Chapter 14
Origin of Oxygenic Photosynthesis from Anoxygenic Type I and Type II Reaction Centers

John F. Allen

Abstract All anoxygenic photosynthetic bacteria currently known have photosynthetic reaction centers of only one type, either type I or II. In contrast, all oxygenic photosynthetic systems—of plants, algae, and cyanobacteria—have both type I and type II reaction centers. Molecular oxygen is the oxidation product of water in a type II reaction center that is connected, in series, with a type I reaction center. Around 2.4 billion years ago, the evolutionary origin of this series connection initiated biological water oxidation and began to transform our planet irrevocably. Here I consider the question of how separate type I and type II reaction centers diverged from a common ancestor. How they later became linked together, to become interdependent, is also considered, and an answer proposed. The "redox switch hypothesis" for the first cyanobacterium envisages an evolutionary precursor in which type I and type II reaction center genes are present in the genome of a single anoxygenic bacterial lineage, but never expressed at the same time, their gene products forming different reaction centers for light energy conversion under different growth conditions. I suggest that mutation disrupting redox control allowed these two reaction centers to coexist—an arrangement selected against prior to the acquisition of a catalyst of water oxidation while having a selective advantage thereafter. Predictions of this hypothesis include a modern, anoxygenic descendent of the proto-cyanobacterium whose disabled redox switch triggered the Great Oxidation Event, transforming both biology and Earth's surface geochemistry.

Keywords Electron transport • Photochemistry • Evolution • Molecular oxygen • Redox switch hypothesis • Gene expression • Biogeochemistry

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14.1 Two Light Reactions in Photosynthesis, Isolated or Connected

The primary process in photosynthesis is light-induced separation of electrical charge across a membrane [1]. In photochemical reaction centers this charge separation lasts long enough for its recombination to occur only after a number of secondary reactions have taken place. These secondary reactions include electron transport in a chain of coupled oxidation-reduction reactions, proton translocation to establish delocalized transmembrane gradients of pH (proton concentration) and electrical charge, and synthesis of ATP from ADP and inorganic phosphate. These reactions are together sufficient for complete photosynthesis, defined as the conversion of radiant energy into biologically useful chemical free energy. One or more assimilatory reactions, acting on environmental and inorganic substrates, are usually coupled, in turn, to the secondary reactions of photosynthesis. Thus photosynthesis is often linked to assimilation of carbon dioxide in photoautotrophy or to assimilation of molecular nitrogen in photodiazotrophy, providing essential inputs not only of energy but also of elemental carbon or nitrogen into living cells, organisms, and ecosystems. Biological, ecological, and geochemical nitrogen, carbon, and oxygen cycles can be viewed as the eventual, long-term return of electrons to a photooxidized chlorophyll that is the primary electron donor, P, in a photosynthetic reaction center. At its simplest:

$$\mathrm{DPA} + h\nu \rightarrow \mathrm{DP^*A} \rightarrow \mathrm{DP^+A^-} \rightarrow \mathrm{D^+PA^-}$$

where D is an electron donor, A stands for a chain of acceptors, P is the primary donor, and P* is its excited state. A committed reductionist might summarize ensuing reactions as follows:

$$\mathrm{D^+PA^-} \rightarrow \mathrm{DPA} + \textit{biology}$$

While all photochemical reaction centers use light to separate charge, moving an electron across a membrane, reaction centers can be divided into two distinct types according to the chemical identity of their immediate secondary electron donors and acceptors, each serving to stabilize the charge separation in a different way. The characteristics of the two types of reaction centers, and the relationship between them, are outlined in Fig. 14.1.

Type I reaction centers take an electron from a donor such as a cytochrome or the copper protein, plastocyanin, and use the energy of an absorbed quantum to pass the electron, through transient intermediates, to an iron-sulfur acceptor (a ferredoxin) on the opposing side of the membrane. Bacterial type I reaction centers (Fig. 14.1) then drive a linear electron transfer from any one of a range of inorganic donors to reduced ferredoxin, which supplies its electron, at low potential, to drive one or more of the coupled assimilatory reactions. In chloroplasts of cyanobacteria, plants, and algae, the type I reaction center is the core of photosystem I, which supplies electrons, also via ferredoxin, to $NADP^+$ and H^+, giving NADPH, which is oxidized again in the reactions of CO_2 assimilation. In photosynthetic bacteria, a hydrophilic

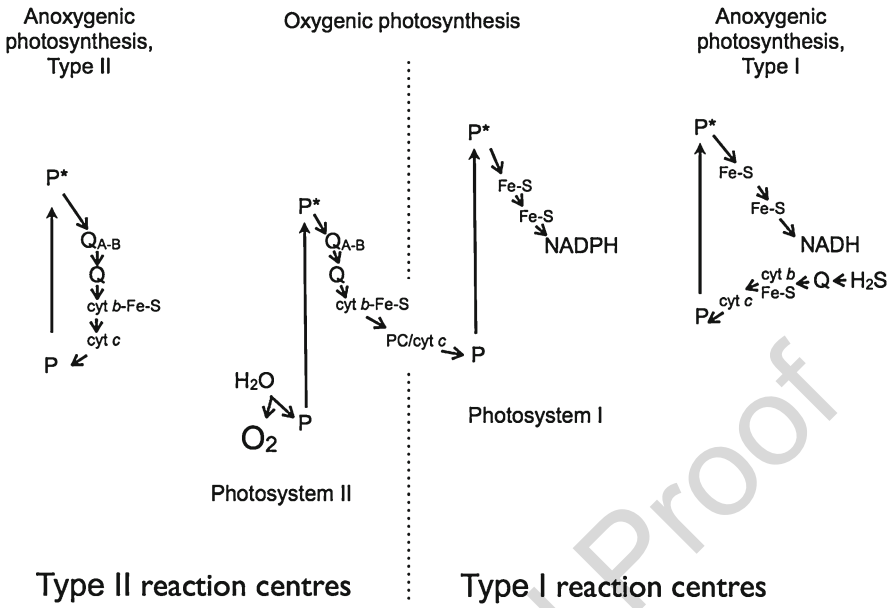

Fig. 14.1 Pathways of photosynthetic electron transport through reaction centers of type I and type II. *Arrows* indicate the direction of transfer of an electron, or liberation of oxygen. The vertical scale is one of redox potential—the tendency to donate or accept the electron. Light generates the excited state, P*, of the primary electron donor, P. P* is a strong electron donor, and loses an electron. In type II reaction centers, the electron passes to a pair of membrane-intrinsic quinones, Q_A and Q_B. In type I reaction centers, the electron passes to a chain of membrane-bound iron-sulfur centers, Fe-S, including water-soluble ferredoxin. Oxygenic photosynthesis couples the type II reaction center of photosystem II to the type I reaction center of photosystem I, allowing oxidation of water to supply electrons, and electrons pass through both reaction centers, reducing $NADP^+$ as the terminal electron acceptor. Anoxygenic photosynthesis has either a type I or a type II reaction centers, but never both. In all cases, a central cytochrome *b*-c_1 (or *b*-*f*)–Fe-S complex oxidizes a quinone pool, Q, in a proton-translocating cycle that transduces the free energy of the chemical redox reactions to a chemiosmotic, transmembrane proton-motive force, the intermediate between electron transport and ATP synthesis. *Cyt* cytochrome, *PC* plastocyanin

type I acceptor, ferredoxin, supplies electrons directly into assimilatory reactions with soluble components as intermediates in CO_2 fixation and nitrogen fixation. Type I photosynthesis is essentially a linear, or noncyclic, electron transfer, though the electron may return to re-reduce the primary donor after a few steps in a cyclic pathway in special circumstances, driving ATP synthesis without coupled net oxidation-reduction of any external substrate.

Type II reaction centers, in contrast, have lipophilic quinones as secondary electron acceptors. In anoxygenic bacterial type II reaction centers, the secondary donor is a cytochrome, which is re-reduced with electrons from the proton-translocating cytochrome *b*-c_1 complex, itself reduced by the reduced quinone (Fig. 14.1). The overall pathway of anoxygenic type II electron transfer is therefore cyclic, again with no net substrate-level oxidation or reduction. While type II

photosynthesis is cyclic in anoxygenic bacteria, the type II reaction center of cyanobacteria and of chloroplasts of plants and algae has a predominantly noncyclic pathway. In the latter, electrons are obtained from an inorganic donor—water. The oxidation product is free, molecular oxygen—photosynthesis is then *oxygenic*. The type II reaction center of oxygenic photosynthesis forms the core of photosystem II. Its eventual electron acceptor, from its reduced quinone secondary acceptor, plastoquinone, via the cytochrome *b-f* complex, is the type I reaction center of photosystem I.

Oxygenic photosynthesis takes electrons from water at a standard redox potential (H_2O/O_2) of +810 mV to $NADP^+$ at a standard redox potential ($NADPH/[NADP^+ + H^+]$) of −320 mV. The energy required to move an electron through more than 1.1 V comes from two photochemical reaction centers, one type II and the other type I. Their series connection means that they have the same electrical current, while their electrical potentials are added. Thus oxygenic photosynthesis of cyanobacteria and chloroplasts always requires two separate photosystems, photosystem II and photosystem I (Fig. 14.1). The terminology derives from the pigment systems I and II, proposed by Hill and Bendall [2] as components of electron transport in "the chloroplast reaction." The reaction center terminology of type I and type II derives from the evident biophysical and structural similarity of the reaction centers of photosystems I and II with each of the two major types of single, isolated reaction center found in anoxygenic photosynthetic bacteria. Anoxygenic photosynthesis uses just one reaction center of either type I or type II, and therefore has a quantum requirement of 1 for transfer of one electron. In contrast, oxygenic photosynthesis requires the coupling of the two distinct reaction centers of photosystem I and photosystem II, and therefore has a corresponding quantum requirement of 2. For assimilatory reactions such as CO_2 fixation, requiring four electrons, these differing quantum requirements are equivalent to 4 per CO_2 molecule for anoxygenic photosynthesis and 8 for oxygenic photosynthesis.

If anoxygenic photosynthesis requires half the number of quanta, why has the less quantum-efficient, *oxygenic* form of photosynthesis come to dominate biological energy flux and the global carbon cycle? The answer lies in the universal availability of the electron donor, water, in contrast to the potentially limiting supply of more easily oxidized electron donors such as hydrogen sulfide, hydrogen, Fe^{2+}, and reduced carbon compounds. Furthermore, inorganic electron donors other than water must have become less abundant after the advent of oxygenic photosynthesis, as oxygen began to suffuse the atmosphere [3]. Once water oxidation and oxygen evolution appeared and began to distribute oxygen as the energetically preferred terminal electron acceptor for respiration, then electron donors originally useful to single-photosystem, anoxygenic photosynthetic bacteria became restricted to special environments. Donors such as H_2S are now provided either from localized or transient geochemical efflux or as products of anaerobic respiration. Once photosynthesis had begun to produce oxygen, there was no turning back.

14.2 Anoxygenic Type I and Type II Reaction Centers: Divergence from a Common Ancestor

A wealth of spectroscopic evidence has long supported the resemblance, summarized in Fig. 14.1, between anoxygenic type I and the reaction center of photosystem I, and between anoxygenic type II and the reaction center of photosystem II [4–8]. This resemblance turned out to have a deep evolutionary foundation when it was seen that core protein subunits in the newly resolved structure of a purple bacterial, type II, reaction center exhibit functional amino acid sequence similarities with proteins of chloroplast photosystem II, as deduced from the nucleotide sequence of chloroplast DNA [9, 10]. An emerging structure of photosystem I from a cyanobacterium then indicated that the type I-type II division extends to the architecture and disposition of the central, membrane-spanning α-helices that traverse the membrane, holding the donors and acceptors in place for light-driven charge separation [11]. Today it is indisputable that the cores of photosystems I and II are examples of reaction centers of types I and II, respectively [12–16].

In evolutionary terminology, two structures are said to be homologous if they share a common ancestor. There can be no doubt that type I and type II reaction centers are homologous. What did their common ancestor look like, what were its electron donors and acceptors, and which sort of photosynthesis did it perform—cyclic or noncyclic? Figure 14.2 depicts reaction centers spanning a membrane, with divergence and specialization of type I and II reaction centers arising from a single ancestral and more versatile form that combined features of both. The prototype reaction center is depicted as having been capable of both cyclic, proton-motive electron transport and noncyclic electron transport with H_2S and ferredoxin as donor and acceptor, respectively. It should be noted that some authors favor the idea of the common ancestor having been a type I center [17] while others favor type II [18], each viewing the alternative type as a subsequent derivative of the favored precursor.

Vectorial electron transport—donor and acceptor lying on, or near, opposing sides of a membrane—is fundamental to biology, and not unique to photosynthesis. It is relevant and natural to ask how a vectorial electron carrier, predating light capture and conversion, might first have acquired a photoelectrochemical component, driving a reaction that had previously depended on an existing transmembrane redox gradient. This is an open question, and a fundamental one for understanding life on Earth and, perhaps, our prospect of our discovering life elsewhere. The answer may incidentally help to resolve the priority dispute between type I and type II reaction centers. At present it seems that a case can be made for either type I or type II coming first, while Fe-S centers, the hallmarks of type I, are likely to be more ancient electron carriers than quinones and cytochromes [19, 20]. With some exceptions, photosynthetic bacteria that are

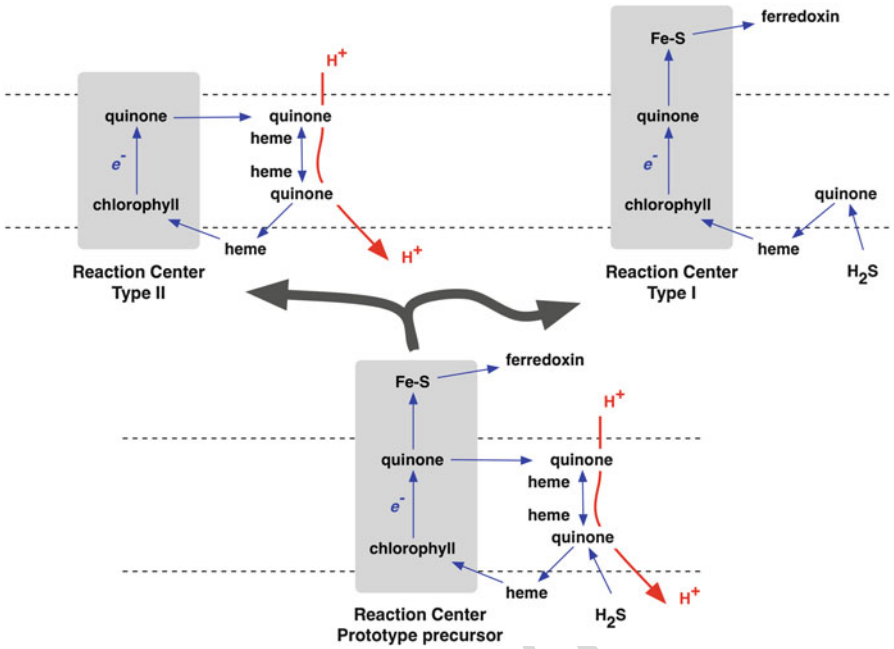

Fig. 14.2 Divergence of reaction center structure and function. A prototype photosynthetic reaction center diverges to give separate, type I and type II reaction centers, each preserving a subset of the original reaction center's functions. The primary electron donor is a chlorophyll molecule. The type I reaction center becomes adapted to noncyclic, H_2S-oxidizing electron transport with the iron-sulfur protein ferredoxin as the dominant secondary electron acceptor. The type II reaction center in turn becomes committed largely to cyclic electron transport, re-reducing the quinone. In the type II center a quinone is the predominant electron acceptor and also serves in a proton-translocating Q-cycle involving cytochrome hemes as electron carriers, eventually returning electrons to chlorophyll. Adapted from [59]

dependent on type I centers alone are also typically obligate anaerobes—still in hiding, as it were, from oxygen. Type II anoxygenic bacteria have adapted to survive aerobic environments by temporarily abandoning photosynthesis completely, becoming transiently chemotrophic. In facultatively phototrophic and anoxygenic bacteria, a redox genetic switch controls expression, on illumination and anoxia, of the apparatus of type II photosynthesis [21, 22]. This versatility may have been a later evolutionary acquisition, in which case type II came second, and modern type I anoxygenic photosynthetic bacteria more closely resemble the common ancestral form. However, there is a case for redox genetic switching being no novel innovation, being present even in the first living cells [23]. "Which came first?" remains a question for future research.

14.3 How Did the Two Divergent and Isolated Reaction Centers, Type I and Type II, Reconnect, and so Become Interdependent?

Two central electron transport pathways (Figs. 14.1 and 14.2), each with its own reaction center, must have become coupled together in series to comprise the oxygenic "Z-scheme" [24]. While photosynthetic production of oxygen from water occurs at the electron donor side of photosystem II, there is no oxygen evolution without photosystem I, which acts as the electron acceptor of photosystem II. Without exception, this series connection of a type I and a type II photochemical reaction center is necessary for sustained oxygen-evolving photosynthesis, where each photosystem depends absolutely on the other. Thus there is no single-reaction-center oxygenic photosynthesis. In fact, the resulting quantum requirement, for oxygen evolution, of 8 [25] is a minimum to which oxygenic photosynthetic systems approximate by means of both posttranslational [26] and transcriptional [27] mechanisms for optimal distribution of absorbed light energy between the two photochemical reaction centers. For maximal quantum yield of oxygen, redistribution of excitation energy and adjustment of photosystem stoichiometry occur in proportion to the varying and interrelated capacity of the two reaction centers to utilize this energy in photochemistry [28, 29]. A redox genetic switch, perhaps initiating oxygenesis itself, clearly found new applications following the onset of two-light-reaction photosynthesis.

Since type I and type II reaction centers evolved by diverging, under natural selection, from a common ancestor (Fig. 14.2), it follows that oxygenic photosynthesis, which depends on their coming together again, was a later addition to the photosynthetic repertory. The conclusion is that oxygenic photosynthesis appeared later, and evolved from anoxygenic photosynthesis. There is now abundant, diverse, and independent geochemical evidence that the Earth's atmosphere was largely anoxic from the planet's formation at 4.6 Gigayears, through a billion years or more of early life, metabolism, and ecology [30], up until the "Great Oxidation Event" at about 2.4 Gigayears (Fig. 14.3). Thus the emergence of oxygenic photosynthesis changed everything, imposing a requirement for oxygen tolerance on biochemical metabolism that is, to this day, fundamentally anaerobic. A self-renewing supply of free oxygen also meant the appearance of an abundant terminal electron sink for energetically favored aerobic respiration, eventually creating the conditions for complex, multicellular life. Molecular oxygen coincidentally allowed photo-conversion of diatomic oxygen to ozone in the upper atmosphere, creating a long-pass filter to attenuate ionizing ultraviolet radiation and making possible the colonization of the land.

A number of suggestions have been made concerning the eventual coupling of two anoxygenic reaction centers to give the oxygenic Z-scheme, with its interdependent photosystems I and II [6]. One idea with wide support is lateral gene transfer between different species and lineages, either from a type II-containing genetic donor to a type I recipient, or vice versa, from a type I genetic

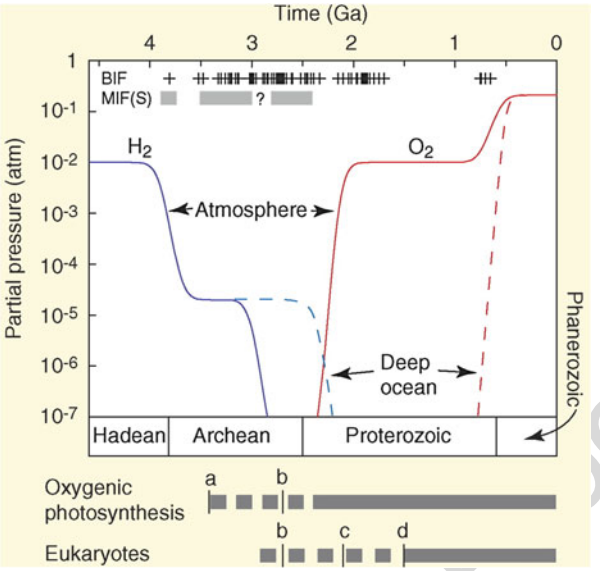

Fig. 14.3 Geochemical evolution of the atmosphere and oceans. BIF: banded iron formations. MIF(S): mass-independent fractionation of sulfur isotopes. Approximate time points: (**a**) the earliest evidence for anoxygenic photosynthesis; (**b**) the earliest known occurrence of steranes and 2-methylhopanes, taken as markers for aerobic metabolism; (**c**) the first putative eukaryotic microfossils; and (**d**) the first known assemblages of diverse eukaryotic microfossils in shallow marine sediments. Reproduced from [71]

donor to type II recipient. The complexity of a photosystem, correctly regulated and assembled by means of protein assembly factors and molecular chaperones, might make lateral gene transfer an implausible explanation for the coming together of photosystems I and II; the probability of every imported component being synthesized and fitting in place may be small. Nevertheless, it is notable that anoxygenic photosynthetic bacteria carry photosynthesis genes packaged into operons [22], so plasmid or viral [31] vectors can transfer a compatible and integrated set of photosystem genes. Concerted migration of a complete and active genetic system, coupled with its own membrane-bound metabolism, might be more likely to achieve such a result. This is a process now thought to lie, much later, at the endosymbiotic origin of chloroplasts and mitochondria in eukaryotic cells.

Another suggestion for the origin of the Z-scheme is that type I and type II reaction centers survived as functional entities within one or more distinct anoxygenic bacterial lineages [32, 33], eventually to hit upon the trick of water oxidation at the donor side of the type II center. A problem with this hypothesis is that the two separate modes of anoxygenic electron transport would have had to take place in separate membranes, or even in metabolically isolated subcellular compartments. One reason for this requirement is that if the two modes were present in the same membrane at the same time, then linear electron transport by

type I reaction centers would destroy redox poise required for sustained cyclic electron transport around the type II reaction center. Cyclic electron transport requires each electron carrier to be available as both a donor and as an acceptor. A linear electron transport pathway intersecting a cyclic one causes over-reduction (absence of acceptors) or over-oxidation (absence of donors) unless some compensatory mechanism exists to balance electron influx and efflux [34]. Such mechanisms are now found in oxygenic photosynthesis, where photosystem I on its own can cycle a proportion of electrons to drive proton pumping and ATP synthesis independently of NADPH production [35, 36]. In anoxygenic photosynthesis, however, it is difficult to see any additional benefit of controlled interaction of type I photosynthesis with type II photosynthesis, given that type I electron transport is capable, on its own, of electron cycling through the cytochrome complex in the absence of an external electron donor. The need for a mechanism achieving redox poise of type I cyclic electron transport arises primarily only after the advent of molecular oxygen, which competes with $NADP^+$ for electrons from photosystem I [37], and which results in inhibition by over-oxidation if electron input from photosystem II is restricted for any reason.

If both lateral gene transfer and simultaneous type I and type II photochemistry are unlikely, then what is left? There is a third hypothesis, an alternative to coordinated DNA transfer as well as to the proposition that anoxygenic type I and type II centers somehow functioned, and survived, in a shared membrane. This third hypothesis envisages a redox switch to select between *genes* for type I and type II reaction centers. These genes are proposed to have been continually present in a single genome but never expressed at the same time—not, at least, without disastrous consequences. One consequence happened to be photo-oxidation of manganese and then of water, permanently emancipating phototrophy from localized, fleeting, or irregular supplies of H_2S. The reaction product, oxygen, was difficult to live with. In due course, however, oxygen became impossible for many organisms to live without.

14.4 The Redox Switch Hypothesis for the First Cyanobacterium

Bacteria are usually highly versatile in their ability to use different energy sources, coupling them to any of a variety of sources and sinks for carbon, nitrogen, and electrons [38]. Thus the divergence, indicated in Fig. 14.2, of type I and type II reaction centers from a common ancestor need not have depended on loss of the complementary reaction center and its genes. Figure 14.4 describes a sequence of events in which the *capacity* for either type I or type II photosynthesis was retained within a single lineage of cells. Metabolic flexibility in anoxygenic photosynthesis might be particularly advantageous in environments with fluctuating supplies of H_2S, as found today in the vicinity of hydrothermal springs [39]. In the Archaean

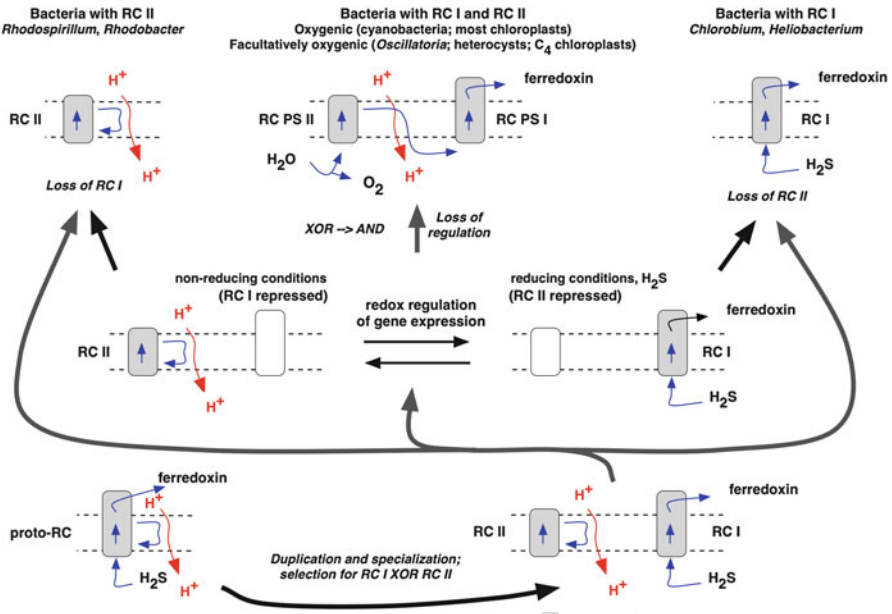

Fig. 14.4 Retention of genes for both type I and type II centers in a single genome, selection between their expression by means of redox regulation, and oxygenic photosynthesis as the accidental consequence of a broken switch. Type I (RC I) and type II (RC II) reaction centers separate, allowing specialization and eventual loss of the redundant reaction center in photoautolithotrophic (type I-containing) lineages (e.g., *Chlorobium*, *Heliobacillus* spp.) and in photoorganotrophic (type II-containing) lineages (e.g., *Rhodobacter*, *Rhodospirillum* spp.). A versatile, facultatively chemoautotrophic photosynthetic bacterium retains genes for both type I and type II reaction centers. In this proposed ancestor of cyanobacteria and chloroplasts, expression of type I center genes in the presence of H_2S is accompanied by repression of type II genes. In the absence of H_2S, type II genes are induced, and type I genes become repressed. Subsequent impairment of regulatory control allows coexistence of type I and type II reaction centers, with complementary functions. In place of H_2S, the type II center, as photosystem II (PS II), oxidizes water, liberating oxygen, and donates electrons to the type I center, as photosystem I (PS I). The proposed loss of the redox regulatory switch replaces the logical (Boolean) relation "Type I ***XOR*** Type II" (each type excluding the other) with "Type I ***OR*** Type II" (either is, and both are, allowed). This in turn leads to "Type I ***AND*** Type II" when interdependency of photosystems I and II is established in the noncyclic electron transport chain of oxygenic photosynthesis. Adapted from [59]

aeon, such environments are likely to have been common, with varying H_2S concentration a normal state of affairs. Before the advent of O_2 as a respiratory electron acceptor, geochemical H_2S will have been widespread, but periodically diluted by rainfall in lakes, rivers, and streams, and additionally by rising tides in littoral rock pools [3, 40].

The scheme in Fig. 14.4 proposes that redox regulation of gene expression, all under strictly anoxic conditions, determines whether type I or type II reaction center genes are expressed in a single anoxygenic bacterial cell whose genome carries them both. Quinone-level redox control provides a suitable mechanism, given the

established redox regulatory control of gene transcription in both phototrophic [21, 41–43] and chemotrophic [44–47] bacteria. An inducible type II reaction center is retained at the core of photosystem II in the cyanobacterium *Oscillatoria limnetica*, which exhibits anaerobic type I photosynthesis in the presence of H_2S, but oxygenic, two-light reaction photosynthesis in its absence [48].

In the absence of H_2S, selection would have favored opportunistic use of weak environmental reductants, including organic substrates, to allow slow, catalytic donation of electrons into a cyclic chain that would otherwise become over-oxidized. It is possible that the inorganic catalyst of photosynthetic water oxidation [49, 50] first served such a poising role for purely anoxygenic, type II photosynthesis, and that this occurred in the inducible type II photosynthesis of the bacterium ~~which~~ also housed temporarily unexpressed genes for a reaction center of type I (Fig. 14.4).

The universal inorganic catalyst of photosynthetic water oxidation is Mn_4CaO_5, a well-defined cluster of five metal and five oxygen atoms [15]. The cluster seems to have no independent existence, and dissociates without its amino-acid side-chain ligands [51]. Its biological assembly suggests that environmental Mn^{2+} itself was an initial substrate and electron donor [20]. Bicarbonate enhances and stabilizes light-induced electron transfer from Mn^{2+} to P^+ in isolated type II reaction centers and may have itself been a precursor secondary electron donor [51]. An additional possibility for the initial advantage of association of a type II center with manganese lies in the latter's UV-absorbing property, providing a screen for ionizing radiation [52]. Mn^{2+} ions will donate electrons readily to a biochemically exposed P^+ in photosystem II (P_{680}) [53] and to an engineered purple bacterial type II reaction center (P_{870}) [54]. Re-reduction of higher oxidation states of manganese by the superoxide anion radical, or by H_2O itself, liberates O_2.

Geochemical data on drill cores from an early Paleoproterozoic succession at 2.415 Gigayears preserved in South Africa indicate substantial enrichment with manganese carbonate [55]. These results are interpreted as evidence that the extensive oxidation of manganese predated the rise of atmospheric oxygen, providing support for the hypothesis that the water-oxidizing complex of photosystem II evolved from a photosystem originally driving oxidation of manganese [55]. The advantage of moving to water as the initial electron donor would have been to free the bacterium from the need for substrate quantities of Mn^{2+}, provided that sufficient manganese could be assimilated to maintain the catalytic complex. Water oxidation might initially have been a minor pathway, since the reaction would have been slow, and the product, molecular oxygen, was toxic. A trickle of oxygen, produced as a by-product of useful regulatory water oxidation, would have been scrubbed from the immediate environment by dilution, or by acting as a respiratory electron sink for neighboring chemoheterotrophs. Subsequent selection, however, can be expected to have increased the redox midpoint potential of the primary chlorophyll electron donor by tuning its protein environment [54, 56, 57] while more effectively coupling the water-oxidation complex to re-reduction of the primary donor, today exemplified by the redox-active tyrosine that links the oxygen-evolving Mn_4CaO_5 cluster with P_{680}^+ [56–58].

Once a mechanism for water oxidation was in place, any mutation producing constitutive expression of both type I and type II genes would provide entirely new functions for each of the two reaction centers, previously forbidden from operating at the same time. Coupling of type II and type I centers simultaneously through a single, shared quinone pool would have allowed the two centers to function in series, and therefore in cooperation—the acceptors for the type II center, oxidizing water, became identical with the donors for the type I center, reducing ferredoxin. This coupling would have provided the first oxygenic bacteria with the advantages of both modes of photosynthesis—ATP synthesis and reduction of soluble, low-potential ferredoxin—while also releasing them from dependency on transient supplies of H_2S for photoautolithotrophic growth. It is proposed (Fig. 14.4) that the origin of the "Z-scheme" of two light reactions, connected in series, occurred by these means [20, 52, 59, 60].

14.5 What Was the Precursor?

The advent of oxygenic photosynthesis had global, irreversible impact, and can be viewed as the most far-reaching event in the history of life, second only to its origin [61]. The redox switch hypothesis for the genesis of the cyanobacteria suggests the persistence today of a two-light-reaction, phototrophic anaerobe retaining genes for both type I and type II reaction centers. This proto-cyanobacterium, a versatile anoxygenic phototroph, should be able to switch between sulfide-oxidizing, photolithotrophic, type I photosynthesis, and sulfide-independent, photoorganotrophic, type II photosynthesis. This organism can be autotrophic, assimilating carbon dioxide, in both modes of photosynthetic metabolism, since the proton-motive cytochrome b-c_1 complex acts not only to provide energy for ATP synthesis. In modern purple non-sulfur anoxygenic photosynthetic bacteria, the same proton-motive force supplies energy for reverse respiratory electron transport from succinate, reducing $NAD(P)^+$ to NAD(P)H for CO_2 and N_2 assimilation.

The green, filamentous, anaerobic phototroph *Chloroflexus aurantiacus* grows in environments with variable sulfide content [39]. *Chloroflexus aurantiacus* has genes only for type II reaction center core proteins (PufLM) and not for type I (PscA) [62] contrary to a previous suggestion [60]. It is uncertain whether this conclusion will hold for all *Chloroflexus* species. In addition, *Chloroflexus* has a major, peripheral, membrane-extrinsic light-harvesting antenna, the chlorosome, originally discovered and characterized in the type I reaction center-containing bacterium *Chlorobium* [38]. *Chloroflexus* may therefore be a close relative both of cyanobacteria and of the anoxygenic phototroph predicted by the hypothesis proposed here and depicted in Fig. 14.4. Figure 14.5 shows a scheme in which the proposed, metabolically versatile proto-cyanobacterium is the last common ancestor of *Chlorobium*, *Chloroflexus*, and cyanobacteria. Facultative type I and type II-plus-type I photosynthesis is seen in the cyanobacterium *Oscillatoria*

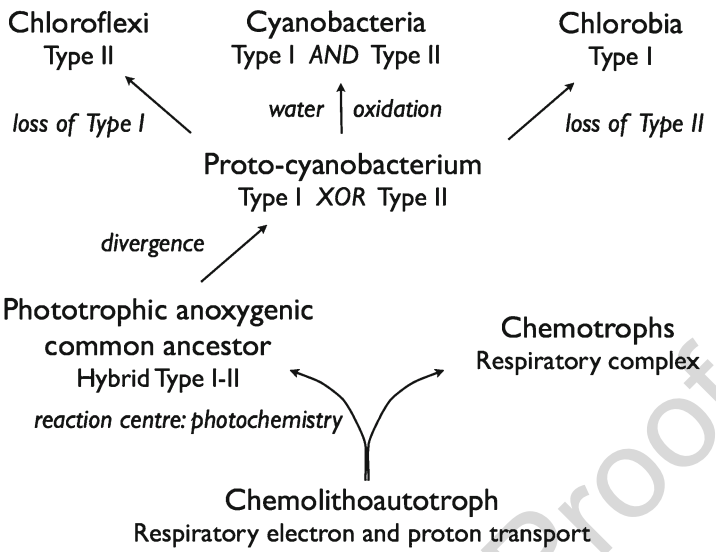

Fig. 14.5 Proposed evolutionary development of photochemical reaction centers from a respiratory chain complex. Vectorial electron transport and proton translocation originate in a respiratory complex. Photochemical charge separation is introduced to form a combined type I-type II reaction center in the common ancestor of all phototrophs, and the prototype reaction center shown in Fig. 14.2. Type I and II reaction centers themselves then diverge, being refined by natural selection operating on products of genes expressed under different growth conditions while retained within single genomes of versatile phototrophic lineages. These lineages give rise to the proposed proto-cyanobacterium, from which loss of type I reaction center genes gave Chloroflexi, loss of type II genes gave Chlorobia, and retention of both type I and type II genes gave cyanobacteria

limnetica, which has inducible photosystem II and reaction center core proteins homologous to PscA and PufLM [63]. 374 375

The redox switch hypothesis (Fig. 14.4) predicts specific, sulfide-responsive redox regulatory control in an anoxygenic, phototrophic bacterium containing genes for both type I and II reaction centers. Such an organism could be expected to share some of the characteristics of *Chloroflexus* and *Oscillatoria*. Suitable habitats still exist. It is therefore to be expected that this bacterium is either an undiscovered or a known species as yet incompletely characterized. Such a bacterium will be a modern example of the species in which photosynthetic oxygen evolution originated, and from which cyanobacteria, and their eventual chloroplast descendants, evolved (Fig. 14.4). It can also be considered whether the redox switch will be found to share components in common with the quinone redox regulatory mechanisms involved in control of respiration and photosynthesis in bacteria, as well as in state transitions and control of photosystem stoichiometry in cyanobacteria [41] and chloroplasts [64]. 376 377 378 379 380 381 382 383 384 385 386 387 388

14.6 Evidence and Evidence Required: An Anoxygenic Phototroph That Switches Between Type I and Type II

Meta-analysis of genome sequences concludes that there is no deep division between type I and type II anoxygenic bacteria with respect to enzymes of chlorophyll biosynthesis [65]. Such a division might be expected if a unique origin of cyanobacteria occurred from within a lineage represented by one or the other group of extant anoxygenic bacteria. Therefore chlorophyll synthesis seems to argue against a fusion of preexisting reaction centers to give photosystems I and II. This conclusion [65] implies that the two original reaction center types were supplied with chlorophyll by a single biosynthetic pathway, as they are today in oxygenic phototrophs. The obvious inference is that a versatile cyanobacterial progenitor retained the capacity to synthesize both type I and type II reaction centers, as depicted in Fig. 14.4.

Comparative genomics points to an origin of cyanobacteria within modern Subsection V, which contains filamentous, N_2-fixing, heterocyst-bearing, freshwater forms [66]. This finding is consistent with the proposed affinity of the proto-cyanobacterium with species of the genera *Chloroflexus* and *Oscillatoria*. This study [66] also lends weight to the assumption that the first oxygenic phototroph lived under conditions of low salinity, where further freshwater periodically diluted the H_2S in its habitat. A further implication of these results [66] is that the advantage of water oxidation might have been that it provided essentially limitless reductant, not for carbon fixation, as often supposed, but for nitrogen fixation, as shown in the inclusive scheme for type I electron transport in Fig. 14.6. The continuation of water-oxidizing diazotrophy from the Archaean into the Proterozoic may also provide a new perspective on the endosymbiosis that gave rise to the chloroplasts of eukaryotic algae and plants.

The redox switch hypothesis (Fig. 14.4) predicts specific, sulfide-responsive redox regulatory control in an anaerobic, phototrophic bacterium retaining genes for both type I and II reaction centers. Anoxic lakes with a varying H_2S influx are known [67–70]. It is therefore to be expected that a recognizable descendant of the proto-cyanobacterium is either as yet undiscovered or else a known species, incompletely characterized. In the early Archaean, the whole biosphere was anoxic (Fig. 14.3), and the proposed precursor of oxygenic cyanobacteria may have been a dominant primary producer, adapted to surface light intensities rather than to low-light environments to which its direct descendants may be confined today. It is likely to have contained chlorophyll rather than bacteriochlorophyll.

It is now a realistic prospect to take samples from anoxic, low-light environments for metagenomic sequencing in order to see if type I and type II genes indeed ever cohabit a single genome. Looking beyond the anticipated success of such a finding, enrichment culture conditions can easily be envisaged. These could begin by setting up a cyclical influx of H_2S at concentrations that vary at a frequency found in the bacterium's natural habitat. A wealth of information and insight would then be forthcoming concerning primary photochemistry, biophysics,

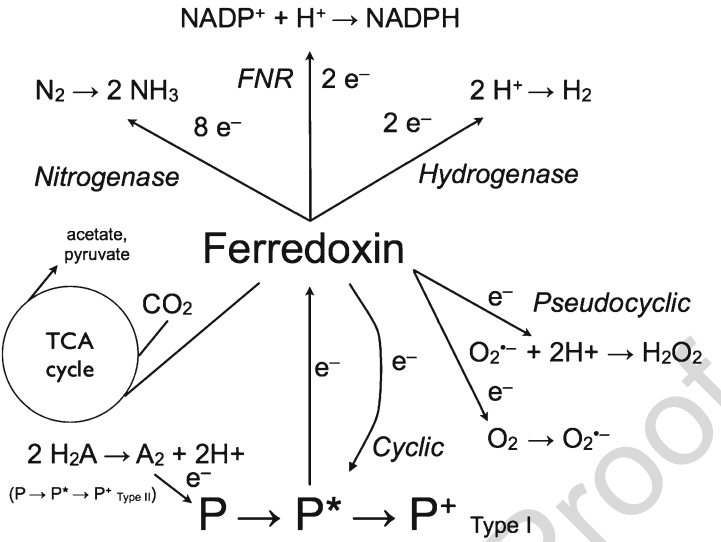

Fig. 14.6 The versatility of type I photosynthetic reaction centers. Pathways of electron transfer from the primary donor, P. Soluble ferredoxin is a major branch point in transferring electrons onwards from membrane-bound Fe-S centers to reduce substrates involved in a range of pathways, notably terminating with assimilation. CO_2 can be fixed since ferredoxin is oxidized directly in the reductive TCA or citric acid (Krebs) cycle or indirectly via NADPH supplied to the reductive pentose phosphate pathway (Benson-Calvin cycle). Reduced ferredoxin can also transfer electrons to molecular nitrogen, hydrogen, and oxygen. Ferredoxin can ~~also~~ pass electrons back to P^+ through a cyclic pathway. The secondary donor to P^+ may be a cytochrome or a plastocyanin, in turn reduced by a donor represented by H_2A. Organic examples of H_2A are succinate, pyruvate, and acetate. Inorganic H_2A may be H_2, Fe^{2+}, Mn^{2+}, H_2S, or H_2O. In the case of H_2O, molecular oxygen is liberated and electrons are supplied through a second reaction center of type II, with its own primary donor, P, as depicted in Fig. 14.1

light-harvesting mechanism(s), biochemistry, and physiology. We might also then help to solve the mystery of a planetary revolution, the single most decisive step in biogeochemical, biological, and evolutionary history [61].

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