PHOTOSYSTEM 0

A POSTULATED PRIMITIVE PHOTOSYSTEM THAT GENERATES ATP IN FLUCTUATING LIGHT

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Summary:

Photosystem 0 (PS0) is a theoretical, simple, dynamic, membrane-based mechanism for free energy gain—in the form of ATP—from fluctuating light. The PS0 reaction centers have no reducing power, as the centers do not transfer charge across the membrane; instead, light induces metastable dipoles within the reaction centers, and a membrane potential is generated by these inner-membrane dipoles. Fluctuating light causes cyclic changes of the dipole potential, and thus of the membrane potential. This permits ATP gain by an ATPsynthase that, when the membrane potential is high, discharges the membrane at a low H^+/ATP ratio, and, when the potential is low, recharges the membrane at a high H^+/ATP ratio. An alternative ATP gain mechanism is possible in flashing light in which (1) after the flash, at high potential, the membrane discharges through ATPsynthase, (2) in the dark the dipole potential decays, and (3) the resulting negative potential dissipates by conduction. PS0 is a plausible progenitor of modern photosynthesis, permitting a model for its evolution. Moreover, PS0 gives new viewpoints on electrochromic shifts, ‘localized’ and ‘delocalized’ protons in membranes, inactive PSII reaction centers, oscillations in chloroplasts, State 1-State 2 transitions and effects of fluctuating light in standard photosynthesis.

Abbreviations and mathematical symbols:

a	:	antenna size: number of Chl molecules per reaction center
A	:	membrane area per reaction center
A_{RC}	:	membrane area covered by one reaction center; note $A_{RC} < A$
BChl	:	bacteriochlorophyll
bc_1	:	proton pumping complex in mitochondria and bacteria
BPh	:	bacteriopheophytin
b_6f	:	proton pumping complex in chloroplasts that corresponds to bc_1 in photosynthetic bacteria
BRC	:	bacterial photosynthetic reaction center
CC	:	central core or core complex of an RC or an BRC
Chl	:	chlorophyll
C_m	:	electrical capacity per unit membrane area
CSP	:	standard photosynthesis potential: the potential caused by charge separation, i.e. charge transfer across the membrane
CF_oF_1	:	CF_oF_1-ATPsynthase, the chloroplast ATP-synthase
cyt	:	cytochrome
d	:	membrane thickness
D	:	Debye, unit of dipole moment: two elementary charges of opposite sign at a distance of 0,1 nm constitute a dipole moment of 4,8 Debye
DCMU	:	3-(3,4-dichlorophenyl)-1,1-dimethylurea: inhibitor of electron transport from Q_A to Q_B
e	:	elementary charge
f	:	electrostatic screening factor
I	:	light intensity
K	:	number of RC excitations per second
LHC, LHCI, LHCII	:	light harvesting complex, resp. light harvesting complex of PSI, resp. of PSII
LH1, LH2, LH3	:	light harvesting complexes occurring in photosynthetic bacteria
m	:	H^+/ATP ratio of the ATPsynthase reaction in a certain mode
MDP	:	membrane dipole potential: the potential across the membrane due to dipoles within the membrane
MTS	:	thermosynthesis based on temperature dependency of the electrical polarization of a membrane
MTS/PS0	:	a system with both MTS and PS0 capabilities
n	:	H^+/ATP ratio of the ATPsynthase reaction in a certain mode; $n < m$
n_e	:	number of charges separated in one reaction center in a flash of light
N	:	number of dipoles or reaction centers per unit membrane area
OEC	:	oxygen evolving complex
pF_1	:	hypothetical progenitor of the F_1 subunit of ATPsynthase; non-membrane bound pF_1 synthesized ATP during thermal cycling.
pRC	:	common ancestor of RCI and RCII
P	:	primary electron donor during photosynthesis
PDP	:	photosynthetic dipole potential
PS	:	‘standard photosynthesis’: photosynthesis based on charge transfer across a membrane
PS0, PSI, PSII	:	photosystem 0 (photosynthesis based on dipole formation within a membrane), resp. photosystem I, photosystem II
P_R, P_{PS0}, P_{PS}, P_{MTS}	:	specific power (power per unit mass) of respiration, PS0, photosynthesis, membrane-associated thermosynthesis
Q	:	quinone \ ubiquinone

Q_A, Q_B	:	quinone A, B
RC, RC0, RCI, RCII	:	reaction center, reaction center of photosystem 0, I, II
$RCII_\alpha$, $RCII_\beta$	:	RCII reaction center with, resp. without a peripheral LHC
t_{cyc}	:	cycle time of thermal cycling or fluctuating light
t_r	:	recombination time, inverse of the recombination rate of a first order reaction; $t_r = 1{,}443\ t_{1/2}$
$t_{1/2}$	:	halftime of a decay process; $t_{1/2} = 0{,}693\ t_r$
V_i	:	equilibrium potential of the ATPsynthase reaction in a mode *i*, i.e. at an H^+/ATP ratio of *i*
x	:	displacement of excited electron within the membrane after charge separation
Z	:	secondary electron donor in PSII
ΔQ	:	charge transported across the membrane per unit area
$\Delta\Psi$	:	membrane voltage, the electrical potential across the membrane
$\Delta\Psi_{CS}$	:	photosynthesis potential, potential due to charge separation across the membrane
$\Delta\Psi_D$	:	dipole potential across the membrane due to molecular dipoles (this dipole potential is normally screened by counterions)
$\Delta\Delta\Psi_D$	:	change in dipole potential across the membrane (not instantaneously screened by counterions)
$\Delta\Psi_{PDP}$	:	photosynthetic dipole potential (= PDP), potential due to dipole formation within the membrane
ε	:	dielectric permittivity
μ	:	magnitude of a dipole: product of separated charges and the separation
$\Delta\mu$	:	change in dipole moment of a pigment upon excitation

I. INTRODUCTION

The evolution of photosynthesis has attracted much interest (Granick, 1957; Gaffron, 1962; Olson, 1970; Broda, 1975; Gest, 1980; Scherer, 1983; Olson and Pierson, 1987a, 1987b; VanGorkom, 1987; Pierson and Olson, 1989; Awramik, 1992; Blankenship, 1992; Boxer, 1992; Hartman, 1992; Mauzerall, 1992; Larkum, 1992). Until now, models of the first photosynthesizers have consisted of a biomembrane that contains pigments which upon excitation transfer charge across the membrane (Ilani *et al*., 1989). Thus substrates are reduced and the membrane potential, $\Delta\Psi$, rises. The latter effect can, however, also result from the formation of a large number of dipoles within the membrane, since a potential difference is present across a layer of dipoles (Fig. 1) (Jackson, 1975; Schuhmann, 1990).

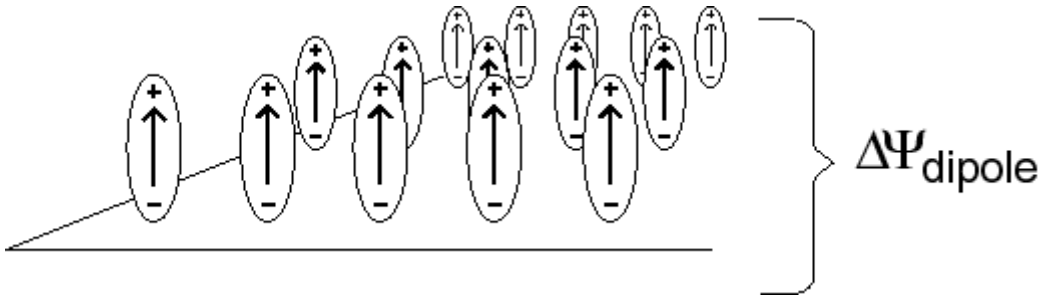

Fig. 1. The dipole potential: across a layer of dipoles a potential difference is present.

This study presents a theoretical mechanism for the first photosynthesizers in which a voltage arises by such a dipole potential, caused by temporary charge displacement *within* the membrane (Fig. 2). In contrast with previous models for the first photosynthesizers, the proposed mechanism only yields ATP, and reducing power is absent. Reduction of intramembrane charge carriers in the light is temporary, and is followed by their reoxidation in the dark.

In chloroplasts, for example, the pertinent dipoles could consist of reaction centers containing oxidized pigments, and reduced quinones that would remain bound. In the dark the quinones are reoxidized by return of the electron to the oxidized pigment. 'Inactive' PSII reaction centers, in which the quinone Q_A cannot reduce the quinone Q_B (Lavergne and Leci, 1993), can function as the light-induced dipoles described above. The contribution of these dipoles to the $\Delta\Psi$ across the membrane has been experimentally demonstrated (Chylla and Whitmarsh, 1989, 1990).

The proposed mechanism uses fluctuating light. The dipole potential formed upon illumination is called the 'photosynthetic dipole potential' (PDP or $\Delta\Psi_{PDP}$). The PDP is proportional to the dipole density in the membrane. When the density is high enough, the PDP permits ATP synthesis by an ATPsynthase, presumably active in a low H^+/ATP ratio, or, a 'low mode'. In the dark, the dipoles decay and $\Delta\Psi_{PDP}$ and $\Delta\Psi$ decrease. The protons that have crossed the membrane through the ATPsynthase either return by conduction—a process especially suited during illumination by flashes, because of the long dark period—or the ATPsynthase recharges the membrane at a high H^+/ATP ratio, or, a 'high mode', which costs less ATP than was obtained during the preceding discharge. A dark-light-dark cycle thus results in a net gain of ATP.

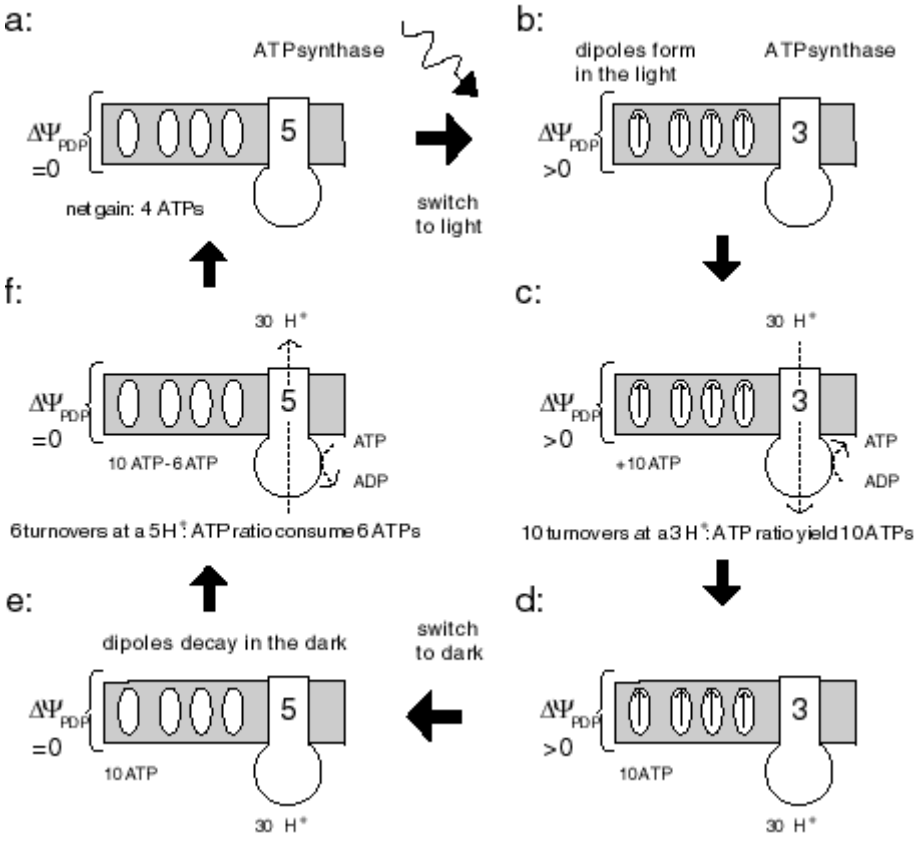

Fig. 2. Basic principle of the Photosystem 0 (PS0) mechanism. A membrane contains reaction centers and an ATPsynthase that can charge and discharge the membrane at different H^+/ATP stoichiometries or modes, here 3 and 5 H^+/ATP. Upon illumination (a→b) electric dipoles are formed in the PS0 reaction centers. The dipoles result in a dipole potential of the membrane (c), which in turn increases the potential across the membrane. The ATPsynthase discharges the membrane in 10 turnovers, transporting 30 protons, yielding, in mode 3, 10 ATP molecules (d). After the light-dark switch the electric dipoles decay again (d→e), causing the dipole potential to vanish and the membrane potential to drop (e). The ATPsynthase charges the membrane, but now at a high H^+/ATP mode of 5. In 6 turnovers, 30 protons are transported, at a cost of 6 ATP molecules (f). At the end of the light-dark cycle (a), a net profit has been made of 10 – 6 = 4 ATP molecules.

Hereafter it will be shown that $\Delta\Psi_{PDP}$ equals $\mu/(\varepsilon A)$, where μ equals the dipole magnitude (the product of distance, *l*, and separated charge, $n_e e$ (number of electrons times elementary charge)), ε, the dielectric constant and A, the membrane area per reaction center (RC; bacterial RC: BRC). The particles in the thylakoid membrane that are visible under the electron microscope and that are associated with Photosystem II, consist of RCII dimers (Boekema *et al*., 1994; Santini *et al*., 1994); from a particle density of one per 900 nm^2 (Staehelin, 1986), a surface area per RCII monomer of 450 nm^2 follows. With two electrons as separated charge, a $\Delta\Psi_{PDP}$ of 80 mV is calculated[1], which is larger than 63 mV, the difference of the equilibrium membrane potentials associated with ATPsynthase mode 3, 157 mV (470:3), and mode 5, 94 mV (470:5). These values thus indeed permit ATP gain in the manner depicted in Fig. 2.

[1] Assume l 4 nm, ε_r 4. Then $\Delta\Psi_{PDP} = 2 \times 1{,}6\ 10^{-19} \times 4\ 10^{-9} / 450\ 10^{-18} / 4 / 8{,}854\ 10^{-12} = 0{,}080$ V.

A variable stoichiometry and a variable $\Delta\mu_{H^+}/\Delta G_P$ ratio—where $\Delta\mu_{H^+}$ equals the electrochemical proton gradient across the membrane and ΔG_P the phosphorylation potential—of ATPsynthase have often been reported (Wikström and Krab, 1980; Ferguson and Sorgato, 1982; Westerhoff *et al*., 1984; Haraux, 1985; VanWalraven *et al*., 1990). Mode changes were applied in a theoretical mechanism for a biological heat engine based on thermal cycling that was named membrane-associated thermosynthesis (MTS). In MTS the orientation of molecular dipoles within the biomembrane changes during a thermotropic phase transition (Muller, 1993, 1994). The essence of MTS is (1) reversal of a high-mode process, resulting in charging of the membrane, (2) increase of the membrane potential due to a dipole potential change, $\Delta\Delta\Psi_D$, caused by a temperature change, (3) discharge by a low-mode process, which closes the cycle. Just as in Fig. 2, the mode of ATPsynthase switches between two values. Other schemes for alternating ATPase/ATPsynthase activities also permit free energy gain from cyclic membrane potentials (Chapter 2).

The photosynthetic mechanism presented is named ‘Photosystem 0’ (PS0). PS0 can be applied in a stepwise model for the early evolution of photosynthesis. Photosynthesis would gradually evolve from thermosynthesis by increasing PS0 activity in MTS/PS0 systems, systems having both thermosynthesis and PS0 capabilities. These systems would gain energy from the combined thermal and light-intensity cycling occurring during circulation in a convection cell (Fig. 3). At first, acquisition of PS0 ability may have given only a slight advantage to an MTS system, as both the halftimes and the magnitude of the metastable light-induced dipoles were small. Next, PS0 would evolve stepwise—by stepwise addition of intermembrane charge carriers—towards standard photosynthesis, in which charge is transported across the membrane, and charging by ATPsynthase in the backwards mode or by conduction is superfluous. At that point photosynthesis has obtained reducing power

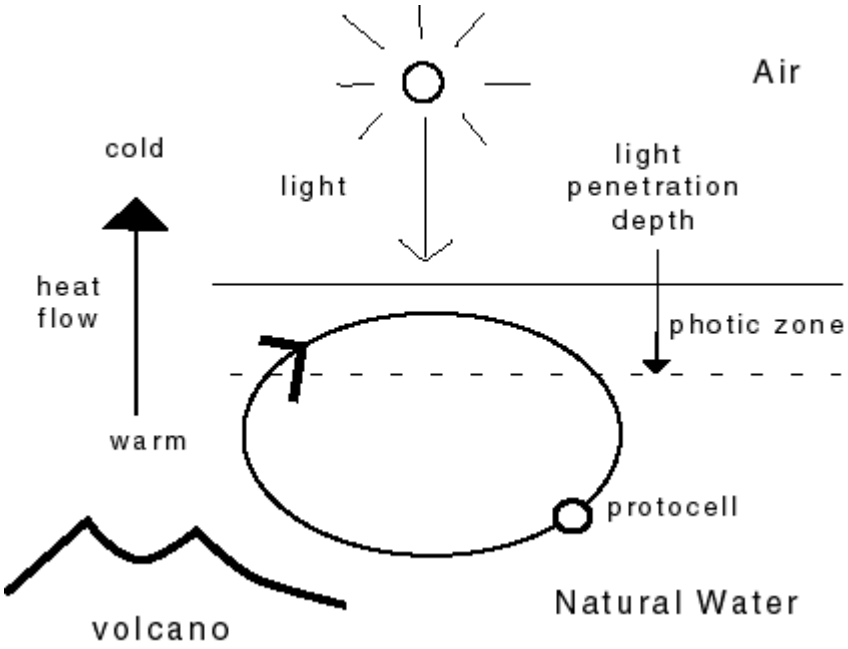

Fig. 3. A primordial cell is thermally cycled while being carried along by a convection current. The cell is also light cycled, as it moves in and out of the photic zone. Combined thermal/light cycling makes combined thermosynthesis/photosystem 0 activity (MTS/PS0) possible.

PS0 is a biological analog of a proposed macroscopic device (Glazebrook and Thomas, 1982) for the direct conversion of fluctuating light into electrical energy. Its basic principle is the photodielectric effect, the ability of light to change the electrical polarization of a dielectric. A capacitor containing a slab of the dielectric is charged by a battery. In the light the displaced electron moves downhill with respect to the electric field between the plates, increasing the polarization (Fig. 4A). The energy gain corresponding to the potential increase following the polarization decrease caused by the light-dark switch, is directly passed on to a load. The polarization decrease in the dark is due to the decay of the polarization caused by light-induced metastable excited states.

Placing the light sensitive dielectric on top of a suitably oriented electret, a material with a constant electrical polarization (Sessler, 1980; Axe and Lines, 1991; for electrets consisting of biomaterials, see Mascarenhas, 1980) yields a system in which the potential increases upon illumination (Fig. 4B). In the light the displaced electron moves downhill with respect to the electric field of the electret, but uphill with respect to the electric field due to the charges on the plates.

Devices similar to Fig. 4A have been described in which free energy is gained from thermal cycling (Fig. 4C) (Margosian, 1965; Gonzalo, 1976; Pulvari and Garcia, 1978), one way to effect thermal cycling being cyclic illumination with visible or infra red light (Hadni, 1981). The electrical polarization of the dielectric in these systems depends on the temperature, a phenomenon called pyroelectricity (Hadni, 1981; Abrahams, 1991). MTS is a biological analog of these devices. Placing the light-sensitive dielectric on top of a pyroelectric (Fig. 4D) yields a system that gains energy from both thermal cycling and fluctuating light. At constant temperature the pyroelectric in such a combined system can function as the electret in Fig. 4B. Obviously, a combined MTS/PS0 system is a biological analog.

Conceptually, PS0 is obtained by replacing the capacitor with a biomembrane, the battery/load with an ATPsynthase working at a variable stoichiometry, and the light-sensitive dielectric with a biomembrane containing photosynthetic RCs; the electret can be replaced by molecular dipoles, or by the surface dipoles in one of the two monolayers of an asymmetric biomembrane (Muller, 1993).

Does PS0 occur in modern photosynthesizers? Could modern photosynthesizers yield the parts for a PS0 system? The photosynthesis literature is reviewed with these questions in mind. In Chapter 2 the *in situ* occurrence of fluctuating light, light-induced dipoles, the potential formed by a layer of light-induced dipoles, the required ATPsynthase, the operating cycle, and the power of PS0 are considered. Chapter 3 applies the PS0 concept in a model for the evolution of photosynthesis, in the order *thermosystem→PS0→bacterial photosynthesis*. Chapter 4 considers the ramifications of PS0 for chloroplasts, showing that (1) only a few small changes suffice to make chloroplasts function as a PS0, (2) the peculiar properties of ATPsynthase are consistent with PS0, (3) chloroplast oscillations resemble PS0 oscillations, and (4) several phenomena such as State 1-State 2 transitions and temperature dependency of charge transfer reactions may be vestiges from MTS/PS0.

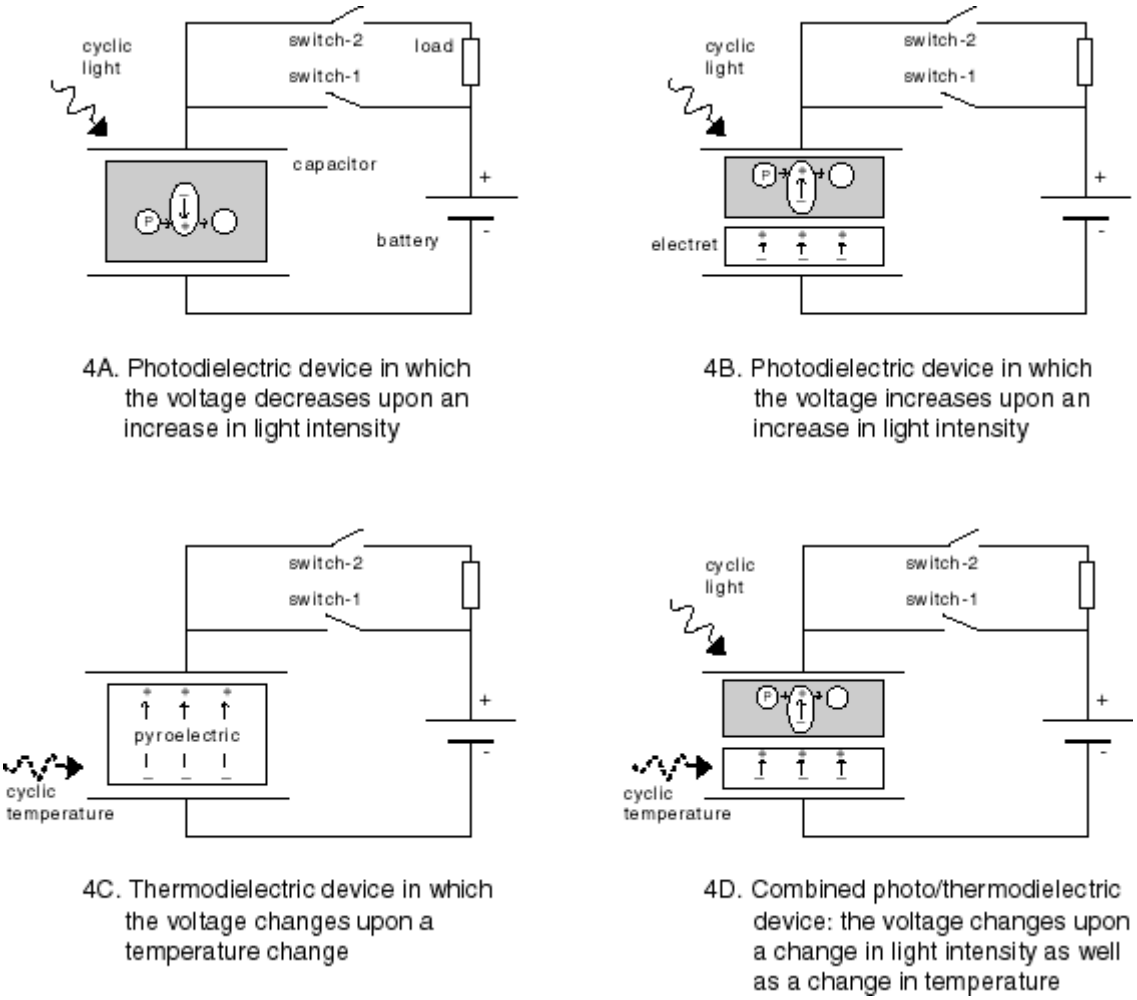

Fig. 4. The photodielectric energy converter (see Glazebrook and Thomas, 1982). The converter consist of a battery, a capacitor of which the capacitance varies, two switches, and a load, which takes up the gained free energy.
In *A*, at the start of the operating cycle, the dielectric in the capacitor is in the dark and switch-1 is closed. Upon illumination excited states formed in the photodielectric increase its polarization. The voltage across the capacitor decreases, and the battery charges the capacitor. Before the end of illumination switch-1 is opened. In the dark the polarization decays, the capacity decreases, and the potential across the capacitor increases. Closing switch-2 then causes a current to flow through the load, which takes up electrical work. Upon opening switch-2 and closing switch-1 the system returns to the start of the cycle.

In *A* the voltage across the capacitor decreases upon illumination. *B* shows a device in which the voltage increases upon illumination: the light-sensitive dielectric is placed on top of a dielectric with a permanent dipole that is antiparallel to the electric field between the plates. By using a dielectric with a temperature dependent polarization (a pyroelectric) a thermodielectric energy converter is obtained (*C*) (Pulvari and Garcia, 1978). A combined system that can gain energy from both temperature and light intensity changes is obtained by combining a pyroelectric with a light-sensitive dielectric (*D*).

II. PHOTOSYSTEM 0: THE MECHANISM

1. *Fluctuating Light*

At constant light intensity, the dipole potential due to membrane-embedded light-induced dipoles will eventually be screened by counterions that have leaked across the membrane. In order to gain free energy by means of such dipoles fluctuating light is required. Many of the studies on the effect of fluctuating light upon photosynthesis (Pollard, 1970; Kriedemann *et al*., 1973; Lakso and Barnes, 1978; Quéguiner and Legendre, 1986; Walsh and Legendre, 1983, 1988; Knapp and Smith, 1990; Pearcy, 1990) discuss its *in vivo* occurrence. Fluctuating light can have many causes.

A fluctuating light intensity will occur in the biomembranes of organisms in convecting hot springs (estimated t_{cyc} ~10-100 s (Muller, 1985)) or in turbulent natural waters, or in biomembranes circulated by the cytoplasm stream (estimated t_{cyc} ~10 s (Muller, 1993)) in the light gradient within the leaf cell (Terashima and Saeki, 1985; Melis *et al*., 1987: Fig. 3; Vogelmann, 1989; Evans *et al*., 1993). Within natural waters, surface waves cause fluctuating irradiation by variable focusing and defocusing of the incoming light (Schenck, 1957; Dera and Olszewski, 1967; Dera, 1970; Snyder and Dera, 1970; Weinberg, 1976). The oscillations have the same magnitude as the mean irradiance (Snyder and Dera, 1970). Just below the sea surface, the main power of the spectrum of the fluctuations has cycle times (t_{cyc}) between 0,1 and 10 s (Quéguiner and Legendre, 1986; Walsh and Legendre, 1983, 1988).

On land, fluctuating irradiation is caused by variable shading or movement within a light gradient. The cycle-time range of these fluctuations is ~0,1-100 s (Pollard, 1970; Desjardins *et al*., 1973; Kriedemann *et al*., 1973). Below canopies, much CO_2 is assimilated during fluctuating irradiation, caused by shading by clouds, variable selfshading during plant movement by the wind, and sunflecks moving because of the earth's rotation (Chazdon, 1988; Pearcy, 1990). In a soybean canopy, sunflecks contribute 40-90% of the daily photon flux density; about one third of these sunflecks lasts less than 10 s (Pearcy, 1990). Similarly, in understories the sunfleck contribution can be 20-80% (Pearcy, 1990). Vibrations of leaves can result in light fluctuations with cycle times between 33 to 200 ms. Membranes within plants such as the aspen, in which even a small breeze causes extensive leaf quaking, must in particular be subject to strongly fluctuating irradiation (Pollard, 1970).

In a fungus cell, strong fluctuations in the local—on the scale of microns—light intensity due to variable shading by particles carried along by the cytoplasm stream (Dennison and Vogelmann, 1989) have been observed. On the shade side of chloroplasts self shading may occur, and rotation diffusion (Atkins, 1986) or rotation due to shearing forces caused by the cytoplasm stream (Hayama *et al*., 1979) then must cause fluctuating illumination of thylakoids. All these causes of fluctuations add up. Much *in vivo* chlorophyll will be subjected to strongly fluctuating light.

In photosynthesis research, intermittent light is a standard tool (Rabinowitch, 1956), used to investigate the efficiency of photosynthesis (Warburg, 1919, 1920), to demonstrate the existence of RCs (Emerson and Arnold, 1932b), and to distinguish between light and dark processes (Witt, 1960). It is advantageous for PS0, just as for the photodielectric converter (Glazebrook and Thomas, 1982), when the light arrives as flashes, the long dark intervals between the flashes giving the metastable states time to decay. A similarly enhanced efficiency of photosynthesis in flashing light has been observed (Warburg, 1919, 1920; Rabinowitch, 1956; Emerson and Arnold, 1932a). Flash-induced membrane potential increases are commonly observed in the laboratory, and are able to drive ATP synthesis (Nishimura, 1962a, 1962b; Witt, 1979; Petty and Jackson, 1979a, 1979b, 1979c).

2. *Formation of Metastable Dipoles*

The photosynthetic dipole potential is a good quantitative parameter for PS0. The PDP depends on the magnitude and the lifetime of dipoles formed in RCs, the number of electrons displaced per RC, and the local dielectric constant.

(a) *Light-induced RC dipoles and their metastability*
During photosynthesis light induces dipoles in RCs. In PS0, these dipoles must decay again, preferably in the dark. Decay during the light period is acceptable, as long as reexcitation causes the time-averaged dipole moment to remain significant. The required dipole metastability is indeed commonly observed in RCs. It is associated with prompt and delayed fluorescence, luminescence, thermoluminescence, phosphorescence and states with temporary local charge separations (Jursinic, 1986; Sane and Rutherford, 1986; Bilger and Schreiber, 1990). Metastability can be functional in standard photosynthesis as well, protecting against a high light intensity.

The largest dipoles will be formed in states with reduced acceptors at one side of the membrane, and oxidized donors at the other side; many different quinone acceptors can be present ('acceptor heterogeneity' (Hansson and Wydrzynski, 1990)). As yet, not all charge carriers of metastable states have been identified (Johnson *et al*., 1994). Decay times vary from several minutes—such as in luminescence—to milliseconds, and even much smaller values during prompt fluorescence. For the purpose of the present study metastable states with decay times between 1 ms and 10 s are of interest, because *in situ* fluctuating light has comparable cycle times.

Assuming an extinction coefficient of 5 mM^{-1} cm^{-1} (obtained by averaging over all wavelengths), the absorbance cross section of one Chl molecule is 2 10^{-17} cm^2 (Clayton, 1980) or 1150 /N_A m^2, where N_A is Avogadro's number[2]. A photon irradiance of 1000 μmol m^{-2} s^{-1} results in an average 1,15 photons being absorbed by one Chl molecule per second, each photon resulting in one exciton. The *in situ* light intensity varies widely. At low light intensity, the exciton production is proportionally less.

The antenna, which may contain as many as 600 Chl molecules (Melis, 1991: Table 2) funnels the excitons into the RC, which therefore can be excited ~700 times a second at a light intensity of 1000 μmol m^{-2} s^{-1}. Again, at a smaller antenna size or a smaller light intensity the RC excitation rate must be proportionally less.

Which RC decay time is significant for PS0 depends on the RC reexcitation rate. Provided decayed RC states are promptly reexcited, states with decay times as short as 1 ms can contribute, although this would require a high light intensity during the light period and a large antenna. Short decay times have the advantage of a small chance of significant reexcitation when the dark period is not completely dark. Contribution to PS0 of states with long decay times requires, on the contrary, a low light intensity during the dark period, and a small antenna. The light-induced dipole state should decay before the end of the dark period, and should not be reexcited in the dark, even not by very little light. During the light period a small light intensity would suffice.

The legend of Table 1 gives data on light intensities, and on antennas and decay times of RCs of several organisms.

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[2] It follows from Beer's law that the decrease in light intensity $I_o \rightarrow I_x$ of a beam due to absorbance by a dissolved pigment can be described by: $I_x = I_o\ 10^{-\varepsilon c x} = I_o\ e^{-2{,}303\,\varepsilon c x}$, where c is the concentration (M), x the distance of the beam in the medium (cm) and ε the extinction coefficient, here 5 mM^{-1} cm^{-1} = 5000 M^{-1} cm^{-1}. Each molecule has a cross section σ cm^2 for photon absorption. Since the number of molecules per cubic cm equals (C/1000) N_A, Beer's law can also be written as: $I_x = I_o\ e^{-\sigma c N_A x / 1000}$. It follows that $\sigma = 2{,}303\ \varepsilon\ 1000/\ N_A = 1{,}92\ 10^{-17}\ cm^2 = 1{,}92\ 10^{-21}\ m^2$. Irradiation with 1 mol m^{-2} s^{-1} then results in $1{,}92\ 10^{-21} \times N_A = 1150$ absorptions per second (see also Clayton, 1980).

Table 1. Light intensity thresholds [μmol m^{-2} s^{-1}], estimated from Eqn 1, for stabilization of a semi-quinone by a following excited electron as a function of the Chl/RC ratio and the decay time of the semi-quinone. '>' indicates a threshold larger than 3000 μmol m^{-2} s^{-1}.

decay time [ms]	**N: antenna size:[Chl/RC ratio]**						
	1	**6**	**30**	**60**	**100**	**300**	**600**
2	>	>	>	>	>	1400	720
7	>	>	>	2100	1200	410	210
20	>	>	1400	720	430	140	72
70	>	2100	410	210	120	41	21
200	>	720	140	72	43	14	7
700	1200	210	41	21	12	4	2

Background light levels Chazdon (1988) gives much numerical data on sunflecks. Sunfleck threshold levels are difficult to define; depending on the circumstances the range appears to be 5-150 μmol m^{-2} s^{-1}. These sunfleck thresholds and the values of the table should be compared with *in situ* continuous background light levels, which cover a large range. Directly in the sun, the level can be ~2000 in the tropics and ~1800 μmol m^{-2} s^{-1} at temperate latitudes (Hart, 1988). Under an overcast sky the rate can be reduced by a factor 40, yielding ~50 μmol m^{-2} s^{-1} (Hart, 1988). The author estimates that shading by leaves may reduce it by a factor of ~5 (Melis *et al*., 1987: Fig. 3), yielding ~10 μmol m^{-2} s^{-1}, shading within a leaf may further reduce it by another factor ~5, ~2 μmol m^{-2} s^{-1}. Pearcy (1990) gives a range of 15-50 μmol m^{-2} s^{-1} for forest understories, and a level up to 200 μmol m^{-2} s^{-1} for crop canopies. Plants growing under < 100 μmol m^{-2} s^{-1} are said to grow under low solar radiation, and obligate shade species live under < 20 μmol m^{-2} s^{-1} (Melis, 1991). An understory plant has been observed with a maximal photosynthetic rate at 4,1 μmol m^{-2} s^{-1}, and the very low compensation point of 1,7 μmol m^{-2} s^{-1} (Pearcy and Calkin, 1983). Many cyanobacteria grow best at 50-60 μmol m^{-2} s^{-1} (Wyman and Fay, 1987). Under polar sea-ice the light intensity may be 0,05 μmol m^{-2} s^{-1}, the lowest recorded compensation point value being 0,22 μmol m^{-2} s^{-1} (Andersen, 1989). The compensation values of algae within ice are 1-10 μmol m^{-2} s^{-1}. A lower light intensity limit for photosynthesis of 0,4 μmol m^{-2} s^{-1} has been estimated from the proton leakage rate across the thylakoid membrane (Raven and Beardall, 1982). As a comparison, the intensity of moonlight is 0,005 μmol m^{-2} s^{-1} (Hart, 1988).

Antenna size The antenna size *N* varies widely. Obviously, the minimal possible value is 1. The central core, the 'CC', of the BRC of photosynthetic bacteria contains 4 BChl and 2 bacteriopheophytins (BPh) (Straley *et al*., 1973; Clayton, 1973; VanderRest and Gingras, 1974; Thornber *et al*., 1980), i.e. has an *N* of 6, a value also proposed for a common progenitor of the RC of purple and green bacteria (Nitschke and Rutherford, 1991). A functional *Rb. capsulatus* mutant with this small antenna has been observed (Dörge *et al*., 1990). Other reported ranges for bacteria are 50-100 (Clayton, 1980) and 25-250 (Zuber, 1985); for chlorosomes of green bacteria, a value of 1000 (Zuber, 1985). For *Rb. sphaeroides* a value of 89 was reported (Packham *et al*., 1978). Purple bacteria can contain three types of LHCs: some contain only a proximal LH1 (B870 in *Rb. capsulatus*, B880 in *Rp. rubrum*, or B1015 in *Rp. viridis*): CC + LH1 ~150 Chl, some also contain a distal LH2 (B800-850 in *Rb. capsulatus*): ~55 BChl, and, lastly, some contain an LH3 (Glazer and Melis, 1987; Hunter *et al*., 1989).

The center complex of the RC of higher organisms consists of a D1-D2-cyt b_{559} complex (Nanba and Satoh, 1987) which contains 6 Chl *a* and 2 phaeophytins

(Kobayashi *et al*., 1990; Gounaris *et al*., 1990). Addition of the CP43 and CP47 proteins, the 'core antennas' or 'proximal antennas', yields a system with 40-50 Chl *a*, called the 'core complex', 'central core', 'PSII-core' or '$PSII_\beta$' (Thornber, 1986; Glazer and Melis, 1987; Bassi *et al*., 1990; Hansson and Wydrzynski, 1990; Melis, 1991). A *Synechococcus* PSII with an antenna of 31 has been observed (Dekker *et al*., 1988). In intermittent light (1-120 min LD) a barley mutant yields antennas of 37 Chl in PSII and 95 Chl in PSI (Glick and Melis, 1988). The core complex is surrounded by one or more rings of LHCs, which results in antennas of 200-300 (Zuber, 1985), 100-600 (Melis, 1991), and 600-800 (Mauzerall and Greenbaum, 1989). A complete PSII, a '$PSII_\alpha$', contains ~230-250 Chl (*a*+*b*) or more (Ghirardi and Melis, 1984; Melis, 1991). After removal of 'peripheral LHCII', ~120 Chl (*a*+*b*), a core remains, $PSII_\beta$, with ~130 Chl (*a*+*b*) (Melis, 1991). Further division yields again the Central Core, ~50 Chl *a*, and 'intrinsic LHCII', ~80 Chl (*a*+*b*) (Glazer and Melis, 1987; Hansson and Wydrzynski, 1990; Melis, 1991).

Similarly, several sizes are found for PSI cores: from ~60, ~90 till ~130 Chl *a*. LHCI contains ~70 Chl (*a*+*b*) (Glazer and Melis, 1987). For PSI itself an antenna of ~200 has been reported (Ghirardi and Melis, 1984; Glazer and Melis, 1987). Other values given are CPI: 90 Chl *a*, LHCI-730: 88 Chl (*a*+*b*), LHCI-680: 32 Chl (*a*+*b*), LHCII (RCI bound in State 2): 93 Chl (*a*+*b*) (Bassi *et al*., 1990).

RC decay times The literature on RC decay times is extensive and the range of observed RC decay times wide. In BRCs, primary charge separation has a $t_{½}$ of ~70 ms (Baccarini-Melandri *et al*., 1981). During the decay of $(BChl)_2{}^+Q_A{}^-$ and $(BChl)_2{}^+Q_B{}^-$ in Langmuir-Blodgett monolayers of BRCs the slow fraction has a decay time of 0,1-2 s, the fast fraction a decay time of 2-10 ms (Alegria and Dutton, 1991). In *Rb. sphaeroides* strain R-26, $t_{½}$ is ~80 ms in the absence of Q_B (Blankenship and Parson, 1979), but in its presence the decay is slower: $t_{½}$ ~0,8-1 s (Blankenship and Parson, 1979). The simplest explanation for biphasic decay is partial occupation of the Q_B binding sites (Blankenship and Parson, 1979), other explanations are protonation of the quinone (Crofts and Wraight, 1983) or the existence of several RC conformational states (Baciou and Sebban, 1992). In *Rb. sphaeroides* 50-90% of the electrochromic signal decays with a $t_{½}$ of ~2 s (Jackson *et al*., 1975; Clayton, 1980). For purple bacteria Gunner (1991) gives 33 ms for the recombination time t_r —defined as the inverse of the recombination rate—of $(BChl)_2{}^+Q_A{}^-$ in the absence of Q_B. In mutants the t_r of $D^+Q_B{}^- \rightarrow DQ_B$, where D is the electron donor to $(BChl)_2$, ranged between 120 ms and 12 s (Okamura and Feher, 1992; Fig. 8).

In *Rp. viridis* decays are faster. Quinone protonation diminishes the decay rate: the $t_{½}$ of $P^+Q_A{}^- \rightarrow PQ_A$ is ~1 ms, but the $t_{½}$ of $P^+Q_A(H) \rightarrow PQ_A$ is ~100 ms (Crofts and Wraight, 1983). For positive charges stored in the high-potential cytochromes in the stalk the decay time of $Q_A{}^-$ covers the range 0,1-10 s in the absence of Q_B (Gao *et al*., 1990). In chromatophores and proteoliposomes the two occurring BRC conformations have $P^+Q_A{}^-$ t_r values of 0,6 ms (~50% of all BRCs) and 2,2 ms (other ~50%) and $P^+Q_B{}^-$ t_r values of 120 ms (~35%) and 480 ms (~65%) (Baciou *et al*., 1990; Sebban *et al*., 1991; Gao *et al*., 1991).

In higher organisms, heterogeneity of RCs is indicated by variable fluorescence and variable electron transport from Q_A to Q_B, in particular its absence in the inactive RCII fraction. Decay of inactive RCII follows the $S_2Q_A{}^- \rightarrow S_1Q_A$ scheme and takes a few seconds (Lavergne and Leci, 1993). Both the BRC of photosynthetic bacteria and the RCII of chloroplasts have fast and slow decaying states.

In inactive PSII or RCII, i.e. Q_B non-reducing photosystems (Chylla *et al*., 1987; Chylla and Whitmarsh, 1989, 1990; Ort and Whitmarsh, 1990; Guenther *et al*., 1990; Krause and Weis, 1991; Melis, 1991; Lavergne and Leci, 1993) a dipole is constituted by a negative reduced Q_A and a positive S_2 state in the oxygen evolving complex (OEC). The inactive PSII fraction can be 75-80% in dark-adapted green algae, 20-35% in other photosynthetic membranes (Guenther *et al*., 1990), and 10-25% in higher organisms (Lavergne and Leci, 1993). Inactive PSII, with an antenna size of 110 (Chylla and Whitmarsh, 1990), may overlap with $PSII_\beta$ (Melis, 1991), which has a smaller antenna (Melis, 1985). In inactive PSIIs the electrochromic shift at 518 nm, and the fluorescence, decay slowly: $t_{½}$ ~1,7 s (Chylla and Whitmarsh, 1989; Lavergne and Leci, 1993). The function of inactive PSII is still controversial (Demmig-Adams and Adams, 1992). Since it is electrogenic (Chylla and Whitmarsh, 1989), inactive PSII may be PS0 active.

(b) *Distance traversed within the RC by the excited or separated electron, x.*
Two types of light-generated dipoles are of interest for the present purpose: (1) a single molecule that upon excitation has a different dipole moment (Middendorf *et al*., 1993) and/or has 'charge-transfer character' (Friesner and Won, 1989), and (2) the dipole arising from electron transfer from one molecule to another. Electrochromic absorbance shifts constitute quantitative evidence of dipole changes, $\Delta\mu$, of excited states of photosynthetic pigments. The shifts can be explained by the mixing of states with a charge-transfer character with the excited state (Friesner and Won, 1989). An enhanced polarizability of the excited state, in combination with an electrostatic field due to the local environment, would also cause a change of the dipole moment (Middendorf *et al*., 1993).

During the charge separation that can follow excitation, the dipole moment μ is defined as the product of separated charge and distance, its unit being the Debye (D); 1 Debye is equivalent to the dipole of a positive and negative elementary charge at a distance of 1/48 nm (Cramer and Knaff, 1991). Since most large molecules already have a dipole moment in the ground state, the change in dipole moment upon excitation, $\Delta\mu$, is often the significant quantity. Electrochromic absorbance shifts yield dipoles as $\Delta\mu / f$, where f is an electrostatic screening factor (Lockhart and Boxer, 1987), commonly assumed to equal ~1,2-1,4 (Gottfried *et al*., 1991c). In order to allow comparison with dipoles due to displaced electrons, reported $\Delta\mu$ values in Debye units are converted to equivalent charge displacements in nm by division by 48 and 1,3, the assumed screening factor.

Stark effect studies show large increases of μ by Chl dimers upon excitation. Whereas the Chl monomer has a $\Delta\mu$ of only 1,0 D (~0,02 nm), the excited state of $(Chl.ethanol)_2$ in ethanol has a $\Delta\mu$ of 5,2 D (~0,08 nm) (Krawczyk, 1991). A BChl dimer is involved in the excited state of the BRC of *Rhodobacter sphaeroides*, which has a $\Delta\mu$ of 8,6 D (~0,14 nm) (Lockhart and Boxer, 1987). A recent study (Middendorf *et al*., 1993) gives the somewhat lower value of ~4,5 D/ f (~0,07 nm); the lower value results from invoking the effect of enhanced polarizability of the excited state.

Within RCs the excited electron can separate from the pigment, and can next jump along several stepping stones to the reducing side of the membrane. The remaining vacancy can similarly move along one or more stepping stones to the oxidizing side. Thus the distance between the vacancy and the electron, and the length of the associated dipole, increases until it equals the membrane thickness. For the chromatophore membrane a thickness of 4-5 nm has been observed (Pape *et al*., 1974) and a value of 3,0 nm assumed (Packham *et al*., 1978). For the thylakoid a value of 4 nm has been reported (Mühlethaler, 1966; Kirk, 1971); a value of ~4,5 nm is commonly assumed. For comparison, the membrane thickness of the egg-phosphatidylcholine lipid bilayer is 6,9 nm (Ververgaert and Elbers, 1971).

The BRC of *Rhodopseudomonas viridis* is a remarkable example of charge separation over a large distance within an RC. The dipole length can be about 13 nm, since the BRC contains a stalk, the C protein subunit, consisting of cytochromes (cyt), that extends far into the periplasmic space (Fig. 5) (Deisenhofer and Michel, 1989; Gunner, 1991). Because water has the high relative dielectric constant of 80 (for bound water the value is probably less, a value of ~30 has been assumed (Lakhdar-Ghazal *et al*., 1983)), the contribution of the stalk to the formed dipole will depend strongly on its hydration. The stalk also occurs or has occurred in *Chromatium vinosum* and more generally, in the RCs of several green and purple bacteria and their progenitors (Nitschke and Rutherford, 1991).

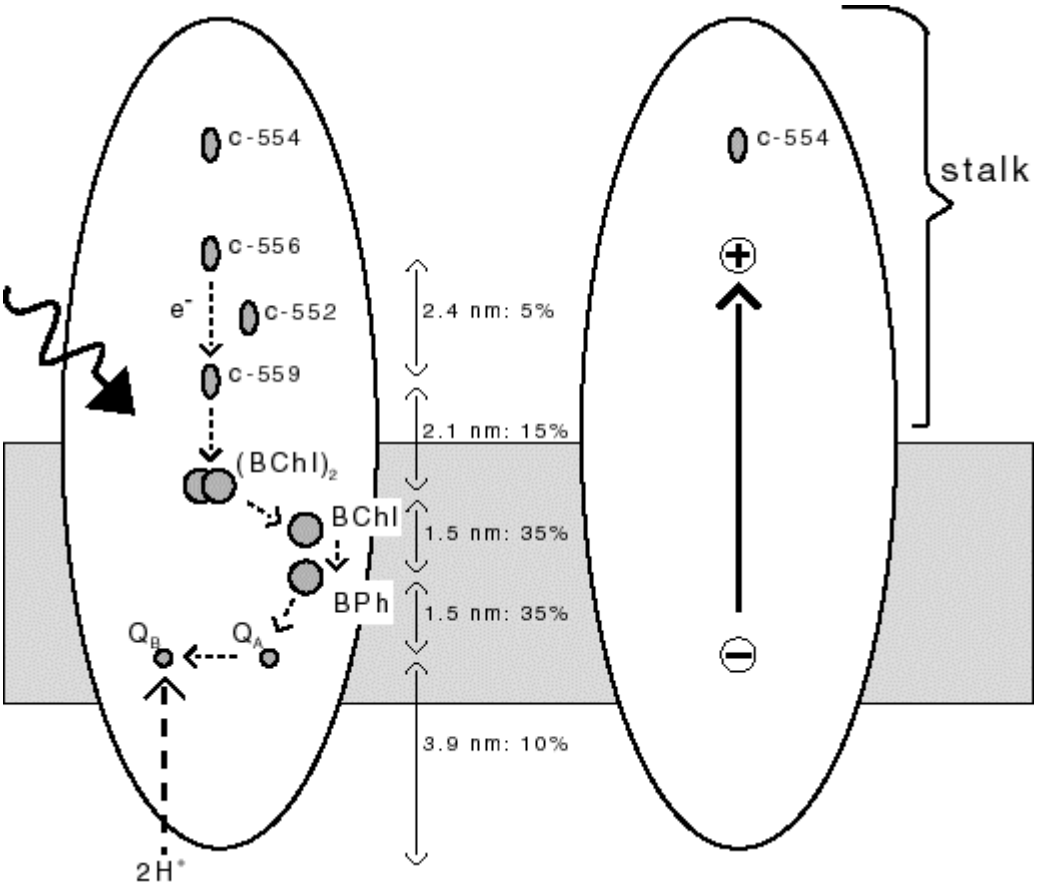

Fig. 5. Chain of charge carriers in the reaction center of *Rheudopseudomonas viridis* (after Dracheva *et al*., 1988: Fig. 11). The reaction center contains a stalk. Upon excitation of a pigment, the $BChl_2$ dimer, an electron leaves the pigment and jumps to the first charge carrier, bacteriopheophytin. The resulting hole in the pigment is filled up by the electron from another carrier, cyt c_{559}. Subsequent electron and hole jumps result in the electron and the hole being separated by a large distance: a large dipole is formed. The jumps are electrogenic; the percentages indicate the relative contribution to the membrane potential. In the PS0 model for the evolution of photosynthesis the charge carriers are added one after the other to the RC (Fig. 12).

The prosthetic groups that constitute the charge carriers are larger than shown here: see Deisenhofer *et al*. (1984: Fig. 4).

Light harvesting complexes (LHCs) absorb photons, converting their energy into excitons that are supplied to the RC, which also can convert photons into excitons on its own. LHCs and RCs therefore have common properties, reason to compare LHCs with the model of Fig. 4B. LHCs can contain carotenoid molecules, which are long enough to span a biomembrane. The binding of a carotenoid to the B800-850 LH2 light harvesting complex of *Rb. sphaeroides* can strongly influence the carotenoid's optical properties (Gottfried *et al*., 1991a, 1991b). Upon excitation, the carotenoid spheroidenone shows a $\Delta\mu$ of ~35 D/ *f* (~0,6 nm) (Gottfried *et al*., 1991a, 1991b). Just as in Fig. 4B, local electric fields may increase the displacement of the excited charge, i.e. enhance the polarizability, and could cause the observed electrochromic shifts in the absorption spectrum of the carotenoid (Jackson and Crofts, 1969; Schmidt *et al*., 1972; Wraight *et al*., 1978; Witt, 1979; Vredenberg, 1981; Kakitani *et al*., 1982) while it is bound to an LHC (DeGrooth and Amesz, 1977; Symons *et al*., 1977; Webster *et al*., 1980). The local fields may be due to charges or dipoles (Kakitani *et al*., 1982), i.e. charged amino acid residues (Eccles and Honig, 1983), dipolar amino acid residues (Fowler *et al*., 1992), electric

quadrupoles of aromatic residues (Burley and Petsko, 1989), the dipole of a protein with an α-helix conformation (Sitkoff *et al*., 1994), surface charges with opposite sign at the two sides of the membrane (Witt, 1979) or of the surface dipole of the monolayers of a biomembrane (Gross *et al*., 1994). Replacement of a *Tyr* by a *Phe* residue in the BChl800-850 LH2, i.e. essentially removing the oxygen atom from a hydroxyl group, or, essentially removing a dipole, results in a blue shift (Fowler *et al*., 1992), showing that single molecular dipoles can indeed strongly influence the optical properties of nearby pigments. This finding has been explained by exciton interactions among nearby chlorophyll molecules, but an alternative explanation is an electrostatic interaction by electric quadrupoles of aromatic residues (Burley and Petsko, 1989) with the hydroxyl group, which enhances its polarization, and increases the dipole moment of the excited state as well.

(c) *Number of separated electrons per RC, n_e*

The larger the number of displaced electrons in an RC, the larger the dipole. In theory, both quinones in the BRC of purple bacteria can be doubly reduced, allowing storage of four electrons, although in practice Q_A becomes doubly reduced only under extreme conditions (Clayton, 1980; Hansson and Wydrzynski, 1990; Gunner, 1991) such as a high light intensity, and absence of Fe in the RC (Okamura and Feher, 1992). Similarly, the cytochromes in the stalk of *Rp. viridis* can store several positive charges (Gao *et al*., 1990). RCII can also accumulate charge at the oxidizing as well as reducing side. The Z-electron donor must be able to store up to 4 positive charges to make oxygen evolution possible (Diner and Joliot, 1977), and on the reducing side the Q_A and Q_B quinones together may also store 4 electrons. Doubly reduced Q_A quickly takes up 2 protons (VanMieghem *et al*., 1992). The protonated quinol state is presumably more stable with respect to decay by electron transport to the oxidized side of RCII. Thus n_e can vary between 1 and 4.

(d) *Contrast*

In nature, light intensity does not vary in an on-off manner, but varies instead upon a continuous background. It is advantageous for PS0 when the PDP *vs.* light intensity graph shows a threshold, or, in photographic terms, 'contrast'. Ideally, contrast results in two distinct potentials and states, a light and a dark state, even when the amplitude of the light fluctuations is small with respect to the continuous background. Contrast could be obtained by apt stabilization and destabilization of the light-induced dipole. Being essentially non-linear, contrast could result in hysteresis.

An optimal efficiency of photon capture 'by a small relaxation of the dipoles of the local environment' has been proposed (Warshel and Schlosser, 1981). The inverse relation between prompt fluorescence and thermoluminescence between 180 and 260°K in chloroplasts (Sane and Rutherford, 1986: Fig. 3) can be explained as a freezing out of this relaxation at low temperatures. The lipid requirement for charge-separation stabilization (Eckert *et al*., 1987; Hansson and Wydrzynski, 1990) suggests involvement of lipids in this relaxation. Lipids are plausible agents for such effects, as lipid and protein structural states can be coupled (Heimburg and Marsh, 1993). The RCII-bound lipids, ~150 molecules, can be removed stepwise. After a first removal of lipids and proteins, 10 lipid molecules remain in the PSII core complex; a second removal leaves 1 lipid molecule in the center complex (Murata *et al*., 1990).

Interaction between lipids bound to different RCs could, especially at a high RC density, enable cooperative charge-separation stabilization. If the interaction is associated with a thermotropic phase transition then contrast can be related to the overall fluidity of the membrane, and thus to MTS. The cooperation can explain the two conformational states of the BRC of *Rp. viridis*, which have different recombination times, one state being associated with the cold, a high solvent viscosity and a high lipid rigidity, the other with a high temperature, low solvent viscosity and low lipid rigidity (Sebban and Wraight, 1989; Sebban *et al*., 1991; Gao *et al*., 1991; Baciou and Sebban, 1992). The same biphasicity has been observed in *Rb. sphaeroides* (Sebban, 1988). A sharp transition temperature is, however, absent. Cooperation between RCs could lead to separate domains containing cooperatively stabilized and cooperatively destabilized dipoles.

The 'Kok effect' (Kok, 1949), a light intensity threshold for photosynthesis, can be associated with contrast, which mechanically could be obtained by cooperative charge stabilization. At the

oxidizing side of RCs, the Z-donor can assume several states of increasing positive charge, S_0, S_1, S_2, S_3, S_4 (Joliot *et al*., 1969). Oxygen is evolved during the $S_4 \rightarrow S_0$ step. S_3 and S_2 are metastable with a $t_{½}$ of 1-100 s (Diner and Joliot, 1977). The $S_3 \rightarrow S_2$ deactivation has a $t_{½}$ ~3 s; at a low light intensity this decay may limit the photosynthesis rate (Radmer and Kok, 1977). State decay is associated with reduction by quinones. A structural RC transition around 21°C has been invoked from temperature dependency of this $S_3Q_B^- \rightarrow S_2Q_B$ decay (Messinger and Renger, 1990).

At the reducing side, charge stabilization occurs by double reduction of bound quinones (Larkum, 1991), which is a condition for double protonation of the quinone; the process is a 'two-electron gate' (Bouges-Bocquet, 1973; Velthuys and Amesz, 1974; Vermeglio, 1977; Wraight, 1977, 1982; Vermeglio and Clayton, 1977; Barouch and Clayton, 1977; DeGrooth *et al*., 1978; Baccarini-Melandri *et al*., 1981; Crofts and Wraight, 1983; Gunner, 1991). The gate is operative in the R-26 strain of *Rb. sphaeroides*, but not in the Ga-strain (Barouch and Clayton, 1977). In the operative gate at the RC reducing site, after a first electron has been transferred to a quinone, forming a semiquinone, a second transfer can occur before the semiquinone decays; thus both transfers can be stabilized. Obviously, the chance of a second electron arriving in time increases with the light intensity. PS0 requires that quinones upon double reduction and double proton binding do not dissociate from the RC; this could be effected by strong binding of the quinone—such a *Rb. sphaeroides* mutant has been described (Shinkarev *et al*., 1993)—or by hindrance of quinone diffusion across the membrane by a lessened membrane fluidity (Poore and Ragan, 1982).

Let N equal the Chl/RC ratio, i.e. the antenna size, and I the light intensity or fluence rate (μmol m^{-2} s^{-1}). Then, since a photon irradiance of 1000 μmol m^{-2} s^{-1} results in 1,15 photons being absorbed by one Chl molecule per second, the number of RC excitations per second, K, equals 1,15 N (I/1000) (Ghirardi and Melis, 1987). The mean arrival time between two RC excitations is 1/K. Going from a long to a short mean arrival time, cooperative stabilization occurs when 1/K becomes equal to the RC decay time. It follows that the light intensity T of

$$T = 1000 / (1{,}15 \text{ t } N) \ \mu\text{mol } m^{-2} \ s^{-1} \qquad (1)$$

is an estimate for the light intensity threshold for cooperative stabilization. Whether the system is near the contrast threshold thus depends on the three independent variables RC decay time, antenna size, and light intensity.

Table 1 gives the contrast thresholds for several combinations of antenna sizes and RC decay rates. Photosynthetic bacteria with an antenna of 6 and an RC decay time of 700 ms would have a T of 210 μmol m^{-2} s^{-1}. Conversely, at an antenna of 300, light intensities as high as 1400 and as low as 4 μmol m^{-2} s^{-1} permit a threshold for RC decay times of resp. 2 ms, typical of Q_A^- decay, and 700 ms, typical of slow Q_B^- decay. For a decay time of 70 ms the antenna range of 6-600 yields contrast at a threshold range of 2100-21 μmol m^{-2} s^{-1}, a range embraced by natural environments. In these environments contrast is associated with the light intensity range 15-1500 μmol m^{-2} s^{-1}, which requires antennas of 100 or less. Obviously, these estimates can be bettered, for instance by taking the effects of decay of doubly reduced states (decay times ~1-10 s) into account, by applying Poisson statistics (Mauzerall and Greenbaum, 1989), and by implicating S_i transitions (Radmer and Kok, 1977) or even proton leakage across the membrane (Raven and Beardall, 1981, 1982). The basic concept shall however be clear. Photosynthesizers could adapt the threshold to the environmental light intensity by varying the antenna size or RC decay times. The decay times depend on the amino acid composition of the RC proteins (Okamura and Feher, 1992), and *in situ* mutants with decay times optimal for PS0 could be selected.

Since in pea chloroplasts many photophysiological parameters (composition, structure and function: Chl a/ Chl b ratio, electron transfer rates, etc.) change as function of the light intensity at 200 μmol m^{-2} s^{-1} (Leong and Anderson, 1984a, 1984b), this value is a candidate for the proposed contrast threshold.

The formation is therefore plausible in contemporary organisms of metastable dipoles within RCs with decay times in the range 0,1-10 s, a time scale range agreeing with natural light fluctuations.
Obviously, the formation is therefore also plausible in early organisms.

3. *The Photosynthetic Dipole Potential*

(a) *The Dipole Potential*

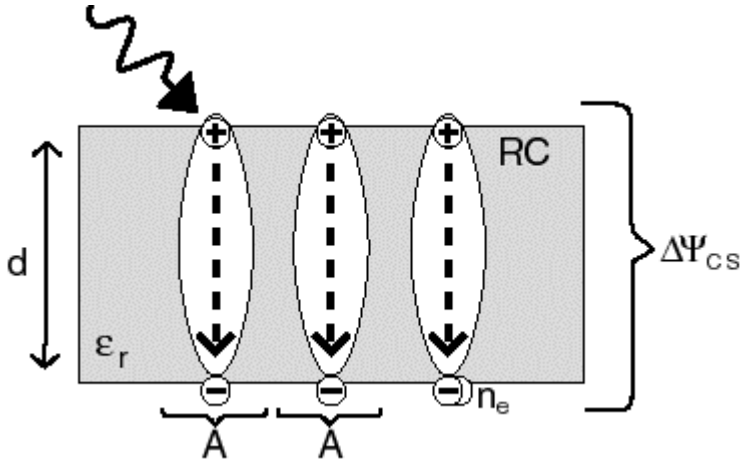

Fig. 6. The standard photosynthetic potential (CSP) is caused by charge separation *across* the membrane. When each reaction center puts n_e electrons across the membrane, the potential difference across the membrane equals $\Delta\Psi_{CS} = n_e\, e\, d/A\, \varepsilon_o\, \varepsilon_r$, where e equals the elementary charge, d the thickness of the membrane, and A equals the surface area per reaction center. The broken arrows indicate the paths followed by the displaced electrons; the associated dipoles are oppositely oriented.

In standard photosynthesis the biomembrane acts as a capacitor (Fig. 6). The increase in potential across this membrane after a saturating flash of light, $\Delta\Psi_{CS}$, follows from the charge transported across the membrane per unit area, ΔQ, and the electrical capacity per unit membrane area, C_m:

$$\Delta\Psi_{CS} = \frac{\Delta Q}{C_m} \qquad (2).$$

ΔQ equals the product of N, the surface density of the RCs, n_e, the number of electrons transported per RC per flash, and e, the elementary charge; C_m equals ε / d, where $\varepsilon = \varepsilon_r\, \varepsilon_o$ is the dielectric constant of the membrane and d its thickness. Substitution in Eqn. 2 gives:

$$\Delta\Psi_{CS} = \frac{N\, n_e\, e\, d}{\varepsilon_o\, \varepsilon_r} \qquad (3a).$$

N is the inverse of A, the membrane area per RC. Hence one can write Eqn. 3a also as:

$$\Delta\Psi_{CS} = \frac{n_e\, e\, d}{A\, \varepsilon_o\, \varepsilon_r} \qquad (3b),$$

a similar expression has been given by Junge (1977) and Witt (1979). $\Delta\Psi_{CS}$ is called here the 'Charge-Separation Potential' (CSP), to distinguish it from the photosynthetic dipole potential (PDP).

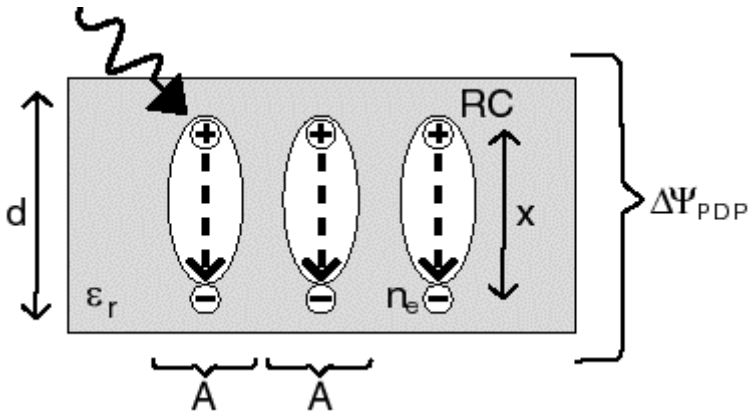

Fig. 7. The photosynthetic dipole potential (PDP) is caused by dipole formation *within* the membrane. When in each reaction center n_e electrons are displaced over a distance x within the membrane, then the resulting potential difference across the membrane equals $\Delta\Psi_{PDP} = n_e\, e\, x/A\, \varepsilon_o\, \varepsilon_r$.

The PDP is formed when the electrons excited during a flash do not move across the membrane, but instead halt within the membrane after having traversed a distance x (Fig. 7). The RCs with the separated charges then constitute electrical dipoles, and the Helmholtz formula applies (Gaines, 1966; Jones, 1975; Schuhmann, 1990): the potential across a layer of dipoles with dipole moment μ, placed at a surface density N in a medium with dielectric constant ε, equals

$$\Delta\Psi_{dipole} = \frac{N\,\mu}{\varepsilon} \qquad (4).$$

μ equals the product of the separated charge and their distance: $n_e\, e\, x$. Substitution in (4) gives,

$$\Delta\Psi_{PDP} = \frac{N\, n_e\, e\, x}{\varepsilon_o\, \varepsilon_r} \qquad (5a),$$

or,

$$\Delta\Psi_{PDP} = \frac{n_e\, e\, x}{A\, \varepsilon_o\, \varepsilon_r} \qquad (5b).$$

In an asymmetric membrane a similar dipole potential can be present due to oriented molecules, e.g. lipids, that constitute dipoles: this potential has been called the 'Membrane Dipole Potential' (MDP or $\Delta\Psi_D$) (Muller, 1993). The MDP is normally screened by counter ions.

It has been recognized for some time (Hagins and McGaughy, 1967; Jackson and Dutton, 1973; Evans and Crofts, 1974; Renger, 1974; Huebner *et al*., 1982; Hong, 1994) that the membrane potential can increase due to intramembrane charge displacement, i.e. dipole formation. Since dipole formation precedes charge transfer across the membrane during standard photosynthesis, a PDP precedes the CSP. This quick PDP formation explains the prompt electrochromic shift of chromatophores and chloroplasts after a dark-light transition (Jackson and Dutton, 1973; Witt, 1979). Comparison of Eqn. 5 with Eqn. 3 shows that for x equal to d, $\Delta\Psi_{PDP}$ equals $\Delta\Psi_{CS}$. It follows for the ratio of $\Delta\Psi_{PDP}$ and $\Delta\Psi_{CS}$:

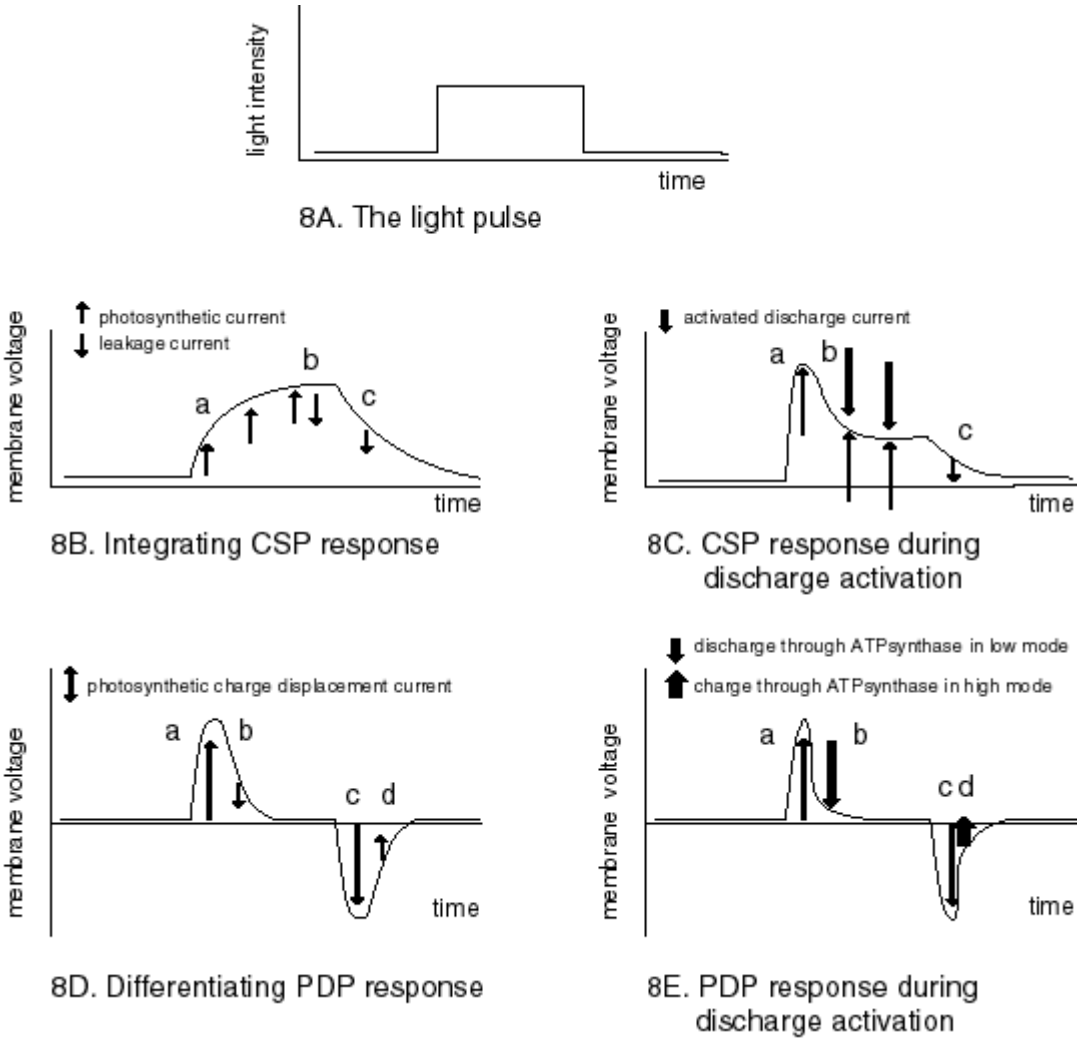

Fig. 8. Different responses to a light pulse for charge-separation driven and dipole driven potentials.
A. The light pulse.
B. Integrating response of the standard photosynthetic potential (CSP), obtained by charge separation. a: At low potential leakage is small; the potential increases. b: At a higher potential the photosynthetic current equals the leakage current. c: In the absence of a photosynthetic current in the dark the CSP dissipates.
C. CSP response during discharge activation. a: The potential initially increases to a high value. b: Discharge current has been activated. CSP decreases, but stabilizes at a finite value, while the two currents cancel each other. c: CSP dissipates.
D. Differentiating response of the photosynthetic dipole potential (PDP). a: Dipoles are formed after the dark-light switch, and a PDP is formed. b: Due to charge leakage across the membrane, the membrane potential dissipates: the light-induced dipoles are being screened by counter-ions. c: After the light-dark switch the dipoles decay quickly. The still present screening charge causes a potential of opposite sign, which then dissipates (d).
E. The PDP is screened by protons moving through ATPsynthase. Different H^+/ATP ratios of the ATPsynthase during forwards (b) and backwards (d) activity result in a net ATP gain from a light pulse.

$$\frac{\Delta\Psi_{PDP}}{\Delta\Psi_{CS}} = \frac{x}{d} \qquad (6).$$

A similar expression has been proposed by Jursinic *et al*. (1978) for the potential of the state that causes delayed light emission in chloroplasts; see also Höök and Brzezinski, 1994 (Eqn. 9). Expressions resembling Eqn. 5a have been given by Popovic *et al*. (1986: Eqn., 19) and Höök and Brzezinski (1994: Eqn. 1) for the potential across a layer of reaction centers. Trissl and Läuger (1972) and Berns (1976) discussed the potential of a dipole layer adjacent to the photosynthetic membrane, a layer formed during illumination, but gave no expression for it. Baker *et al*. (1979) gave an expression similar to Eqn. 5, but considered a different system: dyes, charged during illumination, entering the membrane and causing the membrane voltage to change.

CSP and PDP formation respond differently to a light pulse (Fig. 8) (Trissl and Läuger, 1972; Ullrich and Kuhn, 1972: Fig 6). During standard photosynthesis (Fig. 8B) a steady-state CSP is formed provided the pulse lasts long enough: the photosynthesis current in the steady state equals the membrane discharge or leakage current (b). After a light-dark switch the photosynthesis current disappears, the leakage current continues and the potential dissipates (c). If a discharge current is activated (Fig. 8C) then the steady-state potential (b) is lower than the initial one, but the sign of the decaying potential remains the same after the light-dark switch (c). An activated ATPsynthase might result in such a response. In the PDP response (Fig. 8D) there is an initial potential increase (a) that subsequently dissipates (b): the PDP becomes screened by counterions before the light-dark switch. The switch causes decay of the PDP, while the remaining counterions cause a potential of opposite sign that also subsequently dissipates (c). A caveat is that such a negative overshoot could be the result from proton diffusion potential generated upon the light-dark switch of a CSP system, the diffusion potential being the result of the proton gradient formed in the light across the membrane (Vredenberg and Tonk, 1975; Vredenberg, 1976); such an effect will in general have a magnitude different from the initial potential increase. In the absence of the latter effect, the sign of the CSP response thus remains the same throughout, whereas the PDP response to a light pulse consists of two transients of different sign but equal magnitude. In electronic terms, the CSP gives an integrating, the PDP a differentiating response.

Experiments on a monolayer of BRCs are consistent with PDP formation (Tiede *et al*., 1982), oxidized bacteriochlorophyll (BChl) constituting the positive and reduced ubiquinone the negative pole of the formed dipoles. The dipole decays by return of the electron on the Q to the BChl, $t_{½}$ 80 ms. The experiments of Ullrich and Kuhn (1969, 1972) on lipid bilayers are also consistent with PDP formation (Trissl and Läuger, 1972), just as the experiments on bilayers containing BRCs (Barsky *et al*., 1976; Drachev *et al*., 1975, 1976; Schönfeld *et al*., 1979: Fig. 1B, Table 1; Packham *et al*., 1982, 1988). Other plausible PDP systems are the squid retina (Hagins and McGaughy, 1967), the impaled thylakoid membrane (Vredenberg and Tonk, 1975), asymmetric bilayers (Berns, 1976: Fig 3), the plasma membrane of leaf cells (Jeschke, 1976), BRCs upon a collodion film (Drachev *et al*., 1981; Skulachev, 1982; Dracheva *et al*., 1988) or teflon film (Höök and Brzezinski, 1994) or at the heptane/water interface (Trissl, 1983), and layers of rhodopsin and bacteriorhodopsin (Trissl *et al*., 1977; Miyasaka *et al*., 1992: Fig. 2). The initial potential increase (a) in Fig. 8D would correspond to the fast $\Delta\Psi$ increase observed upon a dark-light switch; for photosynthetic bacteria this increase is called ‘phase I’ (Jackson and Dutton, 1973; Wraight *et al*., 1978), for chloroplasts ‘phase 1’ (Vredenberg, 1976). In addition the PDP can be associated with the Maxwell displacement current (the current due to dipole formation (Jackson, 1975)) observed in azobenzene monolayers (Iwamoto *et al*., 1991). The PDP and CSP can also be associated with the ‘AC photoelectric effect’ and the ‘DC photoelectric effect’ (Hong, 1994).

The surface charge of the inner and outer side of the thylakoid membrane changes with opposite sign upon a dark-light switch, the lumen side becoming positive, the stroma side negative (Witt, 1979: Fig. 12). Such charging is equivalent to the formation of a membrane-spanning dipole. The thylakoid pigments showing electrochromic shifts are, in addition to the electric field due to the potential difference between the bulk solutions at the two sides of the membrane, also subject to the field due to the membrane surface charge changes. The associated potential difference is indicated by Witt as ‘$^{ss}\Delta\Psi$’ (‘ss’ stands for ‘steady state’); he gives as its value ~80 mV. The increase of the internal surface charge is attributed to protonation of acid groups as the lumen pH increases. It will hereafter be shown

that $\Delta\Psi_{PDP}$ could have a similar magnitude. There are other similarities, but there are also differences: $\Delta\Psi_{PDP}$ is independent of the pH, whereas $^{ss}\Delta\Psi$ depends on the pH at both sides of the thylakoid, which changes relatively slowly upon illumination, on the time scale of seconds. According to Witt, the dipole potential, $^{1}\Delta\Psi$ —which arises after a single saturating flash due to surface charge changes similar to $^{ss}\Delta\Psi$ —is nil, whereas in PS0 the PDP reaches its maximal value in one or two saturating flashes. Care should be taken to distinguish between the two dipole potentials during the interpretation of experimental results.

(b) *Surface area per RC*
The surface area per RC, A, follows directly from the RC density in the membrane as visible in the electron microscope, often given as the number of RC particles per square micron (Staehelin, 1986). The lower limit of A equals the surface area of the RC, A_{RC}. Removing LHCs decreases A_{RC} and a variable number of LHCs can explain the variety in reported A_{RC} values. For a standard chloroplast, Barber (1972) gives a value of 16,7 cm^2 thylakoid membrane per µg chlorophyll, equivalent to a surface area of 2,5 nm^2 per chlorophyll molecule[3]. For an antenna size *N*, of 250 an A value of 625 nm^2 results. Table 2 gives pertinent data on A and A_{RC}, which both are seen to vary strongly. A may be 25 nm^2 for crystallized central cores, 120 nm^2 for thylakoids with crystallized RCs, 150 nm^2 for BRCs in chromatophores. Since the particle visible under the electron microscope is a dimer (Boekema *et al*., 1994; Santini *et al*., 1994) old reported values of RCII densities in chloroplasts and cyanobacteria can be doubled: thus for chloroplasts an A value of 450 nm^2 is obtained. Small A values will occur particularly when RCs crystallize.

Sometimes the literature suggests high RC densities, but does not give explicit values. For instance, the wild type of *Rb. capsulatus* contains 450 pmol RC/mg membrane protein (Schmidt *et al*., 1993), from which a maximal RC molecular weight of 2200 kDa follows. An antenna-deficient mutant contains even more RCs: 1637 pmol RC/mg membrane protein, equivalent to a maximal RC molecular weight of 611 kDa. In this mutant RCs must constitute much of the membrane protein, and their density must accordingly be high. Photoinactivated RCIIs constitute another example: ‘Consideration suggests that, under chronic irradiation stress, a significant surface area of the thylakoid membrane area functions to house photoinactivated centers’ (Vasilikiotis and Melis, 1994).

(c) *Relative dielectric constant of the membrane and the RC*
For lipid membranes ε_r equals 2 (Schnitzer and Lambrakis, 1991), whereas for membrane proteins a value of 4 ‘seems to work quite well’ (Sharp and Honig, 1990). From the often reported specific membrane capacity C_m value of 1 µF/cm^2, and a membrane thickness *d* of 4 nm, a value $\varepsilon_r = C_m\, d\, /\, \varepsilon_o = 4{,}5$ follows. Packham *et al*. (1978) estimated the C_m of the *Rb. sphaeroides* chromatophore membrane to be ~1,1 µF/cm^2, and gave an ε_r of 3,8. For the BRC of *Rp. viridis* an ε_r of 6 has been estimated (Gao *et al*., 1990: Fig 10).

Several studies discuss the validity of idealizing the polarization behavior of membranes in terms of a homogeneous dielectric with a single dielectric constant (Warshel and Russell, 1984; Rogers and Sternberg, 1984; Gao *et al*., 1990). Some models subdivide the membrane in parts with different dielectric constants (Nakamura *et al*., 1988; Gao *et al*., 1990), others (Schnitzer and Lambrakis, 1991) let the value of ε_r at the lipid-water interface vary continuously over a distance of 1 nm from 2 to 89, the dielectric constant of water (bound water, water near an interface such as a biomembrane, has however an ε_r of ~30 (Lakhdar-Ghazal *et al*., 1983)) and the most detailed models simply sum the contributions of individual atoms to the polarization, an approach justified by the finding that removal of one oxygen atom in a residue can result in a red shift of the BChl850 of LH2 of *Rb. sphaeroides* (Fowler *et al*., 1992). Unidirectionality of electron transport in the BRC along one of the two present protein chains has been explained by a lower dielectric constant in one chain (Steffen *et al*., 1994).

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[3] $2{,}5 = 16{,}7 \times 10^6 \times 10^{14}$ (nm^2/cm^2) × 890 (M_W chlorophyll) / 6,0 10^{23}. When the ratio is valid is, however, uncertain, and Kirk (1971) gives, in contrast, a range of 0,9 - 3,8 nm^2/Chl molecule.

Table 2. Determined surface areas per RC, A, or determined surface area of the RC itself, A_{RC}. In a few cases the surface area calculated from the observed density of RCII particles was halved since these particles consist of RCII dimers (Boekema *et al*., 1994; Santini *et al*., 1994); this correction is indicated by an '*'.

PHOTOSYNTHETIC BACTERIA	A [nm^2]	A_{RC} [nm^2]	references
• chromatophore of *Rp. viridis*; hexagonal array with period of ~10 nm	~173	~173	Giesbrecht and Drews, 1966
• tubular membrane of *Thiococcus*; hexagonal array with period of ~12,5 nm	~270		Eimhjellen *et al*., 1967
• chromatophore of *Rb. sphaeroides*: 4200 μm^{-2}	240	~133	Reed and Raveed, 1972
• *Rb. Sphaeroides* BRCs labeled by antibodies	175	~71	Reed *et al*., 1975
• chromatophore of *Rb. sphaeroides*	370		Packham *et al*., 1978
• membrane of *Rps capsulata*: 5170 μm^{-2}	193	~71	Golecki *et al*., 1979
• *R. rubrum*: cytoplasmic membrane:			Golecki and Oelze, 1980:
under chemotrophy: 4700 μm^{-2}	213	~62	Table 1
under phototrophy: 6264 μm^{-2}		~69	
chromatophores: only during *Rb. sphaeroides*:	147	~80	
cytoplasmic membrane: 5708 μm^{-2}	172		
chromatophores: 6674 μm^{-2}	150	~47	
R. tenue: exoplasmic face		~48	
cytoplasmic membrane: 3765 μm^{-2}	266	~102	
• *R. rubrum*: 5500 μm^{-2}; particle diam. ~13 nm	182	~133	Meyer *et al*., 1981
• monolayers of *Rb. sphaeroides* BRCs	22		Tiede *et al*., 1982
• BChl *b* containing bacteria with			Engelhardt *et al*., 1983
(1) membranes for 10-25% covered by 2D crystals of BRCs of *Ectothiorhodospira halochloris*	1150-2900	144 25	
(2) membranes of *Thiocapsa pfenniggi* for 100% covered by 2D crystals of BRCs	275	(RC core) 275	
• *Rps viridis*: rectangular crystals of BRCs 4,5x6 nm^2	~27		Miller and Jacob, 1983
• *Rps viridis*: hexagonal array with period of 13 nm; core visible	146	64 (core)	Stark *et al*., 1984
• *Heliobacterium chlorum*; contains Bchl *g*.	174		Fuller *et al*., 1985: Fig. 1
• X-ray analysis RC core of *Rps viridis*; double axes elliptical cross section: 3 and 7 nm	~25		Deisenhofer and Michel, 1989

• monolayer of *Rb. sphaeroides* BRCs monolayer of *Rp. viridis* BRCs	~125 ~ 50		Alegria and Dutton, 1991

ALGAE AND CHLOROPLASTS			**A [nm^2]**	**A_{RC} [nm^2]**	**references**
• 2D crystals of 'quantasomes', unit cell dims 15,5x18,5 nm^2; one quantasome contains 4 RCIIs			72	72	Park and Biggins, 1964;Park, 1965
• 2D crystals of tetramers of RCIIs (possibly without LHCs: Bassi *et al*., 1989)			108		Miller *et al*., 1976
• RCII particle density		~1100 μm^{-2}	450 *		Armond *et al*., 1977
• thylakoid membranes of spinach and barley with 2D crystals of RCs, unit cell dims 17,5x20 nm: tetramers			86	86	Clayton, 1980, p. 117
• RCII particles	grana	1200-1700 μm^{-2}	295- 415 *	~80-130 (RCI)	Staehelin, 1986: Fig. 31
	whole membrane	850-1300 μm^{-2}	700-1620 *	~50 (RCI core)	
RCI particles:	grana	250- 400 μm^{-2}	385- 600 *	~95 ($RCII_\beta$), 80-230	
A_{RC}values calculated from reported diameters			2500-4000	($RCII_\alpha$)	
			300- 480		
			625-1000		
• RCII particle in etiolated chloroplasts, grown in intermittent light (2-118 min LD).				~50	Staehelin, 1986
Upon transfer to continuous light LHCs are acquired (Armond *et al*., 1977), and A_{RC} increases stepwise				50→90→130→200	
• intramembrane particle densities in algae, most probably RCs:			400-3300		Staehelin, 1986
• red algae *Spermothamnion turneri*:					Staehelin, 1986
RCII particles		960 μm^{-2}	520 *		
RCI		3570 μm^{-2}	280		
• cyanobacterium *Synechococcus*, trimer of RCI; A_{RC} of 280 nm^2				~95 (RCI)	Boekema *et al*., 1987
• monomer of oxygen evolving complex of *Synechococcus* double axes 10,5 and 15,8 nm				130 (RCII)	Rögner *et al*., 1987
• green algae *Dunaliella tertiolecta*					Sukenik *et al*., 1987: Table 4
at	100 μmol m^{-2} s^{-1}		790		
at	1900 μmol m^{-2} s^{-1}		1620		
A refers to the density of the electron transport chain: PSI+PSII+b_6f					
• *Synechococcus*; RCII dimer with dimensions 15x12,8 nm^2				96-121	Dekker *et al*., 1988: Fig. 9
• 2D crystals of tetramers of RCIIs, unit cell dims 17,8x26,7 nm^2				120	Bassi *et al*., 1989
• PSI core + 8 LHCIs: oval 16x12 nm^2				151	Boekema *et al*., 1994

Middendorf *et al*. (1993) analysed the polarizability of excited states of pigments in terms of electron wave functions, thus giving an even more involved description. The problem of modeling the electrical polarization of an RC from first principles is similar to the problem of modeling the charge separation within an RC from first principles. The latter problem is still unresolved, although many approaches have been tried (Friesner and Won, 1989). One expects that a good description of the electrical polarization of an RC will only be obtained after solving the latter problem.

Obviously, for biomembranes both the applicability and, when applicable, the value of a single relative dielectric constant are uncertain.

(d) *Estimated values*
Eqn. 5b can be rewritten as[4]:

$$\Delta\Psi_{PDP}\,(mV) = 18070\ \frac{n_e\, x[nm]}{\varepsilon_r\, A[nm^2]} \qquad (7).$$

Substituting for n_e 4, x 4, ε_r 2 and A 25, yields 5800 mV, whereas substituting n_e 1, x 1, ε_r 4,5 and A 500 yields 8 mV, showing the large latitude of possible $\Delta\Psi_{PDP}$ values.

Comparable $\Delta\Psi_{CS}$ values have previously been calculated and observed. For the *Rb. sphaeroides* chromatophore membrane Packham *et al*. (1978) implicitly or explicitly assumed values of n_e 1, x 3, ε_r 3,8 and A 311. Applying Eqn. 7 results in 46 mV, the observed value in their single saturating flash experiments. For chromatophores of *Rb. sphaeroides* Jackson and Crofts (1969) have reported a $\Delta\Psi_{CS}$ of 430 mV directly after a dark-light switch; for a single RC turnover, Wraight *et al*. (1978) calculate a $\Delta\Psi_{CS}$ of 100-140 mV. Substituting for A 150 nm^2, the value for photosynthetic bacteria, and taking for the other variables the values assumed by Witt (1979) for chloroplasts, n_e 2, x 3, ε_r 2, yields 360 mV.

For these chloroplasts Witt assumed A 1000, resulting in a CSP of 55 mV. A similar value was found in experiments using flashing light. Zimányi and Garab (1982) assumed for chloroplasts n_e 1, x 7, ε_r 2 and A 970, which yields 65 mV. Correction for the PSII particle being a dimer doubles $\Delta\Psi_{CS}$. For crystallized RCs, or more generally, for RC densities enhanced by a factor 4, values of ~200 mV are calculated. Potential increases upon a dark-light switch have been determined by using microcapillary glass electrodes, or the electrochromic shift determinations of an oxonol probe. Reported values are 70-75 mV (Bulychev *et al*., 1972), 4-12 mV (Vredenberg *et al*., 1973; Vredenberg and Tonk, 1974), 40 mV (Bulychev *et al*., 1976), 15-35 mV, with single RC turnover (Schapendonk and Vredenberg, 1977) and ~90 mV, with 50 mV in the steady state (Schuurmans *et al*., 1978).

In the discussion of the power of PS0 that follows, $\Delta\Psi_{PDP}$ values of 70, 100, 150, 200, 400 and 600 mV are considered (Table 3). A membrane voltage of ~600 mV can cause electrical breakdown of the membrane (Coster and Zimmermann, 1975). Charge can however be transferred across the membrane by an ATPsynthase or by conduction before such a high potential is reached. The PDP then yields a measure for the amount of charge dQ that can be transferred across the membrane in one PS0 light cycle: $dQ \sim \Delta\Psi_{PDP} / C_m$, rather than a measure of the actually assumed membrane voltage.

[4] $18070 = 10^3 \times 1{,}6\ 10^{-19} \times 10^{-9} / 8{,}854\ 10^{-12} / 10^{-18}$

The concept of a dipole potential is not new to the discipline of photosynthesis, and it even has already been treated quantitatively. The concept is however not well known in this discipline. Several of the studies referred to in the foregoing do not recognize the dipole potential as such, are vague on the issue (see also Case and Parson (1973), who otherwise discuss the problem clearly), and are not consistent in their terminology. Terms as ‘electrogenicity’, ‘photoelectric current’, ‘displacement current’ are either vague, or misnomers when all pertinent effects are due to static dipoles, and not to the dynamic formation of dipoles. Moreover, the similarity to the dipole potential studied in surface science (Gaines, 1966; Haydon and Hladky, 1972) becomes obvious after identification of the light-induced potential as a dipole potential. Wraight *et al*. (1978) formulate an explicit question:

> ‘Placing the initial charge separation within the dielectric matrix of the membrane, rather than right across it, raises the question when and if delocalization of the electric field occurs. A delocalized field, or membrane potential, is necessary if subsequent coupling to ATP synthesis is to occur in a chemiosmotic manner’.

The present study gives as answer that the local dipole results, with the dipole potential $\Delta\Psi_{PDP}$ as an intermediate, in the delocalized membrane potential $\Delta\Psi$.

Distinguishing between the dipole potential and the potential due to charge translocation across the membrane, helps to clarify their differences: (1) the PDP can diminish by both dipole decay and leakage across the membrane, the CSP only by leakage; (2) the PDP increases with a high dipole density, the CSP by a large number of charge translocations across the membrane, and (3) the PDP is formed upon a dark-light switch, the CSP in constant light.

In PS0, ATP is synthesized upon a single saturating flash, while the excited electron is not displaced across the membrane. If it would move across the membrane, then the PDP would be even higher, and one saturating flash then would certainly have to be sufficient for ATP synthesis. For chromatophores of photosynthetic bacteria this is indeed the case (DelValle-Tascon *et al*., 1978; Petty and Jackson, 1979a, 1979b; Melandri *et al*., 1980; Baltscheffsky *et al*., 1982), but in chloroplasts two saturating flashes are required (Graan *et al*., 1981). In standard photosynthesis a low RC density is possible, for instance when ATPsynthase needs activation by a threshold potential (Junge, 1970a, 1970b): the RCs then make multiple turnovers before phosphorylation starts. In contrast, in PS0 the effects of multiple turnovers do not add up, and the RC density must be high to obtain the potential required for ATP synthesis, provided such a threshold potential is indeed operative.

4. ATPsynthase

Charging and discharging of the membrane at a different H^+/ATP ratio are essential for the MTS (Muller, 1985) and the PS0 mechanism. How plausible is such a process? A variable ratio could most easily be effected by an ATPsynthase with a variable stoichiometry, and appropriate on/off-switching, but there are other possibilities, which shall now be discussed.

Consider first the reactions of ATPsynthase in a constant single mode *n*, i.e. at a stoichiometry of *n* H^+ translocated across the membrane per ATP molecule (see Schlodder *et al*., 1982). The reaction can be written as:

$$n\ H_p^+ + ADP + P_i \quad \rightarrow \quad n\ H_n^+ + ATP.$$

The membrane voltage $\Delta\Psi$ is taken between the positive p-side of the membrane and the negative n-side, the side with the F_1 part of the enzyme. ATPsynthase can in a mode *n*, synthesize ATP for $\Delta\Psi > V_n$ and hydrolyze ATP for $\Delta\Psi < V_n$: the equilibrium potential of a mode *n* equals $V_n = V_1 / n$. The value of V_1 follows from the ΔG for ATP hydrolysis, ΔG_P: $V_1 = \Delta G_P / F$, where *F* is Faraday's constant. For the reaction ADP + $P_i \rightarrow$ ATP + H_2O a $\Delta G_P°$ value of 31,5 kJ/mol has been reported (for further details see Rosing and Slater, 1972). For the ΔG_P of resp. darkened intact chloroplasts, illuminated intact chloroplasts and illuminated broken chloroplasts (Giersch *et al*., 1980) values of 41,8, 45,6 and 60 kJ/mol have been determined, equivalent to V_1 values of 430, 470 and 620 mV. From now on for V_1 a constant value of 470 mV is assumed in quantitative estimates, although this value is somewhat arbitrary, ΔG_P values varying strongly. Schlodder *et al*. (1982) assume a value of 360 mV.

In the presence of a ΔpH across the membrane, a term involving ΔpH must be added to $\Delta\Psi$, yielding the $\Delta\mu_{H^+}$ or 'electrochemical proton gradient' across the membrane. At equilibrium $\Delta G_P / \Delta\mu_{H^+}$ equals *n*. Whether ATP is synthesized or hydrolyzed thus depends on the $\Delta\mu_{H^+}$; one can call this dependency thermodynamic regulation. The rate of ATP synthesis, the kinetic regulation, can however also depend on $\Delta\mu_{H^+}$. At low $\Delta\mu_{H^+}$, ADP binding can inactivate CF_0F_1, but at a high $\Delta\mu_{H^+}$ the enzyme can be reactivated with simultaneous release of the bound ADP (Junesch and Gräber, 1987).

It is difficult to agree on a value for *n*. For ATPase/synthase values as low as 1, and as high as 9 (Guffanti *et al*., 1984; VanWalraven *et al*., 1990) have been reported. In contrast to the stoichiometry of standard chemical reactions, the stoichiometry of the ATPsynthase reaction seems to be variable (Westerhoff *et al*., 1984; Haraux, 1985; Gerst *et al*., 1994). But is it variable? Apparent variability must either involve (1) errors in the measurement of $\Delta\mu_{H^+}$ or ΔG_P (this possibility is further ignored, although such measurements may be difficult), (2) variation of the stoichiometries of the relevant proton pumps, or (3) inapplicability of the chemiosmotic mechanism: the membrane embedded electron transfer chain would directly couple protons to ATPsynthase, instead of generating the $\Delta\mu_{H^+}$ intermediate.

For the present purpose, four schemes for mode variation during charging and discharging of the membrane are of interest: (a) a single mode and conduction, (b) switching between two different modes, (c) flexible coupling, and (d) local coupling.

(a) *Single mode and conduction*
In this scheme the membrane is discharged by an ATPsynthase in the single mode *n*, and is recharged by conduction through the membrane. The enzyme is kinetically regulated in such a manner that it can only be forwards active. At the start of the cycle $\Delta\Psi$ equals zero. Upon illumination the $\Delta\Psi_{PDP}$ formed is larger than V_n, and ATP is synthesized, and the membrane discharged, until $\Delta\Psi \simeq V_n$. Upon the light-dark switch $\Delta\Psi_{PDP}$ vanishes. Since after the start of the cycle, a charge C ($\Delta\Psi_{PDP} - V_n$), where C is the electrical capacity of the membrane, has been translocated through ATPsynthase to the other side

of the membrane, $\Delta\Psi$ becomes negative. By conduction the charge leaks away until $\Delta\Psi$ equals zero, and the cycle is closed. In this scheme charging by an ATPsynthase operating backwards in the high mode is superfluous.

The scheme is especially advantageous when the duration of the induced high voltage phase is short, i.e. when it resembles a voltage spike, and there is much time for the negative charge to leak away. The high temperature phase in Fig. 3 is indeed short, and in PS0 light can arrive as flashes separated by long dark intervals. During a short high-voltage phase, conduction will dissipate little energy, but at a longer high-voltage phase it will dissipate more.

Oxidized CF_oF_1 is suited to act as the described ATPsynthase, since it becomes (kinetically) activated by a high $\Delta\mu_{H^+}$ (Junesch and Gräber, 1985, 1987; Ort and Oxborough, 1992), so that it will not be backwards active. During acid-base phosphorylation a $\Delta\mu_{H^+}$ threshold is commonly observed (Westerhoff *et al*., 1984). Evidence of the operation of a threshold-regulated ATP-synthase in chromatophores of intact cells of *R. capsulata* has also been given. The flash-induced potential decays much faster above a threshold potential (Cotton and Jackson, 1982; Taylor and Jackson, 1987). In chloroplasts, with uncouplers absent, a flash-induced potential decays faster above a certain threshold potential as well (Junge, 1970a). Saha *et al*. (1970) however could not detect the threshold reported by Schwartz (1968). It is therefore not clear whether a light-intensity threshold exists for photophosphorylation; the absence of a threshold points to a variable mode of ATPsynthase. According to Gräber and Witt (1976) the observations can be reconciled: at low ΔpH a threshold would be operative, but at a large ΔpH it would be absent.

(b) *Two different modes*

The simplest scheme for a variable mode of ATPsynthase is assumption of two values, say *n* and *m*, with $n < m$: note that each mode has its own equilibrium potential at which the ATP synthesis is in equilibrium with ATP hydrolysis, V_n and V_m, with $V_n > V_m$.

Many different values of the H^+/ATP ratio of CF_oF_1 have been published: Hall (1976) and Reeves and Hall (1978) give a table with more than 10 references - the most frequently reported value was 2. For chromatophores, early reported mode values were derived from the voltage decay after a light flash and were typically low, ~2 (Petty and Jackson, 1979c). More recent studies determined mode values in pH jump experiments (Mills and Mitchell, 1982; Junesch and Gräber, 1987). Gräber *et al*. (1987) and Rumberg *et al*. (1990) gave mode values of 4, Bogdanoff (1990) a value of 5 for reduced CF_oF_1, and a value of 3 for the oxidized enzyme.

The occurrence of mode switching is not commonly accepted by CF_oF_1 researchers. Ort and Oxborough (1992), for instance, assume a mode of 3 for both the oxidized and reduced enzyme. Mode determinations by pH jumps in the dark yield higher values (Mills and Mitchell, 1982). A low mode value directly after flashes and a higher one later in the dark obviously agree with the PS0 mechanism.

Measuring the mode by determining changes in H^+ and ATP concentrations is difficult, and mode values may be suspect. Each mode *n* is however also associated with a V_n, and strongly variable $\Delta\mu_{H^+}$ threshold values for ATP synthesis have indeed been found. Provided ΔG_P was constant, these variations are indirect evidence for the occurrence of a variable mode. CF_oF_1, for instance, requires a much lower ΔpH for ATP synthesis when reduced than when oxidized (Mills and Mitchell, 1982; Kramer and Crofts, 1989; Cramer and Knaff, 1991; original data from Junesch and Gräber, 1987). Uncouplers typically lower the threshold, whereas inhibitors of ATPsynthase (oligomycin) or of bc_1 (antimycin) enhance it (Melandri *et al*., 1977: Fig. 4). Such experiments (Baccarini-Melandri *et al*., 1977; Kell, 1979; Giersch, 1981, 1983; Giersch and Meyer, 1984; Adam *et al*., 1987; Sigalat *et al*., 1988, 1993) in which, at a fixed $\Delta\mu_{H^+}$ near a threshold value of ATP synthesis, addition of ionophores *increases* the phosphorylation rate, are naturally explained by an increase in the mode value: the larger distance from equilibrium, $\Delta\mu_{H^+} - V_m$, of the higher mode *m* results in a larger current and ATP synthesis rate, compensating for the smaller ATP yield per proton in the new mode (Melandri *et al*., 1974).

Mechanistically, the mode increase can be explained by the uncoupler enabling, during the catalytic cycle, proton exchange between the bulk medium and an otherwise shielded, membrane embedded proton binding site within the proton translocator of CF_oF_1, a site placed near the medium-membrane interface (see also Giersch and Meyer, 1984; Sigalat *et al*., 1988, 1993). The pertinent proton would cross the $\Delta\mu_{H^+}$ barrier upon carrier translocation, but would require an uncoupler to reach the aqueous medium at the other side. In contrast to models of localized proton coupling, long range coupling between RCs and CF_oF_1 by inner-membrane bound protons would be absent.

Even in the absence of changes in the redox state of ATPsynthase, mode changes may occur simultaneously with the phase transitions that occur in the lipids bound to ATPsynthase (Solaini *et al*., 1984). CF_oF_1 requires lipids (Pick *et al*., 1984, 1987). A mode dependency on the membrane composition has been reported (Guffanti *et al*., 1984; VanWalraven *et al*., 1990; Bakels *et al*., 1993). An increase of the mode value of CF_oF_1 with the temperature was proposed by Nishizaki (1973), who found in acid-base jump experiments a lower ATP production at 20°C than at 4°C. Similar results have been found during photosynthesis in flashing light in the range 5-50°C (Graan *et al*., 1981). Junge (1970b) has explained a membrane potential threshold for ATPsynthesis by different conformational states, with different electrical dipole moments, of CF_oF_1. If the proton carrier of CF_oF_1 also assumed distinct conformations under the influence of these dipoles, then different mode values at different potentials could result from pK changes of the amino acid residues that carry the pertinent protons.

In MTS/PS0 similar mode changes are postulated (Fig. 2). In particular it is postulated in the two different modes scheme that the high mode reaction can occur only backwards, by a kinetic regulation by $\Delta\mu_{H^+}$. By both charging and discharging of the membrane by ATPsynthase, but at different and distinct stoichiometries, free energy is won from membrane potential fluctuations. The modes are kinetically regulated ($n < m$): the enzyme is active in mode n only for $\Delta\Psi > V_n$ and active in mode m only for $\Delta\Psi < V_m$. Both modes are inactive for $V_m < \Delta\Psi < V_n$, an interval in which the enzyme switches its mode. A varying membrane potential of which the maximum is higher than V_n and the minimum is lower than V_m permits net ATP gain, since the same number of transported protons yields more ATP during forwards mode n activity than is consumed during their backwards return by mode m. Inactivity for $V_m < \Delta\Psi < V_n$ has to be postulated in order to avoid discharge of the membrane in the high mode when $\Delta\Psi$ rises.

The postulated enzyme with its low and high mode abilities can be associated with CF_oF_1 with its oxidized and reduced modes. At first sight, association of reduced CF_oF_1 with the high mode enzyme in MTS/PS0 may seem absurd since CF_oF_1 is not kinetically inactivated for $\Delta\Psi > V_m$: CF_oF_1 can, and in practice often does, strongly catalyze both the backward and the forward reactions (Schlodder *et al*., 1982; Ort and Oxborough, 1992). CF_oF_1 is regulated by redox state variation, thresholds varying with the redox state (Mills and Mitchell, 1982; Mills, 1984) and inactivation by ADP binding (Junesch and Gräber, 1987; Bogdanoff, 1990). The redox state of the ubiquinone pool, or of ferredoxin, determines the redox state of ATPsynthase; in particular ferredoxin may reduce it (Mills *et al*., 1980; Mills, 1984). By inactivating CF_oF_1, while it is reduced at a low potential, using the regulatory power of ADP generated by reverse action, CF_oF_1 can mimic the kinetic inactivation of the MTS/PS0 F_oF_1 at a low $\Delta\mu_{H^+}$. ATP hydrolysis, i.e. backward activity, by reduced CF_oF_1 has indeed been observed after a light-dark switch (see Carmeli and Lifshitz, 1972; Admon *et al*., 1982; Schlodder *et al*., 1982; Lemaire *et al*., 1985; Shigalowa *et al*., 1985, and the references therein; Joliot and Joliot, 1989).

An advantage of the scheme involving two different modes is that ATP can be won from an alternating membrane voltage even when its amplitude is small, provided n and m are large. For example, since V_8 equals 60 mV, V_{10} 47 mV, ATP could be won from an alternating voltage with a top-to-top amplitude a little larger than 13 mV. Another advantage is that a path can be indicated for the evolution of an ATPsynthase with the postulated mode variability from a primordial proton pump that worked during thermal cycling (Muller, in press).

(c) *Flexible coupling*
Instead of varying between two values, the mode may also vary discretely between several values, resulting in a seemingly continuous mode variation for an ensemble of ATPsynthases. Such 'loose coupling' or 'flexible coupling' may occur during both charging and discharging. For experimental evidence of such a continuous mode increase in chloroplasts, see (Schwartz, 1968: Fig. 7; DelCampo *et al*., 1968: Fig. 1; Gräber and Witt, 1976; Hall, 1976), in mitochondria, see (Petronilli *et al*., 1986: Fig. 5; Fitton *et al.*, 1994), in *Halobacterium halobium*, see (Michel and Oesterhelt, 1980; Helgerson *et al*., 1983). For other membrane systems, see Westerhoff *et al*. (1984), who also refers to cases where such flexible coupling not seems to be applicable. For a flexible coupling in V-ATPase see Davies *et al.* (1994). Ferguson (1985):

> 'Hence it can be argued that the failure of Δp [$\Delta\mu_{H^+}$] and ΔG_P to change in strict proportion is indicative of a variable stoichiometry with high values of H^+/ATP at low values of Δp. There is no direct experimental evidence against this possibility, but it is considered improbable, at least in the context of chemical reaction mechanisms. A variable stoichiometry would make the task of relating the steady-state kinetics of ATP synthesis to changes in Δp very difficult indeed.'

The mentioned improbability may follow from a presumed difficulty of envisaging the mechanism of a variable stoichiometry on the molecular scale. This can however be done by assuming that the F_o proton translocator—identified with subunit *a* (Fillingame, 1992)—can bind a variable number of the present proton-binding subunits—identified with subunit *c*, since its *Asp*/*Glu*-61 residue may carry the pertinent proton (Hoppe *et al.*, 1980, 1982; Fillingame, 1990, 1992). The number of *c*-subunits bound to subunit *a* would equal the mode value (in contrast, Cross and Taiz (1989) assume that the mode value equals the ratio of the number of *c* units and the number of catalytic units in F_1). As many as 10 *c*-subunits per *a* subunit can be available (Foster and Fillingame, 1982), which explains high mode values. Another possibility is that all *c* subunits are bound, but that only a few subunits can assume the proton-translocating conformation. An increased mode value upon uncoupler addition as reported in the foregoing is then explained by the uncouplers permitting proton exchange between the medium and the *c* subunits with inactive conformations.

A completely different model for flexible coupling in ATPsynthase has been given, based on analogies with models for the flagellar motor and for the sliding of myosin on actin during muscle contraction (Oosawa and Hayashi, 1986; Vale and Oosawa, 1990). During this sliding the displacement:ATP ratio seems to be variable[5]; this sliding model is, in turn, based on an idea of Feynman *et al*. (1963) concerning a microscopic heat engine, which uses a thermal gradient instead of thermal cycling. A detailed discussion of Oosawa's model, which is dissipative, even near equilibrium, falls however outside the scope of the present study.

Flexible coupling permits matching when the intensities of energy input and output differ, a clear advantage (Oosawa and Hayashi, 1986; Gerst *et al*., 1994). A gear box similarly matches different torques, a transformer different voltages. Flexible coupling is therefore a physical rather than a chemical concept. In standard chemical reactions with their fixed stoichiometries such matching is not possible. The vectorial character of ion translocation across a membrane and the continuous variability of the $\Delta\mu_{H^+}$ permits such flexible coupling in theory. Acquisition by organisms during evolution of flexible coupling, however involved, would obviously have given these organisms a selective advantage.

[5] Note the similarity between muscle contraction and ATP synthesis: during muscle contraction actin and myosin slide with respect to each other, while in ATPsynthase the proton translocator F_o may slide through the membrane.

(d) *Local coupling*
In order to explain some experiments, large increases in mode values, required for ATP synthesis at small $\Delta\mu_{H^+}$ values, would have to be accompanied by large increases in the mode values of proton pumps such as cytochrome oxidase; for evidence of such a mode increase by the cytochrome oxidase of mitochondria, see Wikström *et al*. (1981). In alkalophilic bacteria, for example, the $\Delta\mu_{H^+}$ decreases as the pH of the medium increases while ΔG_p remains the same and ATP synthesis continues (Krulwich and Guffanti, 1992). Other bacteria, and chloroplasts, appear to be able to synthesize ATP even in the absence of a $\Delta\mu_{H^+}$. This has led to the proposal that Mitchell's chemiosmotic mechanism may in some cases not apply, as the $\Delta\mu_{H^+}$ would be too small to be an intermediate in ATP synthesis. Instead, a local interaction or 'local coupling' between energy transducing machinery and ATPsynthase, in the form of direct intramembrane proton and free energy transport would occur (Westerhoff *et al*., 1984; Haraux, 1985; Sigalat *et al*., 1985, 1988; Ferguson, 1985; Dilley, 1991). In particular it has been proposed that chloroplasts can reversibly be brought in a localized, resp. a delocalized proton-transferring state by storing them in low, resp. high salt (100 mM KCl) (Sigalat *et al*., 1985; Beard and Dilley, 1986; Renganathan *et al*., 1993).

Variable local proton transfer to an ATPsynthase may be hard to distinguish from mode variability of the ATPsynthase (Muller, 1993); the localized and delocalized proton state would be equivalent with resp. a high and low mode ATPsynthase, the localized proton in the higher mode binding directly to F_o. This possible identification of the localized state with a high mode, and the delocalized state with a low mode, follows from the lower $\Delta\mu_{H^+}$ threshold for ATP synthesis in the localized state (Renganathan *et al*., 1991). Local coupling thus may be apparent, i.e. may be a limiting case of a functioning in a delocalized high mode.

Real cases of local coupling can also be implicated in MTS/PS0. Reversed local coupling would result in combined reversed proton and electron transport in the pertinent proton pump and reduction of a redox couple. The mechanism thus becomes rather involved. The forwards and backwards moving protons would have to move by a different H^+/ATP stoichiometry during the backward and forward processes of the MTS/PS0 cycle.

When only localized protons are involved in ATP synthesis/ hydrolysis, a changed dipole potential cannot result in free energy gain from thermal or light cycling by a membrane dipole-based mechanism, since the change of the membrane dipoles cannot drive the MTS/PS0 cycle by means of the membrane potential change. Instead, a conformational change is required that would for instance change the midpoint potential of a charge carrier. An example is cyt b_{559}, which can cycle between a high potential form, E_0' ~400 mV, and a low potential form, E_0' ~75 mV (Cramer and Whitmarsh, 1977). Another possibility is charging of the system during thermal cycling by oxidation/reduction of two redox couples, with an increase in the difference of their redox potentials: the difference in E_0' values of the couples would then have to be temperature dependent.

If ATPsynthase translocated a variable mixture of local and delocalized protons (Wikström and Krab, 1980), then free energy could be won from a cyclic dipole potential: the scheme is (1) by backwards localized action: appropriate reduction and oxidation of two redox couples, (2) switch to delocalized activity, (3) reoxidation and rereduction of the two redox couples, with a simultaneous build up of a $\Delta\mu_{H^+}$, (4) increase in dipole potential, and (5) discharge of the membrane by delocalized-functioning ATPsynthase. It is suggestive that local protons can be removed by a short heat treatment (Baker *et al*., 1981).

Clearly, a scheme based on local-coupling could support MTS/PS0, but the scheme would be rather involved.

The next Chapter discusses a model for local coupling based on dipole-dipole interactions.

At present the cause of the observed mode variations is still unclear. This problem may be solved when, just as the structure of F_1 (Abrahams *et al*., 1994), the structure of F_o becomes available. At this moment it is difficult to choose between the four given schemes. There may be no single generally applicable scheme, and ATPsynthases of different species or cells may operate differently, or they may adapt their ‘modus operandi’ to the circumstances. Whatever the detailed mechanisms, free energy could be won from externally caused membrane voltage changes, by charging and discharging the membrane differently, which is permitted by all four schemes. With the exception of a consideration of local coupling during a discussion of the early evolution of ATPsynthase, only the two different modes scheme is considered from now on.

5. The Photosystem 0 Cycle

Figure 9 gives the trajectory of the membrane voltage $\Delta\Psi$ on a $\Delta\Psi$ - $\Delta\Psi_{PDP}$ plot during one PS0 cycle. The cycle is similar to the thermosynthesis cycle (Muller, 1993: Fig. 3), the PDP replacing the temperature as a variable in the plot. Both the $\Delta\Psi_{PDP}$ and the membrane voltage $\Delta\Psi$ rise with the light intensity until the ATPsynthase is activated in a low mode. Further increase in $\Delta\Psi_{PDP}$ results in a discharge of the membrane through the ATPsynthase, which keeps $\Delta\Psi$ at the activation potential for the low mode. Lowering the light intensity causes the $\Delta\Psi_{PDP}$, and as a consequence, $\Delta\Psi$, to decrease, in turn inactivating the low mode. Upon a further $\Delta\Psi$ decrease the enzyme is reactivated in the backwards direction in the high mode. Backwards reactivation stabilizes the low $\Delta\Psi$ until the end of

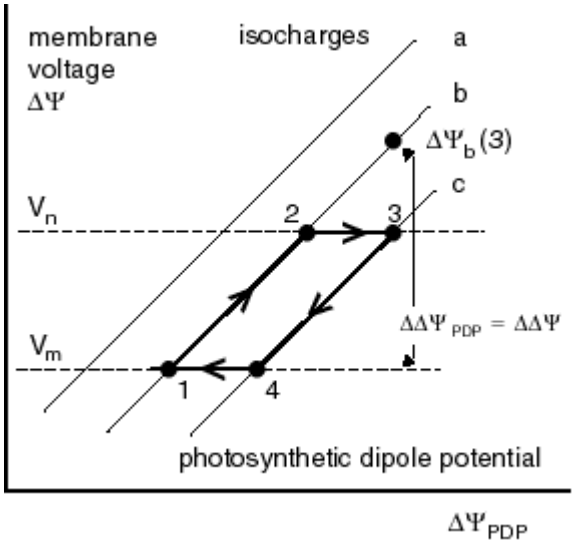

Fig. 9. The Photosystem 0 cycle: the membrane voltage - photosynthetic dipole potential ($\Delta\Psi - \Delta\Psi_{PDP}$) cycle of the newly proposed mechanism. Changes in $\Delta\Psi_{PDP}$ are the result of a changing light intensity. The graph of the cycle consists of isocharges and isopotentials. Isocharges (a, b and c) result from light dependency of the $\Delta\Psi_{PDP}$ in the absence of ATPsynthase activity. V_n and V_m are the equilibrium potentials for mode *n* (*n* H^+/ATP) and mode *m* (*m* H^+/ATP) activities. Isopotentials result from an ATPsynthase activity switched-on or -off by the voltage: ATPsynthase is active either for $\Delta\Psi \geq V_n$ in mode *n*, or for $\Delta\Psi \leq V_m$ in mode *m*; this activity restricts $\Delta\Psi$ to $V_m \leq \Delta\Psi \leq V_n$.

The cycle starts in 1, with $\Delta\Psi = V_m$, where a light intensity increase inactivates mode *m* because it has increased $\Delta\Psi$ by means of a $\Delta\Psi_{PDP}$ increase. Isocharge b is followed until V_n is reached (2) and mode 3 is activated. $\Delta\Psi_{PDP}$ continues to rise, but charge transfer by ATPsynthase keeps $\Delta\Psi$ at V_3 ($2 \rightarrow 3$). The free energy obtained as ATP by the charge transfer equals dQ V_n, in which $dQ = C_m (\Delta\Psi_b(3) - V_n)$ is the charge transferred. Subsequent decrease in light intensity in 3 decreases $\Delta\Psi$, which inactivates mode *n*. Isocharge c is followed until $\Delta\Psi$ drops below V_m, and mode *m* is activated (4). Along isopotential V_m the membrane is recharged ($4 \rightarrow 1$), at a cost of dQ V_m, until point 1 is reached again. The net free chemical energy gained as ATP is dQ (V_n - V_m).

the cycle. In Fig. 9 the total charge transferred along the isopotentials, dQ, follows from the difference between the voltage on isocharge b above point 3, $\Delta\Psi_b(3)$, and the voltage in (3), V_n:

$$dQ = C_m (\Delta\Psi_b(3) - V_n) = C_m \{ \Delta\Delta\Psi_{PDP} - (V_n - V_m) \}.$$

The work done per square cm membrane in one cycle on ATP/ADP equals:

$$W = dQ\, V_n - dQ\, V_m$$
$$= C_m \{ \Delta\Delta\Psi_{PDP} - (V_n - V_m) \} (V_n - V_m) \quad (8).$$

Table 3 gives values of W calculated with this expression, using several n and m combinations, and several estimated PDP values. W is also calculated for the case that recharging occurs by conduction. The tradeoff is that a high *n* value insures free energy gain even at a low $\Delta\Delta\Psi_{PDP}$, but that it results in a low W at a high $\Delta\Delta\Psi_{PDP}$, whereas at a low *n* no free energy gain is possible at a low $\Delta\Delta\Psi_{PDP}$ but the free energy gain at high $\Delta\Delta\Psi_{PDP}$ is high.

Switched modes		**$\Delta\Delta\Psi_{PDP}$ [mV]**					
n	**m**	**70**	**100**	**150**	**200**	**400**	**600**
1	2	<	<	<	<	39	86
1	5	<	<	<	<	9	84
2	3	<	1,7	5,6	9,5	25	41
2	5	<	<	1,3	8,3	36	65
3	5	0,5	2,3	5,5	8,6	21	34
3	8	<	0,2	5,1	10,0	30	49
1	∞	<	<	<	<	<	61
2	∞	<	<	<	<	39	86
3	∞	<	<	<	6,8	38	69
5	∞	<	0,6	5,3	10,0	29	48
7	∞	0,2	2,2	5,6	8,9	22	36
10	∞	1,1	2,5	4,8	7,2	26	26

Table 3. Free energy gain [nJ/cm^2] in one PS0 cycle for several mode value combinations and several $\Delta\Delta\Psi_{PDP}$ values. Recharging by conduction is indicated by a mode value '∞'. The free energies gained have been calculated using Eqn. 8. Assumed value for C_m 1 μF/cm^2, for V_1 470 mV. $V_i=V_1/i$: hence V_2=235, V_3=157, V_4=118, V_5=94, V_7=67, V_8=59 mV; obviously V_∞ = 0 mV. '<' indicates that $\Delta\Delta\Psi_{PDP}$ is too small to span the difference between V_n and V_m.

W is a function of *n* and *m*; its optimum is $\frac{1}{2}\ C_m\ \Delta\Delta\Psi_{PDP}{}^2$, reached for $V_n - V_m = \frac{1}{2}\ \Delta\Delta\Psi_{PDP}$. The foregoing shows that in theory $\Delta\Psi_{PDP}$ can reach values of hundreds of millivolts. A value of 740 mV, and *n* and *m* values of 1 and 5, yield 137 nJ/cm^2 for W. Table 3 shows that a $\Delta\Delta\Psi_{PDP}$ of 200 mV, with *n* and *m* values of 3 and 8, yield 10 nJ/cm^2, a value used for comparison. The possible range of W is estimated to be 0,5-100 nJ/cm^2. Approaching values of ~100 nJ/cm^2 is more probable for PS0 than for MTS, because a high $\Delta\Psi_{PDP}$ is plausible for the observed membranes with crystallized RCs.
The specific power, power per unit mass, of PS0, follows from

$$P_{PS0} = SD\ W / t_{cyc}\ \rho \quad (9),$$

where SD is the surface density of the membrane, i.e. the surface area per unit volume, t_{cyc} the cycle time, and ρ the mass density of the organism, 1,0 g/cm^3. The cycle time may be as small as seconds, since electron transport within the RC occurs in milliseconds, and *in vivo* the ATPsynthase of chloroplasts can be activated and deactivated within milliseconds and seconds, respectively (Harris and Crofts, 1978; Inoue *et al*., 1978).

Specific respiration power rates, P_R, of organisms have been calculated from published oxygen consumption rates (Muller, 1985) and were corrected (Muller, 1993) to obtain P_R in terms of ATP synthesized. Comparisons with P_{PS0} are of interest although of limited validity because of the inaccuracy in the values of the pertinent variables. Organisms in natural waters and biomembranes carried along by the cytoplasm stream in leaf palisade cells are now considered.

Organisms in natural waters. The cycle times of fluctuating light were estimated to lie between 0,1 and 10 s. The membrane SD in microorganisms may be as large as 500 000 cm^{-1} (Muller, 1985). For the unicellular green algae *Dunaliella tertiolecta* SDs of 145 000 cm^{-1}, at 100 μmol m^{-2}s^{-1}, and 56 000 cm^{-1}, at 1750 μmol m^{-2} s^{-1}, have been reported (Sukenik *et al*., 1987: Table 2). These values were taken with respect to the volume of the whole cell. With respect to the volume of the chloroplast, the SDs are 438 000 cm^{-1} and 217 000 cm^{-1}; since chloroplasts can be considered to be symbiotic cyanophyta, similar values may apply to cyanophyta. The value of 500 000 cm^{-1} and a t_{cyc} of 1 s yield a P_{PS0} of 5 mW/g (possible range: 0,25-50 mW/g), a t_{cyc} of 10 s a P_{PS0} of 0,5 mW/g (possible range: 0,025-5 mW/g). Comparison with the P_R ranges of phycophyta (0,05-0,63 mW/g), cyanophyta (0,9-7,7 mW/g) and bacteria (3,1-154 mW/g), shows that P_{PS0} is comparable to P_R in all microorganisms.

Palisade cells in leaves. The cytoplasm stream (Kachar and Reese, 1988) circulates the organelles in the light gradient within palisade cells. The velocity of the cytoplasm stream varies widely; most values lie around 10 μm/s, but some are as high as 1 mm/s (Kamiya, 1962). For Characean internodal cells of *Nitella*, the velocity is 25 μm/s at 10°C, and 75 μm/s at 25°C (Plieth and Hansen, 1992: Fig. 7). A cell length of 50 μm results in a t_{cyc} of 10 s for a velocity of 10 μm/s, and 2 s for 50 μm/s.

Table 4 gives data on the surface density SD, showing a strong variation, which may be due to the strongly variable size of the vacuole in plant cells. Dehydration during fixation and embedding can decrease the volume of the plant cell by 73% (Winter *et al*., 1993), resulting in an SD increase by a factor of 2,7. Because of the uncertainty in the extent of such shrinkage, published SDs should be used with caution.

An SD value of 100 000 cm^{-1} yields a P_{PS0} of 0,1 mW/g (possible range of 0,005-1 mW/g), which overlaps the P_R range of most leaves, 0,06-1,5 mW/g (Muller, 1985). P_{PS0} could become significant when the light intensity approaches the compensation point or respiration rate, for which a range of 0,5-3 μmol CO_2 m^{-2} s^{-1} has been given (Jones, 1992), equivalent to[6] 1,25-7,5 mW/g. Higher cytoplasm stream velocities would increase P_{PS0}. Rotation diffusion of a chloroplast may have a t_{cyc} of ~1 s; the associated P_{PS0} is 1 mW/g (possible range 0,1-10 mW/g).

[6] The ΔG for CO_2 fixation can be estimated in several ways. The ΔG for the $CO_2 + H_2O \rightarrow$ glucose + O_2 reaction is about 478 kJ/mol-C (Muller, 1985). For plant material, a value of 525 kJ/mol-C has been given (Jones, 1992), for biomass 560 kJ/mol-C (Westerhoff *et al*., 1982). For simplicity a value of 500 kJ/mol-C is assumed. Assimilation of 1 μmol CO_2 m^{-2} s^{-1} is then equivalent to a power per square meter leaf of 0,5 W/m^2. Taking for the mass density of a leaf 20 mg/cm^2 (Shull, 1930), i.e. 200 g/m^2, results in a specific power of 2,5 mW/g. The uncertainty in the mass density constitutes a large inaccuracy, since much higher and much lower values have been determined. Pearcy (1990), for instance, reports a water content of 50-100 g H_2O m^{-2} for shade-grown leaves.

species	value [cm^{-1}]	method	reference
average 200 species	43 000	Chl	Chl (*a*+*b*) conc. ~0,26% FW; Rabinowitch, 1945: data from Table 15-1
cucumber (on aging)	37 000 → 14 000	EM	Forde and Steer, 1976: data from Table 1
sunflower	120 000 90 000 stacked 30 000 unstacked	EM	Fagerberg, 1983: data from Table 1
soybean	50 000	Chl	Chl conc. 0,6 g/m^2; from a leaf thickness of 0,2 mm a Chl density of 3000 $\mu g/cm^3$ follows. Gutschick, 1984
sunflower	~90 000	EM	Fagerberg, 1987: data from Table 1
pea	35 000	Chl	Lee and Whitmarsh, 1989: Fig. 5
barley	22 000	Chl	1,3 mg Chl/g FW. Winter *et al*., 1993

Table 4. Surface densities of the thylakoid membrane. The surface density can be measured directly from EM pictures, or can be estimated from Chl content, since 1 µg Chl may be equivalent to a membrane surface area of 16,7 cm^2 (Barber, 1972).

In PS0 about one or two excitons, or maybe 10, are used per RC per second, whereas in PS this number is typically ~100 s^{-1} (Kok and Cheniae, 1966). The estimated P_{PS0} must then be a factor of 10-100 smaller than the observed P_{PS}. Published maximal CO_2 fixation rates at atmospheric CO_2 concentrations are 14-40 for C_3 plants, 18-55 for C_4 plants and ~7 µmol CO_2 m^{-2} s^{-1} for CAM plants (Jones, 1992: Table 7.1), from which maximal P_{PS} ranges of 35-100 (C_3), 45-140 (C_4) and ~17 mW/g (CAM plants) are calculated. At low light intensity P_{PS} must of course be less. Hence P_{PS0} could be comparable with the - low -specific power of photosynthesis in CAM plants, but, again, in general its power must be much smaller than the power of standard photosynthesis.

PS0 uses fewer parts that standard PS, and thus is a simple device for free energy gain from light intensity fluctuations. PS0 invokes no new physico-chemical or biochemical principles. It makes use of quick changes, and its investigation may therefore require new experimental methods.

III EVOLUTION MODEL FOR PHOTOSYNTHESIS

Thermosynthesis is an alternative (Muller, in press) to the commonly presumed primeval energy sources of fermentation and photosynthesis (Broda and Peschek, 1979). A model for acquisition by the first, thermosynthesizing organisms of the hereditary apparatus and an ATPsynthase with a variable stoichiometry has previously been given (Muller, in press). A model in which Photosystem 0 is an intermediate during the stepwise evolution from thermosynthesis to present day photosynthesis is presented here. In contrast with Boxer's model (1992) for the evolution of photosynthesis, the first RCs are already functional.

1. *The Evolution of ATPsynthase*

In the proposed model ATPsynthase is present in the first MTS/PS0 systems. In the beginning its catalytic cycle may have been synchronized with thermal or light intensity cycling, and its turnover time may accordingly have been large, ~1 s. ATPsynthase may have coevolved with photosynthesis (Blankenship, 1992), in particular with RCs, towards the present day enzyme with a turnover time that can be as small as ~2 ms (Junge *et al*., 1992); the typical value is 10-100 ms. The synchronization may have been indirect, for example by the environmental cycling changing the polarization of another enzyme or a lipid domain, and this polarization change in turn effecting ATPsynthase. During evolution the coupling to the temperature or to other enzymes loosened, resulting in ATPsynthases that coupled to their environment by means of the $\Delta\mu_{H^+}$ across the membrane only.

A previous study (Muller, in press) proposed a progenitor of the F_1 part of F_oF_1, a pF_1, that synthesized ATP during thermal cycling and that was not membrane bound. Analogous to the binding change mechanism for tightly bound ATP in present day F_oF_1 (Boyer, 1989), which uses a conformation change in F_o to change the conformation—and thus the nucleotide binding strength—in F_1, pF_1 uses a thermally induced binding change. pF_1 became membrane bound as it acquired lipid domains undergoing, during thermal cycling, thermotropic transitions that assisted in its catalytic cycle. The pertinent interactions between lipid and protein structural states would be similar to those in lipid-cyt *c* complexes (Heimburg and Marsh, 1993). Contemporary CF_oF_1 interacts with bound lipids (Rawyler and Siegenthaler, 1989) and is stimulated by unsaturated lipids (Pick *et al*., 1984).

Modern ATPsynthase may contain a large dipole (Junge, 1970b, 1972; Deléage *et al*., 1986). Monolayers of the possibly related enzyme Na^+/K^+-ATPase show a significant dipole potential (Zaitsev and Möbius, 1994). The present study describes the evolution of photosynthesis in terms of light-induced dipoles formed within the membrane, dipoles that during the course of evolution lengthened until they could transfer charge across the membrane. Just as in photosynthesis, charge transfer across the membrane may at first have been absent in membrane-bound pF_1: instead, it may have been driven by the coulomb interaction of its dipole with other dipoles. Such an interaction would constitute a local coupling that could evolve to delocalized coupling. When the number of membrane embedded PS0 dipoles that were clustered around pF_1 increased stepwise, the distance became larger than the Debye length, 9,5, 3,0 and 0,9 nm for resp. 1, 10 and 100 mM KCl (see Atkins, 1986). The direct dipole-dipole coupling gave way at that point to coupling by means of the delocalized membrane dipole potential, i.e. essentially a membrane potential. There thus would have been selective pressure for both RCs and pF_1 to utilize charge translocation across the membrane. This model for the evolution of F_oF_1 differs from an MTS based model for acquisition by pF_1 of proton translocation and energy transduction between ATP and $\Delta\mu_{H^+}$ that was given in the foregoing study (Muller, in press); therein the first membrane-bound pF_1 only functioned as a proton pump during thermal cycling and did not synthesize net ATP.

Present day occurrence of remaining dipole-dipole coupling can explain the enigma of localized coupling (Slater, 1977; Hitchens and Kell, 1982; Westerhoff *et al*., 1984; Dilley, 1991), i.e. coupling attributed to local proton transfer. A free energy gain by ATPsynthase from coupling by coulomb interaction with dipoles of neighbouring proteins, including electron transport chains, has been proposed (Westerhoff *et al*., 1986; Astumian *et al*., 1987; Tsong and Astumian, 1987). The disappearance of local coupling in chloroplasts at a high salt concentration, 100 mM KCl (Beard and Dilley, 1986; Renganathan *et al*., 1993), is consistent with a screening by the salt of the coulomb

interaction that presumably causes local coupling. Thus, whatever the occurrence and mechanism of local coupling at present, a similar free energy gain from synchronization of the catalytic cycle of pF_1 with environmental cycles by such interaction may very well have occurred during early evolution.

However, both ATPsynthases with a variable mode and ATPsynthases with local coupling ability may have been present during early evolution, just as ATPsynthases with the other capabilities mentioned in Chapter 1. Hereafter the early presence of an ATPsynthase with a variable mode is assumed.

2. *The Thermosystem*

(a) *The Thermosystem and the Reaction Center*

Parsimony analysis of 16S rRNA has shown that the last common ancestor of all present day organisms was probably an archaebacterium that grew at the boiling point of water (Woese, 1987). Nowadays no photosynthesizers grow above 73°C (Sirevag, 1991), which suggests that photosynthesis came late during evolution. Late emergence also follows from the complexity of photosynthesis (Calvin, 1969). Photosynthesis is absent in most archaebacteria, and when it is present (the halobacteria) it is insignificant for power generation; in the eubacteria, in contrast, photosynthesis is widespread and significant (Woese, 1987).

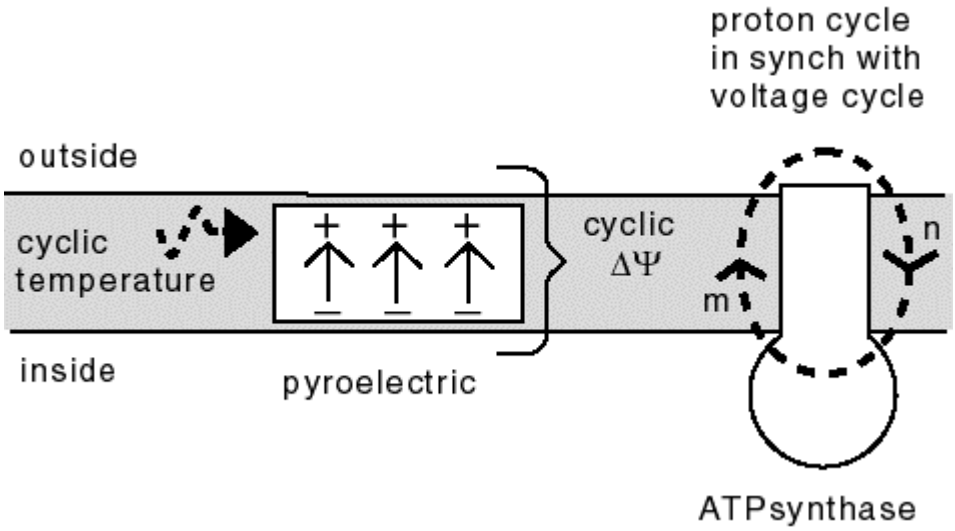

Fig. 10. The thermosystem. Cyclic changes in polarization result in cyclic changes in membrane polarization, which in turn result in cyclic changes in membrane potential. By charging and discharging the membrane at different H^+/ATP ratios *n* and *m* (Fig. 9) net ATP is gained.

The primordial cell is assumed to have acted as a heat engine, converting, during thermal cycling, heat into the chemical energy of ATP (Muller, 1985, 1993, 1994, in press) by means of thermosynthesis. Such a primordial thermosynthesizing organism is called a 'thermosystem' (TS) (Fig. 10). Thermosynthesis is a suitable primordial energy source as it requires only one enzyme, an ATPsynthase. The necessary temperature dependence of the electrical polarization of the membrane is caused by thermotropic phase transitions of lipids, or conformational changes with the temperature (Muller, 1993) of membrane proteins with charge anisotropy across the membrane (Sitaraman *et al*., 1992), or a cooperative transition of both lipids and proteins (Heimburg and Marsh, 1993). The temperature can affect in particular the electrical dipole of a protein by changing its α-helix content: the α-helix conformation is associated with a large electrical dipole (Sitkoff *et al*., 1994). The membranous TS-supporting protein, the primordial MTS/PS0 RC, is called 'RC0'; its density in the membrane was high. PS0 emerged upon the binding of lipid pigments such as BChl to RC0.

It is of interest to compare RC0 with the BRC of *Rp. viridis*. This BRC contains 8 negative charges on one side and 6 positive charges on the other side of the membrane, and thus constitutes a large dipole (Deisenhofer and Michel, 1989: Fig. 16). Application of Eqn. 5b to a monolayer of such BRCs—substituting 6 for *n*, 4 nm for *x*, 25 nm^2 for A (Deisenhofer and Michel, 1989), 6 for ε_r (Gao *et al*., 1990)—yields a MDP value of 482 mV. A change in thickness of this BRC must result in a change of

the MDP (a change of 1,6 nm would result in a change of 200 mV), and it would be interesting to study the thickness of the BRC and its constituting L and M proteins during thermal cycling. Collagen, for instance, can contract by a factor 4 upon a temperature rise of a few degrees (Gustavson, 1956). It was already mentioned in Chapter 2 that the *Rp. viridis* BRC can assume two different conformations, of which one is associated with a low temperature or a high lipid rigidity, the other with a high temperature and high fluidity (Sebban and Wraight, 1989; Sebban *et al*., 1991; Gao *et al*., 1991; Baciou and Sebban, 1992), and in *Rb. sphaeroides* a similar biphasicity has been observed (Sebban, 1988).

Conformational transitions of BRCs (Kleinfeld *et al*., 1984) upon illumination may be a vestige of MTS transitions[7]. An entropy decrease is part of the heat engine cycle, and would also have to occur in RC0 during thermosynthesis. Interestingly, during electron transport within the BRC large changes in entropy occur. From temperature dependency of midpoint potentials and flash induced volume changes it has been concluded that in *Ch. vinosum* and *Rb. sphaeroides* formation of the cyt c_{555}^{+}-Q_A^{-} dipole is associated with an entropy decrease that accounts for all free energy remaining from the excitation (Case and Parson, 1971; Callis *et al*., 1972). Upon electron transfer to Q_B the entropy, and the enthalpy, increase again (Arata and Parson, 1981a, 1981b, 1982), just as in a phase transition caused by a temperature increase: a BRC therefore mimics upon photon absorption the temperature increase in a heat engine cycle[8]. An associated conformation change has been observed by FTIR spectroscopy (Nabedryk *et al*., 1990). Enthalpy and entropy contributions to free energy changes in BRCs can also be deduced from temperature dependency of recombination rates (Gunner, 1991); this method yields however values different from those obtained by the methods just described (Arata and Parson, 1981b, 1982). The reported free energy storage in BRCs as negative entropy is therefore somewhat suspect.

(b) *Power*
Assuming a cycle time of 10 s, and a membrane potential difference of 200 mV, the MTS power per square cm membrane equals 1 nW/cm^2 (Table 3: n = 3, m = 8). Can organisms live on so little power? Consider the specific MTS power of a bacterium with the shape of a cube with an edge of 1 µm. Let MTS occur in the cytoplasmic membrane, which has in this example a surface area 6 10^{-8} cm^2. At a density of 1 g/cm^3 the mass then equals 10^{-12} g, and the specific power equals
$6\ 10^{-8} \times 10^{-9} / 10^{-12} = 0{,}06$ mW/g.

For bacteria the Y_{ATP}, the yield in dry biomass per mol ATP, can be 10 g/mol-ATP (Bauchop and Elsden, 1960). Tempest and Neijssel (1984) give a value of 12 g/mol-ATP; at 100% efficiency the Y_{ATP} would have to be 25 g/mol-ATP (this value follows from the free energy of biomass), i.e. the actual efficiency is ~48%. A value of 38% has been called a ‘good efficiency for many energy conversions’ (Lawlor, 1993).

From a ΔG_P of ATP hydrolysis in bacteria of ~40 kJ/mol (Vink *et al*., 1984), it follows that 0,25 g dry mass, or 0,926 g wet mass (assuming 27% of the bacterium mass is ‘dry weight’ (Winkler and Wilson, 1966)) is synthesized per kJ free energy. Conversely, the doubling of one gram biomass costs 1080 J/g. More generally, at a power of 0,06 mW/g, doubling takes 18 000 000 s, or 208 days. The lowest growth rate determined in a chemostat may be ~0,006 hr^{-1} (Raven and Beardall, 1981), equivalent to a generation time of 7 days. The power of MTS is therefore very low.

On the other hand one may expect a very low power for the first organisms. Nowadays some bacteria still grow in many habitats under very low energy conditions (Gotschall, 1990): *in situ* generation times in deep seawater can be 210 days and in soil 20 days (Matin *et al*., 1989). For dormant cells at very low

[7] Thickness changes of BRCs upon charge separation are not relevant for our purpose. The volume decrease is 20 ml/mol (Arata and Parson, 1982: Table I), equivalent to 3,3 10^{-2} nm^3/RC ($20\ 10^{-6} \times 10^{27}$/ $6{,}0\ 10^{23}$). Assuming an A_{RC} of 33 nm^2, the thickness decrease equals a negligible 0,001 nm.

[8] The photocyle of bacteriorhodopsin also contains a step with a large entropy decrease (Váró and Lanyi, 1991a, 1991b).

growth rates maintenance energy (Pirt, 1965) may approach zero (Pirt, 1987). The available data suggests therefore that MTS can generate sufficient power to permit the same doubling times as some extant microorganisms have. MTS/PS0 and PS0 would yield even more power.

Except for plants surviving desiccation, all higher organisms are continuously metabolically active, even when dormant. In this aspect these organisms resemble machines that are continuously switched on. The flywheel never stops. Lower organisms are however often dormant. The first organisms may have gained energy only intermittently, during irregular environmental changes; energy which was not stored but which was used at once. Obviously, it makes no sense to apply the concept of power to organisms that gain and use energy only piecemeal, and that are metabolically inactive most of the time. During continuously fluctuating conditions, one can however apply the concept.

The switch from intermittent free energy gain to continuous energy gain would have made it much easier for organisms to perform work continuously. Strictly speaking, continuous energy gain is not necessary for continuous work: when one fills a bathtub intermittently with buckets one still can obtain an almost constant flow through the drain, but this constant flow is more easily attained by filling the tub with a tap that is constantly open. The ability to perform work continuously is of interest since it permits the attribution of a dissipative structure (Prigogine, 1969; Glansdorff and Prigogine, 1971; Nicolis and Prigogine, 1977; Haken, 1978) to the organism *regulated by the organism itself*. This capability may be a necessary condition for calling an organism ‘alive’. In this narrow, thermodynamic sense one may pinpoint the origin of life as the moment when organisms acquired an energy reservoir together with a self-regulated dissipative structure (Kuhn, 1994).

(c) *Chlorophylls*
Photosynthesis requires pigments. Synthesis of porphyrins under prebiotic conditions has been demonstrated (Hodgson and Ponnamperuma, 1968), but MTS based organisms must have developed their own source of porphyrins, probably by using enzymes that descended from a nitrogenase. Nitrogenase is homologous to the enzymes that synthesize BChl, with nitrogenase being the older enzyme (Burke *et al*., 1993). Nitrogen fixation consumes much energy and requires a high membrane voltage (Haaker and Klugkist, 1987). Interestingly, nitrogen fixation has been named as a possible application of the macroscopic thermodielectric converter, which is based on a capacitor (Gonzalo, 1976). An ancestral microorganism with a nitrogenase can similarly have utilized thermal cycling, with its membrane functioning as a capacitor. RCI may have originated from an RC0/nitrogenase combination: both nitrogenase and RCI contain an FeS cluster, and both have reducing ability (Burke *et al*., 1993). The possible descendency of RCI from nitrogenase suggests that RCI preceded RCII, and that a progenitor of RCII lost its FeS cluster (Olson and Pierson, 1987). The nitrogenase may have helped in the synthesis of BChl (Burke *et al*., 1993), permitting BChl to assist in its own synthesis.

Chl forms monolayers that have a surface dipole potential of a few hundred mV (Hanson, 1939; Bellamy *et al*., 1963; Reinach *et al.*, 1973). Between 4 and 20°C the Chl monolayer shows a thermotropic phase transition (Heithier *et al*., 1983) that could change the surface dipole potential; Chl monolayers may therefore support MTS.

3. *From Thermosystem to Photosystem 0*

An MTS carried along by a convection current would move in and out of the photic zone, and thus would experience cyclic changes in light intensity (Fig. 3). The energy won by MTS was enhanced when a pigment-protein complex formed in the light a metastable dipole that added to the polarization change caused by the temperature change. In addition to the direct contribution to the polarization because of the charge separation within the RC, the complex may also have added indirectly: upon excitation a primordial RC0 could decrease the local fluidity, and change the polarization of an associated lipid domain.

The new system would act as a combined MTS/PS0 device. The pigment would be placed in the vicinity of the MTS dipoles, and be subject to their electric field. Present day electrochromic shifts in the spectrum of photosynthetic pigments may be a remnant of this field. During evolution the relative contribution of PS0 grew with respect to that of MTS, as the stability and dipole length of the excited state increased, for instance by (1) enhanced stability by triplet formation, (2) quick reexcitation after deexcitation, and (3) the introduction of temporary charge storage.

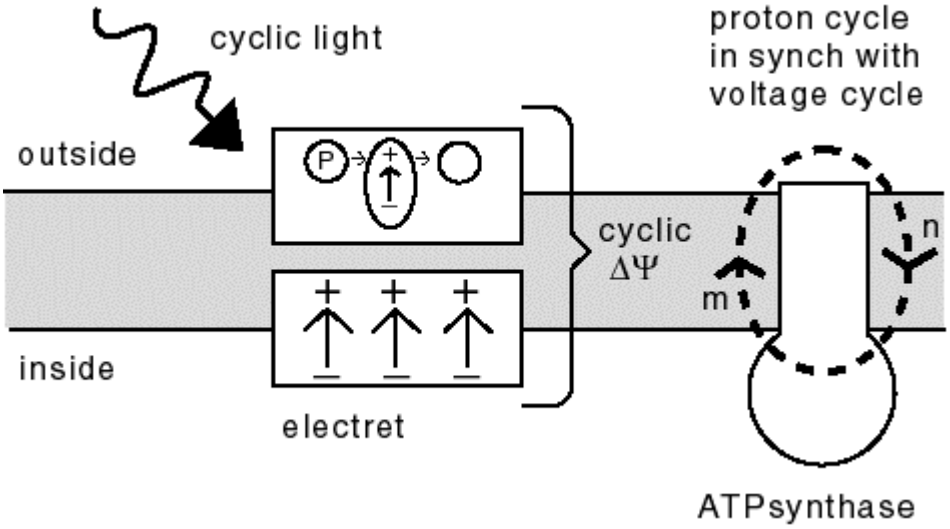

Fig. 11. Photosystem 0. Upon illumination of the reaction center an electron is temporarily displaced. Averaging over many reaction centers and recombinations results in an average dipole moment during illumination. In the figure the electron moves in the electric field caused by the dipole layers associated with thermosynthesis; their electric field causes an electrochromic shift in the absorbance spectrum of the pigment.

Two types of combined MTS/PS0 systems are possible, since the light phase can occur during either the cold or the warm phase of the thermal cycle: ‘cold-light MTS/PS0’ and ‘warm-light MTS/PS0’. The cold-light case applies to organisms carried along by convection, since in convection a high temperature is associated with the dark (Fig. 3). For organisms such as photosynthetic bacteria (Joss *et al.*, 1994) and algae that migrate vertically in natural waters containing a thermocline, the warm-light MTS/PS0 case would have to apply. Previous studies (Muller, 1985, 1993) gave an argument in favor of the applicability of the warm-light case in leaves: in palisade leaf cells the heating by sunlight on the sun side and the cooling by transpiration on the shade side (Lange, 1959) cause a temperature gradient (Perrier, 1971), and membranes will be carried along by the cytoplasm stream in these cells, causing illumination when the chloroplasts are warm. If a temperature gradient exists between the leaf interior (warm) and both its surfaces (cold) (see Rabinowitz, 1956) then the cold-light MTS/PS0 case would be applicable when the cytoplasm stream circulated the chloroplasts in this gradient. In leaves the palisade cells are sometimes found in one layer, and sometimes in two; clearly, one cell layer is suited for the warm-light case, two cell layers for the cold-light case.

(a) *Intramolecular Charge Separation*
Pure Chl monolayers do not fluoresce because of concentration quenching, but mixed Chl-lipid monolayers do fluoresce, showing the occurrence of metastable excited states (Tweet *et al*., 1964a, 1964b; Beddard and Porter, 1976). Upon illumination the surface dipole potential of Chl monolayers does however not increase (Bellamy *et al*., 1963). Excited chlorophyll dimers, or 'special pairs', do have larger $\Delta\mu$ values (Krawczyk, 1991; Middendorf *et al*., 1993). When present in phospholipid vesicles, chlorophyll shows an enhanced fluorescence upon a thermotropic phase transition to the fluid state; this is attributed to the lessened concentration quenching (Colbow, 1973), and demonstrates enhanced stability of metastable states under this condition.

Consider a monolayer of RCs containing only excited dimers of BChl or Chl, and apply Eqn. 7. In that case A could be small, ~25 nm^2. Since x ~0,1 nm, and $n_e = 1$, an ε_r of 2 results in a $\Delta\Psi_{PDP}$ of ~36 mV. Even if the excited state is properly stabilized, then a layer of such dipoles forms only a small, but not insignificant, membrane voltage; the high RC density can compensate for the small magnitude of the dipoles. The dipole would however have to be stabilized, for instance by triplet formation (Hoff, 1986; Budil and Thurnauer, 1991; VanKan, 1992; Breton and Nabedryk, 1993). For proteins the halftime of triplets varies considerably, and values of ~0,1-10 s are common (Vanderkooi and Berger, 1989). Triplets have been invoked in photoinhibition of PSII (Vass *et al*., 1992).

The PDP of about tens of millivolts, caused by *intra*molecular charge transport in excited pigments is small compared to the PDP that can be obtained by *inter*molecular transport. The contribution of the PDP to the dipole potential of the membrane is thus expected to have been small at first, the main contribution to the dipole potential changes assumably originating from MTS: the evolution of photosynthesis may have begun by piggybacking on MTS, for which large potential changes, ~100 mV, are much more plausible (Muller, 1993). PS0 could however have functioned during membrane potential changes of a few tens of millivolts, provided the mode of ATPsynthase was large or varied between large values.

Another possibility is use of carotenoids (x ~0,6 nm), which would result in a $\Delta\Psi_{PDP}$ of ~220 mV, provided, again, that their excited states were sufficiently stable. Both archaebacterial lipids and carotenoids can span a membrane. Upon excitation carotenoid-protein complexes show $\Delta\mu$ values as high as $35/f$ D (Gottfried *et al*., 1991a, 1991b). When this dipole was metastable and was present at a high density it could easily have supported a large PDP. It has been suggested that carotenoids became functional upon the acquisition of oxygen evolution (Clayton, 1980), with the function of deactivating singlet oxygen (Demmig-Adams, 1990); they could just as well have been part of an ancient PS0, instead.

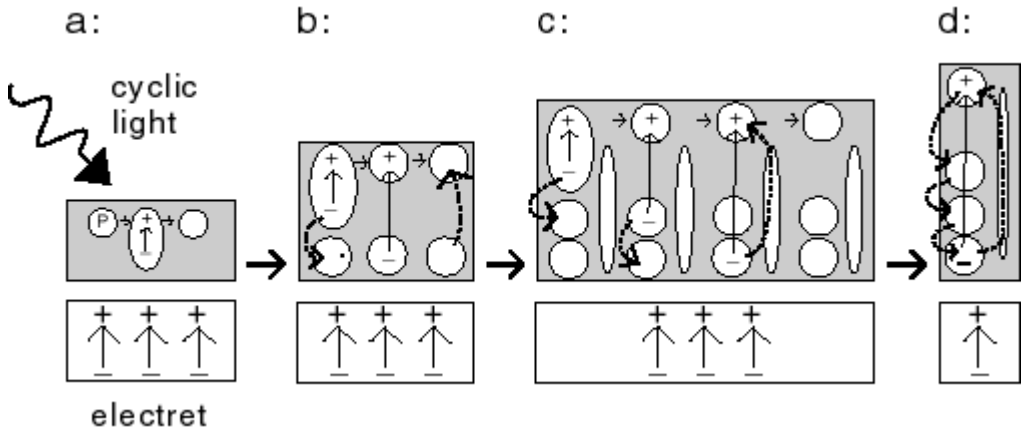

Fig. 12. During evolution an electron transfer chain developed as stepping stones were added to the system described in Fig. 11. The chain evolves into a membrane-spanning path for the electron excited by light absorption. A distinct path for the backward reaction of the displaced electron may have evolved simultaneously (elongated ellipse in c and d).

(b) *Intermolecular Charge Separation*
The first step in the evolution of electron transfer chains was intermolecular charge transport by reduction of charge carriers by excited chlorophylls (Krasnovsky, 1971). An electron transfer chain can have arisen by stepwise addition to RC0 of acceptors that allowed the excited electron a temporary stop at a larger distance from the pigment, and of donors that allowed the temporary loan of an electron for reduction of the oxidized pigment (Figs 11-12). Thus *x*, the distance between the separated charges, increased, simultaneously with the PDP. By giving the charge carriers appropriate midpoint potentials, free energy could temporarily be stored as redox power.

The Reducing Side
At the reducing side of an RC many different quinones can accept electrons in contemporary photosynthesizers (Hansson and Wydrzynski, 1990); all these quinones are candidates for primordial electron acceptors. Temporary charge acceptors may also have been present outside the membrane. Addition of a temporary electron acceptor, $Fe(CN)_6^{4+}$, to Chl monolayers resulted in increases of the surface dipole potential of up to ~33 mV (Seta *et al*., 1987: Figs 2-4). In the primordial acceptors metastability of the reduced state remained required: in fluctuating light the electron should have returned to the pigment, or its electron donors before the end of the dark period. In Fig. 11 the light-induced dipoles are parallel to the electric field of the local dipole but are antiparallel to the field of the voltage across the membrane: this manner permits evolution towards a system with charge separation ability. In the device of Glazebrook and Thomas the formed dipole is parallel to the field of the external voltage (Fig. 4A); in such a device the voltage can increase during illumination, using the local field of a pyroelectric (Fig. 4B). The addition to RC0 of bound quinones allowed the temporary storage of more displaced electrons, and the addition of the progenitor of the OEC similarly allowed the simultaneous temporary storage of several positive charges. This increased the PDP by an increase in n_e.

As discussed above, quinones also permit contrast in the PDP light response, which further increases the efficiency of PS0. The quinone pair in the BRC may have arisen (Deisenhofer and Michel, 1989) by duplication of a monomeric RC (Blankenship, 1992), yielding a primordial RC with two ubiquinone reducing electron transfer chains: the L and the M chains that contain resp. Q_B and Q_A. The L chain lost the ability to transfer charge to Q_B. Thereafter excited electrons in this chain moved instead to the quinone of the M chain, Q_A, from where these electrons could double-reduce Q_B, where the charge was stabilized in the absence of a direct return path along the L chain. Closing and opening of electron pathways along the chains can be attributed to changes in the local dielectric constant (Steffen *et al*., 1994).

The bacterial counterparts of RCI and RCII may descend from a common ancestor (Nitschke and Rutherford, 1991). RCI also contains two quinones (Golbeck, 1987; Golbeck and Bryant, 1991), among which the A_1 acceptor (Nitschke and Rutherford, 1991), and although the structure of RCI has been less well elucidated than that of RCII, the same contrast mechanism as in RCII may occur or may have occurred in RCI and its progenitors. In contrast, Nitschke and Rutherford (1991) assume the absence of a two-electron gate in RCI and its progenitors: electrons could move directly from a quinone to the FeS cluster.

Quinones appear in the present model after the acquisition of PS0 active chlorophyll-dimers; this contrasts with the idea that photosynthesis started with a quinone-iron complex (Boxer, 1992).

The Oxidizing Side
The stalk (Fig. 5) at the oxidizing side of the BRC of *Rp. viridis* (Deisenhofer and Michel, 1989) and other purple and green bacteria (Nitschke and Rutherford, 1991) extends far into the medium adjacent to the membrane. In PS0 it has the function of increasing the dipole length. The independent loss of the stalk by many species during evolution (Matsuura and Shimada, 1990; Nitschke and Rutherford, 1991) may have occurred after the PS0 ∅ PS transition. The loss may have occurred recently: upon the transfer from the natural environment with its fluctuating light to the laboratory with its continuous light.

In PS the magnitude of the dipole formed upon light absorption is less important. Long stalks are superfluous; a thin membrane suffices.

(c) *PS0 diversification*

In PS0 the dipole formed in the light must decay again in the dark. Plain backreactions along the chain of charge carriers in the RC may account for this decay. A precursor of cyt b_{559} in the RC may also have had the function of enabling the backreaction (Demmig-Adams, 1990; Andersson and Styring, 1991; Barber *et al*., 1992). The alternative return path for the charge separation decay from Q_B would also protect chlorophyll against overoxidation (Thompson and Brudvig, 1988). This reaction occurs especially above 18°C; at lower temperatures, however, the reaction requires Mg^{2+} (Yamamoto and Ke, 1979). cyt b_{559} can also reduce, on the time scale of seconds, the *Tyr*-160 residue of the D2 protein in PSII (Vass *et al*., 1990). In the BRC of *Rp. viridis* the decay of cyt $b_{55X}{}^{+}$ $Q_A{}^{-}$ is indeed possible by both a move of the separated electron along its previously followed path that includes the $(Chl)_2$ pigment, and by a direct return pathway (Gao *et al*., 1990). During evolution, the outbound way and the return pathway would have had to develop simultaneously (Fig. 12).

Dipoles can also decay by reduction of an electron acceptor in the medium adjacent to the membrane and oxidation of a donor adjacent to the other side. These redox processes allow for PS0 diversity: a type-II PS0 is expected to have evolved in an environment with a high redox potential, a type-I PS0 in an environment with a low redox potential. Type-II PS0 is associated with RCII, BPh and quinone, and purple bacteria, type-I PS0 with RCI, FeS cluster presence and green bacteria (Blankenship, 1992); it could therefore function as a reductor. The type-I PS0 still could not function as an ATP synthesizing power generator in continuous light; in the absence of quinones that could diffuse across the membrane it could not yet function as a proton pump.

In the model presented here the photosynthetic function of power generation, i.e. ATP synthesis, is acquired before the acquisition of photosynthetic reducing power, just as proposed previously (VanGorkom, 1987). Cyclic-electron transport thus appears before non-cyclic electron transport (Blankenship, 1992).

(d) *Power*

An increasing power production, with its competitive advantage, is the assumed driving force for the evolution of PS0 from MTS towards PS. Assuming an equal dipole potential of ~200 mV for MTS and PS0, the energy won per unit area in one cycle is identical for PS0 and MTS (~10 nJ/cm^2). The cycle time of fluctuating light (~1 s) can however be ~10 times shorter than the cycle time of thermal cycling (~10 s), which results in a specific power of 0,6 mW/g for the previously considered cubic bacterium after its acquisition of PS0 capability.

4. *From Photosystem 0 to Standard Photosynthesis*

(a) *Quinone Diffusion*

The next step in evolution is the acquisition of the ability for the quinone Q bound to RC0 to detach itself and to diffuse across the membrane after it has been reduced and has taken up two protons from the adjacent medium. After diffusion, the quinone reduces the primary electron donor in the RC and releases the protons at the other side of the membrane. Addition of a return path, such as given by cyt *b* and cyt c_2 in purple bacteria, for the electron from the diffused quinone to the primary electron donor would further enhance the reduction rate of the oxidized donor (Fig. 13). The return path may have been part of the cyt *c* containing stalk of the RC. The proton pump constituted by the RC/Q combination makes ATPsynthase mode switching unnecessary. The obtained system has the advantage of functioning in continuous light, i.e. it performs standard photosynthesis.

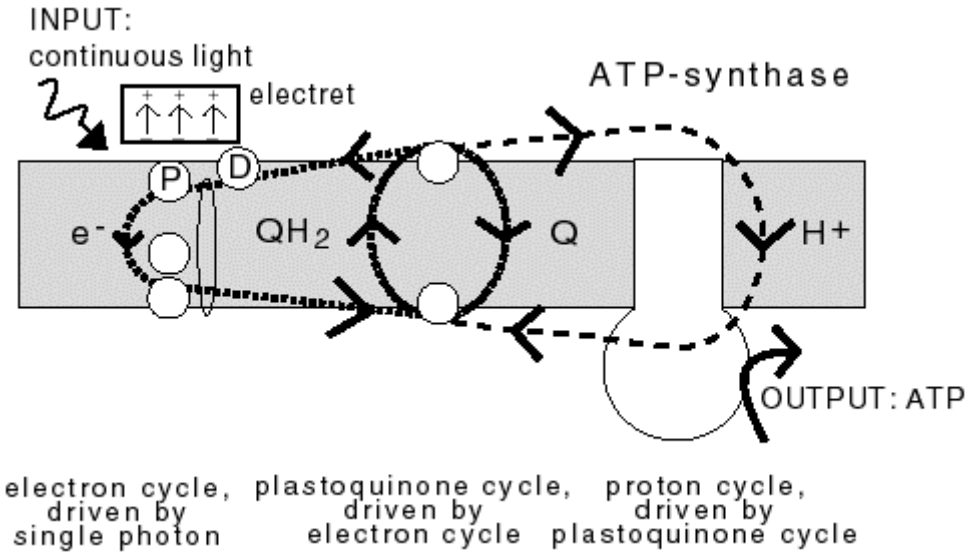

Fig. 13. A photosystem that can perform 'standard photosynthesis', i.e. photosynthesis in continuous light, is obtained by addition to the PS0 reaction center in Figs 11-12 of a quinone that can diffuse across the membrane after uptake of two electrons from the reaction center and two protons from the medium.

In modern chloroplasts $\Delta\Psi$ contributes most to the $\Delta\mu_H^+$ in the first 15-200 ms after a dark-light switch, but thereafter the quinone-induced ΔpH contribution is largest (Graan and Ort, 1983). The initial large $\Delta\Psi$ contribution (Vredenberg and Tonk, 1975; Witt, 1979) can be interpreted as a relic of the light PS0 state. The late, large ΔpH contribution may accordingly be interpreted as a state that has inherited characteristics of the dark PS0 state. Diffusion across the membrane is faster at higher temperatures, in particular upon a thermotropic phase transition (Poore and Ragan, 1982; Tikhonov *et al*., 1984: Fig. 5). Quinone diffusion and the ΔpH build-up can therefore be associated with the warm phase of the MTS cycle; the warm-dark association suggests applicability of the cold-light MTS/PS0 case.

It was already mentioned that the mode of CF_0F_1, directly after a light flash, may very well be lower than later in the dark. An ATPsynthase that supported PS0 could evolve into an ATPsynthase that supported standard photosynthesis by permitting it to function forwards in the high mode; this could be effected by increasing the $\Delta\mu_H^+$ upper activity threshold in this high mode, or by regulation, in the same manner as described above for CF_0F_1, by $\Delta\mu_H^+$, ADP and the redox state.

In chloroplasts, an increase in ΔpH acts as a fluorescence quencher (q_E quenching) (Krause and Weis,

1991). The associated decay of excited RC states would be functional in excited state decay during the dark phase of the PS0 cycle, helping in obtaining contrast. Oxygen may similarly have destabilized excited states, since it is also associated with fluorescence quenching (Walker *et al*., 1983). In particular it may, being a paramagnetic molecule, destabilize triplet states (Witt, 1979): in PS0 oxygen evolution may have destabilized triplet states, also as a means to obtain contrast[9]. Obviously, free energy dissipation at a high light intensity in extant organisms may descend from such quenching, suggesting an association between the dark PS0 state and the high light-intensity PS state, as opposed to a low light intensity PS state.

The first LHC may have been a PS0 RC capable of contributing excitons to the RC. LHCs allow energy gain under darker conditions (Larkum, 1991). In PS0 LHCs are expected to be superfluous, as a few chlorophylls in the whole PS complex suffice for RC excitation. Before the acquisition of diffusible quinones the presence of LHCs would have been a disadvantage, since the larger coverage of membrane area lowers the RC density, diminishing the PDP.

The first LHCs may have been related to hydrophobic phycobilisomes that could reversibly detach themselves from RCs, forming a separated membranous layer that bulged into the stroma and that did not function as a dielectric that separated two conducting solutions. When these separated LHCs would bind lipids, the effective thylakoid surface would decrease and the effective RC density in the remaining functioning membrane would increase, which would favor PS0 activity. The modern chloroplast thylakoid can behave similarly: after a temperature increase, peripheral LHCIIs can concentrate in the grana and RCII cores in the non-appressed area (Anderson and Andersson, 1988).

Both the incidence of stacking in chloroplasts (Anderson, 1982) and the density of phycobilisomes in cyanobacteria are higher at a low light intensity (Staehelin, 1986). It is proposed that this state corresponds to the light state of PS0. At very low light intensity, LHCs returned from the granum to the RCs, helping in catching the excitons, at the price of lowering the RC density; this would correspond with the dark state of PS0.

The switch from PS0 to PS may have been continuous. Light fluctuations are maximal in the upper 1-2 m of natural waters (Walsh and Legendre, 1988). PS may have evolved as a method of gaining energy from light in a new niche, the darker depth, where the fluctuations in light intensity are smaller.

The evolution of photosynthesis after the acquisition of an RC capable of charge separation across the membrane has been discussed by Pierson and Olson (1989).

(b) *Photosystem Combination; Addition of the* bc_1 *Complex*

During the evolution of PS there would have been selective pressure for the evolution of two populations of photosystems with overlapping but different redox spans (Olson, 1970), the reason being that electron donors of low E_h were depleted (Olson and Pierson, 1987; Larkum, 1991, 1992). The two types of RCs evolved into the green (~RCI) and the purple bacteria (~RCII). It was already mentioned that these two types of RCs may have had their counterparts in two types of RC0s.

By combination of the two types of RCs a system was obtained capable of photosynthesis according to the well known Z-scheme (Hill and Bendall, 1960; Duysens *et al*., 1961; Broda, 1975; Blankenship, 1992) in which electrons are transferred by quinones from RCII to RCI. The proton pump constituted by the bc_1 complex (called the b_6f complex in chloroplasts) would however still be absent. Similar to the RC, which contains hemes in the form chlorophyll, bc_1 also contains hemes in the form of cytochromes (Cramer and Knaff, 1991; Knaff, 1993) which constitute an electron transport pathway from the o-center (b_L heme) to the i-center (b_H heme) that is parallel to the electron transport pathway in the RC. In a Q-cycle (Mitchell, 1975; Selak and Whitmarsh, 1982; Hangarter *et al*., 1987b; Robertson and Dutton, 1988; DeVries *et al.*, 1988; Gennis *et al*., 1993) one electron moving from the reducing side of the RC to the oxidizing side sets two protons across the membrane. In the process a semi-quinone formed at the negative side of the membrane takes up a proton from the medium at that

[9] In present day photosynthetic systems the relation between oxygen evolution and fluorescence quenching may be different, since fluorescence quenching therein preceeds—by 10 s—oxygen evolution (Walker *et al*., 1983).

side of the membrane and an electron from the i-center. The formed quinol diffuses across the membrane, reduces the o-center, and releases two protons to the positive side of the membrane. One electron moves to the reducing side of the RC, the other from the o- to the i-center within the bc_1. The resulting quinone diffuses to the other side of the membrane as well, where it can again form a semi-quinone by uptake of an electron from the RC and a proton from the medium (for further details, and modified versions of the Q-cycle, see the references). The electron must have sufficient energy, and the $\Delta\mu_H^+$ be sufficiently small in order to make the pumping energetically possible (Hangarter *et al*., 1987b).

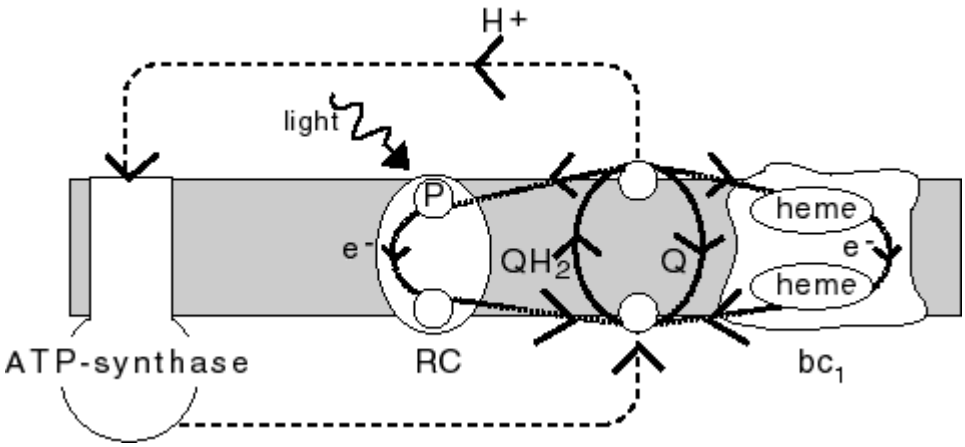

Fig. 14. Evolution of the Q-cycle within an RC, yielding a bc_1 bc_1 evolves from a duplicated RC as a specialized microenvironment that permitted higher diffusion rates for quinones across the membrane and that no longer required light for electron transfer any more: a low electrochemical potential of the incoming electron sufficed to drive the quinone across the membrane.

Electrogenic phases are: (1) electron transfer in the RC, (2) reduction of oxidized pigment in RC, and (3) electron transfer in bc_1 (Jackson and Dutton, 1973).

From the similarity of (1) the charge transfer by hemes in RCII and bc_1, (2) the interaction with quinones, and (3) the inhibitor sensitivity at the quinone binding sites (DegliEsposti, 1989; DegliEsposti *et al*., 1993), it has been proposed that bc_1 descends from an RC (see VanGorkom, 1987), although no homology between bc_1 and RC proteins is known. In the pertinent RCs the bound quinone may not have functioned as a two-electron gate, just as in the Ga strain, as opposed to the R-26 strain, of *Rb. sphaeroides* (Barouch and Clayton, 1977). The quinone could pick up one electron from a bc_1 and one from an RC, and a quinol instead of a semi-quinone would take part of the Q-cycle. The turnover time of bc_1 can be small (Cramer and Knaff, 1991) but still rate limiting. The bc_1 complex may have evolved from a pRC that constituted a microenvironment for quinones that permitted an optimal diffusion rate across the membrane (Fig 14).

Addition of a bc_1 pump to a PSII results in the bacterial photosynthetic system, insertion of bc_1 to the system following the Z-scheme mentioned in the foregoing in a functional cyanobacterium or chloroplast. The combined RCII/bc_1/RCI system may tune the NADPH/ATP ratio, by oscillating between two states with a different NADPH/ATP ratio, to the value required for CO_2 fixation, a proposed function of chloroplast oscillations (Horton, 1985).

(c) *Power*
Because of the smaller RC turnover time, PS has a higher power than PS0. This power can be estimated in several ways. In leaves of *Beta vulgaris*, for instance, the maximum photosynthesis rate at a total Chl (*a*+*b*) of 60 μg cm^{-2} is 106 mg CO_2 dm^{-2} h^{-1} (Terry, 1980: Fig. 4). At this Chl (*a*+*b*) concentration the number of P700 pigments is 98 10^{12} molecules cm^{-2} (Spiller and Terry, 1980): hence the fixation rate is 41 CO_2 molecules per P700 per second[10]. Since reduction of one CO_2 molecule requires four electrons, the turnover time of the electron transport chain is 6,1 ms or less. In *Chlorella* turnover times of 4-14 ms have been reported (Myers and Graham, 1971; Wilhelm and Wild, 1984). A range of 200-300 turnovers s^{-1}, corresponding to a turnover time of ~4 ms, was given for RCII (Chylla *et al*., 1987), and, similarly, in the green algae *Dunaliella tertiolecta* a turnover time of 3-14 ms for the electron transport chain (Sukenik *et al*., 1987). A commonly used value is 10 ms (Kok and Cheniae, 1966).
Compared to PS0, this smaller turnover time increases the power by a factor 100. Moreover, in PS0 the membrane is charged and discharged by the ATPsynthase, but in PS charging by ATPsynthase is superfluous, which increases W with a factor ~3. On the other hand LHC presence decreases the surface density of RCs, say also by a factor ~3 (150 → 450 nm^2). The power of PS then is ~100 times the power of PS0, i.e. 1 μW/cm^2. For the previously considered cubic bacterium a specific power of $1\ 10^{-6} \times 6\ 10^{-8} / 10^{-12}$ = 60 mW/g results, for leaf chloroplasts, from a thylakoid density of 30 000 - 100 000 cm^2/g, a P_{PS} of ~30-100 mW/g. This range agrees well with the maximal ranges given above, 35-100 mW/g, for the P_{PS} of C_3 plants. Such high values imply an efficiency of the conversion of light energy of ~25% (Björkman and Schräfer, 1989).

Diffusion of quinones across the membrane may limit photosynthesis (Stiehl and Witt, 1969; Witt, 1979; Harbinson and Hedley, 1989; Laisk and Oja, 1994). The diffusion (half) time is ~20 ms, and one quinone carries two charges, hence the turnover per charge is indeed ~10 ms. Reported turnover times of bc_1 vary considerably. Cramer and Knaff (1991) give ranges of 2-300 s^{-1} for *Rb. sphaeroides*, value of 5 s^{-1} for cyanobacteria, 2-100 s^{-1} for chloroplasts and 70-4000 s^{-1} for mitochondria. Obviously, rates in the lower ranges would decrease the specific power. A turnover of 5 s^{-1} would bring the power in the range of that of PS0.

A lower specific power than that calculated from turnover times of the electron transfer chain would result if the CO_2 fixation reaction by the rubisco enzyme limited photosynthesis; there is evidence for such limitation, especially at a high light intensity (Gutschick, 1984; Sukenik *et al*., 1987; Gutteridge, 1990).

[10] $106\ 10^{-3} / 44 \times 6{,}0\ 10^{23} / 100 / 3600 / 98\ 10^{12} = 41$

IV. PHOTOSYSTEM 0 AND THE CHLOROPLAST

The literature on chloroplasts is reviewed in the present study from the perspective of the questions whether PS0 plays a role in the chloroplast or whether some of its properties are relics of PS0 activity.

1. *The Relations between Light Intensity, Membrane Potential, Redox State of ATPsynthase and the Q-Cycle*

(a) *Mode Changes*
In chloroplasts (Jagendorf, 1977)—just as in bacterial chromatophores (Baccarini-Melandri *et al*., 1981)—photophosphorylation during flashes differs from photophosphorylation during continuous light. During flashes it is driven by the membrane potential ($\Delta\Psi$ ~100 mV), whereas in continuous light it is driven by the difference in pH ($\Delta\Psi$ ~30 mV). A different response during flashes is compatible with the PS0 hypothesis.

Mode variability as in PS0 could be advantageous in standard photosynthesis as well. The low mode is more efficient, since the ATP yield per proton—and per photon—is higher. Under light limitation the low mode is therefore the most favorable. In *Chlorella* kept in the dark for a long time the membrane potential generated by a flash decays with a time constant of ~100 ms, whereas when it is kept in continuous light the decay time is ~10 ms (Witt and Moraw, 1959): in dark-adapted *Chlorella* a high potential remains present for a longer time, as required for low mode activity.

Besides efficiency, speed can also be important in metabolism. The phosphorylation rate of CF_oF_1 is higher in the reduced state, allowing the enzyme to keep up with a high light intensity (Morita *et al*., 1983; Ort and Oxborough, 1992; Gabrys *et al*., 1994). The mode of the reduced state may be higher (Bogdanoff, 1990); if this is indeed the case, then by reducing CF_oF_1, efficiency can be traded for speed (Westerhoff *et al*., 1982).

Again, when light arrives as flashes, the higher yield per proton in the low mode is favorable. A slowly active, i.e. oxidized CF_oF_1 would stabilize $\Delta\Psi$ at the high value required for a low mode activity. Thus by switching to a forwards high mode at continuous high light intensity, and returning to a forwards low mode at a low light intensity, a chloroplast can process incoming light as needed. On going from low light to the dark it could switch from forwards low mode to backwards high mode (Mills and Mitchell, 1982; Shigalowa *et al*., 1985)—maintaining a small potential, taking the chance that illumination would start again, leading to a quick high potential—before switching off. If the light intensity upon a dark-light switch remains small, then a significant $\Delta\mu_{H^+}$ cannot be built up, and the high mode would remain favorable. CF_oF_1 indeed remains oxidized at a light intensity of 2 μmol m^{-2} s^{-1} but becomes reduced at < 5-22 μmol m^{-2} s^{-1} (Kramer *et al*., 1990: see also Altvater-Mackensen and Strotmann, 1988; Ort and Oxborough, 1992).

It may be difficult to determine a low mode value experimentally at a high membrane potential because of the high leakage across the membrane at such a high potential (Gräber and Witt, 1976; for bacteria, Jackson, 1982), a leakage that apparently increases the mode (Gräber and Witt, 1976; Bogdanoff, 1990). In the high mode the chloroplast operates at a lower membrane potential, which lessens leakage (Morita *et al*., 1983). In the presence of uncouplers, which enhance leakage, the high mode is obviously more favorable. Moreover, as already mentioned, uncouplers may increase the mode directly by interfering with proton translocation by CF_oF_1.

The proposed function of CF_oF_1 oxidation in ATP synthesis, permitting a higher efficiency of processing of light flashes, could be experimentally verified. At present, the common opinion on the function of oxidation of CF_oF_1 seems to be that the associated higher $\Delta\mu_{H^+}$ activity threshold protects against unwanted backwards activity of the enzyme in the dark (Gabrys *et al*., 1994).

(b) *The Q-cycle*
A complication in experimental verification of mode changes is that at a low $\Delta\mu_{H^+}$ the Q-cycle can become activated, which, by pumping an additional proton across the membrane, compensates for the lower ATP yield per proton at a low $\Delta\mu_{H^+}$; as a consequence the overall P/e ratio at low and high $\Delta\mu_{H^+}$ can be similar. The Q-cycle would become in particular active during the proposed lowered $\Delta\mu_{H^+}$ at a high light intensity. The higher cyt b_6f content in algae and leaves growing under light-saturating conditions (Wilhelm and Wild, 1984: Figs 6-9; Anderson, 1986: Fig. 2; Melis, 1991) is in agreement with this higher activity. The Q-cycle would become active at a light intensity of 80-100 μmol m^{-2} s^{-1}; only above this threshold the b_6f inhibitor antimycin diminishes O_2 evolution and the associated electron transfer along the RCII/b_6f/RCI chain (Furbank and Horton, 1987). This threshold is higher than the just mentioned light-intensity threshold for CF_oF_1 reduction.

The occurrence of the Q-cycle in chloroplasts is controversial. Bouges-Bocquet (1981) (see also Fowler and Kok, 1976; Velthuys, 1978) reports operation only at a low $\Delta\mu_{H^+}$, and Ivanov *et al*. (1985) report an H^+/e^- ratio of 3—a value associated with an operative Q-cycle—at 200 W m^{-2} (~400 μmol m^{-2} s^{-1}) and a ratio of 2 at 0,9 W m^{-2} (~1,8 μmol m^{-2} s^{-1}) in dark adapted chloroplasts. In preilluminated chloroplasts the ratio remained however 3 at the low light intensity. In contrast, Rich (1988) claims that the Q-cycle is operative at all light intensities. A counterexample to the proposed occurrence of the Q-cycle at high light intensity is the study of Rathenow and Rumberg (1980: Fig. 2), which gives a *decrease* in the H^+/e^- ratio as the light intensity increases from 5 to 300 W m^{-2} (~10 → ~600 μmol m^{-2} s^{-1}).

Such deviations can be explained by distortions of the proper redox potential required by the Q-cycle or by non-recognition of the occurrence of two transitions: dark → low light and low light → high light, as proposed here.

As one approaches the present in the literature, it becomes more difficult to draw firm conclusions: some studies support an increasing activity of the Q-cycle at increasing light intensities, others a decreasing activity (see also Gerst *et al.*, 1994). If, after taking different proton leakage and Q-cycle activity into account, the P/e ratios become identical for the two states with a high and low $\Delta\mu_{H^+}$ and an oxidized and reduced CF_oF_1, then no advantage in terms of efficiency would result, as has indeed been reported (Hangarter *et al*., 1987a). The advantage would be that a degree of freedom had been obtained: changes in the $\Delta\mu_{H^+}$ could be used for the regulation of other purposes, as the efficiency of power generation would, in a first approximation, not be affected by the $\Delta\mu_{H^+}$. Concomitant stoichiometry changes of CF_oF_1 and the Q-cycle would result in a mimicking by the chloroplast of the gear ratio changes in the automatic transmission of a car engine: both the protons and the teeth of the gears would transduce a varying amount of energy.

2. *Combined PS0/PS activity*

In an experimental demonstration of PS0 one would have to be careful to distinguish PS0 from standard PS with a variable mode. The same ATPsynthase that could support PS0—i.e. had a variable mode, regulated by ADP, the redox state and the $\Delta\mu_{H^+}$—could also enhance the efficiency of a standard photosystem in fluctuating light. The essential difference between PS0 and standard photosynthesis lies in the absence of charge transport across the membrane in PS0. Charge shuttles back and forth within RC0, whereas in PS charge is transferred across the membrane. Another difference is that PS0 can use fluctuating light but not continuous light, whereas PS can use them both.

Since PS0 and PS can use the same ATPsynthase, combined PS0/PS systems are possible. In such systems a light pulse will increase $\Delta\Psi$ by both causing a PDP by the PS0 component and by charge transport across the membrane by the PS component:

$$\Delta\Psi = \Delta\Psi_{PDP} + \Delta\Psi_{CS}$$

Inactive PSII could be PS0-active. Since DCMU blocks electron transport between Q_A and Q_B, a combined PS0/PS system can be obtained by poisoning part of the RCIIs with DCMU. Upon illumination the ATP content of DCMU poisoned cells is indeed enhanced (Bulté *et al*., 1990). During photoacoustic experiments using flashing light (~1-1000 Hz) DCMU addition—or heat inactivation—does not completely inhibit the conversion of light into free chemical energy (Carpentier *et al.*, 1990; Cha and Mauzerall, 1992: Figs 4-5); this has been explained by cyclic electron flow around RCII, but this result can also be explained by PS0 activity.

PS0 activity may also be related to the RCII states associated with energy-dependent fluorescence quenching during light saturation (Weis and Berry, 1987). These states differ from inactive RCII: no Q_A^- accumulates. The incidence of these states rises proportionally to the light intensity, and they have a low reducing power.

High efficiency of photosynthesis in fluctuating light has been observed, and has been studied extensively (Warburg, 1919, 1920; Emerson and Arnold, 1932a, 1932b; Rabinowitch, 1956 (a review); Witt, 1960; Nishimura, 1962a, 1962b; Fredrickson and Tsuchiya, 1970; Pollard, 1970; Döhler, 1973; Kriedemann *et al*., 1973; Lakso and Barnes, 1978; Quéguiner and Legendre, 1986; Walsh and Legendre, 1988; Knapp and Smith, 1990). The explanation of the high efficiency during fluctuating light is the absence of a light requirement for dark reactions such as the ATPsynthase reaction. By saturating RCs with a flash, and then next letting the dark reactions occur, the same product yield is obtained as when a high light intensity had been present during the dark period; this additional light cannot be processed, and is therefore dissipated. The product yield per amount of incoming light is therefore larger when light comes in as flashes, and CO_2 uptake is higher (Pearcy, 1990). For light pulses of 5 s the increase can be 30-80%, the increase diminishing with the pulse time. Pearcy (1990):

> ‘The highest efficiencies are attained with brief (1 s or less) high-light periods separated by somewhat longer low-light intervals.’

and

> ‘High efficiencies mean that an equivalent total flux of *high* light is utilized more efficiently when it is given in short intervals than when it is continuous.’

but

> ‘... the best utilization of photons for assimilation occurs when they are spread out uniformingly over the whole available period ...’

Obviously, high efficiency of photosynthesis in fluctuating light is consistent with mode variability: the light pulse would slowly but efficiently be processed by an ATPsynthase in the low mode, with the enzyme still being active during the begin of the dark period. These two mechanisms for enhanced efficiency in fluctuating light based on dark reactions and on mode changes, mechanisms which could simultaneously be operative, show the need of careful observation in an experimental demonstration of PS0/PS. Nevertheless, the difference between PS0 and PS shall be clear—PS0 cannot work in continuous light.

3. *Combined MTS/PS0/PS Activity*

About 5-10% of the thylakoid lipids undergo a thermotropic phase transition between 10 and 40°C (Low *et al*., 1984), a transition that is also an essential part of MTS. Just as PS0, MTS uses an ATPsynthase with a variable stoichiometry. Since RCIIs can become inactive by heating to ~45-53°C, increasing fluorescence (Bukhov *et al*., 1990; Cao and Govindjee, 1990), and since RCII may also be less active at low temperatures, combined MTS/PS0/PS, MTS/PS0 or MTS/PS activity is in theory possible in chloroplasts. When the contributions to the dipole potential of thermal changes and light intensity changes add up, the energy gained in a combined MTS/PS0 cycle is obtained by adding to the $\Delta\Psi_{PDP}$ in Eqn. 8 the change in membrane dipole potential with the temperature, $\Delta\Delta\Psi_D$, previously estimated to be ~100 mV:

$$\Delta\Psi \quad = \quad \Delta\Delta\Psi_D + \Delta\Psi_{PDP} + \Delta\Psi_{CS}$$

In combined MTS/PS0 both the cold-light and the warm-light case can apply. With the addition of PS as a possibility the number of possible combinations multiplies. For instance, when quinone would diffuse across the membrane and oxygen would evolve in the OEC only at higher temperatures, PS0 would occur mainly at low temperatures and PS at high temperatures. A suggestive combined effect of light and temperature changes is reported by Havaux *et al.* (1991): a short heat exposure (40°C) of pea leaves in the dark causes a decrease in oxygen evolution and PSII fluorescence, whereas the same exposure in the light has no such effect.

During *in situ* photosynthesis thermal cycling can occur. Many leaves try to regulate their temperature to 33°C by varying their transpiration rate (Raschke, 1975). This yields a constant average temperature around which thermal cycling can occur, the temperature regulation required by MTS. In a sunfleck the leaf temperature can increase up to 8-20°C; in thin shade-grown leaves the initial temperature rise can be as fast as 1-2°C s^{-1} (Pearcy, 1990). In soybean leaves, light cycling, with a period of 160 s, between 100 and 1500 µmol m^{-2} s^{-1} resulted in a thermal cycle with an amplitude of 5°C (Gaudillere *et al*., 1987). Clearly, in sunflecks the warm-light MTS/PS0 case would have to apply.

(a) *Dipole Potentials*

In contrast to the surface charge potential, the membrane or surface dipole potential $\Delta\Psi_D$ is not well appreciated in biochemistry (Gross *et al*., 1994). Haydon and Hladky (1972) remarked on this:

> ‘However, without detracting from the obvious importance of surface charge, it must be emphasized that a large proportion of the potential change across a single interface (e.g. of a membrane) may arise from oriented dipoles. Although well known to surface chemists, the influence of this potential on membrane properties has received comparatively little attention. Where both surface charge and dipole contributions occur together they are very difficult to separate in satisfactory manner—impossible, in fact by a study of equilibrium properties. Indeed, as yet no such separation has been possible by any means.’

Presence of dipole potentials in thylakoids is plausible, but has not been demonstrated. Barber (1980a) argued during a discussion of surface potentials of thylakoids that effects of the dipole potentials considered by Haydon and Hladky are difficult to distinguish from the effects of surface potentials, and that dipole potentials therefore would not have to be investigated in depth. It follows that, conversely, surface dipoles may just as well be the cause of the many effects attributed to surface charges (Barber, 1980a, 1982; Conjeaud and Mathis, 1986; Corazza *et al*., 1992), listed by Nakatani and Barber (1980) as: chlorophyll fluorescence, thylakoid stacking, electron transport kinetics, electrochromic absorption change at 515 nm, membrane conformational change, action of fluorescence probes and pH indicating dyes.

A temperature transition of the surface potential at 18°C has been reported. The zeta potential may be proportional to the surface potential or the sum of the surface and surface dipole potential (Muller, 1993). For phosphatidylcholine liposomes the zeta potential and the membrane lipid fluidity indeed

change simultaneously as the temperature changes (Tatulian, 1987). For chloroplasts, both in the light and in the dark the zeta potential strongly depends on the temperature: in the dark it is -50 mV at 4°C, then it decreases to -84 mV at 20°C, whereafter it remains constant; in the light the zeta potential is -63 mV at 4°C, and it decreases to -104 mV, whereafter it also remains constant (Torres-Pereira *et al.*, 1984). The difference of the zeta potentials in the light and in the dark shows a transition at 18°C.

In the dipole layers constituted by lipid monolayers on water the air has in general a positive potential with respect to the water; obviously, the hydrophobic side of the monolayer is directed to the air. The hydrophobic interior of bilayers and membranes is similarly in general positive with respect to the adjacent water. This makes it easy for anions, and difficult for cations, to diffuse across membranes (Gross *et al*., 1994): the charge carriers of electrical conduction across membranes are mostly anions. The formation of a light-induced membrane potential is followed by anion transport, in particular chloride transport, on the timescales of tenths of seconds to seconds (chloride is of interest since it stimulates electron transport in RCII (Izawa *et al.*, 1969; Critchley *et al.*, 1982; Akabori, 1984; Krishan and Mohanty, 1984)). The protons transported by quinones are also electrically neutralized by these anions, which permits the ΔpH rise to continue. Subsequent attraction of water—most biomembranes are permeable to water—by the salt may result in swelling of the thylakoid when the medium has a low osmolarity.

(b) *Effects of Different Constant Temperatures*
Recording its temperature dependency is a standard method for investigating a physiological process. For the purpose of the present study temperature dependency of photosynthetic processes becomes of interest when it is strong. Strong dependency has been observed for fluorescence, delayed light emission, cyt b_{559} oxidation, S_i-states decay, proton movement, electron transport during NADP reduction, P-700 rereduction, electrochromic shifts, membrane potential decay and phosphorylation (Murata *et al*., 1975; Murata and Fork, 1975; Yamamoto and Ke, 1979; Meier *et al*., 1981; Schuurmans *et al*., 1984; Bilger and Schreiber, 1990). Temperature dependency of electron transport will cause temperature dependency of dipole stability.

The luminescence induction curve shows the intensity of delayed light emission vs time. A spike in the curve during the first second depends on $\Delta\Psi$, and is eliminated by the uncoupler valinomycin. An exposure of 5 min to higher temperature decreases the spike: exposure to 33-34°C diminishes it by 50%, (Bilger and Schreiber, 1994) which suggests a concomitant decrease with the temperature of the associated metastable dipoles.

In delayed light emission in the < 1 ms range a lipid phase transition strongly enhances the decay rate (Jursinic and Govindjee, 1977); the dipole potential associated with the pertinent metastable states must be much more stable below ~15°C. Complexes containing cytochromes such as cyt b_{559} or bc_1 could can function as MTS/PS0 dipoles when these cytochromes are reduced.

Membrane-bound enzymes can be sensitive to lipid fluidity (Raison *et al*., 1971). The bulk of the lipids in the thylakoid membrane does not undergo a thermotropic phase transition at physiological relevant temperatures (Martin, 1986), but about 5-10% of all lipids do undergo such a transition between 10-40°C (Low *et al*., 1984). Moreover, many breaks of slope changes in Arrhenius plots in the range 15-20°C (Meier and Bachofen, 1981; Meier *et al*., 1981) or at 9, 20 and 29°C (Nolan and Smillie, 1976, 1977) are explained by phase transitions that enzyme- or RC-bound lipids would undergo (Gräber and Witt, 1974; Solaini *et al*., 1984). Enzymes and reaction centers may also undergo a thermal transition on their own (Kaiser and Oelze, 1980; Quinn *et al*., 1980), which could change $\Delta\Psi_D$.

Phase transitions by RC-bound lipids have already been mentioned in the context of a contrast mechanism for PS0. Lipids are required for charge stabilization in RCs and undergo phase transitions during temperature or membrane potential changes (Eckert *et al*., 1987; Hansson and Wydrzynski, 1990). Conversely, the presence of RCs changes the thermotropic phase transition temperature of lipids: for long lipids it is increased, for short ones decreased (Riegler and Möhwald, 1986).

In the absence of digalactosyl diacylglycerols and phosphatidylcholine the $P680^+$-Q_A^- dipole is unstable (Eckert *et al*., 1987). Oxygen evolution requires lipids (Akabori *et al*., 1984). Temperature dependency of photosynthetic oxygen yields and permeability changes (Renger, 1974), and an increase in the rate of cyt b_{559} oxidation (Yamamoto and Ke, 1979) have been explained by a thermotropic phase transition of RC-bound lipids at 18°C. At this temperature the decay rate of the electrochromic shift in flash experiments increases as well (Gräber and Witt, 1974). Above 21°C the decay rate of the S_3 state increases strongly (E_a 160 kJ/mol) (Messinger and Renger, 1990; see also Koike *et al.*, 1987).

The thermotropic phase transition temperature of lipids increases with the saturation of the constituent fatty acids. Increasing the saturation of these lipids by catalytic hydrogenation diminishes electron transport through the RCs and increases the fraction of inactive RCs (Horváth *et al*., 1986, 1987: Fig. 3).

Ascorbate cannot reduce cyt b_{559} between 20 and 40°C, but it can do so above and below this range (McEvoy and Lynn, 1972): during cycling around these limits, the charge on this cytochrome and the associated dipole could therefore vary strongly. 0,5 M Mg^{2+} removes the strong temperature dependency of the cyt b_{559} oxidation rate (Yamamoto and Ke, 1979). Divalent ions such as Ca^{2+} and Mg^{2+} can also regulate the phase transition temperature of lipids (Träuble and Eibl, 1974; Quinn, 1981). An effect of these ions on chloroplasts has been found (Chu *et al*., 1992), and may be caused by an effect upon the fluidity of bound lipid domains.

Many of the just described temperature effects must result in temperature dependency of the light-induced $\Delta\Psi$, and this is indeed observed. Between 2-25°C there is little temperature dependency of the flash-induced membrane potential (Bulychev *et al*., 1972, 1976), but at 28°C the potential is much smaller (Admon *et al*., 1982: Fig. 7). Gräber and Witt (1974) find that the initial potential upon a single turnover flash decreases linearly with the temperature above 27°C, while it is constant below this temperature. The effect is large; at 35°C the potential is 40% less (Gräber and Witt, 1974: Fig. 1). Calibrating the potential against other results of Witt (1979) results in a potential of 55 mV below 27°C, and a value of 33 mV at 35°C.

Below 12°C the decay of the flash induced membrane potential has a slow component (Yamamoto and Nishimura, 1977).

For ATPsynthase, Nishizaki (1973) observed a lower mode at 4°C than at 20°C. The higher yield of flash-induced ATP synthesis at low temperatures (Graan et al., 1981: Fig. 2) is consistent with a low mode of ATPsynthase combined with a high generated membrane potential.

Clearly, in chloroplasts many temperature effects, in particular the magnitude of light-induced potentials, are consistent with the occurrence of cold-light MTS/PS0 rather than with warm-light MTS/PS0.

(c) *Effects of a Cyclically Varying Temperature*

There are few studies on the effect of combined thermal and light cycling upon photosynthesis. It is suggestive that quick heating (ΔT >8°C) of pre-illuminated chloroplasts leads to light emission (Mar and Govindjee, 1971; Jursinic and Govindjee, 1972). A quick (t < 1 ms) and slow ($t_{½}$ ~0,2 s) proton release by chloroplasts upon a temperature increase of 3,2°C (12→15°C) are reported (Takahama *et al*., 1976, 1977). DCMU diminishes—which implicates RCII—and nigericin and gramicidin suppress the slow release. Illumination during the jump diminishes the proton release as well; this suggests that the cold-light case is applicable. Baker *et al*. (1981) also report proton release upon a transient exposure at 30°C of chloroplasts kept at 10°C; the protons are identified with the protons responsible for local coupling. These protons could however also be the result of other effects, for instance a decrease in pK values of amino acid residues in proteins.

Hysteresis during one thermal cycle (10-25°C) of the chloroplast absorption spectrum—cooling gives a red shift, suggesting a voltage increase— (Brody and Singhal, 1979) could be due to hysteresis in the membrane potential as in MTS. In the red alga *Cyanadium caldarium* a similar hysteresis in fluorescence has been observed between 10-30°C (Fork and Murata, 1977). Slawinski and Popp (1987) have observed strong hysteresis in the luminescence generated in the dark during thermal cycling. All the observations may however be due to transients: the observations should be extended so that a single cycle is followed after many cycles have already occurred.

4. *Possible relics of MTS/PSO activity*

Even if MTS/PS0 would not occur in contemporary chloroplasts, many phenomena in chloroplast still can be interpreted as relics of MTS/PS0. The phenomena concern the occurrence of some special reactions, and etiolation. Moreover, the two states of PS0 and the transitions between these states is a suitable point of departure for the ontogenesis of induction, chloroplasts oscillations and State 1-State 2 transitions. MTS/PS0 may allow for a natural model for the evolution of the behaviour of present day chloroplasts.

(a) *Special Reactions*

Intermittent shade can result in enhanced net photosynthesis, stomatal conductance and xylem pressure potential (Knapp *et al.*, 1989).

PS0 has the advantage that high membrane voltages can be reached, which may permit special reactions. A possible example is the synthesis of a 17,5 kDa translation intermediate of the D1 protein of RCII, which requires a light-dark switch for its synthesis: it is formed neither during continuous darkness nor during continuous illumination (Inagaki and Satoh, 1992). Another special reaction may be protochlorophyllide synthesis in etiolated leaves, which similarly requires a light-dark switch (Sironval *et al*., 1969). Other special metabolic reactions are those depending on a high membrane voltage such as the nitrogen fixation already mentioned, which requires much energy (Haaker and Klugkist, 1987; Kim and Rees, 1994). Reports of enhanced primary productivity under fluctuating light, values approaching the value measured *in situ* (Joiris and Bertels, 1985), can be explained by the occurrence of reactions requiring fluctuating light; in continuous light the reactions would only be effected by a large expenditure of free energy.

(b) *Etiolation*

Submitting growing chloroplasts to intermittent light (2-118 min LD) stops their development in the so-called etiolated phase, in which the antenna is smaller (Glick and Melis, 1988) and the RC density higher (Akoyunoglou, 1977). Such etiolated, or IML (intermittent light) thylakoids are fully photosynthetic competent, and can perform the PSI and PSII reactions. Chl *b* is absent, and the ratio of total fluorescence (F_{max}) to prompt fluorescence (F_0) is much higher than after placement in continuous light. IML thylakoids show thermoluminescence at -10°C, whereas mature thylakoids show it around 25°C: the stability of the pertinent metastable dipoles increases upon maturation (Faludi-Dániel *et al*., 1986).

Submitting growing chloroplasts to flashing light (1 ms-12 min LD) results in thylakoids that still can perform, in the presence of suited reductors and oxidators, the standard PSI and PSII reactions including phosphorylation; only the acquisition of the capability of oxygen evolution requires an induction time of several minutes (Strasser and Sironval, 1972; Strasser and Butler, 1976a, 1976b).

In IML thylakoids the RCII particle covers a surface of ~50 nm^2 (Staehelin, 1986). Upon transfer to continuous light LHCs are acquired (Armond *et al*., 1977), and the surface covered by the RC increases stepwise from ~50, ~90, ~130 till ~200 nm^2 (Staehelin, 1986). From the viewpoint of the PS0 model for the evolution of photosynthesis (Chapter 3), these effects of intermittent light suggest recapitulation of LHC acquisition during etiolation.

In contrast to the PS0 model, IML thylakoids show—as determined by electrochromic shifts—no spike in the membrane potential upon a dark-light switch (Jahns and Junge, 1992: Fig. 4). This absence has been attributed to a lower Chl *b* content of IML thylakoids, the Chl *b* containing LHCs having been associated with the electrochromic shift that is used in determining the membrane potential (Jahns and Junge, 1992). Thus $\Delta\Psi$ may nevertheless increase upon the switch in IML thylakoids.

(c) *Stacking and Swelling*

After transfer to continuous light, IML thylakoids form grana, concomitant with the appearance of LHCs (Armond *et al*., 1977). In grana about two thirds of the thylakoid membrane is stacked, binding tightly to the neighbouring thylakoid (Armond *et al*., 1977). LHCs cause the stacking (Ryrie *et al*., 1980). When stacked LHCs bind lipid membrane material, stacking may increase A, the area per RC, since RCs are concentrated in the stroma-exposed membrane, and the stacked area may not function as a dielectric that separates the lumen and the stroma (Williams, 1978; Barber, 1979). Inactive RCII—and the small $RCII_\beta$, which may overlap with inactive RCIIs—is found mainly in the stroma exposed

region of the thylakoid (Guenther and Melis, 1990), active RCII mainly in the grana (Staehelin and Arntzen, 1979: Fig. 10). Upon heat treatment RCII cores move out of the appressed area to the stroma, while LHCs remain behind (Staehelin, 1986; Anderson and Andersson, 1988), effectively increasing the RC density in the stroma exposed membrane. Thus the PS0 model permits a new answer to the question why plants have grana (Staehelin and Arntzen, 1983; Anderson and Andersson, 1988): to store LHCs when a thylakoid increases the RC density in the stroma exposed part of the membrane, the part that functions as a capacitor. Destacking then would be equivalent to an increase of capacitor area, and a lesser RC density, as suffices for operating in continuous light. From the MTS/PS0 viewpoint destacking can be associated with a switch from MTS/PS0 to PS.

(d) *Induction*

Upon a dark-light switch chloroplasts show transients and oscillations, a phenomenon called induction (VanDerVeen, 1960). Just like ontogeny can follow phylogeny, the PS0 model suggests that both during a dark-light switch in the present and during the evolution of PS in the past, a PDP is generated first, and that quinones start to diffuse across the membrane later.

After a dark-light switch the ATP yield is maximal during the first 15 ms (Graan and Ort, 1981, 1983), and a high ATP concentration builds up. This activates CO_2 fixation at a high rate. During the first minute after a dark-light switch an overshoot occurs in the synthesis of ATP and triose phosphate (Lilley *et al*., 1977) and in CO_2 fixation (VanDerVeen, 1960; Laisk *et al*., 1991). Pools of sugars are formed, at a high cost of ATP (Horton, 1985). The ATP becomes depleted, as the high ATP synthesis rate is not maintained. Thereafter induction may become intertwined with chloroplasts oscillations or State 1-State 2 transitions. PS0 seems a suitable guide for reaching a better understanding of induction.

(e) *Chloroplast Oscillations*

Just as PS0, chloroplasts show oscillations. The oscillations of oxygen evolution, for example, have a t_{cyc} range of 4-60 s (Bannister, 1965), a range that overlaps the range associated with PS0. The oscillations comprise many phenomena: changes in fluorescence, ATP synthesis and hydrolysis (Laisk *et al*., 1991), ΔpH, stacking and swelling, oxygen evolution and CO_2 assimilation (Bannister, 1965; Ogawa, 1982; Walker *et al*., 1983; Sivak *et al*., 1985; Peterson *et al*., 1988; Veljovic-Voljanovic and Cerovic, 1991; Walker, 1992). Walker (1992) even declares that ‘everything’ oscillates! In the first half of the ‘chloroplast cycle’ the following parameters increase, in the order (Sivak *et al*., 1985):

(1) the ΔpH across the membrane (associated with enhanced light scattering, in turn associated with thylakoid swelling),
(2) $\Delta\Psi$, as determined by 518 nm absorbance shifts,
(3) O_2 evolution and CO_2 assimilation (upon an increase in ATP concentration),
(4) fluorescence quenching.

In the second half of the cycle the parameters decrease again in the same order. During the first half of the cycle all parameters therefore indicate a large rate of photosynthesis, whereas during the second half photosynthesis is diminished.

The oscillations have not yet been satisfactorily explained (Walker, 1992). The concentration of ATP or of phosphate may play a role. A proposed function of the oscillations is the tuning of the ratio of the synthesis rates of ATP and NADPH to the ATP/NADPH demand of the CO_2 fixation process (Ogawa, 1982; Laisk *et al*., 1991). Another function may be regulation of primary PS processes by downstream metabolic processes, for instance by processes related to malate metabolism (Scheibe, 1994); the latter processes involve, just as for CF_oF_1, regulation of enzymes by oxidation and reduction of sulfur containing residues.

Horton (1985) points out that a variable coupling or variable mode of ATPsynthase, as proposed in the present study for PS0, would permit a variable ATP/NADPH ratio of the photosynthetic machinery.

In the present context the mechanism behind strong hysteresis in oxygen evolution during light intensity cycling of algae on the time scale of minutes may also be of interest (Falkowski and Owens, 1978).

Clearly, PS0-like processes may be involved in the oscillations, and may permit a model for their biogenesis.

(f) *State 1-State 2 Transitions*
The two photosystems in the Z-scheme, PSI and PSII, are sensitive to different colors. When irradiated with light preferably absorbed by resp. PSI and PSII, different states are assumed, named State 1 and State 2 (Staehelin and Arntzen, 1983; Fork and Satoh, 1986; Williams and Allen, 1987; Allen, 1992). The two states differ strongly. In a particular state the absorption cross section of the complementary RC increases, in State 1 for instance the absorbance by PSII. State changes thus result in equal turnover of the two photosystems, an obvious and commonly accepted function of the changes. The opinion on the control of state changes seems to be divided, since it is still unclear whether one causal factor for state changes exists (Fork and Satoh, 1986). Proposed causal factors are the phosphorylation state of LHC (Allen, 1992), the redox state of the quinone pool, the membrane surface charge (Barber, 1980b), ATP demand (Bulté *et al*., 1990) and temperature (Weis, 1985).

In red algae State 1 can be assumed in 2-5 s, State 2 in 10-20 s; in *Porphyridum* the transition can be complete in 1-2 s. In cyanobacteria and green algae the transitions are slower (Williams and Allen, 1987), in higher organisms they take minutes (Staehelin, 1986). Times in the order of seconds are in the range of environmental light fluctuations.

The two states of PS0 might be extended to include State 1 and State 2, as an oxidized quinone-pool is associated with both State 1 and oxidized CF_oF_1, in turn associated with a low mode. Barber (1980b) has distinguished two salt-related states of chloroplasts. At low salt there is poor electrostatic screening, randomisation of particles in the thylakoid membrane, low fluorescence, good spillover between RCII and RCI, and the membrane is unstacked. In the high-salt state there is good electrostatic screening, domain formation in the thylakoid membrane, high fluorescence and poor spillover. Granal and stromal lamellae are formed.

Table 5 gives for chloroplasts a tentative, even more extensive, association between physiological states that includes changes of CF_oF_1; a similarity of chloroplasts states to the states of cold-light MTS/PS0 is assumed.

A relation between states and PS0 states is supported by the recent observation that in the cyanobacterium *Synechococcus* 6301 the light intensity determines the state (Rouag and Dominy, 1994). Cyanobacteria and green algae assume State 2 in the dark. State 2 (low PSII fluorescence) is found in the dark or deep shade (< 5 μmol m^{-2} s^{-1}), State 1 (high PSII fluorescence) in moderate shade (20 - 60 μmol m^{-2} s^{-1}), and State 2 again for higher light intensities (> 200 μmol m^{-2} s^{-1}). Note that these associations between states and light intensity are opposite to those in Table 5, which concerns chloroplasts.

Further experimental and theoretical research may clarify the validity of the proposed generalization of the State 1-State 2 concept. In the course of evolution the number of physiological states assumed by photosynthesizing organisms may have increased, resulting in deviations from the simple proposed scheme of only two states that show synchronized changes of several variables.

Many phenomena in chloroplasts and many properties of thylakoid components such as CF_oF_1 and RCs thus are consistent with the occurrence of combined MTS/PS0/PS activity, or can be interpreted as relics of such combined activity. The chloroplast contains all the components required for acting during combined changes in temperature and light intensity as an MTS, a PS0, or PS, or as a combination of these systems. The contribution to power of PS0 can however only be minor.
The standard, ‘Mitchellian’ model for photosynthesis is intrinsically static, and therefore cannot deal with chloroplast dynamics. The PS0 model, on the contrary, being intrinsically dynamic, may yield explanations in terms of function and biogenesis for time dependent phenomena such as induction, oscillations and state transitions, and variable stoichiometries.

The function of chloroplast oscillations and state transitions is still unresolved. It is commonly assumed that their function is to permit adaptation to mutual imbalances in the input of the photosynthesis process, constituted by the internal and external variables of light intensity, light colour, temperature, redox potential, pH, concentrations of CO_2, O_2, H_2O, salt, individual cations and anions, in particular phosphate, chloride, ATP, malate, etc. During evolution selection would have been in the direction of a system that could balance the effects of variations of these variables —of which several may often change synchronously —in such a manner that photosynthesis remained active over the widest range of these variables. Ideally, the resulting system would be able to adapt to all fluctuations of *external* variables by utilizing changes of as many *internal* variables as necessary or feasible. The resulting system could however have become so complex that in the regulation process (Foyer *et al.*, 1990) cause and effect are now difficult to discern experimentally. In a bottom-up approach, the MTS/PS0/PS model may permit the delineation of the evolution of the resulting complex system, and, concomitantly, the delineation of the system itself.

Table 5. Tentative extension of the State 1-State 2 concept to the two states of the MTS/PS0/PS model for chloroplasts: changes between two states, for instance between a state with an active Q-cycle and a state with an inactive Q-cycle, may descend from the two primordial MTS/PS0 states. It is *not* claimed that all phenomena in one column necessarily occur together, but rather that they *tend* to do so. The classification is in some places inconsistent: a dark state cannot be associated with photosynthesis. Other associations of the dark state then still can apply. The references of the table give examples of reported associations. The table can be used as a mnemonic device.

	State 1	State 2
physico-chemical factors		
temperature	low	high
light presence	light	dark
light intensity	low	high
light color	~700 nm	~680 nm
redox potential	high (aerobiosis)	low (anaerobiosis)
salt concentration	high	low
overal properties		
PSII/PSI activity	PSII	PSI
PS0/PS activity	PS0	PS
fluorescence	non-quenched	quenched
energy storage	electrical: charge in capacitor - $\Delta\Psi$	chemical: protons in lumen - ΔpH
dominating process	ATP synthesis	NADPH reduction
P/e ratio	high	low
electron transport	cyclic	non-cyclic
limiting factor	light	CO_2
ATPsynthase		
redox state	oxidized	reduced
H^+/ATP ratio	low (high ATP yield per proton)	high (low ATP yield per proton)
$\Delta\mu_{H^+}$ threshold for activation	high	low
forwards/backwards active	almost always only forwards	in PS0 only backwards; in PS both forwards and backwards
type of coupling	delocalized	localized
ATP concentration	high	low
ATP synthesis rate	low	high
oligomycin	present	absent
uncoupler	absent	present
reaction centers		
(phospho)-LHCII binding	to RCII	to RCI
increased absorbance	by RCII	by RCI
peripheral LHC	dephosphorylated	phosphorylated
RCII	inactive (non-reducing)	active (reducing)
DCMU	present	absent
cyt b_{559}	oxidized	reduced
quinone pool and b_6f		
redox state	oxidized	reduced
diffusion rate across membrane	low	high
Q-cycle	not operative	operative
membrane		
voltage	high	low
fluidity	low	high
Mg^{2+}	uptake	release
light scattering	strong	weak
shape	shrunk/stacked	swollen/destacked

References to Table 5

Reported associations consistent with the proposed classification:	
ATP ~ shrinkage	Packer, 1966
amine uncouplers + light ~ swelling; amine uncouplers + dark ~ shrinkage; atebrin ~ shrinkage; salt ~ shrinkage; shrinkage ~ light scattering	Izawa and Good, 1966a
salt ~ stacking	Izawa and Good, 1966b; Staehelin and Arntzen, 1979
Mg^{2+} ~ (PSII) fluorescence	Murata, 1969
salt (Cl^-) ~ PSII activity	Izawa *et al*., 1969; Critchley *et al*.,1982; Akabori, 1984; Krishnan and Mohanty, 1984
light ~ shrinkage ~ stacking; ANS fluorescence ~ light scattering	Murakami and Packer, 1970; Sundquist and Burris, 1970
high temperature (> 10-15°C) ~ ΔpH rise ~ light scattering	Kraayenhof *et al*., 1971
fluorescence ~ shrinkage	Krause, 1973
state 2 ~ dark	Williams and Salamon, 1976
low temperature ~ fluorescence	Murata and Fork, 1975
salt ~ inoperative Q-cycle	Bulychev and Vredenberg, 1976
salt ~ stacking ~ fluorescence ~ grana formation	Barber, 1980b
low membrane fluidity ~ shrinkage ~ light ~ ΔpH	Nesbitt and Berg, 1980: Fig. 2; 1982
salt ~ absence of steady-state ΔΨ required for PS	Tiemann and Witt, 1982
high temperature (> 35°C) ~ destacking ~ low P/e ratio	Stidham *et al*., 1982
high temperature ~ destacking	Gounaris *et al*., 1983, 1984
state 2 ~ LHC phosphorylation ~ destacking ~ reduced quinones	Staehelin and Arntzen, 1983
state 1 ~ ATP ~ fluorescence	Schreiber, 1984
low temperature (~ 10°C) ~ high P/e	Schuurmans *et al*., 1984: Fig. 4
state 1 ~ low temperature (< 15°C); state 2 ~ high temperature (> 25°C)	Weis, 1984, 1985: Fig. 2; Sane *et al*., 1984
fluorescence ~ low membrane potential low light ~ low H^+/e^- ~ Q-cycle not operative high light ~ high H^+/e^- ~ Q-cycle operative	Bulychev *et al*., 1985: Fig. 2; Ivanov *et al*., 1985
Mg^{2+} ~ fluorescence	Krupinska *et al*., 1985
salt ~ delocalized coupling	Sigalat *et al*., 1985; Beard and Dilley, 1986
state 1 ~ oxidized quinone pool ~ aerobiosis ~ oxygen presence ~ Mg^{2+} uptake state 2 ~ high temperature (> 25°C) ~ low fluorescence ~ low ATP level ~ reduction of the quinone pool ~ anaerobiosis ~ Mg^{2+} removal	Fork and Satoh, 1986
DCMU ~ oxidized quinone pool ~ state 1 uncouplers ~ ATPase inhibitor ~ state 2	Williams and Allen, 1987
state 1 ~ dephosphorylated LHCII ~ stacking state 2 ~ phosphorylated LHCII ~ destacking	Staehelin and Arntzen, 1988; Allen, 1992
state 1 ~ high fluorescence ~ ATP	Bulté *et al*., 1990; Gans *et al*., 1990
state 2 ~ high temperature ~ PSI activity	Ruban and Trach, 1991
low membrane fluidity ~ Mg^{2+} ~ Ca^{2+}	Chu *et al*., 1992
low fluorescence ~ high temperature (> 35°C) ~ high light intensity	Agathi *et al*., 1995
Reported associations inconsistent with the proposed classification:	
anaerobiosis ~ shrinkage	Heber, 1969
high temperature (~ 50°C) ~ oxygen evolution	Emmett and Walker, 1969
high temperature (> 10-15°C) ~ light scattering ~ fluorescence	Kraayenhof *et al*., 1971
fluorescence ~ membrane potential	Dau *et al*., 1991; Dau and Sauer, 1991, 1992
low fluorescence ~ low temperature (25°C → 14°C)	Agathi *et al*., 1995

V. DISCUSSION

This study completes a series of studies (Muller, 1983, 1985, 1993, 1994, in press) in which the evolution of biological energy conversion is modelled by using postulated biochemical heat engines as intermediates. The result is an overall, stepwise model for the evolution of photosynthesis. The model consists of seven stages. The first five stages are (Muller, 1994):

1. the wet/dry-thermosynthesis (WDTS) stage, in which amino acids and phosphates are randomly polymerized during combined thermocycling and cyclic wetting and drying;
2. the pF_1-associated thermosynthesis (F_1TS) stage, free energy gain from thermal cycling of a protein, a precursor of the F_1 subunit of ATPsynthase;
3. the membrane-associated thermosynthesis (MTS) stage, free energy gain from thermal cycling of an asymmetric membrane, using a F_oF_1 with a different mode during charging and discharging of the membrane;
4. the photosystem 0 (PS0) stage, free energy gain from cyclic illumination;
5. the standard photosynthesis (PS) stage, free energy gain in constant light.

Chapter 4 suggests two additional stages:

6. the combination of PSI- and PSII-type RCs and a b_6f complex, yielding a functional electron transport chain as present in the chloroplast;
7. the acquisition of the ability to synthesize ATP and NADPH at the ratio required by CO_2 fixation, during fluctuations of light colour, light intensity, temperature, redox potential, pH, concentrations of CO_2, O_2, H_2O, salt, cations and anions, phosphate, ATP, malate, etc.

Present day components of the photosynthetic apparatus are acquired in the sequence:

1. during the WDTS and F_1TS stages: F_1 moiety of ATPsynthase, the first enzyme;
2. during the MTS stage: asymmetric biomembrane, F_o moiety of ATPsynthase, surface protein RC0;
3. during the PS0 stage: stepwise RC acquisition by component addition to RC0: Chl_2 dimer, phaeophytin, temporary charge carriers, cyt *c*-containing stalk, RC-bound quinones;
4. acquired during the PS stage: quinones with ability to diffuse across the membrane, bc_1 complex: derived from the RC, RC duplication and diversification yields two RC types.

The continuity between MTS and PS0 is obvious. Clearly, the concept of energy gain from mode and activity switching by ATPsynthase, synchronized with changes in electrical polarization of a membrane, can not only be applied to thermosynthesis but to photosynthesis as well. In theory, photosynthesis is possible in the absence of charge transfer across a membrane.

Pearcy (1990) emphasizes that photosynthesis should be considered as a dynamic process, rather than a static process,. The PS0 mechanism shows the possible importance in biology of dynamic processes that are far from thermodynamic equilibrium. Perhaps due to the use of radioactive tracers, metabolism is often pictured as a set of metabolic pathways that are in a static steady-state, a picture somewhat modified to include cyclic pathways and feedback from farther downstream; it is pictured as a continuous process that is near thermodynamical equilibrium. MTS and PS0, on the contrary, resemble batch processes, are typically far from equilibrium, and are dynamic. Efficiency is less, dissipation is enhanced, but profit is made from transient environmental changes by use of simple machinery. Given the diversity of life and of natural environments, it is to be expected that some organisms profit now or have profited in the past from such an essentially different energy source.

The physiology of chloroplasts is complex, and has not yet been completely resolved. The PS0 concept may very well be the missing concept required for unscrambling the physiology. The concept integrates many scattered observations into one logical pattern, explaining many aspects of the dynamics of present day photosystems, in particular variable stoichiometries of ATPsynthase, inactive RCIIs, State 1-State 2 transitions, electrochromic shifts, effects of intermittent light, thermoluminescence, etiolation, energy dissipation at high light intensity and chloroplast oscillations.

Moreover, the concept allows the formulation of new, testable hypotheses for photosynthetic phenomena - the necessary basis for experiments (Krebs, 1966): it clearly suggests new experimental investigations of the interactions between light intensity fluctuations, the membrane potential, ATPsynthase, the b_6f complex, and active as well as inactive reaction centers.

It is of interest to compare PS0 with models used to explain experiments with flashing light on bacterial chromatophores (Jackson and Dutton, 1973; DelValle-Tascon *et al*., 1978; Jackson *et al*., 1978; Petty and Jackson, 1979b, 1979c). Some of these models consider light-induced, membrane embedded dipoles, and their effects—maybe by a local coupling—on ATPsynthase. PS0 extends these models, by showing the possible mimicking by flashing light during experiments of environmental light fluctuations, the importance of the ‘delocalized’ light-induced membrane dipole potential, the critical role of the dipole density in the membrane in this dipole potential (see also Packham *et al*., 1982), and the role of mode and activity switching by ATPsynthase for ATP gain from fluctuating light.

Just as for thermosynthesis (Muller, 1993), it is up to observers and experimentalists to establish the occurrence and importance of PS0 in the world around us. The close connection of the previously proposed mechanism for regulation of the ATP:NADPH ratio in chloroplasts (Horton, 1985) in terms of the same H^+/ATP variations of ATPsynthase as assumed in the PS0 model is very suggestive in this regard. PS0 is especially expected to occur in photosynthesizing bacteria with a high RC density in their membranes. Chloroplasts would only have to inactivate their RCs in order to become functional PS0 systems. The thylakoids in plants restricted to habitats with intermittent illumination (Knapp and Smith, 1990), in mutants of barley that contain few LHCs (Glick and Melis, 1988) or that contain RC crystals (Clayton, 1980; Kühlbrandt, 1987), and of course of the aspen (Pollard, 1970), are also good PS0 candidates. PS0 would allow free energy gain in fluctuating light but not in continuous light; this can be used to test, or select, organisms for PS0.

VI. CONCLUSION

Variable dipole potentials of membranes deserve more attention. Photosystem 0, which is based on such a variable dipole potential, yields a new point of view on many observations and experimental findings: effects of fluctuating light, inactive RCIIs, chloroplast oscillations and State 1-State 2 transitions. The concepts presented could be tested in, or applied to, biomembranes in organisms, and artificial membranes. PS0 allows a stepwise model for the early evolution of photosynthesis: even when MTS/PS0 would not occur in contemporary organisms, it would remain of interest as it gives a theoretical solution to the unsolved problems (Scherer, 1983) of the biogenesis of cyclic photosynthetic electron transport, the nature of the first photosynthetic reaction centers (Blankenship, 1992) and why things happen in reaction centers as they do (Gunner, 1991).

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REFERENCES

Abrahams, S.A. (1991) Pyroelectricity. In: *Encyclopedia of Physics* (2nd ed.), pp. 967-968 (eds R.G. Lerner and G.L. Trigg). VCH Publishers, New York.

Adam, V., Virgili, M., Venturoli, G., Pietrobon, D. and Melandri, B.A. (1987) Photosynthetic control and ATP/electron ratio in bacterial photophosphorylation. In: *Cytochrome systems: molecular biology and bioenergetics*, pp 777-784 (eds A. Papa, B. Chance and L. Ernster). Plenum press, New York.

Admon, A., Shahak, Y. and Avron, M. (1982) Adenosine-triphosphate generated transmembrane electric potential in chloroplasts. *Biochim. biophys. Acta* **681**, 405-411.

Agati, G., Mazzinghi, P., Fusi, F. and Ambrosini, I. (1995) The plant F685/F730 chlorophyll fluorescence ratio as a tool in plant fluorescence: response to physiological and environmental factors. *J. Plant Physiol.* **145**, 228-238.

Akabori, K., Imaoka, A. and Toyoshima, Y. (1984) The role of lipids and 17-kDa protein in enhancing the recovery of O_2 evolution in cholate-treated thylakoid membranes. *FEBS Lett.* **173**, 36-40.

Akoyunoglou, G. (1977) Development of the photosynthetic II unit in plastids of bean leaves greened in periodic light. *Arch. Biochem. Biophys.* **183**, 571-580.

Alegria, G. and Dutton, P.L. (1991) I. Langmuir-Blodgett monolayer films of bacterial photosynthetic membranes and isolated reaction centers: preparation, spectrophotometric and electrochemical characterization. *Biochim. biophys. Acta* **1057**, 239-257.

Altvater-Mackensen, R. and Strotmann, H. (1988) Contents of endogenous adenine nucleotides and ATPase activity of isolated whole chloroplasts. *Biochim. biophys. Acta* **934**, 213-219.

Andersen, O.G.N. (1989) Primary production, chlorophyll, light, and nutrients beneath the Arctic sea ice. In: *The Arctic Seas*, pp 147-191 (ed. Y. Herman). VanNostrand, New York.

Anderson, J.M. (1982) The significance of grana stacking in chlorophyll-*b* containing chloroplasts. *Photobiochem. Photobiophys.* **3**, 225-241.

Anderson, J.M. (1986) Photoregulation of the composition, function, and structure of thylakoid membranes. *A. Rev. Plant Physiol.* **37**, 93-136.

Anderson, J.M. and Andersson, B. (1988) The dynamic photosynthetic membrane and regulation of solar energy conversion. *Trends biochem. Sci.* **13**, 351-355.

Andersson, B. and Styring, S. (1991) Photosystem II: molecular organization, function and acclimation. *Cur. Top. Bioenerg.* **16**, 1-81.

Arata, H. and Parson, W.W. (1981a) Enthalpy and volume changes accompanying electron transfer from P-870 to quinones in *Rhodopseudomonas sphaeroides* reaction centers. *Biochim. biophys. Acta* **636**, 70-81.

Arata, H. and Parson, W.W. (1981b) Delayed fluorescence from *Rhodopseudomonas sphaeroides* reaction centers. Enthalpy and free energy changes accompanying electron transfer from *P*-870 to quinones. *Biochim. biophys. Acta* **638**, 201-209.

Arata, H. and Parson, W.W. (1982) Enthalpy and volume changes accompanying electron transfer from P_{870} to the primary and secondary quinones in photosynthetic reaction centers. In: *Function of Quinones in Energy Conserving Systems*, pp 199-212 (ed. B.L. Trumpower). Academic Press, New York.

Armond, P.A., Staehelin, L.A. and Arntzen, C.J. (1977) Spatial relationship of photosystem I, photosystem II, and the light-harvesting complex in chloroplast membranes. *J. Cell Biol.* **73**, 400-418.

Arnold, W.A. and Azzi, J.R. (1977) Two effects of electrical fields on chloroplasts. *Plant Physiol.* **60**, 449-451.

Astumian, R.D., Chock, P.B., Tsong, T.Y., Chen, Y.-D. and Westerhoff, H.V. (1987) Can free energy be transduced from electric noise? *Proc. natn. Acad. Sci. U.S.A.* **84**, 434-438.

Atkins, P.W. (1986) *Physical chemistry*, p. 242, 621. Oxford University Press.

Awramik, S.M. (1992) The oldest records of photosynthesis. *Photosynth. Res.* **33**, 75-89.

Axe, J.D. and Lines, M.E. (1991) Ferroelectricity In: *Encyclopedia of Physics* (2nd ed.), pp 373-376 (eds R.G. Lerner and G.L. Trigg). VCH Publishers, New York.

Baccarini-Melandri, A., Casadio, R. and Melandri, B.A. (1977) Thermodynamics and kinetics of

photophosphorylation in bacterial chromatophores and their relation with the transmembrane electrochemical potential difference of protons. *Eur. J. Biochem.* **78**, 389-402.

Baccarini-Melandri, A., Casadio, R. and Melandri, B.A. (1981) Electron transfer, proton translocation, and ATP synthesis in bacterial chromatophores. *Cur. Top. Bioenerg.* **12**, 197-258.

Baciou, L., Rivas, E. and Sebban, P. (1990) $P^+Q_A^-$ and $P^+Q_B^-$ charge recombinations in *Rhodopseudomonas viridis* chromatophores and in reaction centers reconstituted in phosphatidylcholine liposomes. Existence of two conformational states of the reaction centers and effects of pH and *o*-phenanthroline. *Biochemistry* **29**, 2966-2976.

Baciou, L. and Sebban, P. (1992) Influences of the lipidic environment on the reaction centre's function. In: *Trends in Photosynthesis Research*, pp 157-167 (eds J. Barber, M.G. Guerrero and H. Medrano). Intercept, Hanover, Hampshire.

Bakels, R.H.A., VanWalraven, H.S., Krab, K., Scholts, M.J.C. and Kraayenhof, R. (1993) On the activation mechanism of the H^+-ATP synthase and unusual thermodynamic properties in the alkalophilic cyanobacterium *Spirulina platensis*. *Eur. J. Biochem.* **213**, 957-964.

Baker, J.A., Duchek, J.R., Hooper, R.L., Koftan, R.J. and Huebner, J.S. (1979) Lipid and salt effects on carbocyanine dye-induced photo-voltages in bilayer membranes. *Biochim. biophys. Acta* **553**, 1-10.

Baker, G.M., Bhatnagar, D. and Dilley, R.A. (1981) Proton release in photosynthetic water oxidation: evidence for proton movement in a restricted domain. *Biochemistry* **20**, 2307-2315.

Baltscheffsky, M., Baltscheffsky, H. and Boork, J. (1982) Evolutionary and mechanistic aspects on coupling and phosphorylation in photosynthetic bacteria. *Top. Photosynth.* **4**, 249-272.

Bannister, T.T. (1965) Simple oscillations in photosynthetic oxygen evolution. *Biochim. biophys. Acta* **109**, 97-107.

Barber, J. (1972) Stimulation of millisecond delayed light emission by KCl and NaCl gradients as means of investigating the ionic permeability properties of the thylakoid membranes. *Biochim. biophys. Acta* **275**, 105-116.

Barber, J. (1979) In: *Symposium on Chlorophyll Organization and Energy Transfer in Photosynthesis*, Ciba Foundation Symposium 61, New Series, p 174. Excerpta medica, Amsterdam.

Barber, J. (1980a) Membrane surface charges and potentials in relation to photosynthesis. *Biochim. biophys. Acta* **594**, 253-308.

Barber, J. (1980b). An explanation for the relationship between salt-induced thylakoid stacking and the chlorophyll fluorescence changes associated with changes in spillover of energy from photosystem II to photosystem I. *FEBS Lett.* **118**, 1-10.

Barber, J. (1982) Influence of surface charges on thylakoid structure and function. *A. Rev. Plant Physiol.* **33**, 261-295.

Barber, J., Malkin, R. and Roncel, M. (1992) Photoinduced oxidation and reduction of cytochrome *b*559 in the isolated photosystem two reaction centre indicates its role in cyclic electron flow. In: *Trends in photosynthesis research*, pp 125-134 (eds H. Barber, M.G. Guerrero and H. Merano). Intercept, Andover, Hampshire.

Barsky, E.L., Dancshazy, Z., Drachev, L.A., Il'ina, M.D., Jasaitis, A.A., Kondrashin, A.A., Samuilov, V.D. and Skulachev, V.P. (1976) Reconstitution of biological molecular generators of electric current. *J. biol. Chem.* **251**, 7066-7071.

Barouch, Y. and Clayton, R.K. (1977) Ubiquinone reduction and proton uptake by chromatophores of *Rhodopseudomonas sphaeroides* R-26. Periodicity of two in consecutive flashes. *Biochim. biophys. Acta* **462**, 785-788.

Bassi, R., Magaldi, A.G., Tognon, G., Giacometto, G.M. and Miller, K.R. (1989) Two-dimensional crystals of photosystem reaction center complex from higher plants. *Eur. J. Cell Biol.* **50**, 84-93.

Bassi, R., Rigoni, F. and Giacometti, G.M. (1990) Chlorophyll binding proteins with antenna function in higher plants and green algae. *Photochem. Photobiol.* **52**, 1187-1206.

Bauchop, T. and Elsden, S.R. (1960) The growth of micro-organisms in relation to their energy supply. *J. gen. Microbiol.* **23**, 457-469.

Beard, W.A. and Dilley, R.A. (1986) A shift in chloroplast energy coupling by KCl from localized to

bulk phase delocalized proton gradients. *FEBS Lett.* **201**, 57-62.
Beddard, G.S. and Porter, G. (1976) Concentration quenching in chlorophyll. *Nature* **260**, 366-367.
Bellamy, W.D., Gaines, G.L. and Tweet, A.G. (1963) Preparation and properties of monomolecular films of chlorophyll *a* and pheophytin *a*. *J. chem. Physics* **39**, 2528-2538.
Berns, D.S. (1976) Photosensitive bilayer membranes as model systems for photobiological processes. *Photochem. Photobiol.* **24**, 117-139.
Bilger, W. and Schreiber, U. (1990) Chlorophyll luminescence as an indicator of stress-induced damage to the photosynthetic apparatus. Effects of heat-stress in isolated chloroplasts. *Photosynth. Res.* **25**, 161-171.
Björkman, O. and Schäfer, C. (1989) A gas exchange-fluorescence analysis of photosynthetic performance of a cotton crop under high-irradiance stress. *Phil. Trans. Roy. Soc. Lond. Ser. B* **323**, 309-311.
Blankenship, R.E. (1992) Origin and early evolution of photosynthesis. *Photosynth. Res.* **33**, 91-111.
Blankenship, R.E. and Parson, W.W. (1979) The involvement of iron and ubiquinone in electron transfer reactions mediated by reaction centers from photosynthetic bacteria. *Biochim. biophys. Acta* **545**, 429-444.
Boekema, E.J., Dekker, J.P., VanHeel, M.G., Rögner, M., Saenger, W., Witt. I. and Witt, H.T. (1987) Evidence for a trimeric organization of the photosystem I complex from the thermophilic cyanobacterium *Synechococcus* sp. *FEBS Lett.* **217**, 283-286.
Boekema, E.J., Boonstra, A.F., Dekker, J.P. and Rögner, M. (1994) Electron microscopic structural analysis of photosystem I, photosystem II, and the cytochrome b_6/f complex from green plants and cyanobacteria. *J. Bioenerg. Biom.* **26**, 17-29.
Bogdanoff, P. (1990) *Untersuchungen zur Protonenstöchiometrie bei der ATP Synthese und der Aktivierung der ATPsynthase in Thylakoiden mittels elektrochromer Absorptionsänderungen.* Thesis, Technische Universität Berlin.
Bouges-Bocquet, B. (1973) Electron transfer between the two photosystems in spinach chloroplasts. *Biochim. biophys. Acta* **314**, 250-256.
Bouges-Bocquet, B. (1981) Factors regulating the slow electrogenic phase in green algae and higher plants. *Biochim. biophys. Acta* **635**, 327-340.
Boxer, S.G. (1990) Mechanisms of long-distance electron transfer in proteins: lessons from photosynthetic reaction centers. *A. Rev. Biophys. biophys. Chem.* **19**, 267-299.
Boxer, S.G. (1992) Some speculations concerning the evolution of photosynthetic function. *Photosynth. Res.* **33**, 113-119.
Boyer, P.D. (1989) A perspective of the binding change mechanism for ATP synthesis. *FASEB J.* **3**, 2164-2178.
Breton, J. and Nabedryk, E. (1993) $S_0 \rightarrow T_1$ infrared difference spectrum of the triplet state of the primary electron donor in *Rb. sphaeroides* photosynthetic bacterial reaction centers. *Chem. Physics Lett.* **213**, 571-575.
Broda, E. (1975) *The evolution of the bioenergetic processes.* Pergamon Press, Oxford.
Broda, E. and Peschek, G.A. (1979) Did respiration or photosynthesis come first? *J. theor. Biol.* **81**, 201-212.
Brody, S.S. and Singhal, G.S. (1979) Spectral properties of chloroplast membranes as a function of physiological temperatures. *Biochem. biophys. Res. Comm.* **89**, 542-546.
Budil, D.E. and Thurnauer, M.C. (1991) The chlorophyll triplet state as a probe of the structure and function in photosynthesis. *Biochim. biophys. Acta* **1057**, 1-41.
Bukhov, N.G., Sabat, S.C. and Mohanty, P. (1990) Analysis of chlorophyll *a* fluorescence changes in weak light in heat treated *Amaranthus* chloroplasts. *Photosynth. Res.* **23**, 81-87.
Bulté, L., Gans, P., Rebéillé, F. and Wollman, F.-A. (1990) ATP control on state transitions in vivo in *Chlamydomonas reinhardtii*. *Biochim. biophys. Acta* **1020**, 72-80.
Bulychev, A.A., Andrianov, V.K., Kurella, G.A. and Litvin, F.F. (1972) Micro-electrode measurements of the transmembrane potential of chloroplasts and its photoinduced changes. *Nature* **236**, 175-177.
Bulychev, A.A., Andrianov, V.K., Kurella, G.A. and Litvin, F.F. (1976) Photoinduction kinetics of electrical potential in a single chloroplast as studied with micro-electrode technique. *Biochim. biophys. Acta* **420**, 336-351.
Bulychev, A.A., Niyazova, M.M. and Turovetsky, V.B. (1985) Evidence for the delayed

photoactivation of electrogenic electron transport in chloroplast membranes. *Biochim. biophys. Acta* **808**, 186-191.

Bulychev, A.A. and Vredenberg, W.J. (1976) The effect of cations and membrane permeability modifying agents on the dark kinetics of the photoelectric response in isolated chloroplasts. *Biochim. biophys. Acta* **423**, 548-556.

Burke, D.H., Hearst, J.E. and Sidow, A. (1993) Early evolution of photosynthesis: clues from nitrogenase and chlorophyll iron proteins. *Proc. natn. Acad. Sci. U.S.A.* **90**, 7134-7138.

Burley, S.K. and Petsko, G.A. (1989) Electrostatic interactions in aromatic oligopeptides contribute to protein stability. *Trends Biotechn.* **7**, 354-359.

Callis, J.B., Parson, W.W. and Gouterman, M. (1972) Fast changes of enthalpy and volume on flash excitation of *Chromatium* chromatophores. *Biochim. biophys. Acta* **267**, 348-362.

Calvin, M. (1969) *Chemical evolution.* Clarendon Press, Oxford.

Cao, J. and Govindjee (1990) Chlorophyll *a* fluorescence transient as an indicator of active and inactive Photosystem II in thylakoid membranes. *Biochim. biophys. Acta* **1015**, 180-188.

Carmeli, C. and Lifshitz, Y. (1972) Effects of P_i and ADP on ATPase activity in chloroplasts. *Biochim. biophys. Acta* **267**, 86-95.

Case, G.D. and Parson, W.W. (1971) Thermodynamics of the primary and secondary photochemical reactions in *Chromatium*. *Biochim. biophys. Acta* **253**, 187-202.

Case, G.D. and Parson, W.W. (1973) Redistribution of electric charge accompanying photosynthetic electron transport in *Chromatium*. *Biochim. biophys. Acta* **292**, 677-684.

Cha, Y. and Mauzerall, D.C. (1992) Energy storage of linear and cyclic electron flows in photosynthesis. *Plant Physiol*. **100**, 1869-1877.

Carpentier, R., Leblanc, R.M. and Mimeault, M. (1990) On the nature of the photosynthetic energy storage monitored by photoacoustic spectroscopy. *Photosynth. Res.* **23**, 313-318.

Chazdon, R.L. (1988) Sunflecks and their importance to forest understorey plants. *Adv. ecol. Res.* **18**, 1-63.

Chu, Z.X., Song, C.P., Mu, M.H. and Huang, F. (1992) Influence of Mg^{2+} and Ca^{2+} on the fluidity of the chloroplast. *Photosynth. Res.* **34**, 119.

Chylla, R.A., Garab, G. and Whitmarsh, J. (1987) Evidence for slow turnover in a fraction of Photosystem II complexes in thylakoid membranes. *Biochim. biophys. Acta* **894**, 562-571.

Chylla, R.A. and Whitmarsh, J. (1989) Inactive photosystem II complexes in leaves. *Plant Physiol.* **90**, 765-772.

Chylla, R.A. and Whitmarsh, J. (1990) Light saturation response of inactive photosystem II reaction centers in spinach. *Photosynth. Res.* **25**, 39-48.

Clayton, R.K. (1973) Primary processes in bacterial photosynthesis. *A. Rev. Biophys. Bioeng.* **2**, 131-156.

Clayton, R.K. (1980) *Photosynthesis. Physical mechanisms and chemical patterns*, p. 31, 36, 73, 170, 268. Cambridge University Press.

Colbow, K. (1973) Chlorophyll in phospholipid vesicles. *Biochim. biophys. Acta* **318**, 4-9.

Conjeaud, H. and Mathis, P. (1986) Electron transfer in the photosynthetic membrane. Influence of pH and surface potential on the P-680 reduction kinetics. *Biophys. J.* **49**, 1215-1221.

Corazza, A., Melandri, B.A., Venturoli, G. and Casadio, R. (1992) Evaluation of the surface potential at the membrane-solution interface of photosynthetic bacterial systems. In: *Electrified interfaces in physics, chemistry and biology*, pp 509-532 (ed. R. Guidelli). Kluwer, Dordrecht.

Coster, H.G.L. and Zimmermann, U. (1975) The mechanism of the electrical breakdown in the membranes of *Valonia utricularis*. *J. membr. Biol.* **22**, 73-90.

Cotton, N.P.J. and Jackson, J.B. (1982) The kinetics of the carotenoid absorption changes in intact cells of photosynthetic bacteria. *Biochim. biophys. Acta* 679, 138-145.

Cramer, W.A. and Knaff, D.B. (1991) *Energy transduction in biological membranes. A textbook of bioenergetics*, p. 165, 327, 358. Springer, New York

Cramer, W.A. and Whitmarsh, J. (1977) Photosynthetic cytochromes. *A. Rev. Plant Physiol.* **28**, 133-172.

Critchley, C., Baianu, I.C., Govindjee and Gutowsky, H.S. (1982) The role of chloride in O_2 evolution by thylakoids from salt-tolerant higher plants. *Biochim. biophys. Acta* **682**, 436-

445.
Crofts, A.R. and Wraight, C.A. (1983) The electrochemical domain of photosynthesis. *Biochim. biophys. Acta* **726**, 149-185.
Cross, R.L. and Taiz, L. (1990). Gene duplication as a means for altering H^+/ATP ratios during the evolution of F_oF_1 ATPases and synthases. *FEBS Lett.* **259**, 227-229.
Dau, H. and Sauer, K. (1991) Electric field effect on chlorophyll fluorescence and its relation to Photosystem II charge separation studied by a salt-jump technique. *Biochim. biophys. Acta* **1098**, 49-60.
Dau, H. and Sauer, K. (1992) Electric field effect on the picosecond fluorescence of Photosystem II and its relation to the energetics and kinetics of primary charge separation. *Biochim. biophys. Acta* **1102**, 91-106.
Dau, H., Windecker, R. and Hansen, U.-H. (1991) Effect of light-induced changes in thylakoid voltage on chlorophyll fluorescence of *Aegopodium podagria* leaves. *Biochim. biophys. Acta* **1057**, 337-345.
Davies, J.M., Hunt, I., and Sanders, D. (1994) Vacuolar H^+-pumping ATPase variable transport coupling ratio controlled by pH. *Proc. natn. Acad. Sci. U.S.A.* **91**, 8547-8551.
DegliEsposti, M. (1989) Prediction and comparison of the haem-binding sites in membrane haemoproteins. *Biochim. biophys. Acta* **977**, 249-265.
DegliEsposti, M., DeVries, S., Crimi, M., Ghelli, A., Patarnello, T. and Meyer, A. (1993) Mitochondrial cytochrome *b*: evolution and structure of the protein. *Biochim. biophys. Acta* **1143**, 243-271.
DeGrooth, B.G. and Amesz, J. (1977) Electrochromic absorbance changes of photosynthetic pigments in *Rhodopseudomonas sphaeroides* II. Analysis of the band shifts of carotenoid and bacteriochlorophyll. *Biochim. biophys. Acta* **462**, 247-258.
DeGrooth, B.G., VanGrondelle, R., Romijn, J.C. and Pulles, M.P.J. (1978) The mechanism of reduction of the ubiquinone pool in photosynthetic bacteria at different redox potentials. *Biochim. biophys. Acta* **503**, 480-490.
Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) X-ray structure analysis of a membrane protein complex. *J. mol. Biol.* **180**, 385-398.
Deisenhofer, J. and Michel, H. (1989) The photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis. Science* **245**, 1463-1473.
Dekker, J.P., Boekema, E.J., Witt, H.T. and Rögner, M. (1988) Refined purification and further characterization of oxygen-evolving and Tris-treated Photosystem II particles from the thermophilic *Cyanobacterium synechococcus* sp. *Biochim. biophys. Acta* **936**, 307-318.
DelCampo, F.F., Ramírez, J.M. and Arnon, D.I. (1968) Stoichiometry of photosynthetic phosphorylation. *J. biol. Chem.* **243**, 2805-2809.
Deléage, G., Roux, B. and Marion, C. (1986) Structural aspects and orientation mechanism of mitochondrial F_1 adenosinetriphosphatase. Evidence for a negative electric birefringence due to a permanent dipole perpendicular to the long axes of the particle. *Biochemistry* **25**, 2854-2858.
DelValle-Tascon, S., VanGrondelle, R. and Duysens, L.N.M. (1978) Flash-induced photophosphorylation in *Rhodospirillum rubrum* chromatophores. I. The relationship between cytochrome *c*-420 content and photophosphorylation. *Biochim. biophys. Acta* **504**, 26-39.
Demmig-Adams, B. (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. *Biochim. biophys. Acta* **1020**, 1-24.
Demmig-Adams, B. and Adams, W.W. (1992) Photoprotection and other responses of plants to high light stress. *A. Rev. Plant Phys. Plant mol. Biol.* **43**, 599-626.
Dennison, A.S. and Vogelmann, T.C. (1989) The *Phycomyces* lens: measurement of the sporangiophore intensity profile using a fiber optic microprobe. *Planta* **179**, 1-10.
Dera, J. (1970) On two layers of different light conditions in the euphotic zone of the sea. *Acta Geophys. Pol.* **18**, 287-294.
Dera, J. and Olszewski, J. (1967) On the natural irradiance fluctuations affecting photosynthesis in the sea. *Acta Geophys. Pol.* **15**, 351-364.
Desjardins, R.L., Sinclair, T.R. and Lemon, E.R. (1973) Light fluctuations in corn. *Agron. J.* **65**, 904-

908.
DeVries, S., VanHoek, A.N. and Berden, J.A. (1988) The oxidation-reduction kinetics of cytochromes *b*, c_1 and *c* in initially fully reduced mitochondrial membranes are in agreement with the Q-cycle hypothesis. *Biochim. biophys. Acta* **935**, 208-216.
Dilley, R.A. (1991) Energy coupling in chloroplasts: a calcium-gated switch controls proton fluxes between localized and delocalized proton gradients. *Cur. Top. Bioenerg.* **16**, 265-318.
Diner, B.A. and Joliot, P. (1977) Oxygen evolution and manganese. In: *Photosynthesis I. Encyclopedia of Plant Physiology, New Series, Vol 5*, pp 187-205 (eds A. Trebst and M. Avron). Springer, Berlin.
Döhler, G. (1973) Wirkung intermittierender Belichtung auf die ^{14}C-markierten Produkte während der Photosynthese-Induktion von *Chlorella vulgaris*. *Z. Pflanzenphysiol.* **69**, 142-151.
Dörge, B., Klug, G., Gad'on, N., Cohen, S.N. and Drews, G. (1990) Effects on the formation of antenna complex B870 of *Rhodobacter capsulatus* by exchange of charged amino acids in the N-terminal binding domain of the a and ß pigment-binding proteins. *Biochemistry* **29**, 7754-7758.
Drachev, L.A., Frolov, V.N., Kaulen, A.D., Kondrashin, A.A., Samuilov, V.D., Semenov, A.Y. and Skulachev, V.P. (1976) Generation of electric current by chromatophores of *Rhodospirillum rubrum* and reconstitution of electrogenic function in subchromatophore pigment-protein complexes. *Biochim. biophys. Acta* **440**, 637-660.
Drachev, L.A., Kondrashin, A.A., Samuilov, V.D. and Skulachev, V.P. (1975) Generation of electric potential by reaction center complexes from *Rhodospirillum rubrum*. *FEBS Lett.* **50**, 219-222.
Drachev, L.A., Semenov, A.Y., Skulachev, V.P., Smirnova, I.A., Chamorovsky, S.K., Konokenko, A.A., Rubin, A.B. and Uspenskaya, N.Y. (1981) Fast stages of photoelectric processes in biological membranes. III. Bacterial photosynthetic redox system. *Eur. J. Biochem.* **117**, 483-489.
Dracheva, S.M., Drachev, L.A., Konstantinov, A.A., Semenov, A.Y., Skulachev, V.P., Arutjunjan, A.M., Shuvalov, V.A. and Zaberezhnaya, S.M. (1988) Electrogenic steps in the redox reactions catalyzed by photosynthetic reaction-centre complex from *Rhodopseudomonas viridis*. *Eur. J. Biochem.* **171**, 253-264.
Duysens, L.N.M., Amesz, J. and Kamp, B.M. (1961) Two photochemical systems in photosynthesis. *Nature* **190**, 510-511.
Eccles, J. and Honig, B. (1983) Charged amino acids as spectroscopic determinants for chlorophyll *in vivo*. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4959-4962.
Eckert, H.-J., Toyoshima, Y., Akabori, K. and Dismukes, G.C. (1987) The involvement of lipids in light-induced charge separation in the reaction center of photosystem II. *Photosynth. Res.* **14**, 31-41.
Eimhjellen, K.E., Steensland, H. and Traetteberg, J. (1967) A *Thiococcus* sp. nov. gen., its pigments and internal membrane system. *Arch. Mikrobiol.* **59**, 82-92.
Emerson, R. and Arnold, W. (1932a) A separation of the reactions in photosynthesis by means of intermittent light. *J. gen. Physiol.* **15**, 391-420.
Emerson, R. and Arnold, W. (1932b) The photochemical reaction in photosynthesis. *J. gen. Physiol.* **16**, 191-205.
Emmett, J.M. and Walker, D.A. (1969) Thermal uncoupling in chloroplasts. *Biochim. biophys. Acta* **180**, 424-425.
Engelhardt, H., Baumeister, W. and Saxton, W.O. (1983) Electron microscopy of photosynthetic membranes containing bacteriochlorophyll *b*. *Arch. Microbiol.* **135**, 169-175.
Evans, E.H. and Crofts, A.R. (1974) The relationship between delayed fluorescence and the carotenoid shift in chromatophores from *Rhodopseudomonas capsulata*. *Biochim. biophys. Acta* **333**, 44-51.
Evans, J.R., Jakobsen, I. and Ögren, E. (1993) Photosynthetic light-response curves. 2. Gradients of light absorption and photosynthetic capacity. *Planta* **189**, 191-200.
Fagerberg, W.R. (1983) A quantitative study of daily variation in the cellular ultrastructure of palisade chlorenchyma from sunflower leaves. *Ann. Botany* **52**, 117-126.
Fagerberg, W.R. (1987) Effect of short-term shading on the cytology of palisade tissue in mature leaves of the sunflower *Helianthus annuus*. *Am. J. Bot.* **74**, 822-828.

Falkowski, P.G. and Owens, T.G. (1978) Effects of light intensity on photosynthesis and dark respiration in six species of marine phytoplankton. *Mar. Biol.* **45**, 289-295.

Faludi-Dániel, Á., Mustárdy, L.A., Vass, I. and Kiss, J.G. (1986) Energization and ultrastructural pattern of thylakoids formed under periodic illumination followed by continuous light. *Photosynth. Res.* **9**, 229-238.

Ferguson, S.J. (1985) Fully delocalised chemiosmotic or localised proton flow pathways in energy coupling? A scrutiny of experimental evidence. *Biochim. biophys. Acta* **811**, 47-95.

Ferguson, S.J. and Sorgato, M.C. (1982) Proton electrochemical gradients and energy-transduction processes. *A. Rev. Biochem.* **51**, 185-217.

Feynman, R.P., Leighton, R.B. and Sands, M. (1963) T*he Feynman lectures on physics.* pp 46: 1-9. Addison Wesley, Reading.

Fillingame, R.H. (1990) Molecular mechanics of ATP synthesis in F_1F_0-type H^+-transporting ATPsynthases. In: *The bacteria*, Vol. 12, pp 345-391 (ed. T.A.Krulwich). Academic Press, New York.

Fillingame, R.H. (1992) Subunit *c* of F_1F_0 synthase: structure and role in transmembrane energy translocation. *Biochim. biophys. Acta* **1101**, 240-243.

Fitton, V., Rigoulet, M., Ouhabi, R. and Guérin, B. (1994) Mechanistic stoichiometry of yeast mitochondrial oxidative phosphorylation. *Biochemistry* **33**, 9692-9698.

Forde, J. and Steer, M.W. (1976) The use of quantitative electron microscopy in the study of the lipid composition of membranes. *J. exp. Bot.* **27**, 1137-1141.

Fork, D.C. and Murata, N. (1977) The relationship between changes of the physical phase of membrane lipids and photosynthesis in the thermophilic alga *Cyanidium caldarium*. *Plant Cell Physiol.* Spec. Issue: 427-436.

Fork, D.C. and Satoh, K. (1986) The control by state transitions of the distribution of excitation energy in photosynthesis. *A. Rev. Plant Physiol.* **37**, 335-361.

Foster, D.L. and Fillingame, R.H. (1982) Stoichiometry of subunits in the H^+-ATPase complex of *Escherichia coli*. *J. biol. Chem.* **257**, 2009-2015.

Fowler, C.F. and Kok, B. (1976) Determination of H^+/e^- ratios in chloroplasts with flashing light. *Biochim. biophys. Acta* **423**, 510-523.

Fowler, G.J.S., Visschers, R.W., Grief, G.G., VanGrondelle, R. and Hunter, C.N. (1992) Genetically modified photosynthetic antenna complexes with blueshifted absorbance bands. *Nature* **355**, 848-850.

Foyer, C., Furbank, R., Harbinson, J. and Horton, P. (1990) The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. *Photosynth. Res.* **25**, 83-100.

Fredrickson, A.G. and Tsuchiya, H.M. (1970) Utilization of the effects of intermittent illumination on photosynthetic microorganisms. In: *Prediction and Measurement of Photosynthetic Productivity*, pp 519-541. Wageningen, PUDOC.

Friesner, R.A. and Won, Y. (1989) Photochemical charge separation in photosynthetic reaction centers. *Photochem. Photobiol.* **50**, 831-839.

Fuller, R.C., Sprague, S.G., Gest, H. and Blankenship, R.E. (1985) A unique photosynthetic reaction center from *Heliobacterium chlorum*. *FEBS Lett.* **182**, 345-379.

Furbank, R.T. and Horton, P. (1987) Regulation of photosynthesis in isolated barley protoplasts: the contribution of cyclic photophosphorylation. *Biochim. biophys. Acta* **894**, 332-338.

Gabrys, H., Kramer, D.M., Crofts, A.R. and Ort, D.R. (1994) Mutants of chloroplasts coupling factor reduction in *Arabidopis*. *Plant Physiol.* **104**, 769-776.

Gaffron, H. (1962) On dating stages in photochemical evolution. In: *Horizons in Biochemistry*, pp 59-89 (M. Kasha and B. Pullman). Academic Press, New York.

Gaines, G.L. (1966) *Insoluble monolayers at liquid-gas interfaces*. Interscience, New York.

Gans, P., Bulte, L., Rebeille, F. and Wollman, F.-A. (1990) ATP deprivation induces state I to state II transition in the dark in Chlamydomonas reinhardtii. In: *Current Research in Photosynthesis, Vol IV*, pp 43-46 (ed. M. Baltscheffsky). Kluwer, Dordrecht.

Gao, J.-L., Shopes, R.J. and Wraight, C.A. (1990) Charge recombination between the oxidized high-potential *c*-type cytochromes and Q^-_A in reaction centers from *Rhodopseudomonas viridis.* *Biochim. biophys. Acta* **1015**, 96-108.

Gao, J.-L., Shopes, R.J. and Wraight, C.A. (1991) Heterogeneity of kinetics and electron transfer equilibria in the bacteriopheophytin and quinone electron acceptors of reaction centers from *Rhodopseudomonas viridis*. *Biochim. biophys. Acta* **1056**, 259-272.

Gaudillere, J.P., Drevon, J.J., Bernoud, J.P., Jardinet, F. and Euvrard, M. (1987) Effects of periodic fluctuations of photon flux density on anatomical and photosynthetic characteristics of soybean leaves. *Photosynth. Res.* **13**, 81-89.

Gennis, R.B., Barquera, B., Hacker, B., VanDoren, S.R., Arnaud, S., Crofts, A.R., Davidson, E., Gray, K.A. and Daldal, F. (1993) The bc_1 complexes of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. *J. Bioenerg. Biom.* **25**, 195-209.

Gerst, U., Schönknecht, G. and Heber, U. (1994) ATP and NADPH as the driving force of carbon reduction in leaves in relation to thylakoid energization by light. *Planta* **193**, 421-429.

Gest, H. (1980) The evolution of biological energy-transducing systems. *FEMS Microbiol. Lett.* **7**, 73-77.

Ghirardi, M.L. and Melis, A. (1984) Photosystem electron-transport capacity and light harvesting antenna size in maize chloroplasts. *Plant Physiol.* **74**, 993-998.

Giersch, C., Heber, U., Kobayashi, Y., Inoue, Y., Shibata, K. and Heldt, H.W. (1980) Energy charge, phosphorylation potential and proton motive force in chloroplasts. *Biochim. biophys. Acta* **590**, 59-73.

Giersch, C. (1981) Stimulation of photophosphorylation by low concentrations of uncoupling amines. *Biochem. biophys. Res. Comm.* **100**, 666-674.

Giersch, C. (1983) Nigericin-induced stimulation of photophosphorylation in chloroplasts. *Biochim. biophys. Acta* **725**, 309-319.

Giersch, C. and Meyer, M. (1984) Uncoupler-induced stimulation of photophosphorylation. Effects of carboxylic ionophores and amines at low concentrations of phosphorylation rate, phosphorylation potential and proton motive force in chloroplasts. *Bioelectrochem. Bioenerg.* **12**, 63-71.

Giesbrecht, P. and Drews, G. (1966) Über die Organisation und die makromolekulare Architektur der Thylakoide „lebender'' Bakterien. *Arch. Mikrobiol.* **54**, 297-330.

Glansdorff, P. and Prigogine, I. (1971) *Thermodynamic theory of structure stability and fluctuations.* Wiley, London.

Glazebrook, R.W. and Thomas, A. (1982) Solar energy conversion *via* a photodielectric effect. *J. chem. Soc., Faraday Trans. 2* **78**, 2053-2065.

Glazer, A.N. and Melis, A. (1987) Photochemical reaction centers: structure, organization, and function. *A. Rev. Plant Physiol.* **38**, 11-45.

Glick, R.E. and Melis, A. (1988) Minimum photosynthetic unit size in System I and System II of barley chloroplasts. *Biochim. biophys. Acta* **934**, 151-155.

Golbeck, J.H. (1987) Structure, function and organization of the Photosystem I reaction center complex. *Biochim. biophys. Acta* **895**, 167-204.

Golbeck, J.H. and Bryant, D.A. (1991) Photosystem I. *Cur. Top. Bioenerg.* **16**, 83-177.

Golecki, J., Drews, G. and Bühler, R. (1979) The size and number of intramembrane particles in cells of the photosynthetic bacterium Rhodopseudomonas capsulata studied by freeze-fracture electron microscopy. *Cytobiologie* **18**, 381-389.

Golecki, J.R. and Oelze, J. (1980) Differences in the architecture and intracytoplasmic membranes of three chemotrophically and phototrophically grown species of the *Rhodospirillaceae*. *J. Bact.* **144**, 781-788.

Gonzalo, J.A. (1976) Ferroelectric materials as energy converters. *Ferroelectrics* **11**, 423-430.

Gottfried, D.S., Steffen, M.A. and Boxer, S.G. (1991a) Large protein-induced dipoles for a symmetric carotenoid in a photosynthetic antenna complex. *Science* **251**, 662-665.

Gottfried, D.S., Steffen, M.A. and Boxer, S.G. (1991b) Stark effect spectroscopy of carotenoids in photosynthetic antenna and reaction center complexes. *Biochim. biophys. Acta* **1059**, 76-90.

Gottfried, D.S., Stocker, J.W. and Boxer, S.G. (1991c) Stark effect spectroscopy of bacteriochlorophyll in light-harvesting complexes from photosynthetic bacteria. *Biochim. biophys. Acta* **1059**, 63-75.

Gottschall, J.C. (1990) Phenotypic response to environmental changes. *FEMS Microbiol. Ecol.* **74**, 93-102.

Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1983) Structural and functional changes

associated with heat-induced phase-separations of non-bilayer lipids in chloroplast thylakoid membranes. *FEBS Lett.* **153**, 47-52.

Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1984) Structural reorganization of chloroplast thylakoid membranes in response to heat-stress. *Biochim. biophys. Acta* **766**, 198-208.

Gounaris, K., Chapman, D.J., Booth, P., Crystall, B., Giorgi, L.B., Klug, D.R., Porter, G. and Barber, J. (1990) Comparison of the D1/D2/cytochrome b559 reaction centre complex of photosystem two isolated by two different methods. *FEBS Lett.* **265**, 88-92.

Gounaris, K., Whitford, D. and Barber, J. (1983) The effect of thylakoid lipids on an oxygen-evolving Photosystem II preparation. *FEBS Lett.* **163**, 230-234.

Graan, T., Flores, S. and Ort, D.R. (1981) The nature of ATP formation associated with single turnovers of the electron transport carriers in chloroplasts. In: *Energy Coupling in Photosynthesis*, pp 25-34 (eds B.R. Selman and S. Selman-Reimer). Elsevier/North-Holland, New York.

Graan, T. and Ort, D.R. (1983) Initial events in the regulation of electron transfer in chloroplasts. *J. biol. Chem.* **258**, 2831-2836.

Gräber, P., Junesch, U. and Thulke, G. (1987) The chloroplast ATP synthase: the rate of the catalytic reaction. In: *Progress in photosynthesis research, Vol III*, pp 2177-2184 (ed. J. Biggins). Nijhoff, Utrecht.

Gräber, P. and Witt, H.T. (1974) The effect of temperature on flash induced transmembrane currents in chloroplasts of spinach. In: *Proceedings of the Third International Congress on Photosynthesis*, pp 951-956 (ed. M. Avron). Elsevier, Amsterdam.

Gräber, P. and Witt, H.T. (1976) Relations between the electrical potential, pH gradient, proton flux and phosphorylation in the photosynthetic membrane. *Biochim. biophys. Acta* **423**, 141-163.

Granick, S. (1957) Speculations on the origins and evolution of photosynthesis. *Ann. N.Y. Acad. Sci.* **69**, 292-308.

Gross, E., Bedlack, R.S. and Loew, L.M. (1994) Dual-wavelength ratiometric fluorescence measurement of the membrane dipole potential. *Biophys. J.* **67**, 208-216.

Guenther, J.E. and Melis, A.M. (1990) The physiological significance of photosystem II heterogeneity in chloroplasts. *Photosynth. Res.* **23**, 105-109.

Guenther, J.E., Nemson, J.A. and Melis, A. (1990) Development of photosystem II in dark grown *Chlamydomonas reinhardtii*. A light-dependent conversion of $PSII_{\beta}$, Q_B-nonreducing centers to the PS II_{α}, Q_B-reducing form. *Photosynth. Res.* **24**, 35-46.

Guffanti, A.A., Fuchs, R.T., Schniere, M., Chiu, E. and Krulwich, T.A. (1984) A transmembrane electrical potential generated by respiration is not equivalent to a diffusion potential of the same magnitude for ATP synthesis by *Bacillus firmus* RAB. *J. biol. Chem.* **259**, 2971-2975.

Gunner, M.R. (1991) The reaction center protein from purple bacteria: structure and function. *Cur. Top. Bioenerg.* **16**, 319-367.

Gustavson, K.H. (1956) *The chemistry and reactivity of collagen.* Academic Press, New York.

Gutschick, V.P. (1984) Photosynthesis model for C_3 leaves incorporating CO_2 transport, propagation of radiation, and biochemistry. 1. Kinetics and their parametrization. *Photosynthetica* **18**, 549-568.

Gutteridge, S. (1990) Limitations of the primary events of CO_2 fixation in photosynthetic organisms: the structure and mechanism of rubisco. *Biochim. biophys. Acta* **1015**, 1-14.

Haaker, H. and Klugkist, J. (1987) The bioenergetics of electron transport to nitrogenase. *FEMS Microbiol. Rev.* **46**, 57-71.

Hadni, A. (1981) Applications of the pyroelectric effect. *J. Physics E* **14**, 1233-1240.

Hagins, W.A. and McGaughy, R.E. (1967) Molecular and thermal origins of fast photoelectric effects in the squid retina. *Science* **157**, 813-816.

Haken, H. (1978) *Synergetics* (2nd ed). Springer, Berlin.

Hall, D.O. (1976) The coupling of photophosphorylation to electron transport in isolated chloroplasts. *Top. Photosynth.* **1**, 135-170.

Hangarter, R.P., Grandoni, P. and Ort, D.R. (1987a) The effects of chloroplast coupling factor reduction on the energetics of activation and on the energetics and efficiency of ATP formation. *J. biol. Chem.* **262**, 13513-13519.

Hangarter, R.P., Jones, R.W., Ort, D.R. and Whitmarsh, J. (1987b) Stoichiometries and energetics of proton translocation coupled to electron transport in chloroplasts. *Biochim. biophys. Acta* **890**, 106-115.

Hanson, E.A. (1939) Some properties of the chlorophyll in relation to its biological function. *Recueil Trav. Botan. Néerl.* **36**, 180-267.

Hansson, Ö. and Wydrzynski, T. (1990) Current perceptions of Photosystem II. *Photosynth. Res.* **23**, 131-162.

Haraux, F. (1985) Localized or delocalized protons and ATP synthesis in biomembranes. *Physiologie Végétale* **23**, 397-410.

Harbinson, J. and Hedley, C.L. (1989) The kinetics of P-700$^+$ reduction in leaves: a novel *in situ* probe of thylakoid functioning. *Plant Cell Environm.* **12**, 357-369.

Harris, D.A. and Crofts, A.R. (1978) The initial stages of photophosphorylation. Studies using excitation by saturating, short flashes of light. *Biochim. biophys. Acta* **502**, 87-102.

Hart, J.W. (1988) *Light and plant growth*, pp 39-41. Unwin, London.

Hartman, H. (1992) Conjectures and reveries. *Photosynth. Res.* **33**, 171-176.

Havaux, M., Greppin, H. and Strasser, R.J. (1991) Functioning of photosystems I and II in pea leaves exposed to heat stress in the presence and the absence of light. *Planta* **186**, 88-98.

Hayama, T., Shimmen, T. and Tazawa, M. (1979) Participation of Ca^{2+} in cessation of cytoplasmic streaming by membrane excitation in *Characeae* internodal cells. *Protoplasma* **99**, 305-321.

Haydon, D.A. and Hladky, S.B. (1972) Ion transport across thin lipid membranes: a critical discussion of mechanisms in selected systems. *Quart. Rev. Biophys.* **5**, 187-282.

Heber, U. (1969) Conformational changes of chloroplasts induced by illumination of leaves *in vivo*. *Biochim. biophys. Acta* **180**, 302-319.

Heimburg, T. and Marsh, D. (1993) Investigation of secondary and tertiary structural changes of cytochrome *c* in complexes with anionic lipids using amide hydrogen exchange measurements: an FTIR study. *Biophys. J.* **65**, 2408-2417.

Heithier, H., Ballschmiter, K. and Möhwald, H. (1983) Liquid-crystalline phase transition of monomolecular layers of chlorophyll *a*. *Photochem. Photobiol.* **37**, 201-205.

Helgerson, S.L., Requadt, C. and Stoeckenius, W. (1983) *Halobacterium halobium* photophosphorylation: illumination dependent increase in the adenylate energy charge and phosphorylation potential. *Biochemistry* **22**, 5746-5753.

Hill, R. and Bendall, F. (1960) Reaction of the two cytochrome components in chloroplasts: a working hypothesis. *Nature* **186**, 136-137.

Hitchens, G.D. and Kell, D.B. (1982) On the extent of localization of the energized membrane state in chromatophores from *Rhodopseudomonas capsulata* N22. *Biochem. J.* **206**, 351-357.

Hodgson, G.W. and Ponnamperuma, C. (1968) Prebiotic porphyrin synthesis: porphyrins from electric discharges in methane, ammonia and water vapor. *Proc. natn. Acad. Sci. U.S.A.* **59**, 22-28.

Hoff, A.J. (1986) Triplets: phosphorescence and magnetic resonance. In: *Light emission by plants and bacteria*, pp 225-263 (eds Govindjee, J. Amesz and D.C. Fork). Academic Press, Orlando.

Hong, F.T. (1994) Photovoltaic effects in biomembranes. *IEEE Engineer. Med. Biol. Magaz.* **13**, 75-93.

Höök, F. and Brzezinski, P. (1994) Light-induced voltage changes associated with electron and proton transfer in photosystem II core complexes reconstituted in phospholipid monolayers. *Biophys. J.* **66**, 2066-2072.

Hoppe, J., Schairer, H.U. and Sebald, W. (1980) The proteolipid of a mutant ATPase from *Escherichia coli* defective in H^+-conduction contains a glycine instead of the carbodiimide reactive aspartyl residue. *FEBS Lett.* **109**, 107-111.

Hoppe, J., Schairer, H.U., Freidl, P. and Sebald, W. (1982) An Asp-Asn substitution in the proteolipid of a mutant ATPase from *Escherichia coli* leads to a non-functional proton channel. *FEBS Lett.* **145**, 21-24.

Horton, P. (1985) Interactions between electron transfer and carbon assimilation. *Top. Photosynth.* **6**, 135-187.

Horváth, G., Droppa, M., Szitó, T., Mustárdy, L.A., Horváth, L.I. and Vigh, L. (1986) Homogeneous catalytic hydrogenation of lipids in the photosynthetic membrane: effects on membrane structure and photosynthetic activity. *Biochim. biophys. Acta* **849**, 325-336.

Horváth, G., Melis, A., Hideg, É., Droppa, M. and Vigh, L. (1987) Role of lipids in the organization and function of photosystem II studied by homogeneous catalytic hydrogenation of thylakoid membrane in situ. *Biochim. biophys. Acta* **891**, 68-74.

Huebner, J.S., Arrieta, R.T. and Millar, D.B. (1982) Aromatic amino acids and ultraviolet induced photo-electric effects in bilayer membranes. *Photochem. Photobiol.* **35**, 467-471.

Hunter, C.N., VanGrondelle, R. and Olsen, J.D. (1989) Photosynthetic antenna proteins: 100 ps before photochemistry starts. *Trends biochem. Sci.* **14**, 72-76.

Ilani, A., Woodle, M. and Mauzerall, D. (1989) Photoinduced electron transfer across lipid bilayers containing magnesium octaethylporphyrin. *Photochem. Photobiol.* **49**, 673-679.

Inagaki, N. and Satoh, K. (1992) Accumulation of a translation intermediate of D1 protein by light-dark transition in isolated spinach chloroplasts. *FEBS Lett.* **300**, 5-8.

Inoue, Y., Shibata, K. and Heber, Y. (1978) Synthesis and hydrolysis of ATP by intact chloroplasts under flash illumination and in darkness. *Biochim. biophys. Acta* **504**, 142-152.

Ivanov, B.N., Shmeleva, V.L. and Ovchinnikova, V.I. (1985) Stoichiometry of proton uptake in isolated pea chloroplasts under different light intensities. *J. Bioenerg. Biom.* **17**, 239-249.

Iwamoto, M., Majima, Y., Naruse, H., Noguchi, T. and Fuwa, H. (1991) Generation of Maxwell displacement current across an azobenzene monolayer by photoisomerization. *Nature* **353**, 645-647.

Izawa, S. and Good, N.E. (1966a) Effects of salts and electron transport on the conformation of isolated chloroplasts. I. Light scattering and volume changes. *Plant Physiol.* **41**, 533-543.

Izawa, S. and Good, N.E. (1966b) Effects of salts and electron transport on the conformation of isolated chloroplasts. II. Electron microscopy. *Plant Physiol.* **41**, 544-552.

Izawa, S., Heath, R.L. and Hind, G. (1969) The role of chloride ion in photosynthesis. III. The effect of artificial electron donors upon electron transport. *Biochim. biophys. Acta* **180**, 388-398.

Jackson, J.B. (1982) Evidence that the ionic conductivity of the cytoplasmic membrane of *Rhodopseudomonas capsulata* is dependent upon membrane potential. *FEBS Lett.* **139**, 139-143.

Jackson, J.B. and Crofts, A.R. (1969) The high energy state in chromatophores from *Rhodopseudomonas spheroides. FEBS Lett.* **4**, 185-189.

Jackson, J.B. and Dutton, P.L. (1973) The kinetic and redox potentiometric resolution of the carotenoid shifts in *Rhodopseudomonas spheroides* chromatophores: their relationship to electric field alterations in electric transport and energy coupling. *Biochim. biophys. Acta* **325**, 102-113.

Jackson, J.B., Saphon, S. and Witt, H.T. (1975) The extent of the stimulated electrical potential decay under phosphorylating conditions and the H^+/ATP ratio in *Rhodopseudomonas sphaeroides* chromatophores following short flash excitation. *Biochim. biophys. Acta* **408**, 83-92.

Jackson, J.B., Greenrod, J.A., Packham, N.K. and Petty, K.M. (1978) Contribution from both electron transport and ion transport to the decay of the carotenoid shift after flash-activation of chromatophores. In: *Frontiers of Biological Energetics*, Vol. 1, pp 316-325 (eds P.L. Dutton, J.S. Leigh and A. Scarpa). Academic Press, New York.

Jackson, J.D. (1975) *Classical Electrodynamics.* (2nd ed) p 38, 218. John Wiley, New York.

Jagendorf, A.T. (1977) Photophosphorylation. In: *Photosynthesis I. Encyclopedia of Plant Physiology, New Series, Vol 5*, pp 307-337 (eds A. Trebst and M. Avron). Springer, Berlin.

Jahns, P. and Junge, W. (1992) Thylakoids from pea seedlings grown under intermittent light: biochemical and flash-spectroscopic properties. *Biochemistry* **31**, 7390-7397.

Jeschke, W.D. (1976) Ionic relations in leaf cells. In: *Encyclopedia of Plant Physiology, New Series, Vol 2*, pp 160-194 (eds U. Lüttge and M.G. Pitman). Springer, Berlin.

Johnson, G.N., Boussac, A. and Rutherford, A.W. (1994) The origin of the 40-50 ºC thermoluminescence bands in Photosystem II. *Biochim. biophys. Acta* **1184**, 85-92.

Joiris, C. and Bertels, A. (1985) Incubation under fluctuating light conditions provides values much closer to real in situ primary production. *Bull. mar. Sci.* **37**, 620-625.

Joliot, P., Barbieri, G. and Chabaud, R. (1969) Un nouveau modele des centres photochimiques du systeme II. *Photochem. Photobiol.* **10**, 309-329.

Joliot, P. and Joliot, A. (1989) Characterization of linear and quadratic electrochromic probes in *Chlorella sorokiniana* and *Chlamydomonas reinhardtii*. *Biochim. biophys. Acta* **975**, 355-360.

Jones, H.G. (1992) *Plants and microclimate.* 2nd ed.p. 195, 204. Cambridge university press.
Jones, M.N. (1975) *Biological interfaces. An introduction to the surface and colloid science of biochemical and biological systems*, p 38. Elsevier, Amsterdam.
Joss, A., Mez, K., Känel, B., Hanselmann, K.W. and Bachofen, R. (1994) Measurement of fluorescence kinetics of phototrophic bacteria in the natural environment. *J. Plant Physiol.* **144**, 333-338.
Junesch, U. and Gräber, P. (1985) The rate of ATP synthesis as a function of ΔpH in normal and dithiothreitol-modified chloroplasts. *Biochim. biophys. Acta*. **809**, 429-434.
Junesch, U. and Gräber, P. (1987) Influence of the redox state and the activation of the chloroplast ATP synthase on proton-transport-coupled ATP synthesis / hydrolysis. *Biochim. biophys. Acta* **893**, 275-288.
Junge, W. (1970a) The necessity of an electric potential difference and its use for photophosphorylation in short flash groups. *Eur. J. Biochem.* **14**, 575-581.
Junge, W. (1970b) The critical electric potential difference for photophosphorylation. *Eur. J. Biochem.* **14**, 582-592.
Junge, W. (1972) On the molecular mechanism of passive and active ion translocation across the thylakoid membrane. In: *Proceedings of the 2nd International Congress on Photosynthesis Research*, pp 1065-1074 (eds G. Fiorti, M. Avron and A. Melandri). Junk, The Hague.
Junge, W. (1977) Membrane potentials in photosynthesis. *A. Rev. Plant Physiol.* **28**, 503-536.
Junge, W., Engelbrecht, S., Griwatz, C. and Groth, G. (1992) The chloroplast H^+-ATPase: partial reactions of the proton. *J. exp. Biol.* **172**, 461-474.
Jursinic, P.A. (1986) Delayed fluorescence: current concepts and status. In: *Light Emission by Plants and Bacteria*, pp 291-328 (eds J. Govindjee and D.C. Fork). Academic Press, Orlando.
Jursinic, P. and Govindjee (1972) Delayed light emission in DCMU-treated chlorella: temperature effect. In: *Proceedings of the IInd International Congres on Photosynthesis Research*, pp 223-232 (eds G. Forti, M. Avron and A. Melandri). Junk, The Hague.
Jursinic, P. and Govindjee (1977) Temperature dependence of delayed light emission in the 6 to 340 microsecond range after a single flash in chloroplasts. *Photochem. Photobiol.* **26**, 617-628.
Jursinic, P., Govindjee, and Wraight, C.A. (1978) Membrane potential and microsecond to millisecond delayed light emission after a single excitation flash in isolated chloroplasts. *Photochem. Photobiol.* **27**, 61-71.
Kachar, B. and Reese, T.S. (1988) The mechanism of cytoplasmic streaming in characean algal cells: sliding of endoplasmic reticulum along actin filaments. *J. Cell Biol.* **106**, 1545-1552.
Kaiser, I. and Oelze, J. (1980) Growth and adaptation to phototrophic conditions of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* at different temperatures. *Arch. Microbiol.* **126**, 187-194.
Kakitani, T., Honig, B. and Crofts, A.R. (1982) Theoretical studies of the electrochromic response of carotenoids in photosynthetic membranes. *Biophys. J.* **39**, 57-63.
Kamiya, N. (1962) Protoplasmic streaming. In: *Encyclopedia of Plant Physiology, Vol 17, part 2*, pp 979-1035 (ed. W. Ruhland). Berlin, Springer.
Kell, D.B. (1979) On the functional proton current pathway of electron transport phosphorylation. An electrodic view. *Biochim. biophys. Acta* **549**, 55-99.
Kim, J. and Rees, D.C. (1994) Nitrogenase and biological nitrogen fixation. *Biochemistry* **33**, 389-397.
Kirk, J.T.O. (1971) Chloroplast structure and biogenesis. *A. Rev. Plant Physiol.* **21**, 161-196.
Kleinfeld, D., Okamura, M.Y. and Feher, G. (1984) Electron-transfer kinetics in photosynthetic reaction centers cooled to cryogenic temperatures in the charge-separated state: evidence for light-induced structural changes. *Biochemistry* **23**, 5780-5786.
Knaff, D.B. (1993) The cytochrome bc_1 complexes of photosynthetic purple bacteria. *Photosynth. Res.* **35**, 117-133.
Knapp, A.K. and Smith, W.K. (1990) Stomatal and photosynthetic responses to variable sunlight. *Physiol. Plant* **78**, 160-165.
Knapp, A.K., Smith, W.K. and Young, D.R. (1989) Importance of intermittent shade to the ecophysiology of subalpine herbs. *Functional Ecol.* **3**, 753-758.
Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H. and Satoh, K. (1990) Chlorophyll a and b-

carotene content in the D_1/D_2/cytochrome *b*-559 reaction center complex from spinach. *FEBS Lett.* **260**, 138-140.

Koike, H., Hanssum, B., Inoue, Y. and Renger, G. (1987) Temperature dependence of S-state transition in a thermophilic cyanobacterium, *Synechoccus vulcanus* Copeland measured by absorption changes in the ultraviolet region. *Biochim. biophys. Acta* **893**, 524-533.

Kok, B. (1949) On the interrelation of respiration and photosynthesis in green plants. *Biochim. biophys. Acta* **3**, 625-631.

Kok, B. and Cheniae, G.M. (1966) Kinetics and intermediates of the oxygen evolution step in photosynthesis. *Cur. Top. Bioenerg.* **1**, 1-47.

Kraayenhof, R., Katan, M.B. and Grunwald, T. (1971) The effect of temperature on energy-linked functions in chloroplasts. *FEBS Lett.* **19**, 5-10.

Kramer, D.M. and Crofts, A.R. (1989) Activation of the chloroplast ATPase measured by the electrochromic change in leaves of intact plants. *Biochim. biophys. Acta* **976**, 28-41.

Kramer, D.M., Wise, R.R., Frederick, J.R., Alm, D.M., Hesketh, J.D., Ort, D.R. and Crofts, A.R. (1990) Regulation of coupling factor in field-grown sunflower: a redox model relating coupling factor activity to the activities of other thioredoxin-dependent chloroplast enzymes. *Photosynth. Res.* **26**, 213-222.

Krasnovsky, A.A. (1971) The evolution of photochemical electron transfer systems. In: *Prebiotic and biochemical evolution*, pp 209-216 (eds A.P. Kimball and J. Oró). North-Holland, Amsterdam.

Krause, G.H. (1973) The high-energy state of the thylakoid systen as indicated by chlorophyll flurorescence and chloroplast shrinkage. *Biochim. biophys. Acta* **292**, 715-728.

Krause, G.H. and Weis, E. (1991) Chlorophyll fluorescense and photosynthesis: the basics. *A. Rev. Plant Physiol.* **42**, 313-349.

Krawczyk, S. (1991) Electrochromism of chlorophyll *a* monomer and special pair dimer. *Biochim. biophys. Acta* **1056**, 64-70.

Krebs, H.A. (1966) Theoretical concepts in biological sciences. In: *Current Aspects of Biochemical Energetics*, pp 83-95 (eds N.O. Kaplan and E.P. Kennedy). Academic Press, New York.

Kriedemann, P.E., Törökfalvy, E. and Smart, R.E. (1973) Natural occurrence and photosynthetic utilisation of sunflecks by grapevine leaves. *Photosynthetica* **7**, 18-27.

Krishnan, M. and Mohanty, P. (1984) Reactivation by chloride of hill activity in heat- and tris-treated thylkoid membranes from Beta vulgaris. *Photosynth. Res.* **5**, 185-198.

Krulwich, T.A. and Guffanti, A.A. (1992) Proton-coupled bioenergetic processes in extremely alkaliphilic bacteria. *J. Bioenerg. Biom.* **24**, 587-599.

Krupinska, K., Akoyunoglou, G. and Senger, H. (1985) Relationship between light-induced state I/II-transition and Mg^{2+}-effect on fluorescence, tested in synchronous cultures of *Scenedesmus obliquus*. *Photochem. Photobiol.* **41**, 159-164.

Kühlbrandt, W. (1987) Photosynthetic membranes and membrane proteins. In: *Electron Microscopy of Proteins*, pp 156-207 (ed. J.R. Harris). Academic Press, London.

Kuhn, H. (1994) Organized monolayer assemblies. Their role in constructing supramolecular devices and in modeling evolution of early life. *IEEE Engineer. Med. Biol. Magaz.* **13**, 33-44.

Laisk, A. and Oja, V. (1994) Range of photosynthetic control of postillumination $P700^+$ reduction rate in sunflower leaves. *Photosynth. Res.* **39**, 39-50.

Laisk, A., Siebke, K., Gerst, U., Eichelmann, H., Oja, V. and Heber, U. (1991) Oscillations in photosynthesis are initiated and supported by imbalance in the supply of ATP and NADPH to the Calvin cycle. *Planta* **185**, 554-562.

Lakhdar-Ghazal, F., Tichadou, J.-L., and Tocanne, J.-F. (1983) Effect of pH and monovalent cations on the ionization state of phosphatidylglycerol in monolayers. *Eur. J. Biochem.* **134**, 531-537.

Lakso, A.N. and Barnes, J.E. (1978) Apple leaf photosynthesis in alternating light. *HortScience* **13**, 473-478.

Lange, O.L. (1959) Untersuchungen über Wärmehaushalt und Hitzeresistenz mauretanischer Wüsten- und Savannenpflanzen. *Flora* **147**, 595-651.

Larkum, A.W.D. (1991) The evolution of chlorophylls. In: *Chlorophylls*, pp 367-383 (ed. H. Scheer). CRC Press, Boca Raton.

Larkum, A.W.D. (1992) Evolution of chlorophylls, light harvesting systems and photosynthetic reaction centers. In: *Research in Photosynthesis, Vol III*, pp 475-482 (ed. N. Murata). Kluwer, Dordrecht.

Lavergne, J. and Leci, E. (1993) Properties of inactive Photosystem II centers. *Photosynth. Res.* **35**, 323-343.

Lawlor, D.W. (1993) *Photosynthesis: molecular, physiological and environmental processes* (2nd ed) p 50. Longman, Harlow.

Lee, W.-J. and Whitmarsh, J. (1989) Photosynthetic apparatus of pea thylakoid membranes. *Plant Physiol.* **89**, 932-940,

Lemaire, C., Girault, G. and Galmiche, J.M. (1985) Flash-induced ATP synthesis in pea chloroplasts in relation to proton flux. *Biochim. biophys. Acta* **807**, 285-292.

Leong, T.-Y. and Anderson, J.M. (1984a) Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll-protein complexes. *Photosynth. Res.* **5**, 105-115.

Leong, T.-Y. and Anderson, J.M. (1984b) Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. II. Regulation of thylakoid membranes of pea chloroplasts, electron carriers, coupling factor (CF_1) activity and rates of photosynthesis. *Photosynth. Res.* **5**, 117-128.

Ley, A.C. and Mauzerall, D.C. (1982a) Absolute absorption cross-sections for photosystem II and the minimum requirement for photosynthesis in *Chlorella vulgaris*. *Biochim. biophys. Acta* **680**, 95-106.

Ley, A.C. and Mauzerall, D.C. (1982b) The reversible decline of oxygen flash yields at high flash energies. Evidence for total annihilation of excitations in photosystem II. *Biochim. biophys. Acta* **680**, 174-180.

Lilley, R.M., Chon, C.J., Mosbach, A. and Heldt, H.W. (1977) The distribution of metabolites between spinach chloroplasts and medium during photosynthesis in vitro. *Biochim. biophys. Acta* **460**, 259-272.

Lockhart, D.J. and Boxer, S.G. (1987) Magnitude and direction of the change in dipole moment associated with excitation of the primary electron donor in *Rhodopseudomonas sphaeroides* reaction centers. *Biochemistry* **26**, 664-668.

Low, P.S., Ort, D.R., Cramer, W.A., Whitmarsh, J. and Martin, B. (1984) Search for an endotherm in chloroplast lamellar membranes associated with chilling-inhibition of photosynthesis. *Arch. Biochem. Biophys.* **231**, 336-344.

Mar, T. and Govindjee (1971) Thermoluminescence in spinach chloroplasts and in Chlorella. *Biochim. biophys. Acta* **226**, 200-203.

Margosian, P.M. (1965) *Parametric study of a thermoelectrostatic generator for space applications.* NASA Techn. Note TN D-2763

Martin, B. (1986) Arrhenius plots and the involvement of thermotropic phase transitions of the thylakoid membrane in chilling impairment of photosynthesis in thermophilic higher plants. *Plant Cell Environm.* **9**, 323-331.

Mascarenhas, S. (1980) Bioelectrets: electrets in biomaterials and biopolymers. *Top. Appl. Physics* **33**, 321-346.

Matin, A., Auger, E.A., Blum, P.H. and Schulz, J.E. (1989) Genetic basis of starvation survival in nondifferentiating bacteria. *A. Rev. Microbiol.* **43**, 293-316.

Matsuura, K. and Shimada, K. (1990) Evolutionary relationships between reaction center complexes with and without cytochrome c subunits in purple bacteria. In: *Current Research in Photosynthesis*, Vol I, pp 193-196 (ed. M. Baltscheffsky). Kluwer, Dordrecht.

Mauzerall, D. (1992) Light, iron, Sam Granick and the origin of life. *Photosynth. Res.* **33**, 163-170.

Mauzerall, D. and Greenbaum, N.L. (1989) The absolute size of a photosynthetic unit. *Biochim. biophys. Acta* **974**, 119-140.

McEvoy, F.A. and Lynn, W.S. (1972) The effects of temperature on photophosphorylation and on the two forms of cytochrome 559 in subchloroplast particles. *Arch. Biochem. Biophys.* **150**, 632-635.

Meier, A. and Bachofen, R. (1981) The temperature dependence of initial events in photophosphorylation of spinach chloroplasts. *FEBS Lett.* **127**, 193-195.

Meier, A., Hofmann, M. and Bachofen, R. (1981) Initial events in photophosphorylation with

chloroplasts and chromatophores: effects of temperature and of valinomycin. In: *Energy Coupling in Photosynthesis*, pp 79-87 (eds B.R. Selman and S. Selman-Reimer). Elsevier-North Holland, New York.

Melandri, B.A., Zannoni, D., Casadio, R. and Baccarini-Melandri, A. (1974) Energy conduction and transduction in photosynthesis and respiration of facultative photosynthetic bacteria. In: *Proceedings of the Third International Congress on Photosynthesis*, pp 1147-1162 (ed. M. Avron). Elsevier, Amsterdam.

Melandri, M.A., Casadio, R. and Baccarini-Melandri, A. (1977) Energy levels and rates of photophosphorylation in bacterial chromatophores. In: *Proceedings of the Fourth International Congress on Photosynthesis*, pp 601-609 (eds. D.O. Hall, J. Coombs and T.S. Goodwin). The Biochemical Society, London.

Melandri, M.A., Venturoli, G., DeSantis, A. and Baccarini-Melandri, A. (1980). The induction kinetics of bacterial photophosphorylation. Treshold effects by the phosphate potential and correlation with the amplitude of the carotenoid absorption band shift. *Biochim. biophys. Acta* **592**, 38-52.

Melis, A. (1985) Functional properties of photosystem II_β in spinach chloroplasts. *Biochim. biophys. Acta* **808**, 334-342.

Melis, A. (1991) Dynamics of photosynthetic membrane composition and function. *Biochim. biophys. Acta* **1058**, 87-106.

Melis, A. and Homann, P.H. (1976) Heterogeneity of the photochemical centers in system II of chloroplasts. *Photochem. Photobiol.* **23**, 343-350.

Melis, A., Spangfort, M. and Andersson, B. (1987) Light-absorption and electron-transport balance between photosystem II and photosystem I in spinach chloroplasts. *Photochem. Photobiol.* **45**, 129-136.

Messinger, J. and Renger, G. (1990) Temperature dependence of O_2-oscillation pattern of spinach thylakoids. In: *Current research in Photosynthesis*, Vol I, pp 849-852 (ed. M. Baltscheffsky). Kluwer, Dordrecht.

Meyer, R., Snozzi, M. and Bachofen, R. (1981) Freeze fracture studies of reaction centers from *Rhodospirillum rubrum* in chromatophores and liposomes. *Arch. Microbiol.* **130**, 125-128.

Michel, H. and Oesterhelt, D. (1980) Electrochemical proton gradient across the cell membrane of *Halobacterium halobium*: comparison of the light-induced increase with the increase of intracellular adenosine triphosphate under steady-state illumination. *Biochemistry* **19**, 4615-4619.

Middendorf, T.R., Mazzola, L.T., Lao, K., Steffen, M.A. and Boxer, S.G. (1993) Stark effect (electroabsorption) spectroscopy of photosynthetic reaction centers at 15 K: evidence that the special pair has a large excited-state polarizability. *Biochim. biophys. Acta* **1143**, 223-234.

Miller, K.R. and Jacob, J.S. (1983) Two-dimensional crystals formed from photosynthetic reaction centers. *J. Cell Biol.* **97**, 1266-1270.

Miller, K.R., Miller, G.J. and McIntyre, K.R. (1976) The light harvesting complex of photosystem II. *J. Cell Biol.* **71**, 624-638.

Mills, J.D. (1984) Thiol modulation of CF_o-CF_1 by thioredoxin: the function and consequences of a redox link between the reactions of electron transport and ATP synthesis. In: *H^+-ATPase (ATPsynthase): structure, function, biogenesis. The F_oF_1 complex of coupling membranes*, pp 349-358 (eds S. Papa, K. Altendorf, L. Ernster and L. Packen). ICSU Press, Bari.

Mills, J.D., Mitchell, P. and Schürmann, P. (1980) Modulation of the coupling factor ATPase activity in intact chloroplasts. *FEBS Lett.* **112**, 173-177.

Mills, J.D. and Mitchell, P. (1982) Thiol modulation of CF_o-CF_1 stimulates acid/base-dependent phosphorylation of ADP by broken pea chloroplasts. *FEBS Lett.* **144**, 63-67.

Mitchell, P. (1975) Protonmotive redox mechanism of the cytochrome *b*-c_1 complex in the respiratory chain: protonmotive ubiquinone cycle. *FEBS Lett.* **56**, 1-6.

Miyasaka, T., Koyama, K. and Itoh, I. (1992) Quantum conversion and image detection by a bacteriorhodopsin-based artificial photoreceptor. *Science* **255**, 342-344.

Morita, S., Itoh, S. and Nishimura, M. (1983) Flash-induced photophosphorylation in chloroplasts with activated ATPase. *Biochim. biophys. Acta* **724**, 411-415.

Mühlethaler, K. (1966) The ultrastructure of the plastid lamellae. In: *Biochemistry of chloroplasts*, pp 49-64 (ed. T.W. Goodwin). Academic Press, New York.
Muller, A.W.J. (1983) Thermoelectric energy conversion could be an energy source of living organisms. *Phys. Lett. A* **96**, 319-321.
Muller, A.W.J. (1985) Thermosynthesis by biomembranes: energy gain from cyclic temperature changes. *J. theor. Biol.* **115**, 429-453.
Muller, A.W.J. (1993) A mechanism for thermosynthesis based on a thermotropic phase transition in an asymmetric biomembrane. *Physiol. Chem. Physics med. NMR* **25**, 95-111.
Muller, A.W.J. (1994) A model for biogenesis based on thermosynthesis. *Origins of Life* **24**, 253-254.
Muller, A.W.J. Were the first organisms heat engines? A new model for biogenesis and the early evolution of biological energy conversion. *Progr. Biophys. mol. Biol.* In press.
Murakami, S. and Packer, L. (1970) Protonation and chloroplast membrane structure. *J. Cell Biol.* **47**, 332-351.
Murata, N. (1969) Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in spinach chloroplasts. *Biochim. biophys. Acta* **189**, 171-181.
Murata, N., Troughton, J.H. and Fork, D.C. (1975) Relationships between the transition of the physical phase of membrane lipids and photosynthetic parameters in *Anacystis nidulans* and lettuce and spinach chloroplasts. *Plant Physiol.* **56**, 508-517.
Murata, N. and Fork, D.C. (1975) Temperature dependence of chlorophyll *a* fluorescence in relation to the physical phase of membrane lipids in algae and higher plants. *Plant Physiol.* **56**, 791-796.
Murata, N., Higashi, S.-I. and Fujimura, Y. (1990) Lipids in spinach photosystem II. In: *Current Research in Photosynthesis, Vol 1*, pp 403-406 (ed. M. Baltscheffsky). Kluwer, Dordrecht.
Myers, J. and Graham, J.-R. (1971) The photosynthetic unit in *Chlorella* measured by repetitive short flashes. *Plant Physiol.* **48**, 282-286.
Nabedryk, E., Bagley, K.A., Thibodeau, D.L., Bauscher, M., Mäntele, W. and Breton, J. (1990) A protein conformational change associated with the photoreduction of the primary and secondary quinones in the bacterial reaction center. *FEBS Lett.* **266**, 59-62.
Nakamura, H., Sakamoto, T. and Wada, A. (1988) A theoretical study of the dielectric constant of protein. *Protein Engineer.* **2**, 177-183.
Nakatani, H.Y. and Barber, J. (1980) Further studies of the thylakoid membrane surface charges of particle electrophoresis. *Biochim. biophys. Acta* **591**, 82-91.
Nanba, O. and Satoh, K. (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome *b*-559. *Proc. natn. Acad. Sci. U.S.A.* **84**, 109-112.
Nesbitt, D.M. and Berg, S.P. (1980) Proton involvement with the light-induced hindrance of spin label motion in the lumen of spinach thylakoids. *Biochim. biophys. Acta* **593**, 353-361.
Nesbitt, D.M. and Berg, S.P. (1982) The influence of spinach thylakoid lumen volume and membrane proximity on the rotational motion of the spin label tempamine. *Biochim. biophys. Acta* **679**, 169-174.
Nicolis, G. and Prigogine, I. (1977) *Self-organization in non-equilibrium systems.* John Wiley, New York.
Nishikazi, Y. (1973) Kinetics of ATP formation and proton efflux by acid-base transition in chloroplasts. *Biochim. biophys. Acta* **314**, 312-319.
Nishimura, M. (1962a) Studies on bacterial photophosphorylation. I. Kinetics of photophosphorylation in *Rhodospirillum rubrum* chromatophores by flashing light. *Biochim. biophys. Acta* **57**, 88-95.
Nishimura, M. (1962b) Studies on bacterial photophosphorylation. II. Effects of reagents and temperature on light-induced and dark phases of photophosphorylation in *Rhodospirillum rubrum* chromatophores. *Biochim. biophys. Acta* **57**, 96-103.
Nitschke, W. and Rutherford, A.W. (1991) Photosynthetic reaction centers: variations on a common structural theme? *Trends biochem. Sci.* **16**, 241-245.
Nolan, W.G. and Smillie, R.M. (1976) Multi-temperature effects on Hill reaction activity of barley chloroplasts. *Biochim. biophys. Acta* **440**, 461-475.
Nolan, W.G. and Smillie, R.M. (1977) Temperature-induced changes in Hill activity of chloroplasts isolated from chilling-sensitive and chilling resistant plants. *Plant Physiol.* **59**, 1141-1145.
Ogawa, T. (1982) Simple oscillations in photosynthesis of higher plants. *Biochim. biophys. Acta* **681**,

103-109.
Okamura, M.Y. and Feher, G. (1992) Proton transfer in reaction centers from photosynthetic bacteria. *A. Rev. Biochem.* **61**, 861-896.
Olson, J.M. (1970) The evolution of photosynthesis. *Science* **168**, 438-446.
Olson, J.M. and Pierson, B.K. (1987a) Origin and evolution of photosynthetic reaction centers. *Origins of Life* **17**, 419-430.
Olson, J.M. and Pierson, B.K. (1987b) Evolution of photosynthetic reaction centers. *Int. Rev. Cytology* **108**, 209-248.
Oosawa, F. and Hayashi, S. (1986) The loose coupling mechanism in molecular machines of living cells. *Adv. Biophysics* **22**, 151-183.
Ort, D.R. and Whitmarsh, J. (1990) Inactive photosystem II centers. *Photosynth. Res.* **23**, 101-104.
Ort, D.R. and Oxborough, K. (1992) In situ regulation of chloroplast coupling factor activity. *A. Rev. Plant Physiol. Plant mol. Biol.* **43**, 269-291.
Packer, L. (1966) Evidence of contractility in chloroplasts. In: *Biochemistry of Chloroplasts*, p 233-242 (ed T.E. Goodwin). Academic, New York.
Packham, N.K., Berriman, J.A. and Jackson, J.B. (1978) The charging capacitance of the chromatophore membrane. *FEBS Lett.* **89**, 205-210.
Packham, N.K., Dutton, P.L. and Mueller, P. (1982) Photoelectric currents across planar bilayer membranes containing bacterial reaction centers. Response under conditions of single electron turnover. *Biophys. J.* **37**, 465-473.
Packham, N.K., Mueller, P. and Dutton, P.L. (1988) Photoelectric currents across planar bilayer membranes containing bacterial reaction centers: the response under conditions of multiple reaction-center turnovers. *Biochim. biophys. Acta* **933**, 70-84.
Pape, E.H., Menke, W., Weick, D. and Hosemann, R. (1974) Small angle x-ray scattering of the thylakoid membranes of *Rhodopseudomonas spheroides* in aqueous suspensions. *Biophys. J.* **14**, 221-232.
Park, R.B. (1965) Substructure of chloroplast lamellae. *J. Cell Biol.* **27**, 151-161.
Park, R.B. and Biggins, J. (1964) Quantasome: size and composition. *Science* **144**, 1009-1011.
Pearcy, R.W. (1990) Sunflecks and photosynthesis in plant canopies. *A. Rev. Plant Physiol.* **41**, 421-453.
Pearcy, R.W. and Calkin, H.W. (1983) Carbon dioxide exchange of C_3 and C_4 tree species in the understory of a Hawaiian forest. *Oecologia* **58**, 26-32.
Perrier, A. (1971) Leaf temperature measurement. In: *Plant Photosynthetic Production. Manual of Methods*, pp 632-671 (eds Z. Sestak, J. Catsky and P.G. Jarvis). Junk, The Hague.
Peterson, R.B., Sivak, M.N. and Walker, D.A. (1988) Carbon dioxide-induced oscillations in fluorescence and photosynthesis. *Plant Physiol.* **88**, 1125-1130.
Petronilli, V., Pietrobon, D., Zoratti, M. and Azzone, G.F. (1986) Free energy coupling between H^+-generating and H^+-consuming pumps. *Eur. J. Biochem.* **155**, 423-431.
Petty, K.M. and Jackson, J.B. (1979a) Correlation between ATP synthesis and the decay of the carotenoid shift after single flash activation of chromatophores from *Rhodopseudomonas capsulata*. *Biochim. biophys. Acta* **547**, 463-473.
Petty, K.M. and Jackson, J.B. (1979b) Kinetic factors limiting the synthesis of ATP by chromatophores exposed to short flash excitation. *Biochim. biophys. Acta* **547**, 474-483.
Petty, K.M. and Jackson, J.B. (1979c) Two protons transferred per ATP synthesized after flash activation of chromatophores from photosynthetic bacteria. *FEBS Lett.* **97**, 367-372.
Pick, U., Gounaris, K., Admon, A. and Barber, J. (1984) Activation of the CF_0-CF_1 ATP synthase from spinach chloroplasts by chloroplast lipids. *Biochim. biophys. Acta* **765**, 12-20.
Pick, U., Weiss, M., Gounaris, K. and Barber, J. (1987) The role of different thylakoid glycolipids in the function of reconstituted chloroplasts ATPsynthase. *Biochim. biophys. Acta* **891**, 28-39.
Pierson, B.K. and Olson, J.M. (1989) Evolution of photosynthesis in anoxygenic photosynthetic procaryotes. In: *Microbial Mats*, pp 402-427 (eds Y. Cohen and E. Rosenberg). American Society for Microbiology, Washington.
Pirt, S.J. (1965) The maintenance energy of bacteria in growing cultures. *Proc. Roy. Soc. Lond. Ser. B* **163**, 224-231.
Pirt, S.J. (1987) The energetics of microbes at slow growth rates: maintenance energies and dormant

organisms. *J. Ferment. Technol.* **65**, 173-177.
Plieth, C. and Hanssen, U.-H. (1992) Light dependence of protoplasmic streaming in *Nitella flexilis* L. as measured by means of laser-velocimetry. *Planta* **188**, 332-339.
Pollard, D.F.W. (1970) The effect of rapidly changing light on the rate of photosynthesis in largetooth aspen (*Populus grandidentata*). *Can. J. Bot.* **48**, 823-829.
Poore, V.M. and Ragan, C.I. (1982) Effects of lipid concentration and fluidity on oxidoreduction of ubiquinone by isolated complex I, complex III, and complex I-III. In: *Function of Quinones in Energy Conversion Systems*, pp 141-151 (ed. B.L. Trumpower). Academic Press, New York.
Popovic, Z.D., Kovacs, G.J., Vincett, P.S., Alegria, G. and Dutton, P.L. (1986) Electric-field dependence of the quantum yield in reaction centers of photosynthetic bacteria. *Biochim. biophys. Acta* **851**, 38-48.
Prigogine, I. (1969) Structure, dissipation and life. In: *Theoretical physics and biology*, pp. 23-52 (ed. M. Marois). North-Holland, Amsterdam.
Pulvari, C.F. and Garcia, F.J. (1978) Conversion of solar to electrical energy utilizing the thermodielectric effect. *Ferroelectrics* **22**, 769-771.
Quéguiner, B. and Legendre, L. (1986) Phytoplankton photosynthetic adaptation to high frequency light fluctuations simulating those induced by sea surface waves. *Mar. Biol.* **90**, 483-491.
Quinn, P.J. (1981) The fluidity of cell membranes and its regulation. *Prog. Biophys. mol. Biol.* **38**, 1-104.
Quinn, P.J., Gomez, R. and Madden, T.D. (1980) Modification of membrane lipids of sarcoplasmic reticulum to probe the influence of bilayer fluidity on Ca^{2+}-activated ATPase activity. *Biochem. Soc. Trans.* **8**, 38-40.
Rabinowitch, E.I. (1945) *Photosynthesis and related processes. Volume I.* Interscience, New York.
Rabinowitch, E.I. (1956) *Photosynthesis and related processes. Volume II*, Part 2, p. 1216. Interscience, New York.
Radmer, R.J. and Kok, B. (1977) Light conversion efficiency in photosynthesis. In: *Photosynthesis I. Encyclopedia of Plant Physiology, New Series, Vol 5*, pp 125-135 (eds A. Trebst and M. Avron). Springer, Berlin.
Raison, J.K., Lyons, J.M., Mehlhorn, R.J. and Keith, A.D. (1971) Temperature-induced phase changes in mitochondrial membranes detected by spin labeling. *J. biol. Chem.* **246**, 4036-4040.
Raschke, K. (1975) Stomatal action. *A. Rev. Plant Physiol.* **26**, 309-340.
Rathenow, M. and Rumberg, B. (1980) Stoichiometry of proton translocation during photosynthesis. *Ber. Bunsengesell. Phys. Chem.* **84**, 1059-1062.
Raven, J.A. and Beardall, J. (1981) The intrinsic permeability of biological membranes to H^+: significance for the efficiency of low rates of energy transformation. *FEMS Microbiol. Lett.* **10**, 1-5.
Raven, J.A. and Beardall, J. (1982) The lower limit of photon fluence rate for phototrophic growth: the significance of 'slippage' reactions. *Plant Cell Environm.* **5**, 117-124.
Rawyler, A. and Siegenthaler, P.A. (1989) Change in the molecular organization of monogalactosyldiacylglycerol between resting and functioning thylakoid membranes. Involvement of the CF_0/CF_1-ATP synthetase. *Biochim. biophys. Acta* **975**, 283-292.
Reed, D.W. and Raveed, D. (1972) Some properties of the ATPase from chromatophores of *Rhodopseudomonas spheroides* and its structural relationship to the bacteriochlorophyll proteins. *Biochim. biophys. Acta* **283**, 79-91.
Reed, D.W., Raveed, D. and Reporter, M. (1975) Localization of photosynthetic reaction centers by antibody binding to chromatophore membranes from *Rhodopseudomonas spheroides* strain R26. *Biochim. biophys. Acta* **387**, 368-378.
Reeves, S.G. and Hall, D.O. (1978) Photophosphorylation in chloroplasts. *Biochim. biophys. Acta* **463**, 275-297.
Reinach, P., Aubrey, B.B. and Brody, S.S. (1973) Monomolecular films of bacteriochlorophyll and derivatives at an air-water interface: surface and spectral properties. *Biochim. biophys. Acta* **314**, 360-371.
Renganathan, M., Pan, R.S., Ewy, R.G., Theg, S.M., Allnutt, F.C.T. and Dilley, R.A. (1991)

Evidence that localized energy coupling in thylakoids can continue beyond the energetic threshold onset into steady illumination. *Biochim. biophys. Acta* **1059**, 16-27.

Renganathan, M., Pfündel, E. and Dilley, R.A. (1993) Thylakoid lumenal pH determination using a fluorescent dye: correlation of lumen pH and gating between localized and delocalized energy coupling. *Biochim. biophys. Acta* **1142**, 277-292.

Renger, G. (1974) Studies on the reaction mechanism of the oxidizing equivalents produced by system II. In: *Proceedings of the Third International Congress on Photosynthesis*, pp 127-144 (ed. M. Avron). Elsevier, Amsterdam.

Rich, P.R. (1988) A critical examination of the supposed variable proton stoichiometry of the chloroplast cytochrome *bf* complex. *Biochim. biophys. Acta* **932**, 33-42.

Riegler, J. and Möhwald, H. (1986) Elastic interactions of photosynthetic reaction center proteins affecting phase transitions and protein distributions. *Biophys. J.* **49**, 1111-1118.

Ryrie, I.J., Anderson, J.M. and Goodchild, D.J. (1980) The role of the light-harvesting chlorophyll *a*/*b*-protein complex in chloroplasts membrane stacking. Cation-induced aggregation of reconstituted proteoliposomes. *Eur. J. Biochem.* **107**, 345-354.

Robertson, D.E. and Dutton, P.L. (1988) The nature and magnitude of the charge-separation reactions of ubiquinol cytochrome c_2 oxidoreductase. *Biochim. biophys. Acta* **935**, 273-291.

Rogers, N.K. and Sternberg, M.J.E. (1984) Electrostatic interactions in globular proteins. Different dielectric models applied to the packing of a-helices. *J. mol. Biol.* **174**, 527-542.

Rögner, M., Dekker, J.P., Boekema, E.J. and Witt, H.T. (1987) Size, shape and mass of the oxygen-evolving photosystem II complex from the thermophilic cyanobacterium *Synechococcus* sp. *FEBS Lett.* **219**, 207-211.

Rosing, J. and Slater, E.C. (1972) The value of ΔG^o for the hydrolysis of ATP. *Biochim. biophys. Acta* **267**, 275-290.

Rouag, D. and Dominy, P. (1994) State adaptations in the cyanobacterium *Synechococcus* 6301 (PCC): dependence on light intensity or spectral composition. *Photosynth. Res.* **40**, 107-117.

Ruban, A.V. and Trach, V.V. (1991) Heat-induced reversible changes in Photosystem 1 absorption cross-section of pea chloroplasts and sub-chloroplast preparations. *Photosynth. Res.* **29**, 157-169.

Rumberg, B., Schubert, K., Strelow, F. and Tran-Anh, T. (1990) The H^+/ATP coupling ratio at the H^+-ATPsynthase of spinach chloroplast is four. In: *Current Research in Photosynthesis, Vol III*, pp 125-128 (ed. M. Baltscheffsky). Kluwer, Dordrecht.

Saha, S., Izawa, S. and Good, N.E. (1970) Photophosphorylation as a function of light intensity. *Biochim. biophys. Acta* **223**, 158-164.

Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee (1984) Heat induced reversible increase in photosystem 1 emission in algae, leaves and chloroplasts: spectra, activities, and relation to state changes. *Photosynthetica* **18**, 439-444.

Sane, P.V. and Rutherford, A.W. (1986) Thermoluminescence from photosynthetic membranes. In: *Light Emission by Plants and Bacteria*, pp 329-360 (eds Govindjee, J. Amesz and D.C. Fork). Academic Press, Orlando.

Santini, C., Tidu, V., Tognon, G., Magaldi, A.G. and Bassi, R. (1994) Three-dimensional structure of the higher-plant photosystem II reaction centre and evidence for its dimeric organization *in vivo*. *Eur. J. Biochem.* **221**, 307-315.

Schapendonk, A.H.C.M. and Vredenberg, W.J. (1977) Salt-induced absorbance changes of *P*-515 in broken chloroplasts. *Biochim. biophys. Acta* **462**, 613-621.

Scheibe, R. (1994) Lichtregulation von Chloroplastenzymen. *Naturwissenschaften* **81**, 443-448.

Schenck, H. (1957) On the focusing of sunlight by ocean waves. *J. opt. Soc. Am.* **47**, 653-657.

Scherer, S. (1983) Basic functional states in the evolution of light-driven cyclic electron transport. *J. theor. Biol.* **104**, 289-299.

Schlodder, E., Gräber, P. and Witt, H.T. (1982) Mechanism of phosphorylation in chloroplasts. *Top. Photosynth.* **4**, 105-175.

Schmidt, S., Reich, R. and Witt, H.T. (1972) Electrochromic measurements in vitro as a test for the interpretation of field indicating absorption changes in photosynthesis. In: *Proceedings of the IInd International Congres on Photosynthesis Research*, pp 1087-1095 (eds G. Fort, M. Avron and A. Melandri). Junk, The Hague.

Schmidt, S., Arlt, T., Hamm, P., Lauterwasser, C., Finkele, U., Drews, G. and Zinth, W. (1993) Time-resolved spectroscopy of the primary photosynthetic processes of membrane-bound reaction centers from an antenna-deficient mutant of *Rhodobacter capsulatus*. *Biochim. biophys. Acta* **1144**, 385-390.

Schnitzer, J.E. and Lambrakis, K.C. (1991) Electrostatic potential and Born energy of charged molecules interacting with phospholipid membranes: calculation via 3-D numerical solution of the full Poisson equation. *J. theor. Biol.* **152**, 203-222.

Schönfeld, M., Montal, M. and Feher, G. (1979) Functional reconstitution of photosynthetic reaction centers in planar lipid bilayers. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6351-6355.

Schreiber, U. (1984) ATP-induced increase in chlorophyll fluorescence. Characterization of rapid and slow induction phases. *Biochim. biophys. Acta* **767**, 70-79.

Schuhmann, D. (1990) Electrical properties of adsorbed or spread films: the effective values of permittivities in the Helmholtz equation (plane distribution of dipoles). *J. Coll. Interf. Sci.* **134**, 152-160.

Schuurmans, J.J., Casey, R.P. and Kraayenhof, R. (1978) Transmembrane electrical potential formation in spinach chloroplasts. *FEBS Lett.* **94**, 405-409.

Schuurmans, J.J., Veerman, E.C.I., Francke, J.A., Torres-Pereira, J.M.G. and Kraayenhof, R. (1984) Temperature dependence of energy-transducing functions and inhibitor sensitivity in chloroplasts. *Plant Physiol.* **74**, 170-175.

Schwartz, M. (1968) Light induced proton gradient links electron transport and phosphorylation. *Nature* **219**, 915-919.

Sebban, P. (1988) pH effect on the biphasicity of the $P^+Q_A^-$ charge recombination kinetics in the reaction centers from *Rhodobacter sphaeroides*, reconstituted with anthraquinones. *Biochim. biophys. Acta* **936**, 124-132.

Sebban, P., Parot, P., Baciou, L., Mathis, P. and Verméglio, A. (1991) Effects of low temperature and lipid rigidity on the charge recombination process in *Rps. viridis* and *Rb. sphaeroides* reaction centers. *Biochim. biophys. Acta* **1057**, 109-114.

Sebban, P. and Wraight, C.A. (1989) Heterogeneity of the $P^+Q_A^-$ recombination kinetics in reaction centers from *Rhodopseudomonas viridis*: the effects of pH and temperature. *Biochim. biophys. Acta* **974**, 54-65.

Selak, M.A. and Whitmarsh, J. (1982) Kinetics of the electrogenic step and cytochrome b_6 and f redox changes in chloroplasts. Evidence for a Q cycle. *FEBS Lett.* **150**, 286-292.

Sessler, G.M. (1980) Introduction. *Top. Appl. Physics* **33**, 1-12.

Seta, P., Bienvenue, E., D'Epenoux, B., Tenebre, L. and Momenteau, M. (1987) Photoinduced volta potential in a porphyrin monolayer. *Photochem. Photobiol.* **45**, 137-143.

Sharp, K.A. and Honig, B. (1990) Electrostatic interactions in macromolecules: theory and applications. *A. Rev. Biophys. Chem.* **19**, 301-332.

Shigalowa, T., Lehmann, U., Krevet, M. and Strotmann, H. (1985) Transient stimulation of light-triggered ATP hydrolysis by preillumination of chloroplasts in the presence of ATP. *Biochim. biophys. Acta* **809**, 57-65.

Shinkarev, V.P., Takahashi, E., and Wraight, C.A. (1993) Flash induced electric potential generation in wild type and L212EQ mutant chromatophores of *Rhodobacter sphaeroides*: Q_BH_2 is nor released from L212EQ mutant reaction centers. *Biochim. biophys. Acta* **1142**, 214-216.

Shull, C.A. (1930) The mass factor in the energy relations of leaves. *Plant Physiol.* **5**, 279-282.

Sigalat, C., DeKouchkovsky, Y., Haraux, F. and DeKouchkovsky, F. (1988) Shift from localized to delocalized protonic energy coupling in thylakoids by permeant amines. *Biochim. biophys. Acta* **934**, 375-388.

Sigalat, C., DeKouchkovsky, Y. and Haraux, F. (1993) Flow-force relationships in lettuce thylakoids. 2. Effect of the uncoupler FCCP on local proton resistance at the ATPase level. *Biochemistry* **32**, 10201-10208.

Sigalat, C., Haraux, F., DeKouchkovsky, F., Hung, S.P.N. and DeKouchkovsky, Y. (1985) Adjustable microchemiosmotic character of the proton gradient generated by systems I and II for photosynthetic phosphorylation in thylakoids. *Biochim. biophys. Acta* **809**, 403-413.

Sirevag, R. (1991) Thermophilic phototrophs. In: *Thermophilic Bacteria*, pp 196-219 (ed. K. Kristjansson). CRC Press, Boca Raton.

Sironval, C., Michel, J.-M., Bronchart, R. and Englert-Dujardin, E. (1969) On the ``primary'' thylakoids of chloroplast grown under a flash regime. *Prog. Photosynth. Res.* **1**, 47-54.
Sitaraman, V., Paranjpe, S.A. and Gangal, A.D. (1992) Charge anisotropy across biological membranes: evidence and implications. *Biochim. biophys. Acta* **1098**, 336-342.
Sitkoff, D., Lockhart, D.J., Sharp, K.A. and Honig, B. (1994) Calculation of electrostatic effects at the amino terminus of an α helix. *Biophys. J.* **67**, 2251-2260.
Sivak, M.N., Dietz, K.-J., Heber, U. and Walker, D.A. (1985) The relationship between light scattering and chlorophyll *a* fluorescense during oscillations in photosynthetic carbon assimilation. *Arch. Biochem. Biophys.* **237**, 513-519.
Skulachev, V.P. (1982) A single turnover study of photoelectric current-generating proteins. *Methods Enzymol.* **88**, 35-45.
Slater, E.C. (1977) Mechanism of oxidative phosphorylation. *A. Rev. Biochem.* **46**, 1015-1026.
Slawinski, J. and Popp, F.-A. (1987) Temperature hysteresis of low level luminescence from plants and its thermodynamical analysis. *J. Plant Physiol.* **130**, 111-123.
Snyder, R.L. and Dera, J. (1970) Wave-induced light-field fluctuations in the sea. *J. opt. Soc. Am.* **60**, 1072-1079.
Solaini, G., Baracca, A., Parenti Castelli, G. and Lenaz, G. (1984) Temperature dependence of mitochondrial oligomycin-sensitive proton transport ATPase. *J. Bioenerg. Biom.* **16**, 391-406.
Spiller, S. and Terry, N. (1980) Limiting factors in photosynthesis. II. Iron stress diminishes photochemical capacity by reducing the number of photosynthetic units. *Plant Physiol.* **65**, 121-125.
Staehelin, L.A. (1986) Chloroplast structure and supramolecular organization of photosynthetic membranes. In: *Encyclopedia of Plant Physiology, New Series, Vol 19*, pp 1-84 (eds L.A. Staehelin and C.J. Arntzen). Springer, Berlin.
Staehelin, L.A. and Arntzen, C.J. (1979) Effects of ions and gravity forces on the supramolecular organization and excitation energy distribution in chloroplast membranes. In: *Symposium on Chlorophyll Organization and Energy Transfer in Photosynthesis*, Ciba Foundation Symposium 61, New Series, pp 147-175. Excerpta medica, Amsterdam.
Staehelin, L.A. and Arntzen, C.J. (1983) Regulation of chloroplast membrane function: protein phosphorylation changes the spatial organization of membrane compounds. *J. Cell Biol.* **97**, 1327-1337.
Stark, W., Kühlbrandt, W., Wildhaber, I., Wehrli, E. and Mühlethaler, K. (1984). The structure of the photoreceptor unit of *Rhodopseudomonas viridis*. *EMBO J.* **3**, 777-783.
Steffen, M.A., Lao, K. and Boxer, S.G. (1994) Dielectric asymmetry in the photosynthetic reaction center. *Science* **264**, 810-816.
Stidham, M.A., Uribe, E.G. and Williams, G.J. (1982) Temperature dependence of photosynthesis in *Agropyron smithii* Rydb. II. Contribution from electron transport and photophosphorylation. *Plant Physiol.* **69**, 929-934.
Stiehl, H.H. and Witt, H.T. (1969) Quantitative treatment of the function of plastoquinone in photosynthesis. *Z. Naturforsch. B* **24**, 1588-1598.
Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Pigment content and molar extinction coefficients of photochemical reaction centers from *Rhodopseudomonas spheroides*. *Biochim. biophys. Acta* **305**, 597-609.
Strasser, R.J. and Butler, W.L. (1976a) Correlation of absorption changes and thylakoid fusion with the induction of oxygen evolution in bean leaves greened by brief flashes. *Plant Physiol.* **58**, 371-376.
Strasser, R.J. and Butler, W.L. (1976b) Energy transfer in the photochemical apparatus of flashed bean leaves. *Biochim. biophys. Acta* **449**, 412-419.
Strasser, R.J. and Sironval, C. (1972) Induction of photosystem II activity in flashed leaves. *FEBS Lett.* **28**, 56-60.
Sukenik, A., Bennett, J. and Falkowski, P. (1987) Light-saturated photosynthesis - limitation by electron transport or carbon fixation? *Biochim. biophys. Acta* **891**, 205-215.
Sundquist, J.E. and Burris, R.H. (1970) Light-dependent structural changes in the lamellar membranes of isolated spinach chloroplasts: measurement by electron spectroscopy. *Biochim. biophys. Acta* **223**, 115-121.

Symons, M., Swysen, C. and Sybesma, C. (1977) The light-induced carotenoid absorbance changes in *Rhodopseudomonas sphaeroides*. An analysis and interpretation of the band shifts. *Biochim. biophys. Acta* **462**, 706-717.

Takahama, U., Shimizu, M. and Nishimura, M. (1976) Temperature-jump-induced release of hydrogen ions from chloroplasts and its relaxation characteristics in the presence of ionophores. *Biochim. biophys. Acta* **440**, 261-265.

Takahama, U., Shimizu, M. and Nishimura, M. (1977) Temperature-jump-induced release of H^+ from chloroplasts: a relationship between the release of H^+ and reverse electron transfer. In: *Photosynthetic Organelles. Plant Cell Physiol.*, Special Issue. No. 3. pp. 149-156.

Tatulian, S.A. (1987) Fluidity-dependence of membrane adhesiveness can be explained by thermotropic shifts in surface potential. *Biochim. biophys. Acta* **901**, 161-165.

Taylor, M.A. and Jackson, J.B. (1987) Adaptive changes in membrane conductance in response to changes in specific growth rate in continuous cultures of phototrophic bacteria under conditions of energy sufficiency. *Biochim. biophys. Acta* **891**, 242-255.

Tempest, D.W. and Neijssel, O.M. (1984) The status of Y_{ATP} and maintenance energy as biologically interpretable phenomena. *A. Rev. Microbiol.* **38**, 459-486.

Terashima, I. and Saeki, T. (1985) A new model for leaf photosynthesis incorporating the gradients of light environment and of photosynthetic properties of chloroplasts within a leaf. *Ann. Bot.* **56**, 489-499.

Terry, N. (1980) Limiting factors in photosynthesis. I. Use of iron stress to control photochemical capacity *in vivo*. *Plant Physiol.* **65**, 114-120.

Thompson, L.K. and Brudvig, G.W. (1988) Cytochrome *b*-559 may function to protect photosystem II from photoinhibition. *Biochemistry* **27**, 6653-6658.

Thornber, J.P. (1986) Biochemical characterization and structure of pigment-proteins of photosynthetic organism. In: *Encyclopedia of Plant Physiology, New Series, Vol 19*, pp 98-142 (eds L.A. Staehelin and C.J. Arntzen). Springer, Berlin.

Thornber, J.P., Cogdell, R.J., Seftor, R.E.B. and Webster, G.D. (1980) Further studies on the composition and spectral properties of the photochemical reaction centers of bacteriochlorophyll *b*-containing bacteria. *Biochim. biophys. Acta* **593**, 60-75.

Tiede, D.M., Mueller, P. and Dutton, P.L. (1982) Spectrophotometric and voltage clamp characterization of monolayers of bacterial photosynthetic reaction centers. *Biochim. biophys. Acta* **681**, 191-201.

Tiemann, R. and Witt, H.T. (1982) Salt dependency of the electrical potential at the photosynthetic membrane and its structural consequence. *Biochim. biophys. Acta* **681**, 202-211.

Tikhonov, A.N., Khomutov, G.B. and Ruuge, E.K. (1984) Electron transport control in chloroplasts. Effects of magnesium ions on the electron flow between two photosystems. *Photobiochem. Photobiophys.* **8**, 261-269.

Torres-Pereira, J.M.G., Wong Fong Sang, H.W., Theuvenet, A.P.R. and Kraayenhof, R. (1984) Electric surface charge dynamics of chloroplast thylakoid membranes. Temperature dependence of electrokinetic potential and aminoacridine interaction. *Biochim. biophys. Acta.* **767**, 295-303.

Träuble, H. and Eibl, H. (1974) Electrostatic effects on lipid phase transitions: membrane structure and ionic environment. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2214-2219.

Trissl, H.-W. (1983) Spatial correlation between primary redox components in reaction centers of *Rhodopseudomonas sphaeroides* measured by two electrical methods in the nanosecond range. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7173-7177.

Trissl, H.-W. and Läuger, P. (1972) Photoelectric effects at lipid bilayer membranes: theoretical models and experimental observations. *Biochim. biophys. Acta* **282**, 40-54.

Trissl, H.-W., Darszon, A. and Montal, M. (1977) Rhodopsin in model membranes: charge displacements in interfacial layers. *Proc. natn. Acad. Sci. U.S.A.* **74**, 207-210.

Tsong, T.Y. and Astumian, R.D. (1987) Electroconformational coupling and membrane protein function. *Prog. Biophys. molec. Biol.* **50**, 1-45.

Tweet, A.G., Gaines, G.L. and Bellamy, W.D. (1964a) Fluorescence of chlorophyll *a* in monolayers. *J. chem. Physics* **40**, 2596-2600.

Tweet, A.G., Bellamy, W.D. and Gaines, G.L. (1964b) Fluorescence quenching and energy transfer in

monomolecular films containing chlorophyll. *J. chem. Physics* **41**, 2068-2077.
Ullrich, H.-M., and Kuhn, H. (1969) Photospannung an bimolekularen Lipid-Farbstoff-Membranen. *Z. Naturforsch.* B **24**, 1342.
Ullrich, H.-M., and Kuhn, H. (1972) Photoelectric effects in bimolecular lipid-dye membranes. *Biochim. biophys. Acta* **266**, 584-596.
Vale, R.D. and Oosawa, F. (1990) Protein motors and Maxwell's demons: does mechanochemical transduction involve a thermal ratchet? *Adv. Biophys.* **26**, 97-134.
Vanderkooi, J.M. and Berger, J.W. (1989) Excited triplet states used to study biological macromolecules at room temperature. *Biochim. biophys. Acta* **976**, 1-27.
VanDerRest, M. and Gingras, G. (1974) The pigment complement of the photosynthetic reaction center isolated from *Rhodospirillum rubrum*. *J. biol. Chem.* **249**, 6446-6453.
VanDerVeen, R. (1960) Induction phenomena. In: *Encyclopedia of Plant Physiology, Vol V, Part 1*, pp 675-688 (ed. W. Ruhland). Springer, Berlin.
VanGorkom, H.J. (1987) Evolution of photosynthesis. In: *Photosynthesis*, pp 343-350 (ed. J. Amesz). Elsevier, Amsterdam.
VanKan, P.J.M., Groot, M.L., Kwa, S.L.S., Dekker, J.P. and VanGrondelle, R. (1992) Chlorophyll triplet states in the CP47-D1-D2-cytochrome *b*-559 complex of photosystem II. In: *The Photosynthetic Bacterial Reaction Center II*, pp 411-419 (eds J. Breton and A. Verméglio). Plenum Press, New York.
VanMieghem, F.J.E., Searle, G.F.W., Rutherford, A.W. and Schaafsma, T.J. (1992) The influence of the double reduction of Q_A on the fluorescence decay kinetics of Photosystem II. *Biochim. biophys. Acta* **1100**, 198-206.
VanWalraven, H.S., Scholts, M.J.C., Koppenaal, F., Bakels, R.H.A. and Krab, K. (1990) Dependence of the proton translation stoichiometry of cyanobacterial and chloroplast H^+-ATP synthase on the membrane composition. *Biochim. biophys. Acta* **1015**, 425-434.
Váró, G. and Lanyi, J.K. (1991a) Kinetic and spectroscopic evidence for an irreversible step between deprotonation and reprotonation of the Schiff base in the bacteriorhodopsin photocycle. *Biochemistry* **30**, 5008-5015.
Váró, G. and Lanyi, J.K. (1991b) Thermodynamics and energy coupling in the bacteriorhodopsin photocycle. *Biochemistry* **30**, 5016-5022.
Vasilikiotis, C. and Melis, A. (1994) Photosystem II reaction center damage and repair cycle: chloroplast acclimation strategy to irradiance stress. *Proc. natn. Acad. Sci. U.S.A.* **91**, 7222-7226.
Vass, I., Deák, Z., Jegerschöld, C. and Styring, S. (1990) The accessory electron donor tyrosine-D of photosystem II is slowly reduced during low-temperature storage of isolated thylakoids. *Biochim. biophys. Acta* **1018**, 41-46.
Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M. and Andersson, B. (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced Q_A species promote chlorophyll triplet formation. *Proc. natn. Acad. Sci. U.S.A.* **89**, 1408-1412.
Veljovic-Jovanovic, S. and Cerovic, Z.G. (1991) Induction of oscillations in chlorophyll fluorescense by re-illumination of intact isolated pea chloroplasts. *Planta* **185**, 397-400.
Velthuys, B.R. (1978) A third site of proton translocation in green plant photosynthetic electron transport. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6031-6034.
Velthuys, B.R. and Amesz, J. (1974) Charge accumulation at the reducing side of system 2 of photosynthesis. *Biochim. biophys. Acta* **333**, 85-94.
Vermeglio, A. (1977) Secondary electron transfer in reaction centers of *Rhodopseudomonas sphaeroides*. Out-of-phase periodicity of two for the formation of ubisemiquinone and fully reduced ubiquinone. *Biochim. biophys. Acta* **459**, 516-524.
Vermeglio, A. and Clayton, R.K. (1977) Kinetics of electron transfer between the primary and the secondary electron acceptor in reaction centers from *Rhodopseudomonas sphaeroides*. *Biochim. biophys. Acta* **461**, 159-165.
Ververgaert, P.H.J.T. and Elbers, P.F. (1971) Ultrastructural analysis of black membranes. *J. mol. Biol.* **58**, 431-437.
Vink, R., Bendall, M.R., Simpson, S.J. and Rogers, P.J. (1984) Estimation of H^+ to adenosine 5'-triphosphate stoichiometry of *Escherichia coli* ATP synthase using ^{31}P NMR. *Biochemistry*

23, 3667-3675.
Vogelmann, T.C. (1989) Penetration of light into plants. *Photochem. Photobiol.* **50**, 895-902.
Vredenberg, W.J. (1976) Electrical interactions and gradients between chloroplast compartments and cytoplasm. *Top. Photosynth.* **1**, 53-88.
Vredenberg, W.J., Homann, P.H. and Tonk, W.J.M. (1973) Light-induced potential changes across the chloroplast enclosing membranes as expressions of primary events at the thylakoid membrane. *Biochim. biophys. Acta* **314**, 261-265.
Vredenberg, W.J. and Tonk, W.J.M. (1975) On the steady-state electrical potential difference across the thylakoid membrane of chloroplasts in illuminated plant cells. *Biochim. biophys. Acta* **387**, 580-587.
Vredenberg, W.J. (1981) P515: A monitor of photosynthetic energization in chloroplast membranes. *Physiol. Plant.* **53**, 598-602.
Walker, D.A., Horton, P., Sivak, M.N. and Quick, W.P. (1983) Anti-parallel relationship between O_2 evolution and slow fluorescense induction kinetics. *Photobioch. Photobiophys.* **5**, 35-39.
Walker, D.A. (1992) Concerning oscillations. Photosynth. Res. 34, 387-395.
Walsh, P. and Legendre, L. (1983) Photosynthesis of natural phytoplankton under high frequency light fluctuations simulating those induced by sea surface waves. *Limnol. Oceanogr.* **28**, 688-697.
Walsh, P. and Legendre, L. (1988) Photosynthetic responses of the diatom *Phaeodactylum tricornutum* to high frequency light fluctuations simulating those induced by sea surface waves. *J. Plankton Res.* **10**, 1077-1082.
Warburg, O. (1919) Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. *Biochem. Zeitschr.* **100**, 230-270.
Warburg, O. (1920) Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen II. *Biochem. Zeitschr.* **103**, 188-217.
Warshel, A. and Russel, S.T. (1984) Calculations of electrostatic interactions in biological systems and solutions. *Quart. Rev. Biophys.* **17**, 283-422.
Warshel, A. and Schlosser, D.W. (1981) Electrostatic control of the efficiency of light-induced electron transfer across membranes. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5564-5568.
Webster, G.D., Cogdell, R.J. and Lindsay, J.R. (1980) Identification of the carotenoid present in the *B*-800-850 antenna complex from *Rhodopseudomonas capsulata* as that which responds electrochromically to transmembrane electric fields. *Biochim. biophys. Acta* **591**, 321-330.
Weinberg, S. (1976) Submarine daylight and ecology. *Mar. Biol.* **37**, 291-304.
Weis, E. (1984) Short term acclimation of spinach to high temperatures. *Plant Physiol.* **74**, 402-407.
Weis, E. (1985) Light- and temperature-induced changes in the distribution of excitation energy between photosystem I and photosystem II in spinach leaves. *Biochim. biophys. Acta* **807**, 118-126.
Weis, E. and Berry, J.A. (1987) Quantum efficiency of photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. *Biochim. biophys. Acta* **894**, 198-208.
Westerhoff, H.V., Lolkema, J.S., Otto, R. and Hellingwerf, K.J. (1982) Thermodynamics of growth. Non-equilibrium thermodynamics of bacterial growth. The phenomenological and the mosaic approach. *Biochim. biophys. Acta* **683**, 181-220.
Westerhoff, H.V., Melandri, B.A., Venturoli, G., Azzone, G.F., and Kell, D.B. (1984) A minimal hypothesis for membrane-linked free-energy transduction. The role of independent, small coupling units. *Biochim. biophys. Acta* **768**, 257-292.
Westerhoff, H.V., Tsong, T.Y., Chock, P.B., Chen, Y.-D. and Astumian, R.D. (1986) How enzymes can capture and transmit free energy from an oscillating electric field. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4734-4738.
Wikström, M. and Krab, K. (1980) Respiration-linked H^+ translocation in mitochondria: stoichiometry and mechanism. *Cur. Top. Bioenerg.* **10**, 51-101.
Wikström, M., Krab, K. and Saraste, M. (1981) Proton-translocating cytochrome complexes. *A. Rev. Biochem.* **50**, 623-655.
Wilhelm, C. and Wild, A. (1984) The variability of the photosynthetic unit in *Chlorella*. The effect of light intensity and cell development on photosynthesis, P-700 and cytochrome f in homocontinuous and synchronous cultures of *Chlorella*. *J. Plant Physiol.* **115**, 125-135.
Williams, R.J.P. (1978) The multifarious couplings of energy transduction. *Biochim. biophys. Acta*

505, 1-44.
Williams, W.P. and Allen, J.F. (1987) State 1/State 2 changes in higher plants and algae. *Photosynth. Res.* **13**, 19-45.
Williams, W.P. and Salamon, Z. (1976) Enhancement studies on algae and isolated chloroplasts. Part I. Variability of photosynthetic enhancement in *Chlorella pyrenoidosa*. *Biochim. biophys. Acta* **430**, 282-299.
Winkler, H.H. and Wilson, T.H. (1966) The role of energy coupling in the transport of β-galactosides by *Escherichia coli. J. biol. Chem.* **241**, 2200-2211.
Winter, H., Robinson, D.G. and Heldtm H.W. (1993) Subcellular volumes and metabolic concentrations in barley leaves. *Planta* **191**, 180-190.
Witt, H.T. (1960) Untersuchungen der Photosynthese bei Anregung mit Blitzlicht. In: *Encyclopedia of Plant Physiology, Vol V, Part 1*, pp 634-671 (ed. W. Ruhland). Springer, Berlin.
Witt, H.T. (1979) Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods. The central role of the electric field. *Biochim. biophys. Acta* **505**, 355-427.
Witt, H.T. and Moraw, R. (1959) Untersuchungen über die Primärvorgänge bei der Photosynthese. I. Mitteilung. *Z. Phys. Chem. Neue Folge* **20**, 253-282
Woese, C.R. (1987) Bacterial evolution. *Microbiol. Rev.* **51**, 221-271.
Wraight, C.A. (1977) Electron acceptors of photosynthetic bacterial reaction centers. Direct observation of oscillatory behaviour suggesting two closely equivalent ubiquinones. *Biochim. biophys. Acta* **459**, 525-531.
Wraight, C.A. (1982) The involvement of stable semiquinones in the two-electron gates of plant and bacterial photosystems. In: *Function of Quinones in Energy Conversion Systems*, pp 181-197 (ed. B.L. Trumpower). Academic Press, New York.
Wraight, C.A., Cogdell, R.J. and Chance, B. (1978) Ion transport and electrochemical gradients in photosynthetic bacteria. In: *The photosynthetic Bacteria*, pp 471-511 (eds R.K. Clayton and W.R. Sistrom). Plenum Press, New York.
Wyman, M. and Fay, P. (1987) Acclimation to the natural light regime. In: *The Cyanobacteria*, pp 347-372 (eds P. Fay and C. VanBaalen). Elsevier, Amsterdam.
Yamamoto, Y. and Ke, B. (1979) A temperature-dependent conformational change in photosystem-II thylakoid membrane. *FEBS Lett.* **107**, 137-140.
Yamamoto, Y. and Nishimura, M. (1977) Characteristics of light-induced 515-nm absorbance change in spinach chloroplasts at lower temperatures. I. Participation of structural changes of thylakoid membranes in light-induced 515-nm absorbance change. *Plant Cell Physiol.* **18**, 55-66.
Zimányi, L. and Garab, G. (1982) Configuration of the light induced electric field in thylakoid and its possible role in the kinetics of the 515 nm absorbance change. *J. theor. Biol.* **95,** 811-821.
Zuber, H. (1985) Structure and function of light-harvesting complexes and their polypeptides. *Photochem. Photobiol.* **42**, 21-844.