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PREBIOTIC EVOLUTION AND THE ORIGIN OF CELLS

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INTRODUCTION

This paper presents an argument, summarized in *Figure 1*, that the earliest autopoietic reproducing systems, ancestral to all contemporary forms of life, were structures formed by catalytic RNA molecules and other chemical species of prebiotic origin that were encapsulated within the boundaries of double-layered liposomes formed by lipids of non-enzymatic origin. According to this scheme, the appearance of reproducing cells preceded the origin of the translational apparatus (genetic code, tRNA, ribosomes, etc.) and its products, i.e., proteins. The latter were initially selected for to increase the catalytic potential of early RNA-cells.

RNA polymerase is hypothesized to have been one of the earliest proteins to appear; vestiges of the original enzyme can still be identified from the comparative studies performed on eukaryotic, archaebacterial and eubacterial DNA-dependent RNA polymerases (Zillig *et al.* 1985a), supporting the concept that both replication and translation are of monophyletic origin.

The appearance of double-stranded DNA as an informational macromolecule in RNA-cells is explained on the basis of selective pressures that favoured an enhanced stability of the genetic information. Thus, it is suggested that the origin and evolution of the biosynthetic pathways leading to the reduction of ribose to deoxyribose, the formation of thymine, and the presence of DNA polymerases with proofreading activity occurred subsequent to the origin of cells when DNA-based genetic systems were selected for to stabilize earlier systems based on RNA replication. The presence of double-stranded DNA allowed the further increase in genetic information content by recombination and gene duplication, although it is likely that these phenomena were operating before the evolutionary emergence of DNA.

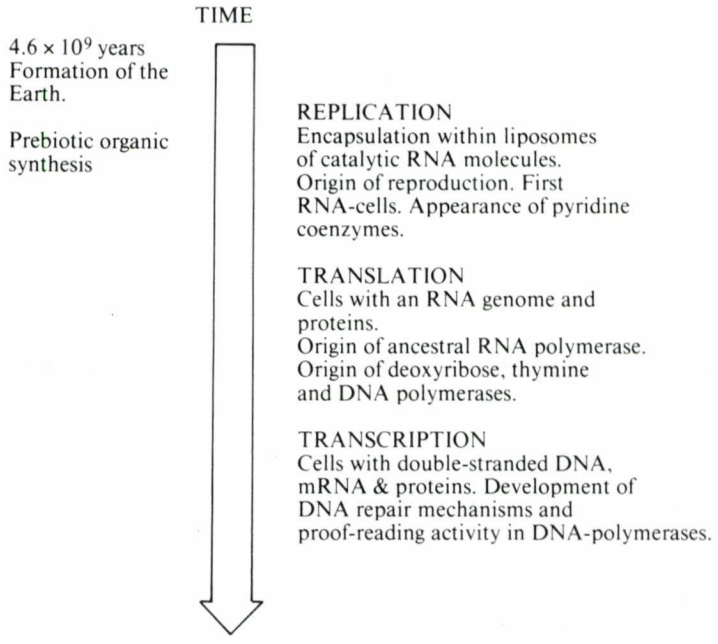

Figure 1

In order to develop this step-wise model of the origin of the earliest cells, the prebiotic synthesis of monomers and oligomers of organic compounds of biochemical significance will be briefly reviewed in Section II, with special emphasis on the non-enzymatic template-directed oligomerization reactions studied by L.E. Orgel and his co-workers. Section III presents the view that the first genomic material was of polyribonucleotide character. This concept is supported by a short review of the catalytic properties of RNA molecules. In Section IV the idea that life emerged due to the spontaneous formation of a single type of molecule – whether protein, DNA or catalytic RNA – is critically rejected. The significance of liposomes in laboratory simulations of the evolution of precellular systems and the step-wise appearance of the first cells are discussed in Sections V and VI, respectively. The origin and early evolution of RNA polymerase are discussed in Section VII. The emergence of double-stranded DNA molecules as informational polymers is discussed in Section VIII, and conclusions are presented in the final section.

PREBIOTIC ORGANIC SYNTHESES

Although the idea of prebiotic organic synthesis as a necessary prerequisite for the appearance of life was suggested by Oparin (1924) and Haldane (1928) more than half a century ago, it was not put succesfully into practice until 1953, when Miller (1953) showed that amino acids and a diverse assortment of other small organic molecules of biochemical significance could be made in the laboratory under environmental conditions thought representative of the early Archean Earth. Since then a wide variety of organic compounds have been experimentally formed from simple molecules such as water, methane, ammonia and HCN. These results have

lent strong support to the idea that a large number of different kinds of organic molecules, including many found universally in contemporary living systems, were formed abiotically during the first epoch of the Earth's history.

Furthermore, many organic compounds have also been found in the interstellar clouds of the Milky Way and other galaxies, in comets and in carbonaceous chondrites; in fact, the production of organic molecules seems to be an inevitable consequence of the interaction of small compounds formed from light elements in the presence of energy sources. What follows is a brief summary of the occurrence of extraterrestrial organic compounds and the non-enzymatic synthesis, under putative prebiotic conditions, of biochemical monomers and oligomers, largely based on material published elsewhere (Lazcano *et al.* 1983).

Although the existence of simple carbon combinations (C_2, CN, CH, CO) in the atmosphere of relatively cool stars had been known about for a long time, it was not until 1969 that microwave techniques led to the detection of NH_3, H_2O and the first organic molecule, H_2CO (Snyder *et al.* 1969). This was quickly followed by the identification of many organic compounds, including acetaldehyde, hydrogen cyanide, cyanamide and the relatively complex linear cyano-oligoacetylenes, such as HC_3N, HC_5N, HC_7N, HC_9N (cf. Turner 1980) and the largest organic molecule identified so far, $HC_{11}N$ (Bell *et al.* 1982). $HC_{11}N$ was found in the circumstellar envelope of the cool carbon star IRC + 10°216, providing evidence that one source of these complex interstellar molecules is cool carbon stars which, through various processes, eject material into the interstellar medium. A similar mechanism was suggested some time ago for the formation of interstellar molecules by Oró (1972). Recently the first truly interstellar ring molecule, cyclopropenyllidene, was shown to exist in relatively large amounts in the Milky Way (Irvine 1985). The list of known interstellar compounds is shown in *Table 1*.

Quite remarkably, a comparison between *Table 1* and *Table 2* shows that among the identified interstellar compounds are many that have been used in prebiotic organic syntheses to obtain most of the biochemical monomers found in contemporary living systems. However, as

TABLE 1
Interstellar molecules*
Number of atoms:

2	3	4	5	6	7	8	9	11	13
H_2	N_2H	NH_3	C_4H	CH_3OH	CH_3CCH	CH_3COOH	CH_3OCH_3	HC_9N	$HC_{11}N(?)$
CO	HCO^+	H_2CO	CH_2NH	CH_3SH	CH_3CHO	CH_3C_3N	CH_3CH_2OH		
CN	HCS^+	H_2CS	NH_2CN	CH_3CN	CH_3CHCN		CH_3CH_2CN		
CS	HCN	HNCO	HCOOH	NH_2CHO	HC_5N		CH_3C_4H		
CH	HNC	C_3H	$CH_2CO(?)$				HC_7N		
OH^+	C_2H	C_3O	HC_3N						
CH^+	H_2O	C_3N							
C_2	SO_2	$HOCO^+$							
NO	H_2S								
NS	HCO								
SO	OCS								
SiS	HNO(?)								
SiO	$HOC^+(?)$								

* As of January, 1986.

TABLE 2
Major prebiotic synthetic pathways*

1. CO H_2	FTT Catalysis	→	Hydrocarbons Fatty acids
2. CH_2O	Base catalysis	→	Ribose (glycerol)
3. CH_3CHO CH_2O	Base catalysis	→	Deoxyribose
4. RHCO HCN NH_3	Strecker synthesis	→	Amino acids Hydroxy acids
5. As 4 above CHL_2S or CH_3SH	Strecker synthesis (+ condensation)	→	Cysteine, methionine
6. HCN	Base catalysis	→	Adenine, guanine
7. HC_3N Urea	Condensation	→	Uracil, cytosine
8. As 7 above CH_2O	Hydrazine or H_2S	→	Thymine
9. Amino acids	Condensation	→	Oligopeptides
10. Mononucleotides	Condensation	→	Oligonucleotides
11. Isoprene	Condensation	→	Polyisoprenoids
12. Fatty acids Glycerols Phosphate Bases	Condensation	→	Neutral lipids Phospholipids

* Adapted from Lazcano Araujo and Oró (1981).

of January 1986 no interstellar amino acid, nucleotide derivative or other metabolite has been reported. The search for interstellar heterocyclic furan and imidazole (DeZafra *et al.* 1971), and for pyrimidines and pyridines (Simon and Simon 1973) has yielded negative results. Glycine, a protein amino acid which is a major product of most abiotic syntheses and one of the most abundant amino acids in meteorites, has been looked for unsuccessfully in several galactic molecular clouds (Brown *et al.* 1979). Hollis *et al.* (1980) have detected a single emission line in the Sgr B2 complex that is coincident with a rotational transition of this amino acid, but this is inadequate for definitive identification.

A large number of interstellar emission lines in the 3-6 mm wavelength region remain to be identified, and it is possible that some of them correspond to biochemicals. This possibility is made plausible by the results of several laboratory simulations of the interstellar enviroment in which icy mixtures of H_2O, CH_4, CO_2, N_2, NH_3 and other simple molecules were irradiated by ionizing radiation (cf. Oró 1963; Lazcano-Araujo and Oró 1981) and ultraviolet light (Greenberg 1981). These experiments have yielded significant amounts of non-volatile organic polymers and monomeric water-soluble biochemical compounds, including amino acids, hydroxyacids, and nitrogenous bases.

Since most of the interstellar organic compounds have been detected in dense cool interstellar clouds where star formation is taking place, it is reasonable to assume that the primordial solar nebula had a similar composition. This assumption is supported by the detection of a number of organic molecules and radicals (C_2, C, CH, CO, CS, HCN, CH_3CN) and other simple chemical species (NH, NH_3, OH, H_2O) in cometary spectra. Comets are relatively minor bodies with diameters of a few kilometers, consisting of ices (CO_2, NH_3, H_2O) and clathrates of simple com-

pounds. They appear to be the most pristine bodies in the solar system (Delsemme 1981; Lazcano-Araujo and Oró 1981). The forthcoming ground-based and satellite exploration of comet Halley which will increase our knowledge of the chemical composition of cometary nuclei, is likely to reveal the presence of more complex organic compounds.

The presence of organic molecules in the solar nebula is further indicated by the large array of amino acids, carboxylic acids, purines, pyrimidines and hydrocarbons which have been found in carbonaceous chondrites. The earlier evidence has been reviewed by Hayes (1967) and Nagy (1975), the results on extraterrestrial D and L amino acids were published by Kvenvolden *et al.* (1970, 1971) and Oró *et al.* (1971a, b), and data on nucleic acid bases (Stoks and Schwartz 1981) and amino acids (Cronin *et al.* 1980; Cronin 1982) have recently been presented. These compounds are presumed to be condensation and hydrolytic products of several of the interstellar compounds (see *Table 1*). This suggests that some of these organic compounds were a part of the material from which the Earth was formed.

There is a relatively close correspondence between the amino acid content of samples from the Murchison CII carbonaceous chondrite and the products of electric discharge experiments with a CH_4-NH_3-N_2-H_2O atmosphere. A suggested interpretation is that the amino acids in the meteorite were synthesized on its parent body by similar processes (Wolman *et al.* 1972; Miller *et al.* 1976). Such a possibility is supported in part by the presence of veins of magnesium sulfate and dolomite in type I carbonaceous chondrites, which in turn has been interpreted by some to indicate exposure to fluid water (DuFresne and Anders 1962; Nagy *et al.* 1963; Richardson 1978; Kerridge and Bunch 1979). In fact, by assuming that both the amino acids and the hydroxyacids in the Murchison meteorite were formed abiotically via Strecker-cyanhydrin synthesis, it has been estimated from the various equilibrium and rate constants involved in this chemical pathway that a HCN concentration of 10^{-3} to10^{-2} M existed in the parental body of the Murchison meteorite (Peltzer *et al.* 1984). Using data on the hydrolysis of HCN, it was concluded that the period of synthesis of organic compounds was as short as 10^4 years (Peltzer *et al.* 1984), which suggests that if similar events took place in the prebiotic Earth, the non-enzymatic synthesis of monomers and oligomers of biochemical significance could have occurred in ten thousand years or less.

It has also been claimed that the meteoritic organic compounds were abiotically formed at the pressures and temperatures of the condensing solar nebula by a Fischer-Tropsch-type catalytic synthesis (Hayatsu *et al.* 1971). But, as Cronin *et al.* (1980) have pointed out, neither this model system nor the Strecker-cyanhydrin synthesis produce an amino acid suite completely comparable to that found in the Murchison meteorite or in other carbonaceous chondrites. A complete correspondence of the model experiments and the meteoritic amino acids should not be expected, since the meteorite organic compounds have probably undergone considerable alteration since their synthesis. In particular, there are indications of considerable thermal decomposition. At any rate, it is quite likely that several processes were involved in the synthesis of the amino acids (or their precursors) in carbonaceous chondrites. These include electric discharges, HCN condensation reactions, catalysis by the alumino-silicates of carbonaceous chondrites, and metal catalyzed Fischer-Tropsch-type reactions (Oró 1965; Hayatsu *et al.* 1968, 1971; Miller *et al.* 1976; Cronin *et al.* 1980).

In the three decades that have gone by

since the first successful synthesis of organic compounds was achieved under the highly reducing conditions of a CH_4-NH_3-H_2-H_2O model atmosphere (Miller 1953), one of the major questions in the understanding of the environment in which life arose has been the detailed composition of the Earth's early atmosphere. It is possible that if intense degassing took place during the accretional phase of our planet formation then the primitive Earth could have had a highly reducing atmosphere (Pollack and Yung 1980). However, the presence of the 3.8×10^9 years old carbonate-rich metasedimentary rocks in Isua, West Greenland (Moorbath *et al.* 1973) shows that even if methane were the dominant form of carbon in the prebiotic atmosphere, a substantial amount of gaseous CO_2 had to be present in the Earth only 800 million years after its condensation (Schidlowski 1978; Lazcano-Araujo and Oró 1981). Probably some gaseous CO_2 was present on the Earth at all times during the Archean even if methane was the predominant carbon-containing gas. This is similar to the situation in CI carbonaceous chondrites, which contain carbonates (Nagy 1975; Richardson 1978) and cometary nuclei which contain CO_2 (Delsemme 1981), even though these two kinds of minor bodies as a whole are quite reducing.

Because of the restrictions that high CO_2 and low H_2 put on abiotic synthesis of organics it was proposed that either icoutgassing and meteoritic bombardment occurred simultaneously for a long enough time to produce sufficiently reducing conditions for the appearance of the relevant biochemicals, or the production of these in low concentrations of CO_2 occurred much more rapidly (less than 10^5 years) than previously realized (Lazcano *et al.* 1983). When neutral atmospheres, primarily CO_2, are used in laboratory simulations, only negligible amounts of a few organic compounds are produced (Abelson 1965). Theoretical calculations by Pinto *et al.* (1979) suggest that significant amounts of H_2CO can be produced from a weakly reducing atmosphere of CO_2 and H_2, yet high partial pressures of H_2 inhibit the synthesis of partially dehydrogenated compounds like purines, pyrimidines (Ponnamperuma *et al.* 1963; Bar-Nun *et al.* 1981) and nitriles (Bossard *et al.* 1982). Electric discharge experiments with a CO_2-N_2-H_2O atmosphere and no added H_2 give only extremely small yields of amino acids (10^{-3} %), formaldehyde and HCN (Miller 1984). Since HCN is essential in the prebiotic synthesis of amino acids, nitrogenous bases and other compounds of prebiotic significance (Oró and Lazcano-Araujo 1981), the early Archean conditions must have favoured its presence.

In an attempt to overcome this dilemma, it has been suggested that the prebiotic starting material was actually derived from cometary nuclei and carbonaceous chondrites accreted by the early Earth (Blake and Carver 1977; Towe 1981). The large number of impact craters which have been identified on the Moon, Mercury and Mars, and on several of the satellites of the outer planets, clearly show that collisions with small interplanetary bodies played a major role in shaping the surface of the Archean Earth (Goodwin 1976; Lazcano-Araujo and Oró 1981). Important amounts of extraterrestrial organic material were probably brought to the Earth by such bodies (cf. Lazcano-Araujo and Oró 1981). However, under the slightly oxidizing conditions proposed by Blake and Carver (1977) and by Towe (1981), a partial photolytic degradation and oxidation of the organic compounds would be expected, as appears to have happened on Mars. In fact, the absence of organic compounds at the two Viking Lander sites on Mars can be explained by the UV-degradation and oxidation of the organic molecules to CO_2 (Oró and Holzer 1979a, b).

Figure 2

An important supply of reduced gases was probably derived from the accretion of cometary nuclei and related volatile rich minor bodies by the early Archean Earth. Shock waves caused by such collisions would in themselves produce organic compounds (Oró *et al.* 1980; Lazcano-Araujo and Oró 1981). Besides comets, reducing gases would have been produced at the trailing edges of plates at spreading centers which probably occurred on the Earth. These mechanisms and others helped provide the necessary precursors and to generate the aqueous and reducing environmental conditions required for the synthesis of organic compounds of biological significance (Lazcano *et al.* 1983).

The wide variety of energy sources (solar radiation, electric discharges, shock waves and others) (Miller *et al.* 1976) and chemical precursors that have been used in experiments of prebiological organic synthesis have been reviewed elsewhere (Oró and Lazcano-Araujo 1981; Lazcano *et al.* 1983; Miller 1982, 1984; Ferris and Usher 1983). Several of these prebiotic model experiments have been performed under extreme concentrations, high inputs of energy (Miller and Van Trump 1981; Chang 1981), or as in the case of the Fischer-Tropsch synthesis of fatty acids, under conditions of pressure and temperature that, although they may have been present in the solar nebulae, are more difficult to envisage on the Archean Earth (Miller *et al.* 1976).

In principle, however, the results of the laboratory non-enzymatic synthesis of organic molecules indicate that comparable processes took place on the early Earth

that eventually led to an accumulation of a complex heterogeneous mixture of monomeric and polymeric organic compounds. For example, the sparking of a gaseous mixture of methane, ammonia, nitrogen and water produces protein and non-protein amino acids (cf. Miller 1984), although if a similar gaseous mixture is irradiated with ultraviolet light the yields are very low (Groth and Von Weyssenhoff 1960). It should also be pointed out that significant quantities of amino acids, as well as ammonia, can be derived from the hydrolysis of HCN condensation products (Oró and Kamat 1961; Ferris *et al.* 1978). A number of protein amino acids can also be produced non-enzymatically starting from formaldehyde and hydroxylamine (Oró *et al.* 1965).

Possible prebiotic routes for the formation of all the components of nucleic acids have been established. A complex mixture of different sugars is readily formed when formaldehyde polymerizes under alkaline conditions (Butlerow 1861). Adenine, a purine that plays a central role in genetic processes and energy utilization in all organisms, can be obtained together with guanine by the condensation of HCN in the presence of ammonia (Oró 1960; Oró and Kimball 1961, 1962). Adenine was also synthesized from aqueous solutions of HCN via UV-irradiation of diaminomaleonitrile to form 4-amino-imidazole-5-carbonitrile (Sánchez *et al.* 1966). Fischer-Tropsch-like reactions using ammonia in addition to CO and H_2 yield purines, pyrimidines and other nitrogenous compounds (Yoshino *et al.* 1971; Yang and Oró 1971). Cytosine is obtained from cyanacetylene (Sánchez *et al.* 1966), and on deamination yields uracil (Ferris *et al.* 1968), and thymine can be formed from the condensation of uracil with formaldehyde under reducing conditions (Stephen-Sherwood *et al.* 1971). The hydrolysis of HCN oligomers releases significant amounts of pyrimidines (including orotic acid, the common biological precursor of cytosine, uracil and thymine), together with adenine and several protein and non-protein amino acids (Ferris *et al.* 1970).

In the presence of linear and cyclic phosphates the phosphorylated forms of the nucleosides of these bases (i.e., the corresponding nucleotides) can be produced non-enzymatically. However, the nucleosides themselves are somewhat difficult to synthesize, and additional studies are required to demonstrate their prebiotic synthesis. Fatty acids may be formed from CO and H_2 in the presence of meteoritic nickel-iron and other catalysts (Nooner *et al.* 1976; Nooner and Oró 1979), and glycerol, a component of fats, readily forms by the reduction of glyceraldehyde, itself a product of the base-catalyzed condensation of H_2CO (Oró 1965).

Several mechanisms for the condensation of amino acids and nucleotides in the absence of enzymes have been studied (Oró and Stephen-Sherwood 1971; Miller and Orgel 1974). These include coupling reactions using activated derivatives, condensation reactions using polyphosphates, and condensation reactions using organic condensing agents such as cyanamide, derived from HCN (Oró and Lazcano-Araujo 1981). Cyanamide, which has been detected in the interstellar medium and was presumably synthesized on the prebiotic Earth from HCN, is one of the best condensing agents. The evaporating pond model provides a geologically plausible and realistic means by which cyclic changes in humidity and temperature in the presence of cyanamide may have yielded significant amounts of oligopeptides (Hawker and Oró 1981), oligonucleotides (Odom *et al.* 1982) and phospholipids (Rao *et al.* 1982). The prebiotic synthesis of acylglycerols (Eichberg *et al.* 1977), phosphatidic acids (Epps *et al.* 1978) and other lipids (Hargreaves *et al.* 1977) has also been accom-

plished. For reviews on the prebiotic synthesis of membrane components see Oró *et al.* (1978) and Hargreaves and Deamer (1978a).

Table 2 summarizes some of the major prebiotic pathways that may have been responsible for the non-enzymatic synthesis of organic molecules on the Archean Earth. The use of cyanamide and other condensing agents in the polymerization of ribonucleotides yields a significant amount of «unnatural» 2'-5' bonds in the phosphodiester backbone of the resulting oligomer (Miller and Orgel 1974; Lazcano *et al.* 1983). However, the hydration/dehydration cyclical pathway for the non-enzymatic synthesis of polynucleotides suggested by Usher and McHale (1976) and Usher (1977) would have led to the preferential hydrolysis of the «unnatural» 2'-5' bonds and the accumulation of molecules linked by 3'-5' bonds. These results are consistent with theoretical studies that have shown that 2'-5' bonds can not support helical structures, while the 3'-5' bonds allow more favorable base-stacking interactions (Dhingra and Sarma 1978; Dhingra 1980).

Polynucleotide formation has been extensively studied by L.E. Orgel and his associates, who have shown that the non-enzymatic template-directed synthesis of activated nucleotides (Lohrman *et al.* 1980) in the presence of Zn^{++} results in an extremely high fidelity in the incorporation of complementary bases (Bridson and Orgel 1980; Bridson *et al.* 1981). In fact, both DNA– and RNA–polymerases are known to be Zn metalloenzymes (Mildvan and Loeb 1979). Since Orgel and his group found no evidence for the direct activation of the 3'-OH group, they have suggested that the Zn ion functions by coordination with the N-7 position of guanine, thereby changing the detailed stereochemistry of the double-helical complex (Inoue and Orgel 1981). A favourable orientation of the double-helix complex was also achieved using a nucleotide activated with 2-methyl-imidazole (Inoue and Orgel 1982). In the absence of metals, guanosine 2-methyl imidazole derivatives which polymerize on a poly (C) template can give rise to over 90 % of 3'-5' linked oligo (G) nucleotides with chain lengths from 2 to approximately 40 bases (Joyce *et al.* 1984). The synthesis of imidazole, 2-methyl-imidazole and 4-methyl-imidazole under putative prebiotic non-enzyme conditions has also been achieved (Oró *et al.* 1984). Recent experiments have also demonstrated the polymerization of 2-methyl-imidazolide derivatives using random poly (C, U) copolymers and templates (Joyce *et al.* 1984).

Oligo– and poly-deoxycytidylate templates can be used for the polymerization of guanosine 5' phospho-2-methyl imidazolide, at a rate similar to that when comparable ribo-analogues are used (Chen *et al.* 1985). These types of template-directed reactions may take place even if deoxyguanoside or deoxyadenosine 3'5' diphosphates are used, but they yield oligonucleotide analogues in which a pyrophosphate group replaces the typical nucleic acid 3'-5' phosphodiester bond (Schwartz and Orgel 1985). From this work I assume that in the early Archean eon the chemical and physical transformations of prebiotically synthesized organic molecules led to an ample variety of template-directed replication-like reactions upon which natural selection worked during the precellular stages of evolution – or perhaps even later.

THE EARLIEST GENOME: RNA PRIOR TO DNA

The existence of an intracellular genetic apparatus able to store, express and, upon reproduction, transmit to its progeny information capable of undergoing evolutionary change is an essential requirement of

all contemporary cells (Hartman *et al.* 1985). Replication schemes lacking nucleic acids have been suggested. For example, Cairns-Smith (1982) has argued that the earliest genetic system was based on cationic substitution in replicating clays. Even if one accepts his views, it is clear that at some point polynucleotides must have become the carriers of genetic information in early forms of life that emerged from the complex mixtures of abiotically synthesized organic compounds described above.

The idea that RNA preceded DNA as genetic material has been expressed independently by many authors (Belozevskii 1959; Brachet 1959; Oparin 1961; Rich 1962; Haldane 1965). This hypothesis is supported by the well-known fact that protein synthesis can take place in the absence of DNA but not of RNA. The existence of viroids (Diener 1982) and RNA viruses (Reanney 1982) shows that replicating systems can use either single– or double-stranded polyribonucleotides to store genetic information.

The existence of a primordial replicating and catalytic apparatus devoid of DNA and protein, and based on RNA was suggested by Crick (1968) and Orgel (1968). Such a possibility is likely. Evidence that supports the hypothesis that RNA molecules were selected for as the primordial genomic material prior to the evolution of the present-day DNA-based system of cells includes, among others, the following:

1. *The 2'-OH group of ribose appears to be involved in a number of prebiotically plausible phosphorylation and condensation reactions.* Using cyanogen and cyanamide as condensing agents in aqueous solutions of D-ribose and orthophosphate, Halmann *et al.* (1969) obtained high yields of 10 to 20 % of the β-D-ribofuranose 1-phosphate molecule. Furanose phosphate was the only phosphorylation product, and 2'-deoxy-D-ribose did not undergo the same reaction.

The condensation of glycine to polyglycine on clay surfaces exposed to temperature and moisture fluctuations was significantly enhanced by the presence of polyribonucleotides, but not by their deoxy-equivalents (White and Erickson 1980).

2. *The biosynthesis of deoxyribonucleotides always proceeds via the enzymatic reduction of ribonucleotides,* i.e., all deoxyribonucleotides are formed, directly or indirectly (as in the case of dTTP), from a cellular pool of ribonucleotides (Henderson and Paterson 1973; Therlander and Reichard 1979; Kornberg 1980; Lammers and Follmann 1983). With the exception of highly specialized cells like sea-urchin eggs or those from plant seeds, where deoxyribonucleotides are stored for the embryo (Lammers and Follmann 1983), neither prokaryotic nor eukaryotic cells accumulate these compounds, but form them by reducing a fraction of the total nucleotides formed *de novo* in purinic and pyrimidinic biosynthetic pathways. This is also true for at least one archaebacterium, *Methanobacterium thermoautotrophicum* (Sprengel and Follmann 1981), where a ribonucleotide reductase system different from that found in eubacteria may exist (Lammers and Follmann 1983).

3. *The ubiquity of pyridine nucleotide coenzymes and similar ribonucleotide cofactors in metabolic pathways.* Approximately 50 % of all catalogued enzymes cannot function without coenzymes (White 1976, 1982), many of which are small molecules of ribonucleic nature whose biosynthesis is intimately related to the metabolism of RNA and its monomers. Since many coenzymes appear to be composed of relatively few organic compounds whose synthesis has been achieved abiotically, it has been assumed that they represent early catalysts that the first cells initially took directly from their aqueous external environment,

until eventually they developed the capability of synthesizing them. According to this view, coenzymes were eventually displaced from their central role in the early metabolic pathways by proteins, whose evolutionary development led to a whole new set of much more efficiently catalyzed reactions (Oparin 1972).

I support an alternative concept that suggests that coenzymes represent the addition of catalytic molecules to a replicating system in which polyribonucleotides with catalytic properties already existed. Thus, coenzymes represent pre-genetic code catalysts that were employed by the early cells before the appearance of proteins (Orgel and Sulston 1971; White 1976, 1982; Reanney 1979; Visser 1981, 1982, 1984a, b). This theory is strongly supported by the recently discovered catalytic properties of RNA molecules (see below). Coenzymes may have resulted from the interaction between catalytic polyribonucleotides and amino acids, polypeptides, sugars, and other organic molecules that perhaps were used as substrates in RNA-catalyzed reactions. A logical consequence of this hypothesis is that early nucleic acid replication must have been based on the mutual interaction of a set of catalytic polyribonucleotides, their derivatives and other molecules of abiotic origin. Histidine, whose imidazole moiety allows it to function either as a proton-donor or acceptor at physiological pH, may be the fossil remnant of a ribonucleotide, since it is the only amino acid whose biosynthesis begins from both ATP and 5-phospo-alpha-ribosyl-1-pyrophosphate (PRPP) (White 1976, 1982). Furthermore, White has also argued that cysteine may be a replacement for what was once a sulfhydryl-containing coenzyme. Partial support for these ideas may be found in the fact that there is still no satisfactory prebiotic synthesis of histidine, although cysteine has been reported from the UV-irradiation at 2537 Å of mixtures of CH_4, C_2H_6, NH_3, H_2S and H_2O (Sagan and Khare 1971).

4. *RNA molecules have catalytic activity.* The existence of catalytic properties in RNA molecules had been suspected for a long time, yet the first experimental confirmation of this idea came only recently with the observation that the precursor rRNA (p-rRNA) from the ciliated protist *Tetrahymena thermophila*, which has an intervening sequence (IVS) 413 nucleotides long, performs upon itself a set of highly specific cleavage and ligation reactions that result in the precise excision of the IVS, followed by the joining of the 3' and 5' ends of the exon, and the further cyclization of the intron (Kruger *et al.* 1982). The self-splicing reaction that p-rRNA performs in aqueous solutions requires only NH_4^+, Mg^{++} ions and guanosine (pG-OH). Both the guanosine moiety and the 2'-hydroxyl appear to be essential, since Cech *et al.* (1982) have shown that the splicing of the IVS can take place if guanosine is substitued by GMP, GDP or GTP, but not if 2'-deoxyguanosine, 2', 3' – dideoxyguanosine, or 3' – GMP are used. These results are, in fact, consistent with the importance of the 2' – OH group in prebiotic simulation experiments discussed above. It was shown that ATP, UTP and CTP are also inactive in the self-splicing reaction.

Additional experiments have established that during the excision of the IVS, the guanosine cofactor is linked to the IVS in a 3'-5' phosphodiester bond (Zaug and Cech 1982). The *in vitro* reactions were further confirmed by inserting the DNA fragment from *T. thermophila* containing the intron sequence into the β-galactosidase gene of *Escherichia coli* in such a way that the auto-excision of the IVS restores the proper reading-frame of the altered bacterial system (Waring *et al.* 1985).

The IVS is autocatalytically converted from a small linear RNA molecule into a

covalently closed circular form (Zaug *et al.* 1983). This cyclization reaction occurs via the transesterification mechanism that also accounts for the IVS excision from the p-rRNA, and which involves a cleavage-ligation reaction. It is likely that this cyclization reaction has been selected for in order to prevent the whole set of splicing reactions from going backwards (Zaug *et al.* 1983). An unsuspected result in the study of these catalytic properties of RNA molecules occurred when the reverse reaction (i.e., IVS RNA + exons → p-rRNA) was attempted. The IVS RNA molecules excized from *T. thermophila* p-rRNA were heated above their denaturation temperature (95° C) in order to disrupt their secondary structure. The subsequent cooling of the solution to 42° C in the presence of Mg^{++} did not restore the original form of the molecules, but resulted in their linkage in circular and linear chains that where actually dimers, trimers and higher products of the original IVS RNA molecules. This experiment shows that small RNA molecules can perform their own oligomerization in the total absence of proteins, by a mechanism that involves recombination of different RNA molecules (Zaug and Cech 1985).

Further evidence of the catalytic properties of RNA was accidentally discovered by Altman and his co-workers (Guerrier-Takada *et al.* 1983), who were able to demostrate that the RNA moiety of RNase P of both *E. coli* and *Bacillus subtilis* cleaves, by itself, the precursor-specific segment from the tRNA precursor (p-tRNA) to generate the mature 5' terminus. The mechanism of this reaction is, in fact, different from that of the autosplicing reaction described above (Marsh and Pace 1983). RNase P is an enzyme that is composed of a protein with a molecular weight of 17,000 and a RNA moiety that has about 400 nucleotides (Altman 1978). Under physiological conditions RNase P requires its two subunits to perform its catalytic function. However, in the presence of high mono– and divalent cation concentrations, the RNase P RNA moiety itself cleaves the p-tRNA very precisely, generating the 5' terminus. The possibility of protein contamination has been completely ruled out by the chemical synthesis of the gene coding for the RNase P RNA (Guerrier-Takada and Altman 1984). It has also been firmly established that the polyribonucleic subunits of *E. coli* and *B. subtilis* have a different primary structure (Altman 1984).

Additional examples of chemical and biochemical reactions catalyzed by RNA will most likely be discovered. Of course, the obvious candidates include the RNA subunits from eukaryotic ribonuclease P (see, for instance, Kline *et al.* 1981) as well as other enzymes with RNA moieties, like potato O-diphenol-oxidase (Balansingam and Ferdinand 1970), and rabbit muscle 1,4-alpha-D glucan: 1,4-alpha-D glucan 6-alpha (1,4-alpha glucano)-transferase (Korneeva *et al.* 1979). Other possibilities may include RNA molecules from ribonucleoprotein particles involved in RNA processing (Altman 1984) and, as suggested by R. Obar (personal comm.), the RNA molecules involved in protein-transport in mitochondria.

THE MYTH OF THE EVOLVING MOLECULE

There is no detailed scheme of the mechanisms that led gradually – but not necessarily slowly – from the prebiotically formed monomers and polymers to the earliest cells. Attempts to solve this problem have sometimes relied on the assumption that life arose due to the spontaneous formation of a single substance (Troland *et al.* 1914 *et seq*; Muller 1929, 1955). Although this reductionist approach is deeply rooted in the intellectual background of

modern biology (Keosian 1974), there is no such thing as a «living molecule». No chemical compound is alive by itself. One can hardly reduce, the recently discovered catalytic properties of RNA molecules notwithstanding, all the characteristics of living systems to a substance that arises suddenly by a lucky combination of atoms.

The assumption that life originated due to the spontaneous formation of a single molecule – whether DNA, RNA or protein – is based on remote chance events and is therefore not susceptible to experimental testing. Could evolution of all forms of life from such a single hypothetical molecular ancestor actually have taken place?

The elegant experiments in which the Qβ-RNA/Qβ-polymerase system has been employed have shown that is possible to select RNA variants more suitable for replication under *in vitro* altered environmental conditions. Under certain expe rimental conditions both Qβ RNA and the replicating variants derived from it can adapt to continuously increasing concentrations of tuberdicin –i.e., 7-deazaadenosine, a chemical analogue of ATP– by point mutations and suppression of the sites where the substitution of adenine can damage the system (Mills *et al.* 1967; Spiegelman 1970, 1971; Saffhill *et al.* 1970). Pieces of variant RNA about 90 nucleotides long form. These can replicate more rapidly than the original Qβ-strand (4,220 nucleotides), with which they compete for the replicase.

In addition, both V2 RNA and MDV-1 RNA, two replicating variants of Qβ RNA that have about 550 and 218 nucleotides respectively, can become resistant to ethidium bromide, as selection leads to the outgrowth of RNA «mutant molecules» that have lost some of the sites where the inhibitor can bind (cf. Orgel 1979). It has also been claimed that Qβ replicase can lead to a whole series of small Qβ RNA variants with different high percentages of GC pairs synthesized *de novo* by the enzyme in the absence of templates (cf. Schuster 1984 and references therein). Such results have been explained in terms of natural selection acting in the test tube over a whole variety of Qβ RNA midivariants (Eigen *et al* 1981). However, the discovery that Qβ replicase is usually contaminated with very small amounts of specific templates appears to rule out this possibility (Hill and Blumenthal 1983).

Nevertheless, the experiments with Qβ replicase have led to rather sophisticated mathematical models of systems where natural selection is assumed to be occurring (Eigen and Schuster 1977; Eigen 1971, 1984). «Darwinian behaviour is observed whenever certain conditions are met, whether the evolving species are living or not. The conditions are metabolism, self-reproduction and mutability» (Biebricher 1983). In fact, these conditions are an independent reformulation of Muller's (1929) definition of life: a catalytic and autocatalytic primordial gene formed spontaneously that evolves by undergoing mutations. However, as argued persuasively by Oparin (1961, 1972 and 1975), Keosian (1974), and others, the evolution of a such a system would not lead to a biosphere. In the optimal situation, it would result only in the accumulation of replicating mutated molecules in the Archean oceans.

The experiments described above and their mathematical description satisfy *some* of the criteria required to call a series of changes «natural selection» in a neo-Darwinian sense. However, this type of selection leads to the formation of replicating systems that are unidirectionally adapted to a particular selective pressure. But this is not the kind of behaviour observed either in organisms or in biological populations. Since the structure of biological systems depends on many components, they tend to exhibit neither linear nor unidirectional responses. As pointed out by

Tomkins (1975) in his posthumously published paper, mutations damaging one element of a living being have pleiotropic effects that may imperil (or benefit) the entire organism, thus leading to differential reproduction.

Furthermore, evolution has lead to populations in constant interaction among themselves and with other populations of organisms, each of which profoundly influences its environment. Living organisms are not passive elements; the environment is under constant modification because of the interdependence of organisms with it. Thus, as Margulis (1972) wrote, «... molecules do not evolve. Chemical evolution, like "stellar" and "cultural" evolution are helpful analogies to the very different process of biological or neoDarwinian evolution. The former are a series of regular complex changes unidirectional in time. Biological evolution is a process only possible in populations of reproducing organisms. Differential survival, the production of permanently damaged or mutated organisms again giving rise to their own kind are necessary elements of biological evolution. There is nothing less complex than a population of living cells that is subject to this kind of evolution.»

LIPOSOMES AND THE ORIGIN OF MEMBRANES

If the origin of life did not depend on the chance appearance of a single molecule, but on the gradual evolution of systems formed by sets of different chemicals of abiotic origin, then it is obvious that a mechanism for keeping them together was required from the very beginning, i.e., a decisive step towards the origin of life must have been the early appearance of membrane-bound polymolecular systems (Oparin 1924 *et seq*; Haldane 1954). That these early membranes were made of lipids of non-enzymatic origin is supported by a variety of recent experimental evidence. (See Oró *et al.*, 1978).

There has been no lack of theoretical and experimental models to study the properties of putative precellular systems; to cite just a few, this list includes coacervates (Oparin 1936); proteinoid microspheres (Fox and Dose 1977); and *sulphobes*, i.e., microtructures made of NH_4SCN, H_2CO and other simple molecules (Herrera 1942).

All these systems are formed under conditions that tend to produce condensation of small molecules into polymers (Deamer and Oró 1980), and their choice as laboratory models of prebiotic systems has rested upon the assumptions about the nature of early life made by each researcher. A critique of these and other putative models of precellular systems may be found in Day (1984), whose detailed analysis has led him to give considerable support to liposomes as the best contemporary laboratory model of precellular structures. Somewhat similar views may be found in Oró *et al.* (1978), Hargreaves and Deamer (1978a), Deamer and Oró (1980) Ferris and Usher (1983), and Oró and Lazcano (1984).

Liposomes are spheres with diameters of 5 to 50 microns, whose amphiphilic components self-assemble into vesicles with a double-layered membrane in the absence of polymerization processes (Deamer and Oró 1980). Although some authors have argued that the formation of membranes resulted after the development of a replicating system (see, for instance, Eigen 1971), the lack of compartments would not only severely limit the possible cooperative interaction between the different molecules forming the replicating apparatus, but would also lead eventually to their dispersal. Membranes are essential to life not only because they allow cells to maintain an internal microenvironment substantially different

from the exterior, but also because any self-replicating and catalytic system lacking them would be unable to undergo preferential accumulation, and eventually, differential replicativ (Oparin 1972, 1975; Oró 1980; Ferris and Usher 1983). In any case, lipidic membranes are an essential component of all contemporary cells, and as Deamer and Barchfeld (1982) have pointed out, « ...at some point in time one or more such (possible replicating) systems must have become encapsulated within a membranous structure containing lipids.»

Although the prebiotic formation of lipids has not been as extensively studied as that of amino acids or nitrogenous, bases, it is possible to non-enzymatically sinthesize neutral lipids and other important amphiphilic molecules found in contemporary prokaryotic membranes (Oró *et al.* 1978; Hargreaves and Deamer 1978). Furthermore, since non-polar molecules capable of forming boundary structures have been extracted from a sample of the Murchison carbonaceous chondrite (Deamer 1985), clearly the potential to form liposomes exists in the absence of life.

Encapsulation of DNA within liposomes formed from phosphatidylcholine has been achieved by dehydration-hydration cycles that may have occurred in intertidal settings during the early Archean eon (Deamer and Barchfeld 1982). The evidence supporting the RNA character of the earliest genomes has led Oró *et al.* (1986) and Lazcano *et al.* (1986 a) to study the encapsulation of polyribonucleotides within liposomes made from dipalmitoyl phosphatidylcholine (*Figure 2*) using the reverse-phase evaporation method described by Szoka and Papahadjopoulos (1978). The efficiency of encapsulation of poly (U) and poly (C) was not affected by the presence of urea – a condensing agent in prebiotic phosphorylation reactions (Osterberg *et al.* 1977) – or cyanamide, which has been used as a condensing agent in non-enzymatic synthesis of polypeptides and oligonucleotides (Oró *et al.* 1978).

Very little is known about the origin and early evolution of transport mechanisms and membrane-bound bioenergetic systems (Stillwell 1980; Wilson and Maloney 1978). If indeed abiotically synthesized phospholipids and other amphiphilic molecules provided the starting material for the earliest membranes, then the appearance of life led to the exhaustion of the abiotic supply of such molecules. When this happened, which early metabolic steps led eventually to the development of the biosynthetic pathways of lipids? Since lipids are an obligate prerequisite to the origins of life, and acetyl-Co A is mandatory in contemporary biosynthesis of fatty acids (Vance and Vance 1985), the activation of acetate – whose prebiotic synthesis has been achieved by several different simulation experiments since Miller (1953) – may have resulted from its interaction with RNA molecules.

Since the biosynthesis of the tetracyclic sterol nucleus is an oxygen-requiring process, it is likely that it did not appear until free-oxygen had become a component of the early Proterozoic atmosphere (Bloch 1985). We still know almost nothing about the evolutionary history of the metabolic pathways leading to other membrane components, including the different types of lipids found in archaebacteria (Tornabene *et al.* 1980). These questions have to be solved, at least in a general way, before an understanding of the role of lipids and liposomes in the origin and evolution of microbial cells can be claimed.

THE FIRST LIVING SYSTEMS: RNA-CELLS

Since fossil prokaryotes at least 3.5×10^9 years old have been identified (Awramik

et al. 1983; Schopf 1983), life must have originated before that time. However, it is unlikely that the early Archean geological record will provide evidence of the transition from the prebiotic organic molecules to the earliest cells. A cladistic approach to this problem is not possible, since all possible intermediates that may have once existed have long since vanished. Therefore, we must rely on the development of reasonable theoretical and experimental models of precellular-to-cellular systems, constrained by information derived from the study of metabolic pathways, amino acid sequences in proteins (Schwartz and Dayhoff 1978), oligonucleotide informational macromolecules (Fox *et al.* 1980) and the rest of contemporary biochemistry.

I agree with several other authors that RNA-based replication evolved *prior* to the appearance of translation and transcription, i.e., before protein synthesis and the use of DNA as a reservoir of genetic information (Crick 1968; Orgel 1968; Orgel and Sulston 1971; Reanney 1979; White 1976, 1982; Visser 1984 a,b; Day 1984). If RNA preceded DNA as the genetic material of the earliest ancestors of all contemporary life forms, then the origin of life was not associated with the appearance of DNA transcription to mRNA. This assumption eliminates immediately the need for primordial genes coding for ribonucleotide reductases, DNA polymerases, DNA topoisomerases, DNA-dependent ATPases that couple the unwinding of double-stranded DNA with the hydrolysis of ATP, etc. (Reanney 1982). Furthermore, if replicaton predated translation (*Figure 1*), then theoretical and experimental models of RNA-dependent replicating systems can be constructed. As shown in *Figure 4*, it is possible to imagine template-mediated synthesis of RNA catalized solely by another RNA molecule (Sharp 1985).

A central argument in this paper is that there was no prebiotic transtational synthesis of proteins. Although there are many experiments in which the non-enzymatic formation of peptide bonds has been demonstrated, I believe that translation evolved within the lipidic boundaries of RNA-cells where reproduction had been mediated until then by catalytic RNA molecules. Although it is not known how protein synthesis appeared, I believe that the basic selective pressure for the origin and stabilization of the translation apparatus was the enhancement of the catalytic activities of the first RNA-cells, in order to guarantee their own stability and survival. RNA may be unique among biomolecules because of its double ability to store and replicate genetic information and to perform catalytic activities, but the latter were probably not very efficient – otherwise, biochemists would have discovered them a long time ago. Moreover, self – maintenance and reproduction of a RNA-cells in which polyribonucleotides were acting as their own replicases were probably hindered by constant hydrolytic cleavage of the RNA phosphodiester backbone.

It is possible that tRNA-mediated polypeptide synthesis may have initially been selected for inside RNA-cells because of the structural stability gained by replicative and catalytic RNA molecules to which these polypeptides associated (White 1982; Lewin 1985). However, the enormous catalytic potential of proteins as compared to RNA molecules and coenzymes must have led rapidly to the preferential survival and reproduction of a new type of cell that had an RNA genome and a translation apparatus capable of synthesizing catalytic polypeptides from amino acids of abiotic origin available directly from the early Archean aqueous environment. Heritability had appeared with RNA-mediated polyribonucleotide replication, but it was enhanced by the evolutionary development of more efficient biological catalysts.

How the first translating system actually

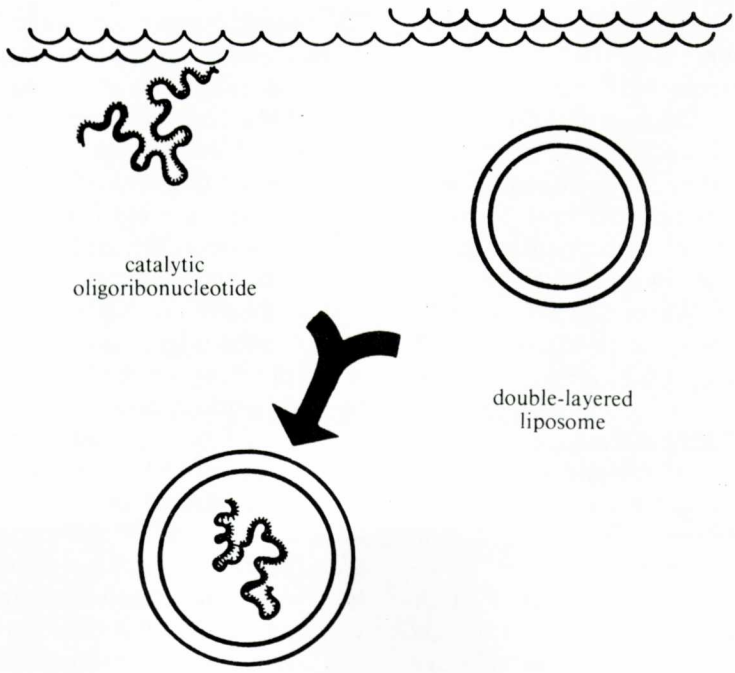

Figure 3

emerged is one of the major problems in the study of the origin of life. Undoubtedly amino acids were among the most abundant products of non-enzymatic chemical synthesis in the prebiotic environment (Miller 1953, 1984; Miller and Orgel 1974), and their coexistence with both non-catalytic and catalytic oligonucleotides must have led to many types of interactions among them, both inside and outside RNA-cells. The monophyletic origin of contemporary tRNAs from what has been called a proto-tRNA is supported by the overwhelming similarities in both size and structure among all contemporary tRNAs (Dayhoff 1969).

The earliest tRNAs may have initially been selected for not because of their present adaptor role, but rather because they were acting as cofactors (Sirlin 1972) in RNA-mediated RNA replication taking place inside the earliest cells. This hypothesis is supported by observations showing that uncharged tRNAs are required as cofactors for the initiation of the reverse-transcriptase catalyzed synthesis of DNA (Aboud and Pastan 1975; Panet *et al.* 1975). If a tRNA-like molecule appeared prior to protein synthesis, then the gene coding for it must have been initially transcribed in a polyribonucleotide-replication-like fashion by catalytic RNA molecules.

Transfer RNA molecules can be considered as rather large coenzymes that participate in the transfer of aminoacyl groups (White 1976, 1982). Whether or not one agrees with this view, Crick's (1976) well-known remark that «...tRNA looks like Nature's attempt to make RNA do the job of a protein» acquires a renewed significance due to the discovery of the catalytic activities of RNA molecules. An enormous number of theoretical models have been developed to explain the origin of the genetic code and of the translation apparatus, but unfortunaly with few significant results. This is also true for the question of

the origin of ribosomes (Crothers 1982), although ribosome-free tRNA-mediated polypeptide systems have been proposed (see, for instance, Crick *et al.* 1976). If the earliest tRNAs auto-catalyzed their covalent attachment to amino acids, with specificity that perhaps resulted from some similar physico-chemical properties of the amino acid side chain and its contemporary anticodon (cf. Weber and Lacey 1978), then it may be possible to discover RNA molecules capable of binding to amino acids in the absence of proteins.

Clues to the early interactions between prebiotic catalytic RNA and other molecules may be found among contemporary biochemical processes. In the precursor-tRNA cleavage effect in *E. coli* and *B. subtilis*, the protein subunit of RNase P modifies the structural characteristics of the RNA moiety, i.e., *in vivo* the protein acts as a cofactor (Altman 1984). The higher rate of this cleavage reaction *in vivo* is due to the stabilizing effect of the protein over the RNA moiety (Guerrier-Takada *et al.* 1983; Kruger *et al.* 1982). This conclusion extrapolated to the time of the origin of life allows the assumption that abiotically synthesized oligo- or polypeptides, even if they lacked catalytic activity, enhanced the catalytic properties of primordial RNAs simply by stabilizing their structures. Other molecules, like polyamines (Wong, personal comm.) could have acted in a similar way. The observation that the secondary and tertiary structures of tRNAs can be stabilized by spermidine (Tropp and Redfield 1983) supports this hypothesis. This organic polycationic molecule also stabilizes the conformation of the anticodon loop of yeast $tRNA^{trp}$ (Nöthig-Laslo *et al.* 1981).

Polyribonucleotides and polypeptides have been mixed in attemps to study precellular evolution. These models include, among others, coacervates with polylysine and RNA or poly (A) (Oparin *et al.* 1964); *«proteinoid complexes»* made from polyribonucleotides and basic proteinoid rich either in arginine or lysine (Yuki and Fox 1964); and the proposal of liposomes that would include catalytic oligopeptides and other components (Oró 1980; Oró and Lazcano-Araujo 1981, 1984).

These models are useful laboratory analogues for the study of: 1) the interaction that may have taken place between abiotically formed oligoribonucleotides (whether self-replicating or not) and polypeptides (with or without catalytic activity) of similar origin, *before* the establishment of the genetic code; and 2) the interactions between the earliest genomes and the proteins they coded, i.e., polypeptides formed *after* the evolutionary emergence of an early translation molecular apparatus. However, I believe that these studies are not relevant to the appearance of nucleotide-triplet-coded protein synthesis. As discussed above, from its very beginning the translation apparatus must have required some unknown type of oligoribonucleotide ancestral to contemporary tRNAs.

Indeed, the mere coexistence – whether within liposomes or not – of polypeptides and polynucleotides outlined above does not guarantee that protein synthesis will develop. Even if an extremely rich supply of abiotic polypeptides with the catalytic or structural characteristics necessary for the maintenance and reproduction of systems based on polyribonucleotide replication were available, in the absence of translation sooner or later these polypeptides would be exhausted. Regardless of how many catalytic polypeptides were formed abiotically in the early Earth, protein synthesis would not have evolved without a replicating mechanism insuring the maintenance, stability and diversification of its basic components, i.e., the formation of peptide-bonds via charged oligoribonucleotides ancestral to contemporary tRNAs that could translate the cryptic

information coded in the base-sequence of RNA molecules that had been until then only templates for non-enzymatic polynucleotide replication.

The hypothesis that both the metabolism and the reproduction of the earliest cells were based entirely on catalytic and replicative polyribonucleotides encapsulated inside liposomes in which translation was later selected for, is largely an updating of Oparin's (1936 *et seq*) concept of precellular evolution. The critical step in the origin of life is thought to have been the encapsulation of self-reproducing polymers within the boundaries of a semipermeable membrane (Haldane 1954). The abiotically produced polyribonucleotides with catalytic and auto-catalytic activities discussed above are simply Haldane's polymers in detail. *Figure 3* is merely a schematic representation of a more complex process in which many other types of organic and inorganic chemical species of abiotic origin participated. Prebiotic encapsulation (Deamer and Oró 1980; Deamer and Barchfeld 1982) probably did not produce uni- or multilamellar liposomes with «pure» internal microenviroments. Aqueous solutions of poly (U) and poly (C) to which amino acids, Zn^{++}, and condensing agents like urea and cyanamide are added can be easily encapsulated within dipalmitoyl-phosphatidyl-choline double-layered liposomes (Oró *et al.* 1986; Lazcano *et al.* 1986). Enhanced encapsulation due to synergistic effects in even more complex mixtures may occur.

A large variety of liposomes and related structures, with membrane composition and internal milieu reflecting the local environments must have formed prior to the appearance of life. All lipidic vesicles formed at the same time and place would have not been identical, as shown by Deamer's experiments using dehydration/hydration cycles, where liposomes produced under similar conditions contained differing amounts of DNA (cf. Trachtman 1984). A considerable diversity must have existed amid the precellular systems that predated the first RNA-cells. Experimental efforts to achieve autopoiesy in the laboratory ought not to expect the non-enzymatic synthesis of systems exhibiting transcription or translation, but should focus instead on the achievement of liposome-bounded systems containing catalytic and auto-catalytic RNA molecules capable of self-maintenance in the presence of inorganic ions, and organic compounds like amino acids already known to be available under putative prebiotic conditions (Lazcano *et al.* in prep.).

THE OLDEST ENZYME

If RNA preceded DNA as the reservoir of cellular genetic information, then RNA polymerase must be one of the oldest proteins. The hypothesis that RNA polymerase was initially RNA-dependent, i.e., was a RNA replicase, is supported by the observation that DNA-dependent RNA polymerases can use polyribonucleotides and RNA molecules as templates (see, for instance, Adman and Grossman 1965; Ono *et al.* 1985; Chamberlin 1976). Contemporary replicase activity in eubacterial RNA polymerases is interpreted as vestigial from before the appearance of double-stranded DNA. The transition from a RNA-dependent to the present DNA-dependent RNA polymerases probably required only minor changes in the enzyme. This is supported by experiments in which *E. coli* DNA-dependent RNA polymerase can accept an RNA template to carry out the enzymatic synthesis if Mg^{++} is replaced by Mn^{++} (Biebricher and Orgel 1973).

The exhaustive comparison between eukaryotic, eubacterial and archaebacterial DNA-dependent RNA polymerases performed by Zillig *et al.* (1985 a), indicates

the existence of significant differences in the number and molecular weights of their subunits. However, I believe that vestiges of the original RNA polymerase can be identified from this study. After immunoblotting techniques were used to study the homologies between the individual subunits of all the RNA polymerases, Zillig *et al.* (1985 a, b) came to the conclusion that «...the second largest component (component A) of RNA polymerase from the thermoacidophylic bacteria archaebacteria is homologous to the largest component of the enzyme from halophylic and methanogenic bacteria, to the largest component of the three eukaryotic RNA polymerases, and to the subunit β' of the eubacterial enzyme» (cf. Gropp *et al.* 1985).

I interpret this result to mean that the common polypeptide, i.e., the eubacterial β' subunit and its homologues, is the oldest component of contemporary RNA polymerases and thus one of the oldest nucleotide-triplet coded proteins. The essentiality of zinc for enzyme activity has been shown for only a few DNA and RNA polymerases. However, the presence of tightly bound Zn^{++} in all DNA and RNA polymerases that have been studied suggests that this metal plays an essential role in all polymerases (Mildvan and Loeb 1979). I suspect that this protein with a RNA substrate contained Zn^{++} at its active site from the beginning. The two Zn^{++} atoms required for catalytic activity are bound only to the β' subunit in *B. subtilis* (Halling *et al.* 1977), and in the *E. coli* β' subunit at least one ZN^{++} atom is found (Wu *et al.* 1977; Speckhard *et al.* 1977; Miller *et al.* 1979). Although it is not known which subunits of the eukaryotic RNA polymerases contain zinc, at least one atom of this element is probably present in the eukaryotic RNA polymerase components that share antigenic determinants with the eubacterial β' subunit.

Genetic studies using mutated strains of *E. coli* showing that both the β and the β' subunits are in the same operon at 89.5 min (Chamberlin 1982) can be interpreted as the result of an early tandem duplication of the gene coding for the β' subunit. The wide distribution among RNA polymerases of different cellular lineages of subunits which are immunologically related to the eubacterial sigma subunit (Zillig *et al.* 1985 a) suggests that this component of the enzyme, which is an initiation factor involved in promoter selection, was selected for after the appearance of the β' subunit. These conclusions are in agreement with previous suggestions that the early proteins must have been small polypeptides with less specificity and accuracy than their contemporary equivalents (Stackebrandt and Woese 1981).

Although reconstitution experiments have shown that all the components of the eubacterial core RNA polymerase are essential for recovering enzymatic activity (Chamberlin 1976, 1982), it can be predicted that under modified laboratory conditions both the eubacterial β' subunit and its homologues from the other enzymes will exhibit at least some minor catalytic activity as replicases in template-directed ribonucleotide polymerization reactions.

It is interesting to note that the β' subunit, which is believed to be involved in DNA binding, is a basic polypeptide rich in arginine, lysine and histidine. However, there is still no satisfactory non-enzymatic synthesis of these three basic amino acids (Miller and Orgel 1974). The molecular weight of the β' components has been determined in various eubacterial RNA polymerases. Their sizes range from 155 Kd in *E. coli* to 86 Kd in *Mycobacterium tuberculosis* (Zillig and Stetter 1980). The latter figure is comparable to the 98 Kd size of the phage T7 DNA-dependent RNA polymerase (Chamberlin and Ryan 1982). β' subunits would provide excellent laboratory models for studying the cataly-

tic activity, amino acid composition, and minimal size of the ancestral RNA polymerase.

THE APPEARANCE OF DNA IN RNA-CELLS

No systematic attempt has been made to explain the evolutionary transition from RNA as the first genomic material to the use of double-stranded DNA molecules as cellular informational polynucleotides, with the exception of Ferris and Usher (1983). The «RNA-first hypothesis» can be further developed by understanding the nature of the selective pressures that led to the biosynthesis of deoxyribose, thymine, and proof-reading DNA polymerases. These additional points are all related to the idea that DNA was selected for in cells to stabilize earlier RNA replicating systems. These arguments are: 1) the 2'-deoxy-containing phosphodiester backbone is more stable in aqueous conditions than its ribo-equivalent; 2) absence of proof-reading activity in RNA polymerases leads to a higher rate of mutations in RNA genomes relative to DNA; 3) information in RNA degrades because of the tendency of cytosine to deaminate to uracil, and the lack of a correcting enzyme; 4) UV-irradiation produces a larger number of photochemical changes in RNA molecules than in double-stranded DNA. Some of these points have been discussed elsewhere, and are further developed below:

1) An important stability difference between DNA and RNA molecules lies in the fact that the 2'-deoxy-containing phosphodiester backbone of the former is much more resistant to basic hydrolysis than its ribo-equivalent (Ferris and Usher 1983). Since the hydrolysis of RNA proceeds by the formation of intermediate cyclic-2', 3' phosphonucleosides, the phosphodiester bonds of DNA, which lack the 2'-OH group of ribose required to form the intermediates, are more stable under basic conditions.

The addition of DNA as an informational polymer must have required the development of the reductive elimination of the 2'-hydroxyl group in ribonucleotides to yield 2'-deoxy-derivatives. As indicated by the universal distribution of DNA in all contemporary forms of life, this must be one of the oldest metabolic pathways. Detailed discussion on the evolution of ribonucleotide reductase activity and the appearance of corrinoids have been discussed elsewhere (Dickman 1977; Follman 1982, 1983; Lammers and Follmann 1983).

2) Almost all DNA polymerases from phages and bacteria are known to exhibit at least some 3'-5' exonuclease activity that allows them to edit the newly synthesized DNA by excising mismatched bases and recopying the information when a mistake is detected (Loeb and Kunkel 1982). Methylation of DNA provides a mechanism by which the newly formed strand can be distinguished from the parental one (Fersht 1983). The only known system lacking this proof-reading activity is DNA polymerase II from *B. subtilis* and from some mycoplasms (Mills *et al.* 1977). Since these obligate parasites probably evolved from more complex eubacteria (Woese *et al.* 1980; Maniloff 1983), the lack of editing activity in some of their DNA polymerases is a secondary loss due to the tendency of mycoplasms toward smaller genomes. No RNA polymerase known possesses 3'-5' exonuclease activity, and there is no evidence of error-suppressing proof-reading mechanisms in them (Reanney 1982; Fersht 1983). This implies, of course, that RNA replication is intrinsically a «noisier» process than DNA replication. The error rate of viral RNA replicases (Eigen and Schuster 1977; Eigen *et al.* 1981) and bacterial RNA polymerases (Springgate and Loeb 1975) have been

demonstrated to be 10^{-3} to 10^{-4} per base transcribed. Wild-type *E. coli* DNA polymerase I in contrast has an error rate of 10^{-6} to 10^{-7} (Eigen and Schuster 1977). Although DNA polymerases appear to have intrinsic fidelity differences, the overall accuracy achieved by prokaryotes when copying their DNA seems to lie between 10^{-7} to 10^{-11} mutations per base transcribed (Loeb and Kunkel 1982).

Unfortunately nothing is known about the origin of the editing activity of DNA polymerases; however, it is obvious that cells capable of using double-stranded DNA replication with proof-reading DNA polymerases would be rapidly selected for. The lack of 3'-5' exonuclease activity in all RNA polymerases implies that RNA genomes can not achieve very large sizes without risking their genetic identity, since the number of misplaced sequences will be proportional to the length of the produced RNA (Reanney 1982). This effect can be observed in the high number of mutations measured in RNA viruses (Holland *et al.* 1982; Reanney 1982). The segmented genomes found among certain RNA viruses may represent an evolutionary strategy that overcomes the noisiness of RNA polymerases (Reanney 1982). The extrapolation of these arguments to the time of the appearance of life suggests the existence of a selective pressure favouring DNA as the repository of genetic information, since the RNA character of the earliest genomes must have strongly limited not only the fidelity of hereditary transmission of genetic information, but also the increase of genomic content.

3) Miller and Orgel (1974) stated that « *...double stranded DNA is more stable to depurination by at least a factor of 10, and this may have been important for the accumulation of DNA.*» Hydrolytic reactions (deamination, depurination and depyrimidination) are one to two orders of magnitude higher in single-stranded DNA prepared from rat liver cells, than in double-stranded DNA from the same source (*Table 3*). Equivalent measurements for RNA molecules have not been made; assuming early RNA genomes had single-stranded regions, it can be assumed that their hydrolytic lability was most likely at least comparable to that of their 2'-deoxy equivalents.

TABLE 3
Rates of hydrolytic reactions in DNA*

Reaction	Events/day/10^{10} bases	
	Double-stranded	Single-stranded
Depurination	$\sim 1.0 \times 10^4$	$\sim 4.0 \times 10^4$
Depyrimidination	$\sim 5.0 \times 10^2$	$\sim 2.0 \times 10^3$
Deamination	$\sim 1.7 \times 10^2$	$\sim 4.3 \times 10^4$

* Adapted from Singer and Kusmierek (1982).

Deamination of cytosine into uracil under physiological conditions is considerably faster than the other deamination reactions suffered by purinic and pyrimidinic bases (Singer and Kusmierek 1982), and therefore information in RNA tends to degrade. Both the replacement of uracil by thymine in DNA molecules, and the correcting enzyme uracil-DNA-glycosylase (Kornberg 1980), which removes uracil from DNA, greatly enhance the stability of information storage of DNA relative to RNA.

The gradual replacement of cytosine by uracil in G-C pairs of double-stranded DNA tends to decrease the duplex stability, but since the resulting G-U pair is relatively undistorted in conformation, the change in the geometry of the molecule does not induce repair (Kornberg 1980). However, if uracil is left unattended it will induce point mutations in the subsequent replication round (Hayawaka and Sekiguchi 1978). This substitution is the most simple known mutational mechanism (Singer and Kusmierek 1982). While the uracil in an A-U pair is normally the result

of its erroneous incorporation into the DNA duplex, the uracil residue in a G-U pair is usually derived from the deamination of the original cytosine (Kornberg 1980).

Uracil in DNA is not lethal. Bacterial mutants are known to persist through several generations with levels of uracil in their DNA as high as one per 100 thymines (Tye *et al.* 1978 a), and an *E. coli* strain is known that remains functional with as much as 30 % of its thymine being replaced by uracil (Warner and Duncan 1978). Nevertheless, there are at least two biological mechanisms which have evolved in order to prevent the permanent incorporation of uracil into DNA.

The first of these mechanisms depends on the presence of dUTPase, a hydrolytic enzyme converting dUTP to dUMP. This enzyme maintains low levels of the endogenous dUTP pool and provides, at the same time, the dUMP substrate for thymidylate synthetase (Kornberg 1980). The key role of dUTPase in the biosynthesis of dTTP has led to the suggestion that this enzyme may have originated to prevent the misincorporation of uracil into DNA (Bertani *et al.* 1963).

The second mechanism depends on the presence of a system that detects uracil incorporated in the place of thymine or derived from the hydrolysis of cytosine, and removes it by the action of an enzyme that catalyzes the hydrolytic cleavage of the uracil-sugar bonds. This enzyme, uracil-DNA-glycosylase, is known to be present in considerable quantities in both prokaryotic and eukaryotic cells (Kornberg 1980; Lindhal 1982). The products of these reactions are free uracil and a DNA chain with a missing pyrimidine--which may be quickly replaced by endonucleolytic incision followed by nucleotide excision-replication by DNA polymerase I (Kornberg 1980).

Since deamination of cytosine to uracil is a common phenomenon both *in vivo* and *in vitro* (Olivera 1978; Shlomai and Kornberg 1978; Tye *et al.* 1978 b), the ubiquity of uracil-DNA-glycosylase can be understood as an evolutionary adaptation that helps to ensure the stability of the genetic message (Kornberg 1980). This enzyme is a highly conserved protein (Lindhal 1982), which must have appeared *after* the development of the thymine biosynthetic pathway, i.e., after the appearance of dUTPase. An equivalent system could never have developed among cells using RNA as informational polymers; since uracil is a natural constituent of RNA, a hypothetical uracil-RNA-glycosylase system would be unable to distinguish between the uracil molecules originally present in RNA and those that resulted from the deamination of cytosine residues.

4) Although there is considerable debate regarding the detailed conditions at the surface of the early Archean Earth, it is generally agreed that because of the lack of substantial amounts of free atmospheric oxygen and the consequent absence of an ozone shield (Lazcano *et al.* 1983; Schopf 1983), the flux of solar UV radiation must have been higher than the contemporary value. Moreover, satellite observations of young T-Tauri stars theoretically resembling the early Sun (Cantó and Mendoza 1983) suggest that young solar-like stars can emit up to 10^4 times more ultraviolet light than the present solar value (Gaustad and Vogel 1982; Canuto *et al.* 1982, 1983). Since there is ample paleontological evidence showing that life appeared in the early Archean more than 3.5×10^9 years ago (Knoll and Barghoorn 1977; Knoll, this volume; Schopf 1983; Awramik *et al.* 1983), these high levels of unattenuated ultraviolet radiation must have led to the early development among prokaryotes of ultraviolet protection mechanisms (Margulis *et al.* 1976; Margulis 1982). This idea is supported by the presence of highly sophisticated enzyme-

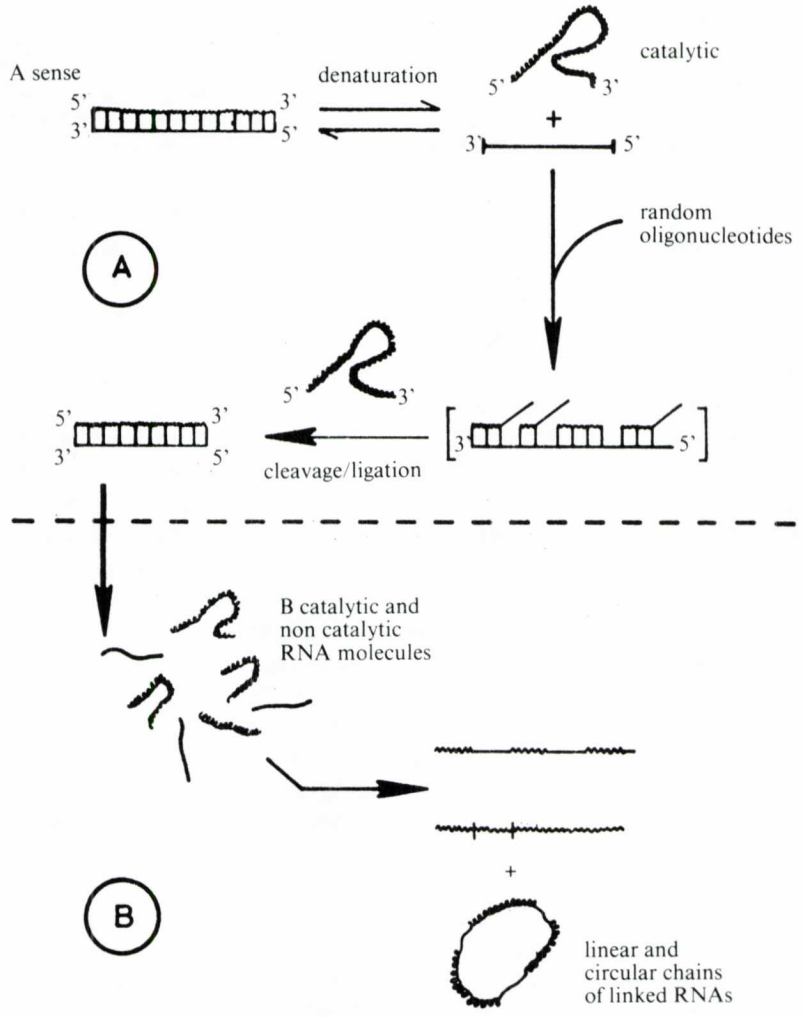

Figure 4

mediated DNA repair mechanisms, like photoreactivation, even among obligately anaerobic visible-light-insensitive bacteria like *Clostridium sporogenes* (Rambler and Margulis 1980; S. Giovannoni, unpublished observations).

Ultraviolet light is known to cause a major number of photochemical changes in nucleic acids. Although the sugars and the phosphate group of both DNA and RNA make a rather insignificant contribution to the absorption spectra at wavelenghts greater than 200 nm (Cantor and Schimmel 1980), the purinic and pyrimidinic moieties show very strong absorption in both RNA and DNA (Shugar 1960). With the exception of uridine, which shows a near-UV absorption maximum at 260 nm rather than at the 268 nm region peak of thymidine, the absorption spectra of the usual deoxyribonucleotides are essentially the same as the corresponding ribo-compounds (Voet *et al.* 1963).

The most important photoproducts of the ultraviolet irradiation of nucleic acid molecules are a cyclobutane ring joining

two sets of 5,6 double bonds of adjacent pyrimidic residues, and the formation of pyrimidine hydrates (Friedberg 1985). UV-irradiation of the RNA phage R17 has revealed the major photochemical product to be uracil hydrate (Remsen and Cerutti 1972). Of other photochemical lesions, which include nucleic acids-protein links, breaks in the phosphodiester backbone, and the formation of cross-links in double-stranded DNA, the only photochemically reversible lesion is the pyrimidine dimer (Setlow 1968; Friedberg 1985).

The extent of the UV-induced photochemical changes in nucleic acids depends, among other things, on the conformation of the molecule. Single- and double-stranded DNA molecules prepared from phage Ø X 174 have very different ultraviolet sensitivities (Cerutti *et al.* 1965). UV-induced dimerization of thymine is significantly higher in the single-stranded DNA than in the native double-stranded DNA from *Enterococcus* (McLare and Shugar 1964). When polyribonucleotides are UV-irradiated, the ultraviolet induced hydration of pyrimidines is significantly suppressed in double-stranded but not single-stranded molecules (Setlow 1968). The formation of uridine dimers is five times greater in poly (U) than in poly (A): poly (U) copolymers. The hydration of uridylic acid in this poly (A): poly (U) duplex is ten times less than in poly (U) polymers (Pearson and Johns 1966).

The genomes of the first Archean cells are hypothesized to have been RNA molecules with small regions of short complementary base-pairing double strands that resulted from random matching processes, and with large portions of single-stranded regions exposed to photochemical lesions. The absence of ultraviolet attenuation during the early Archean imposed an intense selection pressure favouring duplex DNA molecules over other possibilities of storing genetic information (Lazcano *et al.*, in prep.). When UV-irradiated poly (U) was used as mRNA (Grossman 1963) and as template for *in vitro* replicating experiments (Adman and Grossman 1965), uracil hydrate tended to code as cytosine. Cytosine hydrate formed by the UV-irradiation of poly (C) codes as uracil (Wacker *et al* 1964; Ono *et al.* 1965; Adman and Grossman 1967). These results are equivalent to the changes in specificity of base-pairing due to keto-enol and amino-imino tautomeric shifts in uracil and cytosine, respectively. However, under physiological conditions the enol and imino forms are present in only about one in 10^{-4} to 10^{-5} (Saenger 1984), and the mistakes made during DNA replication due to their presence are rapidly corrected by proof-reading DNA polymerases. All the photochemical alterations of RNA molecules induced by UV-irradiation are of little significance in DNA-dependent polymerase systems (Setlow 1968). However, the first living systems must have been exposed to a very high rate of UV-induced mutations due to the ribonucleic character of their genomes. Moreover, since DNA-repair systems work by duplication of the genetic information contained in the complementary strands of duplex DNA (Kornberg 1980; Friedberg 1985), the addition of DNA to stabilize the earlier irreparable RNA systems led to the selection of mechanisms to correct the damage caused by ultraviolet light.

CONCLUSIONS

The first autopoietic systems, i.e., the earliest cells, were based on the catalytic and auto-catalytic activity of RNA molecules encapsulated within the boundaries of lipidic membranes of liposomes. RNA polymerase, ancestor to the contemporary eubacterial β' subunit, evolved inside these RNA-cells, was one of the first proteins.

From RNA-cells with polymerase the t-RNA mediated synthesis of proteins evolved, stabilizing the RNA-based reproduction. Because of RNA's UV sensitivity, inability to elongate and inability to be repaired, as compared to double-stranded DNA, RNA-cells were replaced by DNA-RNA cells from which all extant life then evolved. Pathways of deoxyribose formation and thymine biosynthesis give support to of the assertion that RNA-cells preceded extant DNA-RNA cells.

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