NEWS & VIEWS FEATURE

EVOLUTIONARY BIOLOGY

Out of thin air

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The invention of oxygenic photosynthesis was a small step for a bacterium, but a giant leap for biology and geochemistry. So when and how did cells first learn to split water to make oxygen gas?

The oxygen that gives us the breath of life is renewed by sunlight falling on plants, algae and a particular class of bacterium called cyanobacteria — all of which produce molecular oxygen (O_2) as a waste product of photosynthesis (Box 1)[1]. Biologists agree that cyanobacteria invented the art of making oxygen[2], but when and how this came about remain uncertain.

Oxygenic photosynthesis involves about 100 proteins that are highly ordered within the photosynthetic membranes of the cell. The main players are two molecular machines, photosystem I and photosystem II, that act as electrochemical solar cells. With the help of chlorophyll (the pigment that makes plants green), they transform sunlight into electrical current (Fig. 1). Photosystem II generates an electrochemical potential of + 1.1 volts, enough to remove two electrons from each of two water molecules, making a molecule of O_2 at a cost of four photons — one for each electron moved. Photosystem II performs this remarkable feat only when photosystem I is present to dispose of the electrons. Photosystem I grabs the four electrons and uses four more photons to deposit them, in two pairs, on an electron carrier called $NADP^+$. $NADP^+$ ultimately transfers the electrons to carbon dioxide, thereby providing the energy to make carbon-based sugars and the other molecules of life: light makes life and oxygen out of water and thin air.

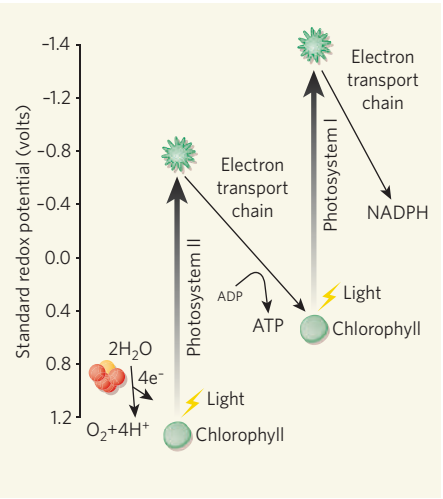


Figure 1 | The two photosystems in photosynthesis. Photosystems I and II absorb light energy, convert it into electrochemical potential, and are connected in series electrically. These two 'light reactions' of photosynthesis form links in a chain of electron (e^-) transfers that is coupled, by means of proton pumping, to synthesis of the energy-storage molecule adenosine triphosphate (ATP). The electron transport chain of photosynthesis, also known as the Hill and Bendall Z-scheme, ends with photosystem I delivering electrons to $NADP^+$, making NADPH. ATP and NADPH drive the 'dark reactions' that transfer the electrons to CO_2 so as to provide the energy to make sugars and the other molecules of life. The chain begins when water is oxidized to oxygen by the very high electrochemical potential of photosystem II. The catalyst of water oxidation (Box 3) is shown here as a cluster of four red spheres and one yellow one.

A matter of time

Oxygenic photosynthesis is the only significant source of O_2 known, so indications for O_2 in the geological record should date its origin. Geochemical evidence suggests that 2.3 billion years ago atmospheric oxygen had risen to more than 10^{-5} of its present concentration[3]. So this date should set a minimum age for cyanobacteria[4]. But might they be older? Combining palaeontology and molecular phylogeny, Tomitani *et al.*[5] claim that they are, arguing that 2.3 billion years maps not to the infancy, but to the middle ages of cyanobacterial evolution. Their argument is based on the presence in certain cyanobacteria of modified cells (heterocysts) that are dedicated to nitrogen fixation. Heterocysts occur only in the more specialized cyanobacterial lineages and offer a virtually O_2-free environment for nitrogen fixation, which is inhibited by O_2. Tomitani *et al.* reason that heterocysts would not have been needed until the advent of atmospheric O_2, and that their origin coincided with the onset of atmospheric O_2 accumulation.

Could cyanobacteria date back to 3.8 billion years ago, or even earlier? Not according to the geochemical data from a 3.4-billion-year-old sedimentary formation in South Africa called the Buck Reef Chert[6]. This massive formation shows evidence of having been deposited through carbon fixation by photosynthesizing organisms, but it contains none of the mineral traces expected from the production of O_2. Thus, the simplest interpretation is that the deposit was laid down by anoxygenic photosynthesis for which the source of electrons was molecular hydrogen or other inorganic

Box 1 | The discovery of photosynthesis

In 1772, Joseph Priestley described how oxygen is consumed by combustion or by respiration using a burning candle, or a live mouse, in a closed glass jar. His discovery predated the term oxygen, which was coined by Lavoisier[20,21]. The results were reported in a paper entitled 'Observations on Different Kinds of Air'[1]:

"...I flatter myself that I have accidentally hit upon a method of restoring air which has been injured by the burning of candles, and that I have discovered at least one of the restoratives which nature employs for this purpose. It is vegetation. In what manner this process in nature operates, to produce so remarkable an effect, I do not pretend to have discovered; but a number of facts declare in favour of this hypothesis...

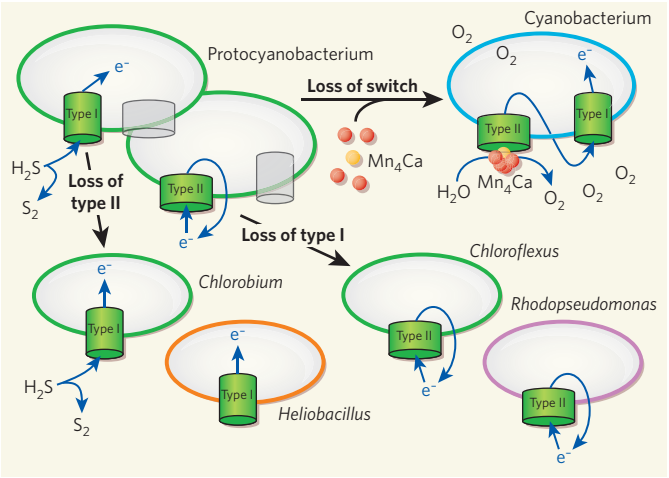
"One might have imagined that, since common air is necessary to vegetable, as well as to animal life, both plants and animal had affected it in the same manner, and I own that I had that expectation, when I first put a sprig of mint into a glass-jar, standing inverted in a vessel of water; but when it had continued growing there for some months, I found that the air would neither extinguish a candle, nor was it at all inconvenient to a mouse, which I put into it.

"...Accordingly, on the 17th of August 1771, I put a sprig of mint into a quantity of air, in which a wax candle had burned out, and found that, on the 27th of the same month, another candle burned perfectly well in it. This experiment I repeated, without least variation in the event, not less than eight or ten times in the remainder of the summer." **J.F.A. & W.M.**

Box 2 | A missing link in the evolution of photosynthesis

A modern anaerobic 'protocyanobacterium' possessing the genes encoding both photosystems involved in photosynthesis, but expressing them differentially, would be a bona fide missing link in the evolution of this vital process. This missing link (top left) would have the ability to switch between a *Chlorobium*-like, type-I photosystem and a *Rhodopseudomonas*-like, type-II photosystem[22]. In the protocyanobacterium, if either photosystem is permanently switched off, its mutation incurs no selective penalty, and its genes are eventually lost. Such a process may have given rise to the familiar, anoxygenic species, represented in the diagram by *Chlorobium* and *Heliobacillus* (type-I photosystems), and *Chloroflexus* and *Rhodopseudomonas* (type-II photosystems).

When both photosystems are being selected for, but under different growth conditions, a mutation in the switch could cause the two photosystems to coexist in the same membrane at the same time. This is usually harmful, and selected against, because the type-I and type-II electron (e^-) transport pathways (blue arrows) share components of the rest of the electron transport chain while being linear and cyclic, respectively, so the two photosystems would interfere with each other.



However, with a catalyst that oxidizes water (producing oxygen and electrons), the situation would be transformed because a continuous, smooth, linear electron flow is assured; the two photosystems would become complementary, and together begin to use light to drive oxygen production by the first true cyanobacterium (top right). The catalyst of water oxidation (Box 3) is shown here as four red spheres (each representing a manganese (Mn) atom) and one yellow one (representing a calcium (Ca) atom).

So, is there anything like a protocyanobacterium around today? One true cyanobacterium, *Oscillatoria limnetica*, turns off its genes for photosystem II in the presence of H_2S, and thus 'reverts' from oxygenic photosynthesis[23] to the kind of anoxygenic photosynthesis seen in *Chlorobium*. Other cyanobacteria retain the ability to use thiosulphate as an electron donor, in place of water, and photosystem II[24]. But the converse switch, turning off photosystem I so as to survive like *Rhodopseudomonas*, has not been seen. *Chloroflexus aurantiacus* has a type-II photosystem in combination with a peculiar kind of light-collecting antenna, called a chlorosome, that is otherwise specific to the type-I-containing *Chlorobium*. This has led to the suggestion that *Chloroflexus* is descended from a protocyanobacterium that lost its type-I photosystem[25]. **J.F.A. & W.M.**

molecules[4,6]. If 3.4 billion years is taken as an upper boundary for the age of O_2 synthesis, the origin of oxygenic photosynthesis would fall within the range of 3.4 billion to 2.3 billion years ago. This range is admittedly rather imprecise, but it is something. Of course, absence of evidence is not evidence of absence, and other authors suggest that O_2 could have been produced as early as 4.0 billion years ago, but was rapidly consumed[7,8].

On the double

So how did oxygen production originate? Some modern bacteria carry out anoxygenic photosynthesis, which is assumed to have been the precursor of the oxygen-producing reaction. But these bacteria use either a protein complex similar to photosystem I, or one similar to photosystem II — never both systems together[9]. For example, *Rhodopseudomonas palustris* is one of the purple photosynthetic bacteria, which possess only a photosystem-II-like reaction centre that drives a cyclic electron transport pathway (Box 2). Purple bacteria are versatile organisms, able to grow in the dark using cellular respiration as a source of energy, or in the light by switching on their photosystem. *Chlorobium tepidum*, on the other hand, is an anaerobic green bacterium that uses only a photosystem-I-like reaction centre to harness light energy, drawing electrons from hydrogen sulphide for linear electron transport.

No tree of bacterial life can readily account for the observed distributions of the two sets of photosystem genes among the species[10]. This has left biologists with little alternative but to suggest that genes encoding the photosystems have moved across species boundaries during evolution, a process called lateral gene transfer. But transfer from what to what? Did a cell containing photosystem I subsequently acquire photosystem II (ref. 11) or vice versa[12]? Or did both photosystems arise in a precursor of cyanobacteria — a 'protocyanobacterium' — later to be exported to other lineages by lateral transfer (Box 2)?

Mulkidjanian and colleagues[13] recently weighed in with evidence for the latter alternative. These authors compared the genome sequences of several cyanobacteria and anoxygenic photosynthetic bacteria, and identified a core set of genes involved in photosynthesis. They suggest that these genes arose in a now-extinct group of anoxygenic bacteria and then moved between species to other distant lineages. Like others[11], they suggest[13] that photosystem I was the ancestral prototype, from which an evolutionary precursor of photosystem II arose. The reaction-centre cores of the photosystems are similar in structure[14], and their divergence probably began with a simple duplication of the associated gene cluster. But before the water-splitting complex evolved, what would a bacterium with two different and specialized, non-oxygenic photosystems have done with them? Probably just what modern bacteria do: express them when needed, with the help of a regulatory switch.

How would a putative photosystem-switching bacterium then smooth the evolutionary path to oxygenic photosynthesis? It would have been only a small step away from the cyanobacterial state of oxygenic photosynthesis (Box 2), provided that it underwent the right mutation — disabling the regulatory switch — and provided that this happened in the right environmental setting and at the right time.

Centre stage

Of course, any proposed evolutionary scheme must explain not only the presence of two photosystems, but also how the oxidation of water came to be coupled to their reactions. This essential catalysis in photosynthetic oxygen production is carried out by metal ions: four oxidized manganese (Mn) atoms and a calcium (Ca) atom. These atoms are held in place by proteins, at a site called the water-splitting complex of photosystem II. One could say that the job of photosystem II in oxygenic photosynthesis is not to oxidize water, but rather to extract, one at a time, four electrons from the Mn_4Ca cluster. Only when this cluster is fully four electrons short does it replace them. It grabs them back in one fell swoop from two water molecules, thereby restoring its original charge state and releasing, in the process, the most useful waste product ever known — O_2.

The Mn_4Ca cluster lies on the surface of photosystem II that faces the environment. Its precise structure and the chemistry of its catalytic reactions have long been sought, not least because of the prospect of mimicking nature to exploit sunlight as a clean and renewable source of energy. At the end of last year, Yano

et al.[15] brought the cluster sharply into focus (Box 3). Their high-resolution structure shows, as some expected[8,16], a similarity to manganese oxide minerals. Might the Mn_4Ca cluster carry an evolutionary imprint of the specific environment where water-splitting arose? And is there anything special about manganese that makes it nature's only solution to water splitting?

It has been known for some time that manganese ions of the type that occur in the water-splitting complex are readily oxidized by ultraviolet light of wavelength less than 240 nanometres[8,17]. In this photooxidation process, ultraviolet light is absorbed by the electron cloud of a manganese atom, causing it to throw off an electron, and leaving the Mn atom in a more oxidized state. A similar photooxidation, but one involving chlorophyll and visible light, occurs in photosynthetic reaction centres. Moreover, it is well known that ultraviolet light damages the photosystem II of modern cyanobacteria through a process called photoinhibition. This involves absorption of ultraviolet light by Mn atoms in the water-splitting complex, which causes them to break away from photosystem II, depriving it of the ability to oxidize water[18]. So the light-absorbing behaviour of Mn ions is a biologically relevant process in modern photosynthesis.

Two into one

Given that there was no ozone layer to filter out the Sun's ultraviolet light before the origin of water splitting, what might have happened if an organism possessing both photosystems, but expressing only photosystem II, was introduced into an ancient, aquatic, Mn-containing environment? Photooxidation of environmental Mn would 'push' electrons into photooxidized chlorophyll of photosystem II. That would have been deadly, as a log-jam of electrons would stall the smoothly running electron transport cycle — unless the protocyanobacterium responded, either physiologically or through mutation, by expressing photosystem I. This complex would bleed off the surplus electrons from photosystem II, and create precisely the flow of electrons seen in cyanobacteria today: a linear flow through two photosystems, and from manganese to carbon dioxide (Box 2). The circuit would hardly have been perfect to start with, but the basic wiring would have been right. Notably, the Mn atoms of the water-splitting complex are bound directly to the proteins of the photosystem II reaction centre, without an intervening protein or electron carrier. This suggests that no major evolutionary invention was required for photosystem II to tap environmental Mn as an electron source.

The final step to the evolution of oxygen would then have entailed the transition from a photosystem exploiting an environmental supply of soluble manganese, where each electron-donating Mn ion reached photosystem II by simple diffusion, to one in which four manganese atoms (and a calcium of as-yet-unknown function) were held in place. A fine-tuning of photosystem II by natural selection to optimize its reduction/oxidation potential[19] would allow it to oxidize its biologically portable manganese reservoir four times in a row.

The best evidence for this evolutionary scheme would be the discovery of a modern-day protocyanobacterium. Although it is possible that all protocyanobacterial lineages have died out, we prefer to think that the missing link is still out there. An organism that possesses and expresses two photosystems, but only one at a time, may have adapted to present-day environments, such as eutrophic, anaerobic lakes with intermittent sulphide influx. An anoxygenic phototroph that switches gene expression between type-I and type-II photosynthesis could still be with us, unchanged from the time when its ancestors gave birth to our familiar, aerobic world[20]. ■

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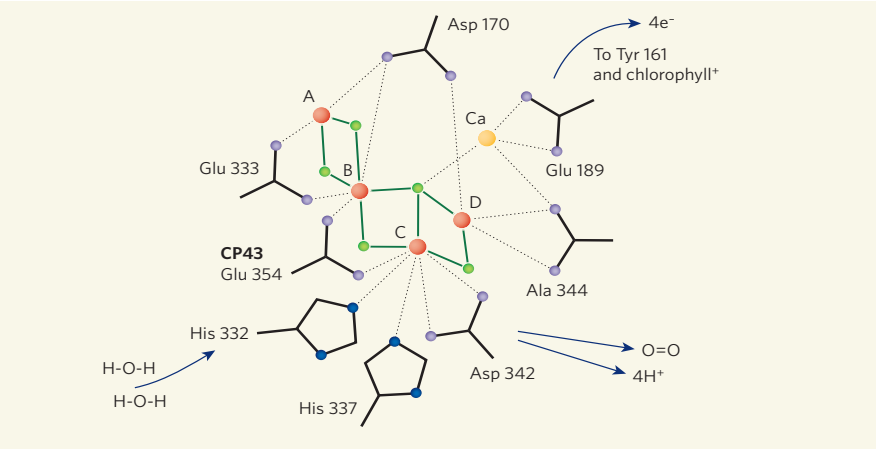
Box 3 | The water-splitting reaction centre



A model of the structure of the Mn_4Ca cluster in photosystem II (structure reproduced with permission from ref. 15). The 'model II' structure, from X-ray spectroscopy of a crystal of the photosystem II reaction centre, shows four Mn atoms (red; A–D) and a Ca atom (yellow; Ca). The figure shows a prediction of how the water-splitting metal-atom cluster fits into the highest-resolution protein structure so far obtained with X-ray crystallography. Predicted non-covalent bonds are shown as dashed lines. The bonds between Mn and O atoms are green. Protein amino-acid side-chains are identified by their three-letter abbreviation and number in the sequence of the 'D_1' reaction-centre protein. One of the side-chains is provided instead by glutamate 354 of a protein called 'CP43'. The Mn_4Ca cluster occupies a position close to the outer surface of the photosynthetic membrane, which would be below the plane of the graphic. When four electrons have been removed, one from each manganese atom, two water molecules become oxidized to give one diatomic oxygen molecule. Water enters and oxygen leaves at the outer surface of the membrane. Each electron is transferred in turn from the cluster to a tyrosine side-chain (Tyr 161; not shown) and moves through the reaction centre of photosystem II to a very strong electron acceptor deep in the reaction centre — the chlorophyll molecule that has been oxidized by light. **J.F.A. & W.M.**

1. Priestley, J. *Phil. Trans.* **62,** 147–264 (1772).
2. Blankenship, R. E. *Molecular Mechanisms of Photosynthesis* (Blackwell Science, Oxford, 2002).
3. Bekker, A. *et al. Nature* **427,** 117–120 (2004).
4. Olson, J. M. *Photosynth. Res.* **88,** 109–117 (2006).
5. Tomitani, A., Knoll, A. H., Cavanaugh, C. M. & Ohno, T. *Proc. Natl Acad. Sci. USA* **103,** 5442–5447 (2006).
6. Tice, M. M. & Lowe, D. R. *Geology* **34,** 37–40 (2006).
7. Kasting, J. F. *Nature* **443,** 643–645 (2006).
8. Russell, M. J. & Hall, A. J. in *Evolution of Early Earth's Atmosphere, Hydrosphere, and Biosphere — Constraints from Ore Deposits* (eds Kesler, S. E. & Ohmoto, H.) 1–32 (Geol. Soc. Am., Boulder, CO, 2006).
9. Bryant, D. A. & Frigaard, N.-U. *Trends Microbiol.* **14,** 488–496 (2006).
10. Raymond, J., Zhaxybayeva, O., Gogarten, J. P. & Blankenship, R. E. *Phil. Trans. R. Soc. Lond. B* **358,** 223–230 (2003).
11. Baymann, F., Brugna, M., Muhlenhoff, U. & Nitschke, W. *Biochim. Biophys. Acta* **1507,** 291–310 (2001).
12. Xiong, J. *et al. Science* **289,** 1724–1730 (2000).
13. Mulkidjanian, A. Y. *et al. Proc. Natl Acad. Sci. USA* **103,** 13126–13131 (2006).
14. Schubert, W. D. *et al. J. Mol. Biol.* **280,** 297–314 (1998).
15. Yano, J. *et al. Science* **314,** 821–825 (2006).
16. Sauer, K. & Yachandra, V. K. *Proc. Natl Acad. Sci. USA* **99,** 8631–8636 (2002).
17. Anbar, A. D. & Holland, H. D. *Geochim. Cosmochim. Acta* **56,** 2595–2603 (1992).
18. Hakala, M. *et al. J. Exp. Bot.* **57,** 1809–1816 (2006).
19. Rutherford, A. W. & Faller, P. *Phil. Trans. R. Soc. Lond. B* **358,** 245–253 (2003).
20. Lane, N. *Oxygen: The Molecule that Made the World* (Oxford Univ. Press, 2002).
21. Govindjee & Krogmann, D. in *Discoveries in Photosynthesis* (eds Govindjee, Beatty, J. T., Gest, H. & Allen, J. F.) 63–105 (Springer, Dordrecht, 2005).
22. Allen, J. F. *FEBS Lett.* **579,** 963–968 (2005).
23. Oren, A. & Padan, E. *J. Bacteriol.* **133,** 558–563 (1978).
24. Koenig, F. *Bot. Acta* **103,** 54–61 (1990).
25. Allen, J. F. & Puthiyaveetil, S. in *Photosynthesis: Fundamental Apects to Global Perspectives* (eds van der Est, A. & Bruce, D.) 753–756 (Alliance Communications Group, Lawrence, KS, 2005).