Light and Life: Building a Cell Part 1

(Behrenfeld, Falkowski et al., Science, 1998)

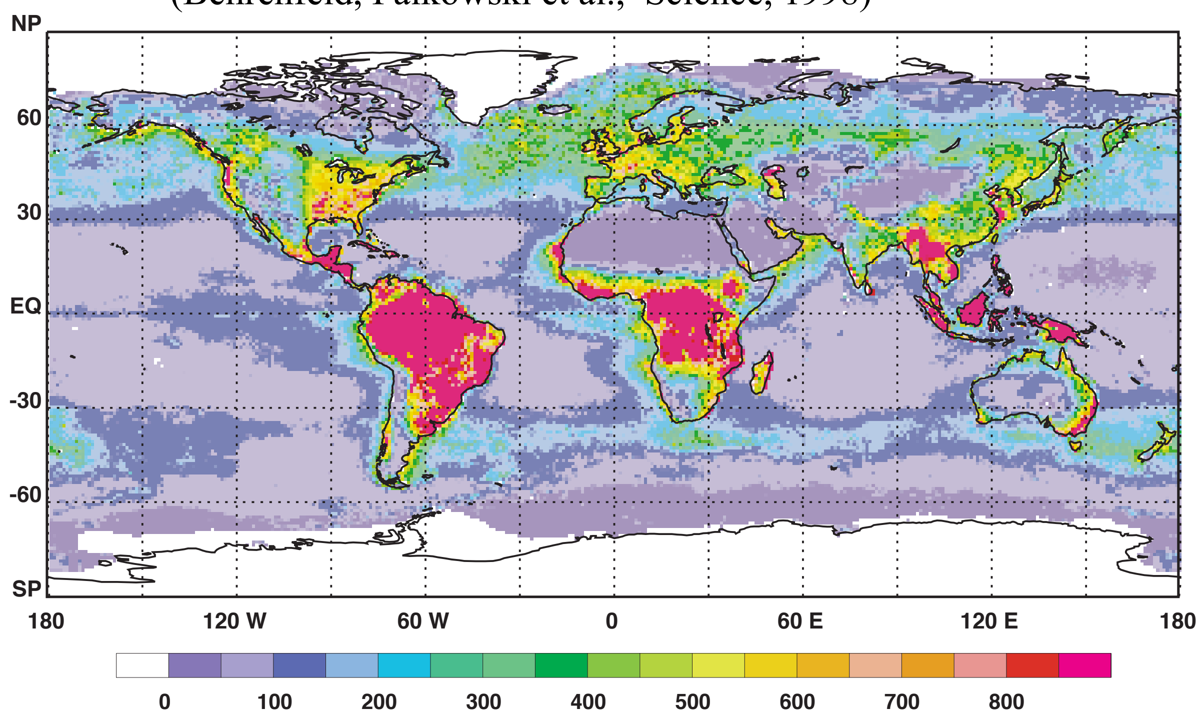


Fig. 1. Global annual NPP (in grams of C per square meter per year) for the biosphere, calculated from the integrated CASA-VGPM model. The spatial resolution of the calculations is 1° × 1° for land and 1/6° × 1/6° for the oceans. Input data for ocean color from the CZCS sensor are averages from 1978 to 1983. The land vegetation index from the AVHRR sensors is the average from 1982 to 1990. Global NPP is 104.9 Pg of C year^{-1} (104.9×10^{15} g of C year^{-1}), with 46.2% contributed by the oceans and 53.8% contributed by the land. Seasonal versions of this map are available at www.sciencemag.org/feature/data/982246.shl. NP, North Pole; EQ, equator; Sp, South Pole.

APh/BE161: Physical Biology of the Cell

Winter 2009

“Lecture # 1” – will take several days

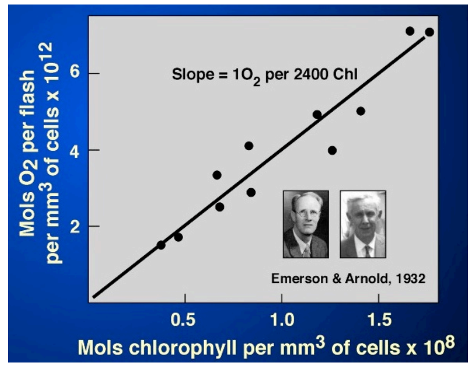
Rob Phillips

Logic of Lecture

- ***"Who am I? Why am I here?"***
- ***Who are you? Why are you here? - the diversity question.***
- ***A few words on what this course is? Style and approach (graduate course in spirit and all that implies – "I don't know", all learning together, open-ended homeworks, trying to find the right questions to ask) – thinking, estimating and calculating biological phenomena. Often start with a) data from some experiment to change your life for and b) show and tell. This is followed by model building and analysis.***
- ***Photosynthesis: show and tell - drinking from a powerpoint firehose.***
- ***Course logistics: when, grades, TAs, expectations, website, etc.***
- ***162??????????????????????????????????***

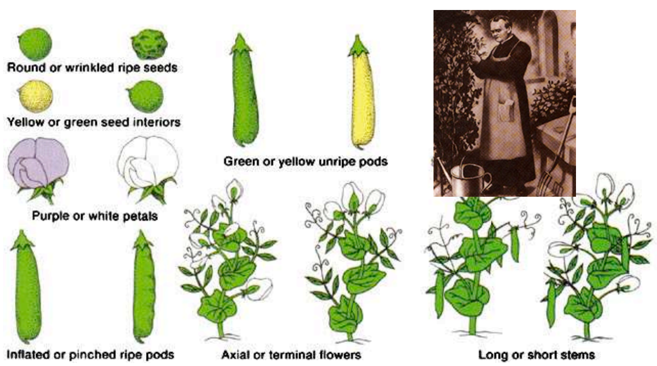
Does Quantitative Insight Lead to Biological Discovery? Not Just Crossing "t"s and Dotting "i"s

Photosynthesis: carbon and oxygen stoichiometry

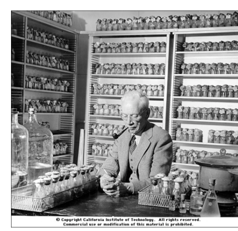


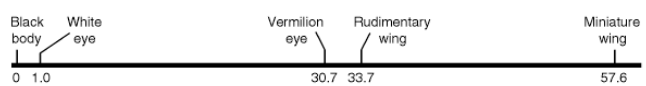
Photosynthesis: photons, light collection and electron transfer

"… no one has concentrated on the number of different forms that appear among the offspring of hybrids… No one has counted them. But doing all this counting and sorting appears to be the only way by which we can finally solve a question whose importance cannot be overestimated." – Gregor Mendel



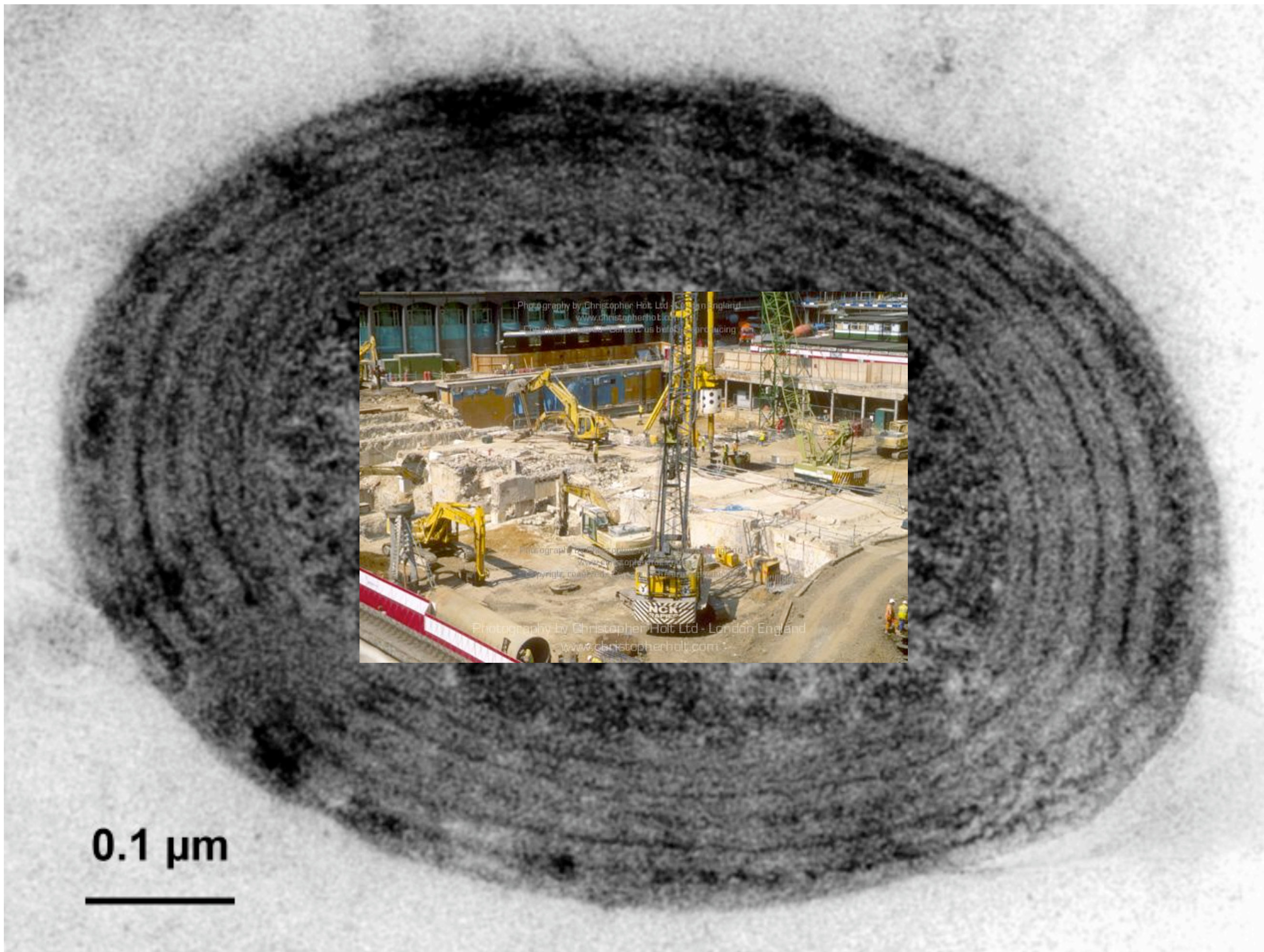
Genetics and counting





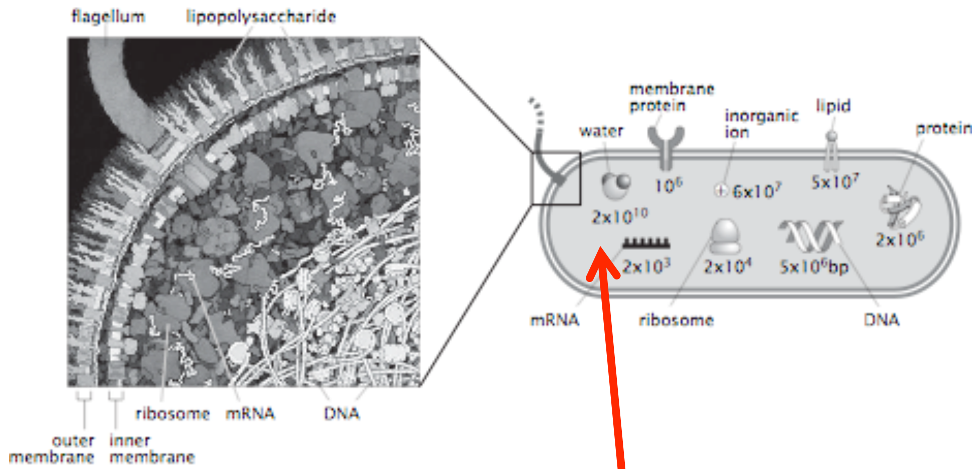
The first map of a chromosome

First Theme: Building a Cell

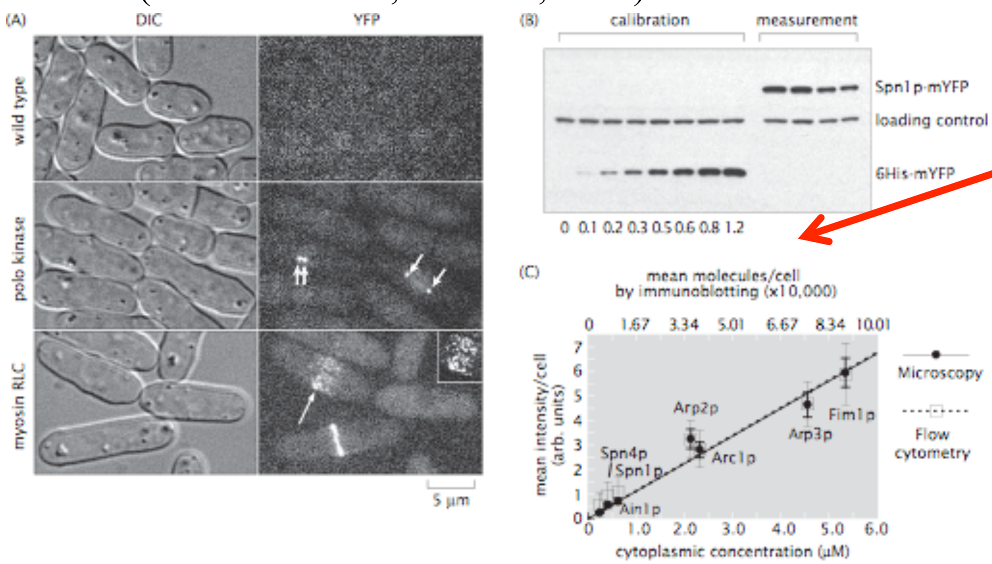


Order of Magnitude Biology: Why the Numbers?

- ***The plan: figure out the number of molecular actors of various kinds in the drama of a cell.***
- ***Reasons why: stoichiometrically correct descriptions of biosynthetic pathways, understanding of binding, regulation and cooperativity, dissection of mechanism, etc.***



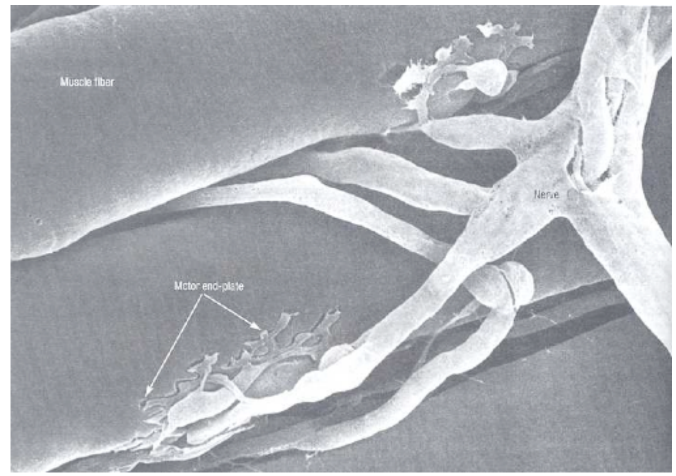
(Wu and Pollard, Science, 2005)

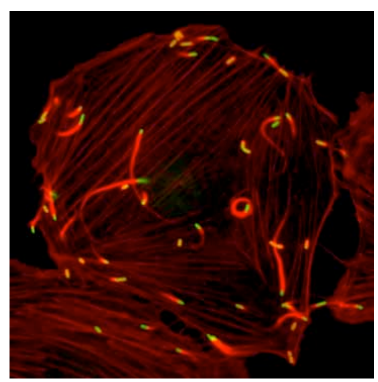


- ***The approach: simple order of magnitude estimates followed by description of how numbers are measured and what their significance is.***
- ***Estimating and measuring the numbers serve complementary purposes.***

Living Organisms are Made of Cells

- ***Why bother? One of the great insights in the history of biology. Cells as the minimal living unit bring us to the heart of some of the great biological mysteries.***
- ***``If in some cataclysm, all of scientific knowledge were to be destroyed, and only one sentence passed on to the next generation of creatures, what statement would contain the most information in the fewest words? I believe it is the atomic hypothesis.. that all things are made of atoms" - Richard Feynman***
- ***Cells are the fundamental units of living organisms.***
- ***"Omnis cellula e cellula" - every cell from a pre-existing cell. Rudolf Virchow.***
- ***Cells are extremely diverse!***





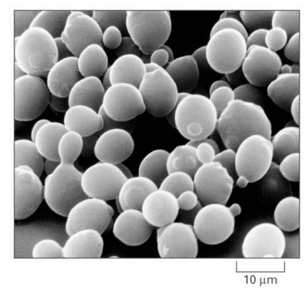
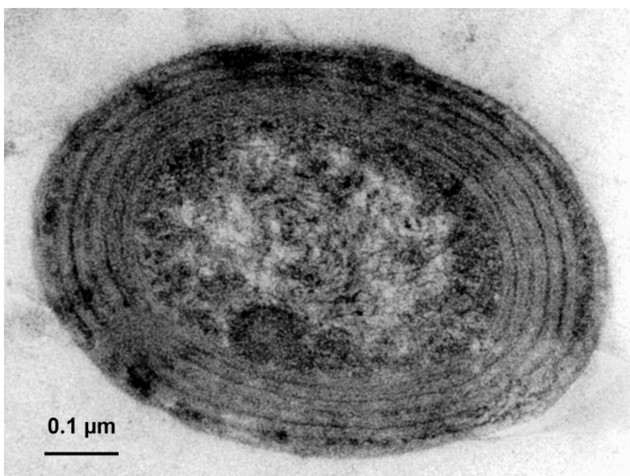


Figure 1-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Architecture of Cyanobacteria

- ***Every time I show you a picture of a cell, ask yourself how the architecture works.***
- ***For cyanobacteria, we are going to examine several remarkable specializations related to their ability to perform photosynthesis.***

(Cannon et al.)



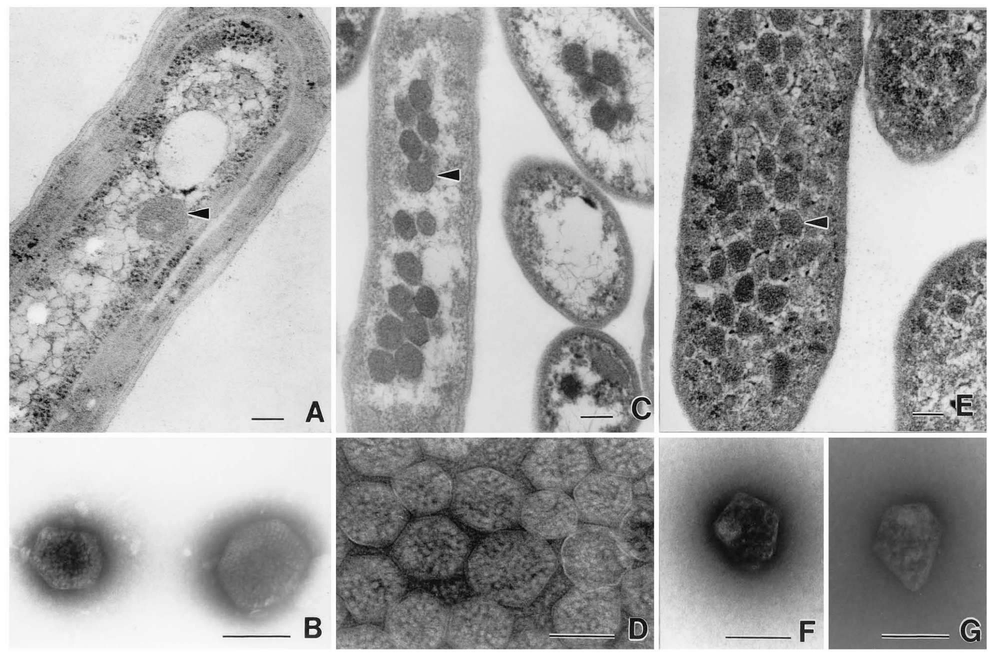
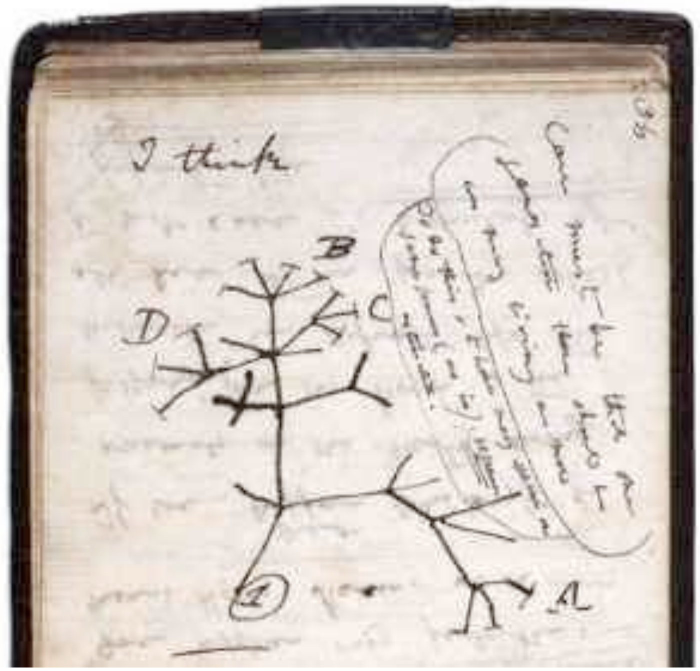


FIG. 1. Transmission electron micrographs of carboxysomes and enterosomes. (A) Thin section of a cell of *Synechococcus* strain PCC7942 (fixed cells kindly supplied by George Espie), showing a typical carboxysome (arrowhead). (B) Negatively stained carboxysomes from lysed cells of *A. nidulans* (now *Synechococcus*). Molecules of RuBisCO are visible inside. Micrograph kindly supplied by Elisabeth Gantt. (C) Thin sections of *H. neapolitanus* grown in air, showing aggregation of carboxysomes (arrowhead) in the nucleoid region of the cell. (D) Negative stain of carboxysomes isolated from *H. neapolitanus*. RuBisCO assemblies are visible inside. (E) Thin section of *S. enterica* serovar Typhimurium LT2 grown on propanediol under aerobic conditions. Many polyhedral bodies (enterosomes [arrowhead]) are visible throughout the cytoplasm. They are less regular than carboxysomes and slightly smaller. (F and G) Negatively stained, isolated enterosomes from *S. enterica* serovar Typhimurium LT2. Note the irregular shape. Contents appear to be of variable sizes. Photographed from preparation kindly supplied by Greg Havemann. Panels A, C, and E are all printed at the same magnification, as are panels B, D, F, and G. Bars, 100 nm.

A Tension: General Ideas or Embracing the Diversity

- ***There is a great charm in both finding the unity of things and in celebrating their differences.***
- ***The diversity of living organisms is thrilling and astonishing.***
- ***Darwin's notebook shows him with the realization of the single biggest unifying idea in biology: descent with modification from a common ancestor.***
- ***Diversity of life gives us a gold mine of beautiful puzzles.***



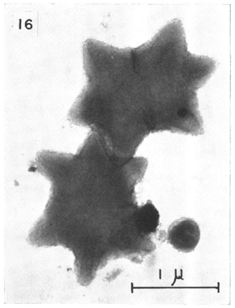
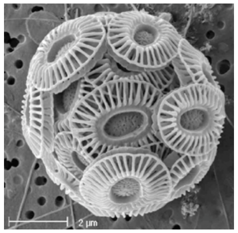


FIG. 16. *Electron micrograph of star-shaped cells (form 8) observed in a negatively stained preparation from a crude culture. Note the division planes.*



“Nothing in Biology Makes Sense Except in the Light of Evolution”

- ***And that so includes the study of: a) how to build a cell, b) photosynthesis – our first two big themes.***

Nothing in Biology Makes Sense Except in the Light of Evolution

THEODOSIUS DOBZHANSKY

AS RECENTLY AS 1966, sheik Abd el Aziz bin Baz asked the king of Saudi Arabia to suppress a heresy that was spreading in his land. Wrote the sheik:

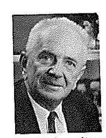
“The Holy Koran, the Prophet’s teachings, the majority of Islamic scientists, and the actual facts all prove that the sun is running in its orbit . . . and that the earth is fixed and stable, spread out by God for his mankind. . . . Anyone who professed otherwise would utter a charge of falsehood toward God, the Koran, and the Prophet.”

The good sheik evidently holds the Copernican theory to be a “mere theory,” not a “fact.” In this he is technically correct. A theory can be verified by a mass of facts, but it becomes a proven theory, not a fact. The sheik was perhaps unaware that the Space Age had begun before he asked the king to suppress the Copernican heresy. The sphericity of the earth had been seen by astronauts, and even by many earth-bound people on their television screens. Perhaps the sheik could retort that those who venture beyond the confines of God’s earth suffer hallucinations, and that the earth is really flat.

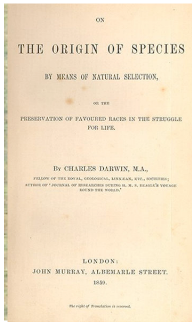
Parts of the Copernican world model, such as the contention that the earth rotates around the sun, and not vice versa, have not been verified by direct observations even to the extent the sphericity of the earth has been. Yet scientists accept the model as an accurate representation of reality. Why? Because it makes sense of a multitude of facts which are otherwise meaningless or extravagant. To nonspecialists most of these facts are unfamiliar. Why then do we accept the “mere theory” that the earth is a sphere revolving around a spherical sun? Are we simply submitting to authority? Not quite: we know that those who took time to study the evidence found it convincing.

The good sheik is probably ignorant of the evidence. Even more likely, he is so hopelessly biased that no amount of evidence would impress him. Anyway, it would be sheer waste of time to attempt to convince him. The Koran and the Bible do not contradict Copernicus, nor does Copernicus contradict them. It is ludicrous to mistake the Bible and the Koran for primers of natural science. They treat of matters even more important: the meaning of man and his relations to God. They are written in poetic symbols that were understandable to people of the age when they were written, as well as to peoples of all other ages. The king of Arabia did not comply with the sheik’s demand. He knew that some people fear enlightenment, because enlightenment threatens their vested interests. Education is not to be used to promote obscurantism.

The earth is not the geometric center of the universe, although it may be its spiritual center. It is a mere speck of dust in cosmic spaces. Contrary to Bishop Ussher’s calculations, the world did not appear in approximately its present state in 4004 B.C. The estimates of the age of the universe given by modern cosmologists are still only rough approximations, which are revised (usually upward) as the methods of estimation are refined. Some cosmologists take the universe to be about 10 billion years old; others suppose that it may have existed, and will continue to exist, eternally. The origin of life on earth is dated tentatively between 3 and 5 billion years ago; manlike beings appeared relatively quite recently, between 2 and 4 million years ago. The estimates of the age of the earth, of the duration of the geologic and paleontologic eras, and of the antiquity of man’s ancestors are now based mainly on radiometric evidence—the proportions of isotopes of certain chemical elements in rocks suitable for such studies.



One of the world’s leading geneticists, Theodosius Dobzhansky is professor emeritus, Rockefeller University, and adjunct professor of genetics, University of California, Davis 95616. Born in Russia, in 1900, he is a graduate of the University of Kiev and taught (with J. Philipchenko) at the University of Leningrad before coming to the U.S., in 1927; thereafter he taught at Columbia University and the California Institute of Technology before joining the Rockefeller faculty, in 1962. He has been president of the Genetics Society of America, the American Society of Naturalists, the Society for the Study of Evolution, the American Society of Zoologists, and the American Teilhard de Chardin Association. Among his many honors are the National Medal of Science (1964) and the Gold Medal Award for Distinguished Achievement in Science (1969). He holds 18 honorary doctorates from universities in this country and abroad. Among his well-known books are *The Biological Basis of Human Freedom* (1956) and *Mankind Evolving* (1963). The present paper was presented at the 1972 NABT convention.


ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

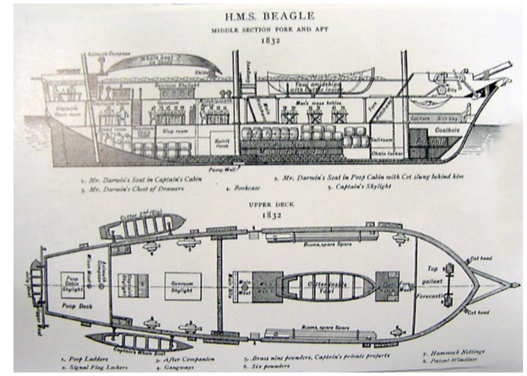
PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

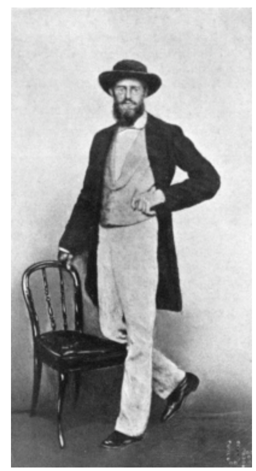
By CHARLES DARWIN, M.A.,

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1859.

Biology's Greatest Idea

- *It is a great moment to reflect on evolution: 200th birthday of Darwin, 150th anniversary of his great work "On the Origin of Species"*
- *Fascinating essay of T. Dhobzhansky entitled: ``Nothing in biology makes sense except in the light of evolution." - the phrase has become hackneyed, but the idea has not.*
- *Darwin found data led to inescapable conclusion, ``it was like confessing a murder" he wrote.*
- *Darwin's "species question" already helps us think about the photosynthetic process.*



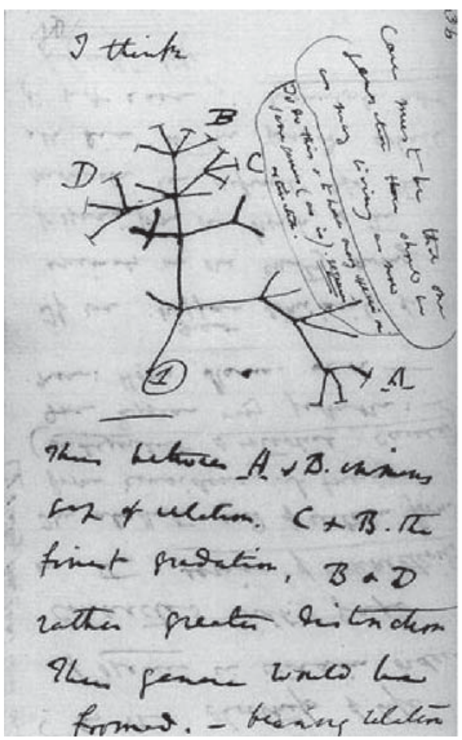


Lagrange on Newton: he was "the most fortunate, for we cannot find more than once a system of the world to establish." The same can be said of Darwin and Wallace.

Darwin's Only Figure: Extinctions and Evolution

- ***The theory of evolution is built up on many different threads of evidence and one of the most important of those threads is the frequent extinctions revealed in the fossil record.***
- ***Species have typical lifetimes measured in millions of years.***
- ***Darwin's one and only drawing in "On the Origin of Species" highlights extinctions.***

(A)



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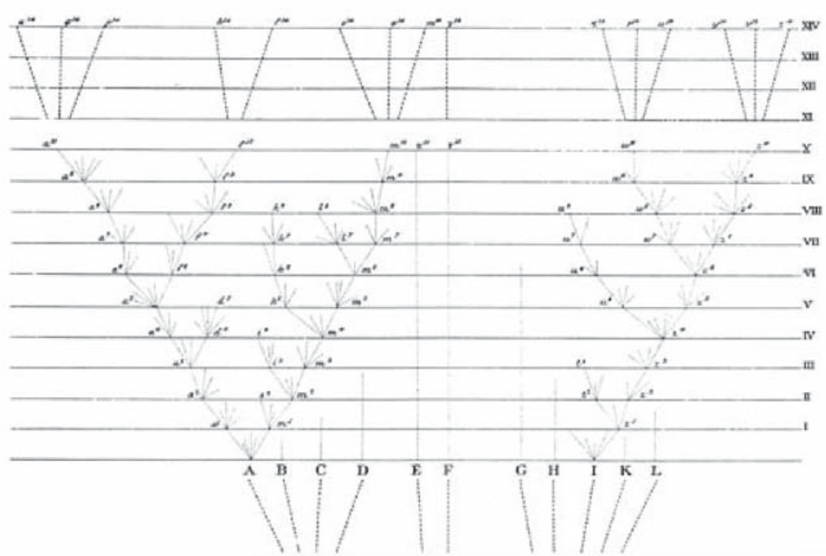
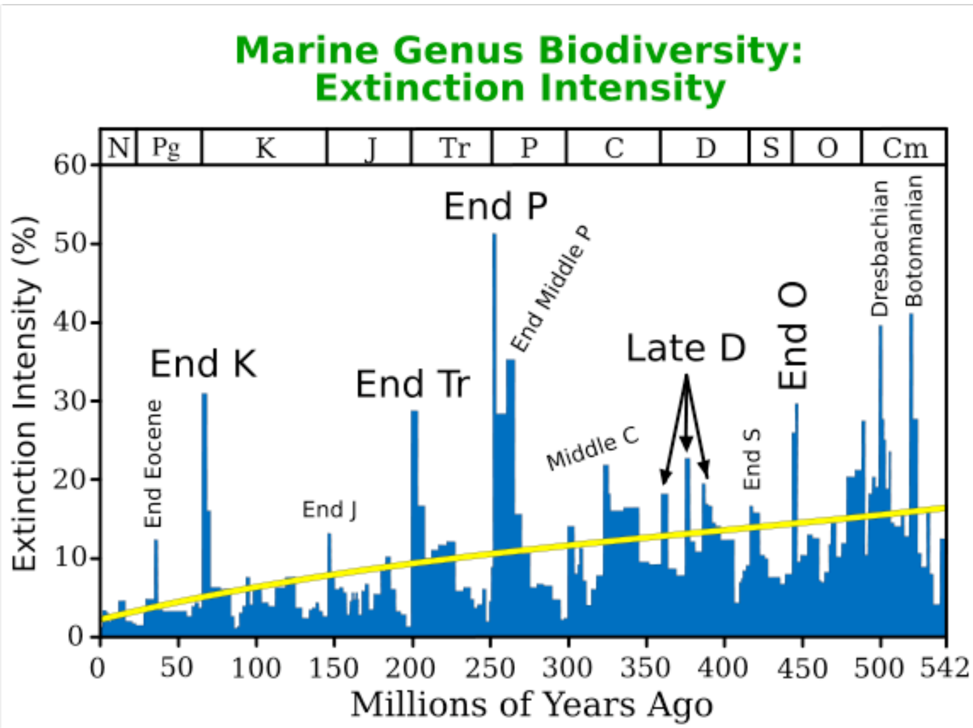


The Great Extinctions

- ***The history of life on Earth has been punctuated by massive extinction events, some of which are famous (i.e. end of dinosaurs), but some of which are much more impressive (i.e. end Permian).***
- ***For the famous dinosaur-ending extinction, a leading hypothesis argues for damage to the ability of photosynthetic organisms to collect sufficient light, with this effect propagating viciously through ecosystems.***



THE MAJOR EXTINCTION EVENTS

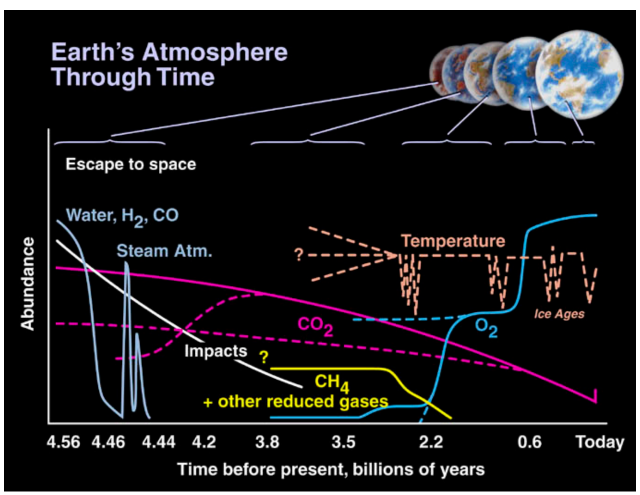
Era	Period	Epoch	Approximate duration of Era, Period, or Epoch (millions of years before present)	Major extinction events*
CENOZOIC	Quaternary	Holocene	present-0.01	←6th major extinction ?
		Pleistocene	0.01-1.6	
	Tertiary	Pliocene	1.6-5.3	
		Miocene	5.3-24	
		Oligocene	24-37	
		Eocene	37-58	
		Paleocene	58-65	
MESOZOIC	Cretaceous		65-144	←5th major extinction (end of Cretaceous; K-T boundary)
	Jurassic		144-208	
	Triassic		208-245	←4th major extinction (end of Triassic)
PALEOZOIC	Permian		245-286	←3rd major extinction (end of Permian)
	(Carboniferous) Pennsylvanian		286-325	
	(Carboniferous) Mississippian		325-360	
	Devonian		360-408	←2nd major extinction (late Devonian)
	Silurian		408-440	
	Ordovician		440-505	←1st major extinction (end of Ordovician)
	Cambrian		505-570	
PRECAMBRIAN			570-4500	

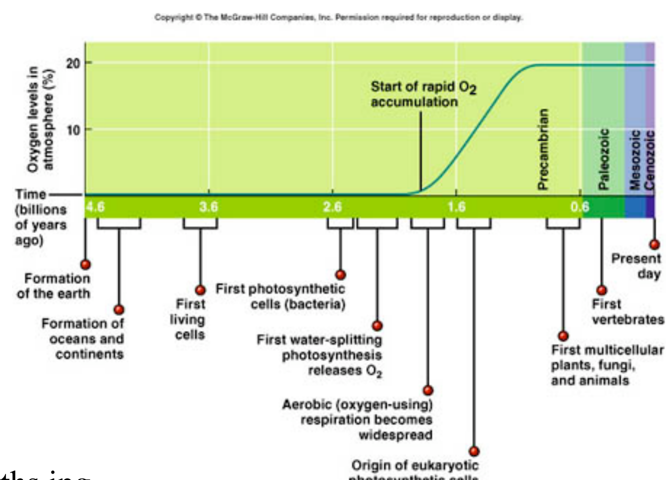
* Many smaller extinction events are not indicated; see Raup (1991: fig. 4-1 for examples).

See "Extinction: Bad Genes or Bad Luck" by David Raup or "Extinction" by Doug Erwin

Losing Light and Life

- ***The leading hypothesis on the K-T extinction event is an asteroid impact in Central America though others argue for increased volcanism.***
- ***One common feature in these different scenarios is a change in the light reaching Earth with a concomitant impact on photosynthetic organisms.***
- ***I bring this up here as an attempt to drive home the importance of photosynthesis to life on Earth.***
- ***For those with an interest in the history of Earth, photosynthesis is also a huge player in the composition of the atmosphere.***



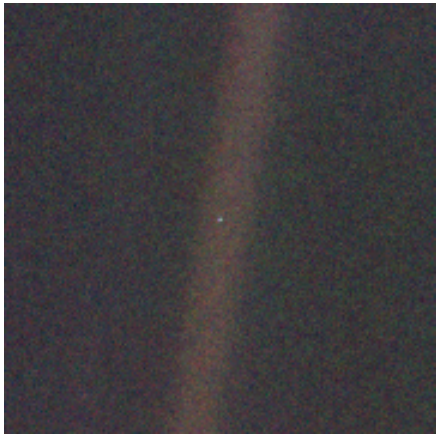


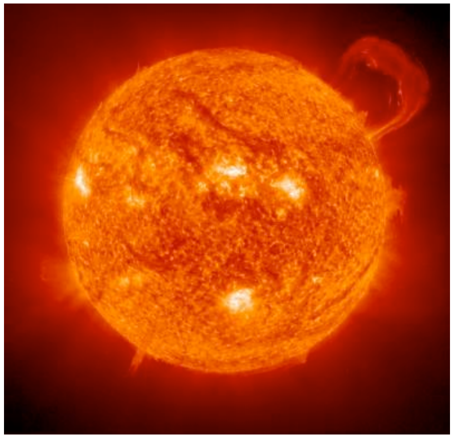
http://science.nasa.gov/headlines/y2002/images/exo-atmospheres/ATM_Time_Earths.jpg

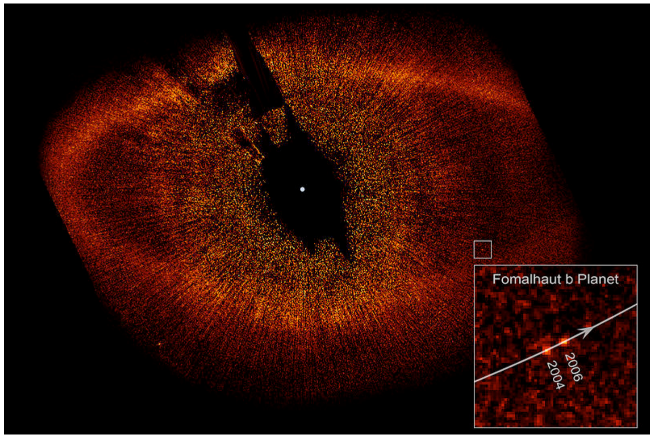
Our Pale Blue Dot: Eating the Sun

"Look again at that dot. That's here. That's home. That's us. On it everyone you love, everyone you know, everyone you ever heard of, every human being who ever was, lived out their lives. The aggregate of our joy and suffering, thousands of confident religions, ideologies, and economic doctrines, every hunter and forager, every hero and coward, every creator and destroyer of civilization, every king and peasant, every young couple in love, every mother and father, hopeful child, inventor and explorer, every teacher of morals, every corrupt politician, every "superstar," every "supreme leader," every saint and sinner in the history of our species lived there-on a mote of dust suspended in a sunbeam."   - Carl Sagan, seen in Al Gore movie

And, every cell (at least my favorite hypothesis) and the 10^45 genomes that have ever existed.

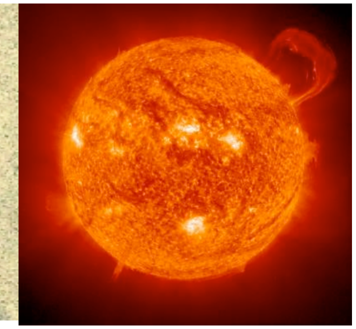




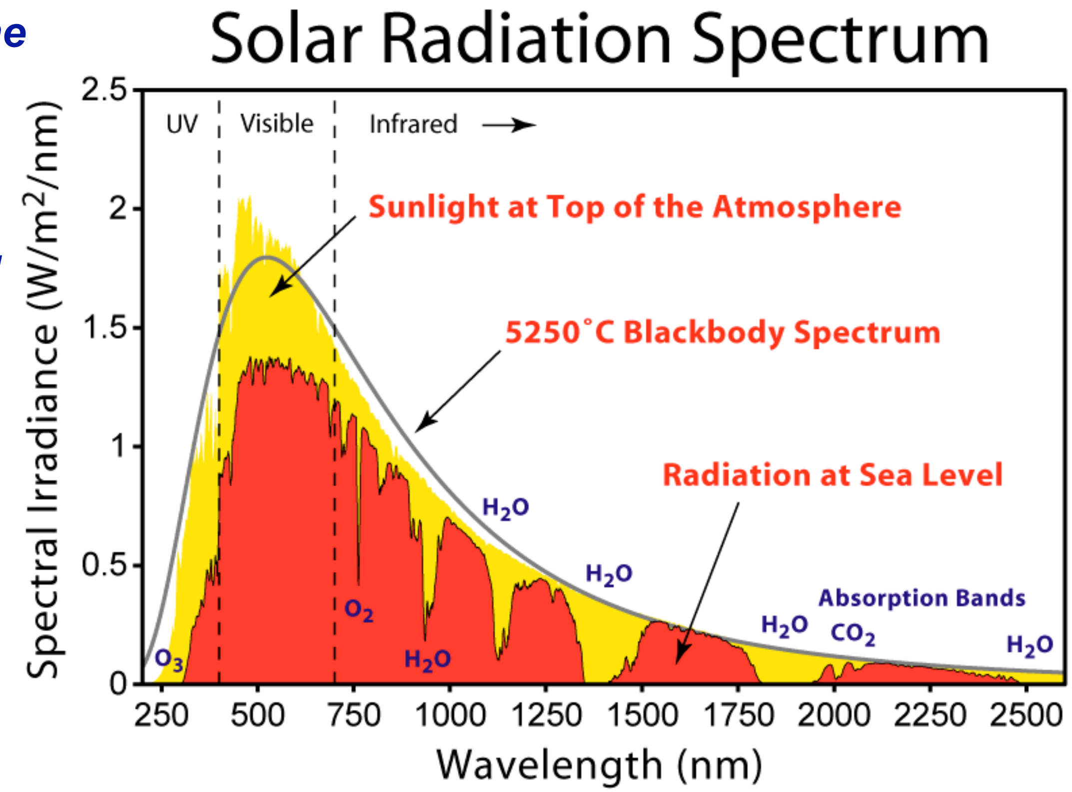


Such pictures make me think of the amazing way that life has learned to eat the sun. (see the book by Oliver Morton with that title)

Sunlight and the Earth



- ***This curve allows us to see how the incident radiation from the sun is partitioned amongst different wavelengths.***
- ***We should carry some important numbers around in our heads and one of them is 1200 W/m^2 as the power available from sunlight.***



"The world looks so different after learning science. For example, trees are made of air, primarily. When they are burned, they go back to air, and in the flaming heat is released the flaming heat of the sun which was bound in to convert the air into tree … These things are beautiful things, and the content of science is wonderfully full of them. They are very inspiring, and they can be used to inspire others." - Richard Feynman

What Do We Know About Eating the Sun and How Do We Know It?

- ***The stoichiometric equation for photosynthesis tells us the key idea of the process.***

$$6CO_2 + 12H_2O + light \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

  - ***Energy of sunlight is converted into useful chemical bond energy in the form of sugar.***
  - ***Broadly speaking, the process can be conceptually divided into a part having to do with harvesting light, a part having to do with shuttling electrons and protons and a part having to do with fixing atmospheric carbon.***
  - ***Our plan: we will start with an overview in words and cartoons and then make some calculations on several key parts of the story.***

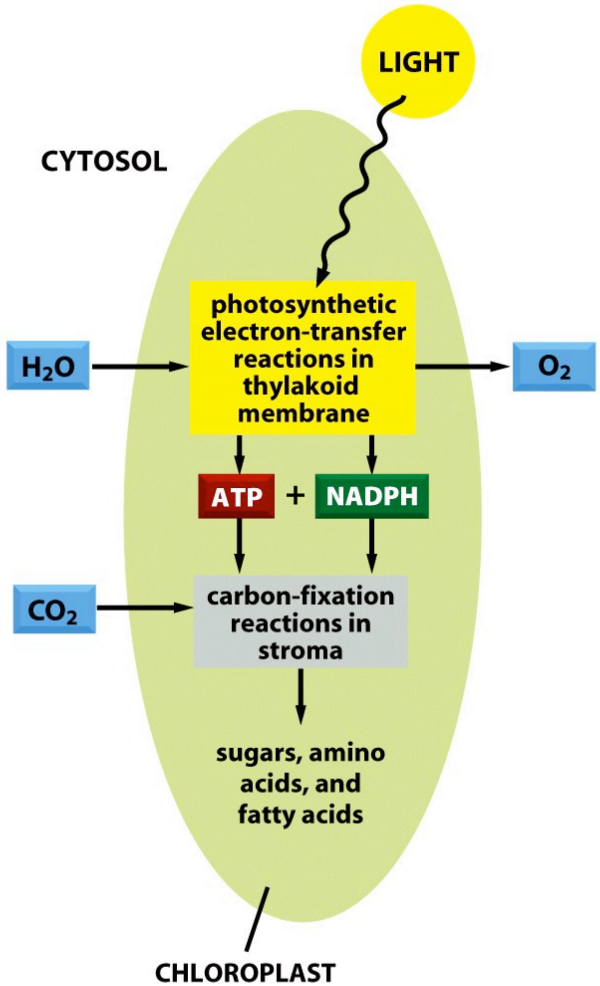
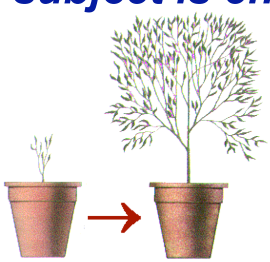
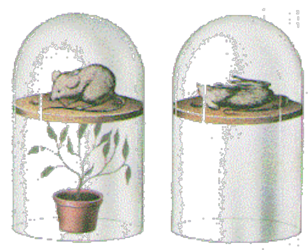


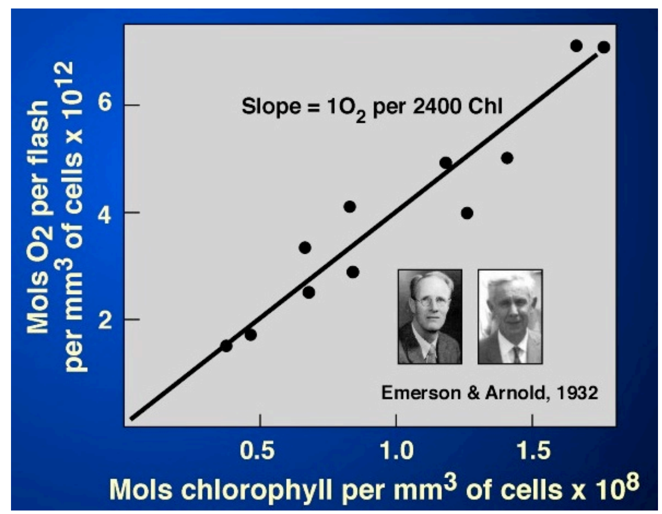
Figure 14-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Key Themes and Key Experiments

- ***The amazing story of photosynthesis passes through the conservation of matter, the discovery of the existence and nature of gases, the conservation of energy, the nature of microbes, the biochemical basis of metabolism and beyond.***
- ***This subject is characterized by a long history of quantitative measurement.***



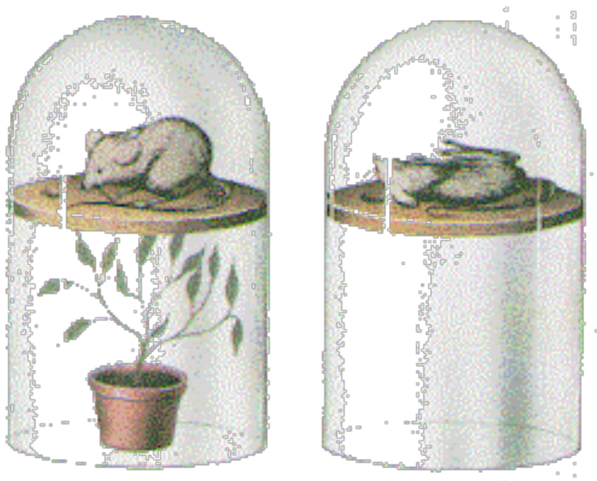




(Govindjee)

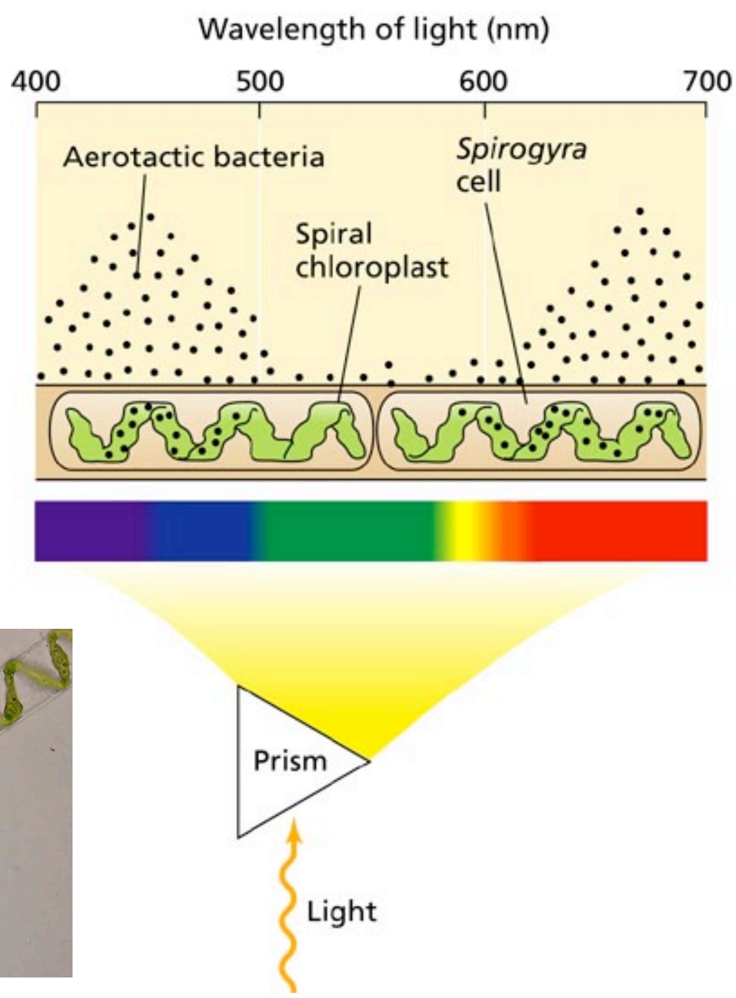
Gases and the Pneumochemists

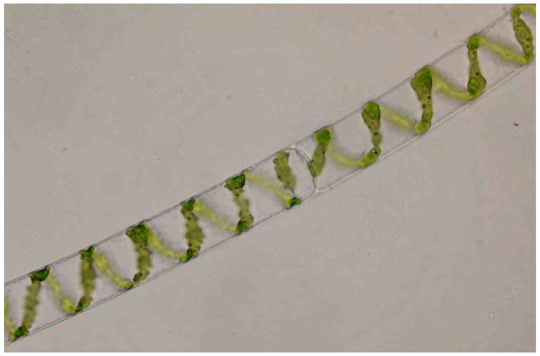
- ***Van Helmont – measurement of mass change in soil during growth. He thought that the key mass transaction was the water. Ironic since he is one of the founders of the study of gases.***
- ***The great "pneumochemists" – Lavoisier, Priestley, de Saussure, Ingenhousz, Senebier and others – the idea: measure the gases required for and liberated by photosyntheis.***
- ***Boussingault – measured ratio of CO_2 taken up and O_2 released.***
- ***Outcome: a stoichiometrically correct equation for the photosynthetic transaction that properly acknowledges the roles of water and CO_2.***



Light in Photosynthesis

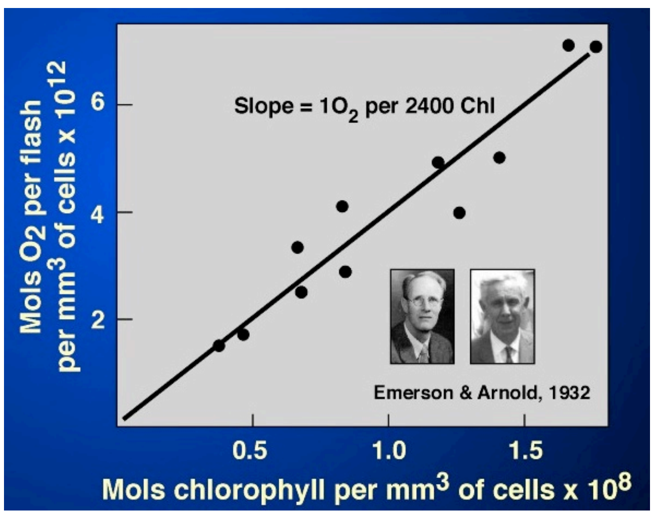
- *A variety of interesting experiments demonstrated that light is required for photosynthesis.*
- *Experiment of T. W. Engelmann – expose Spirogyra (challenged on wiki) (filamentous, green algae) to light of different wavelengths and see where bacteria aggregate. Answer: at places where chlorophyl absorbs. Bacteria provide a "living graph" of absorption spectrum.*
- *Starch production in leaves can be stained. Where starch is synthesized controlled by light exposure.*





Light in Photosynthesis: Emerson and Arnold and Flashing Experiments

- ***A final example: clever, precision measurements of Emerson and Arnold by flashing lights of known intensity for known time periods.***



Published November 20, 1932

THE PHOTOCHEMICAL REACTION IN PHOTOSYNTHESIS

By ROBERT EMERSON and WILLIAM ARNOLD

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

(Accepted for publication, July 13, 1932)

I

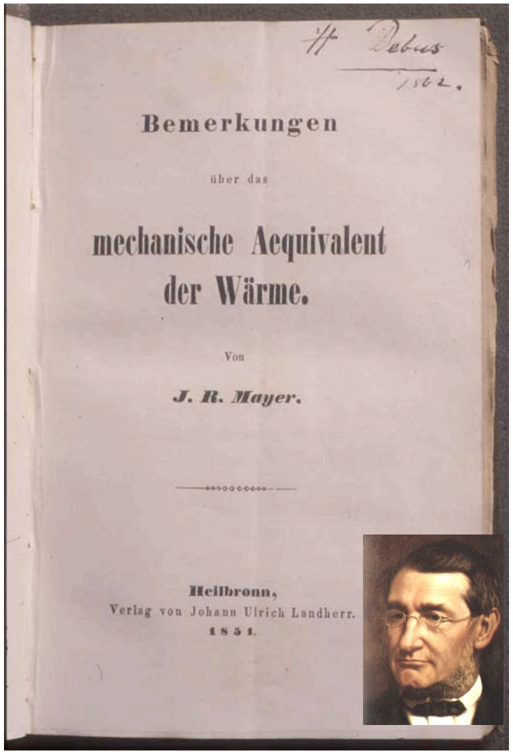
From the experiments of Warburg and Negelein (1923), we know that the green alga *Chlorella pyrenoidosa* can reduce one molecule of carbon dioxide for each four quanta of light absorbed, when conditions permit maximum efficiency. Chlorophyll is clearly the substance absorbing the light quanta, so we may inquire how much chlorophyll must be present for the reduction of one molecule of carbon dioxide.

In a preceding paper (1932) we have presented evidence that the mechanism involved in the photochemical reaction must undergo a slower reaction, the so called Blackman reaction, before it can again take part in the photochemical reaction. Let us consider a cell in flashing light when the dark periods between flashes are so long that each unit activated in a given flash has time to complete the Blackman reaction before the next flash. Increasing the intensity of the flashes should increase the carbon dioxide reduction per flash until each unit capable of undergoing the photochemical reaction does so once in each flash. We say then that the photochemical reaction is saturated with light. The possibility that any unit will undergo the light reaction more than once in a single flash may be neglected, because the time required for the completion of the dark reaction is about 0.02 sec. at 25°C., while the duration of a light flash is 10^{-5} sec.

We define one unit arbitrarily as the mechanism which must undergo the photochemical reaction to reduce one molecule of carbon dioxide. If we can obtain light flashes of sufficient intensity to saturate the photochemical reaction, then the number of units in a sample of cells will equal the number of carbon dioxide molecules reduced per flash. The

The Conservation of Energy and Photosynthesis

- *Though we all take the conservation of energy for granted, it was an idea that was hard won.*
- *Julius Robert Mayer articulated the idea and proposed that photosynthesis is a concrete example with energy from sunlight converted into chemical bond energy.*

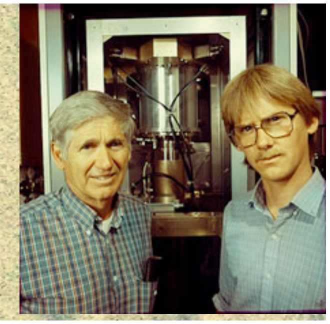


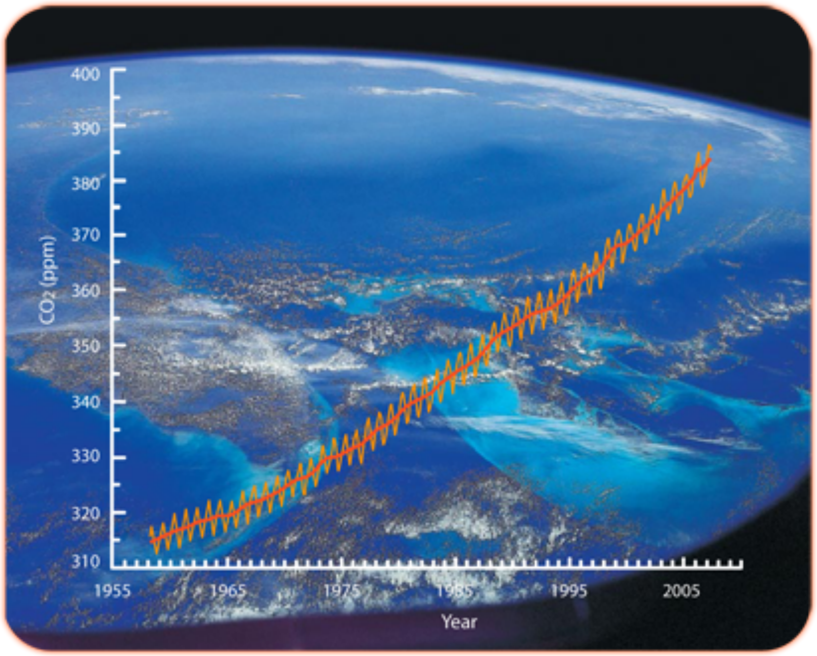
Big themes that passed through photosynthesis: mass conservation, energy conservation, nature of gases, light and life.

Logic of Lecture

- Logical flow of lecture: we have talked about the history of our understanding of photosynthesis (very broad brush strokes) and the themes that emerged.
- Now, we turn to simple estimates about the overall photosynthetic productivity on the Earth and the nature of the cells that do this photosynthesis.

Use the Earth's breathing to formulate an estimate

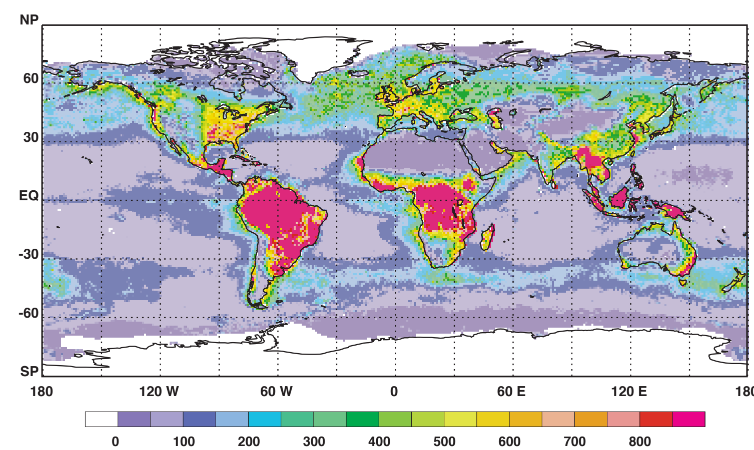




- Building a cell with photons.
- *My daughter's biochemistry book (and many others) tells me: every year, the earth's plants convert 6 x 10^16 grams of carbon to organic compounds.*
- *The point of this estimate is to see how light, CO_2 and H_2O are used to make cells.*

(Behrenfeld, Falkowski et al., Science, 1998)

Fig. 1. Global annual NPP (in grams of C per square meter per year) for the biosphere, calculated from the integrated CASA-VGPM model. The spatial resolution of the calculations is 1° × 1° for land and 1/6° × 1/6° for the oceans. Input data for ocean color from the CZCS sensor are averages from 1978 to 1983. The land vegetation index from the AVHRR sensors is the average from 1982 to 1990. Global NPP is 104.9 Pg of C year^{-1} (104.9 × 10^{15} g of C year^{-1}), with 46.2% contributed by the oceans and 53.8% contributed by the land. Seasonal versions of this map are available at www.sciencemag.org/feature/data/982246.shl. NP, North Pole; EQ, equator; Sp, South Pole.



- *David Keeling spent his entire career making ever more precise measurements of CO2.*
- *In his interviews, he mentions that he initially made two interesting discoveries, one of which is less famous, but extremely relevant to our story.*

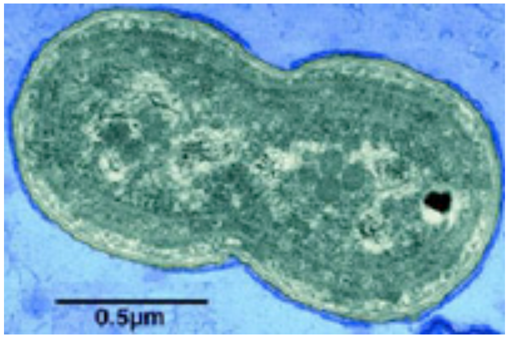
Who Does Photosynthesis and How Much?

Baobob tree

(Image credit: plizzba [flickr])

Mangrove tree

Synechococcus



Arabidopsis thaliana

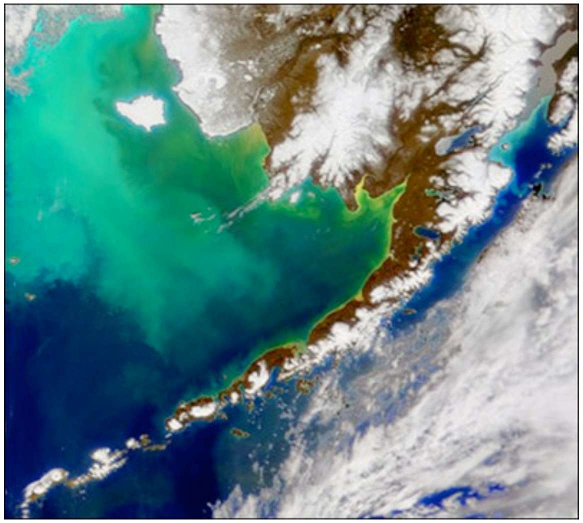
Corn (maize)

Chlamydomonas

60:40 split between land and ocean in net primary production.

Who Does Photosynthesis and How Much? A Wonderful Example!

- ***One of my favorite marine organisms is Emiliana huxleyi, a single-celled, eukaryote that performs photosynthesis to make a living.***
- ***These organisms also have a peculiar morphology (mineral shell) that scatters light and gives characteristic appearance to the ocean from space known as a "bloom".***
- ***Reminder: one of biology's "great ideas" that is easy to take for granted is the cell theory, the idea that all living organisms are made up of cells and: "Omnis cellula e cellula" - every cell from a pre-existing cell. Rudolf Virchow***



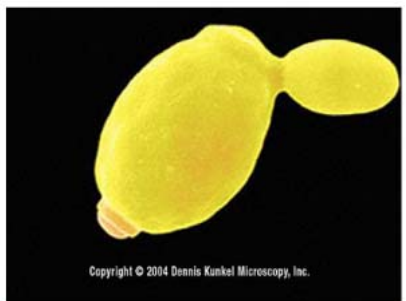
Thinking the Numbers vs Measuring Them

- ***What are the numbers, how should we think about them?***
- ***An ode to estimation (and an argument with the class)!***

B10NUMB3R5

THE DATABASE OF USEFUL BIOLOGICAL NUMBERS

ATP to make one cell: ~55 billion
Volume occupied by RNA: 6%
Number of tRNA/cell: ~200,000
Speed: 50 μm/sec
Ribosomes: 6,800 - 72,000
Proteins: $\sim 3.6 \times 10^6$
Translation rate: 12 - 21 aa/sec
Volume occupied by water: 70%



Generation time: 4 days
Cells in an adult male: 1031
Number of genes: 20,621
Eggs laid during lifetime: 300
Size of Genome: 100Mbp
Life span: 2-3 weeks
Run speed at 20°C: 0.13mm/sec
Cells in hatched larvae: 556

Median haploid volume: 42 μm^3
Number of ribosomes: ~200,000
Nucleus volume: 7% of cell
mRNA out of total RNA: 5%
mRNA in cell: 15,000
Kcat of Pyruvate kinase: 71,400/min
Cell diameter: ~5μm
RNA to DNA ratio: 50

Total number of taste buds: 10,000
Cell divisions in a life-time: 10^{17}
Abundance of p53 per cell: ~160,000
Average brain weight: ~1350g
Hairs on the head: 90,000-150,00
Diameter of erythrocytes: 7.5μm
Weight of skin: 4.1 Kg
Average time between blinks: 2.8 Sec

"If arithmetic, mensuration and weighing be taken away from any art, that which remains will be not much." - Plato

The Numbers: A First Look at RuBisCO and CO_2

- Comments on estimates, playing with numbers, sanity checks, Fermi problems, etc.
- How many molecules of CO_2 in the atmosphere, how many carbons fixed by photosynthesis each year, how many rubisco molecules, etc.?

$$M_{atmosphere} = pA/g \approx (10^5 N/m^2)(4\pi(6\times10^6 m)^2)/10m/s^2 \approx 3.6\times10^{18} kg$$

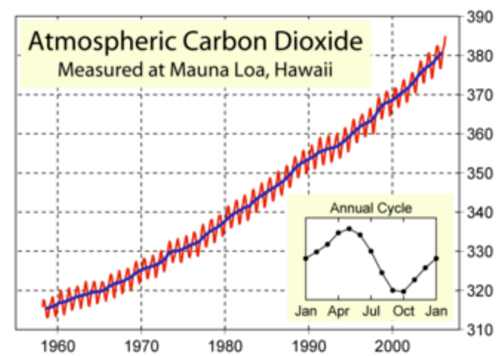
$$\# molecules \approx M/(0.78 m_{N_2} + 0.2 m_{O_2}) \approx 10^{44}\, molecules \qquad \# CO_2 \approx \frac{400}{10^6}10^{44} \approx 4\times10^{40}$$

$$\# CO_2\ fixed \approx \frac{10}{10^6}10^{44} \approx 10^{39} \Rightarrow m_{CO_2} \approx 10^{13} kg$$

- These 10^39 carbons are fixed by rubisco molecules operating at a rate of roughly one carbon fixed per second (average night and day, plants and microbes).

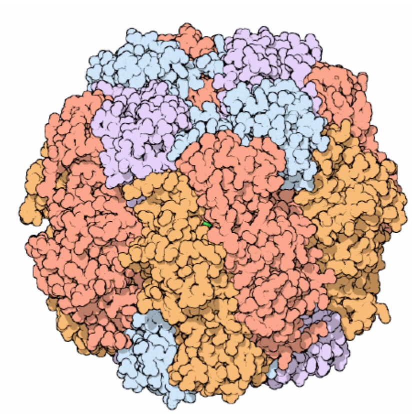
$$rubisco\ rate \approx 10^{39} C/(\pi\times10^7 s) \approx 10^{31} s^{-1} \Rightarrow 10^{31} molecules$$

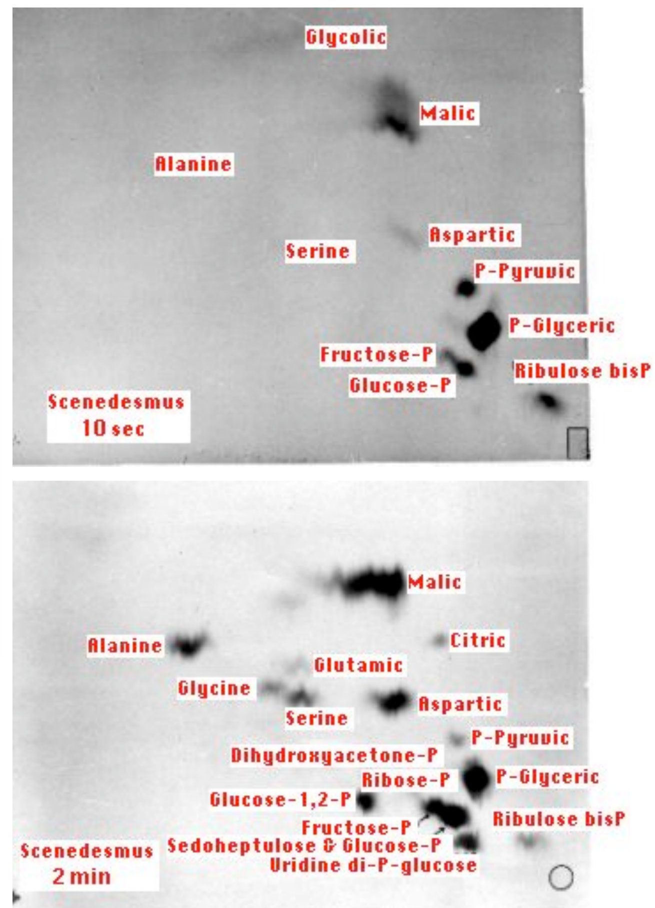
$$\# of\ photons \approx 10\, photons\, per\, carbon\, fixed \Rightarrow 10^{40}\, photons$$



Calvin and Benson: “The Path of Carbon in Photosynthesis” – the role of RuBisCO

- ***The concept: where are the radioactive carbons? NOTE: as usual, when examining these classic experiments, I am struck by how blunt their instruments were and nevertheless, the reach of those discoveries. Radioactivity + chromatography.***
- ***The discovery: rubisco (among other things), the machine responsible for taking atmospheric CO_2 and carrying out the first steps in carbon fixation.***
- ***Books claim “rubisco is the most abundant protein on earth”. Is it true? Such assertions cannot be made without some sort of justification! Let’s check the numbers.***



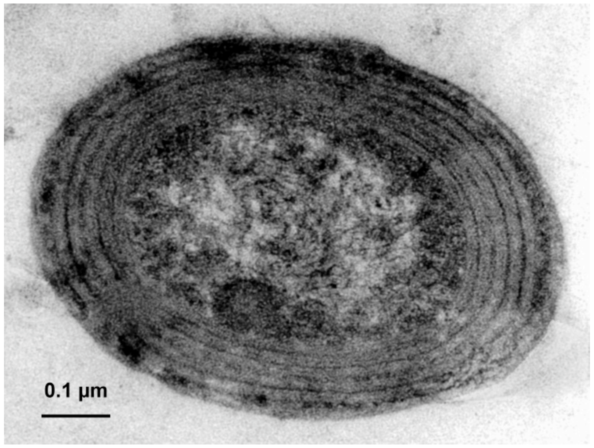


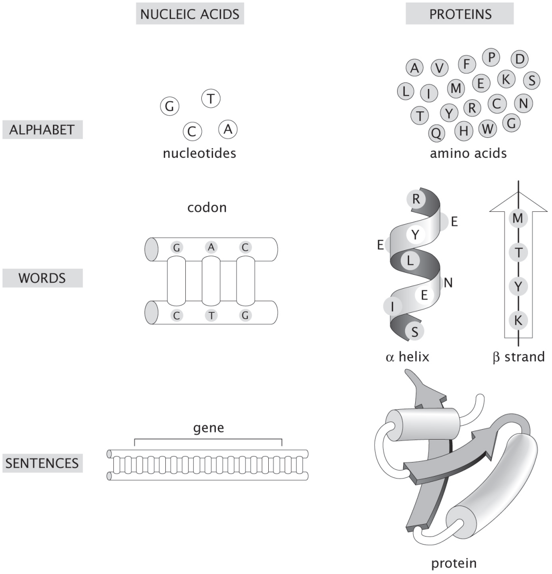
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/Chromatography_paper.html#Autoradiography

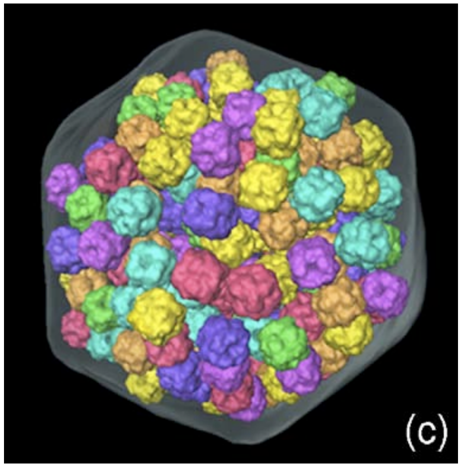
Inventories and Budgets for Some of the Most Abundant Organisms on Earth

- ***We think about the microbes that are responsible for 40% of the overall photosynthesis on Earth.***
- ***Ocean water census tells us between 10,000 and 100,000 cyanobacteria per mL. This yields estimate of roughly 10^{26} cyanobacteria doing 10% (ish) of the overall carbon fixation. Conclusion: 10^4 rubisco per cyanobacterium.***
- ***Using relatively few facts: 1 pg in 1fL with 30% of the mass ``dry''. 30,000 Da "typical" protein tells us 3 x 10^6 proteins.***

Prochlorococcus





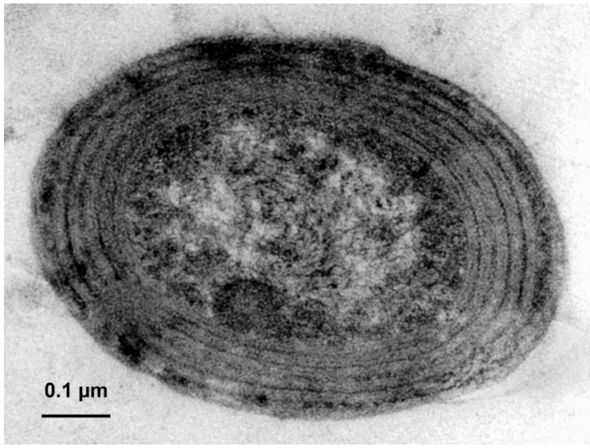


(Iancu et al, JMB, 2007)

Photosynthetic Membranes!

- ***One of the intriguing features of these organisms is their membrane disposition. Membrane area is roughly 5 microns^2. This means that the number of lipids in the outer leaflet of the bilayer is roughly 10^7, yielding a total of roughly 10^8. Membrane management: interesting and challenging.***

Prochlorococcus



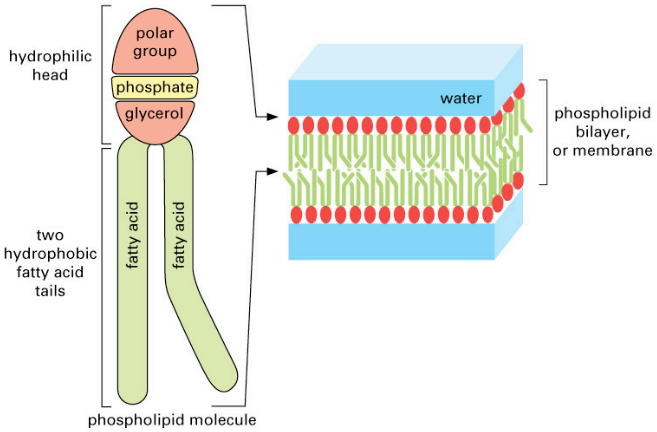
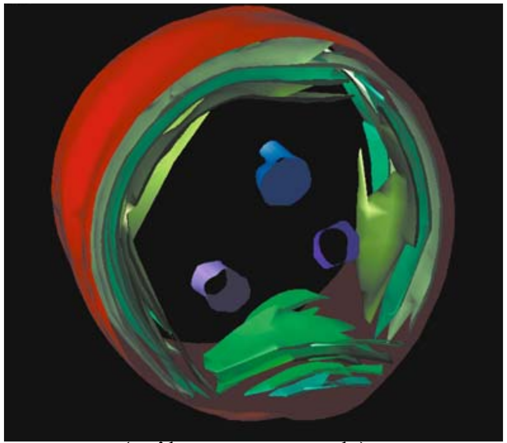


Figure 2-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Thylakoid membrane in Synechocystis



(Liberton et al.)

Logic of Lecture

- ***Next: how light is captured and energy is stored by photosynthetic organisms.***

Electron Microscopy Images of Chloroplasts

- ***We already talked about cyanobacteria. Most familiar photosynthetic organisms are plants. They have internal organelles devoted to photosynthetic process (these organelles are thought to be endosymbionts – how do we know?).***
- ***Chloroplast structure is rich and fascinating, and features a complex membrane system dividing the chloroplast into three distinct spaces.***
- ***Thylakoid membranes are a challenge to our understanding of biological membrane morphology.***

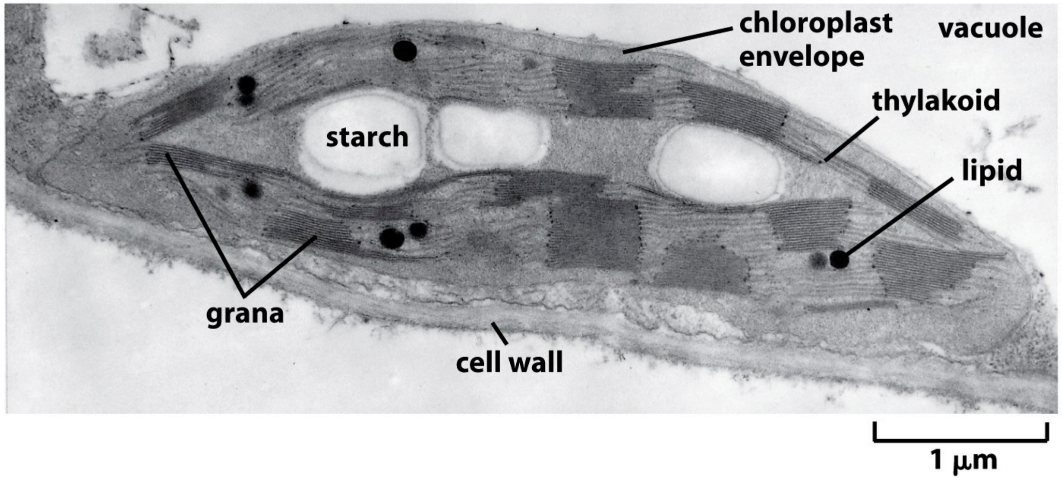


Figure 14-35b Molecular Biology of the Cell 5/e (© Garland Science 2008)

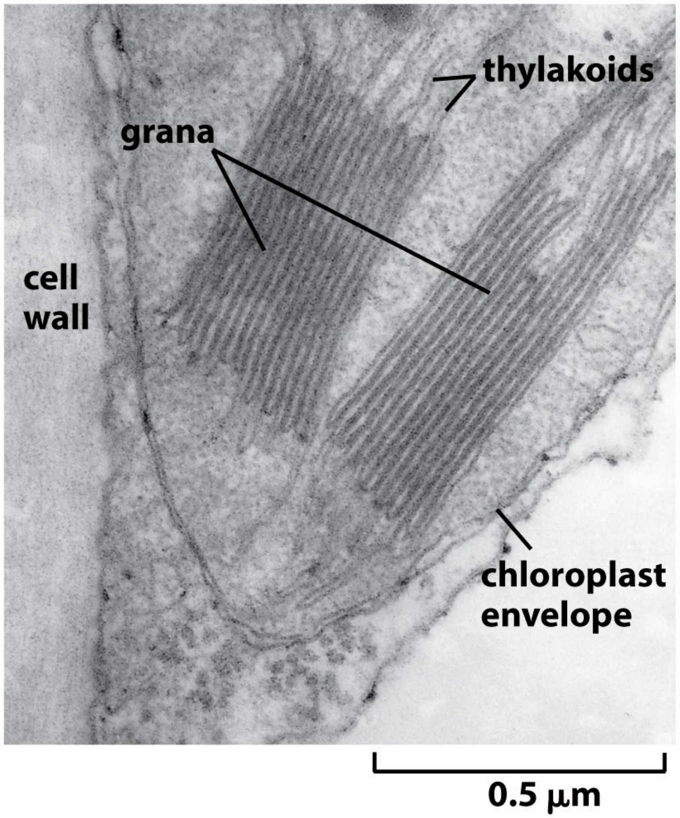


Figure 14-35c Molecular Biology of the Cell 5/e (© Garland Science 2008)

Models of Chloroplast Structure

- *Hierarchical description of the structure of chloroplasts.*
- *This schematic shows the three membrane-bound spaces as well as the thylakoid membrane system.*
- *Note from RP: the formation of maintenance of these membrane structures is fascinating and mysterious.*

From Alberts, MBoC5: This photosynthetic organelle contains three distinct membranes (the outer membrane, the inner membrane, and the thylakoid membrane) that define three separate internal compartments (the intermembrane space, the stroma, and the thylakoid space). The thylakoid membrane contains all the energy-generating systems of the chloroplast, including its chlorophyll. In electron micrographs, this membrane seems to be broken up into separate units that enclose individual flattened vesicles (see Figure 14-35), but these are probably joined into a single, highly folded membrane in each chloroplast. As indicated, the individual thylakoids are interconnected, and they tend to stack to form grana.

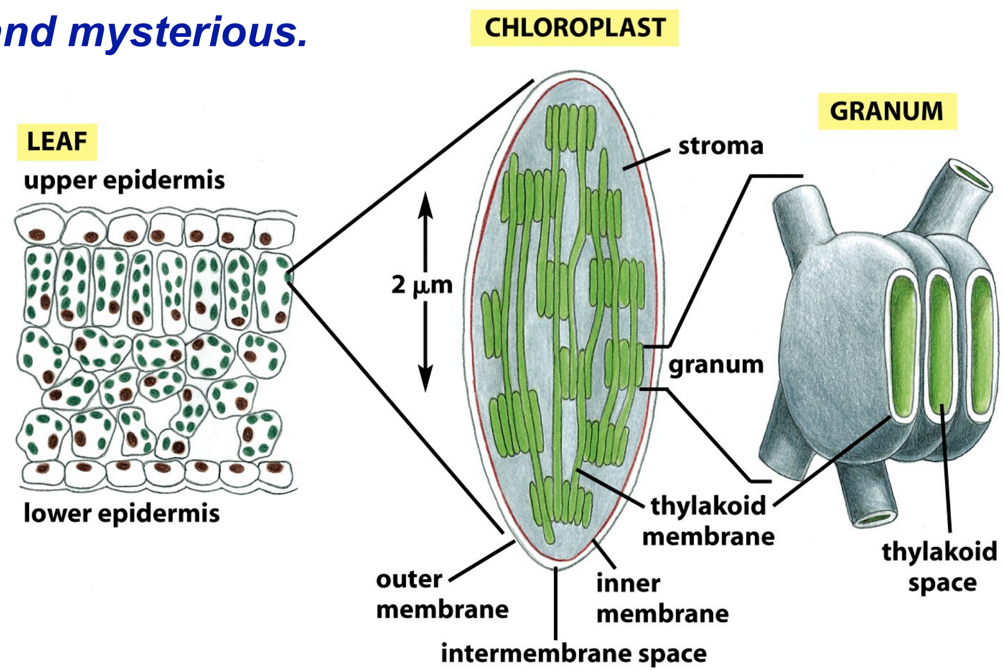


Figure 14-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Comparison of Mitochondria and Chloroplasts: Energy Factories of the Cell

- ***Mitochondria and chloroplasts share several interesting features.***
- ***Foremost, they are both thought to be endosymbionts and have their own DNA to prove it.***
- ***Complex membrane morphologies provide the seat of membrane machines responsible for ATP generation, electron transfer (and charge separation) and light harvesting (chloroplasts).***

From Alberts et al., MBoC5: A chloroplast is generally much larger than a mitochondrion and contains, in addition to an outer and inner membrane, a thylakoid membrane enclosing a thylakoid space. Unlike the chloroplast inner membrane, the inner mitochondrial membrane is folded into cristae to increase its surface area.

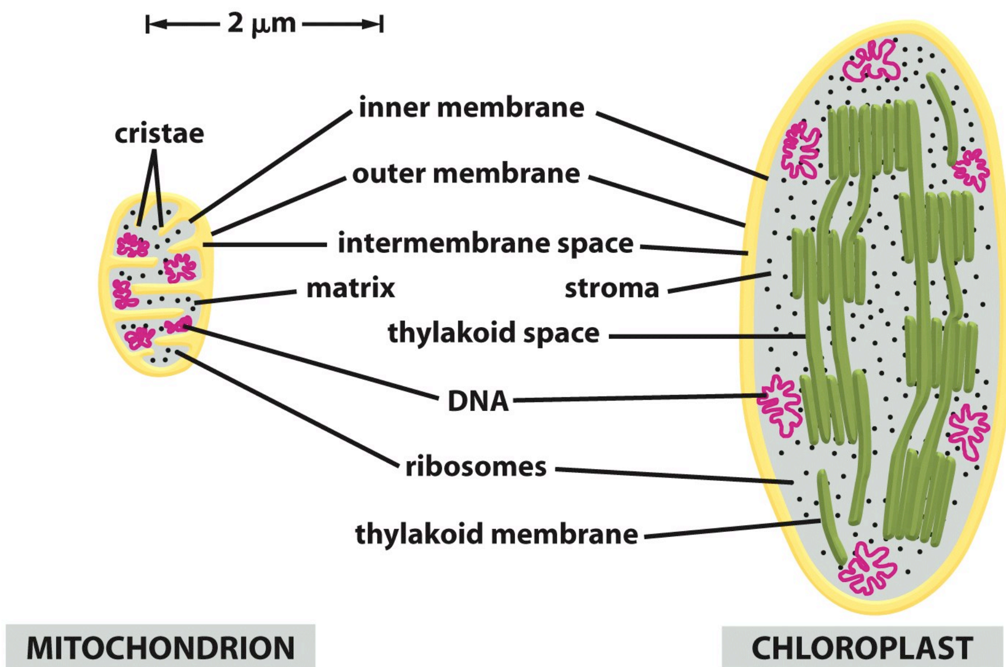


Figure 14-37 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Molecules Responsible for Absorption of Light

- ***Chlorophyll characterized by a porphyrin ring and a hydrophobic tail which anchors the molecule to the membrane.***
- ***The porphyrin ring is host to the electronic states that participate in the interaction with light.***

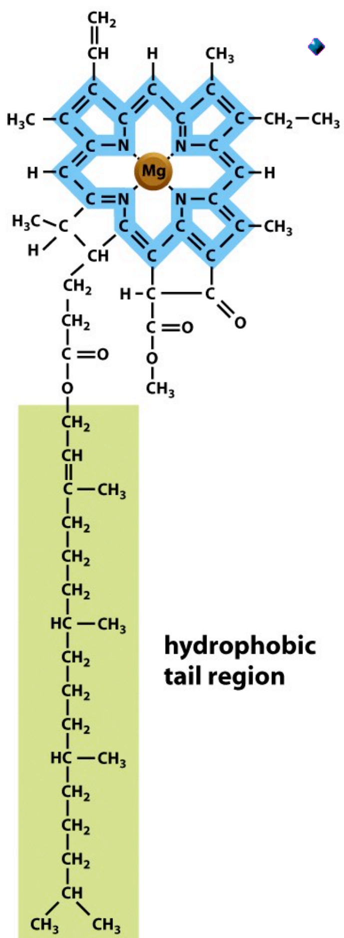


Figure 14-42 Molecular Biology of the Cell 5/e (© Garland Science 2008)

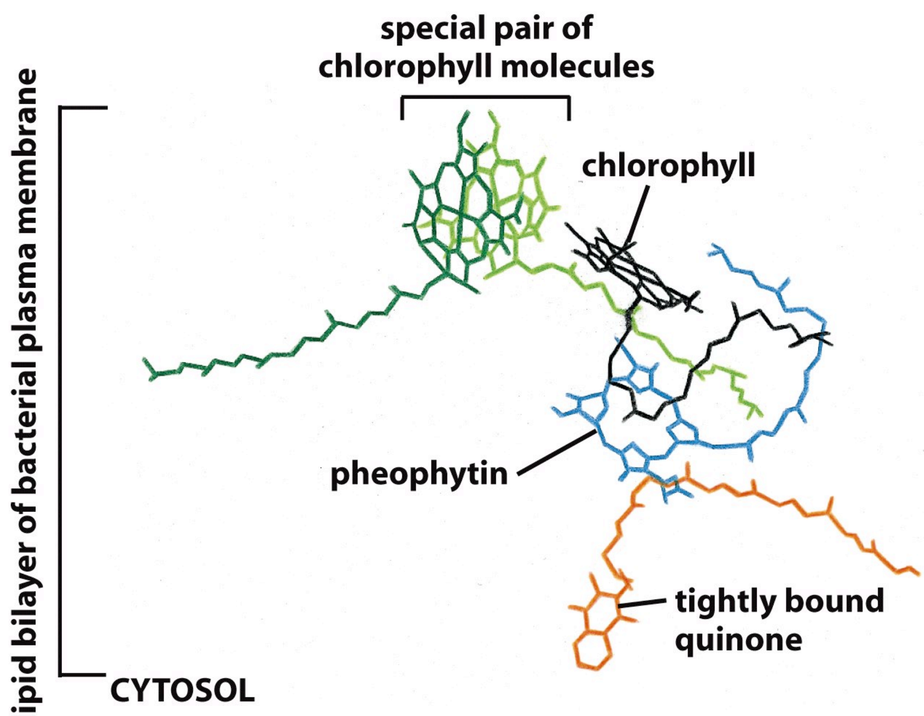
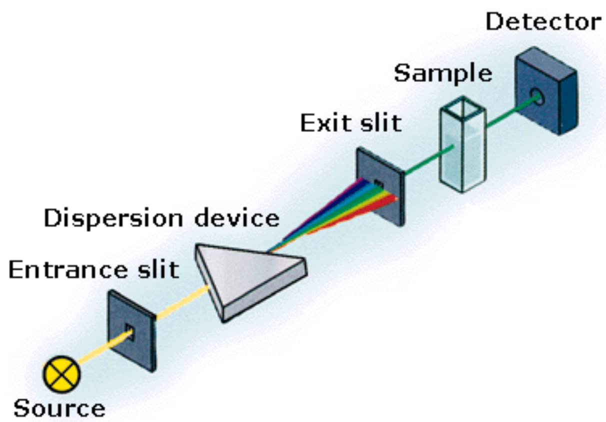
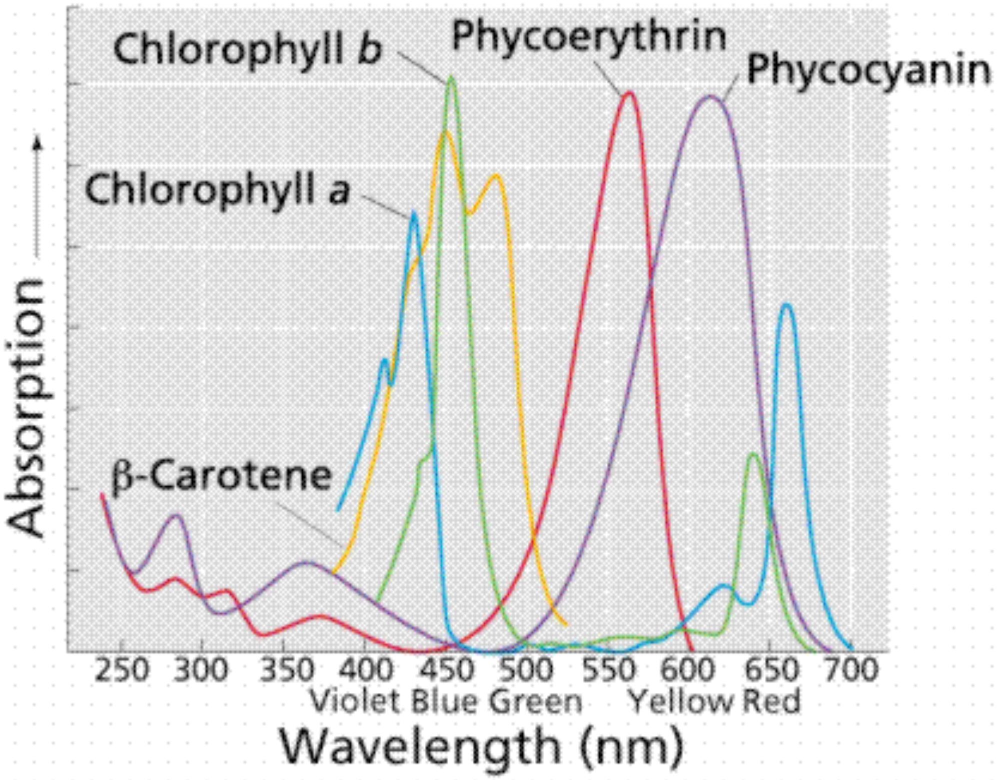


Figure 14-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Absorption Spectra of Biological Pigments

- *The spectrophotometer permits the measurement of absorption as a function of the incident wavelength.*
- *Note that chlorophyll appears green because it absorbs strongly in the blue and the red.*
- *We will be interested in examining the quantum mechanical underpinnings of absorption spectra.*





Pigments in Different Organisms

- ***Chlorophylls and other biological pigments are quite diverse (and can be used to help us classify organisms – think about it, how do you decide on evolutionary relatedness?).***
- ***This table is in case you want to think about these molecules more deeply. (see "Plant Physiology" by Lincoln Taiz and Eduardo Zeiger.***

Web Table 7.2.A
Distribution of chlorophylls and other photosynthetic pigments

	Chlorophylls				Bacteriochlorophylls							
Organism	a	b	c	d	a	b	c	d	e	g	Carotenoids	Phycobiliproteims
Eukaryotes												
Mosses, ferns, seed plants	+	+	−	−							+	−
Green algae	+	+	−	−							+	−
Euglenoids	+	+	−	−							+	−
Diatoms	+	−	+	−							+	−
Dinoflagellates	+	−	+	−							+	−
Brown algae	+	−	+	−							+	−
Red algae	+	−	−	+							+	+
Prokaryotes												
Cyanobacteria	+	−	−	+							+	+
Prochlorophytes	+	+	−	−							+	−
Sulfur purple bacteria					+ or	+	−	−	−	−	+	−
Nonsulfur purple bacteria					+ or	+	−	−	−	−	+	−
Green bacteria					+	−	+ or	+ or	+	−	+	−
Heliobacteria					−	−	−	−	−	+	+	−

http://4e.plantphys.net/article.php?ch=7&id=67

Photon Flux Calculation

- ***The question: How many photons are reaching a molecule each second and what might this tell us about the nature of the photosynthetic reactions?***

How Light Energy Is Funneled Away to Perform Charge Separation

- ***One of the key outcomes of the Emerson-Arnold experiments was the realization that the molecular apparatus came with numbers that had an odd ratio.***

From Alberts et al., MBoC5: The antenna complex is a collector of light energy in the form of excited electrons. The energy of the excited electrons is funneled, through a series of resonance energy transfers, to a special pair of chlorophyll molecules in the photochemical reaction center. The reaction center then produces a high-energy electron that can be passed rapidly to the electron-transport chain in the thylakoid membrane, via a quinone.

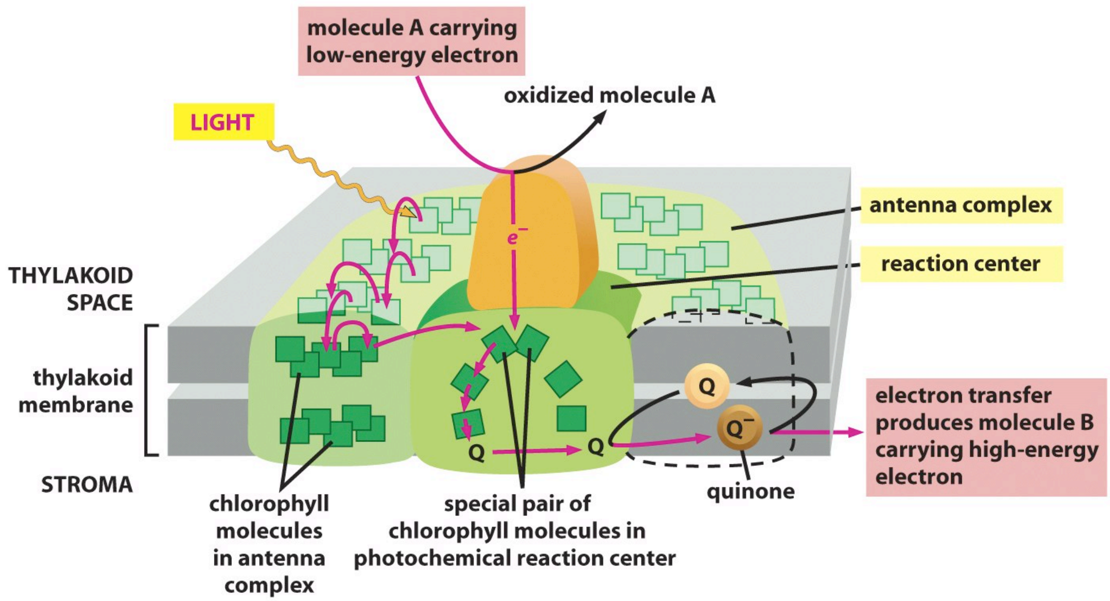


Figure 14-43 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The Molecular Machines of Photosynthesis

Figure 14-47 The structure of photosystem II in plants and cyanobacteria.

The structure shown is a dimer, organized around a two fold axis (red dotted arrows). Each monomer is composed of 16 integral membrane protein subunits plus three subunits in the lumen, with a total of 36 bound chlorophylls, 7 carotenoids, two pheophytins, two hemes, two plastoquinones, and one manganese cluster in an oxygen-evolving water-splitting center. (A) The complete three-dimensional structure of the dimer. (B) Schematic of the dimer with a few central features indicated. (C) A monomer drawn to show only the non-protein molecules in the structure, thereby highlighting the protein-bound pigments and electron carriers; green structures are chlorophylls. (Adapted from K.N. Ferreira et al., Science 303:1831-1838, 2004. With permission from AAAS.)

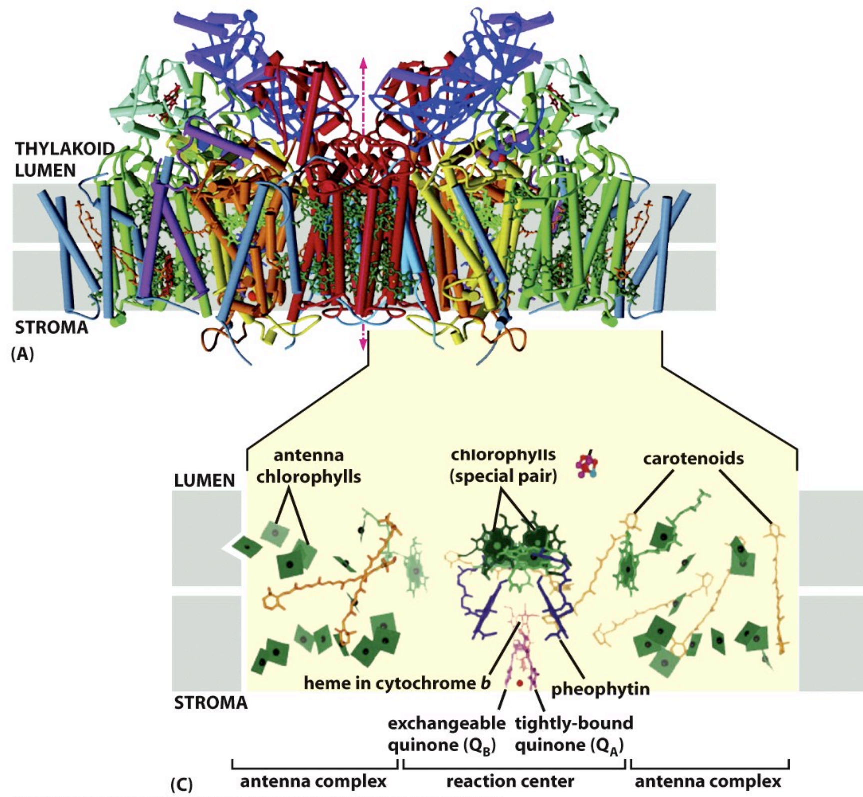


Figure 14-47ac Molecular Biology of the Cell 5/e (© Garland Science 2008)

Schematic of the Electron Transfer Process

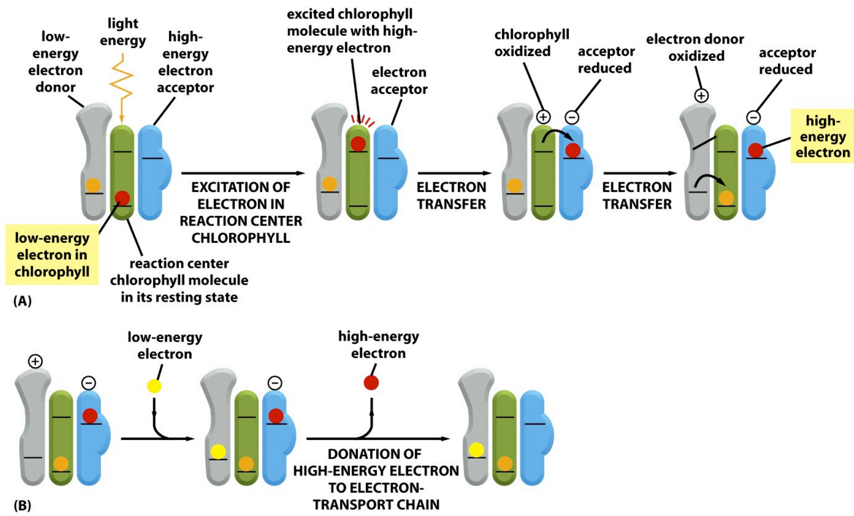


Figure 14-45 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- ***Schematic of the charge transfer process after optical excitation.***

(A) The initial events in a reaction center create a charge separation. A pigment-protein complex holds a chlorophyll molecule of the special pair (blue) precisely positioned so that both a potential low-energy electron donor (orange) and a potential high-energy electron acceptor (green) are immediately available. When light energizes an electron in the chlorophyll molecule (red electron), the excited electron is immediately passed to the electron acceptor and is thereby partially stabilized. The positively charged chlorophyll molecule then quickly attracts the low-energy electron from the electron donor and returns to its resting state, creating a larger charge separation that further stabilizes the high-energy electron. These reactions require less than 10-6 second to complete. (B) In the final stage of this process, which follows the steps in (A), the photosynthetic reaction center is restored to its original resting state by acquiring a new low-energy electron and then transferring the high-energy electron derived from chlorophyll to an electron transport chain in the membrane. As will be discussed subsequently, the ultimate source of low-energy electrons for photosystem II in the chloroplast is water; as a result, light produces high-energy electrons in the thylakoid membrane from low-energy electrons in water.

Schematic of the Electron Transfer Process

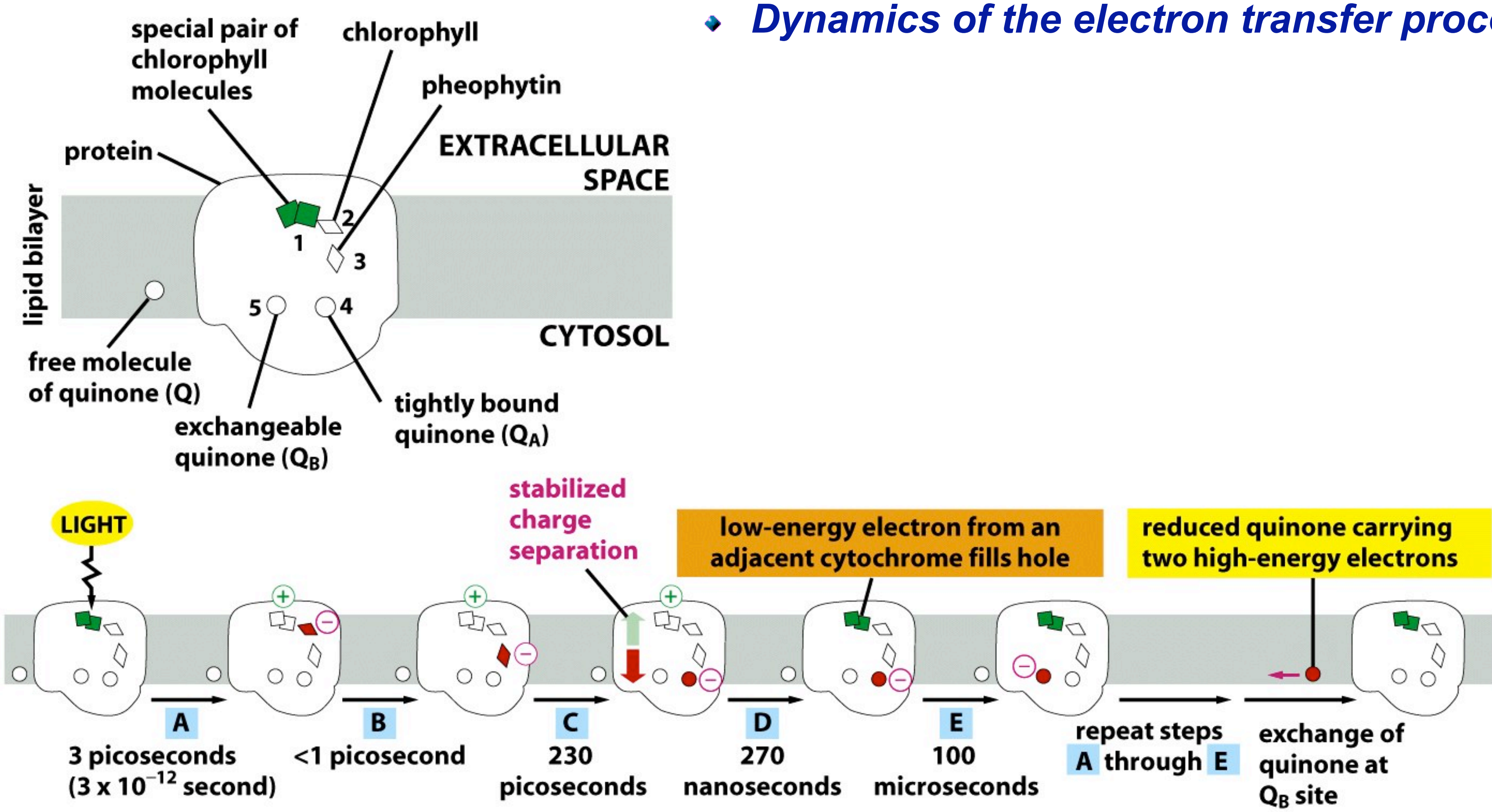
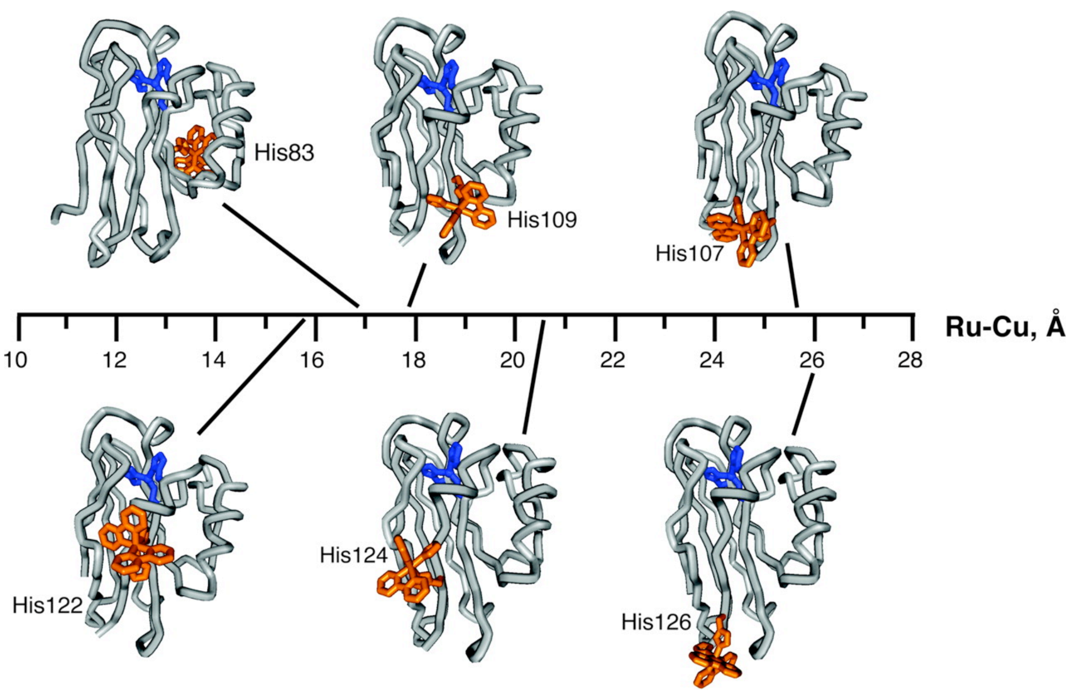


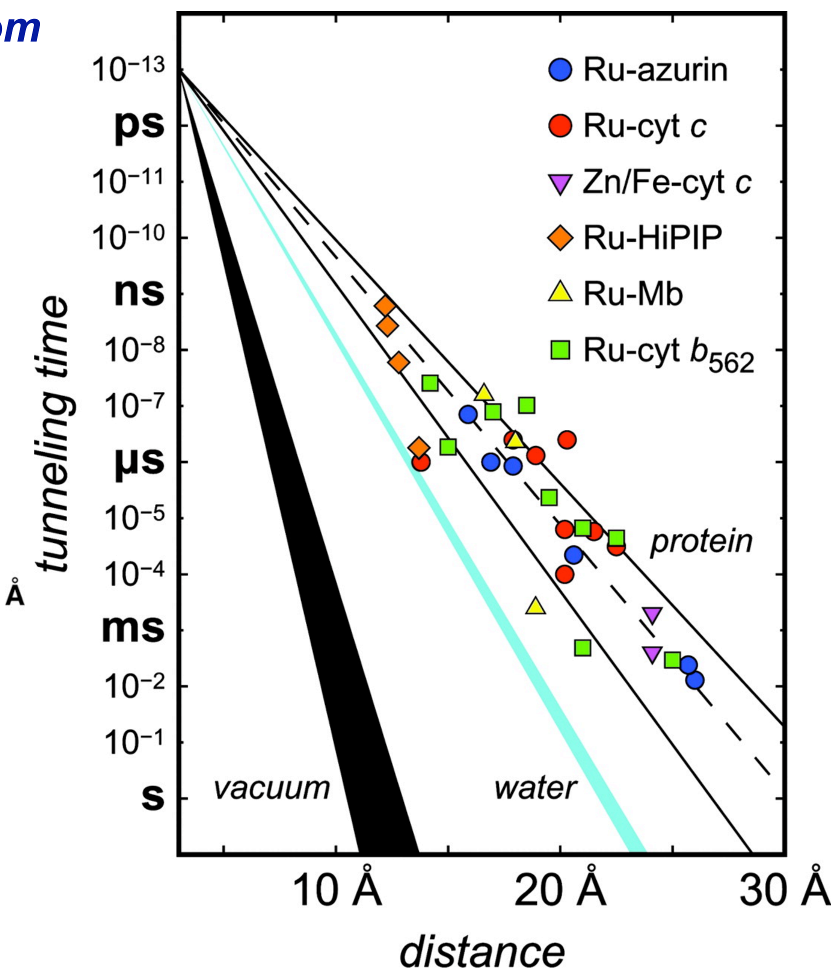
Figure 14-46 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- *Dynamics of the electron transfer process.*

Measuring the Rate of Electron Transfer: the Case of Azurin

- ***Protein engineering permits construction of donor-acceptor pairs at different distances from each other.***
- ***Measure the rate of electron transfer as a function of distance.***





(From our own Prof. Harry Gray – see his papers in PNAS)

"Changes in Redox Potential During Photosynthesis"

- ***The energetics of the light-induced reactions have been worked out.***

The redox potential for each molecule is indicated by its position along the vertical axis. Note that photosystem II passes electrons derived from water to photosystem I. The net electron flow through the two photosystems in series is from water to NADP+, and it produces NADPH as well as ATP. The ATP is synthesized by an ATP synthase that harnesses the electrochemical proton gradient produced by the three sites of H+ activity that are highlighted in Figure 14-48. This Z scheme for ATP production is called noncyclic photophosphorylation, to distinguish it from a cyclic scheme that utilizes only photosystem I (see the text).

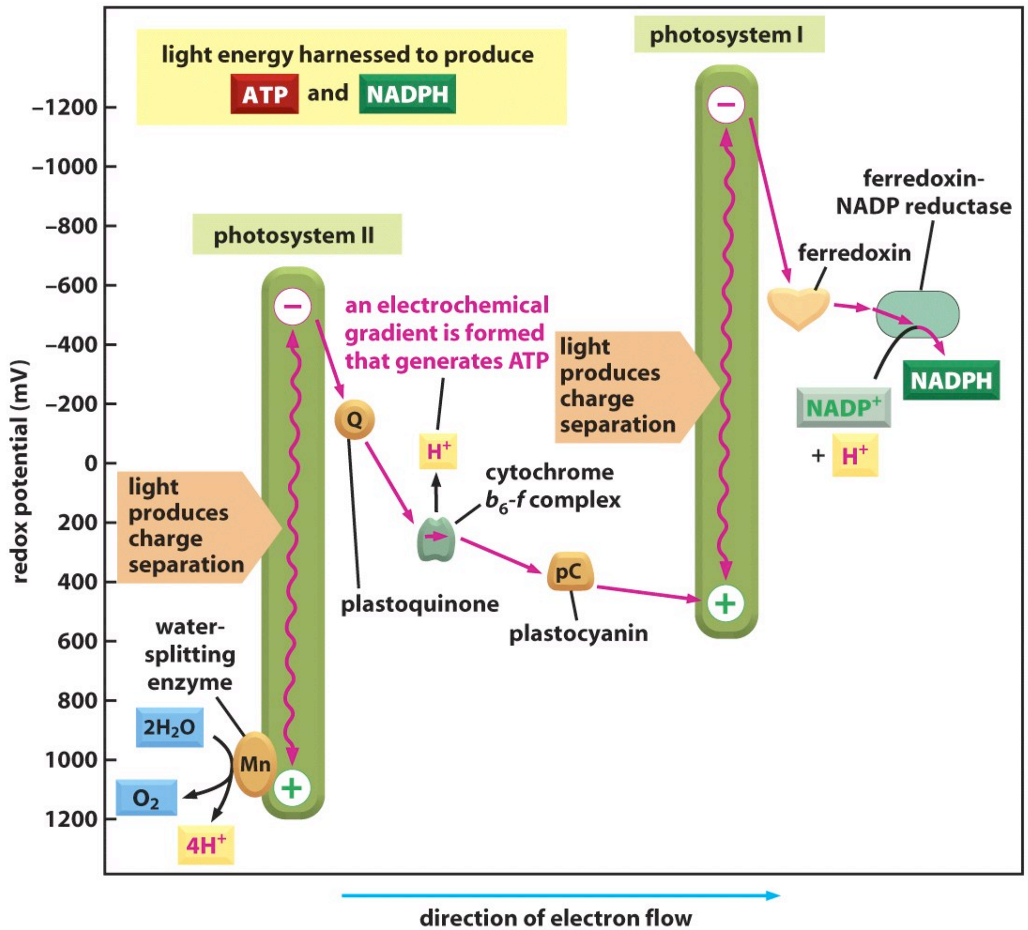


Figure 14-49 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Logic of Lecture

- ***Next: A few words on the chemistry of carbon fixation in photosynthetic organisms.***

The Overall Process of Photosynthesis

- *Harvest light.*
- *Move charges.*
- *Make organic matter (sugars, starch and beyond).*

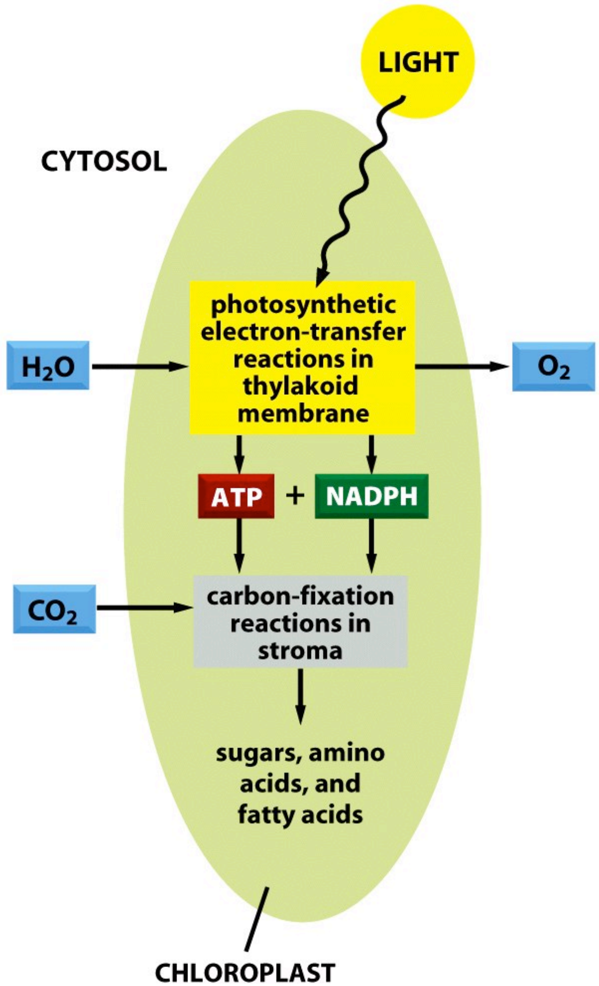


Figure 14-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Carbon Fixation and the Calvin Cycle

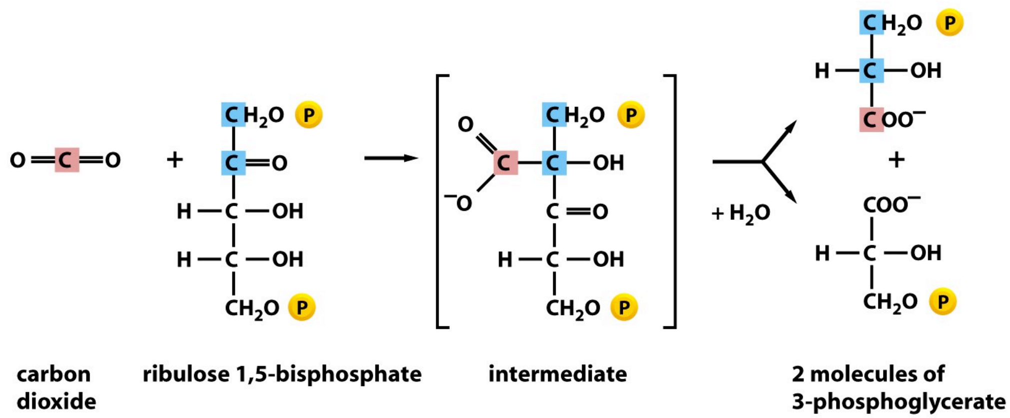


Figure 14-39 Molecular Biology of the Cell 5/e (© Garland Science 2008)

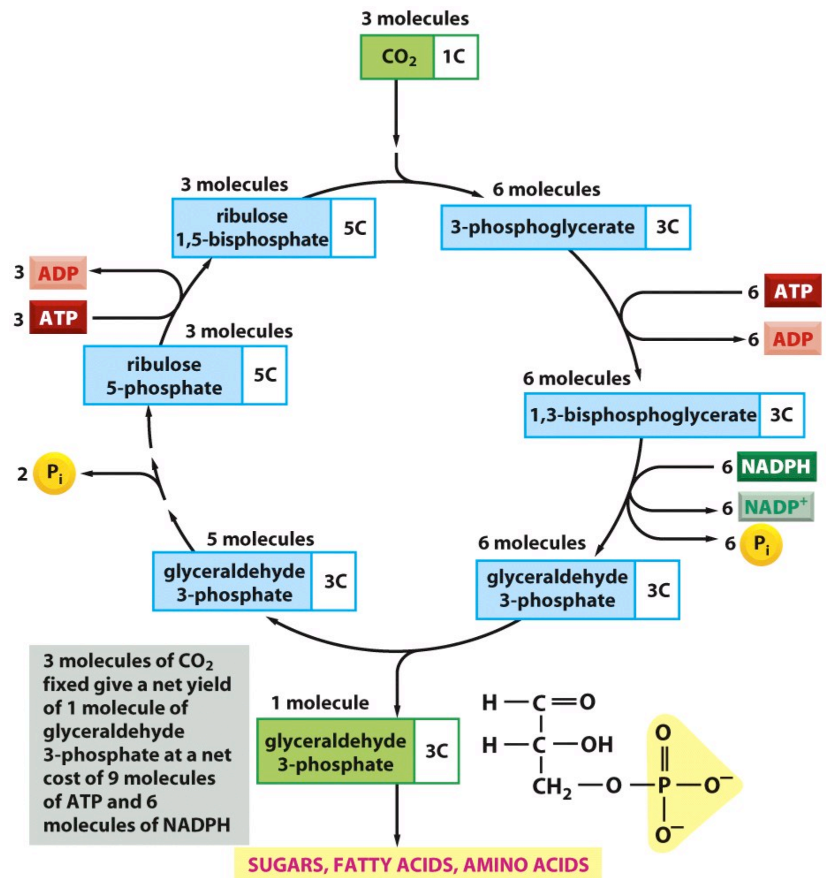


Figure 14-40 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 14-40 The carbon-fixation cycle, which forms organic molecules from CO2 and H2O.

The number of carbon atoms in each type of molecule is indicated in the white box. There are many intermediates between glyceraldehyde 3-phosphate and ribulose 5-phosphate, but they have been omitted here for clarity. The entry of water into the cycle is also not shown.

Different Photosynthetic Specializations

(A) Comparative leaf anatomy in a C3 plant and a C4 plant. The cells with green cytosol in the leaf interior contain chloroplasts that perform the normal carbon-fixation cycle. In C4 plants, the mesophyll cells are specialized for CO2 pumping rather than for carbon fixation, and they thereby create a high ratio of CO2 to O2 in the bundle-sheath cells, which are the only cells in these plants where the carbon-fixation cycle occurs. The vascular bundles carry the sucrose made in the leaf to other tissues. (B) How carbon dioxide is concentrated in bundle-sheath cells by the harnessing of ATP energy in mesophyll cells.

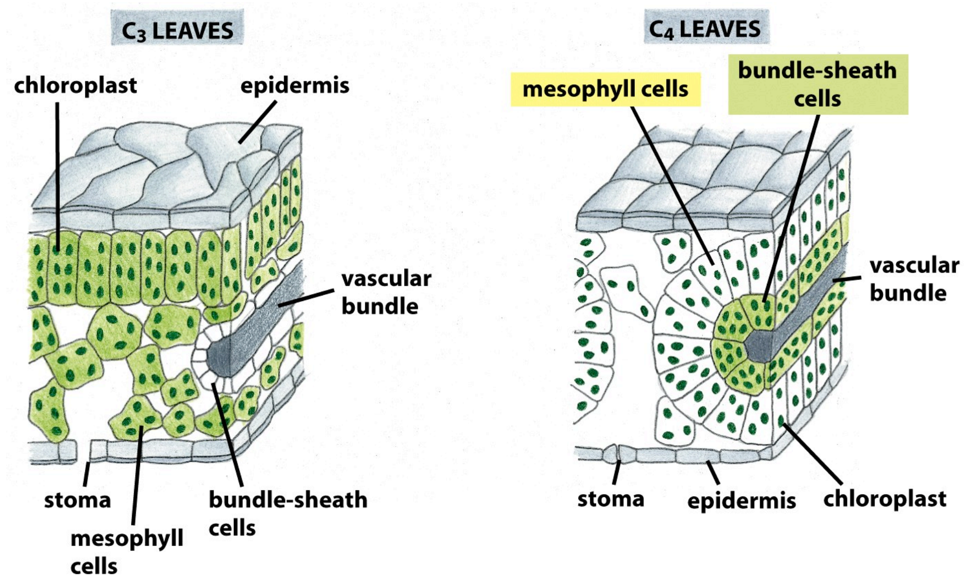


Figure 14-41a Molecular Biology of the Cell 5/e (© Garland Science 2008)

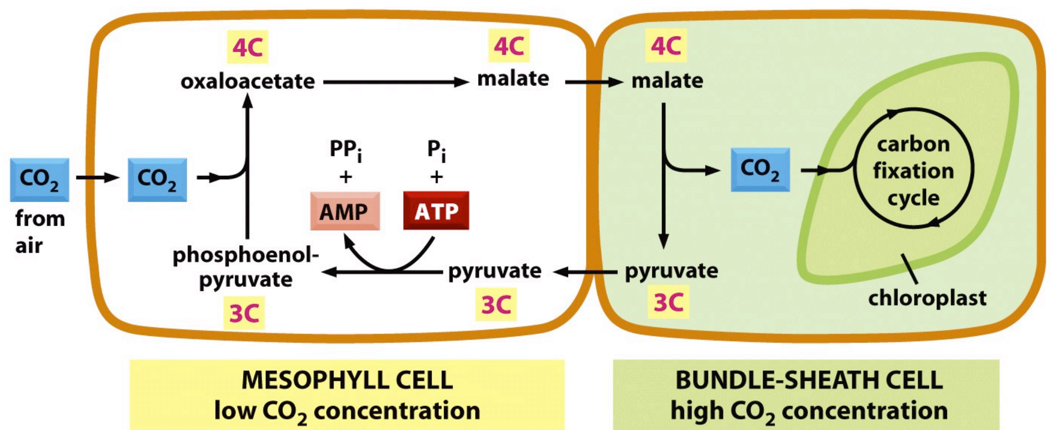


Figure 14-41b Molecular Biology of the Cell 5/e (© Garland Science 2008)