Molecular and Cellular Biology (MCB)
BB 101

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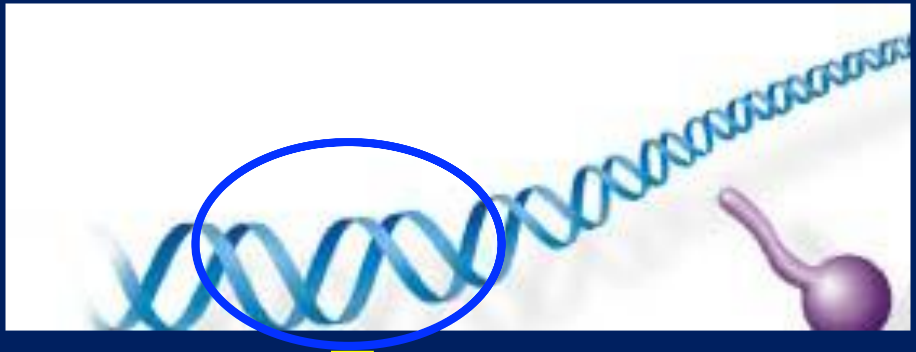
Class 6: learning objectives

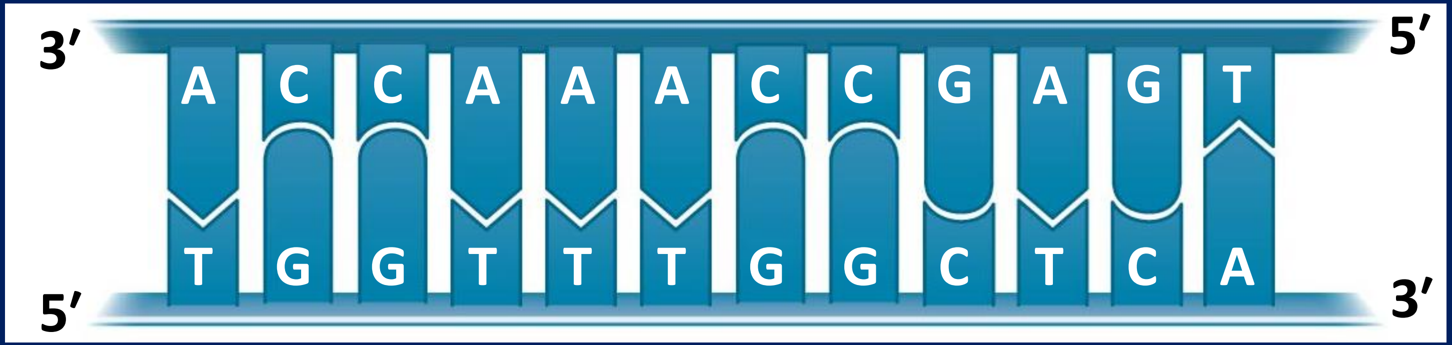
- Inherited DNA leads to specific traits
  - Coat color, white eyes, phenylketonuria
- DNA: packaging, accessing the stored message
  - Book shelves, storage compactors, chromosomes
- DNA to protein – basic principles
  - Transcription and translation
- The genetic code and mutations

How is information stored in DNA?

Figure 16.22



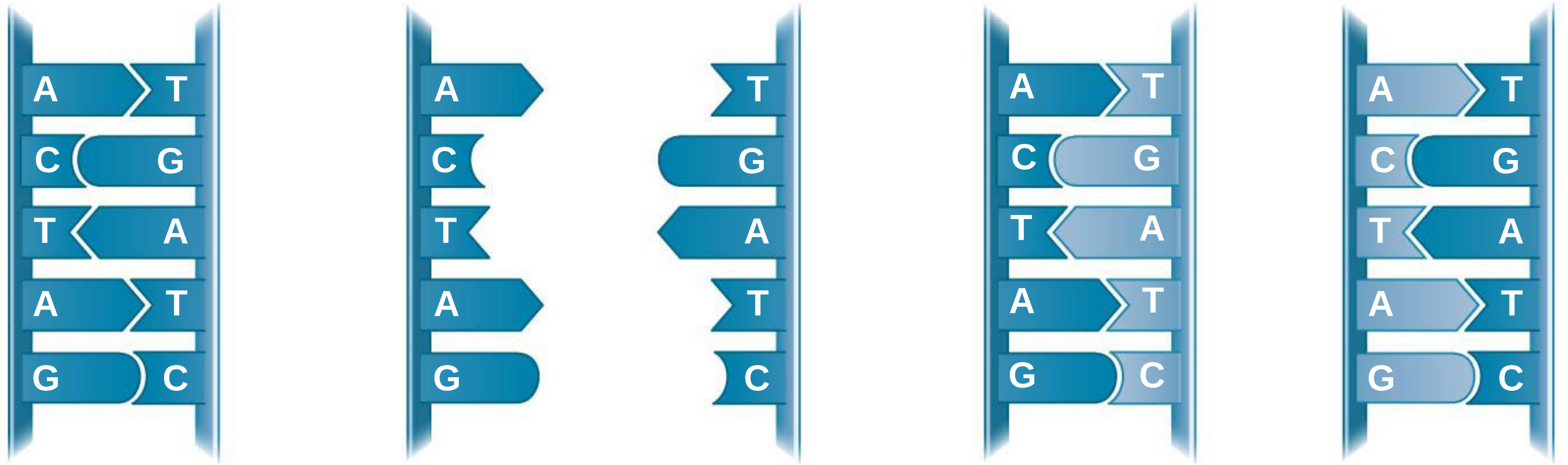
DNA (chromosome)



part of Figure 17.4

Specific sequence of nucleotides along the strands of DNA

Replication of DNA - copying of information



(a) Parental molecule

(b) Separation of parental strands into templates

(c) Formation of new strands complementary to template strands

Figure 16.9

Will there be (or can there be) errors during replication?

If yes, what are the consequences?

Directional synthesis

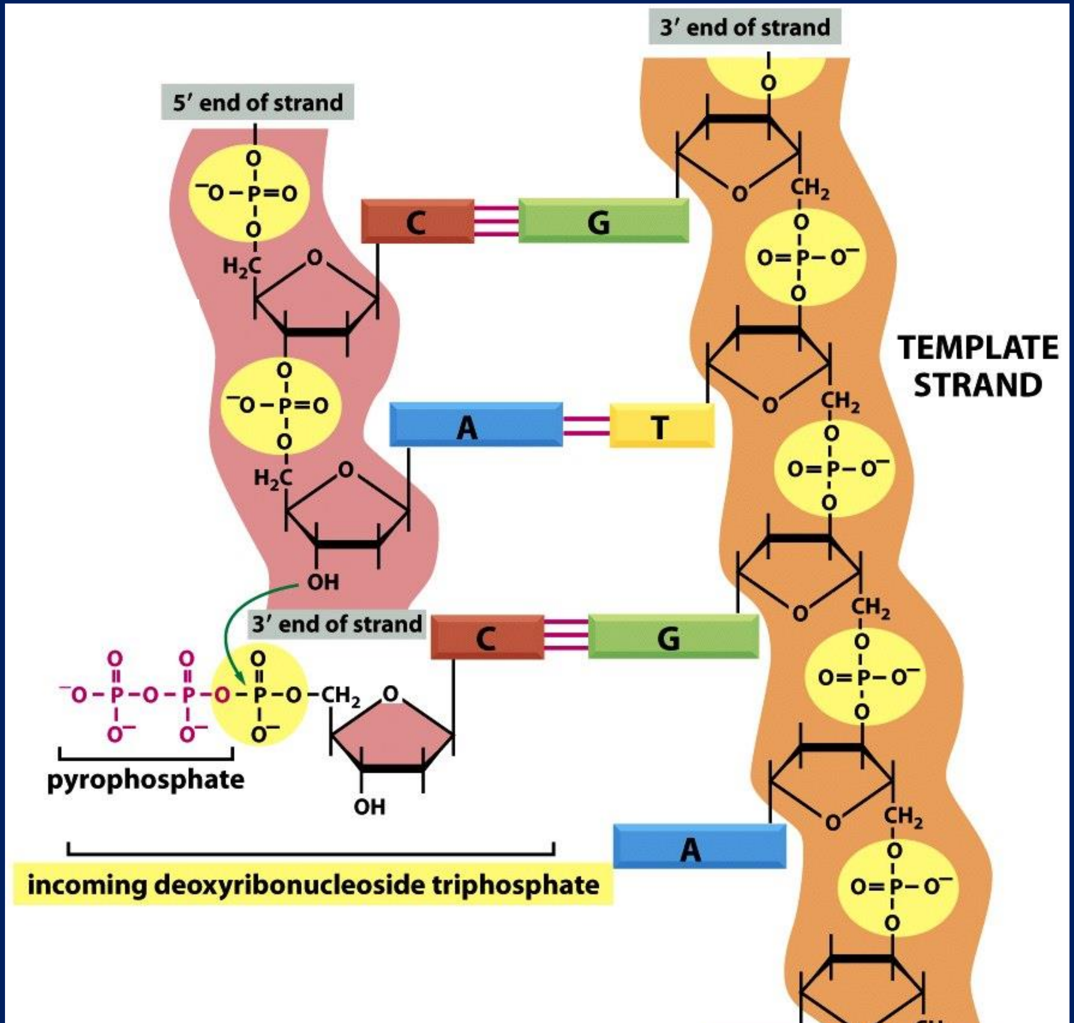


Image is from the book entitled Molecular Biology of Cell by Bruce Alberts and co-authors

DNA synthesis proceeds in 5′ to 3′ direction

DNA polymerases are enzymes which catalyze DNA synthesis

DNA polymerases have error checking and correction activity also

http://www.school-clipart.com/school_clipart_images/clip_art_illustration_of_a_short_pencil_with_an_eraser_0515-1007-2718-0950.html

Mutations

MUTATION

Change in "information"
i.e., change in DNA sequence

Can be natural or induced

Error by DNA polymerase
External causes (e.g., UV light)

MUTATION

Can be silent i.e., no APPARENT effect on the organism

Wild type and mutants

WILD TYPE
The reference (genetic) state of an organism

"trait normally found in a population"

$$\xrightarrow[\text{(genetic change)}]{\text{Mutation}}$$

MUTANT
Organism that has undergone genetic change with respect to the wild type

- Wild type and mutant are two DISTINCT organisms
- Wild type and mutant are WITH RESPECT TO A TRAIT or GENE
- A mutant can have one or more genetic changes with reference to the wild type

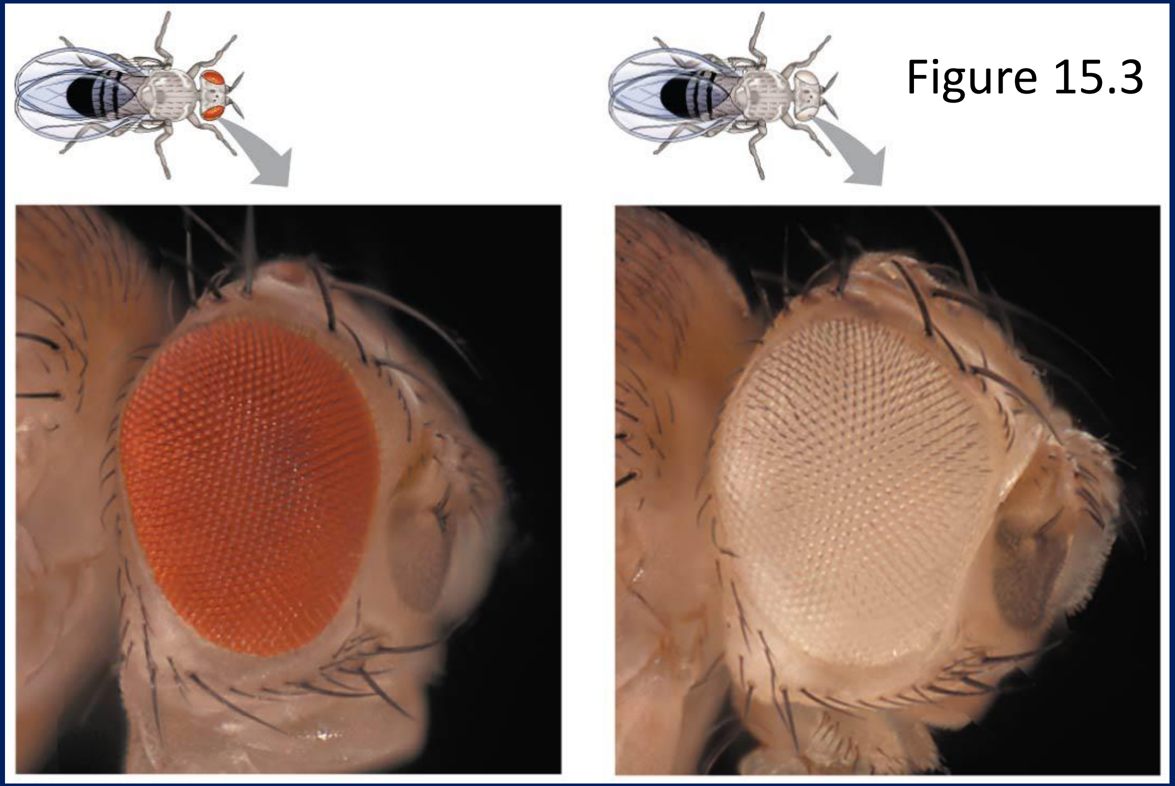
Wild type and mutants

ADP Opens Global ‘Center of Excellence’ in Bucharest - New Jersey Business Magazine (njbmagazine.com)

Who Are The New Mutants In X-Men: Days Of Future Past? | Movies | Empire (empireonline.com)

Wild type and mutants

Figure 17.1
Biology. A global approach



Fruit fly with white eyes

Fruit fly: *Drosophila melanogaster*

Germ cells (gametes) and somatic cells

In multi-cellular organisms, egg and sperm are called germ cells (or gametes)

All other cells are called somatic cells

Only mutations in germ cells are transmitted

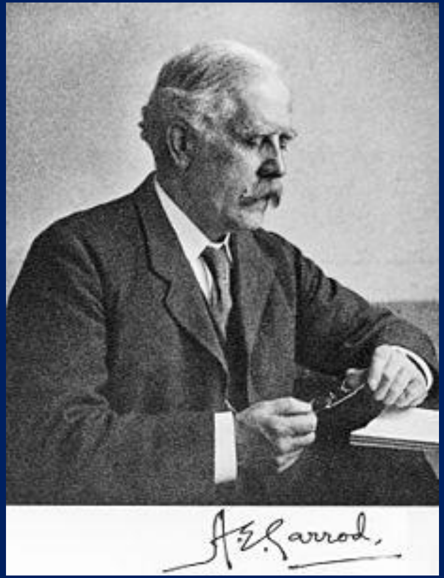
Genetic makeup and observed traits

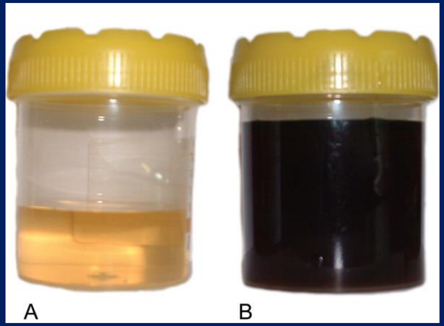
Inherited DNA leads to specific traits

GENOTYPE The genetic content of an organism	$\xrightarrow{?}$	**PHENOTYPE** Inherited traits e.g., albinism

How does the information flow?

Genes dictate phenotype through enzymes





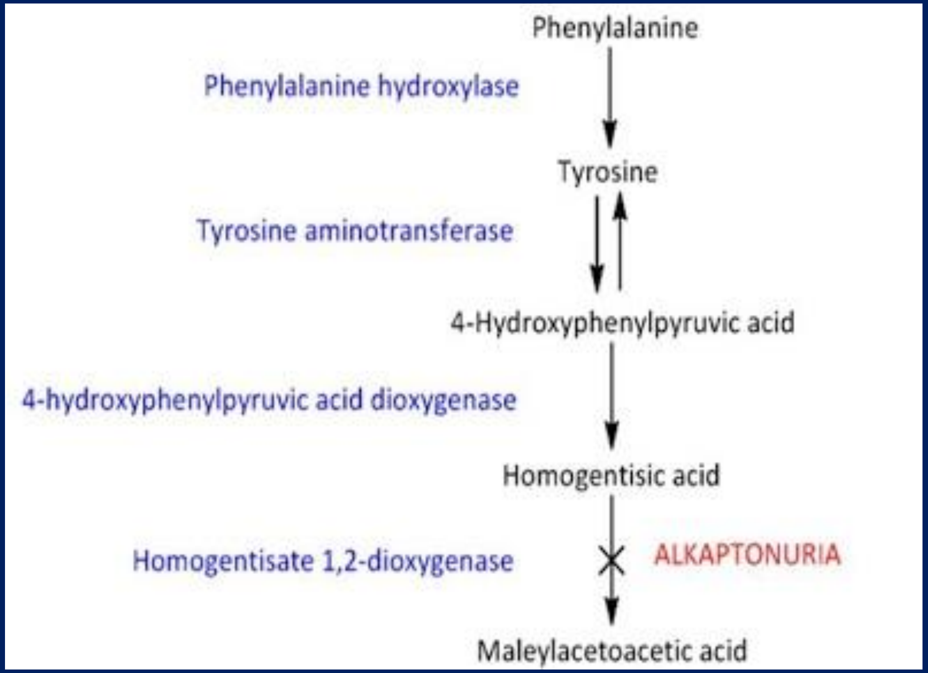
- Urine becomes black because it contains the chemical alkapton
- Most people have an enzyme that converts (i.e., metabolizes) alkapton
- People who do not inherit this enzyme suffer from the disease alkaptonuria
- Garrod named this as an “inborn error of metabolism”

He was ahead of his time!

Archibald Garrod (1857-1936) English Physician
Image of Garrod taken from Wikipedia
Images of urine and pigmented hands taken from https://lrodgers93.wordpress.com/

Metabolic pathways

Gregor Mendel's principles of heredity apply to humans as well as peas



http://www.rarediseasesindia.org/aku

Genes dictate production of specific enzymes

Cells synthesize or degrade organic molecules via metabolic pathways

Each chemical reaction in a pathway is catalyzed by a specific enzyme

Neither the chemical reactions nor the enzymes that catalyze were known

Relationship between genes and enzymes

Which genes are responsible for the

coat color in animals

white eyes of Drosophila

Black-urine in alkaptonuria

…

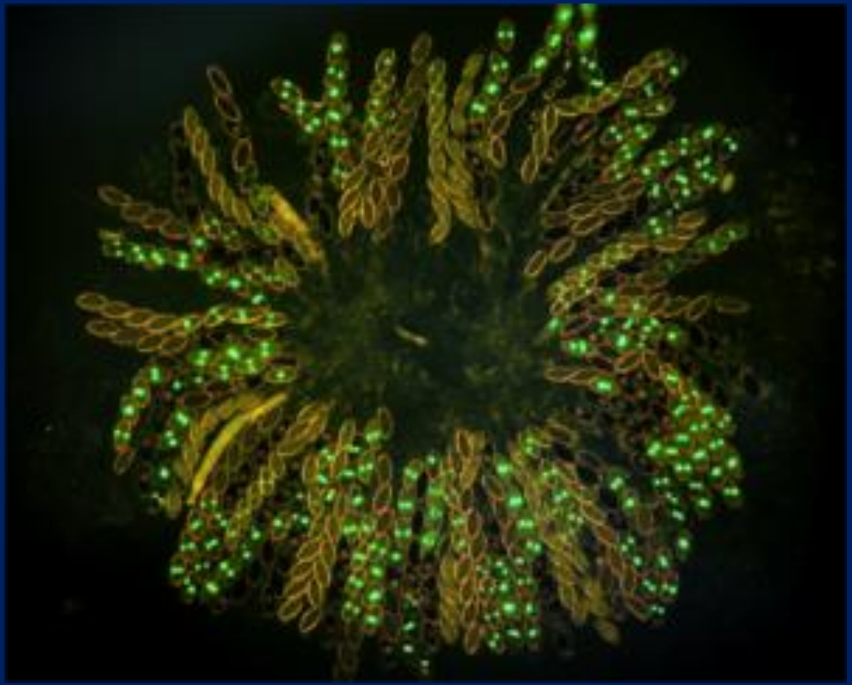
What do genes do?

Relationship between genes and enzymes

Figure 1 | **George Beadle (left) and Edward Tatum (right) receiving their Nobel Prizes.** Image courtesy of the Karolinska Institute, Stockholm, Sweden.

Taken from the article published in the journal Nature Review Genetics (2004) Vol 5, p949

Worked at the Stanford University



It is a bread mold
It is a fungus
Its scientific name is *Neurospora crassa*

Mold is a type of fungus

Relationship between genes and enzymes

Which genes are responsible for the coat color, white eyes, etc.?
What do genes do?

Beadle and Tatum’s approach:
Instead of asking, “which genes are responsible for observed phenotype”
Seek (or look for) genes that control already known reactions

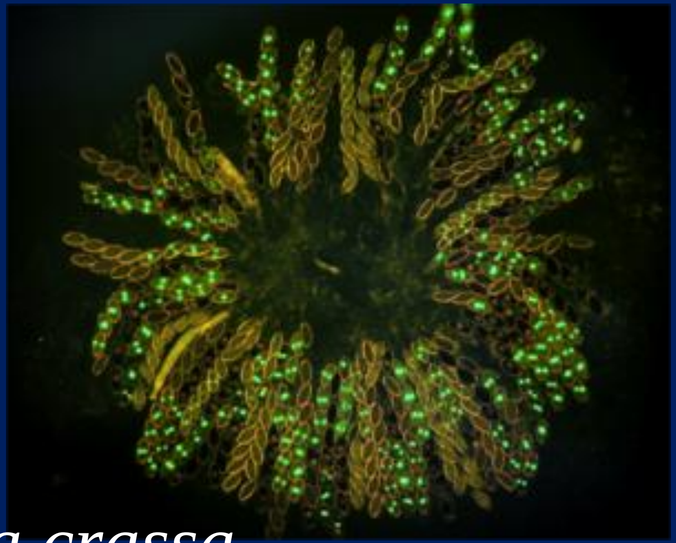
Wild type *Neurospora* can grow on minimal media

Minimal medium consists of
- inorganic salts,
- glucose and
- biotin (a vitamin)

in agar, a support medium

Complete medium consists of
- minimal media,
- all 20 amino acids and
- a few other nutrients

in agar, a support medium



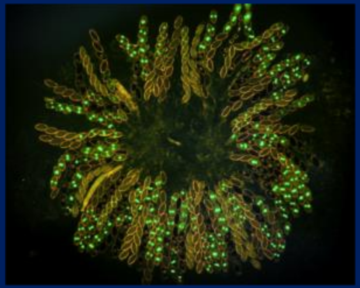
Neurospora crassa

Wild type *Neurospora* can grow even in minimal medium because it can biosynthesize all the other molecules required for growth

Finding out what genes do

- George Beadle and Edward Tatum exposed bread mold to X-rays
- X-ray were known to cause mutations (change in the genetic make up)
- By this process, they isolated mutants that were unable to survive on minimal media

Relationship between genes and enzymes



Neurospora crassa

Exposure to x-rays results in mutants which do NOT grow even in complete medium. Such mutants are discarded

Exposure to x-rays alters the genetic make up (i.e., causes mutations)

Wild type *Neurospora*

X-ray irradiation

Mutant *Neurospora*

Complete medium

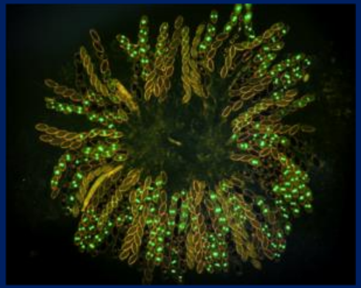
Growth

Minimal medium

No growth

Isolate ONLY such mutants which grow in complete medium but not in the minimal medium

Relationship between genes and enzymes



Neurospora crassa

Wild type *Neurospora*

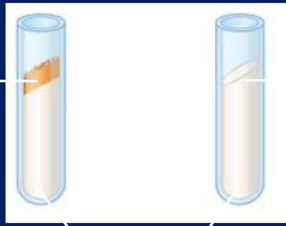
X-ray irradiation

Mutant *Neurospora*

Complete medium → Growth

Minimal medium → No growth

Figure 17.2



Growth: Wild-type cells growing and dividing

No growth: Mutant cells cannot grow and divide

Control: Minimal medium

INFERENCE

Mutant is not able to synthesize one or more essential components

Relationship between genes and enzymes

Wild type *Neurospora*

X-ray irradiation

Mutant *Neurospora*

Complete medium → Grows

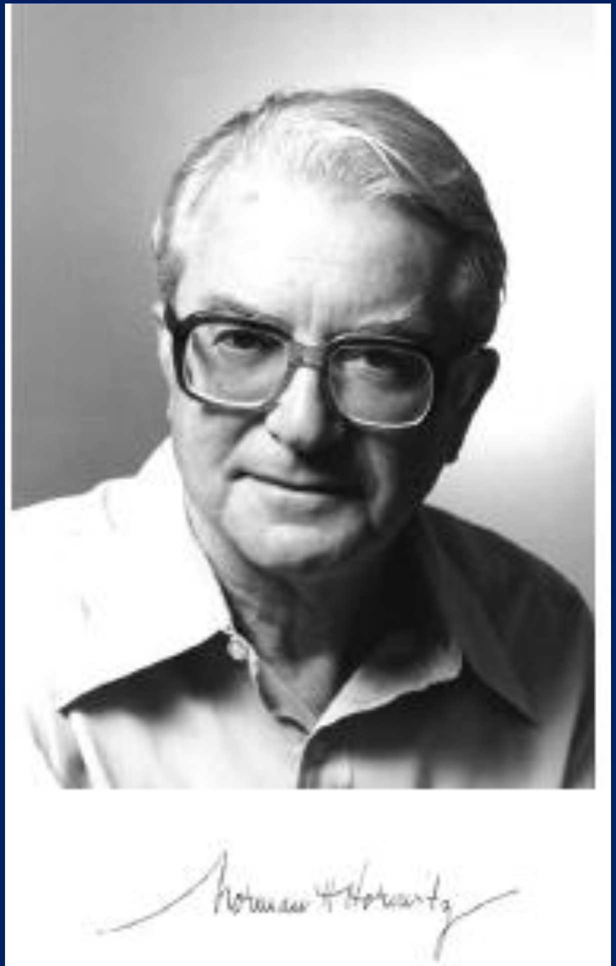
Minimal medium → No growth

	GROWTH
Minimal medium + component #1	No
Minimal medium + component #2 (let us say, arginine)	Yes
Minimal medium + component #n	No

Mutant is NOT able to biosynthesize component #2 (e.g., arginine)

Finding out what genes do

Adrian Srb



Norman Horowitz

Photographs are from the biographical memoirs available at the National Academy of Sciences (www.nasonline.org)

Used Beadle and Tatum's method to investigate the biochemical pathway for arginine biosynthesis

Arginine is one of the amino acids that is found in natural proteins

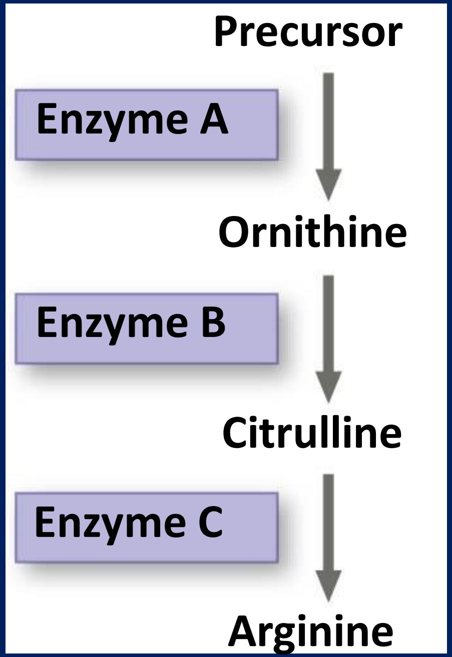
Worked at the Stanford University

A (part of a) metabolic pathway

Experiments of Adrian Srb and Norman Horowitz

Pathway for arginine biosynthesis described in the mammalian liver

Figure 17.2

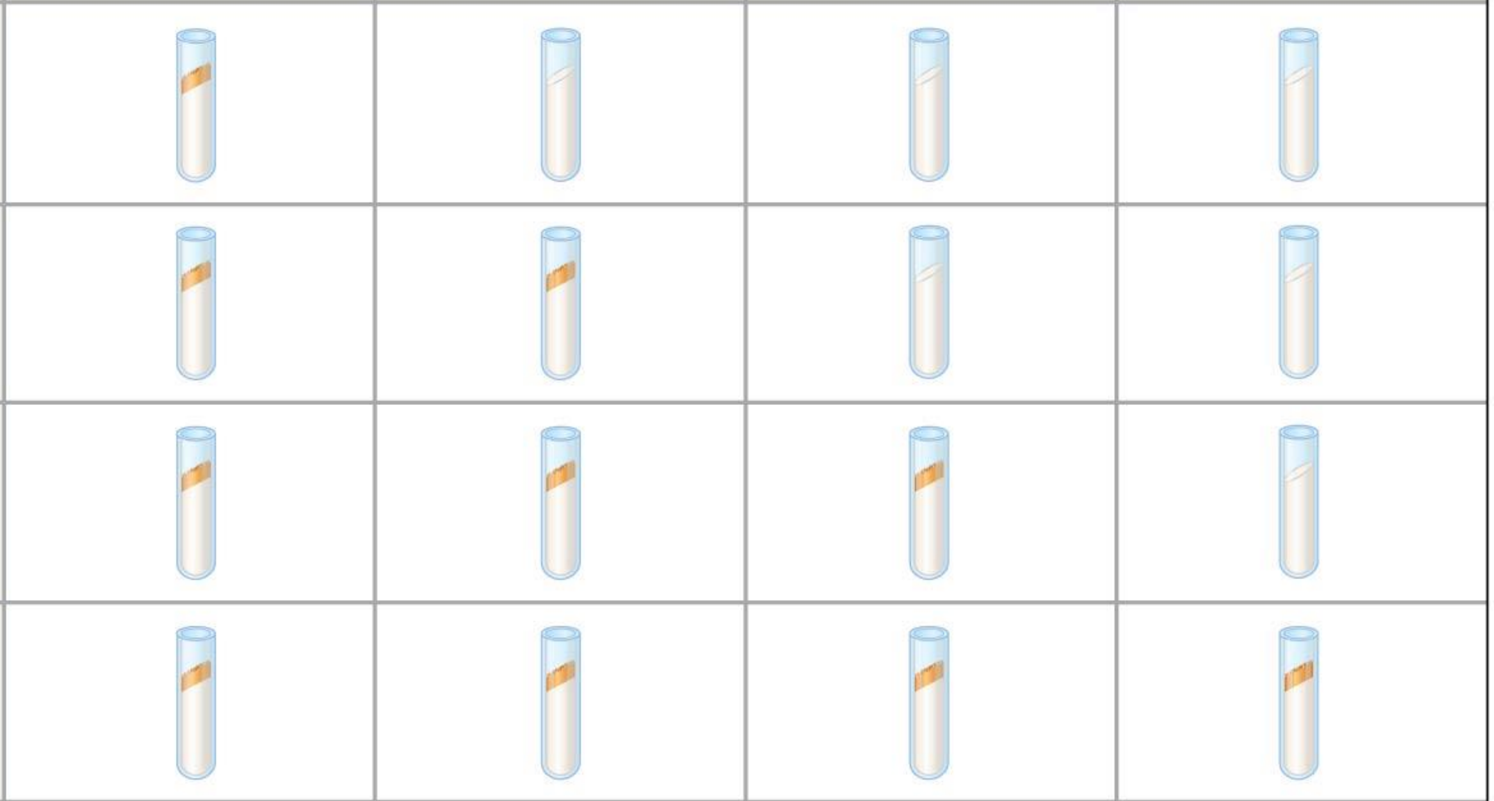


Arginine is one 20 amino acids that make up proteins in ALL organisms

Experimental set up

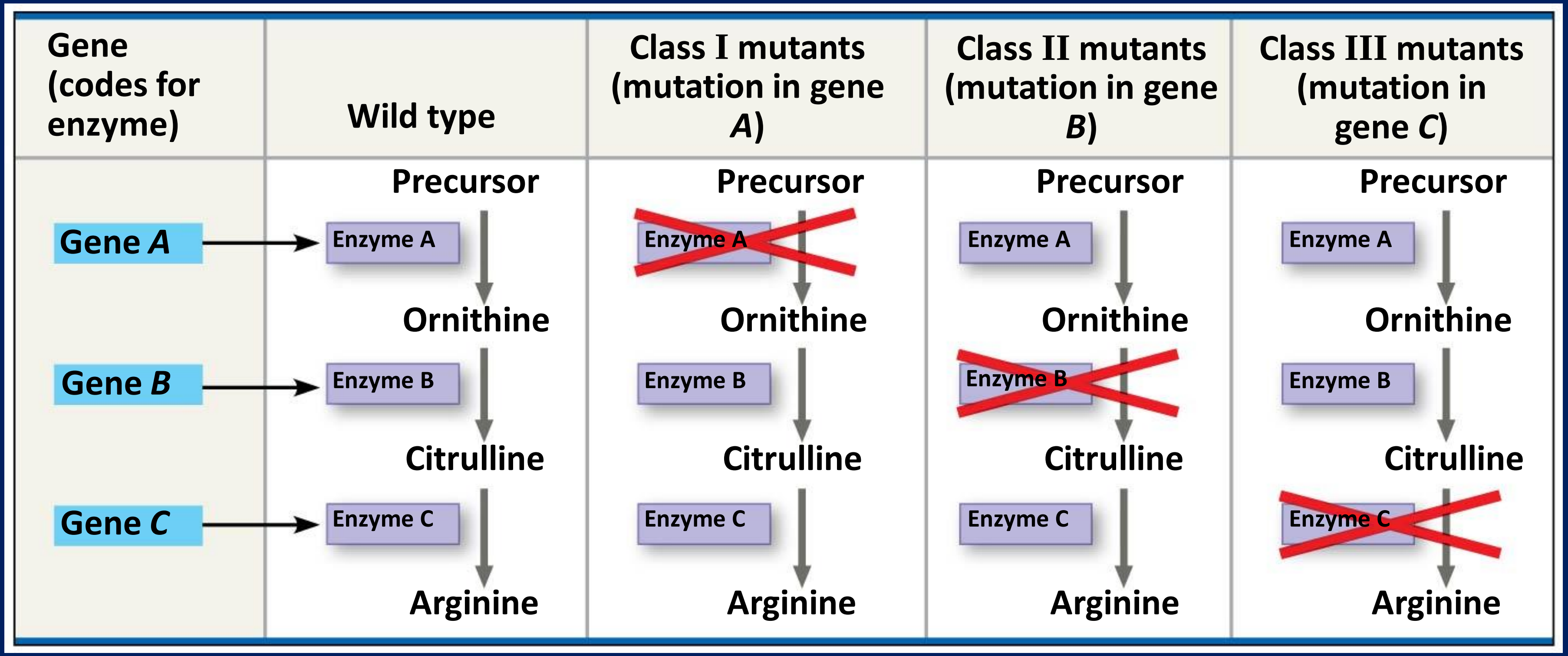
Results Table — **Classes of *Neurospora crassa*** — Figure 17.2

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



Interpretation

Figure 17.2



Information content of DNA and traits

- DNA inherited by an organism leads to specific traits by dictating the synthesis of proteins
- Proteins are the links between genotype and phenotype

GENOTYPE
The genetic content of an organism

Proteins

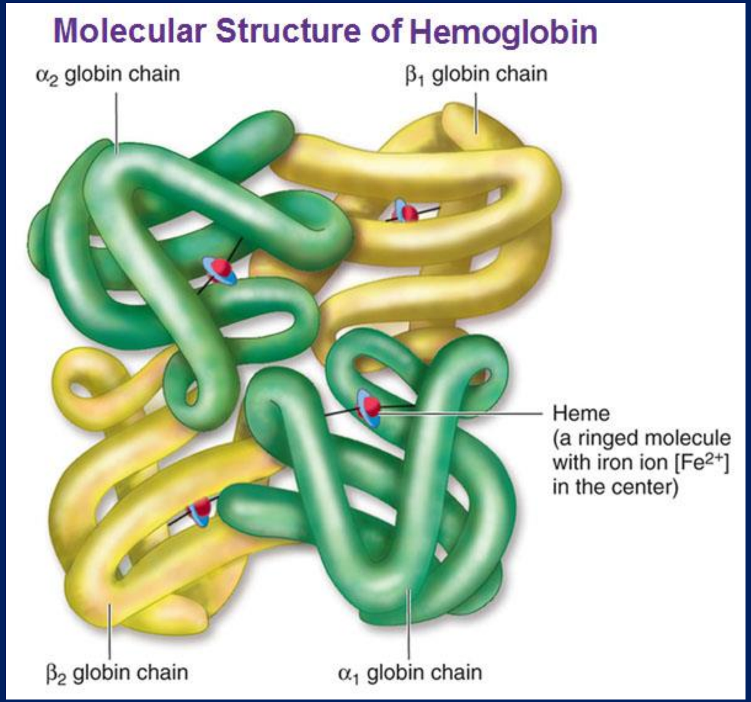
RNA

PHENOTYPE
Inherited traits e.g., albinism

One gene – one polypeptide

- Some proteins are not enzymes… one gene–one protein
- Many proteins have several polypeptides, each with its own gene
- Beadle and Tatum's hypothesis: one gene–one polypeptide

http://antranik.org/blood-components-hemoglobin-typerh-factor-agglutination/



Chromosomes and genes

- Observable traits can be inherited
  - Gregor Mendel's studies with pea plants

    Flower color (white or purple)

    Seed shape (round or wrinkled)

    Pod color (yellow or green)
  - Thoman Morgan's studies on fruit fly
- Inherited traits are determined by genes
- Genes are present in chromosomes

Mendel's : Law of segregation

- Mendel crossed true-breeding purple flowered plants and white flowered plants.
- F1 hybrids were allowed to self-pollinate or were cross-pollinated with other F1 hybrids
- Both purple-flowered and white-flowered plants appeared in the F2 generation, in a ratio of approximately 3:1.
- The "heritable factor" for the recessive trait (white flowers) had not been destroyed, deleted, or "blended" in the F1 generation but was merely masked by the presence of the factor for purple flowers, which is the dominant trait.

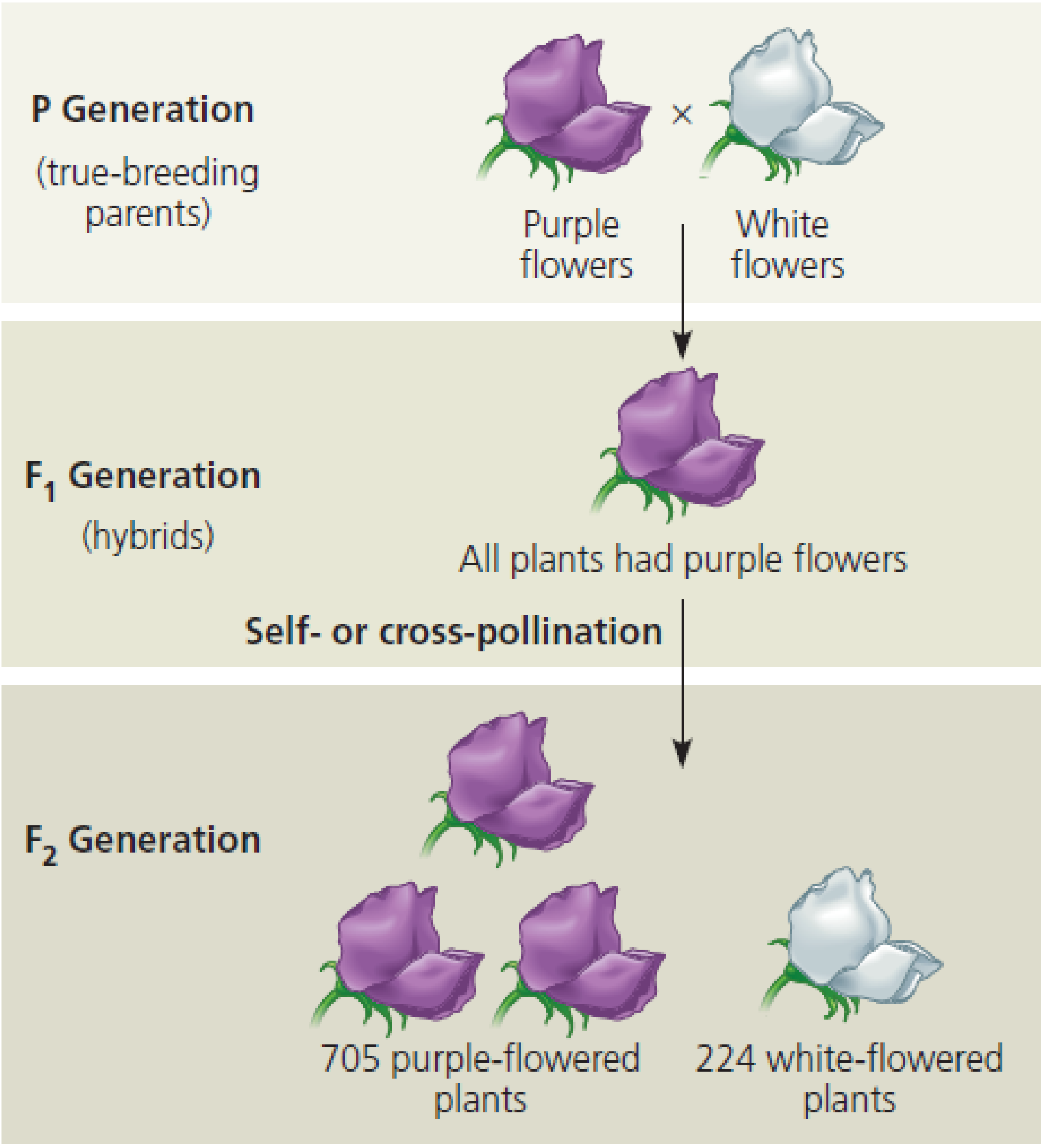


Figure 14.3

Mendel's Law of segregation

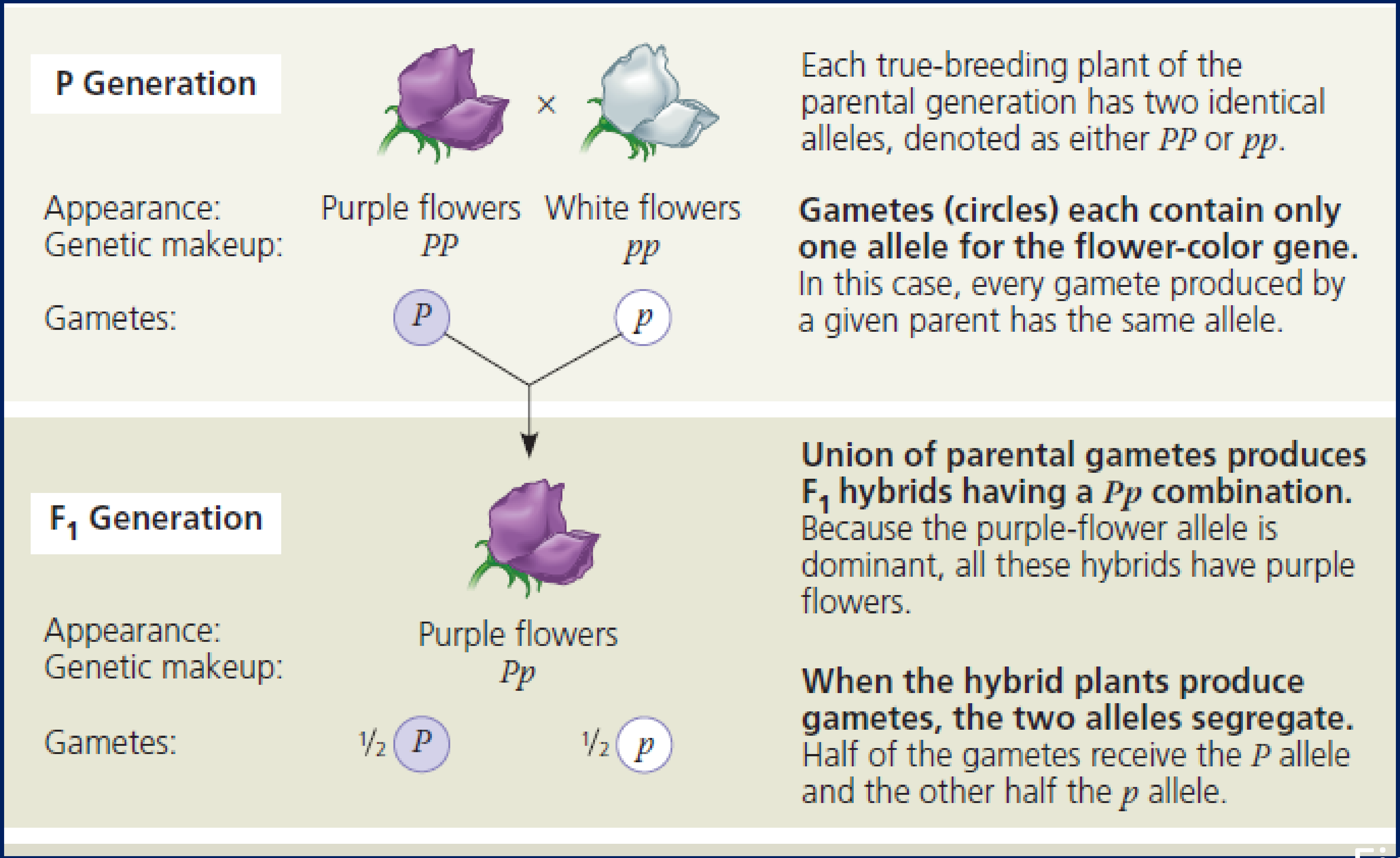
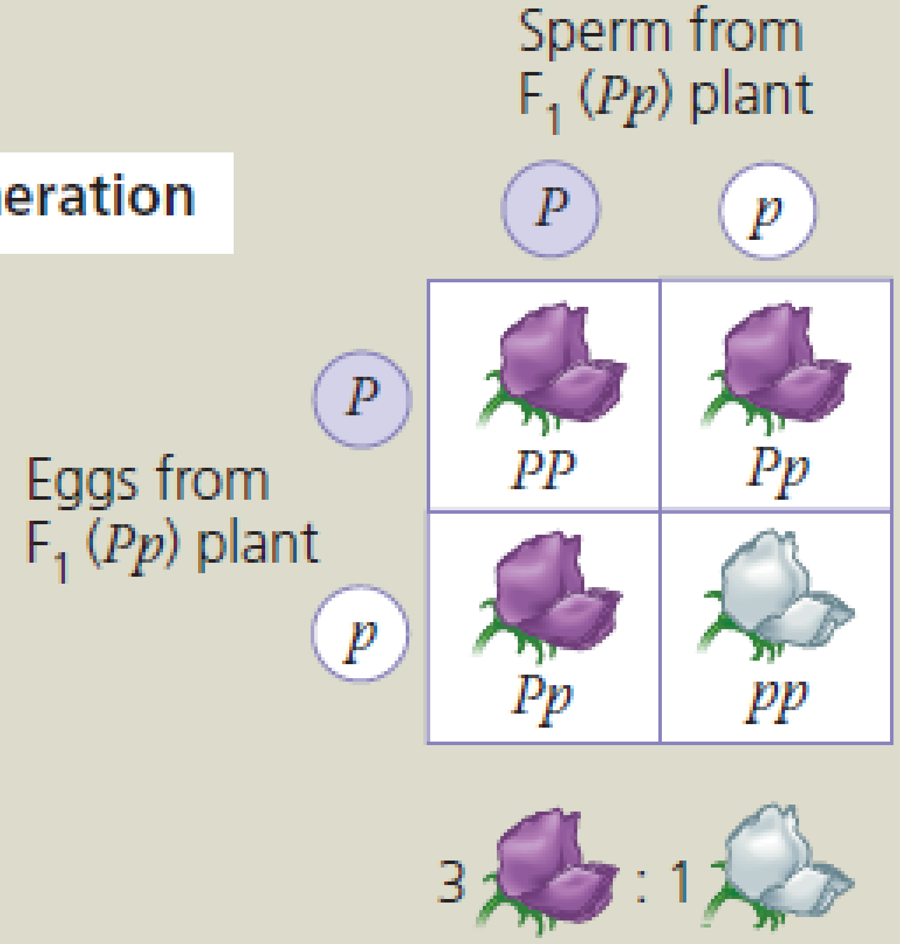


Figure 14.5

Mendel's Law of segregation

F_2 Generation



This box, a Punnett square, shows all possible combinations of alleles in offspring that result from an $F_1 \times F_1$ ($Pp \times Pp$) cross. Each square represents an equally probable product of fertilization. For example, the bottom left box shows the genetic combination resulting from a *p* egg fertilized by a *P* sperm.

Random combination of the gametes results in the 3:1 ratio that Mendel observed in the F_2 generation.

Figure 14.5

Mendel's Law of Independent Assortment

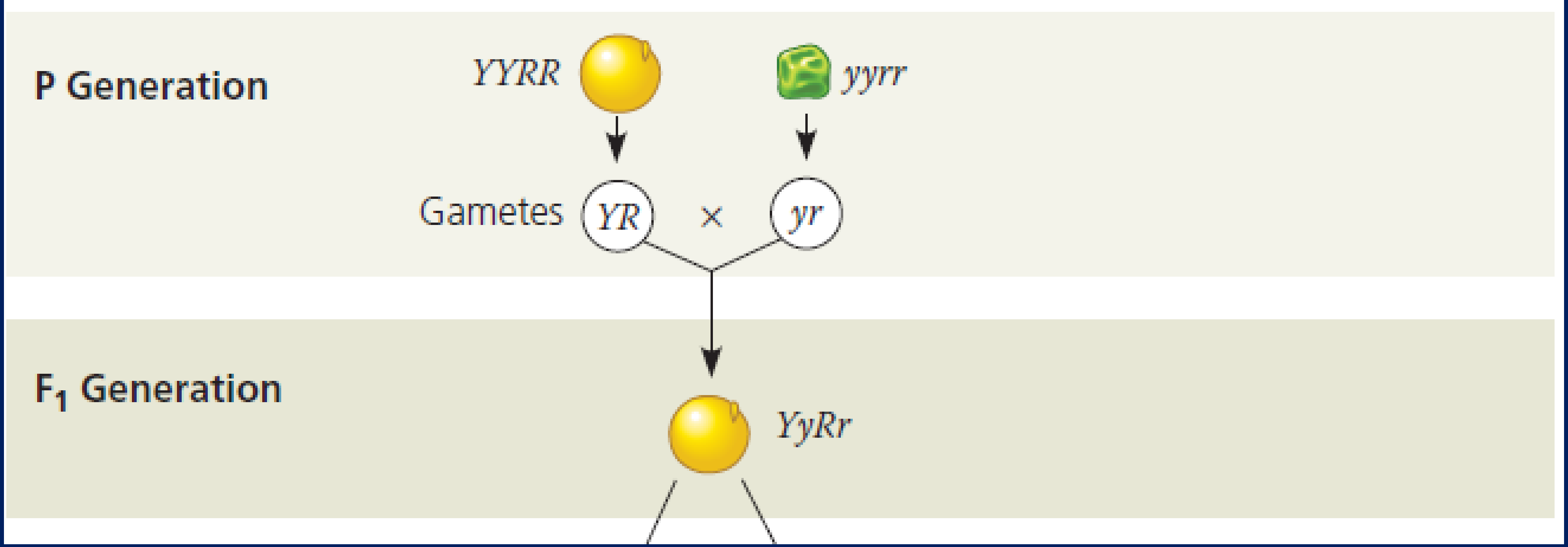


Figure 14.8

Mendel's Law of Independent Assortment

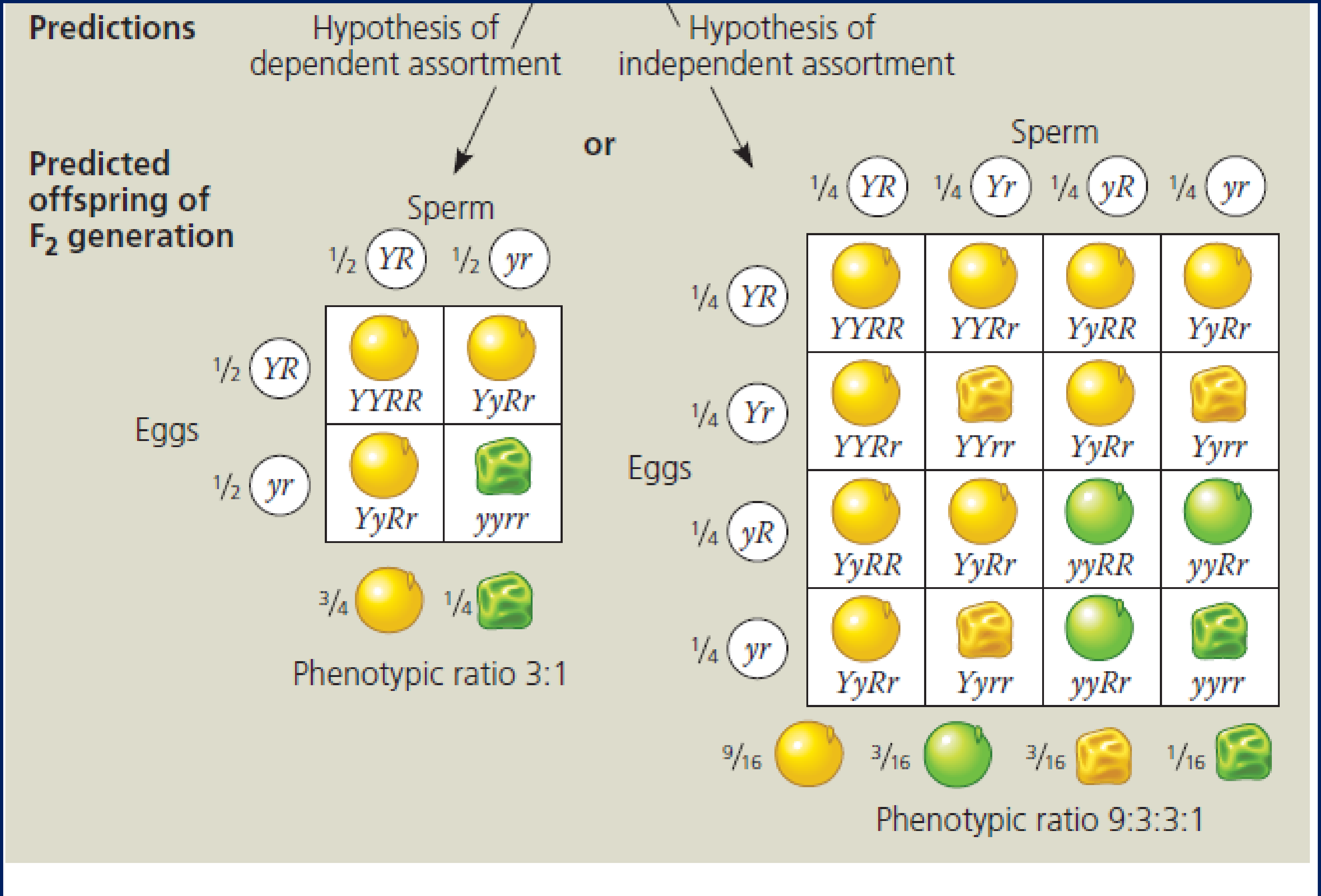


Figure 14.8

Class 4: learning objectives

- Inherited DNA leads to specific traits
  - Coat color, white eyes, phenylketonuria
- DNA: packaging, accessing the stored message
  - Book shelves, storage compactors, chromosomes
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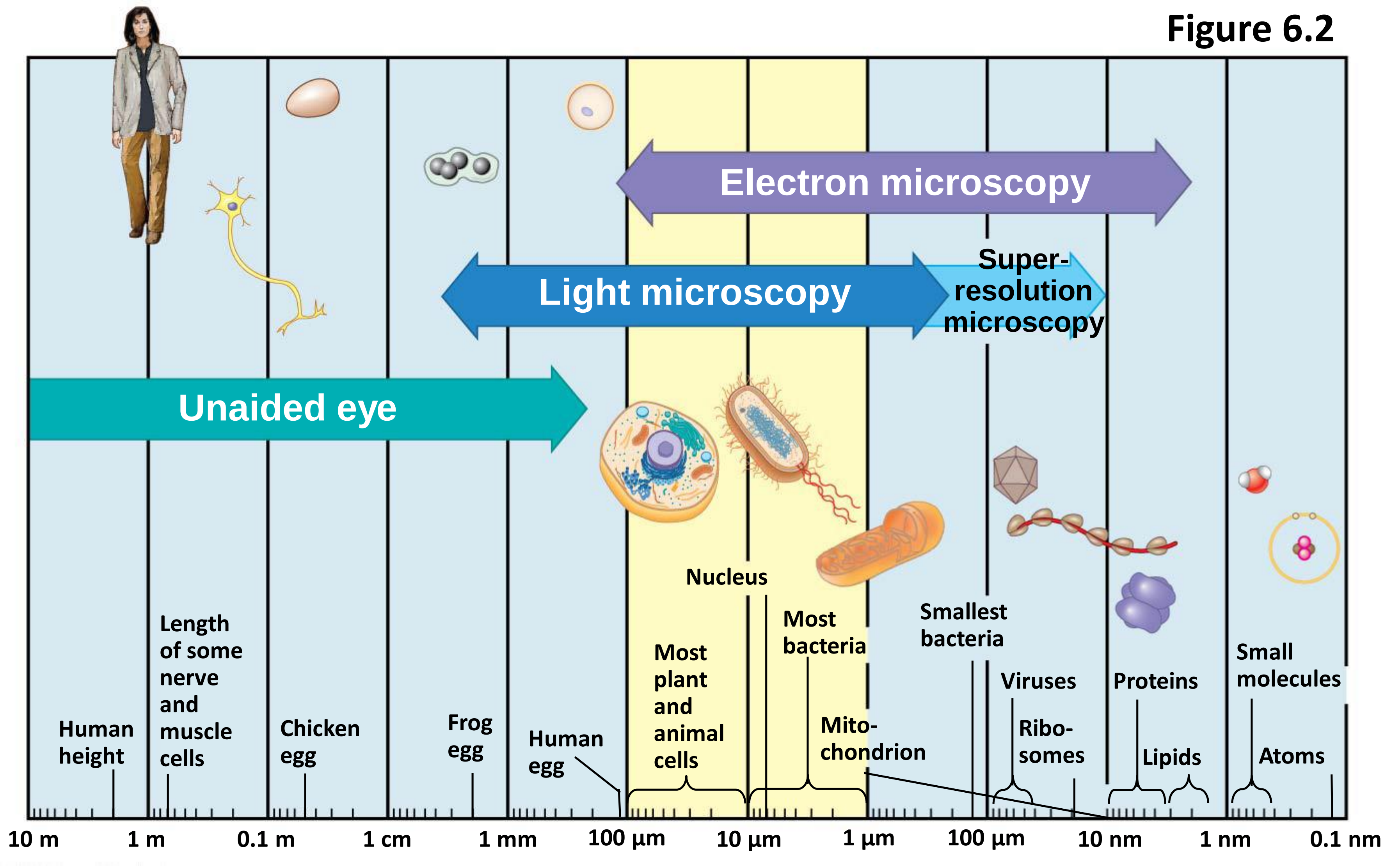
Length comparison: DNA and cell



Logarithmic scale

Length of DNA
Watson-Crick model

10 base pairs: 3.4 nm

Number of bases in human chromosome #1 is approximately 250 million base pairs

Length:
$250 \times 10^6 \times 0.34$ nm
85 mm

Packaging of DNA

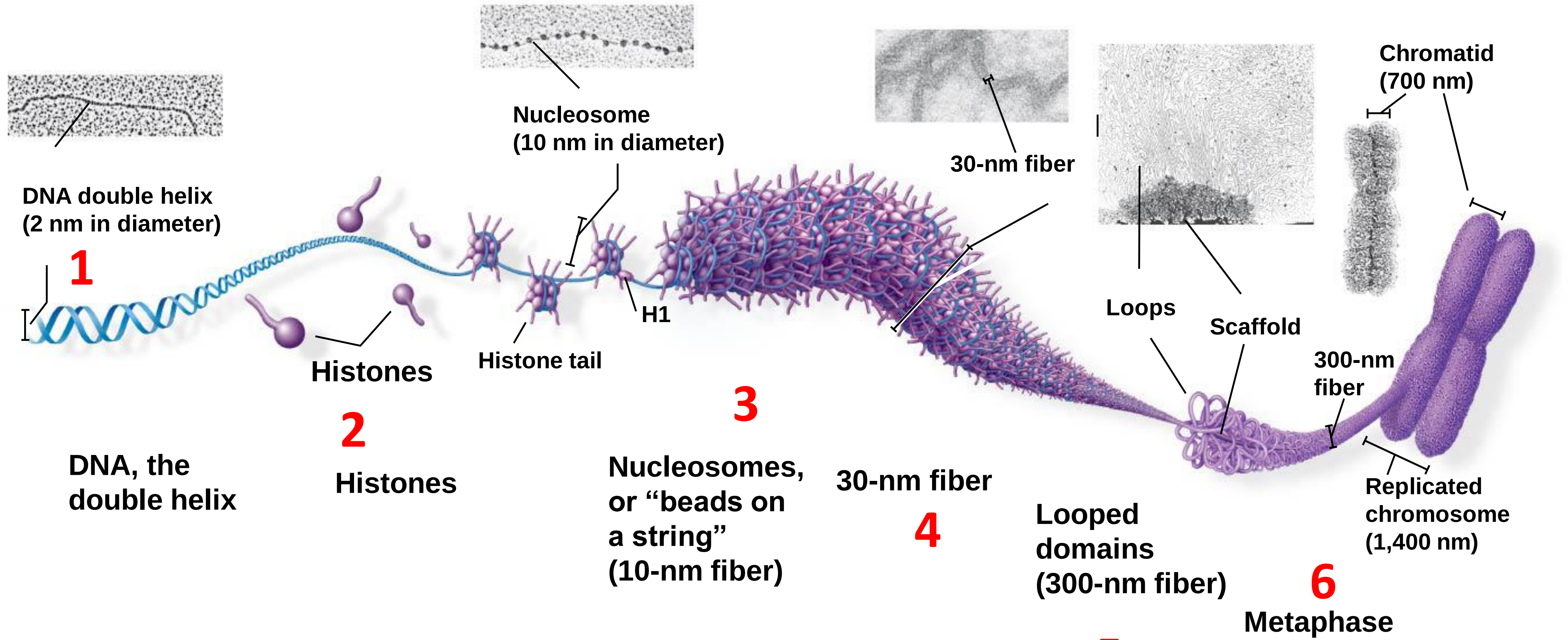


Figure 16.22

1. Ribbon model of DNA. Each ribbon represents a DNA strand

Packaging of DNA

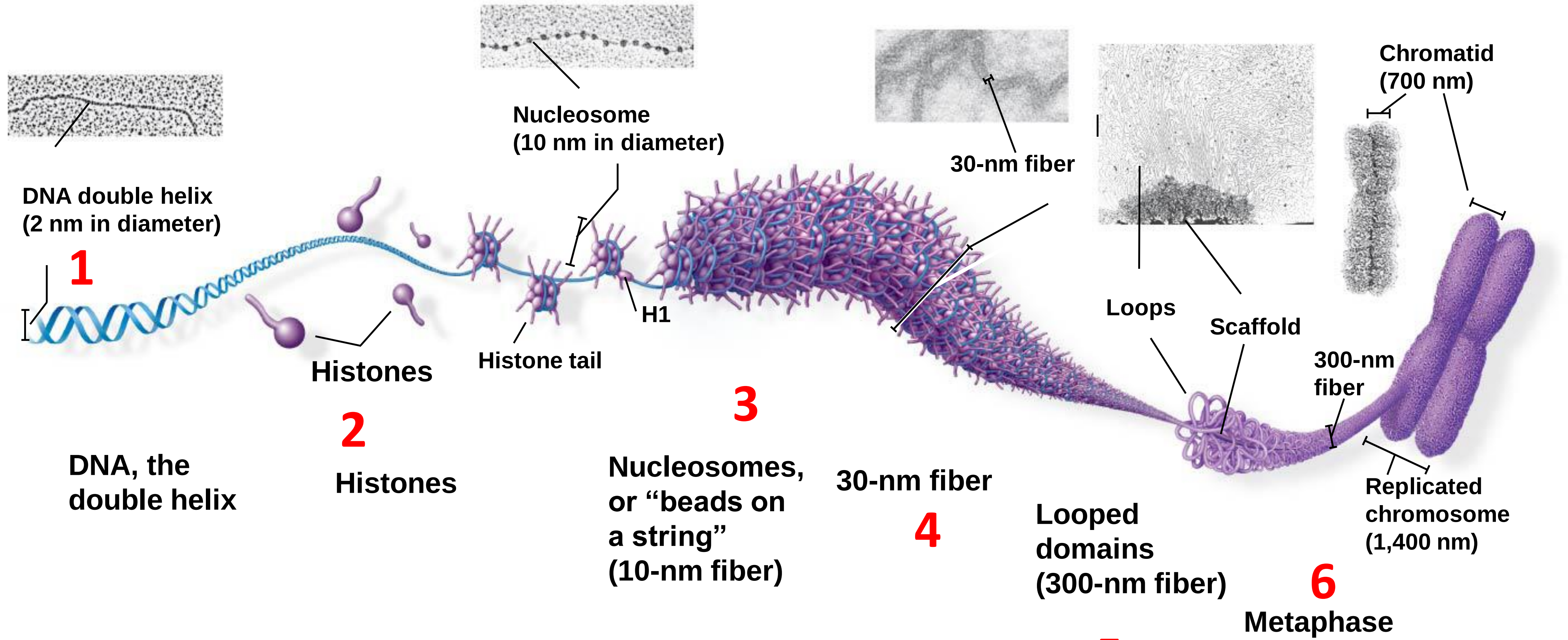


Figure 16.22

Chromatin = protein + DNA complex

2. First level of packing of DNA in the chromatin

Packaging of DNA

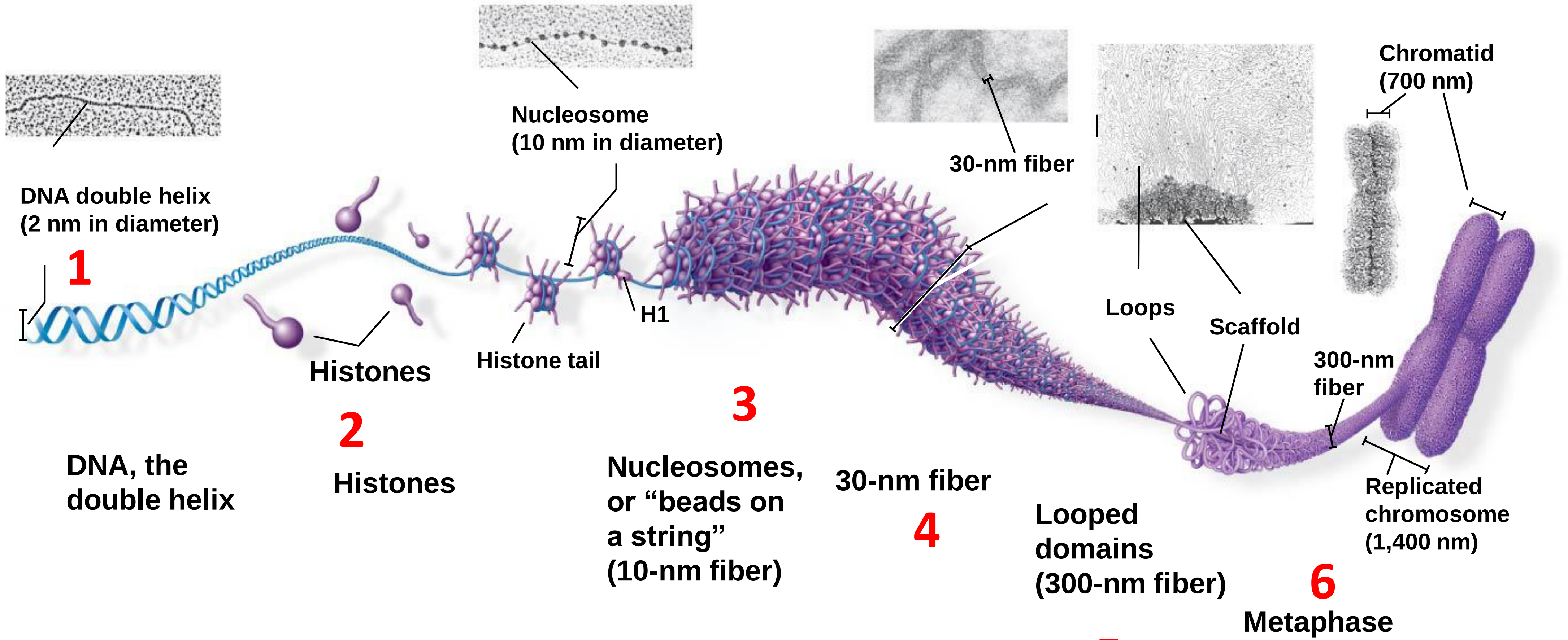


Figure 16.22

Bead = nucleosome; string – linker DNA

3. An aggregate of histones = resembles a ball of wool

DNA is wound twice around histones = nucleosome

Packaging of DNA

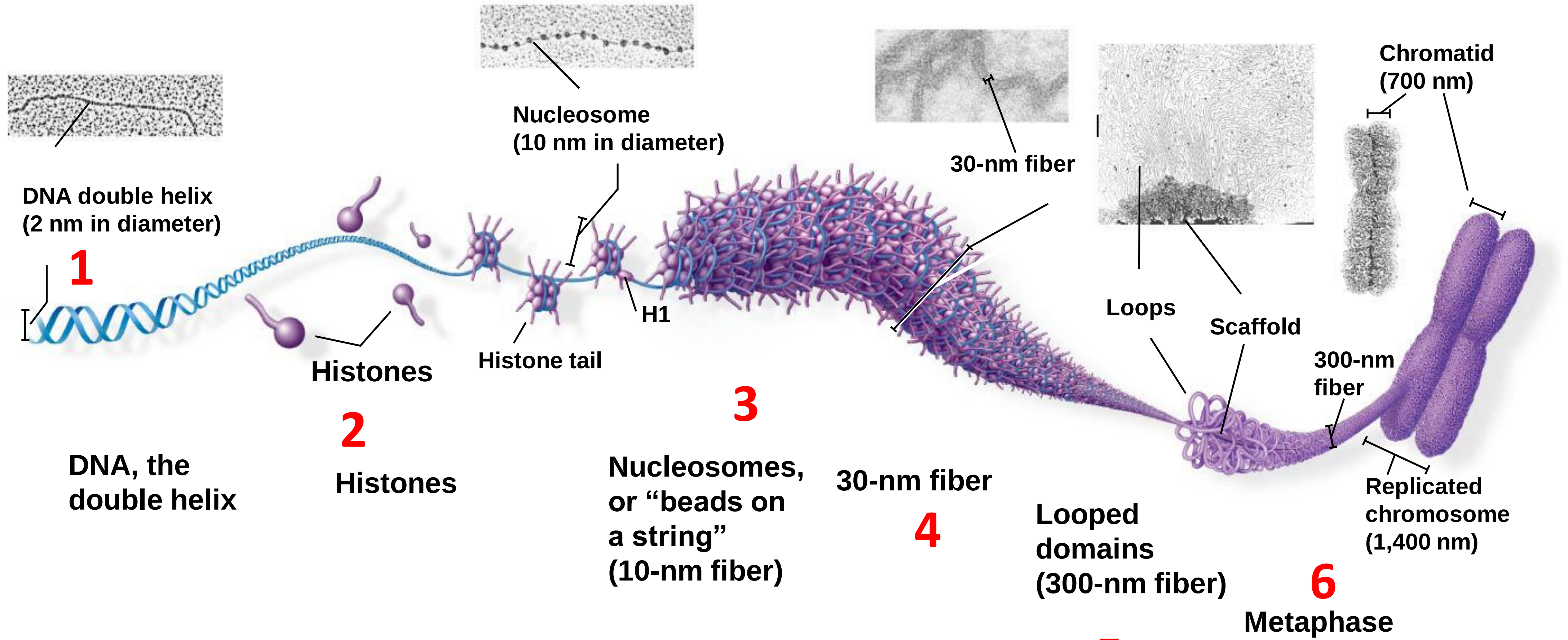


Figure 16.22

4. Second level of packing. Involves interactions of histone tails

Packaging of DNA

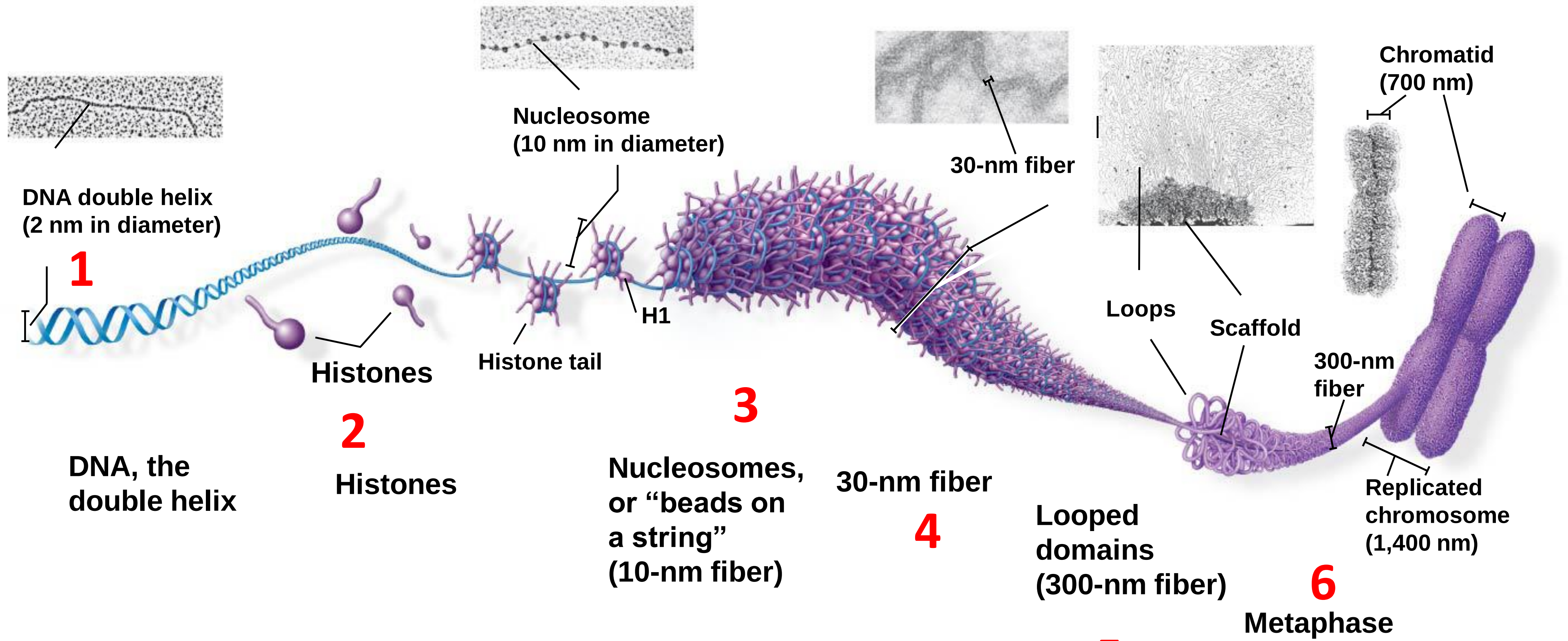


Figure 16.22

5. Formation of loops by 30-nm fibers

Packaging of DNA

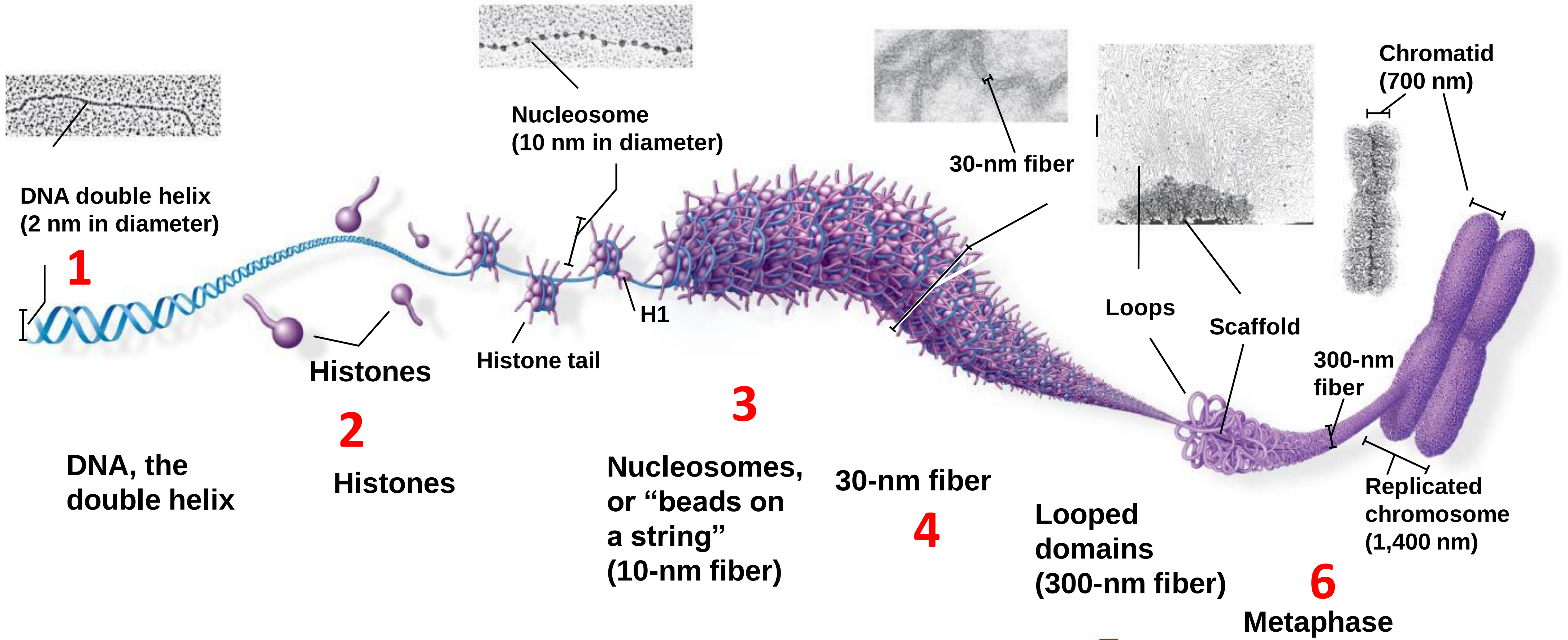
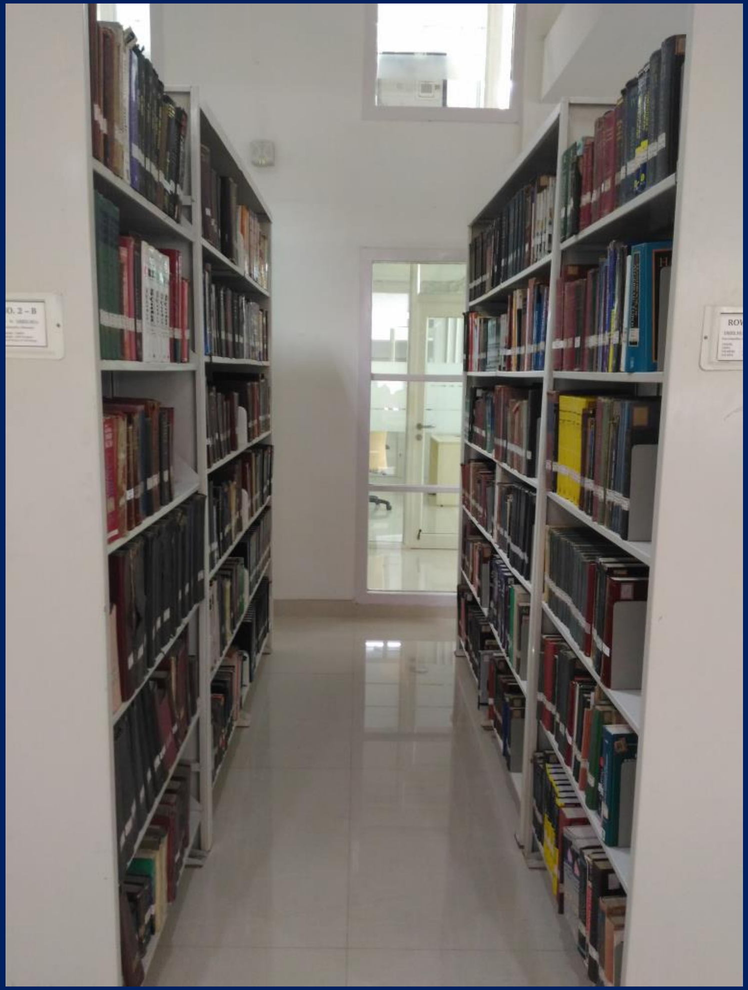


Figure 16.22

6. Coiling and folding of 300-nm fibers leading to further compaction

Accessing books in a library



Books arranged in shelves

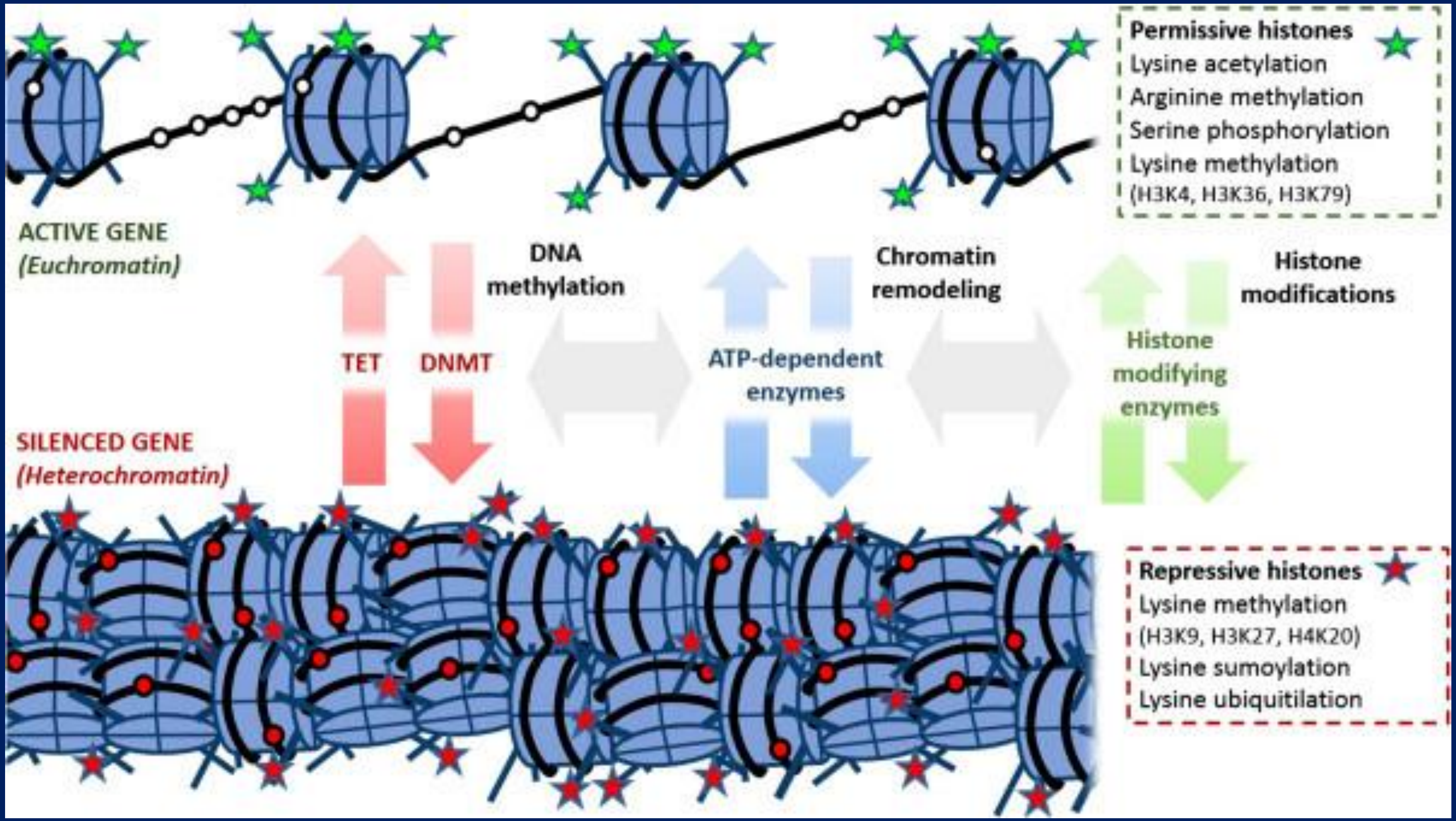
Shelves spaced out to facilitate access

Photo courtesy:
Sri Manju Naika
Central Library
IIT Bombay

Requires a lot of space
Not all shelves are accessed simultaneously
Sub-optimal space utilization

Packed and accessible forms

Euchromatin: dispersed, less compact, accessible form
Heterochromatin: compact form, not possible to access genes

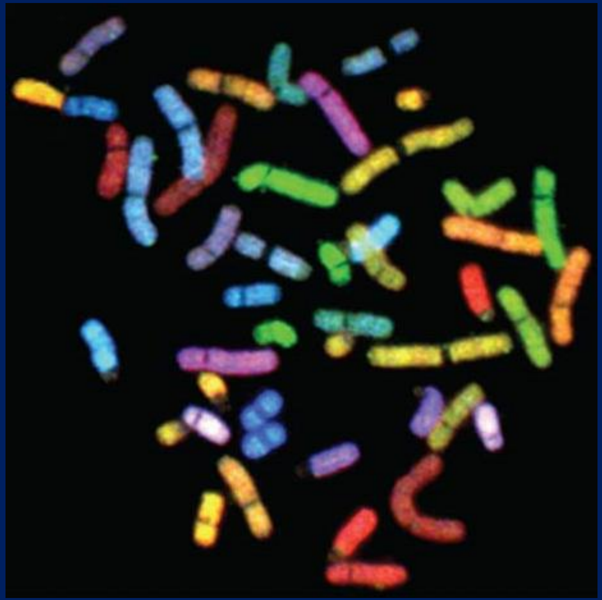


This figure is taken from a research article published in the year 2014
Name of the journal: Allergy Asthma and Clinical Immunology
Volume 10; article # 27.

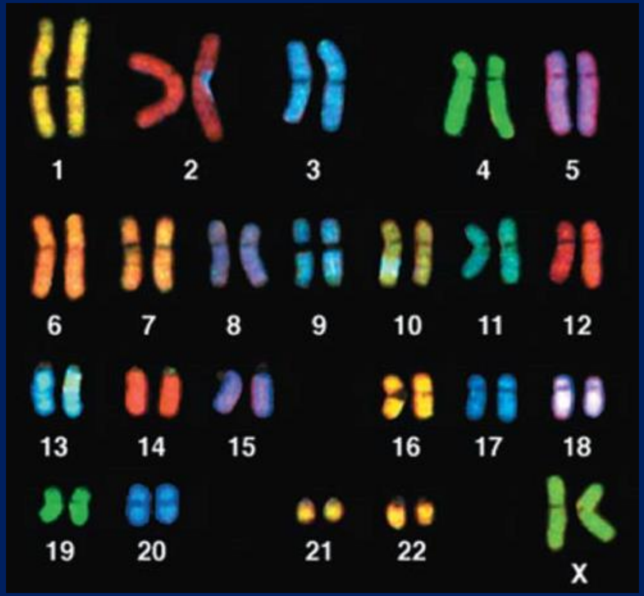
How are human chromosomes arranged?

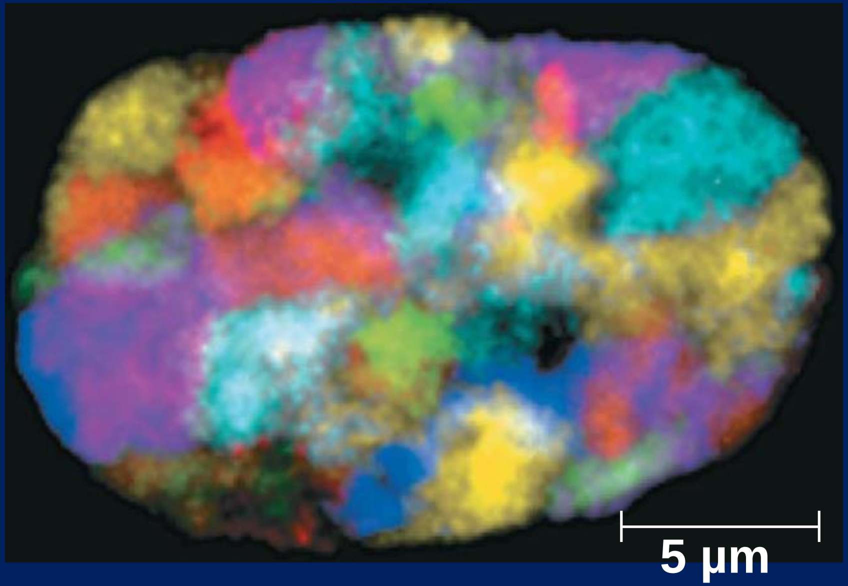
Figure 16.23

Chromosomes spread out



Chromosomes arranged into a karyotype





Tagging each chromosome such that it can be seen in a different color

Arrangement in a cell that is not undergoing cell division

Use as and when required

Encyclopedia comes in several volumes – all volumes are probably of same size

DNA of an organism also comes as ‘volumes’ called chromosomes

Chromosomes are of different sizes

Each chromosome (except XX or XY) come in two copies with ‘slightly’ different sequence

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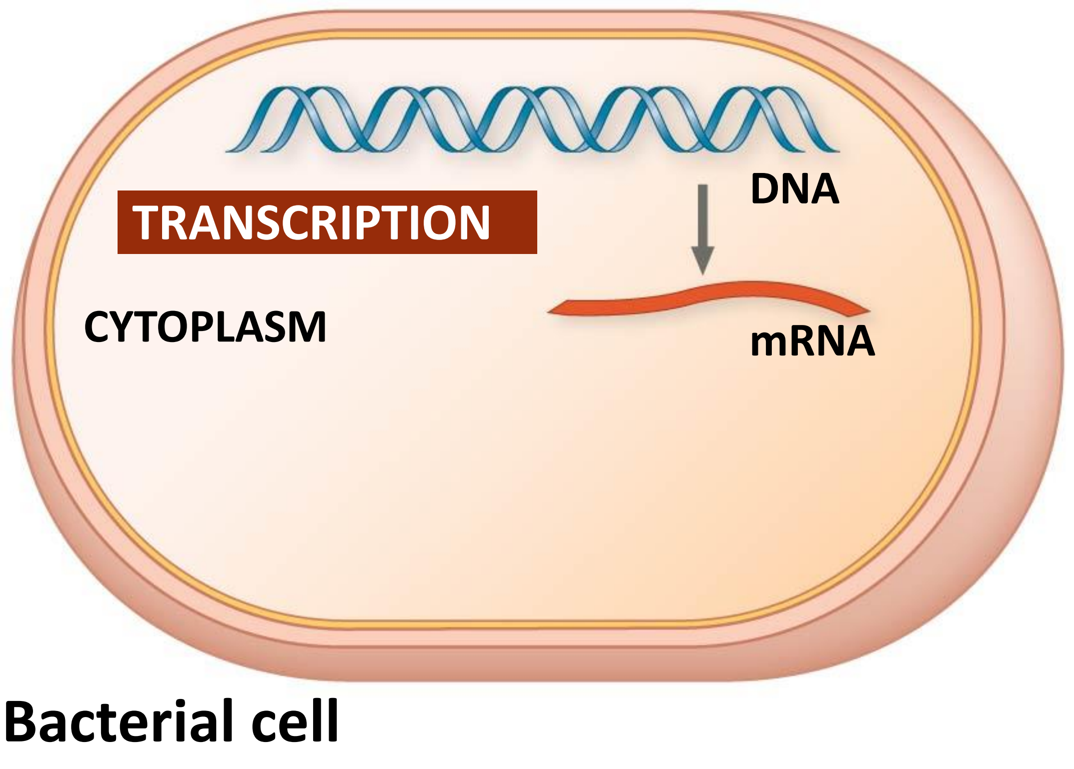
Transcription in prokaryotes

Figure 17.3

Transcription: synthesis of RNA using information in DNA

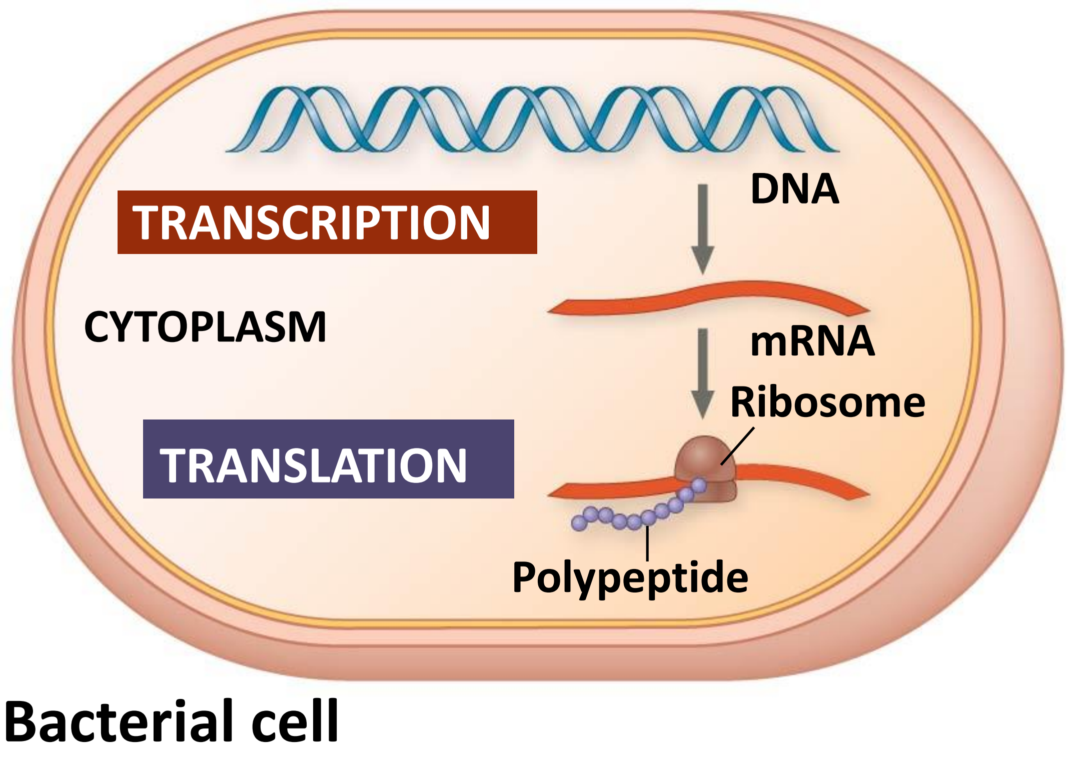


RNA: bridge between genes and proteins that they encode

Transcription produces messenger RNA (mRNA)

Translation in prokaryotes

Figure 17.3



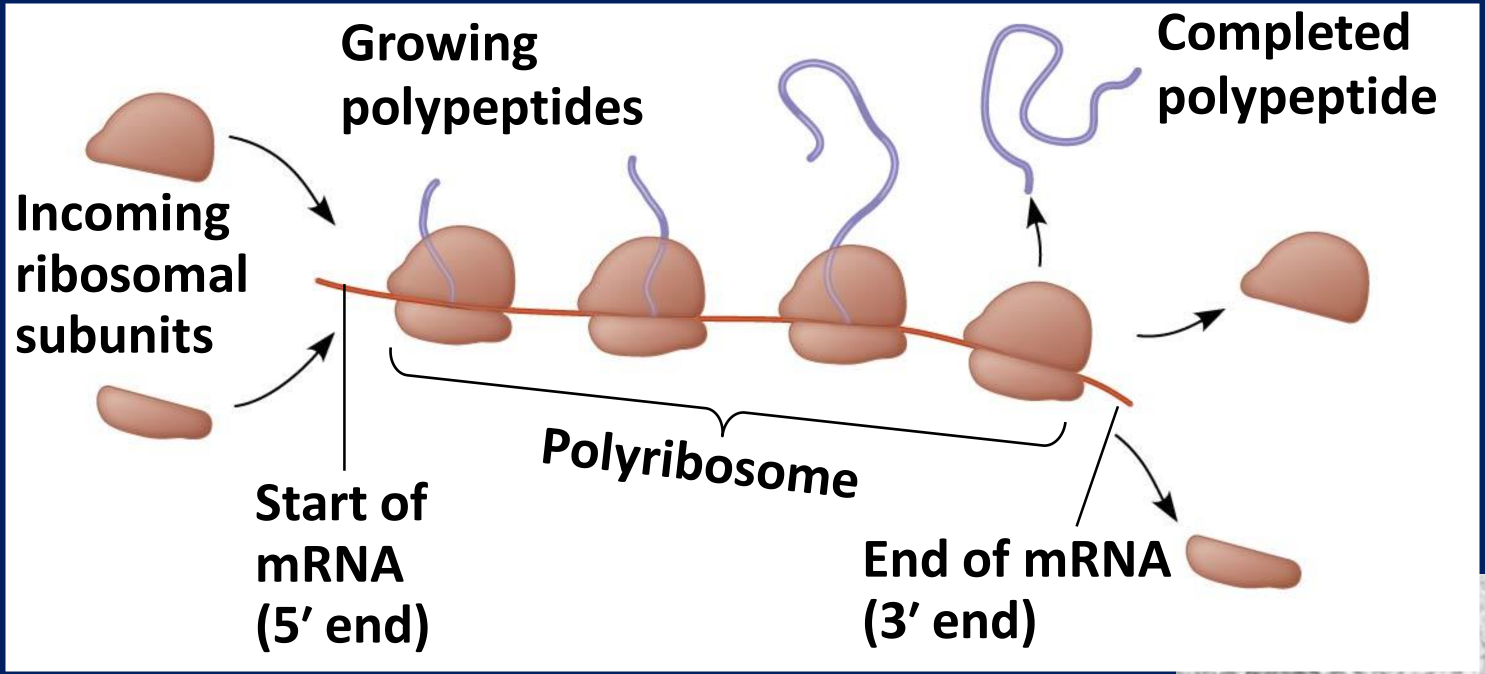
Translation: synthesis of a polypeptide, using information in the mRNA

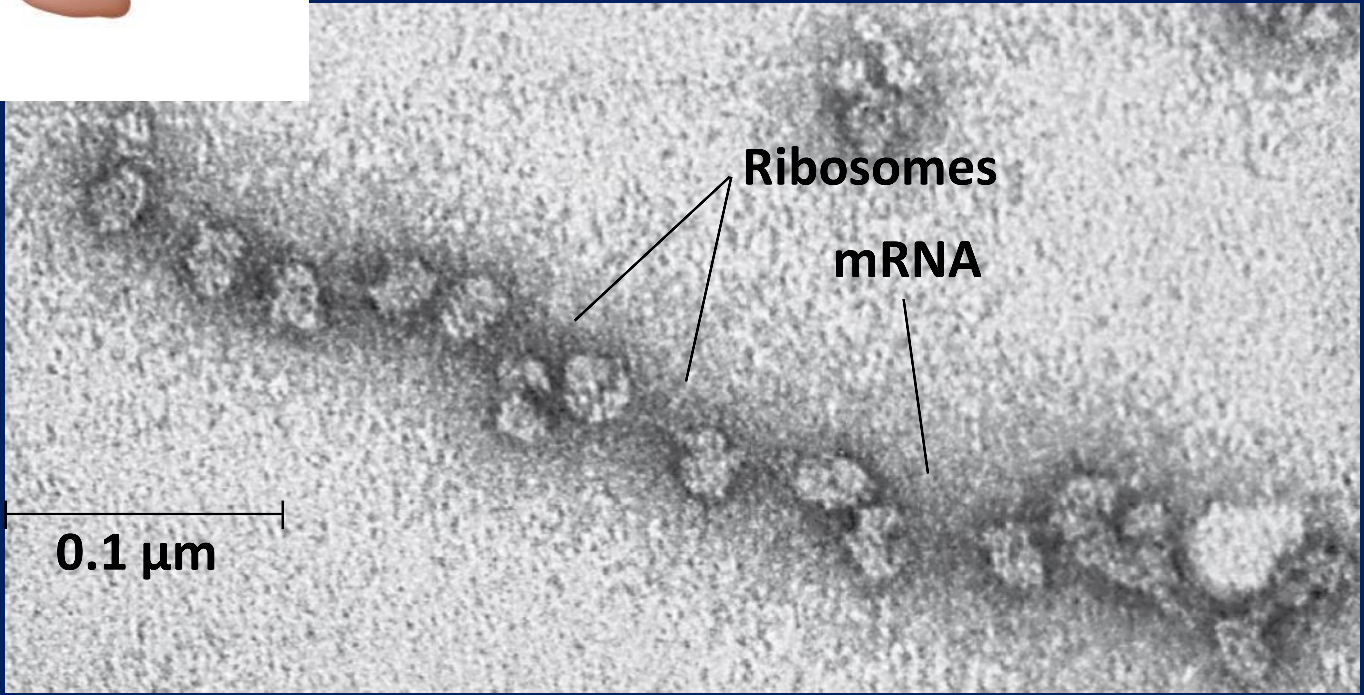
Ribosomes are the sites of translation

Translation can be concurrent with transcription

Poly-ribosomes

Figure 17.21

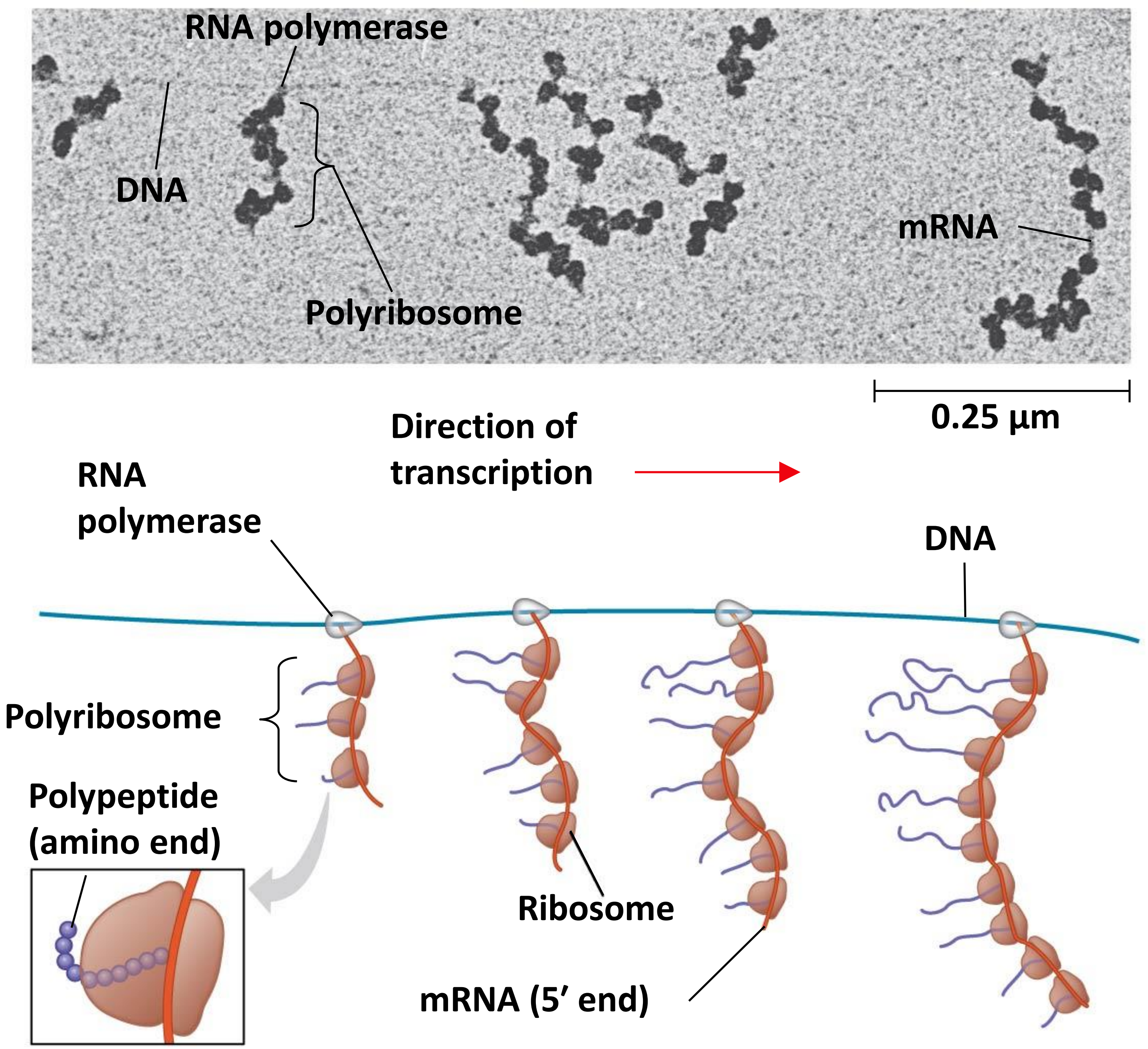




Several copies of a polypeptide chain are made simultaneously

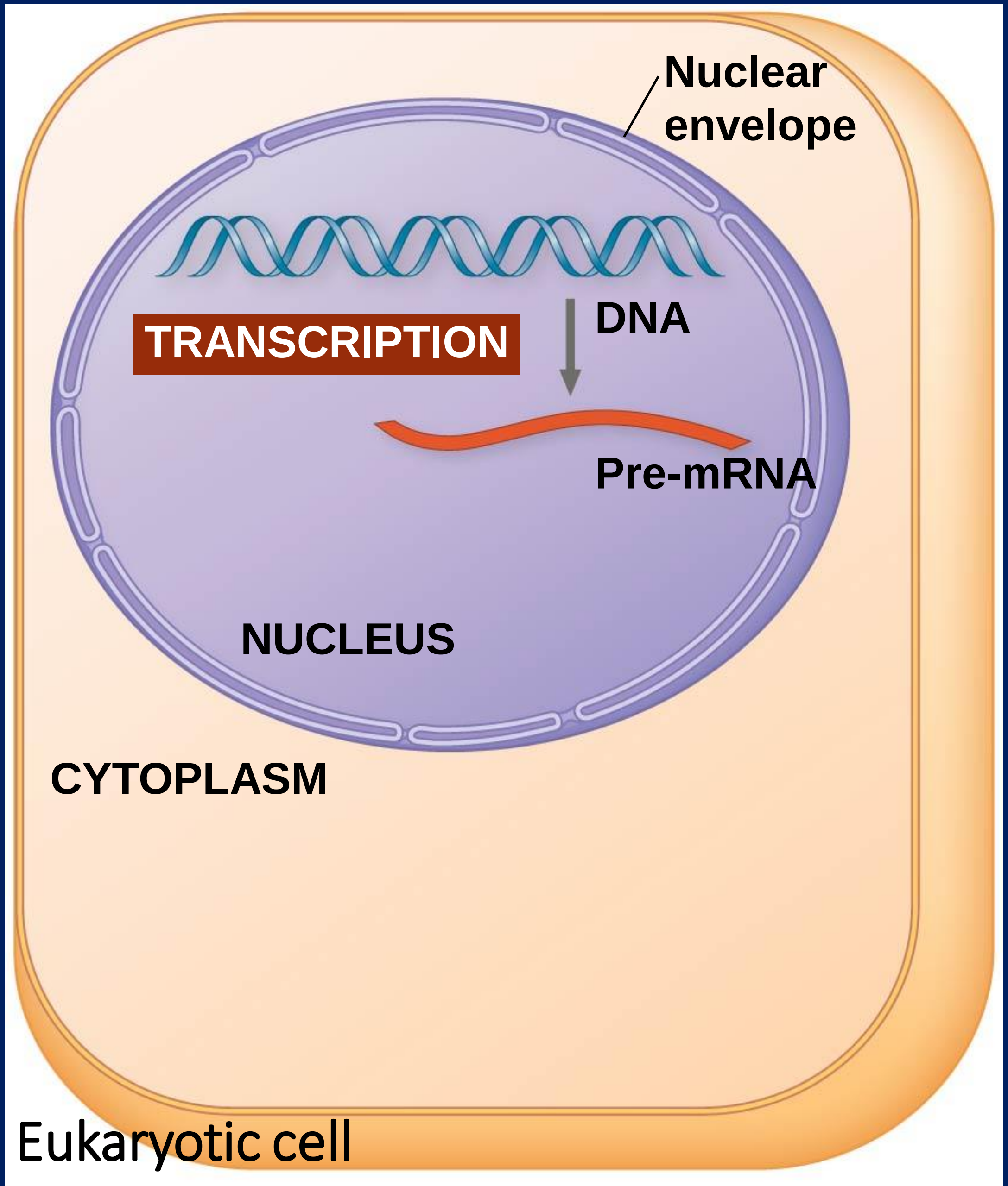
Coupling transcription and translation

Figure 17.25



Transcription in eukaryotes

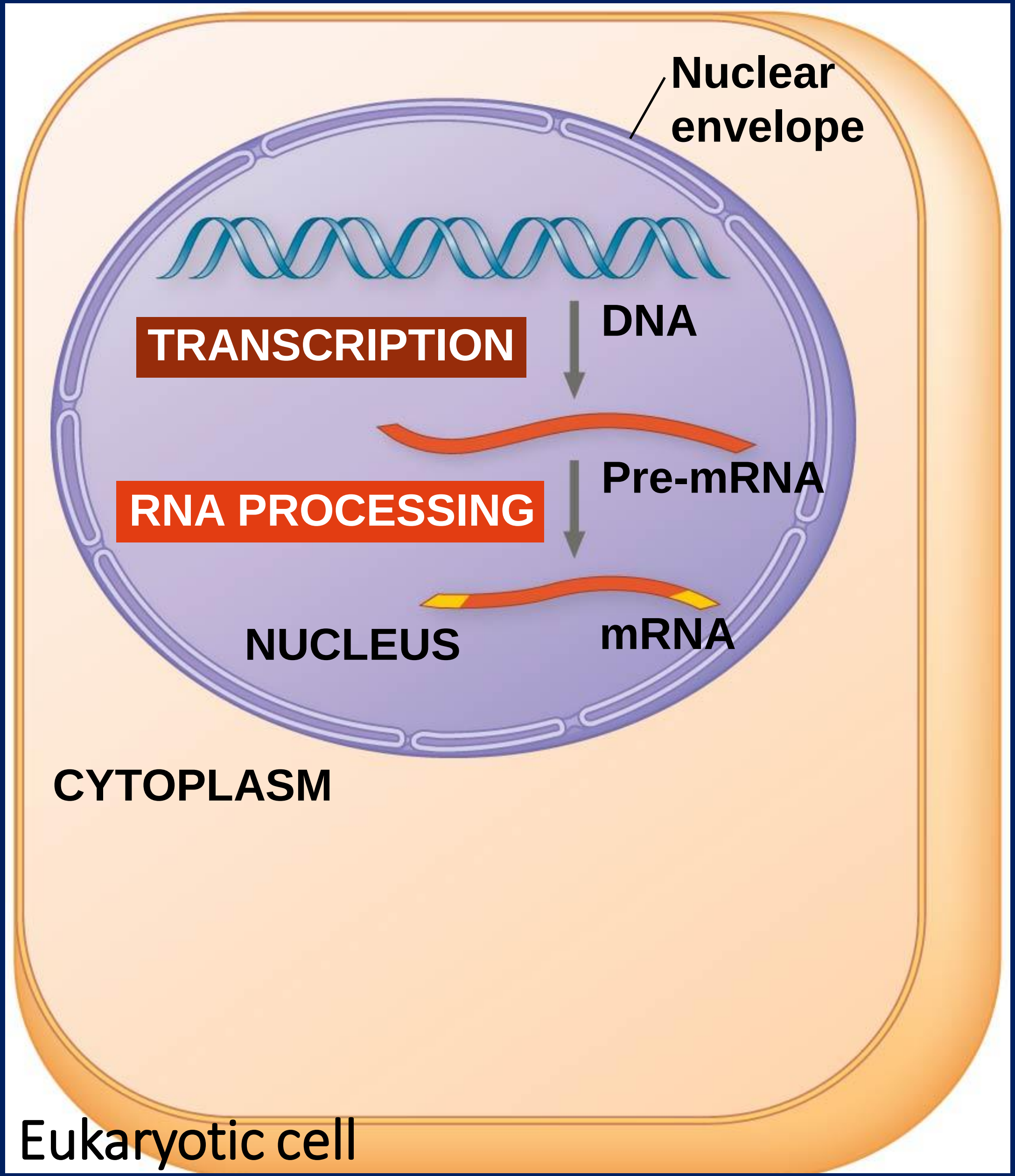
Figure 17.3



mRNA undergoes processing

Figure 17.3

Primary transcript: initial mRNA transcript prior to processing



mRNA is modified through RNA processing to yield the finished mRNA

Translation in eukaryotes

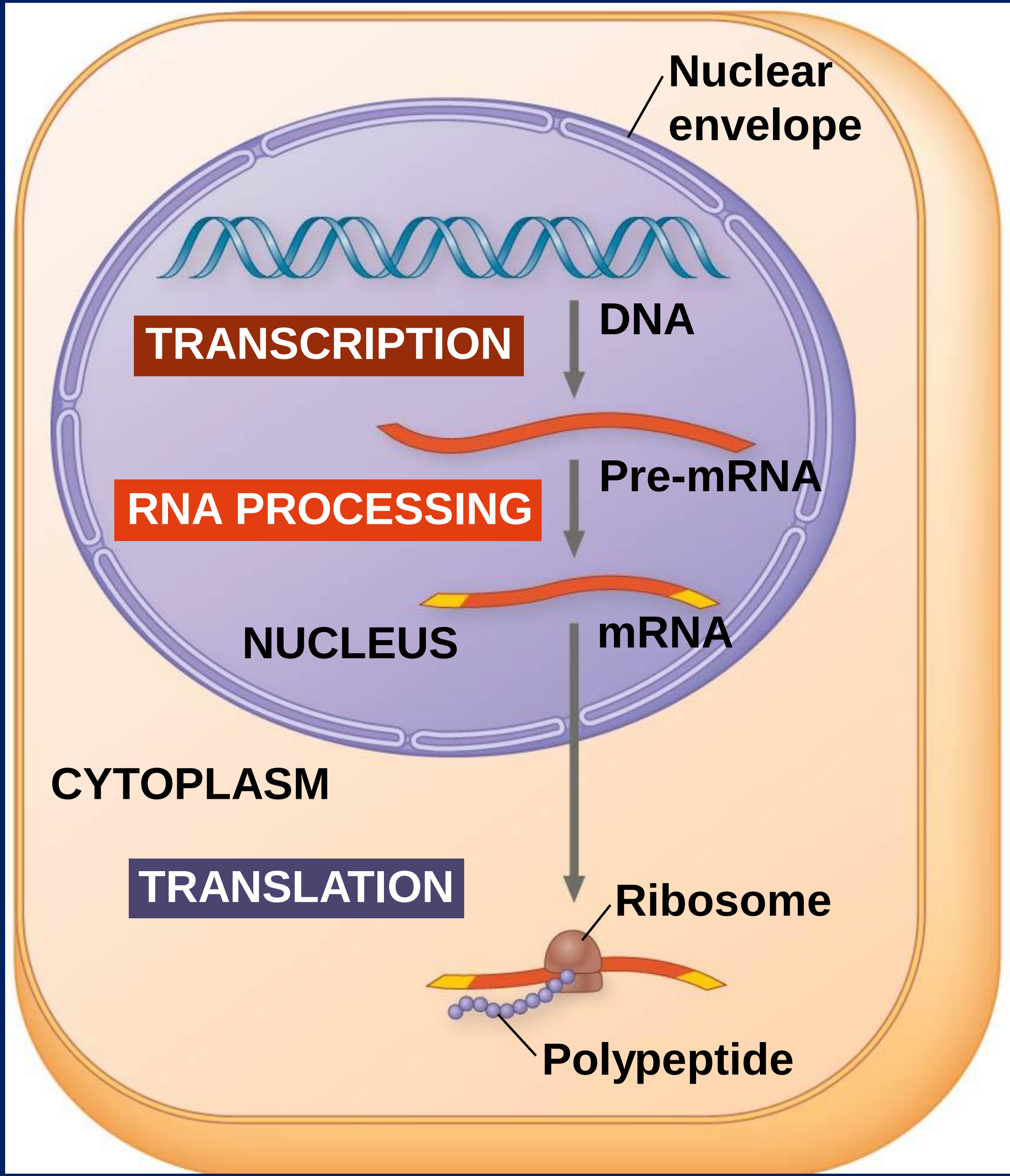
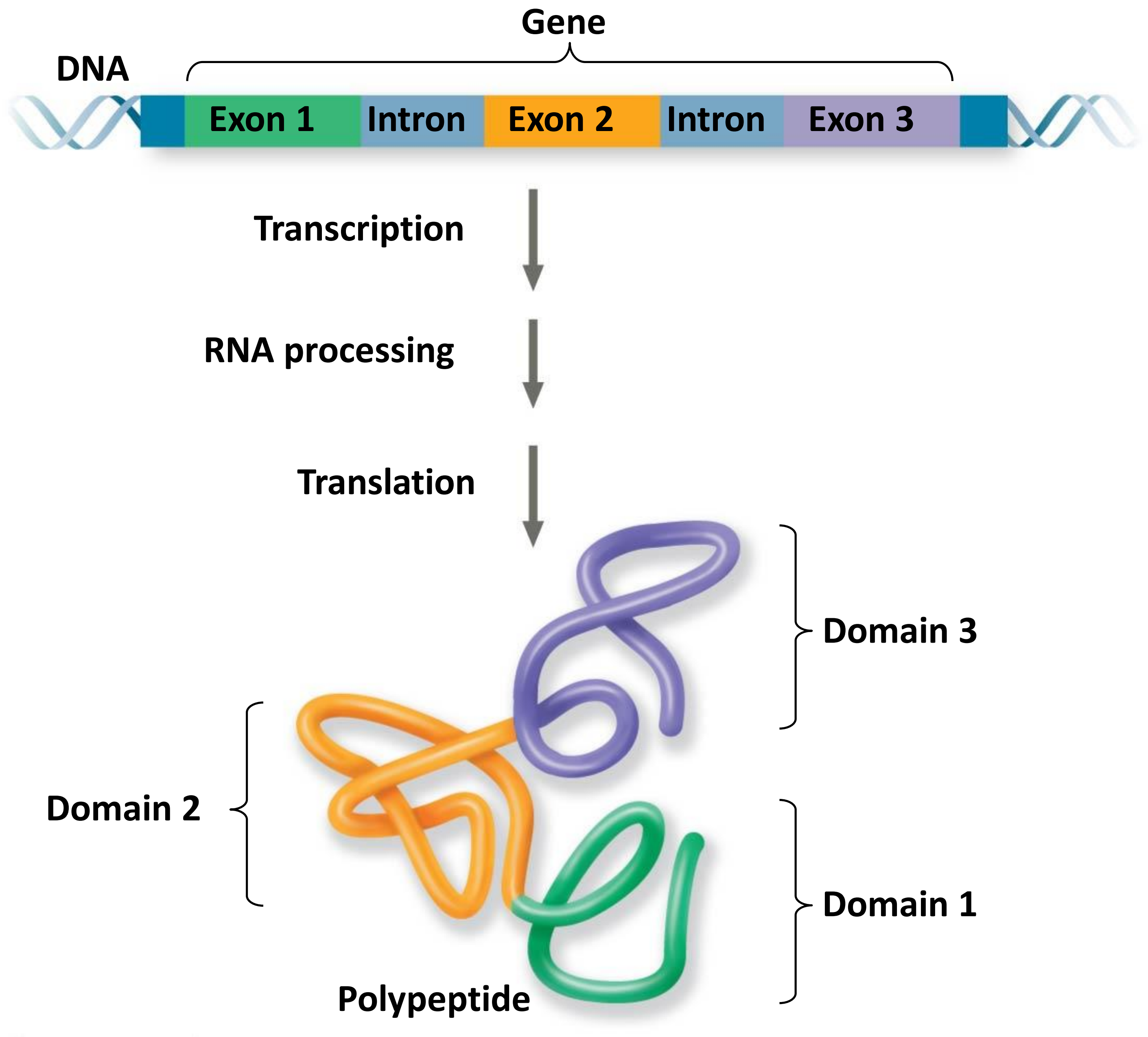


Figure 17.3

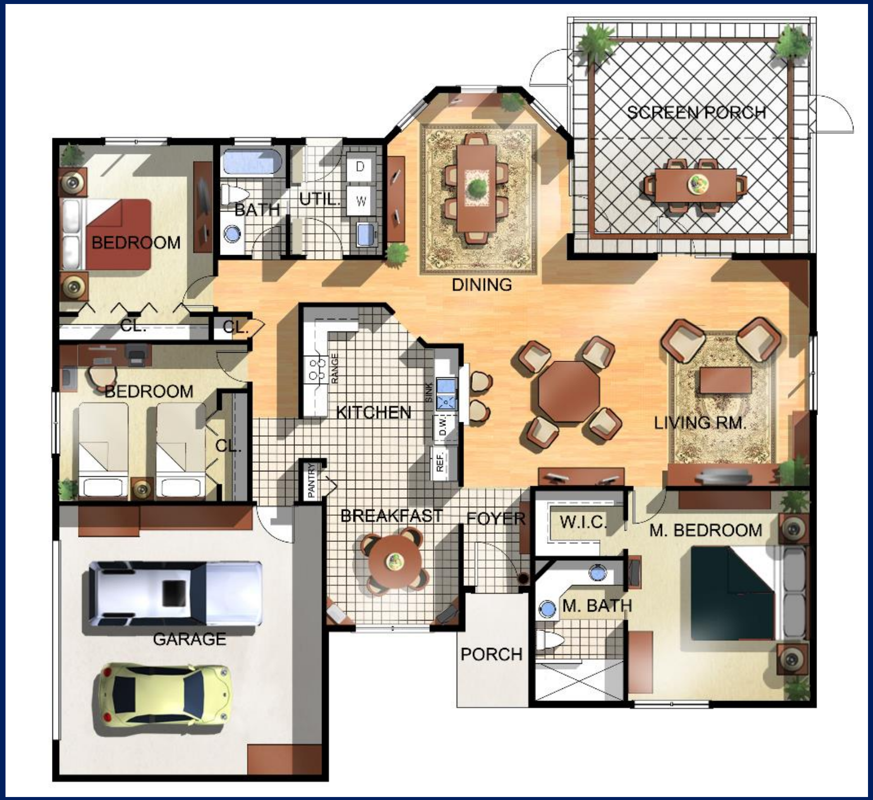
Eukaryotes: nuclear envelope separates transcription from translation

RNA processing



Compartmentalization

Floor plan of an affluent home



http://zenlibs.com/a_floorplan-of-a-house/

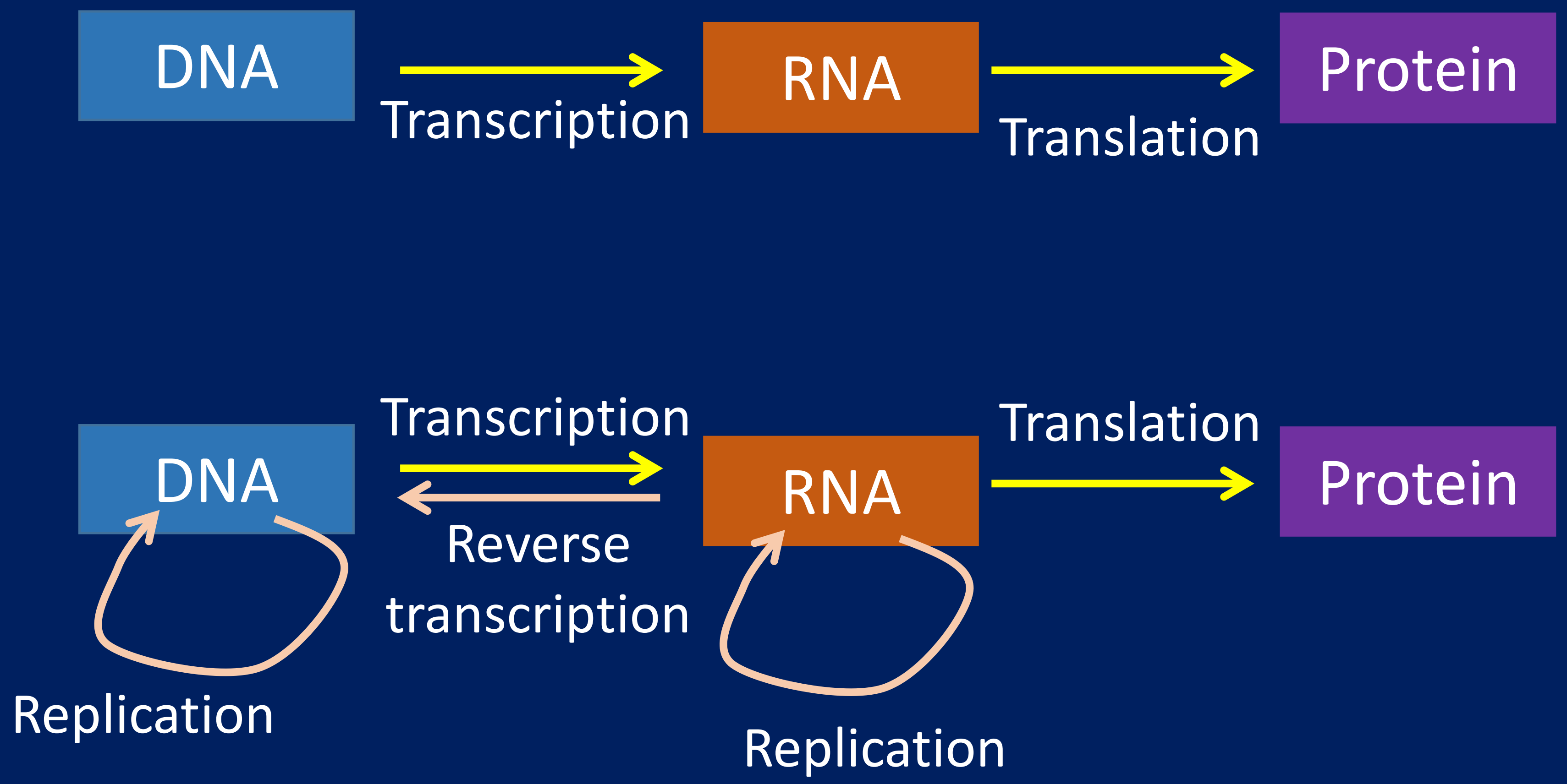
A 1-room tenement

www.studenthandouts.com/01-Web-Pages/01-Picture-Pages/10.07-Industrial-Revolution/1-Riis-Family-Living-in-One-Room-New-York-City-Slum-1890.htm

Tenement: a room that by itself is a residence

Central dogma

Central dogma is the concept that cells are governed by a cellular chain of command



Class 6: learning objectives

- Inherited DNA leads to specific traits
  - Coat color, white eyes, phenylketonuria
- DNA: packaging, accessing the stored message
  - Book shelves, storage compactors, chromosomes
- DNA to protein – basic principles
  - Transcription and translation
- The genetic code and mutations

DNA and protein alphabets

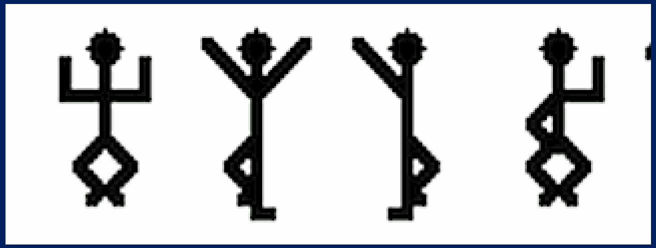
How are instructions for assembling amino acids into proteins encoded into DNA?

The alphabet of English: 26 letters
The alphabet of Hindi (or Assamese or …): __ letters

The alphabet of DNA: 4 letters (A, C, G, T)
The alphabet of protein: 20 letters (names intentionally omitted here)

Decoding the genetic code

Sherlock Holmes in
The dancing men



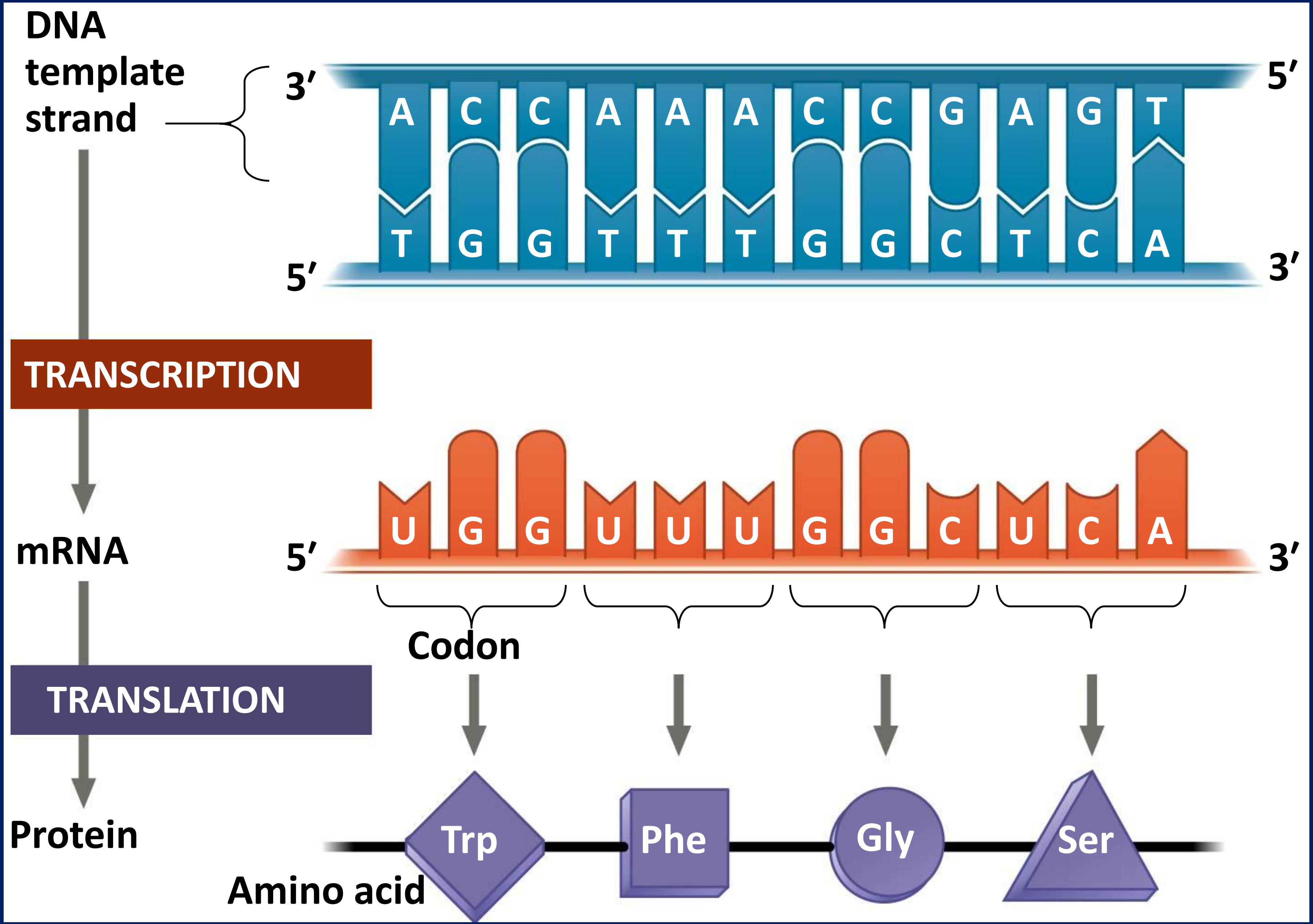
How can one possibly use 4 dancing men to send messages in a script that contains 20 letters?

$$4^1 = 4$$
$$4^2 = 16$$
$$4^3 = 64$$

All images are from
http://www.harsmedia.com/SoundBlog/Archief/00800.php

Non-overlapping triplet code

Figure 17.4



Is there an equivalent of full stop?

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

The “full stop” or stop codons: UAA, UAG and UGA

The triplet genetic code: problem of plenty?

- Number of types of bases in DNA 4
- Number of types of amino acids in proteins 20 + at least 1 full stop
- Number of possible triplet codons 64

What about the additional 44-1=43 triplet codons?

The degenerate genetic code

Codon	Amino acid
UUU	Phenyl alanine
UUC	Phenyl alanine
UUA	Leucine
UUG	Leucine
GUU	Valine
GUC	Valine
GUA	Valine
GUG	Valine

Refer to figure 17.25

Substitution mutation. 1. Silent mutation

Codon	Amino acid
UUU	Phenyl alanine
UUC	Phenyl alanine
UUA	Leucine
UUG	Leucine
GUU	Valine
GUC	Valine
GUA	Valine
GUG	Valine

Refer to figure 17.25

Mutating UUU to UUC

Same amino acid
Mutation is “silent”

Substitution mutation. 2. Missense mutation

Refer to figure 17.25

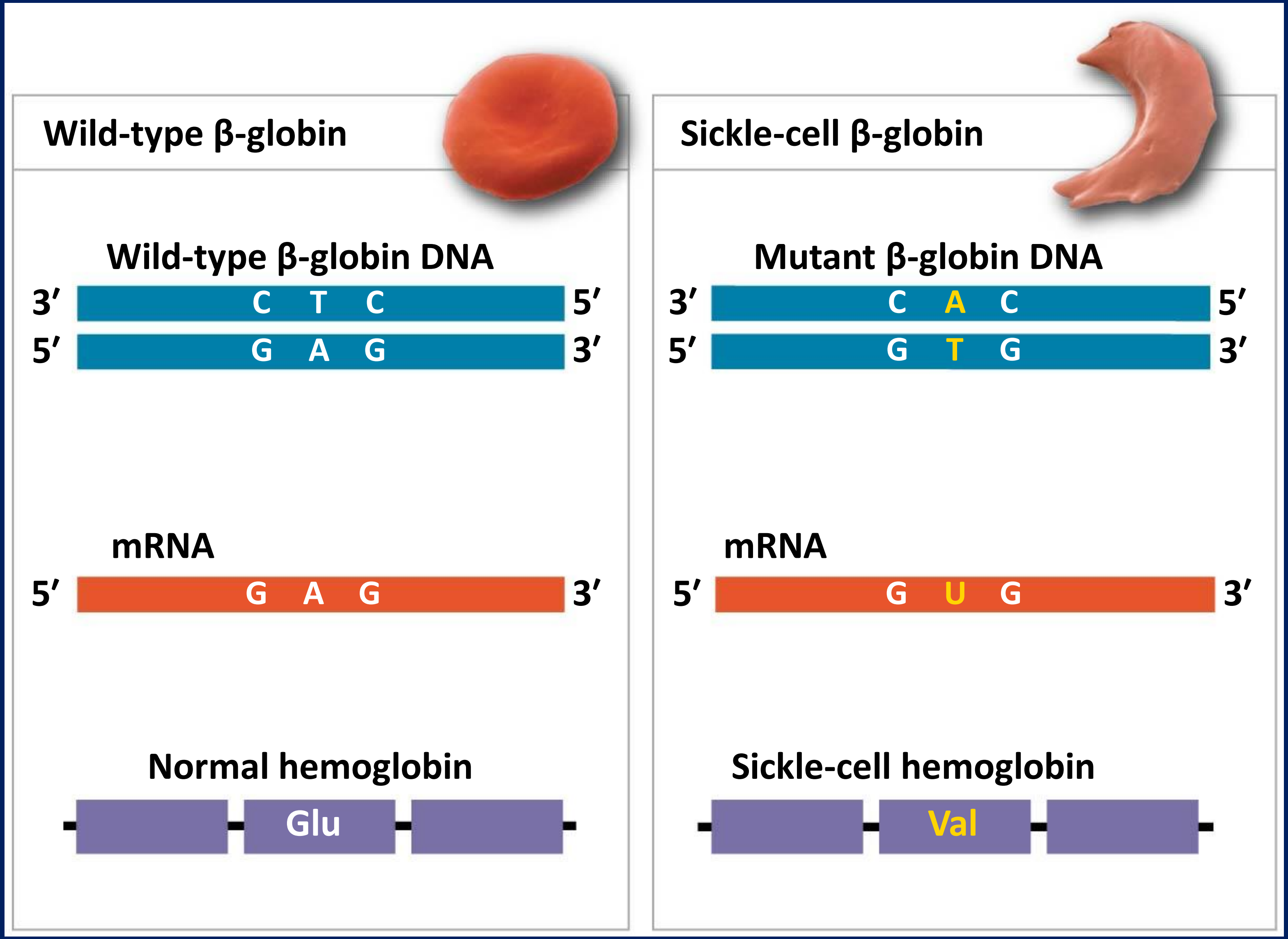
Codon	Amino acid
UUU	Phenyl alanine
UUC	Phenyl alanine
UUA	Leucine
UUG	Leucine
GUU	Valine
GUC	Valine
GUA	Valine
GUG	Valine

Mutating UUU to GUU

Valine in place of phenyl alanine
Mutation is “mis-sense”

Sickle cell anaemia

Figure 17.25



Substitution mutation. 3. Nonsense mutation

Refer to figure 17.25

Codon	Amino acid
UUU	Phenyl alanine
UUC	Phenyl alanine
UUA	Leucine
UUG	Leucine
GUU	Valine
GUC	Valine
GUA	Valine
GUG	Valine

Mutating UUA to UAA

UAA is a STOP codon

Protein synthesis stops
Mutation is termed “non-sense”

Insertion or deletion: frameshift mutation

Refer to figure 17.25

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′

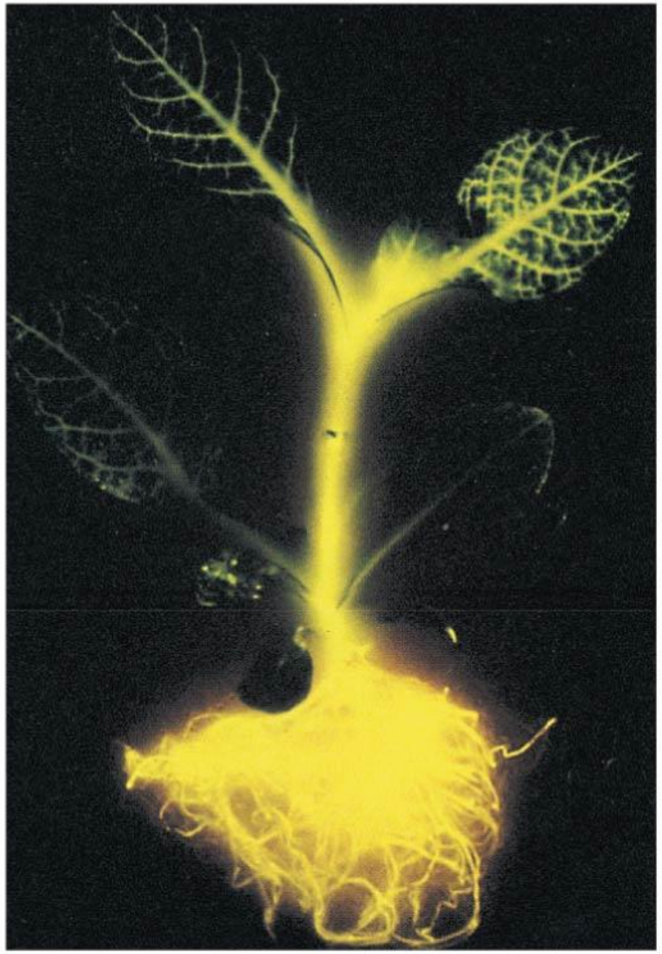
mRNA
5′-A U G A A G U U U G G C U A A-3′

Template strand
3′-T A C G T T C A A A C C G A T T-5′
5′-A T G C 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′
5′-A U G C A A G U U U G G C U A A-3′

Universality of the genetic code

Figure 17.6



(a) Tobacco plant expressing a firefly gene

(b) Pig expressing a jellyfish gene