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The Origin of Primitive Cells, Nutrient Intake, and Non-Enzymatic Elongation of Encapsulated Nucleotides**

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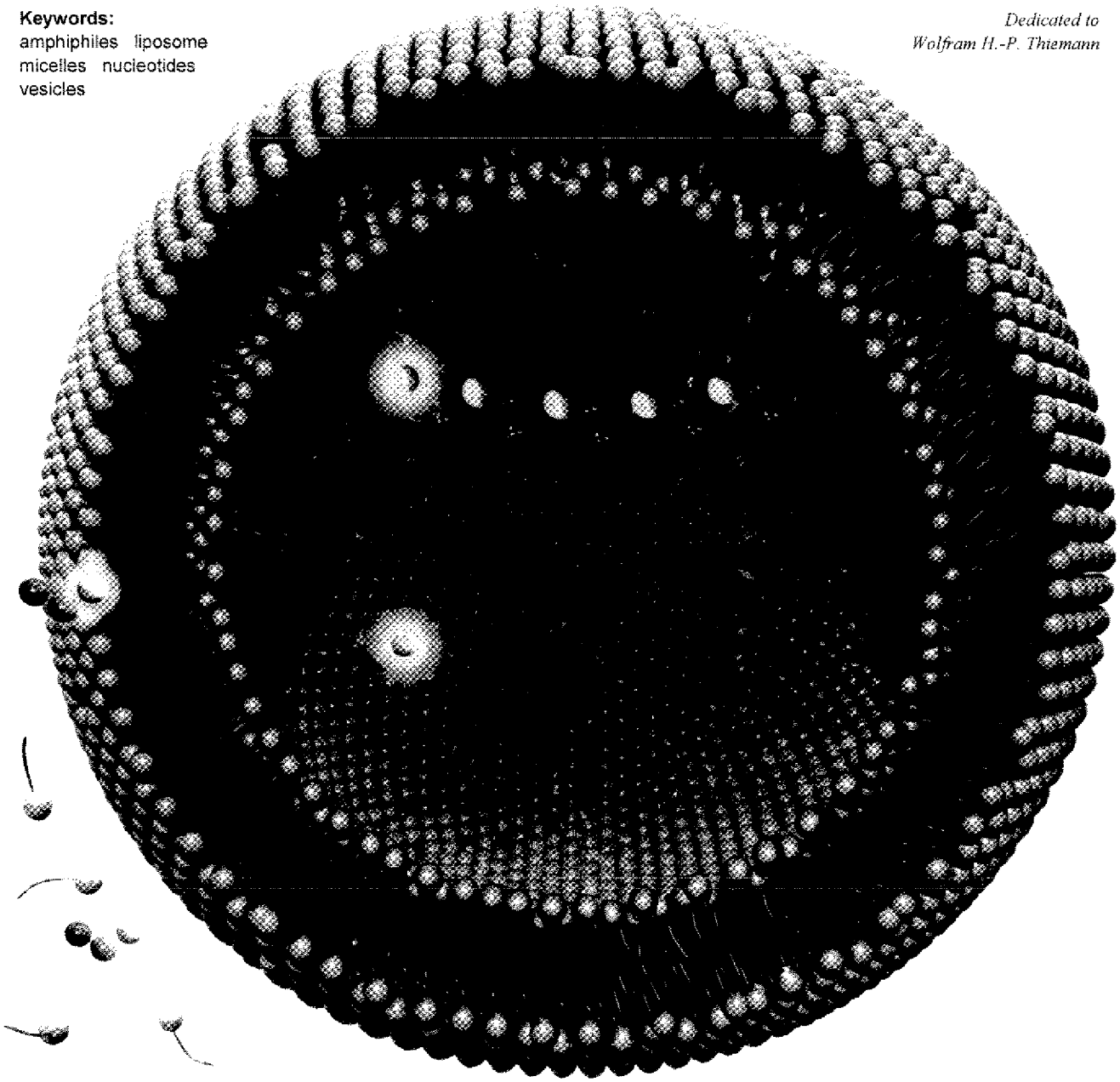
Dedicated to Wolfram H.-P. Thiemann



Fatty acids and fatty alcohols are commonly found in experiments simulating the prebiotic 'soup'. These amphiphiles can be synthesized under prebiotic conditions, at least as long as the molecules are chemically relatively simple and do not need to be enantiomerically pure.[2] In the context of topical origin-of-life theories, two distinct formation pathways for amphiphiles have been described: one related to geophysical sites, such as marine hydrothermal systems, and another to extraterrestrial sources, such as the proto-solar nebula, which was fed by interplanetary and interstellar nebulae. The chemical analysis of each provides individual characteristic challenges.

2.1. Aqueous Fischer-Tropsch-Type Formation of Amphiphilic Molecules

The Fischer-Tropsch-type (FTT) reaction has drawn the attention of geochemists as a potential starting point for the formation of organic molecules, including amphiphiles. The FTT reaction is known to occur in different geological settings such as volcanoes and igneous rocks. For a long time, it was assumed that the FTT process cannot occur in the aqueous phase due to likely inhibition by water, but recent laboratory experiments by the team led by Simoneit have proven that the chemical formation, accumulation, and selection of amphiphiles is feasible via FTT reactions even in the aqueous phase.[5, 6] Aqueous FTT synthesis is important since mid-ocean-ridge hydrothermal systems are discussed with increasing emphasis as a possible starting place for the origin of life on Earth. This is due to the discovery of primitive life forms around hydrothermal vent systems at the bottom of the ocean, where magma (liquid rock) spills through the Earth's crust and reacts with seawater.

Contemporaneous marine hydrothermal systems, however, are dominated by organic compounds derived from all-pervasive biological processes, and therefore hydrothermal vent-simulating laboratory experiments provide the best opportunity for confirmation of the potential for organic synthesis in such systems. Consequently, FTT reactions have been performed under temperature and pressure control in the laboratory, mimicking hydrothermal conditions. Starting with aqueous solutions of either formic or oxalic acid – used as proxies for CO, CO_2, and H_2 in hydrothermal fluids in order to overcome the practical difficulties of adding these volatile gas components to the high pressure reaction vessel – as the carbon and hydrogen sources, the formation of lipid compounds with carbon lengths between C_2 and C_{35}, including *n*-alkanols and *n*-alkanoic acids, was observed inside reaction vessels after cooling, extraction, and gas chromatography-mass spectrometry analyses. The identification of the reaction products was confirmed using ^{13}C labeled reactants. Both formic and oxalic acid carbon sources yielded the same lipid classes with essentially the same ranges of compounds. The optimum temperature window for the formation of alkanoic acids was shown to be 300 °C; higher temperatures reduce the yield due to competing cracking processes.[6] Table 1 presents the relative concentrations and carbon number ranges of alkanoic acids obtained by aqueous FTT synthesis at various temperatures.

Carbon preference index (CPI) values vary from 0.95 to 1.15, showing no carbon number predominance. CPI values close to one indicate that the chain growth of the homologue series is by single carbon units. The aqueous FTT reaction thus proceeds by the transformation of oxalic acid to C1 species such as CO, followed by the insertion of the CO group at the terminal end of a carboxylic acid functionality to form homologue series of alkanoic acids after reduction.[6] This mechanism differs from the classically known industrial FTT process, in which the growth of the hydrocarbon chain relies on the reaction of vapor phase mixtures of CO or CO_2 with H_2 proceeding by surface-catalyzed stepwise polymerization of methylene.[5, 6] Besides amphiphilic molecules, straight-chain alkanols, alkyl formates, alkanals, alkanones, alkanes, and alkenes were identified, and methylalkanes were found above 250 °C with a maximum concentration at 350 °C.

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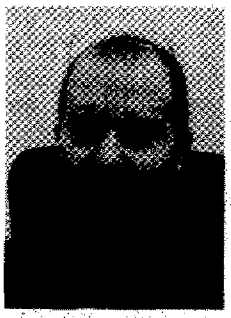
Cornelia Meinert received her diploma in chemistry in 2004 at the university of Leipzig focussing on organic and environmental chemistry. She is currently completing her Ph.D. studies on preparative capillary GC with Werner Brack at the Helmholtz-Centre and became postdoctoral fellow in the group of Uwe Meierhenrich at the University of Nice-Sophia Antipolis. Her research interests focus on the origin of biomolecular asymmetry, especially enantiomer separation using GCxGC techniques.



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Advanced experiments with an encapsulated dye confirmed that the amphiphilic components of the droplets assembled into membranous vesicles providing well-defined interior spaces.

2.3. Identification of Amphiphiles in Carbonaceous Meteorites

Functional organic molecules have been extracted from the carbonaceous Murchison meteorite. Murchison belongs to the CM2-type meteorites, known to contain up to several percent of their mass as organic carbon; it has a complex history and is certainly not of identical chemical composition to the simulated precometary ices presented in chapter 2.2. However, in the case of the Murchison meteorite, enantioenriched amino acids,[13, 18–20] chiral and achiral diamino acids,[21] nucleic bases,[22–23] and amphiphilic molecules have been identified. Chloroform-methanol extracts of the meteorite sample showed that vesicular structures appear when a phosphate buffer is added to the organic extract. To determine whether the amphiphilic components can assemble into membranous vesicles with interior spaces, Dworkin et al. added a hydrophilic pyranine dye via an approved dehydration-rehydration cycle[24] to an extract of the Murchison meteorite.[15] As shown in Figure 2, among oil droplets and other morphologies, micrometer-sized vesicular structures encapsulating the fluorescent pyranine dye in interior spaces were formed.

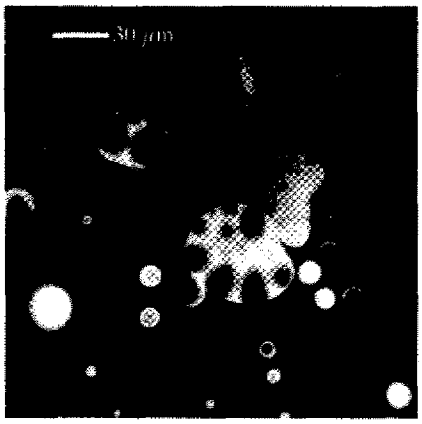


Figure 2. Meteoritic compounds seen in a new light: pyranine dye encapsulated in vesicles made from an extract of the Murchison meteorite. Vesicles show interior spaces with sizes in the micrometer range, but oil droplets and inverse emulsions are also visible.[15] Copyright (2001) National Academy of Sciences, U.S.A.

Because the Murchison meteorite extracts are available only in limited quantities, the exact composition of the membrane-forming amphiphiles was not established in this study.[10]

New work of Pizzarello et al. revealed via solid phase micro-extraction (SPME) sample preparation that low molecular weight monocarboxylic acids are the most abundant water-soluble organic compounds in the Murchison and many other carbonaceous meteorites.[25] More than 50 monocarboxylic acids were identified in 11.3 g taken from the inside of the meteorite, representing quantities 10 to 100 times greater than those of amino acids. Compound-specific isotopic analyses performed with isotope ratio gas chromatography including a combustion system (GC-c-IRMS) present new opportunities to better define the origins and formation pathways of meteoritic organic compounds. They showed δD and $\delta^{13}C$ values verifying an interstellar origin of the amphiphilic molecules.[25] Besides linear chain monocarboxylic acids with carbon chains up to C_{10}, a large diversity of nearly randomly substituted branched chain monocarboxylic acids was identified. This complex mixture of branched monocarboxylic acids was proposed to have originated by exothermic and thermodynamically favored interstellar gas phase radical reactions proceeding from 10 to 100 K. More than 30 years ago, comparatively primitive analytical studies identified 18 monocarboxylic acids in the Murray and Murchison CM2 carbonaceous meteorites, identical to the core analytes now detected by the Pizzarello group.[26]

In 1989, extracts from the interior of a 90 g sample of Murchison showed evidence for surface activity involving both the formation of monomolecular films at air-water interfaces and self-assembly into membranous vesicles with encapsulated polar solvents.[16] This research presented novel observations where amphiphilic molecules extracted from the Murchison meteorite were chemically identified, and they showed lipid-like behavior and self-organized into vesicles, suggesting that extraterrestrial materials could exhibit a far greater range of chemical properties and behaviors than previously thought.[15] Amphiphilic molecules can be delivered to planetary surfaces such as the early Earth, where they mix with endogenous compounds synthesized on the planet.[10]

The relevance of fatty acid vesicles to origin-of-life scenarios is recognized because they are chemically simple versions of amphiphiles, in contrast to phospholipids used in contemporary biological cells. We conclude that fatty acids and other amphiphilic compounds present in carbonaceous meteorites can participate in membrane self-assembly processes, as can carboxylic acids synthesized by aqueous FTT reactions.[27]

2.4. Designing the First Cell: Self-Assembly of Amphiphiles into Cell-Like Vesicles

Amphiphilic molecules linking a single saturated hydrocarbon chain to a polar headgroup will, when dispersed in an aqueous phase, self-assemble into different phases depending on concentration, chain length, head group characteristics, and environmental factors such as temperature, counter ions, and pH. Amphiphiles including medium- and long-chain monocarboxylic acids, alcohols, amines, alkyl phosphates, and alkyl sulfates,[1] as well as organic-inorganic nanoparticle hybrid systems[28, 29] typically form spherical micelles above the Krafft temperature[30] and above the critical micellar concentration (*cmc*). They can form bilayers and vesicles at a critical concentration for vesicle formation (*cvc*, sometimes abbreviated *cbc* for critical bilayer concentration)[31, 32] in rapid dynamic equilibrium with single molecules and micelles. We point out that the *cvc* is usually much higher than the *cmc*. Nonmicellized or nonvesiculized amphiphiles are always present together with micelles and vesicles.[32]

Lipid vesicles, also called liposomes (liposomes are, strictly speaking, vesicles made out of lipids),[33] or often simply vesicles,[34] are quasi-spherical shells composed of lipid bilayers encapsulating an aqueous space.[35–37] Unilamellar and multilamellar vesicles are generally formed upon dispersion of amphiphilies (or mixtures thereof) that self-organize in water into lamellar phases. These quasi-spherical supramolecular structures are composed of thousands to millions of individual molecules[1] with diameters

existence of a lamellar region D in the phase diagram, as illustrated in Figure 3. Such a growth process of cell membrane-like bilayers is driven by the rapid equilibrium between individual amphiphiles, micelles, and bilayers resulting in the uptake of the micellar components/micelles into the bilayer structure and the concomitant dissolution of the micelles.

In principle, the simplest mechanistic models of carboxylic acid vesicle growth would be: 1) the direct fusion of micelles with vesicles in a single step, 2) the dissolution of micelles into carboxylic acid followed by incorporation into the preformed membrane, or 3) vesicle-vesicle fusion.[47] The direct observation of the growth mechanism of fatty acid vesicles was for a long time inaccessible due to the lack of an appropriate analytical methodology but – due to scientific advancement in this field – was deciphered recently.

3.1. Vivat, Crescat, Floreat: Vesicle Growth

Cryotransmission electron microscopy (cryo-TEM) was applied in the first pioneering study clearly demonstrating the growth of vesicles after the addition of fatty acid micelles.[34] Here, the water-soluble protein ferritin, which, due to its dense iron core, can be detected by cryo-TEM, was entrapped in the internal aqueous volume of preformed vesicles. The size distribution of filled (ferritin-containing) and empty vesicles could be distinguished, and the cryo-TEM data – obtained from frozen vesicle suspensions – gave evidence for the growth of vesicles upon the addition of fresh surfactant, as well as evidence of fission processes of larger vesicles that lead to a large number of small vesicles. Unfortunately, this cryogenic method could not be used to follow membrane vesicle growth in real time.[47]

Recently, the laboratory of Szostak applied an innovative methodology based on membrane-localized fluorescence resonance energy transfer (FRET) dyes to follow fatty acid vesicle growth to distinguish between vesicle growth by direct micelle-vesicle fusion versus vesicle growth by the incorporation of free molecular fatty acids. Hence, a membrane-implemented FRET donor-acceptor pair allowed for measurement of the increasing vesicle surface area during controlled vesicle growth by the well-adapted addition of micelles. The FRET efficiency decreased as the surface density of the FRET dyes decreased when incorporating additional fatty acid. In contrast to former experimental approaches, this methodology provided the advantage of allowing for a) the quantitative measurement of the growth of preformed vesicles even when new vesicles were formed simultaneously and b) such measurements to be made in real time during the process of controlled membrane formation.[47] Kinetic data revealed that none of the three above mentioned mechanistic models of vesicle growth is appropriate, and a new pathway involving previously unsuspected intermediate aggregates was proposed. The structure of these metastable intermediates could not be elucidated; candidate structures are bilayer patches, cup-like membrane structures, and long cylindrical micelles. The sizes of heterogeneous intermediate aggregates could be determined by dynamic light scattering to have a hydrodynamic radius of about 45 nm, much larger than spherical micelles.[47]

A time-resolved structural study on the micelle-to-vesicle transition had already shown that intermediate metastable states occur, which were described as cylindrical wormlike micelles that finally evolve via disks into vesicles.[49] Membrane patches and discs had been reported to be short-lived intermediates in a micelle-to-vesicle transition in a model bile,[50] and cup-like particles or open bilayers partially rolled into lipid tubules had been identified during vesicle formation using the elastic bending energy approach.[51] Spontaneous formation and growth of vesicles in a micelle solution had moreover been studied by small-angle neutron-scattering experiments (SANS), opening up the possibility for few hundred millisecond resolution experiments. These data revealed that cylindrical micelles form before their continuous transition into vesicles[52] and that the number of micelles required to produce a vesicle is about 25–50.[53] Studies on the phase behavior of the reverse vesicle-micelle transition applying the cryo-TEM methodology had revealed, too, that not only spherical micelles but also long cylindrical micelles form as intermediate nanostructures during the solubilization of phospholipid vesicles by surfactants.[54]

Vesicle formation was furthermore observed to be mediated by minerals. It was shown that montmorillonite clay[42] and different minerals and surfaces such as quartz, pyrite, and gold nanostructures[55] accelerated the conversion of fatty acid micelles into bilayer membrane vesicles. Even silica particles of 6 nm diameter, a diameter smaller than the smallest possible vesicle, promoted vesicle formation. Nucleation most likely involved the formation of small patches of membrane that can continue to grow at their edges independently of the silica spheres. This type of surface-assisted vesicle formation was observed in real time, enabling researchers to see the formation of vesicles streaming off a microsphere just after micelle addition.[55] The authors assumed that a layer of positively charged cations associated with or adjacent to the montmorillonite surface attracts negatively charged micelles or free fatty acid molecules, increasing their concentration locally and thus facilitating their aggregation into a bilayer membrane.[48]

3.2. Dynamic Properties of Vesicles

In contrast to micelles, membrane vesicles are described as systems not to be at chemical equilibrium. They are thermodynamically unstable, requiring energy to form.[33] In recent years it became more evident that nonequilibrium structures appear at all levels in biology, and, as Kondepudi and Prigogine stated, "we cannot describe Nature around us without an appeal to nonequilibrium situations."[56] In this context it was shown that different size populations of vesicles can coexist for several days in the same solution without a tendency to fuse with each other to search for an energy minimum. The different vesicle sizes correspond to energy minima, but no tendency for a homogeneous size distribution was observed after mixing. However, the individual amphiphilic molecules were observed to be in local equilibrium with the vesicular structure. Luisi's team concluded that two populations of different vesicle sizes can not only coexist but also, due to larger uptake rates of bigger vesicles for amphiphilic monomers present in the surrounding solution, compete with each other, for example for the uptake of reagents.[33]

Bilayer vesicles are dynamic systems, and individual molecules can easily enter and leave the vesicular structure. Fatty acids in a bilayer membrane are in rapid exchange with the aqueous environment. Amphiphilic monomers can exchange from two different layers within one vesicle.[1] They were observed to flip from the outer shell into the inner shell and vice versa.[57] This behavior will be important for the nutrient intake and the metabolite outtake of cell-like vesicles via bilayer membranes.

3.3. A New Generation of Cells: Controlled Vesicle Redivision

In the absence of the complex machinery that controls the division of modern cells,[58–59] the redivision of growing vesicles must rely on the intrinsic properties of the vesicle and the physico-chemical forces of the environment.[45] In R&D, where vesicles are used as model membranes and in pharmaceutical applications where vesicles are applied as nanoscale containers for drug transport and delivery,[60] the most widely used method to prepare vesicles under controlled conditions in the laboratory is by extrusion of vesicle suspensions through small pore filters. For "division", a vesicle enters a membrane pore under pressure, transforms into a sphero-cylindrical shape, and fragments into smaller vesicles with a diameter somewhat smaller or larger than the pore diameter, depending on the ratio between vesicle size and pore diameter.[36] Even though this method is widely applied, the actual mechanism by which vesicles break up into smaller vesicles remains unclear.[36–37]

Szostak's group distinguishes between two distinct mechanisms for vesicle division: 1) the parent vesicle can be disrupted into smaller membrane fragments, subsequently resealing into a new generation of smaller vesicles, or 2) by the pinching-off of smaller vesicles resulting in insignificant dilution of the vesicle contents.[48] A fluorescent dye (calcein) was therefore encapsulated into 90 nm sized myristoleate vesicles that were grown to a size of 140 nm through slow micelle addition, then extruded through 100 nm pores to a final mean size of 88 nm. It was found that during extrusion 55 % of the dye had been lost from the vesicles.[48] The results show that the myristoleate vesicle division proceeds with only a slightly greater loss of internal contents than that demanded by the geometric constraints of deriving two daughter spheres from one larger parent.

In advanced studies Szostak et al. repeated cycles of growth and division by growing a population of extruded myristoleate vesicles by slow feeding with myristoleate micelles and then dividing by extrusion. The amount of encapsulated calcein was followed after each growth period and each extrusion. As expected, essentially no dye was lost during any of the five growth phases, whereas 40 % of the dye was lost after each extrusion. These experiments constitute a proof-of-principle demonstration that vesicle growth and division can result from simple physicochemical forces, without any complex biochemical machinery.[48] Furthermore, environmental shear forces can cause vesicles to divide.[45] In a recent experiment, a template replication inside a cell-type model vesicle followed by the observed random segregation of the replicated genetic material lead to the formation of daughter protocells (see section 5).[57]

4. Towards Life's Dynamics: Nutrient Uptake through Bilayer Membranes

4.1. Encapsulation During Vesicle Formation By Dehydration/Rehydration

Successfully integrating functional chemical systems into the interior space of vesicles is a key challenge in biophysics.[42] The dehydration/rehydration method is among the most efficient encapsulation methods. By the dehydration/rehydration method, nutrients and functional target molecules can be sequestered into the interior space of vesicles at the stage of vesicle formation. Such a phenomenon might well have triggered the appearance of cell-type vesicles on the early Earth.

Recent studies have shown that vesicles made from a decanoic acid-decanol mixture are capable of encapsulating and retaining a variety of organic macromolecules such as fluorescent dyes (Figure 4). The formation of vesicles in the presence of a dye resulted in the capture of the dye molecules within the vesicles. Subsequent size exclusion chromatography allowed for the separation of the vesicles from unencapsulated dye, thus releasing dye-enclosing vesicles for further investigations.[31] Not only dyes but also enzymes such as catalase, as well as oligonucleotides, can be encapsulated in fatty acid vesicles using the dehydration/rehydration method (Figure 4).[24, 31]

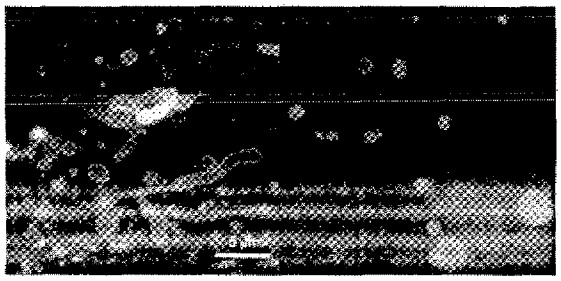


Figure 4. Decanoic acid-decanol vesicles stained with fluorescent rhodamine (left). 600mers of DNA encapsulated in vesicles of decanoic acid alone by the dehydration/rehydration method (right). DNA was stained with 3,6-dimethylaminoacridine (acridine orange), a nucleic acid-selective stain used to enhance the contrast in the microscopic image. Image courtesy: David Deamer, UC Santa Cruz.

As described in chapter 3.1. montmorillonite was observed to accelerate the conversion of fatty acid micelles into vesicles. The surface-mediated bilayer membrane organization allowed for the vesicular encapsulation of catalytically active surfaces such as montmorillonite. By previous loading of the montmorillonite surface with adsorbed RNA, RNA oligonucleotides were incorporated into the vesicles.[48] The observed encapsulation of mineral particles within vesicles thus brought the catalytic potential of the RNA-tagged mineral surfaces into the vesicle.

Photoactive semiconducting particles, such as titanium dioxide particles in the 20-nm size range, were incorporated into vesicles via the dehydration/rehydration method, retaining photoactivity by allowing incident light to drive photoelectrochemical reactions in a comparable manner to contemporaneous photosynthesis possibly relevant for the origin of life on Earth.[61]

4.2. The Static Solubility-Diffusion Theory

Phospholipid membranes of extant biological cells show limited permeability to ionic nutrients such as amino acids, nucleotides, and phosphate with measured permeability coefficients $P \approx 10^{-12}$ cm s^{-1}.[62] Deamer et al.[10] raised the question "how might an early form of cellular life gain access to nutrient solutes?" We are confronted with the paradoxical situation that required vesicular membranes to a) be permeable enough to enable the intake of nutrients and b) act as a barrier prohibiting the loss of the encapsulated primitive

catalytic and genetic system. Without the barrier function, newly synthesized substances would diffuse into the surrounding bulk phase, and the potential for interactive systems and speciation would be lost.[63] For increasing membrane permeability to solutes, one should use membranes in a fluid (liquid-crystalline) state rather than in a gel (crystal) state. Another solution is to reduce the membrane thickness. These goals can be achieved by reducing the length of the lipophilic chains in the membrane-constituting amphiphiles,[10, 64] by introducing *cis*-unsaturation or branching in the chains, and/or by adding amphiphiles with larger head groups.[57]

Various mechanisms have been proposed to describe the uptake of nutrients through bilayer membranes. The static solubility-diffusion theory interprets the bilayer membrane as a liquid hydrocarbon phase separating two aqueous phases. Permeating molecules will partition into the hydrophobic region, diffuse across, and leave by redissolving in the opposite aqueous phase, driven by its concentration gradient (this is also known as the passive diffusion mechanism). Permeability coefficients can hence be calculated if appropriate partition and diffusion coefficients as well as the membrane thickness are known. The solubility-diffusion theory is applicable for uncharged molecules, because of their relatively high solubility in the intermediate hydrocarbon phase. This theory also explains that uncharged amino acid methyl esters permeate lipid bilayers orders of magnitude faster than their zwitterionic parent compounds, the former being much more lipophilic than the latter. Transmembrane pH gradients are used for the active and quantitative loading into vesicles and are based on concentration gradients as well.[62]

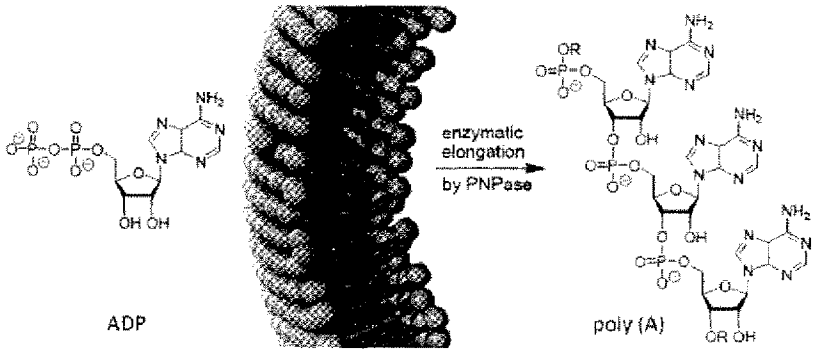
4.3. The Dynamic Pore Mechanism

Discrepancies between predicted and measured permeabilities were observed for small ions penetrating thinner bilayer membranes. The alternative dynamic pore mechanism suggests that the permeation of ions through bilayer membranes occurs through pores or cavities that are hydrated transient defects produced by thermal fluctuations within the bilayer causing disturbances in the lipid packing order.[65] Small ions can enter into these pores located in the headgroup region of the amphiphiles and pass through such hydrated defects, evading the high-energy barrier associated with partitioning into the hydrophobic membrane interior.[64] If membranes are sufficiently thin, pores provide the dominant permeation pathways for ions. Ionic substrates such as the nucleoside triphosphate ATP were shown to permeate DMPC-based vesicular bilayers at the gel-fluid main phase transition temperature of 23.3 °C, at rates capable of supplying an encapsulated template-dependent RNA polymerase.[63] Permeation was observed to be greatest at the phase transition temperature. At 37 °C, the optimal temperature for enzyme catalyzed reactions, permeability decreased by two orders of magnitude. Even if the authors proposed the dynamic pore mechanism for ATP permeation, the flip-flop mechanism cannot be excluded for explaining the observed results.

Alternatively to the dynamic pore mechanism, charged molecules can coordinate on the external shell of the vesicular membrane to the polar headgroups of the amphiphilic molecules. Amphiphiles can flip from the outer/inner shell into the inner/outer shell to be capable of releasing the charged molecules to the vesicle's interior/exterior space (see chapter 3.2.). This phenomenon is most important at the main phase transition temperature of the bilayer and in the fluid state compared to the gel state.

The functional enzyme catalase was encapsulated in decanoic acid-decanol vesicles, and its substrate, hydrogen peroxide, was added to the external aqueous environment. The bilayer membrane was shown to be permeable to hydrogen peroxide, releasing oxygen inside the vesicle by maintaining the catalytic function of catalase and protecting the enzyme in the vesicular internal space against external influences, e.g. catalase-degradading protease.[31] Similarly, polymerase enzymes encapsulated with their substrates in a cell-type vesicle led to polymeric products, which were protected from degradation by hydrolytic enzymes present in the external medium.[63] Walde et al. entrapped PNPase enzymes in oleic acid/oleate vesicles, followed by external addition of ADP. The nutrient ADP that carries three negative charges at pH 9 was observed to permeate across the vesicular bilayer into the interior space, where PNPase catalyzed the formation of poly(A), a stretch of ribonucleic acid that was retained inside the membrane vesicle (Scheme 1).[44]

We have seen that under well-defined physicochemical conditions, amphiphilic molecules can form a population of bilayer membrane vesicles that "replicate" through processes of growth and division and have the ability to entrap macromolecules while remaining permeable to smaller polar solutes.[16, 47] The dynamic pore and flip-flop mechanisms might have allowed early cells to have access to functional ionic nutrients from the external environment.



Scheme 1. ADP permeates across the vesicular bilayer into the interior space of oleic acid/oleate vesicles. Catalyzed by polynucleotide phosphorylase (PNPase), intraprotocellular enzymatic ADP elongation is shown, resulting in poly(A), a stretch of RNA that is prevented from escaping into the extracellular medium.[44]

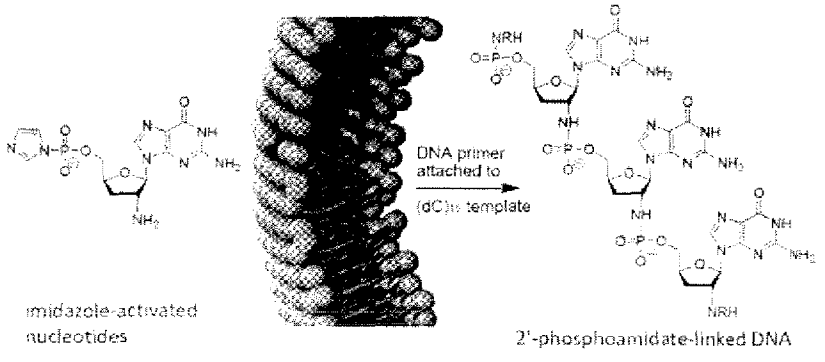
5. Non-Enzymatic Elongation of Encapsulated Nucleotides Inside Cell-type Vesicles

It was previously assumed that the encapsulation of mineral particles within membrane vesicles allows for the use of the catalytic potential of the mineral surface for the elongation of encapsulated nucleotides.[48]

Recently, the elongation of an encapsulated genetic polymer was observed inside cell-like vesicles with neither mineral surface nor enzymatic support: synthetic single-strand DNA molecules with cytosine bases were trapped inside membrane vesicles, acting as

primers and templates for their own elongation. Activated nucleotides containing the complementary guanosine bases were added to the surrounding medium of the vesicles. The mixture of molecules composing the vesicle membranes, including carboxylic acids, their corresponding alcohols, and monoglycerides, was optimized for maximal permeability to ribose, the sugar component of RNA, but minimal permeability to polymers such as DNA.[66] The optimized cell-like vesicles showed an elongation of the synthetic DNA primer as guanosine-containing imidazole-activated nucleosides were added one by one to the external medium! In contrast, in experiments with POPC vesicles that were run in parallel, the authors observed no elongation.[57] The authors assumed that permeation of the imidazole-activated and negatively charged nucleotide across the membrane was driven by the interaction of its polar functional group with the amphiphile head group, whereas non-polar regions of the nutrient interacted with the hydrophobic chains of the amphiphiles. The amphiphile-nutrient complex then flips from the outer to the inner membrane shell (see Chapter 4.3) carrying the nutrient to the internal space of the vesicle. This experiment shows that prebiotically plausible membranes composed of fatty acids provide surprisingly high permeabilities to charged molecules such as nucleotides, which can thus be incorporated from an external source of nutrients to take part in efficient template copying in the protocell interior (Scheme 2).

The decoded non-enzymatic elongation of encapsulated nucleotides inside protocells has far-reaching consequences, suggesting that a heterotrophic origin of life is feasible and that early living organisms or systems incorporated carbon-containing nutrients already available in the environment. The authors argue that early protocells made of fatty acid membranes could not have been autotrophs because internally generated metabolites would leak out.[57] Cellular life might first have sourced energy and nutrients from the environment, and more complex autotrophic lifestyles appeared at a later stage of evolution.[66] These experimental data again highlight that fatty acid membrane vesicles seem to be a suitable model for a protocell during early evolution leading to cellular life.[63]



Scheme 2. Negatively charged imidazole-activated nucleotides cross the vesicular membrane and participate in non-enzymatic copying of an oligo-dC DNA template. Membrane vesicles were composed of decanoic acid, decanol, and decanoic acid glycerol monoester.[57]

Cellular evolution continued to progress. A typical protocell is assumed to encapsulate not only an information-bearing template but also a polymerase or replicase composed of amino acids, so that sequence information in the template can be transcribed to a functional molecule.[10] Recently, oligopeptide synthesis from amino acid monomers inside vesicles made of fatty acids or phospholipids in a simulated hydrothermal environment was reported. It was found that encapsulation of the glycine monomers enhanced oligomerization.[67] Amino acid nutrients for polymerase and replicase architecture are moreover required to cross the membrane barrier to enter the interior space of the cell-like vesicles. Controlled conditions that not only allow for the passage of charged nucleotides but also the uptake of zwitterionic amino acids while retaining polymerized nucleic acids inside vesicles will hopefully enlighten our understanding of the crucial steps of the origin of life in the near future.

Discussions are ongoing regarding whether, when fed with amphiphiles and precursors for membranes, replicases, RNA synthesis, and membrane vesicles will grow and divide, and whether improved replicases[68] will evolve.[45] Szostak et al. pointed out that a vesicle carrying an improved replicase would itself not have an improved capacity for survival or reproduction. It would not be called "alive". For this to happen, an RNA-coded activity is needed that imparts an advantage in survival, growth, or replication for the membrane component providing internal control of cell division.[45] A ribozyme that synthesizes amphiphilic lipids and thus enables the membrane to grow would serve as an example. The membrane and the genome would then be coupled, and the 'organism' as a whole could evolve as vesicles with improved ribozymes would have a growth and replication advantage.[45]

6. Summary and Outlook: From Amphiphiles to Living Cells

Endogenous aqueous FTT-type syntheses and exogenous delivery via meteorites and comets are potentially important sources of prebiotic and biogenic molecules to the early Earth. Both processes provide amphiphilic molecules that, under well-defined physicochemical conditions, assemble into membrane vesicles. Vesicles are assumed to have harbored potential prebiotic catalysis. With compartmentalization, the encapsulated replicase component is not only capable of, but also inevitably subject to, variation, natural selection, and thus Darwinian evolution.[45] Based on experimental studies in the laboratory, we can assume that cell-like membranous compartments composed of bilayers appeared wherever organic compounds became concentrated, within which molecules were trapped. Life began when one or more of the assemblies found a way not only to grow but also to reproduce by incorporating a cycle involving catalytic functions and genetic information. Lipid vesicles may have served as a physical container housing informational polymers such as DNA and RNA and a metabolic system that chemically regulates and regenerates cellular components.[42]

Some authors have suggested that a lipid world may have preceded an RNA world.[1] Nonetheless, at some point in prebiotic evolution assemblies of lipid-like molecules likely began to incorporate monomers of present-day life, such as nucleotides and amino acids. After oligomerization, catalysis and templating capacities would be enhanced within the assemblies.

An important goal for future origin-of-life research will be to systematically explore the physicochemical parameters under which cell membrane-like 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, followed by the synthesis of templating molecules in the interior space of vesicles.[1] In this context, it is widely believed that the design of an artificial cell, namely a highly simplified version of a biological cell, might be achievable in the near future[4, 69] as an imaginable goal.[45] If these predictions are right, we should be hearing about some dramatic findings very soon. The question of the most likely early technological applications of artificial cell research remains as yet unanswered. In time, research will eventually produce dramatic new technologies, such as self-repairing and self-replicating nanomachines. With metabolisms and genetics unlike those of existing organisms, such machines would literally form the basis for a living technology possessing powerful capabilities and raising important social and ethical implications.[42] Experimentally, the potential exists to supply a population of cells with random RNA sequences to observe and determine what new ribozyme activities were most accessible and advantageous for evolving simple cells.[45] In the long run, it might even be possible to observe at least some aspects of the evolution of protein synthesis, possibly with different sets of amino acids.[45]

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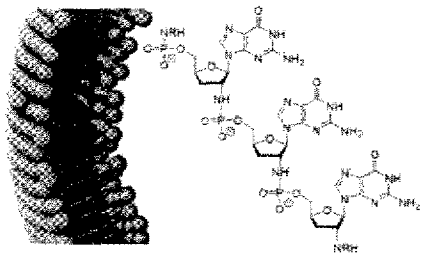
Entry for the Table of Contents

Origin of Life

U. J. Meierhenrich,* J.-J. Filippi, C. Meinert, P. Vierling, J. P. Dworkin _____ **Page – Page**

The Origin of Primitive Cells, Nutrient Intake, and Non-Enzymatic Elongation of Encapsulated Nucleotides



A primitive cell remodeled: Bilayer membrane vesicles provide a multi-faceted microenvironment in which protometabolic reactions could have been triggered. Here, we report on the formation of membrane vesicles via the self-assembly of amphiphiles. When nucleotides were added to the extra-vesicular medium, the intake of these 'nutrients' was observed, followed by their participation in non-enzymatic DNA primer elongation in the vesicular interior.