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Hypothesis

Symmetry Breaking of Phospholipids

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Abstract: Either stereo reactants or stereo catalysis from achiral or chiral molecules are prerequisite to obtain pure enantiomeric lipid derivatives. We reviewed a few plausible organic syntheses of phospholipids under prebiotic conditions with a special attention 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 term of specific recognition, optimal functions of enzymes, membrane fluidity and topological packing. All biological membranes contain enantiomeric 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 lipid metabolism reveals that all modern cells including *archaea* synthetize enantiomeric 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 enantiomeric pure lipids. The occurrence of two genes encoding for G1PDH and G3PDH, served to build up an evolution tree and the basis of our review 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 evolution tree than *eukarya*. *Archaea* and *bacteria* evolved probably separately, due to their distinct respective genes coding for G1PDH and G3PDH. The suggested hypothesis is that catalysis of homochiral G1P or G3P from DHAP are more efficient than those leading to racemic G1P and G3P, since there are no enzymes able to synthesize racemic G1P and G3P from DHAP. We propose that G1PDH or G3DPH, which are not "image mirror enzymes" but belonging to distinct family of proteins, emerged separately during evolution. They were probably selected for their efficient catalytic activities during evolution from large libraries of vesicles containing various biopolymers, including amino acids, carbohydrates, nucleic acids, lipids, and meteorite components to induce chemical 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 phospholipids symmetry breaking occurred, is intended to complement our experimental paper on racemic phospholipids [1] for origin of life published in the special issue entitled "Chirality and the Origin of Life". Researches 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 trough the assembly of metabolic and non-metabolic networks[11,12]. The origin of chirality was considered only at 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.

2. The "*advantage*" to be homochiral

Homochirality has an effect with respect to heterochirality, most strongly on an aggregate or polymer level[19]. Chemical and physical properties of homo- or hetero-chiral monomers are not sufficiently distinct to 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 organization as in lipid rafts and in membrane permeability [1,15]. Phospholipids are aggregates organized as bilayer membranes [20]. The "*advantage*" of being homochiral instead of being heterochiral leaded to optimal functions of peptides and proteins[21,22], since fluidity of bilayer membranes can affect enzymatic activities. In addition, specific recognition of lipids with various ligands and lipid raft organization are essential properties to finely tune up enzymatic activities that are significantly different in homo and in hetero chiral membranes. However, recent investigations showed that homochirality packaging of phospholipids in prebiotic protocells [23] was not necessary a prerequisite, since 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. Such 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 answer of this question forms the basis of our hypothesis. One of the key precursors for the biosynthesis of phospholipids in living species is dihydroxy acetone phosphate (DHAP). Any type of oxidative catalysis from DHAP would lead to racemic species as well as to pure enantiomeric species. One possible answer to this question is that the catalytic oxidation of DHAP is more efficient to lead pure enatiomeric species: either *sn*-glycerol-1-phosphate (G1P) or *sn*-glycerol-3-phosphate (G3P) than to lead racemic species. Consistent with this, the evolution did not retain any biological catalytic reaction from DHAP toward the formation of both racemic species G1P and G3P. Usually only G1P derivatives are present in *archaea* while usually only G3P derivatives, precursors of phospholipids esters, are present in *bacteria* and *eukarya* underlining that in all actual living species only homochiral phospholipids are components of biological membranes.

Molecules react according to materials and conditions in their proximity. Modern cells are well evolved biochemical machines and the chemical processes are carried on by enzymes, which determine the path of the reactions. However, prebiotic mechanisms in primitive last common ancestor (LCA) protocells [23–26], were probably not necessarily similar to the actual ones. System protobiology suggests that lipids played a fundamental role into life emergence. Thus, in a hypothetical racemic lipid word, life emerged thanks to the compartmentalization of simple lipophilic or poor hydrophilic small proteins that showed a catalytic role together with auto-replicative functional nucleic acids[27,28].

3. Prebiotic scenarios for the chemical imbalance of amino acids, phospholipids and carbohydrate precursors

Speculation about where and how life emerged from a primordial soup of abiotic mixtures of molecules is extremely well reviewed and summarized including some aspects on the biological origin of chirality [5,29]. One main conclusion was that all chiral molecules can be formed in both enantiomeric types suggesting that a chemical imbalance between the two possible stereoisomers occurred. Prebiotic symmetry breaking scenarios were depicted using mathematical models only[19].

Concerning the synthesis of life's building blocks, Meierhenrich and co-workers showed that exposure of circular polarized light (CPL) in simulated interstellar racemic alanine can induce a chemical imbalance between the formations of L- or D- alanine where the imbalance depends from the wavelength of the incident CPL and sense of rotation [30,31]. Further investigations, performed in simulated conditions, proven that glyceroaldehyde (**1**, Scheme 1), the first chiral product of the "formose" reaction[32] – one of the chemical pathways for the synthesis of carbohydrates– is present in comets and other space bodies [31]. It is probable that the chemical imbalance between the two possible stereoisomers of **1** occurred before seeding the Earth by asteroids or comets impact[33] creating the conditions for a deracemization before the prebiotic polymerization of peptides and formation of nucleic acids. Glyceraldehyde (**1**), dihydroxyacetone (**2**) and glycerol (**3**) together

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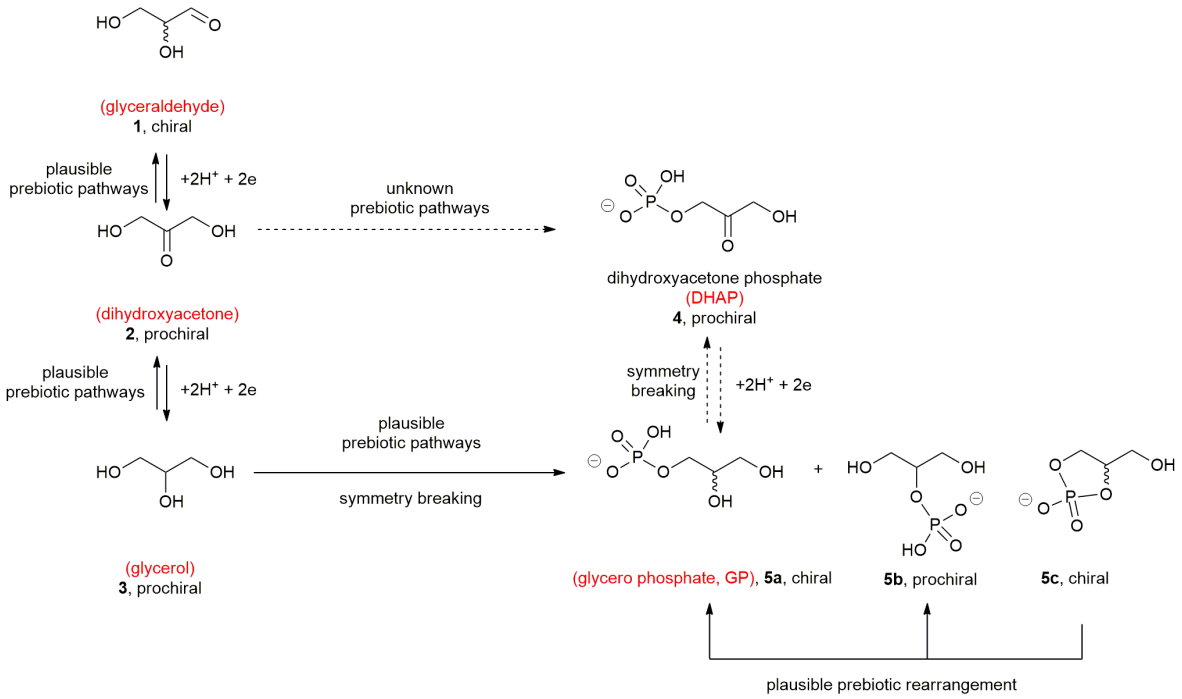
with their phosphate derivatives (**4** and **5a**–**c**) are the most plausible chemical precursors of glycero phospholipids such as phospholipid esters and ethers (Scheme 1).

In a well-studied prebiotic scenario, Sutherland and co-workers, among others, shown that **1** can be one of the plausible precursor of **5a** together with ribonucleosides and a few amino acids such as valine and leucine[34]. DHAP (**4**), instead, was hypothesized to be a key intermediate in the prebiotic synthesis 3-pentulose and racemic mixtures of erythrulose[35].



Scheme 1. Plausible prebiotic pathways for glycerophosphates (**5a–5c**), precursor of phospholipids, from glyceraldehyde, dihydroxyacetone or glycerol (**1–3**). Plausible prebiotic pathway for DHAP (**4**) from **1** was not explored.

Glyceraldehyde (**1**) can interconvert into or from dihydroxyacetone (**2**) (double arrow in Scheme 1) while glycerol (**3**) can interconvert into or from dihydroxyacetone (**2**). The redox reactions (Equations 1—6) that occur should be a key step for stereochemistry imbalance during the phosphorylation or oxidation of glycerol[36] (**3** → **5a** and **5c**, Scheme 1). The synthesis of DHAP (**4**) under plausible prebiotic reaction is not reported, while the synthesis of glycerol (**3**) in interstellar ices was simulated instead [37] suggesting that glycerol is plausibly present in space bodies.

The oxidation reaction can be summarized as a loss of electrons whatever chemistry or biochemistry. In several biological reactions, as in lipid beta oxidation, glycolysis and 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 the evolution maintained. The oxidation and the reduction of **2** and **3** can be written in analogy with respect to NAD^+/NADH processes. Concerning 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 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 $[Cu(CN)_3]^{2-}$ is supposed to be a source of electrons for oxidoreduction of glyceroaldehyde (**1**, Scheme 1) in enzyme free conditions.[38,39] The hydrogen cyanide–cyanocuprate photochemistry have been proven to be effective for the 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 chemical imbalance of **1** for the absence of any chiral inductor, irrespective that model reaction proven that deracemization or interconversion occur using photocatalysis [40].

Iron (III)-sulfur-L-glutathione complexes are able to oxidize NADPH in catalytic networks and inside model protocells made of (*R*)-POPC and oleic acid [41]. This suggests that simple but effective catalytic networks probably existed into protocells, before the advent of LCA. Ferredoxins are one of the most known metallo-proteins and their sequences are well known in three of them isolated from fermentative bacteria [42]. The presence of a high percentage (>64%) of plausible prebiotic aminoacids in their sequence[43] such as glycine, alanine, valine, proline, glutamic and aspartic acids together with cysteine[44], indicates that short hydrophobic peptides, able to complex iron (III) should have been precursors of ferrodoxins in LCA. These peptides in the presence of iron (III) formed aggregates[45] able to perform redox reactions as those with NAD^+/NADH in evolved cells. Such peptides may have been formed from scalemic mixtures of amino acids due to the chemical imbalance induced possibly by meteorite and comet seedings. The scalemic ratio between each D- and L-amino acid was plausibly improved by CLP[33] or from the presence of enantiomorphic crystals ~~as~~. Thus, amino-acid sequences within peptides ~~have been~~ 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. paragraph 4). Non-functional sequences were probably 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 induce more alpha-helix structures than hetero peptides with alternate or stochastic D- and L-amino acids (LDLD… or…LDLL… or …DDLD… etc..). Not only the structural topology of homo peptides is 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 witch 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[23] occurred before the rise of full-fledged cells, since vesicles form spontaneously in aqueous solution from a variety of surfactants.[46] Closed membranes exert confinement and protection of an internalised chemical network including reactions on their hydrophobic region[47–49]. According to the current view, early membranes were more likely formed from derivatives of alkanols [50] fatty acids, [51] mono-alkyl phosphates, [52] and isoprenoids[53]. Most probably they were composed by a mixture of components[54] (Figure 1).

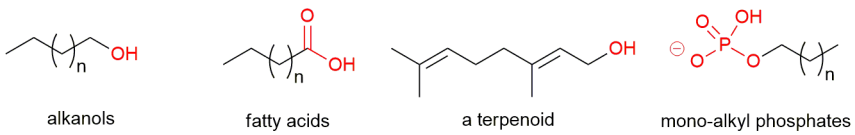


Figure 1. Plausible prebiotic lipid derivatives. Red colour is used to indicate the polar head group.

4.2. Racemic amphiphiles and their precursors

Several plausible prebiotic synthesis were explored, however all the proposed pathways, carried out in enzyme free conditions from glycerol (**3**) yield racemic phospholipids (**6–8**, Scheme 2-A)[50,52,55–59] or racemic mixtures of glycerol phosphates (**5a**–**c** and **9**–**10**, Scheme 2-B [34,60–64]. In addition, the chemical imbalance between the *R*:*S* ratio of mono- and di-alky phosphates (**6**, **12** scheme 2-C) and cyclic glycerophosphates (cGP, **13**, scheme 2-D)[65,66] from di-acyl glycerols **12** or glycerol **3**, respectively, were not reported or investigated either. Remarkably, all the phospholipids crude mixtures containing **6–8**, **12** and **13** are able, using appropriate

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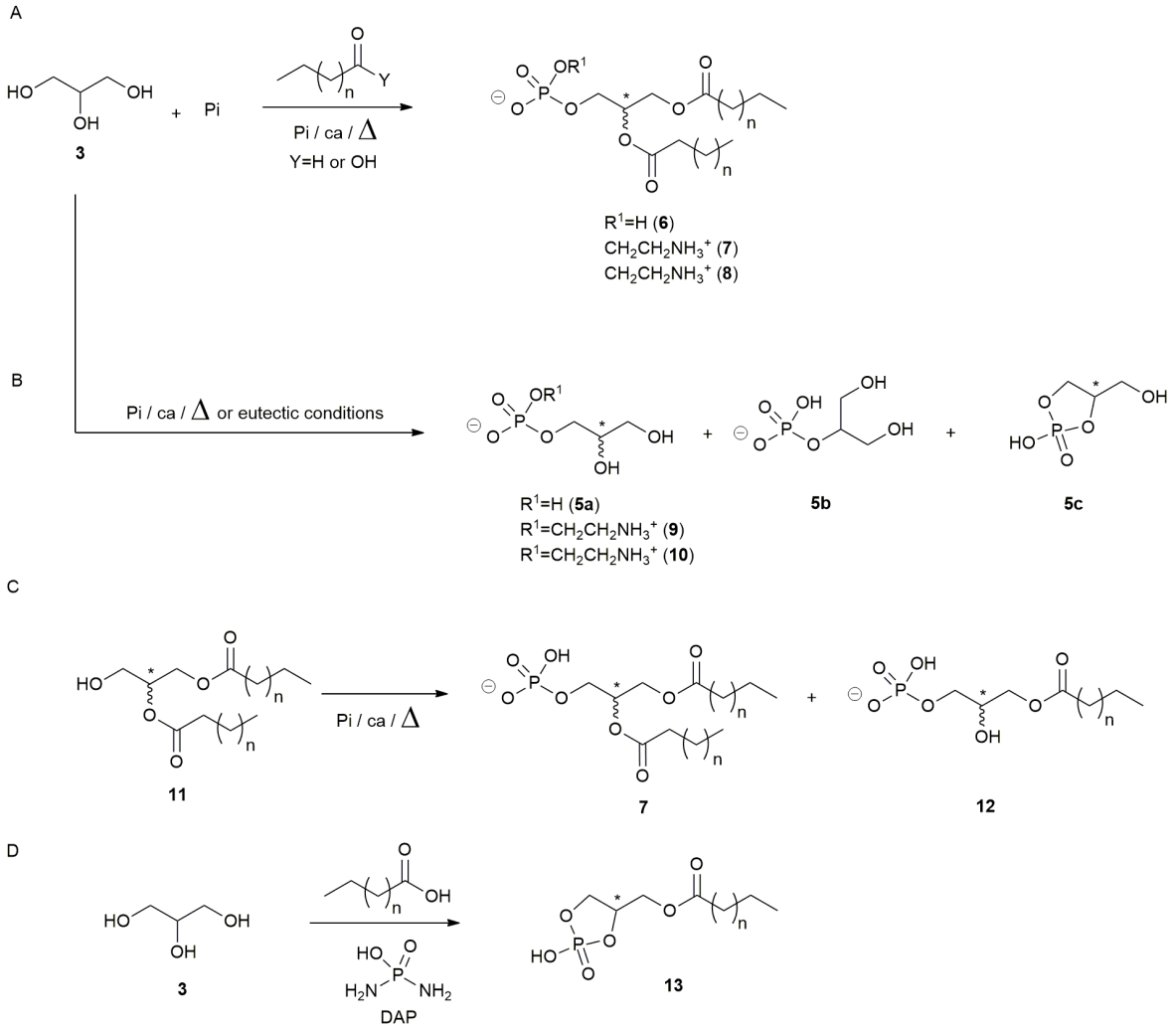
buffers, to form giant vesicles that per sizes and membranes properties are similar to that of lipid bilayer of modern cells.



Scheme 2. A few relevant prebiotic pathways that allows the formation of phospholipid esters and glycerol phosphates. The asterisk (*) indicates the stereogenic carbon C2 of any phospholipids and phospholipids precursors ; Pi stands for any phosphorous salt or plausible phosphate containing mineral able to promote phosphorylation of primary or secondary alcohols[36]; ca, stands for any condensing agents[50]; Δ, stands for temperatures between 65 and 130°C; DAP stands for diamidophosphate[66]; for eutectic conditions see the recent works of Menor-Salvan and Pasek [67,68].

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 compartmentalization of bioactive molecules[69–71]. 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)[72] or 2R – in the Cahn-Ingold-Prelog formalism)[73,74]. The opposite configuration is L-diacylglycerol phosphate, 2,3-diacyl-*sn*-glycerol-1-phosphate or (2S), which occurs mostly in *archaea* membranes. The *archaea* phospholipids contain usually isoprenoid glycerol ethers instead of hydrocarbon glycerol esters[75].

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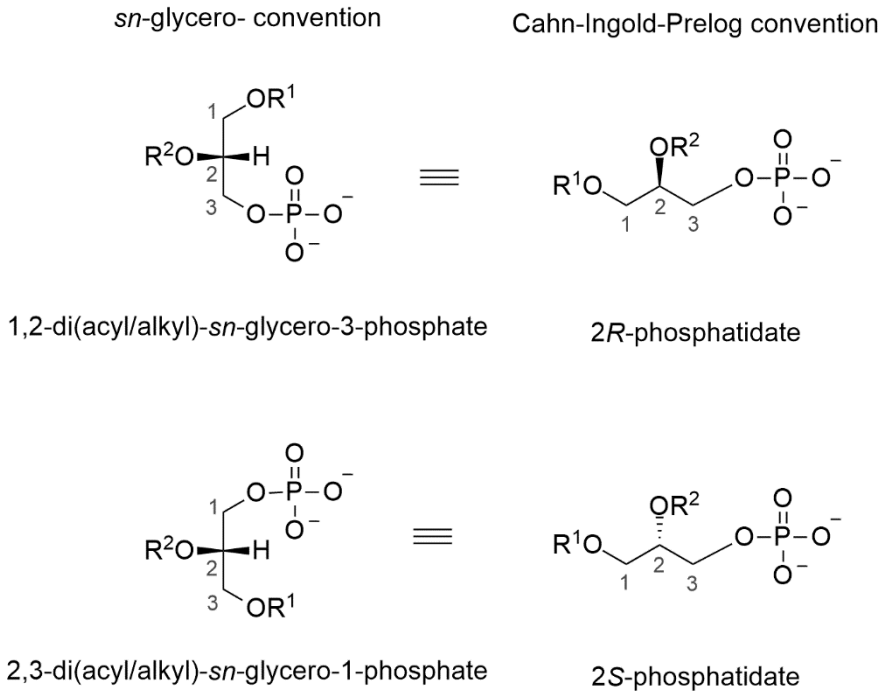

Figure 2. Phosphaditate enantiomers and their *sn*-glycerol and Cahn-Ingold-Prelog nomenclatures. 1,2-diacyl-*sn*-glycero-3-phosphate is the enantiomer of 2,3-diacyl-*sn*-1-glycerophosphate: the stereo numbering (*sn*-glycerol) is based on the position of the second oxygen of the glycerol moiety to the 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.

The last common ancestor (LCA), or *Commonote Commonote* (*C. Commonote*)[76], lived probably under a sulfur-rich atmosphere [77–79] around 1-2 Gy ago. 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*[48,80]. *C. Commonote* had archaeal and bacterial characteristics,[81–84] while *eukarya* evolved from *archaea*, [81,83,84]. 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* [85] or other evolution models [80,84]. 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[86] [80,84]. 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* [80,84]. *Archaea* membranes contain usually phospholipids having G1P moiety and isoprenoid hydrocarbon chains ether-linked to the G1P moiety [80], 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) [48,80].

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 extremophile LCA, was 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, probably later in the evolution, in *archaea* or in *bacteria*, signals a selective catalytic activity that could have initiated by non-enzymatic or enzymatic ways [73].

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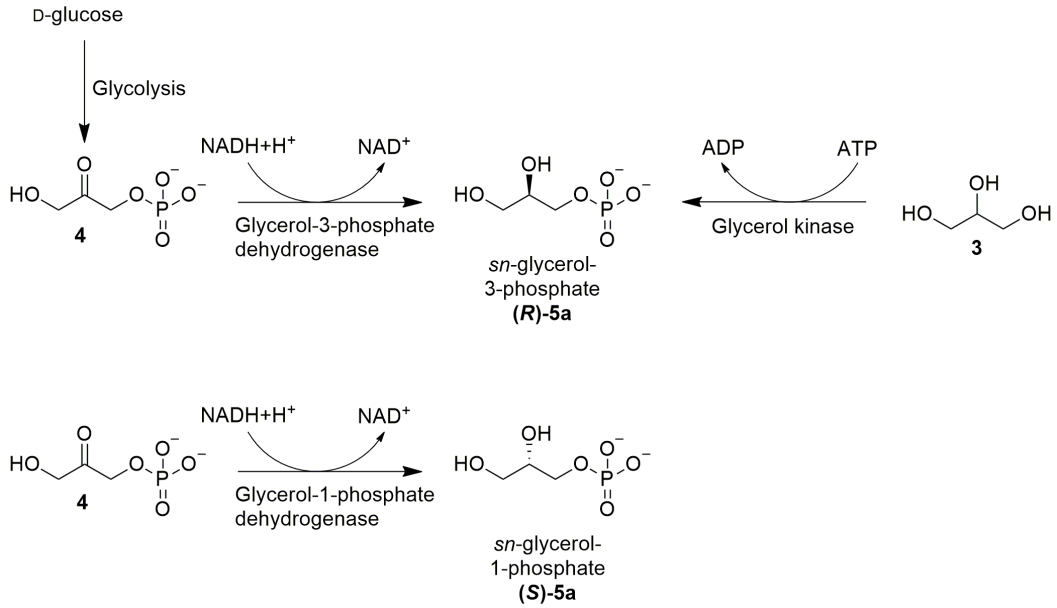
Pro-chiral DHAP, (**4**), is a starting material for the synthesis of lipids in all the three domains of life: *archaea*, *bacteria* and *eukarya*. The first step to obtain phospholipid precursors in *archaea* is the hydrogenation catalysed by G1PDH which gives G1P with NADH + H^+ as proton donors (Scheme 3-B). In *bacteria* and *eukarya*, the first step to obtain the phospholipid precursors is catalysed by a G3PDH giving rise to G3P (Scheme 3-A)[48,80].



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

Generally there are two biosynthetic pathways to obtain G3P in *bacteria* and in *eukarya*, (Scheme 3-A) while there is only one to obtain G1P in *archaea* (Scheme 3-B).[48,80]. Alternatively, G3P can be produced from glycerol and is catalysed by a glycerol kinase (GK) in bacteria and eukarya, while *archaea* lacks GK.[21,65] To the best of our knowledge, there is no GK producing G1P. So far, the catalytic activity of one and the same enzyme producing both G1P and G3P from glycerol, if it existed, was not retained during the evolution. The genes coding for G1PDH, G3PDH and GK in *archaea* and *bacteria* are used to construct phylogenetic trees.[47,80,87,88] This reveals possible evolutions of synthetic pathways from a common ancestor[85]. Several models, based on the occurrence of G1P-lipids or G3P-lipids, were inferred from the presence of either G1PDH or G3PDH.[48,80,89,90]. We are summarizing a few facts from these reports in this section.

The first chiral membranes, in primitive cells, were probably catalytically synthetized separately either by G1DPH or G3DPH (Figure 3). Among the *Commonote* ancestors having chiral membranes, *Commonote archaea* (*C. archaea*) were probably the first biological entities to be formed, since they could live under H_2S with little concentration of O_2. The archaeal descendants are phylogenetically related to each other, while they share very little phylogenetic characteristics with *bacteria*, [82,83,91,92] 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*[81,83,93], consistent with the eocyte hypothesis [85]. *Lokiarchaeta* is closely related to *eukarya* because of the absence of gene for coding G1PDH, and the presence of a gene coding for G3PDH [93,94] ,which is rarely observed in *archaea* (Figure 3). This supports the evidence that *eukarya* originated from *archaea*.

Later in the evolution path, genes coding for G1DPH or G3DPH were horizontally exchanged (grey dashed lines between two arms of the evolution tree in Figure 3). G1PDH and G3PDH may coexist in *C. bacteria* or in *C. archaea* (Figure 3), although it is rarely observed. We may hypothesize 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 by an evolutionary invention. 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 "image-mirror" enzymes since both had L amino acids. Indeed, G1PDH is a part of 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 [48,80].

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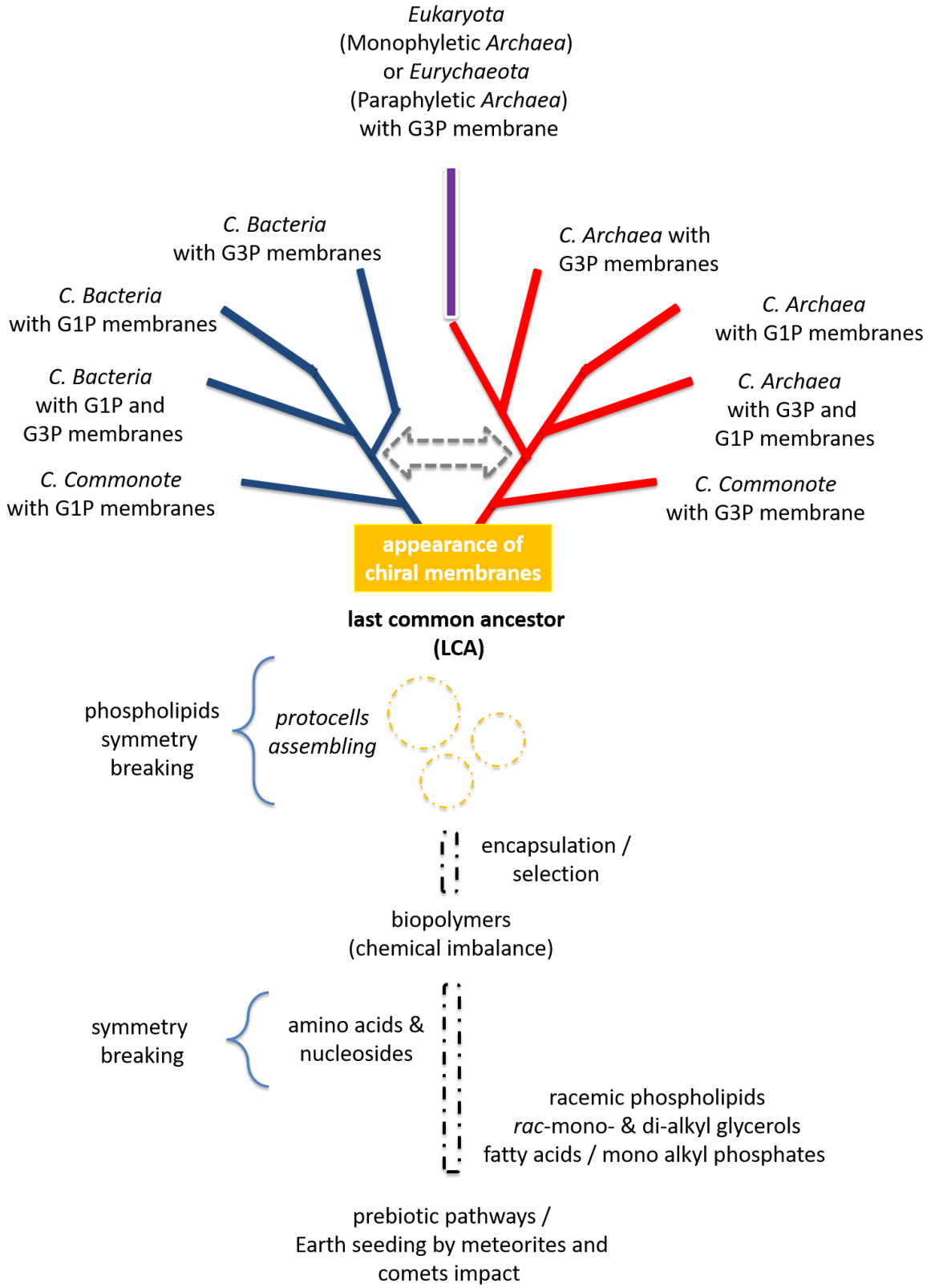

Figure 3. Hypothetic phylogeny of the Last Universal Ancestor (LCA) and *Commonnote Commonote* and their evolution into *Archaea, Bacteria* and *Eukarya* or *Euryarchaeotae* from prebiotic pathways.

Conclusions

The question why living species did not retain racemic lipids to form their membranes during the evolution path remains unanswered and only mathematical models were 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. GIDPH and G3DPH evolved apart from each other since they are structurally different and are not "image mirror" enzymes. Apparently racemic membranes do not have the same properties than those in enantiomeric membranes due to their distinct ability to form lipid rafts, recognition process and packing organizations. From a chemistry perspective, several aspects of this problem could be tackled. Firstly, organic synthesis from DHAP yielding to 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 enantiomeric 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 evolutions of proteins[95–97], as catalysts allowed the biosynthesis of enantiomeric lipids. [27] Large libraries of vesicles containing biopolymers including amino acids, carbohydrates, nucleotides or other meteorite materials served as possible sources of chemical imbalance (Figure 4).

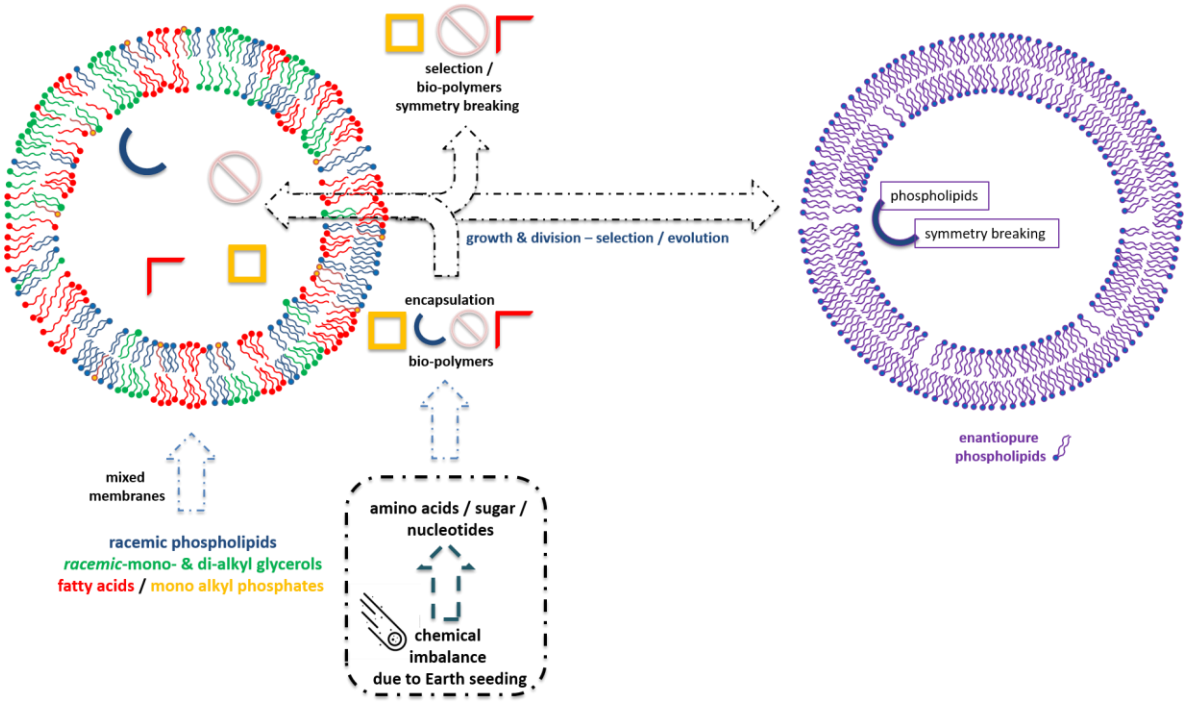

Figure 4. A hypothetical pathways allowing the selection troughs the formation of enatiopure phospholipids and deracemization of mixed protocell membranes upon encapsulation of enantiopure biopolymers (geometrical forms) followed by growth and division of membrane bilayers.

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). Growth and division of lipid boundaries[98] and formation of enantiomeric pure vesicles, drastically contributed to the selection processes. Further studies on 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[98–102]. The compartmentalization of primitive enzyme-free or enzyme molecular replicators, inside the organelles and/or protocells, was probably one of several strategies that evolution retained for Darwinian selection processes.

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authors contribute equally to the funding acquisition; Both authors have read and agreed to the published version of the manuscript.

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