RNA world and protocells

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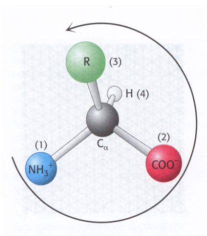
Origin of the homochirality of biological molecules

- Understanding the origin of homochirality may cast light on the early stages of prebiotic chemistry
- The general idea is that a slight enantiomeric eccess was produced by some prebiotic process
  - At a later stage, the enantiomeric eccess would have been amplified up to the point of attaining homochirality



The hypothesis of an interstellar origin of a prebiotic enantiomeric eccess

- The hypothesis of an enantiomeric eccess of astronomical origin is taken into consideration
  - Motivated by the discovery of the weak enantiomeric eccesses in the Murchison meteorite
- A possible scenario:
  - A circularly polarized interstellar radiation field may have affected the early prebiotic chemical reactions in interstellar space, leading to a small eccess of molecules with one type of symmetry
- Laboratory tests can be perfomed using circularly polarized light produced in synchrotron experiments

Last stages of prebiotic chemistry

- The last stages of prebiotic chemistry involve the formation of nucleic acids and proteins starting from monomers (e.g. nucleic bases and amino acids) synthesized in previous steps
- The formation of nucleic acids is central in these type of experimental studies
- The last stages involved in the formation of RNA are:
  - Activation of monomers

    Phosphorylation

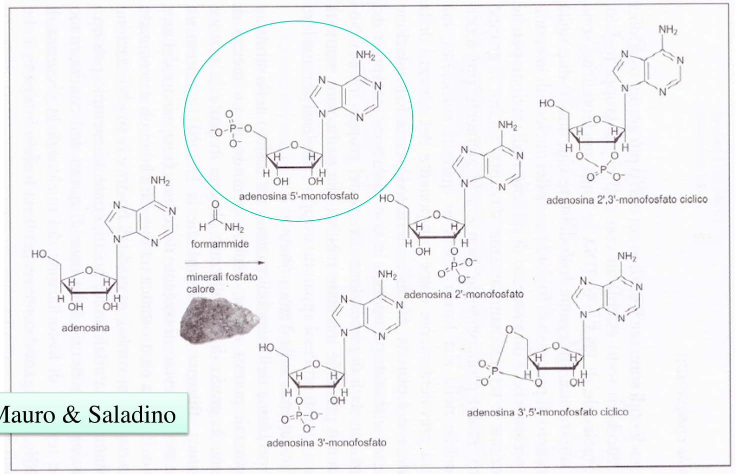
    Phosphorylation is the addition of a phosphoryl group (PO_3^{2-}) to a molecule
  - Polymerization

    Formation of phosphodiester bonds

    - Phosphodiester bonds make up the backbone of the strands of nucleic acids

Phosphorylation

In presence of phosphate-rich minerals, formamide provides natural routes of phosphorylation of nucleosides, including the form used in the RNA (in the circle)



Di Mauro & Saladino

The problem of phosphorus abundance

- The role of phosphorus is central in terrestrial biomolecules
  - Phosphate groups are essential in nucleic acids, as well as in ADT and ATP
- The abundance of phosphorus is low in the universe and is particularly low in the terrestrial crust
  - Phosphorus is a siderophile (rather than lithophile) element and is expected to be differentiated in the iron-rich core of the Earth, explaining its low abundance in the crust
- The fact that phosphates play a central role in spite of the low phosphorus abundance suggests that *molecular selection* is more important than elemental abundance in the succesful pathways of prebiotic chemistry
- Phosphorus for prebiotic chemistry might have been provided by iron-rich meteorites or in phosphorus-rich environments on the Earth crust

Polymerization

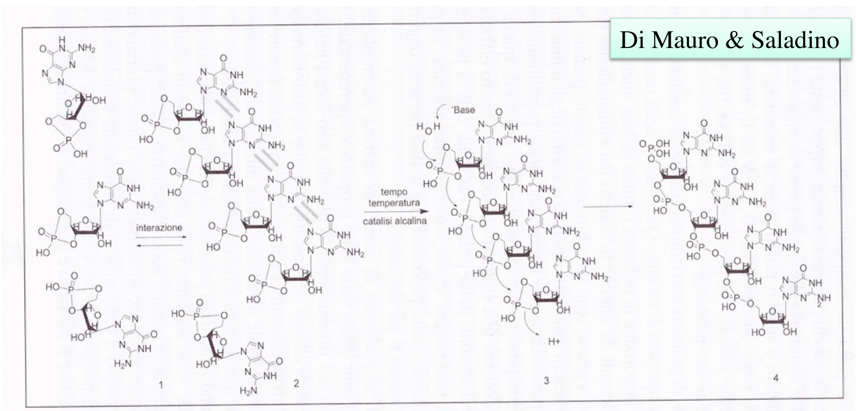
Polymerization is one of the bottlenecks of prebiotic chemistry

The spontaneous formation of the first nucleic acids involves:

(1) naturally activated (phosphorylated) nucleosides,

(2) a spontaneous structural arrangement of the nucleosides,

(3) a reaction of polymerization



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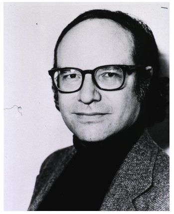
Origin of replication and metabolic properties

- Conceptual "chicken-egg" problem
  - In present-day cells, nucleic acids and proteins are responsible for replication and metabolic functions, respectively
  - The formation of each one of these two types of macromolecules requires the existence of the other one

    The synthesis of nucleic acids is catalyzed by proteins (enzymes)

    The synthesis of proteins requires the instructions stored in nucleic acids
- Who came first?
  - Proteins or nucleic acids ?
  - In other words: metabolic or genetic functions ?
- Different approaches have been adopted to tackle this problem
  - Old approach: "Metabolism first" <u>or</u> "genes first"
  - Modern approach: <u>search for macromolecules able to perform both tasks</u>

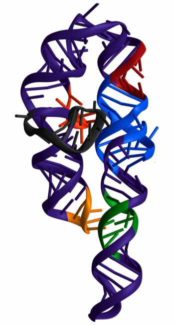
The "RNA world"



- Present-day, main stream theory in studies on life's origin
- Introduced by Walter Gilbert (1986) after the discovery of ribozymes
  - RNA molecules with catalytic properties
- According to this theory, the genetic system is the first to emerge, but with self-catalytic properties
  - Present-day ribozymes would be a sort of molecular fossiles of an ancient "RNA world"
- Present-day DNA-world would have emerged at a later stage because of its advantages
  - DNA provides greater genetic stability

    E.g. the lack of an oxygen atom in the sugar (deoxyribose instead of ribose) makes DNA less reactive than RNA
  - The DNA-proteins world has an extremely greater flexibility

    due to the introduction of proteins specialized in a large variety of metabolic functions



Life as a kinetic state of matter

Addy Pross

Example of the kinetic power of self replication

A, *B*: reactants

X: catalyst

- Comparison between normal and self-catalytic reactions
  - start with 1 molecule of catalyst *X*
  - assume reaction rate 1µs in both cases

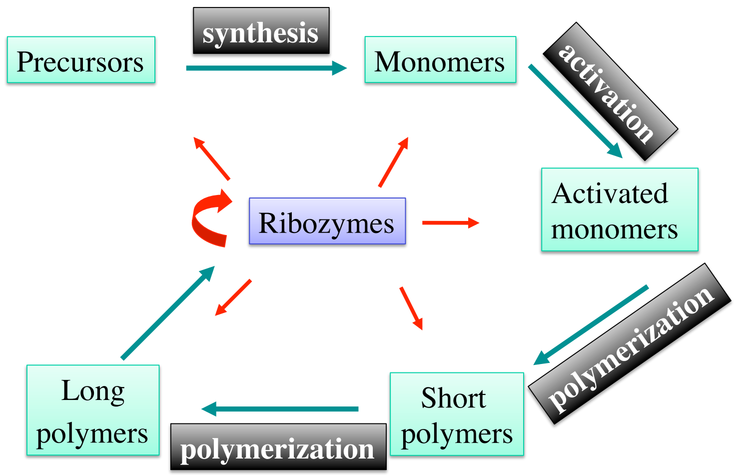
$$A + B \xrightarrow{X} C$$

- Time required to build up a mole of products (6 x 10^{23})
  - Normal case: 20 billon years
  - Self-catalytic case: 79 µs
- The kinetic control of chemical reactions could be the key for understanding the origin of life (in chemistry, the term "kinetics" is related to the rate of chemical reactions)
  - see literature by Addy Pross

$$A + B \xrightarrow{X} X$$

Steps of prebiotic chemistry leading to the RNA world

The ambient physico-chemical requirements may change in different steps



Replication and molecular evolution

Imperfect replication and chemical selection are supposed to be the ingredients of evolution that has lead to the molecular machinery that we see today

In a broad sense, *molecular replication and chemical selection is an extension of the concept of Darwinian evolution* which, strictly speaking, takes place only after the first living organisms are born

Darwinian evolution works *a posteriori*, in the sense that it favours the most suitable variations for a function that already exists

Molecular replication is probably the key function for the initial selection

Compartments

In order to develop protocells, the early products of the RNA-world must be enclosed in compartments

Compartmentalization is required to prevent the dispersion of genetic information and to concentrate the action of cooperative biochemical processes in an enclosed space

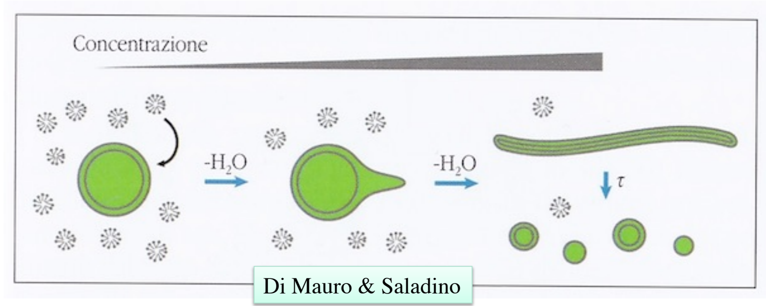
Membranes delimit a set of structures and reactions that can be transmitted as a specific heritage, paving the road for the onset of Darwinian evolution

Protocellular vesicles

Laboratory experiments demonstrate that simple amphiphilic molecules, resulting from prebiotic processes, can give rise to vesicle structures

Variations of the *concentration* of amphiphilic molecules and of *ambient conditions* drive the formation and destruction of vesicle structures that can grow and duplicate

Jack Szostak demonstrated that protocellular vesicles better replicate if they contain RNA and, at the same time, RNA better replicates if it is enclosed in lipidic vesicles



Di Mauro & Saladino

Early membranes

In present-day life the compartments are provided by the phospholipid bilayers of the cell membranes

Phospholipds are the result of an evolutionary process, and their synthesis requires enzymatically catalyzed reactions not available for the first protocells

Early membranes could have been constituted by simple fatty acids

Simple fatty acids can be spontaneously generated in prebiotic chemistry, as demonstrated, for example, by their presence in the Murchison meteorite

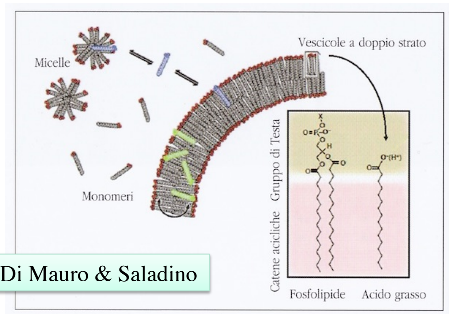


Figure: Di Mauro & Saladino

From amino acids to proteins

Several routes have been investigated for the spontaneous polymerization of amino acids

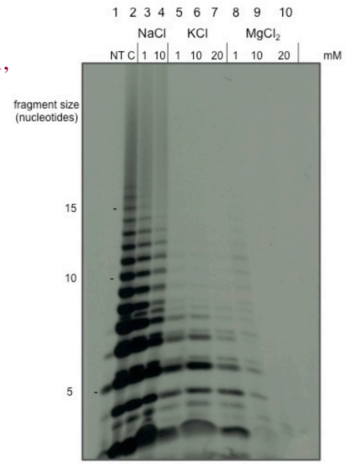
Small oligopeptides are relatively easy to obtain, while polypeptides are harder to produce

A solution rich of KCl seems to favour the polymerization of aminoacids (Dubina et al. 2013)

The physico-chemical requirements for the spontaneous polymerization of amino acids are generally different from the requiments for the polymerization of nucleic acids

For instance, KCl seems to inhibit the polymerization of nucleotides (in figure)

In the RNA-world scenario, proteins appear at later stage, as a result of synthesis of amino acids driven by the RNA



Di Mauro, priv. comm.

Casting light on the first living cells

The chemistry conservation principle

Mulkidjanian & Galperin (2007)

The chemical traits of organisms are more conservative than the changing environment and hence retain information about ancient ambient conditions

In absence of geological record of the first cells, the chemistry conservation principle can be used to cast light on the most ancient organisms and the last stages of prebiotic chemistry from the study of present-day organisms

Casting light on the first living cells

- "Top-down" approach
  - From the study of present-day terrestrial organisms, we try to characterize the properties of the first unicellular organisms proceeding backwards in evolution
- One of the methods being employed is the comparison of genetic sequences of present-day living organisms
  - Thanks to this comparative analysis, we can trace backwards the evolution at the molecular level
  - The results are visualized in the "phylogenetic tree", where the distances between different species are proportional to the differences found in the genetic sequences

Genetic sequences and classification of organisms

The techniques of molecular biology allow us to classify organisms on the basis of their genetic sequences, rather than on their morphology or phenotype (composite of observable traits and behaviour of organisms)

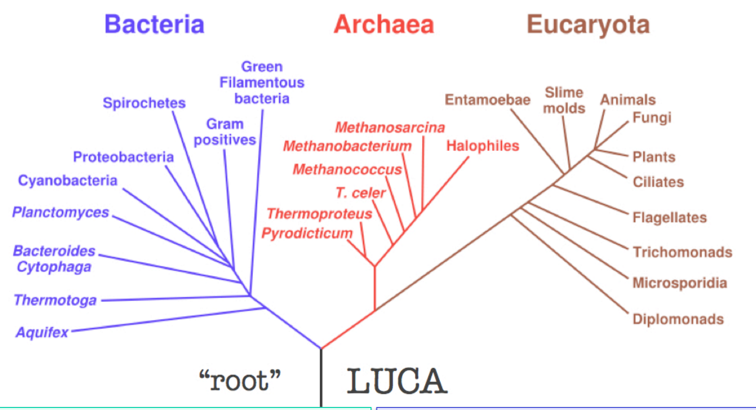
The classification based on genetic sequences has revolutionized our understanding of unicellular organisms

The classification based on genetic sequences has lead to distinguish three different types of unicellular organisms:

archaea, eubacteria and eukaryotes

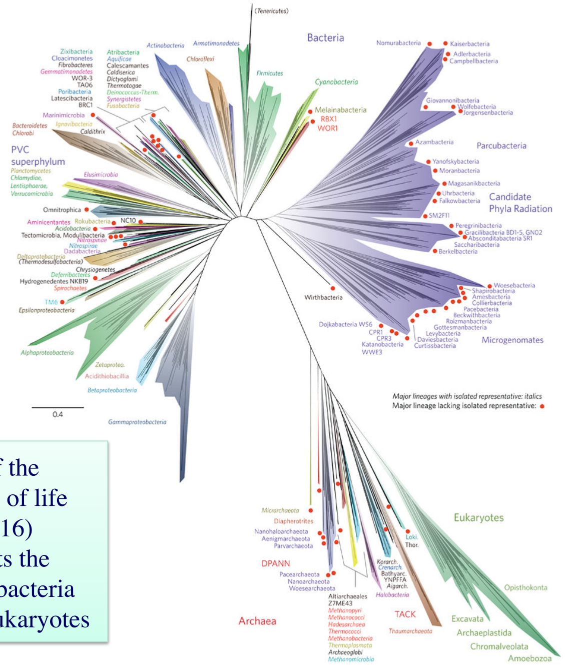
Archaea have been discovered through genetic classification

The phylogenetic tree of life



LUCA = Last Universal Common Ancestor of present-day living organisms, also called Cenancestor

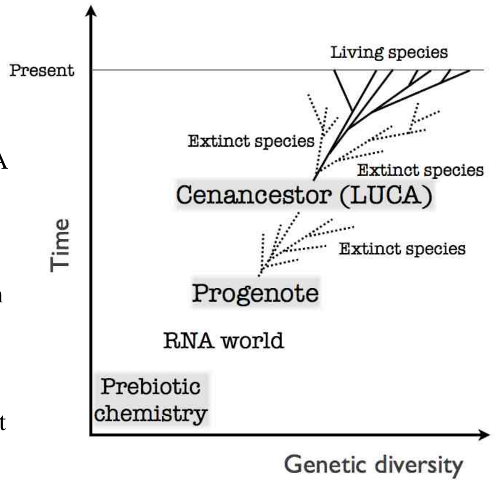
Close to the "root" of the tree, we find thermophilic Archaea and Bacteria



A new view of the phylogenetic tree of life (Hug et al. 2016) which highlights the predominance of bacteria over archaea and eukaryotes

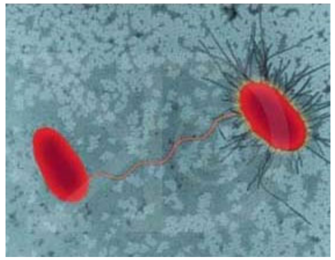
The gap between the RNA world and the LUCA

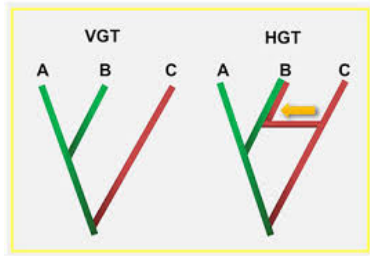
- The root of the phylogenetic tree is not representative of the oldest living cell
  - Other forms of life, extinct in the course of the evolution, must have preceded the LUCA
  - This early form of life is sometimes called the "progenote"
  - The early life could have been a collection of somewhat different cells, rather than a single type of cell
  - Detailed analysis suggests that *early life was mesophilic, rather than thermophilic*



Horizontal gene transfer
(also called Lateral Gene Transfer)

- Bacteria can exchange genetic material not only during their reproduction ("vertical gene transfer", VGT) but also via direct exchange from one cell to another ("horizontal gene transfer", HGT)
- The existence of HGT complicates the reconstruction of the philogenetic tree, which is based on the VGT scenario
- HGT must have played an essential role in the early stages of life, providing a simple mechanism to exchange genetic material before more complex mechanisms of "vertical" transmission were set in place





Heterotrophic versus autotrophic hypothesis on the origin of life

Heterotrophic hypothesis

The first organisms were harvesting organic material and energy from prebiotic molecules that were already present in the environment. This hypothesis does not require a specific enviromental niche since the molecular ingredients of terrestrial life could have been delivered on Earth from space and could also have been synthesized on the primitive Earth.

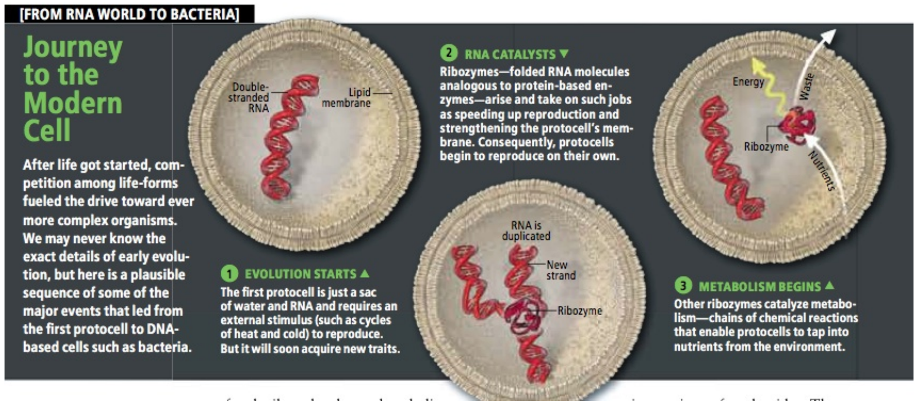
Autotrophic hypothesis

Requires the production of energy and organic material from the abiotic world. The early life forms would have emerged in the proximity of redox or pH gradients, using the harvested energy to feed biosynthesis reactions. These processes require extremely reactive chemical environments. The autotrophic scenario can only take place in specific thermodynamical niches.

The cradles of life

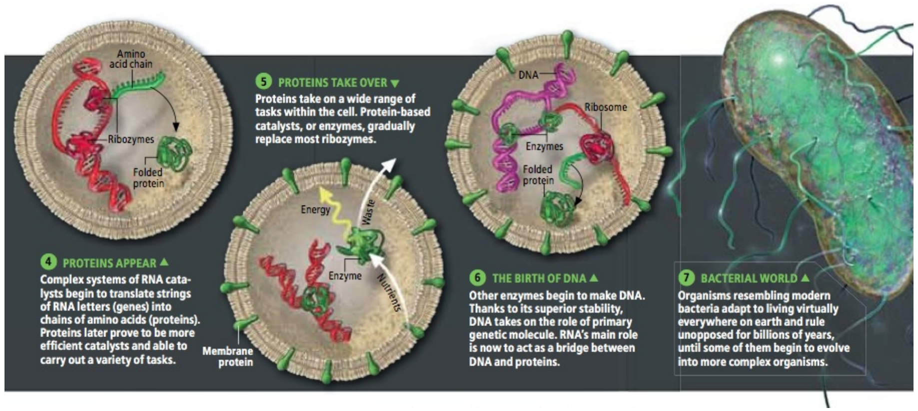
- Deep sea hydrothermal vents
  - In line with the *autotrophic hypothesis*, hydrothermal vents provide inorganic compartments, versatile catalysis and sources of organic matter
  - An origin in the oceans poses the problems of the containment of the reactions in an open water environment
  - The presence of Na salts, typical of the oceans, may hinder the formation of biological membranes (Natochin 2010)
- Anoxic geothermal fields (example
  - In line with the *heterotrophic hypothesis*, supported by geochemical data and phylogenomic analysis (Mulkidjanian et al. 2012)
  - Geothermal fields are conducive to condensation reactions and enable the involvement of solar light as an energy source and as a selector factor of stable nucleotides
  - Geothermal vapour is enriched in phosphorus compounds that could be essential for the emergence of the first RNA-like oligomers

Filling the gap from RNA world to bacteria



Ricardo & Szostak (2009)

Filling the gap from RNA world to bacteria



Ricardo & Szostak (2009)