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Lecture 5

ribosomal RNA central role in catalysis (actual making of the protein)

This is the basic machinery and people use information about this machinery (encoded in genes) to understand how related different organisms are (paper discussion)

EVOLUTIONARY DISTANCE IS MEASURED IN THE ACTUAL DIFFERENCE IN THE NUCLEOTIDE AND AA SEQUENCE (NUMBER OF SEQUENCE DIFFERENCES IS PROPORTIONAL TO THE NUMBER OF STABLE MUTATIONAL CHANGES)

THE right chronometer: universal molecule, functionally homologous, regions of homology:

ribosomal RNAs = ancient, functionally constant, universally distributed and moderately well conserved, large so there's a large number of possible sequences – similarities really indicate relationship (AAATA) and AATAA may not mean much but 1500 nucleotides is more (for example a ten-letter sequence has 4^{10} possibilities = a little over a million, a bacterial genome has 2-5 million base pairs so there could be such a sequence in every bacterium – a 1000 bp sequence would better distinguish unique characteristics

smallest genome – *Carsonella ruddi* (160 000 bp – 182 genes) – a bacterial symbiont within an insect

largest genome – a lungfish (and some protozoa, but the way it is measured may measure the mitochondrial DNA)

16s and 23s: sedimentation unit (70 s the whole ribosome, 30s and 50s, 30-16s, 50- 23 and 5s)

16S EASIER TO MANIPULATE

80s RIBOSOME IN EUKARYOTES, BUT 70S IN CHLOROPLASTS AND MITOCHONDRIA (what does this suggest?)

aligned sequences are used to generate trees (graphic representation of the distance between organisms): Bacteria, Eukarya and Archea (Chapter 3 – reader ABOUT THE COMPARISON OF THE CELLULAR PROPERTIES)

TREE OF LIFE PICTURE

PAPER DISCUSSION

evolution or presence of different proteins: do they follow the basic tree based on 16s RNA

question: what came first? chicken and egg

SKETCH - MYCELLES

RNA world (SKETCH):

PREBIOTIC SYNTHESIS, LIPOPROTEIN VESICLES (ARTIFICIAL CELLS), RNA AND PROTEIN, DNA to make RNA: RIBOSE, PHOSPHATE (rocks), NUCLEOTIDE (RIBOSE FROM formaldehyde and glycolaldehyde), NUCLEOTIDE FROM HCN (N2 and methane)

- Late 1960s: Woese (U. Illinois), Crick (England), Orgel (Salk Inst, San Diego) concurrently proposed RNA may have preceded proteins & catalyzed all reactions for survival & replication of 'last common ancestor'. The 'RNA World' hypothesis born.

Virtually all biologists now agree that bacterial cells cannot form from nonliving chemicals in one step. If life arises from nonliving chemicals, there must be intermediate forms, "precellular life."

Of the various theories of precellular life, the most popular contender today is "the RNA world."

RNA has the ability to act as both genes and enzymes. This property could offer a way around the "chicken-and-egg" problem. (Genes require enzymes; enzymes require genes.) Furthermore, RNA can be transcribed into DNA, in reverse of the normal process of transcription. These facts are reasons to consider that the RNA world could be the original pathway to cells. James Watson enthusiastically praises Sir Francis Crick for having suggested this possibility (1):

The time had come to ask how the DNA→ RNA→ protein flow of information had ever got started. Here, Francis was again far ahead of his time. In 1968 he argued that RNA must have been the first genetic molecule, further suggesting that RNA, besides acting as a template, might also act as an enzyme and, in so doing, catalyze its own self-replication.

But Carl Woese had something to do with this as well.

- It was prescient of Crick to guess that RNA could act as an enzyme, because that was not known for sure until it was proven in the 1980s by Nobel Prize-winning researcher Thomas R. Cech (2) and others. The discovery of RNA enzymes launched a round of new theorizing that is still under way. The term "RNA world" was first used in a 1986 article by Harvard molecular biologist Walter Gilbert (3): 1983: Thomas Cech (U.

Colorado) & Sidney Altman (Yale) independently discovered *ribozymes*, enzymes made of RNA.

Heritability & reproducibility possible with a single molecule.

RNA CATALYZES THE PRODUCTION OF MORE RNA, BUT

Because synthesizing nucleotides and achieving replication of RNA under plausible prebiotic conditions have proved so challenging, chemists are increasingly considering the possibility that RNA was not the first self replicating molecule..." (9).

It has been postulated that there was a time in protobiological evolution when RNA played a dual role as both genetic material and a catalytic molecule ("the RNA world"). However, this appealing concept encounters significant difficulties. RNA is **chemically fragile and difficult to synthesize abiotically**. The known range of its catalytic activities is rather narrow, and the origin of an RNA synthetic apparatus is unclear.

Another idea is the "PNA world." Because starting the RNA world is so difficult, there probably needs to be a pre-RNA world. PNA, or peptide nucleic acid, might have some of the properties necessary to constitute that world (21). This would be *pre*-precellular life.

SO WHAT WERE THE FIRST BUILDING BLOCKS?

AA in meteorites, small hydrocarbons

- left and right-handedness of molecules (50-50%) life is not like that

PICTURE OF CHIRALITY (AA LEFT, SUGARS RIGHT): THE ORIGIN OF THIS???

- It may have occurred in the solar nebula during the formation of the solar system.
- Amino acids with a slight L-enantiomeric excess is observed in the Murchison & Murray meteorites

- (Although beware of contamination, since all Earthly aa's begin with L configuration. But note: during natural decomposition processes, protein aa's revert to a 50-50 (racemic) mixture over time.)

  Crystal faces have surface structures that are mirror-images. Experiments show that crystal faces can select L or D amino acids quite efficiently (40% excess) (Hazen, 2001). While this mechanism can explain the propagation of the L or D configuration, it cannot explain the *origin* of that preference

  chirality in biology is a consequence of enzymatic processes and is tied into biochemistry

  slight enrichment of L over D in meteorites – propagation would generate more chiral molecules

- New observations with the Anglo-Australian Telescope (above) have shown suprisingly high circular polarizations (the red and white regions in the image) in the infrared light from reflection nebulae in the star forming regions Orion OMC1 (a region in the Orion nebula M42) and NGC 6334. Although we can only observe these regions at infrared wavelengths which can penetrate the thick dust clouds in which they are embedded, it is predicted that circular polarization should also be present at the ultraviolet wavelengths needed for asymmetric photolysis of molecules such as amino acids. If our own solar system formed in such a region of high circular polarization, it could have led to the excess of L-amino acids which we see in meteorites and to the homochirality of biological molecules. It is possible that without such a process operating it would not be possible for life to start. This may have implications for the frequency of occurrence of life in the universe. PICTURE OF POLARIZATION

MINERAL SURFACES: SELECTIVELY ADSORB AND CATALYZE (PICTURE)

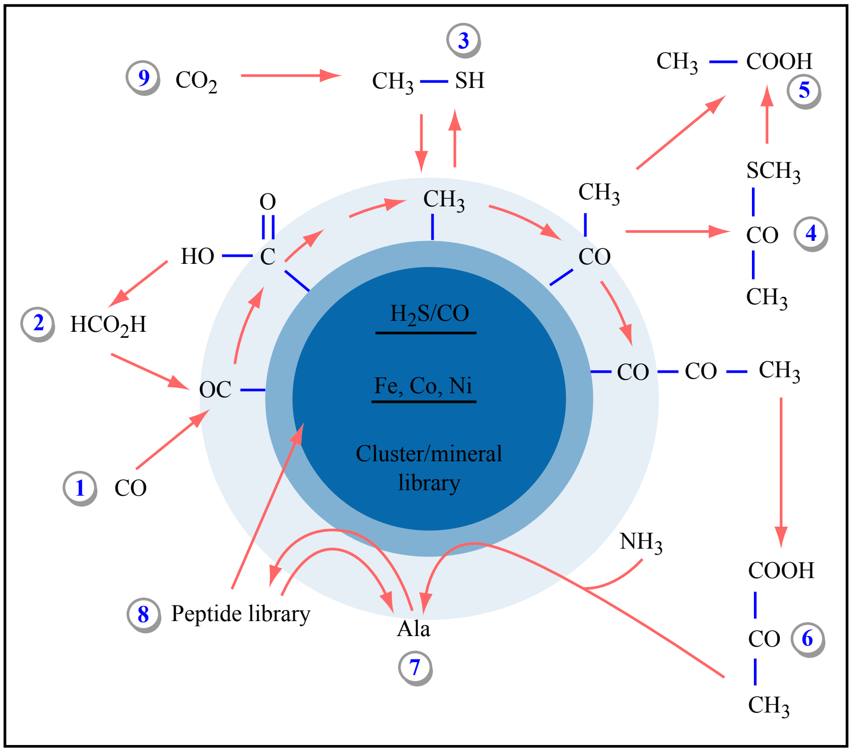


Figure by MIT OpenCourseWare.

- 1988: Günter Wächtershäuser (German patent lawyer!) theorizes that Fe & Ni sulfide *minerals* at hydrothermal vent systems provided the template & catalyst for formation of biological molecules.
- 1977: Hydrothermal vents on the seafloor discovered teaming with diverse life. Suggests possibility life may not have evolved at the surface.
- 1996: Everett Shock hypothesizes that chemical disequilibria existing where seawater reacts with hot rocks are conducive to organic synthesis from CO_2 or H_2CO_3.
- 1997: Jay Brandes (Carnegie Inst.) demonstrates that N_2 is converted to NH_3 in the presence of H_2 & magnetite (Fe_3O_4), at T & P typical of hydrothermal vents. Mineral surfaces and HT vent environments can produce biologically-useful form of N.

- 2000: George Cody et al. demonstrate synthesis of pyruvate using mineral catalysis under hydrothermal conditions. Pyruvate is branch point for many extant biosynthetic pathways.
- 2003: McCollom demonstrate organosynthesis from $FeCO_3$ at 300C (Martian meteorite – organics could be expected)

MAKING AN ORGANISM: PHOSPHOLIPID MEMBRANE

what does a cell need to live?

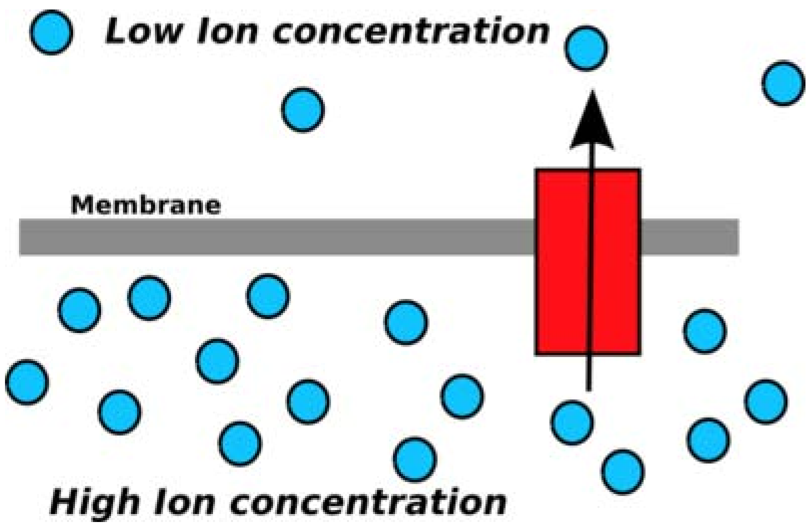
CHEMIOSMOTIC THEORY:

MICROBES ARE LIKE BATTERIES: GENERATION OF ENERGY IS BASED ON THE SEPARATION OF CHARGES (IN MOST CASES THESE CHARGES ARE PROTONS, not electrons like a normal battery)

Figure – draw Fig. 3.1

protons are translocated out of the cell by energy-producing reactions (respiration, photosynthesis or ATP hydrolysis)

membrane potential would be outside positive



even if protons accumulate electroneutrally (for example, a cell gets Na in and H out), there is a pH gradient

Chemiosmosis is the diffusion of ions across a **selectively-permeable membrane, so protons do not enter by leakage, but through specialized transporters**. More specifically, **it relates to the generation of ATP by the movement of hydrogen ions across a membrane during cellular respiration.**

membrane is a capacitor (1 microfarad/cm2)

HW – how many protons are needed outside to generate about -200 mV

ATP figure

As ATP is an unstable molecule it tends to be hydrolysed in water, and if ATP and ADP are allowed to come to chemical equilibrium, almost all the ATP will be converted to ADP. Any system that is far from equilibrium contains potential energy, and is capable of doing work. The cell maintains the ratio of ATP to ADP at a point ten orders of magnitude from equilibrium, with ATP concentrations a thousandfold higher than the concentration of ADP. This displacement from equilibrium means that the hydrolysis of ATP in the cell releases a great deal of energy.[5] ATP does not contain any special "high-energy bonds" and any other unstable molecule would serve equally well as a way of storing energy if the cell maintained its concentration far from equilibrium.

The amount of energy released can be calculated from the changes in energy under non-natural conditions. The net change in heat energy (enthalpy) at standard temperature and pressure of the decomposition of ATP into hydrated ADP and hydrated inorganic phosphate is −20.5 kJ/mol, with a change in free energy of 3.4 kJ/mol.[7] The energy released by cleaving either a phosphate (Pi) or pyrophosphate (PPi) unit from ATP, with all reactants and products at their standard states of 1 M concentration, are:

ATP + H_2O → ADP(hydrated) + Pi(hydrated) + H^+(hydrated) ΔG° = -30.54 kJ/mol (−7.3 kcal/mol)
ATP + H_2O → AMP(hydrated) + PPi(hydrated) + H^+(hydrated) ΔG° = -45.6 kJ/mol (−10.9 kcal/mol)

ELECTROCHEMICAL ENERGY OF PROTONS

EQUATIONS FROM PAGE 69

Δp is the force pulling protons across the membrane into the cell toward the lower electrochemical potential (generated by reactions that produce energy)

average Δp is -140 to -200 mV but the values for respiring bacteria are higher than the values for fermenting bacteria

if y protons move over Δp volts, y Δp eV is done (work), yF Δp J of work

membrane potential exists ΔΦ (pSI IN- PSI OUT, in V), negative when the inner membrane surface is negative

if a proton moves across, the work done is Δpsi eV

chemical energy is added to electrical
Δμ (electrochemical energy) G=Go+ RTln[H+] is the chemical energy of a proton, R is the ideal gas constant and G0 is the standard free energy when the concentration of everything is 1M

the change in free energy that accompanies the transfer of one mole of protons between H out and H in is the difference
ΔG= RTln[H+] in / [H+] out

ΔG/F = 0.06 log10 ([H+] in / [H+] out) or 60(pH out- pH in) = -60 ΔpH mV (pH outside is lower than the pH inside because the membrane is energized – there are more protons outside)

An Ion gradient has potential energy and can be used to power chemical reactions when the ions pass through a channel (red).

Hydrogen ions (protons) will diffuse from an area of high proton concentration to an area of lower proton concentration. Peter Mitchell proposed that an electrochemical concentration gradient of protons across a membrane could be harnessed to make ATP. He likened this process to osmosis, the diffusion of water across a membrane, which is why it is called *chemiosmosis*.

ATP synthase is the enzyme that makes ATP by chemiosmosis. It allows protons to pass through the membrane using the kinetic energy to phosphorylate ADP making ATP. The generation of ATP by chemiosmosis occurs in chloroplasts and mitochondria as well as in some bacteria.

ATP synthase -

ATP Synthase is made up of two main parts: F1 and Fo.

F1 contains the catalytic center

Fo couples F1 to the membrane and is in charge of transporting hydrogen ions.

Fo has three types of subunits: proteins 'a', 'b', and 'c'. Of these, Fo has one 'a', two 'b's, and nine-twelve 'c's (Walker *et al*, 1990).

F1 has five subunits: three alphas, three betas, one gamma, one, delta, and one epsilon. The beta subunit is where synthesis of ATP actually occurs.

The gamma, delta, and epsilon subunits are not symmetrical. This becomes important in discussing the function of ATP Synthase and it's parts.

In their nobel prize winning work on ATP Synthase structure, Boyer and Walker (Royal Swedish Academy, 1997) found that instead of the synthesis of ADP to ATP requiring energy, it was the binding of ADP to and the phosphate to the enzyme and the release of ATP that actually required the energy.

Enzymes typically bind and release substrates spontaneously (Walker *et al*, 1990), for this, the overall reaction will require energy. This is how ATP Synthase differs from many enzymes. Here, the asymetrical structure comes into play. Each of the three beta subunits has different couplings to the gamma, delta, and epsilon subunits, yet they function in the exact same way. Boyer discovered that gamma, delta, and epsilon each operate in a cylinder formation that is comprised of alpha and beta subunits (Royal Swedish Academy, 1997). The rotation of these cylinders is hypothesized to be caused by the hydrogen ion flow across the membrane (Walker *et al*, 1990). Figure 3 illustrates Boyer's discovery, which is now coined "Binding Change Mechanism".

The rotation of alpha and beta subunits causes structural changes in the enzyme which lead to functional changes.

With each rotation, the binding ability of the F1 part of the enzyme changes. The interaction of the gamma subunit with the alphas and betas forces "their active surfaces to assume different three-dimmensional structures" (Walker *et al*, 1990).

separation of charges across the membrane: many proteins involved – significant metabolic differences