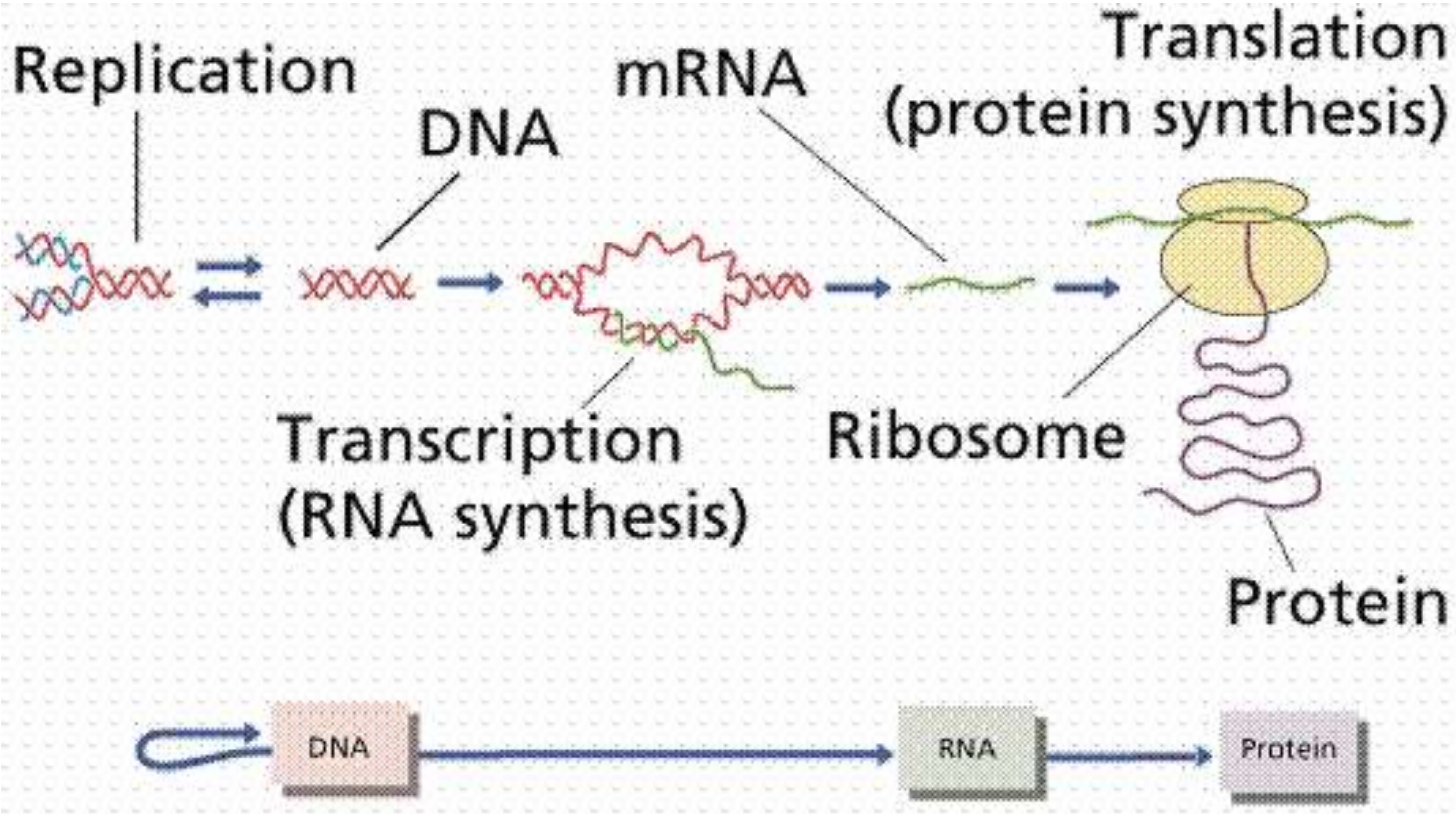
Chapter 17

From Gene to Protein

Modified by YJ Chuang at NTHU-MS

Central Dogma of Life

A term first used by **Crick**



http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html

Overview: The Flow of Genetic Information

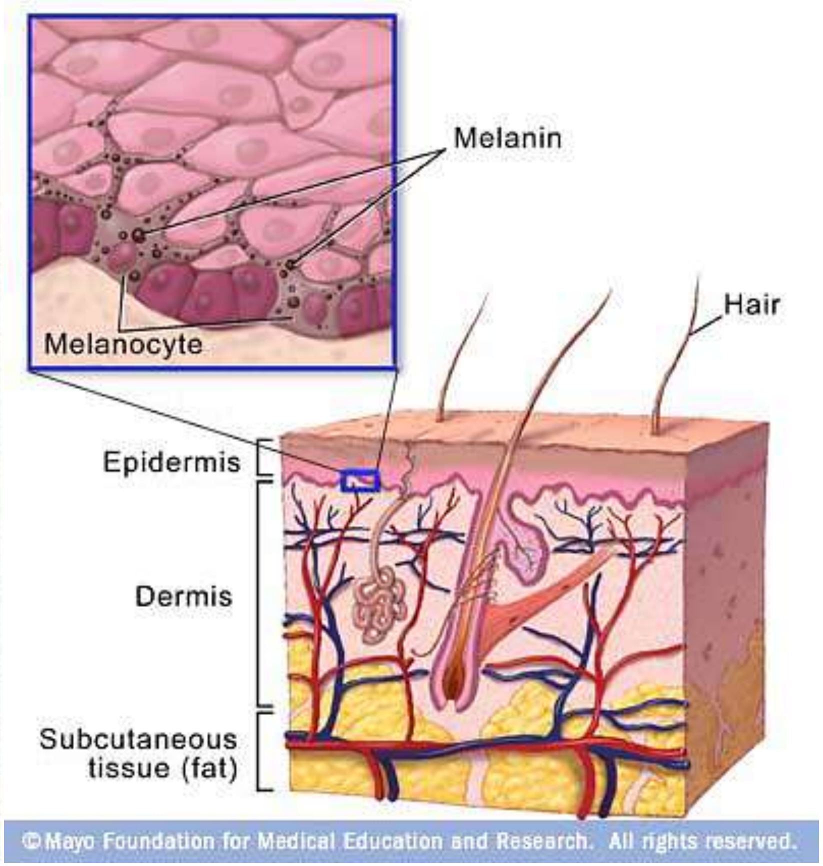
- The information content of DNA is in the form of specific sequences of nucleotides

- The DNA inherited by an organism leads to specific traits by dictating the synthesis of mRNAs and proteins

- Proteins are the links between genotype and phenotype

- **Gene expression**, the process by which DNA directs protein synthesis, includes two stages: transcription and translation

Question: How does a single faulty gene result in the dramatic appearance of an albino raccoon?

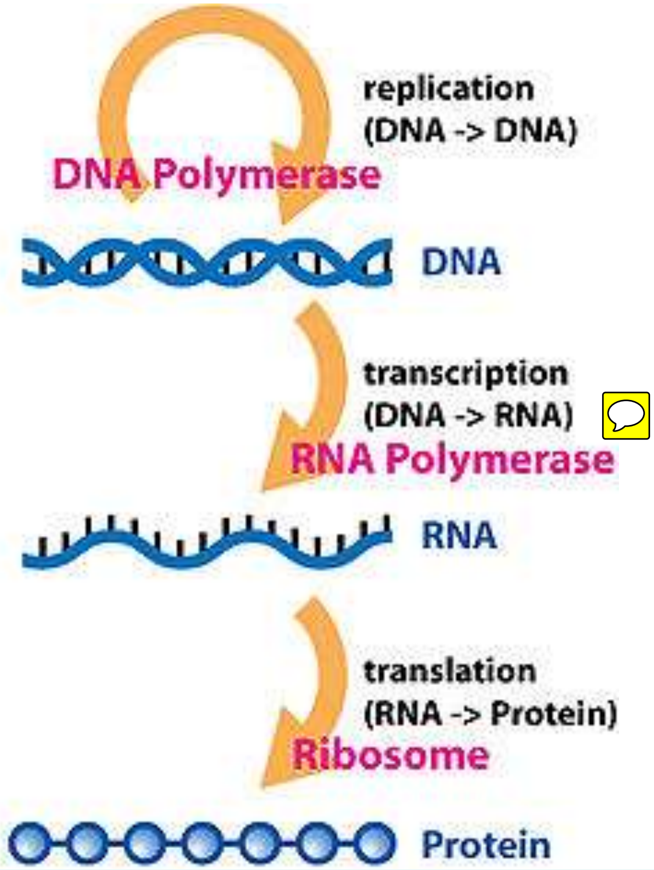


© Mayo Foundation for Medical Education and Research. All rights reserved.

Mutation in gene(s) involved in the production of a pigment called melanin.

Concept 17.1: Genes specify proteins via transcription and translation

- How was the fundamental relationship between genes and proteins discovered?



Evidence from the Study of Metabolic Defects

- In 1909, British physician Archibald Garrod first suggested that genes dictate phenotypes through **enzymes** that catalyze specific chemical reactions

- He thought symptoms of an inherited disease reflect an inability to synthesize a certain enzyme

- Linking genes to enzymes required understanding that cells synthesize and degrade molecules in a series of steps, a metabolic pathway

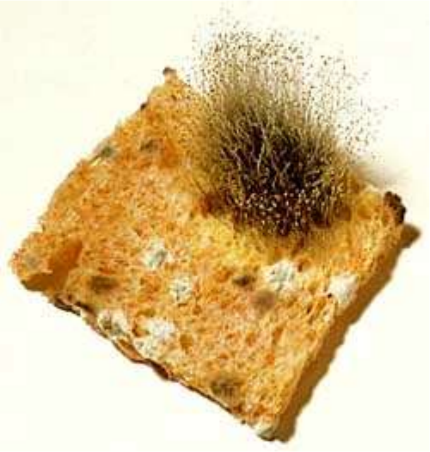
Nutritional Mutants in Neurospora: Scientific Inquiry

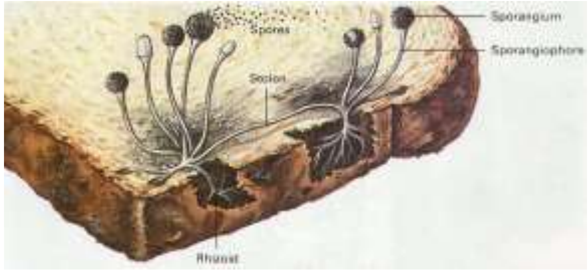
- George Beadle and Edward Tatum exposed **bread mold** to X-rays, creating mutants that were unable to survive on minimal medium as a result of inability to synthesize certain molecules
- Using crosses, they identified three classes of arginine-deficient mutants, each lacking a different enzyme necessary for synthesizing arginine
- They developed a *one gene–one enzyme hypothesis*, which states that each gene dictates production of a specific enzyme

Fig. 17-2a

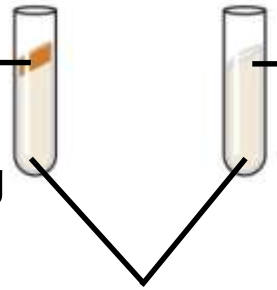
Do individual genes specify the enzymes that function in a biochemical pathway?

Bread Mold $\xrightarrow{\text{X-ray}}$ Mutated Molds





EXPERIMENT

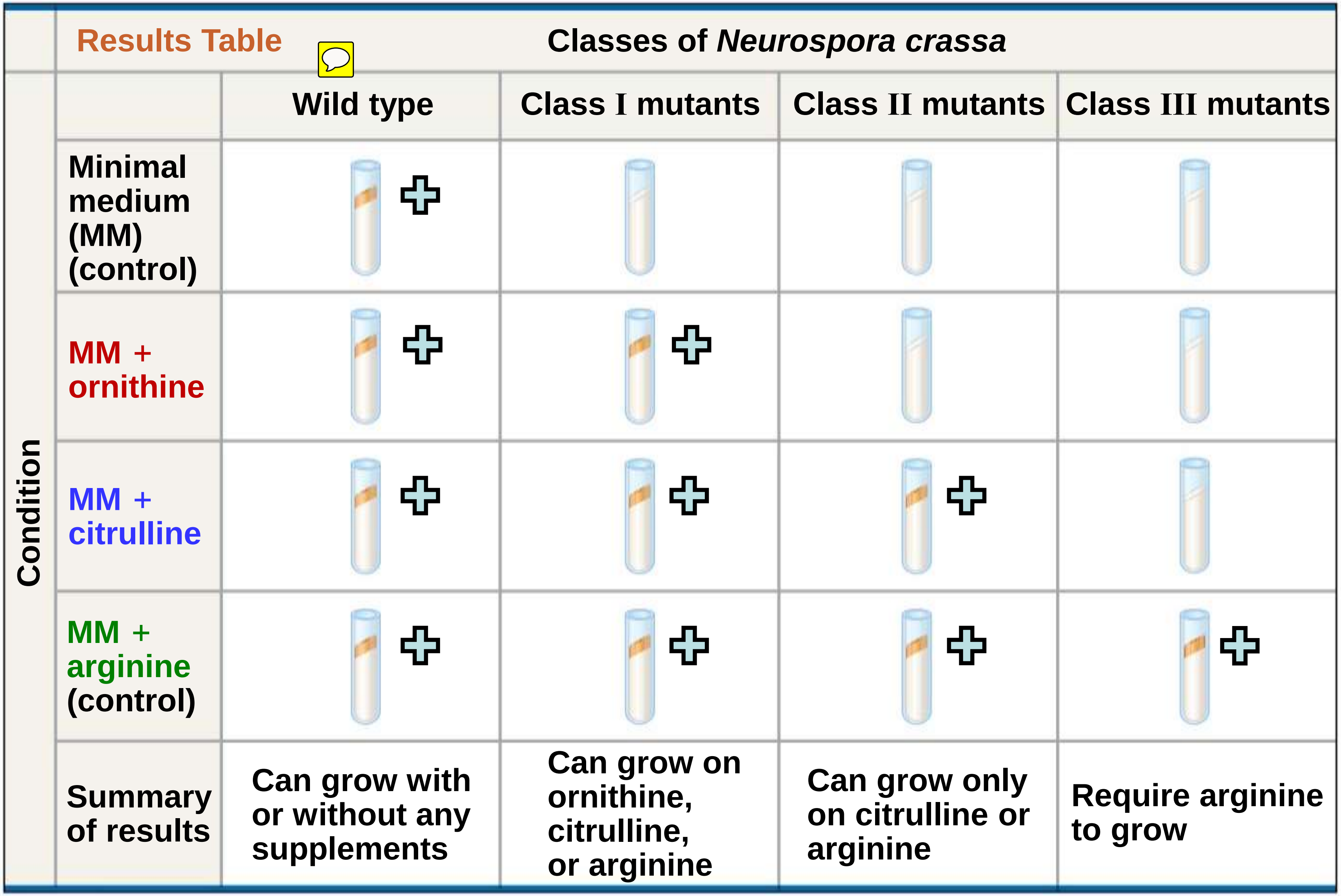


Growth:
Wild-type cells growing and dividing

No growth:
Mutant cells cannot grow and divide

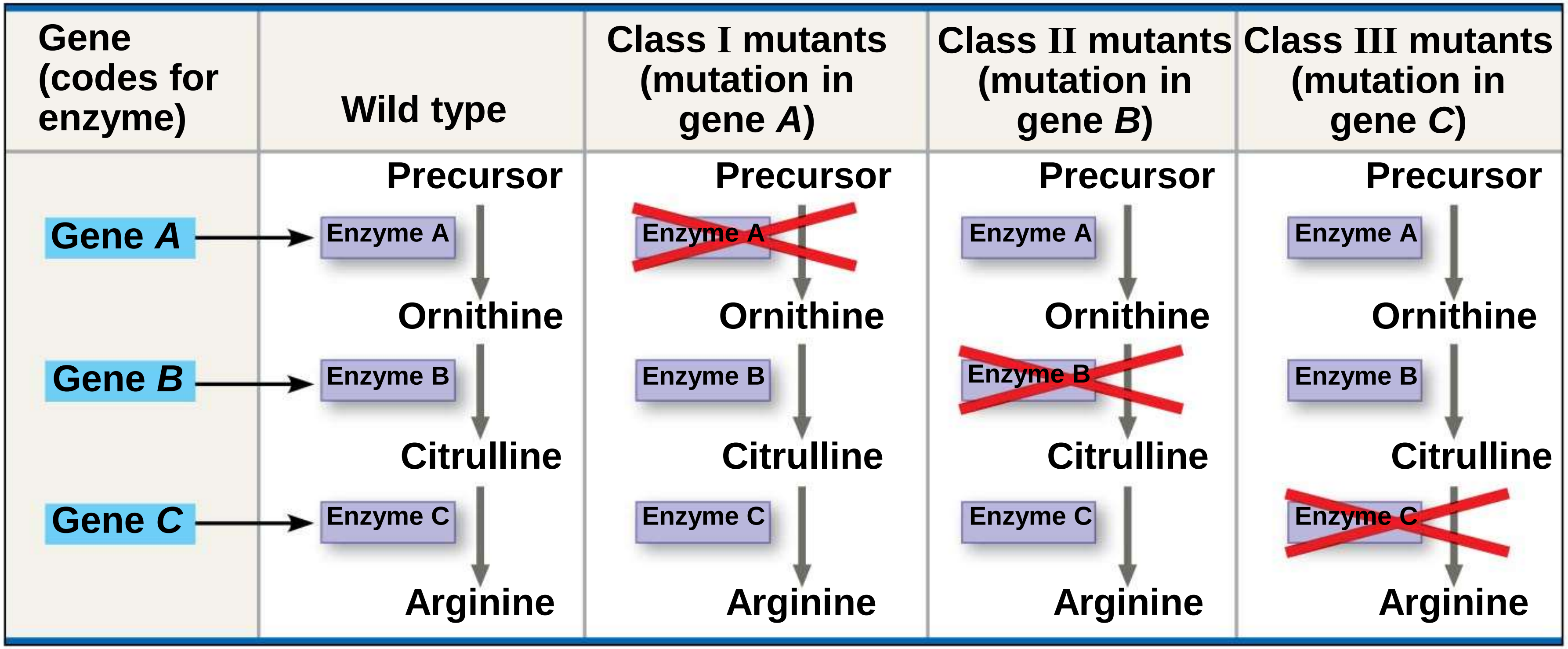
Minimal medium

Figure 17.2c



	Results Table	Classes of *Neurospora crassa*			
Condition		Wild type	Class I mutants	Class II mutants	Class III mutants
	Minimal medium (MM) (control)	+			
	MM + ornithine	+	+		
	MM + citrulline	+	+	+	
	MM + arginine (control)	+	+	+	+
	Summary of results	Can grow with or without any supplements	Can grow on ornithine, citrulline, or arginine	Can grow only on citrulline or arginine	Require arginine to grow

Figure 17.2d



Gene (codes for enzyme)	Wild type	Class I mutants (mutation in gene *A*)	Class II mutants (mutation in gene *B*)	Class III mutants (mutation in gene *C*)
	Precursor	**Precursor**	**Precursor**	**Precursor**
Gene *A* →	Enzyme A ↓	~~Enzyme A~~ ↓	Enzyme A ↓	Enzyme A ↓
	Ornithine	**Ornithine**	**Ornithine**	**Ornithine**
Gene *B* →	Enzyme B ↓	Enzyme B ↓	~~Enzyme B~~ ↓	Enzyme B ↓
	Citrulline	**Citrulline**	**Citrulline**	**Citrulline**
Gene *C* →	Enzyme C ↓	Enzyme C ↓	Enzyme C ↓	~~Enzyme C~~ ↓
	Arginine	**Arginine**	**Arginine**	**Arginine**

Alternative Summary

補充資料

Arg-1 mutants
Can grow if given ornithine <u>or</u> citrulline; lack enzyme A

Arg-2 mutants
Can grow if <u>only</u> given citrulline; lack enzyme B

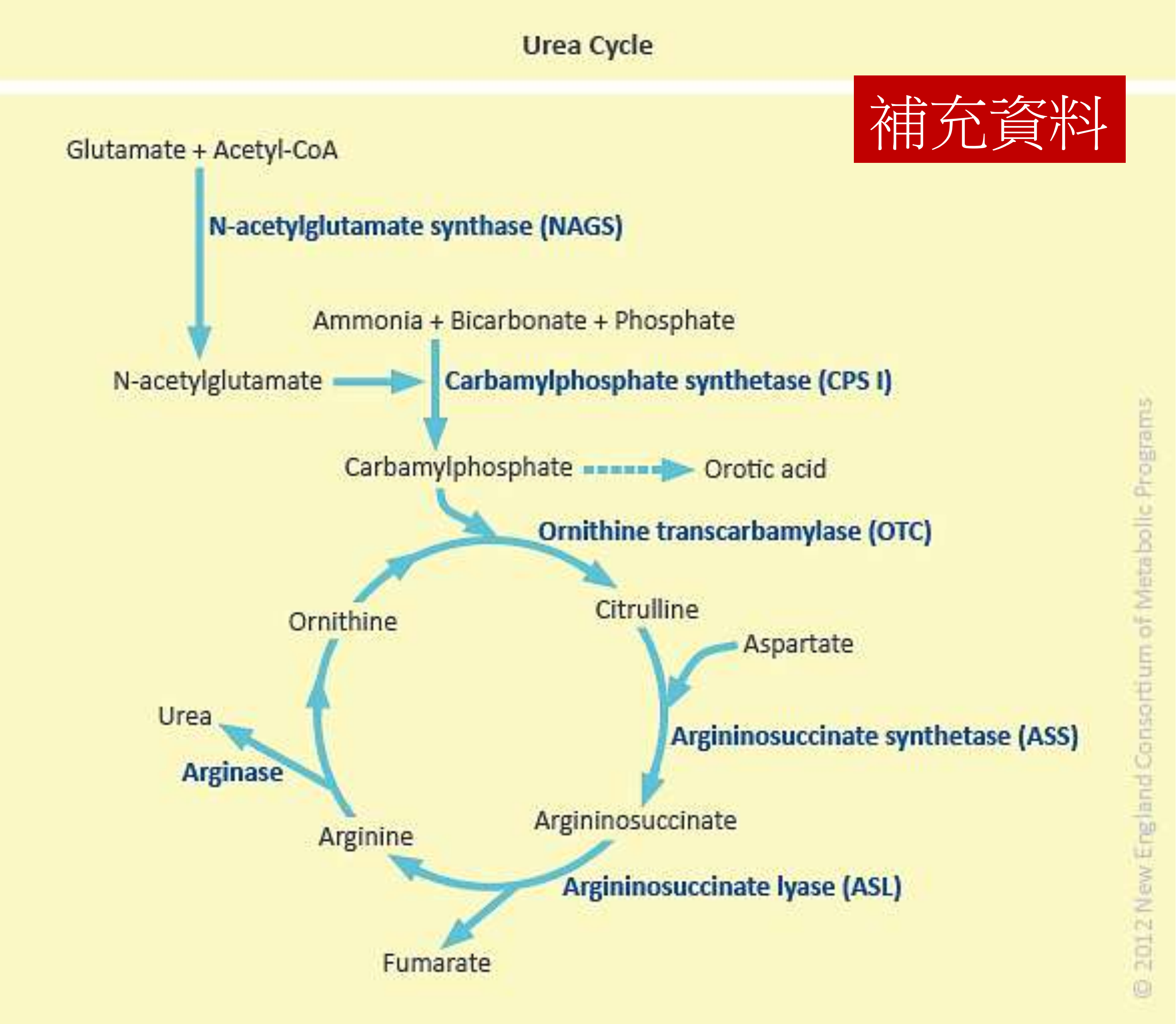
Arg-3 mutants
Can grow if <u>only</u> given arginine lack enzyme C

Conclusion:
Each gene in an organism is responsible for making a different enzyme.

Found that one gene mutation was responsible for one enzyme being defective; enzymes are proteins thus, this lead to the:

1 gene - 1 protein (polypeptide) hypothesis

補充資料



http://newenglandconsortium.org/for-professionals/acute-illness-protocols/urea-cycle-disorders/ornithine-transcarbamylase-deficiency-otc/

The Products of Gene Expression: A Developing Story

- Some proteins aren't enzymes, so researchers later revised the hypothesis: *one gene–one protein*
- Many proteins are composed of several polypeptides, each of which has its own gene
- Therefore, Beadle and Tatum's hypothesis is now restated as the *one gene–one polypeptide hypothesis*
  - Note that it is common to refer to gene products as proteins rather than polypeptides

Basic Principles of Transcription and Translation

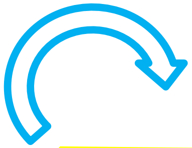
- **RNA** is the intermediate between genes and the proteins for which they code
- **Transcription** is the synthesis of RNA under the direction of DNA
- Transcription produces **messenger RNA (mRNA)**
- **Translation** is the synthesis of a polypeptide, which occurs under the direction of mRNA
- **Ribosomes** are the sites of translation

- In prokaryotes, mRNA produced by transcription is immediately translated without more processing

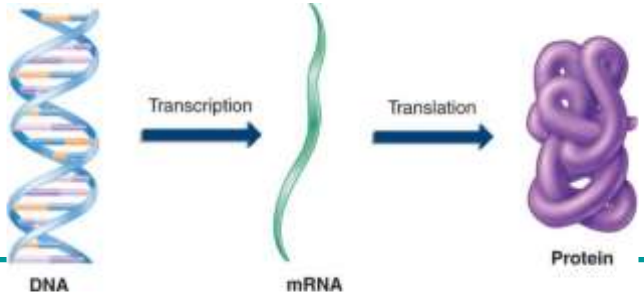
- In a eukaryotic cell, the nuclear envelope separates transcription from translation

- Eukaryotic RNA transcripts are modified through **RNA processing** to yield finished mRNA

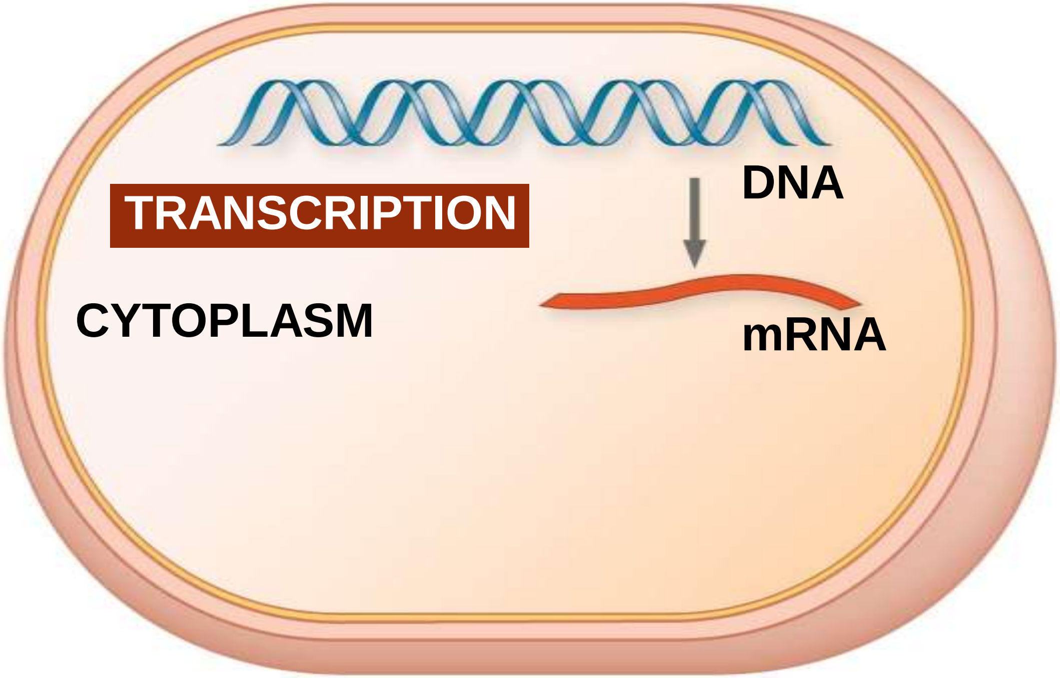
- A **primary transcript** is the initial RNA transcript from any gene
- The central dogma is the concept that cells are governed by a cellular chain of command:



DNA → RNA → Protein



(a) Bacterial cell



(a) Bacterial cell

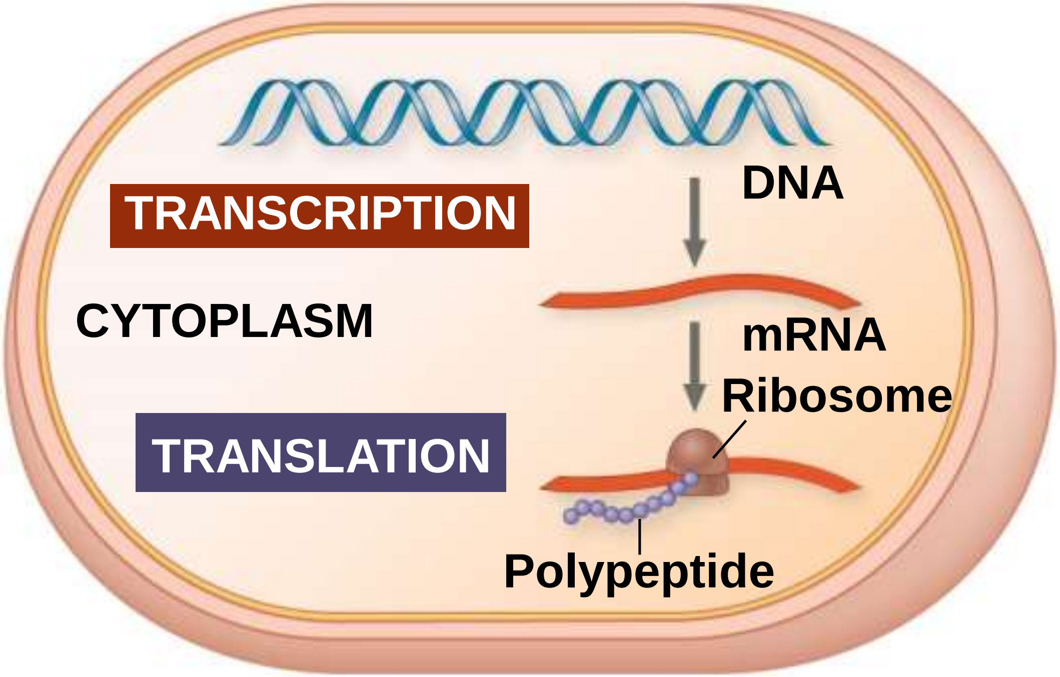
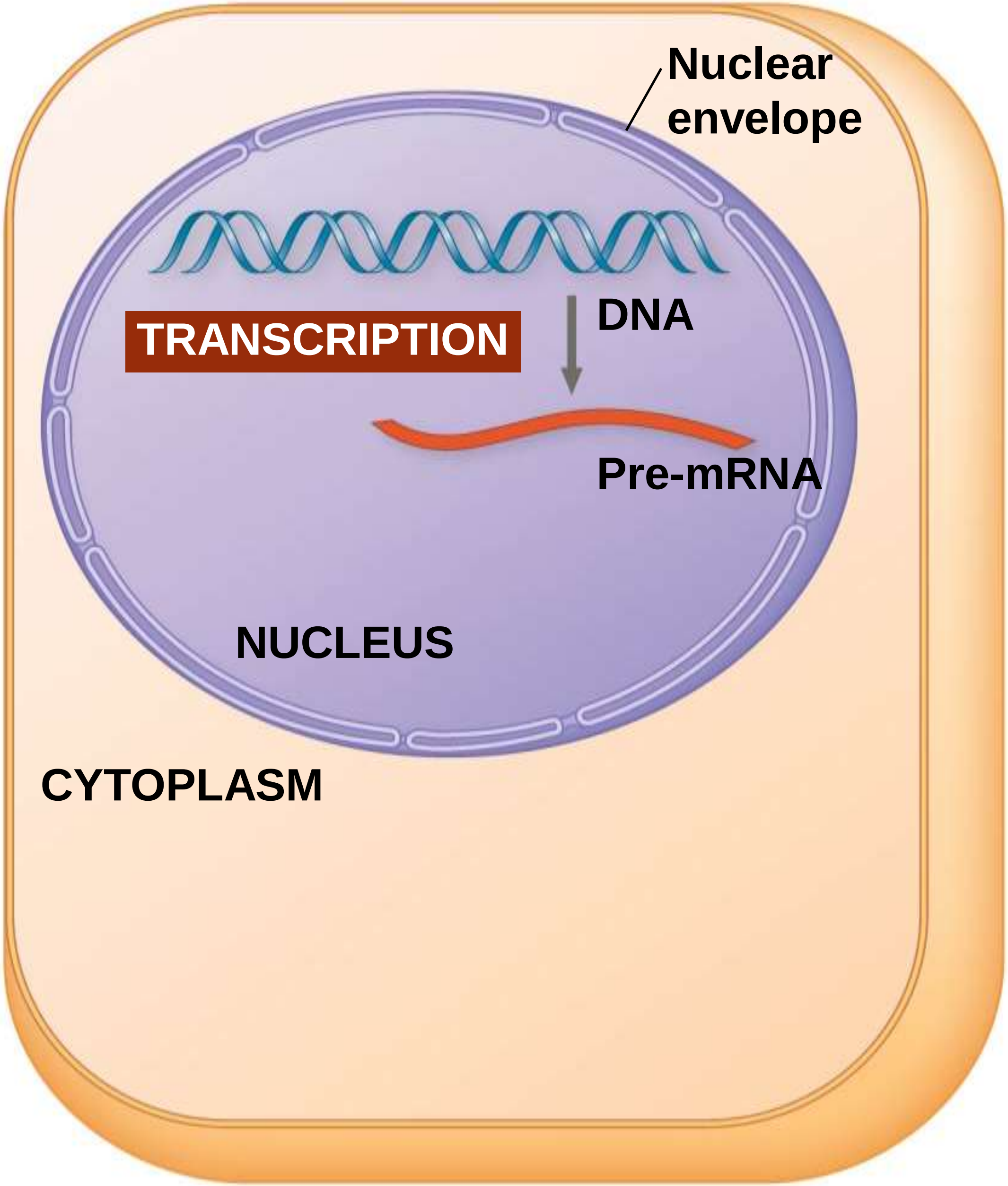
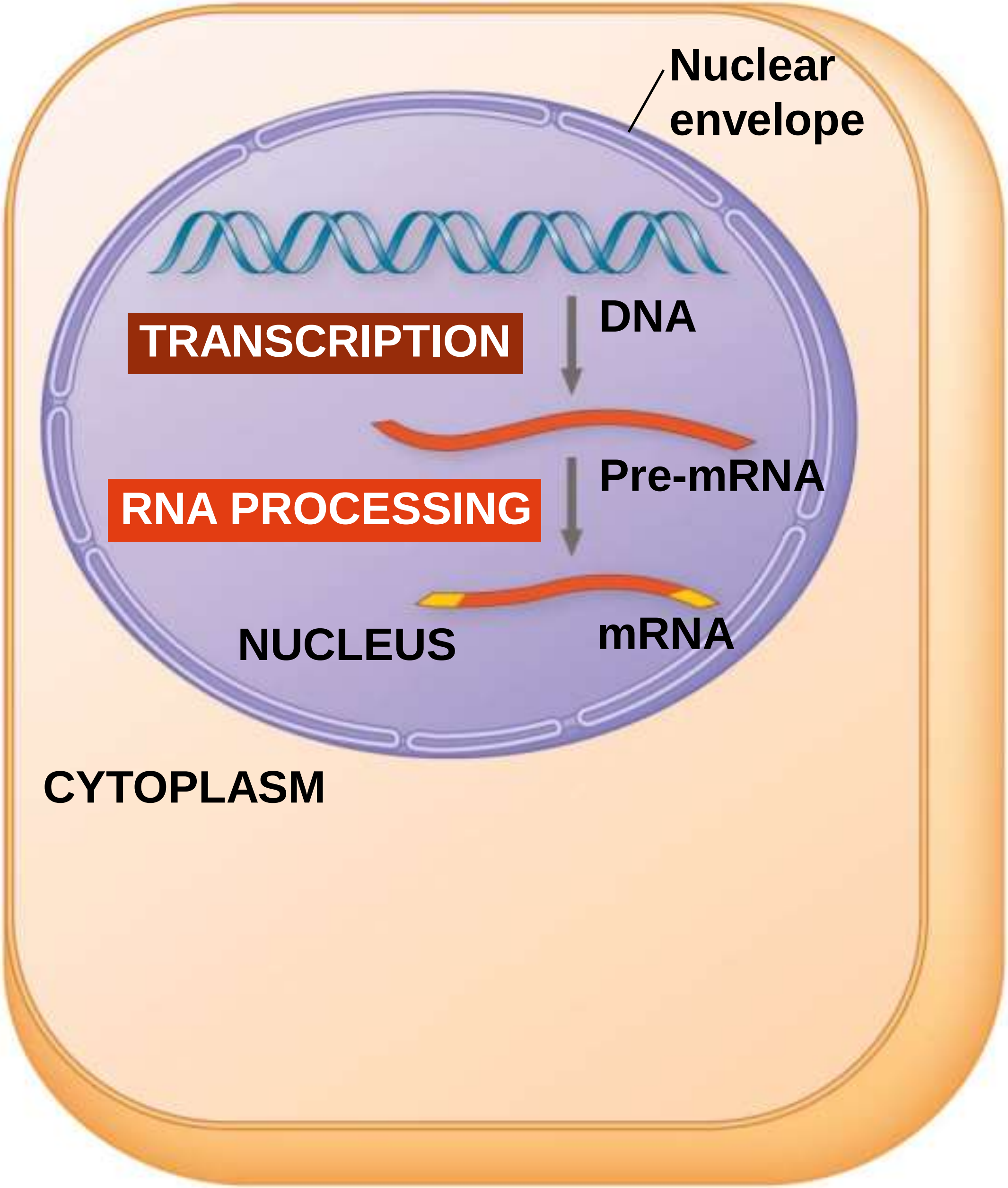


Figure 17.3b-1

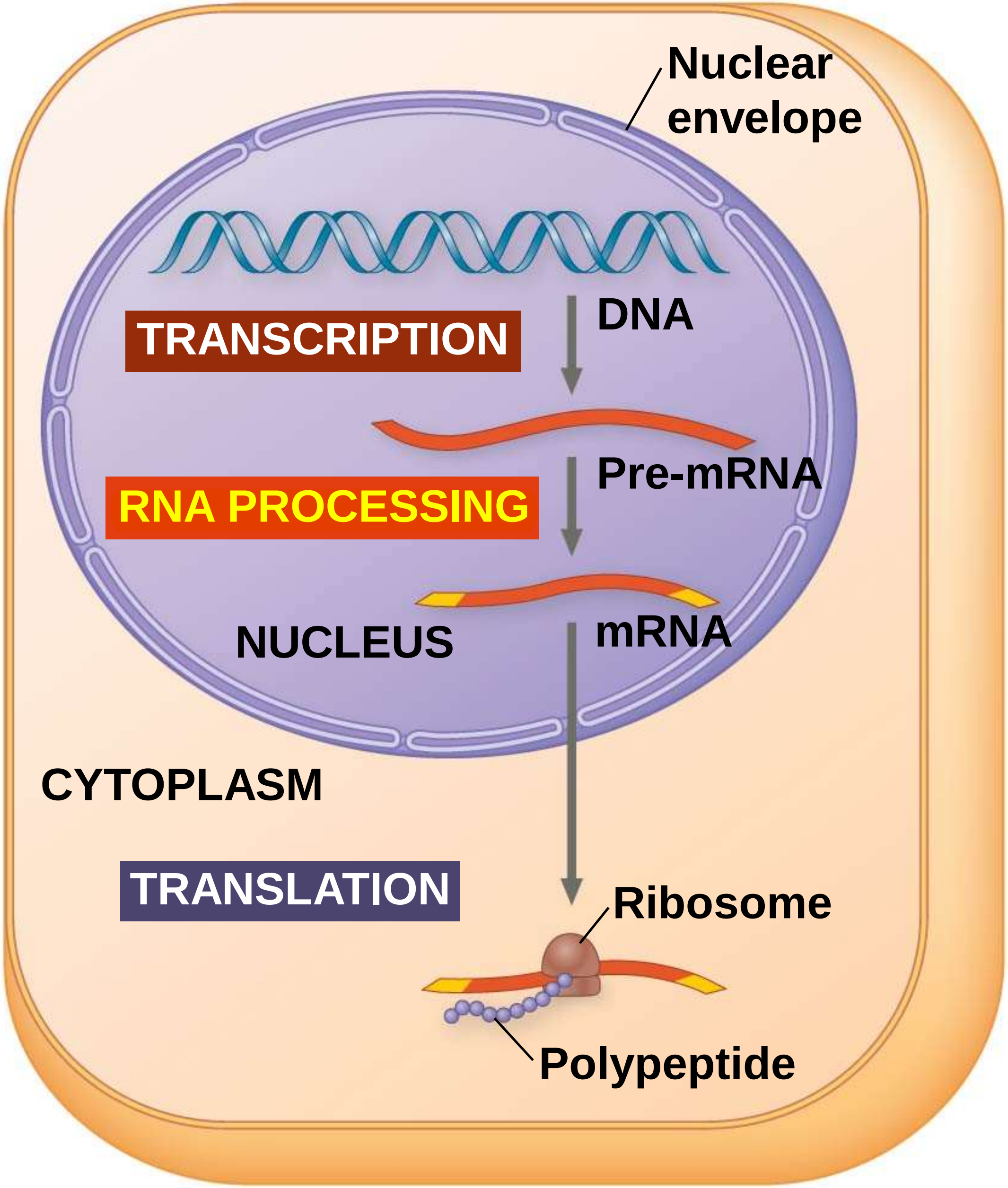


(b) Eukaryotic cell

Figure 17.3b-2



(b) Eukaryotic cell



(b) Eukaryotic cell

The Genetic Code

- How are the instructions for assembling amino acids into proteins encoded into DNA?
- There are 20 amino acids, but there are only four nucleotide bases in DNA
- How many bases correspond to an amino acid?

Codons: Triplets of Bases

- The flow of information from gene to protein is based on a **triplet code**: a series of nonoverlapping, three-nucleotide words
- These triplets are the smallest units of uniform length that can code for all the amino acids
- Example: AGT at a particular position on a DNA strand results in the placement of the amino acid serine at the corresponding position of the polypeptide to be produced

- During transcription, one of the two DNA strands called the **template strand** provides a template for ordering the sequence of nucleotides in an RNA transcript

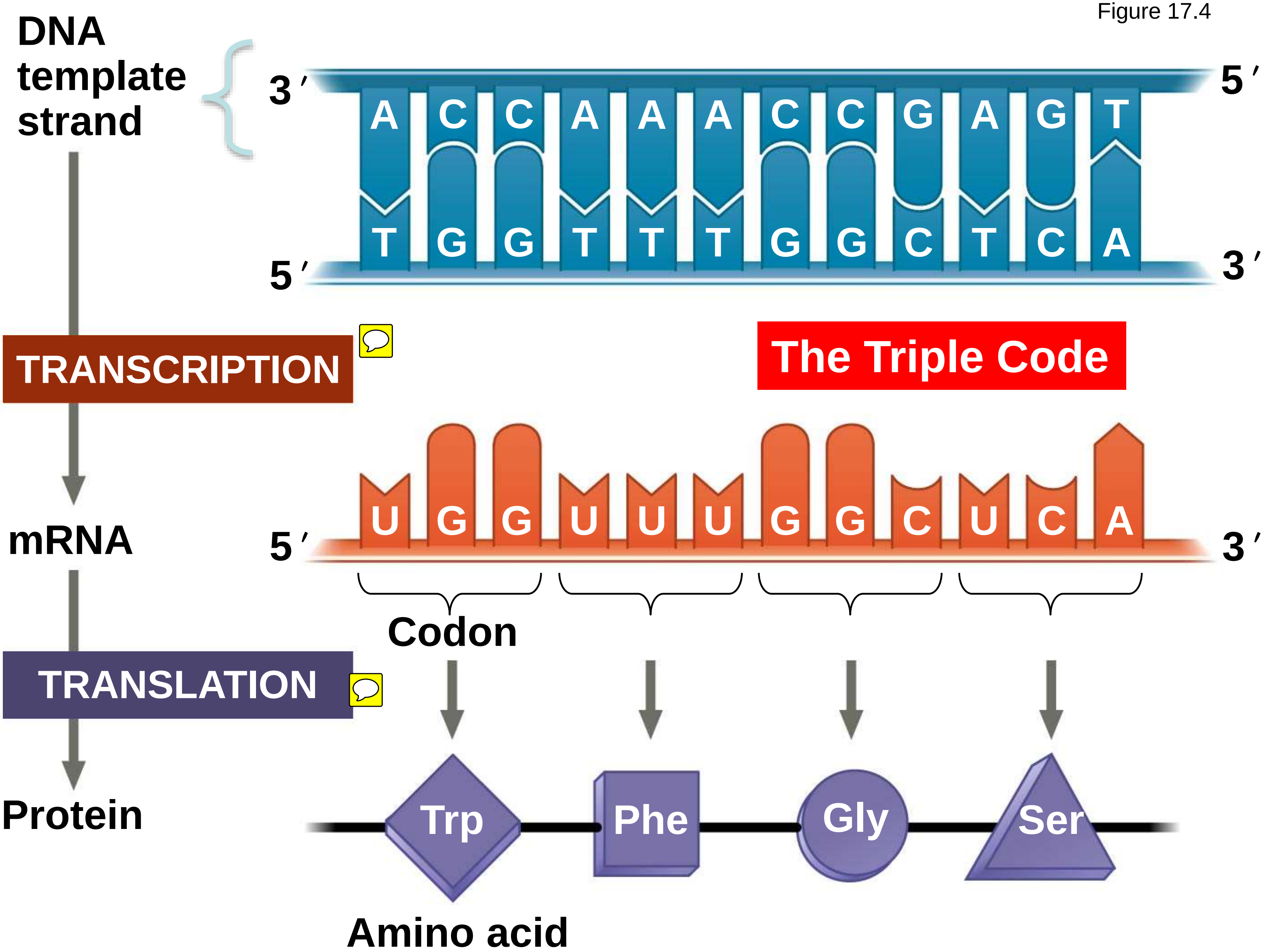
- During translation, the mRNA base triplets, called **codons**, are read in the 5′ to 3′ direction

- Each codon specifies the amino acid to be placed at the corresponding position along a polypeptide

- Codons along an mRNA molecule are read by translation machinery in **the 5′ to 3′ direction**
- Each codon specifies the addition of one of 20 amino acids

Figure 17.4

The Triple Code



DNA template strand

TRANSCRIPTION

mRNA

Codon

TRANSLATION

Protein

Amino acid

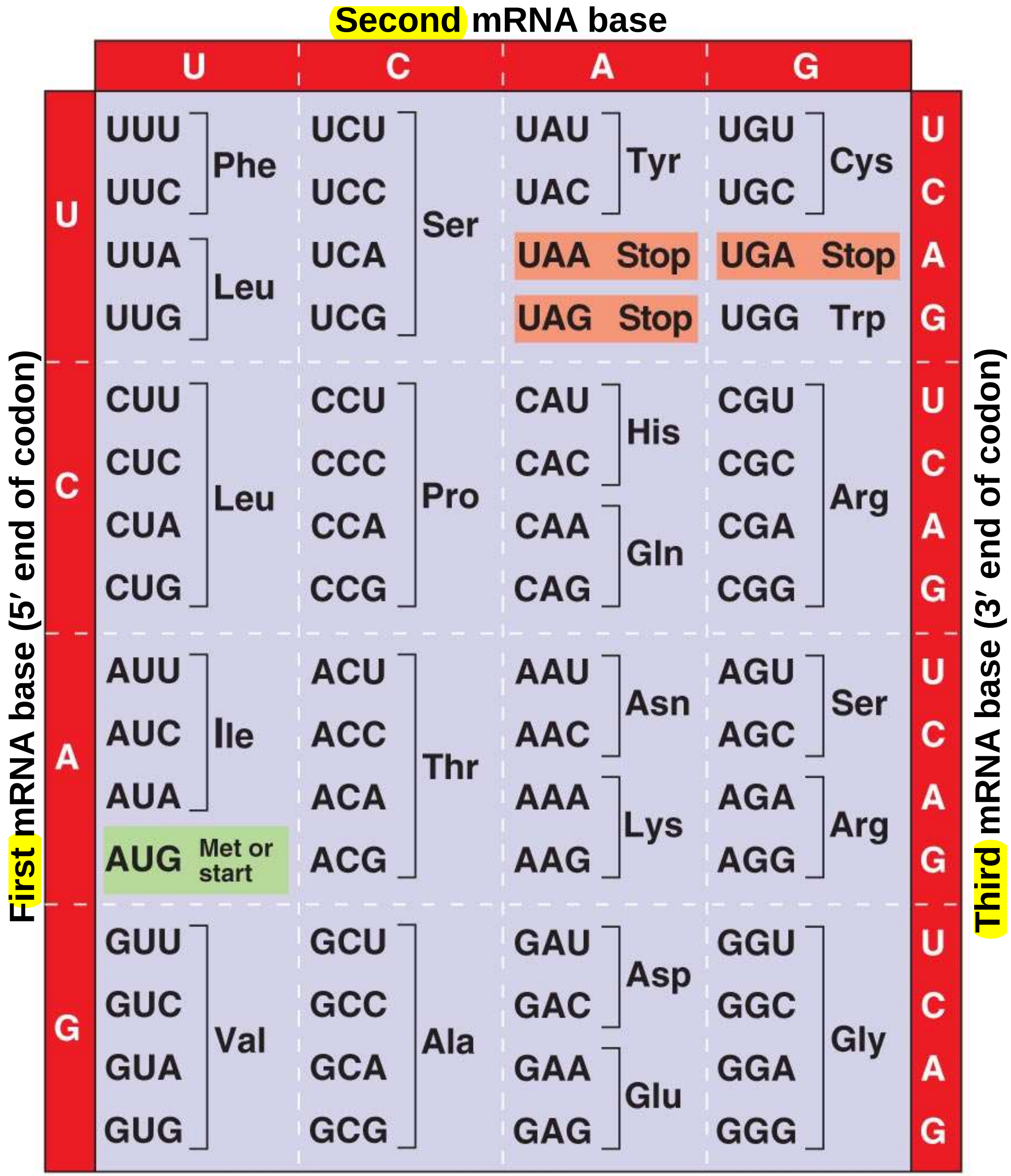
Cracking the Code

- All 64 codons were deciphered by the mid-1960s

- Of the 64 triplets, **61** code for amino acids; 3 triplets are “stop” signals to end translation

- The genetic code is redundant but not ambiguous; no codon specifies more than one amino acid

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



First mRNA base (5′ end of codon)	Second mRNA base								Third mRNA base (3′ end of codon)
	U		C		A		G		
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 or start	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

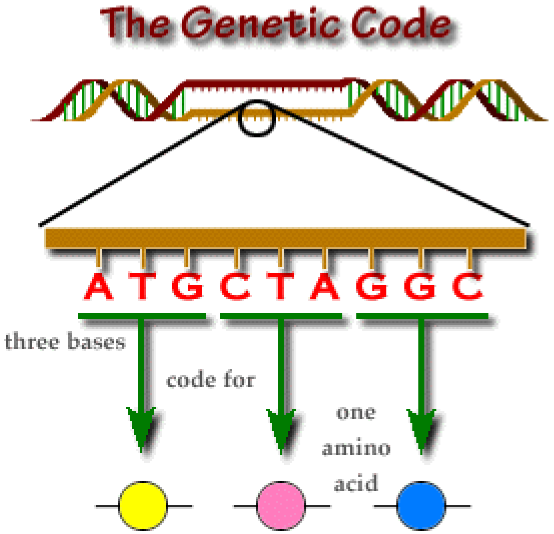
Fig. 17-5
Codon table of mRNA

思考題:

Why has nature invented three stop codons of DNA and only one start codon?

Evolution of the Genetic Code

- The genetic code is nearly universal, shared by the simplest bacteria to the most complex animals (除極少數的例外，遺傳密碼是通用的)
- Genes can be transcribed and translated after being transplanted from one species to another (遺傳密碼的通用性，讓基因可轉殖至不同物種)



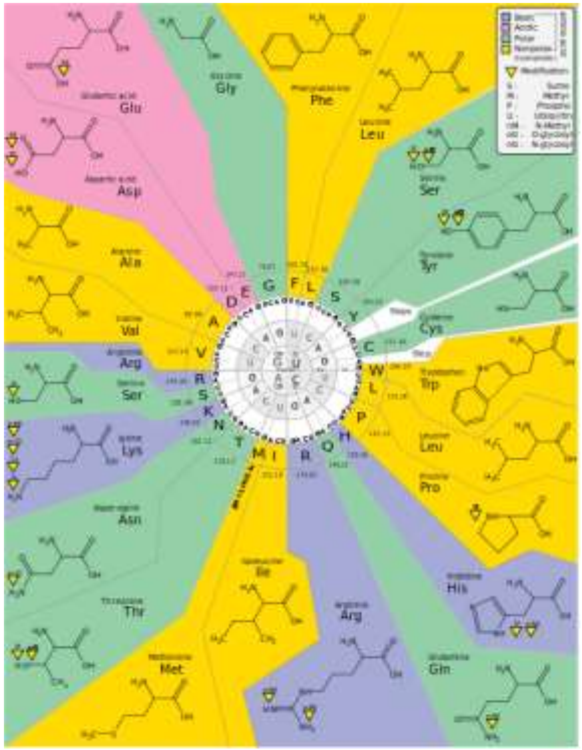
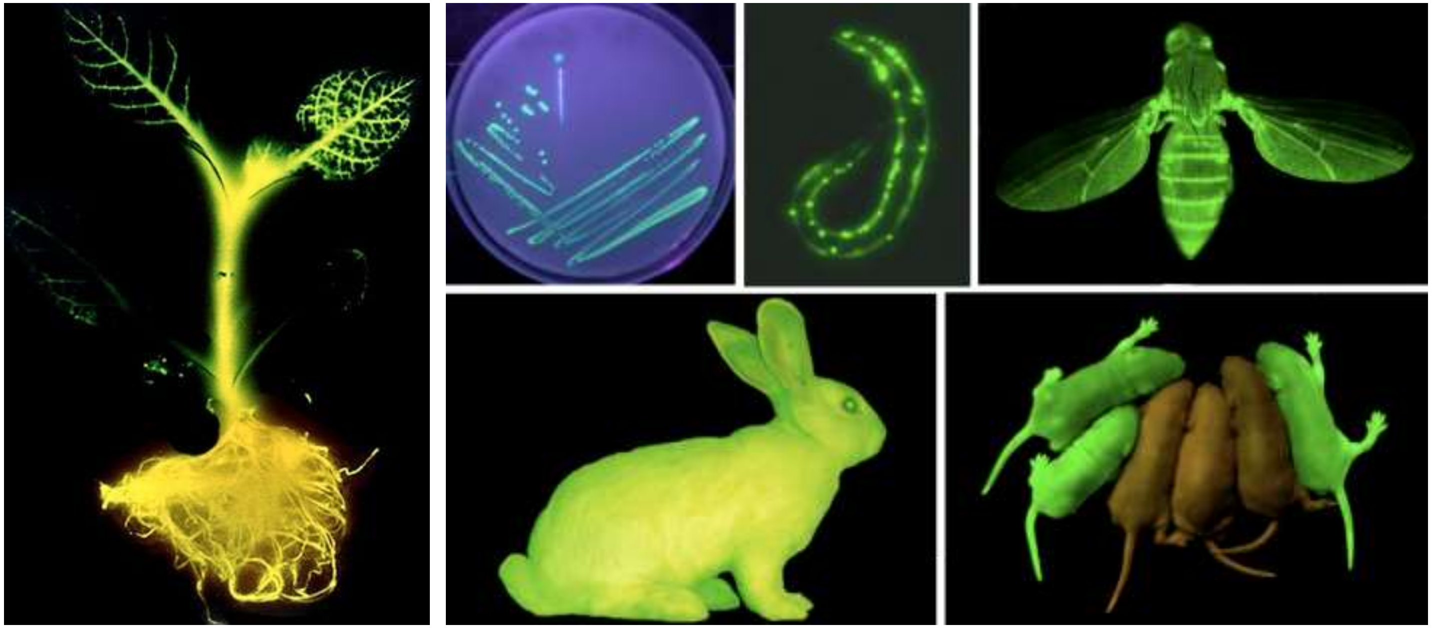
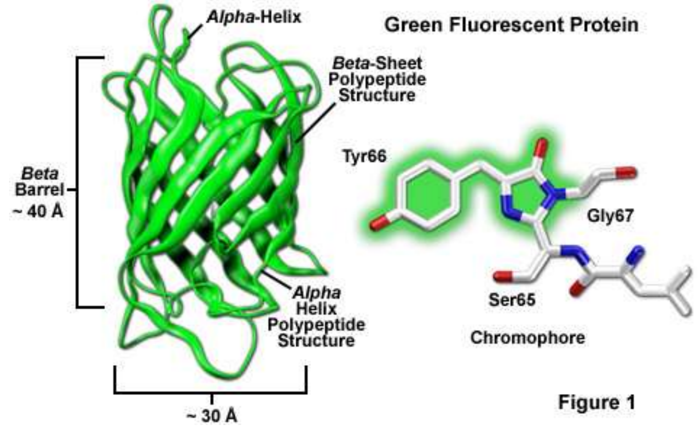
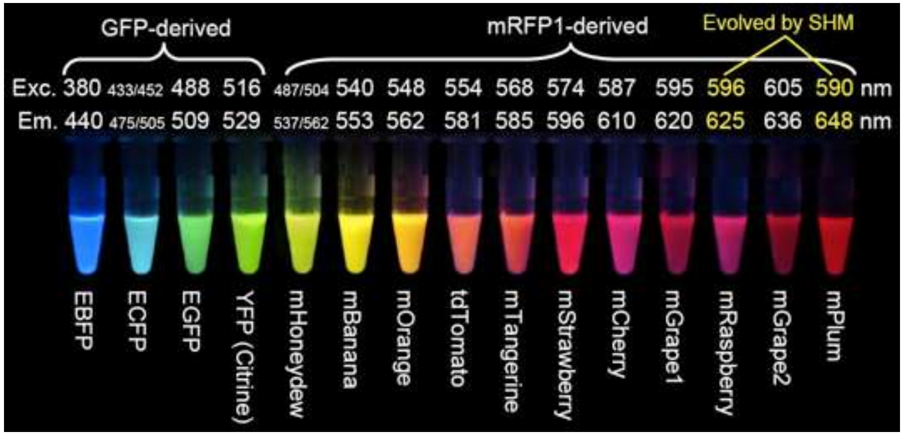


Fig. 17-6

Example: Tracking gene/protein expression in different species



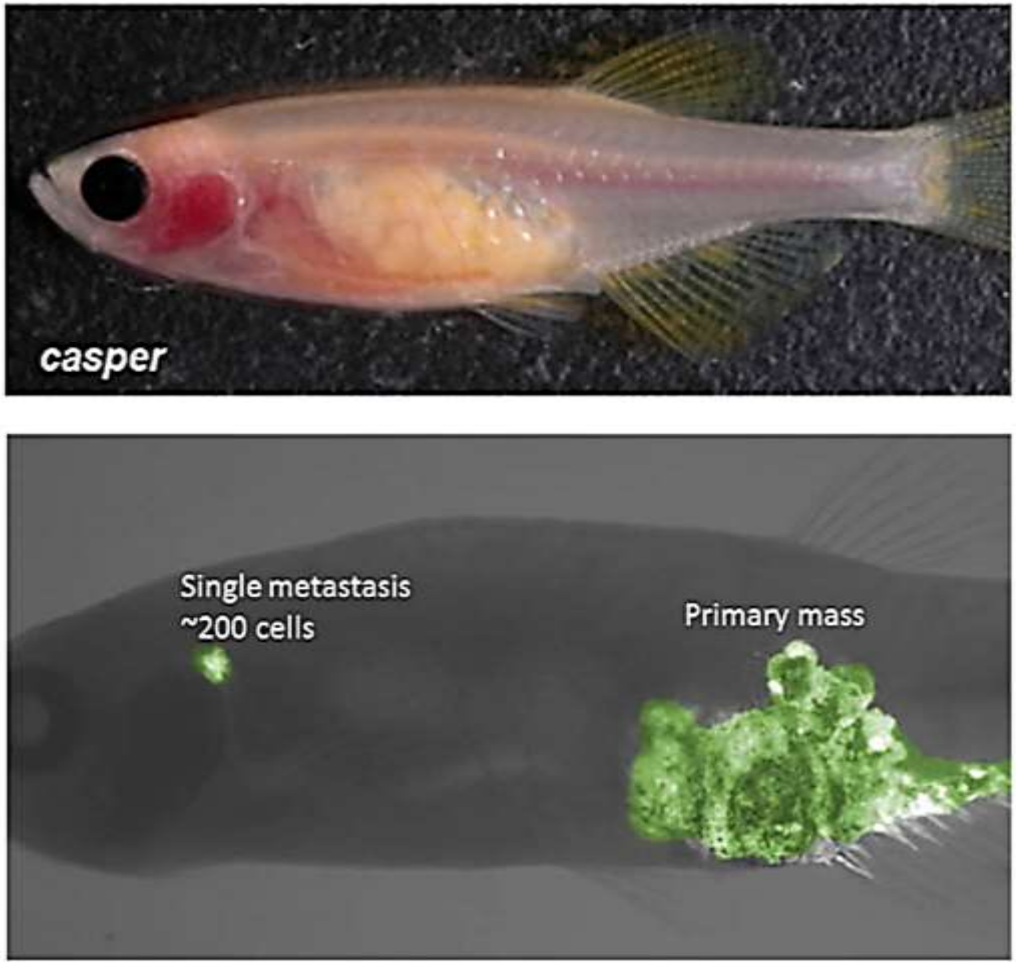




Application of bio-imaging in cancer research

Normal

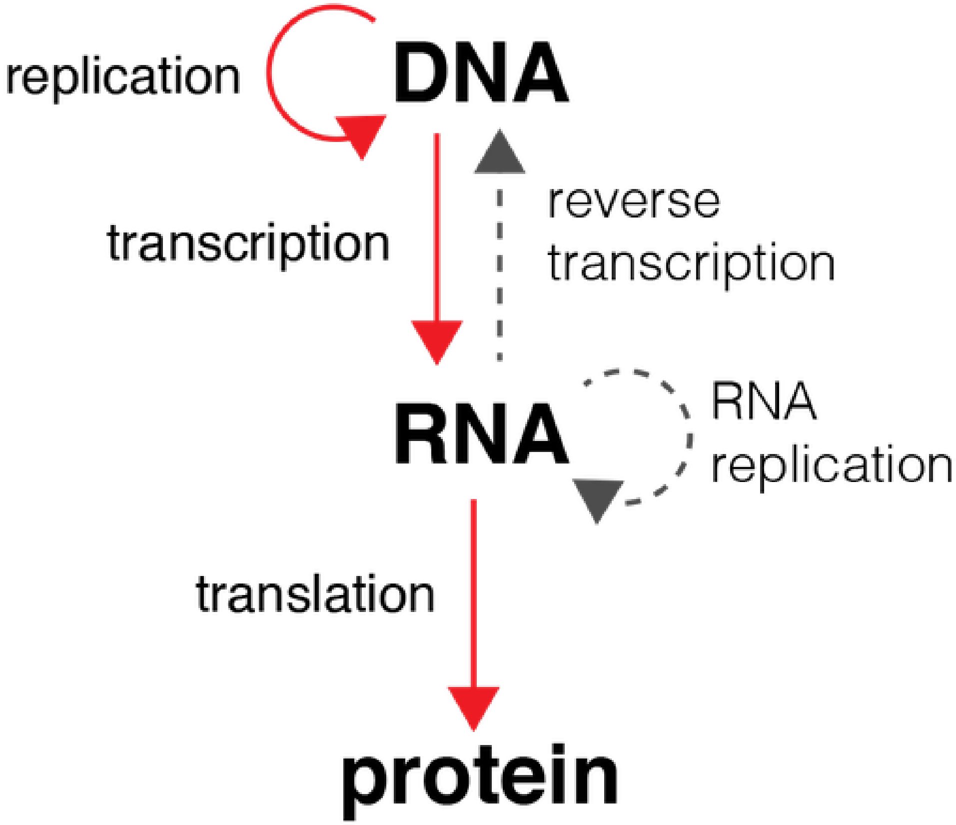
Melanoma 黑色素瘤



The *casper* zebrafish (top) is translucent throughout its adult life cycle due to a lack of melanocytes and iridophores. The **transplantation of GFP-melanoma cells** into the flank of a *casper* recipient yields both primary and metastatic growth (bottom).

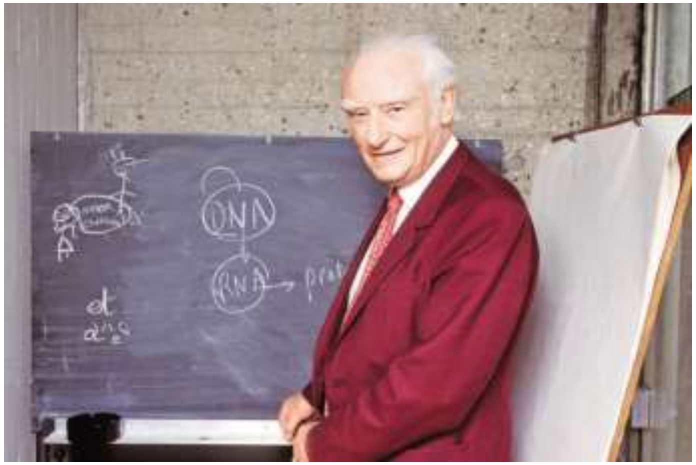
http://www.mskcc.org/research/lab/richard-white

Central dogma of life


replication
DNA
transcription
reverse transcription
RNA
RNA replication
translation
protein

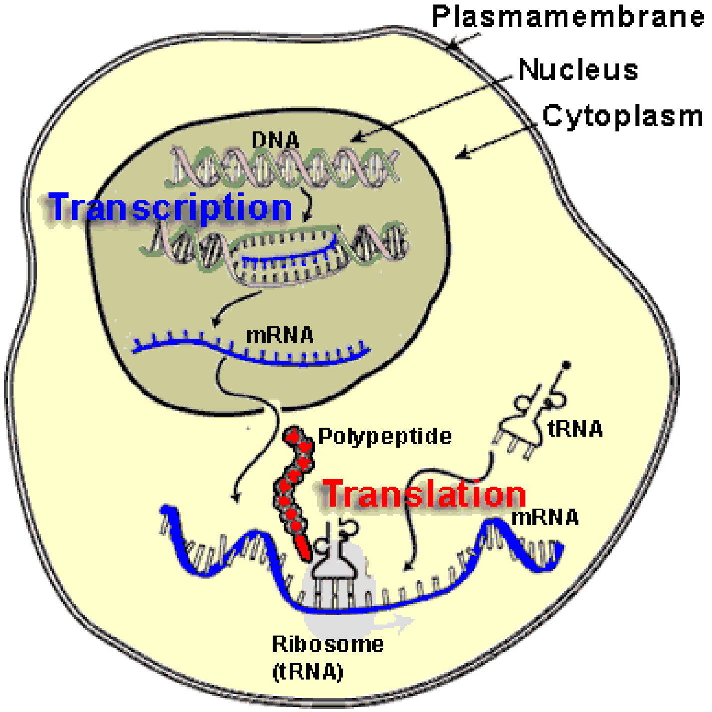
“The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.”

- Francis Crick, 1958



Concept 17.2: Transcription is the DNA-directed synthesis of RNA: *a closer look*

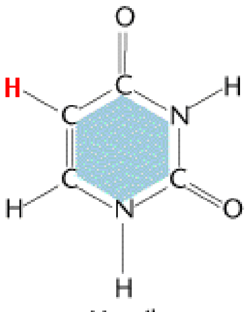
- **Transcription 轉錄**, the first stage of gene expression. - 依循”鹼基配對”原則，以任一DNA為模板，合成一條與之互補的RNA副本的過程。



Molecular Components of Transcription

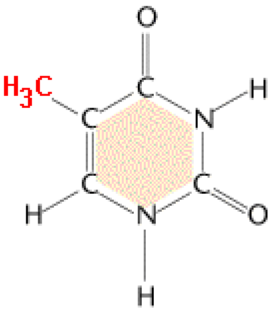
- RNA synthesis is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
- RNA synthesis follows the same base-pairing rules as DNA, except **uracil** substitutes for **thymine.**

RNA **U** ← **T** DNA



Uracil

A**U**GC ATGC



Thymine

Promoter = specific DNA Sequence during transcription

- The DNA sequence *where RNA polymerase attaches* is called the **promoter**
  - In bacteria, the sequence signaling *the end of transcription* is called the **terminator**
- The *stretch of DNA* that is transcribed is called a **transcription unit**

PLAY

Animation: Transcription

Synthesis of an RNA Transcript

- The three stages of transcription:
  - **Initiation**
  - **Elongation**
  - **Termination**

Figure 17.7-1

The stages of transcription

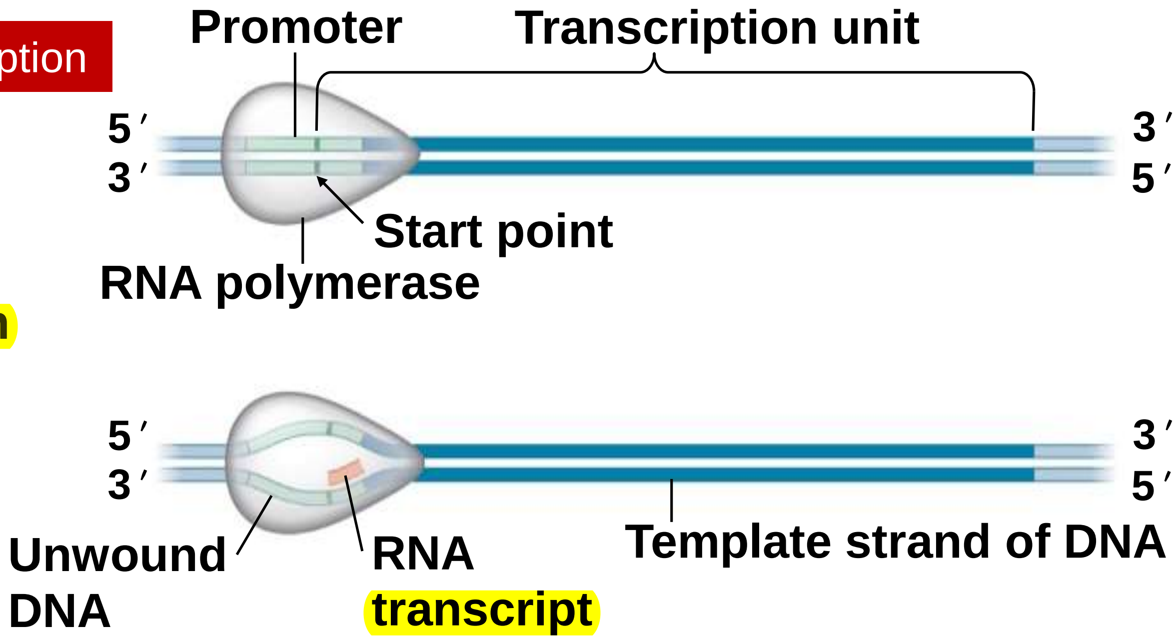
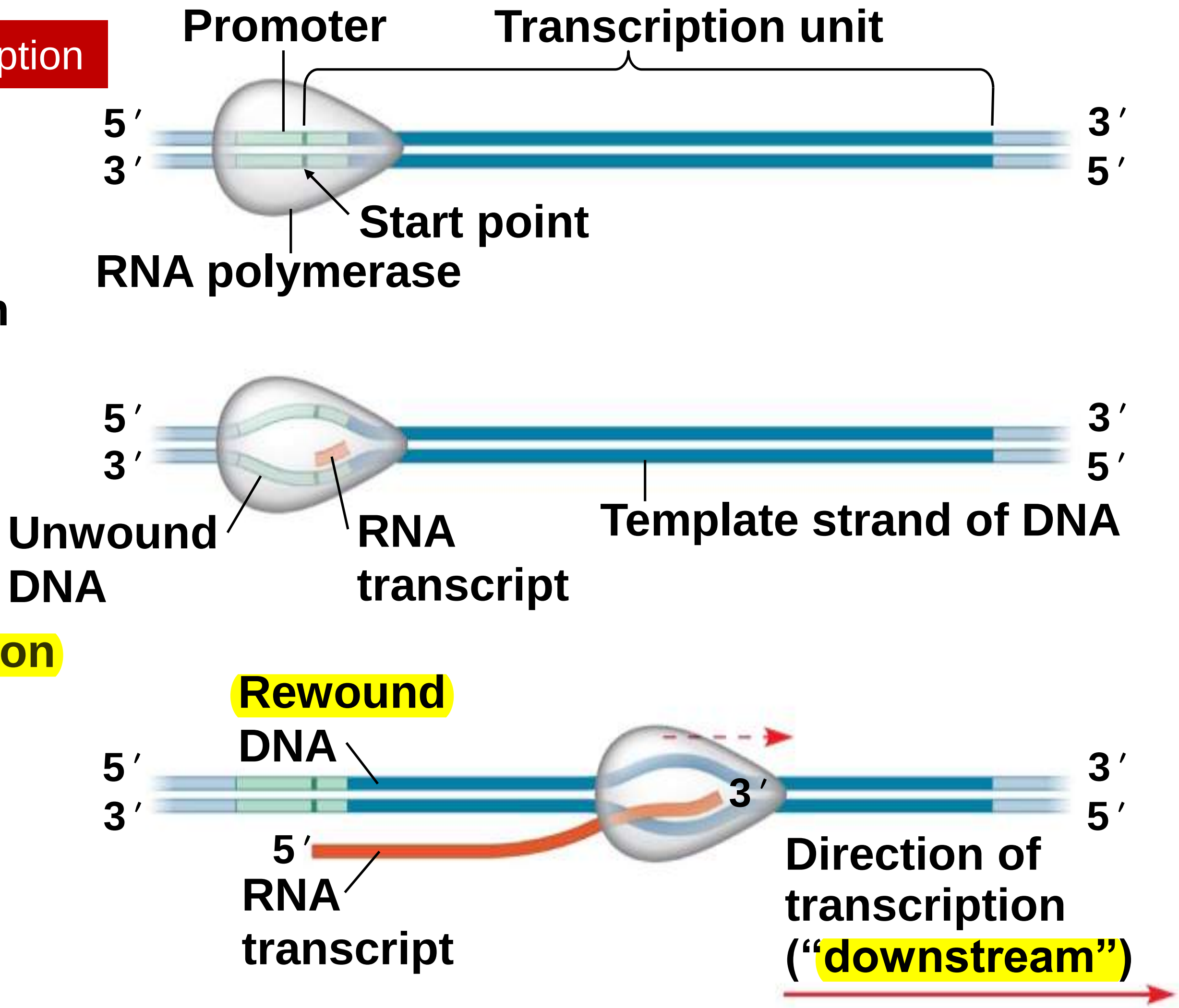


Figure 17.7-2

The stages of transcription



Promoter

Transcription unit

5′ 3′ 3′ 5′

Start point

RNA polymerase

① **Initiation**

5′ 3′ 3′ 5′

Unwound DNA

RNA transcript

Template strand of DNA

② **Elongation**

Rewound DNA

5′ 3′ 3′ 5′

3′

5′

RNA transcript

Direction of transcription (“downstream”)

Figure 17.7-3

The stages of transcription

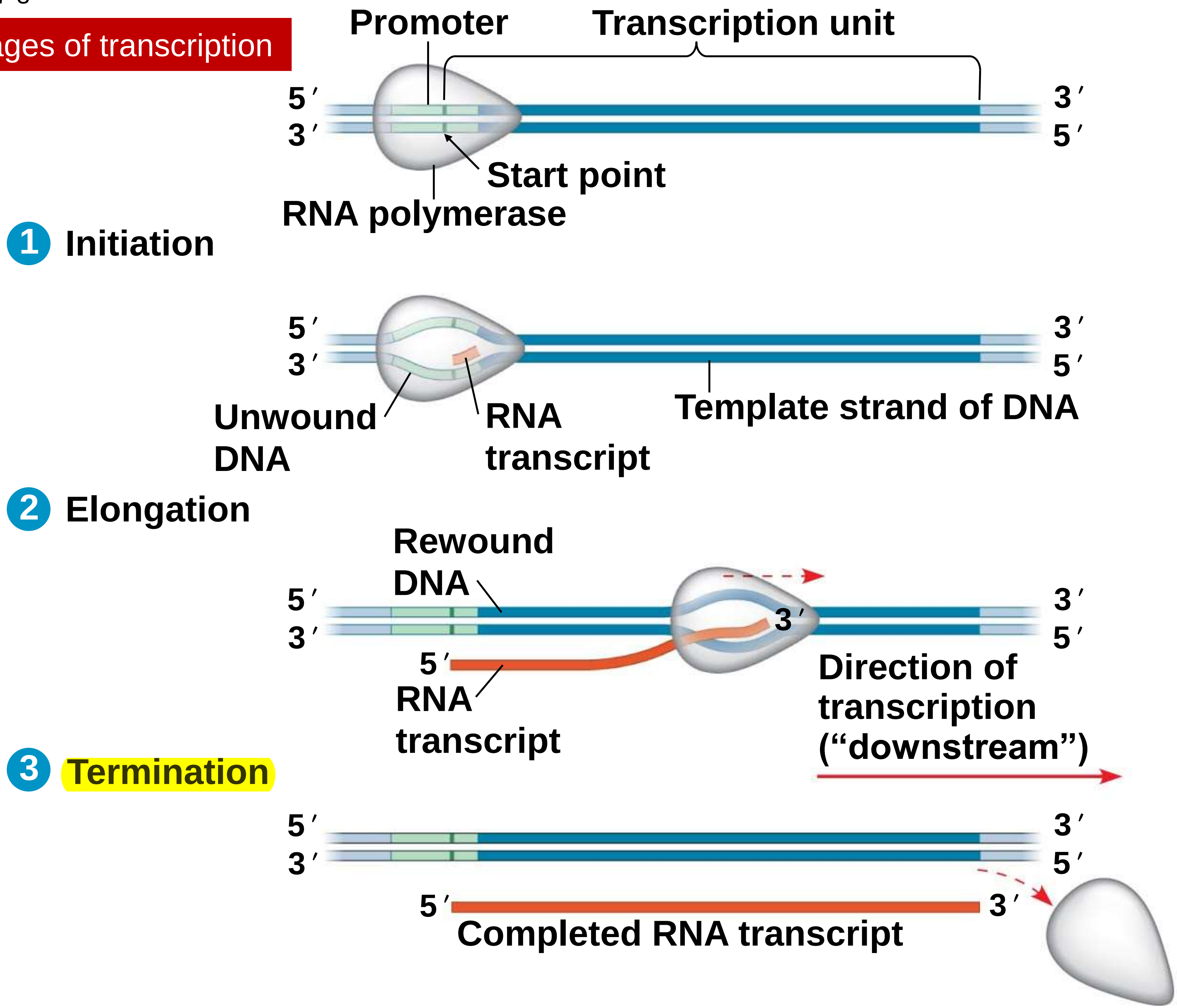
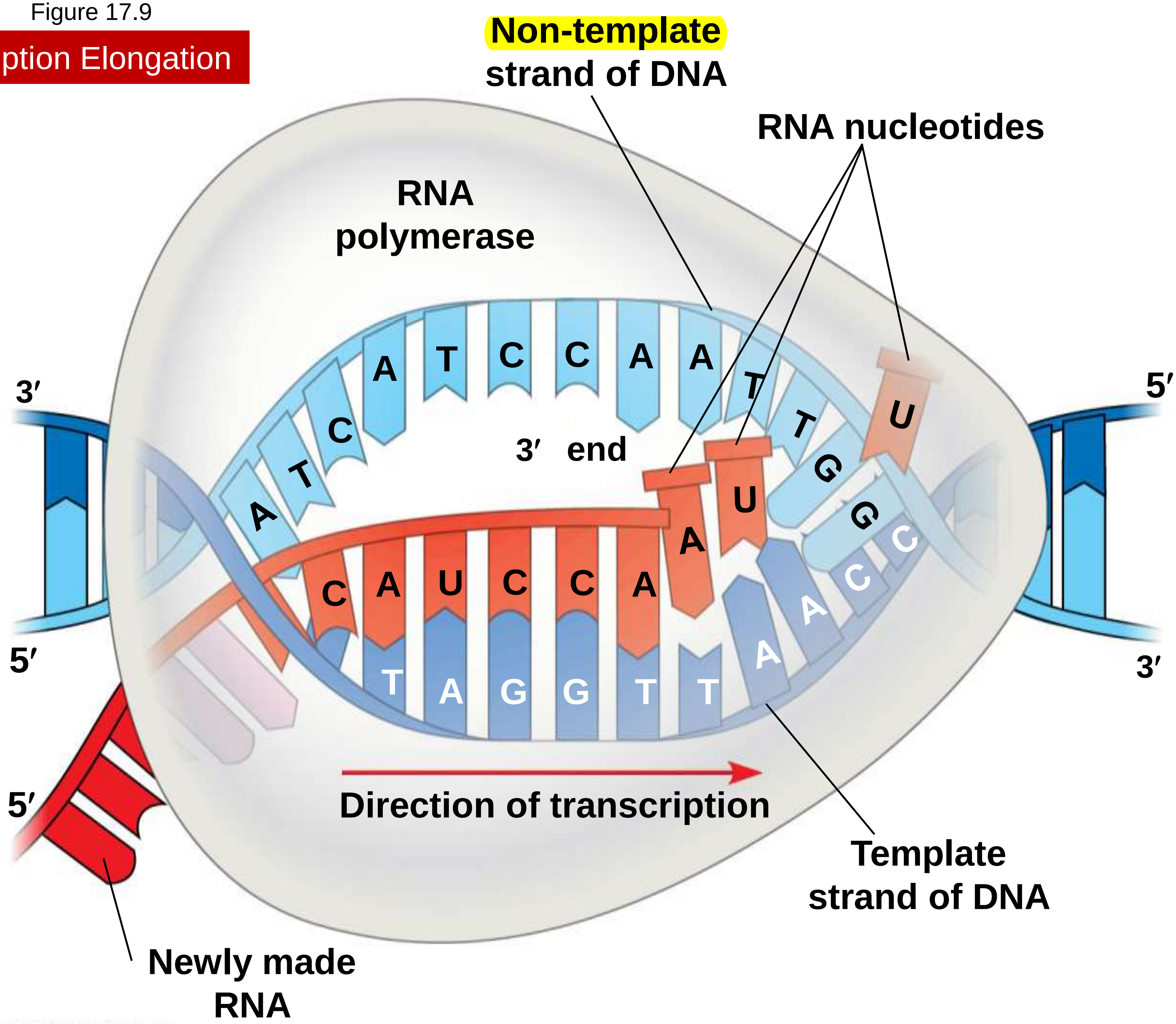


Figure 17.9

Transcription Elongation



1st stage:

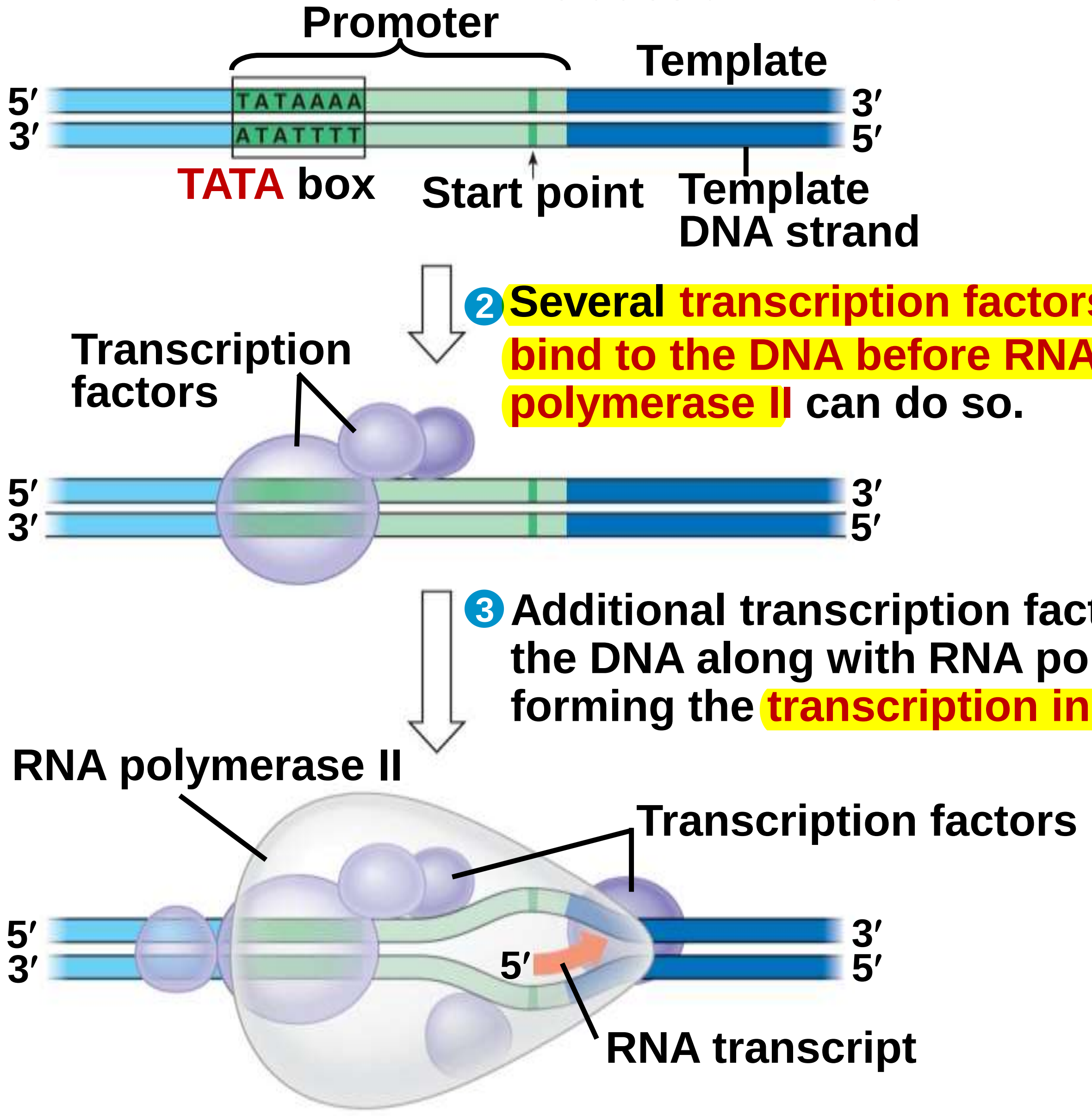
RNA Polymerase Binding and Initiation of Transcription

- Promoters signal the initiation of RNA synthesis
- **Transcription factors** (which are proteins) mediate the binding of RNA polymerase and the initiation of transcription
- The completed assembly of transcription factors and RNA polymerase II bound to a promoter is called a **transcription initiation complex**
- A promoter called a **TATA box** is crucial in forming the initiation complex in eukaryotes

Eukaryotic Transcription

Fig. 17-8

The initiation of transcription at a eukaryotic promoter



① A eukaryotic promoter includes a **TATA box**

② Several **transcription factors must bind to the DNA before RNA polymerase II** can do so.

③ Additional transcription factors bind to the DNA along with RNA polymerase II, forming the **transcription initiation complex**.

“Transcription initiation complex”

2nd Stage: Elongation of the RNA Strand

- As RNA polymerase moves along the DNA, it un-twists the double helix, 10 to 20 bases at a time
- Transcription progresses at a rate of 40 nucleotides per second in eukaryotes
- A gene can be transcribed simultaneously by several RNA polymerases

3rd stage:
Termination of Transcription

- The mechanisms of termination are different in bacteria and eukaryotes
  - In bacteria, the polymerase stops transcription at the end of the terminator
  - In eukaryotes, the polymerase continues transcription after the pre-mRNA is cleaved from the growing RNA chain; the polymerase eventually falls off the DNA

HHMI video clip

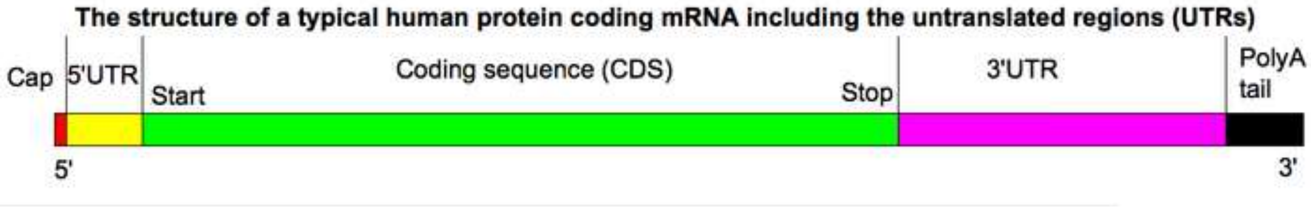
Concept 17.3: Eukaryotic cells modify RNA after transcription

- Enzymes in the eukaryotic nucleus modify pre-mRNA before the genetic messages are dispatched to the cytoplasm
- During RNA processing, **both ends** of the primary transcript are usually altered
- Also, usually some interior parts of the molecule are cut out, and the other parts spliced together

Keywords: **Exon, Intron, Splicing**

Alteration of mRNA Ends

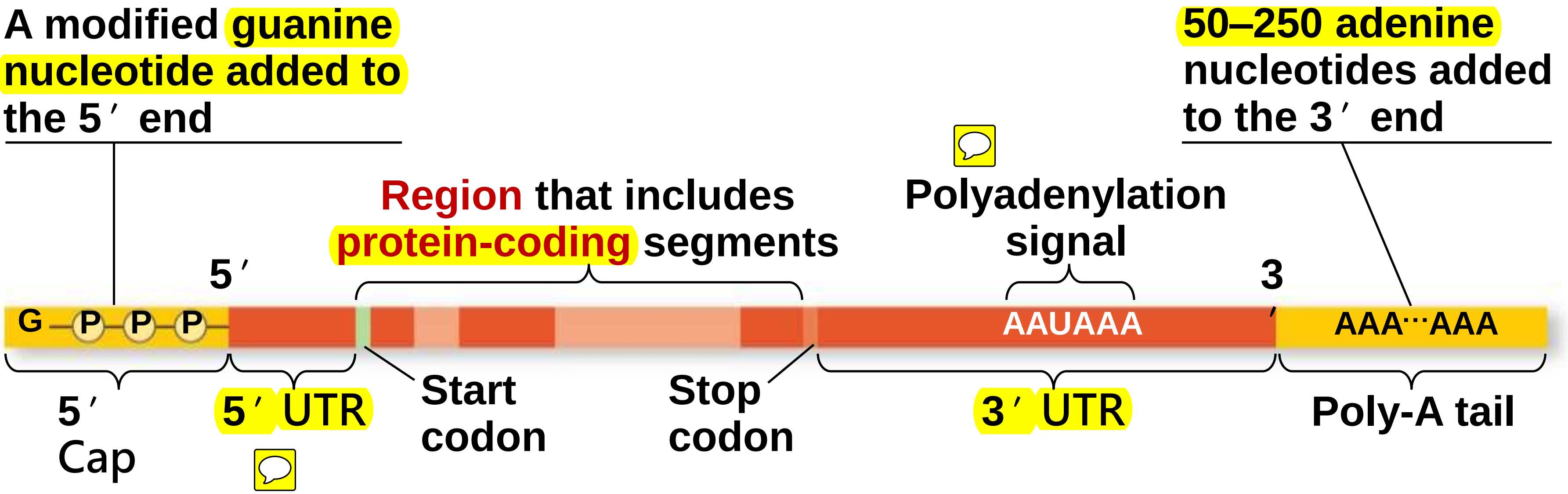
- Each end of a pre-mRNA molecule is modified in a particular way:
  - The 5′ end receives a modified nucleotide **5′ cap**
  - The 3′ end gets a **poly-A tail**


The structure of a typical human protein coding mRNA including the untranslated regions (UTRs)

- These modifications share several functions:
  - facilitate the export of mRNA
  - protect mRNA from hydrolytic enzymes
  - help ribosomes attach to the 5′ end

Figure 17.10

RNA processing: addition of the 5′ cap and poly-A tail



Reminder: Functions of the modifications and UTRs

1. facilitate the export of mRNA
2. protect mRNA from degradation
3. help ribosome binding

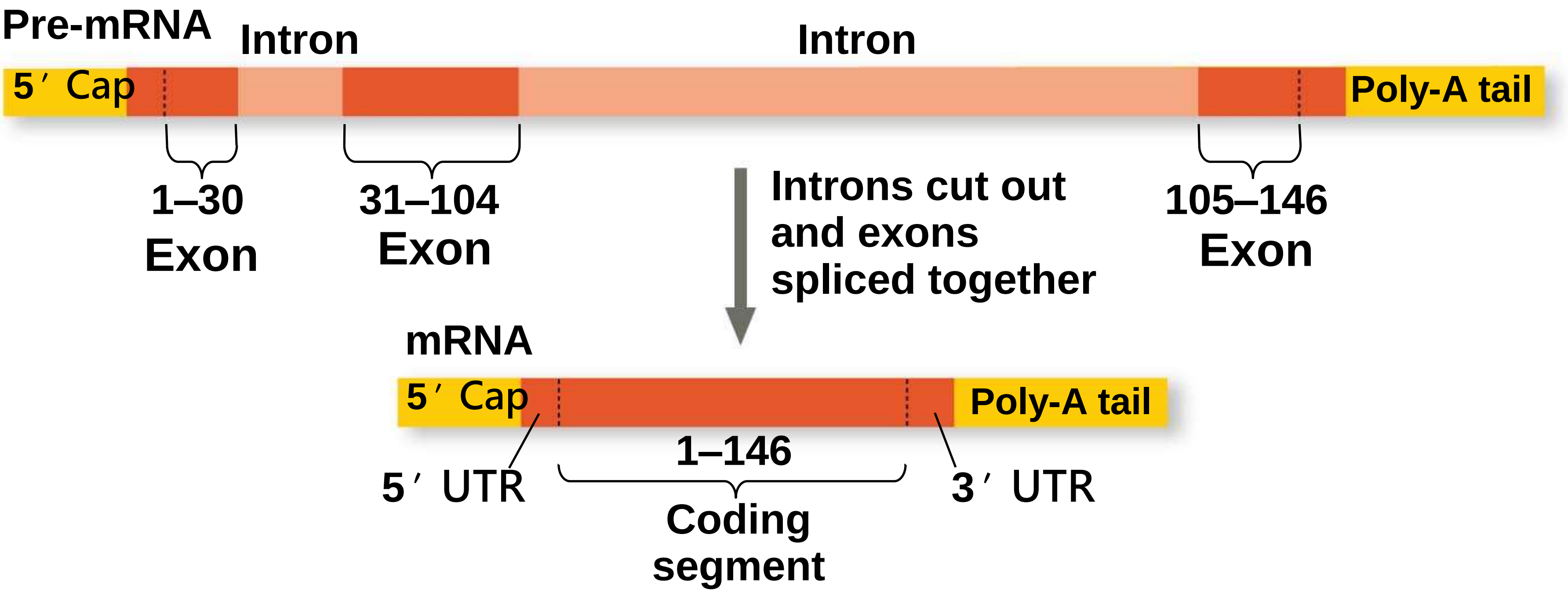
Split Genes and RNA Splicing

Important!

- Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions
- These noncoding regions are called intervening sequences, or **introns**
- The other regions are called **exons** because they are eventually expressed, usually translated into amino acid sequences
- **RNA splicing** removes introns and joins exons, creating an mRNA molecule with a continuous coding sequence

Figure 17.11

RNA processing: RNA splicing



This example is B-globin; numbers under the RNA refers to codons; β-globin is 146 aa long.

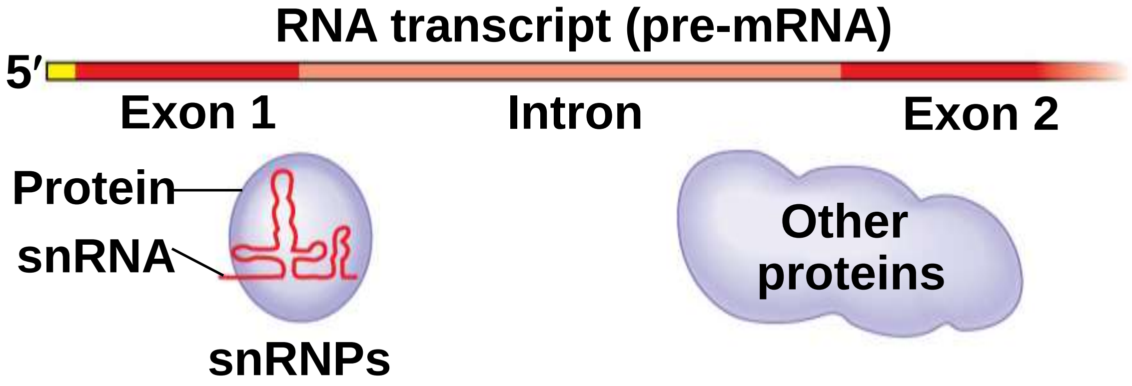
Spliceosome

- In some cases, RNA splicing is carried out by spliceosomes
- **Spliceosomes** consist of a variety of proteins and several small nuclear ribonucleoproteins (snRNPs) that *recognize the splice sites*

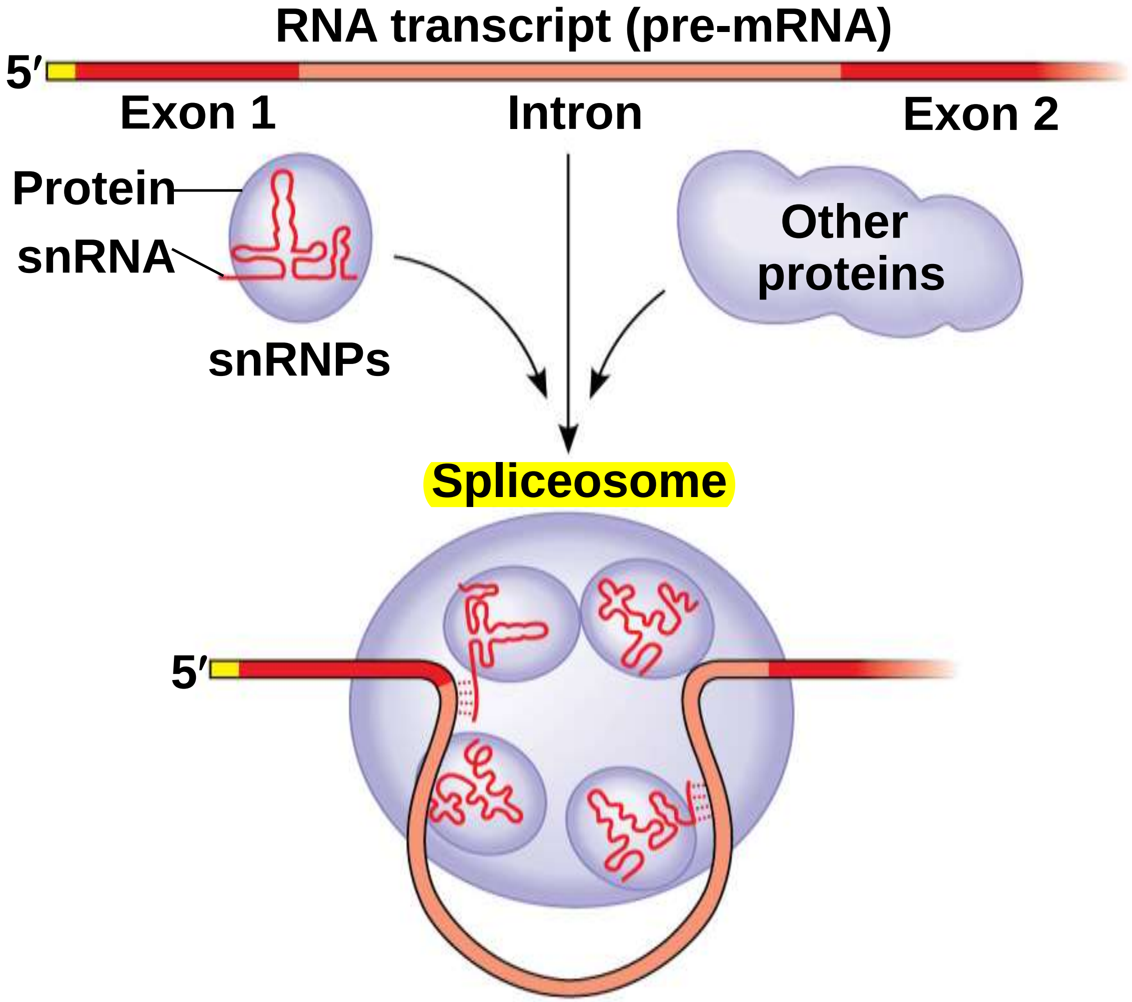
Cartoon next page

Figure 17.12

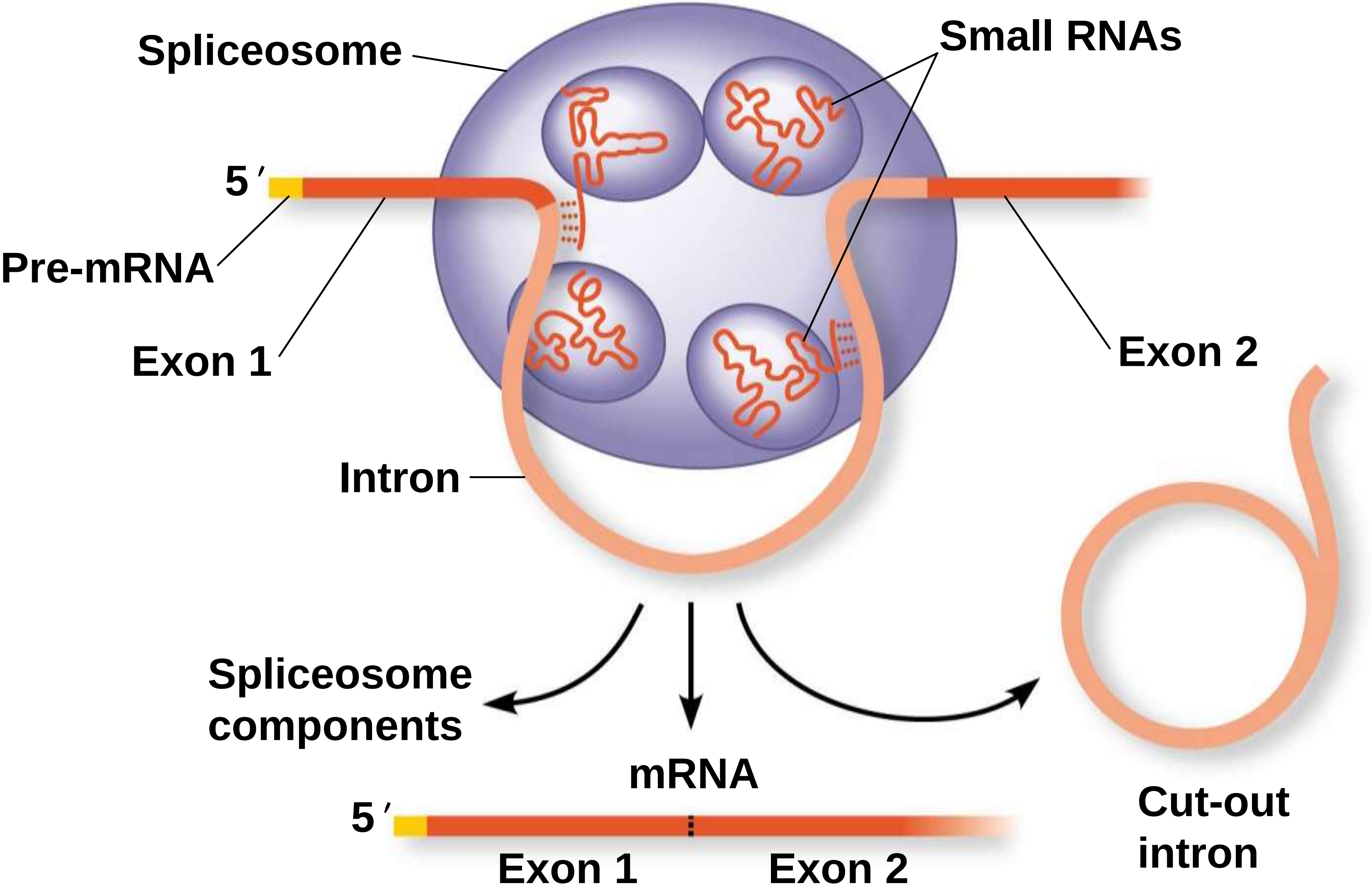
The roles of snRNPs and spliceosomes in pre-mRNA splicing



The roles of snRNPs and spliceosomes in pre-mRNA splicing



A spliceosome splicing a pre-mRNA



Ribozymes

- **Ribozymes** are catalytic RNA molecules that function as enzymes and can splice RNA
- The discovery of ribozymes rendered *obsolete* the belief that *all biological catalysts were proteins*

所以酵素不一定是蛋白質！

RNA as an exzyme

- Three properties of RNA enable it to function as an enzyme

  1) It can form a three-dimensional structure because of its ability to base pair with itself
  2) Some bases in RNA contain functional groups
  3) RNA may hydrogen-bond with other nucleic acid molecules

G≡C ; A=T/U

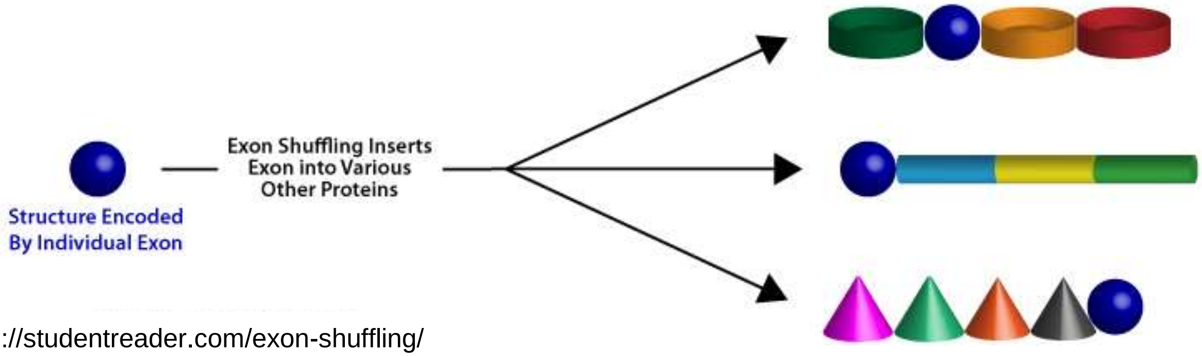
The Functional and Evolutionary Importance of Introns

- Some genes can encode more than **one** kind of polypeptide, depending on which segments are treated as exons during RNA splicing
- Such variations are called **alternative RNA splicing**
- Because of alternative splicing, *the number of different proteins an organism can produce is much greater than its number of genes*

蛋白質數目遠大於基因數目！

Protein Domain

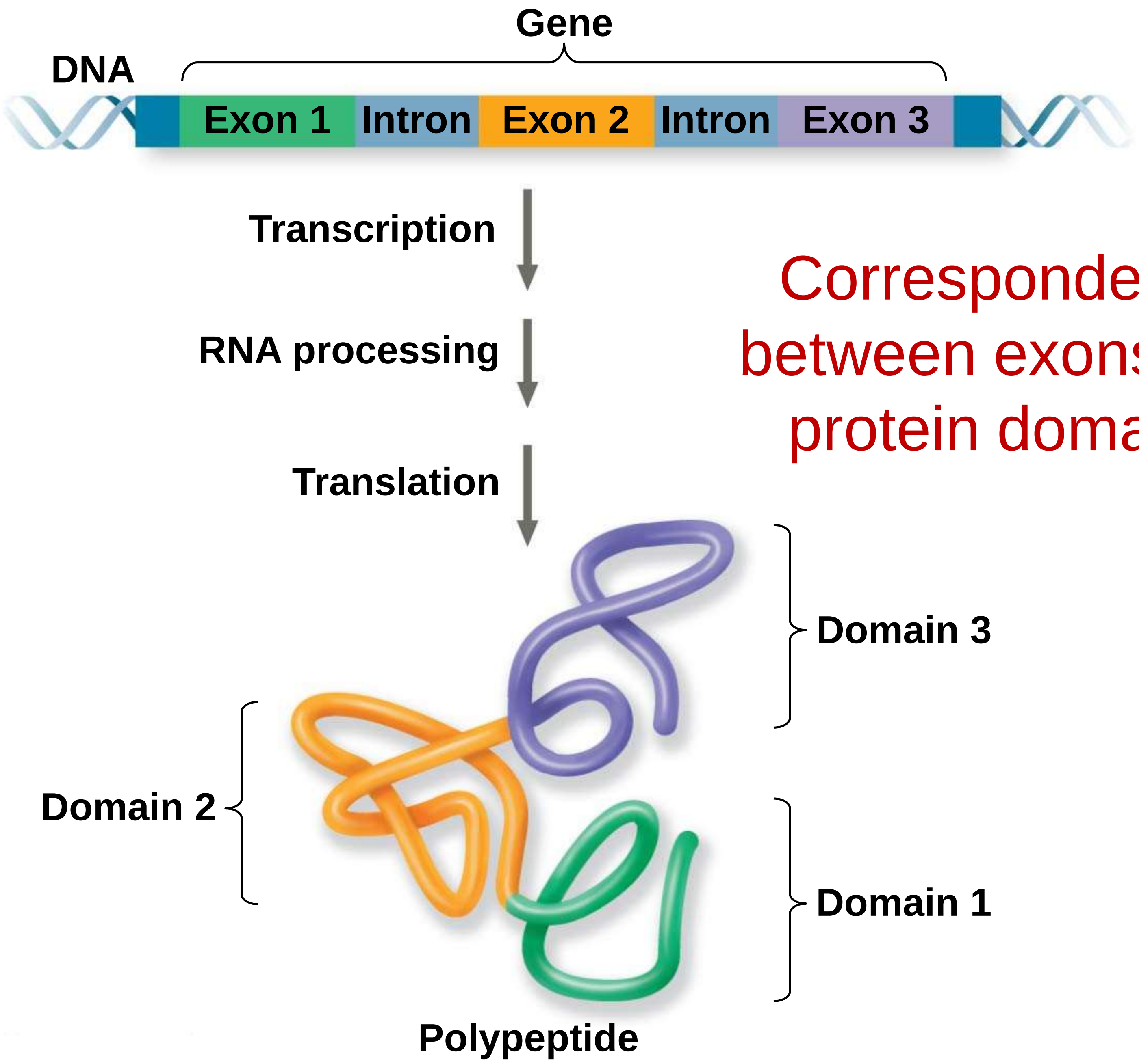
- Proteins often have a modular architecture consisting of discrete regions called **domains**
- In many cases, different exons code for the different domains in a protein
- Exon shuffling may result in the evolution of new proteins

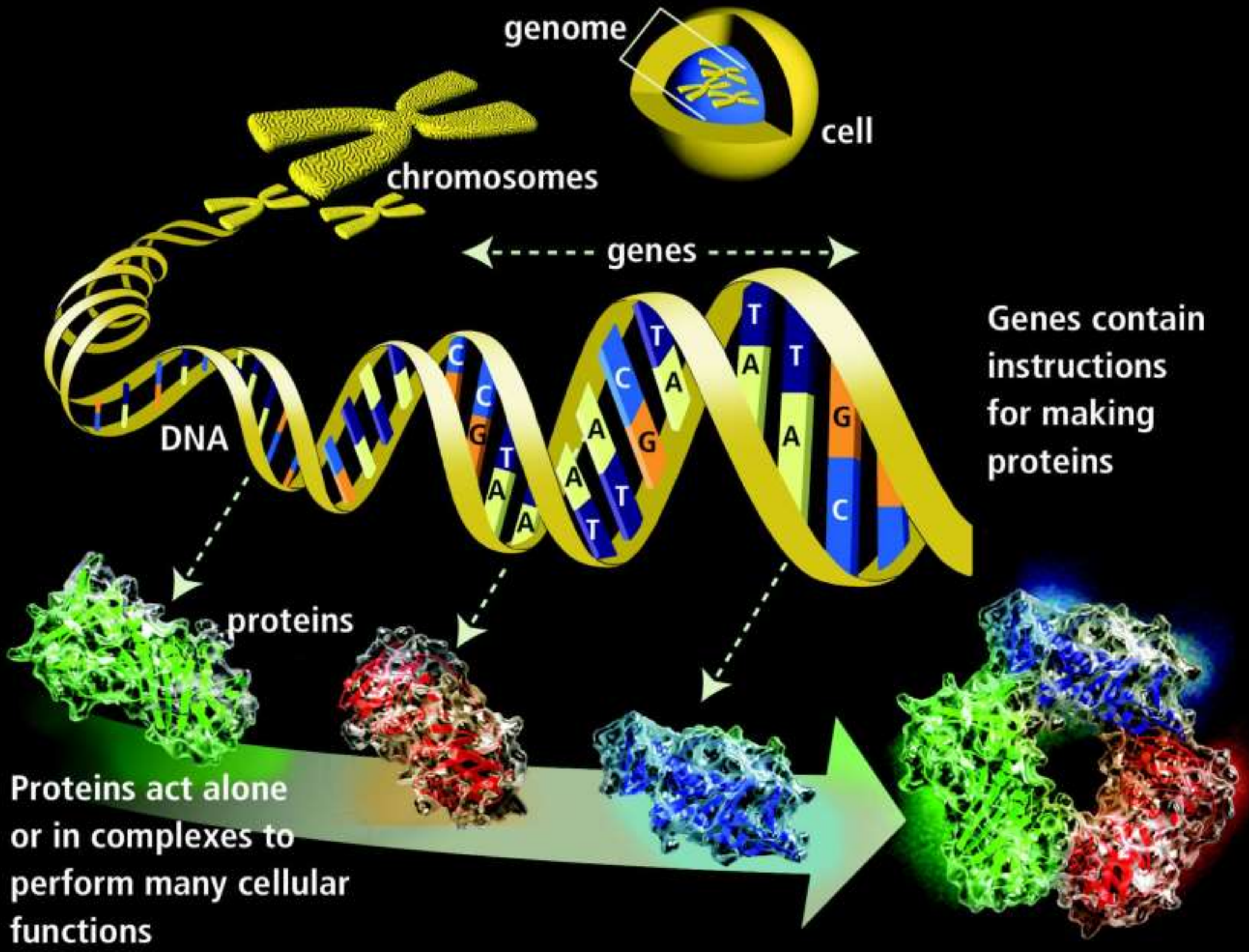


http://studentreader.com/exon-shuffling/

Figure 17.13

Correspondence between exons and protein domains





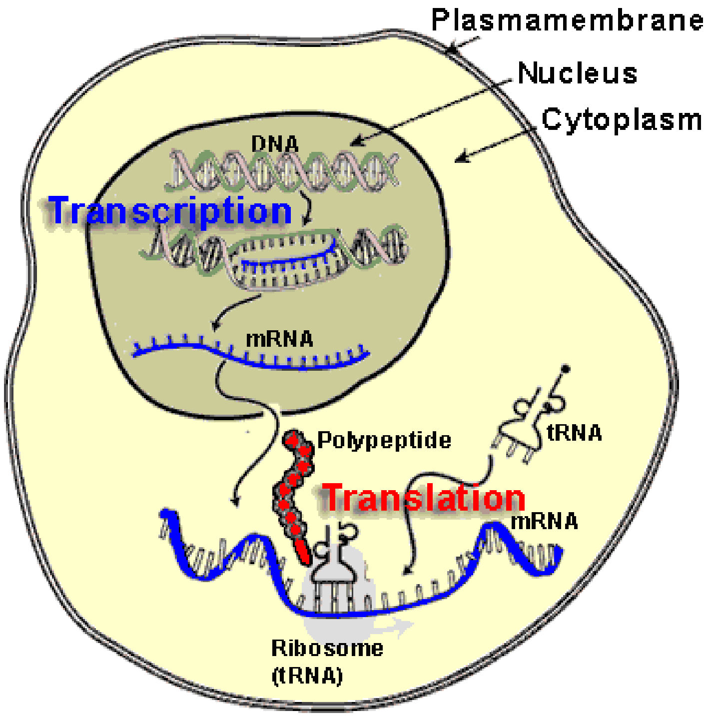
Genes contain instructions for making proteins

Proteins act alone or in complexes to perform many cellular functions

U.S. DEPARTMENT OF ENERGY

Concept 17.4: Translation is the RNA-directed synthesis of a polypeptide: *a closer look*

- The **translation** 轉譯 of **mRNA to protein** can be examined in more detail - 以mRNA為模板在核糖體上依序合成 polypeptide 鏈的過程。



Molecular Components of Translation

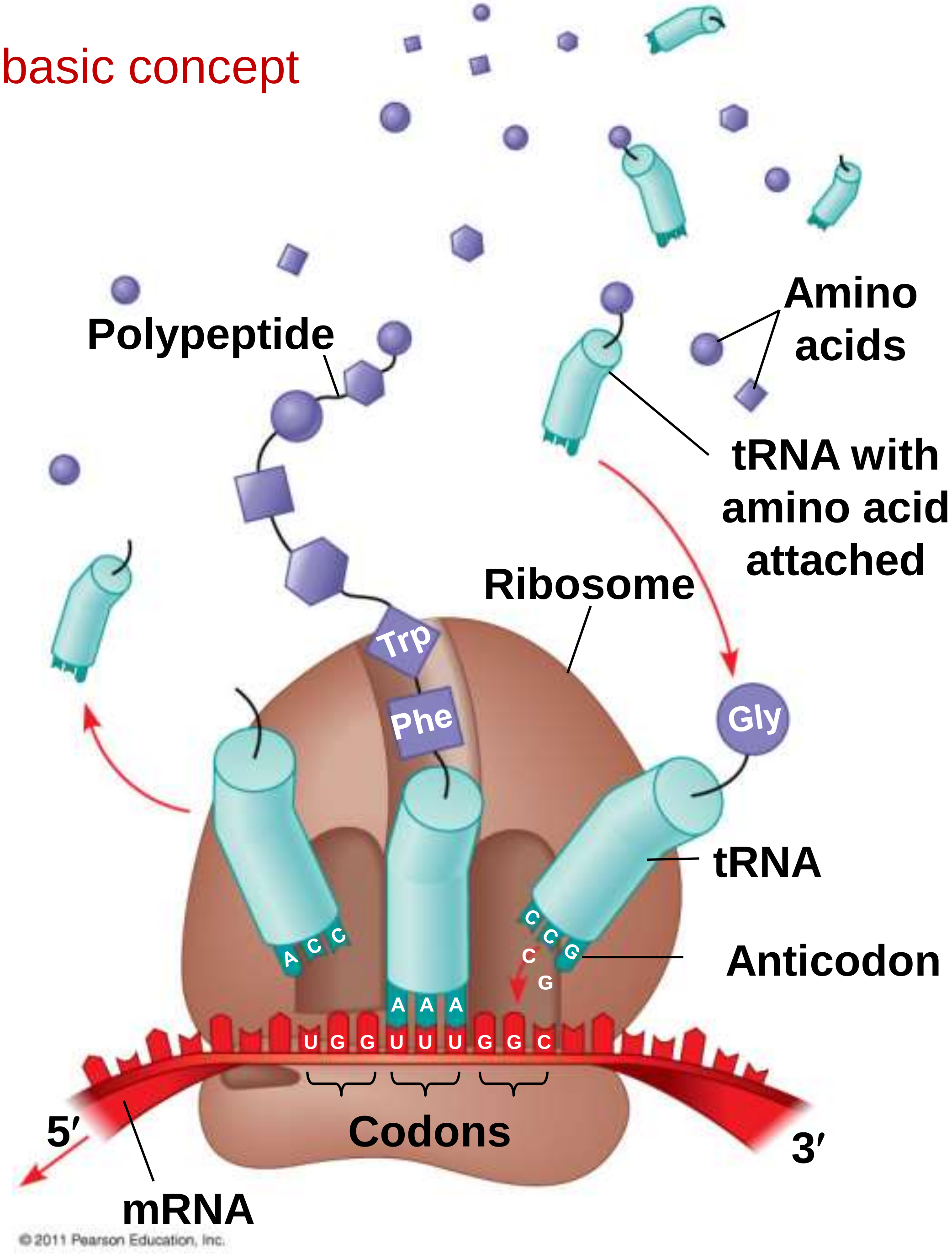
- A cell translates an mRNA message into protein with the help of **transfer RNA (tRNA)**

- Molecules of tRNA are not identical:
  - Each carries a specific amino acid on one end
  - Each has an **anticodon** on the other end; the anticodon base-pairs with a complementary codon on mRNA

PLAY **BioFlix: Protein Synthesis**

Figure 17.14

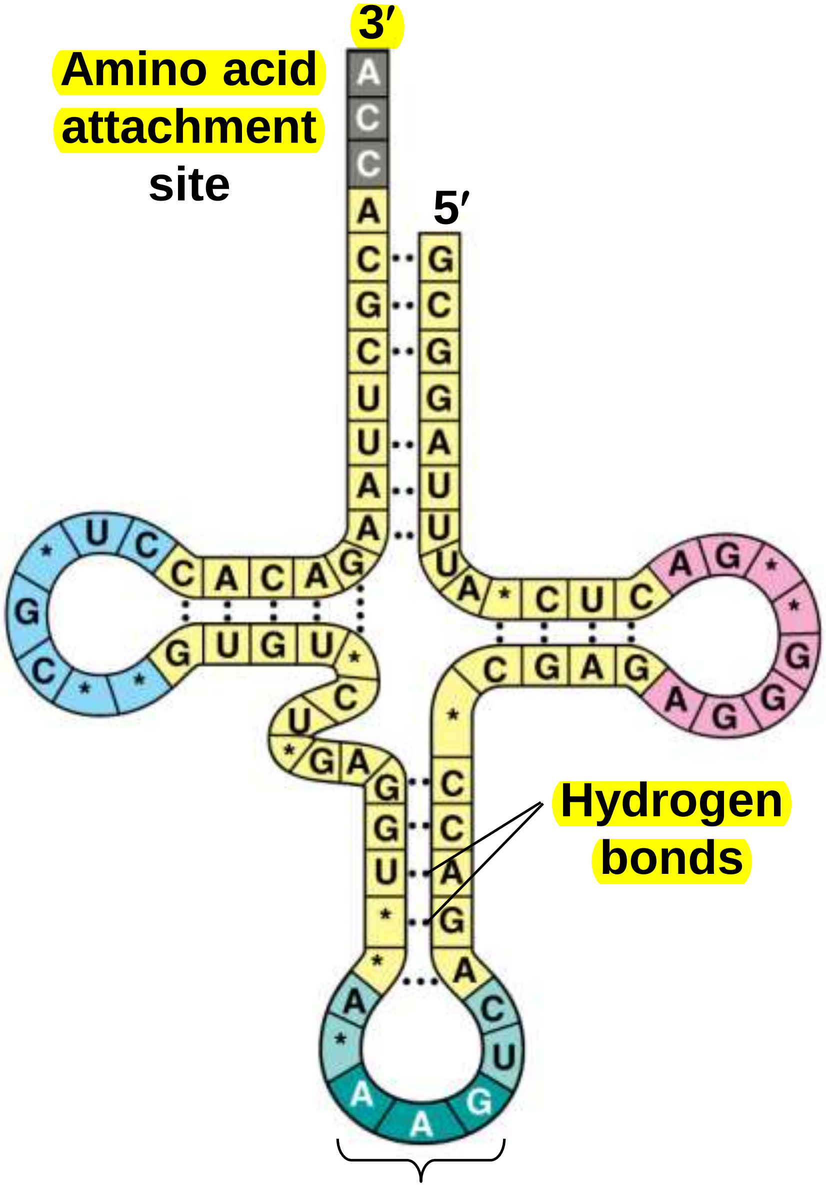
Translation: the basic concept



The Structure and Function of Transfer RNA

- A tRNA molecule consists of a single RNA strand that is only about 80 nucleotides long
- Flattened into one plane to reveal its base pairing, a tRNA molecule looks like a cloverleaf

Figure 17.15a



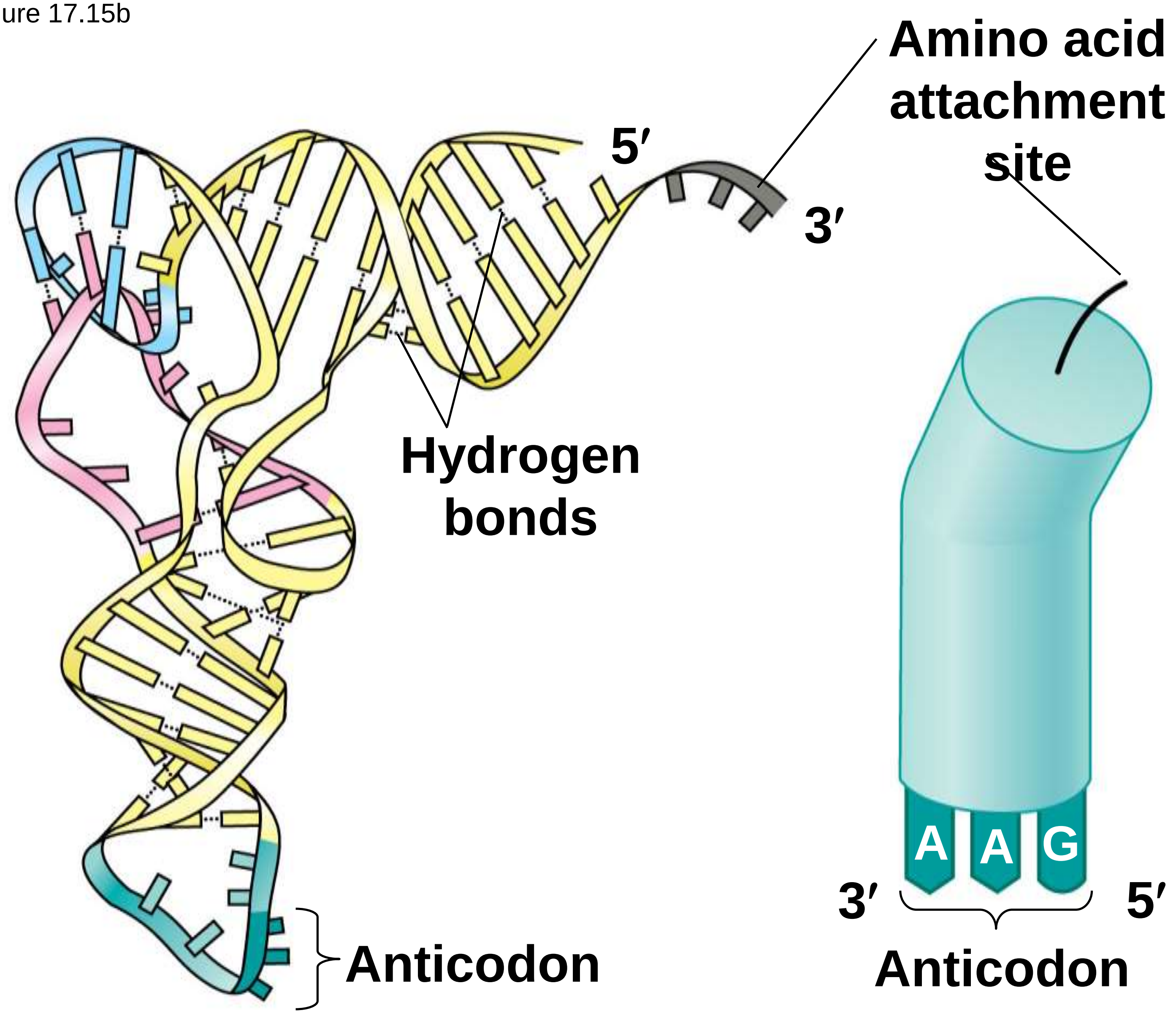
Amino acid attachment site

Hydrogen bonds

Anticodon

(a) Two-dimensional structure

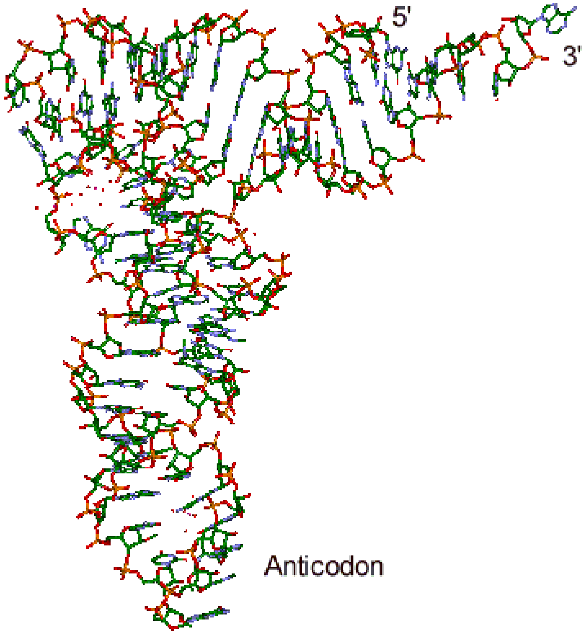
Figure 17.15b



(b) Three-dimensional structure

(c) Symbol used in this book

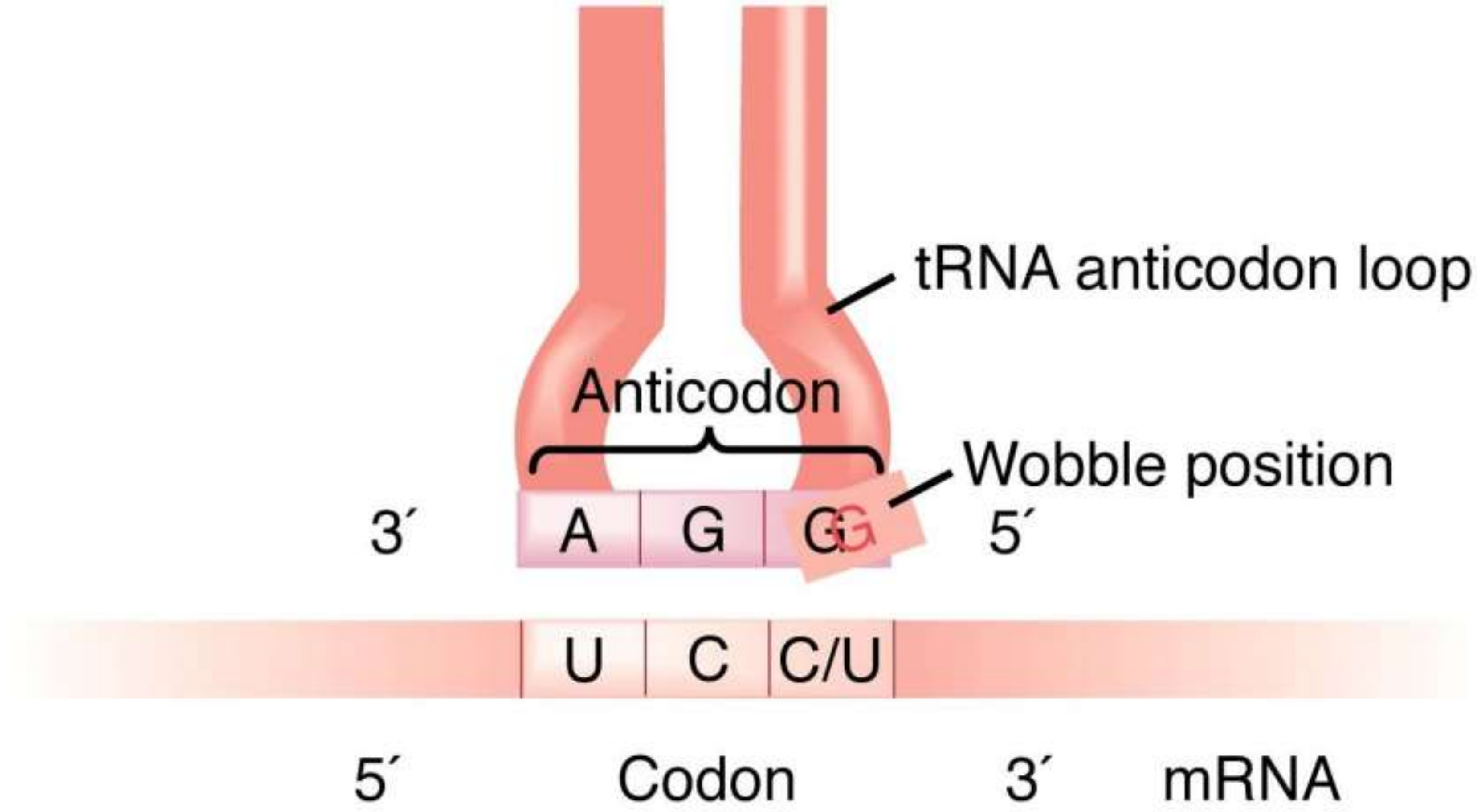
- Because of hydrogen bonds, tRNA actually twists and folds into a three-dimensional molecule
- tRNA is roughly L-shaped



tRNA - Amino acid - mRNA codon

- Accurate translation requires two steps:
  - **1.** A correct match between a tRNA and an amino acid, done by the enzyme **aminoacyl-tRNA synthetase**
  - **2.** A correct match between the tRNA anticodon and an mRNA codon
- Flexible pairing at the third base of a codon is called **wobble** and allows some tRNAs to bind to more than one codon

Wobble base pairing



(Not enough tRNAs to match all 64 codons)

Figure 17.16-1

An aminoacyl-tRNA synthetase joining a specific amino acid to a tRNA

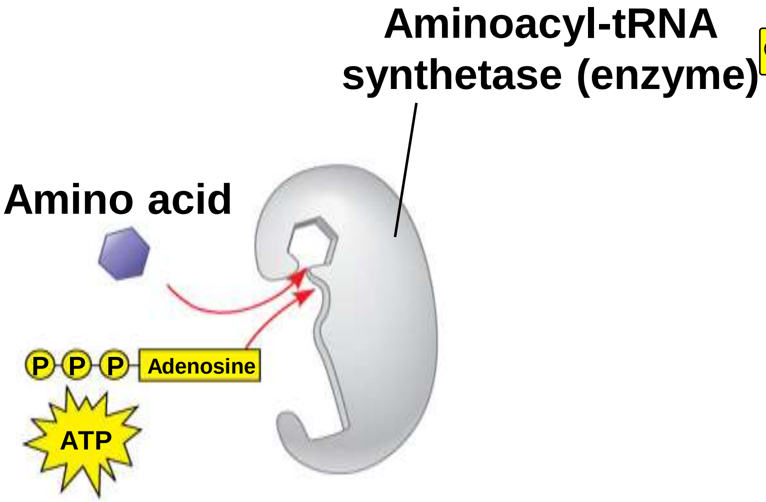


Figure 17.16-2

An aminoacyl-tRNA synthetase joining a specific amino acid to a tRNA

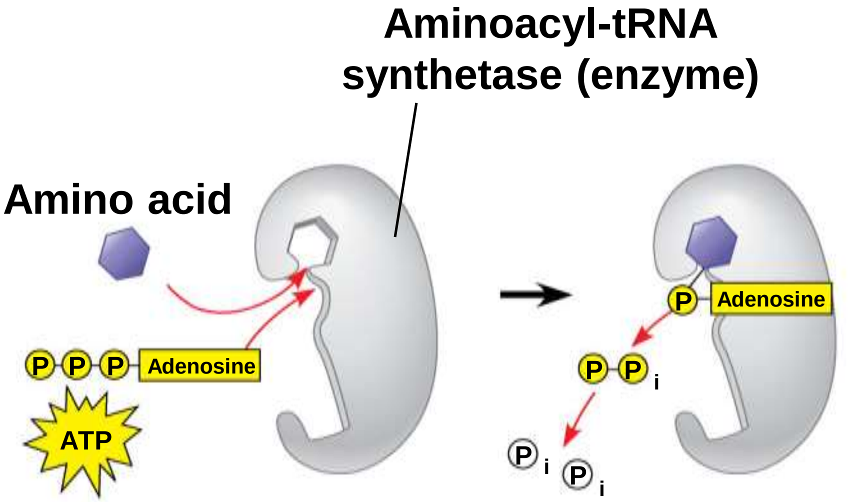


Figure 17.16-3

An aminoacyl-tRNA synthetase joining a specific amino acid to a tRNA

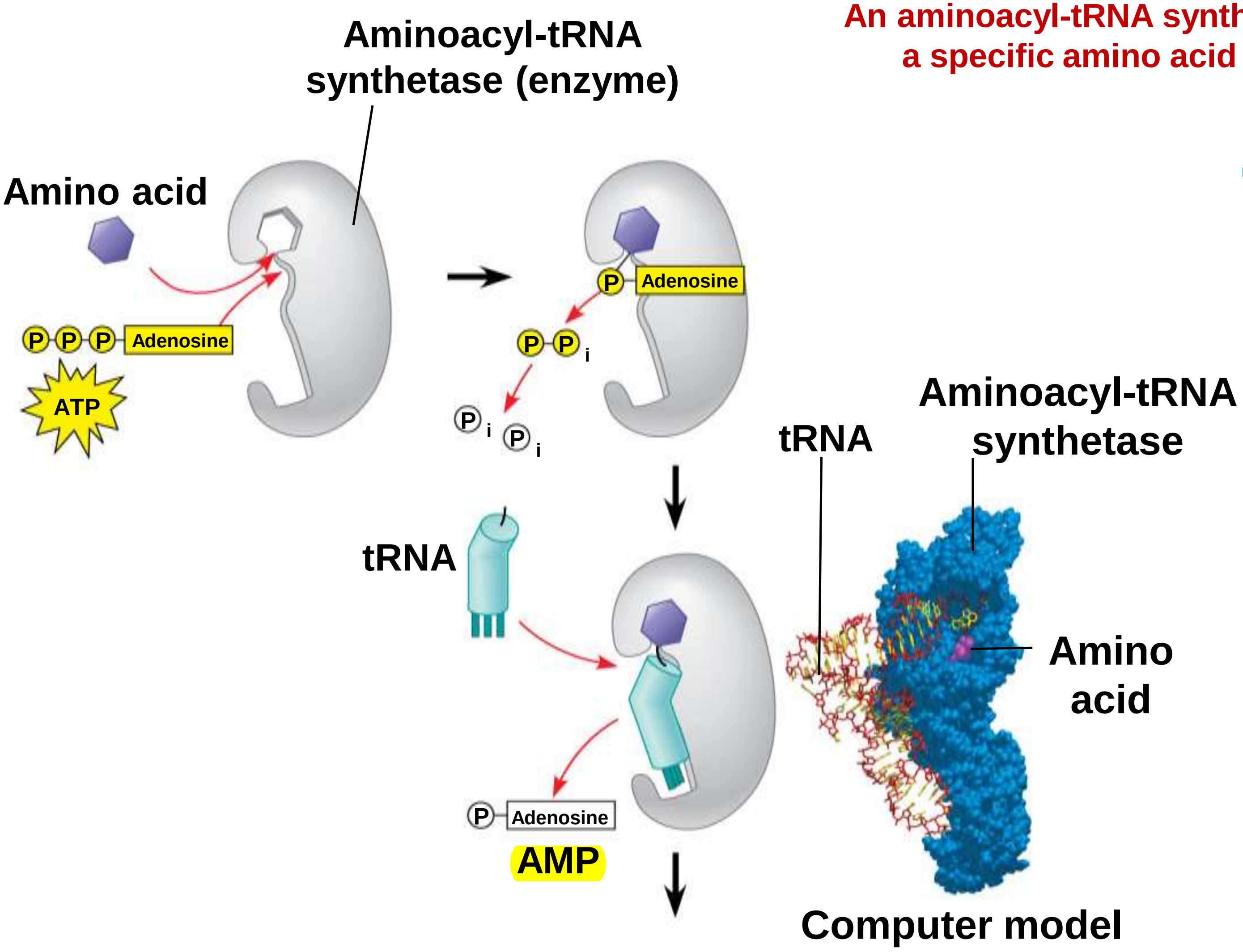
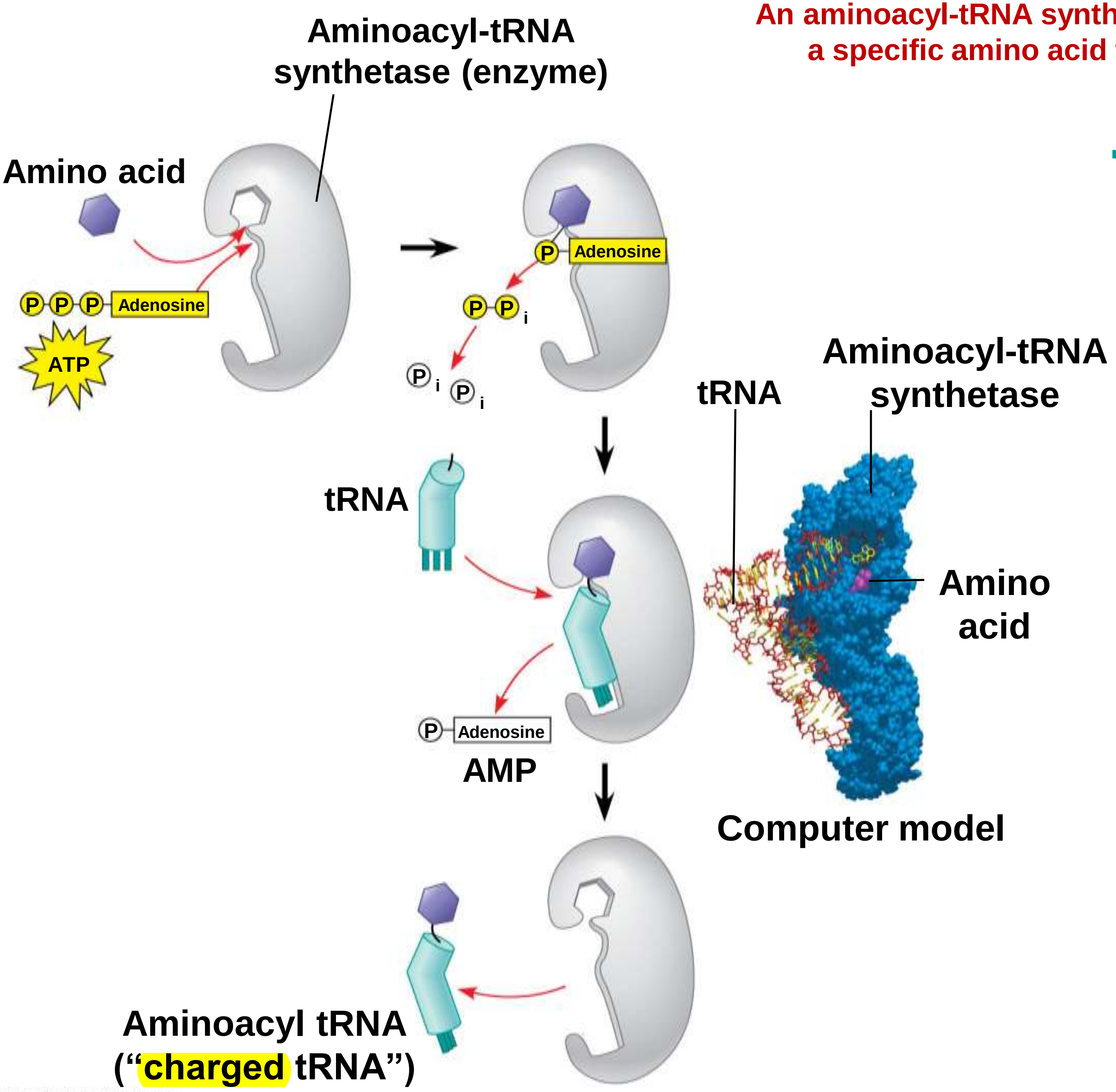


Figure 17.16-4

An aminoacyl-tRNA synthetase joining a specific amino acid to a tRNA



Ribosomes

- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis

- The two ribosomal subunits (large and small) are made of proteins and **ribosomal RNA (rRNA)**

常見錯誤

Ribozyme vs. Ribosome

mRNA Splicing　Protein Synthesis

諾貝爾雙饗宴 大師談蛋白質的形成機制

諾貝爾大師在清華 演講影片

回上頁 【2012.06.29秘書處】

2004年諾貝爾化學獎得主傑哈諾佛教授（Aaron Ciechanover）及2009年諾貝爾化學獎得主尤娜特教授(Ada E. Yonath)兩位諾貝爾大師，6月19日蒞臨本校生科院參訪及演講，受到熱烈的歡迎。因適逢生科院創院20週年，舉辦2012諾貝爾雙饗宴深具意義，當日下午並另舉辦一場生技論壇，由馮達旋副校長主持，邀請二位以色列籍諾貝爾得主及生技界專家參與，除介紹我國生技產品創新研發及跨入國際市場的策略及成功案例，也共同談論台灣與以色列在世界經貿與生技舞臺上扮演的關鍵性角色。

陳力俊校長與尤娜特教授及傑哈諾佛教授合影

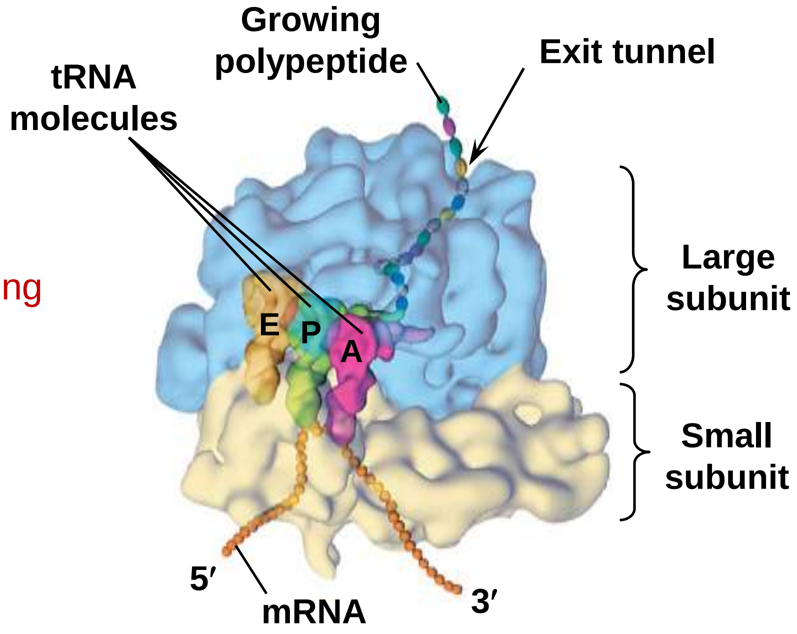
陳力俊校長提到，兩位大師都來自以色列，也有共同特色，從小就具有好奇心與冒險精神，有境外學習經驗，長期與其他國際團隊合作，並且不畏挫折，都是從基礎研究出發而有很高的成就。陳校長說，在國內有機會聽一位諾貝爾獎得主演講已是十分難得，一天早上能聆聽兩位世界級學術大師分享他們的智慧光華，實屬有幸，是名符其實「滿漢全席」級的盛筵。

尤娜特教授為以色列諾貝爾得主中唯一女性，也是中東地區科學類諾貝爾獎第一位女性得主。她專研核醣體數十年，利用X光晶體繞射的方法，成功解得「核醣體」(ribosome)—生物細胞製造蛋白質的工廠—的立體分子結構。她也進一步利用其他生物物理方法，完整解析核醣體合成蛋白質的運作機制。

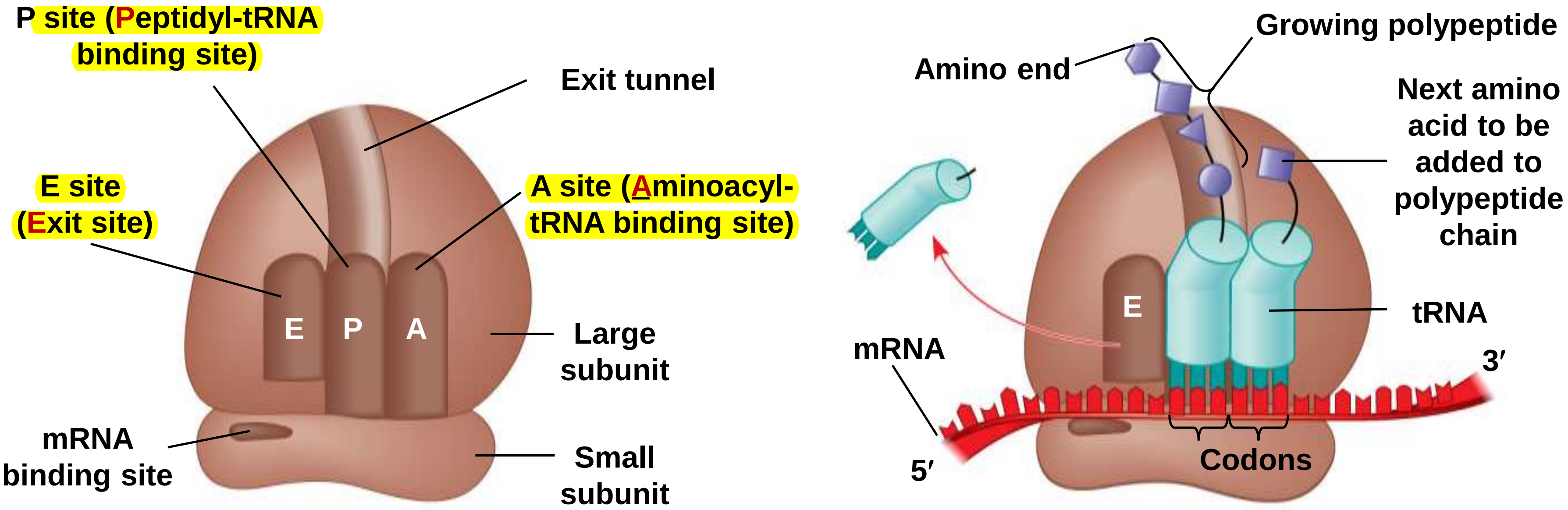
The Nobel Prize in Chemistry 2009 was awarded to Ada E. Yonah
"for studies of the structure and function of the ribosome".

Figure 17.17

The anatomy of a functioning ribosome



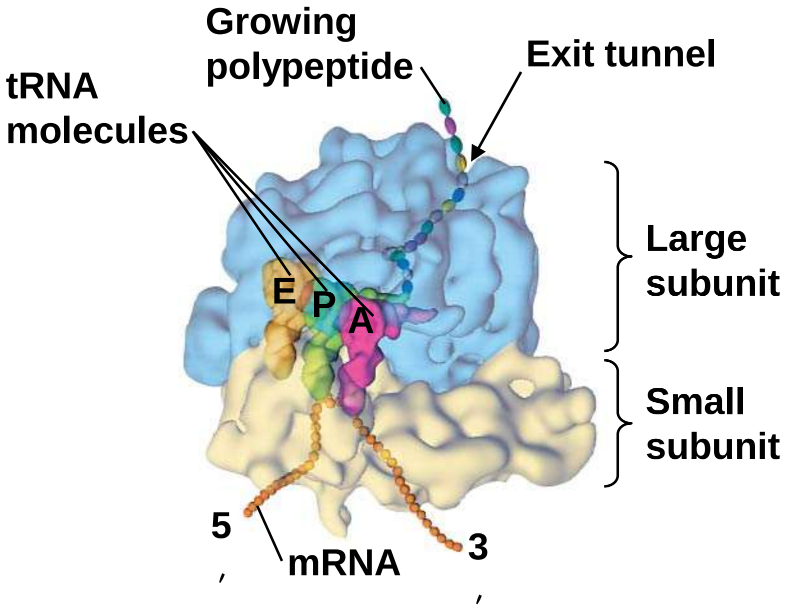
(a) Computer model of functioning ribosome



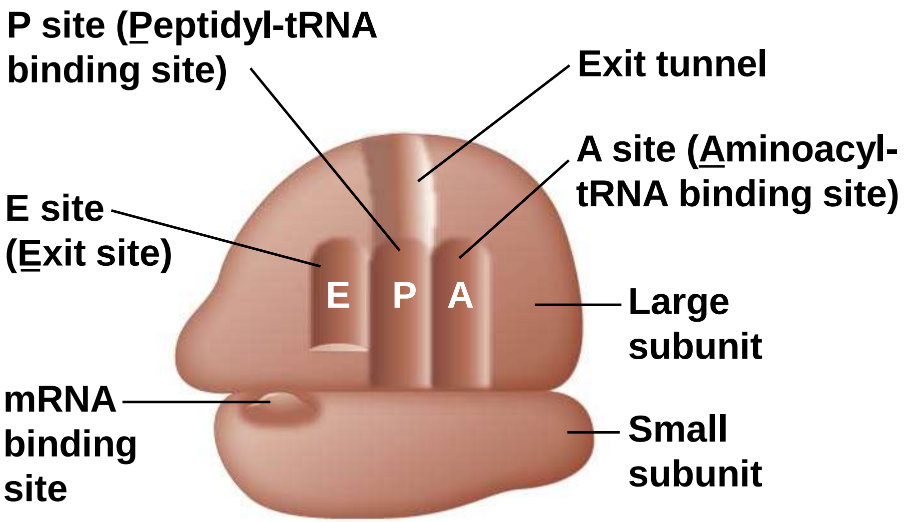
(b) Schematic model showing binding sites

(c) Schematic model with mRNA and tRNA

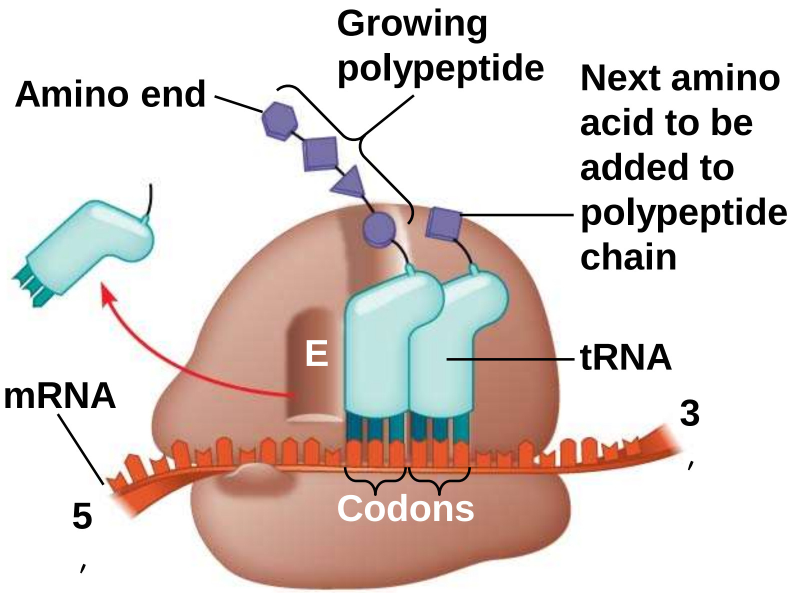
Figure 17.17



(a) Computer model of functioning ribosome

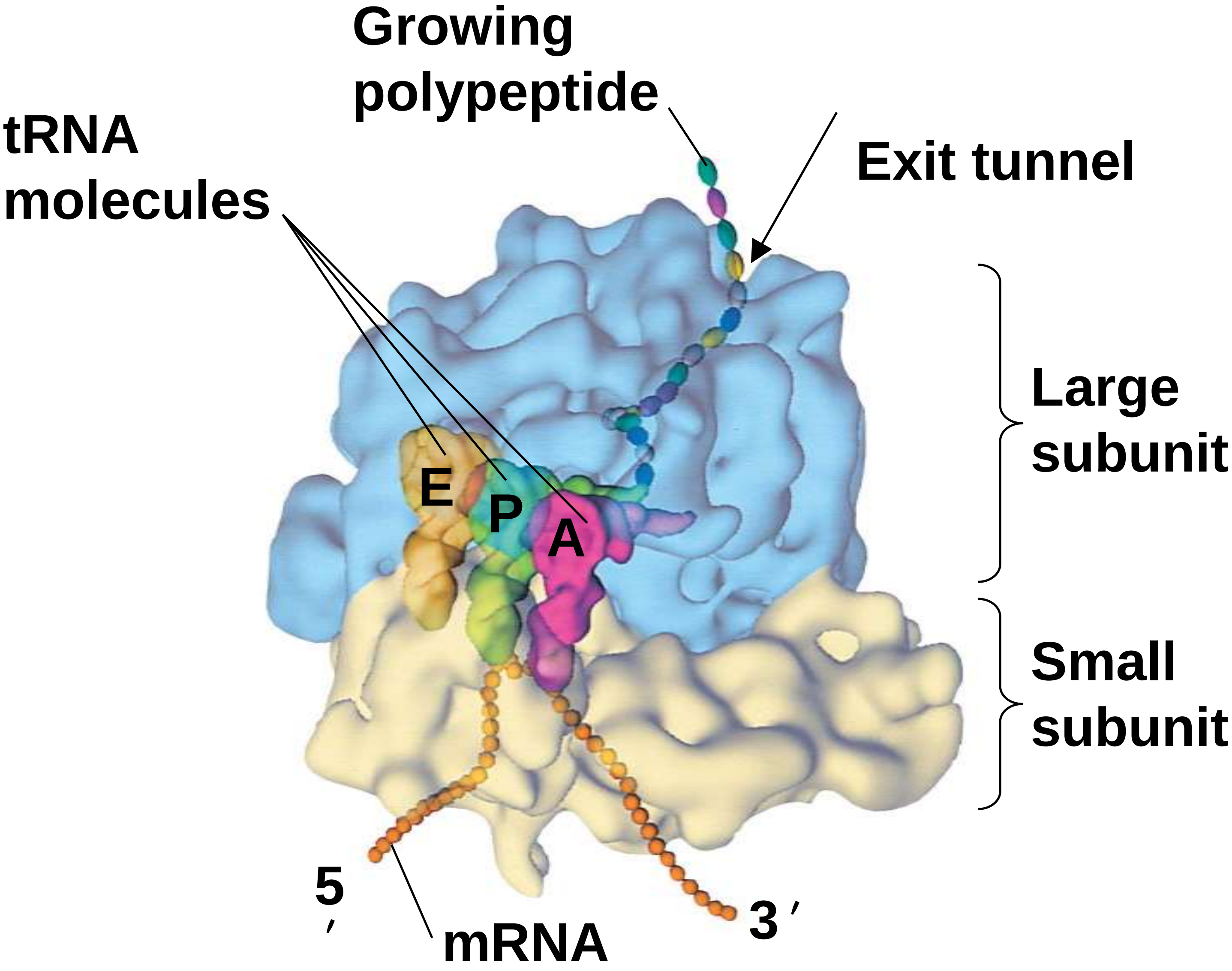


(b) Schematic model showing binding sites



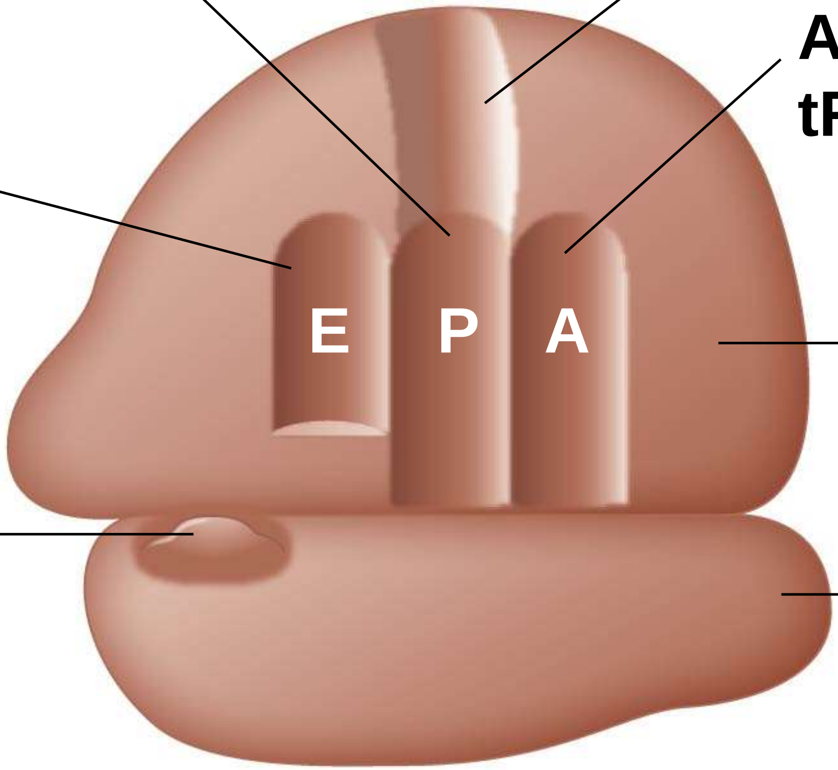
(c) Schematic model with mRNA and tRNA

Figure 17.17a



(a) Computer model of functioning ribosome

Figure 17.17b



P site (Peptidyl-tRNA binding site)

Exit tunnel

A site (Aminoacyl-tRNA binding site)

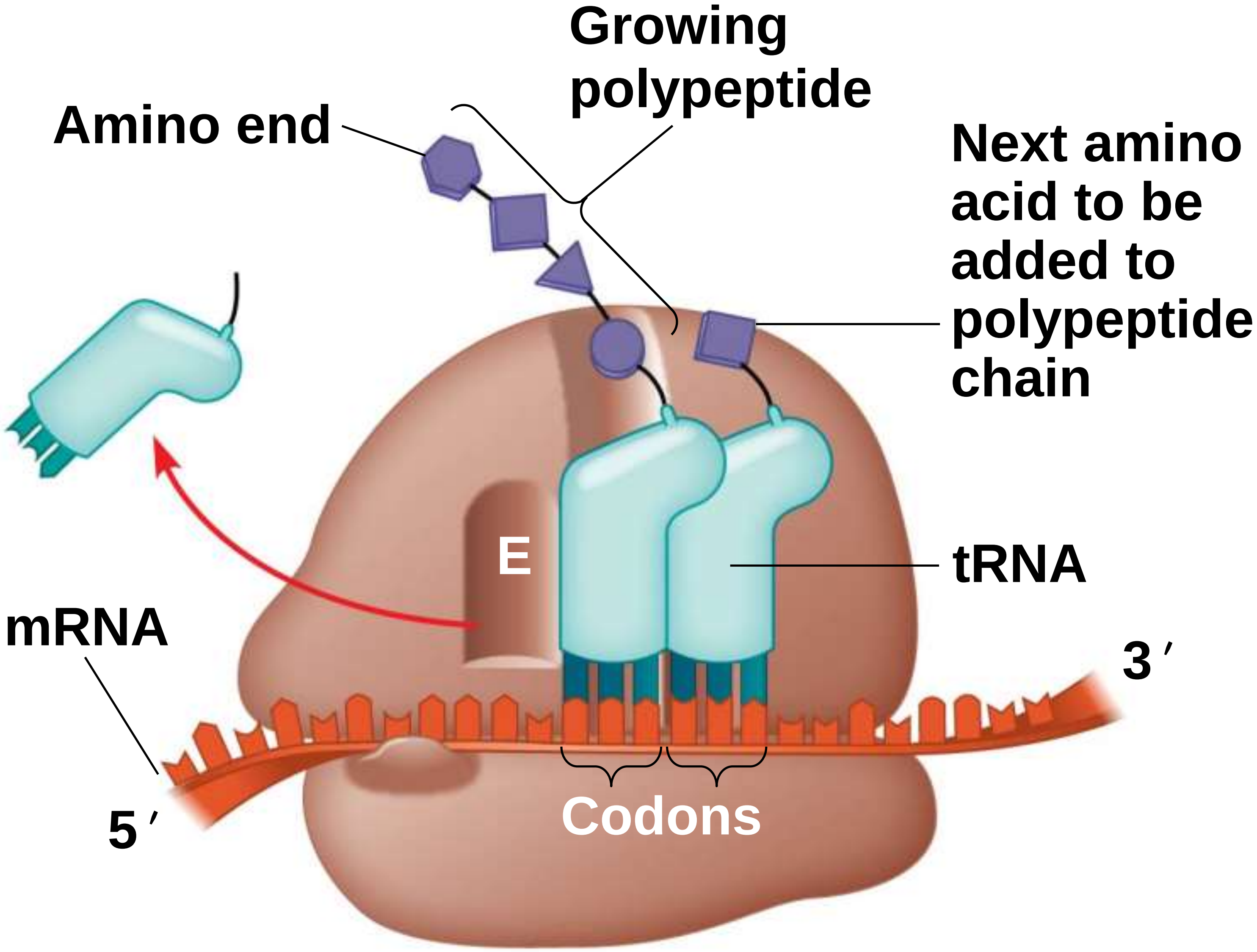
E site (Exit site)

Large subunit

mRNA binding site

Small subunit

(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

Summary: P, A, E sites in Ribosome

- A ribosome has three binding sites for tRNA:
  - The **P site** holds the tRNA that carries the growing polypeptide chain
  - The **A site** holds the tRNA that carries the next amino acid to be added to the chain
  - The **E site** is the exit site, where discharged tRNAs leave the ribosome

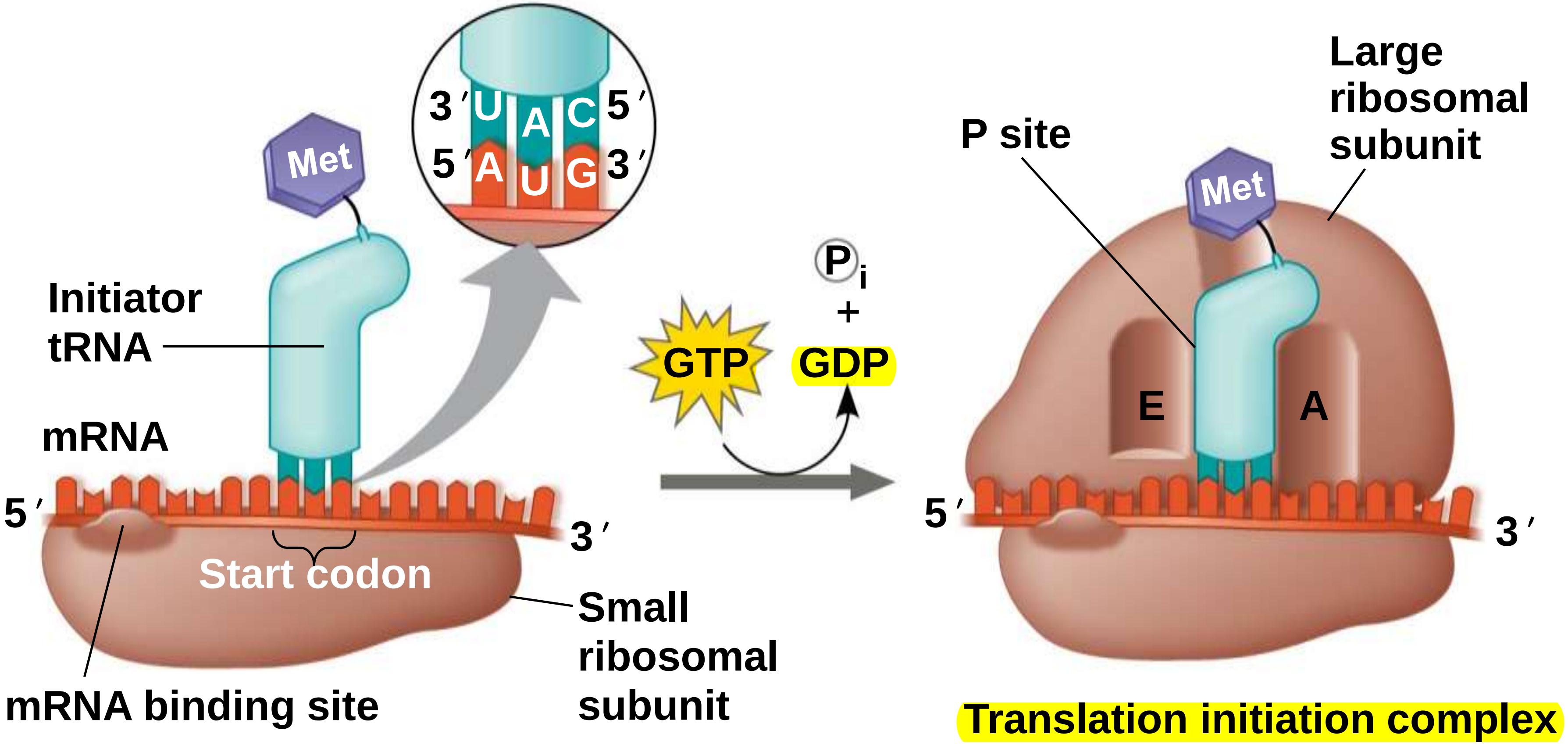
Pick **A**n **E**gg for Protein - 蛋白！

Building a Polypeptide

- The three stages of translation:
  - **Initiation**
  - **Elongation**
  - **Termination**
- All three stages require protein “factors” that aid in the translation process

Compare with the stages of **transcription** and **translation**

Figure 17.18 The initiation of translation



① Small ribosomal subunit binds to mRNA.

② Large ribosomal subunit completes the initiation complex.

Description on next page

Ribosome Association and Initiation of Translation

- The **initiation stage** of translation brings together mRNA, a tRNA with the first amino acid, and the two ribosomal subunits
  - First, a small ribosomal subunit binds with mRNA and a special initiator tRNA
  - Then the small subunit moves along the mRNA until it reaches the **start codon (AUG)**
  - Proteins called initiation factors bring in the large subunit that completes the translation initiation complex

Elongation of the Polypeptide Chain

- During the elongation stage, amino acids are added one by one to the preceding amino acid
  - Each addition involves proteins called elongation factors and occurs in three steps:
    - Codon recognition,
    - Peptide bond formation, and
    - Translocation

Figure 17.19-1

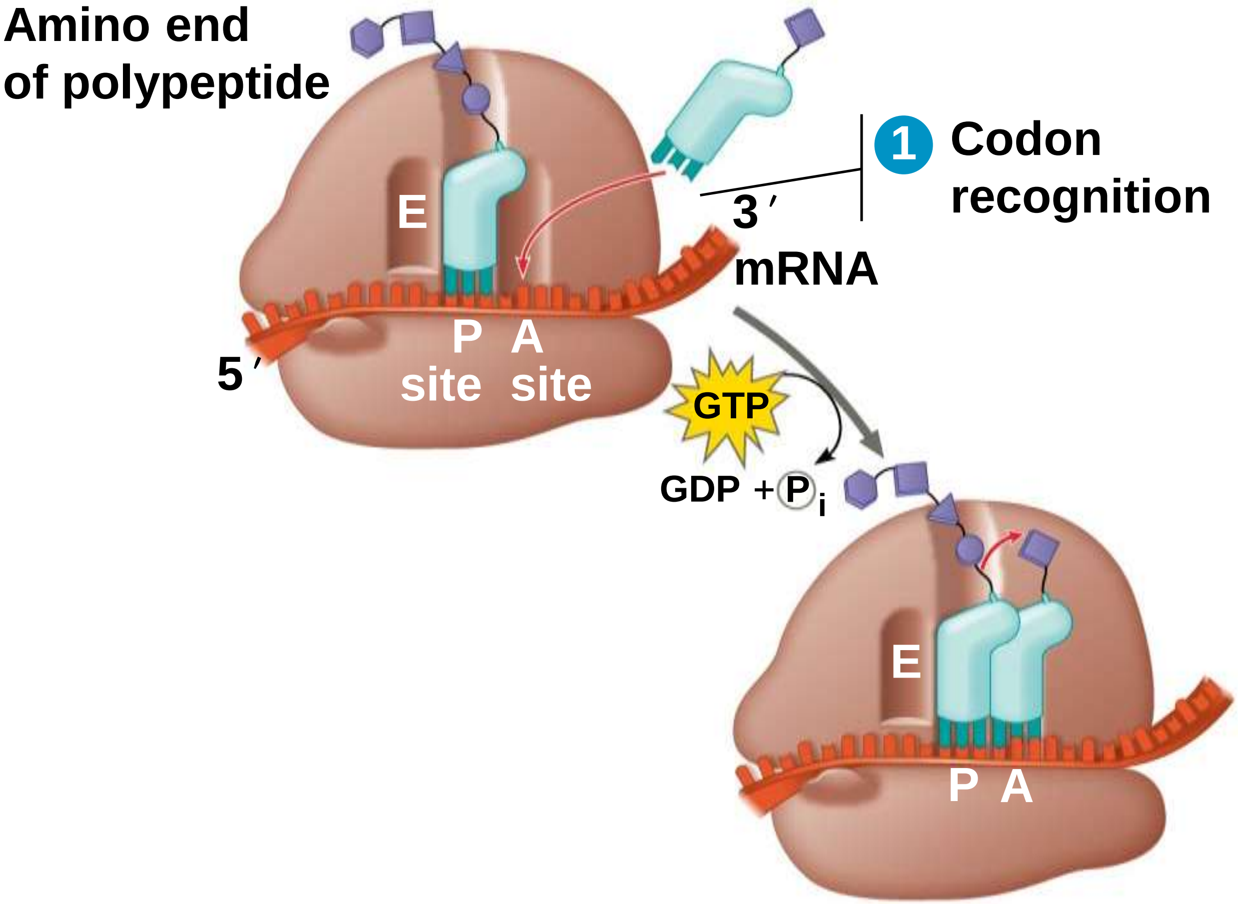


Figure 17.19-2

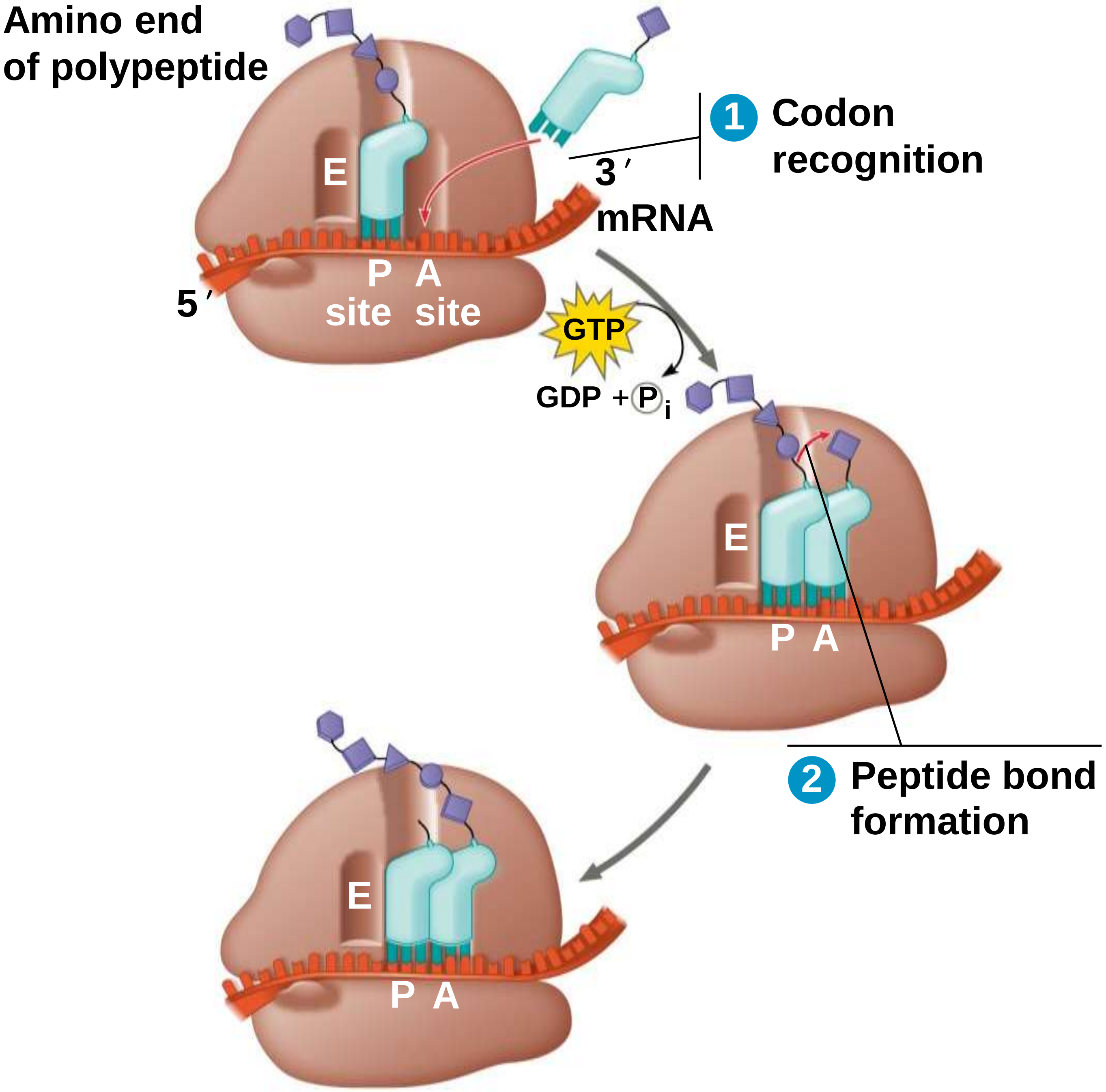
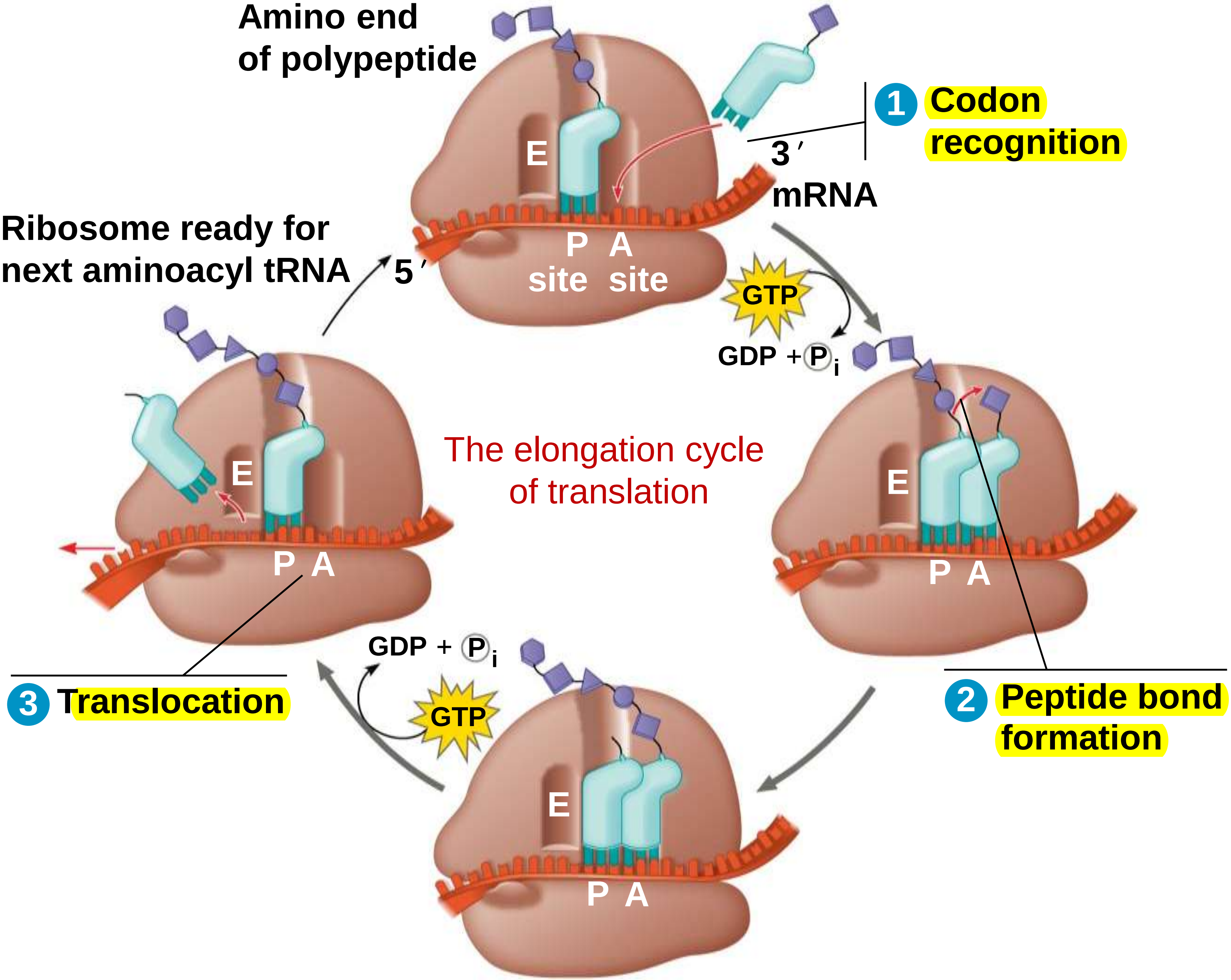


Figure 17.19-3



1 Codon recognition

2 Peptide bond formation

3 Translocation

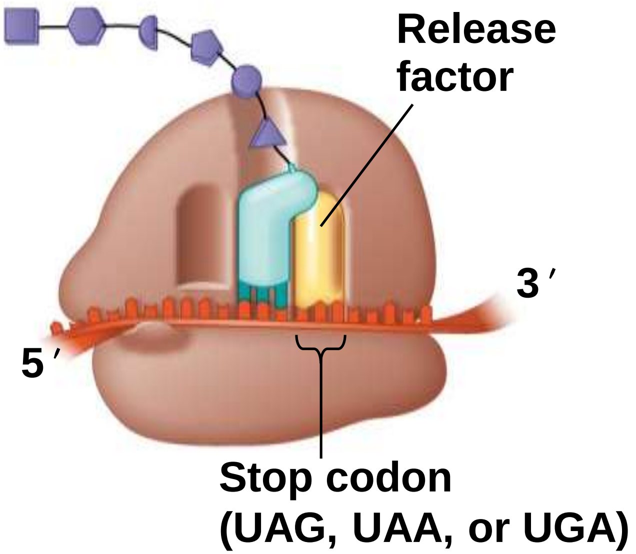
The elongation cycle of translation

Termination of Translation

- Termination occurs when a stop codon in the mRNA reaches the A site of the ribosome
  - The A site accepts a protein called a release factor
  - The release factor causes the addition of a water molecule instead of an amino acid
  - This reaction releases the polypeptide, and the translation assembly then comes apart

Figure 17.20-1

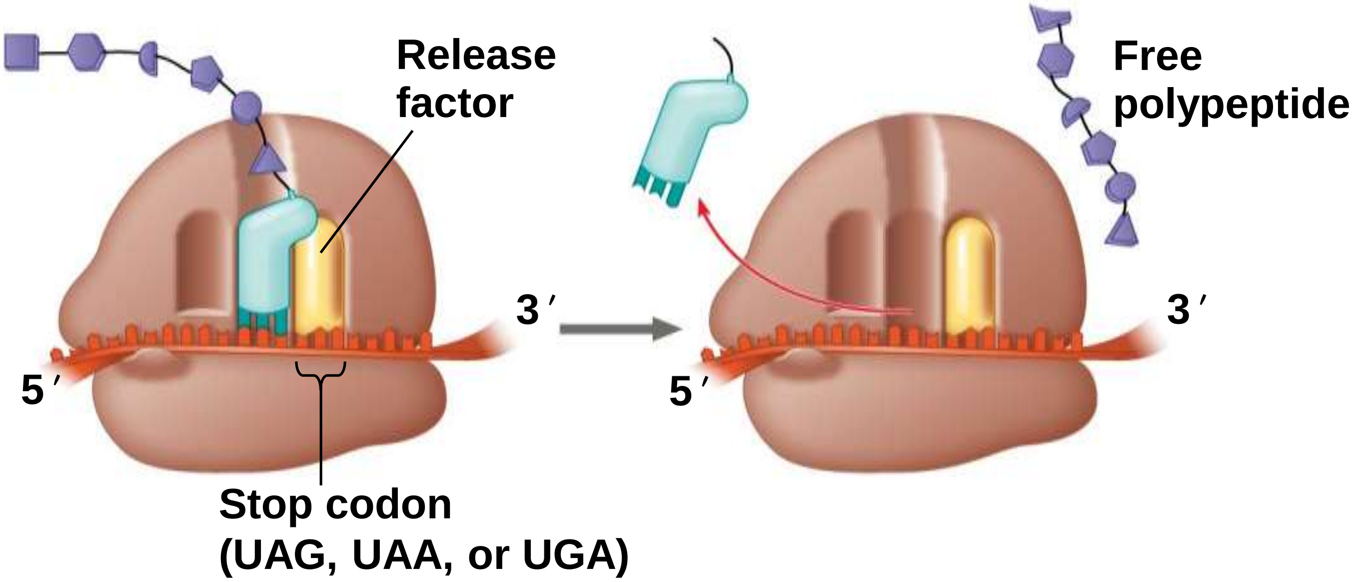
The termination of translation



1 Ribosome reaches a stop codon on mRNA.

Figure 17.20-2

The termination of translation

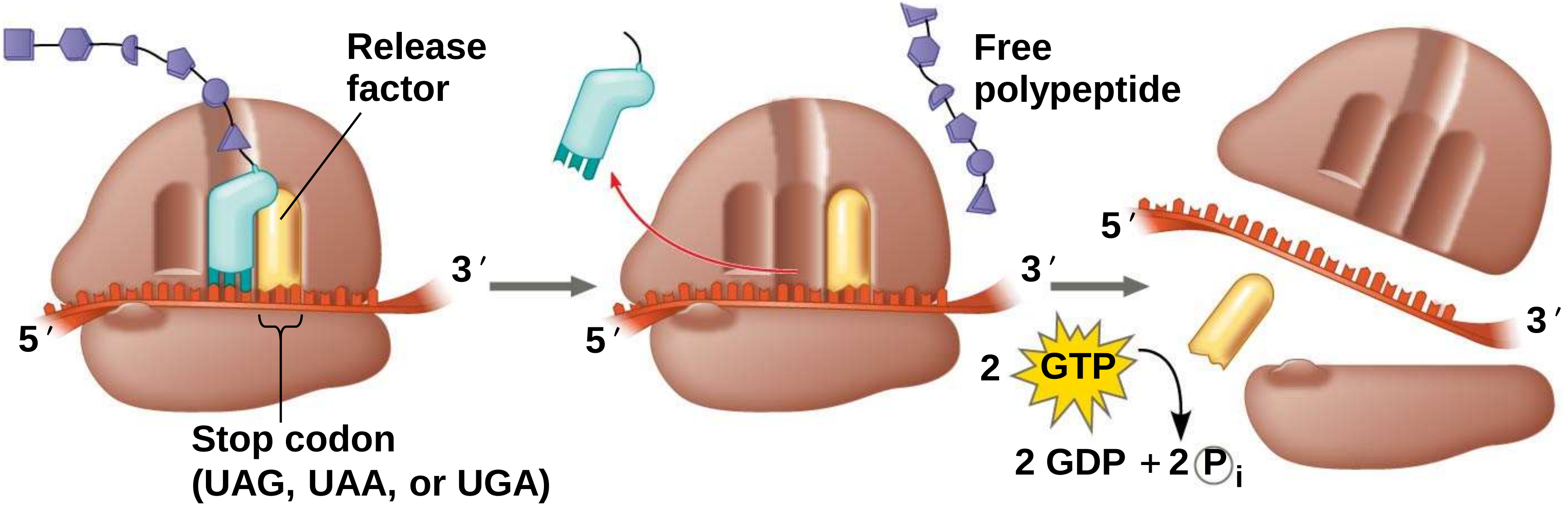


① Ribosome reaches a stop codon on mRNA.

② Release factor promotes hydrolysis.

Figure 17.20-3

The termination of translation



① Ribosome reaches a stop codon on mRNA.

② Release factor promotes hydrolysis.

③ Ribosomal subunits and other components dissociate.

HHMI video clip

Polyribosomes

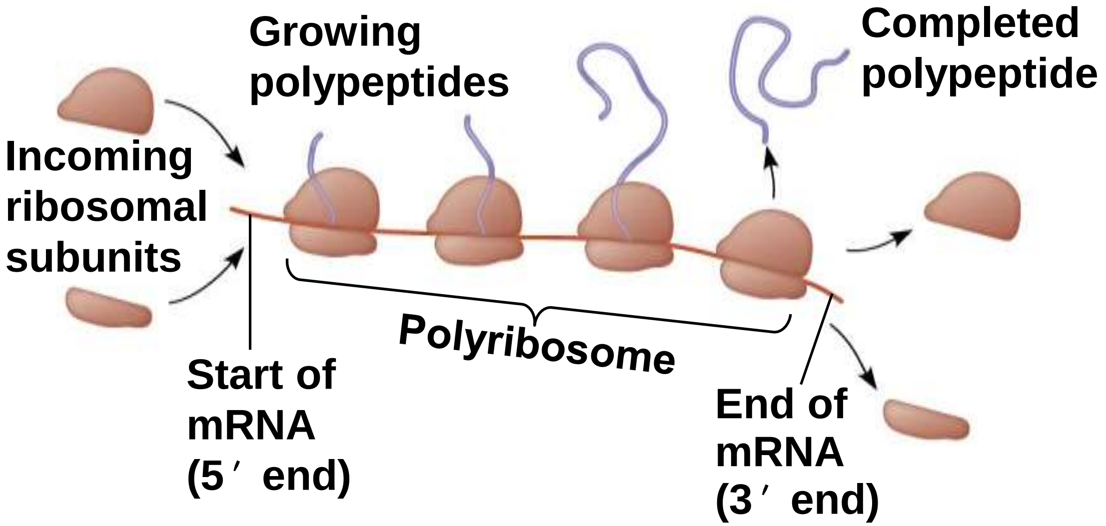
- A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**)

(多個生產線同時開工！）

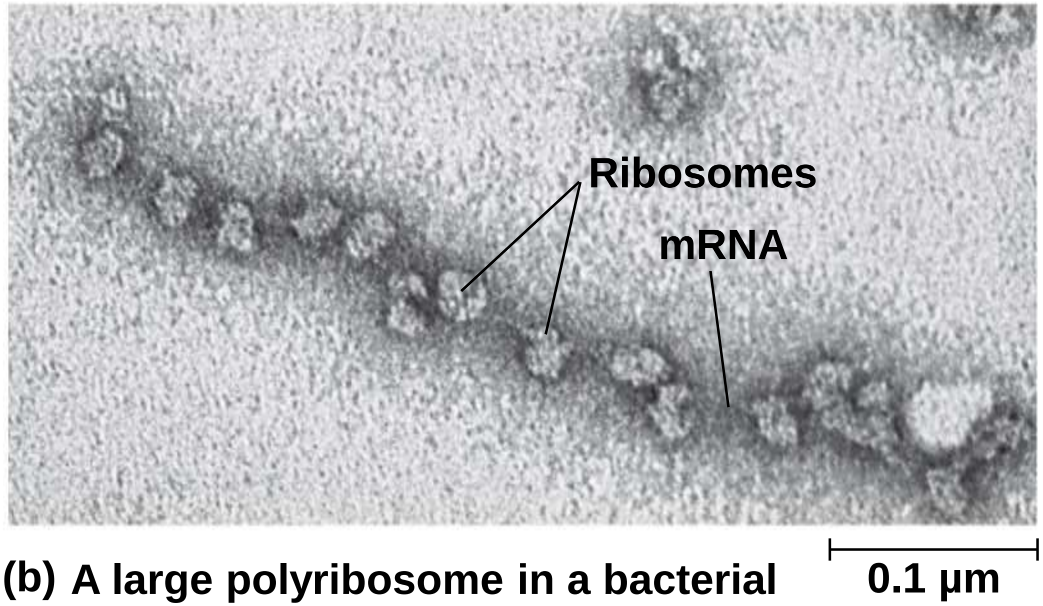
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly

Figure 17.22

Polyribosomes

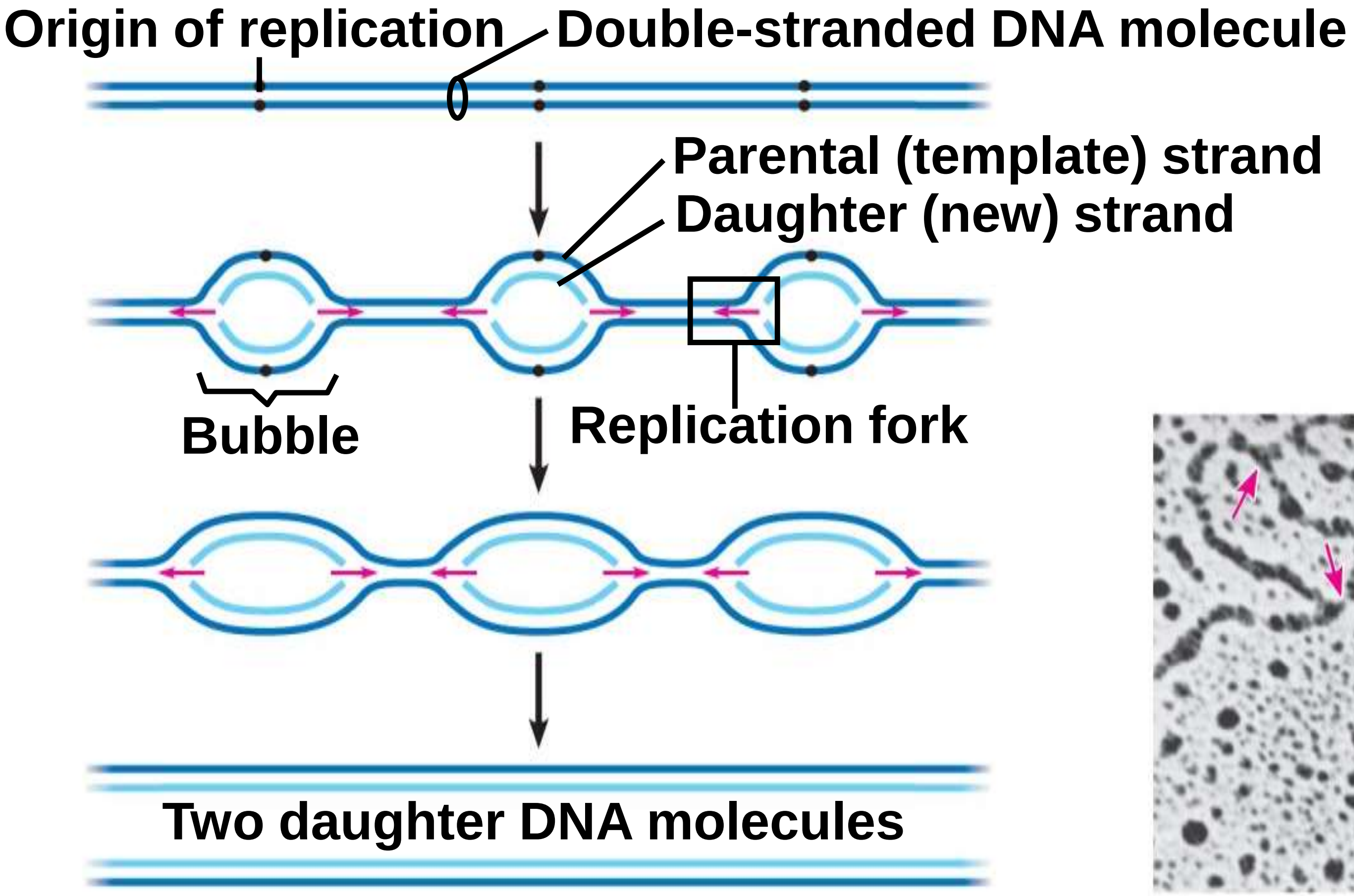


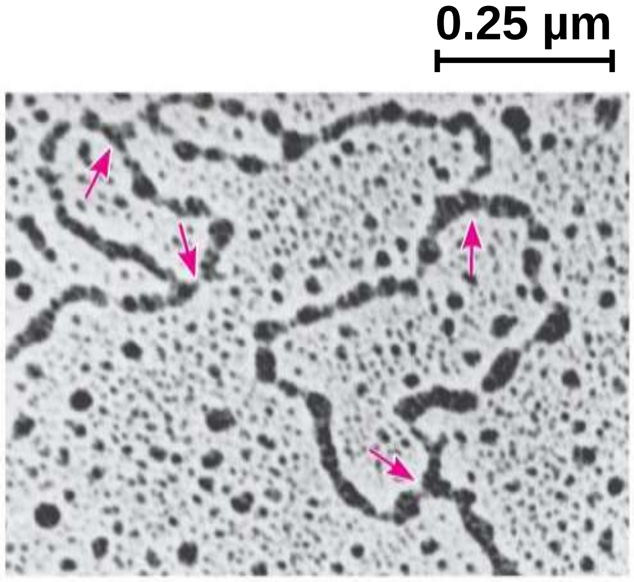
(a) Several ribosomes simultaneously translating one mRNA molecule



(b) A large polyribosome in a bacterial cell (TEM)

Review: Origins of replication in eukaryotes

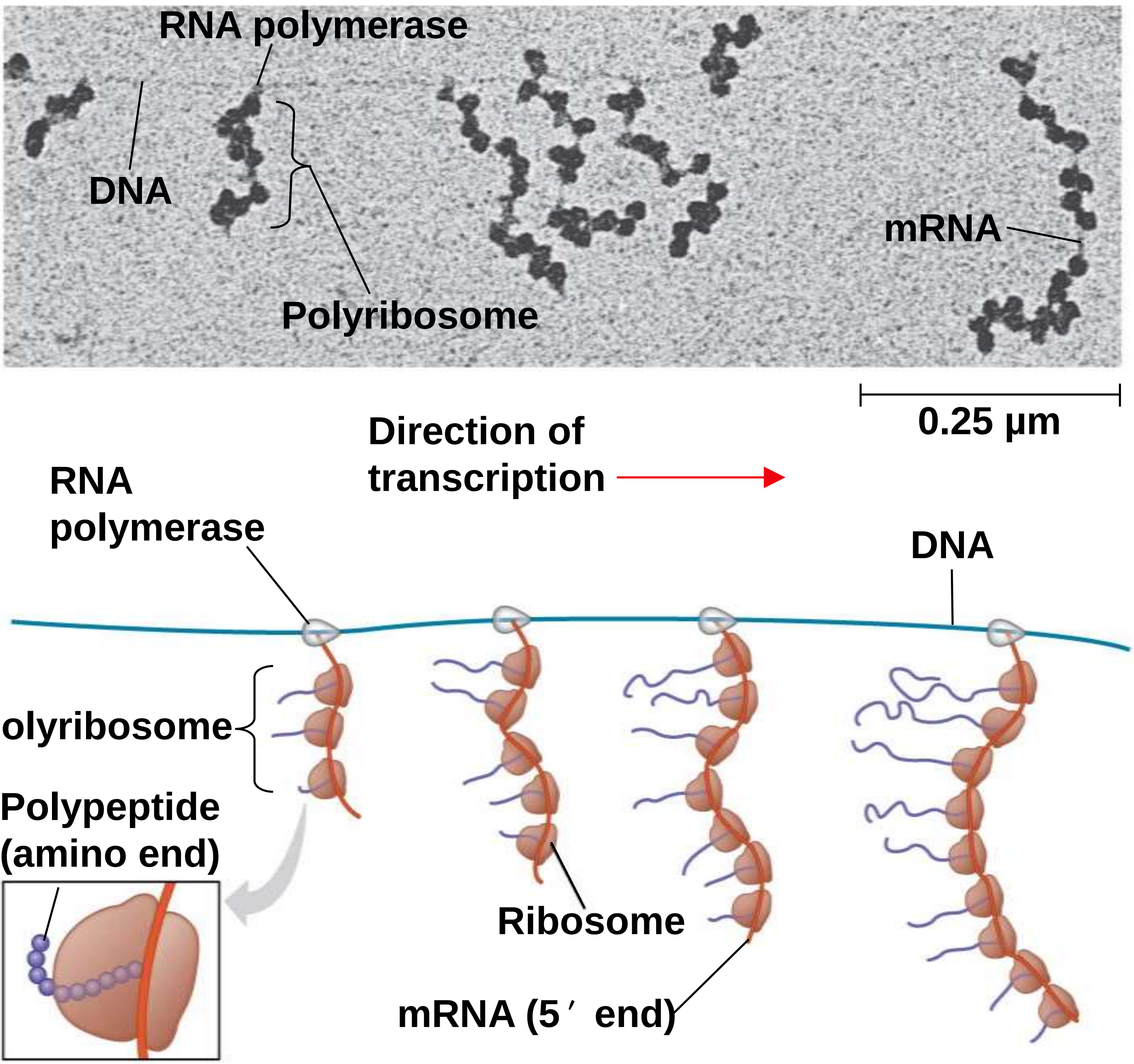




(b) Origins of replication in eukaryotes

Figure 16.12 Origins of replication in *E. coli* and eukaryotes

Figure 17.23



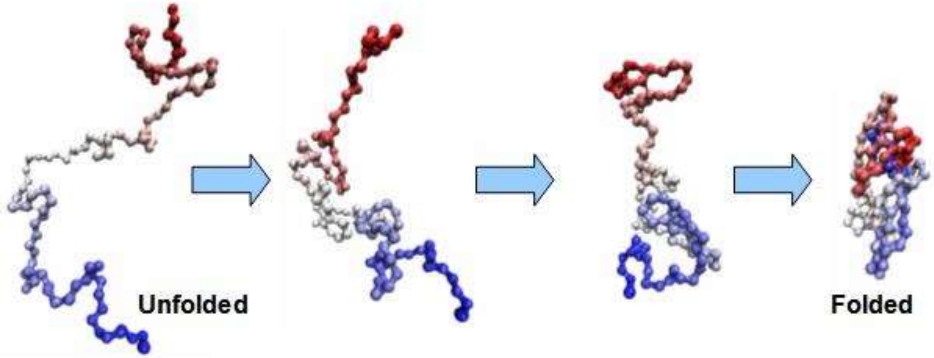
Coupled transcription and translation in bacteria

Completing and Targeting the Functional Protein

- Often translation is not sufficient to make a functional protein
- Polypeptide chains are modified after translation
  - Example: cleavage, addition of other entities (i.e. glyco-, lipo-protein) --後製加工作業!
- Completed proteins are targeted to specific sites in the cell
  - Example: membrane protein, nuclear protein

Protein Folding and Post-Translational Modifications

- During and after synthesis, a polypeptide chain spontaneously coils and folds into its three-dimensional shape



- Proteins may also require post-translational modifications before doing their job
- Some polypeptides are activated by enzymes that cleave them
- Other polypeptides come together to form the subunits of a protein

Targeting Polypeptides to Specific Locations

- Two populations of ribosomes are evident in cells: free ribsomes (in the cytosol) and bound ribosomes (attached to the ER)
    - Free ribosomes mostly synthesize proteins that function in the cytosol
    - Bound ribosomes make proteins of the endomembrane system and proteins that are secreted from the cell
- Ribosomes are identical and can switch from free to bound

Where have all the proteins gone?

- Polypeptide synthesis always begins in the cytosol
- Synthesis finishes in the cytosol *unless* the polypeptide signals the ribosome to attach to the ER
- Polypeptides destined for the ER or for secretion are marked by a **signal peptide**

蛋白質的引路人 **Signal-recognition particle (**SRP)

- A **signal-recognition particle (SRP)** binds to the signal peptide
- The SRP brings the signal peptide and its ribosome to the ER

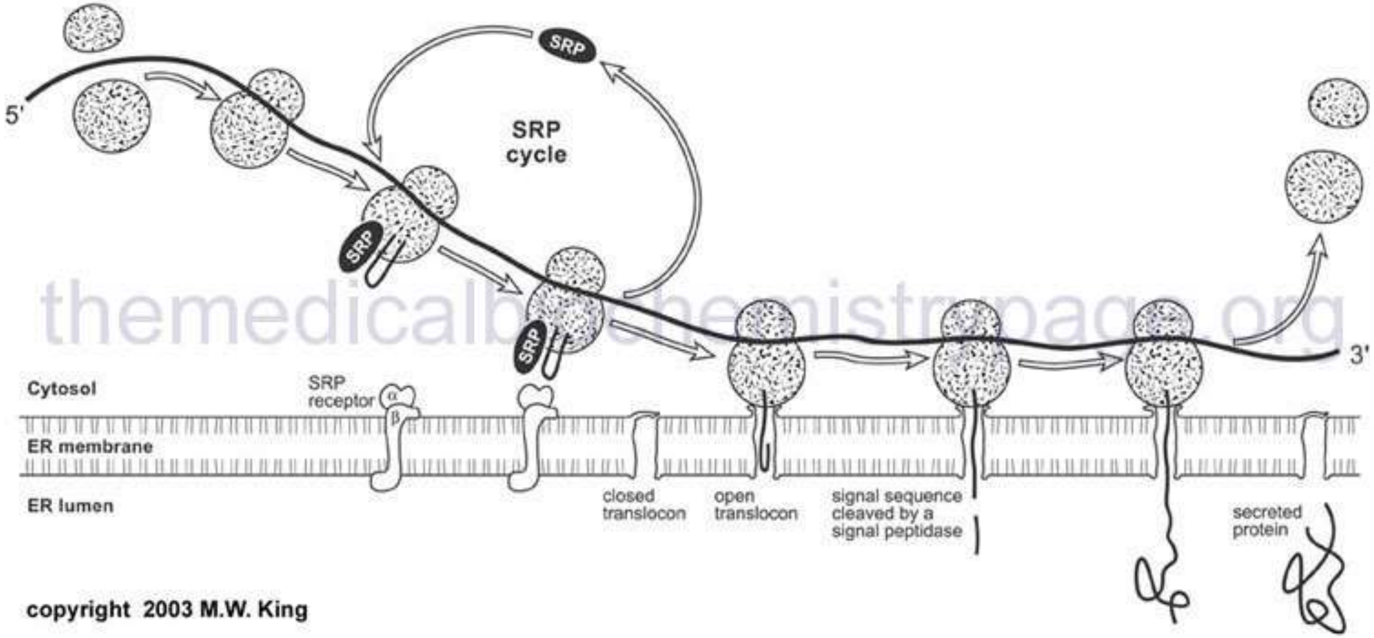


Figure 17.21

① Polypeptide synthesis begins.

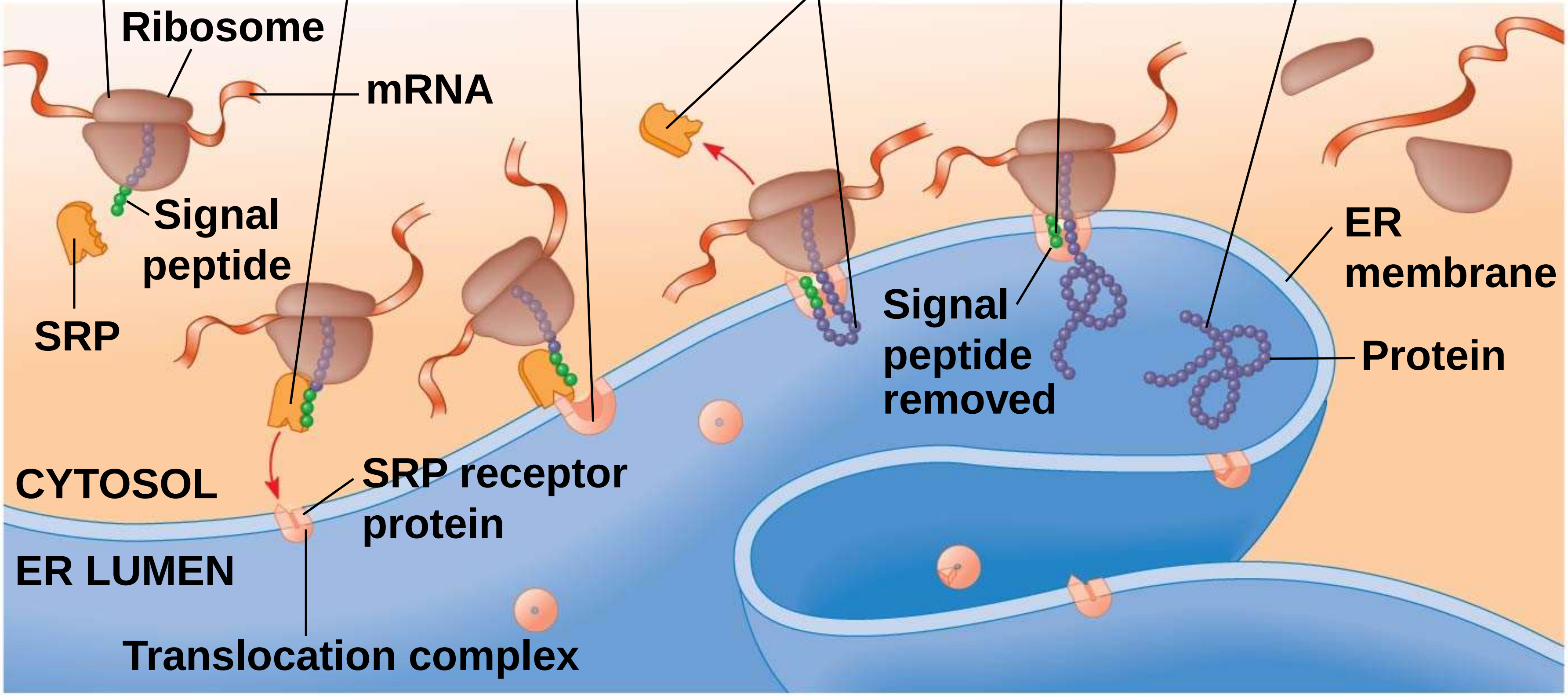
② SRP binds to signal peptide.

③ SRP binds to receptor protein.

④ SRP detaches and polypeptide synthesis resumes.

⑤ Signal-cleaving enzyme cuts off signal peptide.

⑥ Completed polypeptide folds into final conformation.



Concept 17.5: Point mutations can affect protein structure and function

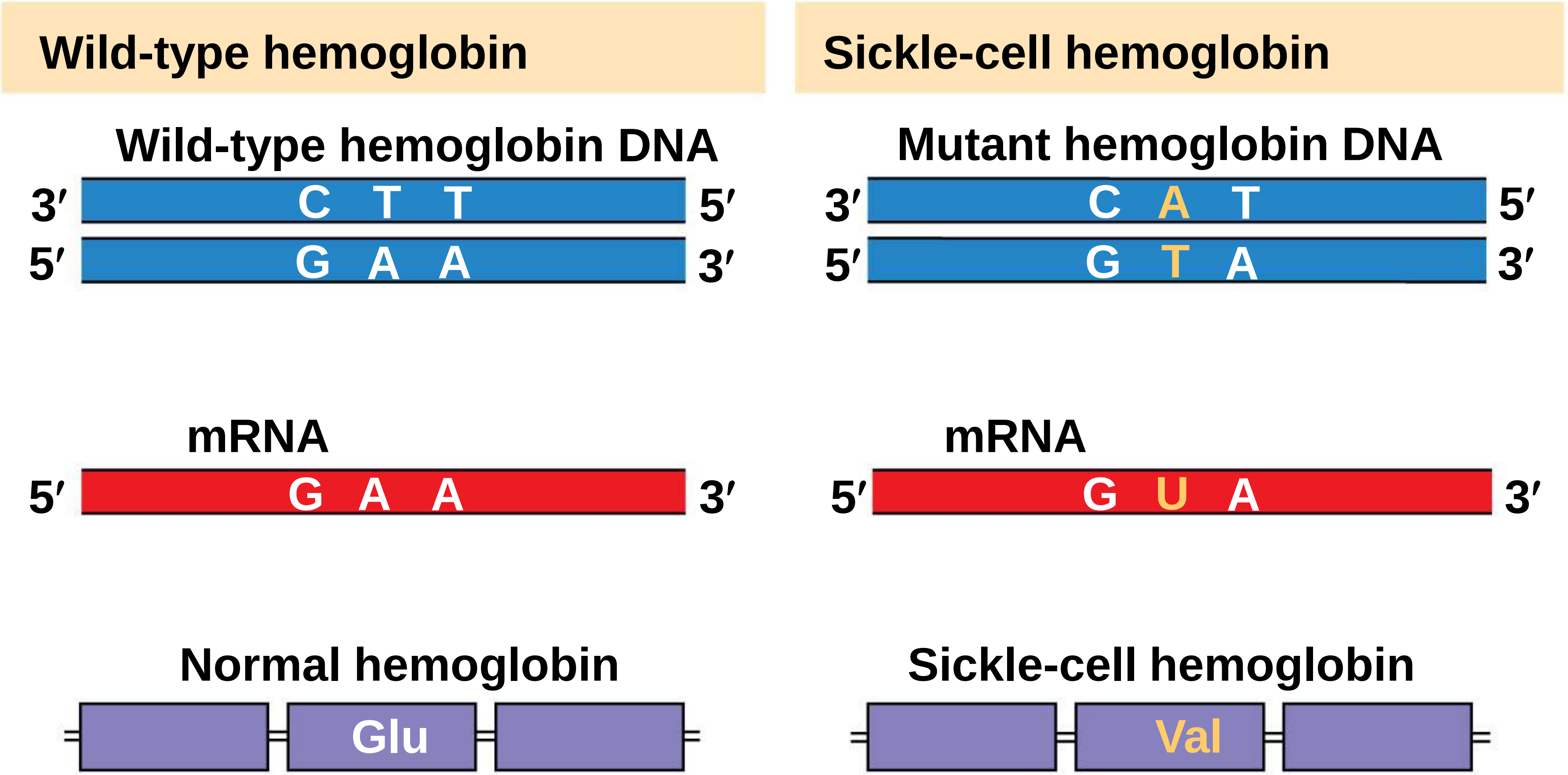
- **Mutations** are changes in the genetic material of a cell or virus

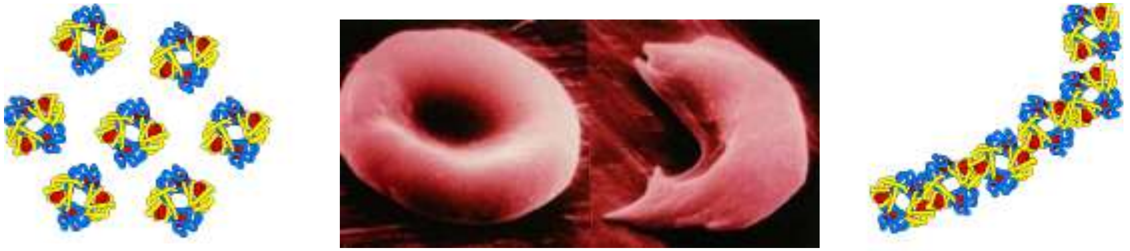
- **Point mutations** are chemical changes in just one base pair of a gene

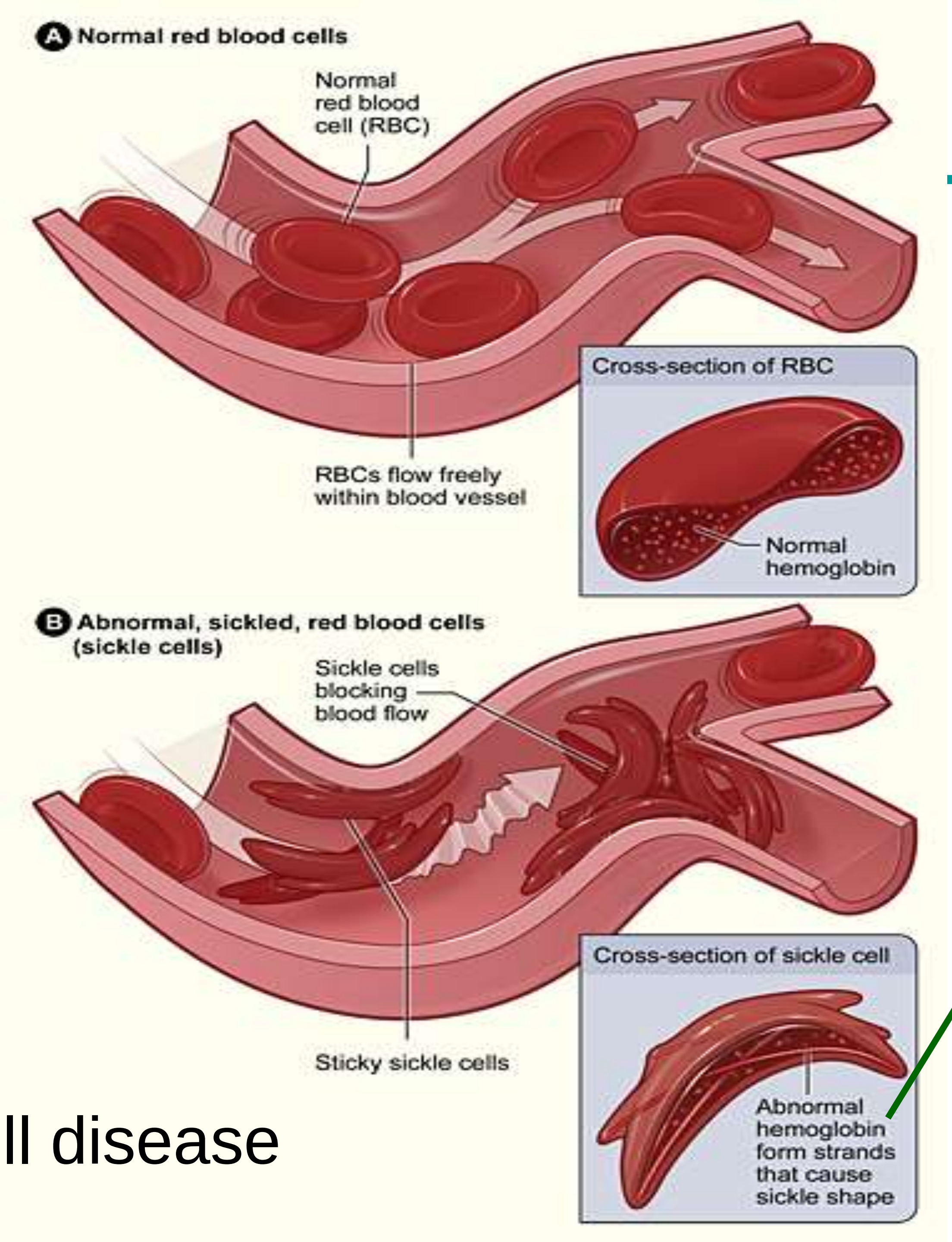
- The change of a single nucleotide in a DNA template strand can lead to the production of an abnormal protein

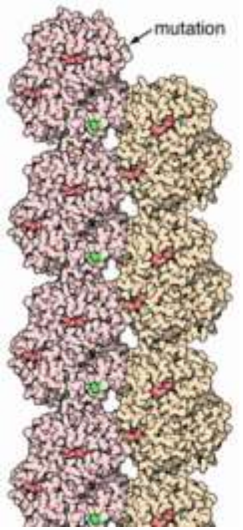
Figure 17.23

The molecular basis of sickle-cell disease: a point mutation







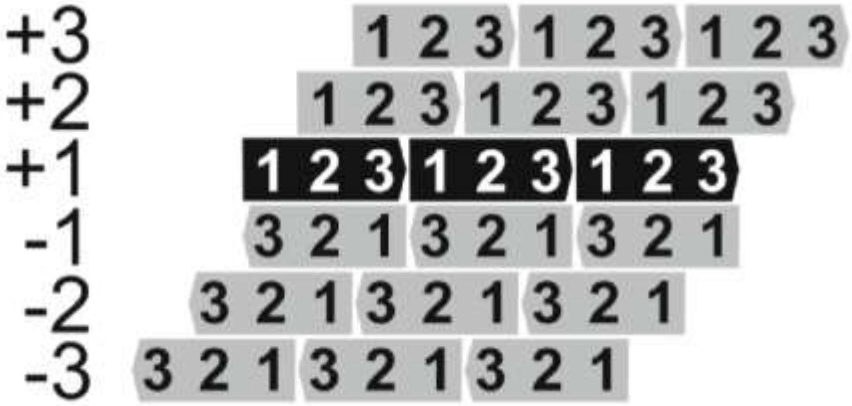


Sickle-cell disease

Types of Point Mutations

- Point mutations within a gene can be divided into two general categories
  - Base-pair substitutions
  - Base-pair insertions or deletions (indel)

Reading Frames



Mutation

- A **nucleotide-pair substitution** replaces one nucleotide and its partner with another pair of nucleotides
- **Silent mutations** have no effect on the amino acid produced by a codon because of redundancy in the genetic code
- **Missense mutations** still code for an amino acid, but not the correct amino acid
- **Nonsense mutations** change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein

Figure 17.24a

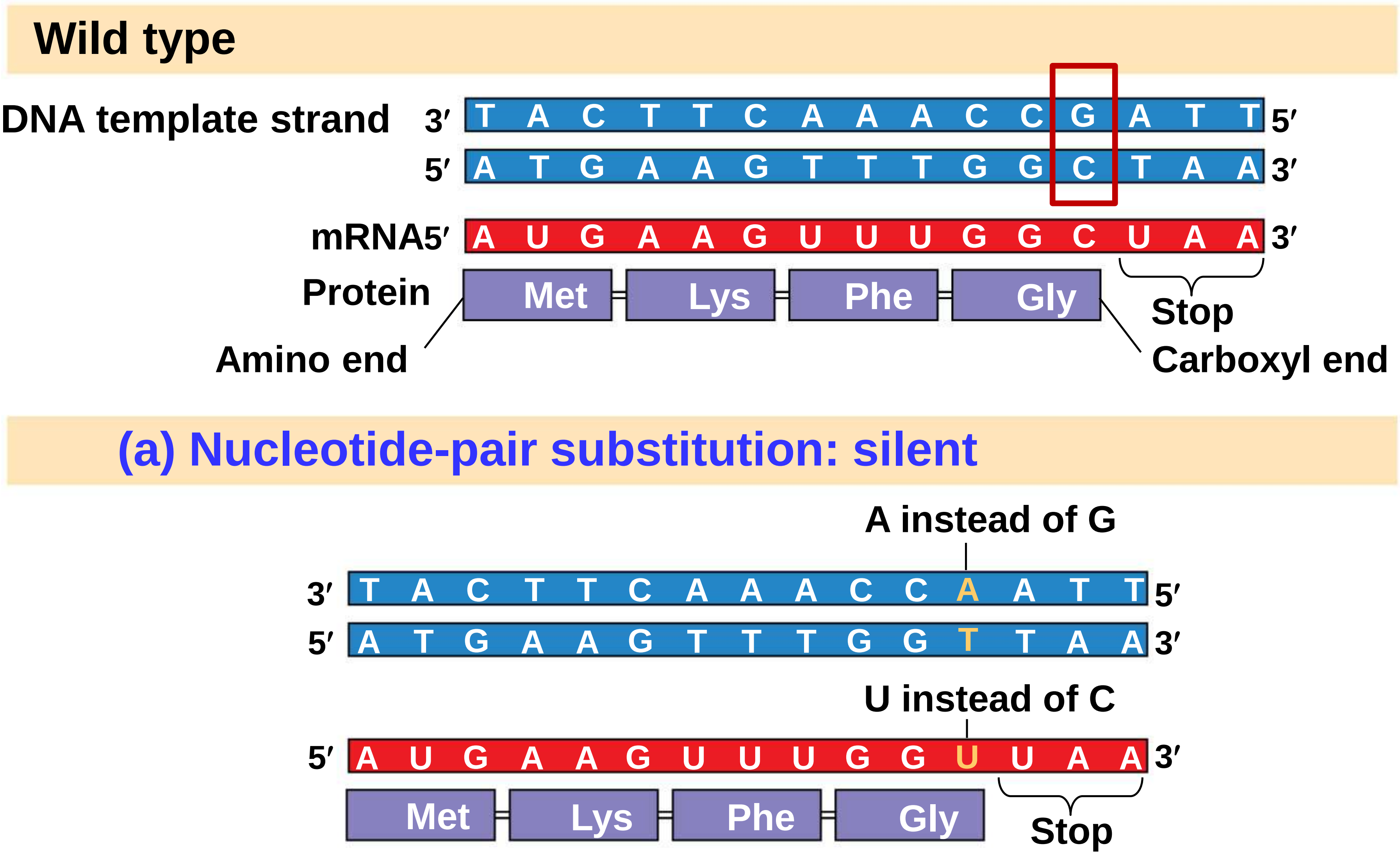
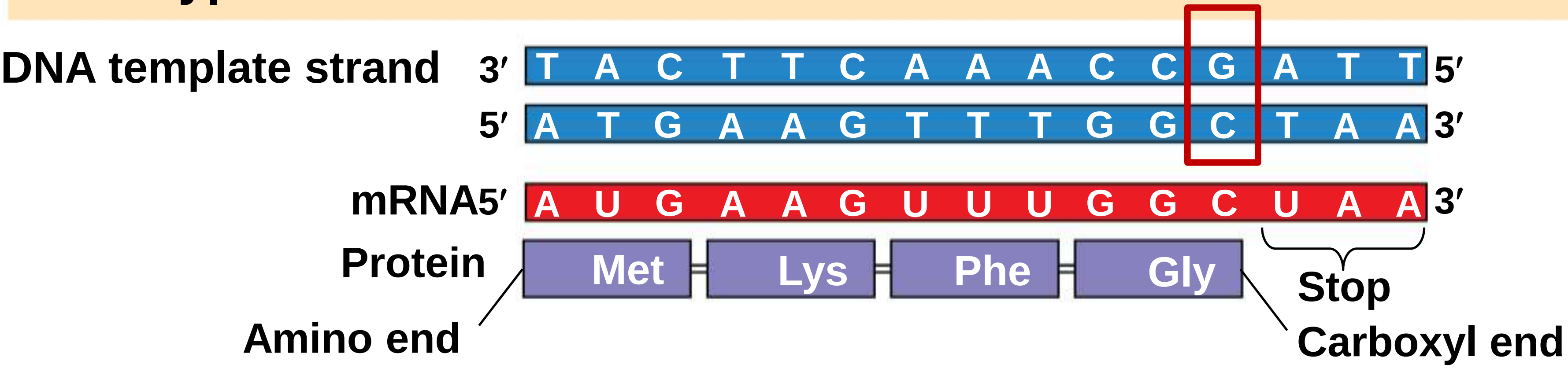


Figure 17.24b

Wild type



DNA template strand 3′ T A C T T C A A A C C G A T T 5′

5′ A T G A A G T T T G G C T A A 3′

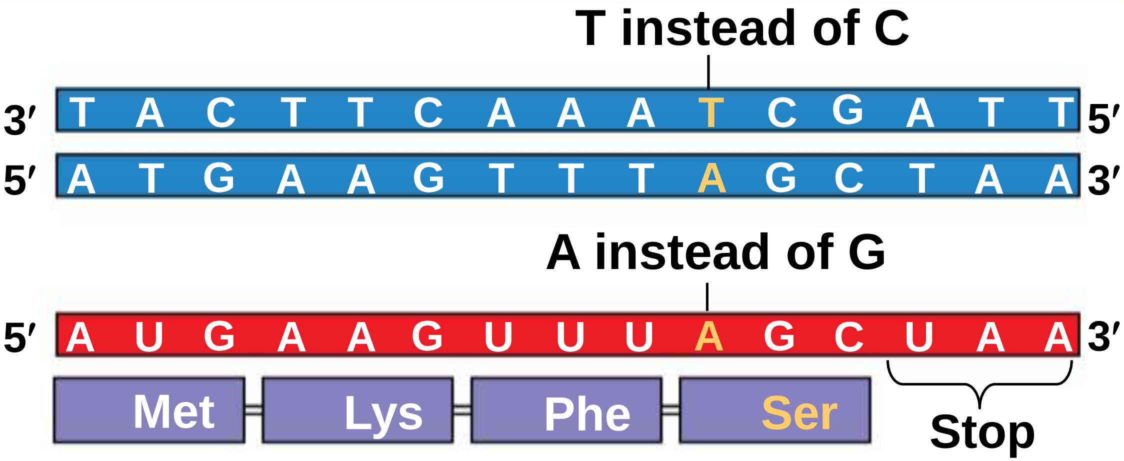
mRNA 5′ A U G A A G U U U G G C U A A 3′

Protein Met Lys Phe Gly Stop

Amino end

Carboxyl end

(a) Nucleotide-pair substitution: missense



T instead of C

3′ T A C T T C A A A T C G A T T 5′

5′ A T G A A G T T T A G C T A A 3′

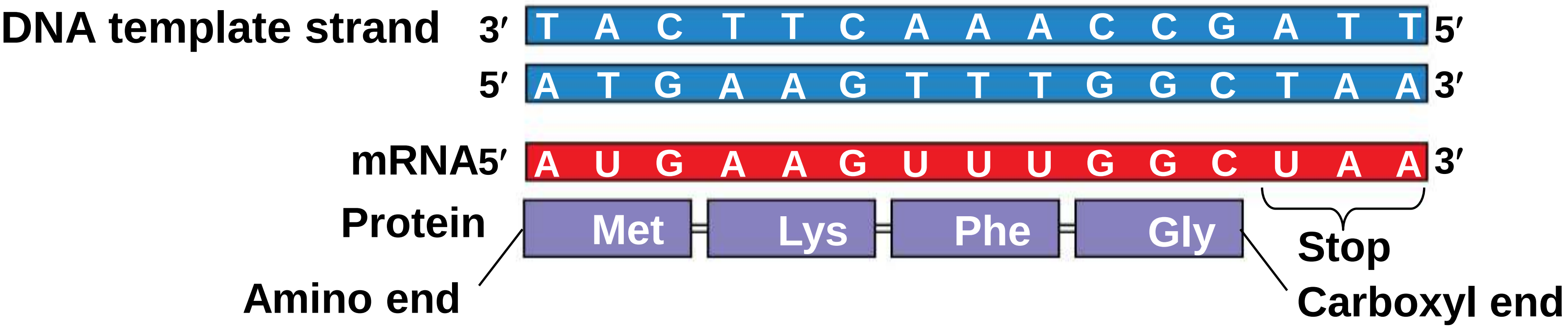
A instead of G

5′ A U G A A G U U U A G C U A A 3′

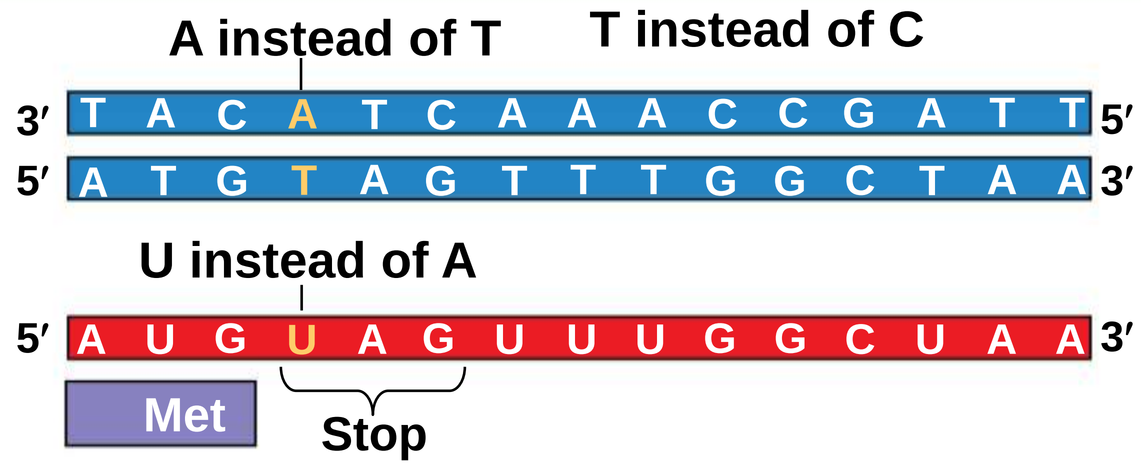
Met Lys Phe Ser Stop

Figure 17.24c

Wild type



(a) Nucleotide-pair substitution: nonsense

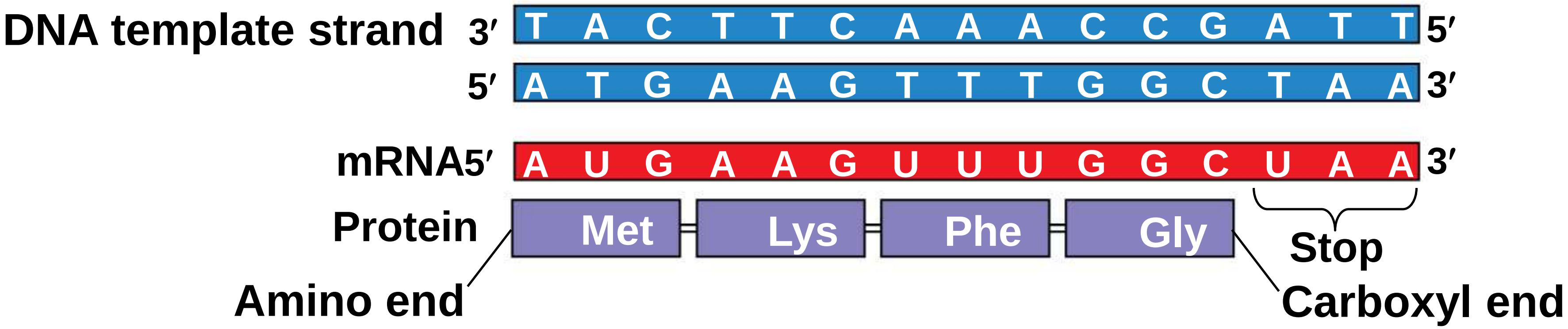


Insertions and Deletions

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene
- These mutations have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame, producing a **frame-shift mutation**

Figure 17.24d

Wild type



DNA template strand 3′ T A C T T C A A A C C G A T T 5′

5′ A T G A A G T T T G G C T A A 3′

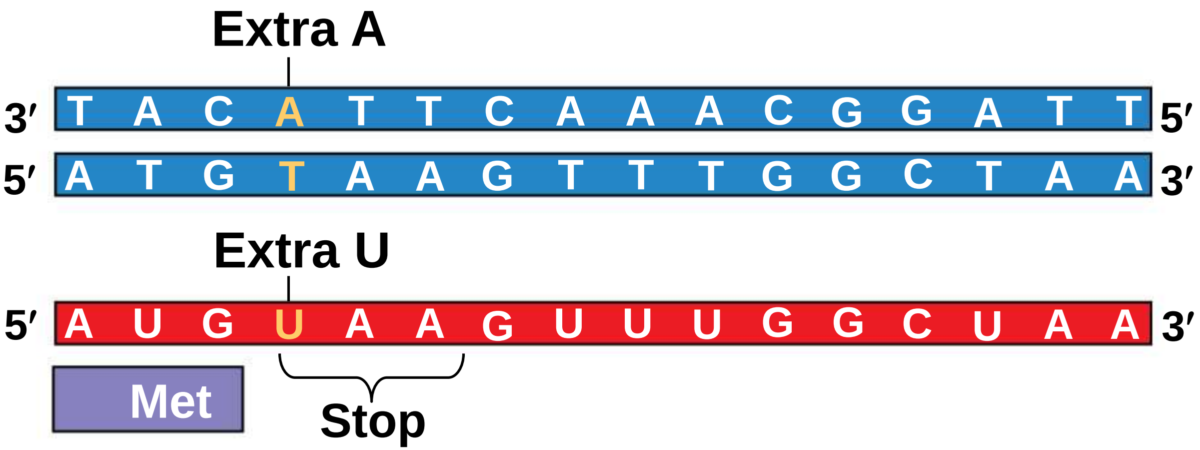
mRNA 5′ A U G A A G U U U G G C U A A 3′

Protein Met Lys Phe Gly Stop

Amino end

Carboxyl end

Nucleotide-pair insertion or deletion: frameshift causing immediate nonsense



Extra A

3′ T A C A T T C A A A C G G A T T 5′

5′ A T G T A A G T T T G G C T A A 3′

Extra U

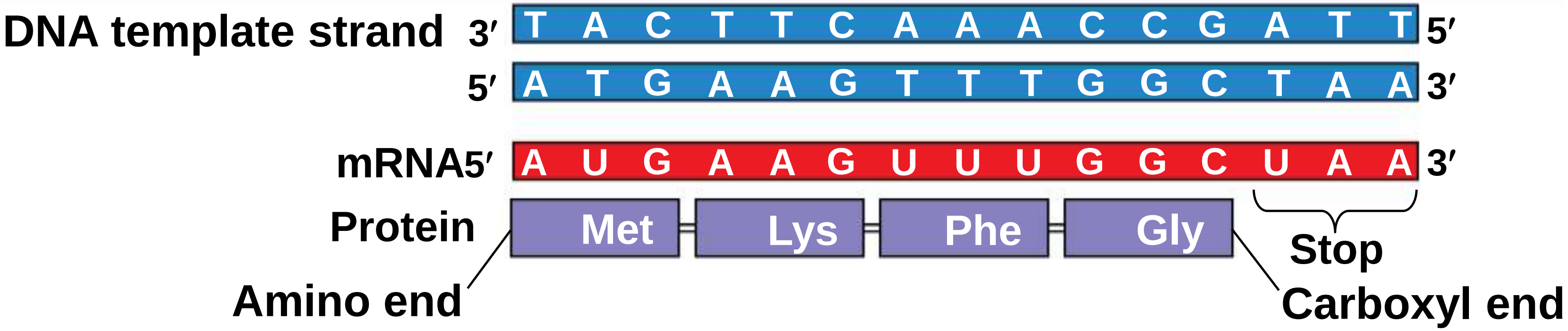
5′ A U G U A A G U U U G G C U A A 3′

Met Stop

1 nucleotide-pair insertion

Figure 17.24e

Wild type



DNA template strand 3′ T A C T T C A A A C C G A T T 5′
5′ A T G A A G T T T G G C T A A 3′

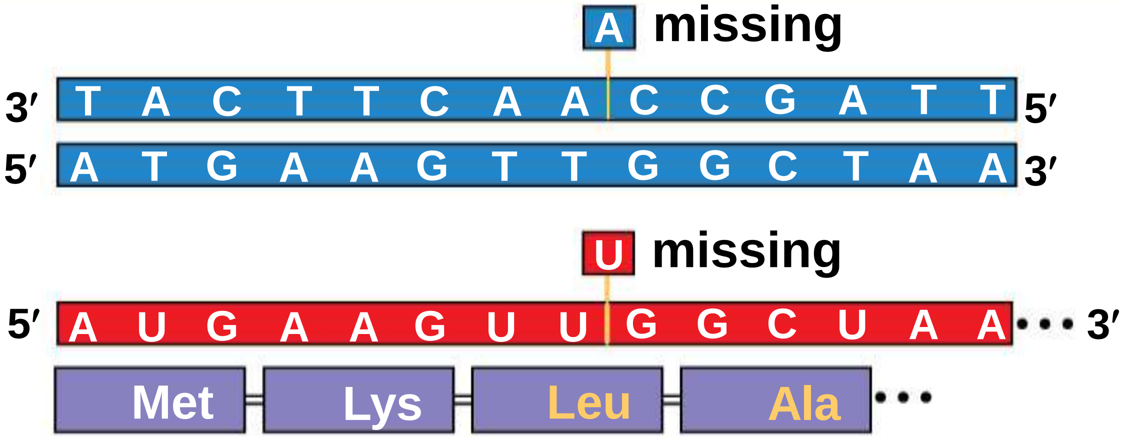
mRNA 5′ A U G A A G U U U G G C U A A 3′

Protein Met Lys Phe Gly Stop

Amino end

Carboxyl end

Nucleotide-pair insertion or deletion: frameshift causing extensive missense



A missing

3′ T A C T T C A A C C G A T T 5′
5′ A T G A A G T T G G C T A A 3′

U missing

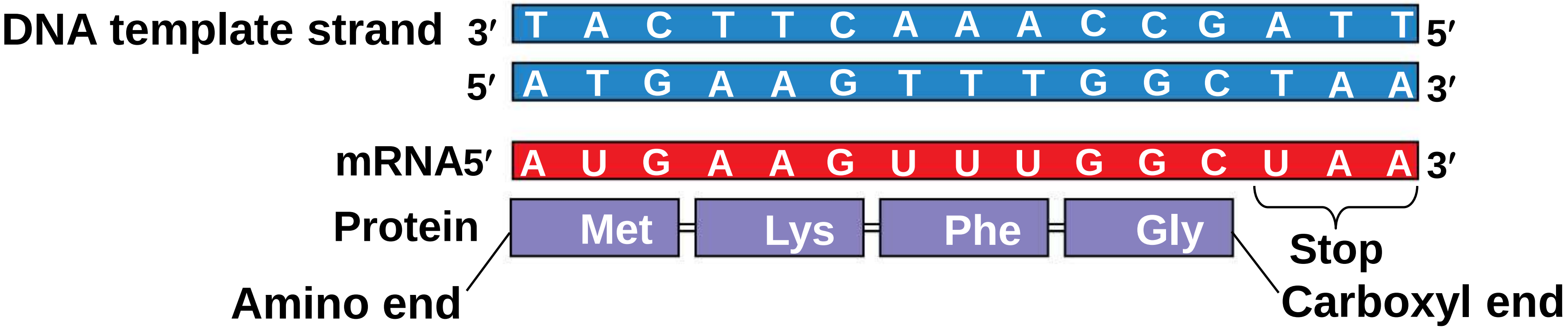
5′ A U G A A G U U G G C U A A • • • 3′

Met Lys Leu Ala • • •

1 nucleotide-pair deletion

Figure 17.24f

Wild type



DNA template strand 3′ T A C T T C A A A C C G A T T 5′

5′ A T G A A G T T T G G C T A A 3′

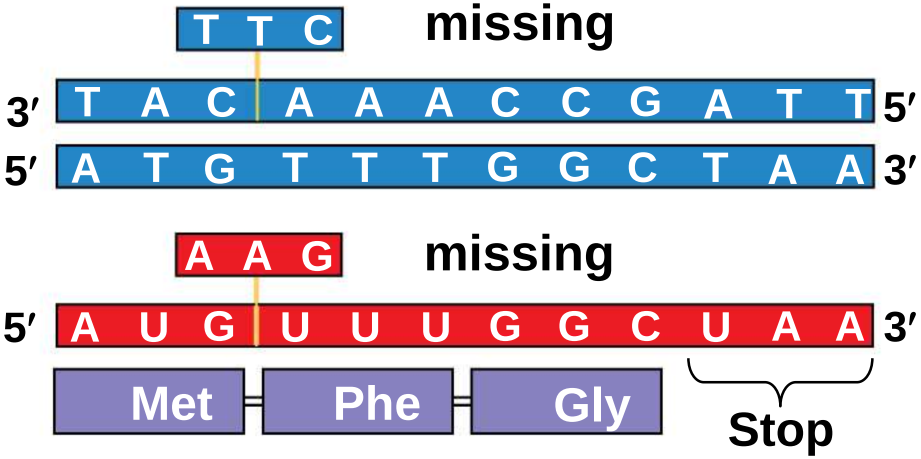
mRNA 5′ A U G A A G U U U G G C U A A 3′

Protein Met Lys Phe Gly Stop

Amino end

Carboxyl end

Nucleotide-pair insertion or deletion: no frameshift, but one amino acid missing



T T C missing

3′ T A C A A A C C G A T T 5′

5′ A T G T T T G G C T A A 3′

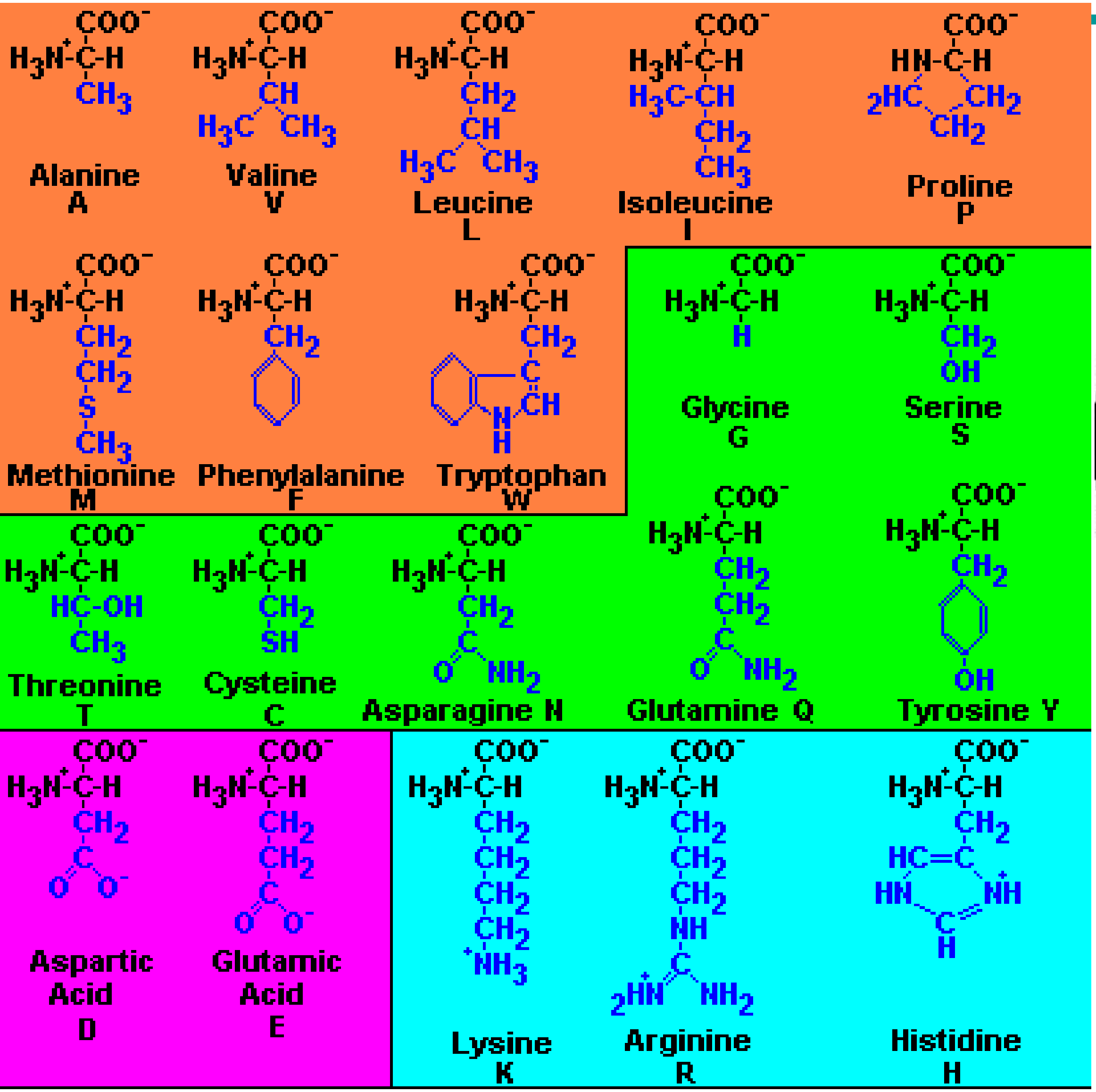
A A G missing

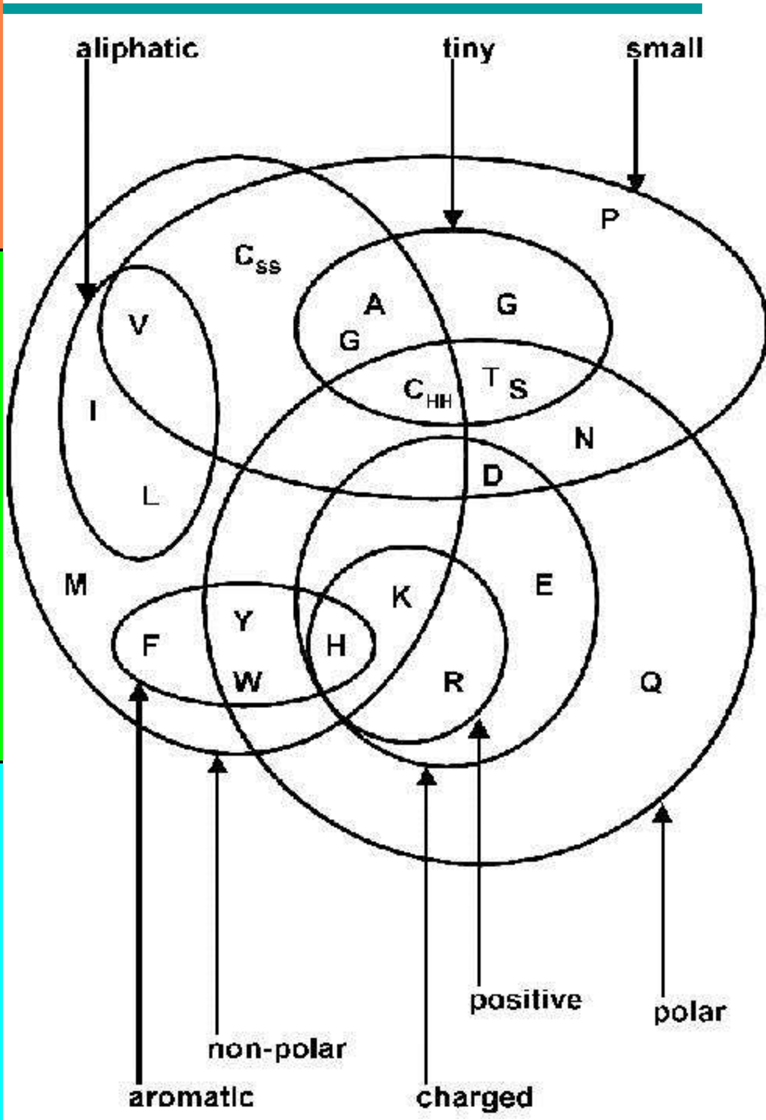
5′ A U G U U U G G C U A A 3′

Met Phe Gly Stop

3 nucleotide-pair deletion

20 Essential Amino Acids

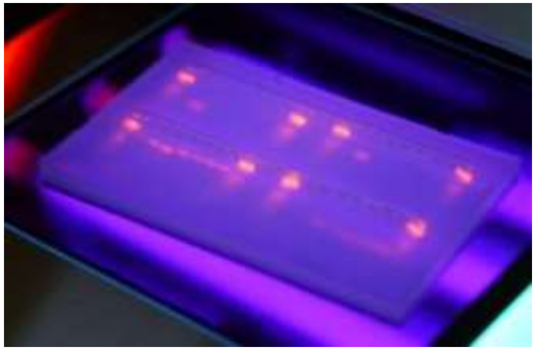




Mutagens

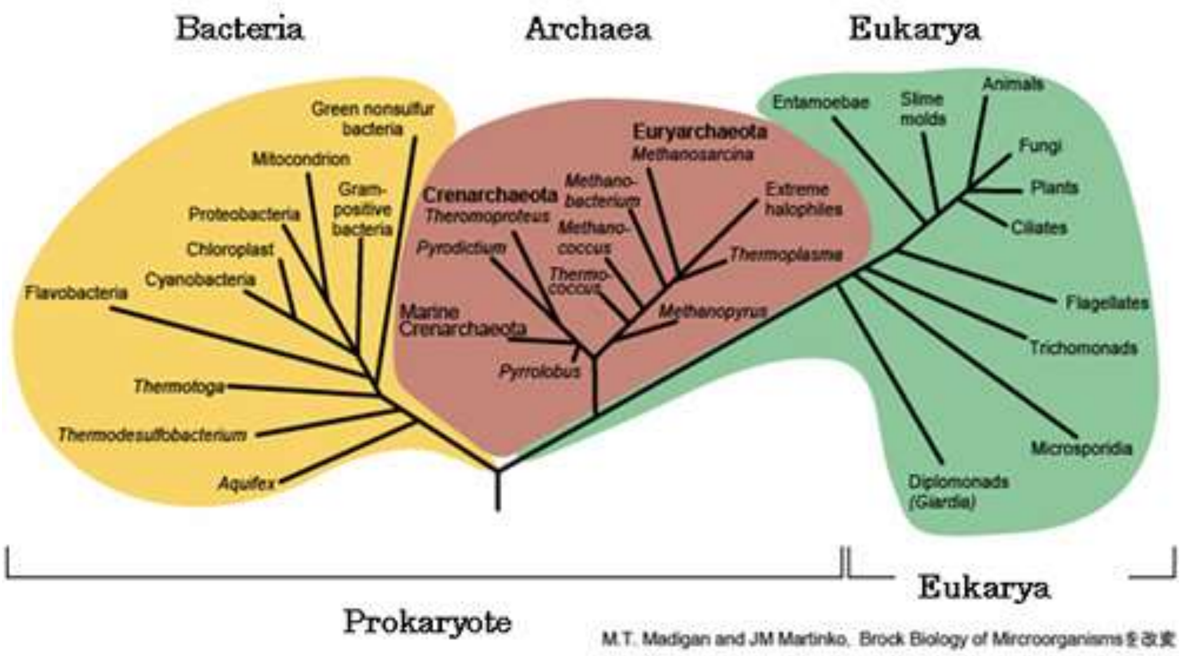
- Spontaneous mutations can occur during DNA replication, recombination, or repair

- **Mutagens** are physical or chemical agents that can cause mutations
  - **EtBr (Ethidium bromide)** 是法定致癌物，它會嵌入 核酸的氮基之中，無論是DNA or RNA
  - Ethidium bromide 會堆疊在 DNA nucleotides 之間， 形成 DNA/EtBr complex ，吸收 300 nm 的紫外線, 然後放出螢光 590 nm. 這就是用 EtBr 染色的原理.



Concept 17.6: While gene expression differs among the domains of life, the concept of a gene is universal

- Archaea **[ahr**-kee-on**]** are prokaryotes, but share many features of gene expression with eukaryotes



M.T. Madigan and JM Martinko, Brock Biology of Mircroorganisms を改変

http://www.jamstec.go.jp/e/about/press_release/20080722/

Comparing Gene Expression in Bacteria, Archaea, and Eukarya

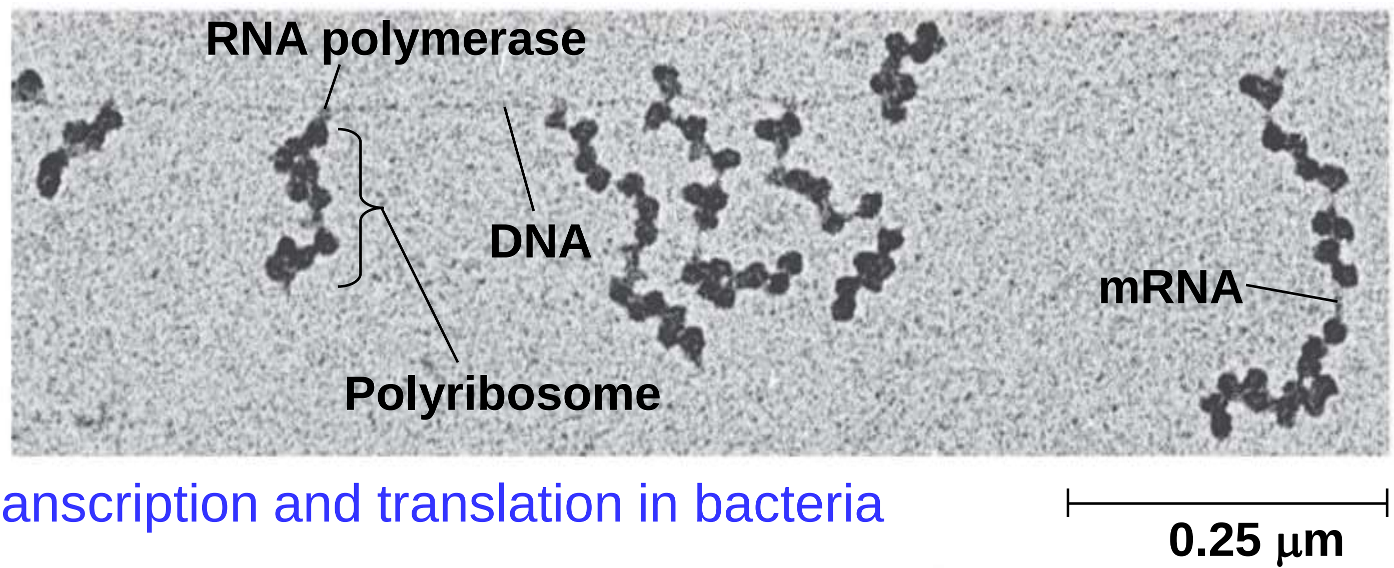
- **Bacteria** and **eukarya** differ in their RNA polymerases, termination of transcription and ribosomes; **archaea** tend to resemble **eukarya** in these respects

- **Bacteria** can simultaneously transcribe and translate the same gene

- In **eukarya**, transcription and translation are separated by the nuclear envelope

- In **archaea**, transcription and translation are likely coupled

Figure 17.25



Coupled transcription and translation in bacteria

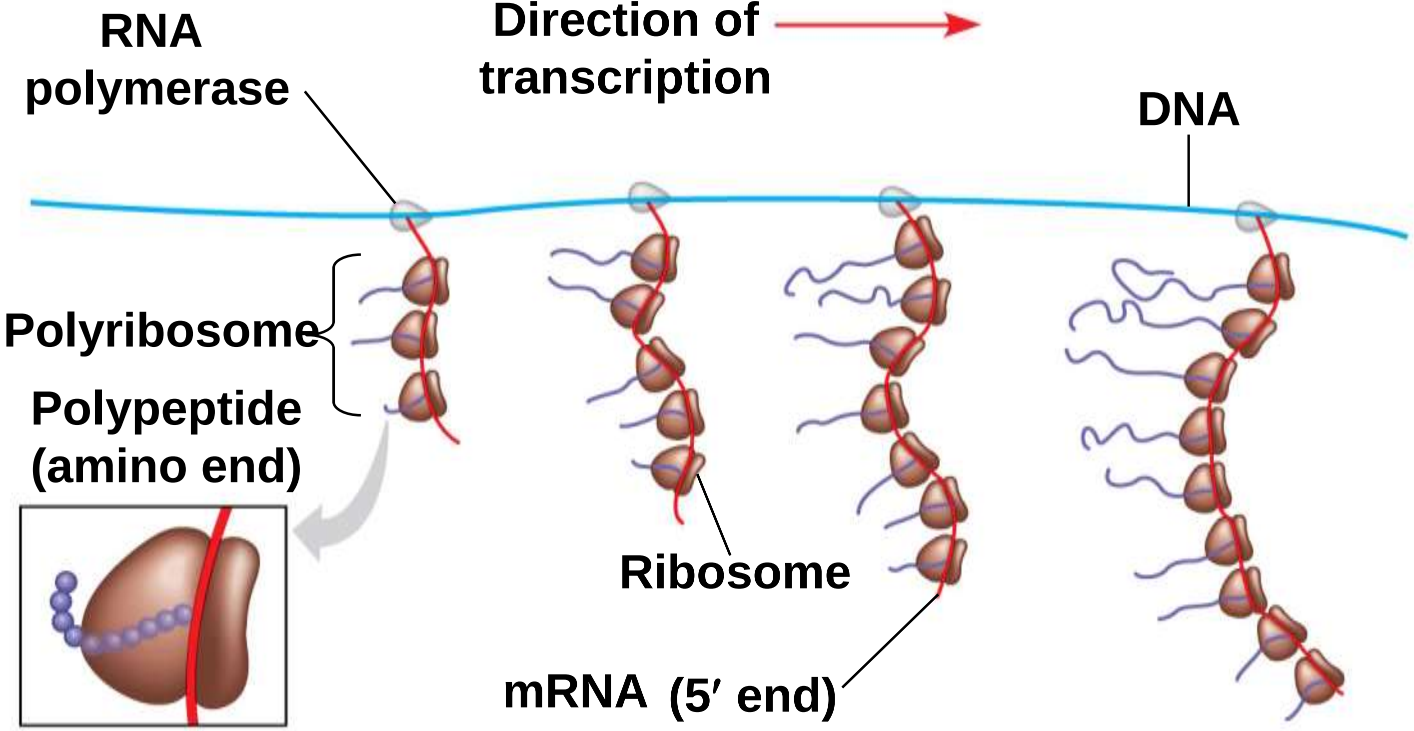
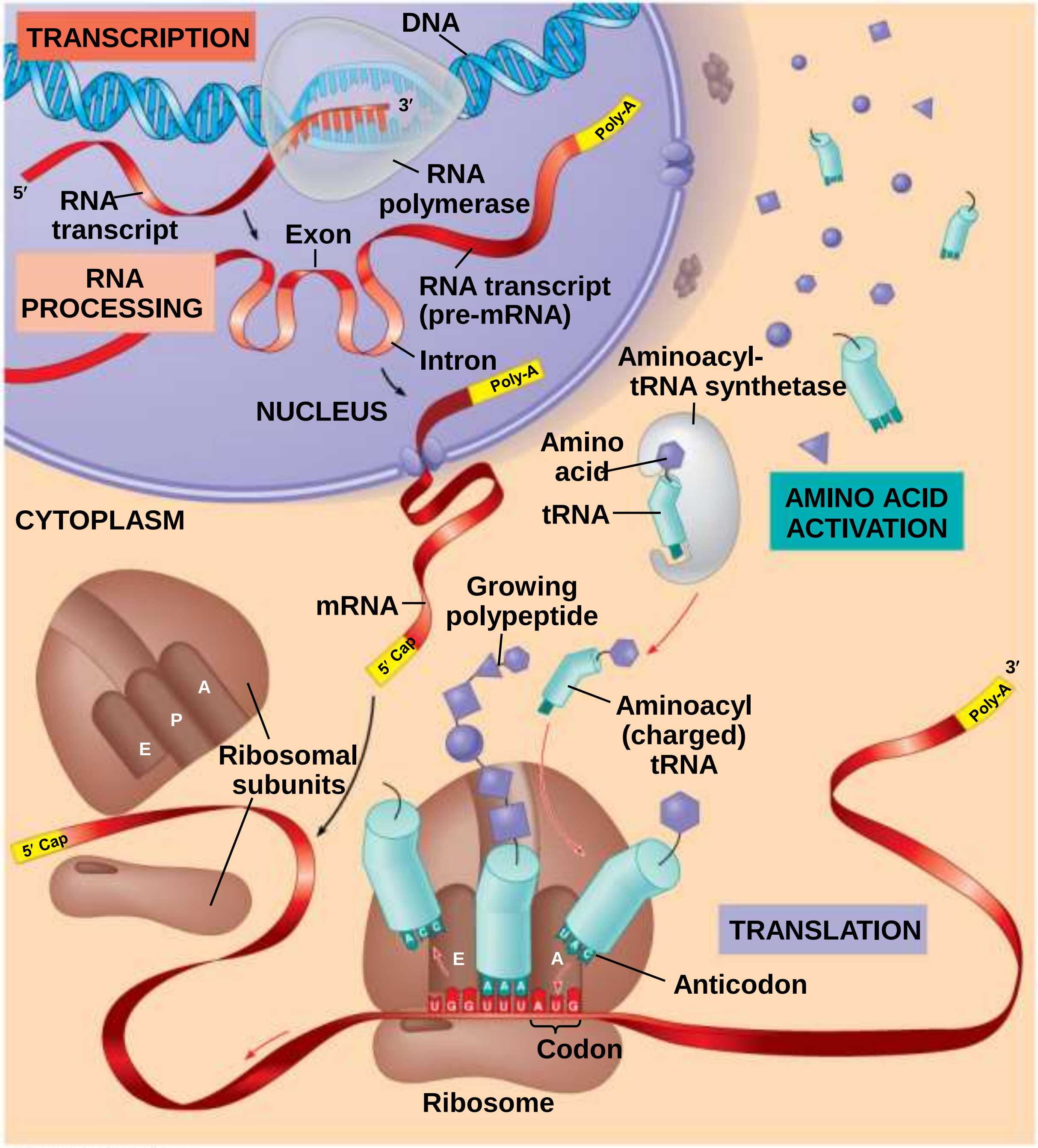


Figure 17.26

Summary



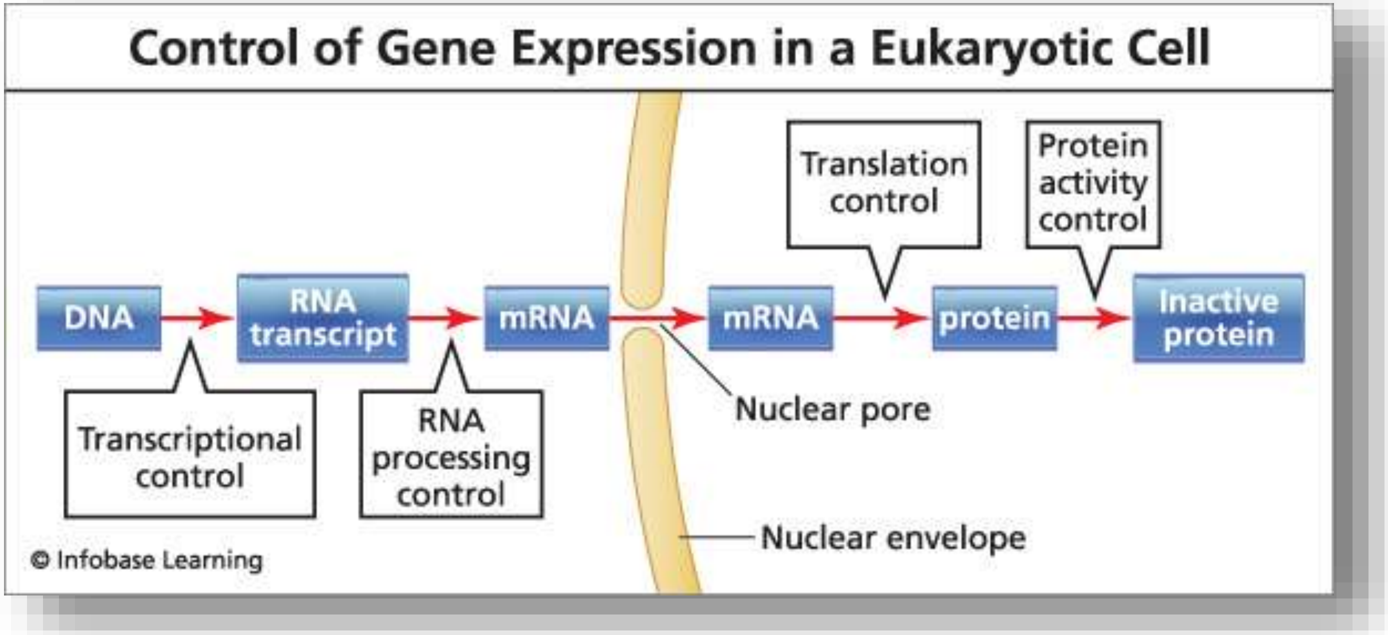
What Is a Gene? *Revisiting the Question*

- The idea of the gene has evolved through the history of genetics
- We have considered a gene as
  - A discrete unit of inheritance
  - A region of specific nucleotide sequence in a chromosome
  - A DNA sequence that codes for a specific polypeptide chain

Gene as a Region of DNA Sequence

The next big question is:

How is gene expression regulated?

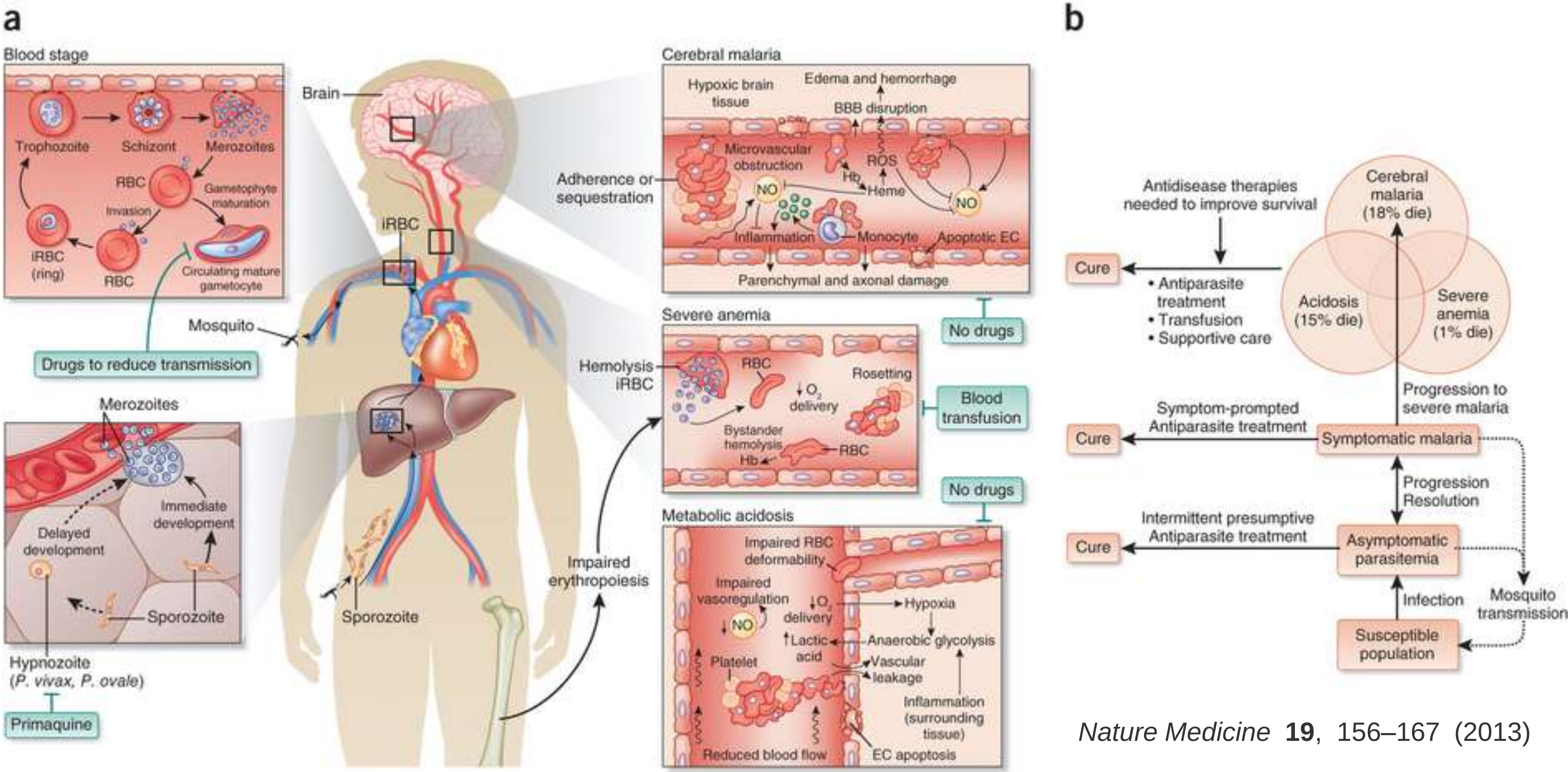


You should now be able to:

1. Describe the contributions made by Garrod, Beadle, and Tatum to our understanding of the relationship between genes and enzymes
2. Briefly explain how information flows from gene to protein
3. Compare transcription and translation in bacteria and eukaryotes
4. Explain what it means to say that the genetic code is redundant and unambiguous

5. Include the following terms in a description of transcription: mRNA, RNA polymerase, the promoter, the terminator, the transcription unit, initiation, elongation, termination, and introns
6. Include the following terms in a description of translation: tRNA, wobble, ribosomes, initiation, elongation, and termination

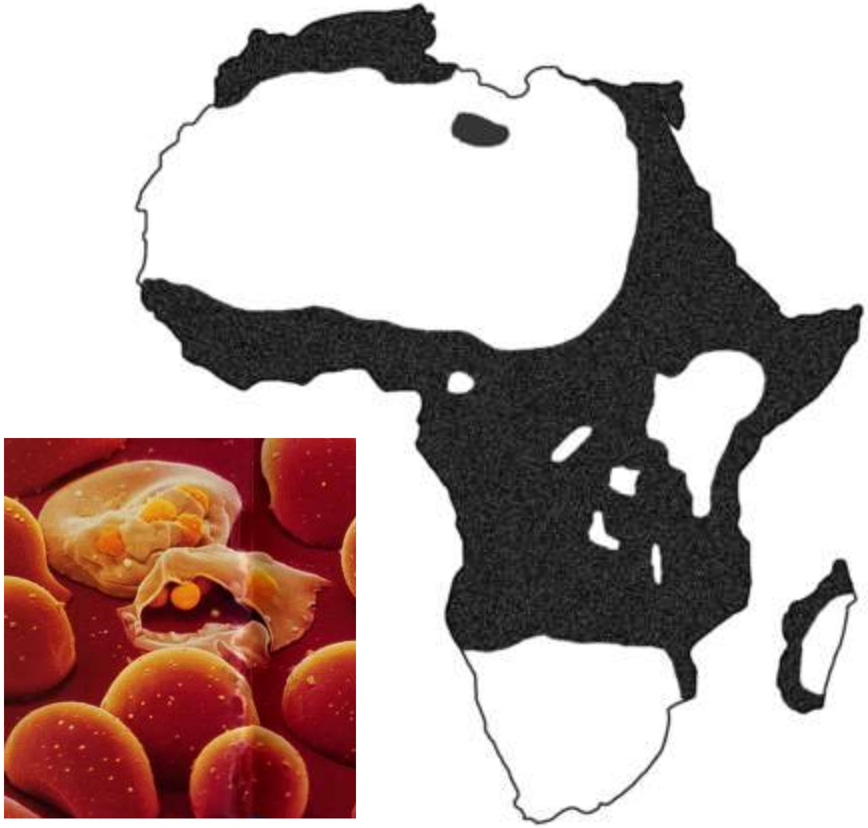
Malaria biology and disease pathogenesis: insights for new treatments



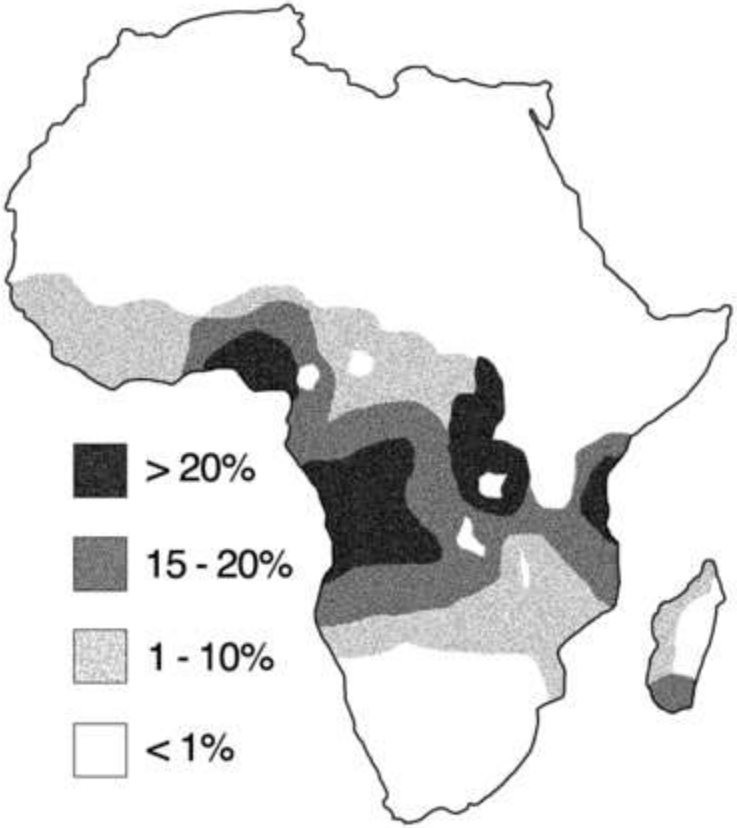
Nature Medicine **19**, 156–167 (2013)

Thinking Question

Does sickle cell trait has any evolutionary advantage?



Frequency of
malaria in population



Frequency of
sickle cell trait in population