

Photosystem II is a chimera of reaction centers

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A complete scenario for the evolution of photosynthesis must account for the origin and diversification of photochemical reaction centers. Two lively debated questions are how the distinct types of reaction centers evolved and how cyanobacteria acquired two distinct reaction centers—Photosystem I and Photosystem II—in the path towards the origin of light-driven water oxidation; or in other words, towards the evolution of oxygenic photosynthesis (Hohmann-Marriott and Blankenship 2011; Fischer et al. 2016). Here I show how the chimeric structure of Photosystem II provides unambiguous answers to these questions.

Multiple independent lines of evidence, provide support to the hypothesis that having two distinct reaction centers, Type I and Type II, side by side, is an ancestral state of the last common ancestor of photosynthetic bacteria. Firstly, the evolution of the chlorophyll synthesis pathway suggests that Type I and Type II reaction centers originated from an ancestral gene duplication event (Sousa et al. 2013); secondly, proteome similarity networks of a diverse group of prokaryotes provided a similar result (Harel et al. 2015). But earlier genome comparisons had lent support to the hypothesis that the last common ancestor to all photosynthetic organisms had already evolved two distinct reaction centers from an early gene duplication event (Mulkidjanian et al. 2006). Such a result has usually been followed by the question: “Which bacteria were the first phototrophs?” as asked by Mulkidjanian et al. (2006), or “in which lineage did the gene duplication take place?” as questioned by Sousa et al. (2013). Because cyanobacteria are the only known bacteria to display both types of reaction centers, the above questions lead to the hypothesis that photosynthesis originated in cyanobacteria or a protocyanobacterium. This interpretation is in conflict with phylogenomic data, which consistently do not locate cyanobacteria as a particularly early branch in comparison with other phototrophic groups (Ciccarelli et al. 2006; Segata et al. 2013; Marin et al. 2016). Therefore, the

evidence at hand (Mulikidjanian et al. 2006; Sousa et al. 2013; Harel et al. 2015) might be better reinterpreted as evidence for two distinct reaction centers as a trait of the ancestral populations of photosynthetic bacteria. Today, this trait is *retained*, not acquired, in cyanobacteria. From this perspective it can be deduced that this trait has been retained in cyanobacteria since the origin of photosynthesis and was later transferred to photosynthetic eukaryotes after primary endosymbiosis.

Further and more direct evidence for the ancient origin of two distinct reaction centers in a single ancestral bacterium is derived from the structure of Photosystem II. The Mn_4CaO_5 cluster of Photosystem II is not only coordinated by the D1 core subunit, but the CP43 antenna subunit also offers two ligands to the cluster: residue E354 coordinates two Mn atoms and R357 offers a hydrogen bond to two of the μ -oxo bridges. Site-directed mutagenesis of these two residues severely impairs the water oxidation cycle (Hwang et al. 2007; Service et al. 2011). What is more, both the CP43 and CP47 antennae play key roles in water and proton access into or out of the cluster. This means that the tight cooperation of a Type I and a Type II reaction center were required to evolve efficient water oxidation; this is because CP43 and CP47 share a common origin with Type I reaction centers (Mix et al. 2005), and for all intents and purposes are Type I reaction center proteins. From this observations I deduce that in order to evolve the Mn_4CaO_5 configuration of the cluster, a close interaction between an ancestral Type I and ancestral Type II reaction center was needed. Put in another way, Photosystem II is structurally not just a Type II reaction center, but more precisely, it is a chimera of Type I and Type II reaction centers.

The evolutionary link of the CP43 and the CP47 subunits with the core subunits of Photosystem II goes deeper. The D1 and D2 core reaction center proteins coordinate each a peripheral chlorophyll that allows excitation energy transfer from the core antenna to the reaction center dimer, coordinated by homologous histidine ligands, D1-H118 and D2-H117. Figure 1 shows that these are absent in anoxygenic Type II reaction center proteins (L and M) but they have been retained in Photosystem I and anoxygenic Type I reaction centers from heliobacteria and green sulfur bacteria (Cardona 2015). I pointed out before that sequence and structural homology in this region strongly suggest that these peripheral pigments are a trait present in the most ancestral photochemical reaction centers before the divergence of Type I and Type II reaction centers (Cardona 2016). Now, the fact

that these pigments have been retained in Photosystem II, as energy connectors between the reaction center (Type II) and the core antenna (Type I), argues definitively that the close interaction between the two type of reaction centers is continuous since the origin of photosynthesis, throughout the early diversification of Type I and Type II reaction centers, and until the origin of the Mn_4CaO_5 cluster in Photosystem II.

The origin of oxygenic photosynthesis required the evolution of two photosystems linked in series to shuttle electrons from water to NADP^+ and to power the regeneration of ATP. But it also meant that two linked photosystems were required to evolve Photosystem II and the Mn_4CaO_5 cluster. If two reaction centers is a trait of the last common ancestor to all photosynthetic organism, one question arises: what was the selective pressures at the earliest stage in the evolution of photosynthesis driving the divergence of both reaction centers and the specialization of their respective chemistries? Taking into consideration that: 1) there are no anoxygenic phototrophic bacteria with two distinct types of reaction centers, 2) the two distinct types of reaction centers linked in series is a unique trait of oxygenic photosynthesis, and 3) Photosystem II itself, the water oxidizing enzyme of oxygenic photosynthesis, is a chimera of the two distinct types of reaction centers: it is starting to look increasingly likely that the oxidation of water to oxygen may have been this selective pressure. If this is the case, it would place water oxidation early during the evolutionary history of photosynthesis, which is consistent with geochemical evidence for oxidative elemental cycling by 3.7 Ga (Rosing and Frei 2004; Frei et al. 2016) and henceforward (Lyons et al. 2014).

It is a long standing paradigm that anoxygenic photosynthesis predates oxygenic photosynthesis. I believe now there are multiple independent lines of evidence to start considering seriously the possibility that the evolution of light-driven water oxidation may have been the main driving force behind the origin and diversification of photosynthesis on Earth.

Acknowledgement

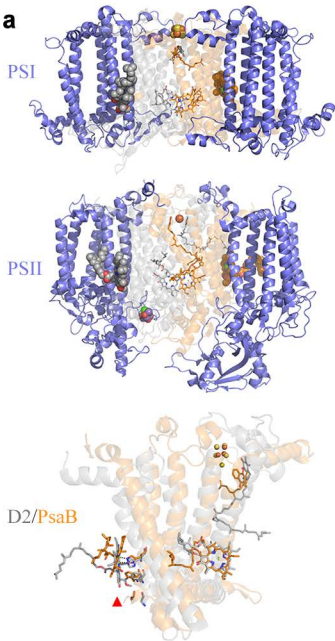
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References

- Cardona T (2015) A fresh look at the evolution and diversification of photochemical reaction centers. *Photosynth Res* 126:111-134. doi:10.1007/s11120-014-0065-x
- Cardona T, Murray JW, Rutherford AW (2015) Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria. *Mol Biol Evol* 32:1310-1328. doi:10.1093/molbev/msv024
- Cardona T (2016) Reconstructing the origin of oxygenic photosynthesis: Do assembly and photoactivation recapitulate evolution? *Front Plant Sci* 7:257. doi:10.3389/fpls.2016.00257
- Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. *Science*, 311:1283-1287. doi:10.1126/science.1123061
- Fischer WW, Hemp J, Johnson JE (2016) Evolution of oxygenic photosynthesis. *Annu Rev Earth Planet Sci* 44:647-683. doi:10.1146/annurev-earth-060313-054810
- Frei R, Crowe SA, Bau M, Polat A, Fowle DA, Døssing LN (2016) Oxidative elemental cycling under the low O₂ Eoarchean atmosphere. *Sci Rep* 6:21058. doi:10.1038/srep21058
- Harel A, Karkar S, Cheng S, Falkowski PG, Bhattacharya D (2015) Deciphering primordial cyanobacterial genome functions from protein network analysis. *Curr Biol* 25:628-634. doi:10.1016/j.cub.2014.12.061
- Hohmann-Marriott MF, Blankenship RE (2011) Evolution of photosynthesis. *Annu Rev Plant Biol* 62:515-548. doi:10.1146/annurev-arplant-042110-103811
- Hwang HJ, Dilbeck P, Debus RJ, Burnap RL (2007) Mutation of arginine 357 of the CP43 protein of Photosystem II severely impairs the catalytic S-state cycle of the H₂O oxidation complex. *Biochem* 46:11987-11997. doi:10.1021/bi701387b
- Lyons TW, Reinhard CT, Planavsky NJ (2014) The rise of oxygen in Earth's early ocean and atmosphere. *Nature* 506:307-315. doi:10.1038/nature13068
- Marin J, Battistuzzi FU, Brown AC, Hedges SB (2016) The timetree of prokaryotes: new insights into their evolution and speciation. *Mol Biol Evol* 34:437-446. doi:10.1093/molbev/msw245
- Mix LJ, Haig D, Cavanaugh CM (2005) Phylogenetic analyses of the core antenna domain: investigating the origin of Photosystem I. *J Mol Evol* 60:153-163. doi:10.1007/s00239-003-0181-2
- Mulkidjanian AY et al. (2006) The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci USA* 103:13126-13131. doi:10.1073/pnas.0605709103

- Rosing MT, Frei R (2004) U-rich Archaean sea-floor sediments from Greenland - indications of >3700 Ma oxygenic photosynthesis. *Earth Planet Sci Lett* 217:237-244. doi:10.1016/S0012-821x(03)00609-5
- Segata N, Bornigen D, Morgan XC, Huttenhower C (2013) PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. *Nat commun* 4:2304. doi:10.1038/Ncomms3304
- Service RJ et al. (2011) Participation of glutamate-354 of the CP43 polypeptide in the ligation of manganese and the binding of substrate water in Photosystem II. *Biochem* 50:63-81. doi:10.1021/bi1015937
- Sousa FL, Shavit-Grievink L, Allen JF, Martin WF (2013) Chlorophyll biosynthesis gene evolution indicates photosystem gene duplication, not photosystem merger, at the origin of oxygenic photosynthesis. *Genome Biol Evol* 5:200-216. doi:10.1093/Gbe/Evs127

Fig. 1 Structural comparison of Photosystem II and Photosystem I core proteins. **a** The antenna domain of Photosystem I and the antenna subunits of Photosystem II are shown in blue, while the core domain of PSI and the core subunits of PSII are shown in transparent grey and orange. The grey and orange chlorophyll molecules shown in spheres are the peripheral chlorophylls Chl_Z and Chl_D, also known as ChlZ_{D1} and ChlZ_{D2}, which are coordinated from the second transmembrane helix of D1 and D2, and the respective pair in Photosystem I. The bottom image shows an overlap of D2 and the core domain of the PsaB subunit of Photosystem I. **b** Primary structure of the second transmembrane helix of Type II reaction center proteins and the homologous region in Type I reaction center proteins. L and M represent Type II reaction center core proteins from purple and green non-sulfur bacteria. Several D1 and D2 subunits from cyanobacteria and a photosynthetic eukaryote (*Arabidopsis thaliana*) were also included in the alignment. Representative D1 sequences are marked G0, G1, G2, G3, and G4, for the different types of D1 as described by Cardona *et al.* (2015). A and B are sequences from the PsaA and PsaB subunits of Photosystem I respectively, H represents the PshA sequences of heliobacteria, and C represents the PscA subunits of green sulfur bacteria and the acidobacterium *Chloracidobacterium thermophilum*. Overall the level of sequence identity between the two types of reaction centers is low, yet there is conservation preserved in the primary and tertiary structures. The red arrow highlights the ligating histidine that coordinates the peripheral chlorophylls



b

L *Chloroflexus* sp. Y-400-f1
 L *Roseiflexus castenholzii*
 L *Methylobacterium* sp. 88A
 L *Blastochloris sulfoviridis*
 M *Chloroflexus* sp. Y-400-f1
 M *Kouleothrix aurantiaca*
 M *Rhodopseudomonas palustris*
 M *Hoeflea phototrophica*
 G4 *Arabidopsis thaliana*
 G4 *Nostoc* sp. PCC 7120
 G4 *Gloeobacter violaceus*
 G3 *Cyanothece* sp. ATCC 51142
 G2 *Chroococcidiopsis thermal.*
 G1 *Oscillatoriales cyanobact.*
 G0 *Gloeobacter kilauensis*
 D2 *Arabidopsis thaliana*
 D2 *Nostoc* sp. PCC 7120
 D2 *Gloeobacter violaceus*
 B *Arabidopsis thaliana*
 B *Nostoc* sp. PCC 7120
 B *Gloeobacter violaceus*
 A *Arabidopsis thaliana*
 A *Nostoc* sp. PCC 7120
 A *Gloeobacter violaceus*
 H *Heliobacterium modesticald.*
 H *Heliobacterium gestii*
 C *Chlorobium limicola*
 C *Prosthecochloris aestuarii*
 C *Chloracidobacterium thermo.*

PGFAWQ**M**T**V**L**F**A**T**I**A**FFG**W**M**R**Q**V**D
 PGFF**F**W**L**T**M**V**A**A**T**I**A**FV**G**W**L**L**R**Q**I**D
 EG**G**LW**Q**I**I**T**F**C**A**I**G**S**F**V**S**W**A**L**R**E**V**E
 EG**G**FW**Q**A**I**T**V**C**A**I**G**A**F**V**S**W**M**L**R**E**V**E
 EG**G**W**W**L**I**A**T**F**F**L**T**V**S**I**F**A**W**M**H**I**Y**T
 N**G**G**N**W**L**A**A**T**F**F**L**H**L**S**V**L**A**W**A**R**V**Y**S**
 EG**G**W**W**L**I**A**G**F**F**L**T**V**S**I**L**L**W**W**V**R**T**Y**R**
 D**G**G**W**Y**I**I**S**S**V**F**L**L**A**S**V**L**L**W**A**R**I**Y**V**
 N**G**G**P**Y**E**L**I**V**L**H**F**L**L**G**V**A**C**Y**M**G**R**E**W**E
 N**G**G**P**Y**Q**L**V**I**F**H**F**L**T**G**V**F**C**Y**L**G**R**E**W**E
 N**G**G**P**Y**Q**L**V**V**F**H**F**L**I**G**I**F**A**Y**L**G**R**E**W**E
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 P**L**G**T**A**D**F**L**V**H**H**I**H**A**F**T**I**H**V**T**V**L**L**I**L
 A**L**G**T**A**D**F**L**I**H**H**I**H**A**F**T**I**H**V**T**V**L**L**I**L
 H**L**G**T**A**D**F**M**V**H**H**I**F**A**L**C**I**H**V**T**V**L**L**I**L
 K**G**S**I**S**E**F**V**A**A**H**A**I**A**G**L**H**F**T**M**V**P**M**W**
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 D**G**E**I**N**D**A**I**F**F**W**L**M**I**G**G**W**L**F**G**F**I**P**L**L

