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REVIEW

The three-dimensional structures of bacterial reaction centers

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Abstract This review presents a broad overview of the research that enabled the structure determination of the bacterial reaction centers from *Blastochloris viridis* and *Rhodobacter sphaeroides*, with a focus on the contributions from Duysens, Clayton, and Feher. Early experiments performed in the laboratory of Duysens and others demonstrated the utility of spectroscopic techniques and the presence of photosynthetic complexes in both oxygenic and anoxygenic photosynthesis. The laboratories of Clayton and Feher led efforts to isolate and characterize the bacterial reaction centers. The availability of well-characterized preparations of pure and stable reaction centers allowed the crystallization and subsequent determination of the structures using X-ray diffraction. The three-dimensional structures of reaction centers revealed an overall arrangement of two symmetrical branches of cofactors surrounded by transmembrane helices from the L and M subunits, which also are related by the same twofold symmetry axis. The structure has served as a framework to address several issues concerning bacterial photosynthesis, including the directionality of electron transfer, the properties of the reaction center-cytochrome c_2 complex, and the coupling of proton and electron transfer. Together, these research efforts laid the foundation for ongoing efforts to address an outstanding question in oxygenic photosynthesis, namely the molecular mechanism of water oxidation.

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Abbreviation

P865	Bacteriochlorophyll dimer in reaction centers from *R. sphaeroides*
BChl	Bacteriochlorophyll
BPhe	Bacteriopheophytin
P680	Primary electron donor in photosystem II
Q_B	Secondary quinone
EPR	Electron paramagnetic resonance
ENDOR	Electron nuclear double resonance

Introduction

Photosynthesis is a biological process that converts sunlight energy into chemical energy and drives biochemical reactions in plants, algae, cyanobacteria, purple bacteria, and green bacteria. The initial discoveries concerning photosynthesis were in the 1600s and 1700s as van Helmont measured the mass of trees as they grew and Priestly discovered the production of oxygen by plants. The term "photosynthesis" was originally called "photosyntax" by (Barnes 1893) and later changed to "photosynthesis" by MacMillan (Gest 2006). Since these initial studies, the pathway of understanding photosynthesis has involved an enormous number of research groups who have unraveled the chemical nature of the reactions as well as the structures of photosynthetic pigment-protein complexes (Blankenship 2002; Govindjee et al. 2006). Photosynthesis can be divided into two general categories, oxygenic as performed by algae, cyanobacteria, and plants, and anoxygenic as performed by

bacteria. In this paper, we focus on anoxygenic photosynthesis, in particular on the cyclic pathway carried out in purple bacteria (Blankenship et al. 1995; Hunter et al. 2009). In purple bacteria such as *Rhodobacter sphaeroides* (formerly *Rhodopseudomonas spheroides* and *Rhodopseudomonas sphaeroides*), light initially excites bacteriochlorophylls (BChls) in the antenna complexes. The light energy is transferred to the site of the primary photochemistry, the reaction center, leading to the transfer of an electron from the primary electron donor, the bacteriochlorophyll dimer P865, through intermediates to the secondary quinone, Q_B. After the oxidized bacteriochlorophyll dimer, $P865^{\bullet+}$, is reduced by cytochrome c_2, it can be excited again by light resulting in the transfer of a second electron to Q_B, which is coupled with proton transfer. The resulting quinol leaves the reaction center and binds to the cytochrome bc_1 complex, leading to oxidation of the quinol and electron transfer to the hemes and iron-sulfur centers of the cytochrome bc_1 complex. This electron transfer in the cytochrome bc_1 complex results in proton transfer across the cell membrane, which drives the formation of energy-rich compounds such as ATP and NADPH. The cyclic electron transfer pathway is completed by the transfer of electrons from the cytochrome bc_1 complex back to the reaction center through cytochrome c_2.

In this article, we first review some of the historical developments that enabled the determination of the structure, namely the identification of antenna complexes and photosystems as well as the isolation and characterization of the bacterial reaction center. Then, we discuss how biochemical and spectroscopic results facilitated the determination and interpretation of the three-dimensional structures of the reaction center from *Blastochloris viridis* (formerly *Rhodopseudomonas viridis*) and *R. sphaeroides*. Finally, we discuss how the molecular mechanism of reaction centers has been elucidated by a combination of structural, mutagenesis, and spectroscopic experiments with the specific examples of proton transfer to the secondary quinone, electron transfer from the exogeneous cytochrome c_2 to the primary electron donor, and the use of reaction centers as a framework to understand photosystem II.

Identification of pigment-protein complexes in photosynthetic organisms

The development of an instrument that could illuminate samples using flashes of light by Emerson and Arnold (Emerson and Arnold 1932) provided the opportunity to precisely measure oxygen release from photosynthetic organisms due to illumination. One outcome was the postulation of a minimal structural unit capable of performing light-induced electron transfer. Another was the realization that the ratio of oxygen production per chlorophyll molecule was very low, which today is recognized as resulting from the large number of chlorophylls that serve as antenna molecules. These observations led to questions concerning the efficiency of light conversion in photosynthesis. To address those questions, Emerson and coworkers measured the dependence of the efficiency on the excitation wavelength and showed that the photosynthetic rate dramatically dropped with the use of red light, with this effect becoming known as the red drop. Unexpectedly, a dramatic enhancement of the rate was observed when two beams of red light with different wavelengths were used simultaneously (Emerson et al. 1957). The increase in the rate of oxygen production was much higher than the sum of the rates based upon the individual wavelengths and was termed the enhancement effect.

An outcome of these and other experiments was the conceptual formulation that oxygenic photosynthesis uses two sequential photosystems, with one photosystem providing the molecules that serve as substrates for the other (Hill and Bendall 1960). This concept received a key boost by an experimental observation by Duysens and coworkers (Duysens et al. 1961). His laboratory had built sensitive optical spectrometers and demonstrated that the oxidation state of cytochrome *f* could be measured optically. Cytochrome *f* was reduced under dark conditions but oxidized under illumination as measured using difference absorption spectra, namely light-minus-dark spectra (Duysens 1954, 1989). The use of different wavelengths for excitation showed a pattern of oxidation of the cytochrome with infrared light but a reduction of the cytochrome with green light (Fig. 1). These opposite outcomes from the different excitation wavelengths, termed the antagonistic effects, were dramatic and provided clear support for the concept of two independent photosystems. The infrared light is largely absorbed by photosystem I resulting in oxidation of cytochrome *f* with reduction of $NADP^+$. In the red alga *Porphyridium cruentum*, the antenna complexes preferentially absorb the green light and direct that light energy to photosystem II resulting in oxidization of water with reduction of cytochrome *f*. The interpretation of these experiments has now been solidified as the Z scheme that links together the involvement of the different pigment-protein complexes in the overall electron transfer pathway.

Over the same period of time, the photosynthetic process in anoxygenic prokaryotes was being investigated. In addition to his work on oxygenic photosynthesis, Duysens characterized purple bacteria using the optical spectroscopy techniques (Duysens 1951, 1952). This research provided direct evidence of light-induced changes in the optical spectrum that later were identified as arising from the reaction center. Through these studies, it was found that the efficiency of energy transfer could be estimated by

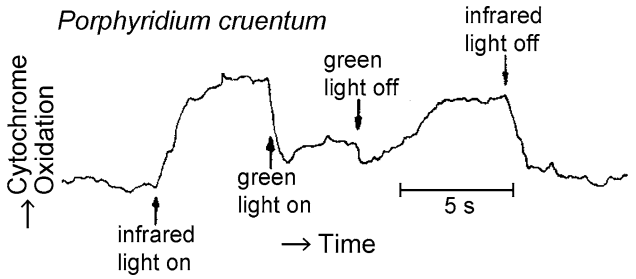

Fig. 1 Cytochrome *f* oxidation/reduction changes due to light excitation with green light and infrared light. The cytochrome oxidation state was measured by monitoring absorption changes at 420 nm from samples of the red alga *Porphyridium cruentum*. Excitation with near infrared light results in cytochrome oxidation while reduction is observed for excitation using green light. These antagonistic effects arise from the different roles of cytochrome *f* in the Z scheme. Excitation using green light (centered at 562 nm) results in the preferential excitation of photosystem II, which reduces cytochrome *f*, while the use of near infrared light (>680 nm) predominately excites photosystem I, which results in oxidation of cytochrome *f*. Figure modified from (Duysens et al. 1961)

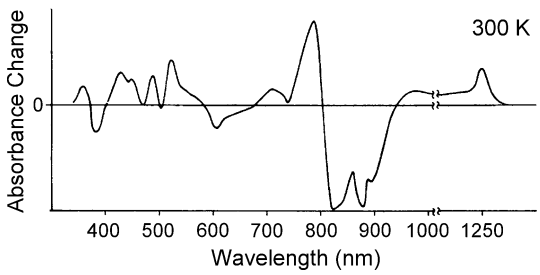

Fig. 2 Light-minus-dark difference optical spectrum of chromatophores of *R. sphaeroides* measured at 300 K. The spectrum shows the optical regions of the spectrum that are altered because of illumination, including loss of absorption at 865 nm due to oxidation of the primary electron donor P865. Modified from Arnold and Clayton (1960)

comparing the amplitudes of the fluorescence spectrum to the absorption spectrum. By measuring the fluorescence as a function of temperature in *R. sphaeroides*, the efficiency of energy transfer from antenna pigments to the reaction centers was determined to be nearly 100 % (van Grondelle et al. 1978). The antenna pigments were later found to be BChl cofactors of the light-harvesting I and II complexes (Cogdell et al. 1999).

Isolation and characterization of bacterial reaction centers

Clayton began an investigation of the light-induced optical changes of *R. sphaeroides* around 1960 (Clayton 1988, 2002). Chance and Nishimura had shown that the light-dependent oxidation of cytochrome in *Chromatium vinosum* still occurred at liquid nitrogen temperatures (Chance and Nishimura 1960). For these early studies, experiments were performed on membrane fragments termed chromatophores, which are small vesicles formed after the cells are broken by a French press or other device. To examine the effect of low temperatures, chromatophores from different purple bacteria were measured and light-induced absorption changes were observed even at liquid helium temperatures (Arnold and Clayton 1960). The absorption spectrum of chromatophores from *R. sphaeroides* showed a large number of light-induced spectral changes in the light-minus-dark difference spectrum, notably including an absorption decrease at 865 nm (Fig. 2). These spectral differences were assigned as arising from oxidation of BChl and cytochrome and reduction of quinone. These findings were in agreement with earlier observations of samples from *R. rubrum* that showed light-induced absorption changes at ~870 nm (Duysens 1952). The studies by Clayton were boosted by the development of a carotenoid-less mutant, termed R-26, which has a simpler optical spectrum than wild type due to the lack of one of the light-harvesting complexes (Clayton and Smith 1960) and was found to be easier to work with biochemically. The name reaction center was proposed by Clayton (Clayton 1963) as the complex that catalyzed the light-driven photosynthetic electron transfer.

Beginning in 1968, Feher started research on bacterial photosynthesis after a career in solid state physics that included experiments using electron paramagnetic resonance (EPR) spectroscopy and the development of electron-nuclear double resonance (ENDOR) spectroscopy (Feher 1998). These techniques proved to be very suitable for examining photosynthetic processes as they are sensitive probes of cofactors, such as the primary electron donor and electron acceptors, when they are oxidized or reduced. His laboratory obtained cultures of *R. sphaeroides* R-26 and characterized the reaction centers using EPR spectroscopy (McElroy et al. 1969). The major light-induced changes in the EPR spectrum and the absorption at 865 nm in the optical spectrum were assigned to the primary electron donor, P865. Similar conclusions were also reached by Loach and Sekura (1967), Parson (1968), and Clayton and coworkers (Bolton et al. 1969).

The early experiments were limited by the use of chromatophores, which have reaction centers as well as other complexes such as the light-harvesting antenna and cytochrome bc_1 complex. The many spectroscopic signatures provided the opportunity to determine the conditions needed to isolate pure and active reaction centers. By treating chromatophores of *R. sphaeroides* with Triton X-100, Clayton and Reed successfully isolated the first batch of crude reaction centers (Reed and Clayton 1968). Feher replaced Triton X-100 with another detergent,

lauryldimethylamine oxide, which resulted in a significantly improved preparation of the reaction centers (Feher 1971). Modifications of these initial preparations led to the ability to obtain large quantities of very pure protein (Clayton and Wang 1971; Feher and Okamura 1978). The availability of pure preparations of reaction centers from purple bacteria led to these reaction centers being intensively studied and consequently serving as a model system for photosystems I and II, which have many more cofactors and protein subunits.

The availability of purified reaction centers enabled one of the notable discoveries using EPR, namely the identification of the primary electron donor as a BChl dimer. Due to the unpaired electron, the oxidized primary electron donor has an EPR signal (Fig. 3). In 1971, Norris measured the EPR signals from chloroplasts and chromatophores from different organisms and noted that the narrower linewidths observed for oxidized BChls and Chls in photosynthetic systems compared to isolated $BChl^+$ or Chl^+ in solution could be explained as arising from the unpaired electron being shared over two tetrapyrroles rather than residing on only one tetrapyrrole (Norris et al. 1971). This postulate was confirmed with ENDOR experiments showing a reduction of the hyperfine couplings that was consistent with the distribution of electrons over two BChls (Feher et al. 1975; Norris et al. 1975). Thus, the combination of EPR and ENDOR revealed P865 to be a BChl dimer.

The three-dimensional structures of bacterial reaction centers

The primary structure of the reaction center was obtained by sequencing the genes encoding the protein subunits. Through a collaboration between Feher and Steiner, the amino-terminal regions of the three subunits of reaction centers from *R. sphaeroides* were determined, allowing the construction of oligonucleotide probes that were used to identify the genes (Sutton et al. 1982; Williams et al. 1983, 1984, 1986). At the same time, the sequences of the genes encoding the reaction centers from *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) were determined by Youvan and coworkers (Youvan et al. 1984). For reaction centers from both organisms, the sequences of the L and M subunits each showed the presence of very hydrophobic regions of 20–25 amino acid residues consistent with the presence of five transmembrane helices. The H subunit showed a much different sequence, with only one long hydrophobic stretch of amino acid residues followed by a water-soluble domain. Also revealed was a sequence homology of the L and M subunits of reaction centers with the D1 and D2 subunits of photosystem II, demonstrating an evolutionary relationship between

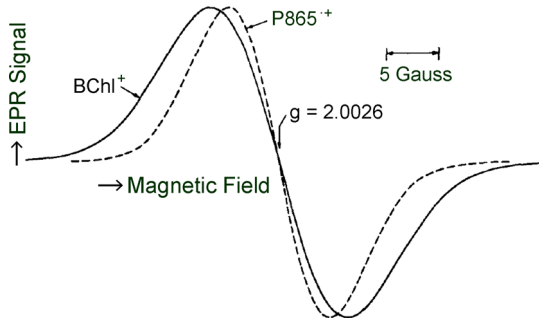

Fig. 3 EPR signals from the oxidized primary electron donor from reaction centers (*dashed line*) and isolated oxidized BChl (*solid line*) measured at a temperature of 77 K. Upon illumination, P865 is oxidized resulting in an EPR signal with a derivative shape centered at a *g*-value of 2.0026. Oxidation of BChl in solution also generates an EPR signal at this *g*-value but with a much larger linewidth. The difference in linewidth arises due to the distribution of electrons over two BChls in P865 compared to one BChl for the isolated molecule. The reduced quinone also has an EPR signal that is evident only when measurements are performed at liquid helium temperatures. Modified from (Feher et al. 1975)

anoxygenic and oxygenic photosynthesis (Williams et al. 1983; Youvan et al. 1984).

While the sequences delineated the general organization of the protein in the cell membrane, determination of the three-dimensional organization of the protein and cofactors required the use of X-ray diffraction. At that time, these experiments were considered to be not feasible as they make use of crystals and despite success with hundreds of water-soluble proteins, no membrane protein had been successfully crystallized. A critical reason for the lack of crystals of membrane proteins was the difficulty in obtaining the abundant amounts of pure protein needed for the trial-and-error approach of crystallization experiments. The availability of pure preparations of bacterial reaction centers that could be isolated in large quantities from bacterial cultures provided the opportunity to test numerous combinations of conditions allowing the crystallization of bacterial reaction centers to be pursued in several laboratories. In 1982, Michel reported the crystallization of reaction centers from *B. viridis* (Michel 1982) that was soon followed by the crystallization of reaction centers from *R. sphaeroides* (Allen and Feher 1984). These efforts demonstrated the feasibility of crystallizing membrane proteins using combinations of detergents to solubilize membrane proteins and small amphiphiles to supplement the detergents (Michel 1983).

With the availability of the crystals, the determination of the three-dimensional structures of the reaction centers from *B. viridis* and *R. sphaeroides* fell into place (Fig. 4) (Deisenhofer et al. 1985, 1995; Allen et al. 1987a, 1987b; Chang et al. 1991). The three-dimensional structure revealed the presence of five long helices, in each of the L and M subunits, that correspond to the long regions of

hydrophobic residues, thus the identification of the helices as being transmembrane was straightforward (Yeates et al. 1987). The H subunit has a large hydrophilic domain on the cytoplasmic side of the membrane while the reaction centers from *B. viridis* also have a large tetraheme subunit on the periplasmic side of the membrane. One of the striking features of the structures is the approximate two-fold symmetry axis of the reaction center. The cofactors are arranged into two branches with the symmetry axis passing from the primary electron donor to the non-heme iron. The symmetry axis also relates the long transmembrane helices of the L and M subunits. For both reaction centers, the cofactors are buried in the L and M subunits that form the core of the protein. For their efforts in the elucidation of the structure of the reaction center from *B. viridis*, Deisenhofer, Huber, and Michel received the Nobel Prize in Chemistry in 1985.

A number of predictions concerning the reaction center were directly confirmed by the structures (Feher et al. 1989). For example, an early outcome from EPR and ENDOR experiments was that the primary donor is a BChl dimer. The structure clearly shows the presence of two BChls that closely overlap at the ring A position and are separated by only ~ 3 Å (Fig. 5). The structural arrangement of P865 next to a BPhe, followed by the primary quinone and Q_B, was consistent with the electron transfer pathway predicted from spectroscopic measurements. These structures also confirmed the predictions of long transmembrane helices and that the L and M subunits form the core of the protein (Williams et al. 1983, 1984, 1986; Youvan et al. 1984; Debus et al. 1985).

The strong structural homology between the reaction centers from *B. viridis* and *R. sphaeroides* (Fig. 4) suggested that the core of the L subunit, M subunit, and cofactors was structurally conserved in reaction centers from other bacteria. The combination of the structural studies with comparisons of the sequences of the reaction center and photosystem II led to the postulate that the D1 and D2 subunits constitute the core of photosystem II with a similar structural arrangement as found for the reaction center (Trebst 1987; Michel and Deisenhofer 1988). This idea was soon verified by several experiments, including the isolation of a core complex of photosystem II consisting of the D1, D2, and cytochrome *b559* subunits (Nanba and Satoh 1987).

The presence of two symmetry-related branches was an unexpected outcome and raised questions about the directionality of electron transfer along the two branches. The structures also provided the framework for addressing outstanding questions concerning the function of the reaction center, including the coupling of proton transfer with electron transfer and the molecular nature of the reaction center-cytochrome c_2 complex. Investigations into these topics were facilitated by use of site-directed mutagenesis in which amino acid residues near the cofactors were altered. Since these types of mutations often result in decreased electron transfer rates and yields, mutants are grown under non-photosynthetic conditions to prevent selection for other more efficient reaction centers. The organisms *R. capsulatus* and *R. sphaeroides* are able to grow in the dark by respiration but suitable conditions for

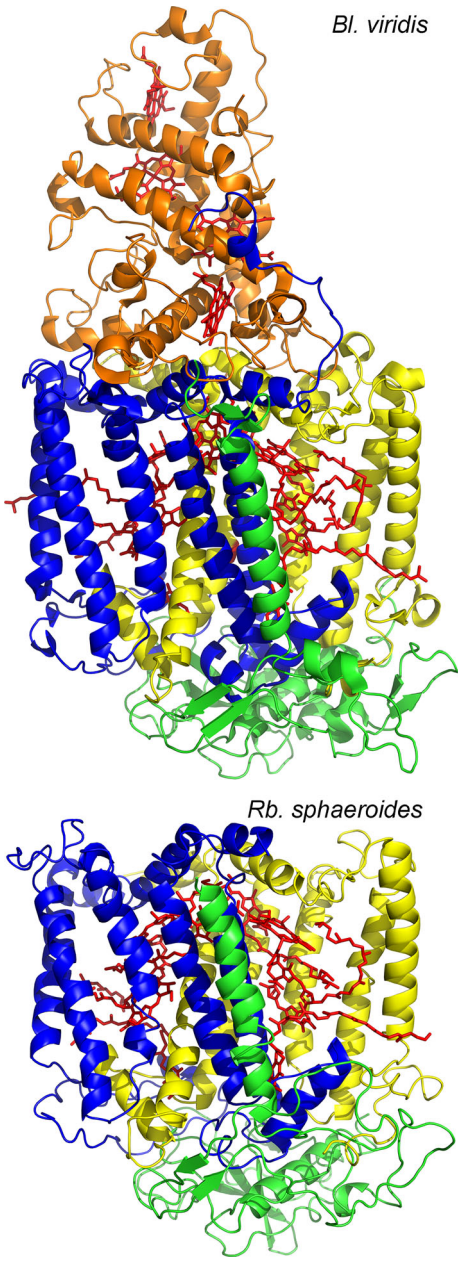

Fig. 4 Three-dimensional structures of the reaction centers from *B. viridis* and *R. sphaeroides*. Shown are the cofactors (*red*) and protein subunits (L *yellow*, M *blue*, H *green*), including the tetraheme cytochrome in *B. viridis* (*orange*). The twofold symmetry axis runs vertically in the plane of the paper (Deisenhofer et al. 1985, 1995; Allen et al. 1987a, 1987b). (PDB codes 1PRC and 4RCR)

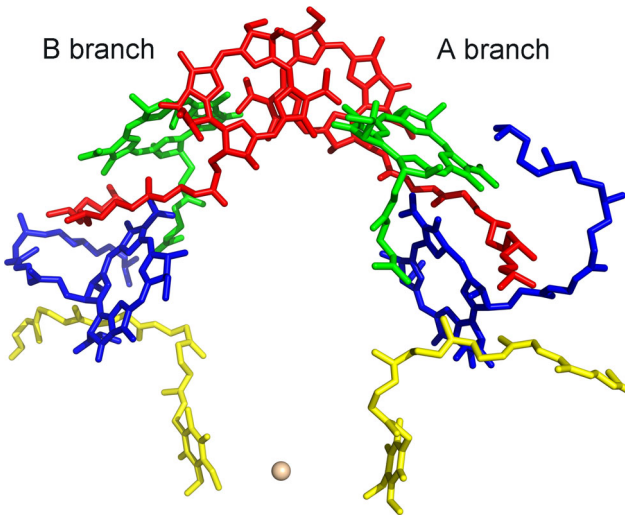

Fig. 5 Structural arrangement of the cofactors of the reaction center from *R. sphaeroides*. The cofactors are arranged in two branches, identified as the A and B branches. Each branch contains one of the BChls of P865 (*red*), followed by a BChl monomer (*green*), a Bphe (*blue*), and a quinone (*yellow*). The cofactor P865 is seen to be formed by two BChls that overlap at the ring A position with a separation of approximately 3 Å. The branches are related by an approximate twofold symmetry axis that runs from P865 through the non-heme iron (wheat) in the plane of the paper (Allen et al. 1987a) (PDB code 4RCR)

B. viridis were not established (Williams and Taguchi 1995). Consequently, mutagenesis studies on reaction centers from *B. viridis* were minimal despite its prominence in the earlier structural work. The utilization of structures and mutants with spectroscopic measurements to address these questions is presented below, followed by a discussion of current research into how these insights into the structure and function of the reaction center are being used to probe the mechanism of water oxidation by photosystem II.

Directionality of electron transfer

The structural arrangement of the cofactors raised the question of whether electron transfer proceeds along one or two of the symmetrically arranged branches of cofactors (Woodbury and Allen 1995). The primary evidence is provided by the optical monitoring of the two BPhe cofactors that have distinct optical absorption bands (Kellogg et al. 1984; Breton et al. 1986; Kirmaier and Holten 1987; Lockhart et al. 1990). These measurements showed that the absorption of light energy from the surrounding antenna complexes results in the excitation of P865. Within a few picoseconds, an electron is transferred from the excited state of P865, P865*, through the BChl monomer on the A branch to the BPhe on the A branch, and then to the primary quinone and finally to Q_B with a quantum yield of nearly 100 %. Despite the near symmetry of the two branches of cofactors, these optical measurements demonstrated that electron transfer in wild-type reaction centers proceeds along only the A branch cofactors with no measureable electron transfer along the B branch.

To address the question of why electron transfer occurs only along one of the two branches, the amino acid residues surrounding P865, the BChl monomers, and the BPhe monomers were altered using site-directed mutagenesis, and the electron transfer properties of the mutants were characterized, primarily using transient optical spectroscopy. For example, the removal of one of the histidines coordinating P865, either His L173 or His M202, results in formation of a BChl–Bphe heterodimer (Bylina and Youvan 1988; Allen et al. 1996). Despite large changes in the distribution of electrons over the BChl–BPhe heterodimer compared to the BChl homodimer found in wild type and the corresponding alterations of energetics, the directionality of electron transfer remained largely unchanged compared to wild type (Kirmaier et al. 1988; McDowell et al. 1991). Nor was there any significant directionality change observed when the introduction of a His near the active BPhe resulted in the incorporation of a BChl in this position (Kirmaier et al. 1991). However, a combination of such mutations with the placement of several charged residues along the A branch resulted in measurable electron transfer along the B branch (Heller et al. 1995). Together, these experiments revealed that the asymmetry of electron transfer is primarily due to differences in the energetics of the different BChl cofactors as well as the protein dynamics that can limit the electron transfer process (Wang et al. 2007).

In the three-dimensional structures of the reaction center, a BChl monomer is located in a position where it can bridge electron transfer from P865* to the BPhe along the A branch (Fig. 5). After the determination of the structures, two possible mechanisms for the role of the BChl monomer were extensively discussed. In one mechanism, the BChl monomer served as a true intermediate electron acceptor, and in the second the BChl monomer primarily facilitated the coupling between P865* and BPhe. Resolving this question was made difficult by the complexity of the changes in the optical spectra immediately after excitation, with no significant optical signal associated with a reduced BChl during the initial electron transfer (Kirmaier and Holten 1987; Woodbury and Allen 1995). Careful analysis of spectra obtained using improved spectrometers yielded the conclusion that the formation of the intermediate $P865^{+}BChl^{-}$ state was occurring (Zinth and Wachtveitl 2005). The electron transfer rate from $BChl^{-}$ to BPhe can be construed as being much faster than the rate from P865* to BChl, resulting in the loss of the $P865^{+}BChl^{-}$ state

before it can accumulate. Thus, the amount of this state during electron transfer is always small. Subsequently, alteration of the binding site of the active BChl monomer by mutagenesis resulted in loss of this cofactor and electron transfer proceeded through the normally inactive BChl monomer to the BPhe on the B branch (Carter et al. 2012). The initial electron transfer process continues to be probed through the combination of mutagenesis studies and transient optical spectrometers with improved sensitivities.

Proton-coupled electron transfer

Another outstanding question was how electron transfer was coupled to proton transfer in reaction centers (Okamura and Feher 1995). The energy from light is converted into proton transport across the cell membrane due to proton-coupled electron transfer involving the reaction center. Light absorbed by the reaction center results in the transfer of an electron from P865 to Q_B, resulting in a charge separation across a distance of approximately 22 Å (Fig. 5). The characteristics of Q_B have been examined in detail using optical spectroscopy by the laboratories of Feher/Okamura and Wraight (Okamura et al. 2000; Wraight and Gunner, 2009). These studies established that the primary quinone served as a shuttle between the BPhe monomer and Q_B while Q_B served as a two-electron gate, with the second electron transfer being coupled with proton transfer. However, the molecular mechanism of coupling electron and proton transfer remained elusive until the determination of the structure that revealed the interactions between Q_B and the surrounding protein environment. The keto groups of the quinone form hydrogen bonds with the side chains of Ser L223 and His L190, which also serves as a ligand for the non-heme iron (Fig. 6). The binding site for Q_B is hydrophilic, with several protonatable amino acid residues, namely His L190, Glu L212, Asp L213, and Ser L223, that each could serve as proton donor groups.

To understand the role of the protonatable residues in electron transfer, the amino acid residues found in the binding site of Q_B were systematically altered using site-directed mutagenesis, and the mutants were characterized using optical and EPR spectroscopy. A reduction in the electron transfer rate from the primary quinone to $Q_B^{\bullet-}$ was observed when either Glu L212 or Asp L213 was altered showing that these residues play critical roles in the protonation of Q_B during electron transfer (Paddock et al. 1989, 1994; Takahashi and Wraight 1990, 1991). Many of the amino acid residues further away from Q_B were then mutated and in a number of cases the mutants showed pronounced decreases in the rates, consistent with loss of the proton pathway from the cytoplasmic environment to Q_B. The presence of well-defined proton pathways was

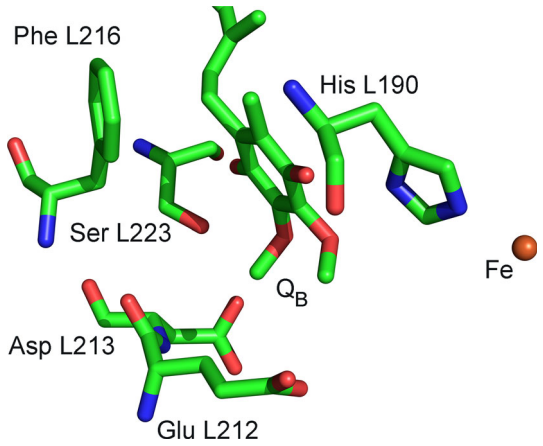

Fig. 6 Structure of the Q_B site of the reaction center from *R. sphaeroides*. Shown are nearby amino acid residues His L190, Glu L212, Asp L213, Phe L216, and Ser L223 (*colored* by atom type) and the non-heme iron (*orange colored sphere*). The quinone is positioned by hydrogen bonds to the side chains of His L190 and Ser L223 with Glu L212, Asp L213, and Ser L223 participating in proton transfer. (Stowell et al. 1997) (PDB code 1AIG)

confirmed by the observation of a Zn^{2+} binding site on the surface of the protein, which when occupied blocked proton transfer (Utschig et al. 1998; Axelrod et al. 2000). All of these studies present a picture of a complex mechanism of electron transfer involving not only the properties of the electron donor and acceptor but also interactions with the amino acid residues forming the cofactor binding sites.

Electron transfer from cytochrome c_2

Although the structures of the reaction centers helped in the unraveling of electron and proton transfer within the reaction center, how electrons were transferred from cytochrome c_2 to the reaction center remained an unsolved question at a molecular level. In *R. sphaeroides*, cytochrome c_2 serves as the secondary electron donor to $P865^{\bullet+}$ while in other purple bacteria, such as *B. viridis*, a bound tetraheme cytochrome serves as the secondary donor. The initial observations of cytochrome oxidation upon illumination were in the 1950s (Vernon and Kamen 1953; Duysens 1954; Chance and Smith 1955) but the determination that cytochrome c_2 serves as the secondary electron donor was not achieved until time-resolved optical spectroscopic experiments by Parson demonstrated that the fast oxidation of BChl was followed by its reduction in 2 μs by cytochrome (Parson 1968). Since cytochrome c_2 is water soluble and not bound to the reaction center, the rate of electron transfer is limited by the binding of the cytochrome to the reaction center, which is embedded in the cell membrane (Crofts and Wraight 1983).

To elucidate the nature of the reaction center-cytochrome complex, models were proposed based upon the individual structures of the reaction center and cytochrome c_2 (Salemme et al. 1973; Axelrod et al. 1994). The periplasmic surface of the reaction center contains many negatively-charged residues that can serve as the binding interface for the positively-charged residues found on the heme edge of cytochrome c_2. The models placed the cytochrome c_2 onto this binding interface by adjusting the orientations to maximize the interactions between the positively- and negatively-charged residues (Allen et al. 1987b; Tiede 1987; Adir et al. 1996). These models had different positions and orientations of the cytochrome c_2 as each model also incorporated some additional information, for example the model by Tiede made use of the relative orientations of the heme compared to P865 based upon optical measurements (Tiede 1987). The precise arrangement of the two proteins was established with the determination of the three-dimensional structure of the reaction center-cytochrome complex using protein crystallography (Axelrod et al. 2002). The cytochrome is positioned in the center of the periplasmic surface of the reaction center with the solvent-exposed heme positioned directly over P865 (Fig. 7). This heme position is similar to the location of the heme closest to the BChl dimer in *B. viridis* (Fig. 4).

With the structure of the complex determined, the role of different interactions in binding the cytochrome was investigated by site-directed mutagenesis of the amino acid residues at the binding interface (Axelrod and Okamura 2005). These studies were performed using cytochrome c_2 and reaction centers from *R. sphaeroides* as the three-dimensional structure of the complex provided a platform for using mutagenesis to investigate a range of interactions at the binding interface, including charge–charge interactions, hydrogen bonding, and hydrophobic interactions. Electrostatic interactions were shown to play a key role as changes of charged residues at the binding site resulted in large decreases in the binding affinity of the cytochrome to the reaction center. Computational simulations of the association process identified transition states with cytochrome orientations that differ compared to the final active state of the complex and established the involvement of water molecules (Autenrieth et al. 2004; Miyashita et al. 2004, 2005). These studies provide a general picture of how water-soluble proteins interact with membrane-bound proteins in photosynthetic as well as other organisms.

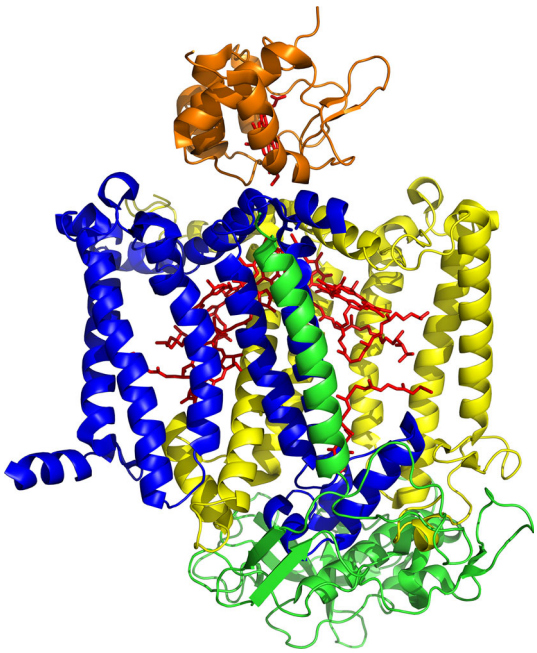

Fig. 7 Structure of the reaction center from *R. sphaeroides* with a bound cytochrome c_2. Shown are the protein subunits of the reaction center, L (*yellow*), M (*blue*), and H (*green*), the subunit of the cytochrome (*orange*), and cofactors (*red*). The cytochrome c_2 binds directly above P865 with heme of the cytochrome being located approximately on the twofold symmetry axis of the reaction center, approximately at the same location as the heme closest to the BChl dimer in reaction centers from *B. viridis* (Fig. 4). The view is approximately the same as Fig. 4. (Axelrod et al. 2002) (PDB code 1L9 J)

Implications for water oxidation

While many questions concerning the bacterial reaction center have now been answered, the mechanism of water oxidation remains one of the major unsolved questions in photosynthesis (Wydrzynski and Satoh 2005). As was true for bacterial photosynthesis, the development of optical spectrometers and transient flash light sources provided the experimental means to investigate oxygenic photosynthesis. For example, experiments in the laboratories of Joliot and Kok led to an understanding that water oxidation proceeds by the accumulation of four-oxidizing equivalents that are driven by a series of four excitations of the primary electron donor, P680, in what is termed the S cycle (Joliot et al. 1969; Kok et al. 1970). Investigations into the mechanism of water oxidation are now reaping the benefits of the determination of three-dimensional structures for photosystem II from the cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* (Zouni et al. 2001; Ferreira et al. 2004; Loll et al. 2005; Yano et al. 2006; Umena et al. 2011). These structures show that the complex can be thought to have an outer domain that harvests light and surrounds a core domain that performs the primary electron transfer reactions and contains the cofactors involved in the primary photochemistry. The core domain is formed by two protein subunits, D1 and D2, that are evolutionarily related to the L and M subunits of the reaction center and exhibit the same pattern of five

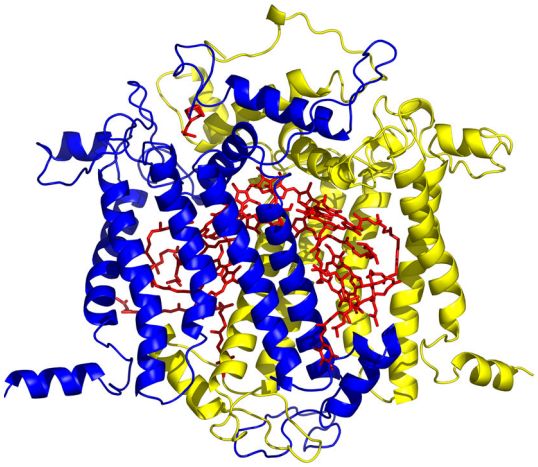

Fig. 8 Three-dimensional structure of the core domain of photosystem II from *T. elongatus*. The twofold symmetry axis that relates the two branches of cofactors (*red*) and transmembrane helices of the D1 (*blue*) and D2 (*yellow*) subunits is in the plane of the paper. The structure shows a notable structural homology to the core subunits of reaction centers (Fig. 3). The site of water oxidation, the Mn_4Ca cluster, is located approximately 10 Å from the primary donor P680. (Ferreira et al. 2004) (PDB file 1S5L)

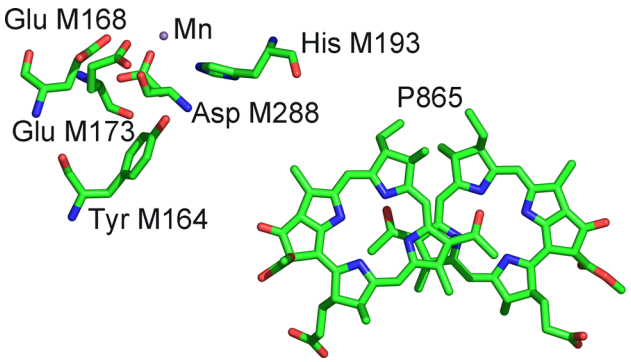

Fig. 9 Structure of modified reaction centers from *R. sphaeroides* with a bound Mn-cofactor. Shown are the Mn-cofactor (purple sphere), the BChl dimer P865, and nearby amino acid residues Tyr M164, Glu M168, Glu M173, His M193, and Asp M288. Some of the amino acid residues binding the mononuclear Mn are not present in wild type but were introduced by mutagenesis in a highly oxidizing mutant. The Mn-cofactor serves as an efficient secondary electron donor to $P865^{\bullet+}$. The view is approximately the same as Fig. 4. (Thielges et al. 2005) (PDB code 1Z9 J)

transmembrane helices related by a twofold symmetry axis (Fig. 8). One of the remarkable aspects is the strong structural conservation of the arrangement of the D1 and D2 subunits of photosystem II compared to the L and M subunits as well as the organization of the cofactors into two branches.

Deciphering the mechanism of water oxidation involves spectroscopic investigations while using the three-dimensional structure as the foundation to investigate the impact of altering amino acid residues that interact with the cofactors. The key cofactor is the Mn_4Ca cluster that is located approximately 10 Å from P680, with a redox active tyrosine, termed Y_Z, serving as a bridging cofactor (Tommos and Babcock 2000; Rappaport and Diner 2008). The Mn_4Ca cluster is the site of water oxidation and collects the four electron equivalents during the S cycle. The Mn_4Ca cluster has a distorted cubane configuration and is coordinated by several carboxylates, histidines, and four bound water molecules. These amino acid residues have been altered and their roles in electron transfer are being investigated (Debus 2001).

The close structural similarity of the core complexes of reaction centers and photosystem II provides the opportunity to modify the reaction center such that it gains the functional properties of photosystem II. The reaction center has been altered to be highly oxidizing with a mononuclear manganese cofactor located at the position analogous to that of the Mn_4Ca cofactor in photosystem II (Thielges et al. 2005; Allen et al. 2012). Upon illumination, the Mn-cofactor serves as a rapid secondary electron donor to $P865^{\bullet+}$. Using X-ray diffraction, the structure of the reaction center with the bound manganese cofactor has been determined and found to bind the manganese at the designed site with carboxylate and histidine ligands (Fig. 9). Research is underway to develop reaction centers with more complex manganese cofactors to provide an alternative means to investigate how such cofactors perform multielectron transfer that is coupled with proton transfer.

Using the spectroscopic and biochemical techniques pioneered by Duysens, Clayton, and Feher, the properties of photosystem II are being unraveled (McEvoy and Brudvig 2006; Rivalta et al. 2012). These ongoing studies should provide insight into how the Mn_4Ca cluster of photosystem II efficiently oxidizes water. In addition, the studies of reaction centers and photosystem II should yield insight into the evolutionary process by which ancient primitive phototrophs, which performed anoxygenic photosynthesis, evolved into organisms containing complexes capable of performing water oxidation. These studies will not only benefit our fundamental understanding of photosynthesis, but also potentially lead to bio-inspired development of solar devices that can efficiently convert light energy into fuels such as hydrogen and oxygen.

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