Biology 255
CELL & MOLECULAR BIOLOGY

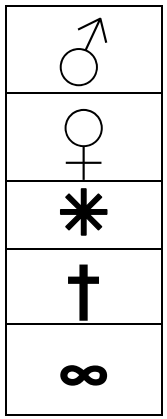
Dr. Luis Glaser and Dr. Charles Mallery

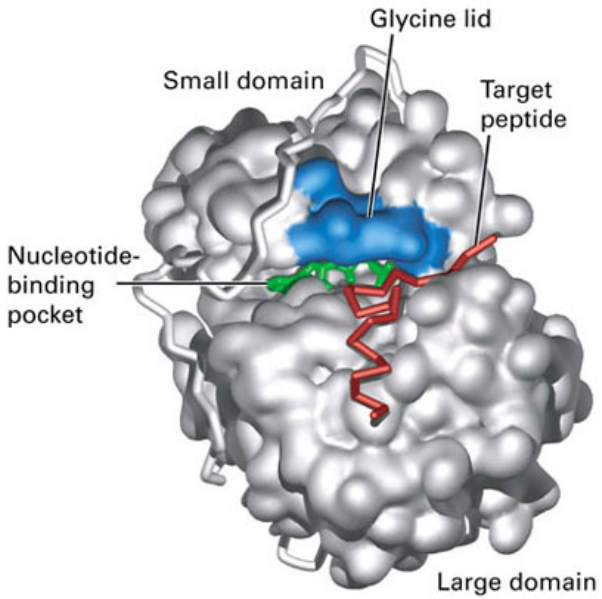
Fall 2009 - Section S : **TR 3:30pm to 4:45pm in Cox 145**

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? "

Matrin Olomucki *the chemistry of life*. NY, McGraw Hill, 1993.

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

Rene Thom, 1989.

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

[re: HGP].... **John M. Thomas**, 1994.

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

Erwin Chargaff 1989.

Bil 255
Cell & Molecular Biology

a brief description of CMB

3 credits Fall & Spring Semester & Second Summer Session

Structure, molecules, and functions of cells.

Prerequisite: One year of general biology with laboratory.

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.

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**, and **microbiology**.

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

required reading… class web link to a text description of Cell Biology

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."

[re: HGP].... John M. Thomas, 1994.

Instructors

Luis Glaser,
Ashe Bldg. 240 – Professor & Special Assistant to President.
Charles Mallery,
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

Textbook (spring 2006) - "*Molecular Cell Biology*" **6th edition**
by Lodish et al, W.H. Freeman & Co., NY (ISBN 0-7167-7601-4).

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.

Bil 255 – CMB

Introduction
a brief history of CMB

basic properties of cells
& some milestones

Bil 255 - Cell and Molecular Biology...

structure, function, & the molecules of cells with
Professors Glaser and Mallery - Spring Semester

text : Molecular Cell Biology, 6th Edition
by: Lodish et al, Publisher: W.H.Freeman, NY, 2007

read chapter 1 pg 1-30

some web resources:

The Virtual Library of Biochemistry & Cell Biology &
Access Excellence - a national biology education resource of the
National Health Museum (originally sponsored by GENENTECH, Inc.,)
it contains some of excellent graphics: AE STUDENT RESOURCES*

and Mallery's web links for CMB RESOURCES*.

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

Bil 255 - Foundations of Cell Molecular Biology

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

CMB gave us... the Central Dogma of Molecular Biology
DNA --> RNA --> Protein mcb fig 4.1*

"Life begets Life " - is now seen at the molecular level,
as the faithful replication of DNA...

CMB asks... what do all cells have in common...
the answer = "their molecules & chemical reactivity"
their biochemistry, thus
we need to understand Molecular Biology to see how life works

CMB is about energy & reactivity, movement & change, action & reaction;
almost everything that happens in life (happens in cells)...
which likely boils down to ENZYME CATALYSIS.
CMB replaces the gross anatomy and physiological studies of the 17th & 18th century,
with the biology of molecules & molecular systems in 21st century.

but as Erwin Chargaff (former Chair of Bioc @ Columbia U; pic Heineken Prize winner)
has said, " CMB... is the practice of Biochemistry without a license "

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**"...
'small, membrane bounded compartments, filled with
concentrated aqueous solutions of reactive chemicals'
"All organisms are believed to have descended from
a common ancestral cell [LUCA] selected for its better fitness
through the processes of evolution, via Natural Selection"
Some cell links Cell Theory Origins
Schleiden (pic) & Schwann

Consequences of Cell Theory

VITALISM	**vs.**	**MECHANICALISM**
living	**vs.**	**non-living**
organic	**vs.**	**non-organic**
Vital Force	**vs.**	**no vital force**

Top 10 Properties of Cells*

Cell Theory replaces Vitalism... the mainstream scientific thought of 17th century,
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.**

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 procaryotes [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 form a common progenitor[NAS-1], 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 prokaryotes 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 prokaryotic cells (fig 1.10*)
(cocci, bacilli, spirochetes)

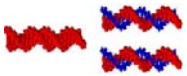
EUKARYOTIC [eu -true karyon -nucleus...]
cell plan of multi-cellular organisms
eukarya: include the fungi, algae, protozoa, slime molds, & all plants & animals.

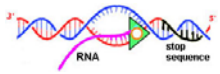
7 CHARACTERISTIC of EUCARYOTES: **panel 1-2: animal* & plant* cells**

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)
presence of **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

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**

5. **all cells regulate rate of gene transcription/translation, so only a portion of full repertoire of possible RNA's/proteins are copied, thus hereditary info dictates not only nature of cell's proteins, but also when/where they are to be made so called, differential gene activity.**
6. **all cells use protein catalysts (enzymes) to make/break covalent bonds**
   E + S <---> ES <---> E + P
7. **all cells metabolize - consume free energy and are far from chemical equilibrium. Consumption of free energy creates covalent bonds (which resist the disordering effects of thermal motion), thereby creating hereditary info in a DNA sequence.**
   the methods that cells have evolved to obtain free energy include:
   heterotrophy - oxidation of foods (covalent bonds)
   autotrophy - capture of light energy via pigments photosynthesis)
   lithotrophy - chemical electron donors provide energy
   N_2 & CO_2 are stable & unreactive & reduction to NH_2 & CH_2O uses energy
8. **All cells are enclosed in a spontaneously aggregating amphiphilic phospholipid bilayer: membranes regulate nutrient/water transfer, concentrate molecules internally.**
   All membrane have embedded protein transport molecules.

What cells types will we be looking at? [see pg 25 - 28]*
[a freshman review of Prokaryote, Eukaryote, & Virus]

Model Organisms* in Cell & Molecular research include:

Bacteriophages - virus infects bacteria; today used as cloning vectors
Escheriichia coli - bacteria common to human colon; work horse
Giardia - primitive eukaryotic cell, anaerobic protozoan cell with 2 nuclei
other eucaryotic models -
single celled - *Saccharomyces cerevisiae* - yeast [pic]
plants - mustard plants *Arabidopsis thaliana* [pic]
nematode - *Caenorhabditis elegans* - nematode [2002 Nobel]
animals - fruit fly *Drosophilia melangaster*
[Mickey] mouse - *Mus musculus* - common house mouse & its genes

Single cell culture models
for genetic & embryonic development model systems...
Hela cells (pic) - (George & Margaret Gey at JHU)
human - fibroblast- connective tissue easily grown in tissue culture
immortal stem cells (Stem Cell Journal)

Bil 255 – CMB

A Journey through the Cosmos of the Cell

Origins of Life

Have you ever seen an individual living cell ?

Hela cells: ATTC 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 pics*
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 rxs),**
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 (H_2O) - 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 rxs:

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

$$\Delta G = \Delta H - T\Delta S$$

6) Life is manifest by the absence of the living condition...

cells die: a lack of the properties of the living state is itself definitional of living state. Death is a deterministic event, because all living beings will eventually die. [death & taxes]

Cell death is the collapse of the quantum state which has allowed living matter to take energy from the environment, while preventing an increase local entropy and delaying the tendency of energy to be dispersed or diffused. Thus, death is an irreversible final state. Dead organisms will never return to life because they would be violating the Law of Entropy.

LAW OF ENTROPY CAN BE TEMPORARILY BLOCKED (Life), BUT IT CAN'T BE VIOLATED.

6) Life is manifest in a CELL - the fundamental unit of living systems.

Three fundamental cell types have evolved:
bacteria, archea, & 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)] [animal* & plant* cells (fig 9.1 pg 373 - 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
cells metabolize - capable of 1,000's of reactions (via ENZYMES – in pathways)
cells are capable of self regulation
- 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)

Origins of Cells & Life Mallery 9

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, CO_2, & CH_4

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
1953 - Miller & Urey ---> abiotic making of organics in lab experiments
> 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...

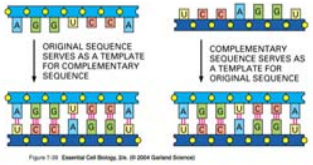
C) 1990's - astrobiological origins for biomolecules... Great 20th Century Discoveries
> Space Debris... space dust, meteorites, asteroids
may have deposited organics on newly formed planet Earth.
> Comets are mostly ice crystals on cores of silicates & carbon
contain about 10% CO, CO_2, CH_4, CH_3OH, and NH_3
> 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 rxs
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.

complementary templating*

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 vesicleized his replicase 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).

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 ???

Could basic physiochemical properties acting in elementary artificial protocells have given rise to essential cellular behaviors?

Evidence to date includes:

- fatty acids have been 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*). such "genomic fitness" may have evolved into cellular fitness.
- 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.

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 it 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. It's circular chromosome has 580,073 base pairs, the smallest known genome of any free-living organism determined. M.g. has a total of only 525 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 Genomic Research (TIGR) 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

Some (unexplained) Events in Chemical Evolution of Eukaryotes

the evolution of the eukarya was single most important step in evolution of mutlicellular life forms & was a key step that lead to plant & animal life.

1. **cell membrane encapsulates genetic DNA... development of nucleus**
   greatest evolutionary invention - it internalized the genome
2. **loss of a rigid cell wall...**
   cells developed ability of phagocytosis - allowed engulfing of foods
   also allowed cells to clump together --> multicellularity --> tissues
3. **evolve a selectively permeable membrane...**
   protects cell, allows uptake nutrients & exchange with environment
4. **evolve a cytoskeleton...**
   provides framework- allowed cell to grow larger, move, & permitted metabolism; eukarya are 10x larger that bacteria
5. **evolve aerobic respiration... more efficient energy transformation**
6. **develop various organelles... (maybe by endosymbiosis)...**
   a sub-cell part that catalyzes a specific metabolic function
7. **development of sexual cell cycles... (transposons - moveable genes)...**
   a method to shuffle genes along chromosomes favored cellular evolution.

BIL 255 – CMB

Mircroscopy
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 Abbe 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 GPF (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 tissues, or biological 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)
1998 Live Cell Imaging confocal microscopy by PerkinElmer, Inc. (image of scope)
2007 Live Cell Video Microscopy (5.5 min - view at home)

Electron Microscopy

mcb fig 9.20* ecb panel 1.1

TEM 1931 Ruska - 1st transmission EM TEM-photo & mcb6eFig1.2b* & mcb9.5a*
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)
2000 computer image averaging allows 3D modeling - models of ribosome & Ca pump*

fFEM (cryoelectron microscopy) - an aqueous specimen is frozen in liquid N_2 (-196^{0}C)
1964 Steere & Muhlethaler - develops freeze fracture EM - prep* & pics (scroll down)
2004 cryoelectron tomography – specimen rotated in electron beam & individual images are computationally fit into 3D reconstruction (tomogram) - nuclear pores*

SEM (scanning electron microscopy) - neuron* & virtual 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

Tagging - 1981 antibody tagging with gold particle in electron microscopy - fig 9.21*
1974 Nobel Prize to G. Palade, C. deDuve, A. Claude - for their "inner workings of cells"

Interpret EM's* & Microscopy provides for size relationship analyses*

RESULTS of MICROSCOPY...
Investigations of Cells - some major EUKARYOTIC ORGANELLES
a tour through a Virtual Cell*

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 microscopy uses a focused electron beam on fixed sectioned of 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.

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

Double Membrane Bound Organelles:

1. **nucleus**...
synthesizes DNA, rRNA, tRNA, primary transcript (mRNA preccursor)
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 (mcb 8.20a*)
- regulates nucleoplasm-cytoplasm exchange
via NLS of 7 aa sequence @ C-terminus (pro-lys-lys-lys-arg-lys-val)

nucleolus - regions of rDNA that makes rRNA

nucleoplasm - 'cytoplasm' of the nucleus

heterochromatin - condensed (**dark EM color**) = inactive DNA mcb6.33a*
euchromatin - non-condensed (light EM color) = active DNA

lamins - fibrous proteins adjacent to inner nuclear membrane
- form frame for nuclear shape

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*) pic1 & pic2

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 **chlorophyllous** pigments
   converts light energy into ATP & NADPH (chemiosmosis)
   reduces CO_2 into CH_2O

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 mcb9.5*
   - 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 mcb9.5b*

   medial - where modifications (glycosylations) occur

   trans - vesicle packages & budded off here for secretion mcb9.6*

Single Membrane Bound Organelles:

6. **endosomes**... membrane bound vesicles of ectra-cellular milieu internalized by ENDOCYTOSIS
   a. **endocytosis** - cathrin 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
   mcb9.2a & endosomes & lysosomes
7. **lysosomes**... several hundred single membrane bound vesicles (exclusive to animals- **plants use vacuoles**)
   have acid pH environment to help denature proteins
   (H+ATPases* & Cl transporters --> HCl)
   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 ganglosides, glycolipids 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. mcb9.7*
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 O_2 to oxidize (removes e-'s) from molecules as H_2O_2 (& other toxins). degrade FA's to acetyl groups - used to make cholesterols (esp. impt in liver/kidney cells).
X-linked adrenoleukpdystrophy (ADL): no FA digestion occurs, leads to several neuro-linked defects & death. mcb9.4*

plants contain **glyoxysomes** which oxidize lipids (very similar to peroxisomes).

Mallery Microscopy & Methods in CMB 13

Bil 255 - CMB

Molecules of Living Systems
Chemical Makeup of Cells

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.

Molecules of Living Systems

chapter 2 pg 31-54

the chemical properties of the living state

The structure of biological molecules
how their shape determines the roles they play in the complex chemical processes of life.

Even the most complicated biological molecules can be divided into smaller and smaller functional groups

REDUCTIONISM

A Web Resource that gives 3-D shapes of Biomolecules

A Site of the Molecules of Life mwk

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 30 of 92 elements OCCUR IN LIVING SYSTEMS...
- 99% of LIVING MATTER is made of C H O N P S
  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 majors groups of small biomolecules:

a. SUGARS - compounds with formula $[CH_2O]_n$

aldoses vs. ketoses*, rings*, α & ß-links*,
isomers: glucose vs. galactose*
glucose + glucose = mono-, disacc-*, tri-, poly- saccharides*
& long chain polymers of monosaccharides

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

c. NUCLEOTIDES* parts
nitrogen containing "ring" compounds…
a nitrogenous-base linked to a 5-carbon sugar
(ribose & deoxyribose) & a phosphate
pyrimidines = **C, T, U** purines = **A, G**

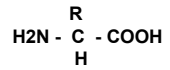
nucleotides form the energy rich compounds
of cells (as ATP & GTP), as well as the nucleic acids.

d. AMINO ACIDS* pics 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



20 common amino acids – mcb 2.14 p42 & panel 2.5

lys-arg-his-asp-glu-ala-val-leu-ile-pro-phe-met-trp-gly-cys-ser-thr-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.

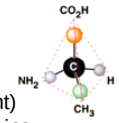
4-hydroxy proline	**occurs in plant cell wall proteins**
5-hydroxy lysine	**occurs in fibrous proteins as collagen**
N-methyl lysine	**occurs in myosin contractile proteins**
γ-carboxy glutamate	**occurs in prothrombin**

Amino Acids... structures & chemical properties of AA's [m.w.king]

1st amino acid discovered was **asparagine** (1806 in asparagus)
last 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

classes of amino acids [classified... by R-Groups]

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**

Peptide Bond...

formed by condensation reaction between
amino of one aa... & carboxyl of another aa ... mcb 6e fig 3.3

substituted amide covalent bond –

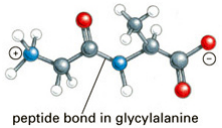
dipeptide has partial double bond character –

shorter & stronger than C-C longer, yet weaker than C=C

no free rotation (group in same plane, but TRANS)

results is zig-zag planar molecule figure*

peptide bonds* & a polypeptide*



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 **a**-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]

insulin - two polypeptides... controls carbohydrate metabolism 1. alpha chain of 30 aa's & 2. beta chain of 21 aa
glucagon -pancreatic hormone 29 aa... opposes insulin action
Nutra Sweet - a dipeptide (2aa) of L-aspartyl-phenylalanyl-methyl
corticotropin - 39aa - anterior pituitary hormone... stimulates adrenal cortex
oxytocin - 9aa - hormone of posterior pituitary... stimulates uterine contractions
bradykinin - 9aa – hormone acts on smooth muscle... vasodilatation/inflammation
angiotensin octapeptide (derived from angiotensinogen by kidney enzyme renin) - increases blood pressure ACE Inhibitors block AT & lower bp. [sport]
thyrotropin relasing factor (TSH) 3 aa's of hypothalmus... - stimulates thyroid to release thyroid hormone
enkephalins - either of two penta-peptides with opiate & analgesic activity, occurs naturally in brain & have marked affinity for opiate receptors… compare endorphins

Amino Acids Mallery 13

Biological Activity & Shapes of Molecules:

Structural Chemistry: orientation of covalent bonds in space.

molecular configuration results in specific bond angles and molecular geometry

methane	**CH_4**	109.5°	- a tetrahedron	with free rotation
formaldehyde	**$H_2C=O$**	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 (tetrathedron)
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
chiral animation

stereoisomers may have mostly identical chemical properties,
but often rotate plane of polarized light via different angles.
LEVOROTARY* (L) - rotate light left (**- negative optical rotation**)
DEXTRORTATORY (D) - rotate light right (**+ positive optical rotation**)

and likely have **different BIOLOGICAL ACTIVITY...**
Parkinson's Disease **& dihydroxyphenylalanine L-DOPA** figure*

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]*

BIOLOGICAL ACTIVITY & the Shape of Biomolecules

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

see Panel 2.7 pg 78

Non-covalent Electrostatic Interactions*... (in the 10-150 cal/mol)

IONIC bonds* - charged small ions (atoms which gained/lost e-'s) which attract (+/-); w/o water they are very strong (crystals of NaCl)
DIPOLES* - attractions via asymmetrical, internal distribution of charges in a molecule, which has no net charge (opposite poles +/- attract)
DISPERSION* (van der Waal's) 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-'s on an acceptor group: O-H & N-H with O- & N-

Covalent Molecular Forces of Life

see table 2.1 pg 46

TYPE of BOND	ENERGY (Kc/mol)
SINGLE COVALENT BONDS	
O - H	110
H - H	104
C - H	99
C - O	84
C - C	83
S - H	81
C - N	70
C - S	62
DOUBLE BONDS	
C = O	170
C = N	147
C = C	146

TYPE of INTERACTIONS	ENERGY (Kc/mol)
NON-COVALENT BONDS	
IONIC BONDS	1.0 - 5.0
HYDROGEN BONDS	1.0 - 2.0
VANDER WAALS	0.1 - 1.0
HYDROPHOBIC	0.1 - 1.0

mcb 6e fig 2.6 bond energies

Biological Design
or How Weak Molecular Forces & Shape Build Form

Is there 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 (???)

...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 stabilizedby 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

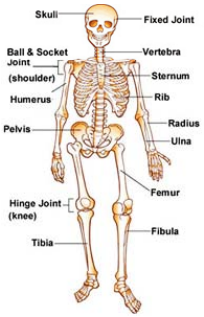
- Prestress Structures **(Snelson pic) -**

  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]
Donald Ingber's Tensegrity Model of a Cell

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 & conformation result in biologically active molecules
   figure 2.1a
2. small molecules are the building blocks of larger molecules
   monomers make **polymers**, then supramolecular complexes, then **organelles**.
   fig 2.1b

items 3 and 4 below will be covered under metabolism.

3. chemical reaction are reversible depending on rate constants and the [P] & [R]
   fig 2.1c
4. source of cellular chemical energy is the hydrolysis of ATP, when high energy phosphoanhydride bonds are broken by addition of water (hydrolysis).
   fig 2.1d

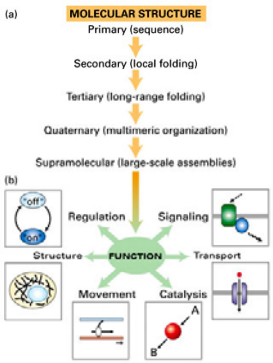
Mallery Molecules of Cells 13

Bil 255 – CMB

proteins & their properties

The work horses of cell metabolism

PROTEINS... work horses of cell metabolism



PROTEOME:

entire complement of an organisms proteins:
yeast ≈ 6,000 proteins
human ≈ 32,000 proteins

We'll look at how **Structure gives rise to Function**

a) structure: primary, secondary, teritary, & quarternary
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 of ligaments (tropoelastin), keratin of hair, feathers, & nails, fibroin 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.

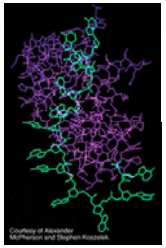
Nomenclature - classes of proteins

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

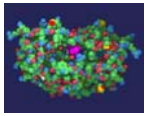
Common terminology:

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 20^{300} different linear arrays of aa's
that's [10^{390} different proteins]



1st protein sequenced was Beef Insulin *
by Fred Sanger - 1958 Nobel Prize winner
2 polypeptides [21/30 aa's] Humulin & ADA

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 (lysozyme)
repeated peptide bonds form the back bone 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
H_2N-C-C-N-C-C-N-C-C-N-C-C-N-C-C-N-C-COOH

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 environs interactions

Primary sequence… & some consequences

Polymorphism… proteins may vary in primary sequence but have the same function. ex: enzymes H_2O_2 --> 2 H_2O + 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)

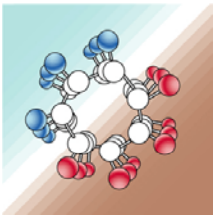
Homologous Proteins: evolved in related fashion & perform the same cellular function in different species ex: cytochrome-C :
in duck & chickens = 2 variants & in yeast & horses = 48 variants

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
- R-groups radiate outward
- 3.6 aa per 360° turn
- single repeat turn of helix (360°) = 0.54 nm
- right handed helix - (counterclockwise)
- helix formed from H-bond interactions
- H^+ of N (of any aa) & $-C=O^-$ (of 4th aa)
- ¼ of aa's in globular proteins occur in alpha helix
- flexible - wool is stretchable (breaks H-bonds)



mcb fig 3.4

Secondary structure

- BETA SHEET (fig 4.10) (model = silk protein fibroin)
a linear extended ZIG-ZAG pleated sheet formed by H-bonds
intra- & inter-chain

resist pulling (tensile) forces = strength of silk fibers
non- α/β regions = hinges, turns, loops, etc = flexibility
ribbons & sheets*

turns - mcb 3.6

MOTIFS combos of recurring arrangements of **α**-helix and/or **β**-sheets in unrelated proteins.... such as:
hairpin beta motif... antiparallel beta-sheets joined by

Structural MOTIFS: regular 3D conformations or folds within secondary or tertiary structure common to many different proteins...
indicative of a particular 3-D architecture & associated with specific function...
same structure is present in different proteins that have similar functions;
recurring arrangements of **α**-helix and/or **β**-sheets in unrelated proteins....
such as:

EF hand... two short helices connected by a loop; a Ca^{+2} ion binder region of hydrophilic residues present in over 100 Ca^{+2} binding proteins.
fig 3.9b*

helix loop helix... commonly bind gene transcription factors to DNA

zinc finger... 1 **α** and 2 **β** strands with antiparallel orientations.
forms fingers bound by Zn ion that often link to DNA * &RNA
fig 3.9c*

coiled coil... **α** helicies, where the hydrophobic amino acids wind together forming a coil; also called leucine zippers:
common to transcription factors.
fig 3.9a*

Tertiary level

most responsible for 3-D orientation of proteins in space
- thermodynamically most stable conformation
- weak non-covalent interactions [fig 4.4*] & S-S bridges [fig 4.29*]
- hydrophobic interior & hydrophilic exterior

Protein Folding... **forms 3D shapes & binding sites**
occurs via H-bonds* fig 4.31* & fig 4.9*

some examples:
Myoglobin **MW 16,700 - animal muscle protein - stores O2**
Cytochrome-C **MW 12,400 - heme binding single polypeptide of 100 aa's in ETS of mitochondria**
Lysozyme **MW 14,600 enzyme; egg white & human tears pdb-lysozyme 124 aa's with 4 S-S; that hydrolyses polysaccharies in bacterial cell walls = bactericidal agent pic catalog**
Ribonuclease **MW 13,700 enzyme of 124 aa w 4 S-S**

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. ecb 5.12* & ecb 5.13*

3 classes of domains:

functional domain - region with particular activity characteristic of a protein CATALYSIS:
ex: kinase domains add PP to other molecules.

structural domain - region of 40+ aa's in a stable 2nd or 3rd-ary conformation (repeatable).
ex: 1. hemagglutinin: - a surface protein on influenza viruses, that is made of 3 quaternary identical subunits composed of 2 polypeptides (HA_1 & HA_2); each HA peptide has two domains... globular domain & a fibrous domain
mcb 3.10a*
2. EGF (Epidermal Growth Factor) domain - small soluble peptide hormone that binds to embryonic cells in skin/connective tissue & promotes cell division. EGF is generated by proteolytic hydrolysis of the domain from several other proteins, all of which have an EGF domain as a structural part.
mcb 3.11*

topological domain - distinctive spatial relationships to rest of a protein;
ex: membrane proteins with extrinsic (cytoplasmic domain) and intrinsic (membrane spanning domain).

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 knowns as homologs and homologous proteins belongs to a "family"
taxonomic cladistics (tree diagrams) of sequence analysis are used to show homologies

ex: 1. **serine proteases ecb fig 4.21**- proteolytic enzymes with nearly identical amino acid sequences all with a SER at the active site
2. **globins** - gene slowly diverged into animal and plant lineages
myoglobin - monomeric oxygen binder of muscle fig 3.13a*
hemoglobin - tetrameric oxygen binder of blood fig 3.13b*

Today, computer modeling is used to predict function of yet unisolated proteins by comparing known sequence homologies
sequence analysis = 2ndary structure

QUARTERNARY structure:

multiple polypeptides each with 3-D conformations
ex: hemoglobin, RNA polymerase, ASP-trans-carbamylase

Some Common Quarternary Level Protein Shapes...
1. **dimers** - self recognizing symmetrical regions
- bind together @ identical binding sites
[Catabolic Activator Protein] homodimers - 2 identical subunits
heterodimers - non-identical subunits (PDH)

2. **tetramers** - 4 identical subunits... [neuraminidase]

3. **filaments** - polymers of subunits each bound together in an identical way forming a ring or helix see fig 4.24*

4. **colied-coil** - 2 parallel helicies forming a stiff filament, linked via a stripe of hydrophobic aa's. figure* [keratin-fig 4.16*]

Multi-Enzymes Complexes : pyruvate dehydrogenase picture* & pic
ATP-synthase figure*

Multimeric proteins have Quartnerary Structure...

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

HA (hemagglutinin A) is a trimer of 3 idential polypeptide subunits held together by the weak electrostatic 3o level forces fig 3.10a* creates a globular domain and a fibrous domain

Some proteins form **Macromolecular Assemblies**...
very large > 1m Da in ma), 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

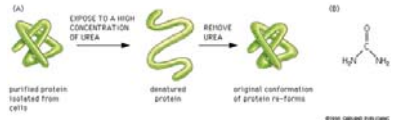


fig 4.7 p124

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 is 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 2^0 structure (**a & β**), then structural motifs & assembly of complex domains, followed by 3^0 level forces and/or 4^0 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 (25º --> 32ºC) 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*

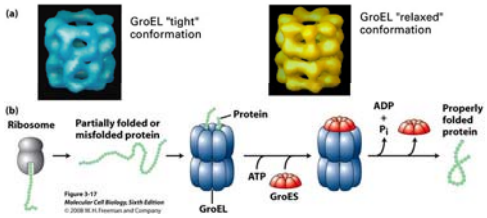
Classes of Heat Shock Proteins: Hsp -40, -60, -70, -90 & -100.
Hsp are named according to the molecular weights (Hsp-70 = 70 kilodaltons)

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;
OPEN conformation has hydrophobic pocket for new unfolded protein...
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-fig 3.17*

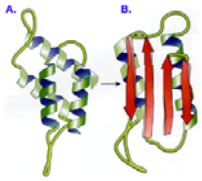


Misfolded Proteins & Disease

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, Alzeheimer'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.

figure of the UMP

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

panel 4.3 pg 160

Crude Cellular Homogenates How to break open cells *

grind cells in...

mortar & pestle, tissue grinders, homogenizers, cell disruptors, and blendors (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

centrifugation - applies centrifugation force to separate

produces a pellet (solid part) & supernatant (liquid portion) : pellet/supernatant*

- 1924 T. Svedberg invents analytical ultracentrifugation (failed rotors)
- 1938 G. Beherens isolates nuclei by centrifugation
- 1954 C. deDuve isolates lysosomes

2 major types: differential centrifugation (mcb3.34a*) & Rate-Zonal (mcb3.34b*)
speeds from 100 x g to 600,000 x g
clinical centrifuges - fixed angle vs. swinging bucket
ultracentrifuge - pic failed rotors

- results: by differential centrifugation mcb9.25*
repeated centrifugations at increasingly higher speeds
separates organelles by their mass and density
300g = whole cells & nuclei,
12,000g = mitochondria/chloroplasts
100,000g = microsomes, ribosome, etc...
& by size velocity sedimentation in a sucrose gradient*

Protein Separation Procedures…

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

CHROMATOGRAPHY... (Color Writing) journal - JCS
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… (panel 4.3 pg 162)

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

Kinds of column chromatography (see pg 97)

ion exchange chromatography… charged ligands [panel 4.4*]
matrix retards passing proteins of opposite charge mcb 3.37b

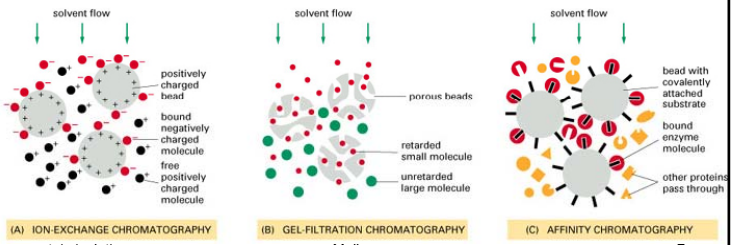
DEAE cellulose [dimethylaminoethyl cellulose] (+)
CM-cellulose [carboxymethyl cellulose] (-) [figure]

gel filtration… size exclusion chromatography [panel 4.4*]
"sized" media (beads) retards smaller size proteins... mcb 3.37a
sample columns and [figure]

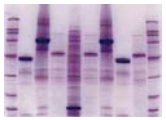
affinity chromatography panel 4.4 pg 162 ecb* & mcb3.37c
based on biological activity, an inert polymer with ligand (antibody, enzyme subst) binds a specific protein [figure]

molecular imprint polymer chromatography
manufacture of specific shaped and contoured chromatography media for high yield isolation of solutes

high pressure liquid chromatography (HPLC)
sample is vaporized and injected; moves through a column containing stationary liquid phase under high pressure; separates mixture into compounds according to their affinity for the stationary phase.



Identification of a protein & quantification



Electrophoresis... mcb pg 94 & ecb panel 4.5 pg 163
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 (panel 4.5*) (gels & staining)

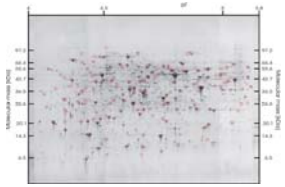
SDS-electrophoresis (SDS-PAGE)... (panel 4.5*)
a detergent - Sodium Dodecyl Sulfate... binds to protein @ 1 SDS/2 aa's thus it is proportional to a protein's molecular weight

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

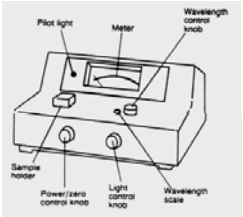
DNA Electrophoresis*: separates polynucleotide strands by charge (pic)

3 procedures are commonly used to help determine peptide structures:

1. **Mass Spectrometry detects exact mass of small peptides (mcb3.40)**
   - **a purified protein is treated with trypsin to produce peptides**
     trypsin cleaves polypeptides on COOH side of LYS & ARG residues]
   - **peptides are dried onto metal plate, blasted with laser,**
     vaporizing them as peptide ions,
   - **peptide ions flow through electric field & time it takes to pass a detector**
     is function of their charge & mass (fig 4.11)
2. **X-Ray Crystallography mcb3.42 determines 3-D shape mathematically**
   - **1st crystallize a purified protein (large, ordered, conformational array)**
   - **crystal scatter beam of X-rays (fig 4.12) forms diffraction pattern**
   - **with 25,000 spots, computer program interprets patterns atom structure**
3. **NMR Spectroscopy: magnetic signal shows distances between atoms**
   - **atom nuclei are "magnetic"... that is influenced by adjacent atoms**
   - **protein is placed in strong magnetic field; bombarded with radio waves**
   - **hydrogen nuclei generate NMR signals (fig 4.13) indicating distances between atoms**
   - **allows computation of 3D structure of molecules**

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 - rx's w amino = blue color (10-9M) (CSI)
Biuret test = mg quantities… based on Copper ion binds stiochiometrically = violet color prepare standard curve
Bradford test = ug amounts [Biorad]
based on dye Coomassie blue - binds to peptide
Fluoroescamine 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 micromole of substrate to product per min at 25^{0}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 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]

not previously isolated nor purified.

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

* 1 unit of activity = 1 micromole H_2O_2 --> H_2O & H^+ per min

** 1,500 fold purification of peroxidase

Bil 255 – CMB

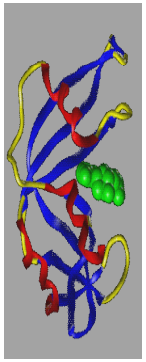
enzymology

procedures and protocols
For assaying enzyme activity

ENZYMOLOGY

pages 79-86; 88-92

Enzyme… gk "in" "leaven" (yeast)



- regulate metabolic reaction rates
- i.e., control metabolism
  - molecules (mostly protein) that accelerate or catalyze chemical reactions (**A--->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

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

  $2\ NH_2\text{-}C\text{-}NH_2 + 2\ H_2O \text{ -----> } 4\ NH^+_4 + 2\ CO_2$
  - Sumner's bioassay - injects rabbits with urease & the ammonia produced killed bunnies

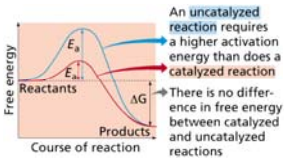
- **to date just over 1000 enzymes purified**

  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**
- **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

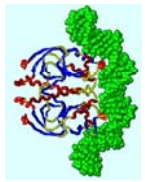
REACTION PATH

$E + S \text{ <---> } [ES] \text{ <---> } E + P$

enzymes catalyze reactions by lowering the energy of activation… Ea



Catalase

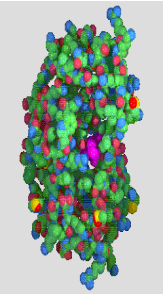


$2\ H_2O_2 \text{ ---> } 2\ H_2O + O_2$

condition	Ea	Rate (lt/mol/sec)
no catalyst	18,000	10^{-7}
Fe catalyst	10,000	56
catalase	2,000	$4x10^6$

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 / form 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 : **acscorbate, cyanocobalamin, folic acid,etc**
- **Prosthetic Group**: large complex organic molecules, which may have catalytic activity (**heme**)



Terminology continued

- **active site**: portion of enzyme which precisely fits the contours of a substrate by weak electrostatic interactions
- **What does an ES Complex do?**
  - holds substrate out of aqueous solution
  - holds substrate in specific orientation, close to Transition State
  - reduces ability of Free Rotation & molecular collisions with non-reactive atoms
  - allows amino acid side chains to alter local environment: changes ionic strength, pH, adds or removes H-bonds to substrate figure*
    - **Analogy**: a nut & bolt held in your hand decreases the Entropy of their eventual binding over a random mix of nuts and bolts in a toolbox.

Mechanism of Enzyme Action:

chemical reaction scheme by which enzymes acts on substrates.

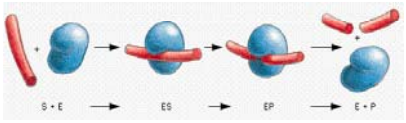
3 examples…

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.

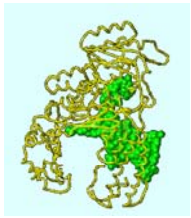
2. Serine Protease hydrolysis of peptide bonds mcb3.25a*.
   Catalytics ite ser195, asp102, & his57 - OH of ser195 attacks the C=O of peptide bond & transition is held by H-bonds.
   e's break peptide bond release part of protein, H-O-H is split & other half released.

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 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

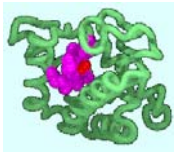


- International Enzyme Commission
  - IUBMB

    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]

     $[aa\text{-}aa]_n + H_2O$ ----> $[aa\text{-}aa]_{n-1}$ + aa

EC MAJOR CLASSES

4. **Lyases** ....
   add or remove groups to C=C bonds
   - **Pyruvate decarboxylase** [EC 4.1.1.1]

     pyruvate -----> acetaldehyde + CO_2
5. **Isomerases**… [mutases]
   catalyze isomerizations
   - **Maleate isomerase** [EC 5.2.1.1]

     maleate ----------> fumarate
6. **Ligases**…
   condensation of 2 substrates with splitting of ATP
   - **Pyruvate Carboxylase** [EC 6.4.1.1]

     PYR + CO_2 + ATP ----> OAA + ADP + P

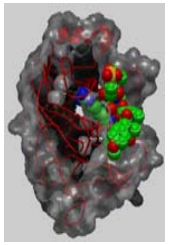
Definitions of Enzyme Activity

- **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**

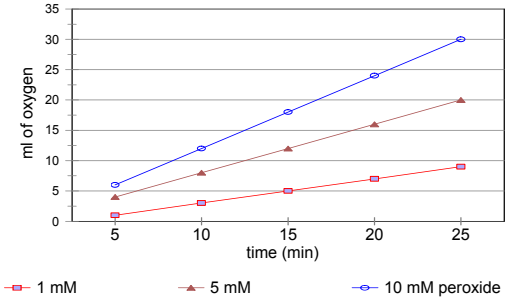
1 unit MOLECULAR ACTIVITY
- **# units per umole of purified enzyme**



Enzyme Kinetics…

mathematical and/or graphical expression of the reaction rates of enzyme catalyzed reactions

- Catalase $2\ H_2O_2$ ----> $2\ H_2O$ + O_2

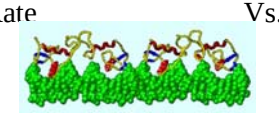


Characteristic Enzyme Curves:

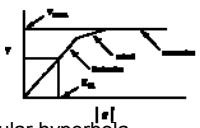
or how to determine if the reaction A —> B is enzymatic

Observed Enzyme Kinetic Reaction Curves :

1. Rate **(0.8 ml O_2/min)** Vs. **[E]**
2. Rate Vs. **pH**
3. Rate Vs. **Temperature**
4. Rate Vs. **[S]**



- **most CHARACTERISTIC curve…**
  plot of v vs. [S] 5.30 p 171
  - curve defines a **rectangular hyperbola**
  - at low [S], rate is directly proportional to [S]
  - at higher [S], rate declines giving a rectangular hyperbola



- **1st & 2nd order reaction kinetics are NOT sufficient to describe the rectangular hyperbola of enzyme reactions**

(see handout)

$A \xleftrightarrow{k_1} B$ 1st order Rx $dP/dt = k_1[A]$

$A + B \xleftrightarrow{k_1} C$ 2nd order Rx $dP/dt = k_1[A][B]$

k_2

1913 Leonor **MICHAELIS** & Maude **MENTEN Kinetics**

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

$$E + S \underset{k_2}{\xleftrightarrow{k_1}} ES \underset{k_4}{\xleftrightarrow{k_3}} E + P$$

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

$$v = (dP/dt) = k_3 [ES]$$

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 : $v = \frac{Vmax\ [S]}{Km + [S]}$

SEE LINK for DERIVATION HANDOUT

Enzymology Mallery 17

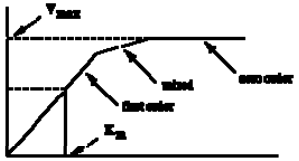
Km - the Michaelis Constant

- is a mathematical interpretation of an enzyme action
- is **substrate concentration** at which rate is equal to ½ **Vmax**
- 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 ***afffinity"*** 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… ½ **Vmax**
  - 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

Enzymology Mallery 18

ways to determine Km

- by extrapolation from M & M standard curve **v vs. [S]**



- by transformation of M & M curve

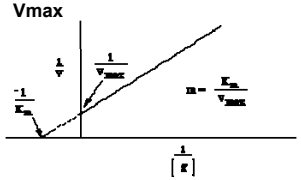
LINEWEAVER- BURKE Plot

- take the reciprocal of both sides of M&M equation & plot

$$\frac{1}{v} = \frac{Km}{Vmax} \times \frac{1}{S} + \frac{1}{Vmax}$$

EADIE - HOFSTEE Plot

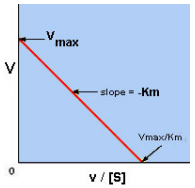
v vs. v/[S]

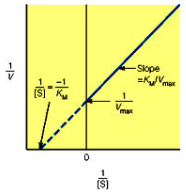


SEE GRAPHICAL PLOTS

Enzymology Mallery 19

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

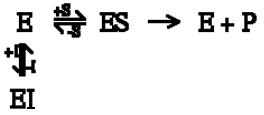




An advantage of an Eadie-Hofstee plot over a Lineweaver Burk plot (which plots 1/V versus 1/S) is that the Eadie-Hofstee plot does not require a long extrapolation to calculate Km.

Enzymology Mallery 20

Enzyme Inhibition



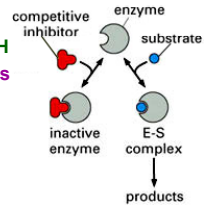
2 classes of inhibitors

- **IRREVERSIBLE** -
  - inhibitor molecule can not be easily removed from enzyme
    i.e, 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 and are thus treatable by M & M kinetics
  - 2 major types of reversible inhibitions
    - **COM PETITIVE**
    - **NON-COM PETITIVE**

Enzymology Mallery 21

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**

Enzymology Mallery 22

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 & favor 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.

Enzymology Mallery 23

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 Angiotensis II (8 amino acids).

Angiotensin II promotes hypertension (high blood pressure - hbp)
in 1960's John Vane discovered TEPROTIDE in Brazalian pit viper venoms, 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 "prils"... (lisinopril, captopril, trandolapril, moexipril, ramipril, etc.)

Enzymology Mallery 24

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, mitochondria
3. by proteolytic clevage - converting inactive peptides to active enzymes
   - often involves hormones and digestive proteases
   - pancreas makes zymogens… (an inactive enzyme large precursor)
     ex: trypsionogen & chymotrypsinogen
   - enterokinase, an aminopeptidase from lining of small intestine hyrolyzes trypsionogen to trypsin (active form), which itself hydrolyzes chymotrypsinogen in chymotrypsin

Enzymology Mallery 25

4. by adjusting reaction rates of existing enzyme (ala M&M kinetics)

a) **STIOCHIOMETRIC** controls - limit amount substrate present

b) **ALLOSTERY** - [allosteric kinetics]
   - binding of a ligand results in a change of 3o/4o conformations
   - common in multimeric proteins/enzyme complexes
     ATCase aspartate transcarbamylase
   - allosteric proteins have 2 binding sites: active site = substrate
     allosteric site = regulator ligand
   - active form = + catalysis & inactive conformation = - catalysis
   - ligand often serves as substrate, activator, or inhibitor (or all 3)

Examples of Ligand induced Allostery

a) **Cooperative Binding**: binding of 1 ligand affects subsequent bindings
   if + = enhanced subsequent bindings
   if - = sequential binding is inhibited
   ex: hemoglobin: binding of 1 O2 to a heme = Δ-local conformation lowering Km of binding of additional O to other subunits (mcb3.30*) chains

Enzymology Mallery 26

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 (mcb15.23a*)
   thus a hormone signals --> cAMP --> active PKA dimer
   without PKA we have an inactiver tetramer (mcb15.23b*)

2. **GroEL chaperonin**: is 2 multi-subunit rings (mcb3.17*)
   binding of ATP and GroES to GroEL reults 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-**7**M.
   to increase cytosol Ca calmodulin, a helix-loop-helix protein is used
   4 Ca ions bind = **Δ**-conformation - now binds target proteins switching on

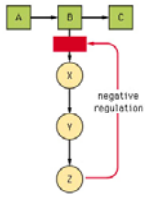
Enzymology Mallery 27

4. **GTPase super family**: a group of allosteric plasma membrane proteins switching between active/inactive, includes Ras & G-proteins mcb3.32*
   which invloves 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 mcb 3.33*
       tyrosine kinases add P to TYR residues of enzymes de-activating them
       serine/theronine 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

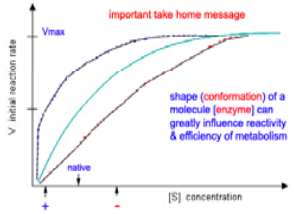
Enzymology Mallery 28

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*)

Enzymology Mallery 29

Bil 255 – CMB

the design of metabolism

Cellular energetics & chemical equilibrium

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 a 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 U**235** --> 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 equlibrium...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)

➡ Cell reactions = increased order within cell with release of heat

➡ 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.

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

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 $\Delta G = \Delta H - T\,\Delta S$
free energy enthalpy entropy

ΔG is a numerical measure of how far a reaction is from equilibrium
ΔG 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
(4° to ≅ 45°) and thus $\Delta H = 0$

What Gibbs showed was that…

"cell chemical systems change, so that Free Energy is minimized"

thus, ΔG can PREDICT..... the Direction of Cellular Reactions.....
TOWARD EQUILIBRIUM and to Maximum ENTROPY

Any natural process occurs spontaneously, if and only if,
the associated change in G for the system is negative ($\Delta G < 0$).
when ΔG is negative a reaction is spontaneous, R --> P,
& there is a decrease in entropy

Likewise, a system reaches equilibrium when the associated change in G
for the system is zero (ΔG = zero),

& no spontaneous process will occur, if the change in G is positive ($\Delta G > 0$).

CHEMICAL REACTION A <---> B Which Way?

J. Willard Gibbs (1839-1903)

$\Delta G = \Delta G0' + \mathbf{R}\,\mathbf{T} \ln [\mathbf{p}]/[\mathbf{r}]$

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.,

ΔG = actual free energy
$\Delta Go'$ = standard free energy [change under std conditions]
R = gas constant (2 x 10^{-3} Kc/mol)
T = absolute temp (-273°K)
ln = natural log (conversion log**10** = 2.303)

at equilibrium $\Delta G = 0$ and [p]/[r] = Keq
if we solve above equation for $\Delta Go'$ we can see relationship* of Keq to $\Delta G0'$

Free Energy Equation...

$$\Delta G = \Delta G^{0'} + RT \ln \frac{[P]}{[R]}$$

@ equilibrium $\Delta G = 0$

thus rearranging $\Delta G^{0'} = -RT \ln \frac{[P]}{[R]}$

@ equilibrium $\frac{[P]}{[R]} = Keq$

@ 250C ... $-RT \ln Keq = -(2.0)(298)(2.303) \lg_{10} Keq$

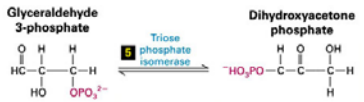
thus..... $\Delta G^{0'} = -[1364] \lg_{10} Keq$

The difference between... ΔG and $\Delta G^{0'}$

$\Delta G^{0'}$ 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 **Δ** G cab be used to predict the direction of a reaction at a given time.

ΔG 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 unfavored reactions

products / reactants	K_{eq}		$\lg_{10}$	$\Delta G0'$ cal/mole [$\lg_{10}$ x -1364]	
1/1000	.001	10^{-3}	-3	+ 4092	[R] > [P]
1/100	.01	10^{-2}	-2	+ 2728	
1/10	.1	10^{-1}	-1	+ 1364	
1/1	1.0	0	0	0	[R] = [P]
10/1	10	10^{+1}	+1	- 1364	[P] > [R]
100/1	100	10^{+2}	+2	- 2728	
1000/1	1000	10^{+3}	+3	- 4092	



Which way this reaction goes is dependent upon existing concentrations?

$\Delta G^{0'}$ @ cell [equilibrium] K_{eq} DHAP/G3P = 22.4
$\Delta G^{0'} = -[1364] \lg_{10} 22.4 = -[1364] \lg_{10} 1.35 = -1842$ cal/mole

$\Delta G = \Delta G0' + RT \ln[P]/[R]$ but when DHAP = 0.001M & G3P = 0.1M
$\Delta G = -1842c/m + (-1364)(\lg_{10} 0.01) = (-1842)+(-1363)(-2) = +886$ 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 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 ---> CO_2 & H_2O

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

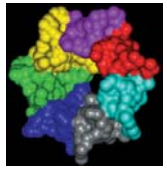
ENDERGONIC REACTION - requires input of energy for A --> B
Product [B] **>>> energy** **Reactant** [A]
ex: photosynthesis - (autotrophy)
glucose made from CO_2 + H_2O --light---> $C_6H_{12}O_6$
energy poor vs. energy rich

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

[enzyme catalyzed metabolic pathways] fig 3.2

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

Design of Metabolism:

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 ---> CO 2 + NADH ---> H 2O**
ADP + P ---> ATP

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

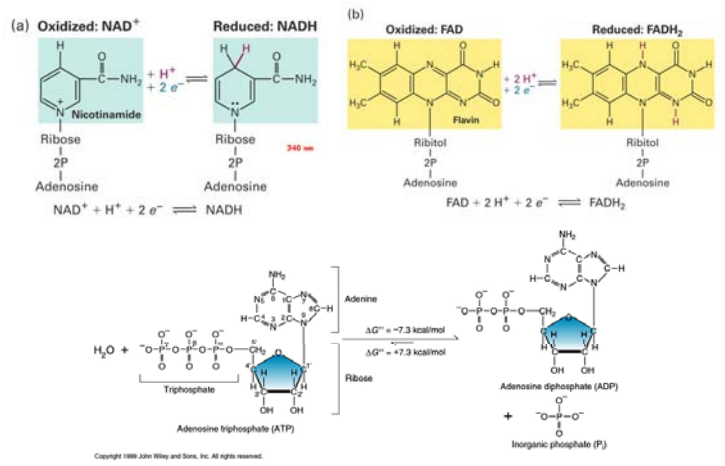
H_2O ◄ e- / H^+ ► NADPH + ATP ; CO_2 ▲ ▼ CH_2O — oxidized form / reduced form

Heterotrophs:

food stuffs more energetically stable

$[CH_2O]n$ + NAD^+ ◄ ► CO2 + H_2O + ATP + NADH

Key Cell energy intermediates - NADH & NADPH, FAD, & ATP*



Design of metabolism…

OXIDATION / REDUCTION - Redox Reactions

e- &/or H+ transferred between oxidized & reduced forms

AH ⇐⇒ A + e^- + H^+

oxidation - removal of e- from substrate

reduction - gaining of e- (& often a proton, H^+) fig 3.12

$$6O_2 + C_6H_{12}O_6 \xrightarrow[\text{NADP+ photosynthesis NADPH}]{\text{NAD+ respiration NADH}} 6CO_2 + 6H_2O$$

fig 3.10

KEY METABOLIC REACTIONS:

6 major categories of bio-chemical reactivity

Bio-chemical reactivity is bond breaking & reforming

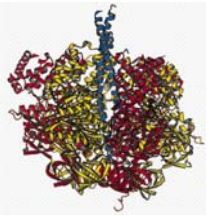
these are violent events inside cells, carefully controlled by ENZYMES

1. redox reaction (oxid/reduction) PGAld + NAD+ <--> 1,3di-PGA + NADH
   oxidoreductases (dehydrogenases)
2. functional group transfers glu + ATP <--> G6P + ADP
   transferases
3. Hydrolysis glu-glu(n) + H_2O <--> glu-glu(n-1)
   hydrolyases
4. C-C breaking or re-formation fruc1-6bP <--> DHAP + 3PGAld
   lyases
5. rearrangement (isomerizations) glucose-6P <--> fructose-6P
   isomerases
6. Condensations protein(n) + aa1 <--> protein(n+1) + H_2O
   transferases

Bil 255 – CMB

how cells make ATP

CELLULAR ENERGETICS



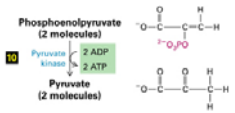
How Cells Make ATP

- **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

- **Chemiosomosis** (Oxidative Phosphorylation)
  subst-H + NAD ---> NADH + subst
  NADH ----> H+ proton motive force → ATP
- **Photosynthetic Phosphorylation**
  light + NADP ---> NADPH ----> H+

Key metabolic reaction = **REDOX Reaction**

$AH + BO \leftrightarrow A + BOH$

$C_6H_{12}O_6 + 6O_2 \leftrightarrow 6CO_2 + 6H_2O + e's$

Cellular Respiration

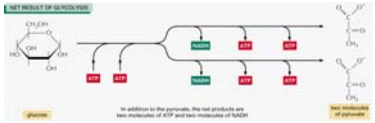
Evolution of aerobic metabolism was a major step in the history of life on planet Earth

Cell Respiration - series cytoplasmic & mitochondrial
- linked enzymatic pathways
- 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 --> monomers
2. Production of AcoA --> glycolysis & FAoxidation
3. Oxidation of AcoA to CO_2 & H_2O --> KC & ETC

4 Cellular Pathways:



- Glyco-lysis

  glucose --> pyruvate + NADH + ATP

- Kreb's Cycle

  AcoA --> CO_2 + NADH + GTP +$FADH_2$

- Electron Transport Chain (ETC)

  passage of e's from NADH to O_2 ---> H_2O + H^+ gradient

- ATP synthase

  mitochondrial membrane protein which makes ATP as H^+ move into mitoplasm

Mallery Cellular energetics = ATP 5

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 [mcb12.3 steps 6 & 7 & 10]
  - redox reaction (glyceraldehyde3-PDH)
  - substrate level phosphorylation

Mallery Cellular energetics = ATP 6

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 $FADH_2$

  - glycerol-P shuttle - skeletal muscle/brain ($FADH_2$)
  - malate shuttle - liver, kidney, heart muscle (NADH)

Mallery Cellular energetics = ATP 7

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

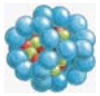
2 PYRUVATE

Fermentations & Shuttles

Mallery Cellular energetics = ATP 8

Krebs Cycle, Citric Acid Cycle, Tricarboxylic Acid Cycle

a cyclical biochemical pathway resulting in aerobic oxidation of cell fuels, as CH_2O, fatty acids, & amino acids, while making CO_2, H_2O, & 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

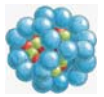
Mallery Cellular energetics = ATP 9

Overall reaction:

acetyl-CoA + 3NAD + E-FAD + GDP + P + $2H_2O$ -->
CoASH + 3NADH + E-$FADH_2$ + GTP + $2CO_2$

ENZYMES of KREBS CYCLE

- 5 dehydrogenases- ISDH, αKGDH, SDH, MDH, & PDH
- 2 hydrolyases- aconitatse & fumarase
- 1 thiokinase- succinyl thiokinase
- 1 synthetase- citrate synthatase
- 2 multi-enzyme complexes (f4.8 p118)
  each with 60 proteins & 5 coenzymes
  1. pyruvate dehydrogenase &
  2. alpha ketoglutarate dehydrogenase



Mallery Cellular energetics = ATP 10

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 $FADH_2$)
2 CO2's are released

3 parts of Mitochondrial Oxidation of PYR

1. PYR --> CO_2 + H_2O --> NADH/$FADH_2$ Krebs
2. e^- of NADH/$FADH_2$ --> O_2 to make H_2O ETS
3. ADP + P ---> ATP Chemiosmosis

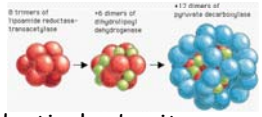
Mallery Cellular energetics = ATP 11

Pyruvate Dehydrogenase Complex

Oxidative decarboxylation of alpha-Keto acid

$$HOOC\text{-}C(=O)\text{-}CH_3 \quad \text{-----> } CoA\text{-}S\text{-}C(=O)\text{-}CH_3 + CO_2$$

3 enzymes (4.8p118)

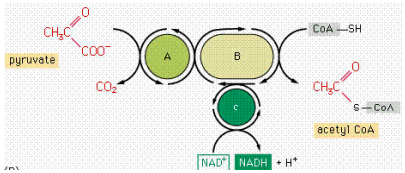


a. pyruvate decarboxylase
   12 dimers = 24 identical subunits
b. dihydrolipoyl transacetylase (reductase)
   8 triamers = 24 identical subunits,
   each 3 lipoates
c. dihydrolipoyl dehydrogenase
   6 dimers 12 subunits with FAD

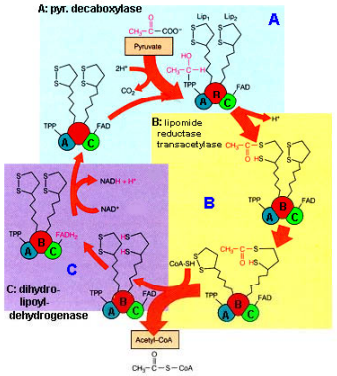
Mallery Cellular energetics = ATP 12

PDH COMPLEX

5 coenzymes



1. CoASH ecb8.8 p309 — pantothenate
2. Lipoate — lipoic acid
3. Thiamine pyrophosphate — thiamin (B1)
4. E-FAD 2.26 p54 — riboflavin (B2)
5. NAD+ 2.26 p54 — niacin



Mechanism of Action of PDH Complex

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

B: lipomide 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+ --> NADH

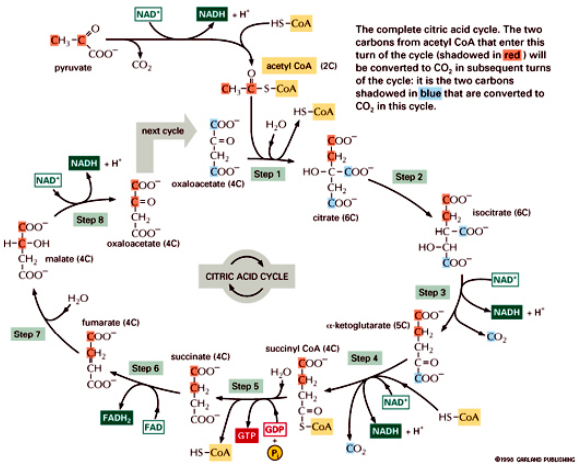
Key Metabolic Reactions of KREBS CYCLE [+ PDH reaction]

1. NAD is reduced (NADH)
2. substrate level phosphorylation occurs
   GDP + P --> GTP (@ 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

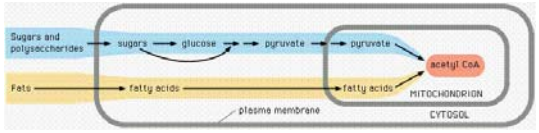


FATTY ACID Metabolism [beta-oxidation]

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 <--> FAcoA + AMP + PP
   converts cyotplasmic FA to Fatty-acyl-coA

2. **carnitine acyl-transferase 1** (outer mito memb.)
   fattyCoA + carnitine <-> Fatty acyl-carnitine + CoASH
   transfers FAcoA to carnitine for transport across mito

3. **carnitine acyl-tranferase 2** (in mitoplasm)
   fatty acyl-carnitine + CoASH <--> FAcoA + carnitine
   releases FAcoA inside the mitoplasm

4. fatty acyl-coA dehydrogenase fig 13.9b

"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 clevage 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 and 1 $FADH_2$ = 2 ATP

Cell Respiration	glucose	beta-OXIDATION 6C-FFA (c-c-c-c-c-c)
to start - GLYCOLYSIS	- 2 ATP	-1 ATP @ ligase
glyceraldehyde DH	+ 2 NADH	
PGA kinase (via SLP)	+ 2 ATP	
pyruvate kinase (via SLP)	+ 2 ATP	
Krebs Cycle per each PYR		per 2 cycles @ Fatty-AcoA-DH
PDH - $2CO_2$	+ 2 NADH	+ 3 AcoA
per each AcoA		+ 2 $FADH_2$ = 4 ATP
ISDH -$2CO_2$	+ 2 NADH	+ 2 NADH = 6 ATP
KGDH -$2CO_2$	+ 2 NADH	----------
thiokinase	+ 2 GTP	+ 10 ATP
SDH	+ 2 $FADH_2$	
MDH	+ 2 NADH	
Totals		6C FFA
1 glucose = 2 PYR = 2 AcoA --------->		B-OX = 10 ATP
-6CO2 + 2ATP + 10NADH + 2FADH2 + 2GTP		Kreb's (3 AcoA) = 72 ATP
		+ 82 ATP
total ATP = 36-38		
ATP via 2 AcoA alone = 24		82 - 1 = 81 - 38 = + 43 ATP net

Regulation of Krebs Cycle

controls flow of intermediates [in & out] - 4.18 & 4.19

- substrate availability - mass action
- allosteric inhibition – PFK-1&2 & **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/Ca^{+2} -ATP**
- alpha-keto gluatarate dehydrogenase — **+Ca^{+2} -ScoA & NADH**

Mallery Cellular energetics = ATP 21

Bil 255 – CMB

mitochondrial membranes
&
electron transport chain

Mitochondrial Membrane Transport

membrane = **impermeant** to most everything, esp to **H+**

outer membrane - **porins** - molecules 5,000 -10,000d

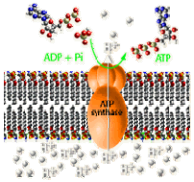
inner membrane - 70% protein & 30% lipid

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**

mtDNA - 16,569 np's... code for 20% mito proteins

mcb 6.23

Mitochondrial encoded DNA functions:

5 subunits of NADH dehydrogenase (complex I),
cytochrome oxidase subunits I, II, III (complex IV),
ATP synthase : subunits 6 & 8 (complex V),
RNA polymerase, & 22 tRNA's & 2 rRNA's

Nuclear encoded components include:

lipid, metabolism, nucleotide metabolism, aa
metabolism, carbo metabolism, heme synthesis, Fe-S
synthesis, ubiquinone synthesis, proteases,
chaperones, signal pathways, & DNA repair & replication.

1,000's copies per cell; maternally inherited;
frequent point mutations; used in sequence analysis

mtDNA & Human Evolution **genetic variation among peoples**
forensic uses of Mito-DNA **mitochondrial diseases**

How Electron Transfer Works

- **REDOX POTENTIAL** (how measured – panel 13.1)

  empirical measure of tendency to gain e's

  - strong reducing agent has negative -ΔEo'
  - strong oxidizing agent has positive +Δ Eo'

  $\Delta Go' = -nf\,\Delta Eo'$

  $NADH \leftrightarrow NAD^+ + H^+ + 2e^-$ -0.32V

  $H_2O \leftrightarrow \frac{1}{2} O_2 + 2H^+ + 2e^-$ +0.82V

  $\Delta Go' = -(1)(0.023)(1.14) = -26.2$ Kcal

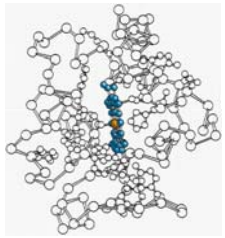
- **Electron Transfer Chain's Order**

  --> Increasing Redox Potential (from - to +)

  see fig 12.18 p500

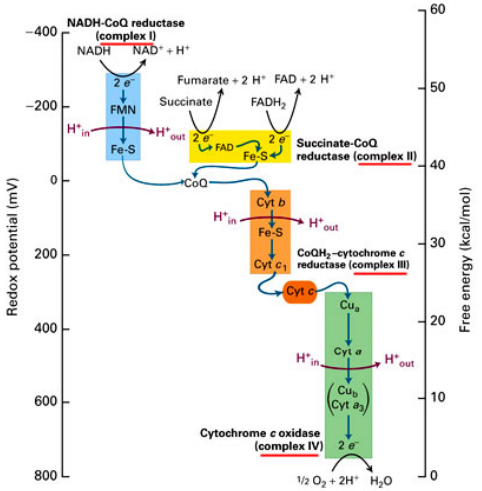
Components of the ETC

- **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** 12.14b 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 [ferrric vs. ferrous]
  iron porphyrin (heme) bound protein carriers



How Oxidative Phosphorylation Works – fig 12.18 p500

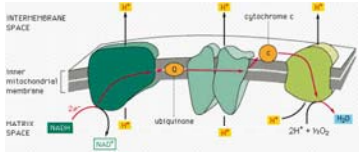
mcb 6e
Fig 12.18
Pg 500



Respiratory Assemblies - Mitochondrial Components

Respiratory Assemblies:

- NADH-Q reductase
- Succinate dehydrogenase
- Cytochrome-C-Reductase
- Cytochrome Oxidase



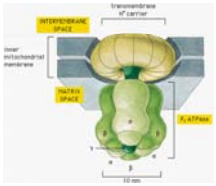
Proton Motive Force:

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***.

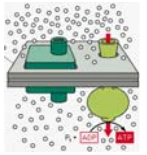
ΔpH = 1.0 - 1.4 pH units & Δcharge = 140mV in(-) vs. out(+)

ATP Synthase: creates a hydrophilic 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 O_2 to make H_2O
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...

'mushroom' shaped complex composed of 2 membrane subunits

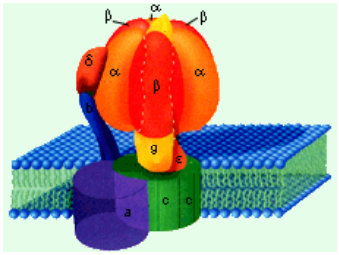
F1 (extrinsic) & F0 (intrinsic)

Humbeto Fernandez (60's) sees lollipops on inner mito membranes
Efraim Racker (1966) isolates lollipop - Coupling Factor 1 - F1

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)



Mallery electron transport chain 9

Binding Charge Mechanism of ATP Synthesis - A Rotary Motor Paul Boyer – 1979

1. H^+ movement changes binding affinity of synthases's active site, thus
when ADP & P bind to active site, they readily condense into ATP
(removed from aqueous solution Keq = 1
and ΔG 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°

Mallery electron transport chain 10

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 30o CCW
next C in ring picks up H+ & thus the ring cycles thru 360o
release of H+ into matrix happens at end of cycle Karp 5.29*

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

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

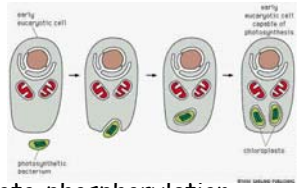
Biovisions animation of ATP synthase

Mallery electron transport chain 11

Bil 255 – CMB

photosynthesis

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 CO_2 to CH_2O

2 Fundamental Reaction Mechanisms

LIGHT Reactions (photo-chemical reactions)

- molecular excitation **chl** by light... charge separation
- generation of proton motive force (H^+ gradient)
- reduction of NADP to NADPH via an ETS

DARK Reactions (thermo-chemical reactions)

- CO_2 fixation (reduction) stages
  - carboxylation CO_2 RuBP --> 2 PGA
  - reduction PGA + NADPH --> PGAL
  - regeneration of RuBP via HMP path ---> RuBP

$$6CO_2 + 12H_2O^* \rightarrow C_6H_{12}O_6 + 6H_2O + 6O^*_2$$

Evolutionary Basis of Photosynthesis

1st autotrophic cells probably used H_2S as e- source

purple-sulfur bacteria of today

$$CO_2 + H_2S \rightarrow CH_2O + H_2O + 2S$$

cyanobacteria - **oxygenic photosynthetic procaryotes**

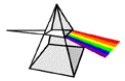
$$CO_2 + H_2O \rightarrow CH_2O + H_2O + O_2$$

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

$$CO_2 + H_2A \rightarrow CH_2O + H_2O + 2A$$

Molecular Excitation of Chlorophyll

- Absorption of Light Energy
  - blue light [440nm] = 71.5 Kc/einstein
  - red light [700nm] = 40.9 Kc/einstein



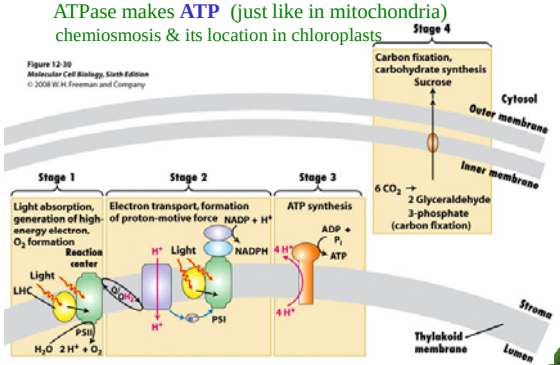
- Ground State -
  - paired e's with opposite spin = stability
  - absorption moves non-bounded e's to higher orbitals
    - 1st excited singlet state
    - 2nd excited singlet state
    - 1st long-lived state

FATES of Absorbed Light Energy

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

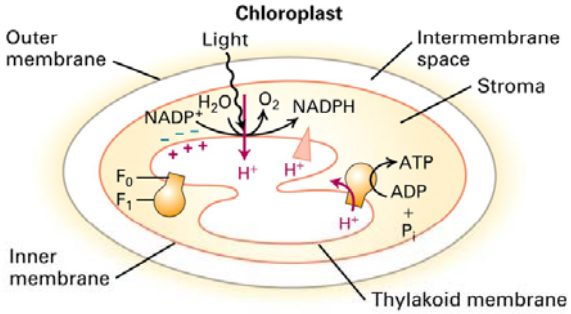
Photosynthetic Electron Flow - stages of photosynthesis

Photosystems – LHC: chlorophylls, reaction center, primary acceptor
PS 1 and PS 2 - path of e- flow (cyclic vs. non-cyclic)
release of O_2 & capture of e- into coenzyme $NADP^+$ ---> **NADPH**
ATPase makes **ATP** (just like in mitochondria)
chemiosmosis & its location in chloroplasts



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) CO_2 into CH_2O (sugars)

3 different pathways to make sugar

C3 - CALVIN cycle

- 1 CO_2 + 5C RuBP ---> (2) 3C sugars (PGA)
- (2) 3C sugars combine ---> 1 net glucose
- RuBP carboxylase [50% of leaf protein]
- Photo-respiration
  - inhibition by O_2

PHOTORESPRATION... **RUBISCO** catalyzes two different reactions:

a **carboxylase** activity... adding **CO2** to ribulose bisphosphate -
an **oxygenase** activity... adding **O2** to ribulose bisphosphate -

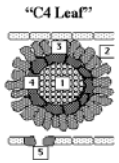
Karp fig 6.19*

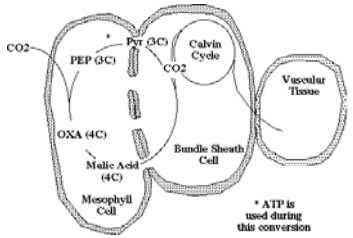
which one predominates depends on the relative concentrations of O2 & CO2 with
high CO_2 : low O_2 favoring the carboxylase action
high O_2 : low CO_2 favoring the oxygenase action

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 O_2 by RUBISCO forms two 3-carbon molecules: [reaction]
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.

C4 - Hatch & Slack pathway

- 4C acid ---> 3C + CO_2 in bundle sheath
- 1 CO_2 + 3C PGA --> 4C acid (mesophyll cells)
- CO_2 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.