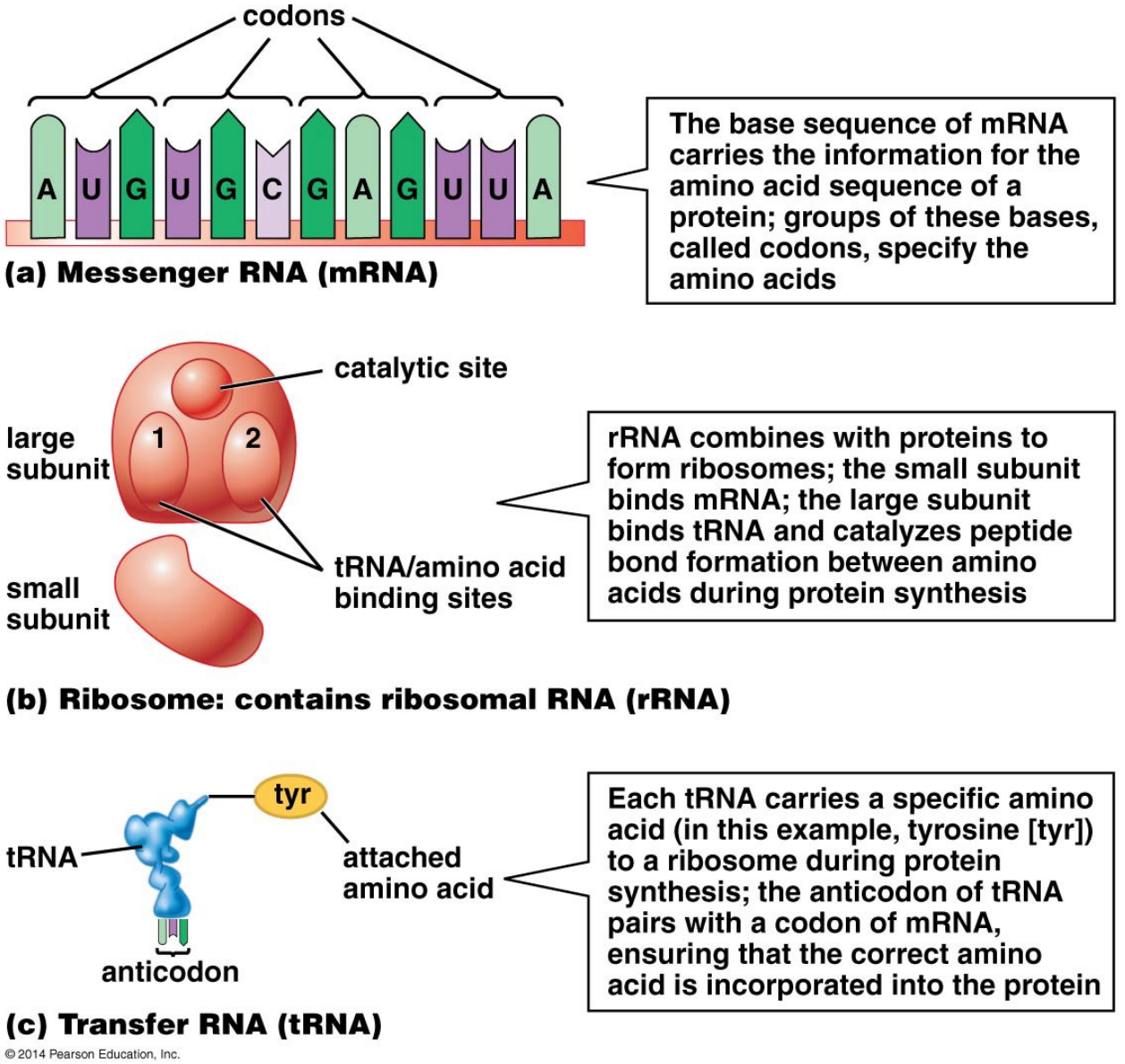
	DNA	RNA
Sugar	 Deoxyribose	 Ribose
Nucleotide bases	Adenine (A), Guanine (G), Cytosine (C), Thymine (T)	Adenine (A), Guanine (G), Cytosine (C), Uracil (U)
Form	 Double-stranded	 Generally single-stranded
Functions	Stores RNA- and protein-encoding information; transfers information to daughter cells	Carries protein-encoding information; helps to make proteins; catalyzes some reactions

Table 10-1 A Comparison of DNA and RNA

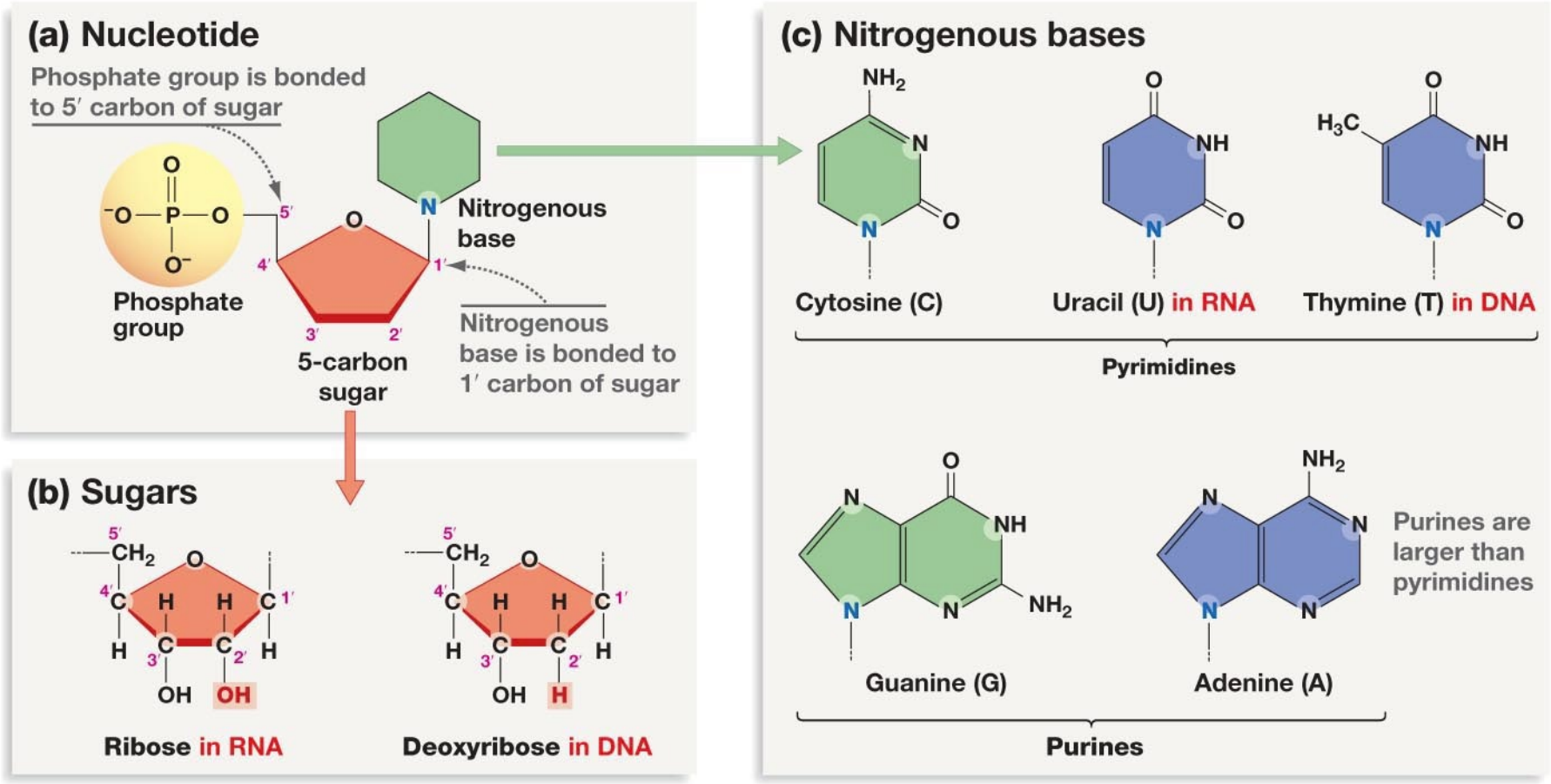
	DNA	RNA	
Strands	2	1	
Sugar	Deoxyribose	Ribose	
Types of Bases	adenine (A), thymine (T) cytosine (C), guanine (G)	adenine (A), uracil (U) cytosine (C), guanine (G)	
Base Pairs	DNA–DNA A–T T–A C–G G–C	RNA–DNA A–T U–A C–G G–C	RNA–RNA A–U U–A C–G G–C
Function	Contains genes; sequence of bases in most genes determines the amino acid sequence of a protein	**Messenger RNA (mRNA):** carries the code for a protein-coding gene from DNA to ribosomes	
		Ribosomal RNA (rRNA): combines with proteins to form ribosomes, the structures that link amino acids to form a protein	
		Transfer RNA (tRNA): carries amino acids to the ribosomes	

RNA

- **Transcription** is the synthesis of RNA under the direction of DNA
- Transcription produces **three types of RNAs**
  - Messenger RNA (mRNA): carries DNA gene information to the ribosome
  - Transfer RNA (tRNA):brings amino acids to the ribosome
  - Ribosomal RNA (rRNA):is part of the structure of ribosomes



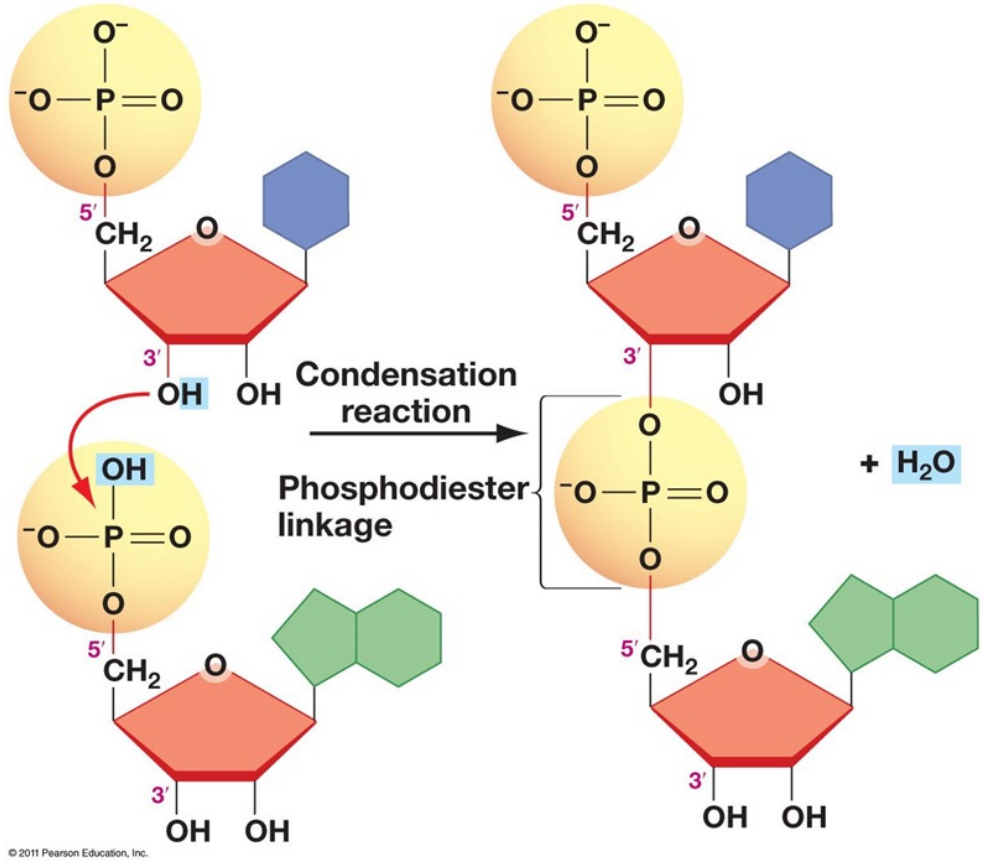
Nucleotides in DNA & RNA



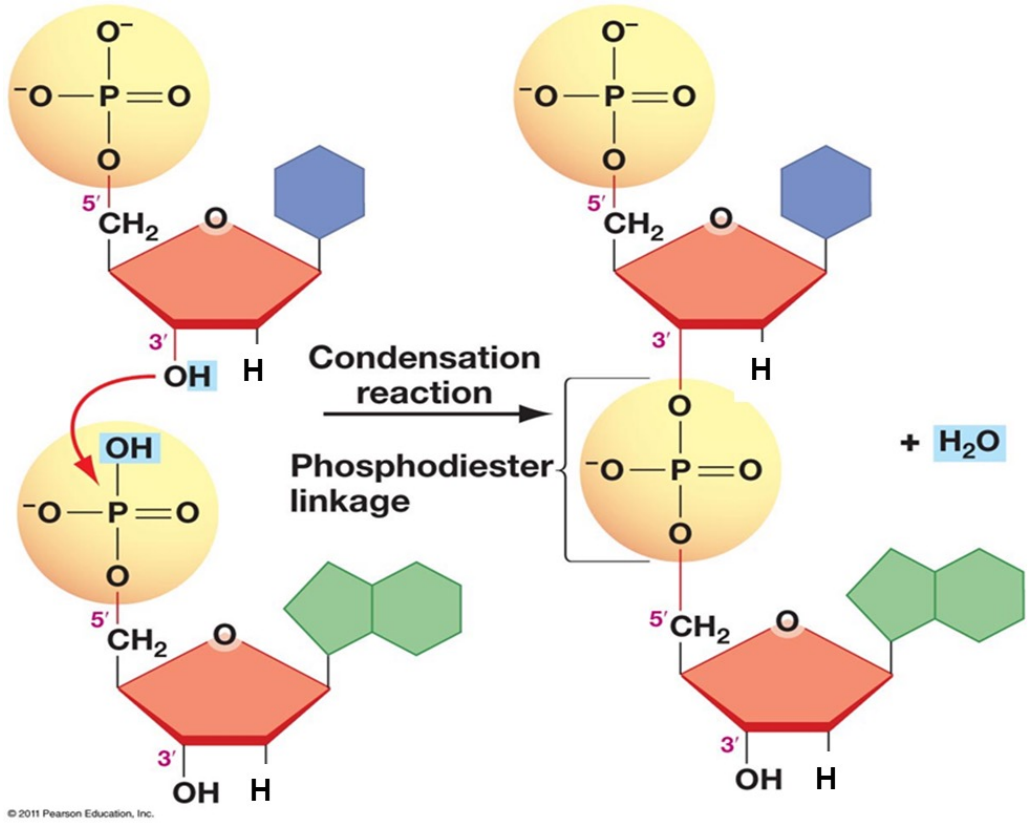
DNA: **D**eoxyribo**N**ucleic **A**cid
RNA: **R**ibo**N**ucleic **A**cid

Synthesis of RNA

RNA



DNA



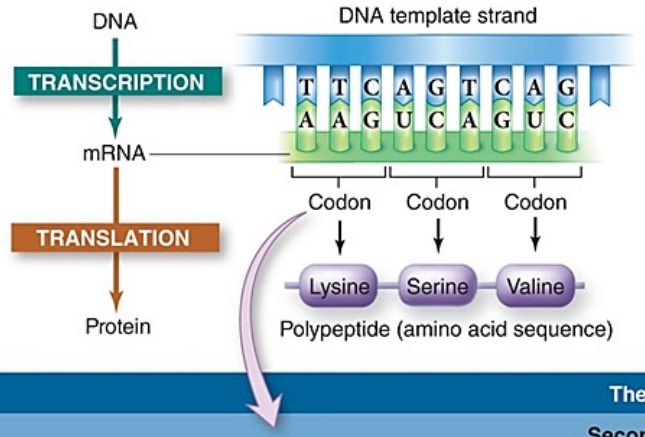
Genetic Code

- Links mRNA to protein
  - Translates sequence/order of bases in nucleic acids to sequence/order of amino acids in proteins
- 20 different amino acids but 4 bases
  - Smallest number of combined bases for each of 20 amino acids – three
  - Three-base code (triplet) has 64 possible combinations
- Codons – triplets of mRNA bases (not DNA)
- Universal
  - Nearly all species use same genetic code
  - Evolution from common ancestor

Genetic Code (cont.)

- Unambiguous (exclusive)
  - Each codon encodes only one specific amino acid
  - No codon encodes two or more amino acids
- Degenerate (redundant)
  - 64 codons for 20 amino acids
  - Most amino acids encoded by two or more codons
- Punctuation codons
  - Start codon – AUG (methionine)
  - Stop codons – UAA, UAG and UGA – do not encode any amino acid

Genetic Code (cont.)



During translation, the mRNA base triplets, called codons, are read in the 5′ to 3′ direction

The Genetic Code

First letter of codon	Second letter of codon: U	C	A	G	Third letter of codon
U	UUU, UUC — Phenylalanine (Phe; F) UUA, UUG — Leucine (Leu; L)	UCU, UCC, UCA, UCG — Serine (Ser; S)	UAU, UAC — Tyrosine (Tyr; Y) UAA — Stop UAG — Stop	UGU, UGC — Cysteine (Cys; C) UGA — Stop UGG — Tryptophan (Trp; W)	U C A G
C	CUU, CUC, CUA, CUG — Leucine (Leu; L)	CCU, CCC, CCA, CCG — Proline (Pro; P)	CAU, CAC — Histidine (His; H) CAA, CAG — Glutamine (Gln; Q)	CGU, CGC, CGA, CGG — Arginine (Arg; R)	U C A G
A	AUU, AUC, AUA — Isoleucine (Ile; I) AUG — Start Methionine (Met; M)	ACU, ACC, ACA, ACG — Threonine (Thr; T)	AAU, AAC — Asparagine (Asn; N) AAA, AAG — Lysine (Lys; K)	AGU, AGC — Serine (Ser; S) AGA, AGG — Arginine (Arg; R)	U C A G
G	GUU, GUC, GUA, GUG — Valine (Val; V)	GCU, GCC, GCA, GCG — Alanine (Ala; A)	GAU, GAC — Aspartic acid (Asp; D) GAA, GAG — Glutamic acid (Glu; E)	GGU, GGC, GGA, GGG — Glycine (Gly; G)	U C A G

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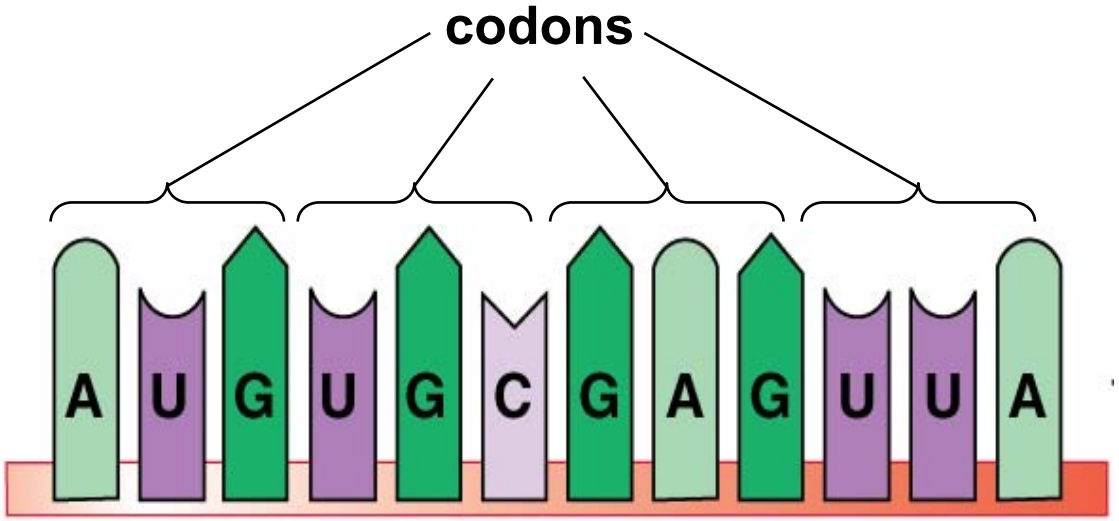
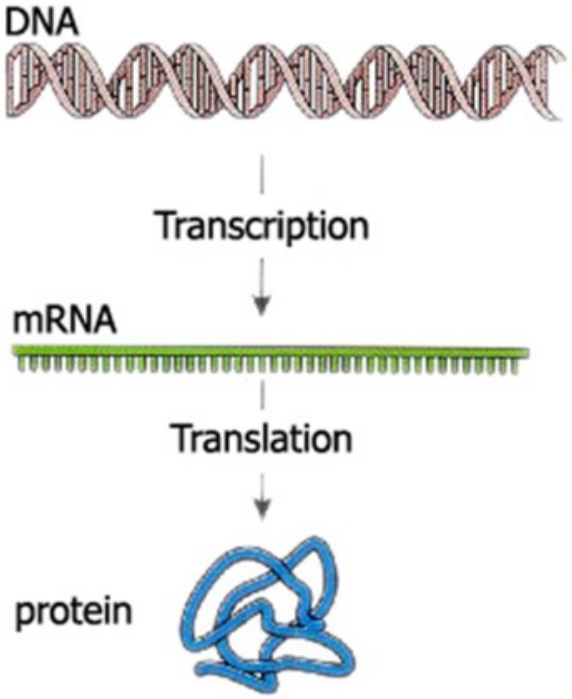
mRNA





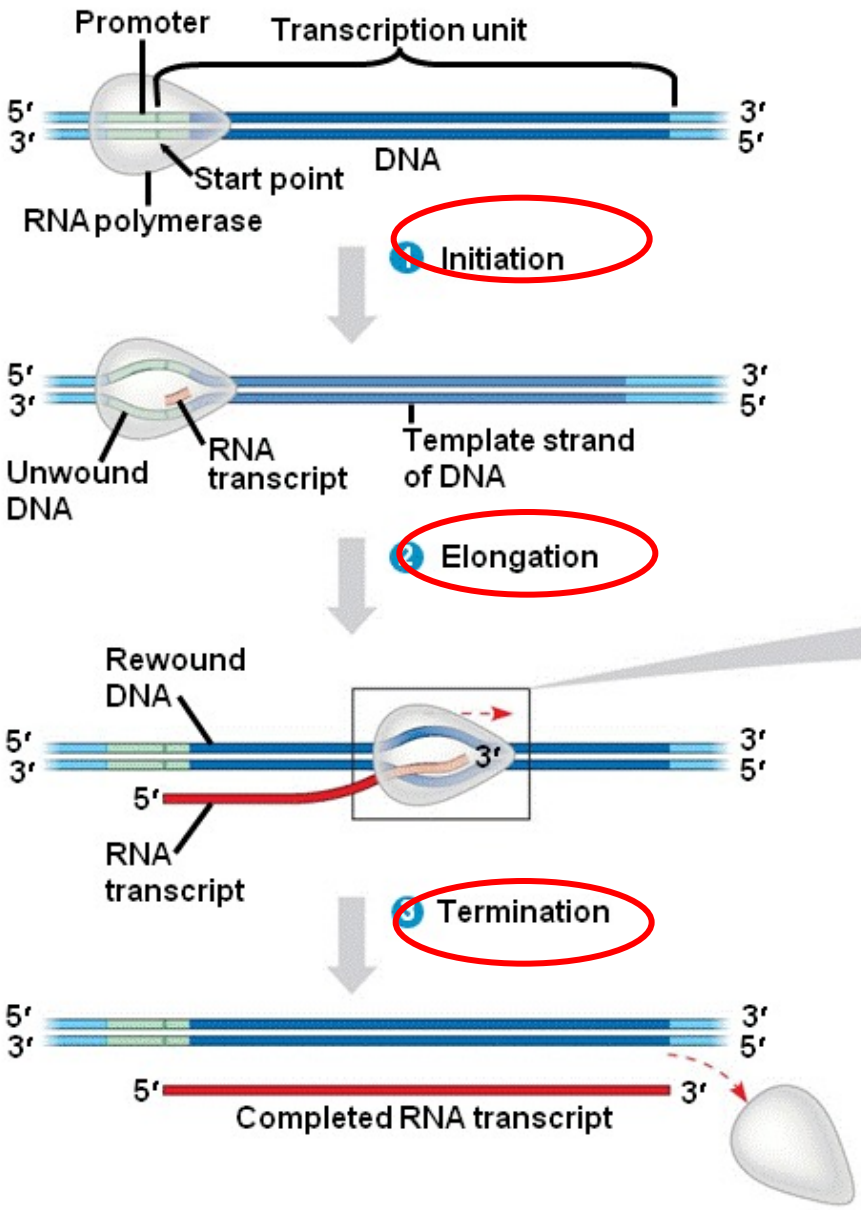
The base sequence of mRNA carries the information for the amino acid sequence of a protein; Each group of three mRNA bases in a row forms a codon, specify the amino acids

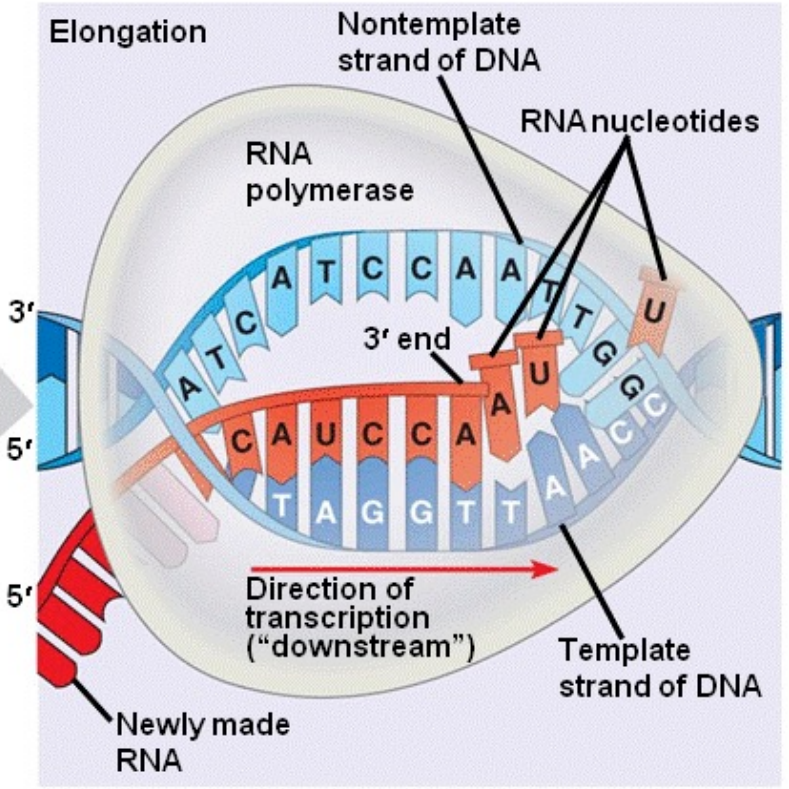
Transcription Process

Transcription of a DNA gene into RNA has three stages

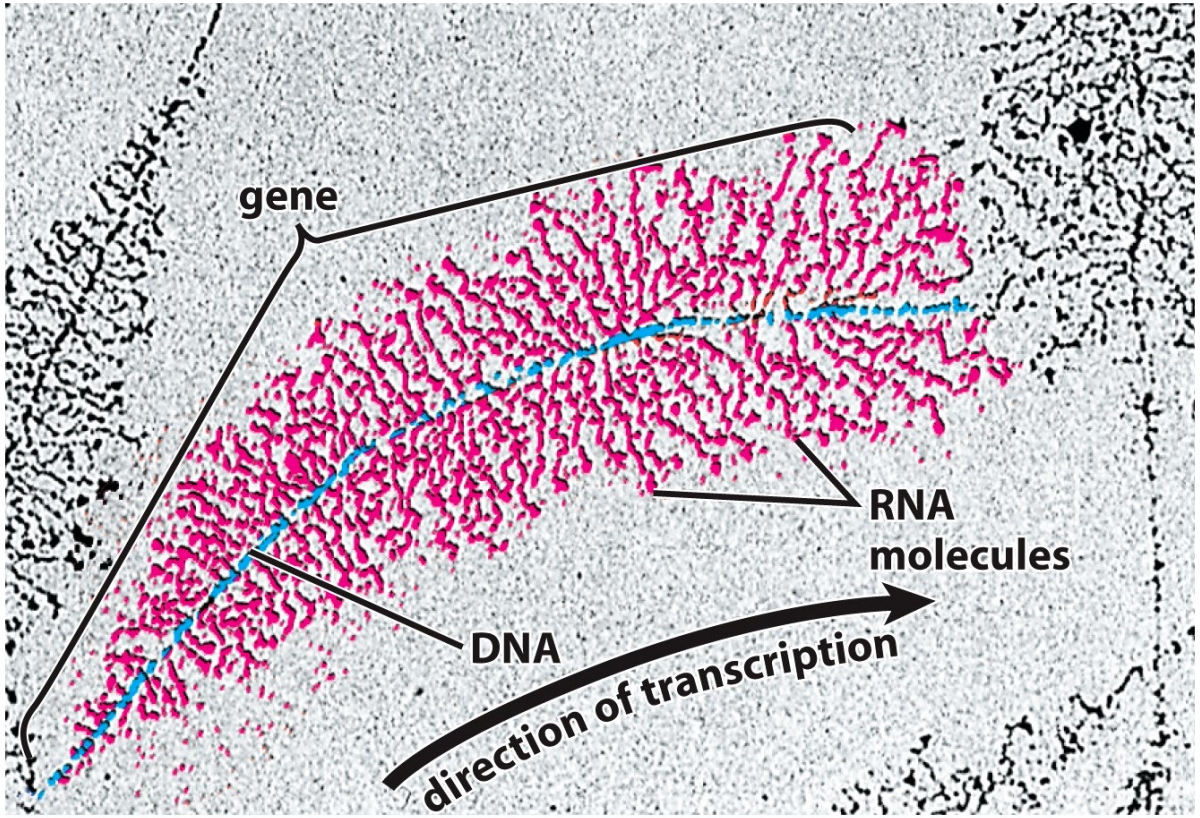
1. Initiation: RNA polymerase binds to the promoter region of DNA near the beginning of a gene, separating the double helix near the promoter.
2. Elongation: RNA polymerase travels along the DNA template strand (blue), unwinding the DNA double helix and synthesizing RNA by catalyzing the addition of ribose nucleotides into an RNA molecule (red). The nucleotides in the RNA are complementary to the template strand of the DNA.
3. Termination: At the end of the gene, RNA polymerase encounters a DNA sequence called a termination signal. RNA polymerase detaches from the DNA and releases the RNA molecule.

Transcription





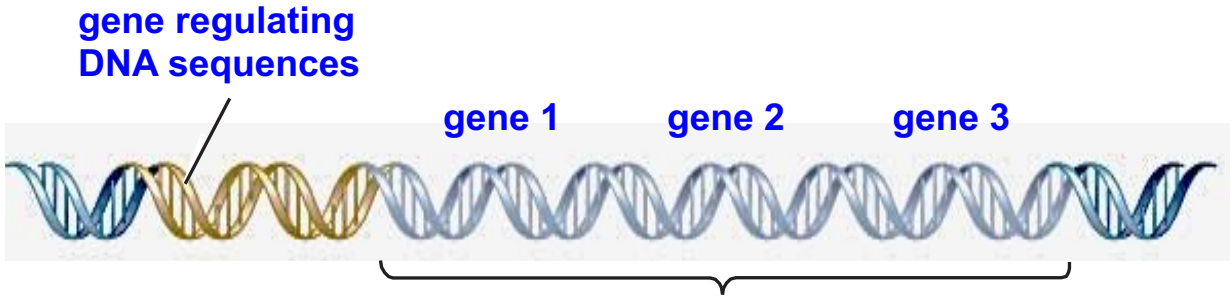
RNA transcription in action



This colorized election micrograph shows the progress of RNA transcription in the egg of an African clawed toad. A series of RNA polymerase molecules (too small to be seen in this micrograph) is traveling down the DNA, synthesizing RNA as they go. The beginning of the gene is on the left.

Prokaryotic Cells

- All the nucleotides in a gene usually code for the amino acids in a protein;
- Genes for related functions are adjacent (sit side by side) and are transcribed together



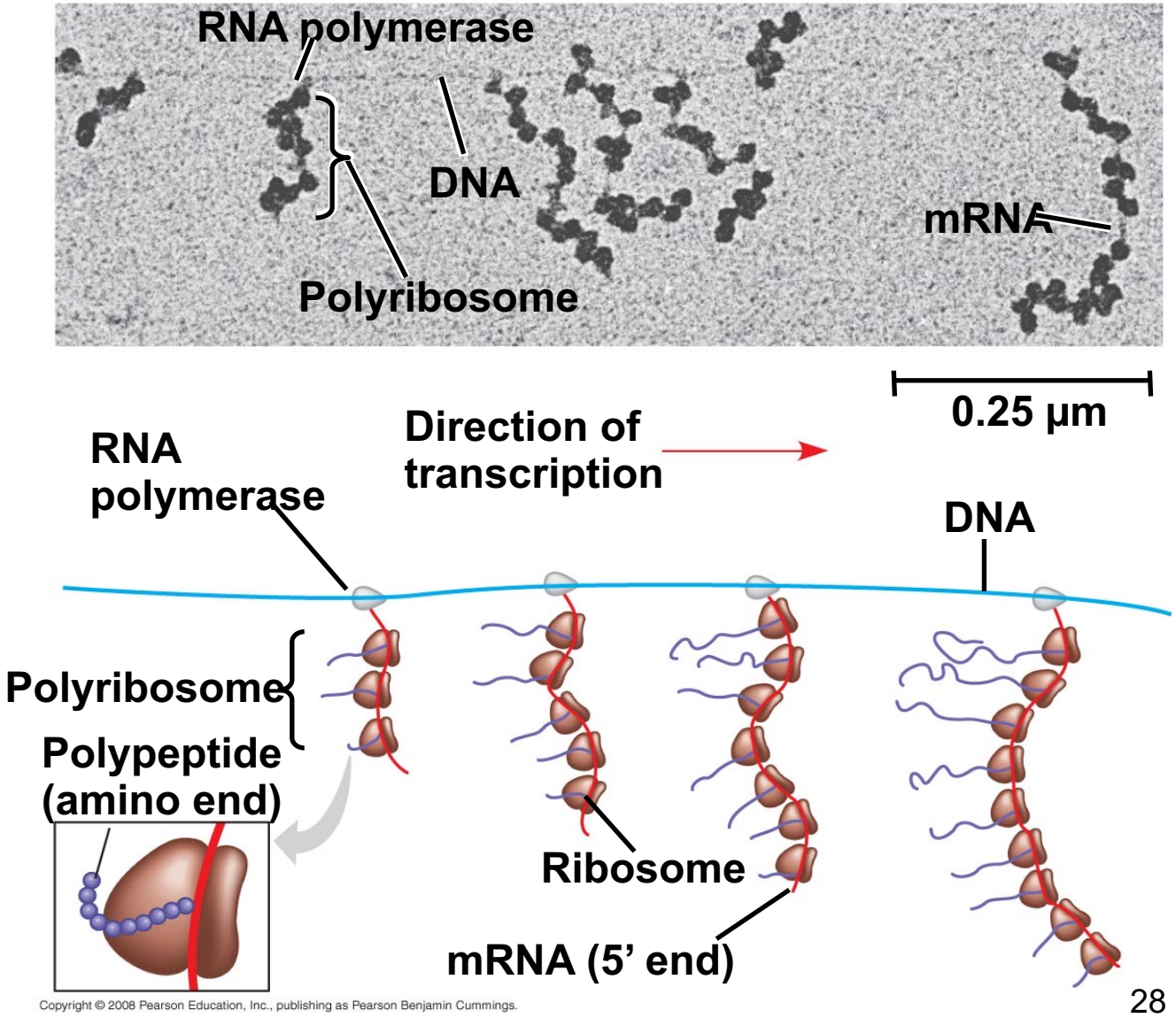
Gene organization on a prokaryotic chromosome

- Because prokaryotes have no nuclear membrane, translation and transcription usually occur at the same place and time.
- Ribosomes begin translation at the free 5' end of mRNA, even as RNA polymerase is elongating the mRNA at its 3' end

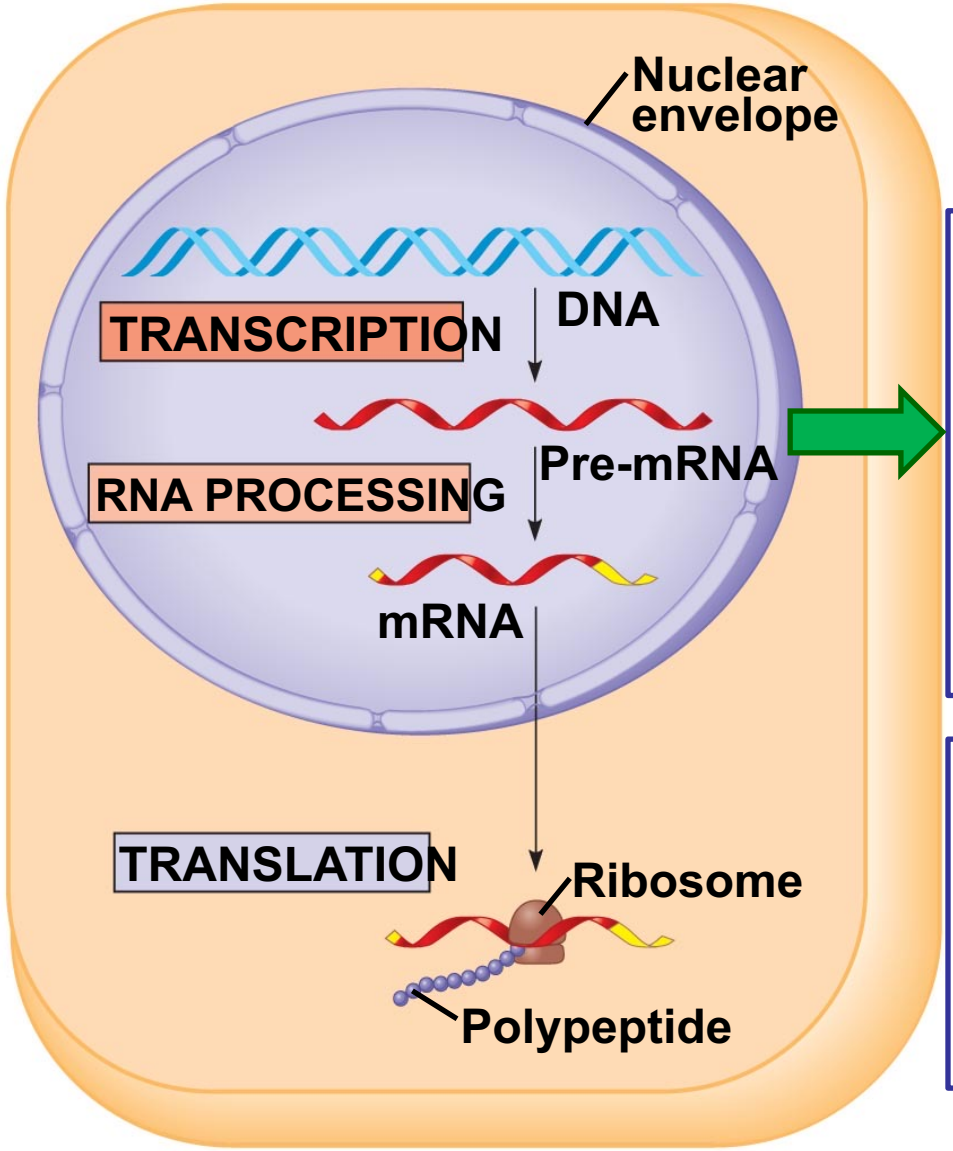
Prokaryotes: mRNA is directly used for protein synthesis

DNA not within nucleus

- Translation and transcription not separated in space or time
- In many cases, as mRNA molecule begins to separate from DNA during transcription, ribosomes immediately begin translating mRNA into protein



Eukaryotes: mRNA synthesis and maturation



Each end of a pre-mRNA molecule is modified in a particular way:

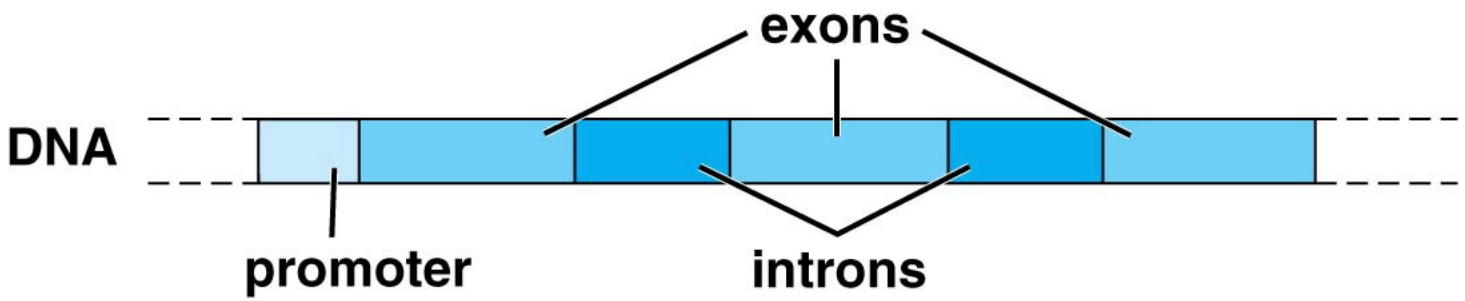
- The 5′ end receives a modified nucleotide **5′ cap**
- The 3′ end gets a **poly-A tail**

RNA splicing removes introns and joins exons, creating an mRNA molecule with a continuous coding sequence

Eukaryotic cells

- Pre-mRNA modified before leaving nucleus as mature mRNA

- Long pre-mRNA strand composed of exons and introns
  - Exons – nucleotide sequences that encode proteins, that will be expressed
  - Introns – nucleotide sequences that are not translated, that will not be expressed

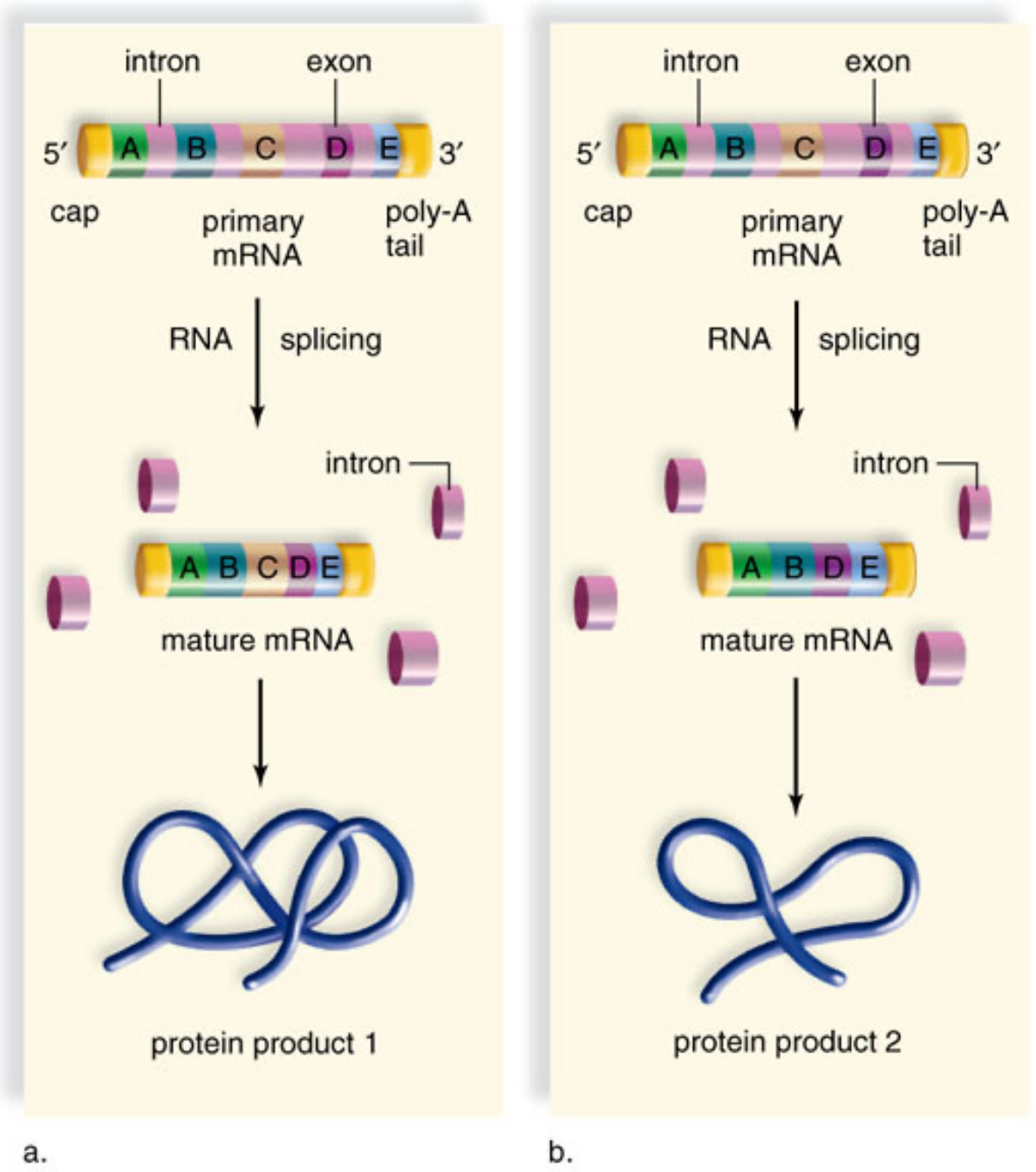


(a) Eukaryotic gene structure

Alternate splicing in eukaryotes

- RNA splicing
  - Introns excised and removed
  - Remaining exons spliced back together

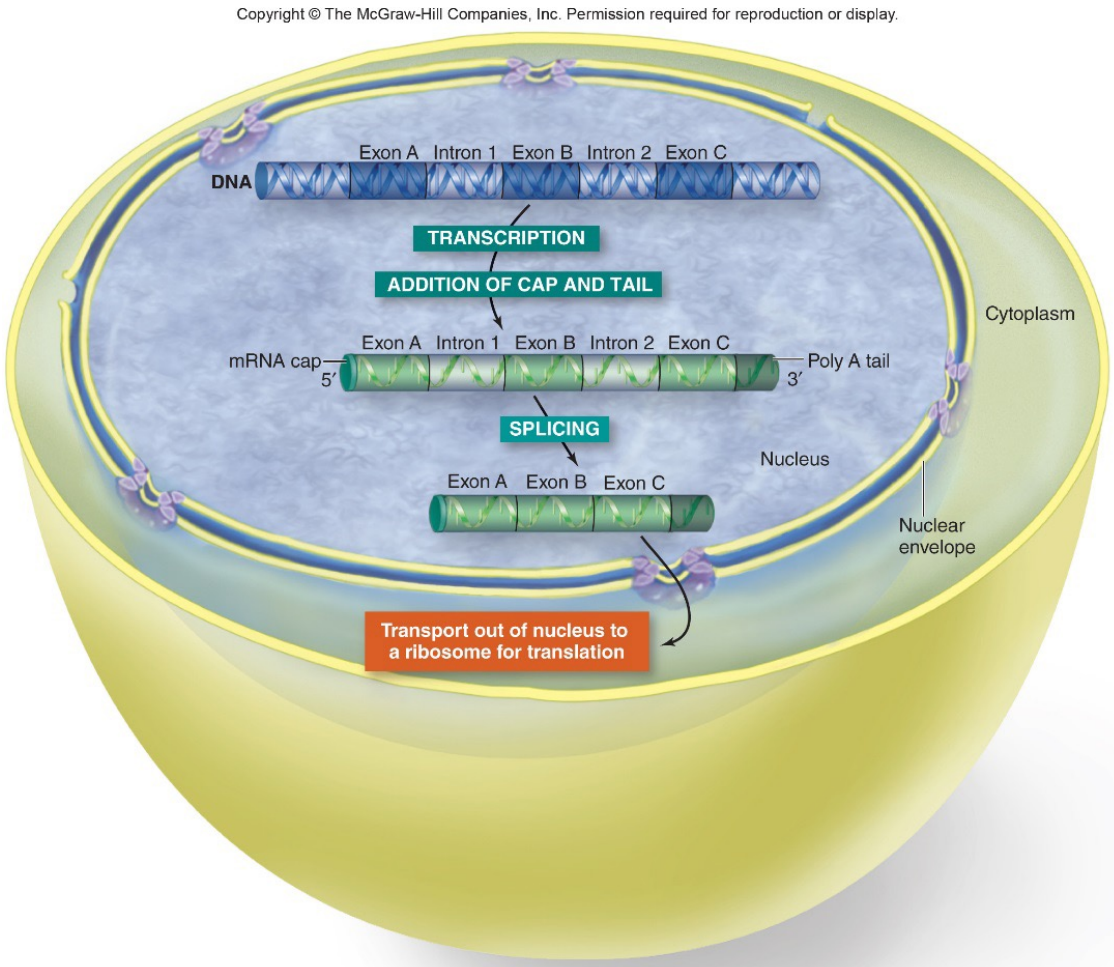
- Alternative splicing – allows one gene to express different proteins
  - Some exons removed
  - Other exons spliced together in different combinations



Eukaryotic Cells

- Modifications also made to ends of pre-mRNA
  - Modified guanine cap at 5' end – facilitates mRNA binding to ribosome
  - Poly-A tail of 150-200 adenines at 3' end facilitates mRNA transport out of nucleus, prevents mRNA breakdown by cellular enzymes

- Mature mRNA exits nucleus through nuclear pore



A summary of transcription

- Like DNA replication
  - Require a template DNA
  - New strand grows in 5′ to 3′ direction
  - Both happen in the nucleus
- Unlike DNA replication
  - Only small section of DNA as template
  - Only RNA polymerase involved
  - Does not need a primer
  - Product is single strand of RNA

Transcription vs. Replication Features

RNA

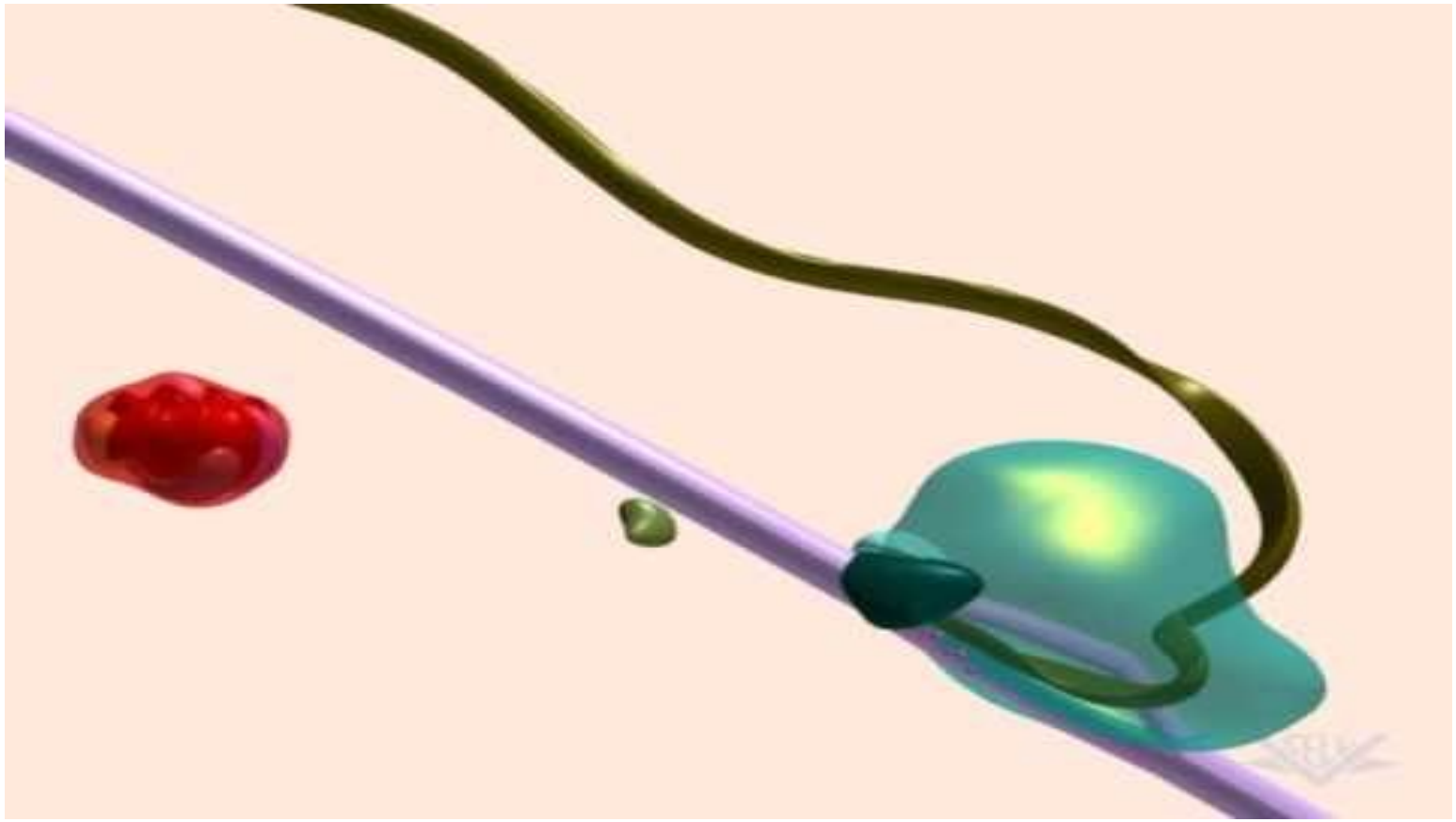
- Uses **(1) strand of DNA** as a template for making RNA during **Transcription**
- **Transcription** occurs in **cytoplasm** of **prokaryotic** cells
- Transcriptions occurs in **nucleus** of **eukaryotic cells**
- **Many** RNA molecules are made from a **(1)** gene

DNA

- Uses **(2) strands of DNA** as a template during DNA **Replication**
- **Replication** occurs in **cytoplasm** of **prokaryotic** cells
- Replication occurs in **nucleus** of **eukaryotic cells**
- **(2) Identical** DNA copies made from **(1) DNA**

Animation

Transcription and mRNA processing (video)



https://www.youtube.com/watch?v=WsofH466lqk

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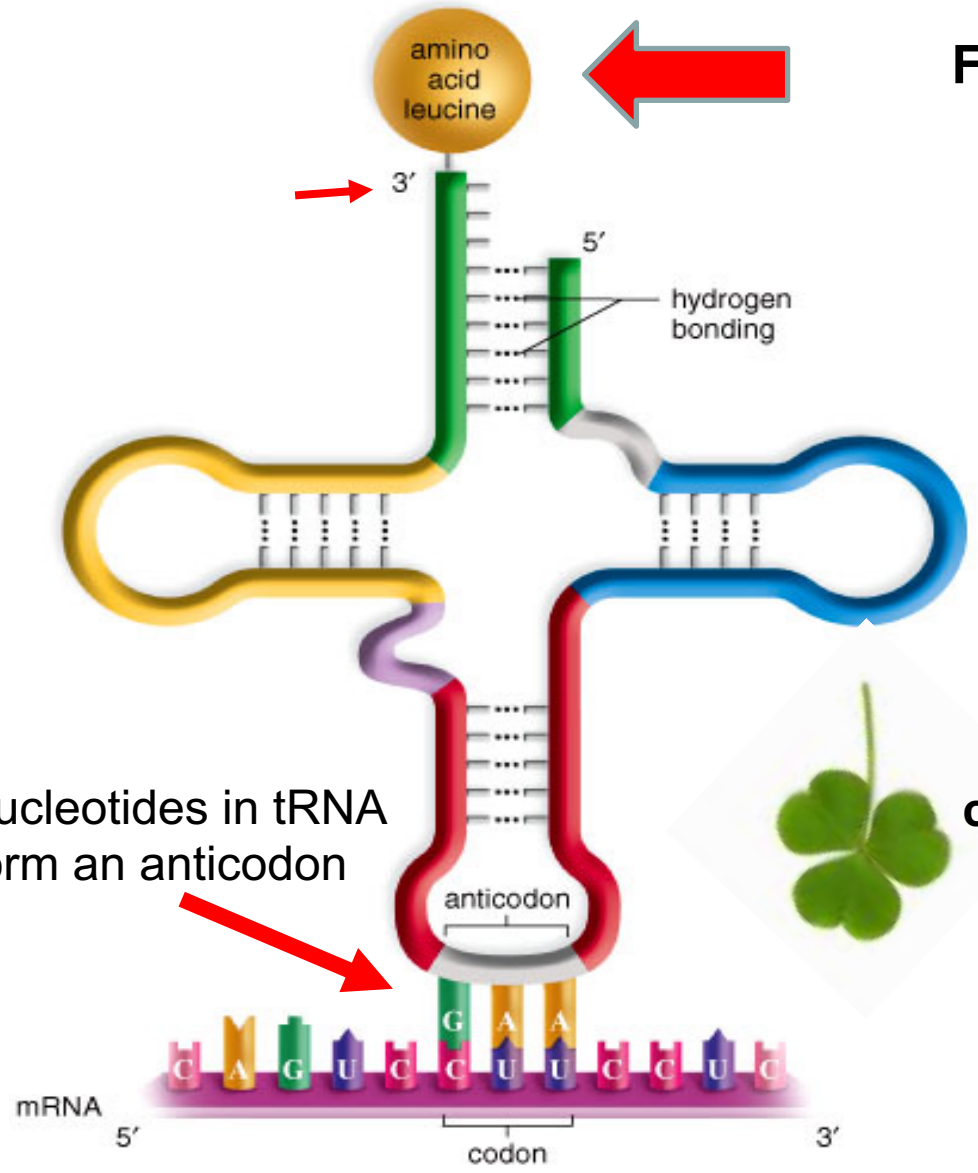
tRNA

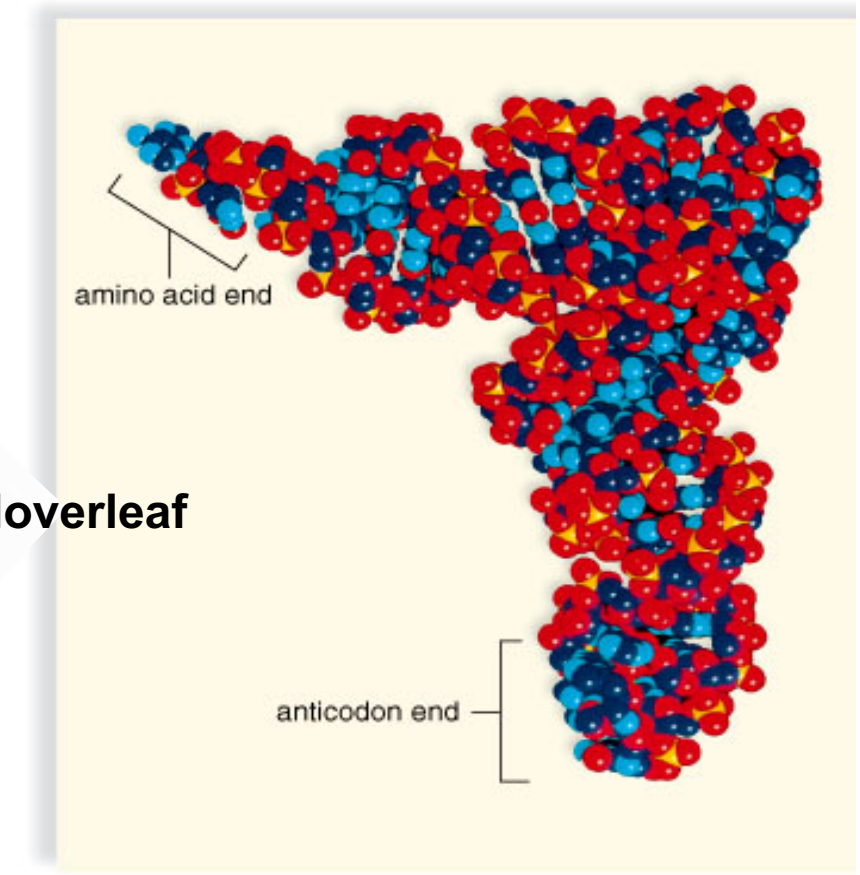
- Each tRNA carries a specific amino acid at its 3’ end to a ribosome during protein synthesis (Adapter molecules)
- Each tRNA has 3 exposed bases called anticodon, which pairs with a codon of mRNA, ensuring that the correct amino acid is incorporated into the protein.
- At least one tRNA assigned to carry each of the 20 amino acids

tRNA



3 nucleotides in tRNA form an anticodon

Functions like an adaptor

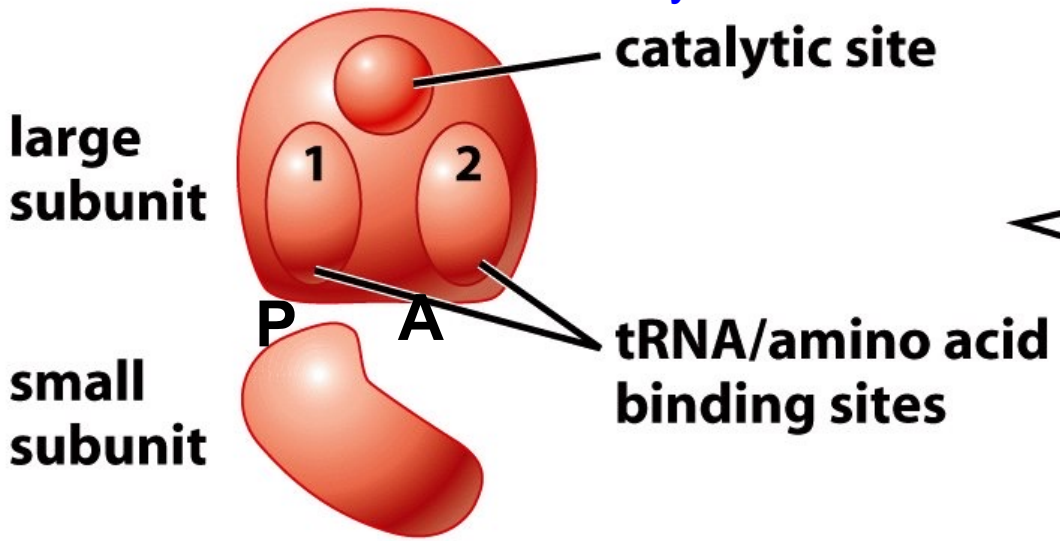


cloverleaf

rRNA

Ribosome: contains ribosomal RNA (rRNA)

Where rRNA catalyzes the formation of the peptide bonds, named ribozyme



rRNA combines with proteins to form ribosomes. The small subunit binds mRNA. The large subunit binds tRNA and catalyzes peptide bond formation between amino acids during protein synthesis.

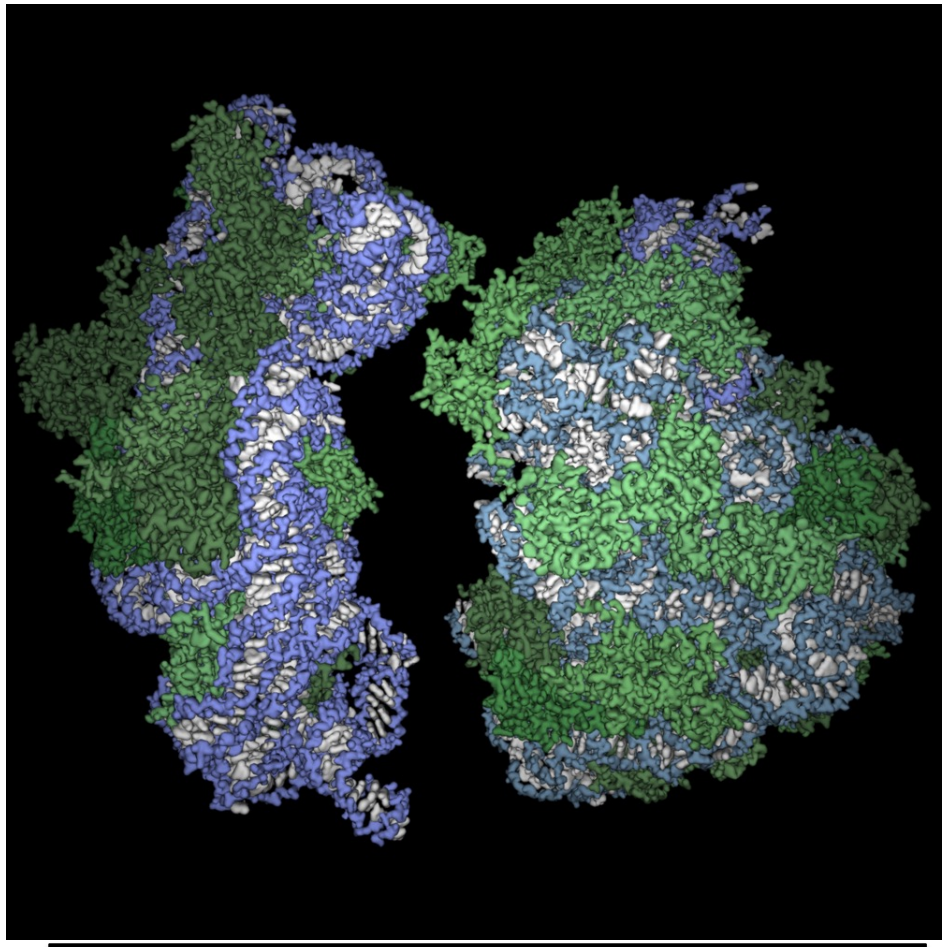
Figure 10-2b Biology: Life on Earth, 8/e

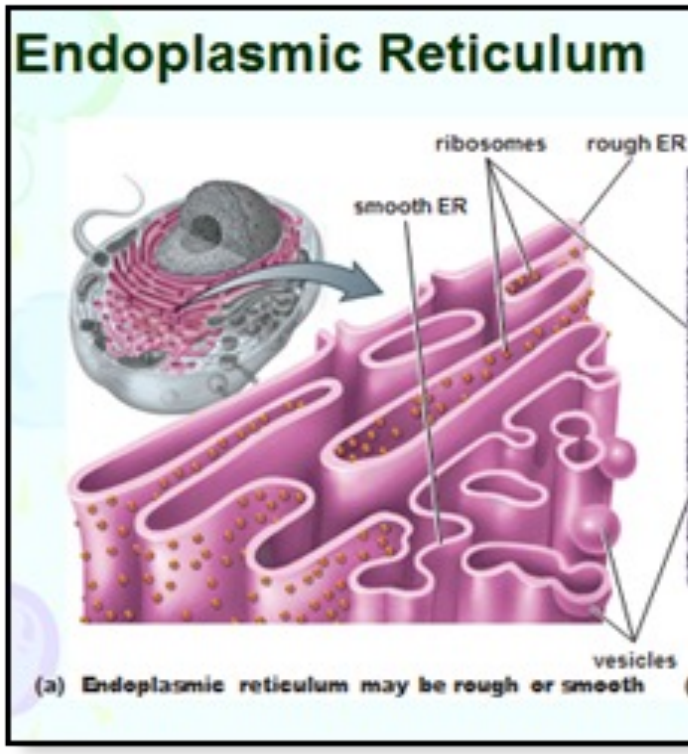
http://exploringorigins.org/ribozymes.html

P site, peptidyl-tRNA binding site

A site, aminoacyl-tRNA (acceptor) site

Ribosome





Proteins are shown in green, and RNA is shown in blue and white

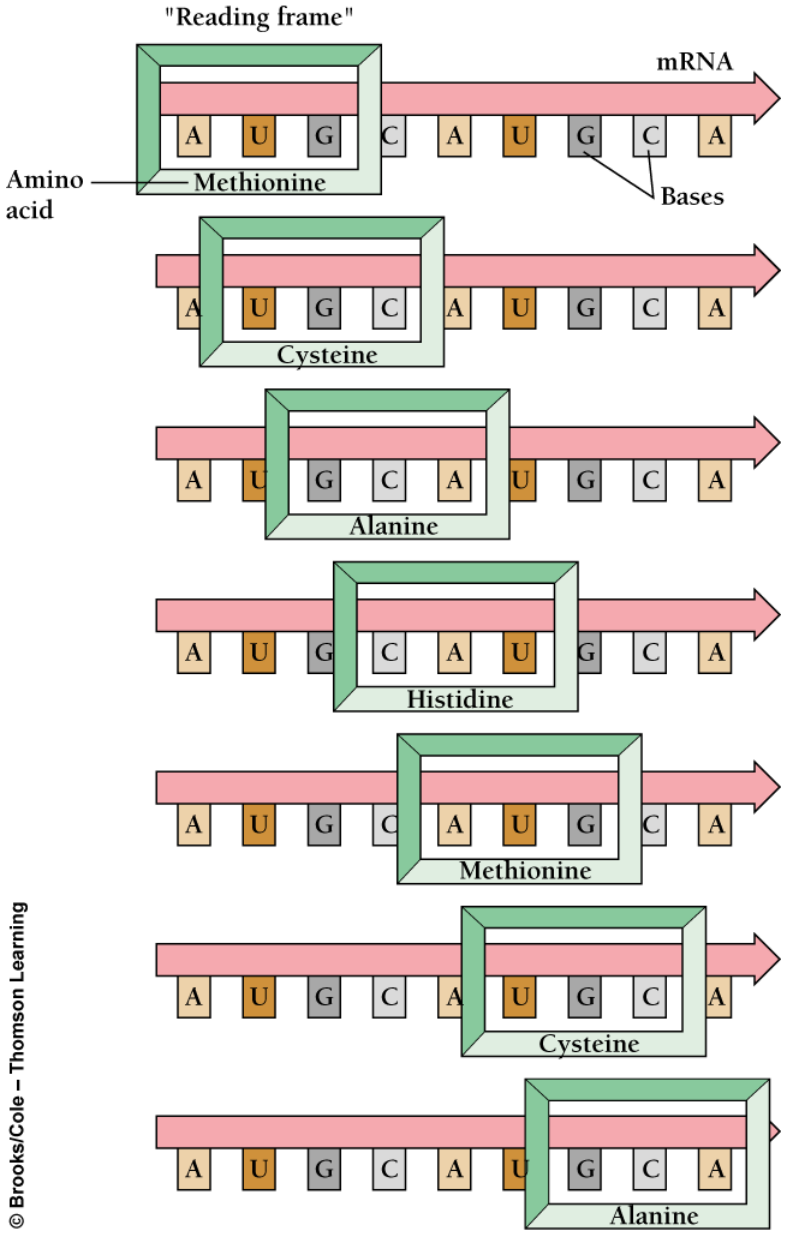
http://exploringorigins.org/ribozymes.html

Reading Frame

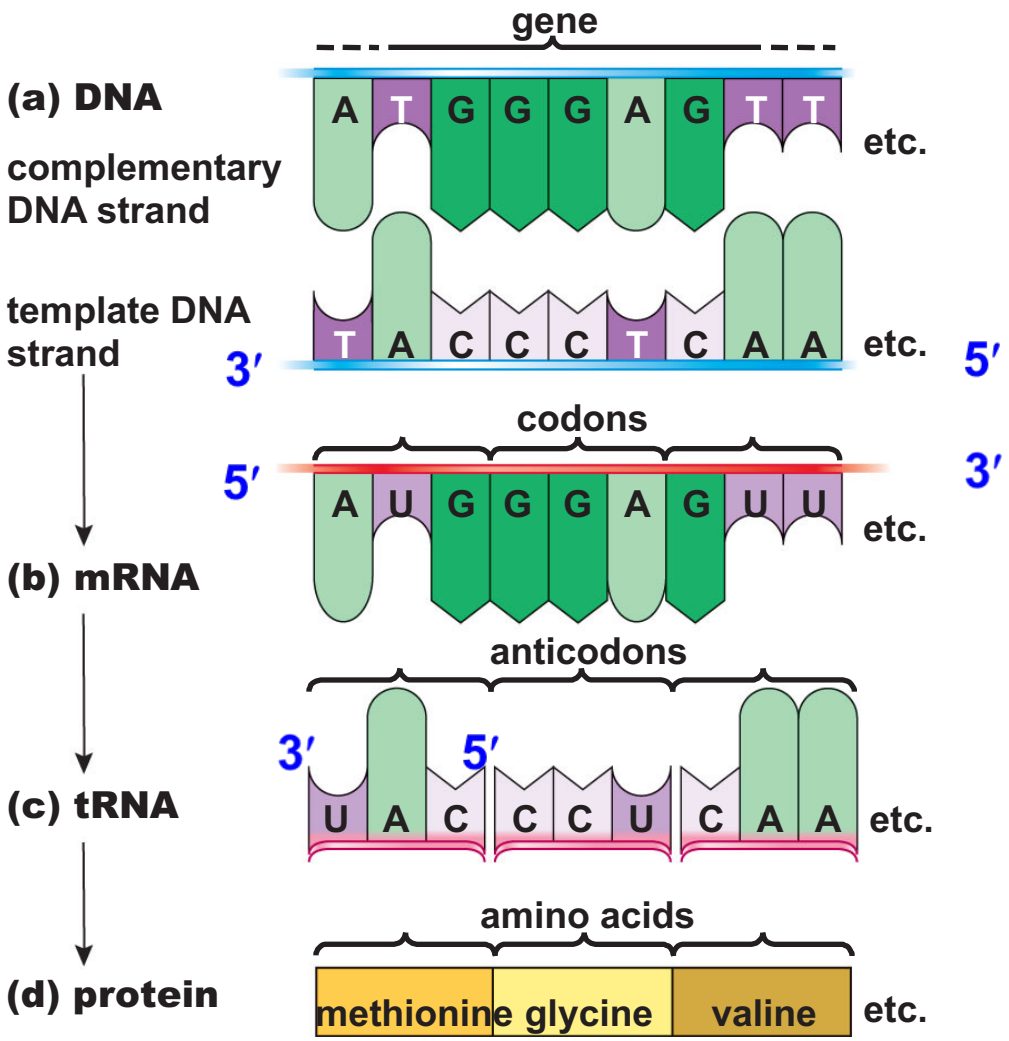
- A reading frame is a way of dividing the sequence of nucleotides in a nucleic acid (DNA or RNA) molecule into a set of consecutive, non-overlapping **triplets**.

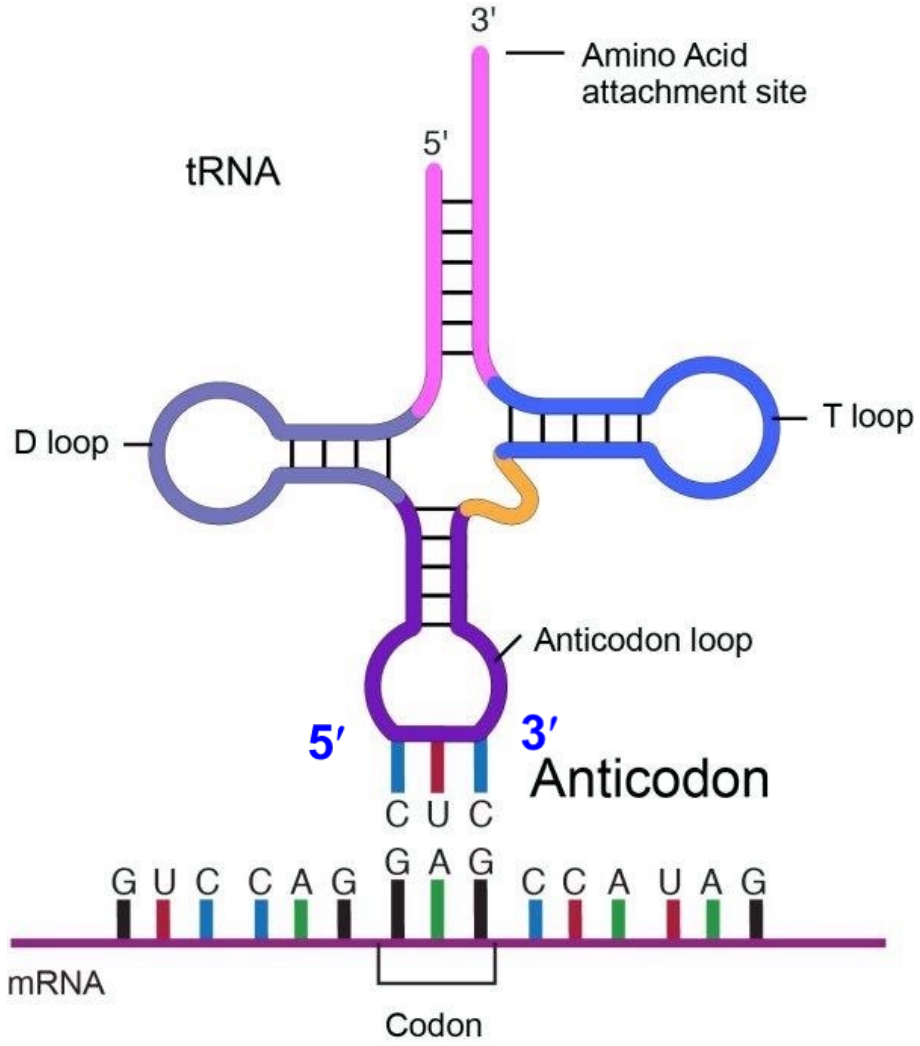
- Codons must be read in the correct **reading frame** (correct groupings) in order for the specified polypeptide to be produced.

- A **frameshift** mutation is a genetic mutation caused by a deletion or insertion in a DNA sequence that shifts the way the sequence is read



Complementary Base-Pairing Is Critical to the Process of Decoding Genetic Information





Translation Process

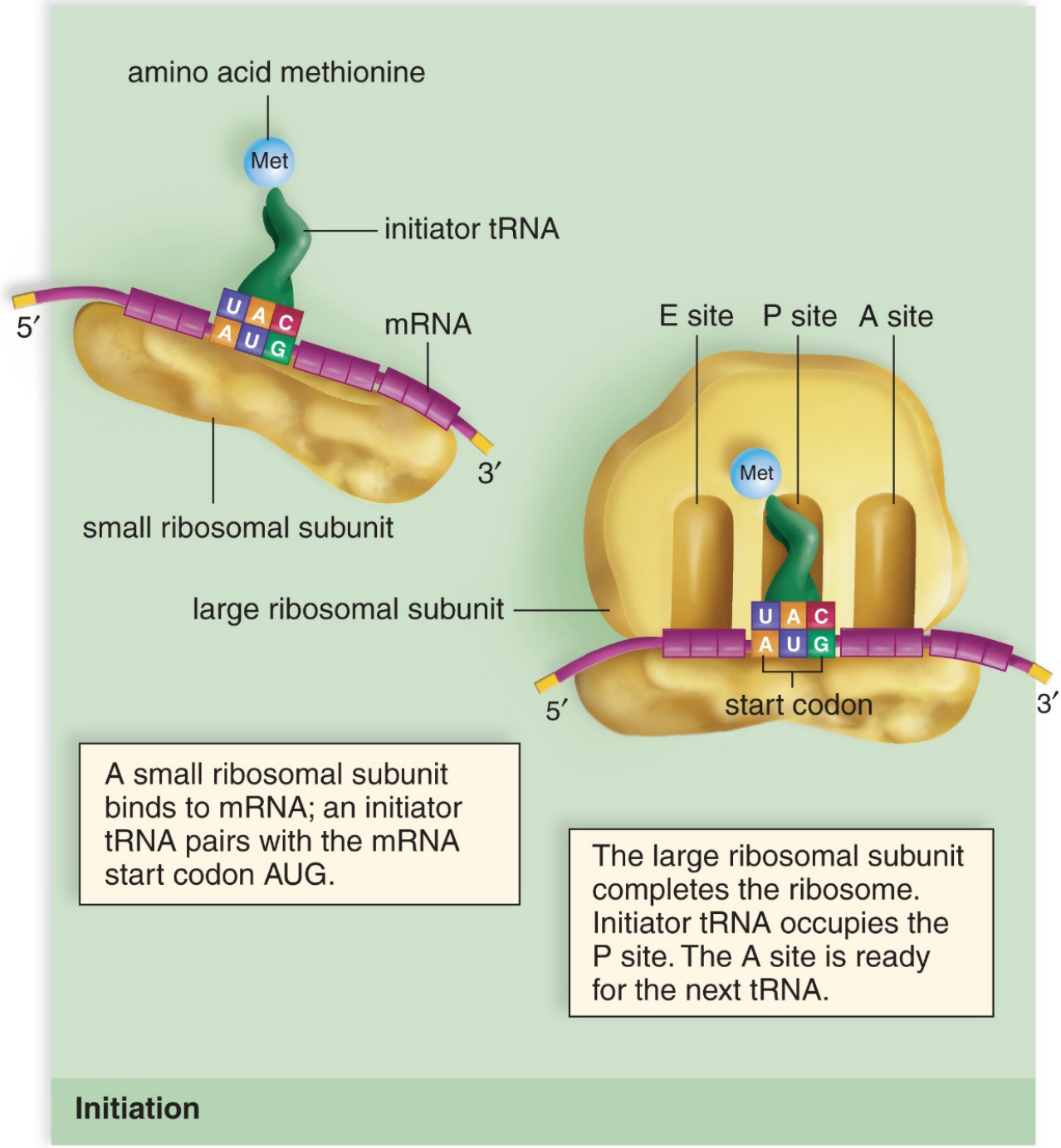
Ribosomes, tRNA, and mRNA cooperate in protein synthesis.

Like transcription, translation has three steps

- Initiation
- Elongation
- Termination

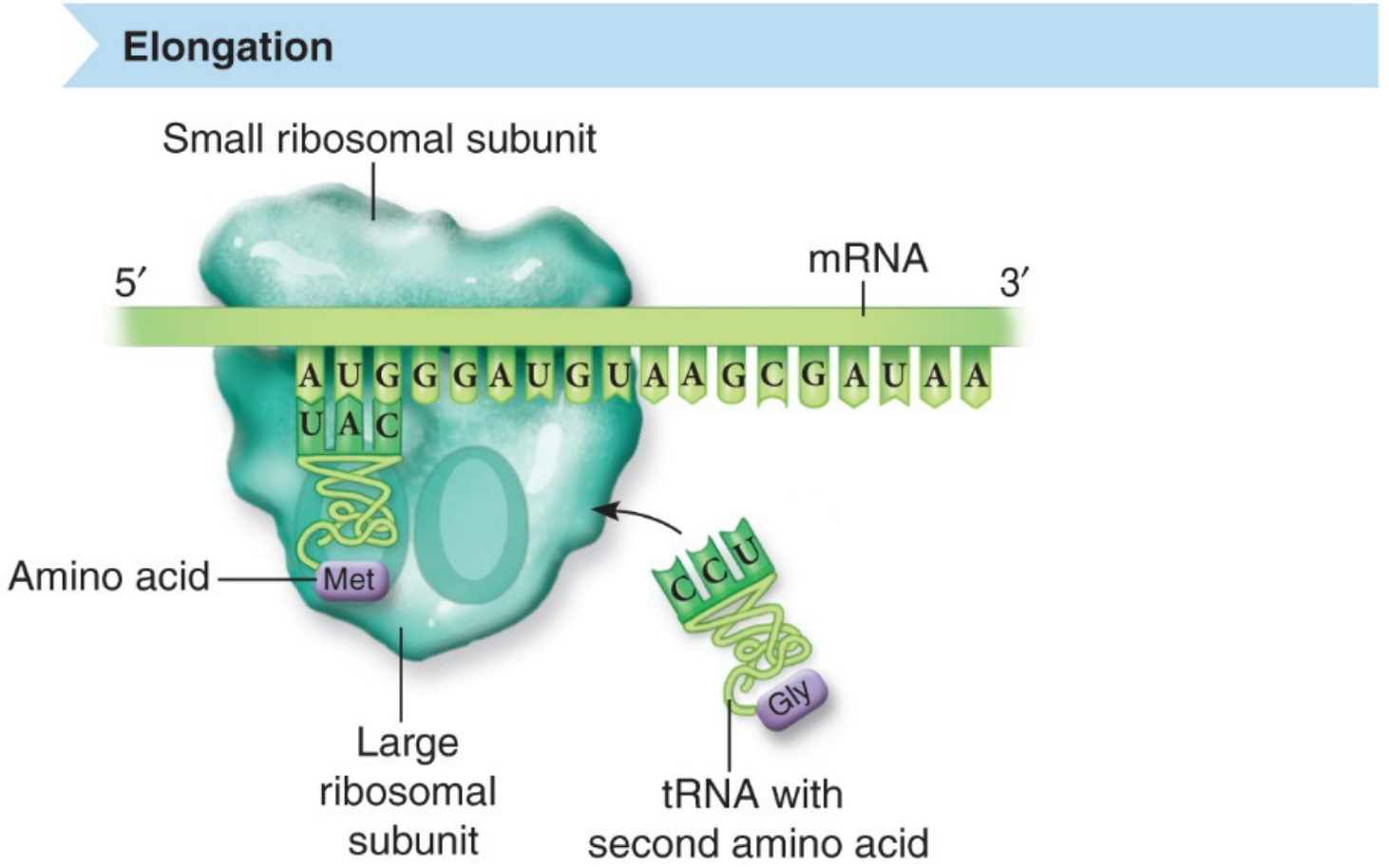
Initiation

- Formation of initiation complex
  - Small ribosomal subunit and initiation factors bind to 5' end of mRNA transcript
  - UAC anticodon of initiator tRNA base-pairs with AUG start codon o mRNA
  - Initiator tRNA, which carries aminc acid methionine, attaches to P site
  - Large ribosomal subunit attaches t small subunit



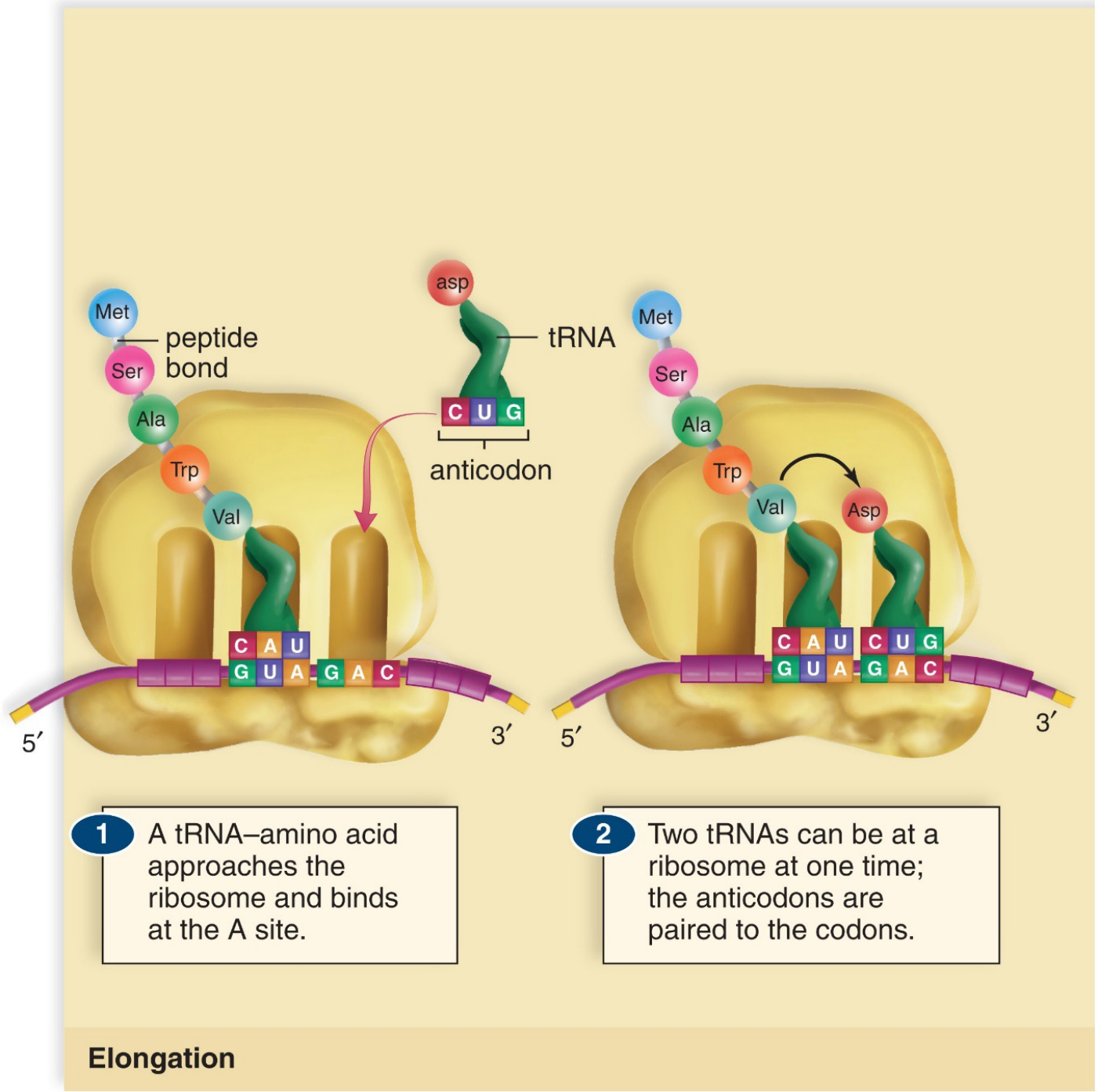
Elongation

- Ribosome reads second codon on mRNA
  - Only allows tRNA with anticodon complementary to second codon to attach to A site



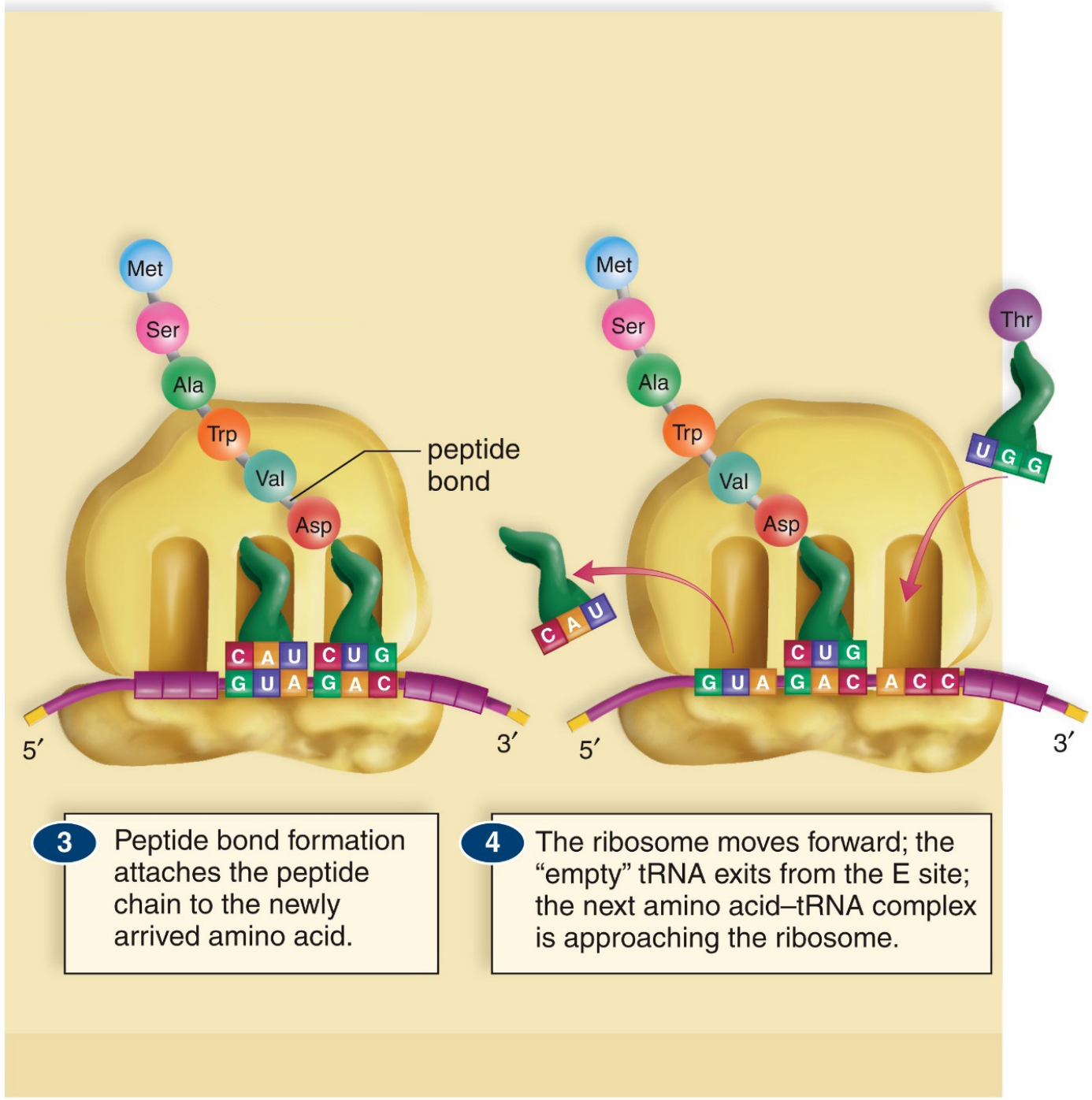
Elongation

- Catalytic site of large subunit
  - Breaks bond between methionine and initiator tRNA
  - Forms peptide bond between methionine and amino acid attached to second tRNA



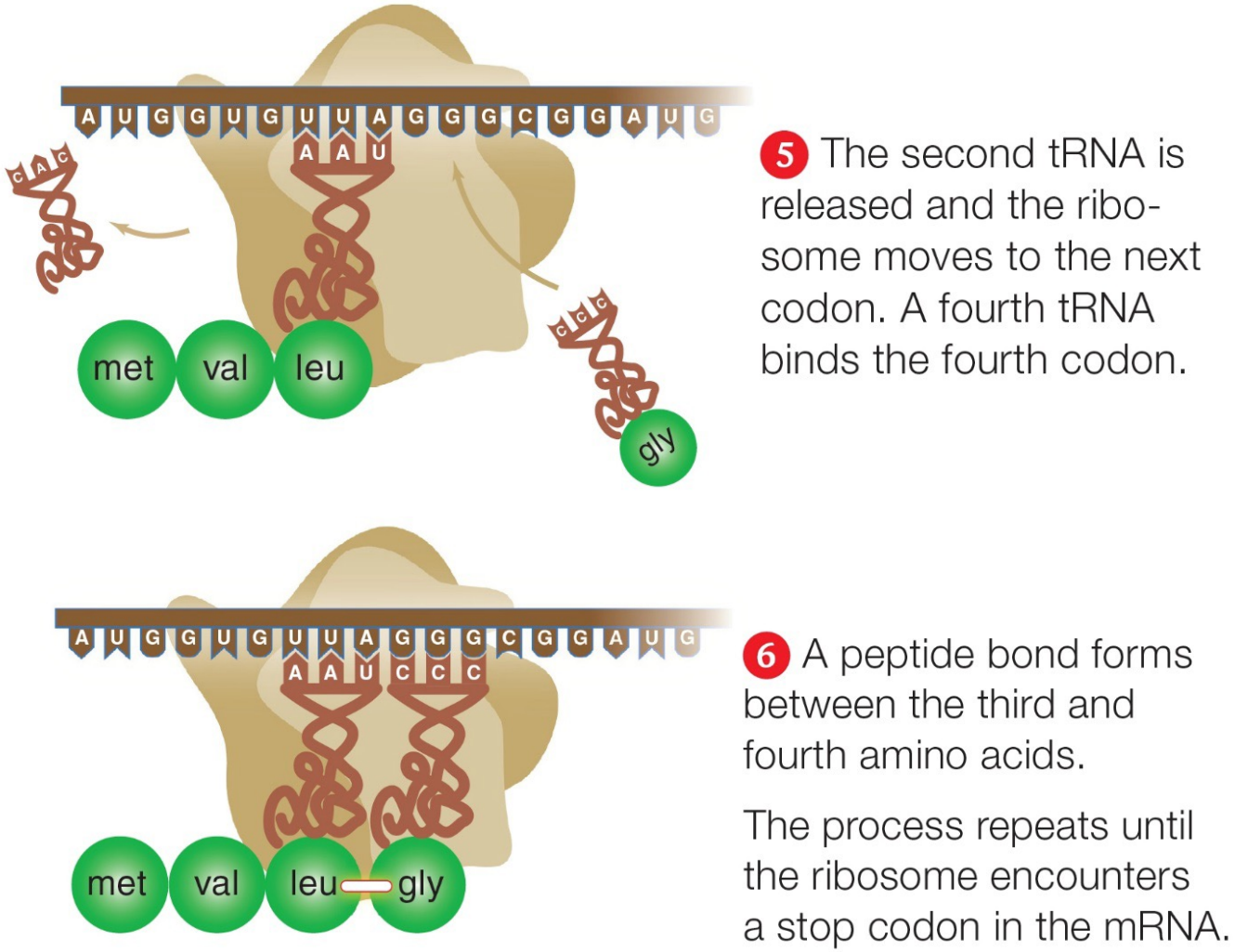
Elongation

- Ribosome moves down mRNA by one codon
  - Empty initiator tRNA in P site is released and leaves ribosome
  - Second tRNA (with growing amino acid chain) moves to P site
  - A site is empty awaiting another tRNA



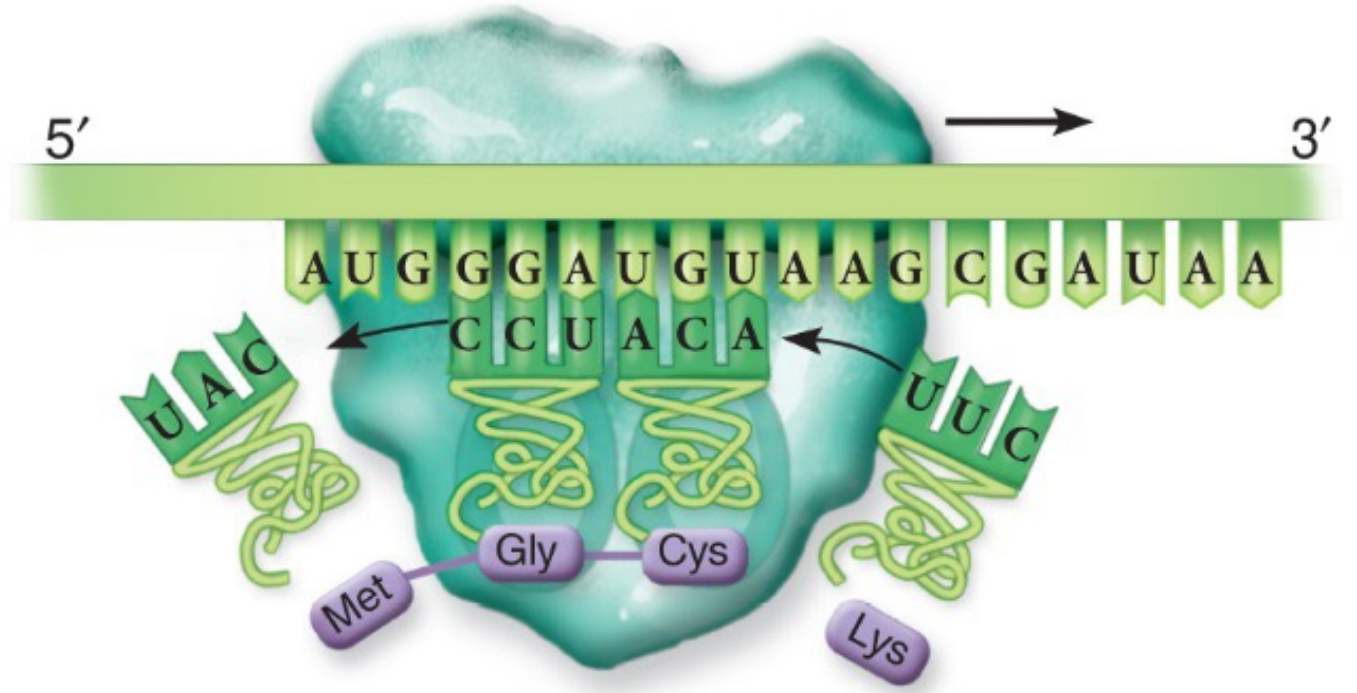
Elongation

- Ribosome reads next codon on mRNA
  - New tRNA with anticodon complementary to third codon joins ribosome at A site and base-pairs with codon on mRNA
  - Amino acid of third tRNA covalently linked to second amino acid of dipeptide through peptide bond by catalytic site on large subunit



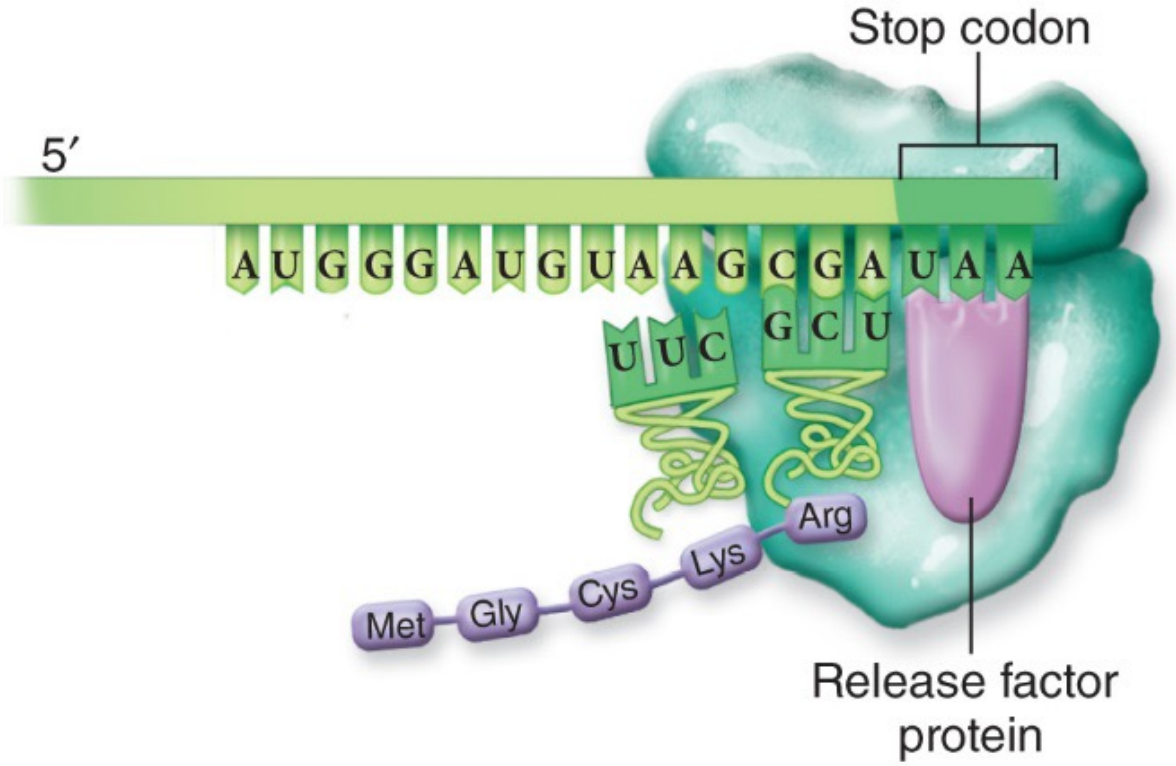
Elongation

- Ribosome shifts to next codon on mRNA
  - Empty second tRNA leaves ribosome
  - Elongation process repeats
  - One codon each time until stop codon
- Empty tRNAs reloaded with appropriate amino acids
  - Catalysed by enzymes in cytoplasm



Termination

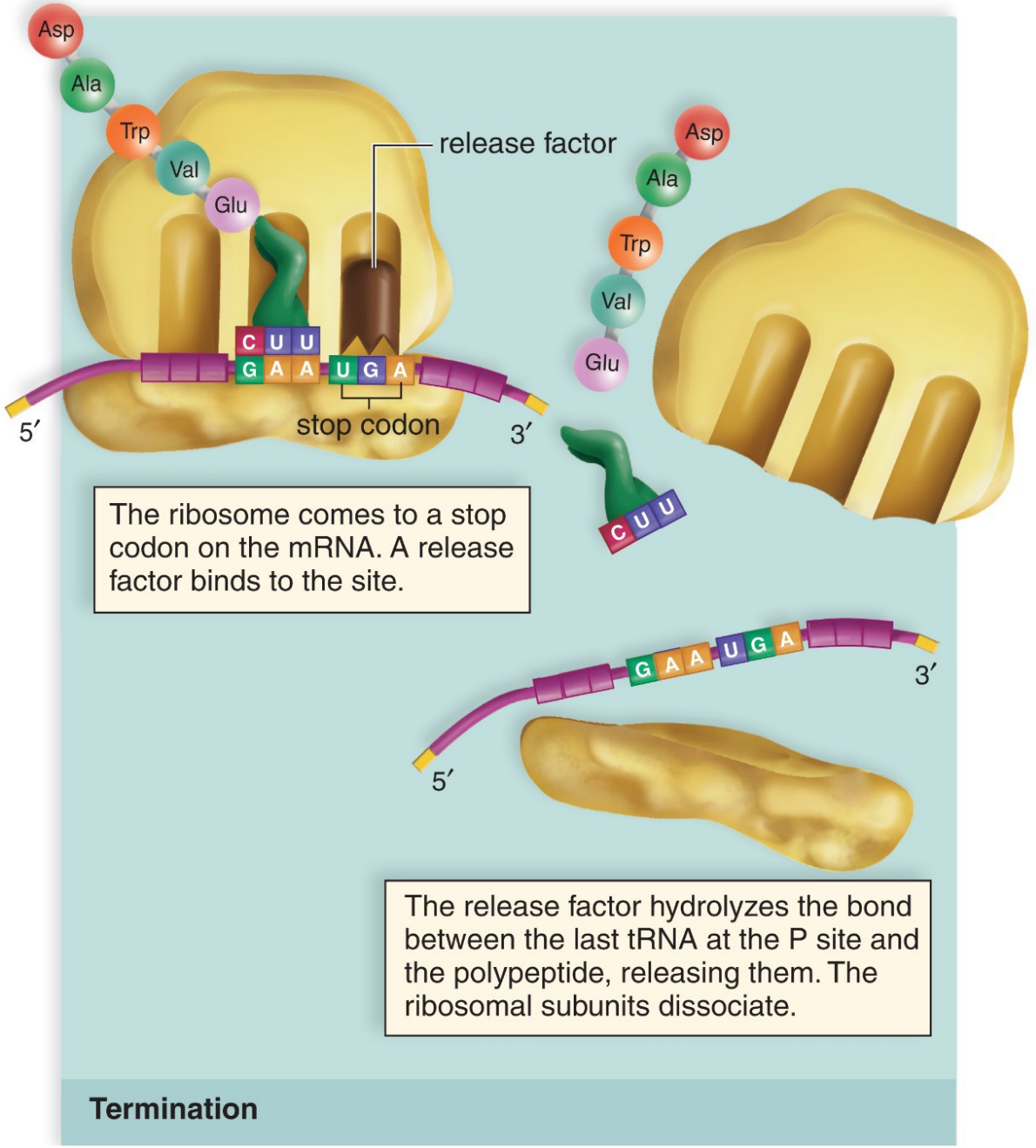
- Ribosome reaches a stop codon at end of mRNA
  - No tRNA molecules bind to stop codons
  - Protein called release factor binds to stop codon

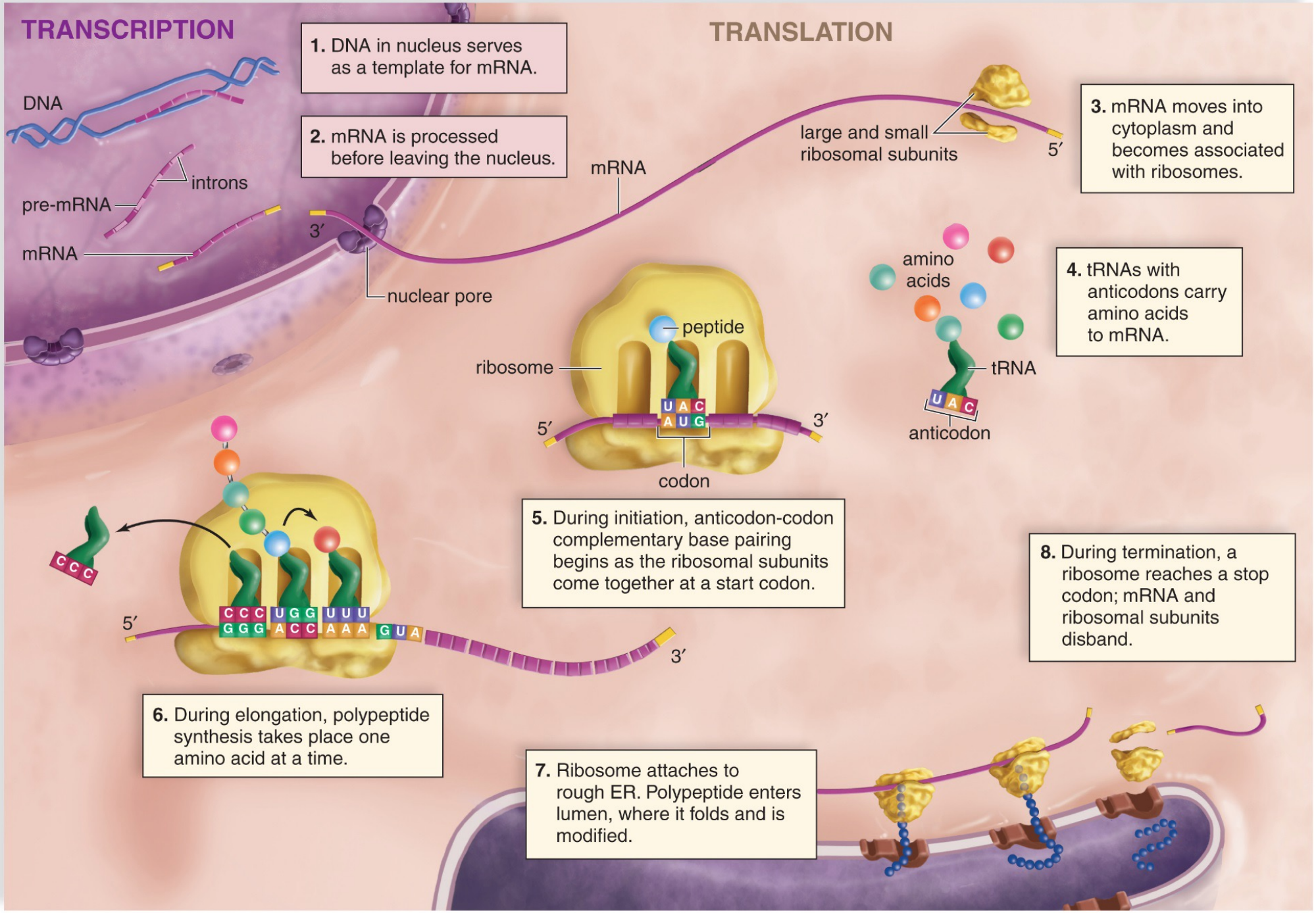


Termination

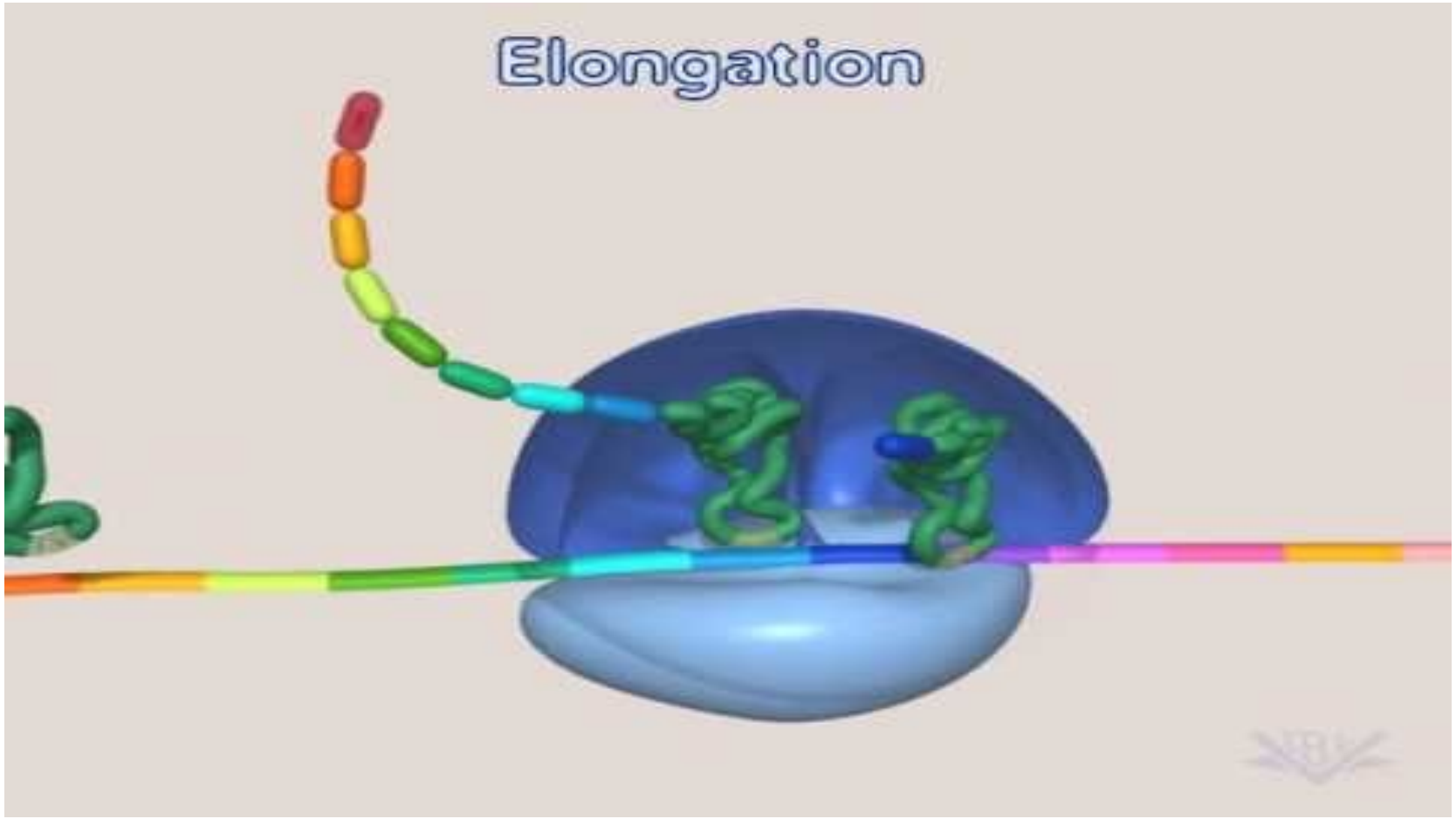
- Releases factor triggers cleavage of polypeptide chain from last tRNA
  - Ribosome releases mRNA
  - Ribosome disassembles and dissociates into large and small subunits

- Released mRNA may can be read by another ribosome to synthesise another polypeptide chain





Animation
Translation (video)



https://www.youtube.com/watch?v=5bLEDd-PSTQ

Outline

- DNA to Proteins
  - RNA
  - Genetic code
- Transcription
  - Prokaryotic cells
  - Eukaryotic cells
- Transfer RNA
- Ribosomes
- Translation
  - Initiation
  - Elongation
  - Termination
- Regulation of Gene Expression
  - Prokaryotic regulation
  - Eukaryotic regulation
  - Androgen insensitivity syndrome

Regulation of gene expression

- The human genome contains about 20,500 genes
  - Other than reproductive cells, all other cells in our bodies have the same DNA with same genes
- A given cell ‘expresses’ only a small number of genes at any given time
  - Rarely more than 5-10%
- Some genes are expressed in all cells
  - As some proteins have to be made all the time

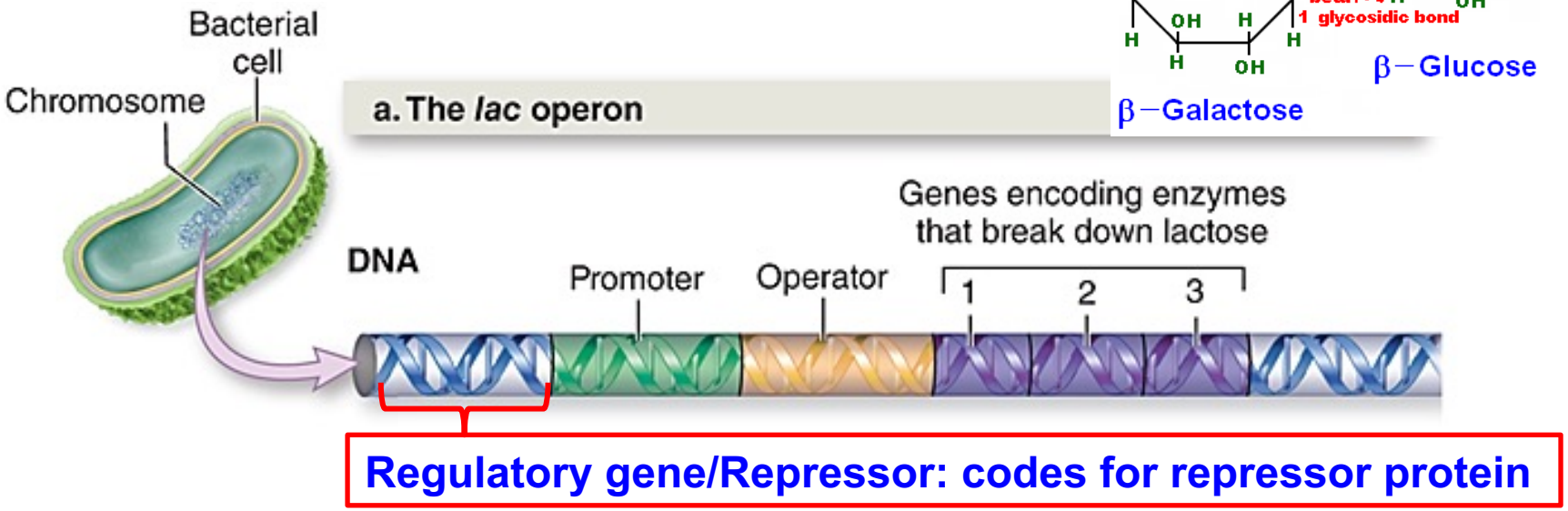
Regulation of gene expression

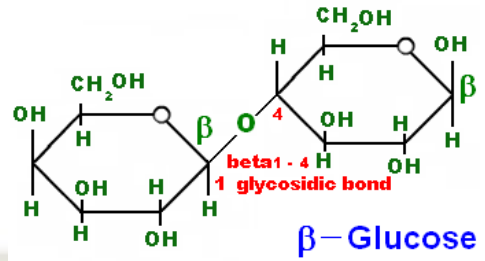
- To save energy, other genes are expressed exclusively
- Some are expressed in certain types of cells
  - As each type of cell expresses different subset of genes
- Some are expressed at certain times in an organism's life
  - As which genes are expressed at a particular time are crucial for proper growth and development
- Some genes are expressed under specific environmental conditions
  - As it provides flexibility to respond to changing conditions by governing the kinds and amounts of substances in cell at any given time

Gene Regulation in Prokaryotes

- Prokaryotic DNA is organized into units called **operons**, which contain functionally related genes
- Whole operons are regulated as units, so that functionally related proteins are synthesized simultaneously when the need arises
- Each operon consists of
  - A **regulatory gene**, which controls the transcription of other genes
  - A **promoter**, which RNA polymerase recognizes as the place to start transcribing
  - An **operator**, which governs access of RNA polymerase to the promoter
  - The **structural genes**, which encode for related proteins

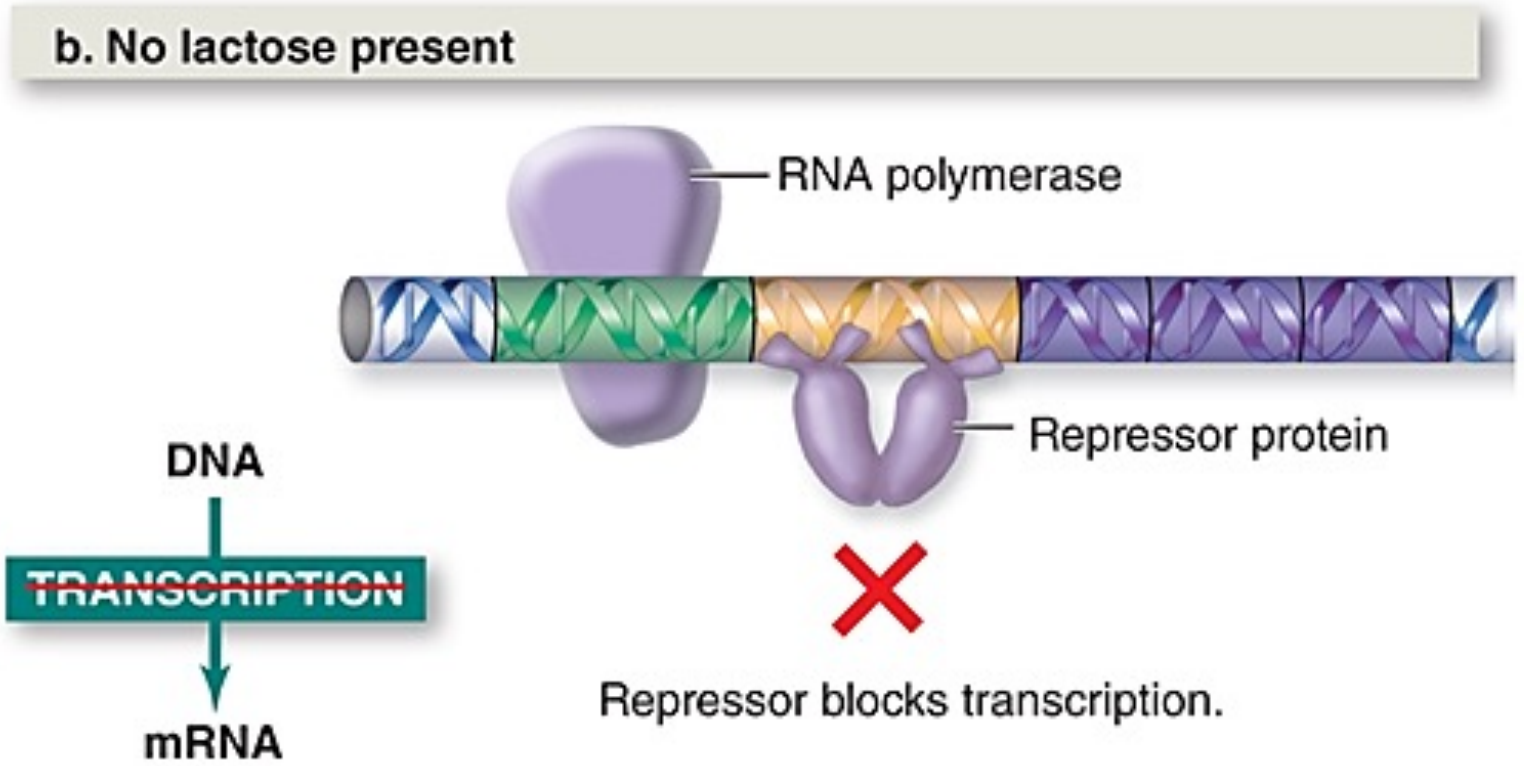
Structure of the lactose operon



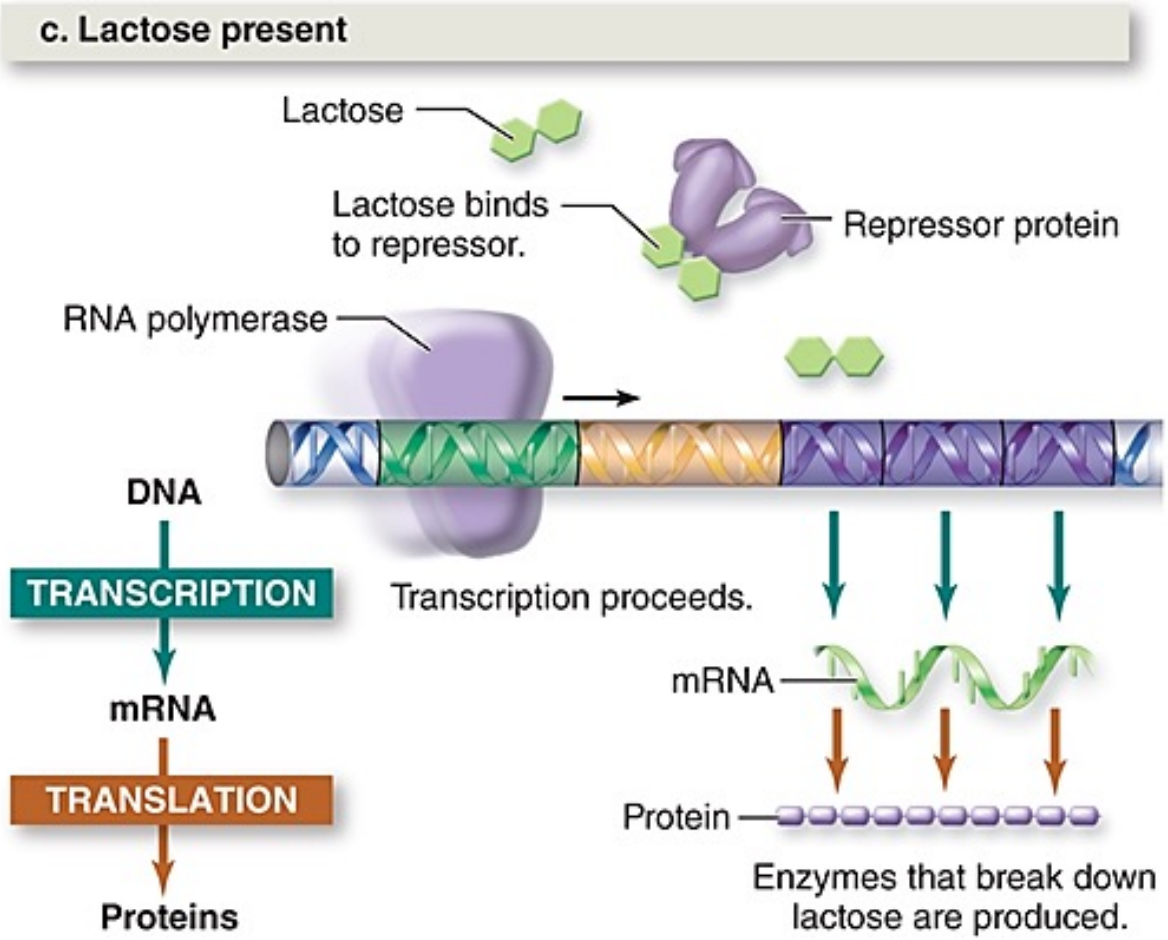


Promoter: RNA polymerase binds to
Operator: Repressor protein binds to

The lactose operon consists of a regulatory gene, a promoter, an operator, and three structural genes that code for enzymes involved in lactose metabolism. The regulatory gene codes for a protein, called a repressor, which can bind to the operator site under certain circumstances.

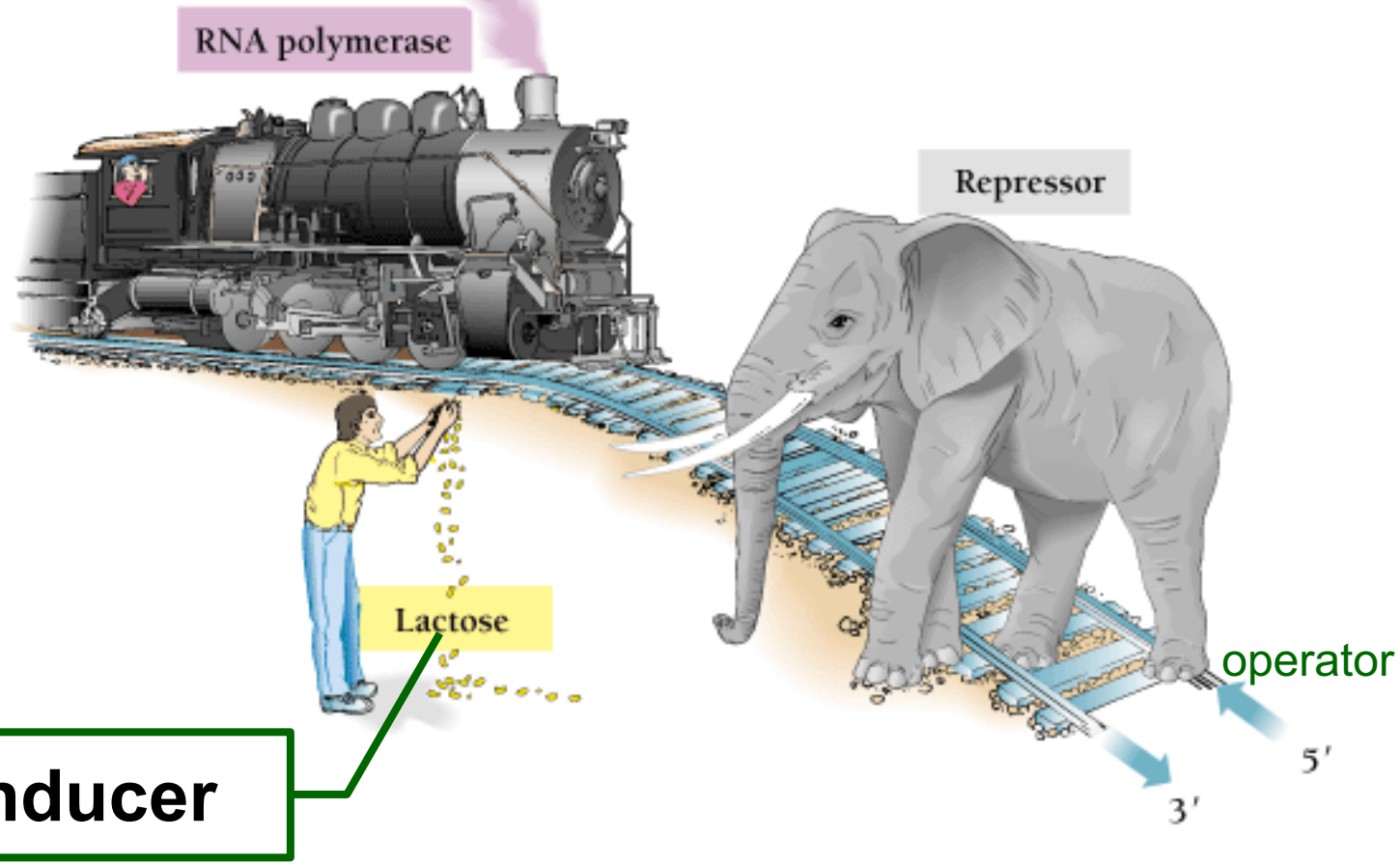


When lactose is not present, repressor proteins bind to the operator of the lactose operon. When RNA polymerase binds to the promoter, the repressor protein blocks access to the structural genes, which therefore cannot be transcribed.



When lactose is present, it binds to the repressor protein. The lactose-repressor complex cannot bind to the operator, so RNA polymerase has free access to the promoter. The RNA polymerase transcribes the three structural genes coding for the lactose-metabolizing enzymes.

Prokaryotic Regulation



Without the repressor in the operator, RNA polymerase is able to reach the promoter and begin transcription of the genes needed to metabolize lactose

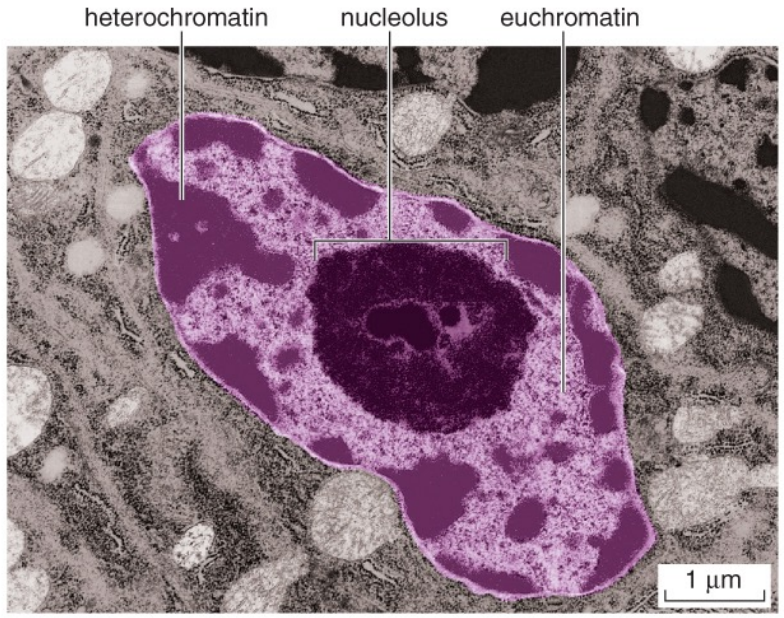
Gene Regulation in Prokaryotes

https://www.youtube.com/watch?v=oBwtxdI1zvk

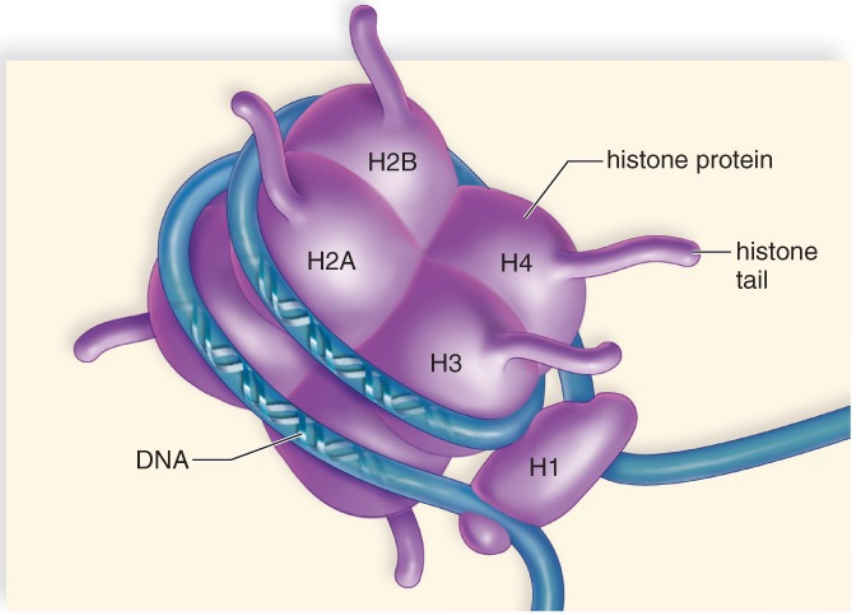
Eukaryotic regulation

- In eukaryotes, regulation of gene expression at many levels, at any step of protein production
- Chromatin structure
  - In eukaryotic chromosomes, DNA is wound around histone proteins
  - Condensed or tightly wound regions of DNA – genes inaccessible to RNA polymerase, will decondense when genes are needed
  - Chemical modifications affect accessibility of RNA polymerase to genes – addition of acetyl groups to histone proteins encourage transcription, addition of methyl groups prevent transcription

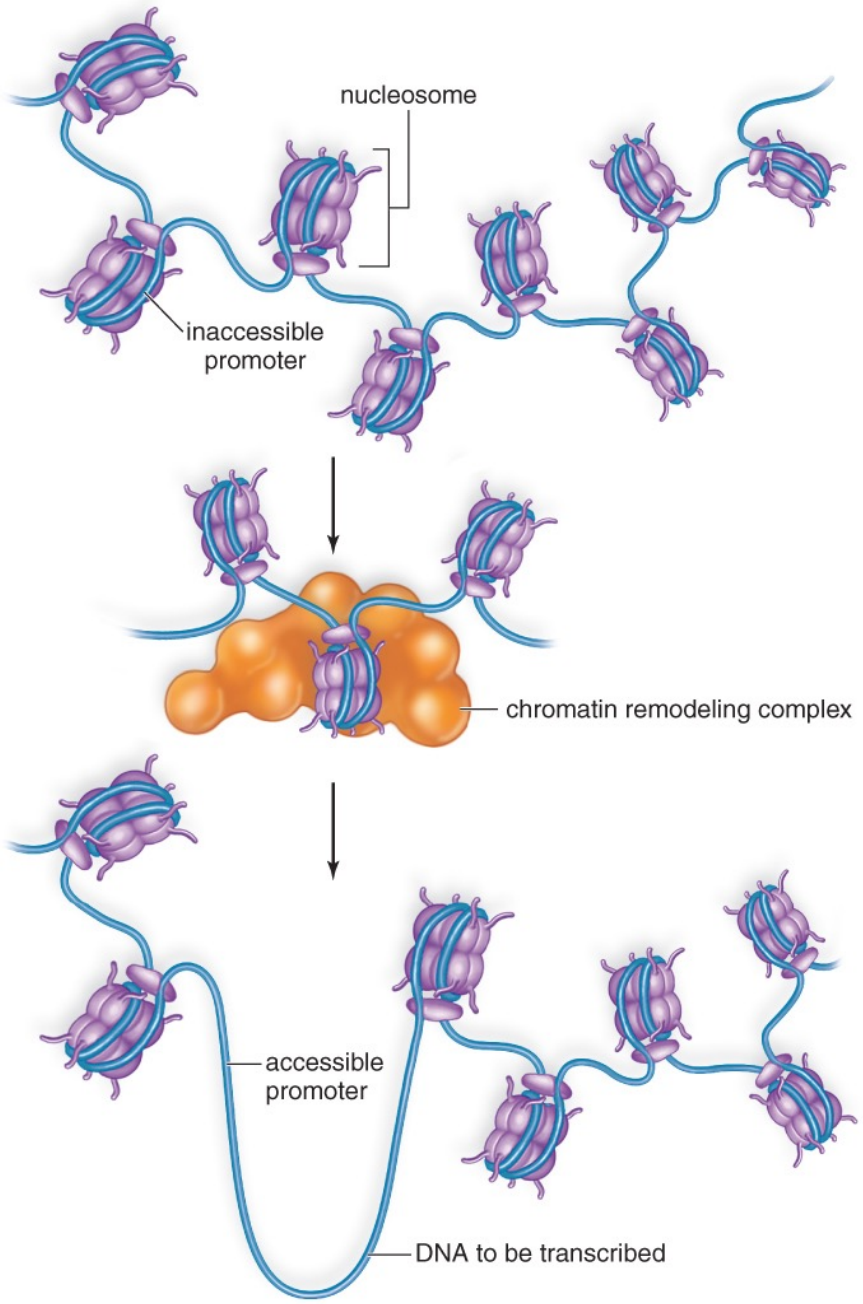
Eukaryotic regulation



a. Darkly stained heterochromatin and lightly stained euchromatin



b. A nucleosome



c. DNA unpacking

a: © Dennis Kunkel Microscopy, Inc./Visuals Unlimited

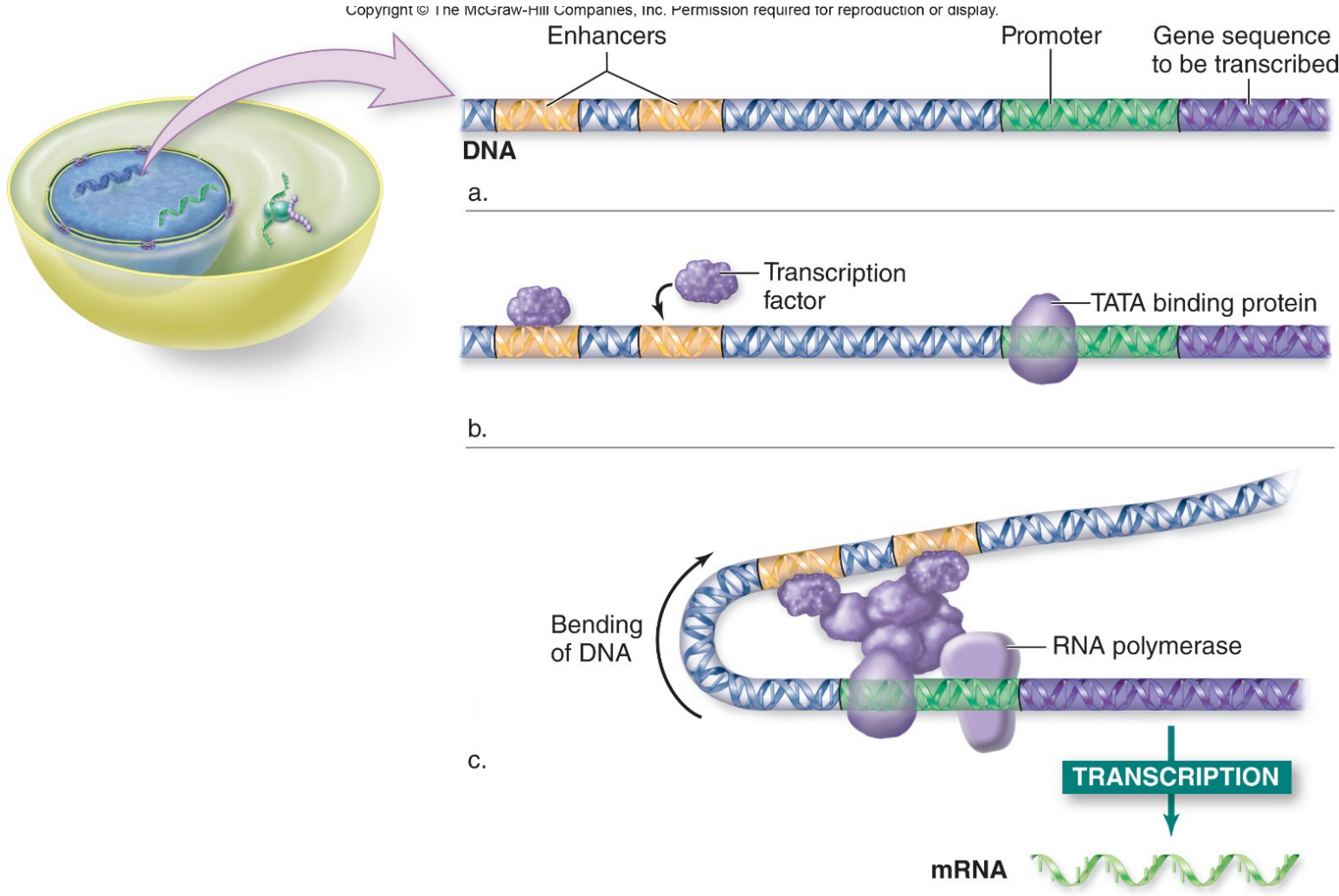
Eukaryotic Regulation

Gene expression is regulated at a number of points

- Transcription
- Post-transcription
- Translation
- Post-translational modification
- Life span of mRNA and proteins

Eukaryotic Regulation

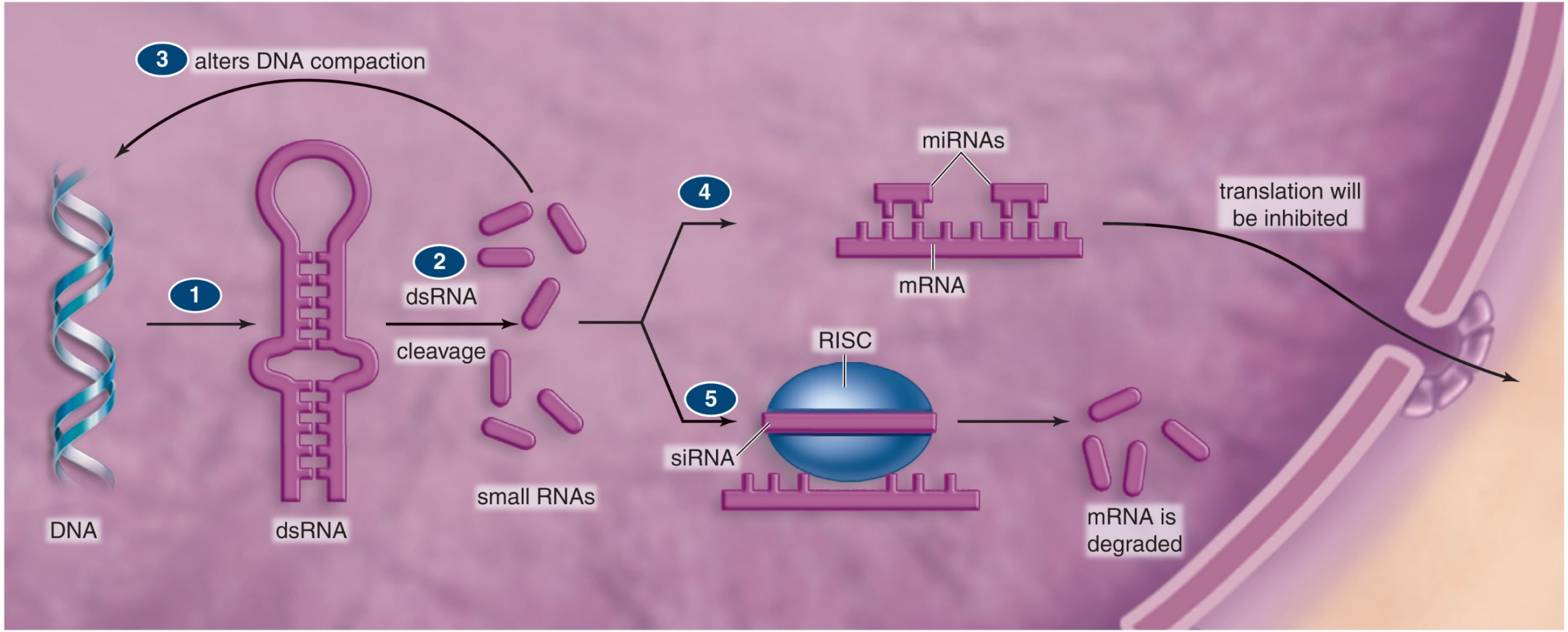
- Transcriptional control
  - By regulatory proteins called transcription factors by binding to regions of DNA
  - Some transcription factors must be activated before binding to DNA
  - Regions of DNA – promoter, enhancers (distant from gene)



Eukaryotic Regulation

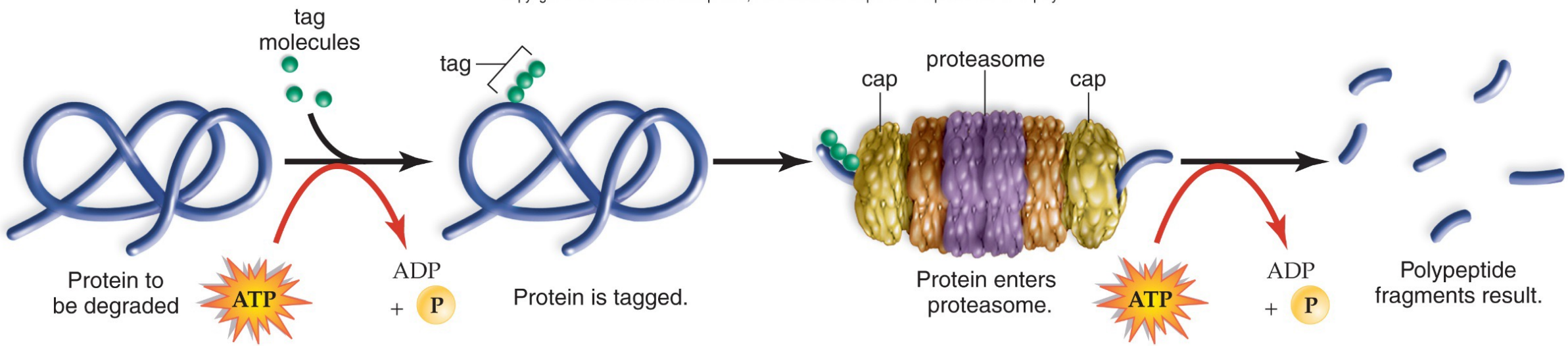
- Post-transcriptional control
  - Alternative splicing produce different mature mRNA from same gene – regulating types of protein synthesised
  - Transport of mRNA from nucleus to cytoplasm – regulating rate of protein synthesis
- Translational control
  - Control of stability and translation of mRNA into protein
  - How long mRNA remains active regulated by presence of 5' cap, length of poly-A tail
  - RNA interference by microRNA (miRNA) regulate translation by blocking translation of some mRNAs or destroying some mRNAs before translation

Eukaryotic Regulation



Eukaryotic Regulation

- Post-translational control
  - By regulating activity of proteins
  - Some proteins require modifications to be activated – through excision of a segment of the protein, through addition or removal of phosphate groups
- Activity also regulated by preventing or promoting degradation of proteins



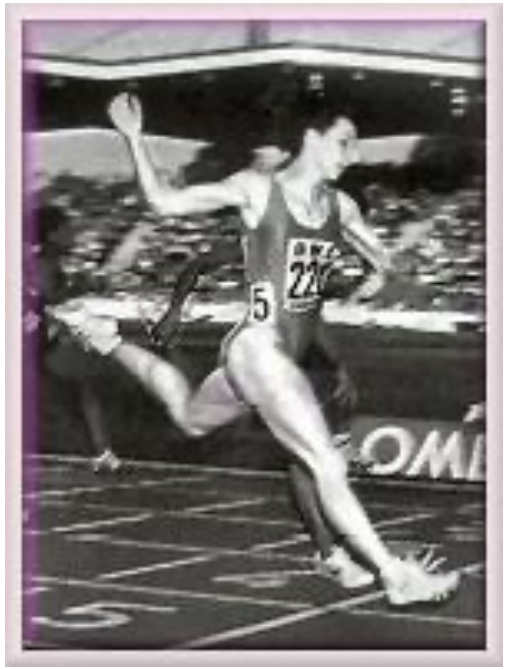
Gene Regulation in Eukaryotes

https://www.youtube.com/watch?v=xh5k6r-oscE

Androgen Insensitivity Syndrome

- Androgens (like testosterone) bind to androgen receptor proteins
  - Resulting androgen-receptor complex binds to DNA
  - Genes related to development of secondary male characteristics expressed
- Mutation in gene for androgen receptor protein
  - Androgen unable to bind to receptor protein
  - Secondary female instead of male characteristics expressed
  - XY female

Case study: Maria Jose Martinez Patino



Intersex athletes

(http://www.instruction.greenriver.edu/kmarr/biology100/Lectures/Maria%20Patino%20Story/Maria%20Patino%20Story.HTM)

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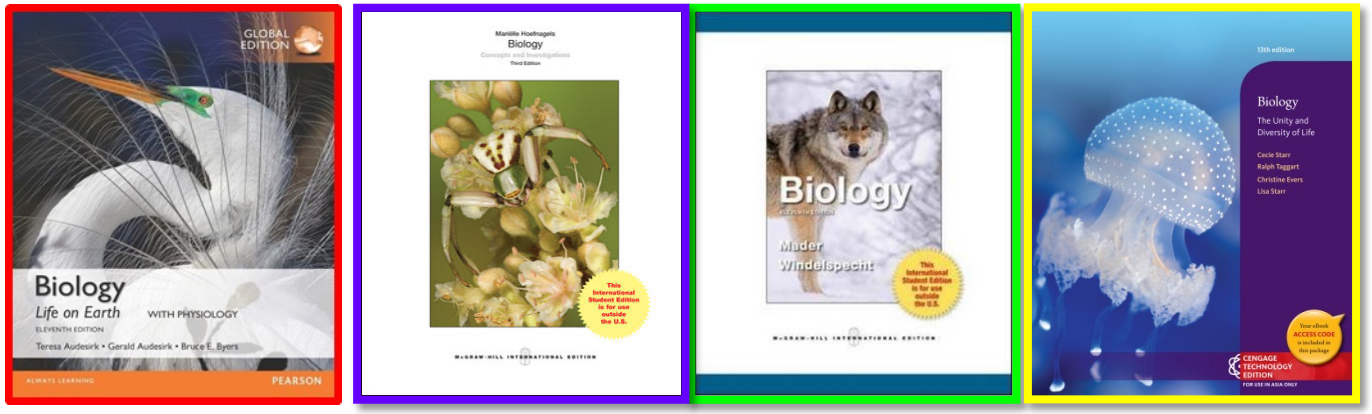
Text Books/References



This Lecture: Gene Expression

Chapters 13	Chapter 3, 7, 8	Chapters 13	Chapter 9, 10

Next Lecture: Biotechnology

Chapter 14	Chapters 11	Chapters 14	Chapters 15

Announcements/Reminders

- Midterm review by Dr. Nalini (after lecture today)
- Laboratory assignment 5 is due tomorrow (30th March, 23.59 hrs)
  - Make sure to check the Similarity Report
- Practical 6 is this Thursday (31th March)
  - Please wear long pants and shoes
  - Bring labcoat or purchase one
  - Read the handout before coming to lab