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Minireview

Electron donors and acceptors in the initial steps of photosynthesis in purple bacteria: a personal account

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Abstract

The discovery by Louis N. M. Duysens in the 1950s that illumination of photosynthetic purple bacteria can cause oxidation of either a bacteriochlorophyll complex (P) or a cytochrome was followed by an extended period of uncertainty as to which of these processes was the 'primary' photochemical reaction. Similar questions arose later about the roles of bacteriopheophytin (BPh) and quinones as the initial electron acceptor. This is a personal account of kinetic measurements that showed that electron transfer from P to BPh occurs in the initial step, and that the oxidized bacteriochlorophyll complex (P^+) then oxidizes the cytochrome while the reduced BPh transfers an electron to a quinone.

Abbreviations: BChl – bacteriochlorophyll; P – the BChl complex that serves as electron donor in the initial electron-transfer step of photosynthesis in purple bacteria; P^+ – the oxidized form of P

The primary electron donor

As a predoctoral student at the University of Utrecht, Louis N. M. (Lou) Duysens measured the ability of purple photosynthetic bacteria to use light of various wavelengths for photosynthesis (Duysens 1952). A photograph of the cover of his classical thesis appears in Govindjee et al. (this issue). Comparisons with the excitation spectra for bacteriochlorophyll (BChl) fluorescence indicated that light absorbed by other pigments was used for photosynthesis only after the energy was transferred to BChl. Duysens found that the bacteria contain BChl complexes with a variety of absorption spectra, and that energy appeared to migrate quickly to complexes that absorbed at the longest wavelengths. He suggested the initial chemical steps of photosynthesis occur when the energy reaches a 'reaction center' or 'trapping' pigment (P), which he estimated might be present in a concentration ratio of about 1/200 relative to the antenna BChl.

To look for absorbance changes that would reveal activities in the hypothetical reaction center, Duysens built a differential spectrophotometer that could measure changes of less than 1% in the absorbance of bacterial cell suspensions in the near infrared. He found what he was looking for. When suspensions of *Rhodospirillum rubrum* or *Chromatium vinosum* cells were exposed to light, there were small, reversible changes in the absorption spectra in the region of 750 to 900 nm.

Following Duysens' discovery, William (Rod) Clayton (1962a, 1963) found that the BChl complex that gave rise to the light-induced signals remained functional in aged bacterial cultures after most of the BChl had been degraded to bacteriopheophytin. Irwin (Tac) Kuntz, Paul Loach and Melvin Calvin (Kuntz et al. 1964) and Clayton (1966) found that P also

survived destruction of the bulk BChl by strong oxidants such as K_2IrCl_6. William (Bill) Sistrom and Rod Clayton showed further that the light-induced absorbance changes did not occur in a mutant strain of *Rhodobacter sphaeroides* (then called *Rhodopseudomonas spheroides*) that was unable to grow photosynthetically (Clayton et al. 1965; Sistrom and Clayton 1964; for a historical minireview, see Clayton 2002). The picture thus began to take shape that P was a special BChl-protein complex that differed significantly from the antenna complexes and was an essential component of the photosynthetic apparatus.

Duysens, however, had found that illuminating bacterial cell suspensions also caused oxidation of a *c*-type cytochrome (Duysens 1954). Pursuing this observation, Britton (Brit) Chance and Lucille Smith showed that illumination or aeration of *Rs. rubrum* cell suspensions caused oxidation of several different cytochromes (Chance and Smith 1955). Chance and Mitsuo Nishimura (Chance and Nishimura 1960) then made the remarkable discovery that photooxidation of cytochrome C_{552} in *Chromatium (Ch.) vinosum* occurred rapidly even at 77 K, and John Olson and Britton Chance found that that the quantum yield of cytochrome photooxidation at room temperature was close to 100% (Olson and Chance 1960a, b, 1962). To Chance and his coworkers, these observations suggested that cytochrome oxidation was driven directly by light and thus was likely to be the initial electron-transfer reaction of bacterial photosynthesis. Because the process occurred when the cells were illuminated at wavelengths that excited BChl, an excited BChl molecule presumably initiated the reaction by extracting an electron from the cytochrome.

A photograph of Chance is shown in Figure 1. A photograph of Duysens can be found in a paper by Delosme and Joliot (2002).

Whether P played any role in the cytochrome photooxidation was not clear. For one thing, the absorbance changes associated with P appeared to reflect BChl oxidation, not reduction. Joop H. C. Goedheer (1958, 1960), Duysens (1958), Clayton (1962a) and Kuntz et al. (1964) showed that similar absorbance changes occurred on the addition of chemical oxidants, and that the absorbance changes induced by either light or oxidants could be reversed by reductants. In addition, the photochemical bleaching of P was not seen at all in intact cells, except at very high light intensities or in the presence of oxidants or inhibitors that abolished cytochrome photooxidation (Duysens et al. 1956). Under more moderate conditions, photooxidation of P became detectable only after the cytochrome was already oxidized (Arnold and Clayton 1960; Beugeling and Duysens 1966; Clayton 1962b; Duysens 1952; Duysens et al. 1956).

There were two equally plausible interpretations of these observations. If the 'primary' photochemical reaction in purple bacteria was oxidation of a *c*-type cytochrome, as Britton Chance and Mitsuo Nishimura had suggested, BChl oxidation might be a side reaction that occurred only when cytochrome oxidation was blocked or already completed. Alternatively, photooxidation of P could be the primary process, as Lou Duysens and Rod Clayton proposed, if the oxidized BChl (P^+) was reduced rapidly by the cytochrome. I remember hearing a lively exchange on this issue in 1964 at the International Congress of Biochemistry in New York, where Rod Clayton presented evidence that P formed the 'reaction center' of photosynthetic bacteria. In the question period after Rod's talk, Brit pointed out that the rapid oxidation of the cytochrome at cryogenic temperatures provided compelling evidence that *this* was the photochemical step. Rod replied with a smile that he liked to maintain two conflicting opinions on controversial scientific questions. I think he attributed this precept originally to Bill Arnold. (See Govindjee et al., 1996, for a special issue dedicated to Arnold's discoveries.) As a biochemistry graduate student working in a remote area of metabolism, I never imagined that I might soon have an opportunity to resolve the issue myself.

When I arrived at the University of Pennsylvania's Johnson Foundation as a postdoctoral fellow a year later, much of the research going on there revolved around the theme of developing new instrumentation to address biophysical questions. Although my prior experience of electronics consisted almost solely of having built a hi-fi amplifier from a commercial kit, I found this spirit exciting. I watched with awe as Brit tinkered with a stopped-flow apparatus and Don DeVault worked out the circuit for a new preamplifier. (See Parson, 1989, for a tribute to Don, and Figure 1 for a photograph; a better photograph appears in the 1989 special issue of *Photosynthesis Research* dedicated to him.) With help from Don, I began to read specifications for transistors and photomultipliers with interest and to look forward to each new issue of *Review of Scientific Instruments*. But when I sounded Brit out on exactly what biophysical question I should address, all he would say was 'Take your time. You'll think of something interesting sooner or later!'

Figure 1. A photograph of Britton Chance (standing) and DeVault (sitting, second from left). Others in the background are Tomoko Ohnishi and Jane Vanderkooi. This photograph was provided to Govindjee by Brit Chance.

As I read more about photosynthesis, it seemed increasingly critical to clarify the role of P in the cytochrome photooxidation. The way to do this was not hard to see. If P oxidation was the primary step, P^+ should appear quickly after a short pulse of light and then should disappear as cytochrome *c* went oxidized. The conversion of P to P^+ should be measurable as a transient bleaching of the absorption band in the region of 870 nm, with the exact wavelength depending on the species of bacteria selected for the experiment. It would be important to make the measurements under conditions in which the quantum yield of cytochrome oxidation was close to 1, and to determine whether the transient photooxidation of P had the same high quantum yield. If both processes could be driven by absorption of a single photon, then they must occur sequentially rather than by competing pathways.

It was clear that the measurements would require high time resolution in order to probe the true kinetics of the cytochrome oxidation. However, this was a moving target. In their landmark 1960 study, Chance and Nishimura (1960) had measured a half-time of several seconds for cytochrome photooxidation at both room temperature and 77 K, which appeared to show that the rate was independent of temperature. However, Wim Vredenberg and Lou Duysens (Vredenberg and Duysens 1964) later found that this was the case only if the excitation light was relatively weak; with stronger excitation, the rate decreased at low temperatures. The kinetics that Brit and Mitsuo had measured evidently were limited by the rate of excitation. But the half-time of about 0.1 s that Vredenberg and Duysens measured for cytochrome oxidation at high light intensities also was limited by their instrumentation, and Chance and DeVault (1964) soon measured a startlingly shorter half-time of 20 μs by using a Q-switched laser flash for the excitation. By 1966, further improvements in the apparatus had reduced the apparent half-time of the reaction to 2 μs (DeVault and Chance 1966).

The main difficulty in detecting the transient photooxidation of P was that excitation of bacterial cell suspensions with a short pulse of light elicited a burst of fluorescence in the same region of the spectrum where I wanted to measure the absorbance changes. The fluorescence overloaded the photomultiplier and preamplifier, and the recovery from this overload could take many microseconds. Worse, the fluorescence artifact could easily be mistaken for a transient bleaching of P. The solution to this problem again was simple enough. If the measuring light beam was made stronger, the sensitivity of the photomultiplier could be reduced. I therefore replaced the continuous measuring lamp that I had been using by a xenon flash lamp. This was a logical extension of Don DeVault's idea to boost the current through an ordinary tungsten lamp for measurements on millisecond time scales (DeVault and Chance 1966). Placing an inductance in series with the flash lamp stretched the discharge sufficiently to give a relatively flat signal on the microsecond time

scale. Fortuitously, xenon flash lamps have a strong emission line at 881.9 nm, which coincides perfectly with the main absorption band of P in *Ch. vinosum*. Although the peak intensity of the light incident on the chromatophores was about 40 times that given by a tungsten lamp, the short duration of the flash kept the integrated irradiance small enough to make the actinic effect negligible (Parson 1968).

To maximize the fraction of the measuring light that reached the photomultiplier, I decided to use chromatophores (membrane fragments) rather than the turbid suspension of whole cells that had been the subject of most previous studies. This required showing that cytochrome photooxidation occurred with a quantum yield near 1.0 in chromatophores as well as in intact cells, which turned out to be the case as long as the cytochrome was poised in the reduced state before the excitation pulse (Parson 1968). Using the optically clear chromatophore suspensions made it possible to put a monochromator between the sample and the photomultiplier, and this helped considerably to discriminate against fluorescence.

But even with these measures, fluorescence artifacts still obscured the signals at short times after the excitation flash. The main trick to reducing these artifacts further lay in the electronic circuitry that followed the photomultiplier. I tried numerous home-built and commercial preamplifiers before Jack Leigh showed me the tiny Fairchild μA702 preamplifier in a fluorescence-lifetime apparatus he had built for Warren Butler. Like many of the integrated circuits that became available in the late 1960s, the μA702 cost only a few dollars but was astonishingly fast and robust. With some attention to minimizing the capacitance at the input and the addition of an emitter-follower to reduce the output impedance, it recovered quickly enough from the fluorescence spike so that I finally could measure absorbance changes on sub-microsecond time scales.

Using Don DeVault's Q-switched ruby laser for the excitation, I found that the absorption band of P was bleached in less than 0.5 μs after the flash (Parson 1968). The absorbance of P then reappeared with a time constant of about 2 μs at room temperature, and a c-type cytochrome ('C422') was oxidized in synchrony with this recovery (Figure 2). Both processes occurred with a quantum yield close to 1 (Figure 3).

Although these measurements showed that photooxidation of P preceded photooxidation of the cytochrome, Brit was pleased to see the question settled in a way that he found convincing, and he congratulated me heartily on the results. Reading the 1968 paper now, I am struck by the figure entitled 'Measurements of Artifacts,' which today probably would fall under the editor's ax or disappear into a supplementary section. Distinguishing transient absorbance changes from instrumental artifacts was the heart of the problem.

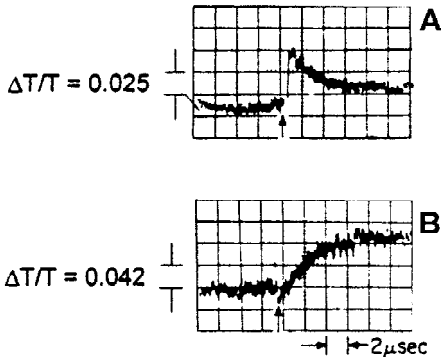

Figure 2. Kinetics of P photooxidation and reduction (A) and cytochrome C422 oxidation (B) in *Chromatium vinosum* chromatophores. The upward deflection of the trace in A shows the transient decrease in the absorbance of P at 882 nm caused by a 30-ns excitation flash (arrow). The absorbance reappears with a time constant of about 2 ms. The measuring light from the Xe flash lamp gave a curving baseline that was measured separately in the absence of the excitation flash (not shown). A fluorescence artifact drove the trace off-scale for about 0.5 μs after the flash. The artifact was measured separately in the absence of the measuring light, and also by using chromatophores in which photooxidation of P was blocked by prior reduction of the electron acceptor. The measurements in B were made with continuous measuring light at 422 nm, where oxidation of the cytochrome causes a longer-lived absorbance decrease (upward deflection of the trace). The cytochrome oxidation parallels the return of P to the reduced state. The small, initial absorbance increase (downward deflection) at 422 nm reflects an absorption band of P^+ that peaks near 430 nm. Reproduced from Parson (1968).

My wife Polly and I moved to Seattle with our daughters Christy and Wendy shortly after I submitted the paper on the photooxidation of P for publication. (See Figure 4 for a photograph of Christy, Wendy and myself shortly after our move.) At the University of Washington, I tried exciting *Ch. vinosum* chromatophores with pairs of laser flashes separated by an adjustable delay (Parson 1969a, b). If the delay was less than about 10 microseconds, little photooxidation of either P or cytochrome was seen on microsecond time scales after the second flash. The energy of this flash instead appeared to be lost as fluorescence and heat. With longer delays, P^+ could be generated again by the second flash and it then oxidized a second cytochrome heme with kinetics similar to those seen after the first flash. The delay between the flashes evidently

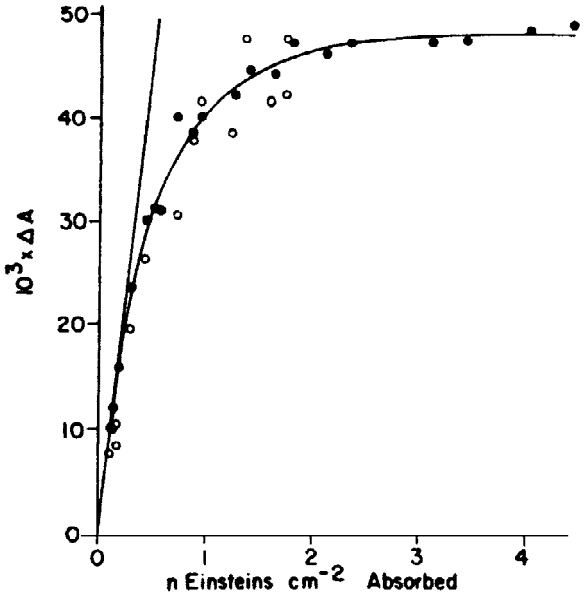

Figure 3. Amplitudes of the absorbance changes associated with photooxidation of P (●) and cytochrome C422 (○) in *Chromatium vinosum* chromatophores, as functions of the intensity of the excitation flash. Photooxidation of P was measured from the transient absorbance decrease at 882 nm and was corrected for fluorescence artifacts; cytochrome photooxidation was measured from the longer-lived absorbance decrease at 422 nm. The change in the molar extinction coefficient at the measuring wavelength was estimated to be approximately 90 mM^{-1} cm^{-1} for both molecules. The initial slopes of the plots indicate that both processes have a quantum yield close to 1.0, which means that they must occur sequentially rather than as competing reactions. Reproduced from Parson (1968).

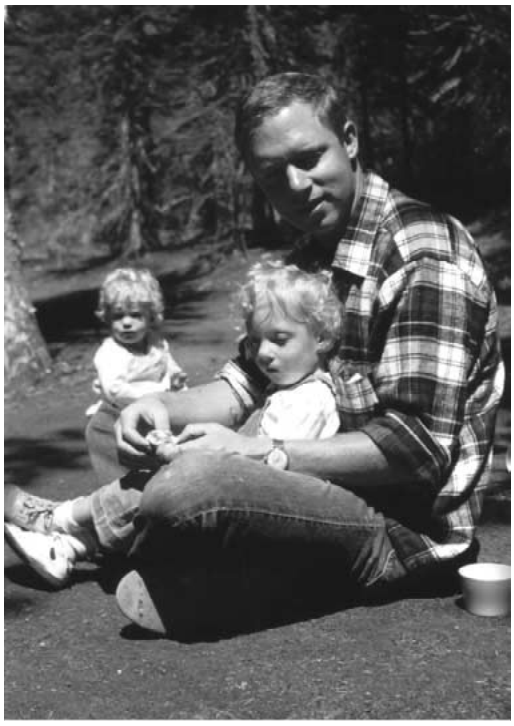

Figure 4. A 1968 photograph of the author (Bill Parson) with daughters Wendy and Christy in Mt. Ranier National Park. Photo by Polly Parson.

was needed in order for the electron acceptor that removed an electron from P to return to its resting state by passing an electron on to a secondary acceptor. These and subsequent studies (Parson and Case 1970; Seibert and DeVault 1970) showed that P^+ can draw electrons sequentially from four hemes with a range of midpoint redox potentials, and the same turned out to be true in *Blastochloris* (*Bl.*, formerly *Rhodopseudomonas*) *viridis* (Case et al. 1970; Holten et al. 1978; Ortega et al. 1993).

When the crystal structures of the *Bl. viridis* (Deisenhofer et al. 1985, 1995) and *Thermochromatium tepidus* (Nogi et al. 2000) reaction centers were solved, both were found to have a bound cytochrome with four hemes stretching out in a zigzagged chain from P (see Figure 7). Although *Ch. vinosum* reaction centers have not yet been crystallized, they are functionally similar to the reaction centers of these species and probably have very similar structures.

Figure 5 shows a current scheme of the initial electron-transport steps discussed in this paper. Further discussion of these reactions can be found in Ke (2001).

Electron acceptors

The double-flash technique also provided a way to measure the kinetics of electron transfer between the 'primary' and 'secondary' electron acceptors (Parson 1969b). Further studies showed that *o*-phenanthroline inhibits the secondary electron-transfer reaction (Parson and Case 1970), and experiments in which we extracted lyophilized chromatophores with organic solvents led to the identification of the secondary acceptor as ubiquinone (Halsey and Parson 1974). Studies by several other investigators converged on the identification of the primary acceptor as a quinone at about the same time (Clayton and Straley 1970; Loach and Hall 1972; Cogdell et al. 1974; Okamura et al. 1975, 1976; Verméglio and Clayton, 1977; Wraight 1977). However, Rod Clayton and his coworkers pointed out a puzzling discrepancy in the effects of blocking photoreduction of the primary quinone (Q_A). When the photoreduction was blocked by lowering

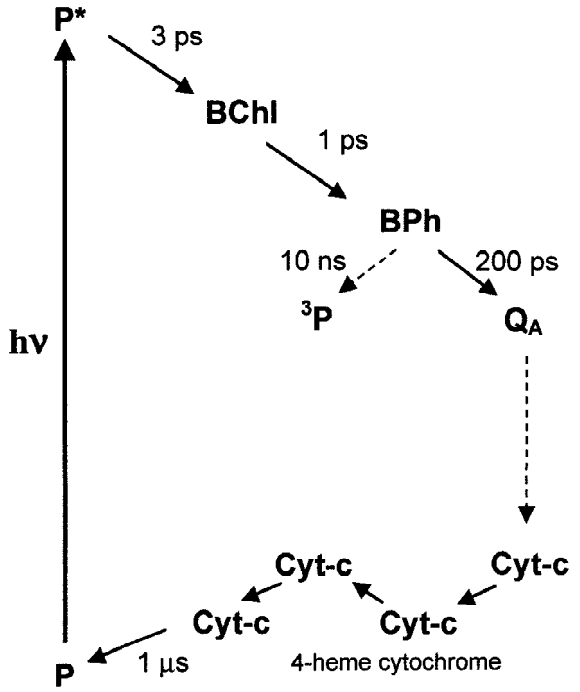

Figure 5. The path of electron flow in the initial steps of photosynthesis in purple bacteria. The excited BChl dimer (P^*) reduces a BPh by way of another BChl. The reduced BPh transfers an electron to a quinone (Q_A), and the oxidized dimer (P^+) draws an electron from a *c*-type cytochrome. Reaction centers of *Chromatium vinosum*, *Thermochromatium tepidum* and *Blastochloris viridis* have a bound cytochrome with four *c*-type hemes; *Rhodobacter sphaeroides* and most other nonsulfur purple bacteria use cytochrome c_2, which has a single heme and dissociates more freely from the reaction center. The primary quinone returns an electron to the cytochrome by way of a secondary quinone (Q_B) and the cytochrome bc_1 complex. The triplet state of P (^{3}P or P^R) can be formed if electron transfer from BPh^- to Q_A is blocked. This step requires spin rephrasing in the P^+BPh^- radical pair (P^F).

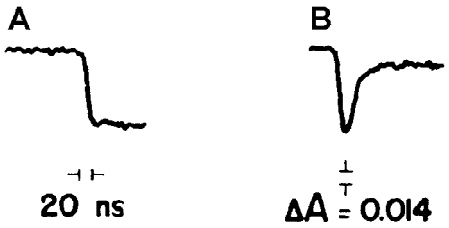

Figure 6. Absorbance changes at 430 nm caused by exciting *Rhodobacter sphaeroides* reaction centers with 20-ns, 834-nm flashes at room temperature. In (A) Q_A was in the oxidized state before the flash; the downward signal (an absorbance increase) reflects the 430-nm absorption band of P^+, which lives for about 100 ms under the conditions of the measurements. In panel (B) Q_A was reduced before the flash; the short-lived downward signal represents P^F (P^+BPh^-); the small, longer-lived signal comes from the triplet state of P (P^R). At room temperature, the triplet state has a lifetime of about 6 μs. At low temperatures, the decay kinetics of the different zero-field sublevels of the triplet state can be resolved (Parson and Monger 1976). Reproduced from Parson et al. (1975).

the ambient redox potential to reduce the quinone beforehand, the yield of fluorescence from the excited BChl complex (P^*) increased by only about a factor of three to four (Zankel et al. 1968; Reed et al. 1969; Clayton et al 1972). The increase in the fluorescence yield on the second of two closely-spaced flashes was similar (Parson 1969b). Since Colin Wraight and Rod Clayton had shown that photooxidation of P occurred with a quantum yield of at least 0.98 in unblocked reaction centers (Wraight and Clayton 1974), blocking this process would be expected to increase the fluorescence from P^* by at least a factor of 50. The discrepancy suggested that the initial electron-transfer step could involve another electron acceptor preceding the quinone. Alternatively, electron transfer might occur from a triplet state of P, rather than from the excited singlet state that gives rise to fluorescence.

There were several reasons for thinking that the initial electron-transfer reaction might proceed through a triplet state. Most importantly, P. Leslie (Les) Dutton, Jack Leigh and Michael (Mike) Seibert (Dutton et al. 1972; Leigh and Dutton 1974) had detected EPR signals indicative of a BChl triplet state when they illuminated reaction centers in which electron transfer to the primary quinone was blocked. Robert Uphaus, James (Jim) Norris and Joseph Katz confirmed these observations, and pointed out that the triplet state had an unusual spin polarization that could not be explained well by the ordinary mechanism of intersystem crossing (Uphaus et al. 1974). In addition, most of the photochemical reactions of BChl or chlorophyll in solution were known to involve triplet states. However, this observation was not necessarily pertinent to photosynthetic reaction centers, where the close proximity of the initial electron donor and acceptor could allow electron transfer in the excited singlet state to compete much more favorably with intersystem crossing.

Kinetic studies of the of reaction centers in which Q_A was already reduced required further improvements in the electronics to decrease the response time to about 5 ns. Rod Clayton, Richard Cogdell and I then found that exciting reduced reaction centers with a 20-ns flash generated a transient state ('P^F') that decayed with a time constant of about 20 ns (Parson et al. 1975). Figure 6 shows a typical measurement of the absorbance changes induced by flashes before and after reducing the quinone. The new state was

formed with a quantum yield near 1.0 at both room- and cryogenic temperatures, and thus appeared to be a candidate for an intermediate between P^* and $P^+Q_A^-$. State P^F differed spectroscopically from P^+, particularly in the region around 540 nm where one of the two bacteriopheophytin (BPh) molecules in the reaction center absorbed. The eventual identification of P^F as a P^+BPh^- radical-pair state was aided greatly by Jack Fajer's studies of the electrochemical and spectroscopic properties of BPh anion radicals in solution (Fajer et al. 1975; a historical minireview by J. Fajer is in press in Part 3 of these historical issues). The broad absorption band of P^F at 680 nm is characteristic of such radicals.

Vladimir Shuvalov and Vyacheslav (Slava) Klimov (Shuvalov and Klimov 1976, Klimov et al. 1977) and David Tiede and Les Dutton (Tiede et al. 1976a, b; Dutton et al. 1976) showed that the BPh^- radical accumulated in the absence of P^+ when reaction centers from *Ch. minutissimum*, *Ch. vinosum* or *Bl. viridis* were illuminated continuously at low redox potentials. Each time the P^+BPh^- radical pair is formed, its 20-ns lifetime provides a brief opportunity for the bound cytochrome to reduce P^+, trapping the BPh in the reduced state. Continued illumination thus can drive virtually all the reaction centers into this state. This finding made it possible to obtain the EPR and optical absorption spectra of the BPh^- radical, which helped to solidify the identification of P^+BPh^- and provided information on the interactions of the BPh with other components of the reaction center.

Measurements of the flash-induced absorbance changes in reduced reaction centers also revealed the formation of another, longer-lived state ('P^R') as the P^+BPh^- radical pair decayed (Parson et al. 1975). Judging from its absorption spectrum, P^R was the triplet state of P that Dutton et al. (1974) had detected by EPR. P^R was formed in a high quantum yield at cryogenic temperatures, but its yield decreased to about 0.1 at room temperature, indicating that the triplet state was unlikely to be an intermediate in the ordinary photochemical electron-transfer reactions. Robert (Bob) Blankenship, Tjeerd Schaafsma and I subsequently found that the quantum yield decreased further in the presence of relatively weak magnetic fields, as would be expected if the triplet state formed by charge-recombination after rephasing of the upaired electron spins of P^+ and BPh^- in the P^+BPh^- radical-pair (Blankenship et al. 1977). The direction of this effect, and the unusual spin polarization seen in the EPR spectrum showed clearly that P^+BPh^- was created initially in a singlet state. Further studies of the effects of magnetic fields by the late Arnold Hoff, and by Mary Elizabeth (Maibi) Michel-Beyerle, Klaus Schulten, Steven (Steve) Boxer, Marion Thurnauer, Jim Norris, and their coworkers unearthed a rich source of information on the electronic coupling and energetics of the P^+ and BPh^- radicals, and led to some of the initial suggestions that there might be still another electron carrier between P and the BPh (see, e.g., Thurnauer et al 1975; Hoff et al. 1977; Haberkorn and Michel-Beyerle 1977; Werner et al. 1978; Haberkorn et al. 1979; Boxer 1983). In reaction centers that contained carotenoids, the BChl triplet state decayed on nanosecond time scales by transferring energy to a carotenoid (Cogdell et al. 1975; Parson and Monger 1976; Shuvalov and Klimov 1976).

Although the high quantum yield with which P^+BPh^- was formed in reduced reaction centers suggested that the BPh might serve as the initial electron acceptor in functional reaction centers, this remained to be proved. Again, it was possible that reduction of the BPh was a side reaction that came into play only when the normal pathway was blocked by reducing the quinone. As with photooxidation of P and the cytochrome, it was not hard to imagine a kinetic experiment that would distinguish between the two schemes. But at this point we seemed to have hit the practical limit of real-time kinetic measurements. If P^+BPh^- was indeed an intermediate in functional reaction centers, it evidently passed an electron on to Q_A on a sub-nanosecond time scale that was shorter than any instrumental response time we could achieve. Fortunately, a different experimental approach was just being developed in several laboratories, including those of Peter Rentzepis, Charles Shank and others at Bell Laboratories and Maurice Windsor at Washington State University. In this approach, the sample is excited with a short pulse of light and a similar pulse is used to measure the sample's absorbance as a function of time after the excitation. The time resolution of such measurements is limited mainly by the widths of the excitation ('pump') and measuring ('probe') pulses and the reproducibility of the delay between pumping and probing, and not by the instrumental response to the transmitted probe light. The discovery that mode-locked lasers could emit pulses with widths of only a few picoseconds thus opened the door to studies of processes that occur on a whole new time scale. In a typical experiment, a weak probe pulse is split off from the pump pulse by a beam splitter, and the delay

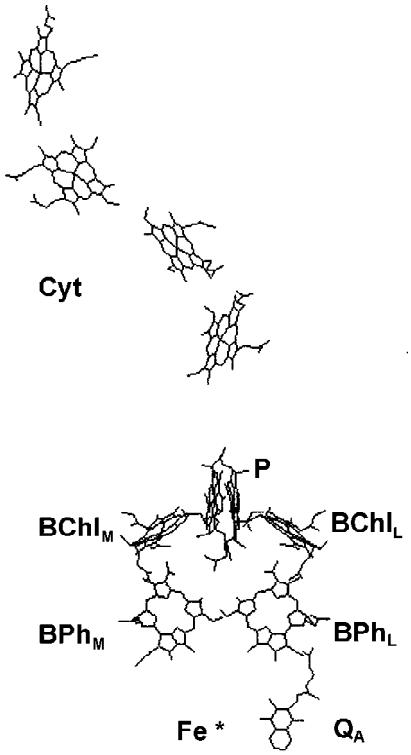

Figure 7. Electron carriers in the reaction center of *Thermochromatium tepidum*. The coordinates are from Protein Data Bank file 1EYS.pdb (Nogi et al. 2000). The prenyl side chains have been removed or truncated for clarity. The primary quinone (Q_A) is menaquinone in *Thermochromatium tepidum*, *Chromatium vinosum* and *Blastochloris viridis*, and ubiquinone in *Rhodobacter sphaeroides* and most other non-sulfur purple bacteria. The secondary quinone (Q_B) is bound less tightly and is missing in the *Thermochromatium tepidum* crystal structure; its binding site is on the opposite side of the nonheme Fe atom from Q_A.

between the pulses is controlled by using a mirror on a translation stage to change the path that one of the pulses follows before it reaches the sample. Because light travels at a known velocity, the delay can be measured with nothing more than a ruler.

Using pump and probe pulses with widths of about 8 ps, Mark Rockley, Maurice Windsor, Richard Cogdell and I (Rockley et al. 1975), and Ken Kaufmann, Katherine Petty, Les Dutton, Britton Chance and Peter Rentzepis (Dutton et al. 1975; Kaufmann 1975, 1976) independently found that state P^F (P^+BPh^-) was formed transiently in high yield in functional reaction centers of *Rb. sphaeroides*. Dewey Holten, Maurice Windsor, Philip Thornber and I subsequently obtained similar results with reaction centers of *Bl. viridis* (Holten et al. 1978). In both species, the P^+BPh^- radical pair formed within 10 ps after the excitation and then decayed with a time constant of about 200 ps, leaving a state that we presumed to be $P^+Q_A^-$. Although the kinetics of reduction of the quinone could not be measured directly on this time scale, it seemed safe to identify Q_A as the acceptor that removed an electron from BPh^-, since pre-reducing or extracting the quinone reversibly blocked the rapid reoxidation of the BPh^-. This assignment proved to be consistent with the reaction center crystal structures (Figure 7), in which Q_A sits close to one of the two BPh molecules (Deisenhofer et al. 1984, 1995; Allen et al. 1987; Chang et al 1991). However, a tryptophan side chain is inserted between the BPh and the quinone and appears to play an important role in the electron-transfer reaction. Replacing the tryptophan by tyrosine or phenylalanine weakens the binding of Q_A to the reaction center and slows the reoxidation of BPh^- (Stilz et al. 1994).

One of the most remarkable features of the reaction center crystal structure is its symmetry (Deisenhofer 1984,1995; Allen et al. 1987; Chang et al 1991; Lancaster and Michel 1999). The BChl dimer that makes up P has a two-fold axis of approximate symmetry, so that a 180 degree rotation around this axis approximately interchanges corresponding atoms of the two BChls. The same symmetry axis also relates homologous residues in the L and M polypeptides, and two chains of monomeric BChl, BPh and quinone molecules extend out from P symmetrically on either side of this axis. Proceeding away from P along either of these chains, one encounters first a monomeric BChl ($BChl_L$ in one chain or $BChl_M$ in the other), then a BPh (BPh_L or BPh_M), and finally a quinone (Q_A or Q_B) (see Figure 7). The rotational symmetry, however, is only approximate. Because the keto group of BPh_L forms a hydrogen bond with a glutamic acid residue while the keto group of BPh_M is not hydrogen bonded, the two BPh molecules have slightly different absorption spectra (Deisenhofer 1984,1995; Allen et al. 1987; Chang et al 1991; Bylina et al. 1988; Peloquin et al. 1990). The spectroscopic signatures of BPh^- and P^+BPh^- show clearly that only BPh_L normally undergoes photoreduction (Parson et al. 1975; Kaufmann et al. 1976; Tiede et al. 1976b; Shuvalov and Klimov 1976 Kirmaier et al. 1985a, b; Robert et al. 1985), although Su Lin, Neal Woodbury and their coworkers have found that electron transfer to BPh_M can occur with high-energy excitation (Lin et al. 1999, 2001).

The high specificity for reduction of BPh_L under ordinary conditions probably is determined in the

earliest step of the electron-transfer process, in which P* passes an electron preferentially to $BChl_L$ rather than $BChl_M$. Electrostatics calculations (Parson et al. 1990; Alden et al. 1995; Gunner et al. 1996; Warshel and Parson 2001) and kinetic studies in a variety of modified reaction centers (Arlt et al. 1996a, b; Heller et al. 1995; Jia et al. 1993; Katilius et al. 1999; Kirmaier et al. 1995, 2001, 2002; Nagarajan et al. 1993; Schmidt et al. 1994) indicate that $P^+BChl_L^-$ is probably close to or slightly below P* in energy, while $P^+BChl_M^-$ lies considerably higher. Craig Schenck, Chris Kirmaier, Dewey Holten, Neal Woodbury, Arnold Hoff, Dieter Oesterhelt, Wolfgang Zinth and others have shown that mutations that lower the energy of $P^+BChl_M^-$ or raise the energy of $P^+BChl_L^-$ can increase the yield of electron transfer to the normally inactive pigments. Although the formation of $P^+BChl_L^-$ is difficult to resolve in wild-type reaction centers, these studies of modified reaction centers have provided increasingly convincing evidence for this state as an intermediate between P* and $P^+BPh_L^-$ and have highlighted the importance of energetics in the specificity of charge separation. However, differences between the electronic coupling of P* to $P^+BChl_L^-$ and $P^+BChl_M^-$ probably contribute to the specificity. The coupling to $P^+BChl_L^-$ appears to be significantly stronger than that to $P^+BChl_M^-$ (Ivashin et al. 1998; Kolbashov and Scherz 2000; Zhang and Friesner 1998). The relative importance of this contribution is still not entirely clear (Bixon and Jortner 1999; Warshel and Parson 2001; Kirmaier et al. 2001, 2002).

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