Biology 150 - GENERAL BIOLOGY

Dr. Charles Mallery [cmallery@miami.edu]

**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 depend upon
   a. number of protons & neutrons in its nucleus
   b. number of electrons in its orbital shells (outermost = valance shell)
3. Atoms with incomplete valence 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 Waals' interactions
4. Metabolism is CHEMICAL REACTIONS, which MAKE/BREAK 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
- euukaryotic cells have internal membranes that compartmentalize their functions
- euukaryotic 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 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 multi-enzyme complex that converts the energy of a hydrogen ion gradient to phosphorylation of ADP
3. Glycolysis and Krebs cycle are key intermediy 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.

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

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...
3. Meiosis I and Meiosis 2
4. Reduction/division occurs... diploid → haploid
daughter cells ½ number of parent chromosomes
5. Stages have same nomenclature as Mitosis: prophase, metaphase, anaphase, telophase,
6. Only one S phase, where DNA is duplicated;
aften may be no interphase between M1 & M2
7. Homologs separate in Meiosis 1 Chromatids separate in Meiosis 2 (mitotic-like)
8. Random Assortment occurs...... homologs align at equatorial plates independent of each other
9. Crossing over... may occur in Prophase I...
   Synapsis: pairing homologs allows exchange chiasma: point exchange of sister chromatids

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 major 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.
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 totipotent or pluripotent.

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 of slow twitch - 100 msec - type 1 - aerobic
   Fast twitch - 50 msec - type 2a/2x - anaerobic.
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 are 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?

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 practitioners 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.

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

♀ ♂ ♠ † ∞
**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

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**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, typhoid fever, diphtheria, TB, malaria, dengue fever, & yellow fever.

Environmental changes can significantly affect "zoones" 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?

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**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.
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

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**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.

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...

involved:

critical thinking ... nothing is accepted as fact

deuctive reasoning ... if...then logic

birds have wings, robin is a bird, robins have wings

ductive 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 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?
BIOLGY - Bios (life) + Logy (study of )
the scientific study of life & living things

LEVELS of Biological ORGANIZATION

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 cellulose 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 cellulose cell walls; often motile
Journey To "Cosmos" of the Cell

Course is divided into three (3) parts:
1) Cell and Molecular Biology
2) Cell Reproduction & Genetics
3) Homeostasis & Physiology

three Unifying Concepts .i.e., Unity of Life

Cell Doctrine  Evolution  Bioenergetics

1. Cell Theory...
1665 - 1st cells described by R. Hooke [cork]
1670 - Marcello Malphigi plant tissues may be cellulars
1805 - Lorenz Oken claims all organisms consist of cells
1839 - Cell Theory formally proposed...
Matthais Schleiden & Theodor Schwann
1858 - Rudolph Virchow: paradigm of the cell theory...
"All cell arise from pre-existing cells"

thus: 1) all individuals derived from a single cell (egg cell)
2) all organisms are related genetically

Some important facts about cells:
diverse: many sizes, shapes, and types (165+ in humans)
similarities: have same basic structural plan
- surrounded by cell membrane
  - contain nuclei (bacteria = genophore)
  - similar sub-cell parts (ORGANELLES)
  - all contain same macromolecules

2. EVOLUTION...
"Descent with modification"
1858 Charles Darwin & Alfred Russell Wallace...
2 new ideas about biological patterns in the natural world
- argue all species (past & present) descended from
  common ancestor
...hypothese all species come from pre-existing species &
they change through time
...individuals in a population vary in hereditary traits...
  - result in vast biological diversity we see in
Living things change gradually over time and
  become new forms...
  change from one form ..........> to another form

MECHANISM of EVOLUTION -

via Natural Selection (SURVIVAL of FITTEST)
- individuals in a population vary in hereditary traits...
- some traits help individuals survive better & reproduce more
- Natural Selection picks individuals better fit to a specific
  environment....
  only those with superior traits ... (physical, behavioral,
  biochemical) are more likely to survive and REPRODUCE.

UNITY comes from fact that all living things
  evolved by same rules, the Laws of Evolution

3. BIOENERGETICS - EQUILIBRIUM THERMODYNAMICS...
Science of energy flow and transformation
within, through, and between organisms...
"cells do not produce energy - they consume energy"

UNITY comes from fact that the mechanisms & processes
of energy change & flow in cells are NOT different,
but are the SAME, among different life forms,

Metabolic pathways used by cells are all the same
in bacteria, plants and animals
  glycolysis,
  Kreb's cycle,
  oxidative phosphorylation, &
  photosynthesis.

CELL is functional unit of life and all living systems

CELL THEORY is to Biology as Atomic Theory is to physics

a Definition of a Cell:
A particular organization of matter, bounded by a
selectively permeable membrane, that is capable of self-
reproduction, without the presence of other living
organism (eliminates viruses).

"Living organisms are composed of inanimate molecules...
and nothing is alive in a cell except the whole of it?"
**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

**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 know 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. Arthressius (1908) radiation emitted by stars carried microbes thru space.
    - (supported by F.Crick)
  - **Murchison Meteorite** - AUS - contains PHA's (polycyclic aromatic hydrocarbons) that when mixed with water from capsule-like droplets [John Deamer UCSC]
  - **ALH84001** - Martian meteorite from Antarctic contains PHA's & microscopic looking microbes.

**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 an 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**

4. **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: 4.0 bya
   - 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)
     - (eucaryotes)
     - (multicellularity)
   - 1.0 bya
   - 0.7 bya

5. **Evolutionary Origin ideas are based upon**
   - 1) today's known "living" molecules made from small number of chemical functional groups (OH, NH, 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...

6. **Experimentally testable... Whose goal may be...**
   - "creation of artificial cell, as model of a life system"
1. **classical chemical evolution** approach:
   Search for sources of early precursor organic molecules of Life??

2. **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: H2=O & CC=N leads to organics including amino acids & sugars
     1960's - Sid Fox - makes polypeptides & protobionts
   - cycle of chemical reactions that produce energy.

   **b. Deep dwelling (ocean) hydrothermal vents** (discovered in 1979)
   - minerals spewing up from pressurized, hot springs is source of 1st biomolecular chemical.
   - bioorganic chemical reactivity may have originated in near hydrothermal vents, before genetics.

3. **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.

4. **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 concentration 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.

5. **c. Space Debris:**
   dust, meteorites, asteroids, & comets deposit organics 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, asteroids 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.
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 (PI) 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 CONDENSAION 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 multicellular life forms that lead to animal life.
1. cell membrane encapsulates DNA
devlopment of nucleus greatest evolutionary invention - internalized genome
2. loss rigid cell wall
developed ability of phagiosysis - 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, 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 --> B --> C --> D --> E

One definition of Life may be:
carefully orchestrated chemical reactions.

5. Self-replication (single most definitive property of life)
an inanimate counterpart 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
  Divide
  Differentiate
  increase in mass),
  increase in cell number), &
  become structurally, functionally &
  biochemically different
  fertilized egg --> to adult

10. Die - reveal absence of properties of life

by Thomas Ray @ U. Delaware - TERRA

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 & 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 Terrains 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

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
   - 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 \( N_2 \rightarrow NH_3 \) & some can fix \( CO_2 \rightarrow CH_2O \)
7. use same molecular building blocks for biochemical rxns
8. are enclosed by an amphipathic lipid plasma membrane

Life and a Living Cell may be described as:

self contained,
self assembling,
self adjusting,
self perpetuating,
iso thermal 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...

**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 \( ^{12}\text{C} \)
- 1% < carbon-13 \( ^{13}\text{C} \)
- 1% < carbon-14 \( ^{14}\text{C} \)

half-life C - 5,730y

unstable = radioactivity

n \( \rightarrow p + e^- \)

\( ^{12}\text{C} \)

isotopic tracing of biomolecules

**14C Dating** - also called **RADIOCARBON DATING**

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

- **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 \( ^{14}\text{C} \), so that the original amount of \( ^{14}\text{C} \) present in its tissues at death steadily decreases.

- \( ^{14}\text{C} \) 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 (\( ^{14}\text{C} \) to \( ^{14}\text{N} \)).

Because \( ^{14}\text{C} \) 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.

**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

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

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

bulk vs. vicinal (structural water ?)

Physical Properties water... exists in 3 forms

gas \( \sim \) liquid \( \sim \) solid

- high : surface tension.......... cohesiveness
- specific heat............... heat 1 gm 1C
- heat of vaporization........ 540 cal/gm
- heat of fusion............... 79 cal/gm
- density on freezing........ less dense

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
"HOPKIN'S NaFe 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>steroids/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>phospholipids</td>
<td>glycogen</td>
<td>energy-animals</td>
</tr>
<tr>
<td>Lipid (fats)</td>
<td>triglyceride</td>
<td>oil, fat</td>
<td>energy storage</td>
</tr>
<tr>
<td></td>
<td>(3 fatty acid &amp; 1 glycerol)</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>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... GLYCOSIDIC bond
- sucrose - alpha, 1,2 glu-fruc → sugar
- maltose - alpha, 1,4 glu-glu → amylose
- cellbiose - 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"

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

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
Proteins are Classified by Function

Protein - a polymer of amino acids with biological activity
Enzymes - catalytic activity and functions
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, fibrin of silk & webs
Defensive - protect: antibodies (IgG), fibrinogen & thrombin, snake venoms
Regulatory - regulate metabolic processes, hormones, transcription factors & enhancers

peptide bond - covalent link between carboxyl end of aa1 & amino end of aa2 forms a dipeptide shorter & 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 releasing factor - 3aa of hypothalamus that stimulates release of thyrotropin
enkephalins - CNS peptides that bind to brain cell receptors = analgesic reaction of pain deadening
NutraSweet - dipeptide of L-aspartyl-phenylalanin (methyl ester)

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 % ionized & % non-ionized

R (side) groups... TYPES of Amino Acids
ACIDIC ... negatively charged - ASP & GLU
BASIC ... positively charged - LYS, ARG, HIS
POLAR UNCHARGED ... SER, THR, TYR, ASN, GLN
NON-POLAR ... GLY, ALA, VAL, LEU, ILE, PRO

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
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

2-dimensional 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 20100 different
linear arrangements [1.268 x 10^30]

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 - α & β
tertiary complete 3-D shape of a peptide
quaternary spatial relationships between
other polypeptides or subunits

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
[Hof N (of any aa) & O=C (of 4th aa)]
⅛ 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)

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

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)

Evolutionary Invariants...
primary sequences don’t vary
ubiquitin & histones

Site Specificity...
some sequences determine intracellular 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 5-S] hydrolizes polysaccharides in bacterial cell walls.

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
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 crystals 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: $E + S \leftrightarrow ES \leftrightarrow E + P$

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 --- 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]

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.

Michaelis-Merten

A plot of rate (amount of product per unit time) vs [S] i.e., rate vs. substrate concentration saturates..... at [S] = Vmax (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 Vmax

Inhibition - where action of inhibitor is ...reversible competitive... inhibitor binds to active site lower Km same Vmax
noncompetitive... binds to allosteric site same Km, but lower Vmax
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 rxns, 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 iso thermal 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 CO2 + H2 → CH4
HALOPHILES live in Dead Sea & Great Salt Lake
THERMOPHILES in acid hot springs, deep ocean geyers
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 - Streptomycin = streptomycin
Penicillius = penicillin

CYANOBACTERIA - are photosynthetic eubacteria
Archeaens/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:
coci, 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 eukaryotes:
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 - metazoaon - heterotrophic feeder plant - metaphytan - 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
- Selective staining:
  - Types: bright field, phase-contrast, Nomarski, dark-field

**Electron Microscopy**
- Resolution = 0.2 nm
- 3D = Orange Bowl cross section
- TEM - Transmission
- SEM - Scanning
- FEM - Freeze fracture

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**
- **1 st described by Robert Brown** in 1831 – in the stamens of Tradescantia
- **1 st 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 nucleolus
  - 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... nucleoplasm made in cytoplasm... he radioactively tagged it & autoradiographically followed its movement
    - showed nucleoplasm enters nucleus
    - suggests protein has an aa sequence helps mobility
    - aa signal is in tail

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

**Review chromosome structure:**

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**MITOCHONDRIA...**

**role:** convert bond energy in foods to ATP...........
couples redox transfer of e- & H+ to ATP synthase
**site of:** cellular respiration
- redox rxn's: [CH₄O] → CO₂
- gas exchange in cell: - CO₂ released & O₂ reduced
- Krebs cycle: PYRUVATE → CO₂ + H₂O
- Respiratory ETC chain & oxidative phosphorylation

**1 st described 1900's:** Vital (req living) stains as Janus Green B; today = fluorescent dyes as daammine
**structure:** elongate cylinders to oblate spheroids
- 3-Sum 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 (SK)
- peri-mitochondrial space - where H+ accumulate
- inner membrane – impermeant; req carrier proteins cristae-
- inner membrane holds resp. assemblies ETC
- mitoplasts - 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

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

**LEUCOPLASTS...** non-pigmentous, 2x5 um, variable shape
- 3 types: AMYLOPLASTS, ALEUROPLAST, ELAIOPLASTS

**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 (oblate spheroid –stellite-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
**GOLGI**... Part of the ENDOCYTIC 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)

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**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 $\rightarrow$ 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 caspase proteolytic activity.

Endomembrane system (Endocytic pathway) – includes SER & RER, Golgi, lysosomes, & vacuoles used in transfer of membranous vesicles throughout the cell.

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**ENDOSYMBIOTIC 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 (e.g.), 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, chiplast to cyanobacteria
- Ribosomes are same size as bacterial (70s)
- Double membrane bound = a phagocytic engulfment?
**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 eukaryotic 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 heterogeneous 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:** 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 plasmodesma

**Apoplastic 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 hypotonic.

Cells and Solutions -

<table>
<thead>
<tr>
<th>Source of water movement</th>
<th>Full movement of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertonic inside</td>
<td>into the cell</td>
</tr>
<tr>
<td>Hypotonic outside</td>
<td>out of the cell</td>
</tr>
</tbody>
</table>

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...

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
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.
DIFFUSION - net thermal motion of solute down a concentration and/or electrical gradient

PASSIVE... requires no expenditure of energy

Nernst Equation defines passive equilibrium

\[ E_{mv} = \pm \frac{RT}{nF} \log \frac{C_o}{C_i} \]

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)

Comparison of passive vs active transport

CARRIER MEDIATED TRANSPORT
Facilitated Diffusion ... protein mediated passive 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

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 responses

SOME EXAMPLES of CELL SIGNALING SYSTEMS...

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

- direct Cell to Cell contact examples:
  1) gap junctions & plasmodesma
  2) cytoplasmic continuity favors cellular interactions

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

a 2nd example of membrane receptors & responses

2. Tyrosine Kinase receptors...
- receptor proteins that have kinase activity...
  - i.e., they can add a -PO4 group to tyrosine residues of inactive proteins making them active -> cell response
  - includes many growth factors function via tyr-kinases they stimulate cell division & growth

- mechanism of action...
  - binding of growth factor (signal ligand)
  - causes 2 single tyrosine kinases to aggregate
  - the tyrosine dimers, now each phosphorylate the others tyrosine residues via ATP kinase activity
  - the activated-phosphorylated dimer binds relay proteins, activating them...
  - which in turn (by cascade effect) can active up to 10 others, etc...

- net result...
  - 1 signal molecule can trigger many proteins and multiple pathways.
**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 (fodds)

2) GLYCO-YSIS → AcCo [splitting of sugar]

3) oxidation of AcCo → CO₂ + NADH → H₂O

**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

**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: synthesis, 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

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 measure amount energy in 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 \( \longrightarrow \) B Which Way?

\[ \Delta G = \Delta G^\circ + RT \ln \left[ \frac{[p]}{[r]} \right] \]

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 99o)

\[ \Delta G = \text{actual free energy} \]

\[ \Delta G^\circ = \text{standard free energy} \]

\[ R = \text{gas constant} \ (2 \times 10^{-3} \text{ Kc/mol}) \]

\[ T = \text{absolute temp} \ (\text{-273OK}) \]

\[ \ln = \text{natural log (conversion log10 = 2.303)} \]

at equilibrium \( \Delta G = 0 \) and we call \([p]/[r] = K_{eq} \)

solve above equation to see relationship of \( K_{eq} \) to \( \Delta G^\circ \)
**Free Energy Equation**

\[ \Delta G = \Delta G^0 + RT \ln \left( \frac{[P]}{[R]} \right) \]

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

thus rearranging \( \Delta G^0 = -RT \ln \left( \frac{[P]}{[R]} \right) \)

@ equilibrium \( [P] / [R] = \text{Keq} \)

\& @ 25°C... \(-RT \ln \text{Keq} = -(2.0)(298)(2.303)\log_{10} \Delta G^0 = -[1372] \log_{10} \Delta G^0\)

thus...... \( \Delta G^0 = -[1372] \log_{10} \Delta G^0\)

<table>
<thead>
<tr>
<th>Products / Reactants</th>
<th>Keq</th>
<th>log10</th>
<th>( \Delta G^0 ) cal/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>[R] &gt; [P]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1000</td>
<td>0.001</td>
<td>-3</td>
<td>+4116</td>
</tr>
<tr>
<td>1/100</td>
<td>0.01</td>
<td>-2</td>
<td>+2744</td>
</tr>
<tr>
<td>1/10</td>
<td>0.1</td>
<td>-1</td>
<td>+1372</td>
</tr>
</tbody>
</table>

1/1

10/1

100/1

1000/1

([P] > [R])

**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

- \( \text{Glu} + \text{Fruc} \rightarrow \text{Suc} \)
  \( \Delta G = +5.5 \text{ Kc/m} \)
- \( \text{ATP} \rightarrow \text{ADP} + \text{P} \)
  \( \Delta G = +7.3 \text{ Kc/m} \)
- \( \text{Glu} + \text{ATP} \rightarrow \text{G}-1-\text{P} + \text{Fruc} \rightarrow \text{Suc} + \text{P} \)
  \( \Delta G = -1.8 \text{ Kc/m} \)

**COUPLED REACTIONS - linking hydrolysis of ATP**

(a favored rx) to a thermodynamically unfavorable 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) \( \Delta G^0 = -7.3 \text{ Kc/m} \)

**CHEMICAL REACTIONS**

A \( \rightarrow \) B Which way & Why?

**EXERGONIC REACTION** - is one which releases energy

Product (B) \( \ll \) Reactant (A) [stored in covalent bonds]

ex: burning wood (cellulose)

- glucose monomers: potential energy breaks bonds, release heat & light \( \rightarrow \text{CO}_2 \) & \( \text{H}_2\text{O} \)

- cell respiration - cellular burning of glucose

slow, multi-step process to capture released energy.... as ATP

**ENERGONIC REACTION** - requires input of energy A \( \rightarrow \) B

Product (B) \( \gg \) Reactant (A)

ex: photosynthesis (autotrophy)

- glucose made from \( \text{CO}_2 \) & \( \text{H}_2\text{O} \) \( \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 \)
  - energy poor
  - energy rich

**CELL METABOLISM is then a mix of...**

Exergonic & Endergonic reactions that occur in cells

**So how do cells make ATP?**

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

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

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's...energy transferred into new molecule

OXIDATION = removal of electron &/or proton
removes an e from a food molecule
adds an e to an acceptor molecule

REDUCTION = gaining electron &/or proton
becomes oxidizing becomes reducing

A-H + B-O ===> A + B-O-H
redox reducing oxidizing becomes becomes
agent acceptor H acceptor donor

Thus: heterotrophic metabolism is stepwise oxidation of foods
if aerobic - requires oxygen as electron acceptor
if anaerobic - requires no oxygen (other e acceptor)

Metabolic Pathways of Cell Respiration...
Glycolysis
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
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 fermentation (fermentation)
Shuttles
malate shuttle (liver, kidney, heart) = NADH
Glycero-P shuttle (muscle/brain) = FADH2

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
**Summary: cell respiration & heterotrophic metabolism**

1. **Substrates** = sugars, amino acids, acids, fatty acids
2. Glyco-lysis, Krebs Cycle, & ETC are **Universal** to all cells
3. **Products** = \( \text{CO}_2, \text{H}_2\text{O} \), and energy as NADH, \( \text{FADH}_2 \), & ATP
4. Process is **anaerobic** respiration \( (-\text{O}_2 ; \text{GLYCO-LYSIS}) \)
   - alcohol & lactate fermentation (anaerobic)
   - aerobic \( (+\text{O}_2 ; \text{glycolysis} & \text{Krebs Cycle}) \)
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

**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 mitoplas
- involves coenzyme CoASH → acetyl coenzyme A [AcoA]
- rx’s = decarboxylation [\(-\text{CO}_2\)] & redox reaction

**Key Reactions of KREBS CYCLE**
- NAD is reduced
- substrate level phosphorylation occurs [GTP]
- decarboxylation [\(-\text{COOH}\)]
- 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 \( (\text{H}^+) \)
- as e^- flow thru the ETC

**OVERVIEW of Cell Respiration**
- How much ATP is made per Glucose?
- Substrates [carbs, proteins, fats]
- Regulation [phosphofructokinase of glycolysis]
PHOTOSYNTHESIS – Autotrophic Metabolism

**GREEN MEN** - Why do metazoan cells not photosynthesize?
- chlorophyll vs. hemoglobin (leghemoglobins)
- mutants = hemoglobins that capture light energy

<table>
<thead>
<tr>
<th>Plant photosynthetic rates</th>
<th>20 mg hexose/dm²/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average human surface area</td>
<td>170 dm²</td>
</tr>
<tr>
<td>Hexose productivity</td>
<td>60.8 gm/d</td>
</tr>
<tr>
<td>1 mole glucose = 183 gm</td>
<td>686 Kc/mol</td>
</tr>
<tr>
<td>BMR = 2,000 Kc/d</td>
<td>about 8.5 % of need</td>
</tr>
</tbody>
</table>

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 through conversion of light energy into covalent bonds.
  - a. **Chemoletic**...
    - oxidation of small inorganics
  - b. **Phototropic**...
    - use light energy to make organics

2 Fundamental Reaction Mechanisms of Photosynthesis:

**LIGHT Reactions** - photochemical reactions
- molecular excitation of chlorophyll 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
  - regeneration HMP path → PGAL

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
- ALEOPLASTS - contain stored protein (crystals)
- ELAIOLASTS contain oil-fat globules – fat synthesis
- CHROMOPLASTS...found in flower petals, ripe fruit, senescent leaves

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

**6CO₂ + 12 H₂O* --- C₆H₁₂O₆ + 6H₂O + 6O₂**
**CO₂ + 2H₂A ---> CH₂O + H₂O + 2A**

Source C + e donor organic C oxidized donor

Morphological basis of chloroplasts...

**CHLOROPLAST** - ubiquitous to all green plants

SHAPE variable (ellipsoid 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 (prokaryotic ribosomes)
- naked DNA - 2 to 10 fentograms of DNA/chl
- 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...

**ABSORPTION SPECTRA**
- Light absorption by pigments is a graphical plot of amount light absorbed vs. wavelength

**ACTION SPECTRA**
- Engelman's action spectrum is a plot of physiological activity (O₂ released) vs. wavelength

**MOLECULAR EXCITATION of CHLOROPHYLL**

**FATES of Absorbed Energy**
1. Re-radiates as vibrational heat
2. Reradiated as fluorescence (red light)
3. Reradiated as phosphorescence (far red)
4. Induced Resonance - vibrational excitation transfer
5. Photolysis - chlorophyll loses an electron to acceptor = ionized chl^""
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

Nuclear 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

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
- 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

Chromosomes
- bacteria = 3,000 genes
  - 1 chromosome
- human = 25,000 genes (?)
  - 46 chromosomes

Genes occur in chromatin of nucleus, which condense into chromosomess 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, one that switches on/off target proteins by phosphorylating them...
  inactive (ADP > ATP) ➔ active-P

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 (chrn # = 46)
meiosis ➔ haploid gametes (half chrn # = 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

Prophase I ➔ chromosomes condense
Metaphase I ➔ chromosomes align at equator
  homologs PAIR together - synapsis
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

SUMMARY OF MEIOSIS

1. Nuclear division phase of sexual cell reproduction
2. Two successive divisions, results in 4 daughter cells...
   Meiosis I and Meiosis II
3. Reduction/division occurs...
4. cells halve the number of parent cell chromosomes
   diploid ➔ haploid
5. Stages have same nomenclature as Mitosis
   prophase, metaphase, anaphase, telophase, M1 & M2
6. Only one S phase, where DNA is duplicated
   often is no interphase between M1 and M2
7. Homologs separate in Meiosis I
   chromatids separate in Meiosis 2 (mitotic-like)
8. Random Assortment occurs
   homologs align at equitorial plates
   independent of each other
9. 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 discernable as “particles”, ‘factors”
which were:
discrete entities within 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
- 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 x rr
gametes
F1 self pollinate Rr
F2 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>phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>round</td>
</tr>
<tr>
<td>Rr</td>
<td>wrinkled</td>
</tr>
<tr>
<td>rr</td>
<td>wrinkled</td>
</tr>
</tbody>
</table>

Phenotype: round, wrinkled
Starch content: high, medium, low
Starch grains: numerous, median, few
Reducing sugar: low, high, median
UDP-glucose phosphorylase: high, median, low

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

P1: WWGG x wwgg
F1: WwGg (x WwGg self cross)
F2: parental red green, non-parental white yellow

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

NEW COMBINATION NOT SEEN IN PARENTS: TRAITS SORT INDEPENDENT of EACH OTHER genes occur on DIFFERENT CHROMOSOMES

<table>
<thead>
<tr>
<th>genotype</th>
<th>flower</th>
<th>seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>W_ G_</td>
<td>W_ g_</td>
</tr>
<tr>
<td>Ab</td>
<td>W_ G_</td>
<td>W_ g_</td>
</tr>
<tr>
<td>aB</td>
<td>W_ g_</td>
<td>W_ G_</td>
</tr>
<tr>
<td>ab</td>
<td>W_ g_</td>
<td>W_ g_</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 parents: 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

**CHROMOSOMAL 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 vs. X^a Y

ex: red green colorblindness (males = 8% and females 1%) hemophilia, albinism, myopia, Duchenes’ 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 doses of gene activity,
while XY = 1 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.

Linked genes, crossing over & mapping
F1 Rryy round and yellow
Testcross Rryy x rryy
if linked ry if non-linked ry mixed

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>RY</td>
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<td>RY</td>
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<td>Rry</td>
</tr>
<tr>
<td>ry</td>
<td>ryry</td>
<td>ryry</td>
</tr>
</tbody>
</table>

1:1 1:1:1:1

BUT; we do a F1 testcross and actually get :
round & yellow 41.5% R_Y_ parental
wrinkled & green 41.5% rryy parental
round & green 8.5% R_y_ non-parental
wrinkled & yellow 8.5% rryy non-parental

How ???
CROSSING OVER

A B C
a b c
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

R Y
17cm

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...

<table>
<thead>
<tr>
<th>Genetic Interaction</th>
<th>Phenotype</th>
<th>Genotype</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple flower color &amp; protein compliment</td>
<td>F1: P_Pcc</td>
<td>F2: 9 P_C_ purple, 3 ppC_ white, 3 ccP_ white, 1 ccpp white</td>
<td>9:3:3:1</td>
</tr>
</tbody>
</table>

Epistasis - works in similar fashion (c = melanin gene)
but, a gene at one locus alters the phenotype of other gene at a second locus = altering dihybrid ratio

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 C_B_</td>
<td>3 C_bb</td>
<td>black</td>
</tr>
<tr>
<td>3 C_B_</td>
<td>1 C_bb</td>
<td>brown</td>
</tr>
<tr>
<td>3 ccP_</td>
<td>1 ccpp</td>
<td>white</td>
</tr>
</tbody>
</table>

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 more Human Genetics...

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

PHENYLKETONURIA [PKU] pp 1/18,000
disfunctional phenylalanine hydroxylase
PHE → PHE-phenylpyruvate → 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.

Some Human Genetic Traits...

Pedigree Analysis symbols

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Blood Groups - 4 phenotypes O, A, B, and AB
due to carbohydrates on RBC surface

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ii</td>
<td>O, A, AB</td>
</tr>
<tr>
<td>AA</td>
<td>A, B, AB</td>
</tr>
<tr>
<td>BB</td>
<td>B, A, AB</td>
</tr>
<tr>
<td>AB</td>
<td>A, B, AB</td>
</tr>
</tbody>
</table>

Polygenic Inheritance - ex: height, weight, etc...
multiple genes → 1 phenotype
usually quantitative traits w 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^YX^Y X^OX^O X^YX^Y
X^OX^Y can we have a colorblind female...some crosses

Chromosomal aberrations due to alterations
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 XXX X Y
XO YO

Turner Syndrome dies 1 / 5,000
female appearance sterile
1 / 2,000 symptoms sterile
turner syndrome male

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, & snRNPs

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

The next 2 pages may be omitted—
list for class announcements

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
(q) = q
then by definition
p + q = 1

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

In population of Canettes...
Orange is dominant (GG) to Green (gg)
of 1000 Canettes
40 are Green (gg) & 960 are Orange (GG or Gg)
q^2 = freq homozygous recessive (green gg) = 0.04
40 / 1000 = [0.04] x 1000 = 40
q = freq of recessive allele
0.04 = 0.20
p = freq dominant allele [G] = 1 - q = 1 - 0.2 = 0.80
2pq = freq of heterozygote
= 2 (0.2) (0.8) = 0.32 x 1000 = 320
q^2 = freq of homozygous dominant
(0.8)^2 = 0.64 x 1000 = 640

Cystic fibrosis example

\[
\begin{align*}
\text{GG} & \quad 96 \\
\text{Gg} & \quad 320 \\
\text{gg} & \quad 40
\end{align*}
\]
MOLECULAR GENETICS

Gene --> 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 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.

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 - 15N & 14N
Enzyme that makes DNA is DNA polymerase
req: deoxy-TP'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 prismsome) - 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

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 [phase 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 (polymers 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

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
triptet 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 (snRNPs)** - 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.
  
  ![siRNA](siRNA.png)

**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 [ribosomes, mRNA, aa-tRNA] - **INITIATION**
3. adding new aa’s - **elongation**
4. stopping the process - **termination**

**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
  
  ![Genetic Code](Genetic_Code.png)

**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] = [CTA]
  
  - substitutions:
    
    - non-sense = change to no amino acid (STOP)
    
    - UCA --> UAA = ser to stop
    
    - 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 F) primitive sex-like reproduction
  
  5. **Viral Transduction** - via a viral vector

- general transduction - pieces of bacterial DNA are packaged with viral DNA during viral replication restricted transduction - a temperate phage goes lytic carrying adjacent bacterial DNA into virus particle

- **DESIGNER GENES**

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*Note: *snRNPs* = small nuclear ribonucleoproteins*
**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 - diploidic cuts at unique DNA sequences, including palindromes

<table>
<thead>
<tr>
<th>5'</th>
<th>3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAATTC</td>
<td>CTAAAG</td>
</tr>
<tr>
<td>CTACAA</td>
<td>GATTTA</td>
</tr>
</tbody>
</table>

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 fluorescently 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
   - disease/infection diagnosis:
     - PCR & labeled probes from pathogens help identify microbe types: [RT-PCR] - HIV RNA - RT - cDNA - PCR - probe can ID AIDS infection
   - RFLP - Restriction Length Fragment Analysis - markers often inherited with disease
     - what is RFLP* genetic testing & polymorphism → RFLP markers & disease* & MST II cuts Sickle Cell*
   - Paternity testing via DNA fingerprinting
   - 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 human individ. 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 (fetal-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 = 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 protein, i.e., protein
- increase in enzyme activity = gene action?
  - but, pre-existing inactive enzymes $\rightarrow$ active forms
  - ZYMGENS - peptidogenogen $\rightarrow$ peptisin
  - tyrospigenogen $\rightarrow$ 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 $\rightarrow$ lactose
- alpha -beta-galactosidase $\rightarrow$ beta-galactosidase
- lactose + galactose

Operon = series of mapable-linked genes controlling synthesis of protein
- promoter - binds RNA polymerase
- Operator - makes repressor protein
- structural - make enzyme proteins

![Gene Regulation Diagram](image)

**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 - $\frac{1}{3}$ life mRNA*
    & 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 Diagram](image)

**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-oncopogene gene*) = 30% human cancers
  - is a G-protein that promotes cell division proteins
  - a Ras mutation $\rightarrow$ hyperactive Ras protein $\rightarrow$ 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 $\rightarrow$ active p53 $\rightarrow$ p51 gene $\rightarrow$ protein binds to cyclin dependent kinase stops cell division] thus a p53 mutation $\rightarrow$ 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,
& snRNPs

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

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.....
a. digestive
b. respiratory
c. cardiovascular
d. lymphatic & immune
e. excretory
f. endocrine
g. reproductive
h. nervous
i. muscular
j. skeletal &
k. integumentary

4 Fundamental tissue of verts -
epithelial connective muscle nerve
ectoderm mesoderm endoderm
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/histocytes - 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
...bonds & packs - holding organs & tissue in place

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

**BASEL 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"** - (claus bernard - Fr. 1880's)

- the interstitial fluids filling spaces between cells
- the milieu exchanges nutrients w blood

**Constancy of human milieu**

- body temp: 39° C ± 1° C
- pH: 7.4 ± 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 & Hypothalmus 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**

- CO₂ + H₂O → H₂CO₃
- H₂CO₃ → H⁺ + H₂CO₃

- Hb pick up H⁺ ions buffering blood

- if pH blood drops ([H⁺]) then H₂CO₃ → H₂CO₂

3. Calcium homeostasis (in blood - range is 9 to 11 mg%)

   - Ca²⁺ 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 → parathroid 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

- SW fish - internal solutes less... thus constantly loses water

**Other marine vertebrates** - birds & sharks
VERTEBRATE DEVELOPMENT PATTERNS

Embryology - study of development of the embryo

5 major stages

1. **Gametogenesis** - gamete production - (meiosis)
   - **Spermatogenesis** - in seminiferous 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, mid, & flagella
     - must penetrate... 1) egg’s jelly coat, 2) vitellin layer... (glycoproteins), 3) membrane
   - acrosome reaction...
   - monospermy = plasma membrane / vitellin layer
   - permeable? hardens forming fertilization membrane

3. **Cleavage** - 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 blastocoele
   - **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
   - **Ectoderm** - outer epidermal layers of organs, skin
   - **Endoderm** - digestive tract tissue
   - **Mesoderm** - fills in space in between: muscle
   - gastrointestinal obliterations the blastocoele - 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....

**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)
- Electrical properties 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
- autonomic nervous system - signals regulate internally -
- under involuntary control

FUNCTIONAL TYPES -
- Sensory neurons... (afferent 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
- **Nodes of Ranvier**
  - space between successive Schwann cells - opens nodes
  - speed of conduction = w/myelin (100 m/sec or 200 ml/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

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
- Frog muscle fibers - 90 mV
- Nitella - 150 mV
- Valonia - + 15 mV

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

\[ E_{rest} = \pm 62 \log_{10} \left( \frac{[C]\text{[Na]}-[K]}{[C]\text{[Cl]-}} \right) \]
**CONDUCTION** of an AP along an axon...

- Local spreading of electric charge = change in membrane permeability of adjacent region leads to an autocatalytic reaction - "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 

- removal of stimulus – acetylcholine esterase (AChase [enz]) destroys transmitter

**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

**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
**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
   - osmo-receptors: of hypothalamus which monitors blood osmotic pressure
   - photo-receptors: light eye, eyespots, infrared receptors of snakes, etc...
   - thermo-receptors: radiant energy
   - electro-receptors: detect currents...
   - mechano-receptors: mechanical forces
     - hair cell - deflection = depolarization
     - stretch receptors of muscles
     - equilibrium receptor of inner ear
     - 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.

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**MUSCLE PHYSIOLOGY**
model: skeletal neuromuscular junction (see web fig)
an innervated muscle fiber
muscles can only contract (pull)
4 parts of a Muscle twitch
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

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2 TYPES of MUSCLE FIBERS
determined both genetically and functionally based on how fast they can produce a contractile twitch
Every muscle composed of varying % composition of two types

**TYPE I SLOW TWITCH**
Tonic muscle (red)
Leg muscles
slower contraction times (110 msec)
continuous use muscles
for endurance performance (marathoners)
good for long slow-sustained contractions
and prolonged performance
not easily fatigued
contain myoglobin (red)
more capillary beds larger max VO2
smaller in size
lower glycogen content
poor anaerobic glycosis
predominant aerobic enzymes
& aerobic metabolism
higher fat content
more mitochondria-
 Beta Oxidation high poorly formed sarcoplasmic reticulum
slower release of Ca = slow contractions
tropin has lower affinity for Ca

**TYPE IIa/IIX FAST TWITCH**
Tetanic muscles (white)
Pectoral muscles
faster contraction times (50 msec)
one time use muscles
for power & speed (sprinters)
good in rapid contraction short time
and brief performance
easily fatigued
no myoglobin (white)
less capillary beds
larger in size
higher glycogen content
predominant anaerobic glycosis
easily converts glycogen to lact w/o O2
some aerobic capacity
lower fat content
fewer mio... Beta Oxidation low well formed sarcoplasmic reticulum
quick release of Ca = rapid contractions
tropin - higher affinity for Ca
**Vertebrate Skeletal Muscle** - structure
sarcomere - repeat unit of striated muscle,
delimited by Z lines
- "clear zone" around Z-line (isotropic)
- "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 & affinity to bind actin
- G-actin - globular protein which polymerizes into
  thin filament, contains a myosin binding site
- tropomyosin - fiber-like protein which helically wraps
  around actin thin filament
- troponin - globular protein which binds Ca+2

**Muscle Contraction Cycle & Role of Ca** - review

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**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.

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**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 viral (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 that blocks muscle growth, promotes atrophy and slow muscle cell growth, and 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 - a human case study - 2004.

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 epithelium 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 epithelium, 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 eyeglasses:
- 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 to 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:

```
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
```

Images: [Human eye diagram] [Sensory physiology diagrams]