THE LIPID WORLD

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Abstract. The continuity of abiotically formed bilayer membranes with similar structures in contemporary cellular life, and the requirement for microenvironments in which large and small molecules could be compartmentalized, support the idea that amphiphilic boundary structures contributed to the emergence of life. As an extension of this notion, we propose here a 'Lipid World' scenario as an early evolutionary step in the emergence of cellular life on Earth. This concept combines the potential chemical activities of lipids and other amphiphiles, with their capacity to undergo spontaneous self-organization into supramolecular structures such as micelles and bilayers. In particular, the documented chemical rate enhancements within lipid assemblies suggest that energy-dependent synthetic reactions could lead to the growth and increased abundance of certain amphiphilic assemblies. We further propose that selective processes might act on such assemblies, as suggested by our computer simulations of mutual catalysis among amphiphiles. As demonstrated also by other researchers, such mutual catalysis within random molecular assemblies could have led to a primordial homeostatic system displaying rudimentary life-like properties. Taken together, these concepts provide a theoretical framework, and suggest experimental tests for a Lipid World model for the origin of life.

Keywords: compositional information, GARD, lipozyme, membrane mimetic chemistry, micellar catalysis, mutual catalysis, origin of life, prebiotic evolution, self-reproduction

1. Introduction: Did Life Processes Necessarily Begin with Biopolymers?

The molecular systems from which life emerged were likely subject to the same physical and chemical laws that guide self-assembly processes in contemporary living systems (Tanford, 1978). While primitive prebiotic systems were not endowed with the highly evolved information-coding and replication mechanism found in contemporary cellular life, it is imperative that some forms of information storage and transfer have been at work. Since the transition from microscopic chemical mechanisms to the macroscopically detectable emergent properties that characterize life remains unresolved, there is little knowledge of what such primitive information-prone mechanism must have been. This is despite the fact that the principles underlying present-day biochemistry and molecular biology are well understood.

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The first attempt to bridge this gap were Oparin's experiments with coacervate droplets (Oparin, 1957; Oparin *et al.*, 1976). Although these are considered an important historical step (Walde *et al.*, 1994) the attention of investigators today has shifted from colloid phenomena and protein chemistry to nucleic acids. Many researchers maintain that the first living cells had predecessors in an RNA world (Gilbert, 1986; Joyce *et al.*, 1987; Gesteland *et al.*, 1999). This scenario for life's origin is supported by striking experimental evidence that RNA can display catalytic activity (Cech, 1993), and under defined conditions can be made to evolve new catalytic activities through molecular Darwinian selection (Eigen and Schuster, 1982; Beaudry and Joyce, 1992; Wilson and Szostak, 1994). Still, this does not imply that early evolutionary processes followed the same path. Indeed, RNA itself seems unlikely to have emerged spontaneously in a primordial environment (Shapiro, 1984). Scenarios in which replication is a collective property of a loose molecular assembly could be as likely to reflect the earliest stages of biogenesis (Dyson, 1985; Farmer *et al.*, 1986; Morowitz, 1992; Kauffman, 1993; Segré *et al.*, 1998a; Dyson, 1999; Segré *et al.*, 2000a). The notion that lipids and other amphiphiles could serve as intermediates in prebiotic evolution has also been specifically elaborated (Ourisson and Nakatani, 1994; Norris and Raine, 1998; Luisi *et al.*, 1999). In particular, it has been suggested that lipid membranes may have a hereditary potential, as most membranes are generated from other membranes but not created de novo (Cavalier-Smith, 1995; Szathmáry, 1999). These alternative approaches have received less attention, probably because of the relative paucity of experimental support, and because of the lack of a scenario explicitly involving information storage and evolution therein.

Here we critically analyze some known physical and chemical properties of intermediate-size organic molecules capable of forming non-covalent assemblies. From this analysis, we propose an alternative view in which an original, high-probability 'Lipid World' later gave rise to a world populated by the complex, relatively improbable biopolymers that are ubiquitous in all life today. The advent of the RNA world concept was initiated by the realization that nucleic acids have catalytic capacities beyond their specific role as information carriers. We review here the evidence that catalysis is not restricted to proteins and RNA, and that lipids and other simple amphiphiles, normally considered to be chemically inert and to have primarily a structural role, have substantial catalytic capacities. We will also explore the possibility that non-covalent assemblies of amphiphilic molecules could be endowed with a capacity to store and propagate information, and to undergo selection and evolution.

2. Lipid-like Amphiphiles Are Highly Diverse

In living cells today, biological membranes have numerous roles, including compartmentalization, energy transduction (photosynthesis and oxidative respiration)

nutrient and ion transport, signal transduction, and enzyme-catalyzed metabolic reactions. The latter include the synthesis of the membrane lipids themselves to allow growth of the bilayers in different cellular compartments. Yet membrane lipids are still commonly regarded primarily as providing physical partitions, while the embedded proteins allow them to subserve diverse functions. We argue here that the active role of membranes is not a late stratagem of advanced cellular life, but instead has an evolutionary continuity with the earliest life forms, in which lipid-like molecules may have had a more active metabolic role.

A present-day eukaryotic cell incorporates three primary classes of lipid: phospholipids, sphingolipids and sterols. When these are further differentiated with respect to head groups, hydrocarbon chains and linking bonds, hundreds of different membrane lipids can be defined. The number grows even larger if we take into account the variant lipids of prokaryotic cells. A similar diversity of lipophilic organic compounds may be generated along other chemical pathways, such as terpenoids (Ourisson and Nakatani, 1994). Although not all of these molecular species are necessarily functionally distinct, it is significant that the combinatorics of molecular structures can easily generate a large diversity (Figure 1A). The diversity of primitive amphiphiles could be responsible for the rise of a multiplicity of functions in a lipid monolayer or bilayer, through a form of combinatorial chemistry, even in the absence of classical biopolymer combinatorics (Figure 1B) (cf. also (Lehn, 1995)). It should be pointed out that present day organisms carry in their circulation micellar particles with defined but highly heterogeneous compositions, in the form of lipoproteins (Esterbauer, 1995). These could serve as models for the proposed heterogeneous amphiphile assemblies discussed in this paper.

3. Amphiphiles Undergo Self-Assembly

Among the large number of molecular species expected to be found on prebiotic Earth, lipid-like molecules have a distinct property: an ability to undergo spontaneous aggregation to form droplets, micelles, bilayers and vesicles within an aqueous phase, through entropy-driven hydrophobic interactions (Tanford, 1978; Safran, 1994). While such property seems remote from complex biological functions such as self-replication or information coding, it may underlie some of the earliest processes that led to the emergence of biological complexity.

The concentration of organic compounds in the water bodies of prebiotic Earth has been estimated to be approximately 1 micromolar (Lasaga *et al.*, 1971), potentially too low for typical covalent chemical reactions. Yet, because of their spontaneous tendency to aggregate, some hydrophobic and amphiphilic molecules would constitute local phases of highly concentrated organic compounds within a dilute solution of organic species, and therefore could enhance concentration-dependent chemical interactions. It should be stressed again that this self-concentration advantage does not require complex evolution: it is a molecular property that depends

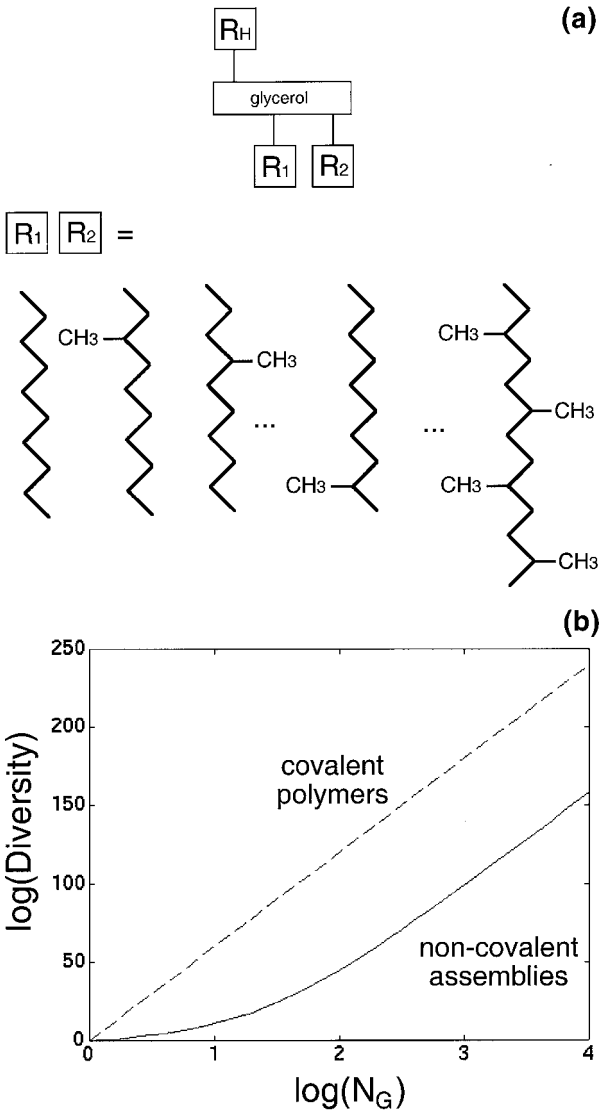

Figure 1. Diversity of lipids and of their assemblies. (a) Diversity at the level of single molecules, examplified by a glycerolipid. The two acyl chains (R_1 and R_2) can vary in terms of length, unsaturation and chain branching (as, for example, in prokaryotic lipids). The lipid head groups (R_H) are also composed of a variety of molecular species, so that literally thousands of different lipid species can be produced by combinations of the hydrophobic tails and hydrophilic head groups. (b) Diversity of non-covalent assemblies compositions. Given a number N_G of monomer species, the number of non-branched polymers of length N that could virtually form is $(N_G)^N$ (N=60, dashed line). A similar evaluation of combinatorial diversity can be performed for an assembly composed of N=60 amphiphiles chosen out of N_G possible types. The number of different compositions is $(N_G+N-1)!/(N!\ (N_G-1)!)$ (continuous line), a remarkably large quantity, although smaller than the correspondent polymers diversity.

solely on physical attributes of the organic molecules.

Vesicles are composed of thousands to millions of individual molecules, held together by weak non-covalent interactions driven by the hydrophobic forces. The combined energy of these interactions constitutes an appreciable free energy barrier that opposes the disruption of the entire assembly. Therefore, the lifetime of a given amphiphilic assembly may be long, of the order of hours, days or even months (Kaler *et al.*, 1989). Yet, individual molecular components can enter and leave the assembly with relative ease, in contrast to the situation of a covalent polymer, in which monomer exchange is subject to a very high energy of activation. It should be pointed out, however, that molecular exchanges within amphiphilic assemblies still have a finite free energy barrier, and may be subject to rate enhancement by catalytic effects.

The 'soft-matter' properties of lipid aggregates, which make them suitable for rapid material exchange, also render them quite different from crystals, which are much more rigid in structure, and have defined atomic and molecular coordinates. While bearing considerable similarity to inorganic clay minerals in term of a potential capacity to underlie the rudiments of life's origin (Cairns-Smith, 1982) organic lipid assemblies, similar to other phase-separated organic systems such as proteinoid microspheres (Fox, 1976), may be much more diverse and chemically labile, and they also do not come into conflict with the continuity principle (Fry, 1995; Lahav and Nir, 1997)

4. Lipid-Like Amphiphilic Molecules Predated the Origin of Life on the Earth

While it is reasonable to assume that the first cellular life forms used amphiphilic molecules for boundary membranes, as well as for other functions, the origin and diversity of amphiphiles on the early Earth remains to be elucidated. Theoretical and experimental estimates of prebiotic diversity of organic molecules are available (Chyba and Sagan, 1992; Morowitz, 1992; Schwartz, 1996), but these do not directly address the abundance and variety of prebiotic amphiphiles. One of the reasons is that many of the experiments on prebiotic organic synthesis concentrated on specific compounds occurring in living systems today, rather than relating to the entire spectrum of possible primordial molecules.

Several potential prebiotic reactions that could produce simple organic compounds on early Earth have been proposed (Miller and Urey, 1959; Schlesinger and Miller, 1983; Wächtershäuser, 1988; de Graaf *et al.*, 1995; Huber and Wächtershäuser, 1997). Energy sources available to drive such reactions range from volcanoes and hydrothermal vents to solar photochemistry and pyrite-dependent reduction. Some of these prebiotic syntheses have been shown to include the formation of lipid-like amphiphilic molecules, long-chain hydrocarbons and their derivatives (Hargreaves *et al.*, 1977; Leach *et al.*, 1978; Rao *et al.*, 1982). For example,

Fischer-Tropsch synthesis which produces organic compounds from simple gasses such as CO, H_2 and CO_2 (McCollom *et al.*, 1999) may lead to long chain fatty acids and fatty alcohols. It should also be stressed that in many cases, in addition to water-soluble organic compounds that are easily analyzed, insoluble residue (e.g. 'tar' or 'tholins' (McDonald *et al.*, 1996; Bernstein *et al.*, 1999)) are reported in prebiotic synthesis simulations (Miller, 1953; Schlesinger and Miller, 1983). These are likely to contain sparingly soluble hydrophobic and amphiphilic compounds.

A second possible source for prebiotic organic material is delivery to the early Earth by meteoritic and cometary infall. Anders (Anders, 1989) and Chyba and Sagan (Chyba and Sagan, 1992) concluded that interplanetary dust particles (IDP) were the most abundant source of extraterrestrial organic carbon in the late accretion phase of the early Earth. Comets would contribute smaller amounts, while carbonaceous meteorites would be a negligible source. The total amount of delivery over a period of 100 million years of the late Hadean – early Archean eras is estimated to be on the order of 10^{16} – 10^{18} kg (Chyba and Sagan, 1992). For comparison, the total organic carbon in the present biosphere is 6×10^{14} kg. It follows that organic material delivered as extraterrestrial infall was likely to have been a significant contribution to the organic inventory of the early Earth environment.

Because the parent bodies of carbonaceous meteorites accreted from the same molecular cloud as comets and IDP (Bernstein *et al.*, 1999) it is possible that similar organic compounds would be found in all three forms of extraterrestrial material. One can therefore use the organic components of carbonaceous meteorites as a guide to the organic inventory plausibly available for chemical evolution on the primitive Earth. Fischer-Tropsch syntheses have been proposed also as one of the sources of the hydrocarbons found in carbonaceous meteorites (Studier *et al.*, 1972).

Using the Murchison meteorite as a guide, the most abundant organic material delivered to the early Earth would be a complex aromatic hydrocarbon polymer ($\sim$90% of total organic content) followed by a variety of soluble organic acids, aliphatic and aromatic hydrocarbons, amino acids, ureas, ketones, alcohols, aldehydes and purines (Cronin *et al.*, 1988). Of these, both the longer chain monocarboxylic acids and certain polycyclic aromatic hydrocarbons (PAHs) derivatives have amphiphilic properties, i.e. solubility in organic solvents and a capacity to form aqueous micelles as well as monolayers at the air-water interface. This suggests the possibility that amphiphilic compounds present in carbonaceous meteorites could self-assemble into membranous vesicles under appropriate conditions, and in fact such structures have been experimentally demonstrated to be produced from extracted compounds (Deamer, 1985; Deamer and Pashley, 1989) (see (Deamer, 1997) for review).

Assuming that a significant amount of the organic substances in cometary and meteoritic infall survived atmospheric entry, most of the material would presumably enter the oceans and be released over a period of many years. One mechanism for the release of organic components from extraterrestrial infall is thermal ex-

traction (Mautner *et al.*, 1995): over half of the organic compounds present in a Murchison meteorite sample were shown be released by hydropyrolysis at elevated temperatures and pressures. For our purposes, we will assume that the primitive earth, at some point, contained a diverse array of organic chemicals with varying complexity, including hydrocarbon derivatives with lipid-like properties. While on a global scale, organic compounds concentrations were probably rather low (Stribling and Miller, 1987), their self-aggregation properties could lead to local high concentrations within the assemblies. Given that amphiphilic molecules were present, it is possible to discuss their catalytic and self-assembly processes, likely to have played a role in the emergence of primitive precursors of life.

5. Prebiotic Synthesis and Assembly of Membranes

One aspect of early cellular life that is often disregarded is that primordial membranes would need to continuously add amphiphilic components in order to accommodate the growth and replication of the encapsulated macromolecular system or of the lipid aggregate itself. To understand this process, it is useful to study the properties of abiotic amphiphilic structures. Bangham *et al.* (Bangham *et al.*, 1965) first reported that phospholipids have the capacity to self-assemble into vesicular structures now called liposomes, in which lipid bilayers act as permeability barriers to the free diffusion of polar and ionic solutes. Hargreaves *et al.* (Hargreaves *et al.*, 1977; Hargreaves and Deamer, 1978) extended these observations to the prebiotic environment, asking what minimal properties are required for amphiphiles to form membranes. It was found that a variety of single-chain amphiphiles can in fact self-assemble into bilayers under certain conditions. Examples of single chain amphiphiles include medium- and long-chain monocarboxylic acids (fatty acids), alcohols, amines, alkyl phosphates, and alkyl sulfates. The minimal chain length for the microscopic appearance of closed vesicles at physiological temperatures was 10 carbons. An appropriate balance between charge and hydrophobicity was also required. For instance, sodium dodecyl sulfate, a 12-carbon alkyl sulfate, is micellar, but equimolar additions of 1-dodecanol permitted the mixed system to form very robust membranes.

Hydrocarbon-based amphiphiles would be expected to concentrate at air-water interfaces. Because sunlight is a primary energy source in the contemporary biosphere, and presumably was equally abundant on the early Earth, it seems reasonable to ask whether photochemical synthesis of amphiphiles could occur under simulated prebiotic conditions. Klein and Pilpel (1973) first demonstrated that amphiphiles can be synthesized by a light-dependent reaction using PAHs as photosensitizers. This reaction can easily be demonstrated with mixtures of normal hydrocarbons and PAHs either in the form of aqueous dispersions or as films at aqueous interfaces (Deamer, 1992; Volkov *et al.*, 1995). In this work, hexadecane was used as a model aliphatic hydrocarbon, and pyrene, fluoranthene, and anthra-

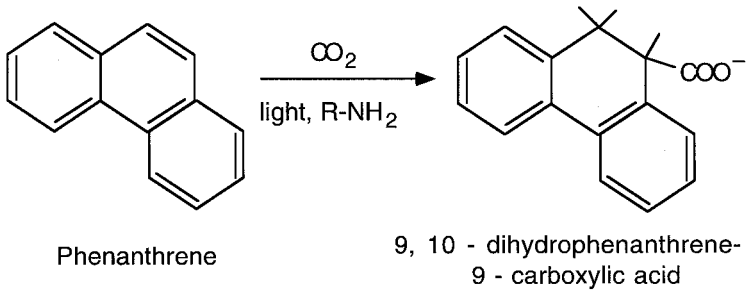

Figure 2. Photocarboxylation of phenanthrene, a model for primitive photosynthetic reactions involving PAH as pigments. Similar reactions occur with other PAH such as naphthalene, anthracene andpyrene (Tazuke and Ozawa, 1975). Carbon fixation reaction, where COOH derivatives of the PAH are synthesized.

cene derivatives were used as model polycyclic aromatic hydrocarbons. All of the latter are present in the Murchison meteorite as free hydrocarbons (Basile *et al.*, 1978; Cronin *et al.*, 1988). Upon illumination of such mixtures, a variety of surface-active compounds were rapidly produced, both in air and under anaerobic conditions. Analysis by gas chromatography – mass spectrometry (GC/MS) indicated that 1-hexadecanol and 2-hexadecanone were major products of the reactions, together with oxidized derivatives of the PAH. Significantly, if CO_2 was present in the absence of oxygen, a form of carbon fixation reaction can occur (Tazuke and Ozawa, 1975), in which carboxylic acid derivatives of the PAH are synthesized (Figure 2).

These results clearly show that UV illumination can drive oxidation of alkanes to more polar amphiphilic compounds, particularly long chain alcohols, which are surface-active. Pyrene, fluoranthene, and 2-ethyl anthracene, models for polycyclic aromatic hydrocarbons present in carbonaceous meteorites, are able to act as photosensitizers. The rate-limiting step does not depend on oxygen concentration, because carrying out the reaction under aerobic conditions did not markedly increase the rate or yield. It follows that polycyclic aromatic hydrocarbons derived from meteoritic sources could have served as primitive pigment systems to capture light energy and drive synthesis of amphiphilic compounds.

The reactions described above do not require a catalyst, but instead are driven by photochemical activation. In the Lipid World scenario, the lipid assemblies themselves could provide a weak catalytic activity to enhance the rates of specific synthesis reactions. One such generalized catalytic activity has been described by Luisi and co-workers (Blocher *et al.*, 1999; Luisi *et al.*, 1999). One caveat is that photochemical oxidation of hydrocarbons so far has been shown to produce only long-chain alcohols and ketones. Neither of these molecules by itself can form stable membranes, although both are able to contribute to membranes as a structural component, just as cholesterol does in contemporary cell membranes.

There are two challenges for future research in this area. The first is to find a plausible synthetic pathway for hydrocarbons with 10 or more carbons in their chains. Such chains must also have modifications, e.g. chain branching, that will allow them to be fluid at the permissive environmental temperature. Second, reactions must be established by which both polar and ionic character can be added to the hydrocarbon chains. This might include oxidation of the chains to long-chain carboxylic acids. Alternatively, chains oxidized to alcohols could conceivably esterify with phosphate to produce the necessary ionic characteristics.

For instance, Ourisson and Nakatani (Ourisson and Nakatani, 1994) suggested that isoprenoids such as isopentenol can condense to form acyclic polyprenol chains that might serve as hydrophobic moieties in primitive lipids, and showed that in fact dipolyprenol phosphates are able to self-assemble into bilayer membrane structures. They also note that terpenoid and hopanoid derivatives are commonly present as molecular fossils, clearly suggesting that microorganisms utilized such compounds. Another possible source of longer chain amphiphilic molecules is the Fischer-Tropsch type synthesis recently reported (McCollom *et al.*, 1999) in which fatty acids and fatty alcohols are produced. Other investigators have demonstrated that such compounds, either individually or as mixtures, are able to produce stable bilayer vesicles (Hargreaves and Deamer, 1978; Walde *et al.*, 1994). This is exemplified by the stable vesicles produced from a mixture of a carboxylic acid, an alcohol, and pyrene, a PAH derivative (Figure 3).

6. Lipozymes: Non-covalent Amphiphilic Aggregates with Catalytic Properties

Several authors have reported cases of chemical reactions whose rates can be enhanced by the presence of certain lipid micelles or vesicles (Fendler and Fendler, 1975; Cuccovia *et al.*, 1982; Kust and Rathman, 1995). For example, Cuccovia *et al.* have reported a 10^6-fold rate enhancement for ester thiolysis catalyzed by n-heptyl mercaptan in dimethyl di-(n-alkyl) ammonium chloride (Cuccovia *et al.*, 1982) (Figure 4). A particularly rich compendium of lipid-mediated rate enhancements has been published by Fendler (Fendler and Fendler, 1975) (Figure 6b). Although most reported cases of micellar catalysis involve hydrolytic reactions, rate enhancement of synthetic reactions has been demonstrated as well, for example in non biological surfactants synthesis (Kust and Rathman, 1995) and in liposome-catalyzed oligomerization of amino-acids (Luisi *et al.*, 1999). In a different realm of investigation, Luisi has extensively demonstrated the capacity of amphiphile assemblies to enhance the rate of hydrolytic reactions, in ways that lead to the autocatalytic growth of such assemblies (Bachmann *et al.*, 1992)

An analogous system is one in which enzyme-catalyzed synthesis of a phospholipid, allows self-assembly into vesicular membranes. Acyltransferase, a common enzyme in most biological membranes of eukaryotic cells, transfers acyl groups

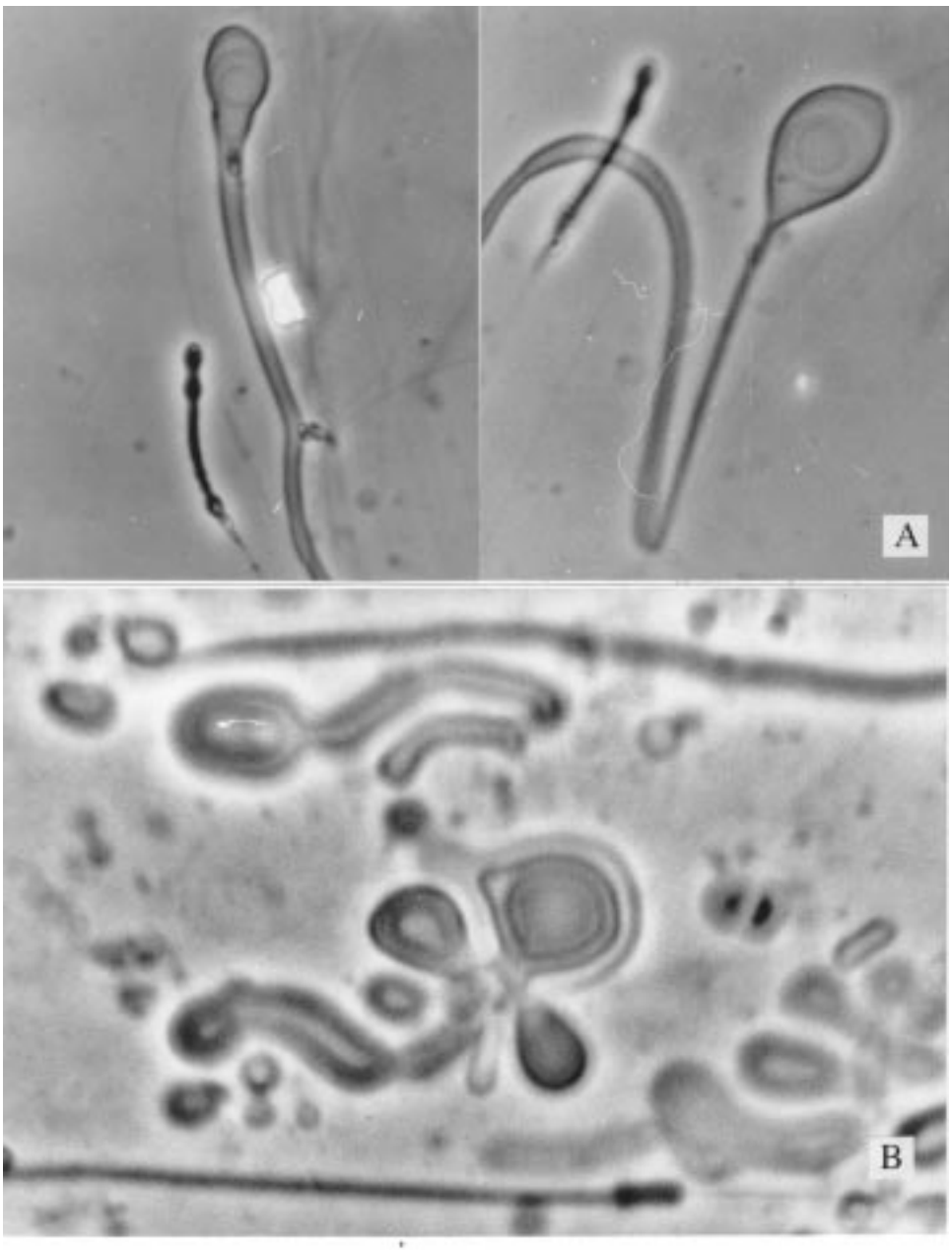

Figure 3. Bilayer vesicles produced by the mixture of amphiphilic compounds isolated from the Murchison carbonaceous meteorite (A) and by nonanoic acid, a component of the mixture (B) (see Deamer, 1997).

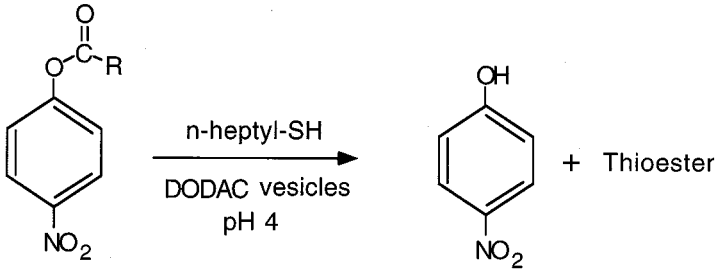

Figure 4. Thiolysis is markedly catalyzed by lipid vesicles. In the presence of DODAC vesicles (dioctadecyldimethylammonium chloride) thiolysis rates are increased by 10^6 fold (Cuccovia *et al.*, 1982).

from acyl-CoA derivatives to lysophospholipids. Since both acyl CoA and lysophosphatidylcholine form micellar dispersions rather than bilayers, the enzyme-catalyzed reaction reduces the concentration of the detergent-like substrates while simultaneously producing a double-chained amphiphile. Membranes appear when the micellar substrates fall below the critical micelle concentration of the mixed system, with the result that the bilayer-forming double-chain product predominates (Deamer and Boatman, 1980; Gavino and Deamer, 1983) (Figure 5). The lesson gleaned from this experiment is that vesicular membranes can assemble from non-membranous components in a relatively simple one-step enzyme-catalyzed reaction, which might, under some conditions, be mimicked by the amphiphilic structures themselves.

Membrane mimetic chemistry (Fendler, 1982) does not necessarily display the same features classically manifested in enzyme catalysis. Concentration of reactants in a dimensionally restricted environment has been held responsible for rate enhancement in some cases (Cuccovia *et al.*, 1982), while the active role of chemical groups bound to a membrane was considered essential in others (Dugas and Penney, 1981). In general, both aspects can combine to render lipid aggregates with an effective rate enhancement capacity. Similar kinetic effects can also be envisaged for non covalent reactions, e.g. for joining of a free molecule to a previously formed aggregate or for the transition between two different layers within it (Zachowsky *et al.*, 1989).

The above reactions may also not strictly conform to the standard attributes of enzymatic catalysis, such as the degree of turnover, the definition of the transition state and the stereospecificity of bond formation. Still, in a broader sense, they could be regarded as manifesting a form of lipid-mediated catalysis.

Can these observations have any implications to the study of the emergence of life? The earlier studies of living systems have led to the notion that biological catalysis is mainly attributed to protein enzymes. Subsequently, the discovery that RNA molecules can act as biological catalysts eliminated this constraint, and ribozymes (Been and Cech, 1988) are now seriously considered as candidate key components in a primordial ‘RNA world’ (Gesteland *et al.*, 1999). With further

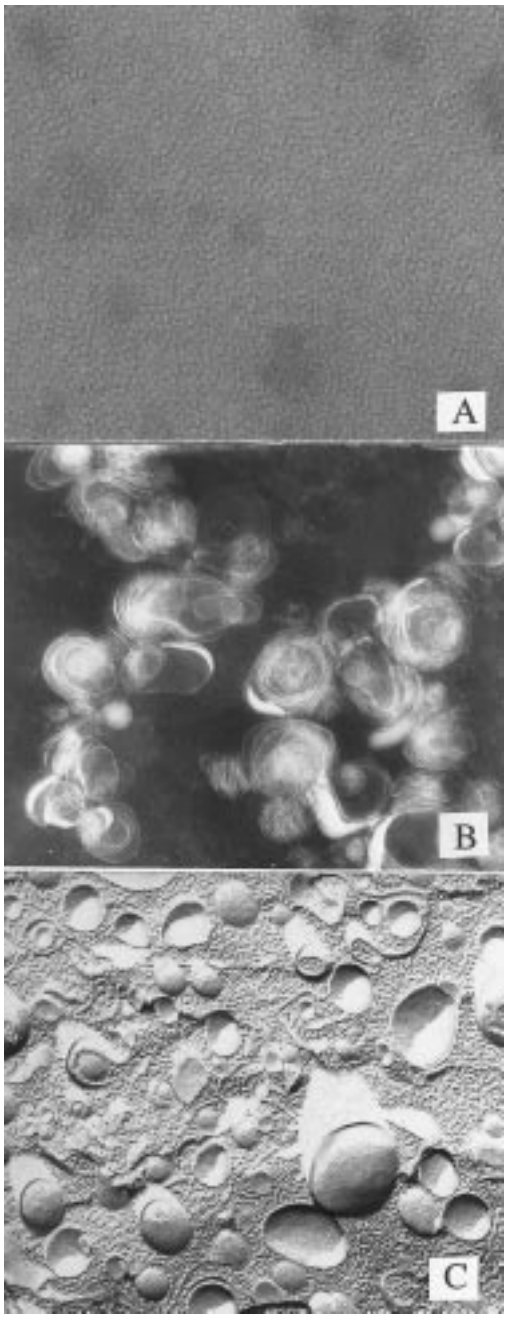

Figure 5. Bilayer synthesis from soluble reactants. Acyltransferase catalyzes the transfer of a fatty acid from acyl CoA derivatives to lysophosphatides, in this case, lysophosphaticylcholine. The resulting phosphatidylcholine self-assembles into lipid bilayers. (A) Negative stain of the original dispersion showing mixed micelles of the two detergent-like substrate molecules. (B) Negative stain of the mixture after 1 hour incubation, showing large numbers of membranous vesicles. (C) Freeze-fracture image of the lipid vesicles in B. Original magnification 40 000 X.

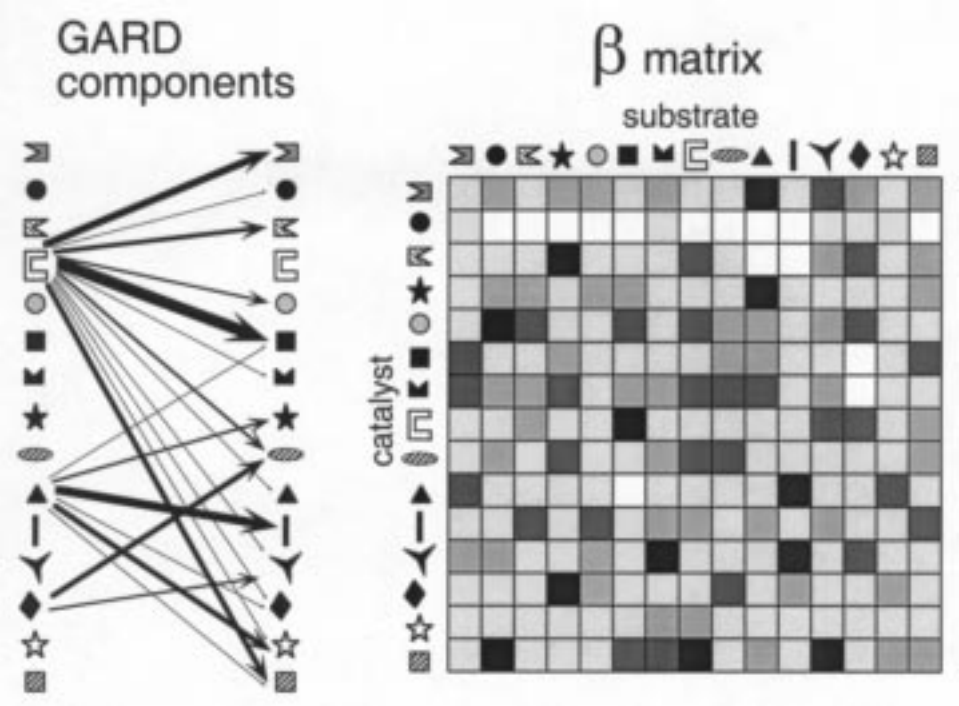

Figure 6a. The different molecules in a GARD system may have varying mutual catalytic enhancement values, shown by the thickness of arrows in the schematic depiction on left and by a gray level scale in the β matrix representation on the right. The propeties of the mutually catalytic network depend on the values of the β matrix components, derived from a statistical distribution as shown in *Figure 6b*.

extrapolation, it is possible to conceive amphiphilic assemblies as harboring potential prebiotic catalysis. Based on the above mentioned rate enhancement properties of amphiphiles as well as on the previously explored concepts of heterogeneous micelles as structures that bear tangible similarity to globular proteins (Lipowsky, 1995), we introduce here the term 'lipozymes' to indicate lipid aggregates that have a rate enhancement capacity for chemical reactions, either endogenous or exogenous.

7. Autocatalytic Lipozymes May Self-reproduce

The capacity of a system to transform part of its surrounding environment into its own similes is considered as one of the fundamental properties of living organisms. This may be the property of individual molecules, that can replicate autocatalytically (Ballester and Rebek, 1990; Orgel, 1992; Li and Nicolaou, 1994; Rebek, 1994; Lee *et al.*, 1996; Lifson, 1996). However, the phenomenon of reproduction is not necessarily confined to single molecules. Assemblies of molecules might

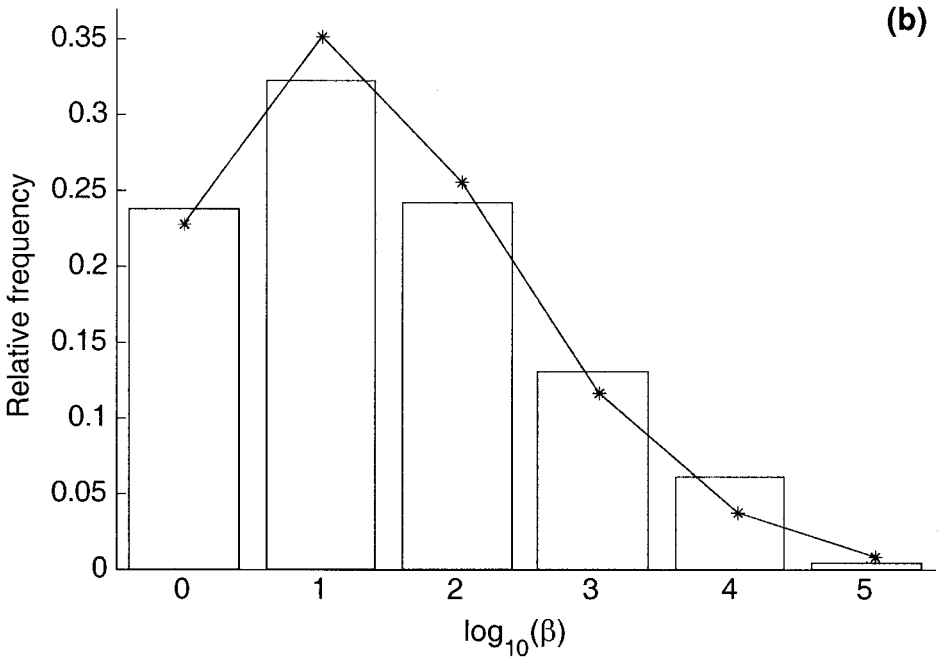

Figure 6b. Distribution of catalytic enhancement factors (β), from Fendler's list of lipid-catalyzed reactions (Fendler, 1982). The 260 values were taken from tables 12.1, 12.2, 12.3, 12.4, 12.5 of Fendler's book, and they correspond to ratios between the catalyzed and the uncatalyzed rates of different reactions (mostly hydrolytic) that take place in aquaeous micelles. The relative frequency of these rate enhancement factors (bars) is superimposed here to a theoretical estimate (line) based on the Receptor Affinity Distribution (RAD) model (Lancet *et al.*, 1993; Lancet *et al.*, 1994; Lancet *et al.*, 1994) for the statistics of receptor-ligand recognition. The RAD probability function is defined by three parameters: α, the free energy contribution per elementary subsite interaction; B, the number of subsites, and S, the number of differnt types of subsite configurations. In the curve plotted here the parameters used are: B=18, S=13, α/RT=1.

also produce new assemblies by a growth and division process, even if none of the molecular components is capable of reproduction (Oparin, 1957; Dyson, 1985; Morowitz, 1992; Dyson, 1999). Such collective self-replication of mutually catalytic species has been demonstrated experimentally (Sievers and Von-Kiedrowski, 1994; Lee *et al.*, 1997), and investigated theoretically (Farmer *et al.*, 1986; Kauffman, 1986; Bagley and Farmer, 1991; Jain and Krishna, 1998; Segré and Lancet, 1998; Segré *et al.*, 1998a; Segré *et al.*, 1998b).

Assemblies composed of a single type of amphiphile have been shown to undergo replication-like behavior (Bachmann *et al.*, 1992; Kust and Rathman, 1995), due to rate-enhancement effects between the lipid micelles and the precursors of their constituents. These assemblies may be considered as special cases of autocatalytic lipozymes. These and similar autopoietic systems (Luisi and Varela, 1989; Bachmann *et al.*, 1992) have been suggested to represent paradigms of early life,

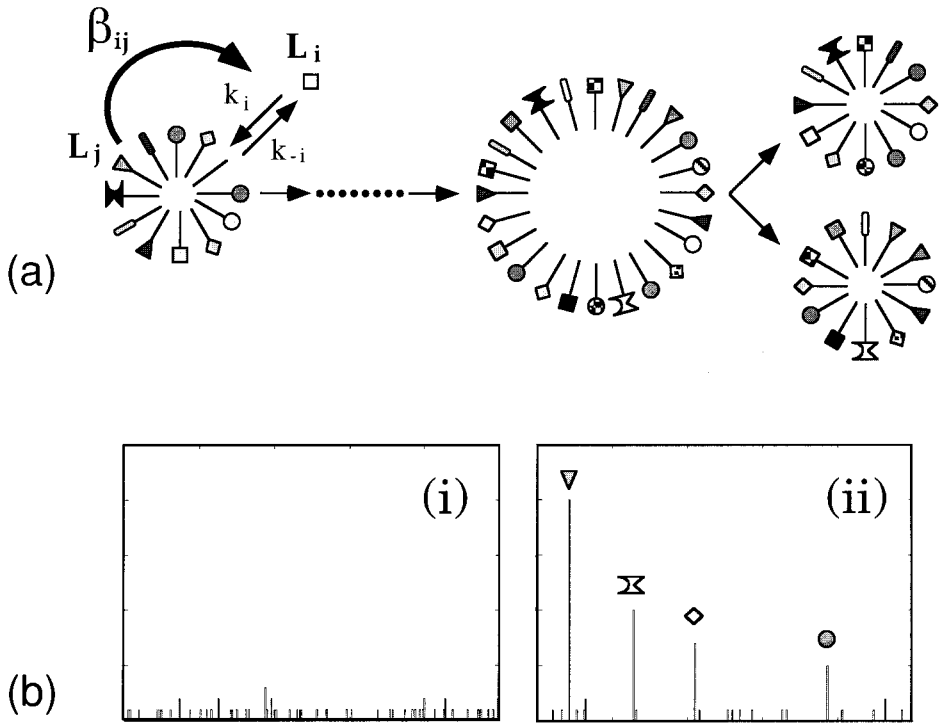

Figure 7a–b. A multicomponent mutually catalytic micelle (a) A schematic view of the proposed mechanism of amphiphile Graded Autocatalysis Replication Domain (A-GARD). Micelles are composed of amphiphiles with polar head groups (geometric shapes) and hydrophobic tails (sticks). A chemical reaction that generates an amphiphile L_i is catalyzed by the presence of an amphiphile L_j within the assembly. Numerous such reactions lead to the growth of the micelle. The rate of growth depends on the summated action of numerous catalytic events, governed by the matrix β, whose element β_{ij}, when multiplied by the basal rate constants k_i and k_{-i}, produces the catalyzed rates. Upon reaching a critical size, the micelle may split, giving rise to progeny. (b) A starting composition (i) has most compounds represented scantly and more or less equally. The gradual evolution towards the establishment of a mutually catalytic network in one of many possible micellar compositions may result in a highly biased composition (ii), whereby a few species are highly represented, and the rest are rather low in abundance.

i.e. enclosure and self-reproduction. But they have been argued not to embody some of the properties essential for initiating an evolutionary process, since they lack information carriers such as nucleic acids or peptides. Coupling of lipid enclosure with nucleic acids replication (Chakrabarti *et al.*, 1994; Luisi *et al.*, 1994) or with peptide oligomerization (Blocher *et al.*, 1999; Luisi *et al.*, 1999) has therefore been investigated as a more advanced alternative to the simplest autopoietic unit.

Here we delineate an alternative scenario, whereby amphiphiles, besides displaying self-assembly and self-reproduction, may also embody the diversity and

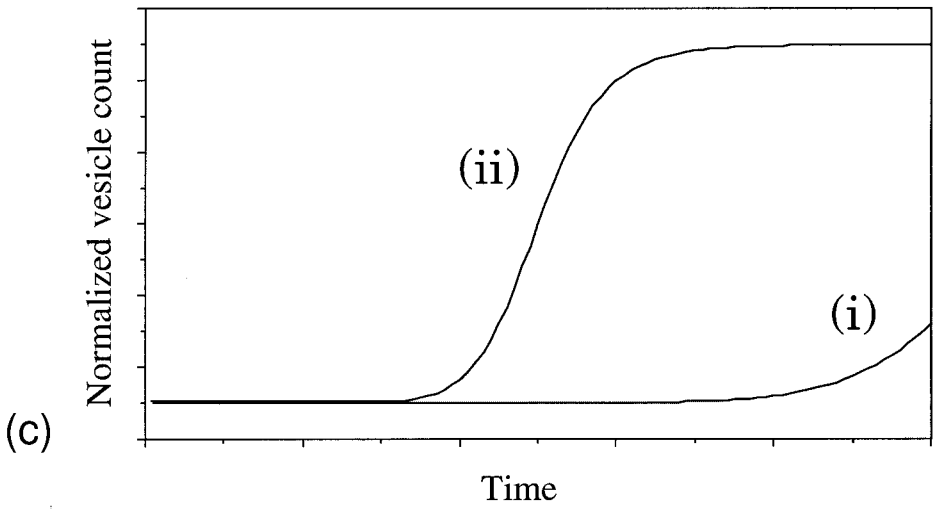

Figure 7c. Schematic depiction of a scenario merging the results of the chemical kinetics of the A-GARD model (Segré *et al.*, 1998a; Segré *et al.*, 2000b) with the experimental outcome for autocatalytic growth of amphiphilic micelles and vesicles (Bachmann *et al.*, 1992; Walde *et al.*, 1994). It is surmised that if a complex mixture of starting compounds would be used instead of one amphiphile-generating species, a kinetic behavior as schematically shown in curve (ii) (cf. (Bachmann *et al.*, 1992; Walde *et al.*, 1994)) would obtain. This is because a GARD mutually catalytic network has been demonstrated to behave in a similar manner to a single autocatalyst (Segré *et al.*, 1998a). Assemblies with compositions deviating considerably from the optimal mutual catalytic network, including uniform compositions of single species with low autocatalytic capacity, would display a kinetic behavior as shown in curve (i).

information content needed for the emergence of life. We propose that crucial steps in the origin of life might have been carried out by lipid-like molecules alone, potentially prior to the emergence of polynucleic acids and polypeptides. We suggest that heterogeneous autocatalytic lipozymes with defined internal compositions might have been gradually selected out of an initial highly complex repertoire of micelles and vesicles formed spontaneously by abiotic processes. This would entail information content as well as a capacity to undergo natural selection, as delineated below (Figure 7). In the classification proposed by Szathmáry (Szathmáry, 1999), autocatalytic lipozymes would belong to the class of phenotypic replicators, because of their functional rather than digital inheritance.

8. Statistical Properties of Lipozyme Catalysis: the Importance of Diversity

When the values for the catalytic enhancement factors (k_{cat}/k_{uncat}) for many dif-

ferent lipid-related reactions (Fendler, 1982) are plotted as a frequency histogram (Figure 6B), certain features are revealed which are often observed in the realm of random or combinatorial chemistry (Hassan *et al.*, 1996; Borman, 1997; Collins, 1997). Catalysis is in principle a gradative phenomenon, and assays measuring the catalytic potency of different compounds may display a broad spectrum of values. General properties of the resulting probability distribution may be inferred both from experimental data and from theoretical considerations. Along these lines, we have previously proposed and investigated a model for the distribution of binding affinities in multireceptor repertoires (Lancet *et al.*, 1993; Lancet *et al.*, 1994; Rosenwald, 1998). We have subsequently argued that similar statistical considerations can be extended to catalysis, and are a viable way for looking at prebiotic scenarios without questioning the details of each single chemical species (Lancet *et al.*, 1994; Segré *et al.*, 1998a; Segré and Lancet, 1999). Thus, we demonstrated that the catalytic efficiency values conform to a distributions as manifested in the elements β_{ij} of the β-matrix (Figure 6A). These may be used as a universal tool for describing and computationally simulating the kinetic behavior of large molecular repertoires, as exemplified by the Graded Autocatalysis Replication Domain (GARD) model (Segré *et al.*, 1998a). In this context, it is significant that the distribution of kinetic values in Figure 6B indeed generally conforms to the proposed statistical model.

In reality, compounds do not always exert mutual rate enhancements, and some may manifest inhibitory effects upon the recruitment and/or synthesis of others. In the model described herein, inhibition is not included, i.e. only positive β_{ij} values are used. Embodiments of the GARD model, in which kinetic inhibition is addressed, are currently being explored, and it is believed that this will not modify the central conclusions in a major way.

9. Mutually Catalytic Networks Within Complex Lipozymes

Mutually catalytic networks have been described as primordial metabolism-based entities which may have preceded the appearance of informational biopolymers (Dyson, 1982; Kauffman, 1986; Morowitz, 1999). It was further shown that, if a network of chemical reactions manifests ‘catalytic closure’, it will acquire properties akin to self-replication (Farmer *et al.*, 1986; Kauffman, 1986). We present here a case for lipid assemblies as realistic chemical embodiments of such mutually catalytic networks. Simple lipozymes which behave autocatalytically (Bachmann *et al.*, 1992; Walde *et al.*, 1994; Luisi *et al.*, 1999) provide an important demonstration of the principle: when an amphiphile is formed through an endogenously catalyzed reaction, vesicle growth becomes a consequence of the catalytic action. Such principle may be extended to a multi-component scenario. Instead of a single amphiphile, we consider a large number (N_G) of different components, for example precursors of vesicle-forming compounds. Chemical conversions could initially occur spontaneously, giving rise to mixed micellar structures. Subsequently, such

micelles might catalyze further reactions in a way that could be described by a mutually catalytic network formalism. It is conceivable that specific micellar compositions would display faster growth as compared to others. If the conditions are such that the system is kept far from equilibrium (for example by a continuous availability of fresh precursors, and the random elimination of some of the micelles), transients of specific compositions could be kept in a homeostatic fashion. The compositional bias that ensues would constitute a rudimentary form of transferable chemical information, as described herein.

It should be borne in mind that in some catalytic networks, certain chemical species may be detrimental to the system, as they catalyze side reactions that might disrupt the homeostatic characteristics of the network. It may however be envisaged that as chemical evolution proceeds, such elements will be selected against, and 'good' networks, even if initially rare, will prevail. Alternatively, inhibitory effects might serve as regulatory mechanisms through negative feedback loops in a network, and could therefore be incorporated fully in the dynamics of a homeostatic system. In future extensions of the current models these questions could be addressed quantitatively, and the effects of inhibition on mutually catalytic networks could be better understood.

Several embodiments of this scenario may be envisaged, in terms of the types of chemical reactions and the source of free energy. The latter could include photochemically driven reactions, that might help keeping the system away from equilibrium. It is important to stress the contrast between two scenarios: (a) an 'engineered' chemical systems, in which a specifically designed chemical architecture underlies the emergence of *molecular* self-replication, and (b) a naturally occurring mixture of chemicals, where random interactions within large repertoires of arbitrary organic molecules lead to the appearance of self-reproducing *assemblies*. Future experiments could be initiated with a mixture resembling a possible prebiotic amphiphile repertoire, and while keeping the system far from equilibrium, the composition of individual assemblies would be monitored. Such experiments are still very challenging and could be characterized by utterly slow rate constants of months and years. For this reason we have resorted to a mathematical modeling approach, based on computer simulations of the kinetics of self-assembly in complex molecular mixtures (Segré and Lancet, 1998; Segré and Lancet, 1999; Segré *et al.*, 2000b) (cf. also (Mayer and Rasmussen, 1998)).

The fluid nature of micellar structures may harbor important advantages in a prebiotic milieu. While for an evolved polymer structure, such as a folded protein, the emergence of a new function would require changes in covalent bonds, a lipozyme might acquire different functions as a consequence of facile joining of new amphiphiles, or rearrangements of its constituent molecules (Moss *et al.*, 1976; Sackmann and Feder, 1995). Therefore, the same lipozyme could serve as a polyfunctional endogenously catalytic system and entail the capacity of combinatorially giving rise to different rate enhancement effects. Consider for example a catalytic triad composed of the active head groups A, B and C diffusing as amphiphiles

within a micelle. While an encounter of all three groups to form the ABC triad would be much less likely than in a folded protein enzyme, it would still be much more probable compared to the situation in a dispersed solution. Also, diffusion encounters that form pairs such as AB or AC could still harbor some catalysis. This flexibility in the modulation of functions could allow spontaneous 'screening' and 'tuning' of different catalytic networks with no need for complex evolutionary processes such as sequence mutations within biopolymers, and a complex coding apparatus.

10. Compositional Information in Lipozymes

Legitimate concerns about the likelihood of a prebiotic Lipid World scenario could derive from the seeming discontinuity between catalytic lipid aggregates and present-day biopolymer-based cellular life. Biochemical information transfer is traditionally identified with sequences of the four letters alphabet of nucleic acid polymers. Could any information transfer system be envisaged for catalytic lipid aggregates? We have recently formally analyzed the idea that information may be transferred in the form of specific molecular compositions (Segré *et al.*, 1997; Segré *et al.*, 1998a; Segré and Lancet, 1999), an idea initially hinted upon in earlier studies (Oparin, 1957; Morowitz, 1967; Morowitz, 1999). We have now extended this concept of compositional information to amphiphile assemblies and have provided examples of how it may be utilized in computer simulations of prebiotic evolution (Segré and Lancet, 1998; Segré *et al.*, 2000b).

Imagine a population of idiosyncratic assemblies formed randomly out of a large number of available amphiphiles. If the typical count of molecules N within a typical assembly is considerably smaller than the number of molecular species types N_G, then practically every assembly will be different. Some of these assemblies may harbor superior lipozyme characteristics, e.g. a capacity to catalyze the recruitment or the synthesis of further amphiphiles. These lipozymes would tend to increase in size faster and eventually divide through physical forces (Koch, 1985; Sackmann and Feder, 1995), giving rise to 'daughter' assemblies. These might display varying degrees of similarity to the original composition, depending on the organization of the catalytic network and on the fate of each molecule upon splitting. The process described above implies the transfer of a compositional information from one assembly to its progeny. A capacity for high fidelity transfer of compositional information could be acquired gradually, after many growth and division cycles, leading to a process akin to self-reproduction (Segré and Lancet, 1998; Segré *et al.*, 2000b).

It is important to point out that when present-day cells divide, they too transmit considerable elements of compositional information (including specific gamuts of lipids, proteins and RNA) which are 'inherited' from the mother cell. Higher level structures may also be inherited epigenetically, as exemplified by the concept of

genetic membranes (Cavalier-Smith, 1995; Szathmáry, 1999). This is in addition, and in parallel to the classical self-replication of DNA sequence information.

11. Evolution of Autocatalytic Lipozymes

The number of compositionally different assemblies that can be formed from a set of possible amphiphiles can reach very high values (Figure 1B). In a primordial environment, where millions of different chemical species might have been present, a huge variety of random compositions should be expected, provided that the assemblies are smaller than a critical value (Segré *et al.*, 1998b; Segré and Lancet, 1999). In this respect, autocatalytic lipozymes might be viewed as replicators with unlimited hereditary potential (Szathmáry, 1999). The capacity of 'trying out' many different combinations, and of searching the fitness landscape for successfully propagating compositions, is probably a fundamental characteristic of a life-nurturing environment. The reduction of the many possible compositional configurations to few specific compositions has been considered as a crucial step in the emergence of life (Smith and Morowitz, 1982; Morowitz, 1992; Segré *et al.*, 2000b; Segré and Lancet, 1999).

One concern about a prebiotic Lipid World is how any additional progress could eventually be made in the absence of an alphabet-based coding system. In other words, can natural selection act on non-covalent structures that replicate with very low fidelity? An answer may be provided by our computer simulations of the amphiphile-GARD (A-GARD) model, in which several kinds of amphiphilic molecules join or leave preformed assemblies, with probabilities that depend on the assembly's composition. In these simulations, assemblies with different compositions arise, grow and divide. Under constant population conditions (Küppers, 1983), some specific compositions prevail, suggesting a form of selection and evolution. It should be pointed out that this happens without the introduction of an explicit fitness parameter (cf. (Lifson and Lifson, 1999)).

We have performed an analysis asking what might be the difference in behavior between a set of autocatalytic molecules and a true mutually catalytic network. We assigned the β matrix in two different ways, one in which the diagonal elements are artificially selected as dominant (a set of autocatalysts) and the other in which assignment was random. The simulations indicated that in the first case one species, the best autocatalyst, ended up dominating the system, as previously described (Eigen and Schuster, 1982; Küppers, 1983; Lifson and Lifson, 1999). On the other hand, when a 'natural' matrix is used, the result is a simulated amphiphile assembly that harbors a mutually catalytic network, and display multiple stationary states that provide a substrate for a more diverse selection and evolution process (Ben-Eli, 2000). It is likely that if in an experimental setup a variety of different amphiphile precursors are mixed, the second scenario will prevail, as it is rather unlikely,

by statistical considerations, that most amphiphiles will provide the highest rate enhancement to their own kind.

In a further elaboration (Segré, Ben-Eli *et al.*, 1999; Segré *et al.*, 2000b), it is predicted that within such mutually catalytic assemblies of monomeric amphiphiles, dimers and higher oligomers could gradually form. These could replace some of the monomers, assuming their catalytic roles in the network. We show that rearrangements of monomers within the oligomers would make the new assemblies more successful in propagating their compositions. In addition to this selective advantage, oligomers could also exhibit higher mutually catalytic potencies because of a 'combinatorial library' effect. Our computer model utilizes a fitness function that balances the thermodynamic price of polymerization with the advantages of oligomer formation. In preliminary simulations, a defined size of monomer alphabet was reached and a hierarchy of oligomer 'words' crystallized (Segré *et al.*, 2000b). The large diversity of the molecular components thus generated would enhance the capacity of a GARD assembly to embody an unlimited hereditary potential (Szathmáry, 1999), which could eventually lead to the combinatorial nucleic acid 'takeover', and to the emergence of a genetic code (Szathmáry and Maynard-Smith, 1995).

12. Summary

The possible role of RNA as the initial carrier of catalytic capacity and genetic information faces several difficult problems. The monomers of RNA are not readily synthesized under prebiotic conditions; it is difficult to imagine ways in which they could be assembled spontaneously into polymeric structures of sufficient complexity; and RNA has no ability to capture energy from the environment and cannot readily contribute to organized supramolecular structures.

In contrast, amphiphilic molecules were likely to have been relatively abundant in the prebiotic environment, given that virtually any molecule that contains a long enough hydrocarbon moiety and a polar group is an amphiphile. Furthermore, self-assembly of amphiphilic molecules into complex supramolecular structures is spontaneous. The plausibility that such structures were present in the prebiotic environment is supported by the occurrence of amphiphilic molecules in carbonaceous meteorites and the demonstration that they can assemble into membrane vesicles. Vesicle structures have the capacity to capture light energy by incorporation of pigment molecules that partition into the bilayer, or redox energy by mediating electron transport reactions across the membrane so that electrochemical potentials are produced. Assemblies of amphiphilic molecules also have the capacity to act as catalysts for a variety of reactions pertinent to the living state, including synthetic reactions leading to growth of the bilayer structure from precursors. Finally, as demonstrated by the GARD model in computer simulations,

assemblies of amphiphilic molecules have the capacity to contain compositional information and to undergo an evolution-like process.

We conclude that a high-probability Lipid World may have preceded a low probability RNA world. Nonetheless, at some point in prebiotic evolution assemblies of lipid-like molecules likely began to incorporate additional chemical moieties. Head groups could diversify, to include monomers of present-day life, such as nucleotides and amino acids. These could oligomerize in the plane of the monolayer or the bilayer, thus giving rise to entities more akin to contemporary biopolymers, which would enhance the catalysis and templating capacities within the assemblies. Some molecules could detach and become soluble, perhaps enclosed in a vesicular lumen. It is at this stage that a scenario akin to the RNA world could be initiated, although this does not imply by any mean that RNA chemistry was exclusively present.

An important goal for future research will be to provide an additional experimental basis for the Lipid World scenario. One could explore ways in which amphiphilic micelles and vesicles could constitute a suitable microenvironment in which diverse chemical reactions could occur. This would include rudimentary photosynthesis, as well as the generation of RNA and protein monomers, that could be synthesized in a chemically active form, followed by oligomerization into highly catalytic forms. An energy-transducing vesicular system containing templating molecules as well as catalysts for reactions leading to amphiphile synthesis would be a clearly recognizable intermediate in the pathway leading to the origin of life.

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