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REVIEW

Photosynthetic and Respiratory Electron Flow in the Dual Functional Membrane of Facultative Photosynthetic Bacteria

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1. Introduction

Although photosynthetic bacteria represent a very heterogenous group for their morphological, biochemical, and physiological characteristics, they are unified by the common property of being able to use light energy under anaerobic conditions. All three groups in which they have been subdivided [1] (Chlorobiaceae, Chromatiaceae, and Rhodospirillaceae) possess membranes endowed with photosynthetic pigments, characteristically different for every group, which allow the capture of photons and their utilization for the formation of the chemical bond of ATP. Bacterial photosynthesis is a unique process, differing from plant photosynthesis in the source of reducing equivalents, which can be of inorganic (H_2, H_2S, thiosulfate, etc.) or of organic nature (succinate, malate, etc.); water cannot be used and therefore no oxygen evolution accompanies bacterial photosynthesis.

Apart from the Chlorobiaceae and the Chromatiaceae, which can only live at the expense of light energy in anaerobiosis, stands the third group (Rhodospirillaceae) which can be probably considered as the most flexible and versatile group of microorganisms in the biological world. In fact, in addition to the photoautothrophic and photoheterothrophic conditions of

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growth, they can also adapt to aerobiosis, developing the ability to form ATP by respiration. The possibility of using alternative electron acceptors in anaerobic respiration, such as nitrate [2], or of living on substrate fermentation has also been reported [3–6]; in these last two cases lower growth rates are observed. Anaerobic dark growth in the presence of dimethyl sulfoxide has recently been described in *Rps. capsulata* [6]; in this regard the involvement of an electron transport through cytochromes has not been ruled out. To their outstanding efficiency in utilizing different sources of energy must also be added the important capability of high rates of atmospheric nitrogen fixation [7], which can be used as the only nitrogen source, under photosynthetic growth conditions. The nitrogenase complex, as in most cases, is subjected to repression and derepression control, and under optimal conditions [8] (in the presence of an organic carbon source and an amino acid nitrogen source) H_2 photoevolution, catalyzed by the nitrogenase [9], has been found to operate at quite high rates [10].

A large number of mutant strains of these bacteria can readily be isolated, which are blocked in pigment biosynthetic pathways [11–13] or in photosynthetic reaction center formation [14, 15] or in electron-transport components [16]. The discovery of a genetic exchange system in *Rhodopseudomonas capsulata* [17, 18] will offer a powerful tool for approaching research studies in energy transduction in the future.

From this brief introductory description the complexity and the difficulty in covering all the multiple aspects of photosynthetic bacterial metabolism appear quite evident. In this review we will focus our attention on the photosynthetic and respiratory electron flow leading to ATP synthesis in some genera of the Rhodospirillaceae family (*Rhodopseudomonas* and *Rhodospirillum*) in an effort to point out the interrelationships between these two processes in one and the same membrane system.

Several reviews dealing with the morphology, biochemistry, and physiology of photosynthetic bacteria have appeared in the literature in the last few years [19–25].

2. Modulation of Membrane Formation

A brief outline of the morphology of cells grown under different conditions and of the membrane fractions obtained therefrom will be given here in order to clarify the terminology used in the subsequent sections. More detailed information dealing with the arrangement, development, and composition of the membranes can be found in the reviews by Lascelles [19] and Oelze and Drews [21].

During photosynthetic conditions of growth with or without an organic source of carbon (anaerobic, light), cells of the Rhodospirillaceae exhibit a very extensive development of intracytoplasmic membranes, arranged in vesicular or lamellar structure, often characteristic for each type of bacterium. These lipoprotein membranes possess, in addition to photopigments (bacteriochlorophyll *a* or *b* and acyclic carotenoids), components involved in electron transport and energy transduction. Evidence based on different experimental approaches [26–30] strongly indicate that all parts of the intracytoplasmic membranes are connected to each other, forming a continuum with the external cytoplasmic membrane. Spheroplasts can readily be obtained by these Gram-negative bacteria by a conventional EDTA-lysozyme treatment [31] and have been used in general for studies concerning the topography and polarity of the cytoplasmic membrane [32].

If one disrupts the bacterial cells by physical means (French pressure cell, alumina grinding, sonication, etc.) closed vesicles, which retain the ability to carry on electron flow coupled to ATP synthesis, can be isolated by differential centrifugation. These particles (about 600 Å in diameter) are commonly called chromatophores and show an inverse polarity in respect to whole cells or spheroplasts [33, 34]. These vesicles are lined with knoblike particles (120 Å) [35] analogous to similar structures observed in mitochondria and chloroplasts and identified as the morphological representation of the coupling ATPase [32, 36]. Preparations conserving a polarity similar to that of whole cells and spheroplasts have been also obtained using different procedures. Hochman et al. [37] have reported a procedure involving passage of whole cells of *Rps. capsulata* through a Yeda press at relatively mild pressures, which give preparations designated as heavy chromatophores; on several grounds, such as proton translocation and localization of peripheral proteins [32], it has been suggested that this fraction has an orientation opposite to that of normal chromatophores [37].

Successful preparations of homogeneous membrane vesicles with a right-side-out polarity have only been recently achieved by Michels and Konigs [38] by means of osmotic lysis of spheroplasts from *Rps. sphaeroides*. The orientation of these preparations has been demonstrated by freeze-etch electron microscopy, by studies on the localization of cytochrome c_2 and of ATPase, and by observations on active transport mediated by light-dependent cyclic electron transport. To facilitate osmotic lysis of spheroplasts, cells grown at high light intensity, which decreases the formation of extensive invaginations of the cytoplasmic membrane, have been utilized. A detailed characterization of the properties of these particles and a direct comparison with the chromatophore vesicles have been carried out [38].

If the conditions of growth are switched from anaerobiosis in the light to aerobiosis in the dark, a marked inhibition of bacteriochlorophyll synthesis and a repression of the development of intracytoplasmic structures take place. However, it has been observed that pigmented membrane invaginations, sometimes quite extensive, can be found also in darkness [28, 39]. This is due to the modulation of membrane differentiation not only by light intensity, but also by the oxygen partial pressure in the medium. For example, in *Rps. capsulata* [28] only very high oxygen partial pressure ($pO_2 \approx 400$ mm Hg) inhibits effectively intracytoplasmic membrane formation, whereas at the oxygen partial pressure of a normal air-saturated medium ($pO_2 \approx 150$ mm Hg) only a partial inhibition is observed.

In addition to other evidence (see reviews [19, 21]), the more direct correlation between membrane invaginations and bacteriochlorophyll synthesis rests on the demonstration that mutants of *R. rubrum* [40] or of *Rhodopseudomonas* [41], blocked in bacteriochlorophyll biosynthesis, are unable to produce an extensive arrangement of intracytoplasmic membranes, also at very low oxygen partial pressure. A quite complex regulation of membrane development and also of the two modes of energy transduction, i.e., photosynthesis or respiration, can occur; very often neither of the two functions is completely repressed. On the same membrane, light- or substrate oxidation-dependent ATP synthesis can operate, although at different relative rates. Moreover, as will be shown later in this review, evidence points to a strict and mutual interaction between the two electron transport chains.

3. Photosynthetic Electron Flow

Cyclic electron flow, i.e., a transfer of electrons in the absence of net production of oxidized or reduced chemical species, is a typical feature of photosynthesis in Rhodospirillaceae. This electron flow is coupled directly to production of ATP and is thought to be involved also in the production of reduced pyridine nucleotide via an energy-requiring inversion of the respiratory electron transport.

Light is first absorbed by an antenna bacteriochlorophyll–protein complex and transferred to a photochemical reaction center P 870 (P indicating pigment and 870 the wavelength at which bleaching of a major absorption band takes place upon oxidation), where charge separation occurs: P 870 becomes oxidized and a primary electron acceptor (X) reduced. After this

first photochemical act, electrons are transferred in a cyclic way through a number of redox components (quinones, nonheme iron proteins, and cytochromes). These electron-transport carriers are thought to be arranged within the chromatophore membrane in such a way as to provide for an active proton movement from the external to the internal aqueous compartment.

The nature of the different components found in photosynthetic membranes and their possible role in cyclic electron flow will be illustrated in the following paragraphs.

3.1. Reaction Center (P870)

In the primary electron-transfer reaction P 870 functions as electron donor [42–44]. Studies on the nature and the function of this reaction center has spread rapidly since the successful isolation of a reaction-center complex, free of the bulk antenna bacteriochlorophyll, was first reported by Reed and Clayton [45]. This reaction center, isolated from a carotenoidless mutant of *Rps. sphaeroides* (R26) by treatment with the nonionic detergent Triton X-100, was shown to have a molecular weight of 650,000 daltons and to contain ubiquinone, iron, and copper together with P 870, P 800, and cytochromes [45]. Subsequently more refined purification procedures have developed: Preparations with a smaller molecular weight (around 100,000 daltons), free of cytochromes and containing ubiquinone and Fe (1:1), have been reported [47, 48]. The most convenient procedure [49] utilizes the zwitterionic detergent lauryldimethylamine oxide, centrifugation in a sucrose gradient, fractionation with ammonium sulfate, and chromatography on agarose column. The properties and composition of the different reaction-center preparations from various bacterial strains have been recently reviewed [23, 49, 50]. In addition to proteins, nonheme iron, and ubiquinone, the reaction centers contain four molecules of bacteriochlorophyll and two of bacteriopheophytin [51, 23]. Two of the bacteriochlorophyll molecules (a bacteriochlorophyll dimer), responsible for an absorption band near 870 nm, undergo oxidation upon excitation by a quantum of light, functioning as the primary electron donor [52]. The function of the other two molecules showing an absorption band at 800 nm is still not clearly understood [53]. The recent isolation of a mutant strain of *R. rubrum,* which lacks P 800 but is capable of phototrophic growth, might be of help in the elucidation of the function of P 800 in the reaction center [54].

Analysis by SDS polyacrylamide gel electrophoresis indicates the presence in the reaction center of three subunits of approximate molecular

weights of 21,000 (subunit L), 24,000 (subunit M), and 28,000 (subunit H) daltons [55, 56]; 70% of the amino acids present in these subunits are nonpolar in nature [56]. Studies utilizing immunological methods indicated that the H subunit is partially exposed to the cytoplasmic phase of the chromatophore membrane [56–58]. Recently, by iodination with ^{131}I of chromatophores from which purified reaction centers were prepared or by direct iodination of reaction centers from *R. rubrum,* Zürrer et al. [59] have shown that the H subunit is heavily labeled in comparison with the two smaller subunits. These observations can suggest an arrangement of the reaction center where the L and M subunits are more buried in the core of the membrane, while subunit H is located partially on the membrane surface facing the cytoplasm; since sonication during the iodination treatment did not result in an increase in the label of the H subunit, it was suggested that this subunit is accessible from both sides of the chromatophore membrane. However, iodination of spheroplasts results in a low label of chromatophores and reaction centers prepared therefrom [59].

The oxidation-reduction potential of the P 870/P^+ 870 couple has been reported to have a value of +440 mV in *Rps. sphaeroides* and to be pH independent between pH 6.0 and 8.0 [60].

3.2. Primary and Secondary Electron Acceptor

Although many studies have been devoted to the elucidation of the nature of the primary acceptor, which is reduced by P 870 with a half time of 200 psec [61, 62], following a single turnover flash, its nature and its operational midpoint potential are still debated (for recent reviews see [22, 23, 63]).

Many observations suggest that the primary acceptor is formed by an iron-ubiquinone complex (X) where ubiquinone is present in a tightly bound form [64, 65]. This view is supported by the observations that a single turnover flash can generate both an optical signal (A450) [66, 67] possibly related to that of the ubisemiquinone radical [68] and an iron sulfur EPR signal ($g = 1.82$), detectable at liquid-helium temperature [69].

Reaction-center preparations from several species of Rhodospirillaceae contain both ubiquinone and nonheme iron [70]. In chromatophores and reaction-center preparations of *Rps. sphaeroides*, removal of ubiquinone by isooctane extraction in the presence of 0.1% methanol appears to abolish primary photochemistry, which can be restored by addition of purified quinones [71]. Evidence has also been presented that depletion of iron from reaction centers allows extraction of the bound form of ubiquinone

also by isooctane alone [65]. The thermodynamic properties of the primary acceptor have also been the subject of considerable controversy. The midpoint potential has been shown to be pH dependent in chromatophores from several species of Rhodospirillaceae [23] (in contrast to the behavior in reaction centers, where it appears to be pH independent). Since its reduction does not involve a proton, on a fast time scale, it has been speculated [72] that its operating midpoint potential is quite negative, as would be dictated by the pK of the reduced form. This apparent pK has been found [73] to be around pH 9.0–10.0 where the E_m is −180 mV both in *R. rubrum* and *Rps. sphaeroides,* suggesting an operational midpoint potential of the couple X/X^- around −180 mV. However, lower values have been reported by Loach et al. [74], Govindjee et al. [75] and by Silberstein and Gromet Elhanan [76].

The presence of a large pool of quinones [77] (mainly ubiquinone-10) in chromatophores of Rhodospirillaceae and the observation that reduction of endogenous ubiquinone occurs in the light [78–80] can be taken as evidence of an involvement of this electron-transport carrier in the cyclic electron flow. Moreover, depletion of ubiquinone by isooctane extractions of chromatophores from *R. rubrum,* G9 [81] and *Rps. capsulata,* Ala pho^+ [82] decreases markedly the rate of photophosphorylation, which can be reconstituted by preincubation of depleted particles with pure ubiquinone-10. The reconstituted activity is sensitive to Antimycin A. However, direct evidence on the role, location, and mode of reaction of quinone in the chain is still lacking.

It is generally assumed that UQ plays the role of a secondary electron acceptor from X, as has clearly been demonstrated to occur in lyophilized chromatophores of *Chromatium vinosum* (Chromatiaceae) by extraction-reconstitution techniques [83]. Observations based on the same technical approach and pointing to the same conclusions have been reported by Clayton et al. [84], but only for reaction-center preparations of *Rps. sphaeroides.* In chromatophores from *Rps. capsulata* Ala pho^+ isooctane extraction induces a decrease in the rate of cytochrome *b* photoreduction, measured under continuous light and in the presence of Antimycin A, which could support the role of ubiquinone on the reducing side of this redox carrier [82]. Other indirect evidence comes from studies on rapid hydrogen ion binding in chromatophores from *Rps. sphaeroides,* GA. The kinetics of this phenomenon, as detected by pH indicator techniques, were found compatible with the rate of electron-transport reactions and associated with two sites where hydrogen ion binding occurs [85, 86]. The requirement for a carrier, which becomes protonated upon reduction in the

submillisecond range, in the presence of Antimycin A and having an E_m of about 5 mV at pH 7.5 was taken by Cogdell et al. [87] as an indication of the possible involvement of ubiquinone, which binds a proton following its reduction by the primary acceptor.

The same conclusions were drawn by Petty and Dutton [88] from a detailed analysis of the mechanism of flash-induced proton binding in *Rps. sphaeroides,* GA. Their studies indicated that the proton uptake occurs with a half time of 150 μsec at pH 7.0 and with a stoichiometry of 1 ± 0.1 H^+/e^- when observed in the presence of Antimycin A and at pH lower than the p*K* observed for the proton uptake. The apparent p*K* was found to be variable from 8.5 to 7.5 and related to the redox state of the electron-transfer chain. Recently oscillations of the redox state of quinones, after a discrete number of flashes, have been observed [89, 90]. These oscillations, detected by absorbance changes at 450 nm (which is consistent with the spectrum of ubiquinone radicals), have been reported in purified reaction centers supplemented with ubiquinone-10 [91] and in chromatophores of *Rps. sphaeroides,* R 26. Odd-numbered flashes of light give rise to the formation of the anionic ubisemiquinone whereas fully reduced ubiquinone is formed on even-numbered flashes. This phenomenon has been also correlated with H^+ binding, which occurs only on even-numbered flashes [92]. This conclusion is in disagreement with the results of Petty and Dutton described above [88], which were obtained, however, on a different strain of *Rps. sphaeroides*; on the other hand, the ambient redox potential appears to play an important role in these quinone oscillations [93], possibly by affecting the rate of cytochrome *b* oxidation.

3.3. Cytochromes

Different types of cytochromes of *b* and *c* have been described in chromatophores from photosynthetically grown cells of Rhodospirillaceae. However, only in *Rps. capsulata,* Ala pho^+ and *Rps. sphaeroides,* GA has a thermodynamic characterization "in situ" of these electron-transport components been correlated with their possible role in light-induced electron flow [94–98]. For the sake of clarity and since a complete description of all the different cytochromes detected so far is beyond the scope of this review, we will only present a short survey of the different kinds of cytochromes found in photosynthetic membranes, giving more particular emphasis to the results obtained in the two organisms studied more thoroughly and mentioned above. For more details on the structure and function of the variety of cytochromes present in photosynthetic bacteria, we refer to the excellent reviews of Kamen and Horio [99], Horio and Kamen [100] and of Parson [22].

A common feature in chromatophores from Rhodospirillaceae, as in other photosynthetic bacteria, is the presence of one or more *c* type cytochromes, which can be very rapidly photooxidized [101] and act as direct electron donors to the reaction-center Bacteriochlorophyll. In *Rps. viridis* and *Rps. gelatinosa* two *c type* cytochromes, with low and high potential, both capable of reducing directly P 870 by parallel pathways, have been found [102, 103]. In these two organisms light-induced cytochrome *c* oxidation at low temperatures has been reported by Kihara and Chance [104] and by Dutton [105]. In the carotenoidless mutant G9 of *R. rubrum,* Kakuno et al. [106] and Dutton and Jackson [96] described a cytochrome with an α band at 552 nm having an $E_{m,7}$[1] close to +300 mV. A cytochrome with similar midpoint potential appears to be present also in *Rps. capsulata,* Ala pho$^+$ [94] and in *Rps. sphaeroides,* GA [98]. It is generally accepted that these components are identical with the soluble cytochrome c_2 purified by Bartsch [107] from several sources.

While in *Rps. sphaeroides* cytochrome c_2 appears to be the only pigment of *c* type present [96], in *Rps. capsulata,* Ala pho$^+$ Evans and Crofts [94] detected, by dark redox titrations, another two *c* type cytochromes with midpoint potential at pH 7.0 of +120 and 0.0 mV. Of these three *c* type cytochromes only cytochrome c_2 ($E_{m,7}$ = +340 mV) [and possibly cytochrome *c* ($E_{m,7}$ = 0.0 mV)] has been shown to be involved in cyclic electron flow, as judged by measuring the appearance and attenuation of laser flash-induced changes at 551–540 nm as a function of the ambient redox potential [95].

A different *c* type cytochrome, cytochrome *cc'*, is widely distributed among photosynthetic bacteria: Its characteristics and function is extensively discussed in Section 4.2 of this review.

A cytochrome which is present both in *Rps. sphaeroides,* GA and in *capsulata,* Ala pho$^+$ and shown to be involved in cyclic electron flow is a cytochrome of *b* type with an $E_{m,7}$ around +50 to +60 mV [96, 94]. Two other *b* type cytochromes are also present in *Rps. sphaeroides* ($E_{m,7}$ = +155 and −90 mV); their function, however, is still not understood (for more details on cytochrome b_{155}, see Section 4.3). A low-potential cytochrome *b* ($E_{m,7}$ = −25 mV) has been detected in *Rps. capsulata* [94]. Also in *R. rubrum* more than one *b* component has been found: Two cytochrome *b* with $E_{m,7}$ = +20 and −160 mV have been reported by Kakuno

[1] $E_{m,pH} = E_m$ at a pH other than zero, defined as follows:

$$E_{m,pH} = E_0 - n \cdot 0.59 pH$$

where E_0 is the half reduction potential at pH 0 and n is the number of protons per electron involved in the redox reaction.

et al. [106], and three *b* with $E_{m,7} = +160$, -50, and -10 mV by Dutton and Jackson [96].

3.4. Pathways of the Cyclic Electron Flow

In *Rps. sphaeroides,* GA, cytochrome c_2, which appears to be present at a concentration of two molecules per reaction center [98], is photooxidized following a single turnover flash in a biphasic way, with each phase being of similar extent, and with half times of 20 and 300 μsec respectively [98]. Analogous results are also found in *Rps. capsulata.* Following this fast oxidation, electrons are donated to cytochrome c_2 in a cyclic way by the pool of the secondary acceptor, ubiquinone, through an electron-transport chain involving cytochrome b_{50} and possibly other still unidentified components as discussed below. The flow of electrons through this segment of the cyclic system (UQ–cytochrome c_2) is involved in energy conservation and has therefore been correlated with the phenomena of light-induced binding of protons from the outer phase and of proton release into the inner phase of chromatophores, which can be only observed in the presence of uncouplers.

In extensively uncoupled chromatophores the reduction of cytochrome c_2 appears to match kinetically the oxidation of cytochrome *b*, suggesting that either cytochrome *b* is a direct electron donor to cytochrome c_2 or that any possible intermediate is not kinetically limiting [97]. Discrepancies, however, arise under coupled conditions: In fact, the rate of electron transport from cytochrome b_{50} and to cytochrome c_2, which is sensitive to Antimycin A, is highly dependent upon the state of coupling of chromatophores and is a function of the ambient redox potential [108, 109].

The mechanism of the reduction and oxidation of cytochrome b_{50} has been extensively studied in *Rps. sphaeroides,* GA by examining directly the pH dependence of the midpoint potential of this component and indirectly the release of protons related to electron transport [110]. Cytochrome b_{50} shows a shift in its midpoint potential as a function of external pH (60 mV per pH unit) with an apparent p*K* of 7.4 [110]. Since, as mentioned earlier [88], a functional p*K* for flash-induced proton binding, possibly related to ubiquinone reduction, has been determined at pH 8.4, only below pH 8.4 should a reductive protonation of ubiquinone take place. Therefore a release of protons should occur upon ubiquinone reoxidation below pH 8.4; on the contrary, the pH dependence of proton release in the presence of Antimycin A shows that, when cytochrome *b* oxidation is inhibited, the proton release into the inner phase, which is indeed observed in the presence of uncouplers below pH 8.4, decreases to

negligible levels below pH 7.4, which corresponds to the functional pK of cytochrome b_{50} [110]. On this basis it has been proposed [110] that the proton donated by ubiquinone is held on cytochrome b_{50} and that, in the absence of Antimycin, it is released into the inner phase during electron flow from cytochrome b_{50} at some point in the cycle before cytochrome c_2. Similar conclusions were reached also for *Rps. capsulata* by Crofts et al. [111].

Several studies on the electron transport from cytochrome b to cytochrome c_2 of both *Rps. capsulata* and *Rps. sphaeroides* have been reported, in particular by the groups of Crofts and Dutton respectively. In *Rps. capsulata*, Ala pho$^+$, detailed kinetic analysis and thermodynamic characterizations of cytochrome b and cytochrome c_2 changes lead Evans and Crofts [95] to suggest the presence of a component (Z) mediating electron flow between cytochrome b and c_2, with midpoint potential of +130 mV at pH 7.0. In subsequent works [108, 111] it has been proposed that this component could be identified with a redox couple of ubiquinone, possibly UQH·/UQH$_2$, which might operate as an obligate redox mediator in the b–c_2 segment. In this model the UQ/UQH· and UQH·/UQH$_2$ couples are considered as two pools of electron carriers operating independently, at least in a kinetic sense, in a linear electron-transport scheme, as reductants and oxidants of cytochrome b_{50}. These ideas are summarized in Fig. 1a. The possibility of extracting ubiquinone-10 from lyophilized chromatophores of *Rps. capsulata*, Ala pho$^+$ and of reconstituting ATP synthesis by preincubation of depleted particles with pure ubiquinone-10 have allowed studies on the role of this carrier in light-induced electron flow [82]. The data, obtained under continuous illumination, are consistent with a possible role of ubiquinone between cytochrome b and cytochrome c_2, in addition to its function on the reducing side of cytochrome b [82]. These results are also compatible with the effects of the quinone competitive inhibitor, dibromothymoquinone, observed in unextracted chromatophores of *R. rubrum* [112]. However, definitive conclusions should be reached possibly by following the redox changes of cytochromes in single turnover flashes and at appropriate redox potentials in ubiquinone-extracted and reconstituted membranes.

Also the studies by Dutton and co-workers in *Rps. sphaeroides*, GA have indicated the presence of an electron carrier between cytochrome b_{50} and cytochrome c_2 [109]. Similar to *Rps. capsulata*, this redox component has a pH-dependent oxidation reduction potential with a value of +155 mV at pH 7.0, indicating the involvement of one proton per electron transferred. In a recent paper the posible nature of this carrier has been

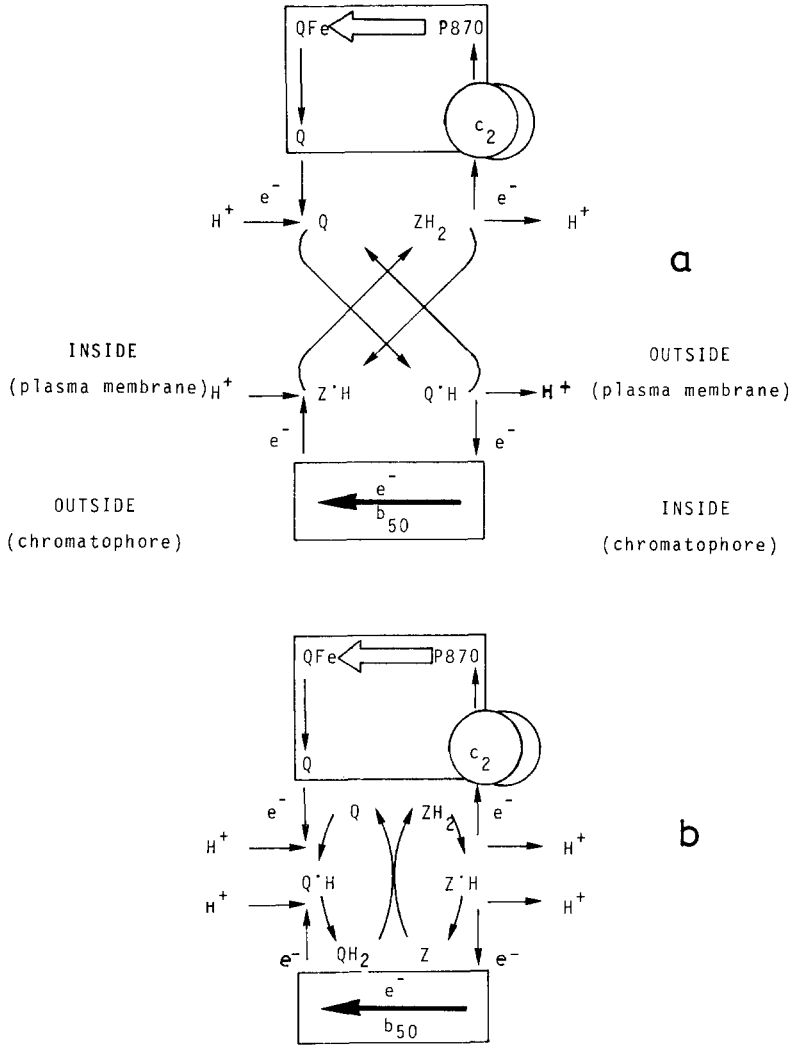

Figure 1. Possible mechanisms of cyclic electron transport in Rhodospirillaceae modified and simplified from refs. 114 and 162. Q is ubiquinone-10; QFe is a ubiquinone–iron complex. The open arrow represents the light reaction; the heavy arrow represents electrogenic electron translocations.

discussed extensively by Prince and Dutton [109]: The authors have taken into account the thermodynamic properties of this carrier and compared them with those of other electron components, known to be present in these photosynthetic membranes (cytochrome b_{-90}; cytochrome b_{+50}; cytochrome b_{+155}; a Rieske type iron sulfur protein, characterized by EPR absorbance at $g = 1.90$; another iron sulfur protein characterized by EPR absorbance at $g = 1.95$; and ubiquinone). All these components, except ubiquinones, are considered as unlikely candidates for the role of a redox mediator in the b–c_2 oxidoreductase region. In the authors' opinion, however, the thermodynamic properties of quinones are still too uncertain for a definitive conclusion and therefore the identity of Z remains an open problem. A scheme proposed by Prince and Dutton [109] for electron flow in *Rps. sphaeroides,* GA which postulates a redox interaction between Q and Z is reported below (Fig. 1b).

This nonlinear model of electron transport bears similarity with one version of the "Q" cycle proposed by P. Mitchell [113], although Q and Z are, in this model, considered as components not necessarily identical and part of the unique ubiquinone pool.

Both schemes (Figs. 1a and 1b) require the existence of a second phase of proton binding which must be sensitive to Antimycin A. This second phase was indeed detected by Cogdell et al. [37], but only in the presence of valinomycin and KCl. Recently Petty et al. [114] have reported that, also in the absence of valinomycin, an Antimycin-sensitive proton is bound to chromatophores of *Rps. sphaeroides,* Ga, with a half time close to 1.5 msec at a redox potential of +130 mV, which fits very well with the current schemes of electron transport in this region. However, discrepancies in kinetics and extent of this second phase of proton binding have also been observed in other redox conditions of the system; these discrepancies are not easily explainable at the present time.

4. Respiratory Electron Flow

Most members of the Rhodospirillaceae family, grown aerobically in the dark, synthesize a membrane-bound respiratory electron-transport system [115–119] that can be considerably more complex than the system generally found in mitochondria from eukaryiotic cells. The variability of such pathways, as far as the nature and number of alternative oxidases is concerned, appears to be quite large, although few species have been studied extensively. It is important to underline in this context that very often

the composition of the respiratory system is dependent upon the conditions and the phase of growth of the culture so that, also for the same strain, it is not always proper to compare or extrapolate results obtained in different laboratories unless special care is taken in reproducing identical conditions of growth.

4.1. The Terminal Oxidases

The *Rhodospirillaceae* family usually possesses oxidases of *b* and/or a/a_3 types.

Oxidases of *b* type which bind CO are classified as cytochromes *"O"* [120] and differ from the classical *b* cytochrome principally in their rapid autooxidability and their property of combining with carbon monoxide and with cyanide in their native state.

Carbon monoxide difference spectra attributed to cytochrome *"O"* (α band at 578–565 nm, β band at 540–535 nm, Soret band at 410–420 nm) have been found in *Rhodospirillum rubrum* [117], *Rhodopseudomonas sphaeroides* [121–123], *Rhodopseudomonas viridis* [124], *Rhodopseudomonas palustris* [125], and *Rhodopseudomonas capsulata* [126]. In *Rhodopseudomonas sphaeroides*, however, cytochrome *"O"* appears to be present only in semiaerobically grown cells in the dark or photosynthetically grown cells [121].

An unusual *b* type cytochrome *c* oxidase, not able to bind CO, has been found in *Rps. capsulata,* St. Louis [127]. This cytochrome shows spectral characteristics of a *b* type component and is not spectrally detectable in photosynthetically grown cells [94]. In membrane particles obtained from aerobic cells, its redox potential at pH 7.0 is about +410 mV and its presence is associated with the cytochrome *c* oxidase activity of *Rhodopseudomonas capsulata* cells [127]. In fact, this component is completely absent in a mutant strain (*Rps. capsulata,* strain M7) that lacks cytochrome *c* oxidase activity, but is still able to grow aerobically [16, 127]. This last property of the M7 strain demonstrates that a second terminal oxidase, alternative to cytochrome *c* oxidase, is present in *Rps. capsulata,* as also suggested by the observation that NADH and cytochrome *c* oxidases of the wild type strain of this organism exhibit large differences in their sensitivity towards KCN [128].

Indeed a CO difference spectrum with broad bands at 420, 539, and 572 nm of chromatophores obtained from aerobic cells of *Rps. capsulata,* M7 after lysozyme and EDTA treatment was attributed to an alternative oxidase [129]. This oxidase was tentatively identified as a *b* type cytochrome with a midpoint potential at pH 7.0 of about +270 mV on the basis of an

observed shift of its redox potential in the presence of CO [129] and can be classified as a classical cytochrome *"O."* A respiration-deficient mutant (M6 strain) with a specific lesion on this oxidase is also available.

In a bacteriochlorophyll-less mutant of *Rps. sphaeroides,* two *b* type cytochromes of high potential can be detected ($E_{m,7}$ = +390 and +255 mV) [130]. These data suggest some similarity between b_{390} and b_{255} of *Rps. sphaeroides* and the two high-potential *b* type cytochromes present in *Rps. capsulata.* However, neither of the *b* components of *Rps. sphaeroides* appears to shift its redox potential in the presence of an atmosphere of nitrogen (50%) and CO (50%); therefore, the function of these two high-potential cytochromes in aerobic cells of *Rps. sphaeroides* still remains obscure [130].

Among members of *Rhodospirillaceae,* only *Rps. sphaeroides* shows a cytochrome *c* oxidase with the same spectroscopic properties as a/a_3 cytochromes of mitochondria [122]. Redox titrations performed on aerobic membranes of *Rps. sphaeroides* at 607 nm showed that this absorption band corresponds to two components with midpoint potentials at pH 7.0 of +200 and +375 mV, respectively [131]. The requirement of copper for the full development of the oxidase was also established suggesting the expected role of copper moieties in the oxidase mechanism [131].

The presence of an *a* type cytochrome, in addition to cytochrome *"O,"* was also reported by Eley et al. [132] in *Rps. palustris* cell-free extracts prepared from cells growing photoautotrophically on thiosulfate. However, no *a* type components could be found when *Rps. palustris* was grown either aerobically or semiaerobically in the dark or anaerobically in the light [125]. Also in this organism a branched respiratory system has been demonstrated by inhibition studies with KCN [125].

4.2. c Type Cytochromes

Cytochrome c_2 (α band of absorption at about 550 nm) seems to be the typical *c* type component common to the *Rhodospirillaceae.* Its redox potential ($E_{m,7}$) is very close to +300 mV, ranging between +288 mV in *Rps. molischianum* [133] and +342 mV in *Rps. capsulata* [126].

It has been suggested that in *R. rubrum,* even if grown aerobically in the dark this component functions only in photosynthetic electron transport and plays a minor role in the main respiratory chain, since it seems not to be readily oxidized following aeration [117, 118]. In membranes prepared from dark aerobically grown *Rps. palustris* cytochrome c_2 can be fully reduced by NADH or succinate and reoxidized by aeration; however, since in the presence of Antimycin it remains reduced even under aerobic steady-

state conditions [125], it was suggested that this component is involved only in a minor branch of the respiratory chain (see also Fig. 2).

In contrast, there are many indications that in *Rps. sphaeroides* [134] and *Rps. capsulata* [126, 129], cytochrome c_2 is localized in the main respiratory phosphorylating pathway, and it is the physiological electron donor to the cytochrome oxidase in these organisms (see, however, ref. 135). Recently, in spheroplasts prepared from *Rps. capsulata,* strain M6 (the respiratory mutant endowed only with a cytochrome oxidase system and lacking the alternative branch [16, 126], the role of cytochrome c_2 in the respiratory chain has been unequivocally demonstrated [136]; see also Section 5. In addition, in aerobic cells obtained from *Rps. capsulata,* strain MT 113, where cytochrome c_2 is completely absent, the capability to oxidize exogenous cytochrome *c* and the ascorbate–DCPIP couple is completely lost (Zannoni et al., in preparation).

Another hemoprotein which exhibits a broad band (550–560 nm) when reduced and combined with CO has been found in large amounts in all species of nonsulfur purple bacteria. This cytochrome has a peculiar spectrum which resembles that of myoglobin, and under acid denaturating conditions it reverts to a typical high-spin hemocromogen spectrum indicating that the heme is the normal *c* type. On the basis of these characteristics, this CO-binding pigment was classified as *c′* cytochrome. This class was originally thought to consist of two groups: a monoheme *c′* class and a diheme *cc′* class. Kennel and co-workers [137] showed that this class distinction does not exist and that *cc′* cytochrome is a dimer of identical subunits with molecular weight around 12,000. However, in order to maintain continuity in the original literature, this nomenclature has been retained. CO-binding pigments classified as *cc′* cytochromes have been found in photosynthetically and aerobically (dark) grown intact cells of *R. rubrum* [116, 138], *Rps. molischianum* [139], *Rps. sphaeroides* [140], and *Rps. capsulata* [141]. Dark grown cells of *Rps. palustris* [139] and *Rps. gelatinosa* [139] contain the monoheme variant of this subclass of cytochrome *c* (*c′* cytochrome).

The periplasmic localization of *cc′* cytochrome has been demonstrated in *Rps. capsulata, St. Louis,* grown aerobically in the dark, by means of lysozyme and EDTA cell-wall digestion [129]. Its midpoint potential at pH 7.0 in the soluble fraction is approximately 0 mV, while $E_{m,7}$ of *Rps. palustris c′* is notably higher ($E_{m,7} = +150$ mV). The physiological and functional role of cytochromes *c′* and *cc′* remains obscure. One possibility is that *c′*-like pigments are terminal oxidases for oxygen respiration. Against this point of view is the fact that the midpoint potential is too low for an

oxidase cytochrome; however, this objection may be countered by the argument that the midpoint potential could be different in the lipid environment of the membrane. In fact, preliminary measurements performed by Dutton (personal communication) on *cc'* cytochrome bound to membrane liposomes indicated a midpoint potential of about +150 mV. Recently the presence of *cc'* cytochrome in aerobically (dark) grown cells of the c_2 mutant of *Rps. capsulata,* strain MT 113 has been associated with the presence of a cytochrome absorbing in the 550–560 nm region, with an apparent midpoint potential ranging between 115 and 140 mV at pH 7.0 (Zannoni et al., in preparation).

Cytochromes of *c* type detectable spectrophotometrically by means of redox titrations performed at 551–540 nm, with a midpoint potential at pH 7.0 very close to +100 mV, have been found in *Rps. capsulata* [126] and *Rps. sphaeroides* [131] grown aerobically in the dark, but it has not been possible to assign functional roles to them. Other *c* cytochromes which have been identified only by their absorption spectra in whole cells or extracts are present in many aerobically (dark) grown nonsulfur purple bacteria. In some cases the multiple absorption bands can be explained as the soluble and the membrane-bound form of the same component, but usually the composition of *c* type cytochromes in the respiratory transport chain in the *Rhodospirillaceae* family is indeed more complex (see refs. 99, 100, and 142 for a complete review of this topic).

4.3 b Type Cytochrome

Cytochromes *b* with midpoint potentials at pH 7.0 of about +185, +44, and −104 mV were reported by Connelly, Jones, Saunders, and Yates [134] in *Rps. sphaeroides* grown aerobically in the dark. In addition, two other *b* type components with a high midpoint potential ($E_{m,7}$ = +390 and +255 mV) have been found by Saunders and Jones [130] in aerobic cells of *Rps. sphaeroides.* With increasing aeration of the culture medium the relative concentration of these two *b* type cytochromes diminished; their presence in low aerated membranes of *Rps. sphaeroides* has not been associated so far with any functional role in the respiratory electron transport system of this organism (see also Section 4.1).

Five *b* type cytochromes distinguished on the basis of their redox potentials ($E_{m,7}$ = +410, +270, +140, +60, and −30 mV at pH 7.0) have been characterized in aerobically (dark) grown *Rps. capsulata* St. Louis [126]. Two of these components, cytochromes b_{410} and b_{270}, seem to be associated with the cytochrome *c* oxidase and the alternative oxidase ac-

tivity, respectively [127, 129] (see Section 4.1). The three *b* type cytochromes at medium-low potential are also present in photosynthetically grown cells; however, only the role of cytochrome b_{60} in the photosynthetic electron-transport flow of *Rps. capsulata* has been clearly demonstrated [95] (compare Section 3.4).

A cytochrome *b* with midpoint potential at pH 7.0 around +50 mV appears to be the major component of the cytochrome *b* pool present in aerobic cells of *Rps. capsulata* St. Louis [126] and *Rps. sphaeroides* [130]. Zannoni et al. [126] showed that a *b* type cytochrome, most likely cytochrome b_{60}, is involved in both branches of the respiratory chain present in *Rps. capsulata,* St. Louis and appears to be in thermodynamic equilibrium with the quinone pool.

The *b* type components of the respiratory chain of *Rps. palustris, Rps. viridis, Rps. molischianum,* and *R. rubrum* have not been as well characterized on the basis of redox titrations as those of *Rps. capsulata* and *Rps. sphaeroides*; however, a low *b* type component ($E_{m,7} = -167$ mV) seems to be common to photosynthetic and aerobic cells of *R. rubrum* [143].

4.4. Quinones

Studies on the function and localization of quinones in the respiratory electron-transport system of heterotropically grown nonsulfur purple bacteria have been performed mainly for *Rps. capsulata* [126] and *Rps. palustris* [144]. The quinone complement of *Rps. capsulata* and *Rps. palustris* has been demonstrated to be confined essentially to a single species of quinone, ubiquinone-10. In *Rps. palustris* the sites of membrane interaction for ubiquinones are structurally different for NADH and succinate oxidase systems [144]; in fact, the site for ubiquinone in the NADH oxidase has, in contrast to the site in succinate oxidase system, a specific requirement for high hydrophobicity of an isoprenoid side chain and for phospholipids [144]. Ubiquinone is also clearly involved in the respiratory chain of dark grown *Rps. capsulata* and seems to be located at the site of branching of the oxidative electron-transport system present in this organism [126].

4.5. Model Schemes of Respiratory Chains

Although it is extremely difficult to generalize and bring order to the confusing array of data so far available, we have selected four models which represent a summary of the aerobic systems found in facultative photosynthetic bacteria (Fig. 2).

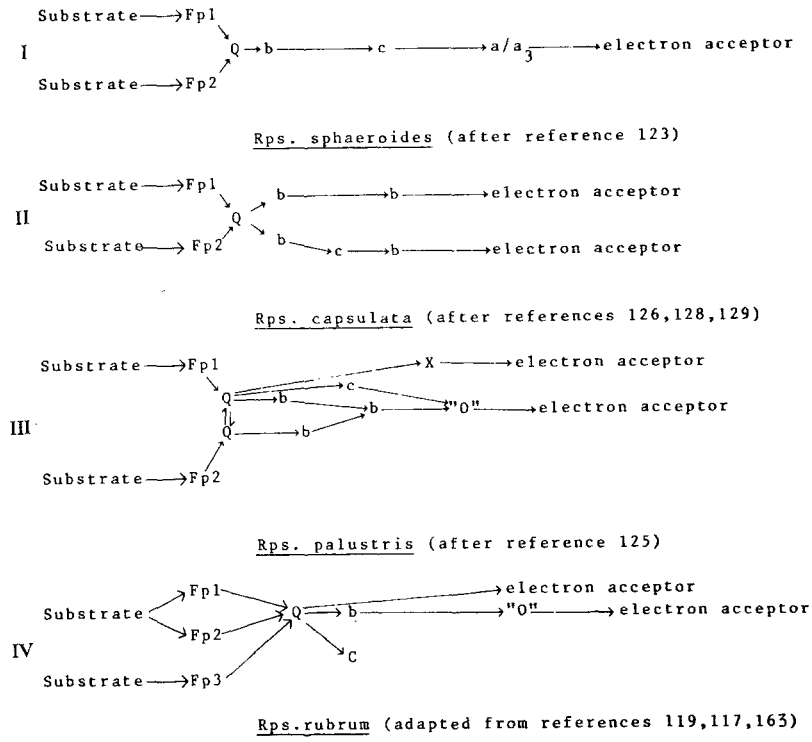

Figure 2. Some models of respiratory electron-transport chains in facultative photosynthetic bacteria: (I) linear; (II) branched; (III) branched and parallel interconnecting; (IV) multibranched not interconnecting; abbreviations: Fp, flavoproteins; Q, ubiquinone; *b*, *c*, *"O,"* *a*, a_3, cytochromes.

It has to be emphasized again that the pathways presented in Fig. 2 are relative to minimal schemes of the respiratory chains, deduced from experimental data obtained under specific conditions of growth, not always completely detailed. Several indications (see, for example, ref. 21) are, however, available which suggest that the biosynthesis of the respiratory apparatus of Rhodospirillaceae can be under strict induction-repression control by the parameters of growth, so that drastic differences are to be expected when other culture conditions are adopted. At least for aerobically grown *Rps. capsulata* it has been shown that the two alternative respiratory pathways differ greatly in their efficiency in oxidative phosphorylation, being the branch operating through cytochrome b_{410} (cytochrome *c* oxidase) but not that operating through cytochrome b_{270}, coupled

to ATP synthesis [128]. Thus the regulation of the relative rates of the two alternative branches in the overall respiration can conceivably be used as a physiological tool for an independent control of the two main functions of the respiratory apparatus, i.e., dissipation of reducing power and synthesis of ATP. For example, when, as a consequence of a decrease of the partial pressure of oxygen, the differentiation of the photosynthetic apparatus is induced and consequently the energy charge in the cell can be kept at high levels by photosynthetic phosphorylation, the excess reducing power (generated especially under particular photoheterotrophic conditions) can be effectively dissipated by the alternative nonphosphorylating branch without the constraints by the respiratory control. Indeed a regulation of this type can be suggested on the basis of preliminary studies in our laboratory [145].

5. Functional Interactions between Photosynthetic and Respiratory Electron Transport Chains

Several components showing similarities in their chemical, thermodynamic, or immunological properties are present in membranes prepared both from photosynthetically and aerobically grown cells of Rhodospirillaceae. For some of them a direct involvement in the two processes has been clearly shown.

The participation of cytochrome c_2 ($E_{m,7}$ around +340 mV) as direct reductant of P 870 (see Section 4) and its localization on the inner part of chromatophore membranes [32] have been clearly established. However, conflicting results on its involvement in respiration have been reported. Recently [136] by means of immunological studies it has been shown that this component undoubtedly plays a role in one of the branches of the respiratory chain of *Rps. capsulata.* A monospecific antibody against homogeneous cytochrome c_2, purified from photosynthetically grown cells, inhibits quite effectively succinate respiration in spheroplasts of cells of the M6 mutant of *Rps. capsulata,* a strain which lacks alternative oxidases, which could obscure the function of this carrier. These experiments indicate clearly that cytochrome c_2 is an obligate component of one branch of the respiratory chain and that, in analogy with photosynthetically grown cells, it is located on the periplasmic phase of the cytoplasmic membrane. Thus cytochrome c_2, in the respiratory and photosynthetic system, is, at least in *Rps. capsulata,* not only thermodynamically indistinguishable but also immunologically cross reacting.

In addition to cytochrome c_2 the presence in photosynthetic and respiratory systems of other carriers of similar, if not identical, nature has also been proved. Quinone coenzymes are universally present in the photosynthetic cyclic electron system of Rhodospirillaceae; in the organisms studied so far, *Rps. palustris* and *Rps. capsulata,* ubiquinone-10 has also been found to be part of the respiratory systems [126, 144]. Photosynthetic or aerobic differentiation of the cell does not alter in a significant way the nature of the quinone pool nor its size, on a membrane protein basis. Given its lipophilic nature and its relative abundance in the membrane, coenzyme Q could conceivably act as a diffusible pool of redox carriers, shared by a photosynthetic and a respiratory chain coexisting on the same membrane system. A cytochrome *b* with midpoint potential at pH 7.0 around +60 mV is clearly involved in photosynthetic electron flow both in *Rps. capsulata* and *sphaeroides* (Section 3), and circumstantial evidence suggest also a role for it in the respiratory chains of these organisms (Section 4). The identity in photosynthetic and oxidative phosphorylation of the ATP-synthesizing complex [or at least of its intrinsic part (F1)] has also been clearly established [146–148] by means of immunological techniques, structural studies of the purified protein, and reconstitution experiments in heterologous membrane systems.

Thus a substantial segment of the electron-transport chains, from ubiquinone-10 to cytochrome c_2, and the ATP synthetase coupled to it, is quite likely identical in photosynthetically or aerobically differentiated cells. This possibility can easily be seen by looking at Fig. 3 where, as an illustrative example, simplified schemes of the photosynthetic and respiratory electron-transfer chains of *Rps. capsulata* (already discussed in Sections 3 and 4) are compared. From this figure it appears self-evident that the ubiquinone–cytochrome c_2 oxidoreductase region can be shared between the two energy-transducing systems.

This situation offers obviously relevant biosynthetic advantages, since the induction of the respiratory electron chains could be limited only to that of the terminal oxidases and of the dehydrogenase region, and conversely the photosynthetic differentiation could be envisaged as the insertion in a preexisting respiratory system of the photosynthetic reaction center complex and of the associated "antenna" pigments. Indeed attempts to reproduce "in vitro" this possible mode of interaction have been performed. Jones and Plewis [149] and Hunter and Jones [150] have shown that incubation of membranes from aerobically grown cells of a mutant strain of *Rps. sphaeroides,* unable to synthesize bacteriochlorophyll, with purified reaction centers from the blue green mutant of the same organism

leads to the formation of a reaction center–membrane complex, in which photoinduced cytochromes *b* and *c* redox changes, sensitive to Antimycin A, could be observed. Light-induced phosphorylation, which could offer an unequivocal proof of the successful reconstitution of an energy-transducing photosynthetic electron flow, has not been reported so far by the authors. On the other hand, Garcia et al. [151], using a similar approach, have reported on the reconstitution of light-induced ATP synthesis, although at very limited rates.

Several examples of interactions both "in vivo" and "in vitro" between the respiratory and photosynthetic apparatuses in facultative photosynthetic bacteria can be found in the literature. The partial inhibition of respiration by light in intact cells of Rhodospirillaceae has long been known [152]; in cell-free systems, however, the situation is rather confused since both inhibition and stimulation by light of NADH and succinate respiration has been reported by different authors [119, 153–156]. In a detailed study on membrane fragments from photosynthetically grown *R.*

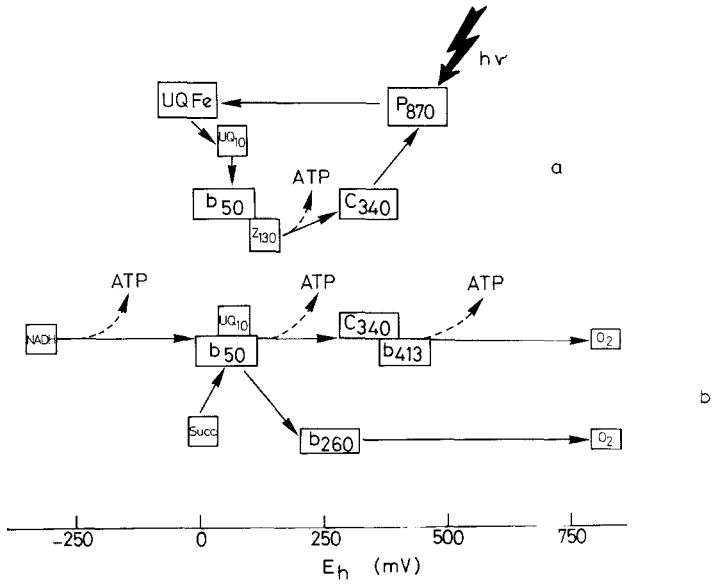

Figure 3. Simplified schemes of the photosynthetic (a) and respiratory (b) electron-transport chains of *Rps. capsulata* (from refs. 95, 126, and 129).

rubrum, Thore et al. [119] have shown that NADH oxidation was inhibited by 35–50% in the presence of light and that this inhibitory effect was reversed by Antimycin A; further addition of uncouplers increased the oxidase activity well above the dark control, especially if a branch of the respiratory pathway was also at the same time inhibited by KCN. The authors interpreted these results by proposing a branched respiratory system (see also Fig. 2) which shares a common segment (UQ–cytochrome c_2) with the cyclic electron flow system and a direct redox interaction between the two apparatuses.

In addition to direct redox reactions a more indirect kind of interaction between the two systems has been demonstrated: Light-driven reduction of pyridine nucleotide from such electron donors as succinate, ascorbate-dichlorophenolindophenol, and even molecular hydrogen has generally been attributed to an energy-dependent reversal of electron flow in the NADH dehydrogenase region of the respiratory chain, exploiting the energy derived from the exergonic reactions of photosynthetic electron flow [157–159]. This view is substantiated by the value of the midpoint potential of the primary acceptor of photosynthesis, which appears too positive to be consistent with a direct photoreduction of NAD^+, by the inhibition of NADH photoreduction by uncouplers, or by the competition with a phosphate acceptor system and by its stimulation in the presence of oligomycin [160]. Although this interpretation is widely accepted, there have been arguments against it [25], based mainly on reported more negative values of the midpoint potential of the primary acceptor (see also Section 3.2) and in some cases on unclear response to inhibitors.

All these studies are therefore consistent with the presence in the membranes of Rhodospirillaceae of a dual functional electron-transport chain competent both for the respiratory and photosynthetic electron transport (similar conclusions are also reported in ref. 161).

The chemical, thermodynamic, and immunological identity of the electron-transport carriers cannot, however, be considered sufficient for the demonstration of this hypothesis, since no conclusive data are so far available on the kinetic competence of the electron-carrier pools for both the photoinduced and oxygen-linked electron flow in the same membrane system. The complexity of branched respiratory systems and the lack of detailed knowledge of the nature and properties of the electron-transport carriers involved has prevented so far these kinds of studies. The availability of respiratory mutants in which only a linear respiratory pathway is operative offers now a possibility of experimental approach to these problems.

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