THE PRIMARY STEPS OF PHOTOSYNTHESIS

The two important initial steps of photosynthesis—electron transfer and energy transfer—occur with great speed and efficiency. New techniques in laser optics and genetic engineering are helping us to understand why.

Graham R. Fleming and Rienk van Grondelle

Photosynthesis, the process by which plants convert solar energy into chemical energy, results in about 10 billion tons of carbon entering the biosphere annually as carbohydrate—equivalent to about eight times mankind's energy consumption in 1990. The apparatus used by plants to perform this conversion is both complex and highly efficient. Two initial steps of photosynthesis—energy transfer and electron transfer—are essential to its efficiency: Molecules of the light-harvesting system transfer electronic excitation energy to special chlorophyll molecules, whose role is to initiate the directional transfer of electrons across a biological membrane; the electron transfer, which takes place in a pigment–protein complex called the reaction center, then creates a potential difference that drives the subsequent biochemical reactions that store the energy. (Higher plants use two different reaction centers, called photosystems I and II, while purple bacteria make do with a single reaction center. The difference is that the bacteria do not generate oxygen in the photosynthetic process.) Both the elementary energy transfer and the primary electron transfer are ultrafast (occurring between 10^{-13} and 10^{-12} seconds), leading to the trapping of excitation energy at the reaction center (on a 100-picosecond timescale) and subsequent electron transfer in about 3 picoseconds with almost 100% quantum yield.

In this article, we describe current theoretical and experimental efforts aimed at understanding photosynthetic electron and energy transfer. Quantum dynamics—particularly the subtle interplay between timescales set by electronic coupling between molecules, their internal nuclear motions and the coupling of the system to the dissipative bath (in this case, the protein scaffolding)—is emerging as a key factor in understanding the efficiency and rapidity of these two processes. Such an understanding has implications for fields ranging from the design of herbicides to the construction of molecular electronic devices.

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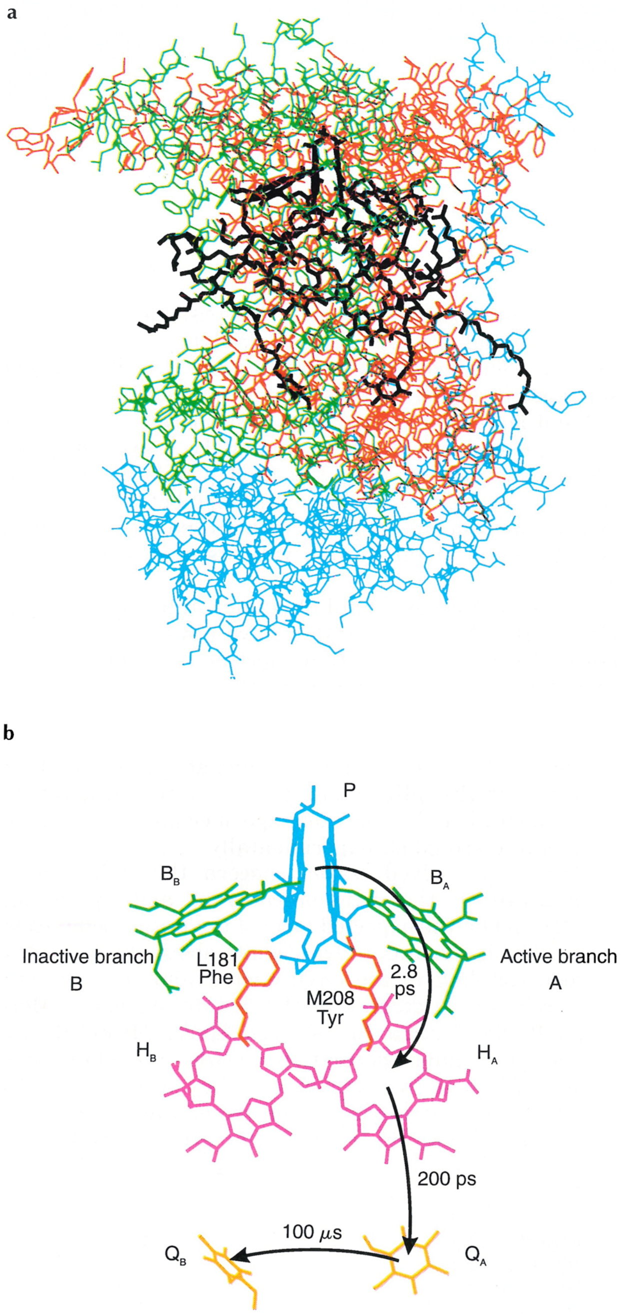
Electron transfer

In 1989 Hans Deisenhofer and Hartmut Michel determined the molecular structure of a bacterium's photosynthetic reaction center to atomic resolution (figure 1).[1] This remarkable achievement spurred great interest in the mechanism of the primary electron-transfer step in photosynthetic bacteria. Developments in ultrafast lasers allow experimenters to observe electron transfer in real time; genetic engineering allows controlled changes to be made in the protein structure; and advances in theory and computer simulation have brought into focus many challenging questions, some of which we hope to illustrate—but not resolve—in this article.

Pioneering work by P. Leslie Dutton, Peter Rentzepis, William Parson and Maurice Windsor in 1975 established the sequence of events in the reaction center: Following optical excitation of the "special pair," labelled P in figure 1b, charge separation occurs within about 3 psec to give the oxidized special pair P^+ and a pheophytin anion H_A^-. The electron then hops to a quinone molecule Q_A in about 200 psec and on to a second quinone Q_B in about 100 μsec. This entire sequence is repeated (after the special pair has been restored to neutrality by a cytochrome molecule), and the Q_B molecule leaves the protein as Q_BH_2 to take part in the chemical reactions that lead to the generation of an electrochemical potential gradient across the biological membrane in which the complex sits. Eventually the two electrons of the reduced quinone molecule are returned to the cytochrome, leading to the transport of four protons across the membrane. The cumulative energy stored in the electrochemical gradient is sufficient



Structure of the reaction center protein of the purple bacterium *Rhodobacter sphaeroides*. **a:** The complex consists of three protein subunits called M, L and H (shown in red, green and blue, respectively), with the molecules responsible for electron transfer indicated in black. The complex spans the biological membrane. **b:** The active constituents of the reaction centers (shown here with the protein scaffold stripped away) are the special pair P (blue), the two bacteriochlorophyll molecules B (green), the two pheophytin molecules H (pink) and the two quinone molecules Q (orange). The reaction center is made up of active branch A and the inactive branch B. Two amino acids at sites L181 and M208 have been modified by genetic manipulation; in the natural (wild-type) system they are phenylalanine and tryptophan, respectively. The rates of the forward electron-transfer steps in the wild-type system are indicated by arrows. (Courtesy of James R. Norris, Chong-Hwan Chang, Ossama El-Kabbani, David Teide and Marianne Schiffer, Argonne National Laboratory.) **Figure 1**

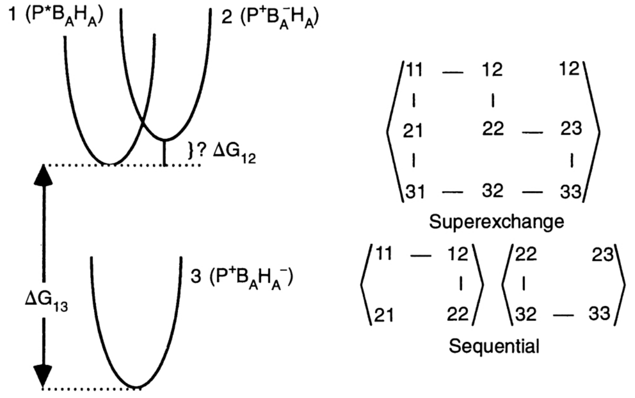
for green plants and bacteria to synthesize adenosine triphosphate and other molecules used as energy sources by living organisms. If unused, the charge returns from Q_B to P within a few seconds.

There are many remarkable aspects about the process just described. The quantum yield of electron transfer is close to 100%, and about 40% of the input photon energy is stored in the transmembrane charge separation. As figure 1b shows, there appear to be two equally good paths for the electron to travel, yet no evidence has been found for electron transfer along the inactive branch (labelled B in the figure). Experiments by David Tiede have shown that the active branch A is favored by at least 200:1.[2] The center-to-center distance between P and H_A (or H_B) is 17 Å, and the observed electron-transfer rate between the two molecules is at least 1000 times faster than the expected rate over that distance in a vacuum. Naturally, these findings have focused attention on the role played in electron transfer by the bacteriochlorophyll molecule B_A and the protein scaffolding, as we shall discuss below. Another striking finding is that the primary electron-transfer step, far from being thermally activated, actually speeds up slightly as the temperature is lowered from 300 to 10 K. Furthermore, the forward and backward electron-transfer rates differ by many orders of magnitude. For example, if Q_B is removed, electron recombination from Q_A to P takes 100 msec, six orders of magnitude slower than the forward process P → Q_A (via H_A).

Electronic states

A look at the reaction center's active constituents, stripped of their protein scaffold, raises the question of how to describe the electronic states of such a system: Are they localized on a single molecule or delocalized over several? Almost 20 years before the x-ray work of Deisenhofer and Michel, James Norris and Joseph J. Katz concluded from electron paramagnetic resonance data that the primary donor cation was indeed a dimer, a *pair* of molecules. Steven Boxer, Gerald Small and their coworkers have used hole-burning spectroscopy at 1.5 K (see the article by Dietrich Haarer and Robert Silbey, PHYSICS TODAY, May 1990, page 58) to probe the electronic states of the reaction center and their interactions with the protein.[3] The special pair is indeed "special" in its spectroscopy, showing a strong coupling of the optical excitation to low-frequency vibrational modes, which is not observed in the spectra of monomeric chlorophylls. Resonance Raman spectroscopy by Boxer, Richard Mathies and their coworkers reveals that these modes are likely intramolecular in origin.[4] It is, of course, tempting to speculate that the nuclear motions coupled to optical excitation also mediate electron transfer, but no one has yet confirmed this hypothesis.

Small has analyzed nonphotochemical hole burning



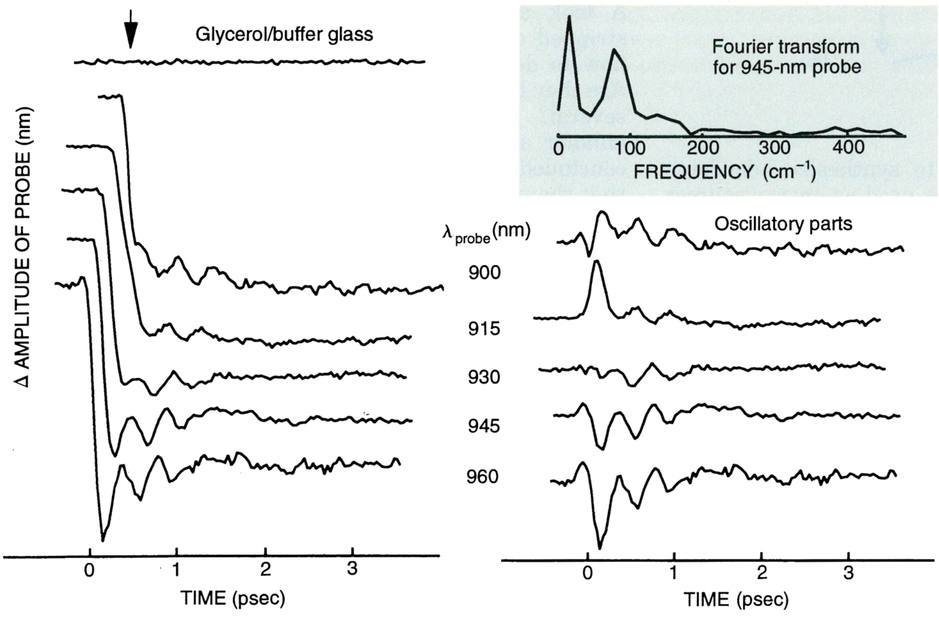
Electron transfer. Left: Free-energy surfaces for the three states believed to be involved in the primary electron-transfer step from the special pair. The location of state 2 ($P^+B_A^-H_A$) is not yet known: It may lie above or below state 1 ($P^*B_AH_A$). The free-energy gap ΔG_{13} between states 1 and 3 ($P^+B_AH_A^-$) is approximately 2000 cm^{-1}. Right: The diagrams illustrate the distinction between the sequential and superexchange mechanisms for electron transfer. The left and right numbers designate the ket and bra of states 1, 2 or 3. The angle brackets denote ensemble averaging; the sequential process involves two uncorrelated steps, whereas the superexchange process is fully coherent. **Figure 2**

using an effective Hamiltonian, proposed by Youngdo Won and Richard Friesner,[5] in which all six pigments are coupled excitonically. This model implies that only the pheophytin states can be considered monomeric. However, many fundamental aspects of the quantum dynamics in such a complex system remain unclear.

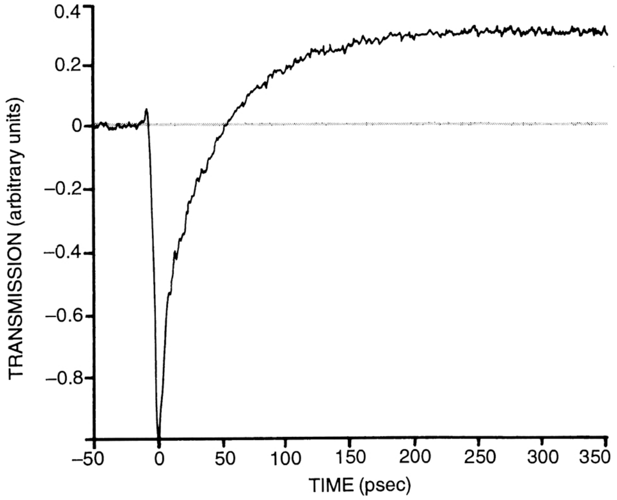
The most basic (and certainly the most controversial) question concerns the two possible mechanisms for the electron transfer from P to H_A. One possible mechanism is a sequential transfer process: $P^*B_AH_A \rightarrow P^+B_AH_A^- \rightarrow P^+B_AH_A^-$. The second is a virtual process (called superexchange) in which B_A acts to mix the electronic states of P and H_A. This mechanism must operate if the state $P^+B_A^-H_A$ is higher in energy than $P^+B_AH_A^-$, and coherence must be maintained until the final population state is reached. (See figure 2.) The competition between the two proposed mechanisms depends on both the energetics and the interaction of the system with its environment (the protein).

Rudolph Marcus, Shaul Mukamel, Julian Joseph and William Bialek,[6] and Chi Mak have advanced the theoretical description of virtual electron transfer in condensed phases. The interest in these two mechanisms has stimulated development of the spin-boson model with three electronic states. Detailed analysis suggests that the spectral features of the intermediate state might be observable in the fully virtual process, which means that the sequential and superexchange mechanisms may be difficult to distinguish experimentally.

Other unresolved issues concern the influence of temperature on the ratio of coherent and incoherent transfer and on the expected form of the initial population decay. Much remains to be learned about the interplay between coupling to the environment and the coherent and incoherent processes in three-state systems. Mordechai Bixon, Joshua Jortner and Maibe Michel-Byerle offer an intriguing speculation on why the plant may bother with both mechanisms: They argue that the coexistence of superexchange and sequential mechanisms allows for efficient electron transfer over a broad range



Decay of the excited state of the special pair of the D_{LL} mutant produced by stimulated emission from a probe beam shows that no electron transfer occurs in this mutant. The oscillations result from vibrational wavepackets created by the excitation pulse. Because of the coherent vibrational motion, the probability for interaction with the probe light shows a periodic modulation that depends on the wavelength of the probe light. The Fourier transformation of the oscillatory components reveals frequencies at 15 cm^{-1} and 77 cm^{-1}. (Adapted from ref. 14) **Figure 3**



Trapping dynamics in photosynthesis of a mutant of the photosynthetic purple bacterium *Rhodobacter sphaeroides;* the mutant lacks the peripheral light-harvesting antenna. This process can be studied using low-intensity, single-wavelength picosecond pump-probe measurements. Overlapping absorption bands and relaxation processes in the antenna give rise to complicated dynamics: At 860 nm there is a very small transmission increase followed by a strong absorption increase due to the excited state absorption of the antenna, which decays, roughly monoexponentially, in 50 psec to a state with a residual transmission increase. The 50-psec decay time represents the average time for an excitation to reach the reaction center and initiate electron transfer. This final transmission increase results from a spectral change in the reaction center when the charge-separated state is formed. (Adapted from ref. 17) **Figure 4**

of free-energy gaps.[7] Thus the element of redundancy results in a system that is less susceptible to environmental perturbations.

Computer simulations based on classical mechanics have provided important insights into the functioning of the reaction center. Klaus Schulten, Arieh Warshel, William Parson, David Chandler and their coworkers all have carried out largescale simulations.[8] These calculations show that the response of the protein to the change separation is extremely rapid (50–100 fsec) even at low temperature. Chandler's group has shown that although the free-energy surfaces are harmonic, the protein response does appear to show detectable nonlinear behavior.

Unfortunately, findings related to the energy levels of the relevant states are less clear cut: Warshel, Parson and coworkers find that the states $P^*B_AH_A$ and $P^+B_A^-H_A$ are very similar in energy, whereas Chandler and coworkers find that $P^+B_A^-H_A$ is significantly higher in energy than $P^*B_AH_A$, which would point to the coherent process as the only viable mechanism. Chandler's simulations further imply that electron transfer along the "inactive" branch to H_B is only slightly endothermic, whereas Warshel and Parson find that $P^+B_B^-H_B$ lies significantly above P^*BH, which would allow one to use either the sequential *or* the superexchange mechanism to rationalize the lack of electron transfer along the inactive branch.

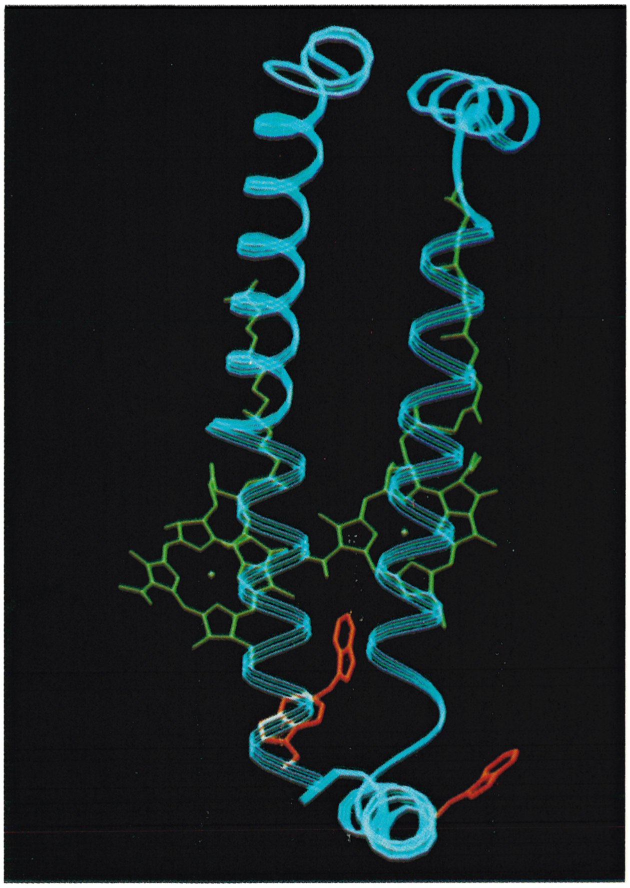
And the experimenters have fared no better at reaching a consensus: Wolfgang Zinth concludes that a two-step mechanism is consistent with his data,[9] while Christine Kirmaier and Dewey Holten find no evidence for an intermediate state and so favor the superexchange process.[10]

Experiments by Boxer and coworkers exploit the sensitivity of electronic absorption bands to external electric fields to probe the effective dielectric constant in the vicinity of the chromophores. Their results support the argument that intermediates such as $P^+B_A^-$ and $P^+H_A^-$ are lower in energy than $P^+B_B^-$ and $P^+H_B^-$. Boxer's group suggests that the dielectric constant is higher along the functional, or active, pathway and argues that this determines the directionality of electron transfer. The effect appears to be a collective one involving many amino acids, so that changing a small number of amino acids would be unlikely to alter the direction of electron flow.

Mutant reaction centers

The methods of genetic engineering, which allow the controlled modification of specific amino acids, make the reaction center a laboratory for studying the protein's role in electron transfer. Douglas Youvan and coworkers swapped an entire transmembrane helix on the active branch with the homologous helix from the inactive branch, which contains 13 different amino acids. The resulting mutant (known as D_{LL}) is missing the pheophytin H_A. In a remarkable demonstration of the electron's reluctance to proceed down the inactive branch, Jean-Louis Martin and Jacques Breton showed that in D_{LL}, the electron refuses to go down *either* branch.[11] The excited state of the special pair simply relaxes back to its ground state, and the quantum yield of electron transfer is zero. This mutant, however, has proved extremely valuable for studies of vibrational coherence in the reaction center, as described below.

Graham Fleming's group (Chi-Kin Chan, Theodore DiMagno and Yi-Wei Jia), in collaboration with James Norris, Deborah Hansen, Marrianne Schiffer and their coworkers, have investigated the role of two particular amino acids, L181 and M208 (figure 1b).[12] In the natural system, M208 (on the active branch) is a tyrosine residue and L181 (on the inactive branch) a phenylalanine. Naively supposing that the tyrosine might control the directionality of the electron transfer, we chose to reverse the locations of the two amino acids. We found that the electron-transfer rate is essentially unchanged by this modification, still proceeding along the active branch. Even more surprisingly, the electron-transfer rate increased slightly when "symmetry" was restored to the reaction center by making both L181 and M208 tyrosine residues—in spite of the fact that this change modified only the "inactive" side. Studies of a set of ten different mutations on these two sites led us to conclude that the amino acids in these positions affect the redox potential



Light harvesting system in a photosynthetic bacterium. The proposed arrangement of the $\alpha\beta$-heterodimer shows two bacteriochlorophyll molecules (green) and the membrane-spanning helices (blue). The tryptophan residues (red) significantly influence the position of the absorption spectrum of the bacteriochlorophyll molecules. (Courtesy of Neil Hunter, Sheffield University, UK.) **Figure 5**

of the special pair—that is, the difference in free energy between P and P^+. The dependence of the electron-transfer rate on the redox potential of the special pair implies that changes in the protein structure as a result of electron transfer (and hence the reorganization energy) are extremely small. Nuclear magnetic resonance studies by Huub de Groot and coworkers also suggest that the reaction center protein is extraordinarily rigid. In fact, the electron-transfer theory of Rudolph Marcus (see PHYSICS TODAY, January 1993, page 20) requires a rigid nonpolar protein if the electron transfer is to proceed at an optimal rate, given the very low free-energy drop between $P^*B_AH_A$ and $P^+B_AH_A^-$. If the protein reorganized substantially as a result of the electron transfer, the transfer would be slow and strongly thermally activated.

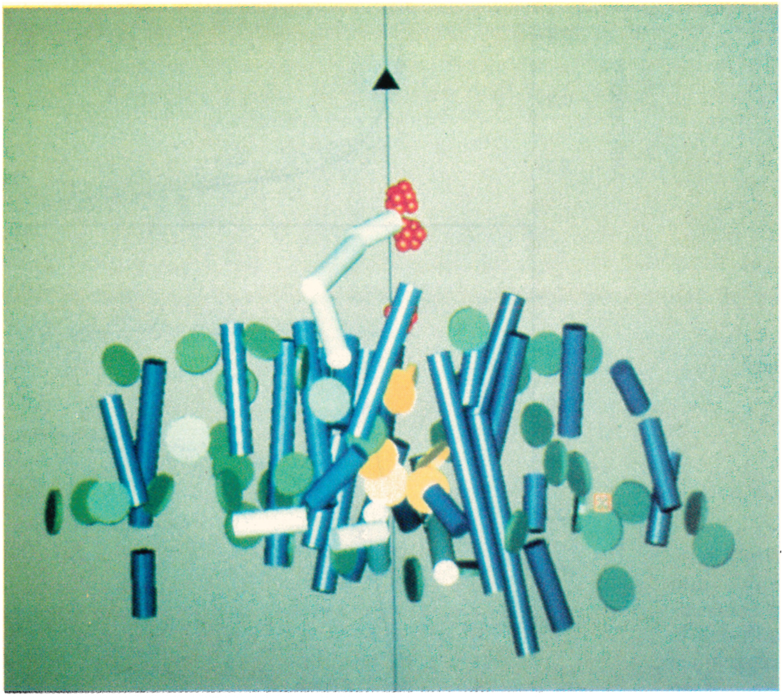
It is now possible for experimenters to generate light pulses that are short compared to the timescales of vibrational motion in molecules. This allows abrupt optical excitation of electronic transitions in which vibrational wavepackets are generated in both ground and excited electronic states. The subsequent motion of the excited state and ground state wavepackets imposes periodic modulation on the transmission of a probe beam, permitting one to probe the nuclear motion in real time. Ahmed Zewail and coworkers have used this technique extensively in the gas phase, and Fleming, Sandford Ruhman and others have used it to study small molecules in solution. It was a considerable surprise, then, when Martin, Breton and their collaborators first reported observing coherent nuclear motion in such a large, multidimensional system as the reaction center.[13] Figure 3 shows their results for the stimulated emission of the special pair in the D_{LL} mutant.

Observation of an oscillatory contribution to the signal calls into question the conventional assumption that vibrational dephasing and relaxation occur on much shorter timescales than does the electron-transfer step. Jose Onuchic and coworkers have suggested[14] that the primary electron transfer occurs in a different regime from the traditional nonadiabatic regime conventionally described by the Golden Rule. (See the article by Fleming and Peter Wolynes, PHYSICS TODAY, May 1990, page 36.) They propose that the electronic mixing between the initial and final states is of the same order of magnitude as the width (due to relaxation and dephasing) of the final states, thus making the electron-transfer rate competitive with relaxation in the final state. They show that in this regime the rate is rather insensitive to changes in the system parameters (free-energy gaps, coupling strengths, relaxation rates or vibrational frequencies).

John Jean, Richard Friesner and Fleming also investigated coherence effects and the influence of slow energy flow between the reaction coordinate and its surroundings. They quantified the breakdown of the Golden Rule that results from slow relaxation and quantum interference effects. A full description of these effects, incorporating a realistic model of the system–bath interaction (for example, at the so-called spin-boson level), is a formidable problem and remains an area of active theoretical development.

Energy transfer

The reaction centers of bacteria and plants are highly optimized devices that leave little room for improvement. However, on their own they would be of limited significance to the plant or bacterium, because the solar energy flux is too low to justify the investment a cell must make to synthesize this complex equipment. In nature, reaction centers are surrounded by a light-harvesting system, generally consisting of chlorophyll and carotenoid molecules complexed to proteins. The light-harvesting system allows the cell to greatly improve the absorption cross section of each reaction center and make optimal use of its energy-converting capacity. The functions of the light-



Transmembrane helices and chlorophyll molecules in photosystem I. The green discs represent the light-harvesting chlorophyll molecules surrounding the electron-transfer components, shown in yellow. Only about half of the total number of chlorophyll molecules have been located to date, which means that the complete structure must be extremely densely packed with antenna chlorophyll molecules. Once an antenna molecule is excited, the probability of the excitation reaching the primary electron donor (a pair of chlorophyll molecules known as P700) is at least 99%. (From ref. 22.) **Figure 6**

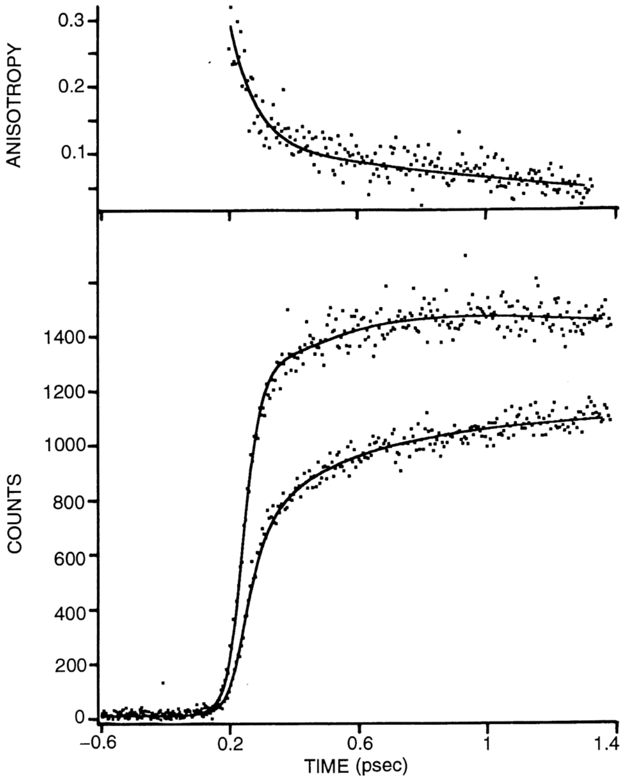
harvesting system are to absorb light over a broad range of wavelengths and to transfer the excitation energy to the special pair of the reaction center. The light-harvesting system typically contains between 24 and 300 (and in some exceptional cases more than 1000) pigments per reaction center. The size of the light harvesting system, often called the antenna, implies that the excitation must visit many molecules before finally being trapped at the reaction center, and thus individual transfer steps must be highly optimized.

Intermolecular energy transfer is generally described by the Förster dipole–dipole resonance mechanism. The rate of energy transfer scales with the inverse of the sixth power of the distance between pigments, along with a factor that accounts for the orientation of the dipoles in space and another factor that measures the overlap between the emission spectrum of the excitation donor and the absorption spectrum of the excitation acceptor. For a pair of chlorophyll *a* molecules separated by a distance of 15 Å (not unlike the values found in nature), the rate of energy transfer may be faster than 10^{12} per second. For these short distances and fast rates, the Förster equation has only limited applicability. The point-dipole approximation is likely to be inadequate and, as in the case of electron transfer, the excitation transfer possibly occurs from a vibrationally unrelaxed state. Additionally, in densely packed chlorophyll protein systems, excitonic interactions between the pigments leads to a distinct splitting of the excited state energies. In this case it is more appropriate to describe the short-time portion of the energy transfer as a relaxation between the different exciton levels of the system. Hole-burning studies have suggested that these times can be 100 fsec or less.[15]

Light trapping

Because the excited state of an individual chlorophyll molecule lives for about 10^{-9} seconds, trapping of the excitation energy must occur in less than 100 picoseconds to produce a quantum yield greater than 90%. Arvi Freiberg and coworkers (using fluorescence techniques) and Villy Sundström, Rienk van Grondelle and coworkers (using absorption methods) have made direct measurements of the excitation trapping time in photosynthetic purple bacterium, which have a relatively simple light-harvesting antenna. These measurements showed that for most of the systems studied, the trapping time is about 50 picoseconds.[16,17] Figure 4 shows a typical example of the kinetics of excitation trapping, measured in a bacterium consisting of only the reaction center surrounded by a ring of antenna pigments (the core antenna) absorbing, like the special pair, near 870–900 nanometers. Others have found the timescale of excitation trapping in other, more complex photosynthetic systems to be rather similar: Fleming's group measured a trapping time of about 30–40 psec for the core antenna of photosystem I; Alfred Holzwarth and coworkers measured an 80-psec trapping time for the core antenna of photosystem II.[18] Based on these findings, along with the known numbers of light-harvesting pigments per reaction center and their spectral distribution and using a theoretical expression derived by Robert Pearlstein, the estimated single-step energy-transfer time in these light-harvesting antennas is about 200 fsec or less. Analyzing the efficiency of biexcitonic annihilation processes following excitation with an intense laser pulse, van Grondelle and coworkers and Leonas Valkunas obtained a similar estimate for the single-step energy-transfer time.[19]

No high-resolution crystal structure is available for the core light-harvesting antenna of the photosynthetic bacterium. However, through a comparison of the amino acid sequences of the polypeptides binding the bacteriochlorophylls, invaluable structural information is now available. Herbert Zuber and coworkers discovered that in all photosynthetic purple bacteria, the light-harvesting chlorophyll molecules are noncovalently bound to a pair of small polypeptides called α and β. Zuber suggested that the $\alpha\beta\mathrm{BChl}_2$ heterodimer is the basic structural element for the bacterium's light-harvesting antenna. Although the precise structure of the unit is not known, biochemical, spectroscopic and electron microscopy data provide a detailed view of the arrangement of the two polypeptides and the bacteriochlorophyll molecules bound



Femtosecond spontaneous fluorescence signals observed from the LHCII antenna system of green plants. Energy transfer between differently oriented chlorophyll molecules leads to depolarization of the fluorescence. The bottom panel shows the parallel (upper) and perpendicular (lower) decay data from which the fluorescence anisotropy (top panel) is constructed. The anisotropy clearly contains both ultrafast (250-fsec) and slower (11-psec) decay components. The 250-fsec decay may represent energy transfer between nearest neighbors, while the longer timescale represents transfer over longer distances. **Figure 7**

to them. Figure 5 depicts the structure of the light-harvesting unit. In vivo, heterodimer units assemble into ring-like aggregates that surround the reaction centers.

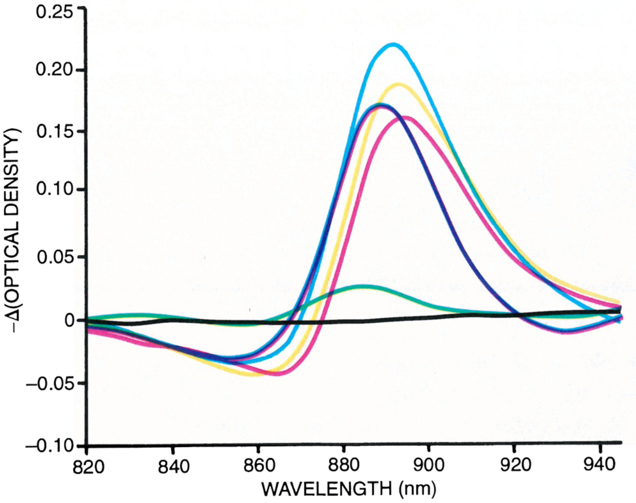
One phenomenon that so far remains unexplained is the dramatic shift in color that the pigments experience upon assembly of the light-harvesting antenna. Free bacteriochlorophyll in an organic solvent absorbs near 770 nm, while the intact light-harvesting antenna may be red-shifted to 870–900 nm. Although excitonic interactions between the quasidegenerate excited states contribute to the red shift, a large part of the shift is not excitonic, but is probably due to specific electronic changes in the immediate environment of the pigments.

Similar concepts apply for many other light-harvesting pigment proteins. As early as 1975 Roger Fenna and Brian Matthews crystallized the BChl *a* complex of a green photosynthetic bacterium,[20] and today we know its structure to a 1.9-Å resolution. The complex consists of a trimer of identical subunits assembled with C_3 (threefold rotational) symmetry; each subunit contains seven BChl *a* molecules arranged in a rather nonsymmetric fashion. The absorption properties of the complex are characteristic of strong excitonic coupling. The BChl *a* molecules within each monomer are excitonically coupled, with interaction energies up to $200\ \mathrm{cm}^{-1}$; the distances between BChl *a* molecules in adjacent subunits are sufficiently short to give rise to excitonic interactions on the order of $20\ \mathrm{cm}^{-1}$. Hole-burning spectroscopy by Small[15] and singlet–triplet spectroscopy by van Mourik[21] have shown that all the absorption bands of the complex are indeed coupled. Small identified at least eight excitonic components and concluded that the excitonic splitting exceeds by far the distribution of aborbance wavelengths of the various sites (inhomogeneous broadening). Moreover, both Small and van Mourik were able to distinguish in the lowest-energy absorption band of the complex, excitonic components parallel and perpendicular to the C_3-axis. On the basis of these results, Small and coworkers have suggested that in this complex, the phonon-induced scattering between various exciton levels is the dominant mechanism for energy transfer.

Structural information is beginning to appear for the photosynthetic proteins of plant systems. Photosystem I of plants and cyanobacteria consists of an antenna system, which contains about 100 chlorophyll molecules combined with the reaction center.[22] (See figure 6.) Horst Witt, Wolfram Saenger and coworkers have located 45 chlorophyll molecules in the 6-Å-resolution structure. Among those is the primary electron donor, a chlorophyll dimer known as P700.

Werner Kühlbrandt has used electron microscopy to obtain the structure of the major light-harvesting complex associated with the oxygen-evolving photosystem II of plants.[23] Known as LHCII, this complex binds about half the chlorophyll on Earth and is composed of trimeric assemblies of monomer units, each containing 14±1 chlorophyll molecules: 8±1 Chl *a*'s and 5±1 Chl *b*'s. Within the monomer the chlorophylls are organized in two layers, and the center-to-center distances between the chlorophylls fall in a narrow range of 9–14 Å. From structural studies it is evident that, as for the green bacterium, excitonic interactions between pigments have a decisive influence on the spectral properties of LHCII.

All the structural information available to date is consistent with ultrafast energy transfer within chlorophyll protein complexes. Recently, advances in laser technology have allowed researchers to monitor directly the early energy-transfer events. By measuring time-dependent fluorescence depolarization, Mei Du, Sunney Xie and Yiwei Jia in Fleming's lab observed the elementary



Transient absorption spectra of the light-harvesting antenna of the photosynthetic bacterium *Rs. Rubrum.* The spectra are recorded at 0 fsec (black), 166 fsec (green), 333 fsec (purple), 500 fsec (blue), 1166 fsec (yellow) and 1833 fsec (red). Following the rise in the first four spectra, resulting from the apparatus response time, the spectra shift to progressively longer wavelengths as the excitation begins to approach a Boltzmann distribution among the excited states. Similar shifts are seen in the stimulated emission (positive optical density change, plotted negative). **Figure 8**

timescale of energy transfer for the photosystem I antenna and the major light-harvesting complex of plants, LHCII.[24] In both cases, depolarization occurs on a timescale of 150–300 fsec. Figure 7 shows some of Du's data: Depolarization of the fluorescence results from excitations jumping from the initially excited chlorophyll molecule to other molecules with different spatial orientations. In the LHCII system further depolarization is observed on a 5-psec timescale, perhaps corresponding to energy transfer between different units of the whole assembly. Similarly, Matthieu Visser, in van Grondelle's lab, has demonstrated recently that equilibration of an initially created distribution of excitations occurs on a timescale of a few hundred femtoseconds: Figure 8 shows the time-resolved difference spectra (after a 200-fsec laser pulse) of the light-harvesting antenna of purple bacteria. Effective localization of excitation on the red-most fraction of the pigments causes the dynamic red shift of the spectrum and the increase in excited-state absorption.

Thus, energy transfer in the complete photosynthetic apparatus spans a range of three orders of magnitude in time, from perhaps a few tens of femtoseconds up to hundreds of picoseconds. The process may initially involve coherent migration, in which the excitation is delocalized over several molecules, while the longer timescales correspond to incoherent hopping from molecule to molecule. As for electron transfer, current theories will need to be extended to describe and interpret that process.

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