Chapter 1
Experimental Systems to Explore Life Origin: Perspectives for Understanding Primitive Mechanisms of Cell Division

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Abstract Compartmentalization is a necessary element for the development of any cell cycle and the origin of speciation. Changes in shape and size of compartments might have been the first manifestation of development of so-called cell cycles. Cell growth and division, processes guided by biological reactions in modern cells, might have originated as purely physicochemical processes. Modern cells use enzymes to initiate and control all stages of cell cycle. Protocells, in the absence of advanced enzymatic machinery, might have needed to rely on physical properties of the membrane. As the division processes could not have been controlled by the cell's metabolism, the first protocells probably did not undergo regular cell cycles as we know it in cells of today. More likely, the division of protocells was triggered either by some inorganic catalyzing factor, such as porous surface, or protocells divided when the encapsulated contents reached some critical concentration.

1.1 Studies of the Origin of Cellular Life

There is no commonly accepted definition of life. Most scientists working on the problem agree that life can be defined by the set of functions and features that must be possessed by the system to be called alive. Yet, the specificity of these functions remains undefined (Luisi 1998). Since there is no indisputable definition of life, it is also hard to define the event of the origin of life. For the purpose of this work, it will be assumed that the origin of life was the process during which the chemical reactions spontaneously arranged into a homeostatic system, and the newly formed living cells started undergoing spontaneous cell cycle of growth and division. As a

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beginning of cellular life, we understand a compartmentalized system capable of self-maintenance owing to a self-regeneration process from the inside.

Life originated on Earth at least 3.6 billion years ago. The oldest known traces of fully developed life are dated to approximately 3.465 billion years (Schopf 1994), and some evidences show a possibility of biochemical cycles existing as early as 3.8 billion years ago (Schidlowski 2001). The time between the origin of Earth's crust and primordial ocean 4 billion years ago (Morbidelli 2000) and the first known traces of life (date back to 3.8 billion years) is the time when all processes of the origin of life must have occurred. This leaves approximately 200–500 million years for the chemical evolution processes.

Various possible environments are considered as possible site of the prebiotic evolution and the origin of life. Prebiotic Earth provided many different sites for possible prebiotic chemistry reactions, including open water of the ocean, lagoons, surfaces of various minerals, thin layers of organic compounds, gaseous phase of the atmosphere, or submarine hydrothermal vents. Different prebiotic processes proposed in the literature are placed in different conditions. Nevertheless, the origin of life on Earth might not have been a singular accident; only one protocell lineage succeeded and survived, proliferating into all known forms of life. There is no reason to assume that our cells' metabolism represents the only possible type of metabolic process; yet, all the evidence suggests that all known life comes from a single ancestor.

The above mentioned ancestral cell, or population of cells, must have already some sort of functioning cell cycle, consisting of growth and division of the membrane and cell contents, driven by metabolic processes and genetically encoded. The exact nature of processes that have led to the development of the cell cycle is a subject of intensive studies. It is not impossible that the origin-of-life processes are still occurring, although it is much more difficult on the oxidized environment and on the planet absolutely possessed by one type of biological organisms; it is practically unimaginable to expect any other form of metabolism to grow enough to successfully compete with "our type" of life. Therefore, no effective biogenesis processes are observed today (Delaye and Lazcano 2005).

The chemical reaction system undergoing cycles of growth and division, selection and evolution, must have originated as a result of a long series of simpler, more primitive processes. These processes, chemical reactions leading to organic molecules, not based on any biological catalysts, are the subject of interest in prebiotic chemistry. To understand the mechanism of origin of modern cell cycle, simple models, the so-called protocells, have been studied (Luisi et al. 1999).

1.2 Protocell Membrane

To study the origin of elements of the cell cycle, particularly growth and division of protocell membrane, model protocell vesicles are commonly used (Chakrabarti et al. 1994; Walde et al. 1994; Segre et al. 2001). The self-assembled bilayer

membranes, semipermeable to small organic molecules and able to encapsulate bigger, polar compounds, are a good model of a prebiotic protocells.

Several authors, including the group of D. Deamer, proposed that at the earliest stage of the prebiotic bilayer membrane formation, membranes consisted of simple, long chain carboxylic acids (Fig. 1.1). The open question about the nature of the membrane in Last Universal Common Ancestor (LUCA) leaves many possible routes to the origin of lipid membranes during the earliest stages of protobiological evolution. In modern cells, apart from compartmentation, membranes perform several other functions, including energy transduction and transport of organic and inorganic compounds, and they are the docking site of many enzymes. Presumably, the very first role of the membranes was simple encapsulation – isolation of the reaction cycles (i.e., genetic materials or enzymatic peptides) from the environment. This could be done by the simplest amphiphiles, possibly available under the prebiotic conditions: medium-sized (up to C10) chain carboxylic acids (Fig. 1.2).

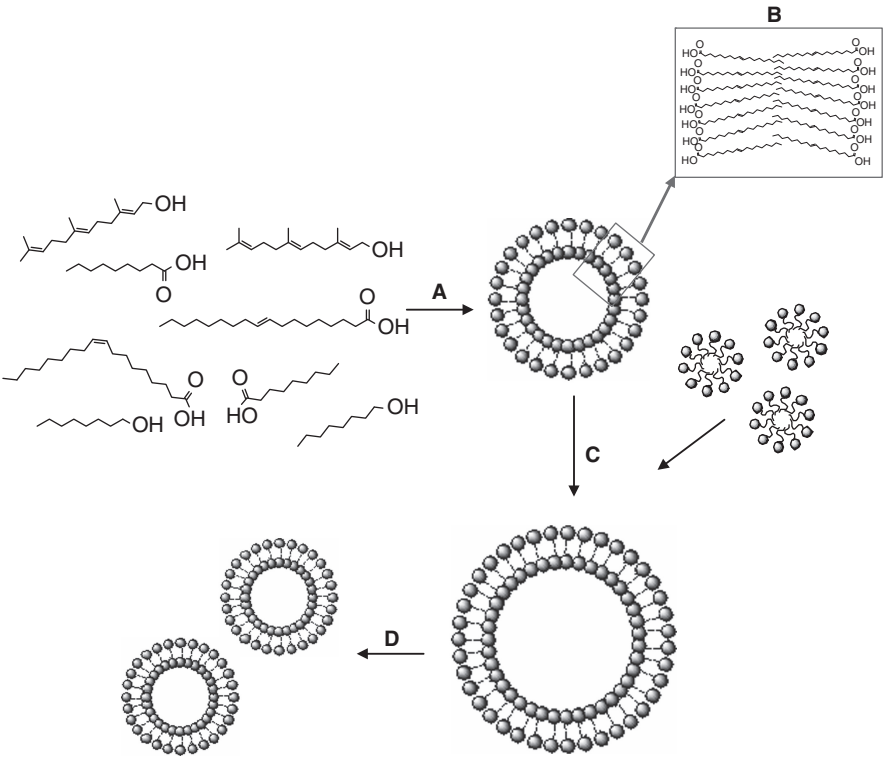

Fig. 1.1 Vesicles. (**a**) Vesicles are spontaneously forming from the amphiphilic monomers; (**b**) bilayer membrane of the vesicle, with polar, hydrophilic headgroups directed outside, and aliphatic, hydrophobic chains inside; (**c**) vesicles can grow upon addition of micelles; (**d**) vesicles can be forced to divide into daughter vesicles

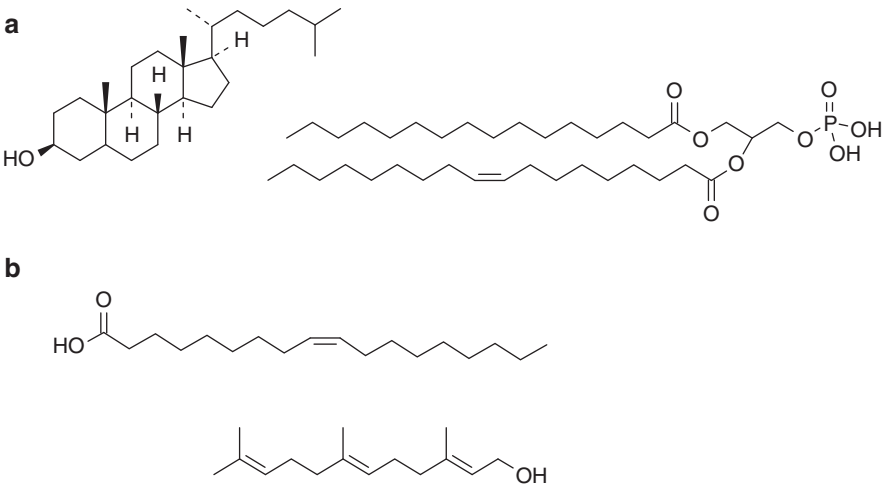

Fig. 1.2 Amphiphilic compounds building the membranes. (**a**) Modern cell's membrane building block; (**b**) possible prebiotic amphiphiles

The main building blocks of modern cells' membranes are phospholipids and sterols. Phospholipid glycerol esters and sterols are too complex to be synthesized under abiotic conditions. However, all these compounds can be derived from simplest building block – sterols from isoprene units and lipid derivatives from simple unsaturated carboxylic acids. The simple lipids might have been synthesized under prebiotic Earth conditions (Yuen et al. 1981; Allen and Ponnamperuma 1967), including environment of the underwater hydrothermal vents (McCollom et al. 1999). Simple amphiphiles were also detected in carbonaceous chondrite meteorites (Yuen and Kvenvolden 1973; Deamer 1985).

Compounds based on these simplest units could have formed the first membranes encapsulating biochemical cycles of the protocell. In a water solution, with the pH close to the polar headgroup p*K*a, the simplest amphiphiles spontaneously self-organize into bipolar membrane sheets that close into spherical vesicles (Apel et al. 2002).

Vesicles are commonly accepted as an approximation of the compartments of the earliest protocells (Walde 2006). Vesicle-like bilayer membranes were even observed in amphiphiles organic material from Murchison carbonaceous chondrite (Deamer 1985; Deamer and Pashley 1989), making its availability on prebiotic Earth more probable.

Vesicle structures can grow (Chen and Szostak 2004), divide (Hanczyc et al. 2003), and selectively take up compounds from the environment (Chen et al. 2004). Therefore, investigating properties of the different vesicle systems can give insight into possible routes to the origin of protobiological compartmentalization.

1.3 Models for Studying Protocell Growth and Division

Protocell vesicles can undergo cycles of growth and division based on simple physical properties of the bilayer membrane. Unlike modern cell cycle, the protocell size and shape changes are caused by external factors, such as addition of amphiphiles or pressure applied to the membrane, and not the internal metabolic processes (Oberholzer and Luisi 2002).

1.3.1 Growth of Vesicles

Simple prebiotic vesicles can grow upon addition of micelles, but the growth is triggered by addition of lipids from the external source, not as a result of reactions occurring inside protocells. The process of growth of simple fatty acid vesicles upon addition of micelles was first described by P.L. Luisi and coworkers (Fig. 1.3). Addition of fatty acid micelles in alkaline solution to buffered solution of vesicles causes vesicles to grow. Fatty acid micelles are stable only under highly alkaline

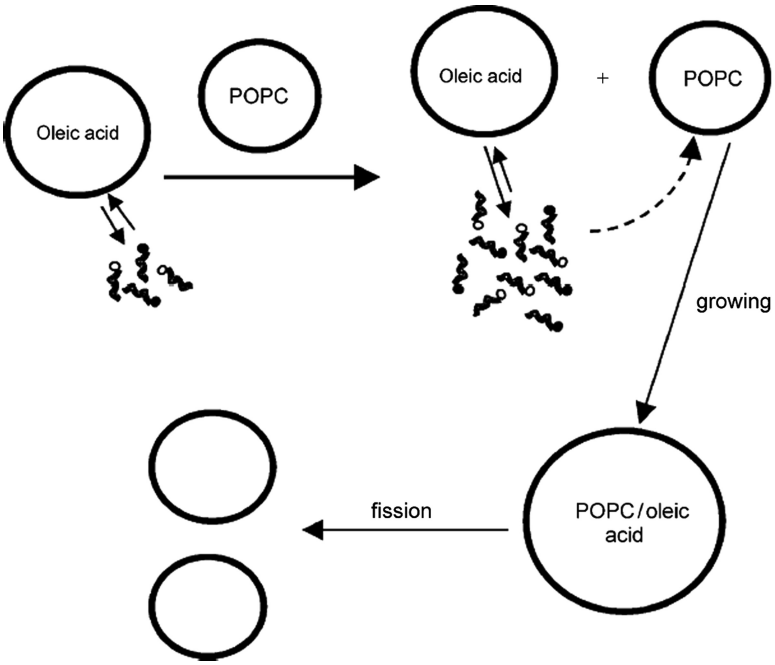

Fig. 1.3 Schematic representation of protocell vesicles competetive growth and division (from Cheng and Luisi 2003)

pH; when micelle solution is added to solution of vesicles, at pH slightly alkaline (i.e., pH 8 for oleic acid vesicles), micelles become thermodynamically unstable and either lipids from added micelles are taken up by the existing vesicles or de novo vesicles are formed. (Luisi et al. 2004; Berclaz et al. 2001; Blochliger et al. 1998; Rasi et al. 2003).

Addition of micelles is a plausible prebiotic model for vesicle growth. It is possible that lipids, such as simple fatty acids, were synthesized in one place on prebiotic Earth, and then transported to other place with lower pH, where they organized into vesicles or fuelled preexisting protocell vesicle population. This must have been caused by the arrival of lipids from external source, and not by processes of the protocells' internal metabolism. Thus, we can model the process of growth, necessary for the origin of cell cycle of growth and division.

Protocell vesicles can undergo competitive growth: when two populations of protocell vesicles are mixed, one made of simple oleic acids and the other made of phospholipids, the phospholipid vesicles grow on account of the oleic acid vesicles. (Cheng and Luisi 2003). This is also a good example of possible origin of competition on the protocell level.

Another process of competitive growth of simple prebiotic vesicles was described by J.W. Szostak and coworkers (Chen et al. 2004). One population of simple fatty acid vesicles can grow, on the expense of another population of vesicles made of similar amphiphiles, if there is a difference in osmotic pressure between those vesicles. Furthermore, the concentration gradient necessary for the competitive growth can be achieved with nucleotides and RNA molecules. That opens up the possibility of coupling two of the essential elements of cell cycle: growth of protocell vesicles in connection with the presence of genetic material.

Myelin-like giant multilamellar vesicles can divide in response to changes of osmotic pressure (Takakura and Sugawara 2004). This is not a particular prebiotically plausible example, since compounds used to build those vesicles are not simple lipids that can synthesize abiotically in aqueous environment. However, it is an interesting example of physical mechanism driving vesicle's membrane shape and size change.

1.3.2 Protocell Vesicle's Division

The first laboratory evidence of possible controlled division of protocell vesicles came from Luisi and colleagues (Berclaz et al. 2001); upon addition of oleic acid micelles to phospholipid vesicles, the diameter of the vesicles increased; at the end of the experiment, there were more vesicles present in the pool. The electron-dense protein ferritin was used as a marker of internal size of the vesicles. After the micelle addition, the vesicles were found not to contain any ferritin, or containing significantly less. This suggests the formation of new protocell vesicles during the process by the division of the grown original vesicles. This simple vesicle's division

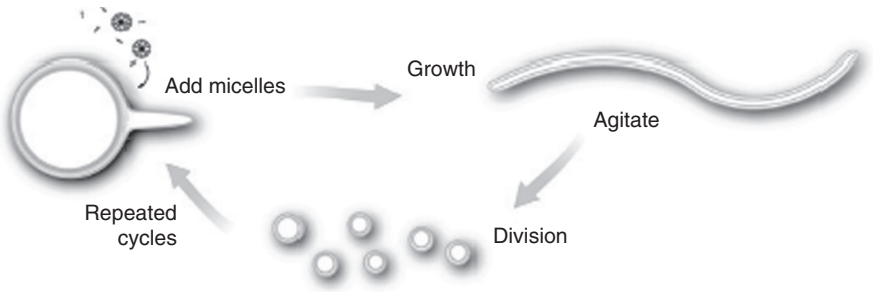

Fig. 1.4 The proposed mechanism of prebiotically plausible division of protocell vesicles (from Zhu and Szostak 2009)

is a proof of principle demonstration that protocell vesicles can be divided in a purely physical process, without any use of cell metabolism.

One of the simplest methods of protocell vesicle division is extrusion through porous cellulose membrane. This allows precise control of the size of daughter protocell vesicles, but a significant part of the contents encapsulated within vesicles is lost during the extrusion process. (Hanczyc et al. 2003) The extrusion is most commonly used during protocell "replication" experiments.

Upon addition of fatty acid micelles to previously formed large multilateral vesicles made of the same amphiphiles, vesicles develop thread-like structures, after gentle agitation, that separate and form new generation of protocell vesicles (Fig. 1.4). This process conserves the encapsulated contents of vesicles (Zhu and Szostak 2009) couples model protocell growth (by addition of micelles) and division.

If the acyltransferase enzyme is delivered inside the giant phospholipid vesicles, the 1-palmitoyl-sn-glycerol-3-phosphate is synthesized in the vesicles, and the change in membrane composition causes shrinkage of the parent vesicle. Also, small daughter vesicles are formed on the inner surface of the original giant vesicle (Wick and Luisi 1996). The inner protocell metabolism can be therefore coupled with the changes of the membrane shape and size and with the production of next generation of vesicles.

1.4 Conclusions

The cell cycle of modern cells, driven by complex networks of metabolic processes, must have originated in much simpler form in the protocell populations. The studies of protocell model systems can give insights into the origin and the underlying mechanisms of the modern cell cycle. Also, knowing the processes that have led to the origin of cellular life can help in the future in modifying cell cycle in different organisms and may be even designing entirely artificial cells.

References

Allen WV, Ponnamperuma C (1967) A possible prebiotic synthesis of monocarboxylic acids. Curr Mod Biol 1:24–28

Apel CL, Deamer DW, Mautner MN (2002) Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers. Biochim Biophys Acta 1559:1–9

Berclaz N, Muller M, Walde P, Luisi PL (2001) Growth and transformation of vesicles studied by ferritin labeling and cryotransmission electron microscopy. J Phys Chem B 105:1056–1064

Blochliger E, Blocher M, Walde P, Luisi PL (1998) Matrix effect in the size distribution of fatty acid vesicles. J Phys Chem B 102:10383–10390

Chakrabarti AC, Breaker RR, Joyce GF, Deamer DW (1994) Production of RNA by a polymerase protein encapsulated within phospholipid vesicles. J Mol Evol 39:555–559

Chen IA, Roberts RW, Szostak JW (2004) The emergence of competition between model protocells. Science 305:1474–1476

Chen IA, Szostak JW (2004) A kinetic study of the growth of fatty acid vesicles. Biophys J 87:988–998

Cheng Z, Luisi PL (2003) Coexistence and mutual competition of vesicles with different size distributions. J Phys Chem B 107(39):10940–10945

Deamer DW (1985) Boundary structures are formed by organic components of the Murchison carbonaceous chondrite. Nature 317:792–794

Deamer DW, Pashley RM (1989) Amphiphilic components of carbonaceous meteorites. Orig Life Evol Biosph 19:21–33

Delaye L, Lazcano A (2005) Prebiological evolution and the physics of the origin of life. Phys Life Rev 2(1):47–64

Hanczyc MH, Fujikawa SM, Szostak JW (2003) Experimental models of primitive cellular compartments: encapsulation, growth, and division. Science 302:618–622

Luisi PL (1998) About various definitions of life. Orig Life Evol Biosph 28:613–622

Luisi PL, Stano P, Rasi S (2004) A possible route to prebiotic vesicle reproduction. Artif Life 10:297–308

Luisi PL, Walde P, Oberholzer T (1999) Lipid vesicles as possible intermediates in the origin of life. Curr Opin Colloid Interface Sci 4:33

McCollom TM, Ritter G, Simoneit BT (1999) Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. Orig Life Evol Biosph 29:153–166

Morbidelli A et al (2000) Source regions and time scales for the delivery of water to earth. Meteorit Planet Sci 35(6):1309–1320

Oberholzer T, Luisi PL (2002) The use of liposomes for constructing cell models. J Biol Phys 28:733–744

Rasi S, Mavelli F, Luisi PL (2003) Cooperative micelle binding and matrix effect in oleate vesicle formation. J Phys Chem B 107:14068–14076

Schidlowski M (2001) Carbon Isotopes as biogeochemical recorders of life over 3.8 Ga of earth history: evolution of a concept. Precambrian Res 106:117–134

Schopf JW (1994) New evidence of the antiquity of life. Orig Life Evol Biosph 24(2–4)

Segre D, Ben-Eli D, Deamer DW, Lancet D (2001) The lipid world. Orig Life Evol Biosph 31:119–145

Takakura K, Sugawara T (2004) Membrane dynamics of a myelin-like giant multilamellar vesicle applicable to a self-reproducing system. Langmuir 20:3832–3834

Tawfik DS, Griffiths AD (1998) Man-made cell-like compartments for molecular evolution. Nat Biotechnol 16:652–656

Walde P (2006) Surfactant assemblies and their various possible roles for the origin(S) of life. Orig Life Evol Biosph 36:109–150

Wick R, Luisi PL (1996) Enzyme-containing liposomes can endogenously produce membrane-constituting lipids. Chem Biol 3:277–285
Yuen GU, Kvenvolden KA (1973) Monocarboxylic acids in Murray and Murchison carbonaceous meteorites. Nature 246:301–303
Yuen GU, Lawless JG, Edelson EHJ (1981) Quantification of monocarboxylic acids from a spark discharge synthesis. Mol Evol 17:43–47
Zhu TF, Szostak JW (2009) Coupled growth and division of model protocell membranes. J Am Chem Soc 131(15):5705–5713