Biology 255
CELL & MOLECULAR BIOLOGY

Dr. Luis Glaser and Dr. Charles Mallery

Fall 2009 - Section S: TR 3:30pm to 4:45pm in Cox 145
Spring 2010 - Section Q: TR 12:30pm to 1:45pm in Cox 126

Lecture Outlines and Handouts
http://henge.bio.miami.edu/mallery/255/
Some quotable quotes about Cell and Molecular Biology:

“The aim of modern (CMB) BIOLOGY is to interpret the properties of the living organism within the structure of its molecules”... paraphrased from Francois Jacob - in The logic of Life, 1973.

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


"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 ."


"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."


" CMB is the practice of biochemistry without a license."

Erwin Chargaff 1989.
Cell and Molecular Biology

is the study of life & the living cell through the analysis of the constituent molecules found within cells.

"Living organisms are composed of inanimate molecules... and nothing is alive in a cell except the whole of it." Matrin Olomucki (1993) *The Chemistry of Life*. NY, McGraw Hill

CMB is designed to construe the properties of the organism by understanding the structure of its constituent molecules.

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Cell & molecular biology...

is a vibrant & exciting discipline.

Today, CMB forms a bridge between such basic disciplines as:

- biochemistry
- developmental biology
- physiology
- neurobiology
- molecular genetics
- immunobiology
- microbiology.

Cell & molecular biology provides a natural connection between basic biological research and medicine.

More complete description:

The goal of cell biology is to understand the molecular basis of cell function and the fundamental cellular processes ranging from cell division and protein trafficking to signal transduction and cell migration, and to the formation of tissues during development and wound healing. The experimental approaches used in studying cell regulation and function are multidisciplinary and include: biochemical and biophysical approaches and molecular and genetic manipulation of function at both the cellular and organismal levels.

CMB is the ultimate reductionist philosophy... the methodological approach of 20th century

Reductionism is a fundamental research protocol of CMB

i.e., "knowing the parts may explain the function of the whole"
Procedures: web lecture notes and outlines

Class material presented in a Web based format is designed to allow individuals in the class to meet their own unique learning requirements. The web pages give "baseline-needed material" which we all must learn, including "starred links", that are designed to enhance directed learning, and explain in greater detail a concept presented on the base page.

If a web link is starred*, then you are responsible for content at that link; if a figure is listed [fig 7.1] or figure* then you are responsible for it.

Additionally, there is immersion-learning links that are not starred, about cell-molecular biology topics, which allow a learner (you) to delve into an area of self-interest, build your knowledge base, and increase your biology productivity.

If a web-link is NOT starred, you're NOT responsible for its content.

Fundamental Questions Asked via CMB

what does it mean to be living?
what is definition of Life?

Life is manifest in the cell... So
what are the origins of life?
what causes the great diversity of life?
what are the properties of cells?
how does life work - why do we get sick, grow old, die?
how does an organism develop from single fertilized egg?

Course Goals -

1. examine the details of the cell, stressing the fundamental & relationships between structure & function
2. generate an appreciation for how the properties of molecules lead to the living condition

"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."


Instructors

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Ashe Bldg. 200 - Associate Dean, College of Arts and Sciences.

Office Hours - please call their secretary’s for an appointment:
Mallery 284-3188 or Glaser 284-2056 or 4015


Prerequisites - Biology 150 and 160; a background in genetics (Bil 250) & organic (CHM 201) is recommended.

Lecture - fall
- 3:30pm to 4:45pm - Tues & Thurs - in Cox 145
- spring Q
- 12:30pm to 1:45pm - Tues & Thurs - in Cox 126.
The goal of cell biology is to understand the molecular basis of cell function and the fundamental cellular processes ranging from cell division and protein trafficking to signal transduction and cell migration, and to the formation of tissues during development and wound healing. The experimental approaches used in studying cell regulation and function are multidisciplinary and include: biochemical and biophysical approaches and molecular and genetic manipulation of function at both the cellular and organismal levels.

CMB is the ultimate reductionist philosophy...
the methodological approach of 20th century

Reductionism is the fundamental research protocol of CMB
i.e., "knowing the parts may explain the function of the whole"

The aim of Modern CMB is to interpret the properties of life and organisms through the structure of their constituent cellular molecules.

1980's and 1990's are the dawn of the modern Cell & Molecular Biology Age and is the content of Biology 255

my mother (1906): auto, airplane, radio, T.V... man on the moon
me (1943): heart transplants (who was 1st?), antibiotics,
DNA & transgenic animals,
artificial genes & manipulation, cloning, human genome....

CMB is part of our modern culture - Movies: Species, Jurassic Park

Nobel Prizes in Physiology & Medicine and Chemistry

to repeat.....
Cell Theory Origins

CMB is rooted in the 2 major theories of Biology

1. Evolution - Darwinian Natural Selection
changes in the allele frequency of a population's gene pool from one generation to another generation... as influenced by a habitat, which enhances population's reproductive fitness, & leading to progressively better adaptation via Natural Selection*
The principles of morphological change and natural selection, applied repeatedly over millions of cell generations, are basis of evolution Voyage of Beagle* Snoppy Darwin's books & publications

2. Cell Theory...
"All living things are made of cells"...
Some cell links
Cell Theory Origins
Schleiden (pic) & Schwann

Consequences of Cell Theory

VITALISM vs. MECHANICALISM
Vital Force vs. no vital force

Top 10 Properties of Cells*

VITALISM was school of scientific thought, that attempts to explain the nature of life as resulting from a vital force, "a soul", peculiar to living organisms and different from all other physical forces found outside living things.

MECHANICALISM

Mechanists believed that life is essentially a mechanical process, it can be explained entirely by the workings of laws of physics and chemistry without a 'vital force'.

"There are no Laws of Chemistry or Physics unique to the living condition."
The cell is the fundamental unit of all life, and though man and mouse have very different anatomical structure, their cells & organelles are the same, thus by studying cells in one organism has direct application to other organisms.

Cell Types... (refer to chapter 1) All Living Organisms are grouped into...

EUBACTERIA - true bacteria
ARCHAEA - ancient proaryotes [Collage]
EUCARYA - modern eucaryotes

Carl Woese... (interview) compared the nucleotide sequences of small-unit rRNA from many species... rRNA is found in all cells and therefore, if all cells are derived from a common progenitor, their sequence changes over time can indicate divergence (loss of relatedness) through phylogeny. The RNA phylogeny tree produced, by comparing similar & divergent sequences, a tree with 3 distinct branches (Domains) (fig 1.29*)

there are only 2 successful Plans of Cellular Organization distinguished primarily by size & type of internal structure (organelles)

PROKARYOTE - "before nucleus"
today proaryotes includes blue green algae & bacteria... lack membrane bound organelles genes "naked DNA" - no "chromosomes?" little to no internal compartmentation figure* + panel1.2 + E.coli*

size range - 0.1 to 10 µm diameter
3 primary forms of shape of proaryotic cells (fig 1.10*) (cocci, bacilli, spirochetes)
**EUKARYOTIC** [eu -true karyon -nucleus...]
cell plan of multi-cellular organisms

**eu:** include the fungi, algae, protzoa, slime molds, & all plants & animals.

### 7 CHARACTERISTIC of EUCARYOTES:  
**panel1-2:** animal & plant cells

- **nucleus** - single greatest step in evolution of higher animals
  - genes in *chromosomes* [colored bodies... made of DNA + protein]
  - contains more DNA (1,000x more) than procaryotes
- **presence of organelles** - significant internal compartmentalization of function
  - organelle - a subcell part that has a distinct metabolic function
- **presence of flexible cell walls** (allows phagocytosis)
  - *cytoskeleton* (provides framework to be larger)
- **usually larger - cell volume 10X > than bacteria - size 5.0 to 20 µm diameter**
- **extensive internal membranes**
- **reproduce sexually**

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**Universal Characteristics of Cells (all Life)**

1. **all cells store their hereditary information in DNA**
2. **all cells replicate their hereditary info via templated polymerization**
3. **all cells transcribe hereditary info into intermediate RNA via templated transcription**
4. **all cells translate RNA in same mechanistic way via codon:anticodon**
   - “Chargaff” pairing: **A : T and G : C**

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**What cells types will we be looking at?**  
* [see pg 25 - 28]*

- **Model Organisms** in Cell & Molecular research include:
  - **Bacteriophages** - virus infects bacteria; today used as cloning vectors
  - **Escherichia coli** - bacteria common to human colon; work horse
  - **Giardia** - primitive eukaryotic cell, anaerobic protozoan cell with 2 nuclei
  - other eucaryotic models -
    - **Saccharomyces cerevisiae** - yeast
    - **Arabidopsis thaliana** - plants - mustard plants
    - **C. elegans** - nematode
    - **Drosophila melanogaster** - fruit fly
    - **Mus musculus** - common house mouse & its genes

- **Single cell culture models**
  - for genetic & embryonic development model systems...
    - **Hela cells** (pic)
    - **embryonic stem cells** (Stem Cell Journal)

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**Origins of Cells & Life**

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**Some Milestones in CMB**

- **single cell culture models**
  - for genetic & embryonic development model systems...
    - **Hela cells** (pic)
    - **embryonic stem cells** (Stem Cell Journal)
Have you ever seen an individual living cell?

Hela cells: ATCC CCL 2 - FROZEN AMPUOLES @ -321 0F
American Tissue Culture Collection - Rockville, MD

no signs of life, not even simple chemical metabolism,
if warmed to room temp - "resurrection" seem to come back to life"
they move about, feed and metabolize, maybe reproduce

Human Life... is sum of lives of many individual cells.
for centuries, life was defined in the unit of the whole organism...
a cat, a bird, a human being...
Now life is defined in terms of the individual CELL.

The Attributes of Life & the Living State:
So what exactly defines Life: life exhibits certain "QUALITIES" ...

1) Autonomous Replication: Self-Replication Mitosis & Meiosis
   two whole copies of genome (maternal & paternal copies :
   (backup-redundancy - based in the semi-conservative
   replication of DNA (complementary templating)
   most defining trait of the living state...

2) Life had an Origin
   - Life begets Life... "all cells are derived from preexisting cells"
   Rudolph Virchow states this cell theory paradigm in 1858.
   eliminates Spontaneous Generation (Redi & Pasteur exp.

All living things have evolved from a common ancestor,
through processes that include natural selection and genetic drift acting
on heritable genetic variation.

LUCA - Last Universal Common Ancestor -

All cells are derived from a single PRIMORDIAL cell

This hypothesis is based upon the circumstantial evidence such as,
the commonality that occurs in all current living organisms (life forms)...

1. all living things are composed of very similar organic molecules:
   the same proteins, lipids, carbohydrates, etc...

2. all proteins (biological catalysts responsible for life's chemical rxns),
   are made from one set of 20 standard amino acids...

3. all contemporary organisms carry their genetic information in nucleic
   acids [DNA/RNA] and use the same genetic CODE.
3) Life exhibits EMERGENT PROPERTIES...
   a large scale, group behavior in a system, which doesn't seem to have any clear explanation in terms of the system's constituent parts.
   - oxygen - colorless, odorless, tasteless, reactive GAS that supports combustion
   - hydrogen - colorless, odorless, tasteless, reactive GAS that is flammable
   - water (H₂O) - non-flammable chemically reactive polar LIQUID (exist in 3 phases)
Emergent properties are unexpected, nontrivial results of relatively simple interactions by relatively simple components. Emergent properties seem to be a consequence of complexity from which unpredicted behaviors and patterns emerge.

From mix of biomolecules emerges a complexity that exhibits properties we call Life

4) Life requires a Critical Level of COMPLEXITY...
   Structural complexity and information content are built up according to current paradigm by combining simpler subunits into multiple complex combinations.

   A single cell has no concept of the whole. A cell runs by the chemical rules built into its molecules. A single cell can't do much without interaction with other cells, but in combination cells can produce complex results such as consciousness.

5) Life exhibits biochemical autonomy, i.e., it carries on metabolism
   biochemical activities in cells make energy (ATP) & molecules to sustain cells
   cell energetics occurs via...
   1. cellular redox reactions,
   2. capture of light energy in photosynthesis,
   3. electron flow through carrier proteins,
   4. H+ ion pumps.

Living systems are far from equilibrium:
   they utilize energy, largely derived from photosynthesis, which is stored in high-energy bonds or ionic concentration gradients & release this energy by coupling it to thermodynamically unfavorable reactions to drive biological rxn:

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

   there are no unique Laws of chemistry or physics just for the Living State.

6) Life is manifest in a CELL - the fundamental unit of living systems.
   Three fundamental cell types have evolved: bacteria, archa, & eukaryotes.

   Information encoded in DNA is organized into genes, and these heritable units use RNA as info intermediates to encode proteins, which become functional on folding into distinctive 3-D shapes.

   In some situations RNA itself has catalytic activity.

   Unlike atoms and simple molecules studied in chemistry and physics, no two cells are identical.

   SO WHERE DID the FIRST CELLS COME FROM ?

Thus Some Basic Properties of Life & thus Cells... or How Cells Work

1. cells have an evolutionary origin - all cells are derived from other cells... originally from a single primordial cell [LUCA some 3 bya] via Chem.Evolution?
2. cells: highly complex mix of biomolecules (C, O, H, N) --- structural complexity show structural complexity - review [ figure 9.5a pg 376 (mcb)]
3. cells come in 2 fundamental types - prokaryote & eukaryote
   (read pgs 1-4: mcb6e)
   - cells obey laws of chemistry & physics (the laws of Universe) cells build and degrade numerous molecules, generally via use of ATP
   - fig 1.14* cells extract energy from environment & maintain stasis far from equilibrium cells acquire and utilize energy -via metabolic pathways:
     - Glycolysis, Krebs, ETC
     - series of ordered reactions that are self-adjusted cells divide, grow, & differentiate leading to cell Form & Function
     - cells osmoregulate - control what gets in/out of membranes (organelle or plasma)
4. Cells are motile... cells are involved in numerous mechanical activities
   assembly, disassembly, movement of organelles, motor proteins –
   all via the cytoskeleton fig 1.15 + webinar
   vesicle & organelle walking*view@home

5. cells respond to stimuli - via external surface or internal cell receptors
   - fig 1.16*

6. cells grow and divide...

7. cells use nucleic acids for genetic information

8. cells are capable of self-replication:
   - Mitosis & Meiosis - fig 1.17*

9. cells regulate their gene expression (RNA and protein synthesis)

10. cells die - absence of life may be a most defining characteristic of
    living? Ex: apoptosis - programmed cell death due to
     absence of certain growth signals - fig 1.19*
     via cysteine-aspartic acid proteases (caspases)
What is the Origin of Life... a paradigm question for CMB...
or what is the origins of the Primordial Cell...
Was it a chemical evolution or an astrobiological event?

**CURRENT PARADIGM**
most experimental evidence favors a chemical evolutionary origin of life...
"Simple chemical self-assembly has lead to complex self-replicating systems"

Earth forms 4.5 billion years ago,
between 4.5 to 4.0 bya - asteroids bombard & sterilize planet's surface
then by 4.0 bya - first fossil evidence of microscopic life
Initial chemical event may have been evolution of CARBON BASED MOLECULES
Ancient atmosphere (was reducing) with single carbon gases... CO, CO₂, & CH₄

Is origin of Research Life Experimentally Testable?

4 experimental approaches used in today's Origin of Life Research

1st approach: Search for bioorganic precursor molecules of life...

A) formed from a chemically reactive soup... in early oceans of Earth
   > H₂O, NH₃, CH₄, & H₂ make HCN & formaldehyde: then amino acids, nucleotides, & sugars
   link to timeline of experimental organic syntheses & origins of life*

B) 1979 - Deep dwelling (ocean) hydrothermal vents... (deep sea volcanic plumes)
   > vents are full of organically rich molecules --> life... [tube worms & bacteria]
Speculation: life may have originated in vents regions...

C) 1990's - astrobiological origins for biomolecules... Great 20th Century Discoveries
   > Space Debris... space dust, meteorites, asteroids
   may have deposited organics on newly formed planet Earth.
   > Comets are mostly ice crystals on cores of silicates & carbon
   contain about 10% CO, CO₂, CH₃, CH₂OH, and NH₃
   > Asteroids contain molecules as... kerogen [a PAH], nucleobases, quinones,
   COOH's, amines & amides = some 70 amino acids, with 8 of common 20.

D) 2007 > repeat of Miller-Urey famous experiments

2nd experimental approach: MODEL MOLECULAR REPLICATIVE SYSTEMS

Evolution of an RNA world... (which came 1st DNA or RNA)

In 1989 Sidney Altman and Thomas Cech showed that RNA molecules RIBOZYMES had CATALYTIC ACTIVITY
i.e., these RNA's catalyze hydrolysis & condensation rxns of phosphodiester bonds.

If RNA's can be a template and also catalyze polymerization of like molecules, i.e., replicate itself,
then RNA molecules may have been the 1st SELF-REPLICATING living entity.

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

SELF-REPLICATING MOLECULES...
are an experimental bridges between molecules & living organisms...
the origin of stable self-replicating molecules represents a fundamental obstacle to our understanding of the events in the origin of life.

Ribozymes/an RNA World... Gerald Joyce, et al (@ Scripps, LaJolla)
Joyce & Wright used a test tube of ribozymes that can reproduce indefinitely, some with mutations, which improved rate of replication... Scripps Report
and studied ribozyme molecular selection...
"...with a starting ribozyme molecule, with barely detectable DNA-cleavage activity,
after 63 "generations" of in vitro selection for catalysis, showed a number
variants of ribozymes, that cleave single-stranded DNA with high efficiency and specificity.
These ribozymes had accumulated an average of 27 mutations relative to the wild type ribozymes and had improved their ability to cleave DNA
by 10^6-fold"...

In Jan 2009 Tracey Lincoln & Gerald Joyce demonstrated a pair of RNA ribozymes each of which could make copies of the other by joining together two shorter RNA strands. In 30 hours, they found a population of RNA molecules could grow 100 million times bigger. Unfortunately, success in their experiments required the presence of preexisting RNA pieces that were far too long and complex to have accumulated spontaneously. Still, the results suggest that RNA has the raw catalytic power to catalyze its own replication... Lincoln and Joyce kept their RNA molecules in beakers... to demo the evolution of artificial replicating RNA molecules they should be packed into cells...
Jack Szostak (Mass. General Lab) - REPLICASE...
Molecular Replication Systems...
Protocells
He investigates how fatty acids (lipids) might have trapped RNA producing...
“evolving” new ribozymes.
Szostak started with trillions of random RNA sequences, selected ones that had catalytic properties, & made copies of those. At each round of copying some of the new RNA strands underwent mutations that turned them into more efficient catalysts. He was able to produce ribozymes that can catalyze the copying of relatively short strands of other RNAs. He then vesicelized his replicate RNA molecular complex that had the ability to make a copy of itself and direct other RNA molecules to replicate themselves...
His "vesicles" can add new fatty acids & grow and leaky enough to bring in new nucleotides, making a first "protocell"
BUT - No self-replicating artificial RNA molecules exists naturally today,
However, lab experimentation may be able to establish that it was feasible, and that RNA molecules can be selected for via Darwinian evolutionary mechanism (natural selection).

Could basic physiochemical properties acting in elementary artificial protocells have given rise to essential cellular behaviors?
Evidence to date includes:
- fatty acids have been found in meteorites & have been made under a variety or prebiotic conditions & self assemble.
- artificial protocells may be made in the lab by encapsulating a self-replicating genome inside a chemically simple self-replicating membrane vesicle.
- artificial vesicles encapsulating active genome replicators do generate an osmotic pressure, which causes a vesicle to "steal" membrane fragments from other vesicles with less active genome pieces. (figure*)
- as fatty acid vesicles grow larger micelles, a transmembrane pH gradient can be generated, due to faster flip-flop of protonated FA's on outer leaflet.
- Acidification of a vesicle's interior stores energy in form of a pH gradient, a primary metabolic system of living cells.

3rd approach:
PROTOBIONTS... chemically made artificial cells
Sidney W. Fox University of Miami (1912 - 1998)
Director of the NASA supported Institute for Molecular Evolution at UM.
His laboratory conducted analyses of the first moon rock samples...
- produced proteinoids from amino acids... dropped on hot lava rock, sand or clay.
- definition of Protobiont - an aggregate of abiotic made, chemically reactive molecules
- internally... chemically different from their environment, & are metabolically active.
Some Examples of Protobionts
coacervates made of polypeptides, polysaccharides & nucleic acids and lipids
- form liposomes that are enzymatically active
Proteinoid microspheres - are selectively permeable & have membrane potentials
liposomes made from Lipids - are microscopic spherical vesicles that form when phospholipids are hydrated; can engulf smaller proteinoids making more active ones
It's a big jump form protobionts to what a eukaryote of today is ???

4th experimental approach
Synthetic Biology & Protocell Research...
a. bottom-up approach: one can't truly understand what one can't build
goals: to assemble all the components to synthetically form life to understand why & how matter can self-organize... and become living an artificial man-made cell?????
Synthetic Biology is constructing fully functional cells from scratch... the engineering of new genetic circuits, entire genomes, or organisms to make complex biological machines taking genetic elements to the level of engineering a cell and altering gene content & arrangements to make novel designer genes
i.e., artificial creation of DNA molecules, genes, viri, & cells that mimic, or surpass, natural systems.
Some examples of what has been done in Synthetic Biology so far:

1. Synthetic Polio Virus: July 2002: Molecular Origin of Life Research
   E. Wimmer from the University of New York at Stony Brook used the poliovirus' widely known genetic sequence to synthesize the virus from shelf chemicals. They followed a recipe they downloaded from the internet and used gene sequences from a mail-order supplier. The artificially constructed virus appears identical to its natural counterpart; when injected into mice the animals were paralyzed and died.

2. Phi X-174 virus synthesized - November 2003:
   Craig Venter and colleagues created an artificial version of Phi X-174 by piecing together synthetic DNA ordered from a biotechnology company. They used a technique called polymerase cycle assembly (PCA) to link the strands of DNA together.

3. The 1918 Spanish Flu Virus is Reconstructed - October 2005:
   Jeffery K. Taubenberger, a molecular pathologist at the Armed Forces Institute of Pathology and his colleagues were able to piece together the virus's genes from two unusual sources:
   1) lung tissue removed at autopsy from a 21-year-old soldier and
   2) the frozen body of an Inuit woman who died of influenza in November 1918 and was buried in the Alaskan permafrost.

   These sources provided intact pieces of viral RNA that could be analysed and sequenced. The virus's has eight "RNA gene segments" and by gene sequencing and PCR they reassembled the virus. Two of the 8 genes: Hemagglutinin-A type [H5] and Neuraminidase type 1 [N1] are protein surface coatings.

   There are at least 16 different HA antigens, which binds the virus to the host cell. Neuraminidase is an antigenic glycoprotein enzyme found on the surface of the flu virus. Nine neuraminidase subtypes are known, which aid in the efficiency of virus release from infected cell.

b. synthetic biology top-down-up approach:
   looking for a minimalist essential genome required to make a cell...

   J. Craig Venter, a principle investigator (P.I.) of the Human Genome Project is attempting to make a synthetic new type of bacterium using DNA manufactured in the lab; using the sequenced genes of a bacterium Mycoplasma genitalium, a gram-positive parasitic bacterium, whose primary infection site may be the human urogenital tract that causes non-gonococcal urethritis. Its circular chromosome has 580,073 base pairs, the smallest known genome of any free-living organism determined. M.g. has a total of only 25 genes (482 encoding for proteins; & 43 RNA genes).

   > Venter's researchers began systematically removing genes [so called knock-out cells] to determine how many genes are essential for life... In 1999, they published the narrowed the needs of M. genitalium to between 265 & 350 genes.

   > How many genes does it take to make an organism? What is the minimum genes a cell needs? The scientists at The Institute for the Human Genome Research (1b) who determined the Mycoplasma genitalium sequence followed this work by systematically destroying its genes (by mutating them with insertions) to see which ones are essential to life and which are dispensable. Of the 480 protein-encoding genes, they conclude that only 265-350 of them are essential to life.

   > next step: to artificially assemble these 300+ genes & create SYNTHETIC CELL
Microscopy and Methods, Protocols & Instrumentation
for observing cells
in Cell & Molecular Biology

Methodologies, Techniques, & Procedures...
for observing Cell Structure in CMB
web links : use class web pages to hyperlink:
History of Microscopy, Optical Microscopy, Microscopy on Web,
Nobel links to microscopy, Wikipedia, Virtual Library-microscopy,
Zeiss, Inc, History of EM (CZ), the Transmission-EM, EM-stock pics,
EM-Wikipedia, TEM-Wikipedia.
Cell Biology Dictionaries
A Table of Glossaries
Glossary of Techniques
National Human Genome, Glossary
General Procedures & Protocols - Cell Bio
General Procedures & Protocols - Molecular Biology
Mallery’s CMB Resources
mcb(5/e) pages 184-193 & 165-173

Early Methodology in CMB - 1910 to 2010
Equipment advances of last 50 years are the epitome of modern scientific age
MICROSCOPY is the technical field using microscopes to view cellular objects:
development of microscopy revolutionized biology & remains essential tool of CMB
2 major classes of microscopy: Light (optical) microscopy and electron microscopy

Light Microscopy: - produces magnified images of small objects with compound lens
objective lens - next to object (100x) and ocular lens (10x) = 1,000x magnification
(technically complicated) - mcb fig 9.10*
types of light microscopy* (technically complicated & mcb fig 9.10*)
1876 Abbé optimizes microscope designs (lens & condensers)
1886 Zeiss - lens RESOLUTION near limits of light (0.2 um** = 200 nm)

Specimen preparation:
1900's - killing, fixing, embedding & sectioning: microtome* (1 to 10 um thin tissue sections)
selective staining: stains attach to specific molecules (picture*)

Tracing with molecular precursors & light microscopy:
autoradiography - 1924 Lacassagne - produces an autoradiograph, which is an light microscope
image on photographic film or emulsion produced by the pattern of radioactive decay
emissions (e.g., beta particles or gamma rays) from a distribution of a radioactive substance.
methods & preparation*, images*, tracking*
fluorescence microscopy* - 1941 Coons - form of light microscopy where component of interest
in a specimen has been specifically labeled with a fluorescent molecule as GFP (Green
Fluorescent Protein or fluorescein). mcb 9.opener*
immunofluorescence microscopy - fluorescently tagged antibodies bind specifically to a
 corresponding antigen as a probe for identifying a particular molecule in cells, tissues, or
 fluids: ex. rat intestine*
confocal fluorescence microscopy* - 1957 Minsky - confocal microscopes uses pinpoint
illumination of a fluorophore in one focal plane to eliminate out-of-focus fluorescence.
Since only fluorescence in a narrow focal plane is detected the image resolution is greatly
enhanced providing a sharper image
1980 Alexrod - TIRF (total internal reflection fluorescence) eliminates background light (pics)
1988 Live Cell Imaging - confocal microscopy by PerkinElmer, Inc. (image of scope)
2007 Live Cell Video Microscopy (5.5 min - view at home)
**Electron Microscopy**

**TEM**
- 1931 Ruska - 1st transmission EM
- TEM passes e's through a specimen onto a viewing screen (resolution theoretical = 0.005nm, but effective resolution is ≈ 0.1 to 0.2 nm)
- 1952 Palade / Porter - 1st TEM pics & EM stains – image due to differential scattering of e's in specimen (stains as heavy metals - osmium tetroxide for membranes) stain = dark.
- Specimens must be thin = 50 nm thick; cut via microtome
- 1957 Robertson - unit membrane hypothesis (all membranes look alike in EM)
- 1952 Palade / Porter - 1st TEM pics & EM stains – image due to differential scattering of e's in specimen (stains as heavy metals - osmium tetroxide for membranes)
- Specimens must be thin = 50 nm thick; cut via microtome

**IFEM**
- cryo-electron microscopy - an aqueous specimen is frozen in liquid N2 (-196°C)
- 1964 Steere & Muhlethaler - develops freeze fracture EM
- 2004 cryoelectron tomography - specimen rotated in electron beam & individual images are computationally fit into 3D reconstruction (tomogram)

**SEM**
- 1965 Charles Oatley - 1st scanning EM (Stereoscan) uses metal shadowing to coat sample & bombardment with e's releases 2ndary e's when focused onto detector reveals 3D surface details
- 1974 Nobel Prize to G. Palade, C. deDuve, A. Claude - for their “inner workings of cells”

**Tagging**
- 1981 antibody tagging with gold particle in electron microscopy - fig 9.21

**RESULTS of MICROSCOPY...**
Investigations of Cells - some major EUKARYOTIC ORGANELLES

The light microscope, so called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology. Live cells lack sufficient contrast and internal cell structures are colorless and transparent. Contrast is increased by staining with selective dyes, which involves killing and fixing the sample, which can introduce artifacts.

The electron microscope uses a focused electron beam on fixed sectioned cells, which are static (mcb9.5a) to describe organelles, mostly by presence or absence of membranes...

The section on CELL ORGANELLES below is a general review of freshman biology cell structure. Please REVIEW this material on your own, and we will question you on the material during testing.

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**Double Membrane Bound Organelles:**

1. **nucleus**
   - synthesizes DNA, rRNA, tRNA, primary transcript (mRNA precursor)
   - largest double membrane bound – outer membrane contiguous with ER
   - **peri-nuclear space** (2-5nm) is contiguous with lumen of ER contains pores of protein complexes (mcb8.20a)
   - regulates nucleoplasm-cyttoplasm exchange via NLS of 7 aa sequence @ C-terminus (pro-lys-lys-lys-arg-lys-val)

2. **nucleolus**
   - regions of rDNA that makes rRNA

3. **nucleoplasm**
   - “cytoplasm” of the nucleus

4. **heterochromatin**
   - condensed (dark EM color) = inactive DNA

5. **euchromatin**
   - non-condensed (light EM color) = active DNA

6. **laminas**
   - fibrous proteins adjacent to inner nuclear membrane
   - form frame for nuclear shape

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**the Results of Microscopy : Investigations of Cells....**

**some major EUKARYOTIC ORGANELLES**

microscopy has used fixed sectioned cells which are static (mcb5.22a) divide organelles by presence or absence of membranes

Links to reviews of major cell organelles of animal & plant cells:

- mcb5.19*(ans) & mcb5.19*(ans) – Quick Review of Major Eukaryotic Cell Organelles
2. **mitochondria...** conducts ATP production of cell via oxidative metabolism of glucose & fatty acids
   - outer membrane (50:50 lipid/protein)
   - contains porin (mcb10.18) transports most ligands < 10K
   - inner membrane (20:80 lipid/protein)
   - strictly regulates most transport into mitoplasm
   - cristae - infoldings of inner membrane (mcb9.8 & 12.6)

3. **chloroplast...** largest green plant cell organelle (0.5-2.0 µm by 10 µm)
   - double membranes
   - with extensive inner membrane-limited sacks called thylakoids (mcb9.9)
   - absorbs light energy via chlorophylous pigments
   - converts light energy into ATP & NADPH (chemiosmosis)
   - reduces CO₂ into CH₂O

**Similarities of Mitochondria & chloroplasts...**
1. make ATP/NAD(P)H via same mechanism - chemiosmosis: oxidative creation of H⁺ gradient coupled to ATP synthase
2. show mobility throughout cell
3. divide by fission independent of cell's division
4. autonomously replicate their own DNA
   - [mito: 16,569 nucleotide pairs: about 37 genes]
   - [chlp: 10fg or 120 genes - highly supercoiled & repetitive-up to 6 copies]
5. both contain 70s - bacterial size ribosomes
6. synthesize their own proteins on own protein synthesizing machinery

4. **endoplasmic reticulum...** network of closed-flattened membrane sacks called cisternae
   - found in all nucleated cells; involved in protein/lipid biosynthesis
   - 2 types: SER (smooth) - lacks ribosomes mcb10.5
     - makes FA & lipids (esp. in hepatocytes)
     - detoxifies hydrophobic chemical including carcinogens & pesticides
   - RER (rough) - membranes bound w ribosomes mcb9.4
     - makes plasma membrane proteins & exportable proteins of ECM
     - abundant in cells making - antibody protein (plasma cells)
     - pancreas (digestive enzymes & hormones)

5. **Golgi Complex...** series of flattened membrane sacks (cisternae)
   - that take up ER transport vesicles and process contents via glycosylation (adding carbohydrate residues)
   - three divisions:
     - cis - where ER vesicles enter mcb9.5
     - medial - where modifications (glycosylations) occur
     - trans - vesicle packages & budded off here for secretion mcb9.6

6. **endosomes...** membrane bound vesicles of extra-cellular milieu internalized by ENDOCYTOSIS
   a. endocytosis - catherin protein "coated" membrane pits, pinch of endosome vesicles
   b. phagocytosis - whole cells engulfed & passed to lysosomes for digestion
   c. autophagy - worn-out organelles fuse with lysosome mcb9.2 & endosomes & lysosomes

7. **lysosomes...** several hundred single membrane bound vesicles (exclusive to animals- plants use vacuoles)
   - have acid pH environment to help denature proteins
   - contains hydrolytic enzymes (nucleases, proteases, phosphatases, glycosylases)
   - cytosolic & nuclear proteins are not digested within lysosomes, but rather *proteasome*
   - Tay-Sachs (tt): defective lysosomal enzyme degrades gangglisodides, gangliolipids buildup in neurons ≈ dementia, blindness, and death
8. **plant vacuole**... membrane limited interior space (up to 80% cell volume) containing membrane transporters that accumulate ions, nutrients, & wastes. Lumen holds digestive enzymes (acid pH optima). **tonoplast membrane** permeable to water influx, helps establish turgor pressure (5-20 ATM)

9. **peroxisomes**... spherical (0.2-1.0 µm) organelle containing oxidases (catalase) that use O₂ to oxidize (removes e-')s from molecules as H₂O₂ (& other toxins). degrade FA's to acetyl groups - used to make cholesterol (esp. imp't in liver/kidney cells). X-linked adrenoleukodystrophy (ADL): no FA digestion occurs, leads to several neuro-linked defects & death.

plants contain **glyoxysomes** which oxidize lipids (very similar to peroxisomes).
Lecture Topic #5 - Chemistry of Life -

is a review of your freshman biology course material that described the structure of the fundamental biomolecules (sugars, lipids, nucleotides, & amino acids) that make up the major macromolecules of cells: starches/glycogen, triglycerides, phospholipids, nucleic acids and proteins.

YOU ARE RESPONSIBLE for reviewing topic # 5 (below) on your own and I will quiz you on this material during our tests.

The chemicals of life...

ELEMENTS - substances composed of atoms all having an identical number of protons...
- can’t be reduced to simpler substances by normal chemical means
- only 92 elements OCCUR IN LIVING SYSTEMS...
- 99% of LIVING MATTER IS made of C, H, O, N, & P...
- all have low atomic numbers & are easily reactive & form covalent bond

Molecular composition of cells...

Water (H2O)…………………………………………           70 %
Inorganic ions (Na, K, Cl, PO4)……………………………  1 %
Small molecules (aa’s, sugar, nucleotides),…..  5 %
Macromolecules (protein, n.a., etc)………………….. 24 %

Biomolecules, Weak Forces, & Design of Metabolism

1. BIOMOLECULES... (carbon skeletons)
   - mostly carbon compounds are found in living systems...
   - WHY Carbon? - easily forms 4 covalent bonds...
   - thus makes many small biomolecules
   - allows 3-D shapes that can evoke biological activity
   - possesses great chemical reactivity...
   - interacts with common chemical functional groups*

   *Functional Groups - groups of atoms, acts as a unit, give organic molecules their physical properties, chemical reactivity, & solubility in aqueous solutions. *common functional groups*

   In bio-molecular chemistry, the concept of functional groups is useful, as a basis for classification of large numbers of compounds according to their chemical properties and reactivity.

   most groups possess electronegative atoms [ O, N, P, S]
   - key bonds are : ester C-O-C & amide -C-N-
   - most are ionizable at physiological ( pH 6.8 to 7.4) read pages 40-50
2. small Biomolecules [monomers]

Four major groups of small biomolecules:

a. SUGARS - compounds with formula \([\text{CHO}]_n\)
   - aldoses vs ketoses
   - \(\alpha\) & \(\beta\)-links
   - glucose + glucose = mono-, di-, tri-, poly-saccharides

b. FATTY ACIDS - long chain hydrocarbons
   - saturated vs. unsaturated
   - lipids (triacylglycerols = animal fats) & phospholipids of membranes
   - easily self-assembly into soap micelles & bilayers
   - steroid & cholesterol (4-ring skeleton) are lipids because they're insoluble

c. NUCLEOTIDES - nitrogen containing "ring" compounds
   - pyrimidines = C, T, U
   - purines = A, G
   - nucleotides form the energy rich compounds of cells (as ATP & GTP), as well as the nucleic acids.

d. AMINO ACIDS - pictures Fig 2.21 peptide bond
   - hundreds known, but only 20 common in proteins of cell.
   - once established in the "primordial cell", certain small biomolecules, as covalent themes, seem to have been preserved throughout evolution (i.e., they were favored energetically)

Amino Acids & their role in Proteins...

Proteins - the penultimate molecules?
- structurally complex
- functionally sophisticated
- long repeats of individual monomers (amino acid's)
- most abundant molecule in cells
- 15% of cell's dry mass

Amino Acids

\[\text{R} \quad \text{H}2\text{N} - \text{C} - \text{COOH} \quad \text{H}\]

20 common amino acids - mcb 2.14 p42 & panel 2.5

lys-arg-his-asp-glu-ala-val-ile-pro-phe-met-tyr
k-r-h-d-e-a-v-l-i-p-f-m-w-g-c-s-t-y

why only these 20?
- all are structurally similar
- alpha-amino acids and the L-stereoisomers...
- it may be an evolutionary anomaly...
- there are some unusual aa's...
- and all play structurally important roles.

Amino Acids... structures & chemical properties of AA's [m.w.king]
1st amino acid discovered was **asparagine** (1806 in asparagus)
lst amino acid found was **threonine** (1938)

**STRUCTURE** - amino acids have a carboxyl group (-COOH) &
amino group (-NH2) bound to an asymmetric carbon

20 ubiquitous aa's have 4 groups in a tetrahedron shape

2 stereo-isomers (enantiomers = mirror images)

- levo-rotatory (left) & dextro-rotatory (right)
- only **L**-amino acids occur in living cell proteins

**Zwitterion** - (an ampholyte) holds 2 groups of opposite sign

**Isoelectric Point** - pH where no net charge in molecule

**pK** - pH where groups are 50% ionized & 50% non-ionized

**ACIDIC... negatively charged** ASP & GLU
R group with 2nd COOH that ionizes above pH 7.0 mcb 2.14*

**BASIC... positively charged** LYS, ARG, HIS
R group with 2nd amide that protonates below pH 7.0

**POLAR UNCHARGED...** SER, THR, TYR, ASN, GLN
are soluble in water, i.e., hydrophilic

**NON-POLAR...** (aliphatic) ALA, VAL, LEU, ILE,
contain only hydrocarbons R groups = hydrophobicity

**AROMATIC** (hydrophobic non-polars) PHE, MET, TRP, GLY, PRO, CYS
contain R groups with ring structures & others

There are only 3 known ways to make a peptide bond...
1. chemical abiotic synthesis in the laboratory
2. genetic engineering cloning mechanisms
3. biologically in cells... (@25aa/sec in prokaryotic cells)

Some common terminology:

dipeptide, tripeptide, oligopeptide, polypeptide
protein - polymer of α-L-amino acids joined by peptide bonds

**whale myoglobin** - ecb panel 4.2 pg 132-133
some naturally occurring small oligopeptides
[many are vertebrate hormones]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Two polypeptides... controls carbohydrate metabolism</td>
</tr>
<tr>
<td></td>
<td>- 1. alpha chain of 30 aa's &amp; 2. beta chain of 21 aa</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Pancreatic hormone 29 aa... opposes insulin action</td>
</tr>
<tr>
<td>Nutra Sweet</td>
<td>A dipeptide (2aa) of L-aspartyl-phenylalanyl-methyl</td>
</tr>
<tr>
<td>Corticotropin</td>
<td>39aa - anterior pituitary hormone... stimulates adrenal cortex</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>9aa - hormone of posterior pituitary... stimulates uterine contractions</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>9aa - hormone acts on smooth muscle... vasodilatation/inflammation</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>Octapeptide (derived from angiotensinogen by kidney enzyme renin)</td>
</tr>
<tr>
<td></td>
<td>- Increases blood pressure ACE Inhibitors block AT &amp; lower bp. [sport]</td>
</tr>
<tr>
<td>Thyrotropin</td>
<td>Releasing factor (TSH) 3 aa's of hypothalamus... stimulates thyroid to release thyroid hormone</td>
</tr>
<tr>
<td>Enkephalins</td>
<td>Either of two penta-peptides with opiate &amp; analgesic activity, occurs naturally in brain &amp; have marked affinity for opiate receptors... compare endorphins</td>
</tr>
</tbody>
</table>
Biological Activity & Shapes of Molecules:

**Structural Chemistry:** orientation of covalent bonds in space.

molecular configuration results in specific bond angles and molecular geometry

- methane $\text{CH}_4$ 109.5° - a tetrahedron with free rotation
- formaldehyde $\text{H}_2\text{C}=\text{O}$ 120° - same plane with no free rotation

one key to shape is the ASSYMETRIC CARBON...
a carbon atom bound to 4 dissimilar atoms
in a nonplanar configuration (tetrahedron)
results in 2 different spatial orientations producing CHIRAL molecules
ones that are mirror images of each other (optical or stereoisomers)

CHIRALITY & ENANTIOMERS... are molecules that are non-superimposable mirror images of one another
called Stereoisomers... two molecules are not equivalent or identical, & have 2 molecular orientations or mirror images
an optically active, CHIRAL*, is not superimposable on its mirror image
stereoisomers may have mostly identical chemical properties, but often rotate plane of polarized light via different angles.

- LEVOROTATORY* (L) - rotate light left (- negative optical rotation)
- DEXTROROTATORY (D) - rotate light right (+ positive optical rotation)

and likely have different BIOLOGICAL ACTIVITY...
Parkinson’s Disease & dihydroxyphenylalanine L-DOPA

Biological Activity & the Shapes of Biomolecules

Biological activity... is catalytic ability of molecules to do work
There are 2 properties of biomolecules, which gives them their unique FITNESS for Biological Activity & the Living State

A. **CONFIGURATION:** the spatial arrangement of atoms in molecules...
configuration can’t be inter-converted w/o breaking bonds based upon COVALENT BOND* - sharing of outer orbital e-s between two atoms thereby forming a molecule

examples of Covalent Configurations:
- isomers... based upon covalent bond configurations [ glu v gal ]*

B. **CONFORMATION** [or shape] - surface outline or contour or 3-D orientation of chemical groups that are free to assume different positions in space without breaking any bonds
- do primarily to...
  - FREE ROTATION of atoms about a single chemical bond
  - WEAK NON-COVALENT FORCES hold atoms in spatial arrays-
- consequences of conformations...
  - different isomeric shapes (forms) of molecules can exist, only one of which may be biologically active (others aren’t)
  - ENZYMES can distinguish between biologically active forms (isomers) based upon the "SHAPE" of that isomer
Weak Molecular Forces of Life

Non-covalent Electrostatic Interactions* ...

(see Panel 2.7 pg 78 in the 10-150 cal/mol)

**IONIC bonds** - charged small ions (atoms which gained/lost e-) which attract (+/-); w/o water they are very strong (crystals of NaCl)

**DIPOLAR** - attractions via asymmetrical, internal distribution of charges in a molecule, which has no net charge (opposite poles +/- attract)

**DISPERSION** (van der Waals) Forces - electrostatic attraction based upon closeness of atoms, is important in macromolecular interactions for 3-D shapes

**HYDROPHOBIC Interactions** - repulsion of electrostatic dipoles of water by non-polars - "fatty-hydrocarbon" groups self assembly

**HYDROPHILIC Interactions** - substances that dissolve readily in water (ions & polar molecules) water, as a dipole, surrounds & solubilizes a solute molecule

**HYDROGEN bonds** (fig*) - electrostatic attraction between H of one atom and a pair of non-bonded e- on an acceptor group: O-H & N-H with O- & N-

Biological Design
or How Weak Molecular Forces & Shape Build Form

Is there a fundamental architectural principle that guides biological organization... in practice?

Some common-universal rules of molecular assembly must exist... one sees recurring patterns of spirals, triangulated forms, & pentagons in everything from crystals to proteins, viruses to plankton, paramecia to protozoa.

**Tensegrity** - is an architectural principle that may influence biological shape & form.

How individual groups of molecules assemble themselves within whole living organisms is a fundamental question of the living condition (???)

Covalent Molecular Forces of Life

(see table 2.1 pg 46)

<table>
<thead>
<tr>
<th>TYPE of BOND</th>
<th>ENERGY (Kcal/mol)</th>
<th>TYPE of INTERACTIONS</th>
<th>ENERGY (Kcal/mol)</th>
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<tr>
<td>SINGLE COVALENT BONDS</td>
<td>NON-COVALENT BONDS</td>
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<tr>
<td>O - H</td>
<td>110</td>
<td>IONIC BONDS</td>
<td>1.0 - 5.0</td>
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<tr>
<td>H - H</td>
<td>104</td>
<td>HYDROGEN BONDS</td>
<td>1.0 - 2.0</td>
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<tr>
<td>C - H</td>
<td>99</td>
<td>VANDER WAALS</td>
<td>0.1 - 1.0</td>
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<tr>
<td>C - O</td>
<td>84</td>
<td>HYDROPHOBIC</td>
<td>0.1 - 1.0</td>
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<tr>
<td>C - C</td>
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<tr>
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</tr>
<tr>
<td>C - S</td>
<td>62</td>
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<td>DOUBLE BONDS</td>
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<td>C = O</td>
<td>170</td>
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<tr>
<td>C = N</td>
<td>147</td>
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</tr>
<tr>
<td>C = C</td>
<td>146</td>
<td></td>
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</table>

*PRINCIPLE of SELF-ASSEMBLY...

molecules join to form larger & more stable structures, often with new & non-predicted properties or emergent properties...

macromolecules -> organelles -> cells -> tissues -> organs

The answer may lie in the principles of **tensegrity**...

the application of general architectural principles to biomolecules & living systems

**TENSEGRITY** defines the mechanical rules and how structures are stabilized by balancing forces of internal tension and compression.
TENSEGRITY may be a fundamental aspect of SELF-ASSEMBLY - an architectural system, mechanically stable, yet dynamic, where the forces of tension and compression balance. "tension & compression are complementary elements in any structure"

- Geodesic Domes (Buckminster Fuller)
  - entire structure distributes its mechanical stresses...
  - frames of rigid struts connected into triangles, pentagons, or hexagons...
  - each of which bears tension or compression

- Prestress Structures (Snellson pic)
  - struts that bear tension are distinct from ones that bear compression.
  - Compression members can provide rigidity while remaining separate, not touching one another, held in stasis only by means of tensed wires. In both of these structures tension is continuously transmitted across all structural members.

Tensegrity in Biological Systems...
'Architecture of Life' by Don Ingber

Organismal Level (examples)
- bones are the compression struts and muscles, tendons, & ligaments are the tension bearing wires.

Cell (1970's view)... membrane bound viscous gel (molasses filled balloon)
- (today)... cytoskeletal awash in a viscous gel, surrounded by a membrane & cytoskeletal elements as: microtubules... act as compression "girders" & microfilaments exert tension, pulling on a cell's part.
- Cytoskeleton is then a hard-wired molecular system that stabilize cell form & shape.

Biological Tensegrity suggests -
- that the structure of cell's cytoskeleton can be changed by altering the balance of physical forces transmitted across the cell's surfaces.
- for example: cultured cells on glass [flat] vs a flexible surface [round]

Tensegrity further suggests -
- Since many enzymes and other substances that control protein synthesis, energy conversion, & growth in the cell are physically immobilized upon the cytoskeleton, changing the cytoskeletal geometry & mechanics may affect biochemical reactions & even alter the genes which are activated and thus the proteins that may be made.

Binding a signal molecule (as a hormone) to a receptor, which traverses cell membrane into a cell, MAY CAUSE conformational changes at the opposite end of the receptor, which in turn may trigger a cascade of molecular restructuring inside a cell, including reorientation of the cytoskeleton.

SELF-ASSEMBLY...
of molecules into organelles and/or cells into tissue is not much different from self-assembly of atoms into compounds.

The shape a molecules assumes is characteristic of the way the structure as a whole will behave in 3-D space, and maybe cells respond in a similar way according to rules of Tensegrity.

Fully triangulated tensegrity structures, once self assembled, may have been selected for through evolution because of their structural efficiency, their high mechanical strength, & minimal use of materials.

Tensegrity may be the most economical and efficient way to build cell structure.
SUMMARY:

A few fundamental principles of chemistry are essential for understanding cellular processes at the molecular level:

1. **Covalent and non-covalent electrostatic forces** control molecular shape. Forces of configuration and conformation result in biologically active molecules.

2. Small molecules are the building blocks of larger molecules. Monomers make polymers, then supramolecular complexes, then organelles.

   ![Figure 2.1a](image)

   ![Figure 2.1b](image)

   Items 3 and 4 below will be covered under metabolism.

3. Chemical reactions are reversible depending on rate constants and the [P] & [R].

   ![Figure 2.1c](image)

4. Source of cellular chemical energy is the hydrolysis of ATP, when high energy phosphoanhydride bonds are broken by addition of water (hydrolysis).

   ![Figure 2.1d](image)
Proteins & their structure

PROTEINS... work horses of cell metabolism

PROTEOME: entire complement of an organism’s proteins:
yeast = 6,000 proteins
human = 32,000 proteins

We’ll look at how Structure gives rise to Function
a) structure: primary, secondary, tertiary, & quaternary
b) protein folding – chaperones
c) degradation/turnover – proteosomes
d) molecular motors
e) enzyme kinetics

pages 63-78

- Proteins - classified by functions
  - Transport Proteins: bind & carry ligands
  - Enzymes: catalytic activity and function
  - Storage Proteins: ovalbumin, gluten, casein, ferretin
  - Contractile (Motor): can contract, change shape, elements of cytoskeleton (actin, myosin, tubulin)
  - Structural (Support): collagen of tendons & cartilage, elastin in ligaments (tropoelastin), keratin of hair, feathers, & nails, fibrin of silk & webs
  - Defensive (Protect): antibodies (IgG), fibrinogen & thrombin, snake venoms, bacterial toxins
  - Regulatory (Signal): regulate metabolic processes, hormones, transcription factors & enhancers, growth factor proteins
  - Receptors (Detect stimuli): light & rhodopsin, membrane receptor proteins and acetylcholine or insulin.

Nomenclature - classes of proteins

Based on SOLUBILITY of PROTEINS: Two classes - Simple & Complex

SIMPLE PROTEINS include:
1. Albumins: soluble in water, globular, mostly enzymes
2. Globulins: soluble in dilute aqueous solutions; insoluble in pure distilled H2O
3. Prolamins: insoluble in water; soluble in 50% to 90% simple alcohols
4. Glutelins: insoluble in most solvents; soluble in dilute acids/bases
5. Protamines: not based upon solubility; small MW proteins with 80% Arginine & no Cysteine
6. Histones: unique/structural - complexed w DNA high content basic aa’s - 90% Arg, Lys, or His
7. Scleroproteins: insoluble in most solvents fibrous structure - cartilage & connective tissue

Collagen = high Glycine, Proline, & no Cysteine when boiled makes gelatin
Keratins = proteins of skin & hair high basic aa’s (Arg, His, Lys), but w Cys
Complex Proteins:

- **lipoproteins** - blood, membrane, & transport proteins
- **glycoproteins** - antibodies, cell surface proteins
- **nucleoproteins** - ribosomes & organelles

Common terminology:
- **peptide** = short chain of amino acids (20-30)
- **dipeptide** = 2 amino acids
- **tripeptide** = 3 amino acids
- **polypeptide** = many amino acids (up to 4,000)
- **protein** = polypeptide with well defined 3D structure

Structure of Proteins

The Variety of Protein Structures may be INFINITE...

- Average protein has 300-400 amino acid’s & has a MW of 30 to 45kD
- A protein of 300 amino acids made with 20 different kinds of amino acids can have $2^{300}$ different linear arrays of aa’s that’s ~10^900 different proteins

1st protein sequenced was **Beef Insulin** by Fred Sanger - 1958 Nobel Prize winner
- Humulin & ADA
- 2 polypeptides [21/30 aa’s]
- To date about 100,000 protein have been sequenced
- Only about 10,000 structures known [2K/yr]
- E. coli make about 3,000 proteins; humans about 100,000

4 levels of protein structure are recognized

- **Primary** - linear sequence of aa’s
- **Secondary** - regular, recurring orientation of aa in a peptide chain due to H-bond
- **Tertiary** - complete 3-D shape of a peptide
- **Quaternary** - spatial relationships between different polypeptides or subunits

Primary Sequence is...

Linear sequence of amino acids in a polypeptide (zymogen)
- Repeated peptide bonds form the backbone of the polypeptide chain
- R side groups project outward on alternate side

Chain... one end polypeptide chain has free (unlinked) amine group: N-terminus
- Other end has a free (unlinked) carboxyl group: C-terminus

Size... protein size is specified by mass (MW in daltons = 1 amu)
- Average MW of a single amino acid ≈ 113 Da
- Thus if a protein is determined to have a mass of 5,763 Da ≈ 51 amino acids
- Average yeast protein = 52,728 Da [52.7 kDa] with about 466 amino acids

Protein Primary Sequence today is determined by reading GENOME Sequence
- Function is derived from the 3D structure (conformation) specified by the primary amino acid sequence and the local environments interactions
Primary sequence... & some consequences

**Polymorphism**... proteins may vary in primary sequence but have the same function. ex: enzymes $\text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$

inter-specific: between species [diff. aa sequences]

intra-specific: within a species [liver vs. kidney]

**Invariants**... don't vary significantly in aa sequence
- examples: ubiquitin (proteosomes) & histones (chromosomes)
- Site Specificity... sequences determine intra-cellular location
- signal sequences, prosthetic binding sites, etc...

**Families of proteins**:
- different but related functions evolved from a single ancestral protein, 30% + commonality of sequence... serine proteases (trypsin, chymotrypsin, elastase)
- **Homologous Proteins** evolved in related fashion & perform the same cellular function in different species
  - ex: cytochrome-C in duck & chickens = 2 variants & in yeast & horses = 48 variants

**Mutation** - change in primary aa sequence = defective protein
- **SICKLE CELL**

Secondary structure - 3D conformation of portions of polypeptide chains

**Alpha helix**
- described by Linus Pauling 1954 Nobel
- using X-ray diffraction technique
- peptide backbone around long axis core
- rigid cylinder
- $\alpha$-helix height = $\frac{0.54 \text{ nm}}{\text{3.6 } \text{aa per 360° turn}}$
- $\alpha$-helix - (counterclockwise) right handed helix
- $\beta$-strands = hinges, turns, loops, etc = flexibility
  - $\beta$-strands - inter- & intra-chain
  - ribbons & sheets*
- turns - mcb 3.6
- MOTIFS: combos of recurring arrangements of $\alpha$-helix and/or $\beta$-sheets in unrelated proteins...
- such as:
  - hairpin beta motif... antiparallel beta-sheets joined by
  - EF hand... two short helices connected by a loop, a $\text{Ca}^{2+}$ ion binder region of hydrophilic residues present in over 100 $\text{Ca}^{2+}$ binding proteins.
- zinc finger... 1 $\alpha$ and 2 $\beta$ strands with antiparallel orientations. forms fingers bound by Zn ion that often link to DNA * & RNA
- coiled coil... $\alpha$ helices, where the hydrophobic amino acids wind together forming a coil; also called leucine zippers: common to transcription factors.
**Tertiary level**

Most responsible for 3-D orientation of proteins in space
- Thermodynamically most stable conformation
- Weak non-covalent interactions (H-bonds) & S-S bridges
- Hydrophobic interior & hydrophilic exterior

**Protein Folding**... forms 3D shapes & binding sites
occurs via H-bonds & S-S bridges

**Some examples:**
- **Myoglobin**
  - MW 16,700 - animal muscle protein - stores O2
  - 124 aa's with 4 S-S; that hydrolyses polysaccharides in bacterial cell walls = bactericidal agent
- **Ribonuclease**
  - MW 13,700 enzyme of 124 aa w 4 S-S

**Protein Folding**

**Protein Families** – proteins with a common evolutionary ancestry
Function derives from 3D structure that is due to primary sequence, thus some proteins have many identical or chemically similar amino acids in identical sequence positions each may contain domains that closely resembles that of other proteins.

Proteins with common ancestors are known as homologs and homologous proteins belongs to a "family"

**Taxonomic cladistics** (tree diagrams) of sequence analysis are used to show homologies.

**Ex.:**
- **Serine proteases**
  - Ecb figure 4.21
- **Globins**
  - Gene slowly diverged into animal and plant lineages

Today, computer modeling is used to predict function of yet unisolated proteins by comparing known sequence homologies.

**Quaternary structure:**
Multiple polypeptides each with 3-D conformations
- **Hemoglobin**
- **RNA polymerase**
- **ASPC-trans-carbamylase**

**Some common quaternary level protein shapes:**
1. **Dimers** - self-recognizing symmetrical regions
   - bind together @ identical binding sites
2. **Tetramers** - 4 identical subunits...
3. **Filaments** - polymers of subunits each bound together in an identical way form a ring or helix
4. **Colled-coil** - 2 parallel helices forming a stiff filament

**Multi-Enzyme Complexes:**
- **Puruvate dehydrogenase**
- **ATP synthase**

**Domains**
- Distinct modules or structural element of the tertiary level of protein structure...
- Compact folded regions in a polypeptide of 100-150 amino acids, often self-forming, self-stabilizing, that often fold independently.

**3 classes of domains:**
- **Functional domain** - region with particular activity characteristic of a protein CATALYSIS:
- **Structural domain** - region of 40+ aa’s in a stable 2nd or 3rd-ary conformation (repeatable).
- **Topological domain** - Distinctive spatial relationships to rest of a protein;
Multimeric proteins have Quaternary Structure...

3D shape of a protein... involving more than one polypeptide or subunits of a protein

HA (hemagglutinin A) is a trimer of 3 identical polypeptide subunits held together by the weak electrostatic 3o level forces creates a globular domain and a fibrous domain

Some proteins form Macromolecular Assemblies... very large (> 1m Da in mass), 30-300 nm in size, & 10-100 individual peptides examples include: viral capsids, some cytoskeletal complexes, molecular machines, & mRNA transcription complex (some 60 proteins - fig 3.9)

Selected examples of some Molecular Machines can be seen in Table 3.1 we will look at some of these in greater detail later

Protein Conformation is critical to Biological Function

DENATURATION loss of 3-D conformation by heat, pH, organic solvents, detergents

RENATURATION - regaining of biological activity via self-assembly

protein shape & conformation...

the NATIVE Protein CONFORMATION is the...

3-D SPATIAL ORIENTATION that’s MOST thermodynamically STABLE & has the lowest free energy expenditure, and forms spontaneously

3 most common conformations

HELIX - a spiral staircase-like shape
FIBER - elongated bound monomers
GLOBULAR - roughly a sphere

the Native Conformation of most enzyme proteins is GLOBULAR:

an interior pocket of hydrophobics exterior surface of hydrophilics - maximizes the number H-bonds that form fig 5.5

the PHYSICAL forces include mostly weak electrostatic bonds*: non-covalent bonds, H-bonds, hydrophobic & hydrophilic interactions, & covalent bonds (as in peptide bonds & disulfide bonds)... results in a variety of protein shapes & sizes - fig 4.9 pg 127

How does 3D protein folding come about? "FUNCTION follows FORM"

peptide bond is PLANAR (partial double bond character) as are all the atoms bonded to it all occur in same plane & thus there is no free rotation - restricts protein conformations the native folded conformation is most stable, i.e., in lowest free energy state, often dictated by R-group properties (size, hydrophobicity) hydrophilicity, ionic strength, etc... folding involves: changes in 3D conformations: - by orderly steps in a sequential way, each step facilitating the next - first 2D structure (α & β), then structural motifs & assembly of complex domains, followed by 3D level forces and/or 4D shapes. fig 3.15

Unless protected during folding, proteins would interact with all the other molecules in a cell.

Cells makes 2 sets of proteins that facilitate folding: CHAPERONES...

Molecular Chaperones - which bind and stabilize newly made unfolded proteins preventing these proteins from self aggregating and/or being denatured before folding.

Chaperonins - which makeup a small folding chamber into which unfolded proteins are moved to provide a proper environment favoring native folding of a protein.
MOLECULAR CHAPERONES - are families of proteins to help "properly fold" a new protein... multiple ones bind to newly made proteins and include: Hsp70 (of cytosol & mitoplasm); BiP (of the E.R.); & DnaK (of bacteria).

1st discovered by heat shock treatment (under temperature elevation (25°C -→ 32°C) cells make heat shock proteins (HSPs); mutant bacteria didn't make Hsp's nor assemble normal proteins. when bound with ATP = OPEN conform w hydrophobic pocket for new unfolded protein ADP conform closes around protein and aids native folding... mcb6e.3.16*

Classes of Heat Shock Proteins: Hsp -40, -60, -70, -90 & -100. Hsp are named according to the molecular weights (Hsp-70 = 70 kilodaltons)

Hsp-40 binds new protein amino acid chains & carries it to Hsp-70

Hsp-70 grabs proteins by an open cleft when ATP is bound to Hsp-70; in its ADP conform closes around protein and aids native folding... mcb6e.3.16*

Hsp-90 receives partially folded proteins from Hsp-70's and other chaperones... helps join polypeptides into larger quaternary proteins forming multi-subunit proteins, such as cellular receptors.

CHAPERONINS or Foldase - small folding CHAMBERS into which unfolded proteins are moved to provide a proper environment favoring native folding.

- a molecular Machines made of chaperone proteins hsp70's & hsp60's form a barrel shaped structure made of 14 polypeptides (from GroEL gene) in 2 donut rings with a cap (from GroES gene) that opens an inner chamber, where a cell's new protein enters & is folded. barrel chamber has 2 conformations: tight & relaxed; new polypeptides is inserted into cavity of GroEL chamber & conformational changes favor native protein folding; ATP hydrolysis = relaxed state & release of a native 3D-protein mcb6e-fg 3.17*

Misfolded Proteins & Disease

CJD: Creutzfeld-Jacob disease, genetic based or acquired -(eating "mad cow" tissue) fatal neurological disease due to misfolded PRPc protein.

Spongiform Encephalopathy (SE) = vacuolation (holes) in brain nerve tissue

PRION: a defective protein agent (PrPsc) due to mis-coded gene (PRNPc) native prion protein is PrPc & resides on nerve cell surfaces... defective protein PrPsc accumulates forming aggregates that lead to CJD & SE's

Both PRION proteins can have identical aa sequence, but may fold differently [are known as conformers = proteins differ only in conformation]

A. normal (PrPc) protein... mostly α-helix foldings - remains soluble
B. abnormal PrPsc protein... 45% β-sheet - insoluble & protease insensitive produces cell surface aggregates that kill cells

PROTEIN DEGRADATION (Digestion/Turnover)
cells often contain specialized mechanisms or pathways to digest cell proteins...

1. that rapidly turnover proteins with short half-lives
2. that recognize & eliminate damaged or misfolded proteins that can lead to diseases as Huntington's, Alzheimer's, and Creutzfeldt-Jacob disease.

many proteins are degraded in cytosol using proteases hydrolyze peptide bonds some proteins are degraded in the lysosomes via phagocytosis, but most proteins are degraded by large complexes of proteolytic enzymes in structures known as PROTEASOMES by ubiquitin-mediated proteolysis (UMP)

short half-life proteins hold a signal sequence targeting proteins for UMP and misfolded proteins seem to be recognized for degradation by the UMP
Discovered by Alfred Goldberg & Martin Rechsteiner in 1980’s

**PROTEOSOMES** are large multi-enzyme complexes (fig 7.36*)

Average human cell holds between 20,000 & 30,000 proteasomes.
Each proteasome is a barrel shaped complex (2,400kD) made of 4 parts

1) a Lid of 9 proteins,
2) a Regulatory Cap that lets in only ubiquitinized proteins,
3) a Base of 4 stacked protein rings with protease activities, and
4) a Base Cap.

**Protein Digestion...** begins when cells add small polypeptide (ubiquitin)-to protein to be degraded.

**Ubiquitin:** globular protein of 76 aa (virtually identical aa sequence in bacteria, yeast, or mammals). 3 ubiquitin ligase enzymes [E1, E2, E3] add Ubiquitin to proteins to be degraded, a ubiquitinized protein is targeted for entry into a Proteasome’s central chamber, where proteases with chymotrypic, tryptic, & caspase-like proteolytic activity cleave the protein into peptides. The ubiquitin is recycled.  

---

**Protein Engineering...**
producing novel proteins, with unique shapes, via artificial means

1. **use proteomics...**
   make artificial proteins of desired sequence to functions as “drugs”  
   vaccine protein - binds to viral surface and inactivates it  
   simplistic idea - but it’s hard to make connection from 1o to 3o

2. **modify existing proteins via site directed mutagenesis**
   isolate a gene, alter its sequence in precise way,  
   clone the protein product  
   - can be used to study effect of one amino acid change on 3D-folding  
   - often done with clinically useful proteins to enhance efficiency (Km)

3. **structure based drug design**
   make drug molecules with high binding affinity to known proteins  
   [to remove it] use computers to design ‘virtual’ drug to fit into a protein rendering it inactive

4. Bionanotechnology - viruses made to order (A. belcher of MIT)
Bil 255 – CMB

enzyme isolation techniques, procedures, and protocols

PROTEIN BIOCHEMISTRY

Protein Chemistry - Techniques & Procedures for Isolating Proteins

Methodologies, Techniques, and PROTEIN PROCEDURES for Isolation & Purification of "PROTEINS"

Protein Chemistry Journals and books

procedures based upon physical properties of proteins - size, charge, solubility

Cell Biology Protocols & Methods COOK BOOK

Protein Isolation Techniques

Crude Cellular Homogenates How to break open cells

grind cells in...

mortar & pestle, tissue grinders, homogenizers, cell disruptors, and blenders (Waring) or in beaters or sonicators

in an osmotically, buffered medium w enzyme's substrate &/or in an isotonic media

Results in...

ruptured cells producing a liquified homogenate

Protein Isolation Techniques

panel 4.3 pg 160

how to break open cells

Crude Cellular Homogenates

Protein Isolation Techniques

Panel 4.3 pg 160

Crude Cellular Homogenates

Protein Isolation Techniques

Panel 4.3 pg 160

Crude Cellular Homogenates

Protein Isolation Techniques

Panel 4.3 pg 160

Crude Cellular Homogenates

Protein Isolation Techniques

Panel 4.3 pg 160

Crude Cellular Homogenates

Protein Isolation Techniques

Panel 4.3 pg 160

Crude Cellular Homogenates
Protein Separation Procedures...

Proteins are separated based upon their physical properties - size, charge, affinity for ligands, shape, etc...

**CHROMATOGRAPHY**

- separation of molecules based on differences in their structure &/or physical properties interacting with a stationary support media

**PARTITION chromatography**

- developed by R.L.M. Synge
- small MW molecules are partitioned between phases of 2 different solvents (water/alcohol) on a support media

**PAPER chromatography**

- uses cellulose as support media [chlorophylls*]

**THIN LAYER chromatography**

- media is silica gel on glass plates [alpha]

**COLUMN chromatography**

- done in cylindrical glass column, on permeable support media, which retards flow of selected molecules, while others pass through

**Kinds of column chromatography**

- ion exchange chromatography...
  - charged ligands in matrix retards passing proteins of opposite charge
  - DEAE cellulose [dimethylaminoethyl cellulose] (+)
  - CM-cellulose [carboxymethyl cellulose] (-)

- gel filtration...
  - size exclusion chromatography
  - "sized" media (beads) retards smaller size proteins...
  - sample columns and sample media

**Identification of a protein & quantification**

**Electrophoresis**

- proteins migrate in an electrical field at rates that depends upon their net charge, size, and shape

- Gel Electrophoresis - [PAGE] media is porous gel (starch/polyacrylamide)
  - separation is by size & charge

- SDS-electrophoresis (SDS-PAGE)... (panel 4.5*)
  - a detergent - Sodium Dodecyl Sulfate... binds to protein @ 1 SDS/2 aa's
  - thus it is proportional to a protein's molecular weight

**Isoelectric focusing**

- a pH gradient in a glass column of gel, proteins move to point of its pI, i.e., no charge

**2-dimensional electrophoresis**

- combines isoelectric focusing with SDS-electrophoresis
  - an I.F. gel is turned at right angle & SDS-PAGE is done
PROTEOMICS – total protein expressions in a cell...

* PEPTIDE MAP - a protein fingerprint...
  treat a purified protein with proteolytic enzymes...
  analyze distinctive fragments by SDS-electrophoresis

PROTEOMICS -
  is the science of protein expression
  of all the proteins made by a cell.

Proteome - all the proteins being made according to the transcriptome
  Human Proteomics Initiative (HPI)
  highly curated database of human protein sequences
  Proteomics Symposia
  Confocal microscopy, mass spectroscopy, & other techniques
  Proteomics & Drug Therapies

DNA Electrophoresis*: separates polynucleotide strands by charge (pic)

Identification of protein’s presence & its amounts

Identification –
  is often done by spectrophotometry

spectrophotometers measure intensity of light
  beam before & after light passes through a liquid solvent with sample dissolved in it,
  (in a cuvette)… compares the two light intensities over a range of wavelengths.  figure*

Percent transmittance…
  ratio of intensity of light passing through the sample
to the intensity of light shining on sample multiplied by 100%.

Absorbance…
  is the log of the transmittance … an instrument = Spectronic 20

SPECTROPHOTOMETRIC METHODS of DETECTING PROTEINS...

UV absorbance at 280 nm. (measures aromatic aa’s - figure*)
colorimetric reactions - colored dye binds to amino acids
  Ninhydrin reaction - rxn’s w amino = blue color (10-9M)  (CSI)  
  Biuret test = mg quantities… based on Copper ions
  binds stoichiometrically = violet color  prepare standard curve
  Bradford test = ug amounts  (Biorad)
  based on dye Coomassie blue - binds to peptide
  Fluoresceamine dye = pg quantities… (10^-12 M)

Quantification of amounts of protein present
  is based on BEER-LAMBERT Law

linear relationship between… light Absorbance vs. Concentration (figure*)
  Protein Standard Curve (figure*)
Quantification by Biological ENZYME ACTIVITY...

1 (international) UNIT of enzyme activity...
that amount of protein which converts 1 µmole of substrate
to product per min at 25°C at optimal pH
UREASE - 1 unit will liberate 1.0 µmole of ammonia from
urea per minute at pH 7.0 at 25°C [equivalent to 1.0 I.U.]

1 unit SPECIFIC ACTIVITY...
number of micromoles converted per min per mg protein
  i.e., Units (as above) of enzyme activity per mg protein

1 unit MOLECULAR ACTIVITY...
number of units of enzyme activity per µmole of enzyme

---

**Purification Table for a "NEW" Enzyme**

**Horse Radish Peroxidase**

<table>
<thead>
<tr>
<th>STEP</th>
<th>Fraction Volume (ml)</th>
<th>Total Protein (mg)</th>
<th>Activity (units*)</th>
<th>Specific Activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.  Homogenate</td>
<td>1,400</td>
<td>10,000</td>
<td>100,000</td>
<td>10</td>
</tr>
<tr>
<td>2.  Precipitate</td>
<td>280</td>
<td>3,000</td>
<td>96,000</td>
<td>32</td>
</tr>
<tr>
<td>3.  Ion-exchange chromatography</td>
<td>90</td>
<td>400</td>
<td>80,000</td>
<td>200</td>
</tr>
<tr>
<td>4.  Gel filtration</td>
<td>80</td>
<td>100</td>
<td>60,000</td>
<td>600</td>
</tr>
<tr>
<td>5.  Affinity chromatography</td>
<td>6</td>
<td>3</td>
<td>45,000</td>
<td>15,000**</td>
</tr>
</tbody>
</table>

* 1 unit of activity = 1 micromole H₂O₂ --- H₂O & H₂O₂ per min
** 1,500 fold purification of peroxidase
Enzymology

**Enzymology**

- regulate metabolic reaction rates
- i.e., control metabolism
  - molecules (mostly protein) that accelerate or catalyze chemical reactions (A $\rightarrow$ B) in cells by breaking old covalent bonds and forming new covalent bonds
- a biological catalyst......
  - but, different from a chemical catalyst -
    - have complex structure (sequence of aa's)
    - act only upon a specific substrate
    - do not change direction (energetics) of rx

**cAMP Protein kinase A** - [2.7.1.37]
- a group of enzyme that phosphorylate proteins

---

**REACTION PATH**

$E + S \rightarrow [ES] \rightarrow E + P$

enzymes catalyze reactions by lowering the energy of activation...Ea

**Catalase**

$2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ea</th>
<th>Rate (lt/mol/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no catalyst</td>
<td>18,000</td>
<td>$10^{-7}$</td>
</tr>
<tr>
<td>Fe catalyst</td>
<td>10,000</td>
<td>56</td>
</tr>
<tr>
<td>catalase</td>
<td>2,000</td>
<td>$4 \times 10^6$</td>
</tr>
</tbody>
</table>

---

**Some important dates in early Enzyme History**

- 1833 Payen & Peroz - alcohol precipitate of barley holds heat labile components - convert starch to sugars
- 1878 Kuhn - coins term "enzyme" : Greek "in leaven"
- 1898 Ducleaux - uses suffix "ASE" for enzyme names
- 1900 E. Fischer - stereospecificity of enzymes discovered

---

**1st enzyme crystallized UREASE, 1926 James Sumner**

$2 \text{NH}_2\text{C-NH}_2 + 2 \text{H}_2\text{O} \rightarrow 4 \text{NH}_4^+ + 2 \text{CO}_2$

- Sumner's bioassay - injects rabbits with urease & the ammonia produced killed bunnies
- to date just over 1000 enzymes purified about 100* crystallized, out of some $10^6$
  - except for ribozymes all catalytic agents are proteins
  - proof something is enzyme has usually been to note the loss of biological activity due to proteolytic digestion

---

**Bil 255 - CMB**

enzymology

procedures and protocols

For assaying enzyme activity
Terminology

- **Substrate, Product, Enzyme**
- **Cofactor**: small organic ions and mostly metal ions: Cu, Mg, Mn act as activators & inhibitors
- **Coenzymes**: small non-protein ligands catalyze reactions... +/- electrons, transfer a group, break a bond
  - *Lipoic acid*: oxidative de-COOH alpha-keto acid
  - *NAD* (*NADP*): dehydrogenation; H+ carrier and/or electron transfer
  - *CoASH*: acyl carrier via sulfhydryl (-SH)
- **Vitamins**: ascorbate, cyanocobalamin, folic acid, etc.
- **Prosthetic Group**: large complex organic molecules, which may have catalytic activity (heme)

**Mechanism of Enzyme Action**

Chemical reaction scheme by which enzymes act on substrates.

**3 examples...**

1. Catalytic action of cAMP dependent Protein Kinase A mcb3.18*
   - e's of ATP delocalized by LYS & Mg+2: new bond forms between SER-OH & γP; bond between βP-γP broken = ADP + P-protein.

2. Serine Protease hydrolysis of peptide bonds mcb3.25*
   - Catalytic site ser195, asp102, & his57 - OH of ser195 attacks the C=O of peptide bond & transition is held by H-bonds. e's break peptide bond release part of protein, H-O-H is split & other half released.

3. Another example *Lysozyme*: pg 146-149*
   - An enzyme that cuts polysaccharides (substrate*) by hydrolysis (adds H2O)
   - Breaks glycosidic bond (… -C-O-C- …) via bond strain of glu & asp
   - Active site is a long groove, holding six sugar units...
   - Has 2 acidic side chains (ASP & GLU) hold substrate
   - Binding of substrate, bends bonds from a stable state, lowering Ea.
   - Acidic side group of GLU provides [H+] ions = acid hydrolysis, & negative charged ASP stabilizes + charge* of the transition state.
Classification of Enzymes

- International Enzyme Commission
  - IUBMB
    4 digit Numbering System [1.2.3.4.]
    - 1st #... Major Class of Enzyme Activity
    - 2nd #... a subclass (type of bond acted upon)
    - 3rd #... a subclass (group acted upon, cofactor required, etc...)
    - 4th #... serial number ... order in which enzyme was added to list

EC MAJOR CLASSES

1. Oxidoreductases... [dehydrogenases]
catalyze oxidation reduction reactions, often using coenzyme as NAD+/FAD
   - Alcohol dehydrogenase [EC 1.1.1.1]
     ethanol + NAD+ --------> acetaldehyde + NADH

2. Transferases....
catalyze the transfer of functional groups
   - Hexokinase [EC 2.7.1.2]
     D-glu + ATP ----------> D-glu-6-P + ADP

3. Hydrolases .... catalyzes hydrolytic reactions
   adds water across C-C bonds
   - Carboxypeptidase A [EC 3.4.17.1]
     [aa-aa]_n + H_2O -----> [aa-aa]_n-1 + aa

Definitions of Enzyme Activity

- Measured by relative rate substrate ---> product
  1 unit activity that amount protein which converts
    - 1 umole substrate per min at 25°C & optimal pH
  1 unit SPECIFIC ACTIVITY
    • # units per mg protein present
  1 unit MOLECULAR ACTIVITY
    • # units per umole of purified enzyme
Enzyme Kinetics... mathematical and/or graphical expression of the reaction rates of enzyme catalyzed reactions

- **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 the reaction $A \rightarrow B$ is enzymatic

**Observed Enzyme Kinetic Reaction Curves**:

1. Rate $(0.8 \text{ ml O}_2/\text{min})$ vs. $[E]$  
2. Rate vs. pH  
3. Rate vs. Temperature  
4. Rate vs. $[S]$

**1913 Leonor MICHAELIS & Maude MENTEN Kinetics**

- proposed mathematical modeling of enzyme reactions  
  i.e., an algebraic expression of rectangular hyperbola

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P$$

**assumptions**

1) rate formation ES complex from $E + P$ is negligible  
   i.e., can ignore the rate constant $k_4$
2) rate LIMITING step is disassociation of ES to $E + P = k_3$
3) important state of the ENZYME is termed **FREE ENZYME**

- free enzyme = $E_t - ES$  
- bound enzyme = $ES$  
- total enzyme = $E_t = [E - ES] + [ES]$
Derivation of Michaelis-Menten Kinetics

derivation of equation occurs at a time when the rate of formation of
ES complex is equal to rate of destruction (break down).
– i.e., at equilibrium, when [S] >>>> [E] so that total E is bound in
ES complex
– as a 1st order reaction enzyme catalyzed reaction
\[ v = \frac{dP}{dt} = k_3 [ES] \]

Let’s measure the concentration of [ES] …..spectrophotometer
– so then the derivation of M&M kinetics was to be able to express [ES]
in terms of E & S alone…..
M & M equation is then : \[ v = \frac{V_{max} [S]}{K_m + [S]} \]

SEE LINK for DERIVATION HANDOUT

Km - the Michaelis Constant

• is a mathematical interpretation of an enzyme action
• is substrate concentration at which rate is equal to \( \frac{1}{2} V_{max} \)
• is a characteristic physical property for each different enzyme
• is independent of [E]
• if there's more than 1 substrate, then each has its own Km
• measures "RELATIVE affinity" of an enzyme for its substrate
  – one enzyme with 2 substrates with following Km's - 0.1 M & 0.05 M
  one takes more substrate to reach same rate... \( \frac{1}{2} V_{max} \)
  – many enzymes have individual steps in a complex reaction
  sequences, each with their own Km's.....
  – i.e., Km is a complex function of many rate constants
• not all enzymes are treatable by M & M kinetics…
  – most regulatory enzymes (multi-subunits) are not treatable

ways to determine Km
• by extrapolation from M & M
  standard curve \[ v \text{ vs. } [S] \]
• by transformation of M & M curve
  LINEWEAVER- BURKE Plot
  • take the reciprocal of both sides of M&M equation & plot
  \[ \frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{S} + \frac{1}{V_{max}} \]
  EADIE - HOFSTEE Plot
  \[ v \text{ vs. } \frac{v}{[S]} \]

SEE GRAPHICAL PLOTS

The Eadie-Hofstee plot is a way of plotting kinetic enzyme data
so as to yield a straight line for reactions obeying Michaelis-Menten
kinetics. This is done by plotting reaction velocity (V) versus
velocity/substrate concentration (V/[S]). The slope of the line is
equal to -KM and the x-intercept is Vmax

An advantage of an Eadie-Hofstee plot over a Lineweaver Burk
plot (which plots 1/V versus 1/[S]) is that the Eadie-Hofstee plot
does not require a long extrapolation to calculate Km.
Enzyme Inhibition

2 classes of inhibitors

- **IRREVERSIBLE** -
  - inhibitor molecule cannot be easily removed from enzyme
  - e.g., enzyme is physically altered by binding of inhibitor
    - alkylating agents like iodoacetamide (bind to -SH's)
    - organophosphorous compounds - nerve gases (SER)

- **REVERSIBLE** -
  - enzyme activity may be restored by removing the inhibitor
  - 2 major types of reversible inhibitions
    - COMPETITIVE
    - NON-COMPETITIVE

Some Native Examples of Enzyme Inhibition:

Irreversible Enzyme Inhibition & Mechanism of Action of an Antibiotic...

**Antibiotic** - a natural molecule (often made by bacterial cells) that can kill other bacterial cells (& without hurting eukaryotic cells: they're insensitive)

**Penicillin** - any one of a group of **antibiotics** derived from the fungus *Penicillium*. The action of natural penicillin was first observed in 1928 by British bacteriologist Alexander Fleming, and recognized as anti-bacterial by Howard Florey and others.

Penicillin is a **substrate-like molecule** of bacterial peptidases, that naturally cross-links bacterial proteoglycans in the bacterial cell walls & favors rigidity. penicillin works by IRREVERSIBLE inhibition via binding to active site of peptidases, forming covalent link, removing enzyme, reducing Vmax; weak bacterial walls eventually rupture & cells die.

---

**COMPETITIVE**

- inhibitor often *looks like substrate...* fools active site & binds
- inhibitor binds to E forms an [EI] complex at the **active site**
- extent of inhibition is **concentration dependent**
  - i.e., can be overcome if [S] is very high, [S] >> [I]
  - classical example: malonic acid inhibition of SDH
  - easy to demonstrate: via Lineweaver-Burke plots
  - shows Vmax is same & Km is higher

**NON-COMPETITIVE**

- inhibitor binds to E, forms an [EI] complex but, **not at active site**
- inhibitor often bears no structural relationship to substrate
- removes a net amount of active enzyme, i.e., lowers total [E]
- can NOT be overcome, even if [S] is very high
  - easy to demonstrate via Lineweaver-Burke plots
  - shows Km is same & Vmax is different

Competitive Enzyme Inhibition & Mechanism of Drug Action

**ACE Inhibitors** - drugs that bind to enzyme active site & reduce its activity

ACE - Angiotensin Converting Enzyme: a proteolytic enzyme that cuts Angiotensin I protein (10 amino acids) to Angiotensin II (8 amino acids).

**Angiotensin II** promotes hypertension (high blood pressure - hbp) in 1960's John Vane discovered TEPROTIDE in Brazilian pit viper venom, which functioned as ACE competitive inhibitors, by binding to the active site of ACE.

Today there are a number of synthetic peptide **ACE inhibitors**, all called "prls"... (lisinopril, captopril, trandolapril, moexipril, ramipril, etc.)
Mechanisms of Protein & ENZYME REGULATION...
4 approaches commonly employed used by cells…

1. by controlling number of enzyme molecules present (gene action)
2. by sequestering (compartmentalizing) - for example into lysosomes, mitochonrdria
3. by proteolytic cleavage - converting inactive peptides to active enzymes
   - often involves hormones and digestive proteases
   - pancreas makes zymogens… (an inactive enzyme large precursor)
   - enterokinase, an aminopeptidase from lining of small intestine.
   - hyrolyzes trypsionogen to trypsin (active form), which itself hydrolyzes chymotrypsinogen in chymotrypsin
4. by adjusting reaction rates of existing enzyme (ala M&M kinetics)
   a) STIOCHIOMETRIC controls - limit amount substrate present
   b) ALLOSTERY - [allostic kinetics]
   - binding of a ligand results in a change of 3o/4o conformations
   - common in multimeric proteins/enzyme complexes
   - binding of a ligand results in a change of 3o/4o conformations
   - common in multimeric proteins/enzyme complexes
   - enterokinase, an aminopeptidase from lining of small intestine.
   - hyrolyzes trypsionogen to trypsin (active form), which itself hydrolyzes chymotrypsinogen in chymotrypsin

b) Ligand-induced activation of catalysis: (ex - PKA)
1. inactive PKA is activated by cAMP...
   - binding of cAMP induces Δ-conformation, so tetramer dissociates into 2 active monomers & a dimeric regulatory subunit
   - thus a hormone signals -> cAMP --> active PKA dimer
   - without PKA we have an inactive tetramer
2. GroEL chaperonin: is 2 multi-subunit rings binding of ATP and GroES results in a tight peptide binding complex, which opens the folding cavity allowing efficient folding of nascent proteins
3. Calmodulin: ER membrane pumps Ca into ER lumen leaving cytosol @ 10^-7M.
   - to increase cytosol Ca calmodulin, a helix-loop-helix protein is used
   - 4 Ca ions bind = Δ-conformation - now binds target proteins

4. GTPase super family: a group of allosteric plasma membrane proteins switching between active/inactive, includes Ras & G-proteins which involves COVALENT MODIFICATION of existing enzyme...
   - addition of P to an inactive enzyme --> activate enzyme via P transfer [reversible phosphorylation changes protein conformation]
   - done by
     - PROTEIN KINASES, which transfer P from ATP
     - tyrosine kinases add P to TYR residues of enzymes de-activating them
     - serine/threonine kinases add P to SER or THR residues
     - PROTEIN PHOSPHATASES... dephosphorylate, thus inactivating GTP Binding Proteins (G Proteins)
   - are Active when GTP is bound to protein
   - Inactive when GTP is hydrolyzed to GDP
Net RESULTS of Protein Regulatory mechanisms...

feedback inhibition (negative regulation)
an initial enzyme is inhibited by end product
prevalent in amino acid biosynthetic pathways - *fig*

Balancing inhibition & stimulation
(ex: glycogen metabolism via cAMP)
epinephrine stimulated increase in cAMP, which activates PKA
converts glycogen to G-1-P
a. inhibits glycogen synthesis
b. stimulates glycogen degradation

Primary mechanism of action is altering enzyme's activity (both negative or positive*)
The Design of Metabolism... Biological Order and Cell Energy Transformations

Cells do obey laws of chemistry & physics...
cells possess potential energy by having different bonds

2 kinds of traditional energy:
1. Potential Energy: stored energy, due to mass in position
2. Kinetic Energy (energy of movement)
   ex: heat (thermal) energy which flows from higher heat or greater molecular motion to lower heat content;
   radiant energy: kinetic energy of photons (light);
   when molecules absorb light radiant → thermal chlorophyll → light → ATP in photosynthesis
   mechanical energy: push/pull of cytoskeletal filaments
   electrical energy: energy of moving electrons

Energy in cells is housed in molecules chemical bonds

Cells possess chemical potential energy

It occurs in such forms as:
- chemical concentrations gradients across membranes
can diffuse from [higher] to [lower]
- electrical gradients (potential differences) across membranes
  a separation of charge
  as much as 200,000 volts per cm

Thermodynamics: science of energy transformations

1st Law of Thermodynamics...
Energy can neither be created nor destroyed, but is convertible.
[nuclear blast - mass of U235 → heat/light]

All forms of energy are inter-convertible
& thus all are expressed in same units of measure
Joule, but biologists use more common calorie
Calorie is amount of heat needed ↑ 1gm 1°C
1 Kcal = 1,000 cal = 4,184 Joule [1 cal = 4.184 J]

2nd Law of Thermodynamics...
Entropy is commonly referred to as a measure of degree of order of the Universe,
& thus its randomness (entropy - disorder) can only increase
Entropy is maximum disorder..... “heat”
Events in the Universe have a direction → max entropy
The Rules of the Universe are simple: Cities crumble, Stars go Supernova, & we are all equilibrium...izing (dying)

Yet, WOW! ... Cells are highly ordered... wings of a bird, human eye, spider’s web and all cells - feed, grow, and differentiate

HOW... in light of the 2nd law of thermodynamics?

FOOD (light energy & covalent bond energy)

HEAT = overall increased entropy

Entropy must increase (heat); yet disorder within one part of Universe can decrease at the greater expense of the Total Surroundings.

What we need to be able to do is measure Energy in systems, esp. energy able to do work

Willard Gibbs (1839–1903) applied the principles of Thermodynamics to chemical systems to determine the energy content and changes within a chemical reaction and derived the...

FREE ENERGY EQUATIONS

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

\( \Delta G \) is a numerical measure of how far a reaction is from equilibrium
\( \Delta G \) is measure amount energy in system able to do work
(to stay away from equilibrium)...
Disorder increases (thus entropy increases) when useful energy, that which could be used to do work, is dissipated as heat...
biological systems are are ISOTHERMAL, e.g., held at constant temp/pressure

\[
\text{CHEMICAL REACTION } \text{A} \leftrightarrow \text{B} \quad \text{Which Way?}
\]

\[ \Delta G = \Delta G^0 + R T \ln \frac{[p]}{[r]} \]

J. Willard Gibbs (1839–1903)

change in free energy content of a reaction...depends upon:
1. energy is stored in molecule’s covalent bonds
2. remember, temperature is negligible... cells are isothermal, i.e.,
\[ \Delta G = \text{actual free energy} \]
\[ \Delta G^0 = \text{standard free energy \text{[change under std conditions]}} \]
\[ R = \text{gas constant \ (2 x 10^{-3} Kc/mol)} \]
\[ T = \text{absolute temp \ (-273oK)} \]
\[ \ln = \text{natural log \ (conversion log_{10} = 2.303)} \]

at equilibrium \( \Delta G = 0 \) and \( \frac{[p]}{[r]} = \text{Keq} \)
if we solve above equation for \( \Delta G^0 \) we can see relationship of Keq to \( \Delta G^0 \)
Free Energy Equation...

\[ \Delta G = \Delta G^\circ + RT \ln \frac{[P]}{[R]} \]

@ equilibrium \( \Delta G = 0 \)

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

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

@ 250°C \( -RT \ln K_{eq} = -(2.0)(298)(2.303) \lg 10 K_{eq} \)

thus............ \( \Delta G^\circ = -[1364] \lg 10 K_{eq} \)

The difference between... \( \Delta G \) and \( \Delta G^\circ \)

\( \Delta G^\circ \) is a fixed value for a given reaction & indicates in which direction that reaction will proceed under standard conditions. standard condition do not exist within a cell, thus \( \Delta G \) cab be used to predict the direction of a reaction at a given time.

\( \Delta G \) is determined by the concentrations present at that time & is a measure of how far a reaction is from equilibrium then.

Cell metabolism is essentially a non-equilibrium condition. Metabolism works by changing the relative concentrations of reactants & products to favor the progress of unfavorable reactions.

<table>
<thead>
<tr>
<th>products</th>
<th>( K_{eq} )</th>
<th>( \lg K_{eq} )</th>
<th>( \Delta G^\circ ) cal/mole</th>
<th>([\lg_{10} \times -1364])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/100</td>
<td>.001</td>
<td>10^-3</td>
<td>-2</td>
<td>+4092</td>
</tr>
<tr>
<td>1/100</td>
<td>.01</td>
<td>10^-4</td>
<td>-2</td>
<td>+2728</td>
</tr>
<tr>
<td>1/10</td>
<td>.1</td>
<td>10^-1</td>
<td>-1</td>
<td>+1364</td>
</tr>
<tr>
<td>1/1</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10/1</td>
<td>10</td>
<td>10^1</td>
<td>+1</td>
<td>-1364</td>
</tr>
<tr>
<td>100/1</td>
<td>100</td>
<td>10^2</td>
<td>+2</td>
<td>-2728</td>
</tr>
<tr>
<td>1000/1</td>
<td>1000</td>
<td>10^3</td>
<td>+3</td>
<td>-4092</td>
</tr>
</tbody>
</table>

Which way this reaction goes is dependent upon existing concentrations?

\( \Delta G^\circ = \lg_{eq} [DHAP/G3P] = 22.4 \)

\( \Delta G^\circ = -[1364] \lg_{10} 22.4 = -2728 \) cal/mole

\( \Delta G = \Delta G^\circ + RT \ln \frac{[P]}{[R]} \) but when DHAP = 0.001M & G3P = 0.1M

\( \Delta G = -1842 \text{c/m} +(-1364)(\lg_{10} 0.01) = (-1842)+(-1363)(-2) = +886 \text{ c/m} \)

Thus under standard condition the reaction is favored from G3P toward DHAP (-\( \Delta G \)), but under specific cellular condition, where the ratio of reactant & products is changed, the reaction isn’t favored & goes in other direction from DHAP to G3P

This is what happens in \textit{glycolysis}*, but the pathway shifts ratios and pulls it to G3P
CHEMICAL REACTIONS  A <-> B  Which way & Why?

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

cell respiration - (heterotrophy) - cellular burning of glucose
slower, multi-step process to capture & release
energy... as ATP

ENDERGONIC REACTION - requires input of energy for A --> B
Product [B] >>> energy Reactant [A]
ex: photosynthesis - (autotrophy)
glucose made from CO₂ + H₂O --light--> C₆H₁₂O₆
energy poor vs. energy rich

How does Metabolism create more order in chemical reactions?

COUPLED REACTION via ATP hydrolysis:
if ΔG for the reaction B + C --> D is +,
but less than the ΔG of ATP hydrolysis,
then the reaction can be driven to completion by coupling
it to the hydrolysis of ATP.

ATP hydrolysis energy can be coupled to:
conformational changes in enzyme,
as kinases, which phosphorylate proteins (add –P)
converting then from inactive to active (& vice versa);
energy gained in the stressed
conformation is released,
when the protein relaxes.

Design of Metabolism:

2 Categories of metabolic reactions

Catabolic - cell respiration in heterotrophs  fig 3.3
oxidation (removal) of e-‘s from foodstuffs
3 steps: 1. Digestion of polymers (foods) into monomers
2. GLYCO-LYSIS --> AcoA  splits sugar monomers
3. Oxidation of AcoA --> CO₂ + NADH --> H₂O
   ADP + P --> ATP

Anabolic - biosynthesis in autotrophs

coupling reactions that are energetically unfavorable
with reactions that are energetically favored
done by linking hydrolysis of ATP (favored) to reactions
linking atoms together (not favored), creating new biological order

FREE ENERGY EQUATIONS  ΔG = ΔH - T ΔS
a numerical measure of how far a reaction is from equilibrium
Design of Metabolism... or how biological order comes about
Organisms are classified by the nutritional habits...

**Autotrophs:**
- light energy... is converted into covalent chemical bond energy

\[
\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\text{e}^-} \text{NADPH} + \text{ATP} \\
\text{H}^+ & \quad \text{oxidized form} \\
\text{CO}_2 & \quad \text{reduced form} \\
\end{align*}
\]

**Heterotrophs:**
- food stuffs more energetically stable

\[
\begin{align*}
[\text{CH}_2\text{O}]_n + \text{NAD}^+ & \xrightarrow{\text{CO}_2 + \text{H}_2\text{O} + \text{ATP} + \text{NADH}} \\
\text{Key Cell energy intermediates} & \quad \text{NADH, NADPH, FAD, \& ATP}^* \\
\end{align*}
\]

**OXIDATION / REDUCTION - Redox Reactions**
- e- \&/or H+ transferred between oxidized & reduced forms

\[
\begin{align*}
\text{AH} & \xleftrightarrow{\text{oxidation}} \quad \text{A} + \text{e}^- + \text{H}^+ \\
\text{reduction} & \quad \text{gain of e- \& often a proton, H}^+ \\
\end{align*}
\]

\[
\begin{align*}
\text{6O}_2 + \text{C}_6\text{H}_{12}\text{O}_6 & \xrightarrow{\text{NAD}^+ \text{respiration}} \quad \text{6CO}_2 + 6\text{H}_2\text{O} \\
\text{NADPH} & \quad \text{photosynthesis} \\
\end{align*}
\]

**KEY METABOLIC REACTIONS:**

6 major categories of bio-chemical reactivity
Bio-chemical reactivity is bond breaking & reforming
these are violent events inside cells, carefully controlled by ENZYMES

1. redox reaction (oxid/reduction) PGAlld + NAD+ \xleftrightarrow{} 1,3di-PGA + NADH
2. functional group transfers glu + ATP \xleftrightarrow{} G6P + ADP
3. Hydrolysis glu-glun(n) + H2O \xleftrightarrow{} glu-glun(n-1)
4. C-C breaking or re-formation fruc1-6bP \xleftrightarrow{} DHAP + 3PGAld
5. rearrangement (isomerizations) glucose-6P \xleftrightarrow{} fructose-6P
6. Condensations protein(n) + aa1 \xleftrightarrow{} protein(n+1) + H2O
Bil 255 – CMB

how cells make ATP

CELLULAR ENERGETICS

How Cells Make ATP
- **Autotrophic Metabolism**
  - Photosynthesis
  - Photophosphorylation
- **Heterotrophic Metabolism**
  - Cell Respiration
    - Oxidation of Foods
      - Aerobic & Anaerobic
      - Oxidative Phosphorylation

... primarily by phosphorylation

Primary Mechanisms of Phosphorylation
- **Substrate Level Phosphorylation**
  - mcb 12.3 pg 482
  - steps 7 & 10
- **Chemiosmosis** (Oxidative Phosphorylation)
  - subst-H + NAD \( \rightarrow \) NADH + subst
  - NADH \( \rightarrow \) H+ proton motive force \( \rightarrow \) ATP
- **Photosynthetic Phosphorylation**
  - light + NADP \( \rightarrow \) NADPH \( \rightarrow \) H+

Key metabolic reaction = REDOX Reaction
- \( AH + BO \rightarrow A + BOH \)
- \( C_{6}H_{12}O_{6} + 6O_{2} \rightarrow 6CO_{2} + 6H_{2}O + e\)'s

Cellular Respiration

Evolution of aerobic metabolism was a major step in the history of life on planet Earth

**Cell Respiration** - series cytoplasmic & mitochondrial
- stepwise OXIDATION food molecules - makes ATP
  - physiological view: uptake of \( O_{2} \) & release of \( CO_{2} \)
  - biochemical view: \( O_{2} \) consumption, \( CO_{2} \) production

3 Stages:
1. Digestion - food polymers \( \rightarrow \) monomers
2. Production of AcoA \( \rightarrow \) glycolysis & FAoxidation
3. Oxidation of AcoA to \( CO_{2} \) & \( H_{2}O \) \( \rightarrow \) KC & ETC
4 Cellular Pathways:

- **Glyco-lysis**
  
  glucose --> pyruvate + NADH + ATP

- **Kreb's Cycle**
  
  AcoA --> CO₂ + NADH + 6TP +FADH₂

- **Electron Transport Chain (ETC)**
  
  passage of e’s from NADH to O₂ --> H₂O + H⁺ gradient

- **ATP synthase**
  
  mitochondrial membrane protein which makes ATP as H⁺ move into mitoplasm

---

**GLYCO-LYSIS**

Embden, Meyerhof, Parnas Pathway

Greek (glykos) - "sweet" + "splitting"

- anaerobic = no requirement of oxygen
- cytoplasmic location

- 10 step enzymatic pathway
  
  hexose --> 2 PYR + 4ATP (2 net) + 2NADH
  
  * energy investment phase (coupled Rx’s)
    
    phosphorylation of low energy intermediates
  
  * energy capture phase [mbc12.3 steps 6 & 7 & 10]
  
  * redox reaction (glyceraldehyde3-PDH)
  
  * substrate level phosphorylation

---

**GLYCO-LYSIS** and Ancillary Pathways

Fates of PYRUVATE

if anaerobic - 1. alcoholic fermentation via alcohol dehydrogenase

2. lactic acid respiration - LDH

if aerobic - Krebs Cycle

**Shuttles**

purpose to move e’s from cytoplasmic NADH to mitochondrial NADH or FADH₂

- glycerol-P shuttle - skeletal muscle/brain (FADH₂)
- malate shuttle - liver, kidney, heart muscle (NADH)

---

**KEY REACTIONS of GLYCOLYSIS** (panel 13.1)

substrate level phosphorylation (steps 7 & 10)

redox reaction involving NAD (step 6)

**Summary of GLYCOLYSIS**

2 ATP to initiate pathway

2 substrate level phosphorylations makes 2 ATP (net),

2 NADH, and

2 PYRUVATE

Fermentations & Shuttles
**Krebs Cycle, Citric Acid Cycle, Tricarboxylic Acid Cycle**

A cyclic biochemical pathway resulting in aerobic oxidation of cell fuels, as CH₂O, fatty acids, & amino acids, while making CO₂, H₂O, & ATP.

**HISTORY**

1910's - enzymatic nature learned - dehydrogenases
1930's - substrates identified = di-COOH's
experiments on minced flight muscle prep's
1937 - Sir Hans Krebs - citrate synthetase
acetyl-coA + OAA --> citrate + CoASH
1948 - cycle localized within the Mitochondria
1961 - Peter Mitchell proposes Chemiosmosis

**Overall reaction:**

acetyl-CoA + 3NAD + E-FAD + GDP + P + 2H₂O -->
CoASH + 3NADH + E-FADH₂ + GTP + 2CO₂

**ENZYMES of KREBS CYCLE**

- 5 dehydrogenases - ISDH, αKGDH, SDH, MDH, & PDH
- 2 hydrolyases - aconitate & fumarase
- 1 thio-kinase - succinyl thio-kinase
- 1 synthetase - citrate synthetase
- 2 multi-enzyme complexes (4.8 p118)
each with 60 proteins & 5 coenzymes
  1. pyruvate dehydrogenase &
  2. alpha ketoglutarate dehydrogenase

**Pyruvate Dehydrogenase Complex**

Oxidative decarboxylation of alpha-Keto acid

\[
\begin{align*}
\text{HOOC-C-CH₃} & \quad \rightarrow \quad \text{CoA-S-C-CH₃} + \text{CO₂} \\
\text{O} & \quad \text{O}
\end{align*}
\]

3 enzymes (4.8 p118)

- a. pyruvate decarboxylase
  12 dimers = 24 identical subunits
- b. dihydrolipoyl transacylase (reductase)
  8 trimers = 24 identical subunits,
each 3 lipoates
- c. dihydrolipoyl dehydrogenase
  6 dimers 12 subunits with FAD

**Key Metabolic Reactions of KREBS CYCLE**

NAD is reduced
substrate level phosphorylation occurs
decarboxylation [-COOH]
acylation via CoASH

Each turn of the cycle
4 protons passed to coe’s (3NADH & 1 FADH₂)
2 CO₂’s are released

3 parts of Mitochondrial Oxidation of PYR
1. PYR --> CO₂ + H₂O --> NAD/FADH₂ → Krebs
2. e⁻ of NAD/FADH₂ --> O₂ to make H₂O → ETS
3. ADP + P --> ATP → Chemiosmosis
Mechanism of Action of PDH Complex

A: pyruvate dehydrogenase – thiamine pyrophosphate (TPP) removes COOH from pyruvate leaving 2 carbon fragment bound to the TPP.

B: lipoamide reductase transacetylase – lipoate the 2 carbon group is transferred to one lipoamide arm, and then the other to position for CoASH transfer.

C: dihydrolipoyl dehydrogenase - CoASH, FAD, NAD+ acetyl group is transferred to CoASH; the reduced lipoamides transfer 2H's to FAD --> FADH2, and FADH2 passes H's to NAD+ --> NAD

Key Metabolic Reactions of KREBS CYCLE [+ PDH reaction]

1. NAD is reduced (NADH)
2. substrate level phosphorylation occurs GDP + P --> GTP (equivalent to ATP)
3. decarboxylation (-COOH)
4. acylation via CoASH (ACoA)

Each turn of the cycle:
4 protons passed to coe's (3 NADH & 1 FADH2)
2 CO2's are released
1 GDP is phosphorylated to GTP (equivalent to ATP)

fig mcb6e 13.2
FATTY ACID Metabolism [beta-oxidation]

Oxidation Fatty Acids to Acetyl-CoA

3 Steps in Fat Oxidation Cycle

1. oxidation of COOH end of free fatty acid
2. transport of fatty acyl-CoA into mitoplasm
3. oxidation of 2 carbon fragments as AcoA

4 enzymes of beta-oxidation

1. **fatty acyl-coA ligase** (on outer mito. membranes)
   
   \[
   FA-COOH + ATP + CoASH \rightarrow FAcOA + AMP + PP
   \]

   converts cytoplasmic FA to Fatty-acyl-coA

2. **carnitine acyl-transferase 1** (outer mito memb.)
   
   \[
   \text{fattyCoA} + \text{carnitine} \leftrightarrow \text{Fatty acyl-carnitine} + \text{CoASH}
   \]

   transfers FAcOA to carnitine for transport across mito

3. **carnitine acyl-tranferase 2** (in mitoplasm)
   
   \[
   \text{fatty acyl-carnitine} + \text{CoASH} \leftrightarrow \text{FAcoA} + \text{carnitine}
   \]

   releases FAcOA inside the mitoplasm

4. **fatty acyl-coA dehydrogenase**
   
   "Beta-Oxidation Cycle"

   Four steps for these dehydrogenase enzymes...
   
   a) dehydrogenation w FAD --> FADH$_2$
   b) hydration - addition of water
   c) dehydration w NAD --> NADH
   d) thiol cleavage w CoASH

   - releases a 2c piece = AcoA

   **Net result**: each turn of the cycle shortens a long chain fatty acid by 2 carbons generating 1 AcoA, 1 NADH and 1 FADH$_2$

Balance Sheet Aerobic Oxidation glucose vs 6C FFA

Rule of Thumb... the P to O ratio

- 1 NADH (via mito ETC) = 3 ATP
- 1 FADH$_2$ = 2 ATP

<table>
<thead>
<tr>
<th>Cell Respiration</th>
<th>glucose</th>
<th>beta-oxidation 6C-FFA (c-c-c-c-c-c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>to start - GLYCOLYSIS</td>
<td>-2 ATP</td>
<td>-1 ATP @ ligase</td>
</tr>
<tr>
<td>glyceraldehyde DH</td>
<td>+ 2 NADH</td>
<td></td>
</tr>
<tr>
<td>pyruvate kinase (via SLP)</td>
<td>+ 2 ATP</td>
<td></td>
</tr>
<tr>
<td>Krebs Cycle per each PYR</td>
<td></td>
<td>per 2 cycles @ Fatty-AcoA-DH</td>
</tr>
<tr>
<td>PDH -2CO$_2$ per each AcoA</td>
<td>+ 2 NADH</td>
<td>+ 3 AcoA</td>
</tr>
<tr>
<td>ISDH -2CO$_2$</td>
<td>+ 2 NADH</td>
<td>+ 2 FADH$_2$ = 4 ATP</td>
</tr>
<tr>
<td>KGDH -2CO$_2$</td>
<td>+ 2 NADH</td>
<td>+ 2 NADH = 6 ATP</td>
</tr>
<tr>
<td>thiolkinase</td>
<td>+ 2 GTP</td>
<td>+ 10 ATP</td>
</tr>
<tr>
<td>SDH</td>
<td>+ 2 FADH$_2$</td>
<td></td>
</tr>
<tr>
<td>MDH</td>
<td>+ 2 NADH</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>6C FFA</td>
</tr>
<tr>
<td>1 glucose = 2 PYR = 2 AcoA</td>
<td></td>
<td>8-OX = 10 ATP</td>
</tr>
<tr>
<td>-6CO$_2$ + 2ATP + 10NADH + 2FADH$_2$ + 2GTP</td>
<td></td>
<td>Kreb's (3 AcoA) = 72 ATP</td>
</tr>
<tr>
<td>+ 82 ATP</td>
<td></td>
<td>+ 82 ATP</td>
</tr>
<tr>
<td>total ATP</td>
<td>= 36-38</td>
<td>82 - 1 = 81 - 38 = + 43 ATP net</td>
</tr>
<tr>
<td>ATP via 2 AcoA alone</td>
<td>= 24</td>
<td></td>
</tr>
</tbody>
</table>

Net result: each turn of the cycle shortens a long chain fatty acid by 2 carbons generating 1 AcoA, 1 NADH and 1 FADH$_2$
Regulation of Krebs Cycle

controls flow of intermediates [in & out] - 4.18 & 4.19
substrate availability - mass action
allosteric inhibition - PFK-1 & feedback inhibition model
covalent modification - reversible phosphorylation...
protein kinases & phosphoprotein phosphatases

4 key enzymes are involved in regulation...
PDH SER-P by kinase - inactive
citrate synthetase +ADP - ATP/NADH/cit/ScoA
isocitrate dehydrogenase +ADP/Ca²⁺ - ATP
alpha-keto glutarate dehydrogenase +Ca²⁺ - ScoA & NADH
Mitochondrial Membrane Transport

membrane = *impermeant* to most everything, esp to H+
outer membrane - porins - molecules 5,000 -10,000d
inner membrane - 70% protein & 30% lipid
holds
  a. redox proteins of ETC
  b. ATP synthase
  c. carrier proteins-
      phosphate translocases
      ADP/ATP translocases,
      pyruvate/H+ symporter 13.16
  d. glycerol-P & malate shuttles

How Electron Transfer Works

- **REDOX** POTENTIAL (how measured – panel 13.1)
  empirical measure of tendency to gain e’s
  - strong reducing agent has negative $-\Delta E_0'$
  - strong oxidizing agent has positive $+\Delta E_0'$

$$\Delta G_0' = -nF \Delta E_0'$$

<table>
<thead>
<tr>
<th>Chemical Reaction</th>
<th>$\Delta G_0'$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH $\leftrightarrow$ NAD$^+ + H^+ + 2e^-$</td>
<td>-0.32V</td>
</tr>
<tr>
<td>H$_2$O $\leftrightarrow$ O$_2 + 2H^+ + 2e^-$</td>
<td>+0.82V</td>
</tr>
</tbody>
</table>

$$\Delta G_0' = -(1)(0.023)(1.14) = - 26.2 \text{ Kcal}$$

- **Electron Transfer Chain’s Order**
  -> Increasing Redox Potential (from - to +)
  see fig 12.18 p500
Components of the ETC

- **Pyridine nucleotides** NAD+ 2.33
  - enzyme bound hydrogen carriers
  - accepts 2e’s and/or protons
  - shows spectral shift @ 340nm

- **Flavoproteins** FMN & FAD 2.33b
  - protein bound hydrogen carriers
  - spectral shift @ 340, 370, & 460 nm

- **Iron sulfur proteins** FeS 12.14 p495
  - non-heme iron electron carriers

- **Ubiquinone** CoQ 12.15 p496
  - semiquinone & hydroquinone
  - mobile membrane bound non-protein hydrogen carriers

- **Cytochromes** (a, a3, b562, b566, c1, c) 12.14 p495 & above
  - "colored proteins" with bound Fe atoms [ferric vs. ferrous]
  - iron porphyrin (heme) bound protein carriers

How Oxidative Phosphorylation Works - fig 12.18 p500

Respiratory Assemblies - Mitochondrial Components

**Respiratory Assemblies:**
- NADH-Q reductase
- Succinate dehydrogenase
- Cytochrome-C-Reductase
- Cytochrome Oxidase

**Proton Motive Force:**
- an electrochemical concentration gradient of protons across a membrane coupled to ATP synthase to make ATP... likened to process of **osmosis**, the diffusion of water across a membrane thus **chemiosmosis**.

**ATP Synthase:** creates a hydrophobic channel for H+ flow makes 100 ATP per 300 H+ per sec [ADP + Pi --- ATP]

Fo – membrane piece & stalk
F1 – soluble piece; 5 proteins rotational models

Oxidative Phosphorylation - Making of ATP

**Synthesis of ATP made via a proton motive force**
H+ gradient generated by transfer of e’s
H’s passed to O2 to make H2O through series of redox proteins

**Mechanism - Chemiosmotic Coupling - Mitchell 1961**
- fundamental mechanism - arose early in evolution - was retained

**3 steps** fig 12.22
- ETC - passage of e thru membrane carrier proteins
  - electron flow (hydride ion H+ --- H+ + 2e-)
  - generates a proton motive force gradient (pH difference)
  - pH = 1.0 units [8.0 matrix vs. 7.0 peri-mito. space]
  - & a membrane potential - charge [140mV in(-) out(+)]
- ATP Synthase - which links ADP & P... making ATP
  - uncouplers as DNP destroy H+ gradient = no ATP
## ATP Synthase Structure...

A mushroom-shaped complex composed of 2 membrane subunits

### F1 (extrinsic) & F0 (intrinsic)

- **ATP synthase of liver mitochondria**
  - About 15,000 present

- **F1**
  - 5 polypeptides (nuclear DNA):
    - 3α, 3β, 1γ, 1δ, 1ε
  - Arranged like sections of grapefruit
  - 3 catalytic sites for ATP synthesis
    - One on each β subunit

- **F0**
  - 3 polypeptides in ratio of:
    - 1a, 2b, and 12c (C-ring)

### Binding Charge Mechanism of ATP Synthesis - A Rotary Motor

Paul Boyer – 1979

1. H+ movement changes binding affinity of synthase's active site, thus when ADP & P bind to active site, they readily condense into ATP (removed from aqueous solution $K_{eq} = 1$ and $\Delta G$ close to zero, thus ATP forms easily)

2. Active site (β subunits) changes conformation thru 3 successive shapes:
   - L - loose - ADP & P loosely bound to site
   - T - tight - ADP & P tightly bound favoring condensation without water
   - O - open - site has low affinity to bind ATP - thus releases it

3. Conformational changes result in rotation of subunits relative to central stalk (γ)
   - α & β subunits of F1 form hexagonal ring that rotates around central axis
   - γ stalk extends from Fo & interacts with 3 β's differently as it rotates 360°

### Pathway of the Protons through Fo

- Rotational model of C-ring & γ stalk

- 12 C-proteins reside in lipid bilayer (C-ring)
  - C-ring is attached to γ stalk of F1
  - H+ diffuse through Fo rotating the 12c's of Fo ring
  - Each C protein has a half-channel space with a charged ASP-
  - C's bind H+ (& via shape changes) C-rotates 30° CCW
  - Next C in ring picks up H+ & thus the ring cycles thru 360°
  - Release of H+ into matrix happens at end of cycle

- 4 H+ moves ring 120° (γ stalk) shifts 120° → β's change
- 4 H+ result in ATP being made

- Rotation of C-ring drives γ stalk through 360° & 3 conformations of F1 (L-T-O) to make ATP

### Biovisions animation of ATP synthase
Bil 255 - CMB

**PHOTOSYNTHESIS**

- **Light driven phosphorylation** - production of ATP via photo-phosphorylation
- **Cellular process** - bacteria, blue-green, and eucaryotic cells with chloroplasts
- **Capture of light energy by pigments** - chlorophylls & accessory pigments
- **Capture e's as reducing power in NADPH**
- **Reduction of CO₂ to CH₂O**

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**2 Fundamental Reaction Mechanisms**

**LIGHT Reactions** (photo-chemical reactions)
- Molecular excitation of chlorophyll by light... charge separation
- Generation of proton motive force (H⁺ gradient)
- Reduction of NADP to NADPH via an ETS

**DARK Reactions** (thermo-chemical reactions)
- CO₂ fixation (reduction) stages
  - Carboxylation: CO₂ + RuBP → 2 PGA
  - Reduction: PGA + NADPH → PGAL
  - Regeneration of RuBP via HMP path → RuBP

**Evolutionary Basis of Photosynthesis**

1st autotrophic cells probably used H₂S as e- source

- **Purple-Sulfur bacteria of today**
  \[ \text{CO}_2 + \text{H}_2\text{S} \rightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{S} \]
- **Cyanobacteria - oxygenic photosynthetic procaryotes**
  \[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} + \text{O}_2 \]

**Van Neil equation** [gs@SU] Phts is a REDOX reaction

\[ \text{CO}_2 + \text{H}_2\text{A} \rightarrow \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{A} \]
Molecular Excitation of Chlorophyll

- **Absorption of Light Energy**
  - Blue light [440nm] = 71.5 Kc/einstein
  - Red light [700nm] = 40.9 Kc/einstein

- **Ground State**
  - Paired e's with opposite spin = stability
  - Absorption moves non-bounded e's to higher orbitals
  - 1st excited singlet state
  - 2nd excited singlet state
  - 1st long-lived state

FATES of Absorbed Light Energy

1. Re-radiated as **vibrational heat**
2. Re-radiated as **fluorescence**
   - Emission of light of longer wavelength
   - 700 nm -> 710 nm in time frame 10-9 sec less energetic
3. Re-radiated as **phosphorescence**
   - Emission of light much longer wavelength
   - 700 nm -> 720 nm in real time (1 sec)
4. **Induced resonance**
   - Vibrational e excitation inducing like vibrations in adjacent molecules causing their excitation
5. **Photoionization**
   - Enters into the photochemical reactions
   - Loses electron to acceptor = ionized chl+

Photosynthetic Electron Flow - stages of photosynthesis

Photosystems – LHC: chlorophylls, reaction center, primary acceptor

Photosystems 1 and 2 - path of e- flow (cyclic vs. non-cyclic)

Release of O₂ & capture of e- into coenzyme NADP⁺ → NADPH

ATPase makes ATP (just like in mitochondria)

Photosynthetic Electron Flow - stages of photosynthesis

Photosynthetic Proton Motive Force

The extrusion of protons via electron transport creates a proton concentration gradient across thylakoid membranes & an ATP synthase uses this gradient to energize the synthesis of ATP.
Dark Reactions of Photosynthesis

- occur in stroma (chloroplasm)
- consume ATP and NADPH made in light reactions
- reduces (fixes) CO₂ into CH₂O (sugars)

3 different pathways to make sugar

**C₃ – CALVIN cycle**
- 1 CO₂ + 5C RuBP --- (2) 3C sugars (PGA)
- (2) 3C sugars combine ---> 1 net glucose
- RuBP carboxylase [50% of leaf protein]
- Photo-respiration
  - inhibition by O₂

**C₄ – Hatch & Slack pathway**
- 4C acid --- 3C + CO₂ in bundle sheath
- 1 CO₂ + 3C PGA --> 4C acid (mesophyll cells)
- CO₂ into Calvin cycle (as above)

**CAM Pathway (C₄- Crassulacean Acid Metabolism)**
- CAM plants... are also C₄ plants but do not separate C₄ & C₃ pathways in different parts of the leaf (spatial), but rather separate them in time instead. CAM was 1st studied in members of the plant family Crassulaceae.

**At night**, CAM plants take in CO₂ through open stomata at night, when the succulents are in a cool environment. The CO₂ joins with PEP to form the 4-carbon oxaloacetic acid. OAA is converted to 4-carbon malic acid that accumulates during the night in the central vacuole.

**In the morning**, stomata close (thus conserving moisture as well as reducing the inward diffusion of oxygen). Accumulated malic acid leaves the vacuole and is broken down to release CO₂ that is taken up into the Calvin (C₃) cycle.

**PHOTORESPARATION**... RUBISCO catalyzes two different reactions:
- a carboxylase activity... adding CO₂ to ribulose bisphosphate -
- an oxygenase activity... adding O₂ to ribulose bisphosphate -

which one predominates depends on the relative concentrations of O₂ & CO₂ with:
- high CO₂: low O₂ favoring the carboxylase action
- high O₂: low CO₂ favoring the oxygenase action

The light reactions liberate O₂ & more O₂ dissolves in the cytosol of the cell at higher temps. Thus, high light intensities & high temps (above ~ 30°C) favor the oxygenase second reaction. The uptake of O₂ by RUBISCO forms two 3-carbon molecules:

1) one is 3-phosphoglyceric acid [3PGA] just as in the Calvin cycle
2) the other is 2P-glycolate.

and involves 3 organelles: chloroplast, peroxisome, and mitochondria.