Biology 150 - GENERAL BIOLOGY

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Fall (section PT) and
TR - 11:00 am to 12:15 pm - Lecture
T - 5:00 pm to 6:15 pm - Workshops
room - Cox 126

Summer 1 (section AB)
MWR - 8:30 am to 9:45 pm - lecture 1
- 10:00 am to 11:15am - lecture 2
MWR - 11:15am to 11:30am - workshops
room - Cox 145

Lecture Handouts and Outlines
Quotable quotes about Biological Sciences:

“The fundamental properties of the living condition at the cellular, genetic, organismal, and population levels of organization. An analysis of the constituent molecules found in cells. The aim of modern BIOLOGY is to interpret the properties of the living organism within the structure of its molecules”... paraphrased from Francois Jacob, 1973 - in The logic of Life

"Biology belongs to one of the surprising sciences, where each rule must always be supplemented with several exceptions (except this rule, of course)".


"If we admit a priori that science is just the acquisition of knowledge that is, building an inventory of all observable phenomena in a given disciplinary domain, then, obviously, any science is empirical."

Rene Thom, 1989

"Although concepts and ideas occupy a central place in the grand sweep of our understanding of the nature of the world around us, it is a mistake to imagine that they play a greater role than tools and techniques in achieving scientific progress. Few scientific revolutions are concept driven."

a paradigm is:
currently accepted, outstanding clear model, or archetypal example of the interpretation of the data.

it is the current set of practices that define a scientific concept...
what has been observed, the kinds of questions asked, and how the data should be interpreted.

it is the outstanding clear current model, and what we should learn.
Scientific Method in Biology - Paradigm Concepts:

1. Biology explores Life and its properties
2. Biology uses many forms of INQUIRY to study Life
3. Science (Biological Science) is experimentally testable
4. Scales (spatial & temporal): questions arise on different scales (NRC #23)
5. What is included (the systems variables) depends upon the questions addressed, as does the hierarchical level in which the problem is framed (e.g. molecular, cellular, organismal) (NRC #24)
6. There are only a few basic data types - numerical, ordinal, categorical - but these may often be interconnected & expanded (e.g., as vectors or arrays) (NRC #39)
7. Consistency of the units with which one measures a system is important (NRC #40). Precision is important with measuring physical quantities, units, time/ length/mass (NRC # 86)
8. A variety of stat methods exist to analyze data sets and make comparisons(NRC #41)
9. There are diverse methods to display data, simple line graphs are often not sufficient, nonlinear transformations (NRC #44-45)
10. a SCIENTIFIC THEORY that has stood the test of time is a TRUTH.
Paradigm Concepts about Life:

1. Life exists from the microscopic (cell) to the global level (ecosystems)

2. Organisms include a great diversity of species

3. Evolution accounts for Life's Diversity and its Unity

4. Biological systems (Life) is much more than the sum of its parts

5. Biologists use scientific method & hypothesis-based testing to explore life

6. There are several THEMES that connect the CONCEPTS of Biology & Life
Unifying Themes in Biology of Life:

1. **Cell** - Cell Theory is basis of all known life
2. **Heredity** - DNA is basis of inheritance & information transfer
3. **Emergent Properties** - interaction and complexity lead to unexpected properties
4. **Regulation** - maintains a steady state (homeostasis) far from equilibrium
5. **Interactions with environment** - cells exchange matter/energy with surroundings
6. **Energy** - all cells perform work requiring energy (mechanical, osmotic, electrical)
7. **Unity/Diversity** - similar molecules, Genetic Code vs. 3 Domains
8. **Evolution** - core theme allows adaptations to environs via reproductive success
9. **Structure** - form/function are correlated at all levels of biological organization
10. **Scientific Method** - observational based discovery & hypothesis testing
CHEMISTRY - some paradigms:

1. **Matter** consists of chemical elements in pure form & in combinations called compounds

2. An element's properties depends upon
   a. number of protons & neutrons in its nucleus
   b. number of electrons in its orbital shells
      (outermost = valance shell)

3. **Atoms** with incomplete valance shells can form chemical bonds by sharing, gaining or losing electrons
   a. in a covalent bond 2 atoms share a pair of electron
   b. an ionic bond is the attraction between oppositely charged ions
   c. weak bonds include hydrogen bonds and van der Waal's interactions

4. **Metabolism** is CHEMICAL REACTIONS, which MAKEs/BREAKs chemical bonds.
WATER - Key Concepts

1. Water has a **tetrahedral shape**, which influences in physical properties.

2. **Polarity** of water is due to hydrogen bonds

3. Four **emergent properties** of water (all due to H-bonds)
   - cohesion
   - solvency
   - temperature (Specific Heat & Heat of Vaporization)
   - provide insular properties (resistance to heat change in large bodies)

4. **Dissociation** of water leads to acid/base conditions that affect life (pH)
Chemical Makeup of Life Paradigms -

1. Organic Chemistry –
   *carbon compounds* are the basis of life's molecules

2. Carbon atoms can **form 4 covalent bonds**, making the complex molecules of life

3. **Functional groups**
   
   [OH, COOH, NH2, CH3, PO4, SH, and C=O]

   are key to the chemical reactivity of life's molecules

4. Life's molecules are **POLYMERS**:
   
   a. Carbohydrate's fuel life,

   b. lipids are hydrophobic,

   c. proteins have multiple shapes resulting in broad functionality,

   d. nucleic acids store hereditary information.
Key concepts and paradigm’s about CELLS:

ancient prokaryotic bacteria (*Archaea*) and modern (*Eubacteria*) both lack significant membrane structure

Gram +/- staining differentiate bacterial wall structures

eukaryotic cells have internal membranes that compartmentalize their functions

eukaryotic genetic info is housed in a membrane encapsulated *nucleus*

the *endomembrane system* regulates protein traffic and performs metabolic functions

mitochondria & chloroplasts change energy from one form to another

the *cytoskeleton* is a network of protein fibers that organize cell structures and activities

extracellular components and connections between cells help coordinate cellular activity

virus genome can only reproduce within a host cell
Cell Membranes - Paradigms

1. Cell membrane is a **fluid mosaic** made of proteins and lipids

2. Cell membrane is **selectively permeable**, regulating molecular transport in/out

3. **Passive diffusion** (high to low) requires no expenditure of a cell ‘s energy

4. **Active transport** (low to high) requires energy, often coupled to ATP hydrolysis or cotransport

5. **Bulk transport** (large pieces of molecules) occurs via exo/endocytosis.
Cell Communication - paradigms

1. **External** molecular **SIGNALS** (molecules such as hormones) trigger intracellular responses

2. **RECEPTION**: signal molecules bind to cell membrane receptor proteins causing shape changes and subsequent metabolic reactions

3. **TRANSDUCTION**: cascades of molecular relay reactions convert inactive/active forms of molecules and vice versa

4. **RESPONSE**: cell signals lead to regulation of cytoplasmic activities and/or gene transcription.
Paradigms of Cell Metabolism

1. Cell metabolism transforms matter & energy in accord with Laws of Thermodynamics (NRC#2 & #94-95)
2. Living systems are far from equilibrium. (NRC#5)
3. Release of Free Energy indicates that a cellular reaction will occur spontaneously
4. ATP hydrolysis powers cellular work by making and/or breaking chemical bonds
5. a Coupled Reaction works by linking the exergonic release of energy to an endergonic cellular reaction (NRC#5)
6. Enzymes regulate rates of metabolic reactions
7. Enzymes function by lowering the Energy of Activation of a reaction (its inertia)
8. Michaelis/Menten kinetics define enzyme action
9. Km is measure of affinity of enzyme for its substrate. Vmax is enzymes maximum velocity
10. Regulation of Metabolism is via controlling the rate of enzyme reactions via subtle changes in the shape & efficiency (Km) of enzymes.
Making ATP - Paradigms

1. Cell respiration uses 3 catabolic pathways to oxidize sugars to yield energy as ATP.
   A. Glycolysis: in the cytosol, anaerobically converts (oxidizes) glucose to pyruvate makes 2 ATP (net) & 2 NADH.
      Subsequent pathways include:
      fermentations producing lactate or ethanol, while recycling NADH.
   B. Citric acid cycle (Krebs cycle):
      in mitochondria; occurs in 3 steps aerobically:
      1st: conversion of pyruvate to acetyl-CoA by PDH.
      2nd: which is oxidized to CO2 by cycle enzymes that make 3 NADH, 1 FADH2, & 2 GTP;
      3rd: couples electron transport & ATP synthesis.
   C. Oxidative phosphorylation: inner mitochondrial membranes
      - passages of electrons thru protein carriers to O2 forming water; protons are pumped to peri-mitochondrial space creating a proton gradient for ATP synthesis

2. ATP Synthase is a multienzyme complex that converts the energy of a hydrogen ion gradient to phosphorylation of ADP

3. Glycolysis and Krebs cycle are key intermediary pathways of all other metabolic pathways.
Photosynthesis - Paradigms.

1. Photosynthesis converts light energy (photonic electrons) into chemical bond energy

2. Light reactions convert light energy into ATP/NADPH

3. Calvin Cycle (dark reactions) use ATP/NADPH to reduce CO2

4. C4 cycles (Hatch-Slack & CAM) reduce CO2 to organic acid, then again to PGA, without the interference of photorespiration.
CELL CYCLE – paradigm’s

Summary of MITOSIS
1. asexual cell division results in genetically identical progeny
2. the 3 phases of life cycle of a cell are:
   a. Interphase (G1 - S - G2)
   b. mitosis - nuclear division
   c. cytokinesis
3. the cell cycle is controlled by regulatory (kinase active) proteins & passes through Checkpoints

Summary of MEIOSIS
1. Nuclear division phase of sexual cell reproduction
2. Two successive divisions, results in 4 daughter cells... Meiosis 1 and Meiosis 2
3. Reduction/division occurs... diploid ----> haploid
daughter cells ½ number of parent chromosomes
4. Stages have same nomenclature as Mitosis:
   prophase, metaphase, anaphase, telophase,
5. Only one S phase, where DNA is duplicated;
   often may be no interphase between M1 & M2
6. Homologs separate in Meiosis 1
   Chromatids separate in Meiosis 2 (mitotic-like)
7. Random Assortment occurs...... homologs align at equitorial plates independent of each other
8. Crossing over... may occur in Prophase I...
   *synapsis*: pairing homologs allows exchange
   *chiasma*: point exchange of sister chromatids
Genetics & Inheritance

1. Mendel used scientific methodology to identify
   2 Laws of Inheritance
   a) Law of Segregation –
      genes separate during gamete formation
   b) Law of Independent Assortment -
      genes sort independent of each other
2. statistical probability governs Mendelian Inheritance
3. many human traits obey Mendel's Laws

Molecular Genetics

1. DNA is the genetic material
2. genes specify proteins via transcription & translation
3. eukaryotic cells modify RNA after transcription
4. mutations may affect protein structure & function
5. Individual bacteria respond to environmental change
   by regulating their gene expression
6. chromosome structure is based upon successive
   levels of DNA packing
7. gene expression in eukaryotes is regulated at many levels.
   but key step is transcriptional control
8. eukaryotic genomes contain much non-coding DNA
9. duplications, rearrangements, & mutations of DNA
   contribute to genomic evolution
10. DNA cloning can produce multiple copies of genes
11. entire genome can be mapped at DNA level
ANIMAL STRUCTURE & FUNCTION Paradigms

Chordates have a notochord and a dorsal, hollow nerve cord

Vertebrates are craniates that have a backbone

Craniates are chordates that have a head

Vertebrates contain 4 majors tissues: epithelial, connective, nerve, and muscle

Blood is a connective tissue with cells suspended in plasma

The endocrine system and the nervous system act individually and together in regulating an animal’s physiology
REPRODUCTION and DEVELOPMENT

1. gametes are formed via MEIOSIS in specialized tissues

2. fertilization depends on molecular recognition between sperm & egg of same species

3. development proceeds in continuous steps: cleavage, gastrulation, & organogenesis

4. morphogenesis in animals involves changes in cell shape, migration, and adhesion

   morphogenesis in plants depends more upon plane of cell division

5. all cells have the same genetic potential, i.e., they are are totipotent or pluripotent

6. cells differentiate via differential gene activity
NEUROPHYSIOLOGY PARADIGMs

Nervous systems consist of circuits of neurons and supporting cells

Ion pumps and ion channels maintain the resting potential of a neuron

Action potentials are the signals conducted by axons

Neurons communicate with other cells at synapses via chemical diffusion of neurotransmitters
SENSORY PHYSIOLOGY &
MUSCLE PHYSIOLOGY

Sensory receptors transduce stimulus energy and transmit signals to the central nervous system.

The mechanoreceptors involved with touch detect changes by deflection of hairs in response to force.

Similar mechanisms underlie vision throughout the animal kingdom.

Muscles move skeletal parts only by contracting.

AP is 2-3 msec and muscle twitch 50-100 msec. Muscle are typed by contraction time:
- slow twitch - 100 msec - type 1 - aerobic
- fast twitch - 50 msec - type 2a/2x - anaerobic
"The fundamental properties of the living condition at the cellular, genetic, organismal, and population levels of organization".

Bil 150 is an analysis of the constituent molecules found in cells. The aim of modern BIOLOGY is to interpret the properties of the living organism within the structure of its molecules"...

paraphrased from Francois Jacob, 1973 - in *The logic of Life*
I. INTRODUCTIONS - SEE class web Pages
http://henge.bio.miami.edu/mallery/150/
Instructor, Time & Place,
Syllabus fall*/ summer*, Tests & Workshops...

some rules:
if a web link is starred*,
then you are responsible for the content at that link;
if a figure is listed [ fig 7.1 ] or a figure*
then you are responsible for its content.
if a web-link is NOT starred,
then you NOT responsible for its content;*

What are your reasons for taking course
What you would like to get out of the course

II. PEDAGOGICAL THEMES of COURSE

Learn a BROAD BASE of SCIENTIFIC FACTS

Become BIOLOGICALLY LITERATE
names, terms, definitions,
concepts all biologists should know
the Great Experiments of Biology

Learn the SCIENTIFIC METHOD
begin to think as a scientist/biologist

Cost of science in America
Myth of scientific certainty
We'll use an **INQUIRY** Based approach to the Science of Biology...

- the emphasis will be placed upon **inquiry** & **experimentation**

- look at case history's of experimentation & its data for interpretations,

- look for competing hypotheses & identify what is yet unknown.

- the facts of an introductory course will change over time, but the analytical skills you learn here will serve you for a lifetime.

an inquiry based approach means...

we'll  **ASK QUESTIONS** & **ANALYZE DATA** to find answers

- for each new concept or experiment...
  
  ASK YOURSELF these questions

  1. What **motivated** this experimental study?
  2. How were the experiments **designed**?
  3. What **new methods** or analytical techniques were used?
  4. How **surprising** was the outcome?
  5. How did a discovery influence the **future course** of Science of Biology?
Learn & appreciate **HISTORICAL CONTEXT** of biology...
science changes society (we live in a technocratic age of science)
egotism may drive science; 20th century = age of physicist & chemist;
but 21st is the biologist's

To see the **RELEVANCE** of Biology...
how does your own body work
how/why did family member(s) get a certain disease

Learn Biology for **SOCIETAL REASONS**...
where do we fit into Nature?
what may I do to protect our Earth?
prior concepts? – are my prior concepts correct or incorrect?
to enhance our own knowledge base.

We'll use Social Learning **Communities...**  **Workshops**:
The Workshops are Social Learning Communities where student participation is the basis for learning. Your role is that of a learner and also a teacher, to help others in your learning community benefit from your knowledge.

**Mastering biology** involves "learning" the content of the subject matter, but also "learning to be" a biologist... a full participant in the field of biology, which means acquiring the practices and norms established by the practioneers of biology, kind of like apprenticing or supervised graduate study. The idea is to engage new students, as yourself, in learning by productive inquiry.

Your Social Learning Community will do **practice problem sets** that are designed to help you become more familiar with the material presented in class in a student oriented environment. The Workshops employ Peer Led Team Learning - a concept where other student Biology majors facilitate a series of practice problems for currently enrolled students. As you review the lecture material, your **PEER MENTOR** will encourage engagement by asking questions or initiating discussions about the material. Lecture provides the content, the Peer Mentor stimulates earning interaction & you gain a better understanding by focused conversation.
III. **BOOK - Biology**: by Neil Campbell & Jane Reece - UCR  
*Pearson Education - Benjamin Cummings*, 7th edition, 2005  
[to buy]  
55 chapters and 1231 pages - (way too much)  
Book has three main objectives...  
1. to explain biological concepts clearly & accurately  
   within context of unifying themes  
2. to help you as student (learner) *develop* a more positive  
   & realistic impression of science & how it is done  
3. to stress *inquiry based learning* by looking at how biologists think,  
   by presenting real data to be interpreted by the student,  
   offering evidence for competing hypotheses,  
   and referring to work in progress,  
   and noting what biologists do not know.  

We'll use book this semester to look at  
**2 main themes of biology...**  

1. Biological Principles *COMMON to ALL* organisms  
   a) cell chemistry  
   b) cell structure  
   c) cell function  
   d) cell genetics  
2. How particular *organisms work*, in their specific habitats,  
   especially some Vertebrate examples

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Fall & Summers  
Biology 150 - About
GROWTH of the Earth's Population:
with no limits on resource = exponential growth

but resources do limit growth = sigmoid curve
What is likely Carrying Capacity of planet Earth?

Replacement Level Fertility
Number of children each woman should have in order to do no more than replace herself and her mate: global average should be: 2.1 - 2.5 children.

Population pyramids - age/sex diagrams
a graph of male vs. females by age groups. note reproductive year categories.

CURRENT Projections suggest by 2050 = 9,050,494,208
EMERGENT & ZOONOTIC Diseases

Emerging infectious fatal diseases of the late 20th century have alarmed the health care professions to the dangers of...

EMERGENT & ZOONOTIC DISEASES.

Most emerging diseases are caused by changes in "microbial traffic". An environmental change (natural or manmade, including weather changes, deforestation, earthquakes, etc...) affect a class of diseases referred to as "vector-borne diseases", where infectious agent is typically carried out in an arthropod or mollusk.

Emergent examples: cholera, thyphoid fever, diptheria, TB, malaria, dengue fever, & yellow fever

Environmental changes can significantly affect "zoonoses" - communicable diseases of animals that may be transmitted to man. Zoonotic diseases are caused by species jumping infectious agents, which now infect humans.

Zoonotic examples: SARS, Ebola, BSE's, AIDS?
Greenhouse Gases & Their Effect

Solar radiation reaching the earth's surface is largely absorbed resulting in surface warming. Much of this absorbed energy is eventually re-radiated in longer infrared wavelengths. As it leaves this energy interacts with the atmosphere and is reflected back to the earth's surface by atmospheric molecules. This reflected energy further warms the surface of the earth.

The molecules responsible for this phenomenon are called greenhouse gases, i.e. water (H2O), nitrous oxide (N2O), methane (CH4), and carbon dioxide (CO2) because they act like the glass in a greenhouse, trapping re-radiated energy. Without these gases most life on earth would not be possible, as the surface temperature would likely be about 60°F colder.

Greenhouse gases act like an insulator or blanket, keeping the heat in. Increasing the concentration of these gases in the atmosphere increases the atmosphere's ability retain heat. Therefore too great a concentration of greenhouse gases can have dramatic effects on climate and significant repercussions upon the earth.
the Scientific Method and the Scientist

"Equipped with his five senses, man explores the Universe around him and calls the adventure Science."

Edwin P. Hubble
astronomer - 1948
Gk: **Bios** = life    **Logy** = study of

the term **biology** was coined by Gottfried R. Treviranus in 1802.

**Physics** is the study of atomic particles that interact by a relatively simple set of rules that may be distilled into predictive formulas.

**Chemistry** is a more complicated application of physics & its rules,

**Biology**, then could be a more complicated application of chemistry, which also might be reducible to predictive physical formulas.

but as we shall learn, Biology's rules are messy and its molecular interactions are almost impossible to faithfully predict.

**Scientific Theories have 2 components:**

1. one component describes a **pattern** seen in the natural world

2. other component identifies a **process or mechanism** responsible for the pattern
Scientific Method – includes the formulation of an **HYPOTHESIS**, (which is a tentative answer to a question) and doing experiments from which may deduce a general answer to the hypothesis...

- employs **rigorous methodology** and
- devises **experiments** to validate observations...

**Observations** .... from previous studies or directly

- students in back of lecture hall sleep
- ask questions about how’s & why’s of observations
- search literature for previous questionings?

**Postulating a premise** (pose a question):

- the posing of a critical and experimentally testable question
- critical thinking
- does back of classroom promote sleep

**Formulate a hypothesis** ... A tentative explanation

- a proposed explanation for a phenomena that is experimentally testable...
  - sitting in back of room promotes sleep
  - critical thinking can suggest alternative hypotheses
  - a boring lecture promotes sleep

**Experimental Prediction**... states expected results from

- observational or an experimental test
Designing Experimental Tests ....

experiments are supposed to test a premise (hypothesis)...

a Hypothesis can be refuted (proven wrong) or falsified,

but a hypothesis can never be proven right,

experiment purpose is to disprove, not prove something

moving one student to front will = awake

experiments must define the variables...

dependent variables:

such as - number of parts, growth weight, etc...

measurable & observable things;

the variable modified by treatment

independent variable:

only one variable to be manipulated, which may change

height, weight, age, sex, time, amount of drug, etc.

the treatment

controlled variables:

those kept constant and not allowed to change

all experiments must have a control...

standard for comparison (a challenge)

scientists rarely say “the results prove...“

but rather say... "results suggest...“

or "results provide support for..."

Draw a conclusion...

a Theory - set of rules about what we observed...
Methodology of Science

"Science is what scientists do, and there are as many scientific methods as there are individual scientists. The scientific method is what working scientists do... "


Hypothetico-Deductive Thinking... includes the formulation of an HYPOTHESIS, (a tentative answer to a question) and doing experiments from which one may deduce a general answer to the hypothesis...

Involves:
critical thinking ... nothing is accepted as fact unless it is demonstrable
deductive reasoning ... if...then logic
  birds have wings, robin is a bird, robins have wings
inductive reasoning ... To deduce intuitively/creatively
  object falls to ground = force acts on object = gravity
cumulative.... results of earlier studies serve as initial
  observations of new studies
adaptability.... Results must fit with known facts

collection-Interpretation of data...
  variability in experiments must be estimated...
  statistical analysis: some common statistical tests include...
    t-test  - compares the means of two groups
    ANOVA  - compares means of three (3) or more groups
    chi square  - compares how closely the observed or
                  measured data is to the expected results
                  (ex: genetic crosses)
organizing data... tables, figures (line graphs, bar graphs)

- **tables**: often used to emphasize numbers themselves, rather than a trends
- **figures**: are graphs (trends), pictures, photos, and diagrams (visuals)
- **line graphs** - show effect of independent variable (X-axis) on the dependent (measured) variable (Y-axis)
- **bar graphs** - compare sets of data that may be discontinuous i.e., maybe different groups

repetition... consistent results from test to test

adaptable... not all scientific experiment are clear cut

error...
- **random error**... intrinsic to instruments of measurement
- **non-random** or **systematic error**... may bias results, esp:
  - **Human error**: failure to follow procedures, inconsistent measurements by 2 researchers, math errors, ignoring data that do not support hypothesis, a scientist's personal bias, etc.

**Bias** can be overcome... via open communication

**Publish or Perish…..**

**limited**... science doesn't deal w hypotheses that aren't testable...... IRONIC SCIENCE ...
  e.g., **Is there other life in the Universe?**
**Drawing a Conclusion**

A **hypothesis** is a limited statement regarding cause and effect in specific situations.

A **scientific theory** or law represents a hypothesis, or a group of related hypotheses, which has been confirmed through repeated experimental tests over a long period of time. \[\text{theory} = \text{truth}\]

**All Scientific Knowledge is Tentative, Open to Challenge**

**What makes a scientist?**

Lab coat? Pipette? Curiosity and Creativity?
A **scientist's job** is to figure out how the world works.

**Two vital ingredients** seem to be necessary to make a scientist:

1) **curiosity** to seek out mysteries and
2) **creativity** to solve them.

**Scientists** often delve deeper into a basic question showing a passion for knowledge for its own sake. **Curiosity** is a sensitivity to small discrepancies in an otherwise ordered world. A great imagination and the **ability to improvise** are important parts of the scientist's arsenal. **Creativity** has an analogy with child development. Scientists use the same strategies for investigating the world as an infant does discovering his/her surroundings for the first time, which makes scientific curiosity part of the basic 'tool kit' of the scientist.
National Academy of Science
was signed into being by President Lincoln on March 3, 1863
- to advise U.S. govt. on science and technology matters
- has 2,340 current members (as of April 2005).
- NAS is composed of 4 separate Academies or Divisions:
  National Academy
  National Research Council
  National Academy of Engineering
  the Institute of Medicine
- Proceeding of National Academy (PNAS)
  is its print journal and PNAS Online web link

Royal Society of London
- British equivalent, founded in 1660
- currently some 1240 members
- Royal Society publications includes its International scientific journals:
  Proceedings A & Proceedings B
  quality submitted research papers
  Transactions A & Transactions B
  focuses upon commissioned themes & reviews.
  Notes and Records - covers the history of science.
BIOLOGY - Bios (life) + Logy (study of)
the scientific study of life & living things

LEVELS of Biological ORGANIZATION [reductionism]

biosphere... all the environments of planet Earth that are inhabited by life
ecosystem ... (ex: tropical rain forest)
biotic factors = all organisms living in an area
abiotic factors = physical components of environment
community ... all the organisms
population ... all interbreeding individual of one species
organism ... an individual living entity

organ systems... circulatory, nervous, etc
organ... parts of organ system : brain, spinal cord, nerves
tissue... individual groups of similar cells (w specific function)

CELL... ultimate unit of living matter, bounded w membrane
macromolecule ... polymers of biological molecules
    carbohydrates, lipids, proteins, nucleic acids
molecules... a cluster of atoms with unique properties
atom... smallest unit of matter with same atomic number
protons, neutrons, electrons...
Biological Diversity

Classification of Living Things

All Living Organisms are Grouped into...

3 DOMAINS

- **EUBACTERIA** - true bacteria
- **ARCHAEA** - ancient procaryotes
- **EUCARYA** - modern eucaryotes

defined by Carl Woese –
using small subunit rRNA sequences homology

and 5 KINGDOMS

1. **MONERA** - single celled, microscopic bacteria
   most successful of all living organisms
2. **PROTISTA** - algae/protozoans; unicellular eucaryotes
3. **PLANTAE** - contains plants, photosynthetic organisms
   with cellulosic cell walls
4. **FUNGI** - contains molds, yeasts, mushrooms organisms
   that decompose dead organisms
5. **ANIMALIA** - kingdom that contains animals organisms
   that eat other organisms & lack cellulosic cell walls; often motile
Journey To "Cosmos" of the Cell

Modern Biology is often looked upon as.....
the Biochemistry of the Cell

Unifying Concepts i.e., Unity of Life

Properties of Life...
Top Ten Things that Characterize Cell as Living

2. Cells are Highly Structured
3. Cells have an Evolutionary Origin
4. Cells Metabolize
   process nutrients, possess metabolic pathways, self adjust for metabolic regulation
5. Cells Self-Replicate (divide)
6. Cells Osmoregulate
7. Cells Communicate
8. Cells show Animation (cyclosis)
9. Cells Grow, Divide, & Differentiate
10. Cells Die
Properties of Life

Basic unit of life is CELL...

an inanimate mix of biomolecules -selected for fitness
to perform certain cell/biochemical functions of life.

1. OBEY Physical/Chemical LAWS of Universe

all living things are parts of larger systems of matter &
energy, & matter continually recycles through systems as
energy flows thru the systems.

there are NO Unique laws defining the Living State

Cells can transform energy... by:

a) extracting energy from environment
   autotrophs (light) & heterotrophs (food)]

b) transform energy into biological work
   osmotic, mechanical, electrical

c) cells constantly expend energy to maintain a
   ordered state

   HOW: 1) capture light energy, 2) redox rx, 3) e- flow

2. Cells are Highly Structured

all living things maintain a high degree of order & complexity

Diverse, yet similar (all w membrane, nuclei, organelles)

Uniqueness is structural organization brought about by
interaction of natural elements selected by living systems

Chemical elements mix to give emergent property of life.
3. All cells are derived from a single PRIMORDIAL cell

Origins of Life...

a) Special Creation... benevolent supreme being suspends laws of physics & chemistry to create life.

b) Extraterrestrial... life was formed or evolved elsewhere and was seeded on the newly formed planet

some extraterrestrial experimental systems...

SETI - Universe has $10^{20}$ stars similar to our sun's
if 10% have planetary systems &
if 1/10,000 has a planet equal in size & properties to Earth, then life as we known it (carbon based life forms) might occur on as many as $10^{15}$ planets

Panspermia - idea that living microbes drifted in from space and colonized Earth; S. Arrhenius (1908) radiation emitted by stars carried microbes thru space.
(supported by F.Crick)

Murchison Meteorite – AUS - contains PAH's (polycyclic aromatic hydrocarbons) that when mixed with water from capsule-like droplets [John Deamer UCSC]

ALH84001 - Martian meteorite from Antarctic contains PAH's & microscopic looking microbes.
c) chemical evolution - in the beginning........ BIG BANG

Beginning of the Universe 12.0 billion yr ago + 10%  
(Hubble Constant = 70 km per sec per 3.26 mil ly)
Formation of Earth 4.5 billion yr ago
Earth coalesced from space dust. 4.5 bya
Bombarded by interplanetary comets 4.5 to 4.0 bya
water source for oceans
Heavy comet bombardment stops &
----> life begins 3.9 bya
Oldest fossil rocks 3.8 bya
Life (anaerobes) 3.5 bya
(advent of oxygen evolution aerobes) 2.0 bya
(eucaryotes) 1.0 bya
(multicellularity) 0.7 bya

Evolutionary Origin ideas are based upon
1) today's known "living" molecules made from small number chemical functional groups (OH, NH₃, C=O, COOH, etc.)
2) these functional groups easily form monomers..... molecules as - amino acids, nucleotides, sugars
3) these monomers make polymers or macromolecules which favored the energy transforming & self-replicating features that define today's cell...

Experimentally testable... Whose goal may be...
"creation of artificial cell, as model of a life system“
4 experimental approaches used in origins research

1. classical chemical evolution approach:
   Search for sources of early precursor organic molecules of Life ???

4 possible sources for 1st bioorganic molecules of Earth

a. Classical chemical evolutionary mechanism:
   - molecules formed in a chemically reactive soup
     1922 - Oparin & Haldane = reducing atmosphere early Earth rich in NH3, CH4, & H2O = organics
     1953 - Miller & Urey - abiotic synthesis: HC=O & CC=N leads to organics including amino acids & sugars
     1960's - Sid Fox - makes polypeptides & protobionts

b. Deep dwelling (ocean) hydrothermal vents*
   (discovered in 1979)
   - minerals spewing up from pressurized, hot springs is source of 1st biomolecular chemical s.
   - bioorganic chemical reactivity may have originated near hydrothermal vents, before genetics:
     a cycle of chemical reactions that produce energy.
c. Space Debris:
  dust, meteorites, asteroids, & comets deposit organics on
  on newly formed planet Earth.

Comets = ice crystals on core of silicates & carbon
  contain about 10% CO, CO2, CH4, CH3OH, and NH3.

Asteroids: We know that asteroids that hit Earth contain
  molecules as: Kerogen [a PAH], Nucleobases,
  Quiniones, Carboxylic acids, Amines & Amides.

Space debris anomalies:
  Some 70 aa, including 8 of common 20 aa's of today proteins
  can be found in space debris.

While Miller & Urey's soup had 50%/50% mix of D & L aa's
  isomers, asteroid aa's have a surplus of L-aa's,
  as are found in today's proteins (argues for asteroid origin).

Max Berstein (NASA - 1999) has modeled cryochemistry
  reactions to look at molecular events in comets.
  reactions can occur even in very low temps [25K (-400oF)],
  UV can break bonds - has formed ketones, ester, quinones
  (for e- transport), and alcohols.

  Some molecules formed spherical capsule-like droplets when
  exposed to water, with properties akin to cell membranes.

  Suggestion...
    extraterrestrial amino acids built the 1st proteins
    and there may be a possible role for minerals?
Role of Mineral in early abiotic molecular evolution...

**Minerals** (calcite, feldspar, magnetite, clay, etc...) may have fostered organic chemistry of early life.

1998 **Robert Hazen** showed that amino acids decompose at 200°C under pressure, but when FeS (iron-sulfur) minerals are added, amino acids remain intact.

Idea is one of **SCAFFOLDING SUPPORT** for reactivity...
an easy way to assemble molecules in dilute solution is to concentrate the molecules on a flat surface...

**feldspar** - houses microscopic pits that could shelter life's precursor molecules from UV radiation & destruction...
allows concentrating components & chemical reactivity

**magnetite** (iron oxide) - triggers combination of nitrogen & hydrogen into ammonia (NH3), a reduction reaction essential for organic life

**clay** - layered clays can trap organics between sheets; held close together these molecules can form more complex molecules.

**Minerals may answer one chemical anomaly....**
the selection of only 1 optical isomer [L-amino acids] out of 2 (D & L) **enantiomers**... in the making of cellular proteins... which contain only L-amino acids.

Miller's experiment produces 50-50 mix of 2 isomers, the D & L amino acids...

**calcite** - attracts D & L- amino acids to different crystal faces; life could have selected one over the other.
2. Deep dwelling hydrothermal vents:
Deep dwelling (ocean) hydrothermal vents (discovered in 1979) with minerals spewing up from pressurized, hot springs...
vent areas are full of organically rich molecules ---> life (tube worms @ vents).
and bacteria living in hydrothermal vents

speculation is that life may have originated in vents regions.

3. Origins of Self-Replicating Systems...
Evolution of an RNA world... (which came 1st DNA or RNA) in 1989 Sidney Altman & Thomas Cech - received Nobel Prize by demonstrating that RNA molecules have CATALYTIC ACTIVITY (RIBOZYMES)
i.e., these RNA's catalyze hydrolysis & condensation rxs of phosphodiester bonds.
If RNA can be a template & also catalyze polymerization of like molecules, i.e., replicate itself, then RNA molecules may have been 1st SELF-REPLICATING living entity.

No self-replicating RNA molecules exists naturally today, but lab experimentation may establish that it was feasible, and that RNA molecules can be selected for via Darwinian evolutionary mechanisms (natural selection).
4. **Knock-out Cells**...

By modifying a simple microbe, scientists hope to create a new form of single cell life.

> **J. Craig Venter**, a principle investigator (P.I.) of the Human Genome Project is attempting to make a new type of bacterium using DNA manufactured in the lab;

> using the sequenced the genes of a bacterium called *Mycoplasma genitalium*, a gram-positive parasitic bacterium, whose primary infection site may be the human urogenital tract. It probably causes non-gonococcal urethritis. It is also one of the simplest known microbes with only 1 chromosome & 517 genes.

> researchers began systematically removing genes to determine how many genes are essential for life. In 1999, they published a paper that narrowed the needs of *M. genitalium* to between 265 and 350 genes.

> a genomic goal will be to learn on a molecular level the minimum genes a cell needs to thrive and reproduce and how to artificially make those and other genes.

> In 2008 Venter & Smith constructed a synthetic BAC chromosome that is 381 genes (580,000 bp) long using lab-made chemicals. They hope to transplant it into bacterial cell = **new synthetic life form**
Pre-Biotic Chemical Evolution (current paradigm)

origin of life was not a single event...
   it was most likely a gradual sequence of modest
chemical formations, which added a degree of order
and complexity to molecular structure.

1st step - formation of building blocks
   either by... Miller & Urey or hydrothermal vents or
   by 'space debris'

2nd step - minerals provide structural basis for
   concentrating, confining, ordering, & selecting
   molecules; may have lead to first primitive
   self-replicating systems

next step - competition for limited resources leads to

MOLECULAR NATURAL SELECTION

5 Steps in Chemical Evolution of Life

"It was a Dark and Stormy Night"

1. **Abiotic synthesis** of small organics
   HCN & O=CH make sugars, aa's, nucleotides, etc...

2. **Autocatalytic assembly** of **polymers**
   via **CONDENSATION REACTION**

3. **Origin of Heredity...** 'most probably RNA'
   unique sequences, complementary templating
   polymeric catalysts, errors in replicating process

4. **Translation of RNA sequence** into **amino acid sequence**

5. **Membranes** probably define First Cell
Unknown, but necessary evolutionary steps of eucarya
single most important step in evolution of plants & animals was establishment of mutlicellular life forms that lead to animal life.

1. cell membrane encapsulates DNA
development of nucleus greatest evolutionary invention - internalized genome

2. loss rigid cell wall
developed ability of phagosytosis - engulf foods allows cells to clump-->multicellularity--> tissues

3. evolved selectively permeable membrane
protects cell, allows uptake gases & nutrients

4. evolved a cytoskeleton
provides a framework to allow cell to grow larger, move, and permit metabolism
eucarya are 10x larger that bacteria

5. evolved aerobic respiration
more efficient energy transformation

6. developed various organelles - (endosymbiosis)
a sub-cell part that catalyzes a specific metabolic function

7. development of sexual cell cycles - (transposons)
a method to shuffle genes along chromosomes
Top 10 things that characterize cells as living... (continued)

4. All cells METABOLIZE
- chemical processes in living cells where some substances are broken down to yield energy for vital processes & other molecules are made

When classifying organisms biology often looks at mode on Nutrition, i.e., how cells obtain energy & carbon from their environment

**AUTOTROPHS** - capable of synthesizing all their organic molecules using CO₂ as sole C source
1) photosynthetic = use solar light energy
   ... capture light by chlorophyll
   transfer e- from donor (at first H₂S, now...H₂O) to CO₂ to reduce it to CH₂O
2) chemotrophic = use simple inorganics as energy

**HETEROTROPHS** - obtain energy from foods, i.e., chemical fuel systems (sugars, fats, proteins) unable to synthesize all needed molecules - i.e., obtain nutrients by dietary means
cells possess Metabolic Pathways.....

**ANABOLIC** - synthetic reactions  
larger from  smaller

**CATABOLIC** - degradatory  
breakdown larger into smaller

**Metabolic Pathways show**:
  economy & efficiency  
controlled by ENZYMES  
are Self-Regulating  
negative feedback & dynamic steady state  
rate of synthesis = rate of degradation  
are integrated = work in a coordinated fashion  
everything at the right time

A  ---&gt;  B  ---&gt;  C  ---&gt;  D  ---&gt;  E  
e1  e2  e3  e4

**One definition of Life may be:**
carefully orchestrated chemical reactions.
5. **Self-replication (single most definitive property of life)**
   an inanimate counterparts is growth crystals in chemistry computer simulation models of evolution *(Tierra)*
   a key molecular property --> self assembly...
   individual macromolecules with affinities for each other form supramolecular complexes (organelles)
   genetic info is DNA - asexual cell division = **mitosis** &
   - sexual cell division = **meiosis**

6. **Osmoregulate**
   regulate exchange material across cell membrane with the environment - solvents & solutes in/out

7. **Communicate** - intra (within) & inter (between)
   hormones/neurons

8. **show Animation**
   cytoplasmic streaming, motility, & cyclosis

9. **cells Grow** increase in mass),
   **Divide** increase in cell number), &
   **Differentiate** become structurally, functionally &
   biochemicaly different fertilized egg ---> to adult

10. **Die** - reveal absence of properties of life
by Thomas Ray @ U. Delaware - **TIERRA**

Small computer programs of assembly code
how to copy self
like a computer virus
compete for cpu time & memory space

Primordial program = 80 instructions
  stored in cpu (lived), consumed cpu cycles
  (metabolized), copied itself (reproduced), moved
  up in que (animation) & was removed (died)

New programs emerged (mutated & evolved)
first had 79 instructions
one had 22 instruction..replicated 6x faster
some had only 45 lines & lost ability to replicate
  but borrowed instruction from other = parasites
some programs became defensive = immunized
  against parasites

Are the Tierrans alive ?
Is "Star Trek's Cloud" alive ?
"Borg" - term used by Mallery for the premise by
  some cosmologists that artificial
  intelligence may be the evolutionary
  consequence for human-kind
Life is manifest in the structure we call the CELL

all cells are presumed to derive from single primordial cell born some 4 billion years ago, it out reproduced its contemporary competitors, and has a family resemblance to today's cells
...all use DNA
...all have same genetic code
...all possess same basic molecule types
...all have similar properties & functions
Universal Properties of All Cells:

1. store their hereditary information in a linear DNA molecular code

2. replicate their hereditary information by templated polymerization

3. transcribe hereditary information into RNA intermediate

4. translate RNA into protein by same mechanisms
   - fragment of hereditary information for 1 protein is 1 gene
   - small cells can have fewer than 500 genes
   - new genes come from preexisting genes via intragenic mutations, gene duplications, fragment shuffling, and intercellular transfers.

5. use proteins as catalysts

6. life (cells) require free energy - free energy sources for cells include:
   - heterotrophy (foods), phototrophy (light), and lithotrophy (inorganic reactions)
   - some cells can fix N₂ --> NH₂ & some can fix CO₂ --> CH₂O

7. use same molecular building blocks for biochemical rxns

8. are enclosed by an amphiphatic lipid plasma membrane
Life and a Living Cell may be described as:

self contained,
self assembling,
self adjusting,
self perpetuating,
isothermal mix of biomolecules,

held in a 3-D conformation by weak non-covalent forces,
which can extract raw materials (precursors) & free energy from its surroundings,
that can catalyze reactions with specific biocatalysts (enzymes), that it makes,
which shows great efficiency & economy of metabolic regulation,
that maintains a dynamic steady state far from equilibrium,
and that can self-replicate, using the linear information molecule DNA.
The Chemistry and Molecules of Life

Cells are made of molecules & molecules are made of matter

MATTER... occupies space and has mass (weight)

made of ELEMENTS.... which are composed of ATOMS

NUCLEUS... PROTONS & NEUTRONS
ELECTRON... ORBITAL CLOUD... Orbital Stability
    valance = # bonds element can form

ATOMIC NUMBER = # protons present
ATOMIC MASS = # of protons + # of neutrons
    also called atomic weight
    compares atom to atom
    units are called Daltons or amu
        1 amu = 1/12th mass of carbon 1.0073

ION - electrically charged atom
    loss of e⁻ = OXIDATION
    gain of e⁻ = REDUCTION

ISOTOPE - atom with same # protons, but more neutrons
    99% < carbon-12 ¹²C₆
    1% < carbon-13 ¹³C₆
    1% < carbon-14 ¹⁴C₆  half-life C - 5,730y
    unstable = radioactivity   n --> p + e⁻   ?¹⁴
    isotopic tracing of biomolecules


**$^{14}$C Dating - also called RADIOCARBON DATING**

is a method of age determination that depends upon the decay of radiocarbon (carbon-14) to nitrogen.

$^{14}$Carbon- is continually formed in nature and is absorbed by plants and then passed on to animals through the food chain. Radiocarbon spontaneously decays slowly in a living cells & the amount lost is continually replenished as long as the organism takes in air or food. Once it dies, however, it ceases to absorb $^{14}$C, so that the original amount of $^{14}$C present in its tissues at death steadily decreases.

**$^{14}$C has a half-life of 5,730 +/- 40 years**

i.e., half the amount present at any given time will undergo spontaneous disintegration during the succeeding 5,730 years.

A half-life is the time it takes for one-half of the parent isotope to decay to its daughter isotope ($^{14}$C to $^{14}$N).

Because $^{14}$C decays at this constant rate, an estimate of the date at which an organism died can be made by measuring the amount of its residual radiocarbon present now.
MOLECULE... a group of like or dissimilar atoms held together by a CHEMICAL BOND (an electrostatic attraction).

**TYPES of BONDS:** (energies 0 to > 1,000 cal/mol)

**NON-COVALENT** - electrostatic interactions (10-150 cal/mol)

1. **IONIC Bonds** - small ion attractions by charge [+/-] (CATION s/ ANIONs)... $^{11}\text{Na}^{(2,8,1)}$ & $^{17}\text{Cl}^{(2,8,7)}$
2. **DIPOLES** - attractions via asymmetrical internal distribution of charge without a net charge
   - Ionic Charge - DIPOLE - DIPOLE - DIPOLE
   - Induced DIPOLE - Charge
   - Induced DIPOLE - DIPOLE
3. **DISPERSION** (van der Waal) **FORCES** - attraction based upon closeness of atomic nuclei
4. **HYDROGEN BOND** - electrostatic attraction between hydrogen of one atom & pair of non-bonded e's on an acceptor group electrostatically attract...

Individual non-covalent forces very weak, but billions can... hold two atoms together very tightly, forge a 3-D shape, that holds specific biological activity

**COVALENT** - sharing of electrons between atomic nuclei such as: C-C and H-H and C-H
**CHEMICAL ARCHITECTURE of CELLS**

**WATER – Biological Role of Water**

70% of mass of a cell is water (H2O)

role of water ... solvent/product of reactions

\[
6\text{CO}_2 + 6\text{H}_2\text{O} \rightleftharpoons \text{C}_6\text{H}_1\text{2O}_6 + 6\text{O}_2
\]

its location in cells ... is the soluble phase of the cell

bulk vs. vicinal (structural water ?)

**Physical Properties water... exists in 3 forms**

<table>
<thead>
<tr>
<th>Form</th>
<th>Gas</th>
<th>Liquid</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>high</td>
<td>surface tension</td>
<td>cohesiveness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>specific heat</td>
<td>heat 1 gm 1C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heat of vaporization</td>
<td>540 cal/gm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heat of fusion</td>
<td>79 cal/gm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>density on freezing</td>
<td>less dense</td>
<td></td>
</tr>
</tbody>
</table>

**STRUCTURE of WATER...**

a tetrahedral shape with unequal distribution of charge

i.e., it's a molecular dipole (magnet-like) +/-

Primary force – is its weak electrostatic interactions...

HYDROGEN BOND... not a "bond" at all
CHEMICAL ARCHITECTURE of CELLS

INORGANICS...
95% of Mass of cells is ONLY 6 ELEMENTS - C H O N P S
"C HOPKIN'S CaFe Mg"
Why These?

Role of inorganics in cells...
mainly - bone, exoskeleton, vitamins, etc...
learn the pH scale

ORGANIC MOLECULES... molecules of C and H

<table>
<thead>
<tr>
<th>CLASSES</th>
<th>Biological Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbohydrate (CHO)</td>
<td>structure &amp; energy molecules</td>
</tr>
<tr>
<td>fats (lipids) (CHO)</td>
<td>structure &amp; energy molecules</td>
</tr>
<tr>
<td>phospholipids (CHO-P)</td>
<td>membrane structure</td>
</tr>
<tr>
<td>steriods/sterols</td>
<td>membrane parts - hormones</td>
</tr>
<tr>
<td>proteins</td>
<td>structural, enzymatic (catalytic)</td>
</tr>
<tr>
<td>nucleic acids</td>
<td>informational, genetic role</td>
</tr>
</tbody>
</table>
## Main Classes of Biological Macromolecules

<table>
<thead>
<tr>
<th>Class</th>
<th>Sub Types</th>
<th>Ex:</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>monosaccharide</td>
<td>glucose</td>
<td>energy</td>
</tr>
<tr>
<td>approx. formula</td>
<td>simple sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₂O</td>
<td>disaccharide (2)</td>
<td>sucrose</td>
<td>transport sugar</td>
</tr>
<tr>
<td></td>
<td>polysaccharide</td>
<td>starch</td>
<td>energy -plants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glycogen</td>
<td>energy -animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cellulose</td>
<td>structure -plants</td>
</tr>
<tr>
<td>Lipid (fats)</td>
<td>triglyceride</td>
<td>oil, fat</td>
<td>energy storage</td>
</tr>
<tr>
<td>high C &amp; H</td>
<td>(3 fatty acid &amp; 1 glycerol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>wax (FA+ alcohols)</td>
<td>waxes</td>
<td>waterproof plants</td>
</tr>
<tr>
<td></td>
<td>phospholipids</td>
<td></td>
<td>membranes</td>
</tr>
<tr>
<td></td>
<td>(1P, 2FA, 1X, 1glycerol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>steroids</td>
<td>cholesterol</td>
<td>membranes</td>
</tr>
<tr>
<td></td>
<td>(isoprenoid ring)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic Acids</td>
<td>nucleotide polymer</td>
<td>DNA</td>
<td>genetic material</td>
</tr>
<tr>
<td></td>
<td>(S,,P, base)</td>
<td>RNA</td>
<td>virus genes</td>
</tr>
<tr>
<td></td>
<td>single nucleotides</td>
<td>ATP</td>
<td>short term energy</td>
</tr>
<tr>
<td>Protein</td>
<td>amino acid polymer</td>
<td>hemoglobin</td>
<td>varied</td>
</tr>
</tbody>
</table>
A. CARBOHYDRATES..... CH₂O

MONOSACCHARIDES ... simple sugars

Molecular or empirical formula vs. structural formula
stick and space filling models
ISOMERS - structural, geometric, and optical

DISACCHARIDES ...
condensation rx... GLY COS IDIC bond
sucrose  - alpha 1,2 glu-fruc ---> sugar
maltose  - alpha 1,4 glu-glu ---> amylose
cellibiose - beta 1,4 glu-glu ---> cellulose

POLYSACCHARIDES ....
complex sugars ...polymeric chains
STARCH (AMYLOSE) AMYLOPECTIN GLYCOGEN
CELLULOSE CHITIN

TAKE HOME MESSAGE here...

"Structure is critically important to function, & structure relates to "ORIENTATION" of covalent bonds in 3D space"
Importance of Molecular Shape

2. UNIQUE properties of BIOMOLECULES that give them unique FITNESS for the Living State

1. configuration -
Spatial arrangements of atoms in a molecule configuration can't be interconverted without breaking bonds isomers are based upon covalent configurations

**Asymmetric Carbon** atom [4 diff. groups attached]
stereoisomers - CHIRALS - [enantiomers =mirror images]
isomers with identical chemical properties, but, rotate plane polarized light at different angles

levorotatory = left handed  dextrorotatory = right handed

**Enantiomers** are molecules that have opposite spatial configuration are said to be optically active. One enantiomer will rotate polarized light a set number of degrees to the right. This is called the dextrorotatory isomer or (+) isomer.

The other enantiomer will rotate the plane polarized light the same number of set degrees in the opposite left direction. This isomer is said to be a levorotatory isomer or (-) isomer.

**Double covalent bonds**  \( C = C \)
fix atoms ABOVE & BELOW plane of molecule

maleic (cis) and  fumaric (trans)

11-cis-retinal  11-trans-retinal
2. conformation [3D-shape]

- surface outline or contour of a molecule
  3-D orientation of groups made without breaking any bonds
due to free rotation of atoms about a single chemical bond
different isomeric forms.....
only one of which may be biologically active

- ENZYMES can distinguish between biologically active forms of molecule based on their "3D-SHAPE"
B. FATS and LIPIDS

TRIGLYCERIDE ... 1 GLYCEROL and 3 FATTY ACIDs
SATURATED vs. UNSATURATED
glycerol end = HYDROPHILIC (POLAR)
fatty acid end = HYDROPHOBIC (NON-POLAR)

PHOSPHOLIPIDS...
1 glycerol, 2 fatty acids, PO4, & an organic
MICELLES........ BILAYERS

CHOLESTEROLS.... insoluble in water
anabolic steroids

C. NUCLEOTIDES
aromatic base, ribose sugar, phosphate
polynucleotide - polymer of ATCG (U)
RNA - ribose nucleic acid
DNA - deoxy ribose nucleic acid
- double helix of DNA
**FUNCTIONAL GROUPS**

Groups of atoms, acting as a unit, that give organic molecules their physical properties, chemical reactivity, & solubility in aqueous solutions.

Most possess electronegative atoms (N, P, O, S)

Key bonds are: ester C-O-C & amide -C-N-

Are ionizable at physiological pH

- **OH**  HYDROXYL = alcohol
- **NH2**  AMINE = amino acid
- **COOH**  CARBOXYL = acid
- **CH3**  METHYL = hydrocarbon
- **C=O**  CARBONYL = aldehyde/ketone
- **SH**  SULFHYDRYL = disulfide
- **PO4**  PHOSPHORYL = phosphate

**Consequences of Substitution w Functional Group**

- Ethane CH3-CH3 toxic, flammable gas
- Ethanol CH3-CH2-OH ethyl alcohol, potable
- Propionate CH3-CH2-COOH propionic acid
- Ethanethiol CH3-CH2-SH "rotten eggs - onions"
  - smell of natural gas
Proteins are Classified by Function

Protein - a polymer of amino acids with biological activity

Enzymes - catalytic activity and functions
  A       ------->      B

Transport Proteins - bind & carry molecules

Storage Proteins - ovalbumin, ferretin, casein

Contractile - can contract, change shape,
  elements of cytoskeleton & muscles

Structural - support .... collagen of tendons,
  elastin of ligaments, keratin of hair & feathers,
  fibroin of silk & webs

Defensive - protect: antibodies (IgG),
  fibrinogen & thrombin, snake venoms

Regulatory - regulate metabolic processes,
  hormones, transcription factors & enhancers
Structure and Properties of Proteins

PROTEINS - polymer of amino acids w biological activity

STRUCTURE of Amino Acids
- alpha amino acid (20)
- aa's have a carboxyl group (-COOH) & amino group (-NH2)
- bound to an asymmetric carbon
- 20 ubiquitous aa's

ZWITTERION - 2 groups of opposite sign in same molecule
Isoelectric Point - pH where there is no net charge in molecule
pK - pH at which groups are 50% ionized & 50% non-ionized

R (side) groups... TYPES of Amino Acids
ACIDIC ... negatively charged - ASP & GLU
  R group with 2nd COOH that ionizes above pH 7.0
BASIC ... positively charged - LYS, ARG, HIS
  R group with 2nd amide that protonates below pH 7.0
POLAR UNCHARGED ... SER, THR, TYR, ASN, GLN
  are soluble in water, i.e., hydrophilic
NON-POLAR ... GLY, ALA, VAL, LEU, ILE, PRO
  all contain only hydrocarbons; R groups = hydrophobicity
AROMATIC (hydrophobic)... PHE, MET, TRP, CYS
  all contain R groups with ring structures
peptide bond - covalent link between carboxyl end of aa1 & amino end of aa2 forms a dipeptide shorten & stronger than C-C; longer & weaker than C=C no free rotation (attached group in same plane)

Some examples of naturally occurring oligopeptides

insulin - 2 polypeptides  alpha chain of 30 aa’s beta  chain of 21 aa
glucagon -pancreatic hormone of 29 aa, opposes insulin action
corticotropin -39 aa anterior pituitary hormone that stimulates adrenal cortex
oxytocin - 9 aa hormone pf posterior pituitary that stimulates uterine contractions
bradykinin - 1 aa hormone that inhibits inflammation
thyrotropin relasing factor - 3aa of hypothalmus that stimulates release of thyrotropin
enkephalins -CNS peptides that bind to brain cell receptors = analgesic reaction of pain deadening NutraSweet - dipeptide of L-aspartyl-phenylalanyl (methyl ester)
Protein Fractionation Techniques

Isolation & Purification of a "new" protein....

**Crude Homogenates** - grind up cells in blenders & sonicators

**Differential Centrifugation** - subcell fractionation by **centrifugal gravity**... centrifuge speeds to 250,000xgravity [supernatant & pellet]

**Fractionations – by Column Chromatography**

**gel filtration** – by size in exclusion chromatography

sephadex - porous carbohydrate polymer beads

**ion exchange** – held by ion charge on column’s media

**affinity chromatography** - polymeric beads with special ligands (as substrate) to bind protein

**gel electrophoresis** ...porous gel by size & charge

SDS-electrophoresis (sodium-dodecyl-sulphate) **bymass-mw**

**isoelectric focusing** - migration to point of pi

**2-dimentional electrophoresis** -

isoelectric focusing & SDS electrophoresis

**Identification - colorimetric tests** [Biuret & Bradford]

a protein is reacted with a colored dye, as Biuret or Coomassie blue; amount of color density is measured in a spectrophotometer; units are absorbance given at specific wavelengths (say 595nm) & absorbance is plotted vs. protein amount & results in a linear plot.
PROTEIN STRUCTURE

Variety of Amino Acid Sequences is infinite.....
Average prot = 300-400 aa's & MW 30,000 to 45,000

A protein of 100 amino acids made w 20 different kinds aa's can have $20^{100}$ different linear arrangements [1.268 x $10^{130}$]

Fred Sanger - 1958 Nobel prize for INSULIN sequence
to date 1,000 protein sequences are known in computer data bases vs. e. coli makes about 3,000 proteins ; humans about 100,000 proteins

4 levels of protein structure are recognized
primary linear sequence of amino acids
secondary regular, recurring orientation of aa’s in a peptide chain - $\alpha$ and $\beta$
tertiary complete 3-D shape of a peptide
quaternary spatial relationships between different polypeptides or subunits
PRIMARY SEQUENCE…
linear Sequence of Amino Acids… some consequences

Polymorphism…
proteins may vary in primary sequence, but
have the same biological function. ex: enzymes
H₂O₂ --> 2 H₂O + O₂
inter-specific: between species – diff. aa sequence
intra-specific: within a species (liver vs. kidney)

Invariants… primary sequences don't vary
ubiquitin & histones

Site Specificity…
some sequences determine intracell location
Signal Sequences, Prosthetic Binding sites, etc..

Homologous Proteins…
evolved in a related fashion
perform same cellular function in diff species
Hb & cyto-C ex: in duck & chickens = 2 variants
in yeast & horses = 48 variants

Example - LYSOZYME:
Functions as a bactericidal agent (breaks cell walls);
an enzyme found in egg whites & human tears;
MW = 14,600 & 129 aa's with 8 CYS residues [4 S-S]
hydrolyses polysaccharides in bacterial cell walls.
Secondary Level

**alpha Helix** - peptide backbone wound around a long axis; core forms a rigid helix cylinder
- R-groups radiate out
- 3.6 aa per turn
- right handed helix - (counterclockwise)
  formed by H-bonds
  
  \[
  \text{Hof N (of any aa) } \& \text{ -O=C (of 4th aa)}
  \]
  \[
  \text{¼ of aa's in globular proteins}
  \]

**Beta sheet** - a linear extended ZIG-ZAG pleated sheet
  formed by H-bonds intra- \& inter-chain

Tertiary Level

**3D shape of a protein** ...
- often the most stable conformation
- involves weak forces:
  - H-bonds, hydrophilic \& hydrophobic interactions
  - \& stronger bonds as: ionic bonds \& disulfide bonds

**Denaturation** vs. **Renaturation** (loss biological activity)

Quarternary Level

- shape between more than one polypeptide or subunit of a protein
  - hemoglobin, RNA polymerase, ASP-transcarbamylase
Nomenclature of Proteins

Two classes - Simple & Complex
Based on solubility of Proteins in Solvents... esp. Water

**SIMPLE**
1. **Albumins** - soluble in water; globular; many enzymes
2. **Globulins** - soluble in dilute aqueous solutions;
3. **Prolamines** - insoluble in water; soluble in 50% to 90% simple alcohols
4. **Glutelins** - insoluble in most solvents; soluble in dilute acids/bases
5. **Protamines** - not based upon solubility;
   low MW proteins w 80% Arg & no Cys
6. **Histones** - unique - complexed w DNA
   high [ basic aa's ] - 90% Arg, Lys, or His
7. **Scleroproteins** - insoluble in most solvents
   fibrous structure - cartilage & connective tissue
   Collagen = high Gly, Pro & no Cys
   when boiled makes gelatin
   Keratins - proteins of skin & hair -
   high basic aa's w Cys

**COMPLEX**
lipoproteins... blood, membrane, & transport proteins
glycoproteins... antibodies, cell surface proteins
nucleoproteins... ribosomes & organelles
ENZYMEs - “in yeast”

1878 1st described on Pasteur's lab
1926 Sumner crystalizes 1st enzyme enzymesUrease

regulate metabolic reaction rates: controls metabolism molecules (mostly protein) that accelerate or catalyze chemical reactions (A ---> B) in cells by breaking old covalent bonds and forming new covalent bonds. biological catalyst... but, differs from metal catalysts:

1. have complex, specific structure (sequence of aa's)
2. act only upon a specific substance (substrate)
3. do not change direction (energetics) of a reaction
4. function by lowering Energy of Activation \( E_a \)
catalyzes reactions by:
   gains/loses e- ; transfers group ; breaks bond.
many require cofactor or coenzyme
   cofactor - small inorganic ion that catalyzes reaction
       Cu+2 ; Mg+2 ; Mn+2, etc...
   coenzyme - smaller, non-protein ligand which

Reaction path:  \[ E + S \rightleftharpoons ES \rightleftharpoons E + P \]

Active site - portion of enzyme protein that holds the substrate by means of weak chemical bonds (H-bonds, ionic bonds, hydrophobic forces, etc...)

lock & key vs. induced fit.
ENZYME KINETICS
mathematical and/or graphical expression of the reaction rates of enzymes

Catalase \[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

Characteristic Enzyme Curves:
or how to determine if a reaction \( A \rightarrow B \) is enzymatic

1. Rate (0.8 ml O2/min) Vs. [E]
2. Rate (optimum) Vs. pH
3. Rate (optimum) Vs. Temperature
4. Rate (saturates) Vs. [S]
A plot of rate (amount of product per unit time) vs \([S]\) i.e., rate vs. substrate concentration saturates..... at \([S] = V_{\text{max}}\) (maximum velocity)

\(K_{m}\) = substrate concentration at which rate is \(\frac{1}{2}\) of the maximal velocity (in above \(K_{m} = 2\) mg) is a measure of affinity of enzyme for its substrate i.e., amount of \([S]\) needed to reach 1/2 \(V_{\text{max}}\)

**Inhibition** - where action of inhibitor is ...reversible competitive... inhibitor binds to active site lower \(K_{m}\) same \(V_{\text{max}}\)

**noncompetitive**... binds to allosteric site same \(K_{m}\), but lower \(V_{\text{max}}\)
Regulation of Metabolism via **Allosteric Regulation**
regulates enzyme activity by changing protein shape

**FEEDBACK INHIBITION** and **POSITIVE FEEDBACK:**
an end product inhibits an initial pathway enzyme
by altering efficiency of enzyme action and
an end product stimulates an early enzymatic step.

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![Diagram of enzyme pathways with feedback inhibition and positive feedback](image)

**Important take home message**

- **Shape (conformation) of a molecule [enzyme] can greatly influence reactivity & efficiency of metabolism**

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Fall & Summer Bil 150 - Proteins & Enzymes
ENZYME NOMENCLATURE...
4 digit Number System [1.2.3.4.] Enzyme Commission #
1st Major Class of Activity
2nd Subclass (type of bond acted upon)
3rd Subclass (group acted upon, cofactor required, etc...)
4th Serial number ... sequence order

MAJOR CLASSES
1. Oxidoreductases [dehydrogenases] ....
catalyze oxidation-reduction rxs, often w coe NAD+/FAD
   Alcohol dehydrogenase [EC 1.1.1.1]
   ethanol + NAD+ -------> acetaldehyde + NADH
2. Transferases... catalyze transfer of functional groups
   Hexokinase [EC 2.7.1.2]
   D-glu + ATP -------> D-glu-6-P + ADP
3. Hydrolyases... catalyze hydrolysis –
   adds water across C-C bonds
   Carboxypeptidase A [EC 3.4.17.1]
   [aa-aa]n + H2O ------> [aa-aa] n-1 + aa
4. Lyases.... add or remove groups to C= bonds
   Pyruvate decarboxylase [EC 4.1.1.1]
   PYR -------> acetaldehyde + CO2
5. Isomerases [mutases] .... catalyze isomerizations
   Maleate isomerase [EC5.2.1.1.] (cis-trans isomerization)
   maleate -------> fumarate
6. Ligases... condenses 2 substrates w splitting ATP
   Pyruvate carboxylase [EC 6.4.1.1.]
   PYR + CO2 + ATP -------> OAA + ADP + P
A definition of a cell?

A living cell is a ..... 

self contained 
self assembling 
self adjusting 
self perpetuating 
isothermal mix of biomolecules 

held in a 3-D conformation by weak 
non-covalent forces 
which can extract raw materials (precursors) 
& free energy from its surroundings 
that can catalyze reactions with specific 
biocatalysts (enzymes), which it makes 
that shows great efficiency & economy of 
metabolic regulation 
that maintains a dynamic steady state far 
from equilibrium 
that can self-replicate using the linear 
information of a molecule of DNA.
How Cells are ORGANIZED:

3 Basic Parts of a Cell
1. membrane (selectively permeable - in/out)
2. a DNA region (nucleoid or nucleus)
3. the cytoplasm & its parts organelles

within the three Biological Domains
- **Eubacteria** - true bacteria
- **Archaea** - ancient prokaryotes
- **Eucarya** - modern multi-cellular systems

2 successful Cellular Plans of Organization

1. PROKARYOTE - "before nucleus"
   - includes... Unicellular forms  blue green algae & bacteria
   - primitive, simple, versatile, common unicellular forms
   - most successful life form -
     - 80% to 90% of total biomass of planet
     - 2500 different species known
   - characteristics:
     - lack membrane bound organelles
     - genes "naked DNA" - no "chromosomes"
     - little to no internal compartmentation
     - size 0.1 to 10 µm diameter
Two major forms of prokaryotes exist today:

Archaebacteria and Eubacteria [ancient & true]

**ARCHAEBACTERIA** ...

living archaebacteria include:

- the **extremophiles** – those living in extreme environments
  - METHANOGENS: \( \text{CO}_2 + \text{H}_2 \rightarrow \text{CH}_4 \)
  - HALOPHILES: live in Dead Sea & Great Salt Lake
  - THERMOPHILES: in acid hot springs, deep ocean geysers
  - ACIDOPHILES & ALKALIPHILES: acid & base loving

**EUBACTERIA** (all other living bacteria modern form + flagella)

- many cause diseases - Bacillus anthracis = anthrax
  - Clostridium botulinum = botulism
  - Staphylococcus aureus = food poisoning
  - Salmonella = food poisoning & typhoid
- many make antibiotics - Streptomyces = streptomycin
  - Penicillus = penicillin

**CYANOBACTERIA** - are photosynthetic eubacteria

Archaean/eubacteria ... are highly conserved - living fossil forms and solve many environmental challenges (problems) by their chemistry and by evolving new metabolic solutions.

**procaryotic bacteria are found in 3 common shapes:**

- **cocci,**
- **bacillus,**
- **spirochetes**
current paradigm...
eukaryotes evolved from simpler prokaryotes

2. EUKARYOTIC  eu - true  karyon - nucleus
   plan of multicellular organisms...
   many internal membrane bounded organelles
   **organelle** = a subcell part that has a distinct metabolic function

7 common major characteristics of eucaryotes:
   nucleus - single greatest step in evolution of animals
   genes in "chromosomes" [colored bodies + protein]
   contains more DNA (1,000 x more)
   presence of **organelles**- internal compartmentation
   presence of flexible cell walls (allows **phagocytosis**)
   presence of **cytoskeleton**
   reproduce sexually
   usually larger - cell volume 10X greater than bacteria)
   - size 5.0 to 20 µm diameter
   extensive internal **membranes**

2 basic types of eukaryotic cells:
   animal - metazoan - heterotrophic feeder
   plant  - metaphytian - autotrophic producer
   chloroplasts, large vacuoles, cellulosic cell wall
Where do the VIRUSES fit?

obligatory intracellular parasites:
- pathogens of made of a protein capsid (capsule) & genetic material (ss or ds RNA or DNA)

**VIRION** - virus outside of host
**VIROID** - RNA pathogen (virus w/o capsid) 240-600 n's

Extreme viruses:
Viruses *(like extremophiles)* can live in Earth's most extreme environments.

**Origin of Viri(?)...** small pieces of cell chromosomes, that maintained an autonomous existence within cells. Overtime these genetic elements acquired protein coats & ability to transfer to other hosts (and became infective) ???

**What are PRIONS –** protein infectious pathogens
- biological activity without RNA or DNA ? How ???
- cause diseases as: **encephalopathies**...
  - scrapie, Creutzfeldt-Jacob, mad-cow disease.
- all are due to --> **misfolded proteins**

**Nanobes??** are tiny filamental structures found in some rocks and sediments;
- smallest are just 20nm long. May be crystal growth, but they're purported to hold **DNA**;
- look similar to life-like structures found in ALH84001
How do we identify subcell parts?

**Light Microscopy**
resolution = distance by which distinguish 2 dots = 0.2 um
killing/fixing samples: formaldehyde & glutaraldehyde
sectioning: microtome (1 to 10 um thick)
selective staining:
types: bright field, phase-contrast, Nomarski, dark-field

**Electron Microscopy**
resolution = 0.2 nm
TEM - Transmission
SEM - Scanning
FfEM - Freeze fracture

3D = Orange Bowl cross section

Size relationships of parts -

Cell Isolation & Culture ..... HeLa cells

Homogenization - Fractionation & Centrifugation
Major Eukaryotic sub cell ORGANELLES

NUCLEUS: envelope, chromatin, nucleolus, nucleoplasm

MITOCHONDRIA: peri-mitochondrial space, cristae, matrix

CHLOROPLAST: peri-chloroplast space, thylakoids, stroma

RIBOSOME: small unit, large unit, polysome

ENDOPLASMIC RETICULUM: smooth & rough

GOLGI BODY: sided - cis & trans; endomembrane pathway

LYSOSOME: hydrolytic enzymes

MICROBODIES: peroxisome & glyoxysome

CYTOSKELETON:
   microfilaments, microtubules, intermediate filaments

CENTROSOME: centriole, basal body, flagella, cilia

INTRACELLULAR JUNCTIONS:
   tight junctions, desmosomes, gap junctions, plasmodesmata

PLANT CELL VACUOLE:
   surrounded by membrane; stores waste, balance osmosis

CELL MEMBRANE:
   surrounds cell & regulates what gets in/out
Major Eukaryotic Organelles...

**NUCLEUS**

1st described by Robert Brown in 1831 – in the stamens of Tradescantia
1st isolated by Frederick Meischer in 1871 – in wounds

Largest organelle:
- maximum dia 10 um,
- volume to 40 um3 (10% of cell),

found in all euc's (except erythrocytes-sieve tubes)
evolutionary origin= mesosome?

**Components**
- nuclear envelope - double membrane bound
- pore complexes -
- chromatin - DNA (5x10-12gm) + histones & ANP's
  - heterochromatin (condensed & inactive)
  - euchromatin (less dense & active)
- chromosome structure
  - nucleosome & supercoiling
- nucleolus - rDNA genes makes rRNA
- nucleoplasm - soluble phase
  - enzymes, RNA's. solutes, chromatin, etc...
Nuclear transport & pores...

Experiments to determine Nuclear Transport & Pore Sizes

1960's - Feldherr injects gold particles in unicell amoeba's
   TEM's show particles near nuclear pores with a minute
   in 10 min, then gold particles in nucleoplasm

1970's - used fluorescent tagged proteins -
   showed proteins < 60,000 MW passed

1980's - How do large proteins get in/out?
   Laskey - used nuclear protein... nucleoplasmin made
   in cytoplasm... he radioactively tagged it &
   autoradiographically followed its movement
   > showed nucleoplasmin enters nucleus
   suggests protein has an aa sequence helps mobility
   > aa signal is in tail

Conclusion: nucleoplasmin holds a special
   17 amino acid sequence that targets transport into nucleus
   called  NUCLEAR LOCALIZATION SIGNAL  (NLS)

Review chromosome structure:
MITOCHONDRIA...

**role**: convert bond energy in foods to ATP.......... couples redox transfer of e- & H+ to ATP synthase

**site of**: cellular respiration
- redox rx's [CH$_2$O $\rightarrow$ CO$_2$]
- gas exchange in cell - CO$_2$ released & O$_2$ reduced
- Krebs cycle: PYRUVATE $\rightarrow$ CO$_2$ + H$_2$O
- Respiratory ETC chain & oxidative phosphorylation

**1st described 1900's**: Vital (req living) stains as Janus Green B; today = fluorescent dyes as dasmine

**structure**: elongate cylinders to oblate spheroids
- 3-5um by 0.5-1.0 um dia, "shape-shifters", mobile.

**number**: 20 to 1,000 per cell ; > # in more active cells
- 20% of cell's volume

**double membrane bound organelle**: outer membrane - holds transport protein porin (5K)
- peri-mitochondrial space - where H+ accumulate
- inner membrane – impermeant; req carrier proteins
cristae- inner membrane holds resp. assemblies ETC

**mitoplasm**: aqueous compartment of mito...
- holds DNA, ribosomes, KC, etc.
PLANT PLASTIDS...

- all are double membrane bound organelles found in all higher plants produce organics required by metazoan cells [sucrose]

PROPLASTID... precursor plastid to all the other plant plastids found in apical meristems - dividing cells of root/shoot tips cell environment defines type plastids to be made

ETIOPLAST .....develops in dark, interior array of crystalline-membranes & yellow-chlorophyll precursor-like molecules

LEUCOPLASTS .... non-pigmentous, 2x5 um, variable shape

3 types: AMYLOPLASTS, ALEUROPLAST, ELAIIOPLASTS

CHROMOPLASTS ... water soluble pigments, flower colors...

CHLOROPLAST .....develops in light from etioplasts , site of autotrophic metabolism ... i.e., PHTS, O₂ evolution, CO₂ reduction

shape - variable shape (oblite spheroid –stellate-reticulate)

size - 2-3 um dia by 5-10 um long ;

number - 15/20-100's/cell

contents = STROMA (chloroplast)

1) 70s ribosomes (bacterial size)
2) lipid droplets
3) DNA pieces: supercoils & repetitive
4) starch granules & pyrenoids ->
5) enzymes of CO₂ fixation (reduction)
6) internal membrane system = THYLAKOID Disks
   GRANA Stacks and INTERGRANAL membranes
**ENDOSYMBIONT HYPOTHESIS...**

Proposed by Lynn Margulis - 1981

"Mitochondria & Chloroplasts are derived from prokaryotes that were once free living & have joined symbiotically with eukaryotic aerobes during cellular evolution"

**Some Evidence:**

Many of today's single celled eukaryotes live in oxygen poor places (gut), lack mito, & function anaerobically

*Pelomyxa palustis* (euc amoeba) that lacks mito & holds aerobic bacteria in its cytoplasm (symbiosis)

*Chloroplasts* share a common molecular ancestry with the cyanobacteria (1st photosynthetic procaryotes)

**Number striking similarities of Bacteria & Mito/Chlp**

- semiautonomous: derived from themselves (by fission)
- replicate independently from cell
- have own DNA & protein biosynthetic systems
- DNA sequence homology:
  - mitochondria to aerobes, chlplast to cyanobacteria
  - ribosomes are same size as bacterial (70s)
  - double membrane bound = a phagocytic engulfment?

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[Diagram of endosymbiosis process]
**RIBOSOME ...**

subcell ribonucleo-particle  
site protein synthesis  
spheroid - 17 to 23 nm dia  
composed of 2 PARTS  
small subunit and a large subunit  
composition - 35% protein and 65% rRNA  
occur free in cytosol or membrane bound,  
on outer surface of E.R. membranes,  
to mRNA molecule = POLYSOME [polyribosome]

**ENDOPLASMIC RETICULUM...**

found in all eukaryotic cells with a nucleus  
structural continuity with nucleus (contiguous)  
makes up 50% of all cell's membranes  
composed of convoluted network  
enclosing internal spaces flattened sheets, sacs, & tubes  
lumen - internal compartment  
[up to 10% cell's volume]  
**Rough E.R.** (w ribosomes) &  
**Smooth E.R.** (without ribosomes)

**Functions:**

RER: makes, transports, & packages protein vesicles  
SER: lipid & bile biosynthesis, drug detoxification

**SIGNAL SEQUENCE:** aa's @ N-term, bind, release into lumen
GOLGI … Part of the ENDOCYTOTIC Pathway

cell's internal membrane system for **endocytosis**...
packaging of extracellular molecules for internal digestion
& **exocytosis** (secretory) - delivery of newly synthesized proteins/carbo's for extra-cellular secretion

**size** : 1 to 3 um dia x 4 to 7 membranes high

**Number** : up to 100 per cell

two sided - three parts:
- **CIS** side [entry side]...faces R.E.R
  - Proteins made in R.E.R. lumen
    --> vesicles ---> cis Golgi
- **MEDIAL** cisternae elements
  - proteins are modified by adding sulfate, carbohydrates & lipids;
    these modifications help address vesicle to its destination
- **TRANS** [exit side] ...golgi side
  - modified vesicle leave as ... export vesicles, lysosomes, membrane bound vesicles

LYSOSOME …

cytoplasmic single membrane bound vesicle containing hydrolytic enzymes
with acid pH optima (pH 5.0);
lysosomal membranes have ATP driven membrane H+pump (in)
diverse shapes, mostly spherical
functions in intracellular digestion
  (autophagy - phagosome)
PROTEASOME - a protein digesting "organelle?"

large multi-enzyme complex (molecular motor) in all eukaryotes & archaea that digest endogenous proteins... Such as transcription factors, cell cycle cyclins, virus coded proteins, and improperly folded proteins to short peptides, followed by --> hydrolysis of these function: Protein Digestion...

begins when cells add a small polypeptide (ubiquitin) to a protein to be digested;
addition of ubiquitin targets a protein's entry into a Proteasome complex.

Proteasomes are located in nucleus & cytoplasm and is a barrel-shaped structure made of a lid, a base and 4 stacked protein rings with trypsin, chymotrypsin, and caspace proteolytic activity.

Endomembrane system (Endocytotic pathway)—
includes SER & RER, Golgi, lysosomes, & vacuoles used in transfer of membraneous vesicles throughout the cell.
CYTOSKELETON...

network of protein fibers running throughout the cytoplasm that give a cell its shape & provide a basis for movement composed of 3 kinds of protein fibers –
which are universal in eucaryotic cells

microfilaments (actin)... 7 to 8nm dia & indefinite length
actin is universal eukaryotic protein (from protists to verts)
5% of total cell protein
filaments of globular protein G-actin (conserved)
a polypeptide of 375aa + 1 ATP molecule
3 types of G-actins: α-actins of muscle cells,
β- & γ- actins of nonmuscle cells

intermediate filaments... 10nm dia vimentin & lamin
protein fibers [rope-like] with intermediate diameter
span cytoplasm providing framework for mechanical strength made from a heterogenous family of filament proteins

microtubules... 25nm dia tubulins (conserved)
rigid - hollow rods or spiral tubes 21-25 nm dia,
up to several um long - make long fibrillar protein complexes form spontaneously
repeating globular units: 2 different proteins α- & β-tubulin

Centrosome: MTOC = centriole: 9 MT triplets around 2 MT’s,
forms spindle fibers during cell division in animal cells
Basal Body: a centriole at base of flagella or cilia
Flagella: 9 doublets surrounding 2 = locomotion & feeding
Intercellular junctions...
Cell surface regions specialized for intercellular contact esp., prominent in epithelial cells...
impermeabilize areas, adhering junctions, communication

**Tight Junctions** - (impermeabilizes)
prevents leakage of materials between cells )
fibrillar network at apical ends of cells
"SIX-PACK MODEL"

**Desmosome** - adhering junctions (anchors cells together)
spot desmosome - spot weld with tonofilaments
belt desmosome (zona adherens)

**Gap Junctions** -
intercellular channels for communication [0.2nm]
allows ions, electric impulses, etc... to pass between

**Plasmodesmata**
cytoplasmic strands between plant cell walls [70nm]
makes cells coenocytic (“one-celled compartment”)

**Extracellular Matrix** - protein and polysaccharide complex
embedding and protecting cells.

**VACUOLE:** is a membrane-bound [tonoplast] sac that
plays roles in intracellular digestion & the release
of cellular waste products.

**Endodermis...** innermost layer of cells (cortex of plant root)
contains a waterproof **Casparian Strip** : results in
**SYMPLASTIC ROUTE** - internal via plasmosdesma
**APOLPLASTIC ROUTE** - external via intercellular space
HOW THINGS GET IN/OUT of CELLS

Cell Membranes
1. Unit Membrane Hypothesis - "all membranes look alike"
2. Source for study (plasma membrane - RBC ghosts)

Two Ways to Study Membranes & Transport

NATURE OF MEMBRANE ITSELF
- "its molecular makeup"

PERMEABILITY STUDIES
- "physiological Properties of Membranes"

A. Current structural model - Fluid Mosaic model
1. lipids = phospholipids
2. proteins = a) Integral (intrinsic proteins) - denatured on release
   b) Peripheral - easily extractable
3. Functions of Membrane Proteins
4. Extra-cellular Matrix [common to animal cells]
   glycoproteins secreted by cell - make a cell "wall"

B. Physiological Properties of Membranes

Solute Movement...
movement of molecule across membrane
based upon lipid solubility –
Partition Coefficient vs. Permeability
WATER TRANSPORT – an anomaly?
not lipid soluble, yet readily permeable across membranes

Bulk flow... mass flow of water due to potential energy - rivers from area greater water potential to area of lesser water potential

Osmosis - net movement of water from [high] ---> [low]
passive transport of water, no energy

SOLUTIONS - hypertonic solution = greater [solute] - less water cell is said to be hyperosmotic.

hypotonic solution = less [solute] - more water cell is said to be hypoosmotic.

Cells and Solutions -

<table>
<thead>
<tr>
<th>Condition</th>
<th>Net movement of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>External solution</td>
<td>into the cell</td>
</tr>
<tr>
<td>is hypotonic to cytosol</td>
<td></td>
</tr>
<tr>
<td>External solution</td>
<td>out of the cell</td>
</tr>
<tr>
<td>is hypertonic to cytosol</td>
<td></td>
</tr>
<tr>
<td>External solution</td>
<td>none</td>
</tr>
<tr>
<td>is isotonic to cytosol</td>
<td></td>
</tr>
</tbody>
</table>

plant vs. animal
plasmolysis - loss of cytoplasmic structure due to loss water turgid - swollen due to water gain
AQUAPORINS – Water Channels

Osmotic permeability of some epithelial cells is much too large to be accounted for by simple diffusion...

In 1992 a "water channel" protein was identified by Peter Agre (2003 Nobel), while studying membrane proteins of RBC’s.

Agre compared cells with and without the protein. He used artificial membranes (liposomes); which he found to become permeable to water only if the aquaporin protein was implanted in their artificial membranes.

**Aquaporins** form tetramers in cell membranes, & have 6 membrane-spanning pieces, which facilitate the transport of water molecules. The pores are completely impermeable to charged species, as H+.

A probable mechanism of action of aquaporin channels is studied using supercomputer simulations. Water molecules worm their way through narrow channel by orienting themselves in electrical field formed by atoms of the channel wall. The strictly opposite orientations of the water molecules keep them from conducting protons, while still permitting a fast flux of water molecules.
How do Things Get Across a Membrane

4 Ways

1. through a PORE ... ions and small hydrophilics

2. by DISSOLVING IN membrane... hydrophobic solutes

3. by CARRIER PROTEINS...
   protein receptors w specificity for a solute
   transport solute through a lipid bilayer

4. by membrane VESICLES...
   ENGULFING/RELEASING particles ...

   ENDOCYTOSIS = phagocytosis & pinocytosis
   uptake [in] of solutes/particles by vesicles
   EXOCYTOSIS =
   releases (out) bulk material to outside...
DIFFUSION - net thermal motion of solute down a concentration and/or electrical gradient

PASSIVE.... requires no expenditure of energy

Nernst Equation defines passive equilibrium
\[ E_{mv} = (+/-) \, 62 \log \frac{C_o}{C_i} \]

CARRIER MEDIATED TRANSPORT
Facilitated Diffusion ... protein mediated passive transport
ACTIVE TRANSPORT - expends energy moving solute against a concentration gradient

animals- Na-Pump : NaK-ATPase
plants- Proton Pump : H-ATPase

[electrogenic = voltage]

COTRANSPORT... movement of 2 solutes together - moving 1 solute passively & other actively

ex: 1) H-pump coupled with Sucrose (H+ symport)
2) Na-glucose transport model (see Handouts)

uniport - single solute in one direction
symport - 2 solutes simultaneously in same direction
antiport - 1 solute in & 1 solute out - opposite directions

Comparison of passive vs active transport
Exocytosis
releases bulk material to outside - see micrographs

Endocytosis
taking in solutes/particles by vesicles
- phagocytosis - solid particle uptake
- pinocytosis - liquid uptake
- receptor mediated endocytosis
  see micrographs

Exocytosis

Endocytosis
CELL COMMUNICATION...

How do cells Communicate...
- cell membranes contain specific protein-receptors, which bind & transmit an extra-cellular signal molecule, converting the signal into a specific cellular response.

- in multi-cell organisms cell-to-cell contact is critical

some UNIVERSAL PRINCIPLES of cell communication...
- though many different molecules may be involved
- only few mechanisms survived throughout evolution
- an analogy: from auto industry...
  cars basically have same parts (engines, fenders, lights) but the variety of different patterns is boundless

cell to cell contact is most often done through cell signaling, where an exogenous molecule is received by a cell & converted into a response by the receiving cell.

pattern is remarkably similar in all cells, probably evolved early, before first multi-cellular systems (maybe in single cell prokaryotes) has been highly conserved in today's ancestral cells.
Local vs. Distant signaling...

PARACRINE (local) SIGNALING
local regulator chemical messengers are targeted to specific receptors & often includes:
growth factor proteins, which promote cell division & growth & neurotransmitters,
move across synapses other neurons

ENDOCRINE (distant) SIGNALING
specialized cells release molecules (often hormones) into blood vessels of circulatory system,
hormones move to target cells... elicit response

SOME EXAMPLES of CELL SIGNALING SYSTEMS...

mating in yeast cells
sex-1 is "a"-cell - releases a-factor (peptide of 12 aa's)
  - which binds to sex-2’s receptors
sex-2 is “α”-cell - releases α-factor
  - which binds to sex-1’s receptors
  - result: fusion of 2 cells - mating produces a diploid cell

direct Cell to Cell contact examples:
  1) gap junctions & plasmodesma
cytoplasmic continuity favors cellular interactions
  2) cell surface contacts: receptor protein specificity
3 Stages of Cell Signaling Mechanisms
i.e., Properties of a Signal Transduction Pathway...

RECEPTION, TRANSDUCTION, RESPONSE

1. Reception... not unlike recognition of enzyme & substrate
   forms an ES-like complex
   similar to lock-&-key hypothesis of enz-sub recognition
   ... ligand molecules (usually water soluble)
   are recognized by only one receptor protein bound
   within a membrane layer

2. Transduction... leads to conformation change in receptor
   ... shape change results in receptor interacting with
   other intra-cellular molecules
   ... may result in multiple, structural changes in other
   cellular proteins
   ... inactive enzymes ---> active enzymes, etc...

3. Response... usually a cellular activity - enzyme catalysis,
   or rearrangement of cytoskeleton,
   or specific gene activity.
Some types of membrane receptors & responses

1. G-Protein Receptors...
    are receptor proteins that bind GTP/GDP
    & convert between active & inactive forms...

    inactive protein [G-(GDP)]
    signal molecule binds receptor, --> conformation change
    now favors G-protein to bind, which then binds GTP
    (replacing GDP)... forming active protein [G-(GTP]
    protein stimulates an inactive enzyme, which then
    hydrolyzes GTP --> inactives G-protein

G-proteins structure...
    7 transmembrane α-helix
    + site for receptor - see fig

Some specific examples of cellular responses:
    ... Gene activation & transcription factors...
    ... Steroid hormones & new muscle proteins...
    ... Ligand gated ion channel receptors -
        post-synaptic membranes & neuron responses...
    ... IP3-DAG and Ca 2nd messenger signaling

    net result... one signal molecule = a cellular response
Design of Metabolism
or How Biological Order Comes About

What is Metabolism?
It is catalytic reactions (run by enzymes) in cells.....

2 Categories catalytic reactions -
CATABOLISM - Cell respiration: oxidation of food stuffs
3 steps: 1) digestion of polymers (foods)
2) GLYCO-LYSIS ---> AcoA [splitting of sugar]
3) oxidation of AcoA ---> CO₂ + NADH --> H₂O
   ADP + P ---> ATP

ANABOLISM - biosynthesis
coupled reaction - energetically unfavored w favored

ENERGY - capacity or ability to do work - kinds:
KINETIC – motion
HEAT - assoc w movement molecules in a body of matter; most random form of energy (wasted)
POTENTIAL - stored energy; capacity to do work eventually

molecules in living cell have potential energy to do work because of the arrangement (orientation) of their atoms in space... we call this chemical energy & the energy is stored in the covalent bonds of the molecules.

EXAMPLES: general: heat, light, sound, mechanical
biological: synthetic, osmotic, mechanical,

HOMEOSTASIS = energy needed to maintain steady state
BIOENERGETICS - study of energy transformations (changes)

EQUILIBRIUM THERMODYNAMICS

1st LAW = Conservation of Energy
energy is a constant,
energy can't be created or destroyed,
only transformed.
caloric data says 1st law is true

2nd LAW = energy transformations reduce order of universe
entropy is directional ->
  moves toward equilibrium (maximize disorder)

ENTROPY = amount of disorder in a system

ice melting is a good example
of entropy increasing – a gain
in disintegration of the
molecular order of the ice

The Rules of Universe are:
  Cities crumble, Stars go Supernova, &
  we're all dying.... (Equilibrium...izing)

Degree of disorder of the Universe can only increase.
  [its randomness - its ENTROPY]
There are no unique laws covering cells...

**Cells do obey Laws of Chemistry & Physics**

yet **CELLS**... WOW ! ... become highly **ORDERED**...

- wing of bird, a spider's web, the human eye, ....etc & cells Feed, Grow, & Differentiate = HOW?

for a system to become more ordered [lose entropy]
  (such as a cell),
  its surroundings must become more disordered (gain entropy)

**FOODs** (light & covalent bond energy)

- cell reactions give **increased order** within cell
- with release of **HEAT**

ENERGY IN ----> CELL STRUCTURE ----> ENERGY OUT

FREE ENERGY   \[ \Delta G = \Delta H - T \Delta S \]

\[ \Delta G \] is then a numerical measure of how far a reaction is from equilibrium
\( \Delta G \) is a measure of the amount of energy in the system able to do work...

Disorder increases (entropy increases) when useful energy, that which could be used to do work, is dissipated as heat.

PREDICTS .....the Direction of Cellular Reactions......

TOWARD EQUILIBRIUM... Towards Maximum ENTROPY

CHEMICAL REACTION..... A \( \rightleftharpoons \) B  Which Way?

\[ \Delta G = \Delta G^0' + RT \ln \frac{[p]}{[r]} \]

The change in free energy content of a reaction...depends upon:
1. energy is stored in molecule's covalent bonds
2. temperature is negligible... ells are isothermal, i.e., function within narrow range (40 to 990°)

**\( \Delta G \) =** actual free energy
**\( \Delta G^0' \) =** standard free energy
\( R = \) gas constant (2 x 10^-3 Kc/mol)
\( T = \) absolute temp (-273OK)
\( \ln = \) natural log (conversion log10 = 2.303)

At equilibrium \( \Delta G = 0 \) and we call \([p]/[r] = \text{Keq}\)

Solve above equation to see relationship of Keq to \( \Delta G^0' \)
Free Energy Equation

\[ \Delta G = \Delta G' + RT \ln \left( \frac{[P]}{[R]} \right) \]

@ equilibrium \( \Delta G = 0 \) ....

thus rearranging \( \Delta G' = -RT \ln \left( \frac{[P]}{[R]} \right) \)

@ equilibrium \( [P] / [R] = K_{eq} \)

& @ 25^\circ C...

\(-RT \ln K_{eq} = -(2.0)(298)(2.303)\ln K_{eq} = -[1372] \ln K_{eq} \)

thus...... \( \Delta G' = -[1372] \ln K_{eq} \)

<table>
<thead>
<tr>
<th>Products / Reactants</th>
<th>Keq</th>
<th>log10</th>
<th>dG0' cal/mole*</th>
</tr>
</thead>
<tbody>
<tr>
<td>[R] &gt; [P]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1/1000</td>
<td>0.001</td>
<td>-3</td>
<td>+4116</td>
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<td>1/100</td>
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<td>0.1</td>
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<tr>
<td>[P] &gt; [R]</td>
<td></td>
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<tr>
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<td>1000</td>
<td>3</td>
<td>-4116</td>
</tr>
</tbody>
</table>
CHEMICAL REACTIONS  A <----> B  Which way & Why?

EXERGONIC REACTION - is one which releases energy
  Product (B) << Reactant (A) [stored in covalent bonds]
  
ex: burning wood (cellulose)
  glucose monomers= potential energy
  breaks bonds, release heat & light ---> CO₂ & H₂O

  cell respiration - cellular burning of glucose
  slower, multi-step process to capture released
  energy.... as ATP

ENDERGONIC REACTION - requires input of energy A-->B
  Product (B) >> Reactant (A)

  ex: photosynthesis (autotrophy)
  glucose made from CO₂ + H₂O --light---→ C₆H₁₂O₆
  energy poor               energy rich

CELL METABOLISM is then a mix of ...
  Exergonic  &  Endergonic  reactions that occur in cells
How does metabolism really work energetically?

**COUPLED REACTIONS:**
for RX's which share one or more intermediates....
the free energy change $\Delta G$ is the sum of indiv $\Delta G$'s

$$\begin{align*}
\text{Glu + Fruc} & \rightarrow \text{Suc} \quad \Delta G = + 5.5 \text{ Kc/m} \\
\text{ATP} & \rightarrow \text{ADP} + \text{P} \quad \Delta G = - 7.3 \text{ Kc/m} \\
\text{Glu + ATP} & \rightarrow \text{G-1-P + Fruc} \rightarrow \text{Suc + P} \quad \Delta G = -1.8 \text{Kc/m}
\end{align*}$$

**COUPLED REACTIONS** - linking hydrolysis of ATP
(a favored rx) to a thermodynamically unfavored reaction
creates biological order (greater molecular structure).

**WHY ATP?** and not other nucleotides.

the **ENERGY MOLECULE of CELLS** is ATP
over evolution... cells favored enzymes that bind
ATP & use its hydrolysis to drive endergonic reactions

- adenosine triphosphate
- its structure
- how it works
- phosphorylation, energy coupling

**HYDROLYSIS of ATP**

$$\text{ATP} \rightarrow \text{ADP} + \text{P (exergonic)} \quad \Delta G^0' = -7.3 \text{ Kc/m}$$
So how do cells make ATP?

By **PHOSPHORYLATION**... adding phosphate to ADP

\[ \text{ADP} + \text{P} \longrightarrow \text{ATP} \]

a) **substrate level phosphorylation** –
where a substrate molecule (X-p) donates its P to ADP making ATP

b) **Chemiosmosis** - [oxidative phosphorylation]...
food substrates donate e- & protons to acceptors protons are pumped out of mito/chlp protons diffuse back into mito thru ATP synthase ATP synthase makes ATP

c) **photophosphorylation** –
e- of light energy are captured to make a proton gradient across the chloroplast membranes protons move through chloroplast ATP synthase to make ATP
Heterotrophic Metabolism organisms that consume foods

we say our bodies oxidize (consume) foods to make energy
where is energy in foods? it's in covalent bonds (& e\(^{-}\)s)

OXIDATIVE METABOLISM...
Metabolism is then, cells capturing e\(^{-}\) from food to make ATP

REDOX Reaction –
e\(^{-}\) passed from one molecule to another in chemical rx's
called REDOX rx .....energy transferred into new molecule

**OXIDATION** = removal of electron &/or proton
removes an e\(^{-}\) from a food molecule

**REDUCTION** = gaining electron &/or proton
adds an e\(^{-}\) to an acceptor molecule

\[
\text{A-H} + \text{B-O} \quad \text{<----->} \quad \text{A} + \text{B-O-H}
\]

<table>
<thead>
<tr>
<th>donor</th>
<th>acceptor</th>
<th>oxidizing agent</th>
<th>reducing agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>变成氧化剂</td>
<td>acceptor</td>
<td>becomes oxidized</td>
<td>becomes reduced</td>
</tr>
</tbody>
</table>

Thus: heterotrophic metabolism is stepwise oxidation of foods
if **aerobic** - requires oxygen as electron acceptor
if **anaerobic** - requires no oxygen (other e\(^{-}\) acceptor)
**CELL RESPIRATION...**

oxidation of GLUCOSE to CO₂ + H₂O & reduction O₂ to H₂O

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{e}^- \rightarrow 36\text{ATP} \]

- 686 Kc/mole

called **oxidation**.....because e- are removed from glucose
called **reduction**.....because e- passed to O₂ making water

**Fuller definition:**

series of enzyme rx's (pathways) in cytoplasm & mitochondria which...

- remove e- from chemical bonds of substrates (as glucose)
- pass e- to acceptor molecules [CoE's] as NAD+ & FAD which become reduced [NADH & FADH₂]
- reduced CoE [NADH & FADH₂] pass e- to other acceptors a series of protein electron carriers [cytochromes]
- electron carriers [cytochromes] pass e- to O₂----> H₂O
- cytochromes also pump protons (H+) out of mito into space between membranes
- protons move back into mitochondria thru a special enzyme protein (ATP synthase) & make ATP
METABOLIC PATHWAYS OF CELL RESPIRATION...

**Glyco-lysis**
- converts glucose (C6) to pyruvate (C3)
- makes 2 pyruvate, 2 NADH & 2 ATP (net) in cytoplasm
- alcoholic fermentation = glucose --> alcohol
- lactic acid fermentation = glucose --> lactic acid

**KREBs Cycle**
- converts 2 pyruvate to CO₂ + H₂O
- makes 8 NADH, 2 ATP, 2 FADH₂
- releases 6 CO₂ in mitochondria

**ETC - Electron Transport Chain**
- passes e⁻ & H⁺ from NADH & FADH₂ to O₂ to make water
- generates a proton gradient (chemiosmosis) in mitochondria

**ATP synthase**
- enzyme of mitochondrial membrane
  that lets H⁺ back into mitoplasm & makes ATP
KEY REACTIONS of GLYCOLYSIS -
substrate level phosphorylation
Redox reaction involving NAD

Fates of NADH -
   alcoholic fermentation
   lactic acid respiration (fermentation)

Shuttles
   malate shuttle (liver, kidney, heart) = NADH
   Glycero-P shuttle (muscle/brain) = FADH$_2$
Fates of PYRUVATE -
  if anaerobic
    alcoholic fermentation
    lactic acid respiration (fermentation)
  if aerobic
    Krebs Cycle

Summary of GLYCOLYSIS
- 2 ATP to initiate
  2 substrate level phosphorylations
  makes 2 ATP (net), 2 NADH, and 2 PYRUVATES
Fermentations & Shuttles

PYRUVATE DEHYDROGENASE Reaction
  occurs in mitoplasm
  involves coenzyme CoASH --> acetyl coenzyme A [AcoA]
  rx’s = decarboxylation [-CO₂] & redox reaction

Key Reactions of KREBS CYCLE
  NAD is reduced
  substrate level phosphorylation occurs [GTP]
  decarboxylation [-COOH]
  acylation via CoASH
OXIDATIVE PHOSPHORYLATION & ELECTRON TRANSFER

coupling of oxidation of substrates (-e) to the phosphorylation of ADP to make ATP involves "protein carriers" - gain/lose e-'s & protons occurs in 3 submitochondrial cell membrane complexes:
  a) NADH Reductase,
  b) Cytochrome Reductase, &
  c) Cytochrome Oxidase

Chemiosmosis & ATP Synthase
- creation of a hydrogen ion gradient (H+)
as e- flow thru the ETC

- some carriers release protons to outside
- H+ diffuse back into mitoplasm thru ATP synthase --> ATP

OVERVIEW of Cell Respiration

How much ATP is made per Glucose ?
Substrates [carbs, proteins, fats]
Regulation [phosphofructokinase of glycolysis]
Summary: cell respiration & heterotrophic metabolism

1. Substrates = sugars, amino acids, fatty acids

2. Glyco-lysis, Krebs Cycle, & ETC are Universal to all cells

3. Products = CO₂, H₂O, and energy as NADH, FADH₂, & ATP

4. Process is anaerobic respiration (−O₂; GLYCO-LYSIS)
   alcohol & lactate fermentation (anaerobic)
   & aerobic (+O₂; glycolysis & Krebs Cycle)

5. Reactions include:
   oxidation, reduction, decarboxylation, phosphorylation,
   & hydrolysis (dephosphorylation)

6. Energy capture is via electron transfers & proton pumps

7. Regulation is by:
   feedback inhibition & allosteric modulation of key enzymes as phosphofructokinase

8. Intracellular compartmentation:
   glycolysis is in the cytoplasm
   Krebs Cycle is mostly in the matrix of mitochondria
   ETC is in the cristae membranes of mitochondria
PHOTOSYNTHESIS – Autotrophic Metabolism

GREEN MEN - Why do metazoan cells not photosynthesize?

chlorophyll vs. hemoglobin (leghemoglobins)
mutants = hemoglobins that capture light energy

plant photosynthetic rates = 20 mg hexose/dm²/hr
average human surface area = 170 dm²
hexose productivity = 40.8 gm/d
1 mole glucose = 183 gm = 686 Kc/mol
41 gm = 153 Kc/mol
BMR = 2,000 Kc/d = about 8.5 % of need

they evolve, i.e., increased surface area, remain sessile,
peristalsis becomes vestigial, circulation replaced
... "We are a plant"

PHOTOSYNTHESIS ... is a light driven phosphorylation
...it is AUTOTROPHIC Metabolism, and occurs in
organisms, which produce all their organic nutrients
from inorganic materials thru conversion of light energy
into covalent bonds.

  a. Chemotrophic...
     oxidation of small inorganics
  b. phototrophic...
     use light energy to make organics
What is PHOTOSYNTHESIS ...?

it is a cellular process - requires a living cell
it occurs in prokaryotes - bacteria, blue-green, and eukaryotes - cells w chloroplasts
it captures light energy via pigments - chlorophylls and accessory pigments (carotenes & phycobilins)
it is a REDOX reaction - produces oxidizing power = O₂ via PHOTOLYSIS
it captures e- into coenzymes, as in cell respiration produces reducing power = NADPH
it produces ATP via photophosphorylation
it couples e⁻ transfer to H⁺ gradients & ATP synthase
it reduces CO₂ to CH₂O

\[
6\text{CO}_2 + 12\text{H}_2\text{O}^* \longleftrightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2
\]

\[
\text{CO}_2 + 2\text{H}_2\text{A} \longleftrightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{A}
\]

Source C + e donor organic C oxidized donor
2 Fundamental Reaction Mechanisms of Photosynthesis:

**LIGHT Reactions** - photochemical reactions
molecular excitation of chl by light = charge separation
generation of proton motive force (gradient)
reduction of NADP via an ETS

**DARK Reactions** - thermochemical reactions
CO₂ fixation (reduction) stages
- carboxylation: CO₂ + RuBP → 2 PGA
- reduction: PGAL + NADPH → PGAL
- regeneration: HMP path → RuBP

**Morphological Basis of Photosynthesis**

**PLASTIDS** - double unit membrane bound organelles
classified by pigment content (functional)

**PROPLASTIDS** ... plastid in MERISTEMATIC cells
gives rise to all other plastids

**LEUCOPLASTS** - amyloplasts - synthesize & store starch
aleuoplasts - contain stored protein (crystals)
elaioplasts - contain oil-fat globules – fat synthesis

**CHROMOPLASTS** - found in flower petals, ripe fruit, senescent leaves
Morphological basis of chloroplasts...

**CHLOROPLAST** - ubiquitous to all green plants

**SHAPE** - variable (elipsoid to ovoid; & lenticular or stellate)

**SIZE** - 2 to 3 um dia by 5 to 10 um long

**NUMBER** - 15 to 20 perr mesophyll cell  [400,000/cc]

**VOLUME** - often much larger than mitochondria

**CHLOROPLASM** - (Stroma)

- pyrenoids - which are starch coated protein granules
- 70s (procaryotic ribosomes)
- naked DNA - 2 to 10 femtograms of DNA/chlp
  - about equal to bacterial cell DNA
  - highly supercoiled & repetitive (6 copies)
- enzymes of CO₂ fixation and lipid droplets
LIGHT ABSORPTION

PIGMENTS

Accessory Pigments
- Carotenoids - carotenes, xanthophylls
- Phycobilins - chromophore + a protein
  phycoerythrin & phycocyanin
- Chlorophylls - a, b, c, d, etc... [side chain differences]

ABSORPTION SPECTRA - is a graphical plot of amount light absorbed vs. wavelength

ACTION SPECTRA - (by Engelman) is a plot of physiological activity [O₂ released] vs. wavelength

MOLECULAR EXCITATION of CHLOROPHYLL

FATES of Absorbed Energy,
  i.e., blue light and red light
1. re-radiates as vibrational heat
2. reradiated as fluorescence (red light)
3. reradiated as phosphorescence (far red)
4. induced resonance - vibrational e excitation transfer
5. photoionization - photochemical reactions...
  chlorophyll loses an electron to acceptor = ionized chl⁺
THYLAKOIDS MEMBRANES & ELECTRON FLOW

the Photosystems

complexes of chlorophylls, reaction centers, and primary acceptors

PS 1 and PS 2

release of O₂ by oxygenase in photolysis
capture of e- into coenzyme NADP⁺ \( \rightarrow \) NADPH

path of e- flow (cyclic vs. non-cyclic)

chemiosmosis (location in chloroplasts)

ATP synthase makes ATP (just like mitochondria)
DARK REACTIONS
occur in stroma (chloroplasm)
consume ATP and NADPH mad in light reactions
reduces (fixes) CO₂ into CH₂O (sugars)

Three (3) different pathways to make sugar

C₃ - CALVIN cycle
1 CO₂ + 5C RuBP --→ (2) 3C sugars (PGA)
(2) 3C sugars --→ 1 net glucose
RuBP carboxylase
Photorespiration - inhibition by O₂

C₄ - Hatch & Slack pathway
1 CO₂ + 3C PGA --→ 4C acid (mesophyll cells)
4C acid --→ 3C + CO₂ in bundle sheath
CO₂ into Calvin cycle (as above)
RuBP carboxylase + PEP Carboxylase
spatial separation of acid & sugar production

C₄ - CAM Pathway
same as C₄, but within the same cell
RuBP carboxylase + PEP Carboxylase
temporal separation of acid & sugar production,
not spatial differences.
GENETICS & DEVELOPMENT

Physical Basis of Inheritability - Cell Division

Cell Reproduction
cells reproduce identically, yet with variation (new traits)
"All living cells arise from pre-existing cells"

Genetics asks
how at cellular & molecular level

Development reveals – the life cycle of organisms
1. gametic cells divide ---> egg & sperm cells
2. reproduction mechanisms of organisms - fertilization
3. growth of organism..... zygote to adult
   cell differentiation
   differential gene activity
   totipotency & cloning

METHODS of CELL REPRODUCTION
Fission - binary = 2 equal halves (bacteria)
Budding - outgrowths detach = new organism
Asexual - Mitosis = identical cell copies
Sexual - Meiosis = produces sperm & egg
Asexual Cell Division...
duplication of DNA & division of chromosomes
(liver cells - 1x/yr vs. epithelial cells - 1x/day)

CELL CYCLE - Life Cycle of a Cell........[ 3 Stages ]

Interphase - between successive divisions (3 parts)
G1, before DNA synthesis (S), & G2 period after
Cytokinesis - physical division of cell into two parts
Nucler Division Phase – MITOSIS

Stages of Mitosis
Interphase - DNA (chromatin) duplicates
Prophase - chromatin condenses
each homolog has 2 chromatids
Metaphase - chromosome align at equator
homologs align independently
separates chromatids of homolog
Anaphase - MT attach to kientochore
pull chromatids apart
Telophase - chromosome decondense

Time determination of stages done by Pulse-Chase exp
Identify the typical stages of mitosis

PMAT in figure of onion root cell mitoses above
Chromosomes

**bacteria** = 3,000 genes
- 1 chromosome

**human** = 25,000 genes (?)
- 46 chromosomes

Genes occur in *chromatin* of nucleus, which condense into **CHROMOSOMES** at time of cell reproduction

Human:
- has 46 chromosomes,
- 23 HOMOLOGOUS pairs

in mitotic division:
- each homolog
- has 2 **chromatids**
- which separate into 2 cells.
Control of Cell Division & Cell Cycle  2001 Nobel prize

Regulated by "Growth Factors" - 
proteins that promote cell division

MPF - mitotic promoting factor...
  a protein complex* of cdk + cyclin
MPF is a kinase enzyme, ones that switches on/off 
target proteins by phosphorylating them...
  inactive  ----------------->  active-P
  ATP ----> ADP

MPF - promotes mitosis by phosphorylating other proteins 
including ones that leads to destruction of cyclin itself

  cdk - another cell division control protein...
    - a cyclin dependent kinase;
      active only when bound to cyclin;

  cyclin - a protein whose amount varies cyclically*;
    when in high concentrations*,
    binds to cdk makes MPF...

  [cyclin + cdk = MPF]...  favors Mitosis

Cell Division is also regulated via critical CHECK POINTS...
1. **G1** checkpoint - cell size adequate, polymerases
2. **G2** Checkpoint - chromosome replication successful
3. **metaphase** checkpoint - chromosome attached to spindle fibers.
SEXUAL CELL REPRODUCTION - meiosis

Where does meiosis occur in sexual cell reproduction
haploid gametes --> fertilization --> diploid (chrm # = 46)
meiosis -------> haploid gametes (half chrm # = 23)

Stages of Sexual Cell Division
are same as asexual (interphase, cytokinesis, nuclear)
but, 2 Divisions  →  Meiosis I & Meiosis II  1 = 2 = 4 cells

Names of stages are same & have analogous functions
Prohase I  =  chromosomes condense
Metaphase I  =  chromosomes align at equator
  homologs PAIR together - synthesis
crossover exchange at chiasmata
Anaphase I  =  chromosomes migrate toward poles
Telophase I  =  chromosome at poles

Meiosis I  --> separated homologs of homologous pair
Meiosis II  is just like mitosis  --> separates chromatids of
  one homolog of a homologous pair
Comparison of Mitosis/Meiosis

**mitosis** – separates chromatids

**meiosis** – separate homologs, then chromatids

**Independent Assortment** - random alignment homologs

**Crossing Over** - exchange of chromosome material
SUMMARY OF MEIOSIS

1. **Nuclear division** phase of sexual cell reproduction
2. Two successive divisions, results in 4 daughter cells... Meiosis 1 and Meiosis 2
3. **Reduction/division** occurs....
4. cells halve the number of parent cell chromosomes diploid ---- > haploid
4. Stages have same nomenclature as Mitosis prophase, metaphase, anaphase, telophase, M1 & M2
5. Only one S phase, where DNA is duplicated often is no interphase between M1 and M2
6. **Homologs separate** in Meiosis 1 chromatids separate in Meiosis 2 (mitotic-like)
7. **Random Assortment** occurs homologs align at equitorial plates independent of each other
8. **Crossing over** may occur in Prophase I
   - synapsis = close pairing of homologs to allow exchange chiasmata = points of exchange of sister chromatids

**Consequence of sex**... new gene/chromosome combos that did not exist in either parent, which will become the stuff of evolution
Science of HEREDITY - Mendelian Genetics

is the study of the form (morphology)
and appearance (phenotype) of an organism
as established by its genes & influenced by environment.

Essence of heredity
ability of a cell to faithfully copy its
DNA instructions into identical progeny cells

Mendelian Genetics -
quantitative analysis of inheritance in organisms
experiments which established the basic principles
& laws that predict the pattern of inheritance
from generation to generation

Molecular Genetics -
physical properties of molecules (DNA & RNA)
as they effect patterns of inheritance

Central Dogma of Molecular Biology
DNA --- transcription --> RNA --- translation --> Protein
Mendelian MONOHYBRID Cross
produces progeny from an experimental genetic cross between true breeding parents (homozygous) for a single genetic character with 2 different phenotypes (appearances)

Parental round seed x wrinkled seed
F1 round seed [x themselves]
F2 3 round seed to 1 wrinkled seed

Mendel's 7 true breeding traits in crosses of pea plants

Mendel’s Particulate Inheritance
Inheritance is discernible as "particles", ‘factors"
which were:
discrete entities w own integrity (no blending of traits),
preserved through inheritance
occur within the individual in PAIRS (diploid)
some particles mask expression of others

Mendel’s Law of Segregation
when gametes are formed
2 particles in an individual segregate from each other
each gamete receives 1 particle from each parent
Interpretation of Mendel’s results...

Particles are GENES, definable in molecular terms referred to by alpha taxonomy = A and a, B and b, etc... occur in different forms called alleles 

alleles - a gene which codes for different forms of a similar protein, that governs the character, trait, or phenotype

each individual possess 2 alleles for a specific trait

RR  -  homozygous dominant
Rr  -  heterozygous
rr  -  homozygous recessive

alleles occur on chromosomes at GENE LOCI - a corresponding area on each chromosome each individual possess 2 chromosomes (homologs) one from each parent

maternal homolog - the chromosome from the mother paternal homolog - the chromosome from the father

Traits can mask expression of others - dominance & recessive

recessive - trait that disappears in the F1 generation

dominant - trait that does not disappear in the F1
Parental cross pollinate RR round x rr wrinkled gametes

F1 Rr self pollinate

F2 3 round to 1 wrinkled egg sperm

PHENOTYPE 3 round to 1 wrinkled
GENOTYPE 1 (RR) : 2 (Rr) : 1 (rr)

Mendel's hypothesis basically is that the F1 is heterozygote = Testcross/backcross
**Link between phenotype & genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RR</th>
<th>Rr</th>
<th>rr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype</td>
<td>round</td>
<td>round</td>
<td>wrinkled (dry seed)</td>
</tr>
<tr>
<td>starch content</td>
<td>high</td>
<td>median</td>
<td>low</td>
</tr>
<tr>
<td>starch grains</td>
<td>numerous</td>
<td>median</td>
<td>few</td>
</tr>
<tr>
<td>hold water &amp; swell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>reducing sugar</td>
<td>low</td>
<td>median</td>
<td>high</td>
</tr>
<tr>
<td>UDP-glucose phosphorylase</td>
<td>high</td>
<td>median</td>
<td>low</td>
</tr>
</tbody>
</table>

Thus:  
R gene codes for an enzyme **UDP-glucose phosphorylase** which makes starch that is hygroscopic and absorbs water, making the seed appear round in the dry seed state, or wrinkled, if the gene is recessive.

Also: Gibberellic acid gene – promotes stem elongation.
## Dihybrid cross - Law of Independent Assortment

<table>
<thead>
<tr>
<th>flower color</th>
<th>DOMINANT</th>
<th>RECESSIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>flower color</td>
<td>( W = ) red</td>
<td>( w = ) white</td>
</tr>
<tr>
<td>seed color</td>
<td>( G = ) green</td>
<td>( g = ) yellow</td>
</tr>
</tbody>
</table>

**P1**  
\[ \text{WWGG} \times \text{wwgg} \]

**F1**  
\[ \text{WwGg} \quad (x \text{ WwGg self cross}) \]

**F2**  
<table>
<thead>
<tr>
<th>phenotypes</th>
<th>flower</th>
<th>seed</th>
<th>counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>parental</td>
<td>red</td>
<td>green</td>
<td>9 of 16</td>
</tr>
<tr>
<td>non-parental</td>
<td>white</td>
<td>green</td>
<td>3 of 16</td>
</tr>
<tr>
<td>non-parental</td>
<td>red</td>
<td>yellow</td>
<td>3 of 16</td>
</tr>
<tr>
<td>parental</td>
<td>white</td>
<td>yellow</td>
<td>1 of 16</td>
</tr>
</tbody>
</table>

Each trait alone  
red = 12 of 16  
white = 4 of 16  
green = 12 of 16  
yellow = 4 of 16

3 to 1

NEW COMBINATION NOT SEEN IN PARENTS
TRAITS SORT INDEPENDENT of EACH OTHER

genes occur on DIFFERENT CHROMOSOMES

<table>
<thead>
<tr>
<th></th>
<th>AB</th>
<th>Ab</th>
<th>aB</th>
<th>ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>AABB</td>
<td>AAbb</td>
<td>AaBB</td>
<td>AaBb</td>
</tr>
<tr>
<td>Ab</td>
<td>AABb</td>
<td>AAbb</td>
<td>AaBb</td>
<td>Aabb</td>
</tr>
<tr>
<td>aB</td>
<td>AaBB</td>
<td>AaBb</td>
<td>aaBB</td>
<td>aaBb</td>
</tr>
<tr>
<td>ab</td>
<td>AaBb</td>
<td>Aabb</td>
<td>aaBb</td>
<td>aabb</td>
</tr>
</tbody>
</table>
Mendel’s Law of Independent Assortment

Dihybrid Cross - involves 2 characters or traits
new combinations of traits not exhibited by either parent

parentals - progeny look like parents
non-parentals- progeny don't look like either parent

traits are distributed into progeny independent of each other therefore, genes must occur on different chromosomes

Incomplete Dominance - neither gene is dominant, both are needed to express the trait

Chromosomal Theory of Inheritance

Sutton 1903 - looked at spermatogenesis in grasshoppers
- discovered meiosis
- hereditary traits carried in sperm & egg cells
- on chromosomes
- chromosomes obey Mendel's laws
- chromosomes occur in pairs, as "particles"
- chromosomes segregate at meiosis
- members of chromosome pair sort independently
CHROMSOMAL LINKAGE

Thomas Hunt Morgan...
U of Columbia geneticist - fruit fly

Genes & their traits are linked on specific chromosome
SEX chromosomes (X and Y)
heterogametic sex & homogametic sex
AUTOSOMES - non-sex chromosomes

KARYOTYPE –
photographic map of a species chromosomes

SEX LINKED Recessives & HEMIZYGOUS Cells

\[ X^a X^a \quad \text{vs.} \quad X^a Y \]

ex: red green colorblindness (males = 8% and females 1%)
hemophilia, albanism, myopia, Duchen's M.D.

Autosomal Linkage

two traits are carried on a single chromosome
do not sort independently
crossing over will allow us to map position of genes
mapping - frequency of cross over exchange is proportional to relative distance between 2 genes
**Y chromosome**

- is much smaller than its homolog, the X chromosome
- holds only a dozen or so genes (X holds 2,000-3,000 genes)
- involved in male fertility
  (codes for testes proteins - many are repeat amplified)
- holds some "housekeeping genes" (†)
  & genes with homology to the "X" (‡) fig*
- holds lots of "junk" DNA & STR's (Short Tandem Repeats)
- holds a gene - SRY (sex determining region) encodes a protein that triggers formation of testes by activating genes on several autosomes.

**X & Y** evolved from a matching pair of autosomes some 240 to 320 millions years ago

tips of X & Y can engage in recombination, but rest of Y can not

the degeneration of Y chromosome occurred after evolution of SRY gene, because of a loss of ability to recombine during meiosis... without recombination genes on Y mutated, stopped working, & degenerated
**Barr Body - an Inactive X-chromosome**

\[ XX = 2 \text{ doses of gene activity,} \]

\[ Xy = 1 \text{ dose of X genes} \]

yet, expression of males & females is about the same...

**HOW?** an inactive X-chromosome in mammals is common

one of the 2 XX's becomes transcriptionally inactive

[which X is inactive seems to be randomized]

In interphase cells the inactive X-chromosome is visualized

as a dark chromatin spot & is called a BARR BODY.

Inactivation of the X chromosome occurs due to chromatin

condensation via:

1. **methylation** of 5'Cysteine
2. presence of chromatin proteins that promotes
   **heterochromatinization**
3. presence of a single gene on one X that is active...
   
   **Xist gene** (only lightly methylated) -->
   makes **RNAi** (interference RNA; not a mRNA)
   **Xist-RNAi** binds to X-chromosome from which it is
   transcribed & favors inactivation of X-chromosome.
   once inactivated all progeny cells are also inactivated.

**How do active X-chromosomes prevent the action of RNAi?**

anti-Xist gene called **Tsix** is involved

Tsix makes an **RNAi** that binds complementarily to the
**RNAi-Xist** molecule, making a double stranded RNA &
thereby inactivating it from protein synthesis.
Animal Structure and Function -

VERTEBRATE PHYSIOLOGY...

- structure & function of cells, tissues, & organs of verts
- hierarchy: cell --> tissue --> organ --> organism

VERTs have a number of similarities.....
- all have same basic body plan & same sort of organs
- skeleton - with bony skull (cranium) surrounding brain
- jointed bones - ball & socket, hinge, & pivot joints
- vertebrate column around the dorsal nerve cord
- internal tube called coelum (mouth to anus)
  a) thoracic cavity - holds heart & lungs of verts
  b) abdominal cavity - holds stomach, intestines & liver

Evolutionary innovations of CHORDATES: basic body plan
- bilateral symmetry, cephalization, notochord & spinal cord, gill slits, tail, a fully lined body cavity thoracic and abdominal cavities, gut tube (coelom), and segmented development (myomeres).

Model vertebrate could be ourselves - the human
- a warm blooded vert that regulates its internal temperature at some constant value.
- cold blooded verts - do not (snakes, lizards, etc...)
- has hair instead of scales & feathers
- has birthing process instead of laying eggs
- human has about 165 different kinds of cells in its body
4 Fundamental tissue of verts -
epithelial  connective  muscle  nerve
ectoderm  mesoderm  mesoderm  ectoderm

EPITHELIAL - prevents dehydration (loss of H₂O)
- permeability barrier  - sensory surfaces - secretory layer
- typed by shape: squamous, cuboidal, columnar, stratified

CONNECTIVE - bind and support other tissues
Adipose tissue - fat, which pads & insulates body
Blood - matrix (not solid) fluid of RBC & WBC's = transport
...plasma (H₂O, salts, dissolved proteins)
...transports substances to tissues
...WBC (lymphocytes/leukocytes - macrophages)
Fibrous connective dense matrix collagen fibers, ...forms
...tendons - attach muscles to bone
...ligaments - join bones together at joints
Cartilage - strong, but flexible skeletal material at bone end
...collagen fibers embedded in rubbery matrix
Bone - rigid connective tissue
...collagen fibers embedded in Ca-salts = hardness
Loose connective - loose weave of fiber proteins
...binds & packs- holding organs & tissue in place
**MUSCLE Tissue** - made from mesoderm
contains proteins: actin & myosin = in filament forms
3 kinds:  
a) **smooth**.... non-striated  
b) **skeletal**.... striated
    assembled into fibers called myofibrils  
c) **cardiac**.... striated, but branched

**NERVE Tissue** - cells that conduct electrical impulses
2 kinds  
a) **neurons**  
b) **glial cells** - surround, support, insulates, & protects neurons

**ORGANS** - systems made of the 4 types of tissue above,
which catalyze a physiological process (specific function)

**The ORGAN SYSTEMS.....**  
Table (web)  
a. digestive  
b. respiratory  
c. cardiovascular  
d. lymphatic & immune  
e. excretory  
f. endocrine  
g. reproductive  
h. nervous  
i. muscular  
j. skeletal &  
k. integumentary
METABOLIC RATE:

Animal Bioenergetics...
energy costs.... to do vertebrate physiology
energy costs.... to walk, run, swim, or just to be...

BASAL METABOLIC RATE - total energy used per unit time measured in calories - amt of heat energy raise 1g H₂O 1°C determined by O₂ consumption.
equipment = respirometer, stress test & cycle ergometer, swimming flume, etc
minimal = that required for functions of life
maximal = peak metabolic activity - Olympic swimmer influenced by variables that make up science of Physiology
age, sex, body size, temp, food level, time of day
size of organism, hormonal balance, available O₂

BMR - endotherms @ rest w/o stress
an animal that derives its body heat from its
own metabolism ex: humans
males 1,600 - 1,800 Kc/d
females 1,300 - 1,500 Kc/d
Lance Armstrong = 6,500Kc/d & 10,000Kc/d for mountains
heart is 1/3 larger, @ rest = 32 bpm & @ max = 200 bpm, w stroke volume of 200 (2x avg)

SMR - standard metabolic rate - ectotherms @ given temp
an animal that warms itself by absorbing
heat from its surroundings
**HOMEOSTATIC MECHANISMS...**

Animals regulate their internal environment

**HOMEOSTASIS...** maintenance of a steady state internal environment (constancy) in face of a changing external environment

**Physiological Compensation...** short term physiological adjustments or adaptations to environmental changes, i.e., homeostatic compensation

**Internal "milieu"** - (claude bernard - Fr. 1880's)
the interstitial fluids filling spaces between cells
the milieu exchanges nutrients w blood

**Constancy of human milieu**

- body temp $39^\circ$ C $\pm 1^\circ$ C
- pH $7.4 \pm 0.1$
- blood sugar $0.1\%$

**Homeostatic Regulation:**
is mechanisms that cells have evolved to remain constant

A homeostatic Regulator - 3 parts *

- **receptor** .... detects a change
- **controller** ... processes info = response
- **effector** ... produce the response

not unlike earlier signal transduction model
Examples of Homeostatic Regulations:

1. Room temperature controllers - see model & Hypothalamus regulation of body temperature

2. pH regulation of the blood
   pH 7.4 +/- 0.1  a shift of 0.4 pH unit = death
   'Andromeda Strain' - space microbe infects people -
   die by blood clotting; growth curve of virus-microbe
   has narrow pH range.  (see web)
   Only 2 survived......
   a crying baby = alkalosis - blows off CO₂ lowers acidity
   & drunk = acidosis - bleeding stomach ulcers

Carbonic anhydrase

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{H}_2\text{CO}_3 \]

Hb pick up H⁺ ions buffering blood
if pH blood drops \([\text{H}^+]\) then \(\text{H}_2\text{CO}_3\rightarrow \text{H}_2\text{CO}_3\)

3. Calcium homeostasis (in blood - range is 9 to 11 mg%)
Ca⁺² needed for nerve function, muscle contraction, blood clotting, etc.

antagonistic hormones

thyroid  --> calcitonin hormone - lowers Ca levels
causes Ca to be deposited in bone
reduces intestinal absorption of Ca
reduce Ca uptake by kidney

parathyroid --> parathyroid hormone - raises Ca levels
stimulates release Ca from bone
increase Ca uptake by intestine & kidney
4. **Blood Glucose balance**
   pancreas makes **insulin** and **glucagon**
   antagonistic hormones

5. **Osmoregulation** - water balance of organism
   **osmosis** - net movement of water hypotonic to hypertonic
   terrestrial animal.... gain water water from food & drink
   lose water by urinating, defecating, & evaporation
   aquatic animals....
   **osmoconformer**... internal [solute] same as environment
   **osmoregulator**... internal [solute] maintain constant level

**fresh water vs. seawater fish**

**FW fish** - internal solutes greater... thus gains water thru its
   body surface, its gills, and food
   compensates does not drink water
   excretes large amounts dilute urine
   gills take up lost ions [Na, Cl, K]

**SW fish** - internal solutes less... thus constantly loses water
   compensates drinks SW
   pumps ions [Na, Cl, K] out via gills
   urinates ions

**Other marine vertebrates** - **birds & sharks**
Linked genes, crossing over & mapping

F1  \( \text{RYry} \) round and yellow

Testcross  \( \text{RYry} \times \text{ryry} \)

<table>
<thead>
<tr>
<th>if linked</th>
<th>ry</th>
<th>if non-linked</th>
<th>ry</th>
<th>mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{RY} )</td>
<td>( \text{RYry} )</td>
<td>( \text{RY} )</td>
<td>( \text{RYry} )</td>
<td>( \text{RrYy} )</td>
</tr>
<tr>
<td>( \text{ry} )</td>
<td>( \text{ryry} )</td>
<td>( \text{Ry} )</td>
<td>( \text{Ryry} )</td>
<td>( \text{Rryy} )</td>
</tr>
<tr>
<td>( \text{rY} )</td>
<td>( \text{rYry} )</td>
<td>( \text{ry} )</td>
<td>( \text{ryry} )</td>
<td>( \text{rrYy} )</td>
</tr>
<tr>
<td>( \text{ry} )</td>
<td>( \text{ryry} )</td>
<td></td>
<td></td>
<td>( \text{rryy} )</td>
</tr>
</tbody>
</table>

\( 1:1 \) \hspace{1cm} \( 1:1:1:1 \)

**BUT:** we do a F1 testcross and actually get:

- round & yellow 41.5% \( \text{R}_\text{Y}_\text{ parental} \)
- wrinkled & green 41.5% \( \text{rryy \ parental} \)
- round & green 8.5% \( \text{R}_\text{yy \ non-parental} \)
- wrinkled & yellow 8.5% \( \text{rrY}_\text{ non-parental} \)

**HOW ???**

CROSSING OVER
Frequency of crossover exchange is **GREATER** the **FARTHER** apart 2 genes are.

Frequency of exchange is **proportional to relative distance** between 2 linked genes.

1% crossover frequency = 1 map unit of distance
1% CrossOver = 1 centiMorgan

Genes **R** and **Y** reveal **17% CO frequency** of exchange thus genes are **17 map units** (centiMorgans) apart.

Genes **R** and **X** reveal **5% crossover frequency**
Genes **Y** and **X** can reveal either **12% or 22%**
Some gene interactions with unique progeny ratios

**Complimentary Genes** - mutually dependent on two genes for phenotype expression...

**Purple flower color** & protein compliment

\[
P : \text{PPCC} \quad \times \quad \text{ppcc}
\]

F1

\[
\text{PpCc}
\]

F2

\[
\begin{array}{ccc}
P_C_ & \text{purple} \\
\text{ppC} & \text{white} & 9 : 7 \\
\text{ccP} & \text{white} & \text{not 9:3:3:1} \\
\text{ccpp} & \text{white} \\
\end{array}
\]

**Epistasis** - works in similar fashion \((c = \text{melanin gene})\)

but, a gene at one locus alters the phenotype of other gene at a second locus = altering dihybrid ratio

\[
\begin{array}{ccc}
\text{C_B}_ & \text{black} \\
\text{C_bb} & \text{brown} \\
\text{ccB}_ & \text{white} \\
\text{ccbb} \\
\end{array}
\]

**Polygenic Inheritance** –

2 more genes affect 1 phenotype quantitative characters
- height, weight, etc....

**Pleiotropy** - 1 gene influences many phenotypic traits

mutant defect in collagen gene = deformity
Some Human Genetic Traits...

**Pedigree Analysis symbols**

- Female ♀
- Male ♂

Widow's peak, finger hair, freckles, free ear lobes

**Blood Groups** - 4 phenotypes O, A, B, and AB
  - Due to carbohydrates on RBC surface
  - **Genotypes** = ii, AA, Ai, BB, Bi, AB
  - **Phenotypes** = O, A, A, B, B, AB

**Polygenic Inheritance** - ex: height, weight, etc...
  - Multiple genes --> 1 phenotype
  - Usually quantitative traits with normal dist.

**Linkage** - 2 genes on same chromosome
  - Autosomes vs. sex chromosomes
  - Karyotypes

**Sex linkage** - Trait carried on X-chromosome
  - Red-green colorblindness
  - Carrier = heterozygote $X^R_X^r$, $X^R_X^r$, $X^R_Y$, $X^r_Y$
  - Can we have a colorblind female...some crosses
Some more Human Genetics...

1. Inborn Errors of Metabolism – defective enzymes...

**PHENYLKETONURIA [PKU]** pp 1 / 18,000
disfunctional phenylalanine hydroxylase
PHE --> PHE-pyruvate --> degraded & excreted
accumulates - blood/brain barrier = mental retardation

**ALKAPTONURIA** aa Garrod in 1908
homogentisic acid oxidase ----> ALKAPTON
1st suggestion effect of genes was oxidizes black
to make proteins (disease = defective)

**TAY SACHS** tt 1 / 300,000
defective lysosomal enzyme = swell burst --> death
Ashkenazic Jews show 1/ 3,600 births 1 / 28 is Tt

**SICKLE CELL** ss 1 / 6,000 births
defective beta polypeptide of Hb
improper folding of Hb --> clogs capillaries

2. Chromosomal Abberations
   **Mistakes of Meiosis**
   aneuploidy - variations in chromosome #
due to non-disjunction, which can lead to
   Downs Syndrome (chromosome 21) and
   a number of sex chromosomes syndromes.
Chromosomal aberrations due to alterations

Diagram showing deletion, duplication, inversion, and reciprocal translocation.
Aneuploidy = Variation in chromosome #
monosomy       - 1 less than normal
disomy         - normal (diploid)
trisomy        - 1 more than normal

Aneuploidy in the sex chromosomes

Gametes  XX  --->  O  &  XX
          & X or y  X    Y
           XO    YO    XXX    XXY

Turner Syndrome  dies  Triple X  Kleinfelter
1 / 5,000 1 / 1,000 1 / 2,000
female appearance  meta female  appear male
sterile  no visible symptoms  sterile

Eugenics - euploidy & criminal inheritance
XYY of 197 violents - 7 were XYY
Richard Speck (Chicago nurse serial killer)

Amniocentesis: monitoring for genetic defects
Definitions of a Gene...

Mendel's Particles
a unit of heredity responsible for a phenotype

Morgan's Loci
placed gene on a chromosome, i.e.,
it is a cellular entity, part of a chromosome

Watson & Crick
is a region of specific nucleotides
along length of a DNA molecule

Modern Molecular Biology functional definition:
DNA sequence coding for a specific polypeptide

Split Genes - Introns & Exons:
eukaryotic genes contain non-coding segments
with no corresponding proteins

Others... any definition should also include:
promoter sequences, enhancers, segments that code
for rRNA, tRNA, & snRNP's

"A GENE is a region of DNA that CODES for an RNA"
Hardy-Weinberg Equilibrium (population genetics)  
1908  G.H.Hardy, English mathematician  
G.Weinberg, German physician  

Law of Genetic Equilibrium:  
describes gene pool (i.e., all alleles present) mathematically  
defines ideal case of a NON-evolving populations  
criteria:  
must be used in large populations (large sample sizes)  
which exhibit random mating  
absence of forces which can change allele frequencies  
  no migration (in/out)  
  no mutation  
  no selection  
  each allele is equally viable (no lethals)  

law states -->  
original percentage of a genotypes alleles remain constant  
HW is defined algebraically by the binomial expansion  
any gene with 2 allelic form  
A & a  
then frequency of one allele  
(A) = p  
& frequency of other allele  
(a) = q  
then by definition  
p + q = 1  

HW equation  
(p + q)^2 = p^2 + 2pq + q^2 = 1  
GG  
Gg  
gg  

Fall & Summer  
Bil 150 - Mendelian Genetics
MOLECULAR GENETICS

Genes --> Enzyme --> Metabolism
Central Dogma of Molecular Biology
DNA --> RNA --> Protein

GENES = ? DNA is the genetic material...
(what about, retroviruses, as HIV & TMV contain RNA)
a discrete piece of deoxyribonucleic acid is ....
- linear polymer of repeating nucleotide monomers
  nucleotides - A adenine,  C cytosine
  T thymidine,  G guanine
  letters of the genetic alphabet (code)
- unit of information is CODON = genetic 'word'
  triplet sequence of nucleotide CAT
  3 nucleotides = 1 codon (word) = 1 aa
- definition of word = amino acid

Size of Human Genome:
~ 3,000,000,000 base pairs or 1.5b in single strand genes
~ 500,000,000 codons (words or amino acids)
  average page your textbook = approx 850 words
  thus, human genome is equal to 590,000 pages
  or 470 copies of bio text book
  reading 3 bases/sec = about 47.6 years @ 8h/d - 7d/w
WOW... nanotechnology to the extreme.
Proof DNA is the Genetic Material

1. Transformation Exp's of F. Griffith....
   R-strain (benign) absorbs heat killed DNA is transformed

2. Avery, Macleod, McCarty.... (1940's)
   Transforming substance was active DNA

3. Alfred Hershey & Martha Chase Experiment 1952 *
   ... VIRAL REPLICATION [ phage infection ]
   a genetically controlled biological activity
   ... novel experiment (1st real use radioisotopes in biology)

CONCLUSION - DNA is genetic material because ----
   32p (nucleic acid) not 35s (protein) controls viral replication

Structure of DNA... Double Helix.
   people - Rosy Franklin, Maurice Wilkins, JD Watson,
   Francis Crick, Erwin Chargaff, etc....

   double stranded, helical, polynucleotide chains, made of
   4 nucleotides - A,T,G,C (purine & pyrimidines)
   2 polynucleotide strands (polymer chains)
   held together via complimentary pairing -
   Chargaff's rule ..... A:T  G:C
   A + G / T + C = 1.0
   head-tail polarity [5'-----3'] - antiparallel
Replication of DNA  (Arthur Kornberg - 1959 Nobel)

copying of DNA into DNA is structurally obvious

Patterns of Replication -
conservative, semiconservative, & dispersive
Matt Meselson & Frank Stahl experiment (1958) –
used heavy isotope of N to label DNA - $^{14}$N & $^{15}$N

Enzyme that makes DNA is DNA polymerase
req: deoxy-XTP's and ssDNA template piece
reads template and makes a complimentary copy
reads 3' to 5' and synthesizes in 5" to 3' direction

Replication forks - bidirectional synthesis
Primase activity - RNA polymerase required
Continuous & Discontinuous replication simultaneously
other enzymes required include:

- **helicase** - untwists DNA
- **topoisomerase** [DNA gyrase] - removes supercoils,
- **binding proteins** - stabilize replication fork
- **primase** (bacterial primosome) - makes RNA primer
- **Pol III** - synthesizes new DNA strands
- **Pol I** - remove RNA primer & adds DNA bases
- **ligase** - repairs Okazaki fragment holes

Okazaki fragments
DNA Repair
GENE EXPRESSION -

**Transcription** - copying of a DNA sequence into RNA

**Translation** - copying of RNA sequence into protein

Flow of genetic information [Central Dogma Molecular Bio.]
DNA sequence ---> RNA sequence ---> amino acid sequence

TAC  AUG  MET

Triplet sequence in DNA --> codon in mRNA ----> amino acid in protein

Information : triplet sequence = genetic word [codon]

Compare Events:
- Procaryotes vs. Eucaryotes = Separation of labor
- Differences DNA vs. RNA

**Transcription** - RNA polymerase
- RNA polymerase binds to promoter DNA region
- transcription factors read DNA sequence - make RNA copy
- makes a complimentary copy of one of 2 DNA strands

**Kinds of RNA**
- tRNA - small, 80n, anticodon
  - function = picks up aa & takes it to ribosome
- rRNA - piece of RNA that make up organelle = ribosome
- RNA Processing -
- mRNA - intermediate size - 100n to 400n
  - function - codes for amino acid sequence
- hnRNA - heterogeneous nuclear RNA
  - Primary Transcript
  - function - precursor of mRNA in eukaryote
Some other types of RNA molecules:

**small nuclear RNA (snRNP's)** -
plays a structural and catalytic role in spliceosome*

**SRP (signal recognition particle) RNA** -
a component of the protein-RNA complex that recognizes signal sequence of polypeptides targeted to ER

**small nucleolar RNA (snoRNA)** -
aids in processing of pre-rRNA transcripts for ribosome subunit formation in the nucleolus

**small interfering RNA (siRNA)** - also called microRNA;
short (20-24 nucleotide) RNAs present in MODEL eukaryotic organisms as: roundworms, fruit flies, mice, humans, & plants (arabidopsis) helps regulate gene expression by controlling timing of developmental events also inhibits translation of target mRNAs.
ex: siRNA - c7-fig 19.9*
INTRONS:

What are Introns? and What is the Role of Intron DNA? is it DNA Junk or sophisticated Genetic Control Elements?

in 1977 Phillip Sharp & Richard Roberts discovered that DNA contains introns ...
intervening DNA segments that do NOT code for proteins

Presence of Introns:
mostly absent in prokaryotes:
  have few non-coding DNA pieces, but as eukaryotic complexity grew, so did a few non-coding DNA pieces.

  now makes up greater than 95% of the DNA, i.e., less than 1.5% of human genome encodes proteins

  40% of human genome is Transposons & repeat genetic elements.

Evolutionary Origins?
  may have been self-splicing mobile genetic elements that inserted themselves into host genomes

Advent of Spliceosomes:
  a primary RNA transcript is processed by splicing to assemble protein coding exons
  spliceosome = catalytic RNA/protein complexes that snip RNAs out of mRNAs, would encourage introns to proliferate, mutate, evolve
TRANSLATION
- process of making a protein in a specific amino acid sequence from a unique mRNA sequence

Sequence of Steps in Translation
1. add an amino acid to tRNA = aa-tRNA - ACTIVATION
2. assemble parts [ribosome, mRNA, aa-tRNA] - INITIATION
3. adding new aa's - peptidyl transferase - ELONGATION
4. stopping the process – TERMINATION
   a polypeptide is built on the Ribosome on a polysome review process and figures.

GENETIC CODE
- sequence of nucleotides in mRNA that specifies sequence of amino acids in protein
  - Coding Ratio = # n's = 1 aa = 3 nucleotides
  - S. Ochoa (1959) - polynucleotide phosphorylase
  - M. Nirenberg (1968 Nobel) - synthetic mRNA's
    1 n = U 5'-UUU-3' = phe

CODE is:
universal (minor anomalies),
1 initiator codon (AUG),
redundant but not ambiguous,
and exhibits wobble.
GENETIC CHANGE:

**Mutation** - change in DNA nucleotide sequence results in a different codon = different amino acid sequence
- Point mutation - single to few nucleotide changes
  - deletions, insertions, frame-shift mutations [CAT]
  - substitutions -
    - non-sense = change to no amino acid (STOP)
      UCA --> UAA  ser to non
    - mis-sense = different amino acid
      UCA --> UUA  ser to leu
- effects = no effect, detrimental (lethal),
  +/- functionality, beneficial
  ex: Sickle Cell Anemia - a mis-sense mutation...

Recombination - Recombinant DNA
  genotype change by inserting NEW DNA into recipient cell
  1. fertilization  n + n = 2n  sperm into egg cell
  2. exchange of homologous chromatids (crossing over)
  3. transformation - absorption of DNA by recipient cells
  4. BACTERIAL CONJUGATION - plasmids (F+ and R)
    primitive sex-like reproduction
  5. VIRAL TRANSDUCTION - via a viral vector
    general transduction - pieces of bacterial DNA are packaged w viral DNA during viral replication
    restricted transduction - a temperate phage goes lytic carrying adjacent bacterial DNA into virus particle
  6. DESIGNER GENES
DESIGNER GENES & BIOTECHNOLOGY

- Recombinant DNA Technology
  collection of experimental techniques, allows for isolation, copying, & insertion of new DNA sequences into host-recipient cells by artificial methodologies

Restriction Endonucleases - diplotomic cuts at unique DNA sequences, including palindromes

5' GAATTC 3'  ->  5' G AATTC 3'
3' CTTAAG 5'  ->  3' CTTAA G 5'

DNA cut as such can be recombined (reannealed) or spliced w other DNA molecules to produce new genes

What's Being Done?

Cloning of Genes...
1. Plasmids... genome libraries & BAC’s
2. Probes via cDNA & reverse transcriptase
3. Polymerase Chain Reaction.... O.J. & Jurassic Park

Locating Genes - electrophoresis & restriction maps DNA fingerprints, hybridization,

Southern Blots - a technique for detecting specific DNA sequences separated by gel electrophoresis via hybridization to a previously radioactively labeled nucleic acid probe.

Microarrays - passes cDNA of the cell's mRNA over slide with ssDNA copies of all a cell's genes; DNA microchips are made by high speed robotics akin to Intel chip making; cDNA (mRNA's) are flourescently tagged so easy to see in slide's wells.

Gene Sequencing - Human Genome Project & dideoxy DNA.
Some Practical Applications of DNA Technology
- What's been Done...

1. Medical... disease may involve changes in gene expression
   a. disease/infection diagnosis:
      PCR & labeled probes from pathogens help identify
      microbe types: [RT-PCR] - HIV RNA -RT-> cDNA -PCR->
      probe can ID AIDS infection
   b. RLFp - Restriction Length Fragment Analysis -
      markers often inherited with disease
      what is RFLP* genetic testing & polymorphism --->
      RFLP markers & disease* & MST II cuts Sickle Cell* &
      Dde-I cuts Sickle Cell*
      paternity testing via DNA fingerprinting
   c. Gene Therapy... idea is to replace defective genes
      - microinjection of DNA* & fig 20.16*
      & (ADA Deficiency & Ashanti DeSilva update)
      - SCID (Severe combined immunodeficiency -
      a single gene enzyme defect):
      clinical trials in 2000 resulted in 2 of 9 cured, but
developing lukemia: the retroviral vector inserted
repair gene near bone marrow cell genes involved
in blood cell division, thus lukemia. trials stopped.

2. Pharmaceutical Products... manufactured drugs
   protropin (an ethical dilemma)* &
   Recombinant bacteria* = Humulin
3. **Forensics...**

- **DNA fingerprinting** - example judicial modus operandi
  a murder case* & a rape case*
  DNA fingerprinting usually looks a 5 RFLP markers and blood is tested via Southern Blotting (20.10) using probes for these alleles

- **Simple Tandem Repeats** (short- 5n to 6n) - trinucleotide (3n) repeats can undergo an increase in copy number by a process of dynamic mutation; # of tandem repeats is unique to a genetic indiv. Variation in the length of these repeats is polymorphic. Individual A has ACA repeated 65 times @ loci 121, 118, & 129 individual B has different repeat pattern at these loci.

**STR's** can cause genetic diseases as well:
  CCG trinucleotide occur in fragile sites on human chromosomes (folate-sensitive group).
  fragile X (FRAXA)- responsible for familial mental retardation. Another FRAXE is responsible for a rarer mild form of mental retardation.
  mutations of AGC repeats gives a #- of neurological disorders.

4. **Environmental Clean-up...**

  bacteria can extract heavy metals (Cu, Pb, Ni) from the environment & convert them into not toxic compounds genetically modified bacteria may be the "miner's" of the future.
5. Franken Food...
   genetically modified (GM) animals & agricultural crops

Transgenics –
   organisms with inserted foreign DNA in their genomes

Animals* - GFP novelties* + Dolly
   - animal cloning companies --->
   - "pharm" animals (20.18*) - sheep carry human
     human blood protein gene that inhibits enzyme
     in cystic fibrosis patients

Plants* - genetically modified crop plants - fig 20.19*
   - to get Ti plasmids in = a DNA gun*
   - Frankenfood & Edible Vaccines
   - National Plant Genome Initiative Plan update

An overview of biotechnology
   History of Biotechnology
   Human Genome Project & Biotech Companies
Control of Gene Expression -

How do we know a gene has been active (turned on)
look for gene's product, i.e., protein
increase in enzyme activity = gene action?
no enzyme activity = no gene action
but, pre-existing inactive enzymes --> active forms
ZYMOGENS - pepsionogen ---> pepsin
- tyrpsinogen ----> trypsin

2 circumstances  1) pre-existing inactive enzyme
  2) de novo (new) enzyme synthesis

Mechanism of Gene Action  (turning on/off genes)

Jacob and Monod - prokaryotes  -  Lactose Operon

E. coli

glucose
NO beta-galactosidase

lactose
beta-galacotsidase

lactose  ----->  glucose + galactose

Operon = series of mapable-linked genes controlling
synthesis of protein

promoter  -  binds RNA polymerase
Operator  -  binds repressor protein
Regulator  -  makes repressor protein
structural  -  make enzyme proteins
Mechanism of Gene Action (turning on/off genes)

**Eukaryotes** -
more complex (more DNA - nuclear compartment)
**promoter** - site where RNA polymerase binds
**enhancer** - sites where enhancers/transcription factors bind
**transcription factors** - proteins that help transcription

Some examples: Eukaryotic gene expression controls

**Differential Gene Activity**... selective expression of genes
i.e., different cell types express different genes
liver vs. lens cell
1. role of **activators** in selective gene expression (DGA)

2. molecular turnover - ½ life mRNA's*
   & longevity of some proteins*

3. **steroid hormones** (figure*)

4. **Processing of RNA transcript** (figure*)
   cut/spliced in nucleus and capped for transport
   **intron** - pieces cut out (non gene-proteins)
   **exons** - pieces transported to cytoplasm
   alternative **splicing** (next page)*
ALTERNATIVE SPLICING

at the beginning of the 3rd millennium, the estimates of the number of human genes was 153,000 making about 90,000 proteins;

by the first draft of the Human Genome Sequence (summer of 2005) the number had shrunk to ~ 20-25,000 protein coding genes.

The current estimates of the NHGRI puts the number of human genes at less than 25,000. (& there is actually a betting pool)

But, humans still make about 90,000 proteins. How from only 25,000 genes????

In 2004 the mouse genome was sequenced and we learned it also has 25K genes (the same as man) and we both share many of the same exons and introns.

If Mice and Men are so genomically similar, what makes then so vastly different?

alternative splicing ?
Cancer & Gene Expression

cancer often results from gene changes affecting cell cycle control cancer genes, such as adenomatous polyposis coli causes 15% of colorectal cancers
is a tumor suppressor gene, a type of Oncogenes*

2 kinds of human cancer genes:
**Ras** (proto-oncogene gene*) = 30% human cancers
is a G-protein that promotes cell division proteins
a Ras mutation --> hyperactive Ras protein --> division
fig 19.12a*

**p53** (tumor suppressor gene* = 50% human cancers)
fig 19.12b*
p53 is a transcription factor promoting cell cycle inhibiting proteins.
DNA damage --> active p53 --> p51 gene --> protein binds to cyclin dependent kinase stops cell division]
thus a p53 mutation --> excess cell division (cancer).

other cancer genes can lead to new gene actions as:
**BRCA1** and **BRCA2** (tumor suppressor genes)
are involved in 50% of breast cancers in humans
Definitions of a Gene:

**Mendel's Particles...** unit of heredity responsible for phenotype

**Morgan's Loci...** placed gene on a chromosome, i.e.,
   it's a cellular entity, that is part of chromosome - mapable

**Watson & Crick...** is sequence of specific nucleotides along the length of DNA molecule

**Molecular Definition** -
   1 nucleotide = 0.34 nm thus tRNA = 81n x 0.34 = 27.5 nm
   1 nucleotide = 340 amu thus tRNA = 81 x 340 = 27,540 amu

**Modern Molecular** - a functional definition:

**Biological...** DNA sequence coding for a specific polypeptide

**Split Genes...** Introns & Exons :
   euc genes contain non-coding segments (introns) with no corresponding protein
   & coding segments (exons) = proteins

**Others pieces ...** any definition should also include:
   promoter sequences, enhancers, regulator gene, operators, CRP...
   segments that code for rRNA, tRNA, & snRNP's

"A GENE is a region of DNA that CODES for an RNA"
VERTEBRATE DEVELOPMENT PATTERNS

Embryology - study of development of the embryo

5 major stages

1. gametogenesis - gamete production - (meiosis)
   spermatogenesis - in semiferous tubule
   spermatocytes --meiosis 1 & 2 --> sperm cells
   oogenesis - in ovary
     every 28d FSH (pituitary) stimulates dormant follicle
     oocytes -meiosis-> produce 2nd oocyte & polar body
     LH (pituitary) triggers ovulation

2. Fertilization - union of sperm & egg cell --> 2n zygote
   parts of a sperm - acrosome, head, mito, & flagella
   must penetrate... 1) egg's jelly coat, 2) vitellin
   layer .. (glycoproteins), & 3) membrane
   acrosome reaction...
   monospermy = plasma membrane/ vitellin layer -
   impermeable? hardens forming fertilization membrane

3. Clevage - rapid succession of cell divisions.......-
   without growth - no increase in size, only cell #
   forms hollow ball of cells called blastula, w internal fluid
   filled cavity is the blastocoel
   animal pole - portion of embryo primary tissues
   vegetal pole - portion of embryo with "yolk“
4. **Gastrulation** - period of cell migrations around blastopore, which converts embryo from hollow ball of cells into a 3 layered stage called gastrula embryo forms 3 primary germ cell layers:
   - **ectoderm** - outer epidermal layers of organs, skin
   - **endoderm** - digestive tract tissue
   - **mesoderm** - fills in space inbetween: muscle

Gastrulation obliterates the blastocoel - forms new cavity called **archenteron** - forms digestive cavity

5. **Organogenesis** .... Organ Formation

   ex: **neuralation** in frog –
   formation of nervous system

   - **neural plate** (ectoderm) - flat tissue surface that migrates to form tube = neural tube = brain & spinal cord
   - **notochord** (mesoderm) cartilage-like = backbone

   Organs form --> flat plates into tubes --> 3D shape

**Key differences**: plant & animal development...

- **animals** - cell migrations & embryonic induction - where one group of cells influences development of an adjacent group of cells - ex: lens induction in eye

- **plants** –
  plane of cell divisions (cytokinesis)
  influences width vs. height
Genetic Basis of Development....

is Differential Gene Activity...
cells become structurally, functionally, & biochemically
different by expressing different genes at different times
during development

Totipotency...
demonstration that all cells of organisms have a full
genetic complement
i.e., differentiation does not proceed by loss of genes
Genomic Equivalency: experiments -
1. F.C. Steward (1950) w carrot grows full plant via 1 cell
2. Briggs & King (1952) & J.B.Gurdon (1974) show same in frogs
3. John Wilmut (1997) clones Dolly (mammals) – see fig

Stem Cells...
unspecialized cells (in form & shape) that can reproduce
indefinitely under appropriate conditions -->
differentiate into one or more cell types.
fertilized egg cells are totipotent (= all) embryonic stem cells
vs. adult stem cells (pluripotent) = many, but not all)- see fig

Transcriptional Regulation...
results in expression of Tissue Specific Proteins – see fig
ex: muscle cell determinism – see fig
NEUROPHYSIOLOGY...

Electrical Properties of Nerve cells (neurons)
Electro-physiology of neurons lie in **Membrane Physiology**
Model organisms is **Squid Giant Axon** (SGA)
diversity of Nervous systems

NERVOUS SYSTEM FUNCTIONS -
1. gathers sensory input  (sense organs via Peripheral NS) -->
2. integrates information  (CNS - brain & spinal cord)-->
3. responds with motor output  (effector organs - muscle)

PARTS -

central nervous system - brain and spinal cord
  nerve- bundle of neurons wrapped in connective tissue
  ganglia- cluster of cell bodies of neurons
peripheral nervous system - carries signals in/out of CNS
somatic nervous system - carries signal to skeletal muscle
  under conscious control
autonomic nervous system - signals regulate internally-
  under involuntary control

FUNCTIONAL TYPES -

  Sensory neurons...  (affarent neurons)
    - carry external stimuli from receptors to CNS
  Interneurons...
    - integrate & relay sensory input to motor neuron
  Motor Neurons...  (efferent neurons)
    - convert signals to effector cells = response
Structure of a vertebrate Neuron

- **Dendrites**
  short outgrowth of Cell Body
  carry signal into Cell Body

- **Cell Body** - is main part of cell w cytoplasm & organelles

- **Axon**
  long outgrowth of cell body - carry signal to next nerve

- **Schwann cell**
  cells surrounding axon in vertebrates - produce myelin
  (sheath) membrane-like insulation surrounding axon

- **Nodes of Ranvier**
  space between successive Schwann cells - opens nodes
  speed of conduction - w/myelin (100 m/sec or 200 mi/hr)
  w/o myelin speed is less (5 m/sec)

  Multiple Sclerosis - degeneration of myelin sheaths

- **Synaptic Knob** - enlarged end of neuron
  holds neurotransmitters in synaptic vesicles

**Reflex Arc** - unconscious response to external stimulus
  knee-jerk reflex -

neuro-muscular junction is the model for neurophysiology
  see web EM's
  1. neuron innervating muscle
  2. synapse
  3. muscle fiber
  4. myofibril
The electrical properties of cells:

RESTING POTENTIAL - the characteristic electric charge exhibited by a cell at rest... most often negative (-)

potential - (in electrical terms) is amount of electrical charge at one point in an electric circuit compared to some other point in the same circuit measured with a volt-meter (multimeter)

How to measure resting potential in cells inside Vs outside of cells - microelectrode

SGA - 65 to -70 mV i
Frog muscle fibers - 90 mV i
Nitella - 150 mV i
Valonia + 15 mV i

Causes of Resting Potential... all make inside (-)

1. active transport of Na & K = high Na out & High K in
2. differential permeability Na (slower in) & K (faster out)
3. lots of protein anions (-) inside
4. diffusion of Cl- in

Nernst \( E_{mv} = +/- \ 62 \ log_{10} \ [C_o]/[C_i] \)
**ACTION POTENTIAL** - a self-propagating change in the voltage across plasma membrane of a nerve cell. Name given to changes in electrical charges that occur during the stimulation of a nerve cell, usually visualized graphically from an oscilloscope recording.

**PROPERTIES of an AP**
- requires a living cell, i.e., requires O$_2$ for metabolism
- .... eliminated by metabolic poisons as cyanide
- measured using microelectrodes impaled into cells
- has threshold - amount of stimulus needed to "fire" an AP
  "all-or-none-phenomena"
- rapid - time course = 2-3 msec

**EVENTS DURING an AP**
- **depolarization** - goes from negative to positive
  - Na channel opens - Na floods in = -70mV to +50mV
- **repolarization** - Na channels close & K channels open
  - K floods out
- **hyperpolarization** - overshoot of resting potential
- **refractory period** - time before another AP can 'fire'
**CONDUCTION** of an AP along an axon...
local spreading of electric charge = change in membrane permeability of adjacent region leads to an autocatalytic - "domino effect"

Saltatory Conduction - node to node vertebrate conduction

**Synapse**-
functional connection between neurons.
allows transmission of AP's between cells

- **synaptic cleft** - space between neurons across which a chemical transmitter diffuses
- **synaptic knob** - site of vesicles holding neurotransmitter
- **vesicle** - holds neurotransmitter (ex: acetylcholine)
- **pre-synaptic side** - releases neurotransmitter
- **post-synaptic side** - a receptor binds transmitter.... ion channels open - change potential charge of post-synaptic membrane ----> new AP

removal of stimulus –
**acetylcholine esterase (ACHase [enz])** destroys transmitter
Post-Synaptic Responses...

Excitatory neurons -- open Na channels = + = AP
Inhibitory neurons -- open Cl channels = - = no AP

**EPSP** - excitatory post-synaptic potential (-15mV)
excitatory neurons --> open Na channels --> + --> AP

**IPSP** - inhibitory post-synaptic potential (-75mV)
inhibitory neurons --> open Cl channels --> more (-)

**AP** - all or none 120mv polarizations (-65 to +55mV)

Integration of impulses - review figures
Summation of Impulses - review figures

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(a) 
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Neurotransmitters ...

neuro-muscular junction - **acetylcholine** (contractions)

biogenic amines (CNS)

epinephrine & norepinephrine - increase Heart Rate
serotonin & dopamine - affect mood, attention & learning
psycho active drugs (LSD/mescaline)
  function by binding to serotonin/dopamine brain cell receptors
Parkinson's = lack of dopamine
schizophrenia = too much dopamine
depression = reduced epinephrine/norepinephrine
Prozac (antidepressant) blocks removal of
  serotonin from synaptic cleft

**amino acids** - ASP and GLU - excitatory (CNS)

...Chinese Restaurant Syndrome

GLY & GABA - inhibitory

**peptides** (small proteins)

endorphins - decrease perception of pain
substance P - excitatory transmitter - signaling pain

Stimulants - chemicals that increase activity of CNS

cocaine - prevents re-uptake of Ach by synaptic vesicle

caffeine - increases post-synaptic threshold (Cl in = -)
  stimulates HR & breathing rate

barbiturates & Valium - intensify GABA (inhibitory) effects

Poisons like strychnine - prevent loss of transmitter = tetanus
SENSORY PHYSIOLOGY... Sensations & Perceptions

sensation - is an awareness of sensory stimuli in brain
perception - meaningful interpretation or conscious understanding of sensory data.

Sensory Signaling – Reception -> Transduction -> Response

5 Components of Sensory Physiology:

1. Sensory Receptors - structures that detect changes in external & internal environment modified neurons or epithelial cells eyes, ears, that respond to stimuli

Classes of Receptors

chemo-receptors: chemicals
  sense solutes in solvents, taste, smell
osmo-receptors of hypothalmus which monitors blood osmotic pressure
photo-receptors: light
eye, eyespots, infrared receptors of snakes, etc...
thermo-receptors: radiant energy
phono-receptors: sound
electro-receptors: detect currents...
lateral line of fish, electric eels, etc..
noci-receptors: pain receptors...
naked dendrites of skin
mechano-receptors: mechanical forces
  (1) hair cell - deflection = depolarization
  (2) stretch receptors of muscles
  (3) equilibrium receptor of inner ear
  (4) touch receptors of skin
2. Reception - 
ability of receptor to absorb energy of a stimulus

3. Transduction – 
conversion of stimulus energy into membrane potential, a Receptor Potential... change in permeability of a post-synaptic membrane. 
is graded = proportional to strength of stimulus may be amplified and may be summed

4. Transmission -
receptor potential transmitted via AP's to CNS

5. Integration -
processing of the frequency of receptor potentials received via summation

Sensory information is encoded by FREQUENCY of AP’s

Sensory Adaptation – decreased responsiveness by receptor to continual stimulation 
a uniformly maintained stimulus of constant intensity is perceived as progressively weaker with time, while a variable intensity stimulus of short duration is perceived as stronger.
another example: **MUSCLE PHYSIOLOGY**

model: skeletal **neuromuscular junction** (see web fig)

an innervated muscle fiber
muscules can only contract (pull)

4 parts of a Muscle twitch
[ a **CONTRACTION** cycle ]

1) **latent period** - 5 msec
   time between application of AP & initiation of contraction

2) **contraction** - 40 msec
   muscle shortens & does its work

3) **relaxation** - 50 msec
   muscle elongates & returns to original position

4) **refractory period** - 2 msec
   time of recovery between stimulations

**Summation** - a 2nd contraction before the 1st subsides

**Tetany** - sustained contractions

**Fatigue** - under repeat stimulation, contraction get feebler, lactate accumulates, fatigue, contractions stop

**Shivers** - involuntary-summed muscle contractions which release waste heat, that warms body
2 TYPES of MUSCLE FIBERS

determined both genetically and functionally
based upon how fast they can produce a contractile twitch

Every muscle composed of varying % composition of two types

<table>
<thead>
<tr>
<th>TYPE I</th>
<th>TYPE IIA/IIx</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLOW TWITCH</td>
<td>FAST TWITCH</td>
</tr>
<tr>
<td>Tonic muscle (red)</td>
<td>Tetanic muscles (white)</td>
</tr>
<tr>
<td>Leg muscles</td>
<td>Pectoral muscles</td>
</tr>
<tr>
<td>slower contraction times (110 msec)</td>
<td>faster contraction times (50 msec)</td>
</tr>
<tr>
<td>continuous use muscles</td>
<td>one time use muscles</td>
</tr>
<tr>
<td>for endurance performance( marathoners)</td>
<td>for power &amp; speed (sprinters)</td>
</tr>
<tr>
<td>good for long slow sustained contractions and prolonged performance</td>
<td>good in rapid contraction short time and brief performance</td>
</tr>
<tr>
<td>not easily fatigued</td>
<td>easily fatigued</td>
</tr>
<tr>
<td>contain myoglobin (red)</td>
<td>no myoglobin (white)</td>
</tr>
<tr>
<td>more capillary beds greater max VO2</td>
<td>less capillary beds</td>
</tr>
<tr>
<td>smaller in size</td>
<td>larger in size</td>
</tr>
<tr>
<td>lower glycogen content</td>
<td>higher glycogen content</td>
</tr>
<tr>
<td>poor anaerobic glycolysis</td>
<td>predominant anaerobic glycolysis</td>
</tr>
<tr>
<td></td>
<td>easily converts glycogen to lac w/o O2</td>
</tr>
<tr>
<td>predominant aerobic enzymes &amp; aerobic metabolism</td>
<td>lower fat content</td>
</tr>
<tr>
<td>higher fat content</td>
<td>fewer mito. - Beta Oxidation low</td>
</tr>
<tr>
<td>more mitochondria-Beta Oxidation high</td>
<td>well formed sarcoplasmic reticulum</td>
</tr>
<tr>
<td>poorly formed sarcoplasmic reticulum</td>
<td>quick release of Ca = rapid contractions</td>
</tr>
<tr>
<td>slower release of Ca = slow contractions</td>
<td>troponin - higher affinity for Ca</td>
</tr>
<tr>
<td>tropinin has lower affinity for Ca</td>
<td></td>
</tr>
</tbody>
</table>
Vertebrate Skeletal Muscle - structure
sarcomere - repeat unit of striated muscle,
delimited by Z-lines
  I band - "clear zone" around Z-line (isotropic)
  A band - “dark region” in center of sarcomere (anisotropic)
  M line - mid point of sarcomere
  H zone - clear region in center of sarcomere around M line

SLIDING FILAMENT THEORY of Muscle Contraction
  A band remains constant in size
  H Zone becomes denser
  I band varies in length becoming shorter

Muscle Cell Proteins
  myosin - 2 polypeptides forming a helix with globular end,
           which has ATPase activity & an affinity to bind actin
  THICK FILAMENT
  G-actin - globular protein which polymerizes into
            THIN FILAMENT, contains a myosin binding site
  tropomyosin - fiberlike protein which helically wraps
                 around actin thin filament
  troponin - globular protein which binds Ca+2

Muscle Contraction Cycle & Role of Ca - review
The end
The material in the slides below on:

Myosin isoform types
Insulin like growth factor
Myostatin
and the physiology of the human eye

may or may not be covered in lecture.

Listen for specific announcement to see if you are responsible for this material.
The Performance Enhancing Drugs of the Future... not steroids, but the introduction of artificial genes:

What kind of genes

1. genes for myosin type transcriptions factors, which activate genes for dormant myosin isoforms...
   for ex: say an ancient type IIb isoform that's faster than any known Type II isoform of today

2. or IGF-I* (insulin-like growth factor)
   IGF-I is a growth factor structurally related to insulin. IGF-I is produced in response to GH & induces subsequent cellular activities, particularly on bone growth. IGF-I has autocrine and paracrine activities, & like the insulin receptor, it has intrinsic tyrosine kinase activity. Owing to their structural similarities IGF-I can bind to the insulin cell membrane receptor.

Normal Muscle Cell Growth includes:

1. satellite cell recruitment... divide & fuse with muscle cells
2. growth factors as IGF-I... promotes satellite cell proliferation
3. growth inhibition factors, such as myostatin.

Current research - H.L. Sweeney at U. Penn... has used adeno-associated virals (AAV) to infuse IGF-I gene into muscle cells in normal mice: overall size & growth rates up 15% to 30% in mice genetically engineered to overproduce IGF-I: injection of AAV-IGF-I into one leg of lab rats with weight training program:
   = 2x increase in strength in treated leg
   = longer period before gained strength is lost
   = sedentary rats showed 15% increased strength
Myostatin... is a muscle inhibitory growth factor
blocks muscle growth, promotes atrophy and slow
muscle cell growth,
may function antagonistically with IGF-I,

discovered by Se-jin Lee at Johns Hopkins in 1997
Belgian Blue cattle* are due to defective myostatin gene
defective myostatin genes = considerably larger muscle mass

may be useful in muscle
debilitating muscle diseases as:

muscular dystrophy –
sarcopenia - age related muscle loss
cachexia - aggressive muscle loss
in cancer & HIV patients
myoclonus - abnormal muscle
contractions

Wyeth pharmaceuticals is at work
on myostatin inhibitors
1st drugs to date are antibodies
to myostatin and some clinical
trials are set to begin
in M.D. patients
Example of Sensory Organ - the Human Eye

**EYE** - a specialized sensory organ capable of light reception in vertebrate animals, formed visual images are then carried to the visual center of the brain = perception.

**Parts** - of a simple eye -
- roughly spherical with opaque sides & back, with transparent front & interior
- **lens** - focuses light on rod & cone cell of retina - cuboidal epithelia
- **retina** - layer of nerve tissue of millions of light receptor cells
- **rod & cone cells** - transmits signals of varying light intensity
- **fovea** - structure near center of retina, where cone cells give max sharpness of vision
- **optic nerve** - retinal cells record light images & transmit to optic nerve, which exits eyeball behind the optic disk (blind spot) to the visual centers of brain.
- **sclera** - tough outer shell of eyeball, made of dense fibrous tissue
- **cornea** - stratified squamous epithelia, chief refractory part of eye allows light to pass & aids in focusing.
- **vitreous humour** - transparent jellylike material, helps eye keep its spheroid shape.
- **aqueous humour** - anterior chamber, filled with a watery fluid
- **iris** - muscular curtain that opens/closes to regulate amount of light entering eye through the **pupil** (opening of iris into eye)-
Some common vision disorders - correctable by eye glasses

**myopia** (near-sightedness) -
  lens' point of focus falls within the vitreous body, so that when light reaches the retina it is out of focus

**hyperopia** (farsightedness) -
  point of focus falls behind the retina (out of focus)

**astigmatism** - results from defects in the corneal curvature rays of light don't form a point of focus on the retina.

**night blindness** - lack of chromophore retinal

**color blindness** - lack of trichromatic pigments

**glaucoma** - result of increased pressure of fluids in the eye, produces defects in field of vision, lead sto vision loss

**Optical Illusions**
  - illustrate difficulty of perception & understanding what you see is actually what you see?

Seen as **columns** of Xs and Os rather than **rows** of alternating Xs and Os.

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X O X O X O X O X O
X O X O X O X O X O
X O X O X O X O X O
X O X O X O X O X O
X O X O X O X O X O
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Fall & Summer Bil 150 - Sensory Physiology