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MDPI

Hypothesis

Symmetry Breaking of Phospholipids †

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Abstract: Either stereo reactants or stereo catalysis from achiral or chiral molecules are a prerequisite to obtain pure enantiomeric lipid derivatives. We reviewed a few plausibly organic syntheses of phospholipids under prebiotic conditions with special attention paid to the starting materials as pro-chiral dihydroxyacetone and dihydroxyacetone phosphate (DHAP), which are the key molecules to break symmetry in phospholipids. The advantages of homochiral membranes compared to those of heterochiral membranes were analysed in terms of specific recognition, optimal functions of enzymes, membrane fluidity and topological packing. All biological membranes contain enantiomerically pure lipids in modern bacteria, eukarya and archaea. The contemporary *archaea*, comprising of methanogens, halobacteria and thermoacidophiles, are living under extreme conditions reminiscent of primitive environment and may indicate the origin of one ancient evolution path of lipid biosynthesis. The analysis of the known lipid metabolism reveals that all modern cells including *archaea* synthetize enantiomerically pure lipid precursors from prochiral DHAP. *Sn*-glycerol-1-phosphate dehydrogenase (G1PDH), usually found in *archaea*, catalyses the formation of *sn*-glycerol-1-phosphate (G1P), while *sn*-glycerol-3-phosphate dehydrogenase (G3PDH) catalyses the formation of *sn*-glycerol-3-phosphate (G3P) in *bacteria* and *eukarya*. The selective enzymatic activity seems to be the main strategy that evolution retained to obtain enantiomerically pure lipids. The occurrence of two genes encoding for G1PDH and G3PDH served to build up an evolutionary tree being the basis of our hypothesis article focusing on the evolution of these two genes. Gene encoding for G3PDH in *eukarya* may originate from G3PDH gene found in rare *archaea* indicating that *archaea* appeared earlier in the evolutionary tree than *eukarya*. *Archaea* and *bacteria* evolved probably separately, due to their distinct respective genes coding for G1PDH and G3PDH. We propose that prochiral DHAP is an essential molecule since it provides a convergent link between G1DPH and G3PDH. The synthesis of enantiopure phospholipids from DHAP appeared probably firstly in the presence of chemical catalysts, before being catalysed by enzymes which were the products of later Darwinian selection. The enzymes were probably selected for their efficient catalytic activities during evolution from large libraries of vesicles containing amino acids, carbohydrates, nucleic acids, lipids, and meteorite components that induced symmetry imbalance.

Keywords: symmetry breaking; dihydroxyacetone phosphate; *sn*-glycerol-1-phosphate dehydrogenase; *sn*-glycerol-3-phosphate dehydrogenase; membrane evolution

1. Introduction

This hypothesis, focusing on how phospholipid symmetry breaking occurs, was intended to complement our experimental paper on racemic phospholipids for the origin of life published in the Special Issue entitled "Chirality and the Origin of Life" [1]. Our hypothesis is based on the evolution

of lipid synthesis from raw materials leading to racemic lipids toward the actual occurrence of chiral lipids in all living species. The background of our hypothesis is divided in four parts: (1) the advantage to be homochiral; (2) prebiotic scenarios for the symmetry imbalance of phospholipid precursors; (3) achiral and racemic amphiphiles; and (4) biological synthesis in archaea, bacteria and eukarya. We speculated that the biosynthesis of racemic lipids is less efficient than that of enantiomeric lipids. We will discuss critically the hypothesis by providing scientific evidence to support it.

Studies on the origin of life have been carried out in several directions including dynamic combinatorial chemistry [2], self-assembly and self-organization [3,4], prebiotic chemistry [5–8], minimal self-replicating molecules [9], autocatalytic systems [10], and the assembly of metabolic and non-metabolic networks [11,12]. The origin of chirality was considered only on a theoretical level with a few exceptions for the abiotic formation of nucleotides [13,14]. A few examples were reported for phospholipids and model membranes [15,16]. In evolved cells, enantiopure membranes are produced in living organisms, which are supramolecular chemical systems that maintain persistent structures and reaction networks through reproduction rather than thermodynamic stability [17]. Although enantiomorphism in crystals is one of the most supposed sources of homochirality of organic compounds on Earth [18], alternate theories have been proposed, such as the enantiomeric cross inhibition [19], for example.

2. The "Advantage" of Being Homochiral

Homochirality has an effect with respect to heterochirality, most strongly at the aggregate or polymer level [20]. Chemical and physical properties of homo- or heterochiral monomers are not sufficiently distinct from each other unless they form aggregates or polymers. The strongest effect is expected to be exerted by dense packing. Crystals can be enantiomorphic in 100% [18], exacerbating more distinct properties than in case of racemic crystals. Heterochiral and homochiral membranes have significant distinct properties in packaging organizations as in lipid rafts and in membrane permeability [1,21]. Phospholipids are aggregates organized as bilayer membranes [22]. Recent investigations showed that the homochirality packaging of phospholipids in prebiotic protocells [20] was not a necessary prerequisite to build up the first protocells. It was sufficient to have compartment property of the heterochiral membranes [1]. For example, bilayers and vesicles composed of heterochiral lipids have a useful permeability, since these bilayers are looser than the more compact homochiral bilayers [1,21]. Such a permeability property could serve to filter and select possible materials to build up primitive organic components, including carbohydrates, lipids, nucleic bases, amino acids and their derivatives. So why are biological membranes made of homochiral phospholipids? The "advantage" of membranes being homochiral rather than heterochiral is that it probably leads to optimal functions of lipophilic peptides and transmembrane proteins. Indeed, the fluidity of the membrane, the specific recognition of lipids with various ligands and lipid raft organization finely tune up enzymatic activities that are significantly different in homo and in heterochiral membranes [21,23]. Lipid composition can modulate and affect enzyme activity, even within homochiral membranes [24].

Several theories concerning membrane compositions in the primitive last common ancestor (LCA) were reviewed [25,26]. Among them, it was proposed that earlier life forms were dependent on the presence of membrane lipids with an isoprenoid hydrocarbon core, especially since fatty acid metabolism is underdeveloped in archaea. Other theories advocated that the most divergent feature is the glycerophosphate backbone [26,27]. Indeed, there is one chiral centre in the glycerol moiety leading to either G1P or G3P phospholipid derivatives, forming the basis of the "lipid divide" theory [26,27]. The key precursors for the biosynthesis of phospholipids in living species is the prochiral dihydroxy acetone phosphate (DHAP) [26–28]. Any type of oxidation from DHAP would lead to racemic species as well as to pure enantiomeric species.

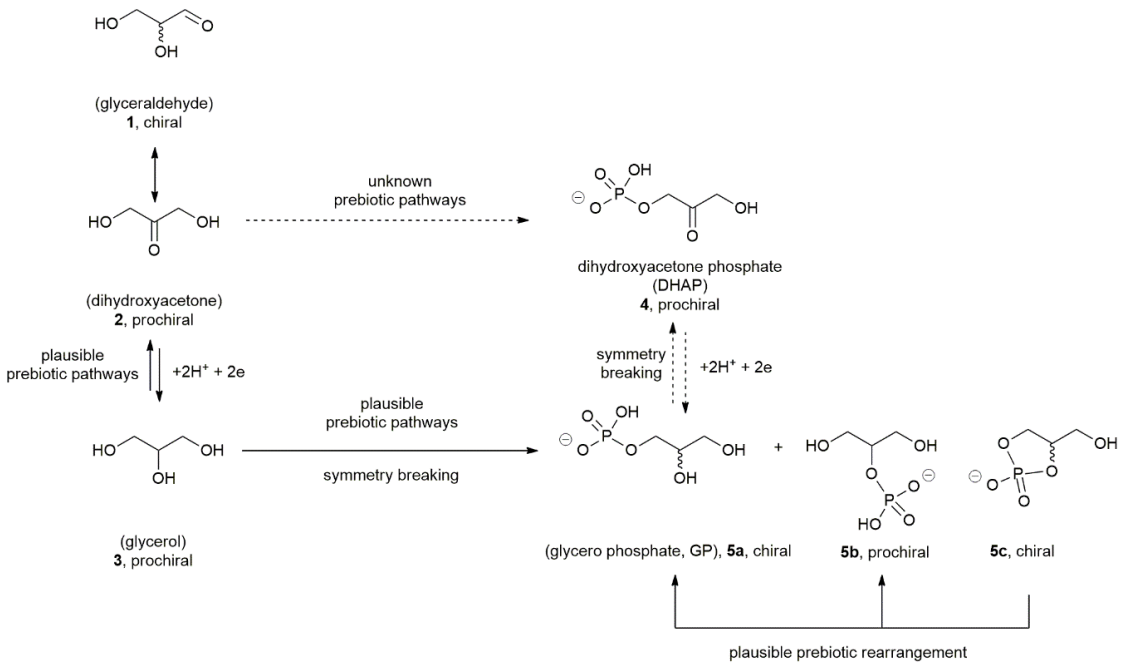
Molecules react according to materials and conditions in their proximity. Modern cells are well evolved biochemical machines and the chemical processes are carried out by enzymes, which determine

the path of the reactions. However, prebiotic mechanisms in LCA protocells [29–32], were probably not necessarily similar to the actual ones. System protobiology suggests that lipids played a fundamental role in the emergence of life. Thus, in a hypothetical racemic lipid word, life emerged thanks to the compartmentalization of simple lipophilic or poorly hydrophilic small proteins that showed a catalytic role together with auto-replicative functional nucleic acids [33,34].

3. Prebiotic Scenarios for the Symmetry Imbalance of Phospholipids Precursors

Speculations about where and how life emerged from a primordial soup of abiotic mixtures of molecules are extremely well reviewed and summarized including some aspects on the biological origin of chirality [5,35]. One main conclusion was that all chiral molecules can be formed in both enantiomeric types suggesting that a symmetry imbalance between the two possible stereoisomers occurred. Prebiotic symmetry breaking scenarios were depicted using mathematical models only [20].

Concerning the synthesis of life's building blocks, Meierhenrich and co-workers showed that the exposure of circularly polarized light (CPL) in simulated interstellar media can induce a mirror symmetry breaking between the formation of L- or D-alanine where the imbalance depends from the wavelength of the incident CPL and sense of rotation [36,37]. Further investigations showed that glyceraldehyde (**1**, Scheme 1), the first chiral product of the "formose" reaction [38]—one of the chemical pathways for the synthesis of carbohydrates—is present in comets and other space bodies [37]. It is probable that the symmetry imbalance between the two possible stereoisomers of **1** occurred before seeding the Earth by asteroids' or comets' impact [39] creating the conditions for deracemization before the prebiotic polymerization of peptides and the formation of nucleic acids. Glyceraldehyde (**1**), dihydroxyacetone (**2**) and glycerol (**3**) together with their phosphate derivatives (**4** and **5a–c**) are the most plausibly chemical precursors of glycero phospholipids such as phospholipid esters and ethers (Scheme 1).



Scheme 1. Plausibly prebiotic pathways for glycerophosphates (**5a–5c**), precursor of phospholipids, from glyceraldehyde, dihydroxyacetone or glycerol (**1–3**). Plausibly prebiotic pathway allowing DHAP (**4**) from glyceraldehyde (**1**) remains unexplored.

In a well studied prebiotic scenario, Sutherland and co-workers, among others, showed that **1** can be one of the plausible precursors of **5a** together with ribonucleosides and a few amino acids such as

valine and leucine [40]. DHAP (**4**), instead, was hypothesized to be a key intermediate in the prebiotic synthesis 3-pentulose and racemic mixtures of erythrulose [41].

Glyceraldehyde (**1**) and its tautomer (**2**) (double arrow in Scheme 1) are precursors of glycerol (**3**) which is the reduced form of **2**. The redox reactions (Equations (1)–(6)) that occur should be a key step for stereochemistry imbalance during the phosphorylation or oxidation of glycerol [42] (**3**→**5a** and **5c**, Scheme 1). The synthesis of DHAP (**4**) under plausibly prebiotic reaction conditions is not reported, while the synthesis of glycerol (**3**) in interstellar ices was simulated instead [43], suggesting that glycerol is plausibly present in space bodies.

The oxidation reaction can be summarized as a loss of electrons in either chemistry or biochemistry. In several biological reactions, as in lipid beta oxidation, glycolysis and the Krebs cycle, the electrons are transferred via cofactor FAD or $NAD(P)^+$. The reduction process is conducted via $FADH_2$ or NAD(P)H which can recycle the cofactors for a next round of oxidation. These reactions can be found everywhere in archaea, bacteria and eukarya, suggesting that it was one of the most efficient oxidation or reduction mechanisms that evolution maintained. The oxidation and the reduction of **2** and **3** can be written in analogy with respect to NAD^+/NADH processes.

Concerning the reduction of **2**, where R^1 and R^2 are CH_2OH, respectively:

$$R^1\text{-}C{=}(O)\text{-}R^2 + 2H^+ + 2e^- = R^1\text{-}CH(OH)\text{-}R^2 \quad (1)$$

$$NAD(P)H + H^+ = NAD(P)^+ + 2e^- + 2H^+ \quad (2)$$

$$R^1\text{-}C{=}(O)\text{-}R^2 + NAD(P)H + H^+ = R^1\text{-}CH(OH)\text{-}R^2 + NAD(P)^+ \quad (3)$$

Concerning the oxidation of **3**, where R^1 and R^2 are CH_2OH, respectively:

$$R^1\text{-}CH(OH)\text{-}R^2 = R^1\text{-}C = (O)\text{-}R^2 + 2H^+ + 2e^- \quad (4)$$

$$NAD(P)^+ + 2e^- + 2H^+ = NAD(P)H + H^+ \quad (5)$$

$$R^1\text{-}CH(OH)\text{-}R^2 + NAD(P)^+ = R^1\text{-}C{=}(O)\text{-}R^2 + NAD(P)H + H^+ \quad (6)$$

Tricyanocuprate $[Cu(CN)_3]^2$ and tetracyanocuprate $[Cu(CN)_4]^2$ are supposed to be a source of electrons for the oxidoreduction of glyceraldehyde (**1**, Scheme 1) in enzyme-free conditions [44–46]. The hydrogen cyanide–cyanocuprate photochemistry has been proven to be effective for synthesis in abiotic conditions of glyceraldehyde precursors starting the oxido-reduction of **1** into **2** then **3**, respectively (Scheme 1). However, this system cannot lead to any symmetry imbalance of **1** in the absence of any chiral inductor even if deracemization or interconversion can occur using photocatalysis reaction conditions [47]. Iron (III)-sulfur-L-glutathione complexes are able to oxidize NADPH in catalytic networks across the membrane of model protocells made of (*R*)-POPC and oleic acid [48]. This suggests that simple but effective catalytic networks probably existed in protocells, before the advent of the LCA. Ferredoxins are one of the most known metallo-proteins and their sequences are well known as three of them were isolated from fermentative bacteria [49]. The presence of a high percentage (>64%) of plausibly prebiotic amino acids in their sequence [50] such as glycine, alanine, valine, proline, glutamic and aspartic acids together with cysteine [51], indicates that short hydrophobic peptides, able to complex iron (III) could have been precursors of ferredoxins in LCA. These peptides in the presence of iron (III) formed aggregates [52] that could perform redox reactions as those with NAD^+/NADH in evolved cells. Such peptides may have been formed from the scalemic mixtures of amino acids due to the symmetry imbalance possibly induced by meteorite and comet seedings. The scalemic ratio between each D- and L-amino acid was plausibly improved by CPL [39] or from the presence of enantiomorphic crystals. Thus, amino acid sequences within peptides were selected on the basis of their emerging functions or properties. Their selections should have occurred in large libraries of vesicles containing various biopolymers during evolution (cf. Section 4). In our hypothesis, non-functional sequences were discarded in favour of enantiopure sequences probably

due to their distinct structural properties. For example, homo peptides with either pure D- or L amino acid sequences more favourably induce alpha-helix structures than hetero peptides with alternate or stochastic D- and L-amino acids (LDLD ... or ... LDLL ... or ... DDLD ... etc.). The emblematic case is Gramicidin A, containing alternating L and D residues, which does not form alpha-helix structures but beta-helix structures with C–O moieties of the L residues parallel to helix axis, whereas for the D residues, they are antiparallel to it [53]. This is due to the fact that bulky side chains shall be positioned to the outside helix axis, otherwise bulky side-chain residues positioned inside the helix destabilize the helical structure. Not only is the structural topology of homo peptides different from that of hetero peptides, but their possibilities to interact with charged groups or to form hydrogen bonds are distinct due to the positions of polar groups. The enantiomeric excess in the peptide might have been amplified by autocatalytic pathways, gradually favouring the formation of a peptide containing the first dominating enantiomer [11,30,50–52] yielding chemical environments in which the predominance of one enantiopure sequence of peptides was preferred.

4. Achiral and Racemic Amphiphiles

4.1. Non-Chiral Amphiphiles

Obviously, the formation of large vesicles, precursors of protocells [29], occurred before the rise of full-fledged cells, since vesicles form spontaneously in aqueous solution from a variety of surfactants [54]. Closed membranes exert the confinement and protection of an internalised chemical network including reactions on their hydrophobic region [25,28,55]. According to the current view, early membranes were more likely formed from derivatives of alkanols, Ref. [56] fatty acids, Ref. [57] mono-alkyl phosphates, Ref. [58] and isoprenoids [59]. Most probably, they were composed of a mixture of components [60] (Figure 1).

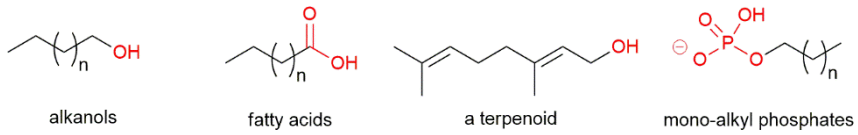
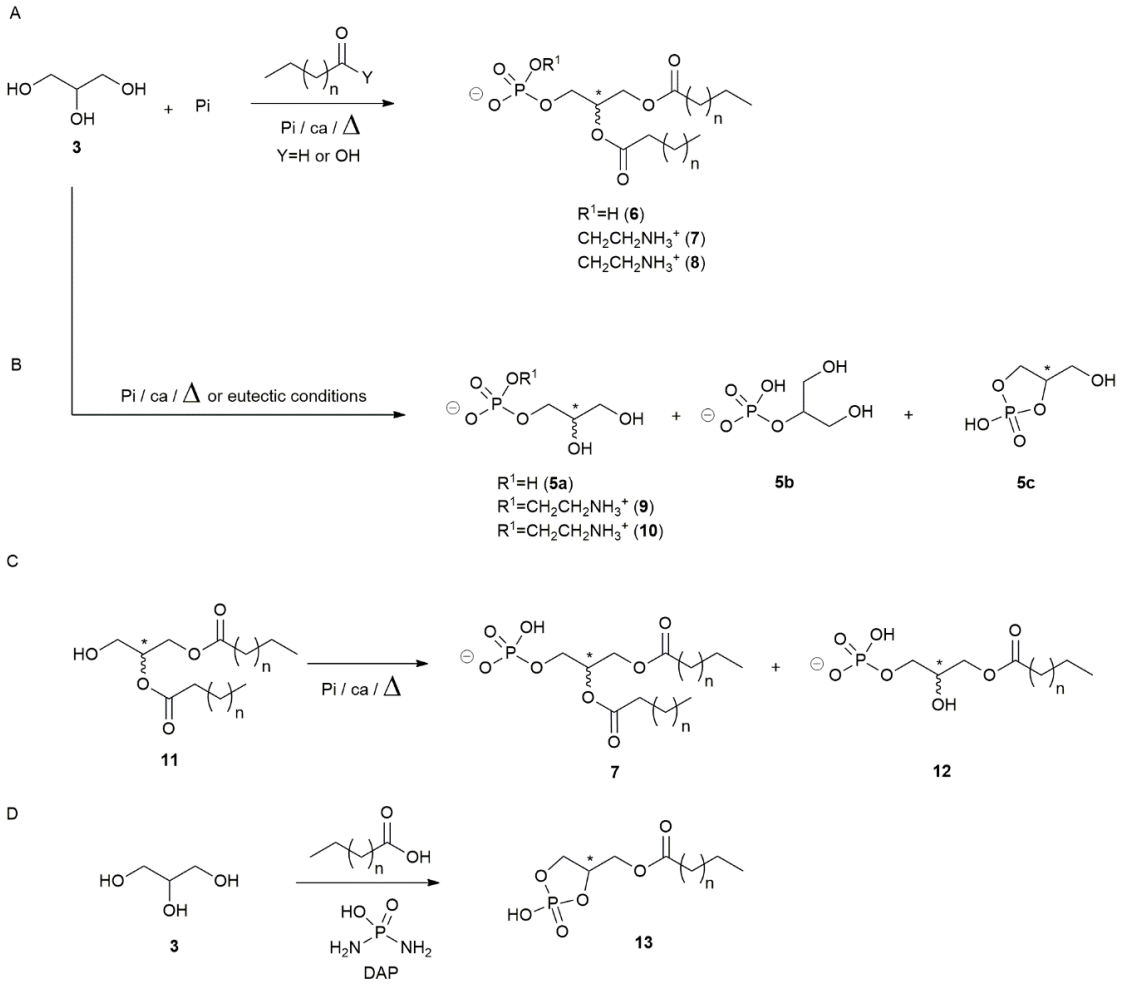


Figure 1. Plausibly prebiotic lipid derivatives. Red colour is used to indicate the polar head group.

4.2. Racemic Amphiphiles and Their Precursors

Several plausibly prebiotic syntheses were explored, however all the proposed pathways, carried out in enzyme free conditions from glycerol (**3**), yielded racemic phospholipids (**6**–**8**, Scheme 2A) [56,58,61–65] or racemic mixtures of glycerol phosphates (**5a**–**c** and **9**–**10**, Scheme 2B [40,66–70]. In addition, the symmetry imbalance between the *R*:*S* ratio of mono- and dialky phosphates (**6** and **12**, Scheme 2C) and cyclic glycerophosphates (cGP, **13**, Scheme 2D) [71,72] from di-acyl glycerols **12** or glycerol **3**, respectively, were not reported or investigated either. Remarkably, all the crude mixtures containing **6**–**8**, **12** and **13** were able, using appropriate buffers, to form giant vesicles that per sizes and membrane properties are similar to those of the bilayer of modern cells.



Scheme 2. A few relevant prebiotic pathways that allow the formation of phospholipid esters and glycerol phosphates. (**A**) Summary of the prebiotic pathways explored during pioneering research (1977–1982); (**B**) phosphorylation of glycerol; (**C**) recent results obtained in phosphorylation of diacylglycerols and (**D**) concomitant acylation of glycerol in the presence of fatty acids and diamidophospahte. The asterisk (*) indicates the stereogenic carbon C2 of any phospholipid and phospholipid precursors; **Pi** stands for any phosphorous salt or plausibly phosphate-containing mineral able to promote the phosphorylation of primary or secondary alcohols [42]; **ca**, stands for any condensing agents [56]; **Δ**, stands for temperatures between 65 and 130 °C; **DAP** stands for diamidophosphate [72]; for eutectic conditions see the recent works of Menor–Salvan and Pasek [73,74].

5. Biological Synthesis in *Archaea*, *Bacteria* and *Eukarya*

5.1. Lipid Characteristics in Archaea, Bacteria and Eukarya

One essential characteristic of living species is their ability to create a compartmentalization of bioactive molecules [75–77]. The natural enantiomer of all phosphatidate derivatives, in *eukarya* and in most of *bacteria*, is D-diacylglycerol phosphate (Fischer convention), 1,2-diacyl-*sn*-glycerol-3-phosphate (*sn*-glycerol nomenclature) [78] or 2*R*—in the Cahn–Ingold–Prelog formalism (Figure 2) [79,80]. The opposite configuration is L-diacylglycerol phosphate, 2,3-diacyl-*sn*-glycerol-1-phosphate or (2*S*), which occurs mostly in *archaea* membranes. The *archaea* phospholipids usually contain isoprenoid glycerol ethers instead of hydrocarbon glycerol esters [81].

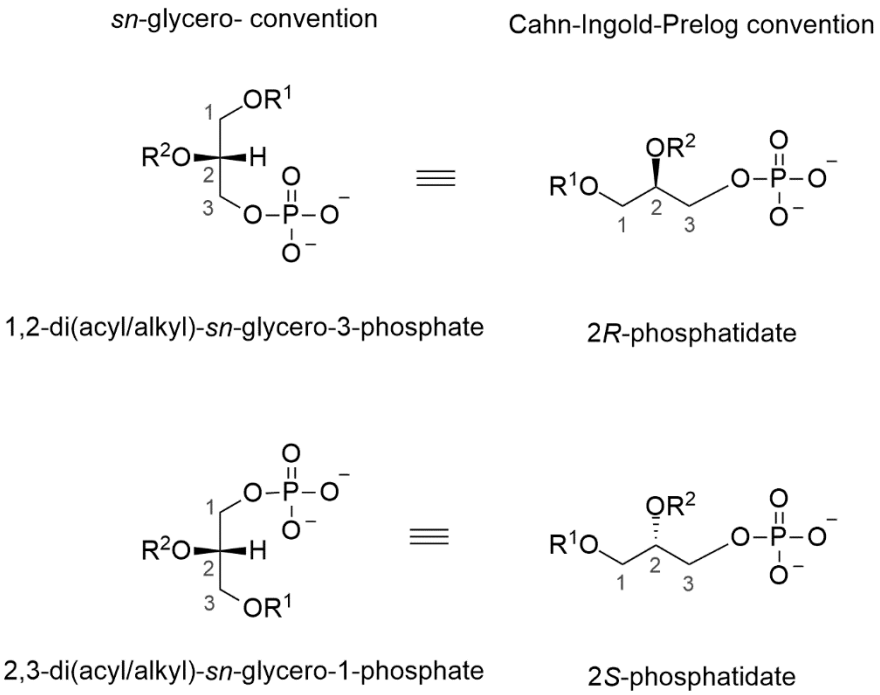


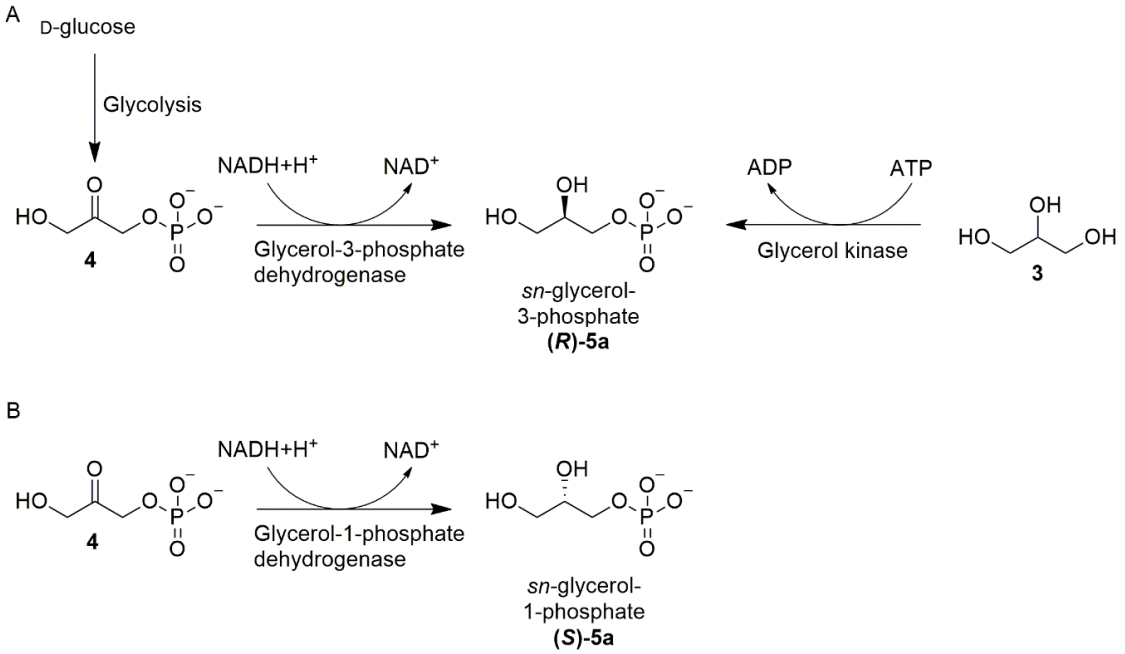
Figure 2. Phosphaditate enantiomers and their *sn*-glycerol and Cahn–Ingold–Prelog nomenclatures. 1,2-diacyl-*sn*-glycero-3-phosphate is the enantiomer of 2,3-diacyl-*sn*-1-glycerophosphate: the stereo numbering (*sn*-glycerol) is based on the position of the second oxygen of the glycerol moiety to the left side in the Fisher representation, with the top carbon numbered as one, second as two and the bottom carbon numbered as three, the enantiomer changes the order of numbers of glycerol moiety due to the opposite position of the second oxygen.

Last common ancestor (LCA) or *Commonote Commonote* (*C. Commonote*) [82], lived at around 3.5–3.8 Ga. There are still controversies about the environment where the LCA lived [83]. A sulphur-containing atmosphere [84–86] together with CO_2, H_2, N_2 and CH_4 [87] is the most probable. Contemporary *archaea*, comprising of methanogens (which generate actually around 85% of the methane in Earth's atmosphere), halobacteria and thermoacidophiles are living under extreme conditions reminiscent of this primitive environment. These descendants are phylogenetically related to each other, while they share very little phylogenetic characteristics with *bacteria* and *eukarya* [25,26]. *C. Commonote* had archaeal and bacterial characteristics [88–91], while *eukarya* evolved from *archaea* [88,90,91]. There is an open debate between three domains of life, *archaea*, *bacteria* and *eukarya* which evolved separately from LCA versus Eocyte hypothesis where *eukarya* are descendent of prokaryotic *Crenarchaeota* [92] or other evolution models [26,91]. The origin of the controversy lies in the inconsistencies of the phylogenetic distributions and in the selection of appropriate genes to build up the phylogenetic tree [26,91,93]. Here, we focus on the phylogenetic tree based from the genes that encode *sn*-glycerol-1-phosphate dehydrogenase (G1DPH) or *sn*-glycerol-3-phosphate dehydrogenase (G3DPH), enzymes catalysing, respectively, *sn*-glycerol-1-phosphate (G1P) and *sn*-glycerol-3-phosphate (G3P) from pro-chiral DHAP. The reason to focus on the two genes for encoding G1DPH and G3DPH in this review is that G1P and G3P are key precursors of phospholipids and are essential to determine the mechanisms of symmetry breaking. The lipid composition in *archaea* is distinct from those in *bacteria* and *eukarya* [26,91]. *Archaea* membranes contain usually phospholipids having G1P moiety and isoprenoid hydrocarbon chains ether-linked to the G1P moiety [26], whereas membranes in *bacteria* and *eukarya* are usually composed of phospholipids derived from G3P and alkanoyl chains ester-linked to the G3P moiety (Figure 3) [25,26].

5.2. Appearance of Homochiral Membranes Based on Phylogenetic Analysis on Enzymes Forming sn-Glycerol-1-phosphate or sn-Glycerol-3-phosphate

One likely path of lipid synthesis at the appearance of the extremophile LCA is the geochemical production of racemic lipids via non-catalytic or catalytic, but enzyme-free pathways, giving rise to racemic membranes (Figure 3). Then, the appearance of homochiral membranes from, probably later in the evolution, in *archaea*, signals a selective catalytic activity that could have been initiated by non-enzymatic or enzymatic ways [77].

Pro-chiral DHAP, (**4**), is a starting material for the synthesis of lipids in all the three domains of life: *archaea*, *bacteria* and *eukarya*. The first step to obtain phospholipid precursors in *archaea* is the hydrogenation catalysed by G1PDH which gives G1P with NADH + H^+ as proton donors (Scheme 3B). In *bacteria* and *eukarya*, the first step to obtain the phospholipid precursors is catalysed by a G3PDH giving rise to G3P (Scheme 3A) [25,26].



Scheme 3. Biosynthetic pathways leading to G3P (**A**) and G1P (**B**) from prochiral glycerol (**3**) or DHAP (**4**).

Generally, there are two biosynthetic pathways to obtain G3P in *bacteria* and in *eukarya*, (Scheme 3A) while there is only one to obtain G1P in *archaea* (Scheme 3B) [25,26]. G3P can be produced from glycerol and is catalysed by a glycerol kinase (GK) in bacteria and eukarya, while *archaea* lacks GK [26]. To the best of our knowledge, there is no GK producing G1P. To date, the catalytic activity of the one and the same enzyme producing both G1P and G3P from glycerol, if it existed, was not retained during evolution. The genes coding for G1PDH, G3PDH and GK in *archaea* and *bacteria* are used to construct phylogenetic trees [26,28,94,95]. This reveals possible evolutions of synthetic phospholipid pathways from a common ancestor [92]. Several models, based on the occurrence of G1P-lipids or G3P-lipids, were inferred from the presence of either G1PDH or G3PDH [25,26,96,97]. The separate evolution of G1PDH or G3PDH is supported from several phylogenetic analyses [26,28,94,95]. We summarize a few facts from these reports in this Section. According to the phylogenetic analyses, it seems that there is no any direct evidence that *C. commonote* could form G1P lipids via enzymatic reactions [26]. Among the *Commonote* ancestors having chiral membranes, *Commonote archaea* (*C. archaea*) were probably the first biological entities to be formed, since they could live with little concentration of O_2. The early stage of the archaeal lineage, had G3DPH (GLpA/GlpD) leading to G3P lipid membranes instead of G1P lipid membranes (Figure 3) [26]. In the next stage of the evolution

path, the archaeal lineage acquired G1PDH (Egs A) probably leading to a population of archaeal lineage mixed with GIPDH and G3PDH [26]. The archaeal descendants are phylogenetically related to each other, while they share very little phylogenetic characteristics with *bacteria*, Refs. [89,90,98,99] suggesting that both living organisms *C. archaea and Commonote bacteria (C. bacteria)* evolved separately (Figure 3). *Commonote eukarya* (*C. eukarya*) could have appeared much later than *C. archaea* since it was suggested that *eukarya* originated from *archaea* [88,90,100], consistent with the eocyte hypothesis [92]. *Lokiarchaeta* is closely related to *eukarya* because of the absence of gene for coding G1PDH, and the presence of a gene coding for G3PDH [100,101], which is rarely observed in *archaea* (Figure 3). This supports the evidence that *eukarya* originated from *archaea*.

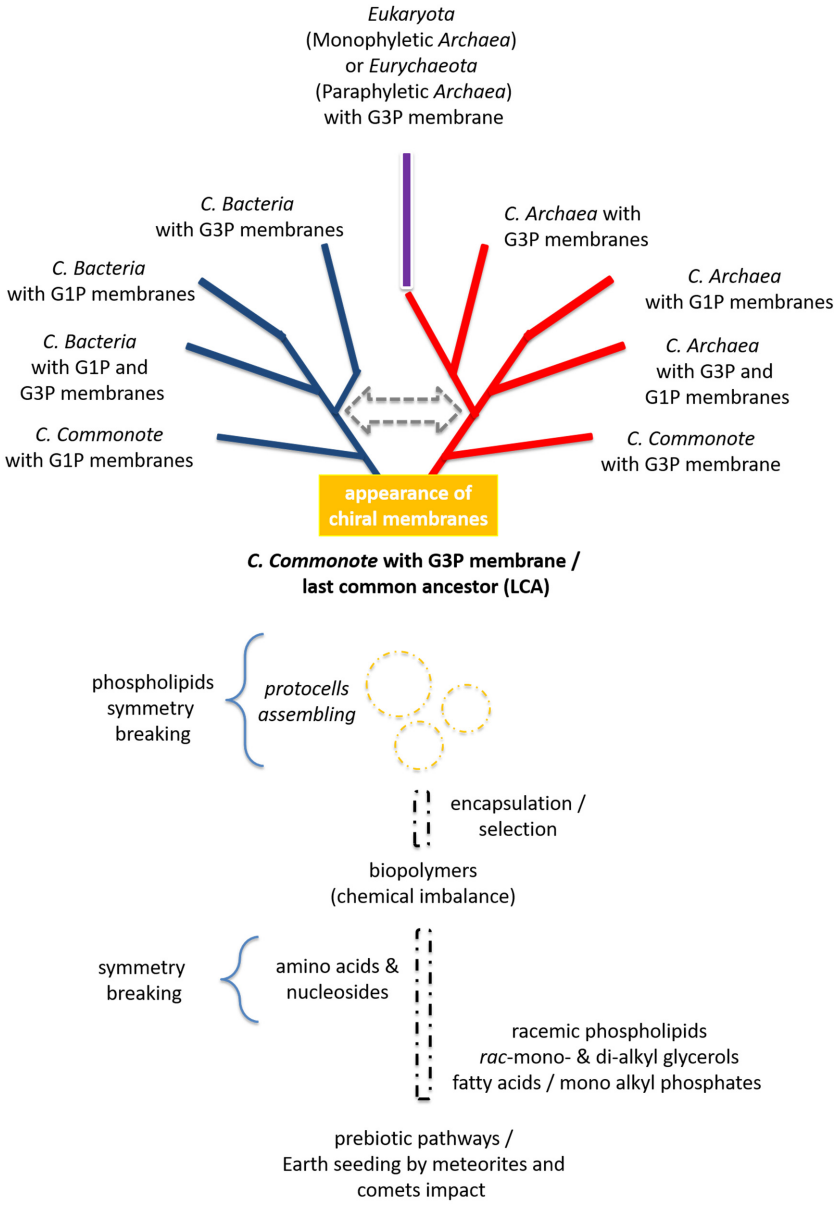


Figure 3. Hypothetic phylogeny of the last common ancestor (LCA) and *Commonnote Commonote* and their evolution into *Archaea*, *Bacteria* and *Eukarya* or *Euryarchaeotae* from prebiotic pathways, adapted from [26]. The early stage of the archaeal lineage had G3PDH so that the ancestor *C. commonote* had a G3P polar lipid membrane rather than G1P lipid membranes, giving rise to *C. archaea.* Then, *C archaea*, had G1P lipids, probably mixed with G3P lipids [26]. *C. bacteria* appeared later than *C archaea* [26]. Hypothetical horizontal gene transfer (indicated by dashed grey arrows) may have occurred [102]. *Eukarya* was significantly distinct from *bacteria* and may have originated from *Archaea* [88,90,100].

During, the evolutionary path, there is the possibility that genes could have been horizontally exchanged (grey dashed lines between two arms of the evolution tree in Figure 3) [102]. This is supported by the fact that in certain bacterial lineages GIPDH (Egs A) having stereospecific synthesis of G1P from DHAP, was acquired as another lineage GIPDH (AraM) [26]. On the other hand, in certain archeal lineages G3PDH (GpsA), which is the major G3PDH of modern bacterial species, was acquired via horizontal transfer [26]. G3PDH homologs such as GlpA and GlpD, found in various eukaryotic cells, are involved in glycerol shuttle and not in the formation of G3P in cellular membrane [26]. Therefore, the horizontal transfer of genes coding for the enzymes could have resulted in a shift of enzymatic activity. G1PDH and G3PDH may coexist in *C. bacteria* or in *C. archaea* (Figure 3) [26]. Although it is rarely observed. These observations suggest that the choice of which kind of chirality, that of G1P or that of G3P, was not accidental but resulted from an efficient catalytic activity that was retained during the evolutionary process from LCA and *C. commonote*. Of interest, the evolution retained both catalytic activities. However, G1PDH and G3PDH genes are different, suggesting that *archaea* and *bacteria* evolved apart from one another (Figure 3). This is supported by the fact that G1PDH and G3PDH are not "mirror-image" enzymes since both had L amino acids. Indeed, G1PDH belongs to a larger structurally related superfamily comprising of NAD(P)H-dependent hydrogenases, including alcohol dehydrogenase, UDP-glucose 6-dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and dehydroquinate synthase, which are all unrelated to G3PDH [25,26]. Since G1PDH and G3PDH are not homologs, then one shall ask what is the evidence of convergent evolution? The convergence lies in the fact that both enzymes use the same substrate that is DHAP to yield either G1P or G3P phospholipid derivatives [26]. Furthermore, both enzymes have L amino acids indicating a common origin. Taken together, these facts indicate that G1PDH and G3PDH occurred much later in the evolution path than LCA and were retained during Darwinian evolution (Figure 3).

6. Conclusions

The question of why living species did not retain racemic lipids to form their membranes during the evolution path remains unanswered, as thus far only mathematical models have been used. We speculate that the biosynthesis of racemic lipids is less efficient than that of enantiomeric lipids. Indeed, only G1DPH and G3DPH enzymes leading to their respective enantiomeric G1P and G3P are actually observed in all living systems, while there are no enzymes producing a racemic mixture of G1P and G3P from DHAP. G1DPH and G3DPH evolved apart from one another since they are structurally different and are not "mirror image" enzymes. These enzymes, which appeared much later in the evolution path than the LCA, are essential, since they catalyse the formation of phospholipid precursors G1P and G3P from the pro-chiral DHAP. Indeed, DHAP is a key molecule for phospholipid metabolism. What is missing is why the Darwinian selection retained only these enzymes, G1DPH and G3PDH? To fill the gap between LCA, that probably possessed racemic membranes, and protocells with homochiral membranes, several hypothesises could be formulated. Apparently racemic membranes do not have the same properties as those in enantiomeric membranes due to their distinct ability to form lipid rafts, recognition process and packing organizations [21,23]. From a chemistry perspective, several aspects of this problem could be tackled. Firstly, organic synthesis from DHAP yielding racemic and enantiomeric lipid precursors under prebiotic conditions shall provide more insight into their mechanisms and efficiencies. Secondly, further analysis on the physico-chemical properties of vesicles made either from racemic or enantiomerically pure lipids may support the notion that the overall property of membranes made either by racemic and or enantiomeric lipids are distinct.

To conclude, our hypothesis speculates that the chemical evolution of proteins [103–105] allowed the biosynthesis of enantiomeric lipids [33]. Large libraries of vesicles containing biopolymers including amino acids, carbohydrates, nucleotides or other meteorite materials may have served as possible sources of symmetry imbalance (Figure 4).

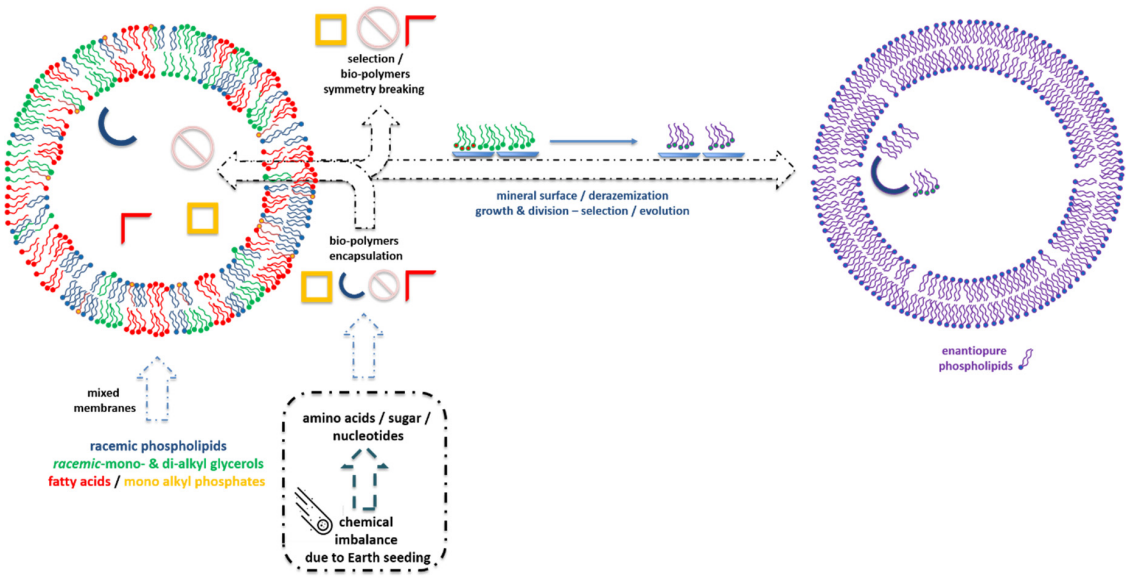


Figure 4. A hypothetical pathway allowing the selection trough the formation of enantiopure phospholipids and deracemization of mixed protocell membranes upon the encapsulation of enantiopure biopolymers (geometrical forms) followed by the growth and division of membrane bilayers. Colour code is used to better highlight the symmetry imbalance from racemic (green) to enantiopure (violet).

Not only homochiral vesicles, but also vesicles made of racemic phospholipids or mixed achiral amphiphiles may contribute to the selection process of retaining the best enzymes able to catalyse a reaction from achiral DHAP to form enantiopure lipid precursors (Figure 4). The synthesis of enantiopure phospholipids occurred firstly in the presence of chemical catalysts, before being catalysed by enzymes which are the products of a later evolution stage. Symmetry imbalance in the deracemization of racemic mono- or di-alkyl glycerol could appear at different stages of the evolution process, driven by interaction on mineral surfaces [106] such as graphene. The growth and division of lipid boundaries [107] and the formation of enantiomerically pure vesicles drastically contributed to this selection process. Further studies on the symmetry breaking of phospholipids in protocell membranes can be carried out using synthetic protocells where the transmission of catalytic protein can be controlled under selection processes upon growth and division experiments [107–111]. The compartmentalization of primitive enzyme-free or enzymic molecular replicators, inside the organelles and/or protocells, was probably one of several strategies that evolution retained for the Darwinian selection processes.

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