7 – ENZYME ORIGIN AND EVOLUTION

The resolution of the three-dimensional structures of proteins has led to an ever more precise understanding of the topology of active sites in a great number of enzymes and in some even, the molecular motion associated with it. The architectural complexity of currently known enzyme molecules prompts the following two questions:

- How have such structures, endowed with catalytic activity, progressively formed and been selected? A corollary to this question would be: why one particular structure and not another?
- How are they formed today in vivo from the information contained within a cell?

The origin of enzyme function practically overlaps with the origin of life. For a long time, it was thought that discussions relating to the origin of life arose more in lounge conversation than in serious science. It is true that this problem will always be of a speculative nature, as none of us was present at the beginning of time to witness it. However, experimentation has enabled simulation of some aspects of the formation of biological molecular structure; furthermore, much progress has been made in dating fossils with the help of isotopes allowing information to be obtained about the emergence of enzymes. We are therefore in a position to suggest plausible hypotheses about the appearance of these molecules, enzymatic activity and even the first cells. We are faced with diverse hypotheses, however, which we shall consider and discuss.

7.1. TIME-SCALE OF EVOLUTION

The Earth is estimated to be 4.6×10^9 years old. For a long time, authors interested in the origin of life assumed that the primitive atmosphere was most likely a very reducing atmosphere containing H_2O, CH_4, NH_3, H_2, N_2 and CO. Progressively, some of these molecules, in particular H_2, NH_3 and CH_4, disappeared and the atmosphere became less reducing. However, this hypothesis has had to be reconsidered. Current geochemical models increasingly indicate that the principal source of carbon was CO_2 and that there was very little or no methane. Ferrous ions would have played a key role in the photoreduction of CO_2 (BOROWSKA & MAUZERALL, 1988). However, all hypotheses about the origin of life assumed the absence of oxygen and the presence of SH_2 in the primitive atmosphere.

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Numerous observations suggested that the first organic molecules were formed from inorganic molecules in the atmosphere under the influence of UV radiation, electrical discharges or heat. The first organic molecules were dissolved in the sea, which at the time covered nearly all of the Earth's surface. This period of chemical evolution is estimated to have taken about 10^9 years, which represents a fifth of the Earth's history. The oldest organic material was discovered in schistose deposits in Fig Tree, South Africa. This material was composed of carbohydrates, porphyrins, purines and pyrimidines. The use of isotopes enabled the substance to be dated back 3.1×10^9 years. The same isoprenoid components that were found are present in cells today. Additionally, in these sediments analogues of cells were revealed, 0.6 mm in length. It is probable that the first living cells were heterotrophic anaerobic organisms capable of using dissolved organic substances from the sea. When these substances became scarce the only organisms to survive were those able to use simple carbon components. It is believed that the first photosynthetic organisms, blue algae, appeared a little later. Fossils of blue algae have been discovered in the same schistose sediments from South Africa and dated to 3.1×10^9 years ago. Current understanding suggests that until their appearance, there was little or no oxygen in the atmosphere. A further 100 thousand million years were needed before the development of anaerobic vertebrates and only in the last two thousand million years did *Homo sapiens* make its appearance on Earth. Table 7.1 illustrates the time-scale of evolution.

Table 7.1 Time-scale of evolution

Time	*Geological period*	*Approximate origin*	
-2×10^6	Phanerozoic	Man Mammals, birds Terrestrial plants Fish	
-5.6×10^6	Proterozoic	Invertebrates Multi-cellular organisms Eukaryotic cells	*Biological evolution*
-10^9	Archaea	Aerobic bacteria First fossils First photosynthetic prokaryotes	
-4×10^9		First cells	*Chemical evolution*
-4.4 to -4.5×10^9	Formation of the Earth		
-15×10^9		*Big Bang*	

The first theories about the origin of life were based on the hypothesis of a highly reducing primitive atmosphere, and from this an entire prebiotic chemistry was developed aiming to simulate the composition of the **'*original soup*'** of precursor

molecules consisting of proteins and nucleic acids. The experiments began with methane, which produces cyanide, this in turn being the precursor of biomolecules. The **"original soup"** theory was strongly criticised using thermodynamic, chemical and geochemical arguments. An alternative was proposed in 1988 by WÄCHTERSHÄUSER, the **theory of surface metabolism,** which favours the notion that triose phosphates are at the origin of metabolism.

7.2. PREBIOTIC CHEMISTRY ACCORDING TO THE "ORIGINAL SOUP" HYPOTHESIS

7.2.1. FORMATION OF SOME SIMPLE ORGANIC MOLECULES

Laboratory experiments were destined to simulate prebiotic conditions. In particular, they were carried out in a reducing atmosphere. The energy sources used were either ionising or ultraviolet radiation, electrical discharges or indeed heat (temperatures were probably higher near to volcanic sites). To these experiments we associate the names OPARIN, MILLER, FOX and ORGEL. Generally, in this type of experiment an electrical discharge is applied to a mixture of NH_3, CH_4, H_2 and H_2O. As early as 1920, OPARIN had suggested that the first organic molecules likely to generate more complex biological molecules were formed under these conditions. MILLER, in 1953, performed experiments of this type and observed the formation of biochemical components; in particular he obtained: glycine, alanine, sarcosine, β-alanine, γ-amino butyric acid, N-methylamine, and aspartic, glutamic, iminodiacetic, formic, acetic, glycolic, lactic, β-hydroxybutyric and succinic acids, urea and methylurea. A series of reactions generated these molecules; among the intermediates, cyanide and nitriles played a critical role. Here are the principal chemical reactions leading to the formation of alanine when an electrical discharge is applied:

$$CH_4 + NH_3 \longrightarrow HCN + 3H_2$$

$$C_2H_4 + HCN \longrightarrow CH_3CH_2CN \quad \text{(nitrile)}$$

$$CH_3CH_2CN \longrightarrow CH_3CH_2COOH \quad \text{(propionic acid)}$$

$$CH_3CHOHCN \longrightarrow CH_3CHNH_2CN \quad \text{(aminopropionitrile)}$$

$$CH_3CHNH_2CN \longrightarrow CH_3CHNH_2COOH \quad \text{(alanine)}$$

Some studies have shown that cyanide is at the basis of the formation of pyrimidines and purines, but also amino acids. The following scheme (from LEHNINGER) summarises the principal reactions that cyanide may undergo. It is interesting to notice that the elementary molecules obtained during experiments simulating prebiotic conditions are the same as those found in ancient rocks and sediments and also in certain meteorites.

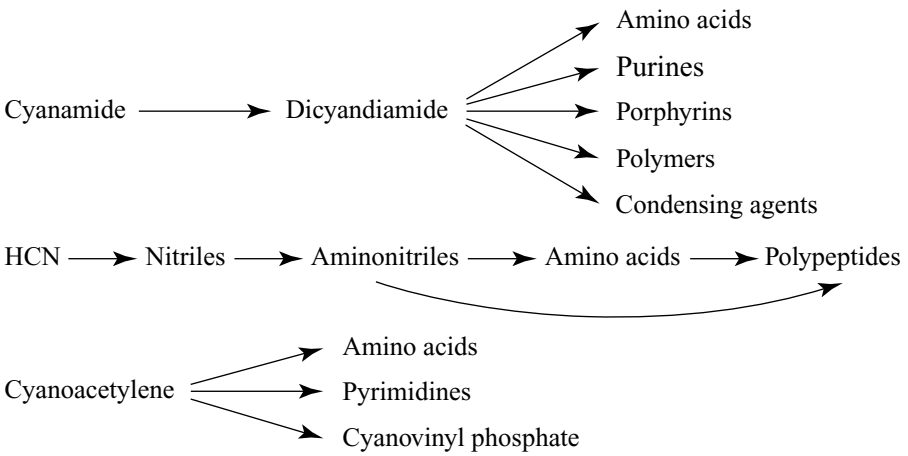

Table 7.2 below gives an idea of the sources of energy existing on the Earth's surface and their respective values.

Table 7.2 Sources of energy, average values at the Earth's surface

(Reprinted from *Biochemistry*, LEHNINGER A.L., chapter 24.
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Source	*Calories/cm^2/year*
Solar radiation	260 000
Ultraviolet light	660
Electrical discharges	4
Radioactivity	0.8
Volcanic activity	0.13
Cosmic radiation	0.0015

The simple molecules thus formed can be the dominant products because their formation takes place along favourable energy pathways or because they are more stable than other products under defined conditions.

7.2.2. FORMATION OF MACROMOLECULES

The following step is the condensation reaction that leads to the formation of oligomers and polymers. During condensation water molecules are liberated. However, the bonds formed are hydrolysable; the peptide and glycosidic bonds are unstable in aqueous solution under prebiotic conditions. They tend to be hydrolysed, therefore, only a small number of molecules would succeed in being formed. These molecules can appear in anhydrous conditions, for example, at temperatures higher than the boiling point of water. FOX thus carried out the polymerisation of amino acids. The role played by ATP in the formation of peptide bonds during protein biosynthesis led to the suggestion that polyphosphates might be involved in the process of condensation in prebiotic conditions. Substances such as carbodiimides could have played an analogous role.

7.2.2.1. ABIOTIC FORMATION OF POLYPEPTIDES

FOX and other investigators obtained proteinoids from a mixture of amino acids either by heating to 170°C for several hours, or by a more gentle heat treatment (50–60°C) in the presence of polyphosphates for a longer time.

Starting from a mixture of all amino acids he obtained polymers of high molecular weight able to exceed 10 000 D and which did not form by chance. There was a mix of acidic, basic and neutral polymers produced which could be separated by chromatography. Besides, these polymers possessed properties that are common to all proteins such as precipitation at the isoelectric point, "salting in" and "salting out". Similarly, the incubation of a mixture of aminoacyl adenylates at pH 9 led to the formation of proteinoids with a high molecular weight; this reaction was carried out at mild temperatures. It is possible to synthesise proteinoids successfully by the polymerisation of aminoacetonitriles, suggesting that such a reaction could be produced under prebiotic conditions.

Some proteinoids thus appearing in abiotic conditions manifest a weak though significant biological activity. For example, certain proteinoids possess a **catalytic activity.** Their presence accelerates the hydrolysis of paranitrophenyl acetate, or increases the rate of decarboxylation of pyruvic acid. It is true that even imidazole is able to catalyse the hydrolysis of paranitrophenyl acetate. Furthermore, the activities of these proteinoids present pH profiles comparable to those observed in enzymatic reactions and obey MICHAELIS' law. Of course, these activities are much weaker than those of the corresponding enzyme (proteases or pyruvate decarboxylase). The proteinoids having an activity have been pointed out, for instance, an MSH (melanocyte-stimulating hormone) activity associated with a proteinoid has been observed.

7.2.2.2. ABIOTIC FORMATION OF NUCLEOTIDES AND NUCLEIC ACIDS

The experiments destined to recreate prebiotic conditions also ended with the formation of nucleotides from their corresponding bases (adenosine, deoxyadenosine). PONNAMPERUMA and co-workers managed to simulate the formation of AMP, ADP and ATP by heating or irradiating adenine and ribose in the presence of a phosphorylating agent. The condensation of mononucleotides was achieved by heating to 50–65°C in the presence of polyphosphoric acid. In abiotic conditions the 2′-5′ bond is formed predominantly; the 3′-5′ bond seems to be more difficult to create. Possibly the most interesting experiments were those carried out in the presence of template molecules, such as the polynucleotides from WATSON and CRICK's model of the double helix. In this way, the specificity in the pairing of bases, cytosine-adenine and uracil-guanine, was reproduced. MILES and TS'O showed that mononucleotides have a tendency to associate preferentially according to this complementarity and that a helical structure is formed along the template strand in which the nucleotides are covalently linked. ORGEL continued with the same type of experiment, but added a carbodiimide as a condensing agent and, using polyuridylic acid (poly U) as a template and AMP, he obtained poly A. Even under these conditions the polynucleotides form with 2′-5′ bonds.

Some amino acids are liable to associate to nucleotides with a certain selectivity. Poly-L-lysine and poly-L-arginine readily associate to guanylic and adenylic acids.

Due to this property, it was suggested that an α helix composed of a poly-amino acid could bind to adenylic acid and form helical structures in which a residue R would correspond to three nucleotides, thus foreshadowing the genetic code.

7.2.3. DISCUSSION OF THE NATURE OF THE FIRST BIOLOGICAL MOLECULES

Due to the highly speculative nature of the subject, it is not surprising that diverse theories exist all seeking to explain how life appeared on Earth. There is considerable discrepancy in the various suggested explanations, covering the nature of the first molecules and even the mechanisms of life's appearance. Two contrasting theories propose that life began either with proteins or with nucleic acids.

The first hypothesis, attributable to OPARIN and FOX, suggested that life started without nucleic acids, with the formation of droplets called ***protobionts*****:** microspheres containing proteins and possessing an elementary metabolism sometimes limited to a single reaction. According to OPARIN, the first cells existed when a membrane formed around a few macromolecules including catalytically active proteins. In OPARIN's view, the development of the genetic machinery was a late event in evolution. A cellular phase would have been formed from the primitive soup by coacervation. In the protobiont model, OPARIN and co-workers attempted an experiment with systems forming coacervates. Concentrated polymer solutions (5–50%) of polypeptides and polysaccharides in aqueous media were able to form visible droplets under the microscope; they had volumes of 10^{-8} to 10^{-6} cm^3. One of these coacervates containing glycogen phosphorylase was immersed in a solution of glucose-1-phosphate and was able to produce starch. When amylase was added to the system, maltose was formed. Similarly, a coacervate containing NADH dehydrogenase could reduce a solution of an oxidised dye (Fig. 7.1).

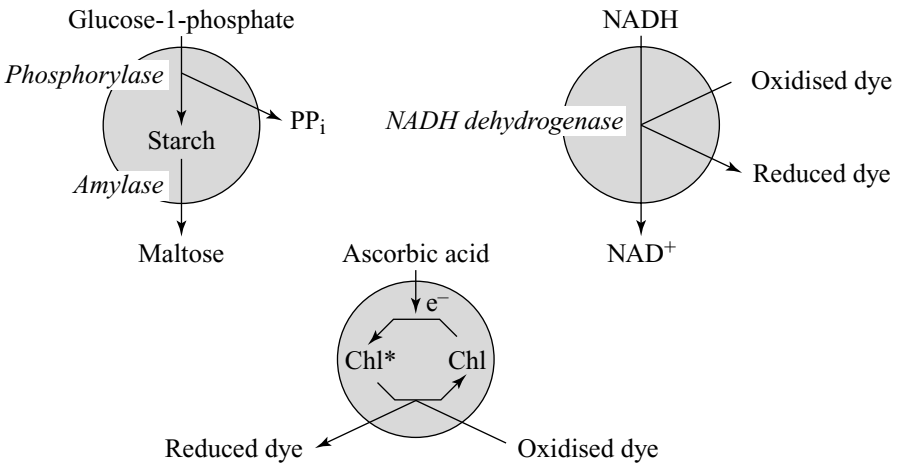

Fig. 7.1 Enzymatic activities within coacervates

FOX obtained microspheres by the progressive cooling of a hot and concentrated solution of proteinoids. When the pH was suitably adjusted, a bilayered structure analogous to a membrane was formed in the absence of lipids. These microspheres were

about 2HM thick and capable of dividing both in the presence of $MgCl_2$ and following a change in pH. The phenomenon of budding, similar to that observed with yeast, was sometimes produced. It is also thought that the action of wind at the sea surface helped to bring about membrane formation (lipid bilayers) which, upon closing up again, encapsulated proteins inside the vesicles (Fig. 7.2).

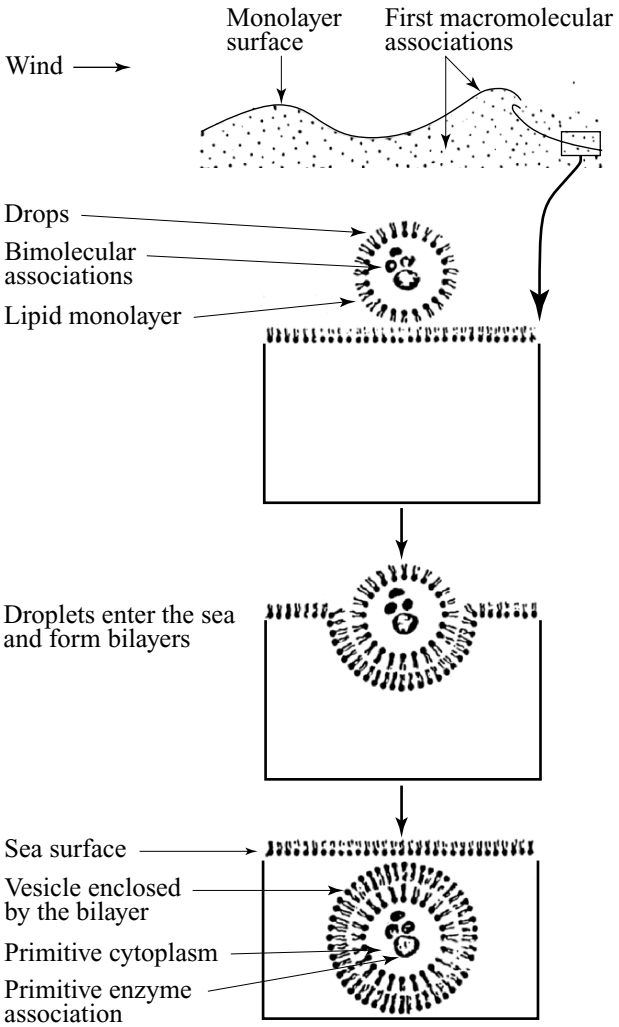

Fig. 7.2 Hypothetical mechanism for the formation of vesicles that encapsulate proteins from the sea surface

The stability of these vesicles is maximal around a particular size. The difference between the changes in internal volume and those at the external surface led them to divide like living cells by a purely physical phenomenon resulting from the variation in surface tension. It is possible to imagine that late in evolution the vesicles containing active molecules (enzymes and catalysts) and those containing information molecules (nucleic acids) fused, giving rise to structures similar to living cells and

capable of dividing. Whatever the case, without a genetic machinery to conserve the information, these first molecules would not have been able to replicate themselves.

Over time, the genetic hypothesis gave way to the notion of genetic machinery. MILLER, in 1929, proposed that life began with the abiotic formation of one or more genes. He considered that the minimum properties of a living organism, metabolism and reproduction, are potentially present within genes; when these are placed in an adequate molecular environment at the heart of a cell, they can give rise to a daughter cell. The genetic theory, which remained for a long time incompletely developed, was taken up again with the advent of genetic knowledge by various authors including CRICK, ORGEL and HOROWITZ. Membrane formation and the appearance of catalysts are considered to be late events in this paradigm. The genetic hypothesis was supported by the capacity of viruses to self-replicate and by modern developments in molecular genetics. CRICK and ORGEL suggested that the origin of life is allied to the origin of ribosomes, tRNA and the genetic code (tRNA anticodon triplets). The partisans of the genetic hypothesis postulated that before the appearance of activating enzymes the first forms of tRNA and rRNA filled one of the functions of enzyme molecules, namely, they provided specific binding sites for amino acids. The association of amino acids to tRNA in the initial conditions would have been non-covalent. Only later would ribosomes have acquired the ability to synthesise an enzyme capable of catalysing the formation of peptide bonds. In line with this hypothesis, it is noteworthy that RNA (and not DNA) possesses a tertiary structure; DNA supposedly appeared much later in evolution. As we shall see further on, the discovery in 1986 by ZAUG and CHECH that certain RNAs have a catalytic activity has reinforced the genetic hypothesis. **The capacity to self-organise into a three-dimensional structure seems to be vital for the origin of life**. Whatever the nature of the first biological molecules that appeared in the origin, proteins or nucleic acids (and here we find ourselves faced with the chicken-and-egg problem), the systems have subsequently evolved in response to environmental changes.

It is clear that, as interesting as these simulations of prebiotic chemistry are, they all build on the preconceived idea of an "original soup" created under strongly reducing conditions. This theory has been widely criticised by a number of authors, in particular CAIRNS, SMITH, WÄCHTERSHÄUSER and DANCHIN, who considered that the formation of biological macromolecules from an "original soup" is highly improbable from a thermodynamic, chemical and geochemical viewpoint. Furthermore, among the many molecules thus formed, as many "poisons" as "viable molecules" would have been present. The problem that seemed important for these authors is that of selectivity and selection during the formation of the first biological molecules. Also, as an alternative to the preceding hypotheses, WÄCHTERSHÄUSER proposed a theory of surface metabolism at life's origin. ◢

7.3. THEORY OF SURFACE METABOLISM

The theory of surface metabolism suggested by WÄCHTERSHÄUSER (1988) arises from the hypothesis that life in the beginning was autotrophic and consisted of autocatalytic metabolism confined to an organic bilayer. Anionic "metabolists"

were linked to positively charged surfaces such as pyrites, at the interface with hot water. Their association at the surface had to have been strong; the surface must have possessed positive charges due, for example, to polyvalent metals (Mg^{++}, Ca^{++}, Fe^{++}, Mn^{++} and Zn^{++}) and capable of forming insoluble salts. The organic constituents had to have been anionic possessing at least two negative charges in order to bind strongly enough to the surface. Thus, WÄCHTERSHÄUSER suggested that all polyanionic constituents from ancient metabolic pathways are ancient surface metabolites; for example, surface binding may have been an ancient function of phosphate groups. From a thermodynamic point of view, the reactions taking place on a surface are favoured by a positive entropy value which can compensate for the negative ΔH values that are otherwise unfavourable for these reactions. Surface metabolism therefore favours the formation of large molecular structures from less reactive groups with respect to the system in solution. For example, phosphotriose molecules cannot form intermolecular hemiacetal bonds in aqueous solution, but theory predicts that this would be possible on a surface. Figure 7.3 shows the condensation of glyceraldehyde phosphate and dihydroxyacetone phosphate to form structures named "phosphotriboses" by the author.

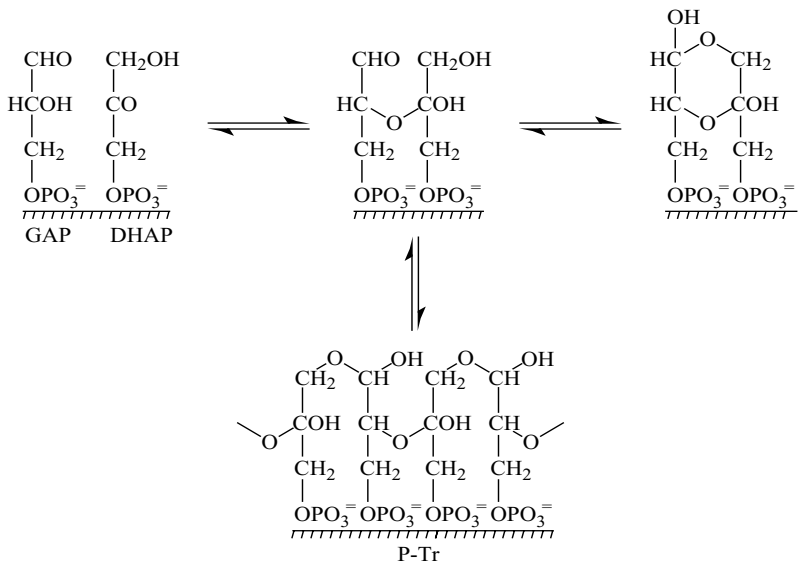

Fig. 7.3 Formation of phosphotriose (P-Tr) from glyceraldehyde phosphate (GAP) and dihydroxyacetone phosphate (DHAP) linked to a positively charged surface

(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 452, reproduced with permission from American Society for Microbiology)

These structures tightly bound to the surface are highly stable. The author suggested that phosphorylated sugars are the precursors to nucleic acids and coenzymes comprising a purine base. With similar reasoning, polypeptide formation from amino acids such as aspartic and glutamic acids or phosphoserine is less unfavourable on a surface than in solution. Surface metabolism offers several advantages,

not only thermodynamic, but also kinetic since it is a quasi-intramolecular process and therefore accelerates reaction rates; furthermore, it enables a high selectivity. It requires high temperatures, which were likely to have prevailed in primitive conditions.

7.3.1. AUTOTROPHIC SURFACE "METABOLISTS"

Surface metabolism gives rise to several types of transfer reaction, which are not observed in solution and which have been preserved in part in existing metabolic pathways. Small molecules like CO_2, HCOOH, CH_2O, CH_3COOH, CH_3CHO, $CH_2OH–CHO$, NH_3^+ and H^+ can be transferred from one molecule to another linked at the surface. This explains the formation of coenzymes such as pyridoxal phosphate, thiamine pyrophosphate, NAD^+ and haems. A sort of self-sufficient "life" would have established itself on a single-layered surface supporting the absorption of nutrients such as CO_2 and autocatalytic production of constituents, which would have spread out over the available surface.

This theory assumes that the first reactions were oxidoreduction reactions, in which a plausible electron source would have come from the formation of iron pyrite:

$$Fe^{++} + 2H_2S \longrightarrow FeS_2 + 4H^+ + 2e^-$$

Iron pyrite is able to reduce CO_2, CO or COO^- as shown in Fig. 7.4, via the formation of a thio acid.

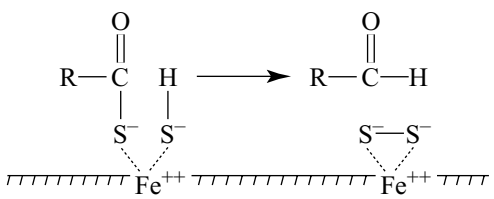

Fig. 7.4 Mechanism for the reduction of a carboxylic acid by iron pyrite via the formation of a thio acid
(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 466, reproduced with permission from American Society for Microbiology)

WÄCHTERSHÄUSER considered that this suggestion was in agreement with geochemistry. Indeed SH_2 was, and still is, abundant in the emissions of the Earth; besides, ferrous irons are ubiquitous and have been found in ancient sediments. Pyrite formation is anaerobic, which is consistent with the absence of oxygen in prebiotic conditions. Thereafter, nitrogen and phosphorous would have participated in this surface metabolism; C–N bonds would have been formed and ammonia, being insoluble in water, would have been instantly eliminated. The universal implication of ferredoxins and other iron-sulphur proteins in the electron transport chain and additionally the central role of cysteine residues and coenzyme A in numerous metabolic processes are probably relics from this primitive metabolism. It is remarkable that, even today, ferredoxins are short anionic polypeptides and their ancestral sequences contain only 11 of the 20 amino acids and none of the more complex amino acids that appeared later in evolution, namely: Met, His, Trp, Tyr, Phe, Leu, Thr, Lys and Arg. This signifies that the ancestral sequence of ferredoxins would

have pre-existed in the translation process. SH_2 would have played a double role, that of electron source and of nucleophilic agent, functions that would have become subsequently separated. The nucleophilic function would have been ensured by the cysteinyl groups on peptides linked to the surface, the activation of the carboxylate would have taken place via the formation of a thio acid generating a variety of peptides. Afterwards, a family of components would have been formed with acetyl-CoA.

Evolution would have progressed by **innovation and selection.** New metabolic cycles would have emerged by leaps. It has often been suggested that certain current coenzymes might be the vestiges of a pre-enzymatic metabolism. Many coenzymes are polyanionic, others like vitamin B12 and the quinones are derivatives of polyanionic biosynthetic precursors. The theory of surface metabolism suggests that coenzymes bound to the surface are not only catalytic for a class of reactions, but also **autocatalytic for their own synthesis from components bound to the surface**. Thus, the synthetic pathway for nicotinamide coenzymes would have involved the anaerobic formation of quinolinic acid from dihydroxyacetone phosphate and aspartic acid in the presence of an oxidant. Later, quinolinic acid would have been ribosylated by phosphoribosyl pyrophosphate (PRPP) to form $NADP^+$, as illustrated in Fig. 7.5.

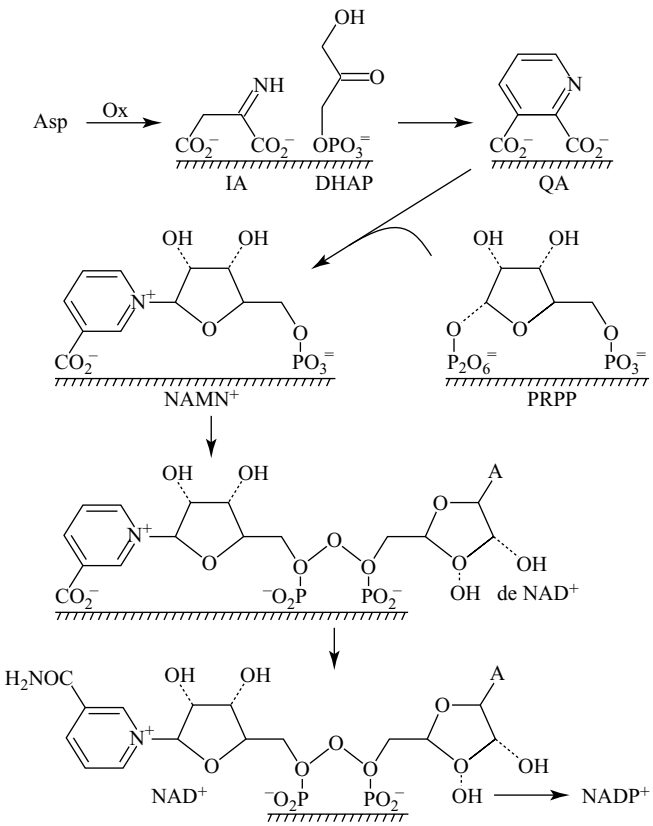

Fig. 7.5 Example of the anaerobic formation of coenzymes by surface metabolism *Quinolinic acid (QA) is formed from iminoaspartate (IA) and dihydroxyacetone phosphate (DHAP) and, in the presence of PRPP, gives rise to nicotinic acid mononucleotide ($NAMN^+$) and then to deamido-NAD^+ (from NAD^+) which can be phosphorylated to give $NADP^+$.*
(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 458, reproduced with permission from American Society for Microbiology)

Thiamine pyrophosphate would have been formed in a similar manner. It is clear that no surface metabolism has been found in nature. Furthermore, WÄCHTERSHÄUSER considered that the process has disappeared and proposed a phylogenetic model, which relates step by step the hypothetical precursor to systems currently existing.

7.3.2. THE CHANGE TOWARDS CELLULAR METABOLISM

A change in the environment could cause a sudden change in one organism, which may then acquire transmissible characteristics, whereas others may perish. The cellular revolution and genetic control profoundly modified evolutionary mechanisms. On an open surface, metabolism and evolution are identical. The change towards cellular metabolism marked a separation between *ontogenesis* and *phylogenesis*. On an open surface, all processes involving a detachment are eliminations. The appearance of closed cells represents an entirely different means of selection with their metabolic control systems. At this stage, evolution brought about a dichotomy between the organism and the environment.

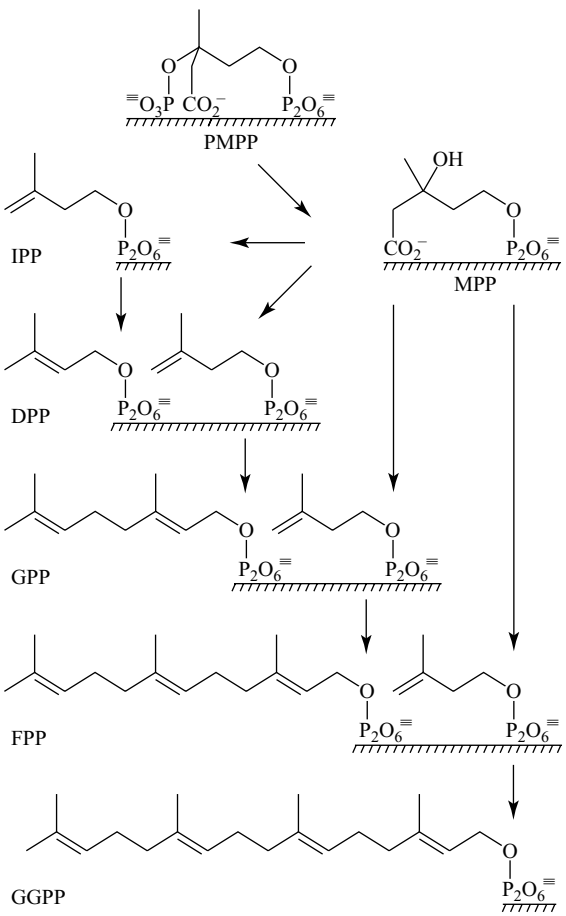

Fig. 7.6 Mechanism of stepwise extension of an isoprenoid lipid chain fixed to a surface, from 3-phosphomevalonate 5-pyrophosphate (PMPP), via mevalonic acid pyrophosphate (MPP), isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMPP), geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP)
(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 463, reproduced with permission from American Society for Microbiology)

The appearance of lipids and membranes therefore constitutes the next step in primitive metabolism. WÄCHTERSHÄUSER assumed that surface metabolites produced isoprenoid lipids (Fig. 7.6 opposite) that remained attached to the surface and then accumulated as regular membranes.

The hydrophobic environment created by the accumulation of lipids had the effect of shifting the equilibria towards the formation of large molecules (polypeptides and nucleic acids). Lipid membranes fixed to the surface became detached to form *semi-cellular structures* still retaining a mineral support and leading to a cytosolic metabolism (Fig. 7.7). The change would have been helped by the emergence of reactions enabling the elimination of charged groups bound to the surface.

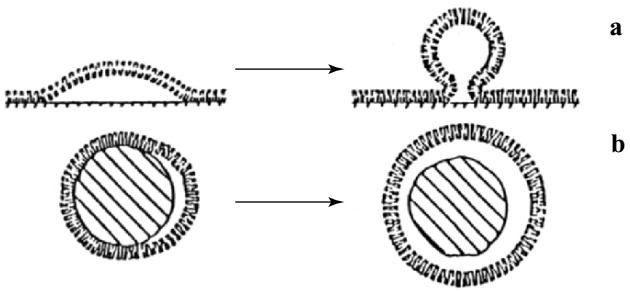

Fig. 7.7 Formation of primitive cells
(a) by constriction – (b) by circular detachment and mineral inclusion
(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 458,
reproduced with permission from American Society for Microbiology)

Evolution might have proceeded therefore by surface metabolism, semi-cellular metabolism and finally cellular metabolism. The genetic apparatus, enzymes, membrane pumps and electron transporters would have been established before the appearance of actual cells lacking a mineral support. WÄCHTERSHÄUSER believed that his theory was consistent with the emergence of three kingdoms in cellular life as proposed by WOESE: archaebacteria, eubacteria and eukaryotes. Isoprenoid lipids are still found in archaebacterial membranes. Other biological membranes arose from the metabolic pathway of fatty acids and would have been formed by enzymatic action.

With cellularisation, certain peptide precursors would have been incorporated into enzyme primary structures and then refolded into a three-dimensional structure. Coenzyme A would have become a true coenzyme by forming a covalent bond with a protein serine residue and thus becoming acyl carrier protein.

During evolution electron transporters bound to the membrane, tetrapyroles, seem to have played an essential role early on with the formation of haems, whereas bacteriochlorophyll would only have appeared much later on; it only exists in eubacteria and chloroplasts.

7.3.3. EVOLUTION OF THE GENETIC APPARATUS

The proposed model hypothesised an increasingly complex evolution of enzymes having a three-dimensional structure and replication machinery, as well as capable of translating nucleic acids – a process also suggested to have evolved in stages. Phosphotrioses were to play an important role; they are able to form polymeric structures, phosphotriboses, by binding carbon after activation by a thio acid. Purines would have been formed first, in the initial phase of surface metabolism, and pyrimidines last during the semi-cellular phase of evolution. Figure 7.8 opposite shows the hypothetical pathway of purine formation on a surface, which implicates the participation of imidazole. The structures formed from phosphotrioses and imidazole bound to the surface have been termed *tribonucleic acids* (TNA). Thus, a variety of purine-containing TNA structures would have been produced with novel structural properties and ultimately leading to complementary base-pairing.

Figure 7.9a below gives an example of a structure with two anti-parallel strands linked to the same surface. The bases would have appeared in the following order: U, A, G, C. The phosphoribose moieties produced by transfer of C2 and then a rearrangement would have formed glycosidic bonds with the purine bases by the same mechanism as the phosphotriboses.

The theory of surface metabolism assumed that translation would have preceded replication. Ribonucleosides would have associated with the TNA by base-pairing. These constituents, coupling to amino acids via ester linkages to the ribose moiety, could catalyse the formation of peptide bonds. From the outset, TNA molecules would therefore have catalysed the formation of peptide bonds without there being translation. The first translation process would likely have been autocatalytic, with TNA and ribonucleotides ensuring the synthesis of their own bases. TNA would have grown by extension and sequence changes brought about by modification of the purines. With the appearance of phosphoribosides, a new type of nucleic acid would have been formed by polymerisation, the TNA serving to position the elements in an orientation favourable to the formation of phosphodiester bonds. The TNA-TNA ribbon structure would have been replaced by a TNA-RNA hybrid (Fig. 7.9b). RNA would have thus inherited stereoregularity from the TNA. Linked in this way to TNA, it would have blocked the TNA and hence acted as a repressor. The situation would have changed during folding and as a result of RNA detachment; transcription would then have become differentiated. Later evolution would have been marked by the appearance of new amino acids and new bases. The first replication process would thus have involved RNA.

The appearance of DNA, considered to be a late event in the "original soup" hypothesis due to the instability of deoxyribose, is accepted as a relatively early event in the theory of surface metabolism. DNA would have been formed at the same time as the accumulation of surface lipids which would have influenced the formation of phosphoanhydrides. The deoxyribonucleotide pyrophosphates thus formed are capable of oligomerising on an RNA or TNA template (the former being reverse transcription).

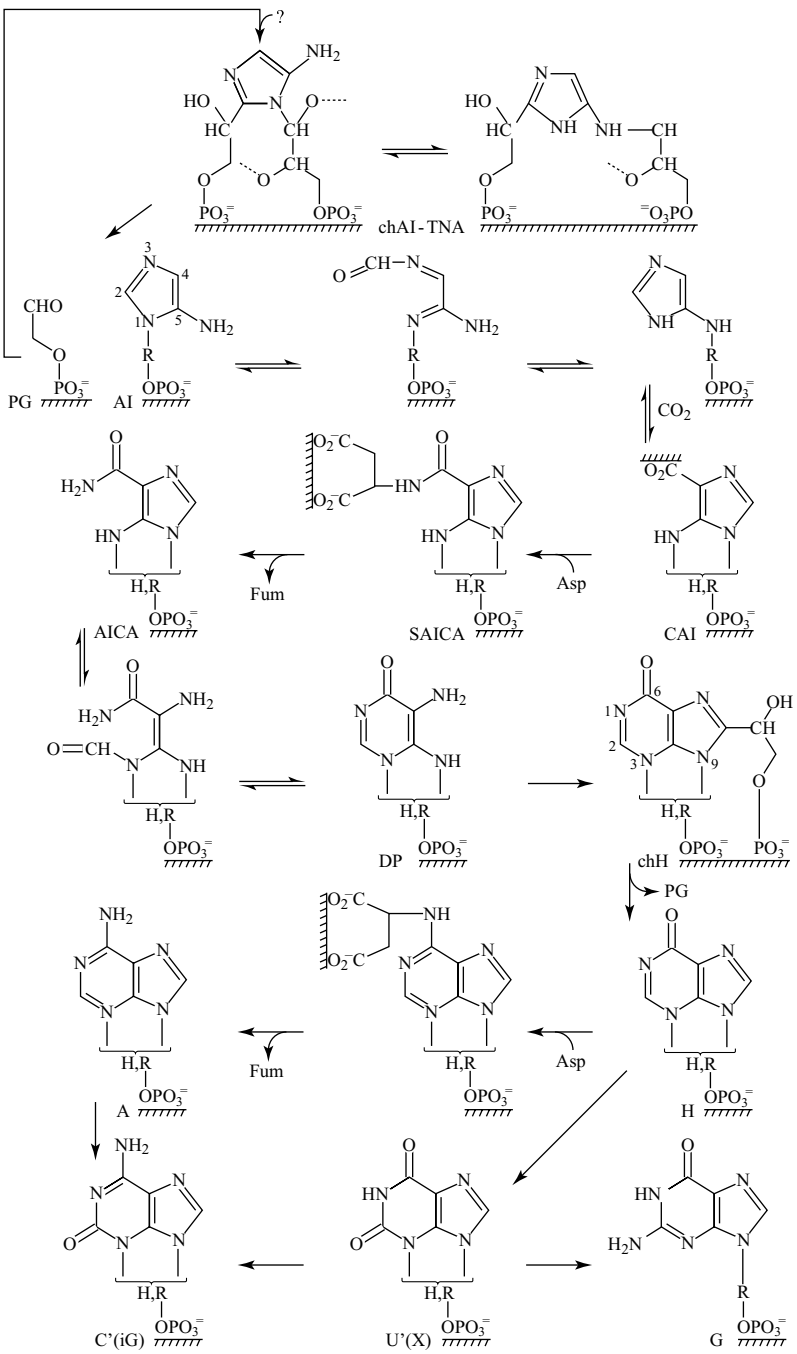

Fig. 7.8 Hypothetical pathway for the formation of purines on a surface
chAI: charged aminoimidazole; PG: phosphoglyceric aldehyde; CAI: carboxyaminoimide; SAICA: succino-aminoimidazole carboxamide; AICA: carboxamide derivative; Fum: fumaric acid; iG: isoguanine. (From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 470, reproduced with permission from American Society for Microbiology)

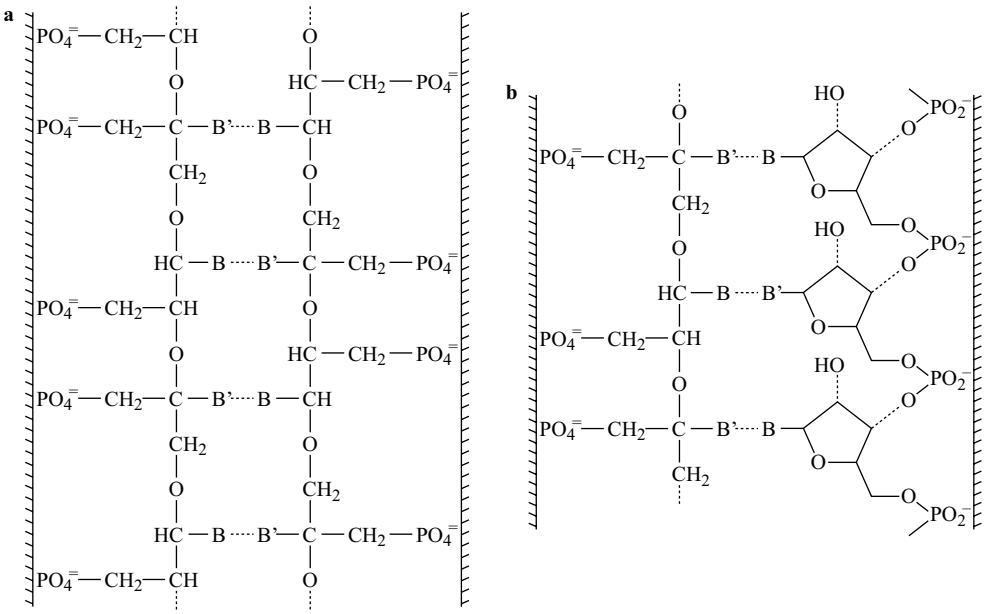

Fig. 7.9 (a) ribbon structure of two TNA strands linked to the same surface (b) hybrid structure of TNA-RNA linked to the same surface

(From *Microbiol. Mol. Biol. R.*, 1988, **52**, WÄCHTERHAÜSER G., 472 and 474, reproduced with permission from American Society for Microbiology)

In parallel to the evolution of transcription and replication, the nature of the bases changed, increasing their structural stability and with an improvement in folding. In this theory, an important role is assigned to phosphoribosyl pyrophosphate in pyrimidine synthesis which is thought to have been produced after the formation of semi-cellular structures. Finally, DNA would have folded itself, encircling the grains of ferrite (Fig. 7.7b) to form a closed, circular DNA molecule. The disappearance of ferrite grains in the cellular stage would therefore have led to the formation of the double helix.

The first peptides would have been formed by translation from building blocks having anionic side-chains (Asp, Glu, P-Ser). Certain amino acids would have undergone conversion e.g. phosphoserine to serine or cysteine. The sequences that ensured a strong bond from each anionic group with the surface would have been selected. Their configuration and conformation would have been determined by their surface association; only the amino acids with the L configuration would have been retained. Over time, more and more amino acids would have been incorporated into polypeptides, first of all hydrophilic amino acids, then smaller hydrophobic ones and lastly aromatic and cationic amino acids. WÄCHTERSHÄUSER favoured the presence of β and β-turn structures in early protein folding. The last amino acids would have entered into the genetic code after the appearance of synthetases. Specifically, these were histidine (derived from imidazole), tryptophan and methionine, which was perhaps the very last. The most recent experiments

done by WÄCHTERSHÄUSER and co-workers demonstrated the plausibility of this theory. The investigators showed in particular that a co-precipitate of FeS and NiS reduced carbon monoxide (HUBER & WÄCHTERSHÄUSER, 1997). Furthermore, they obtained peptides by the activation of amino acids with CO on co-precipitated (Fe,Ni)S (HUBER & WÄCHTERSHÄUSER, 1998; HUBER et al., 2003). These experiments took place in conditions that reproduced primordial temperatures. The existence of volcanic faults in sea depths, where spurts of very hot water rich in transition metals and sulphur mixed with cold sea water, makes this a very attractive theory. These zones are indeed regions of highly abundant biological activity.

Thus, according to WÄCHTERSHÄUSER's theory, evolution progressed in parallel co-evolution to amino acids and the genetic code, and RNA appeared before DNA. The central role of RNA has been previously emphasised in the theories of CRICK and ORGEL. The most recent data on the catalytic properties of RNA molecules seem to support the hypothesis of their particular role since the origin, but do not enable us to decide if they arose before proteins.

7.3.4. CATALYTIC PROPERTIES OF RNA

The discovery by ZAUG and CHECH, in 1986, indeed indicates that RNA possesses a catalytic activity. In their article entitled "The intervening sequence of Tetrahymena is an enzyme", they showed that splicing of this particular RNA sequence takes place without protein involvement, in the presence of micromolecular quantities of GTP or even simply guanosine which attacks RNA at position 413, at its 5′ extremity. This is a transesterification reaction in which the guanosine hydroxyl group attacks the phosphodiester bond between the 3′ end of the first exon and the first nucleotide of the intron. The guanosine remains attached to the 5′ end of the intron. Then, the 3′ end of the liberated exon attacks the extremity of base 413 from the intron to bring together the exon's two ends (Fig. 7.10 below). Thus, the mechanism involves a series of transesterifications. The intron itself undergoes a cascade of spontaneous reactions, which are rapid and relatively specific. This was remarked upon by WESTHEIMER (1986), as many organic molecules are able to undergo internal rearrangements without the aid of a catalyst. WESTHEIMER cites, for example, the cyclisation of 2-hydroxyphenyl propionic acid into dihydrocoumarin. The pentamethyl derivative cyclises 10^{10} times faster than the non-substituted derivative and hence with a high efficiency, and without considering the phenolic acids to be enzymes. However, in the reactions discovered by CHECH, firstly, the distance between the catalytic groups and the hydrolysable bond is great, which imposes a particular folding constraint on these RNAs. Secondly, the reaction is produced at a precise site even though other possible sites exist on the molecule. In the presence of this "truncated sequence", different polynucleotides are hydrolysed at a rate of 2 min^{-1}, which is slow, yet much quicker than the spontaneous hydrolysis of RNA and roughly corresponds to the rate of DNA hydrolysis by restriction enzymes or by non-specific phosphodiesterases. This reaction not only constitutes

autocatalysis, but also true catalysis since the intron can act on some other RNAs. These introns are classed as group I introns, and since their discovery a second class, group II introns, has been found. Their mechanism of excision and splicing proceeds slightly differently, in particular, the presence of guanosine is not required; the attack is carried out by the 2′OH group of a specific adenylate in the intron.

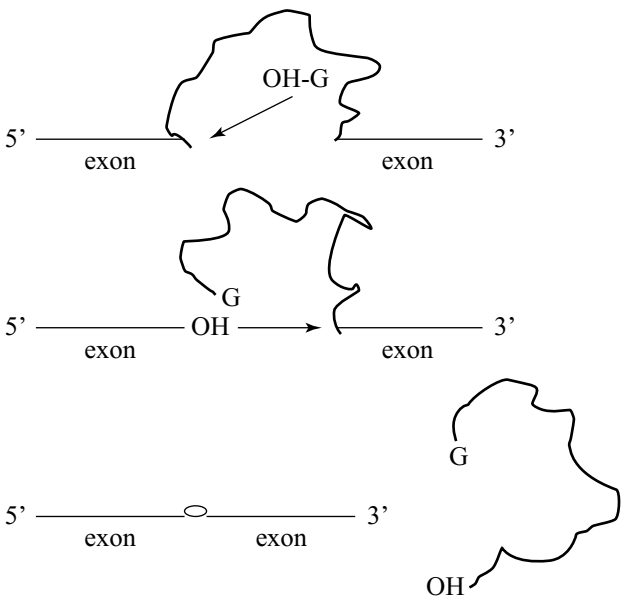

Fig. 7.10 Schematic representation of the catalytic activity of RNA

Over the last few years, numerous RNAs possessing catalytic activities have been discovered in diverse organisms. In addition, many synthetic RNAs capable of binding metabolites specifically and cofactors have been produced in the laboratory. Besides, from a catalytic RNA, M.C. MAUREL and co-workers obtained a variant whose activity is dependent on the presence of an adenine cofactor, thus showing that ribozymes can, like protein enzymes, function by means of a prosthetic group (MELI et al., 2003). The great plasticity and adaptability of RNA, which is demonstrated by these various observations, brings supportive arguments to the implication that an “RNA world” would have preceded the nucleoprotein world in the processes governing the appearance of life (see *La Naissance de la vie* [The birth of life], M.C. MAUREL, 2003).

Once more it is important to stress that the molecules harbouring a catalytic activity require a three-dimensional structure, whereas information storage is one-dimensional, which facilitates reading the code and copying the message. The fact that RNA can have catalytic properties argues for a primordial role of RNA in the origin, but does not necessarily signify that it appeared before proteins. **Whatever the scenario chosen to attempt to explain the origin of life and the first macromolecules, there is unanimous agreement that the existence**

of three-dimensional structures was vital for the expression of biological activity such as enzyme catalysis.

Returning to the theory of WÄCHTERSHÄUSER, life would have begun with metabolism on surfaces in contact with water at neutral pH and high salinity, at high temperatures and probably high pressure in an environment containing SH_2, CO_2, N as well as ferrous ions and other catalytic metals. Whereas the "original soup" favoured an initial appearance of proteins or nucleic acids according to its proponents, in the theory of surface metabolism, metabolic pathways, which resulted in protein and nucleic-acid formation, evolved in parallel. The existence of submarine, volcanic, hydrothermal sources has revived this theory, which today interests many experts investigating the origin of life.

7.4. CHIRALITY OF BIOLOGICAL MOLECULES

Biological molecules are asymmetric. Natural amino acids have the L configuration. Figure 7.11 shows the configuration of amino acids. In order to recall it, the rule is simple: if we look from the hydrogen along its bond linking the asymmetric carbon, the three other groups R, N, C′ appear clockwise in that order in the L configuration; in the D configuration they appear anti-clockwise. Trioses, such as glyceraldehyde, which only possess one asymmetric carbon, have the D configuration; the same goes for pentoses and hexoses.

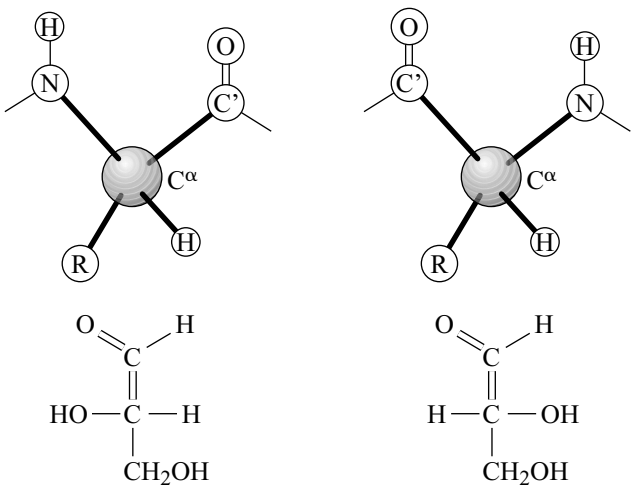

Fig. 7.11 Chirality of biological molecules

Diverse hypotheses have been proposed to explain the chirality of biological molecules. According to the "original soup" idea, these molecules supposedly existed

as racemic mixtures. The three-dimensional structure of polymers is only stable, however, if these mixtures are formed exclusively from L or D elements. Both polymer types must have existed initially. It has been suggested that the L form of amino acids was selected by chance. This might imply, consequently, that all cells derive from the same primitive cell or very similar cells. Molecular asymmetry would result either from the polarisation of light at sea surfaces, or from the chirality of the Universe. Using surface metabolic theory, the chirality of biomolecules (the first selection process) can be explained by considering the nature of the surface itself.

7.5. OTHER THEORIES ON THE ORIGIN OF LIFE

While discrepancies exist about the nature of the first molecules present in the beginning as we have just described, the mechanisms by which they are formed from original chaos are also the subject of controversy. In addition to the previously described models, various others based on different theories have been proposed.

For PRIGOGINE and his school, the origin of life can be described in terms of the spontaneous formation of structures in solution far from equilibrium (see Chap. 3). This is produced when, in a series of reactions, a flux of reactants exists along with a regulatory feedback loop (retroinhibition, retroactivation), the products disappearing when a steady state develops. Following instability at any given moment, the system can oscillate in time as well as in space, and dissipative structures may form. Currently, in many fields in physics and for the past few years in astronomy, in celestial mechanics and even in biology, chaos theories have aroused a growing interest. Indeed, environmental fluctuations may constitute an important aspect of a system's dynamics.

EIGEN, in his hypercycle theory, adopts quite a similar position. He considered that the spontaneous formation of a tRNA molecule is plausible; with mRNA serving as the adaptor, the spontaneous synthesis of a polynucleotide from individual nucleotides is conceivable. He thought that the initial apparatus was thus formed before a mechanism capable of removing the errors could be put in place. According to EIGEN, the filtering mechanism appeared later by coupling two or more cycles, introducing cooperation. The resulting hypercycle dominated and allowed the selection of information from all possible errors. For EIGEN, the first appearance of cooperative mechanisms was the mutual interaction of cycles that produced replicases. He suggested that three conditions were nevertheless necessary to overcome the loss of information across all possible replication errors:

- each replicative unit must selectively maintain its information in competition with its own error distribution;
- the competition between replicative units belonging to the same cooperativity must cease to be operational;
- the functional unit must be capable of competition between alternative units.

Other authors, in particular KUHN and WASER, refused the notion of such a spontaneous appearance of structures resulting from instability. They proposed an appearance

in stages, each stage having a reasonable probability and averaging quite long time periods. They suggested that, first of all, short segments would be formed, which are then condensed into longer segments, the first molecules being RNAs. Erroneous replication would have been rejected during the formation of aggregates, which are favoured by cooling. ◢

In summary, different scenarios have been proposed to attempt to explain the appearance of biological macromolecules in the beginning, and they have given rise to several controversies. The first assumed a very reducing atmosphere and through the effects of electrical discharges, the molecules present, including methane, would have generated an ensemble of biological molecules forming the "original soup". Then the macromolecules would have appeared, certain authors giving precedence to proteins, others to nucleic acids. The "primitive soup" hypothesis has been much criticised on chemical, geochemical and thermodynamic grounds. The most recent theory of surface metabolism developed by WÄCHTERSHÄUSER offers a more appealing alternative. It stems from the hypothesis that life's origin was autotrophic and consisted of an autocatalytic mechanism confined to a bilayer, the anionic components being linked to positively charged surfaces such as pyrites, and thus ensuring molecular selection. Diverse coenzymes would have been formed in this period along with polymeric components, the *phosphotriboses*. With the formation of isoprenoid lipids, changes would have proceeded in stages, firstly towards semi-cellular structures, then towards cellular structures. The pathways for the formation of proteins and nucleic acids would have evolved in parallel, and RNA appeared prior to DNA.

It is difficult to come up with proof for the theories of life's origin, which can only suggest more or less plausible scenarios. These theories evolve with progress in the understanding of currently existing biological systems. In this sense, it is reasonable to wait for useful information arising from studies into archaebacteria living in conditions of extreme temperature and pressure.

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