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1 **Fortuitous events in the evolution of Light-dependent Protochlorophyllide**
2 **Oxidoreductase**

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7 **Running Title**. Evolution of Light-dependent Protochlorophyllide Oxidoreductase

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26
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28 **Highlights**
29

30 1. Protochlorophyllide oxidoreductase is an important photo-enzyme in angiosperms that
31 needs light as a substrate for the synthesis of chlorophylls. Therefore, angiosperms cannot
32 green in dark although several algae and lower green plants can synthesize chlorophyll in
33 dark due to the presence of light-independent protochlorophyllide oxidoreductase (LIPOR).

34 2. In response to climate change, during the great oxygenation event light-dependent
35 protochlorophyllide oxidoreductase (LPOR) evolved due to the O_2-induced selection pressure
36 that inactivated the oxygen-sensitive LIPOR.

37 3. Increased O_2 concentration, changing light quality and quantity at different depths of
38 ocean, gene reorganization during several endosymbiotic events, selective LIPOR gene loss
39 and multiple duplication events played a major role in the evolution and diversification of
40 LPOR and its isoforms in phototrophs.

41 4. Phylogenetic studies indicate that LPOR genes have been overwhelmingly
42 horizontally transferred between phototrophs and also non phototrophic organisms.

43 5. Presence of LPOR in non-photosynthetic organisms, Mycobacterium and certain
44 fungi suggests that LPOR may have some other reductive functions in these organisms.

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53

54 **Abstract**

55 Light-dependent protochlorophyllide oxidoreductase (LPOR) is a nuclear-encoded
56 photoenzyme in many photosynthetic organisms. LPOR originated in primitive
57 cyanobacterial ancestors during the great oxygenation event that was detrimental to the
58 existence of the oxygen-sensitive LIPOR that prevailed in anoxygenic Earth. Both LIPOR
59 and LPOR catalyse reduction of protochlorophyllide to chlorophyllide in the penultimate step
60 of chlorophyll biosynthesis. Except for angiosperms and gnetophytes several oxygenic
61 phototrophs harbour both LIPOR and LPOR. The coexistence of LIPOR and LPOR in
62 certain phototrophs provides niche spaces for organisms in unconducive environment. The
63 selection pressure of increased O_2 concentration, changing light quality and quantity at
64 different depths of the ocean, nutrient status of water, gene reorganization during several
65 endosymbiotic events, horizontal gene transfer, LIPOR gene loss and multiple duplication
66 events played a major role in the evolution and diversification of LPOR and its isoforms in
67 photosynthetic and non-photosynthetic organisms. In the absence of LIPOR angiosperms
68 become vulnerable to protochlorophyllide-sensitized and light-induced oxidative stress
69 mediated by singlet oxygen. To overcome the photo-damage PORA was expressed
70 abundantly in the plastids of etiolated plants. PORB evolved to take over the function of
71 vanishing PORA isoform in light. Brassicales evolved PORC to protect plants from high light
72 and other environmental stresses.

73

74 **Keywords:** chlorophyll biosynthesis, climate change, endosymbiosis, gene duplication,
75 horizontal gene transfer, photosynthesis, protochlorophyllide oxidoreductase

76 **Abbreviations:** endosymbiotic gene transfer (EGT), horizontal gene transfer (HGT), light-
77 dependent protochlorophyllide oxidoreductase (LPOR). light-independent
78 protochlorophyllide reductase (LIPOR),

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82 **1. Introduction**

83 Photosynthesis involves the conversion of the solar energy into chemical energy using readily
84 available raw materials. What makes photosynthesis truly exceptional is that not only does it
85 synthesize the building blocks of life, but it also provides the critically important oxygen for
86 the much-needed survival of higher forms of living organisms (Björn, 2009; Blankenship,
87 2010). The status quo of photosynthetic processes was not always as we see it now.
88 Evolutionary processes have left an indelible imprint on the timeline of photosynthesis. When
89 the first photosynthetic organisms originated around 3.4 billion years ago (bya), earth was
90 dominated by anoxygenic phototrophs that used hydrogen sulphide or other substrates as
91 electron donors without the evolution of oxygen. The prokaryotic oxygenic phototrophs that
92 originated in anoxygenic earth created the great oxygenation event (GOE) approx. 2.2 to 2.7
93 bya (Björn, 2009; Blankenship, 2010; Hohmann-Marriott and Blankenship, 2011; Buick,
94 2008; Schirrmeister et al., 2013; Fujita and Uesaka, 2022). This established the oxygenic
95 photosynthesis on earth. The primary endosymbiotic event that gave rise to the classical
96 double membrane bound organelles chloroplast and mitochondria occurred around 1.5 bya.
97 Endosymbiosis culminated into the evolution of present day eukaryotic oxygenic
98 photosynthetic organisms that use light energy for the oxidation of water, thereby releasing
99 oxygen (Blankenship and Hartman, 1998; Archibald 2009;2015; Blankenship, 2010; Rebeiz
100 et al., 2010; Hohmann-Marriott and Blankenship, 2011; Cardona, 2019).

101 Photosynthesis takes place with the help of the tetrapyrrolic light absorbing pigment
102 molecules bacteriochlorophylls (BChls) and chlorophylls (Chls) in anoxygenic and oxygenic
103 phototrophs respectively (Chen et al. 2010; Nascimento et al. 2016). The light-dependent
104 reaction takes place in photosynthetic membranes and begins with photon absorption by
105 pigment molecules associated with antennae proteins, followed by excitation energy transfer
106 to the reaction centres (RC). The absorbed solar energy is conserved in the form of high-
107 energy bonds of adenosine triphosphate (ATP), and a strong reductant, the reduced
108 nicotinamide adenine dinucleotide phosphate (NADPH) (Rabinowitch, 1965; Rabinowitch
109 and Govindjee, 1969; Bryant et al., 2020). There are many different types of Chls (Chla,
110 Chlb, Chlc, Chld, Chle, Chlf, Chlg) and Bchls (Bchl a, Bchl b, Bchl c, Bchl d, Bchl e, Bchl f,
111 Bchl g) (Chen et al., 2010; Kobayashi et al., 1998; Fujita and Yamakawa, 2017; Bryant et al.,
112 2020). In oxygenic phototrophs Chl a is the most abundant Chl species which is a product of
113 several enzymatic steps (depicted in Fig 1.) some of which are common for the biosynthesis
114 of Bchl and Chl molecules (Chen et al., 2010; Scheer, 2006, Tripathy and Pattanayak, 2012).

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116 5-Aminolevulinic acid (ALA) is the first committed precursor for the synthesis of Mg-
117 tetrapyrroles such as the Chls, Fe-tetrapyrroles, heme, cobalamin (vitamin B12), siroheme,
118 and coenzyme F430 (Battersby, 2000; Hunter and Ferreira, 2009). In several bacteria and
119 higher phototrophs ALA is synthesized from glutamyl-tRNAGlu by the activities of two
120 distinct enzymes glutamyl-tRNA reductase (GluTR) and glutamate 1-semialdehyde
121 aminotransferase (GSA-AT). However, ALA is synthesised from succinyl CoA and glycine
122 in β proteobacteria, fungi and animals catalysed by ALA synthase. The biosynthesis of Chls,
123 Heme or Bilin is similar till the formation of non-conjugated macrocyclic tetrapyrrole
124 protoporphyrin IX from ALA (Beale, 1999; Tripathy and Dalal, 2013). At the onset
125 condensation of two ALA molecules results in the synthesis of a 5-membered heterocyclic
126 ring of porphobilinogen. Thereafter four molecules of porphobilinogen assemble to form the
127 linear tetrapyrrole hydroxymethylbilane (Tanaka and Tanaka, 2007; Tripathy and Pattanayak,
128 2012; Bryant et al., 2020). Cyclization of hydroxymethylbilane forms uroporphyrinogen III
129 with the inversion of the pyrrole D ring (Tanaka and Tanaka, 2007; Tripathy and Pattanayak,
130 2012; Brzezowski et al., 2015). Uroporphyrinogen-III is decarboxylated to produce
131 coproporphyrinogen III. In addition to the synthesis of Chl, uroporphyrinogen-III serves as
132 the substrate for siroheme biosynthesis. The two propionate side chains on the rings A and B
133 of coproporphyrinogen III are oxidatively decarboxylated to produce protoporphyrinogen IX
134 (Bollivar, 2006; Rebeiz et al., 2010). With the removal of 6 electrons from the macrocycle of
135 protoporphyrinogen IX, aromaticity is conferred to the macrocycle to generate
136 protoporphyrin IX. Protoporphyrin IX is the branch point for heme and Chl biosynthesis. The
137 iron branch begins with the insertion of Fe^{2+} to protoporphyrin IX for heme biosynthesis
138 (Tanaka and Tanaka, 2006; Scheer, 2006; Granick and Beale, 1978; Castelfranco and Beale,
139 1983; Carey et al., 1985). The insertion of Mg^{2+} ion into protoporphyrin IX converts it into
140 Mg-protoporphyrin IX. Mg-protoporphyrin IX is esterified to form Mg protoporphyrin IX
141 monomethyl ester that is subsequently metabolised by addition of a fifth isocyclic ring to
142 synthesise Divinyl Protochlorophyllide (DV Pchlide). Protochlorophyllide (Pchlide) is the
143 last common intermediate to a vast array of Chls (including Chl a, Chl b and BChls). It is also
144 the bifurcation point for the formation of the Chl c family. In many phototrophs DV Pchlide
145 is often acted upon by vinyl reductase to form Monovinyl protochlorophyllide (MV Pchlide)
146 (Monovinyl plants) mostly during night time. MV Pchlide and DV Pchlide are acted upon by
147 one of the key enzymes of Chl biosynthesis pathway, protochlorophyllide oxidoreductase

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148 (POR) that catalyses the reduction of C17 – C18 double bond of D ring of Pchlide to
149 synthesise Divinyl Chlorophyllide a (DV Chlide *a*) and Monovinyl chlorophyllide *a* (MV
150 Chlide *a*) respectively. DV Chlide *a* so formed is swiftly converted to MV Chlide *a* by vinyl
151 reductase (Duggan and Rebeiz, 1982; Tripathy and Rebeiz, 1986; 1988; Tripathy and
152 Pattanayak, 2012). Chlorophyllide *a* (Chlide *a*) is metabolised to chlorophyllide *b* (Chlide *b*)
153 by chlorophyllide a oxygenase (CAO) and both Chlide *a* and Chlide *b* are esterified with
154 phytol to form Chl a or Chl b (Duggan and Rebeiz, 1982; Tripathy and Rebeiz, 1986; 1988).

155

156 **2. Protochlorophyllide oxidoreductase**

157 POR exists in two different non-homologous enzymatic forms in phototrophs- (1) NADPH
158 Light dependent Protochlorophyllide Oxidoreductase (LPOR) and (2) Light Independent or
159 Dark Operative Protochlorophyllide Oxidoreductase (DPOR/LIPOR) (Griffiths, 1978; Fujita,
160 1996; Adamson et al., 1997; Brzezowski et al., 2015; Vedalankar and Tripathy, 2019). Even
161 though both the POR enzymes catalyse the same reaction they differ from each other right
162 from their origin, subunit composition, structure, catalytic mechanism, and their spread and
163 diversity across the photosynthetic organisms (Hunsperger et al., 2015).

164 LIPOR originated under anoxygenic conditions in the reducing atmosphere of primitive earth.
165 It is widely distributed among photosynthetic organisms ancient anoxygenic to oxygenic
166 phototrophs including cyanobacteria, algae, bryophytes, pteridophytes and gymnosperms
167 except gnetophytes and angiosperms (Bauer et al., 1993; Suzuki and Bauer, 1995; Fujita,
168 1996; Boivin et al., 1996; Armstrong, 1998; Walmsley et al., 1999; Breznenová et al., 2010;
169 Reinbothe et al., 2010). The reduction of Pchlide by LIPOR takes place in the absence of
170 light in an ATP dependent reaction using ferredoxin as reductant (Bröcker et al., 2008;
171 Nomata et al., 2016). LIPOR is encoded by three genes (BchL, BchN, BchB/ChlL, ChlN,
172 ChlB) (Burke et al., 1993a ;1993b, Suzuki and Bauer, 1992; Bröcker et al., 2008; Fujita,
173 1996). Structurally LIPOR is homologous to the ancient Nitrogenase enzyme and consists of
174 two separable protein components: the L protein and the NB protein that are analogous to the
175 Fe- protein and MoFe protein complex of the nitrogenase (Armstrong, 1998; Fujita and
176 Bauer, 2000). The reductive L protein complex is a homodimer of bchLbchL/chlLchlL
177 subunits, and the catalytic NB protein complex is composed of a heterotetramer of
178 2bchNbchB/2chlNchlB subunits (Fujita and Bauer, 2000; Bröcker et al., 2010).

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179 In contrast to LIPOR that is coded by the chloroplast genome, LPOR is a nuclear encoded,
180 single polypeptide of approx. 36kda and has an absolute requirement of light for catalysing
181 the reduction of Pchlide to Chlide (Mullet, 1988; Hunsperger et al., 2015). It requires
182 NADPH as a cofactor and having evolved in the oxygenic environment it is prevalent in all
183 oxygenic phototrophs; unlike LIPOR, the LPOR is insensitive to oxygen (Armstrong, 1998;
184 Bollivar, 2006; Yamamoto et al., 2009). LIPOR is believed to have originated prior to GOE
185 and hence it is considered to be the ancient Pchlide reducing enzyme (Yang and Cheng, 2004;
186 Björn, 2009; Schirrmeister et al., 2013). It is widely believed that the GOE triggered the
187 evolution of LPOR during earth's transition from reducing to oxidising atmosphere created
188 by O_2 evolving cyanobacteria that converted oceanic water to oxygen (Buick, 2008).
189 Evolutionarily both the Pchlide reducing enzymes, LIPOR and LPOR coexist in many
190 photosynthetic organisms (Yang and Cheng, 2004; Yamazaki et al., 2006; Vedalankar and
191 Tripathy, 2019). This review discusses the conundrum of origin and evolution of LPOR, a
192 key light-driven enzyme that plays a crucial role in the Chl biosynthesis and plant
193 development. A better understanding of the LPOR would allow us to understand the need for
194 the existence of entirely two different enzymatic processes for Pchlide reduction i.e., the
195 selection pressure that upended the light-independent reduction to a completely different
196 light-dependent process (Suzuki and Bauer, 1995).

197 It has been well established that light is an indispensable component for the activity of LPOR
198 enzyme much like the DNA repair enzyme DNA photolyase (Begley, 1994; Björn, 2018),
199 bacterial chlorophyllide a reductase (COR) (Saphier et al., 2005), cyanobacterial
200 chlorophyllide f synthase (Chen et al., 2010; Galperin et al., 1998; Ho et al., 2016) and fatty
201 acid photodecarboxylase (FAP) (Sorigué et al., 2017). In addition to light, LPOR requires
202 NADPH as a reductant and Pchlide as a target substrate to catalyse the stereospecific
203 reduction of the C17- C18 double bond in the porphyrin D ring of (Pchlide a) - to (Childe-a)
204 (Yang and Cheng, 2004; Gabruk and Mysliwa-Kurdziel, 2015). Photoreduction of Pchlide to
205 Chlide is an ultrafast event that involves transient charge separation across the C17-C18
206 double bond of the pigment leading to the formation of charge transfer intermediates which
207 facilitate the step wise hydride and proton transfer. These intermediates have been analysed
208 on an ultra-fast time scale by time resolved fluorescent measurements. The proton transfer to
209 C18 of Pchlide by charge transfer intermediates occurs at approx. 3 picoseconds during the
210 first dark reaction while the rate limiting hydride transfer step from pro-S face of NADPH to
211 C17 of Pchlide occurs at 400 ps. The rate of hydride transfer in Pchlide photoreduction is

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212 faster in eukaryotes as compared to prokaryotes suggesting that efficient LPOR evolved
213 during the endosymbiotic era (Heyes and Hunter, 2005; Heyes et al., 2006;2021).

214 **3. Epochal events leading to the origin of LPOR**

215 The redox state of the earth's atmosphere changed dramatically around 2.45 Ga years making
216 free O_2 a permanent constituent of the earth's atmosphere mostly due to the recruitment of
217 Mn-containing oxygen-evolving complex proteins and the evolution of chlorophyll by
218 cyanobacteria that utilized the absorbed solar energy for photolysis of water (Van
219 Kranendonk et al., 2012). This oxygenic photosynthesis allowed the evolutionary emergence
220 of eukaryotes, multicellular organisms, and complex life forms as we know them. The
221 cyanobacterial diversity indicates the origin of cyanobacterial ancestors sometime in the
222 Archean era. The rise of oxygen during the GOE exerted evolutionary pressure on the 4Fe-4S
223 center containing the oxygen-sensitive L subunit of LIPOR and triggered the origin of the
224 oxygen-insensitive Pchlide-reducing enzyme LPOR (Olson, 2001; Schoefs and Franck, 2003;
225 Yamazaki et al., 2006; Nomata et al., 2006; Yamamoto et al., 2009; Reinbothe et al., 2010;
226 Hunsperger et al., 2015). It is noteworthy that the oxygen level present during the late
227 Proterozoic era just after GOE was adequate for the evolution of LPOR (Olson, 2001; Björn,
228 2009; Blankenship, 2010; Schirrmeister et al., 2013; Shih et al., 2013). Similarly,
229 cyanobacteria having another O_2-sensitive enzyme nitrogenase, like LIPOR, having a
230 monopolistic dominance in the anoxygenic environment (Fujita and Bauer, 2000) acquired
231 temporal differentiation by carrying out nitrogen fixation in the dark and spatial
232 differentiation by confining nitrogen fixation to specialized cells called heterocysts. These
233 heterocystous cyanobacteria evolved late in the O_2 event to protect themselves from the
234 oxygenic threat (Fujita and Bauer, 2000; Yamazaki et al., 2006; Fujita and Uesaka, 2022).
235 However, a similar mechanism was not observed in LIPOR, and it is speculated that LIPOR
236 acquired some other protective mechanisms such as the water-water cycle to remove oxygen
237 to combat the oxygen sensitivity (Durnford and Falkowski, 1997). The evolution of LPOR
238 did not lead to the extinction of LIPOR. Both the nonhomologous Pchlide-reducing enzymes
239 coexist among the oxygenic phototrophs, the only exceptions being gnetophytes and
240 angiosperms where LIPOR is non-existent (Walmsley et al., 1999; Schoefs and Franck, 2003;
241 Yamazaki et al., 2006; Hunsperger et al., 2015). Why the two enzymes still coexist today
242 even in advanced archegoniate like gymnosperms does not have a precise answer.

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243 Evolution and spread of LPOR took a magnificent jump to higher eukaryotes that acquired
244 photosynthesis via endosymbiosis. The primitive cyanobacterium was established as a
245 photosynthetic organelle called plastid inside the eukaryotic phototrophs about 1.5 bya
246 (Archibald, 2009; Chan and Bhattacharya, 2010; Keeling, 2010).The photosynthetic
247 eukaryotic ancestor gave rise to the complex supergroup Archiplastida that branched out into
248 three separate photosynthetic lineages – the Glaucophyta, Viridiplantae (comprising green
249 algae and land plants), and Rhodophyta –the red algae. Subsequently, the secondary, tertiary,
250 and higher-order endosymbiosis transmitted the plastids amongst eukaryotes to give rise to
251 the present-day diversity of eukaryotic photosynthetic lineages (Tomitani et al., 1999; Chan
252 et al., 2010; Keeling, 2010; Archibald and Keeling, 2002; Archibald 2015). The
253 endosymbiotic process has played a crucial role in the evolution of photosynthetic genes in
254 eukaryotes. A major aftermath of endosymbiosis was a massive transfer of genes from the
255 endosymbiont to the host nucleus (Keeling, 2010). The endosymbiotic genes underwent
256 several recombination events and became integrated into the host nuclear genome. As a
257 result, the size of the plastid genome was significantly reduced compared with the original
258 cyanobacterial endosymbiont. After primary endosymbiosis, the LPOR genes were
259 transferred to the nucleus, while the LIPOR genes remained in the chloroplast genome or
260 were lost in several algal lineages and higher plants in the oxygenic environment (Hunsperger
261 et al., 2015). Until recently only a single endosymbiotic gene transfer event was thought to
262 have occurred that led to the origin of plastids by engulfment of the β-Cyanobacteria
263 possessing β-carboxysomes with Form-IB Rubisco (Kerfeld and Melnicki, 2016). About 60
264 million years ago another independent endosymbiotic event took place in freshwater rhizarian
265 amoeba *Paulinella chromatophora* (Nowack, 2014; Kim and Park, 2016). These organisms
266 retain cyanobacterial prey of the Prochlorococcus and Synechococcus sp PCC 7002 of α
267 Cyanobacteria type which has α-carboxysomes with Form-IA Rubisco (Kerfeld and
268 Melnicki, 2016; Gabr et al., 2020). The endosymbiotic photosynthetic entities in *P.*
269 *chromatophora* are called as chromatophores that absorb solar energy and they retain
270 cyanobacterial features such as carboxysomes, phycobilisomes, peptidoglycan cell wall
271 (Nowack and Grossman, 2012; Kerfeld and Melnicki, 2016; Kim and Park, 2016). This is a
272 chloroplast in making.

273 In prokaryotes, horizontal gene transfer (HGT) is ingrained within the genealogical fabric of
274 the organisms (Treangen and Rocha, 2011; Bock, 2010; Koonin, 2016). The numerous
275 instances of HGT in organisms although sporadic give ample evidence of the important and

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276 pervasive role of lateral gene transfer (Bock, 2010; Brinkmann et al., 2018). The temporal
277 intrusions in HGT are a result of a unique combination of begging or borrowing or stealing
278 genetic information across the natural mating barriers (Ku and Martin, 2016; Bock, 2010).
279 The presence of ancestral, novel genes or paralogs of existing genes or xenologous sequences
280 can be estimated from the phylogenetic analysis depicting the divergence of the recipient
281 from the direct ancestor (Ku and Martin, 2016; Cohen et al., 2011; Hunsperger et al., 2015).
282 The HGT has contributed immensely towards the evolutionary process of LPOR in
283 photosynthetic organisms. LPOR phylogeny follows the identical pattern to the plastid
284 transfer among algae originating from primary endosymbiosis (Hunsperger et al., 2015).
285 Typically, plastids derived from green algae fall into three separate lineages streptophytes,
286 chlorophytes, and primitive prasinophytes (Archibald and Keeling, 2002). The phylogenetic
287 studies reveal that the marine phototrophic amoeboid flagellates chlorarachniophytes evolved
288 from the chlorophyte clade (Chlorophyceae– Trebouxiophyceae– Ulvophyceae-
289 Pedinophyceae) of green algae (Cavalier-Smith, 1998). The chlorarachniophyte plastids
290 typically contain green algal footprints but some members of chlorarachniophyte including
291 *Bigelowiella natans* contain plastid proteomes and nuclear genes heavily infected with LPOR
292 genes originating from phylogenetically divergent red algae or from xenologous sources
293 (Curtis et al., 2012; Hunsperger et al., 2015). The abundance of 'foreign' genes in
294 *Bigelowiella natans* as a result of HGT compensates for the mixotrophic lifestyle (Rogers et
295 al., 2007; Curtis et al., 2012; Burki, 2017). LPOR proteins originating from the green algae
296 lineage due to the secondary endosymbiotic event are found to be sister branches to one
297 another and nested within the prasinophyte algae indicating a possibility of HGT from
298 prasinophyte to the chlorarachniophyte (Hunsperger et al., 2015). The LPOR proteins
299 funneled from the Rhodophyta branch of plastids should belong within the rhodophytic clade
300 but there are numerous instances with most members following a rather reticulate path
301 similar to that observed due to secondary and higher-order symbiosis. The exact origins of
302 the LPOR genes transferred via red algal lineage are complex (Hunsperger et al., 2015).
303 Phylogenetic studies demonstrate that maximum members of stramenopiles demonstrate an
304 affinity to the prasinophytes branch. All sequenced haptophytes and quite a few peridinin-
305 containing dinoflagellates show their origin within the prasinophytic branch of LPOR
306 proteins (Hackett et al., 2004; Minge et al., 2010). Either the primary Rhodophyta LPOR
307 genes have been completely replaced by HGT to the chlorophyte genes. Within the
308 Cryptophytes and Heterokonts bearing rhodophytic LPORs several phylogenetic
309 reconstructions are observed originating from EGT and several independent HGTs (Keeling,

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310 2010; Hunsperger et al., 2015). Diatoms acquired plastids via secondary endosymbiosis from
311 the red algal lineage (Armbrust et al., 2004; Janouškovec et al., 2010). The diatom
312 *Alexandrium tamarense* shows presence of prasinophytic LPOR genes acquired from green
313 alga *Micromonas* sp as a result of HGT (Wisecaver et al., 2013; Hunsperger et al., 2015). The
314 recently sequenced genome of a number of diatoms reveals a chimeric or transgenic genome
315 that shows several traces of green algae footprints and xenologues acquired from a variety of
316 other sources by EGT and HGT (Petersen et al., 2014). This feature enables diatoms to
317 survive in difficult habitats and varying environmental conditions.

318 **4. Migration of LPOR to anoxygenic photosynthetic organisms.**

319 Like millions of oxygenic photosynthetic species possess LIPOR, several aerobic anoxygenic
320 phototrophic bacteria (AAPB) have functional LPOR sequences. AAPBs are a ubiquitous
321 group of marine microbes that possess light harvesting rection centers supplemented with
322 heterotrophic metabolism. a globally conserved structure similar to the well-characterized
323 cyanobacterial LPOR (Biebl et al., 2005; Chernomor et al., 2021). In contrast to anoxygenic
324 photosynthetic bacteria (APB) like *Rhodobacter capsulatus* AAPBs can perform anoxygenic
325 photosynthesis in the presence of atmospheric oxygen (Yurkov and Hughes, 2017).
326 Possession of LPOR by AAPBs is an adaption to increase Bchl synthesis under aerobic
327 conditions. Members of α proteobacteria - *Dinoroseobacter shibae*, *Erythrobacter litoralis*,
328 *Yoonia vestfoldensis*, *Sulfitobacter guttiformis*, *Porphyrobacter dokdonensis* and β
329 proteobacteria – *Limnohabitans* sp. and *Burkholderia* sp acquired LPOR probably due to
330 HGT from cyanobacteria like organisms involving multiple gene transfer events that might
331 have taken place at different time points (Biebl et al., 2005; Brinkhoff et al., 2010; Wagner-
332 Döbler et al., 2010;Kaschner et al., 2014;Wang et al., 2014; Chernomor et al., 2021; Bryant
333 and Frigaard, 2006; Yurkov and Hughes, 2017; Kasalický et al., 2018). Anoxygenic
334 phototroph *Gemmatimonas phototrophica* member of phylum Gemmatimonadetes also
335 acquired LPOR as an inter-phylum HGT from proteobacteria (Zeng et al., 2015; Chernomor
336 et al., 2021). Phylogenetic studies by Chernomor et al. 2021 expand the database of LPOR
337 sequences in AABPs. The refuge of LPOR in AAPBs is part of a design rather than a chance
338 event or an accidental event which turns favourable and this is not restricted to a few
339 proteobacterial species thus firmly establishing the presence of LPOR in AAPBs (Chernomor
340 et al., 2021).

341 **5. Gene duplication**

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342 Gene duplication is another major mechanism that introduces genomic novelty among
343 organisms (del Pozo and Ramirez-Parra, 2015). The duplication event not only increases the
344 copy number of the duplicated gene but with time a copy of the duplicated gene might
345 accumulate mutation and acquire a novel function in the genome (Birchler and Yang, 2022;
346 Savino et al., 2022). In many cases, the duplicated gene ends up as a non-functional
347 counterpart which does not make a functional product due to modifications in the DNA
348 sequence by nucleotide insertions, deletions, frameshifts, or substitutions that disrupt the
349 reading frame or lead to the insertion of a premature stop codon (Hunsperger et al., 2015;
350 Savino et al., 2022). Three algae lineages underwent genome duplications resulting in LPOR
351 duplication: dinoflagellates, chlorarachniophytes, haptophytes, and stramenopiles
352 (Janouškovec et al., 2017).

353 The Brassicales underwent three whole genome duplications and the three isoforms of LPOR
354 in *Arabidopsis* may be the outcome of these events (Oosawa et al., 2000; Pattanayak and
355 Tripathy, 2002). LPOR expansion in different photosynthetic organisms shows that it exists
356 in three different isoforms within the genome of photosynthetic organisms (Yang and Cheng,
357 2004). The LPOR genes in Brassicales are very similar at the nucleotide level which probably
358 reflects recent gene duplication (Oosawa et al., 2000). LPOR gene duplication occurred after
359 the primary endosymbiotic event in eukaryotic plant cells (Archibald, 2015). The LPOR
360 isoforms in some photosynthetic eukaryotes share similar functions and regulations even
361 though they have been shown to arise from unique duplication events during evolution
362 (Hunsperger et al., 2015). The evidence for the gene duplication event comes from
363 phylogenetic trees which show the distribution of each of the two gene copies between two
364 principal branches of the tree (Hunsperger et al., 2015). Studies show that euglenids and
365 chlorarachniophytes arose from two unique duplication events. *Hordeum vulgare* and *A.*
366 *thaliana* LPOR sequences arose independently but they have similar functions in seedling
367 greening and Chl synthesis.

368 Recent studies have identified multiple LPOR genes arising as a result of duplication in
369 several diatom genomes including *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*
370 (Armbrust et al., 2004) *Fragilariopsis cylindrus*, and *Pseudo-nitzschia multiseries*. Two
371 LPOR isoforms (POR1 and POR2) present in *Phaeodactylum tricornutum* genome are the
372 result of gene duplication to enable it to adapt under varying light conditions (Ambrust et al.,
373 2004; Hunsperger et al., 2015). Certain members of cryptophytes show LIPOR gene loss but
374 other members retain functional LIPOR genes. In cryptophyte, *Chroomonas mesostigmatica*

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375 CCMP1168 some of the sequences of chlN occur as a pseudogene, stop codons, and
376 frameshifts in the nuclear genome. The chlL is absent from *Rhodomonas salina*, *G. theta*, and
377 *H. andersenii* plastid or nuclear genome (Fong and Archibald, 2008; Hunsperger et al., 2015;
378 Kim et al., 2017). Dinoflagellates *Heterocapsa triquetra* (Fong and Archibald, 2008) and
379 *Amphidinium* (Lauritano et al., 2017) show the presence of only one subunit of chlL which
380 has shown to be a functional copy by expressed sequence tag surveys. LIPOR subunit in *H.*
381 *andersenii* and *Chroomonas pauciplastida* possess group II introns in different locations
382 making the gene non-functional (Khan and Archibald, 2008). *Arabidopsis thaliana* and *Oryza*
383 *sativa* contain approx 2700 and 5600 well-defined pseudogenes, respectively (del Pozo and
384 Ramirez-Parra, 2015). Pseudogenes are non-functional but still beneficial in evolution as it
385 helps the organism to counter or survive the changed environmental conditions (Treangen
386 and Rocha, 2011). Plant genomes are replete with instances of the presence of pseudogenes
387 that are non-functional but still beneficial in evolution as it helps the organism to counter or
388 survive the changed environmental conditions. Several algae that lack LIPOR, including
389 certain haptophytes and stramenopiles, the LPOR gene is duplicated, and it is hypothesized
390 that this duplication might compensate for the loss of the dark enzyme, potentially by
391 allowing for the differential regulation of the genes of the photoenzyme (Hunsperger et al.,
392 2015).

393 **6. Impact of other Environmental factors on LPOR origin and evolution**

394 Environmental factors often determine the distribution and diversification of genes for
395 appropriate adaptation. Genes are rendered inactive by a mutation or gene loss and become
396 dispensable during evolutionary pressure. Although this might not be a universally adaptive
397 process, it is helpful under certain situations to combat the evolutionary pressure (Helsen et
398 al., 2020). Pchlide reduction has endured various selective pressures across a range of
399 environmental parameters after the transition of photosynthesis from an initial anoxygenic to
400 an oxygenic environment. Some of the environmental factors include a) High altitude, b) Fe
401 deficiency and c) high temperature, d) light intensity, e) light quality (Li and Bridwell-Rabb,
402 2018; Przybyla-Toscano et al., 2021; Abbas et al., 2022; Kees et al., 2022).

403 In the oxygenic environment, O_2-dependent MPE (oxidative) cyclase is responsible for
404 Pchlide synthesis (Figure 1). A decrease in the partial pressure of O_2 reduces the Pchide
405 concentration in plants (Li and Bridwell-Rabb, 2018). Therefore, the POR activity is
406 downregulated and it influences the distribution and accumulation of the Pchlide and its

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407 reducing enzyme POR at different altitudinal clines (Abbas et al., 2022). In the early
408 oxygenic world, the lower partial pressure of oxygen was sufficient to generate stratospheric
409 O3 in the presence of UV light that prevented the UVB penetration and DNA damage of
410 haploid life forms and produce ROS including singlet oxygen ($^1O_2$). ROS was not only
411 responsible for the destruction of Fe-S centers of LIPOR in several species it also promoted
412 the evolution of different isoforms of LPOR. However, this O_2 concentration was limiting for
413 MPE (oxidative) cyclase, and therefore, it impacted Pchlide synthesis, LPOR evolution and
414 Chl synthesis in early oxygenic haploid photoautotrophs.

415 Similarly, nutrient deficiency especially Fe impacted LPOR evolution with concomitant loss
416 of LIPOR. The 4Fe-4S centers of L subunit of LIPOR needs Fe, for its synthesis and
417 assembly. Oceanic diatoms Phaeodactylum tricornutum, Thalassiosira pseudonana and
418 Nannochloropsis oceanica lost LIPOR because they are majorly present in Fe-sufficient
419 oceanic water (Przybyla-Toscano et al., 2021). The low availability of micronutrient Fe
420 encouraged the dispensability of LIPOR and the establishment of LPOR for better
421 adaptability of other oceanic phototrophs that acquired LPOR by horizontal gene transfer
422 (Behrenfeld et al., 2006; Bowler et al., 2010; Cvetkovska et al., 2019)

423 The LPOR activity in different species is also temperature dependent. In Synechocystis the
424 optimum LPOR activity is at 30^0C. However, in thermophilic cyanobacterium
425 Thermosynechococcus elongates the optimum temperature for LPOR activity is between
426 50^0C- 55^0C and it is less active at room temperature (McFarlane et al., 2005). Although
427 cyanobacteria mostly possess both the Pchlide reducing enzymes i.e., LIPOR and LPOR;
428 thermophilic cyanobacteria lack both LIPOR genes and nitrogenase genes. This loss of
429 LIPOR genes is attributed to high temperature-induced genome reduction in thermophilic
430 cyanobacteria (Kees et al., 2022).

431 Light intensity is a factor influencing the light-driven reaction rates. The LPOR disrupted
432 YFP12 mutant of cyanobacteria and wild-type cyanobacteria grow normally under low light
433 conditions (10-25 mu E m^{-2} s^{-1}), but at high light intensity (85-170 mu E m^{-2} s^{-1}), the mutants
434 stop growing and are photo-bleached (Fujita et al., 1998). In contrast YFC2 mutant with
435 disrupted ChlL (LIPOR less) grew rapidly at higher light intensities, suggesting that the
436 contribution of LPOR in chlorophyll biosynthesis increases with increase in light intensity
437 (Fujita et al., 1998). Under medium-light intensities (25–130 mu E m^{-2} s^{-1}) as well as high-
438 light intensities (above 130 mu E m^{-2} s^{-1}) LPOR is majorly and exclusively functional for Chl

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439 biosynthesis (Fujita et al., 1998). LIPOR is more energy-demanding than LPOR and thus has
440 been replaced during the evolutionary process to allow photosynthetic organisms to follow a
441 more conservative lifestyle (Masuda and Takamiya, 2004). The contribution of LPOR
442 towards the Chl biosynthesis increases with increasing light intensity. LPOR acts as a trigger
443 for the germination of seedlings in plants and provokes a marked change in the
444 morphological development of the plant (Ha et al., 2017). At low light intensities, Chl content
445 in cyanobacterium Plectonema boryanum lacking LIPOR was lower, and its growth rate is
446 retarded (Huang et al., 2004). The early cyanobacteria that had only LIPOR most likely
447 adapted to low light intensity for efficient Chl biosynthesis and photosynthesis for survival.
448 In this context, *Chlamydomonas* sp. UWO241 is an intriguing chlorophyceae member as
449 despite inhabiting low light conditions for extended periods it shows loss of LIPOR, as a
450 result, Pchlide reduction is entirely carried out by LPOR (Bowler et al., 2010; Smith et al.,
451 2019). *Chlamydomonas* sp. UWO241 with its reduced chloroplastic genome inhabits
452 perennially ice-covered lakes of Antarctica with high dissolved oxygen content, iron limiting,
453 and hypersaline conditions. The above conditions seem to support the loss of LIPOR
454 (Behrenfeld et al., 2006; Bowler et al., 2010; Cvetkovska et al., 2019; Smith et al., 2019). It
455 seems that the ancestral chlamydomonadalean clade that gave rise to UWO241 seems to have
456 lost the LIPOR genes (Cvetkovska et al., 2019; Smith et al., 2019).

457 The spectral composition of the light changes with topographic factors. Red light which is of
458 longer wavelength is mostly absorbed by the water at the surface. Blue light penetrates deep
459 and turbid water. The red light is highly efficient for the LPOR-mediated photoconversion of
460 Pchlide to Chlide (Dalal and Tripathy, 2012). Nevertheless, the supposed inefficiency of
461 LPOR in deep or turbid water is not surprising as in these conditions only blue light is
462 available due to the scattering. Greenlight that is available deep in the ocean is not efficiently
463 absorbed by many phototrophs (Kehoe and Gutu, 2006). The action spectra of LPOR reveals
464 that it is ineffective in green light, in such cases LIPOR compensates for the low LPOR
465 activity. The members of the (Microchaetaceae) *Fremyella diplosiphon* (Shui et al., 2009;
466 Pattanaik et al., 2011), (Nostocaceae) *N. punctiforme* (Hirose et al., 2013), (Rivulararariaceae)
467 *Gloeotrichia 583* (Stowe et al., 2011) depict an interesting phenotype as an example of the
468 complementary chromatic adaptation by alternating their photosynthetic pigments and
469 enzymes under fluctuating light conditions (Hirose et al., 2013). It would not be hyperbolic to
470 say that they can sense a rainbow of colors ranging from red, blue, green and violet
471 (Grossman, 2003; Bordowitz and Montgomery, 2008; Kehoe, 2010). All these explain the

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472 simultaneous existence of LPOR and LIPOR for sustenance and adaptation to environmental
473 factors.

474 **6.1. Photoprotective role:** The catalytic rate of LIPOR for the dark conversion of Pchlide to
475 Chlide is quite slow. Therefore, under the steady-state conditions of Chl biosynthesis the
476 Pchlide accumulation in the cells of LIPOR-containing organisms is high (Soffe, 2016).
477 Under high light that prevails on the ocean surface, the accumulated Pchlide in LIPOR-
478 containing organisms absorb light and transfer their energy to oxygen to produce highly
479 reactive singlet oxygen ($^1O_2$) (Chakraborty and Tripathy, 1992; Tripathy and Pattanayak
480 2010; Pattanayak and Tripathy, 2011) that causes photooxidative damage to cells. The LPOR
481 bestows photo-protection on the plants by limiting the Pchlide-mediated photo-oxidative
482 damage (Buhr et al., 2008; Tripathy and Pattanayak, 2010, Pattanayak and Tripathy, 2011).
483 Whereas the high light intensity on the surface of the ocean could photodamage the slow
484 LIPOR-containing photoautotrophs, it can cause minimal damage to organisms possessing
485 LPOR that converts Pchlide to Chlide rapidly within 1 millisecond (Sytina et al., 2008; Soffe,
486 2016; Heyes et al., 2021). Thus, LPOR protects the etiolated and green phototrophs by
487 binding to the photosensitive Pchlide pool to keep it in photo-transformable form for very fast
488 photo-conversion of Pchlide to Chlide to minimize generation of singlet oxygen that causes
489 destruction of photosynthetic organisms in high light (Tripathy and Chakraborty, 1991,
490 Chakraborty and Tripathy, 1992, Tripathy and Pattanayak, 2011). In angiosperms during
491 senescence the chlorophyll content rapidly declines, however, POR activity persists to photo-
492 transform protochlorophyllide to minimize singlet oxygen production to ensure leaf survival
493 and the translocation of photosynthates from the source to sink (Hukmani and Tripathy,
494 1994).

495 The free Chls do not accumulate rather they bind to photosynthetic pigment-protein complex
496 to efficiently transfer all of its absorbed energy to the reaction center for utilization. The
497 antenna Chl molecules usually do not generate singlet oxygen ($^1O_2$) unless they are under
498 high excitation pressure (Tripathy and Pattanayak, 2010; Pattanayak and Tripathy, 2011;
499 Buhr et al., 2008). Therefore, high light acted as a selection pressure for the evolution of
500 LPOR to protect photoautotrophs from photo-oxidative damage.

501 The LPOR genes are more widespread among phototrophic taxa and therefore, it is fair to
502 interpret that LPOR genes are superior to LIPOR enabling plants to survive and grow at
503 different environmental conditions. LIPOR genes often become redundant, and they are

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504 discarded under some environmental circumstances /selection pressure. Gene dispensability
505 is often inferred by the higher rate of non-synonymous substitution rates and high ratio of
506 non-synonymous to synonymous substitution. It has been shown previously that ChlL gene of
507 the gymnosperm *Thuja standishii* has high rate of non-synonymous substitution confirming
508 its dispensability (Kusumi et al., 2006). Thus, greater selection pressure acted on LIPOR
509 genes leading to its complete absence from higher gymnosperm, gnetophytes, and
510 angiosperms (Walmsley et al., 1999; Yamamoto et al., 2017). Taken together these findings
511 suggest that LPOR genes seem to be to have higher functionality and importance compared to
512 LIPOR genes in the oxygenic world.

513 **7. Structural basis of LPOR function**

514 **7.1 LPOR a short-chain dehydrogenases/reductases superfamily confrère:** LPOR
515 belongs to a large family of enzymes known as short-chain dehydrogenases/reductases
516 (SDRs) (Yang and Cheng, 2004; Wilks and Timko, 1995; Moummou et al., 2012). SDR is
517 part of a large superfamily of enzymes known as the 'RED' (Reductases, Epimerases,
518 Dehydrogenases) that catalyze a variety of NADP (H) - or NAD(P)+-dependent reactions
519 (Wilks and Timko, 1995; Oppermann et al., 2003; Moummou et al., 2012) involving hydride
520 and proton transfer (Hoeven et al., 2016; Archipowa et al., 2018). This is one of the oldest
521 and most diverse protein families present in prokaryotes and eukaryotes that typically occur
522 as oligomers (Oppermann et al., 2003; Yang and Cheng, 2004). It has a wide range of
523 substrates involved in secondary metabolic routes ranging from polyols, retinoids, sterols,
524 sugars, aromatic compounds, and xenobiotics (Persson et al., 2003). Plant LPORs are
525 assigned to SDR73C family in the SDR superfamily (Dong et al., 2020).

526 The classical SDR family of proteins containing all oxidoreductases has two domains, one for
527 binding of the cofactor and another for binding the substrate (Moummou et al., 2012).
528 Despite the considerably low sequence similarity (15% -30%), SDR family members bear
529 significant structural similarity such as a common a/ß folding pattern with Rossmann fold - a
530 characteristic of SDR family members (Yang and Cheng, 2004). The Rossmann fold consists
531 of a central parallel 6-7 β-sheet sandwiched by two arrays of 2-3 α-helices on either side
532 making up αβα-core for NADPH binding (Dong et al., 2020). The Rossmann-fold and a
533 hydrophobic loop region both are involved in anchoring the enzyme to the membrane
534 (Moummou et al., 2012). SDRs consist of a one-domain subunit of about 250 amino acids
535 with the cofactor binding site in the N-terminal part and substrate binding in the C-terminal

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536 part. The variable C-terminal segment determines the substrate specificity (Dong et al.,
537 2020).

538 Other common structural features include a highly conserved active site with a highly
539 conserved pentapeptide YxxxK in the catalytic motif (YKDSK in LPOR) that participates in
540 the proper coordination with NADPH and Pchlide binding (Lebedev et al., 2001; Gabruk et
541 al., 2016). The N terminal contains the conserved sequence (Gly-X-X-X-Gly-X-Gly) in SDR
542 and GASSGV/LG in all LPORs. This glycine-rich motif is for structural integrity and binding
543 of the pyrophosphate portion with NADPH (Dong et al., 2020). A key feature of the SDR
544 superfamily is its catalytically important tetrad Ser-Asn-Tyr-Lys for proton transfer and
545 stabilization of reaction intermediates. The catalytic triad in (Asn-Ser-Thr-Lys) POR contains
546 Thr 145 instead of Ser residue (Moummou et al., 2012; Dong et al., 2020). Site-directed
547 mutagenesis and in vivo analysis confirm that Tyr and Lys are the most conserved at the
548 catalytic site in all LPOR members and that these are indispensable for the enzymatic
549 catalytic activity (Wilks and Timko, 1995; Suzuki and Bauer, 1995; Lebedev et al., 2001
550 Heyes and Hunter, 2002). The Tyr residue acts as a general acid upon deprotonation and
551 facilitates hydride transfer to or from NAD (P)+/H (Ehrig et al., 1994; Lebedev et al., 2001).
552 The proton at the C-18 position of Pchlide is derived from Tyr and the hydride transferred to
553 the C-17 position is derived from the pro-S face of NADPH (Heyes and Hunter, 2005;
554 Archipowa et al., 2018). The mutation of either Tyr 275 or Lys-279 does not completely
555 abolish the catalytic activity of LPOR. However, mutation of either residue impairs the
556 formation of the ground state ternary enzyme-substrate complex, indicating their key role in
557 substrate binding (Dahlin et al., 1999; Heyes and Hunter, 2002). Both residues have multiple
558 roles in catalysis, involving formation of the ground state ternary enzyme-substrate complex,
559 stabilization of a Pchlide excited state species and proton transfer to the reaction intermediate
560 formed after the light reaction (Menon et al., 2009; Dong et al., 2020) (Figure 2).

561 LPOR contains 14 amino acids unique TFT domain that distinguishes LPOR from other
562 structurally related SDR enzymes (Gabruk et al., 2012). The LPOR homologs are structurally
563 conserved with sequence identities of about 54% - 65% between higher plant, cyanobacterial
564 and algal enzymes (Suzuki and Bauer, 1995; Li and Timko, 1996; Dahlin et al., 1999). The
565 secondary structure analysis of LPOR by CD spectroscopy shows 33% alpha-helix, 19%
566 beta-sheets, 20% turn, and 28% random coil (Birve et al., 1996).

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567 **7.2. Crystal structure of LPOR:** Crystal structure of LPORs in their free form (Zhang et
568 al., 2019) and complexed with NADPH have been solved from *Thermosynechococcus*
569 *elongatus* and *Synechocystis* sp. PCC 6803 at 1.3 Å – 2.4 Å resolution (Zhang et al., 2019;
570 Dong et al., 2020). The above studies highlight the potential importance of hydrogen-bonding
571 networks involving the interaction of LPOR active site residues and Pchlide. The general
572 scaffold of protein remains similar to the typical αβα-topology with a central β-sheet. The
573 crystallographic studies of LPOR demonstrate an 8β-sheet consisting of strands β 3-β 2- β 1-
574 β 4- β 5- β 6- β 7- β 8, the latter being antiparallel. The β-sheets are surrounded by 6 α-
575 helices, (αA, α B, α H) on one side and (αC, α D, α F) on the other (Dong et al., 2020).

576 The LPOR homologs of *Synecocystis* and *T. elongatus* contain four evolutionarily conserved
577 cysteine residues; Cys38, Cys89, Cys199, and Cys226 around the active site implicated in
578 Pchlide binding and catalysis. Cys-38 and Cys-89 locate at the ends of β2 and β4,
579 respectively, and Cys-199 locates within αF (Silva, 2014; Dong et al., 2020). Cys 226 is in
580 the loop between β6 and αG and is found to be essential for LPOR membrane interaction.
581 During the proton relay pathway through the catalytic tetrad abundant intermolecular polar
582 interactions take place among NADPH, LPOR, and surrounding water molecules with the
583 help of functional groups and backbone atoms to stabilize the cofactor (Dong et al., 2020).

584 Near the nicotinamide end, a clam-shaped cavity is formed by predominantly hydrophobic
585 and aromatic residues consisting of Leu232, Phe233, His236, Tyr237, Phe240, Phe243, and
586 Phe246 etc (Dong et al., 2020) (Fig 3. Near here). The extra loop of 33 amino acid segments
587 uniquely present in LPOR and absent in other SDR enzyme superfamily members overlaps
588 with certain fragments of the clam–shaped cavity. It participates in Pchlide binding,
589 formation of pigment-complexed POR aggregates and Chlide release (Birve et al., 1996;
590 Reinbothe et al., 2003; Sameer et al., 2021). The LPOR oligomerization takes place upon
591 Pchlide binding which brings about the interaction of the hydrophobic residues and
592 intermolecular interactions in the two distally located lid regions in the POR monomer active
593 site (Gabruk and Mysliwa-Kurdziel, 2015; Zhang et al., 2019, Zhang et al., 2021). It is known
594 that mostly two long α-helices are involved in POR oligomerization (Dong et al., 2020). A
595 POR octamer has been isolated and its structure investigated by cryo-electron microscopy at
596 7.7 Å resolution. This structure shows that oligomer formation is most likely driven by the
597 interaction of amino acid residues in the highly conserved lid regions (Zhang et al., 2021).

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598 **8. De-etiolation via LPOR:** LPOR was initially isolated and characterized from the etiolated
599 plastids of monocots such as barley, oat, and rye in the prolamellar bodies (PLB) (Apel et al.,
600 1980; Roper et al., 1980; Joyard et al., 1990). It is now known that cyanobacterial LPORs are
601 capable of forming PLBs indicating common features between the cyanobacterial and higher
602 eukaryotic LPOR enzymes (Masuda et al., 2009). When the seed germinates beneath the
603 earth in the absence of light i.e., during skotomorphogenesis, LPOR accumulates to high
604 levels as a macromolecular complex with Pchlide and NADPH in highly organized 3-D
605 lattice-like membrane structure known as prolamellar bodies (PLBs) inside etioplasts (Ryberg
606 and Sundqvist, 1982; Solymosi and Schoefs, 2008; 2010). Both the PLBs and PTs are
607 constituents of the etioplasts which are plastids that have not been exposed to light
608 (Reinbothe et al., 2010).

609 When etiolated leaves are subjected to a flash of light, the large aggregates of POR–Pchlide–
610 NADPH ternary complexes are converted to POR–Chlide–NADPH complexes. Such ternary
611 complexes have higher emission and are slowly dissociated into smaller complexes
612 accompanied by the progressive release of Chlide from the POR catalytic site. This leads to a
613 large blue shift in absorption and emission maxima of Chlide and is called the Shibata shift.
614 The process ends with the formation of Chlide absorbing at 672 nm and emitting at 682 nm
615 (Chlide682). Crosslinking experiments have shown that Chlide 672-682 is partly composed
616 of Childe still bound to POR complexes and partly by Chlide bound to other proteins (Ryberg
617 et al., 1992; Wiktorsson, 1993). Shibata shift is followed by the formation of photoactive
618 photosystem II (PSII) units (Franck et al., 1999). The Shibata shift is arrested in extreme
619 environmental conditions that impair plastid development (El Hamouri et al., 1981; Dalal and
620 Tripathy, 2012).

621 During plant development in dark, both the photo- and non-photoactive pools of Pchlide
622 accumulate at different proportions. The Photoreduction of Pchlide to Chlide is mediated by
623 several short lifetime intermediates, e.g., semi-reduced Pchlide radical species formed by
624 hydrogen transfer from NADPH (Belyaeva et al., 1988; Lebedev and Timko, 1999) and
625 characterized by their very low fluorescence yield (Schoefs, 2000). At least three different
626 spectral forms of Pchlide are recognized in intact tissues based on their fluorescence emission
627 maximum (in nm): Pchlide F631, the short-wavelength Pchlide form thought to be located in
628 the tubular lamellae pro-thylakoids bound in a monomeric form to proteins. F644 due to the
629 association of oligomeric LPOR, and Pchlide F655 due to localization in PLBs with
630 polymeric LPOR (Böddi et al., 1992, 1993). The fluorescence lifetime of Pchlide measured in

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631 plants showed that short- and long-wavelength Pchlide forms have fast (0.3 to 0.8 ns) and
632 slow (5.1 to 7.1 ns) components with different proportions depending on plant species (Apel
633 et al.,1980; Sundqvist and Dahlin, 1997; Böddi et al., 1998; Kis-Petik et al., 1999; Mysliwa-
634 Kurdziel et al., 2003).

635 The main photoactive form present in etiolated plants is Pchlide F655, which after
636 illumination is converted to Chlide and subsequently to Chl (F682) through the formation of
637 long wavelength intermediates (Bodd□di and Franck, 1997; Schoefs et al., 2000). The
638 reaction consists of 3 distinct steps including an initial light-driven step followed by dark
639 steps which occur close to or above glass transition temp of proteins. The reduction reaction
640 occurs at temperatures as low as 193K, and in response to femtosecond manipulation of light
641 pulses, signifying its biochemical novelty (Heyes and Hunter, 2005; Heyes et al., 2006). As a
642 result of the light-induced reduction of Pchlide, PLBs disintegrate and the etioplast develops
643 into the chloroplast. The PTs ultimately transform into well-organized thylakoid membranes
644 (Oliver and Griffiths, 1982; Ryberg and Sundqvist, 1988). The isoforms of LPOR are present
645 at different locations of etio-chloroplasts inner membranes (Grzyb et al., 2013; Kowalewska
646 et al., 2016). After flash illumination, the photoactive Pchlide complex can be regenerated by
647 reloading with non-photoactive Pchlide on a fast time scale with concomitant release of
648 Chlide (Franck et al.,1999; Schoefs et al., 2000). Long-term illumination (i.e., greater than a
649 minute) usually converts non-photo-active Pchlide to photo-active Pchlide.

650 The spectrally different forms of Pchlide in etioplast are attributed to the formation of
651 different size aggregates of LPOR and NADPH. LPORs from different organisms can adopt a
652 variety of soluble oligomeric forms ranging from monomers in cyanobacteria to higher-order
653 oligomers in plant enzymes (Gabruk and Mysliwa-Kurdziel ,2015; Gabruk et al., 2015).
654 Pchlide binding leads to a structural reorganization in the POR enzyme, which in turn induces
655 an interaction of POR monomers (Böddi et al., 1989). POR aggregates account for
656 approximately 95% of total protein in PLBs. LPOR dimerization is not conserved for
657 cyanobacteria species such as SyLPOR and TeLPOR (Dong et al., 2020). In contrast,
658 oligomerization is better conserved in higher plants; species-specific amino acid residues
659 mediate the oligomerization of LPOR. In Arabidopsis thaliana residues 85–88 and 240–270
660 regions are implicated to participate in oligomerization (Gabruk et al., 2016). Glycerolipids
661 monogalactosyl diacylglycerol (MGSG), digalactosyl diacylglycerol (DGDG) and sulfosyl
662 quinoline diacylglycerol (SQDG) account for a total of 80-20 % of the total lipids in the
663 thylakoids membrane (Solymosi and Schoefs, 2010; Gabruk et al., 2017; Fujii et al., 2017;

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664 2018; Gabruk and Mysliwa-Kurdziel, 2020; Heyes et al., 2021). These lipids play an
665 important role in the aggregation of the Pchlide-LPOR-NADPH complexes, and membrane
666 structure organization in etioplast development (Kobayashi, 2016; Heyes et al., 2021).

667 There is a possibility of the presence of species-specific motifs in plant LPORs within the
668 oligomerization region. Numerous studies on leaves and isolated plastids indicate that
669 Pchlide: LPOR: NADPH aggregates interact with the lipids of PLB and are responsible for
670 light-triggered PLB dispersion (Engdahl et al., 2001; Gabruk et al., 2017; Fujii et al., 2017;
671 Gabruk and Mysliwa-Kurdziel, 2020). The exact mechanisms for these processes, however,
672 are still elusive (Gabruk and Mysliwa-Kurdziel, 2020). Prokaryotic LPORs from *Gloeobacter*
673 *violaceus* PCC7421 and *Synechocystis* sp PCC6803 could successfully restore characteristic
674 PLB structures in LPORA knockout mutant of *A. thaliana* even though the size and structure
675 of PLBs were normal, there was a lower ratio of photoactive to non-photoactive Pchlide
676 (Masuda et al., 2009). LPOR overexpression studies in LIPOR deficient cyanobacterium in
677 the dark show the formation of PLB-like ultra-structures in dark. These studies clearly show
678 the intrinsic capability of LPOR to trigger PLBs formation irrespective of its origin in
679 phototrophs (Yamamoto et al., 2020).

680 **9. LPOR diversity and phylogeny**

681 LPOR contains multiple isoforms that exhibit differential subcellular localization, expression
682 pattern, mRNA stability, plastid import pathway and response to light. Although POR
683 proteins were known since a long time, the genes coding PORA and PORB were first
684 identified *in A. thaliana* and *H. vulgare* (Reinbothe et al., 1996). Since then, LPOR sequences
685 have been discovered in a number of phototrophs. In higher plant LPOR isoforms show
686 >70% sequence identity for the precursor polypeptides and >80% sequence identity for the
687 mature proteins. The transit peptide region at the N terminal which is not a part of the mature
688 enzyme shows lowest homology (Dong et al., 2020).

689 In gymnosperms LPOR is encoded by a large multigene family, for instance eleven copies of
690 PORB and two copies of PORA have been identified in (Loblolly pine) *Pinus tadea*, *Pinus*
691 *mungo, Pinus strobus* (Spano et al., 1992; Forreiter and Apel, 1993, Skinner and Timko
692 ,1998, 1999). The function, mechanism, and localization of different LPOR isoforms have
693 been studied and it varies in different tissues during different developmental stages (Masuda
694 and Takamiya, 2004). *A. thaliana* contains three LPOR isoforms (AtPORA, AtPORB, and
695 AtPORC) (Reinbothe et al., 2010; Sousa et al., 2013; Masuda and Takamiya, 2004; Oosawa

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696 et al., 2000; Benli et al., 1991; Armstrong et al., 1995; Su et al., 2001, Pattanayak and
697 Tripathy, 2002). *Zea mays* contains PORA and two PORB orthologs PORB1 and PORB2,
698 latter promoting tocopherol biosynthesis post anthesis. Two POR isoforms are found in
699 *Nicotiana tabacum* (Masuda and Takamiya, 2004), *Lycopersicon esculentum* (Masuda and
700 Takamiya, 2004), *Zea mays* (Horton and Leech, 1975), *Oryza sativa* (Sakuraba et al., 2013;
701 Kwon et al., 2017), *Hordeum vulgare* (Apel et al., 1980; Apel, 1981; Schulz et al., 1989;
702 Holtorf et al., 1995), ornamental plant *Amaranthus tricolor* (Iwamoto et al., 2001) and several
703 other species. A single LPOR gene has been detected in *Synechocystis* sp.strain PCC6803
704 (Suzuki and Bauer,1995; Fujita et al., 1998; Rowe and Griffiths, 1995; Kaneko et al., 1996) ,
705 *Plectonema boryanum* (Fujita et al., 1998) , *Phormidium lamonosum* (Fujita et al., 1998;
706 Rowe and Griffiths, 1995), *Chlamydomonas reinhardtii* (Li and Timko, 1996), *Marchantia*
707 *paleacea* (Takio et al., 1998), *Pisum sativum* (Spano et al., 1992), *Triticum aestivum* (Teakle
708 and Griffiths, 1993; Masuda and Takamiya, 2004; Schoefs and Franck, 2003) , *Avena sativa*
709 (Darrah et al., 1990; Klement et al., 1999), *Musa* (Coemans et al., 2005) and *Cucumis sativus*
710 (Yoshida et al., 1995;Fusada et al., 2000). PORA is exclusively expressed in etiolated
711 seedlings and its mRNA abundance and its expression declines rapidly upon illumination in
712 *Hordeum vulgare* and several other species (Armstrong et al., 1995; Reinbothe and
713 Reinbothe, 1996; Runge et al., 1996; Masuda et al., 2003; Garrone et al., 2015). PORA is
714 light-sensitive, and it majorly accumulates during skotomorphogenesis and plays a critical
715 role in the etioplast development and photomorphogenesis (Paddock et al., 2012; Gabruk and
716 Mysliwa-Kurdziel, 2015). Overexpression studies of PORA in porB-1 porC-1 double mutant
717 restore the Chl synthesis at varying light intensities indicating that transiently active PORA
718 might be capable of functioning at a range of light intensities (Paddock et al., 2010). In
719 essence, PORA expression is negatively regulated on exposure to light. PORA evolved
720 mostly to protect etiolated seedlings from Pchlide-sensitized singlet oxygen-induced
721 photooxidative damage.

722 In contrast, PORB transcripts are majorly present in thylakoid membranes in young dark-
723 grown seedlings and in illuminated seedlings. PORB concentration remains unaffected during
724 the change of illumination conditions from dark to light (Lebedev and Timko, 1999; Ha et al.,
725 2017; Buhr et al., 2017). PORB is present right from the seedling development to throughout
726 the life of the plant in mature tissues. PORB closely resembles PORA but there are
727 significant differences between the two enzymes with respect to gene expression,
728 requirements for import of the precursor into the chloroplast ad stability in light. Thus, PORA

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729 and PORB have unique functions in etiolated seedlings and at the onset of greening
730 (Aronsson et al., 2000; Masuda et al., 2003; Dahlin et al., 1999, Pattanayak and Tripathy,
731 2002, 2011).

732 PORC mostly evolved in Brassicales is additionally light indulgent than either PORA or
733 PORB. PORC is expressed in a light intensity dependent manner, being highly expressed in
734 high light (Oosawa et al., 2000; Su et al., 2001; Pattanayak and Tripathy, 2002). PORC
735 mRNA accumulates only after illumination in etiolated seedlings and is predominantly
736 detected in fully matured green tissues during development and throughout the life of the
737 plant (Su et al., 2001; Pattanayak and Tripathy 2002, 2011; Paddock et al., 2010). Despite the
738 physiological equivalence and a perceived redundancy in PORB and PORC functions in
739 mature plants under normal growth conditions it is seen that PORC is differentially regulated
740 and is not under circadian control like PORB. The PORC transcripts are positively regulated
741 by increasing intensity of light while PORB mRNA decreased partially under high light
742 conditions in *Arabidopsis*. Thus, PORB although constitutively active from the seedling stage
743 to the mature plants it is less active under high light conditions (Masuda et al., 2002).

744 Based on the biochemical analysis and evolutionary studies Gabruk and Mysliwa-Kurdziel
745 2020, proposed two group of LPOR enzymes- a) Z type LPOR - bacterial origin and b) Plant
746 origin LPOR- S type (AtPORC type active enzymatically active without lipids) and L type
747 LPOR (are active when bound to lipid membrane).

748 **9.1. Phylogeny of LPOR isoforms:** In the present study we analysed 270 LPOR protein
749 sequences of AABPs, cyanobacteria, algae, bryophytes, pteridophytes, gymnosperms and
750 angiosperms along with LPOR sequences from non-photosynthetic organisms having the
751 characteristic SDR catalytic motif YxxxK and the conserved sequence (Gly-X-X-X-Gly-X-
752 Gly) from the publicly available databases. A Maximum likelihood phylogenetic tree was
753 constructed with Mega 7 software after aligning the sequences with MUSCLE multi-
754 sequence alignment program (Kumar et al., 2016). The phylogenetic distribution was
755 inferred using the boot strap method (500 replicates) with WAG (Whelan and Goldman
756 matrix) and Freq (+F) model. Similar results were obtained when the above LPOR sequences
757 were analysed by neighbor-joining method and UPMGA (Unweighted pair group method
758 with arithmetic mean) (Whelan and Goldman, 2001) (Figure 4. Near here).

759 LPOR sequences in the present study have been split into 4 major clades, clade 1 shows a
760 deep branching radiation representing LPOR sequences from phylum actinomycetota order

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761 Actinomycetales, Micrococcales, Mycobacteriales) and fungal division Ascomycota (orders
762 Incertae sedis, Leotiomycetes sodariomycetes, Saccharomycetales, Mucorales, Helotiales,
763 Eurotiales, Mortierellales) interspersed with beta proteobacteria Burkholderia, α-
764 cyanobacteria and SAR (Stramenopiles-Heterokonts-Alveolates) supergroup. *Streptomyces*
765 *sviceus*, *S. davaonensis*, *Lactococcus latctis* subsp. lactis lie close to Burkholderiales.
766 Phaeophycean *Ectocarpus siliculous* remains close to beta proteobacterial sequences and
767 fungal LPOR sequences.

768 There is a clear phylogenetic clustering of LPOR sequences from Sporidiobolales,
769 Chaetothyriales, Mucorales, Schizosaccharomycetales, Saccaromycetales, Pezizales, and
770 Kickxellaleslies closer to beta proteobacterial sequences. Thus, members of Actinomycetota,
771 Psuedomonadota lie close forming a distinct group. The anaerobic and facultative anaerobic
772 non photosynthetic bacteria show diversification of LPOR enabled by HGT.

773 LPOR in AAPBs is involved in photoconversion of Bacterioprotochlorophyllide to
774 Bacteriochlorophyllide. LPOR sequences from Gemmatimonadetes, betaproteobacteria
775 Limnohabitans, alphaproteobacteria -*Sulfitobater*, *Loktanella* and *Dinoroseobater* lie close to
776 β-cyanobacterial sequences. PORC sequence from algae *Symbiodinium microadriaticum* and
777 LPOR sequences from chlorophyta, charophyta, bryophytes, marchnatiaophyta, lycophyta
778 and gymnosperms cluster together closely with a high bootstrap percentage. Consistent with
779 the previous studies the LPOR of *Gemmatimonadetes* bacterium clusters within beta
780 proteobacterial LPORs as a sister of Limnohabitans.

781 Presence of LPOR sequences in fungal and other non-photosynthetic bacteria may be a result
782 of infrequent HGT from photoautotrophs to heterotrophs including bacterial and fungal
783 pathogens. *Choanephora. cucurbitarium* LPOR sequence is in all likelihood transferred from
784 prokaryotic or eukaryotic phototroph to fungi as a result of overlapping ecological niche or
785 pathogenic close association (Richards et al., 2009; Tunjić and Korač, 2013; Qiu et al., 2016;
786 Min et al., 2017). LPOR sequences might be involved in Mycobacterium and fungi in certain
787 reduction reactions.

788 Clade 2 majorly includes many sequences from β-cyanobacteria, algae and certain
789 anoxygenic photosynthetic bacteria. The β-cyanobacterial clade remains separated from α
790 cyanobacterial sequences. SAR LPOR sequences from Gillardia, Cyanidioschyzon merolae,
791 Chondrus crispus, Gracilariopsis chorda, Porphyra umbilicalis, Symbiodinium
792 microadriaticum PORA form a distinct group with 98-60 % bootstrap percentage.

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793 The placement of AAPBs close to picocyanobacteria suggests these LPOR sequences have
794 been acquired by AAPBs through HGT from oxygenic phototrophs (Gabruk and Mysliwa-
795 Kurdziel, 2020: Chernomor et al., 2021). LPOR sequences were transferred from
796 picocyanobacteria to α proteobacteria and then transferred to beta proteobacteria (Chernomor
797 et al., 2021). The LPOR of Gemmatimonadetes cluster with Limnohabitans suggesting a
798 HGT from Limnohabitans to Gemmatimonadetes.

799 Members of cyanobacteria- *Gloeobacter, Cyanothece, Hassalia, Tolypothrix, Limnorphais,*
800 *Nostoc, Chlorogloeopsis, Anabaena , Microcoleus , Oxynema , Halomicronema , Calothrix ,*
801 *Fremyella diplosiphon , Thermosynechoccus elongates , Synechocystis , Plectonema boryana*
802 , *Synechococcus* , *Cyanobium* form a separate group in clade 2 with 76% bootstrap
803 percentage. These cyanobacterial species might have evolved LPOR in a certain
804 environmental niche habitat (Pattanaik et al., 2011).

805 The recent endosymbiont *Paulinella chromatophora* that engulfed alpha cyanobacterium
806 retained the alpha cyanobacterial LIPOR in its chromatophore (Marin et al., 2007; Kim and
807 Park, 2016; Vedalankar and Tripathy, 2019). However, its nuclear LPOR sequence is closer
808 to β cyanobacteria *Synechococcus sp. WH5701*, *Cyanobium*, *Plectonema boryanum* and other
809 species. The presence of LPOR in *P. chromatophora* is a result of HGT from β cyanobacteria
810 suggesting a polyphyletic origin of POR in *Paulinella* and possibly in several other
811 organisms. The Paulinella chromatophora LPOR sequence closest neighbour are *Marchantia*
812 *palea* and *Phsycomitrella* are positioned on the same branch and their closest neighbour is
813 *Selaginella*. The PORA and PORB sequences are positioned close to each other but on
814 separate branches. Amongst angiosperm PORB is closer to PORC sequences.

815 The chromatophore genome is highly reduced, encoding 867proteins that represent about
816 one-third of proteins of its free-living counterparts (Nowack et al., 2008). Similar to the
817 EGTs found in Archaeplastida, *P. chromatophora* has relocated> 70 chromatophore genes
818 into the nuclear genome (mostly involved in photosynthesis-related functions) (Nowack et
819 al., 2016; Zhang et al., 2017). By contrast, these genes represent<1% of the *Paulinella*
820 nuclear genome, while in *A. thaliana* some reports have suggested that the genes of
821 cyanobacterial origin can account for upto 18% of the nuclear genes (Martin et al., 2002).

822 The clade 3 and 4 mostly includes eukaryotic LPOR sequences from angiosperms consistent
823 with the other LPOR phylogenetic studies. The angiospermic LPOR sequences are distinctly
824 divided into–eudicots and monocots. Monocots members from Poales, Arecales, Zingiberales

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825 and asparagales lie close together. *Musa acuminate* LPOR lies close to *Zingiber officinale*
826 with a 62% bootstrap percentage. Poales from a distinct group with 88% bootstrap
827 percentage. All the monocot sequences are placed in between two distinct dicot groups.

828 Eudicots group - Brassicales, rosales, myrtales, malpighiales, malvales, gentianales, lamiales,
829 solanales, caryophyllales, fabales, rosales, apiales, saxifragales, asterales, ranunculales,
830 vitales, cucurbitales, ericales, fagales, malvales, oxalidales, celastrales, proteales, sapindales
831 lie close together forming one separate group of eudicots. Certain members of lamiales,
832 gentianales, solanales, saxifragales, ericales, fagales, cucurbitales, sapindales, asterales,
833 cornales and apiales from the second group of eudicots. PORA and PORB sequences are
834 clustered together whereas PORC sequence form a completely distinct subgroup among
835 eudicots suggesting a completely different selection pressure that led to their evolution.

836 Most of the sequences analysed distinctly show that despite a great deal of similarity between
837 the LPOR isoforms. These isoforms form separate nodes with PORC present at a distance
838 from PORA and PORB suggesting that PORA and PORB must have evolved around the
839 same time and PORC evolved later as a result of the selection pressure. In this context it is to
840 note that the wild diploid Brassicales and related hybrids evolved in inhospitable conditions
841 with abilities to withstand drought, heat, high light and salt stresses (Arias and Pires 2012;
842 Folk et al., 2020). This explains the evolution of LPOR in Brassicales in PORC gene is
843 usually expressed in high light conditions (Pattanayak and Tripathy, 2011) to protect plants
844 from ROS mediated oxidative stress.

845 The duplication events gave rise to LPOR isoforms and independent duplication events
846 happened in monocots and dicots. The LPOR gene kept duplicating with the diversification
847 of angiosperm species. Both primary and secondary duplication are responsible for the spread
848 of LPOR. HGT and EGT have played an exceedingly huge role in forging the evolution of
849 LPOR in phylogenetically diverse lineages (Gabruk and Mysliwa-Kurdziel, 2020).

850 **10. Conclusion**

851 Evolutionary studies validate the premise that cyanobacterial and plant LPORs originated in
852 the oxygenic Earth about 1.36 bya from a single common ancestor. LPORs originated in the
853 oxygenic Earth about 1.36 bya from a single common ancestor. The phylogenetic studies
854 clearly show a deep branching pattern in LPOR with a great degree of similarity between the
855 PORA and PORB in different taxa suggesting a closer relationship between them. PORC
856 originated in Brassicales in a separate duplication event as a result of unfavourable climatic

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857 conditions (Gabruk and Mysliwa-Kurdziel, 2020) to protect plants from environmental
858 stresses.

859 LPOR is a more evolved Pchide reducing enzyme and loss of LIPOR gene from several
860 eukaryotic lineages clearly show the importance of LPOR in synthesis of chlorophyll,
861 phytochrome mediated developmental processes and protection of plants from ROS induced
862 oxidative stress. Climatic conditions of increasing oxygen tension in atmosphere, high light,
863 high temp, drought, heat along with nutrient status led to multiple gene losses and gene
864 duplication events that played a big role in the current distribution of LIPOR and LPOR. The
865 evolution of LPOR paved way for the loss of LIPOR from highly evolved photosynthetic
866 organisms. The exclusive inheritance of LPOR in angiosperms is a result of differential
867 plastidic LIPOR gene loss. The coexistence of LIPOR and LPOR ensures that there is a
868 continuous supply of chlorophyll under altered (light, oxygen, temperature and other
869 environmental conditions.

870 It would be a challenge to determine if LIPOR and LPOR originated from a common
871 ancestral prokaryote. The distribution of LPOR is both a result of chance and contingency
872 and the relationship between LPOR and LIPOR seem to be less straightforward when looked
873 through the analysis of a few taxa. Our phylogenetic analysis indicates that LPOR is present
874 in a wide set of photosynthetic (AABPs, Cyanobacteria, algae, bryophytes, pteridophytes,
875 gymnosperms, angiosperms) and non-photosynthetic (bacteria and fungi) organisms. LPOR
876 sequences from non-photosynthetic organisms (actinomycetota, bacillota, ascomycota,
877 basidiomycota, mucoromycota, Zygomycota, zoopagomycota) lie close to prokaryotic LPOR
878 sequences. Although no potential functional significance can be assigned to these LPOR,
879 these might contribute to some other specific physiological role in the lifestyle of the
880 organism. Phylogenetic incongruence indicates that LPOR genes have been overwhelmingly
881 horizontally transferred between not only photosynthetic but also non photosynthetic
882 lineages.

883 In this context it would be interesting to see if the signature SDR motif in the fungi is coding
884 for functions yet to be understood. The identification of the function of these LPOR
885 sequences would give us a clue to the LPOR migration and evolution. Many more cases of
886 intra and interphylum HGT events that are yet to be discovered will shed better light on the
887 evolution of protochlorophyllide oxidoreductase.

888

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896 **Conflict of interest.**

897 The authors declare no conflict of interest.

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Figure legends

Figure 1. The schematic representation of the enzymatic steps involved in chlorophyll biosynthesis pathway starting from Glutamic acid

Figure 2. Proposed proton-relay path from Dong et al. 2020. (A) The hydrogen bond network bridging the Tyr193 ηO and a solvent water molecule within the SyLPOR and TeLPOR structures. The well-positioned water, shown in the red sphere, is fixed by the backbone oxygens of Ala91 and Asn115, and the ε-amino group of Lys197. The hydrogen bonds are shown in dashed lines and the bond lengths (Å) are in blue for SyLPOR and dark green for TeLPOR. (B) A proposed proton-relay path following the hydride transfer from NADPH to C17. The photon energy (hv) is represented by a yellow thunderbolt (Dong et al. 2020).

Figure 3. The crystal structure of SyLPOR and TeLPOR from Dong et al. 2020. Ribbon representation of the overall structures of SyLPOR and TeLPOR. (A) Two side views of SyLPOR. The secondary structure elements are colored in blue except the antiparallel β8 in yellow. The loop region is in gray. The LPOR-specific insertion is colored in black. The NADPH-binding sequence is colored in green. Four cysteine residues are shown in sphere mode. The cofactor NADPH is shown in stick-and-ball mode. (B) Front view of SyLPOR (Left), TeLPOR (Right), and their superimposition (Middle). The secondary structure elements of TeLPOR are colored in deep green except β8 in magenta; the NADPH-binding sequence is colored in cyan. The α-helices are labeled alphabetically, and the β-strands are labeled numerically (Dong et al., 2020).

Figure 4. Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Whelan and Goldman + Freq. model (Whelan and Goldman, 2001). The bootstrap consensus tree inferred from 500 replicates (Felsenstein,1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein,1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein,1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The analysis involved 270 amino acid sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment

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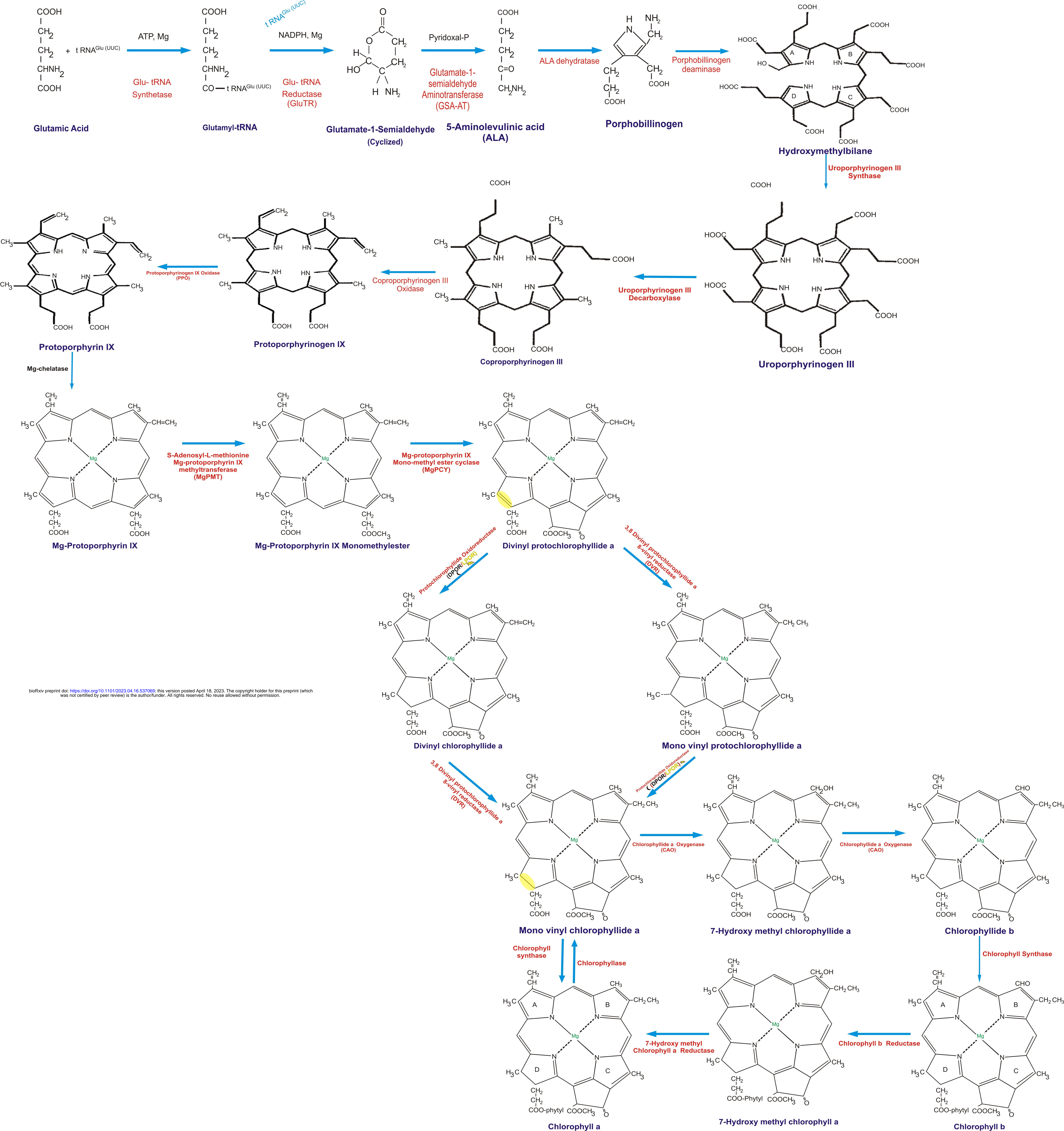
gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 282 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2015). A midpoint rooted circular phylogenetic tree was created and annotated in Interactive Tree of Life (Letunic and Bork, 2016; iTOL, https://itol.embl.de/).



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A
Lys197
Tyr193
Asn115
3.33/3.29
2.90/2.98
2.93/2.66
3.03/3.18
2.74/2.70
Ala91
B
Lys197
Tyr193
Asn115
N^+
H
H
H
O
H
O
H
O
O
H
O
H
hv
18
17
N
HO
2'
N
H
H
O
Ala91
Pchlide
COOH
NH_2
O
NADPH

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A

POR-specific insertion

Cys226 / Cys 4

Cys199 / Cys 3

GASSGL/VG

Cys37 / Cys 1

Cys89 / Cys 2

B

GASSGL/VG

Cys226 / Cys 4

Cys89 / Cys 2

Cys37 / Cys 1

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