Virtual Biology Textbook

(Compiled by H Geckil)

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Life Cycle Diagrams

The contents of these notes are based on Hypertext which provided the basic molecular biology that was a foundation of
MIT's core Biology courses, "7.01x Introductory Biology" (7.012, 7.013 or 7.014).

Virtual Biology Textbook

These pages were retrieved from MIT website in mid 2000s. Now they are no longer posted because the author, Dr. Vernon Ingram, died in 2006 and no longer works for MIT.

Please cite the original author if you use information contained in these documents. For more information you can visit the former MIT virtual biology site and see Dr. Ingram's obituary

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There are a great many useful Biology resources available on the Internet, including:
MIT's OpenCourseWare (see specifically the 7.01x classes in the Biology section.)
Wikipedia on Biology (try links in the introduction such as Microbiology, Cellular Biology, and Molecular Biology). The Online Biology Book, hosted by Estrella Mountain Community College in Arizona, and written and maintained by Michael J. Farabee, PhD.
An online search will net you many more resources, as well.

Introduction - 1

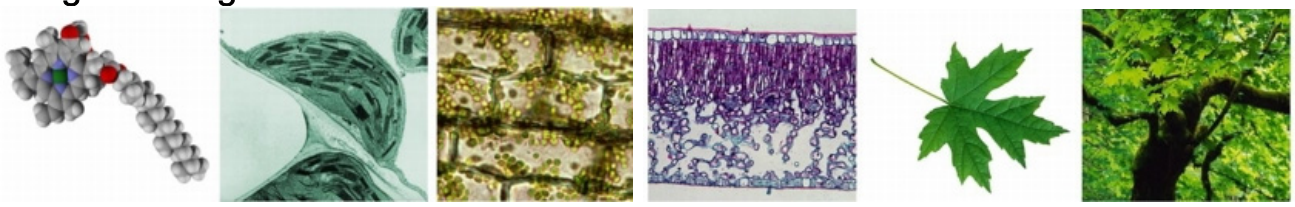
Biology is the subject of life and living organisms. And perhaps, most importantly today, how we humans interact with and impact the lives of each other and the other organisms that share our earth. By studying the living organisms with which humans share this earth, biologists try to answer questions about diversity and about the common characteristics of living organisms. We try, in science to make "sense" of all we see in our world around us.

Living Organisms are virtually everywhere on earth, and are found in all sizes, shapes and colors. From bacteria to aspen groves, blue whales and California redwood trees, there is a remarkable array of living organisms to catalog (or classify) and observe on earth.

All of us have some understanding of what it is to be alive and what non-living stuff is. However, coming up with a good definition of life is not so easy. There are a number of things we can state which are characteristics of living organisms, the sum of which can be of help to us in distinguishing life from non-life:

Although both living and non-living things share the same fundamental properties of matter and energy (which we shall look at) living organisms and non-living materials differ in the degree to which energy is used and materials are organized. To help us determine how life and non-life can be distinguished we can study some of the following common "features" of living organisms:

Biological Organization



Molecule Organelle Cells Tissues Organ Organism

Living organisms have an **organized** structure, with each level of biological structure building on the level below it. Atoms form molecules that are organized to form cell components, called organelles. Organisms may be unicellular or multicellular. Multicellular organisms have structural levels above the cell: tissues, organs and organ systems.

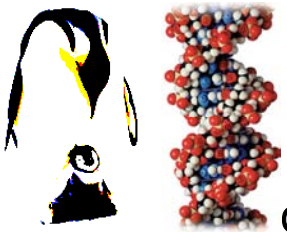
Groups of organisms form populations and groups of different populations (or species) living in the same geographical area form communities and ecosystems. This is our life on earth.

Response to Stimuli and Homeostasis

Organisms constantly sense changes in their surroundings and make controlled responses to those changes. Organisms have specialized **receptors** that detect environmental **stimuli**, and their cells adjust metabolism in response to signals from receptors. This constant monitoring and interaction between cells and their environment is called **homeostasis**.

Energy

All organisms require energy input to maintain the processes of life. Living organisms must have the capacity to obtain and convert energy from their surroundings to grow and maintain themselves. In biology this is known as **metabolism**.



Growth and Reproduction

All living organisms have a **common molecular inheritance** based on the nucleic acid, DNA. DNA contain the instructions for the structure and function of cells, the common structural component of living organisms. DNA guides growth, development and maintenance of tissues and organs of multicellular organisms. DNA instructions are passed from generation to generation (inherited) by the process of reproduction.

Interdependence of Life – Change through Time

Just as the cells of multicellular organisms are dependent upon each other for the survival of the organism, life on earth involves an interdependence of energy and nutrients in ecological processes. Much of biology focuses on the linking of life processes:

- The dependence of life processes on each other
- The interaction of organisms with their environment
- The changes that occur in groups of organisms through time
- The mechanisms of evolution as a foundation for change

While looking for the unity of life processes, we recognize the great diversity of appearance and behavior of species on this earth, as well. Species differ greatly in their adaptations to the many distinct environments on earth. Both the unity and diversity of organisms can be explained by the mechanisms of evolution.

Diversity of Life

For thousands of years humans have categorized living organisms into groups sharing some kind of common features. In the 1700's, Linnaeus proposed a hierarchical scheme, which we continue to follow. For some time, biologists grouped organisms into general groups, called **Kingdoms**, based on broad general features (which are not so easy to see all of the time). Recently, biologists added a new category above Kingdom, called **Domain**. Your textbook uses Domains in its classification of living organisms. There are three Domains:

- Domain Archaea
- Domain Eubacteria
- Domain Eukarya
    - Kingdom Plantae
    - Kingdom Animalia
    - Kingdom Fungi
    - Kingdom Protista

We will discuss a bit about the cellular differences and distinctions are the basis of classification when we discuss cell structure and function.

At times during Biology 160, we will have reason to look a little more closely at the characteristics of these domains and kingdoms, and for those who go on to study diversity in other courses, you'll have the opportunity for greater observations. Unfortunately, we do not have time in Biology 160 to study the wonderful diversity of life on earth in any detail. Biology 162, and Biology 212 and 213 have diversity sections.

Evolution as the Guiding theme of Biology

Evolution is the core of biology. Life has the capacity to change genetically from generation to generation – to evolve. The processes of evolution are fundamental to life on earth. The natural genetic variation found among members of a species provides for the capacity of organisms to respond to changing environmental conditions from generation to generation. Those who have characteristics, or **adaptations**, more favorable in the environment will reproduce more offspring that those with less successful characteristics.

Camouflage Adaptations

These statements, first presented by Charles Darwin and Albert Wallace in the mid-1800s are fundamental to all that we do in biology and our understanding of life.

Diversity and unity are the two faces of life on earth. Biologists have described more than 1.5 million different kinds of organisms, including more than 750,000 insects alone. There are 260,000 plants and 50,000 vertebrates (the animals with which we are most comfortable). There may be millions of additional organisms not yet described by humans

We shall spend some time this term looking at the mechanisms of evolution, as well as seeing the results of evolution as we study the structure and functioning of cells.

How Biologists Ask Questions

Before we leave our introduction, we need to mention how biologists look at the world around them. Each of us is curious about any number of things. Often when we are curious we ask questions to try and find out whatever it is that we are curious about.

Biologists try and find answers to their questions about living things by using the scientific method of problem solving, or some variant of this method, to study the processes of life.

Scientific Principles

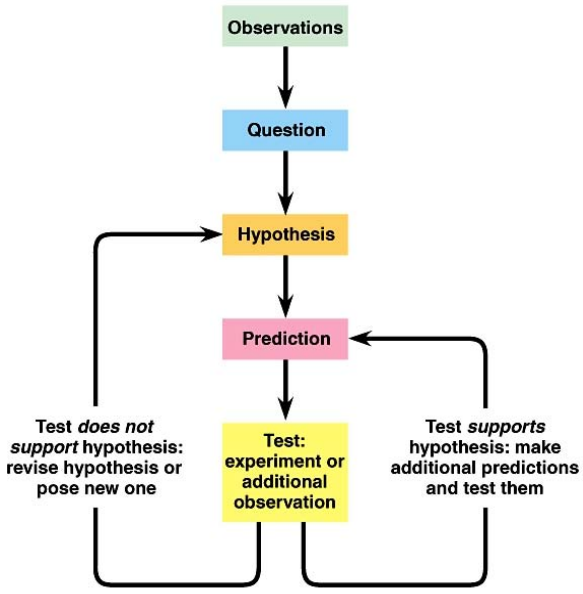
A Scientific Principle is an idea supported by repeated experiments and observations. The assumptions behind which scientific principles are based have been thoroughly tested and found valid over many years.

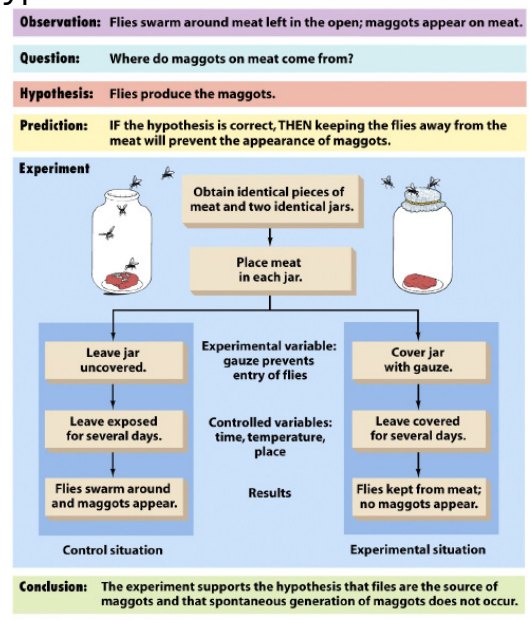
How the Scientific Process Works:

Find something about which you are curious.

- Make observations and ask questions based on your observations to produce a "model" or preliminary explanation for your question.
- Based on your observations and model, make a **testable** hypothesis (reasoned guess) by using the information available to make a general statement (called the hypothesis).
- Predict what will happen if the hypothesis is correct
- Test the hypothesis by models, experiments and/or observations. When possible, science uses **controlled** experiments.

  Science also uses **comparative data**, looking for patterns in nature that are consistent with predictions. Data may be **quantitative**, such as measurements, or **qualitative**, in the form of descriptions of phenomena observed.
- Repeat tests to see if results are consistent with the hypothesis.
- Objectively note results and draw conclusions. Conclusions may support the hypothesis or not support the hypothesis. The purpose of scientific inquiry is to find answers, but not to find only those answers that support the original idea. Examine alternative hypotheses in the same manner.





Scientists work in as many different ways as there are scientists; but all share a critical attitude that requires being shown, not being told, and use logic in their thinking. Conclusions must support evidence and observations.

Science is limited to questions that can be tested. Experimental design is important. When possible, science uses controlled studies, in which the **control** group is a standard for comparison with the **experimental** group. The **variables** of the experiment are aspects, events or objects that may differ or change over time. When testing a hypothesis, scientists are as prepared to find the hypothesis false as they are for validating the hypothesis.

Tested and supported hypotheses in science are known as theories. In this sense, theory is not the same as in some fields where theory means a speculation. A science theory has tested evidence that supports and lacks evidence that disproves it. Other fields may look at issues and ideas that are untestable. These ideas are not appropriate for science.

This term, in Biology 160 we will look at some of these life processes. Chapter One of your text reviews many of the ideas I've mentioned here. Read this chapter with thought. Much of what is written there may help you think more deeply and with greater understanding of what we are to do in Biology 160 as well as in subsequent biology courses you will take.

As indicated in our course introduction, much of Biology 101 emphasizes the study of cells, which structurally and functionally are an aggregate of atoms and molecules (chemicals) working together, and which require the **energy** of these chemicals to stay alive and to function.

Atoms and molecules combine in various ways (to be discussed) to form the structures of the cells and tissues of which living organisms are composed, and provide the energy to sustain these cells and tissues.

In our first unit of lectures we will discuss the basic structure of atoms and molecules to help us understand the biological concepts that will follow in this course.

Atoms

The atom is the fundamental unit of matter. And matter is any substance of the universe (gas, liquid, solid, plasma). More simply, matter is stuff or anything that has mass and occupies space. Living organisms are composed of matter. Matter can be changed from one form to another in a chemical reaction, a process in which different forms of matter combine or break apart.

There are 92 naturally occurring atoms on earth. We have about 108 total different kinds of atoms, because humans have been able to make atoms through nuclear reactions.

Each type of atom is composed of hundreds of smaller, subatomic particles, three of which we shall discuss. The proportion of these subatomic particles in any given atom identifies the kind of atom.

An **element** is a substance composed exclusively of one kind of atom. An element is a pure chemical that cannot be separated into or converted into a simpler substance. An atom is the smallest portion of an element that retains the properties of the element.

Each element (atom) has a name and a one- or two-letter abbreviation. The Periodic Table of Elements shows these, along with other useful information about each element. (See Appendix D of your Biology 101 Handbook.)

Although we have 92 different elements (formed from the 92 different kinds of atoms, there are just a few elements from which we are organized, and even fewer that are abundant in living organisms. We shall get acquainted with some of these.

The Arrangement and Properties of Atoms

Atoms are composed of hundreds of smaller **subatomic particles.** Fortunately, to understand Biology 101, we need only look at three of these basic particles, which are located in two regions of the atom: the **nucleus** and the **surrounding electron orbitals.** The "force" that holds these particles "together" forming the atom is an electrical charge (positive and negative) between subatomic particles.

The nucleus of an atom contains two sub-atomic particles: **protons** and **neutrons**. A third type of particle, **electrons,** are found in orbitals in motion surrounding the nucleus of the atom.

Let's look a little at the structure of the atom.

The Nucleus

- The central portion of the atom
- Contains two types of subatomic particles
- Contains the bulk of the atom's mass, but very little of the space of an atom

Particles in the Nucleus

Proton

- Positively charged particle ($^{+}$)
- Number of protons in the nucleus for a particular element is always constant (and fixed)
- The number of protons ranges from 1 ---> 108 from which the **atomic number** is assigned.
- An element is defined by its number of protons.
- Each proton has a relative mass of "1" (For biology purposes, this is fine. It's actually 1 dalton, which is a relative measure of the mass of the hydrogen atom).

Neutron

- No electrical charge
- Mass of "1" (just a tiny bit heavier than the proton; not enough for us to be concerned about).
- About the same number of neutrons as protons/atom, although the ratio changes with larger atoms...
- The number of neutrons plus the number of protons gives the **atomic mass** (or atomic weight) of an atom.

Isotopes

- While a typical element has a fixed number of protons and neutrons; many elements have forms with a number of neutrons, which differs from the typical number. These are called **isotopes**

e.g. Carbon (6p, 6n)
Carbon (6p, 7n)
Carbon (6p, 8n)

- Isotopes will have the same chemical properties.
- Since mass varies, some slight physical property differences occur.

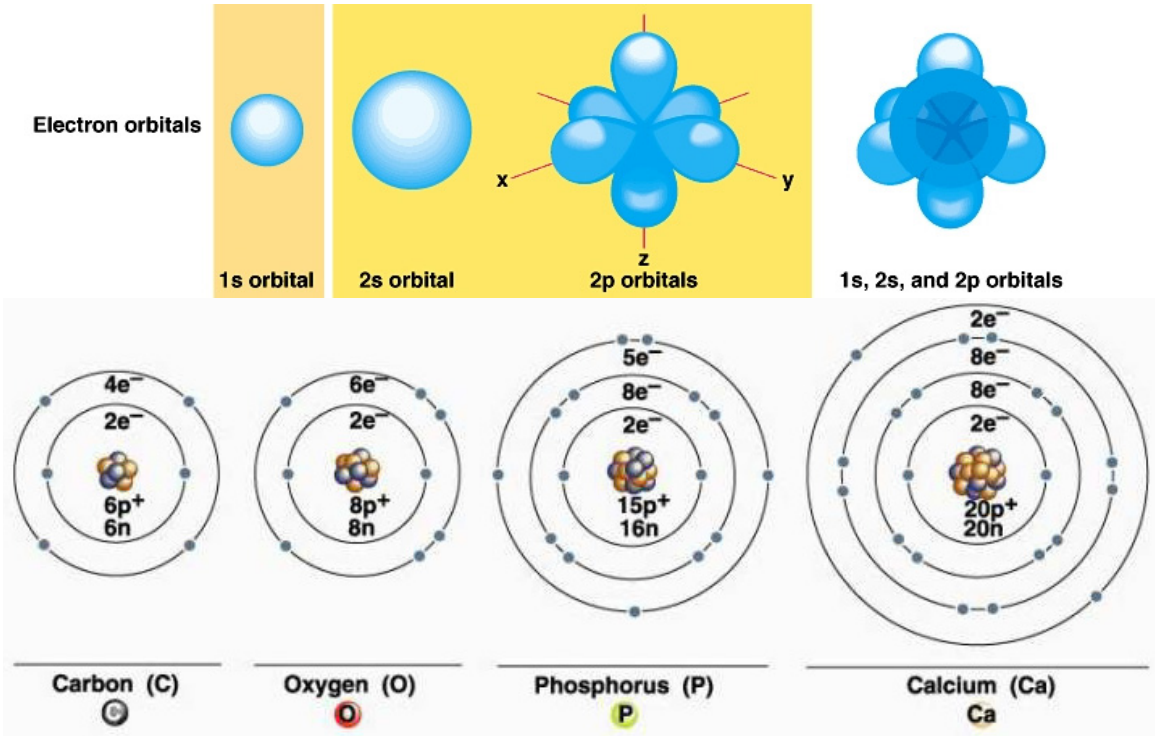
Unstable Isotopes

- Some isotopes have unstable nuclei, where one or more of the neutrons slowly decay, releasing energy in the form of beta particles. Eventually these neutrons decay into a proton (and electron, released as the beta particle).
- Such isotopes are called **radioactive isotopes** and the energy released is radiation.
  - The rate of decay varies for different isotopes.
  - Radioactive isotopes are important in biological research.
  - The rate of decay is measured by the half-life, the amount of time needed for 1/2 of the radioactive isotope to decay.

Electron Properties

The chemical properties of an atom are determined by the arrangement of its **electrons**.

- Negatively charged particle ($^-$)
- Very small mass, (1/2000 the mass of a proton) so small that we can think of an electron as having essentially no mass
- The number of electrons in a pure atom is the same as the number of protons
- Electrons are always in motion, found in **orbitals** located at fixed distances outside of the nucleus called **electron shells**, that correspond to different **energy levels**.
- Each electron orbital holds a maximum of 2 electrons. Each energy level or electron shell has a fixed number of orbitals, and each orbital has a precise pattern. For example, the first electron shell, closest to the nucleus, has one orbital, and it has a spherical pattern.
- The **orbital** of an electron is the most probable location where the electrons might be found. (Orbitals are very important in biology.)
- Electrons can absorb or release energy and when doing so, may change energy levels. This characteristic of electrons is the way we obtain energy for life.



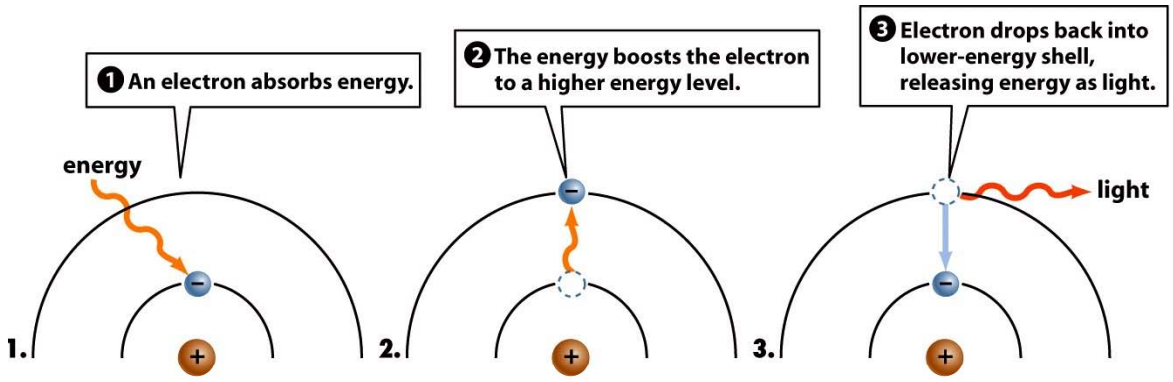
As we look at a hypothetical atom, the electron fill pattern would be:

Shell	orbital(s)	# Electrons
1st	$(1)^2$ one	2
2nd	$(2)^2$ four	8
3rd	$(3)^2$ nine	18

Energy and Electrons

An electron in a given orbital has a characteristic energy. Each electron orbital has a different energy, so that electrons in different shells have different amounts of energy. The **energy level** and motion of electrons has much to do with the stability and reactivity of a given atom.

With reference to the energy of electrons, one might think of electron orbitals and shells like stairs. Electrons can be raised to a higher energy level with additional energy (climbing upstairs), and can release energy when (if) they fall to a lower energy level (going down the steps).



Electron Orbitals and the Stability of Atoms

Generally there are two rules for finding electrons in orbitals

1. Each electron orbital holds 2 electrons
2. Electrons occupy orbitals with the lowest energy level possible

Again, there are a variety of possible energy levels (shells) for the electrons of an atom to occur in, and within each energy level, a set number of **electron orbitals**. The energy levels and orbital patterns are specific characteristics of each type of atom, and are best left to chemistry classes for detailed discussion. But we can have a little lesson.

Electrons fill lower energy level shells first, and then progress to higher shells.

Interactions Between Atoms

The nucleus of the atom tends to provide stability, while electron shells permit interactions between atoms, called bonds. Nuclei of atoms are not affected by normal energy sources, whereas electrons are dynamic; bonds form when electrons from one atom are gained, lost or shared with other atoms. Such interactions are called chemical bonds.

When two or more atoms join together in a chemical bond they form a **molecule**. Chemical bonds are interactions that occur between the outermost energy level electrons of different atoms, details to follow. When molecules are formed from different atoms, they form a **compound**.

As mentioned, different electron orbitals have patterns that are unique and identified by shape. Further, an atom is most stable when its electron shells have **pairs of electrons** in each of the orbitals, and when the orbitals of its outermost energy level are filled.

Atoms that naturally have filled outer energy level orbitals are **non-reactive** and are found in nature as pure elements. (The noble gases were named that way because they were always found in the pure element state. It is not coincidence that the noble gases have filled outer energy levels.)

Most of the atoms naturally have numbers of electrons that do **not** result in filled outer energy level orbitals. These atoms are not stable and tend to undergo chemical reactions, or bonding, with other atoms to form **molecules** or **compounds**, which are more stable. Atoms that have similar electron configurations have similar properties and undergo similar chemical bonding. (The periodic table is organized according to these similar properties.)

By the way, there are some nice "rules" which help determine how an atom will bond. One of these is the **octet** rule. When the outer energy level of an atom has a total of 8 electrons it is especially stable, so most bonds take place to obtain eight electrons in the outer energy level.

Chemical Bonding

When an atom lacks a stable number of electrons In its outer energy level, it will share or transfer electrons to or from other atoms in very precise ways to achieve a stable number of electrons (again, generally 8) in its outer shell. This is the subject of **chemical bonding.** Note that a chemical bond is an energy relationship, involving electrons, and the energy that each electron has.

The energy all living organisms need to sustain life comes from making and breaking chemical bonds. It's vital that we understand how this works!

Types of Bonds

There are two types of common or strong bonds that occur between atoms:

Ionic bonds

One or more electrons are transferred from one atom to a second atom.

When an atom gains or loses one or more electrons, it becomes a charged atom, or an **ion**, because its number of electrons relative to the number of protons has changed.

Covalent bonds

Electron(s) of one atom are shared with electron(s) of a second atom.

Atoms that form covalent bonds do not carry a charge.

Let's looks a little at these two types of bonds.

Ionic Bonds

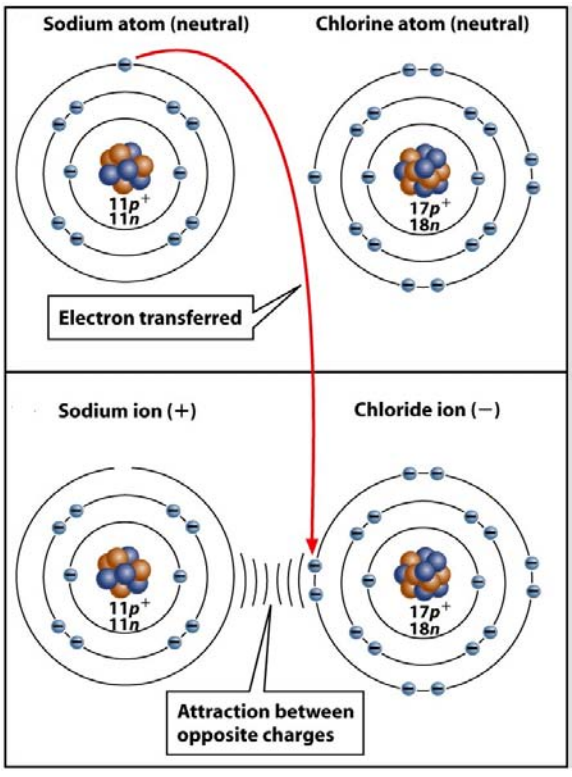
- An atom which has 1 or maybe 2 electrons in its outermost energy level (shell) may donate (give up) these electrons, which results in an outer energy layer with full orbitals.
- This action results in an atom with more protons than electrons, which results in a charged atom, an **ion.**

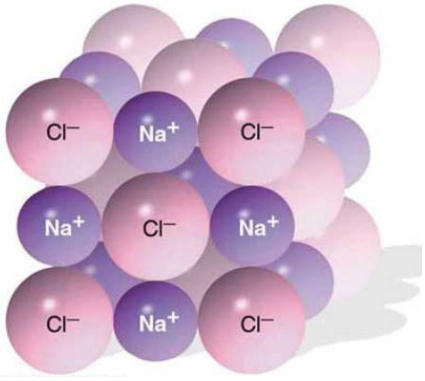
  Example: $Na^{11p\ 11e}$ ---> $Na^{11p\ 10e}$ = $Na^{(+1)}$ ion

- A second atom may have 7 electrons in its outer shell, and may take on a donated electron to complete its orbitals.
- This too, produces a charged atom, or ion, but now one which is negatively charged.

  Example: $Cl^{17p\ 17e}$ ---> $Cl^{17p\ 18e}$ = $Cl^{(-1)}$ ion

- The bond which forms occurs between the **charges** of the respective ions, forming a complex of positive and negative ions. This bond is called the **ionic bond.**





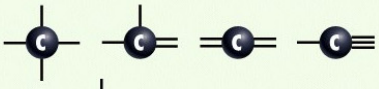
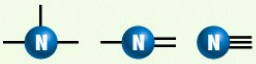
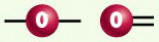
- Ions can be formed from atoms: Na^{+}, Cl^{-} or from molecules: $CO_3^{=}$, NH_4^{+}

Covalent Bonds

The major elements found in living organisms, **carbon, hydrogen, oxygen,** and **nitrogen**, tend to form covalent bonds. Our carbohydrates, proteins, lipids and genetic molecules are formed from these elements, as are the vitamins needed for living organisms. The exception to covalent bonding in the compounds found in living organisms are the minerals, or salts, which form ionic bonds.

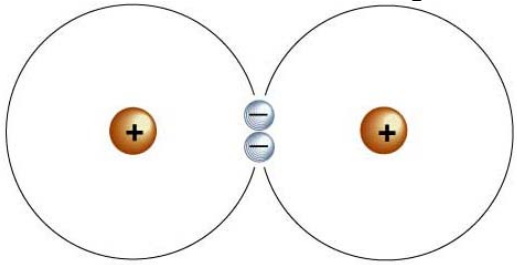
Covalent Bond Characteristics

- A covalent bond is formed when different atoms share one or more of their electrons with each other.
- This sharing causes each atom's orbitals to expand and alter its pattern to include the other atom (more or less). This determines the shape (bonding angles) of the molecule which forms.
- This collectively results in more stable orbitals for both atoms
- Covalent bonds form generally when a transfer of electrons would result in too great of a charge imbalance between the atom's positively charged nucleus and it's negatively charged electron field.
- Depending on the total number of electrons an atom has to share, an atom may covalently bond with one, or with several other atoms.

Atom	Capacity of Outer Electron Shell	Electrons in Outer Shell	Number of Covalent Bonds Usually Formed	Common Bonding Patterns
Hydrogen	2	1	1	
Carbon	8	4	4	
Nitrogen	8	5	3	
Oxygen	8	6	2	
Phosphorus	8	5	5	
Sulfur	8	6	2	

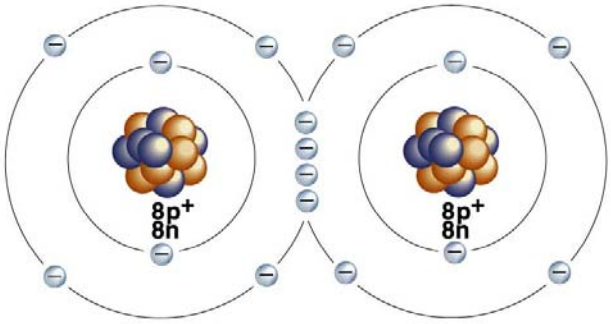
Variations in covalent bonds

Single covalent bond: The 2 atoms are sharing 1 electron pair



Hydrogen (H – H or H_2)

Double covalent bond: The 2 atoms are sharing 2 electron pairs



Oxygen (O = O or O_2)

Triple covalent bond: The 2 atoms are sharing 3 electron pairs

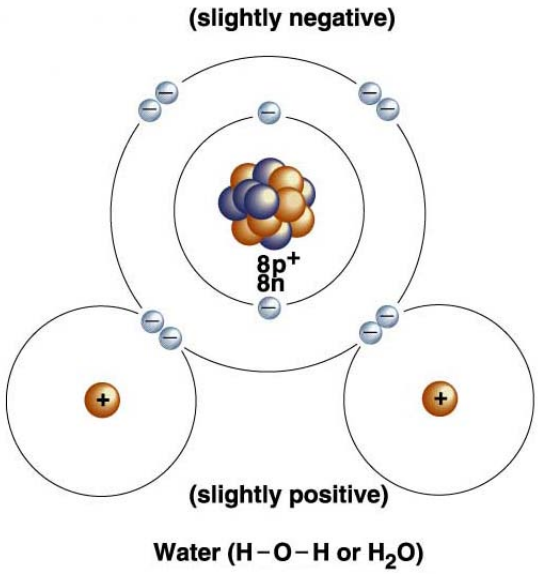
Electronegativity and Polar and Non-Polar Bonds

As stated, in a covalent bond, the electrons of the bonding atoms are shared rather than transferred, so that no charged atoms or ions are formed. We also mentioned that atoms tend to form covalent bonds when a transfer of electrons would result in too great of a charge imbalance between the atom's positively charged nucleus and its negatively charged electron field. All atoms, however, have a property, called **electronegativity**, which is a measure of how strongly the protons of the atom's nucleus attract and hold electrons. The electronegativity of all atoms is not the same.

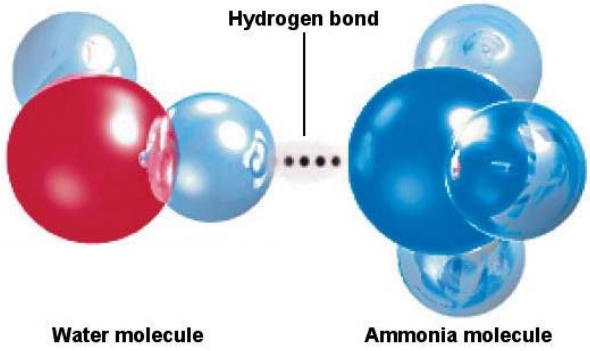
Atoms that form ionic bonds typically have a reasonably high electronegativity. When covalent bonds are formed between atoms which have similar electronegativity, the electrons of the atoms are equally shared and the bond is said to be nonpolar (with no charge attraction).

When covalent compounds are formed between atoms which have very different electronegativities, the electrons of the two atoms are not shared equally. The electrons that are spinning around both atoms will be attracted to the atom which has the stronger electronegativity, so that the electrons spend more time closer to the nucleus of that atom than in the field of the atom with a weaker electronegativity.

A compound (or molecule) formed by the unequal sharing will have one end functionally slightly **negative** (the end with the atom having a strong attraction for electrons) and the other end of the molecule slightly **positive,** resulting in a **polar covalent bond** and molecule.



These slightly charged polar covalent molecules are attracted to other polar molecules (+ attracts -) and form very weak polar bonds. These bonds are weak because the charges are weak and because the electrons are always in motion. (Some of the time anyway, the end which is primarily positive will have the electrons spinning there and lose its polarity for that instant.) The bond that forms between adjacent polar covalent molecules is called a **Hydrogen bond**.



Hydrogen Bonding

- Attraction between adjacent polar molecules
- Common with the element hydrogen (which has a very low negativity) and high electronegativity atoms.
  e.g..: H-N
  H-O
- Hydrogen bonding is very important in the structure of:
  Water
  Proteins
  Nucleic acids

Other Weak Bonds

Hydrophilic (polar) and Hydrophobic (non-polar) molecules separate from each other in aqueous solutions because water is a polar molecule, and water molecules are attracted to other polar substances and repelled by non-polar molecules. Likewise, non-polar molecules are attracted to other non-polar substances and repelled by polar molecules. (Oil and water truly do not mix.)

The hydrophobic interactions of non-polar molecules in solution are reinforced by very weak interactions called van der Waals forces. These interactions are the result of electron motions and the brief attraction a polar molecule will have for another atom's electrons when adjacent to it. This attraction causes a brief charge reaction between the two molecules

Water and Life on Earth

As we know, life on earth is based on the substance, **water**. Water is the most abundant compound found in living organisms (about 80%).

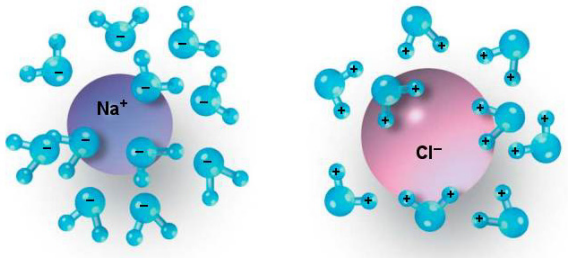
We have just seen that polar and non-polar molecules act differently in water. We have seen too, that water is one of the polar covalent molecules, and hydrogen bonds form between adjacent water molecules.

Lets look now at some properties of water, especially as these properties relate to water's polar nature and the phenomenon of hydrogen bonding.

Properties of Water

Solvent Properties

- Water is an excellent solvent (a fluid in which something can be dissolved) for many substances because of its polar nature.
- Many covalent molecules dissolve in water because covalent molecules often mix with other covalent substances.
- Polar substances and ions dissolve in water because opposite charges are attracted. Ions are attracted to the appropriate ends of water molecules which keep the ions dispersed in the water - or - dissolved.



- Strictly hydrophobic molecules, including most lipids, do not mix well with water
- Some molecules have both hydrophobic and hydrophilic ends. Such molecules are said to be **amphipathic**. Amphipathic molecules make good emulsifiers because they can attract both hydrophobic substances and hydrophilic substances to them.
- Substances dissolved in a solvent are called **solutes.**

Water in Biochemical Reactions

- The breakdown and assimilation of many molecules of living organisms involves water. Water is needed to breakdown carbohydrates, lipids and proteins during digestion. The formation of large biological molecules from smaller building blocks releases water.

Cohesion property of water

- A substance placed under sufficient tension will eventually rupture or be broken apart. Cohesion is the ability to resist rupture.
- The hydrogen bonds of water provide for good cohesion which results in a high surface tension for water.
- Cohesion allows for organisms to "walk on water surfaces" and allows for water to be drawn up to the tops of plants, even the tallest trees.

Adhesion property of water

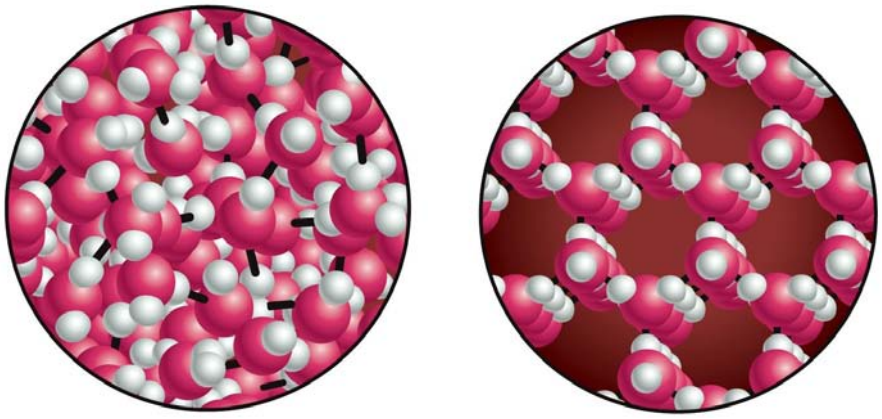
- Water molecules are often attracted to other polar substances, a property known as adhesion. This property provides for capillary action, the manner in which water "creeps" up the surfaces of tiny tubes, or "wicks" up paper and other surfaces.

Temperature moderating property of water

- Temperature is a measure of the rate of molecule movement of some piece of matter
- Heat is a measure of the amount of energy which results from the movement of molecules in some piece of matter.
- Because water has hydrogen bonding it has:
  - A **high specific heat**, the amount of energy needed to increase the temperature of 1 gram of a substance 1 degree Celsius
  - A high **heat of vaporization**, the amount of heat energy needed to change a liquid to a gas (or evaporate)
  - A high **heat of fusion**, the energy needed to convert a substance from a liquid to a solid.

For example:

- Molecules of liquid water, when heated, can not move faster until the hydrogen bonds are broken; therefore it takes more heat energy to increase the temperature of water.
- Because of hydrogen bonding, it takes more energy to convert water to steam at its boiling point. Since it takes lots of energy to evaporate, and that energy must come from the surrounding liquid water, evaporation is a cooling process.
- The constant forming and breaking of hydrogen bonds in water provides for greater movement of the water molecules at lower temperatures. Water molecules do not form a rigid structure until 0 degrees Celsius is reached.
- Water molecules are densest at 4 degrees Celsius, which means that solid water is less dense than liquid water, or ice floats. (This is also because of hydrogen bonding.)



Liquid Water Ice

What do these temperature properties mean for living organisms?

- Water provides a more stable environment, in terms of temperature, both inside and outside of cells, in a world of fluctuating temperatures.
- Life can occur in lakes in temperate areas, since the frozen ice at the top of the lake in winter serves to insulate the water below. Also, if ice sunk, the organisms might find themselves out of a home, flopping on the surface of a solid block of ice...
- The process of evaporation is used by many organisms to maintain appropriate body temperature. (Sweating, for example)

Some special properties of water: Acids, Bases, Salts and Buffers
Recall that ionic bonds dissociate in solution forming ions, but covalent compounds resist dissociation. This is essentially true. However, some polar covalent compounds, including water, do dissociate, and it is important to discuss the meaning of this as it relates to living organisms, and their chemical environment.

A molecule of water is formed by the bonding of 1 oxygen with 2 hydrogen atoms. Oxygen has a very high electronegativity so that water is very polar, so polar that at any given instant, the attraction of the oxygen atom for electrons will literally draw an electron away from a hydrogen, or in fact, the **water molecule ionizes.**

At any time, a fixed proportion (specifically 10^{-7}) of a volume of water will be:
H^+ (Hydrogen ions)
OH^- (Hydroxide ions)
and the rest of the water will be: H_2O (Water molecules)

This phenomenon of water dissociation has bearing on a whole class of substances which contain hydrogen or hydroxide ions:
Acids and **Bases,** Plus **Salts.**

Acid
A substance which liberates H^+ in solution
Base or alkaline substance
A substance which combines with H^+ in solution
A substance which donates OH^- to solution
Salt
- An ionic compound which can be formed which an acid and base react. (Water is also formed along with the salt.)
- Salts dissociate into ions in solution.
- Many minerals needed for living organisms are salts. Some examples:
  Ca^{++}
  Fe^{++} or Fe^{+++}
  K^+
  Na^+
  Cl^-

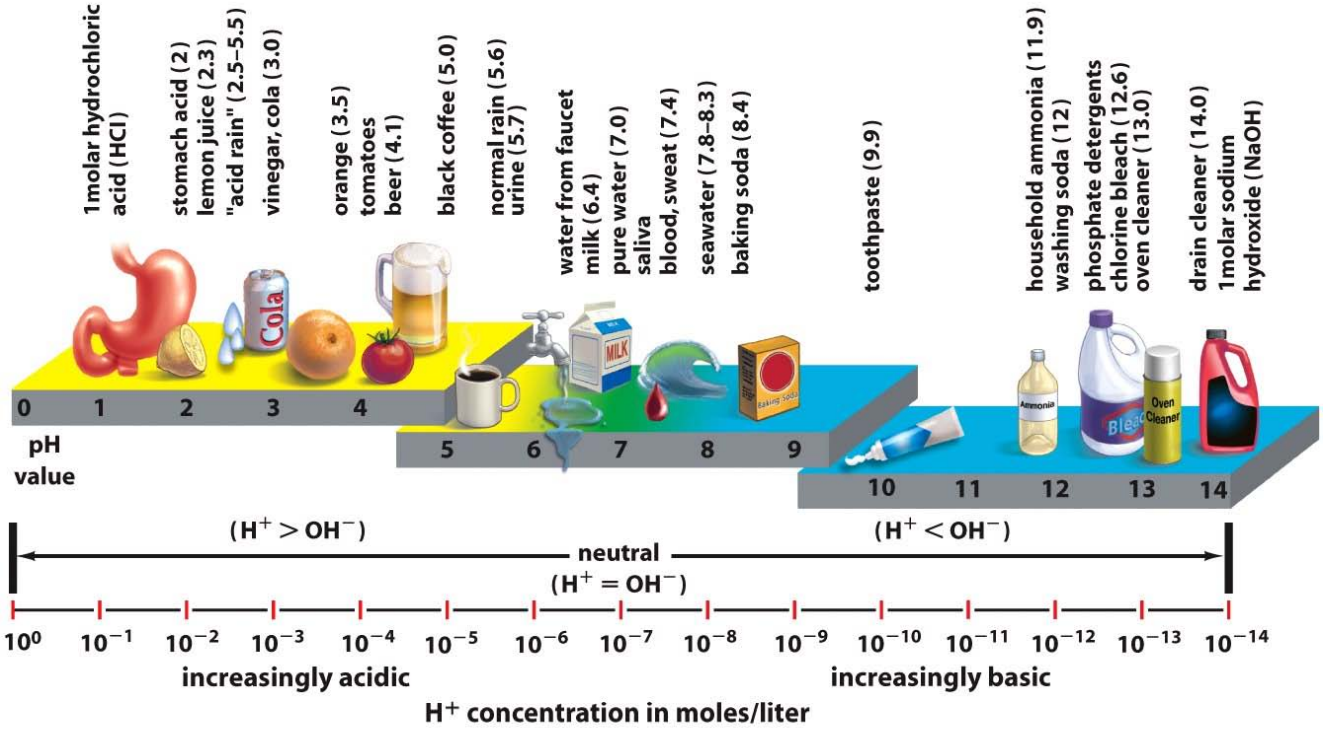
pH, Acids, Bases and Water

How do acids and bases relate to water and its dissociation, and what does this mean to living organisms? We'll answer that in just a minute, after discussing the phenomenon of pH (which is related to H^+ concentration).

Recall that at any given moment, 10^{-7} of any H_2O (Water) will be H^+ and OH^-. This ratio of H^+ to OH^- ions in water is used as a standard to measure the acidity of a substance.

This measurement of H^+ ions has been "translated" to a scale of pH (power of hydrogen).

NOTE: pH = the negative log of H^+ in the substance so that on the pH scale:
1 = maximum H^+, or the most acid.
14 = the most basic
7 = neutral (which is the pH of pure water)



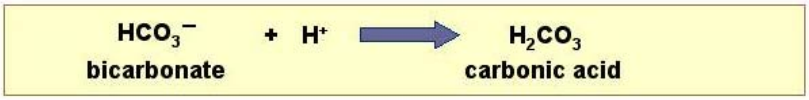
N

ow, why do we care?

Reactions of living organisms are very sensitive to levels of pH. It is therefore critical to maintain proper pH in an environment where cells and tissues are exposed to much variation in H^+ concentration.

How is this resolved? **Buffers**

- Group of materials (generally salts of weak acids and bases) that absorb or release H^+ depending on the condition so that proper pH is maintained.
- One of the most common buffers is the bicarbonate buffer.
    - If a solution is too acidic, bicarbonate can combine with H^+ to form carbonic acid
      ($HCO_3^- + H^+ \rightarrow H_2CO_3$)
    - If a solution is too basic, carbonic acid can combine with OH- to form bicarbonate + water
      ($H_2CO_3 + OH^- \rightarrow HCO_3^- + H_2O$)



- Buffers are critical to the maintenance of life. Buffered systems mean that organisms can maintain a suitable pH environment in their cells and tissues.

Molecules of Living Organisms

We have mentioned that all organisms, from bacteria to Douglas fir trees to humans share a common molecular structure; it's part of the unity of life.

The cells and tissues of virtually all organisms are made up of the same basic molecules. Many of these are substances with which we are familiar: our **carbohydrates, lipids, proteins** and the **nucleic acids.**

These molecules are all compounds with a "backbone" or "skeleton" of **carbon**, or more specifically carbon–hydrogen molecules, which are called **hydrocarbons**. The incredible versatility of carbon accounts for the multitude of different organic molecules, built from the common backbones, which are found in different kinds of organisms. In chemistry, molecules with a backbone of carbon that also contain hydrogen are called **organic molecules**. The other atoms and molecules necessary for life are inorganic. Besides carbohydrates, proteins, lipids and nucleic acids, our vitamins are considered to be organic. (Water, oxygen, carbon dioxide and the minerals needed to sustain life are **inorganic**.)

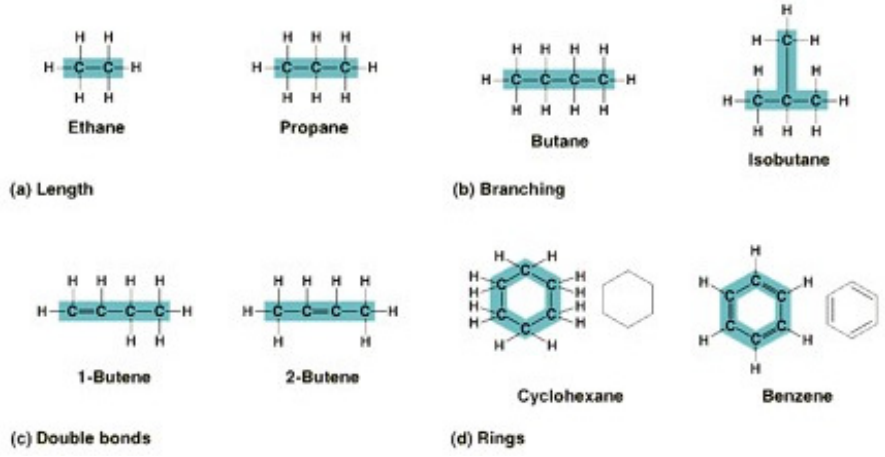
Our organic compounds are responsible for such things as:

- Fuel (energy to do cell work and keep us alive)
- Structure
- Metabolism
- Fuel Storage
- Genetic Information

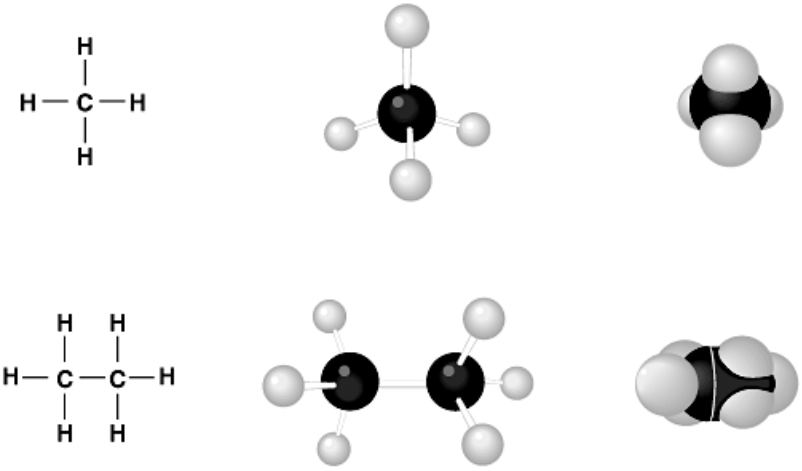
Before understanding the structure of the major groups of compounds of living organisms, we should first study the element, **carbon**, what a **hydrocarbon** is, and also study the molecules called **functional groups**, which bond to hydrocarbons, altering the chemical nature of the resulting compound.

Properties of Carbon

- Carbon is one of the atoms (elements) that forms covalent bonds (joins with other atoms) to become stable. Each carbon atom makes **4 bonds**
- Carbon may make bonds with other carbon atoms forming chains, branching chains or rings of linked carbon atoms.



- Carbon may also bond to different kinds of atoms, most notably hydrogen. In fact, the basic carbon compound is a **hydrocarbon**, formed from carbon and hydrogen.



Properties of Hydrocarbons

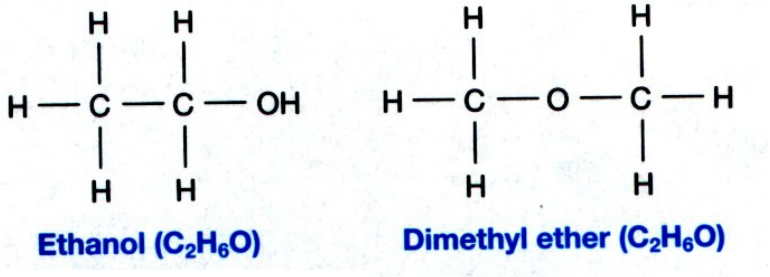
Hydrocarbons, like carbon, typically vary in:

- The number of carbons on the chain
- Straight, branching chains or ring compounds
- What is attached to the carbon chain

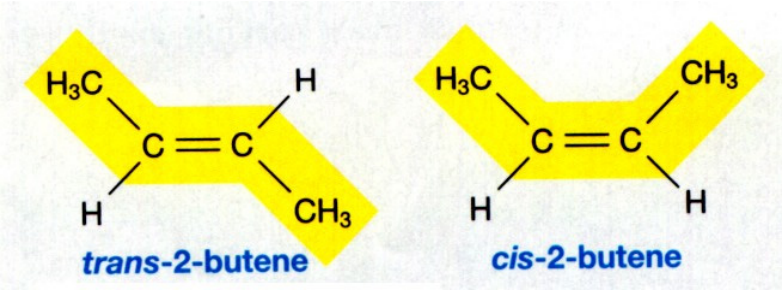
Most hydrocarbons have very similar properties. For example the C—H bond is **energy rich**; so hydrocarbons make good fuels (methane, propane, butane, methanol, alcohol)

Hydrocarbon variations that differ only in the arrangement of atoms are called **isomers**. Isomers are very important in biology, and we shall see many examples of isomers. There are three types of isomers: **structural**, **geometric** and **enantiomers**. (We don't need to worry much about these details in Biology 101, though.)

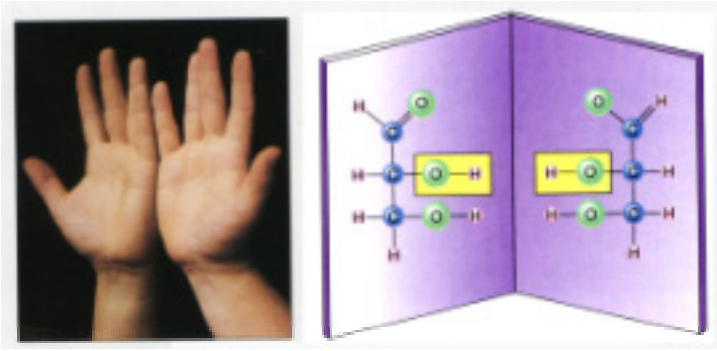
- **Structural** isomers vary in their covalent bonding arrangement.



- **Geometric** isomers share common covalent bonding, but have different shapes. The differing shape of geometric isomers can dramatically affect their biological function. (This is sometimes called the cis-trans difference.) Cis-trans changes occur when one partially hydrogenates fats, forming trans-fatty acids.



- **Enantiomers** are isomers that have the same molecular formula but are mirror images of each other.



Hydrocarbons can also have variations in bonding. Carbon may make double or triple bonds as well as single bonds. The resulting compounds will be different in shape and often function.

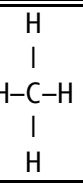
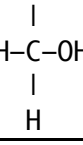
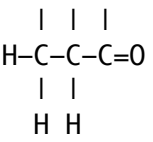
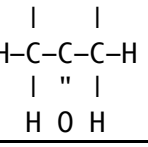
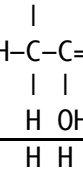
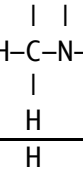
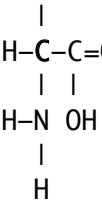
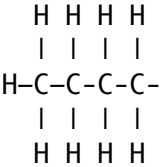
Carbon ring compounds are common in living organisms. The shape of the ring compound is important to its properties. The covalent bond angles in the ring determine the molecule's shape. Two common ring shapes are the "chair" and the "boat"

The major compounds of living organisms are modifications of hydrocarbons with something (very precise) added. These atoms or molecules are called **functional groups,** because they change how the hydrocarbon functions and gives it properties of a carbohydrate, or lipid or protein, etc.

Functional Groups

The functional groups are molecular fragments which, when substituted for one or more hydrogen atoms in a hydrocarbon, confer particular chemical properties to the new compound. The functional group can be said to determine the "behavior" of the molecule. Once you have learned the properties of some functional groups, the major compounds of living organisms are easy!

Some Functional Groups Important in Biological Molecules

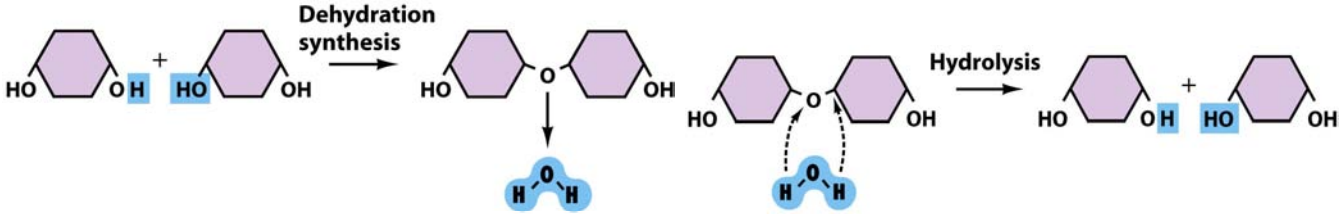
Functional Group Name	Formula	Compound Type	Example
Hydrogen	—H	Alkane	
Hydroxyl	—OH	Alcohol	
Carbonyl	=O	Aldehyde	
Carbonyl	=O	Ketone	
Carboxyl	–C=O | OH	Organic Acid	
Amino	H | –N–H	Amine	
Amino + Carboxyl Note the central alpha carbon to which both amino and carboxyl groups attach to form the amino acid		Amino Acid	
Methyl	H | –C–H | H	Backbone of Hydrocarbon Chains	
Phosphate	O-H | -O-P=O | O-H		Phospholipids Nucleic Acids
Sulfhydryl	-S-H		Found in cysteine

Before discussing the specifics of the molecules of living organisms, we should also be familiar with the chemical processes by which large molecules (**polymers or macromolecules**) are built from smaller molecules (often called **monomers** or **subunits**) that have a common structure.

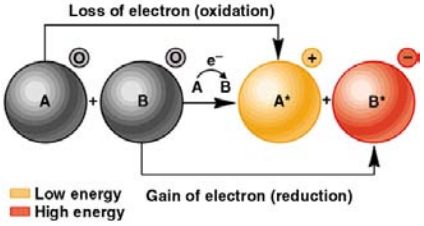
Most of our biological molecules are assembled or broken down using the same types of chemical reactions, used to assemble, rearrange and break apart molecules.

Among the most common of reactions are the chemical reactions that involve **adding or removing water molecules.** Polymers are formed from their subunits by removing molecules of water (a hydrogen (H-) from one subunit and the hydroxyl (-OH) from the second subunit) to join the subunits together. This is called a **dehydration synthesis**, or **condensation reaction**.

When larger molecules are broken down, such as in digestion, water molecules are added in to break the macromolecules into their subunits, a process called **hydrolysis** or sometimes referred to as a **cleavage reaction.**



A second common set of chemical reactions in living organisms involves the transfer of one or more electrons and is known as **oxidation** and **reduction**. An oxidation is the loss of one or more electrons. A reduction is the gain of one or more electrons. Oxidations and reductions are always coupled. A substance that can cause a reduction is called a reducing agent, and one that can cause an oxidation is an oxidizing agent. A substance that prevents something from being oxidized is called an anti-oxidant. Vitamin C and vitamin E both function as anti-oxidants in our cells and tissues. (An anti-oxidant works by being so easily oxidized itself that the oxidizing substance oxidizes the anti-oxidant rather than the "target" molecule that needs "protection".)



In addition, there are rearrangement reactions in which the internal bonds of the molecule are literally, rearranged, which may change the compound from one type to another.

Now we can discuss the major compounds of living organisms: **Carbohydrates, Lipids, Proteins** and **Nucleic Acids.**

Carbohydrates

The word carbohydrate is one of convention, derived from carbon and water, the component elements of the carbohydrate monomers (subunits) or monosaccharides. Carbohydrates include sugars and the complex carbohydrates.

Carbohydrate Functions

- Basic energy source (fuel) for virtually all living organisms
- Structural molecules, especially of plants, most fungi and arthropods (e.g., cellulose, chitin)
- Fuel reserve molecules (e.g., starch, glycogen)

As stated, carbohydrates are composed of one or more **monosaccharides.** The **simple sugars** are formed from one (monosaccharide) or two monosaccharides (called **disaccharides**), and the **complex carbohydrates** (polymers) are formed from long chains of monosaccharides, joined by dehydration synthesis reactions. The complex carbohydrates are also called **polysaccharides** and include starches and fiber. Some plants have **oligosaccharides**, small chain carbohydrates composed of a few monosaccharides. Humans cannot digest oligosaccharides, but the bacteria in our intestines can. Their digestive by-products are often gaseous, something we associate with the consumption of foods that contain oligosaccharides. Our red blood cell coatings that give us our blood types are also oligosaccharide based.

Structure of the monosaccharide

Chemically, monosaccharides contain:

- Carbon
- Hydrogen
- Oxygen

The ratio of atoms in a monosaccharide is: (CH_2O)

e.g. $C_n (H_2O)_n$
$C_6H_{12}O_6$
$C_3H_6O_3$

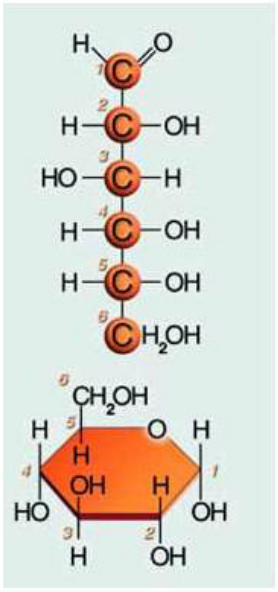
The functional groups of monosaccharides are:

- –OH Hydroxyl
- =O Carbonyl

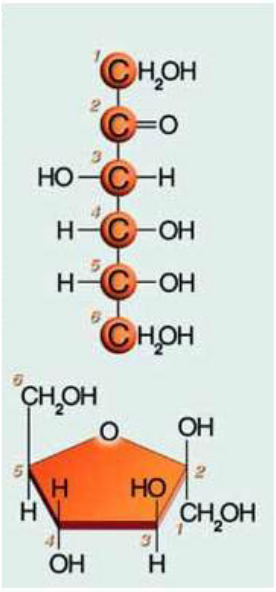
However the arrangement of atoms in the monosaccharide is important. Each monosaccharide is constructed with the following rules:

1. Make a carbon chain
2. Attach the carbonyl group to 1 of the carbon atoms
3. Attach hydroxyl groups to the remaining carbon atoms
4. All remaining open carbon bonds will have hydrogen atoms attached

Given the rules, there are many variations possible with monosaccharides. Many **isomers** are possible and common.



Structure of glucose



Structure of fructose

The common monosaccharides of living organisms are:

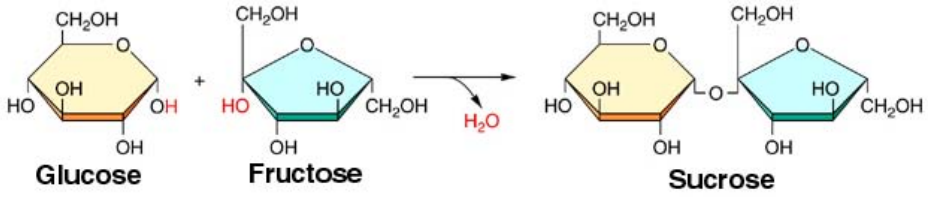
- $C_6H_{12}O_6$ (glucose, galactose, fructose)
- Some 5-carbon (ribose, deoxyribose, ribulose, xylose)

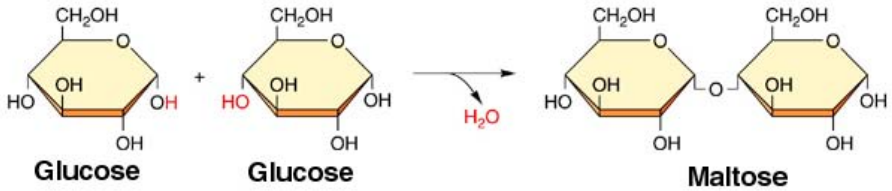
Note: Although we show monosaccharides and other carbohydrates in the chain structure, the carbohydrates in living organisms are found in a ring shape.

Formation of Disaccharides and polysaccharides

Disaccharides

- Disaccharides are 2 monosaccharides joined by a **dehydration synthesis,** or **condensation**, which is the removal of a water molecule. The "H" is taken from a hydroxyl functional group of one monosaccharide and the "OH" from the second. The two molecules are then joined by a C—O—C bond.

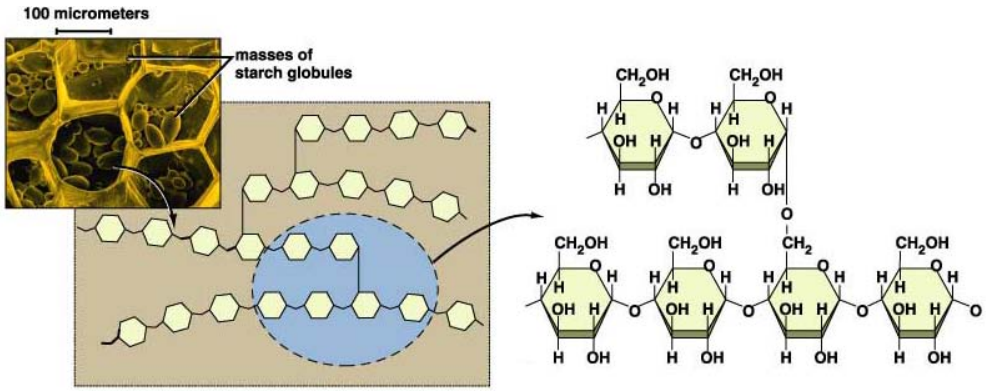




Examples of common disaccharides are **sucrose, lactose,** and **maltose**

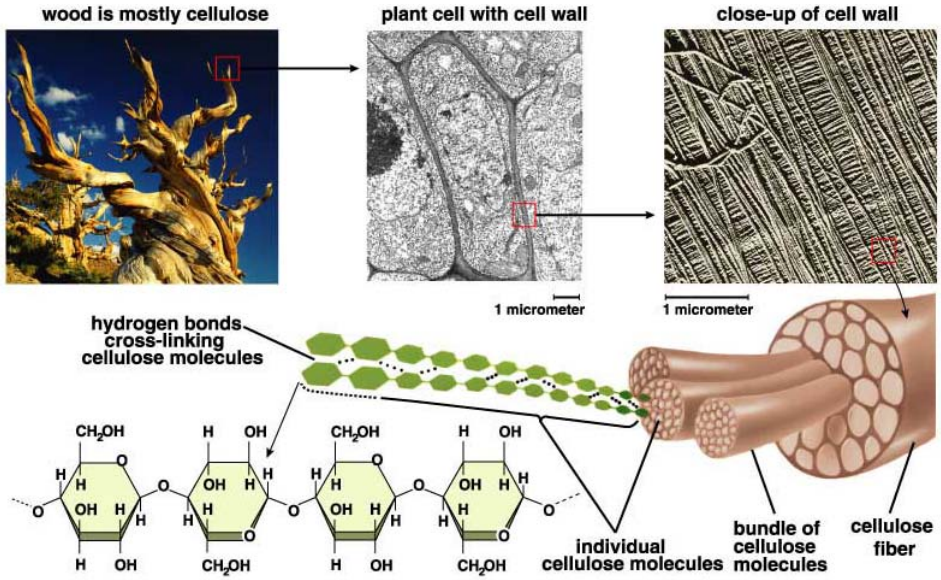
Polysaccharides

- Polysaccharides are formed by joining several monosaccharides, each to the next by a dehydration synthesis.
- The common polysaccharides are:
  - **Starch** (α 1–4 linkage) (boat)

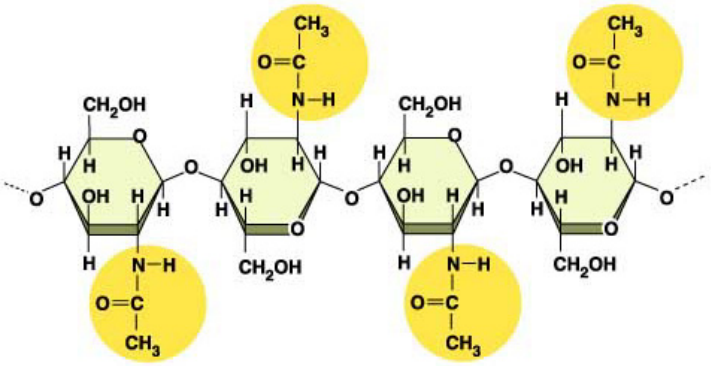


  - **Glycogen**
    - Both starch and glycogen are polysaccharides of glucose. Starch is a very long coiled, unbranched or branching chain, with about 1000 glucose molecules in any branch. Glycogen branches frequently (about every 10 or so glucose units) and is more easily broken down.
    - Starch and glycogen are important fuel storage molecules.

  - **Cellulose** (β 1–4 linkage) (chair)
    - Long chains of glucose
    - Cellulose is for most living organisms, non-digestible. Few organisms have the enzyme needed to break down cellulose. Cellulose and related compounds form most of what we call **fiber**.



- **Chitin**
  - Long modified glucose chains, in which a nitrogen-containing functional group replaces one of the hydroxyl groups on each glucose subunit.
  - Chitin forms the exoskeleton of many invertebrate animals (mostly arthropods)



Disaccharides and polysaccharides can be digested or broken down by **hydrolysis.** (Appropriate enzymes are required for both dehydration synthesis and for hydrolysis)

In addition to the "pure" carbohydrates, glycoproteins, common in plasma membranes, contain carbohydrate, as do protective mucus layers and all nucleic acids.

Lipids

Many of our common substances are lipids, which include fats, oils, and waxes along with a variety of related substances.

Lipid Functions

- Fuel reserve molecules (Lipids are energy rich)
- Structure of cell (plasma) membranes
- Protective surface coatings and insulation
- Many hormones (regulatory chemicals)

Major types of Lipids

1. Triglycerides commonly known as the fats and oils
2. Waxes (similar to triglycerides)
3. Phospholipids
4. Sterols (or steroids)
5. Terpenes

Lipid Characteristics

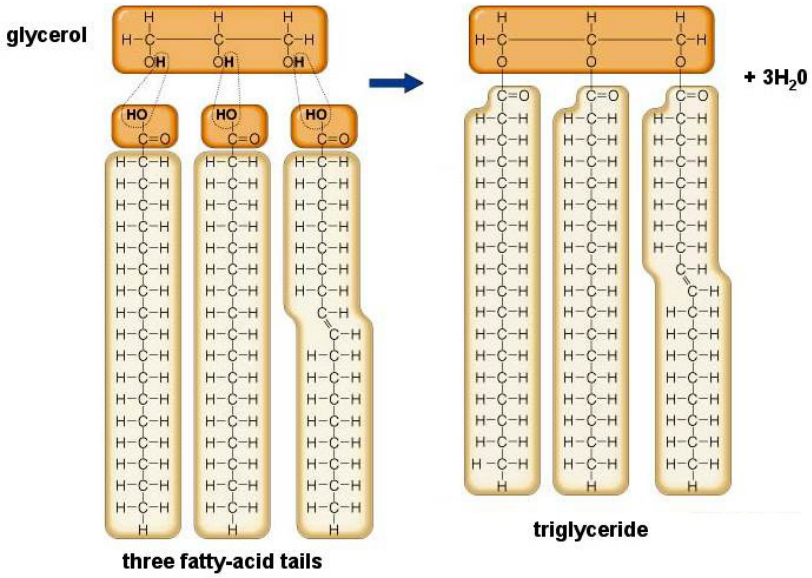
- Most lipids are strictly nonpolar and hydrophobic, so they dissolve in nonpolar substances, but not in water.
- Most lipids feel "greasy"
- Lipids contain large regions of just carbon and hydrogen, as carbon-carbon bonds and carbon-hydrogen bonds

Structure of Lipids

- Lipids contain:
  - carbon
  - hydrogen
  - oxygen
- However – the proportion of oxygen is low, so lipids are mostly **hydrocarbons**
- The chemical structure of our fats and oils, the most common lipids, is based on **fatty acid** building blocks and an alcohol, **glycerol**
- The terms fats and oils are terms of convention
  Fats are "hard" or solid at room temperature
  Oils are liquids at room temperature

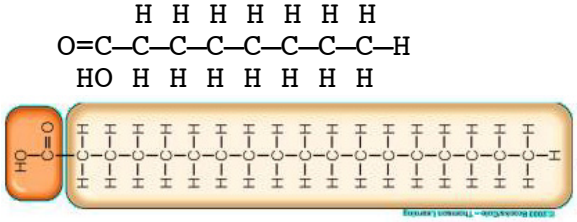
Structure of Fats and Oils (the Triglycerides)

- One molecule of the alcohol, **glycerol**
- Attached to the glycerol (by dehydration synthesis) are **3 fatty acids.** The fatty acids determine the characteristics or properties of the fat. The bond formed between the –OHs of the alcohol and the –OHs of the fatty acid is an ester bond.

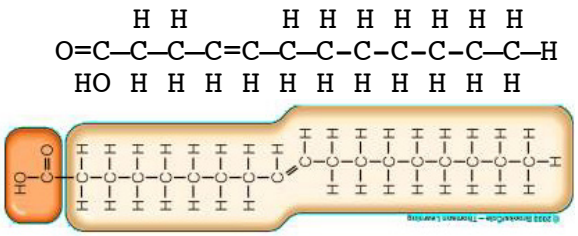


- **Fatty acids** are chains of hydrocarbons 4—22 carbons long with the carboxyl functional (acid) group at end

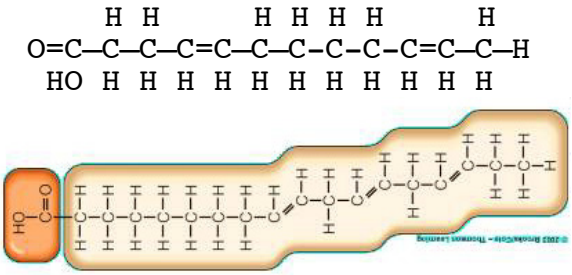
- Each carbon within the chain has 2 spots for bonds with hydrogen
- If each carbon has 2 hydrogens the fatty acid is **saturated**



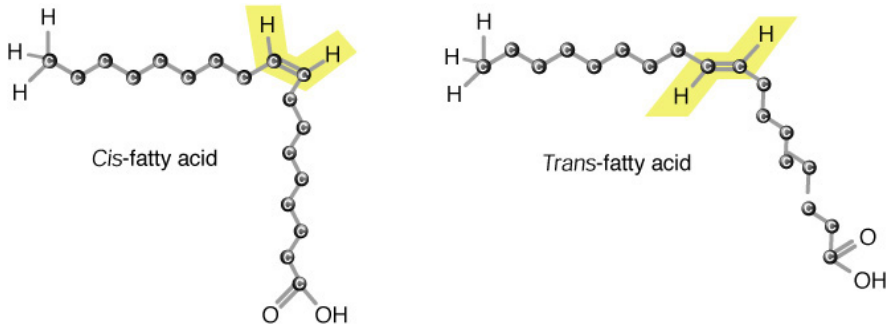
- If two carbon atoms are double bonded, so that there is less hydrogen in the fatty acid, it is **monounsaturated**



- If more than 2 carbon atoms are unsaturated, the fatty acid is **polyunsaturated**



In most fatty acids, the hydrogen atoms attached to the double-bonded carbons are both on one side of the carbon chain (either top or bottom). Such fatty acids are called cis-fatty acids. In trans-fatty acids, the hydrogen atoms attached to the carbons forming the double bond are on opposite sides of the carbon chain. When fats are hydrogenated, trans-fatty acids tend to form. We process trans-fatty acids much the same way as saturated fatty acids.



Let's look at ways that fatty acids are different:

1. Length of chain in fatty acid
   - 4 – 22 carbons long
     - Short chains are more soluble
     - Short chains are more easily broken down
     - Short chains oxidize more easily
2. Degree of saturation
   - Saturated
   - Monounsaturated
   - Polyunsaturated
     - Most plant fats tend to be unsaturated, but fats from tropical plants tend to be very saturated
     - Fish oils tend to be unsaturated (from cold water and salt water fish). Other animal fats tend to be saturated
3. Liquid vs solid
   - Short chains and unsaturated chains are liquid at room temperature (the double bonds distort the molecules so they don't fit close together)
   - Saturated chains are solid (denser) because chains fit together better

Synthetic Fat

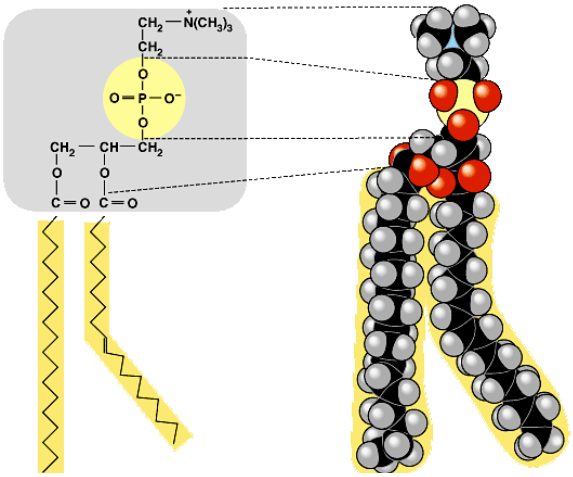
Olestra is a synthetic fat, marketed under the trade name of Olean. It mimics the texture and properties of triglycerides, is fat soluble, but not digestible or absorbed into the body, so all Olestra consumed passes through the digestive tract. Hence, it is considered to be calorie-free. Olestra is a sucrose polyester, composed of fatty acids attached to sucrose rather than glycerol. Six to eight fatty acids are attached to the sucrose molecule so the lipase digestive enzymes can't function to hydrolyze the ester bonds.

Simplesse is a fat substitute that mimics the texture of fat in the oral cavity. It is synthesized from egg and milk proteins. The shape of the simplesse molecule is spherical, resembling miniature marbles, so the product has the slick texture of fat. Simplesse is not heat stable, and cannot substitute for fats in frying or baking.

Phospholipids

Phospholipids are structural molecules forming the major component of **all** membranes of cells.

Phospholipids are composed of a glycerol molecule with two fatty acids attached by ester bonds and a polar phosphate-containing compound attached to the third carbon.



The benefit of the phospholipid structure is that the phosphate region makes the molecule highly amphipathic, ideal for the cell membrane structure

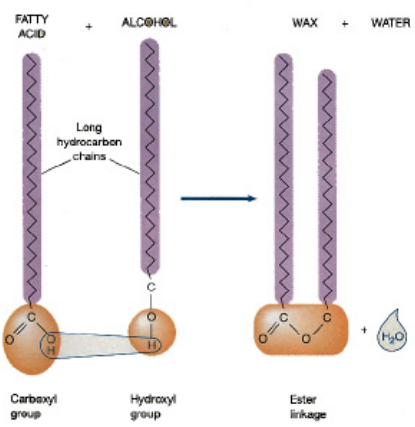
Hydrophilic portion in the phosphate region
Hydrophobic portion in the fatty acid tails

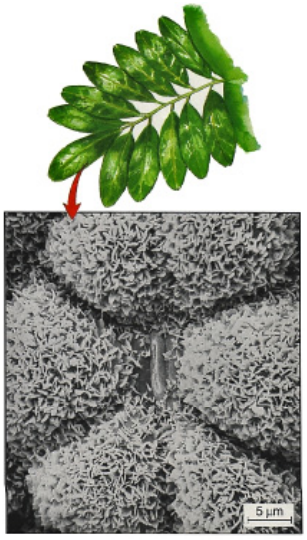
The most common phospholipid is **lecithin**

Phospholipids also make excellent emulsifiers and are used in a number of food and household products.

Waxes

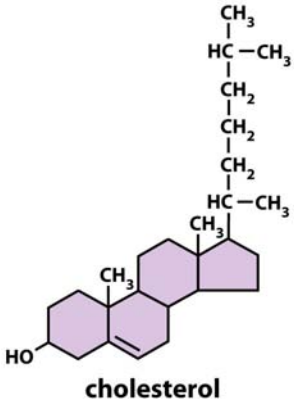
Waxes are similar to triglycerides except they are highly saturated with long-chain fatty acids and have a long-chain alcohol or carbon ring to which the fatty acids bond. They have a rigid, solid structure at "normal" temperatures on earth. Waxes form protective layers on surfaces of many organisms, provide water-resistance, and in some cases, structure. Some organisms can digest waxes for fuel.

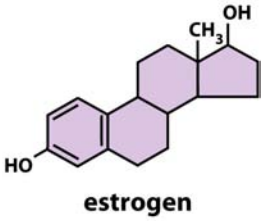


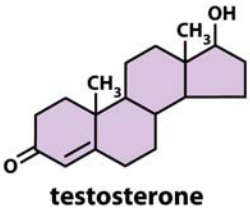


Sterols (Steroids)

All steroids are composed of hydrocarbon chains with four interconnected rings. Although rarely found in plants, certain plant steroids, such as the soy flavinoids, are similar in structure to the estrogen hormones of animals.







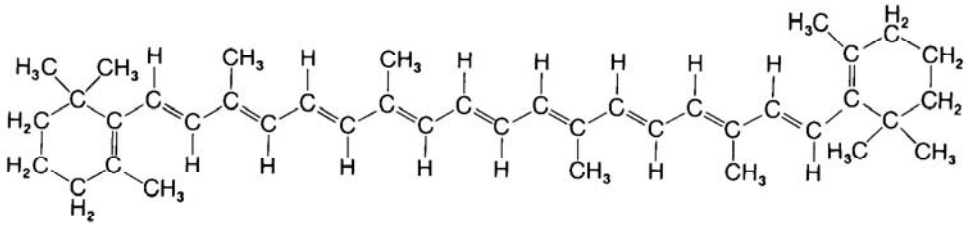
Steroids are used in organisms for a variety of purposes.

- Vitamins A and D
- Hormones (adrenal cortex & sex hormones)
- Cholesterol
  - Precursor to most steroid hormones and vitamin D
  - Necessary for structure of nerve system cells
  - Component of animal cell membranes – not found in plants
  - Cholesterol is made in the liver from digested fatty acids

Terpenes

Over 22,000 different terpenes found in plants, and include some important pigments such as the carotenoid pigments that are responsible for the orange, red and yellow colors of many plants. Chlorophyll, the light absorbing green pigment important in photosynthesis, is a modified terpene.

Many plant aromatic oils are terpenes. Taxol, an extract from yew, is used to treat ovarian cancer, and digitalin is a cardiac medicine. Two plant hormones are also terpenes, as are two important electron transfer molecules. Economically, rubber is an important terpene. Terpenes are lipid soluble and hydrophobic.



β-carotene

Amino Acids and Proteins

Proteins are very large molecules composed of combinations of 20 different amino acids. The precise physical shape of a protein is very important for its function. A single cell may have 10,000 or more different proteins. This diversity of proteins is essential for the functioning of each cell in a living organism.

Functions of Protein

1. Structural
   - Component of all cell membranes
   - Component of cytoplasm "cytoskeleton"
   - Component of movement or contractile structures, such as muscle, cilia and flagella microtubules --- contractile properties
   - Component of hair, nails horns, etc. (Keratin is the main protein of these substances)
2. Metabolic molecules
   - Hormones – regulatory chemicals
   - Energy transfer molecules for cell respiration (cytochromes)
   - Oxygen carrier in circulation (hemoglobin)
   - Antibodies
   - Enzymes
     Probably most "famous"
     Facilitate rate of chemical reaction

Protein Structure

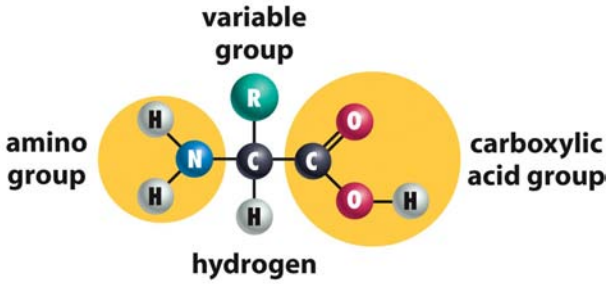
The protein structure is critical for its function. Each protein has a unique shape or **conformation**. However, all proteins are composed exclusively of subunits of amino acids, which join together in long chains called **polypeptides** that fold or coil into the unique shape of the functional protein.

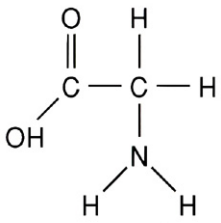
To discuss protein one must
1. Discuss amino acids
2. Discuss formation of protein from amino acid

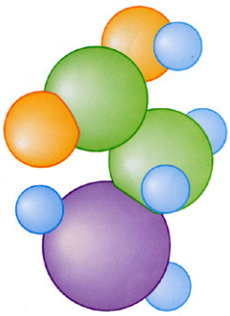
Amino acids

- Amino acids contain Carbon, Hydrogen, Oxygen, Nitrogen, and sometimes Sulfur
- Amino acids have two function groups (both of which are typically in the ionized form)
  NH_2 Amino functional group
  COOH Carboxyl functional group
- Both functional groups attach to a specific carbon, the alpha (α) carbon, of the carbon chain. The third bonding site of the alpha carbon is typically Hydrogen.
- The alpha carbon will have at its fourth bonding site a side chain, or R group which gives the amino acid its unique structure and properties.
- There are 20 + different amino acids in protein. All have a common structure except for the R group.

- Some amino acids have R groups that are polar (so they are hydrophilic), some R groups are nonpolar (and hydrophobic), some have acidic side chains (generally with a negative charge) and some are basic. One, **cysteine**, contains sulfur in the R group, so cysteines can form disulfide bonds **(disulfide bridges)**
- Amino acids are joined together by a dehydration synthesis of amino/carboxyl groups forming a **peptide bond.**

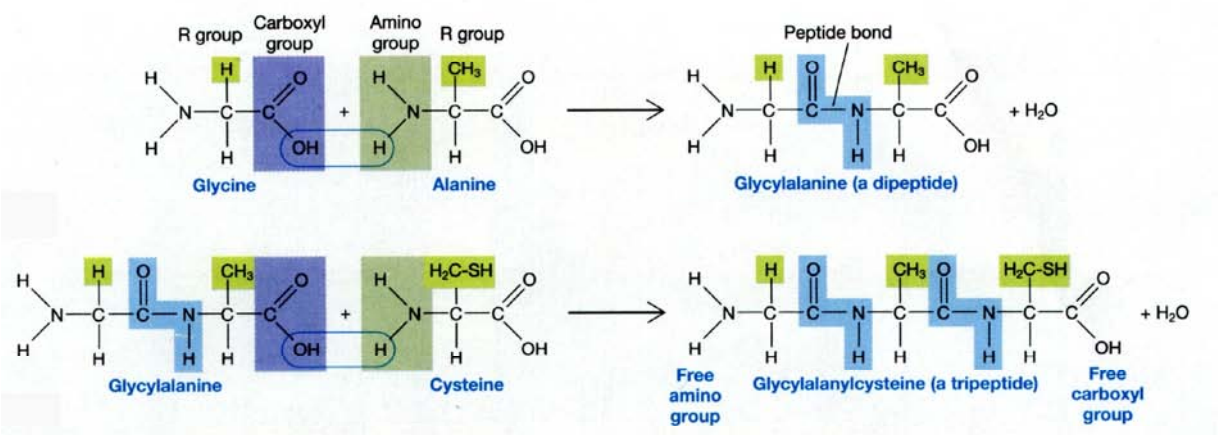






How do amino acids join to make a protein?

1. A protein starts as a chain of amino acids, called a **polypeptide**
2. Amino acids are joined by the **peptide bond,** via dehydration synthesis to form the polypeptide
3. The polypeptide chain is referred to as the **primary structure** of the protein.
4. The specific amino acids in the polypeptide chain will determine its ultimate conformation, or shape, and hence, its function. Even one amino acid substitution in the bonding sequence of a polypeptide can dramatically alter the final protein's shape and ability to function.



Peptide Bond

How do polypeptides vary?

1. Number of amino acids in the chain: 50—1000 or so
2. Which kind of amino acids are in the chain (of the 20 types)
3. How many of each kind of amino acid
4. The bonding order or **sequence** of amino acids

Protein shape and structure

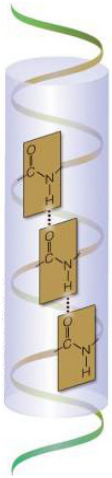
The polypeptide chain is just the beginning of a protein. Functional proteins undergo further processing to obtain a final functional shape. Some proteins are composed of more than one polypeptide. The surface structure of the protein is critical for its function.

The function of many proteins depends on a specific region of the protein that binds to another molecule. Antibodies, critical to the immune system, function by binding to specific regions of the antigen molecules, to deactivate them. An enzyme binds to the substrate (the reactants) at a specific active site on the enzyme.

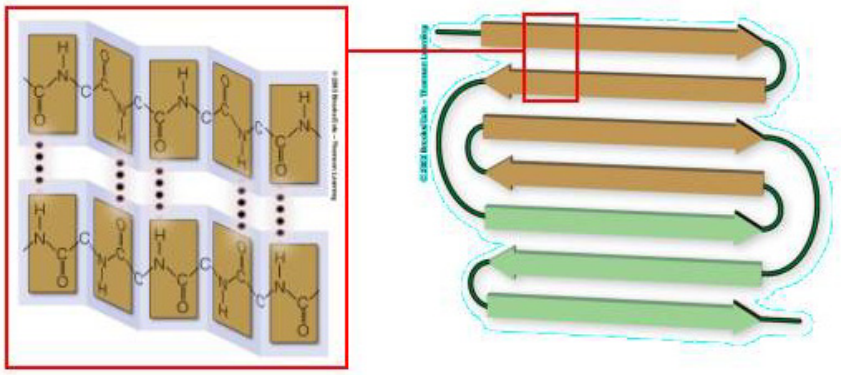
Secondary Structures

The ultimate shape of each protein is determined by bonds that form the secondary and tertiary protein structure. As peptide bonds are formed, aligning the amino acids, hydrogen bonds form between different amino acids in the chain.

This bonding coils the polypeptide into the **secondary structure** of the protein, most commonly the **alpha helix**, discovered by Linus Pauling. The α-helix coils at every 4^{th} amino acid.



α Helix

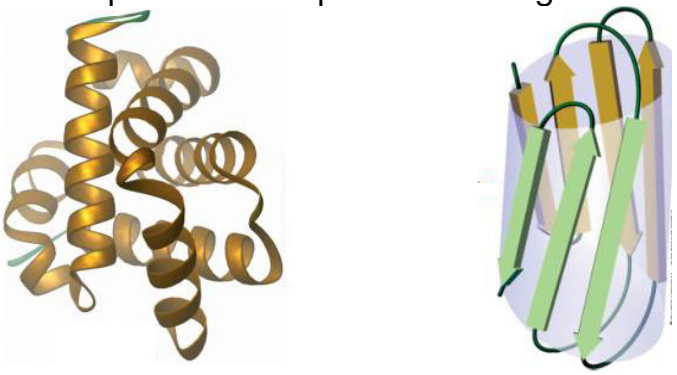


Pleated Protein

Some regions of the polypeptide have portions that lie parallel to each other (still held by hydrogen bonds) instead of in the alpha helix, in which the amino acids' hydrogen bonds form a pleated structure. Fibrous proteins have significant pleated structures.

Domain and Tertiary Structure

Following the secondary shape, openings for bonding along the side chains (the R groups) of amino acids may fold independently into a functional unit called the **domain**. Domains are connected by the rest of the polypeptide. The folding of a protein into its domains is related to the hydrophilic or hydrophobic properties of its amino acids. Domain formation is part of the **tertiary** structure or proteins. **Disulfide bonds** (which are strong covalent bonds) between nearby cysteine molecules are important to the tertiary structure as well, as are **hydrogen bonds**, some **ionic bonds** between charged R-groups and **van der Waals interactions**. The final shape for most proteins is a globular shape.



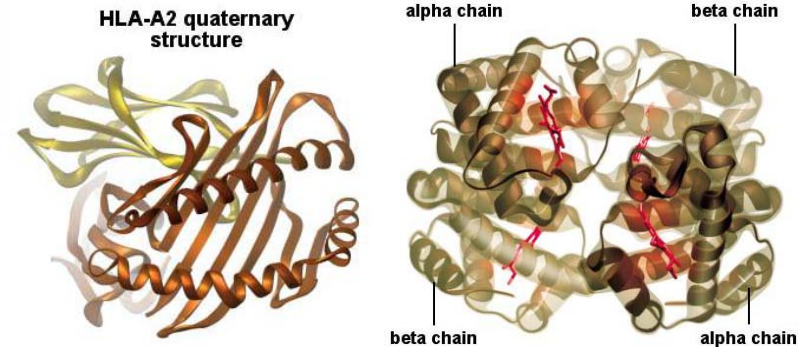
Tertiary Protein Structures

Functionally, domains may perform different functions for a given protein. For example, one domain of an enzyme might be the attachment site for a co-factor and a second domain may function as the active sire of the enzyme.

Quaternary Protein Structure

If two or more polypeptide chains join in aggregates, they form a **quaternary** structure, such as in the protein molecule, hemoglobin. Often quaternary proteins are complexed with a different molecule, often a mineral. Hemoglobin contains iron, for example.

If two or more polypeptide chains join in aggregates, they form a **quaternary** structure, such as in the protein molecule, hemoglobin. Often quaternary proteins are complexed with a different molecule, often a mineral. Hemoglobin contains iron, for example. Other quaternary proteins function in cell defense, with one section anchored in the plasma membrane and a second shaped to catch invaders.



Hemoglobin

Protein Stability

As we have seen, the physical shape of a protein is determined by the amino acid sequence and maintained by weak bonds.

A mistake in the genetic coding of the amino acid sequence may result in a protein whose shape is different, and the protein will not function. One serious example of this is in the hemoglobin protein. An error in the sixth amino acid of the polypeptide chain produces a hemoglobin that causes red blood cells to become sickle-shaped rather than the normal "doughnut" shape.

Many of the bonds that form the tertiary shape of proteins are hydrogen bonds formed from the **polarity** of the amino acids and their "R" groups. If these weak bonds are broken, the protein structure is destroyed and the molecule can no longer function. This process is called **denaturation.**

Things that can denature protein:

1. Heat (as low as 110 F, many @ 130 F)
2. Heavy metals (e.g., silver, mercury)
3. pH changes
4. Salts
5. Alcohols
   Ethyl alcohol least toxic
6. Many proteins will denature if placed in a non-polar substance.
7. Other chemicals

Enzymes are seriously affected by denaturation – but other proteins of the body can also be denatured. Although in most cases, a denatured protein loses its function permanently, in some cases, re-naturation can occur if the substance that promotes the denaturation is removed from the protein. This is more true of chemical denaturants and particularly in experimental environments.

Nucleotides and Nucleic Acids

Nucleic acids are our information carrying compounds -- our genetic molecules. As with many of our other compounds, the nucleic acids are composed of subunits of nucleotides. Nucleotides, in addition have independent functions.

Functions of Nucleotides

- Components of nucleic acids (which are long chains of nucleotides)
- Energy carrier molecules (ATP)
- Energy transport coenzymes (NAD^+, $NADP^+$, FAD^+)
- Chemical intracellular messengers
  (e.g., Cyclic AMP, a cyclic nucleotide that carries messages from the cell membrane to molecules within the cell, to stimulate essential reactions)

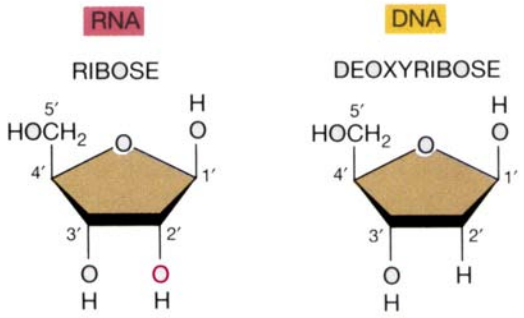
Functions of Nucleic Acids

Storage of genetic information (DNA)
Transmit genetic information from generation to generation (DNA)
Transmit genetic information for cell use (RNA)
DNA self-replication

Most of the information on nucleotides and nucleic acids will be discussed when we discuss genetics and energy relationships of cells. For now we shall just present the basic structure of the nucleotides and nucleic acids.

Nucleotide Structure

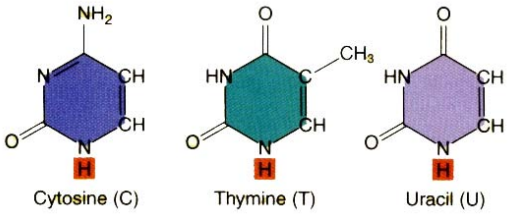
1. 5-carbon sugar component
   Ribose
   Deoxyribose



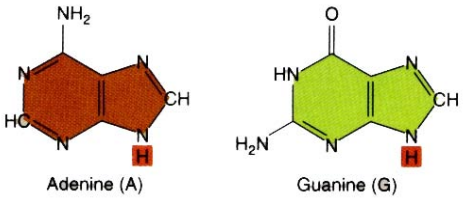
2. Phosphate group
   Attached to the sugar's 5' carbon with a phosphodiester bond

3. Nitrogen Base component attached to the sugar's 1'carbon. There are two types of nitrogen bases:

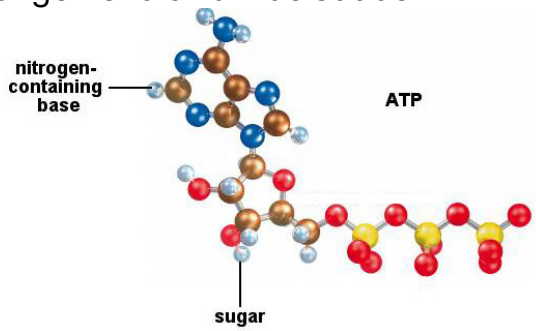
- Single six-sided ring **pyrimidines**
  - Cytosine
  - Thymine
  - Uracil

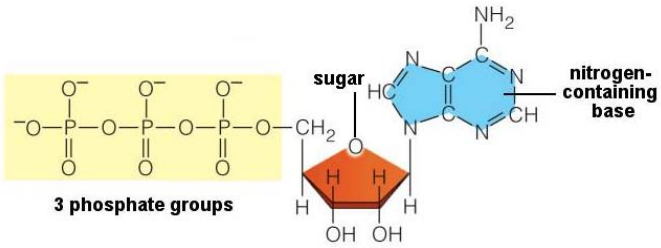


- Double ring **purines** (six- and five-sided)
  - Adenine
  - Guanine

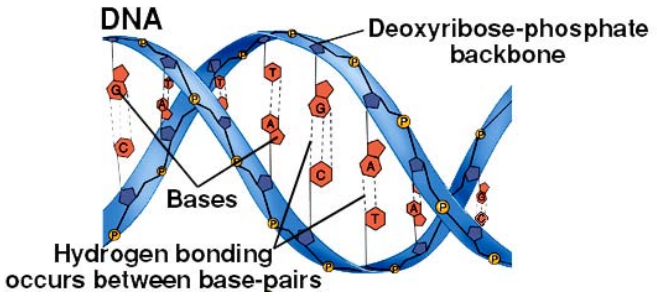


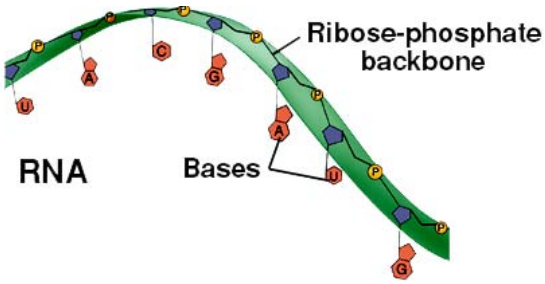
Arrangement of a Nucleotide:





Nucleic acids (polynucleotides) are formed when S–P covalent phosphodiester linkages form long chains. In DNA, a double chain is formed when 2 nitrogen bases hydrogen bond. RNA molecules are single chains.





Genes (specific regions of DNA molecules) contain the hereditary information of an organism. The linear sequence of nitrogen bases of the nucleotides determines the amino acid sequence for proteins in the cells and tissues. As with all of biology, the processes of evolution are validated in DNA information. Organisms more closely related evolutionarily, have more similar DNA.

Just as the atom is the fundamental unit of matter, the cell is the fundamental unit of living organisms. Each cell is unique, composed of carbohydrates, proteins, lipids, and other substances, organized into an orderly structural and functional unit. We shall, in this chapter, see how the structure of cells and, in particular, the structure of cell components, facilitates the functioning of cells.

History of the cell

The study of cells dates back more than three hundred years, coinciding with the development of microscopes. As scientists over the years learned more about cells, a group of common characteristics was developed which we call the **Cell Theory**. Our use today of more sophisticated microscopes and research on biochemical cell activities reinforces these premises.

The Cell Theory

1. Every living organism is made up of one or more cells.
2. Cells are the structural and functional unit of living organisms. The smallest living organisms are single cells, and cells comprise the functional units of multicellular organisms.
3. All cells arise from preexisting cells.

Basic Cell Features (Common to All Cells)

Plasma (cell) membrane

The plasma membrane is the boundary between the cell and its environment. The plasma membrane isolates the cell, regulates what enters and leaves the cell, and allows for interaction with other cells. The plasma membrane is comprised of phospholipid layers with proteins embedded throughout. The diversity of proteins found within membranes is responsible for most membrane activity. We will discuss membrane structure and function a bit later.

Genetic material: nucleus or nucleoid

Each cell contains genetic molecules: (DNA), which stores the instructions for that cell's structure and function, and RNA molecules, which perform a number of functions in cells, including carrying DNA instructions for protein synthesis. The cell's DNA may be found within a membrane - bounded nucleus, (eukaryotic organisms – plants, animas, protists and fungi) or simply concentrated in a region of the cytoplasm called the nucleoid (prokaryotic organisms - Eubacteria and Archaebacteria). Whether DNA is within a nucleus or not is a fundamental cell organizational difference in the classification of life.

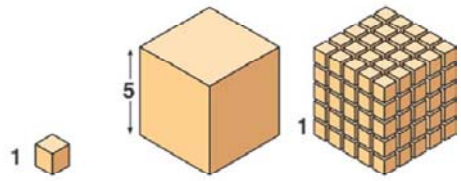
Cytoplasm

Except for its DNA structures, the cytoplasm includes the fluid matrix (called the **cytosol**) inside the plasma membrane in which everything else in the cell, such as internal membranes, particles and membrane-bounded structures, called **organelles**, are suspended.

Cell Organization and Cell Dimensions

Most cells are very small – smaller than we can see with our unaided eye. While the benefits of a cellular organization seem fairly clear, we must look more closely at how a cell functions to understand why most cells are very small, and why multicellular organisms are comprised of many, many microscopic cells, rather than just a few enormous ones.

Each cell needs to perform a number of functions while maintaining a pretty constant internal environment. Cells must exchange materials with the external environment, and undergo any number of chemical reactions, each with specific chemical requirements, in order to stay alive and do their jobs. The more things needed in a cell, the more exchanges have to occur through the membrane. If the volume of a cell becomes too large, there is not enough membrane surface area to accomplish all that needs to be done.



Total surface area (height × width × number of sides × number of boxes)	6	150	750
Total volume (height × width × length × number of boxes)	1	125	125
Surface-to-volume ratio (surface area / volume)	6	1.2	6

The overall limit to cell size seems to be this surface area/volume ratio. As the volume of a cell increases, the cell has proportionally less surface to exchange nutrients, gases and wastes with its environment to sustain the increasing volume. Within the cytoplasm, materials move by diffusion, a passive physical process that can work only for short distances. A large volume would inhibit the rate of movement too much for cells to function. However, cells with minimal metabolic needs can have larger volumes.

Some exceptions are:

- The yolk of a bird is a single cell.
- Some nerve cells run from the spine to the toes of mammals (although the diameter is small and they are microscopic, maintaining a good surface area to volume ratio.)
- Some green algae, such as *Caulerpa* and *Acetabularia* (Kingdom, Protista) have huge cells, and often are multinucleate.

Cell Types and Living Organisms

Every organism is composed of one of two fundamental types of cells: **prokaryotic** or **eukaryotic**.

The cells of **Prokaryotic** organisms do not have their genetic material enclosed within a membrane-bounded structure (no nucleus). Their DNA is concentrated in a region of the cell called the **nucleoid**. Prokaryotic cells also **do not have** membrane-bounded organelles within the cytoplasm of their cells.

The DNA of the cells of **eukaryotic** organisms is contained within a **nucleus**. The nucleus is surrounded by the cytoplasm of the cell, much of which is the semi-fluid matrix, the cytosol, in which organelles are suspended.

As you read in chapter one of your text, the world of life is currently organized into three domains and several Kingdoms. Two of the domains, **Archaea** and **Bacteria** are prokaryotic. The Domain, **Eukarya**, whose members are composed of Eukaryotic cells, is comprised of the **Protista** (an alliance of several candidate Kingdoms) and the Kingdoms **Fungi, Plantae** and **Animalia**.

Brief Review of Domains and Kingdoms – The Diversity of Life

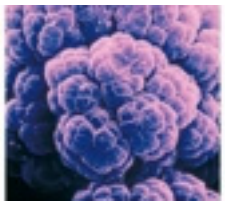
As mentioned in the introductory chapter, the world's organisms are currently organized into Domains and Kingdoms. Two of the Domains, **Bacteria** and **Archaea,** have a prokaryotic cell organization. The Domain, **Eukarya**, is comprised of four Kingdoms: **Protista**, **Fungi, Plantae** and **Animalia**, whose members have a eukaryotic cell organization.

The Domains and Kingdoms are also distinguished from each other on the basis of how they obtain the organic molecules needed for life. **Autotrophs** are organisms that can manufacture their organic molecules from inorganic carbon sources. **Heterotrophs** require organic sources of carbon for their organic molecules.

Current research indicates that the Kingdom, Protista, is likely to be separated into several kingdoms, as will the Bacteria and Archaea.

In brief:


Bacteria


Archaea

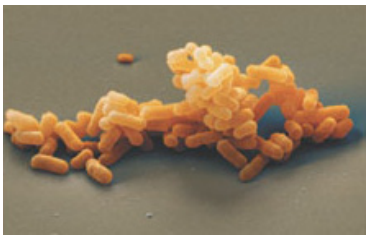

Protista


Plantae


Fungi

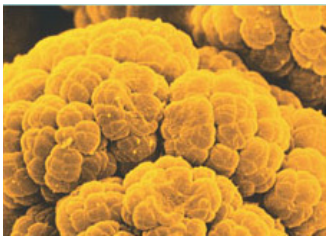

Animalia

Prokaryotic Organisms



Domain and Kingdom Bacteria (Eubacteria)

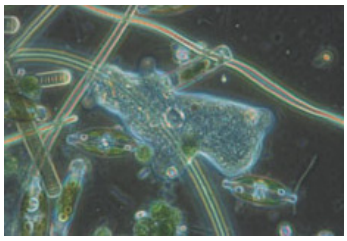
- Cell walls contain peptidoglycan
- Some are autotrophs (photosynthetic or chemosynthetic), but most are heterotrophs (obtain carbon source by processing organic molecules)
- Include Bacteria and Cyanobacteria
- Probably the original organisms on earth



Domain and Kingdom Archaea

- Diverged early in evolution, based on ribosomal RNA studies
- Their cell walls **do not** contain peptidoglycan
- Possess unique common DNA sequences (signature DNA)
- They have unique membrane lipids and rRNA.
- Biochemical versatility in obtaining nutrients (Methanogens)
- Often restricted to harsh environments (Halophiles, Thermophiles)

Eukaryotic Organisms (Domain Eukarya)



Protista

- Organisms that lack "true" tissue development
- Have a variety of means of nutrition
- The unicellular Protista probably had multiple "origins" and are currently being organized into multiple Kingdoms


Plantae

- Photosynthetic Multicellular Autotrophs
- Obtain inorganic materials from the external environment and process them into the organic compounds needed for life.
- Cells secrete a cell wall, comprised of cellulose, exterior to the plasma membrane

Fungi

- Multicellular Heterotrophs
- Obtain organic materials from the external environment and assimilate them for their needs
- Cells secrete a cell wall exterior to the plasma membrane comprised of chitin

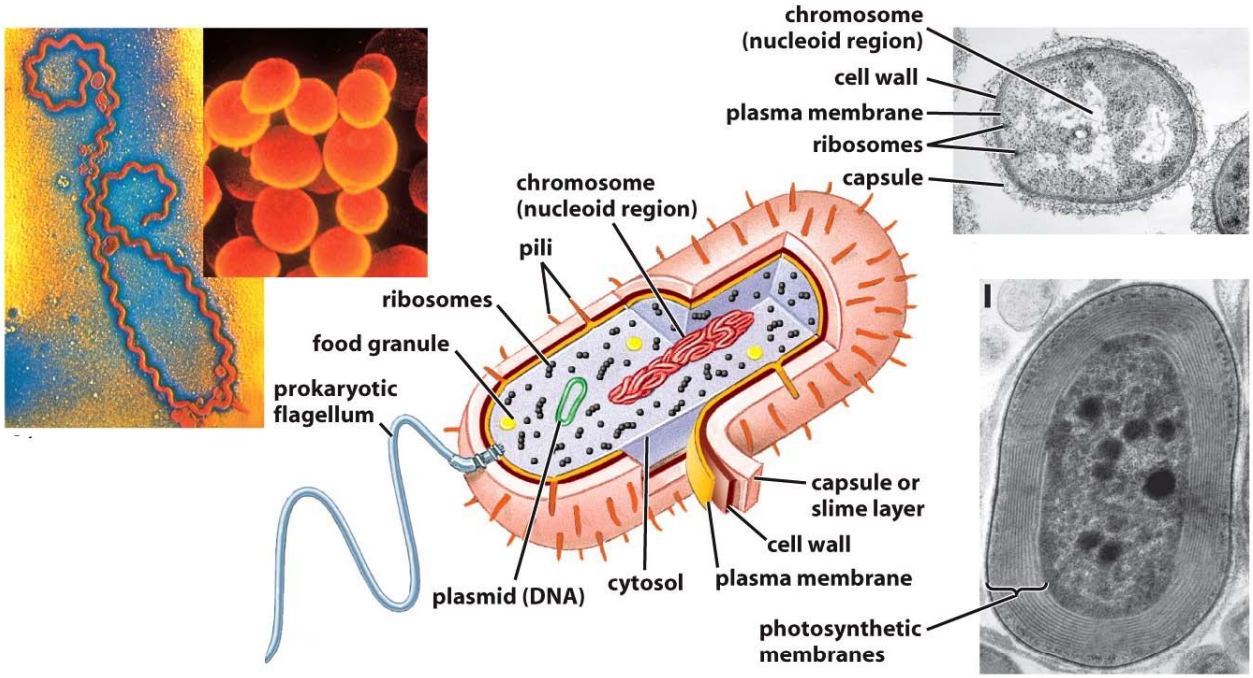
Animalia

- Multicellular Heterotrophs
- Cells lack a cell wall

Most of what we discuss in our introductory Biology class refers to the eukaryotic cell and eukaryotic organisms. Microbiology focuses extensively on bacteria, the major group of prokaryotic organisms. We will take just a little time to discuss the distinguishing feature of prokaryotic cells.

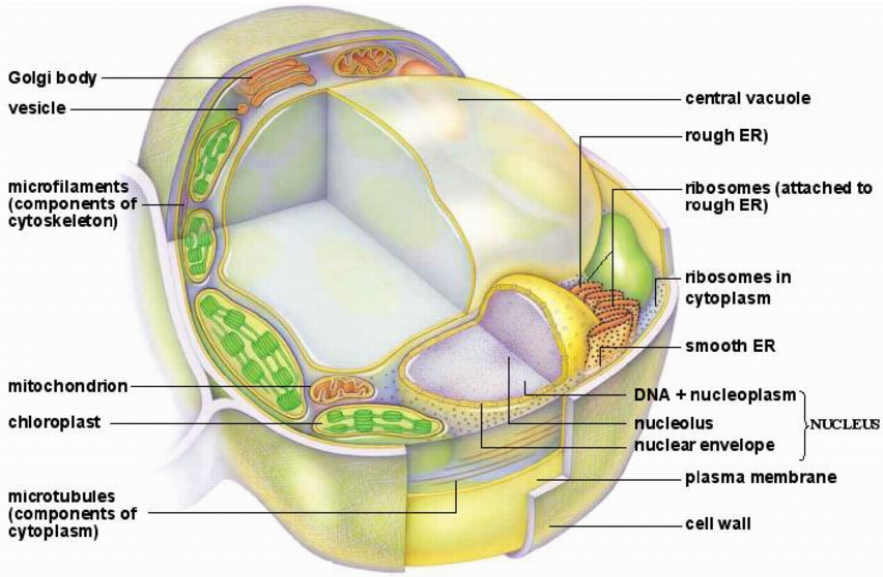
Features of Prokaryotic Cells

- Generally very small and relatively simple (less than 5 μm in diameter.
- External Features
  - Boundary is the plasma membrane
    May have membrane infoldings called mesosomes
  - Rigid wall composed of a unique substance, called **Peptidoglycan**, found only in the walls of the Bacteria (and absent in the Archaebacteria)
    May secrete a slime sheath or capsule for protection
  - May have motile structures called **flagella,** but they are different from the flagella of eukaryotic cells. Their motion is caused by basal rotors.
  - Some have tiny protein projections called **pili**, which help to attach bacteria to surfaces. Some hollow pili are used to transfer genetic material.
- Interior of Prokaryotic Cell
  - Single DNA molecule (circular), concentrated in one area of the cytoplasm called a **nucleoid**. The DNA is not surrounded by protein. Bacteria may have more than one copy of the DNA molecule.
  - May have **plasmids**, independent DNA fragments that carry specific pieces of genetic information. Plasmids can be transmitted from one bacterium to another, or from the environment to a bacterium. Plasmids are important in recombinant DNA research.
  - Cytoplasm
  - Ribosomes, composed of RNA and protein, of 70s density.
  - **NO** internal membrane-bounded structures (organelles)

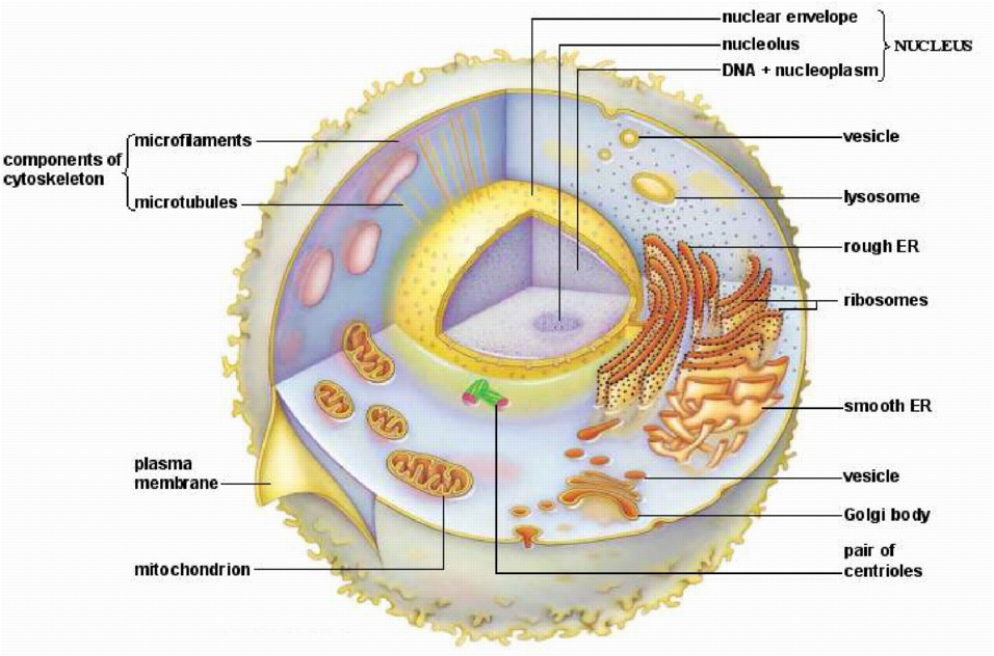


Features of the Eukaryotic Cell

- Eukaryotic cells have a system of internal membrane-bounded structures, called **organelles.**
- Nucleus bounded by the nuclear envelope (Eukaryotic means true nucleus)
- Cytoplasm of cytosol in which specialized organelles are suspended
  - Greater efficiency for cell activities
  - Organelles physically separate different types of cell activities in the cytoplasm space
  - Organelles also allow for separation of cell activities in time, to provide for sequential cell activities
  - May (plants, fungi and some protists) or may not (animals and some protists) secrete an external **cell wall**



Generalized Plant Cell



Generalized Animal Cell

Eukaryotic Cell Components

Nucleus
- Nuclear Envelope
- Chromatin - Chromosomes
- Nucleolus
- Ribosomal sub units (function in cytoplasm)

Cytoplasm of
- Cytosol (fluid matrix)
- Organelles
  - Endomembrane System (Internal Membranes)
    - Nuclear Envelope
    - Rough Endoplasmic Reticulum
    - Smooth Endoplasmic Reticulum
    - Golgi Complex
    - Associated Vesicles and "—somes"
      - Lysosomes
      - Peroxisomes
  - Other Organelles
    - Mitochondria
    - Central Plant vacuole
    - Other Vacuoles
    - Plastids
      - Chloroplast
      - Amyloplast
      - Chromoplast
  - Cytoskeleton (Internal Skeleton)
    - Microfilaments
    - Intermediate Filaments
    - Microtubules
      - Centrioles
      - Cilia and Flagella
      - Basal Bodies

External Structures
- Cell Wall
- Cell Junctions
  - Plasmodesmata
  - Tight Junctions
  - Desmosomes (Anchoring Junctions)
  - Gap Junctions (Communicating Junctions)

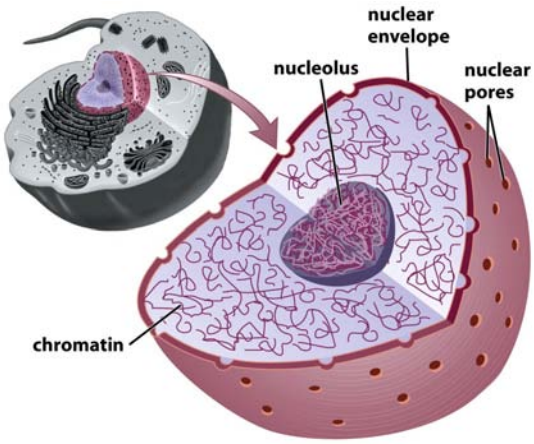
Nucleus

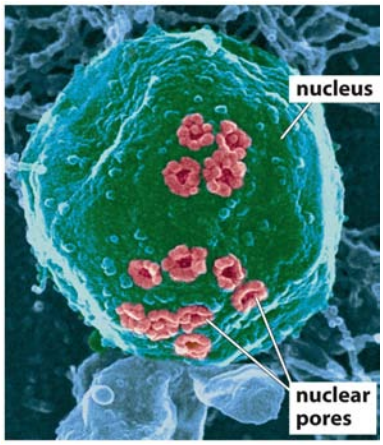
The nucleus is generally the largest or most "conspicuous" (except for when students are trying to find one) structure within the eukaryotic animal cell. In mature plant cells, the central vacuole, which you usually cannot see, takes much more of the volume. The nucleus is spherical and quite dense.

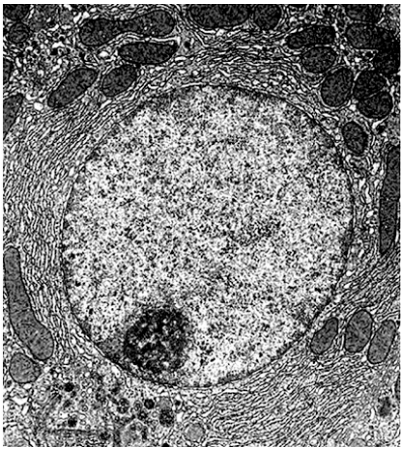
Nucleus Functions

- Contains and stores the genetic information, DNA, that determines how the cell will function, as well as the basic structure of that cell. (A few organelles: mitochondria and chloroplasts, do have some DNA, but the vast majority of a cell's DNA is contained within the nucleus.)
- Manufactures all RNA, including ribosomal, transfer and messenger RNA
- Duplicates the DNA of the cell prior to cell division

Nucleus Structure







Nuclear Envelope

The nucleus is bounded by the **nuclear envelope**, which is a double membrane

- Perforated with pores comprised of RNA and protein that provide channels for exchanging substances with the cytoplasm of the cell. In scanning electron micrographs the surface pores of the nuclear envelope are conspicuous.
- Proteins lining the pores determine which molecules can enter and leave the nucleus.
- The outer surface of the nuclear envelope is coated with ribosomes (see later).

Nucleus Contents

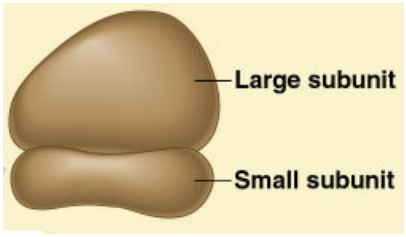
Chromatin

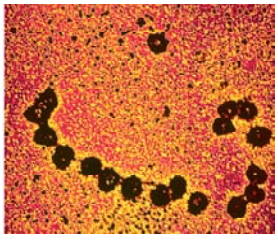
- Chromatin consists of **chromosomes**, long molecules of DNA, surrounded by proteins known as histones.
- Chromatin appears granular when observed with a microscope
- Each type of organism has a set number of chromosomes.
- Some examples:

  Mosquito = 6
  Lily = 24
  Human = 46
  Chimpanzee, Orangutan, Gorilla, and Potato =48
  Amoeba =50
  Horsetails = 216
  Adder Tongue Fern =1262

Nucleolus

- Small concentrated masses DNA, RNA and protein.
- Used in synthesis of **ribosome subunits**
  - **Ribosomes** are the site for the assembly of proteins.
  - Ribosomes consist of two subunits, and are composed of RNA and protein. In eukaryotes, the ribosomes are 80s, whereas the ribosomes of prokaryotes are 70s, one of the reasons some antibiotics are effective against bacteria, and don't harm us.





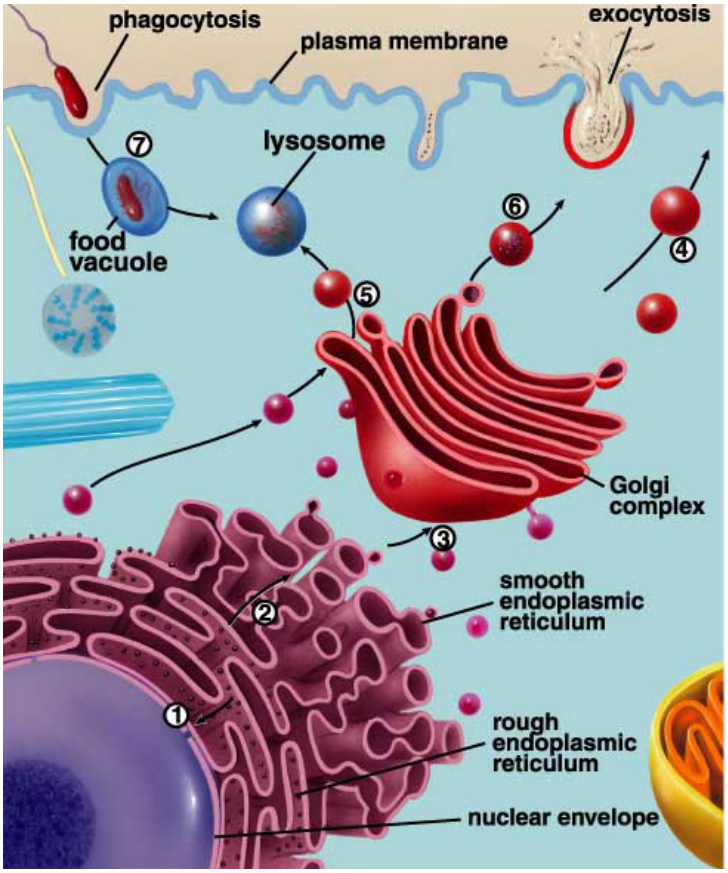
  - The genes needed for the manufacture of ribosomal RNA cluster in the nucleolus, where they direct ribosomal RNA synthesis. Ribosomal subunits are assembled in the nucleolus from rRNA and protein.
  - Completed ribosomal subunits move into the cytoplasm for functioning, where many sit on the surface of rough endoplasmic reticulum. Some ribosomes are located freely in the cytosol.

The Cell's Endomembrane System

Not only do membranes form the boundary of the cell, the plasma membrane, but within the cell we find a membrane system composed of a number of components, each of which may connect to the plasma membrane as well as to the nuclear envelope and to other internal structures. Small membrane fragments may be pinched off forming **vesicles** that are used for transport. Other membranes form vacuoles that hold things for periods of time. Lipids are formed within the endomembrane system and polypeptide chains of proteins are modified and translocated within the membrane system.

Endomembrane Components

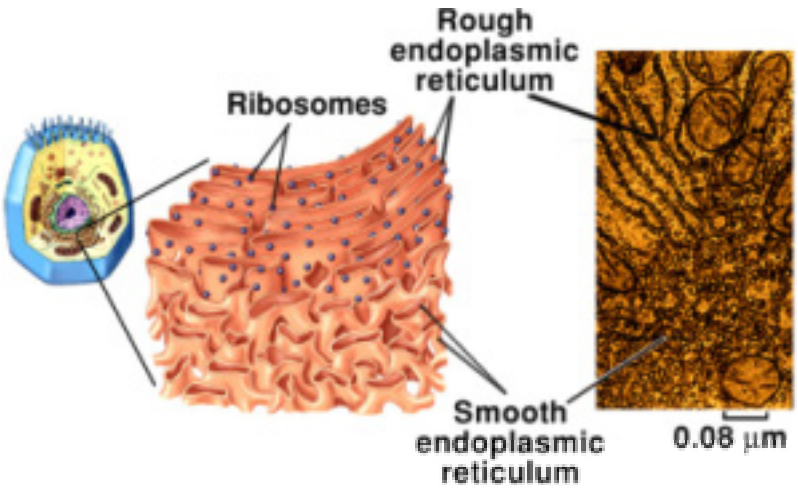
- Rough Endoplasmic Reticulum and associated Ribosomes
- Smooth Endoplasmic Reticulum
- Golgi Complex
- Lysosomes
- Assorted vesicles and vacuoles



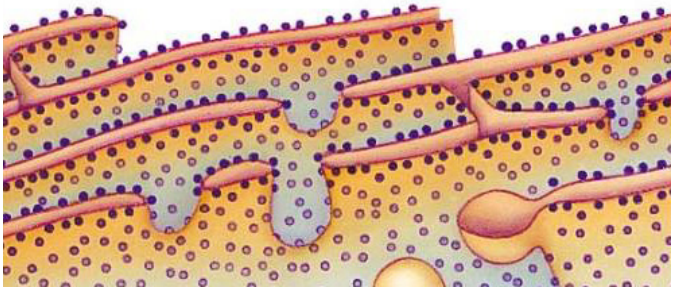
Some components of the Endomembrane System

Endoplasmic Reticulum

- Series of interconnected membrane-enclosed flattened tubes or channels and sacs that compartmentalize the cytoplasm, and run throughout the cytosol. Projections of endoplasmic reticulum connect the nuclear envelope with the endoplasmic reticulum and other projections connect to the plasma membrane.
- Endoplasmic reticulum synthesizes, transports and isolates intracellular contents
- There are two forms of endoplasmic reticulum: **smooth** and **rough**.



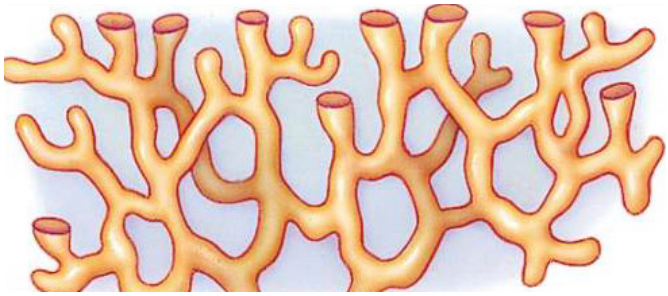
Rough Endoplasmic Reticulum

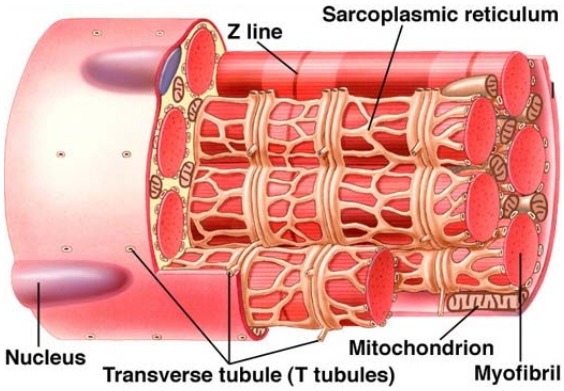


Endoplasmic reticulum that has ribosomes attached to its surface is called **rough endoplasmic reticulum.** This is most abundant in cells that secrete many proteins.

- Rough ER is often found in sheets or layers of flattened sacs within the cytoplasm.
- Rough ER has connections to the nuclear envelope
- Proteins synthesized at the ribosomes of cells that secrete proteins, such as digestive enzymes or hormones, transport the proteins through the rough endoplasmic reticulum channels.
- Small signal peptide sequences during protein synthesis are responsible for moving the polypeptide into the ER for modification and transport. Proteins accumulate in pockets in the ER that break off forming transport vesicles for export.
- Rough endoplasmic reticulum may synthesize itself, and also can be used to maintain and replace nuclear or plasma membrane as needed.

Smooth Endoplasmic Reticulum



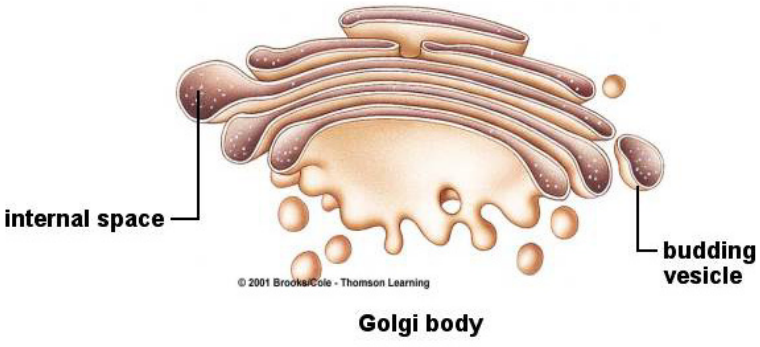


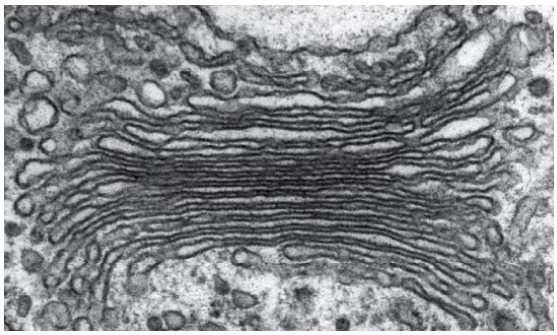
- Smooth endoplasmic reticulum is contiguous with rough, but lacks ribosomes.
- Many enzymes are associated with the surfaces of smooth ER.
- The enzymes needed to synthesize the phospholipids for membranes and the steroid hormones are found on smooth ER.
- Smooth ER is abundant in cells that produce lots of lipids.
- The sarcoplasmic reticulum of muscle tissue is a form of smooth ER. This contains the calcium reservoirs needed to trigger muscle contraction.
- Smooth ER in liver cells of animals contains enzymes for many of the liver's regulatory metabolic functions, including detoxifying alcohol.

Golgi Bodies

The Golgi complex consists of stacks of flattened disk-like membrane sacs that get materials from the endoplasmic reticulum. The Golgi functions as a processing center for materials to be packaged up and distributed in organelles or exported (secreted) from the cell in vesicles pinched off of the tips of the Golgi membranes. Digestive enzymes may be packaged for lysosomes and hormones are packaged into vesicles for secretions. Vesicles formed at ER migrate to the Golgi bodies, merge and pass through the Golgi and are packaged and labeled for export in Golgi vesicles.

Golgi bodies also modify materials prior to export. The carbohydrate portions of glycoproteins, for example, are added in the Golgi body. Polysaccharides are often manufactured in the Golgi, particularly cell wall components.





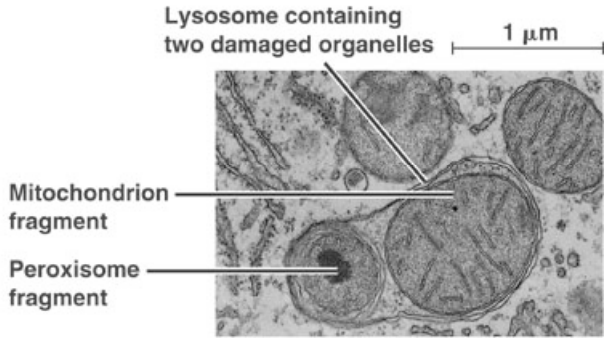
Vesicles: Lysosomes and other "—somes"

Golgi Vesicles

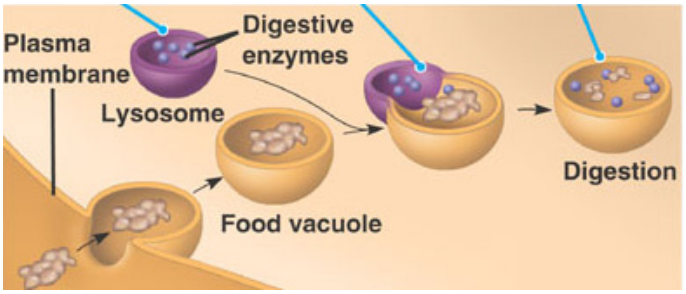
Most Golgi vesicles are temporary structures, formed to transport manufactured molecules for export from the cell. Vesicles may also be formed at the plasma membrane for import of substances into the cell, a process called pinocytosis, which we will discuss later. Other vesicles form organelles, each containing enzymes needed for specialized functions within the cell.

Lysosomes contain hydrolytic enzymes, which can breakdown carbohydrates, proteins, nucleic acids, and many lipids.

- Lysosomes are manufactured from enzymes and membranes of the rough ER and packaged in the Golgi complex.
- The Lysosome is responsible for disassembly or breakdown of cell components when no longer needed or when damaged or in need of recycling. It is a normal part of cell maintenance and renewal.
- Lysosomes can also destroy or degrade bacteria and foreign substances. Macrophages for example, contain large numbers of lysosomes.
- Some protists, such as *Amoeba* feed by a process of phagocytosis. The food vacuole formed merges with lysosomes for digestion.
- Plant cells do not need lysosomes; breakdown products of plants are stored in their central plant vacuoles (see later)



Lysosome



Phagocytosis and Food Vacuole formation

Peroxisomes

Peroxisomes contain enzymes that transfer hydrogen in biochemical reactions to oxygen, forming hydrogen peroxide as a by-product. Since H_2O_2 is toxic, peroxisomes also contain an enzyme, catalase, which breaks down the H_2O_2.

Glyoxysomes

Plant cells, especially in seeds, contain **glyoxysomes**. These cells store oils so that the germinating seed has a fuel supply. During germination, the fatty acids are converted to sugar molecules for the rapid cell respiration needed for successful germination and seedling establishment.

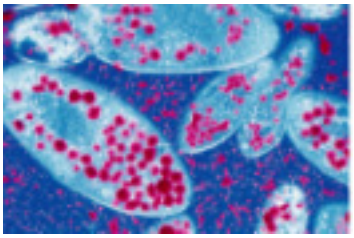
Vacuoles

Vacuoles are also membrane-bounded sacs that hold something (good definition). Vacuoles contain a variety of substances, such as food, wastes, water, etc. Some vacuoles are permanent structures of cells, such as those involved with water balance in protists and the central plant vacuole of plant cells. Others are temporary, such as food and waste vacuoles.

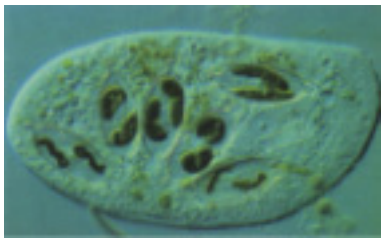
We will mention three vacuoles: **food vacuoles, contractile vacuoles** found in protists, and the **central plant vacuole.**

Food Vacuoles

Organisms that feed by **phagocytosis** surround their prey item with a portion of their plasma membrane, and engulf the item by fusing the membrane around it and moving the now "food vacuole" into the cytosol. Once in the cytoplasm of the cell, the food vacuole is merged with lysosomes for digestion. Digested nutrients are moved into the cytosol for use, and non-digested materials are formed into a waste vacuole that is removed from the cell by a more-or-less reverse process to the initial engulfing.



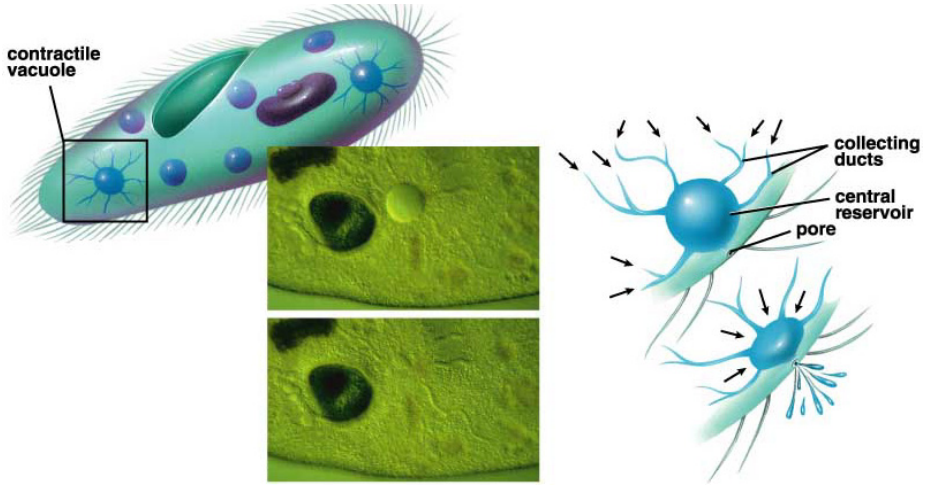
Paramecium **Food Vacuoles filled with carmine-dyed yeast**



Chilodinella **Food Vacuoles containing Diatoms**

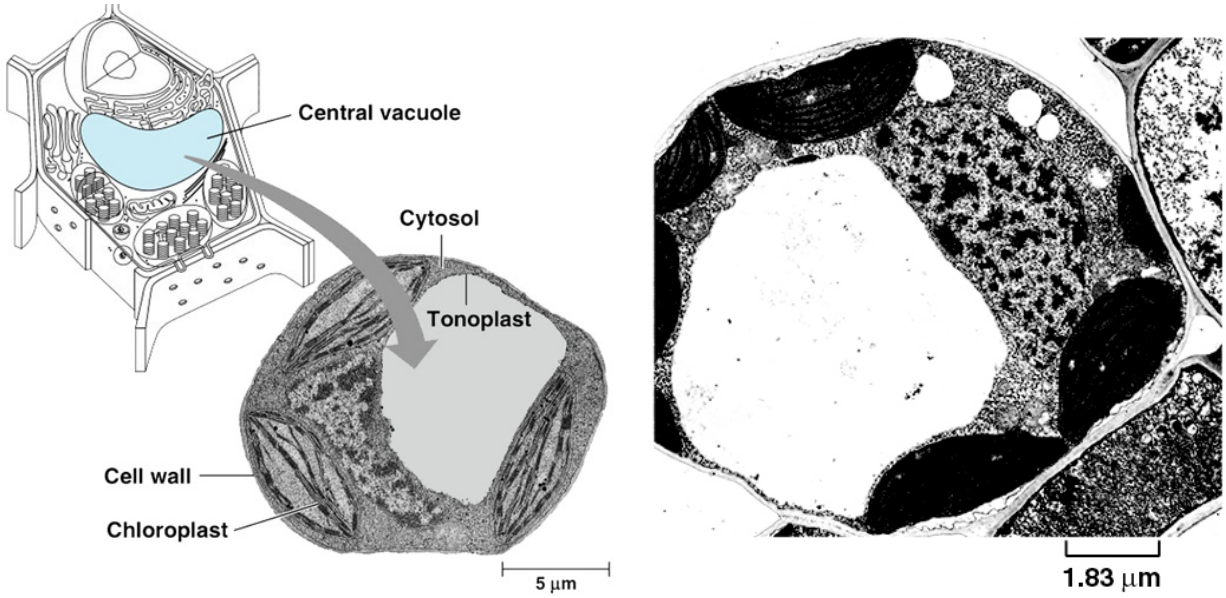
Contractile Vacuoles

Most terrestrial organisms risk dehydration, evaporating water to their surroundings. In contrast, fresh water organisms are in an environment where water tends to move into their cells. Many fresh water protists have **contractile vacuoles,** structures that collect the water moving into their cell from the environment, and periodically expel the collected water to the external environment by contracting the vacuole though a pore, hence the name, contractile vacuole.



Central Plant Vacuole

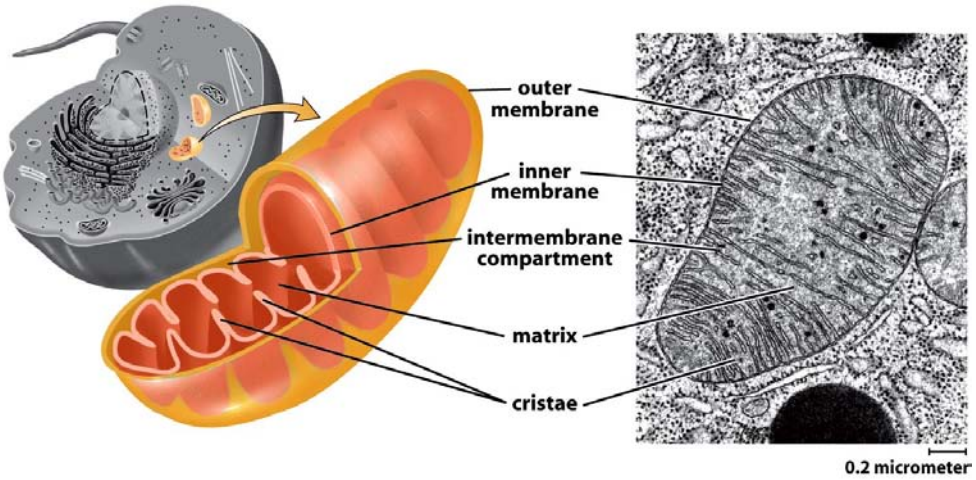
All living, mature plant cells have a large membrane bounded organelle, filled with fluid, called the **Central Plant Vacuole**. The central vacuole occupies as much as 90 - 95% of the volume of the mature cell. The membrane of the vacuole is called the **tonoplast.** The tonoplast is poorly permeable to water and water soluble materials.



Functions of the Central Plant Vacuole

- Stores metabolic products including:
  - many ions and nutrients, such as glucose, amino acids, potassium and chlorine
  - the plant's water soluble pigments (the anthocyanins, including the beet pigment, betacyanin)
  - toxic substances
  - secondary metabolites and, some of which serve to defend the plant against unwanted munching by predators
- Stored substances in the vacuole attract water that increases fluid pressure within the vacuole. This pressure is known as **turgor pressure** and is important in increasing plant cell size and surface area during cell growth. This pressure also forces the cytoplasm against the plasma membrane and cell wall, helping to keep the cell rigid, maintaining a condition of **turgor.** Turgor provides support and strength for herbaceous plants and other plant parts lacking secondary cell walls. When plant cells lose turgor, they wilt, a condition known biologically as **plasmolysis**. "Permanent wilt" is a botanical euphemism for death.

Other Organelles
Mitochondria



Function of Mitochondria:

- Mitochondria contain the enzymes needed to obtain energy stored in carbohydrate and other fuel molecules and use that energy to form **ATP**, the molecule needed to do cell work.
- These processes are a part of aerobic cell respiration, specifically known as the **Krebs Cycle** and **Electron Transport.** We will devote some time to the discussion of these vital metabolic processes of cell respiration in our next unit!

Structure of Mitochondria

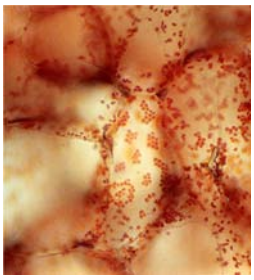
- The mitochondrion has a double membrane system; the outer membrane is smooth; the inner membrane is deeply folded and convoluted, forming **cristae**.
- The double membrane of the mitochondrion forms two compartments filled with fluid: The **intercompartment space** is between the outer membrane and the cristae, and the central **mitochondrial matrix** is formed by the inner cristae membrane. This arrangement facilitates the functions of the mitochondria.
- Cells may have few to many mitochondria, depending on the energy requirements of cell.
- Mitochondria contain their own DNA and ribosomes and can self-replicate. Evidence indicates that they originated as endosymbiont bacteria.

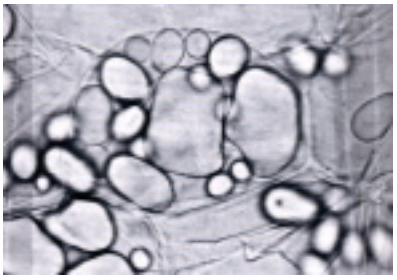
Plastids

Plastids are found in the cells of plants. Animal cells do not contain plastids. In general, a plastid is a membrane-bounded organelle that stores something. (There are actually a number of structures identified as "storing something, such as vesicle, and as we shall see, vacuoles.) There are three common plastids: **chloroplast**, **amyloplast** and **chromoplast**.

Chromoplasts

- Chromoplast means pigmented plastid.
- Store the plant pigments (notably the yellow, orange and red carotenoids) that are not water soluble, and not involved in photosynthesis
- Chromoplasts are abundant in orange, golden and scarlet pigmented regions of plants.


Chromoplasts

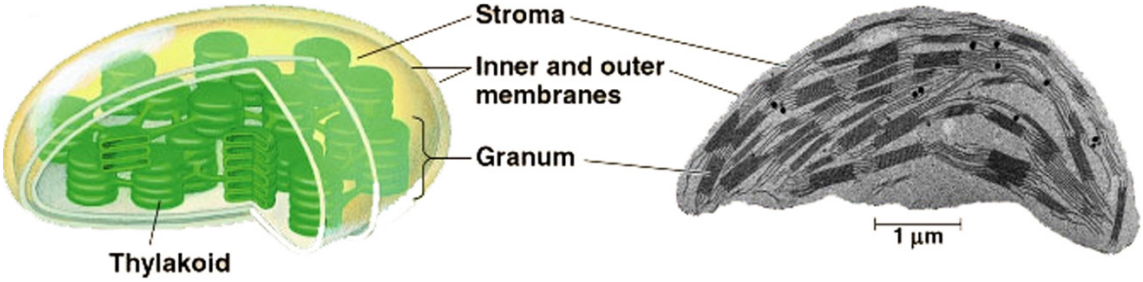

Amyloplasts

Amyloplasts

- Amyloplasts store starch, which is unpigmented. (There is a general term, **leucoplast**, which means unpigmented plastid, but is not as descriptive as amyloplast, which identifies what is stored in the plastid). Amyloplasts are also called starch grains, but not by biology students who know the correct term.
- Amyloplasts vary in size depending on how much starch is being deposited. They are also species specific in overall design; a specialist can identify the source of starch grains.
- Amyloplasts are abundant in the storage cells of most plants.

Chloroplasts

Chloroplasts contain the pigments, including chlorophyll, and the enzymes necessary for **photosynthesis**, the process by which light energy is converted to chemical energy, which is used to manufacture carbohydrate (fuel) molecules. Chloroplasts are found in plants and in some protists. Chloroplasts are not found in heterotrophic organisms. Some bacteria have chlorophyll and can photosynthesize, but lack the membrane-bounded chloroplasts. Some bacteria also have photosynthetic pigments other than chlorophyll.



Typical Chloroplast Structure:

The plant chloroplast is a double-layered membrane bounded organelle, with an inner compartment that contains more membranes. The outer and inner membranes are smooth, and oval shaped in higher plants.

- The internal membranes are disc like in structure and called **thylakoids**. These flattened discs stack up to form **grana.** The photosynthetic pigments are arranged on the grana.
- The fluid in which the grana are suspended is called the **stroma**

This distinctive structure is important for the multiple processes, which occur during photosynthesis, a process that we will discuss in great detail later. Like mitochondria, chloroplasts contain unique DNA and may have evolved similarly as endosymbionts.

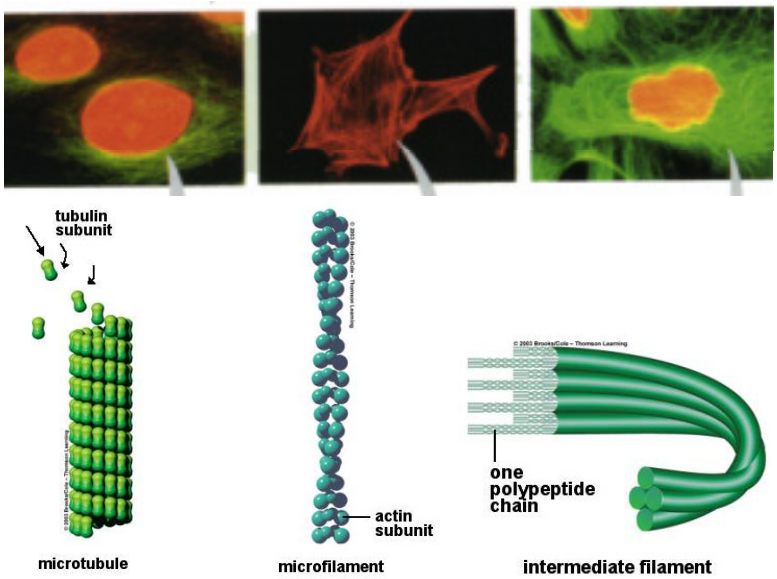
Cytoskeleton

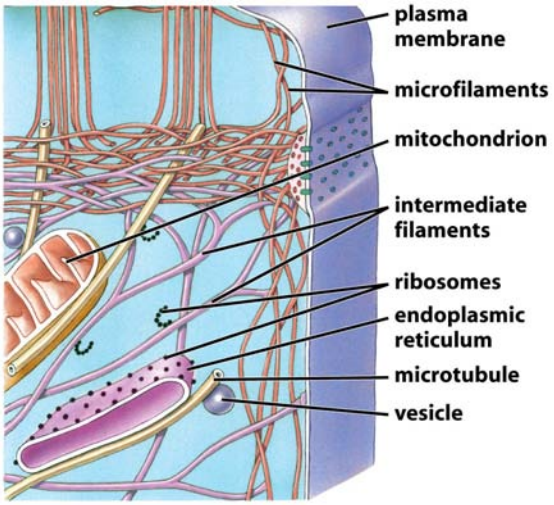
The cytoskeleton is the internal, fibrous framework of cells. Many organelles and some enzymes are organized along this framework.

- The cytoskeleton maintains the shape of cells (animal) by its architectural design and anchors organelles within the cytosol.
- The cytoskeleton is responsible for motility within cells, such as muscle contraction and cyclosis, the internal motion of the cytoplasm.
- Organelles may be transported along cytoskeletal tracks within the cytosol
- Vesicles are moved along cytoskeletal tracks for both import and export.
- The cytoskeleton can also be responsible for motility of cells and external movement such as the amoeboid movement of white blood cells and the migration of cells during development.
- The cytoskeleton also has a role in cell division.

Components of the cytoskeleton

- Microtubules
- Microfilaments (Actin Filaments)
- Intermediate Filaments





Microtubules

- Hollow cylindrical tubules composed of **tubulin**, a dumbbell shaped protein.
- Can generate movement as microtubule aggregates slide past one another.
- In animal cells, microtubules develop from the centrosome or **microtubule organizing center**. The growth of microtubules is determined by the tubulin organization and accessory proteins that cap microtubules to stabilize them.
- Some plants produce toxins that inhibit the production of animal microtubules, blocking cell division. This is an excellent plant defense. Taxol, a terpene produced by the Pacific yew, is used in cancer treatment.
- Microtubules form the **spindle apparatus,** which separates chromosomes during cell division with the assistance of **kinesins**, motor proteins.

Microfilaments

- microfilaments are tiny solid fibers of coiled globular protein, actin.
- Functions
    - Help maintain cell shape along with microtubules.
    - Microfilaments often form a sub-plasma membrane network to support the cell's shape.
    - Muscle contraction (actin filaments alternate with thicker fibers of myosin, an associated motor protein, in muscle tissue)
    - Cyclosis (the movement of cytoplasm contents within the cell).
    - "Amoeboid" movement and phagocytosis.
    - Responsible for the cleavage furrow in animal cytokinesis

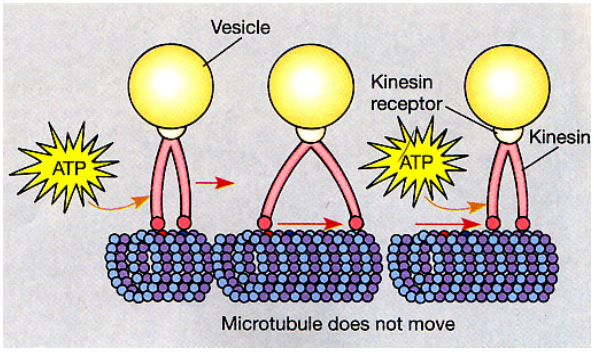
Intermediate Filaments

- Made of fibrous protein forming a solid rope structure
- Intermediate filaments are composed of **keratins.** There are several different keratins.
- Intermediate filaments tend to be fixed in position within the cell, rather than being more "mobile" or transitory as microfilaments and microtubules are.
- Functions
    - Anchor for other cell components, particularly the nucleus
    - Are important in cell attachments (desmosomes)
    - Reinforce cells under tension, maintaining shape.
    - Form the nuclear lamina (a layer beneath the nuclear envelope)

Locomotion and the Cytoskeletal System

Motor Proteins

Most cellular locomotion is generated by motor proteins that associate with cytoskeleton microfilaments or microtubules. M**yosin** is a motor protein that works with actin microfilaments in muscle contraction. Two other motor proteins, **dyenin** and **kinesin** generate locomotion along microtubules for vesicle and organelle transport.

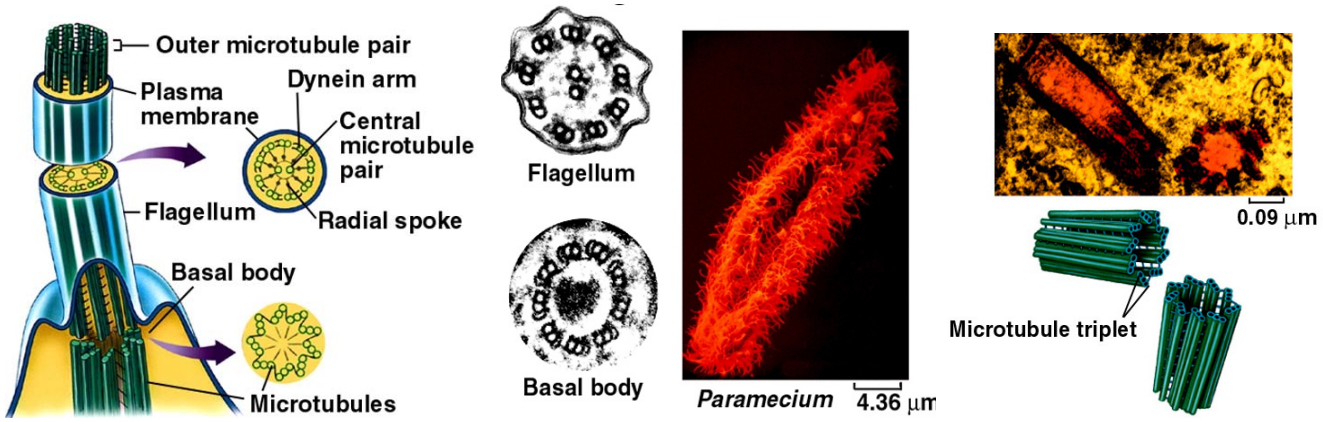


Motor proteins use energy to generate movement. This energy supply is provided by phosphates from the energy molecule, ATP.

Cilia and flagella

Many cells can also generate external locomotion, either by moving their body through the medium, or by moving substances past the surface of their cell. Such locomotion is generated by **cilia** and **flagella**, structures formed from microtubules that are embedded in and extend through the plasma membrane into the external environment. They are coated with plasma membrane material.

Eukaryotic cilia and flagella have an arrangement of microtubules, known as the 9 + 2 arrangement (9 pairs of microtubules (doublets) around the circumference of the cilium and 2 central microtubules).



Flagella and Cilia

Centrioles

Cilia are generally small in length, and a ciliated cell will have many cilia. Flagella are relatively long, and cells will have one or very few.

Cilia and flagella may originate from **centrioles**, also composed of microtubules. Centrioles consist of 9 groups of 3 microtubules (9 X 3 arrangement). Centrioles are self-replicating. A flagellum or a cilium is formed from a **basal body**, which is identical to centrioles and is embedded in the plasma membrane. There is a transition zone where the two microtubules of the cilia/flagella join a third microtubule forming the basal body ring.

(Some prokaryotic cells also have flagella, but their structure and mode of generating motion are very different from the eukaryotic flagella.)

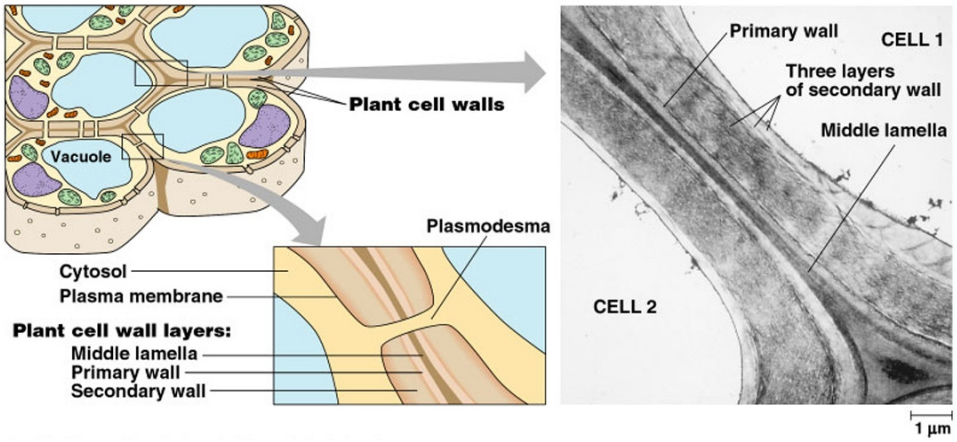
Pseudopodia

External locomotion can also be generated by internal microfilaments that align to form lobes of cytoplasm called pseudopodia. Pseudopodia project outward in one direction while other regions of the cell contract, generating movement. Pseudopodia are also used to surround and capture prey, a process called phagocytosis. The protist *Amoeba* and white blood cells move by pseudopodia and feed by phagocytosis.

The Cell Surface – External Structures

Although the boundary of any cell is its cell or plasma membrane, the cells of many types of organisms, including plants, fungi, bacteria and many protists, have one or more **rigid surface layers** or a **cell wall** exterior to the plasma membrane. (Only animals and some protists and bacteria lack walls.) Cell walls are secreted by the cell they surround, and are composed of a number of different kinds of materials, depending on the Kingdom or Domain. Cell walls support, protect and provide shape to the cells they surround. Let's look a bit at the wall structure of plants.

Plant Cell Wall Layers



Primary Wall

- All plant cells secrete a primary cell wall exterior to the plasma membrane.
- The primary wall is composed extensively of **cellulose**, a polysaccharide of glucose discussed previously. Cellulose is non digestible for most organisms. It is the main constituent of wood, pulp and paper, and fabrics such as cotton, linen, bamboo and hemp. It is also the bulk of the "fiber" of our diets.

Secondary Walls

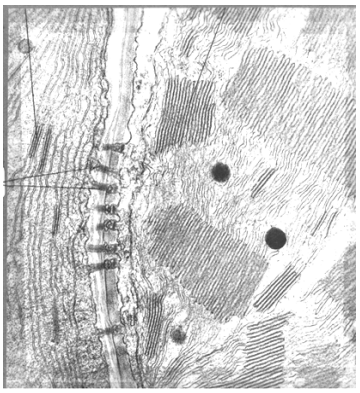
- Secondary walls consist of layers secreted interior to the primary wall and provide additional strength to those cells.
- Secondary walls will have **lignin** as well as cellulose and other polysaccharide materials and are important in woody tissues, and in the support and conducting tissues of plants

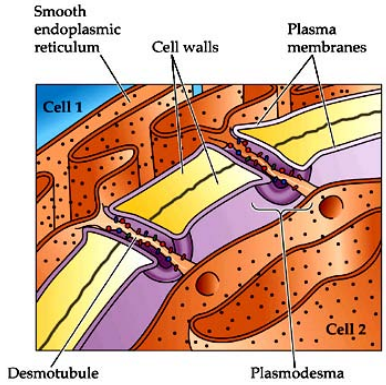
Middle Lamella

When plant cells divide, vesicles secrete pectins (Calcium pectate) and other "gummy" substances between the adjacent cell membranes of the divided cell. This material forms the **middle lamella** that "glues" plant cells together (Unlike membranes, walls are not sticky.)

Plasmodesmata

Plasmodesmata are membrane connections (little channels) between adjacent plant cells that pass through the wall layers. Plasmodesmata provide for intercellular cytoplasm communication.

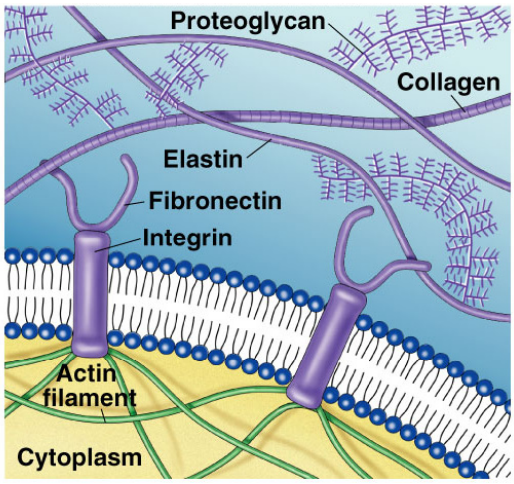




Plasmodesmata

Extracellular Matrix

The surfaces of animal cell membranes have a variety of substances that are attached to the plasma membrane. These materials form the **extracellular matrix.** Many of the molecules of the matrix are **glycoproteins**. **Collagen** fibers are typically embedded into a network in the matrix, and provide strength. Elastin fibers provide flexibility. The matrix molecules are attached to special membrane proteins. This network is important in cell communication.

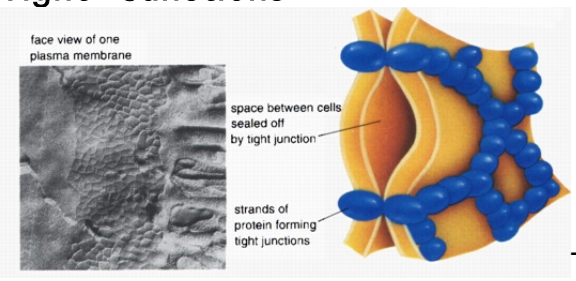


In addition to the extracellular matrix of animal cells, the plasma membranes of all cells have receptor proteins, molecules that chemically detect signals from other cells or chemicals in the environment. For example, hormones often "work" by binding to receptors, or passing through protein channels and binding to chemicals within the cell to direct specific cell functions. Such chemical communications are vital to the functioning of all cells and organisms. We will discuss the membrane proteins in more detail soon.

Cell-to-Cell Attachment and Communication

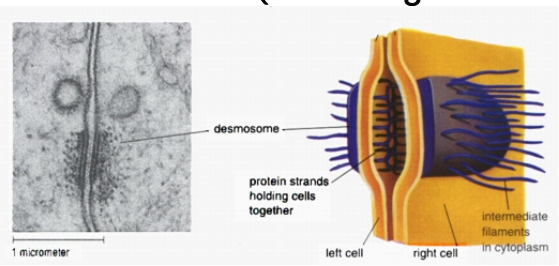
Cells typically have methods of "physically" communicating with adjacent cells using a variety of cell membrane connections. These connections are often called **cell junctions**. There are three common types of cell junctions found in animal cells, as well as the plasmodesmata of plant cells just discussed.

Tight Junctions



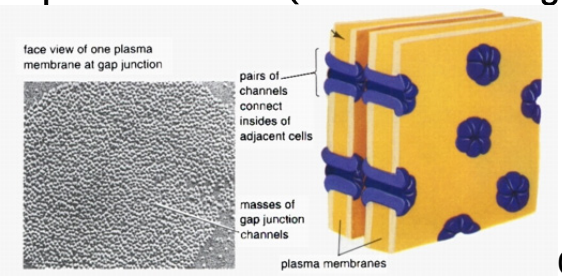
Tight junctions are composed of protein fibers that seal adjacent cells to prevent leakage, something that can be useful in organs such as the bladder and the lining of the digestive tract. Tight junctions literally fuse the cells together forming a sheet of cells that can restrict molecules to one side of the sheet or the other.

Desmosomes (Adhering Junctions)



Desmosomes anchor adjacent cells together by making connections that work like staples or rivets that attach to components of the cytoskeleton. Many epithelial cells must adhere to adjacent membranes to prevent free passage or free movement, and to not break apart under stress. Desmosome filaments are composed of specialized glycoproteins. Intermediate filaments of keratin in the desmosomes help strengthen the junction. Actin microfilaments can also attach to desmosomes, but have less strength.

Gap Junctions (Communicating Junctions)



Gap junctions are **protein channels** between adjacent cells that permit the transfer of small molecules between the cells. They are common in brain cells, forming the synapse, in many glands, and in cells in the heart muscle that coordinate contraction for heartbeat. The plant **plasmodesmata**, discussed previously, are a plant communicating junction.

The Cell Membrane and Interactions with the Environment

Cells interact with their environment in a number of ways. Each cell needs to obtain oxygen and other nutrients (carbohydrates, amino acids, lipid molecules, minerals, etc.) from the environment, maintain water balance with its surroundings, and remove waste materials from the cell. The boundary between any cell and its environment (through which substances must pass) is the **plasma membrane**, composed of phospholipid and protein molecules.

Plasma Membrane

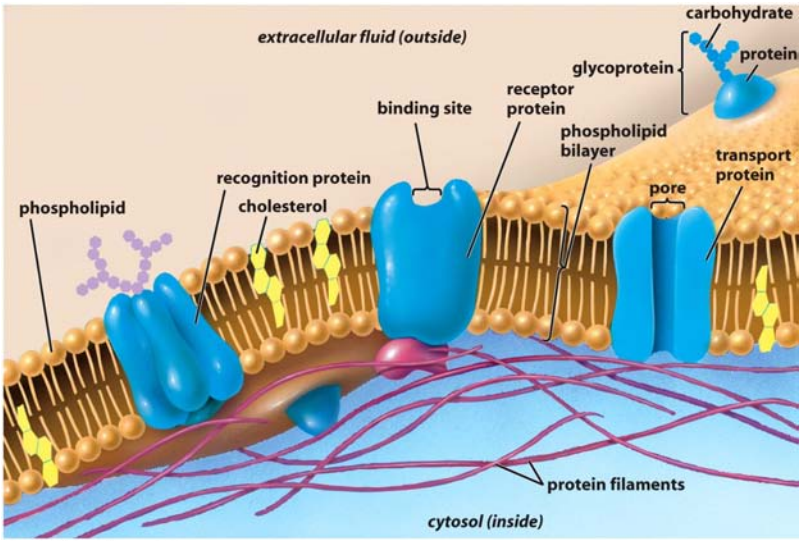
The plasma membrane has a number of functions for a cell.

- Serves as the boundary between the cytoplasm of the cell and the external environment, and selectively isolates the cell from the external environment.
- Maintains the cell's environment by regulating materials that enter or leave the cell. (Anything that enters or leaves the cell must pass through the membrane). We often say that a membrane is selectively or differentially permeable for this reason.
- Provides mechanisms for cell-to-cell communication.
- Provide mechanisms for cell-to-cell and within the cell attachments.
- Genetically unique cell recognition markers embedded in the plasma membrane provide mechanisms for a cell to recognize itself and other cells of its particular individual organism versus non-self (foreign materials). This is important to the immune system and defense of the organism.

Although the plasma membrane forms the boundary of the cell, and surrounds the cell, many internal structures of most cells also have their own membrane boundaries. Much of what we say about membrane structure and function at this time applies to all membranes.

The Fluid Mosaic Membrane Structure

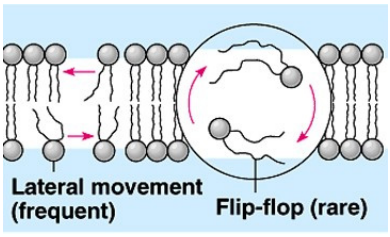
The typical membrane structure consists of a **phospholipid bilayer** with a number of proteins scattered throughout, along with some carbohydrates (glycoproteins), glycolipids and sterols, similar to the way in which one does a mosaic tile, hence the name.



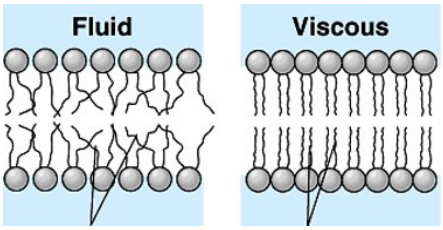
Phospholipid Bilayer

A phospholipid has both polar and non-polar regions. The fatty acid "tails" of the two phospholipid layers are oriented towards each other so that the hydrophilic "heads", which contain the phosphate portion, face out to the environment as well as into the cytoplasm of the cell's interior, where they form hydrogen bonds with surrounding water molecules. Because the individual phospholipid molecules are not bonded to each other, a membrane is flexible (or “fluid”), something which is pretty important to its functions.

The fluidity of a membrane is crucial to its function. In caribou, circulation is reduced in the lower legs to prevent excess heat loss during cold winters. The membranes of the lower legs have more unsaturated fatty acids than those of the upper legs to retain more fluidity in reduced temperatures. Brain cell membranes in ground squirrels become more solid during hibernation. Phospholipids containing more polyunsaturated fatty acids are more fluid than those with fewer polyunsaturated fatty acids. Cholesterol in membranes reduces fluidity as well. There are times when membranes need more or less fluidity, and molecular composition provides for sure membrane flexibility.



Phospholipid Movement



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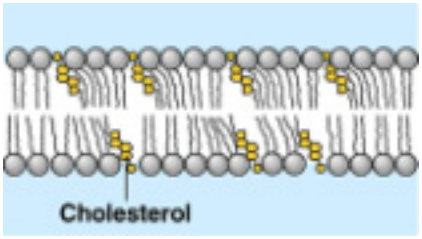


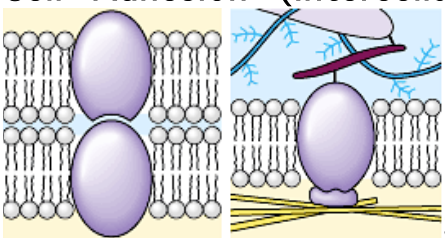
With Cholesterol

Many materials that enter or leave the cell are water-soluble; the fatty acid layers serve as a barrier to their free entry. Proteins in the membrane are required to move these substances through the membrane. Lipids generally pass through the membrane more easily.

Membrane Proteins

The membrane proteins have a number of functions. Some are embedded in the phospholipid layers; others move (literally) throughout the membrane layers. Other membrane proteins are complexed to carbohydrate molecules, forming glycoproteins. Generally, there are a number of different membrane proteins.

Cell Adhesion (Intercellular Joining) Proteins.

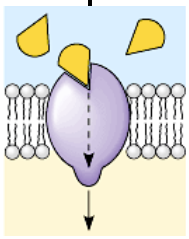


Some proteins are responsible for the cell junctions such as tight junctions that permit cells to adhere to each other. Some adhesion proteins attach to the cytoskeleton or extracellular matrix to help maintain cell shape (particularly for animal cells) and fix into position some membrane proteins. Collagen is an important glycoprotein of the extracellular matrix.

Communication Proteins

Communication proteins are responsible for the cell gap junctions that permit cells to communicate with each other. Gap junctions are common in heart muscle cells so that the contraction can be coordinated.

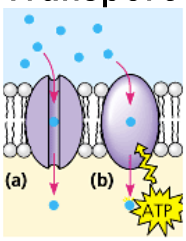
Receptor Proteins



Receptor proteins serve as binding or attachment sites, especially for hormones or other molecular messengers. Once activated, they trigger certain cell responses, often using signal transduction pathways.

Some receptors work in conjunction with carrier proteins, opening gated channels for ion movement. Other receptors are sensitive to nutrient levels, affect metabolic rate or even affect cell division.

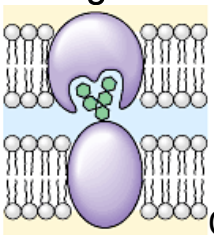
Transport Proteins



Transport Proteins function as carriers, which have binding sites that attract specific molecules. When a molecule binds to the **carrier** protein, the protein changes shape and moves the substance through the membrane. This process often requires energy (ATP), and the ATP complex is a part of the transport protein. When ATP is involved with actively moving molecules through the protein channel the process is called **Active Transport**. Most of our ions (Ca^{++}, Na^{+}, Cl^{-}, K^{+}, etc.), along with amino acids, sugars and other small nutrient molecules are moved through transport proteins.

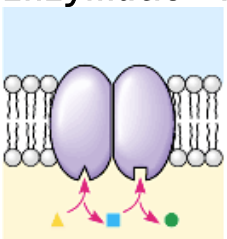
Other transport proteins form **channels** within the phospholipid bilayer, which allows small water-soluble molecules to pass through. **Aquaporins** are important water channels that facilitate the movement of water through membranes. Some channels are gated.

Recognition Proteins



Glycoproteins (carbohydrate-protein hybrids) and some glycolipids serve as surface receptors for cell recognition and identification. They are important to the immune system so that immune system cells can distinguish between one's own cells and foreign cells. Recognition proteins are also used to guide cell attachments/adhesions in developmental processes.

Enzymatic Proteins



Many enzymes are embedded in membranes, which attract reacting molecules to the membrane surface. Enzymes needed for metabolic pathways can be aligned adjacent to each other to act like an assembly line for the reactions.

Moving Materials Through Membranes

A significant part of membrane activity involves transporting materials through it in one direction or the other. Recall that the plasma membrane is selectively, or differentially, permeable. This means that:

- Some materials freely pass - the membrane is permeable to such molecules and whether they are inside or outside of the cell depends on other factors
- Some materials are excluded
- Some materials enter or leave the cell only by the using cell energy

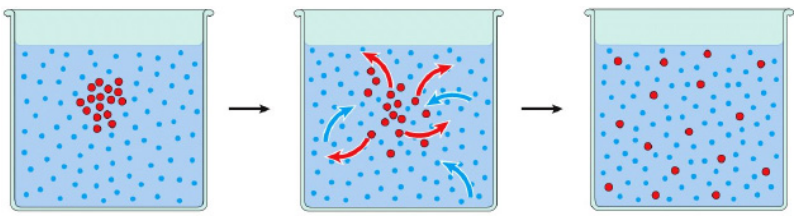
For example, small hydrophobic molecules, such as CO_2, O_2 and small lipids, dissolve in the membrane and pass through readily. Tiny polar molecules, such as H_2O and alcohol, can also slip between the phospholipid molecules. Ions and most nutrient molecules do not move freely through membranes, but are often carried by the transport protein channels, either with or without the use of energy. Most large molecules are excluded and must be manufactured within the cell, or moved by significant alterations of the membrane itself.

Before we talk about how molecules move through membranes, however, it is useful to have some definitions:

- Fluid
  Any substance whose molecules move freely past each other so that they change shape in response to external forces. Gases and liquids are fluids.
- Solute and Solvent
  A solute is a substance that can dissolve (or become individual atoms or molecules) in a usually liquid, solvent. Water is an excellent solvent.
- Concentration
  The number of molecules of a substance in a given volume
- Gradient
  A physical difference between two regions so that molecules will tend to move from one of the regions toward the other. Concentration, pressure and electrical charge gradients are common in cells.

In general, the movement of any substance is subject to “physical rules” of molecule behavior. All molecules are in motion and make random collisions with other molecules. However, when the distribution of molecules is not equal, and we have a gradient, there is a net movement of molecules along the gradient. Many gradients exist between a cell's environment and the cytoplasm of the cell.

Diffusion is the net movement of a substance from where there is more of it down a concentration gradient to where there is less of it, until molecules are equally distributed (and the gradient no longer exists).



Gradients are important in moving materials through membranes, both **passively** (without the use of energy by the cell) and **actively** (transport requiring cell energy).

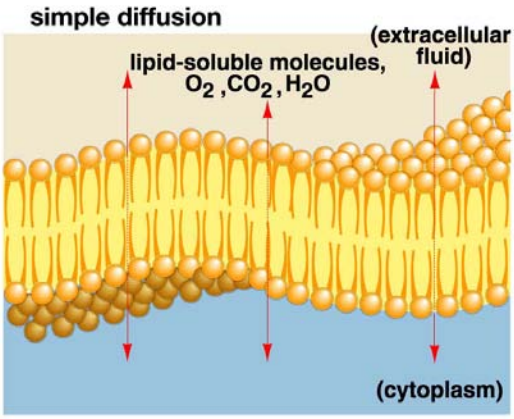
Passive Transport involves moving things through membranes without the expenditure of cell energy down gradients. Passive transport in cells involves the process of **diffusion**.

Simple Diffusion

- Diffusion is a means of passive transport, since no additional energy is expended for the process.
- In terms of cellular activity, diffusion:
    - Requires no energy
    - But the cell has no control over diffusion, and the rate of diffusion is pretty slow and can not cover much distance.
- The Rate of Diffusion can be affected by:
    - Temperature (Higher temperature, faster molecule movement)
    - Molecule size (Smaller molecules often move more easily)
    - Concentration (Initial rate faster with higher concentration)
    - Electrical and pressure gradients of the two regions (Greater the gradient differential, the more rapid the diffusion (again, initially))

Materials that may move through membranes by simple diffusion include:

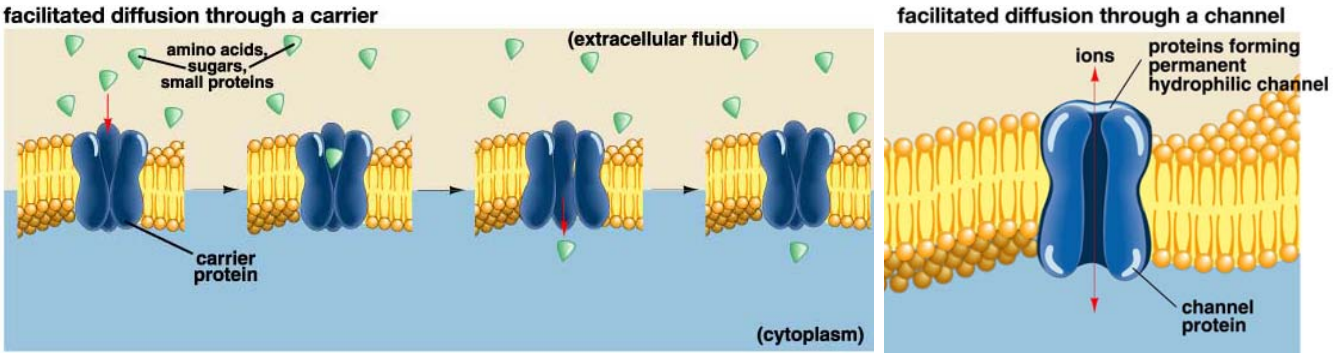
- H_2O (water)
- CO_2 (carbon dioxide)
- O_2 (oxygen)
- Some small lipid-soluble molecules (alcohol)



Note: The movement of water through a differentially permeable membrane in response to solute concentrations, the phenomenon of **osmosis**, is a special case of diffusion that we shall discuss later

Facilitated Diffusion

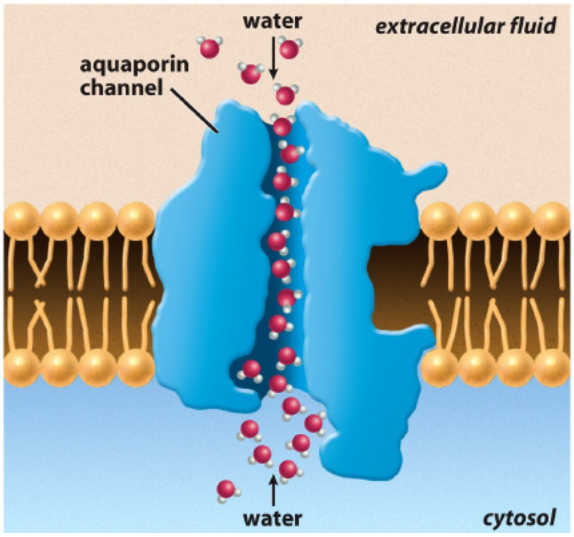
Most molecules can not move freely through the membrane, but do cross membranes with the help of **membrane transport proteins**, which temporarily bind to the substance to be moved through the membrane, a process called **facilitated diffusion** or **passive transport**. No energy is involved, so it is still a passive process. Both carrier proteins and channel proteins are involved in facilitated diffusion.



Materials that move through membranes by facilitated diffusion include:

- Glucose
- Many small ions
- Amino acids

The movement of water through membranes also involves facilitated diffusion. There are special channel proteins, called **aquaporins** that facilitate the movement of water at a rate needed for cell activities.



Now to a Complication of water, membranes and diffusion: Osmosis

Before continuing our discussion of methods of moving materials across membranes, we need to discuss in more depth the impact of water movement through membranes on fluid balance in cells.

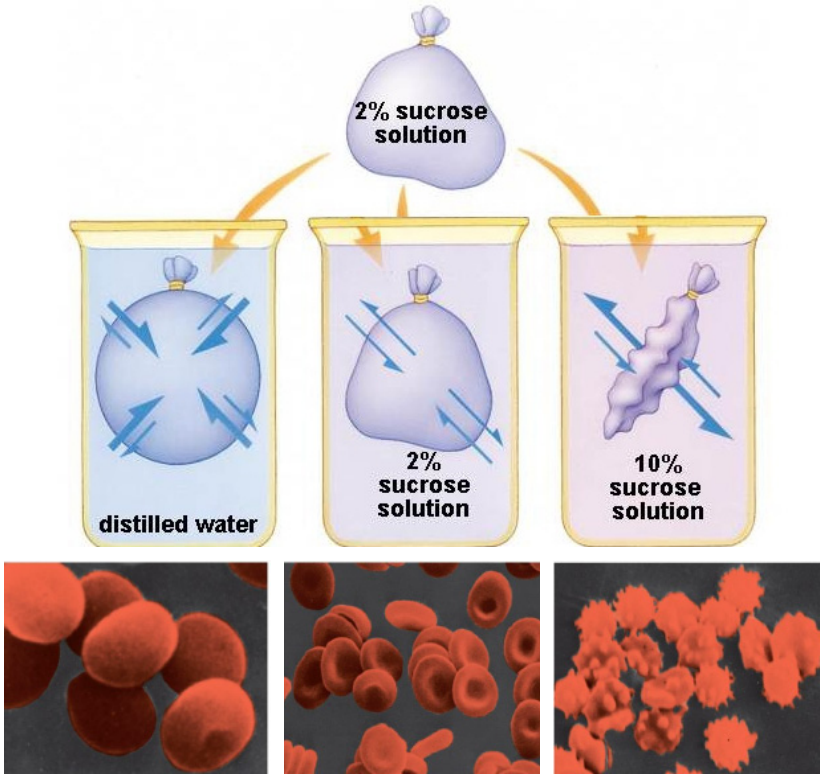
Osmosis is the movement (diffusion) of water across a differentially permeable membrane in response to solute (dissolved substances) gradients that are maintained by the membrane. The "force" to move water through membranes is called **osmotic pressure**. It is comparable to physical pressure. Osmotic pressure may be resisted by the cell membrane (if it is strong enough) or the cell wall, in organisms that have cell walls. The wall or membrane exerts a mechanical pressure. The difference in the osmotic pressure and the wall or membrane pressure is known as water potential. Water potential is very important in a number of processes.

For the process of osmosis:

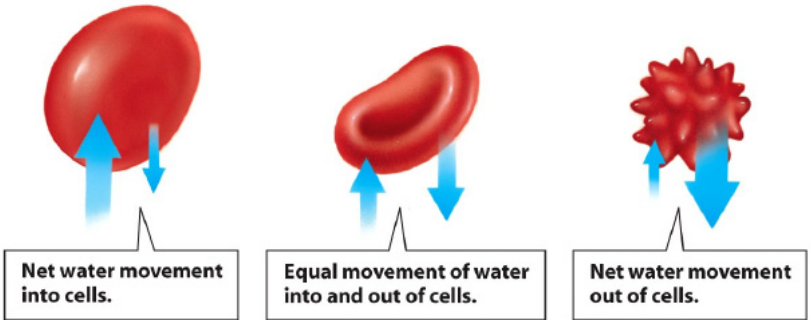
- The membrane is permeable to water.
- The membrane is not permeable to the solute(s), and the solutes will be substances which can "bind" to water, affecting the free flow of water.
- A water gradient exists, in part because dissolved substances always lower the concentration of water in a solution. (Pure water would have the highest concentration of water – any substance that is added to pure water will "displace" some water molecules, lowering the content of the water.)
- Since osmosis depends of the differences in the concentration of water, the specific types of solutes do not matter; it's their collective effect on the concentration of water than counts. Or, it's not so much the number of molecules, or volume, but the proportions of solutes to water.

There are terms that are used to describe the ratio of water to solutes in osmosis, and whether we are discussing the inside condition or the outside condition. They are in your text. (We do not need to use these terms in Biology 101, but we do need to define them in order to understand your book, and to succeed in courses that expect you to understand them):

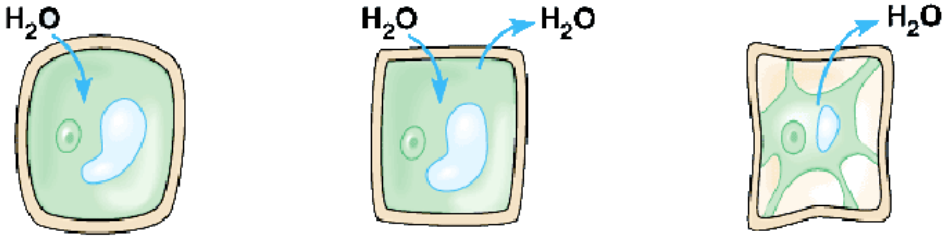
- **Hypertonic** The solution has a higher solute concentration (less water) than the cell
  Hypertonic solutions will cause water to leave cells by osmosis, and cells may shrink.
- **Hypotonic** The solution has a lower solute concentration (more water) than the cell
  Hypotonic solutions will cause water to enter cells by osmosis, causing the cells to swell.
- **Isotonic** Equal proportions of solutes to water on both sides of the membrane.
  Isotonic solutions are osmotically balanced.



Human Red Blood Cells



Typical Plant Cell

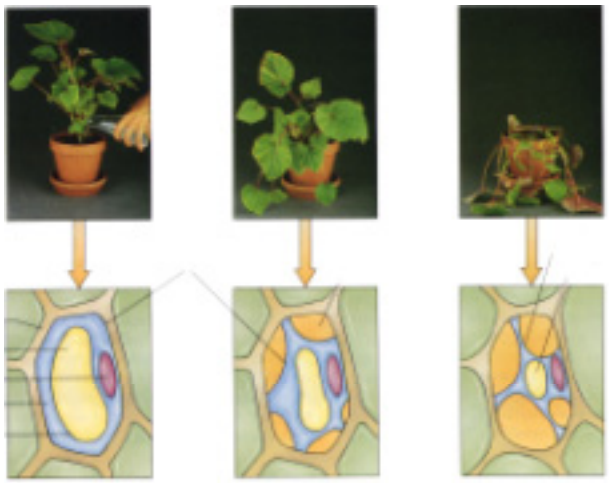


Hypotonic Isotonic Hypertonic

Although we do not need to remember the specific terms which describe the ratio of solutes to water inside and outside of cells, we do need to understand the impact of these different conditions on how cells function. Cells can not afford to either lose water, or gain excess water. They must maintain an equal proportion of solutes both inside and outside of the cells, a condition called **osmotic balance,** to function. The process by which organisms regulate their osmotic balance is called **osmoregulation.** Here are some examples:

Hypertonic Environments

An environment which has a higher proportion of solutes than found inside the cell will cause water to leave the cell. Salt water, for example, is hypertonic to the cells of freshwater organisms. A cell placed in this environment will lose water and shrivel, a phenomenon called **plasmolysis**, unless it has special mechanisms to prevent this.



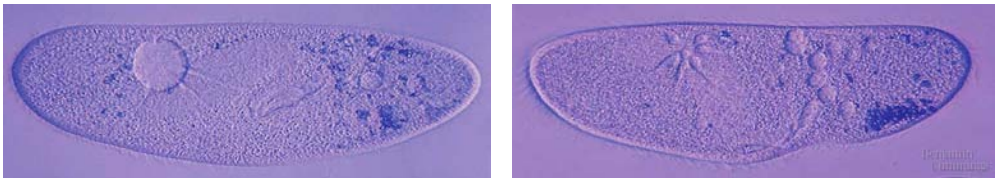
Plasmolysis in Plants

Hypotonic Environments

An environment that has a lower proportion of solutes than found inside the cell will cause water to enter the cell. Fresh water, for example, is hypotonic to the cells of all organisms.

Animal cells may swell to bursting when placed in fresh water. Animal cells, therefore, require some method to prevent this and maintain **osmotic balance.**

One method of doing so is through **vacuoles.** The contractile vacuoles found in protists are used to collect excess water which moves into their cell, and periodically, "spit" the water back out into the environment.



Contractile Vacuole in the *Paramecium*

Full Empty

Plant cells use osmotic pressure to their advantage, using the cell wall and central plant vacuole. As mentioned earlier, stored substances in the vacuole attract water that increases fluid pressure within the vacuole. This pressure forces the cytoplasm against the plasma membrane and cell wall, helping to keep the cell rigid, maintaining a condition of **turgor.** Turgor provides support and strength for herbaceous plants and other plant parts lacking secondary cell walls. When plant cells lose turgor, they wilt, a condition known biologically as **plasmolysis**.

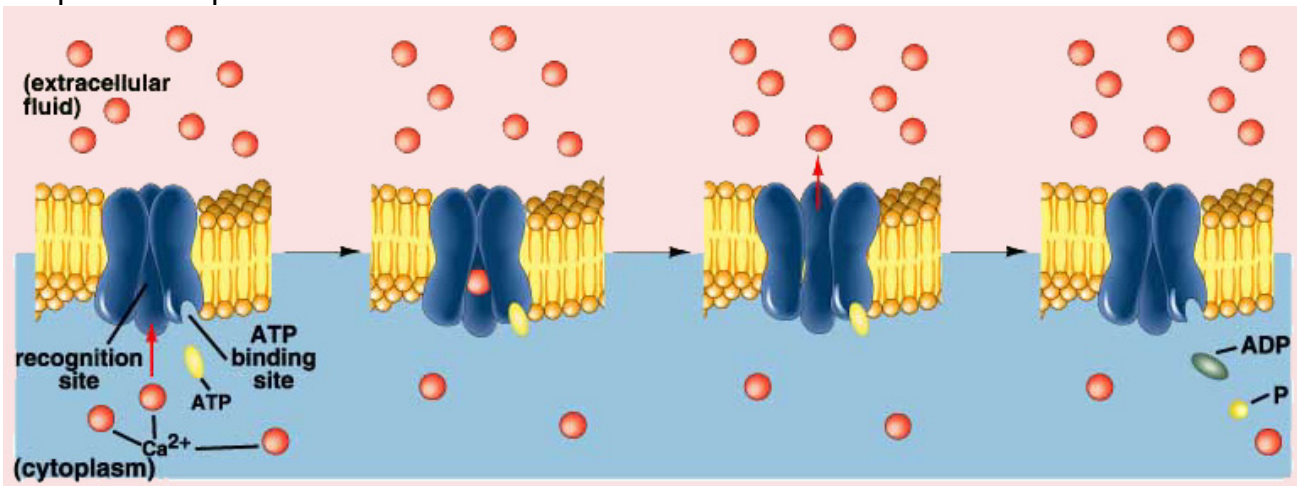
Energy-Requiring Transport Across Membranes

All cells need to move some substances through membrane in a direction counter to the gradient, or move substances that are too large or bulky be moved without the use of cell energy. Cells have a number of ways to move things with the use of energy.

Active Transport

Some transport proteins (carrier proteins) can move substances through the membrane against the concentration gradient. Active transport typically requires two carrier protein active sites: one to recognize the substance to be carried, and one to release ATP to provide the energy for the protein carriers or "pumps". Much energy is expended by the cell to do this!

In other cases, concentration gradients of ions, typically H^+ or Na^+ ions, can be used to provide the energy needed to move something through a membrane. For example, the substance to be moved is "coupled" to the concentration of H^+, and while to H^+ is moving "down" through the carrier channel, the substance is transported "up".



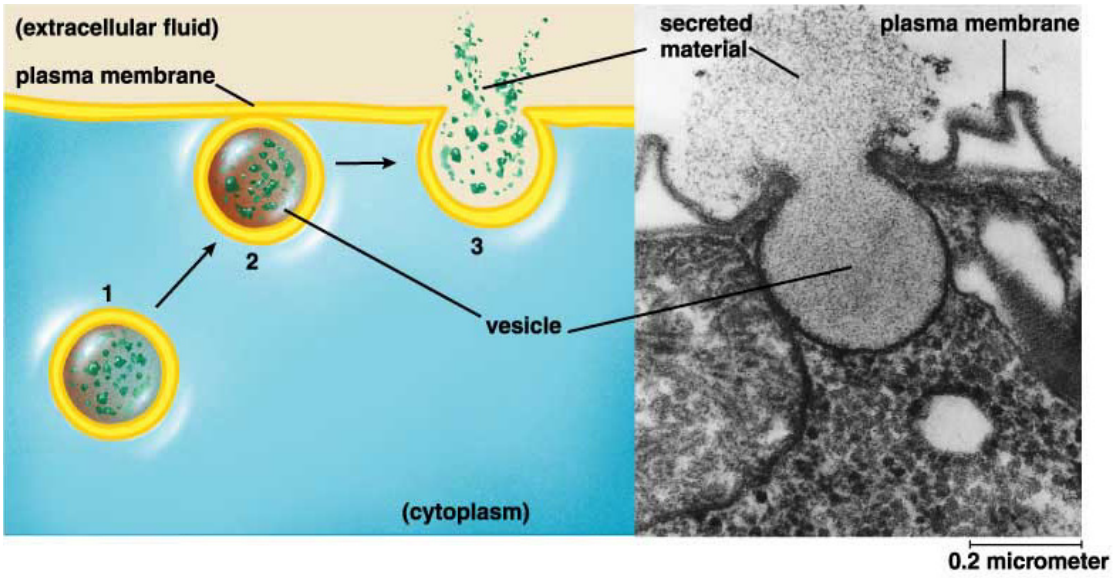
Active Transport

Membrane Interactions with the Environment

Larger substances may require changes in membrane shape and the fusion of the plasma membrane with vesicles containing the substances to successfully move the needed substance. Such changes in membranes occur throughout the lifetime of the cell, so that membrane sections are constantly being formed and reformed in the cell. Movement out of the cell involving changes of the membranes and formation of vesicles is **exocytosis.** Movement of materials into the cell is **endocytosis.**

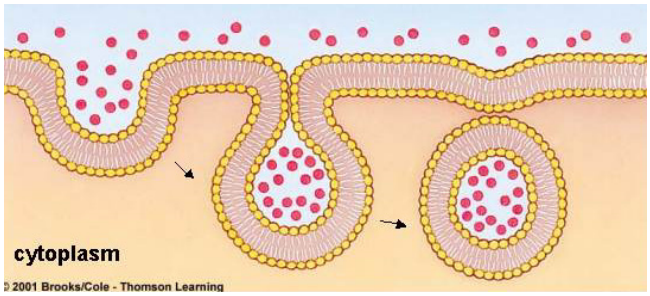
Exocytosis

Materials can be exported from the cell by fusing vesicles with the plasma membrane, a process called **exocytosis.** For example, insulin, made in cells of the pancreas, leaves the cells of the pancreas by exocytosis.



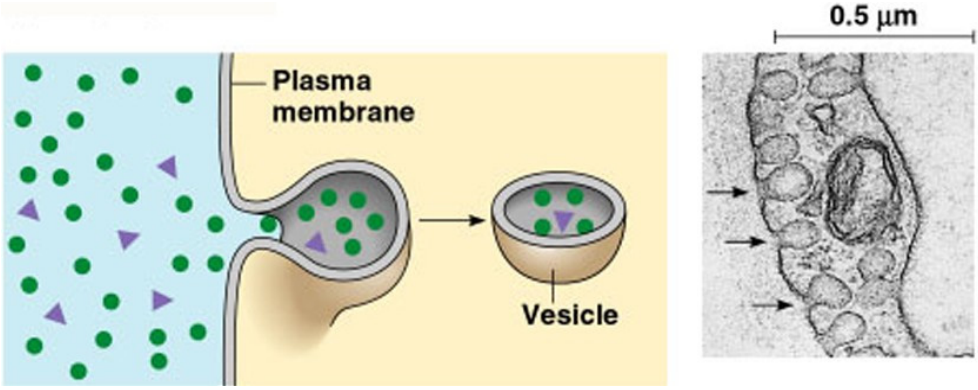
Endocytosis

Substances that enter the cell in this manner move by **endocytosis.** There are a variety of endocytosis processes: **pinocytosis**, **receptor mediated endocytosis** and **phagocytosis.**

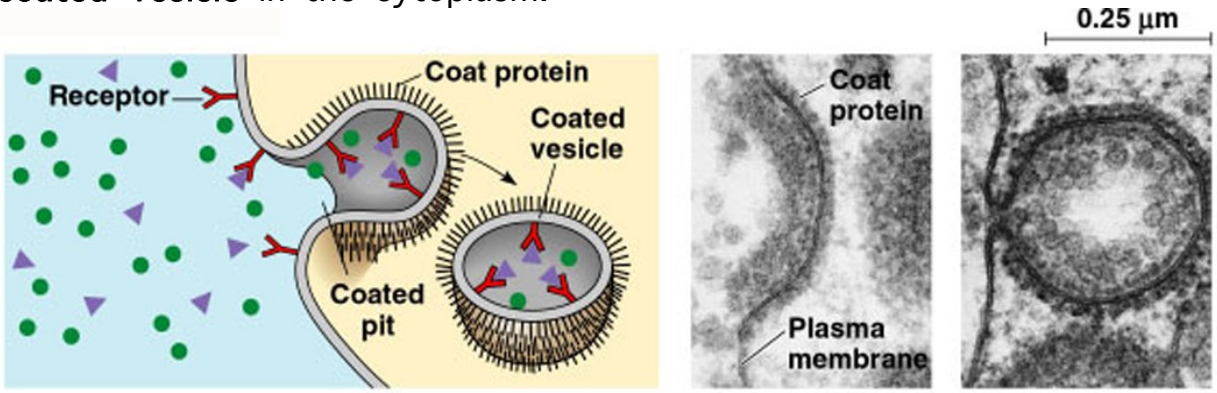


Methods of Endocytosis

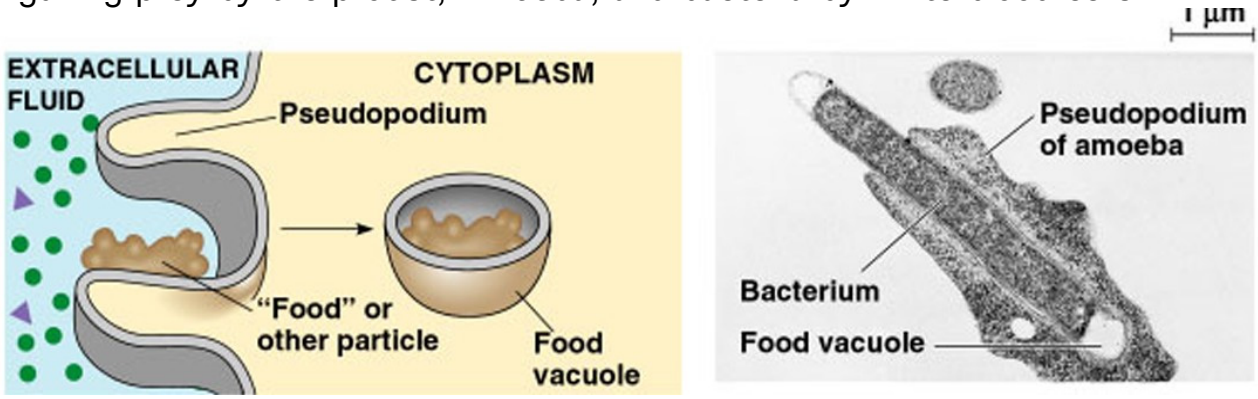
- **Pinocytosis**
  Membrane invaginates, substances "fall" in cavity, used for moving fluids into or out of a cell. Whatever molecules were in the fluid will be moved into the cell.



- **Receptor-Mediated Endocytosis**
  Receptor molecules in the membrane attract the substance to be moved into the cell, creating a membrane depression in that area (or **coated pit**). When sufficient molecules have been attracted, the pocket will be pinched off forming a **coated vesicle** in the cytoplasm.



- **Phagocytosis**
  Membrane surrounds and engulfs object, used for larger objects, such as engulfing prey by the protist, *Amoeba*, and bacteria by white blood cells.



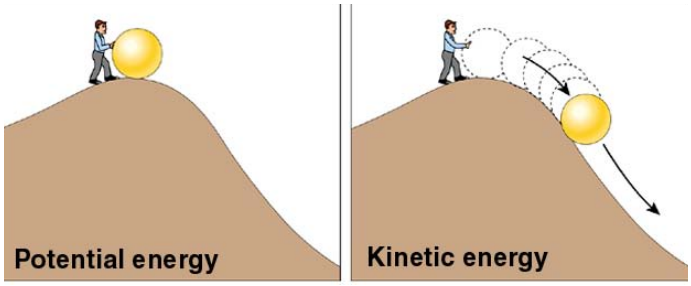
Thousands of chemical reactions occur in our cells and tissues to keep us alive (and hopefully healthy). We have discussed some of the molecules of living organisms (carbohydrates, lipids, proteins, etc.) in the context of the functions they perform in cells and tissues. These coordinated, controlled and orderly activities of cells collectively comprise the **metabolism** of living organisms. Much of metabolism involves energy transfer so it is helpful to have a common knowledge base about energy. The energy used in metabolism is **chemical energy**, the energy of electrons. This energy is used to build and maintain our cells and tissues, to move materials into and out of our cells, to process fuel molecules - to do the work of the cell.

We will briefly look at the physical laws that govern energy flow, how energy flow relates to chemical reactions and how living organisms control the chemical reactions that occur in their cells and tissues. Since **enzymes** catalyze our chemical reactions it's important to take a look at enzyme activity, too.

Defining Energy

Energy is often defined as the capacity to do work. The two fundamental types of energy are **kinetic** (energy at work, or the movement of energy) and **potential** (stored energy waiting to do work, such as the energy in the bonds of chemicals).

Potential energy is energy that matter has because of its structure or location. The water stored behind a dam has potential energy because of its location. Chemical bonds have potential energy because of their structure. The water flowing through the turbines of a dam has **kinetic energy**.



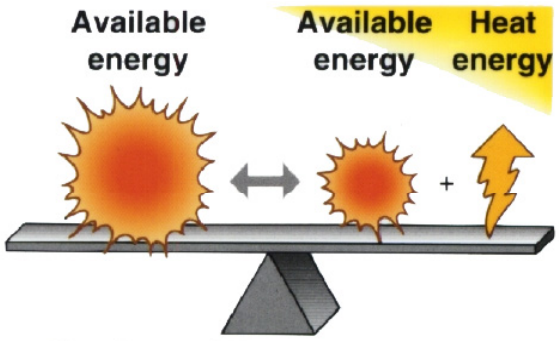
Both kinetic and potential energy can be found in many forms, such as electrical, light, chemical, heat, and mechanical. Under the right conditions, energy can be transformed from one type to another and from one form to another. This is a good thing, since different kinds of work require different kinds of energy, and as it applies to living organisms, it is often thought of as energy flow. Without energy flow, we would have no life on earth.

To make sense of this we have to understand both the **quantity of energy** available and the **usefulness of that energy** to do work, which is the study of **thermodynamics**, the field that studies energy transformations. There are two laws: one addresses the quantity of available energy and the second addresses the usefulness or availability of energy to do work. In addition, thermodynamics involves **a system and its surroundings**. The matter being studied is the system, and everything outside of the system is the surroundings.

The Two Laws of Thermodynamics

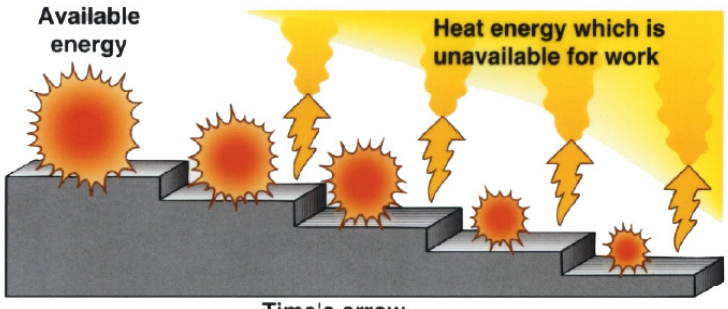
The **First Law of Thermodynamics** states the amount of energy in any process is constant, meaning that energy can neither be created nor destroyed by ordinary processes. But energy can be, and is, converted from one form to another and between potential and kinetic.

When we apply this law to living organisms (or any system) we say that the total amount of energy of a system **and** its surroundings remains constant, because energy transformations occur constantly. With each transformation within any system (such as the cells of your body) some energy escapes to the surroundings as heat energy. Which takes us to the second law.



First Law of Thermodynamics

The **Second Law of Thermodynamics** says something like "the amount of useful energy decreases when energy transformations occur", which means there is a tendency for all systems to reach the lowest possible energy or increase the amount of disorder in the system. This is the famous **"entropy"** law. Entropy is the measure of the amount of disorder (loss of higher level energy) in a system. **Useful** is the critical term here. The amount of energy doesn't change, but the ability of that energy to do the work we need accomplished diminishes. Most entropy is measured in increasing amounts of heat energy released by the system. We can also see increasing entropy when an organism decomposes. The decaying cells are far less "ordered" than when they were alive and functioning.



Second Law of Thermodynamics

To give an example, Apple trees trap light waves of solar radiation in the process of photosynthesis. They use the photosynthetic products to provide energy to maintain their cells and tissues and to produce apple fruits. Apple trees are maintaining their organized structure (and function) by obtaining "fresh" energy through photosynthesis. The apple fruit may lose its energy to you when you choose to eat an apple. The apple becomes seriously disordered as it passes through your digestive system as you process it for nutrients to maintain the organization of your cells.

This is the critical part of the second law of thermodynamics – **order**. Regions of concentrated energy, such as that found in the intact apple, tend to be more orderly than regions of more random energy, found in the individual molecules of the degraded apple in your intestine. A living organism constantly uses energy to maintain order and to prevent disorder of its cells and tissues. It does so at the expense of some other system in which entropy is increasing.

Laws of Thermodynamics, Energy Flow and Chemical Reactions

At first, when we think of the Laws of Thermodynamics, we might think that life can't exist if we must always put energy into a system to sustain it and if all of the chemical reactions that occur in cells are increasing the amount of less useful energy, even when we factor in the transfer of energy from apples to humans. It seems like it's all going downhill.

Enter the Sun

Life on earth relies on the sun. The sun constantly loses energy (the energy of solar radiation) which is trapped by green plants on earth in the process of photosynthesis. Living organisms use the products of photosynthesis to sustain their cells and tissues through the flow of energy within our ecosystems. We survive at the expense of the sun's increasing entropy.

So we can see that while it might seem that we must violate some law of thermodynamics to have life on earth, we really don't.

Now that we've gotten the laws down, let's turn to energy flow and chemical reactions.

Energy Flow in Chemical Reactions

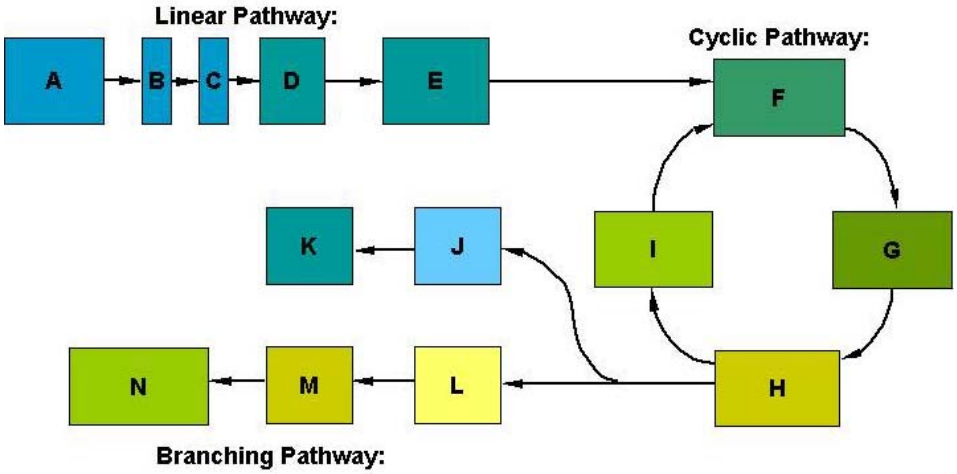
A chemical reaction takes one set of substances, called the **reactants**, (or sometimes, the **substrate**) and converts them to a different set of substances, called the **products**.

Reactants ➜ Products

Many chemical reactions rely on **energy carriers** to activate **enzymes** and other molecules needed to do the reaction. Some enzymes need **cofactors** and **coenzymes** to work. All are important. We will discuss a few of these.

Metabolic Pathways

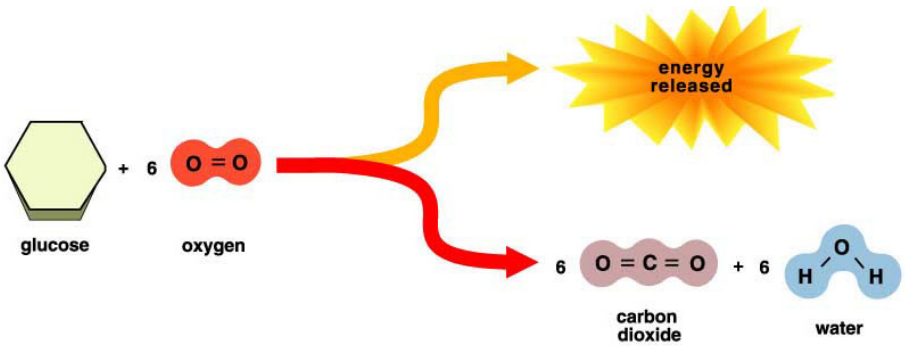
Many of our metabolic reactions take place along pathways in a series of chemical reactions that must be tightly regulated for our cells to function. Metabolic pathways are used to build complex molecules or do complex metabolic activities as well as to degrade. Pathways can be linear, branching or cyclical. Cellular respiration and photosynthesis are but two of the wonderful metabolic pathways of cells.



Direction of Energy Flow

Exergonic Chemical Reactions

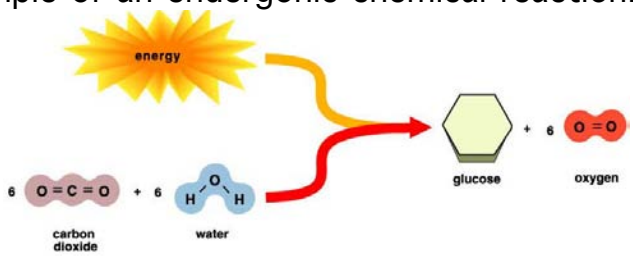
Some chemical reactions release energy -- the amount of energy in the products is less than that of the reactants. Such reactions are said to be **exergonic**.



Exergonic Reactions of Cellular Respiration

Endergonic Chemical Reactions

Some chemical reactions require or consume energy -- the amount of energy in the products is more than that of the reactants. Such reactions are said to be **endergonic**. The dehydration synthesis that forms the peptide bond between amino acids is an example of an endergonic chemical reaction.



Endergonic Reactions of Photosynthesis

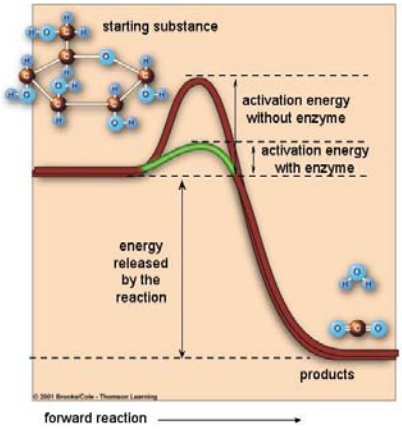
Activation Energy

Given enough time, an exergonic reaction can (and will) occur spontaneously. Endergonic reactions require energy from some other source in order to take place. In living organisms the source of this needed energy is usually ATP, a molecule we will discuss in a few minutes.

For any chemical reaction to get started, the reactants must come together at the right bonding place at the right time. Remember that all atoms and molecules are in motion, so at some time, it is possible that the reactants will come together randomly.

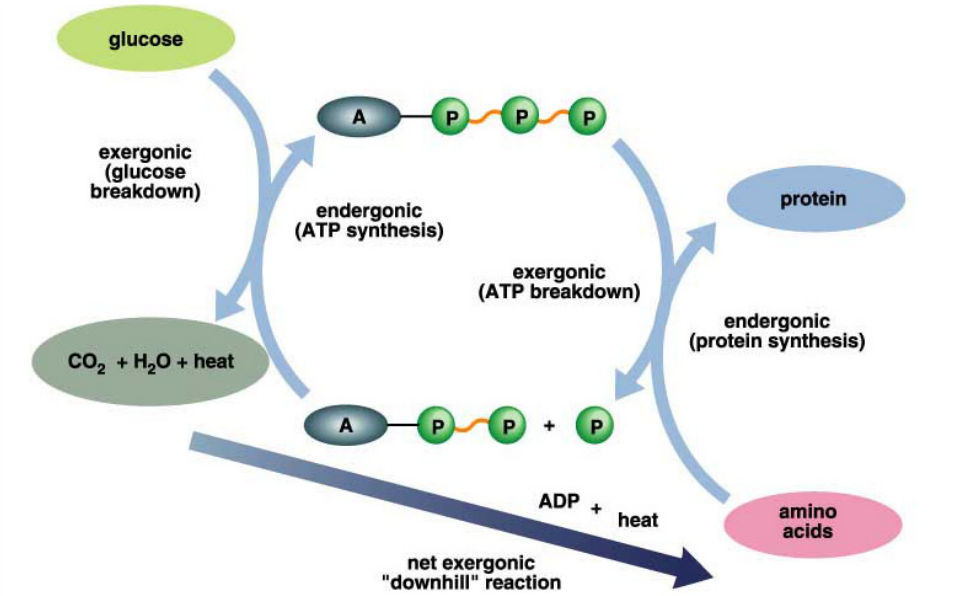
However, the rate of a chemical reaction at normal earth temperatures may be so slow that it is imperceptible. No matter how energetically favorable a chemical reaction is, some energy is needed to get the reaction started. This energy is called the **activation energy**. Activation energy is anything that increases the rate at which the reactants collide (or come together) so that any reaction can occur.

Some chemical reactions occur naturally because the activation energy needed to break the bonds of the reactants can be supplied by ambient heat energy. Most reactions cannot, which is a good thing for life. Reasonably unstable molecules such as nucleic acids and proteins would degrade spontaneously if just a small amount of heat energy was needed to overcome the activation energy.



Applying Energy Flow to Coupled Reactions

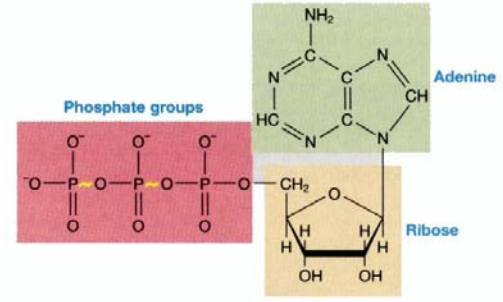
Keeping cells alive takes work and requires energy. This energy comes from exergonic reactions within the cells that provide the free energy to perform the endergonic reactions needed to keep cells and tissues functioning. We refer to these paired reactions as **coupled reactions.** For example, the release of energy from the oxidation of glucose fuel molecules, for example, is coupled to the endergonic assembly of proteins and other macromolecules in cells and tissues.



By far, the most common energy coupler in chemical reactions is the nucleotide, ATP.

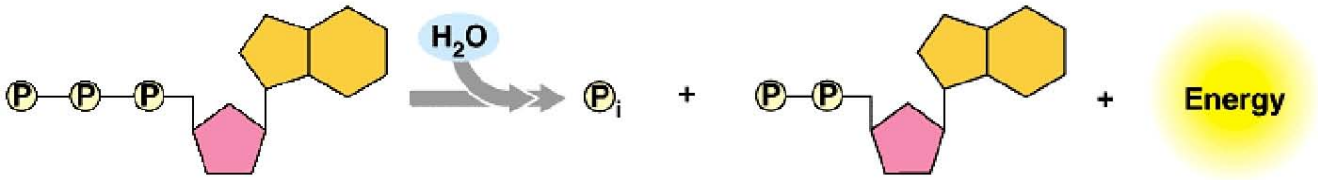
Speaking of ATP

For some reason, in order for energy to be useful to do cell work, it must be in the form of the nucleotide, **ATP**. Cells do not directly use other forms of energy, so that the energy of chemical fuel molecules must be transformed into ATP before cell work can be done. ATP is then used to provide the energy to complete an endergonic chemical reaction.

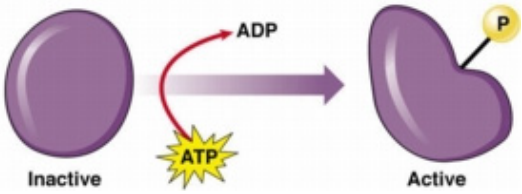


ATP has three "high-energy" phosphate bonds. The second and third phosphate bonds of ATP are unstable. When this phosphate bond is broken by hydrolysis, energy is released (an exergonic reaction). This released energy is just perfect for the amount of energy needed for many cell reactions. This is why we call ATP an energy carrier. It “carries” the energy needed to do the cell work.

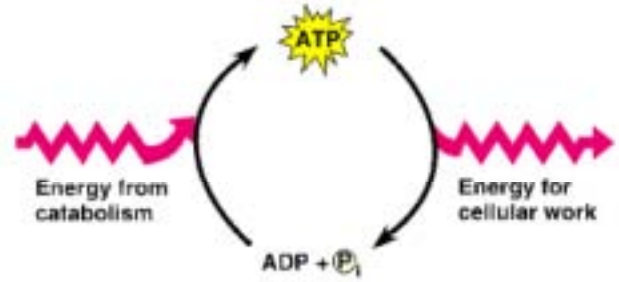
The product of the hydrolysis of ATP is a molecule of ADP and a free phosphate molecule (P_i).



ATP may "energize" a molecule by transferring the ATP phosphate to the molecule, so that the molecule has a more favorable bonding arrangement for its activity.



The exergonic process of cell respiration makes ATP using energy obtained by "burning" or oxidizing fuel molecules, like glucose, to bond a Phosphate (P_i) to the nucleotide ADP. Once synthesized, the ATP is broken to release its energy in the reactions to which it is coupled. This is a constant process in cells. About 88 pounds of ATP is made and broken in 24 hours just for basic cell maintenance. A cell has only about a one-minute supply of ATP at any given time. The unstable ATP cannot be “stockpiled”. Keep in mind, however, that not all of that 88 pounds is useful energy. Much of the energy released when ATP is broken is in the form of less useful heat energy. So in one sense, we can say life is about making and breaking ATP.

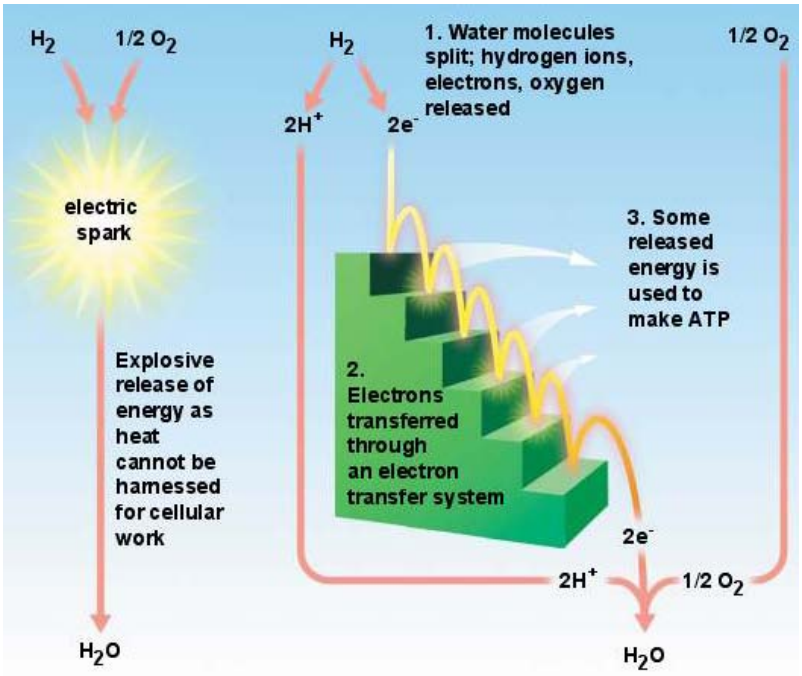


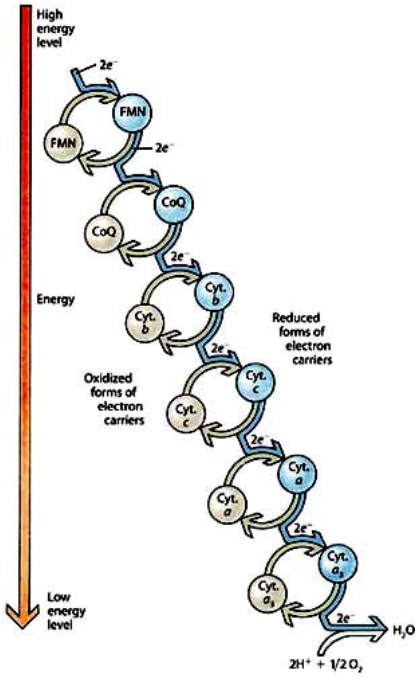
ATP can also work by transferring its third phosphate to the reacting molecule, energizing that reactant. When you are transferring phosphates (P_i) from one thing to another it's called **phosphorylation**, a good term to know.

Electron Carriers

Many coupled reactions involve electron transfers using series of energy carrier molecules embedded in membranes. They are often called **electron transport chains**. These electron transfer chains include a number of nucleotides, such as **NAD^+, $NADP^+$** and **FAD**, along with many other kinds of molecules including proteins and quinones.

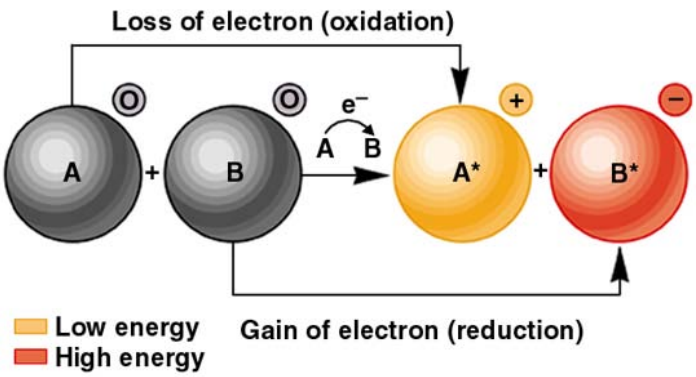
The electron carrier molecules can capture energy released in exergonic reactions and transport it to endergonic reactions so that energy needed to cell work can be controlled and useful, rather than wasted. Often they are described as stair steps, because they can release energy step-by-step along their pathway.





Oxidation-Reduction and Metabolism

Since energy changes in cells involve energy transfers, oxidation-reduction reactions are important. It can be useful to review them. An **oxidation** is the loss of one or more electrons. A **reduction** is the gain of one or more electrons. Oxidations and reductions are always coupled and are useful in controlling the energy released from an exergonic chemical reaction so that it can be made available for cell work. In many cell oxidation-reduction reactions, the electron oxidized is associated with a hydrogen atom, so gaining and losing hydrogens and their electrons is something we will often see in metabolic pathways, such as cell respiration and photosynthesis.



Regulating Metabolism – Activation Energy, Catalysts and Enzymes

We need to be able to control the chemical reactions that occur in our cells and tissues, and we need to be able to do the chemical reactions of metabolism within the temperature, pressure and concentrations of molecules that are found within our cells. Many of our metabolic reactions occur in pathways that must be tightly regulated or our cells cannot function. Much regulation of metabolism depends on the use of **catalysts**, substances that affect the rate of reactions.

Catalysts

An alternative to increasing the heat energy needed to activate a chemical reaction is to stress the bonds of the reactants to make them easier to break. Substances that can do this are called **catalysts**. A catalyst affects the bonds of the reactants in a way to reduce the activation energy needed to break the bonds, thus facilitating the rate of the chemical reaction.

Characteristics of Catalysts

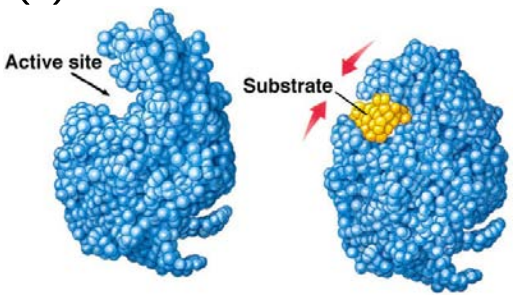
- A catalyst speeds up a chemical reaction
- A catalyst can not cause a reaction to occur that is not energetically favorable
- A catalyst does not change the chemical equilibrium of a reaction.
- A catalyst is not consumed in a chemical reaction

It is important to know that a catalyst is not part of the chemical reaction. It is neither a reactant nor a product. A catalyst facilitates the reaction. In living organisms, we mostly use a special class of catalysts, called **enzymes**. Enzymes are proteins. In addition, some chemical reactions are catalyzed by RNA molecules called **ribozymes**.

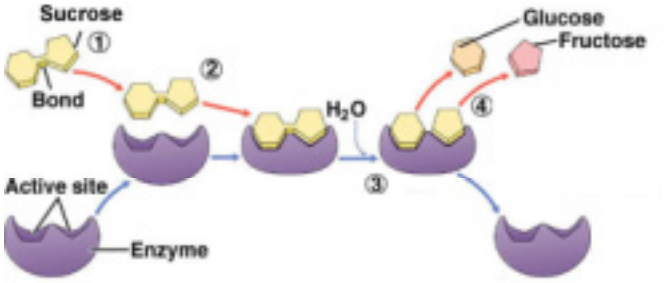
> Note: Activation energy is separate from the energy input needed to sustain endergonic reactions. Any endergonic reaction needs energy added to it to keep it going, along with getting it started.

Enter Enzymes

Enzymes are almost always globular proteins with a place on the surface where the enzyme can bind to the reactant(s). This "notch" is the **active site,** comprised of just a few amino acids (a domain of the tertiary structure). The remainder of the enzyme helps to maintain the integrity of the active site. The active site has a precise size, shape, and electrical charge that exactly complements the reactant(s) or **substrate(s).**



When a substrate binds to the enzyme, it "fits" into the active site, temporarily distorting the reacting molecules; this is called the **induced fit.** This distorted stage of the substrate is called the **transition state** and its bonds are more easily broken (lowered activation energy), promoting the reaction. Once the reaction occurs, the active site is altered, releasing the product. The enzyme is unaffected by the reaction.



Enzymes are highly specific. Each chemical reaction that occurs in cells has its own enzyme. Enzyme shape determines its function. The kinds of enzymes in cells determine what chemical reactions take place and what metabolic activities will occur within that cell.

Although many enzymes are located free within the cytoplasm of the cell, others are found within complexes, typically associated with membranes of specific organelles, such as the enzymes needed for cell respiration found in the mitochondria. Such complexes provide greater efficiency and control in metabolic pathways.

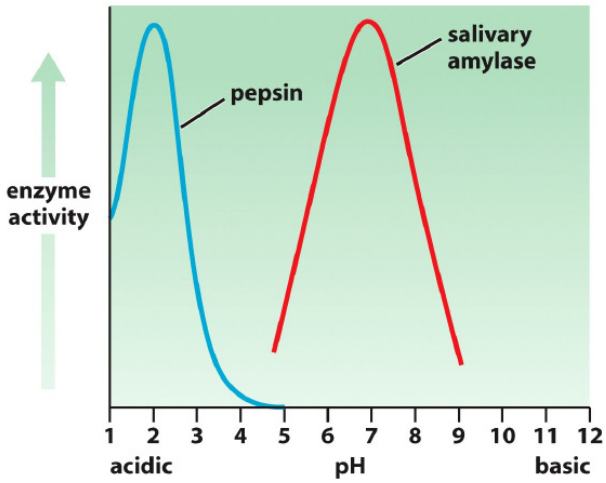
Some enzymes can only work when they have associate molecules, called **coenzymes** (non-protein organic molecules) or **cofactors** (mineral ions) present.

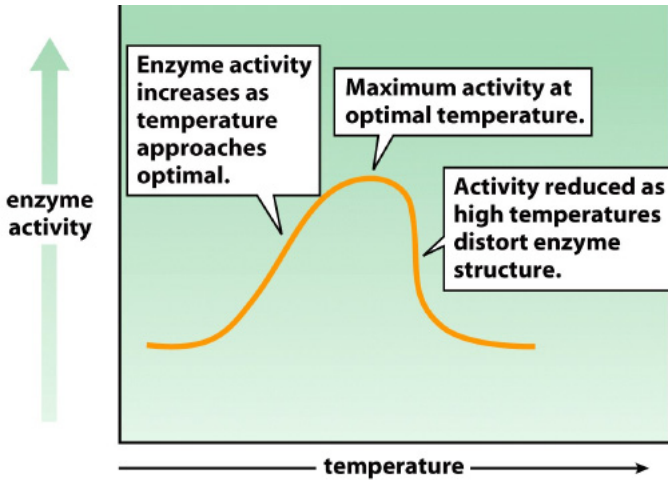
- Many of our vitamins function as coenzymes in electron transport pathways picking up the electrons removed from the substrate and carrying them (and their associated H^+) to the next reaction in the pathway in a series of **oxidation-reduction** reactions.

- Some of our minerals, notably zinc, copper and iron, function as cofactors. Cofactors in the active site attract electrons from the substrate promoting the reaction. Some metals may interfere with enzyme function. Mercury, for example, inhibits the function of many enzymes, partly by blocking the attachment of the needed cofactor.

Enzymes and Their Environment - Enzyme Regulation

Enzyme activity is controlled by conditions of the environment. Each enzyme works at precise pH, temperature and chemical conditions, such as the amount of sodium ions in the cell.





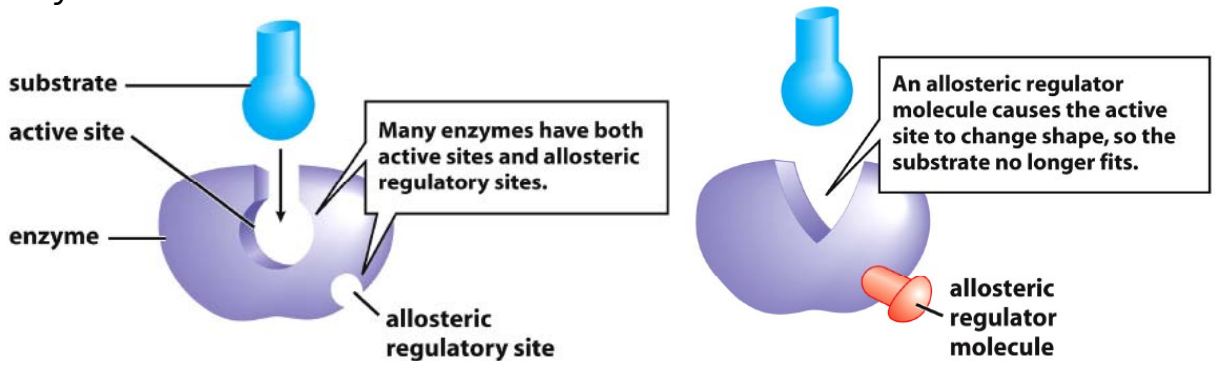
Enzyme Regulation

Enzyme regulation a natural part of how cells function. Homeostasis in cells and organisms involves elaborate feedback mechanisms. They are essential for control of metabolism and the orderly operation of cells. Many of these metabolic controls work by activating or de-activating enzymes at appropriate times. This is the subject of **enzyme regulation.**

- The amount of enzyme present can serve to regulate the rate of some chemical reactions. And the cell's DNA controls whether an enzyme is present.
- Some enzymes are manufactured in an inactive form, and converted to an active form only in the presence of the proper reactant (substrate).
- Regulating enzyme activity often involves "sophisticated" controls.

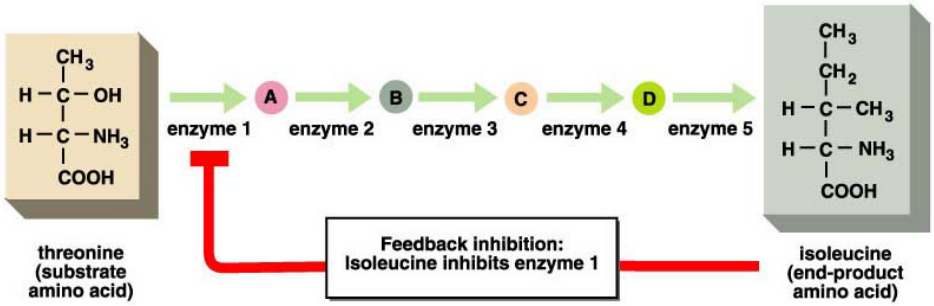
Allosteric Regulation

Some enzymes have specific receptor sites that change its shape to either activate the enzyme or inhibit the enzyme from functioning. These molecules are known as **allosteric regulators**. The receptor site on the enzyme is called the **allosteric site**. One allosteric site may activate by stabilizing the enzyme shape for the active site and another may inhibit by stabilizing an inactive shape of the enzyme.



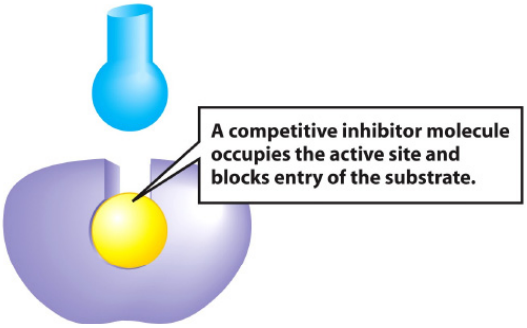
Feedback Inhibition

Many enzymes are regulated by feedback mechanisms that tell the enzyme that its job is finished. Often the product of the reaction serves as this control. When enough product is formed, some of the product blocks the enzyme's activity, stopping the reaction. As the product is used up, its block of the enzyme is removed, and the enzyme can be active again. This process is called **feedback inhibition**.

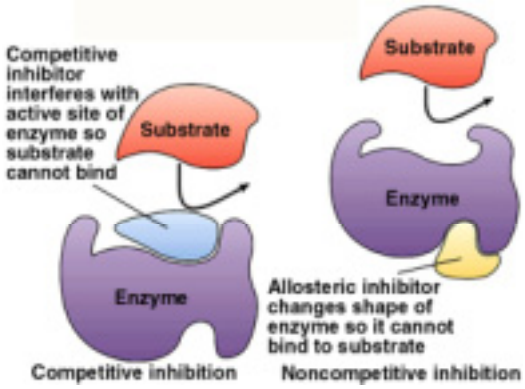


Competitive Inhibition

Some enzyme inhibitors compete with the substrate for the enzyme's active site and wind up blocking the active site since they do not undergo a chemical reaction and get released from the enzyme. Substances that compete for the enzyme's active site are called **competitive inhibitors.** For example, both methanol and ethanol for the active site on the enzyme alcohol dehydrogenase.



Substances that bind to the enzyme at some other place changing its shape so it can no longer function effectively are called **non-competitive inhibitors.** Allosteric inhibitors are non-competitive inhibitors. A number of poisons block enzyme activity in this way. Many pesticides block essential nervous system enzymes. Mercury inhibits the function of many enzymes, partly by blocking the attachment of the needed cofactor.



Using Enzyme Activity in Research

One of the fascinating enzyme actions is on the fluorescent molecule, luciferin. A number of living organisms have enzymes, called luciferases, that cause luciferin to fluoresce (give off light). Fireflies are infamous for this activity, although their use of flashing light is to attract mates, as do some marine worm species. Many Dinoflagellates (photosynthetic protists) fluoresce, probably as a defense activity. Genetic researchers have been able to isolate the genes for luciferase and splice this gene into any number of organisms for a variety of genetic and medical research purposes. A more novel use of this was the creation of the fluorescent rabbit for artist, Eduardo Kac (http://www.ekac.org/). The gene transferred was a fluorescent gene from a jellyfish.

Alba, the fluorescent bunny.
Photo: Chrystelle Fontaine

Some Vocabulary Review

Endergonic
An energy consuming chemical reaction

Exergonic
An energy releasing chemical reaction

Reactants
The substance undergoing a chemical reaction.
Also called: Substrate or Precursor

ATP
Adenosine triphosphate, the energy currency of cells

Metabolism
The chemical reactions which occur in living organisms

Metabolite
Compound involved in a metabolic pathway

Secondary Metabolite
Produced as a by-product or indirect product of a metabolic pathway

Intermediate Metabolite
Compound in the middle of a pathway, or a compound that can branch into alternative metabolic pathways

Enzyme
Protein that serves as a catalyst for a metabolic reaction

Cofactors
Inorganic metal ions that work with enzymes

Coenzymes
Small organic molecules (often vitamins)that work with enzymes

Products
The substance(s) left or produced at the end of a metabolic reaction
A **by-product** is a product not needed for cell use at the end

Oxidation
The loss of electrons (or in cell processes, a Hydrogen ion with its electron)

Reduction
The gain of electrons (or in cell processes, a Hydrogen ion with its electron)

In this unit of Biology 160 we are going to look at the energy transformations that take place to synthesize useful fuel molecules for living organisms, and then at the metabolic processes that cells have to burn (oxidize) fuel to make the ATP needed for all cell work.

The majority of living organisms obtain their fuel molecules, directly or indirectly (recycled, so to speak) from the process of **photosynthesis**. The process of photosynthesis transforms light energy into chemical energy, and uses that energy to manufacture carbohydrate molecules, primarily glucose, and subsequently the other organic molecules needed for growth and development.

In eukaryotic organisms, and in the Cyanobacteria, the process of photosynthesis also produces oxygen. The photosynthetic bacteria produce organic carbon molecules, but do not produce oxygen. We will discuss the primary photosynthetic processes of plants only.

Organisms that obtain energy and carbon from their physical surroundings are **autotrophs.** The majority of autotrophs manufacture their organic molecules by the process of **photosynthesis**. Organisms that obtain their organic fuel molecules pre-formed from the environment are **heterotrophs**. Animals, fungi, many protists and many bacteria are heterotrophs. Most photosynthetic organisms are plants or protists that contain chlorophyll. Some prokaryotes are also photosynthetic. The Cyanobacteria have chlorophyll pigments; some Bacteria, such as the purple sulfur bacteria, have different light-capturing pigments, and slightly different photosynthetic mechanisms. They are studied in microbiology.

Note: Not all autotrophs are photosynthetic; a tiny proportion of our chemical fuel is manufactured by **chemosynthetic** organisms. Chemosynthetic autotrophs, or chemoautotrophs, use energy from chemical reactions involving inorganic atoms and molecules, such as S, Fe, H and N, to make organic compounds. Chemosynthesis is restricted to a very few groups of bacteria. However, chemosynthesis sustains some deep sea-bed ecosystems that surround sulfur vents.

Once organic fuel molecules are manufactured, living organisms must degrade the organic fuel molecules to provide energy to keep their cells alive, the pathways of cellular respiration, the topic of the next chapter.

The Process of Photosynthesis - Requirements

Photosynthesis occurs in all parts of plants that contain the green pigment, **chlorophyll,** which is located in the chloroplasts. In most plants chloroplasts are most abundant in leaves. In the laboratory, we shall look closely at leaf structure as it relates to its function in photosynthesis. Let's now turn to this most important process.

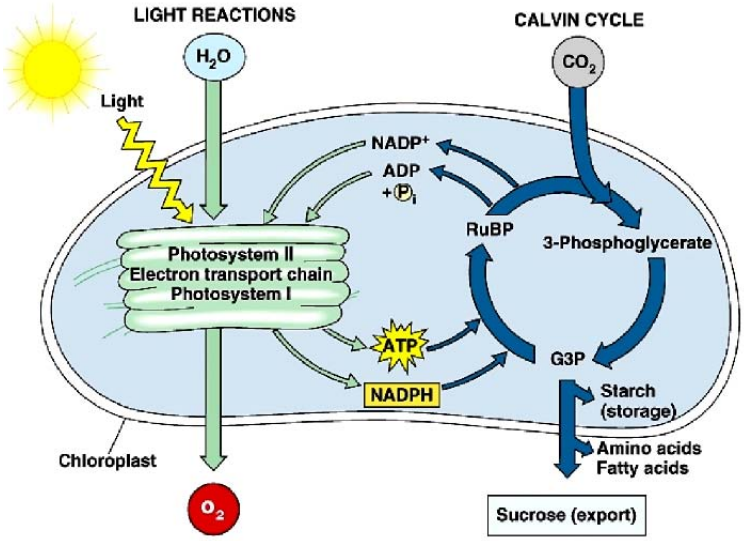
Photosynthesis involves two stages, occurring in separate locations within chloroplasts. In the **light-dependent reactions** of photosynthesis, light energy is transformed into chemical energy in the form of **ATP** and the energy transfer molecule **NADPH** in a series of oxidation-reduction reactions that transfer electrons and hydrogen from water to the energy transfer molecule $NADP^+$, a coenzyme. The light-dependent reactions are known as **photophosphorylations**, because they involve producing **ATP**. They take place on the **thylakoid membranes** of the grana of the chloroplasts.

In the **light-independent reactions** or **Calvin-Benson cycle** (or more simply, the Calvin cycle) the energy from the light reactions is used to manufacture carbohydrate molecules that form glucose. These reactions occur in the **stroma** of the chloroplast and utilize enzyme catalysts.

The overall chemical equation for photosynthesis is:

$$6CO_2 + 12H_2O \xrightarrow{\text{Chlorophyll}} C_6H_{12}O_6 + 6H_2O + 6O_2 + \text{energy}$$

(Carbon dioxide + water + light energy ------> glucose + water + oxygen)



In order to do photosynthesis, water, CO_2, chlorophyll and light energy must be available, along with the energy transfer molecules. We shall briefly look at each of these requirements before we discuss just how this process of photosynthesis works.

Note: ATP/ADP and $NADPH/NADP^+$ constantly cycle between light reactions to the Calvin Cycle.

1. Water

Water is the **hydrogen and electron donor** for the process of photosynthesis.

Water is obtained from the environment, absorbed by roots and conducted throughout the plant in the **xylem** of the vascular system. Water needed for photosynthesis is but one of the demands for water in plants.

Light energy is used to split water molecules, forming **$2H^+$**, **$2e^-$**, and **Oxygen** ($^1/_2O_2$) during the process of photosynthesis.

2. Carbon Dioxide

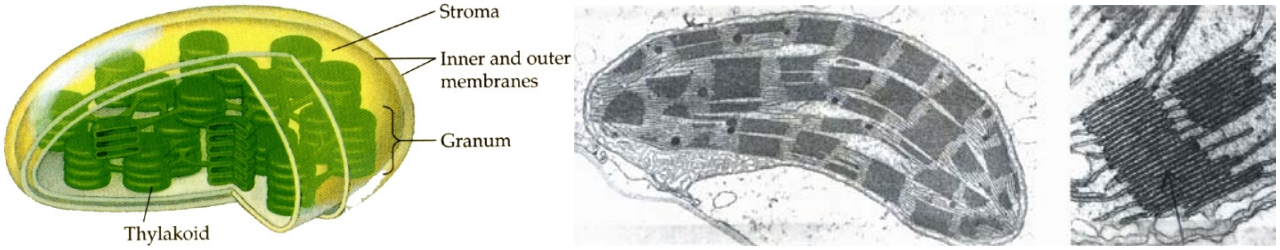
Carbon dioxide provides the **carbon** source for manufacturing the carbohydrates in photosynthesis.

Carbon dioxide diffuses from the atmosphere through pores in leaf surfaces, called **stomata**, which are formed by a pair of guard cells. The carbon dioxide then diffuses to the photosynthetic cells of the **leaf mesophyll**. The rate of diffusion of carbon dioxide and availability of carbon dioxide often limit the rate and amount of photosynthesis that occurs in a plant. Stomata structure will be observed in the laboratory.

3. Chloroplasts

Photosynthesis occurs in chloroplasts. Recall that the chloroplast has a double membrane with a series of internal stacked membranes. Light energy is captured by the photosynthetic pigments located on the special membranes in the chloroplast called **thylakoids,** which are folded into disk-shaped stacks called **grana.** The interior compartments of thylakoids serve as reservoirs for **hydrogen ions** (H^+) that are needed for producing ATP.

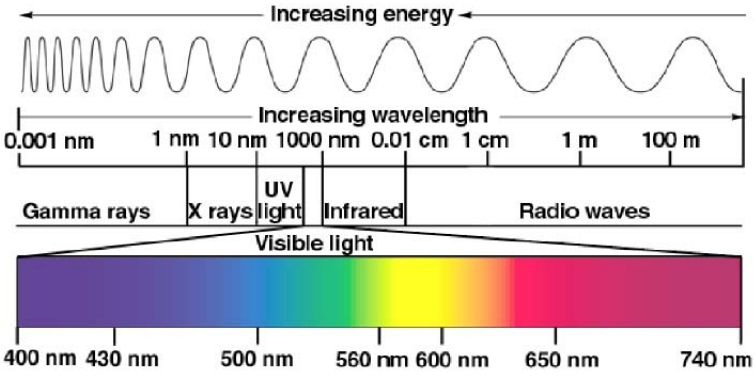
The reactions of photosynthesis that are involved in the transformation of light energy to chemical energy are called the **light-dependent reactions**.



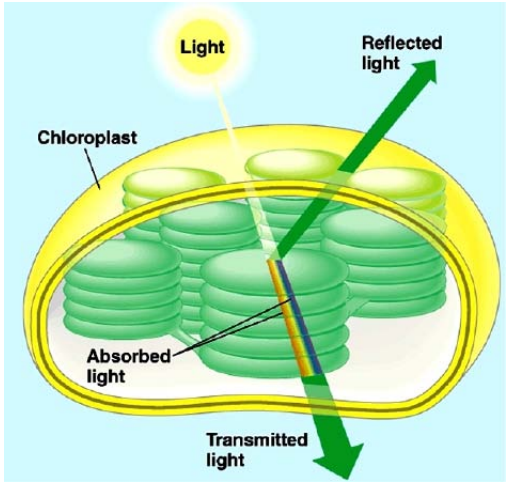
The reactions needed to produce carbohydrates occur in the **stroma** region of the chloroplast. Enzymes are located in the stroma. These reactions are known as the **Calvin-Benson cycle** or light-independent reactions.

4. Light Waves

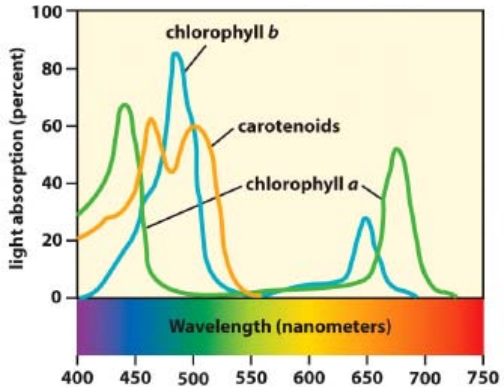
Both light and light-absorbing pigments are needed for photosynthesis. Light is a form of electromagnetic radiation. Visible light is a combination of many wavelengths that we see as different colors (of the rainbow) in the range of 380 - 750 nm. Each wavelength is associated with a specific photon, or particle of energy. In general, shorter wavelengths have more energy.



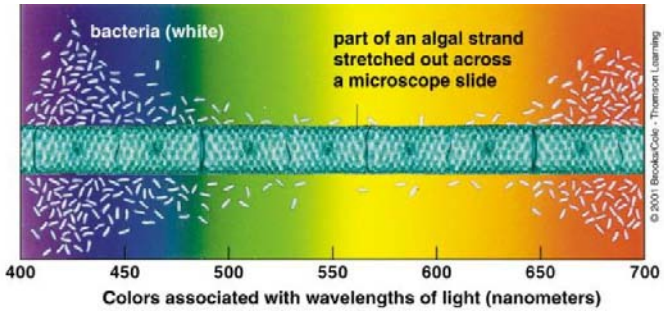
The light-absorbing photosynthetic pigments do not absorb all wavelengths of light equally. Some light energy cannot be absorbed (and is reflected instead) and some is transmitted, or passed through the chloroplasts. The light waves most absorbed and most useful to photosynthesis are reds and blues. Not surprisingly, green light is absorbed poorly.

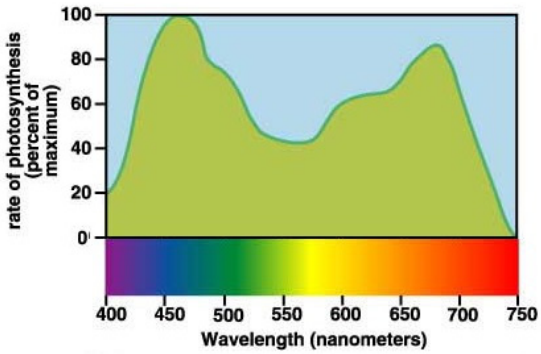


In the laboratory we may do an experiment to demonstrate the absorption of different wavelengths of light by the photosynthetic pigments, an **absorption spectrum.**



One can also measure rates of photosynthesis in different wavelengths to generate an **action spectrum**. This is done by growing plants in light boxes that have just one wavelength of light, or with algae, using a prism to separate light waves passing through a microscope. Action spectra reinforce that photosynthesis occurs more in blue and in red light.



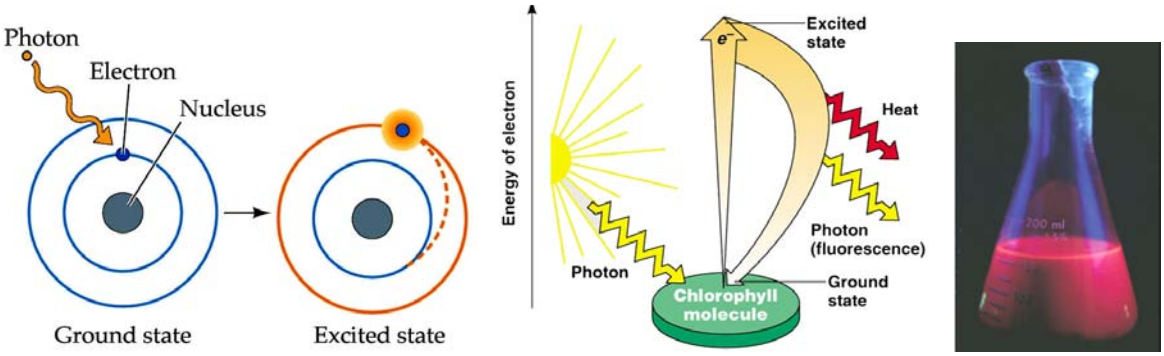


5. The Light Absorbing Pigments of Photosynthesis

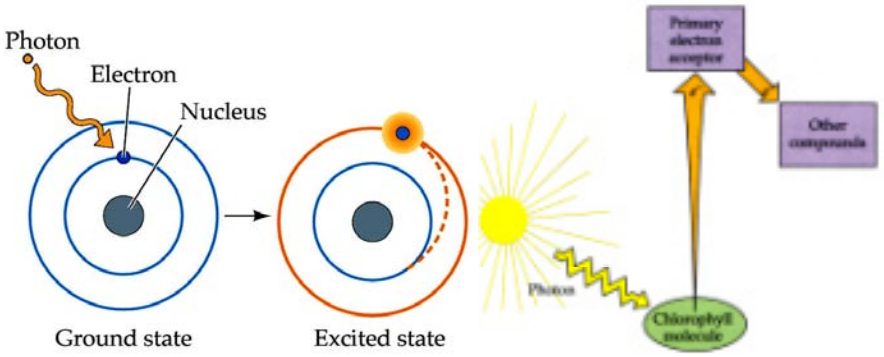
Chlorophyll is the primary pigment that absorbs light energy in photosynthesis. In plants, there are two forms of chlorophyll (a, which has a methyl group, and b, which has an aldehyde group). There are also important **accessory pigments**, the carotenes. Each pigment absorbs certain wavelengths, and collects and concentrates light energy for the photosynthetic process. The red and blue phycocyanin pigments can also absorb light and serve as accessory pigments in photosynthesis.

When pigment molecules absorb energy from light, electrons are excited and move to a higher energy level in their excited state. The energy level of the light absorbed (that photon) and the excited energy level state must match, which is why only certain wavelengths can be absorbed by certain pigments.

This rise in energy is temporary. Electrons fall back to their original energy level if the energy is not transferred elsewhere. When the electrons fall back to their original level, energy is given off as heat, or sometimes as light (fluorescence). Chlorophyll a does both, fluorescing as red light. Fortunately, in photosynthesis, the electrons are transferred elsewhere and their energy is captured.



Excitation of Isolated Chlorophyll Molecules



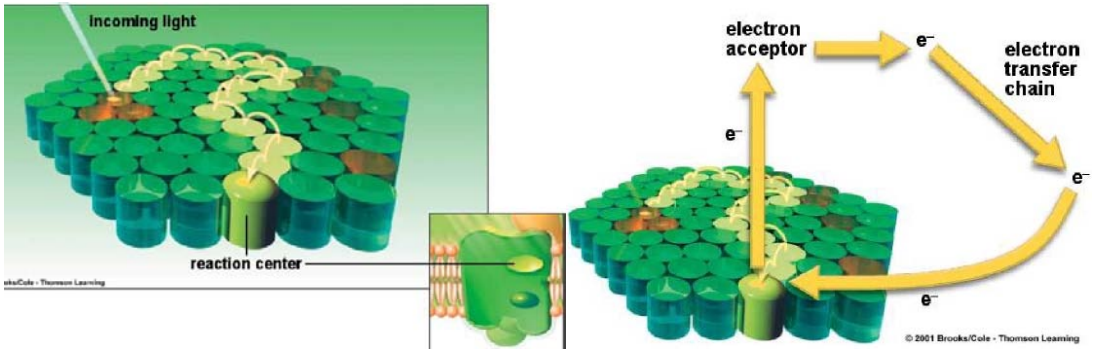
Excitation of Chlorophyll in Chloroplasts

The Photosystems

The photosynthetic pigment molecules do not work alone. They are arranged on the thylakoids of the chloroplast in clusters of about 200 - 300 pigment molecules (plus some protein molecules) in a **light-harvesting complex** (sometimes called the **antenna complex**) that gathers and transfers energy to a **reaction center** that has a special **chlorophyll a** molecule.

There are two such light-harvesting complexes found in the chloroplasts, called **Photosystem I** and **Photosystem II**. The reaction centers of Photosystems I and II are activated by slightly different wavelengths of light.

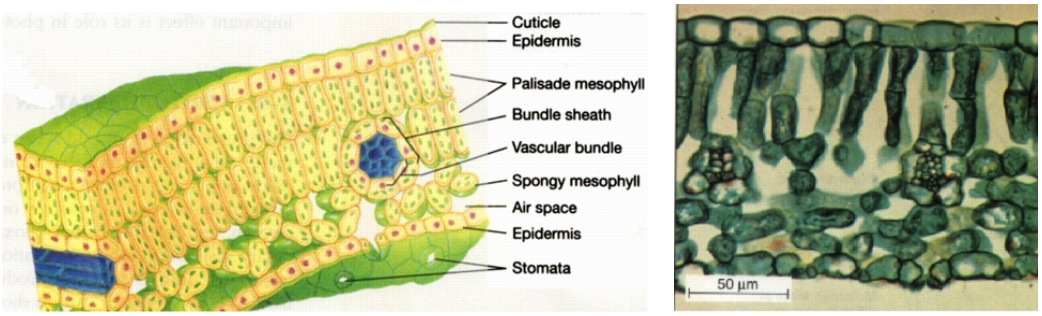
In addition to the light-harvesting complexes, each photosystem has a **primary electron acceptor**, which accepts the electrons released from chlorophyll a, and an associated **electron transfer system**.



6. **Electron Transport System Molecules** (Energy Transfer Molecules)
Many chemical reactions of metabolism are oxidation-reductions that utilize a chain of electron transport molecules. Electron carriers, which are often coenzymes, make it possible for us to trap and use solar energy in photosynthesis by utilizing oxidation-reduction reactions to pass electrons, controlling their energy. In the process of photosynthesis, the electron transport carriers are embedded in the thylakoid membranes.
The most important energy transfer molecule in photosynthesis is the coenzyme, $NADP^+$.

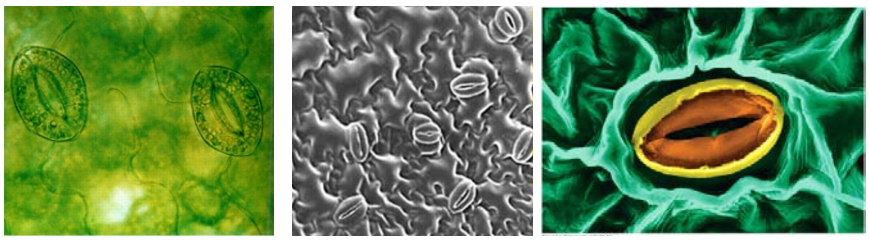
Leaf Structure and Photosynthesis

When one examines a typical leaf, the relationship of its structure to its function in photosynthesis becomes obvious. Water and carbon dioxide must be provided to the chloroplasts, and light must be available. Most leaves are flat providing maximum surface area for sunlight. The leaf surface layer of cells is the **epidermis**, typically with a waxy **cuticle** layer that helps reduce water loss from the leaf surfaces. The lower epidermis layer contains **stomata**, pores that permit diffusion of carbon dioxide into the leaf. The pores are formed by **guard cells** that can open and close the stomata to minimize water loss when photosynthesis is not occurring. **Chloroplasts** are located in the cells of the mesophyll layer of leaves. Most photosynthesis occurs in the upper, or **palisade mesophyll** of the leaf, closest to the light source. The lower mesophyll, the **spongy mesophyll**, contains large **air spaces** to facilitate diffusion of carbon dioxide through the leaf tissue. The **veins** of the leaf deliver water up from roots and transport solutes, particularly sugars, from where they are synthesized to where they are needed or stored within the plant. Veins also provide support and strength for the leaf.



Leaf Cross Section

The stomata found in the leaf surface that permit CO_2 to diffuse into the leaf also permit the diffusion of water and O_2 **out of** the leaf. This loss of water can be significant. As much as 90% of the water absorbed by the plant is lost this way.



Stomata and Guard Cells

Photosynthetic Pathways - Overview.

The two "stages" of photosynthesis (light-dependent and Calvin cycle) are linked by the products of the first stage, the light-dependent reactions. These products are ATP and NADPH. The Calvin cycle uses ATP and NADPH and returns ADP and $NADP^+$.

Stage I: Light-Dependent Reactions.

- The light-dependent reactions transform light energy into chemical energy that is trapped and carried by ATP and NADPH to the Calvin Cycle.
- The light-dependent reactions require chlorophyll and occur in the **thylakoid membranes** of the grana of the chloroplast.
- Light energy is also used to split water (Photolysis) in Photosystem II to:

  $$H_2O \text{ -----> } 2H^+ + 2e^- + {}^1/_2O_2$$

  This produces **oxygen** and provides electrons and hydrogen for the reduction of NADP to NADPH (NADP gains H^+ and electrons; the water is oxidized because it loses the H^+ and e^-).
- The light-dependent reactions are **photophosphorylations** because they involve using light energy to make ATP.
- The light reactions consume H_20 and produce ATP, NADPH and O_2 as a byproduct.

Stage II: Calvin Cycle

- The photosynthetic reactions of the Calvin cycle do not use light energy for their energy source. They use the ATP produced in the light-dependent reactions for their energy source, and the energy transfer molecule, NADPH to provide hydrogen and electrons for a high-energy reduction.
- Carbohydrate molecules are produced in Calvin Cycle in the **stroma** of the chloroplast.
- In the Calvin cycle, carbon dioxide combines with a 5-carbon sugar (Ribulose bisphosphate) and undergoes a reduction to form 3-carbon molecules. These 3-carbon intermediates can be used to regenerate the 5-carbon sugar or metabolized to form the carbohydrate, glucose or other needed organic molecules.

The Photosynthetic Pathways – Details!
The Light Reactions - Cyclic and Non-Cyclic Photophosphorylation

1. Non-Cyclic Photophosphorylation

Uses

Photosystem I
Photosystem II
Electron Transport System

Inputs

Water
Light energy

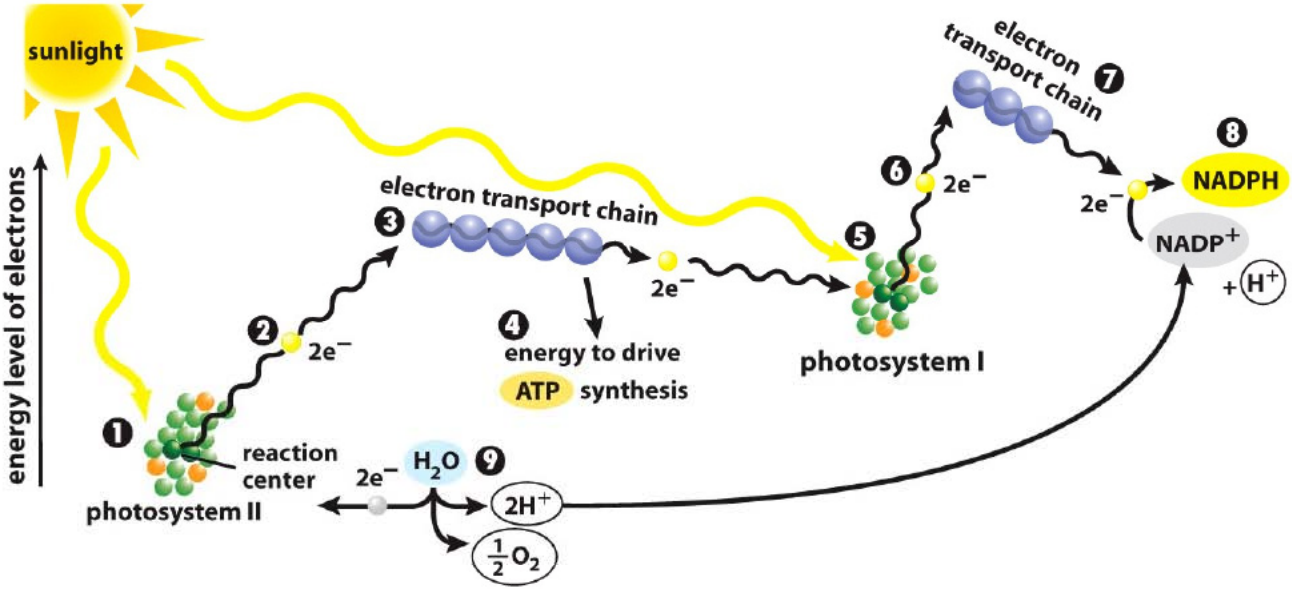
Energy Transfer Molecules

ADP and P
$NADP^+$

Outputs

ATP
NADPH (reduced form) (from $NADP^+$, the oxidized form)
O_2

Process



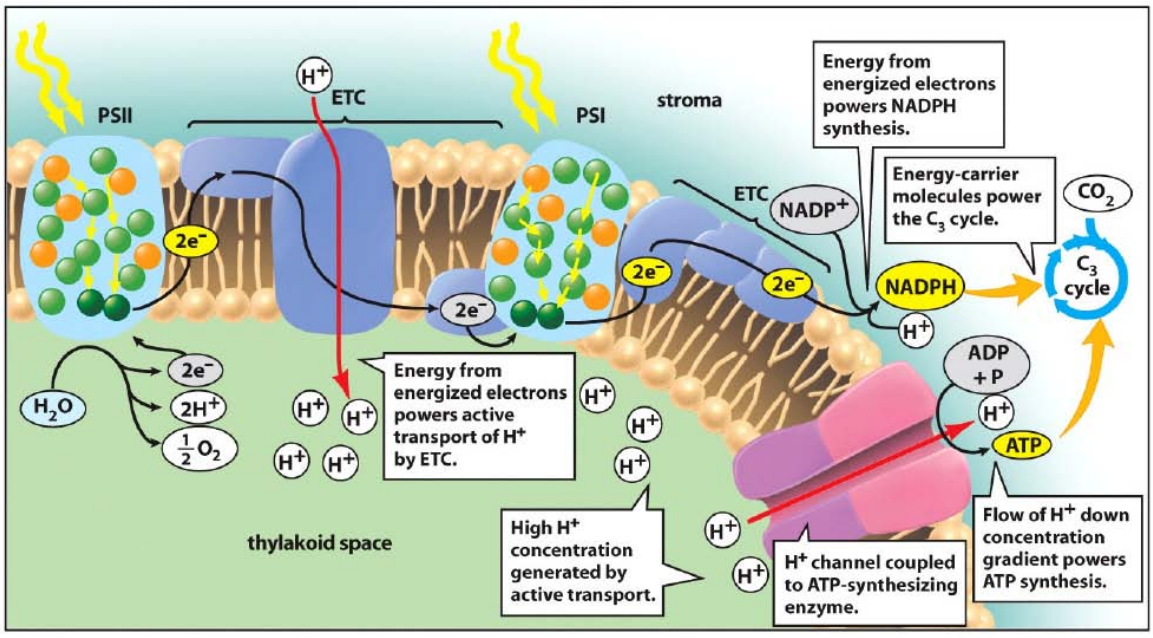
Non-Cyclic Light-Dependent Reactions of Photosynthesis

How it works:

- Light energy splits water molecules (photolysis) in photosystem II producing hydrogen protons, electrons and oxygen.

- Light energy hitting photosystem II excites electrons in its p680 reaction center causing the chlorophyll molecule to lose electrons. They are picked up by electron carriers and passed slowly "down" the chain releasing energy as they go. Some of this energy is used to pump H^+ into the inner thylakoid compartment forming a H^+ gradient. This gradient ultimately drives the mechanism that produces ATP by chemiosmosis. (See a bit later).
- Light energy hitting photosystem I excites its reaction center chlorophyll electrons as well. They are picked up by other electron carriers and transferred to $NADP^+$, which will also pick up a H^+ forming NADPH.
- The electrons released from photosystem II, once they have passed through the electron transport system, are used to replace the electrons lost by photosystem I that were used to form NADPH.
- Water's electrons are passed to photosystem II. The water's H^+ is used in the H^+ gradient (and can be passed to $NADP^+$) and its oxygen is released as oxygen gas molecules.

To summarize, the low energy electrons from water are elevated in energy by passing through both photosystem II and photosystem I on the path to being trapped by $NADP^+$ where their potential energy will be used for the high energy reduction of carbon in the Calvin cycle. Along the way, ATP, needed for the endergonic Calvin cycle, is also produced.



Organization of the Light Reaction Components in the Thylakoid

2. Cyclic Photophosphorylation

Uses

Photosystem I
Electron Transport System
ADP and P

Inputs

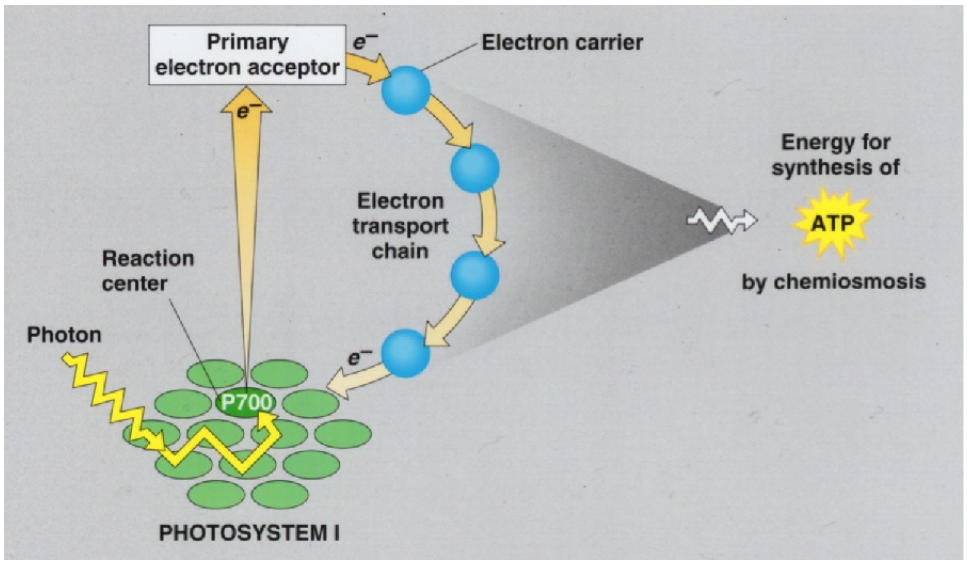
Light energy

Outputs

ATP

Process

Cyclic Photophosphorylation only uses Photosystem I and produces ATP. The earliest prokaryotes, and today's bacteria lack Photosystem II, and use photosynthesis just to produce ATP. All photosynthetic organisms retain the ability to do the cyclic process. In cyclic photophosphorylation, electrons released from the chlorophyll p700 are returned back to Photosystem I after passing through the chain of electron carriers.



Cyclic Photophosphorylation

Before we go much further, we need to discuss how ATP is really made.

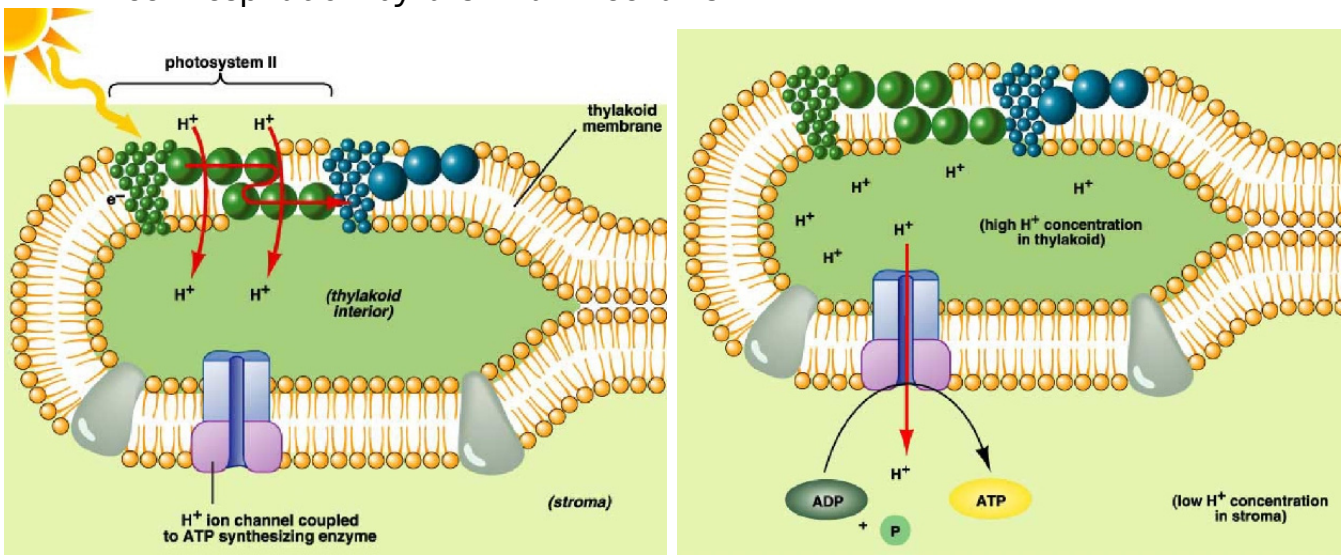
The Chemiosmotic Theory of ATP Synthesis

We have discussed that electrons released from molecules (such as chlorophyll) can travel down an electron transport system, releasing their energy in controlled bits. This energy, as we have said, can be used for the synthesis of ATP.

In photosynthesis, the molecules of electron transport system are located in the thylakoid membrane. The energy released from the electron transport system is used to move Hydrogen ions (H^+) from the stroma into the inner thylakoid compartments by active transport. This concentration of H^+ in the inner compartment of the thylakoid establishes a concentration and a charge gradient in the thylakoid compartment that has a high potential energy.

The accumulated H^+ ions diffuse through ATP synthase protein complex channels in the thylakoid membranes that are coupled to ATP synthesis. As the H^+ ions flow down the gradient in the protein channels, their energy is used to make ATP from ADP and P on the other side of the thylakoid membrane in the stroma.

Note: Peter Mitchell won the 1978 Nobel prize in chemistry "for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory". ATP is synthesized in the mitochondria during cell respiration by a similar mechanism.



Chemiosmotic synthesis of ATP in the Thylakoids

The Calvin-Benson Cycle and Carbon Fixation

The second set of reactions for photosynthesis is known as the Calvin cycle, or the light-independent reactions. They occur in the stroma region of the chloroplast and use the products formed during the light reactions of photosynthesis. All steps of the Calvin cycle are enzyme mediated.

There are many parts to the Calvin cycle:

- Carbon Fixation
- Reduction
- Regeneration
- Surplus (Output)

The requirements for the Calvin cycle are:

- Carbon dioxide (CO_2)
- NADPH from the light-dependent reactions (reducing power, source of electrons and the hydrogen source)
- ATP from the light-dependent reactions (energy source)
- Ribulose bisphosphate, regenerated in the cycle
- Appropriate enzymes for each step in the cycle. Of these, Ribulose bisphosphate carboxylase (Rubisco) is especially important.

The Metabolic Intermediate in Process:

- G3P (Glyceraldehyde 3 Phosphate) (also called Phosphoglyceraldehyde – PGAL)

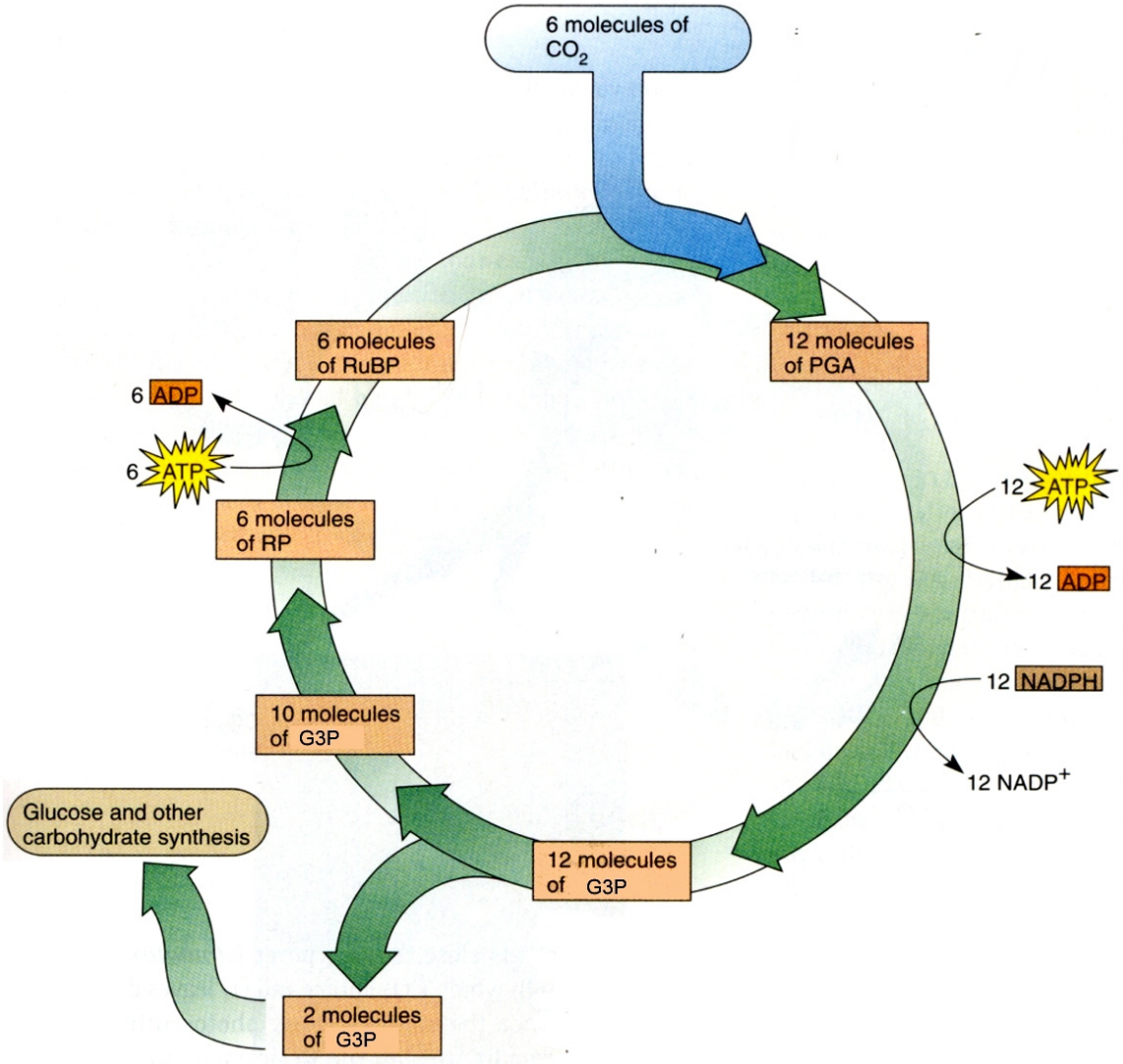
The Calvin cycle produces:

- Glucose (carbohydrate) as the typical end product
- Ribulose bisphosphate, regenerated in the cycle

The details of the Calvin cycle were determined using radioisotopes of ^{14}C. The researchers (Calvin, Benson and others) won a Nobel prize for their discovery. To some extent, the Calvin cycle looks like a carbon cut-and-paste dance. Well, it is. It's easy to follow the maze if one counts carbons, even in more detail than presented in your text. It also helps to remember that without this happening, you'd starve!

The typical Calvin cycle pathway is called **3-carbon photosynthesis** (or C-3) because of the 3-Carbon intermediate that forms in CO_2 fixation. This is to distinguish it from an alternative photosynthetic pathway known as **4-carbon photosynthesis** (or C-4), in which CO_2 is first trapped to form 4-carbon acids, a process to be discussed in a bit.

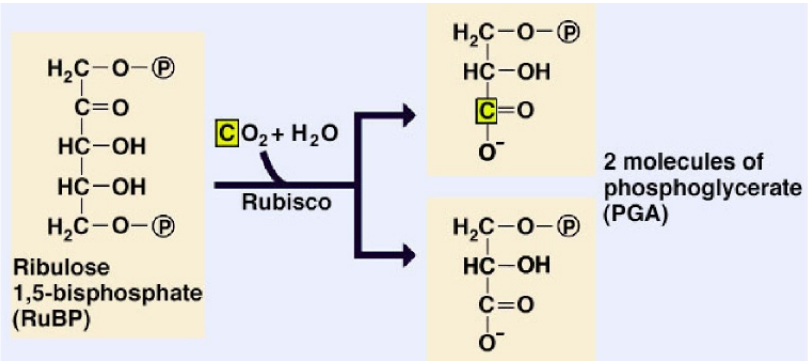
Calvin Cycle Overview



Calvin Cycle – Details!

The Calvin cycle **repeats** 6 times to form 1 glucose

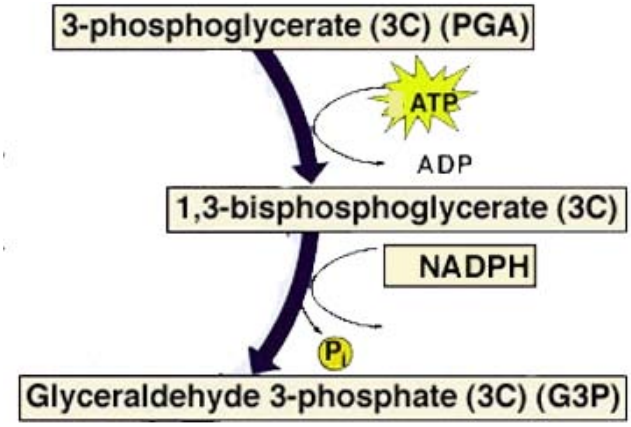
1. Carbon Fixation



CO_2 + RuBP → Hexose Phosphate → 2 PGA

(Ribulose Bisphosphate) → (Phosphoglyceric Acid)

2. Reduction



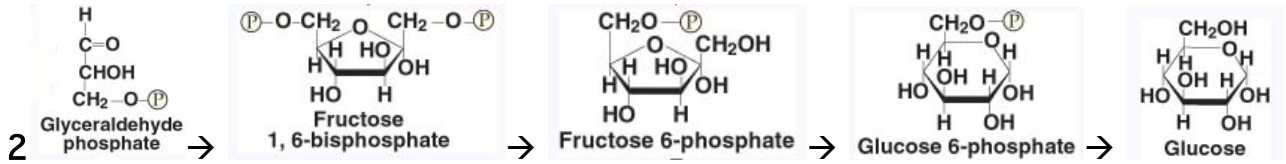
2 PGA (Phosphoglyceric Acid) → 2 G3P* (Glyceraldehyde 3 Phosphate)
(2 ATP + 2 NADPH) → (2 P_i + 2 ADP + 2 $NADP^+$)

A balanced CO_2 Fixation and Reduction uses:
$6CO_2$ + 6 RuBP + 12 ATP + 12 NADPH to produce **12 G3P.**

* G3P has 1 more H atom in its structure than PGA. The H was donated by NADPH during the reduction step. This forms an aldehyde molecule, rather than an organic acid. G3P is also called PGAL for Phosphoglyceraldehyde.

3. The Surplus or Output Phase - Producing Glucose

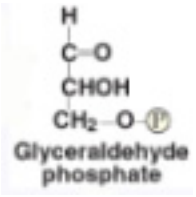
Two of the 12 molecules of G3P are converted into one Glucose molecule.

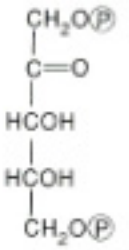


Note: Glucose-6-phosphate often diverts to other carbohydrate synthesis pathways, notably the synthesis of starch, cellulose and sucrose. Little glucose accumulates in plants. G3P can also be converted to starch in the chloroplasts for short-term storage prior to translocation of carbohydrate to permanent storage parts of plants. Sucrose is the carbohydrate most commonly translocated.

4. Regeneration of Ribulose bisphosphate (RuBP) phase

The regeneration of RuBP will use 10 of the 12 G3P molecules from the reduction phase of the Calvin cycle. Water and ADP are given off in the process.



10 (G3P) + 6 ATP → 6 

Ribulose-1,5-bisphosphate (RuBP)

How the regeneration of RuBP works:

- The Regeneration phase of the Calving cycle uses 10 molecules of G3P in 2 sets of 5 each.
- The 3-carbon G3Ps are combined, beheaded, combined differently to form RuMP, which is then converted into the 5-carbon RuBP.
- One ATP is required to convert 1 RuMP to 1 RuBP.
- For each set of 5 G3P molecules:

  Combine 2 G3P to get a 6-C intermediate

  CCC +CCC → CCCCCC

  Remove 2 carbons from the 6-C intermediate→ 2-C + 4-C

  CCCCCC → CC + CCCC

  Combine the 2-C with 1 G3P → 5-C RuMP

  CC + CCC → CCCCC

  The 5-C RuMP + ATP → RuBP

  CCCCC + ATP → RuBP

  Combine the 4-C with 1 G3P → 7-C

  CCCC + CCC → CCCCCCC

  Remove 2 carbons from the 7-C → 5-C RuMP + 2-C

  CCCCCCC → CC + CCCCC

  The 5-C RuMP + ATP → RuBP

  CCCCC + ATP → RuBP

  Combine the 2-C with 1 G3P → 5-C RuMP

  CC + CCC → CCCCC

  The 5-C RuMP + ATP → RuBP

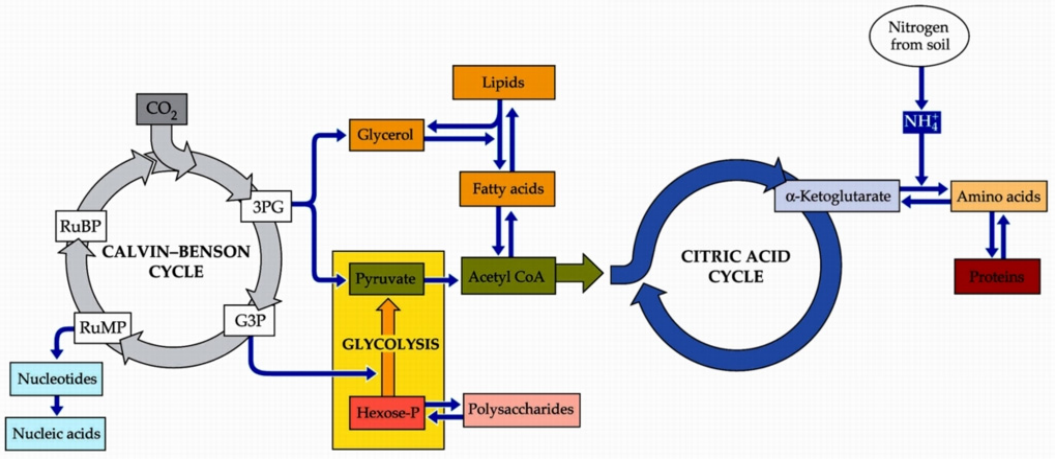
  CCCCC + ATP → RuBP

Repeat the process to regenerate the needed 6 RuBPs.

Synthesis of Other Organic Molecules

Although glucose is the typical end product of photosynthesis, plants do not store or transport glucose. Sucrose (synthesized from fructose-phosphate and glucose-phosphate) is the typical solute translocated throughout the plant, and, as learned, plants typically store starch, including within the chloroplasts.

In addition, plants are capable of synthesizing all of their organic molecules (amino acids, lipids, etc.) from photosynthetic intermediates, notably **G3P** and **glucose phosphate**.

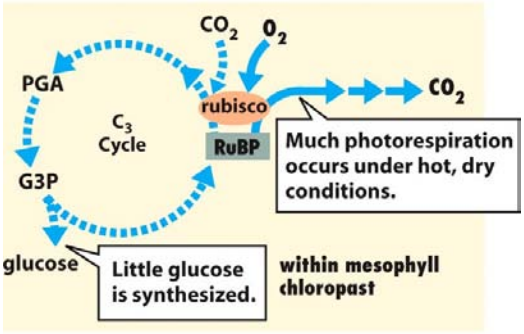


It's important to understand the covalent bonds of the carbohydrate molecules synthesized in the Calvin cycle represent the light energy captured to provide the energy needs for all life!

Photorespiration – Water, CO_2, O_2 and the C_4 Pathway

How much photosynthesis takes place depends in part on climate. If it is too hot or too dry, loss of water through stomata may be too great for the plant, and stomata will close. CO_2 gas is no longer available for photosynthesis. The ratio of oxygen to carbon dioxide in the leaf increases, and this favors a process called **photorespiration**.

Rubisco, the enzyme that brings CO_2 and RuBP together, works only when the concentration of CO_2 is high relative to the level of O_2. When CO_2 levels drop, the enzyme, Rubisco, combines RuBP with O_2 and the Calvin cycle is disrupted. Photorespiration decreases the photosynthetic output of the plant.



Photorespiration in a C_3 plant

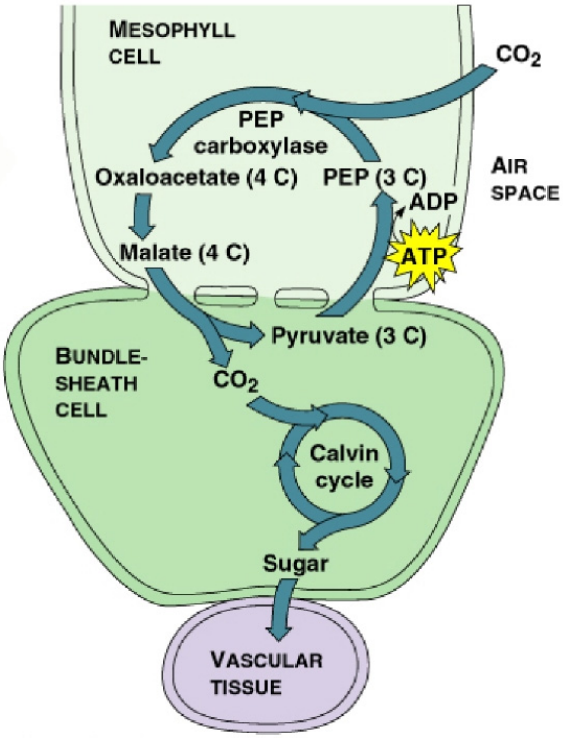
C_4 Photosynthesis

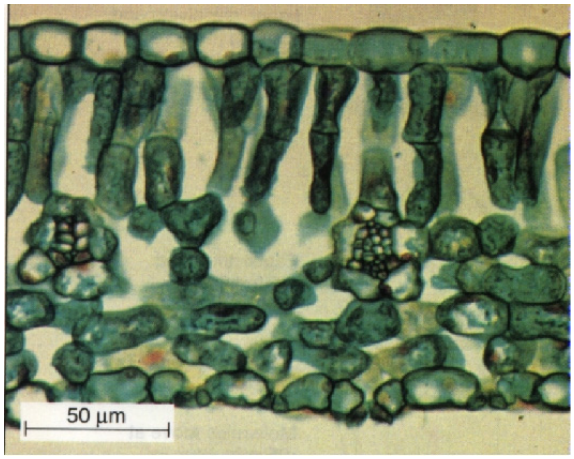
A few specialized plants of hot, dry environments have evolved mechanisms to minimize photorespiration. When CO_2 diffuses into a leaf mesophyll cell, it is combined with a 3-carbon compound, **phosphoenolpyruvate** (PEP), forming a 4-carbon acid, **oxaloacetate** which is converted to malate.

This 4-carbon acid is then transferred to the bundle sheath cells of the leaf. This is a more efficient trap for carbon dioxide since the 4-carbon acid can accumulate during non-light periods, concentrating carbon dioxide when photosynthesis cannot occur, and can be used during periods of low moisture when stomata are closed to prevent water loss. This is called **C_4 photosynthesis** because of the 4-carbon acids formed before the Calvin cycle occurs.

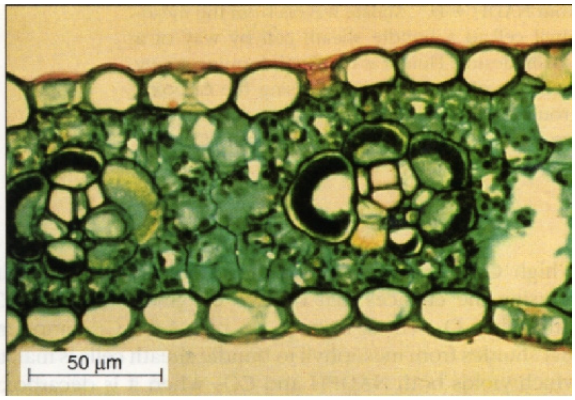
C-4 plants also perform the two stages of photosynthesis in separate cells to keep O_2 away from Rubisco, thereby preventing photorespiration. The light reactions that produce oxygen occur in the leaf mesophyll cells that surround the bundle sheath cells of veins. The chloroplasts of the mesophyll cells have many thylakoids. No Calvin cycle occurs in the mesophyll cells in the absence of CO_2.

The Calvin cycle occurs in specialized **bundle sheath cells** that surround the veins of the leaf. The chloroplasts in the bundle sheath cells have very few thylakoids, but much stroma. The accumulated 4-carbon acids are shunted to the bundle sheath cells. In addition, many plasmodesmata occur between the mesophyll cells and the bundle sheath cells to transport ATP and NADPH produced in the light reactions to the bundle sheath cells for use in the Calvin cycle. ADP, P_i and $NADP^+$ are returned to the mesophyll cells, as is the 3-carbon acid, pyruvate, that forms when the CO_2 is released for carbon fixation in the bundle sheath cell.





C_3 Leaf Structure



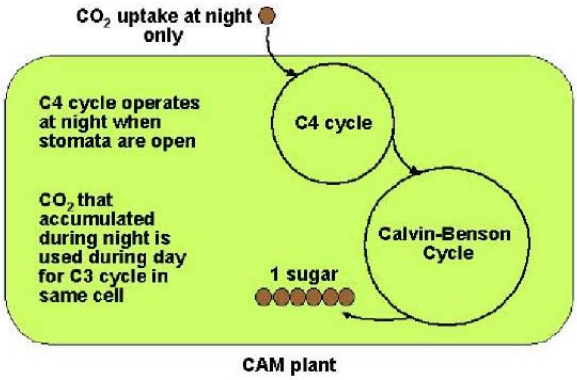
C_4 Leaf Structure

C_4 photosynthesis is highly productive in hot and dry environments. The world's most productive plants, sugar cane and corn, are C_4 plants, as is crab or quack grass, a weedy grass that stays healthy when lawns dry in summer months. However, regenerating PEP requires ATP, so C_4 photosynthesis is not always more productive than the C_3 pathway. Unfortunately, the pathway is genetic, so plants can't choose.

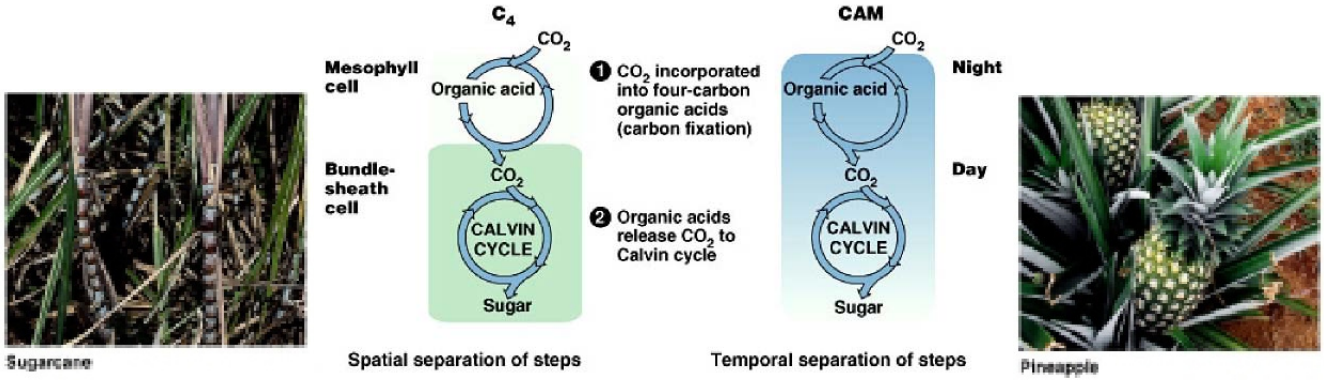
CAM - Another Conservation Mechanism

Some plants can minimize water loss by reversing the time of day when stomates are open. Plants that have reverse stomatal opening can also store CO_2 in 4-carbon acids. They do not, however, have photosynthesis separated into two different cells. In the daytime, the accumulated acids release CO_2 for "normal" mesophyll cell light reactions and Calvin cycle, while the stomata can remain closed to prevent excessive water loss. They do not reduce photorespiration. The name, CAM (for Crassulacean Acid Metabolism) is derived from the types of plants in which it was first discovered, Crassuleans, a plant family of succulents, and for the fact the CO_2 trapped forms acids.

Some CAM plants can even fix CO_2 into acids during prolonged droughts when stomata can't even open at night using the CO_2 produced during aerobic respiration. This is minimal, but sufficient to sustain life. A mature saguaro cactus can survive about 18 months of drought.



C_4 and CAM Plants Compared



Bacterial Photosynthesis

Some bacteria have photosynthetic pigments and a process of photosynthesis. There are some differences, however:

- Bacterial chlorophyll is different from the chlorophyll of other photosynthetic organisms.
- Bacteria do a just cyclic photophosphorylation. They can not do photolysis, and do not produce oxygen.
- The Cyanobacteria, however, have chlorophyll a and photosystem II and perform photosynthesis much the same way as Eukaryotic autotrophs do. They have thylakoid-type invaginations of their plasma membrane.

Chemosynthetic Bacteria

As mentioned in the introduction to this chapter, some bacteria can use inorganic molecules such as Fe^{++}, NH_4^+, S and H to provide energy to "fix" carbon (that is make organic compounds from inorganic sources). In the scheme of life, chemosynthesis plays a small part in energy acquisition. Yet the environmental role of chemosynthetic bacteria, such as in the nitrogen cycle, is critical.

How Productive is Photosynthesis?

It takes 18 ATP and 12 NADPH to make one molecule of glucose. Much of the light energy that hits the surface of plants is not absorbed and not available. And much of the light that hits earth does not hit photosynthetic surfaces of plants. Only about 1 – 3 % of the potential energy of the sun is captured by photosynthesis. C4 plants achieve up to 6% productivity under their ideal conditions.

All cells must do work to stay alive and maintain their cellular environment. The energy needed for cell work comes from the bonds of **ATP**. Cells obtain their ATP by oxidizing organic molecules, a process called **cellular respiration**. Although many organic molecules can be oxidized, glucose, a main product of photosynthesis, is the primary fuel molecule for the cells of living organisms.

Every living organism, autotroph and heterotroph, must do cell respiration. In fact, the metabolic pathways used in the process of cellular respiration are the same in virtually all eukaryotic organisms as well as most prokaryotic organisms. Recall that organisms that do photosynthesis (or properly, manufacture their own fuel molecules) are called **autotrophs**. **Heterotrophs** obtain their fuel molecules "pre-formed" by other organisms. Animals, fungi and many protists are heterotrophs, as are most bacteria. Plants and some protists are autotrophs, as are some bacteria.

Most eukaryotic organisms are **aerobic** (oxygen requiring). **Aerobic cell respiration** is required in order to obtain enough energy (ATP) from the oxidations of fuel molecules for these organisms to survive. In aerobic respiration glucose is oxidized to water and carbon dioxide. Oxygen is required as the final electron acceptor for the oxidations. Most organisms are **obligate aerobic** organisms.

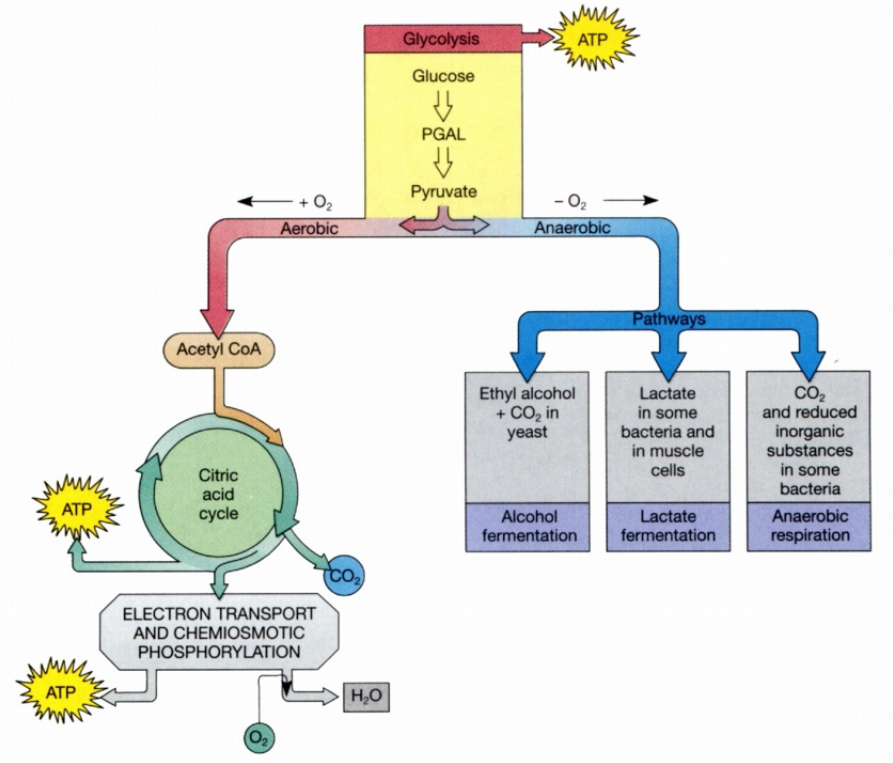
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + 686 \text{ kcal energy (ATP + Heat)}$$

Not all cell respiration is aerobic. All organisms do some type of **anaerobic respiration** during times of oxygen deficit, although it may not be sufficient to sustain the organism's ATP needs for many species. Fuel molecules oxidized without oxygen yield smaller amounts of ATP.

The **fermentations** involve the partial breakdown of glucose without using oxygen. Many prokaryotes have a variety of fermentation pathways, using a number of different fuel molecules. By definition, the end product for the fermentations is an **organic molecule.** In aerobic cellular respiration, the final electron acceptor is **oxygen**, hence, the emphasis on oxygen in aerobic cell respiration. In addition, some prokaryotes use **anaerobic electron transfer respiration** pathways in which their final electron acceptor is an inorganic molecule such as sulfate, iron, or nitrogen compounds.

Some organisms are **obligate anaerobes**. They cannot survive in the presence of oxygen. The *Clostridium* bacteria that cause botulism poisoning, tetanus and gangrene are obligate anaerobes. Other anaerobes are **metabolic anaerobes**; they lack the enzymes needed to do aerobic cell respiration. Many of our intestinal bacteria, such as the *Lactobacillus* bacteria, are metabolic anaerobes. Some organisms will survive nicely in the absence of oxygen but will do aerobic respiration when oxygen is available. Yeast organisms and *E. coli* are two such facultative organisms.

Aerobic and Anaerobic Respiration Pathways

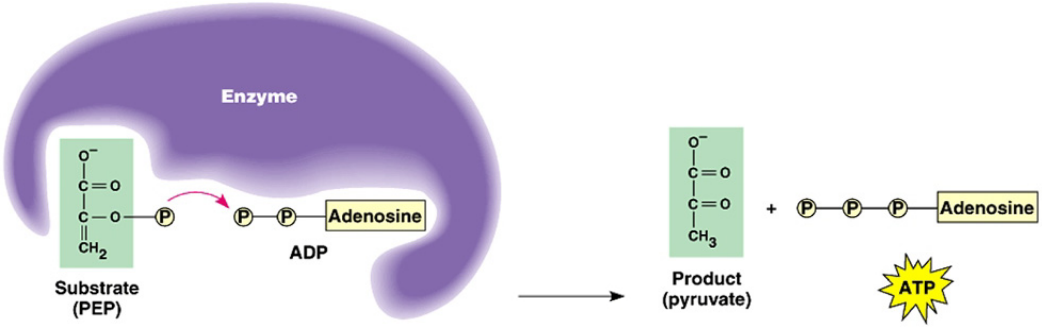


Oxidation-Reduction Reactions in Cell Respiration

The oxidations of fuel molecules in aerobic cell respiration use specialized electron carrier molecules, most of which in eukaryotic organisms are located in the membranes of the **mitochondria**. These electron transport molecules gain and lose electrons at specific energy levels. This is very similar to the electron transport molecules used in photosynthesis.

One of the most important of the electron transport molecules in cell respiration is **NAD^+**. Electrons are passed through an **electron transport chain** to form ATP by chemiosmosis, a process sometimes called **oxidative phosphorylation** or **electron transport phosphorylation**.

Not all of the ATP produced during cell respiration is by chemiosmosis. Some ATP is also synthesized by a direct transfer of phosphate from a substrate molecule to ADP. This process is called **substrate-level phosphorylation**. We will discuss this more when we do the details of the cell respiration pathways.



Cell Respiration - An Overview of the Processes

As with many metabolic processes, cell respiration has a number of stages.

Glycolysis

The initial stage of glucose metabolism, or cell respiration, is a process called **glycolysis**, which splits a glucose molecule into two molecules of **pyruvate**, a 3-carbon compound. Glycolysis occurs in the cytosol of the cell. What follows glycolysis depends on the presence or absence of oxygen and/or the enzymes needed.

If oxygen is available and the organism has the enzymes to do **aerobic respiration**, the pyruvate molecules will be oxidized in the next stages of aerobic respiration. The reactions of aerobic respiration after glycolysis occur in the **mitochondria** and include the **Krebs cycle** and the **electron transport chain.**

If oxygen is not available, or if the organism lacks enzymes needed for aerobic respiration, the pyruvate molecules will proceed with **fermentations**.

In order to obtain sufficient ATP for survival, most organisms must do aerobic cell respiration.

Cellular Respiration - The Pathways

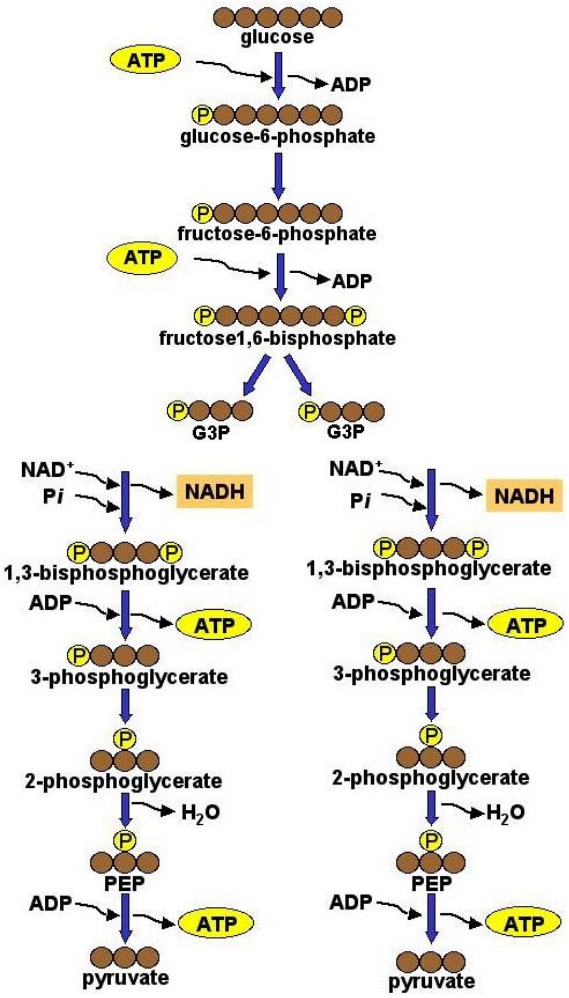
Glycolysis - Overview

- **Glucose** is “activated” for the oxidations by two ATP-consuming reactions. Glucose must be "primed", or destabilized, in order to become reactive.
- Glucose is then broken into two molecules of the 3-carbon compound, **Pyruvate.**
- In addition:
    - Two molecules of NADH are produced.
    - A net of two molecules of ATP are produced. (Four molecules of ATP are produced during glycolysis, but 2 molecules are consumed in activating the glucose.)
- Glycolysis always occurs in the cytosol (cytoplasm) of the cell.
- Glycolysis is the most widespread metabolic pathway in living organisms, today and evolutionarily. The earliest prokaryotes probably had a glycolysis pathway.

Glycolysis Specifics

Glucose + 2ATP + 2NAD^+ + 2ADP + 2P → 2 Pyruvate + 2NADH + 4ATP*

* Net gain of 2ATP



Summary of Glycolysis

Inputs

- Glucose
- 2 ATP*
- (and 2NAD^+ + 2 ADP + 2 P)

Outputs

- 2 Pyruvate
- 2 NADH
- 4 ATP*

* Therefore the **net** energy yield is 2 ATP

- The ATP generated is by **substrate-level phosphorylation**
- All steps are enzyme mediated
- Glycolysis occurs in the cytoplasm of the cell
- Glycolysis is the initial cell respiratory pathway of **all eukaryotic organisms.**

Following glycolysis, the presence of absence of oxygen, and/or the organism's ability to use oxygen in respiration, determines whether pyruvate will be oxidized to yield more energy in **aerobic respiration** or reduced to a stable molecule in the **fermentations**. We will discuss first what happens after Glycolysis when no oxygen is available – The **Fermentations**. Then we will discuss the processes involved in **Aerobic Cellular Respiration**

The Fermentations – Cell Respiration in the Absence of Oxygen

The overwhelming majority of living organisms must do aerobic cellular respiration to stay alive. Fermentations and other anaerobic pathways provide insufficient ATP to sustain life for most organisms. Yet, when no oxygen is available for aerobic cell respiration, eukaryotic organisms, and some prokaryotes, will complete glucose metabolism with the **fermentation** reactions. Fermentations are an alternative pathway for pyruvate after glycolysis occurs.

For some microorganisms, fermentation is a way of life. Some lack the enzymes to do the Krebs cycle; for others, oxygen is toxic. These are the **strict** (or obligate) **anaerobes**. Others, such as yeasts and *E. coli* are **facultative** organisms. When oxygen is available, they do aerobic respiration. When oxygen is not, they perform a fermentation.

NADH carries very high energy electrons, but those electrons can be used to make ATP only in the presence of oxygen. In the fermentations the NADH electrons produced in glycolysis will be used to reduce pyruvate to some other organic molecule, which becomes the final electron acceptor of cell respiration. This is needed to recover NAD^+ for more glycolysis; **no additional ATP energy** is obtained in the fermentation processes beyond the two ATP produced during glycolysis.

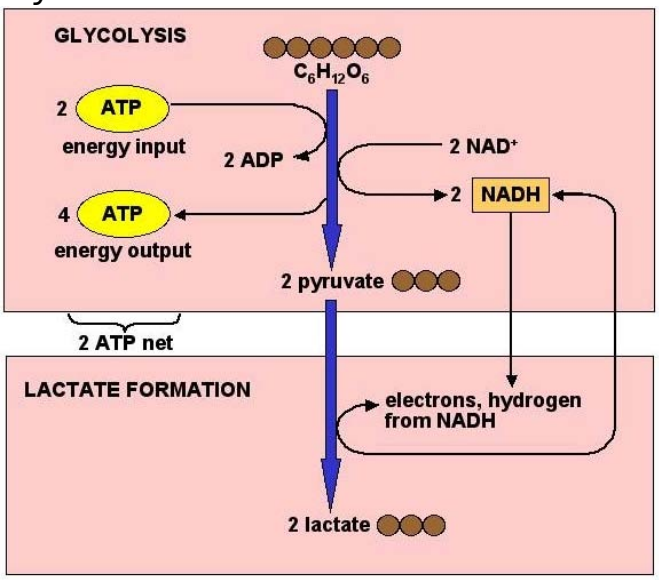
Fermentation Details

- Although a number of different fermentation pathways are found among the bacteria, only two fermentation pathways, which are genetically determined, are found in Eukaryotic organisms.
  - **Alcoholic Fermentation**
  - **Lactic Acid Fermentation**
- Pyruvate from glycolysis functions as the electron acceptor for the NADH produced in glycolysis.
- NADH is used to reduce pyruvate to some stable organic molecule, freeing the NAD^+ (or regenerating NAD^+) for more glycolysis.
- The organic molecule is the final electron acceptor. No additional ATP is produced.
- No additional ATP is produced after the initial ATP production from glycolysis.

Lactic Acid Fermentation

NADH -----> NAD^+

Pyruvate ----------> Lactic Acid



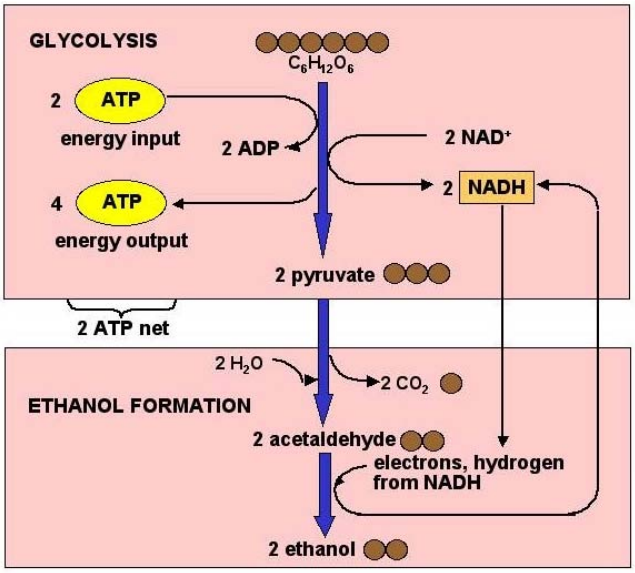
Alcoholic Fermentation

NADH -----> NAD^+

Pyruvate --->Acetaldehyde ----------> Ethanol (Ethyl Alcohol)

\+

CO_2

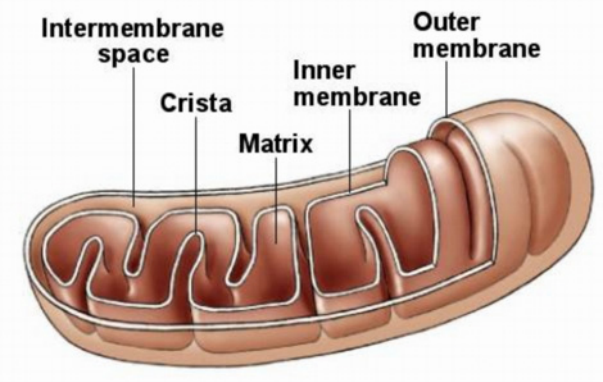


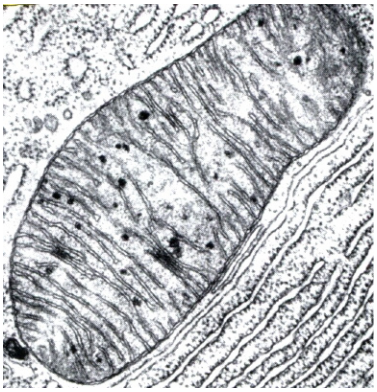
Anaerobic Electron Transport (Those versatile Prokaryotes)

Some anaerobic bacteria have an electron transport system and oxidize a variety of molecules. Some inorganic substance, such as sulfur or nitrogen molecules, becomes the final electron acceptor, rather than oxygen. ATP production is small, but sufficient for the anaerobic bacteria. Aromatic H_2S, hydrogen sulfide, is a common end product.

Aerobic Cellular Respiration

Aerobic Cellular Respiration is comprised of two three stages following glycolysis, that occur in the **mitochondria** of the cell: the oxidation of pyruvate, the Krebs cycle reactions in the mitochondrial matrix (the inner compartment of the mitochondrion) and the electron transport chain reactions that occur in the inner mitochondrial membrane.





The Mitochondrial Matrix (Inner Compartment) Stages

The second and third stages of aerobic respiration comprise the oxidation of pyruvate and the Krebs cycle. Both occur in the mitochondrial matrix.

- Pyruvate molecules are oxidized and lose a CO_2 forming **acetyl**. **NAD^+** picks up the electrons and H^+ from the oxidation forming **NADH.**
- The two-carbon acetyl unites with and is carried to the **Krebs cycle** by coenzyme A (CoA). More oxidations occur in the Krebs cycle, releasing two more CO_2 for each pyruvate molecule and yielding many more NADHs as well as 1 $FADH_2$ and 1 ATP.

The Inner Mitochondrial Membrane Stage

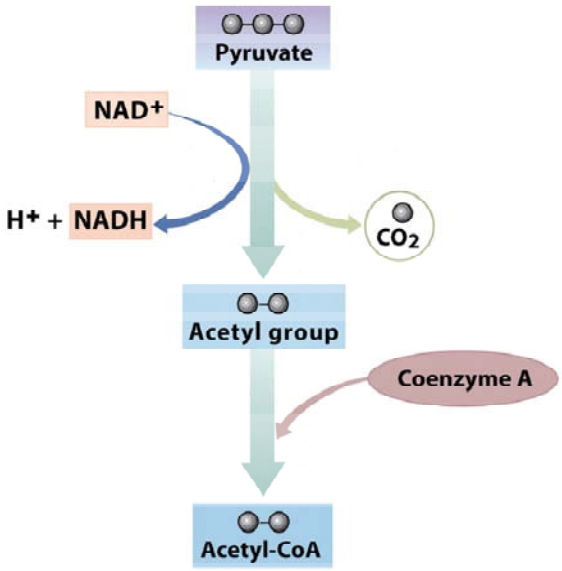
The final stage of aerobic respiration is the **electron transport chain** and the **chemiosmotic** synthesis of ATP. Since the energy to synthesize ATP is from the oxidation-reduction reactions, the ATP formation is also called **oxidative phosphorylation**.

- Oxygen is the final electron acceptor for the oxidation-reductions that start with NADH in the electron transport chain.
- The electron transport chain reactions take place in the inner membrane of the mitochondria.

When oxygen is available, as much as 36 - 38 ATP can be generated from one glucose molecule.

Oxidation of Pyruvate to Acetyl

- The two Pyruvate molecules from the original glucose are transported into the **inner matrix of the mitochondria** by facilitated diffusion
- Each pyruvate is oxidized releasing H^+ to reduce NAD^+ to NADH
- CO_2 is removed producing **Acetyl** (A 2-carbon compound)
- Acetyl combines with Coenzyme A to form **Acetyl-CoA**, which can enter the Krebs cycle.



For one glucose molecule (two pyruvate molecules), we obtain:

- 2 CO_2
- 2 NADH
- 2 Acetyl C0-A

Note: When the level of ATP is high in a cell, the cell can convert acetyl-CoA into lipid molecules that can be stored for later energy use. This is one way that excess calories, no matter the nutrient source, are converted to fat.

The Krebs Cycle

The Krebs cycle is a means to remove energy rich H^+ (with its electrons) (originally part of the glucose molecule) that can subsequently be used to generate ATP in electron transport via chemiosmosis. The leftover carbon is given off as CO_2.

Essentially, the acids of the Krebs cycle are substances that under the right conditions (i.e., The Krebs Cycle) can be **oxidized** (That is donate H^+ with its electrons).

For each glucose molecule, two ATP are produced in the Krebs cycle by **substrate-level phosphorylation**, one for each acetyl Co-A molecule that enters the Krebs cycle. (Recall that the glucose molecule has already gone through glycolysis and has been converted to two molecules of Pyruvate in the cytoplasm prior to starting the Krebs cycle.)

A closer Look at the Krebs Cycle

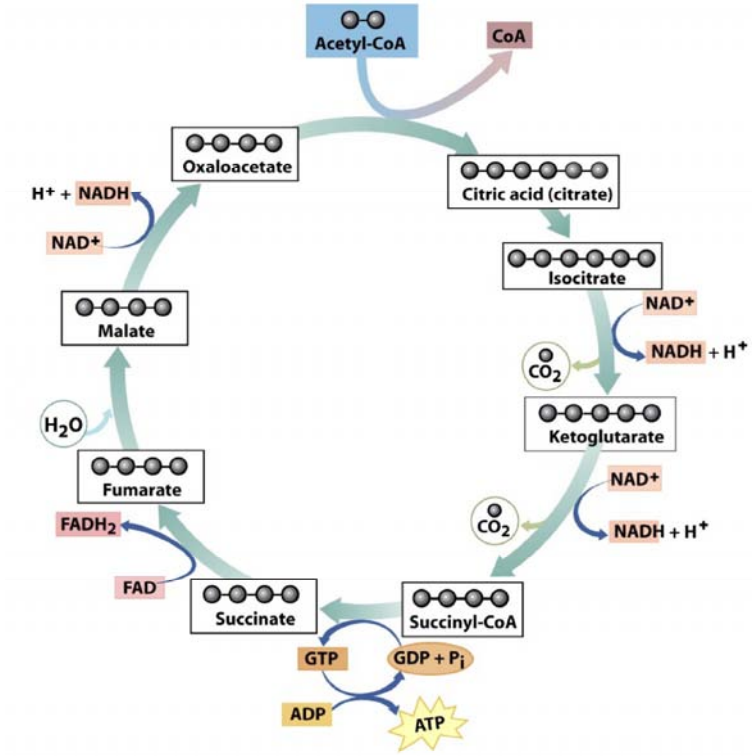
Any cycle requires a substance to start the cycle (which will also be the end of the cycle). For the Krebs cycle the starter is **oxaloacetic acid** (oxaloacetate), a 4-carbon acid that is regenerated at the end of the cycle. The enzymes needed to do the Krebs cycle are located in the **mitochondrial matrix.**

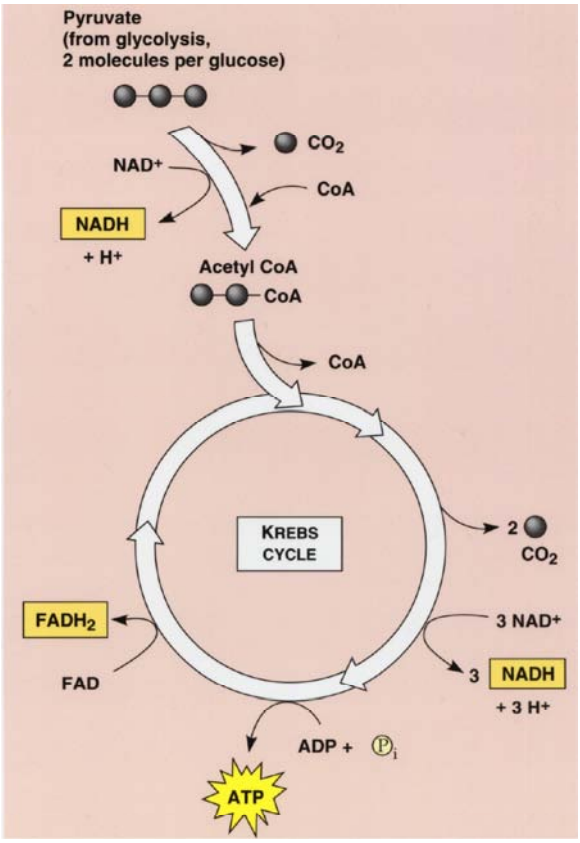
Acetyl-CoA combines with **oxaloacetic acid** to begin the cycle forming the 6-carbon **citric acid** (citrate). Co-A is released to pick up more acetyl.

For each turn of the Krebs cycle we get:

- 2 CO_2 given off (plus 1 from pyruvate oxidation to acetyl) = 3 CO_2
- 1 ATP produced (by substrate phosphorylation)
- 1 $FADH_2$
- 3 NADH (plus 1 from pyruvate oxidation to acetyl) = 4 NADH

The Krebs Cycle – Specifics





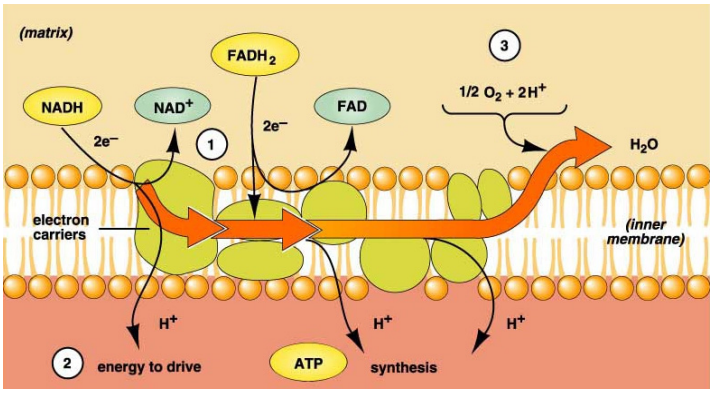
The Krebs cycle will turn **two** times for each glucose molecule. Two turns of the Krebs cycle (including the preparation step of pyruvate → acetyl will produce:

- 6 CO_2
- 2 ATP produced (by substrate phosphorylation)
- 2 $FADH_2$
- 8 NADH

Electron Transport Chain

The enzymes, proteins and electron carriers needed to do electron transport are found in the **inner membranes of the mitochondria**. ATP is produced by using a H^+ concentration gradient to run the ATP synthesis pumps as electrons are passed along the electron transport molecules in a series of oxidations and reductions.

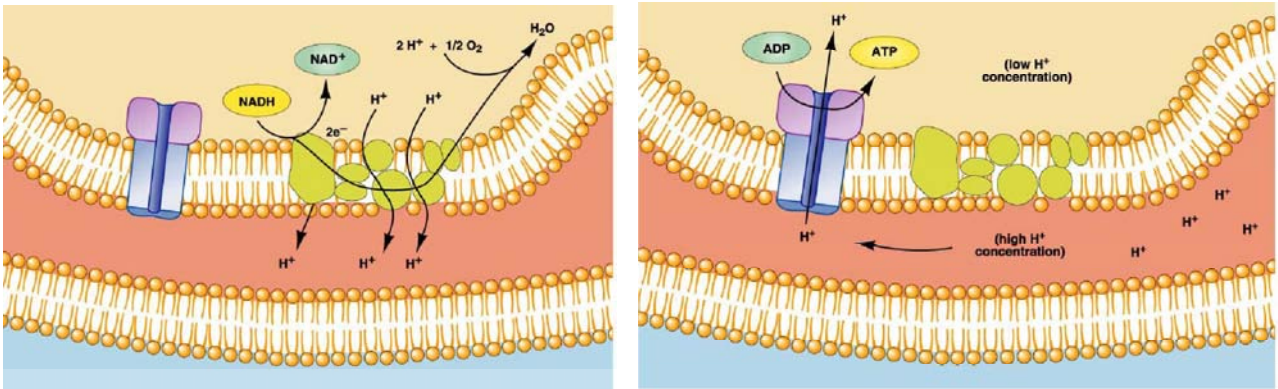
As the electrons are passed from one carrier to the next, the energy released is used to move H^+ ions from the mitochondrial matrix through the inner membrane into the intermembrane space of the mitochondrion. As the H^+ concentration builds, it provides a H^+ gradient that passes through a **protein channel** pore in the membrane that works with the enzyme, ATP synthase, to generate ATP in the mitochondrial matrix. Peter Mitchell won the 1978 Nobel prize in chemistry "for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory". ATP is synthesized in the thylakoid membranes of the chloroplast by a similar mechanism.



The Electron Transport Chain

The Electron carriers, $FADH_2$ and NADH, produced in the Krebs cycle (and in glycolysis), provide the electrons and hydrogen needed for ATP synthesis.

Oxygen is required as the final electron (and hydrogen) acceptor, producing **water** as the end product of aerobic cellular respiration. The H^+ and e^- passed through the carriers combine with oxygen in the final step. (Recall that CO_2 is also a product of aerobic cellular respiration.)



Chemiosmosis in the Mitochondria

How much ATP do we get from oxidizing glucose in aerobic cellular respiration?

- The electrons and H^+ from each NADH produced in the Krebs cycle and the oxidation of pyruvate to acetyl Co-A provides sufficient energy to produce about 3 ATP by chemiosmosis.
- The electrons and H^+ from each $FADH_2$ produced in the Krebs cycle provides sufficient energy to produce about 2 ATP by chemiosmosis
  (FAD is a lower energy electron transfer molecule and enters the transport chain in mid-chain, rather than at the start)
- The electrons and hydrogen from each NADH from Glycolysis provides sufficient energy to produce about 2 ATP
  (The hydrogens and electrons have to be transferred from NADH in the cytoplasm to the mitochondria)

Summary of ATP Production from the complete aerobic metabolism of one glucose molecule

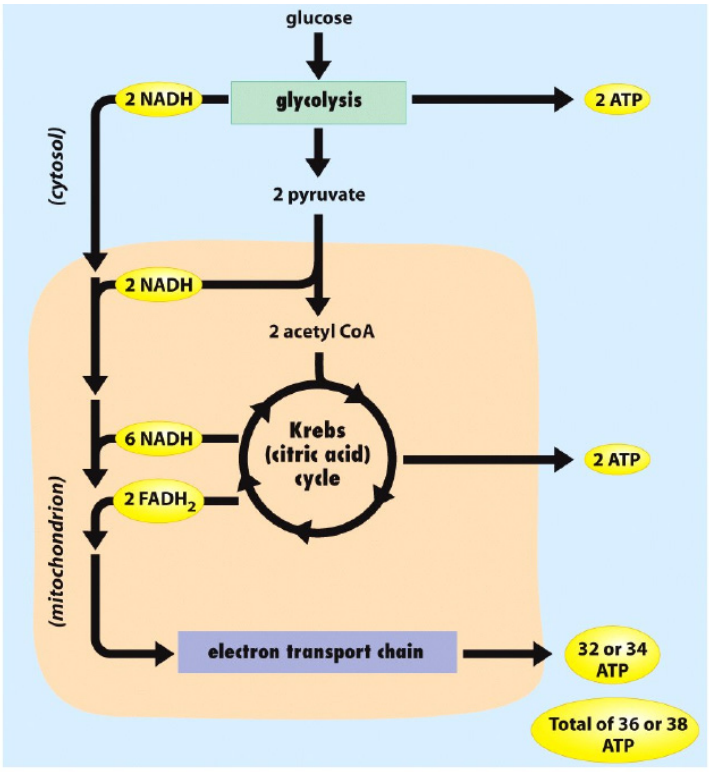
From Electron Transport phosphorylation:

6 NADH from Krebs X 3 ATP each =	18 ATP
2 NADH from Pyruvate to Acetyl X 3 ATP each =	6 ATP
2 NADH from Glycolysis X 2 (3) ATP each =	4(6) ATP
2 $FADH_2$ from Krebs X 2 ATP each =	4 ATP

From Direct ATP synthesis (Substrate phosphorylation)

2 ATP directly from Krebs	2 ATP
2 ATP directly from glycolysis	2 ATP

Maximum Total ATP from 1 glucose = 36(38) ATP



Aerobic (Cellular) Respiration Summary

The complete aerobic respiration of glucose requires the following:

- Glycolysis
- Pyruvate Oxidation and the Krebs cycle
- Electron transport phosphorylation

- **Oxygen**, the final electron acceptor in the electron transport system, combines with Hydrogen to form water.
- Carbon Dioxide (CO_2) is released during aerobic respiration.
- 36 ATP can be produced for each glucose molecule.
- The Krebs cycle and electron transport occur in the **mitochondria. Glycolysis** occurs in the cytosol.
- All steps are catalyzed by enzymes

Generating Heat From Cell Respiration - Thermogenesis

There are times when generating heat rather than ATP is desired. For example, organisms, such as bats, need to increase body temperature rapidly when they wake. There are special proteins, called **uncoupling proteins**, that separate the H^+ proton flow in electron transport from the ATP synthase pump, so that heat is produced instead of ATP.

Plants also have uncoupling proteins for heat generation. Arum lilies attract carrion beetles and flies for pollination by exuding odors that smell like carrion. Skunk cabbage elevates its body temperature as much as 10 - 12° C above ambient air temperature for flowering. Plants emerge through snow in the spring use thermogenesis, too.

Arum Thermogenesis

Versatility of Metabolic Pathways

We present aerobic respiration from its typical start with glycolysis using glucose fuel. We know, however that fats and proteins can also be used to provide energy for cells. How do these molecules fit into cellular respiration?

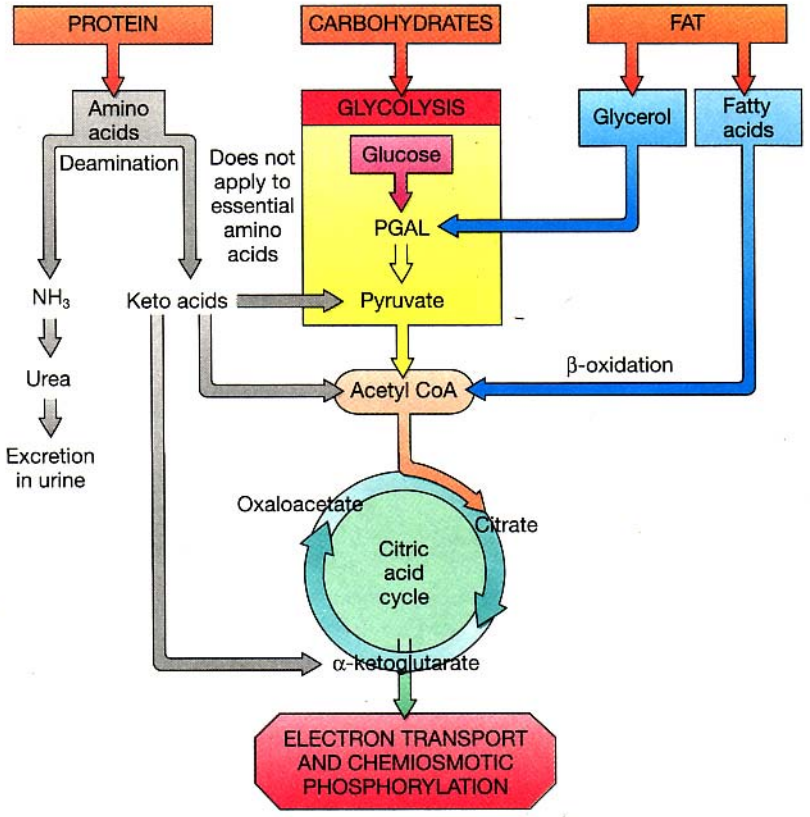
Using other fuel molecules in the energy releasing pathways.

- Other carbohydrates -----> Glucose -----> Glycolysis
- Proteins -----> Amino Acids -------> Pyruvate -------> Krebs Cycle
  or
- Proteins -----> Amino Acids -------> Krebs Cycle
  or
- Proteins -----> Amino Acids** -----> Glucose ------> Glycolysis

  ** Amino acids in this group are converted to pyruvate and metabolized "back" to glucose to provide glucose to brain and nervous system cells and developing red blood cells.

  Note: All amino acids must be deaminated prior to being used for fuel.

- Lipids: Glycerol ------> Glycolysis (Glyceraldehyde 3 Phosphate)
- Lipids: Fatty Acids -----> Acetyl -----> Krebs Cycle
- Alcohol -----> Acetyl -----> Krebs Cycle



Some Notes

- Some of the steps in nutrient inter-conversion can work in both directions. Acids from the Krebs cycle can be used to synthesize some amino acids, and acetyl can be used to synthesize fatty acids. (About half the amino acids are non-essential in this sense; they can be made from other amino acids or from other acids in the cells.)

- Fats are more energy rich than carbohydrates. A gram of fat *potentially* can produce two times as much ATP as a gram of carbohydrate. Most moderate muscle activity, such as breathing and heartbeat, routinely uses a mixture of fats and carbohydrates. However, use of fatty acids for fuel is a strictly aerobic process. All anaerobic respiration must have glucose. Also, fatty acid fragments cannot normally cross the brain membrane barriers so that the brain does not use fats for fuel.

- During starvation or fasting, or when there is insufficient carbohydrate for energy needs, the body uses its protein from body tissues to produce glucose fuel molecules for the brain and red blood cells. (Some amino acids can be converted to pyruvate and by reversing the steps of glycolysis to glucose.)

- When fat reserves are mobilized in response to insufficient calories or insufficient carbohydrate in the diet, some of the fatty acid fragments combine to form ketone bodies rather than acetyl. These ketone bodies enter into circulation. Muscle and some other tissues can use ketone bodies for fuel, and ketone bodies can provide energy to some brain cells. However, some ketone bodies contain carboxyl groups forming keto acids that can cause ketosis, a condition that lowers the pH of the blood and impairs health.

We have briefly discussed that DNA is the genetic molecule of life. In eukaryotic organisms DNA (along with its histone proteins) is found in chromosomes. We have also learned that the metabolic activities of a cell are catalyzed by enzymes, specific proteins, and that the instructions for the synthesis of proteins are found in the structure of DNA.

Moreover, a **gene** is a region of DNA that specifies a certain inheritable characteristic or trait. This region of DNA stores the information in a coded form that specifies the sequence of amino acids that comprise a specific polypeptide. The genes we inherit from our parents determine the polypeptides we synthesize in our cells, which determine the structure and functioning of our cells and tissues.

What DNA is and how DNA works is the subject of this unit of Biology 160. We will look at the structure and functions of DNA, how the information stored in DNA is used to direct cell activities and how cells regulate the activity of their genetic molecules, as well as the mechanism by which DNA molecules duplicate prior to cell division to ensure that all cells of an individual have exactly the same DNA.

The search for the molecule of inheritance spanned a century from the mid-1850's to 1953, when Francis Crick and James Watson announced they had determined the three dimensional structure of DNA. The steps to this discovery are a good example of the process of science.

DNA was first isolated by the Swiss chemist, Meischer, in the 1860's. He identified a phosphorus containing acid found in the nuclei of cells, which he called nuclein. About the same time Feulgen developed a stain that was selective for this material of the nucleus. Fuelgen noted that the volume of the nuclear material was the same for all body (somatic) cells, but gametes had half as much of this material. He also noted that cells that were about to divide had twice as much nuclear material. No one knew how to interpret this information and nothing much happened in molecular genetics until the 1920's.

Although genes, chromosomes and the transmission of genetic information were studied extensively in the first half of the 20th century, the molecular structure of a gene was not known. For most of this period of time, scientists believed that the genetic molecule had to be protein – because of protein's diversity of structures and specificity of functions, and along with that phosphorus-containing acid in the nucleus (and chromosomes), there were proteins.

In the 1920's, Phoebus Levene studied nucleic acid and discovered that there were two, very similar nucleic acids. He determined that both were composed of smaller subunits: a five-carbon sugar, a phosphate group, and a nitrogen base, each found in the same proportions. Levene concluded that the three components bonded together forming repeating units of the nucleic acids. Each repeating unit is a **nucleotide**. Since the nucleic acids were composed of these fairly simple molecules, the means by which DNA could be the genetic molecule was perplexing, and many researchers still favored protein as the potential genetic molecule.

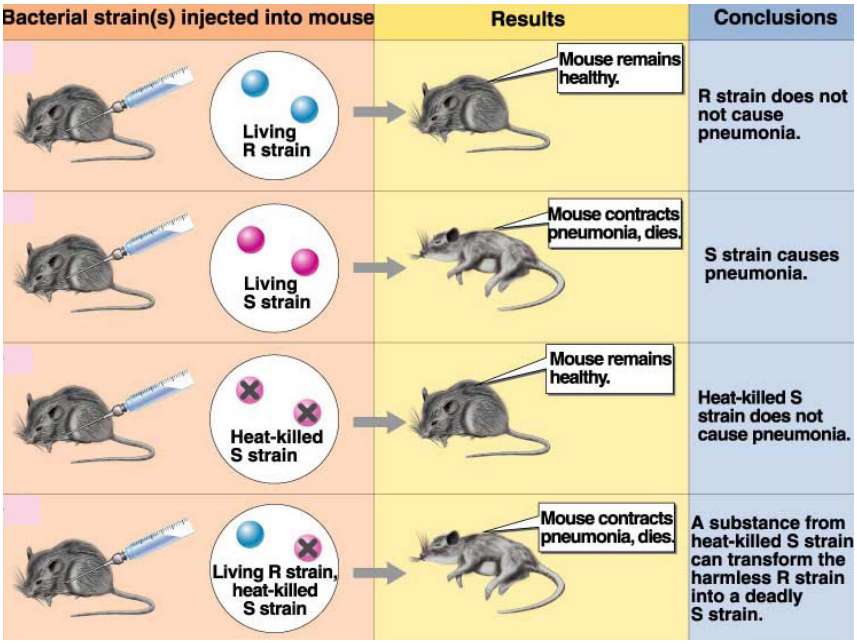
Discovering the Genetic Molecule

But not all were trying to find out how proteins could function as our genetic molecules, and the evidence for nucleic acids accumulated.

Evidence #1

In 1928, the British researcher, Fred Griffith, was trying to find a vaccine to protect against a pneumonia-causing bacterium, *Streptococcus pneumoniae*. He isolated two strains of the bacterium. One had a polysaccharide capsule that gave it a smooth (S) appearance in culture. The other form appeared rough (R) in culture. The S form is a virulent form of the bacterium, since the capsule protects it from harmful things in its environment, which in this case is the immune system of the host.

Griffith injected bacteria into mice, and observed what happened. Mice injected with S forms died. Mice injected wit R forms lived. Mice injected with heat-killed S forms lived. But: Mice injected with a mixture of heat-killed S forms and live R forms died, and when necropsied, contained live S form bacteria.



What did this mean?

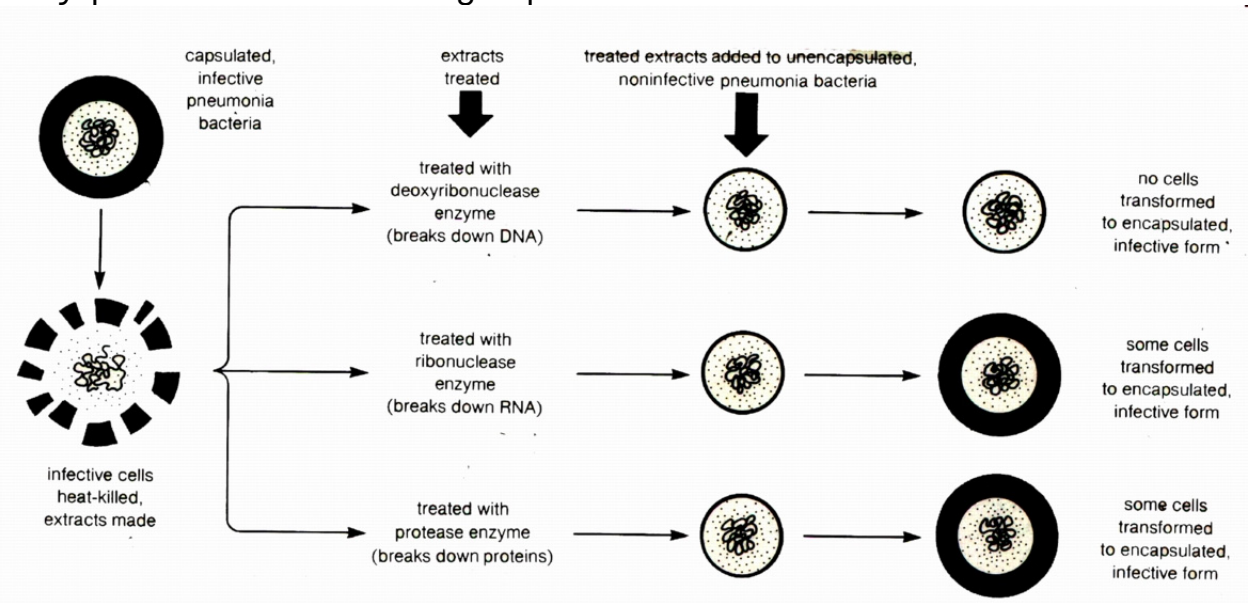
1. The production of a capsule is an inheritable trait that distinguishes the R form from the S form of the bacterium.
2. Somehow, the heat which killed the S cells did not damage the material that had genetic instructions so that this material (instructions on how to make a capsule) could be incorporated into the living R cells (The R cells could pick up this material from the environment) **transforming** these R cells into virulent S forms.
3. Griffith called this the **Transformation Principle.**

Today, **transformation** is defined as the process by which external DNA is assimilated into a cell changing its genotype and phenotype. Transformation is one of the processes used in DNA technologies.

Evidence #2

Starting in the 1930's, a group of microbiologists, headed by Oswald Avery, suspected Griffith's research transformation substance must be the genetic molecule. Avery repeated Griffith's experiments, adding a series of enzymes (from the pancreas) that selectively destroyed DNA, RNA or protein. (Recall Levene's discovery of the two different nucleic acids.)

They performed the following experiments.



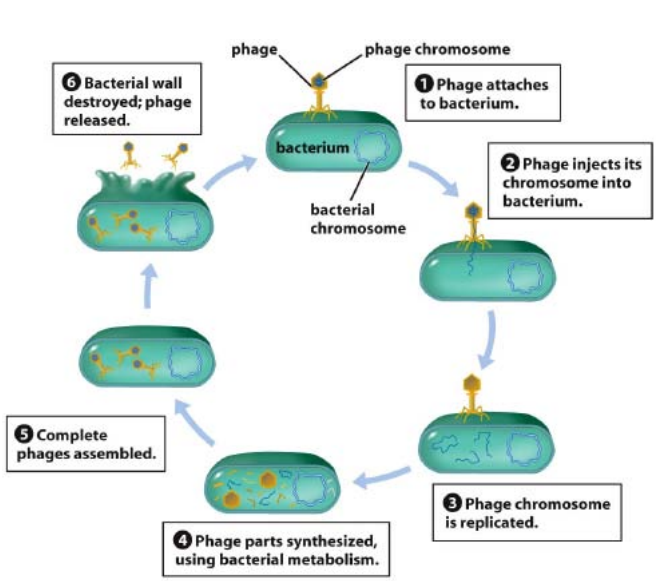
1. Mice + DNA-digesting enzyme + heat-killed S + R ----> Live Mice
2. Mice + RNA-digesting enzyme + heat-killed S + R ----> Dead Mice
3. Mice + Protein-digesting enzyme + heat-killed S + R --> Dead Mice

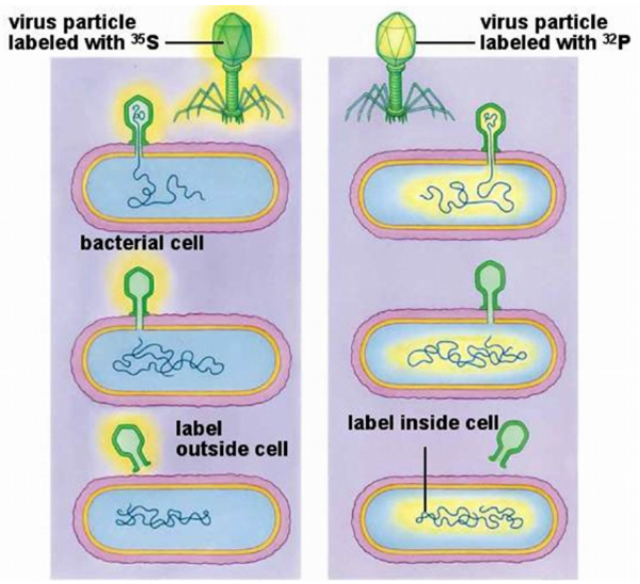
In 1944, **Avery concluded that DNA was the genetic molecule.** Transformation was prevented only when DNA was destroyed. Many scientists still disputed this conclusion, since the structure of DNA was not known, and Avery could not say how DNA might work. Some thought the experiments simply caused mutations in R-strain bacteria.

Evidence # 3

Bacteriophages (viruses that invade bacteria and convert the bacteria into virus making machines) proved to be the means by which the question was finally answered. In 1952, Hershey and Chase (and others) confirmed that DNA was the genetic molecule. Viruses have just DNA (or sometimes just RNA) and a protein coat. Proteins contain sulfur, but not phosphorus and DNA contains phosphorus, but not sulfur.

Hershey and Chase used radioactive sulfur and radioactive phosphorus to "label" T_2 bacteriophages (viruses that infect bacteria). They then tracked the invasion of phages into host bacteria (a strain of E coli) to determine what part of the new generation phages became radioactive. Since only the DNA of the new generation of phages was radioactive, **Hershey and Chase were able to confirm that DNA was the genetic molecule.**





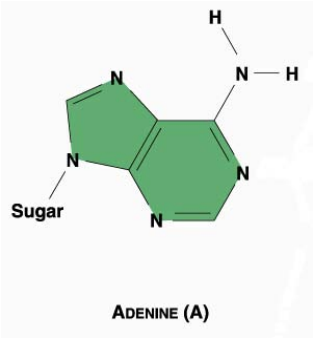
Still, the structure of DNA was unknown, so no one had an explanation for how DNA could do its job. The search continued.

Structural Evidences Supporting DNA as the Genetic Molecule
Demonstrating that DNA was the genetic molecule was one significant part of the solution. To learn how DNA works also required knowledge of the **three dimensional structure** of the molecule.

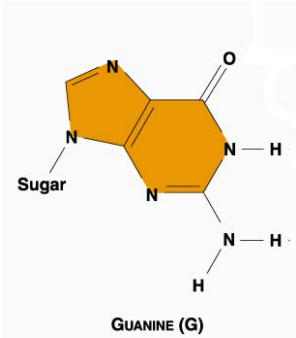
By the early 1950's the following was known about the DNA molecule:

1. DNA was composed of nucleotides. Each nucleotide contained:
   - Phosphate (P)
   - The 5-carbon sugar, deoxyribose
   - One of four different nitrogen-containing bases
     - Two were double ring purines
       - Adenine
       - Guanine
     - Two were single ring pyrimidines
       - Thymine
       - Cytosine

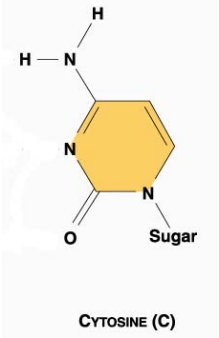
The sugar phosphate formed a backbone with one of the four bases attached to the side of the sugar.



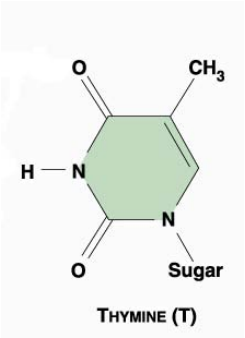
ADENINE (A)



GUANINE (G)

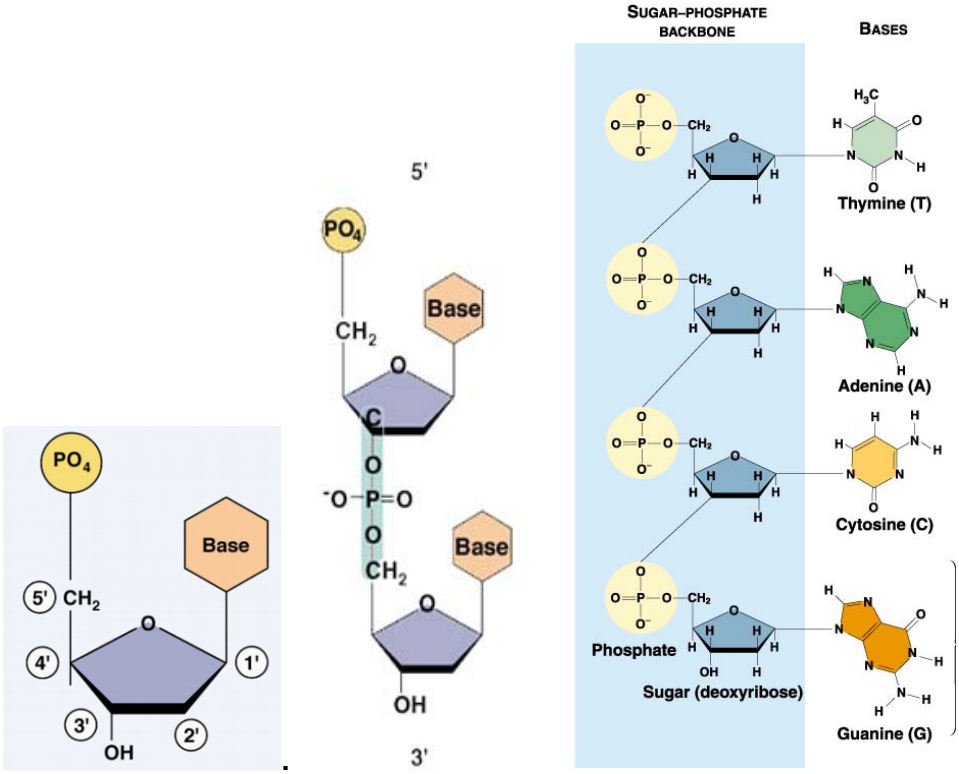


CYTOSINE (C)



THYMINE (T)

Long chains of nucleotides could be formed linking sugar-phosphate backbones with the Nitrogen bases attached to the side of the sugar molecules (S-P-S-P-S-P-S-P, etc.).



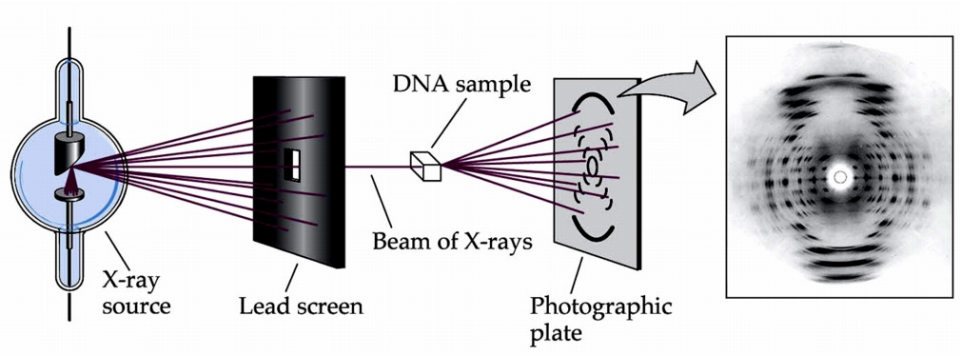
Specifically, the **phosphate bonded to the 5' carbon of the sugar molecule, leaving the 3' carbon of the sugar to attach to the next phosphate.** The nitrogen base attached to the 1' carbon of the sugar molecule. This little detail is important to the structure of DNA. In a carbon compound, each of the carbons is given a number. Deoxyribose is a 5-carbon sugar. Who bonds to what carbon is critical to DNA's structure.

2. Mirsky restated work from the 1850's that determined the relationship of the volume of DNA in the nucleus for normal body cells, cells just prior to division and in gametes. This provided evidence that DNA was the genetic molecule because it corresponded with the behavior of chromosomes in mitosis and meiosis.

3. Erwin Chargaff's work in 1947:
   - The four nitrogen bases were not present in equal amounts
   - The amounts differed in different species
   - But
     - The amount of Adenine was always the same as Thymine
     - The amount of guanine was always the same as Cytosine

     This information is known as **Chargaff's rules**

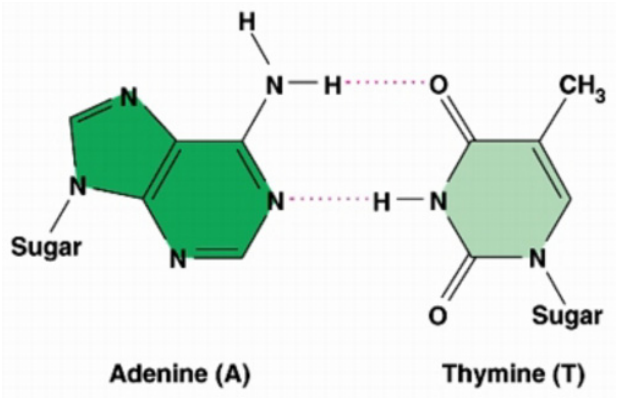
4. X-ray diffraction (best done by Rosalind Franklin at King's College in London) showed that DNA:
   - was long and thin
   - had a uniform diameter of 2 nanometers
   - had a highly repetitive structure with .34 nm between nitrogen bases in the stack
   - was probably helical in shape, like a circular stairway

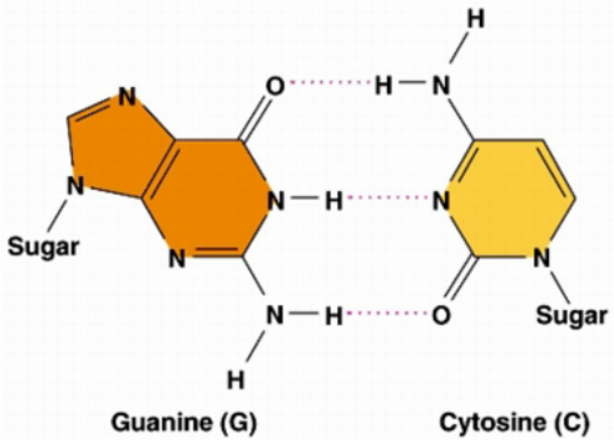


Watson and Crick

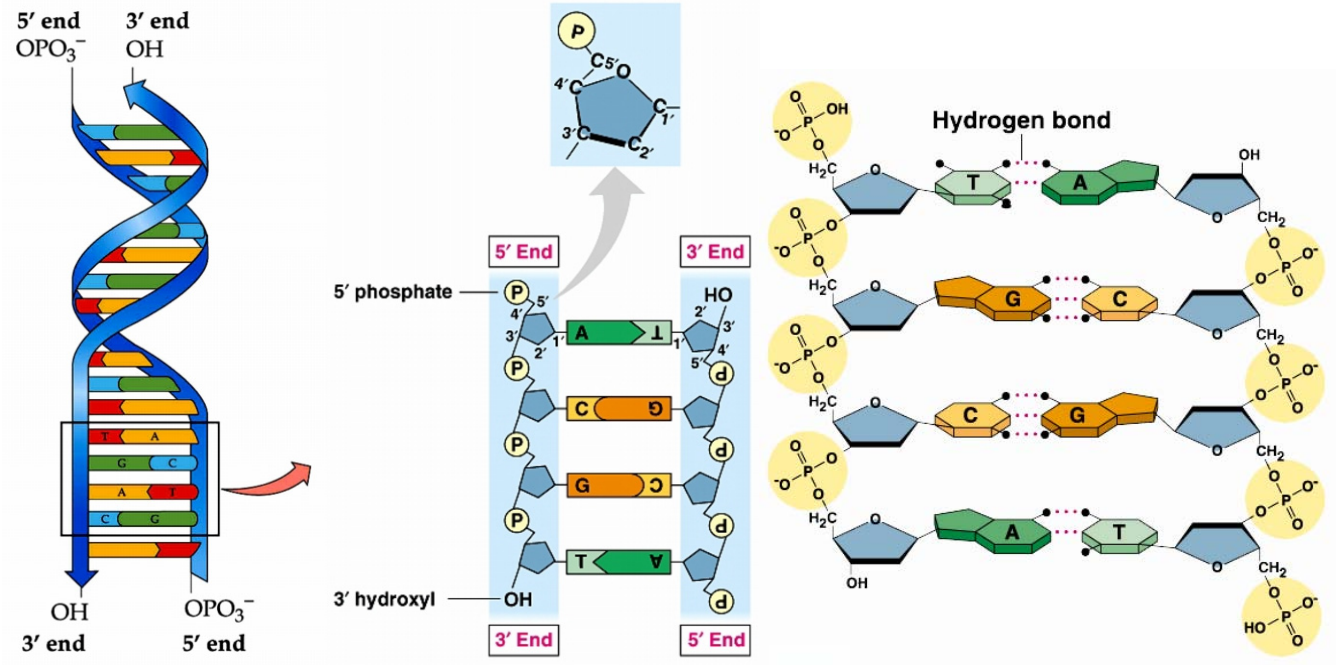
From this information, James Watson and Francis Crick (who died in July, 2004 at the age of 88) determined the structure of DNA in 1953 and published their work in *Nature*. They surmised (and confirmed):

- DNA was double strand (because of the 2 nm diameter) with a helical ladder-like structure.
- To maintain the uniform diameter, a double-ring base probably would pair with a single-ring base along the length of the molecule
  Adenine can hydrogen bond to thymine at 2 places.
  Guanine can hydrogen bond to cytosine at 3 places
  - This explained Chargaff's findings that the amounts of adenine and thymine were the same, and the amounts of guanine and cytosine were the same for a species.

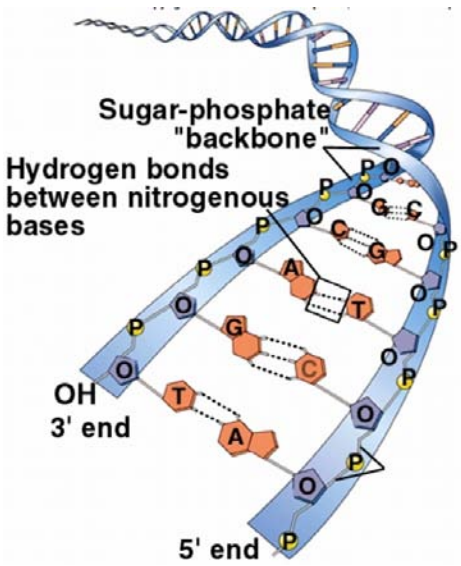


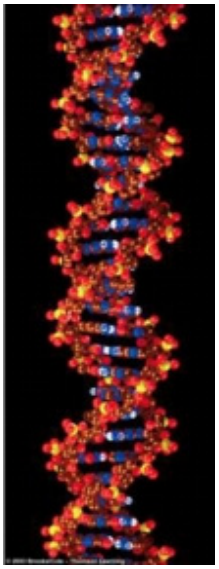


- Two strands of nucleotides, with their bases hydrogen-bonded to each other would form a ladder if, and only if, the **sugar phosphate backbones ran in opposite directions to each other,** or anti-parallel to each other, and twisted to form a double helix. The end of a DNA molecule will have a free sugar (3' end) on one side of the "ladder" and a free phosphate (5' end) on the other side. (Note the importance of the 3' and 5')



The constancy of the **complementary base pairing** is critical to the structure of DNA. DNA of different species and of different genes shows variation in the sequence of base pairs in the DNA chain (which base pair follows the next).



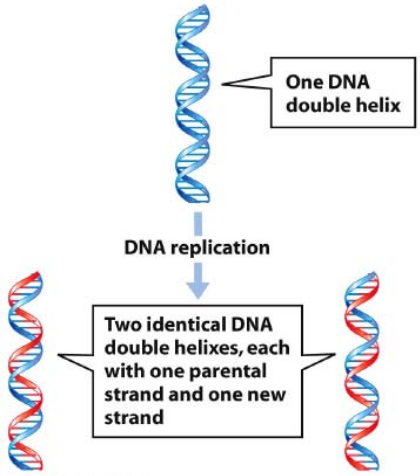


Once the structure of DNA was determined, active research could take place in how DNA can **duplicate** (or replicate) prior to cell division, based on the complementary base pairs, and in how DNA **stores genetic information**, which as we will learn, is in the sequence of nucleotides on the DNA strand.

DNA Replication

- DNA is a double stranded molecule. The two chains (or strands) are attached by hydrogen bonds between the nitrogen bases.
- The two strands are anti-parallel to each other (run in opposite directions). That is, the 3' carbon of the sugar (the free sugar end) starts one strand and the 5' carbon sugar end (the free phosphate end) starts the other. This is necessary for the base pairs to hydrogen bond correctly.
- Adenine must bond to thymine, and guanine must bond to cytosine. The adenine-thymine and cytosine-guanine bonding requirement is known as **complementary base pairing**.

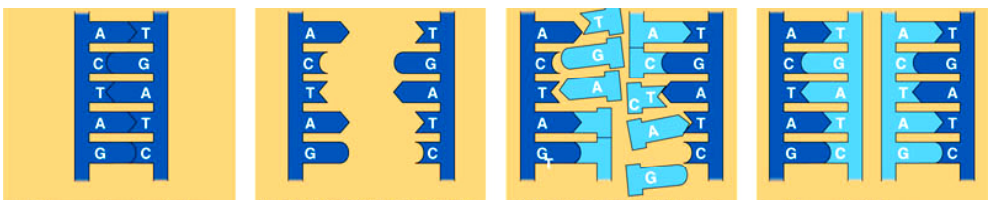
Because of the complementary base pairing, if one side of the double stranded molecule is known, we automatically know what the other half is. This model for DNA replication is known as the **semi-conservative model** for DNA replication.



The process of DNA Replication

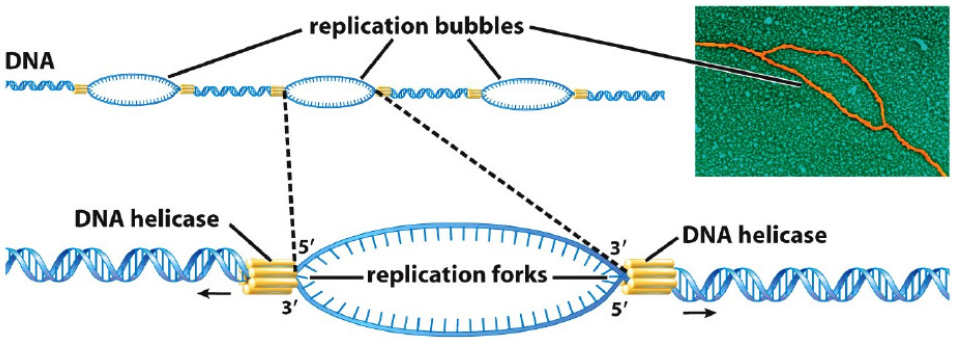
There are three basic steps to DNA replication:

- The two DNA strands of the parental chromosome must unwind and separate.
- Each strand of the parent chromosome serves as a template for the synthesis of a daughter strand. DNA is always synthesized in the 5' → 3' direction from the 3' → 5' parent strand template. **It helps to remember that the 5' end is the free phosphate (PO_4^{-3}) end, and the 3' end is the –OH end of the sugar.**
- The newly synthesized double helix of each parent-daughter combination rewinds to form the DNA chains of a replicated chromosome. Each new DNA molecule is composed of one-half of the parent chromosome and one-half newly synthesized DNA.

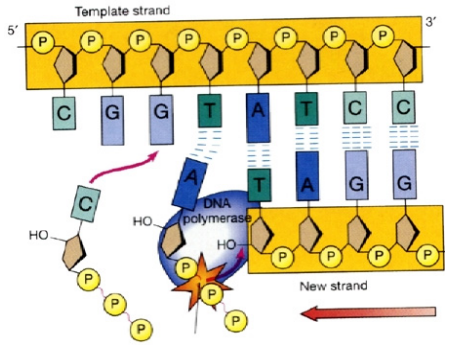


A few details about the process:

- Prior to cell division, the enzyme, **DNA helicase**, facilitates the unwinding of the double-stranded DNA molecule forming **replication bubbles** in the DNA molecule.
- **Replication forks** are formed at the origin of each bubble. New DNA is replicated behind each fork as it progresses along the DNA molecule in both directions from the origin.

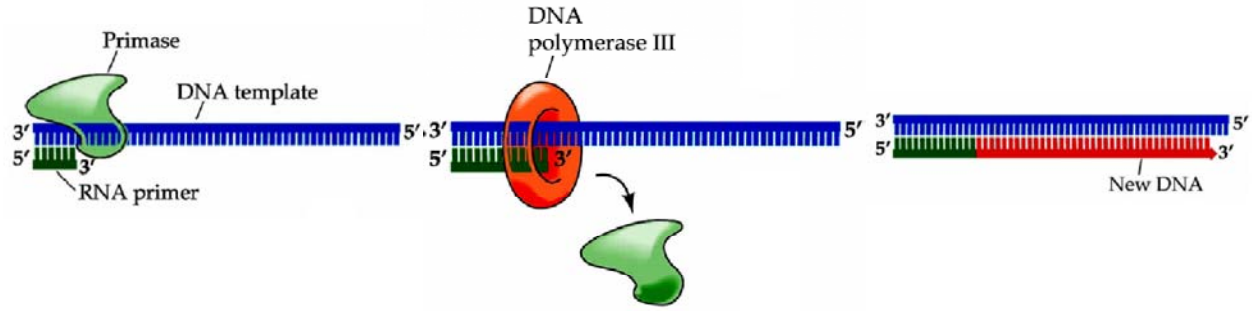


- In eukaryotic organisms, there are many, many replication units involved in the replication of DNA on each chromosome. Each replication unit forms a replication fork at its "origin". As DNA replication progresses in both directions from the origin, replication units join other units when they meet an adjacent replication forks.
- Each of the two strands of the DNA molecule in the fork serves as a template for the attachment of its complement nucleotides (A-T, C-G, G-C and T-A). This takes place on both sides of the replication forks simultaneously, but in opposite directions.
- The enzyme, **DNA polymerase**, promotes the synthesis of the new DNA strands, by recognizing the appropriate complementary base needed and by bonding appropriate daughter nucleotides to the growing DNA molecule.



- DNA polymerase has two limitations:
  1. DNA polymerase cannot add nucleotides until there is a double-stranded starter. It can read the single chain template, but can't bond the nucleotides for the new strand for replication with just the single-stranded template.
  2. DNA polymerase can only work in one direction and the double-stranded DNA has two directions at each replication fork.

- **DNA Polymerase and the Single Chain Template**
  DNA polymerase's inability to add nucleotides to a single chain is solved by starting replication with a **RNA primer** activated by an enzyme called **primase**. Primase catalyzes a short RNA molecule or primer that is used to start the synthesis of DNA.



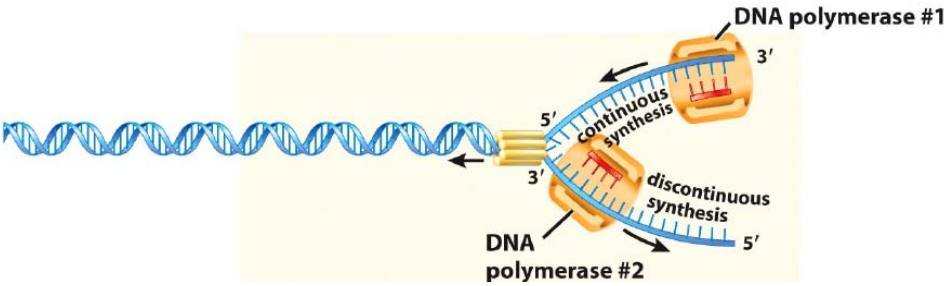
  Once the DNA strand has been "primed" by primase adding the RNA primer, DNA polymerase can go to work adding nucleotides to the available 3' carbon of the growing molecule. (This is sometimes called the free sugar end of the DNA molecule. The opposite end 5' carbon of the nucleotide's sugar that bonds to the phosphate is called the free phosphate end.) DNA polymerase will also eventually replace the RNA nucleotides of the primer with DNA nucleotides.

- **DNA and the Anti-parallel Template Strands**
  For DNA's second limitation, DNA polymerase can only attach a nucleotide to the exposed -OH group on the 3' end of the sugar on the template. **DNA is always synthesized in the 5' → 3' direction from the 3' → 5' template. This sounds stranger than it really is.** During DNA replication, new nucleotides attached must be in a 5' to 3' direction (starting with the phosphate), bonding each new nucleotide to the 3' end of the growing strand (the 3' carbon of the sugar is available to bond to the next nucleotide).

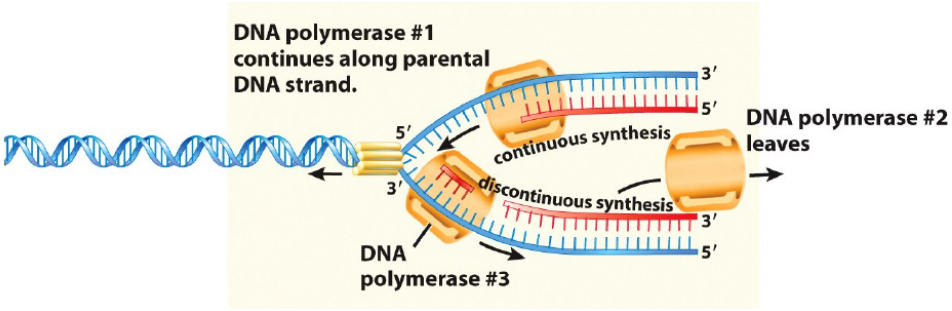
  When DNA helicase initiates the unwinding of the DNA molecule, the two opening strands are in opposite directions at each replication fork: one is 3' → 5' and the opposite is 5' → 3'. This is fine for the 3' → 5' DNA strand of the original molecule, but not for the second strand, which is running in the 5' → 3' opposite direction. The upper 3' (sugar) end of the original DNA molecule is called the **leading strand** of the template because replication starts at that point. The opposite strand is the **lagging strand**, because DNA replication can't readily progress behind DNA helicase and the primer.

- DNA polymerase positions the parent strand of the DNA molecule into a groove and pulls the DNA through the groove as it directs the synthesis of new DNA.

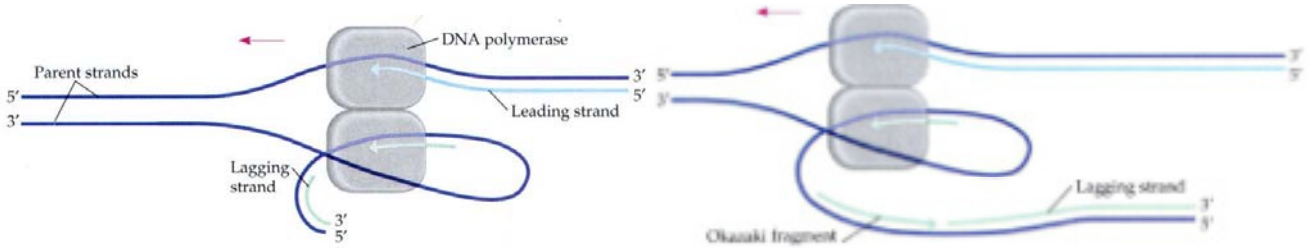
DNA replication is continuous along the **leading strand** of the original template DNA molecule, because the newly synthesized daughter nucleotides can follow the path of DNA helicase.



- However, the **lagging** template strand of DNA is unzipped in the 5' to 3" direction so new DNA synthesis must be discontinuous. Its rate of synthesis lags behind that of the leading strand. Additional DNA polymerase enzymes must be attached at unzipped regions, but the synthesis direction of the lagging strand is opposite the direction of the unzipping DNA helicase enzyme.

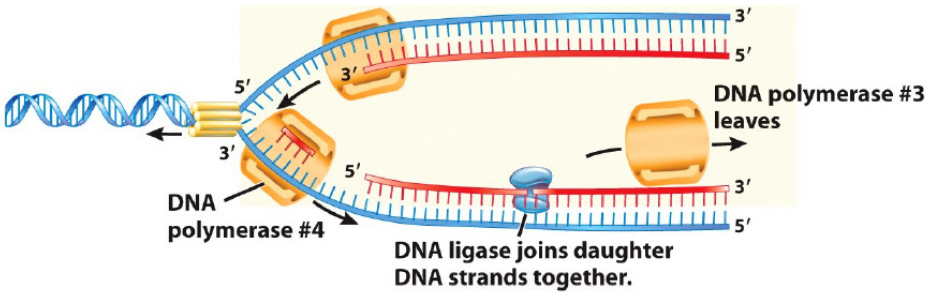


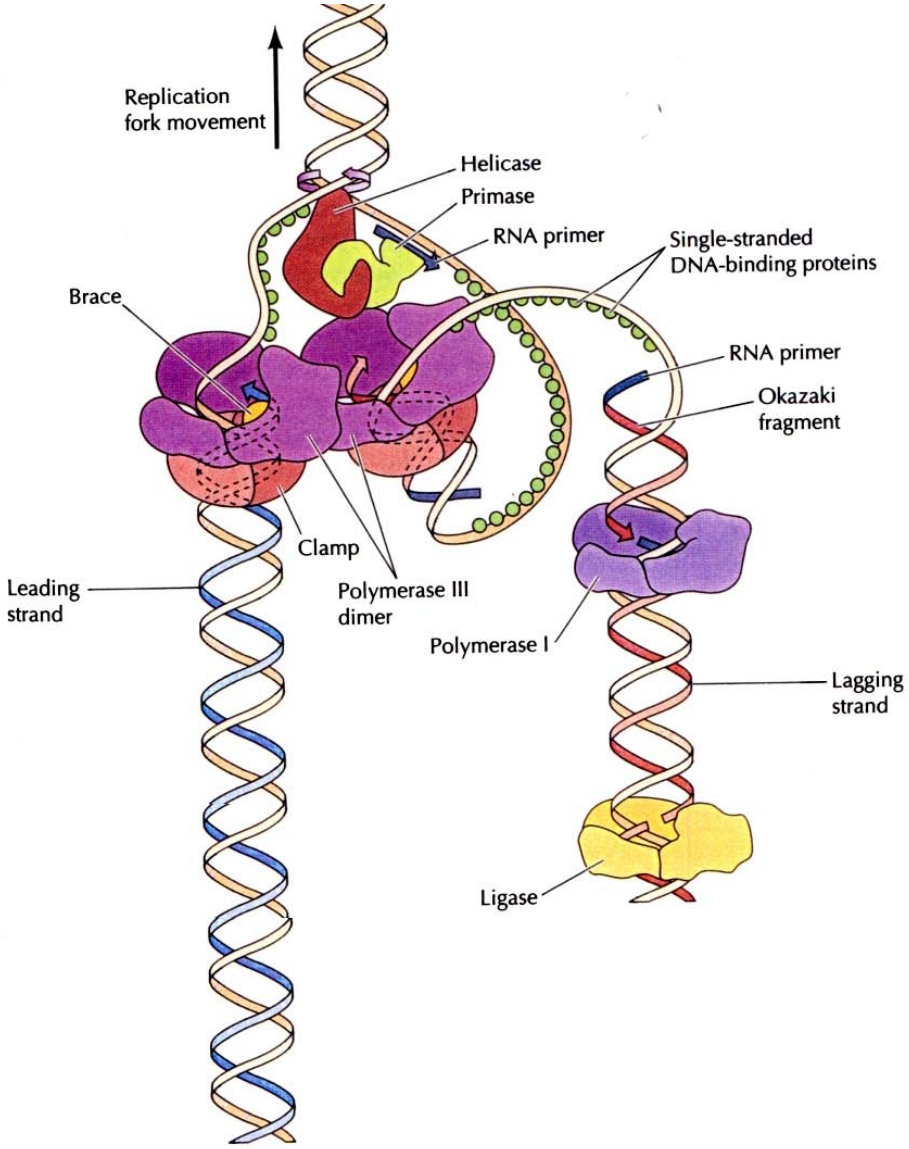
- Since DNA polymerase must do both sides, the lagging strand has to be folded back on itself to "face" the correct 5' to 3' direction to fit into the DNA polymerase sliding rings.



- To do so, the lagging strand must be unwound a greater distance before replication can start, and will be looped to provide the needed orientation for DNA polymerase. New DNA polymerase enzyme molecules have to attach and work on small portions of the **lagging strand** of the unzipped DNA molecule.

  Once segments of the lagging strand, called **Okazaki fragments**, are synthesized, the RNA primer nucleotides are removed and DNA polymerase replaces them with DNA nucleotides. The enzyme, **DNA ligase**, links the short pieces of the lagging side together. Note: Each Okazaki fragment must be initiated by a RNA primer.





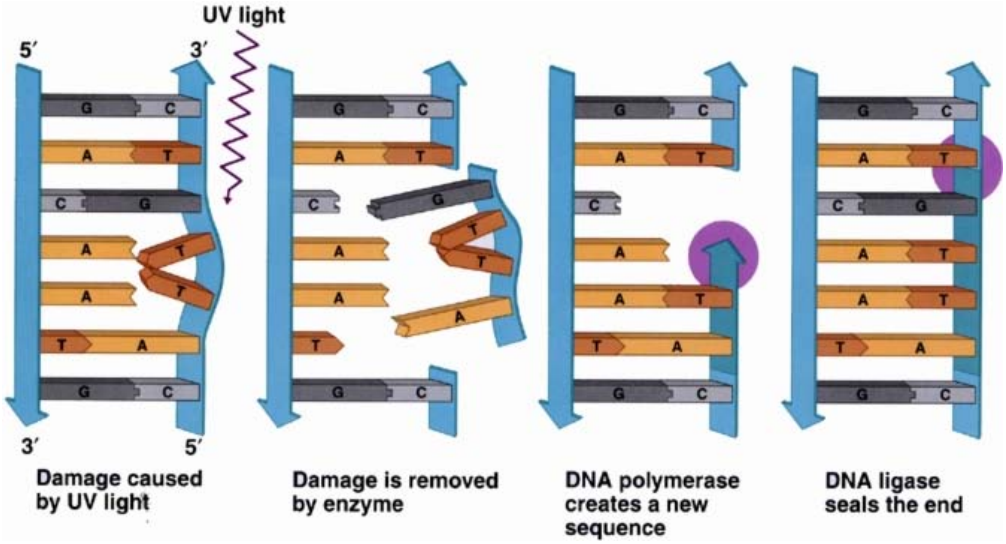
- After the DNA is replicated along its entire length, two DNA molecules have been formed, each half the original and half new nucleotides. Both DNA molecules are identical to each other and to the original.

Proofreading DNA

DNA pairing errors occur during DNA replication. Despite the complementary base pairing, mistakes can and do happen. As you might expect, DNA is proofread by DNA polymerase as it is being replicated. If there is a base-pairing error, it deletes the mistake, and replaces it with the correct nucleotide.

Repairing DNA Damage

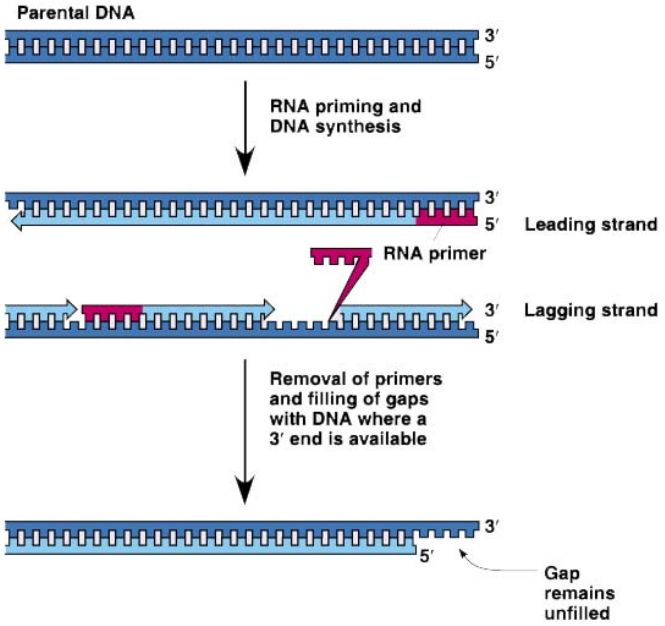
Damage to DNA molecules occurs daily by exposure to routine molecules in the environment, and even to normal body temperatures. DNA repairing enzymes cut out the damaged or mutated DNA and DNA polymerase and ligase fill in the gap with correct nucleotides, assuming there is an undamaged DNA strand to serve as the template. Even so, some mistakes do not get repaired. About 1 in 1 billion DNA base pair errors are not caught. Such DNA changes are known as **mutations**. Deterioration of DNA replication accuracy is likely a contributing factor in aging and in cancer. Exposure to UV light can cause DNA damage, which is why those who tan are at greater risk for skin cancer.



Before we leave the subject of DNA replication, we have one more issue: the end of the DNA molecule and **telomeres**.

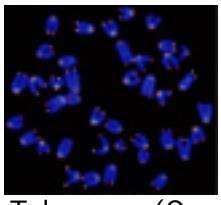
Telomeres

We've just learned that DNA is synthesized in the 5' → 3" direction from the 3' → 5" template, initiated with the RNA primer region. When there is an available 3' open binding site, as there is with the Okazaki fragments, the RNA primer segment is removed and replaced by DNA nucleotides added to the 3' end. Although the initial primer section can be removed from the leading strand, the end is the 5" phosphate so no DNA nucleotides can be added. DNA polymerase cannot "finish" the 5' ends of the "daughter" DNA strands. After each replication, the DNA molecule gets shorter. Why don't we lose valuable genetic information with each DNA replication? Telomeres!



The ends of eukaryotic chromosomes have special nucleotide sequences called **telomeres**. It's the telomere region that gets shorter with each DNA replication. Most cells can divide about 30 times before they run out of telomeres and can no longer replicate DNA without losing codable genes.

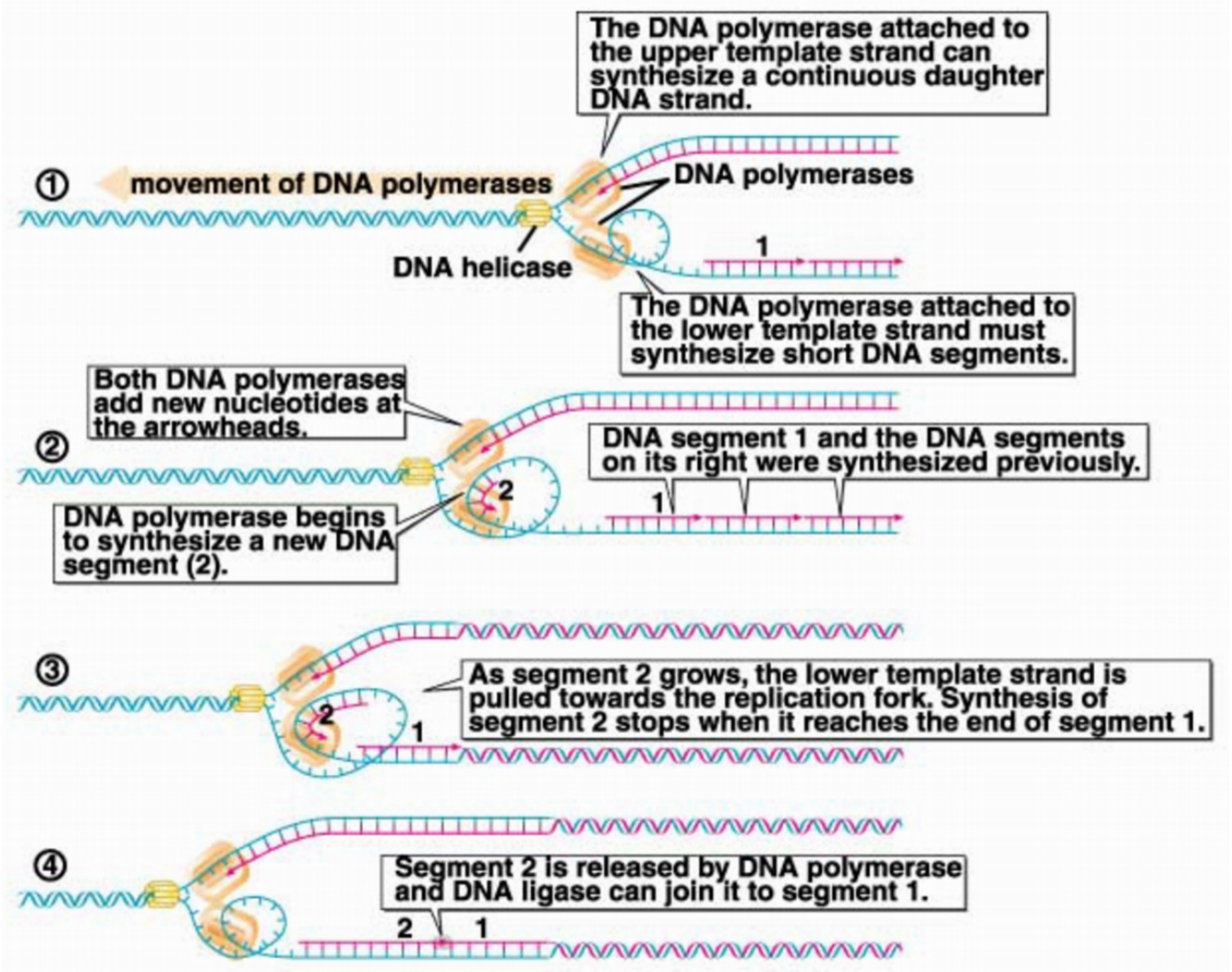
Telomere Ends of Chromosomes



Mouse Telomeres (Orange Tips)

Some tissues have **telomerases**, special enzymes that catalyze new telomere code to chromosomes. In humans, telomerases are normally found only in tissues that produce gametes, but that ensures that gamete chromosomes have long telomeres. Shortening telomeres in tissues may be a factor in aging and lifespan. Regrettably telomerases seem to be abundant in some cancer cells, so that rapid reproduction does not result in shortening of telomeres and cell death from lack of needed genes.

Summary of DNA Replication



Summary of Enzymes Involved in DNA Replication

Double helix unwinds, providing single-stranded DNA templates	**Helicases** and **single-strand binding proteins**

Synthesis of leading strand		Synthesis of lagging strand	
Priming	**Primase**	Priming for Okazaki fragment	**Primase**
Elongation	**DNA polymerase**	Elongation of fragment	**DNA polymerase**
Replacement of RNA primer by DNA	**DNA polymerase**	Replacement of RNA primer by DNA	**DNA polymerase**
		Joining of fragments	**Ligase**

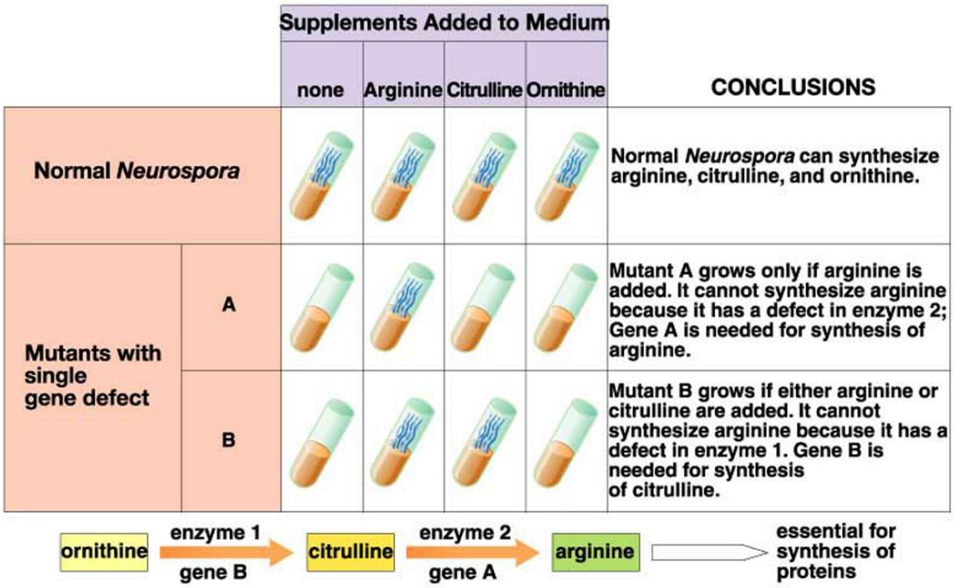
As we have just discussed, the structure of DNA provides a mechanism for self-replication. The structure of DNA also reveals the mechanism for storing the genetic information that determines what a cell is and how it functions: the genetic code. We will look now at how the information stored in molecules of DNA is used to direct the synthesis of proteins (or more properly, polypeptides). It is also important to know how genes are regulated in organisms, so that the appropriate DNA is expressed in each cell, a subject to be addressed a bit later.

Learning What a Gene Does – Garrod's Contribution

In 1909 Archibald Garrod postulated that inherited diseases were caused by the inability of the individual to synthesize a particular enzyme. He was correct; many of our metabolic disorders are caused by not having a specific enzyme. However, it took decades of research to "prove" that a gene's function is to provide instructions on how to synthesize a specific protein, and that metabolic pathways for synthesis and degradation of molecules within a cell (and organism) are catalyzed by specific enzymes at each step of the pathway.

Learning What a Gene Does – Beadle and Tatum and Pink Bread Mold

In the 1940's Beadle and Tatum induced mutations (changes in the genetic code) in *Neurospora*, a pink mold common on bread, and tracked the metabolism of the mutant strains. They mapped chromosome locations of the mutant strains, and then related their chromosome maps to the presence of absence of specific enzymes needed in *Neurospora*'s metabolic pathway for the synthesis or arginine. From their research, Beadle and Tatum postulated the **one gene-one enzyme** theory.



Eventually, we also learned that not all genes must code for enzymes; some code for structural proteins or functional proteins (such as the membrane proteins). Furthermore, quaternary proteins are composed of more than one polypeptide, so the concept of gene has been further refined to be that a **gene codes for a polypeptide**.

But what is the relationship between DNA, genes and protein synthesis in cells? Although DNA most commonly stores information for the synthesis of proteins, some DNA information is used to synthesize the nucleic acid, RNA (ribonucleic acid). At this time, we are going to explore in more detail now DNA information is expressed in cells.

The expression of DNA in the genetic control of the cell - Preview

- DNA contains the instructions for each cell to function, precisely coded in its four-letter "alphabet", A, T, C, and G.
  DNA also has regions of no apparent function and regions of genetic gibberish.
- These instructions are used to direct the synthesis of polypeptides (the primary structures of proteins) for the cell and for the synthesis on RNA molecules. Many proteins become functional enzymes catalyzing the metabolic activities of cells. Others are the structural proteins of cells. Some types of RNA molecules are used in protein synthesis, some are used for gene regulation and some are ribozymes (RNA catalysts).
- DNA is not used directly as a template for protein synthesis, a process that occurs in the cytoplasm at ribosomes. DNA molecules never leave the nucleus of the cell. To carry the information stored in DNA to the cytoplasm, we use the molecule, **RNA (ribose nucleic acid)**.
- DNA is used as a template to build a set of RNA instructions, a process called **transcription**. This occurs in the nucleus.
- RNA molecules, called **messenger RNA** (mRNA) travel from the nucleus to the ribosomes in the cytoplasm of the cell.
- At the ribosomes, a second process, called **translation** occurs. During translation, the information carried by the messenger RNA molecules is used to direct the assembly of specific amino acids into proteins. Two other types of RNA, **transfer RNA** (tRNA) and **ribosomal RNA** (rRNA) are also needed for the process of translation.

  Translation requires the interactions of 3 different types of RNA that convert the original DNA code word instructions (the triplet sequences) into specific polypeptides (unique sequences of amino acids)

However, before we go further in our discussion of transcription and translation we need to look a little more closely at the structure and types of RNA and at the genetic code and how RNA differs from DNA.

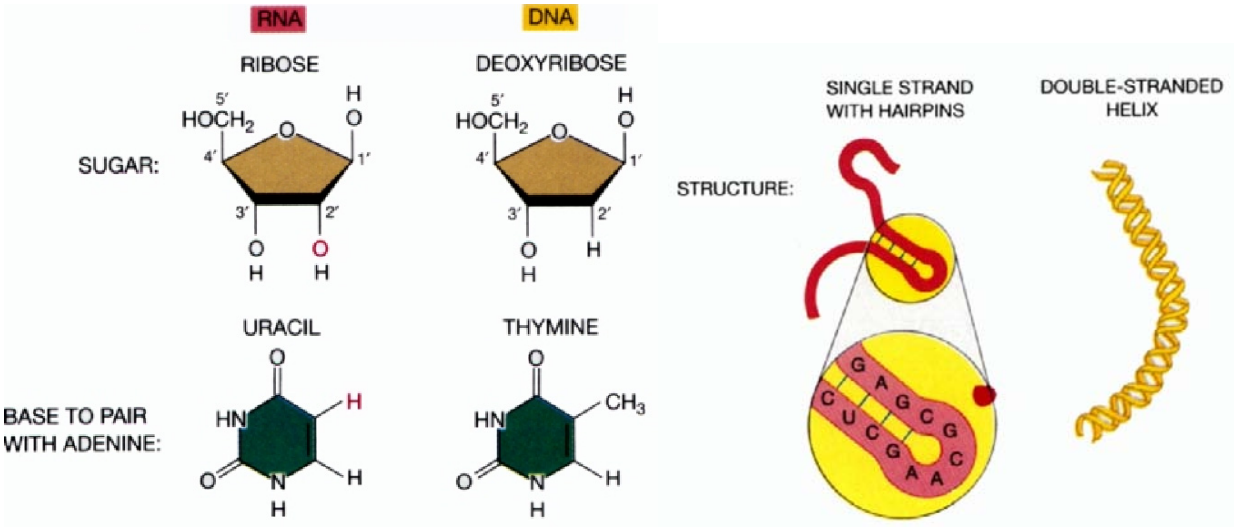
Structure of RNA Molecules

There are three main types of RNA: **messenger RNA** (mRNA), **transfer RNA** (tRNA) and **ribosomal RNA** (rRNA). All are synthesized in the nucleus by the process of transcription.

RNA is composed of:

- Phosphate
- Ribose sugar
- Four nucleotides
  - Adenine
  - Guanine
  - Cytosine
  - Uracil
    Replaces the thymine found in DNA. Uracil bonds to Adenine

Molecules of RNA are single-stranded, not double-stranded as in DNA. However, some RNA molecules fold back on themselves at places, called **hairpins**, forming complementary base pair bonds.



The Types of RNA

1. Messenger RNA (mRNA)

- mRNA is the unique blueprint, or transcript for each protein to be assembled.
- mRNA is manufactured by transcription on demand; that is when a specific protein is needed in the cell.
- mRNA molecules are a precise sequence of nucleotides that complement the DNA template strand.
- A specific mRNA migrates from the nucleus to ribosomes for the process of translation.

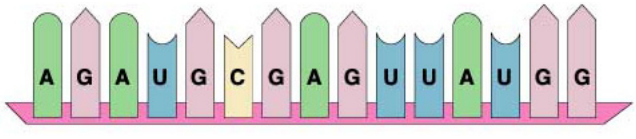
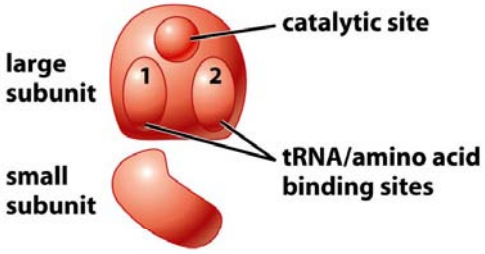
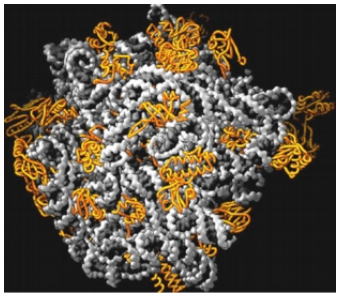


Diagram of mRNA base sequence

2. Ribosomal RNA (rRNA)

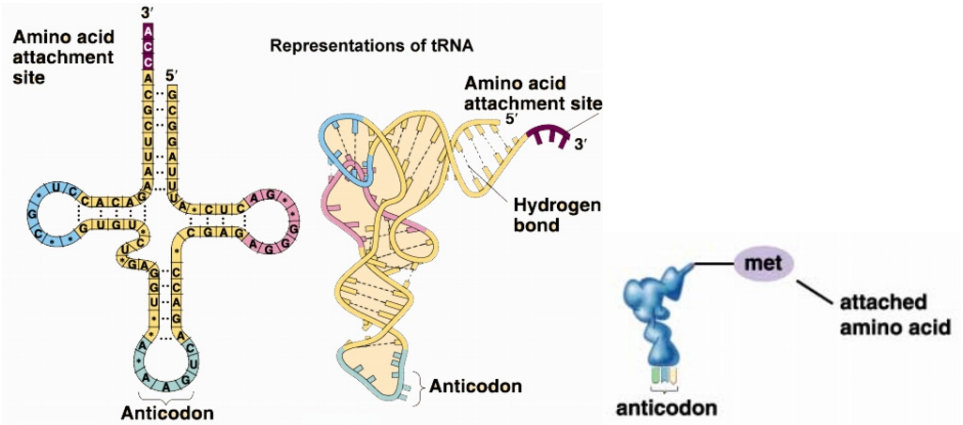
- Ribosomal RNA is a component, with protein, of the ribosomes.
- A ribosome is composed of 2 subunits, a small subunit containing RNA molecules plus proteins, and a larger subunit containing RNA plus proteins and the enzymes needed for protein synthesis.
- The ribosomal subunits are manufactured in the nucleolus, but the complete ribosome is found in the cytoplasm, frequently attached to rough endoplasmic reticulum.
- The small rRNA subunit has a binding site for mRNA molecules during protein synthesis. The larger subunit has three attachment sites for tRNA molecules, the P site, A site and E site (Exit site). During protein synthesis the two subunits bind together.





3. Transfer RNA (tRNA)

- There are a variety of tRNA molecules in the cytoplasm of the cell.
- Each tRNA has hairpin loops in which the RNA is folded back on itself making hydrogen bonds.
- Each different type of tRNA has two important pieces:
  1. An amino acid attachment site at the 3' end, which can attach to one specific amino acid
  2. A special tRNA triplet sequence, which pairs with one specific mRNA triplet sequence. This triplet specifies the precise amino acid that attaches to the attachment site of the tRNA.
- **tRNA is the critical connection between the information carried on the DNA and the amino acids that will be assembled into proteins**



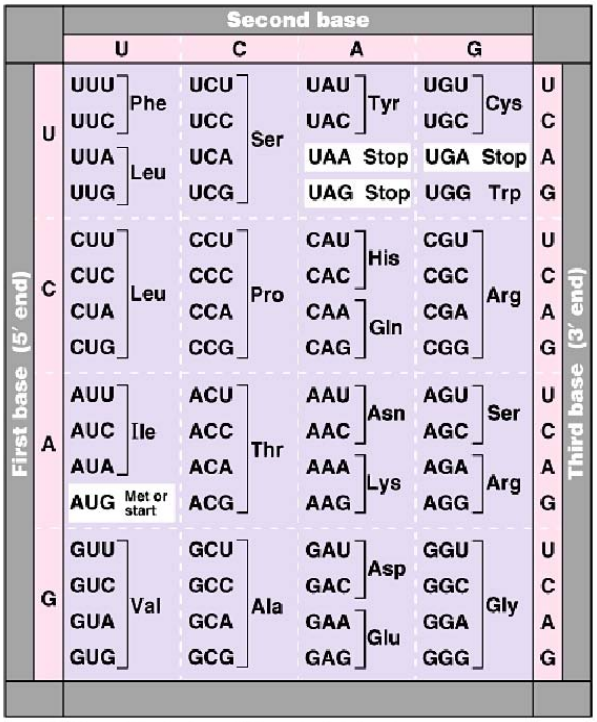
The DNA Code and How It is Used

The information of DNA is coded into three-nucleotide-long sequences (a triplet code) along the length of the DNA molecule. Each triplet sequence is a "code word" for one specific amino acid. DNA molecules contain a linear sequence of triplets that specify which amino acids a protein will contain, and the sequence, or order, in which these amino acids will peptide bond to form a polypeptide. In addition to the triplet sequences, there are start and stop regions of the DNA associated with these instructions for protein synthesis, and regions within the DNA molecule that do not code and are removed by RNA processing after transcription.

The DNA code is non-overlapping and there are no separators between the triplets. Although there can be 64 different DNA code words, (the number of combinations of three of the four different nucleotides) three of code words are "nonsense" and do not code for specific amino acids. The three "nonsense" code words specify the end of a polypeptide coding.

As stated in our preview, DNA is used to synthesize molecules of RNA by the process of **transcription**. A **mRNA nucleotide triplet** (synthesized from a DNA template) that codes for a specific amino acid is a complementary (rather than identical) **codon** to the DNA**.** Synthesis of RNA (transcription) follows the same nitrogen base pair rules that dictate DNA replication. Each mRNA transcript will be a faithful, but complement copy of the nucleotides of the DNA template.

The codons for each of the amino acids are known, as well as specific codons that are used as start and stop messages. To determine which amino acids correspond to which nucleotide triplets, codon tables have been created.

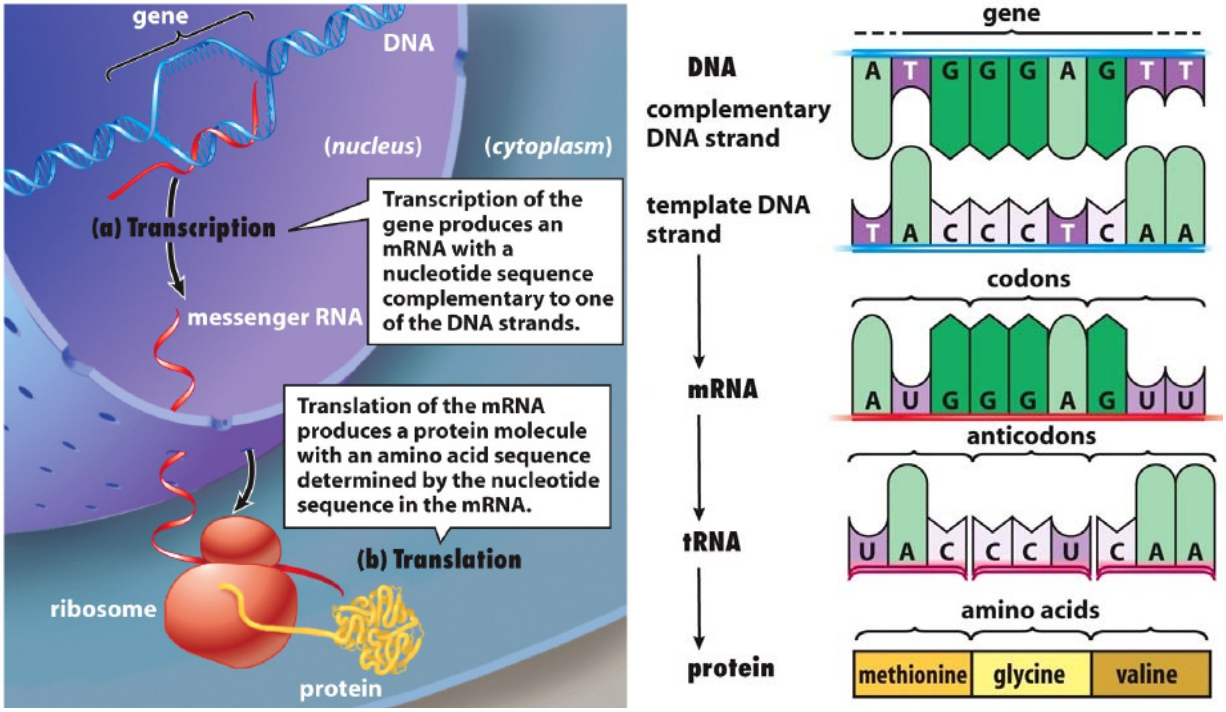


First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC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

When you look at Codon tables, you will see that some amino acids are coded for by more than one codon. Often, only the first two nucleotides of the triplet are essential; the third is redundant. (e.g., CCU, CCC, CCA and CCG all code for the amino acid, proline, and UCU, UCC, UCA and UCG all code for the amino acid, serine.) The ability of amino acids to bond to more than one RNA triplet is known as the "wobble effect". The reverse is not true. One codon can never code for more than one specific amino acid. UCU codes for serine. UCU can never code for any other amino acid.

The process of **translation** requires tRNA molecules that have triplet RNA sequences that match (complement) the mRNA for the amino acid assembly. The only way to match nucleotides is by base pairs that are complements to each other, so the tRNA triplet that codes for and attaches to a specific amino acid is often called the **anticodon.**

Each tRNA has an amino acid binding site that can attach to its specific amino acid. Specific enzymes do this. These attachment sites are also phosphorylated (using ATP) to provide the energy for protein synthesis.



Overview of DNA-mRNA-tRNA and amino acids relationship
(The tRNAs attached to amino acids are not shown on the left diagram)

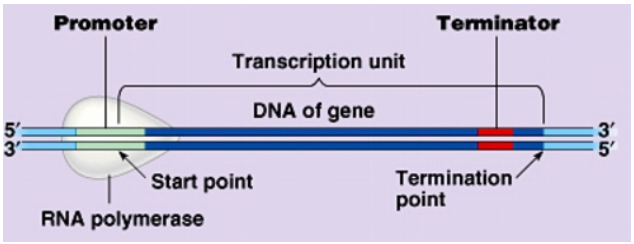
Codon-anticodon (mRNA-tRNA) matches occur at ribosomes where the amino acids, which are attached to the tRNA molecules, can be joined by peptide bonds to form polypeptides. Several ribosomes can function at one time so that several copies of a polypeptide can be made simultaneously.

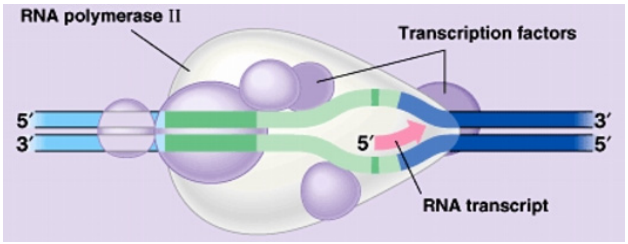
How it works: Details of Transcription and Translation

RNA synthesis, or **transcription**, uses DNA as a template, and occurs in the nucleus. There are three stages in transcription: **Initiation, Elongation** and **Termination.** Recall that all RNA is synthesized by transcription, although we focus on the synthesis of mRNA in our discussions.

Initiation

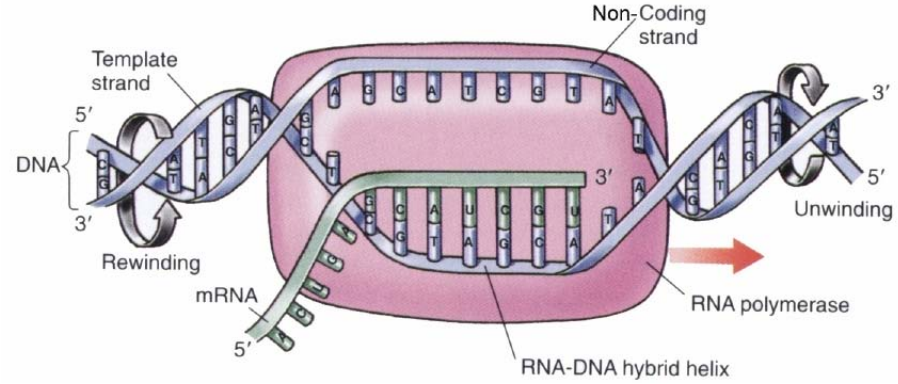
- The region of DNA that codes for the specific gene to be transcribed starts to unwind using the enzyme, **RNA polymerase**, to **initiate** the process.
- Transcription is started at a region of the DNA molecule called the **promoter**, a specific DNA base sequence at the 3' of each gene. A promoter determines the template strand of the DNA and where transcription will start. Special proteins, called **transcription factors**, help RNA polymerase find the promoter regions on the DNA.





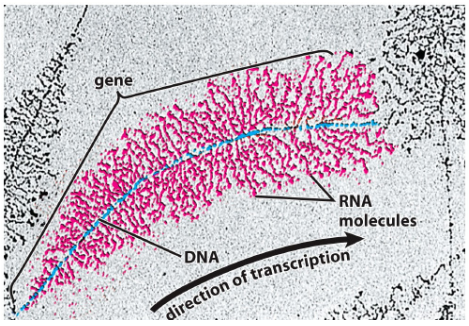
Elongation

- RNA polymerase will move in the 3' to 5' direction along the DNA template during what is now called the **elongation** process of transcription. Like DNA, RNA is synthesized in the 5' to 3' direction from the 3' to 5' DNA template.
- Only one strand of the DNA molecule, the **template strand,** is transcribed. The complement strand, which is not transcribed, is called the non-template strand (or sometimes the complementary strand or non-coding strand).



- RNA Nucleotides are added to the chain according to the complementary base pairing; that is:
  - RNA A - DNA T
  - RNA U - DNA A
  - RNA C - DNA G
  - RNA G - DNA C
- The DNA molecule will start rewinding after about 10 RNA nucleotides have been joined to the mRNA chain.

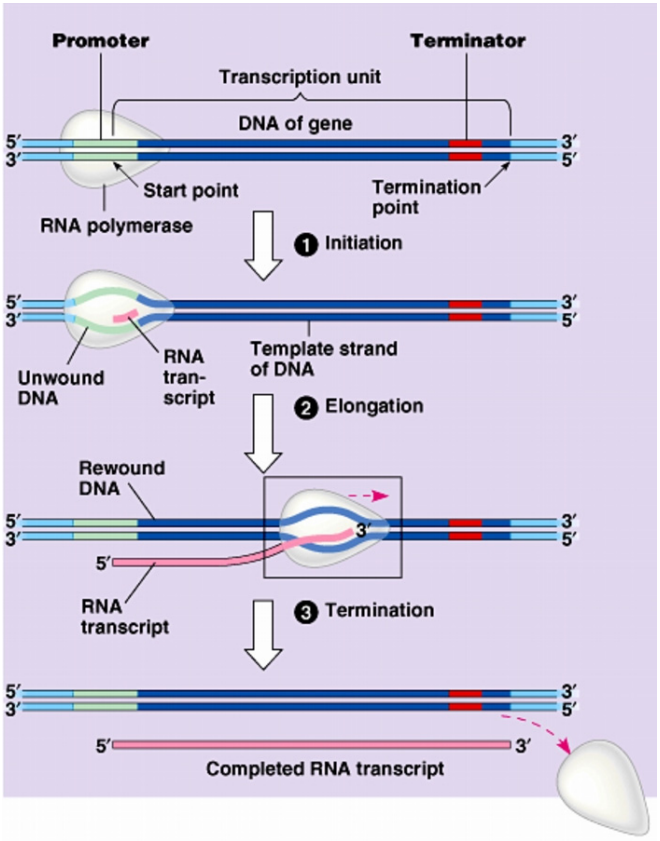
- Several molecules of RNA polymerase can be present so that several mRNA transcripts can be made of the gene (DNA sequence) at one time. As one mRNA is being transcribed, a new RNA polymerase molecule attaches to its transcription factors at the promoter and starts transcribing a second. As the second starts elongating, a third RNA polymerase can attach, until many mRNA molecules are being synthesized along the DNA template.

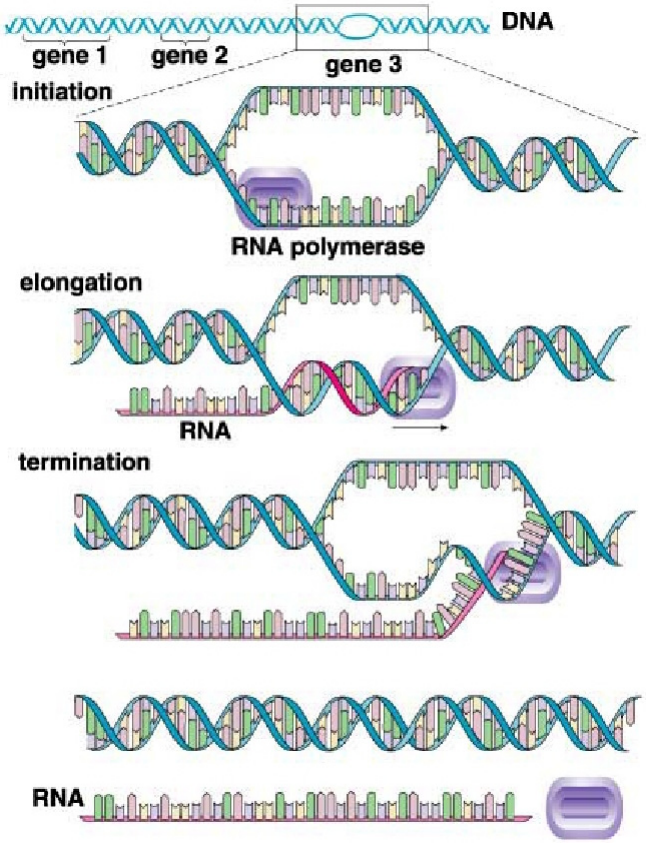


Termination

- There is a **terminator** sequence that tells the RNA polymerase to stop. This is the **termination signal.** RNA polymerase will release the mRNA transcript from the DNA template strand at this point, and the DNA molecule will complete its rewinding. RNA polymerase will be free to bind to another gene's promoter region and initiate a new mRNA transcription.

Note: The process of transcription is also used to synthesize the tRNA molecules and the rRNA of ribosomes.





Processing the mRNA Transcript

Cap and Tail

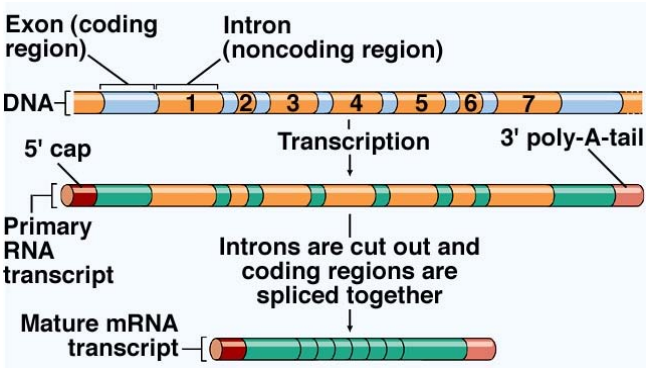
After transcription, the mRNA transcript has both a cap and a tail added. The cap is added to the 5' end of the RNA molecule (the end first synthesized) and a tail of adenine nucleotides is attached to the 3' end of the mRNA transcript.

Both cap and tail help the mRNA attach to the ribosome for translation, and also inhibit enzyme degradation of the mRNA transcript.

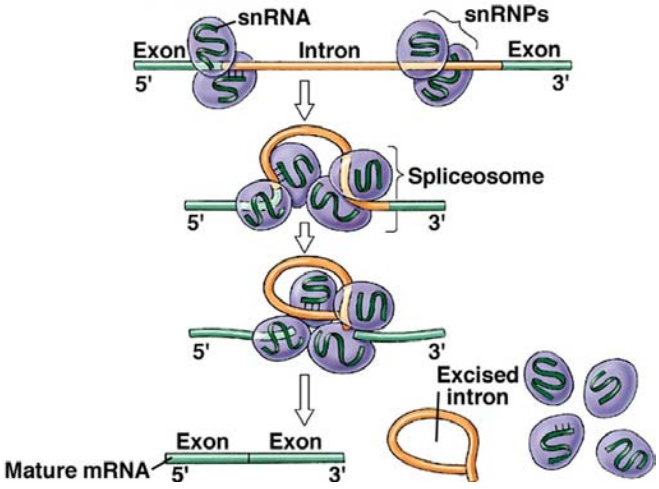
Introns and Exons

The primary mRNA transcript in eukaryotes, when released from RNA polymerase, contains a mix of codable and non-coding RNA nucleotide sequences. The non-coding DNA segments are called **introns** (because they are intervening segments which interrupt the message) and do not code for amino acids.

The regions that do code for amino acids are called **exons** (because they are expressed). Prior to using a mRNA transcript the introns must be removed, which is done during the **RNA processing stage.** This process is called **RNA splicing**, because the introns are cut out and the remaining exons get spliced together.



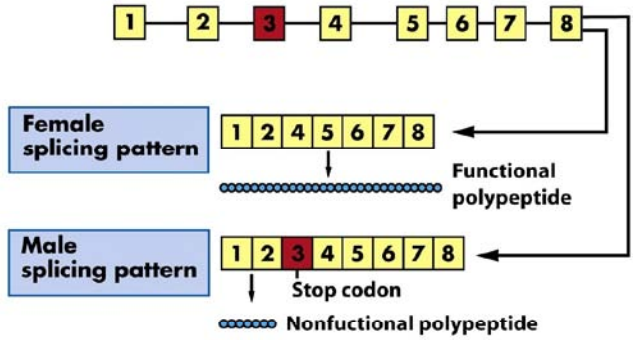
Special enzyme complexes are involved with the removal of introns. These complexes are called **spliceosomes** and are RNA-protein complexes. The removal of introns and splicing together of exons involves both protein enzymes and RNA catalysts. These RNA catalysts are called **ribozymes**, and are an important exception to the rule that all catalysts of living organisms are proteins.



Why Introns?

Why does so much of the DNA molecule contain introns? Some reasons might be:

- One pre-mRNA can be used to code for more than one protein, depending on what is determined to be introns in the processing stage. The same DNA can code for different molecules in different organs depending on the splicing. One might produce a hormone and one a chemical messenger. The proteins that determine gender development in fruit flies have been shown to share a common pre-mRNA.



Selective intron splicing in sex determination in fruit flies

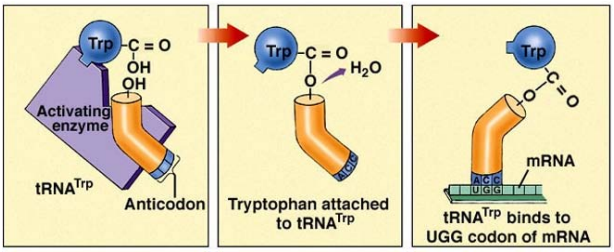
- Introns may help in modifying protein shape. Different introns can affect the location of an active site for an enzyme or the attachment site for a membrane protein. This may affect change in proteins through time, and result in new, different proteins.
- It is also believed that length of introns affects rate of recombination, a process that occurs during meiosis and results in more genetic variation.

Protein Synthesis – The Process of Translation

Once we have a final mRNA transcript, the mRNA is moved from the nucleus of the cell to the cytoplasm where is attaches to the small subunit of a ribosome and we are ready for the process of **translation.** Translation is where the information coded in DNA molecules is interpreted and translated to direct the actual synthesis of proteins. Translation also involves **Initiation, Elongation** and **Termination.**

Amino Acid Attachment

Prior to translation, one additional activity must occur: Amino acids must be attached to their appropriate tRNA molecules. The process of **Amino acid attachment** involves ATP and enzymes specific for each type of amino acid. Both the shape and charge of the tRNA molecules and the amino acids are important for the correct recognition and attachment.

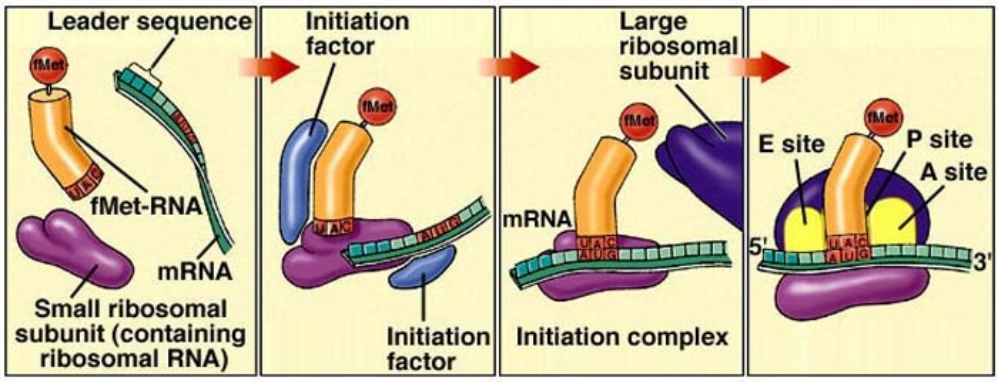


Although theoretically there should be 61 different tRNAs, one for each triplet code word other than the 3 stop triplets, there are only about 45. As mentioned, the triplet code for DNA - amino acids is redundant. Often the third nucleotide is not crucial.

Initiation

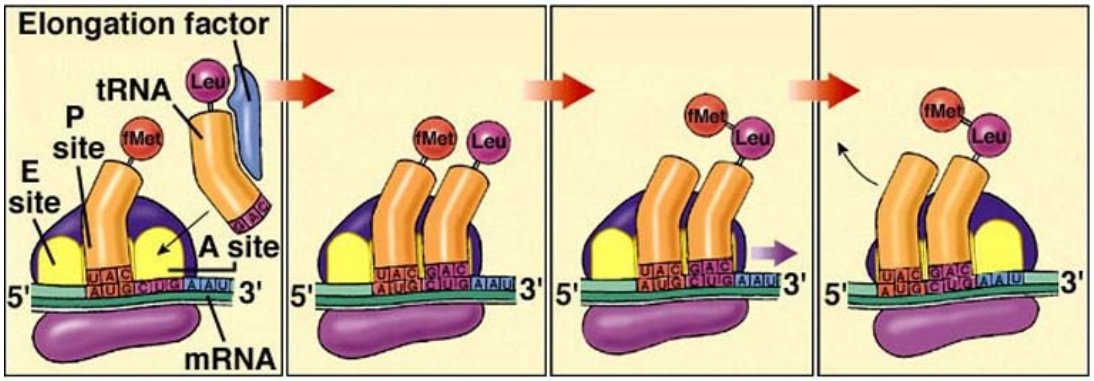
The small rRNA subunit has a binding site for mRNA molecules during protein synthesis and the initiator tRNA. The larger rRNA subunit has three attachment sites for tRNA molecules, the P site, A site and E site. During protein synthesis the two subunits bind together.

- Protein synthesis starts with an **initiation complex** of protein initiation factors, the small ribosome subunit, and the tRNA that has the "initiator" anticodon, UAC and its amino acid, formyl-methionine.
- The initiation complex binds to the cap of the mRNA transcript and moves along the mRNA until it reaches the start codon of the mRNA, AUG.
- The large ribosomal subunit binds to the small subunit, and the initiator tRNA attaches to the **P site** of the large subunit of the ribosome with the assistance of the **protein initiation factors**, bringing the complex together and forming a functional ribosome.
- A functioning ribosome is large enough to hold three mRNA codons. As stated, the first tRNA with its amino acid attaches to the P site. The A site of the larger subunit will be available for the 2nd tRNA molecule's anticodon to bind to the 2nd mRNA codon during elongation. The third codon site is the exit site.
- Note: Polypeptide synthesis is initiated at the amino end of the chain. Amino acids can only be added to the carboxyl end of an amino acid on the ribosome.



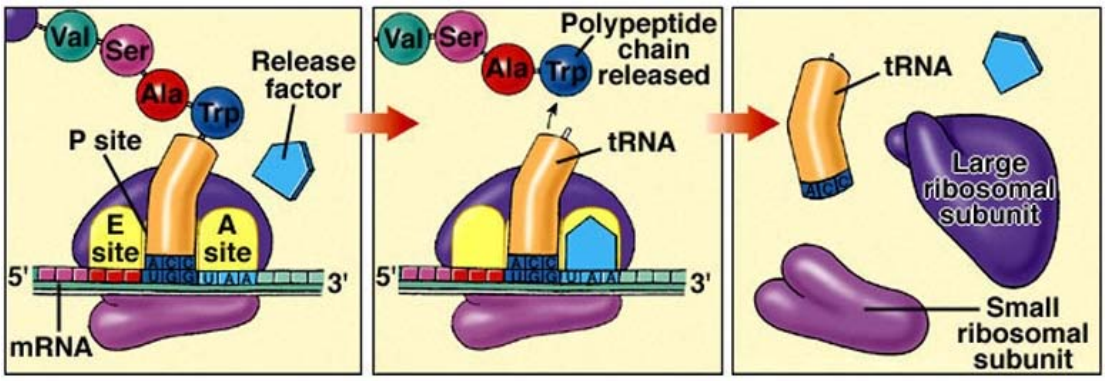
Elongation

- The next tRNA molecule, with its attached amino acid, is brought into place at the ribosome's **A site** with the assistance of elongation factors according to the mRNA codon message. The tRNA anticodon will hydrogen bond to the mRNA codon at this time.
- The positioning of the two tRNA molecules (each with its proper amino acid) at the P and A sites is such that a peptide bond can be formed between the two amino acids that are attached to their respective tRNAs.
- rRNA functions as a ribozyme to catalyze the peptide bond between the amino acid from the P site to the amino acid at the A site at the **peptide bonding site** on the ribosome. This process detaches the P site amino acid from its tRNA; the first amino acid attaches to the second amino acid at the A site. The polypeptide chain always elongates at the A site.
- Once the peptide bond is formed, the A-site tRNA will shift to the P site and the P site tRNA will shift to the E site and be dislodged from the ribosome (which is why the E site is called the exit site).
- A new tRNA that matches the 3rd mRNA codon will be brought into the now vacant A site by elongation factor proteins.
- The codon-anticodon binding, peptide bonding, detachment of tRNA and shifting continues until all of the codons of the mRNA have been matched by tRNA anticodons. The mRNA moves along the ribosome with its 5' cap leading. mRNA moves only in one direction. Ribosomes and mRNA move relative to each other, codon by codon, unidirectionally.

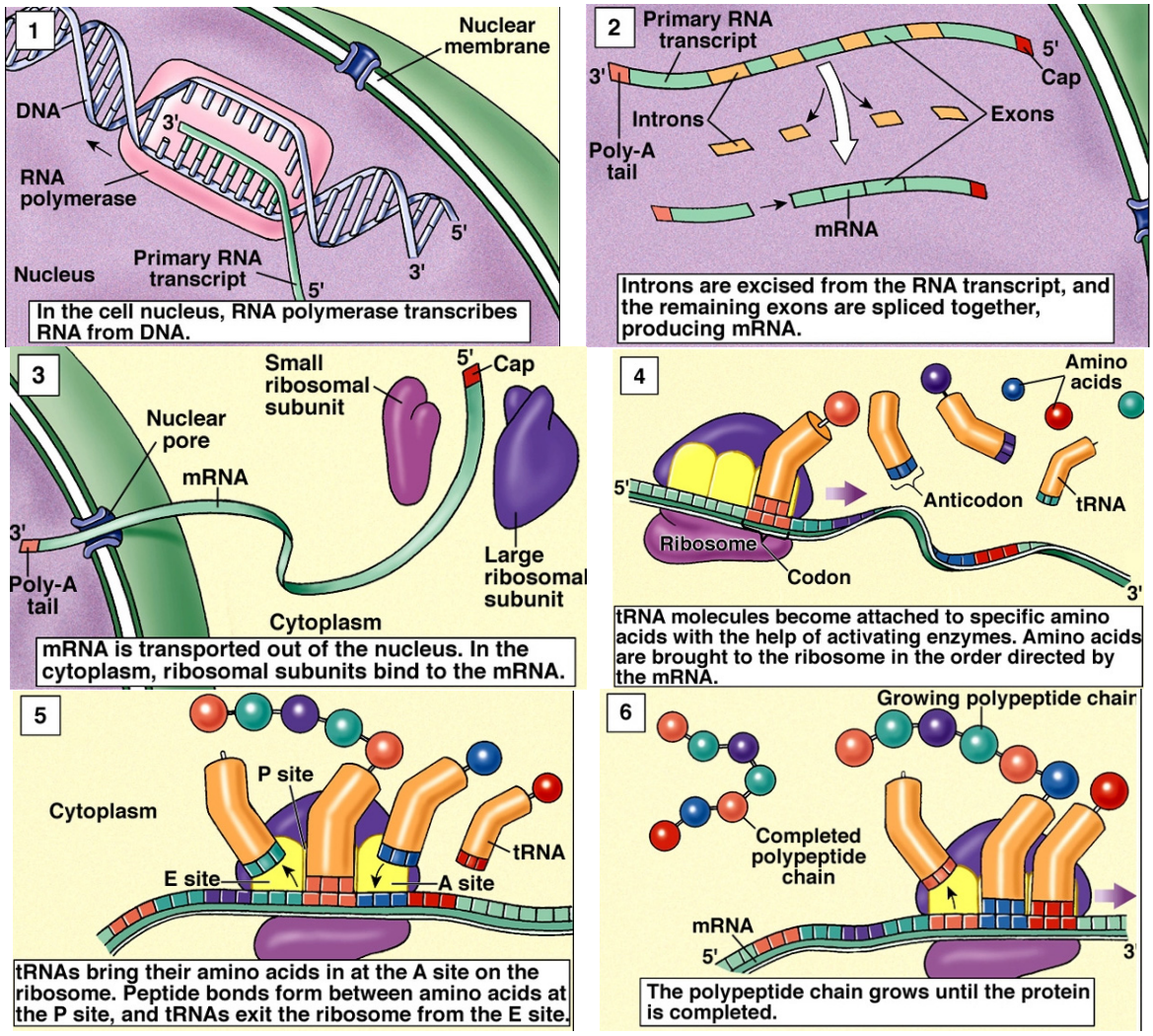


Termination

- The mRNA has a stop codon (UAA, UAG or UGA) which prevents any more tRNA from attaching to the A site. A **releasing factor protein** attaches instead causing the polypeptide to be released from the ribosome.
- The ribosomal subunits dissociate.

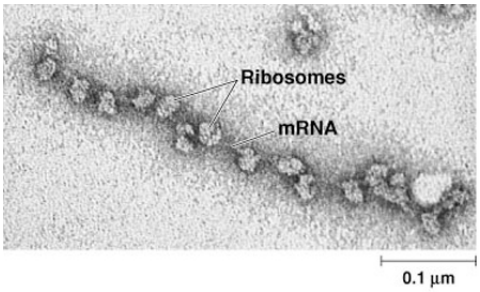


Summary of Transcription and Translation



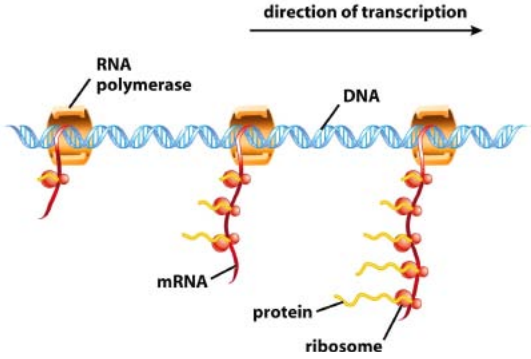
Rate of Polypeptide Synthesis

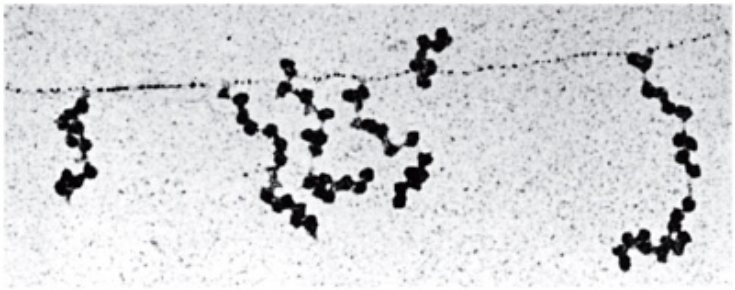
A polypeptide is generally synthesized in about a minute. However, it is typical of mRNA to be working along many ribosomes at a time to direct the synthesis of many polypeptide molecules in sequence. As soon as the 5' cap of a mRNA leaves one ribosome it will attach to the small subunit of an adjacent ribosome to initiate protein synthesis at that ribosome. It is common for one mRNA to have many ribosomes associated at once. Such complexes are called **polyribosomes**.



Prokaryote Transcription and Translation

In prokaryotes, both transcription and translation occur in the cytoplasm of the cell. In addition, virtually all the DNA codes for amino acids; bacterial genes lack introns. Consequently, translation can be initiated as soon as the mRNA transcript is long enough to be attached to ribosomes, and several genes can be transcribed in tandem from groups of related genes on the chromosome. Polyribosomes are common in prokaryotes, too.





Changing the Genetic Message - Mutation

Each gene is a precise combination of DNA. Anything that affects the structure of a chromosome, the structure of DNA, the number of chromosomes typical for a species, or affects the ability of DNA to be transcribed accurately, is known as a **mutation.** Although the processes of DNA replication and RNA transcription are remarkable in their accuracy, sometimes mistakes occur. Mutations occur naturally, caused by errors in DNA duplication, errors in processing DNA, such as in transcription of DNA to RNA, and errors in meiosis and mitosis. Physical damage and chemical damage can induce mutations as well, and are used by researchers to study mutations

Although it seems that we emphasize harmful effects that mutations can cause, many **good** variations also arise from mutations and are passed on within populations. Without such variation, populations would not be able to respond to changing environments and we probably would not have the remarkable array of proteins, and hence, the remarkable array of life processes we have today.

Mutations occur spontaneously as random events in cells. Mutations can also be induced. Substances that can promote mutations are known as **mutagens**. **Ionizing radiation**, such as X-rays and **UV light** can cause mutations. Exposure to radiation is cumulative, so many exposures to low doses can be as harmful as one exposure to a high dose of radiation. Cytosine and thymine are most susceptible to radiation mutations. There are also many chemicals that are mutagens, particularly **alkylating agents**.

When mutations involve chromosomes or numbers of chromosomes they are referred to as **chromosomal mutations.** Mutations involving single genes or nucleotides are known as **point mutations**. We will discuss both chromosomal and point mutations here. The difference between chromosomal and point mutations is not always clear

Chromosomal Mutations

Deletions

A chromosomal deletion is a loss of the chromosome or a large portion of the chromosome (generally a fragment without a centromere). The remainder of the chromosome, with the centromere intact has the deletion. Most deletions cause serious problems and can be lethal, since critical genes are missing.

Duplications

A deleted chromosome fragment can attach to its homologue, thereby duplicating a region of genes on the chromosome to which it attaches. Or, as happens in some genes, a segment of the gene or chromosome undergoes multiple repetitions, so that several copies are located on the chromosome.

Deletion Duplication

A common duplication, the **trinucleotide repeat**, occurs within some abnormal genes, and is responsible for several genetic disorders, including fragile X syndrome and Huntington's disease.

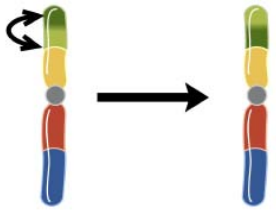
Gene Transfers

We have discussed how recombination between homologous chromosomes changes the specific alleles on a given chromosome and adds to the variations seen in populations. Recombination can also involve **gene transfer**. **Gene transfer** is when genes from one chromosome (or even organism) are transferred to a different chromosome or rearranged within a chromosome. There are two common gene transfers: **inversions** and **translocations** (or transpositions).

Changing Gene Positions – Chromosome Structural Change

Gene Inversions

For an **inversion**, a group of genes can have their order reversed in the chromosome so that a gene sequence that should be A-B-C-D-E-F-G-H is changed to A-B-F-E-D-C-G-H instead. This results in part of the DNA being "backwards" and unreadable. Many gametes are not viable after inversions.

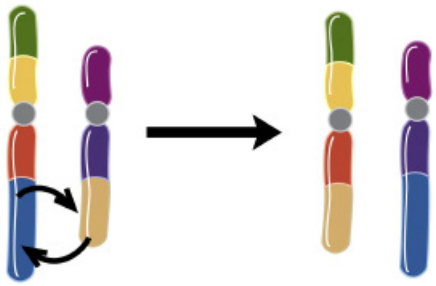


Gene Translocation

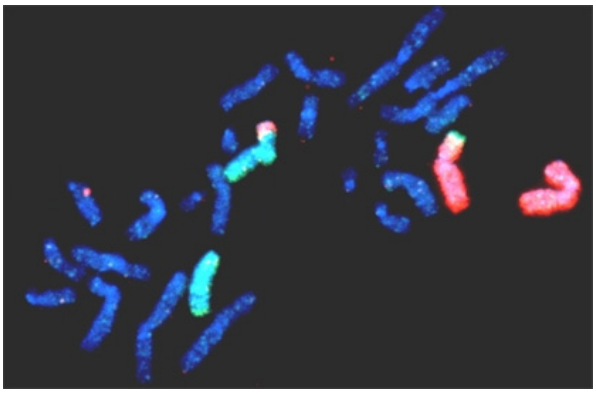
A gene can be **transposed** or translocated (moved) to a different location along the gene, so that the sequence might read A-B-C-E-F-G-D rather than A-B-C-D-E-F-G. Since many genes are read in sequence, altering the sequence may affect the ability to read a gene.

Translocation can also involved transferring a part of a chromosome to a different, non-homologous chromosome. Reciprocal translocations involve exchange of genes between non-homologous chromosomes.

The translocation of a piece of the human #22 chromosome to the #9 chromosome causes a form of leukemia because it interferes with a gene that controls cell division. This abnormal chromosome is called the Philadelphia chromosome, from the city in which the researchers who discovered this abnormality lived.



Translocation



Red and Green illuminated chromosomes illustrate translocation.

Impacts of Chromosome Mutations

The impacts of chromosomal mutations vary depending on when and where the mutation occurs. In some cases, the cell will not work, and dies. In gametes, they will be carried in all cell lines, and there is some evidence that some chromosomal alterations may activate oncogenes, or cancer causing genes. One form of leukemia is caused by a translocation between chromosomes 9 and 22.

Point Mutations

In contrast to chromosome mutations that involve entire genes or regions of chromosomes, **point mutations** are changes that affect one or just a few nucleotides. Point mutation impacts are readily seen in protein synthesis, and we will revisit some of the impacts of mutation on protein synthesis in that section.. Deletions, Insertions, inversions and translocations can all occur as point mutations as well as chromosomal mutations.

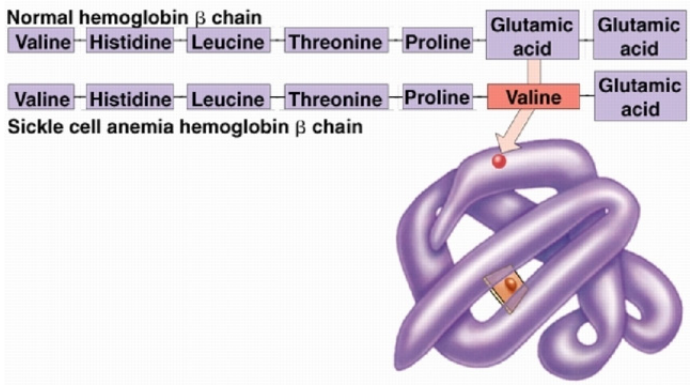
Types of Point Mutations

Base Substitutions

In a base substitution mutation, a single base pair is incorrectly matched, so that A will bond to C or G, rather than to T, for example. The DNA correcting enzymes may find the incorrectly matched pair, but might make the wrong correction, so that a different base pair results. This affects the "reading" of the gene, and may result in DNA instructions that cannot be followed.

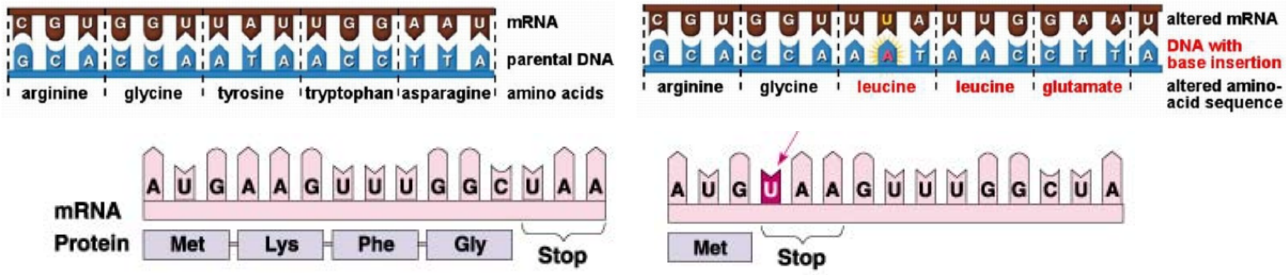
- If the substitution occurs in the third nucleotide of a redundant triplet, such as UCU or UCG, both of which code for serine, there will be no impact on the cell or the organism.
- If the substitution results in coding for one of the stop codons, then transcription will be halted at that point, and no protein can be synthesized.
- In many cases, DNA with a base substitution will result in a substitute amino acid at one location in the polypeptide. If the substitute amino acid is in a non-functional part of the protein, everything may be fine. On the other hand, a base substitution can have a dramatic negative impact, such as is the case with abnormal hemoglobin, or it can result in a protein with an enhanced function.

For example, the DNA for Hemoglobin normally codes for glutamic acid as the #6 amino acid. One hemoglobin mutation codes for valine in this position. The difference is Normal DNA code = CTC or CTT and Abnormal code = CAC or CAA. The result of this base substitution is the genetic disorder sickle cell anemia.



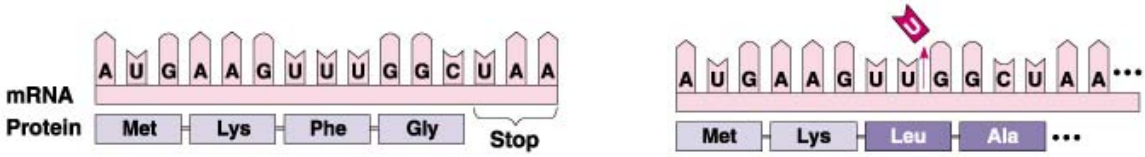
Insertions (Duplications)

If a new base pair is added to the DNA sequence being replicated, an insertion has occurred. The DNA instructions are now altered, and will be misread.



Deletions

In a deletion, a base pair is left out during DNA replication, and the DNA instructions cannot be read correctly.



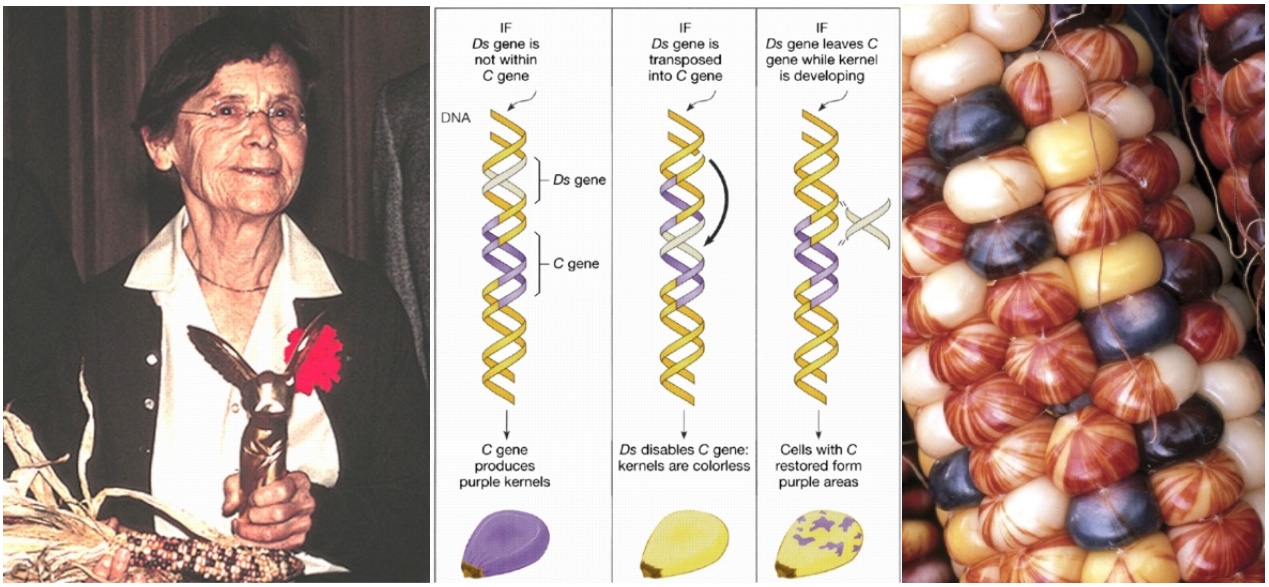
Insertions and deletions will affect the reading of the entire gene past the point of change. (In theory, if an insertion or deletion involved complete triplet nucleotides, there would be just one amino acid affected, and the remaining codes could be read as usual.) In contrast, a base substitution may affect only a short sequence (one nucleotide triplet) of the DNA reading.

Inversions and translocations can also involve just a few nucleotides.

Transposons and Transposition

Some DNA alterations are caused by transposons, genes or small pieces of chromosomes that literally "jump" from one region of a chromosome to a second, or to a different chromosome completely. **Transposons**, also called "jumping genes", often work to inactivate genes in the region where they are inserted.

The "**transposable element**" was first discovered in corn by Barbara McClintock in the 1940's. Different pigment patterns resulted from the "mutations" caused by the jumping gene. When the gene jumped into the middle of the gene to produce pigment, the pigment gene could no longer function and caused the pigment variations observed. She received little support for her discovery at the time; the phenomenon had not been seen in other organisms. However, in the 1970s similar jumping genes, called **transposons**, were described in viruses and bacteria and subsequently found in most organisms. Barbara McClintock was awarded a Nobel Prize for her work in 1983.



Reviewing the Effects of Mutations

The rate of mutation is highly variable, and depends in part of the ability of repair enzymes such as **DNA polymerase** and **DNA ligase** to fix mistakes. Some genes mutate at much greater rates than others. The effect of a mutation is also highly variable. A mutation that does not harm the cell will be perpetuated in the cell line. Mutations that occur in gametes will be passed on to subsequent generations. A mutation in a somatic cell impacts only individual in which is occurs.

While many times we stress that an alteration of the DNA produces harmful effects in the individual, it is by the act of mutation and gene alterations that many **good** variations also arise and can be passed on within populations. Without such variation, populations would not be able to respond to changing environments. Without such changes in the DNA over the millions of years of life on earth, we probably would not have the remarkable array of proteins, and hence, the remarkable array of life processes we have today.

As stated, a mutation may have no effect if a point mutation occurs at a place in the DNA coding that is redundant or if the mutation codes for an amino acid which will not alter the functioning of the protein, such as one in which the shape is not altered or the chemical nature of the protein is unchanged for its job. Such mutations are neutral mutations, since there is no apparent phenotypic impact.

Most commonly, the protein coded for by that gene will not be synthesized, or, if synthesized, cannot function normally, as seen when the base substitution that results in the abnormal hemoglobin that causes sickle-cell anemia. For most mutated genes that code for enzymes, this means that some biochemical activity in the affected cell will not occur. Depending on the specific activity not occurring, the mutation may or may not prove fatal to the cell, and in some cases, the organism.

In addition, if a mutated cell divides, any cells formed from that cell (DNA replication precedes cell reproduction) will perpetuate the DNA mutation. Keep in mind that a mutation may produce a change that improves the survival of the individual rather than being something that is harmful.

If a mutation occurs in the formation of gametes, and that gamete unites with another gamete, all cells of the new individual will have the mutation. The effect on the individual depends entirely on the specific mutation.

Again, mutations that are beneficial are an important source of genetic variation and are critical to the process of evolution.

Regulating Genes

We have been discussing the structure of DNA and that the information stored in DNA is used to direct protein synthesis. We've studied how RNA molecules are used to transcribe and translate DNA information to direct the synthesis of specific proteins. We have also discussed briefly how mutations alter DNA sequences and can affect gene expression.

We also know that each cell of an organism has exactly the same DNA, yet we have many different types of cells and tissues within a multicellular organism. For example, more than 20,000 genes have been identified in the human genome, but only a small portion of those genes are expressed in any given cell. The process of cell differentiation, in which cells become specialized for their specific function, involves selectively activating some genes and repressing others. Many genes in multicellular organisms are activated only at one stage of development, do their job, and function no more. The effects of these genes are not reversible.

How does a cell " "know" what DNA is needed and when? What controls gene activity? Finding answers to these questions is the subject of **gene regulation**.

Some of the answers to how genes are regulated are coming from work on recombinant DNA research, some from genetics and, in particular, from mutant strains of species. Gene regulation is a very active area of research in developmental biology, including stem cell research, the biology of aging, genetic diseases research, and cancer research.

Genes are controlled chemically by molecules that interact with DNA, RNA and/or the polypeptide chains. Both hormone signal molecules and regulatory proteins have effects on gene expression. Genes can also be chemically modified to either enhance or inhibit their readability. Gene controls can be positive – inducing gene activity, or negative – repressing gene activity. Gene expression can be regulated at any step from DNA → Transcription → Translation to the final product's functionality and duration in the cell.

Because gene regulation mechanisms were first studied in prokaryote transcription, much of what is presented deals with transcription control in both eukaryotes and prokaryotes. However, we will also look at some other ways that have been identified that regulate gene activity at the DNA level, as well as pre- and post-translation activities.

Regulating Gene Expression in Prokaryotic Transcription

The early work on gene regulation was done with prokaryotes. It is easier to study activity in prokaryotes because they are less genetically complex, and absent a nucleus, the DNA is accessible to all components of the cell. Much of the research in gene regulation has been accomplished with *Escherichia coli*, the common intestinal bacterium. Moreover, gene expression in prokaryotes is organized into a discreet "package" the operon.

Before we go too far, however, we need our vocabulary. Recall that the typical gene codes for a polypeptide that is used to help the cell function in some way, or codes for some structural protein. A gene that codes for such proteins is a **structural gene**.

Other **Regulatory genes** control how much of and when polypeptide gets formed.

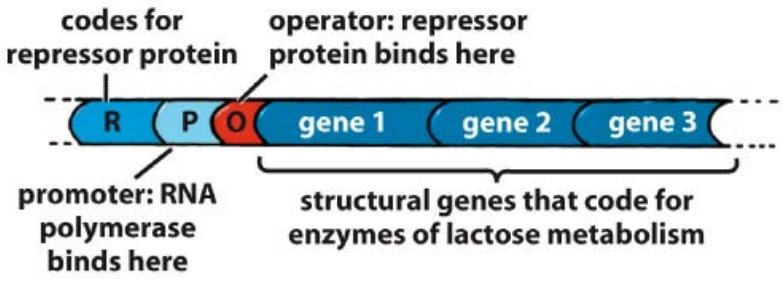
- Some regulatory genes code for small polypeptides that control how other genes get expressed. These polypeptides are called **transcription factors.** There are a number of different types of transcription factors.
- Another type of regulatory gene is a piece of DNA that a transcription factor binds to. These **regulatory sites** of DNA do not actually code for any protein.

The Operon of the Prokaryotic Cell

An active gene (or group of genes) includes the DNA that will be transcribed, the structural gene, along with a promoter and operator. This complex is known as the **operon** and was described in 1961 by Francois Jacob and Jacques Monod. An operon has four parts: **promoter**, **operator** and **structural gene** plus a **regulatory gene** that activates or represses the operon.

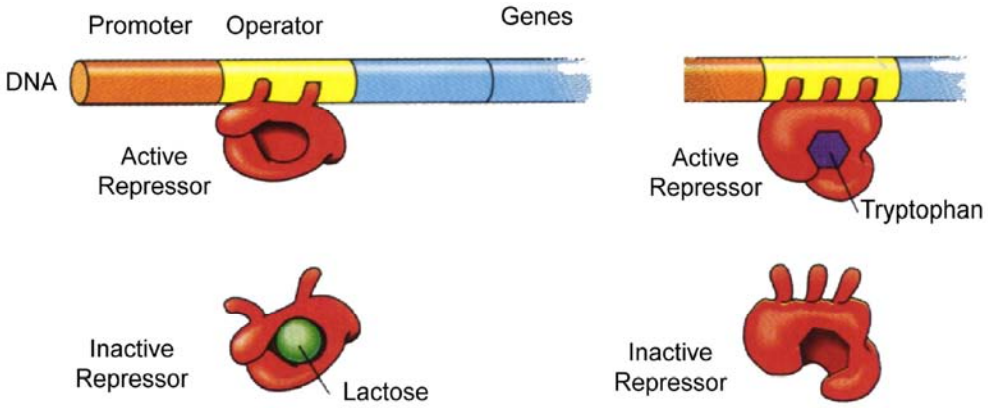
Operon

- **Specific Regulatory Gene**
  Controls how other genes are expressed
- **Promoter**
  Recognized by RNA polymerase as the place to start transcription
- **Operator**
  Controls RNA polymerase's access to the promoter, and is usually located within the promoter or between the promoter and the transcribable gene (or set of genes)
- **Structural (Transcribable) Gene**
  Codes for the needed protein



Regulatory Gene

- A regulatory gene codes for a **repressor protein**. The regulatory gene is located on the DNA some distance apart from the rest of the operon.
- Repressors typically work with **controller** molecules, which typically are substances in the cell. A repressor can be active when attached to its controller molecule or deactivated when attached to a controller molecule.
  - In an **inducible operon**, the gene is normally "off". The repressor actively blocks the gene from transcription. When the controller molecule attaches to the repressor, it removes the repressor from the operator and transcription proceeds.
  - In a **repressible operon** the gene is normally "on" and the gene is being transcribed. The repressor does not blocking gene transcription unless the controller molecule binds to the repressor. When that happens, the repressor, with its controller attached, actively blocks transcription.



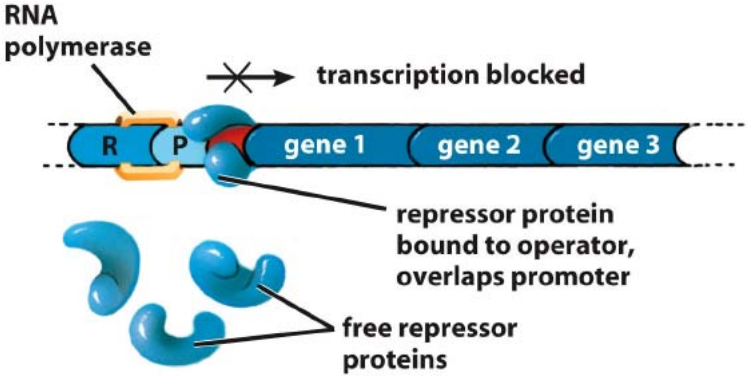
Inducible Operon Repressible Operon

We will look at two prokaryotic operons: the lactose operon, an inducible operon and the tryptophan operon, a repressible operon.

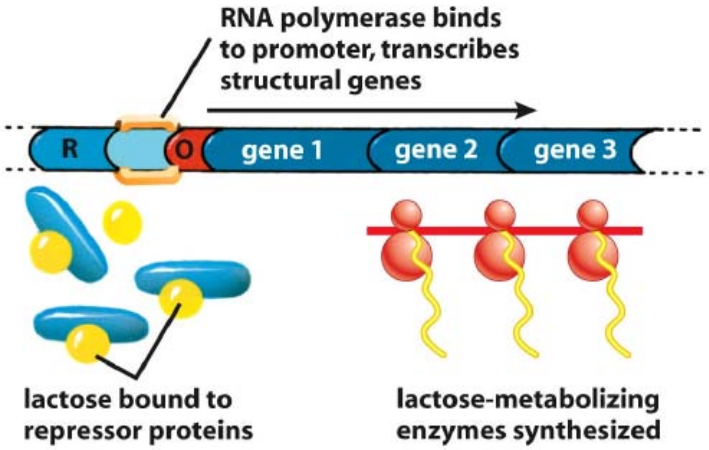
The Lactose Operon – An Inducible Operon

To get an idea of how genes get regulated, we will look at the Lactose operon, described by Jacob and Monod in *E. coli*. The lactose operon contains the three genes that code for the enzymes that degrade lactose.

In the absence of lactose, the controller substance, the repressor inhibits transcription by blocking RNA polymerase from attaching to the promoter.

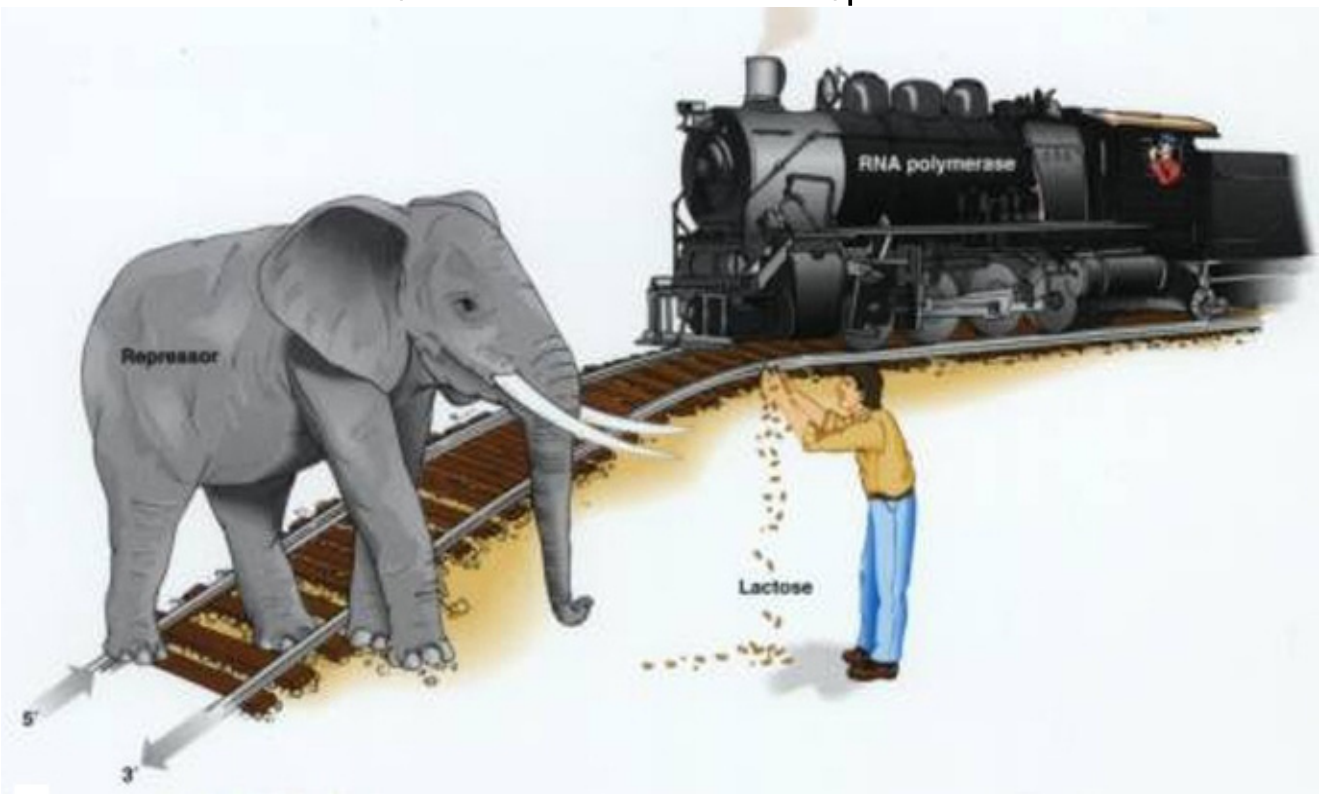


When lactose is present in the cell, some lactose molecules attach to the repressor protein sitting on the operator region of the gene and remove the repressor. The promoter is now available and the genes that code for the three enzymes to digest lactose are transcribed.



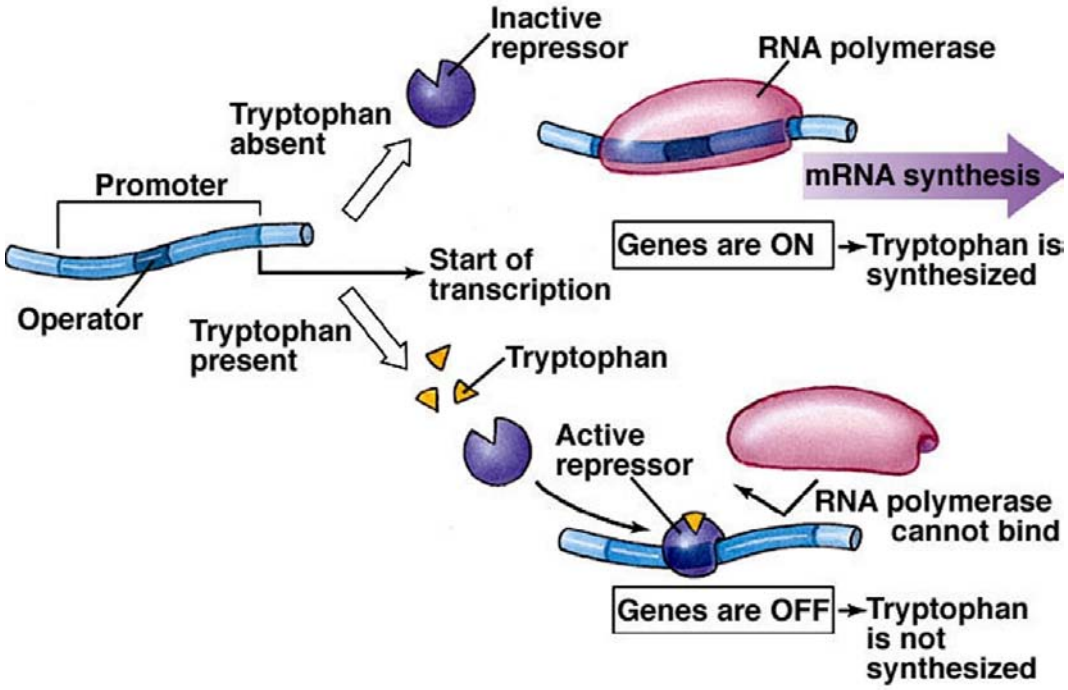
When the enzymes are synthesized, the lactose is degraded, including the lactose molecules attached to the repressor. When lactose is no longer available to bind to the repressor protein, the repressor shuts down the promoter (by sitting back on the operator), which stops transcription.

One view of the Lactose Operon



The Tryptophan Operon – A Repressible Operon

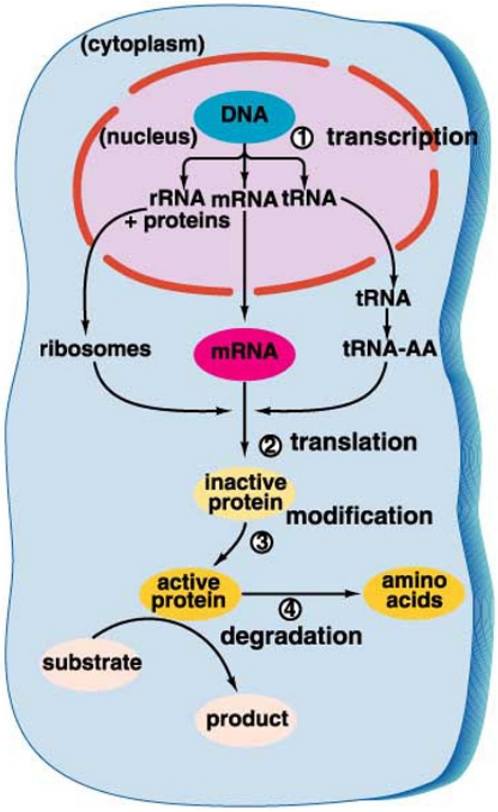
We have just seen how lactose in a bacterium's environment can induce transcription by removing a repressor protein from the promoter-operator region of the operon. Products in the environment can work in reverse too, by **inhibiting** or **repressing** rather than promoting transcription. If a bacterium has sufficient tryptophan in its environment, some of the tryptophan will bind to the tryptophan synthesis gene repressor molecule, but in contrast to lactose that removes the repressor, the tryptophan synthesis repressor needs tryptophan to fit into position and block transcription.



Regulating Gene Expression in Eukaryotic Organisms

Gene expression starts with transcription and "ends" with an enzyme catalyzing a particular chemical reaction, or with a structural/metabolic protein. Gene expression can be controlled at any level of gene activity.

- Making the DNA readable (or not)
- Transcription
  - Activating transcription
  - Frequency and rate of transcription
- Processing the mRNA
  - Selective intron removal
- Translation
  - Stability of mRNA can be blocked in the cytoplasm
  - mRNA access to translation
- Post-translation Protein Modification
- Duration of gene product in the organism
- Enzyme Activity and Feedback Inhibition

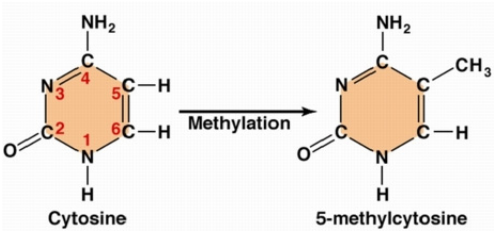


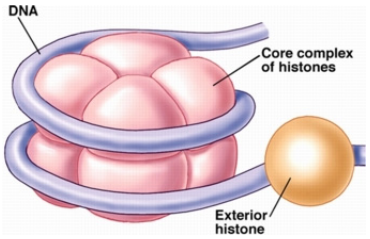
DNA Controls – Pre-transcription

Chemical Modification of DNA

During Interphase, many regions of a chromosome remain tightly condensed and clumped. Highly condensed chromatin regions are called **heterochromatin**. Heterochromatin is too compacted to be transcribed. The less compacted regions of chromatin are known as **euchromatin** and can be transcribed.

Inactive DNA contains nucleotides (especially cytosine) have **methyl groups** ($-CH_3$) attached. (The Barr body *(see later)* is an example of a chromosome that is highly methylated.) Most methylated DNA will remain inactive during differentiation and cell divisions. Methylation keeps some genes permanently turned off.



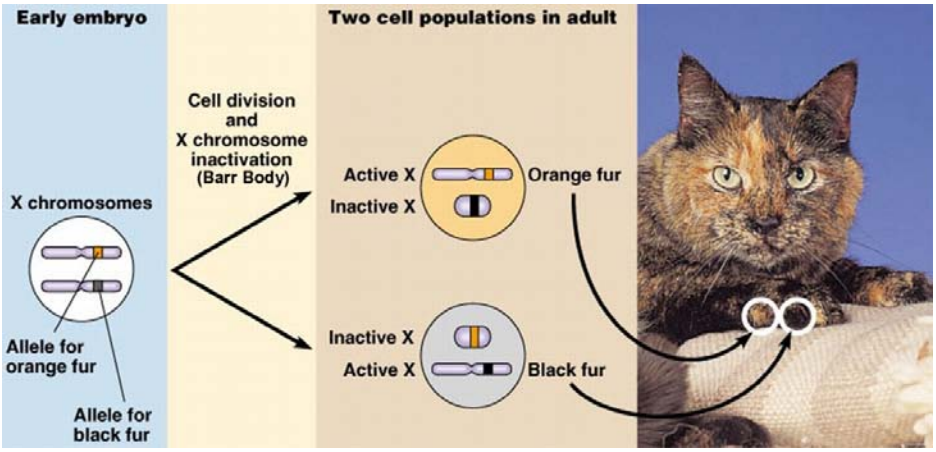


Adding an **acetyl group** ($-COCH_3$) to the histone proteins associated with DNA helps transcription. Histones with acetyl groups bind more loosely to DNA .

Chromosome Inactivation – The Barr Body

Females have two X-chromosomes. In most cells, one of them is deactivated (apparently at random) during embryonic development and forms a tightly condensed object that lines the nuclear membrane, called the Barr body. The DNA of the Barr body is not available for use by the cell, a form of gene regulation by **chromosome inactivation.** The specific alleles for genes carried on the X chromosome that get expressed in any given cell line depends on which X chromosome is not made into a Barr body.

The pattern of the calico cat is an example of Barr body expression. Both orange and black pigment alleles are on the X chromosome. The black patches of fur are from cell lines where the orange X chromosome is a Barr body. Orange patches of fur result when the black X chromosome becomes the Barr body. The patches of white fur are the expression of a different gene.



Eukaryotic Gene Regulation during Transcription

Prior to looking a ways in which transcription in eukaryotes can be controlled or regulated, let's look at the eukaryotic gene transcription complex.

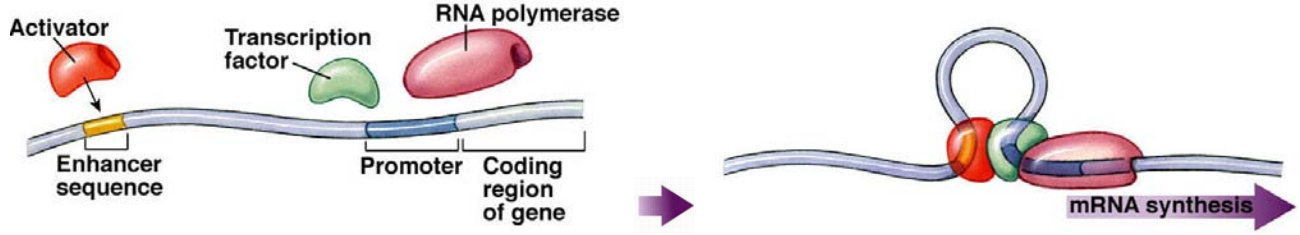
Eukaryotic Gene Transcription Complex

Like the prokaryotic gene, transcription for an eukaryotic gene requires a number of regions, each important to transcription. The components of the eukaryotic transcription complex include:

- **Control elements**
- **The codable gene including introns and exons**
- **A termination sequence.**

Control elements consist of:

- A specific **promoter** region within the control elements, which indicates the starting point for transcription.
- A region called the **enhancer** that stimulates the binding of RNA polymerase to the promoter region. The enhancer region is comprised of non-coding DNA that binds to transcription factors called **activators**. Activators fold the DNA so that the enhancers are brought to the promoter region of the gene where they bind to additional transcription factors

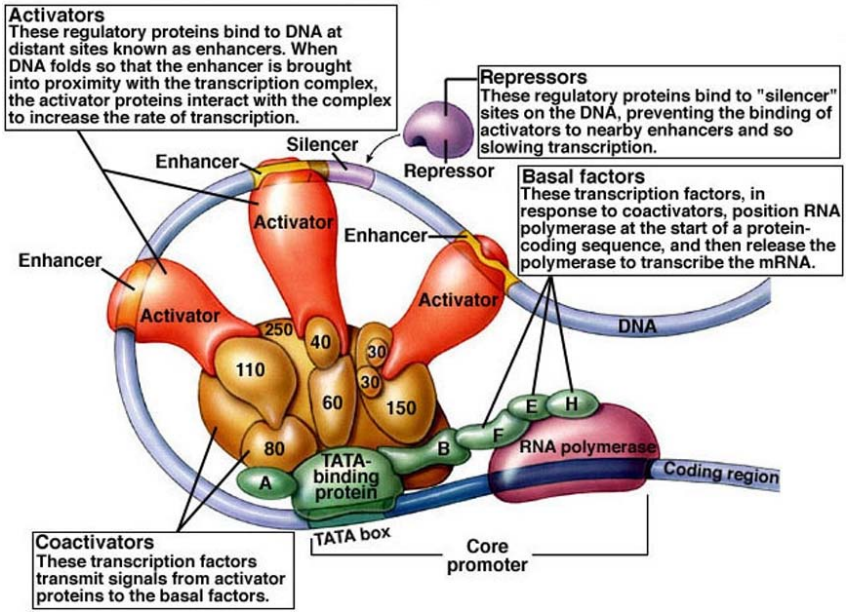


- **Silencers** are control elements that can inhibit transcription. A **repressor** is a transcription factor that binds to a silencer control element and blocks transcription. is called When a repressor is attached to the silencer region, activators are can't bind to enhancers, and transcription is repressed.

Transcription Initiation Complex

Transcription factors bind to the enhancer (or silencer region of the gene if we are going to repress transcription instead) where activator proteins have attached. Hundreds of transcription factors have been identified in eukaryotes, and most likely are the direct control of transcription.

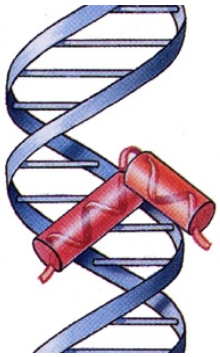
The binding of activators to the enhancer results in **bending the DNA** molecule so that the activator proteins are brought more closely to the promoter region. This serves to attract more transcription factors to form **a transcription initiation complex** into which **RNA polymerase** can fit at the promoter region of the gene. When everything comes together, we get transcription.



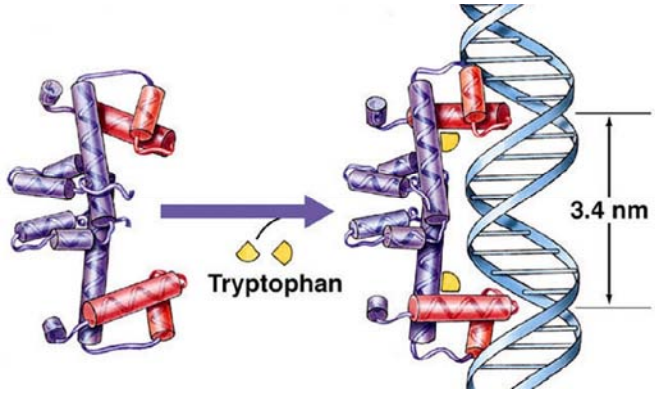
Transcription Factor Binding Sites

Transcription factor proteins have specific binding sites that fit into the DNA molecule at the appropriate location. As expected, the binding sites have specific structural elements, called **motifs** or **domains**, each of which is cleverly named.

One of the more common binding motifs is the helix-turn-helix motif, a protein helix with a bend in it. Regulatory proteins generally have pairs of helix-turn-helix motifs for more strength. Often, repressor molecules attach to the binding motif, changing its shape so it can attach to the DNA molecule repressing its access to RNA polymerase.



Helix-Turn-Helix on DNA

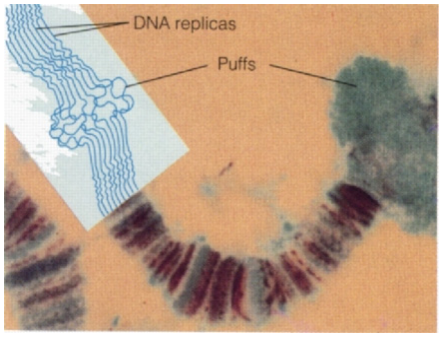


Tryptophan binding motif of paired helix-turn-helix

Examples of Transcription Controls in Eukaryotes

Gene Amplification

It is possible to get multiple copies of genes via **gene amplification**, a process in which a gene, or portion of chromosome, or even entire chromosomes get multiplied many, many times. Salivary glands of many flies have gene amplification of chromosomes forming **polytene** chromosomes, which associated puffs.



Fruit Fly Polytene Chromosome

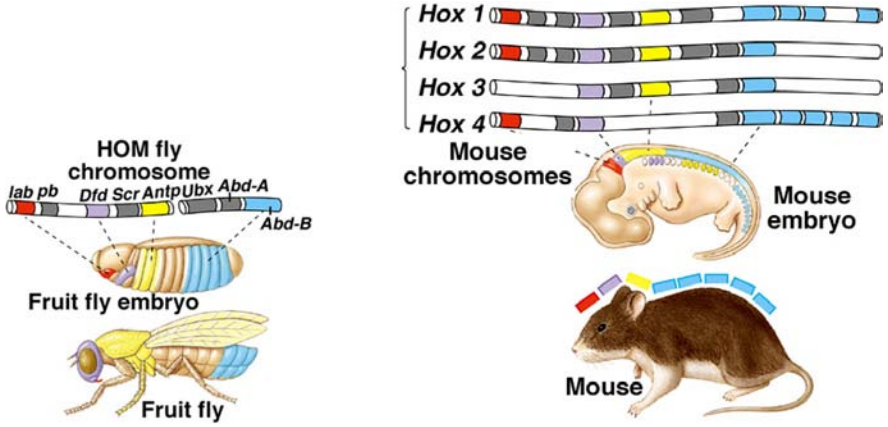
Gene amplification occurs in ovum cells of vertebrates when the genes for ribosomal RNA (rRNA) get replicated millions of times to ensure that the cytoplasm will have the many, many ribosomes needed for protein synthesis activities in early development.

Cancer cells can also have gene amplification, where genes with resistance to the chemotherapy drugs are replicated thousands of times. Increasing the drug concentration results in increasing the resistance of the cancer cell population to the drug by selecting for those cells that have amplified genes for drug resistance.

Pattern Formation and Homeotic Genes

During development different cells become organized in predictable patterns, some of which are determined by organization of the egg cell cytoplasm, some by cell position within the embryo. Plant cell differentiation is almost exclusively positional. Their position in the meristems fixes their ultimate position and function. Animals have pattern formation, too, and often once pattern is induced, it is fixed. Cells transplanted from one position to another in the embryo may develop into the structure to which they were patterned prior to the transplant. Chemical signals are used extensively during pattern formation. The signals trigger cell responses that affect gene transcription of what are called "master genes", genes that control developmental sequences.

Homeotic genes are a group of master genes that determine body-part identity and pattern development. There is a remarkable similarity of homeotic genes among different animals. The HOM genes of the fruit fly and the set of HOX genes in the mouse determine similar pattern development.



Mutations in the homeotic genes produce organisms with misplaced body parts.

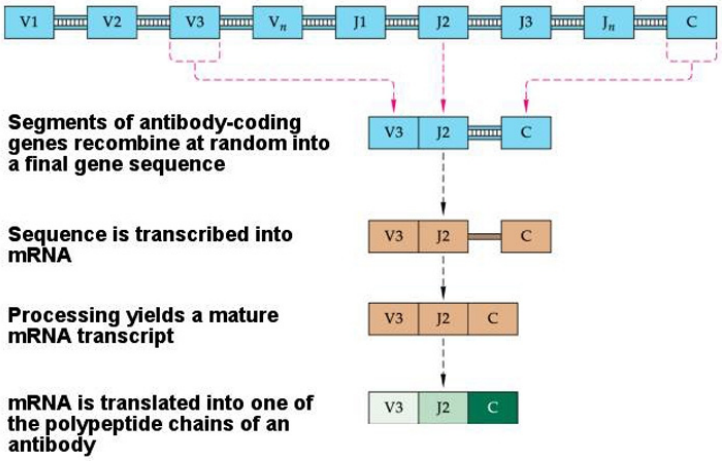

Normal Fly


Mutant Antennapedia Gene

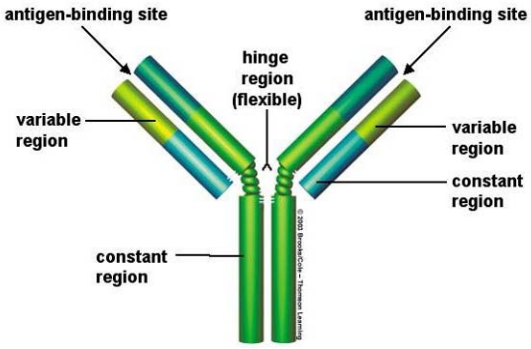

Eye Induction Mutant
Targeted Gene Expression

Gene Rearrangement

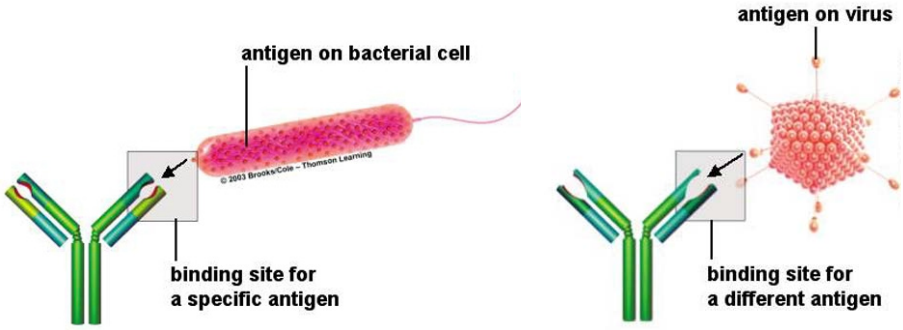
Some genes have a number of different possible arrangements for their nucleotide sequence. The genes that code for antibody formation have multiple possibilities. For each antibody, a core sequence is coded, but additional code, making each antibody unique is pretty much randomly determined.



Coding for Antibodies



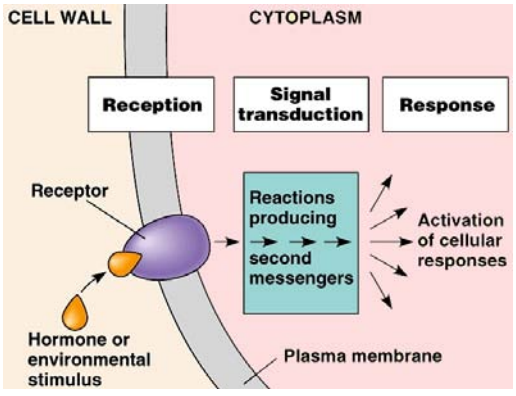
Common Antibody Structure



Two Specific Antibodies

Chemical Activators

Hormones (or other chemicals) can function as signal molecules that trigger **signal transduction pathways** in cells. Signal transduction pathways often result in the synthesis of **transcription factors**. Signal transduction pathways are equally important for chemical messaging in plants and animals. Which transcription factors get synthesized to activate genes depends on the signal molecule, and the transduction pathways triggered.

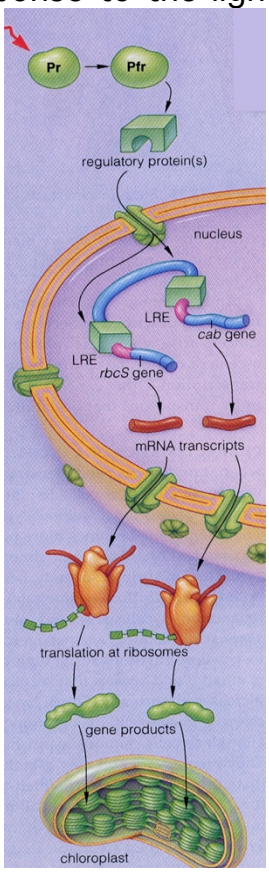


- The hormone, **ecdysone**, found in flies, stimulates the transcription of the genes for the production of **saliva** on the **polytene chromosomes.** Large amounts of saliva are needed to moisten the copious amounts of food the larvae consume.
- **Albumin synthesis** in bird eggs is promoted by an estrogen-protein complex that binds near the enhancer region of the albumin gene. Estrogen is only produced during the breeding season, so no albumin is synthesized when it is not needed.
- The activation of the **molting gene** in insect larvae is controlled by a hormone that activates a regulatory protein on the gene.
- The **androgen receptor** in human males is essential for testosterone to function.

Environmental Activators

Plants in particular have responses to environmental signal activators. Many plant growth activities are activated by light, or the absence of light. **Phytochrome** is a light-sensitive pigment that undergoes a conformational change to trigger signal transduction pathways that affect transcription factors. Phytochrome is involved in flowering of plants, chlorophyll synthesis and etiolation (rapid elongation of cells in the absence of light. Phytochrome is also responsible for signaling anthocyanin production in leaves prior to abscission, which gives the red pigments to leaves in autumn.

Chlorophyll synthesis in response to the light activation of phytochrome



mRNA Processing Gene Regulation

When mRNA is processed, we can get different functional mRNA transcripts depending on which part of the primary transcript is determined to be introns and which exons. These differences can be accomplished via **alternative splicing** by the spliceosomes, thereby controlling gene activity, examples of which were discussed earlier.

Post Transcription Controls of Gene Activity

Duration of the mRNA Transcript

The length of time a mRNA transcript can be read prior to degradation will affect the amount of final product, which will affect cell activity. Some mRNA lasts for weeks; some for hours.

Translation Control

Blocking mRNA attachment to Ribosomes

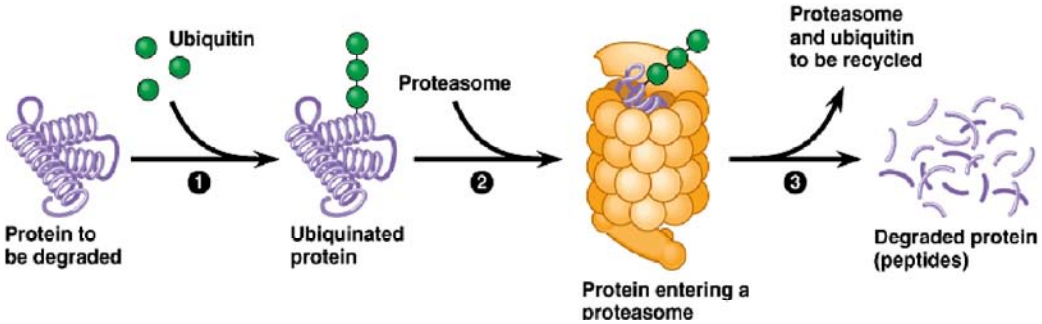
Translation can be controlled by regulatory proteins that block mRNA attachment to the small subunit of the ribosome. In humans, translation of the protein ferritin, an iron carrier, is blocked unless iron is present in the cell.

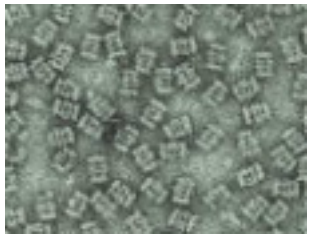
Post Translation Processing

After a polypeptide is synthesized it can be altered by processing. Many metabolic proteins are non-functional until activated by other molecules. Hydrolytic enzymes, in particular, are synthesized in inactive forms; membrane recognition proteins have additional molecules attached to them before they function.

Protein Stability

Proteins targeted for degradation are tagged by a tiny protein that is recognized by huge protease enzymes called **proteasomes.** The tagged protein is readily degraded within the proteasome. In cystic fibrosis, the chloride ion channel protein gets tagged and degraded before reaching the plasma membrane.

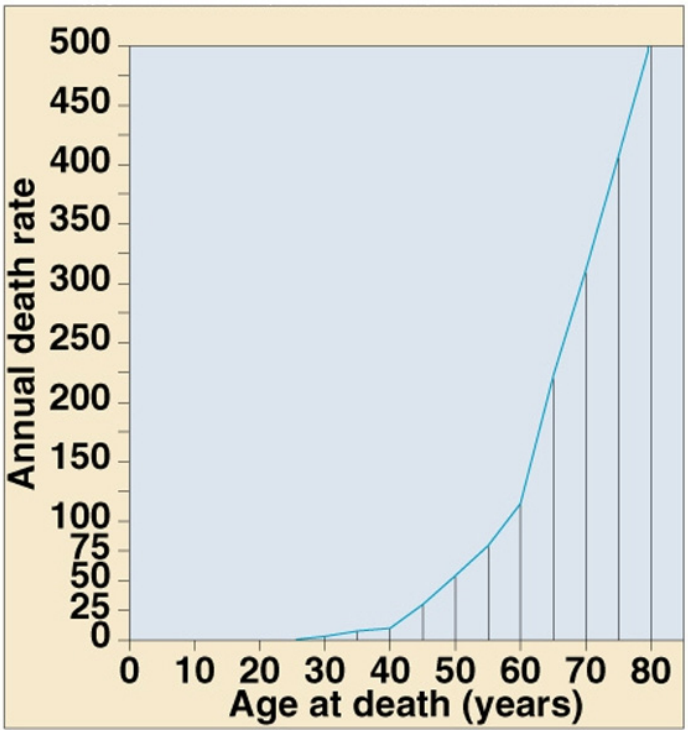


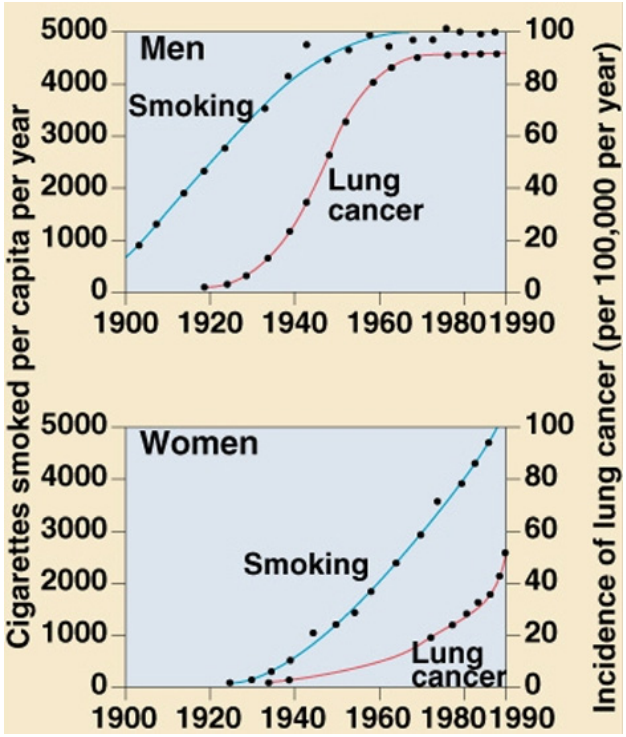


Proteasomes

Cancer and Gene Regulation

Cancer is a disease of uncontrolled and invasive cell reproduction. The current estimates are that a third of the children born now will get some form of cancer in their lifetime. Lung cancer is still a major killer, and the cause of most lung cancers is straightforward: smoking. The three most common cancers are breast cancer (an assortment of cancers), prostate cancer and colon cancer. One in eight will get breast cancer. Almost any male who lives long enough will have prostate cancer.



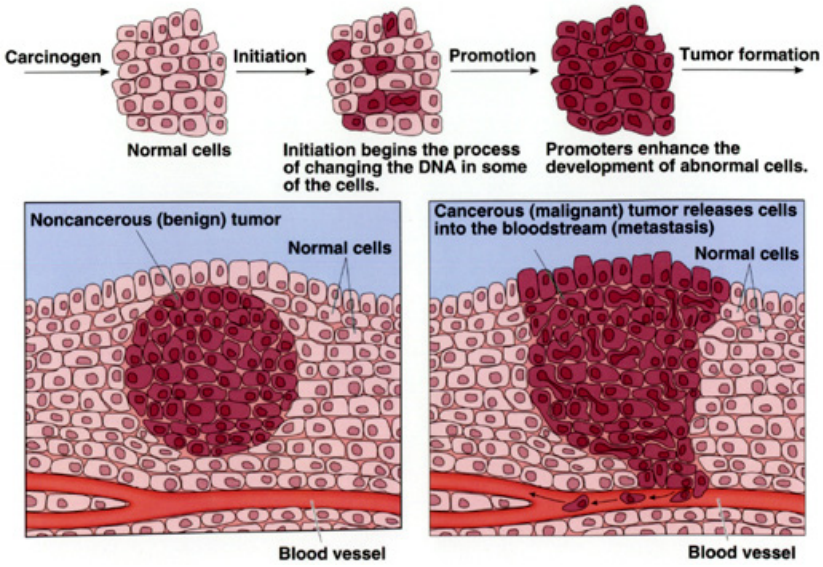


Some cancers develop when the gene regulators are defective, and in all cancers, gene expression is defective. For example, normal cell division has a number of checkpoint controls that ensure that division proceeds correctly. Like everything else, the checkpoint molecules are coded for by genes, and genes can mutate and genes can be suppressed. When checkpoint controls go awry, abnormal division results. Accumulated mutations are a factor in aging and may result cancer. We don't have all the answers.

Cancer cells have abnormal plasma membranes and abnormal cytoplasm. Cancer cells divide rapidly and ignore overcrowding inhibition signals. They can make masses of cells called tumors. Cancer cells promote angiogenesis – new blood vessel development that "feeds" the growing tumors. Once cell controls are not in effect, rapidly dividing cancer cells lose normal positioning and adhesion properties, too. Cancer cells can metastasize – migrate to new areas of the body and start growths in different tissues. Spread of cancer makes it more difficult to treat.

From what we know today, the steps in cancer development include:

- Exposure to a carcinogen from the environment by ingestion, inhalation, etc., naturally or via contamination
- Entry of the carcinogen into a cell
- Initiation of cancer via sufficient DNA changes in cell division control genes
- Promotion and enhancement of cancer via cell transformation
- Tumor formation and uncontrolled cell growth



No one knows why any one person gets cancer. Some cancers are familial, and probably genetic. Some cancers are related to the environment, especially the smoker's environment. A substance that causes a change in DNA that can lead to cancer is a **carcinogen** and exposure to a carcinogen is the first step in cancer.

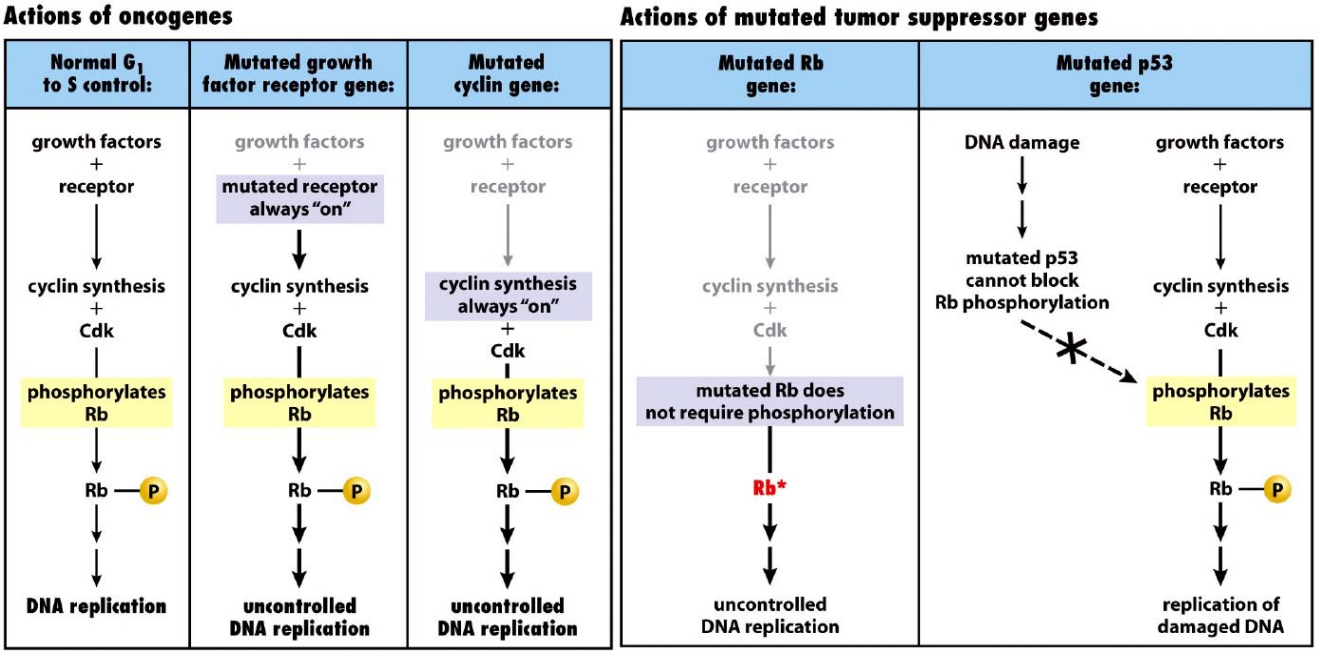
How does one get cancer?

Most believe that the onset of cancer is an accumulation of mutations rather than one single alteration. This correlates with the increase in many cancers with aging. As briefly mentioned during our section on cell reproduction, many cancers are associated with mutations in genes that regulate cell division. Such genes that normally control cell division but when mutated have the potential to induce cancer are called **oncogenes**. They are often called proto-oncogenes when functioning normally.

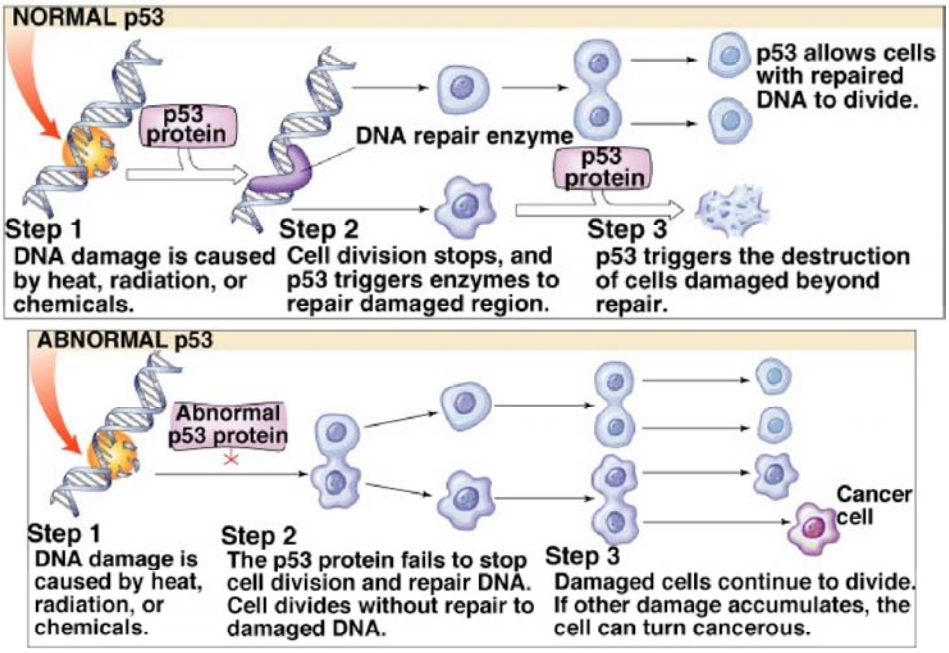
Oncogenes also include genes that normally suppress tumor formation by monitoring DNA and cell division to ensure that all is well. The **P53 tumor suppressor gene** is one such gene mentioned earlier. P53 is a transcription factor for genes that keep a cell's DNA repaired and genes that delay the cell's rate of cell division so that there is time for DNA repair. If the cell is in bad shape, P53 activates cell suicide genes to prevent the harmful mutations from being passed on. Such cell death is called **apoptosis**, and is genetically programmed. When p53 is defective or missing, cancers are more likely because DNA damage isn't caught and repaired.

Any number of things in our surroundings can activate oncogenes. Chemicals that do so are called carcinogens. Radiation and the combustion products from tobacco are two of the most common carcinogens. Asbestos and some heavy metals in particulate form are also carcinogens. Many steroids in higher than normal concentrations are carcinogenic, and a high fat, low fiber diet is also suspected as being cancer promoting. Some viruses promote cancer formation.

A sample of oncogene and tumor suppressor gene normal function and mutated consequences is shown below.

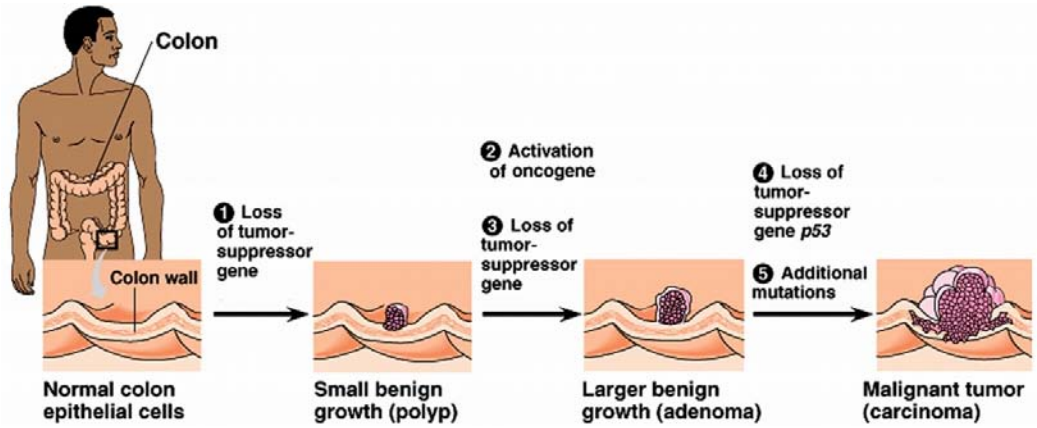


P53 and Cancer



Cancer and the Accumulated Mutations

We still cannot answer why one person will and another will not get cancer after exposure to the same potential carcinogens, but in some familial colon cancers several mutations have been identified in polyp cells that can become colon cancer tumors, including mutations in APC (a gene involved in cell migration and adhesion), Ras (tumor-suppressing gene) and p53. Similar familial oncogene mutations are associated with some breast and ovarian cancers.



Major Factors That Increase the Risk of Cancer

Factor	Examples of Implicated Cancers	Comments
Heredity	Retinoblastoma (childhood eye cancer) Osteosarcoma (childhood bone cancer)	Most cancers are not caused by heredity alone. Persons having familyhistories of certain cancers should follow physicians' recommendations.
Tumor viruses	Liver cancer Adult T cell leukemia/lymphoma Cervical cancer	Five viruses are initiatorsof certain cancers. (See Table 19.1)
Tobacco use	Lung cancer Cancers of the oral cavity, esophagus, and larynx Cancers of the kidney and bladder	Cigarette smoking is responsible for approximately one-third of all cancers. Nonsmokers have an increased risk of smoking-related cancers if they regularly breathe in sidestream smoke.
Alcohol consumption	Cancers of the oral cavity esophagus, and larynx Breast cancer	The combined use of alcohol and tobacco leads to a greatly increased risk of these cancers. The mechanism of action in breast cancer is not yet known.
Industrialhazards	Lung cancer	Certain fibers, such as asbestos, chemicals such as benzene and arsenic, and wood and coal dust are prominent industrial hazards.
Ultravioletradiation from the sun	Skin cancers	Those at greatest risk are fair-skinned persons who burn easily. However, everyone is at risk and should wear sunscreens and protective clothing when in the sun for extended periods of time. All types of UV radiation in tanning beds (UVA, UVB, & UVC) are harmful and may lead to skin cancer.
Ionizingradiation	Related to location and type of exposure	Eliminate unnecessary medical X rays to lower cancer risk. Infantsand children are particularly susceptible to the damaging effects of ionizing radiation. Check your home to detect high levels of radon gas.
Hormones (estrogen and possibly testosterone)	Breast, cervical, ovarian, and prostate cancers	Estrogen-only and estrogen-progesterone hormone replacement therapies both increase the risk of breast cancer. Oral contraceptives increase the risk of breast cancer and cervical cancer, while reducing the risk of ovarian cancer. The role of testosterone in prostate cancer is unclear.
Diet	Breast and prostate cancers (weak association with high-fat diets), stomach and esophageal cancers (nitrites).	Nitrites found in salt-cured, salt-pickled, and smoked foods increase the risk of cancer.

Growth and reproduction are two of the characteristics of life. The cell theory states **"All cells come from preexisting cells by a process of cell reproduction, or cell division".**

Cell division is the process by which all cells of a multicellular organism are formed. Cell division is also responsible for repair and replacement of cells and tissues during one's lifetime. **Asexual reproduction**, a means of making more individuals is from a single "parent" common in protists, fungi, many plants and some animals. Both prokaryotes and eukaryotes have a process of cell division, although the details are a bit different.

The process of cell division must ensure that new cells formed have the genetic information of the original cell and other cellular components from the cytoplasm needed to sustain the cell.

In our discussion of cell reproduction, we shall focus on the processes of cell reproduction (mitosis and cytokinesis) in **eukaryotic** organisms. The process of cell division in **prokaryotic** organisms, **binary fission,** has similarities, but the single molecule of DNA and absence of a nucleus in the prokaryotic cell account for a number of differences in the "mechanics" of the process.

Mitotic Cell Division

We know that all cells of an individual have **exactly the same DNA**, and their DNA is found in structures called **chromosomes.** Each eukaryotic species has a fixed chromosome number, a number that does not change from generation to generation. The DNA must also stay the same from cell to cell within an organism, so that when cells divide, new cells formed will have exactly the same DNA as the original cell.

To ensure that chromosomes and DNA remain the same in new cells, the following must take place when cells divide:

- We must form two new cells from the original cell.
- Since each cell must have all of the genetic material for the organism, we must have a mechanism that exactly **duplicates** the DNA from the original cell and **distributes** the copied DNA equally to the new cells. The distribution of DNA into new nuclei during cell division is called **mitosis**. *(Duplication of DNA is a part of the discussion of structure and function of DNA.)*
- We must also separate the **cytoplasm,** and critical organelles, such as mitochondria and chloroplasts, of the original cell into the new cells formed so that the new cells can survive, grow and function. The separation of cytoplasm into new cells is called **cytokinesis**.

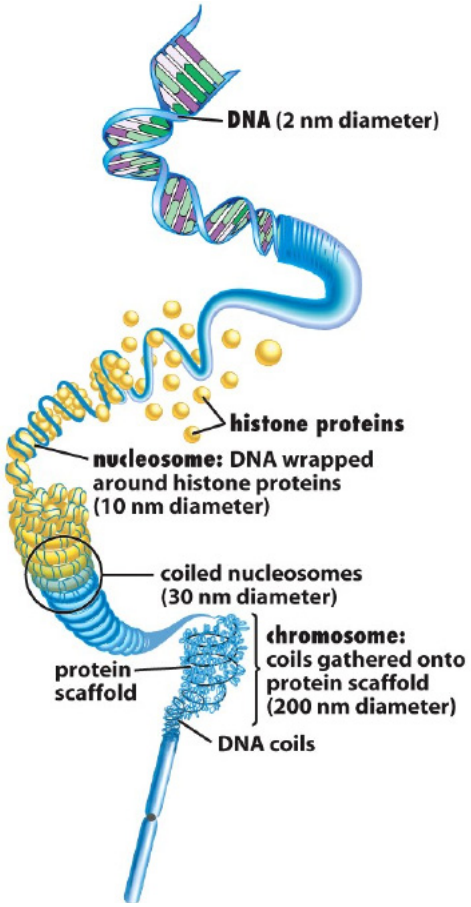
In addition, in sexually reproducing organisms, a variation of cell reproduction, called **meiosis,** occurs at one stage in the organism's life cycle (to form gametes in animals, or to start the gamete producing stage in plants). We will discuss the process of meiosis later.

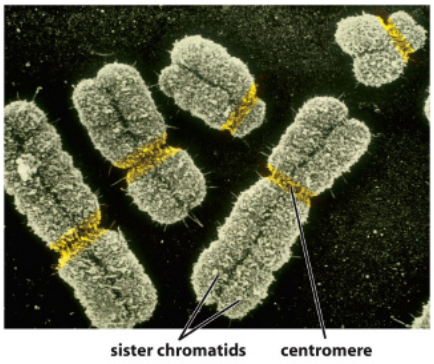
Before discussing how cells divide, it's probably useful to discuss the structure of chromosomes and chromosome terms (of which there are a sufficiency).

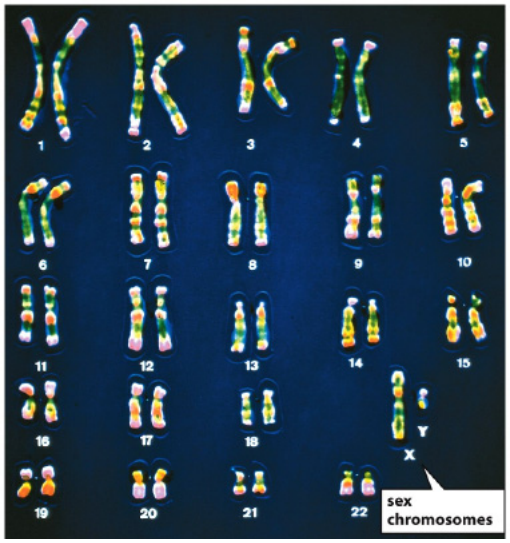
Structure of Chromosomes

Chromosomes are composed of DNA and protein, a mixture called **chromatin.** During the normal metabolic activities of the cell the collective DNA, or chromatin, appears dense and grainy.

DNA is a thread-like double chain of nucleotides. The DNA coils around the histone proteins to form **nucleosomes.** Chromosomes continue to tightly coil and fold back on themselves prior to cell division. Chromosomes are visible only when tightly coiled.





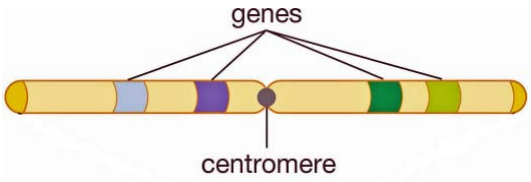


Human Male Karyotype

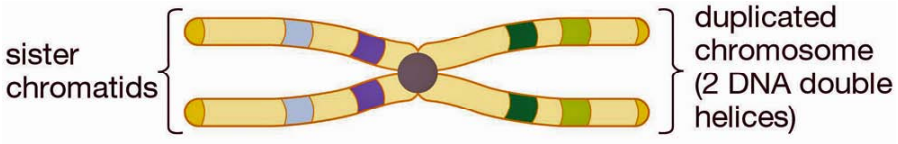
Each species has a characteristic number of chromosomes. Humans have 46 chromosomes; potatoes and chimpanzees have 48 chromosomes. The pea plant, important in Mendelian genetics has 14, the even more famous fruit fly has 8. Some ferns have chromosome numbers exceeding 1000. Chromosomes are self-duplicating and must do so prior to each cell division. There is an essential vocabulary associated with chromosome appearance before and after duplication.

Chromosome Terms before and after Duplication

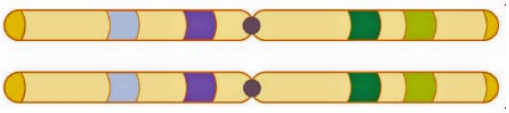
- An unduplicated chromosome is **one chromosome.** A chromosome more or less consists of two arms that extend from a centralized region called the **centromere.**



- When a chromosome duplicates, it becomes **one duplicated chromosome,** and the two copies remain attached to each other. It is still **one chromosome**. The two **exact** copies of the duplicated chromosome, which remain attached at the centromere region, are called **"sister" chromatids**. They are **identical** to each other. **(Remember this; it is essential!)**



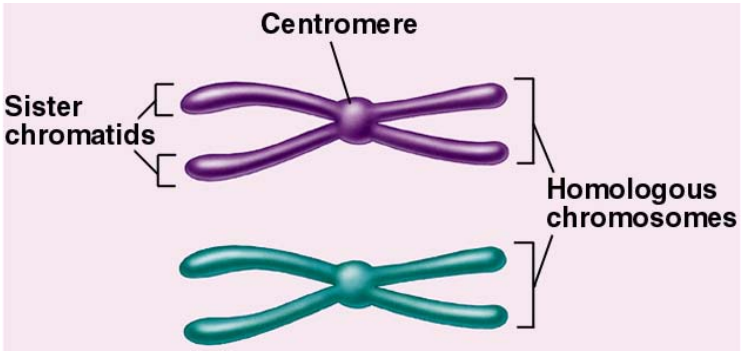
- At the centromere region of the duplicated chromosome, there are structures (made of protein and DNA) called **kinetochores**. The kinetochores attach to microtubules of the spindle during mitosis.
- After the identical sister chromatids are separated during mitosis, each (called a "daughter" chromosome now) becomes a single unduplicated chromosome again.



Rule to remember: A chromatid must be attached to its identical chromatid and the two sister chromatids comprise **one** duplicated chromosome (not a pair of chromosomes – "chromosome pair" is used for something else). **"Sister" chromatids are not two chromosomes. They are one duplicated chromosome that consists of two identical chromatids.** The only time you can use the word chromatid is when you have the two identical chromatids attached to each other.

Homologous Chromosomes

When we look at the chromosomes of most eukaryotic organisms carefully, it can be seen that for each individual chromosome, a second chromosome can be found that physically matches it in length and shape. Closer inspection of the DNA shows that the matching chromosomes have very similar, but **not identical** DNA which carries equivalent genetic information, or genes. These matching chromosomes, called **homologous chromosome**s, with their similar DNA, form the basis of the variation we see in the genetic traits, or genes, of living organisms (*see later*).



A display of homologous chromosome pairs is called a **karyotype**, as shown above. The human karyotype has 23 pairs of chromosomes, 22 pairs of autosomes and 1 pair of sex-determining chromosomes. In contrast to the autosomes, the sex-determining chromosomes do not physically match.

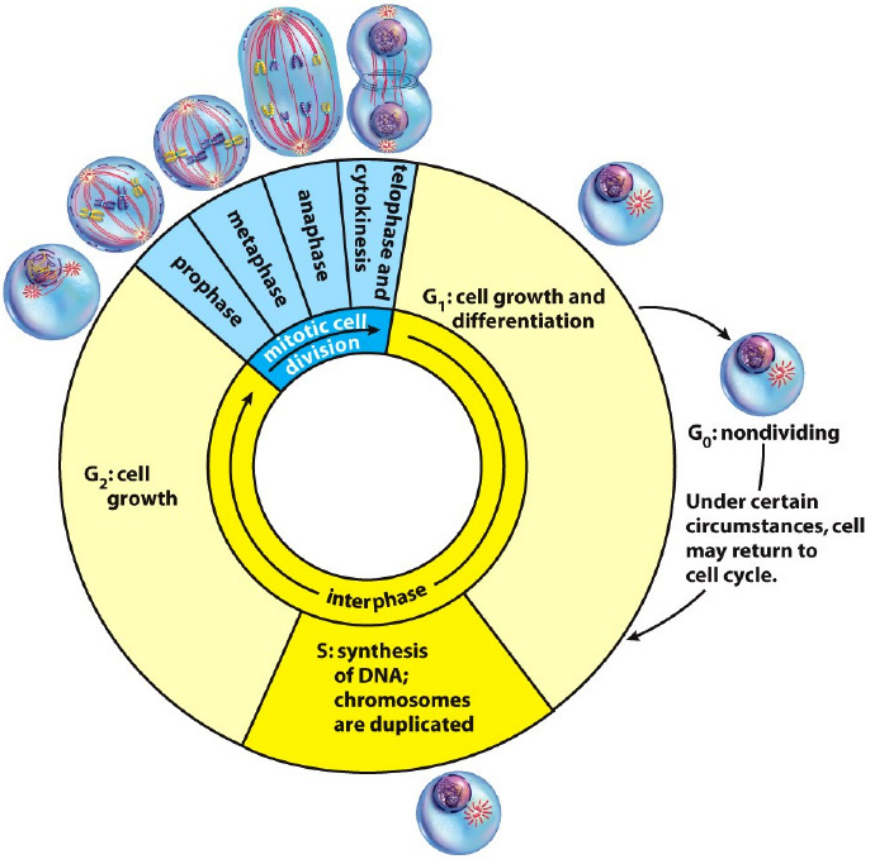
Cells that contain pairs of homologous chromosomes are called **diploid**. For humans, the diploid number of chromosomes is 46. These 46 chromosomes are comprised of 23 homologous pairs of chromosomes. Each pair is physically similar and has equivalent genetic information. As we shall discuss later, the genes on homologous chromosomes do not have to be identical (although they can be). We know for example, that you can inherit either brown eyes or blue eyes; a tongue that curls, or one that cannot curl. The alternative forms that genes can take are called **alleles**. Inheritance looks at how these pieces of DNA interact to produce the traits expressed in individuals.

Homologous chromosomes literally pair up and are separated during the process of meiosis, which will be discussed soon. Cells that have just one of each homologous pair of chromosomes are **haploid** (half of diploid) and contain half the number of chromosomes.

Remember: "Sister" Chromatids are not pairs; they are the two identical parts of one duplicated chromosome. You must make this distinction! The pairs are the homologous chromosomes. Homologous pairs of chromosomes function together during meiosis, as we shall discuss. All chromosomes function independently in mitosis.

The Cell Cycle

Mitosis is a part of the **cell cycle.** The cell cycle starts when a cell is formed and continues until it divides (or dies). Some cells never divide; others are specialized for division (especially in plants, where virtually all cell division occurs in specialized tissue called **meristem**). Cell division is a brief part of the life cycle; most of the life of a cell is spent in normal activities of growth and maintenance.



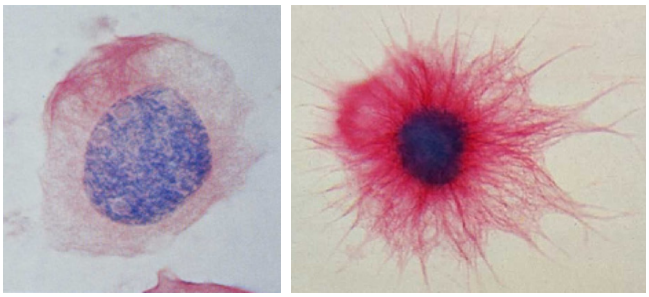
The cell cycle involves the following:

Interphase

Interphase is the period of time for normal cell activities, including:

- **Growth** (G_1 or Gap)
  The newly formed cell does its normal activities.
- **DNA Duplication** (S)
  - DNA duplication (or synthesis) occurs.
  - Once started, DNA duplication cannot be reversed; the cell is committed to divide.
- **Preparation for Division** (G_2)
  - Proteins needed to do cell division are manufactured in preparation for mitosis and cytokinesis.
  - Cells can continue to grow and do their normal cell activities as well.
  - A G_2 checkpoint controls whether or not the cell will go into mitosis.

Note: If a cell never divides, it stays in G_1 "permanently" a state called G_0 (or non-dividing state).



Interphase

Cell Division (or cell reproduction), which includes:

- **Mitosis**
  - Process of distributing the duplicated DNA equally to two new nuclei.
  - Mitosis is divided into 4 stages
    - **Prophase**
    - **Metaphase**
    - **Anaphase**
    - **Telophase**
- **Cytokinesis**
  - Process of separating the cytoplasm contents of the original cell into two new cells.

The events of the cell cycle are carefully regulated at checkpoints in the cycle. Cells that stay in G_0 for example, never receive the appropriate signal at the G_1 checkpoint to proceed into DNA synthesis. There are additional checkpoint signals in G_2 and during mitosis. One is at the start of anaphase.

The Stages of Mitosis

Mitosis is a continuum. Humans have decided to separate the process into stages for the convenience of our discussion. Some humans even separate the stages into sub-stages and intermediate stages.

Properly, mitosis refers to what happens to the chromosomes in the nucleus. Cytoplasmic division occurs during the accompanying cytokinesis.

The Spindle Complex

Since chromosomes are being distributed into new nuclei, a critical component of the process of mitosis is how the chromosomes are moved. Movement of chromosomes involves sets of microtubules, known as the **spindle apparatus**. Microtubules of the spindle complex extend from each pole of the cell and overlap each other at the equator of the cell. Poisons that affect microtubule function block cell division. Spindle formation is one of the events of prophase.

Prophase

Chromosome Condensation

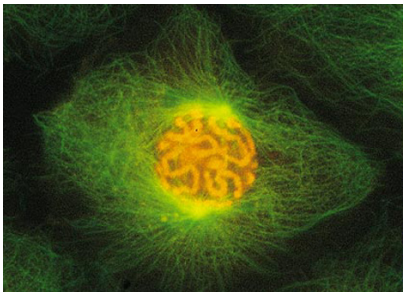
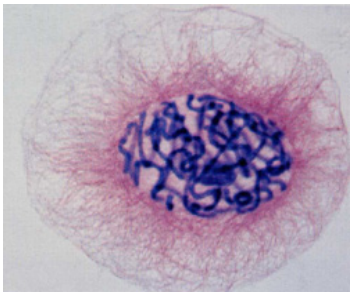
- Duplicated chromosomes start to condense and tightly coil to become visible as threadlike structures as prophase starts. Chromosomes continue to condense and become thicker as prophase progresses.
- The **nucleolus** region (an aggregation of chromosome bits and concentrated RNA and protein) of the nucleus will disassociate.
- The duplicated chromosomes are firmly attached at their centromeres throughout the condensation and coiling.

Nuclear membrane

- The nuclear membrane degrades in later prophase into small vesicles, which can be used to synthesis new nuclear membrane material in the new cells.

Microtubule Organization

- Microtubules initiate **spindle** formation and determine the poles of the cell. The spindle apparatus will extend from the poles of the cell to the center of the cell surrounding the nuclear region and to the opposite pole of the cell.
- Some microtubules from each pole of the cell attach to each duplicated chromosome's **kinetochores** located in the centromere region.
- Other microtubules overlap each other from the poles through the equator region of the cell.
- In many cells, clusters of microtubules form around the **centriole pairs**, which replicated during interphase. Microtubules move centriole pairs to the respective poles of the dividing cell. These regions are sometimes called the **asters**. Centrioles are not essential to mitosis. Cells that lack centrioles still form the spindle complex. It's just a way to ensure that the new cells will have a pair of centrioles in their cytoplasm.

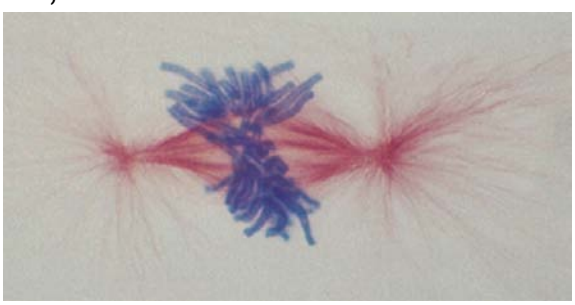
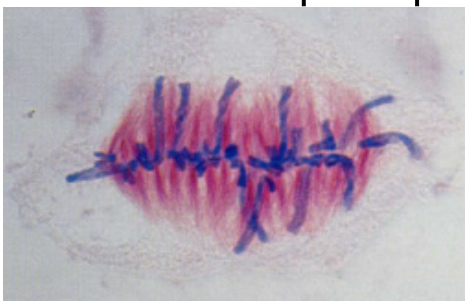



Prophase

Note: some researchers choose to call the events that include the degradation of the nuclear membrane and the attachment of the spindles to chromosomes **prometaphase.**

Metaphase

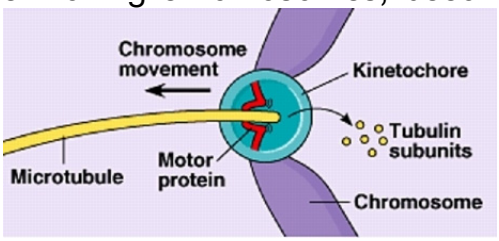
- The spindle apparatus moves the chromosomes to the equator of the cell, aligning the centromeres of each duplicated chromosome along the equator.
- Chromosomes are moved by a combination of pulling and pushing of spindle microtubules.
- The length of the spindle microtubules is regulated by the kinetochores to facilitate the alignment of centromeres at the equator.
- The ultimate alignment of chromosomes along the equator plane of the cell is metaphase, and the chromosomes are often called the **metaphase plate**.

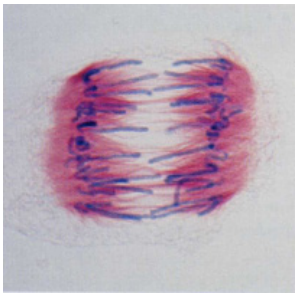
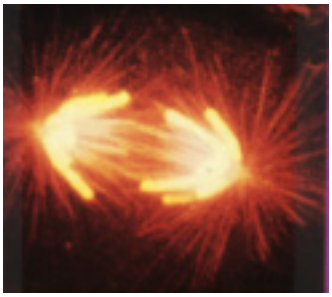
Metaphase

Anaphase

- Centromeres of each duplicated chromosome separate to start anaphase. You can't actually see this; the separating chromosomes are the first visual sign of anaphase.
  - Kinetochore motor proteins pull their chromosomes along the spindle microtubules from the equator to the poles of the cells. The microtubules disintegrate behind the moving chromosomes, becoming shorter.



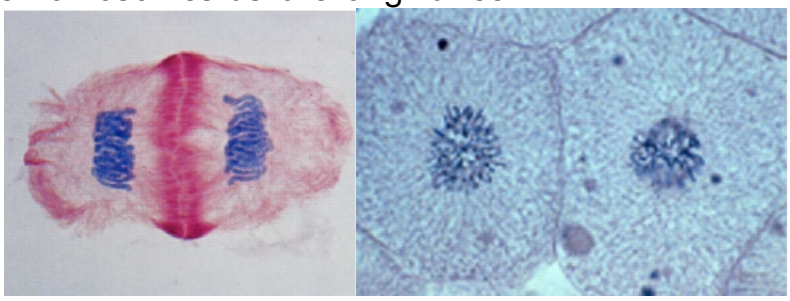
  - The overlapping polar microtubules lengthen moving the poles of the cell further apart, and, in animal cells elongating the cell.
  - Since sister chromatids are identical, each of the two clusters of chromosomes being pulled to the two poles of the cell has one copy of each original chromosome. As the chromosomes are pulled toward the poles, they begin to lengthen out.

Anaphase

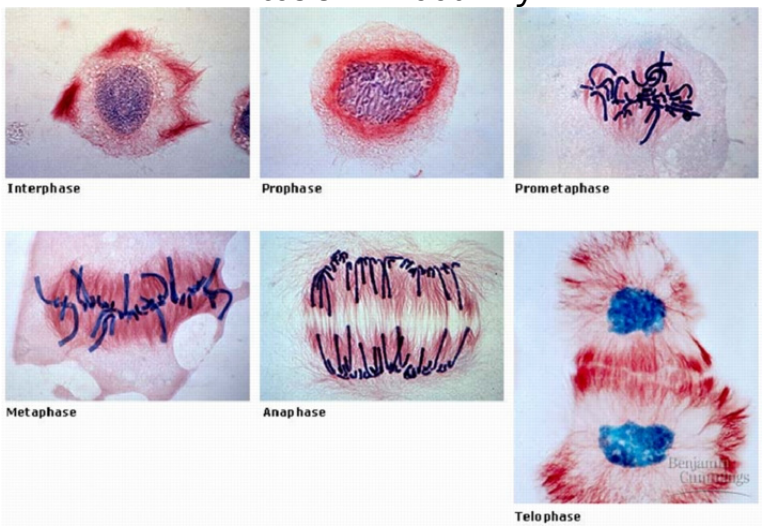
Telophase

- The spindle microtubules disperse and the spindle apparatus disappears.
- Chromosomes stretch back out and become indistinct as chromatin.
- Membrane vesicles form new nuclear membranes around each group of chromosomes (at the two poles).
- Each new nucleus has chromosomes identical to the original cell and the same number of chromosomes as the original cell.

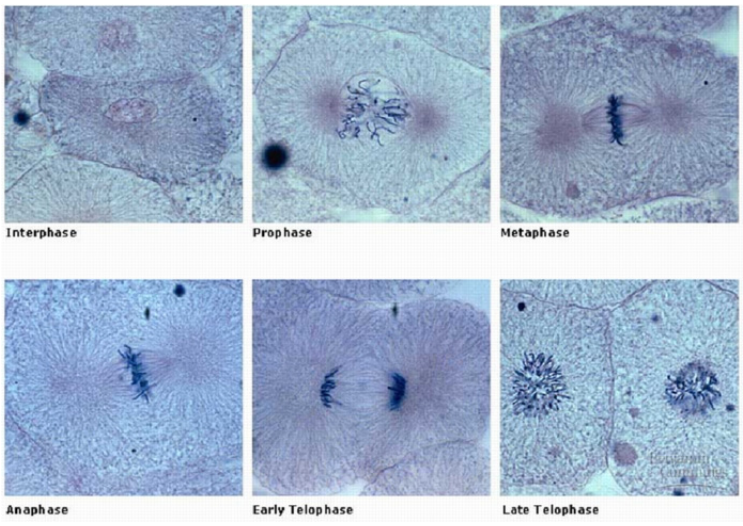


Telophase

Mitosis in Blood Lily



Mitosis in Whitefish Blastula



Cytokinesis: Separation of the Cytoplasmic Contents

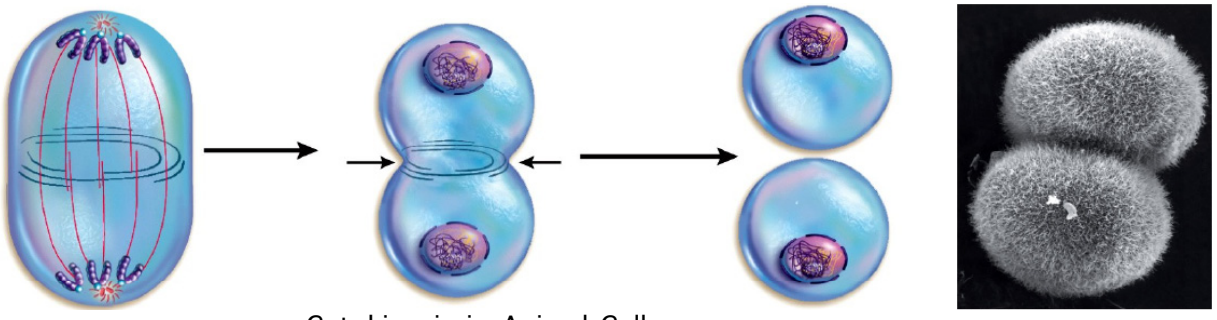
Mitosis describes events of chromosomes and nuclei. Most cells accompany mitosis with **cytokinesis**, the separation of the cytoplasm of the original cell into two new cells. This is not always the case. Some organisms (including many fungi and algae) are "multinucleate", they just have one cell body with many nuclei. Some animal tissues are also multinucleate.

Cytokinesis coincides with the events of telophase or occurs immediately after, so that at the completion of mitosis, the original cell is separated into two cells, each with a nucleus and DNA identical to that of the original cell. Although the end result of cytokinesis is always two new cells, the mechanism of separation is different in plants and animals, so we shall discuss them separately.

Cytokinesis in Animal Cells

The cells of animals lack cell walls. Cytokinesis in animal cells is started with the formation of a **cleavage furrow**, a depression or pinching in of the plasma membrane.

This is caused by a ring of microfilaments (the **contractile ring**), composed of the protein, actin, which forms across the middle of the cell after the chromatids are separated in anaphase. This ring contracts, pinching the membrane toward the center of the cell, which eventually pinches the cell in two. The additional membrane surface needed is supplied by membrane made during interphase.



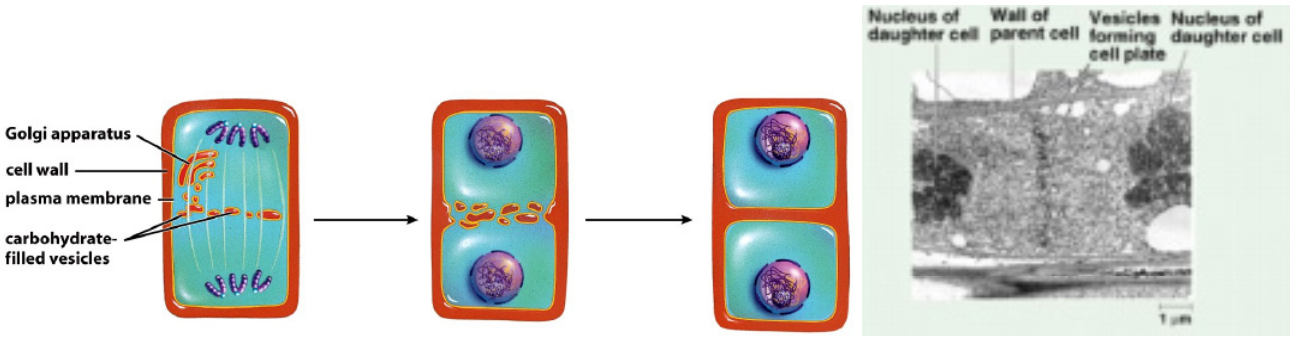
Cytokinesis in Animal Cells

Cytokinesis in Plant Cells

Each cell of a plant is surrounded by a rigid cell wall. During cytokinesis, new wall material must be synthesized along with plasma membrane. The formation of the new cell walls is called **cell plate formation**.

Cell plate formation involves making a cross wall at the equator of the original cell. Golgi vesicles containing wall material fuse along microtubules forming a disk-like structure called the **phragmoplast** or **cell plate**. As cellulose and other fibers are deposited, the cell plate is formed creating a boundary and new cell wall between the two new cells.

Membrane material from the original cell fuses to each side of the cell plate forming new cell membranes on the dividing sides of the original cell.

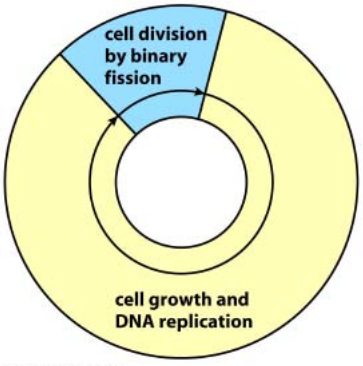


Cytokinesis in Plant Cells

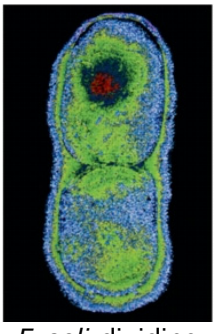
Binary Fission – Cell Division in Prokaryotes

The process of cell division in prokaryotes, is called **binary fission**. Bacteria have just one long continuous, or circular molecule of DNA. That, and the absence of a nucleus in the prokaryotic cell, account for a number of differences in the "mechanics" of the process. Special proteins attached to the DNA molecule facilitate the folding needed for the prokaryote's DNA molecule to fit within the cytoplasm of the cell.

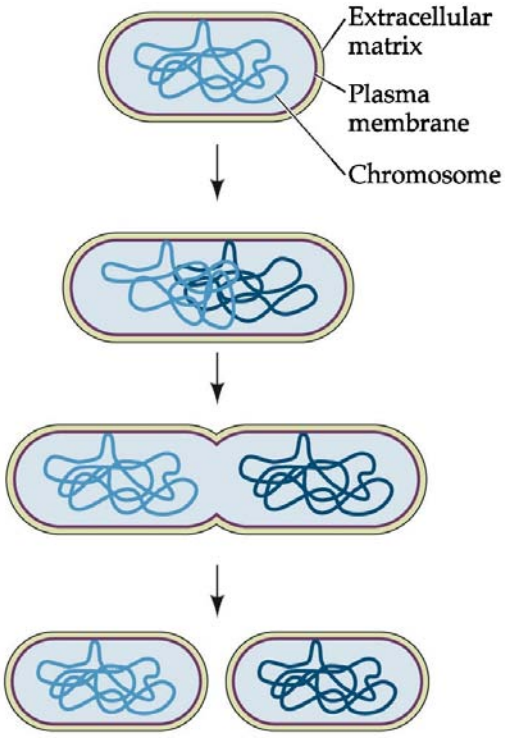
At the beginning of cell division, the single DNA molecule attaches to the plasma membrane at a site on the chromosome identified as the origin. The DNA is duplicated using a complex of enzymes (very similar to that of eukaryotes). While the DNA is being duplicated, the cell elongates by synthesizing new membrane and wall material between the two origin regions, separating the two DNA molecules. After a period of elongation plasma membrane is pulled inward, pinching off the two halves of the original cell. New cell wall material is also synthesized.



Prokaryote Cell Cycle



E coli dividing

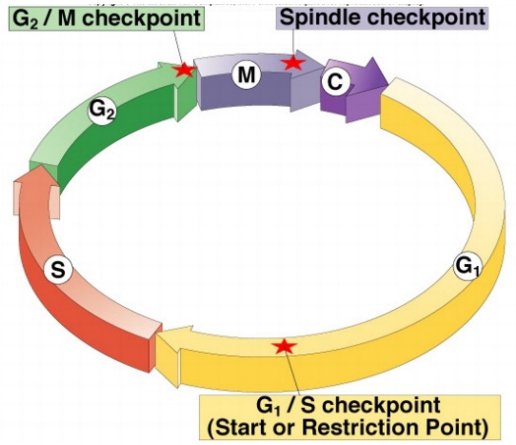


Binary Fission

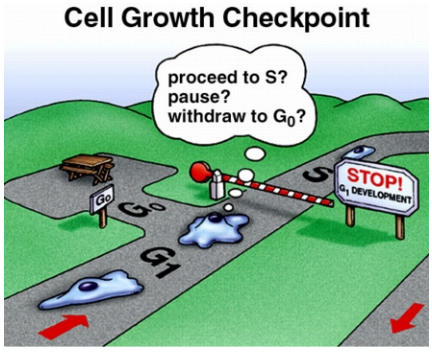
Controlling the Cell Cycle

The control of cell division is one of the most active areas of biological research, in part because cancers are diseases that involve cells that have lost their cell division controls. Some cells divide frequently throughout the lifespan of a multicellular organism, and some rarely, or not at all. Others divide only when damage occurs to the tissue. Cell division is regulated by a series of chemical signals that are an important part of current molecular biology research.

In the 1970's, researchers learned that there are definite **cell-cycle controls** that direct and coordinate the events of the cell cycle. These controls are known as checkpoints. The animal cell cycle has at least three "checkpoints" (in G_1, G_2 and mid-mitosis) where the cell cycle remains in that stage until over-ridden by chemical signals.

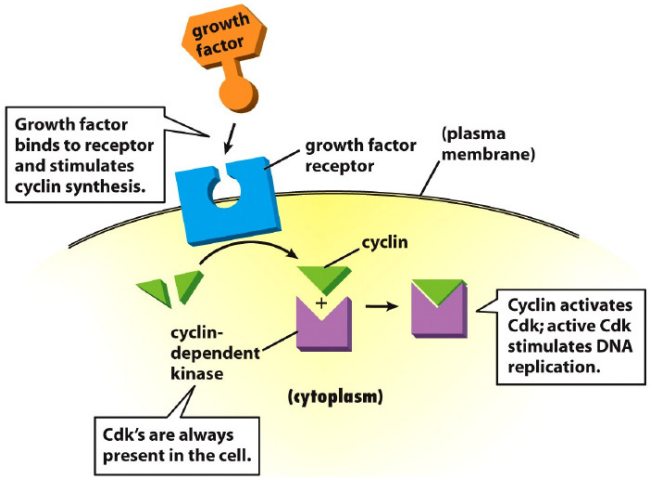


- The first checkpoint, called the **G_1/S** checkpoint, is in G_1 and determines whether DNA replication should proceed. Cells that never leave G_1 are said to be in a non-dividing cycle called G_0. Cells will stay in G_1 until they receive a signal to proceed with DNA duplication.



- The second checkpoint, **G_2/M**, is in G_2 just prior to mitosis and determines if mitosis will begin by ensuring that DNA duplication has been correctly done.
- The third checkpoint, the **spindle** or **APC** (anaphase-promoting complex) checkpoint, occurs in metaphase and ensures that chromosomes are properly aligned on the cell's equator.

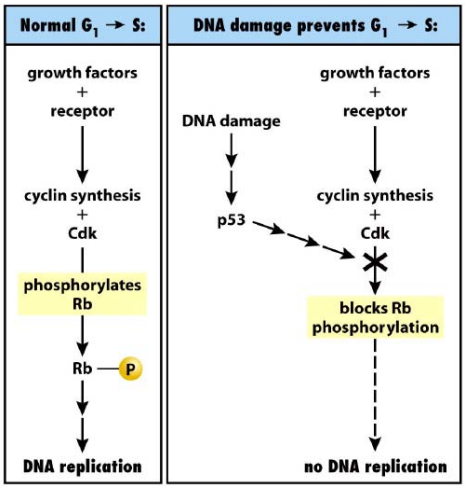
The G_1 and G_2 checkpoint signals involve a set of enzymes, called **cyclin-dependent kinases (Cdks)**, because they are activated by proteins called **cyclins**, whose concentration is cyclical (hence cyclin). Cyclin is a cyclin-dependent kinase allosteric regulator molecule. Cyclin concentration rises and falls during the cell cycle in response to growth factor signal molecules. When levels are high, cyclins combine with the cyclin-dependent kinases (Cdks) forming a complex **(cyclin-Cdk)**.



When activated by their regulator cyclin, the cyclin-dependent kinases phosphorylate specific proteins needed to do mitosis. The phosphorylated proteins change shape to initiate important steps in the cell cycle.

In addition to the checkpoint controls of cell division, the cell has a variety of controls that monitor the processes and regulate checkpoint activities. Some of these regulators are critical in cancer formation, and are the subject of much research.

For example, Rb is one of the phosphorylated proteins, and functions in the G_1 checkpoint promoting DNA synthesis. P53 is a protein that monitors DNA for damage, and when DNA damage is found, normally blocks the activity of Rb and initiates DNA repair activity. If DNA cannot be repaired, P53 initiates apoptosis, a means of cell death. Both defective Rb and defective P53 genes are associated with cancers.



When and Where does Mitosis Occur? Cell Division in Perspective

Growth

All growth (increase in numbers of cells) in individual organisms takes place by mitosis, from the fertilized egg (zygote) to death.

Replacement

Many cells are routinely replaced in organisms. This replacement of cells is done by mitosis. For examples, we replace the cells that line our digestive tract every one to three days.

Repair and Maintenance

Mitosis is used for repair and replacement of damaged cells or tissues, whenever possible. This includes regeneration of lost parts for some organisms.

Non-Sexual (Asexual) Reproduction

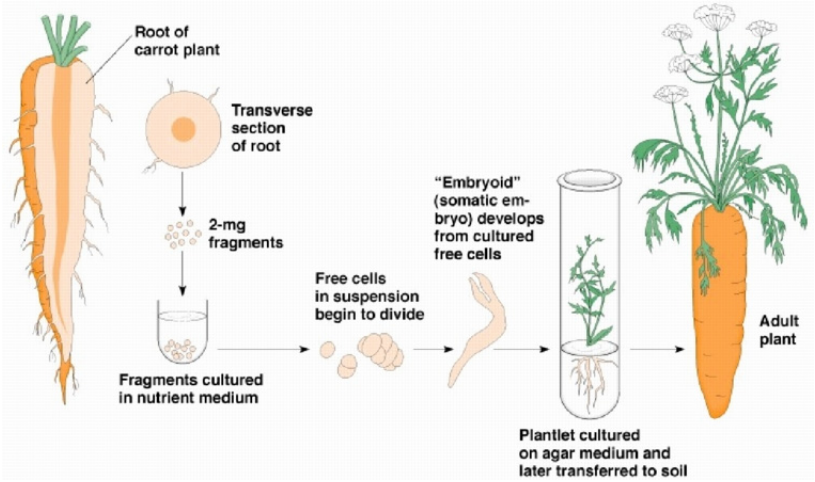
Mitosis is used for all asexual reproduction or **propagation**. This is especially common in plants, fungi and protists. Animals less commonly reproduce asexually. There are many claims for the world's largest organism based on the ability to make more. Asexual reproduction produces offspring genetically identical to the original parent, as would be expected of any mitosis.

Asexual Reproduction in Yeast, Protist, Hydra (an Animal) and Aspen Groves

Cloning, a method of producing genetically identical offspring, uses mitosis, precisely because mitosis duplicates the DNA exactly. Cloning is quite easy to do with many plants; they are easily propagated non-sexually anyway. Many of the agricultural products originate from cloned individuals, such as navel oranges.

Many cells of plants retain the ability to "dedifferentiate" and become embryonic-like. The DNA of their cells remains totipotent (meaning none of the DNA has been "turned off" or inaccessible). Most animal cells, once specialized (or differentiated), cannot do so.

In the 1950's carrots were successfully cultured at Cornell University from single cells taken from the root. This was the first laboratory "clone". Cloning plants turned out to be fairly easy – provide the totipotent cell with the right mix of hormones, nutrients and a growth medium and genetically identical plants grow. It was so successful that in the 1970's one could buy test-tube plants to share with one's friends as a novelty.

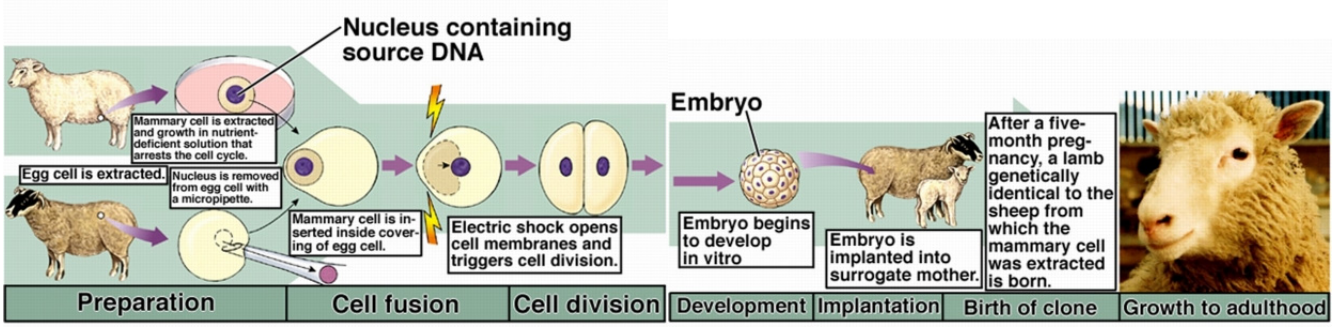


Cloning animals has not been so easy. In contrast to plant cells, the DNA in animal tissues undergoes "permanent" changes during development so that taking one cell and treating it with hormones and nutrients does not result in the cell developing into a new organism.

What passes for cloning in animals involves donor enucleated egg cells and a donor diploid nucleus. This was first accomplished in the 1950's with frogs, but there was little (no) success with mammals for decades.

In 1996, Ian Wilmut cloned a sheep using the nucleus from an altered mammary cell of an adult sheet implanted into an egg cell from which the nucleus was removed. They induced the nuclei of the mammary cells to dedifferentiate by growing isolated mammary cells in a nutrient-poor medium that forced the cells into a G_0 growth phase. Once the diploid egg cell divided and started developing, the embryos were transplanted into a surrogate "mother" and one survived, the now famous Dolly. Since then many mammals have been "cloned" using this technique, and it has been announced that cloned human embryos had been formed. No human clones have been presented to the public, and such research is currently not allowed in the United States.

The success rate of cloning is low. Both donor cell and donor nucleus undergoes trauma, and implantation has additional risks. Newer techniques cause less trauma to the donor nucleus and using a mixture of chemicals from rapidly dividing cells promotes more stable DNA in the donor nucleus. This technique is called **chromatin transfer**.



"Cloning" Dolly

We have just finished looking at the process of mitosis, a process that produces cells genetically identical to the original cell. Mitosis ensures that each cell of an organism has the same DNA as the original fertilized egg or zygote.

Passing chromosomes and genetic information from generation to generation is equally important. A critical role of heredity is to maintain and obtain variation among members of a species. These variations are the result of the specific genes we inherit from our parents through sexual reproduction.

However, chromosome number doesn't double with each generation. Each generation of a species has the same chromosome number as the preceding generations. **Meiosis** is the process that ensures that each new generation has the same chromosome number as the preceding generation.

Meiosis is a process that reduces chromosome number by half and occurs at just one stage in an organism's life cycle (to form gametes in animals, or to start the gamete producing stage in plants, or for some organisms to restore the appropriate chromosome number for the assimilative stage of its life history). **Sexual reproduction** or more properly, **fertilization,** then restores the "typical" number of chromosomes for the next generation.

Meiosis reduces chromosome number by half forming **haploid** nuclei, which have one of each of the homologous chromosomes *(discussed earlier)*. Each haploid cell after meiosis has half as many chromosomes as the diploid cells of the organism. "Ploid" as a general term means a "set", so we can also say that a diploid cell has two sets of chromosomes, or two of each kind of chromosome. A haploid cell has no pairs of chromosomes, just one of each kind. (It's possible to have more than 2 chromosomes of each kind. Polyploids are quite common in agriculture as a result of plant breeding. Polyploids are less common in animals.)

In sexual reproduction, cells from two individuals fuse to form a nucleus with a combination of chromosomes and DNA from each parental cell. The new cell (called a zygote) has a unique set of chromosomes, different from each parent, having obtained one of each homologous chromosome from each parental cell. Without sexual reproduction, the opportunity to express the variations found within the genes of species would be greatly reduced.

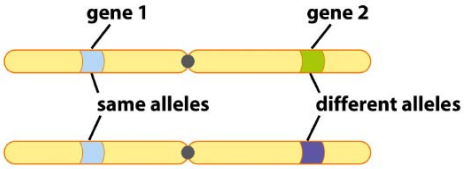
However, despite the fusion of cells from two individuals, chromosome number doesn't double with each generation. Each generation of a species has the same chromosome number as the preceding generations. **Meiosis** is the process that ensures that each new generation of sexually reproducing organisms has the same chromosome number as the preceding generation.

Meiosis and Genetic Variation

Meiosis is necessary to ensure that each new generation has the same chromosome number as the preceding generation, but meiosis has a second function for living organisms: maintaining **genetic variation**. Each time meiosis occurs, followed by, at some point, sexual reproduction, the new individual formed is genetically different from either parent. Because meiosis is involved with genetic variation and is needed for sexual reproduction, we need to mention a few things about genetic inheritance and sexual reproduction to better understand why meiosis is so important.

Gene variations are found on the homologous chromosomes of diploid organisms. As discussed, homologous chromosomes are physically similar and have equivalent genetic information, the information carried on our **genes**. But the genetic information on each homolog may differ in specifics, a primary source of variation among the individuals within a species.

Through **mutation**, the original source of change in the DNA, the DNA on the homologs may be different. As mentioned earlier, specifics alternative forms of genes are called **alleles**. Homologous chromosomes may have identical alleles or different alleles for the gene.



Each parent typically has two "genes" (or more correctly, two alleles of a gene) for each genetic characteristic (one on each of the homologous chromosomes). Each parent passes one of each of its homologous chromosomes (with its particular alleles) to the offspring by meiosis and fertilization. The fertilized egg (**zygote**) will then have two genes (alleles) for each trait, one from each parent. It's important to note that each individual will have a **paternal** set of chromosomes and a **maternal** set of chromosomes. Each homologous pair of chromosomes has one paternal and one maternal origin.

Since parents are not genetically identical, their haploid gametes will have different combinations of genes. Each egg and each sperm (or each spore) is genetically different from the parent's DNA (having only half as much).

In addition, during **meiosis** there is some shifting and recombining of alleles so that gene combinations always occur in the gametes that are different from the parent.

At fertilization, two haploid gametes fuse, each containing one of each type of homologous chromosome, which restores the diploid number of chromosomes. The zygote will now have homologous chromosomes, but one from each of the parents. Since each gamete has a unique combination of chromosomes, each zygote will be unique.

The offspring (children) formed by sexual reproduction will have genetic variation, important for the long-term response of species to their environment. Such variations among offspring lead to physical, behavioral and physiological differences. These differences may be more or less useful in the surroundings of that organism, and are subject to the agents of selection. This variation is an important basis for evolutionary change, which will be discussed later.

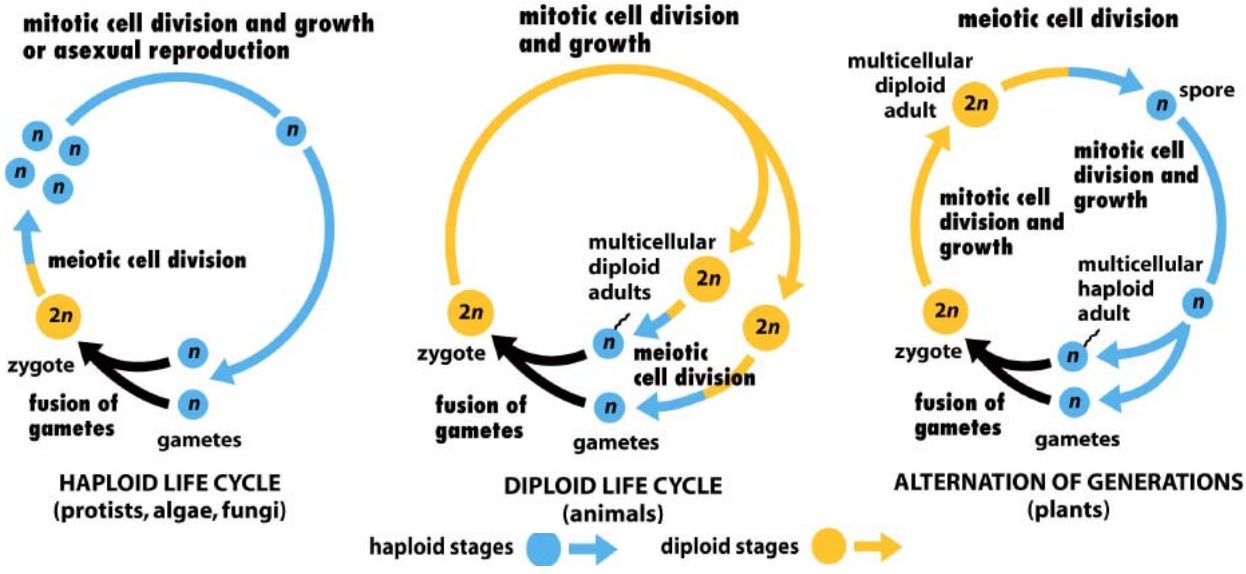
Meiosis and the Life Cycles of Organisms

Meiosis is something that takes place at just one point in any sexually reproducing organism's life cycle. In animals, meiosis generally occurs to form gametes: sperm or eggs. In many other types of organisms, meiosis occurs at some different point in the life cycle, and the products of meiosis may be spores, (as in plants) or the first cells of the next generation (for most protists and most fungi). At some point however, all organisms that sexually reproduce will make haploid gametes. Although we are familiar with, and comfortable with the animal life cycle, it's good to know how different organisms fit meiosis into their life cycles.

Similarly, **fertilization** occurs at one point in an organism's life history. Fertilization occurs between two different haploid cells, called gametes, to form the **zygote**, or fertilized egg. The zygote obtains half its chromosomes from the sperm and half from the egg or from different gametes, the more general term.

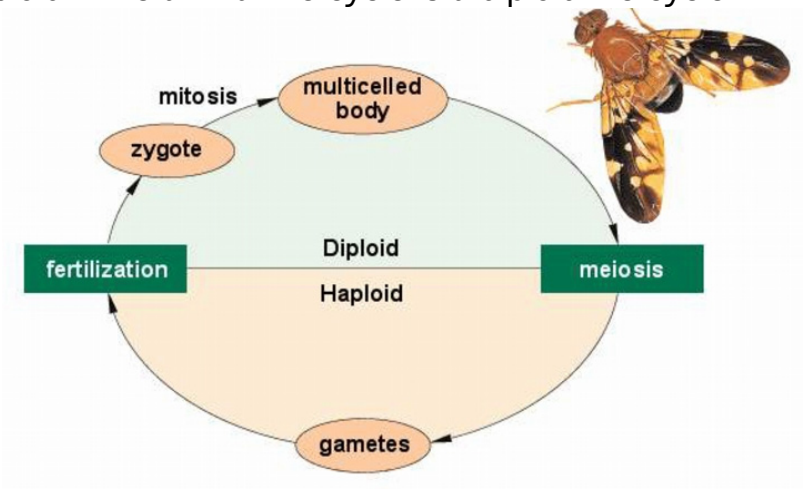
Fusion of gametes restores the diploid number, and in so doing, also restores homologous chromosomes (one of each kind being provided by the sperm and one of each kind coming from the egg). Since each gamete has a unique combination of chromosomes, each zygote will be unique, and genetic variation is both maintained and obtained within the species.

We will now discuss typical life cycle patterns and look at the timing of meiosis in the life cycle.



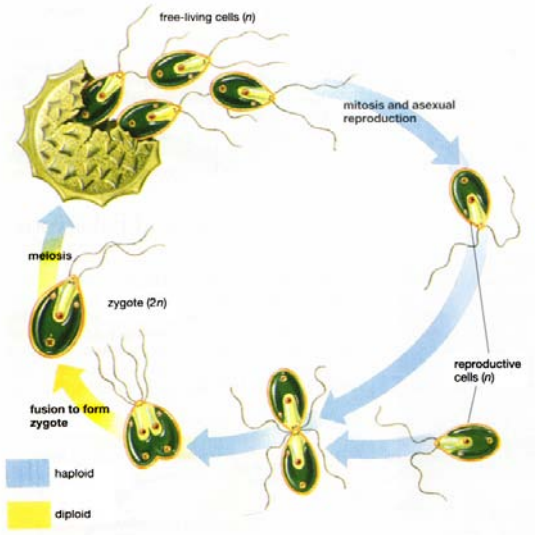
Diploid Life Cycle

- In animals, meiosis generally produces just haploid sex cells, which at fertilization start the next generation. The only haploid cells of the animal are egg or sperm, and the respective maturation processes are called oogenesis and spermatogenesis.
- When gametes fuse, the zygote grows by mitosis producing the adult stage. All cells will be diploid. The animal life cycle is a diploid life cycle.



Haploid Life Cycle

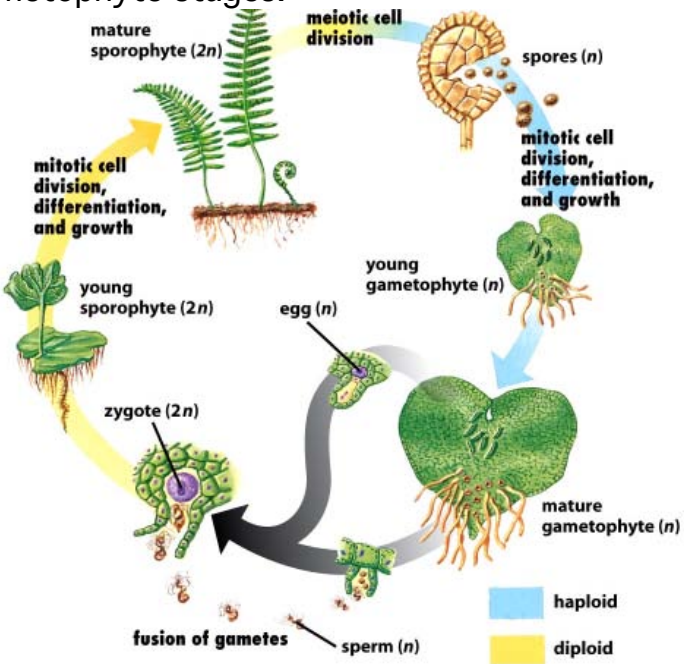
- In many protists, and some fungi, haploid gametes fuse to form a zygote, but the zygote immediately does meiosis forming single haploid cells.
- In protists, which remain single cell organisms, the nucleus is then haploid.
- At some time, the single cells may just decide to become gametes and fuse with another to make a zygote, or haploid cells may do mitosis to make more individuals asexually.
- Haploid life cycles can be more complex. Fungi, and some algae may make multicellular haploid organisms from single-celled meiotic product, by mitosis. At some time, special areas of the haploid body will become gamete-making structures, and haploid gametes are formed by mitosis.



Alternation of Generations

- Most plants have both a multicellular haploid stage and a multicellular diploid stage in their life histories. This is called the alternation of generations.
- In plants, the structure in which meiosis occurs is called a **sporangium.**
- Meiosis does not directly produce gametes, but produces haploid cells, called **spores** that in turn, grow, by mitosis, into multicellular haploid structures called **gametophytes** (gamete-making plant). Gametophytes eventually produce and contain gametes. (The multicellular diploid structures that produce sporangia are called **sporophytes**.)

Which stage (sporophyte or gametophyte) is predominant in the life of a plant varies with different types of plants. Most "higher" plants have predominant sporophytes. A pollen grain of pine tree or flower, for example is the male gametophyte stage of those plants. In ferns, both gametophyte and sporophyte are photosynthetic, but the gametophyte is short-lived and very small. Mosses, in contrast, have predominant gametophyte stages.



Alternation of Generations in Plants

Fern Sporangia

Fern Gametophyte

Non-Sexual Reproduction

As discussed, many organisms have both sexual and non-sexual means of increasing the numbers of individuals. Asexual reproduction can be a good strategy in an environment that is consistent, if a species is well suited to those conditions. Without sexual reproduction, however, there is no genetic variation to adapt to change.

The Process of Meiosis

Homologous chromosome pairs are essential to how meiosis works. In meiosis the homologous chromosomes literally pair up prior to the reduction of chromosome number. In meiosis, one of each type of chromosome (one of each homologous pair) is distributed to each meiotic product, so that the meiotic products have half as many chromosomes as the "parent" cell. This is the crucial difference between mitosis and meiosis, and explains why we can reduce chromosome number and still have all of the genetic information needed to form a new organism. **The homologous pairs of chromosomes in diploid organisms do not interact during mitosis; each chromosome is on its own.**

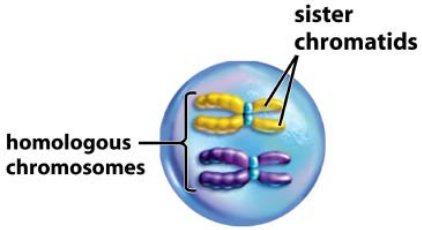
After meiosis, the meiotic products have a **haploid** (half the parental) number of chromosomes, and **no pairs of homologous chromosomes**. Haploid also refers to the cell when there is just one of each kind of chromosome, or the "n" number of chromosomes. (Diploid is the 2n number of chromosomes.)

The diploid number of chromosomes will be restored when two gametes (egg and sperm) unite in sexual fertilization.

The Process of Meiosis - Details

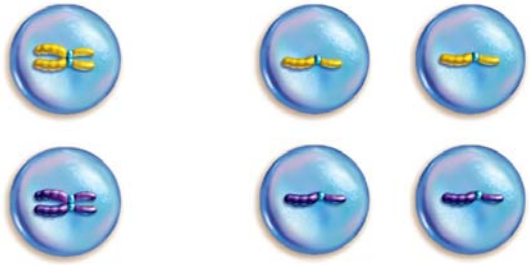
There are three important parts to meiosis:

- Prior to any cell division, chromosomes must undergo DNA duplication.



To achieve the reduction in chromosome number and appropriate distribution of chromosomes, meiosis requires **two divisions**, called **Meiosis I** and **Meiosis II**.

- Pairing of and separating of the homologous chromosomes occurs in **Meiosis I**, reducing the chromosome number.



Meiosis I Meiosis II

- Meiosis II separates the duplicated chromosomes. At the completion of the second division, four cells will typically be produced.

Some Notes:

- The specifics of the meiotic divisions resemble those of mitosis; the differences occur in the matching or pairing of the homologous chromosomes, which occurs during the first division prophase.
- "Sister" Chromatids are not pairs; they are the two identical parts of one duplicated chromosome. You must make this distinction to understand how the process of meiosis works!
- A pair of duplicated homologous chromosomes will have a total of 4 chromatids, two chromatids for each homologue. It's just a fact that prior to any cell division, chromosomes duplicate, so meiosis starts with duplicated chromosomes.

The Meiosis Stages (Or how we break up a continuous process into chunks)

Pre-Meiotic Interphase

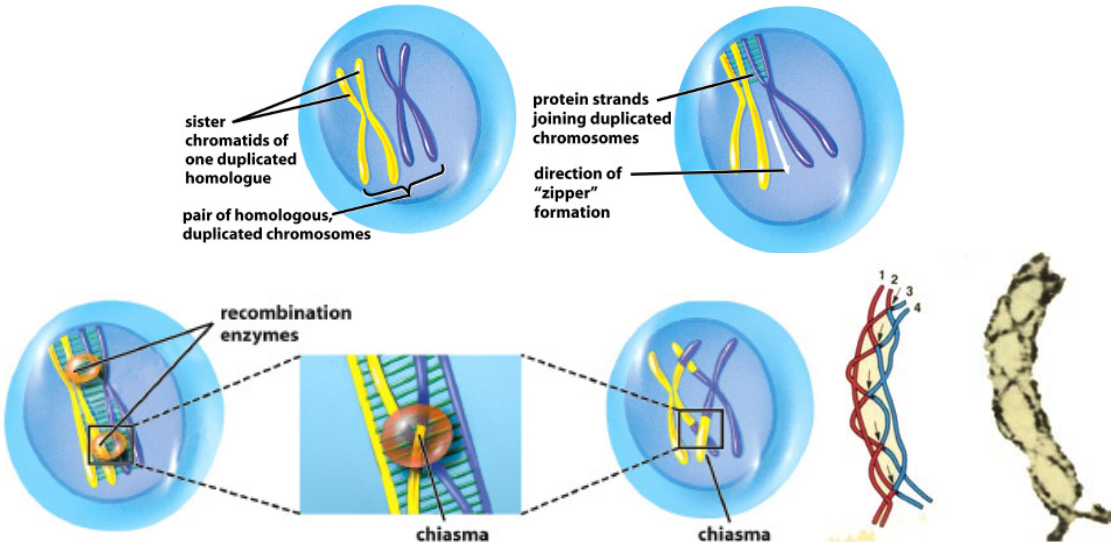
The DNA of the cell* that will do meiosis replicates. (DNA replication must precede any cell division.) The identical sister chromatids of each replicated chromosome are attached at their centromeres and have their kinetochores.

* Again, cells that do meiosis are restricted to specialized structures, such as the sex organs (ovary and testis of animals), sporangia of "lower" plants or anther and ovule (modified sporangia) of "higher" plants. These structures are diploid, just as is the rest of the organism. The products of meiosis (gametes in animals, or the multicellular gamete-producing structures and gametes in plants) are haploid. For organisms that have a haploid life cycle, meiosis immediately follows the formation of the zygote. The zygote is the meiotic structure. The haploid cells produced from meiosis are the first cells of the next generation, which in many protists is the single cell. Multicellular haploid organisms grow by mitosis to become adults, just as diploid organisms do.

Meiosis I

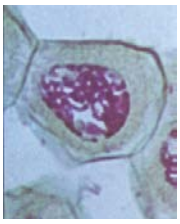
Prophase I

- Homologous chromosomes pair up at the start of prophase I in a process called **synapsis**. This uses proteins along the chromosomes to join the homologues together. The homologues literally join at several points (called **chiasmata**). All four chromatids of the homologous pair are aligned together.
- This arrangement allows for a process of genetic importance to occur. The intertwined chromatids of the homologous chromosomes break at one or more places and exchange equivalent bits of DNA with each other. This exchange is called **crossing over.** This occurs between the non-sister chromatids, and is mediated by enzymes. If the alleles of the homologues were different forms of the genes, than **recombination** occurs. The sister chromatids now have some genetic variation; they are no longer identical.

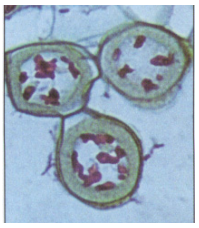


Crossing Over

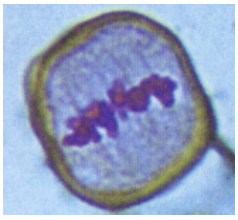
- After crossing over takes place, the still-joined homologues pull apart slightly, although the chromosomes are still attached at the chiasmata.
- All things that we normally think of taking place in a prophase also occur in prophase I of meiosis, including attaching spindle fibers to each chromosome of the attached homologous pairs.



Early Prophase I



Synapsed Homologous Pairs



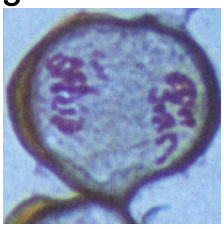
Metaphase I

Metaphase I

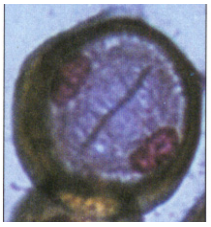
- Synapsed homologous pairs of chromosomes are moved to the equator by the spindle complex.
- The alignment is random; some maternal chromosomes will orient facing one pole along the equator; others face the opposite pole. The random alignment of homologous pairs of chromosomes at metaphase increases genetic variation.

Anaphase I

- The **homologous chromosomes are separated from each other** and pulled toward opposite poles during Anaphase I.
- Replicated chromosomes are not affected during Anaphase I. The sister chromatids are still tightly bound to each other by their centromeres.
- The **chromosome number is officially reduced** at this time because each nucleus that will form at Telophase I will have half the number of chromosomes as the pre-meiotic cell. All of the chromosomes will be replicated. No sister chromatids have separated.
- No homologous chromosome pairs are present at the end of Anaphase I. Each cluster of chromosomes at the respective poles of the cell will have one of each type of homologous chromosome. **The key to reducing chromosome number while maintaining all of the genetic information is the pairing and separation of homologs.**



Anaphase I



Telophase I

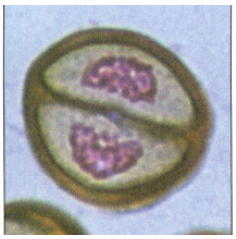
Telophase I and Interkinesis

- Typically two new nuclei are formed, each with one set of the homologous chromosomes
- Cytokinesis will form two cells. Each chromosome is still replicated (which occurred in pre-meiotic interphase), and essentially the cells are just preparing for the second division. Some cells do not bother with cytokinesis here, or even form new nuclear envelopes, which will just have to be degraded during meiosis II, anyway.

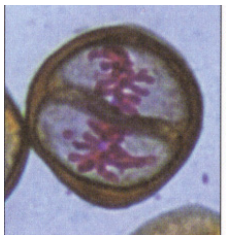
Meiosis II

Prophase II

- New spindle apparatus is formed in each of the two cells from telophase I.
- The still-replicated chromosomes stretch out and recondense.
- Spindle fibers attach to the kinetochores of each of the sister chromatids, one from each pole.
- Nuclear membranes degrade.



Prophase II



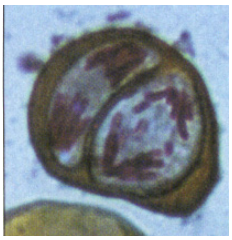
Metaphase II

Metaphase II

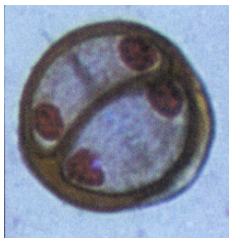
- The replicated chromosomes are aligned along the equator by the spindle complex.

Anaphase II

- Centromeres of sister chromatids are detached from each other.
- The now non-replicated chromosomes are pulled to the poles of the cells.



Anaphase II



Telophase II

Telophase II and Cytokinesis

- Each new nucleus formed has half the number of the original chromosomes but each nucleus has one of each type of homologous chromosome.
- A total of four new cells will be produced.

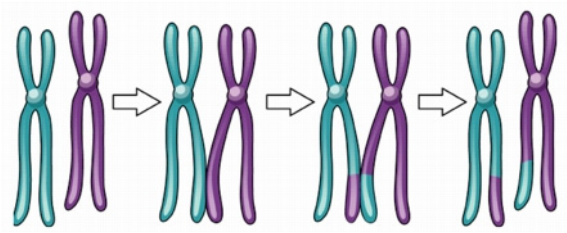
Review of Genetic Importance of Meiosis

To conclude our discussion of meiosis, and to initiate further discussion of genetics and inheritance let us recall that the genetic traits we inherit are in the form of gene alternatives, called alleles. These alleles are located on the homologous chromosomes.

The zygote receives different parental combinations of homologs from the egg and sperm. How these alternatives get expressed is the study of inheritance.

Meiosis plays the following roles in inheritance:

- Recombination or crossing-over between non-sister chromatids during synapsis (Prophase I) produces gametes with greater variation.



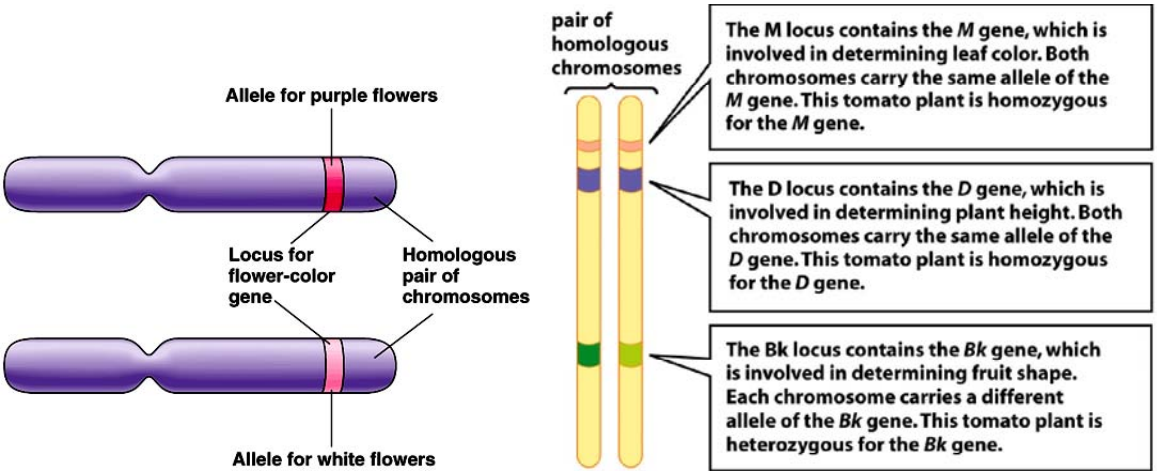
- The independent assortment (or alignment) of maternal and paternal chromosomes at Metaphase I when homologous chromosomes are lined up on the cell equator results in greater genetic variation. For example, with just three pairs of homologous chromosomes, you can have eight different haploid combinations.

- The fusion of genetically different gametes results in new genetic combinations for each generation.
- Meiosis provides a mechanism to preserve chromosome number from generation to generation.

The process by which our genetic information is passed from one generation to the next, or from parent to offspring, is the study of **inheritance**, or transmission genetics. A **gene** is a region of a chromosome (piece of DNA) that contains a set of instructions for synthesizing a protein (polypeptide). These instructions are passed from cell to cell and from generation to generation as inheritable traits or characteristics. Within an organism, mitosis ensures that each cell formed has the precise set of genetic information of its chromosomes. Meiosis and sexual reproduction are the mechanisms used to pass the genetic instructions from parent to offspring.

At this time we will look at how genes are transmitted from generation to generation and how **alleles**, the alternative forms or variations of genes, interact and are expressed in individuals.

As we have learned, the chromosomes of diploid organisms come in pairs, the **homologous chromosomes**, so that each cell of a diploid organism typically has two alleles for each gene (inheritable trait), one on each of the homologous chromosome pairs. The precise location where a gene is found on a chromosome is known as the **locus**.



Three genes of a tomato chromosome

A haploid cell or gamete has one allele of each "gene pair" (or one of each homologous chromosome pair) but not both. The diploid number of chromosomes with its homologous chromosome pairs is restored at fertilization, when two gametes fuse.

We did not always know that genes were located on chromosomes, or that inheritable traits, or genes, came in "pairs". **Gregor Mendel** in 1865 was the first to state that inheritable traits (genes) came in pairs. His work went unappreciated for several decades because no one seemed to understand what it meant. In the early 1900's other researchers independently made the same conclusions about inheritance and Mendel's work was "rediscovered". Soon after, Walter Sutton showed that Mendel's principles of inheritance applied to homologous chromosomes and that chromosomes are the units of heredity.

Prior to Mendel, the subject of inheritance was mostly guesswork. Although the practice of selective animal and plant breeding was well established, virtually nothing was known of the mechanisms of inheritance beyond the presence of an egg and a sperm in animals, and pollen and carpel in plants.

It was generally believed at that time that characteristics of parents were "blended" in offspring since offspring generally had features of both parents. No one went so far as to question why, after several generations, variations still were present, since differences over time should have been thoroughly blended.

The subject of variation among individuals and how different variants were passed on (or apparently not passed on) from generation to generation was very important to science in the 1800's. Mendel's work coincided with the publications of Darwin and Wallace, who addressed variation among the individuals of populations as the foundation for which selective agents could act through time, in the process of evolution. Prior to those publications many others had presented their thoughts on how characteristics were inherited, but none had sufficient evidence to support their ideas.

Gregor Mendel's Contribution to the Subject of Inheritance

Mendel observed how specific traits of the garden pea were transmitted from generation to generation. Mendel kept precise records of the thousands of offspring (and their characteristics) produced in his crosses. He then established mathematical probabilities and explanations to validate his observations.

Although others had studied inheritance, Mendel's educational experiences in math and observing plant variation helped him design and analyze his experiments carefully. Mendel:

- Chose a good organism that had a number of "true breeding" traits easy to observe.
- Designed the experiments carefully. Mendel took plants from true breeding parents (P generation), to first generation (F_1 hybrids), and then self-crossed the first generation offspring to form a second generation (F_2).
- Obtained large sample sizes for good data analysis

Trait	Dominant Form	Recessive Form
Seed shape	smooth	wrinkled
Seed color	yellow	green
Pod shape	inflated	constricted
Pod color	green	yellow
Flower color	purple	white

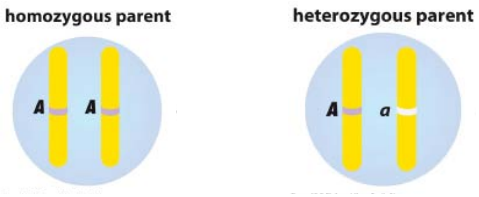
Trait	Dominant Form	Recessive Form
Flower location	at leaf junctions	at tips of branches
Plant size	tall (1.8 to 2 meters)	dwarf (0.2 to 0.4 meters)

Mendel's research led to the following conclusions, two of which are presented as Mendel's Principles:

Mendel's Statements about Inheritance

1. There are alternative forms (or variations) of genes, the "units" that determine inherited traits. The alternative forms of a gene are now called **alleles**. To relate this to what we know about homologous chromosomes, the alleles are located at the same locus on homologous chromosomes. (Specifically, we inherit the alleles for a gene, not the gene).

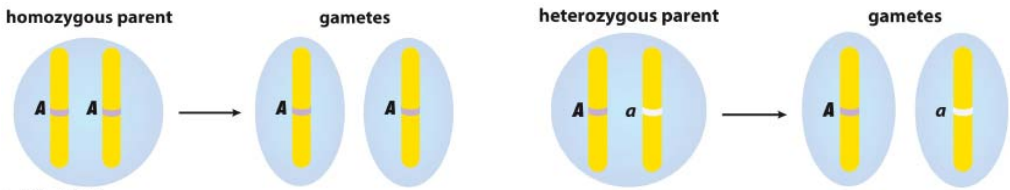
2. An individual will have 2 alleles for each inherited trait. The 2 alleles may be the same, or they may be different. If the two alleles are the same, the individual will be **homozygous** for that trait. If the two alleles are different, the individual will be **heterozygous** for the trait.



When the two alleles for a gene pair are different from each other, one will be expressed, and the second will not affect the organism's appearance. The allele always expressed is said to be **dominant**, and the one that may not be expressed is **recessive**.

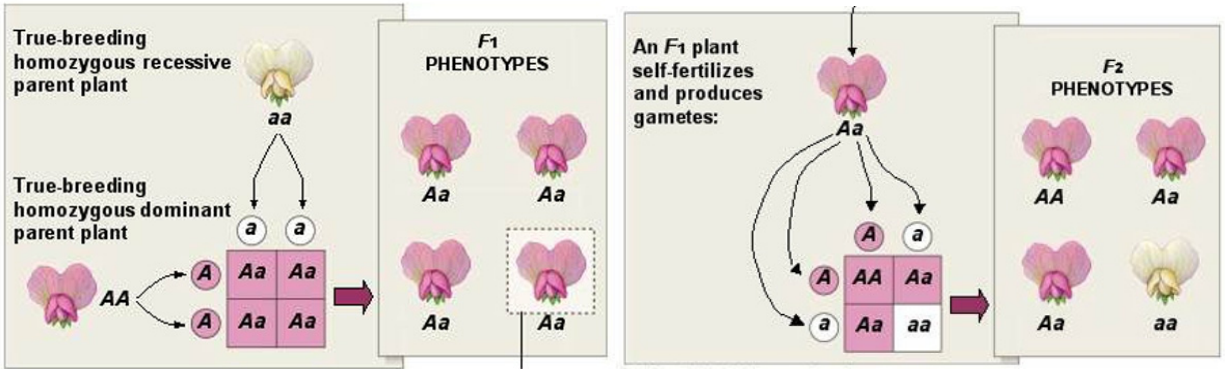
Note: These statements are true for the traits tested in Mendel's peas and for many genes, but are not universally true. Many genes have alleles that are equally expressed, as we shall see, and there are genes that have more than 2 alleles within the population.

3. Gametes have just one allele for each trait, because the allele (gene) pairs are separated (or segregated) during meiosis I when homologous chromosomes pair and then separate. 50% of the gametes receive one allele and 50% of the gametes receive the alternative allele when the alleles are heterozygous. (And as Mendel proposed, fertilization results in restoring the pairs of alleles for the next generation).

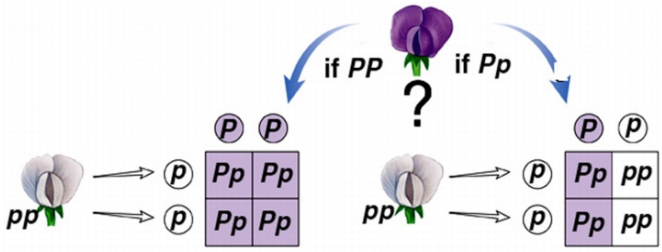


This statement ultimately resulted in Mendel's **Principle of Segregation:** Pairs of genes segregate during the formation of gametes (Meiosis), so that each gamete has one of each gene pair (one allele) but not both. Fertilization restores the gene pairs (on the homologous chromosomes).

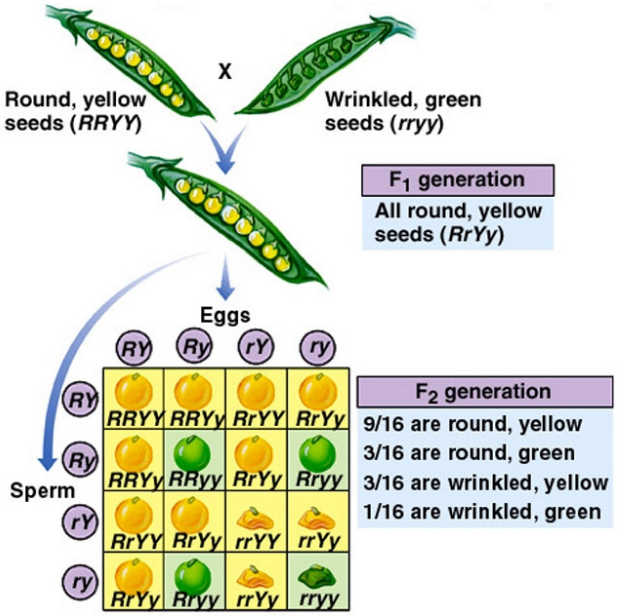
Mendel demonstrated his Principle of Segregation with many **monohybrid** (or single-trait) **crosses**, looking at one characteristic at a time.



Mendel could further validate his Principle of Segregation with the **test cross**, a cross in which the F_1 generation, which appeared dominant, was crossed to the recessive parent. Their offspring would exhibit equal proportions of both dominant and recessive forms.

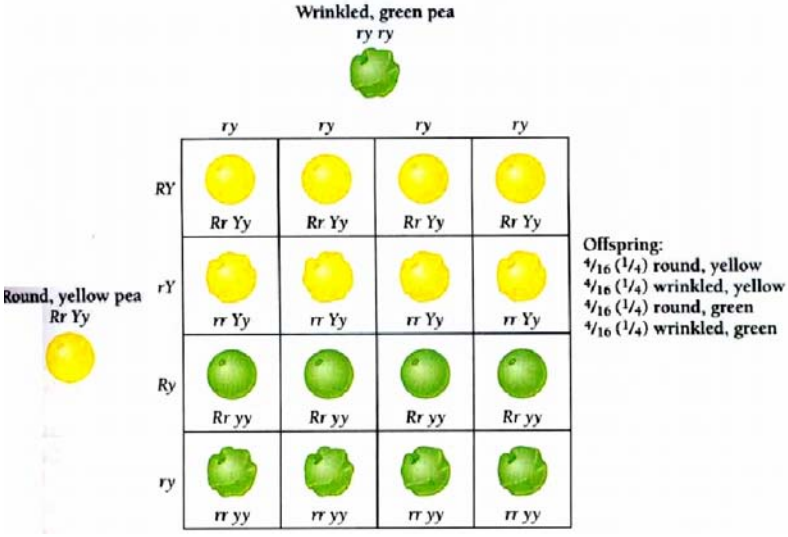


4. Mendel's experiments with crossing two traits at one time, a **dihybrid cross**, resulted in his **Principle of Independent Assortment.** Each gene pair is distributed (assorts) independently of other gene pairs into gametes during meiosis.



We observed this independent assortment during meiosis when the homologous pairs of chromosomes align along the equator at metaphase I. Maternal chromosomes of some pairs align towards one pole some of the time, and the other pole some of the time. Each meiosis event has a different alignment pattern.

Equal numbers of each of the four possible phenotypes result with a **dihybrid test cross**, which crosses an individual heterozygous for both traits with one that is homozygous recessive for both traits.



Dihybrid Test Cross

Beyond Mendel

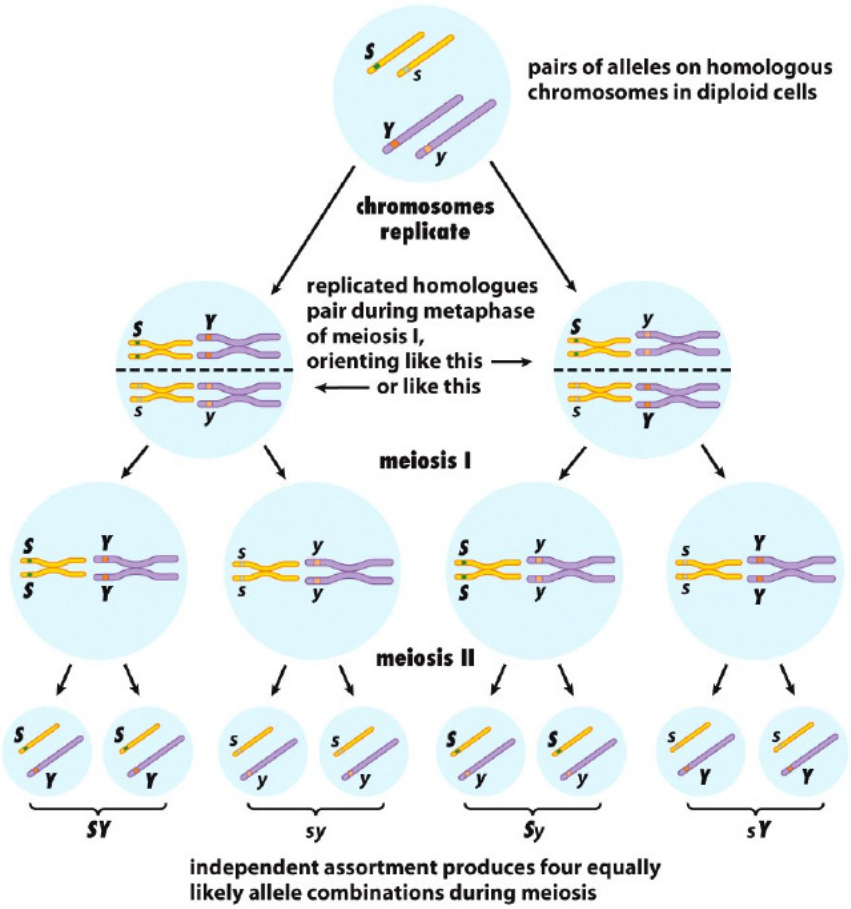
Mendel's research occurred before we had knowledge about chromosomes, molecular genetics, mitosis or meiosis. All of Mendel's genes had dominant and recessive forms, and each of his characteristics was found on different chromosomes. Early on, some inheritance patterns did not match the expectations proposed by Mendel's principles. We shall now turn our attention to some gene actions that go beyond the basic Mendelian predictions.

The Chromosome Theory of Inheritance

Gregor Mendel's work was "rediscovered" in 1900 by three independent geneticists who had done studies that came to the same conclusions that Mendel had made. They had the advantage that the processes of mitosis and meiosis were known explaining how genes could be separated. The next step was accomplished in 1902, when Sutton and Boveri correlated Mendel's conclusions about genes (or inherited traits) to the behavior of chromosomes during mitosis and meiosis. Sutton is credited with first proposing the chromosome theory of inheritance:

- Chromosomes are in pairs
- Homologous Chromosomes separate during meiosis so that alleles are segregated
- Meiotic products have one of each homologous chromosome but not both
- Fertilization restores the pairs of chromosomes

And -- Genes are located on chromosomes.



Chromosomes and Mendel's Laws

Chromosomes and Inheritance

We briefly mentioned the work of Sutton and Boveri who determined that Mendel's principles of inheritance applied to the behavior of homologous chromosomes during meiosis. But the inheritance patterns discussed to date have involved genes located on different chromosomes, so they have followed Mendel's Principle of Independent Assortment.

Each homologous chromosome pair assorts independently, not specific genes. Two genes that are located on the same chromosome will be inherited together, and not assort independently during meiosis.

Chromosomes and Gene Linkage

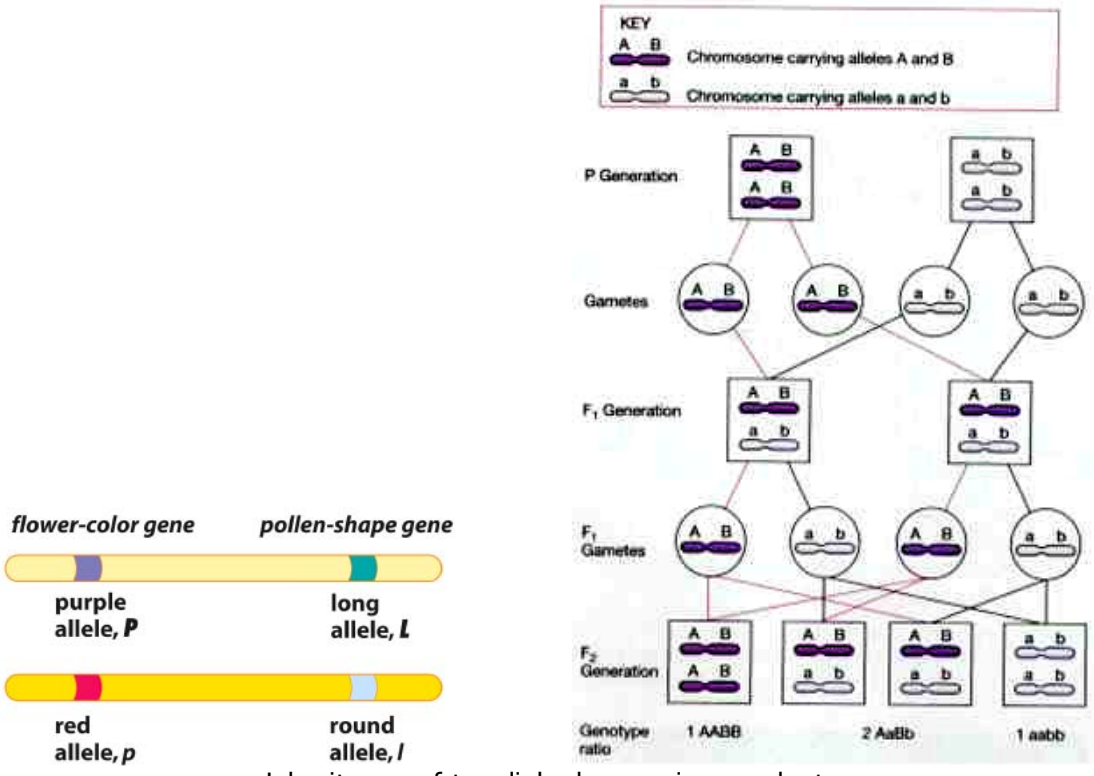
Soon after Sutton and Boveri's connected the behavior of chromosomes to Mendel's pattern of inheritance, it was important to change how we thought about Mendel's conclusions about independent assortment of genes. Although genes that are on different chromosomes indeed assort independently in meiosis, we know that each chromosome contains hundreds of genes and entire chromosomes, not individual genes, are transmitted by meiosis to the gametes. All of the genes located on one chromosome are inherited together – we inherit the whole chromosome not each gene. This is known as **gene linkage**.

This knowledge adds some interesting complications to the predicted patterns of inheritance and also explains why recombination, which we discussed with meiosis, is so important as a source of variation.

In 1908, researchers discovered a dihybrid cross in sweet peas that did not give the predicted Mendelian ratio of 9:3:3:1. They could not explain why their results were closer to 75% and 25% (the 3:1 ratio expected for a monohybrid cross).

Ultimately it was shown that the flower color and pollen length (the genes observed) were on the same chromosome.

Since we inherit entire chromosomes rather than independent genes, all genes on one chromosome are inherited together as a single unit (called the linkage group), and we should expect a 3:1 inheritance ratio for the linkage group. This was just the first time someone had seen this.



Inheritance of two linked genes in pea plant

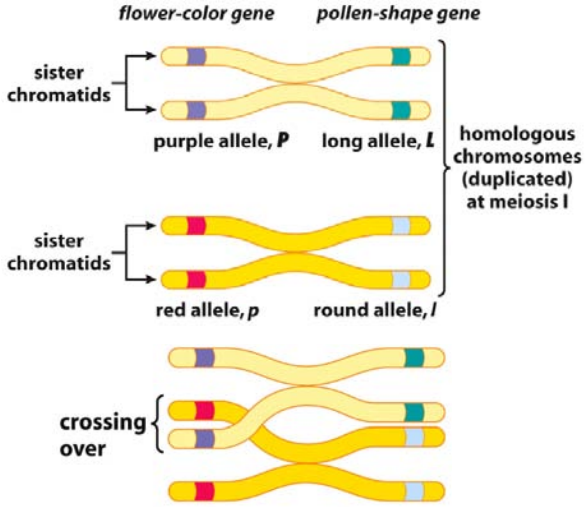
The Effect of Recombination on Gene Linkage and Inheritance

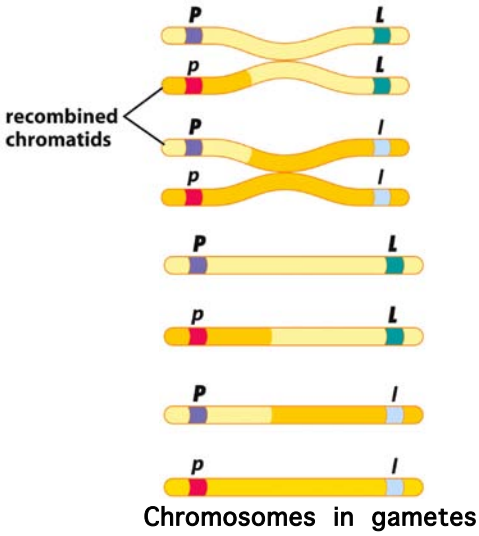
Researchers did numerous crosses of traits to identify gene linkage, and carefully noted percentages and ratios. However, some of the time, the results did show independent assortment of the "linked" genes, not enough to indicate the genes were indeed on separate chromosomes, but frequently enough to search for explanations.

It was known that a phenomenon called crossing over occurred during meiosis. The effect of crossing over on sister chromatids provided an explanation for the "recombined" allele forms in gene linkage experiments.

Crossing over during meiosis **results** in the exchange of bits and pieces of DNA between homologous pairs of chromosomes at the chiasmata during prophase I of **meiosis**. This process of **recombination** results in sister chromatids and gametes (or meiotic products) that are not identical; some of the linkage groups have been changed by the crossing-over. As a result of recombination, new allele combinations are formed, and we have more genetic variation.

Crossing Over and Recombination

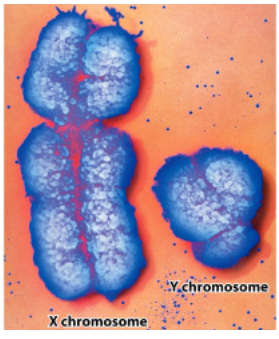


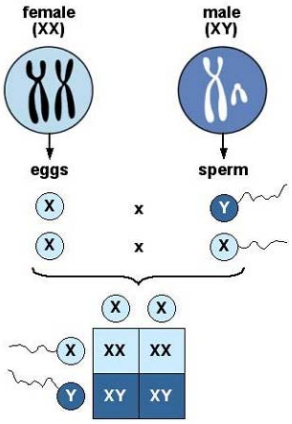


Sex-Determination and Sex-Linked Traits

One of the earliest discoveries about gene linkage related to another significant thing about chromosomes and species, especially animal species. By the early 1900's it was known that males and females of most species have one pair of "not-exactly-matching" homologous chromosomes, which determined the gender of the individual. These chromosomes were called the **sex chromosomes**. (The truly matching chromosomes are the **autosomes**.)

With the sex-determining chromosomes, one sex, usually female, will have two matching chromosomes (XX) and the other sex will have two unmatched chromosomes (XY). At meiosis, all eggs will contain an X chromosome, but half the sperm gametes will have a Y chromosome and the other half will have an X chromosome.





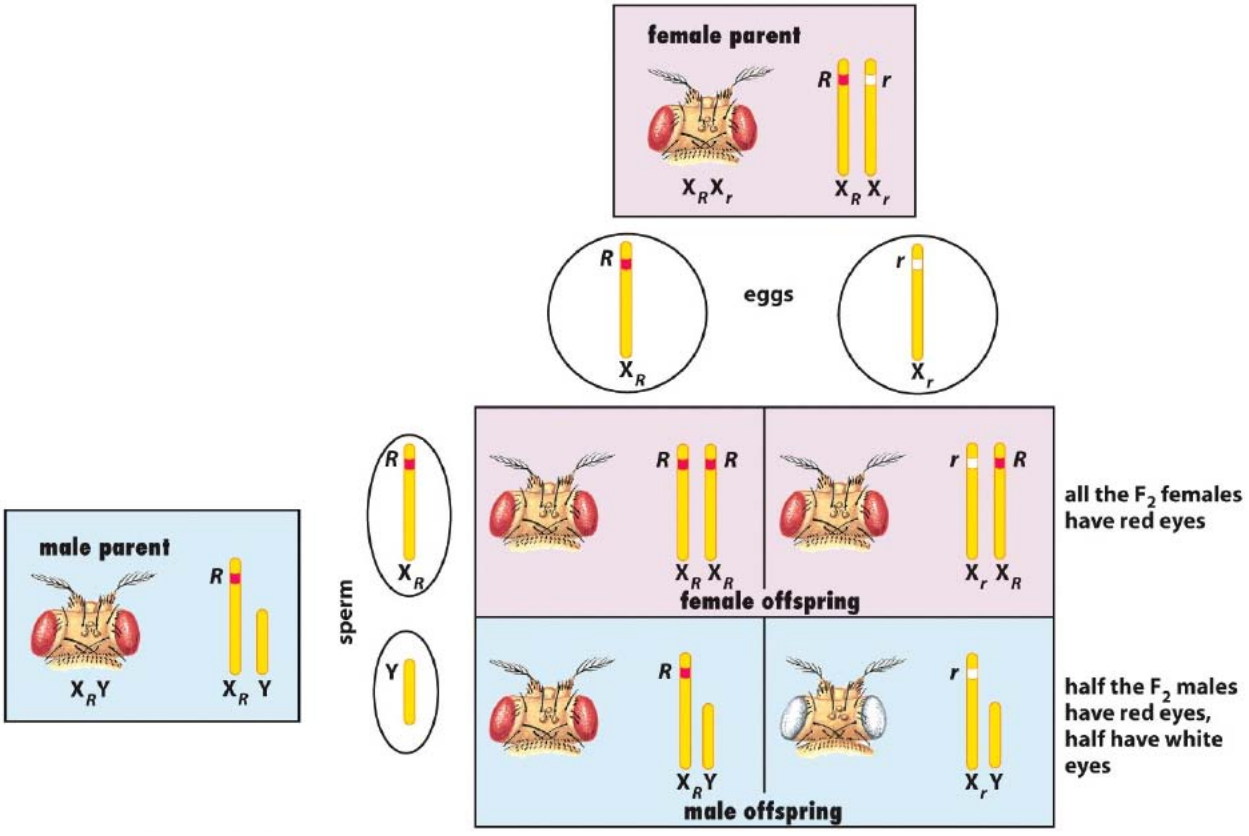
Some species have the reverse pattern of sex chromosomes (male = XX and female = XY), and some species have one gender (female) with a pair of chromosomes and one gender (male) with a single unmatched chromosome. In all cases the gender with the dissimilar pattern will determine the gender of the offspring.

Sex Linkage

In 1910, Thomas Hunt Morgan, who spent much of his career studying inheritance patterns of the fruit fly, *Drosophila melanogaster*, discovered the presence of a white eye in certain individuals. Since this was a distinctive feature, Morgan decided to study the inheritance pattern for this recessive eye color.

Morgan made several crosses using a white-eyed male, expecting the standard Mendelian results. He did not get them. While the ratio of 3:1 was obtained, all of the white-eyed second generation offspring were male flies. All females had red eyes (and 25% of the males also had red eyes).

Morgan did a series of reciprocal crosses of white-eye males with red-eye females and red-eye males with white-eye females. He concluded that the gene for eye color in the fruit fly was located on the X chromosome. Males passed the trait to their daughters (on their solitary X chromosome) and mothers passed the trait to sons. White eyed females could also pass the white eye allele to their daughters, but if the father fly had red eyes, the eye color of the daughters would be red, while the eye color of the sons of white-eyed females would always be white.



Morgan concluded that eye color was related to sex, and that the sex-determining chromosomes also had genes that were unrelated to gender determination. Prior to Morgan's discovery, no one knew that genes unrelated to gender were also located on these chromosomes.

These other traits are said to be **sex-linked** because they are inherited along with the sex of the individual. Because the X and Y chromosome are not exactly matching, the X chromosome can have genes that are not located on the Y chromosome, and vice-versa. Some of these genes are unrelated to the sexual characteristics, but are inherited with the sex-determination. This is referred to as **sex-linkage.**

Some human sex-linked traits are

- Hemophilia (X)
- Hairy ear rims (Y)
- Red-green color blindness (X)
- Duchenne muscular dystrophy (X)

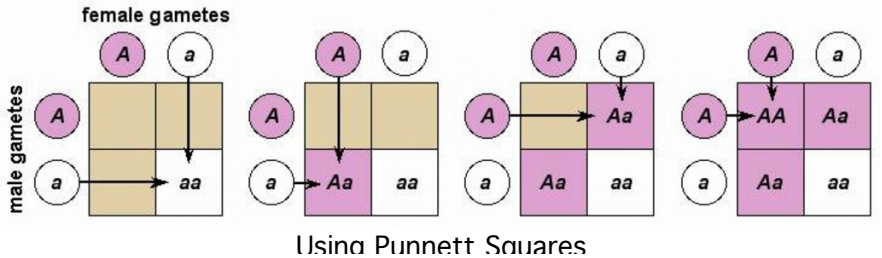
Many human traits follow Mendelian inheritance predictions. You will look at some of those in the laboratory as well as reading about them in your text.

We will not extensively discuss in lecture the specific crosses or predictions that Mendel did which resulted in his conclusions about inheritance. These crosses are discussed in your text and in the laboratory exercise.

You will be responsible for knowing Mendelian inheritance patterns and the predicted inheritance ratios for each outside of lecture. You will also be responsible for other types of inheritance patterns that are in your text or discussed in class.

- Monohybrid Cross with dominance — with a 2nd generation ratio of 3:1
- Monohybrid Test Cross when heterozygous — with an offspring ratio of 1:1
- Dihybrid Cross with dominance — with a 2nd generation ratio of 9:3:3:1
- Dihybrid Test Cross when heterozygous — with an offspring ratio of 1:1:1:1

Note that Mendelian inheritance predictions follow the mathematical laws of probability. Although it is fairly "easy" to diagram a monohybrid test and a dihybrid test using Punnett squares (see figures in text), making predictions and looking at results for increasing numbers of genes or other inheritance observations becomes tedious and time consuming. Applying probability laws is much faster and easier.



Using Punnett Squares

Some terms used in Mendelian Inheritance Tests

True Breeding

- A plant that produces offspring with the same characteristics. The **parental generation** is a true-breeding generation.

Cross Breeding

- A cross between different parental types
- Offspring produced by cross breeding are called **hybrids**

F_1 Generation

- The first generation
- Generally first generation offspring are bred among themselves to produce the second generation. In Mendel's pea plants they self-fertilized.

F_2 Generation

- The second generation
- Mendelian ratios are based on second generation results

Punnett Square

- A method of visualizing Inheritance crosses

Gene

- The physical unit of heredity; the instructions for producing a specific characteristic or trait. For example, the characteristic or gene may be flower color. The alternative forms a gene can have would be the specific flower colors.
- Since diploid organisms have two sets of chromosomes (the homologous chromosome pairs), most "genes" are paired, often called the gene pair

Alleles

- The alternative forms or variations a gene can have, such as brown or blue for eye color, or red or white for flower color.
- A diploid individual will have two alleles for each gene locus.
- Within a population there can be more than two alleles for a gene, but only two alleles will be present in any one diploid individual.

Locus

- The region on a chromosome where a gene is located.
- The alleles of a gene are located at equivalent places (loci) on the homologous chromosomes

Homozygous

- The 2 alleles for a gene are the same in an individual

Heterozygous

- The 2 alleles for a gene are different in an individual

Dominant Allele (loosely and incorrectly called a dominant gene)
- An allele that is always expressed, whether it is homozygous or heterozygous.
- A dominant allele masks or covers the expression of its alternative allele.

Recessive Allele
- An allele that is masked by the presence of its alternative.
- A recessive allele will be expressed only when it is homozygous (when the dominant allele is absent).

Phenotype
- The observable traits of an individual

Genotype
- The specific genetic makeup of an individual, or total combination of alleles present, both those expressed and those not expressed.

You should also review your knowledge of homologous chromosomes and the process of meiosis, since the homologous chromosomes "carry" the alleles, or alternative forms for each gene.

Gene Interactions – Beyond Mendel

The characteristics that Mendel observed had dominant and recessive forms and each inheritable trait was found on different chromosomes. Early on, some inheritance patterns did not match the expectations proposed by Mendel's principles. We shall now turn our attention to some of the gene interactions that go beyond the basic Mendelian predictions.

Single Gene Variations

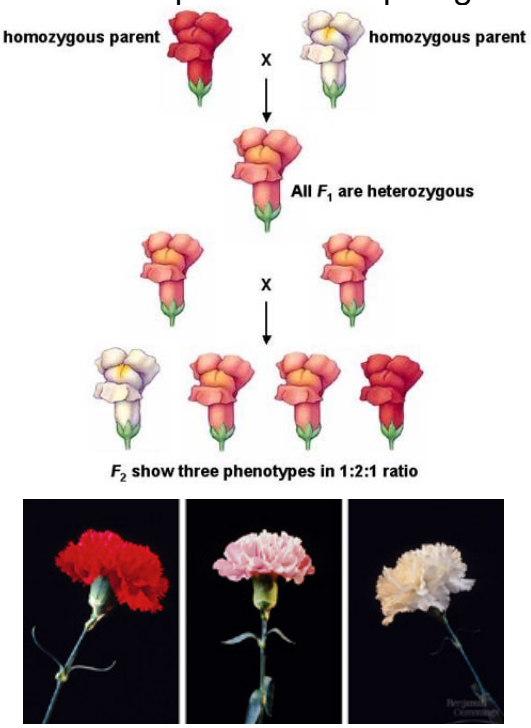
Lack of Dominance

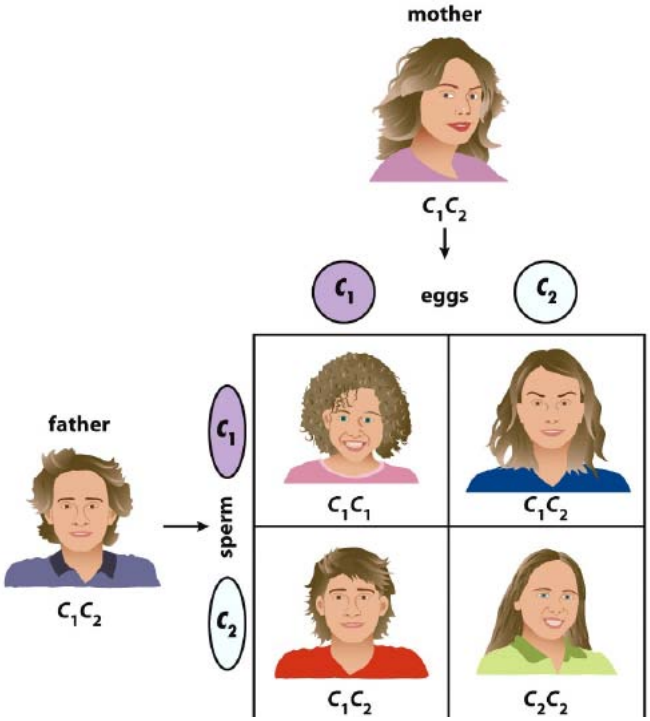
Mendel's F_1 offspring always resembled the dominant parent because each of the genes Mendel chose to study showed complete dominance. That is not always the case with genes and their alleles. For many genes there is no dominant allele. Both alleles are expressed when present and the heterozygote will have a phenotype different from either homozygous form. This is sometimes referred to as an intermediate phenotype.

There are a number of variations in lack of dominance, but each results in heterozygous conditions that have a phenotype different from either homozygous phenotype. In other words, when a gene lacks dominance, there will be three different phenotypes, two homozygous phenotypes (AA and A'A') and a third heterozygous phenotype (AA').

A. Incomplete Dominance

- Failure to completely **mask** or cover the recessive allele
- The heterozygote first generation has some intermediate phenotype between the two homozygous forms, often appearing as a blending of the two alleles.
- Examples: snapdragon flowers and human hair texture





B. Co-Dominance

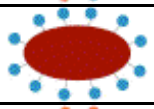
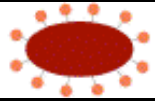
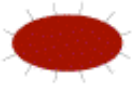
- Both alleles are equally expressed in the heterozygote - both appear in the heterozygous individuals
- Examples
  - Roan Cattle (Red X White cows)
  - Blue Andulusian Fowl (Black X White)
  - A-type and B-type Red Blood Cell Coatings

Lack of Dominance is just one of the many different ways that genes are expressed.

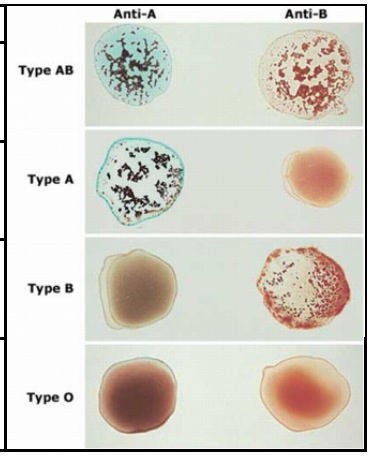
Multiple Alleles of One Gene

The "typical" gene has two alleles, one for each of the homologous chromosomes. This is the same for individuals and within the population. For some genes, however, there can be more than two alternative alleles at the single gene locus among members of the species.

- A diploid individual can inherit just two of the possible alleles (one on each of the homologous chromosomes)
- The effects are shown in the study of the population's variation in phenotypes for the gene.
- Example: A,B,O alleles for human rbc coats, the "I" gene. The I^A (A) and I^B (B) alleles are co-dominant. The i (O) allele is recessive

Blood Type	Genotype	Red Blood Cells
AB	AB $I^A I^B$	
A	AA or AO $I^A I^A$ or I^A i	
B	BB or BO $I^B I^B$ or I^B i	
O	OO ii	

Inheritance of ABO Blood Type

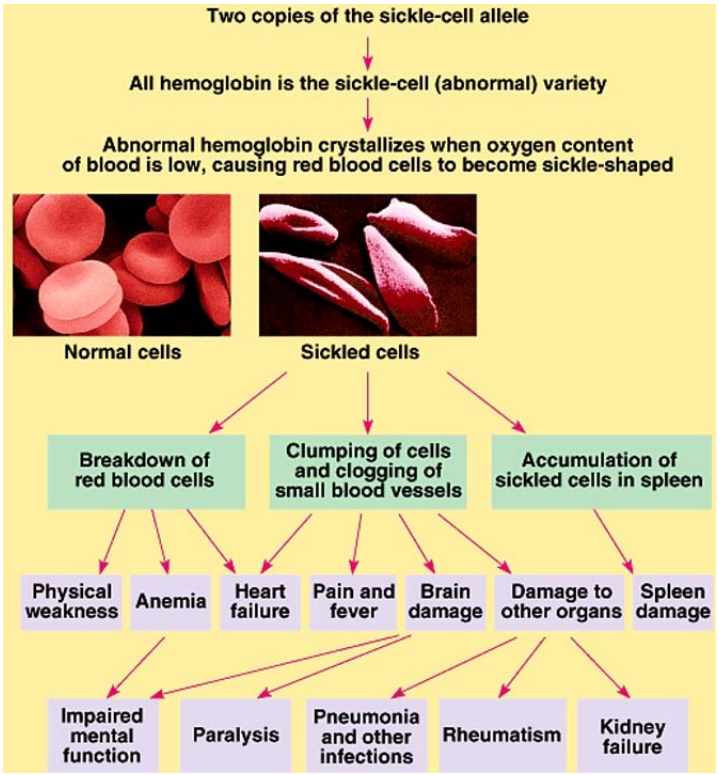


Antigen-Antibody Reactions

One consequence of the inheritance of the ABO blood type is that the A and B coatings are antigens, and can trigger antibody reactions in non-complementary individuals. This is important for blood transfusions, but not in genetics. In reality, there are variant alleles for each of the blood types beyond those discussed in biology classes.

Genes with More Than One Effect - Pleiotropy (Pleio means "more")
Although we typically think of a gene having a single phenotype, many genes are expressed in multiple ways having several effects on the organism. Genes with multiple effects are called **pleiotropic** genes.

- Some Examples
  - Albino condition (No Pigment)
    - Eye and skin sensitivity to light in many animals
  - Frizzle feathers in chickens -- affects feather shape
    - Feathers can't insulate properly
    - Metabolic problems relating to inability to thermoregulate.
  - Abnormal hemoglobin molecule -- affects shape of hemoglobin Protein



  - SRY gene on Y chromosome
    - Codes for the protein that activates the genes that code for testes formation. Testes formation activates hormone production that induces development of other male organs.
  - Cystic Fibrosis in Humans -- affects an ion channel protein
    - Multiple respiratory problems from mucus blockages
    - Blockage of pancreatic ducts
  - Marfan Syndrome
    - Fibrillin, a protein needed for connective tissue formation, is defective, leading to "loose" connective tissues and weakness in the skeleton, blood vessels, tendons and ligaments and joint areas.

Interactions Involving More Than One Gene
Polygenic Inheritance

The traits that we have so far discussed all have phenotypes resulting from the interaction of the alleles of one gene. Two or more genes can interact to produce a greater numbers of phenotypes. When two or more genes interact in ways that result in a number of different phenotypes, we see more variation in the population, with respect to that genetic characteristic. We call this type of inheritance **polygenic inheritance**. The individual phenotype is the result of the combined interaction of all the alleles at all of the gene loci involved.

Inheritance of Eye Color in Humans

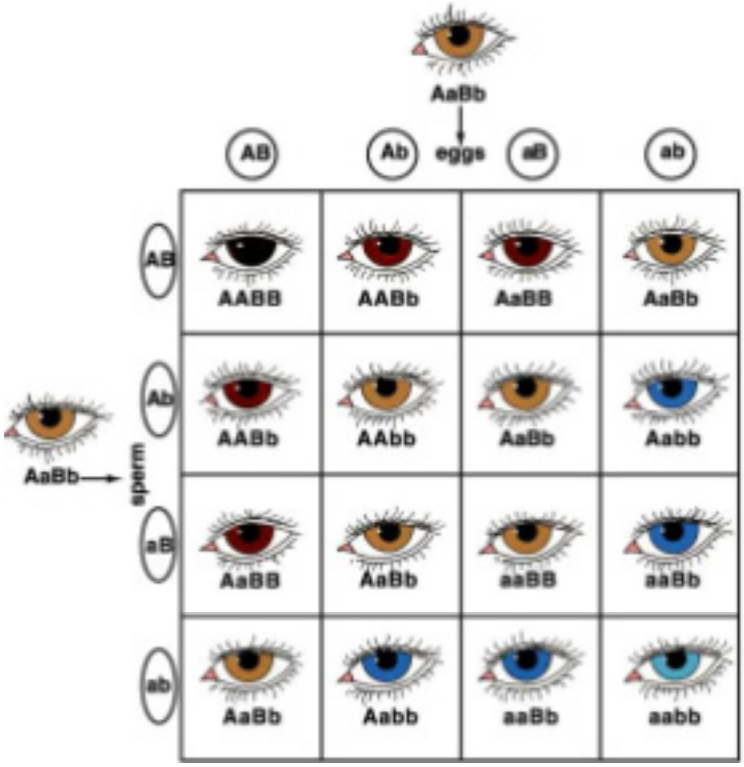
Human eye color involves the polygenic inheritance of at least two genes, each of which lacks dominance. The eye color genes code for the production of a yellow-brown pigment

First Iris Layer Pigment

AA = Produce lots of pigment
Aa = Produce some pigment
aa = Do not produce pigment

Second Iris Layer Pigment

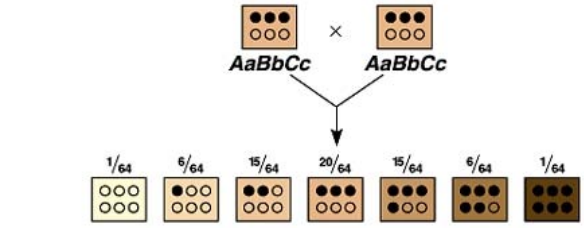
BB = Produce lots of pigment
Bb = Produce some pigment
bb = Do not produce pigment

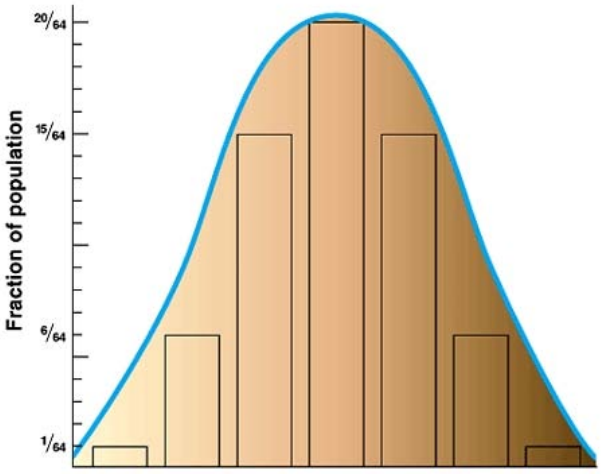


*There is also a **yellow overlay gene** which, when combined with the basic pigment gene alters light brown to hazel and light blue to green.

Continuous Variation in Polygenic Inheritance

When several copies of a gene interact, continuous variation within the population results. Continuous variation can most easily be demonstrated when population data shows a **bell-shaped distribution** pattern when graphed. Skin and hair pigmentation and height are two examples of such polygenic inheritance in humans. It is believed that there are at least 3 independent genes, each of which lacks dominance, responsible for producing the melanin pigment in human skin (and in hair).



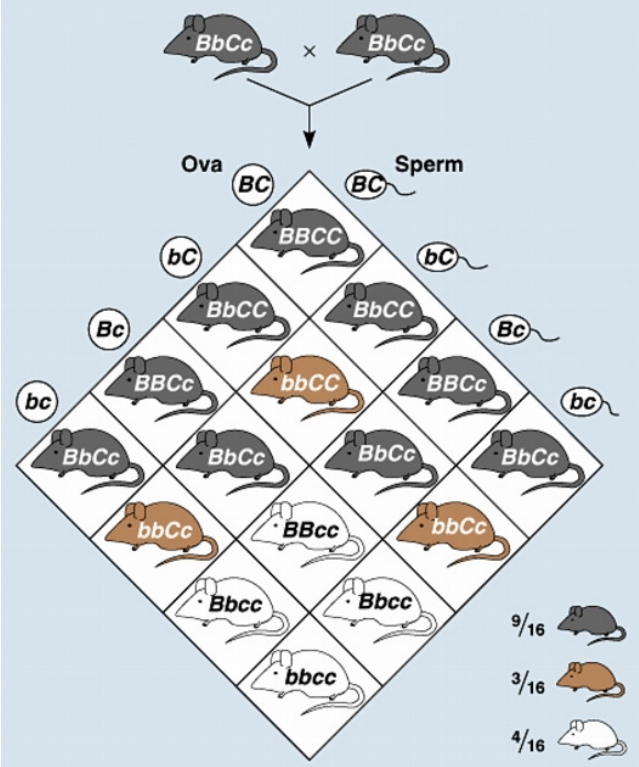


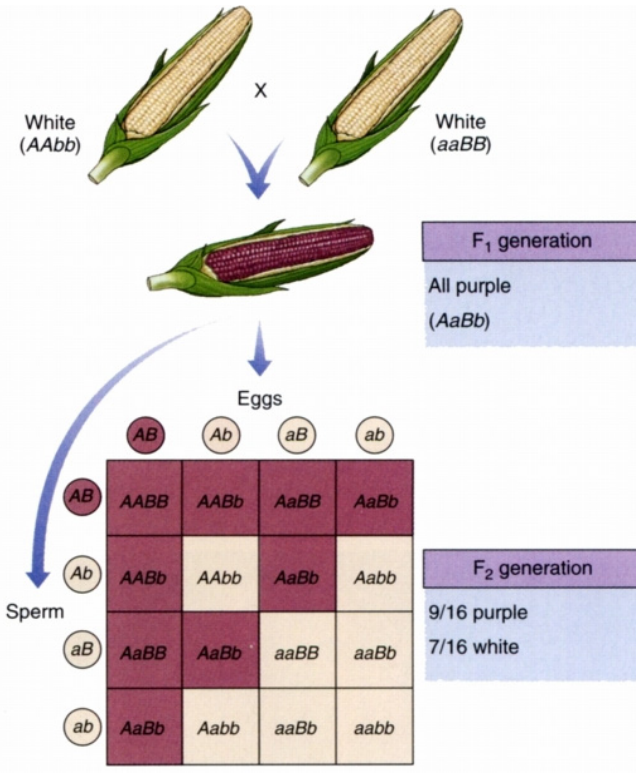
Controlling Genes - Epistasis (means standing upon or stopping)

- We have some gene interactions in which one gene **controls** or alters the expression of a second gene, so that the expected Mendelian phenotypes do not get expressed. A controlling gene is called an **epistatic** gene. Several of the pigment genes are subject to epistasis. The gene to distribute pigment is overridden by a second gene that blocks (inhibits) pigment production.
  - Examples:

    Mice can have black or brown pigmented fur depending on the inheritance of a gene for pigmentation. Black is dominant and brown, recessive. A second, independent gene prevents the distribution of any pigment in the fur. This gene, when recessive, results in white mice.

    In corn, expression of the pigment gene is also controlled by an epistatic gene. Epistatic genes can vary in how they control, resulting in different patterns and inheritance ratios as shown in the diagrams. In corn, distribution of pigment requires the epistatic dominant allele.

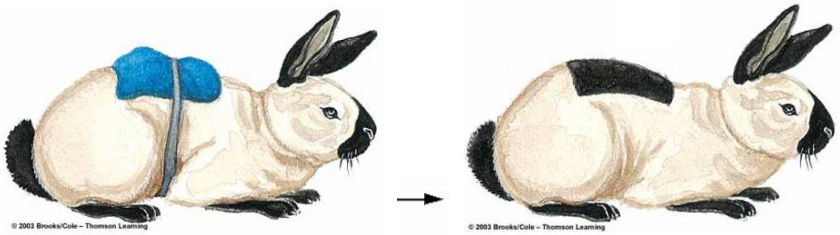




The Influence of the Environment on Gene Expression

Conditions of the environment can often affect the expression of a gene. Or stated differently, the environmental conditions can regulate whether or not a gene gets expressed.

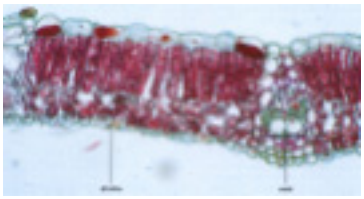
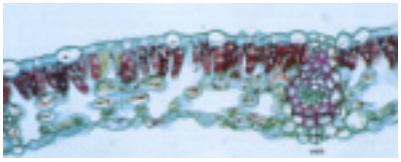
The classic study demonstrating the direct effect environment can have on genetic expression was done with pigmentation gene of Siamese cats and Himalayan cats and rabbits. The pigmentation gene is activated when the temperature falls below a certain point. Typically, the extremities are pigmented while the body core remains unpigmented or cream colored. To demonstrate that the pattern was temperature controlled, the backs of rabbits were shaved and ice packs placed on the shaved portion. When new fur grew, it was pigmented.



The winter/summer pigmentation in ptarmigan, arctic fox and ermine are also temperature controlled.


Conditions of the environment regulate a number of other genes, including:

- Morphology of leaves
  - Sun/shade leaves

  - Submerged/surface leaves in aquatic plants

- Soil pH and flower color

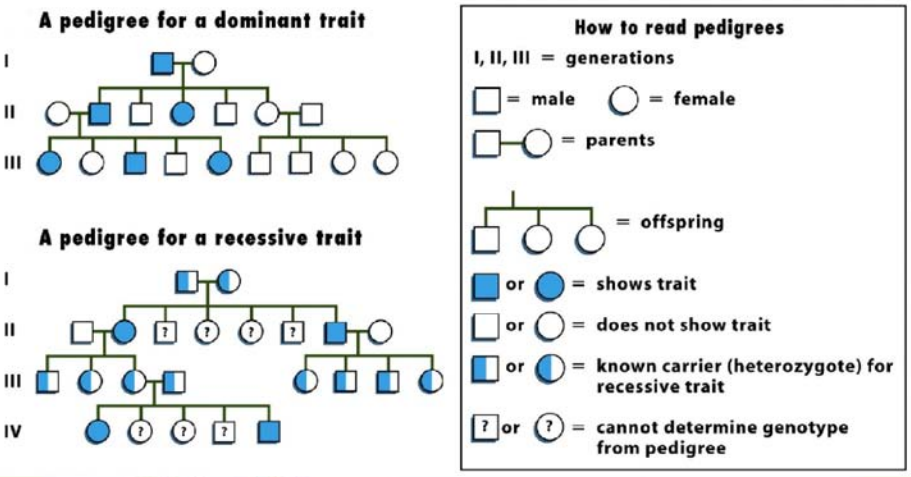
- Nutrition and Growth
  - Height (elongation of bones in growth years)
  - Brain development in first two years
  - Role of exercise in muscle development

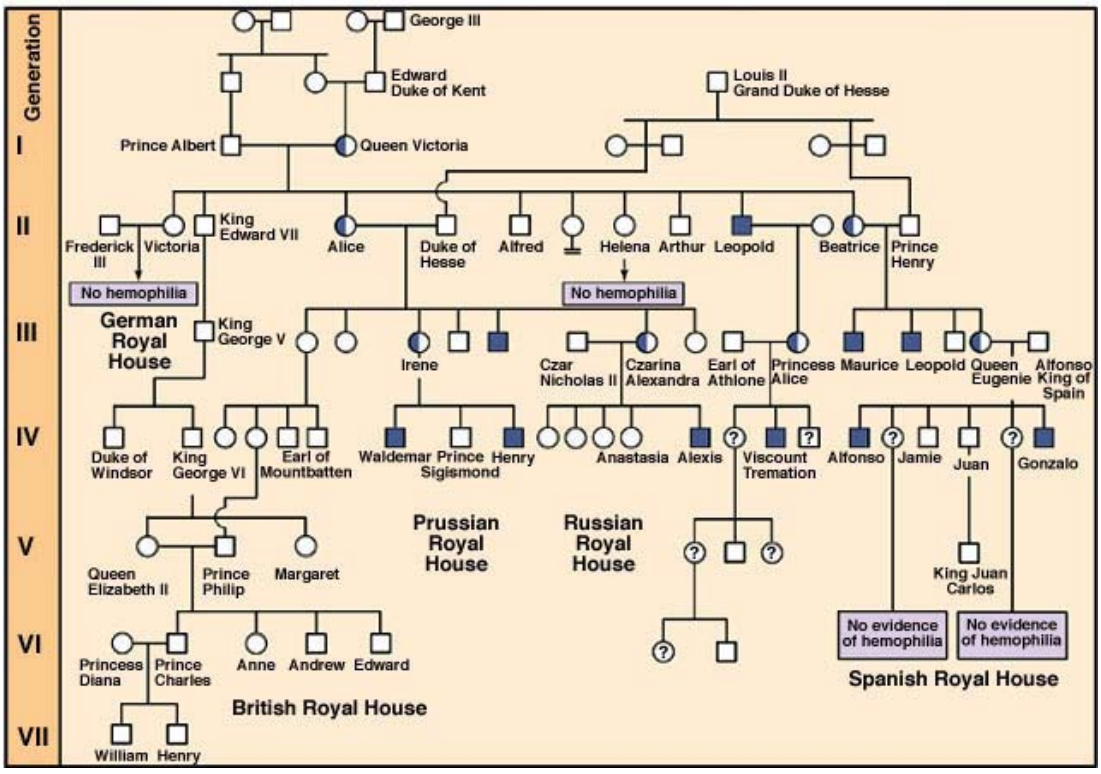
- Sex changes in response to the number of other sex members in the population (Several species of animals)

Ultimately each individual is a combination of his/her genetic potential and response to the multitude of environmental factors to which he/she is exposed.

Investigating Human Inheritance Patterns

Historically, most of our information about human genetics, which, like all organisms, follows basic rules of chromosome inheritance, has come from careful analysis of family histories, or **pedigrees** that sometimes track traits over many generations. It is only within the last generation that advances in molecular genetics have led to much better analyses of the inheritance of specific genetic traits within families.





Hemophilia Pedigree of the European Royal Families

During our recent discussion of gene interactions we have included examples of human inheritance patterns, such as the inheritance of eye color, skin pigmentation, the inheritance of multiple alleles with the ABO blood types, and briefly noted the pleiotropic effects of sickle cell anemia and listed some sex-linked human characteristics.

At this time, we will look a bit at how we study human inheritance and at some of the research in the field of human genetics today. We will also look at some chromosomal alterations that affect human inheritance. In our next unit, we will address some of the ways in which biotechnology is progressing with gene therapy with human genetic disorders.

Most of the research attention focuses on the inheritance of genetic conditions which negatively impact health and well-being, perhaps because these genetic traits are more easily identified, and perhaps because we would like to be able to better treat or prevent these conditions.

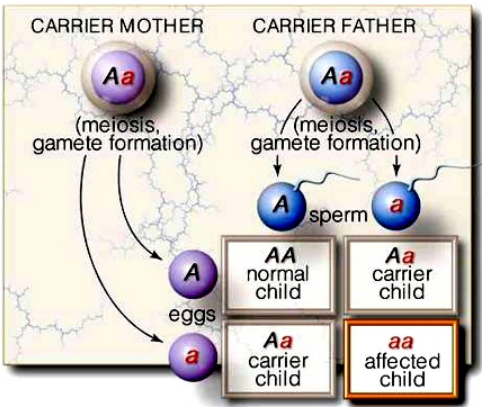
Inheritance of Recessive Alleles

Any alteration of a gene, called a **mutation**, has the potential to inhibit the formation of a needed enzyme or other protein needed for the organism. Gene alterations that affect health are called **genetic disorders**. Those that are just "abnormal" but do not affect physiological health, are called **genetic abnormalities**. When the genetic alteration causes a host of symptoms, it may be called a **syndrome**.

With diploid organisms, however, a mutation most likely affects just one of the homologous chromosomes; the second can still code for the appropriate enzyme with little or no phenotypic effect on the individual. This has been demonstrated in laboratory experiments, and is demonstrated with many single gene inheritance patterns when the heterozygous and homozygous dominant phenotypes are indistinguishable.

Many of our genetic disorders that affect metabolism are the result of the inheritance of recessive alleles that fail to code for the needed enzyme. If this enzyme is critical for survival, affected individuals, those that are homozygous recessive, will die if they cannot be treated.

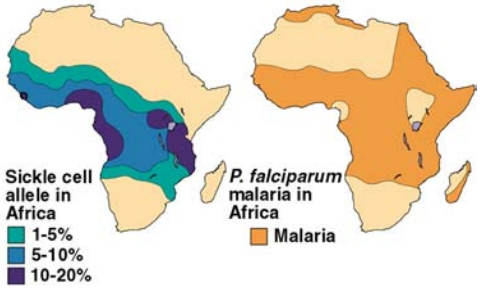
It is difficult to remove recessive alleles from the population since individuals who are heterozygous have the allele but do not exhibit the problem. In human inheritance, individuals who are heterozygous for a genetic "disorder", but do not exhibit symptoms are called **carriers**. Carriers can pass the allele on to the next generation.



Some Examples of Human Recessive Alleles that Cause Problems

- Cystic Fibrosis
  - Affects a chloride ion channel membrane protein. Lack of the protein results in thickened excess mucus production, especially in lungs and pancreas.
  - One recent experimental treatment involved gene therapy using genetically altered cold viruses that contain the normal gene. The viruses are sprayed into the nasal passages. When the virus invades the nasal mucus producing cells, the gene is incorporated into the cells and gets transcribed. The treatment is short-lived, however, since cells are replaced every few weeks.
- Sickle Cell Anemia
  - Hemoglobin takes on a shape in low oxygen conditions that alters the shape of rbcs, causing clumping and rbc fractures that block blood flow.

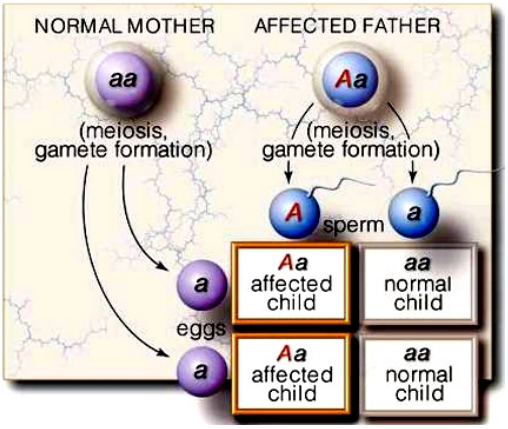
  - Treatment by periodic whole blood transfusions
  - The partial sickle effect of heterozygotes does not negatively affect health, and confers some resistance to the malaria-causing protist. The correlation of sickle cell anemia and malaria is striking.



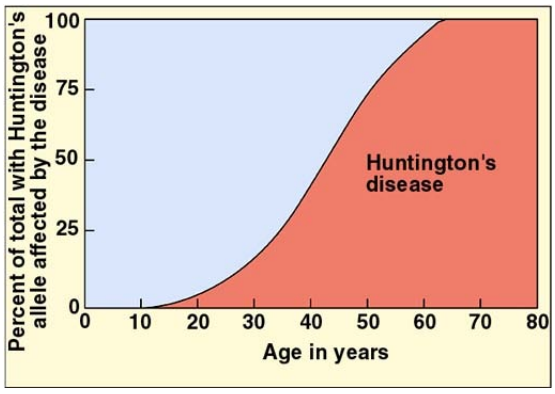
- Tay-Sachs Disease
  - Inability to produce critical lipases, which results in accumulations of particular lipids in the brain. Tay-Sachs is fatal in early childhood. Carriers produce sufficient enzyme to not exhibit symptoms of Tay-Sachs.
- Phenylketonuria
  - Inability to process the amino acid, phenylalanine
  - Protein intake must be critically monitored to prevent buildup of the amino acid to harmful concentrations.
- Albinism
  - Inability to produce any melanin pigment
  - Treatment is to avoid sun and other bright light

Dominant Allele Disorders

It is rare to have serious genetic disorders that are caused by dominant alleles. The dominant is always expressed so individuals who inherit the dominant allele express the genetic problem and often succumb to the effects of the disorder before they can reproduce and pass the trait on to their children. The exceptions are dominant alleles that express themselves post-reproductively, or some that have no negative physiological impact. Rarely, a mutation can result in deactivation of a normal allele.



- Huntington's disease
  - Huntington's causes the brain to deteriorate, a disease which affected Woody Guthrie. Although it is possible to identify and screen for the Huntington's dominant allele, many who have the trait in their pedigree may choose not to go through the testing procedure. It may be difficult to decide if one wants to **know** that he/she will have the symptoms of this brain disease at "mid-life".



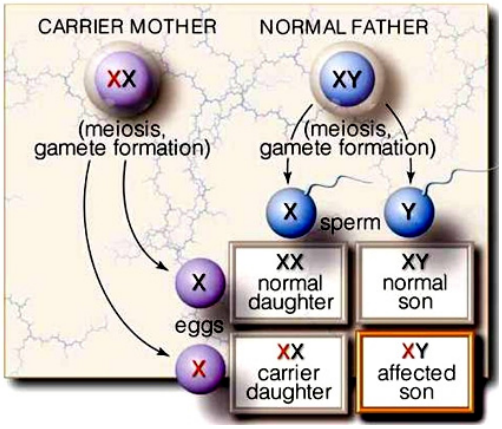
- Acondroplastic Dwarfism
  - Rare in the human population. This dominant allele is not lethal.

Sex-Linked Recessive Problems

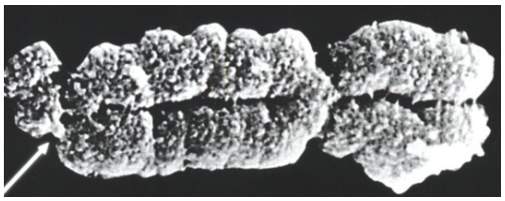
Sex-linked human disorders are caused by non-functioning alleles on the X chromosome. Sex-linked human disorders disproportionately appear in males, who inherit the non-functioning allele from a carrier (heterozygous) mother who does not exhibit the symptoms of the disorder. Fathers pass the allele to their daughters, who, if they inherit a normal allele from the mother will be carriers who do not exhibit symptoms. Females must inherit the non-functioning allele from both parents to have symptoms of the disorder. In a similar fashion, sons cannot inherit the allele from their father, because they inherit the Y chromosome from their father.

Some Sex-Linked Human Disorders

- Hemophilia
  - Hemophilia is caused by the inability to produce a critical blood-clotting factor.
  - Hemophilia is treated by supplying the affected individuals with the clotting factor. If not treated, hemophilia is usually fatal.



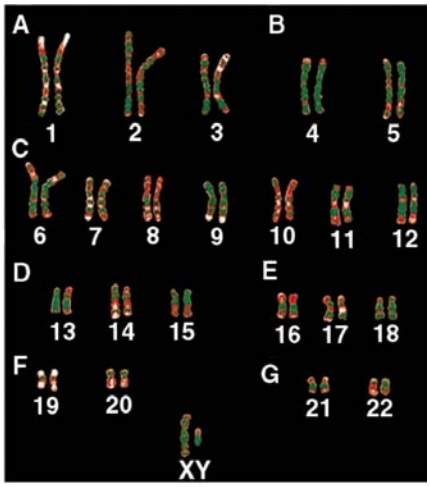
- Duchenne Muscular Dystrophy
  - One of the forms of muscle degeneration
- Fragile X
  - A nucleotide repeat disorder that causes mental impairment



- Red-green colorblindness
- Androgen insensitivity
  - Males do not respond in development to androgen signals resulting in sterility and some "female" traits.

Changes in Chromosome Number and Inheritance

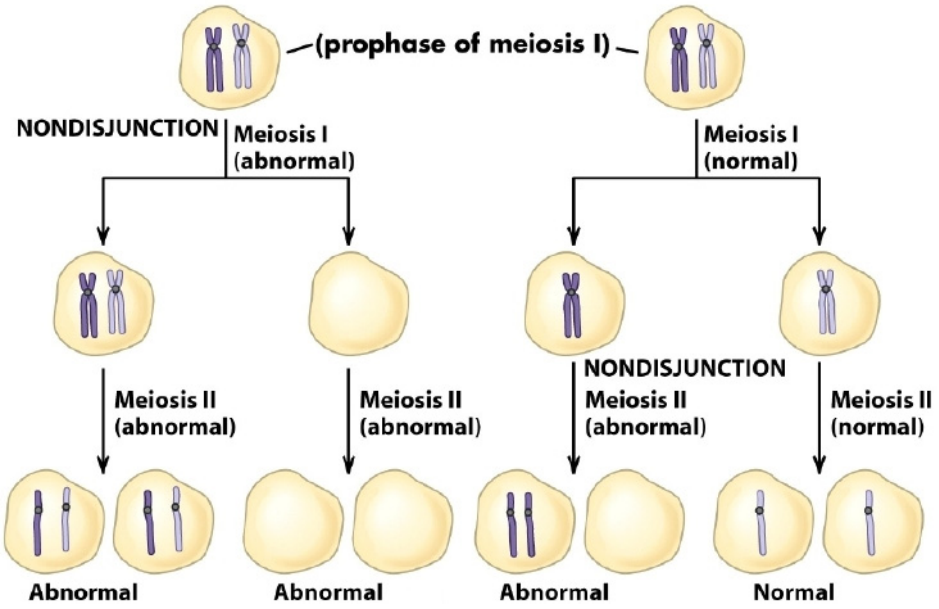
It is fairly easy to observe our 46 human chromosomes and their shapes, because we can obtain a karyotype, or chromosome display during the metaphase stage of cell division. This allows us to see distinct chromosomes, and detect patterns that are not typical.



Aneuploidy

For reasons not understood, occasionally, a homologous chromosome pair will fail to separate during meiosis, resulting in an egg or sperm with one more or one less than the normal complement of chromosomes (trisomy or monosomy). In general, we call this **non-disjunction** or **aneuploidy.**

Most often, a non-disjunction results in a gamete, or if there is successful fertilization, an embryo that does not survive. In some cases, however, some gametes or embryos do survive, producing individuals with abnormal chromosome numbers. Most non-disjunctions have serious genetic consequences. A non-disjunction can affect either the sex-determining chromosomes or autosomes. We will mention a few human examples.



Non-disjunction of sex chromosomes

There are a number of sex chromosome non-disjunctions.

Nondisjunction in Father

Sex Chromosomes of Defective Sperm	Sex Chromosomes of Normal Egg	Sex Chromosomes of Offspring	Phenotype
0 (none)	X	X0	Female—Turner syndrome
XX	X	XXX	Female—Trisomy X
YY	X	XYY	Male—Jacob syndrome
XY	X	XXY	Male—Klinefelter syndrome

Nondisjunction in Mother

Sex Chromosomes of Normal Sperm	Sex Chromosomes of Defective Egg	Sex Chromosomes of Offspring	Phenotype
X	0 (none)	X0	Female—Turner syndrome
Y	0 (none)	Y0	Dies as embryo
X	XX	XXX	Female—Trisomy X
Y	XX	XXY	Male—Klinefelter syndrome

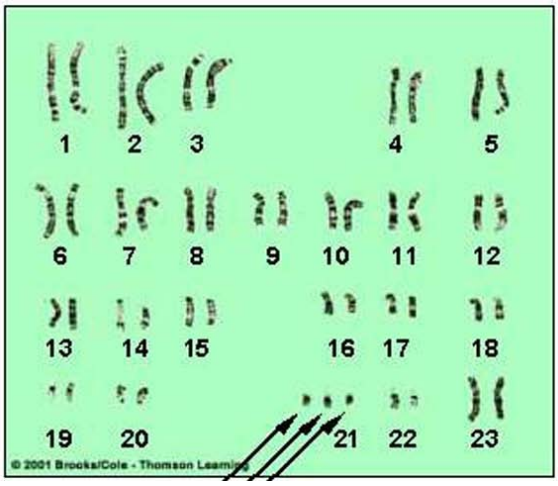
- Monosomy X0
  - Turner syndrome
  - Symptoms include absence of secondary sexual development and sterility because of insufficient hormone production.
  - Turner syndrome is more often the result of non-disjunction in sperm formation (75%) than in egg formation.
- Monosomy Y0
  - Lethal in embryonic development
- Trisomy XXX (female)
  - No detectable problems.
  - Females are usually fertile and bear normal XX or XY children.
- Trisomy XXY (male) (and other multiples with both X and Y, except XYY)
  - Kleinfelter syndrome
  - May have mixed secondary sexual development at puberty and low sperm production; the testes and prostate gland are smaller than normal. Males are generally taller and heavier than average.
  - Two-thirds of the extra Xs come from the egg.
- Trisomy XYY (male)
  - Jacob Syndrome
  - Increased vertical stature
  - May have higher testosterone levels and greater incidence of acne

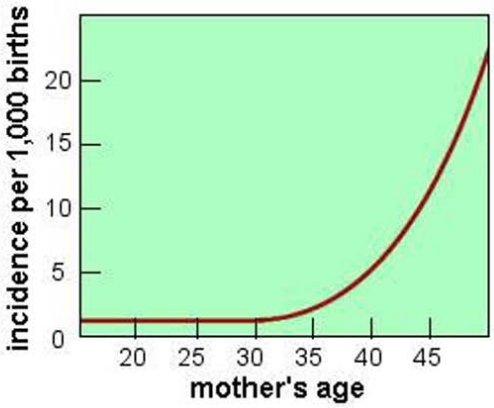
Autosomal Non-Disjunction

Survival with an autosomal non-disjunction is rare. Autosomal Monosomy non-disjunctions result in non-viable embryos. Most trisomy autosomal non-disjunctions are also non-viable, but there are few exceptions, most commonly with chromosome 21 and sometimes 18 and 13.

- Trisomy G or 21 or Down syndrome

  About 75% of Trisomy 21 occurrences are in the egg. As many as 1 in 20 eggs produced after the age of 40 may carry this chromosome abnormality. There is also some increased frequency in sperm associated with aging.





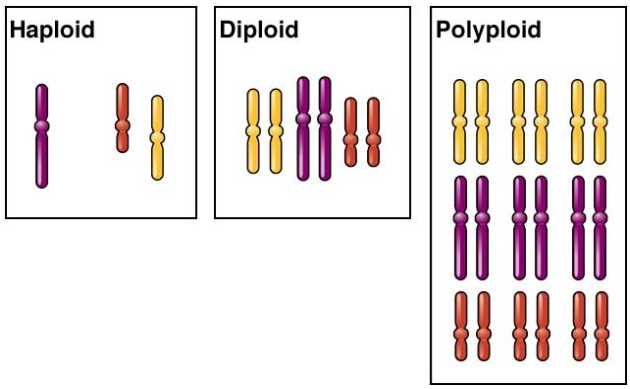
Characteristics:

Poor muscle tone, including the heart muscle
Tongue and mouth not proportioned, affecting speech
Mental retardation is sometimes present
Immune system weakened

Other Chromosome Differences - Polyploidy

Polyploidy is the increase in the **number** of sets of chromosomes, usually resulting from the formation of diploid gametes. Polyploidy occurs naturally in many plants and may produce larger, hardier individuals.

- If a diploid gamete unites with a normal haploid gamete, the triploid hybrid is sterile (no homologous matches at meiosis).
- If both gametes are diploid, the tetraploid individuals are often fertile.
- Polyploidy is used extensively in developing agricultural varieties.



Polyploidy occurs naturally in many plants and may produce larger, hardier individuals. Plants we consume are often hybrid varieties selected for their enhanced productivity. Wheat grown today is a polyploid composite of several ancestral species. A naturally occurring tetraploid rat is found in Argentina.


Hybrid Corn on Right


Tympanoctomys barrerae, 4n Rat

Extra-nuclear gene expression

And for our final note on transmission of characteristics from generation to generation, Mendelian inheritance addresses the behavior of genes on chromosomes.

Organelles, such as mitochondria and chloroplasts (and all plastids) have small circular pieces of DNA, and that DNA is transcribed and translated within the organelle. Mitochondria and chloroplasts are self-replicating. In sexual reproduction, only the egg cell's cytoplasm is passed to the zygote, so only maternal mitochondrial and chloroplast DNA will be transmitted from generation to generation. Some genetic disorders are traced to mutations in mitochondrial DNA that codes for proteins in the electron transport chain. Mutations in mitochondrial DNA may be one reason cells age.

We have discussed ways in which the structure of DNA can be changed in individuals through mutation, and how DNA changes from generation to generation through recombination and independent assortment during meiosis and sexual reproduction.

For thousands of years humans have used selective breeding in agriculture, horticulture and what was once called "animal husbandry" to obtain and maintain desired inheritable traits with many species of plants and animals. In this sense, humans manipulated genes for thousands of years before we had any knowledge about what a "gene" is.

We have taken advantage of the capabilities of many organisms to manufacture foods and beverages we like: yogurt beer, wine and cheese are all examples of natural "biotechnology" which produces things we humans find useful. Drugs, such as penicillin are products of fungi, used for human benefit. The streptomycin drugs are bacterial derivatives. *Penicillium* mold was one of the first organisms deliberately "mutated" to produce better strains of the penicillin drug.

In addition, humans, by our very treatment of our surroundings and those who inhabit our surroundings, have been responsible (deliberately and/or unintentionally) for the loss of hundreds, perhaps thousands, of species, and their unique combinations of genes through extinction.

Today, the field that we speak of as **biotechnology,** (the manipulations of organisms or their components to do something useful) or **genetic engineering** (direct manipulation of genes), effects changes in the DNA molecule and/or in the organism in very precise and directed ways, for research and for industrial, medical, agricultural and commercial applications.

Much of today's DNA research is based on refining natural methods of recombination, and taking advantage of some means of introducing new or different DNA into host cells. Such research is often called **recombinant DNA research. Transgenic** alterations involve DNA isolated from one organism being spliced into a different species. Keep in mind, though, that all DNA is composed of the same four same nucleotides no matter the origin of the unique sequence.

Genes for the fluorescent enzyme luciferase, isolated from naturally fluorescent organisms have been spliced into a several species for a variety of genetic and research purposes. The Nobel prize was recently awarded to several leaders of the use of fluorescent gene markers for genetic engineering.

Fluorescent Rabbit

Fluorescent Tobacco

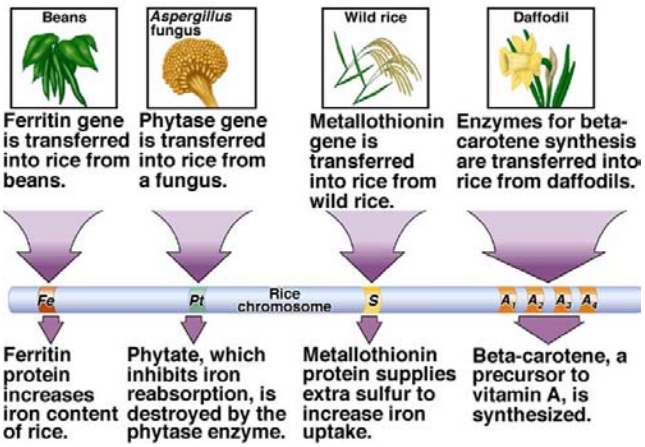
Biotechnology or genetic engineering has many goals, including:

- Improving our understanding of inheritance and genetics with basic research
- Improving our treatment of genetic disorders (and some diseases)
- Using the tools of DNA technology to increase knowledge in all areas of biology
- Facilitating forensics and criminal law by evidence analysis
- Providing economic and health benefits in agriculture, medicine and elsewhere

Some DNA technology research involves altering existing DNA to promote or prevent the expression of certain genes. One example of altered DNA is found in the commercial tomato industry. Biotechnology has been used to suppress the genes that promote ripening and softening of tomatoes so that tomatoes can stay on the plant longer (to develop the proper flavor), but not get soft. Firm tomatoes are necessary for transportation purposes.

Many chemicals are now produced using strains of bacteria or fungi, genetically selected for their ability to produce quantities of the desired chemical, similar to the way the *Penicillium* mold was cultured to obtain a strain that produced good quantities of penicillin. It's much easier today, however, to find strains that produce the desired chemicals than it was in the 1940's.

The golden rice developed by a Swiss consortium is one example of using recombinant DNA techniques. This rice has genes to increase iron content, sulfur content, and, in particular, β-carotene, as well as the enzyme phytase, which destroys the plant phytates that chelate iron. The goal of this project was to make a more nutrient-rich rice, and targeted originally beta-carotene (a vitamin A precursor) that is lacking in diets of those who rely on rice as the diet staple in much of the world. Vitamin A deficiency is a leading cause of blindness in children in "third world nations", and is not reversible. It is estimated that 40 million children suffer vitamin A deficiency. In addition, Iron deficiency affects almost a quarter of the world's population. At the time the first golden rice was ready for market, distribution was withheld for a number of political reasons. Golden rice is still controversial, and its benefits are mixed. Moreover, rice is not always the staple, and the different rice varieties are grown in different climates, factors that affect its use.



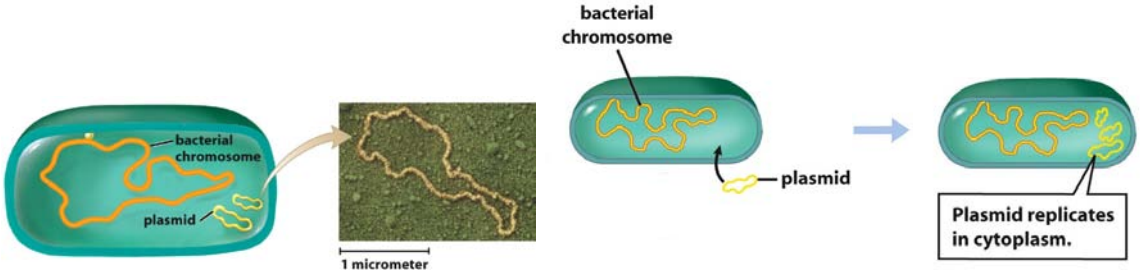
Much of the emphasis of this chapter in your text is to provide some information about the applications of recombinant DNA research, and in biotechnology in general, as well as some of its techniques. We shall look at some of those processes briefly.

Natural DNA Recombination in Prokaryotes

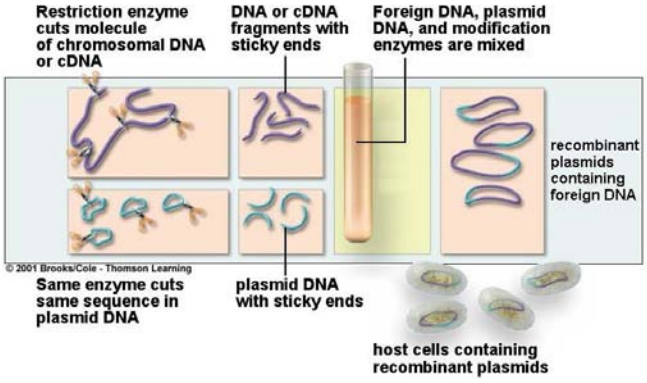
Bacteria are particularly useful for research in biotechnology. Recombination in bacteria is common. **Transformation**, first discovered by Griffith, occurs naturally in many bacteria, and is a good example of recombination.

Bacteria also have recombination using **plasmids**, small independent pieces of DNA incorporated into bacteria directly from the environment.

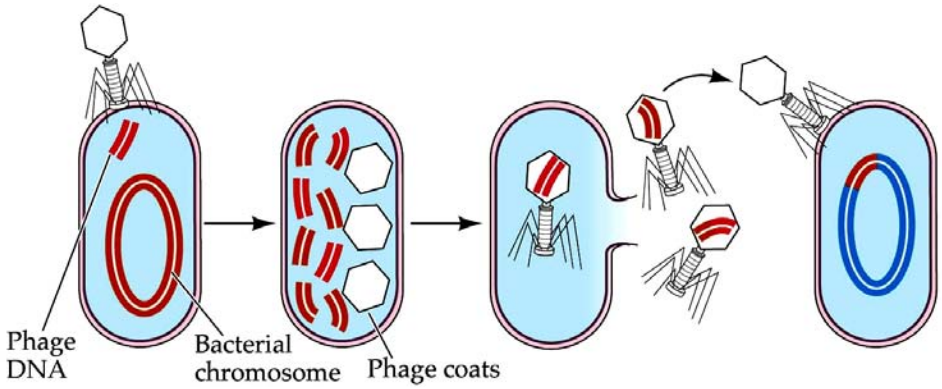
A bacterium may have multiple copies of plasmids, or clones, and when the bacterium dies, its plasmids are released into the environment where they can be incorporated into a different bacterium. Since plasmids carry independent genes, new information is incorporated into the DNA molecule.



DNA technology uses modified plasmids to add desired genes from **plasmid clones** to host cells for research purposes, and for the manufacture of chemicals needed by humans. Often plasmids that have antibiotic resistance, called **R plasmids**, are chosen, because they can also be used to determine if the desired gene has been incorporated into the host bacterium. The modified plasmids that carry DNA to the target host are **cloning vectors**.



Bacterial recombination can also take place by **transduction**, a process involving virus vectors, which can bring bits of DNA which were broken off from a previous host's DNA molecule when the virus left that host and add that DNA to a new bacterium. A virus can also be a cloning vector, and virus clone libraries are maintained.



Natural Recombination in Eukaryotes

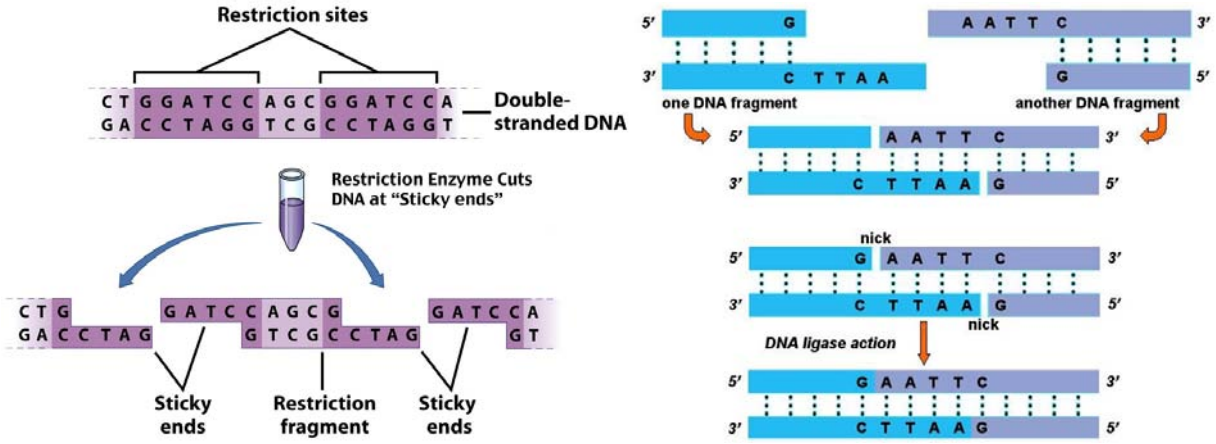
Genetic recombination in eukaryotic organisms naturally occurs between homologous chromosomes with the following characteristics:

- Crossing over occurs anywhere along the chromosome
- Crossing over, except for mutations, is reciprocal and the crossing over distance is fairly extensive.

Genetic recombination can also involve transposons, the jumping or mobile genes discovered in corn by Barbara McClintock in the 1940's, that are able to recognize specific DNA nucleotide sequences on both strands of DNA and make cuts into which they splice the DNA segments they are moving.

In the 1970's, enzymes that could make these cuts were identified in bacteria, along with many transposons. Because each enzyme recognizes a specific DNA sequence the enzymes were called **restriction enzymes** (or more properly restriction endonucleases) because they are restricted to one specific DNA code that they can cut.

Restriction enzymes are used in recombinant DNA work. Restriction enzymes find sections of DNA where the order of nucleotides at one end is the reverse of the sequence at the opposite end. This way a restriction enzyme can cut tiny "sticky ends" of DNA and match that piece of DNA to "sticky ends" of any other DNA that has been cut with the same restriction enzyme. The cut DNA is then spliced into the second DNA molecule. This is what Barbara McClintock's jumping genes were doing. It's believed that the bacterial restriction enzymes are naturally used as part of the bacterium's defense system, to remove unwanted bacteriophage DNA from its genome.



Recombinant DNA Technology Techniques and Applications

In order to make recombinant DNA, a researcher must be able to do the following:

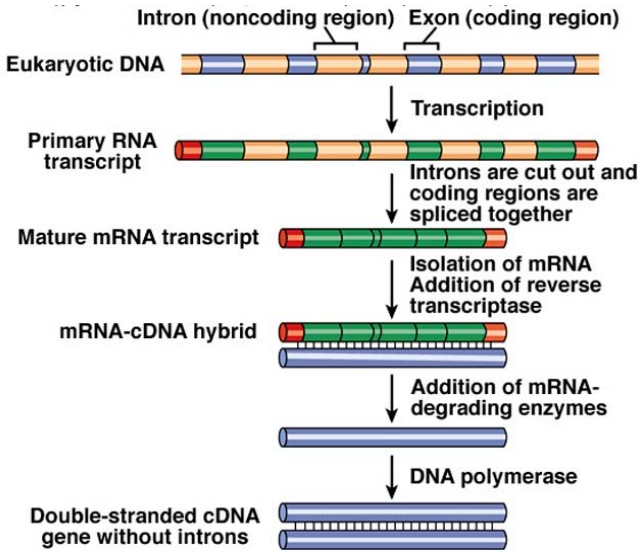
1. The desired gene must be located and isolated (which is why mapping the genome is so important).
    - Restriction enzymes may used to make fragments of DNA more suitable for analysis.
    - The DNA probe is one technique used to obtain desired DNA.
    - Researchers also use complementary DNA (cDNA), which is synthesized from RNA templates, when the DNA sequence can be determined.
2. The target gene or DNA must be made available and in quantities suitable for work.
    - The DNA polymerase Chain Reaction (PCR) is a technique that amplifies the volume of the desired DNA
3. The target gene or DNA must be transferred to the host and incorporated into the host's DNA and expressed.
    - Plasmids and viruses are often gene vectors.
    - Other techniques are also used, including the "DNA gun" and direct uptake of DNA into target cells

Finding the DNA Source – A Critical Step

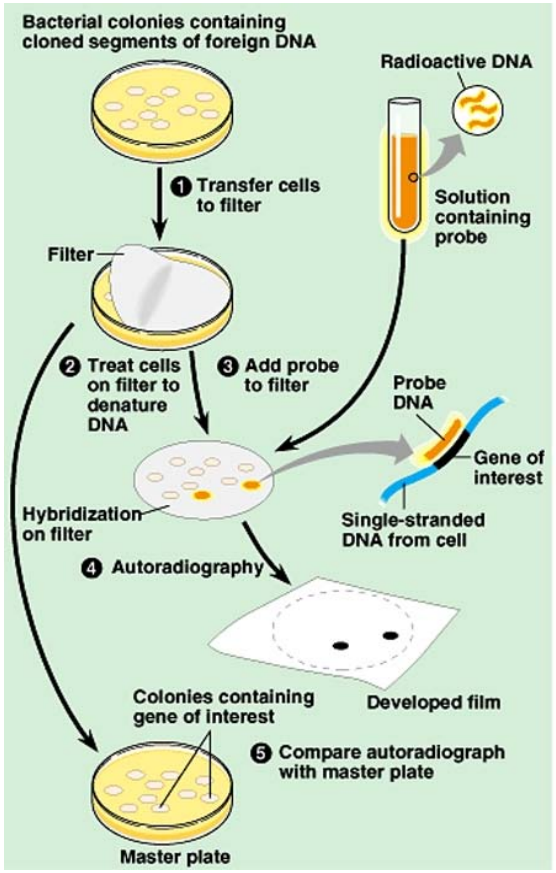
The desired gene that codes for the protein needed must be located and isolated (which is why mapping the genome is so important). It's not easy, and genes with introns, even if successfully recombined, might not get the mRNA transcripts properly spliced and processed for translation in the host. There are a number of ways in which DNA gets isolated for use.

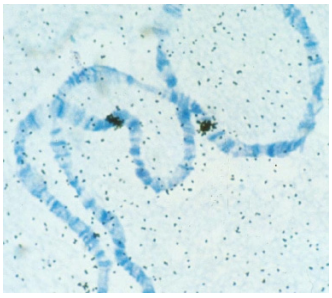
cDNA

To solve the intron problem, some genes used for recombinant DNA work are "artificially" made. The DNA made is known as cDNA or copied DNA using a functional mRNA template. **Reverse transcriptase** enzymes, found in some viruses, can take a strand of mRNA and code a complement DNA strand. The DNA strand can then be used as a template to code its complement to form a double-stranding DNA molecule that can be used to splice into the vector.

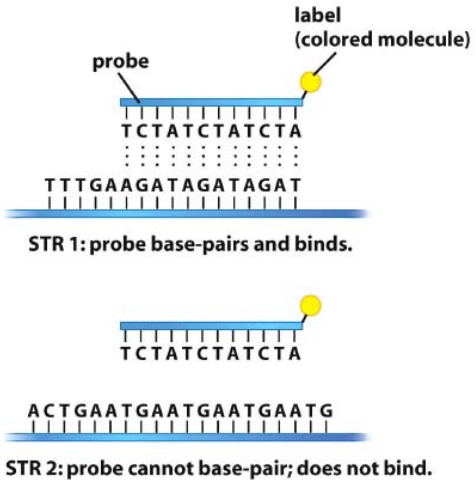


Another way of locating a desired gene is to use a **DNA probe.** A DNA probe is a small piece of single-stranded DNA or RNA with a known nucleotide sequence, and often a radioactive marker. A "**probe**" of a radioactive synthetic single stranded DNA or mRNA can bind to its complement target on the DNA molecule, pin-pointing the target DNA. This works only if a part of the target DNA code is known so that a "matching" probe can be used.

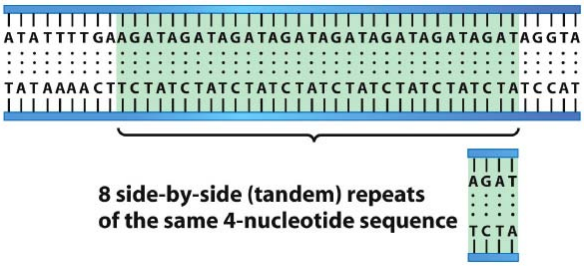




DNA Hybridization on Chromosome

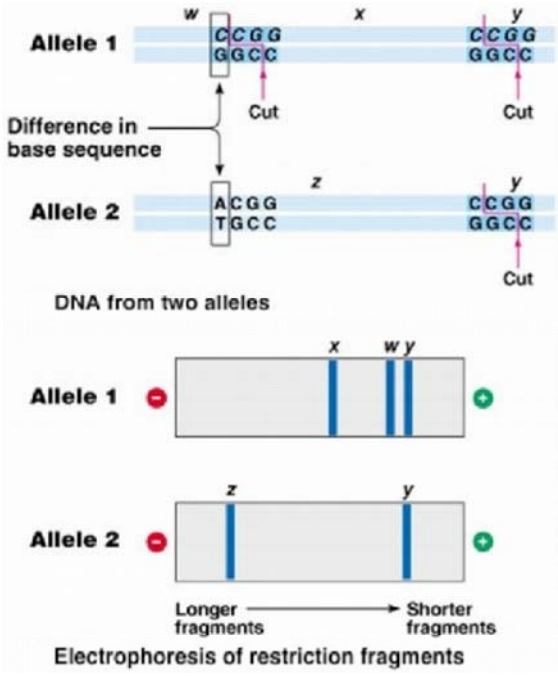


One of the reasons we can use DNA probes is that all individuals have a type of DNA called **short tandem repeats** or STRs. STRs are non-coding DNA that consist of 2 – 5 nucleotide sequences repeated many times (in tandem). Their number and arrangement in human chromosomes is unique to each individual. Forensic medicine, for example, uses STRs in DNA analysis, and have a common set used in their work. When a DNA sample is tested for a set of 10 STRs, the chance of two people having the same profile is less than 1 in 1 trillion.



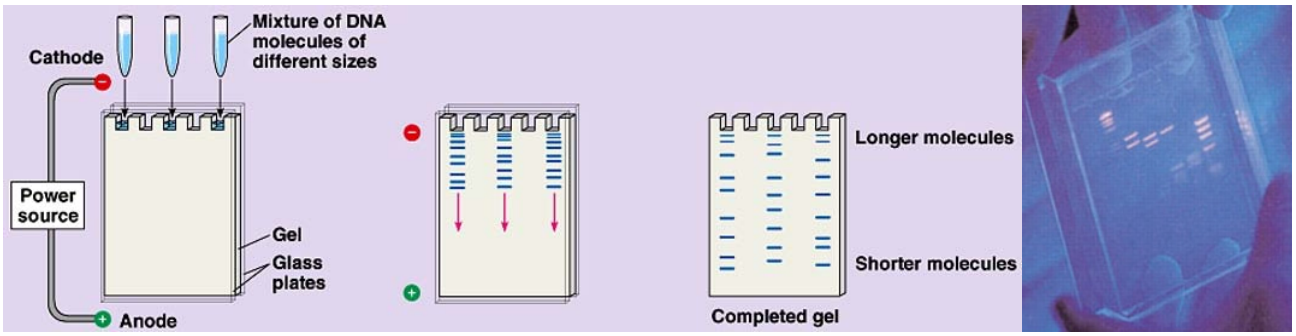
Isolating the Desired DNA from a DNA Gene Library

Target genes can also be isolated using gel electrophoresis. The variations in the DNA sequences between individual DNA samples are determined by differences in restriction enzyme cleavage patterns called **restriction fragment length polymorphisms** or **RFLP**s.)



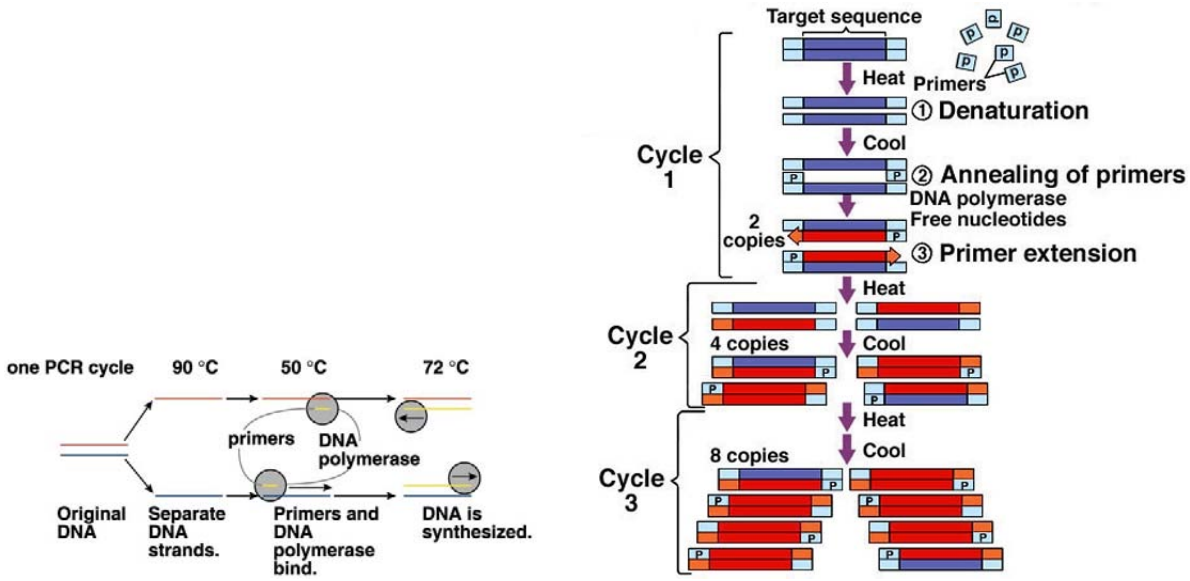
These different DNA fragments can be separated using **gel electrophoresis** and then isolated for closer study using additional techniques. Gel electrophoresis is a common practice in gene analysis, for basic research, for medical research and for forensic study. Gel electrophoresis may be used together with DNA probes and other techniques to more readily identify target DNA sequences.

Gel electrophoresis separates charged molecules based on their molecular weight. An electric current is used to "drive" molecules that are placed in wells made in the gel from the negative electrode of the gel chamber toward the positive electrode. The rate at which molecules move through the gel is relative to their molecular weight. As the molecules are separated they appear as distinct bands on the gel. DNA fragments have a strong negative charge in neutral pH so they are well suited for the technique of gel electrophoresis.



Obtaining A Sufficient Amount of the Target DNA Source – PCR

Once a gene has been located, researchers obtain multiple copies of the gene for their work. One method to obtain sufficient DNA is the **Polymerase Chain Reaction (PCR).** PCR is very valuable when trying to do a detailed analysis of a DNA molecule. PCR is also valuable when there is just a tiny amount of DNA from which to start. This is often the case when one is using DNA materials for potential evidence in criminal investigations, or when one is trying to reconstruct DNA from preserved and fossil materials. Kary Mullis won the Nobel prize for his 1986 development of PCR. PCR uses alternating heating and cooling cycles starting with heated single-stranded DNA, primers that can join complementary DNA and DNA polymerase isolated from thermophilic bacteria to synthesize new molecules.



Polymerase Chain Reaction

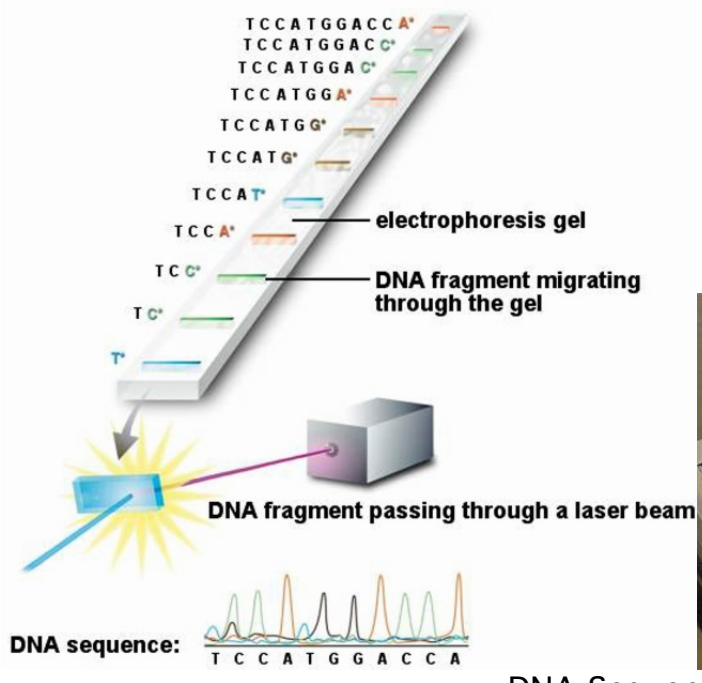
Applications of Biotechnology

Genome Mapping

The techniques of recombinant DNA technology have been used to generate the gene sequences of species, or the species entire **genome**. This has been for several different organisms, including the human genome. The same techniques can also be used to compare genes from different individuals and from different species.

The human genome project, on-going for more than a decade, was deemed completed in summer, 2000. Genome projects include gene mapping using recombination frequencies and DNA restriction fragment mapping, restriction enzymes and DNA probes repeatedly along a chromosome with known markers, along with some special sequencing techniques. Once a genome map is available, genes are studied to identify nucleotide variations for gene disorders, allele differences, and to study the relationship of genes to gene expression. Genomics has been very useful for genetic diseases such as cystic fibrosis and sickle cell anemia.

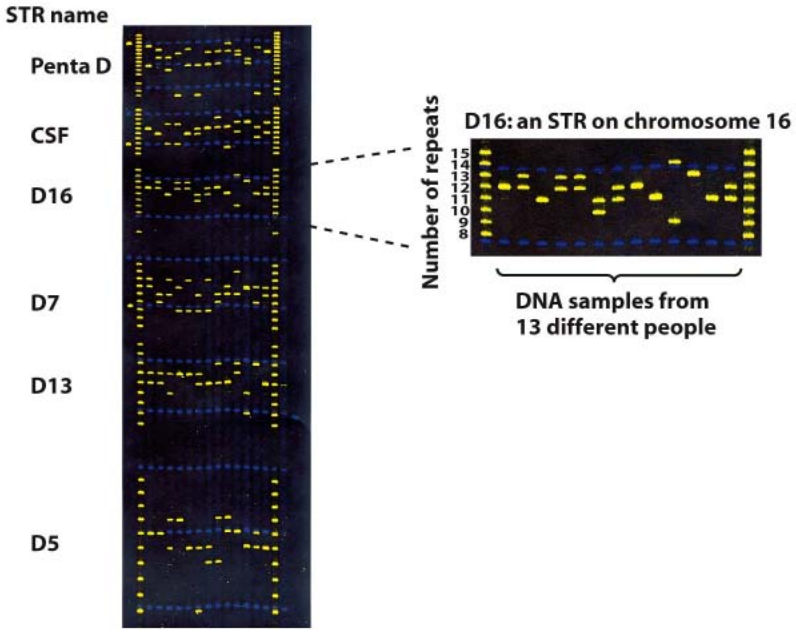
We also use our knowledge of the genome to look for new gene sequences in different organisms. For example, the homeotic genes that affect developmental sequences in all animals can be isolated from a well-known species, such as the fruit fly, and then used as a DNA probe to see if a comparable gene is also in other animals, including humans.



DNA Sequencing

DNA Profiling

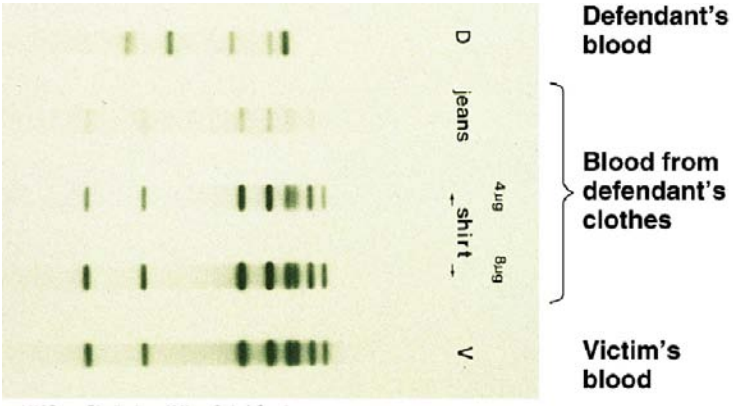
Increasingly, STRs are used for individual profiling (or DNA fingerprinting) precisely because each of us has these unique sequences. Blood or DNA samples provided by those in the military or those convicted of certain crimes are kept in national databases for potential identification purposes.



Use of STR bands for DNA Profiling

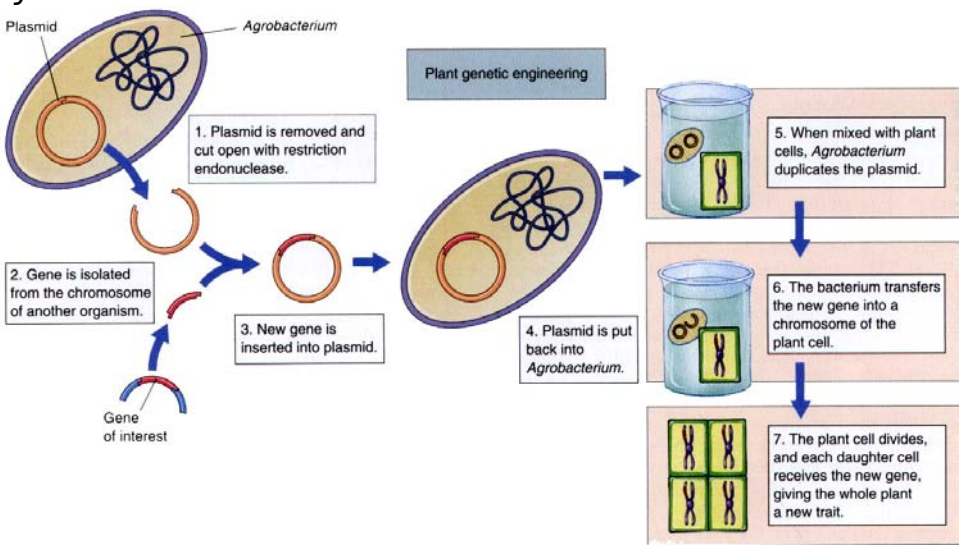
DNA profiling can also be used when an individual's DNA is subjected to a number of different restriction enzymes. Each enzyme recognizes and cuts RFLPs (restriction fragment length polymorphisms) differently. The DNA is then run on a gel, and the patterns produced can be compared to target samples.

Because of the sensitivity of DNA analysis using these STR and RFLPs techniques, forensic cases can use DNA from small samples of skin, hair roots, semen, and even dried blood. The accuracy of these techniques for positive identification is very high. Similarly, a child's DNA "fingerprint" will be a composite of its two parents. DNA fingerprints can also be used to match potential donors and recipients for medical purposes. DNA fingerprints also help establish evolutionary relationships.

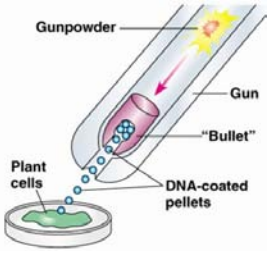


Plant Uses of Biotechnology

Agrobacterium tumefaciens, a natural tumor-causing bacterium of plants, is the host bacterium for much DNA technology in plants. The tumor genes are removed, and a plasmid, the **Ti** plasmid, incorporates desired new genes. The altered *Agrobacterium* "infects" a tissue-cultured plant, which may express the inserted genes normally.



Genes can also be "shot" directly into plant cells with a **"DNA gun"**. The DNA "gun" injects coated DNA particles into the target plant cells.



DNA technology has been used to help plants be resistant to frost, wilting, herbicides such as round-up and to resist insect and fungal pests and viral and bacterial infections.

Many transgenic food crops have been approved. It is estimated that as much as 70% of the foods on our grocery shelves contain ingredients from crops that are genetically modified. The vast majority of these foods come from recombinant corn and soybean products. We eat almost no recombinant corn or soybeans as whole foods, but use oils processed from corn and soy, and high-fructose corn syrup from corn in hundreds of food products.


Wilt-resistant Carnations


Round-Up resistant Petunia


Weevil-resistant peas

Plants as Vaccine Vectors ("Pharm" Plants)

Research is ongoing to introduce vaccines into fruits or vegetables, so that obtaining immunity could be as easy as eating a banana. However, there are huge issues of controlling the amount of vaccine that might be expressed in the plant, or even if the vaccine got expressed in the edible portion of the plant.

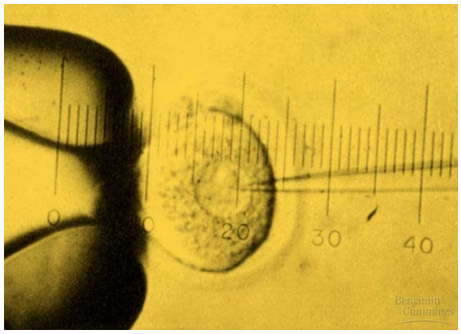
A modified approach is to have plants synthesize the vaccine and then extract and purify the product just as we do today with many gene products synthesized by bacteria into which desired genes have been incorporated.

Research is also ongoing in areas of having plant foods contain anti-bacterial or anti-viral proteins that would "destroy" intestinal viruses or proteins. Again, since most proteins are digested in the stomach and small intestine, success is remote, and normal intestinal bacteria could also be impacted.

Pharm Animals

DNA technology uses virus vectors to incorporate desired DNA into mammalian egg cells that develop into adults that produce certain needed or useful human products.

"Pharm" sheep milk contains a protein that minimizes lung damage-associated respiratory diseases, including cystic fibrosis. The molecule can be extracted from the milk and marketed. Since the protein produced by the pharm animal may not be identical in all respects, testing is essential. Possible allergic reaction is a concern, as it is with genetically modified foods.



Injecting DNA into embryo cell

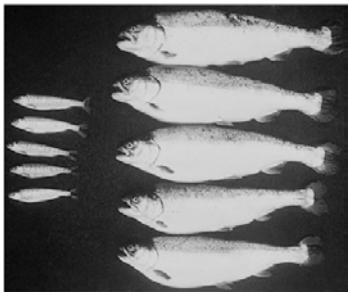

Pharm Sheep

Pharm goats produce spider silk, an incredibly strong protein that is used in protective vests. Other products produced in mammalian milk include blood-clotting factors, red blood cell synthesis promoting factors and factors that break down blood clots that cause heart attacks and strokes.

Growth Hormones

Bovine somatotropic hormone (BST, also known as BGH) has been successfully cloned and its use approved. This hormone increases milk production (something that is desired by many in the dairy industry because the same volume of milk can be produced with fewer cows. BST is also being investigated to see if it increases muscle development in cattle and pigs.

We have a number of transgenic organisms into whose egg cells or early embryos a growth hormone has been injected. Such animals reach maturity much faster than normal so that they can be marketed sooner.



Salmon given growth hormone

Mouse given human growth hormone

Human growth hormone can be produced by bacteria in culture, isolated and purified. The growth hormone can be used to treat children who are genetically lacking this hormone. It helps achieve more "normal" growth patterns. Sometimes parents of children of normal growth request growth hormone treatments thinking it will help their child to become taller and more successful. No hormones should be used frivolously.

Selective Gene Breeding

Genetic engineering is widely being used for selective breeding in cattle, horses and other domesticated animals, saving many generations of breeding to get desired characteristics. Moreover, with cattle, at least, the young totipotent embryo can be "teased" apart so that one zygote can be used to make a dozen identical offspring.

With gene selection, one problem is that the insertion of the target has to be at a gene locus that can be "read" and must be inserted into gametes to be expressed in the whole organism, or at least into the target tissue area. In addition, the inserted gene cannot disrupt normal activity. Since there is little control over where the gene gets spliced into the host egg cells, such mutant or **transgenic** animals often have a low survival rate.

Biotechnology in Health, Medicine and Genetics

Treating Disease

We still have no permanent cures for genetic diseases. We can treat many genetic diseases with substitute substances that can be used to replace the substance the individual cannot synthesis, such as human insulin. Once identified, we can treat some disorders, such as phenylketonuria, with a life-long diet that ensures the impact of the missing gene product does not prove lethal. Many research projects are seeking ways to alter the genetic code to repair faulty genes in affected individuals using cell transplants that carry the "correct" code. But most of our treatments of genetic disorders are much less glamorous than transplanting cells that may survive and produce the needed substances for survival. We will discuss some of these.

Making Molecules

One outcome of DNA technology is that the desired DNA can be used to manufacture needed molecules, such as humulin, clotting factors, interleukin-2, tumor necrosis factors, and a number of vaccines for human health. Although bacteria, and especially E coli, are used most, some products are made from incorporating the genes into yeast cells or even mammal cells.

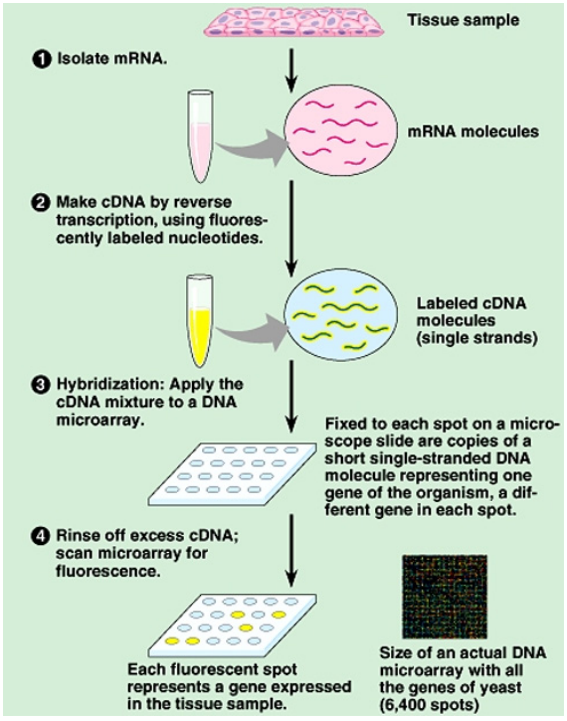
Genetic Screening.

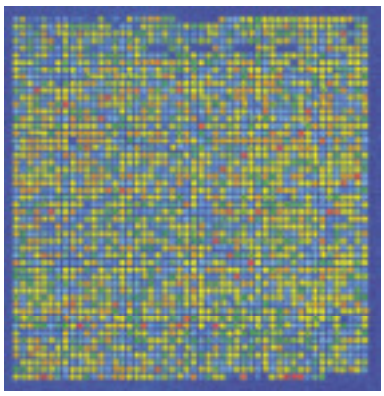
Genetic screening is used to detect a number of genetic disorders, such as Huntington's, Tay-Sach's disease, sickle cell anemia and cystic fibrosis. There are a number of techniques used, from blood tests that can identify certain problems, such as phenylketonuria, to more sophisticated analysis. Prenatal screening can help potential parents determine the risks of genetic disorders in their children, and perhaps, in the future (which is today) apply biotechnology to correct gene defects in the embryo.

In the1980s researchers first succeeded in deactivating a good gene in mice so they could study the effects of a defective version. This has proved useful in studying specific genetic defects. This process is often called genetic **knockouts**. For example, such mice were used to study cystic fibrosis, Huntington's disease, Alzheimer's and some cancers.

DNA Arrays

DNA technology can also be used to produce a **DNA microarray assay** that results in a gene microarray or **biochip**, a small but discrete collection of gene fragments on a chip. With DNA technology, these can be automated and give information about thousands of genes, all located on one array or biochip.



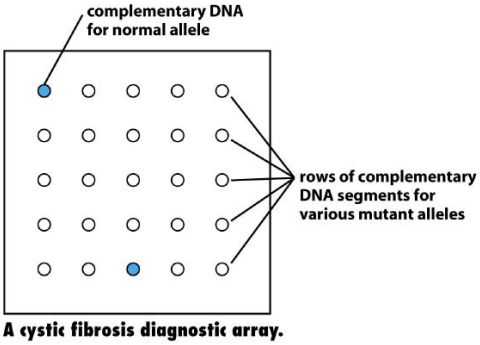


With the use of biochips, researchers can compare an individual's **single nucleotide polymorphisms*** (SNPs), which to the standard from the human genome project, or another biochip, identifying how the individual differs from the standard. Most people have a SNP about one per thousand nucleotides. SNPs may be responsible for many genetic differences, from cystic fibrosis and sickle cell anemia to red hair and cholesterol levels. A database of perhaps 300,000 SNPs is being developed and may be a future way for physicians to screen most accurately for genetic diseases. Your biochip may also uniquely identify all that is you, genetically speaking.

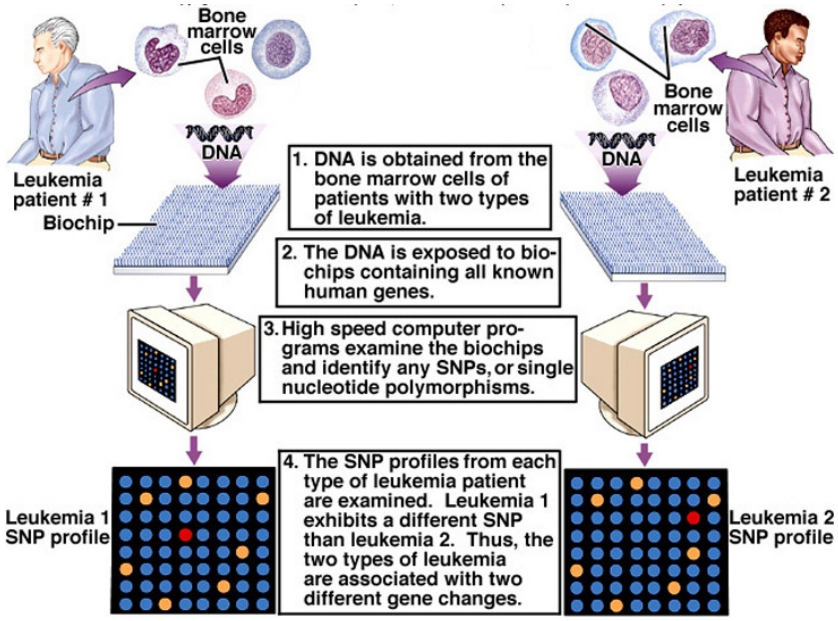
*Single nucleotide polymorphisms are similar to the short tandem repeats (STRs) used in forensics, but they are variations in a single nucleotide between different individuals, different population cohorts, or even one's homologous chromosomes.

Microarray analysis is used in cystic fibrosis screening. Families in which cystic fibrosis is found can be screened to see which family members carry a cystic fibrosis allele.

Microarrays can also be used in cancer screening, for example, to better target a specific treatment to a specific cancer, not just for finding a genetic profile.



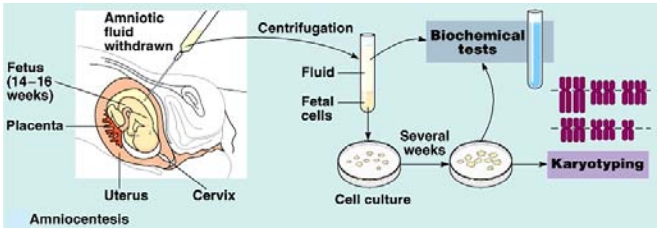
A cystic fibrosis diagnostic array.



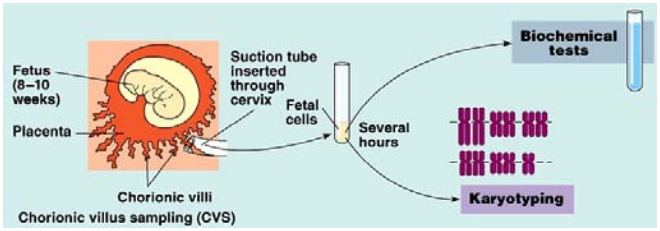
Prenatal Analysis

Prenatal analysis can screen for several abnormalities. There are risks to doing so, however. The decision about what to do if severe abnormalities are detected is one of the most difficult any person can face.

- Amniocentesis obtains fetal cells from the amniotic fluid during the14th to 17th week of pregnancy and requires several weeks to culture the sample.



Amniocentesis



Chorionic Villus Sampling

- Chorionic villus samples fetal tissue from the placenta. It requires more cells than amniocentesis but can be done earlier in the pregnancy.
- Ultrasound can determine several characteristics of the developing fetus
- Fetoscopy uses a thin viewing scope and fiber optic light source inserted through the uterus to view the developing fetus
- Pre-Implantation Analysis – If eggs and sperms are used for in vitro fertilization, cells from very early development can be examined for certain chromosomal abnormalities, such as Huntington's, muscular dystrophy and cystic fibrosis. If no abnormality is detected, the mass of cells can be implanted into the female for development.

Gene Therapy

Gene therapy involves transplanting cells that contain the "normal" gene into tissues of the affected individual. To be effective, transplanted cells must:

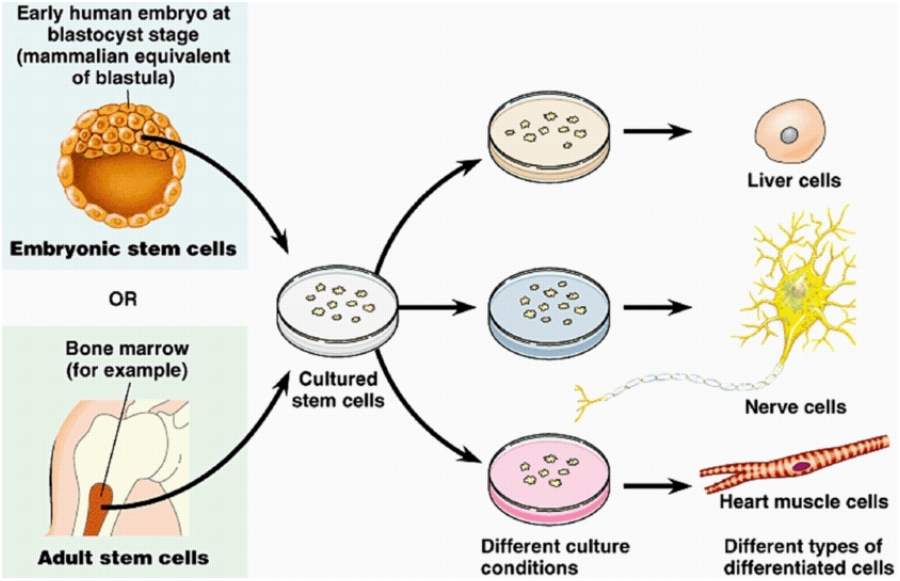
- Not trigger the immune system of the individual to reject them
- Must be in the appropriate tissues
- Must be functional; that is make the substance the gene codes for in the appropriate levels.

All of these things are difficult. There are serious risks when trying to use vectors to splice genes into the chromosomes. Viral DNA may itself cause problems in the chromosome and negatively affect gene expression.

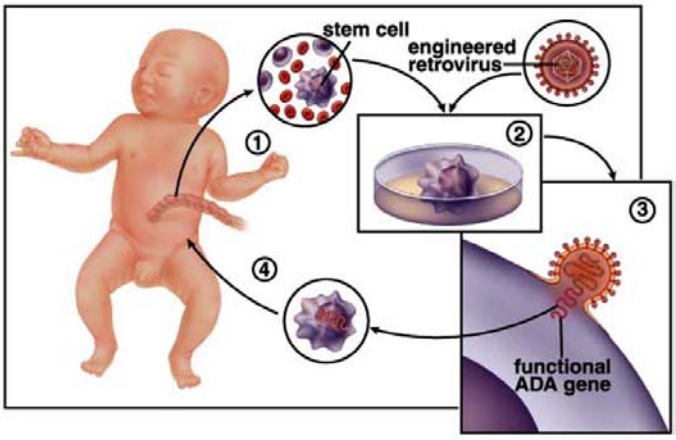
Stem Cell Research

Undifferentiated stem cells hold much promise in gene therapy to restore damaged and lost tissue. Embryonic stem cells (from the early embryo) are totipotent; no differentiation has occurred. Stem cells are also found in differentiated tissues, and are called **tissue-specific stem cells.** As tissues differentiate in development, some cells remain, even in the adult, as stem cells. Bone marrow stem cells are used in leukemia therapy that involves bone marrow transplants. Success varies.

Mouse heart cells have been cultured from embryonic stem cells, and have been successfully transplanted into damaged heart tissue. There is promise that the technique could work in humans, too.



The gene needed for preventing severe combined immunodeficiency disorder has been successfully transferred to bone marrow cells of children who have this genetic disease. (The gene codes for a critical enzyme, ADA, needed to activate the immune system cells.) Stem cells from umbilical cord blood are harvested and the ADA gene is inserted using a retrovirus. Introduced into a genetically "impaired" child, the altered cells carry the needed gene to bone marrow, implant there and produce the needed enzyme.

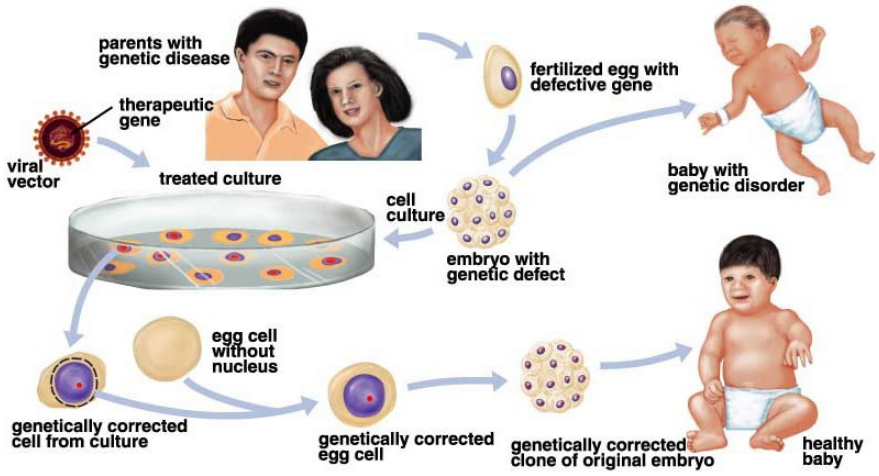


Other diseases in which gene therapy is being attempted include:

- Cystic fibrosis
- Hemophilia
- Muscular Dystrophy
- Some cancers
- Hypercholesterolemia
- Rheumatoid arthritis

Correcting Defective Genes in the Embryo

For the future we can expect similar techniques to be used in conjunction with gene corrections of "defective" genes. A couple with a known genetic disorder can provide fertilized eggs for culture. Embryos can be grown in culture along with a vector that carries the normal gene sequence. Genetically corrected nuclei can be extracted from the embryo culture and implanted in enucleated eggs from the mother. The genetically corrected egg can now be implanted and a "normal" child can result. It was recently announced that an embryo that carried gene markers for Alzheimer's had been "corrected" this way.



Concerns about DNA Technology

Some question the ethics of manipulating a genetic code that has evolved over billions of years just because we humans have reached a stage in our knowledge and technology when we can start to do so. Some of the questions we might ask are:

- What happens if a gene splices into an inappropriate location in the target host's DNA or has negative effects on the host organism? What do we do then?
- Will the genetic alteration affect only the target organism and with the target effect? Or might it affect some non-target organism deleteriously? For example, using genetic engineering in plants to provide pesticide and herbicide resistance means that we can grow crops with less use of the pesticides and herbicides that pollute our air, water and soil. However, pollen from the modified crops can spread to other areas, affecting the gene pool of the plant as well as impacting other organisms. Pollen from corn engineered with BT coats non-target plants in the area. Butterflies that feed on the non-target plants are then affected.
- With recombinant DNA, we can reduce dramatically the amount of pesticides and herbicides that are currently being broadcast into our environment, that affect not just the target pest, but most other organisms related to the target pest. But in a similar fashion, the genetically modified product might also harm a susceptible non-target that eats the product, as mentioned above.

- In agriculture, there is hope of vastly improving plant productivity, by growing plants in areas not poorly suited for agriculture, such as saline soils, or soils with little moisture, or low fertility. What will this mean for the numbers of humans and non-humans who inhabit this earth now? What impact will this have on the earth's other resources? Not to mention the impact on that area not now suited for human agricultural practices.
- Should we have concern that, no matter how remote the likelihood, some altered organism might escape and cause grave harm to our ecosystems? After all, some natural organisms do just that.
- With humans, we are concerned that gene products, which are frequently proteins, might trigger an allergic reaction in someone who unwittingly eats a food containing a protein to which he/she is allergic. (This is actually a risk for anytime anyone eats a protein-containing food that he/she has not yet eaten.)
- With better screening ability, what are the ethical issues associated with screening for life threatening diseases? Should any genetic testing be accompanied by appropriate education and counseling so that families can make informed decisions about what they are discovering? There is always talk about insurance companies or potential employers using genetic screening to "screen out" those who might have expensive to treat problems.
- As mentioned earlier, what about cloning and making more copies of certain individuals? Or choosing the genes one wants his/her children to have?

No matter how we address these concerns, or different concerns that can arise with altering DNA, the research conducted today is under very strict peer and national review, and oversight commissions, as legislated in the United States. These rules were developed in the past, and as knowledge changes, rules and applications must also change.

Each of us, in our lifetimes, as citizens, may be making decisions about the use of DNA technology for medicine, food production and crime, the use of embryonic tissues in research and treatment of diseases, gene experimentation on humans, and even cloning. These are social, political and ethical issues. The more knowledge we have about DNA, genetics, and the application of our basic science research, the better able each of us will be to make informed decisions and contribute intelligently to our political decision-making processes.

Principles of Evolution - 1

We have seen in this course that recombination, segregation of alleles, and independent assortment of homologous chromosomes during meiosis results in the variation that occurs among individuals in populations. We have seen, too, that mutation is a source of increasing variation within populations. Each individual's phenotype depends on how the alleles he or she inherits interact in gene expression.

Some inheritance patterns, such as multiple alleles of a single gene, and the continuous variation resulting from polygenic inheritance, are observed only within the framework of **population genetics**.

We have also learned that the frequency of a gene (or specifically, an allele) affects its appearance in populations. For example, in human blood types, B is a co-dominant allele, though not common within most populations, so that O and A phenotypes are much more abundant.

In the next few lectures we will look at variations that appear within populations with reference to the way an individual "contributes" to the population's genetic mix or **gene pool**, and how this gene pool is changed from generation to generation, which is the study of **evolutionary biology**.

As an introduction, let's begin with a working biological definition of **evolution**, to avoid the confusion and misconceptions that sometimes surround this term.

Evolution is biologically defined as the change in the frequency of a gene's (or allele's) appearance in a population's gene pool from generation to generation (through time). More simply, evolution is inheritable change in organisms over time.

Note the following components to evolution:

- The importance of genetics - the frequency of a gene's appearance
- The importance of time - in generations
- The importance of populations - the aggregate of individuals

It will also help to define what a **species** is: A species is a population of organisms that is naturally capable of interbreeding among themselves, but does not interbreed with other populations of different species. If interbreeding occurs, the offspring are infertile or less viable in some way.

As with most of what we study today in biology, the field of evolutionary biology has interesting beginnings.

Throughout recorded history, humans have been trying to find natural explanations for our observations of events around us, including explanations for the vast diversity of organisms that inhabit this earth with us. Humans of all cultures have always tried to group, or **classify** organisms, in attempts to clarify what has been observed. One of the concerns in classification was to determine what comprised a unique group of organisms, what we now call a **species,** a question still debated to some extent today. In earlier centuries, most considered visual characteristics for defining groups or species; today, we mostly use reproductive isolation as the major determinant of a species. In the 1700's **Linneaus** developed the classification system in use today - binomial nomenclature. Each species is given a binomial. Closely related species share the Genus name, genera are grouped into families, then orders, classes, kingdoms and domains.

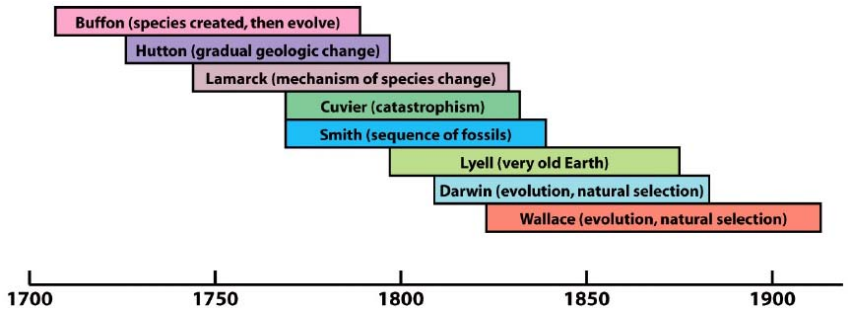
Plato, an early Greek philosopher, proposed that each object on earth was a reflection of its divine “ideal form”. Aristotle (Plato’s pupil) had one of the more interesting systems of classification among the earlier "biologists". He developed a "ladder of nature" from simple to more complex, using standards that made sense at that time.

In the 6th century BC, Anaximander proposed that organisms were not created, but animals changed through time. He proposed that vertebrate animals, for example, descended from fishes.

As centuries went by many schemes existed for classification, but the prevailing thoughts were that each species, or type of organism was seen to have a **fixed** place in the order of things, since the beginning of time, or creation.

The problem became more difficult as European biological exploration encompassed more of the world in the 17th and 18th centuries, and more and more different habitats and types of organisms were described. By the 18th century, the prevailing thoughts on the "cataloging" of living organisms were being questioned, along with the long-held premise that each organism and indeed our earth had been created "as is" all at one time. Why was this so?

Historical Evidences for a natural, scientific, system for explaining species diversity, and for questioning an "as is" creation.
In the 1700's and 1800's there were a number of questions being asked, based on evidence from geology, fossil studies, distribution or organisms on earth and anatomical studies of organisms.



Early Proposals for Organisms Changing Through Time

Buffon

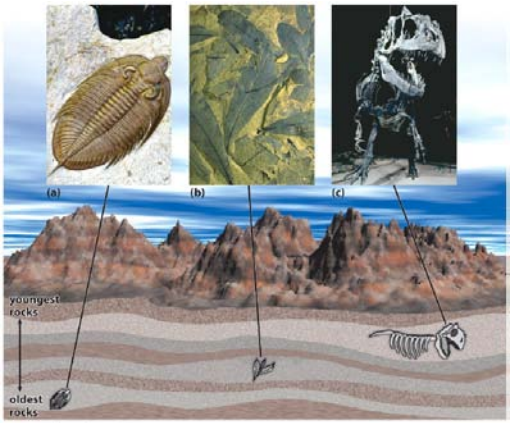
In the late 1700's, a zoologist, **Buffon**, speculated that perhaps there had been several "centers of creation" and that species were "conceived by nature and produced by time". His hypothesis was not widely accepted since he had no mechanism to explain how nature could do this, or how there had been enough time on earth for the modifications to occur. Buffon used **Linnaeus'** classification schemes to support his ideas on how organisms related to each other.

Cuvier

Cuvier proposed that species were created at one time, but a series of **catastrophes** periodically destroyed many species. The survivors of these catastrophes were found in the current world. However, the fossil record did not contain remains of any of the species from the current world, although one would think that at least some of the individuals of the surviving species would have perished in the catastrophes. Agassiz, a follower of Cuvier, proposed multiple creation and catastrophe periods to answer that question, but to do so, he proposed that there had been 50 catastrophes and new creations.

The Geological Time Frame

William Smith recognized in the early 1800's that the same fossil types were always found in the same rock layers, and that the organization of fossils and rocks was consistent.

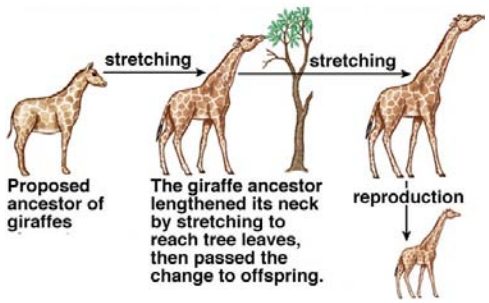


Lyell and **Hutton**, geologists of the 17th and 18th century, both argued that geological events did not require catastrophes, but were natural processes of weather, volcanism and earthquakes.

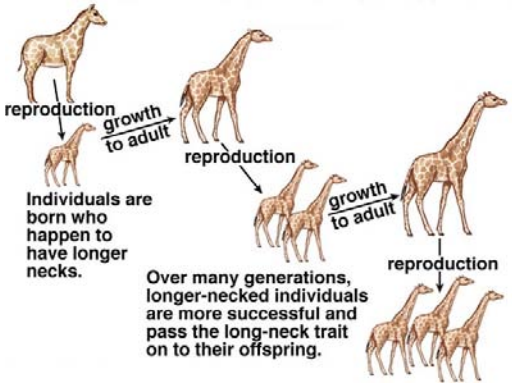
Lyell stated that the processes that formed the earth's surface "today" had also been active in the past, proposing that the **earth was millions of years old**, instead of the few thousands that was believed at the time by most. This meant that the time needed for changes in living organisms could be put into a time frame of many millions of years, long enough for evolution to have occurred. Natural events, repeated over time, explained the findings in rock layers, a proposal given the term **uniformitarianism.**

Lamarck's Contributions

About this same time, Lamarck proposed that all organisms had been created in a simple state and were improved by gradual changes into more complex structures. He was much taken by the gradual changes shown in many organisms in the fossil records. The motivation for the improvement, according to Lamarck, was an innate "drive for perfection". The need to have a better structure resulted in some body force directed to fulfilling that need which was then passed on to one's offspring. Lamarck was the first to propose that one's environment was important to survival, although he erred in proposing how changes occurred, and were subsequently passed on from one generation to the next. (But he also preceded Mendel). Lamarck's work has often been called evolution through the inheritance of acquired characteristics, but that is not exactly what he did.



Lamarck's View of Change



Post-Darwin's View of Change

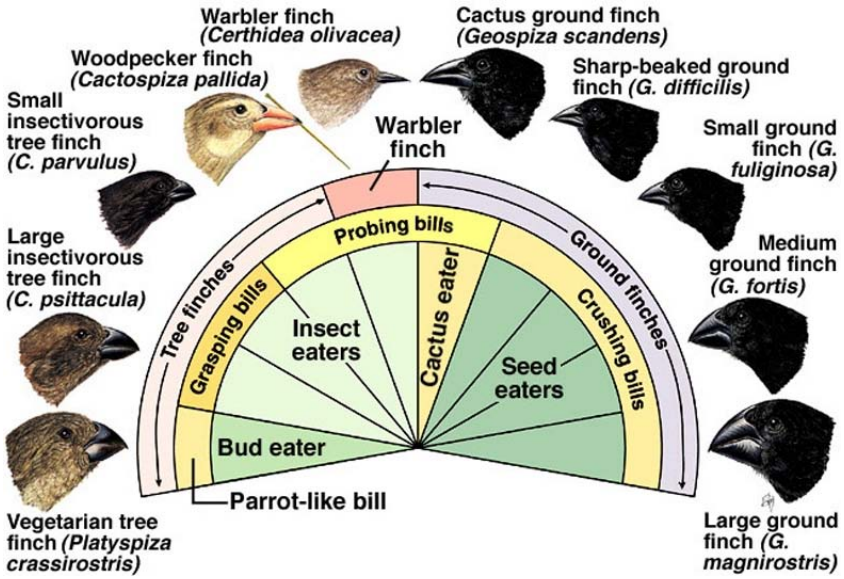
Thomas Malthus

Thomas Malthus made a contribution to the growing question of how we get such variation among organisms when he proposed that all populations reproduce in great numbers but survival is limited by available resources. Individuals compete for those limited resources. As a result of competition, only those best suited survive. Malthus wrote his essay in 1798, and addressed his concerns to the rapidly increasing human population, but the survival of organisms in nature relative to birth rates follows Mathus' predictions.

Darwin and Wallace

In 1858 two naturalists, Alfred Wallace and Charles Darwin, presented a paper to the Linnean Society of London on the origin of species by means of natural selection, followed by Darwin's book *On the Origin of Species by Means of Natural Selection* in 1859. Wallace and Darwin proposed that new species originated from preexisting species through descent with modification driven by natural selection.

They based their paper on their observations of variations among organisms collected while traveling extensively throughout the world as naturalists. Darwin's thinking was influenced by his observations about diversity in the Galapagos Islands and adaptations of animals to the prevailing food sources on different islands. Darwin and Wallace also had the advantage of knowing the works of others who had presented ideas about the origin of species.

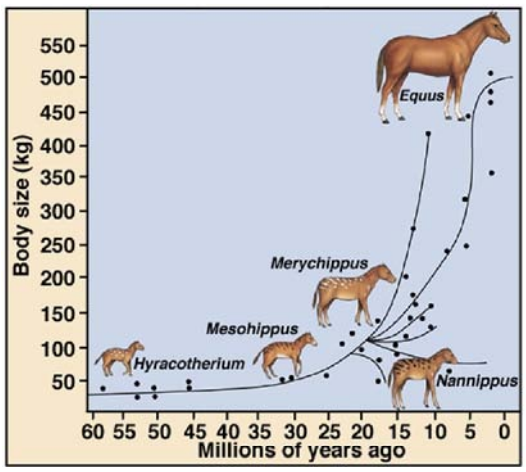


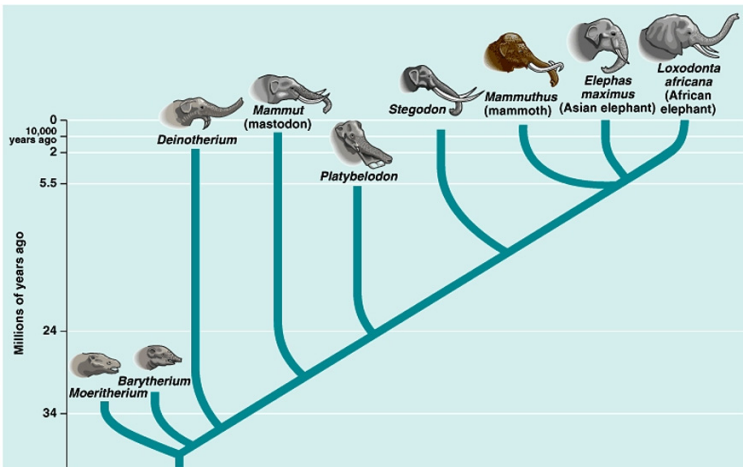
Variation in Galapagos Finch Beak Share

Variation in Mollusk Color Patterns

The theory of Natural Selection was developed to explain adaptations and variations of organisms to their environment based on biological explorations, from the evolutionary evidences of the fossil record, and from anatomical studies. The major statements of this theory are:

- Variations exist among individuals of a species.
- Some of these variations are inherited (genetic).
- Species produce more offspring than can survive.
- Survival depends on ability to compete for food and resources.
- The individuals genetically better adapted to the surroundings survive and reproduce more offspring (therefore passing on the better suited adaptations).
    - Those individuals having less favorable traits produce fewer offspring, so that less successful adaptations are not passed on as frequently.
    - This phenomenon is called **differential reproduction** and successive generations have more individuals with more favorable traits (genetically better adapted to their surroundings).
    - In **natural selection**, inherited traits that are **adaptive** (favorable) will become more frequent in the population at the expense of less adapted traits, which will appear less frequently in passing generations. Populations will change through time in response to environmental factors.





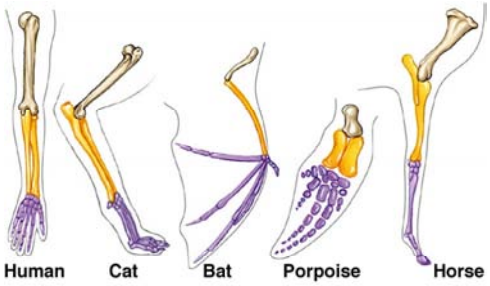
Note the importance of **preexisting variation** to the theory of natural selection, and how better suited variants in a **specific environment** are more likely to survive.

What was missing was an explanation of the **origin** of variation, and the supporting evidence for the process of natural selection. No one could point out the **sources** of variation, because at that time, we did not know how inheritable traits were passed on from parent to offspring (as mentioned briefly in the introduction to genetics. The most controversial component of Darwin's proposals was the idea of gradual change, not the idea of diversity is a result of populations changing through time. Evidence was needed and evidence was forthcoming.

Evidence for Sources of Variations

Comparative Anatomical Studies

Homologous structures – Morphological Divergence such as the arm bones of vertebrates that are specialized different functions

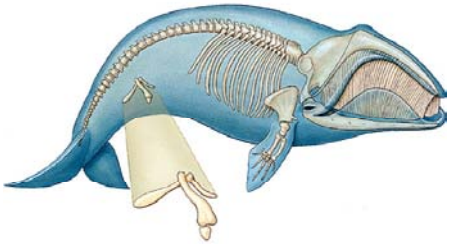


Analogous structures – Convergent Evolution: Morphology that appears similar but has different origins (wings or insects, bats and birds, body shape of seals and penguins, etc.) The similarity of marsupials to placental animals is another example of convergent evolution.

Insect and Bird Wings

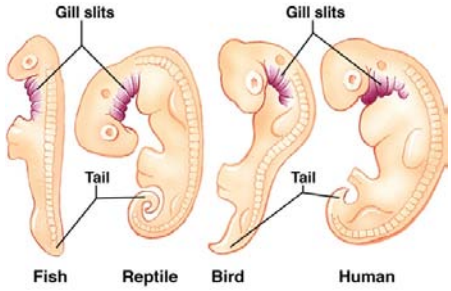
Ocotillo (US) - Allauidia (Madagascar)

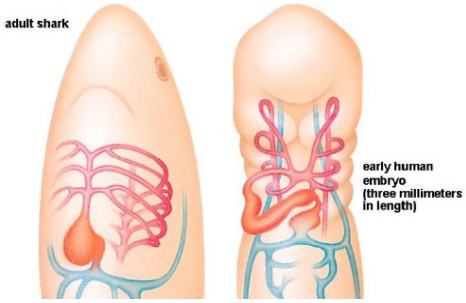
Vestigial structures: Structures that have no "function" but have a common origin, such as pelvic bones in snakes and whales that have no lower appendages, and the human appendix, a vestigial ceacum.



Whale Pelvic Girdle

Common **Embryological** Structures, such as gill slits in vertebrates or heart structural development

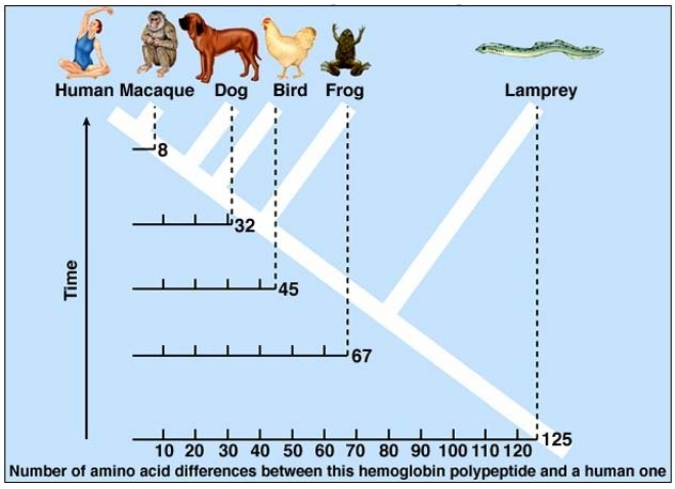




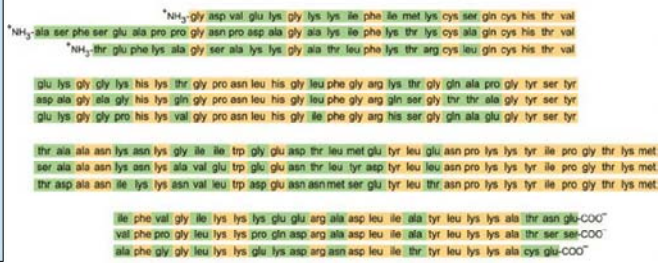
Heart in Shark and Human Embryo

Biochemistry and Molecular Genetics: DNA Homologies and amino acid sequences reveal relationships and can date divergence among groups of similar organisms.

- All cells have DNA as the genetic molecule
- All cells use RNA and about the same genetic code to translate DNA genetic information in the synthesis of proteins, using the same 20+ amino acids
- All cells use ATP as the energy carrier



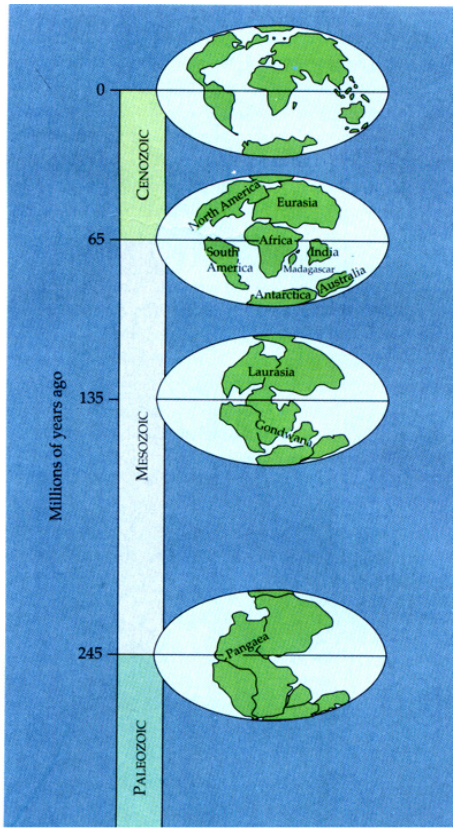
Hemoglobin Comparisons

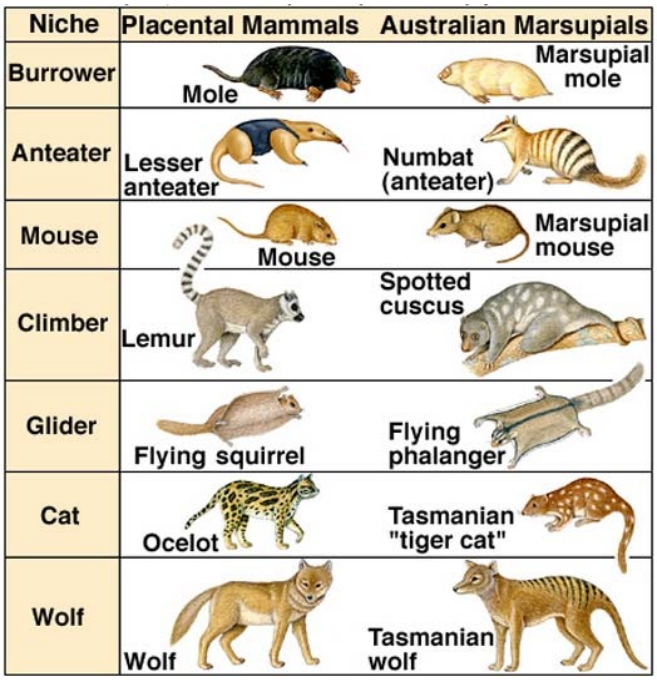


Cytochromes in Yeast, Wheat and Primate

Biogeographical Data

Distribution of organisms based on plate tectonics and continental drift explains diversity of organisms based on divergence and time. The Australian plate separation resulted in separation of marsupials. Placental animals in other areas of the world fill the same environmental niches.





Genetic Origins of Variation

Meiosis and sexual reproduction

- Homologous chromosomes of diploid individuals with gene pairs
- Segregation of homologous chromosomes in meiosis
- Recombination
- Independent assortment of homologous chromosomes during meiosis
- New combinations of alleles with each fertilization

Mutation

- Changes in the DNA sequence which are passed on by subsequent cell divisions

Changes in gene pool frequencies

- Recall that evolution deals with changes which involve populations; an individual does not genetically evolve.
- The gene pool of a population is the total combination of alleles of all of the members of that population.

Evidence for Natural Selection

Darwin and Wallace had presented extensive examples of the variations observed in members of natural populations to propose their theory on origin of species by natural selection. To support this, Darwin also looked at **artificial selection.**

Darwin was much taken with the selective breeding of domestic animals, and used that as evidence for how selection could result in numerous variations. He used the domestic dog as one example of artificial selection. Cabbage, Brussels sprouts, cauliflower, broccoli, kale and kohlrabi are all derived from the same mustard species.

We also have evidences in nature that demonstrate how selection works. During the Industrial Revolution, soot from using coal coated and killed lichens on trees in England, darkening the bark. At that time, the peppered moth came in two variants: dark and light. On trees where soot was not evident, birds were able to see the darker moths, and the lighter moths became more prevalent. In the industrial areas, the soot covered trees made the lighter form more visible to their predators, and the camouflaged darker variant became more abundant. Because the cause of the selection pressure was human impact on the environment, this is often called induced selection.

Evidence demonstrating origins of variations that are passed from generation to generation accumulated in the late 1800s and continues today.
A mutation in cockroaches that resulted in distaste for glucose resulted in populations resistant to a common roach treatment (a poison that contained glucose). It took only a few generations to accomplish this (thanks to the poison killing all roaches lacking the glucose distaste gene that ate the poison and the short generation time needed to build up populations with resistance). Pesticide resistance is common among insects.

Guppies raised in environments without predators have more colorful (to our eyes) patterns than guppies raised with in environments with visual predators.

Antibiotic resistance in bacteria is another good example of contemporary evolution in action. Virus evolution, including the evolution of HIV is also studied.

It's important to always remember the following:

- Variations that are subject to selection must either exist within the population prior to the selection "pressure" or arise by mutation
- Selection favors individuals adapted to a specific environment. A changed environment may favor the same individuals.

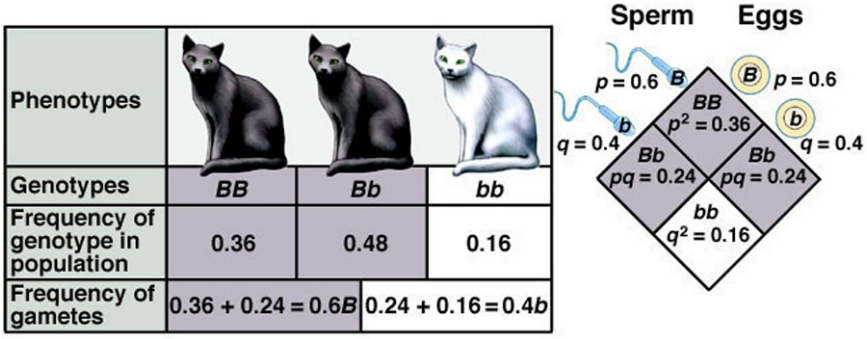
We shall discuss selection as a mechanism for the process of evolution in more detail a bit later. It took a physician and a mathematician to get us started of looking for the agents or mechanisms that could promote **change** in populations by acting on variations in gene frequencies.

The Gene Pool and Genetic Equilibrium

As we stated at the beginning of our discussion on evolutionary principles, evolution involves changes that occur in the frequency of a gene's alleles in a population from generation to generation. Each individual member of a population inherits a set of genes, and for most of these genes, two alleles. He or she cannot evolve or change the alleles inherited. But the contribution he or she makes to the population's gene pool through reproduction, relative to the contribution other members of the population make, can change the population's genetic composition from generation to generation.

The collection of genes (alleles) in a population is called the **gene pool**. For a population's gene pool to change, there must be some mechanism that promotes differential reproduction or differential survival of one allele that is reflected in reproduction. When such change occurs, we have evolution.

A review of the inheritance of a single gene illustrates this. In a population of cats, assume there are two pre-existing alleles for coat color: black and white. Black is dominant. The two coat colors have been reproduced year after year. Then one year, a new nocturnal predator enters the environment. This predator sees the white cats at night and eats them. The white cats rarely reproduce, since they are eaten when young and are more visible to predators. Within a few generations, the frequency of the white allele diminishes significantly. The predator has been the selection force behind the change in coat color allele frequency.



Initial Frequency of coat color alleles

We can see this and explain this today, because we know how genes and alleles are inherited. In the 1800's, they did not know this, and for about 50 years after Darwin's publications, scientists and others searched for mechanisms of evolution.

It took several years to bring together genetics, population biology and natural selection as means of evolution. It started in 1908.

The HARDY-WEINBERG principle for genetic equilibrium

Hardy and Weinberg demonstrated that the equation for a binomial expansion

$$(p^2 + 2\,pq + q^2 = 1)$$

could be used to calculate gene frequencies within a population. They first showed the gene frequency needed for **genetic equilibrium**, the condition in which gene frequency would not change from generation to generation, hence no evolution.

Genetic Equilibrium Formula

Where: p = frequency of 1st allele for gene (A)
q = frequency of the 2nd allele for gene (a)
and:
$p + q = 1$

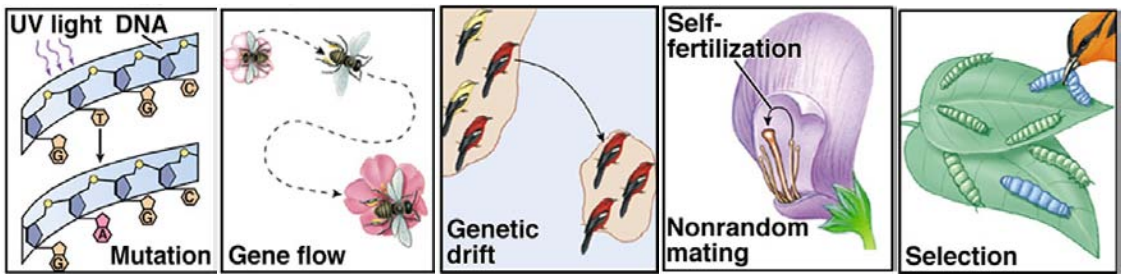
Where: p^2 = homozygous (AA)
q^2 = homozygous alternative (aa)
2pq = heterozygous (Aa)
and:
$p^2 + 2pq + q^2 = 1$

- The allele frequencies and genotype frequencies will be stable (genetic equilibrium) from generation to generation as shown by this equation.
- In an ideal population there would be genetic equilibrium (no change in gene frequencies), and no evolution would occur.
- Hardy and Weinberg then proposed the **conditions** that would be needed in a population to have **genetic equilibrium**:
  - Population must be large enough to eliminate chance or random gene frequency fluctuations
  - Population is isolated from other such populations (no immigration or emigration; no gene flow)
  - Mutation does not occur, or if mutation occurs, forward and reverse mutations are equal, so the gene pool is not modified
  - Mating is random
  - All genotypes are equally viable; natural selection is absent

Any change in gene frequency from generation to generation can then be documented and we can look for the reasons or agents responsible for the change.

As a result of the Hardy-Weinberg Equilibrium, biologists could search for the "agents" of evolution, or those factors that result in the change of gene frequency. You can see why evolution is now defined in genetic terms, since it is a biological phenomenon of population genetics.

Based on their work, there are five major causes or agents of change in populations:



Agents of Evolution: Factors that bring about change

1. Mutation

- Inheritable changes in the DNA sequence
- Can be induced for study
- Original source for infinite numbers of small changes in genes

Recombination

- Natural occurrence in meiosis so that no two gametes are identical

2. Gene Flow or Migration

Migration is the flow of genes from one population of a species to another population. This is also called gene flow.

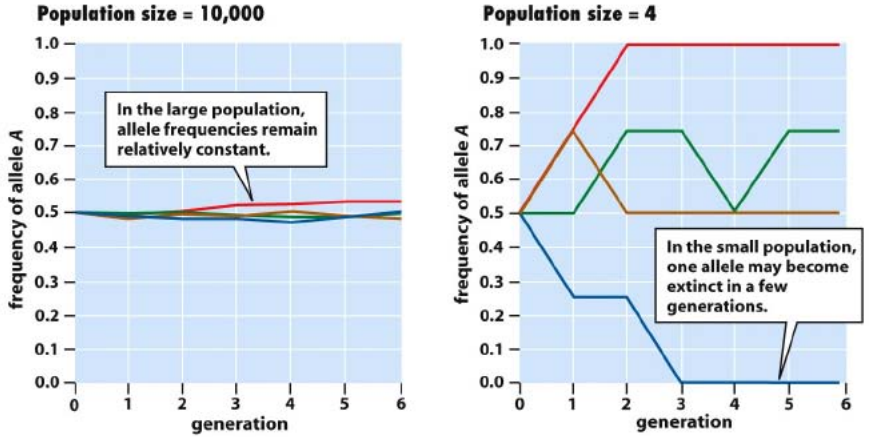
- Dispersal to new geographical areas
  - Fledging of young
  - Transport of pollen, spores, etc.
- Gene flow also maintains a gene pool over larger geographical areas with nomadic patterns of travel. Gene flow usually decreases genetic differences between populations by routinely adding and removing individuals.
- Gene flow tends to keep populations of species from varying too much by continually "mixing" the alleles of the species.
- In contrast, isolated gene pools are important factors in speciation, since they minimize gene flow, and small populations are more subject to random events that limit the gene pool from one generation to the next.

Genetic Drift and Small Populations

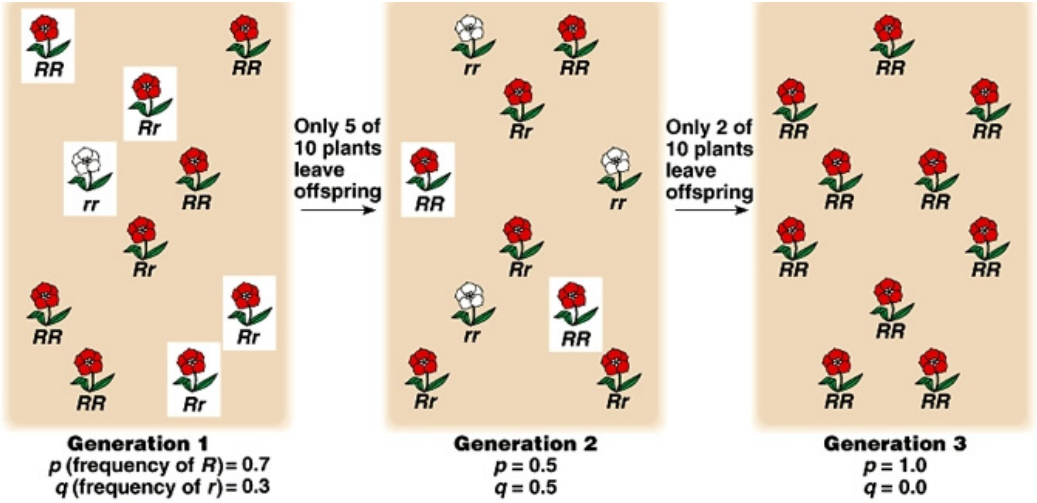
Any population can be subject to rapid and random changes that can be caused by chance events. When a population is large, chance events are less likely to impact the gene frequency, although over generations they can. With small populations random events can have a much greater impact. Such random changes in gene frequencies are known as **genetic drift.**

Characteristics of Genetic Drift

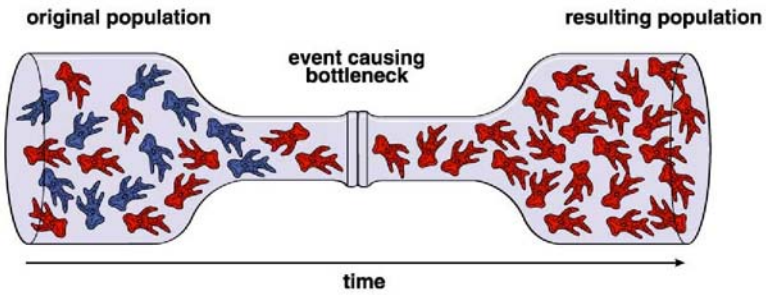
- Rapid and random (chance) changes in gene frequencies of populations can result in a localized reduction in variation for that population.
- More rapid when the gene pool is small and/or isolated



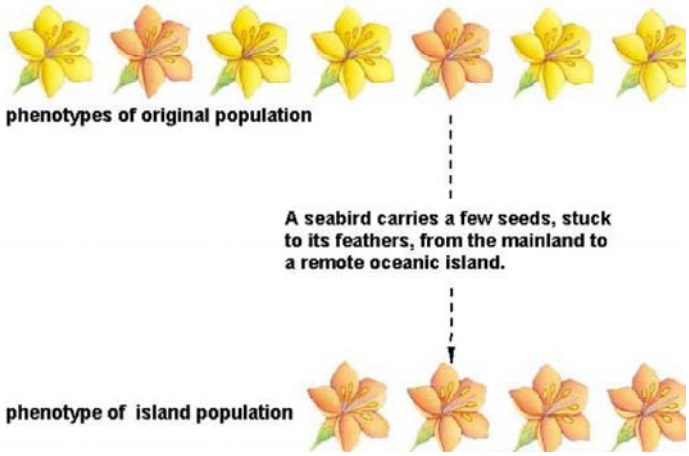
- Genetic drift tends to reduce variation within one population, but may increase genetic variation between different populations.



- Genetic drift includes the **Population bottleneck** in which some catastrophic event causing a "bottleneck" (drastic reduction in size of a large population caused by some unfavorable condition). The few survivors' genotypes will be the source of the subsequent generations. The world's Cheetah population today has a very small gene pool as a consequence of a unknown bottleneck event. The northern elephant seal, hunted almost to extinction, have a very uniform gene pool because of the 20 surviving seals in 1890, just one male fathered all offspring.



  - **In the Founder Effect** a small number of individuals disperse or move to an area **isolated** from the original population. The new population, with a small gene pool, will be established with a preponderance of a few genotypes. (like the bottleneck). The dispersed frequency will determine the character of the new population, which may differ significantly from the original population (which is how the founder effect differs from a bottleneck; in the bottleneck, the gene pool consists of the survivors from the original pool, rather than migrants). An example of this is the frequency of polydactyly in the Pennsylvania Dutch of the United States. Founder populations can lead to adaptive radiation in some environments.



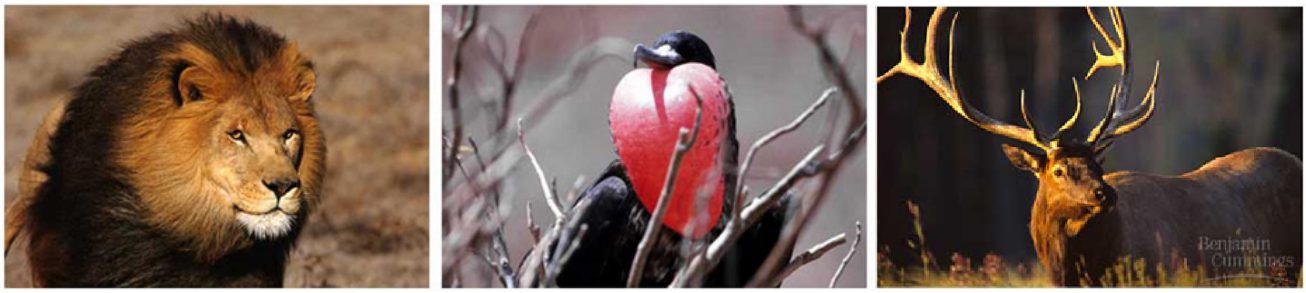
4. Non-Random Mating and Mate Selection

For the Hardy-Weinberg genetic equilibrium to work, each gamete produced by any male would have an equal chance of combining with any gamete made by any female of that species. This occurs very rarely in populations. Differential reproduction is a significant part of the Darwin-Wallace theory, and mate selection is an important part of natural selection.

There are hundreds of examples of mate selection strategies, some of which will be discussed later, as well as species that use other means of non-random mating, such as those animals which are harem forming.

Sexual (or Mate) Selection – A special case of Selection

Sexual selection involves any trait (adaptation) that gives an individual a preferential advantage in mating. Such sexual selection is very important to non-random mating. **Sexual dimorphism**, in which genders differ in morphology is common. Usually the male has a pattern that is used for display.

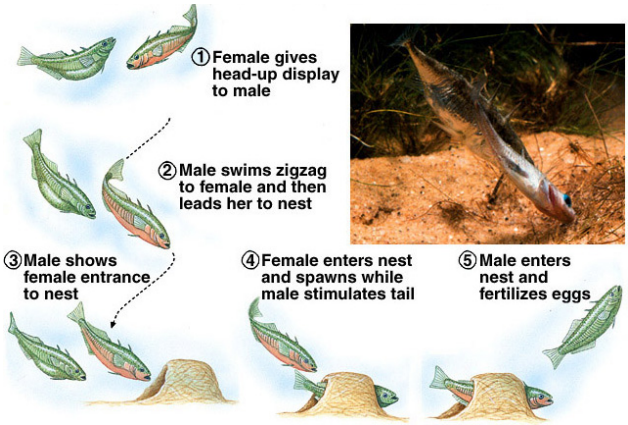


Male Patterns for Display

Mating behaviors, such as the competition for mates among harem animals, and the elaborate courtship displays and mate choice are well-known and popular topics for nature programs.

Bower Bird Bowers

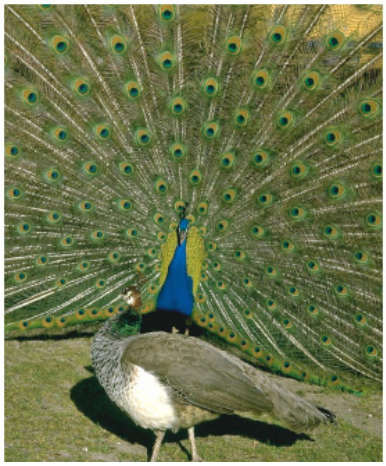
Blue-Footed Booby Courtship



Stickleback Courtship Dance

Gulls Nesting

Snow Geese Dimorphism



Peacock Display

Giraffe Dominance Display

5. Natural Selection (and selection, in general)

In the Hardy-Weinberg equilibrium, all individuals must be equally adaptive in their environment for all of their genetic characteristics. In real populations this is not the case.

- The many variants within a population will have different responses to the common environment in which they live.
- The conditions of the environment enhance the survival of certain phenotypes so these individuals are likely to have greater reproductive success than individuals whose survival is compromised.
- This differential reproduction results in passing more of the successful genes into the gene pool affecting the gene frequency of the next generation
- **Natural selection** is the result of this differential reproduction. Evolution by natural selection occurs whenever these conditions are met in populations. Documentation of this abounds in the field of ecology where studies of competition are common.

Although natural selection acts on phenotypes, recall that the phenotype is the expression of the genotype, and it is the specific alleles that are passed on to the next generation. Natural selection does not cause genetic changes within an individual. An individual cannot evolve. Natural selection acts on the individual. The population evolves as a consequence of differential reproduction.

Moreover, evolutionary change is not, in and of itself, something that is good or more valuable (or bad). Evolutionary change occurs because a specific environmental condition favors one genetic combination over another. If the environment changes, the genetic combination favored may also change.

Adaptation - Traits Subject to Selection

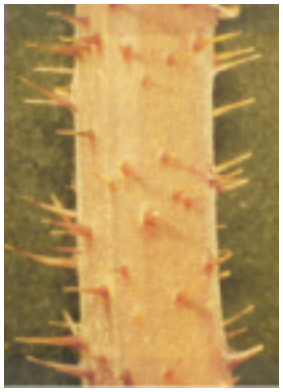
In general, **adaptations** are characteristics that help an individual survive and reproduce in its environment and are therefore important to the process of selection in evolution. The environment includes both the physical environment and interactions with other organisms.

Many of the relationships organisms have with other organisms deal with survival strategies: competition for resources, predator-prey interactions or symbiosis. A few examples will be discussed to illustrate selection. Adaptations can be **morphological, behavioral, physiological** or a combination of these.

Morphological adaptations involve shape, patterns, size, color, etc.

Mechanical Protection

- Spines, thorn, hairs, prickles
- Very common on plants
- Common on some small animals, too



Warning patterns

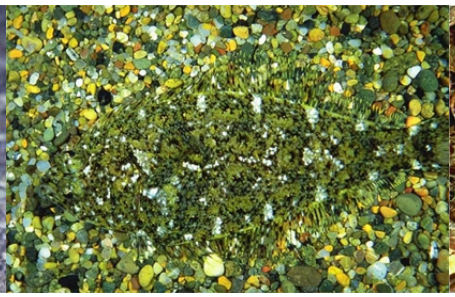
Striking pattern or colors that signal to potential predators that you are to be avoided (don't sample me). Warning coloration is also called aposematic coloration.


"Protective Patterns" (A continuum of sophistication)
Camouflage patterns blend the organism in with its surroundings to be less visible to potential predators, or, to not be visible to potential prey.

Flower and Leaf Mantid Insects

Cactus

Bird



Flounder

Nightjar (Bird)

Larva

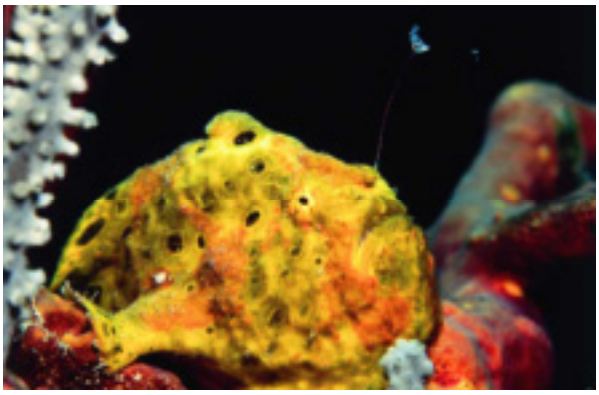
Spider

Leaf Hoppers

Cryptic patterns are similar to camouflage but trick the potential target in nature. Typically a potential prey item resembles something that is not a prey item. Occasionally the predator is the cryptic organism. Some examples include:

- Leaf Hoppers that look like leaf-cutter ants on their trail
- Insect larvae that resembles a snake or insects that resemble crocodile heads
- Trick eyespots or patterns that confuse

EyeSpots

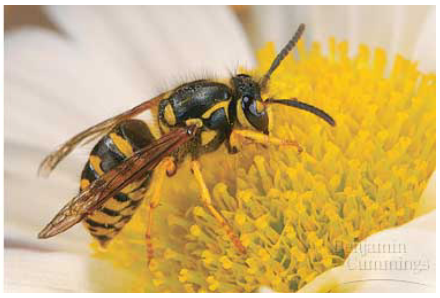


Fish with False Prey

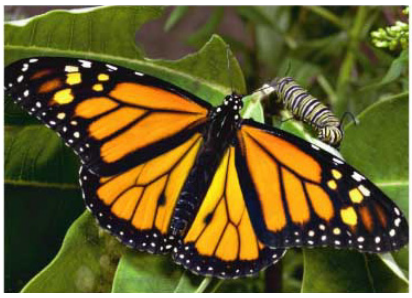
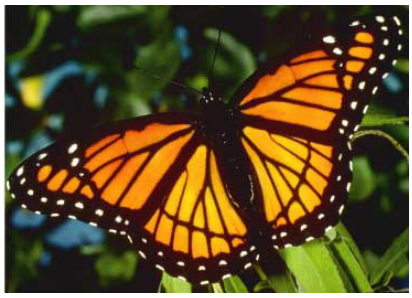
Moth and "Poop"

Mimicry involves many strategies but all involve an organism that resembles something else that has a bad association, so it does not get eaten. The mimic may or may not be most delicious. Mimicry overlaps with cryptic patterns that also allow one to avoid predation.

- Monarch butterflies raise larvae on milkweeds that have distasteful alkaloids. Adult monarchs retain the alkaloids and are not good prey. Viceroys look like monarchs and are not eaten, even though they do not have an alkaloid taste.
- Syrphid flies resemble bees, but have no stinger.
- Some orchid flowers look like and smell like female wasps. They attract male wasps who pollinate the flowers when they attempt to copulate. This has been called pseudocopulation.

Bee and Syrphid Fly

Monarch and Viceroy


Coral and King Snake

Behavioral Adaptations are used to confuse or distract potential predators. There are also behaviors that confer survival benefits such as nocturnal activity in desert habitats in which daytime activity would be dehydrating, using habitat to warm (or cool) such as lizards sunning or insects sunning before moving from flower to flower. Behavior is also used in courtship, as discussed. Some common protective behaviors include:

- Mother Bird with broken wing
- Twisting and turning when fleeing
- Enlarging body or presenting largest dimension to predator
- Rearing up on hind legs to look bigger

- Making warning sounds that indicates that a potential predator might be harmed by encounter. Growling, clicking, or rattle sounds are common.

Altruism is behavior that endangers the individual to protect other members of the population. This occurs most commonly among closely related individuals, and is called **kin selection**. To understand altruism in the evolutionary context you need to remember that what counts in the contribution of alleles to the next generation's gene pool. If "my" behavior results in the next generation's gene pool having a higher frequency of similar alleles to mine, it can be favored. Altruism is observed in many social animals, such as wolves. In a wolf pack, only one pair breeds. All others help to protect and raise the single litter.

Physiological Adaptations promote survival by giving the organism a physiological advantage. Some physiological adaptations are:

- Low metabolic water requirements for desert organisms
- Increased number of rbcs in human populations that live at high altitude
- Insects that circulate antifreeze chemicals for arctic survival
- Heat-resistant enzymes in thermophilic bacteria
- Toxic metabolites (poisons or distasteful chemicals)
  - This works well for plants since animals can sample the plant without causing permanent damage to either. Sampling an animal often causes its death. Animals more often use chemicals for offensive behavior.
  - Examples
    - Slugs and tobacco.
    - Skunks, fire ants, beetles and scorpions that spray or sting


Beetle Offense


Poison Frog


Skunk

- An animal can incorporate a toxin or defense mechanism from something it eats resulting in **second-hand toxins**, too. Nudibranchs eat cnidarians and the cnidarian nematocysts are used by the nudibranch. Monarch larvae get their alkaloid taste from the milkweeds on which they feed.


Monarch on milkweed glycosides

The Adaptive Strategy of Symbiosis

In a symbiosis, two types of organisms become closely associated with each other so that survival depends on both organisms. Symbiosis can be

- Mutually beneficial (**mutualism**)
- Beneficial to one, but neutral to the second (**commensalism**)
- Harmful to one, but beneficial to the second (**parasitism**)

Some examples of **mutualisms** are:

- Cleaner Fish and Sharks
- Nitrogen fixing *Rhizobium* and Legumes
- Corals and Dinoflagellates
- Ants and a variety of mutualistic partners including the acacia trees

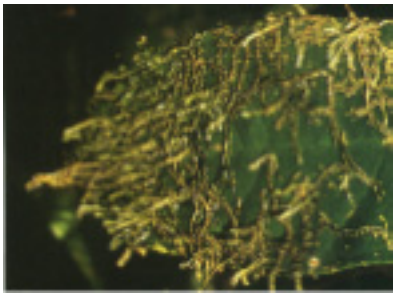
Root Nodules

ClownFish and Anemone

Azteca Ant and the Acacia

Some examples of **commensal** relationships are

- *Demodex*, our facial mites
- Intestinal and skin bacteria
- Decorator crabs, which benefit from the organisms they attach to their carapace to camouflage themselves.
- Thousands of Epiphytes

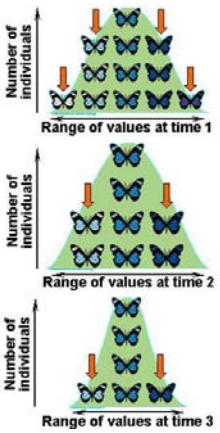


There are "thousands" of examples of **parasitic** relationships. For many humans, the debilitating effects of parasites reduces their survival so that common illnesses become life threatening.

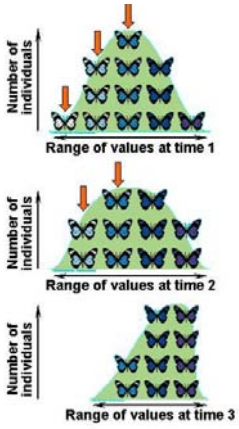
The selection of phenotypes that have beneficial adaptations for their surroundings can result in population changes from generation to generation, so that species can change over time. It's crucial to look at any adaptation in the context of its surroundings. Features with adaptive value in one habitat many be negative in a second. Also, adaptations must be inheritable to be subject to selection and evolution.

How does Natural Selection Affect General Population Patterns?

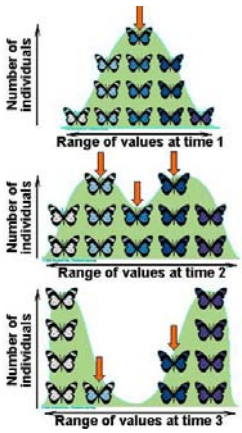
Three basic patterns emerge in populations through time, as a result of selection pressures (or forces): **stabilizing, directional** and **disruptive** selection.



Stabilizing Selection



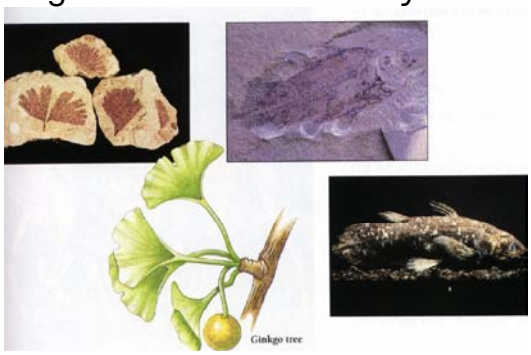
Directional Selection



Disruptive Selection

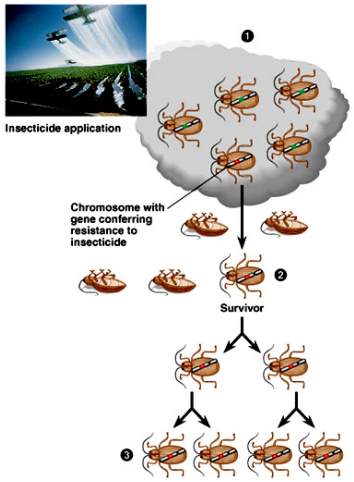
Stabilizing Selection

- In stabilizing selection, a narrow range of phenotypes, often those that are intermediate and usually more common, are favored over extreme variants. Extremes tend to decrease from generation to generation.
- Stabilizing selection is more likely when the environment remains constant.
- Some organisms have been stable for eons, such as the Gingko tree and coelocanth. Horseshoe crabs, found off the southeast coast of the United States have been unchanged for thousands of years.



Directional Selection

- In directional selection the population consistently shifts toward a phenotype that is favored at the expense of others. This differs from stabilizing selection where extremes are reduced at both ends of the variation continuum.
- Directional selection is typically a response to a changing environmental condition or to a new environment.
- Some examples of directional selection include:
  - Peppered moth mentioned earlier is a dimorphic population where the shift has been to the dark form in response to pollution
  - Resistance to pesticides is common in many organisms
  - Most induced selection or artificial selection is directional. We narrow the population for some characteristic.



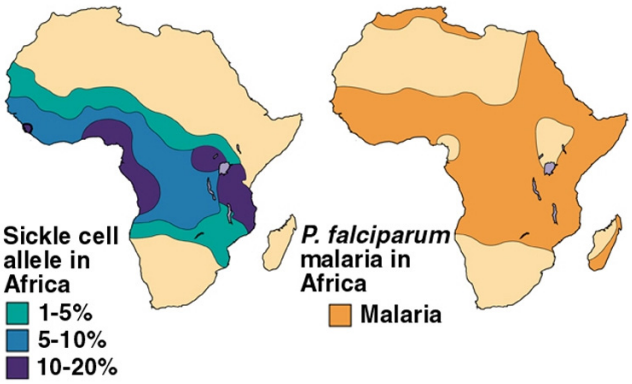
Pesticide resistance

Industrial Melanism

Disruptive Selection

- In disruptive selection extremes are favored at the expense of intermediate forms of a trait, so that the frequencies of phenotypes at the extremes increase.
- A patchy environmental condition promotes disruptive selection
- Polymorphism is a common result of disruptive selection. Adaptive radiation is also a form of disruptive selection.
- It's possible to have polymorphism within a single habitat, if there are balances that favor more than one type. This is called **balanced polymorphism**, and the morphs that are adaptive are stable phenotypes.
- Some examples of disruptive selection include the balanced polymorphism of sickle-cell anemia with malaria in Africa, populations of swallowtail butterflies that have different predators and the Galapagos and African finches with beaks suited to different food sources.

African Finch Beaks



Sickle-cell Anemia and Malaria

Adaptive Radiation is a term that is often used when a single ancestral type eventually evolves into many similar species, each with specific differences, such as the Galapagos finches studied by Darwin and discussed previously.

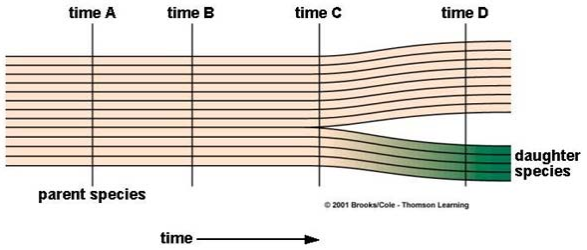
Methods of Speciation - 1

We have been discussing changes that occur in populations through time using the mechanisms of evolution. Speciation results when populations diverge to the point of reproductive incompatibility. Reproductive isolation is a by-product of genetic change. It does not drive genetic change. Recall the definition of species:

- A species is one or more populations whose members interbreed under natural conditions, produce fertile offspring, and are reproductively isolated (can not interbreed) with other populations.
- Non-sexually reproducing organisms and fossils cannot be given species names objectively using this definition. Nonetheless, it's a good working foundation.
- Some molecular systematic biologists are proposing a revised species definition that would incorporate the ability to diagnose descent from a single ancestor. This is a phylogenetic definition, based on genetic relationships and DNA homologies. Such a definition works with organisms that lack sexual reproduction or have not been observed sexually reproducing.

No matter how we ultimately define species, or change our definition of species, in order to have genetic change result in speciation two things must happen:

- Selection pressures that isolate populations must result in gene frequency changes in the genetically divergent populations through time.
- As a result of these isolation mechanisms, gene pools become distinct enough from each other (genetic divergence) so that interbreeding is no longer possible; hence, new species.

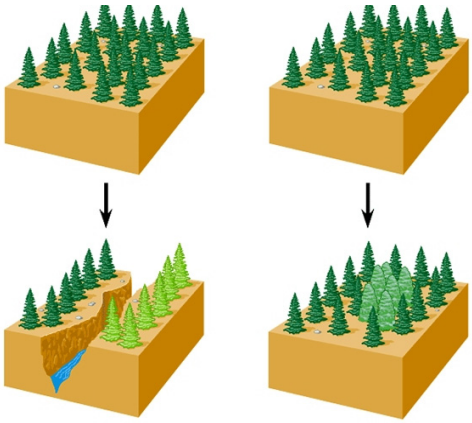


In contrast to the ease with which we can measure gene pool frequencies, for speciation we must search for isolating mechanisms, or barriers, both those that result in reproductive isolations and isolations that separate species: ecologically, behaviorally or in time so that that reproduction is not possible.

We shall look at a variety of reproductive and separating isolating mechanisms in this section. Barriers can also change through time and once-separate species may hybridize, blurring distinct species concepts.

- Isolations that involve geographic isolation are known as allopatric isolation mechanisms and result in **allopatric speciation**.
- Isolations that occur within the same geographic area are sympatric isolation mechanisms and result in **sympatric speciation**.
- The term **parapatric speciation** is used by some for speciation that occurs in adjoining geographic areas.

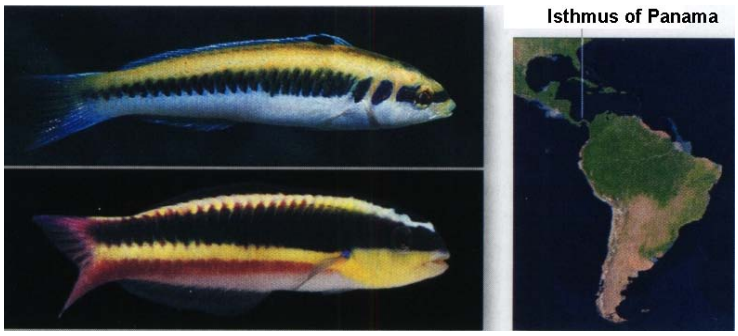
Methods of Speciation - 2



Allopatric (Geographic) Speciation

When individuals are geographically, or physically, isolated gene flow is minimized. When there is little gene flow, changes that occur tend to isolate the gene pools. If something occurs that shuts off gene flow (some barrier), then differential adaptation, genetic drift or some other selection mechanism, accompanied with reproductive isolation can result in divergent selection.

Squirrels at Grand Canyon



Wrasses at Panama Isthmus

Geographic (or Allopatric) Isolation barriers include:

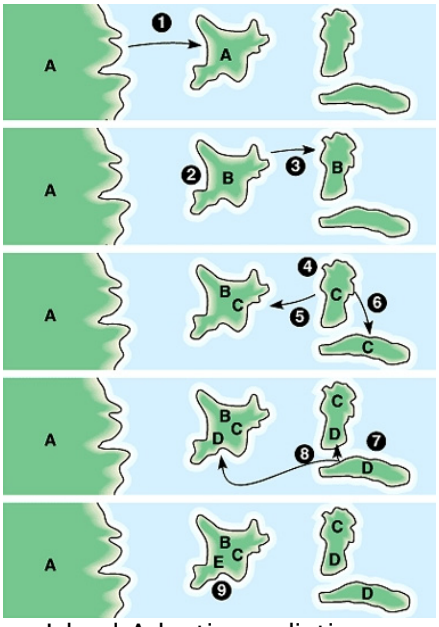
- Rivers and changing river patterns
- Mountain ranges
- Lakes which dry up
- Volcanic eruptions
- Climatic changes, such as the ice ages
- Loss of land bridges

Adaptive Radiation as a Speciation Mechanism

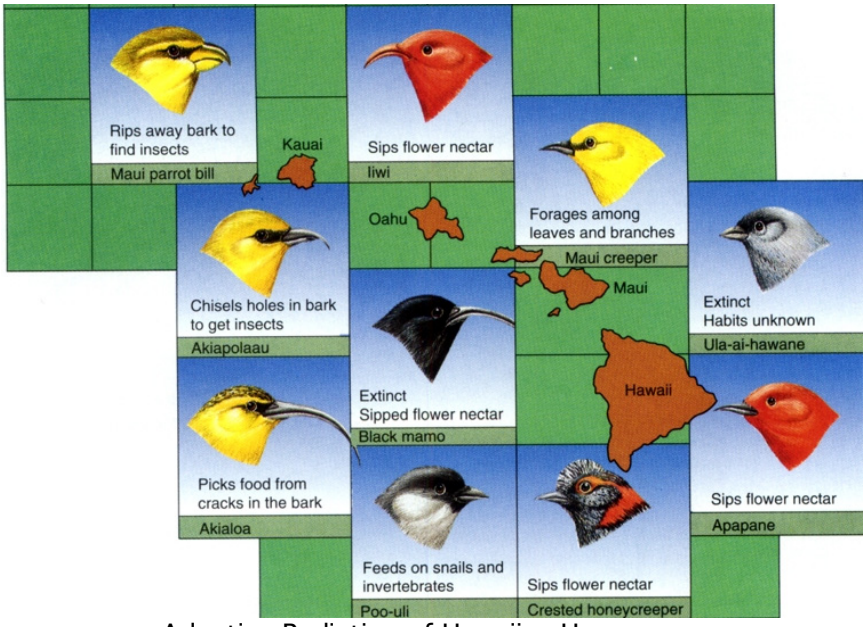
Patterns of speciation that involve **adaptive radiation** have been studied since Darwin's early work with the finch species on the Galapagos Islands. Darwin found fourteen species of similar birds on the islands, each feeding on separate food items, and each with a different beak shape. He speculated that the available food on the different islands "selected" for a specific beak shape. The distance between islands meant that only those birds in one area interbred, which resulted in the separation of species, and divergent evolution. Such multiple divergence is called adaptive radiation, and has been studied with many organisms.

Methods of Speciation - 3

Island archipelagos are excellent areas for the study of adaptive radiation. In addition to Darwin's finches, Hawaiian honeycreepers and Hawaiian fruit flies and the Hawaiian silversword plants provide good research study.



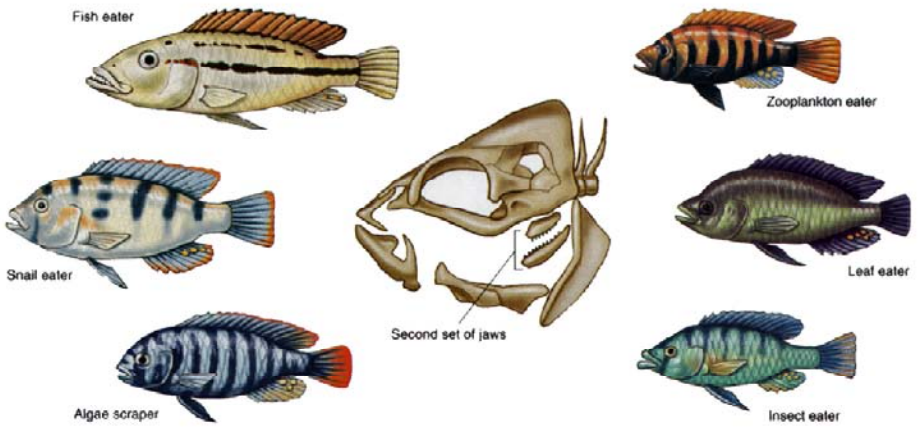
Island Adaptive radiation



Adaptive Radiation of Hawaiian Honeycreepers

Hawaiian Silverswords

Adaptive radiation can also occur without geographic isolation when organisms have different feeding behaviors. The cichlid populations in Africa's Lake Victoria are one example. Unfortunately, introduction of the Nile perch in the 1950's caused rapid decimation of the fish populations of all cichlid species.



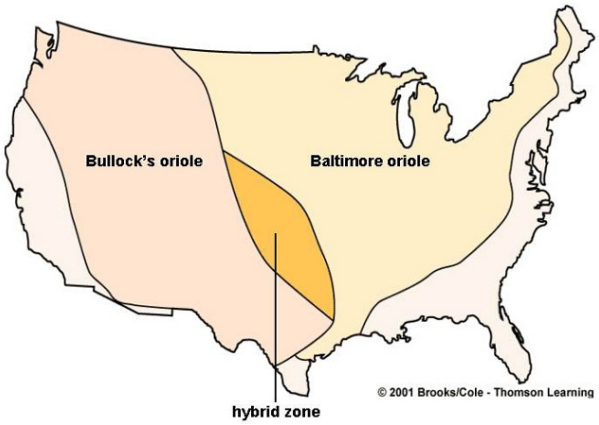
Cichlid species in Lake Victoria

Parapatric Speciation

Species that originate by hybridization in adjoining geographic areas are the result of parapatric speciation. A problematic element of hybridization is whether the hybrids are fertile, and if they reproduce only among themselves or with one or more parent species.

Bullock's Oriole

Baltimore Oriole



Sympatric Speciation

Species that originate in the same geographic area are the result of sympatric speciation. This comes about from ecological, temporal, genetic or behavioral mechanisms that can result in genetic isolation "in place".

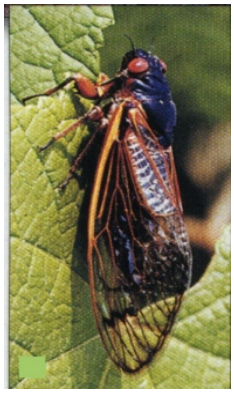
Ecological isolation examples include:

- **Feeding Preferences**
  Organisms may have differences in food preferences, such as fruit flies that feed and live on only hawthorn fruits or only apple fruits
- **Shelter Preferences**
  Organisms may have differences in type of shelter sites
- **Pollinator Preferences** for Flowering Plants
  A flower color or pattern or some other attractant may affect **pollinator visits**. Different pollinators ensure reproductive isolation for the plant. In the case of fig wasps, each species of wasp pollinates just one species of fig. Since wasps lay eggs while pollinating the figs, and their larvae develop at the same time as the fig fruits, the specific mutualism results in both wasp and fig speciation.

Larkspurs with Different Pollinators

Fig Wasp

Temporal isolation occurs if fertility occurs at different times. This can happen because of different hormonal signals, or because adults mature at different times of the year or even in different years.

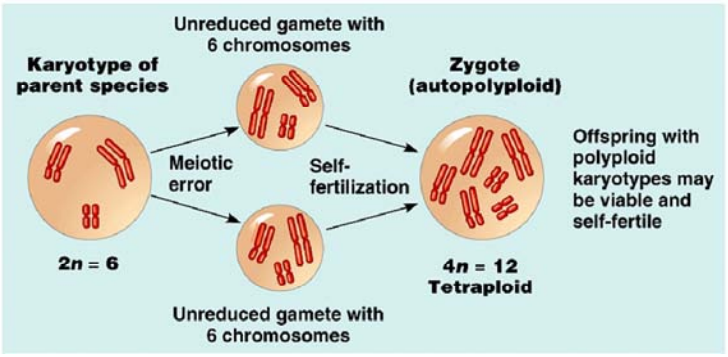


17-Year Cicada

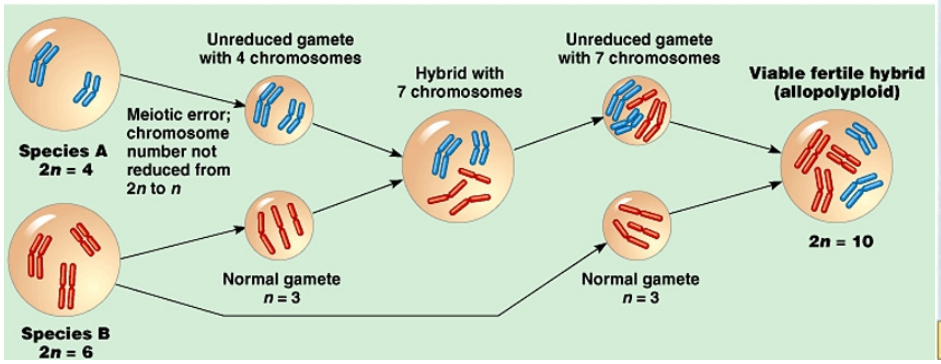
Sage Flowers and Sage Buds of Different Species

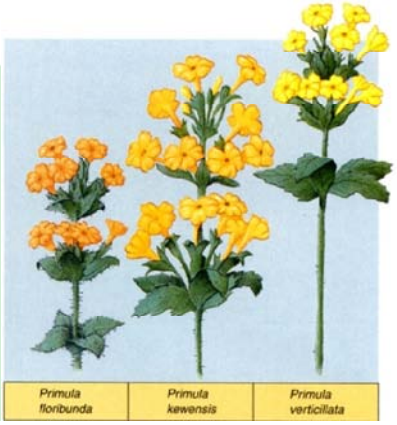
Genetic Isolation

Immediate **Genetic isolation** results from **polyploidy**. In plants it is not unusual for a fertilized egg to have DNA replication but not complete meiosis, resulting in a diploid gamete. If two diploid gametes fuse, tetraploids result that may be fertile. Such polyploidy is known as **autopolyploidy**. Polyploids may result between two closely related species if the first diploid gamete (species A) fuses with a gamete from species B and their fertile offspring also produce diploid gametes that fuse once more with one of the original species. Such polyploids are **allopolyploids** (allo = different).



Autopolyploidy





Allopolyploidy

Behavioral Isolation

Mating behavior, as we have discussed is crucial to successful reproduction in a number of species. Individuals do not recognize courtship patterns or signals from members of populations different from their own will have **behavioral isolation**. Behavioral isolating mechanisms include:

- Visual clues -- patterns and physical movements
- Audio clues
- Chemical clues, such as pheromones
- Pollination attractants

Mechanical Isolation (Mechanical Incompatibility)

There may be physical differences in structure or function of the reproductive organs that prevent copulation between members of different populations, or in the case of some plants, flower shapes that prevent some pollinators from visiting. The domestic turkey has been bred for large breasts to the point that copulation is "impossible". Many Hawaiian fruit flies have copulatory structures that are of different sizes.

Flower Shape and Pollinators

Snails and Coil Shape

Anatomical Change and Speciation

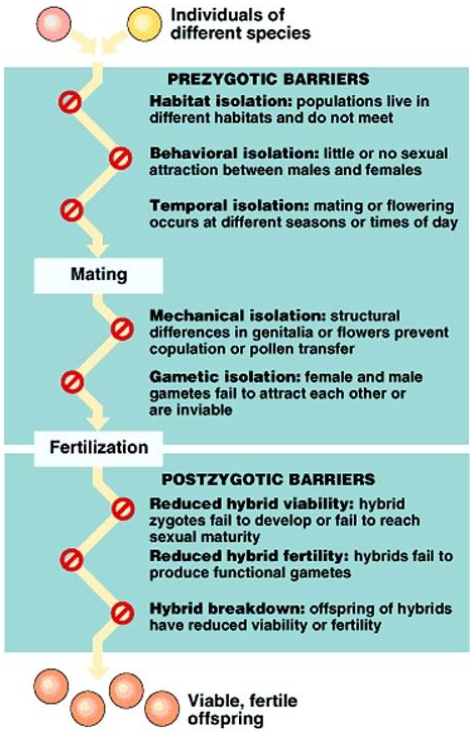
Speciation can occur with little or no anatomical change in organisms. If the organisms do not reproduce, they are biologically different species. Similarly, change in appearance within a species is a natural process and may not lead to speciation in the absence of any isolating factors. Such isolation factors can be random events or the result of natural selection.

Maintaining Reproductive Isolation

Ultimately in order for new species to form, the separation of gene pools by geography, ecology, genetics or behavior, must be accompanied by or followed by reproductive barrier so that interbreeding is not possible, even if the gene pools were to be mixed again. Reproductive isolating mechanisms may prevent successful fertilization (pre-zygotic or pre-mating) or successful development (post-zygotic or post-mating). The isolation mechanisms just discussed of geography, time, behavior and structure are pre-zygotic reproductive mechanisms.

Post-Mating Reproductive Barriers

There are a number of post-mating reproductive barriers that prevent development or successful survival of offspring and serve as isolating mechanisms that keep species separated. The reverse can also be true. If such mechanisms do not prevent isolation, and result in fertile hybrids, we may have just one species.

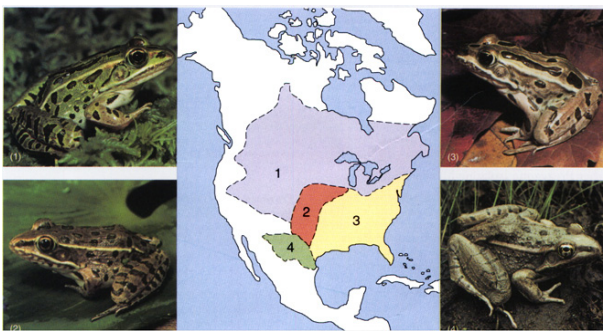


Some post-mating isolating mechanisms include:

Gametic Isolation: Mating (copulation) may occur but fertilization is not successful

- The gametes cannot fuse or are not chemically compatible.
- The sperm cannot survive in the female reproductive tract.
- The zygote cannot develop if fusion does occur.

Non-viable Hybrid: Even if gametes fuse, the offspring can be weakened, and may not survive. There are four ranges of leopard frogs in North American, separated by mating behaviors and incompatibility of embryos if behavior mechanisms are overridden.

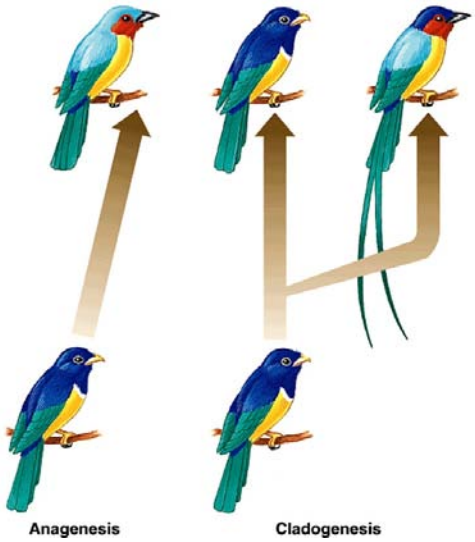


Hybrid Sterility: Successful mating and hybrid survival occasionally occur, but the offspring are sterile, since they lack homologous chromosomes essential for meiosis. The offspring may also appropriate mating signals (behavioral or morphological). The mule hybrid between the horse and donkey is a common example.

Horse Mule Donkey

Evolution – The Big Picture - Genetic Models and Rates of Speciation
How does all of this go together to give a model or models for evolution? As we have seen and read, the process of evolution needs the presence of **inheritable variation**, and variations that can be beneficial within one's immediate surroundings. Adaptations of value in one habitat may be quite negative in others, or even within the same area if conditions change.

It is reasonably easy to determine speciation characteristics associated with changes that occur through time. When fossils from one era are compared to fossils from distant eras, they can be very different. When these differences appear significant, the extremes are called different species. The term, **phyletic speciation or anagenesis** is used for a species that evolves into a new, different, species through time. Anagenesis is associated with directional selection. When new species arise from branches that diverge from an original species, we use the term **cladogenesis**. Cladogenesis is associated with divergent selection.



Darwin's model for evolution, which stresses the process of natural selection **gradually** changing populations, is one way for evolution to proceed, and works well for the changes we see within many species through time.

The **gradualist model** of evolution can be difficult to document in nature (although not in laboratory situations), since we observe the current state, not the past or the future. There is also evidence that suggests that gradualism is not always the way that populations change. Many fossils, for example, "suddenly" appear in sediments, and many organisms remain unchanged for thousands or even millions of years. Even Darwin proposed that changes probably occur in relatively short periods of times, perhaps alternating with longer, more stable periods.

Genetically, the method for such evolution is a series of incremental mutations, each of which can change the organism bit by bit until the accumulation is significant.

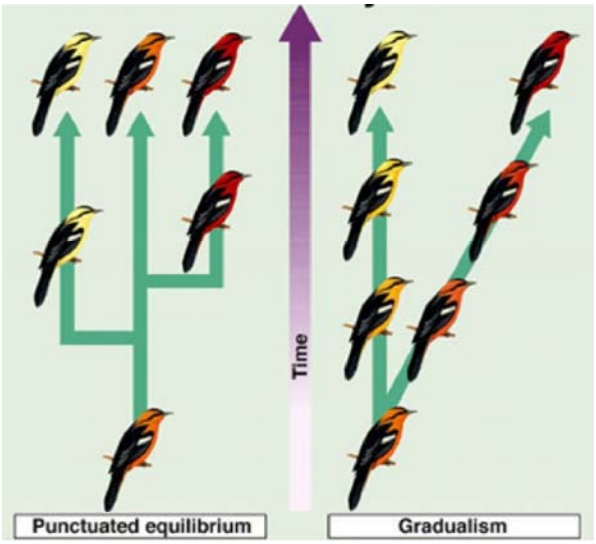
As an alternative, some biologists propose that change occurs through non-frequent major alterations in genes, in particular certain regulatory genes that can control many developmental events. When these changes affect reproduction, new species result.

There is evidence for species changing rapidly, when the stresses of the surroundings (environment) exert strong pressures for particular variants. The finches that Darwin described in the Galapagos Islands exhibit adaptive radiation. Each island has a different habitat and food supplies. To survive, the finches had to be specialized for their specific habitats. Those with variants less able to forage, did not pass on their genes. The induced pressure of pesticides resulted in rapid change in many pest populations. Only variants able to resist the pesticide survived. Significant change can result in just a few generations.

Punctuated Equilibrium

Another model for the process of evolution maintains that such rapid changes in response to intense selective pressures are followed by periods when the populations seem more constant. This model, which offers an alternative to the gradualist model, is known as **punctuated equilibrium** (This is pretty easy to do in laboratory situations, too). Much of what we see as diversifying selection follows the principles of punctuated equilibrium. Punctuated equilibrium can also explain why fossils seem to just appear in layers and not always change. There are some species that have been evolutionarily stable for thousands of years. For punctuated equilibrium, morphology is crucial to change, and good morphology remains constant until some pressure makes that morphology less advantageous. For punctuated equilibrium, chance may have just as an important role in species selection as natural selection has acting on individuals of the species.

Both gradualism and punctuated equilibrium have merit. Evolution can, and does, result from selection pressures. Evolution also takes place in "jumps" consistent with punctuated equilibrium and random events. The differences are in the interpretation of the forces that shape evolution, not in process of evolution as the underlying foundation of biology.



Extinction

Before we leave the subject of life, just as we have new species, we have species that decline in numbers and eventually die out altogether. We discussed a several examples of successful adaptations in this section. What about species that lack adaptations in their environment, and lose to those who are better adapted? Adaptations can affect not just populations of one species, but natural selection can also lead to the loss of a species by extinction.

When we look at extinction, the most significant cause of extinction is **change in habitat**. Today we often discuss loss of habitat, but this loss is generally not a physical loss of geography, but a change in that habitat which results in an area no longer suitable for the individuals who originally lived there. Humans have done much in the past few centuries to alter habitat, to the detriment of thousands of species.

Species that have narrow gene pools (are highly specialized) and/or have restricted distribution are more vulnerable to changing environments. Those who are large-dimensioned and have greater resource demands are more vulnerable. Equally vulnerable are species perceived by humans as physically threatening, in particular the large carnivores, and most particularly cats, wolves and bears.

Karner Butterfly – Lupine restricted

Devil's Hole Pupfish – One Nevada Location

Natural ecological species interactions such as competition and predation impact the ability to survive. Superior competitors may deprive less competitive species' resources to the point where the less competitive can no longer survive.

Geographic changes and accompanying climate changes have also been responsible for loss of populations. Catastrophic geological events such as volcanic eruptions, or massive earthquakes cause major environmental alterations. It is now accepted that a meteor may be responsible for the extinction of dinosaurs, along with numbers of other organisms that lived in that era.

However, the increased rate of extinction we see today on earth is the result of habitat destruction caused by human activities such as deforestation, conversion of native lands to agriculture, over-exploitation of species and pollution of water sources. Tropical rainforest loss each year exceeds the area of the state of Washington. 20 billion pairs of disposable chopsticks made each year come from trees that were once part of forests. The number of acres of trees felled for Sunday newspapers boggles the imagination.

Brazilian Rain Forest Deforestation

Temperate Forest Habitat Loss

Clearing of trees to provide new buildings and parking lots at BCC has resulted in the loss of 90% of the vegetation that once graced this campus, along with the other species that depended on this habitat. The current plantings are a mix of architects' choices and an abundance of a few "natives" grown in nurseries.