

LSM1301 GENERAL BIOLOGY

L1: Looking at the macro- and micro- aspects of life

A/P Henry Mok Yu Keung

S3 level 3

dbsmokh@nus.edu.sg

Course introduction

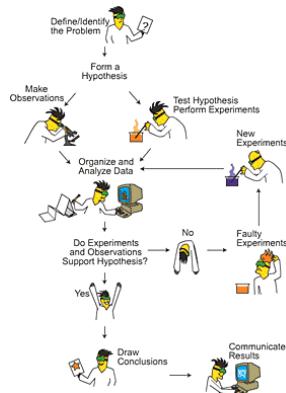
1. Course Management

- Contents
- Schedule
- Learning objectives
- Grading and policies
- Rules & Regulations
- Blended Learning



2. Overview of Science of Biology

- What is life?
- What is the scientific method?
- How we study life?



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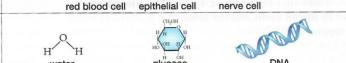
Course contents

Organismal biology

➤ Dr. Zeehan Jaafar

Cell and molecular biology

➤ A/P Henry Mok Yu Keung

Biosphere	All life on Earth and the nonliving portions of Earth that support life	
Ecosystem	A community together with its nonliving surroundings	
Community	Populations of different species that live in the same area and interact with one another	
Species	All organisms that are similar enough to interbreed	
Population	All the members of a species living in the same area	
Multicellular organism	An individual living thing composed of many cells	
Organ system	Two or more organs working together in the execution of a specific bodily function	
Organ	A structure usually composed of several tissue types that form a functional unit	
Tissue	A group of similar cells that perform a specific function	
Cell	The smallest unit of life	
Molecule	A combination of atoms	
Atom	The smallest particle of an element that retains the properties of that element	

Course schedule

Cell and molecular biology

➤ Week 1-6
(Henry Mok)

Organismal biology

➤ Week 7-12
(Zeehan Jaafar)

TIMETABLE FOR SEMESTER I, 2024/25

LSM1301 General Biology

Course Coordinator: Dr Zeehan Jaafar

Lecturers: A/P Henry Mok & Dr Zeehan Jaafar

Email: dbsmokh@nus.edu.sg and jaafarz@nus.edu.sg

Tel: 6516 2858

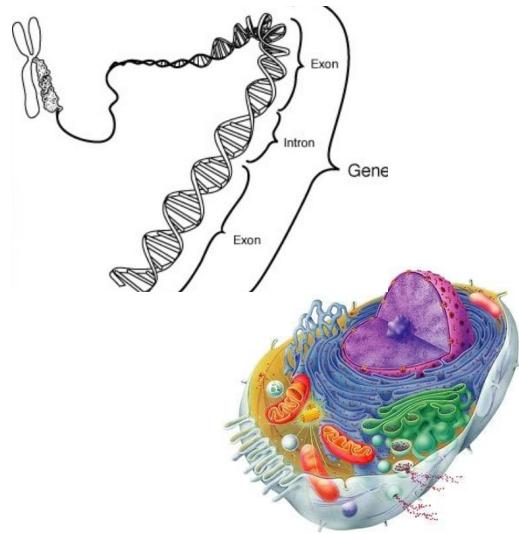
Wk	Month	Lecture		Practical, @S1A-04-Lab3
		Mon, 8-10am LT28	Wed, 10-12nn (B1), 12-2pm (B2), 2-4pm (B3), 4-6pm (B4)	
0.	Aug	Orientation: Mon, 5 Aug – Sat, 10 Aug 2024 (1 week)		
1.	Aug	12 Introduction [HM]	14	
2.		19 Chemistry of Life [HM]	21	
3.		26 Cell Structure and Function [HM]	28 Laboratory 1: Living cells [HM]	
4.	Sep	02 Energy of Life [HM]	04 **DNA and Heredity [HM]	
5.		09 Gene Expression [HM]	11 Laboratory 2: Molecular Biology [HM]	
6.		16 Biotechnology [HM]	18 **Tutorial 1 [HM]	
Recess Week: Sat, 21 Sep – Sun, 29 Sep 2024 (1 week)				
7.	Sep/Oct	30 Continual Assessment 1	02	
8.	Oct	07 Evolution [ZJ]	09 Tutorial 2 [ZJ]	
9.		14 Biodiversity [ZJ]	16 Field Trip: The Diversity of Life [ZJ]	
10.		21 Plant Form & Function [ZJ]	23 Laboratory 3: Flowers & Fruits [ZJ]	
11.		28 Animal Form & Function [ZJ]	30 Laboratory 4: Arthropod Body Plan [ZJ]	
12.	Nov	04 Ecology [ZJ]	06 Tutorial 3 [ZJ]	
13.		11 Continual Assessment 2	13	
Reading Week: Sat, 16 Nov – Fri, 22 Nov 2024 (1 week)				
No Final Examination (100% CA)				
Vacation: Sun, 08 Dec 2024 -Sun, 12 Jan 2025 (5 weeks)				

**Online by zoom.

Learning objectives

Cell and molecular biology

- What are the chemical building blocks of life?
- What is the structure and function of cells?
- How is genetic information transmitted and expressed in living organism?
- How does biotechnology impact our lives?



Learning objectives

Organismal biology

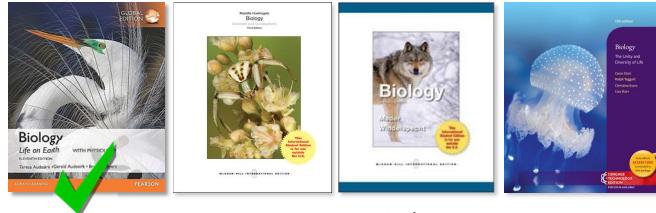
- What are biological species?
- How do populations and species evolve?
- How are different organisms classified?
- How do species interact with the environment and each other?



Course material

- Lecture Notes
- Multimedia (animations, videos on Canvas)
- Online-Lectures/Videos (from websites)
- Assignments
- Quizzes (Examplify, Canvas)

Stay informed of the latest changes
from **Canvas** announcement,
updated course details and FAQ.



Recommended Text Books/References

Grading and policies

100% CA

- Four Lab assignments 36%
- Two tutorial (2 & 3) 8%
- Museum visit 6%
- Two E-exams (20% + 20%)/in-class quiz (5% + 5%) 50%

- ✓ Open book assessments
- ✓ No rote memory
- ✓ Non-graded/graded post-class quizzes

To Note:

- A. Previous exam questions are not released.
However, we will provide non-graded/graded questions on Canvas for practice.
- B. No marks are deducted for incorrect answers (no penalty marking) in the exams.
- C. Students are not allowed to use the internet during the e-exams.
 - E-exam 1 will be held on **30th Sep 2024** during the 8am to 10am lecture slot. E-exam 2 will be held on **13th Nov 2024** during the lecture slot.
 - All E-exam are **in-person** at the Lecture Theater.

Lab assignments

F2F practical sessions.

Read through **FAQ section**
in Canvas carefully

- **Cell and molecular biology**

Lab 1	Living cells (microbes, human and plant cells)	(10%)
Lab 2	DNA extraction, PCR and agarose gel electrophoresis	(10%)

- **(Organismal biology)**

Lab 3	Flowers and Fruits	(8%)
Lab 4	The Arthropod Body Plan	(8%)

Two Points For A Meaningful Learning Experience – NUS Honour Code*

1. Honesty in academic communication

In academia, we pursue truth and knowledge founded on trust that the work is one's own and is accurate, reproducible, and truthful. This trust and the reputation of the individual and NUS are destroyed by dishonest behaviour.



- **Fabrication** – making up data or information (lying)
synthesizing or creating data for unperformed experiments,
constructing graphs and figures unsupported by data or information
- **Falsification** – deliberately manipulating or altering data or
academic/professional credentials (cheating), doctoring photos and graphs,
altering data in tables, using data from one experiment for a different experiment

*<http://www.nus.edu.sg/registrar/adminpolicy/acceptance.html#NUSCodeofStudentConduct>

Two Points For A Meaningful Learning Experience – NUS Honour Code

1. Honesty in academic communication

- **Plagiarism** – submission of ideas, phrases, paragraphs or figures of **others'** as your own (stealing)
 - “**Others**” includes books, journals, internet sources and classmates – they must be acknowledged
 - reusing your own work without attribution
 - The university considers plagiarism an offence and will subject students to disciplinary action
- What is plagiarism and how to avoid it?
 - Short note
 - <http://www.cdtl.nus.edu.sg/success/sl7.htm>
 - E-tutorials on plagiarism
 - <http://emodule.nus.edu.sg/ac>
 - <https://connect.le.ac.uk/p72155629/>



Applies to ALL LSM1301
CA components:
Lab assignments,
museum visit, online
tutorial, e-exams



Department of Biological Sciences
Faculty of Science

E.g.: Online assignments/ practical reports

Turn-it-in

- Whole paragraphs/long sentences highlighted as **identical** to previous semester reports.
- **Word-for-word** from museum exhibit description, Wikipedia or Encyclopedia Britannica.
 - Need to cite the appropriate sources and give credit.
- Several instances of same sentence structure, including **incorrect** answer.
- **50% penalty** if observed in one answer. **Zero mark/"F" grade** if observed in multiple answers.

Excluding single word/species answers

Two Points For A Meaningful Learning Experience
– NUS Honour Code

2. Respecting the rights of others

Not infringing the learning process of fellow students

- **Distractions**, e.g. talking, mobile phone browsing, arriving late
- Uncooperative or failure to listen to others during **group lab work**
- Improper **attire** for field/lab work – resulting in others having to work alone
- Allowing one's work to be **copied** – depriving others opportunity to learn

All assessment should be completed individually.

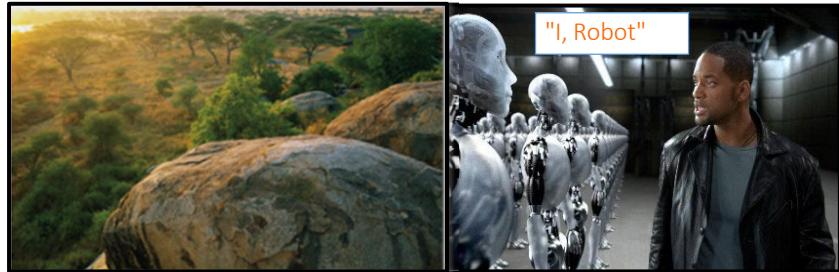
Applies to ALL LSM1301 CA components: Lab assignments, museum visit, online tutorial and e-exams

Blended-learning

Mixed-mode of instruction

1. Exposure to course content prior to class
 - Pre-class videos help students prepare for pertinent topics.
2. Synchronous content delivery via F2F lecture/Zoom (with recording) & F2F practical sessions.
 - In-lecture activities help student access comprehension via PollEverywhere quizzes and real-time Q&As (PollEv.com/mok)
3. Asynchronous non-graded/graded assessment and interaction
 - Post-lecture quizzes (non-graded/graded) and forum discussions help student apply and test their understanding of content.

What is life?



How do we know what is alive or not alive?

Life is intangible and defies simple definition.

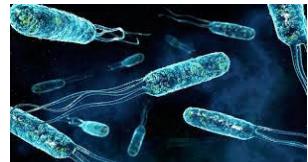
Living things are extraordinary and our quest to define life is one of the most fundamental questions in biology.

The cell is the basic unit of life

Every organism, or living individual, consists of **one or multiple** cells.

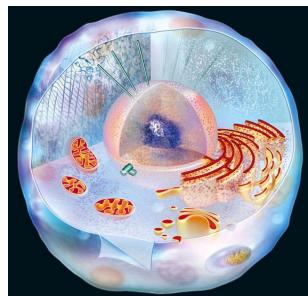
Basic structure of a cell

- Is surrounded by plasma membrane (lipid bilayer)
- Eukaryotic cell has elaborate internal membrane-bound structure (organelles)
- Contains hereditary material DNA



prokaryotic

unicellular



eukaryotic

unicellular
or
multi-cellular

Two cell types

- Prokaryotic ("before nucleus" in Greek)
- Eukaryotic ("true nucleus" in Greek)

Prokaryotic – nucleus absent

Eukaryotic – nucleus present

The Characteristics of Life



All living things share certain characteristics that, taken together, define life:

All living organisms are comprised of cells.

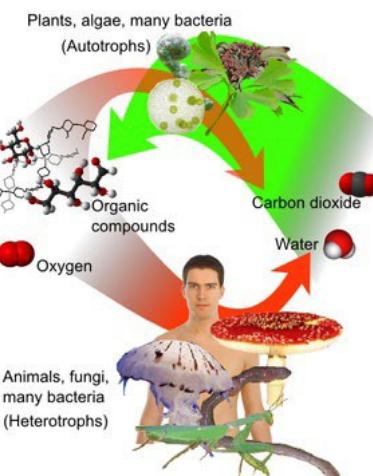
1. Organisms acquire and use materials and energy.
2. Organisms actively maintain organized complexity.
3. Organisms sense and respond to stimuli.
4. Organisms grow and develop
5. Organisms reproduce.
6. Organisms, collectively, evolve.

Nonliving objects may possess some of these attributes, but only living things can do them all.

1. Acquire and use materials and energy

Autotrophs

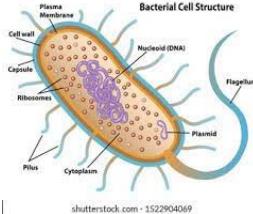
- autos = self; trophe = nutrition
- Self-feeders, producers
- Plants capture light energy to make food
→ photosynthesis



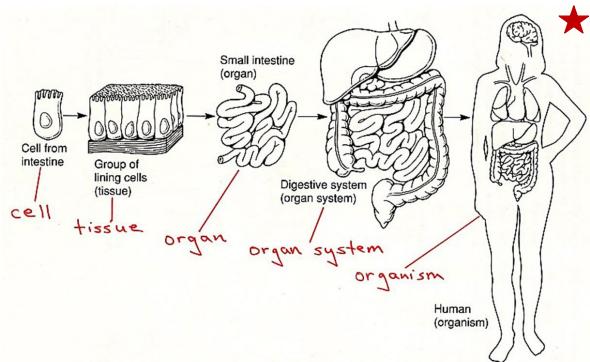
Heterotrophs

- heterone = (an)other
- Other-feeders, consumers
- Most other organisms acquire energy found in molecules of other organisms

2. Actively maintain organised Complexity



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- Different levels of organization
- The complexity and ordered interactions of parts give rise to new properties (emergent properties)
- Even a single cell need to use energy to actively keep its complexity

3. Sense and respond to stimuli

Organisms sense changes in their environments and make responses to changes

- Changes in internal environment
Temperature, water level, blood sugar level, etc.
- Changes in external environment
Food and water, bitterness, light, sound, etc.

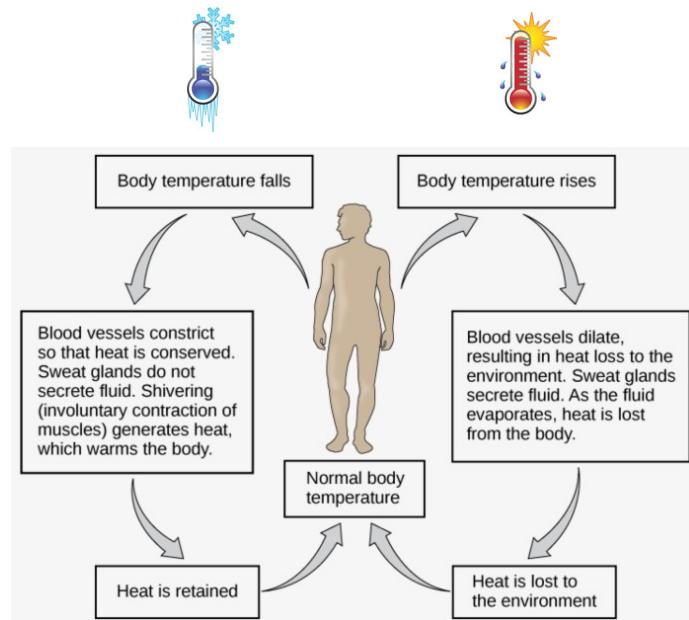
3. Sense and respond to stimuli

- To maintain relatively constant internal conditions
 - **Homeostasis** ("to stay the same" in Greek)
 - Animals regulate temperature, thirst, hunger, sperm production
 - Plants regulate direction of growth (towards light source)
- To grow and **adapt to changes** while maintaining homeostasis

Homeostasis

(e.g. maintain body temperature)

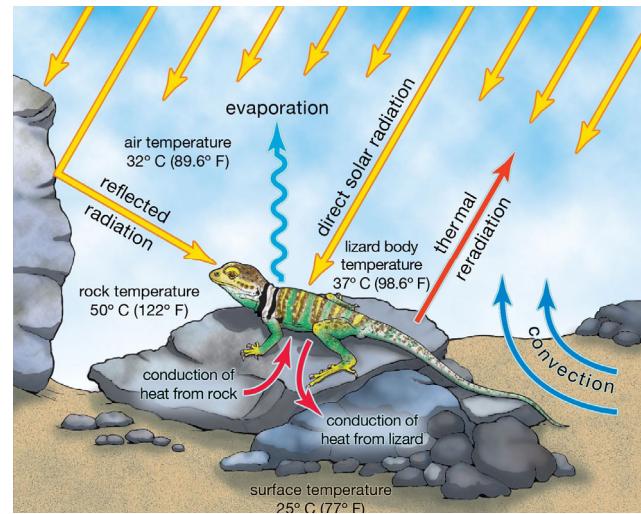
Endothermic organisms (aka 'warm-blooded') maintain body at a metabolically favorable temperature by the use of heat set free by its **internal bodily functions** and not the external environment



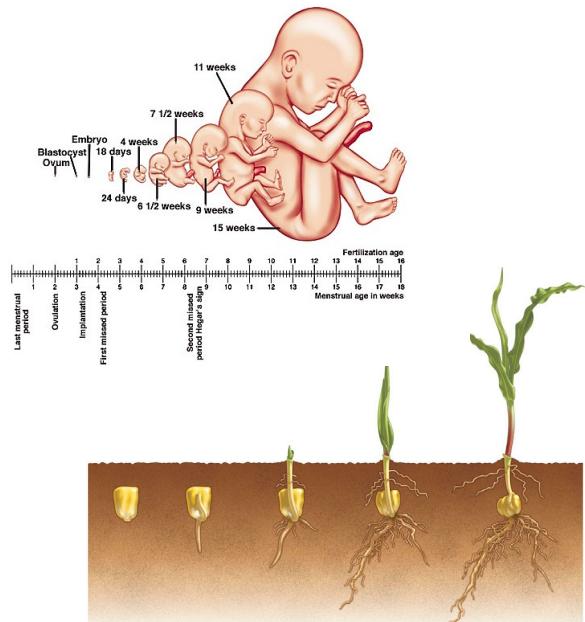
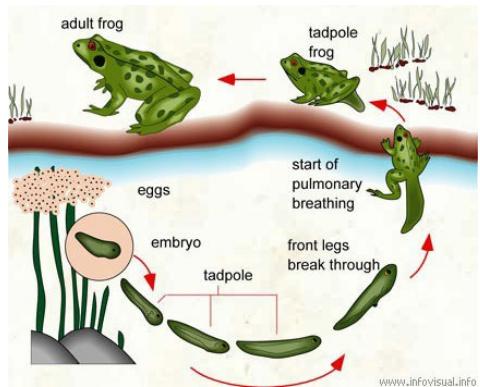
Homeostasis

(e.g. maintain body temperature)

Ectothermic organisms (aka 'cold-blooded') are dependent on external environment for regulation of body temperature such as direct sunlight or heated surfaces



4. Grow and Develop



4. Grow and Develop

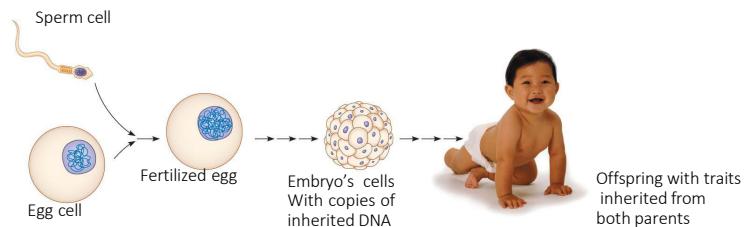
Growth

- Plants and animals grow by producing **more cells** to increase mass and size
- Bacteria grow by **enlarging cells** and **dividing** to make more individuals
- Involves conversion of acquired materials to molecules of organism's body
- Based on **genetic** information, well programmed and coordinated

Development

- The progressive changes in size, shape, and function (**differentiation**) during the life of an organism

5. Reproduce



Sexual reproduction

two genetic contributions
to the formation of a new
individual

Asexual reproduction

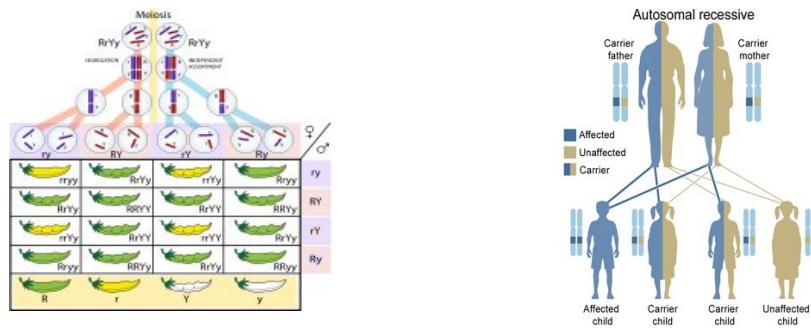
A mode of reproduction by
which offspring arise from a
single parent



5. Reproduce

To maintain population

- Transmission of parents' genetic material to offspring – **continuity of life**
- Offspring may be genetically different from parents – **diversity of life**

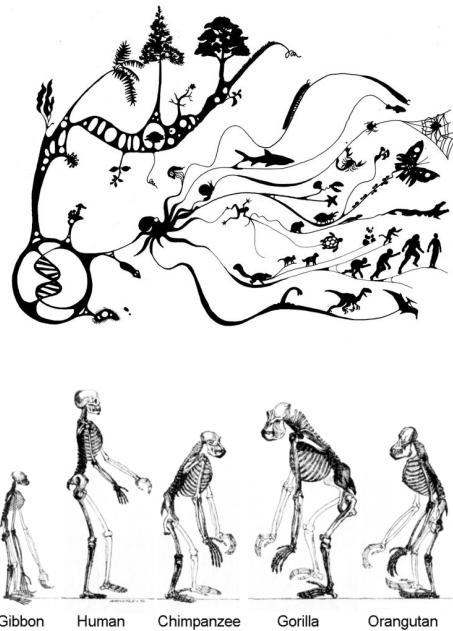


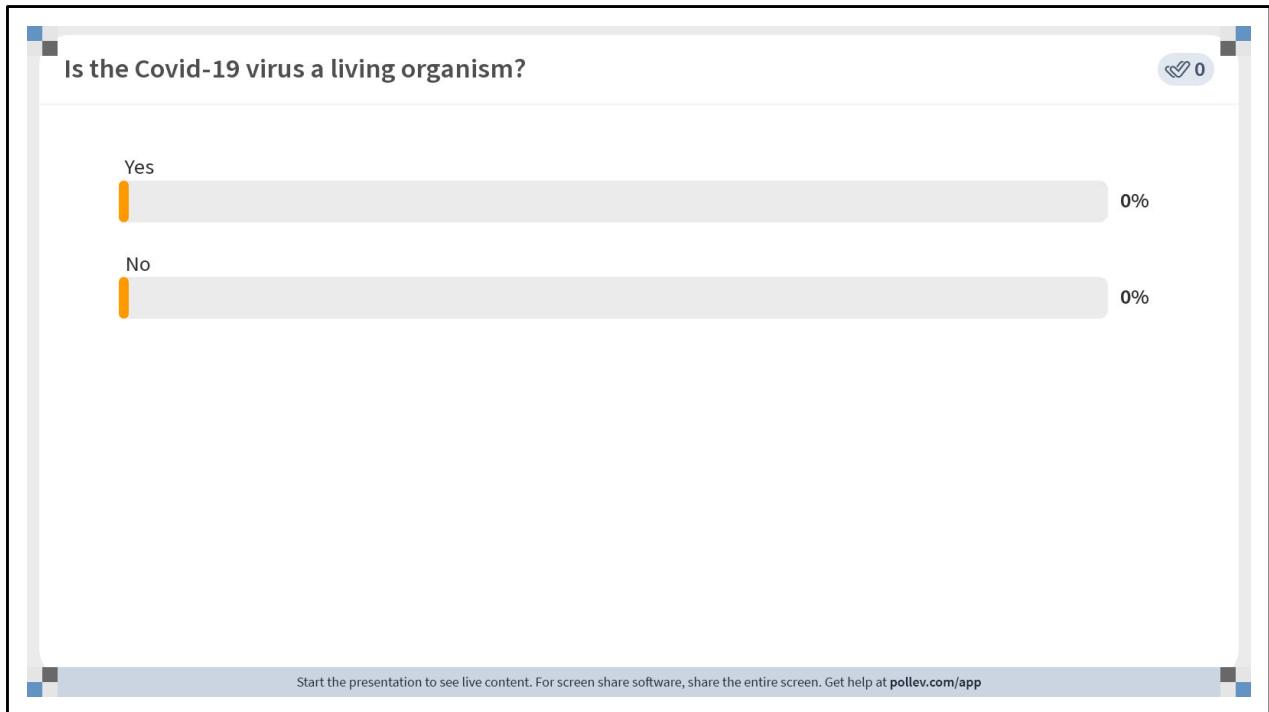
6. Evolve

Evolution is the process by which modern organisms descended, with modifications, from common ancestors

Organisms that best meet environmental challenges leave the most offspring

- **Adaptations** are structures, physiological process, or behaviors that aid in survival and reproduction in a certain environment (**survival of the fittest**).
- Species that cannot adapt to environmental change go extinct e.g. dinosaurs (**natural selection**).



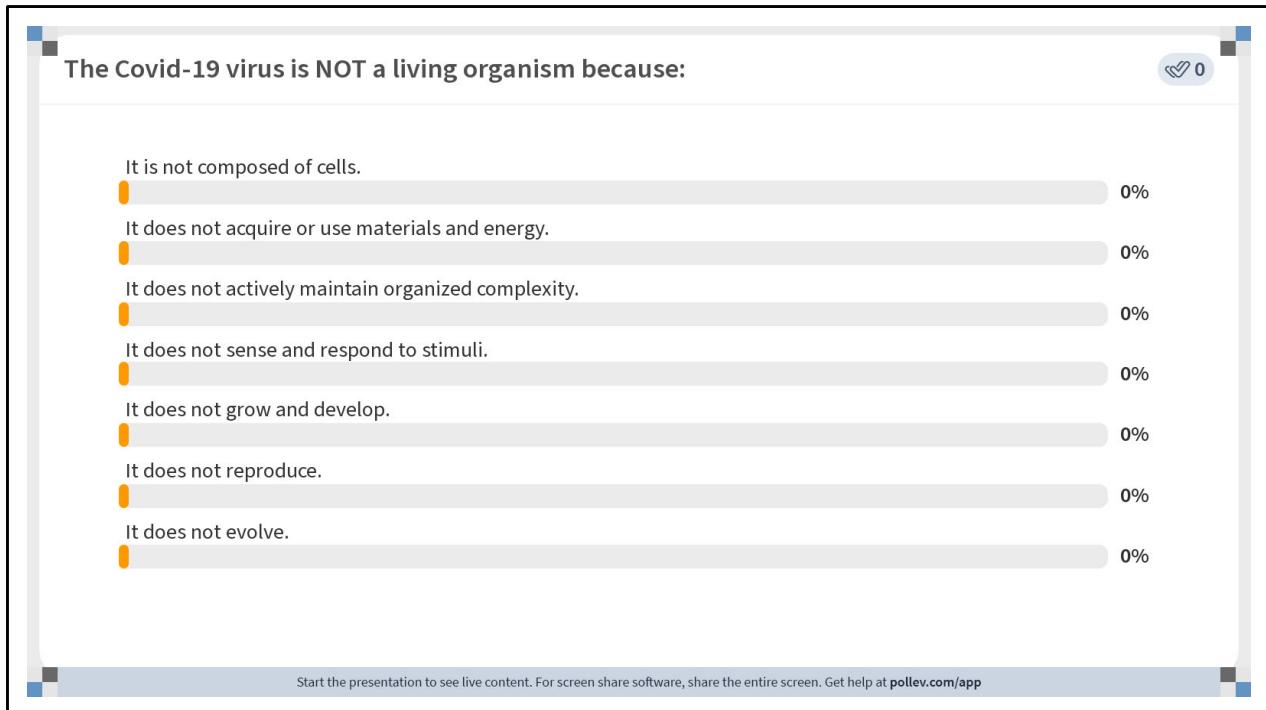


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More info at polleverywhere.com/support

Is the Covid-19 virus a living organism?

https://www.polleverywhere.com/multiple_choice_polls/fkoKv1jgSpbddrgU0ShkW?state=opened&flow=Default&onscreen=persist



a,b,c,d,e

Reproduces thru host

Evolves – different variants available to infect diff hosts

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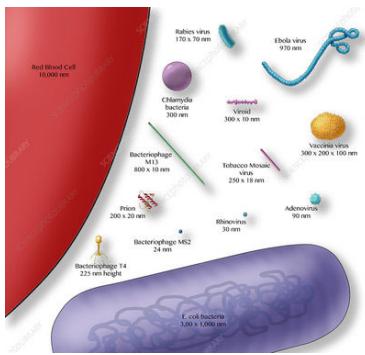
Everywhere activity.

More info at polleverywhere.com/support

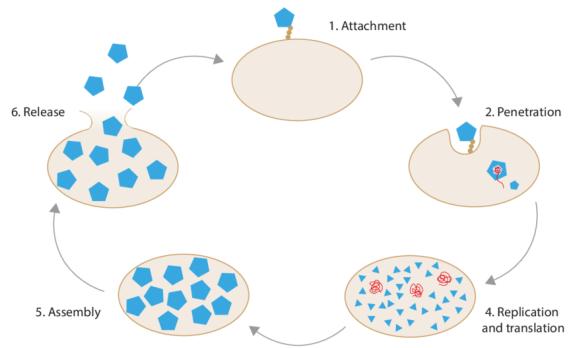
The Covid-19 virus is NOT a living organism because:

https://www.polleverywhere.com/multiple_choice_polls/MTgUobvX9jbQVp2hh6fkZ?state=opened&flow=Default&onscreen=persist

Viruses are not composed of cells and yet possess some characteristics of living things.



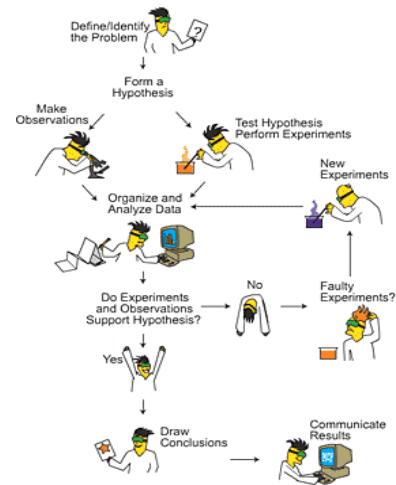
Virus is much smaller than a cell and it does not grow.



Virus **respond** when entering host cell and it use host materials to **reproduce**.

What is the Scientific Method?

- The word *Science* means “to know”
- Inquiry is the search for information and explanation
- There are two main types of scientific inquiry:
 - discovery/descriptive science
 - hypothesis-based science
- Most scientific inquiries combine these two approaches.



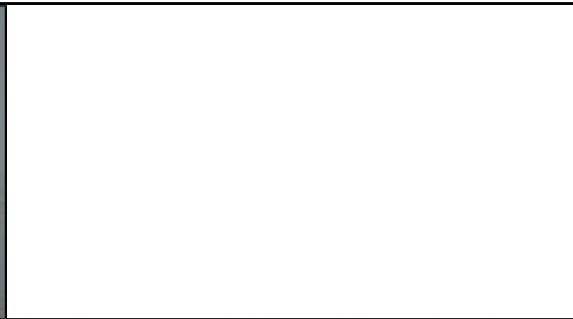
Semmelweis (1856)

Observations

- Childbirth deaths 5x's higher from doctors than nurses due to childbed fever!
- Doctors often did autopsies before attending a birth.

Hypothesis → Doctors transmit 'cadaveric matter' to their patients

Prediction → If doctors washed their hands, it would eliminate cadaveric matter and reduce fever deaths



The Scientific Method



Experimental design

Nothing changes ← **Control:** Doctors not washing hands.

What you measure for results ← **Dependent variable:** # of patients getting childbed fever.

What you change ← **Independent variable:** Doctors washing off 'cadaveric matter'

Correlation ≠ Causation

Rising temps. ≠ more pirates

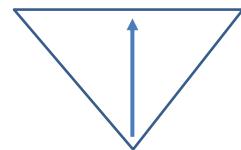
1918 flu
↳ Vaccine



Two types of reasoning

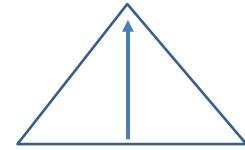
(A) **Inductive reasoning:** The process of creating a broad generalization based on many observations that support it and none that contradict it.

- The “Cell Theory”, many organisms that possess all the characteristics of life are composed of one or more cells and that nothing that is not composed of cells share all of these attributes. All living organisms are composed of cells.
- Every cat that you've observed purrs. Therefore, all cats must purr.



(B) Deductive reasoning: Start with a well-supported generalization and use it to generate hypotheses about a specific experiment or observation that will turn out.

- Based on the “Cell Theory”, if a scientist describe a new entity that exhibit all characteristics of life, he can confidently hypothesized that it will be composed of cells.
- All birds have feathers. All robins are birds. Therefore, robins have feathers.

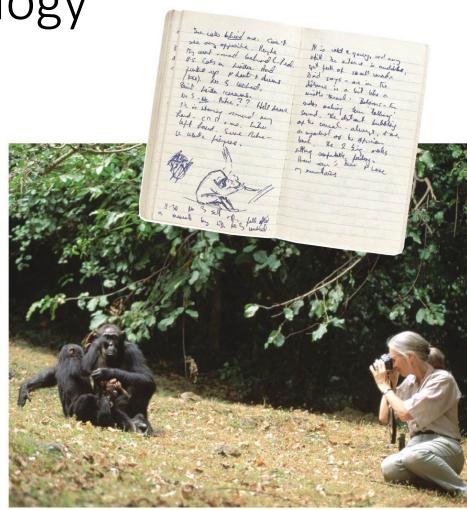




How do we apply the scientific method to study life?

Discovery science in biology

- Describes natural structures and processes
 - This approach is based on **observation** and the analysis of data
 - Lead to conclusions based on **inductive reasoning**/induction



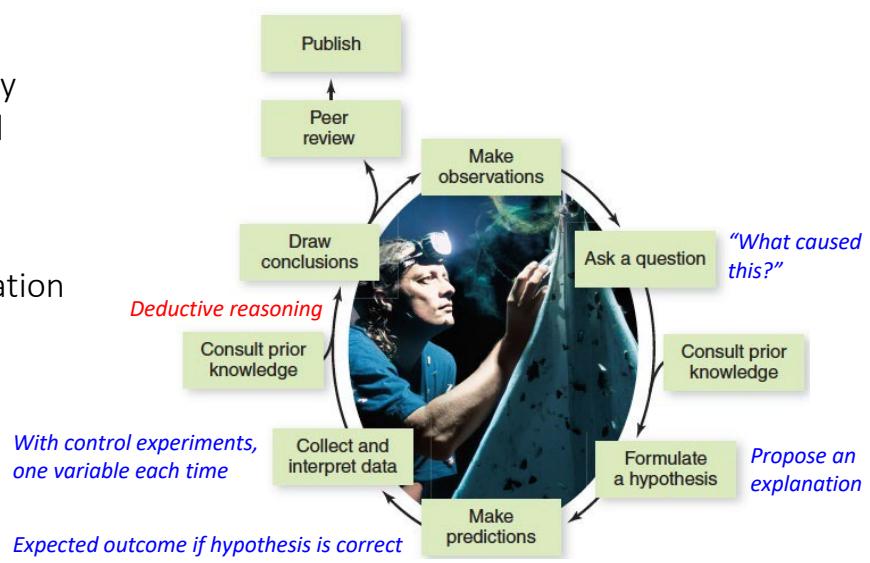
Hypothesis-based science in biology

- Is mostly about explaining nature
- Seek natural causes and explanations for observations, ask questions and propose hypotheses
- A hypothesis is a **tentative** answer to a well-framed question (educated guess)
- A scientific hypothesis leads to predictions that can be tested by experiments
- Lead to conclusions based on deductive reasoning

Hypothesis-based science in biology

In general,
all scientific inquiry
follows a standard
process.

Hypothesis Formation and Testing



Hypothesis and predictions

- Focus on a **single** answerable question — reductionism
- **Tentative** explanation for observed phenomenon (questions)
- Leads to **testable** predictions
- “If/then” predictions that can be **tested** by experiments
- If hypothesis incorrect, experiments would disprove hypothesis: **falsifiable**

Falsifiability is the **capacity** for some proposition, statement, theory or hypothesis **to be proven wrong**. All scientific theories are falsifiable; if evidence that contradicts a theory comes to light, the theory itself is either modified or discarded.

Examples of untestable statements

1. "If you could go from 0 to the speed of light in a millisecond you'd go back in time" since it's virtually impossible for us to do that.
2. "If the earth had no moon all life on earth would die." Since there is no way of removing the moon from earth orbit there is no way to test the hypotheses.

Experiment

Test and challenge the hypothesis under known and controlled conditions

Test the results of the **experimental** group against the **control** group

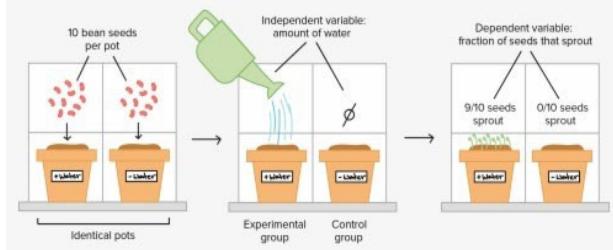
Experimental group

- Group with a **single** variable characteristic to be tested (what you change, i.e. Independent variable)

Control group (Nothing changed)

- Group identical to the experimental group, except for the variable

Hypothesis: Bean seeds require water to sprout



Sampling error should be minimized

- Replicates (usually 3X)
- Random sample (no selection of seed)
- Sample size (more than one seed)

Based on the bean seeds sprouting experiment, the scientist hypothesized that sunlight may also be required for the seeds to sprout. What should be the setup for "control" and "experimental" groups?

0

Experimental: no water, sunlight; Control: water, darkness 0%

Experimental: water, sunlight; Control: water, darkness 0%

Experimental: water, sunlight; Control: no water, darkness 0%

Experimental: no water, sunlight; Control: no water, darkness 0%

Experimental: no water, darkness; Control: water, sunlight 0%

Start the presentation to see live content. For screen share software, share the entire screen. Get help at pollev.com/app

Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

More info at polleverywhere.com/support

Based on the bean seeds sprouting experiment, the scientist hypothesized that sunlight may also be required for the seeds to sprout. What should be the setup for "control" and "experimental" groups?

https://www.pollev.com/multiple_choice_polls/NiWChDqtQ498Gxivq8ByL?state=opened&flow=Default&onscreen=persist

Conclusion

Evaluation of hypothesis in light of **experimental results** that must be **reproducible**, not just by yourself but also by others.

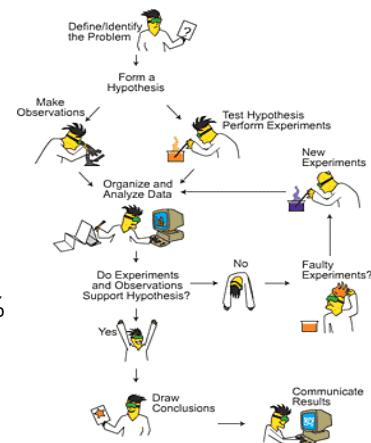
May disprove a hypothesis

- Require redesign of experiments

Use of statistical analyses to provide support for hypothesis

Must remain tentative

- Hypothesis may be supported but cannot be proven 100% correct
- Can never be sure all untested variables are controlled



Limits of the scientific method

Scientific approach cannot provide answers to subjective questions

- Such as social decisions, politics, etc.

Cannot provide moral, aesthetic, or philosophical standards

May result in conflict with other forms of knowledge

Summary

Biology is the scientific study of life.

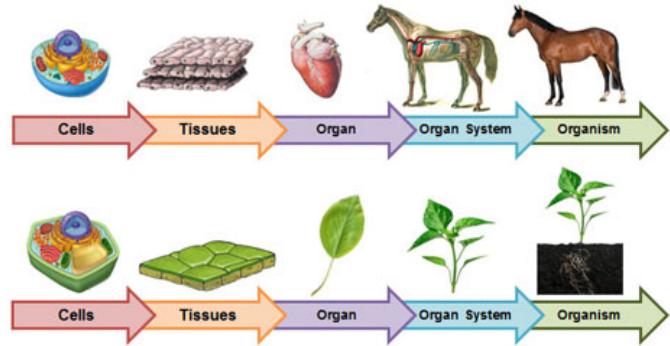
- We can use certain characteristics to classify living things.
- Scientist can use two main forms of inquiry in their study of life.
 - Discovery science
 - Hypothesis-based science

Supplementary material

Optional

- The history of our world in 18 minutes | David Christian
 - http://www.ted.com/talks/david_christian_big_history
 - <https://www.youtube.com/watch?v=yqc9zX04DXs> (watch and read the comments)
- Hidden miracles of the natural world
 - http://www.ted.com/talks/louie_schwartzberg_hidden_miracles_of_the_natural_world
- The Great Debate - What is Life?
 - <http://www.youtube.com/watch?v=xIHMnD2FDeY>

LSM1301



L2: Chemistry of Life

A/P Henry Mok Yu Keung

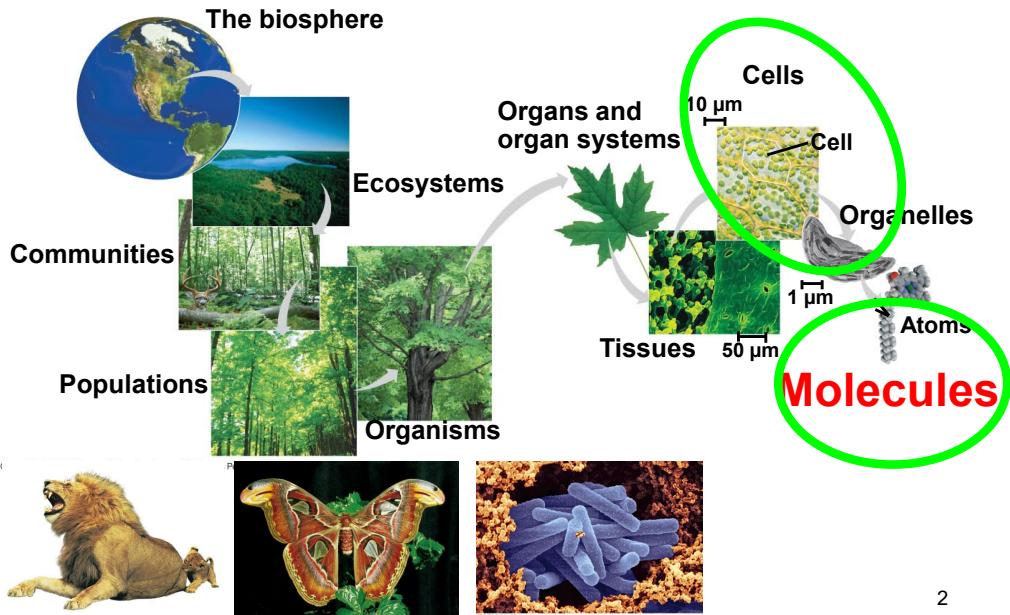
Office at S3-03-01d

dbsmokh@nus.edu.sg

Tel: 65162967

1

Chemistry of Life



Given the rich complexity of life on earth, we might expect organisms to have enormous diversity of molecules..

As homo sapiens, no matter what you are, we have all these molecules in our body.

Visualizing molecules

<http://www.youtube.com/watch?v=nmlBtsAhoKo>

Outline

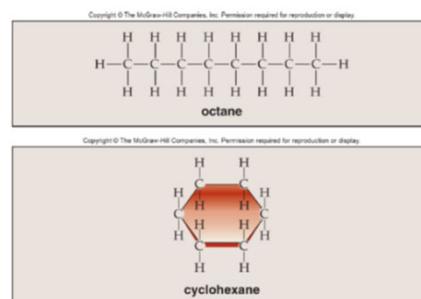
- Organic Molecules
 - Functional groups
 - Synthesis and breakdown
- Carbohydrates
 - Monosaccharides
 - Oligosaccharides
 - Polysaccharides
- Proteins
 - Amino acids
 - Protein structure
- Lipids
 - Triglycerides
 - Phospholipids
 - Waxes
 - Steroids

3

First we start with organic molecules

Organic Molecules

- Contain both carbon and hydrogen atoms
 - Linked by covalent bonds
 - May also be bonded to other atoms
- Each carbon atom
 - Able to bond with up to four other atoms
 - By single, double or triple bonds
- Carbon atoms
 - Able to form chains or rings



4

All living things are made up of organic molecules and it just means that these molecules contain both carbon and hydrogen atoms

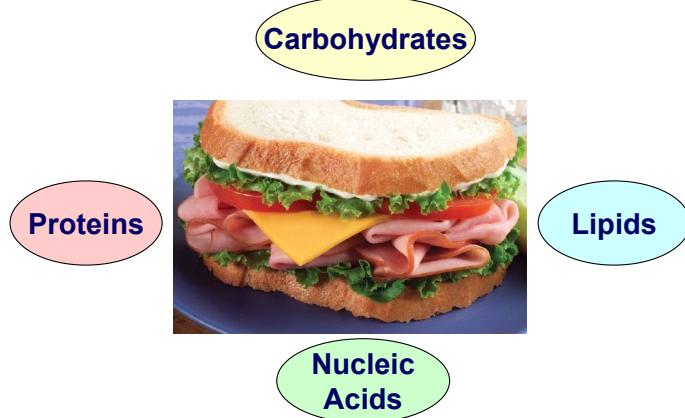
Functional groups

- Clusters of atoms linked to carbon skeleton
- Confer specific chemical properties to molecules
 - Reactivity
 - Polarity

TABLE 3-1 Important Functional Groups in Biological Molecules			
Group	Structure	Properties	Found In
Hydroxyl		Polar; involved in dehydration and hydrolysis reactions; forms hydrogen bonds	Sugars, polysaccharides, nucleic acids, alcohols, some amino acids, steroids
Carbonyl		Polar; makes parts of molecules hydrophilic (water soluble)	Sugars (linear form), steroid hormones, peptides and proteins, some vitamins
Carboxyl (ionized form)		Polar and acidic; the negatively charged oxygen may bond H ⁺ , forming carboxylic acid (—COOH); involved in peptide bonds	Amino acids, fatty acids, carboxylic acids (such as acetic and citric acids)
Amino		Polar and basic; may become ionized by binding a third H ⁺ ; involved in peptide bonds	Amino acids, nucleic acids, some hormones
Sulphydryl		Nonpolar; forms disulfide bonds in proteins	Cysteine (an amino acid), many proteins
Phosphate (ionized form)		Polar and acidic; links nucleotides in nucleic acids; forms high-energy bonds in ATP (ionized form occurs in cells)	Phospholipids, nucleotides, nucleic acids
Methyl		Nonpolar; may be attached to nucleotides in DNA (methylation), changing gene expression	Steroids, methylated nucleotides in DNA

What differentiates these organic molecules from each other are the various functional groups which confer different chemical properties depending on what they are.

The Four Types of Organic Molecules



1. A molecule that is normally found in or produced by living systems.
2. A molecule that typically consists of carbon atoms in rings or long chains, where other atoms (e.g. hydrogen, oxygen, and nitrogen) are attached.

6

Remarkably, however, the critically important large molecules of all living things-from bacteria to animals and plants **fall into just four main classes**

They are carbohydrates, lipids, proteins, and nucleic acids. As humans, or plants or tiny bacteria, all are composed of these four types of biological molecules/biomolecules/or macromolecules, because they are huge on the molecular scale.

Because these complex molecules are produced only by living organisms, they are referred to as biological molecules.

On the molecular scale, they are huge and are thus called macromolecules. For example, a protein may consist of thousands of atoms that form a molecular colossus with a mass well over 100 k daltons. In this lecture, we'll first consider how these biological molecules are built. Then we'll examine their structure and function.

The nucleic acids are not usually utilized by the body as nutrients, and so will not be considered in this exercise, although nucleic acids in the form of DNA and RNA are very important in controlling cellular function and heredity.

Life is made of same stuff

Sandwich,

Macromolecules are polymers, built from monomers

- A **polymer** is a long molecule consisting of many similar building blocks
- These small building-block molecules are called **monomers**
- **Three of the four** classes of life's organic molecules are polymers:
 - Carbohydrates
 - Proteins
 - Nucleic acids



7

Before we go to discuss each type of molecules, let's look at how they are produced in the living organisms.

Three of the four classes of bio-molecules, that is carbohydrates, proteins and nucleic acids, are chain-like molecules called polymers. They are built up from many **similar or identical blocks** linked by **covalent bonds**, much as a train consists of a chain of cars. The **repeating units or module** that serve as the building blocks of a polymer are smaller molecules called monomers.

Synthesis of Polymers

- Monomers are joined together through **dehydration synthesis**
 - An H and an OH are removed, resulting in the loss of a water molecule (H_2O)

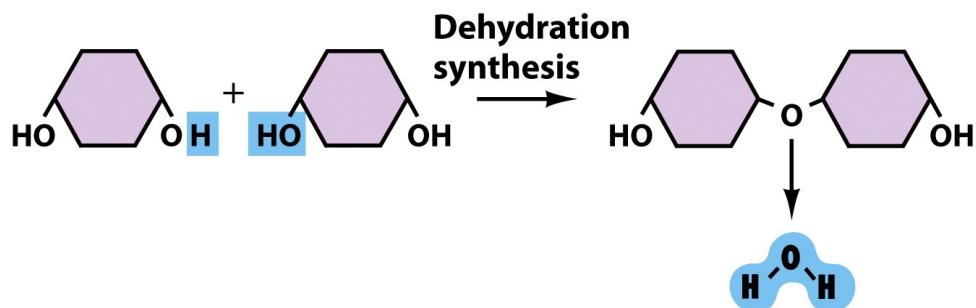


Figure 3-1 Biology: Life on Earth, 8/e
© 2008 Pearson Prentice Hall, Inc.

8

The monomers that make up large bio-molecules almost always join together by means of a chemical reaction called dehydration synthesis or condensation. Literally, they form by removing water. This reaction can be repeated as monomers are added to the chain one by one, making a polymer.

two molecules combine to form a single molecule with the loss of a small molecule; in dehydration reaction, this lost molecule is water.

Hydrogen Sulfide (H_2S)

Breakdown of Polymers

- Polymers are broken apart through **hydrolysis** (“*splitting water*” in Greek)
 - Water is broken into H and OH and used to break the bond between monomers

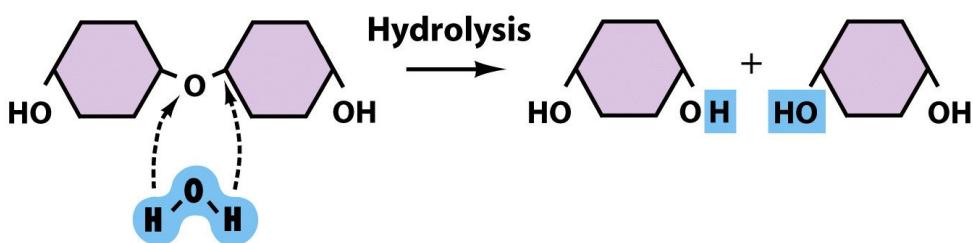


Figure 3-2 Biology: Life on Earth, 8/e
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9

The reverse reaction, hydrolysis, means to break apart with water, splits the molecule back into its original units. An example of hydrolysis working within our bodies is the process of digestion. The bulk of the organic material in our food is in the form of polymers that are much too large to enter our cells. Within the digestive tract, various enzymes attack the polymers, speeding up hydrolysis. The released monomers are then absorbed into the bloodstream for distribution to all body cells. Those cells can then use dehydration reactions to assemble the monomers into new, different polymers that can perform specific function required by the cell.

For example, if you take a bite of a bagel and chew it for a minute or so, you may notice that it gradually tastes sweeter. This is because enzymes in saliva cause hydrolysis of the starch (a polysaccharide) in the bagel into its component glucose molecules, which dissolve in your saliva and stimulate receptors on your tongue that respond to sweetness.

Outline

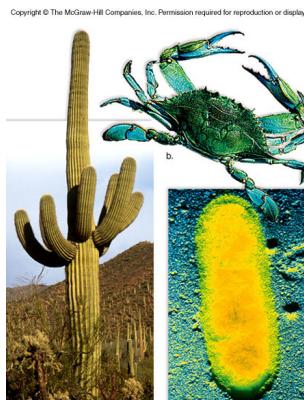
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10

Carbohydrates



Charles D. Winters/Photo Researchers.



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b.

What are they? Why do we need them?

11

With the general understanding how the bio-molecules are formed and broken apart. Let's look at the details of each major classes of large bio-molecules

The first class I am going to introduce is carbohydrates. Carbohydrates are most plentiful in nature. The food you take, the clothe you wear, plants, the shell of crab and the supporting structure of bacteria are all containing carbohydrates. Why we eat carbohydrates?

Carbohydrates

Composition

- C, H, and O in ratio of 1:2:1
- Chemical formula $(CH_2O)_n$ “Carbon + Water”
n is the number of carbons in the backbone
- Single sugar – monosaccharide
- Two sugars – disaccharide
- Several (3-10) sugars – oligosaccharide
- Many sugars – polysaccharide

Function

- Energy source and structural support

12

That's because carbohydrates serve many functions. Two main functions are used as fuel and building materials. Table sugar is an example of a carbohydrate consumed in large quantities as an energy source in the human diet. Energy-providing carbohydrates are stored in plant cells as starch and in animal cells as glycogen, both consisting of long chains of repeating carbohydrate subunits linked end to end. Chain of carbon subunits also form many structural molecules, such as cellulose, one of the primary constituents of plant cell walls.

Carbohydrate molecules are composed of carbon, hydrogen, and oxygen in the approximate ratio of 1:2:1. This formula explains the origin of the word “carbohydrate,” which literally means “carbon plus water.”

The word saccharide comes from the Greek word σάκχαρον (sákkharon), meaning "sugar". While the scientific nomenclature of carbohydrates is complex, the names of the monosaccharides and disaccharides very often end in the suffix -ose.

Blood sugar is the monosaccharide glucose, table sugar is the disaccharide sucrose, and milk sugar is the disaccharide lactose (see illustration).

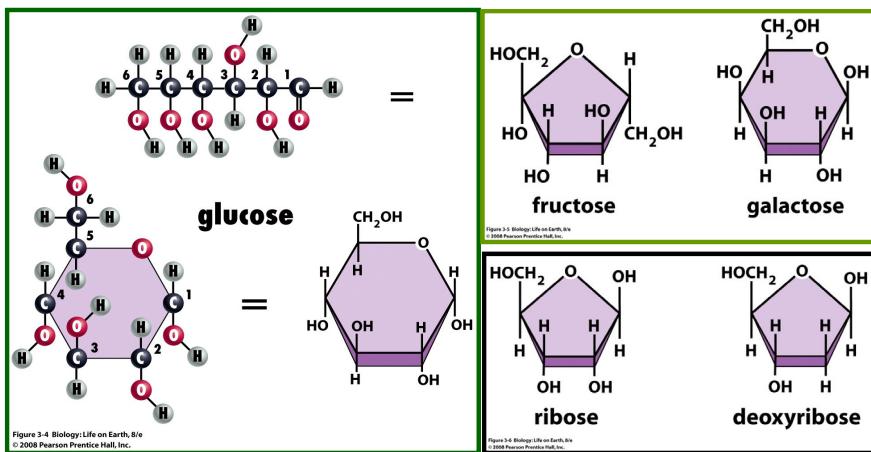
Monosaccharides

- **Single sugar molecule**
 - E.g. Glucose (blood sugar), ribose, deoxyribose
- Most have 5- or 6-carbon backbone
 - 5-carbon sugars – **pentoses**
 - 6-carbon sugars – **hexoses**
- Usually in **ring** form in cells
- Soluble in water
- Most have a distinctly sweet taste

13

Now let's look at each type of carbohydrates. Monosaccharides usually have **a backbone of three to seven carbon atoms**. They are water soluble, so they are easily transported throughout the internal environments of all organisms.

Monosaccharides



Isomers: are molecules with the same molecular formula, but different arrangements of atoms.

14

<http://faculty.lacitycollege.edu/boanta/LAB102/Organic%20Isomers.htm>

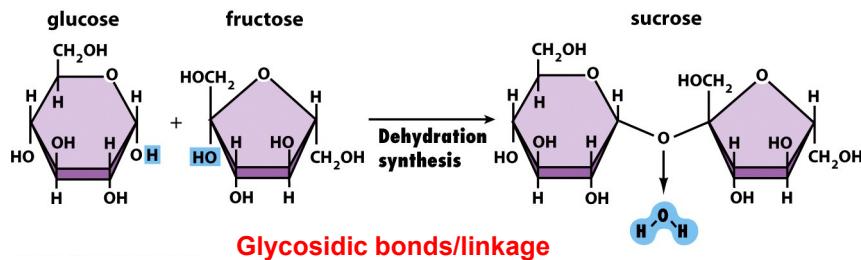
This slide shows you various structures of the common monosaccharide. **Glucose is the most common** monosaccharide in living organisms and is a subunit of many polysaccharides. Fructose in fruits and honey and galactose in milk are other two monosaccharides having the **same chemical formula** as glucose but slightly different structures. These molecules, with the same chemical formula but different molecular structures are called isomers. Other common monosaccharides, such as ribose and deoxyribose with 5 carbons are part of RNA and DNA.

The aldehydic group makes it taste sweet.

A mixture of sugars and other carbohydrates, honey is mainly fructose (about 38%) and glucose (about 32%),^[4] with remaining sugars including maltose, sucrose, and other complex carbohydrates.^[4] Its glycemic index ranges from 31 to 78, depending on the variety.^[80] The specific composition, color, aroma, and flavor of any batch of honey depend on the flowers foraged by bees that produced the honey.

<https://en.wikipedia.org/wiki/Honey>

Disaccharides



- Sucrose (table sugar) = glucose + fructose
- Lactose (milk sugar) = glucose + galactose
- Maltose (malt sugar) = glucose + glucose

15

Disaccharides typically are assembled from two monosaccharides linked together by a dehydration synthesis reaction.

Many foods we eat contain disaccharides. Perhaps you had toast and coffee with cream and sugar at breakfast. You stirred sucrose into your coffee; added milk containing lactose. Maltose is present in the germinating seeds and is a major sugar used in the brewing industry, such as beer and wine (link to E-lab 5).

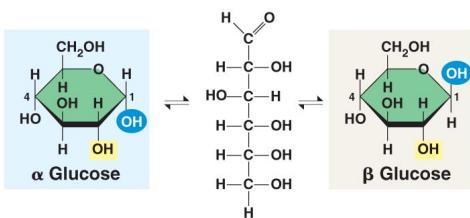
If you are on diet, you may have used

Alpha 1-2 glycosidic linkage in the sucrose.

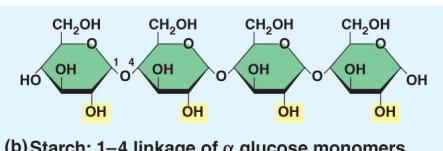
See link: <http://web.virginia.edu/Heidi/chapter7/chp7frameset.htm>

Polysaccharides

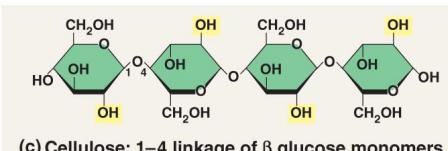
(a) α and β glucose ring structures



(b) Starch: 1-4 linkage of α glucose monomers



(c) Cellulose: 1-4 linkage of β glucose monomers



The designation ' α ' means that the hydroxyl group attached to **C-1** and the $-\text{CH}_2\text{OH}$ group at **C-5** lies on opposite sides of the ring's plane (a **trans** arrangement), while ' β ' means that they are on the same side of the plane (a **cis** arrangement).

16

<http://www.biotoptics.co.uk/JmolApplet/alphabetaoglucose2.html>

Puffed rice or corn is more sweet.

Polysaccharides, are straight or branched chains of many sugar monomers. Some are important energy-storage molecules, such as starch and glycogen, mentioned just now. Some are important structural materials, such as cellulose. Both starch and cellulose are polymers of glucose, but the glycosidic linkage in these two polymers differ. In starch, all monomers are in the same orientation. In cellulose, every other glucose is "upside down" with respect to its neighbors. For most animals, including ourselves, cellulose just passes through the digestive tract, we don't derive any nutrients from it. Then, why we are encourage to eat vegetables? **prevents constipation**

This different structure prevents animals' digestive enzymes from attacking the bonds between glucose subunits. And only a few microbes, can synthesize enzymes to break the bonds and consume cellulose as food.

Starch - a polysaccharide carbohydrate consisting of a large number of glucose monosaccharide units joined together by glycosidic bonds. Amylose is a planar polymer of glucose linked mainly by $\alpha(1 \rightarrow 4)$ bonds. It can be made of several thousand glucose units. It is one of the two components of starch, the other being amylopectin. Amylose structureThe $\alpha(1 \rightarrow 4)$ bonds promote the formation of a helix

structure.

Amylopectin is a highly branched polymer of glucose found in plants. It is one of the two components of starch, the other being amylose. It is soluble in water.

Glucose units are linked in a linear way with $\alpha(1 \rightarrow 4)$ bonds. Branching takes place with $\alpha(1 \rightarrow 6)$ bonds occurring every 24 to 30 glucose units.

2.Glycogen - Glycogen is a highly branched polymer that is better described as a dendrimer of about 60,000 glucose residues and has a molecular weight between 106 and 107 daltons (~4.8 million). Most of the glucose units are linked by α -1,4 glycosidic bonds, approximately 1 in 12 glucos residues also makes alpha-1,6 glycosidic bond with a second glucose, which results in the creation of a branch. So, glycogen is the "animal equivalent"of amylopectin and is more branched.

3.Cellulose - Cellulose is a polysaccharide consisting of a linear chain of several hundred to over ten thousand $\beta(1 \rightarrow 4)$ linked D-glucose units.

Cellulose is the structural component of the primary cell wall of green plants. So it has beta glucose and is linear and not branched.

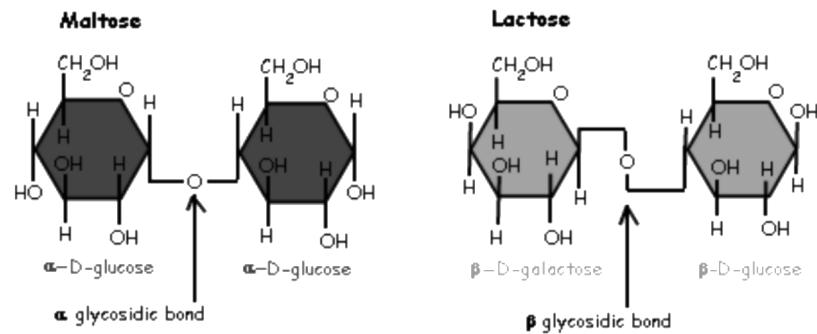
The functional group CH_2OH is hydroxymethyl.

Despite the abundance of cellulose, the nitty-gritty of how it is made is still a mystery," said Nicholas Carpita, professor of plant biology. "Now we're getting down to the molecular structure of the individual enzyme proteins that synthesize cellulose."

Cellulose is composed of several dozen strands of glucose sugars linked together in a cablelike structure and condensed into a crystal. The rigidity of cellulose allows plants to stand upright and lends wood its strength.

"Pound for pound, cellulose is stronger than steel," Carpita said.

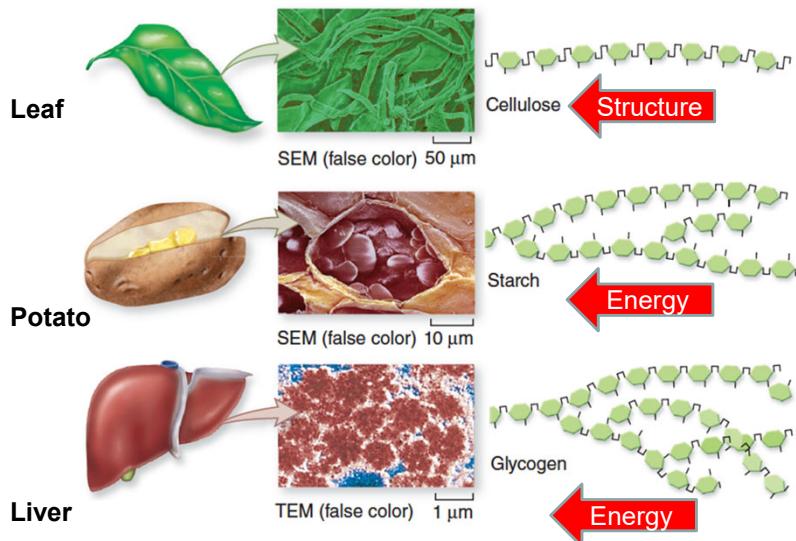
A large protein complex synthesizes cellulose at the surface of the plant cell. The basic unit of this complex is an enzyme known as cellulose synthase. The protein complex contains up to 36 of these enzymes, each of which has a region known as the catalytic domain, the site where single sugars are added to an ever-lengthening strand of glucose that will be fixed in the plant cell wall as one of the strands in the cellulose "cable."



Note the difference in the “shape” of the alpha-1,4 and beta-1,4 glycosidic bonds

Polysaccharides

Polysaccharides are long chains of carbohydrates.



18

Puffed rice or corn is more sweet.

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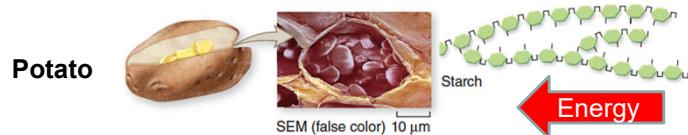
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Cellulose is the structural component of the primary cell wall of green plants. So it has beta glucose and is linear and not branched.

Polysaccharides

Starches

- Starches hydrolyze easily in water and acid to give maltose and finally glucose
- In our bodies, these complex carbs are digested by the enzymes **amylase** in saliva and **maltase** in the intestine
- The glucose units are mostly linked by **alpha 1,4-glycosidic bonds**.

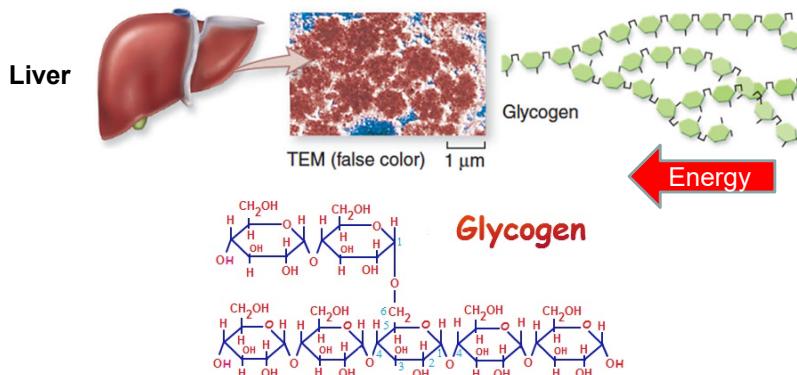


19

Polysaccharides

Glycogen

- Animal starch, polymer of glucose that is stored in the **liver** and muscle of animals
 - It is hydrolyzed in our cells and maintains the blood level of glucose and provides energy between meals
 - In glycogen, the glucose units are joined by **alpha 1,4 glycosidic bond** and **alpha 1,6 glycosidic bonds**: highly branched.

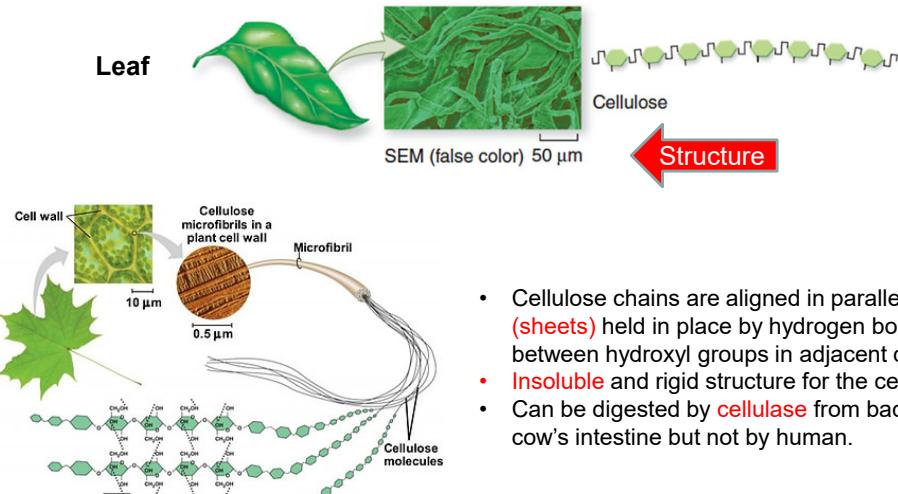


20

Polysaccharides

Cellulose

- Major structural material of wood and plants, cotton
- In cellulose, glucose molecules form a long, unbranched chain, however the glucose units in cellulose are linked by **beta-1,4 glycosidic bonds** (linkage)

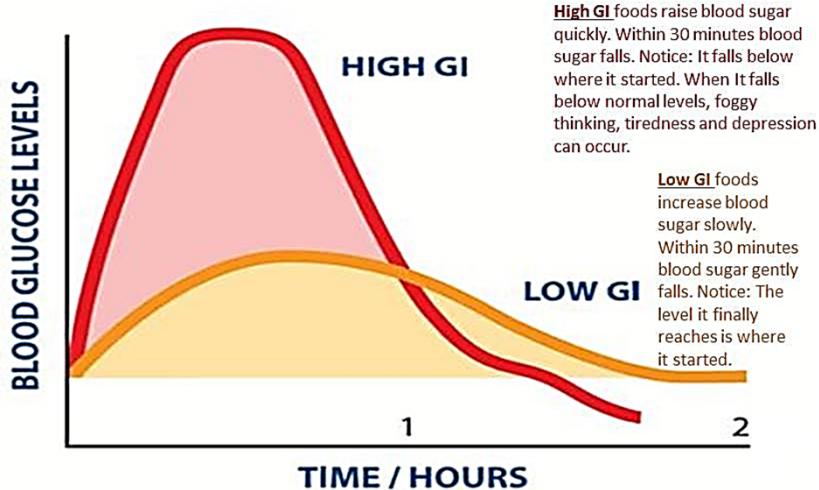


- Cellulose chains are aligned in parallel **rows (sheets)** held in place by hydrogen bonds between hydroxyl groups in adjacent chains.
- **Insoluble** and rigid structure for the cell walls.
- Can be digested by **cellulase** from bacteria in cow's intestine but not by human.

21

Another supportive polysaccharide is **chitin**, which makes up the outer coverings (exoskeletons) of insects, crabs, and spiders. Chitin also stiffens the cell walls of many fungi, including mushrooms. Chitin is similar to cellulose, except the glucose subunits bear a nitrogen-containing functional group

Case study: Glycemic Index



<http://lowglycemichappiness.com/Glycemic-Index-chart.html>

22

Further learning: <https://www.youtube.com/watch?v=Zpb2VHgjCQs>

<http://lowglycemichappiness.com/Glycemic-Index-chart.html>

Glycemic Index Versus the Insulin Index: VERY INTERESTING!

High GI, >70, rapid increase in blood sugar levels

Medium GI, 56-69, moderate increase in blood sugar levels

Low GI, <55, slow increase in blood sugar levels

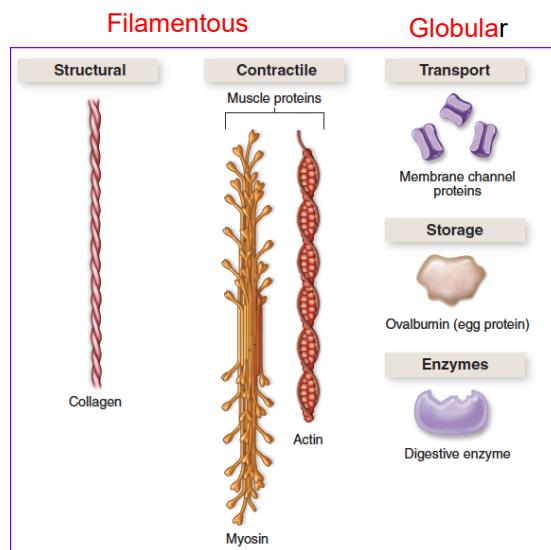
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 - Steroids

23

Proteins

Proteins are polymers composed of building blocks called amino acids. They have more variable **structures** and **functions** than any of the other organic molecules.



Spider silk, hemoglobin, keratin in your nails and hair, actin and myosin in muscle fibers – all these are proteins.

24

A human has tens of thousands of different proteins, each with a specific structure and function; protein, in fact, are the most structurally sophisticated molecules known. Consistent with their diverse functions, they vary extensively in structure, each type of protein having a unique three-dimensional shape.

Now let's turn our attention to Proteins. The word '*protein*' is from a Greek word meaning '**of first importance**'.

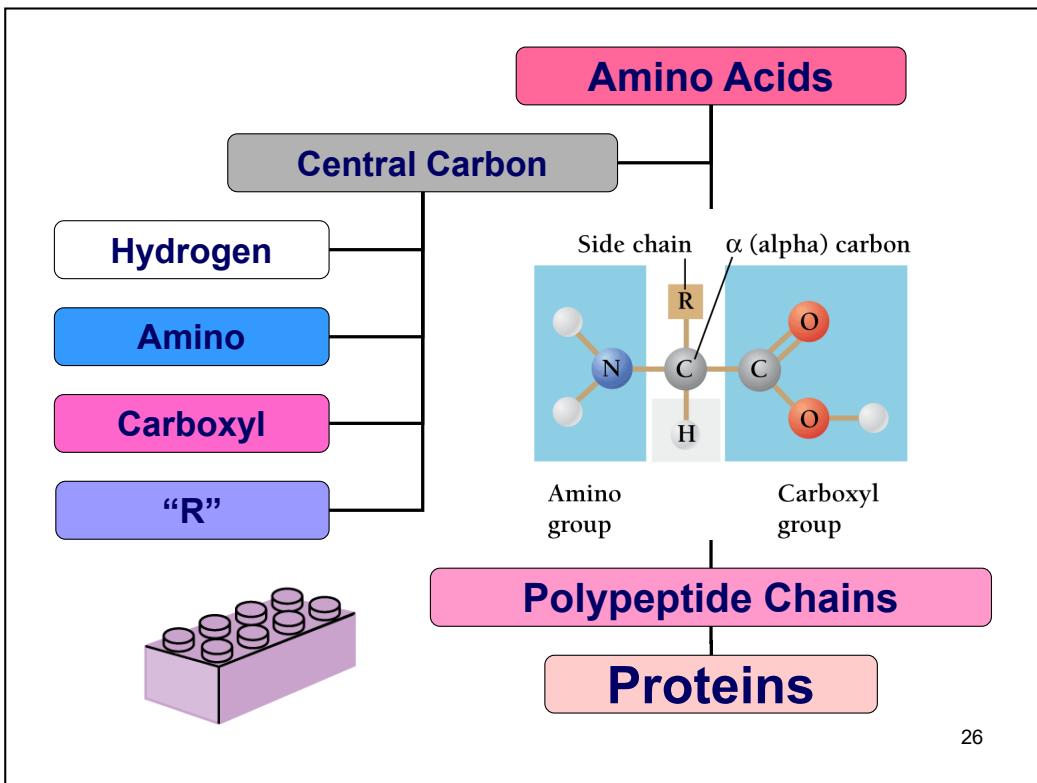
As shown in the table, proteins perform many different functions, and are the most structurally sophisticated.

TABLE 3-3**Functions of Proteins**

Function	Example(s)
Structural	Keratin (forms hair, nails, scales, feathers, and horns); silk (forms webs and cocoons)
Movement	Actin and myosin (found in muscle cells; allow contraction)
Defense	Antibodies (found in the bloodstream; fight disease organisms; some neutralize venoms); venoms (found in venomous animals; deter predators and disable prey)
Storage	Albumin (in egg white; provides nutrition for an embryo)
Signaling	Insulin (secreted by the pancreas; promotes glucose uptake into cells)
Catalyzing reactions	Amylase (found in saliva and the small intestine; digests carbohydrates)

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Table 3-3



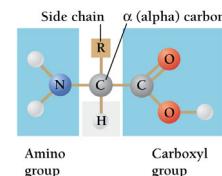
26

Despite their diversity, they are all polymers constructed from the same set of 20 amino acids. In other words, amino acids are the building block of proteins. All amino acids share a common structure. The illustration shows the general formula for an amino acid. At the center of the amino acid is a carbon atom called the alpha carbon. Its four different partners are an amino group, a carboxyl group, a hydrogen atom, and a variable group symbolized by R. The R group, also called the side chain, differs with each amino acid.

R represents “Radical”

Amino Acids

- Subunits of proteins
- 20 different amino acids that make up proteins
- Structure
 - One central carbon (C_α) atom
 - One central hydrogen (H_α) atom
 - One amino ($-NH_2$) group
 - One carboxyl ($-COOH$) group
 - One “R” group, variable group
- “R” groups differ from one amino acid to another
 - 20 different types of “R” groups



27

AMINO ACIDS

Amino acid

© Brooks/Cole – Thomson Learning

○ Essential amino acids

Amino acids with nonpolar side chains

Glycine	Alanine	Valine	Leucine	Isoleucine
Phenylalanine	Methionine	Cysteine	Proline	Tryptophan

○ Sulfhydryl group to produce disulfide linkages (-S-S-) 28

This slide and the next show the 20 amino acids that cells use to build thousands of proteins. Blue boxes highlight R groups, they are different from each other, thus contributing to the unique characteristics of a particular amino acid, affecting its functional role in a polypeptide. For example, sulfhydryl group in Cysteine can interact with other cysteines in different regions of the same protein, or in different proteins, to produce disulfide linkages (-S-S-). The linkages fasten amino acid chains together and help hold proteins in their three-dimensional shape.

Sulfur has been known as the "beauty mineral" because it helps the complexion and skin stay clear and youthful. The hydrogen sulfide gas in onions is what causes tearing. This gas can also be made by intestinal bacteria and is absorbed by the body or released as gas with a characteristic odor.

There are 9 essential amino acids for humans, which we cannot synthesize from other materials.

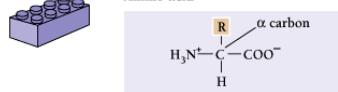
/sʌlf'haɪ drɪl/

Sulfur burps or gas

Many thiols have strong odors resembling that of garlic. Thiols are used as odorants to assist in the detection of [natural gas](#) (which in pure form is odorless), and the "smell of natural gas" is due to the smell of the thiol used as the odorant.

AMINO ACIDS

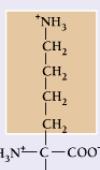
Amino acid



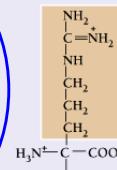
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Amino acids with basic side chains

Lysine

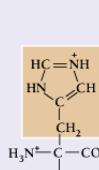


Arginine

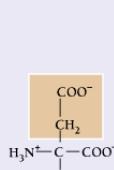


Amino acids with acidic side chains

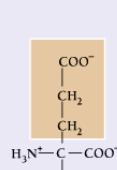
Histidine



Aspartic acid

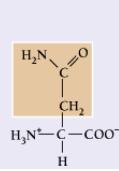


Glutamic acid

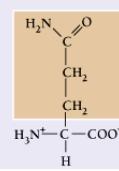


Amino acids with polar uncharged side chains

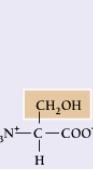
Asparagine



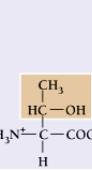
Glutamine



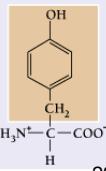
Serine



Threonine



Tyrosine

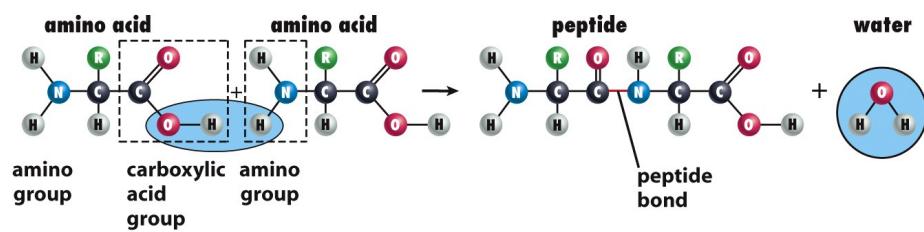


29

Here is another ten amino acids, I do not go into each of them.

Protein Synthesis

- Joined by condensation
 - Carboxyl ($-COOH$) group of one amino acid with amino ($-NH_2$) group of the next amino acid
 - H_2O formed as by-product
- Result in peptide bond (trans)



An amino end (N-terminus) and a carboxyl end (C-terminus).

30

Having examined amino acids, let's see how they are linked to form polymers. When two amino acids are positioned so that the carboxyl group of one is adjacent to the amino group of the other, they can become joined by a dehydration reaction, with the removal of a water molecule. The resulting covalent bond is called a peptide bond. Repeated over and over, this process yields a polypeptide chain, with an amino end (N-terminus) and a carboxyl end (C-terminus). The term protein or polypeptides is often reserved for long chains such as more than 50 amino acid in length, and the term peptide refers to shorter chains.

Like polysaccharides and lipids, proteins are formed by dehydration synthesis. In proteins, the nitrogen in the amino group of one amino acid is joined to the carbon in the carboxylic acid group of another amino acid by a single covalent bond, and water is liberated (FIG. 3-15). This process forms a peptide bond, and the resulting chain of two amino acids is called a peptide, a term used for relatively short chains of amino acids (up to 50 or so).

Additional amino acids are added, one by one, until a long polypeptide chain is completed. Polypeptide chains in cells can be up to thousands of amino acids in length. A protein consists of one or more polypeptide chains.

Protein Structure

Four levels of protein structure

1. Primary – consists of a linear sequence of amino acids linked together by peptide bonds (a polypeptide chain)
2. Secondary – the chain twists into a coil (helix), or a pleated sheet held by hydrogen bonds
3. Tertiary – refers to complex foldings of the protein chain held together by hydrogen bonds, disulfide bridges, and other bonds
4. Quaternary – in which two or more multiple polypeptide chains associate as linked together by hydrogen bonds or disulfide bridges.

31

When a cell synthesizes a polypeptide, the chain generally **folds spontaneously** to form a three-dimensional structure, which determines how it works. With the goal of understanding the function of a protein, we must learn its structure.

In spite of their great diversity, all proteins share **four superimposed levels of structure**, known as primary, secondary, tertiary and quaternary structure.

Hydrophobic Interactions: Soluble globular proteins fold so that hydrophobic side chains are mostly sequestered in the core of the protein. The removal of the non-polar groups from water (i.e. the hydrophobic effect) is the primary force stabilizing tertiary structure.

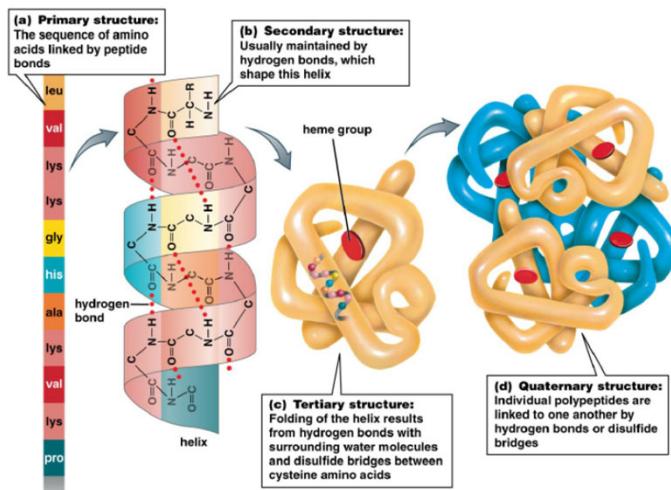
Most polar amino acid side chains are on the outside surface so they can hydrogen bond to water to solubilize the protein.

Salt Bridges: Protein fold so that positively charged side chains are often located adjacent to negatively charged side chains. The salt bridge or ionic bond between the charged functional groups helps stabilize the tertiary structure.

Hydrogen Bonds: Folding is also stabilized by hydrogen bonds that form between the polar hydrogen of one amino acid and a lone pair of electrons on another (note: hydrogen atoms do not usually display in pdb files).

Covalent Bonds: Some proteins are cross-linked with covalent bonds. The most common is a disulfide bond between the sulfur atoms of two cysteines. These are most common in extracellular proteins.

Protein Structure



32

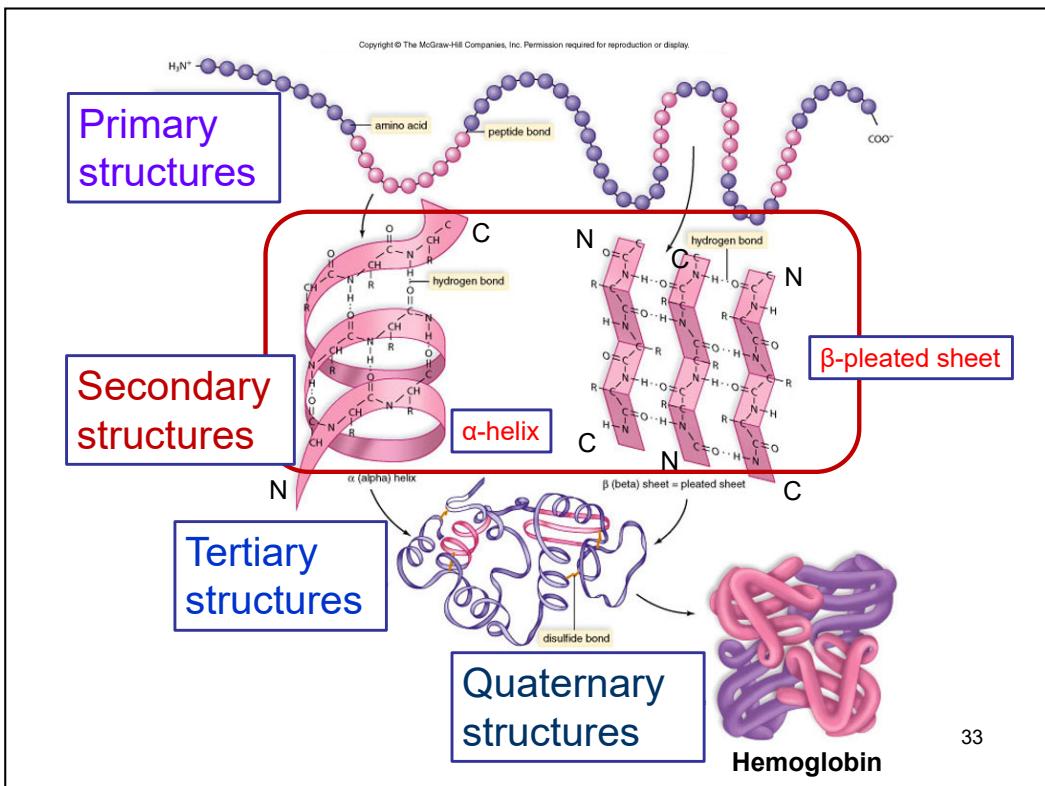
The primary structure of a protein is its unique sequence of amino acids, is like the order of letters in a very long word. The secondary structure emerges as the chain twists, bends, loops and folds. Hydrogen bonding between amino acids makes stretches of the polypeptide chain form a sheet, or coil into a helix a bit like a spiral staircase. Alpha helix and beta sheet are two highly regular secondary structures. A third, less regular arrangement, the random coil or loop, provides flexible regions that allow sections of amino acid chains containing them to bend.

Superimposed on the patterns of secondary structure is a protein's tertiary structure. In other words, a helix, beta sheet and random coil further fold each protein into its tertiary structure.

Many proteins also have a fourth level of organization, or quaternary structure: they consist of two or more polypeptide chains bonded together or in close association. Such as hemoglobin, a protein transporting oxygen in our blood, are globular, with several polypeptide chains folded into a roughly spherical shape.

Alpha helix, beta sheet, pleated sheet,

To be able to perform their biological function, proteins fold into one or more specific spatial conformations, driven by a number of non-covalent interactions such as **hydrogen bonding, ionic interactions, Van der Waals forces, and hydrophobic packing**.



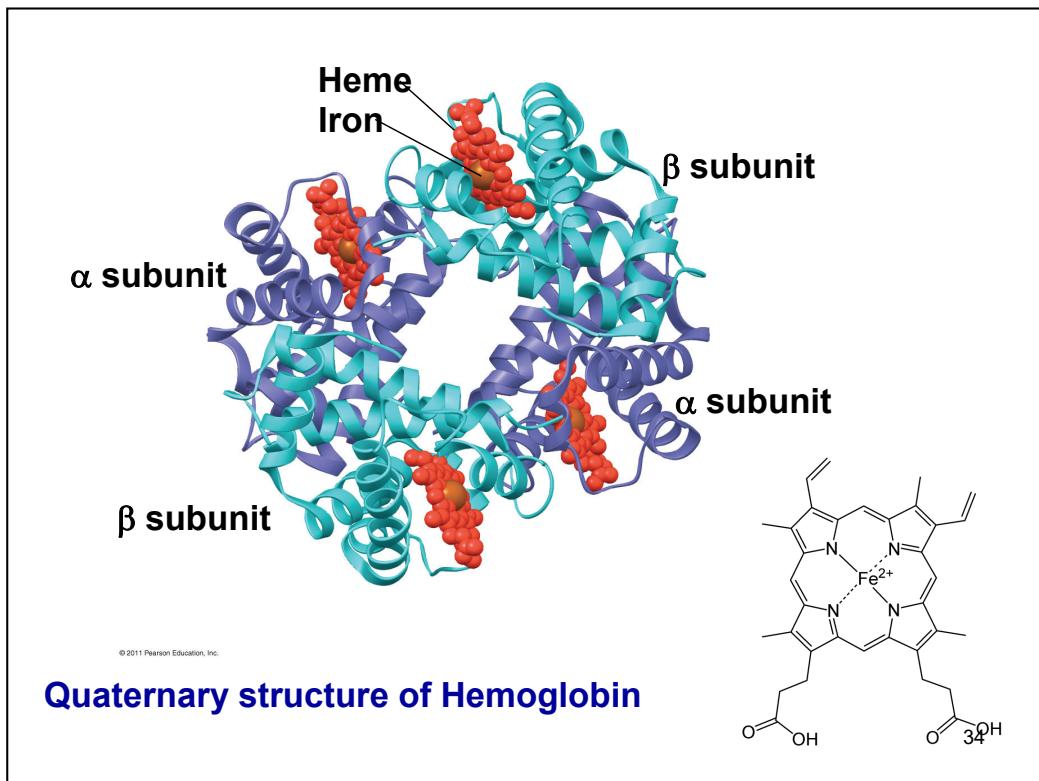


Figure 5.20 Exploring: Levels of Protein Structure

Hemoglobin is a globular protein consisting of four polypeptides: two alpha and two beta chains

is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates

The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane.

In the tetrameric form of normal adult hemoglobin, the binding of oxygen is, thus, a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding. This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex as discussed above; i.e., when one subunit protein in hemoglobin becomes oxygenated, a conformational or structural change in the whole complex is initiated, causing the other subunits to gain an increased affinity for oxygen. As a consequence, the oxygen binding curve of hemoglobin is sigmoidal, or S-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding.

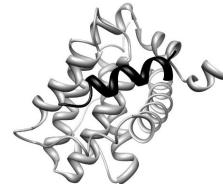
Hence, blood with high carbon dioxide levels is also lower in pH (more acidic). Hemoglobin can bind protons and carbon dioxide, which causes a conformational change in the protein and facilitates the release of oxygen. Protons bind at various places on the protein, while carbon dioxide binds at the α -amino group. Carbon dioxide binds to hemoglobin and forms carbaminohemoglobin.^[47] This decrease in hemoglobin's affinity for oxygen by the binding of carbon dioxide and acid is known as the Bohr effect (shifts the O_2 -saturation curve to the *right*). Conversely, when the carbon dioxide levels in the blood decrease (i.e., in the lung capillaries), carbon dioxide and protons are released from hemoglobin, increasing the oxygen affinity of the protein. A reduction in the total binding capacity of hemoglobin to oxygen (i.e. shifting the curve down, not just to the right) due to reduced pH is called the root effect. This is seen in bony fish.

It is necessary for hemoglobin to release the oxygen that it binds; if not, there is no point in binding it. The sigmoidal curve of hemoglobin makes it efficient in binding (taking up O_2 in lungs), and efficient in unloading (unloading O_2 in tissues)

Protein Structure

The functions of proteins are linked to their
three-dimensional structures

- Precise positioning of amino acid **R groups** leads to **active site** and **binding site**.
- Disruption of secondary and tertiary bonds leads to **denatured** proteins and **loss of function**

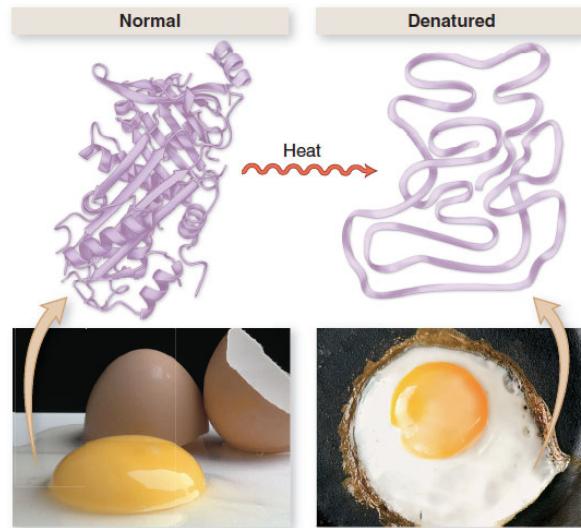


35

Fold paper to show structure and function

Protein Structure & Function

The function of a protein depends on its shape.
When protein structure is changed, its function is lost or changed.



36

For example, egg white consists of albumin protein, which is normally transparent and relatively fluid.

But the heat of a frying pan rips its hydrogen bonds apart, destroying the albumin's secondary and tertiary structure and causing it to become opaque, white, and solid

This is a highly recommended video that you should watch to understand what is a protein.

[PDB-101: Learn: Videos: What is a Protein? \(Video\) \(rcsb.org\)](https://www.rcsb.org/pdb101/learn/videos/what-is-a-protein-video)



37

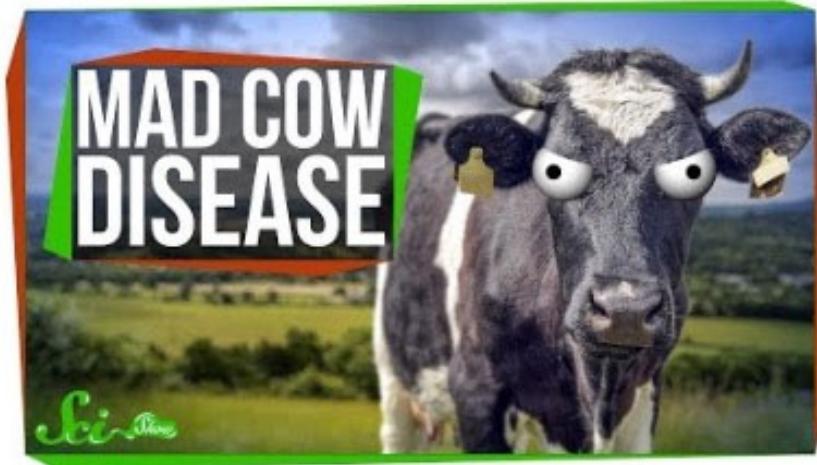
Fold paper to show structure and function

Hydrophobic amino acids – have carbon-rich side chains, don't interact well with water

Hydrophilic amino acids (polar amino acids) – interact well with water (forms hydrogen bonds with water)

Charged amino acids – interact with oppositely charged amino acids or other molecules

Case study: What is mad cow disease? (video)

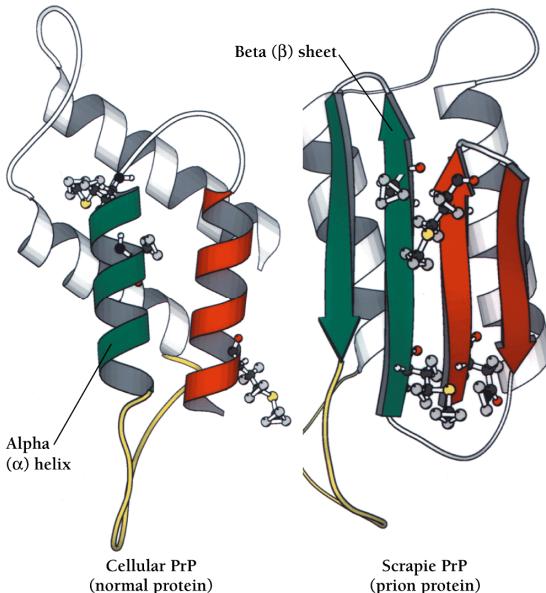


[\(1\) What Happened to Mad Cow Disease? - YouTube](#)

38

Play video on mad cow disease

Case study: Prions and Mad Cow Disease



Stanley B. Prusiner

**The Nobel Prize in
Physiology or
Medicine 1997**

http://nobelprize.org/nobel_prizes/medicine/laureates/1997/prusiner-lecture.html

39

Prions are misfolded proteins with the ability to transmit their misfolded shape onto normal variants of the same protein. Infectious prions such as those that cause mad cow disease

are abnormally folded versions of a protein that is found throughout the body. The secondary structure of the normal prion protein is primarily helical. Infectious prions, however, fold into pleated sheets. The pleated sheets are so stable they are unaffected by the enzymes that break down normal prion protein.

As a result, infectious prions accumulate destructively in the brain. They characterize several fatal and transmissible neurodegenerative diseases in humans and many other animals.

This illustration compares a normal prion protein to a disease-causing form. The two structures exhibit two different, classic protein motifs, called "alpha helices," and "beta sheets." Alpha helices, seen here in the normal prion (left), consist of linked amino-acid building blocks that spiral around like a coiled spring. Beta sheets form when amino acid chains line up in a flat plane within the protein, as in the disease-causing protein shown here. Research discoveries by Stanley Prusiner, MD, Fred Cohen, MD, PhD, and their UCSF colleagues suggest that the propagation of infectious prions arises from a rearrangement of a normal prion protein into a disease-causing form, a process that can be spurred by the initial presence of the infectious protein.

The infectious form of the prion protein (right) arises when alpha-helical regions in the normal protein unfold and beta sheets form within the protein, according to the UCSF researchers. When the protein converts to the form exhibiting more sheet-like regions, it is able to clump together and react in ways that contribute to disease in some as-yet undefined manner.

Prion diseases might one day become preventable or treatable with drugs that stabilize the alpha helices of the normal prion protein and prevent the conversion to the beta structure observed in the disease-causing prion form.

http://nobelprize.org/nobel_prizes/medicine/laureates/1997/prusiner-lecture.html

Nobel Lecture

Banquet Speech

Four residues implicated in the species barrier are shown in ball-and-stick form

Proposed model for replication of prions

1. Prion protein is a normal part of nerve cells
2. Misfolded versions of the normal protein are the infectious particles
3. The misfolded proteins induce normal copies to misfold, too
4. A high concentration of prions in nerve tissue causes cell damage and degeneration

40

Outline

- Organic Molecules
 - Functional groups
 - Synthesis and breakdown
- Carbohydrates
 - Monosaccharides
 - Oligosaccharides
 - Polysaccharides
- Proteins
 - Amino acids
 - Protein structure
- Lipids
 - Triglycerides
 - Phospholipids
 - Waxes
 - Steroids

41

Lipids

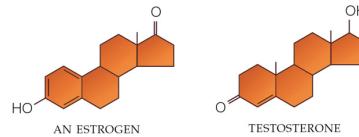
Lipids: Organic substances which are **insoluble** in polar solvents, such as water. Include: **triglycerides** (fats and oils), **phospholipids**, waxes, and **steroids**

Lipids are classified into two major groups

1. Fatty acid lipids: containing fatty acids such as oils, fats, and waxes
2. Non-fatty acid lipids (Steroids) such as cholesterol containing 4 carbon rings

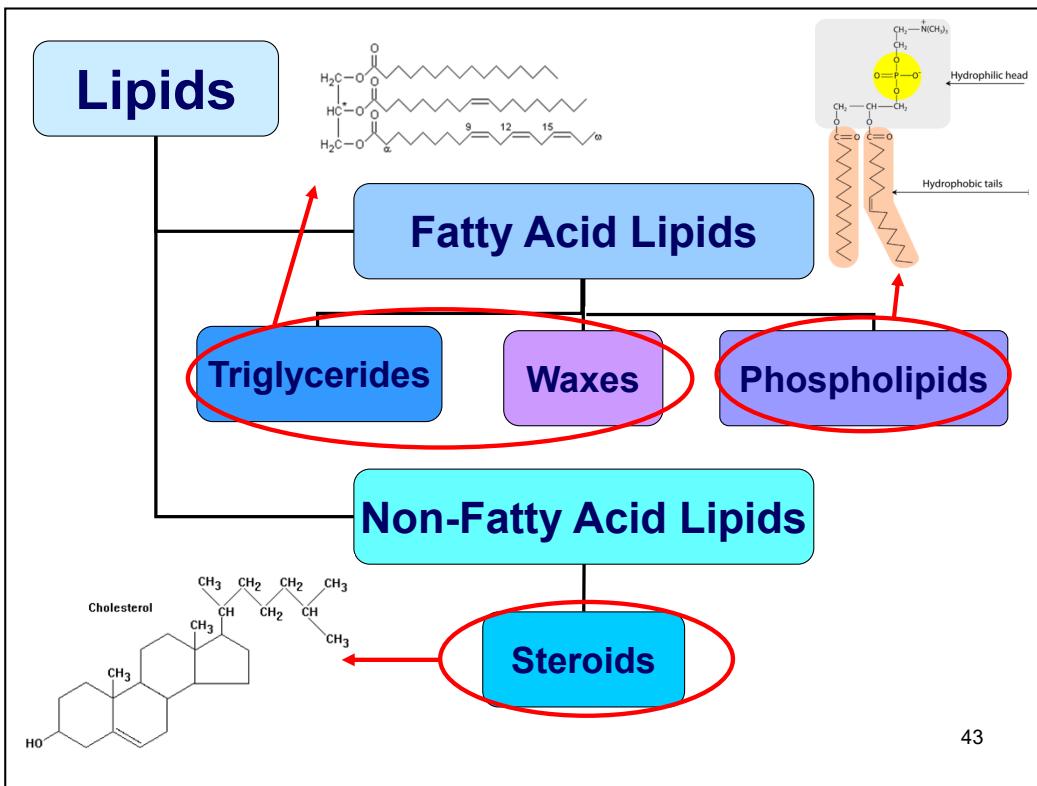


42



Lipids as Energy storage (a) A grizzly bear stores fat to provide both insulation and energy as he prepares to hibernate. If he stored the same amount of energy in carbohydrates, he would

probably be unable to walk. (b) Oily avocado flesh likely originally evolved to entice enormous seed-dispersing mammals (such as giant ground sloths, extinct for about 10,000 years), which would swallow the seeds and excrete them intact.



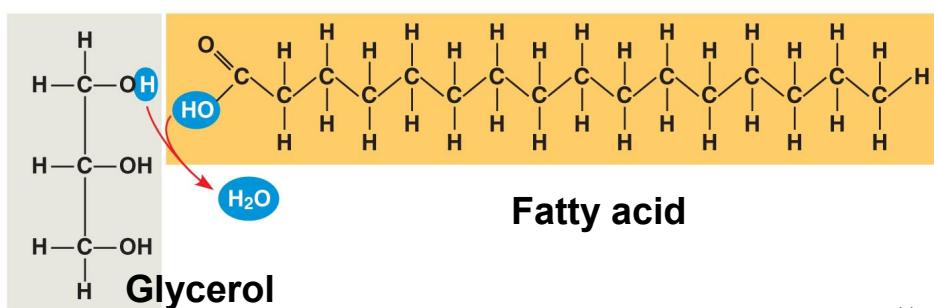
43

Lipids can be classified into three major groups, as shown in the red-lined ovals: (1) triglycerides and waxes, which are similar in structure and contain only carbon, hydrogen, and oxygen; (2) phospholipids, which are structurally similar to oils but also contain phosphorus and nitrogen; and (3) the “fused-ring” family of steroids.

Waxes are highly saturated lipids that remain solid at outdoor temperatures. Bees form wax into the hexagons of honeycomb.

Triglycerids (Oils, Fats)

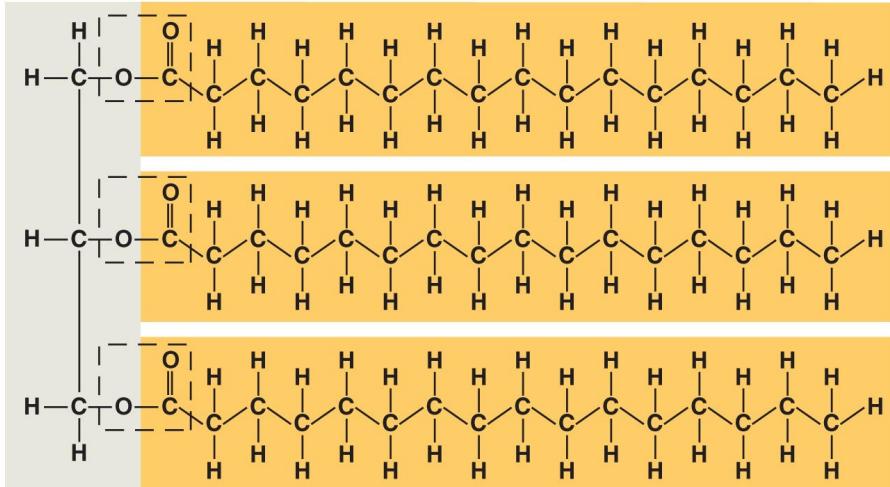
- Are used primarily as energy-storage molecules, containing twice as many calories per gram as carbohydrates and proteins
- Are formed by **dehydration synthesis**
 - Three fatty acids + glycerol → triglyceride



44

A fat is constructed from two kinds of smaller molecules: glycerol and fatty acid, by dehydration. Glycerol is an alcohol with three carbons, each bearing a hydroxyl group. A fatty acid has a long hydrocarbon chain with a carboxyl group at one end. When the two molecules come together, a bond btw hydroxyl group and a carboxyl group, called ester linkage will form to join them

Ester linkage



(b) Fat molecule (triglycerides)

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45

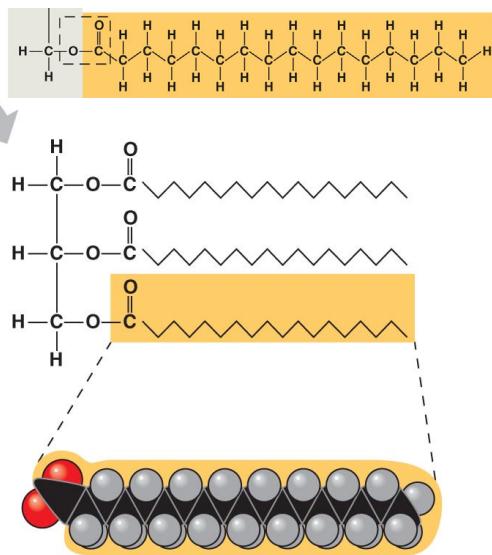
When three hydroxyl groups are jointed with three fatty acids, a triglyceride molecule is formed. This structure gives fats and oils their chemical name: triglycerides.

Figure 5.11 The synthesis and structure of a fat, or triglycerides)

Saturated fat



Fats that are solid at room temperature are **saturated** (the carbon chain has as many hydrogen atoms as possible, and mostly or **all C-C bonds**); for example, beef fat, butter



46

If the hydrocarbon binds the maximum possible number of hydrogen, and all carbons are linked by a single bonds, the fatty acid is said to be saturated with hydrogen atoms, the fat is called saturated fat. Most animal fats are saturated, and tend to remain solid at room temperature.

Figure 5.12 Examples of saturated and unsaturated fats and fatty acids

stearic

[sti5Arik]

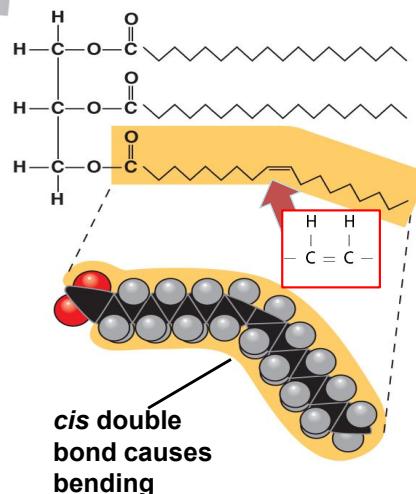
adj.

[化]硬脂的, 似硬脂的, 硬脂酸的, 十八(烷)酸的



Unsaturated fat (oil)

Fats that are liquid at room temperature are unsaturated (with fewer hydrogen atoms, and many **C=C bonds**); for example, corn oil
Unsaturated trans fats have been linked to heart disease

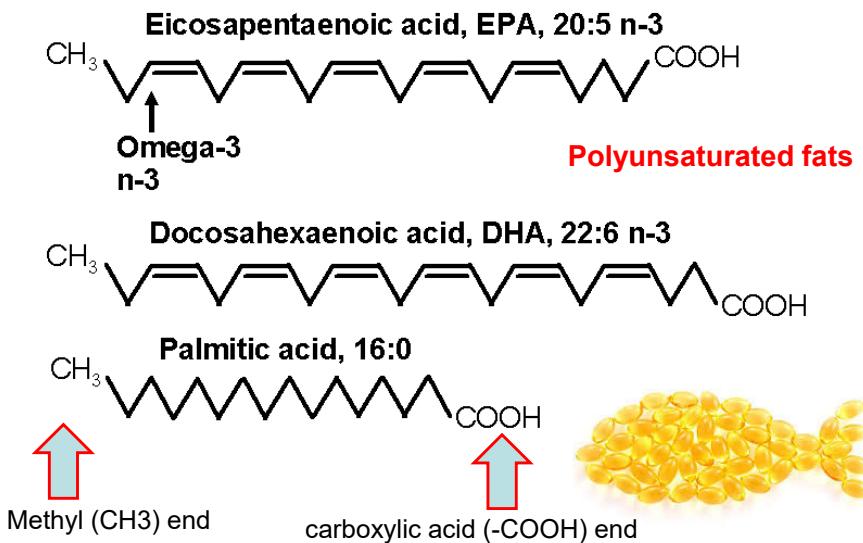


47

If one or more double bonds link the carbons, reducing the number of hydrogen atoms bound, the fatty acid is unsaturated. This double bond creates a kink in the chain, preventing the molecules from packaging together closely enough to solidify at room temperature. We get most of our unsaturated oils from the seeds of plants, where they are stored for the plants' developing embryos, babies. BTW, some waterfowl, such as penguins, secret oil from special glands and spread over their feathers, the oily coating keeps their feathers watertight and dry.

Figure 5.12 Examples of saturated and unsaturated fats and fatty acids

Omega-3 fatty acid ethyl esters



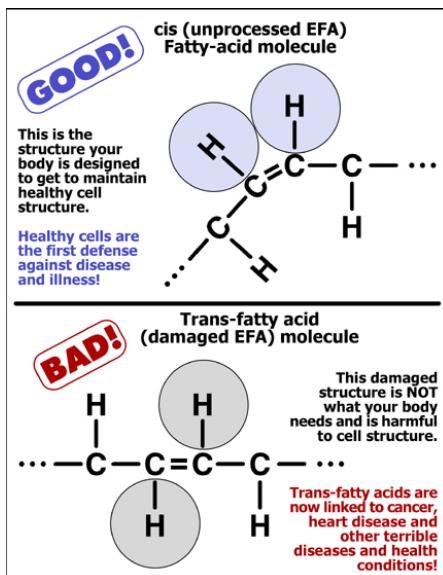
48

Cardiovascular diseases

Omega is the 24th and last letter of the Greek alphabet.

Omega-3 fatty acids (also called ω -3 fatty acids or *n*-3 fatty acids^[1]) are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the end of the carbon chain.^[2] The fatty acids have two ends, the carboxylic acid (-COOH) end, which is considered the beginning of the chain, thus "alpha", and the methyl (CH₃) end, which is considered the "tail" of the chain, thus "omega." The way in which a fatty acid is named is determined by the location of the first double bond, counted from the methyl end, that is, the omega (ω -) or the *n*-end. Omega means the last or ending

Case study: Cis vs Trans lipids



The oil has zero grams of trans fat per serving

<http://www.scienceofhealthindex.com/distorted-efas.html>

49

Come to our daily life, you may consume a lot of french fries, made from oil. Vegetable oils naturally have mostly cis bonds, that means the hydrogen atoms at a double bond are positioned on the same side of the carbon chain, the hydrogen atoms repel each other to create a kink, but if oil is used for frying, some of the cis bonds convert to trans bonds, that means the hydrogen atoms are on different sides of the chain at the double bonds. If the oil is used only once like when you fry an egg, only a few of the bonds do this so it is not too bad. However, if oil is constantly reused, like in fast food French fry machines, more and more of the cis bonds are changed to trans. Recently, however, researchers have become concerned about our consumption of trans fats. Trans fat may cause circulatory system disease, even cancer. As a result, many manufacturers have made efforts to reduce the use of these substances in processed food.

carcinogenic, or cancer-causing. The levels of trans fatty acids in highly-processed, lipid-containing products such as margarine are quite high, and I have heard that the government is considering requiring that the amounts of trans fatty acids in such products be listed on the labels.

ESSENTIAL FATTY ACIDS (EFA)

When the substituent groups are oriented in the same direction, the diastereomer is referred to as *cis*, whereas, when the substituents are oriented in opposing directions, the diastereomer is referred to as *trans*.

Note that, chemically, double bonds may be either of the *cis*-type or the *trans*-type but only the *cis*-type are found in biological systems (the *trans*-type are found in partially hydrogenated vegetable shortening).

The *cis*-type double bond, unlike the *trans*-type double bond, has the property of bending (putting a kink in) the hydrocarbon chain of the fatty acid

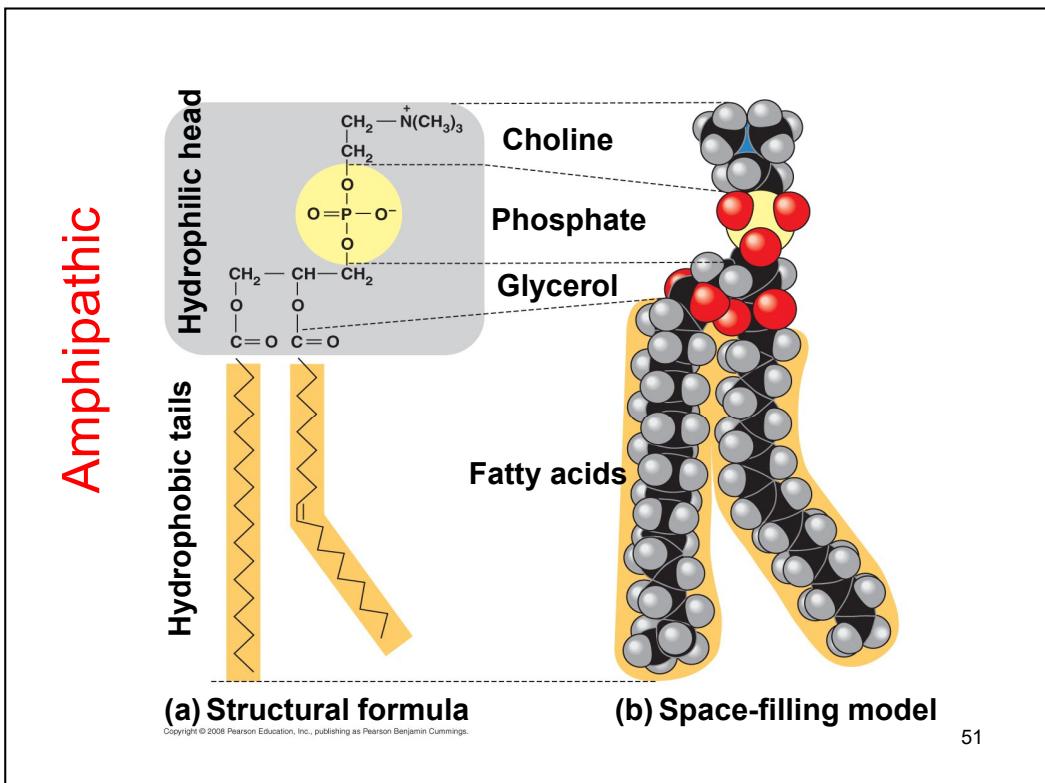
Naturally-occurring unsaturated vegetable oils have almost all *cis* bonds, but using oil for frying causes some of the *cis* bonds to convert to *trans* bonds. If oil is used only once like when you fry an egg, only a few of the bonds do this so it's not too bad. However, if oil is constantly reused, like in fast food French fry machines, more and more of the *cis* bonds are changed to *trans* until significant numbers of fatty acids with *trans* bonds build up. The reason this is of concern is that fatty acids with *trans* bonds are carcinogenic, or cancer-causing. The levels of *trans* fatty acids in highly-processed, lipid-containing products such as margarine are quite high, and I have heard that the government is considering requiring that the amounts of *trans* fatty acids in such products be listed on the labels.

Phospholipids

- The major component of all cell membranes
- Subunits
 - One glycerol
 - Two fatty acids (non-polar and hydrophobic)
 - “Head” with phosphate group (polar and hydrophilic)
- Formed by condensation

50

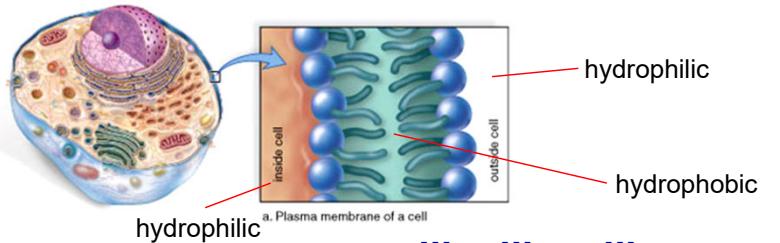
A phospholipid is similar to an oil, except that one of the three fatty acids is replaced by a phosphate group with a short, polar functional group (typically containing nitrogen) attached to the end.



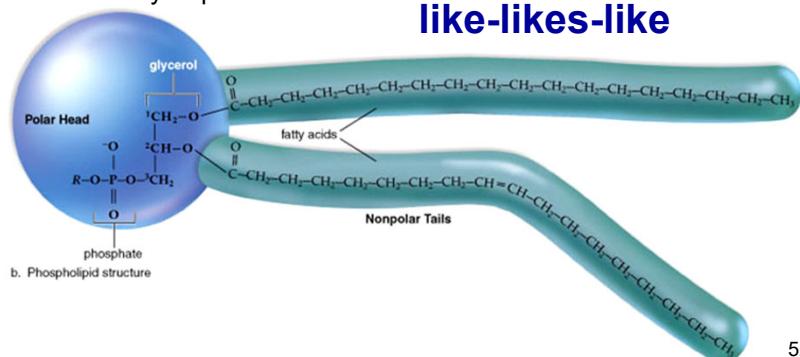
The molecules are shown in this figure. The light yellow circle highlights the phosphate group. It has two dissimilar ends: two nonpolar fatty acid “tails,” which are insoluble in water, and one phosphate-nitrogen “head” that is polar and water soluble.

Figure 5.13 The structure of a phospholipid

Lipid bilayer of cell membranes



like-likes-like



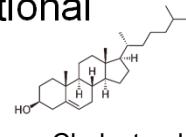
52

Phospholipids are the most abundant lipids in cell membrane, which have two phospholipid layers. Their heads are exposed to watery surroundings, and all of the hydrophobic tails are sandwiched between the heads, that is they are packed together in the interior of the bilayer.

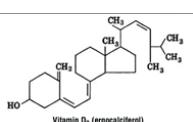
Steroids

- Steroids are composed of **four carbon rings** fused together, with various functional groups protruding from them
- Examples of steroids include:

- **Cholesterol**



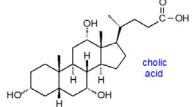
Cholesterol



Vitamin-D

- Vital component of the membranes of animal cells
- The precursor from which the body synthesizes **vitamin D**
- Excessive cholesterol contributes to cardiovascular disease

- **Bile salt (cholic acid)**

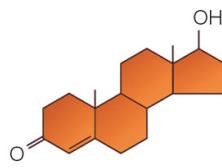
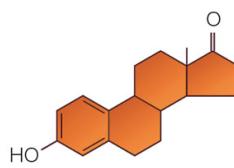
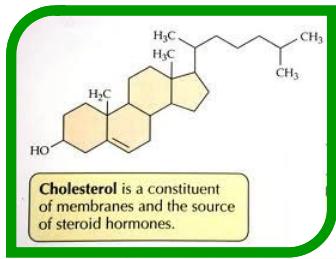


- Break down oil into smaller droplet for digestion

53

It makes up about 2% of the human brain, where it is an important component of the lipid-rich membranes that insulate nerve cells.

Steroids

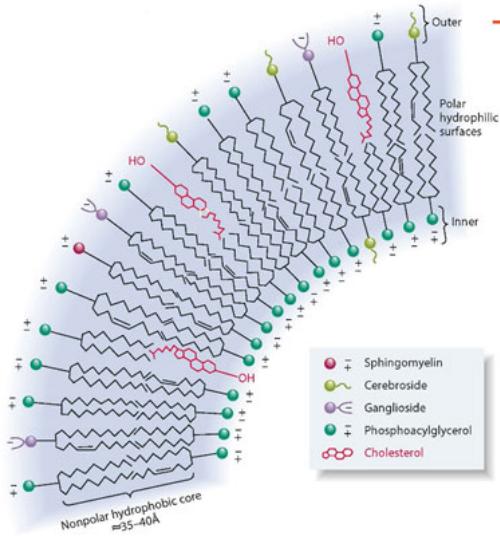


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Male and female **sex hormones** are chemical messengers for development.

54

Steroids are lipids with a rigid backbone of four carbon rings and no fatty acid tails. In animal tissues, cholesterol is the most common steroid. Cholesterol is remodeled into many molecules, such as bile salts (which help digest fats) and vitamin D (required to keep teeth and bones strong). It is also used to synthesize male and female sex hormone.



- Cholesterol (**amphipathic**) is a component of plasma membrane and it maintains **fluidity of lipid bilayer** by preventing close-packing of hydrocarbon tails at **low temperature**.

55

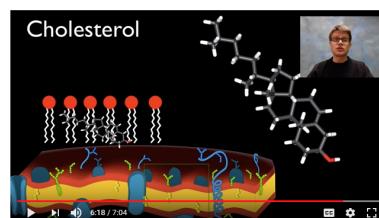
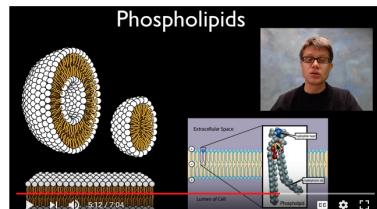
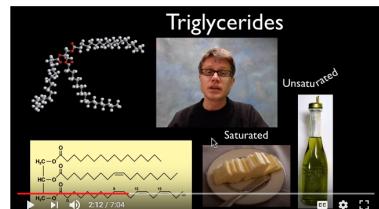
TABLE 3.3**Lipids**

Type	Functions	Human Uses
Fats	Long-term energy storage and insulation in animals	Butter, lard
Oils	Long-term energy storage in plants and their seeds	Cooking oils
Phospholipids	Component of plasma membrane	—
Steroids	Component of plasma membrane (cholesterol), sex hormones	Medicines
Waxes	Protection, prevent water loss (cuticle of plant surfaces), beeswax, earwax	Candles, polishes

Lipids

Online Learning Materials

<https://www.youtube.com/watch?v=VGHD9e3yRIU>



57

Online Resources

****: watch to enhance your understanding of lecture contents**

***: watch to broaden your knowledge related to our topics**

Chemistry of Life

- Carbohydrates**

<https://www.youtube.com/watch?v=M6ZLDJluj6I> (9 min)

- Lipids**

<https://www.youtube.com/watch?v=VGHD9e3yRIU> (8 min)

- Proteins**

[PDB-101: Learn: Videos: What is a Protein? \(Video\) \(rcsb.org\)](https://www.rcsb.org/learn/video/what-is-protein)

https://www.youtube.com/watch?v=2Jgb_DpaQhM (9 min)

- Lipids Digestion and Absorption*

<https://www.youtube.com/watch?v=3J5pNwLYZ7w> (6 min)

- Martin Hanczyc: The line between life and not-life*

http://www.ted.com/talks/martin_hanczyc_the_line_between_life_and_not_life#t-19074 (15 m)

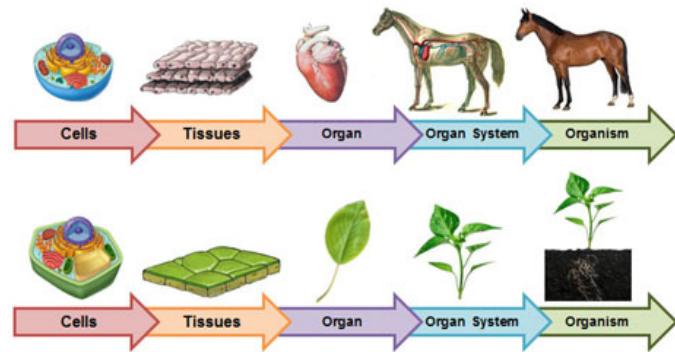
58

All video links are on the Learning flow

Please complete the quiz on Canvas for lecture 2

59

LSM1301



L3: Cell structure and function

A/P Henry Mok Yu Keung

Office at S3-03-01d

dbsmokh@nus.edu.sg

1

Outline

- Cells
 - Cell theory
 - Cell size
 - Structure
- Cell Membranes
 - Membrane structure
 - Movement across membranes
 - Cell walls
- Prokaryotic Cells
- Eukaryotic Cells
 - Nucleus
 - Endomembrane system
 - Energy-related organelles
 - Cytoskeleton

2

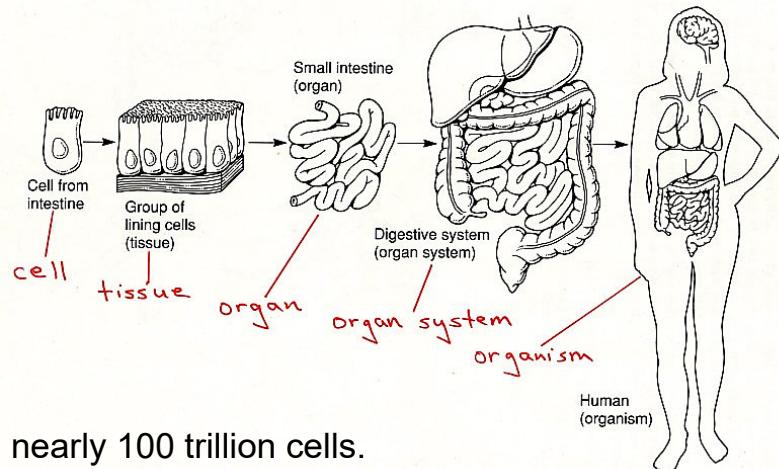
Nearly all cells are so small that they are invisible to the naked eye. No one even knew cells existed until the first microscope was invented. As shown in the figure, human cannot see objects smaller than 0.1 mm in diameter. Light microscope enables us to observe objects around 10 um in diameter while Electron microscopes allow us to observe internal structures at nm scale. (/skeɪl/)

Figure 6.2 The size range of cells



How is life organized around cells?

- Unicellular organisms
- Multicellular organisms



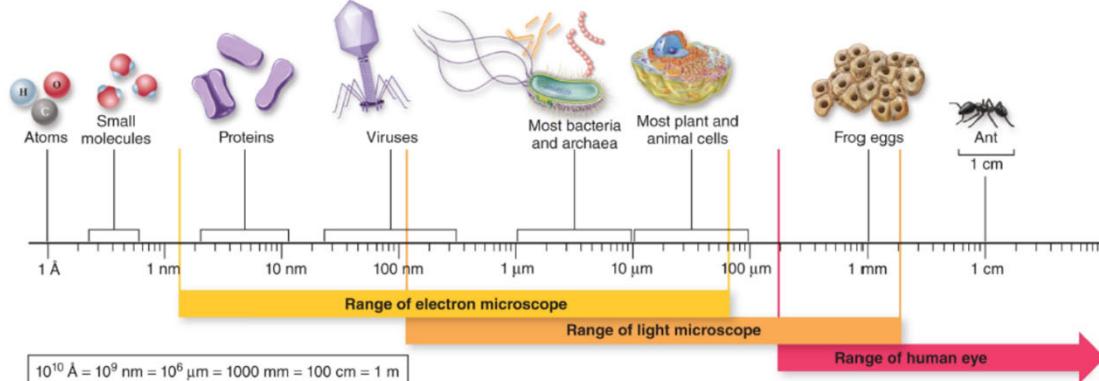
- The human body contains nearly 100 trillion cells.
- There are at least 10 times as many bacteria in the human body as cells.

3

We have known that Cell is the basic unit of life, unicellular organisms are made up of only one cell, while some multicellular organisms are comprised of trillions of cells, such as a human body contains nearly 100 trillion cells, but our body carries even more bacteria in terms of numbers of cells. So from this viewpoint, we are more microbe than we are human. However, in terms of mass, bacteria may take about a few kilograms, because bacteria are much smaller than our body cells. Those bacteria are in our skin, nose, mouth, and especially, in our gut. They are essential to our health.

In multicellular organisms, groups of cells **sharing similar structure** form tissues that carry out a specialized function. Different tissue layers form organs. And separate organs often work together to carry out major bodily functions, such as digestion or circulation. These “teams” of organs are referred to as organ systems. and organ systems of the body work together to maintain the life and health of the organism.

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4

Nearly all cells are so small that they are invisible to the naked eye. No one even knew cells existed until the first microscope was invented. As shown in the figure, human cannot see objects smaller than 0.1 mm in diameter. Light microscope enables us to observe objects around 10 μm in diameter while Electron microscopes allow us to observe internal structures at nm scale. (/skeɪl/)

Figure 6.2 The size range of cells

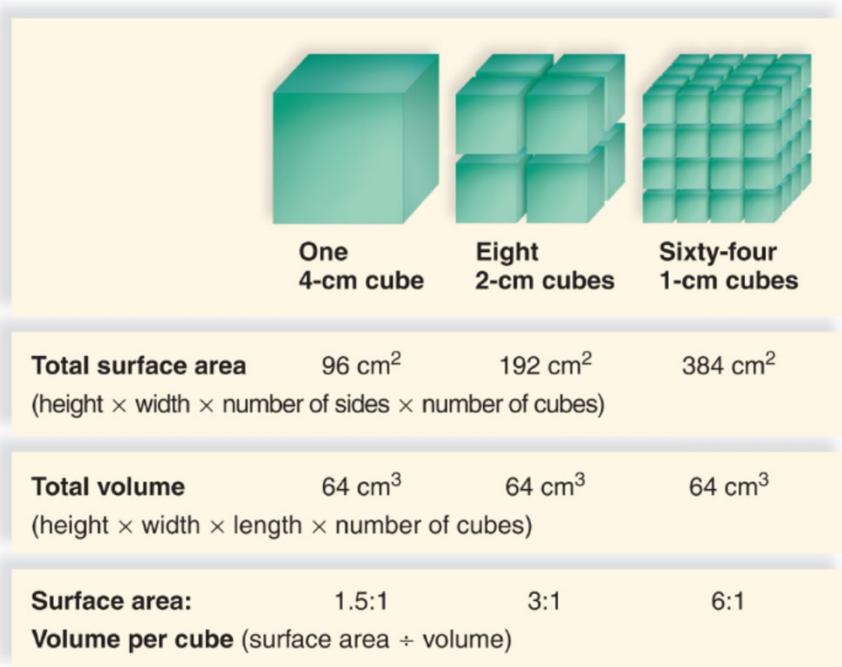
Cell size

- Most cells are small
 - Diameters range from 1 to 100 μm
- Large surface area needed
 - To allow for adequate rate of **exchange** nutrients and wastes with environment
- **Surface-area-to-volume ratio**
 - Larger cells – surface area relative to volume smaller
 - Smaller cells – surface area relative to volume larger

5

Nearly all cells are so small that they are invisible to the naked eye. No one even knew cells existed until the first microscope was invented. As shown in the figure, human cannot see objects smaller than 0.1 mm in diameter. Light microscope enables us to observe objects around 10 μm in diameter while Electron microscopes allow us to observe internal structures at nm scale. (/skeɪl/)

Figure 6.2 The size range of cells



6

Nearly all cells are so small that they are invisible to the naked eye. No one even knew cells existed until the first microscope was invented. As shown in the figure, human cannot see objects smaller than 0.1 mm in diameter. Light microscope enables us to observe an objects around 10 um in diameter while Electron microscopes allow us to observe internal structures at nm scale. (/skeɪl/)

Figure 6.2 The size range of cells

Who discovered the cell?



Robert Hooke
(1635-1703)
England



Peel off dry outer bark of the cork oak, then **thinly slice** a piece of cork

- Observed small boxes, they resembled the tiny rooms, or “cells” occupied by monks
- He coined the word '**cell**' to describe the tiny compartment, later, we know it is the basic unit of life

7

Robert Hooke is a pioneer to make microscopes and observe biological materials. He looked at thinly sliced cork from a mature tree through a microscope, he observed **tiny compartment**.

his *hand-made spherical lenses*

Stopper, a *wine bottle* closure made from the bark of the *cork oak*.
Coin the term “cellulae”

Exceeding thin, piece of cork, a great many little box,
He called the boxes “ cells” because he thought they resembled the tiny rooms, or cells, occupied by monks. He wrote that in the living oak and other plants, “ these cells filled with juices”

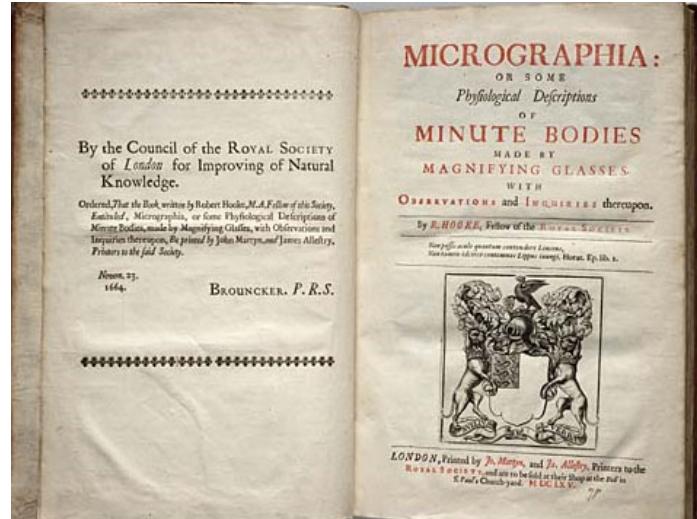
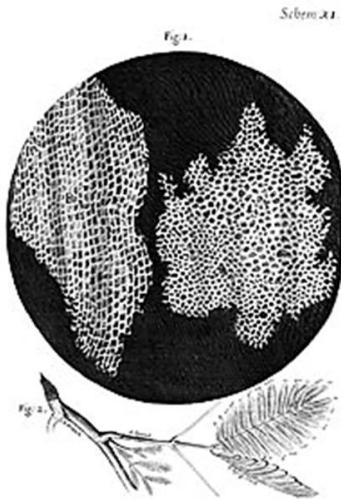
Robert Hooke, the English father of microscopy
In 1665, the English physicist Robert Hooke
<http://www.ucmp.berkeley.edu/history/hooke.html>

Razor Blade

Robert Hooke's Cork Cells

January 01, 1653

First published in 1665



"Exceeding thin, piece of cork, a great many little box"

8

Showing here, is the picture he drew to show what he observed. Hooke was actually looking at the walls of dead cells, later, he also observed living cells, in which he found cells are “filled with juices”, He published a book, and the book became an instant best seller. Hooke had ignited the spark of cell theory and set a trend of scientists making discoveries by looking through microscopes.

<https://bitesizebio.com/166/history-of-cell-biology/>

Cell Theory

Three principles comprise cell theory

- Every living things or organisms are made of **one or more** cells.
- Cells are **functional units** of multicellular organism.
- All cells come from **pre-existing** cells.

9

For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

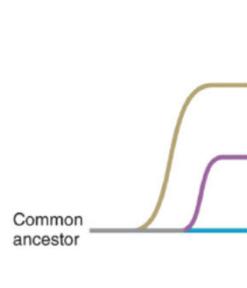
Structure

- Types of cells
 - Prokaryotic (*before nucleus* in Greek)
 - Eukaryotic (*true nucleus* in Greek)

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	Cell Type	Nucleus	Membrane-bound Organelles	Membrane Chemistry	Cell Wall Chemistry	Typical Size
Domain Bacteria	Prokaryotic	Absent	Absent	Fatty acids	Peptidoglycan (if present)	1-10 μm
Domain Archaea	Prokaryotic	Absent	Absent	Nonfatty acid lipids	Pseudopeptidoglycan or protein	1-10 μm
Domain Eukarya	Eukaryotic	Present	Present	Fatty acids	Usually cellulose or chitin (if present)	1-100 μm

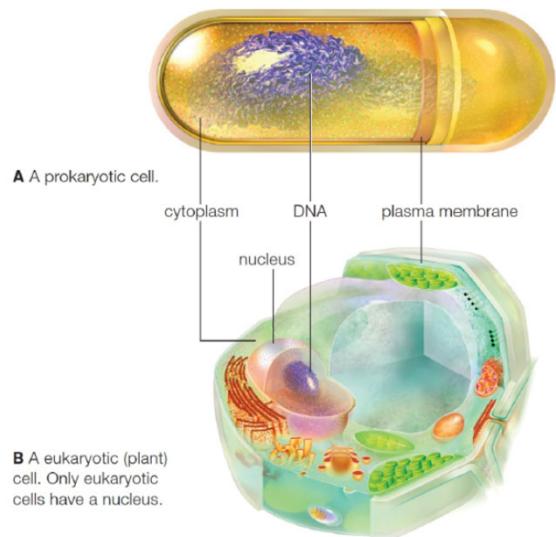
Common ancestor



For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

Structure

- Structural features of all cells
 - Plasma membrane
 - DNA-containing region
 - Cytoplasm



11

For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

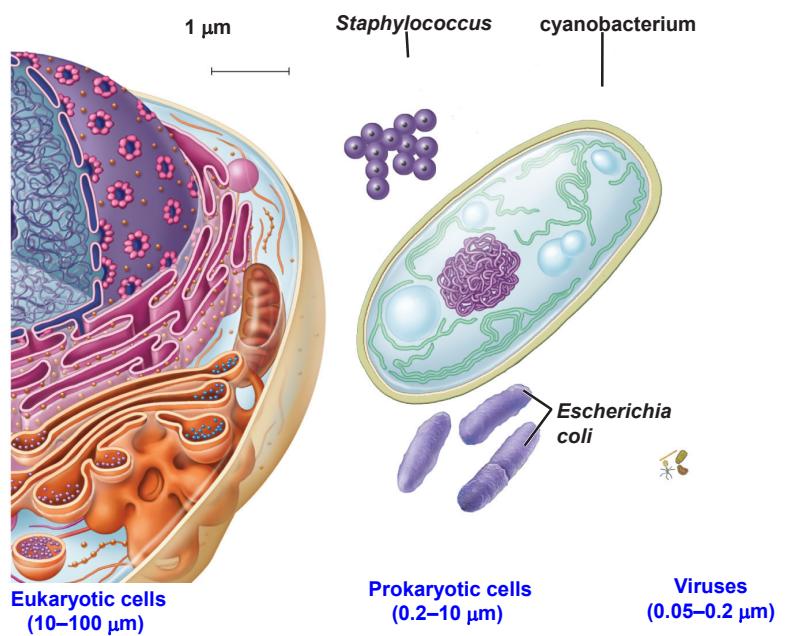
Structure

- Plasma membrane
 - Encloses cell and keeps cell as distinct entity from environment
 - **Boundary** that **controls passage** of substances in and out of cell
- DNA-containing region
 - Nucleus – in eukaryotes
 - **Nucleoid** region – in prokaryotes
- Cytoplasm
 - Fluid portion and structures between plasma membrane and DNA-containing region
 - Fluid portion contains water, salts and organic molecules – cytosol
 - Structures with specific functions – **organelles**

12

For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

The Sizes of Microorganisms



13

Outline

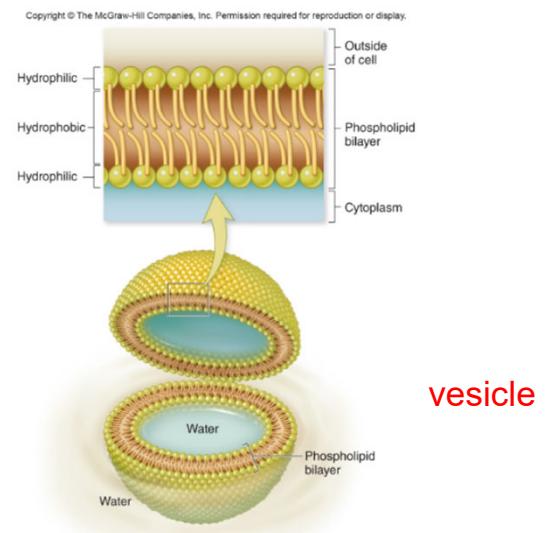
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14

For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

Membrane Structure

- **Fluid mosaic model**
 - Mosaic mixture of phospholipids, steroids and proteins
 - Constantly drifting and moving within viscous fluid of phospholipid bilayer
- **Phospholipid bilayer**
 - Forms **selectively permeable** barrier
 - Some are glycolipids, with attached carbohydrate chains



15

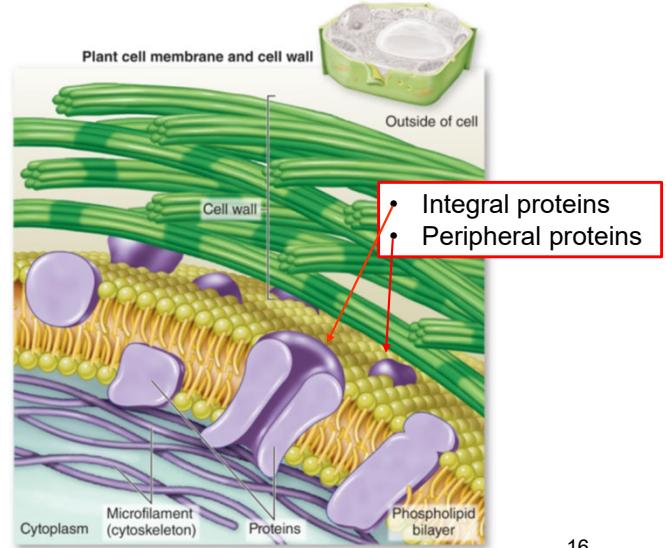
Now let's have a closer look at cell membranes.

As you now know, all cells, as well as many of the organelles within eukaryotic cells, are surrounded by membranes. Cell membranes perform several functions:

Membrane Structure (plant cell)

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- Associated proteins
 - Float around like icebergs on sea
 - Some **anchored** by protein filaments in cytoplasm
 - Cell wall** outside plasma membrane
 - Some are partially or wholly **embedded** in bilayer
 - Some are **temporarily** attached to surface of bilayer



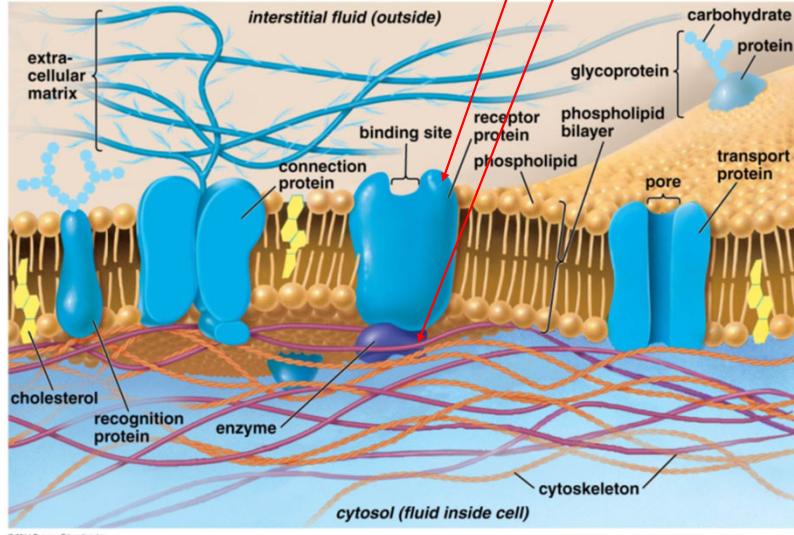
16

Now let's have a closer look at cell membranes.

As you now know, all cells, as well as many of the organelles within eukaryotic cells, are surrounded by membranes. Cell membranes perform several functions:

Membrane Structure (animal cells)

- Associated proteins
 - Responsible for variety of membrane functions – **adhesion, enzymes, receptors, recognition, transport.**
- Glycoproteins
 - Carbohydrates attached to protein



17

Now let's have a closer look at cell membranes.

As you now know, all cells, as well as many of the organelles within eukaryotic cells, are surrounded by membranes. Cell membranes perform several functions:

Cell membranes functions

- Plasma membrane
 - Common to all cells
 - Separates cell's internal cytoplasm from external environment
 - Controls traffic of substances in and out of cell (transporters)
 - Regulates biochemical reactions (receptors)
 - Communicates with other cells (glycoproteins)
 - Creates attachments within (cytoskeleton) and between cells
- Basic structure of all membranes in cells – similar
 - Bilayer of phospholipids with embedded and attached proteins

18

For nearly 200 yrs, Many scientists examined different cells from different organisms, they found three common characteristics among the cells, which together constitute the cell theory.

Case Study: Bites from rattlesnakes and spiders



(a) Justin's rattlesnake bite



(b) Brown recluse spider bite

19

THIRTEEN-YEAR-OLD JUSTIN SCHWARTZ was enjoying his 3-week stay at a summer camp near Yosemite National Park.

But that all changed when, after hiking 4.5 miles, Justin rested on some sunny rocks, hands hanging loosely at his sides.

Suddenly, he felt a piercing pain in his left palm. A 5-foot rattlesnake—probably feeling threatened by Justin's dangling arm—had struck without warning. Justin spent more than a month undergoing 10 surgeries.

These relieved the enormous pressure from swelling in his arm removed dead muscle tissue, and began the long process of repairing the extensive damage to his hand and arm.

Diane had grown accustomed to spiders—which are often harmless—but this was an exception: a brown recluse. The two small puncture wounds seemed merely a minor annoyance until the next day, when an extensive, itchy rash appeared at the site. By the third day, intermittent pain pierced like a knife through her thigh. A physician gave her painkillers, steroids to reduce the swelling, and antibiotics to combat

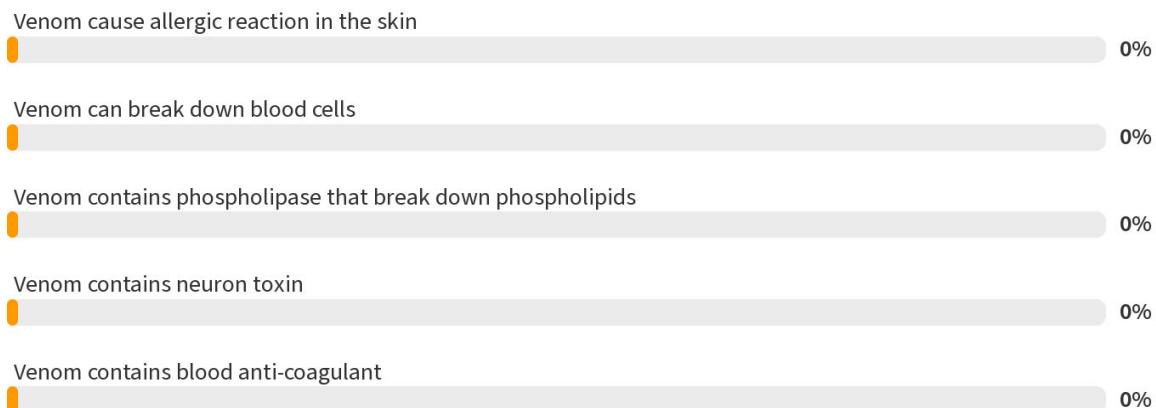
bacteria introduced by the spider's mouthparts.

The next 10 days were a nightmare of pain from the growing sore, now covered with oozing blisters and underlain with clotting blood. It took 4 months for the lesion to heal. Even a year later, Diane sometimes felt pain in the large scar that remained.

How do rattlesnake and brown recluse spider venoms cause leaky blood vessels, disintegrating skin and tissue, and sometimes life-threatening symptoms throughout the body? What do venoms have to do with cell membranes?

How do rattlesnake venom cause leaky blood vessels and disintegrating skin and tissue throughout the victim body?

0



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Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

More info at polleverywhere.com/support

How do rattlesnake venom cause leaky blood vessels and disintegrating skin and tissue throughout the victim body?

https://www.polleverywhere.com/multiple_choice_polls/b5mc493vGLxMPr1g2wy6L?state=opened&flow=Default&onscreen=persist

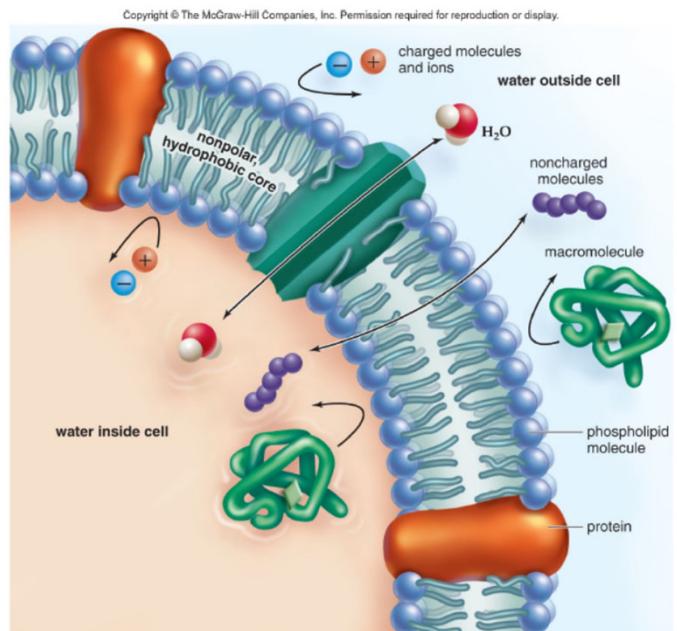
Transport of Substance Across Membranes

I. Passive transport: no energy required

- (a) simple diffusion
- (b) facilitated diffusion (require protein transporter)

II. Active transport: energy-requiring transport

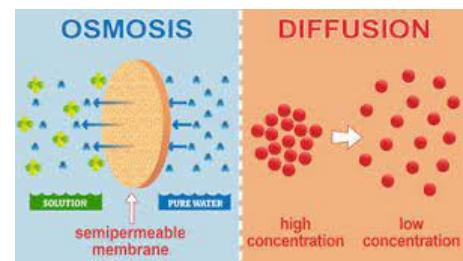
Such as sodium-potassium pump proteins



Having known the membrane structure, let's now focus on one of the most important properties of cell membrane, the ability to regulate transport across cellular boundaries, a function essential to the cell's existence. How structure fits function.

Small solutes/molecules can move across membrane by passive and active transport, however, larger molecules, such proteins and polysaccharides, as well as larger particles, generally cross the membrane involving packaging in vesicles.

I. Passive Transport: Diffusion



- **Diffusion**
 - Net movement of molecules down a concentration gradient (high to low concentration)
- **Osmosis** – special case of diffusion
 - Diffusion of **water** across a **selectively permeable membrane** down its own concentration gradient
 - From high water (low solute) concentration to low water (high solute) concentration

22

Passive transport includes simple diffusion, facilitated diffusion, and osmosis.

Diffusion always occurs down concentration gradients, it requires no expenditure of energy. A concentration gradient drives the diffusion of a solute across a cell membrane, with or without the assistance of a transport protein.

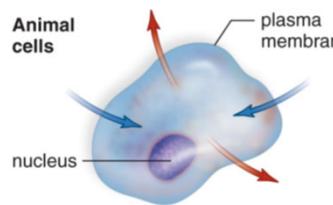
Movement of water across membranes by osmosis

- **Isotonic** solution
 - Solute concentration **equal** on both sides of membrane
- **Hypotonic** solution
 - Solute concentration **lower** than on other side of membrane
- **Hypertonic** solution
 - Solute concentration **higher** than on other side of membrane

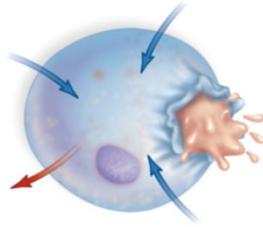
23

Passive transport includes simple diffusion, facilitated diffusion, and osmosis.

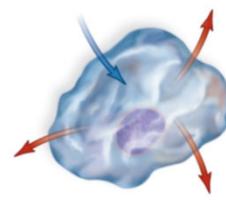
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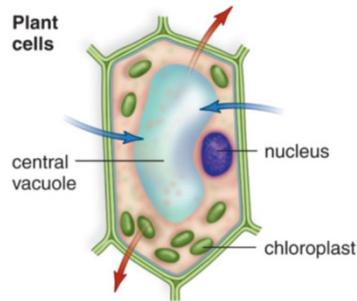
In an isotonic solution, there is no net movement of water.



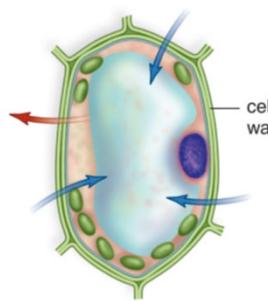
In a hypotonic solution, water mainly enters the cell, which may burst (lysis).



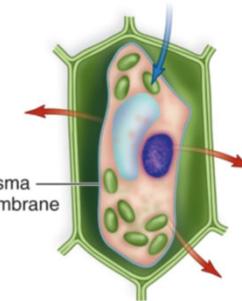
In a hypertonic solution, water mainly leaves the cell, which shrivels (crenation).



In an isotonic solution, there is no net movement of water.



In a hypotonic solution, vacuoles fill with water, turgor pressure develops, and chloroplasts are seen next to the cell wall.



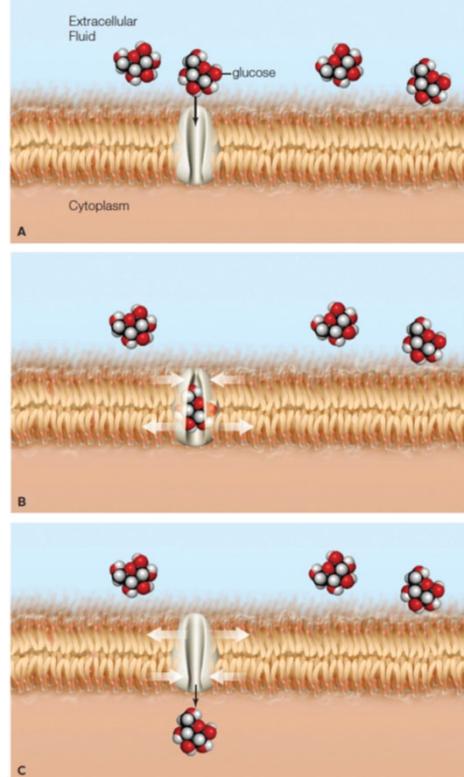
In a hypertonic solution, vacuoles lose water, the cytoplasm shrinks (plasmolysis), and chloroplasts are seen in the center of the cell.

Passive transport includes simple diffusion, facilitated diffusion, and osmosis.

Diffusion always occurs down concentration gradients, it requires no expenditure of energy. A concentration gradient drives the diffusion of a solute across a cell membrane, with or without the assistance of a transport protein.

Movement across membranes

- **Facilitated diffusion** (facilitated transport)
 - Ions and polar molecules
 - **No energy** required
 - **Follow their concentration gradients**
 - Transport proteins (conformational change) required
 - Specialised water-channel proteins, **aquaporins**, enhance water transport



25

Passive transport includes simple diffusion, facilitated diffusion, and osmosis.

Diffusion always occurs down concentration gradients, it requires no expenditure of energy. a concentration gradient drives the diffusion of a solute across a cell membrane, with or without the assistance of a transport protein.

II. Active Transport

The movement of materials across a membrane through the **use of cellular energy**, normally **against** a concentration gradient

- Active transport is performed by specific **proteins (pump)** embedded in the membranes
- Active transport allows cells to maintain concentration gradients that **differ from their surroundings**

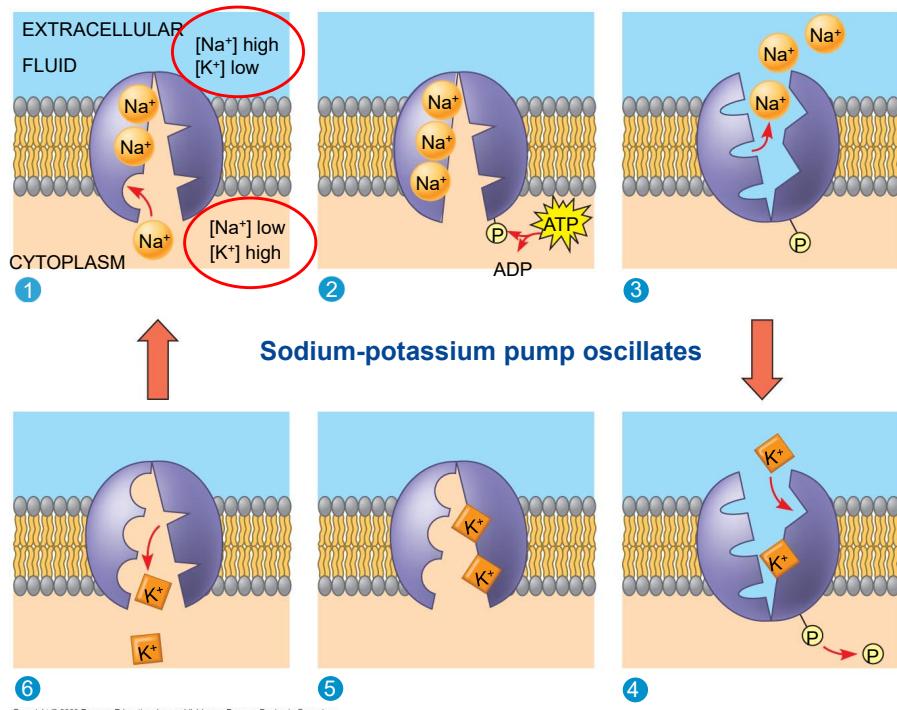
e.g. keep a low concentration of Na^+ inside cell even the Na^+ concentration is very high outside cell.

26

Now let's turn our attention to active transport.

All cells need to move some materials “uphill” across their plasma membranes against concentration gradients. For example, every cell requires some nutrients that are less concentrated in the environment than in the cell’s cytosol. Other substances, such as sodium is maintained at much lower concentrations inside the cell than in the extracellular fluid.

In active transport, membrane proteins use cellular energy to move molecules or ions across the plasma membrane against their concentration gradients.



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27

This figure depicts the sodium-potassium pump: a specific case of active transport.

This transport system pumps ions against steep concentration gradients: sodium ion concentration (represented as Na^+) is high outside the cell and low inside, while potassium ion concentration (K^+) is low outside the cell and high inside. The pump oscillates between two shapes in a pumping cycle that translocates three sodium ions out of the cell for every two potassium pumped into the cell. The two shapes have different affinities for the two types of ions. ATP powers the shape change by phosphorylating the transport protein.

Factors which contribute to a cell's membrane potential (net negative charge on the inside):

Negatively charged proteins in the cell's interior.

Plasma membrane's selective permeability to various ions.
For example, there is a net loss of positive charges as K^+ leaks out of the cell faster than Na^+ diffuses in.

The sodium-potassium pump. This electrogenic pump translocates 3 Na^+ out for every 2 K^+ in - a net loss of one positive charge per cycle.

Electrogenic Pump

A transport protein that generates voltage across a membrane.

Na^+/K^+ - ATPase is the major electrogenic pump in animal cells.

A proton pump is the major electrogenic pump in plants, bacteria and fungi. Mitochondria and chloroplasts use a proton pump to drive ATP synthesis.

Voltages created by electrogenic pumps are sources of potential energy available to do cellular work.

The Na^+/K^+ pump has been shown to control and set the intrinsic activity mode of cerebellar Purkinje neurons.[7] This suggests that the pump might not simply be a homeostatic, "housekeeping" molecule for ionic gradients; but could be a computation element in the cerebellum and the brain. Indeed, a mutation in the Na^+/K^+ pump causes rapid onset dystonia parkinsonism, which has symptoms to indicate that it is a pathology of cerebellar computation.[8] Furthermore, an ouabain block of Na^+/K^+ pumps in the cerebellum of a live mouse results in it **displaying ataxia and dystonia**.[9] The distribution of the Na^+/K^+ pump on myelinated axons, in human brain, was demonstrated to be along the internodal axolemma, and not within the nodal axolemma as previously thought

The pump is pivotal to the body's function

The sodium-potassium pump is a vital enzyme found in all human cells which constantly maintains an optimal ion balance. This uses up a great deal of energy - about a fourth of the body's energy, the so-called ATP, is used to keep the pump going; in the [brain](#) the share is nearly 70%.

The sodium-potassium pump works by pumping two potassium ions into the cell and pumping out three sodium ions using the energy from an ATP molecule. This leads to a build-up of considerable differences in the concentration of the ions on the outside and inside of the cell. Differences which are essential for the communication and transport in and out of the cell of nutrients and other compounds - and for the regulation of the cell's pH and volume. If the pump does not function properly in brain cells, the result is severe neurological conditions such as [migraine](#) with aura, muscle spasms or unilateral [paralysis](#) (hemiplegia).

Knowledge of the pump is therefore crucial for our understanding of the matter and energy balance and of the disease mechanisms which come into play when the pump does not function properly. This knowledge is also important in order to develop new medicines targeting the pump.

Animation of membrane transport (video)

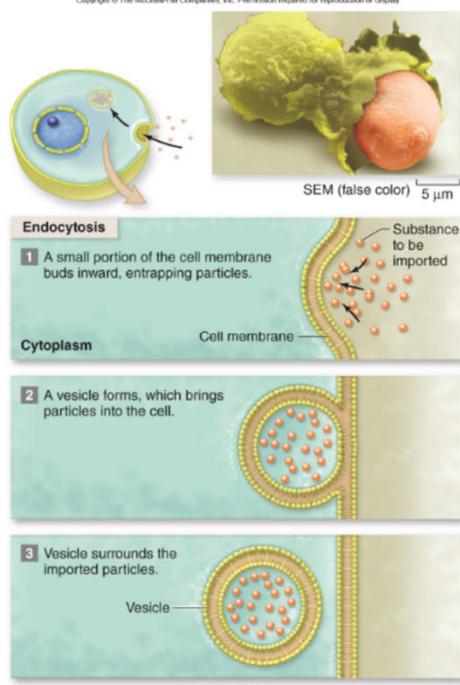
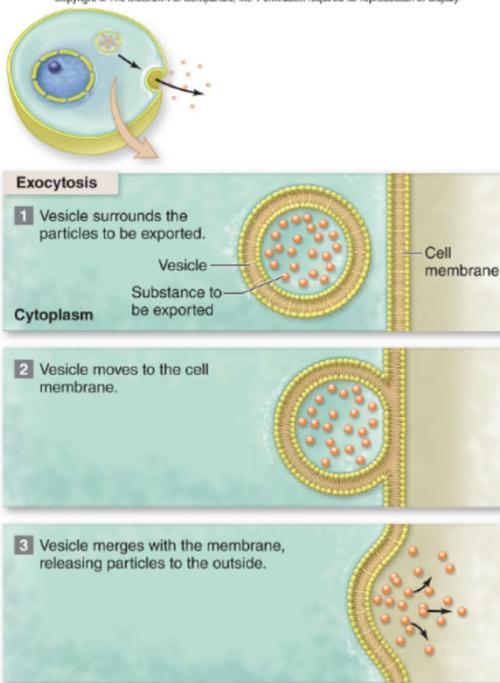


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Play video (2.44 mins)

Movement Across Membranes

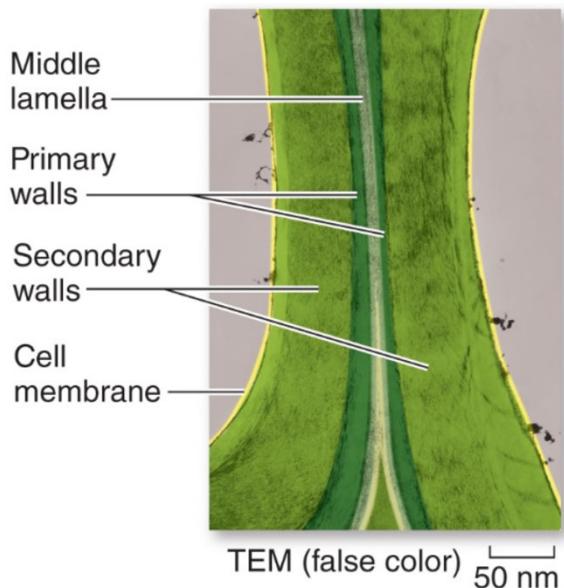
- Large substances are transported into or out of cell inside membrane enclosed pouches (**vesicles**)
 - **Macromolecules** and particles too large for transport proteins
 - Formation and movement of vesicles involve motor proteins and ATP
- **Exocytosis**
 - Vesicle fuses with plasma membrane, releasing contents outside cell
- **Endocytosis**
 - Small patch of plasma membrane **pinched off**, forming vesicle, **engulfing** substances near cell surface, bringing them into cell



Cell Walls

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- Relatively stiff structural coating that surround plasma membranes
 - Strong, flexible and porous
 - Support and protect fragile cell
- Found in nearly all
 - Bacteria
 - Archaea
 - Protists
 - Fungi – chitin
 - Plants – cellulose



Which of the following(s) is/are role of the plasma membrane?

0

- Regulate transport in and out of a cell. 0%
- Receive signal and control activities in the cell. 0%
- Form a boundary between the nucleus and the cytoplasm. 0%
- Modifies, sorts and distributes protein. 0%
- Attachment of cells to extra-cellular matrix. 0%
- Cell-cell recognition and communication. 0%

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Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

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Which of the following(s) is/are role of the plasma membrane?

https://www.polleverywhere.com/multiple_choice_polls/Q3W0I3RPeAOjxvNtys7or?state=opened&flow=Default&onscreen=persist

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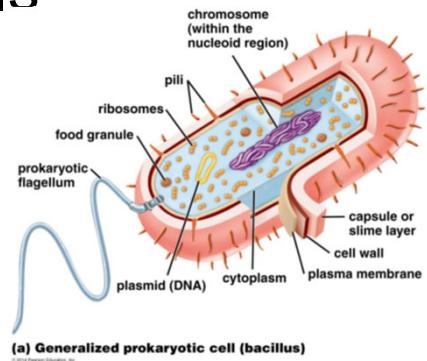
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Specialised surface features

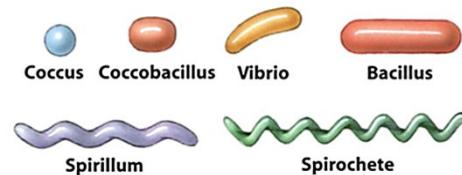
- Cell envelope
- Appendages

Prokaryotic Cells

- Extremely small
 - 1 to 1.5 μm wide
 - 2 to 6 μm long
- Specialised surface features
 - Cell envelope
 - Appendages
- Prokaryotic cells can take several shapes:
 - Rod-shaped
 - Spiral-shaped
 - Spherical



(a) Generalized prokaryotic cell (bacillus)



34

Specialised surface features

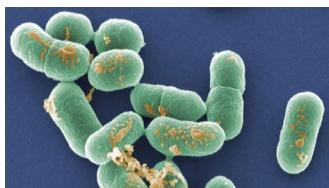
- Cell envelope
- Appendages

Case Study: The National Environment Agency (NEA) has banned the use of all freshwater fish in ready-to-eat raw fish dishes (Yusheng) with immediate effect on Dec 5 2015.

Group B streptococcus (GBS); Sequence Type 283, ST283



Fourth person dies in Australia from contaminated rock melon (The Straits Times, 7 Mar 2018)



35

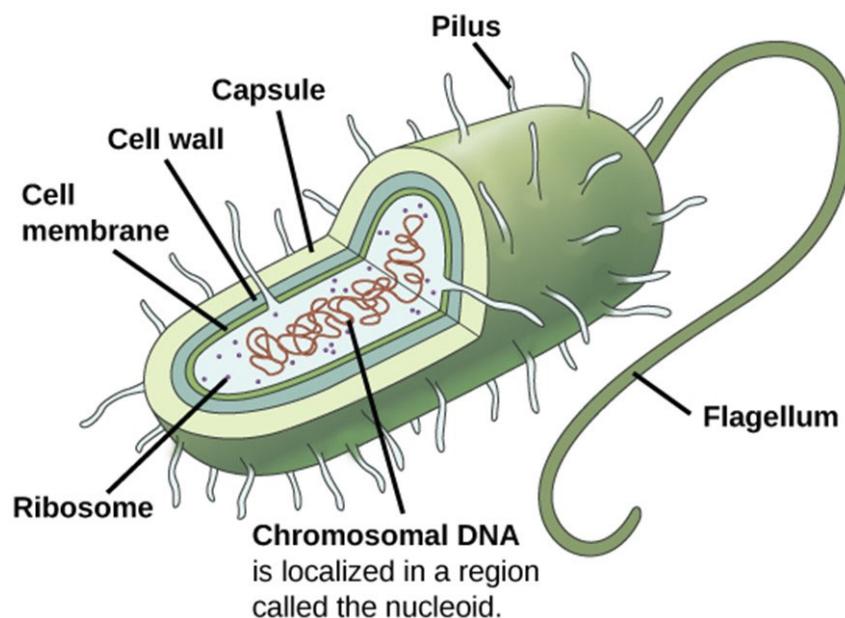
Strep B is a bacterium commonly found in the gut and urinary tract of about 15 to 30 per cent of adults without causing any disease. But it may occasionally cause infections of the skin, joints, heart and brain. It can lead to meningitis, which is an inflammation of the membranes surrounding the brain and spinal cord. Especially dangerous for Babies.

Tests conducted by Agri-Food & Veterinary Authority of Singapore (AVA) and NEA have found such fish to have significantly higher bacterial contamination than saltwater fish, and are likely to present higher risks of infection when consumed raw. "Cooking raw food thoroughly with sufficient heat is still the most effective way to destroy microbial pathogens - ensuring the fishes are safe to eat." All retail food establishments that wish to sell raw fish dishes, such as yusheng dishes, are henceforth to use only saltwater fish intended for raw consumption.

Contaminated Rockmelon: Listeriosis: Listeriosis is a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*. An estimated 1,600 people get listeriosis each year, and about 260 die. The infection is most likely to sicken pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems. Why was there Listeria bacteria on rockmelons?

Adverse weather events listed as heavy rainfall in December prior to harvest, followed by dust storms were blamed for increasing the organic load and amount of Listeria on rockmelons prior to harvest. Another issue is the netted skin of the fruit making it harder to clean and sanitize.

Prokaryotic Cells Are Simpler Than Eukaryotic Cells



36

A rigid cell wall surrounds the plasma membrane of nearly all prokaryotes, and sticky polysaccharides often envelop bacterial cell walls, this layer is named as capsule. These two layers provide protection to the cell interior structure, and are permeable to dissolved substances.

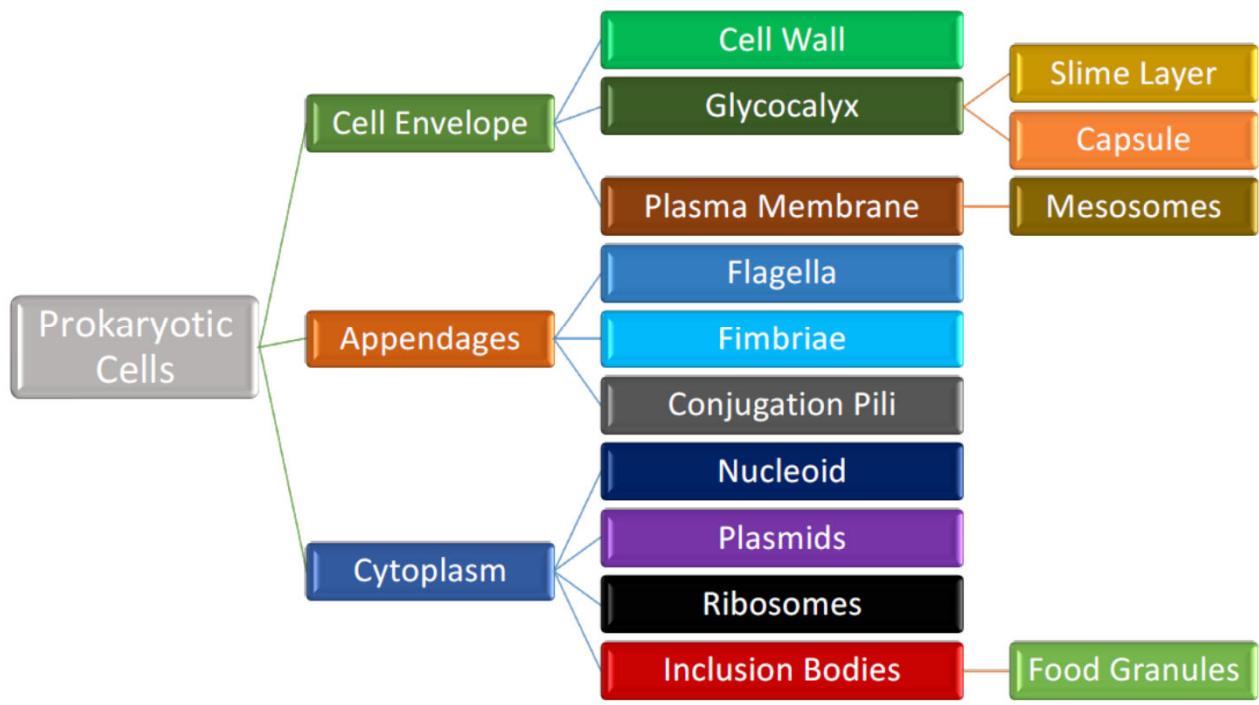
The composition of their cell walls (peptidoglycan) also differs from the eukaryotic cell walls found in plants (cellulose) or fungi and insects (chitin). The cell wall functions as a protective layer and is responsible for the organism's shape. Some bacteria have a capsule outside the cell wall. Other structures are present in some prokaryotic species, but not in others. For example, the capsule found in some species enables the organism to attach to surfaces, protects it from dehydration and attack by phagocytic cells, and increases its resistance to our immune responses. Some species also have flagella used for locomotion and pili used for attachment to surfaces. Plasmids, which consist of extra-chromosomal DNA, are also present in many species of bacteria and archaea.

The plasma membrane selectively controls the flow of substances into and out of the cytoplasm. Its lipid bilayer bristles with protein channels, transporters, and receptors, and it incorporates built-in machinery for reactions.

The cytoplasm contains many ribosomes on which polypeptide chains are built. DNA is concentrated in an irregularly shaped region of cytoplasm called the nucleoid.

Many species have one or more bacterial flagella. They are designed either to move the cell itself or to move substances over or around the cell. In this regard, they are a lot like the legs and arms of humans. Many bacteria also have fimbriae, attachment structures. Some of these may develop into sex pili for gene transfer between bacteria.

Figure 6.6 A prokaryotic cell



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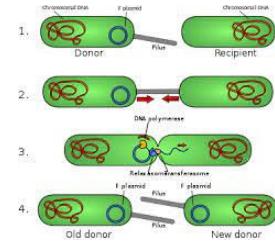
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Figure 6.6 A prokaryotic cell

Prokaryotic cells

- Cell envelope
 - Cell wall – polysaccharides in bacteria, proteins in archaea
 - Glycocalyx – polysaccharide coating outside cell wall – slime layer and capsule (harder to remove)
 - Plasma membrane
- Appendages
 - Flagella – in some bacteria for motility
 - Fimbriae (attachment pili) – bristle-like, short and abundant, for adhesion to surfaces
 - Conjugation pili (sex pili) – tubular, long and fewer, for DNA transfer from cell to cell



38

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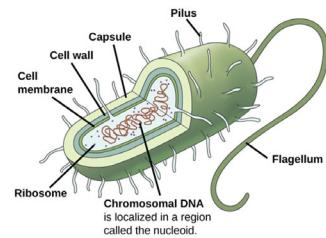
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Figure 6.6 A prokaryotic cell

Prokaryotic Cells



- **Cytoplasm**
 - Fewer specialised cytoplasmic structures
 - **Nucleoid** – irregularly shaped region of single, **circular** chromosome of DNA molecule
 - **Plasmids** – small rings of DNA with few genes that may be advantageous, e.g. resistance to antibiotics
 - **Ribosomes** – organelles on which proteins are synthesized
 - May contain **inclusion bodies**, e.g. food granules

39

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Figure 6.6 A prokaryotic cell

Outline

- Cells
 - Cell theory
 - Cell size
 - Structure
- Cell Membranes
 - Membrane structure
 - Movement across membranes
 - Cell walls
- Prokaryotic Cells
- Eukaryotic Cells
 - Nucleus
 - Endomembrane system
 - Energy-related organelles
 - Cytoskeleton

40

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Figure 6.6 A prokaryotic cell

Eukaryotic Cells

- Much **larger** than prokaryotic cells
- DNA in a **nucleus** that is bounded by a membranous **nuclear envelope**
- **Membrane-enclosed organelles** result in **compartmentalisation**
 - Specialised organelles for specific functions
 - Types and amounts of substances that enter and exit organelles **regulated**
 - Maintains **special internal environments**
 - Metabolic reactions **isolated** from others

Table 4.2 Components of Eukaryotic Cells

Name	Main Function
Organelles with membranes	
Nucleus	Protecting, controlling access to DNA
Endoplasmic reticulum (ER)	Routing, modifying new polypeptide chains; synthesizing lipids
Golgi body	Modifying new polypeptide chains; sorting, shipping proteins and lipids
Vesicles	Transporting, storing, or digesting substances in a cell
Mitochondrion	Making ATP by glucose breakdown
Chloroplast	Photosynthesis in plants, some protists
Lysosome	Intracellular digestion
Peroxisome	Inactivating toxins
Vacuole	Storage
Organelles without membranes	
Ribosomes	Assembling polypeptide chains
Centriole	Anchor for cytoskeleton
Other components	
Cytoskeleton	Contributes to cell shape, internal organization, movement

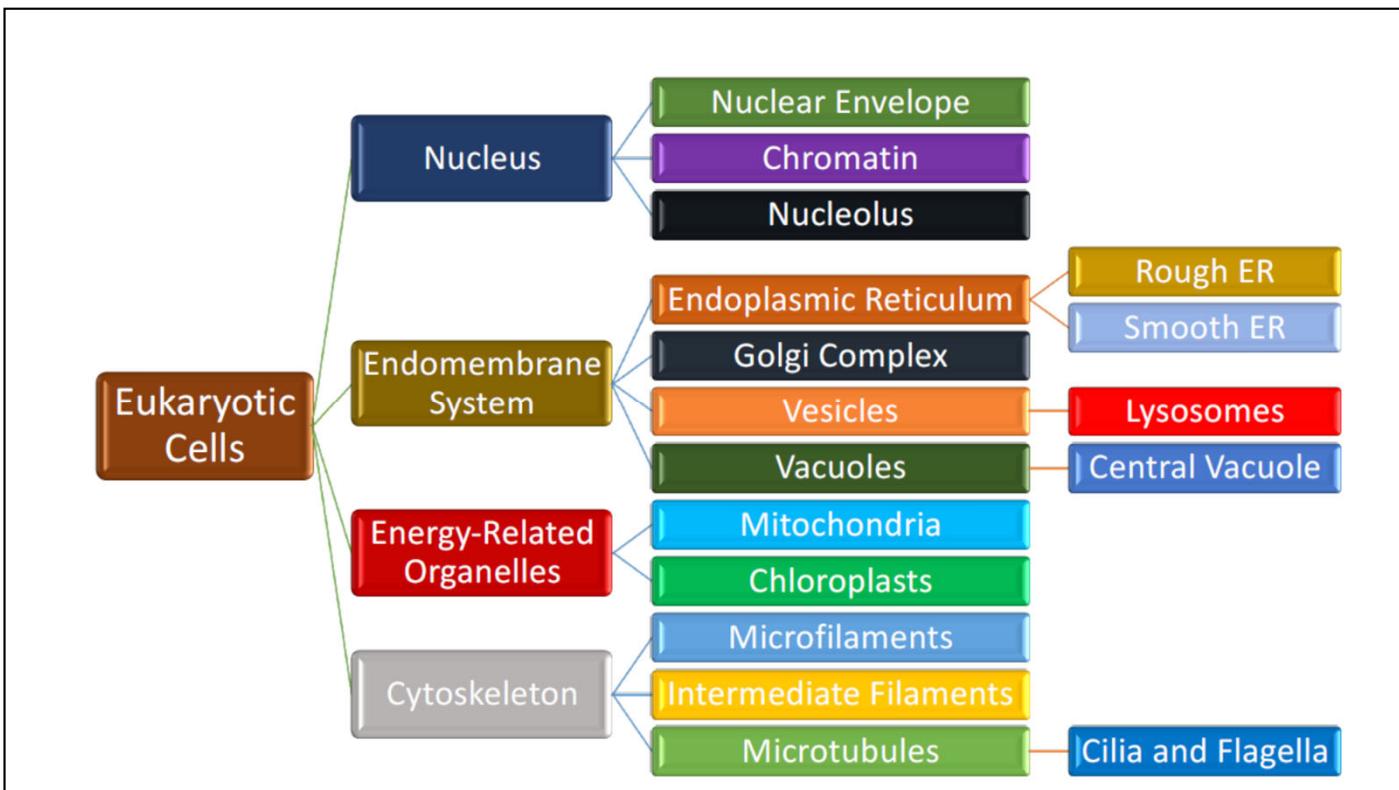
41

Despite their diversity, all cells share common features, that is:

_Each cell is surround by an extremely thin, rather fluid membrane called the plasma membrane.

_All cells use DNA as a hereditary blueprint that stores the instructions for making the other parts of the cell and for producing new cells.

_the cytoplasm consists of all the material and structures that lie inside the plasma membrane, but outside the region of the cell that contains DNA



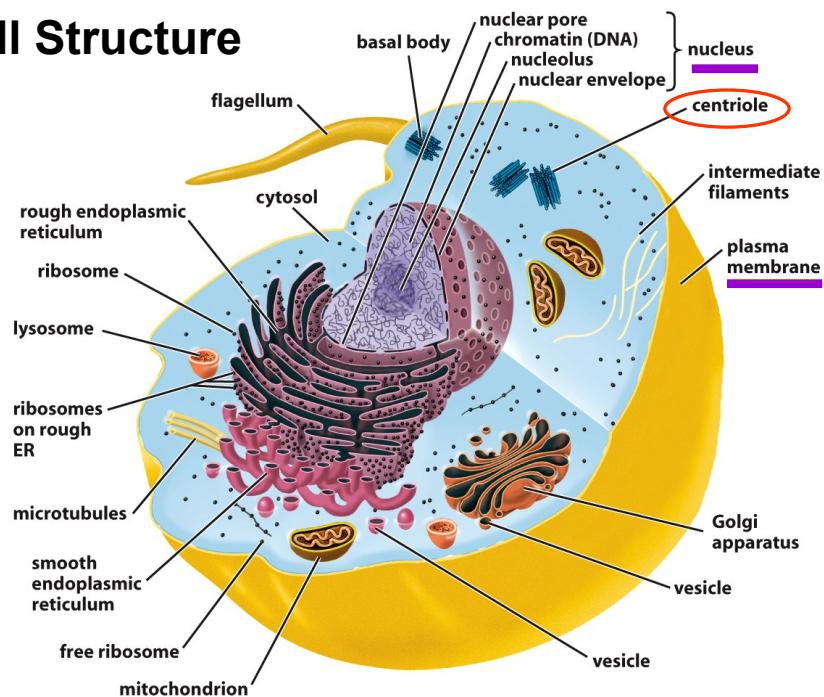
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Animal Cell Structure



43

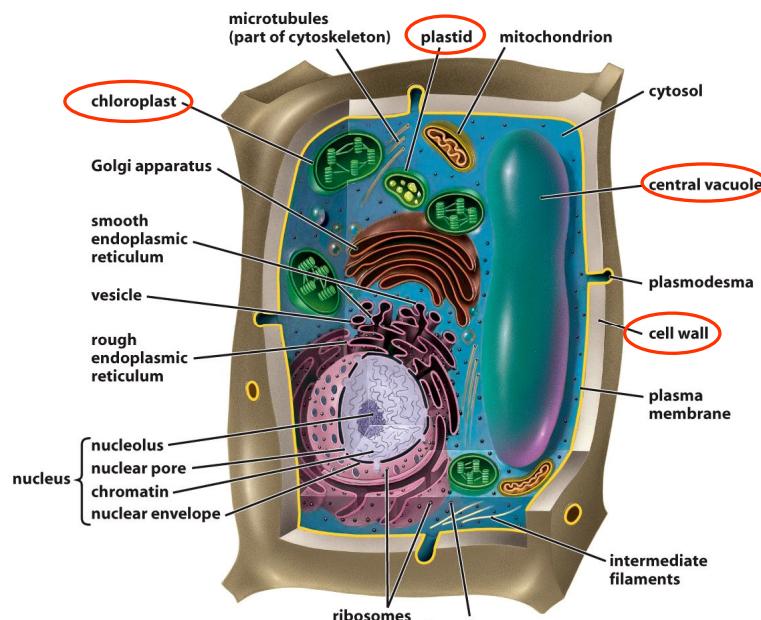
This is a figure showing typical structure of an animal cell.

Centrioles are only found in animal cells and in some plant cells :
Produce the basal bodies of cilia and flagella

A single pair of centrioles is found in animal cells, and these play a role in organizing cytoskeletal proteins during cell division (described in Chapter 9).

FIGURE 4-3 A generalized animal cell

Plant cell Structure



44

Vacuoles are large membrane-bound compartments within some eukaryotic cells where they serve a variety of different functions: capturing food materials or unwanted structural debris surrounding the cell, sequestering materials that might be toxic to the cell, maintaining fluid balance (called turgor) within the cell, exporting unwanted substances from the cell, or even determining relative cell size. The cavity that is the vacuole is considered nonprotoplasmic and the contents classified as ergastic according to some authors (Esau, 1965). Vacuoles are especially conspicuous in most plant cells.

Vacuoles are typically filled with a liquid called **cell sap**, the composition of which can vary (even between vacuoles in the same cell), but is principally water. Water tends to move along concentration gradients into vacuoles. Vacuoles perform different roles in different organisms and these functions include the capture of food, the maintenance of internal hydrostatic pressure (store water), the containment of waste products, the maintenance of an acidic internal pH, the storage of small molecules and finally can enable a cell to elongate rapidly.

Read more:

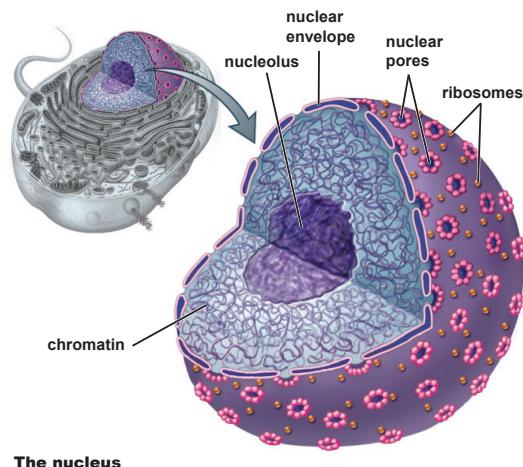
http://wiki.answers.com/Q/What_do_central_vacuoles_do#ixzz1GZCbrAY
8

Here is the figure of a plant cell, its unique structures are highlighted in red circles.

FIGURE 4-4 A generalized plant cell

Nucleus

- Control center of the eukaryotic cell (stores DNA)
- Three major components
 - Nuclear envelope (double membrane)
 - Chromatin
 - Nucleolus (carrying genes for ribosomes production)

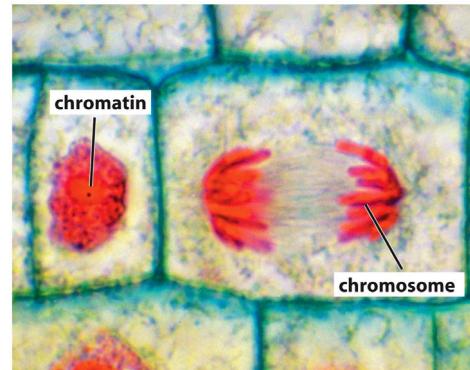


45

Nucleus is generally the most conspicuous organelle in a eukaryotic cell, the nuclear envelope encloses the nucleus, separating its contents from the cytoplasm. It houses most of the cell's DNA. In a cell, the DNA is often associated with proteins, forming a structure named Chromatin.

Chromatin and Chromosome

- Consists of **DNA** and **proteins**
- Chromatin **condenses** to form **chromosomes** during cell division
- Chromatin are **long thin fibres**, but chromosomes are compacted, **thick and ribbon-like**.
- They contain genes that provide a blueprint for a huge variety of proteins



To do this, genetic information in DNA is copied into messenger RNA (mRNA), which travels through the nuclear pores to the cytoplasm, where it directs protein synthesis

46

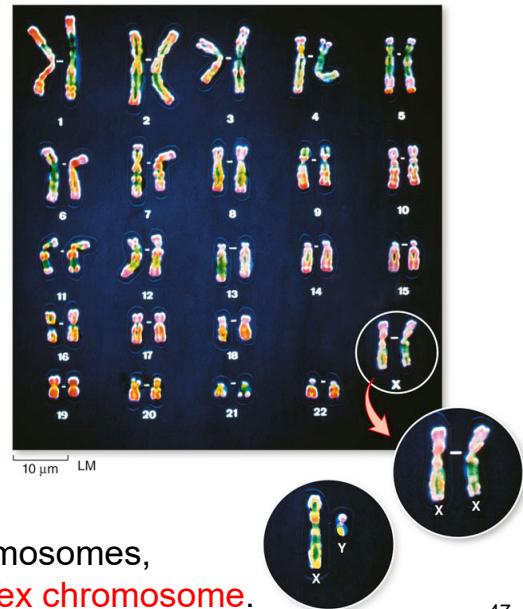
When cells divide, chromatin replicated and condensed to become chromosome, which are easily visible with light microscopes.

In the nucleus, DNA and proteins form genetic material called **chromatin**
Chromatin condenses to form discrete **chromosomes**

Tangled threads (is in a mess)

Chromosomes

- Every eukaryotic species has a characteristic number of chromosomes in each cell nucleus
- Somatic cells** (non-reproductive cells) have **two** sets of chromosomes, one from the father and one from the mother.
- Gametes** (Gametic cells, reproductive cells: sperm and eggs) have **half** as many chromosomes as somatic cells



47

Each of human body cells has **23 pairs** of chromosomes, including 22 pairs of **autosomes** and 1 pair of **sex chromosome**.

Each of our human body cells, we called them somatic cells in biology, has 23 pairs of DNA molecules, they are packaged into 23 pairs of chromosomes. As shown here, the number 1 is the longest chromosome pair, and the number 22 is the shortest. In each pair, one is from mother, the other is from father. There is a special pair of chromosomes, we called sexual chromosomes. It is XX in female, and XY in male.

They are similar but not the same. However, in the gametic cells, ie. Sperms and eggs, there are only half as many chromosomes as somatic cells. In humans, an individual with two X chromosomes is female, and an individual with one X and one Y chromosome is male.

sə'mæt.ɪk

In other living organisms, the chromosome number will be different, for example, Dogs have 39 pairs of chromosomes, rice has 24 pairs, mosquitos have 6 pairs, bacteria usually have one only, but butterflies have more than 100 pairs.

TABLE 4-1**Functions and Distribution of Cell Structures**

Structure	Function	Prokaryotes	Eukaryotes: Plants	Eukaryotes: Animals
Cell Surface				
Extracellular matrix	Surrounds cells, providing biochemical and structural support	Absent	Present	Present
Cilia	Move the cell through fluid or move fluid past the cell surface	Absent	Absent (in most)	Present
Flagella	Move the cell through fluid	Present ¹	Absent (in most)	Present
Plasma membrane	Isolates the cell contents from the environment; regulates movement of materials into and out of the cell; allows communication with other cells	Present	Present	Present
Organization of Genetic Material				
Genetic material	Encodes the information needed to construct the cell and to control cellular activity	DNA	DNA	DNA
Chromosomes	Contain and control the use of DNA	Single, circular	Many, linear	Many, linear
Nucleus ²	Contains chromosomes and nucleoli	Absent	Present	Present
Nuclear envelope	Encloses the nucleus; regulates movement of materials into and out of the nucleus	Absent	Present	Present
Nucleolus	Synthesizes ribosomes	Absent	Present	Present
Cytoplasmic Structures				
Ribosomes	Provide sites for protein synthesis	Present	Present	Present
Mitochondria ²	Produce energy by aerobic metabolism	Absent	Present	Present
Chloroplasts ²	Perform photosynthesis	Absent	Present	Absent
Endoplasmic reticulum ²	Synthesizes membrane components, proteins, and lipids	Absent	Present	Present
Golgi apparatus ²	Modifies, sorts, and packages proteins and lipids	Absent	Present	Present
Lysosomes ²	Contain digestive enzymes; digest food and worn-out organelles	Absent	Absent (in most)	Present
Plastids ²	Store food, pigments	Absent	Present	Absent
Central vacuole ²	Contains water and wastes; provides turgor pressure to support the cell	Absent	Present	Absent
Other vesicles and vacuoles ²	Transport secretory products; contain food obtained through phagocytosis	Absent	Present	Present
Cytoskeleton	Gives shape and support to the cell; positions and moves cell parts	Present	Present	Present
Centrioles	Produce the basal bodies of cilia and flagella	Absent	Absent (in most)	Present

¹Some prokaryotes have structures called flagella, which lack microtubules and move in a fundamentally different way than do eukaryotic flagella.²Indicates organelles, which are surrounded by membranes and found only in eukaryotic cells.

Endomembrane System (Cell's Membrane System)

- Components of the **endomembrane system**:
 - Nuclear envelope
 - Endoplasmic reticulum
 - Golgi apparatus/complex
 - Lysosomes
 - Vacuoles
 - Plasma membrane
- These components are either **continuous** or connected via transfer by **vesicles**/vacuoles

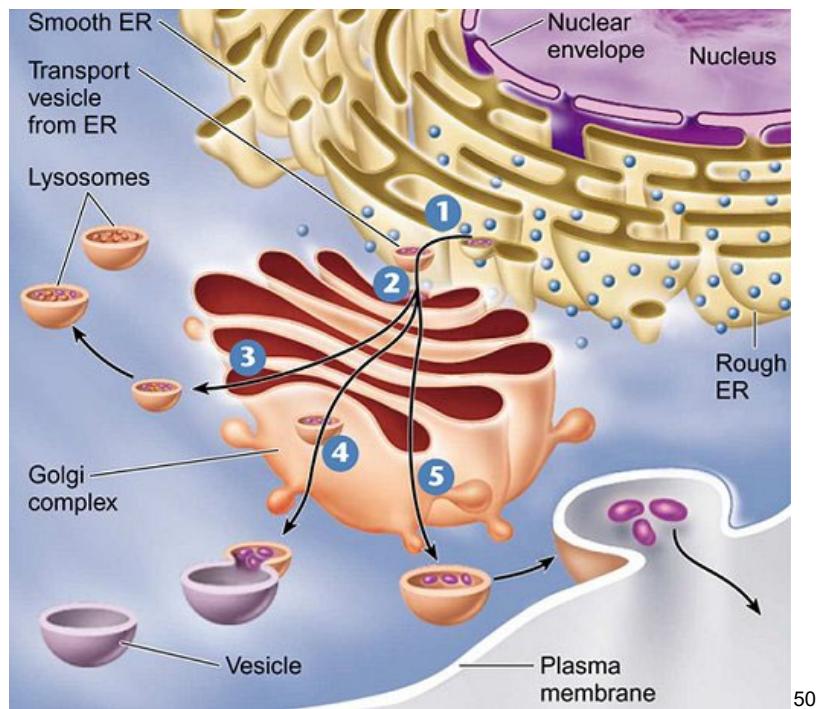
49

Now let's move on to the endomembrane system. It is a group of membranes and organelles in eukaryotic cells that works together to modify, package, and transport lipids and proteins. It includes the nuclear envelope, endoplasmic reticulum, Golgi apparatus, lysosomes, vesicles, and plasma membrane, they are either directly connected or share membrane and contents by trafficking vesicles . The endomembrane system does not include the membranes of either mitochondria or chloroplasts. Let's explore a few common organelles.

Endomembrane System

Functions:

- Synthesizes, modifies, and transports **proteins**
- Synthesizes **lipids**
- Detoxifies the cell of certain toxins



50

Endoplasmic Reticulum (ER)

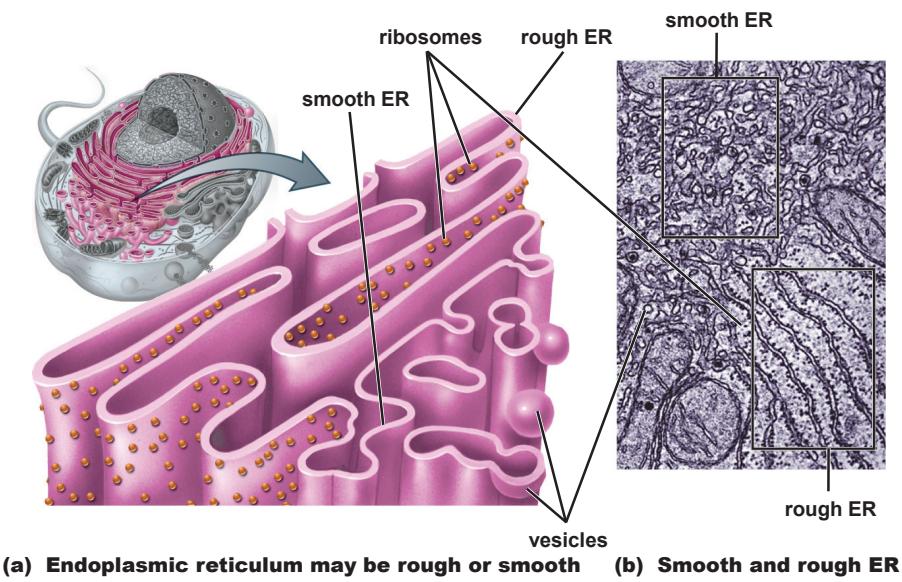
(Endoplasmic means “within the cytoplasm” and reticulum means “network”)

- A network of **sacs** and **tubules** composed of membranes. This complex organelle originates at the **nuclear envelope** and winds throughout the cell.
- Two forms
 - Rough ER
 - Smooth ER

51

Endoplasmic reticulum is an extension of the nuclear envelope, it forms a continuous compartment that folds over and over into flattened sacs and tubes.

Endoplasmic Reticulum



52

There are two kinds of ER, named for their appearance in electron micrographs. One is the Rough ER, many thousands of ribosomes attach to the outer surface of rough ER. The other is smooth ER, without ribosomes on the surface.

Figure 6.12 Endoplasmic reticulum (ER)

I. Rough ER

- Flattened sacs
- Studded **with ribosomes** on outer surface
- **Synthesises, modifies and folds proteins**

II. Smooth ER

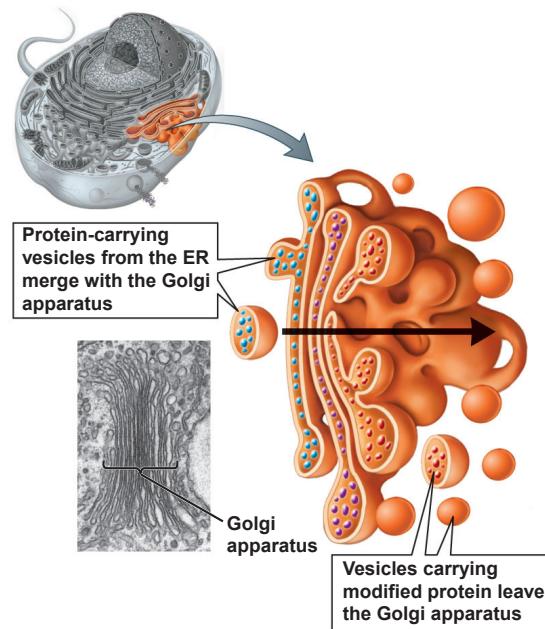
- Series of inter-connected tubules
- **No ribosomes**
- **Synthesises lipids** (like steroid hormones made from cholesterol)
- **Detoxify harmful chemical drugs**

53

The ribosomes synthesize proteins, which fold and take on their tertiary structure inside the ER.

Smooth ER has a variety of functions and is specialized for different activities in different cells, such as detoxify harmful drugs, alcohol, in liver cells. Manufacturing large quantities of lipids such as steroid hormones made from cholesterol in the cells of testes and ovaries.

The Golgi Apparatus

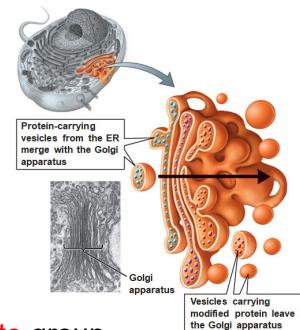


54

These figures show how the Golgi looks like. It has a folded membrane that typically looks like a stack of “roti pratta”. Products moves in and out constantly in vesicles.

Golgi Complex/Apparatus

- Set of stacked flattened, curved sacs
- Receives material from ER in vesicles
- **Modifies some molecules**, such as adding a **carbohydrate** group to proteins, and making glycoproteins; it breaks some proteins into smaller peptides
- **Synthesizes some polysaccharides** used in plant cell walls, such as cellulose and pectin
- **Sort and Packages** finished material in vesicles for shipment to final destinations
 - Some within cell
 - Some exported from cell via **exocytosis**



55

The Golgi apparatus is a specialized set of membranes, derived from the endoplasmic reticulum, that looks like a stack of flattened and interconnected sacs. Its main purpose is to modify, sort, and package proteins produced by the rough ER. The Golgi acts like the finishing room of a factory, where the final touches are put on products and they are packaged and exported.

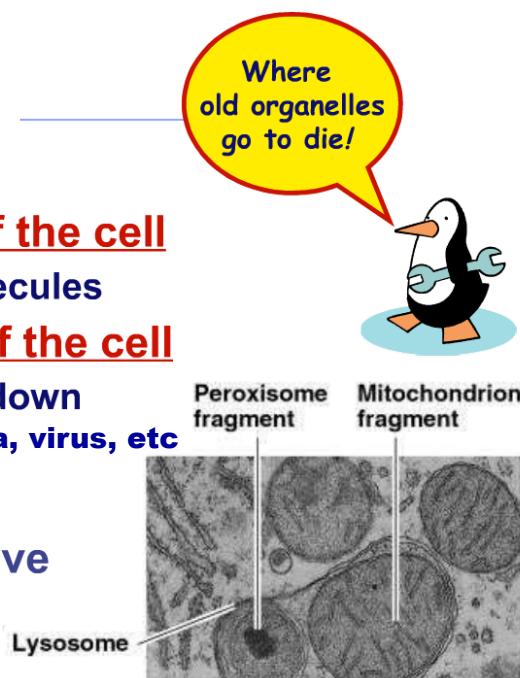
Lysosomes

■ Function

- ◆ **little “stomach” of the cell**
 - digests macromolecules
- ◆ **“clean up crew” of the cell**
 - cleans up broken down organelles, bacteria, virus, etc

■ Structure

- ◆ **vesicles of digestive enzymes**



Lysosomes serve as the cell's digestive system

56

Lysosomes are vesicles that contain powerful digestive enzymes. As we just learnt, many cells “eat” by phagocytosis and forms a food vacuole. Lysosomes recognize these food vacuoles and merge with them. The contents of the two vesicles mix, and lysosomal enzymes digest the food into small molecules such as amino acids, monosaccharides, and fatty acids that can be used within the cell. Lysosomes also digest defective or malfunctioning organelles. Many cells of animals and protists “eat” by endocytosis—

that is, by engulfing particles from just outside the cell 5 .

The plasma membrane with its enclosed food then pinches off inside the cytosol and forms a large vesicle called a **food vacuole**.

Lysosomal Storage Disorders such as Tay-Sachs disease. Mutations in the HEXA gene cause Tay-Sachs disease. The *HEXA* gene provides instructions for making part of an enzyme called beta-hexosaminidase A, which plays a critical role in the brain and spinal cord. This enzyme is located in lysosomes, which are structures in cells that break down toxic substances and act as recycling centers. Within lysosomes, beta-hexosaminidase A helps break down a fatty substance called GM2

ganglioside.

Mutations in the *HEXA* gene disrupt the activity of beta-hexosaminidase A, which prevents the enzyme from breaking down GM2 ganglioside. As a result, this substance accumulates to toxic levels, particularly in [neurons](#) in the brain and spinal cord. Progressive damage caused by the buildup of GM2 ganglioside leads to the destruction of these neurons, which causes the signs and symptoms of Tay-Sachs disease.

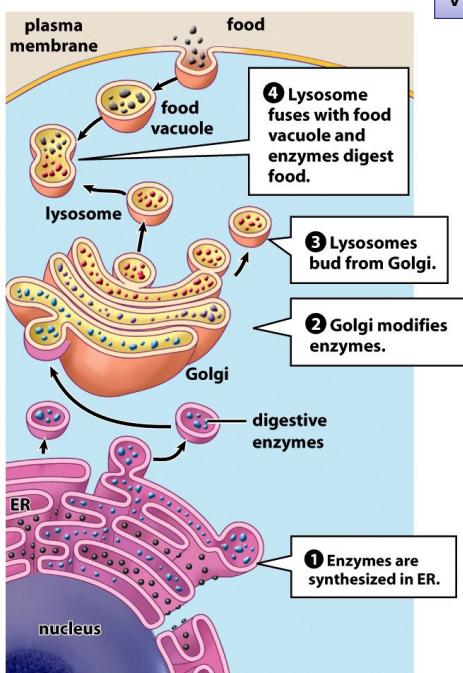
Because Tay-Sachs disease impairs the function of a lysosomal enzyme and involves the buildup of GM2 ganglioside, this condition is sometimes referred to as a lysosomal storage disorder or a GM2-gangliosidosis.

Wornout, defective

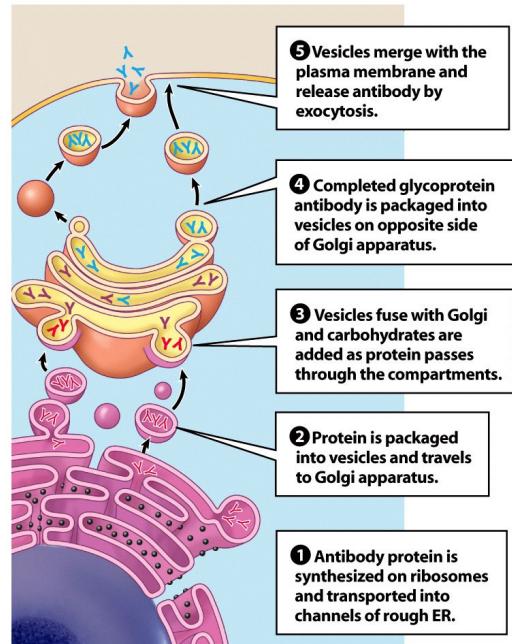
Endomembrane System: A Review

Vesicle traffic in the cytoplasm

Endocytosis



Exocytosis



57

This figure reviews the endomembrane system, which shows the flow of membrane lipids and proteins through the various organelles. As the membrane moves from the ER to the Golgi and then elsewhere, its molecular composition and metabolic functions are modified. The endomembrane system is a complex and dynamic player in the cell's compartmental organization.

Energy-Related Organelles

- ❖ **Mitochondria** are the sites of cellular respiration, a metabolic process that generates ATP, function as the “**powerhouses** of the cell”
- ❖ **Chloroplasts**, found in plants and algae, are the sites of **photosynthesis**
- ❖ Both organelles have their own DNA

58

Now, let's continue our tour of the cell with some membranous organelles that are not closely related to the endomembrane system but play crucial roles in the energy transformations. In eukaryotic cells, mitochondria and chloroplasts are the organelles that convert energy to forms that the cells can use for work.

(maternally inherited diabetes and deafness) at lower mutation loads.

Both mitochondria and chloroplasts are complex organelles with a unique origin. Nearly all biologists accept the **Endosymbiont hypothesis** (see Chapter 18) that both mitochondria and chloroplasts evolved from prokaryotic bacteria. Roughly 1.7 billion years ago, these prokaryotes took up residence within other prokaryotic cells, a process called endosymbiosis (Gk. *symbiosis*, living together).

Both are surrounded by double membranes.
Both have assemblies of enzymes that synthesize ATP, as would have been needed by an independent cell.
Finally, both possess their own DNA and ribosomes that more closely resemble prokaryotic than eukaryotic DNA and ribosomes.

Mitochondria: Chemical Energy Conversion

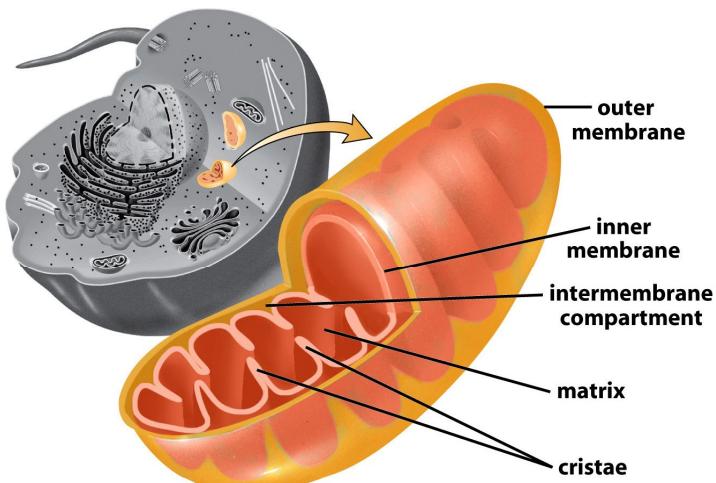


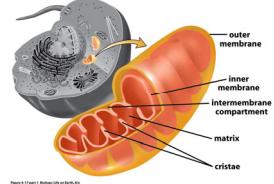
Figure 4-17 part 1 Biology: Life on Earth, 8/e
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59

A mitochondrion has two membranes, outer membrane is smooth, the inner membrane forms deep folds. This arrangement creates two compartments, intermembrane compartment and inner compartment. Some of reactions that break down high-energy molecules occur in the fluid of matrix inside the inner membrane; the rest are conducted by a series of enzymes attached to the membranes of the cristae within the intermembrane compartment. As the highly folded surfaces give the inner mitochondrial membrane a large surface area, thus enhancing the productivity of cellular respiration. This is one example of structure fitting function. The role of mitochondria in energy production will be discussed in next topic.

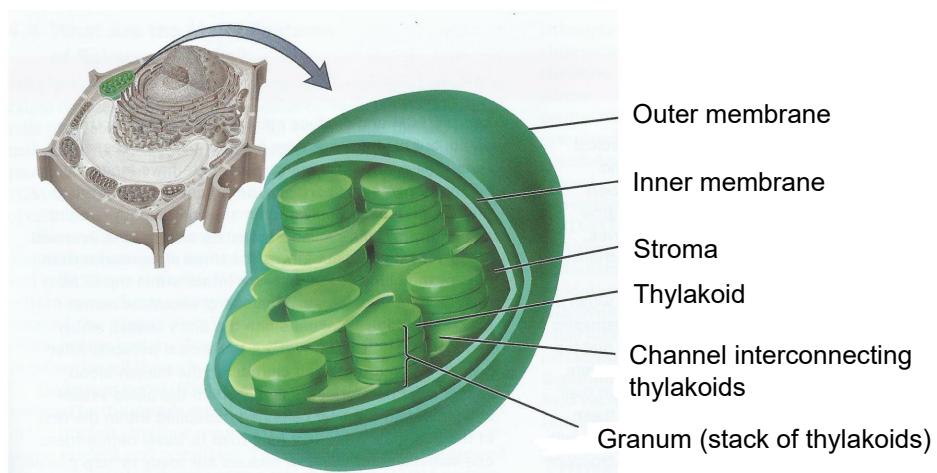
Mitochondria



- Mitochondria are in nearly all eukaryotic cells (**plant and animal**).
- They have a smooth **outer** membrane and an **inner** membrane folded into **cristae**.
- Cristae present a large surface area for enzymes that synthesize ATP.
- The inner membrane creates two compartments: **intermembrane space** and **mitochondrial matrix**.
- Cellular respiration broke down carbohydrate in the mitochondrial matrix to generate **high-energy electron carriers**, e.g. NADH.
- The electron transport chain complexes in inner membrane transfer electron from NADH to O₂ and generate ATP.

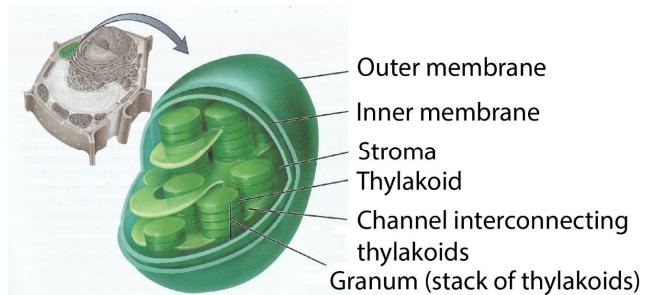
60

Chloroplast: site of photosynthesis



61

Chloroplast



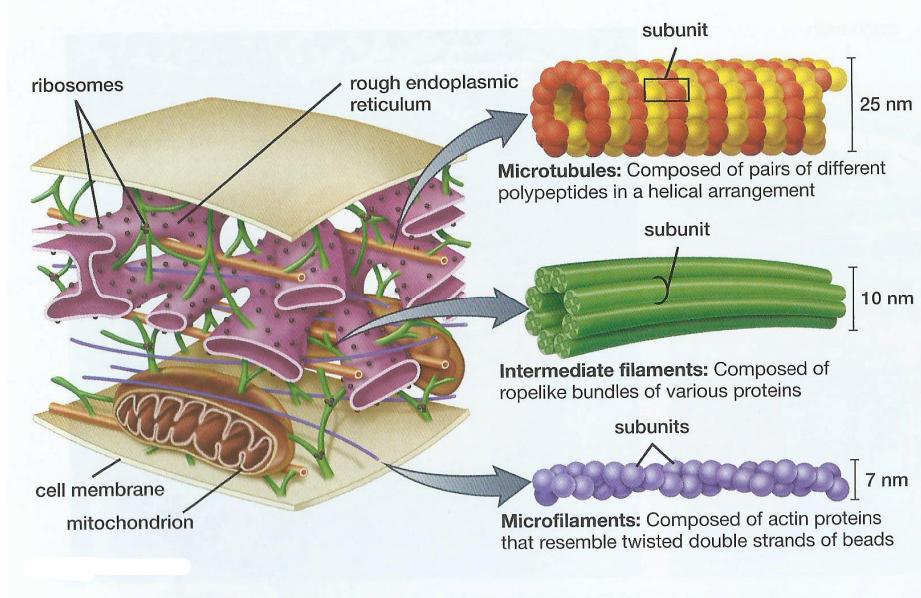
- Chloroplasts are only found in **plant** cell.
- **Thylakoid** membranes contains **chlorophyll** pigment that carry out **light reaction** converting light energy to chemical energy, e.g. ATP
- **Stroma** is the site for **dark reaction**, using ATP to synthesize carbohydrate from CO₂ and water.

62

Light reaction – initial stage of photosynthesis, traps light energy to produce ATP and NADPH

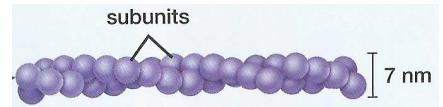
Dark reaction – 2nd step in photosynthesis, uses energy from ATP and NADPH to produce glucose

Cytoskeleton: Shape, support and movement

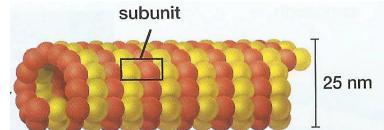


63

Cytoskeleton



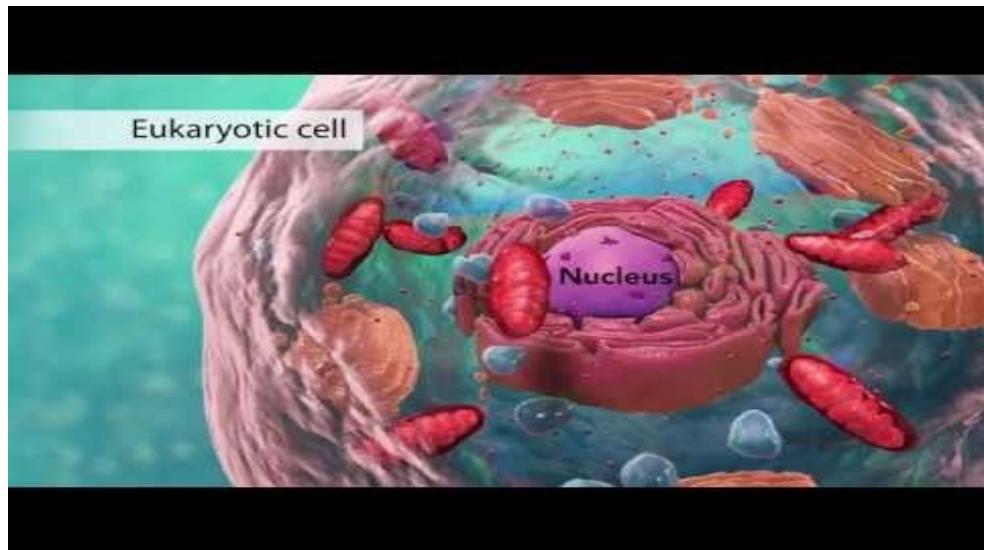
- Microfilament is comprised of actin and provide internal support to the **cell shape**.
- Microfilament can extend by adding subunits at one end and releasing subunits at the other end to allow **cell movement**.
- Microtubule is associated with **motor proteins** to **transport organelles** within the cell.
- Microtubule also **guide chromosome** movements during **cell division**.



64

Summary

Biology: Cell Structure I Nucleus Medical Media



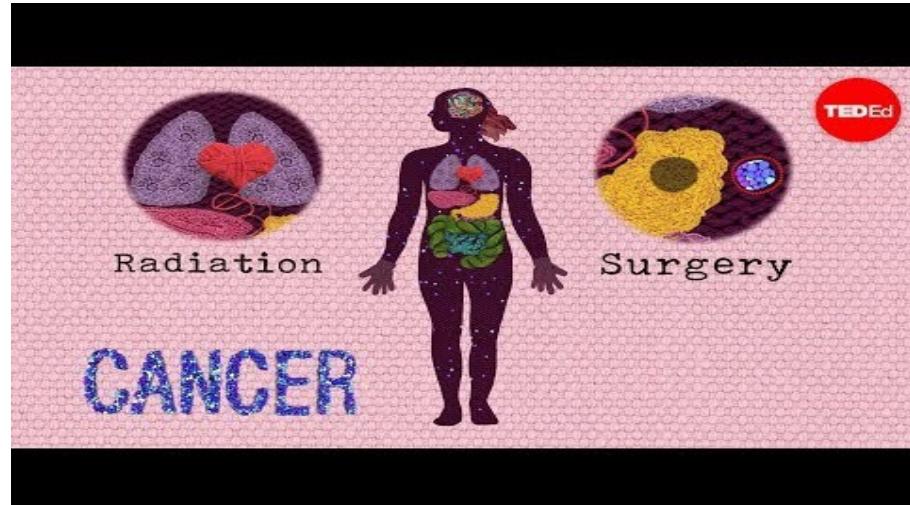
65

<https://www.youtube.com/watch?v=URUJD5NEXC8>

Play Video (7.22 mins) if there is time

Case study: How do cancer cells behave differently from healthy ones?

<https://www.youtube.com/watch?v=BmFEoCFDi-w>



66

Play video (3.5mins)

<https://www.youtube.com/watch?v=BmFEoCFDi-w>

Further questions

- If samples of muscle tissue were taken from the legs of a world-class **marathon** runner and a **sedentary** individual, which would you expect to have a higher density of mitochondria? Why?
- If a cell is treated with **Brefeldin A**, a drug that interferes with the functions of the **Golgi apparatus** and the secretory pathway, what would be the result? Can such a drug be useful in killing diseased or harmful cells?

67

1. Mitochondria density in marathon runners higher as they rely heavily on aerobic metabolism to sustain prolonged periods of physical activity. Their muscles adapt by increasing the number of mitochondria to efficiently produce the large amounts of ATP (energy) required for endurance performance. Sedentary individuals do not engage in such sustained aerobic activity and thus would not require as much mitochondria for energy production.
2. Affects the cell's ability to modify and transport protein
 1. Impairment of secretory functions
 2. Disruption of protein transport
 3. Cell death (apoptosis)
3. Helps with cancer treatment and viral infections though, for diseased or harmful cells where the secretory path plays a critical role
 1. Cancer treatment – some cancer depend on efficient protein synthesis for growth and survival, inhibiting the golgi apparatus and thus the secretory pathway of proteins can induce stress and apoptosis in these cells
 2. Viral infections – many virus hijack the secretory pathway to

67

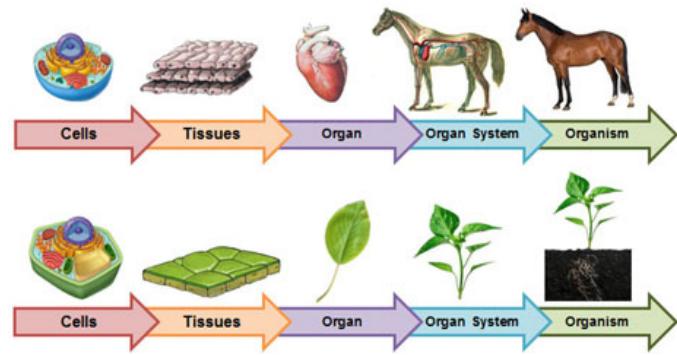
produce and secrete viral proteins, inhibiting it prevents the proper maturation and secretion of these proteins

Please complete the quiz on Canvas for lecture 3

Reminders

- Practical 1 is this Wednesday (23rd August) in LS Lab3 (S1A-03)
 - Please read through practical handout before coming to lab
 - Can be found under “Handouts for tutorials/practical” in “Lab 1 Living Cells” folder
- Be punctual (4 sessions) and properly attired
 - No shorts, slippers, sandals
 - Not allowed to enter lab if not properly attired

LSM1301



L4: Energy of Life

A/P Henry Mok Yu Keung
Office at S3-03-01d
dbsmokh@nus.edu.sg

Outline

- Energy
 - Laws of thermodynamics
- Metabolic Reactions
 - Enzyme
 - Energy carrier molecules
 - Energy release
- Aerobic Cellular Respiration
 - Glycolysis
- Acetyl-CoA formation
- Citric acid cycle
- Electron transfer phosphorylation
- Fermentation
- Food and Energy

2

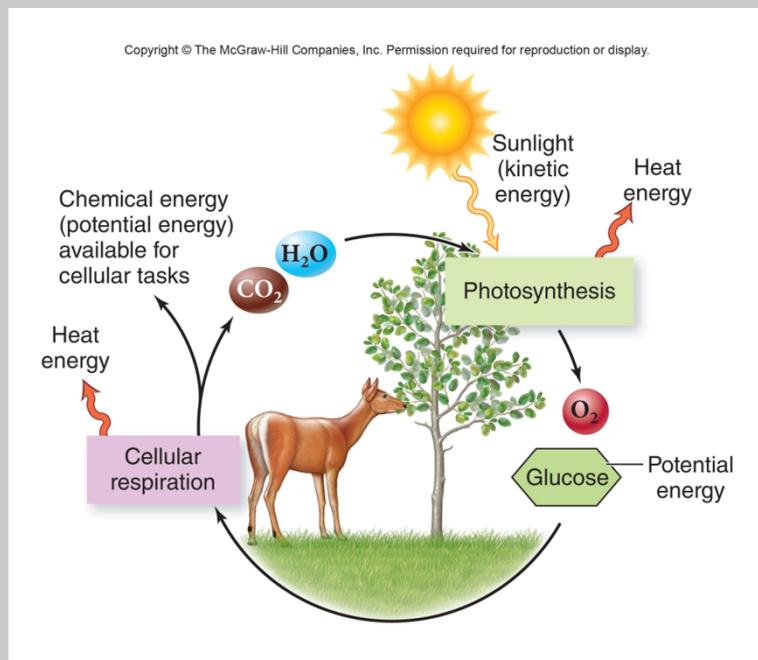
Enzyme – protein, lowers activation energy

Energy

- Energy – the capacity to do work
- Two fundamental forms of energy
 - **Kinetic** energy – energy of motion being used to do work
 - **Potential** energy – stored energy to do work
- One form of energy can be **converted** to another form
 - Plants trap energy from sun via photosynthesis and convert it into **chemical bond energy**
 - All organisms use energy stored in bonds of organic compounds to do work
- Energy stored in chemical bonds – type of potential energy

Energy

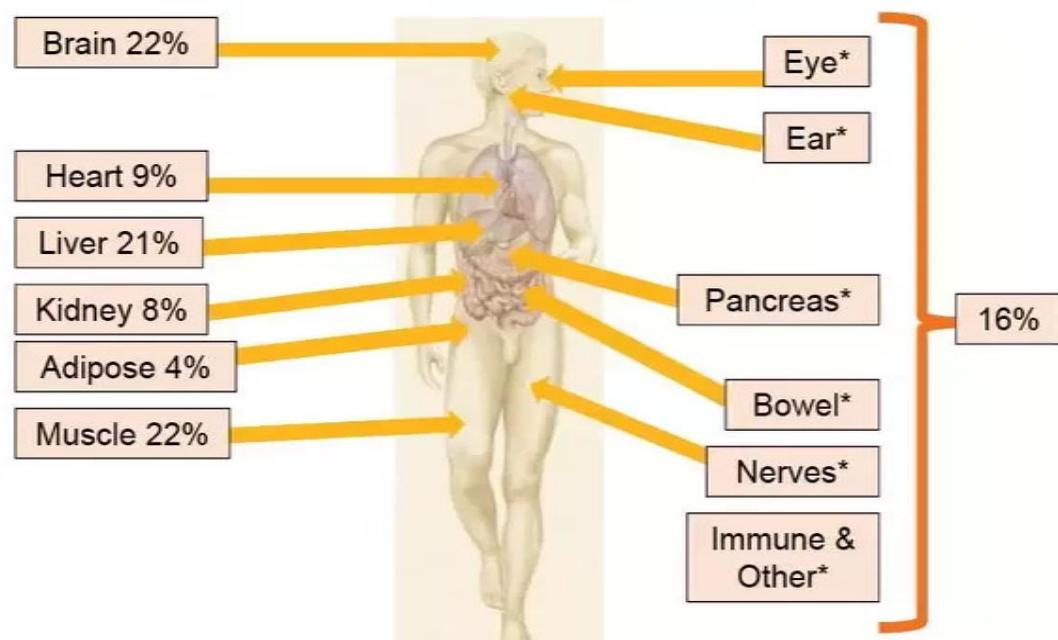
- **Autotrophs**
 - autos = self; trophe = nutrition
 - Self-feeders, producers
 - Plants capture light energy to make food – photosynthesis
- **Heterotrophs**
 - heterone = (an)other
 - Other-feeders, consumers
 - Organisms acquire energy from molecules of other organisms



4

The source of energy for all life comes from the sun, directly or indirectly. Plants make their own food by capturing energy from the sun in a process called photosynthesis, and animals feed on the plants or each other to get the energy. Plants are producers and autotrophs, we are consumers and heterotrophs.

Case study: Where is energy used in the body?



5

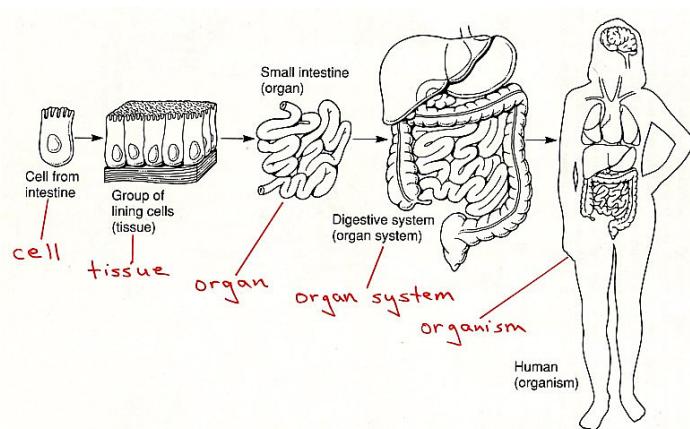
With the brain and muscles consuming a large share of the energy produced, we can now understand why people who are fatigued most often complain of “brain fog”, memory/concentration problems, weakness, and easy fatigability with minimal exertion.

Each Organ Has a Unique Metabolic Profile

<https://www.ncbi.nlm.nih.gov/books/NBK22436/>

Case study: How do we get energy?

The major absorbed end products of food digestion are monosaccharides, mainly glucose (from carbohydrates); monoacylglycerol and long-chain fatty acids (from lipids); and small peptides and amino acids (from protein).



Energy is trapped in the chemical bonds of nutrient molecules. **How is it then made usable for cellular functions and biosynthetic processes?**

6

<https://www.nature.com/scitable/topicpage/nutrient-utilization-in-humans-metabolism-pathways-14234029/>

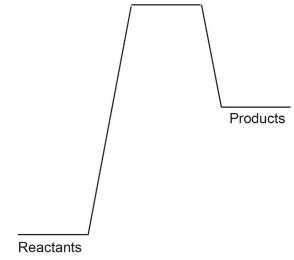
Metabolic reactions

- Metabolism
 - Sum of chemical reactions in cell
- **Reactants**
 - Chemical substances that enter a chemical reaction
- **Products**
 - Chemical substances that form as result of a chemical reaction
- Chemical reactions can be categorised based on energy gain or loss
 - **Endergonic** reactions
 - **Exergonic** reactions

Metabolic reactions

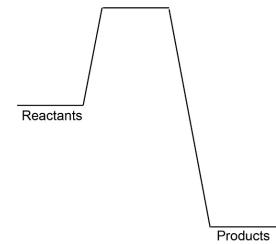
▪ Endergonic Reactions

- Require an **input of energy**
- Products contain more free energy than reactants
- Example – **photosynthesis**



▪ Exergonic Reactions

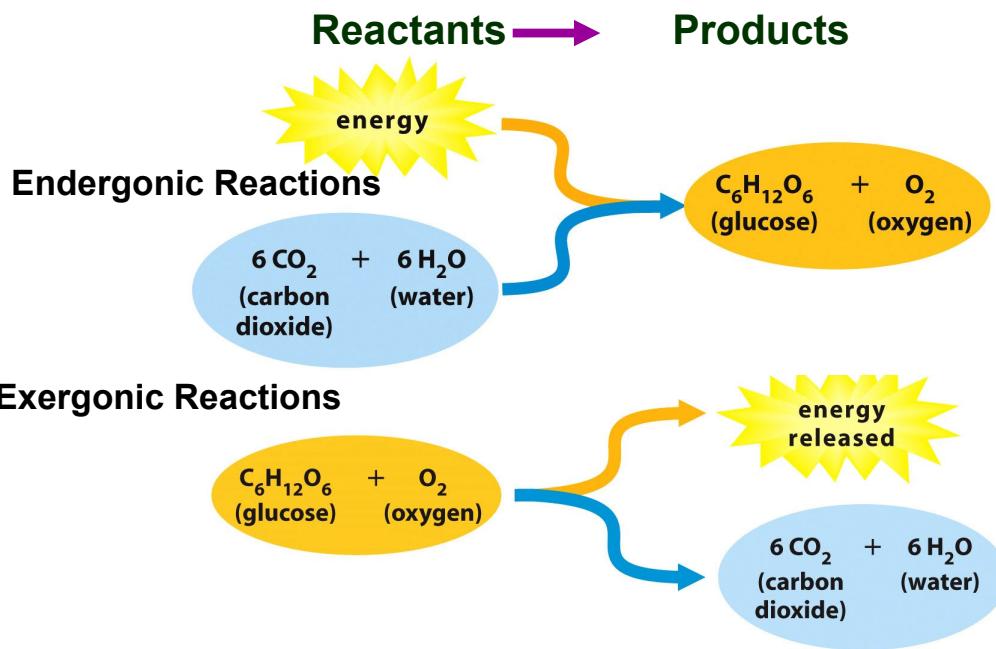
- Net **release of energy**
- Products contain less free energy than reactants
- Example – **cellular respiration**



All reactions require additional energy (activation energy) before reactants become products.

The living cell is a chemical factory in miniature, where thousands of reactions occur within a microscopic space. All chemical reactions either require an overall net input of energy or produce a net release of energy. A reaction is exergonic if it releases energy; a reaction is endergonic if it requires a net input of energy.

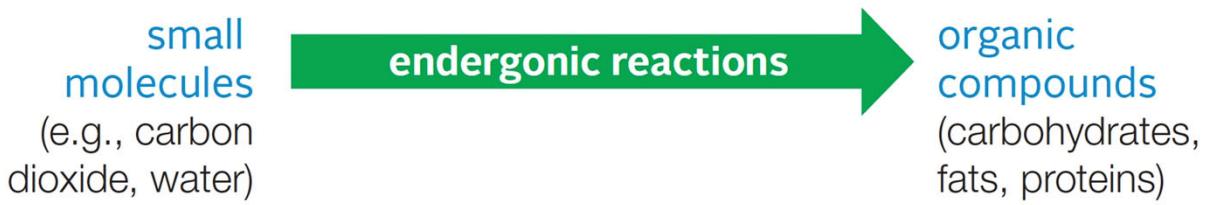
Metabolic Reactions



9

A chemical reaction is a process that forms or breaks the chemical bonds that hold atoms together, the reactants, into another set, the products. This upper figure shows you an endergonic reaction, the lower shows an exergonic reaction.

Energy in glucose cannot be used directly to fuel endergonic reactions
Energy released by glucose breakdown is first transferred to an energy-carrier molecule



A Cells store free energy in the bonds of organic compounds.



B Cells retrieve free energy stored in the bonds of organic molecules.

Metabolic Reactions

- Metabolic reactions occur in sequences – **metabolic pathways**
 - Products of one earlier reaction become reactants of another later reaction
 - Catalysed by enzymes (biological catalysts)
 - Each reaction requires a unique and specific enzyme
- **Catabolism**
 - Breakdown of complex molecules such as food
 - Produces energy (exergonic)
- **Anabolism**
 - Synthesis of complex molecules
 - Uses energy (endergonic)

11

Enzyme required to overcome the required activation energy.

Cells are miniature, incredibly complex chemical factories, but all reactions do not occur haphazardly in cells, they are usually linked in sequences called Metabolic pathway.

If a series of reactions leads to breaking down molecules and release energy, we called it a catabolism pathway.

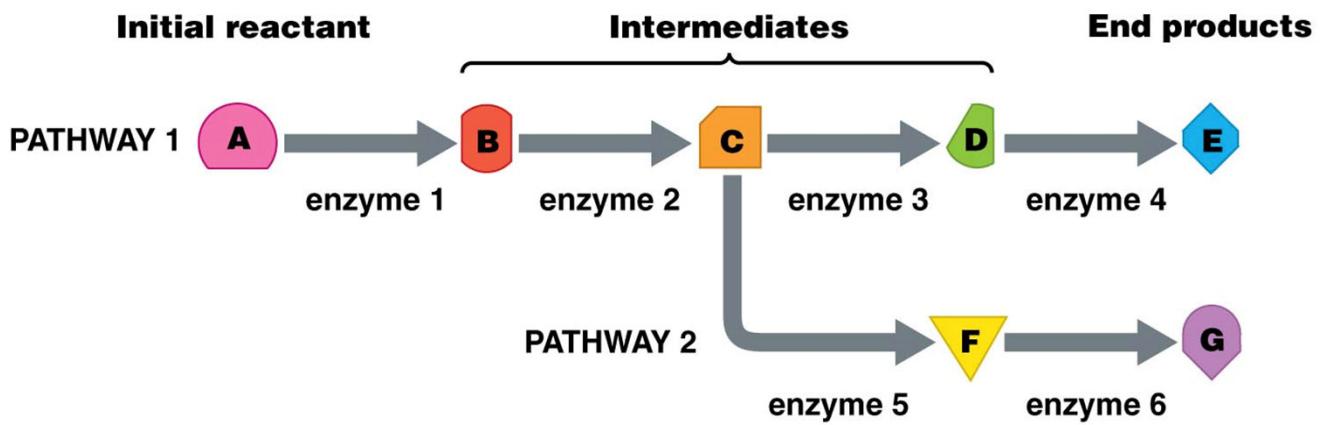
If a series of reactions leads to synthesis large molecules and use energy, we called it a anabolism pathway.

Oxidation refers to the loss of electrons, while reduction refers to the gain of electrons.

The sum of all the chemical reactions inside a cell is its **metabolism**

Many cellular reactions are linked through **metabolic pathways**

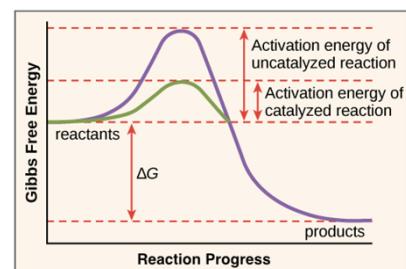
Metabolic reactions



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Enzymes are biological catalysts

- Enzymes are catalytic compounds that drive biochemical reactions in living systems. The **majority of enzymes** are **proteins**.
- Enzymes orient, distort, and reconfigure molecules in the process of **lowering activation energy**
- Activation energy is the minimum energy required to start a chemical reaction.
- Some enzymes require **helper (coenzyme)** molecules to function (e.g. B vitamins, metal ions).
- Enzymes are very **specific** for the molecules they catalyze.
- Enzyme activity is often enhanced or suppressed by their reactants, products and buffer conditions.



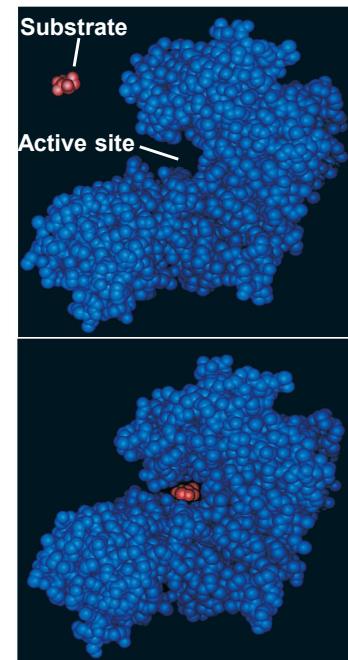
Exergonic reaction

13

Enzyme changes shape of substrate (a molecule that an enzyme reacts with)

Enzyme Structure

- Enzymes have a pocket called an **active site**
- Reactants (substrates) bind to the active site
 - Distinctive shape of active site is **complementary** and **specific** to the substrate (lock and key)
 - Active site amino acids bind to the substrate and **distort bonds** to facilitate a reaction



14

Enzymatic Action

Induced-fit model

- The active site is flexible, not rigid
- The shape of the enzyme, active site, and substrate **adjust** to maximize the fit, which improves catalysis.
- There is a greater **range** of substrate specificity

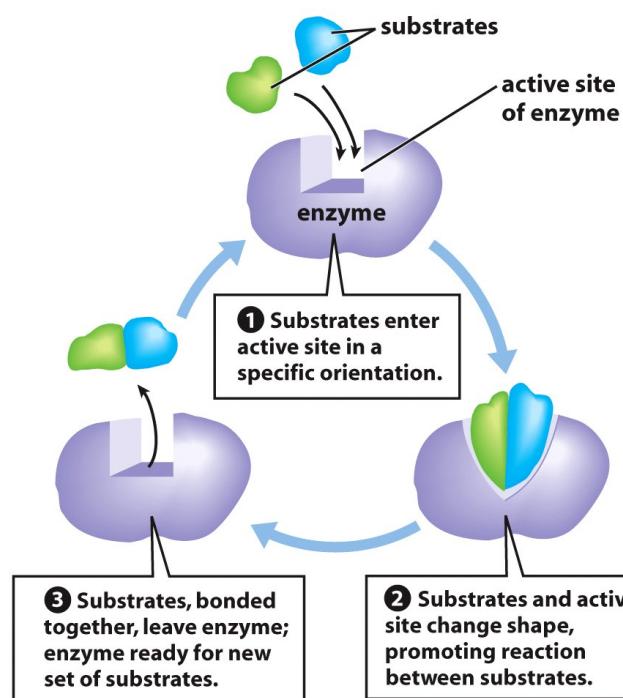
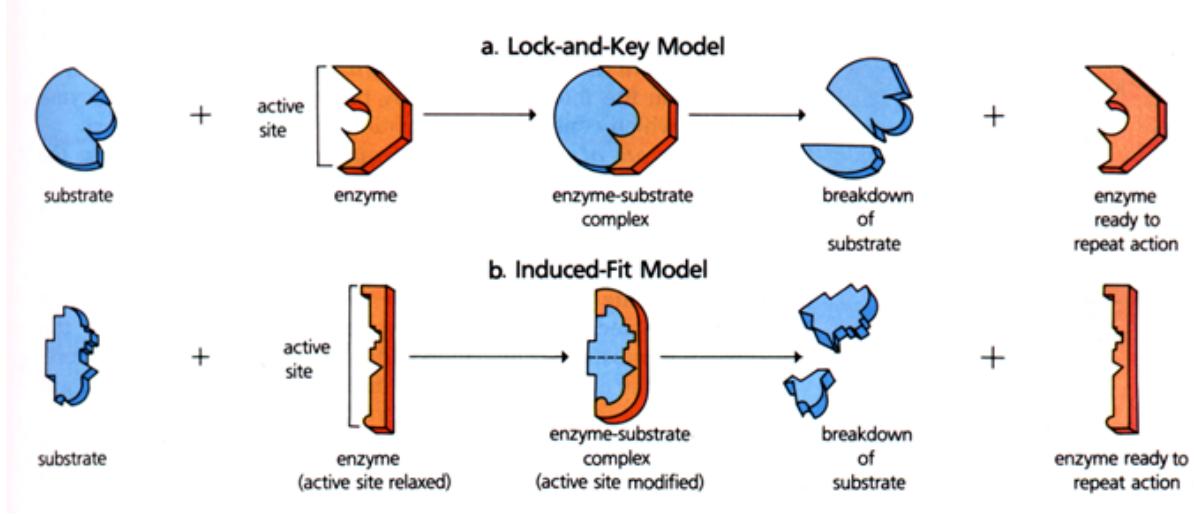


Figure 6-15 Biology: Life on Earth, 8/e
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15

More than 1 type of substrates can fit into the same enzyme

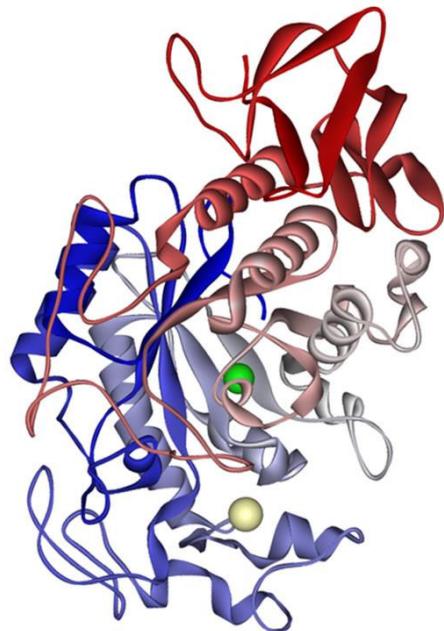
FYI



Case study: Example of enzymes

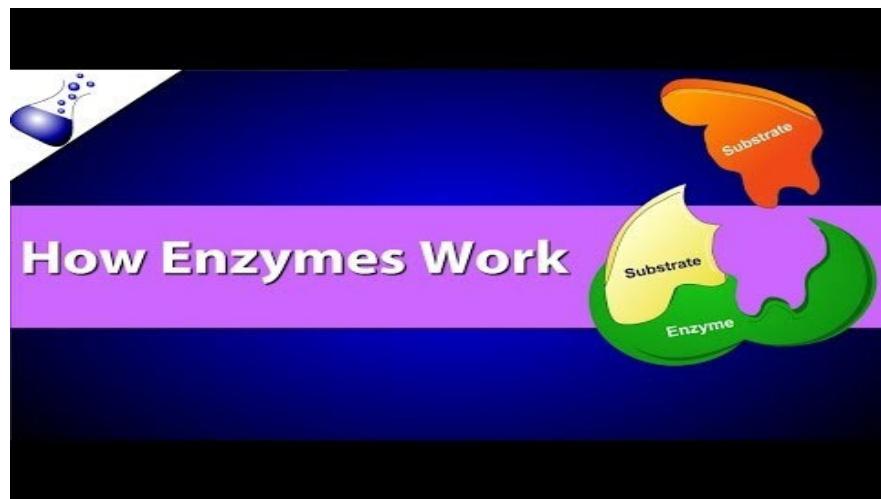
Salivary **amylase** hydrolyses polysaccharides, such as starch, yielding glucose and maltose.

Calcium ion visible in pale khaki, chloride ion in **green**



17

How enzymes work (video)



<https://www.youtube.com/watch?v=UVeoXYJIBtl>

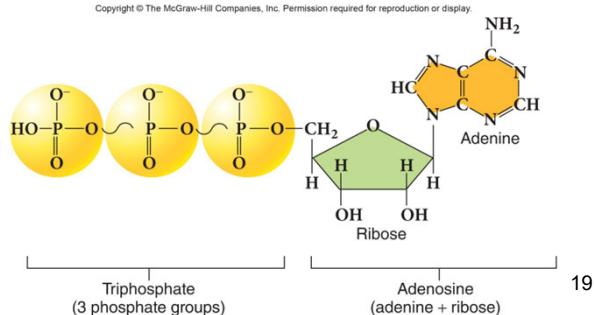
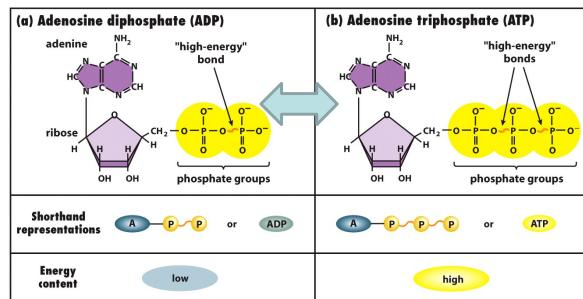
18

Energy Carrier Molecules

- High-energy, unstable molecules
 - Synthesized at sites of **exergonic** reactions
 - Capturing some released energy

Most common:

- Adenosine triphosphate (**ATP**)



19

In the cell, an exergonic reaction often provides the energy needed to drive an endergonic reaction, but the energy must be carried by another molecule, often cannot be used directly. For example, in a runner, the breakdown of sugar releases energy, but this energy cannot be used directly for muscle contraction. Instead, the energy from sugar must first be transferred to an energy-carrier molecule, which provides the muscle with energy to contract. Energy carriers work something like rechargeable batteries. They pick up an energy charge at an exergonic reaction, move to another location within the cell, and release the energy to drive an endergonic reaction. To make an analogy, energy carrier molecules are also works like a currency in our shopping market.

ATP is the most popular currency of energy in a cell. It stores energy in the phosphate group, and carries the energy to the sites where energy is required. When the terminal phosphate bond is broken, a molecule of inorganic phosphate leaves the ATP, which becomes adenosine diphosphate, ADP and releases energy

Energy-carrier molecules are high-energy, unstable molecules that are synthesized at the site of an exergonic reaction, capturing some of the released energy

These high-energy molecules then transfer the energy to an endergonic reaction elsewhere in the cell

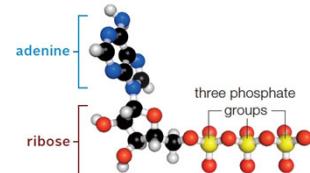
ATP is the most common energy carrying molecule

FIGURE 6-8 ADP and ATP

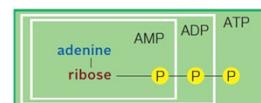
A phosphate group is added to (a) ADP (adenosine diphosphate) to make (b) ATP (adenosine triphosphate). In most cases, only the last phosphate group and its high-energy bond are used to carry energy and transfer it to endergonic reactions within a cell.

Energy Carrier Molecules

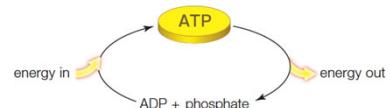
- ATP links endergonic to exergonic reactions through coupled reactions
 - Energy released by **exergonic** reaction used to combine relatively low-energy adenosine diphosphate (**ADP**) and phosphate (**P**) into **ATP**
 - Energy captured in high-energy bonds of ATP
- **ATP used to drive endergonic reaction**
 - Where energy is needed, ATP is broken down into ADP and P
 - Stored energy is released



A Structure of ATP.



B After ATP loses one phosphate group, the nucleotide is ADP (adenosine diphosphate); after losing two phosphate groups, it is AMP (adenosine monophosphate).



C ATP forms by endergonic reactions that phosphorylate ADP. ADP forms again when a phosphate group from ATP is used to phosphorylate another molecule. Energy from such transfers drives endergonic reactions that are the stuff of cellular work.

Coupled reaction: glucose breakdown and protein synthesis

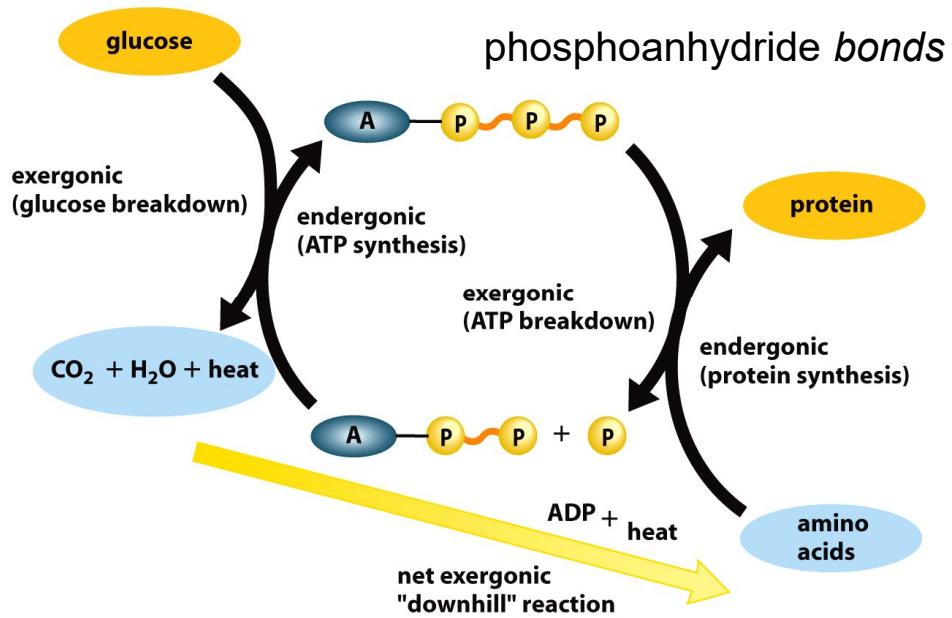


Figure 6-11 Biology: Life on Earth, 8/e
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21

This figure shows a coupled reaction in living cells. Exergonic reactions (glucose breakdown) drive the endergonic reaction that synthesizes ATP from ADP. The ATP molecule can carry its chemical energy to a part of the cell where the energy is used to drive an essential endergonic reaction (protein synthesis).

FIGURE 6-10 ATP breakdown: Energy is released

Energy is stored in the high-energy bond extending to the last phosphate
Heat is given off when ATP breaks into ADP (adenosine diphosphate) and P (phosphate)

The energy released when ATP is broken down into ADP + P is transferred to endergonic reactions through coupling

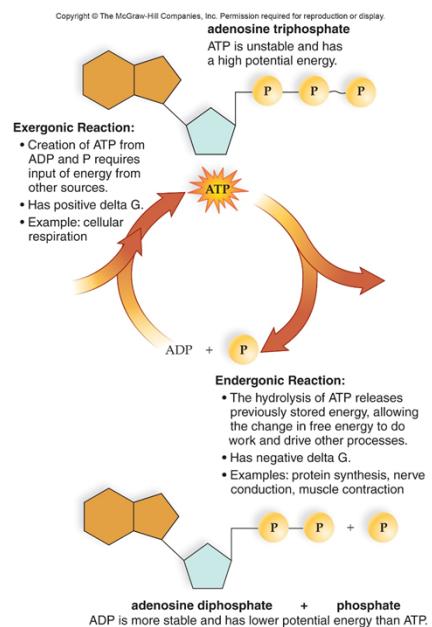
FIGURE 6-11 Coupled reactions within living cells

Exergonic reactions (such as glucose breakdown) drive the endergonic reaction that synthesizes ATP from ADP. The ATP molecule can carry its

chemical energy to a part of the cell where the energy from the breakdown of ATP is needed to drive an essential endergonic reaction (such as protein synthesis). The ADP and phosphate are recycled back to ATP via endergonic reactions. The overall reaction is exergonic, or downhill: more energy is released by the exergonic reaction than is needed to drive the endergonic reaction.

Energy Carrier Molecules

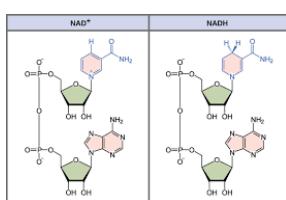
- ATP – **short-term** energy storage
 - Not stockpiled in cells
 - Recycled at furious pace
- **Long-term** energy storage molecules –triglycerides, starch and glycogen



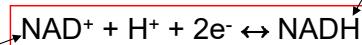
22

Energy Carrier Molecules

- Energy can be transferred to **electrons** in metabolic pathways
 - E.g. cellular respiration, photosynthesis
- **High-energy** electrons then passed from one molecule to another –oxidation-reduction (**redox**) reactions
 - Oxidation – loss of electrons
 - Reduction – gain of electrons
- Both oxidation and reduction reactions occur at same time – one molecule accepts electrons given up by another
 - Molecule that gives up electrons is oxidised
 - Molecule that accepts electrons is reduced



Reduction →



← Oxidation

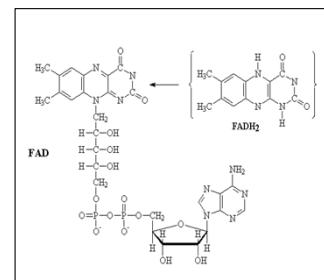
23

Reduction – taking up electrons -> more energy

Oxidation – releasing electrons -> lose energy

Energy Carrier Molecules

- Electron carriers transport high-energy electrons
 - E.g. nicotinamide adenine dinucleotide (**NAD⁺**), flavin adenine dinucleotide (**FAD**)
- Electron carriers then **donate** high-energy electrons to other molecules – membrane-embedded proteins in mitochondria and chloroplasts
 - **Electron transport chain**
- Leads to synthesis of high-energy **ATP**
 - Left with low-energy electrons

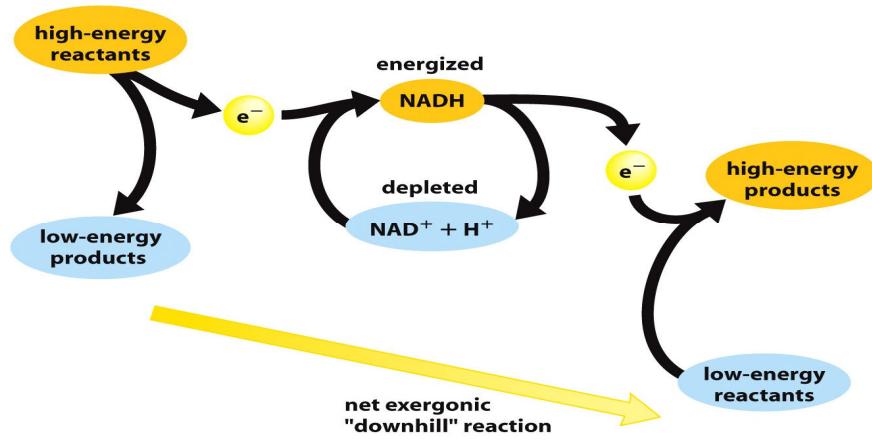


Flavin adenine dinucleotide (FAD)

24

Electron Carriers

1. Nicotinamide Adenine Dinucleotide (**NAD⁺**)
2. Flavin Adenine Dinucleotide (**FAD**)



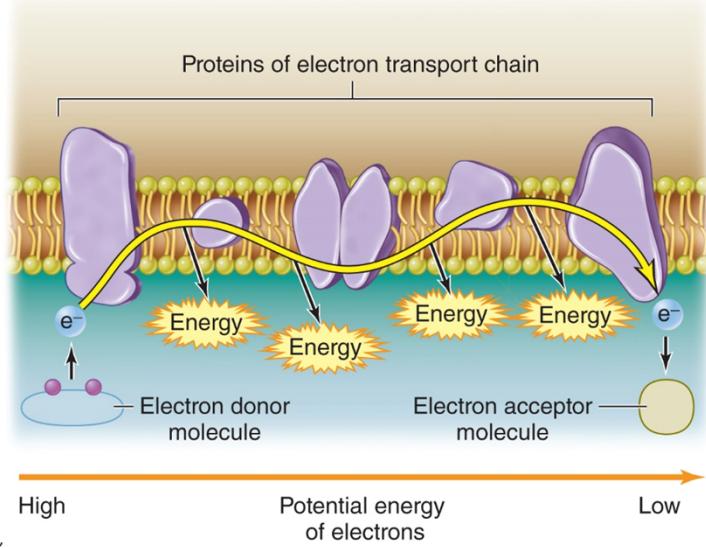
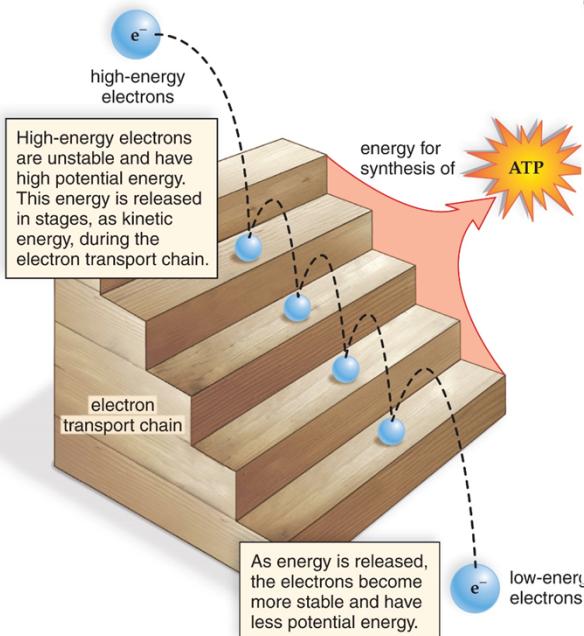
25

In addition to ATP, there are other energy-carrier molecules, such as NAD and FAD, they carry energy by capturing high energy electrons. The low energy molecule "NAD⁺" pick up an high-energy electron and hydrogen ions at the same time, converted to energized NADH. The electron is then transferred, with most of its energy, to another molecule to drive an endergonic reaction, often the synthesis of ATP.

Figure: 19-2 part a

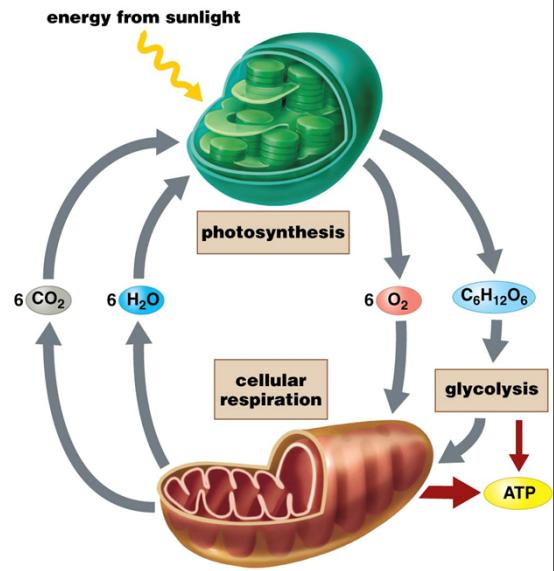
Energy can be transferred to electrons in glucose metabolism and photosynthesis

Electron carriers transport high-energy electrons



Energy Release

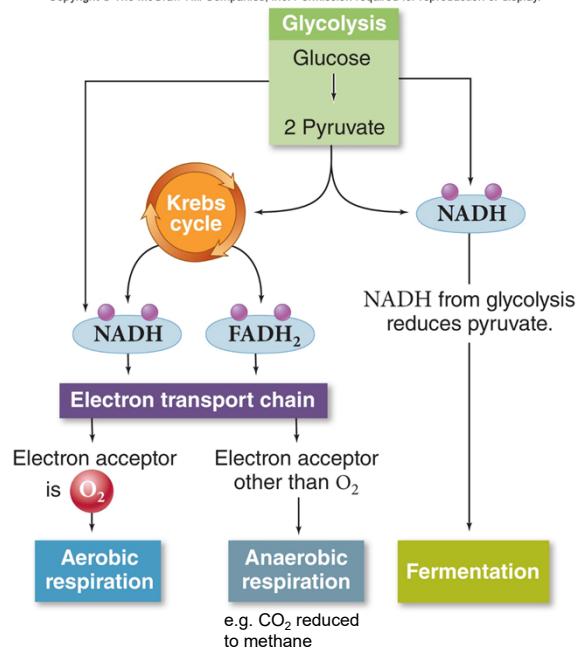
- During photosynthesis – energy of **sunlight** captured, stored in the form of **glucose**
- Glucose produced during photosynthesis
 - Consumed and broken down by nearly all organisms
 - Energy captured in the form of **ATP**
- Main metabolic pathways to generate ATP from glucose breakdown
 - **Aerobic** pathways (need O_2)
 - **Anaerobic** pathways (no O_2)



Energy Release

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- **Aerobic pathways**
 - Require **oxygen**
 - Used by most eukaryotes
 - E.g. aerobic cellular respiration
- **Anaerobic pathways**
 - Do not require oxygen
 - Used by prokaryotes and protists in anaerobic habitats
 - E.g. anaerobic respiration, fermentation



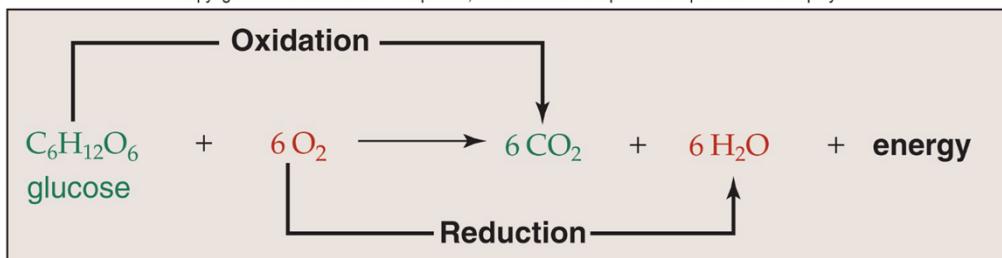
Outline

- Energy
 - Laws of thermodynamics
- Metabolic Reactions
 - Enzyme
 - Energy carrier molecules
 - Energy release
- Aerobic Cellular Respiration
 - Glycolysis
 - Acetyl-CoA formation
 - Citric acid cycle
 - Electron transfer phosphorylation
- Fermentation
- Food and Energy

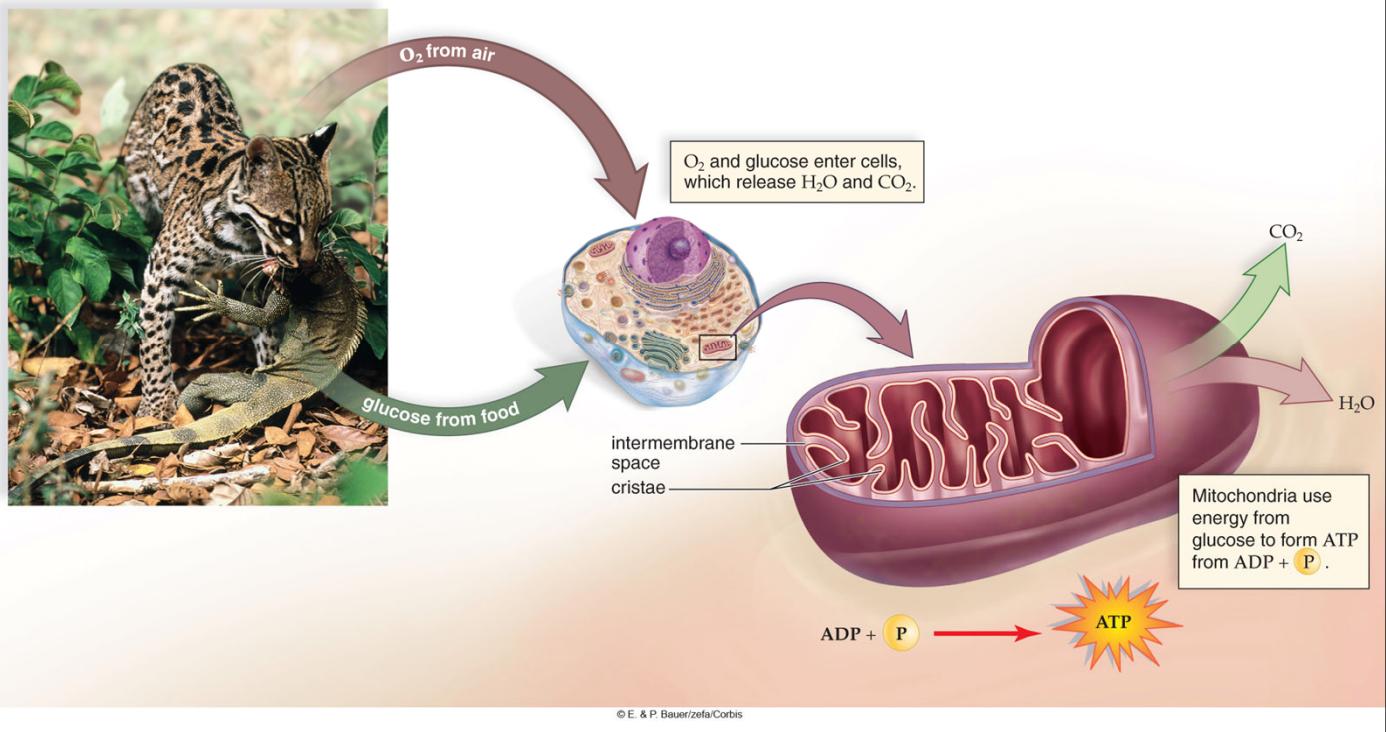
Aerobic Cellular Respiration

- Usually involves breakdown of **glucose** to **CO₂** and **H₂O**
- Energy extracted step-wise from glucose – allows for efficient production of **ATP**
 - **Electrons** removed from intermediates, received by **oxygen**, which combines with hydrogen ions (H⁺) to become water
 - Electrons carried by **NADH** (reduced form of NAD⁺) and **FADH₂** (reduced form of FAD)

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30



Aerobic cellular respiration: 4 stages

- I. **Glycolysis** (occur in cytoplasm, no O₂ needed)
- II. Converting **pyruvate** to **acetyl-CoA**
 - Occurs in cytoplasm (in prokaryotes)
 - Occurs in **matrix** of mitochondria (in eukaryotes)
- III. Citric acid cycle (**Krebs cycle or TCA cycle**)
 - Occurs in cytoplasm (in prokaryotes)
 - Occurs in **matrix** of mitochondria (in eukaryotes)
- IV. Electron transport and **oxidative phosphorylation**
 - Occurs in plasma membrane (in prokaryotes)
 - Occurs in **inner membrane** of mitochondria (in eukaryotes)

32

Glucose metabolism is a cumulative function of 4 stages. The first stage occur in the cytosol. And the rest stages take place within the mitochondrial matrix of eukaryotic cells or simply in the cytosol of prokaryotes.

Now let's look a little more closely at the processes of cellular respiration in the mitochondria.

Glycolysis: (pronounced as “ Gly_+colysis)

Occurs in cytoplasm

Glucose broken down to two molecules of pyruvate

ATP is formed

Transition reaction:

Both pyruvates are oxidized

Electron energy is stored in NADH

Two carbons are released as CO₂

Citric acid cycle (Krebs cycle)

Electron energy is stored in NADH and FADH₂

ATP is formed

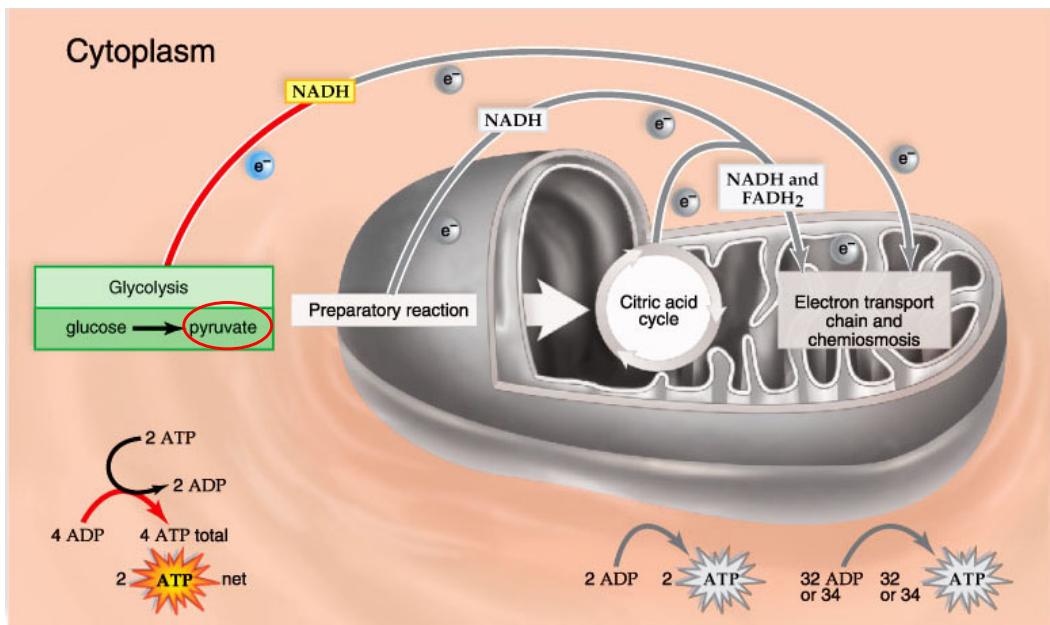
Four carbons are released as CO₂

Electron transport chain:

Extracts energy from NADH & FADH₂

Produces 32 or 34 molecules of ATP

Aerobic cellular respiration



33

Having all those knowledge in mind, now let's examine how cells require a continuous supply of energy to sustain life.

Recall from my second lecture, carbohydrates are the main energy source for the organisms to make ATP by metabolic pathways. Some pathways are aerobic, they use oxygen; others are anaerobic, they occur in the absence of oxygen. Most types of eukaryotic cells either use aerobic respiration exclusively, or they use it most of the time. Many prokaryotes and protists in anaerobic habitats use alternative pathways.

Most cells can metabolize a variety of organic molecules to produce ATP. Today, I focus on the breakdown of glucose for three reasons. First, virtually all cells metabolize glucose for energy. Second, glucose metabolism is less complex than the metabolism of most of other organic molecules. Finally, glucose metabolic pathway is shared by many other organic molecules.

<http://www.youtube.com/watch?v=6AhdTZ03Mvg&feature=PlayList&p=C38DBDA52C455DD0&index=0&playnext=1>

Very good

Glucose serves as primary fuel for all metabolic processes. Energy in its chemical bonds can be used to make other molecules such as proteins, or it can drive movement, transport, etc.

Blood doping

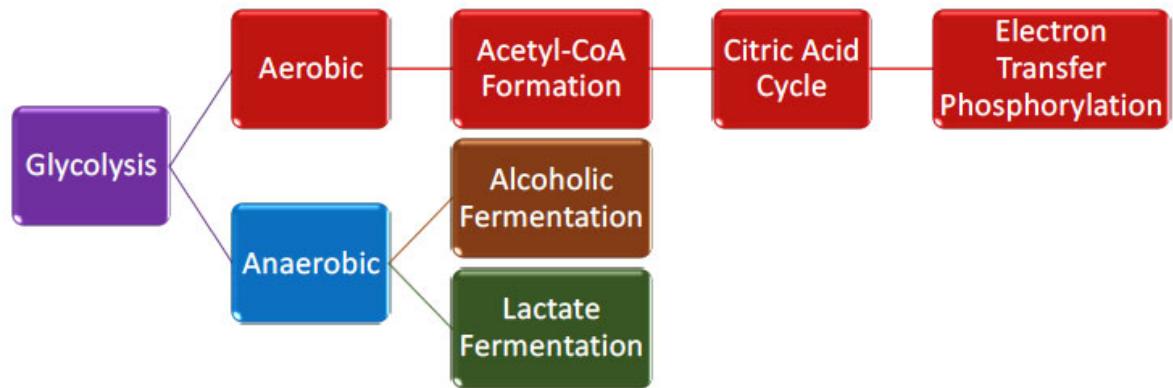
Is a serial of reactions that occur under aerobic conditions and produce a large quantity of ATP.

Breathing vs Respiration

Breathing: taking air in (inspiration) and out of your lungs (expiration); can be consciously controlled (**voluntary** action)

Respiration: the part of a metabolic process; **cellular** activity (occurs inside a cell); the end products are energy molecules, carbon dioxide and water; cannot be consciously controlled (**involuntary** action)

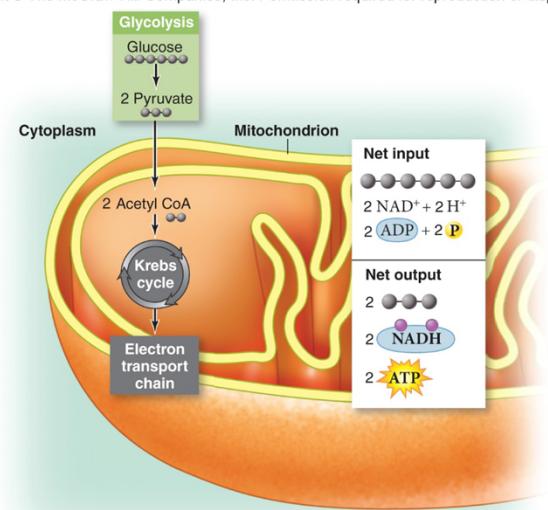
- Aerobic respiration (when oxygen is available)
- Anaerobic respiration (metabolism without oxygen)



Glycolysis

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- Breakdown of one glucose (6-carbon) molecule
 - Into **two pyruvate** (3-carbon) molecules
 - Net yield of **two ATP** and **two NADH** molecules
- Consists of 10 reactions
- **Does not** require oxygen



Impt- steps in glycolysis

I. Glycolysis Splits Glucose

- Occurs in two stages
 - Energy **investment** stage (**activation stage**)
 - Energy **harvesting** stage

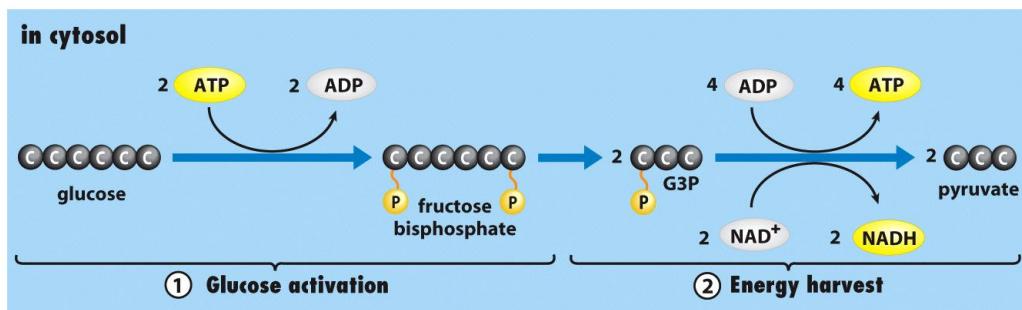


Figure 8-2 Biology: Life on Earth, 8/e
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G3P: glyceraldehyde 3-phosphate

37

Let me use car as an example to explain what is the activation energy

battery is to hold the charge and provide high power **ignition** to start **engine**

The word glycolysis means “sugar splitting”, and that is exactly what happens during this pathway. Glucose, a six-carbon sugar, is split into two three-carbon sugars, called pyruvate.

As shown in the figure, it can be divided into two phases: energy investment or glucose activation and energy harvest.

During energy investment phase, the cell actually spends ATP. This investment is repaid with interest during the energy payoff phase. The net energy yield from glycolysis, per glucose molecule, is 2 ATP plus 2 NADH.

Start a business with a small amount of money,

Start a car with battery,

You have a lot of potential talent
I'll be an enzyme to release your energy,

Energy **investment** stage (Part 1)

Glucose's 6 carbons split into 3-carbon molecules

3-carbon molecules are **phosphorylated**

Uses ATP

Requires energy

Energy **harvesting** stage (Parts 2 and 3)

Phosphates and electrons **removed**

Phosphates added to ADP to make **ATP**

Electrons added to NAD⁺ to make **NADH**

Releases energy

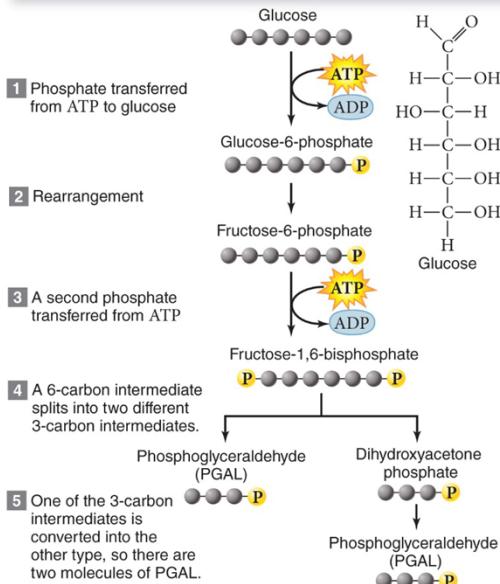
G3P: glyceraldehyde 3-phosphate

Glycolysis

- Energy **investment** stage
 - One glucose (6-carbon) molecule is split into **two** 3-carbon molecules
 - Phosphate groups added to the 3-carbon molecules
 - **Phosphorylation** results in more **reactive** molecule – glucose is relatively stable
 - **Two ATP molecules used to activate one glucose molecule**

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Glucose Activation



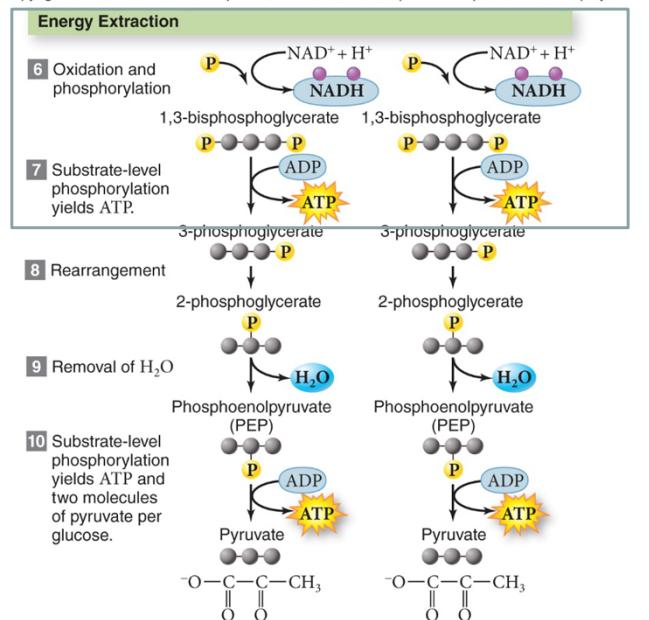
38

Only neeed to know roughly how glycolysis works

Glycolysis

- Energy **harvesting** stage
 - Conversion of phosphorylated 3-carbon molecules to **pyruvate** molecules
 - Phosphorylated 3-carbon molecules **oxidised** by removal of electrons and H⁺
 - Electrons and H⁺ accepted by NAD⁺ resulting in **high-energy electron-carrier molecule NADH**
 - Two phosphorylated 3-carbon molecules produced per glucose molecule – **two NADH molecules** formed

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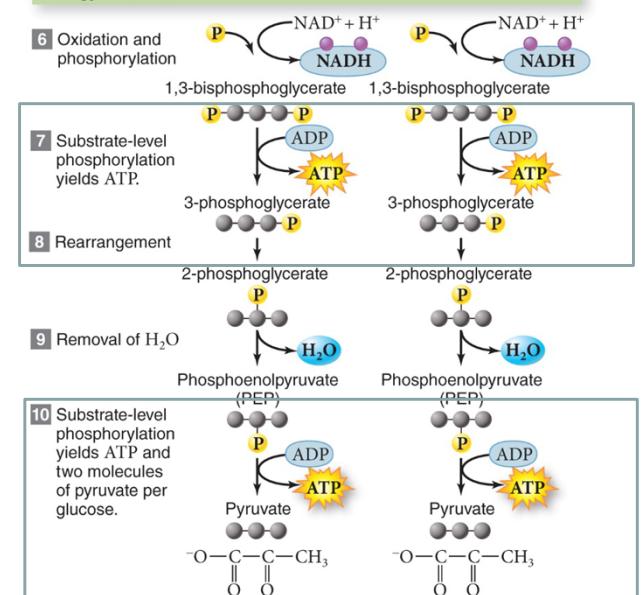


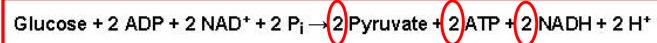
Glycolysis

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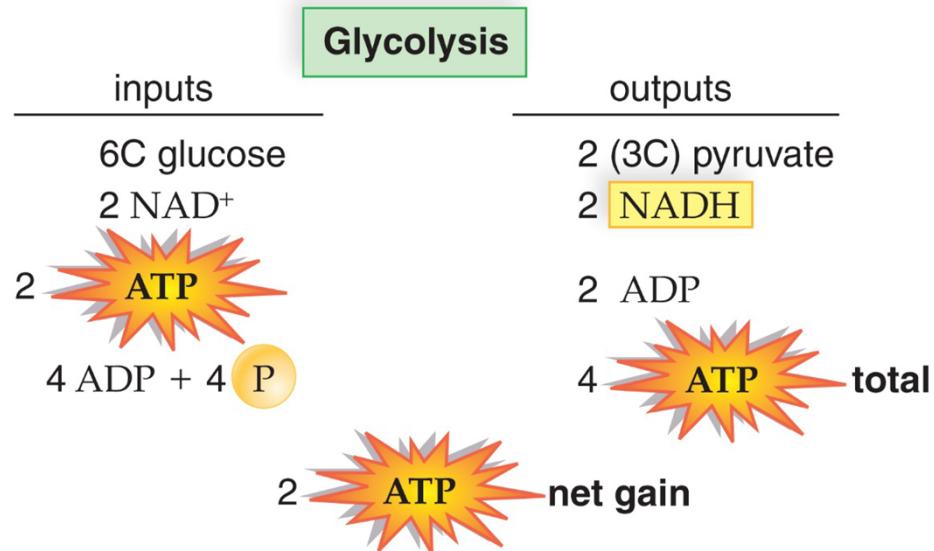
Energy Extraction

- Energy harvesting stage
 - Two ATP molecules generated by **substrate-level phosphorylation** for each molecule of **pyruvate** produced
 - **Two pyruvate** molecules produced per glucose molecule – **4 ATP** molecules generated
 - **Two ATP** molecules used to activate **one glucose molecule** – **net gain of two ATP molecules**

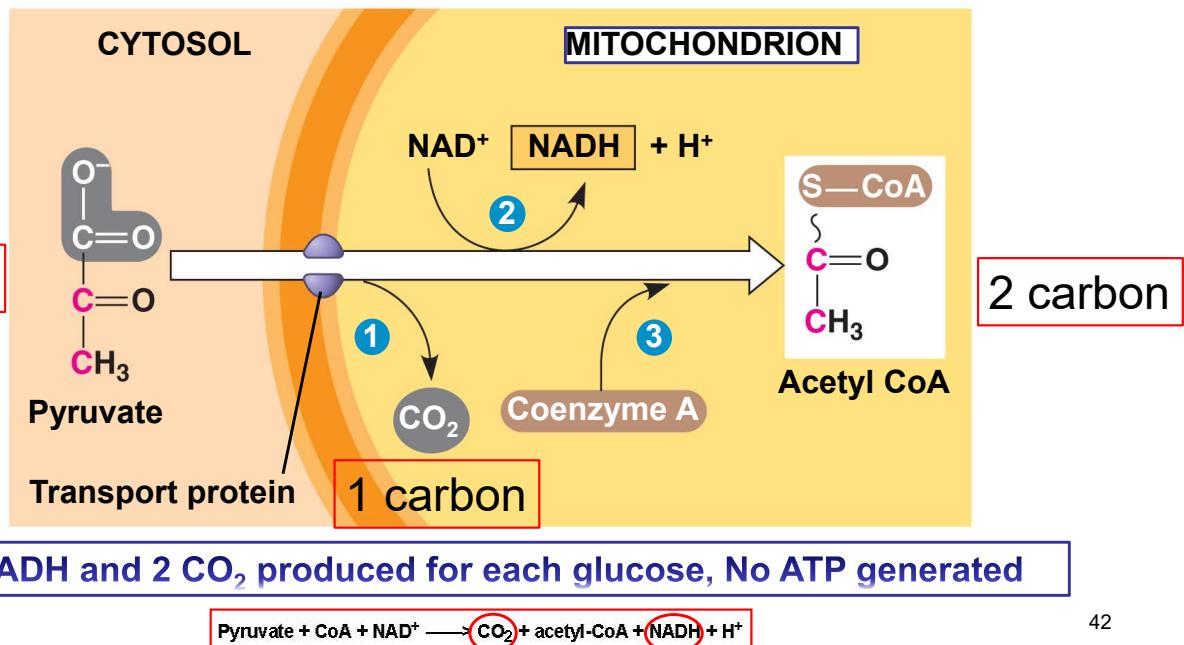




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Formation of Acetyl CoA i.e. Transition reaction



42

In the second stage, the pyruvate diffuses through the mitochondrial membranes until it reaches the mitochondrial matrix, where it reacts with a molecule called coenzyme A, release one CO₂ and form one NADH,

FIGURE 8-7 The reactions in the mitochondrial matrix

- (1) Pyruvate reacts with CoA, forming CO₂ and acetyl CoA. During this reaction, an energetic electron is added to NAD⁺ to form NADH.

Occurs in **mitochondria**

Oxygen needed for this reaction

Aerobic

Pyruvate loses a carbon and 2 oxygen atoms

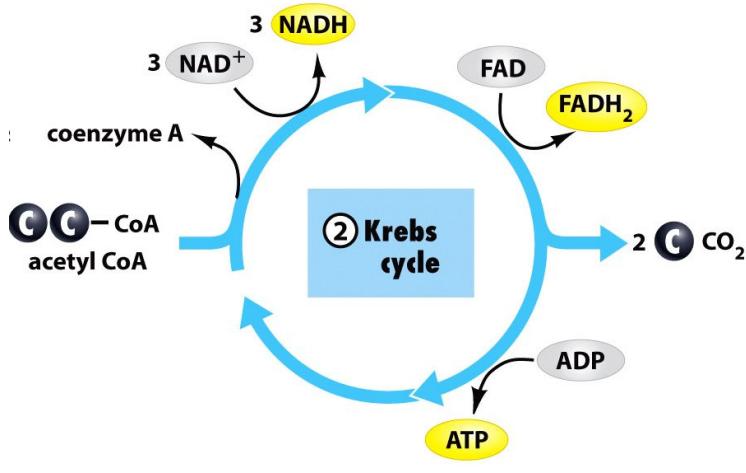
In the form of **carbon dioxide**, CO₂

Two-carbon acetyl unit produced

Linked to **coenzyme A** as **acetyl CoA**

NAD⁺ reduced to **NADH**

Citric acid cycle / Krebs cycle



- Each turn:
- 2 CO₂
 - 1 ATP
 - 3 NADH
 - 1 FADH₂

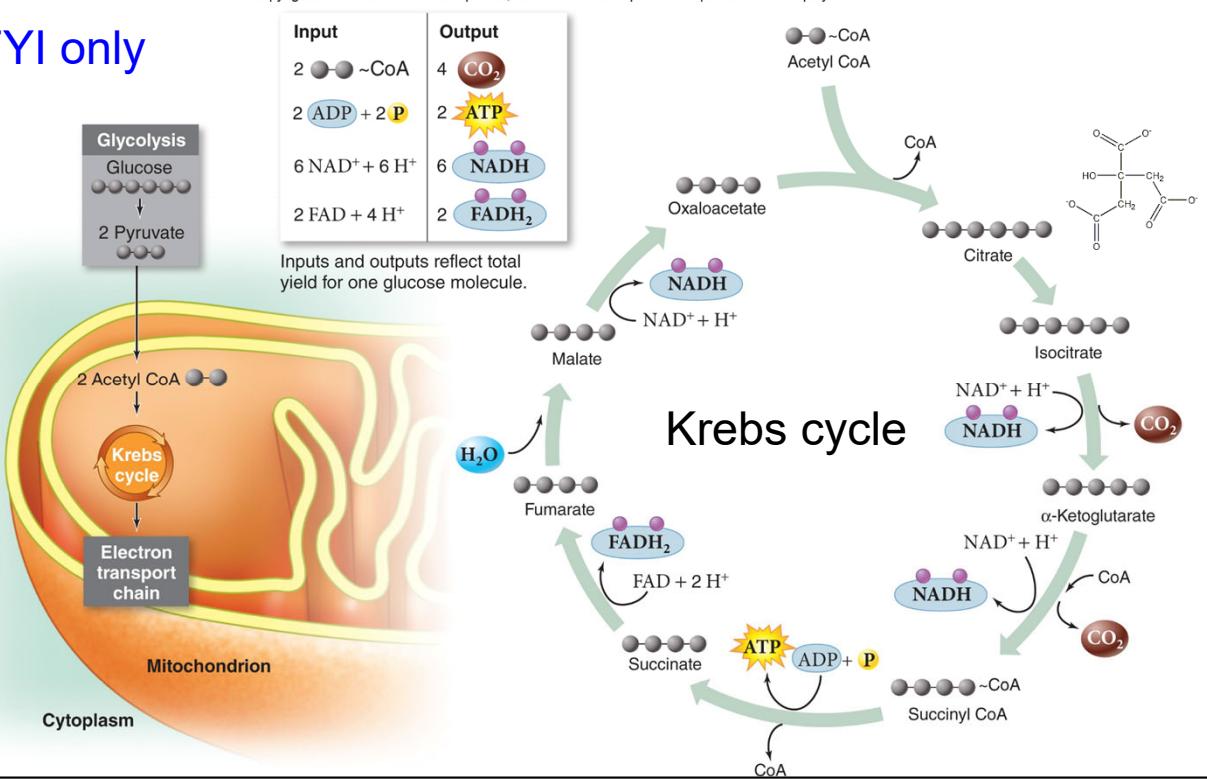
Also called Tricarboxylic acid cycle (TCA cycle)

43

The next stages of the reaction form a cyclic pathway known as the Krebs cycle, named after its discoverer Hans Krebs.

When acetyl CoA enters the Krebs cycle, coenzyme A is released. The Krebs cycle produces one ATP, three NADH, one FADH₂ and two CO₂ for each acetyl CoA. Because each glucose molecule yields two pyruvates, the total energy harvest per glucose molecule in the matrix is two ATP, six NADH, and two FADH₂.

FYI only



<https://courses.lumenlearning.com/boundless-biology/chapter/oxidation-of-pyruvate-and-the-citric-acid-cycle/>

Actual steps for oxidation of pyruvate and citric acid cycle. Just for information. Not tested.

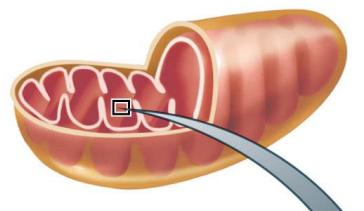
Summary of the Krebs cycle

- The Krebs cycle begins by combining **acetyl CoA** (two-carbon) with a four-carbon molecule to form six-carbon **citrate**, and coenzyme A is released.
- As the Krebs cycle proceeds, enzymes in the matrix break down the acetyl group, releasing **two CO₂** molecules and regenerating the four-carbon molecule for use in future cycles.
- **Each acetyl group produces one ATP, three NADH, and one FADH₂**
- Flavin adenine dinucleotide (FAD), a high-energy electron carrier similar to NAD, picks up two electrons and two H⁺, forming FADH₂
- CO₂ is generated as a waste product. **CO₂** diffuses out of cells and into the blood, which carries it to the lungs, where it is exhaled

45

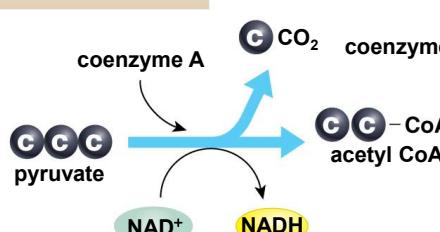
In your body, the CO₂ generated in cells during the stage 1 reactions (Krebs Cycle) diffuses

into your blood, which carries the CO₂ to your lungs. This is why the air you breathe out contains more CO₂ than the air you breathe in.

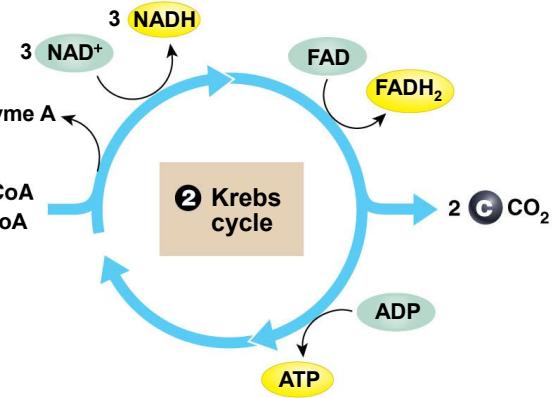


Reactions in the mitochondrial matrix

1 Formation of acetyl CoA



2 Krebs cycle



46

One glucose molecule needs two cycles to break into CO₂

Citric acid cycle

inputs

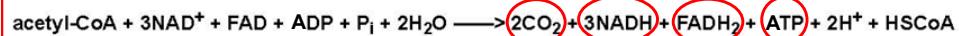
2 acetyl groups
6 NAD⁺
2 FAD

2 ADP + 2 P

outputs

4 CO₂
6 NADH
2 FADH₂

2 ATP

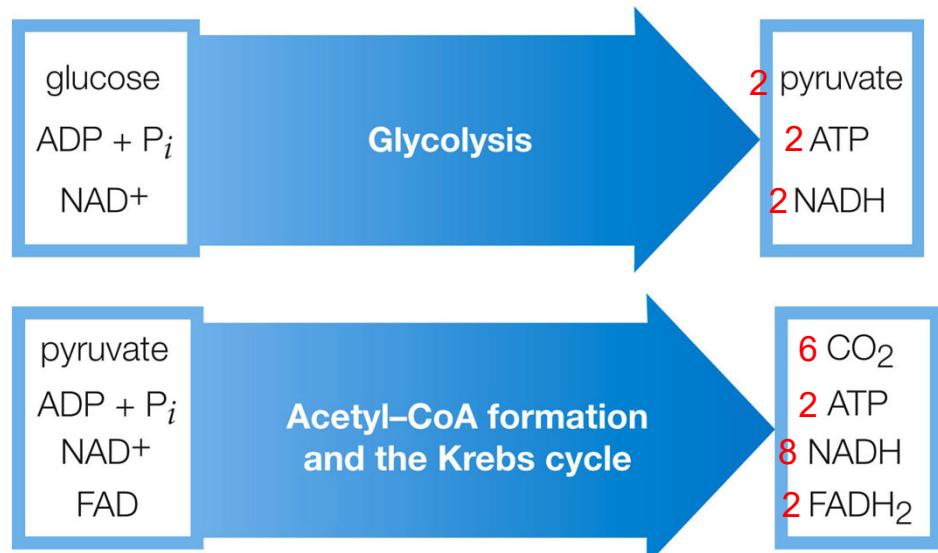


47

Here is the report of inputs and outputs.

Electron Transfer Phosphorylation

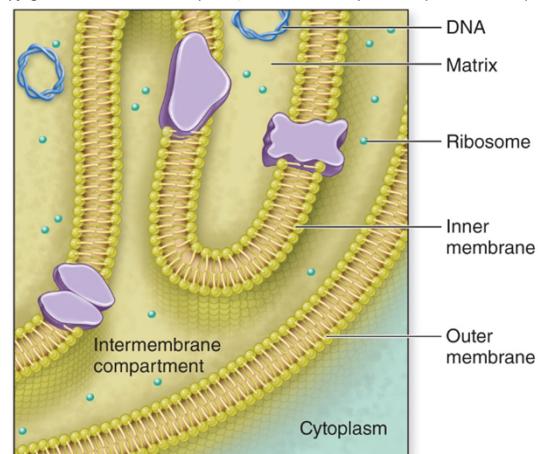
- From glycolysis, acetyl-CoA formation and citric acid cycle
 - High-energy electrons captured by NAD⁺ and FAD
 - For every glucose molecule, **ten NADH** and **two FADH₂** produced



Electron Transfer Phosphorylation

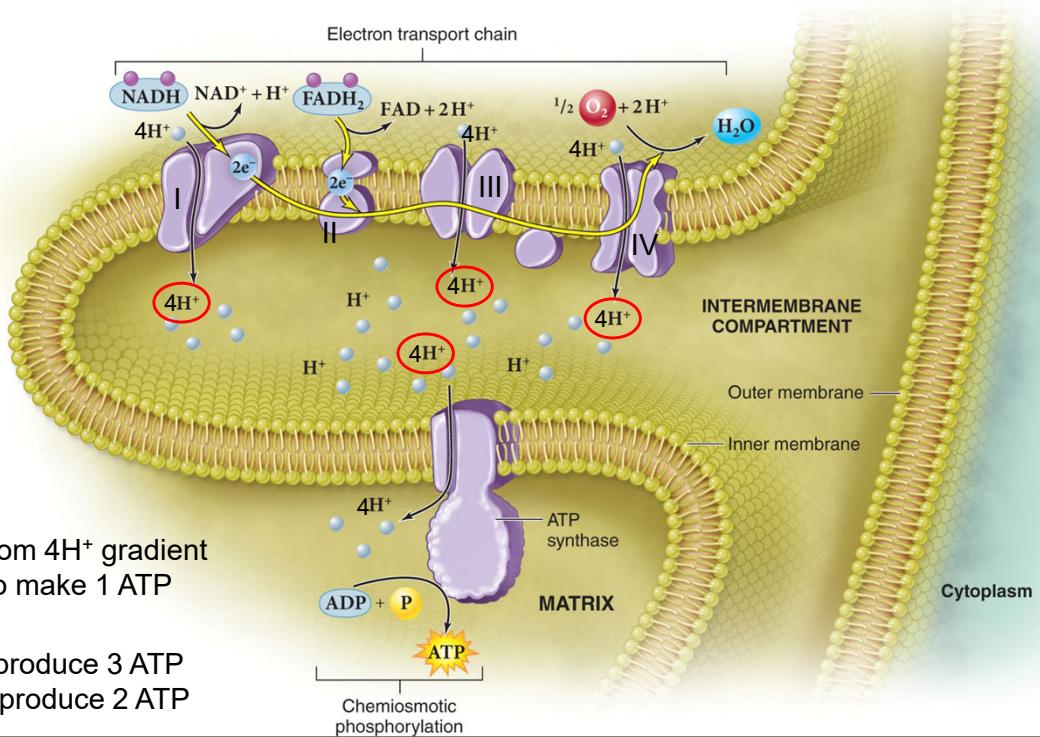
- NADH and FADH₂ release electrons to electron transport chain
- **Electron transport chain** – series of electron carrier proteins
 - In eukaryotes – embedded in **inner mitochondrial membrane**
 - In prokaryotes – embedded in **plasma membrane**
 - High-energy electrons passed successively from one protein to another, losing small amounts of energy at each step

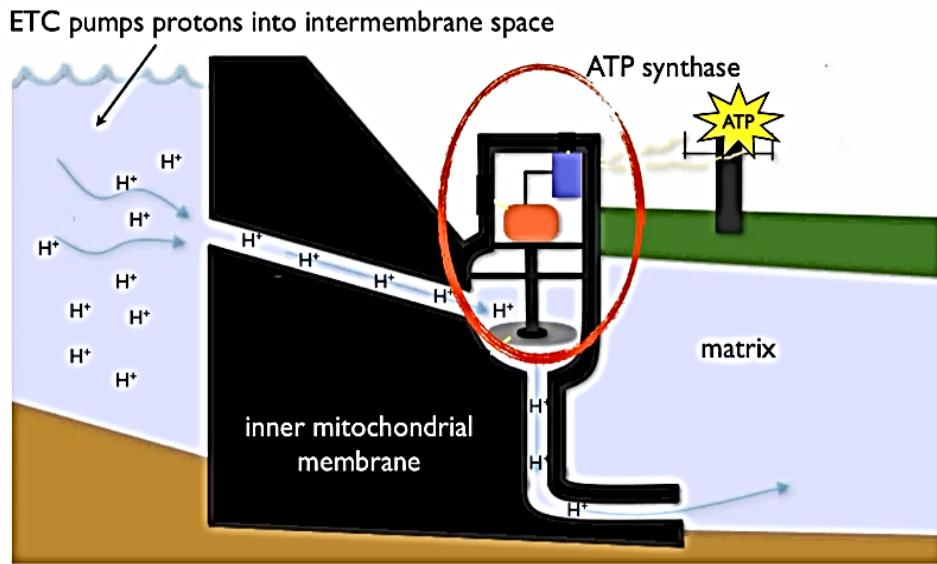
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Electron Transfer Phosphorylation

- Energy lost by electrons harnessed by electron transport chain proteins to **pump H⁺ from matrix into inter-membrane space**
- Higher H⁺ concentration in inter-membrane space
 - **gradient** created
- H⁺ flows down its gradient through **ATP synthase**, which drives synthesis of ATP from ADP and phosphate
 - **Chemiosmosis** – ATP production linked to establishment of H⁺ gradient
- **Oxygen accepts electrons** and combines with H⁺ to form water
 - Final electron acceptor – oxygen





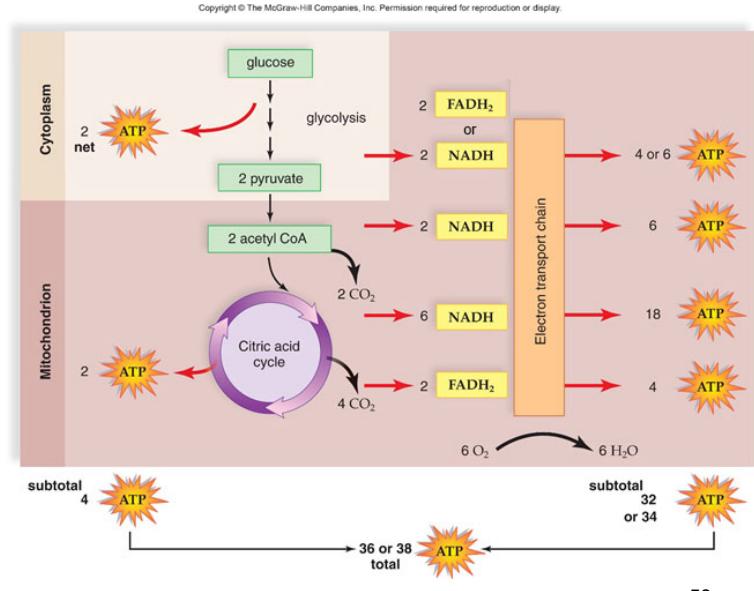
The ATP synthase enzyme complex acts like a proton-powered turbine

52

This proton motive force is a form of stored energy, and protons returning across the membrane down their concentration and voltage gradients release free energy that can be captured by the cell to make ATP. The lipid bilayer membrane is almost impermeable to protons. The ATP synthase enzyme complex acts like a proton-powered turbine, and couples the energy released from the downhill flow of protons back across the membrane through the ATP synthase rotor to synthesize ATP from ADP and inorganic phosphate. The process of generating the proton motive force across the membrane to power ATP synthesis is called [chemiosmosis](#).

Electron Transfer Phosphorylation

- Net yield per molecule of glucose
 - Glycolysis – 2 ATP
 - Citric acid cycle – 2 ATP
 - Electron transfer phosphorylation – 32 or 34 ATP



53

Electron Transport Phosphorylation (Video)



<https://www.youtube.com/watch?v=LQmTKxI4Wn4>

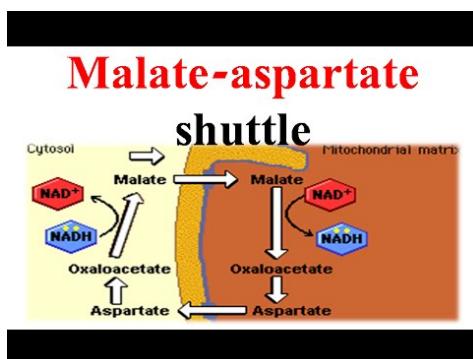
How mitochondria produce energy?

54

Play video (7 mins)

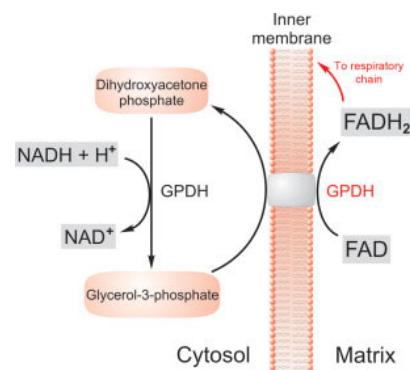
Why 36 or 38 ATP?

- The reason for two different numbers exist for ATP synthesis is because of two different shuttles for NADH (from glycolysis) transfer from cytoplasm into the mitochondrial matrix.



Malate-aspartate shuttle in adults

$$\text{NADH} \rightarrow \text{NADH}$$



Glycerol-phosphate shuttle in infants

$$\text{NADH} \rightarrow \text{FADH}_2$$

55

Net yield per glucose molecule

Glycolysis – 2 ATP

Citric acid cycle – 2 ATP

Electron transport chain – 32 or 34 ATP

In all, about five ATP must be consumed to add a single amino acid to a growing protein. That means that for every glucose molecule that is metabolized, about six amino acids could be added to a protein!

Outline

- Energy
 - Laws of thermodynamics
- Metabolic Reactions
 - Enzyme
 - Energy carrier molecules
 - Energy release
- Aerobic Cellular Respiration
 - Glycolysis
- Acetyl-CoA formation
- Citric acid cycle
- Electron transfer phosphorylation
- **Fermentation**
- Food and Energy

56

Fermentation

- When there is no oxygen after glycolysis, fermentation will occur
 - Processes that **use an organic molecule to regenerate NAD⁺ from NADH are collectively referred to as fermentation.**
 - Do not break glucose down completely to carbon dioxide and water
 - Yield only **2 ATP** from glycolysis
- **Lactate fermentation**
 - In **animals or bacteria** – produces **lactic acid**
- **Alcoholic fermentation**
 - In **yeast** – produces **ethanol**

57

The Joy of Fermentation (youtube)

(<https://www.youtube.com/watch?v=4yupGRIB0iA>) – link on Luminus

Bacteria inhabit deep in mud or soil, animal guts, or some microorganisms are poisoned by oxygen. They rely entirely on the glycolysis to meet their energy needs through a process called fermentation. In other words, fermentation is a way of harvesting chemical energy without using either oxygen and any electron transport chain.

There are two common types of fermentation, alcoholic and lactate.

Pyruvate is processed differently under aerobic and anaerobic conditions

Under aerobic conditions, the high energy electrons in NADH produced in glycolysis are ferried to ATP-generating reactions in the mitochondria, making NAD⁺ available to recycle in glycolysis

Lactate fermentation

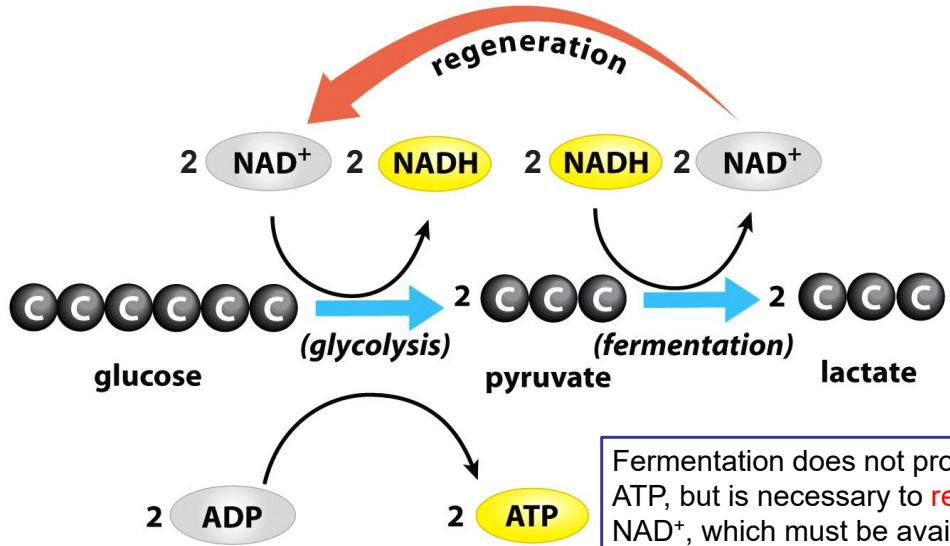


Figure 8-4 Biology: Life on Earth, 8/e
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Fermentation does not produce more ATP, but is necessary to regenerate NAD⁺, which must be available for glycolysis to continue (not sustainable, lactic acid will inhibit enzyme)

58

Glycolysis is the first stage of fermentation, just as it is for aerobic respiration.

Pyruvate is to accept electrons and hydrogen ions and becomes lactate.

This gives one major difference between anaerobic and aerobic glucose breakdown,

Lactic Acid Fermentation

The fermentation method used by animals and certain bacteria, like those in yogurt, is lactic acid fermentation ([link]). This type of fermentation is used routinely in mammalian red blood cells and in skeletal muscle that has an insufficient oxygen supply to allow aerobic respiration to continue (that is, in muscles used to the point of fatigue). In muscles, lactic acid accumulation must be removed by the blood circulation and the lactate brought to the liver for further metabolism. The chemical reactions of lactic acid fermentation are the following:



The enzyme used in this reaction is lactate dehydrogenase (LDH). The reaction can proceed in either direction, but the reaction from left to right

is inhibited by acidic conditions. Such lactic acid accumulation was once believed to cause muscle stiffness, fatigue, and soreness, although more recent research disputes this hypothesis. Once the lactic acid has been removed from the muscle and circulated to the liver, it can be reconverted into pyruvic acid and further catabolized for energy.

FIGURE 8-4 Glycolysis followed by lactate fermentation

Some cells ferment pyruvate to form acids

Human muscle cells can perform fermentation

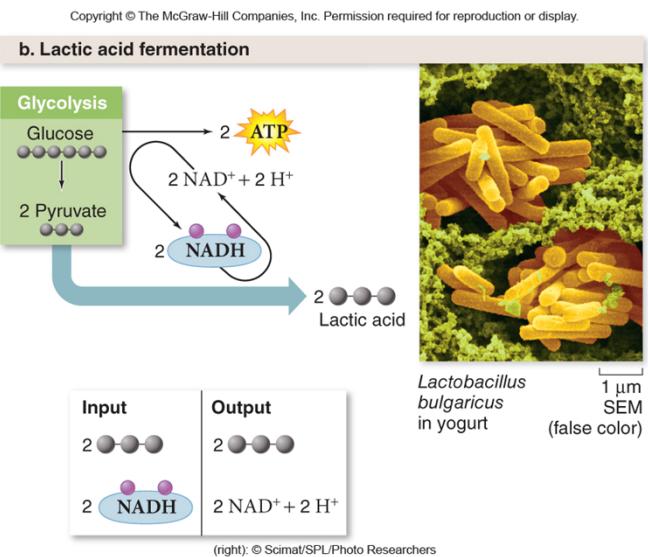
Anaerobic conditions produced when muscles use up O₂ faster than it can be delivered (e.g. while sprinting)

Lactate (lactic acid) produced from pyruvate

What Happens During Lactate Fermentation?

- Carried out by **muscle** cells, certain bacteria and fungi
- Produces **lactic acid**
- NAD⁺ regenerated after NADH transfers electrons to pyruvate
- Used in production of cheese, **yoghurt, sour cream** and **sauerkraut**

What happen if lactate fermentation happen in the brain?



Lab 5

Alcoholic fermentation

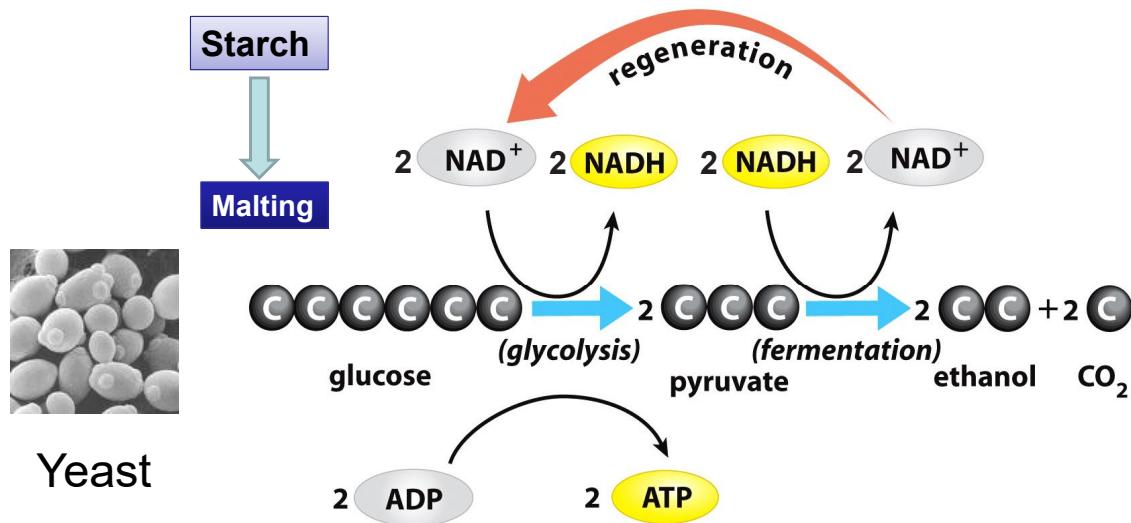


Figure 8-5 Biology: Life on Earth, 8/e
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60

Many microorganisms use another type of fermentation to regenerate NAD under anaerobic conditions: alcoholic fermentation. These organisms produce ethanol and CO₂ (rather than lactate) from pyruate, using hydrogen ions and electrons from NADH.

Starch in malt is hydrolyzed into glucose. (1 marks)

Glucose is converted to pyruvate via glycolysis. (1 marks)

Pyruvate undergoes anaerobic fermentation to produce ethanol and CO₂ (1 marks)

FIGURE 8-5 Glycolysis followed by alcoholic fermentation

Malting grains develops the enzymes that are required to modify the grain's starches into sugars, including monosaccharides (glucose, fructose, etc.) and disaccharides (sucrose, etc.). It also develops other enzymes, such as proteases, which break down the proteins in the grain into forms which can be utilized by yeast

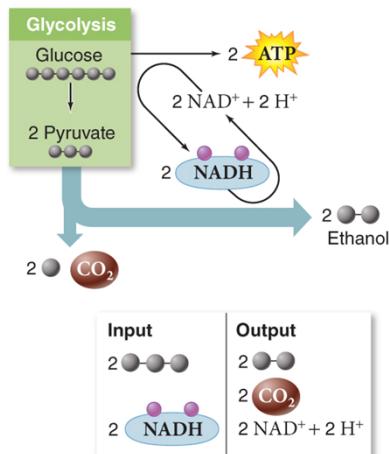
[Link to E-Lab 5](#)

What Happens During Alcohol Fermentation?

- Carried out by microorganisms such as **yeasts** under **anaerobic** conditions
- Produces **CO₂** and **ethanol**
- NAD⁺ regenerated to accept more high-energy electrons during glycolysis
- Used in production of **alcoholic spirits** and **breads**

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a. Alcoholic fermentation



Beer fermentation

© Adam Woolfitt/Corbis

Fermentation

Fermentation keeps ATP production going
when oxygen is unavailable

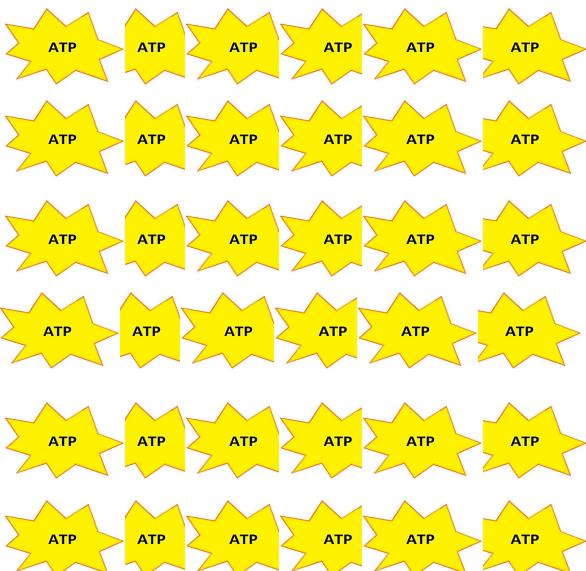


62

Far less efficient than aerobic cellular respiration

- Provides
- Quick burst of energy for muscular activity
- Enough energy for some single-celled anaerobic organisms
- However, lactate and alcohol are toxic
- Lactate changes pH – causes muscle fatigue
- Yeasts die from alcohol produced

Aerobic Respiration



Lactate/Alcoholic Fermentation



FYI only: This yields approximately 380,000 calories (cal) per mole of glucose (ATP ~ 10,000 cal/mole). Thermodynamically, the complete oxidation of one mole of glucose should yield approximately 688,000 cal; the energy that is not conserved biologically as **chemical energy** (or ATP formation) is liberated as **heat** (308,000 cal). Thus, the cellular respiratory process is at best about 55% efficient.

<https://www.ncbi.nlm.nih.gov/books/NBK7919/>

63

Blood doping is the practice of boosting the number of red blood cells in the bloodstream in order to enhance athletic performance. Because such blood cells carry oxygen from the lungs to the muscles, a higher concentration in the blood can improve an athlete's aerobic capacity (VO₂ max) and endurance. Many methods of blood doping are illegal, particularly in professional sports.

People most interested in blood doping would be distance runners from probably 800 meters and up, swimmers in the long races, cyclists for sure, perhaps people in rowing or in the triathlon. So those would be the sports that just kind of pop right out," Joyner said.

By increasing the number of red blood cells, blood doping causes the blood to thicken. This thickening forces the heart to work harder than normal to pump blood throughout the body. As a result, blood doping raises the risk of:

blood clot

heart attack

stroke

An estimated 20 European cyclists are believed to have died as a result of blood doping over the past 25 years.

Outline

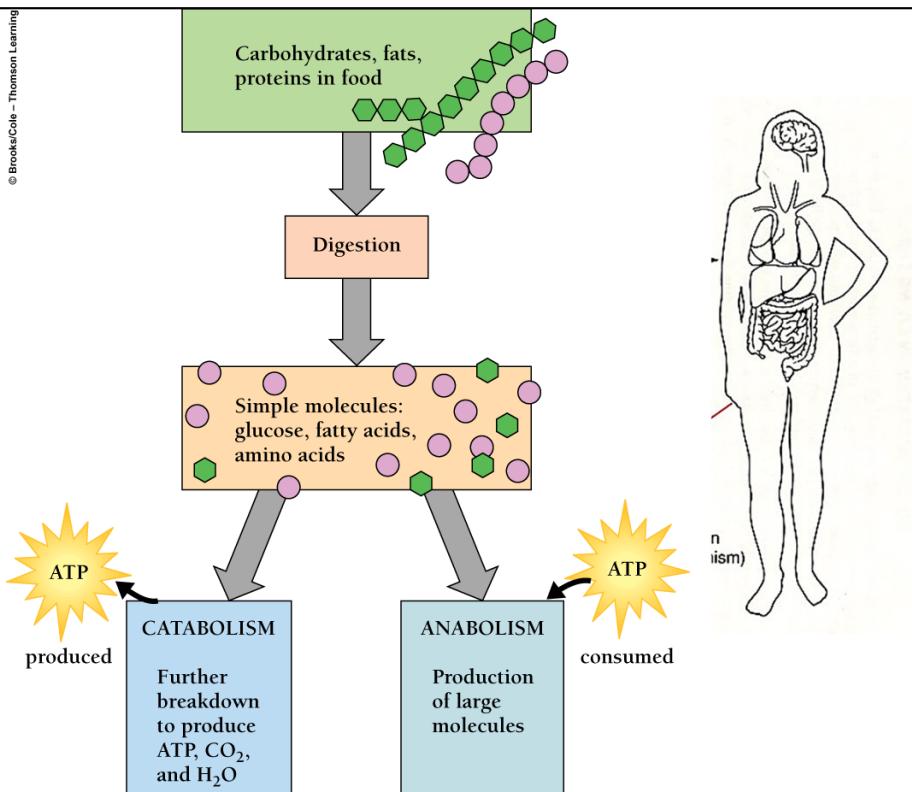
- Energy
 - Laws of thermodynamics
- Metabolic Reactions
 - Energy carrier molecules
 - Energy release
- Aerobic Cellular Respiration
 - Glycolysis
 - Acetyl-CoA formation
- Citric acid cycle
- Electron transfer phosphorylation
- Fermentation
- **Food and Energy**

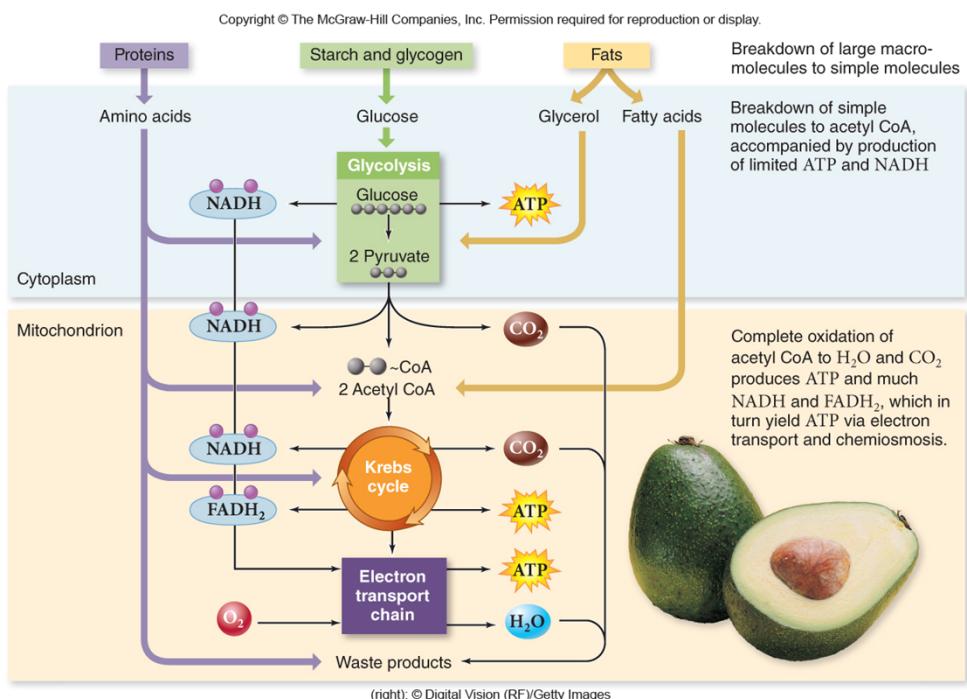
Food and Energy

- Starch and other complex carbohydrates
 - Broken down to monosaccharide subunits that enter glycolysis
- Fats (triglycerides) broken down to glycerol and fatty acids
 - Glycerol enters glycolysis
 - Fatty acids converted to acetyl-CoA that enter citric acid cycle
- Proteins broken down to amino acid subunits
 - Usually used to build proteins but may be used as energy source
 - Amino group removed and converted into waste product eliminated in urine
 - Remaining carbon groups converted to acetyl-CoA, pyruvate or intermediates of citric cycle, depending on amino acid

65

Fats and proteins can go through the aerobic respiration cycle





67

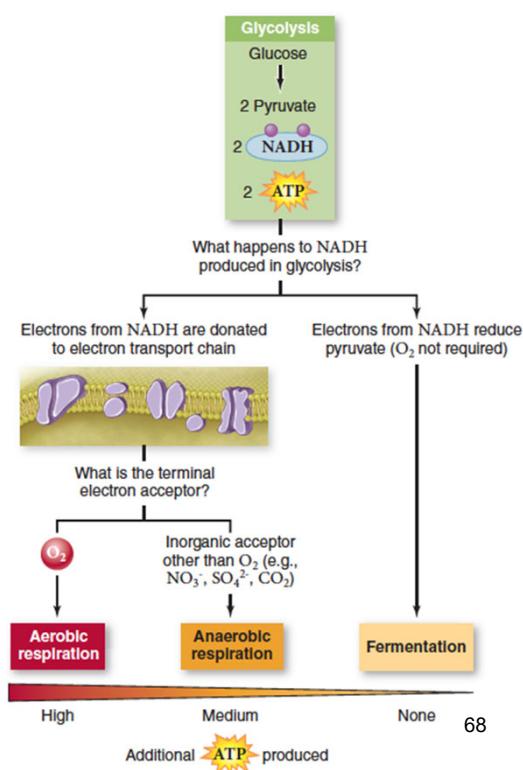
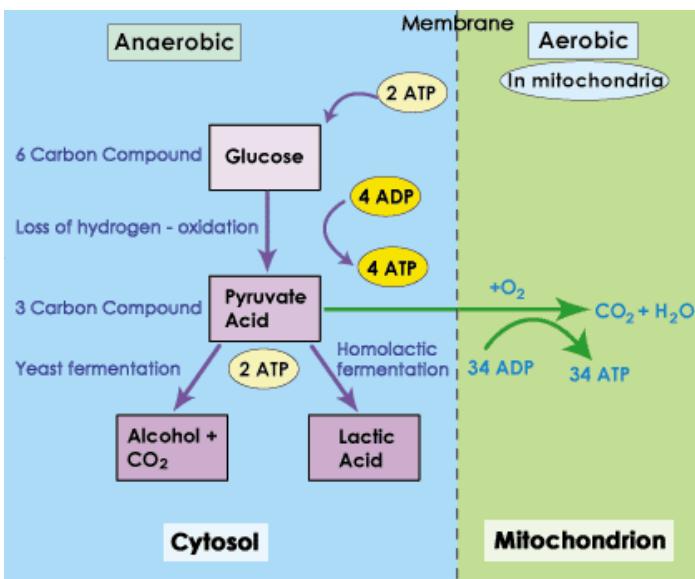
Glucose often enters the body as starch (a long chain of glucose molecules) or sucrose (table sugar; glucose linked to fructose), but the typical human diet also provides considerable energy in the form of fat and some from protein. This is possible because various intermediate molecules of cellular respiration can be formed by other metabolic pathways. These intermediates then enter cellular respiration at various stages and are broken down to produce ATP (FIG. 8-8).

For example, some of the 20 amino acids from protein can be directly converted into pyruvate, and the others can be transformed through complex pathways into molecules of the Krebs cycle. To release the energy stored in fats, the long fatty acid tails (which comprise most of each fat molecule; see Chapter 3) are broken into two-carbon fragments and combined with CoA, producing acetyl CoA, which enters the Krebs cycle.

Different nutrients are digested into simple basic subunits, which are absorbed and delivered into our cells. These molecules are then broken

down to produced energy molecules such as ATP.

Summary



During cellular respiration, some living systems use an organic molecule as the final electron acceptor. Processes that use an organic molecule to regenerate NAD⁺ from NADH are collectively referred to as fermentation. In contrast, some living systems use an inorganic molecule as a final electron acceptor. Both methods are called anaerobic cellular respiration, where organisms convert energy for their use in the absence of oxygen.

Certain prokaryotes, including some species of bacteria and Archaea, use anaerobic respiration. For example, the group of Archaea called methanogens reduces carbon dioxide to methane to oxidize NADH. These microorganisms are found in soil and in the digestive tracts of ruminants, such as cows and sheep. Similarly, sulfate-reducing bacteria and Archaea, most of which are anaerobic (Figure), reduce sulfate to hydrogen sulfide to regenerate NAD⁺ from NADH.

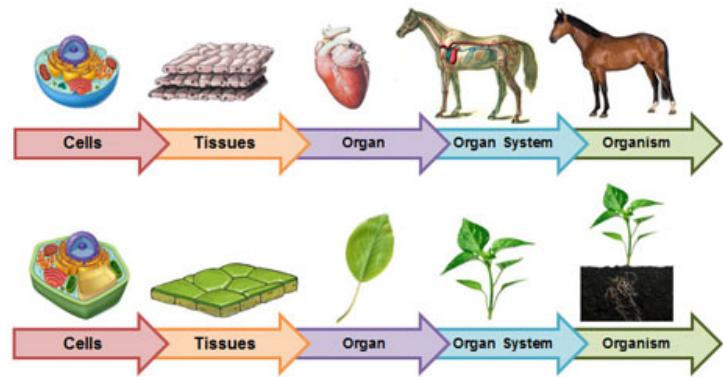
Certain prokaryotes, like Clostridia, are obligate anaerobes. Obligate anaerobes live and grow in the absence of molecular oxygen. Oxygen is a poison to these microorganisms, killing them on exposure.

It should be noted that all forms of fermentation, except lactic acid

fermentation, produce gas. The production of particular types of gas is used as an indicator of the fermentation of specific carbohydrates, which plays a role in the laboratory identification of the bacteria.

Please complete the quiz on Canvas for lecture 4

LSM1301



L5: DNA & Heredity

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Like Father Like Son

Similarities in both physical and behavioral

Could the term of “like mother, like son” be true?



- Like mother, like daughter
- The apple doesn't fall far from the tree

2

I believe you are familiar with this term, “like father, like son” which refers to the various kinds of similarities, both physical and behavioral, that can exist between fathers and sons. There are other similar idioms: “like mother, like daughter,” and the more general “the apple doesn’t fall far from the tree.” But how can we explain it from biological viewpoint, why is it the “like”, but not the “same”? How much do you believe “like mother , like son” is also true? The knowledge of DNA and heredity help us explain these features. we are going to learn in the topic.

From a genetic perspective? Because each person has a direct combination of genes from their biological parents.

There are many different contexts where this idiom might be used, and it can be complimentary or insulting depending on the specific area of comparison. Similar idioms exist which make the same basic comparison, including the female version: “like mother, like daughter,” and the more general “the apple doesn’t fall far from the tree.” Experts suggest that there is often some truth to these idioms, partly for reasons of genetics, and also due to parenting styles and environmental conditions during a child’s developmental years.

From a genetic perspective, each person has a direct combination of genes from their biological parents. They might have similarities in appearance with one parent or both, along with certain temperamental tendencies and identical

physical traits. Generally, parents try to impart some of their values to children, who will often mimic some of their behavior, especially during the developmental stage of life. The term “like father, like son” existed before people really understood much about the reasons for these similarities, but it does generally refer to the common-sense understanding of the basic tendencies in familial similarity. From a scientific perspective, the term “like mother, like son” could just as easily be true, but societies often have a general cultural expectation that sons will be more like their fathers and daughters will be more like their mothers, even though there isn’t really a scientific basis for such a bias.

People use the term “like father, like son” in many different situations and for the purposes of several different comparisons. It could be used to suggest that a father and son have a similar hair color, or a common quirky facial feature. Some people use it specifically to describe behavioral tendencies that seem identical, or hobbies that a father and son may have in common. Most of the time, it is used to point out things that are especially notable and similar in a memorable way. For example, if a child learns a musical instrument, and his dad was a professional musician, people might say, “like father, like son” as a way of referencing that similarity. Other terms, such as “the apple doesn’t fall far from the tree,” and, “like mother, like daughter,” work in basically the same way and fit into similar contexts.



The myths

- **Dominant genes are most often expressed as observable**
- **Recessive gene can be masked by a dominant gene. In order to have a trait that is expressed by a recessive gene, such as Hitchhiker's thumb, you must get the gene from both parents**

Ability to roll tongue



(recessive)



There is little laboratory evidence supporting the hypothesis that tongue rolling is inheritable and dominant.

No Hitchhiker's thumb (**dominant**)



Hitchhiker's thumb (**recessive**)



Cleft chin (**dominant**)



Chin without a cleft (**recessive**)



3

More familiar in our daily life, this is about you.

But please be informed, those are quite complex characteristics, the dominant and recessive relationship are not well proven.

<http://askabiologist.asu.edu/mendelian-trait-humans>

A **recessive** gene is a gene that can be masked by a dominant gene. In order to have a trait that is expressed by a **recessive** gene, such as blue eyes, you must get the gene for blue eyes from both of your parents

The expression of traits, however, is often far more complicated than in those listed above or those which Mendel observed in his garden. Sometimes tens, or even hundreds of genes can play a role in just one trait! In some cases, genes can block or exaggerate processes in the cell which change the visible phenotype. In other cases, environmental factors such as temperature, light, and nutrient levels influence the development of a phenotype. Below is a list of traits in humans involving interaction between multiple genes.

Tongue-rolling: The myth

Some people can roll their tongue into a tube, and some people can't. This is one of the most common traits that biology teachers use to demonstrate basic genetic

principles. Alfred Sturtevant (one of the [pioneers of *Drosophila* genetics](#)) described tongue rolling as a simple two-allele character, with the allele for rolling (usually given the symbol T or R) being dominant over the allele for non-rolling (t or r) (Sturtevant 1940). Many studies have shown that the myth is incorrect, but tongue rolling remains a popular subject in genetics classes.

<https://www.pbs.org/newshour/science/genetic-myth-textbooks-get-wrong>

<https://udel.edu/~mcdonald/mythtongueroll.html>

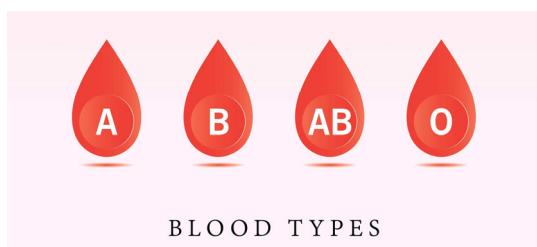
Face freckles (dominant)



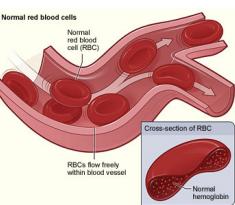
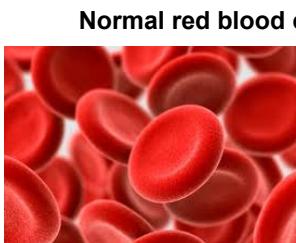
No face freckles (recessive)



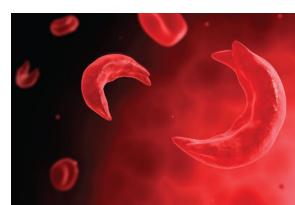
Blood type A/B (dominant)



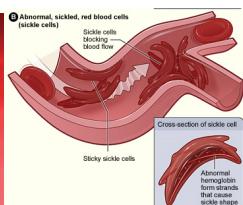
Blood type O (recessive)



Normal red blood cells (dominant)



Sickle blood cells (recessive)



4

The genes that code for freckles are located on chromosome 4q32–q34 and are common in Caucasian and Chinese populations.

The blood type example includes more details: alleles with A and B are codominant with each other and alleles that code for O are recessive to A and B. That's why we have A, B, AB and O blood type.

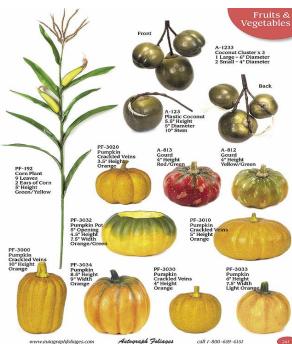
Red blood cells are dominant to sickle blood cells. Normally, the flexible, round red blood **cells** move easily through blood vessels. In **sickle cell anemia**, the red blood are shaped like sickles or crescent moons. These **cells** stick together and can't easily move through the blood vessels. This can block small blood vessels and the movement of healthy, normal oxygen-carrying blood. The **blockage** can cause pain. I will talk more about the mutation that causes this trait later on.

<https://www.nature.com/scitable/topicpage/mendelian-genetics-patterns-of-inheritance-and-single-966/>

Introduction



- **Genetics:** the scientific study of inheritance and its underlying mechanisms.
- **Genetics:** The study of the structure and function of genes and the transmission of genes from parents to offspring.



5

Hope the examples have interested you to study this topic, then, what is genetics? Although there are many versions of definition, but they are essentially the same, Genetics is the scientific study of the structure and function of genes,

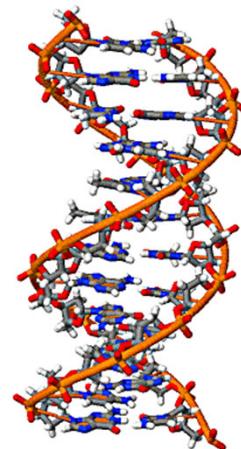
In summary,

A Paradox: Genetics is Hard Because it is Simple

Genetic determinism.

Outline of the topic: DNA and Heredity

- What is DNA?
- How was DNA proved to genetic materials?
- How was DNA structure determined?
-  How does DNA replicate?
- How does DNA inherit?
- How is DNA integrity maintained?



6

Here is the outline of this topic. DNA replication is the key.



- What Is DNA?
- How Did Scientists Discover That Genes Are Made of DNA?

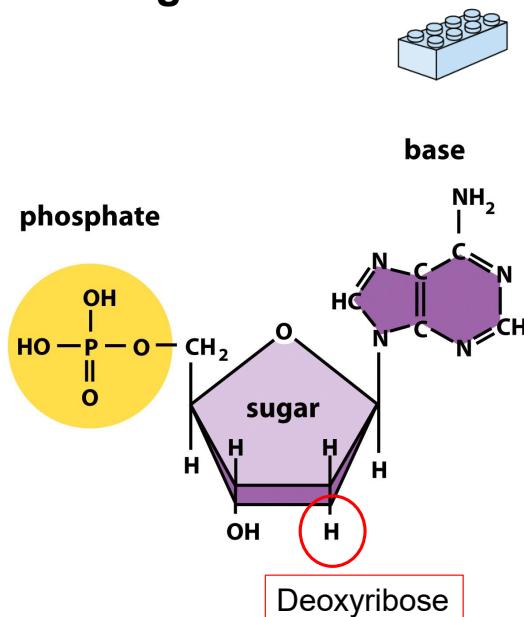
7

Let me introduce important background knowledge first, that is about what is the DNA and how the DNA was proven to be genetic material

What Is DNA?

Deoxyribo Nucleic Acid or DNA is a long chain of nucleotides

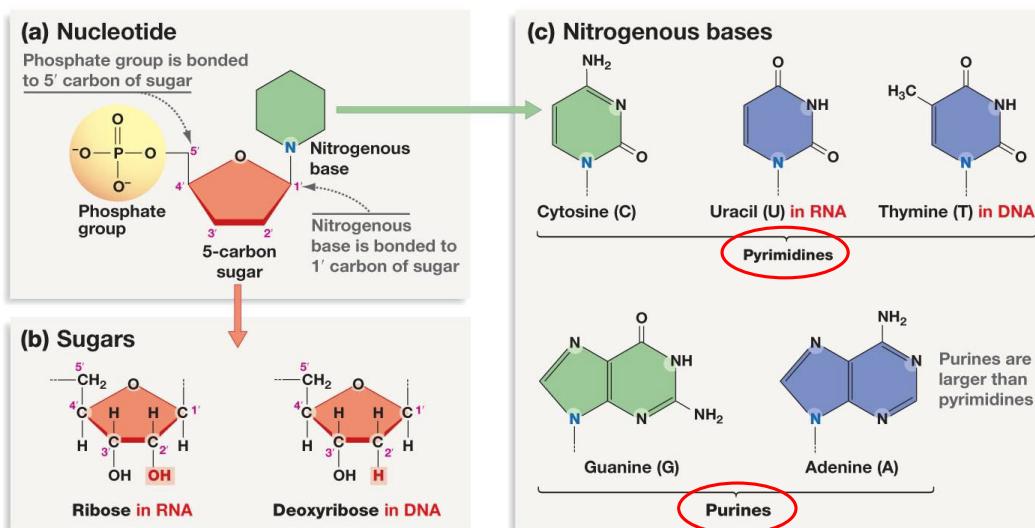
- Nucleotides are the **monomers (building blocks)** of nucleic acid chains
- All nucleotides are made of three parts:
 - Phosphate group
 - Five-carbon sugar
 - Nitrogen-containing base



8

Let me introduce important background knowledge first, that is about what is the DNA and how the DNA was proven to be genetic material

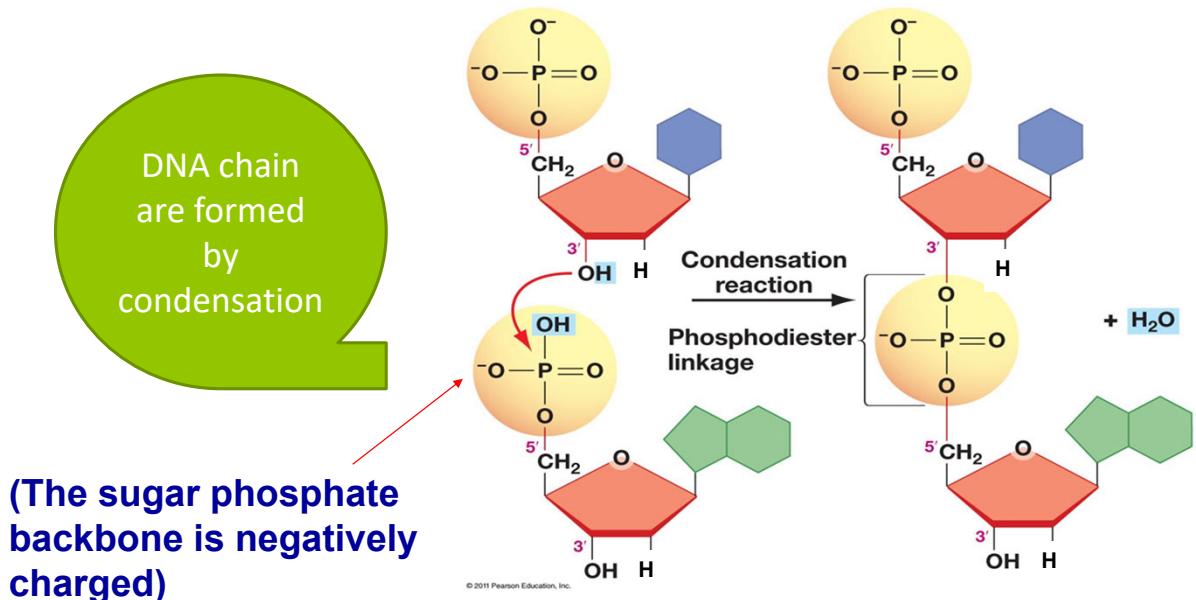
Nucleotides in DNA & RNA



9

Let me introduce important background knowledge first, that is about what is the DNA and how the DNA was proven to be genetic material

DNA Chain Formation



10

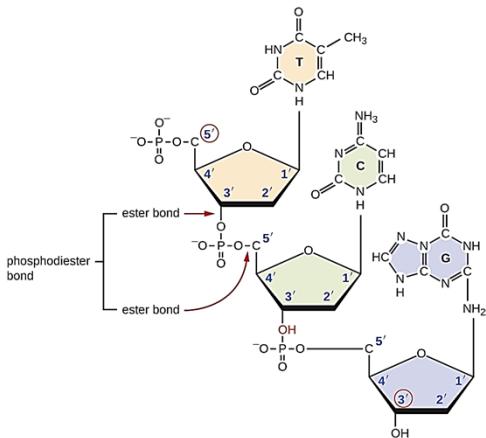
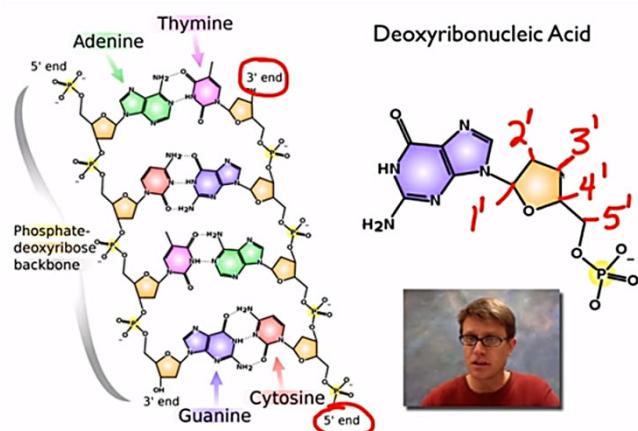
Let me introduce important background knowledge first, that is about what is the DNA and how the DNA was proven to be genetic material

What is DNA?

Online Learning Materials

- What is DNA? ***

[https://www.youtube.com/watch?v=q6PP-C4udkA \(11 min\)](https://www.youtube.com/watch?v=q6PP-C4udkA)



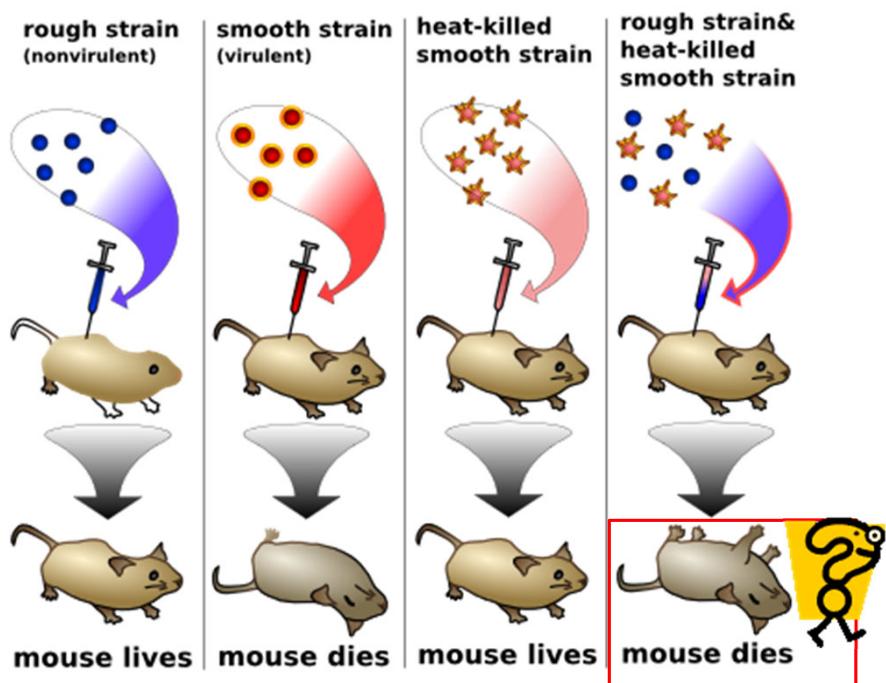
11

Here is the outline of this topic. DNA replication is the key.

How Did Scientists Discover That Genes Are Made of DNA?



Frederick Griffith's transformation experiment using *Streptococcus pneumoniae* in 1928



12

How did

This is an **Awesome Accidental Discovery**, with a lot luck. When Griffith was working on ways to develop vaccine to protect

Lucky accidents have allowed people to discover unexpected but useful side effects, **every dog has its day**

Some scientific discoveries come about after painstaking, goal-oriented lab work finally yields the result that a researcher is trying to find.

But many of the most incredible discoveries in world came about when someone found something they weren't looking for.

At that days, getting pneumonia infection is really bad, 5-6 days after the infection, the patient will have a high fever, and then, one of the two things will happen, either live or die.

In 1928, British bacteriologist **Frederick Griffith** conducted a series

of experiments using **Streptococcus pneumoniae** bacteria and mice. **Griffith** wasn't trying to identify the genetic material, but rather, trying to develop a vaccine against **pneumonia**.

Some substance in the heat-killed S-strain changed the living, harmless R-strain bacteria into the deadly S-strain, a process Griffith called transformation.

the S (smooth) strain, has a polysaccharide coat and produces smooth, shiny colonies on a lab plate; the other, the R (rough) strain, lacks the coat and produces colonies that look rough and irregular. The relatively harmless R strain lacks an enzyme needed to make the capsule found in the virulent S strain.

It happened against all odds

Pandemic

Puzzled him, extracted blood,

Griffith's experiment discovering the "transforming principle" in *pneumococcus bacteria*.

Isolated **two strains** of *Streptococcus pneumoniae* in 1928

Rough (**R**) strain was **harmless**

Smooth (**S**) strain was **pathogenic**

Harmless bacteria could be **transformed** into deadly ones

Mixture of heat-killed S strain bacteria and live R strain bacteria killed the mouse!

Thinking

What caused the transformation?

Eh, it is indeed an interesting question...



13

The substance that causes transformation might be the long-sought molecule of heredity.



Oswald Avery, Colin MacLeod, and Maclyn McCarty in 1940s



Courtesy of the Rockefeller Archive Center.
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"Studies on the **chemical nature** of the substance inducing transformation of pneumococcal types" Journal of Experimental Medicine (1944).

14

About 20 years

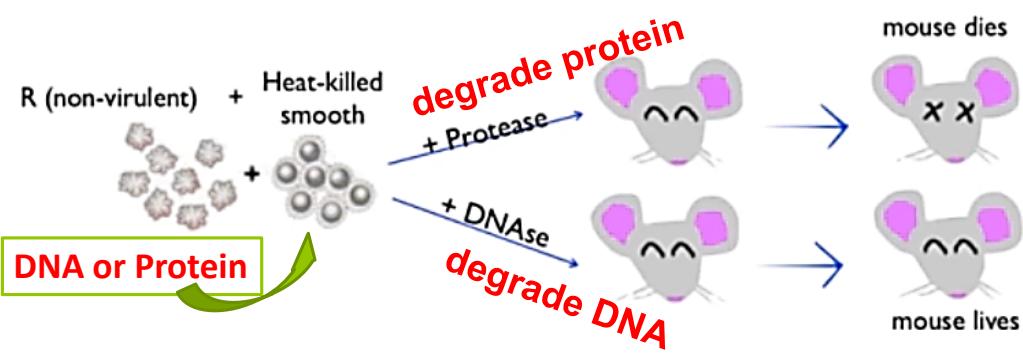
Oswald Avery, Colin MacLeod, and Maclyn McCarty showed that DNA (not proteins) can transform the properties of cells, clarifying the chemical nature of genes.

Avery, MacLeod and McCarty identified DNA as the "transforming principle" while studying *Streptococcus pneumoniae*, bacteria that can cause pneumonia. The bacteriologists were interested in the difference between two strains of Streptococci that Frederick Griffith had identified in 1923: one, the S (smooth) strain, has a polysaccharide coat and produces smooth, shiny colonies on a lab plate; the other, the R (rough) strain, lacks the coat and produces colonies that look rough and irregular. The relatively harmless R strain lacks an enzyme needed to make the capsule found in the virulent S strain.

Griffith had discovered that he could convert the R strain into the virulent S strain. After he injected mice with R strain cells and, simultaneously, with heat-killed cells of the S strain, the mice developed pneumonia and died. In their blood, Griffith found live bacteria of the deadly S type. The S strain extract somehow had "transformed" the R strain bacteria to S form. Avery and members of his lab studied transformation in fits and starts over the next 15 years. In the early 1940s, they began a concerted effort to purify the "transforming principle" and understand its chemical nature.

Bacteriologists suspected the transforming factor was some kind of protein. The transforming principle could be precipitated with alcohol, which showed that it was not a carbohydrate like the polysaccharide coat itself. But Avery and McCarty observed that proteases - enzymes that degrade proteins - did not destroy the transforming principle. Neither did lipases - enzymes that digest lipids. They found that the transforming substance was rich in nucleic acids, but ribonuclease, which digests RNA, did not inactivate the substance. They also found that the transforming principle had a high molecular weight. They had isolated DNA. This was the agent that could produce an enduring, heritable change in an organism. Until then, biochemists had assumed that deoxyribonucleic acid was a relatively unimportant, structural chemical in chromosomes and that proteins, with their greater chemical complexity, transmitted genetic traits.

Chemical Nature



- ❖ To rule out the possibility that a **protein** contaminant was actually causing the transformation, Avery treated samples with **protein-destroying enzymes (protease)** and still induced transformation
- ❖ When **DNA-destroying enzymes (DNase)** were added to the samples, transformation did not occur
- ❖ This suggested that the transforming molecule from the S-strain was **DNA**, not protein

Genes made of DNA

15

Biologists **could not accept** that DNA was the genetic material

Hereditary material originally **believed** to be unknown class of proteins

Reason

Heritable traits are **diverse** and therefore, molecules encoding traits must be diverse

All **DNA identical** in structure, but **proteins** are made of 20 amino acids and are structurally **diverse**

How Did Scientists explain Griffith's transformation experiment?

The experiments can be explained if DNA is the transforming agent

- Heating S-strain cells killed them but did not completely destroy their DNA
- When killed S-strain bacteria were mixed with living R-strain bacteria, fragments of DNA from the dead S-strain cells was absorbed and incorporated into the chromosome of the R-strain bacteria
- If these fragments of DNA contained the genes needed to cause disease, an R-strain cell would be transformed into an S-strain cell
- Thus, Avery, MacLeod, and McCarty concluded that genes are made of DNA.

16

Can watch a review video on Griffith's experiment
(<https://www.youtube.com/watch?v=4LU71ubTCZA>)

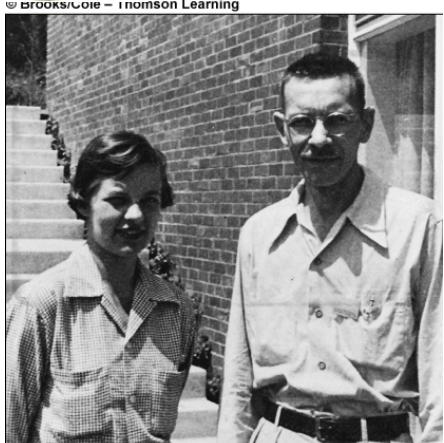
Thinking



**Are you convinced by their experimental data?
Why?**



Martha Chase and Alfred Hershey



© BROOKS/Cole – Thomson Learning

Hershey, A. D. and M. Chase. 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. 36: 39-56

Alfred Hershey was awarded the **Nobel Prize in 1969**. He shared the prize with two other American scientists for "discoveries concerning the replication mechanism and the genetic structure of viruses."

Alfred Hershey and Martha Chase

- Worked with **bacteriophage**
 - Virus that **infects bacteria**
- **Viruses**
 - Composed of **protein coat** (capsid) surrounding **nucleic acid core** (DNA or RNA)
 - Capable of **forcing** host cells to make more viruses
 - **Not living organisms**

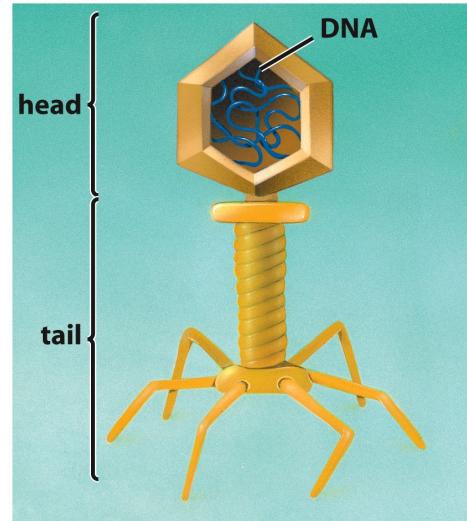


Figure E9-1a Biology: Life on Earth, 8/e
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T2

19

BACTERIOPHAGE LIFE CYCLES

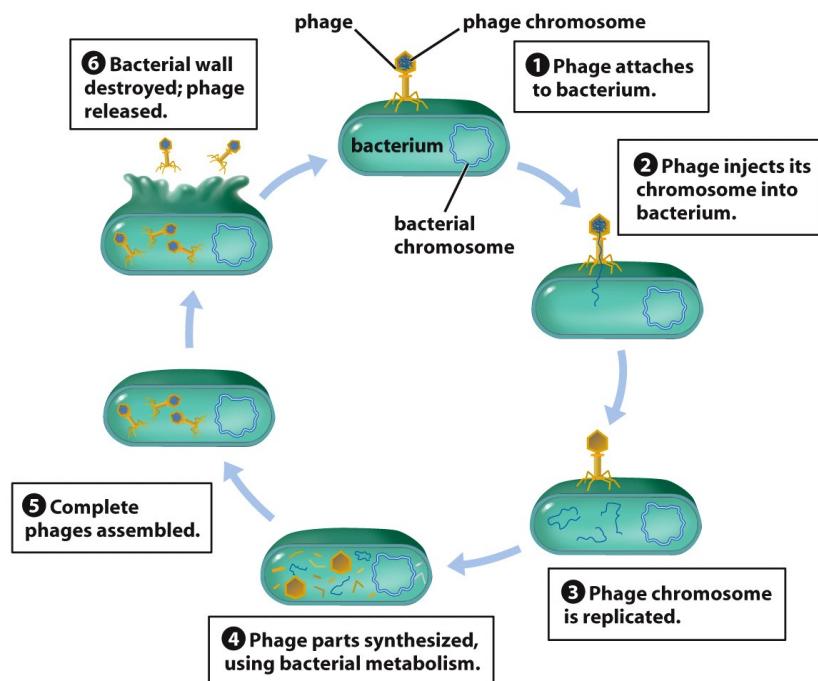
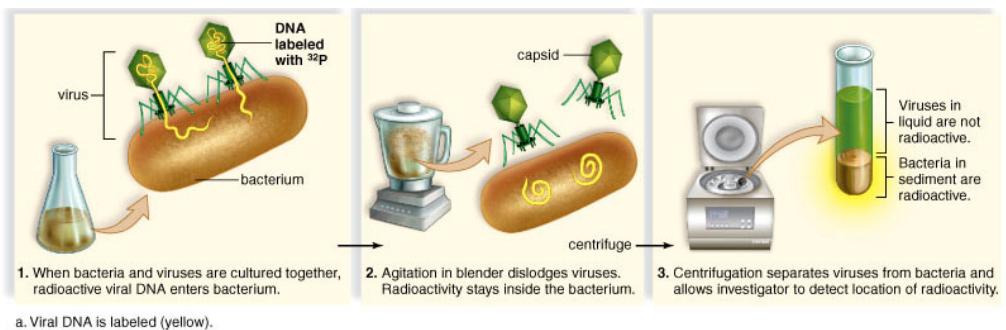
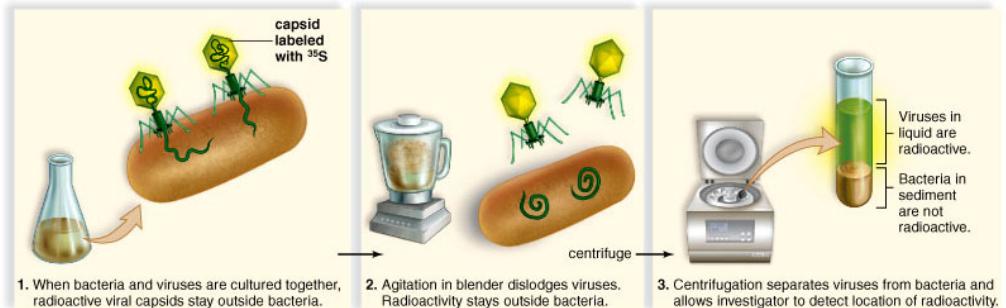


Figure E9-1b Biology: Life on Earth, 8/e
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a. Viral DNA is labeled (yellow).



Radioactive isotopes ^{35}S to track protein and ^{32}P to track nuclei acid , conducted in 1952

21

8 yrs later.

Hershey and Chase forced one culture of phages to synthesize DNA using radioactive phosphorus, thereby labeling the phage DNA. They forced another culture of phages to synthesize protein using radioactive sulfur, labeling the phage protein 1 . Bacteria were infected by one of these two labeled phage cultures 2 . Then the bacteria were whirled in a blender to shake the phage coats off the bacteria 3 , followed by centrifugation to separate the phage coats from the bacteria 4 .

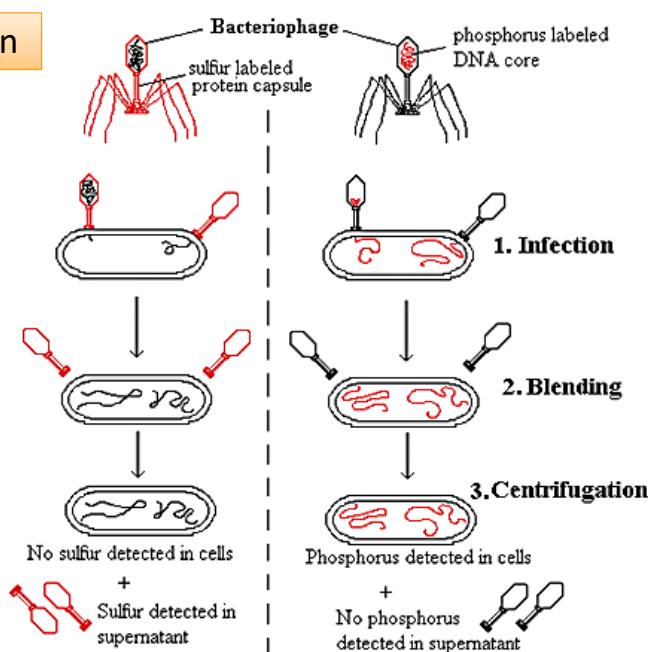
Hershey and Chase found that, if bacteria were infected by phages containing radioactively labeled protein, the resulting phage coats were radioactive but the bacteria were not. If bacteria were infected by phages containing radioactive DNA, the bacteria became radioactive but the phage coats were not 5 . Therefore, the substance injected by the phages into the bacteria was DNA, not protein. Further, the infected bacteria produced new phages, even after the protein coats were

removed, showing that the injected DNA, not the protein in the coat, was the genetic material. In the words of James Watson, this experiment provided “powerful new proof that DNA is the primary genetic material.”

Alfred Hershey and Martha Chase (1952)

- Used radioactive isotopes to label two sets of phage
 - Radioactive sulphur ^{35}S to label protein
 - Radioactive phosphorus ^{32}P to label DNA
- Allowed labelled viruses to infect bacteria
- Location of radioactive labels after infection
 - shown that DNA, not protein, enters and infects bacterium

^{35}S to track protein



^{32}P to track nuclei acid

The Hershey-Chase Experiment

Can we use radioactive ^{13}N to track protein instead of using ^{35}S ?

0

No, because nitrogen is only present in DNA and not proteins

0%

Yes, because nitrogen is only present in proteins and not DNA

0%

No, because nitrogen is present in both proteins and DNA

0%

No, because nitrogen is not present in both proteins and DNA

0%

None of the above

0%

Start the presentation to see live content. For screen share software, share the entire screen. Get help at pollev.com/app

Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

More info at polleverywhere.com/support

Can we use radioactive ^{13}N to track protein instead of using ^{35}S ?

[https://www.polleverywhere.com/multiple_choice_polls/B4h9dV8R2D1TvPLIIphJ3
?state=opened&flow=Default&onscreen=persist](https://www.polleverywhere.com/multiple_choice_polls/B4h9dV8R2D1TvPLIIphJ3?state=opened&flow=Default&onscreen=persist)

What Is the Structure of DNA?

25

Knowing the DNA is genetic materials attracts many scientists to find its structure and replication

Erwin Chargaff (1905-2002)

In 1949, he found **proportions** of bases in many different species

Organism	% Adenine	% Thymine	% Cytosine	% Guanine
Octopus	33.2	31.6	17.6	17.6
Sea Urchin	32.8	32.1	17.3	17.7
Rat	28.6	28.4	20.5	21.4
Grasshopper	29.3	29.3	20.7	20.5
Human	29.3	30.0	20.0	20.7



26

Knowing the DNA is genetic materials attracts many scientists to find its structure and replication. One important finding was

in natural DNA the number of guanine units equals the number of cytosine units and the number of adenine units equals the number of thymine units

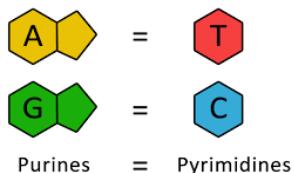
the composition of DNA varies from one species to another, in particular in the relative amounts of A, G, T, and C bases

Unfortunately, he did not recognize the importance of his discovery, and shared his results with Watson and Crick in 1952.

Chargaff's Rules

Rule 1

for a dsDNA



The number of A is equal to T
Adenine (A)= Thymine (T)

In the same DNA molecule

The number of G is equal to C
Guanine (G)=Cytosine (C)



Adenine Thymine

Cytosine Guanine

Rule 2

The relative amounts of A, G, T, and C bases in a DNA molecule varies from one species to another (i.e. differs in different DNA molecules)

27

in natural DNA the number of guanine units equals the number of cytosine units and the number of adenine units equals the number of thymine units

the composition of DNA varies from one species to another, in particular in the relative amounts of A, G, T, and C bases

Unfortunately, he did not recognize the importance of his discovery, and shared his results with Watson and Crick in 1952.

X-ray Diffraction Studies of DNA

Rosalind Elsie Franklin

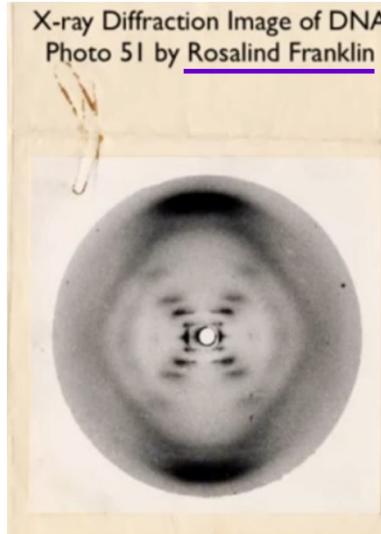


Maurice Wilkins



British, King's College in London

X-ray Diffraction Image of DNA
Photo 51 by Rosalind Franklin



28

Nobel prize is not given/awarded post(h)umously.

Franklin (1920-1958)

Fame and glory

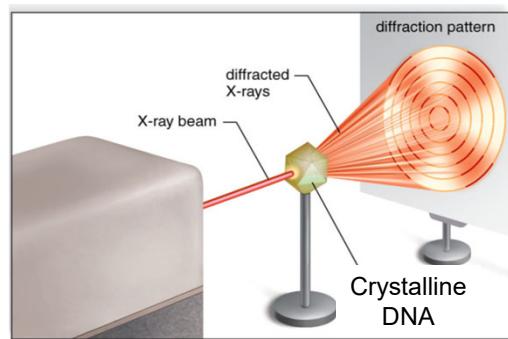
Meanwhile at King's College in London, Maurice Wilkins (b. 1916) and Rosalind Franklin were also studying DNA. The Cambridge team's approach was to make physical models to narrow down the possibilities and eventually create an accurate picture of the molecule. The King's team took an experimental approach, looking particularly at x-ray diffraction images of DNA.

John Randall (physicist), first big boss,

physical chemistry

Linus Pauling, presumed subversive tendencies, the US refused to issue him a passport to leave the US,

From X-ray diffraction patterns, they deduced several qualities of DNA



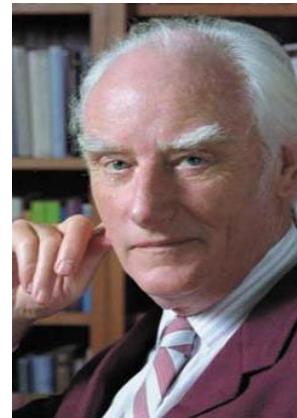
1. It is **helical**; that is, twisted like a corkscrew and consisting of repeating subunits
2. It is long and thin, and has **a uniform diameter of 2 nanometers**

James Watson and Francis Crick combined the X-ray data with bonding theory to deduce the structure of DNA

James Dewey Watson
(American)



Francis Crick
(Englishman)



physical
scientists

Cambridge University

30

In 1947 Crick knew no biology and practically no organic chemistry or crystallography, so that much of the next few years was spent in learning the elements of these subjects. During this period, together with W. Cochran and V. Vand he worked out the general theory of X-ray diffraction by a helix, and at the same time as [L. Pauling](#) and R. B. Corey, suggested that the alpha-keratin pattern was due to alpha-helices coiled round each other.

A critical influence in Crick's career was his friendship, beginning in 1951, with [J. D. Watson](#), then a young man of 23, leading in 1953 to the proposal of the double-helical structure for DNA and the replication scheme. Crick and Watson subsequently suggested a general theory for the structure of small viruses.

At a conference held at the Zoological Station in Naples, he met Maurice Wilkins, whose work convinced him to direct his research towards the structure of nucleic acids and proteins.

In 1950, Watson joined Cavendish Laboratories where many other important people involved in archeology, such as [Francis Crick](#), Maurice Wilkins, and Rosalind Franklin were trying to determine the makeup of DNA.

Brash,
During their inventure,

Francis Crick hired a young mathematician,

James is young, brash, colourful.

Cambridge, X-ray power.

A form, which had not been applied to the B form. Rosalind Franklin did not realise that Watson and Crick were racing to publish first, which they did on the 18th of March, 1953, so beating her because she had not published.

provocative concepts

Watson has repeatedly supported [genetic screening](#) and [genetic engineering](#) in public lectures and interviews, arguing that stupidity is a disease and the "really stupid" bottom 10% of people should be cured.^[53] He has also suggested that beauty could be genetically engineered, saying "People say it would be terrible if we made all girls pretty. I think it would be great."^[53]

On April 25, 1953

Nature published three back-to-back papers on the structure of DNA. It was a momentous day for science.

1. **Watson, J.D. and Crick, F.H.C. (1953)** A Structure for Deoxyribose Nucleic Acid. Nature 171:737-738.
2. **Wilins, M.H.F., Stokes, A.R., and Wilson, H.R. (1953)** Molecular Structure of Deoxypentose Nucleic Acids. Nature 171:738-740.
3. **Franklin, R. and Gosling, R.G. (1953)** Molecular Configuration in Sodium Thymonucleate. Nature 171:740-741.

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.M.S. *Discovery II* for their part in making the observations.

*Young, F. B., Bernstein, H., and Jevons, W. *Phil. Mag.*, **40**, 149 (1949).

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***Vogel, K. *Z. Phys. Chem. (Munich)*, **11**, 153 (1950).

****FRAZER, V. W. *Adv. Met. Letters, Phil. (Oxford)*, **2** (1951) 11005.

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribonucleic Acid

WE wish to suggest a structure for the salt of deoxyribonucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. We have also studied the structure of the various nucleic acids, and we find that our proposed structure is in general agreement with the results obtained by them.

The novel features of our structure are the following: (1) It is built on the same principle as the X-ray diagrams of the salt, the free acid, without the acidic hydrogen atoms. It is not clear what forces would hold the chains together in these cases, as the negatively charged phosphorus near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

An alternative structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is not well defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribonucleic acid. This structure has two main features: (1) the phosphate groups remain on the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain contains one phosphate ester group joining 5'-deoxyribose residues with 3',5' linkages. The two chains run in opposite directions. Both chains follow right-handed helical paths, and the dyed sequences of the atoms in the two chains run in opposite directions. Each chain follows the same path as in the structure of Wilkins' model No. 1; that is, the bases are on the inside of the molecule, and the phosphate groups are on the outside.

The configuration of the sugar and the atoms near it is close to Furberg's model², the sugar being roughly perpendicular to the attached base. There

is a residue on each chain every 3-4 Å. in the α -direction.

We have assumed an angle of 36° between adjacent residues in the same direction, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the axis is taken to be 5 Å. As the phosphates are on the outside, ratios have been calculated.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together. The phosphate groups are perpendicular to the fibre axis, and the planes of the bases are also perpendicular to the fibre axis. These planes are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two heads side with their two co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The pyrimidine bases follow the purines: purine position 1, pyrimidine position 6 to pyrimidine position 6 to purine position 1.

It is assumed that the bases can occur in the same sequence as in the most plausible tautomer form (that is, with the keto rather than the enol configuration), it is found that only specific pairs of bases can bond together. These pairs are adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, it cannot bond with the other, as the other member must be thymine specifically for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way, except that the sequence of bases of each helix formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is also approximately determined.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{3,4} on deoxyribonucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly consistent with the X-ray data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following sections. We are very anxious to receive the details of the results reported there which we devised our structure, which rests mainly though not entirely on published experimental data and theories.

It has not escaped our notice that the specific patterns we have postulated immediately suggests a possible copying mechanism for the genetic material, similar to that proposed by the structure of Wilkins' model No. 1; that is, the bases are on the inside of the molecule, and the phosphate groups are on the outside. The configuration of the sugar and the atoms near it is close to Furberg's model².

It has been found⁵ also Stokes, unpublished that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by a series of Bessel functions. A uniform spacing of points gives a series of Bessel functions corresponding to the helix pitch, the intensity decreasing along the n th layer line being proportional to the square of J_n , the n th order Bessel function. A straight line may be drawn approximately through

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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Twiss, L., and Watson, J. D. *Nature*, **171**, 546 (1943); *Proc. U.S. Natl. Acad. Sci.*, **30**, 81 (1944).

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Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury)¹ show the basic molecular configuration has great simplicity. The purpose of this note is to describe the structure of deoxypentose nucleic acid, and to give some of the experimental evidence for the poly-nucleotide chain configuration being helical, and giving the helical parameters.

The structure of deoxypentose nucleic acid is the same in all species (although the nucleic base ratios differ) and is the same in bacteria, in plant cells, and in purified nucleic acid. The same linear group of polydeoxynucleotide chains may pack together parallel to each other to give crystalline structures or paracrystalline consistencies. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the size of the repeat unit, the other by the spacing of the nucleotides, and the other by the longer spacings of the chain configuration.

It is probable impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

Crystallized paracrystalline deoxypentose nucleic acid structure B' in the following communication by Franklin and Gosling gives a fibre diagram as shown in Fig. 1. Astbury suggested that the strong 3.4-Å. reflection corresponded to the inter-nucleotide repeat along the fibre axis. The ~34 Å. spacing is, however, and not the 3.4 Å. spacing of a deoxynucleoside component, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the intervening water. The absence of reflections on or near the midline immediately suggests a helical structure with axis parallel to fibre length.

Diffracton by Helices

It may be shown² also Stokes, unpublished that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by a series of Bessel functions. A uniform spacing of points gives a series of Bessel functions corresponding to the helix pitch, the intensity decreasing along the n th layer line being proportional to the square of J_n , the n th order Bessel function. A straight line may be drawn approximately through

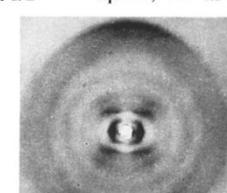


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *E. coli*. Fibre axis vertical.

the innermost maxima of each Bessel function and the origin. The effect of this is to allow an effect of roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_n) on the n th layer line. The helical configuration produces a change on this function at frequency ω , the effect being to reproduce the intensity distribution about the origin around the new origin, on the n th layer line, according to $e^{-jn\omega}$ (Fig. 2).

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a helical molecule, roughly about an axis parallel to the helix axis, the whole helical pattern is given by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives confirmation for the inner-

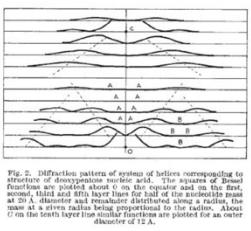


Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions of the first, second, third and fifth orders for half of the nucleotide pass are plotted on a circle of radius 12 Å. The points are plotted at a given radius being proportional to the radius. About C on the fourth layer line points are plotted for an outer diameter of 12 Å.

We wish to thank Prof. J. T. Randall for encouragement; Profs. E. Chargaff, R. Signer, J. A. V. Butler and Drs. J. D. Watson, J. D. Smith, L. Hamilton, J. C. White and G. R. Wyatt for supplying materials; Mr. J. H. Williams for help which was often impossible; also Drs. J. D. Watson and Mr. F. H. C. Crick for stimulation, and our colleagues R. E. Franklin, R. G. Gurney, D. H. Northrop and Dr. J. D. Watson for discussion. One of us (H. R. W.) wishes to acknowledge the award of a University of Wales Fellowship.

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April 2.

¹Archibald, W. T., *Synth. Soc. Rep. Edn.*, 1, Nucleic Acid (Cambridge University Press, 1949).

²Wilcock, D. P., and Oster, G., *Biochim. et Biophys. Acta*, 7, 308 (1951).

³Wilson, M. H. F., Goding, R. G., and Stokes, W. E., *Nature*, 167, 709 (1951).

⁴Archibald, W. T., and Bell, F. O., *Cold Spring Harbor Symp. Quant. Biol.*, 16, 193 (1951).

⁵Archibald, W. T., Crick, F. H. C., and Vand, V., *J. de Phys.*, 8, 581 (1952).

⁶Archibald, W. T., and Randall, J. T., *Biochim. et Biophys. Acta*, 16, 745 (1950).

Molecular Configuration in Sodium Thymonucleate Fibres

SOUDIUM thymonucleate fibres give two distinct types of X-ray diagram. The first corresponds to a crystalline structure, and is described as having about 75 per cent relative humidity. At higher humidities a diffuse halo, which becomes more intense and of higher degree of order, appears and persists over a wide range of ambient humidity. The change from *A* to *B* is reversible. The water content of the fibres in *B* fibres which undergo this reversible change may vary from 40-50 per cent to several hundred per cent of the dry weight. The water content of the fibres in *A* structures *A*, and in these structure *B* can be obtained with an even lower water content.

The X-ray diagram of *B* (see photograph) shows in striking manner the feature characteristic of helical structures, first worked out in this laboratory by Dr. J. D. Watson and myself. In this diagram Dr. Vand, Stokes and Wilkins were the first to propose such structures for nucleic acids as a result of direct studies on the fibres. These structures had been previously suggested by Furberg (thesis, London, 1949) on the basis of X-ray studies of muscle.

While the X-ray evidence cannot, at present, be taken as direct proof that the structure is helical, other evidence strongly supports it, and we believe the existence of a helical structure highly probable.

Structure *B* is derived from the crystalline structure *A* which is obtained when the fibres take up quantities of water in excess of about 40 per cent of their weight. The change is accompanied by an increase of about 10 per cent in the length of the fibre, and by a substantial reduction in the density of the molecule. It therefore seems reasonable to suppose that in structure *B* the structural units of sodium thymonucleate containing groups of nucleotides are relatively free from the influence of neighbouring



Sectional description nucleus from cold syrup. Structure B

molecules, each unit being shielded by a layer of water. Each unit is thus free to take up its least-energy conformation independently of its neighbours and, since the radius of the helix is determined by the sum of the radii of the nucleotides involved, it is likely highly that the general form will be helical¹. If we adopt the hypothesis of a helical structure, we can deduce from the X-ray diagram of structure *B*, to make certain deductions as to the nature and dimensions of the helix.

In the case of a smooth helix the ratio of the third and fifth layer lines lie approximately on straight lines radiating from the origin. For a smooth simple-coiled helix the structure factors on the *n*th layer line is given by:

$$F_n = J_0(2\pi rR) \exp i n(\phi + \frac{1}{4}\pi)$$

where $J_0(n)$ is the zeroth order Bessel function of n , r is the radius of the helix, and ϕ and θ are the radial and azimuthal co-ordinates in reciprocal space²; this expression leads to an approximately linear array of reflections in the form of a series of radial lines, $J_0(J_n)$.

If, instead of a smooth helix, we consider a series of roughly equally spaced along the helix, the transformation in the general case transforms $J_0(J_n)$ into $J_n(J_n)$. Instead Vand is more complicated. But if there is a cylinder number, m , of residues per turn of the helix, possibly some slight inter-penetration of the cylindrical units in the dry state making their effective radius rather less, it is then obvious that the spacing of the layer lines of the measurements alone, whether one repeating unit contains ten nucleotides on each, or two or on each of three nucleotides. (If the spacing of the radial lines in *A* is 8 Å, the cylinder would contain twenty nucleotides.) Two other arguments, however, make it highly probable that the structure is a helix.

In the present case the fibre-axis period is 34 Å, and the very strong reflection at 34 Å. lies on the tenth layer line. This reflection is also very strong from the 3-4-Å. reflection as from the origin as visible on the fifth and lower layer lines, having a maximum intensity on the 10th layer line and a minimum on the 5th layer line. (The strong outer streaks which apparently radiate from the 3-4-Å. maximum are due to the presence of a small amount of water in the fibre.) This argument strongly suggests that there are exactly 10 residues per turn of the helix. If this is so, then from a measurement on the 5th layer line, the radius of the helix, can be calculated. In the present instance, measurements of R_A , R_B , R_3 and R_4 all lead to values of r of about 10 Å.

Since this linear array of maxima is one of the strongest features of the X-ray diagram, we must conclude that a crystallographically important part of the molecule has a helix of this diameter. This can only be the phosphate group of the nucleotides.

If ten phosphorus atoms lie on one turn of a helix of 10 Å., the distance between successive phosphorus atoms in a molecule is 7.1 Å. This corresponds to the 10 Å. P_2O_7 distance in a very extended molecule. We therefore conclude further that the phosphates lie on the outside of the structural unit.

Thus, our conclusions differ from those of Pauling and Corey³, who proposed for the nucleic acids a helical structure in which the phosphate groups form a dense core.

We must now consider briefly the equatorial maxima. For a single helix the series of equatorial maxima is given by $J_m(2\pi rR)$. The maxima on our photograph do not, however, fit this series, the value of r deduced above being 10 Å. and the value of m being 10, 24 Å. and then only a faint sharp reflection at 9.0 Å. and two diffuse bands around 5.5 Å. and 4.0 Å. This is in agreement with the theory, but it is not for us to know that the helix so far considered can only be the most important member of a series of coaxial helices. The non-phosphate parts of the molecule will in this case be helical. However, it can be shown that, whereas these will not appreciably influence the maxima observed on the layer lines, they will have the effect of destroying the maxima on other layer lines.

Thus, if the structure is helical, we find that the phosphate groups or phosphorus atoms lie on a helix of diameter of about 20 Å., and the sugar and base groups are located on the turns of the helix. Considerations of density show however that a cylinder of length 34 Å. and diameter 20 Å. must contain many more than ten nucleotides.

Since structure *B* often exists in fibre with low water content, it is likely that the water in the unit cannot differ greatly from that of dry sodium thymonucleate (160 gm./cm.³), the water in fibres of high water content being about 100 gm./cm.³ of structural unit. On this basis we find that a cylinder of radius 10 Å. and height 34 Å. would contain thirty-two nucleotides. It is therefore possible, possibly with some slight inter-penetration of the cylindrical units in the dry state, making their effective radius rather less, to have a cylinder of 10 Å. diameter containing 32 nucleotides alone, whether one repeating unit contains ten nucleotides on each, or two or on each of three nucleotides. (If the spacing of the radial lines in *A* is 8 Å., the cylinder would contain twenty nucleotides.) Two

other arguments, however, make it highly probable that the structure is a helix.

First, a study of the Patterson function of structure *A*, using superposition methods, has indicated that there is a helical arrangement of the nucleotides in the unit cell in this structure. Since the *A* = *B* transformation is readily reversible, it seems very unlikely that the nucleotides are arranged in a helical structure *A*. Secondly, from measurements on the X-ray diagram of structure *B* it can readily be shown that, whether the number of chains per unit is two or three, the chains are not equally spaced along the

fibres axis. For example, three equally spaced chains would mean that the *n*th layer line depended on $J_{3n}R$, and would lead to a helix of diameter about 60 Å. This is many times larger than the primitive unit length of 10 Å., and is also many times larger than the dimensions of nucleotides. Three unequally spaced chains, on the other hand, would be crystallographically impossible.

Thus, while we do not attempt to offer a complete interpretation of the fibre-diagram of structure *B*, we believe that the phosphate groups lie on the outside of the structural unit, and that the nucleotides are co-axial molecules which are not equally spaced along the fibre axis, their mutual displacement being seen as a series of equatorial maxima observed as a result of the imperfect matching on the layer lines; if one molecule is displaced from the other by about one-eighth of the fibre-axis period, this would account for the absence of the *n*th layer line maxima as the weakness of the sixth. Thus our general ideas are not inconsistent with the model proposed by Pauling and Corey in the preceding communication.

The conclusion that the phosphate groups lie on the outside of the structural unit has been reached previously by quite other reasoning⁴. Two principal lines of argument were invoked. The first derives from the work of Crick and Watson⁵, who showed that even in aqueous solution the $-\text{CO}$ and $-\text{NH}_2$ groups of the bases are inaccessible and therefore cannot be turned towards the interior of the helical axis.

Considerations of density show however that a cylinder of length of about 34 Å. and diameter of 20 Å. must contain many more than ten nucleotides.

Since structure *B* often exists in fibre with low water content, it is likely that the water in the unit cannot differ greatly from that of dry sodium thymonucleate (160 gm./cm.³), the water in fibres of high water content being about 100 gm./cm.³ of structural unit. On this basis we find that a cylinder of radius 10 Å. and height 34 Å. would contain thirty-two nucleotides. It is therefore possible, possibly with some slight inter-penetration of the cylindrical units in the dry state, making their effective radius rather less, to have a cylinder of 10 Å. diameter containing 32 nucleotides alone, whether one repeating unit contains ten nucleotides on each, or two or on each of three nucleotides. (If the spacing of the radial lines in *A* is 8 Å., the cylinder would contain twenty nucleotides.) Two

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Franklin, R. E., and Wilkins, M. H. F., *Acta Cryst.*, A, 8, 392 (1951).

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Goddard, J. M., and Goding, R. G., *Cold Spring Harbor Symp. on Quant. Biol.*, 16, 2 (1951).

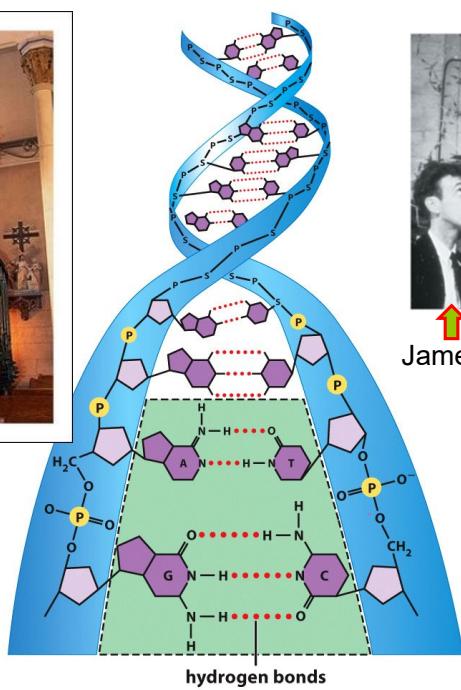
Franklin, R. E., and Wilkins, M. H. F., *Proc. U.S. Natl. Acad. Sci.*, 38, 1014 (1952).

Watson-Crick Model

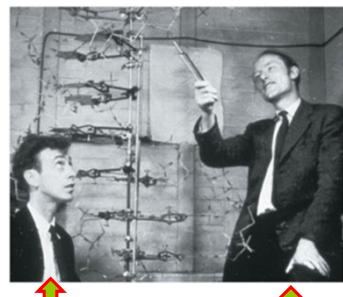
- DNA consists of **two nucleotide chains (two strands)**
 - In **opposite directions (antiparallel)**
- **Double helix**
 - Resemble a ladder twisted around its axis
 - Each rung consisting of **a pair of bases**
 - Deoxyribose and phosphate units linked together alternately into **sugar-phosphate backbone**
- **Hydrogen bonds** between pairs of bases
 - $A=T$
 - $C\equiv G$

34

Strands of DNA wind about each other in a double helix, like a twisted ladder, with the sugar-phosphate backbone forming the uprights and the complementary base pairs forming the rungs.



DNA



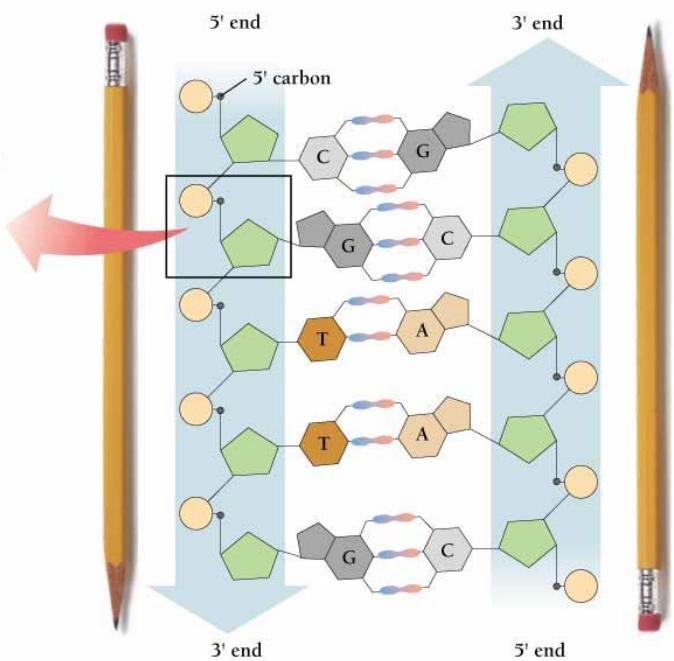
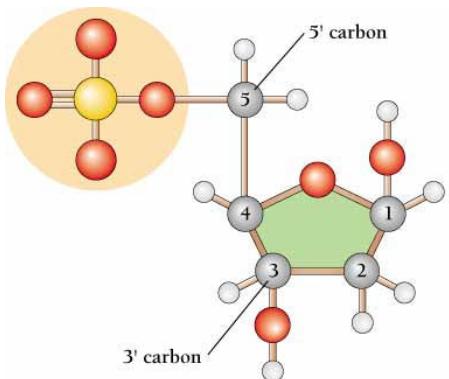
James Watson

Francis Crick

The sides of the ladder are the sugar-phosphate backbones of the two chains, the rungs of the ladder are the nitrogenous bases,

35

Strands of DNA wind about each other in a double helix, like a twisted ladder, with the sugar-phosphate backbone forming the uprights and the complementary base pairs forming the rungs.



Two strands in a DNA molecule are antiparallel

The Nobel Prize in Physiology or Medicine 1962

“...for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material...”



Their paper in Nature (1953 April) opens with the sentence “We wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA). This structure has novel features which are of considerable biological interest.”

37

Read this page:

<http://ba-education.com/for/science/dnadiscovery.html>

How Does DNA Encode Information?

How can a molecule with only four simple parts be the carrier of genetic information?

- The key lies in the **sequence of nucleotides**
- Within a DNA strand, the four types of nucleotides can be arranged in **any linear order**, and this sequence is what encodes genetic information
- The genetic code is analogous to languages, where small sets of letters combine in various ways to make up many different words
- The sequence of only **four nucleotides can produce many different combinations**

A 10-nucleotide sequence can code for more than 1 million (4^{10}) different combinations of the four bases.

38

$$4^{10} = 1048576$$

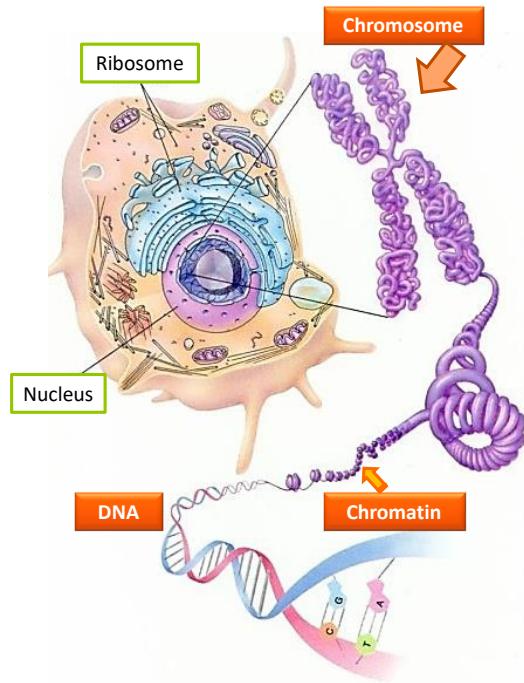
Computer uses binary code for processing all information,

Chromatin vs Chromosome

Both are structures within living cells that contain the genetic material.

Biochemically, chromosomes are composed of

- **DNA**, which is the genetic material
- **Proteins**, which provide an organized structure



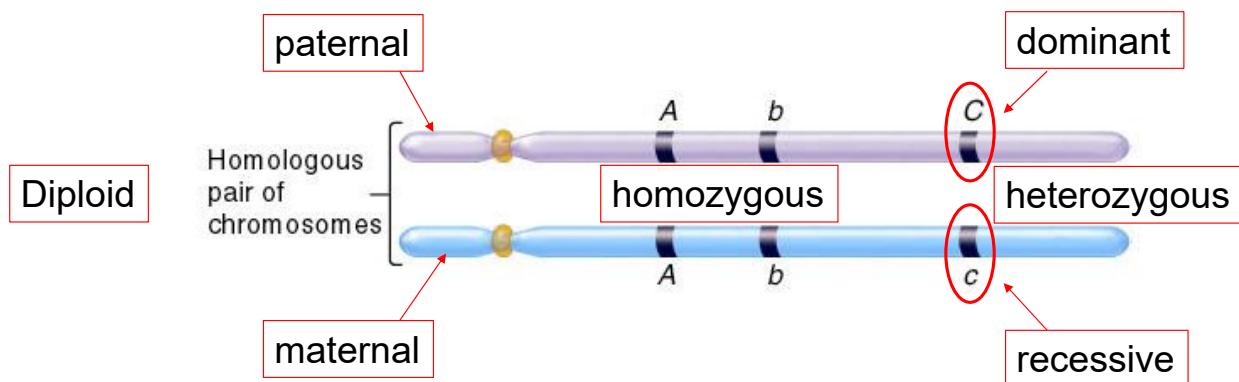
39

How do you send DNA through the mail,

In a nuclear envelope.

Gene vs DNA

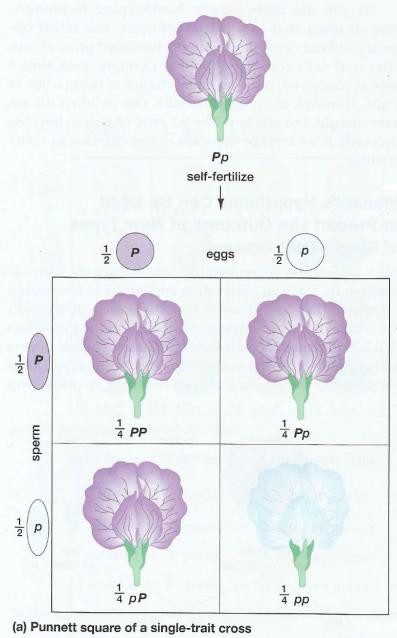
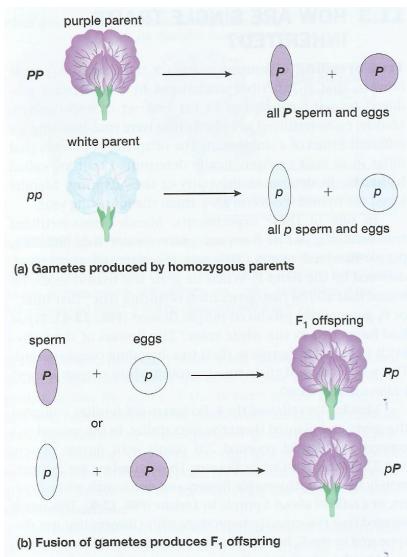
- A **gene** is a specific sequences of nucleotides, is a basic physical and functional unit of heredity, usually corresponding to a **single protein or RNA**



Region of DNA that controls a discrete hereditary characteristic.

Encompassing coding DNA sequences, noncoding regulatory DNA sequences, and introns.

Dominant genes will show phenotypic trait when **homozygous** or **heterozygous**
Recessive genes will only show phenotypic trait when **homozygous**



Number of Genes in Different Organisms

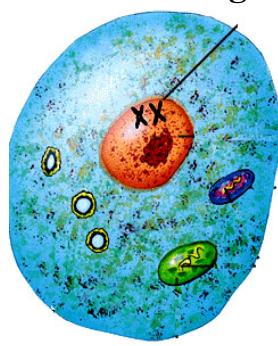
Human ~ 30,000 genes



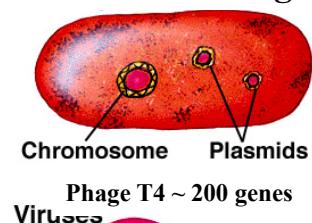
Mouse ~ 30,000 genes



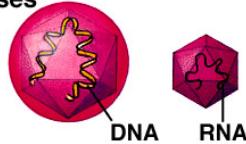
Yeast ~ 6200 genes



E. coli ~ 4200 genes



Phage T4 ~ 200 genes
Viruses



Influenza
~ 12 genes



How big is the human genome?

How many base pairs of human genome: 3 billion

If all A, T, G and C in human genome are compiled in books,
200 books at 1000 pages each would be needed to hold it all.

If you read on a rate of 10 bases per second, it takes you 9.5 yr to finish

If the nucleus is about **10 µm in diameter**, but all DNA molecules in the nucleus will reach about **2 meter in length** if they are stretched from end to end, but it is only about 2 nm diameter.

43

Q. How big is the human genome?

The human genome is made up of DNA, which has four different chemical building blocks. These are called bases and abbreviated A, T, C, and G. In the human genome, about 3 billion bases are arranged along the chromosomes in a particular order for each unique individual. To get an idea of the size of the human genome present in each of our cells, consider the following analogy: If the DNA sequence of the human genome were compiled in books, the equivalent of 200 volumes the size of a Manhattan telephone book (at 1000 pages each) would be needed to hold it all.

It would take about 9.5 years to read out loud (without stopping) the 3 billion bases in a person's genome sequence. This is calculated on a reading rate of 10 bases per second, equaling 600 bases/minute, 36,000 bases/hour, 864,000 bases/day, 315,360,000 bases/year.

Storing all this information is a great challenge to computer experts known as bioinformatics specialists. One million bases (called a megabase and abbreviated Mb) of DNA sequence data is roughly equivalent to 1 megabyte of computer data storage space. Since the human genome is 3 billion base pairs long, 3 gigabytes of computer data storage space are needed to store the entire genome. This includes nucleotide sequence data only and does not include data annotations and other information that can be associated with sequence data.

As time goes on, more annotations will be entered as a result of laboratory findings, literature searches, data analyses, personal communications, automated data-analysis programs, and auto annotators. These annotations associated with the sequence data will likely dwarf the amount of storage space actually taken up by the initial 3 billion nucleotide sequence. Of course, that's not much of a surprise because the sequence is merely one starting point for much deeper biological understanding!



**When
Where
How**

Does DNA Replicate?



When the cell or organelle needs to divide/reproduce

- Prokaryotic cell: replicate in cytoplasm
- Eukaryotic cell: replicate in nucleus and/or mitochondrion, chloroplast

DNA Replication

- Begins when DNA **unwinds** and unzips into two strands
 - By **DNA helicases**, that pull apart the parental DNA double helix at the hydrogen bonds between the complementary base pairs.
 - **Hydrogen bonds** between bases **broken**
 - Formation of **replication bubbles** and **replication forks**

DNA Replication

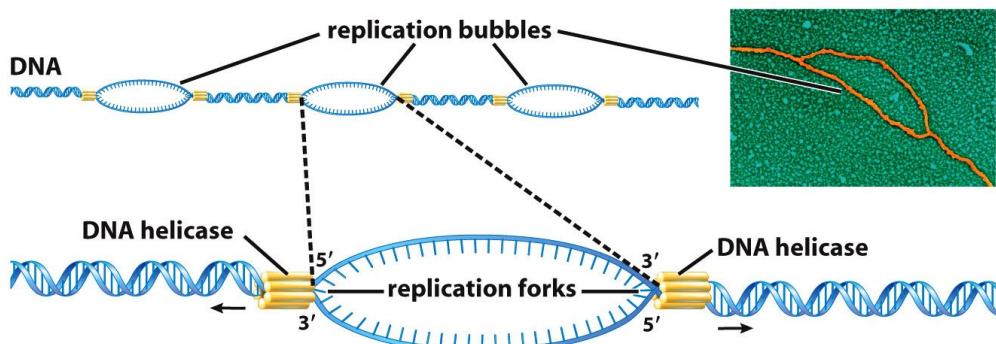


Figure E9-7ab Biology: Life on Earth, 8/e
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A replication bubble is an unwound and open region of a DNA helix where DNA replication occurs.

46

Why make replication bubbles, rather than simply starting at one end of a double helix and copying the DNA in one continuous piece all the way to the other end? Recall that eukaryotic chromosomes are very long: Human chromosomes range from about 50 million nucleotides in the relatively tiny Y chromosome to about 250 million nucleotides in chromosome 1.

Eukaryotic DNA is copied at a rate of about 50 nucleotides per second, so it would take about 12 to 58 days to copy a human chromosome in one continuous piece. To replicate an entire chromosome in a reasonable time, many DNA helicase enzymes open up many replication bubbles simultaneously, allowing many DNA polymerase enzymes to copy the

strands in fairly small pieces all at the same time. Each individual bubble enlarges as DNA replication progresses, and the bubbles merge when they contact one another.

DNA Replication

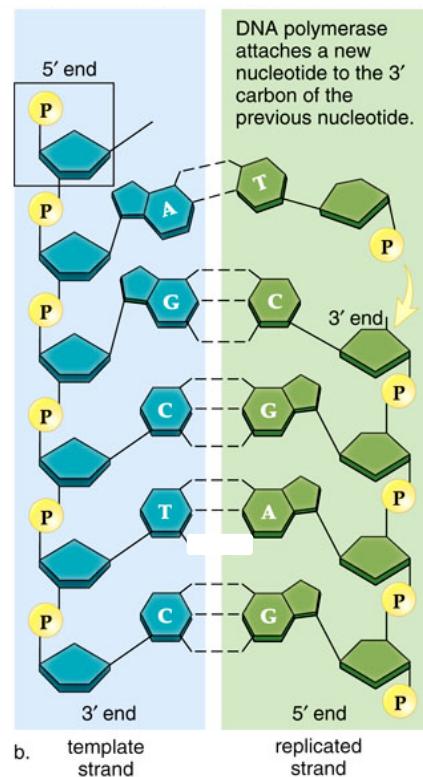
- Short **RNA primer** synthesised first to provide 3' end for DNA polymerase
 - Primer is synthesized by **RNA polymerase (Primase)**
 - A typical primer is about **five to ten** nucleotides long. The primer primes DNA synthesis, i.e., gets it started.
- Each **old strand** serves as **template** for synthesis of new strand
 - By **DNA polymerases**
 - Based on **complementary base-pairing rule**

47

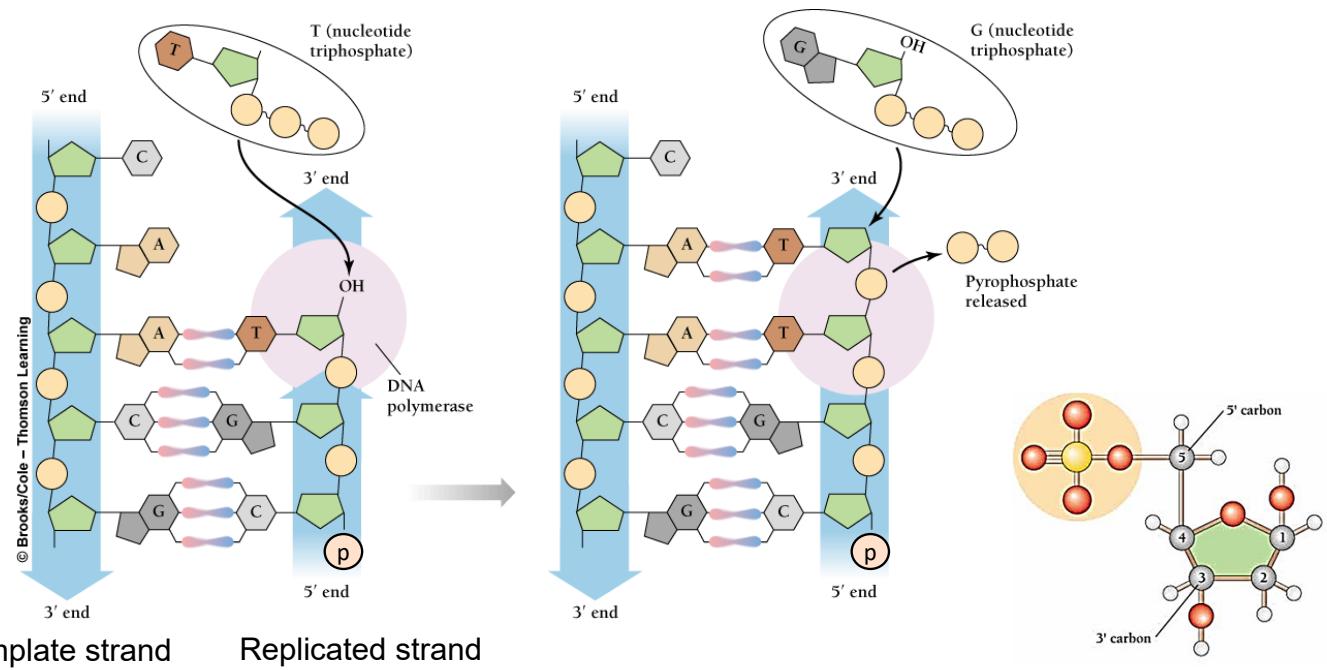
A primer (/ˈpraɪmər/) or undercoat is a preparatory coating put on materials before painting. Priming ensures better adhesion of paint to the surface, increases paint durability, and provides additional protection for the material being painted.

DNA Replication

- DNA polymerase adds nucleotides in **5' to 3'** direction **only**.
 - Can only join new nucleotide to free 3' end of previous nucleotide.



New strand is synthesized in 5' to 3' direction



49

Normal DNA polymerases are 5'-to-3' polymerases. DNA polymerases extend the 3' tail of the DNA molecule but it synthesizes 5'-to-3'. 3' to 5' polymerases would never work because the energy required would be way too high.

Let me explain. In the 5' to 3' polymerase, the 3' OH group of the already synthesized DNA can perform an SN2 nucleophilic attack on the incoming nucleotide because the beta and gamma phosphates of the incoming nucleotides serve as a good leaving group. You might think it is hard for an oxygen-phosphorus bond in the incoming nucleotide to be broken, but the two divalent-cation-bound beta and gamma phosphates help change the charge distribution of the bond.

On the other hand, if you tried to join the new nucleotide in the 3' to 5' direction in a head synthesis reaction, there won't always be a good pyrophosphate leaving group. Why? There can't be a triphosphate on the 5' end because it would spontaneously hydrolyze, but for now, let's just pretend there could be. In this case, the DNA would extend like normal until it accidentally catalyzes a mismatch. The proofreading exo would remove the incorrect nucleotide and you would be left with a monophosphate head. What are you going to do now? It's way too hard to break an oxygen-phosphorus bond when there aren't any phosphate groups bound to magnesium to suck away electrons. Because of this 3' to 5' polymerases would have a hard time working properly or accurately. Here's a nice picture from Essential Cell Biology that describes this.

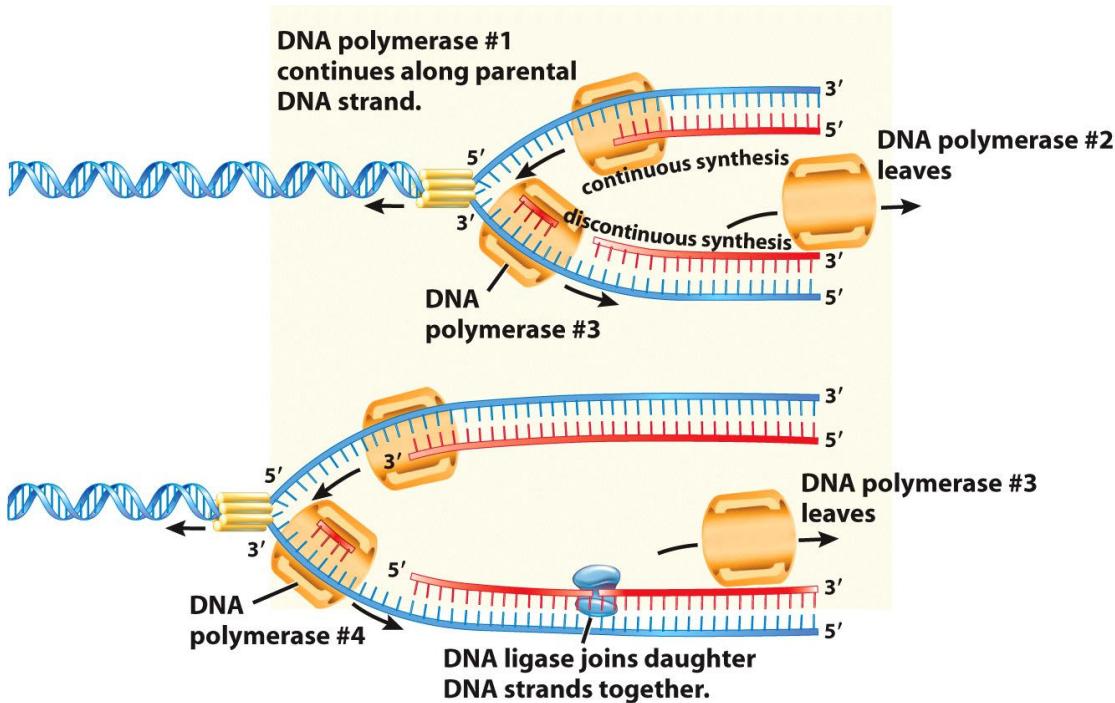


Figure E9-7de Biology: Life on Earth, 8/e
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50

Leading strand (continuous synthesis) Lagging strand (discontinuous synthesis)

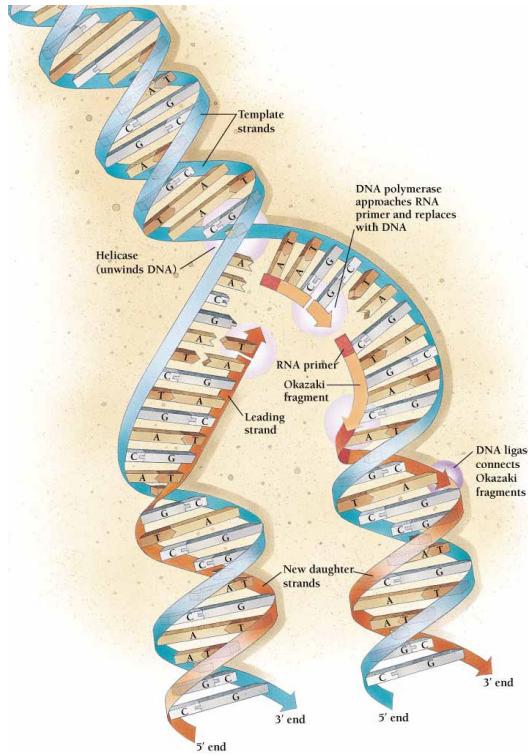
A DNA helicase binds to the double helix and moves along, unwinding the double helix and separating the strands. Because the two DNA strands run in opposite directions, as a DNA helicase enzyme moves toward the 5' end of one parental strand, it is simultaneously moving toward the 3' end of the other parental strand.

Now visualize two DNA polymerases landing on the separated strands of DNA. One DNA polymerase (call it polymerase #1) can follow behind the helicase toward the 5' end of the parental strand and can synthesize a continuous daughter DNA strand until it runs into another replication bubble. This continuous daughter DNA strand is called the leading strand. On the other parental strand, however, DNA polymerase #2 moves away from the helicase: In step , note that the helicase moves to the left, whereas DNA polymerase #2 moves to the right. Therefore, DNA synthesis on this strand will be discontinuous: DNA polymerase #2 will synthesize a short new DNA strand, called the lagging strand, but meanwhile, the helicase continues to move to the left, unwinding more of the double helix

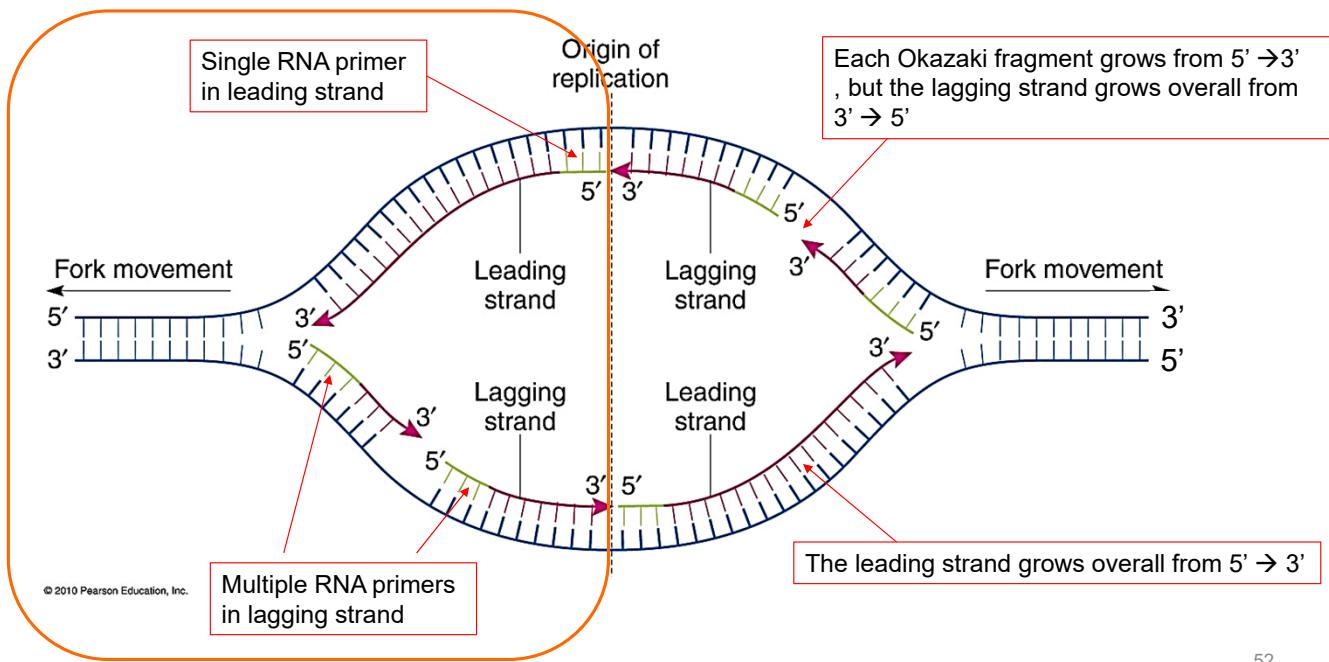
Additional DNA polymerases (#3, #4, and so on) land on this strand and synthesize more short lagging strands.

DNA Replication

- **Leading strand synthesised continuously**
- **Lagging strand synthesised in short fragments**
 - Okazaki fragments
 - Joined together by DNA ligase



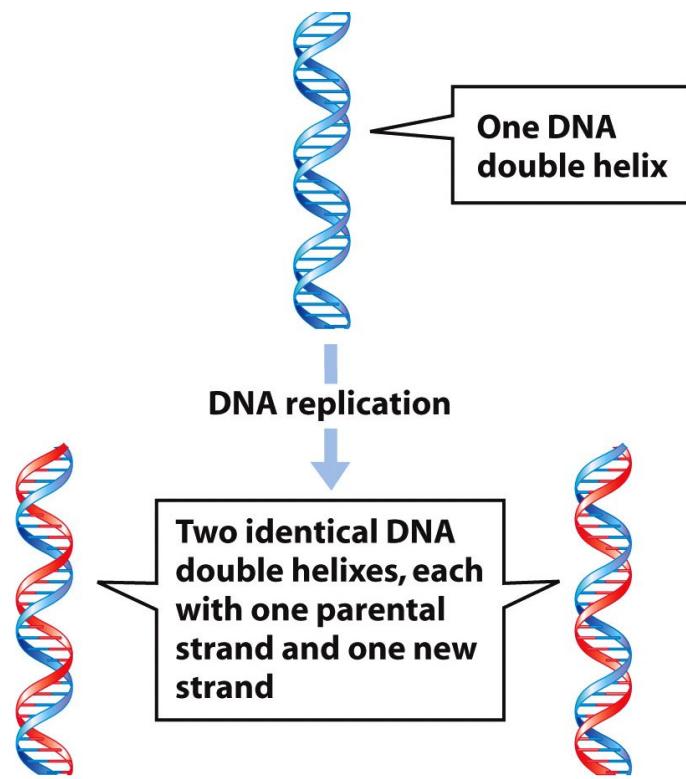
One Replication Bubble and Two Forks



This slide gives you a better view of the leading and lagging strand.

How are all of these pieces sewn together (okazaki fragments)? This is the job of the third major enzyme, DNA ligase ("an enzyme that ties DNA together"). Many DNA

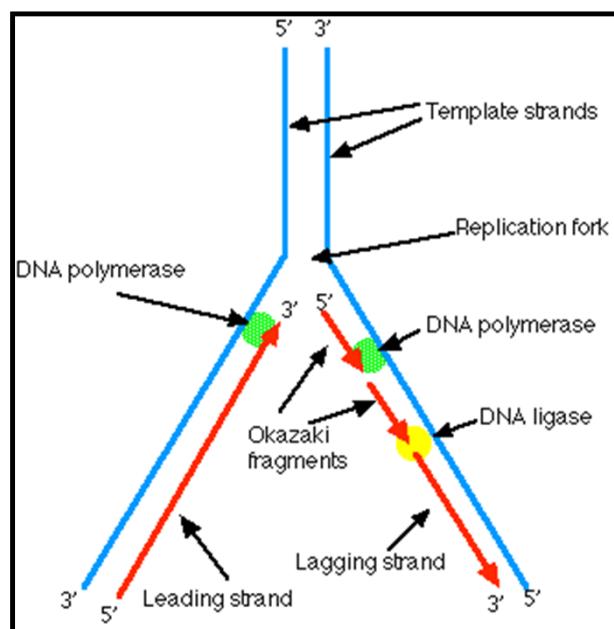
ligase enzymes stitch the fragments of DNA together until each daughter strand consists of one long, continuous DNA polymer.

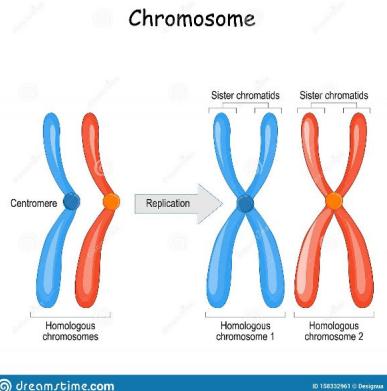


DNA Replication

- **Semi-conservative** replication
- Each strand of double helix acquires a new strand
 - Each “old” parent strand serves as template for complementary “new” daughter strand
- Each new DNA molecule is **half “old” and half “new”**

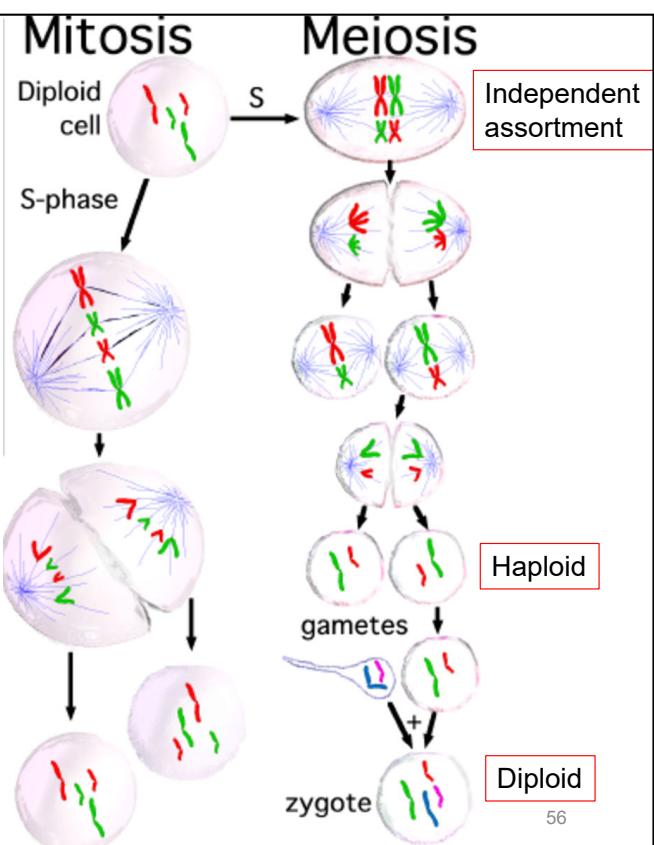
DNA Semi-conserved replication





Mitosis: 1 diploid cell to 2 diploid cells
Meiosis: 1 diploid cell to 4 haploid cells

Independent assortment allow 2^{23} combinations of 23 pairs of chromosomes in human gametes



Mitosis: The process cells use to make exact replicas of themselves. Mitosis is observed in almost all the body's cells, including eyes, skin, hair, and muscle cells.

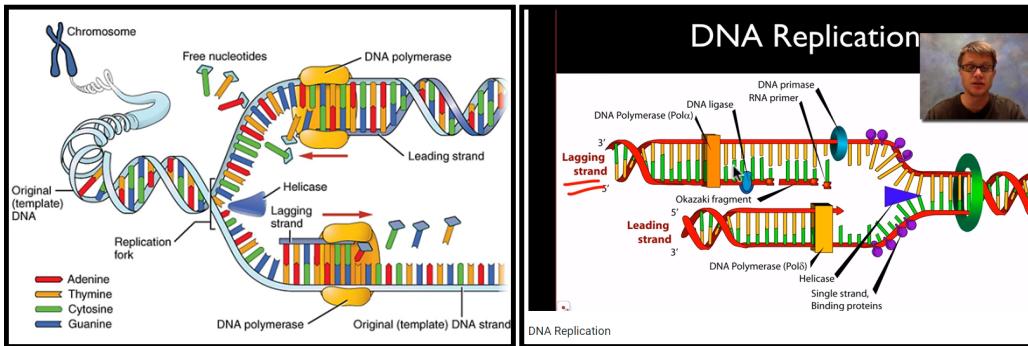
Meiosis: In this type of cell division, sperm or egg cells are produced instead of identical daughter cells as in mitosis.

DNA and its replication

Online Learning Materials

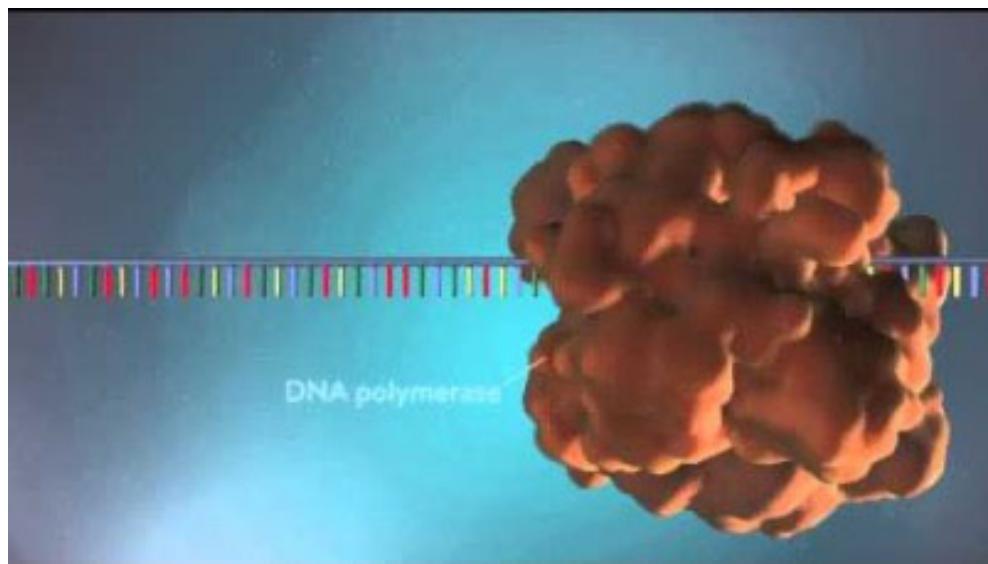
DNA Replication***

[http://www.youtube.com/watch?v=FBmO_rmXxIw \(11 min\)](http://www.youtube.com/watch?v=FBmO_rmXxIw)



Here is the outline of this topic. DNA replication is the key.

DNA Replication (video)



<https://www.youtube.com/watch?v=TNKWgcFPHqw>

58

Play video (3.28 mins)

Watch the PCR video below for a better understanding of the Practical 2. We will discuss it further in the last lecture.

Polymerase Chain Reaction (PCR)

- * Why only the region between the forward and reverse primers is amplified?
- * Why a heat stable DNA polymerase is needed?

What are mutations, and how do they occur?

DNA mutation

- A **mutation** is a change in a cell's DNA sequence, either in a protein coding gene or in a noncoding regulatory region.
- Mutations may have **varying effects** on function
 - Mutations are **often harmful**, and an organism inheriting them may quickly die
 - Some mutations may **have no functional effect**
 - Some mutations may **be beneficial** (rarely) and provide an advantage to the organism in certain environments
 - These advantageous mutations may be favored by natural selection and are the **basis for evolution** of life on Earth

How Do Mutations Occur?

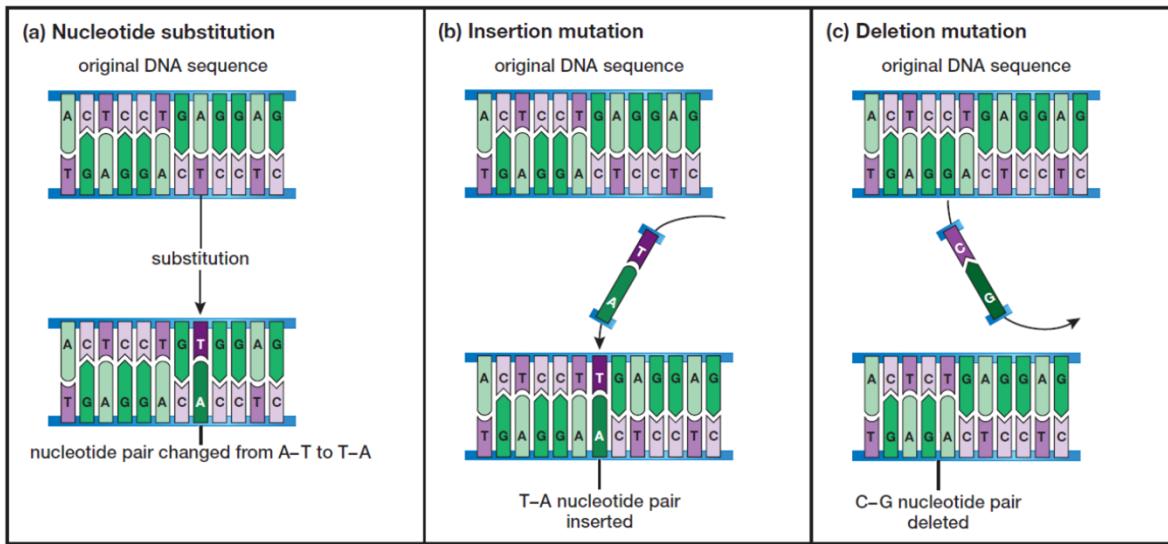
Mistakes do happen: DNA is altered or damaged in a number of ways

- Mistakes are made during normal **DNA replication**
Replicated DNA strands usually contain only about one mistake in every 100 million to 1 billion base pairs
- Certain **chemicals** (some components of cigarette smoke, for example) increase DNA errors during and after replication
- **Ultraviolet radiation** or X-rays also contribute to incorrect base pairing

62

cancerogenic

Types of mutations



63

Sometimes, however, the Enzymes replace the parental nucleotide instead of the Incorrect daughter nucleotide. Although the resulting base pair is complementary, it is different from the original pair; there has been a nucleotide substitution mutation

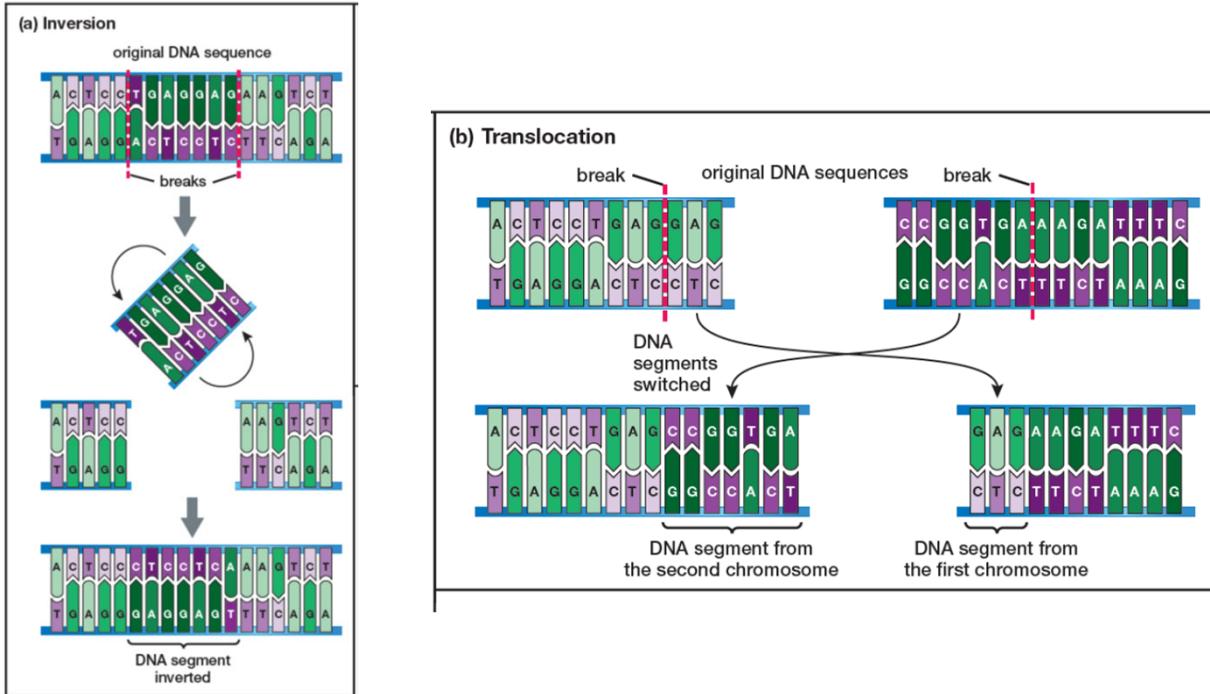
(FIG 12-9a). Because the incorrect base pair is complementary, accurate DNA replication during future cell divisions

will perpetuate the mutation: It has become a permanent part of the chromosome and will be inherited by all the cell's descendants. An insertion mutation occurs when one or more nucleotide pairs are inserted into the DNA double helix (FIG 12-9b). A deletion mutation occurs when one or more nucleotide pairs are removed from the double helix (FIG. 12-9c).

Both insertion and deletion mutations have correctly base-paired DNA, so these mutations will also be permanent.

Tions will also be permanent.

Types of mutations



64

Pieces of chromosomes ranging in size from a single nucleotide pair to massive pieces of DNA are occasionally rearranged. An **inversion** occurs when a piece of DNA is cut out of a chromosome, turned around, and reinserted into the gap (FIG. 12-10a). A **translocation** results when a chunk of DNA, sometimes very large, is removed from one chromosome and attached to a different one (FIG. 12-10b). As with insertions and deletions, the DNA resulting from inversions and translocations has correct, complementary base pairs.

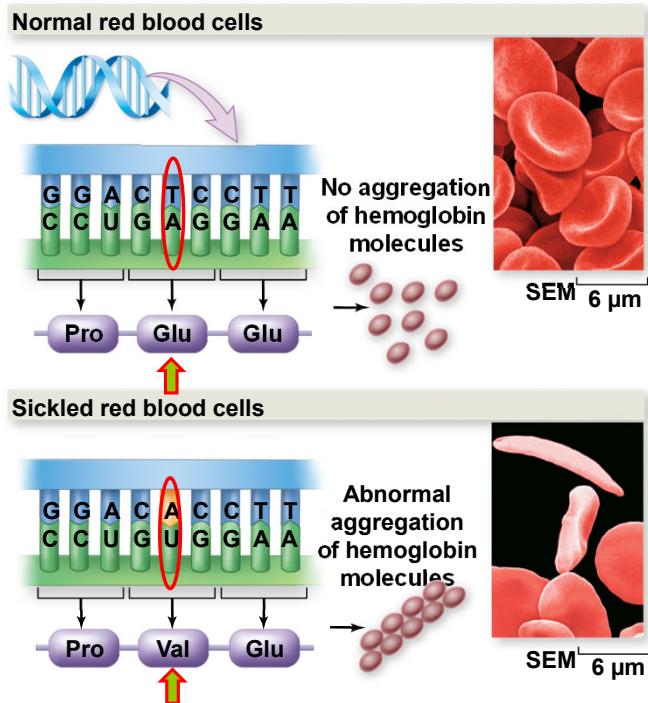
Mutations in DNA (video)



65

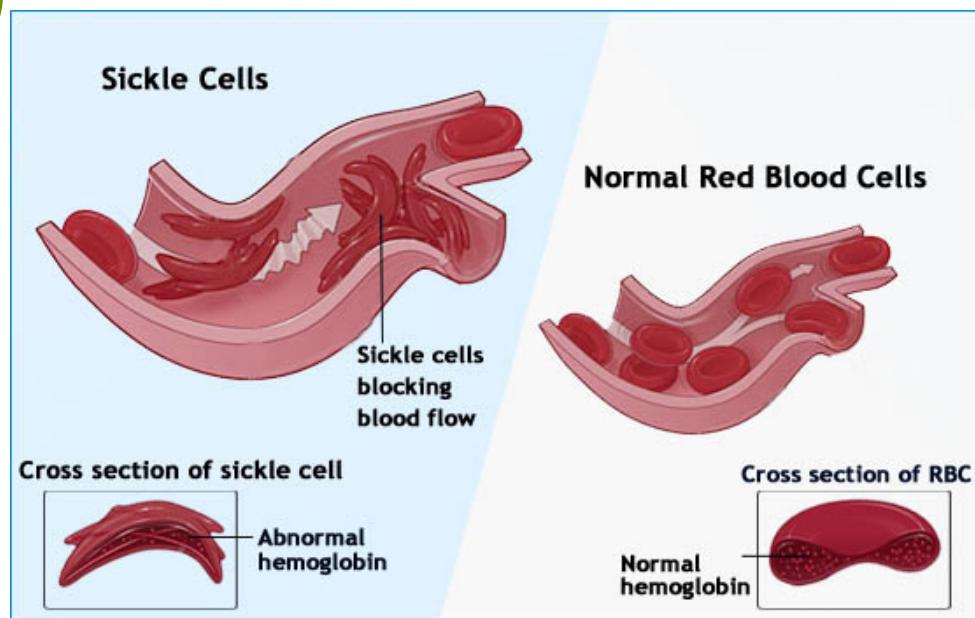
Play video (3 mins)

Mutations Change DNA



A single base substitution in a hemoglobin gene causes blood cells to form abnormally, leading to sickle cell disease (homozygous recessive).

Gene mutation and sickle cell anemia



67

Normal RBCs are disc- or concave -shaped and closely resemble doughnuts minus the holes. They are manufactured in the spongy tissue of the bone marrow and have a life span of about 120 days.

In those with SCA, the hemoglobin sticks together and appears fibre-like, causing the RBCs to stiffen and become C- shaped, like a sickle. Symptoms and problems of sickle cell disease are due to the hemoglobin S (HbS) molecule. It is this form of hemoglobin that gives the abnormal shape to the RBC. Hemoglobin S (HbS) when it loses its oxygen becomes rigid and rod-like and changes the red blood cells into a sickle or crescent shape from the normal disc or concave shape.

These sickled RBCs are extremely fragile and therefore rupture easily (medically called hemolysis) leading to low red blood cell (RBC) count and anemia symptoms. Besides the sickle- celled RBC has a life span of only 10-20 days. The quick turn round time for new RBCs can only happen if the bone marrow can keep pace. As this fails to happen, it results in anemia.

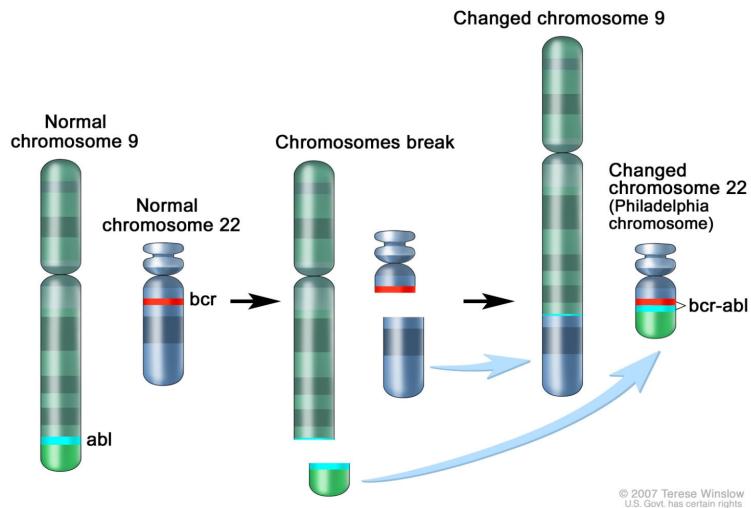
The stiff, sickled RBCs clump together and tend to stick to the wall of the blood vessels. They are circulated with difficulty and can even cause blockage of blood vessels leading to infection, tissue and organ damage along with a great deal of pain.

In patients with SCA, sickling is further triggered by conditions such as low oxygen levels, increased acidity and low volume of the blood resulting from dehydration, injury or when anesthesia is administered.

Read more: Sickle cell anemia—Causes-Symptoms-Diagnosis-Treatment—Prognosis | Medindia

<http://www.medindia.net/patients/patientinfo/sickle-cell-anemia.htm#ixzz2PCQA1iaK>

Gene mutation and leukemia



Philadelphia translocation is a specific chromosomal abnormality that is associated with chronic myelogenous **leukemia** (CML).

68

Leukemia is a cancer of the white blood cells.

CML is a clonal bone marrow stem cell disorder.

The most famous example of an acquired chromosomal change in malignancy is the Philadelphia chromosome (Ph). It was the first chromosomal abnormality to be found in leukemia in 1960 and is now known to be present in 95% of chronic myeloid leukemia (CML) cases. It also occurs in acute leukemia. In acute lymphoblastic leukemia (ALL), the Ph is found in 2–3 % of childhood cases, but in adults it is the most common cytogenetic change, the incidence of which increases with age.

Key Terms and Key Concepts

Key Terms

DNA, RNA, nucleotides, Chargaff's rule, Watson-Crick model of DNA structure, complementary base pairs, sugar-phosphate backbone, double helix, helicase, polymerase, ligase, deletion mutation, insertion mutation, translocation, semi-conservative replication,

Key Concepts

How did scientists discover that genes are made of DNA?

What is the structure of DNA?

How does DNA encode genetic information?

How does DNA replication ensure genetic constancy during cell division?

How does DNA inherit?

What are mutations, and how do they occur?

69

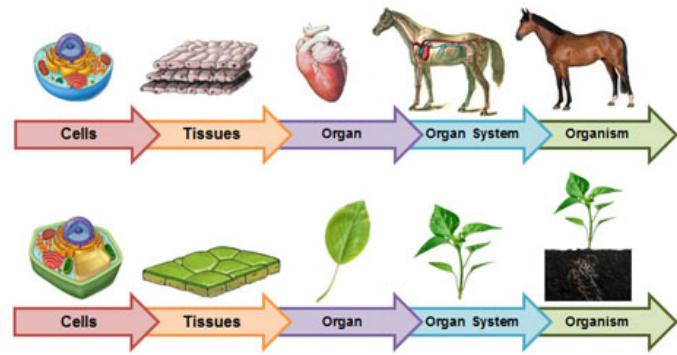
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Please complete the quiz on Canvas for lecture 5

LSM1301



L6: Gene Expression

A/P Henry Mok Yu Keung

Office at S3-03-01d

dbsmokh@nus.edu.sg

1

Outline



How does genetic information flow from DNA to protein? (Central Dogma)

(How does DNA work as the instructions for traits?)

- Transcription: How is RNA produced?



- Translation: How is protein produced?



- How is gene expression regulated?

- If most of cells are genetically identical, why do they look and function differently?

2

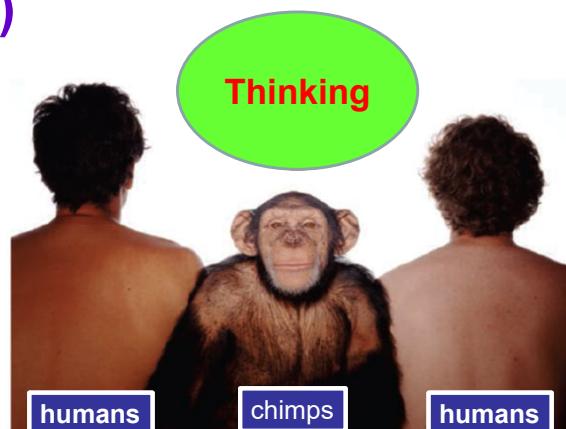
From last talk, we know...., in this topic, we will answer

All in the Family (Hominidae)

<https://genome.cshlp.org/content/15/12/1746.full>

Comparing the human and chimpanzee genomes:
Searching for needles in a haystack

On average only 4% of nucleotide bases differ between chimps and humans.



"The chimpanzee genome sequence is a long-awaited milestone, providing opportunities to explore primate evolution and genetic contributions to human physiology and disease. Humans and chimpanzees shared a common ancestor ~5-7 million years ago (Mya). The difference between the two genomes is actually not ~1%, but ~4%—comprising ~35 million single nucleotide differences and ~90 Mb of insertions and deletions."

3

Genome data reveal a few surprising differences between chimps and humans but overall confirm our close kinship.

The total genetic difference between humans and chimps, in terms of number of bases, sums to about 4% of the genome. What led to the striking differences between us?

Our DNA is also 90% the *same* as a mouse, 60% the *same* as a fruit fly

Are humans and chimps effectively identical in our respective DNA? The short answer is no, no way: not in our DNA, coding and non-coding, not in the way our genes are expressed, how chimps splice their DNA, the existence of human-specific genes, and more, not to mention how this all cashes out in terms of anatomy and behavior. To understand this takes a little sketching in of the relevant scientific background, as Dr. Gauger does, from the basics on up.

Thinking

The chromosomes of a human male

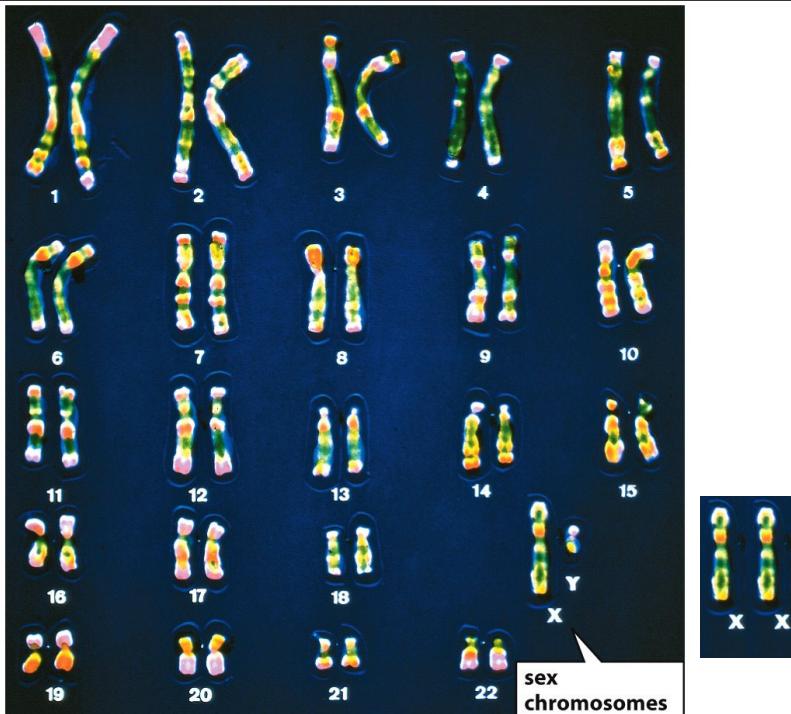
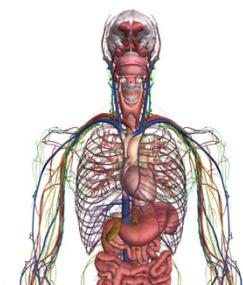
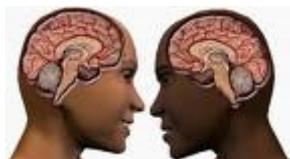


Figure 11-9 Biology: Life on Earth, 8/e
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4

Here is another sample. Human males and females are so alike, yet so different. The physical differences between men and women are pretty obvious, but for a very long time, biologists had only the vaguest ideas about the genetic bases of those differences. It's been less than a century since Theophilus Painter discovered the Y chromosome. Several more decades passed before it was generally accepted that Y chromosomes usually determines maleness in humans and other mammals. But how?

One hypothesis might be that genes on the Y chromosome encode male genitalia, so we would predict that anyone with a Y chromosome will have testes and a penis. But males also have all of the other chromosomes that females have (although males have only one X chromosome, rather than the two that females have). Why, then, don't boys develop both male and female genitalia? Furthermore, many genes needed to produce male sexual characteristics, including the genitalia, are not on the Y chromosome. Girls therefore possess these genes, so why don't girls develop both female and male genitalia?

In boys, the action of a single gene located on the Y chromosome activated the male developmental pathway and deactivates the female developmental pathway. Without this gene we would all be physically female. How can a single gene determine something as complex as the sex of a human being? In this

chapter, we will examine the flow of information from an organism's genes to its physical characteristics.

Just as a house blueprint shows us the layout of all the parts (the boards, bricks, wires, etc.), the genome is a set of instructions from which we can determine the layout of all the proteins used to build and run our body.

Just as information in a book remains hidden until someone opens its cover and reads the text, so too the information in genes may, or may not be used in different organisms, in the various cells of an individual organism, and at different times during the organism's life.

Dog: 39 pairs; elephant 28 pairs; rice 12 pairs; mosquito 3 pairs

Please look at all the chromosomes from a male's cell.

Staining and photographing the entire set of duplicated chromosome within a single cell produced a karyotype.

Genitalia (external sexual organs)

During the eighth week the fetus chooses between two paths: masculine or feminine. If the fetus is genetically male - if it has a Y chromosome - then a "master switch" on the Y chromosome clicks on at this time. This switch, which is a single gene called the testes-determining factor, triggers a whole series of events that will point the fetus in a male direction and culminate in a baby boy - if everything goes as planned. The flipping of this switch is the key step.

If the DNA sequence of the human genome were compiled in books, the equivalent of 200 volumes the size of a Manhattan telephone book (at 1000 pages each) would be needed to hold it all.

Since the human genome is 3 billion base pairs long, 3 gigabytes of computer data storage space are needed to store the entire genome

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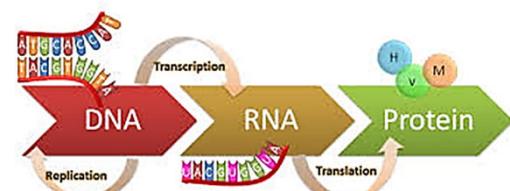
As time goes on, more annotations will be entered as a result of laboratory findings, literature searches, data analyses, personal communications, automated data-analysis programs, and auto annotators. These annotations associated with the sequence data will likely dwarf the amount of storage space actually taken up by the initial 3 billion nucleotide sequence. Of course, that's not much of a surprise because the sequence is merely one starting point for much deeper biological understanding!

If all the DNA in your body was put end to end, it would reach to the sun and back over 600 times (100 trillion times six feet divided by 92 million miles).

How are gene expressed?

Central dogma

- 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

5

How does our body read the genetic instructions and use it to make a protein? DNA cannot be converted into protein directly, but instead, sends a message describing the gene's instruction, to a protein-making machine. Each particular gene can be "transcribed," or copied, into a related molecule called mRNA (messenger ribonucleic acid) and is then transported to a molecular, protein-making machine called a ribosome. The job of the ribosome is to read the mRNA copy of the gene and assemble the appropriate protein.

In other words,

Gene expression is the process by which DNA directs the synthesis of proteins. The gene expression includes two stages: transcription and translation. Transcription is to use the information of DNA to produce an intermediary, a molecule called RNA (ribonucleic acid), which are more directly involved in protein synthesis.

transcription

a sound or television recording (e.g., from a broadcast to a tape recording) something written, especially copied from one medium to another, as a typewritten version of dictation

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

6

As I mentioned just now. Information, by itself, doesn't do anything. Instead, proteins are a cell's "molecular workers." Every cell contains a particular set of proteins. The activities of these proteins control the cell's shape, function, and reproduction, as well as the synthesis of lipids, carbohydrates, and nucleic acids. Therefore, there must be a flow of information from the DNA of a cell's genes to the proteins that actually carry out the cell's function. This information flow is the process of gene expression.

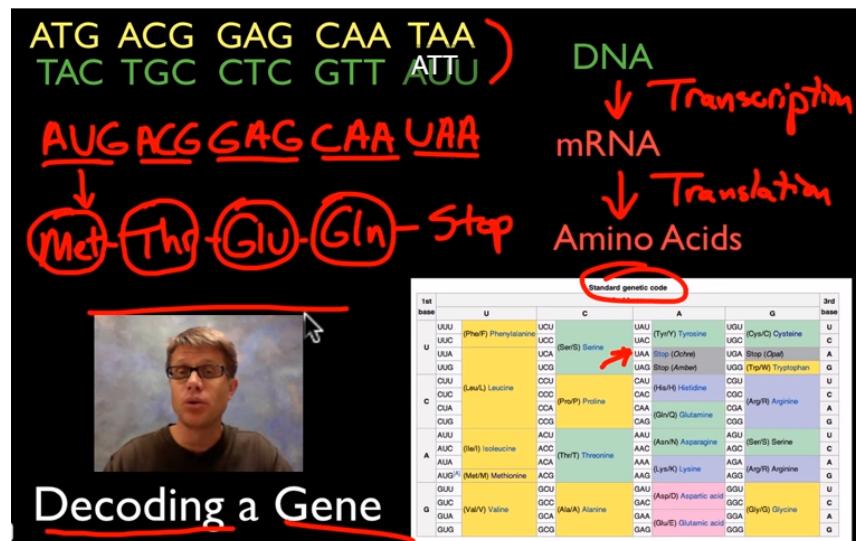
Genotype and phenotype

Gene Expression (transcription and translation)

Online Learning Materials

Transcription and Translation***

[http://www.youtube.com/watch?v=h3b9ArupXZg \(12 min\)](http://www.youtube.com/watch?v=h3b9ArupXZg)



7

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Genotype and phenotype

Transcription

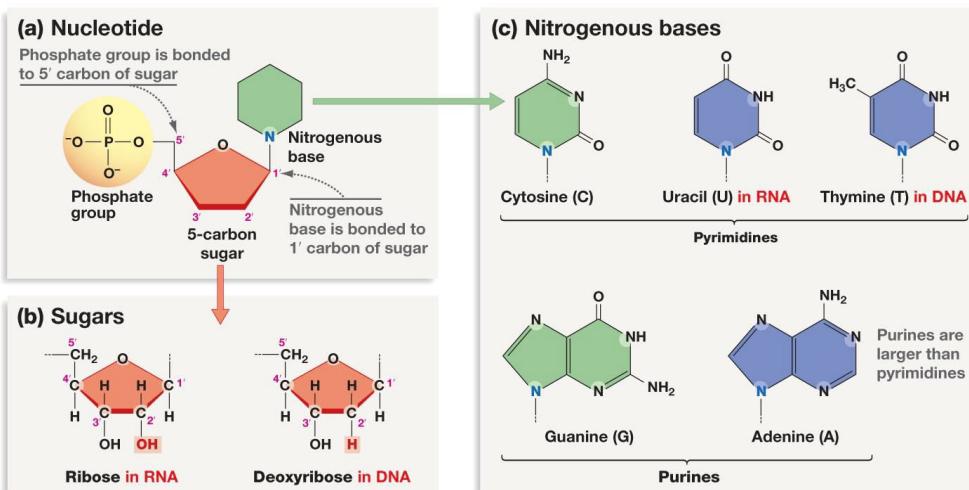
Three types of RNA

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

8

Now let's have a closer look at the first stage of gene expression, transcription and its products: RNAs

Nucleotides in DNA & RNA

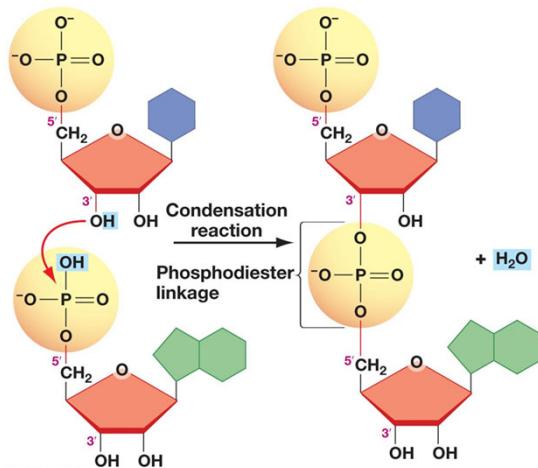


DNA: DeoxyriboNucleic Acid

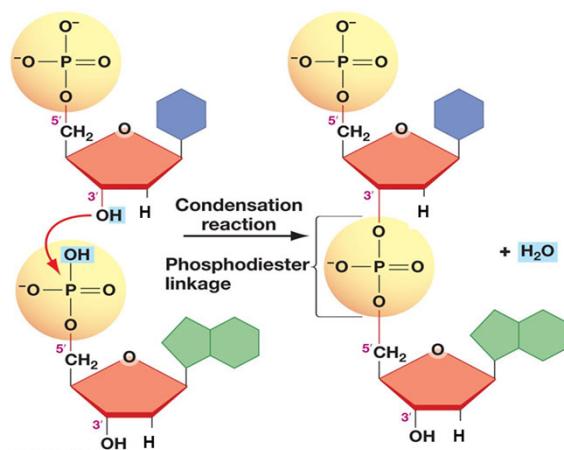
RNA: RiboNucleic Acid

Synthesis of RNA

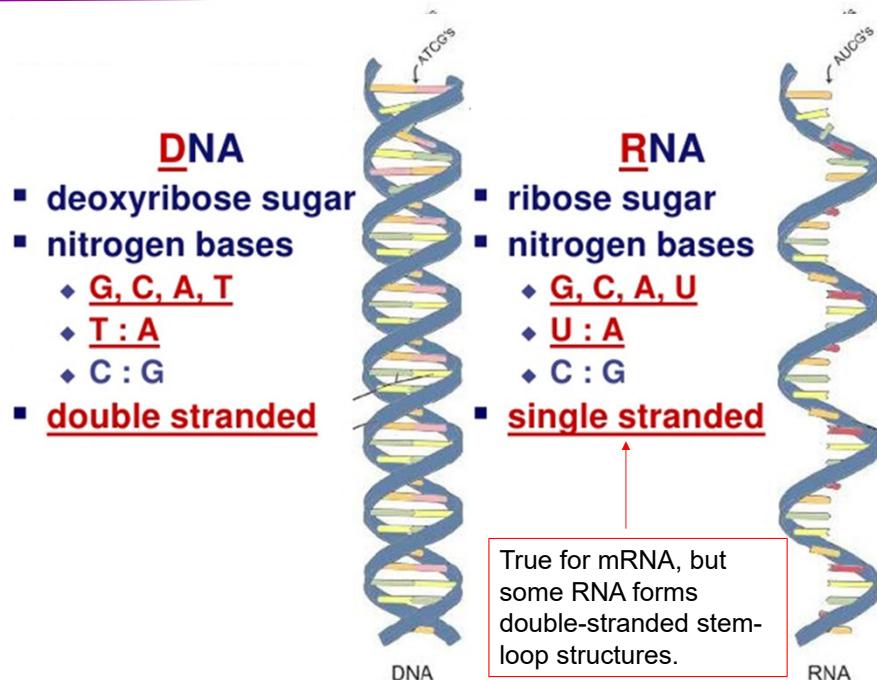
RNA



DNA



DNA vs RNA



11

Rna can fold to form bonds with itself

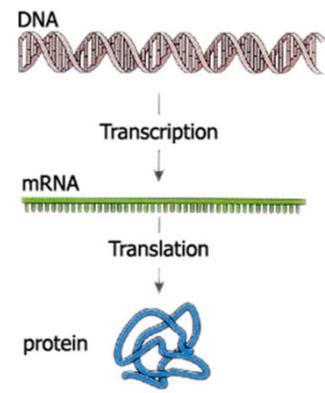
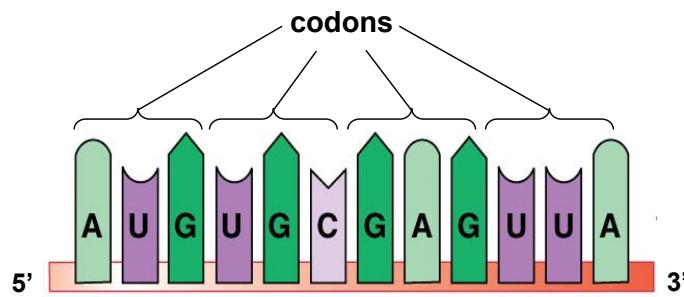
Table 10-1 A Comparison of DNA and RNA

	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
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	

12

- (1). RNA is normally single-stranded;
2. RNA has the sugar ribose (instead of deoxyribose) in its backbone
3. RNA has the base uracil instead of the base thymine found in DNA

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

rRNA

Ribosome: contains ribosomal RNA (rRNA)

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

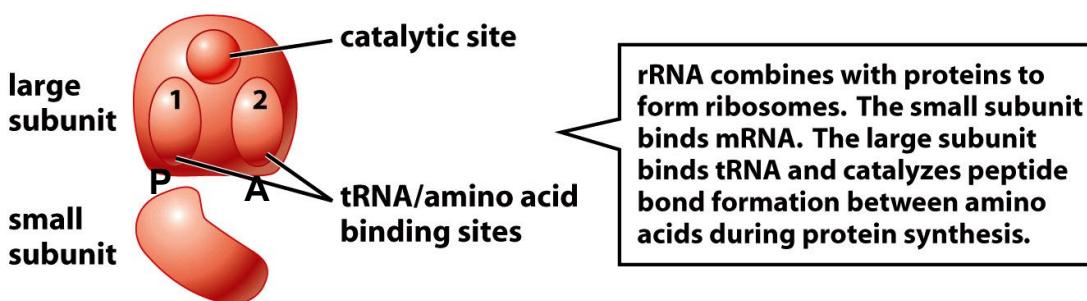


Figure 10-2b Biology: Life on Earth, 8/e
© 2008 Pearson Prentice Hall, Inc.

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.

<http://exploringorigins.org/ribozymes.html>

P site, peptidyl-tRNA binding site

A site, aminoacyl-tRNA (acceptor) site

1989 Nobel Prize in Chemistry.

14

In eukaryotes, ribosomal RNA is produced from a DNA template in the nucleolus of a nucleus. The rRNA is packaged with a variety of proteins into two ribosomal subunits, one of which is larger than the other. Then the subunits move separately through nuclear envelope pores into the cytoplasm, where they combine when translation begins. Ribosomes can remain in the cytoplasm, or they can become attached to endoplasmic reticulum.

In the large subunit, there are three functional sites, named p site, peptidyl-tRNA binding site; holds the tRNA carrying the growing polypeptide chain.

A site, aminoacyl-tRNA site) holds the tRNA carrying the next amino acid to be added to the chain.

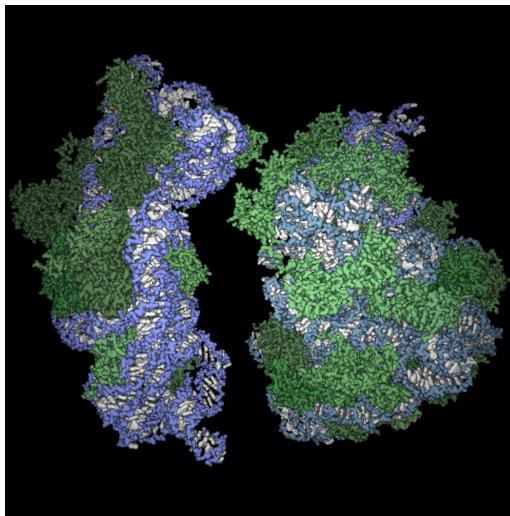
Catalytic site, where rRNA catalyzes the formation of the peptide bonds.

While small subunit bonds to mRNA,

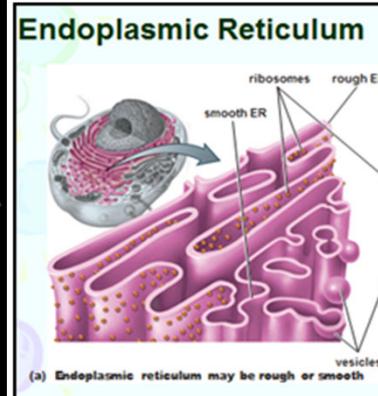
Although the ribosomes of bacteria and eukaryotes are very similar in structure and function, they are different medically. Certain antibiotic drugs can inactivate bacterial ribosomes without inhibiting the ability of

eukaryotic ribosomes to make proteins, these drugs, including tetracycline and streptomycin are used to combat bacterial infections.

Ribosome



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



<http://exploringorigins.org/ribozymes.html>

15

The ribosome, a large molecular machine that drives protein synthesis, is a ribozyme. Roll over to compare the ribosome structure with and without proteins. Proteins are shown in green, and RNA is shown in blue and white.

Not too long ago, most people considered RNA to be just a disposable copy of the really important nucleic acid, DNA. It is the double helix of DNA, after all, that shows up on magazine covers and television; DNA is the material of our genes and chromosomes, the stuff that contains our genetic inheritance. RNA – ribonucleic acid – is a copy of the DNA instructions that serves as a messenger to direct protein synthesis, which is then destroyed after it has fulfilled its function. My research group in Colorado played a role in discovering novel activities of RNA in the early 1980s. Yale University's [Sidney Altman](#), who shared the Nobel Prize in Chemistry with me in 1989, made independent discoveries. We both found that RNA could fold into complex shapes and catalyze biochemical reactions, a function previously thought to be restricted to protein enzymes. Thus, RNA was sometimes an active participant in the chemistry of life, not just a passive messenger. We named these RNA enzymes "ribozymes."

DNA is copied into RNA, which is single-stranded and therefore folds into complex shapes.

An Explosion of Breakthroughs

Within the past few years, RNA research has reached new heights. It's now clear that RNA catalysis has a much more central role in biology than many would have guessed. Furthermore, RNA often controls the expression of genes, another role that had been thought to be at least mostly the domain of proteins called "repressors" and "transcription factors."

An RNA Machine Makes Proteins

One of the most important molecular "machines" in living cells is the ribosome. It translates the message encoded on the mRNA (messenger RNA) to synthesize specific proteins that do most of the work in biology. Proteins can be hormones like insulin or sex hormones; other proteins make muscles contract and hearts beat, and yet other proteins catalyze the digestion of food. Each mRNA encodes a specific protein. The ribosome is remarkable in that it can decode a limitless number of different mRNAs. Think of a videotape: the same tape player can "translate" any videotape into a movie. The tape player is like the ribosome, the tape is the mRNA and the movie is the protein product.

The ribosome is an unusual catalyst, composed of three RNA molecules (four in some species) as well as dozens of proteins. Over the past thirty years there's been a growing body of evidence that RNA might lie at the catalytic "heart" or active site of the ribosome, with the proteins playing supporting roles. Some of the best evidence has come from the laboratory of Prof. Harry Noller (University of California Santa Cruz, USA). But in biochemistry, "one picture is worth a thousand words," and obtaining the actual structure of the ribosome was a hugely challenging project despite years of progress by Prof. Ada Yonath (The Weizmann Institute, Rehovot, Israel).

Then, in 2000, the atomic-level picture of the ribosome emerged, showing the complex fold of the RNA molecules buttressed and supported by numerous proteins. One structure showed the site where amino acids are strung together into proteins (the peptidyl transferase center); it is in fact composed of RNA, with no proteins in the vicinity ([1](#)). This provides the most direct evidence yet that the ribosome is indeed a ribozyme, an RNA catalyst. Other structures showed how the ribosome is inhibited by antibiotics, how it promotes the interaction of mRNA with transfer RNAs, and how the whole assembly is organized ([2](#), [3](#), [4](#)).

The large subunit of the ribosome, starburst, active site for protein synthesis. The active site consists of RNA (white strands), not protein (orange), supporting the conclusion that the ribosome is a ribozyme.

Figure by Dr. Basile Thomas Stoltzfus
Copyright © Howard Hughes Medical Institute This work may seem esoteric, but in fact it has spurred new approaches to developing antibiotics against pathogenic microbes. After all, protein synthesis is essential for life, and human ribosomes are similar but not identical to microbial ribosomes, so it's possible to find drugs that inactivate only the latter.

Riboswitches

The switching on and switching off of genes in response to an organism's needs is one of the most basic of biological control mechanisms. When [François Jacob](#) and [Jacques Monod](#) were mapping out the genetic regulatory circuit that controls metabolism of a simple sugar (lactose) in a bacterium, they thought for a while that the gene repressor might be RNA. Soon afterwards, however, the lac repressor was isolated – it turned out to be a protein, as were the next hundred or so repressors that were identified. So the idea of an RNA gene-repressor lay dormant for decades.

Very recently, however, this idea has been revisited. In another branch of the bacterial kingdom, RNA elements built into messenger RNAs can directly sense the concentration of small metabolites and turn gene expression on or off in response. These riboswitches fold into a specific structure that can switch between two conformations ([5](#)). Three distinct tricks for switching gene expression have been revealed: the RNA element can cause premature termination of transcription of the mRNA; it can block ribosomes from translating the mRNA; or it can even cleave the mRNA and thereby promote its destruction. This last activity involves an RNA unit directly binding a small-molecule metabolite, which switches the RNA into a conformation that activates its intrinsic self-cleavage activity ([6](#)). This "ribozyme riboswitch" represents a new type of biological activity for a catalytic RNA.

RNA Interference

The past few years have witnessed the emergence of double-stranded RNA (dsRNA) as a potent regulator of gene expression in higher organisms including humans ([7](#)). dsRNA had previously received little attention. Although the DNA of our chromosomes is famously double-stranded, only one strand of DNA is typically copied into RNA, so the resulting RNA is normally a single strand. (Because it has no partner strand, it folds back onto itself to form a variety of intricate shapes.) dsRNA was mainly studied as a transient intermediate in the replication of RNA viruses, an intermediate that tipped off animal cells to mount an anti-viral response because, after all, double-stranded RNA was "unnatural".

Now that this dsRNA isn't so unnatural after all, Cells have a sophisticated system for dealing with it: either endogenously, it is made by transcription of their own DNA (or exogenous). First, an enzyme called "dicer" cuts the dsRNAs into 20-base pair segments. One of the two strands is then transferred to a messenger ribosome, a ribosome made of a messenger RNA and an enzyme called "slicer" that cleaves the mRNA at the position of the duplex ([8](#)). The cleaved mRNA is rapidly degraded. In other cellular systems, instead of the mRNA being degraded it stays intact, but the presence of the short RNA duplex renders it somehow untranslatable, so no protein product is made.

On the one hand, the discovery of RNA interference (RNAi) has led to the identification of many small cellular RNAs that do not encode proteins (and therefore escaped identification in the Human Genome Project) but instead act to regulate the expression of other genes ([9](#)). These microRNAs form extensively base-paired "foldback" structures that are then processed by RNA.

On the other hand, scientists have developed robust new technologies for targeted gene inactivation based on RNAi. They synthesize dsRNA with a sequence that matches the intended target; this dsRNA is often called siRNA (small interfering RNA). They then introduce the dsRNA (or DNA encoding it) into cells, where the RNAi machinery takes over and completes the gene inactivation. Thus, RNAi has become a powerful tool for understanding which genes are important in which biological events. There is currently great excitement concerning the possibility that this research tool could be turned into pharmaceuticals directed against disease-causing genes.

Ribozymes at the Chemical Level

Concurrent with all these new RNA discoveries, great progress is also being made in understanding at the detailed chemical level how the original ribozymes work. Chemists think about chemical reactions in the context of detailed atomic structures of molecules, but for many years catalytic RNAs evaded structural analysis. The RNA molecules simply refused to form well-ordered crystals suitable for x-ray diffraction. This problem was first solved for some of the smaller self-cleaving ribozymes ([10](#), [11](#)), revealing unanticipated intricate folding patterns and the use of RNA bases as proton donors and acceptors to speed the chemical reactions.

Finally this year, the first well-resolved structures of self-splicing introns were solved. The long-anticipated catalytic metal ions can now be directly visualized, held into place by RNA phosphate groups that are in turn positioned by the complex fold of the RNA chain ([12](#), [13](#), [14](#)). The metal ions in the active site help to stabilize the mid-point or "transition state" of the reaction by neutralizing charge and by orienting the reacting atoms. Exciting crystal structures of portions of the Ribonuclease P ribozyme have also been announced ([15](#)). Thus, both subjects of the 1989 Nobel Prize in Chemistry are now seen in atomic detail, making them more amenable to mechanistic analysis.

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

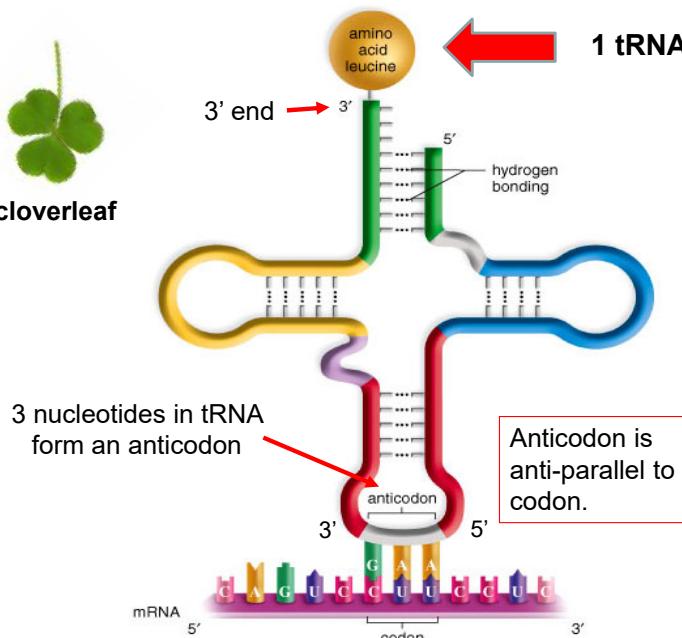
16

Like mRNA and rRNA, tRNA molecules are transcribed from DNA templates in the nucleus of eukaryotes. A tRNA molecule consists of a single RNA strand that is only about 80 nucleotides long. The strand can double back on itself to create regions where complementary bases are hydrogen-bonded to one another.

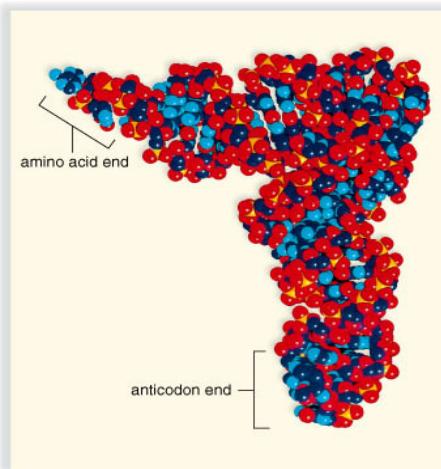
tRNA



cloverleaf



1 tRNA for each of the 20 amino acids



3D structure
of tRNA

17

It looks like a cloverleaf if it is flattened into one plane, to show the base pairing. However, the tRNA actually twists and folds into a compact three-dimensional structure that is roughly L-shaped. There is at least one tRNA molecule for each of the 20 amino acids found in proteins. The amino acid binds to the 3' end. The opposite end of the molecule contains an anticodon, a group of three bases that is complementary to a specific codon of mRNA.

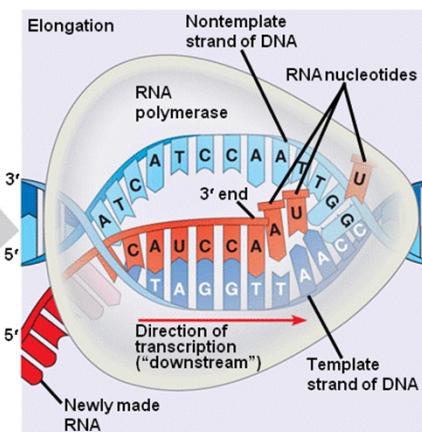
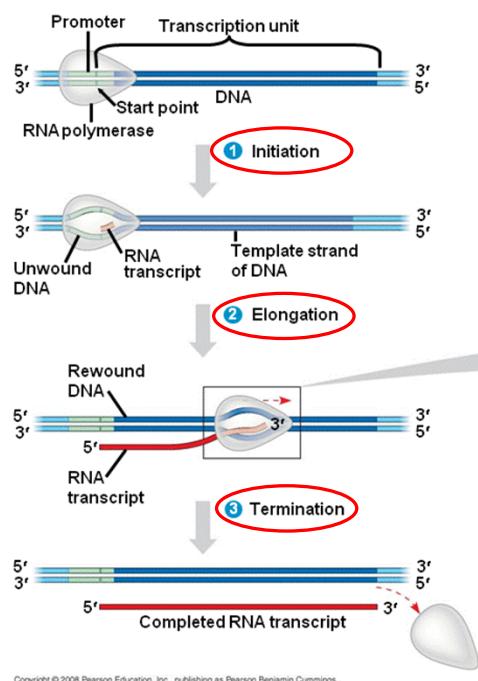
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Transcription Process

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 polymerase **do not** require any primer.

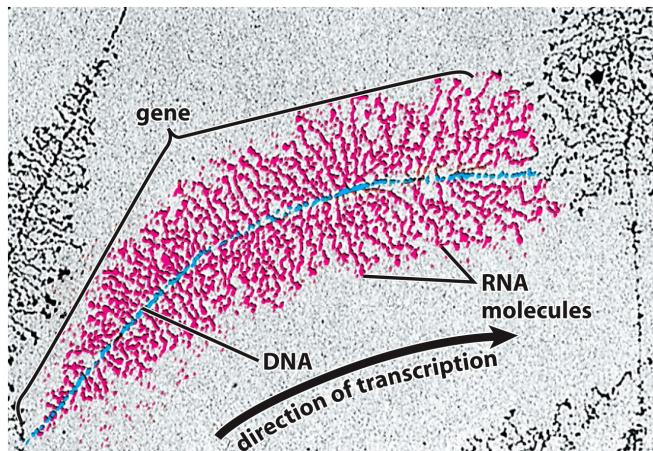
19

Now let's examine the transcription process in detail.

We can view transcription as a process consisting of

1. **initiation:** after RNA polymerase binds to the promoter, the DNA strands unwind, and the polymerase initiates RNA synthesis at the start point on the template strand. Near the beginning of every gene is an untranscribed segment of DNA called the **promoter**.
2. **Elongation:** As RNA polymerase moves along the DNA, it continues to untwist the double helix, and add nucleotides to the 3' end of the growing RNA molecules.
3. **Termination:** When RNA polymerase reaches a sequence of DNA bases known as **the termination signal (terminator)**, RNA polymerase releases the completed RNA molecule and detached from the DNA. The RNA polymerase is then free to bind to another promoter and synthesize another RNA molecule.

RNA transcription in action



This colorized electron 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.

20

A single gene can be transcribed simultaneously by several molecules of RNA polymerase following each other like tracks in a convoy. A growing strand of RNA trails off from each polymerase, with the length of each new strand reflecting how far along the template the enzyme has traveled from the start point. The congregation of many polymerase molecules simultaneously transcribed from it, which helps the cell make the encoded protein in large amount.

Why do you think so many mRNA molecules are being transcribed from the same gene?

Let's do it together!

Quiz

Template sequence
(from problem):

3'-TTCAGTCGT-5'

Nontemplate sequence:

5'-AAGTCAGCA-3'

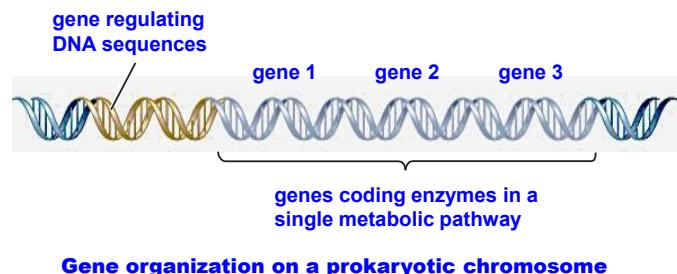
mRNA sequence:

5'-AAGUCAGCA-3'

Antiparallel

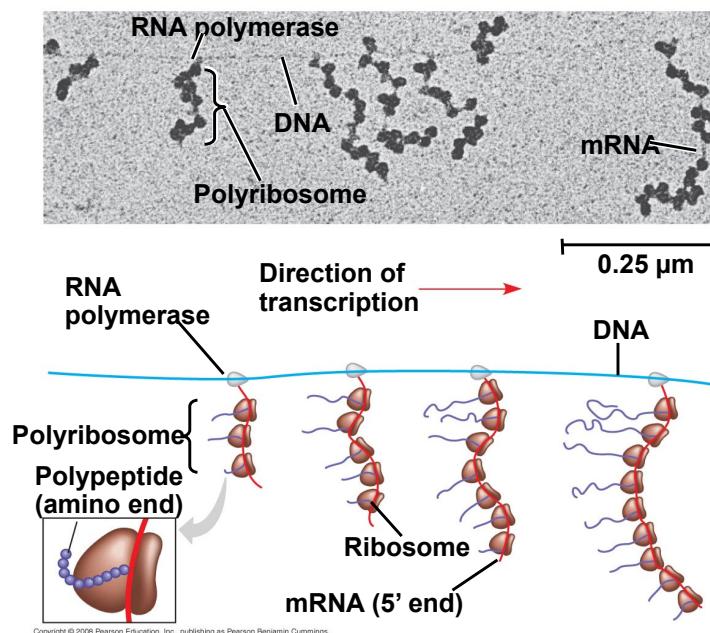
mRNA Synthesis in Prokaryotic Cells

- 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**



- 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

mRNA is directly used for protein synthesis in prokaryotes

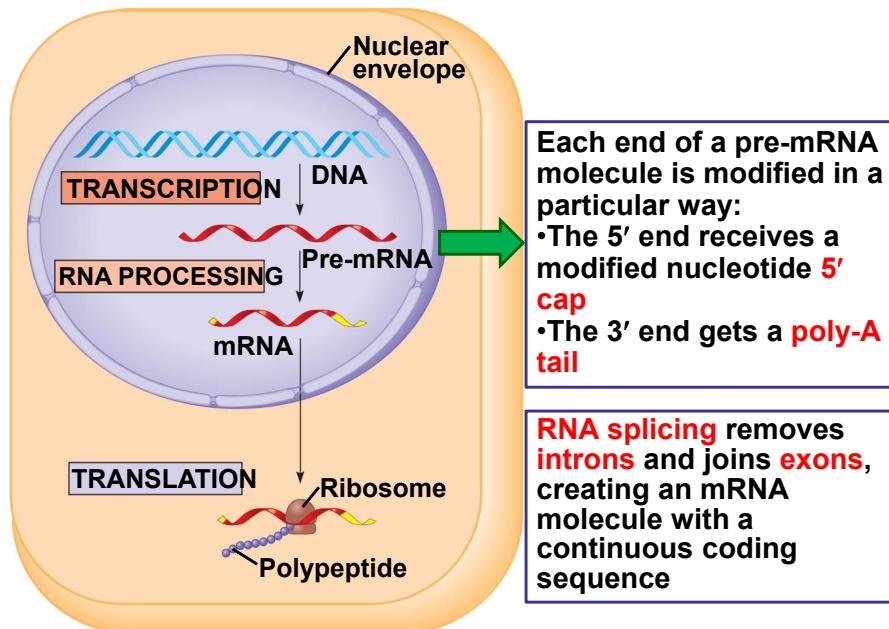


23

In prokaryotes, many or all of the genes for a complete metabolic pathway lie side by side on the chromosome. Therefore, prokaryotic cells commonly transcribe a single, very long mRNA from a series of adjacent genes. Because prokaryotic cells do not have a nuclear membrane separating their DNA from the cytoplasm, transcription and translation are usually not separated, either in space or in time. In most cases, as an mRNA molecule begins to separate from the DNA during transcription, ribosomes immediately begin translating the mRNA into protein.

Figure 17.24 Coupled transcription and translation in bacteria

mRNA synthesis and maturation in eukaryotes



24

Intron – refers to non-coding sequences found in DNA or RNA, not used for amino acid synthesis

Exon – refers to coding portions of DNA or RNA, used for amino acid synthesis

In contrast, the DNA of eukaryotic cells is sequestered in the nucleus, transcription and translation take place in different cellular compartment. In addition, newly formed RNA molecules, called primary mRNA, are modified before leaving the eukaryotic nucleus. For example, mRNA molecules receive a cap at the 5' end and a tail at the 3' end. The cap is a modified guanine (G) nucleotide that helps tell a ribosome where to attach when translation begins. The tail consists of a chain of 150-200 adenine (A) nucleotides. This so-called poly A tail facilitates the transport of mRNA out of the nucleus and also inhibits degradation of mRNA by hydrolytic enzymes.

Figure 17.3 Overview: the roles of transcription and translation in the flow of genetic information

mRNA undergoes maturation steps before it can be used for protein synthesis in eukaryotes

- Each gene consists of two or more segments of DNA that encode for protein, called **exons**, that are interrupted by other segments that are not translated, called **introns**
- Transcription of a gene produces a very long RNA strand that contains introns and exons, which is often called precursor mRNA, or **pre-mRNA**
- More nucleotides are added at the 5' end and 3' end of the pre-mRNA molecule, forming a “**cap**” and “**tail**” -> stabilises the mRNA
 - Cap and Tail assist with moving the RNA through the nuclear envelope, to bind the mRNA to a ribosome, and to prevent cellular enzymes from breaking down the mRNA
- Enzymes in the nucleus cut out the introns and **splice** together the exons to make **mature mRNA**
- The mRNA then exits the nucleus through a nuclear membrane pore and associates with a ribosome

25

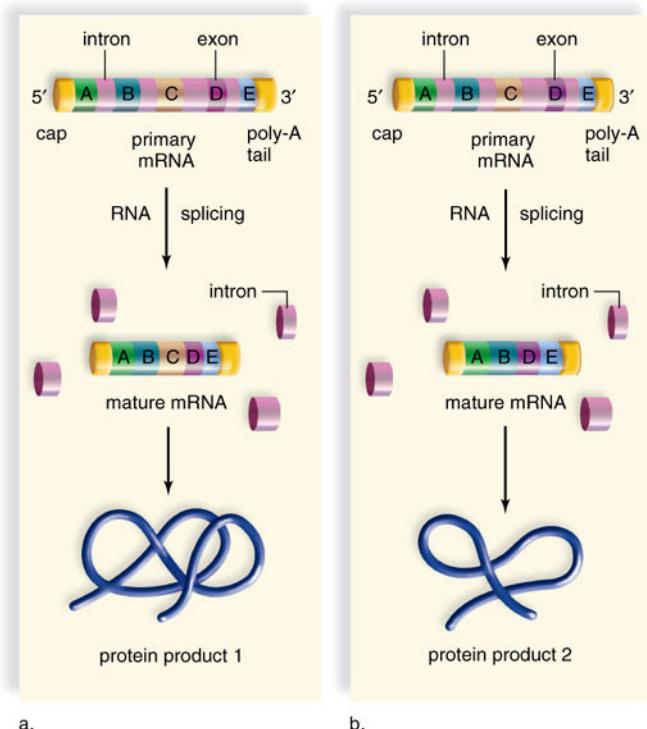
Alternate splicing in eukaryotes

- Exons might combine in different combinations
- Allow different mRNAs and multiple polypeptides from single gene

RNA Splicing: Nobel Prize winning innovation

Thinking

What is biological significance?



26

Splicing the same pre-mRNA can result in different mRNA serving different needs

Another modification is called RNA splicing. Primary mRNA, particularly in multicellular eukaryotes, is composed of exons and introns. The exons of mRNA will be expressed, but not the introns, which occur in between the exons. During RNA processing, the introns are removed, and exons are rejoined together. RNA splicing allows a cell to produce multiple proteins from a single gene by splicing exons together in different ways.

Intervene region

Express

A summary of transcription

- Like DNA replication
 - Require a template DNA
 - New strand grows in 5' to 3' direction
 - Both happen in the nucleus in eukaryotes
- Unlike DNA replication
 - Only small section (gene) of one strand of DNA as template
 - Only RNA polymerase involved
 - Does not need a primer
 - Product is single strand of RNA instead of two identical DNA

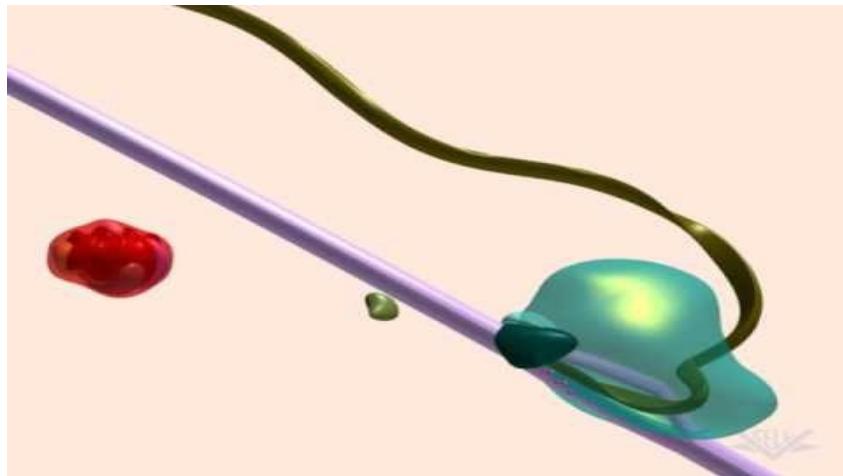
27

Primer is type of paint that is put onto wood in order to prepare it for the main layer of paint.

A *primer* is a strand of nucleic acid that serves as a starting point for DNA replication.

Animation

Transcription and mRNA processing (video)



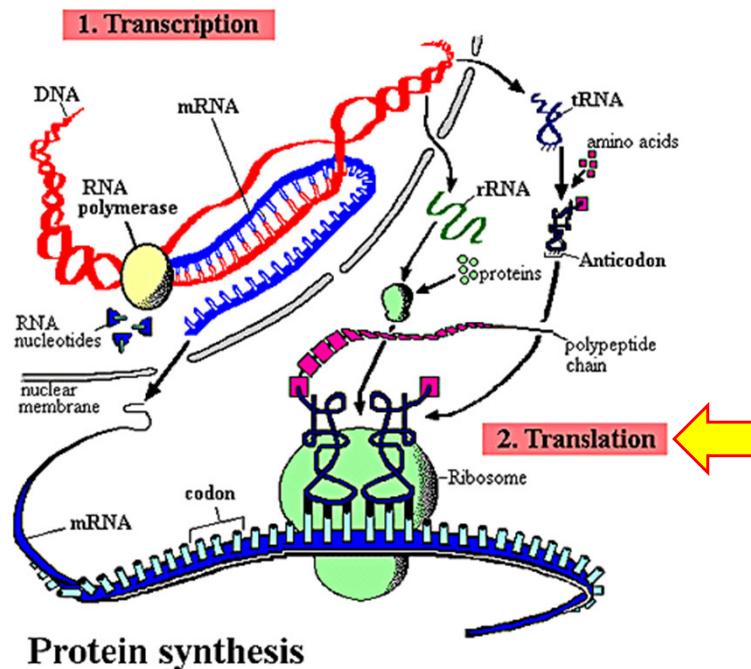
<https://www.youtube.com/watch?v=WsofH466lqk>



28

Play video (2.5 mins)

Translation



29

Genetic Code

- Base sequence in DNA **dictates** sequence and type of amino acids in protein
- There are **20** amino acids, but there are only **four** nucleotide bases in DNA
- The **genetic code** translates the sequence of bases in nucleic acids into the sequence of amino acids in proteins
- The genetic code uses **three** bases (a triplet, **codon**) to specify an amino acid
- Each mRNA also has a **start codon** (AUG) and **one of three stop codons** (UAG, UAA, and UGA)

30

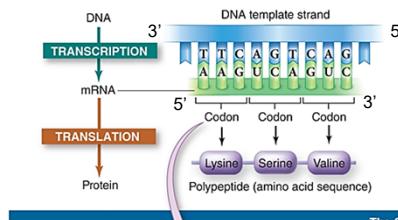
How does the language of nucleotide sequences in messenger RNA translate into the language of amino acid sequences in proteins? This translation relies on a “dictionary” called the genetic code.

Genetic Code (cont.)

- Redundant but not ambiguous
 - 64 (4^3) codons for 20 amino acids
 - Each codon specifies only one specific amino acid; however, some amino acids are specified by as many as six different codons
- Three stop codons do not specify any amino acid
- Almost universal
 - With few exceptions (some codons in bacteria and human are different), all organisms use same genetic code

31

Genetic Code (cont.)



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

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

32

The genetic code translates the sequence of bases in nucleic acid into the sequence of amino acids in proteins. But which combination of bases code for which amino acids? Both DNA and RNA contain four different bases, however the proteins are made of 20 different amino acids, therefore, one base cannot code for one amino acid, because there are simply not enough different types of bases. The genetic code must rely on a short sequence of bases to encode each amino acid. If a sequence of two bases codes for an amino acid, there would be 16 possible combinations, which still isn't enough to code for all 20 amino acids, a three-base sequence, however, gives 64 possible combinations of bases, which is more than enough. Under the assumption that nature operates as economically as possible, biologists hypothesized that the genetic code must be triplet.

Crick's and gene code

Thinking



(a) Tobacco plant expressing a firefly gene



(b) Pig expressing a jellyfish gene

These examples demonstrate the ability of genes from one species to be expressed in a different species. This is possible because of which property of the genetic code?

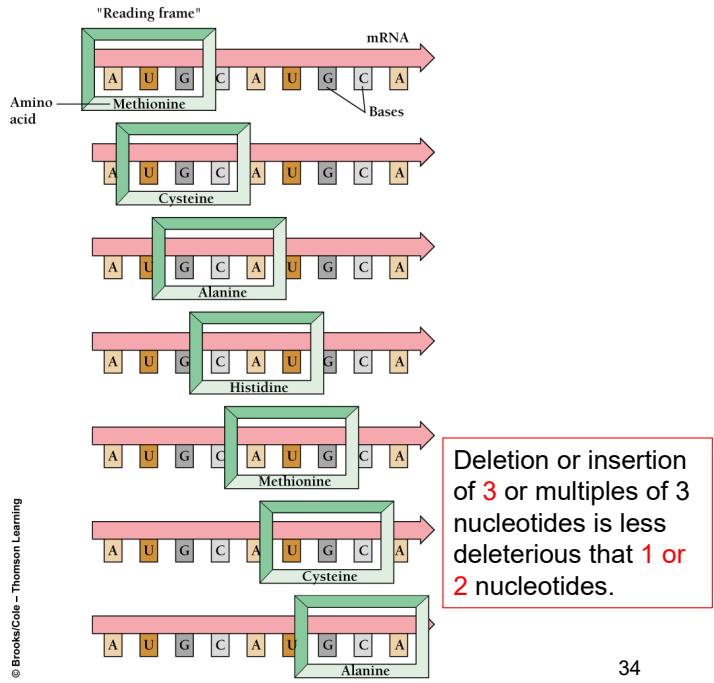
33

Figure 17.6 Expression of genes from different species

The code is universal. Same genetic code is found valid for all organisms ranging from bacteria to man.

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.



34

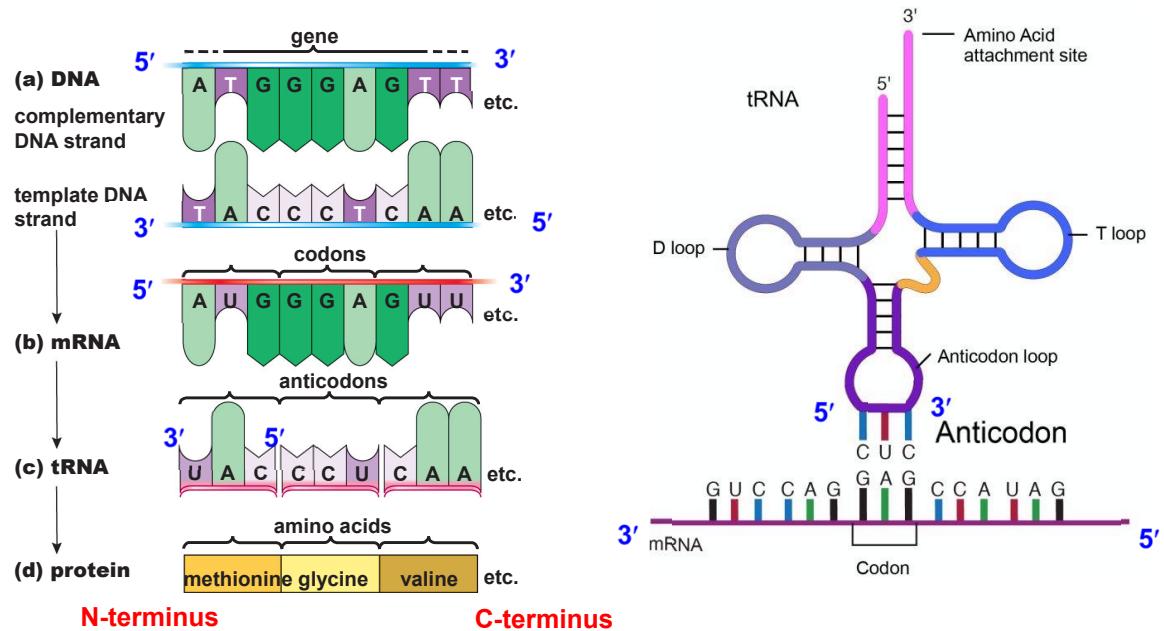
you are not too bad, and I'll aid you.

Add two and one and you may ken the odd key I'll use for now. Not yet? 'Tis not too bad, but I'll aid you. Thy tip: "wee". Now can you spy the key? Yes! You win! But you see how its use can vex the ego, and I'll end. But who can say it's not fun?

Friend, fiend,

http://www.youtube.com/watch?v=x_vlxGFrZLY (Transcription & Translation | MIT 7.01SC Fundamentals of Biology)

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



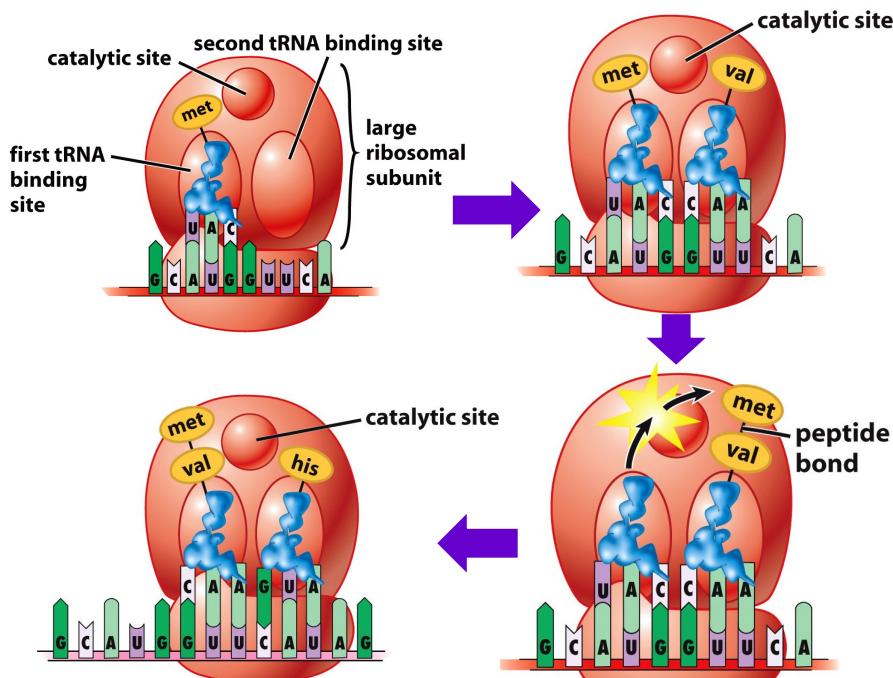
35

Translation Process

Ribosomes, tRNA, and mRNA cooperate in protein synthesis.

Like transcription, translation has three steps

- Initiation
- Elongation
- Termination



37

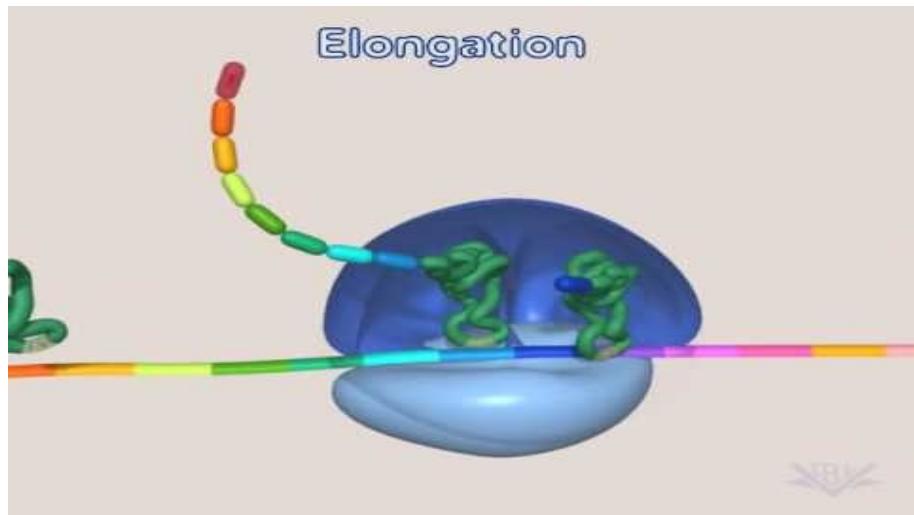
Initiation is the step that brings all the translation components together. Proteins called initiation factors are required to assemble the small ribosomal subunit, mRNA, initiator tRNA, and the large ribosomal subunit for the start of protein synthesis.

Elongation is the protein synthesis step in which a polypeptide increases in length one amino acid at a time. In addition to the participation of tRNAs, elongation requires elongation factors, which facilitate the binding of tRNA anticodons to mRNA codons at a ribosome.

Termination is the final step in protein synthesis. Termination of polypeptide synthesis occurs at a stop codon, that is, a codon that does not code for an amino acid. During the termination, the polypeptide and the assembled components that carried out protein synthesis are separated from one another.

Figure 17.13 Translation: the basic concept

Animation Translation (video)



<https://www.youtube.com/watch?v=5bLEdd-PSTQ>

Play video (3.32 mins)

If the mRNA transcript 5' AUG CGC UGC AAU 3' were to leave the nucleus and undergo translation at a ribosome, what would be the sequence of anticodons translating this nucleotide into protein, and oligo peptide produced?

0

3' UAC GCG ACG UUA 5'; NH2-Met-Arg-Cys-Asn-COOH

0%

3' UAA CGU CGC GUA 5'; HN2-stop-Arg-Arg-Val-COOH

0%

3' TAC GCG ACG TTA 5'; NH2-stop-Ala-Thr-Leu-COOH

0%

3' ATG CGC TGC AAT 5'; NH2-Asn-Cys-Arg-Met-COOH

0%

3' AUG CGC UGC AAU 5'; COOH-Met-Arg-Cys-Asn-NH2

0%

Start the presentation to see live content. For screen share software, share the entire screen. Get help at pollev.com/app

Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

More info at polleverywhere.com/support

If the mRNA transcript 5' AUG CGC UGC AAU 3' were to leave the nucleus and undergo translation at a ribosome, what would be the sequence of anticodons translating this nucleotide into protein, and oligo peptide produced?

https://www.polleverywhere.com/multiple_choice_polls/F21wyvkYUDgyC8p2lhBKa?state=opened&flow=Default&onscreen=persist

Let's do it together!

Quiz

Template sequence
(from problem):

3'-TTCAGTCGT-5'

Nontemplate sequence:

5'-AAGTCAGCA-3'

mRNA sequence:

5'-AAGUCAGCA-3'

tRNA anticodons:

3'-UUC-5' 3'-AGU-5' 3'-CGU-5'

Protein sequence:

NH₂-Lys-Ser-Ala-COOH

40

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

41

All organisms, whether prokaryotes or eukaryotes, must regulate which genes are expressed at any given time. They must continually turn genes on and off in response to signals from external and internal environments.

Because their environment is ever changing, bacteria do not need the same enzymes and possibly other proteins all the time. For example, they need only the enzymes required to break down the nutrients available to them and the enzymes required to synthesize whatever metabolites are absent under the present circumstances.

In 1961, two French scientists proposed the operon model to explain gene regulation in prokaryotes and later received a Nobel Prize for their investigations.

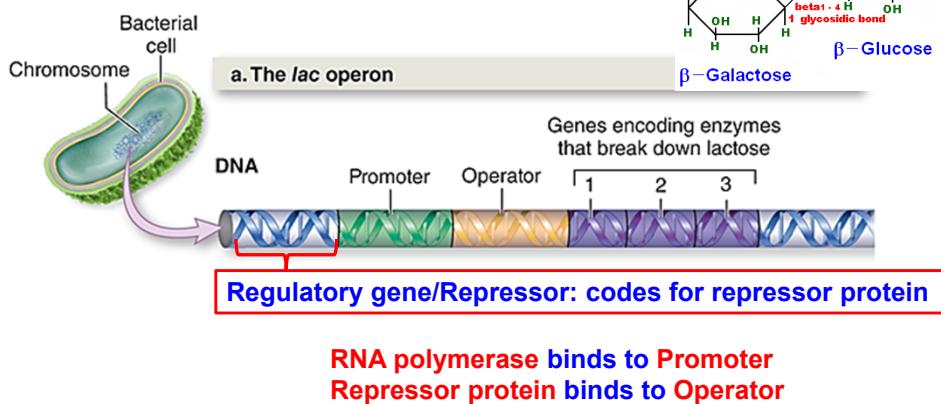
This model consists of a regulatory gene, promoter, operator and structural genes. The regulatory gene works like a switch in an electric circuit, it codes for a repressor that controls whether the operon is active or not.

A promoter is a short sequence of DNA where RNA polymerase first attaches when a gene is to be transcribed. Operator: a short portion of DNA where an active repressor binds. When an active repressor is bound to the operator, RNA polymerase cannot attach to the promoter, and transcription does not occur. In this way, the operator controls mRNA synthesis.

Structural genes: one to several genes, sit side to side, codes for the primary structure of enzymes of a metabolic pathway that are transcribed as a unit.

In prokaryotes it is common to find a group of genes encoding enzymes required for a single metabolic pathway clustered together in the genome, so that they can be transcribed as a single polycistronic mRNA. This allows coordinated regulation of their expression by a single promoter. The term used for such a cluster of genes and their promoter is an **operon**. Using the *E. coli lac* operon as an example, the three co-regulated genes encoding β -galactosidase, lactose permease, and transacetylase are called the *lacZ*, *lacY* and *lacA* genes, respectively. There is also a separate *I gene* (for inducibility) that codes for a protein called the **lac repressor**, and there is a stretch of DNA called the **operator region** to which the *lac* repressor protein can bind. The **promoter** where RNA polymerase binds to begin transcription is flanked by two regulatory DNA sequences, each of which is recognized and bound by a different regulatory protein. The *lac* repressor binds to the **operator** sequence, which lies just downstream of (and partially overlapping) the promoter,

Structure of the lactose operon



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.

Nobel Prize in 1965

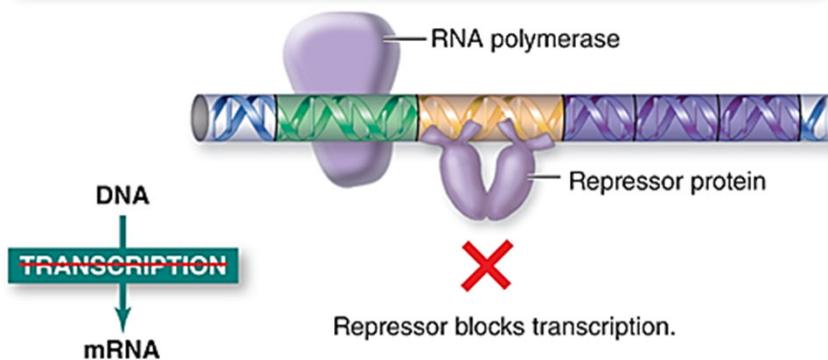
42

In the bacteria, there are different types of operons and are regulated in a variety of ways.

Here shows you the lactose operon. Lactose is the principal sugar in milk. The operon contains three structural genes, each coding for an enzyme that aids in lactose metabolism.

FIGURE 10-10a Regulation of the lactose operon

b. No lactose present



Repressor blocks transcription.

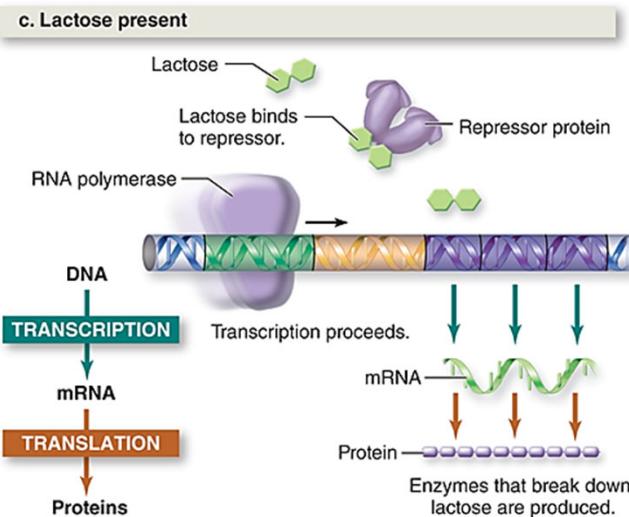
When lactose is not present, repressor proteins bind to the operator of the lactose operon. When RNA polymerase binds to the promoter, the repressor protein blocks access to the structural genes, which therefore cannot be transcribed.

43

The regulator gene codes for a lac operon repressor that ordinarily binds to the operator and prevents transcription of the three genes.

This operon is shut off.

FIGURE 10-10b Regulation of the lactose operon



Operon exert a synchronous and fast regulation of genes belonging to one metabolism process.

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.

44

But when we took a cup of milk, or a baby get from her mother, the lactose molecules enter bacteria in the intestine and bind to the repressor proteins, changing their shape. So that the repressor cannot attach to the operator site, and RNA polymerase can move along the DNA to transcribe the genes and produce the enzymes for lactose metabolism.

Because the presence of lactose brings about expression of genes, it is called an inducer of the lac operon. Without the inducer, the operon would not make enzymes. Why is that beneficial? Because these enzymes need only be active when the nutrients is present.

FIGURE 10-10c Regulation of the lactose operon

Whole operons are regulated as units, so that functionally related proteins are synthesized simultaneously when the need arises

The intestinal bacterium *Escherichia coli* (*E.coli*) lives on what its host eats

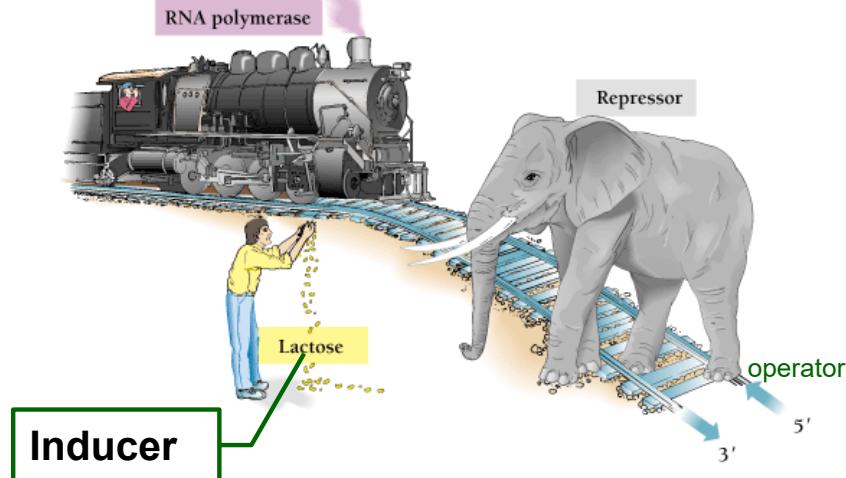
Specific enzymes are needed to metabolize the type of food that comes along

e.g. in newborn mammals, *E.coli* are bathed in milk, containing the milk

sugar lactose

The **lactose operon** contains three structural genes, each coding for an enzyme that aids in lactose metabolism

Prokaryotic Regulation



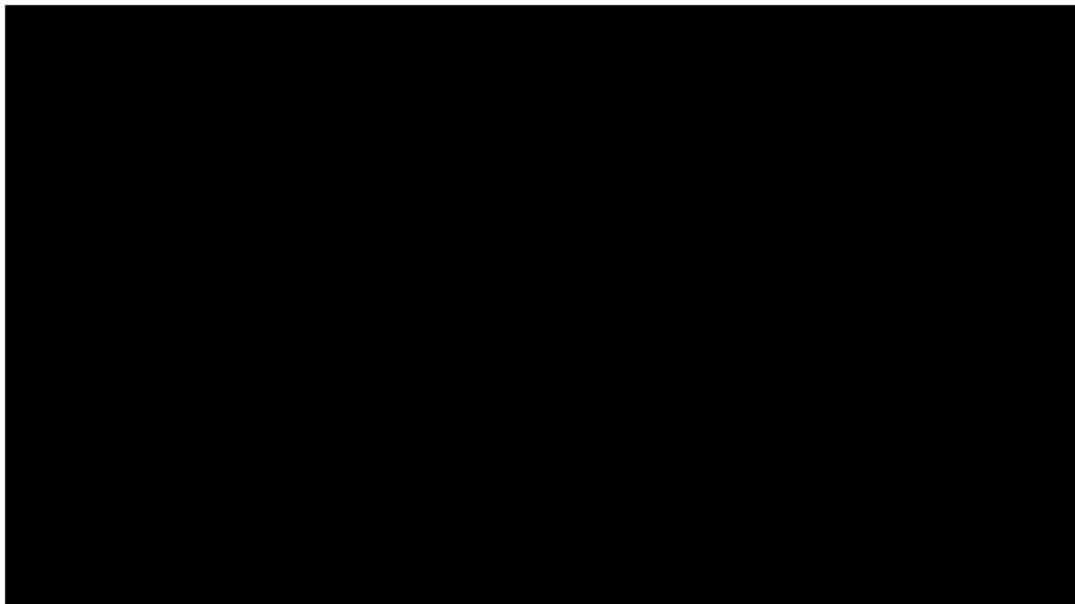
Without the repressor in the operator, RNA polymerase is able to reach the promoter and begin transcription of the genes needed to metabolize lactose.

45

Figuratively, it looks like an elephant, which is the repressor, standing on the railways (DNA), and the train is RNA polymerase, cannot move on if the elephant does not move away. Therefore, you may need some inducers to attract the elephant away from the train.

Gene Regulation in Prokaryotes

<https://www.youtube.com/watch?v=oBwtxdl1zvk>



46

Play video on lac operon (3.25 mins)

I can put on Luminus.

Gene Regulation in Eukaryotes

Gene expression is regulated at a number of points

- Transcription (**transcription factors**)
- Post-transcription (**alternative splicing**)
- Translation (life span of mRNA)
- **Post-translational modification** (protein activity)
- Life span of proteins (**protein degradation**)

47

Unlike prokaryotic cells, a variety of mechanisms regulate gene expression in eukaryotic cells. These mechanisms can be grouped under 5 primary levels of control.

Expression of genetic information by a eukaryotic cell is a multistep process, beginning with transcription of DNA, and ending with a protein that performs a particular function

Eukaryotic gene regulation

DNA is in a membrane-bound nucleus

Variety of cell types in multicellular eukaryotes

The genome is organized differently

RNA transcripts undergo complex processing

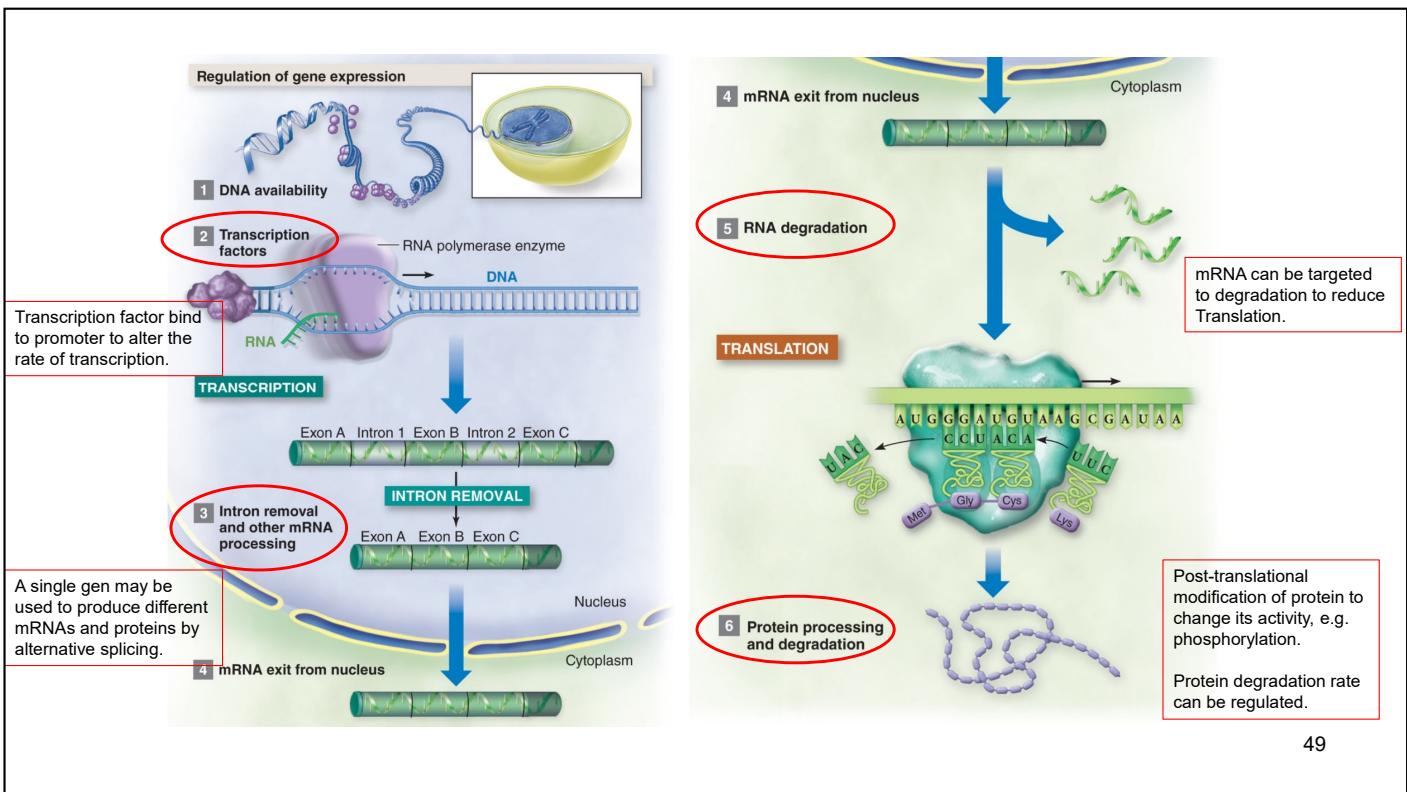
47

Genes Regulation in Humans

- The human genome contains ~ 30,000 genes
- A given cell “expresses” (transcribes) only a small number of genes
- Some genes are expressed in all cells
- Other genes are expressed only
 - In certain types of cells
 - At certain times in an organism’s life
 - Under specific environmental conditions

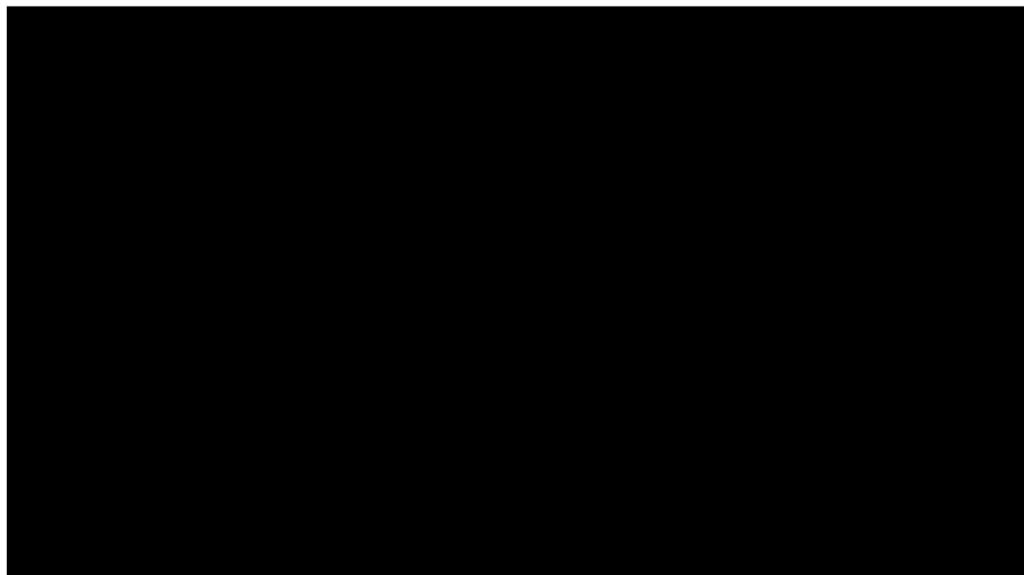
48

All cells contain almost the same DNA with the same genes, but a typical human cell probably expresses about 20% of its genes at any given time. The subset of genes expressed in the cells of each type is unique, allowing these cells to carry out their specific function. Muscle cells, for example, have a different set genes that are turned on in the nucleus and a different set of proteins that are active in the cytoplasm than do nerve cells.



Gene Regulation in Eukaryotes

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



50

Play video on gene regulation in Eukaryotes (2.03 mins).
Will put on Luminus.



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

51

Androgen insensitivity syndrome is a condition that affects sexual development before birth and during puberty. People with this condition are genetically male, with one X chromosome and one Y chromosome in each cell. Because their bodies are unable to respond to certain male sex hormones (called androgens), they may have mostly female sex characteristics or signs of both male and female sexual development.

Androgen

n.

男性荷尔蒙, 男性激素

Tes testosterone

n.

[生化][药] 睾丸激素

Intersex athletes



Maria Jose Martinez Patino



Intersex athletes

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

52

Androgen insensitivity leads to female features. This condition is inherited in an X-linked recessive pattern.

This individual has an X and a Y chromosome. She has testes that produce testosterone, but a mutation in her androgen receptor genes makes her cells unable to respond to testosterone, resulting in her female appearance.

In 1985, when she travelled to Kobe, Japan, to compete in the World University Games, Spanish hurdler Maria Patino got the shock of her life. Like other female competitors there, she had to take a sex test to prove she was not a man in disguise, but she wasn't worried.

She is not a transsexual - that is, someone who has undergone surgery to change from one sex to another.

Psychobiology and Gene Expression

Every individual experiences stressful life events. In some cases acute or chronic stress leads to depression and other psychiatric disorders, but most people are resilient to such effects. Recent research has begun to identify the **environmental, genetic, epigenetic and neural mechanisms** that underlie resilience....

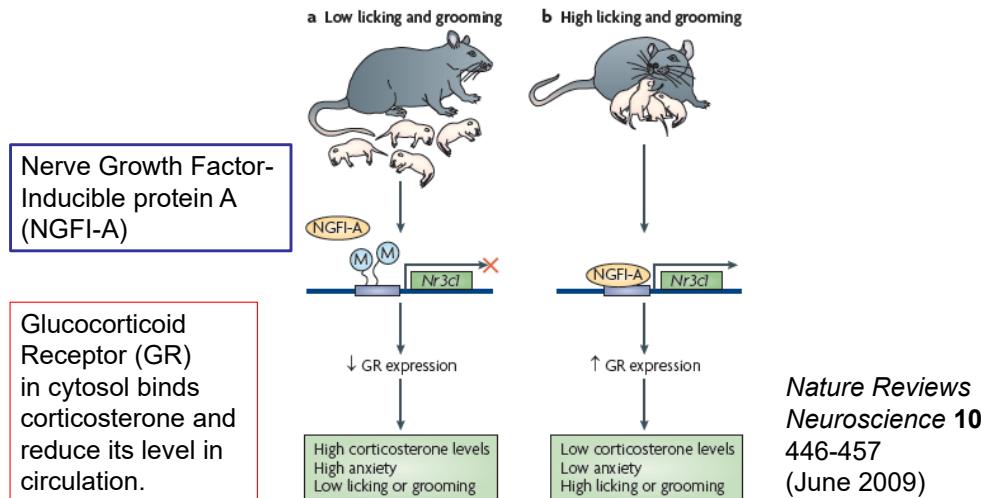


Figure 1 | epigenetic mechanisms of stress responsiveness. Female rats show a range of maternal behaviours, from low levels of licking and other types of grooming of their pups to high levels. These differences during early life can give rise to life-long differences in stress responsiveness^{65,66}. **a | Receiving low levels of** grooming results in low levels of the transcription factor nerve growth factor-inducible protein A (NGFI-A; also known as EGR1) in the hippocampus, which permits increased methylation and repression of the glucocorticoid receptor (GR) gene in this brain region. Lower levels of GR expression in the hippocampus contribute to several traits in adulthood: higher levels of baseline and post-stress glucocorticoid (corticosterone) secretion, higher levels of anxiety-like behaviour and, in females, lower levels of grooming behaviour towards their own offspring. **b | The offspring of high-grooming mothers have higher levels of**

hippocampal NGFI-A, resulting in less methylation of the GR gene and higher GR expression in the hippocampus. In adulthood this is associated with lower levels of baseline and post-stress corticosterone secretion, low anxiety-like behaviour and, in females, high levels of grooming of offspring.

Genetics, physiology, psychology,

Resilience refers to a person's ability to adapt successfully to acute stress, trauma or more chronic forms of adversity.

Sweet Future: Fluctuating Blood Glucose Levels May Affect Decision Making

The results, reported in *Psychological Science*, a journal of the Association for Psychological Science, reveal that people's preferences for current versus later rewards may be influenced by blood glucose levels. The volunteers who drank the regular sodas (and therefore had higher blood glucose levels) were more likely to select receiving more money at a later date while the volunteers who drank the diet sodas (and who had lower blood glucose levels) were likelier to opt for receiving smaller sums of money immediately. These findings are suggestive of an adaptive mechanism linking decision making to metabolic cues, such as blood sugar levels.

Key Terms and Key Concepts

Key Terms

Central dogma, messenger RNA (mRNA), transfer RNA (tRNA), ribosome RNA (rRNA), transcription, translation, RNA polymerase, genetic code, codon, anticodon, start codon, stop codons, promoter, template strand, initiation, elongation, termination, operons, operator, repressor proteins.

Key Concepts

- How is the information in DNA used in a cell?
- What is the information in a gene transcribed into RNA?
- How is the base sequence of mRNA translated into protein?
- How do mutations affect protein structure and function?
- How is gene expression regulated?

54

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Discuss

- In an alternate universe, although proteins are still constructed of combination of 20 different amino acids, DNA is constructed of **six different nucleotides**, not four as on earth. Would you expect the length of a typical gene to be the same, shorter, or longer than that of a typical gene on earth?
- Can a DNA polymerase from one resource replicate the DNA from another source? Explain your answer.

55

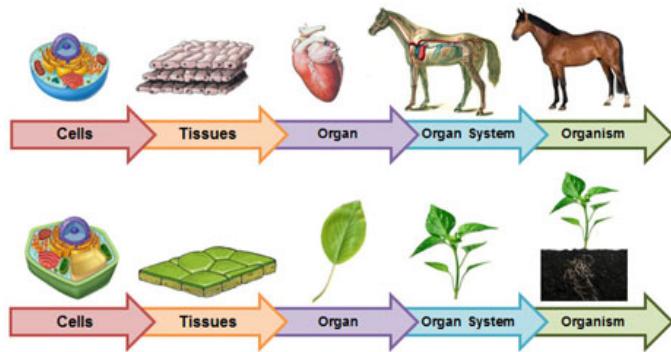
1. 2 bases -> gene will be shorter
2. Yes, with a few exceptions. DNA is universal across all forms of life – all living beings will always be made up of the same nucleotides (A,T,C,G). DNA polymerase is a tool to create DNA molecules by assembling nucleotides through reading the DNA templates, so the same DNA polymerase can be used to replicate DNA from another source.

Please complete Quiz 6 “Gene Expression” in Canvas

Laboratory class on this Wed, 11th Sep, LS Lab 3, 10 am to 6 pm

Remember to bring your lab coat, long trousers and closed shoes.

LSM1301



L7: Biotechnology

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1

Hi everyone, welcome back. I hope everyone had a good recess week.

So I am Dr Mowe and I am going to go into the smaller aspects of biology and away from ecology and organismal biology but into processes, energy production and cell biology and finally DNA

Biotechnology

In its broadest sense, biotechnology is any use or alteration of organisms, cells or biological molecules to achieve specific practical goals.”

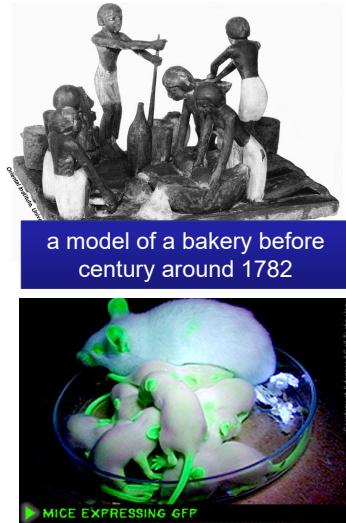
❖ Ancient biotechnology:

Fermentation (beer, bread, wine), domestication, selective breeding

❖ Modern biotechnology:

~ Genetic engineering

manipulates genetic information in an organism



2

In its broadest sense, biotechnology is any use or alteration of organisms, cells, or biological molecules to achieve specific practical goals. Therefore, some aspects of biotechnology are ancient. For example, people have used yeast cells to produce bread, beer and wine for the past 10K yrs.

The figure on the right shows a model of a bakery before century around 1782.

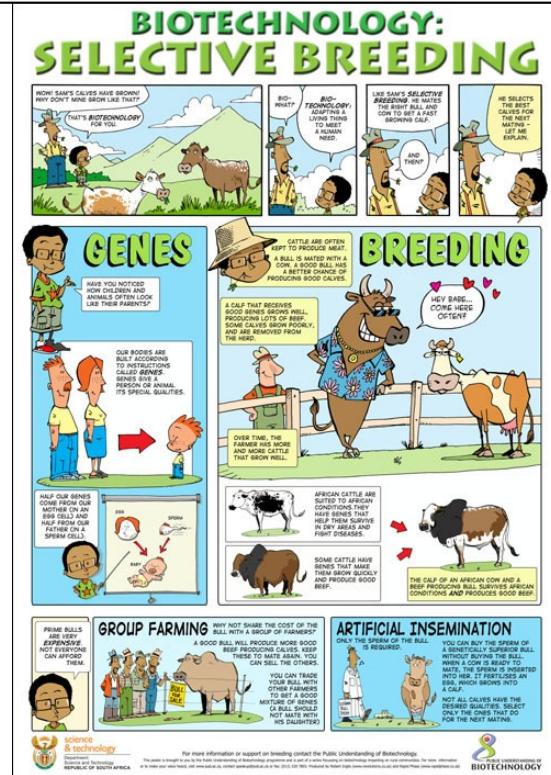
Modern biotechnology, however, also frequently uses genetic engineering, a term that refers to more direct methods for modifying genetic material.

The mouse with a human ear growing on its back proved just how far science could, or would, go. Human cells were added to the mouse to produce a real ear. With an ear-to-body ratio of about eighty percent, he's probably a very good listener.

A model of a bakery, Egypt, middle kingdom

Ancient biotechnology

- Use of biology and living organisms for practical purposes
- Traditional examples
 - Beer-brewing
 - Wine-making
 - Animal breeding
 - Plant breeding



This drawing also shows you selective breeding, an ancient biotechnology, remaining an important tool of biotechnology. It also known as artificial selection, is a process used by humans to develop new organisms with desirable characteristics. Breeders select two parents that have beneficial phenotypic traits to reproduce, yielding offspring with those desired traits. In this example, selected cows and bulls to produce good quantity and quality of milk or meat.

Mini-hearts grown to study disease

<http://www.bbc.com/news/health-28022949>

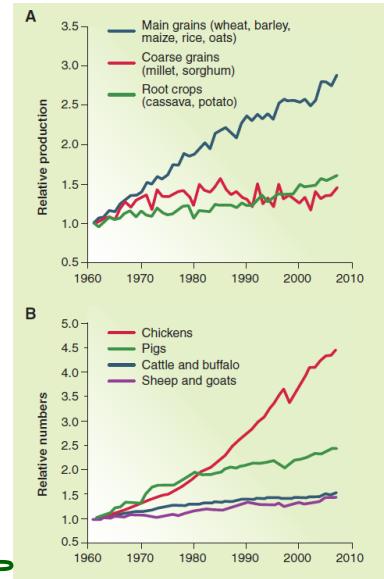
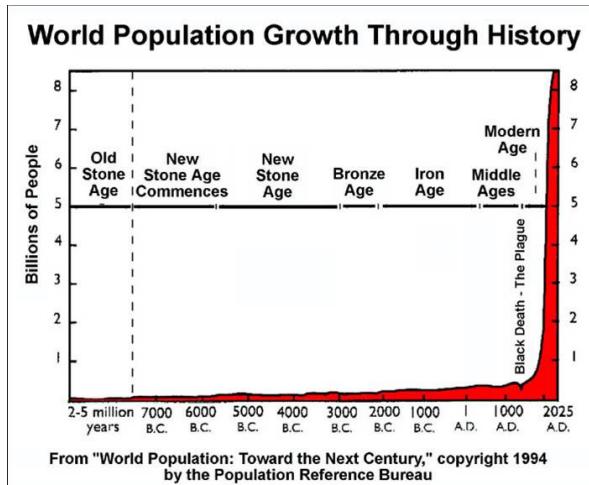


What is the **limitation for the traditional biotechnology?**

4

- Long experiment time
- May not yield results/results are unsatisfactory

Food Security: The Challenge of Feeding 9.8 Billion People by 2050



5

Isreal model,

Grow high-value products, sell them to change for food. For Singapore, it may not be realistic to grow crops like rice, wheat, corn and etc.

By **2050**, with the global **population** expected to reach 9.8 billion, our **food supplies** will be under far greater stress.

Outline of Modern biotechnology



Recombinant DNA

Technology

- Plasmids
- Enzymes
- Transgenic organisms



PCR & DNA agarose gel electrophoresis



DNA Fingerprinting

- short tandem repeats
- Gel electrophoresis
- DNA probes

DNA Chips/Genetic Screening

- Prenatal genetic screening
- Gene therapy

Political and Ethical Issues

6

In today's lecture, we will provide an overview of modern biotechnology. We will emphasize on applications of biotechnology and their impacts on society, we also briefly describe some of the important methods used in those applications. These techniques include Recombinant DNA and polymerase chain reaction.

Recombinant DNA Technology

(DNA Cloning/Engineering)

- Recombinant DNA (rDNA)
 - Contains DNA (usually not found together in nature) from two or more different organisms
- Requires
 - Vector: to introduce recombinant DNA into host cell
 - Plasmids are common vectors (for bacteria and plants)
 - Viruses are vectors for human and animals
 - Enzymes: to introduce foreign DNA into vector DNA
 - Restriction enzyme – to cleave DNA
 - DNA ligase – to link two genes together

7

Recombinant DNA (rDNA) contains DNA from two or more different sources, such as a human cell and a bacterial cell.

To make rDNA, a technician needs a vector, to carry the target gene into a host cell. One common vector is a plasmid.

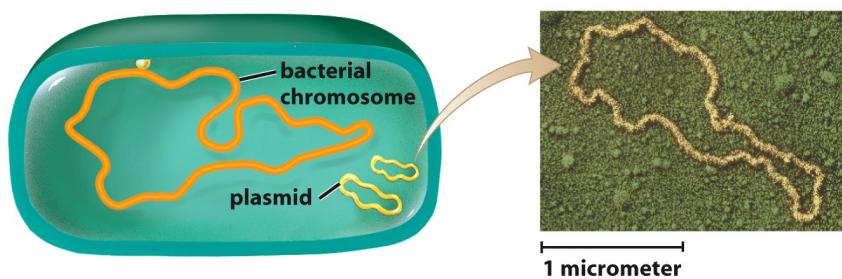
To induce target genes into vector DNA, we need two enzymes. (1) a restriction enzyme, which cleaves DNA, and (2) an enzyme called DNA ligase, which seals the opening created by the restriction.

Plasmids

Small circle DNA in bacterial cell

- Not essential for bacterial growth
- Capable of **autonomous replication**
- Can move from one bacterium to another
- Can **deliver** DNA into another cell

Bacterium



1 micrometer

8

Plasmids are small circular DNA molecules that replicate separately from the bacterial chromosome. A plasmid has only a small number of genes; these genes may be useful when the bacterium is in a particular environment but may not be required for survival or reproduction under most conditions.

PCR

- A cycled reaction that uses a **heat-tolerant form of DNA polymerase** (Taq polymerase) to produce billions of copies of a DNA fragment
- Requires knowledge of nucleotide sequence
 - For design of a pair of short pieces of DNA (called **primers**) complementary to targeted sequence of DNA for amplification
- **DNA replication in test tube**
 - Reaction mix consists of DNA polymerase, template DNA, primer, and **nucleotides**

9

Taq polymerase – able to operate at a high temperature, optimal at 72C

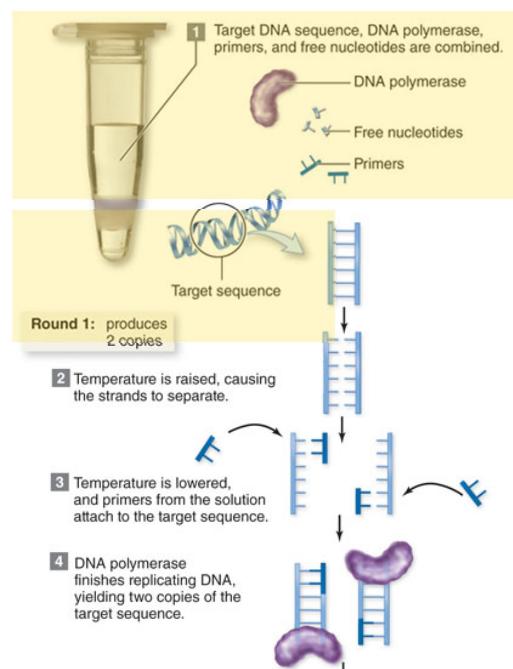
The polymerase chain reaction (PCR), developed by Kary Mullis in 1985, can create copies of a segment of DNA quickly in a test tube. However, PCR requires the knowledge of DNA sequence, without knowing the DNA that is being sequenced, it is very difficult to amplify the DNA.

PCR Replicates DNA in a Test Tube

Denaturation: Targeted DNA sequence to be amplified heated to 90-95°C to separate DNA to single strands

Annealing: Temperature lowered to 50°C to allow primers to bind to single DNA strands

Extension: Temperature raised to 70-72°C for DNA polymerase to uses free nucleotides to synthesize complementary strands



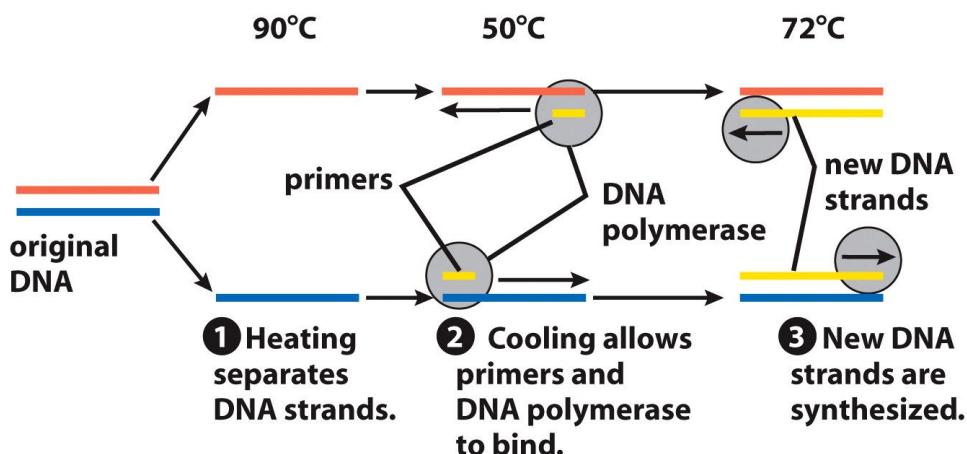
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Annealing temp

Higher -> produces more specific dna strands but less yield

Lower -> produces less specific dna strands but more yield

One PCR cycle

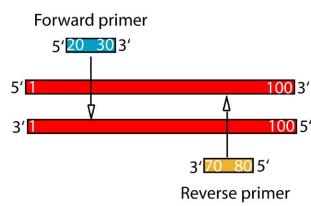


PCR begins with a mixture containing a **DNA template**, a pair of short **ssDNA oligonucleotide primers**, a pool of the four **dNTPs**, and a **heat-resistant DNA polymerase, Taq Enzyme**

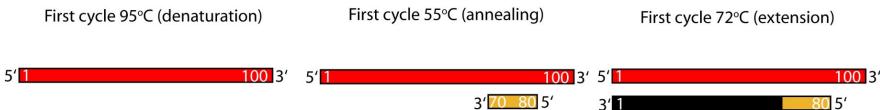
11

This figure shows you the one PCR cycle. Using appropriate mixtures of primers, free nucleotides, and DNA polymerase, a PCR machine automatically runs heating and cooling cycles over and over again. Each cycle takes only 1 or 2 minutes, so PCR can produce billions of copies of a gene or DNA segment in a single afternoon, the DNA is then available for forensics, cloning, making transgenic organisms, or many other purposes.

Amplify the region between bp 20 to 80 of a 100 bp DNA



The locations of the 2 primers determine the final size of the PCR product

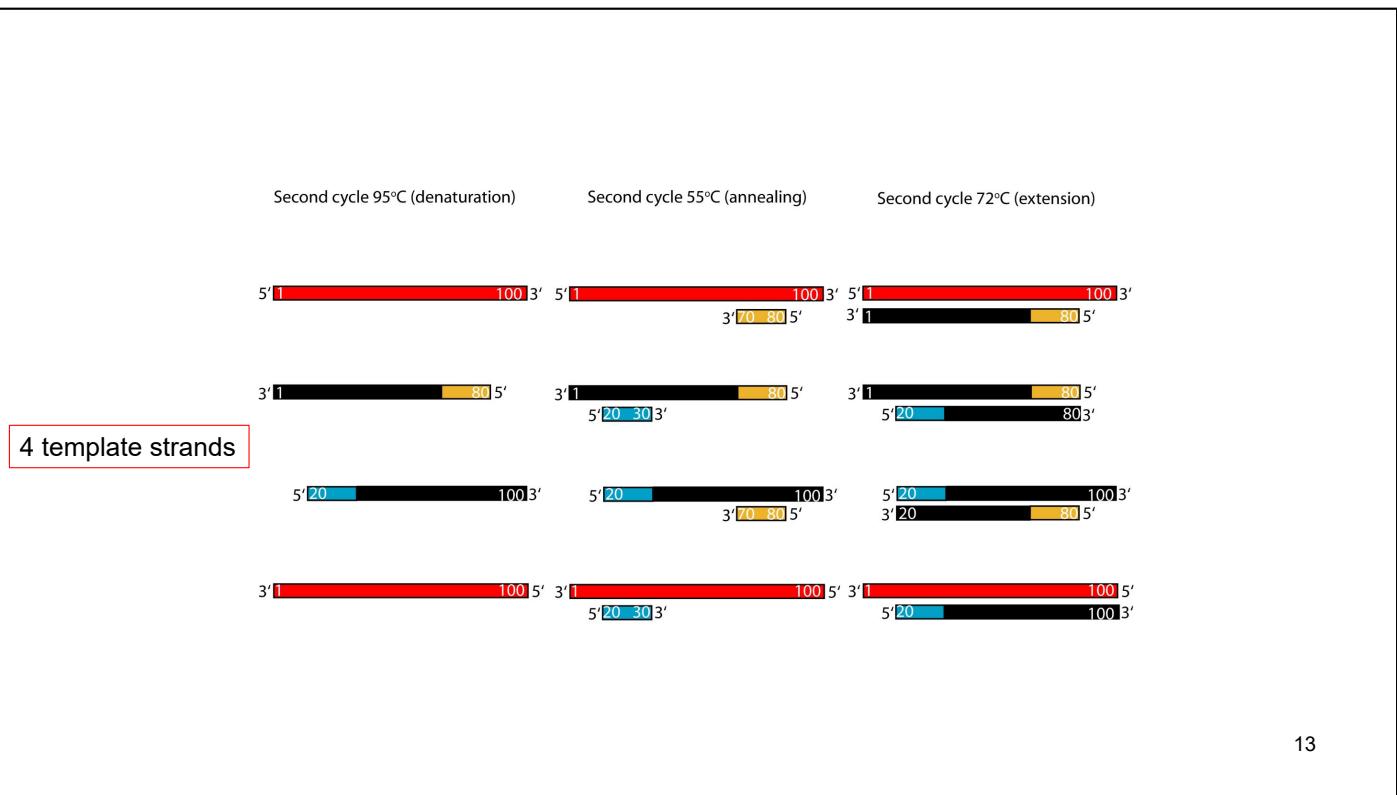


2 template strands

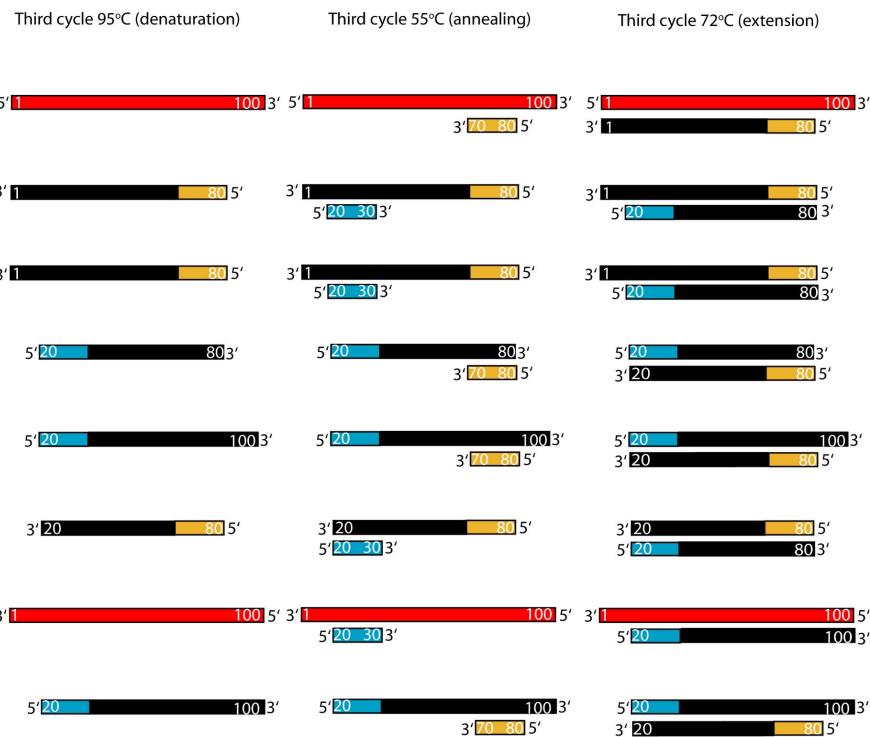


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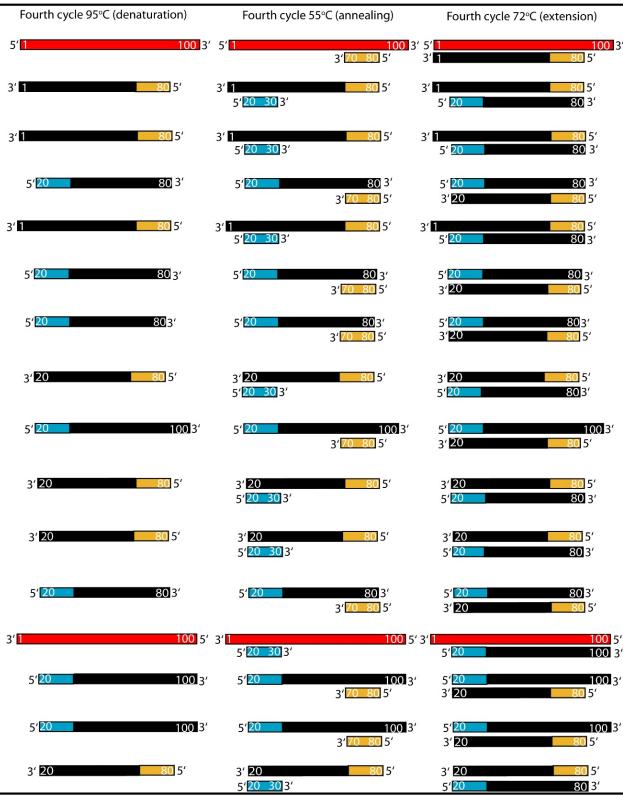


13



14

16 template strands

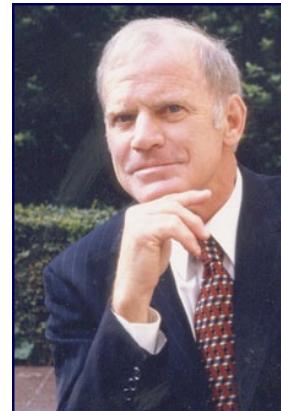


15

15

Polymerase Chain Reaction

- Invented by Kary B. Mullis in 1985
- Awarded the **Nobel Prize** in Chemistry 1993
 - “for his invention of the PCR method”
- Website
 - http://nobelprize.org/nobel_prizes/chemistry/laureates/1993/mullis-lecture.html



How can PCR detect the presence of Covid-19 virus from nose scrub?

16

This is Kary Mullis, he spent quite a number of years, stroll around with a strong interests at Chemistry.

By the time Watson and Crick were being honored here in Stockholm in 1962, I had been designing rockets with my adolescent companions for three years. For fuel, we discovered that a mixture of potassium nitrate and sugar could be very carefully melted over a charcoal stove and poured into a metal tube in a particular way with remarkable results. The tube grew larger with our successive experiments until it was about four feet long (about 1.3 meters) My mother grew more cautious and often her head would appear out of an upstairs window and she would say things that were not encouraging. The sugar was reluctantly furnished from her own kitchen, and the potassium nitrate we purchased from the local druggist.

Back then in South Carolina young boys seeking chemicals were not immediately suspect. We could even buy dynamite fuse from the hardware with no questions asked. This was good, because we were spared from early extinction on one occasion when our rocket exploded on the launch pad, by the very reliable, slowly burning dynamite fuses we could employ, coupled with our ability to run like the wind once the fuse had been lit. Our fuses were in fact much improved over those which Alfred Nobel must have used when he was

frightening his own mother. In one of our last experiments before we became so interested in the maturing young women around us that we would not think deeply about rocket fuels for another ten years, we blasted a frog a mile (1.6 Km) into the air and got him back alive. In another, we inadvertently frightened an airline pilot, who was preparing to land a DC-3 at Columbia airport. Our mistake.

For three months I did sporadic experiments while my life at home and in the lab with Jennifer was crumbling. It was slow going. Finally, I retreated from the idea of starting with human DNA, I wasn't even absolutely sure that the Genentech sequence from *Nature* that I was using was from a single exon. I settled on a target of more modest proportions, a short fragment from pBR322, a purified plasmid. The first successful experiment happened on December 16th. I remember the date. It was the birthday of Cynthia, my former wife from Kansas City, who had encouraged me to write fiction and bore us two fine sons. I had strayed from Cynthia eventually to spend two tumultuous years with Jennifer. When I was sad for any other reason, I would also grieve for Cynthia. There is a general place in your brain, I think, reserved for "melancholy of relationships past." It grows and prospers as life progresses, forcing you finally, against your grain, to listen to country music.

DNA Agarose Gel Electrophoresis

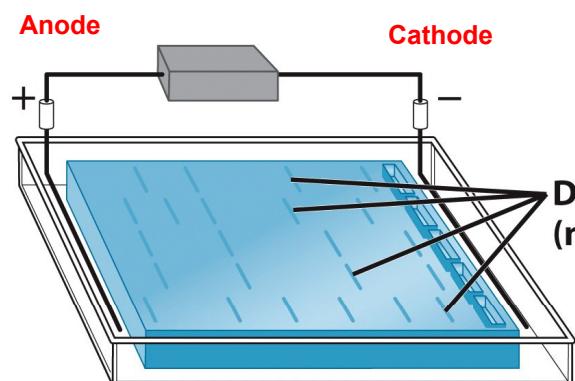
- Technique to separate out mixture of DNA and/or RNA fragments based on their lengths (number of base pairs)
- Steps
 - DNA mixtures placed at one end of gel
 - Electric current applied through gel
 - Negatively-charged DNA fragments move toward positive end of gel
 - Short DNA fragments move faster than long fragments
 - Separated bands of DNA/RNA made visible by stains or DNA probes

17

Gel electrophoresis separates DNA molecules, RNA molecules, or proteins according to their sizes, electrical charges, or other properties through a gel in an electric field. Different gel types and conditions are used for different molecules and types of applications. A common gel for separating large DNA fragments is made of agarose. When agarose is melted and moulded, it can form a gel, which is simply a meshwork of fibers with holes of various sizes between the fibers.

Some of the issues that occurred in your own gel runs were because the gels were not dried in a consistent manner, making the gel uneven, resulting in different speeds of the movement of DNA.

Gel casting



molten agarose

(Agarose) Jelly Food



Electrical current moves DNA segments through the gel. Smaller pieces of DNA move farther toward the positive electrode.

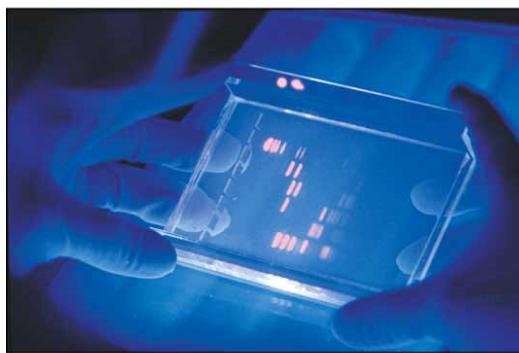
Figure 13-5b Biology: Life on Earth, 8/e
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Recall what you learnt at your practical last week.

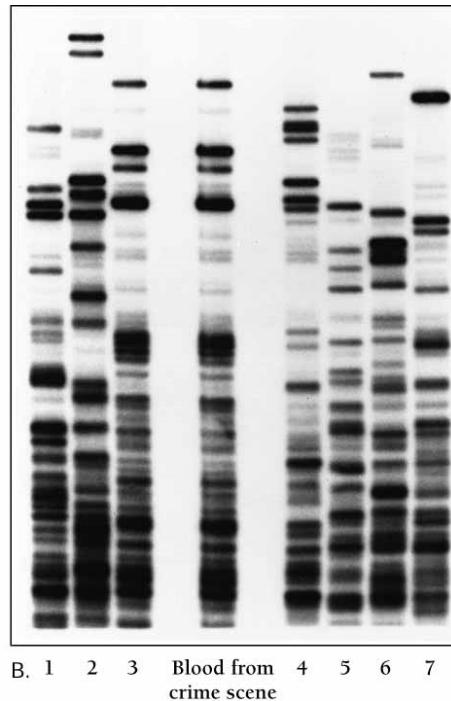
How does the gel separate pieces of DNA when current flows between the electrodes through the gel. Remember, the phosphate groups in the backbones of DNA are negatively charged. When electrical current flows through the gel, the negatively charged DNA fragments move toward the positively charged electrode. Because the smaller sizes of DNA slip through the holes in the gel more easily than larger ones, they move more rapidly toward the positively charged electrode. Eventually the DNA fragments are separated by size, forming distinct bands on the gel.

Visualizing DNA by staining

- Ethidium Bromide (EtBr)
- SYBR® Safe



A.



19

The separated DNA bands can be visualized after staining. The dye can bind to DNA molecules and fluoresce under UV light. Enabling the DNA bands to be seen and photographed. The figure on the left showing an actual gel, and the left is a photograph from an example of blood sample.

The brightness or the intensity of DNA bands are associated with DNA copy number and DNA size (i.e. the number of base pairs in the DNA molecule)

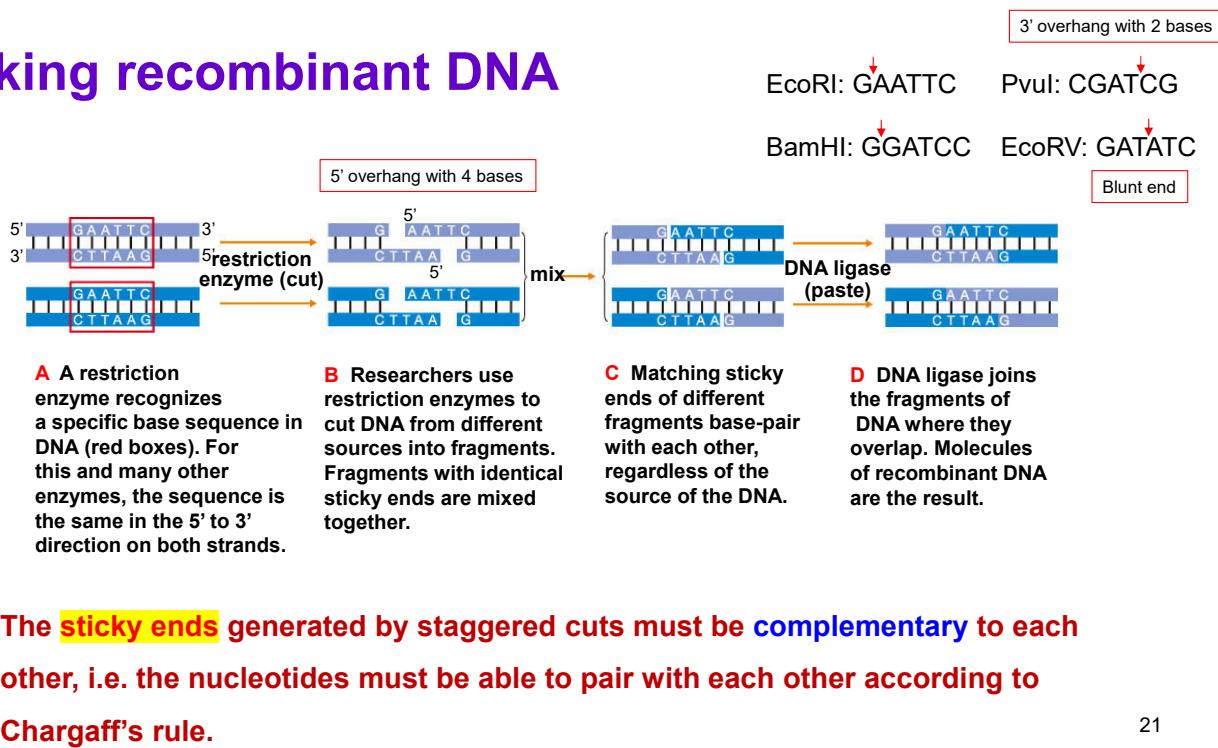
Enzymes

- **Restriction enzymes**
 - Cut DNA at **specific** nucleotide **sequences**
 - Number of cuts in DNA depends on number of times “target” sequence (**restriction site**) occurs
 - Some restriction enzymes cut straight across the double helix and producing **blunt ends**
 - Some restriction enzymes make a staggered cut, snipping the DNA in a different location on each of the two strands and producing **sticky ends**
- **DNA ligase**
 - Produce covalent bond between two DNA ends

20

A **staggered cut** generates two sticky ends, while a straight **cut** generates **blunt ends**.

Making recombinant DNA



The **sticky ends** generated by staggered cuts must be complementary to each other, i.e. the nucleotides must be able to pair with each other according to Chargaff's rule.

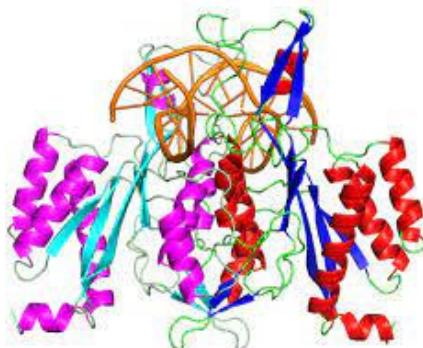
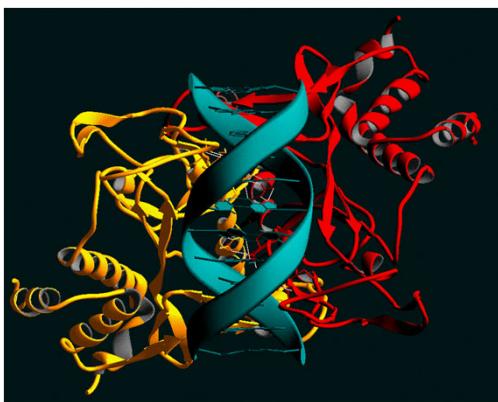
21

Figure 16.2

Making recombinant DNA.

Palindromic sequences

Restriction Endonucleas



Model image of the restriction enzyme EcoRI (red and yellow) binding to DNA (blue). The enzyme is made up of two symmetrical units (**dimer**) that correspond to the **double helix** structure of DNA.

Model image of the restriction enzyme ECO RI (red and yellow) binding to DNA (blue). The enzyme is made up of two symmetrical units that correspond to the double helix structure of DNA.

Restriction enzymes, sometimes called restriction endonucleases, are isolated from prokaryotic organisms that protect the cell from foreign DNA. Several hundred of these enzymes have been isolated from various organisms and most are available commercially. These enzymes recognize specific sequences, usually four to eight nucleotides in length. Different organisms produce enzymes with different sequence recognition sites, called restriction sites. Most organisms only recognize one specific site thus different enzymes will cut the same piece of DNA in different locations. Restriction enzymes cut the DNA strand by binding to the DNA at these specific sites and cleaving the double helix. The cut produces 3'-OH and 5'-P groups at each position. The cuts can either be straight or staggered. Straight cuts are referred to as "blunt" ends and staggered cuts are referred to as "sticky" ends. The piece of DNA that is produced from this cut is called a restriction fragment. There are three different types of restriction enzymes. They will be discussed in section 3. Some common restriction enzymes are **EcoRI**, **HindIII**, **BamHI**, and **TaqI**.

Transgenic Organisms

- **Transgenic organisms** contain foreign DNA that has been introduced using biotechnology. The terms transgenic organism and **genetically modified organism (GMO)** are generally synonymous.
- Genetic modification differs from selective breeding (“traditional biotechnology”) by
 - Genetic engineering is much more rapid
 - Genetic engineering can transfer genes between species
 - Genetic engineering can produce novel genes never seen before on Earth

23

Today, bacteria, plants, and animals are genetically modified in order to have them produce a product desired by humans. The organisms themselves are called transgenic organisms, or Genetically modified organisms (GMO) and the products are called biotechnology products.

genetic makeup has been modified in a laboratory using genetic engineering or transgenic technology.

Transgenic organisms contain foreign DNA that has been introduced using biotechnology. Foreign DNA (the **transgene**) is defined here as DNA from another species, or else recombinant DNA from the same species that has been manipulated in the laboratory then reintroduced. The terms transgenic organism and genetically modified organism (**GMO**) are generally synonymous.

Compared to traditional biotechnology , the modern biotechnology , genetic engineering is more rapid and more creative. It can bring about novel recombination and even artificial genetic products.

Genetic modification differs from selective breeding (“traditional biotechnology”)

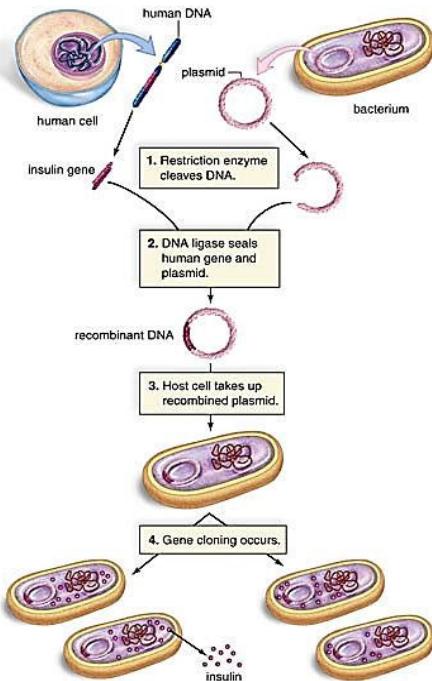
Genetic engineering is much more rapid

Genetic engineering can transfer genes between species

Genetic engineering can produce new genes never seen before on Earth

Transgenic Bacteria

- Used to produce wide range of recombinant DNA products
 - Human insulin
 - Human growth hormone
 - Interferon
 - Vaccines

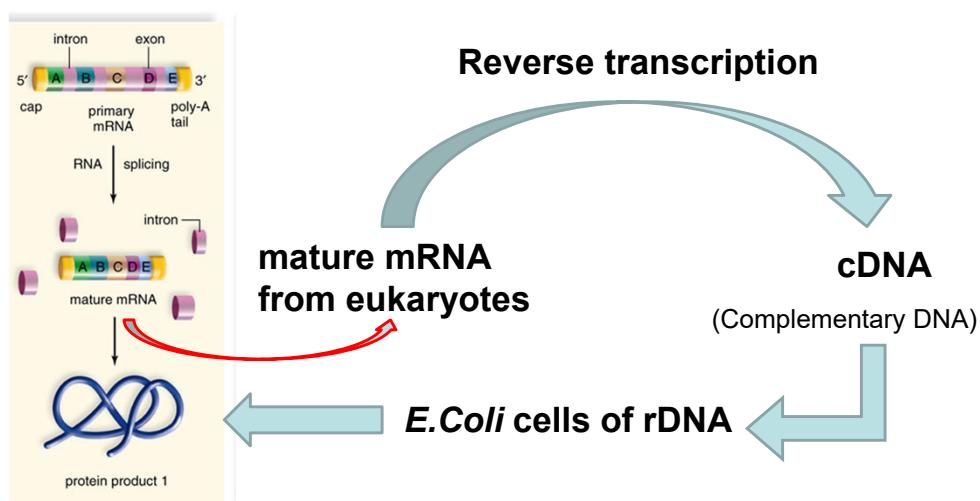


24

Recombinant DNA technology is used to produce transgenic bacteria, which are grown in huge vats called bioreactors. The gene product is collected from the medium. Biotechnology products now on the market that are produced by bacteria include insulin, human growth hormone, and many types of vaccine.

Thinking

What must you consider when you want to get a functional human protein from *E. coli*?



25

Use mature mRNA to make the complementary DNA so there is no intron or exon

Think about it.

Complementary DNA (cDNA)

Intron and exon

Post-translation modification

Protein folding

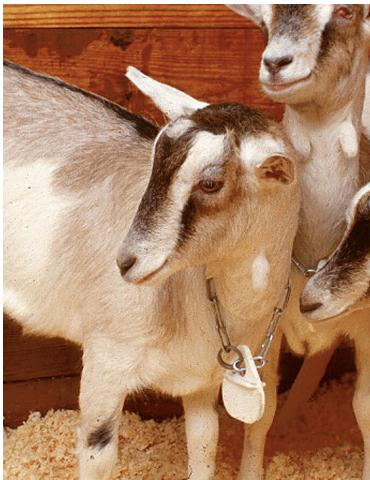
Transgenic Animals

- New DNA **inserted** into **fertilised egg**
 - During development, all cells in body will have the new gene
- **Inefficient** process
 - Success rate of about 1%
- Requires normal expression
 - Must **not disrupt** other essential genes
 - Must be expressed at right cells and right time

26

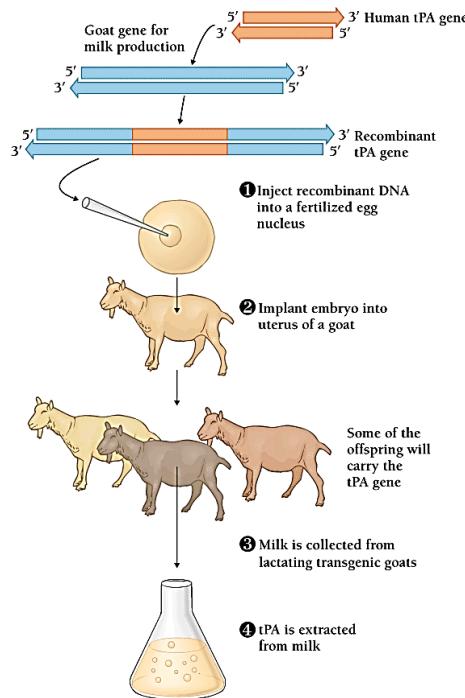
Techniques have been developed to insert genes into the eggs of animals. There are many ways to delivery foreign genes into eggs, such as microinjection and electroporation. When these eggs are fertilized, the resulting offspring are transgenic animals.

Gene pharming



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tPA is Involved in the breakdown
of blood clots



Tissue plasminogen activator (tPA)

27

Tissue plasminogen activator (abbreviated **tPA** or **PLAT**) is a [protein](#) involved in the breakdown of blood [clots](#). Specifically, it is a [serine protease](#) ([EC 3.4.21.68](#)) found on [endothelial cells](#), the cells that line the [blood vessels](#). As an [enzyme](#), it [catalyzes](#) the conversion of [plasminogen](#) to [plasmin](#), the major enzyme responsible for clot breakdown. Because it works on the [clotting system](#), tPA is used in [clinical medicine](#) to treat only embolic or thrombolytic [stroke](#). Use is contraindicated in hemorrhagic stroke and head trauma.

tPA may be manufactured using [recombinant biotechnology](#) techniques. tPA created this way may be referred to as **recombinant tissue plasminogen activator** or **rtPA**.

Another example is to use goat to produce milk containing proteins of spider fibers, which can be processed into fibers. These fibers are extremely light and can be used to make lightweight bulletproof vest. This goat is called spider goat.

Scientists breed goats that produce spider silk



Goats that produce **spider silk protein in their milk** could enable researchers to collect large quantities of the silk proteins for a variety of applications. For instance, due to its strength and elasticity, spider silk fiber could be used for making **artificial ligaments and tendons**, for eye sutures, and for jaw repair. The silk could also have applications in bulletproof vests and improved car airbags.

Read more at: <http://phys.org/news194539934.html#jCp>

28

Normally, getting enough **spider silk** for these applications requires large numbers of spiders. However, spiders tend to be territorial, so when the researchers tried to set up spider farms, the spiders killed each other.

To solve this problem, Randy Lewis, a professor of **molecular biology** at the University of Wyoming, and other researchers decided to put the spiders' dragline silk gene into goats in such a way that the goats would only make the **protein** in their milk. Like any other genetic factor, only a certain percentage of the goats end up with the gene. For instance, of seven goat kids born in February 2010, three have tested positive for having the silk protein gene. When these transgenic goats have kids and start lactating, the researchers will collect the milk and purify the spider silk protein into "much, much higher quantities," Lewis said.

Other than their ability to produce the **spider** silk protein, the goats do not seem to have any other differences in health, appearance, or behavior compared to goats without the gene, the researchers said.

In the future, the scientists plan to incorporate the **silk genes** into alfalfa plants, which they say could produce even larger quantities of silk. They explain that not only is alfalfa widely distributed, it also has a high (20-25%) protein content, making it an ideal crop to produce silk protein.

Read more at: <http://phys.org/news194539934.html#jCp>

<http://www03.usu.edu/sbi/single-blog-spider.html>

Bullet proof shirt

Transgenic Animals

- First transgenic animals in 1974
 - Mouse with a virus gene
- Today
 - Pigs, goats, sheep, monkeys, etc
 - Even fishes (Salmon with growth hormone)

GM salmon inventor



AquAdvantage Salmon is genetically engineered for rapid growth with two stretches of foreign DNA, a growth hormone gene with an antifreeze protein promoter.

GloFish: pollution monitors

**Small Fish Detect Big Problems
Environmental Scientists Use Fish Behavior To Monitor Water Quality**

Gene for **fluorescence protein** inserted downstream of stress response gene.

GloFish acts like a biosensor to detect water pollution.



Fish glowing fluorescent under stress

30

http://www.sciencedaily.com/videos/2007/0303-small_fish_detect_big_problems.htm

Not just used as Ornamental fish

Transgenic Plants

- Easier to modify plants than animals
- Ti (Tumour-inducing) plasmid
 - From plant-infecting bacterium *Agrobacterium tumefaciens*
- When *A. tumefaciens* infects plant cells
 - Ti plasmid is inserted into plant chromosome in nucleus
- Researchers replace plant tumour-causing genes with beneficial genes (e.g. insect resistance)
 - Genes therefore transferred into plant chromosome when *A. tumefaciens* with modified Ti plasmid infects plant cells

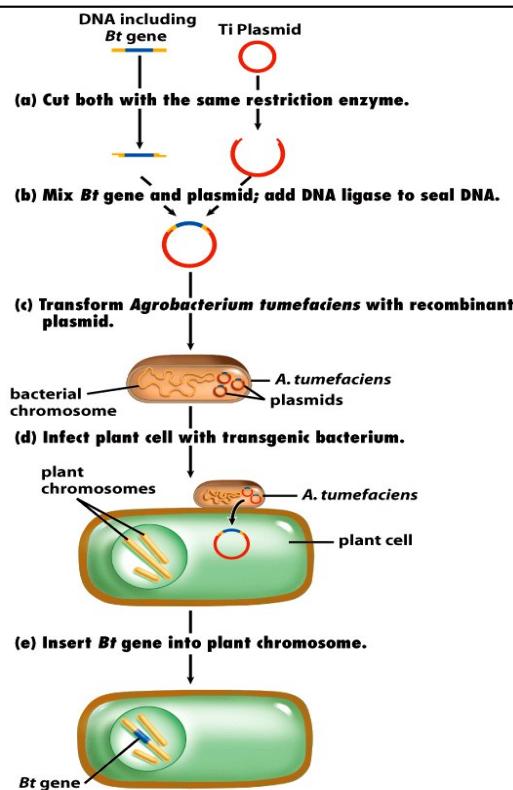
31

We can also develop transgenic plants. For example, using Ti plasmid, which can induce cell division and form a tumour, to deliver a target gene into a plant.

Many Crops Are Genetically Modified



Bt gene (from *Bacillus thuringiensis* bacterium) can be inserted into plants to produce insect-killing protein in crops



32

Roses are red,
Violets are blue, (it may not be true.)
Sugar is sweet,
And so are you

Table 13-1 Genetically Engineered Crops with USDA Approval

Genetically Engineered Trait	Potential Advantage	Examples of Bioengineered Crops with USDA Approval
Resistance to herbicide	Application of herbicide kills weeds but not crop plants, producing higher crop yields	Beet, canola, corn, cotton, flax, potato, rice, soybean, tomato
Resistance to pests	Crop plants suffer less damage from insects, producing higher crop yields	Corn, cotton, potato, rice, soybean
Resistance to disease	Plants are less prone to infection by viruses, bacteria, or fungi, producing higher crop yields	Papaya, potato, squash
Sterile	Transgenic plants cannot cross with wild varieties, making them safer for the environment and more economically productive for the seed companies that produce them	Chicory, corn
Altered oil content	Oils can be made healthier for human consumption or can be made similar to more expensive oils (such as palm or coconut)	Canola, soybean
Altered ripening	Fruits can be more easily shipped with less damage, producing higher returns for the farmer	Tomato

In 2008, about 80% of the corn, 86% of the cotton, and 92% of the soybeans grown in the U.S. were transgenic; that is, they contained the genes from other species

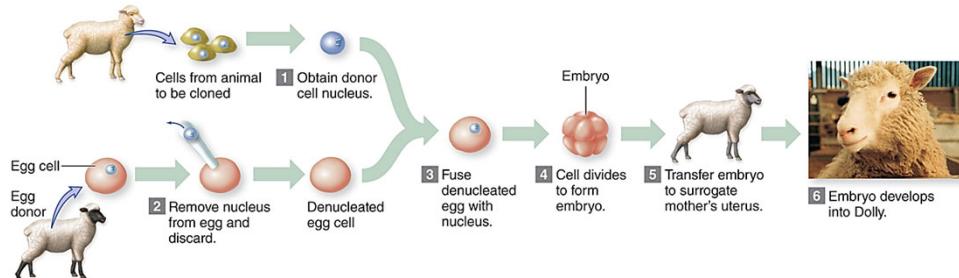
33

Beancurd

Genetic engineering is used both in food crops and in medicine. Golden Rice and almost all the corn and soybeans grown in the United States contain genes from other species. The hepatitis B vaccine is produced by inserting a gene from the hepatitis virus into yeast. The antibodies in ZMapp, currently in clinical trials as an Ebola therapy, are part mouse and part human. Are there scientifically important differences in the use of genetic engineering for food or for medical purposes? Would you accept GMO products for medicine but not food?

Supplementary Information

Cloning of an Organism



Human cloning could help infertile parents have children and could be used to harvest embryonic stem cells. But ethical issues surround cloning of our own species.

Cloning of an Organism (video)

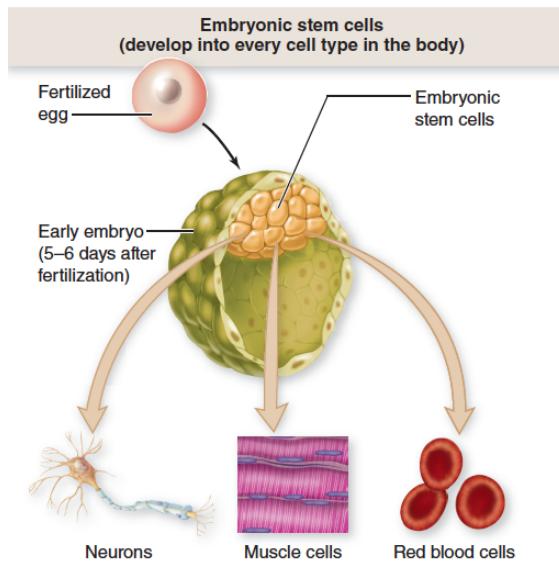


Play video (3.57 mins)

Stem Cells Divide into Multiple Cell Types

Embryonic stem cells give rise to **all** cell types in the body.

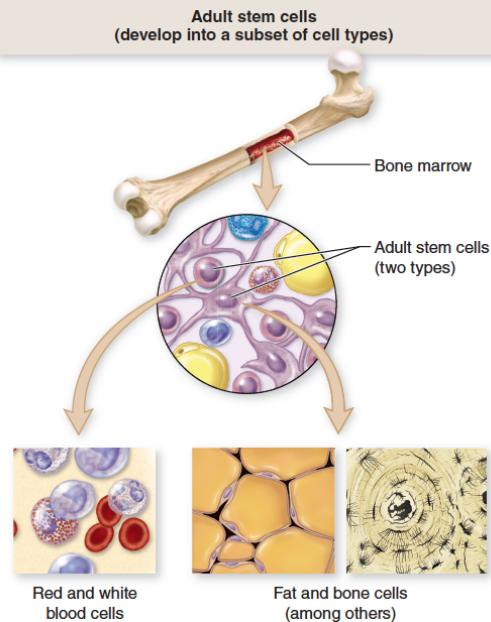
- Replace beta-pancreatic cell in diabetes patient to produce insulin?
- Generate neurons and transplant into patients with Parkinson's disease?



Supplementary Information

Stem Cells Divide into Multiple Cell Types

Adult stem cells differentiate into a **limited number** of cell types. Stem cells in bone marrow, for example, differentiate into all blood cell types.



37

DNA Fingerprinting/Forensic Science

- Forensic scientists have found that small, repeating segments of DNA, called **short tandem repeats (STRs)**, can be used to identify people
- **STRs**
 - STR is short (consisting of 2 to 5 nucleotides), repeated (as many as 5 to 50 times), and tandem
 - STR **does not code** for proteins
 - Many different STRs in genome
 - **Vary greatly** between different people, i.e. each person carries unique combination of **STRs like genetic fingerprints**
- The U.S. Department of Justice established a standard set of 13 STRs, each four nucleotides long (but 5 to 38 repeats), to identify individuals by DNA samples

38

Satellite DNA consists of highly repetitive DNA,[1] and is so called because repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine, and thus have a different density from bulk DNA - such that they form a second or 'satellite' band when genomic DNA is separated on a density gradient.

How would a forensics laboratory know which primers to use? After years of painstaking work, forensics experts have found that small, repeating segments of DNA, called STRs (short tandem repeats) can be used to identify people with astonishing accuracy. Now, it is found that the STR can also be used in other organisms to identify different strains.

What does a DNA profile tell us? As with any gene, every person has two alleles of each STR (see Chapter 11). The two alleles of a given STR might have the same number of repeats (the person would be homozygous for that STR) or a different number of repeats (the person would be heterozygous).

DNA Fingerprinting

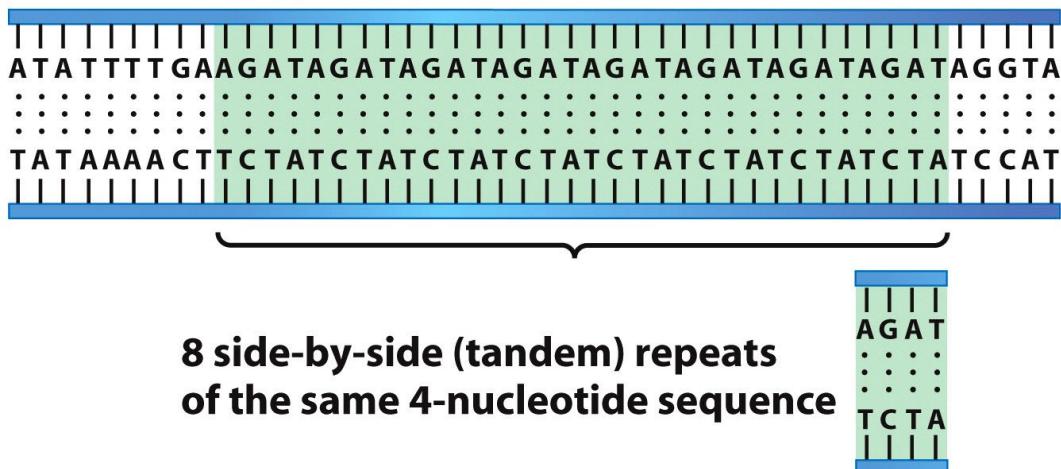
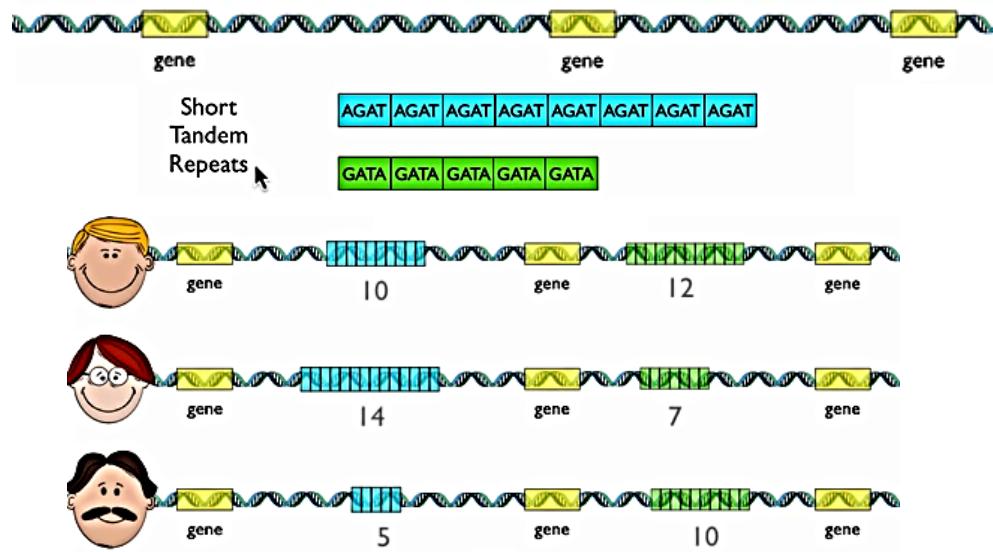


Figure 13-4 Biology: Life on Earth, 8/e
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39

Each STR is short, repeated and tandem. As with any gene, different people may have different patterns of the STRs. A perfect match of 10 STRs in a suspect's DNA and the DNA found at a crime scene means that there is less than one chance in a trillion that the two DNA samples did not come from the same person. What's more, the DNA around STRs doesn't seem to degrade very fast, so even old DNA samples, such as those in the Ruffin case, usually have STR

Non-Coding Regions and STRs



40

Mrs Blonde,

Mrs Red

Mr Moustache

DNA Fingerprinting

- DNA from sample (e.g. crime scene)
 - First amplified by polymerase chain reaction
 - Separated by gel electrophoresis
 - STRs in gel identified by DNA probes/staining
- Match of 13 different STRs between suspect's and crime scene DNA virtually proves that suspect was at crime scene

41

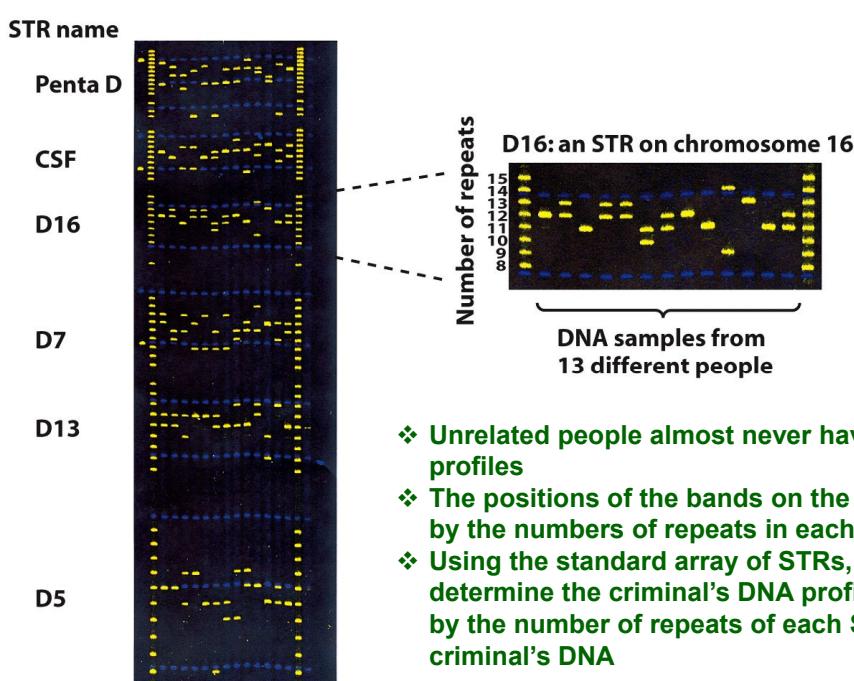


Figure 13-7 Biology: Life on Earth, 8/e
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42

- ❖ Unrelated people almost never have identical DNA profiles
- ❖ The positions of the bands on the gel are determined by the numbers of repeats in each STR
- ❖ Using the standard array of STRs, technicians determine the criminal's DNA profile, which is coded by the number of repeats of each STR found in the criminal's DNA

The lengths of short tandem repeats of DNA form characteristic patterns on a gel. This gel displays four different STRs (Penta D, CSF, D16, and D7). The columns of evenly spaced yellow bands on the far left and far right sides of the gel show the number of repeats in the different STR alleles. DNA samples from 13 different people were run between these standards, resulting

in one or two yellow bands in each vertical lane. The position of each band corresponds to the

number of repeats in that STR allele (more repeats means more nucleotides, so the allele is larger).

However, the odds that anyone who would be a likely suspect in a criminal case being misidentified

are extremely low. Finally, a mismatch in DNA profiles is absolute proof that two samples did not come from the same source.

Link to Life

Daily
Life



43

The king of pop

The star wasn't the father of her two children, Prince Michael and Paris.

S (ChattahBox) – An exclusive by [US Magazine](#) has found out a shocking truth: Michael Jackson was not the father of two of his children.

The father, it is being reported, was Jackson's doctor, Arnold Klein, who was the boss of Michael's ex-wife, Debbie Rowe.

"He and Debbie signed an agreement saying they would never reveal the truth," an anonymous insider was quoted as saying.

It is yet another layer in a story surrounding the late singer's life that has proved to be an unbelievably complicated one.

It isn't yet known how this news, if proven correct, will affect the coming custody issues that are sure to surface. In the meantime, Michael's mother has been granted custody of his three little ones.

Prince Jackson has seemingly admitted that [Michael Jackson](#) may not be his father.

The eldest son of the late King of Pop - who, along with his siblings, have been subject to rumours about his parentage - hit back at a tweeter who claimed he wasn't a Jackson, by making it clear that no matter anything else, MJ was his dad.

Answering a tweet which said: "Everyone knows you are a Rowe-Klein NOT a Jackson!! Yall should stop trying to put on a FAKE front and get real."

Who is the child's father?

- A. Male 1
 - B. Male 2



Kinship Testing

44

http://www.hsa.gov.sg/publish/hsaportal/en/applied_sciences/forensic_science.html

Kinship Testing



Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

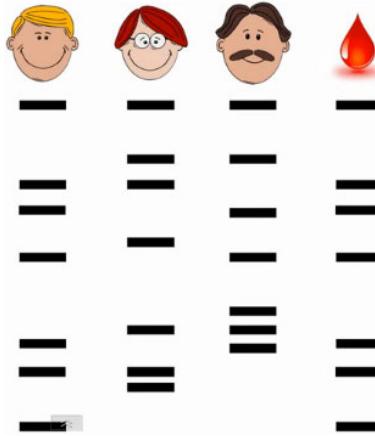
More info at polleverywhere.com/support

Who is the child's father?

https://www.polleverywhere.com/multiple_choice_polls/JoFgpasfwXyAKORqR4W7P?state=opened&flow=Default&onscreen=persist

Who is the criminal?

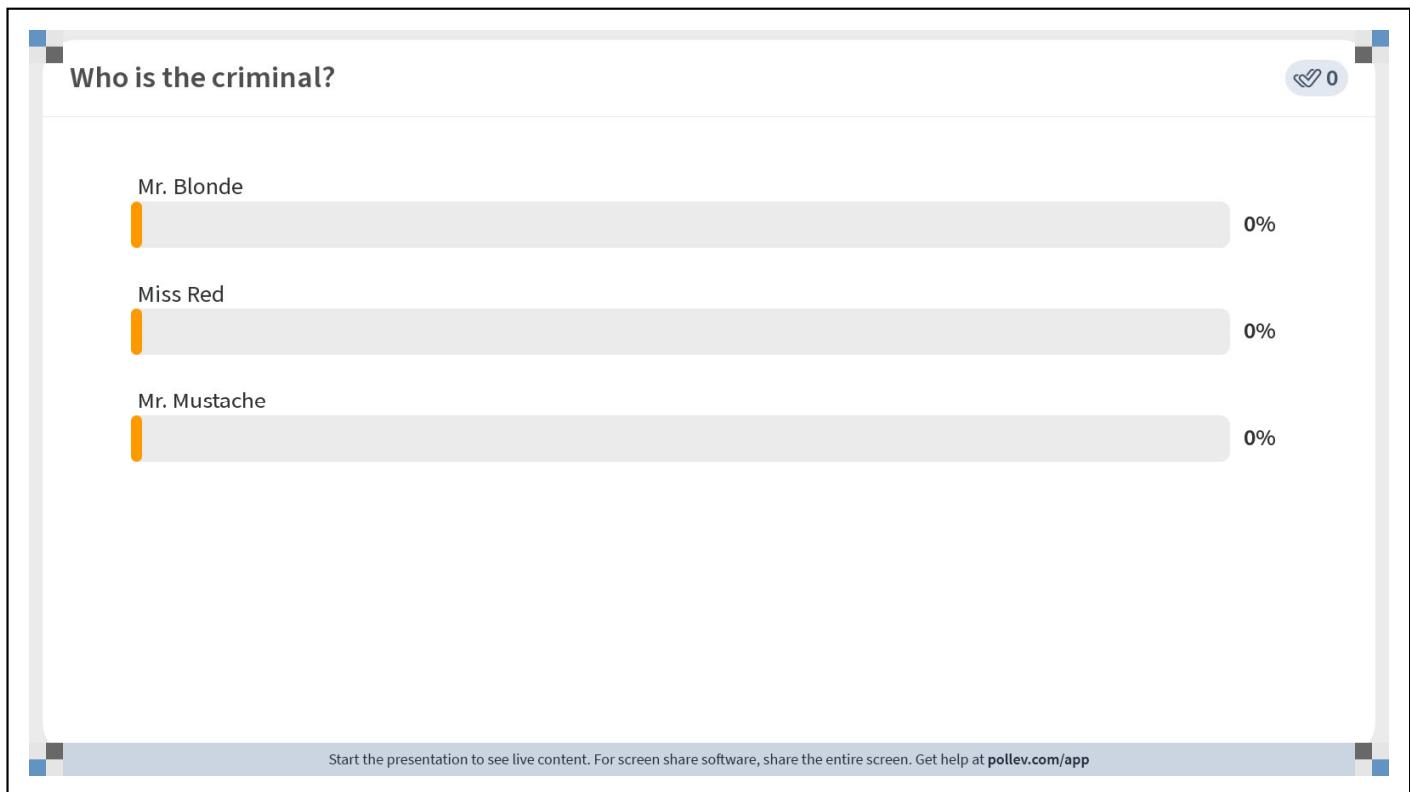
A. Mr. Blonde



B. Miss Red

C. Mr. Mustache

Mr blonde is correct



Poll Title: Do not modify the notes in this section to avoid tampering with the Poll Everywhere activity.

More info at polleverywhere.com/support

Who is the criminal?

https://www.polleverywhere.com/multiple_choice_polls/5iWhdHRzIwXSYuCTnd5v?state=opened&flow=Default&onscreen=persist

DNA Barcoding

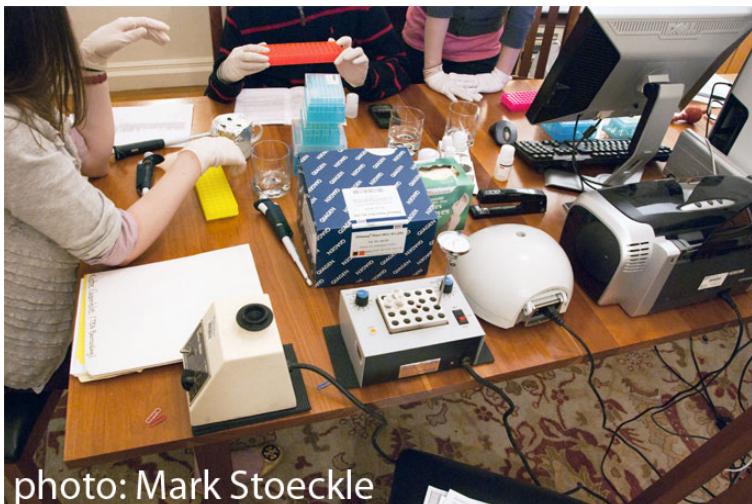


photo: Mark Stoeckle



48

DNA barcoding has been used on bluefin tuna, big-eye tuna, yellowfin tuna, red snapper, Japanese yellowtail, and salmon.

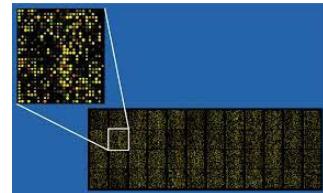
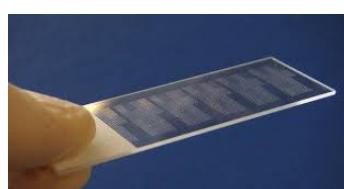
Kate and Louisa visited restaurants and grocery stores and brought home samples of raw fish. They cut off little pieces from each sample, preserved them in alcohol, and sent them off to a lab at the University of Guelph in Canada for barcoding. Surprise! About a quarter of the sushi samples were imposters. And no surprise—the “mistakes” almost always labeled a cheap, readily available fish as a more expensive species. One specimen sold as red snapper was actually Acadian redfish, an endangered species. One “tuna” sushi turned out to be tilapia, a freshwater species often raised in fish farms. Some restaurants had mislabeled half their sushi.

DNA barcoding can also help to stop illegal trafficking in endangered species, which is extremely lucrative (thought to be second only to illegal narcotics).

Identifying the species of origin of meat, skin, feathers, and many other animal parts is often difficult, even for experts, but DNA barcoding can't be fooled. The day may come when barcoding not only verifies your sushi but also puts a stop to the exploitation of endangered species

DNA Chips/Genetic Screening

- Contains up to **thousands** of DNA fragments arranged on a glass plate or slide
- Also known as **DNA microarray**
 - To analyse **genetic profile** of individuals
 - To study **expression** of thousands of **genes** at one time
 - To screen for genetic **abnormalities, pathogens, or cancer**



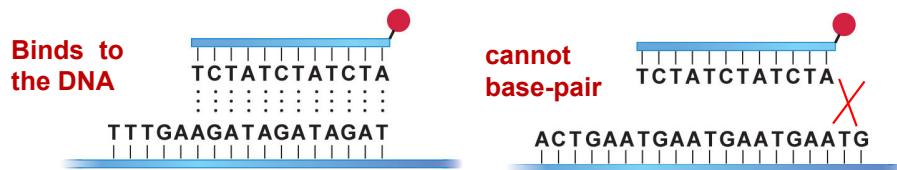
49

DNA chip, also known as DNA microarray, is a high-throughput analysis platform. An expert or company can arrange all-disease-related DNAs onto a small glass plate or paper, a person's DNA is cut into small pieces, separated into single strands, and labelled. The array is then bathed in the resulting solution of labelled DNA fragments. **Under the right conditions, only a perfect complementary strand of the person's DNA will bind to any given spot of DNA on the array;** Even a single "wrong" base will keep the person's DNA from binding. If one's DNA binds to any disease-related DNAs,

DNA Chips for diagnosis

Steps of analysis

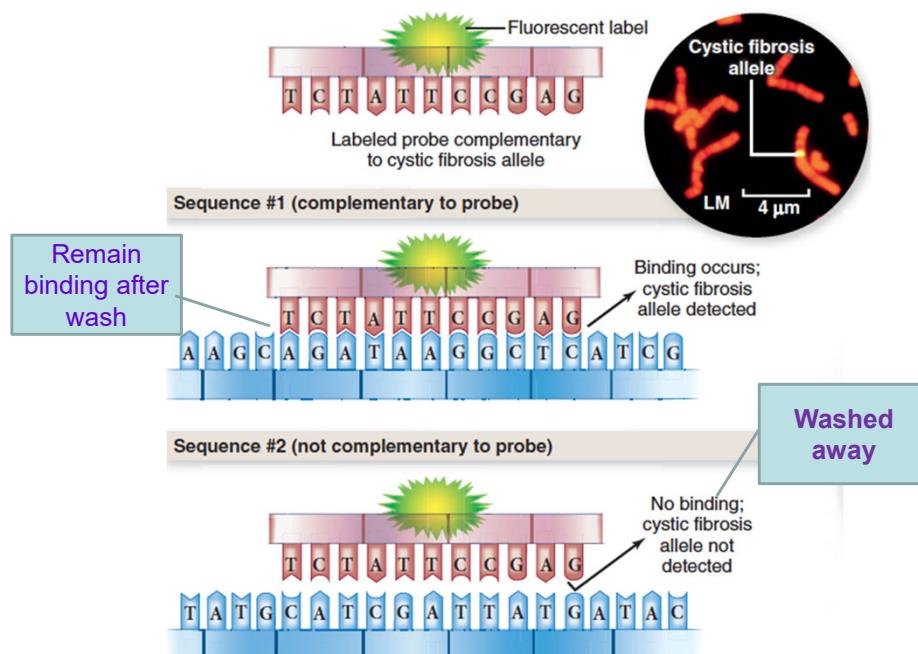
- 1) Isolate DNA from cells
- 2) Amplify by polymerase chain reaction
- 3) Cut into fragments
- 4) Tag with fluorescent dye
- 5) Apply tagged fragments to DNA chip
- 6) Detect the fluorescent signal



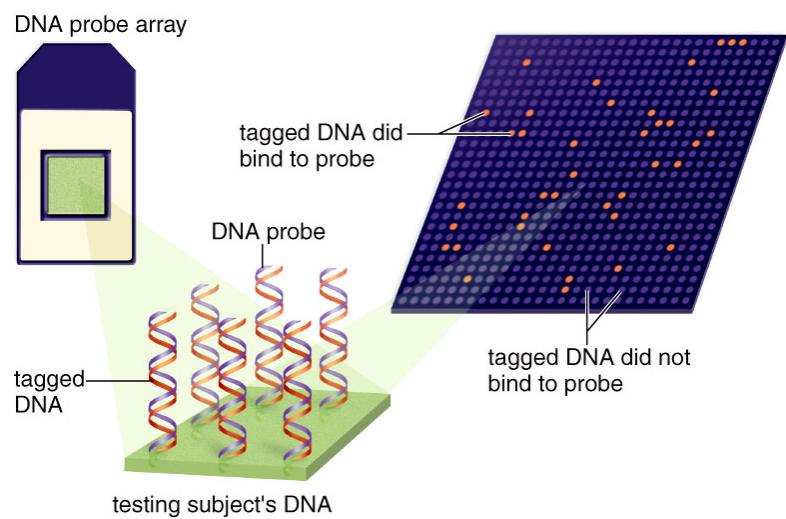
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Red ball is fluorescent dye

Detect Existing Disease



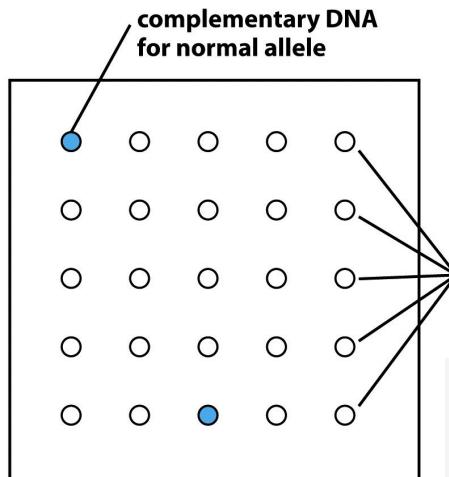
51



DNA microarray technique

52

Such as figure showing you here.

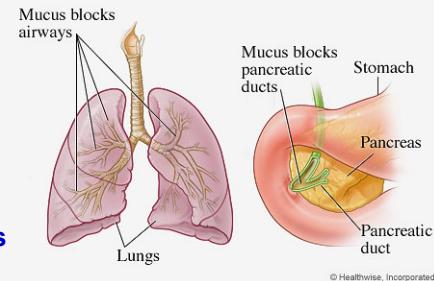


A cystic fibrosis diagnostic array.

affects the exocrine (mucus) glands

Microarray for Disease Screen

rows of complementary DNA segments for various mutant alleles



© Healthwise, Incorporated

53

This shows you an example of DNA arrays in diagnosis of cystic fibrosis, a hereditary disease.

Cystic fibrosis (also known as **CF**, **mucoviscidosis**, or **mucoviscidosis**) is a [hereditary disease](#) that affects the exocrine (mucus) glands of the lungs, liver, pancreas, and intestines, causing progressive disability due to multisystem failure.

Thick mucus production, as well as a less competent [immune system](#), results in frequent [lung infections](#). Diminished secretion of pancreatic enzymes is the main cause of [poor growth](#), [fatty diarrhea](#) and deficiency in fat-soluble vitamins. Males can be [infertile](#) due to the condition [congenital bilateral absence of the vas deferens](#). Often, symptoms of CF appear in infancy and childhood. [Meconium ileus](#) is a typical finding in newborn babies with CF.

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Singapore scientists develop chip that can identify 70,000 viruses



Photo: ST

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Monday, Apr 15, 2013

The Straits Times

By Cheng Jingjie

Photo above: Dr Christopher Wong (left) and collaborator Martin Hibberd with the PathChip, which can identify a pathogen for \$450 in the lab.

SINGAPORE - Scientists here have developed a new wonder chip that can identify 70,000 different viruses and bacteria in one go.

Currently, a typical test can detect fewer than 50 pathogens, and each kit tends to be specific to certain groups.

54

Prenatal Genetic Screening

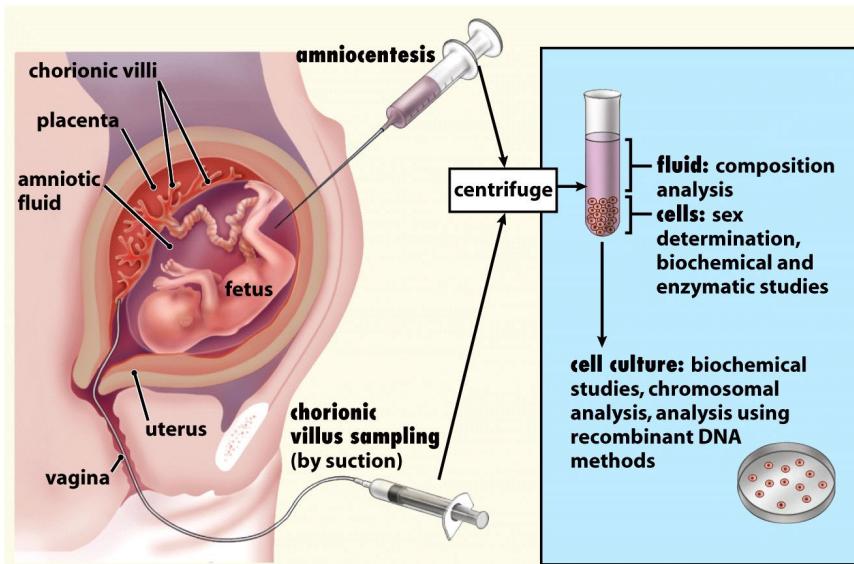


Figure E13-5 Biology: Life on Earth, 8/e
© 2008 Pearson Prentice Hall, Inc.

Down Syndrome is due to
The presence of 3 copies
of chromosome 21
(Trisomy 21)

55

Amniocentesis

The human fetus, like all animal embryos, develops in a watery environment. A waterproof membrane called the amnion surrounds the fetus and holds the fluid. As the fetus develops, it releases various chemicals (often in its urine) and sheds some of its cells into the amniotic fluid. When a fetus is 15 weeks or older, amniotic fluid can be collected by a procedure called amniocentesis.

First, the physician determines the position of the fetus by ultrasound scanning. High-frequency sound is broadcast into a pregnant woman's abdomen, and sophisticated instruments convert the echoes bouncing off the fetus into a real-time image (FIG. E14-3). Using the ultrasound image as a guide, the Physician carefully inserts a sterilized needle through the abdominal wall, the uterus, and the amnion (being sure to avoid the fetus and placenta), and withdraws 10 to 20 milliliters of amniotic fluid (FIG. E14-4). Amniocentesis

carries a slight risk of miscarriage, about 0.5% or less.

Thinking

The world's most precious genes?



<http://www.ebi.ac.uk/fgpt/gwas/#timeseriestab>

<https://www.23andme.com/>

56

now your enemy, we tell ourselves; knowledge is power. Laurie Hunter wanted to know what disease was attacking her daughter Amanda, who by the age of 2 months was not developing normally. Her muscle tone was low. She wasn't lifting her head. She was slow to talk, and she didn't walk until she was 2.

"As a mother, you know that everything that happens to your child is not your fault, yet you still feel responsible," says Hunter, 42, a high school English teacher who lives in Jackson, N.J. "We turned to genetic testing because I wanted answers." The first tests,...

Gene Therapy

An approach to treat disease by either modifying the expressions of an individual's genes or correction of abnormal genes. One of potential approaches is

Targeted gene delivery using viral vector

Watch the above video on Gene Transfer using viral vector

57

By administration of DNA rather than a drug, many different diseases are currently being investigated as candidates for gene therapy. These include cystic fibrosis, cardiovascular disease, infectious diseases such as AIDS and cancer.

Defective, faulty genes.

Transient gene expression (TGE) in mammalian cells is defined as the production of a recombinant protein. (r-protein) over a short time period (1–14 days). Sometimes, techniques can be used that introduce a gene into a cell but do not result in a stable genetic transformation. Such events can result in foreign gene expression for a short period of time.

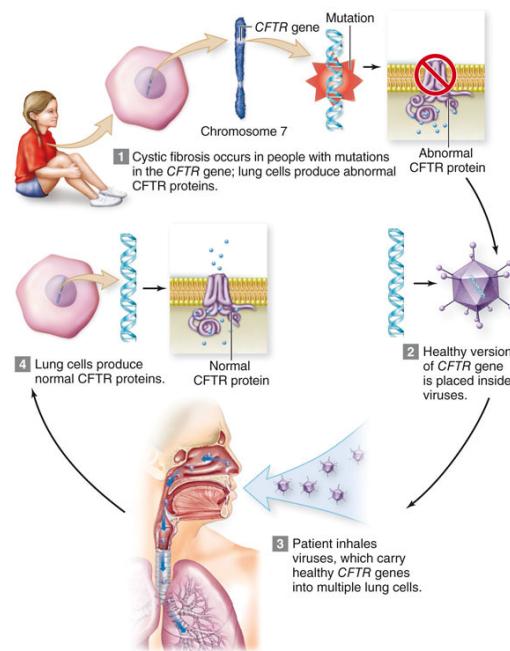
In biology and genetics, the **germline** of a mature or developing individual is the line (sequence) of germ cells that have genetic material that may be passed to a child.

For example, gametes such as the sperm or the egg are part of the germline. So are the cells that divide to produce the gametes, called gametocytes, the cells that produce those, called gametogonia, and all the way back to the zygote, the cell from which the individual developed.

Cells that are not in the germline are called somatic cells. This refers to all of the cells of body apart from the gametes. If there is a mutation or other genetic change in the germline, it can potentially be passed to offspring, but a change in a somatic cell will not be.[1]

Gene Therapy Replaces Faulty Genes

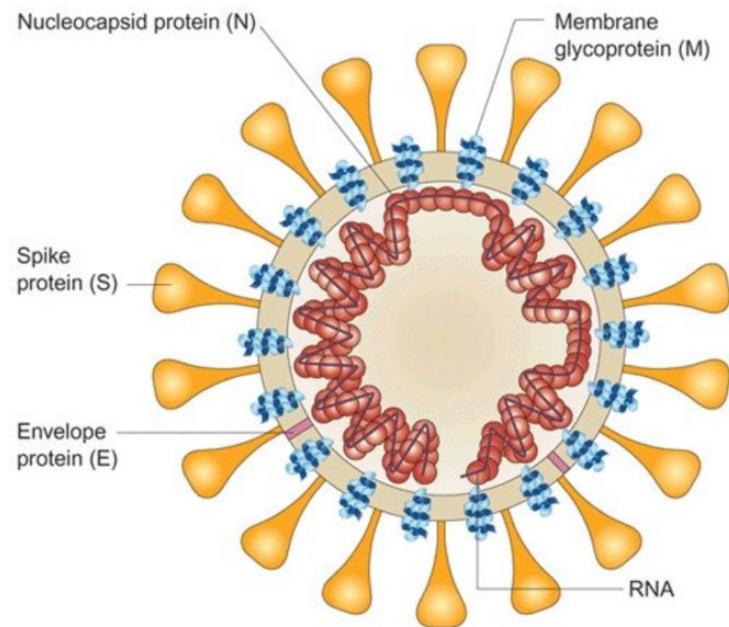
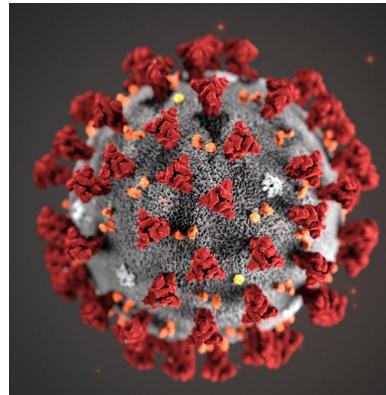
Gene therapy may someday provide new treatment options for genetic diseases by replacing a faulty gene in a person's cells.



58

Cystic fibrosis transmembrane conductance regulator (**CFTR**) is a membrane protein and chloride channel in vertebrates that is encoded by the **CFTR** gene

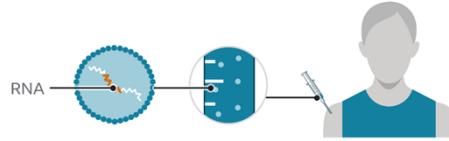
COVID-19
SARS-CoV-2



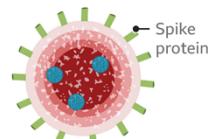
59

How an RNA vaccine works

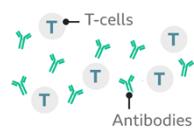
- 1 Scientists take part of the virus's genetic code and turn it into a vaccine that is injected into the patient



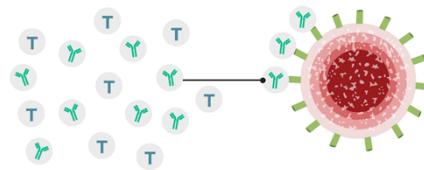
- 2 The vaccine enters the cells and tells them to produce the coronavirus spike protein



The body's immune system reacts, produces antibodies and activates T-cells to destroy cells with the spike protein



- 3 If the patient later catches coronavirus, the antibodies and T-cells are triggered to fight the virus



Source: Nature

BBC

60

Now you know how it works you can explain to your relatives and tell them to get the vaccine if they haven't already done so!

In what instance will this vaccine not work?

Safety and Ethics

Thinking

- **Safety issues**

- Could ingestion of Bt protein in insect-resistant plants be dangerous to humans?
- Are transgenic fish producing extra growth hormone dangerous to eat?
- Could GM crops cause allergic reactions?

- **Legal issues**

- Who has access to my genetic information?
- Should animals be modified to provide organs for human transplants?

- **Environmental issues**

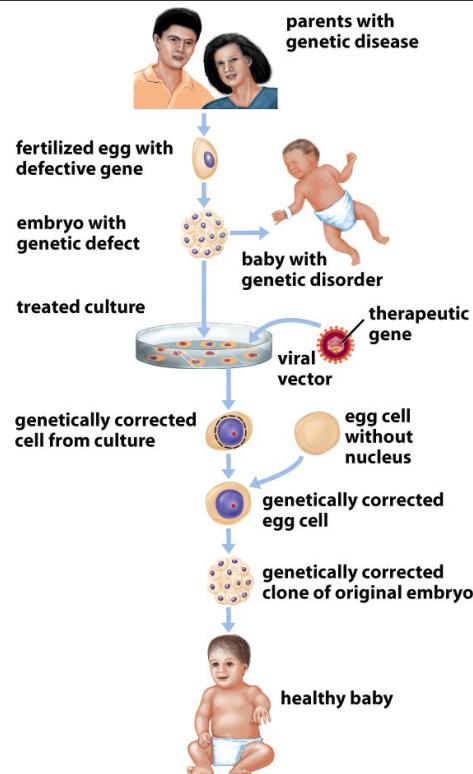
- Could herbicide resistance genes be transferred to weed species creating superweeds?
- Could GM fish reduce biodiversity in wild population if they escape?
 - Reduced diversity in wild makes population of fish more susceptible to catastrophic disease outbreaks

62

What will we do when we do what we can do now?

Ethical issues

- Should parents be given information about the genetic health of an unborn foetus?
 - Should parents be allowed to design or correct the genomes of their offspring?
- **Who decides what should be “corrected”?**
 - Who gets well and who gets enhanced?
- Should humans be cloned?



63

Should parents be allowed to design or correct the genomes of their offspring?

Should parents be allowed to select the genomes of their offspring?

Embryos from *in vitro* fertilization are currently tested before implantation

Many unused embryos are discarded

Child is not for the sake of pleasure as a commodity, like a car, you could choose its color, decor, tyre, and other accessories.

How can we differentiate an improvement from a treatment?



When do we need human gene engineering?

(Modify genetic material of an organism, usually using recombinant DNA technology)

Case 1



Will you select genetic engineering to treat a gene defects in your baby?

Case 2

Pentagon's DARPA (Defense Advanced Research Projects Agency)



Genetically engineered super blood to boost your baby's physical strength?

64

We wanted to create high-value red cells that do more than simply carry oxygen. Here we've laid out the technology to make mouse and human red blood cells in culture that can express what we want and potentially be used for therapeutic or diagnostic purposes,"

Key Terms and Key Concepts

Key Terms

Plasmid, Restriction enzymes, recombinant DNA, STR, genetically modified organism (GMO),

Key Concepts

PCR and DNA agarose gel electrophoresis

How is recombinant DNA made?

How do scientists make GMO?

What are the limitation and ethical issues of GMO?

How is STR used for identification in forensic science?

65

'Term' is lexical: the name you attach to a thing.

'Concept' is cognitive: the idea you have about a thing.

'Notion' is perceptual: the feeling of an idea (or name) you have for a thing.

A term is concrete. It is something you can write down on a piece of paper.

A concept is an idea, a plan of action, but it isn't set in stone yet, and can be changed.

A concept is a non tangible thing, often an idea. For example, love is a concept. A term is another word used for something else. For example, *Felis concolor* is the scientific term used for puma

Please complete the quiz on Canvas for lecture 7

Reminder!

Try quizzes before the on-line tutorial

Review: Chemistry of Life, Cell structure,
Energy of life, DNA & heredity, Gene
expression, Biotechnology; Summary and
Make Connections

**Wed (18th Sep): 8am to 10am
(prepare your questions !)**